Virtual

# VANCOUVER NANOMEDICINE DAY

September 17, 2020

University of British Columbia
Vancouver, BC

## KEYNOTE SPEAKER

**ROBERT LANGER**
Massachussets Institute of Technology, Boston, USA

## INVITED SPEAKERS

**VITO FODERÀ**
University of Copenhagen, Denmark

**LUCIA GEMMA DELOGU**
University of Padua, Italy

**CHRISTINE ALLEN**
University of Toronto


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For questions:
URS.HAFELI@UBC.CA

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# VANCOUVER NANOMEDICINE DAY 2020 - THURSDAY, SEPTEMBER 17, 2020

https://www.nanomedicines.ca/nmd-2020/

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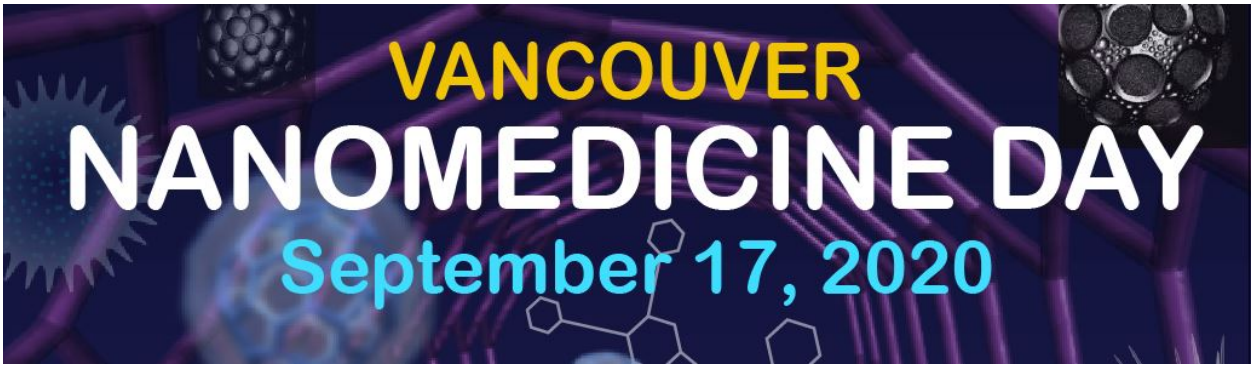
| 8:00 AM | **Urs Hafeli** | **Welcome to the 6th Vancouver Nanomedicine Day 2020** | Pharmaceutical Sciences, UBC |
| | **Session 1** | | **Chair: Urs Hafeli, UBC** |
| 8:05 AM | **Emmanuel Ho** | **Introduction to Nanomedicines** | School of Pharmacy, University of Waterloo |
| 8:25 AM | **Mads Daugaard** | **Targeting Glycosaminoglycans in Solid Tumors** | Vancouver Prostate Centre, UBC |
| 8:40 AM | **INVITED TALK: Vito Foderà** | **Protein self-assembly: the good, the bad and the ugly** | Department of Pharmacy, University of Copenhagen, Denmark |
| 9:20 AM | **SHORT BREAK** | | |
| | **Session 2** | | **Chair: Marcel Bally, UBC + BC Cancer** |
| 9:30 AM | **Karla Williams** | **Nanoscale Flow Cytometry Analysis of Extracellular Vesicles for Liquid Biopsy Development in Cancer** | Pharmaceutical Sciences, UBC |
| 9:45 AM | **INVITED TALK: Christine Allen** | **Complexity and Reality: The Case of Thermosensitive Liposomes** | Leslie Dan Faculty of Pharmacy, University of Toronto |
| 10:25 AM | **SHORT BREAK** | | |
| | **Session 3: Nanomedicines for Drug Delivery** | | **Chair: Christian Kastrup, UBC** |
| 10:30 AM | **Shyh-Dar Li** | **Development and Fabrication of Surfactant-based Liposomes for Drug Targeting** | Pharmaceutical Sciences, UBC |
| 10:45 AM | **Marcel Bally** | **Metals in Liposomes** | BC Cancer & UBC |
| 11:05 AM | **KEYNOTE TALK: Robert Langer** | **Microtechnologies and Nanotechnologies in Drug Delivery** | Massachusetts Institute of Technology, Boston, USA |
| 11:45 AM | **Robert Langer** | **Extended Keynote Q&A** | |
| 11:55 AM | **Diana Royce / Pieter Cullis** | **A Canadian Catalyst: NanoMedicines Innovation Network (NMIN)** | NMIN, Canada |
| 12:00 PM | **LUNCH BREAK & POSTER SESSION** | | |
| | **Session 4: Covid-19 Related Nanomedicines** | | **Chair: Federica Di Palma, Genome BC** |
| 12:40 PM | **INVITED TALK: Lucia Gemma Delogu** | **Nanomaterials, Graphene and Immune Cells - From Biomedical Applications to Fighting COVID-19** | University of Padua, Italy |
| 1:20 PM | **Ying Tam** | **A Novel Vaccine Approach Using Messenger RNA-Lipid Nanoparticles: Preclinical and Clinical Perspective** | Acuitas Therapeutics, Vancouver, BC |
| 1:35 PM | **Ralph Pantophlet** | **Neutralizing Monoclonal Antibodies to SARS-CoV-2 and Prospective Applications** | Health Sciences, Simon Fraser University |
| 1:50 PM | **SHORT BREAK** | | |
| | **Session 5: Rapid Talks (5 min each including discussion)** | | **Chair: Kathy Saatchi, UBC** |
| 2:00 PM | **Daniel Gonzalez-Carter** | **Targeting Nanoparticles to the Brain by Exploiting the Blood/Brain Barrier Impermeability to Selectively Label the Brain Endothelium** | Innovation Center of NanoMedicine, Kawasaki, Japan |
| 2:05 PM | **Maria Poley** | **Chemotherapeutic Nanoparticles Accumulate in the Female Reproductive System During Ovulation Affecting Fertility and Anticancer Activity** | Technion - Israel Institute of Technology, Haifa, Israel |
| 2:10 PM | **Pamela Schimmer / Michael Valic** | **Large Animal Species for Evaluating Long-circulating Nanomaterial Toxicokinetics: Comparisons Between Primates, Canines, and Rabbits** | Princess Margaret Cancer Centre, Toronto, ON |
| 2:15 PM | **Ben Ouyang** | **The Dose Threshold for Nanoparticle Tumour Delivery** | University of Toronto, Institute of Biomaterials and Biomedical Engineering |
| 2:20 PM | **Richard Holland** | **PH-Responsive Endosomal Release Agents to Enhance RNAi Conjugate Activity Across Multiple Cell Types and Receptors** | Genevant Sciences, Vancouver, BC |
| 2:25 PM | **Jayesh Kulkarni** | **Spontaneous, Solvent-Free Entrapment of siRNA Within Lipid Nanoparticles** | Center for Molecular Medicine and Therapeutics, UBC |
| 2:30 PM | **Marta Overchuk** | **Photodynamic Priming as a Means of Enhancing Nanomedicine Delivery and Overcoming Tumour Desmoplasia** | Princess Margaret Cancer Centre, University Health Network, Toronto, ON |
| 2:35 PM | **SHORT BREAK** | | |
| | **Session 6: Debate** | | **Chair: Emmanuel Ho, University of Waterloo** |
| 2:40 PM | **Marcel Bally / Kishor Wasan** | **Debate: Are Nanomedicines Still the Next Big Thing?** | BC Cancer & UBC / UBC & iCo Therapeutics |
| 3:10 PM | **Urs Hafeli** | **Poster Prizes** | Pharmaceutical Sciences, UBC |
| 3:15 PM | **END OF VANCOUVER NANOMEDICINE DAY 2020** | | |




Dear Participants,

It is my great pleasure to welcome you to the 6th **Vancouver Nanomedicine Day 2020**. This time online and virtual. It is an honor to have so many of you check in with us and learn more about the truly amazing field of nanomedicine.

During Nanomedicine Day, you have the opportunity to listen to 18 talks and browse through 93 posters that highlight the discoveries and innovations in nanomedicines. Nanomedicines contribute to global progress in acute, chronic and orphan disease treatment and management. Nanomedicines have allowed us to deliver drugs directly to disease sites, to dramatically improve their efficacy and reduce their toxicity, and to enable gene therapies employing RNA and DNA with the potential to treat most human diseases, including COVID-19. Diagnostics and imaging agents based on nanotechnology will help us to detect disease earlier and to more accurately monitor the effectiveness of therapy.

We are very thankful to our sponsors in life science and startup biotechnology companies, that help with getting a great line-up of speakers, including the keynote by Robert Langer, three poster prizes, and a fun debate at the end of the day.

Please take the occasion of this meeting as a starting point for future collaborations with your Canadian research friends, both in- and outside the co-sponsoring Nanomedicines Innovation Network – NMIN, but also with the many international participants present. The statistics are not final yet, but as of two days before the meeting, we had 850 registered participants from 38 countries. What a lineup!

Thank you all for spending time with us and I hope to see you next year again.

For the organizing committee,

**Urs Hafeli**

Professor, Faculty of Pharmaceutical Sciences, University of British Colombia, Vancouver, BC, Canada urs.hafeli@ubc.ca
Lundbeck Foundation Joint Professor, Department of Pharmacy, University of Copenhagen, Denmark

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## TALKS

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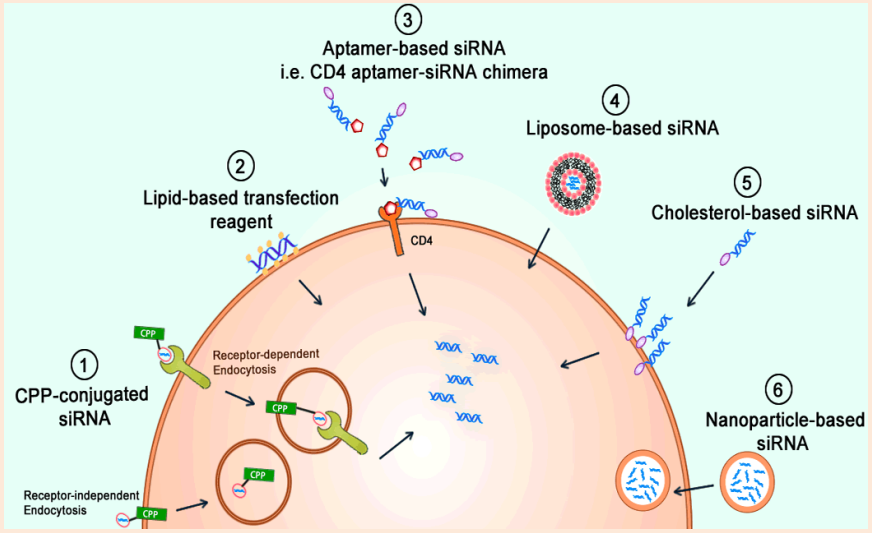


Yang S., Chen, Y., Ahmadie, R. and Ho EA. 2013 J Control Release (167): 29-39

# Introduction to Nanomedicines

Emmanuel A. Ho

School of Pharmacy, University of Waterloo, Kitchener, Ontario Canada

Department of Chemical Engineering, Faculty of Engineering

Waterloo Institute for Nanotechnology

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Nanomedicine is broadly defined as the "application of nanotechnology for the diagnosis, prevention and treatment of disease and to gain increased understanding of the complex underlying pathophysiology of disease. The ultimate goal is to improve quality of life"[1]. For the past several decades, the field of nanomedicine has evolved significantly and rapidly. It is truly an interdisciplinary field that incorporates multiple branches of science including, but not limited to chemistry, pharmaceutical sciences, engineering, physics, biology, etc. Continued development of new nanomaterials has enabled its applications in a broad range of disease states, all with the hopes of improving global health care.

Dr. Ho's research program is focused on the development and characterization of innovative drug delivery platforms and biomaterials for the treatment and prevention of sexually transmitted infections, chronic wounds and ocular diseases. This includes targeted nanomedicines, implantable devices for sustained drug delivery, and biodegradable platforms such as gels and films.

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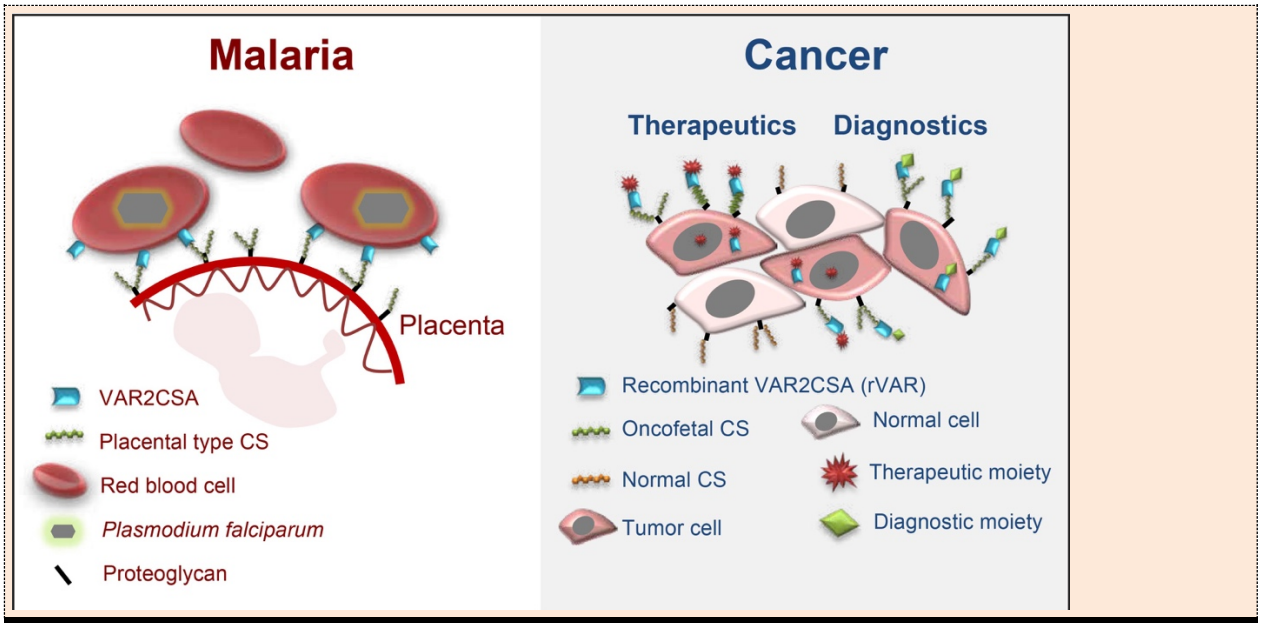


# Targeting glycosaminoglycans in solid tumors

**Mads Daugaard**

Vancouver Prostate Centre| Department of Urologic Sciences | University of British Columbia
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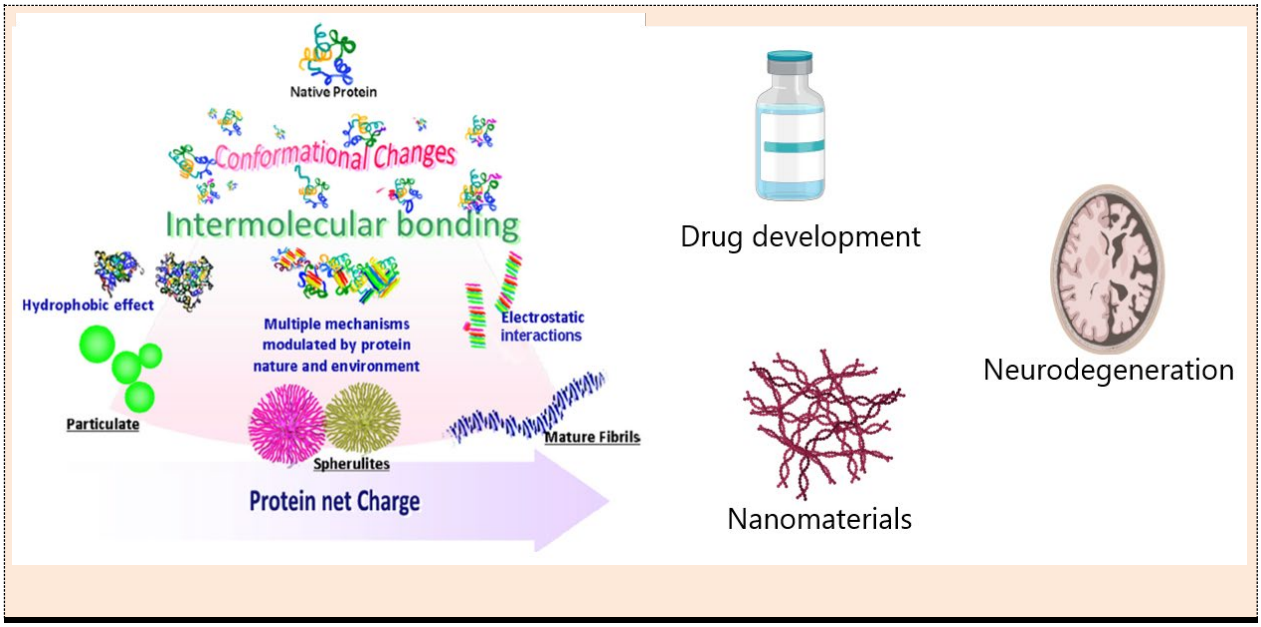
Solid tumors reform expression and composition of glycosaminoglycans to promote tumor progression. Distinct cancer-specific GAGs are candidate targets for therapy, but GAG targeting is challenging due to lack of specific binding technologies. Malignant and placental tissue compartments display a common *oncofetal* chondroitin sulfate GAG subtype that can be targeted by recombinant malarial VAR2CSA (rVAR2) proteins (**Graphical abstract**). In most cancers, oncofetal chondroitin sulfate is redundantly conjugated to a limited repertoire of proteoglycans differentially expressed on the cell surface and in the microenvironment. Accordingly, rVAR2 proteins can be employed in diagnostic and therapeutic applications to detect and target various types of human tumors. Our work exemplifies how evolutionarily refined parasite host-anchor molecules can be conveniently exploited to target specific cancer-associated GAG chains.




# Protein self-assembly: the good, the bad and the ugly

Vito Foderà

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Proteins are intrinsically prone to self-assembly and they may form, both *in vivo* and *in vitro*, a wide range of supramolecular structures. Understanding protein aggregation is crucial in neurosciences, pharmaceutical sciences and nanotechnology. Indeed, deposits of protein aggregates are associated with the onset of pathologies such as Alzheimer´s and Parkinson´s diseases. Equally important is the impact that protein aggregates may have on the quality of a protein drug product. Finally yet importantly, protein aggregates have unique structural properties, making them appealing materials for applications in drug delivery and nanomedicine.

Either one looks at protein aggregation in the context of diseases, drug development or biomaterials, unravelling the mechanisms ruling the self-assembly reaction is of vital importance.

In our team, we have reported the possibility for a large number of proteins to form a variety of protein aggregates, ranging from microparticles and core-shell structures to elongated fibrils (see graphical abstract) [1]. I will present our approach based on advanced fluorescence microscopy, small angle X-ray scattering and spectroscopy and aimed at identifying the key interactions responsible for such variability in structures and morphologies [1-3]. I will also present our platform for the design and production of highly tuneable electrospun protein nanomaterials for the release of antibiotics [4] and as advanced matrices for nicotine replacement treatments [5].

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[2] Vetri, V. et al. **2018 J Phys Chem B**, 122, 3101-3112.
[3] De Luca et al. **2020 J. Coll. Int. Sci.** 574, 229-240
[4] Stie MB et al **2020 ACS Appl. Nanomat.** 3, 2, 1910-1921
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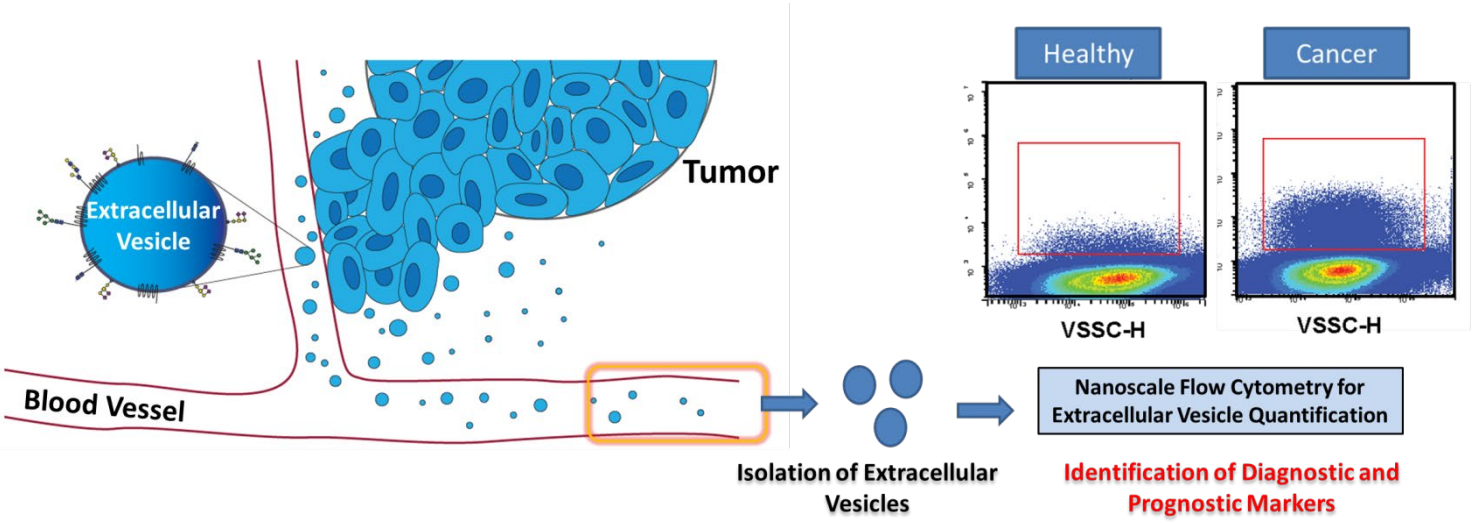


# Nanoscale flow cytometry analysis of extracellular vesicles for liquid biopsy development in cancer

Karan Khanna[1], Nikki Salmond[1], Karla Williams [1]*

[1]Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC V6T 1Z3, Canada; karla.williams@ubc.ca

Extracellular vesicles (EVs) are lipid membrane enclosed nano-sized structures released into the extracellular environment by all cell types. EV constituents include proteins, lipids and nucleic acids that reflect the cell from which they originated. The proteome of cancer cells is distinct as compared to healthy cells of the same tissue type, and this distinct proteome should be reflected by the EVs they release. This makes EVs desirable candidates for blood-based biopsy diagnosis of cancer. EVs can be time consuming to isolate therefore, a technology that can analyze EVs in complex biological samples in a high throughput manner is in demand. We have worked towards the optimization of nanoscale flow cytometry for use in the analysis of EVs in whole, unpurified, plasma samples.[1] Using this technology we have identified circulating STEAP1 (six-transmembrane epithelial antigen of the prostate 1)-positive EVs in the plasma of prostate cancer patients and evaluated its diagnostic and prognostic significance.[2] We find that STEAP1-positive EVs are elevated in prostate cancer and levels are significantly associated with a prostate cancer diagnosis. However, no association was found between STEAP1 EV levels and disease recurrence or overall survival. Our results identify and quantitate STEAP1-positive EVs in plasma and provide rationale for a diagnostic strategy in prostate cancer through the analysis of STEAP1-positive EVs.

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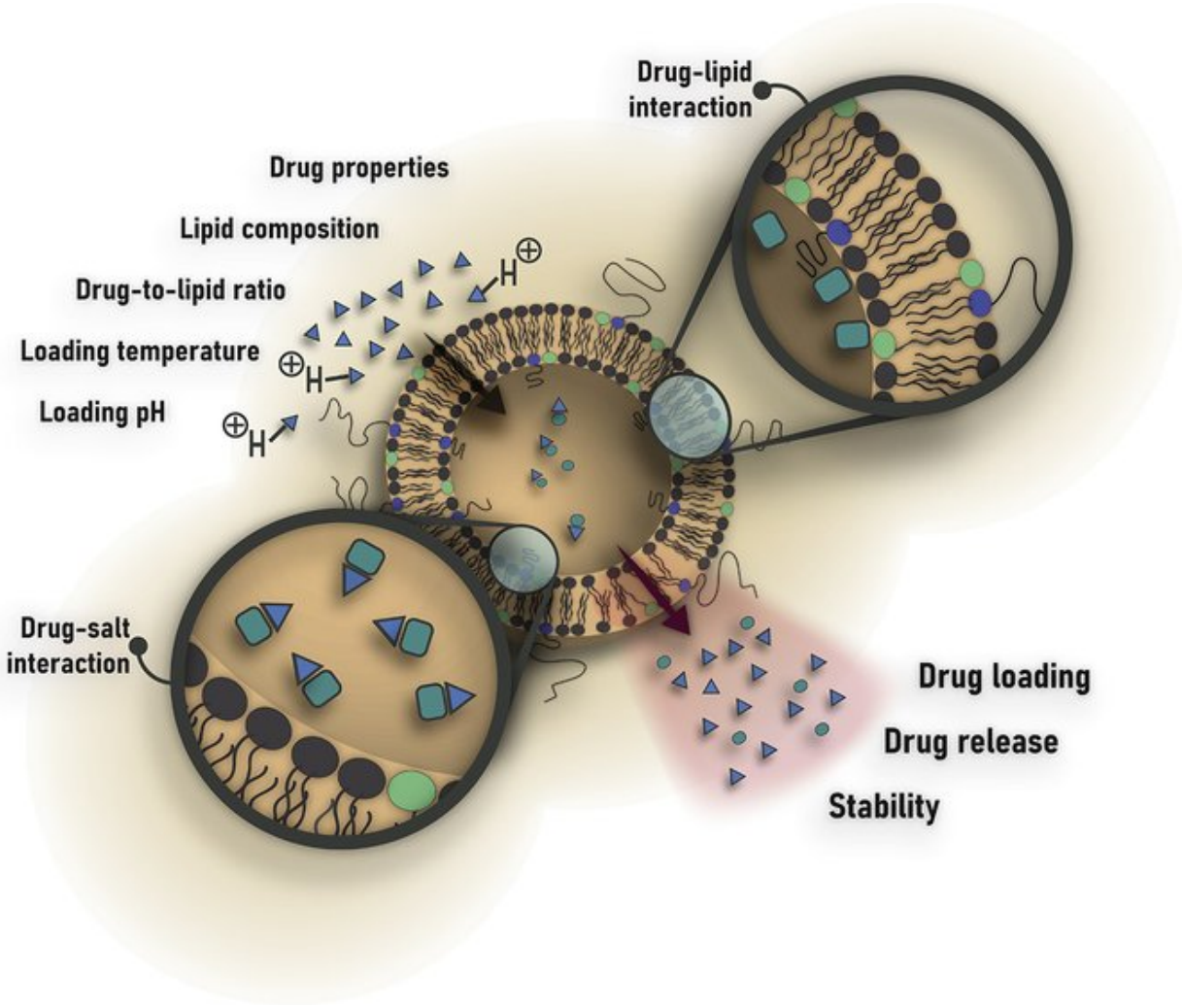


# Complexity and Reality: The Case of Thermosensitive Liposomes

Christine Allen

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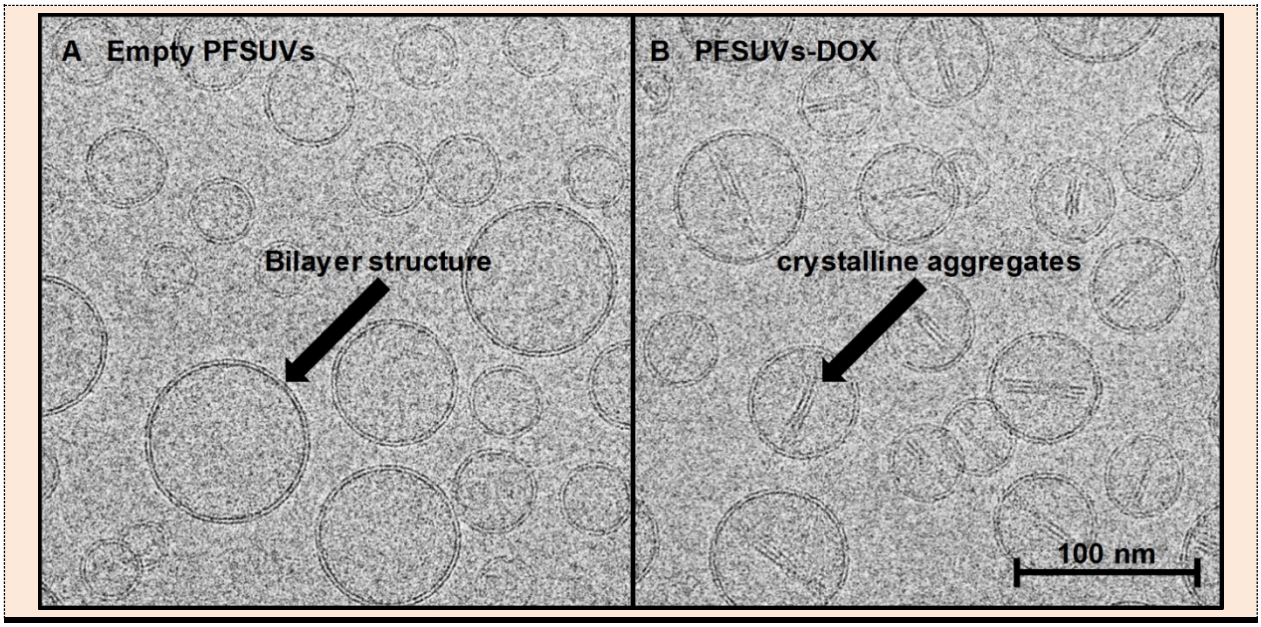


# Development and Fabrication of Surfactant-based Liposomes for Drug Targeting

Shyh-Dar Li

Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC V6T 1Z3, Canada; shyh-dar.li@ubc.ca

Surfactants can be incorporated into liposomes to grant specific functions for drug targeting. An ultra-fast thermosensitive liposomal (uTSL) formulation composed of DPPC and Brij78 (96:4, mol) was developed for targeted delivery of an anticancer drug, doxorubicin (DOX) [1]. This **H**eat-**a**ctivated cyto**T**oxic (HaT) uTSL formulation was stable at 37°C, but burst-released >80% DOX at 42°C in 20 s. The HaT formulation displayed increased DOX release rates at the mild hyperthermic temperatures (39-42°C), ~2-fold increased drug delivery to the locally heated tumor, and significantly enhanced antitumor activity compared to the standard **L**ysolipid **T**emperature-**s**ensitive **L**iposomal (LTSL) formulation. One dose therapy with HaT-DOX effectively shrank the tumor in a murine breast cancer model.

A phospholipid-free small unilamellar vesicular (PFSUV) formulation composed of cholesterol and Tween80 (5:1, mol) was fabricated using microfluidics [2]. PFSUVs displayed a monodispersed particle size of 60 nm and maintained a transmembrane gradient for active loading of weak base drugs (**Figure**), which could be stably retained in PFSUVs when incubated with serum. After i.v. administration, PFSUVs effectively accumulated in the liver in 5 min. Within the liver, 70% and 30% of the hepatocytes and sinusoidal cells were positive with PFSUVs, respectively, indicating the high hepatocyte-selectivity. Preliminary data suggest that PFSUVs target hepatocytes via the LDL receptor pathway. PFSUVs is being investigated as a drug delivery platform for treating various liver diseases.

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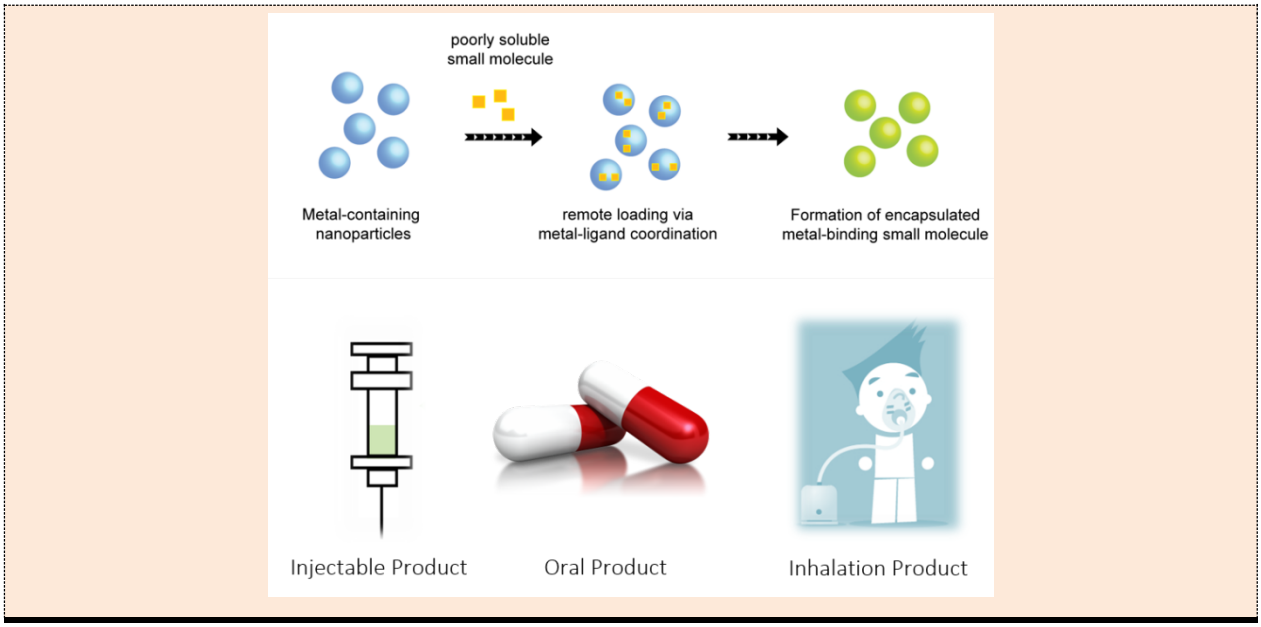


# Metals in Liposomes

Marcel B Bally[1,2,3,5,6], Thomas Redelmeier[2], Mike Abrams[2],
Kevin Sun[2,4], Kent Chen[2,5], and Ada Leung[2]

[1]Experimental Therapeutics, BC Cancer's Vancouver Research Centre, Vancouver, BC, Canada, V5Z 1L3 mbally@bccrc.ca or mbally@cuprous.ca; [2]Cuprous Pharmaceuticals Inc., Vancouver, BC, Canada aleung@cuprous.ca; [3]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada; [4]Interdisciplinary Oncology Program, University of British Columbia, Vancouver, BC, Canada; [5]Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada; [6]Nanomedicine Innovation Network, NCE, Canada

Remote loading technology, where a drug or drug candidate is added to the outside of pre-formed liposomes and is subsequently sequestered into the liposomes, was first described over 35 years ago. The method typically involves the use of a transmembrane pH gradient (inside acid or inside basic) and the addition of a drug candidate that has an ionizable function. If the pH outside the liposome is one where the majority of the candidate drug is in a neutral form, then it can permeate across the liposomal lipid bilayer. Once inside the liposome, the drug candidate encounters a different pH and becomes charged. Since the charged form of the drug candidate is far less permeable across the lipid bilayer it becomes essentially "trapped" inside. The rate of drug loading and the rate of drug release is dependent on a variety of factors including liposomal lipid composition, the stability of the transmembrane ion gradient, and temperature. The rate of drug release in biological assays (e.g. serum containing tissue cultures or following injection into animals) is also influenced by the presence of surface bound proteins (protein corona) and the presence of an external "sink" that can remove any of the candidate drug following dissociation from the liposome. Other approaches have been considered for remote loading drugs, one of which my team has a specific interest in and involves the use of divalent metal ions. One of the challenges with using divalent metals concerned the fact that as the pH increases to >6 metals can bind to hydroxide forming an insoluble precipitate. Thus metal ion gradients are typically formed using low pH solutions. Thus if a selected compound has a metal binding function and an ionizable group it has been difficult to differentiate between whether a compound is remote loaded in response to a pH gradient or a metal ion gradient. One way to address this is to use a metal that is pre-complexed to a compound such as gluconate. Copper gluconate is stable at pH 7 and is able to remote load selected drugs in response to just metal binding. Another way to address this is to select compounds that only have metal binding functions, thus the existence of a pH gradient would have no impact on remote loading potential. This thought lead to the creation of Metaplex technology: a technology where drug candidates can be encapsulated in preformed liposomes in response to a metal gradient (metal inside). This technology is being developed for a variety of applications and some of these will be discussed in the presentation.

Microtechnologies and Nanotechnologies in Drug Delivery

Dr. Robert S. Langer, Sc.D.
Institute Professor
Massachusetts Institute of Technology

There are numerous new technologies being developed that may impact the future of medicine. For example, new drug delivery technologies including microparticles, nanoparticles and nanotechnology promise to create new treatments for cancer, heart disease and other illnesses. Nanotechnology may also be useful in delivering DNA and siRNA as well. Approaches involving polymers, microchips, and lipids will be examined.


# Nanomaterials, Graphene and Immune Cells - From Biomedical Applications to Fighting COVID-19

Laura Fusco [1], Arianna Gazzi [1,2], Marco Orecchioni [3], Lucia Gemma Delogu [1]*

[1]Department Department of Biomedical Sciences, University of Padua, Padua, Italy; luciagemmadelogu@yahoo.it; luciagemma.delogu@unipd.it [2]Department of Chemical and Pharmaceutical sciences, university of Trieste, Trieste, Italy; [3]Department of Chemical and Pharmaceutical sciences University of Sassari, Sassari, Italy

The potential immune modulation induced by nanomaterials, such as graphene-based materials (GBMs) is a key aspect for their biomedical applications. We previously demonstrated that the different nanomaterial physicochemical properties dramatically modulate their impact on human immune cells [1,2]. We pioneered the use of innovative single-cell mass cytometry approaches in the context of carbon-based nanomaterials, to evaluated the impact of a large set of GBMs (graphene oxides, GOs) with different lateral size and functionalization. We demonstrate that the amino-functionalization of GO enhanced the immune compatibility of the material and was able to induce a specific M1-like activation on monocytes, skewing a cytotoxic-like response with the secretion of interleukin-4 and Granzyme-B from B cells. Moreover, combining graphene with inorganic quantum dots containing indium, we enabled its detection using single-cell mass cytometry on a large variety of primary immune cells. Our results demonstrated that monocytes and, unexpectedly, B cells, showed a superior ability to internalize GO compared to the other immune cell subpopulations. Also, we exploited the immune modulation of a specific GO in combination with the osteoinductive capacity of calcium phosphate to design a new nanomaterial (magoCAP) able to induce bone regeneration *in vitro* and *in vivo* [3,4]. Moreover, we reported how the abundance of engineered materials, identifiable by their useful specific physicochemical properties, can offer new approaches to cope with the COVID-19 pandemic [5]. Our results demonstrate that specific design of nanomaterials offers new strategies for the development of new biomedical applications exploiting their immune modulation as well as to fight the COVID-19 pandemic and infectious diseases in general, including future pandemics.

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## A Novel Vaccine Approach Using Messenger RNA-Lipid Nanoparticles: Preclinical and Clinical Perspective

**Ying Tam**[1]

[1]Acuitas Therapeutics, Vancouver, BC V6T 1Z3 Canada

Acuitas is developing lipid nanoparticle systems (LNP) that allow the efficient delivery and expression of mRNA for a variety of therapeutic applications including protein replacement, passive immunization and gene editing. The most advanced therapeutic application is the use of mRNA-LNP as a prophylactic vaccine against infectious disease. In addition to our internal core research program we support multiple industry and academic collaborations. Results from select academic and partnered collaborations describing preclinical and clinical studies of intradermally (id) and intramuscularly (im) administered mRNA-LNP vaccines to provide protection against infectious diseases will be presented. Further, this update will focus on the clinical translation of our mRNA-LNP as a prophylactic vaccine against SARS-CoV2. Information on the potency and safety profile of a id- and im- administered SARS-CoV2 mRNA-LNP vaccine will be presented.

# Neutralizing Monoclonal Antibodies to SARS-CoV-2 and Prospective Applications

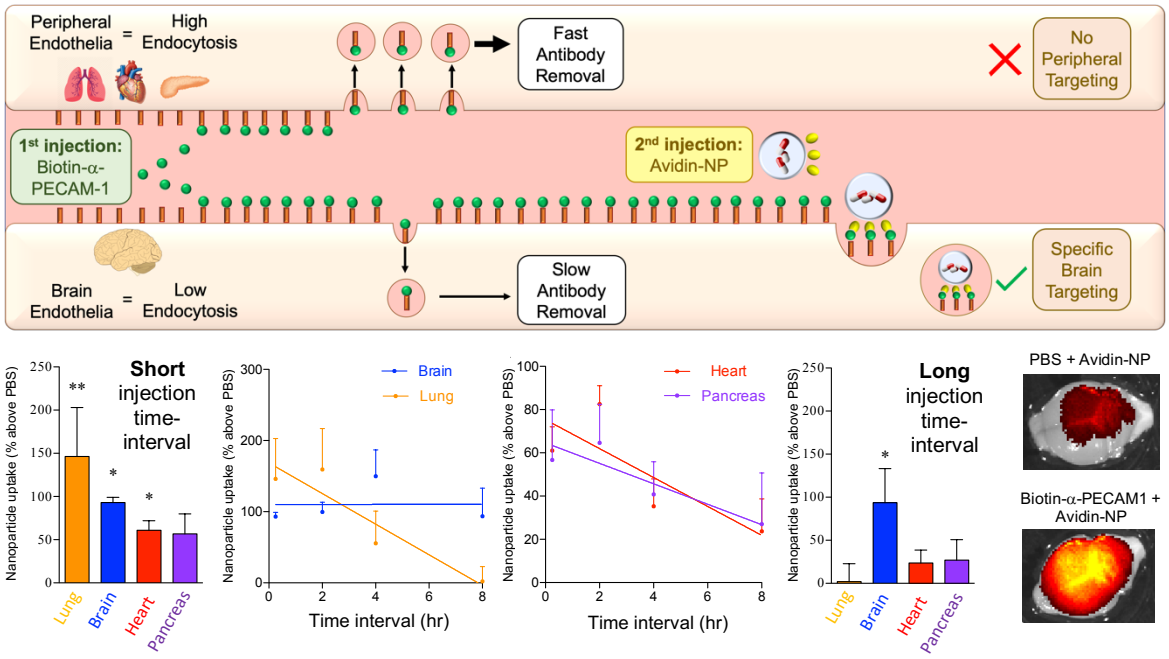
Ralph Pantophlet

Faculty of Health Sciences, Simon Fraser University, Vancouver, BC V5A1S6, Canada; rpantophlet@sfu.ca

More than 27 million people around the world have now been infected with SARS-CoV-2 (https://coronavirus.jhu.edu/map.html). There is general belief that only an effective vaccine will be able to prevent further spread of the virus and significantly curb the pandemic. However, until an effective COVID-19 vaccine is deployed globally, it will be necessary to have therapeutic agents that can at least help to reduce the severity of disease in individuals who become infected and require hospitalization.

In this presentation, I will review current understanding of the antibody response to SARS-CoV-2, which is relevant to vaccine development and immunotherapy with monoclonal antibodies. The talk will focus in particular on so-called neutralizing antibodies (nAbs), which can blunt infection when present at sufficiently high concentration at the time of transmission or limit viremia if administered therapeutically. NAbs against SARS-CoV-2 target the trimeric spike (S) glycoprotein that crowns the surface of virus particles and that is required for viral entry into host cells. Recent studies will be used to highlight important insight into the epitopes targeted by highly potent nAbs. Efforts from my own lab on probing the vulnerability of SARS-CoV-2 glycosylation to nAbs will also be presented.

Scheme 1.




# Targeting nanoparticles to the brain by exploiting the blood–brain barrier impermeability to selectively label the brain endothelium


Daniel Gonzalez-Carter[1*], Xueying Liu[1], Theofilus Tockary[1], Anjaneyulu Dirisala[1], Kazuko Toh[1], Yasutaka Anraku[1,2], Kazunori Kataoka[1,3]

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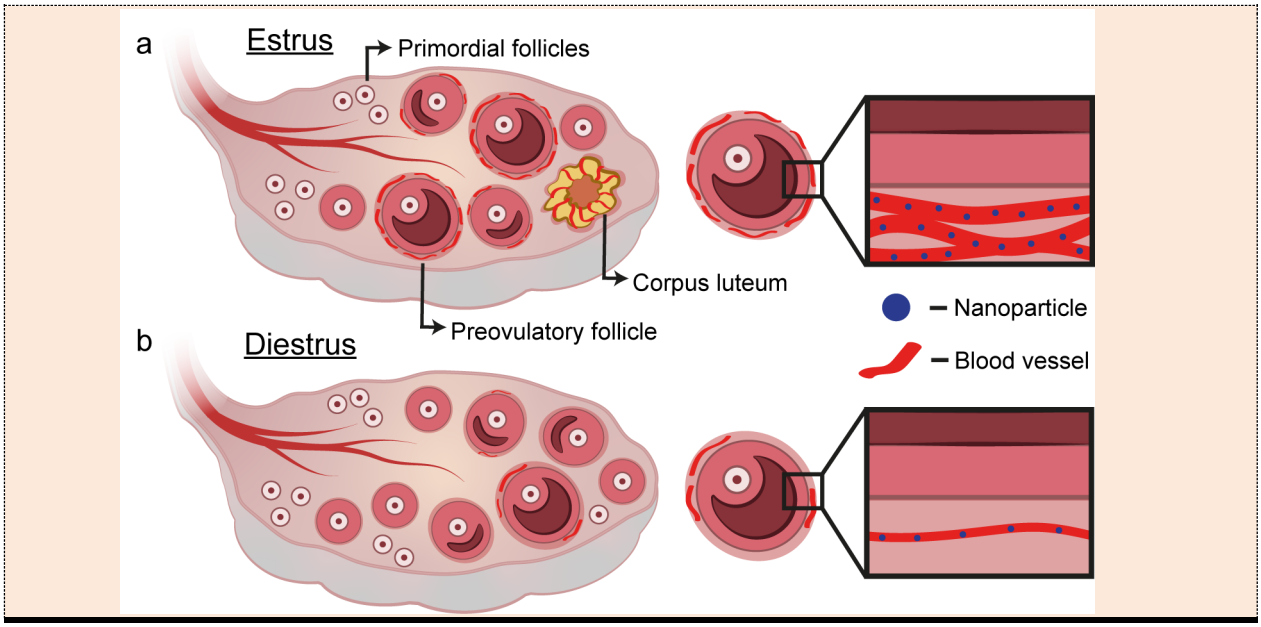
Nanoparticle (NP) brain-delivery strategies targeting proteins overexpressed at the brain micro-vasculature (*e.g.* TfR1, Glut1) have substantial specificity limitations due to significant protein expression in peripheral organs [1]. We have developed a new strategy [2] to target NPs to the brain by instead selectively labelling the brain microvasculature. We exploit the lower endocytic rate of brain endothelial cells (BEC)[3] to promote retention of free ligands (*i.e.* labels) selectively on the surface of BEC. NPs capable of recognizing the endothelial label are subsequently targeted to the brain without peripheral targeting (scheme 1). We demonstrate the *in vivo* feasibility of this strategy by injecting biotinylated α-PECAM1 antibodies (to label endothelial cell surfaces) followed by injection of avidin-functionalized nanoparticles (Avidin-NP) at increasing time-intervals. While short time-intervals result in avidin-NP targeting to the lung, brain, heart and pancreas, long time-intervals result in avidin-NP targeting only to the brain.

The present work therefore provides the basis for a new targeting strategy which exploits the physiology of BEC to generate the required NP targeting specificity.


**References**: [1] Fillebeen, et al. (2019). Blood, 133 (4): 344-355; [2] Gonzalez-Carter, et al. (2020) PNAS doi: 10.1073/pnas.2002016117 (online ahead of print); [3] Ben-Zvi, et al. (2014). Nature, 509 (7501): 507-511.




# Chemotherapeutic Nanoparticles Accumulate in the Female Reproductive System during Ovulation Affecting Fertility and Anticancer Activity

Maria Poley[1], Yael Shammai[1], Maya Kaduri[1], Lilach Koren[1], Omer Adir[1,2], Jeny Shklover[1], Janna Shainsky[1], Irit Ben-Aharon[3,4],Assaf Zinger[5,6,*] and Avi Schroeder[1,*]

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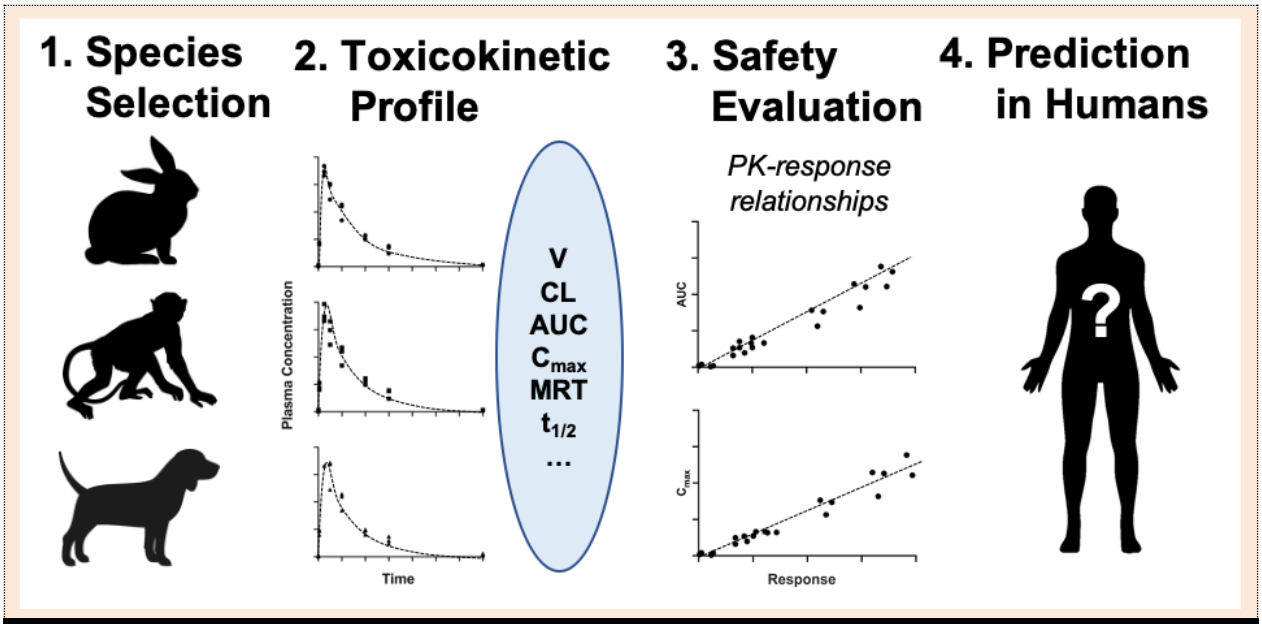
Throughout the female menstrual cycle, physiological changes occur that affect the biodistribution of nanoparticles within the reproductive system. This can have positive or negative effects. We demonstrate a 2-fold increase in nanoparticle accumulation in the ovaries during female mouse ovulation (Fig. 1a) compared to the non-ovulatory stage (Fig. 1b) following intravenous administration. Accumulation in the reproductive system is favored by nanoparticles smaller than 100 nm. Chemotherapeutic nanoparticles administered during ovulation increased ovarian toxicity and decreased short-term and long-term fertility when compared to the free drug. Breast cancer treated with nanomedicines during ovulation results in higher drug accumulation in the reproductive system rather than at the site of the tumor, reducing treatment efficacy. Conversely, ovarian cancer treatment was improved by enhanced nanoparticle accumulation in the ovaries during ovulation. Our findings suggest that the menstrual cycle should be considered when designing and implementing nanotherapeutics for females.




# Large Animal Species for Evaluating Long-circulating Nanomaterial Toxicokinetics: Comparisons between Primates, Canines, and Rabbits

Michael S Valic [1], Carl J Fisher [1], Alexander Gregor [1], Pamela Schimmer [1], Michael Halim [1], Harley Chan [1], Nicholas Bernards [1], Donovan Eu [1], Hong-Zhuan Chen [2], Celina Li [1], Xiao-Ling Gao [2], Kazuhiro Yasufuku [1], Jonathan C Irish [1], Brian C Wilson [1], Gang Zheng [1]*

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The most critical assessment in the preclinical translation of novel nanomaterials is their safety evaluation in large, nonrodent animal species. Conventional routes of testing favour the selection of canine models in single and/or repeated-dose toxicity studies, with nonhuman primates (e.g., macaques) chosen only if canines are deemed scientifically unsuitable. Case in point, questions have been raised regarding the appropriateness of canine models (e.g., beagles) for toxicity and toxicokinetic testing of nanomaterial-based drug delivery systems [1]. Given the risks associated with these pivotal and costly studies for the translational success of nanomaterials, clearer empirically driven guidance on the challenges and considerations for selecting nonrodent animal species for toxicokinetic assessments with long-circulating nanomaterials are highly desired.

Our team performed dose-ranging studies and toxicokinetic assessments with a long-circulating porphyrin-lipid based nanomaterial (Porphysome) in rabbits, beagles, and cynomolgus monkeys. Striking similarities were discovered in the dose-equivalent disposition and pharmacokinetic profiles of Porphysomes in rabbits and monkeys, whereas the profiles in beagles were remarkably different. Specifically, we found greater pharmacokinetic and pharmacologic variability in beagles not observed in the smaller rabbits and monkeys at equivalent doses. Our findings recommend a preclinical translation strategy that employs rabbit and nonhuman primate models in favour of beagles for dose-ranging studies and toxicokinetic assessments of long-circulating nanomaterials.

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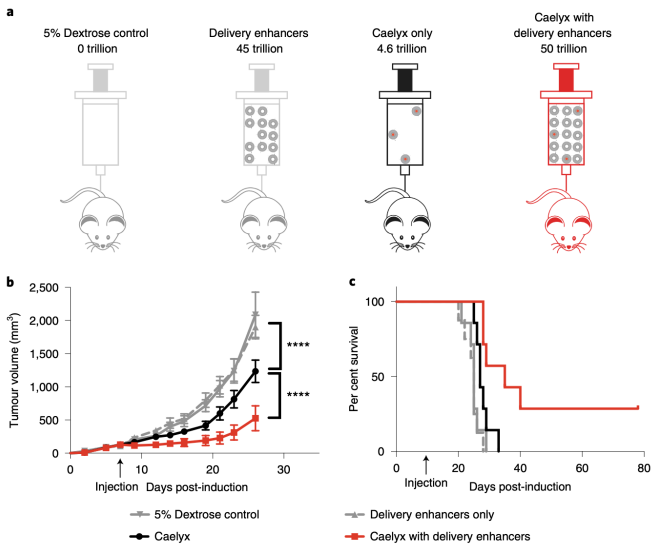


Nature Materials, 2020, 10.1038/s41563-020-0755-z

# The dose threshold for nanoparticle tumour delivery

Ben Ouyang[1,2,3], Wilson Poon[# 2,3], Yi-Nan Zhang[# 2,3], Zachary P. Lin[2,3], Benjamin R. Kingston[2,3], Anthony J. Tavares[2,3,4], Yuwei Zhang[3,5], Juan Chen[6], Michael S. Valic[6], Abdullah M. Syed[2,3], Presley MacMillan[3,5], Julien Couture-Senécal[2,3,7], Gang Zheng[2,6,8], Warren C.W. Chan[* 2,3,9,10,11]

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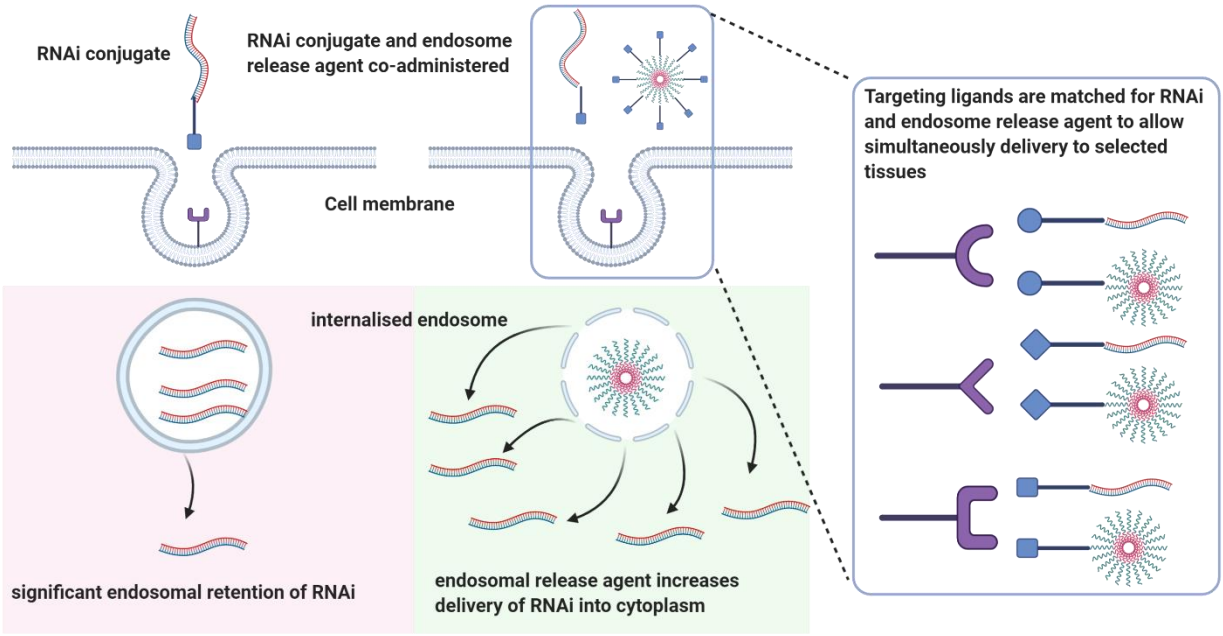
Nanoparticle delivery to solid tumours over the past ten years has stagnated at a median of 0.7% of the injected dose. Varying nanoparticle designs and strategies have yielded only minor improvements. Here we discovered a dose threshold for improving nanoparticle tumour delivery: 1 trillion nanoparticles in mice. Doses above this threshold overwhelmed Kupffer cell uptake rates, nonlinearly decreased liver clearance, prolonged circulation and increased nanoparticle tumour delivery. This enabled up to 12% tumour delivery efficiency and delivery to 93% of cells in tumours, and also improved the therapeutic efficacy of Caelyx/Doxil. This threshold was robust across different nanoparticle types, tumour models and studies across ten years of the literature. Our results have implications for human translation and highlight a simple, but powerful, principle for designing nanoparticle cancer treatments.




# PH-responsive endosomal release agents to enhance RNAi conjugate activity across multiple cell types and receptors

Richard Holland, Mark Wood, Kieu Lam, Lorne Palmer, Xin. Ye, James Heyes

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The great promise of ligand targeted RNA interference (RNAi) therapeutics was further realized in 2019 with the regulatory approval of Givosiran (Givlaari®), a GalNAc-targeted siRNA for treatment of acute hepatic porphyria AHP. Adaptation of this therapeutic modality for cell types beyond hepatocytes has been slow, and, for reasons that are not fully understood, GalNAc mediated delivery appears to be a unique case. Even though conjugates can be targeted to other cell types readily with appropriate ligand selection, little if any of the internalized conjugate escapes the endosome before cellular degradation occurs. This significantly curtails biological activity, in most cases completely.

Genevant has a proprietary, pH-responsive endosomal release agent which can be simultaneously delivered with an RNAi conjugate to cells and tissues of choice by matching the targeting ligand in both entities. Subcutaneous co-administration in nonhuman primate studies has been shown to speed onset of activity and substantially improve both potency and duration of effect of GalNAc conjugates. Moreover, we have a expanding portfolio of extra-hepatocyte ligands with demonstrated biological activity across different cell types positioned to benefit from this unique approach. The modular design of this platform could further enable the realization of RNAi conjugates as therapeutic agents far beyond hepatic diseases.

# Spontaneous, solvent-free entrapment of siRNA within lipid nanoparticles

Jayesh A. Kulkarni[1,2,3,4*], Sarah B. Thomson[2], Josh Zaifman[4,5,6], Yuen Yi C. Tam[4,5], Pieter R. Cullis[1,4*], Terri L. Petkau[2,3], Blair R. Leavitt[2,3]

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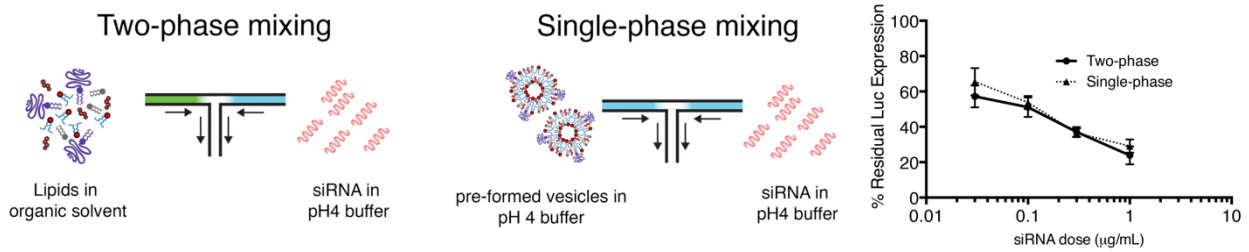
Lipid nanoparticle (LNP) formulations of nucleic acid have established clinical utility and are the enabling technology of the leading vaccines against COVID19, and of Onpattro, the first-ever FDA-approved RNAi therapeutic. These LNPs are composed of ionizable cationic lipids (such as KC2 or MC3), cholesterol, phosphatidylcholine, and poly-ethylene glycol (PEG) lipids and are produced using rapid-mixing techniques where an ethanolic-lipid phase is combined with an acidic aqueous phase containing siRNA. The resulting LNP suspension is prepared for administration by buffer-exchange to neutral pH. The rapid-mixing procedure is a bottom-up manufacturing approach that achieves particle formation and nucleic acid entrapment in a single step. The current paradigm on the mechanism of particle formation suggests that destabilizing agents (such as ethanol or detergents) are essential to achieving efficient entrapment of siRNA, and that specialised mixers are required to improve particle homogeneity.

Recent work using cryo-transmission electron microscopy (cryo-TEM) has shown that rapid-mixing procedures for LNP synthesis generate liposomal structures at pH 4 when produced without nucleic acid. However, when produced with siRNA, a combination of electron-dense and liposomal structures are observed.[1,2] These observations suggested that the presence of siRNA induces the formation of electron-dense structures, but how that occurs was unclear. Specifically, the question remained of whether the empty vesicles at pH 4 are capable of entrapping siRNA and the role of ethanol in that process. Here we show, using cryo-TEM and dynamic light scattering, that ethanol is not required for efficient siRNA entrapment, particle formation likely occurs prior to entrapment, and specialized mixers are not required. Based on data presented here and elsewhere, it is proposed that nucleic acid entrapment at pH 4 occurs through rupture and reformation of positively charged vesicles upon interaction with negatively charged nucleic acid. Finally, we leverage this phenomenon, to demonstrate that unloaded vesicles (at pH 4) can be used as functional genomic screening tools.

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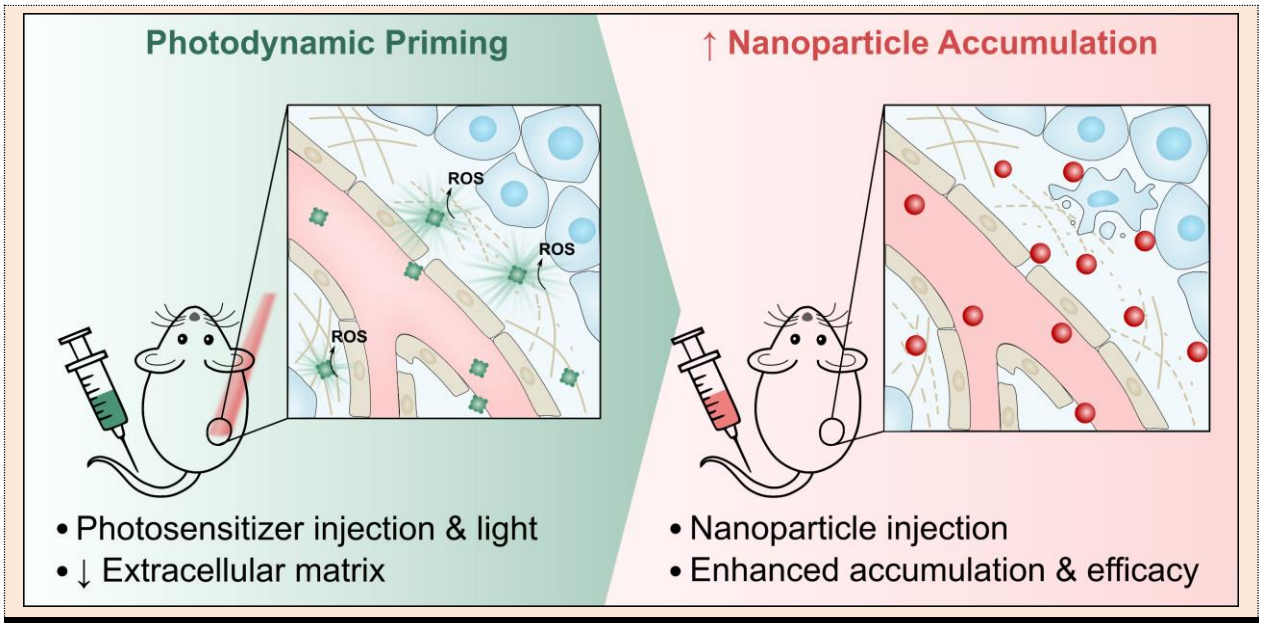


# Photodynamic Priming as a Means of Enhancing Nanomedicine Delivery and Overcoming Tumour Desmoplasia

Marta Overchuk[1,2], Kara M. Harmatys[1], Shrey Sindhwani[2], Abdullah M. Syed[2], Maneesha A. Rajora[1,2], Danielle M. Charron[1,2], Juan Chen[1], Lili Ding[1], Martin G. Pomper[4], Brian C. Wilson[1,5], Warren C.W. Chan[2], Gang Zheng[1,2,5]

[1]Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario M5G 1L7, Canada; gang.zheng@uhnresearch.ca [2]Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario M5G 1L7, Canada; [3]Krembil Research Institute University Health Network, Toronto, Ontario M5G 1L7, Canada; [4]Department of Radiology, Johns Hopkins School of Medicine, Baltimore MD 21205, USA [5]Department of Medical Biophysics, University of Toronto, Toronto, Ontario M5G 1L7, Canada

Excessive extracellular matrix (ECM) deposition is one of the major barriers to nanoparticle extravasation and subsequent penetration in tumours. Tumour photodynamic priming (PDP), which activates photosensitizers with light to locally generate cytotoxic reactive oxygen species (ROS), was proposed as a means to enhance nanomedicine delivery by inciting vascular permeabilization or cancer cell death [1]. However, its effects in the context of tumour ECM remain elusive. Here, we investigate the use of a porphyrin-based photosensitizer and subtherapeutic light irradiation to enhance nanoparticle tumour accumulation and therapeutic efficacy against PSMA+ PC3 PIP subcutaneous mouse prostate cancer xenografts.

PDP-enabled Caelyx® tumour accumulation enhancement resulted in an improved therapeutic efficacy in the absence of off target toxicity, wherein 5 mg/kg was equally effective in delaying tumour growth as 15 mg/kg of Caelyx®. Furthermore, we are the first to demonstrate that subtherapeutic PDP resulted in a ~2-fold decrease in tumour collagen deposition and a significant reduction of ECM density in the subendothelial zone. Overall, this study demonstrated the potential of PDP to enhance tumour nanomedicine accumulation and alleviate tumour desmoplasia, highlighting the utility of PDP as a non-invasive priming strategy that can improve nanomedicine therapeutic outcomes in desmoplastic tumours.


# Debate: Are Nanomedicines Still the Next Big Thing?

Marcel B Bally[1,2,3,4,5], Kishor Wasan[6,7,8]

*Moderated by Emmanuel Ho[9,10]*

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[6]Director of Research, iCo Therapeutics Inc. Vancouver, BC, V6Z 2T3; [7]Adjunct Professor and Distinguished University Scholar, Department of Urologic Sciences, Faculty of Medicine, University of British Columbia; [8]Chief Medical and Scientific Officer, Skymount Medical Group Inc., Calgary, Alberta T3C 0J8, kishor.wasan@ubc.ca

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# POSTER ABSTRACTS

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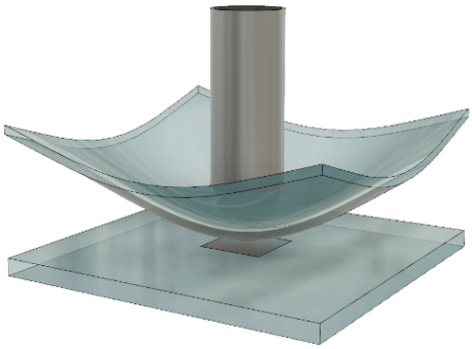
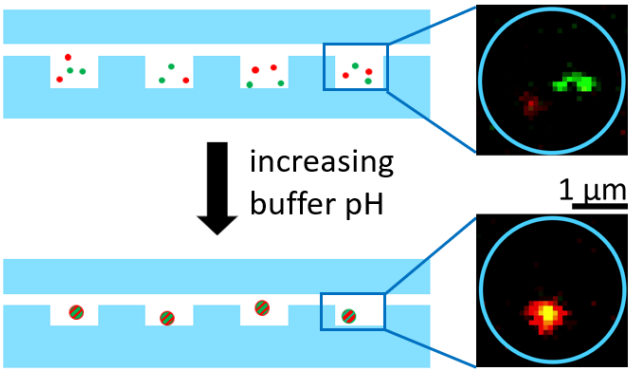


A deflectable flow-cell of the CLiC device




Watching Lipid nanoparticle dynamics – in real-time – as a function of pH

*Kamanzi et al, in preparation (2020)*

# Single-particle imaging of lipid nanoparticles for drug delivery applications

Albert Kamanzi[1], Yifei Gu[1], Romain Berti[1], Zach Friedenberger[1], Raffles Zhu[1], Radin Tahvildari[1]
Dominik Witzigmann[2], Jerry Leung[2], Pieter Cullis[2], Sabrina R. Leslie[1]

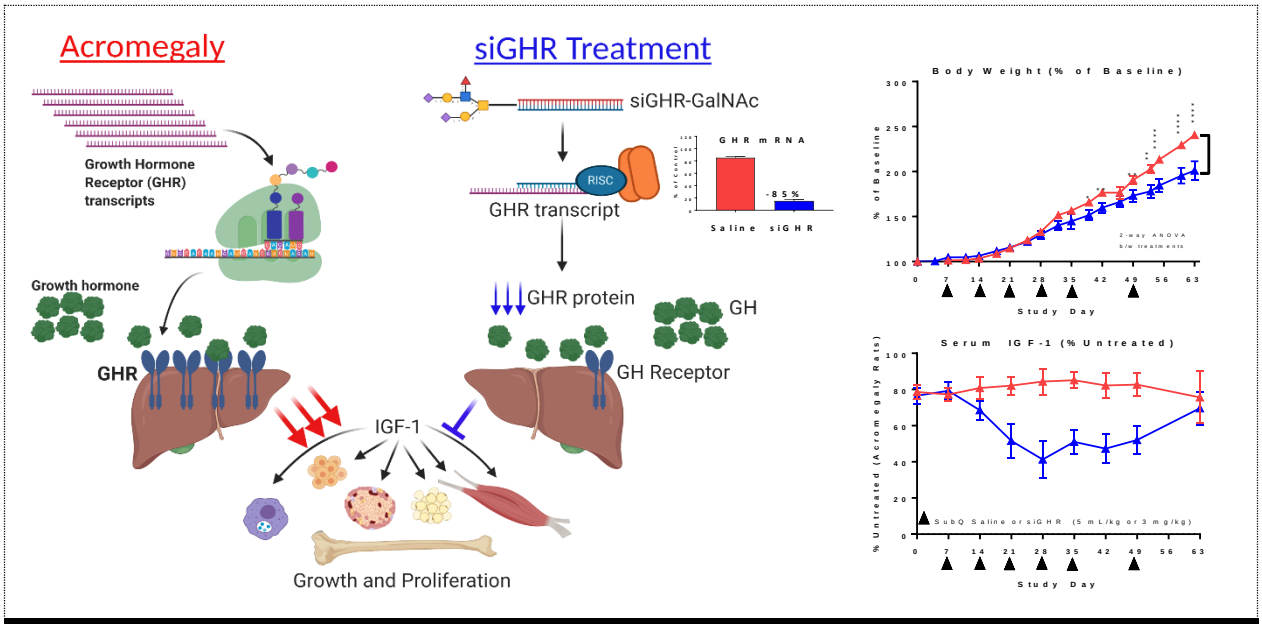
[1]Department of Physics, McGill University, Montreal, QC, Canada

[2]Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, Canada

A wide range of biological nanoparticles are being developed for diverse applications including therapeutics, cosmetics, and textiles. Key challenges in nanoparticle engineering involve resolving, understanding, and predicting important functional properties, such as size and loading, as well as aggregation and interaction properties. Typical characterization tools average over thousands of particles or more to obtain a bulk result, obscuring detailed understanding.

In this work, we introduce a general imaging and analysis method to isolate and track many copies of single diffusing nanoparticles at once. We confine the particles in an array of circular microwells using the CLiC (Convex Lens-induced Confinement) imaging technique. This enables simultaneous measurements of the size and intensity of each particle, without using tethers. We establish agreement between our measurements and the mean particle size reported using other methods such as Dynamic Light Scattering; and provide detailed size and loading distributions.

Further, we investigate the pH-dependent size and dynamic properties of lipid nanoparticles designed for drug delivery, such as real-time measurements of particle fusion. The CLiC platform enables direct investigation of nanoparticle interactions and dynamics under cell-like conditions - such as binding and unbinding, encapsulation and release, and fusion of nanoparticles - and in contexts extending from "glass cells", to model endosomes, living cells, using the same device.




# Silencing of Growth Hormone Receptor by siRNA Ameliorated Disease in a Preclinical Rat Model of Acromegaly

Drew Kondratowicz, Sara Majeski, Kevin McClintock, Christy Esau, Alice Li, Pete Lutwyche, James Heyes

Genevant Sciences Corp., Vancouver, British Columbia, Canada

Acromegaly is a rare and chronic disease characterized by disproportionate skeletal, tissue, and organ growth due to the overabundance of growth hormone (GH) – usually attributed to the presence of a GH-secreting pituitary adenoma. Multiple therapies exist to modulate the excessive secretion of GH and the associated elevations in insulin growth factor-I (IGF-I). Currently approved therapies include surgery and radiation therapy directed at the adenoma and small molecule somatostatin analogs and growth hormone receptor (GHR) inhibitors to inhibit the downstream effects of excess GH[1,2]. Unfortunately, these approaches are often times invasive or provide limited efficacy[1,2]. siRNA-GalNAc conjugates delivered subcutaneously offer the potential for less invasive, more efficacious silencing of the GH pathway, either as standalone therapy or in combination with existing approaches to amerliorate disease.

To evaluate the feasibility of a siRNA-based therapeutic for acromegaly, we established an animal model of disease based on the injection of rat GH overexpressing GC-cells into naive rats. Upon establishment of disease, rats were treated with either siGHR-GalNAc or saline. A significant reduction of GHR mRNA expression (-85%) was noted in the siGHR-GalNAc treated group compared to the saline-treated control. This was accompanied by an up to 50% reduction of functional circulating IGF-1 protein expression compared to control. While serum circulating IGF-1 concentration is one of the main metrics for monitoring therapeutic efficacy in acromegaly patients, we also observed a plateau of body weights in rats suggestive of intervention halting the progression of disease. Observations in this study indicate potent and specific silencing of target genes with siRNA-GalNAc conjugates to treat rodent acromegaly and speak to the potential for the development of clinical siGHR candidates to treat human disease.

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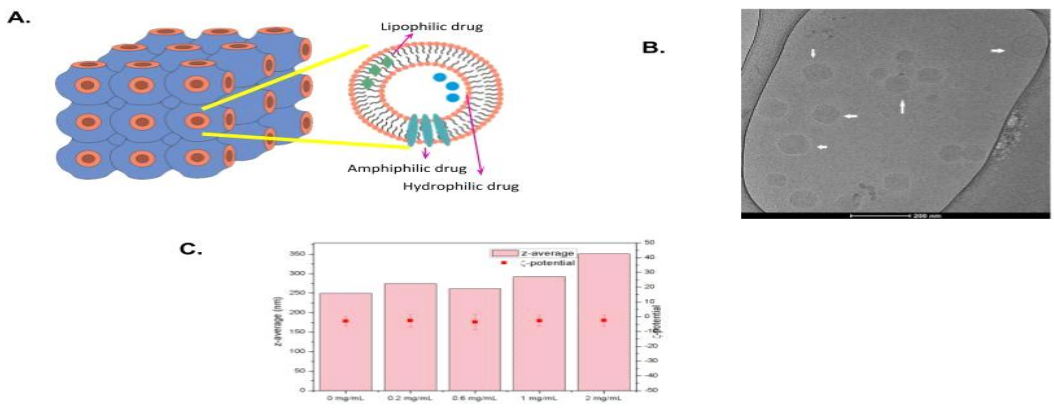


**Figure 1:** (A) Schematic diagram of a cubosome. (B) Cubosome image by Cryo-TEM. (C) DLS and Zeta-potencial results for lysozyme-cubosomes systems.

# The influence of lysozyme in the structural features of cubosomes: a potential drug delivery system

Amanda S. Palma[1], Mayra C. G. Lotierzo[2], Bruna R. Casadei[1] and Leandro R. S. Barbosa [1]*

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[2]Department of Biochemical and Pharmaceutical Technology, University of São Paulo, São Paulo, Brazil

Nanoparticles are becoming more and more important as drug delivery systems. This stem from its capability to increase drug delivery in the some specific targeted area, protect the drug from degradation and it can decrease its side effects. Nowadays, there are several different kinds of nanoparticles, like liposomes, cubosomes, ethosomes and others. Cubosomes are malleable nanoparticles with a three dimensional structure that can encapsulate hydrophilic, hydrophobic and amphiphilic drugs [1]. It can accommodate the drug in the bilayer membrane or in the water channel. Cubosomes usually are composed by lipids and a surfactant to preserve the colloidal stability [2]. In this study, cubosomes are made by phytantriol and Pluronic as nonionic surfactant loaded with lysozyme. This compound is a model protein that can be used as a bactericide. Knowing that proteins aggregate and be degraded in the body, it is interesting to encapsulate it. The lysozyme-cobosome system can be characterized by techniques as dynamic light scattering, zeta-potential, small-angle x-ray scattering and transmission electron microscopy. These techniques can determine size, polidispersity index, potential and shape. Our data showed that cubosomes loaded with lysozyme have the hydrodynamic diameter of 300 nm and are monodisperse (PDI around 0.1). Zeta-potencial experiments showed a zero-like-value for the samples (**Figure 1**). The calculation of encapsulation efficiency demonstrated that this configuration is a promising for drug delivery system.


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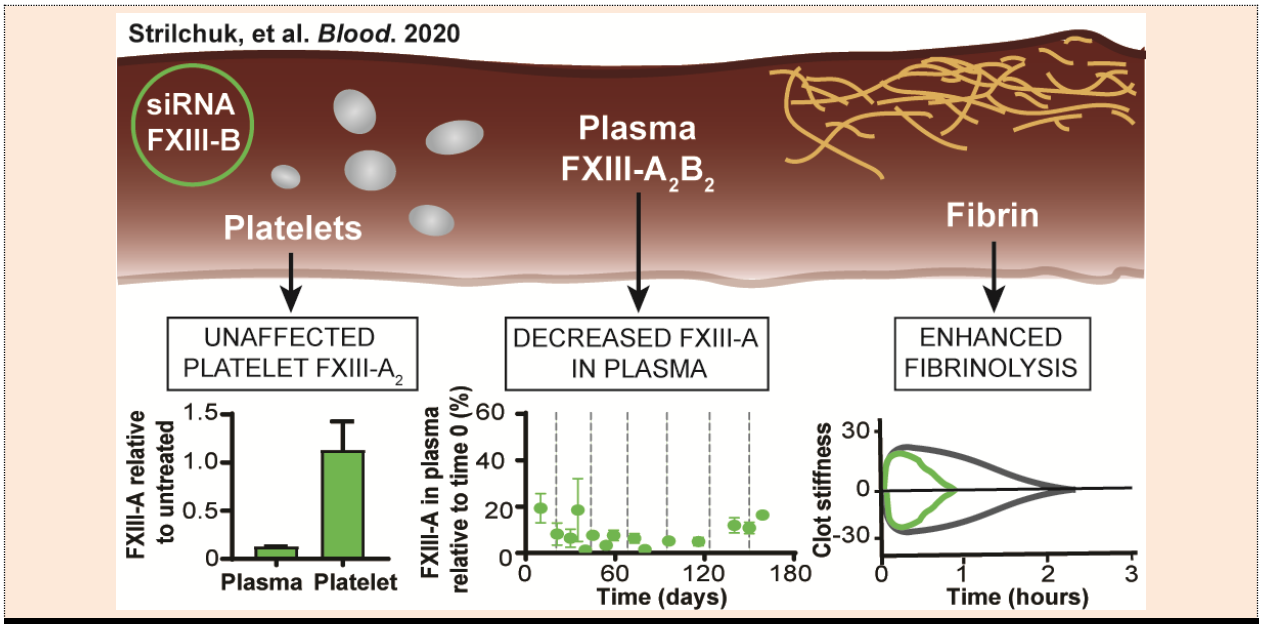


# Transient gene therapy to decrease the stability of thrombi for coagulopathy and thrombosis

Amy W. Strilchuk[1,2,3], Scott C. Meixner[2], Jerry Leung[1,2,3], Nooshin S. Safikhan[2], Jayesh A. Kulkarni[3], Roy van der Meel[3], Michael R. Sutherland[2], Joseph S. Palumbo[4], Edward M. Conway[2], Edward L.G. Pryzdial[2], Pieter R. Cullis[3], and Christian J. Kastrup[1,2,3].

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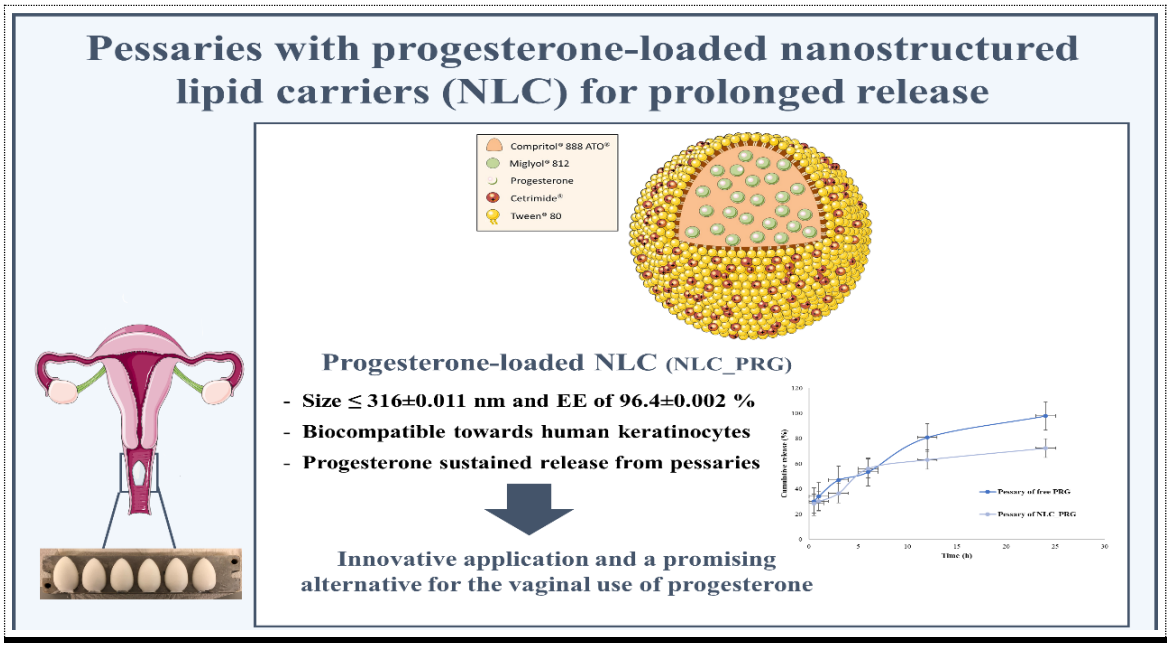
Thrombotic disorders are prevalent and dangerous. Thrombosis involves the formation of unwanted blood clots inside blood vessels, which can impair blood flow and lead to severe cardiovascular events, such as heart attack, stroke, and pulmonary embolism. Current therapies for thrombosis are unsatisfactory in that they require frequent re-administration, and are associated with a significant bleeding risk, a consequence of inhibiting the coagulation cascade upstream of fibrin generation. Coagulation factor XIII (FXIII) acts to stabilize clots, downstream of fibrin generation and polymerization. Though this makes it an ideal target to reduce the burden of thrombosis while maintaining hemostasis, inhibitors suitable for clinical use have not been identified. We have recently published that lipid nanoparticles can be used to deliver siRNA to knock-down FXIII B-subunit and achieve depletion of the enzymatic A-subunit from circulation in mice and rabbits (Strilchuk, et al. *Blood*. 2020). In the current abstract, we discuss the findings that FXIII-A depletion causes less antiplasmin to be crosslinked to clots, resulting in clots that are more susceptible to fibrinolysis. Further, showing that while clots are weaker and easier to clear with thrombolytic therapy, bleeding is not enhanced in mice or rabbits after minor or major injury. In the short term, these novel RNA agents will be valuable tools in investigating the biology of thrombotic disorders. The ultimate goal is to develop this agent into a precise and long-acting prophylactic therapy for thrombotic disorders, that is safer and more effective than current standards of care.




# Pessaries with progesterone-loaded nanostructured lipid carriers (NLC) for prolonged release

A. Correia[1], C. P. Costa[1], R. Silva[2], V. Silva[2], J.M. Sousa Lobo[1], **A.C. Silva[1,3*]**

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Progesterone is responsible for the embryo implantation and for the maintenance of pregnancy and is commonly administered vaginally, avoiding the first-past hepatic first pass. However, vaginal progesterone dosage forms require repeated administrations to ensure maintenance of therapeutic levels [1]. Thus, it is desirable to use alternative systems for the prolonged release of progesterone. Among these, the nanostructured lipid carriers (NLC) have been widely studied, due to their advantages in improve the bioavailability of drugs. In addition, pessaries remain the vaginal forms of choice, given the ease of application and the low cost of production [2].

The objective of this work was to develop pessaries for prolonged vaginal delivery of progesterone. The studies began with the preparation of progesterone-loaded NLC (NLC_PRG), followed by the evaluation of its cytotoxicity. Finally, NLC_PRG were incorporated into pessaries. The results showed NLC_PRG with sizes ≤ 315.60±0.01 nm, an encapsulation efficiency (EE) of 96.42±0.00 %, absence of cytotoxicity and prolonged drug release effect from the pessaries with NLC_PRG. These findings suggest the suitability of pessaries containing NLC_PRG for sustained drug release, which constitutes a promising alternative for the vaginal use of progesterone.

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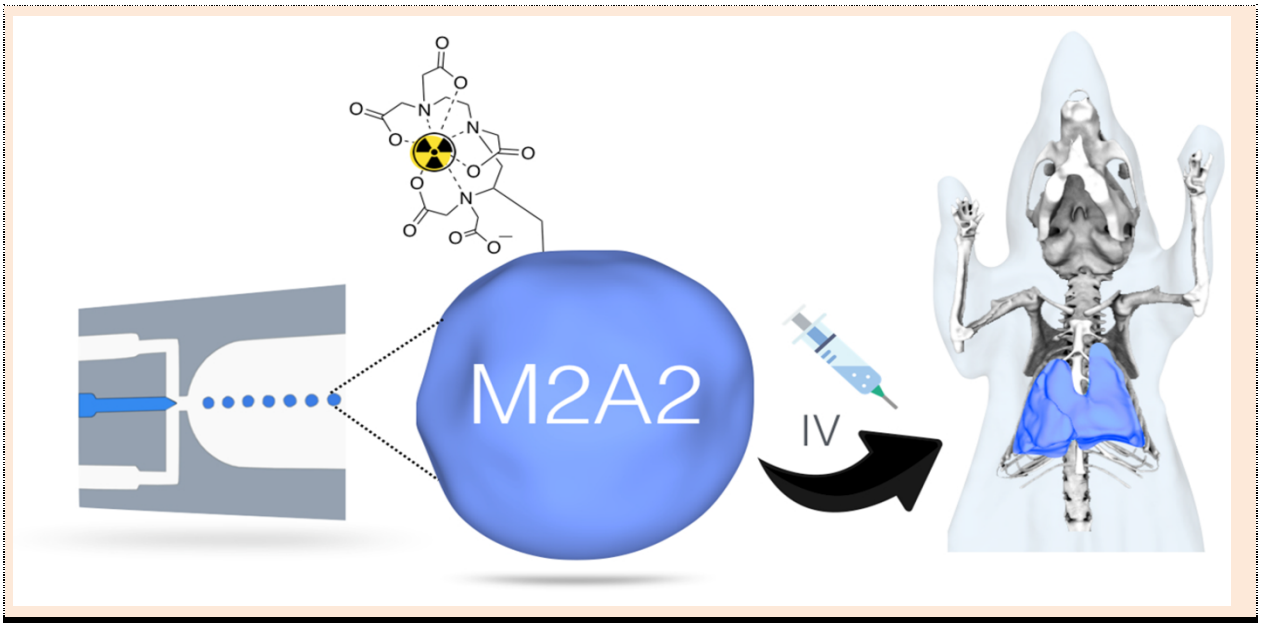


# Preparation of Heat-Denatured Macroaggregated Albumin for Biomedical Applications using a Microfluidics Platform

Tullio V. F. Esposito[1,2,#,*], Helene Stütz[1,3,#], **Colin Blackadar**[1,‡], Cristina Rodríguez-Rodríguez[1,4], Lovelyn Charles[1], Reka Geczy[2], Jörg P. Kutter[2], Katayoun Saatchi[1,*] and Urs O. Häfeli[1,2,*]

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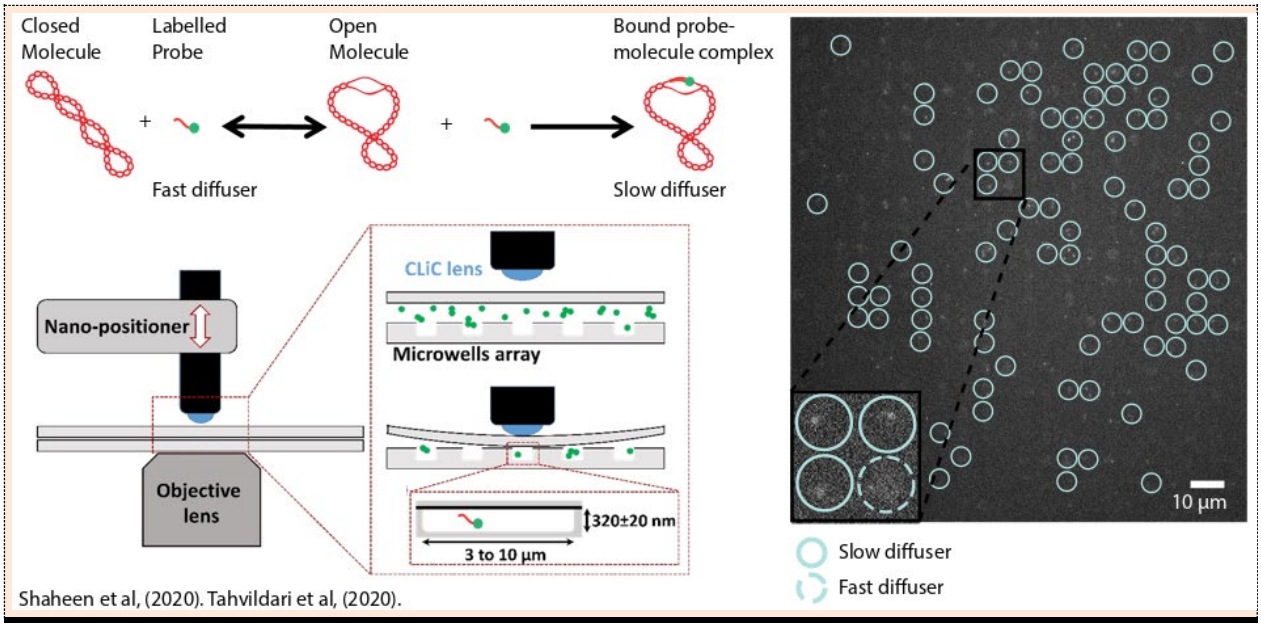
Albumin is widely used to improve the efficacy, decrease the toxicity, or alter the pharmacokinetic profile of active compounds. Macroaggregated albumin (MAA) is a common albumin microparticle, which is predominately used for lung perfusion imaging when labeled with the radionuclide $^{99m}$Tc. These microparticles are formed by heat denaturing albumin in bulk solution; as a result, there is limited control over the size of the particles formed. In this work, we developed an integrated microfluidics platform to create more tunable and precise MAA particles, so-called microfluidic-MAA (M2A2). Prepared using off-stoichiometry thiol-ene chemistry, these chips consist of a flow focusing region followed by an extended and water heated curing channel (85°C). M2A2 particles with diameters between 70 and 300 µm with coefficients of variation between 10-20% were reliably prepared by adjusting the flow rates of the dispersed and continuous phases. To demonstrate a biomedical application of M2A2, particles were labeled with $^{111}$In and their distribution was assessed in healthy mice using nuclear imaging. $^{111}$In-M2A2 behaved similarly to $^{99m}$Tc-MAA; lung uptake was seen early on, and the particles were cleared over time by the renal and reticuloendothelial systems. M2A2 represents an elegant and controllable method to prepare albumin microparticles, and it demonstrates one of many diverse applications for microfluidics in the world of pharmaceutical sciences.




# High-throughput, single-molecule, CLiC analytics of nucleic acid binding kinetics, and applications to oligotherapeutics development

Cynthia Shaheen[1], Cameron Hastie[1], Daniel Berard[1], Shane Scott[1], Fed Kouzine[3], Craig Benham[2], David Levens[3], Sabrina R. Leslie[1]

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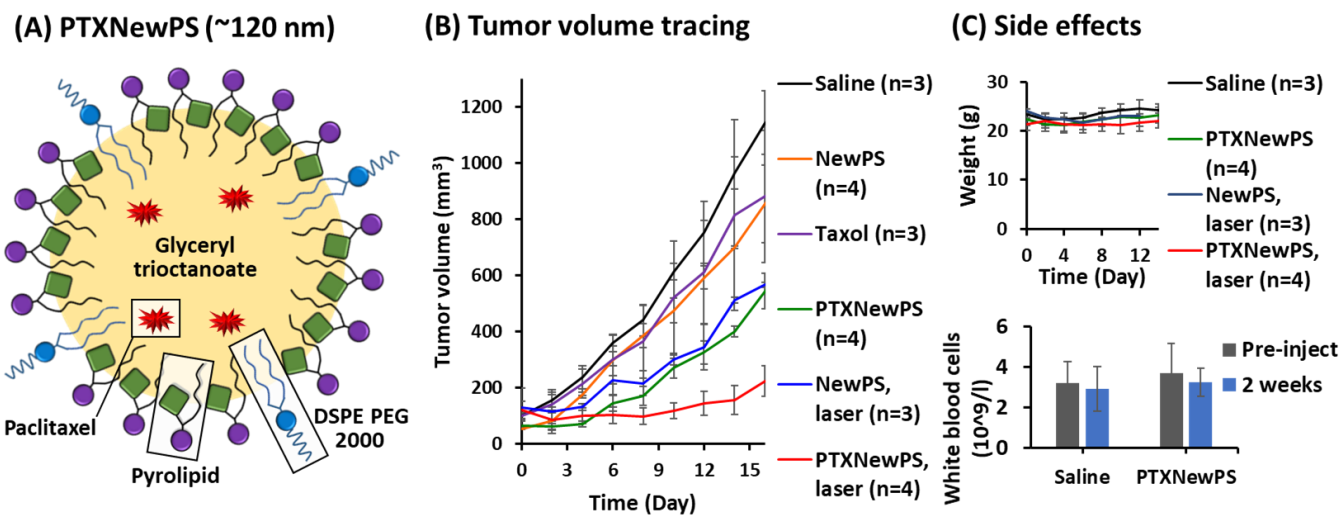
Typical biomolecular assays rely on measuring populations which can overlook fundamental details only detectable on the scale of individual molecules. In contrast to ensemble studies, single-molecule studies identify heterogeneous sub-populations and detect rare events. Understanding such heterogeneity is critical to understanding and treating many diseases.

Ideally, a single-molecule platform would be simple in terms of required hardware, experimental design and data analysis. Towards this end, we developed high-throughput, single-molecule *Convex Lens-induced Confinement* (CLiC) analytics to enable direct imaging, manipulation, and quantification of biomolecules. CLiC enables long observation times (minutes to hours) and uses *untethered and freely diffusing* biomolecules. Furthermore, CLiC provides large numbers of observations yielding *high statistics and high signal-to-noise*. Finally, because it allows reagent exchange during observations, CLiC can *mimic the crowded and confined conditions in cells*.

In this work, we investigate structural heterogeneity at the single-molecule level caused by supercoiling of plasmids, as well as its impact on the binding/unbinding of probes to targets on the plasmids. Using CLiC, we assayed the impact of several biophysical variables (supercoiling, crowding, oligos-probe sequence, etc) on kinetic interaction parameters (on/off rates, site opening/closing rates), and related our observations to statistical physics theory of DNA.

Recently, we have extended the CLiC DNA-binding assay to interrogate and quantify the interaction kinetics of oligonucleotide therapeutics such as ASOs to RNA targets, to understand the mechanisms and efficacies of emerging classes of genetic medicines, and help engineer better drugs. Excited to share our approaches and findings with the UBC nanomedicine community.




# Combination of photodynamic therapy and chemotherapy for cancer treatment by using paclitaxel loaded porphyrin-shelled nanoemulsions

Enling Chang[1,2], Juan Chen[2], Jiachuan Bu[2], Lili Ding[2], and Gang Zheng[1,2] *

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The combination of photodynamic therapy (PDT) and chemotherapy had proved to be an effective tumor treatment in clinical(1). PDT can locally and directly kill tumors whereas chemotherapy could more efficiently eliminate tumor cells beyond PDT, especially for the deep and metastasis tumors that cannot be reached by laser. With the aid of PDT, the effective chemotherapeutic dosages could be reduced, thus reducing unwanted side effects. However, the traditional nanoparticles of the combining PDT plus chemotherapy still showed limited clinic applications due to low drug loading capacity, low serum stability, and toxicity concern(2).

A co-delivery of porphyrin and paclitaxel nanoemulsion system (PTXNewPS) (~120 nm) was created for combinational tumor treatment. The oil core of PTXNewPS could be stabilized by pyrolipid shell with excellent colloidal stability, whereas it gave an amiable matrix for efficient paclitaxel encapsulation. After PEGylation, the in vivo half-life and tumor accumulation of PTXNewPS could significantly be increased on the mouse model. The combination of chemotherapy plus PDT by using PTXNewPS resulted in significant tumor growth inhibition and increased survival rate with 4-times decreased PTX dose (1.8mg/kg) compared to either single chemotherapy (7.2mg/kg) or single PDT treatment. No significant toxicity was observed from the blood biochemistry and CBC tests, body weight tracking, and H&E staining, indicating the safety of PTXNewPS injection. Thus, this nanosystem provides a novel tumor-killing tool for enhanced tumor treatment while overcoming the chemotherapy side effects.

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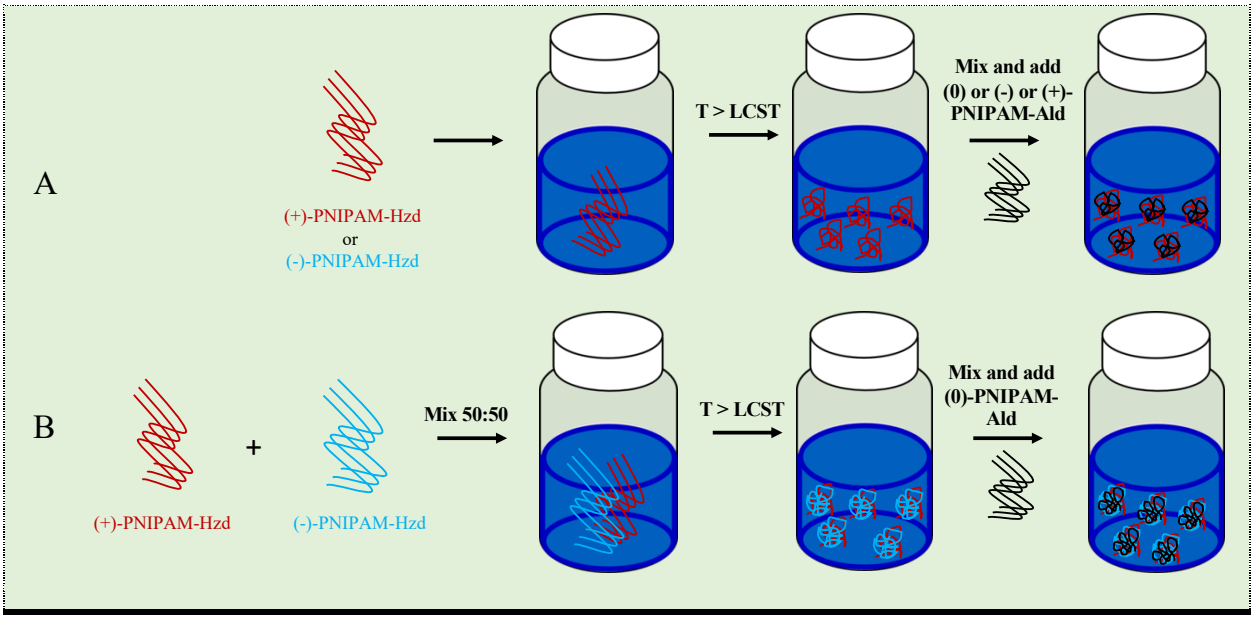


# Cationic, anionic, and amphoteric dual pH/temperature-responsive degradable microgels via self-assembly of functionalized oligomeric precursor polymers

Eva Mueller[1*], Sebastian Himbert[2], Madeline Simpson[1], Ridhdhi Dave[1], Markus Bleuel[3], Maikel Rheinstaedter[2], Todd Hoare[1]

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The application of microgel-based drug delivery *in vivo* necessitates controlled monodispersity and high degradability [1, 2]. However, microgels produced by the conventional free radical precipitation technique contain a backbone of non-degradable C-C bonds. Instead, we have developed a novel fabrication method to create poly(*N*-isopropylacrylamide)-(PNIPAM) based microgels with the potential for safe *in vivo* degradation [5]. Precursor PNIPAM oligomers are functionalized with hydrazide (Hzd) and aldehyde (Ald) reactive groups; heating a solution of PNIPAM-Hzd above the polymer's lower critical solution temperature results in the collapse of the material into nanoaggregates, which are then cross-linked by dropwise addition of PNIPAM-Ald. The resulting microgel solutions have been shown to be highly monodisperse, thermoresponsive, non-cytotoxic, scalable, and degradable (due to the hydrolytically labile hydrazone bonds formed in cross-linking) [5, 6]. This fabrication method is also useful in synthesizing microgels with charge that allows the microgels to respond not only to changes in temperature but also in pH, expanding the range of biomedical applications, and/or enhance affinity loading of the microgel with charged drugs to enhance drug loading efficiency and prolong drug release kinetics.

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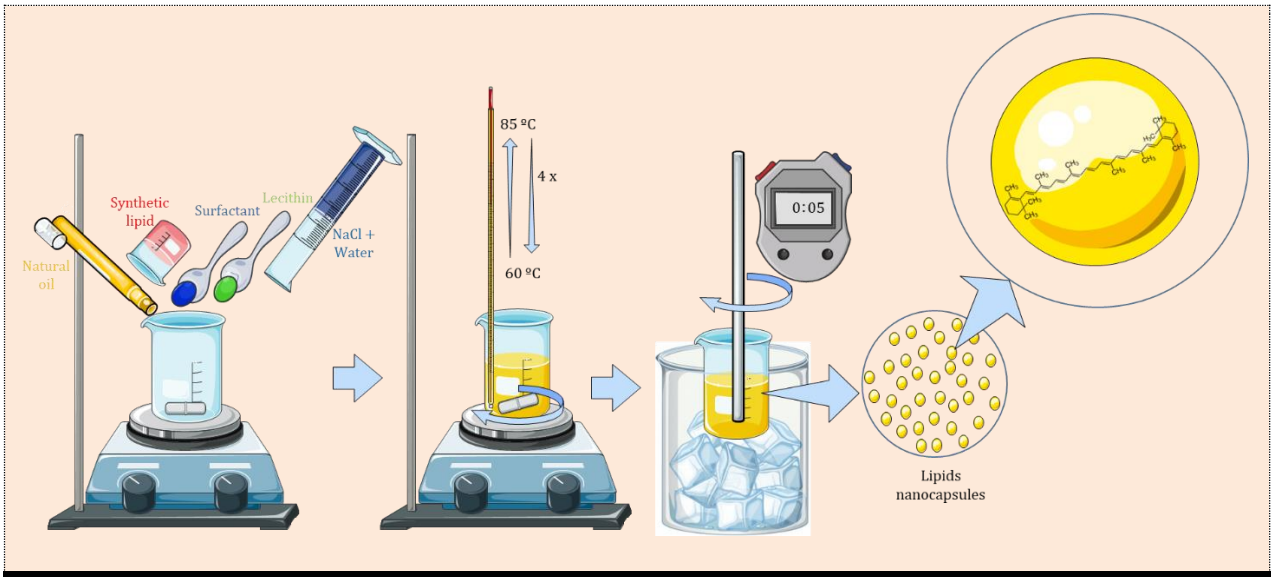


# Development and Characterization of Lipid Nanocapsules with *Attalea Phalerata* Pulp Oil

Fernando Freitas de Lima[1]; Priscila Cordeiro Lima Fernandes[2]; Ludmilla David de Moura[2]; Ivan Pires de Oliveira[3]; Caroline Honaiser Lescano[4]; Leila Maria Spadoti[1]

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The *Attalea phalerata* Mart ex Spreng palm is found in Brazil mainly in the Cerrado region. The fruit of *A. phalerata*, locally known as 'bacuri', has fleshy flesh with a color ranging from yellow to orange. Among the bioactive compounds, bacuri pulp oil (BPO) has a high content of carotenoids (β-carotene). A recent study with BPO demonstrated an anti-inflammatory effect attributed to the high carotenoid content through its oral consumption [1]. However, carotenoids are easily degraded by reacting with oxygen, light, heat and some enzymes. To prevent the degradation of these compounds and improve their bioavailability, technologies have emerged, such as encapsulation in lipid nanocapsules (LNCs). LNC is a new and promising technique used in the Drug Delivery System (DDS). The development of LNCs replacing synthetic oils with a natural oil with a potential natural anti-inflammatory agent is interesting and promising. Given the above, the objective of the work was to develop and characterize (size, polydispersion and zeta potential) of BPO nanocapsules (BPON). The BPON were developed using the phase inversion method [2]. Briefly, liquid lipid, BPO, nonionic surfactant, hydrogenated soy lecithin was mixed at room temperature and NaCl in Milli-Q water. The mixture was subjected to five temperature cycles and an ice bath was given at the end. The results after development, showed LNCs with average sizes of 55.87 ± 0.41 nm, PDI of 0.118 ± 0.066 and zeta potential of -24.80 ± 1.42. The results suggest a system with characteristic sizes for nanosystems. The low PDI (<0.150) and zeta potential distant from zero (negative) suggest stability for the system. Thus, BPON are viable for further characterization studies and later *in vitro* and *in vivo* biological evaluation.

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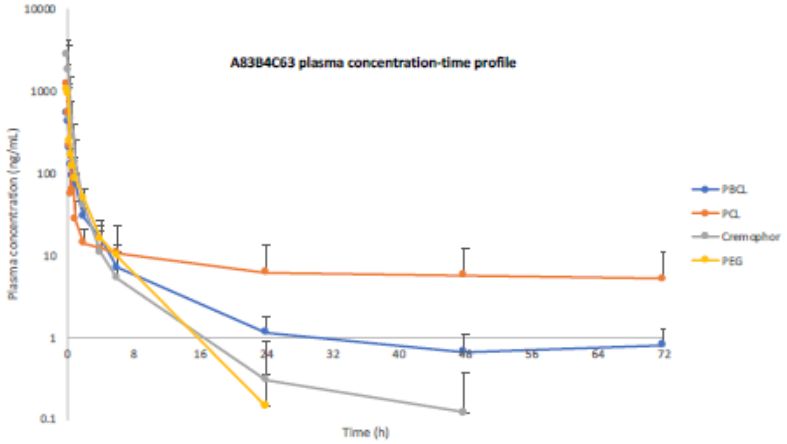
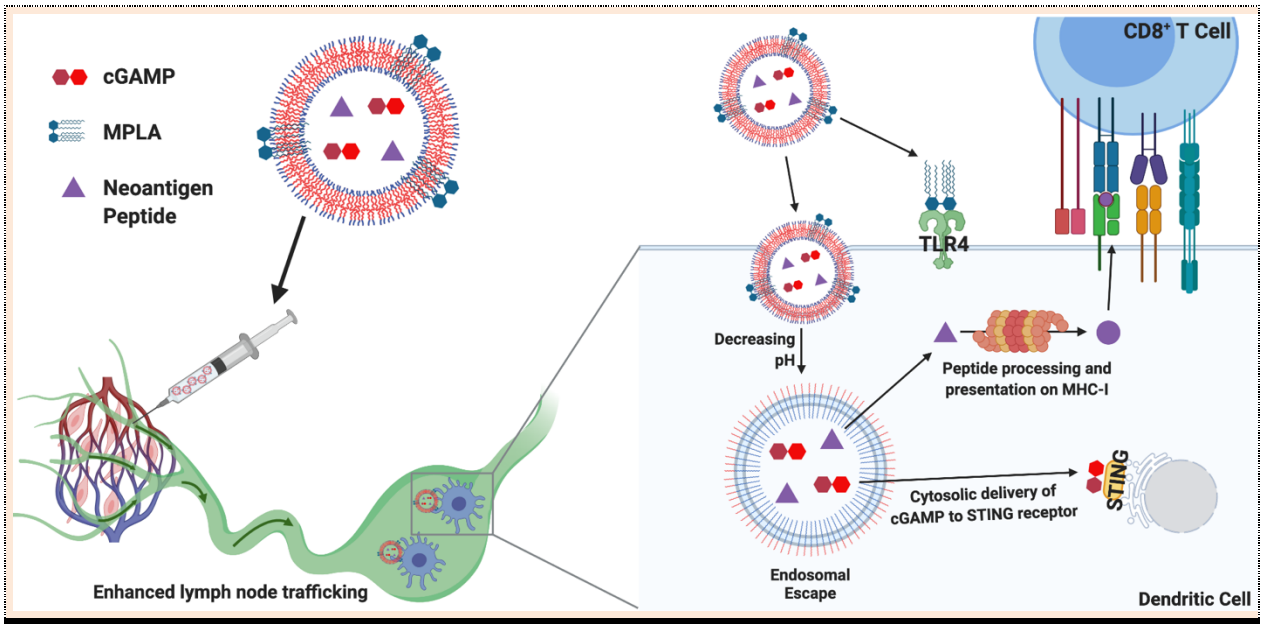



**Pharmacokinetics of Nano versus Conventional Formulations of A83B4C63, a Novel Inhibitor of DNA Repair in Rat**

Forugh Sanaee[1], Marco Paladino[2], Dennis G. Hall[2], Michael Weinfeld[3], Afsaneh Lavasanifar[1*]

[1]Faculty of Pharmacy and Pharmaceutical Sciences University of Alberta, Edmonton, Alberta, Canada, T6G 2N8; afsaneh@ualberta.ca; [2]Department of Chemistry, Faculty of Science, University of Alberta, Edmonton, AB, Canada; [3]Department of Experimental Oncology, Cross Cancer Institute, Edmonton, AB, Canada

**Purpose:** There is an increasing interest in the development of DNA repair inhibitors in order to increase the efficacy of radiation and conventional chemotherapy in cancer treatment. However, in order to avoid reducing the repair capacity of normal tissue, it is highly desirable to target the DNA repair inhibitors to tumor. The long term aim of this study is to develop nanodelivery systems for the encapsulation and tumor targeted delivery of A83B4C63, a novel imidopiperidine-based inhibitor of the DNA 3'-phosphatase activity of polynucleotide kinase/phosphatase (PNKP), which is known to play a critical role in rejoining DNA single- and double-strand breaks[1].Here, A83B4C63 was encapsulated in polymeric micelles of poly(ethylene oxide)-b-poly(ε-caprolactone) (PEO-b-PCL) and PEO-poly(α-benzyl carboxylate-ε-caprolactone) (PEO-b-PBCL) with degree of polymerization of 44 and 26 in their hydrophobic block, respectively. The Pharmacokinetics of A83B4C63 as part of these nano-formulations was then compared to that of conventional water solubilized formulations of this drug in healthy rats. **Method:** PEO-*b*-PCL and PEO-b-PBCL copolymer were synthesized by ring-opening polymerization and used to form micelles encapsulating A83B4C63. Micellar size and polydispersity index (PDI) were determined using Zetasizer Nano ZS. The release of A83B4C63 from Cremophor/ethanol (Control), PEG 400, PEO-*b*-PCL and PEO-*b*-PBCL formulations were assessed using 4% Bovine Serum Albumin (BSA) as recipient phase. Single 10 mg/kg i.v. dose of polymeric micellar and Cremophor/ethanol formulations of A83B4C63 and also A83B4C63 dissolved in PEG 400 were administered to cannulated male Sprague-Dawley rats (n=4/group; Weight: 260 ± 10g). Serial blood samples (200 µL) were collected prior and up to 72 h post-dose and plasma A83B4C63 concentrations were determined using LC/MS/MS. Pharmacokinetic parameters were calculated using a non-compartmental method. **Result:** The A83B4C63 was rapidly released from Cremophor/ethanol formulation (63.18±0.56% release in 24 h) and PEG 400 (92.17±0.41% release in 24h). In comparison, both polymeric micellar formulations showed a significant ability to retain the drug after 24 h (PBCL: 25.31±11.80, PCL: 17.64±6.85 % drug release at 24 h). No significant differences were observed in the release pattern between the two polymeric micellar formulations. Our pharmacokinetic results indicating both micellar formulations produced significantly longer MRT and $t_{1/2}$ when compared to Cremophor/ethanol and PEG formulations.




# A Nanocarrier Platform for Enhancing Immune Responses to Neoantigen-Targeted Cancer Vaccines

Jessalyn Baljon[1,*], Daniel Shae[2], Mohamed Wehbe[2], Lihong Wang-Bishop[2], John T. Wilson[1,2]

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Immune checkpoint blockade (ICB) has revolutionized cancer treatment and led to complete and durable clinical responses, but only for a minority of patients. Resistance to ICB can be largely attributed to an insufficient number and/or function of tumor antigen-specific T cells. Neoantigen-targeted vaccines have the ability to activate and expand this T cell repertoire, but historically, clinical responses have been poor. This is due to the fact that immunity against cancer peptide neoantigens is typically weak, resulting in suboptimal priming, expansion, and function of neoantigen-specific T cells. Therefore, we have designed a nanoparticle vaccine platform that overcomes previous barriers in several regards: 1) Our nanoparticle platform can co-encapsulate the adjuvant cGAMP, a STING agonist, and peptide neoantigens, which coordinates their delivery to the same antigen presenting cell (APC) and improves the downstream T cell response.[1] The nanoparticle size also improves trafficking to APCs in the lymph node, further enhancing the T cell response. Additionally, the nanoparticle is pH-responsive for cytosolic delivery of the antigen, which increases the presentation on MHC class I molecules and improves the $CD8^+$ T cell response. 2) The nanoparticle can co-deliver two potentially synergistic adjuvants – cGAMP and the toll-like receptor 4 (TLR4) agonist MPLA – which can further increase the immunogenicity of the vaccine. This engineered nanoparticle provides a platform to co-load neoantigen peptides and multiple adjuvants, producing a more robust T cell response and improving therapeutic efficacy.

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**PEGylation the key for increased thermostability of biopharmaceuticals: crisantaspase case-study**

TORRES-OBREQUE, Karin[1]; SANTO, João H. P. M. [1], CARRETERO, Gustavo [2], RABELO, Jheniffer [1], MONTEIRO, Gisele [1], PESSOA JR, Adalberto [1], CONVERTI, Attilio [3] and RANGEL-YAGUI, Carlota O [1]*.

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**SIGNIFICANCE STATEMENT**: Thermostability represents an important parameter in the manufacturing process and is critical to the success or failure in the development of a viable drug. In this sense, PEGylation – covalent attachment of poly(ethylene oxide) to a protein surface - is an important strategy to improve protein drugs. It not only reduces the immune system activation but also increases the thermal and long-term stability of proteins. **MAIN FINDINGS:** Circular dichroism (CD) showed that PEGylation preserved the enzyme secondary structure. Thermostability was investigated by unfolding and refolding processes and suggested aggregation of crisantaspase and partial unfolding for PEG-crisantaspase. The thermodynamic study is well described by first-order kinetics. Activation energy ($E$*) was estimated for both native and PEGylated enzyme (22.3 and 11.0 kJ $mol^{-1}$ respectively). Half-life decreases progressively at high temperatures and higher half-life at 50 °C was observed for PEG-crisantaspase (87.74 min) in comparison to the native form (9.79 min). The activation energy of denaturation of PEG-crisantaspase (307.1 kJ $mol^{-1}$) was higher than for crisantaspase (218.1 kJ $mol^{-1}$), which means that more energy is required to overcome the energy barrier of the unfolding process. Finally, higher and positive values of $\Delta H^{\ddagger}$ and $\Delta G^{\ddagger}$ demonstrated higher structural stability of PEG-crisantaspase. **CONCLUDING REMARKS**: Our results demonstrated that site-specific PEGylation of crisantaspase provides protection it from thermal inactivation and improves its thermostability.

**FUNDING:** This work is supported by the State of São Paulo Research Foundation (FAPESP-Brazil, processes numbers: 2013/08617-7, 2016/22065-5 and 2018/25994-2), the National Council for Scientific and Technological Development (CNPq-Brazil, Fellowship # 301832/2017-0) and the Coordination of Improvement of Higher Education Personnel (CAPES-Brazil, process number: 001).

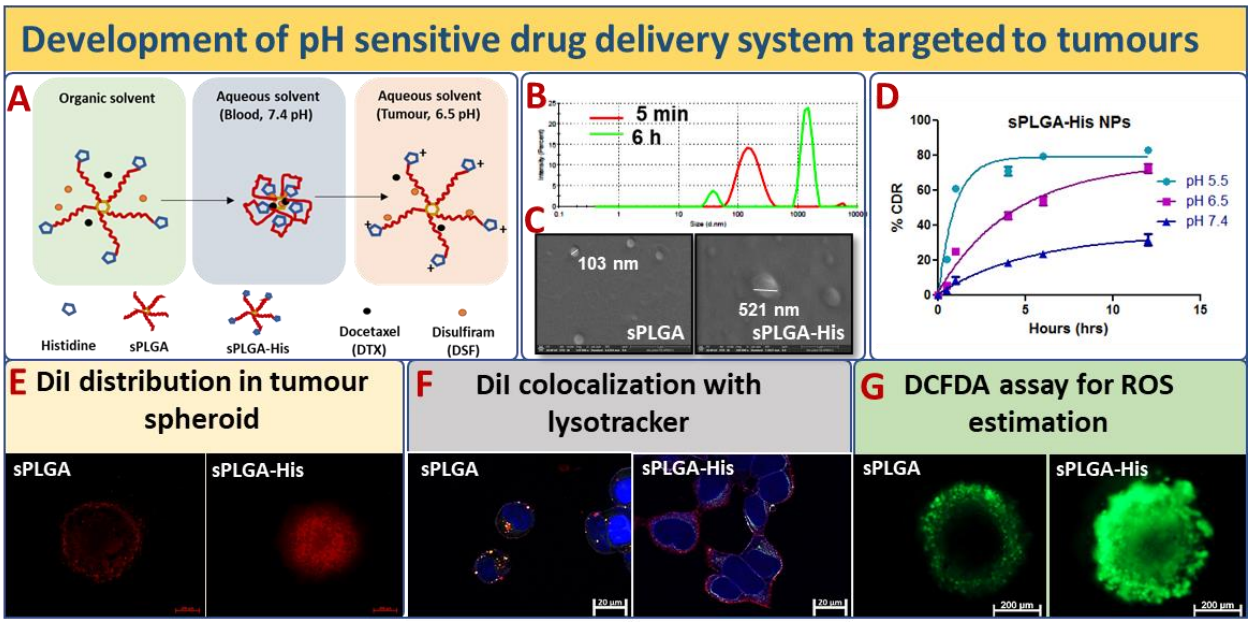


**Figure: A)** Schematic of pH sensitive drug delivery mechanism of sPLGA-His NPs. **B)** Destabilization of sPLGA-His NPs at pH 6.5 analysed by DLS **C)** SEM analysis of NPs at pH-6.5 after 3h. **D)** pH sensitive drug release kinetics of sPLGA-His NPs. **E)** Differential distribution of DiI into tumour spheroids with sPLGA vs sPLGA-His NPs. **F)** Endosomal escape exhibited by sPLGA-His NPs not with sPLGA NPs. **G)** Increase in ROS generation with DTX+DSF loaded sPLGA-His NPs.

# Docetaxel and disulfiram loaded tumor extracellular pH-responsive nanocarrier for targeting cancer stem cells

**K. Laxmi Swetha**, Aniruddha Roy#

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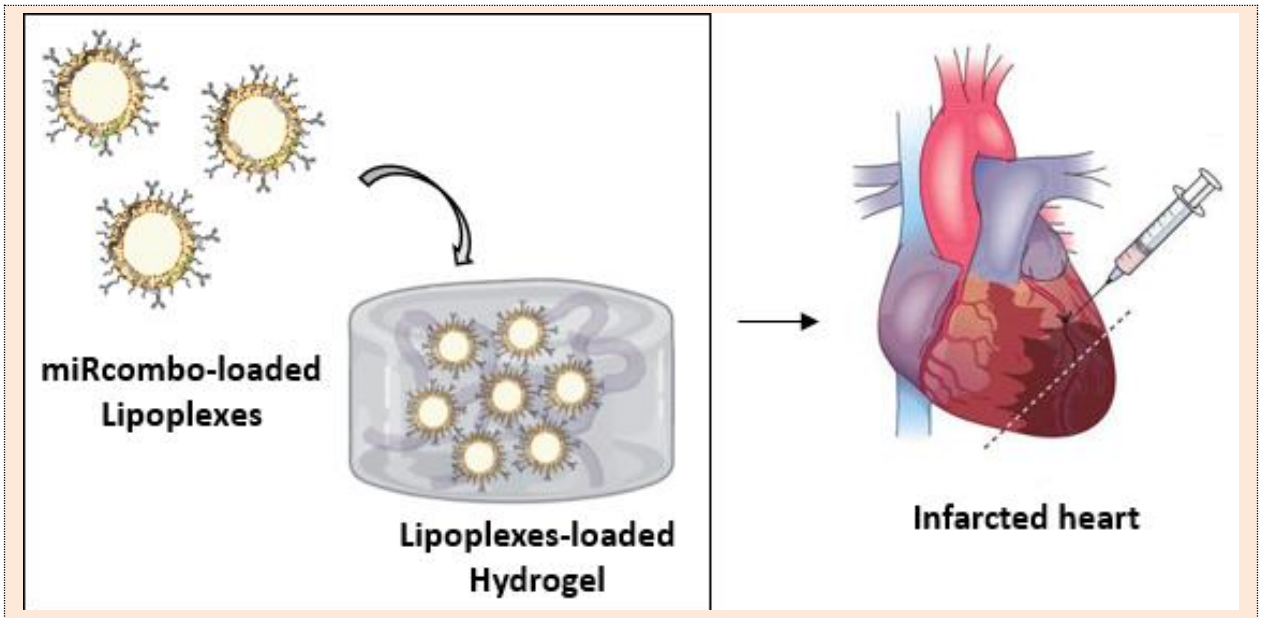
Nanoparticles (NPs) accumulate at tumor site taking advantage of enhanced permeability and retention (EPR) effect. After reaching the tumor site, NPs mostly accumulate in the periphery of the tumor, as their intra-tumoral penetration is prevented due to the low perfusion, high interstitial fluid pressure, and dense matrix. Cancer stem cells (CSCs) that are more abundant at tumor core survives most of the anticancer treatments and causes relapse of tumors. We have developed a pH-sensitive nanocarrier by conjugating histidine to star-shaped PLGA (sPLGA-His) for effective delivery of docetaxel (DTX, a highly potent anticancer agent) and disulfiram (DSF, showing anti-CSC activity) at a predetermined ratio to tumor core. The sPLGA-His NPs exhibited an excellent pH-responsive behavior, with almost 3 times increased drug release at pH 6.5 compared to pH 7.4 in 12 h. *In-vitro* cytotoxicity analysis showed that the pH-sensitive sPLGA-His NPs had enhanced efficacy in both 2D and 3D cell culture models. In the cell uptake study, the sPLGA-His NPs exhibited endosomal escape and uniform cellular distribution, whereas sPLGA NPs were found to be accumulated in the endosomes. In the tumor spheroid model, deep penetration of DiI was observed with the sPLGA-His NPs, while with sPLGA NPs, it was found to be accumulated in the periphery. In the 3D spheroid model, sPLGA-His NPs have shown high induction of ROS compared to sPLGA NPs. Altogether, the sPLGA-His NPs can be used as a tumor extracellular pH-responsive nanocarrier for efficient drug delivery to the tumor.




# Novel lipoplexes for efficient microRNA delivery to human cardiac fibroblasts

Letizia Nicoletti[1], Giulia Tarricone[1], Camilla Paoletti[1], Carla Divieto[2], Ilaria Andreana[3], Barbara Stella[3], Silvia Arpicco[3], Clara Mattu[1], Valeria Chiono[1]*

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[3]Department of Drug Science and Technology, University of Turin, Turin, Italy

Myocardial infarction causes the irreversible loss of cardiomyocytes and the formation of a dysfunctional fibrotic scar. Among advanced therapies for myocardial regeneration, *in situ* release of specific microRNAs (miRNAs) is under evaluation to promote cardiomyocyte proliferation or transdifferentiation of cardiac fibroblasts (CFs) into cardiomyocytes [1, 2].

In this work, new miRNAs-loaded lipoplexes were designed for efficient encapsulation and delivery of miRNAs to human CFs, aimed at triggering their direct reprogramming into cardiomyocytes. Lipoplexes containing negmiR or miR-1 were prepared at different N:P ratios, showing 99% encapsulation efficiency, hydrodynamic diameter ranging from 372 nm to 876 nm and average zeta potential ranging from +40 mV to -26 mV with decreasing N:P ratio from 3.0 to 0.35. Based on stability experiments in different media at different temperatures (4°C and 37°C), lipoplexes with N:P 3 were selected for *in vitro* tests with human CFs, showing more efficient miR-1 release to CFs, as compared to a commercial agent. Direct reprogramming experiments are in progress using miRcombo (miR-1, 133, 208, 499) to validate the newly developed lipoplexes as efficient vectors for direct cardiac reprogramming compared to a commercial agent [2].

This project is supported from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 772168).

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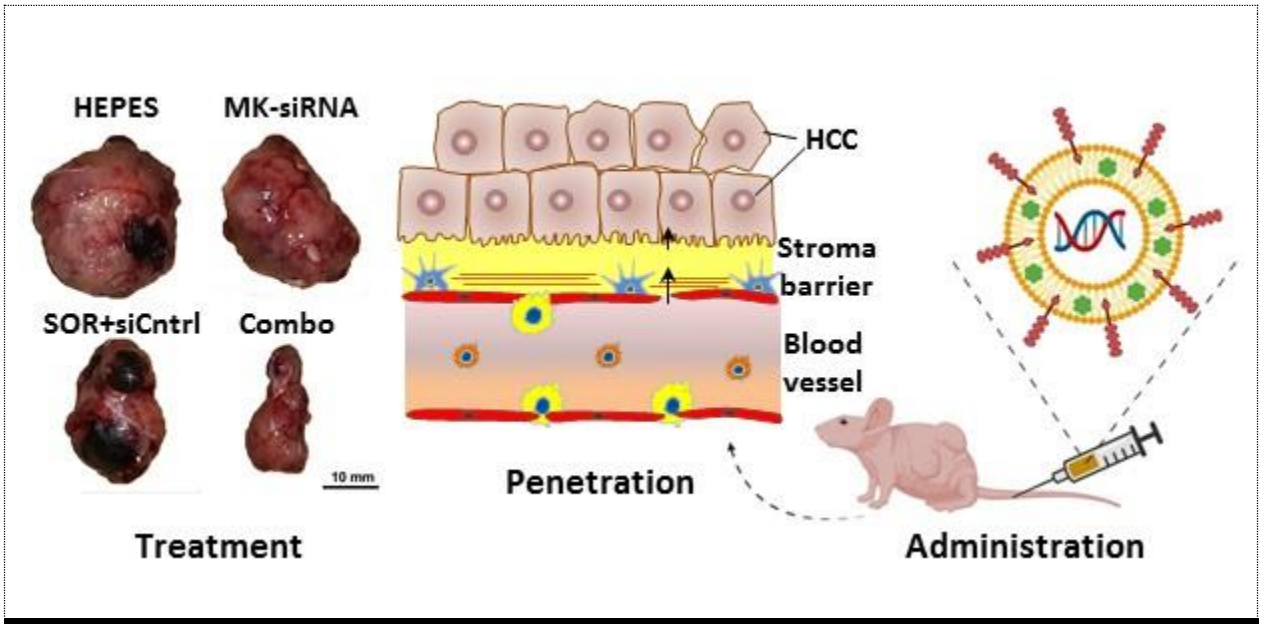
2. Paoletti C, Divieto C, Tarricone G., Di Meglio F, Nurzynska D, Chiono V (2020). **MicroRNA-Mediated Direct Reprogramming of Human Adult Fibroblasts Toward Cardiac Phenotype.** Front Bioeng Biotechnol. 8, 529.




# Manipulation of the Composition and Physico-chemical Properties of Combo Lipid Nanoparticles for Highly-selective Chemo-gene Therapy of Hepatocellular Carcinoma *In Vivo*

Mahmoud A. Younis [1,2], Ikramy A. Khalil [2], Hideyoshi Harashima [1]*

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Hepatocellular carcinoma (HCC) is a global challenge with limited efficient therapies [1]. The stroma-rich tumor microenvironment restricts most nanomedicines from accessing the tumor cells following systemic administration [2]. Combinational therapy based on chemotherapy and gene therapy is a promising strategy for synergistic eradication of the tumor at lower doses. The rational design of nanocarriers dramatically-affect their *in vivo* performance and the fate of treatment [3].

Combo lipid nanoparticles (cLNPs) were designed based on a novel pH-sensitive lipid, a diversity of phospholipids and a Highly-selective targeting peptide. cLNPs were loaded with the cytotoxic drug, sorafenib (SOR), and a small interfering RNA targeting the Midkine gene (MK-siRNA). The lipid composition of cLNPs was tweaked and the physico-chemical properties were manipulated using a novel microfluidic device, iLiNP. The lipid composition and physico-chemical properties of cLNPs significantly-controlled their pharmacokinetics, tumor penetration and gene knockdown efficiency. The optimized cLNPs showed highly-potent gene silencing in the tumor with an siRNA median effective dose of 0.1 mg/Kg following intravenous administration to HCC-bearing mice, compared to minimal effect on the healthy liver. Moreover, the novel combination recruited in this study synergistically-eradicated HCC in mice at surprisingly-low doses of SOR and MK-siRNA. We believe that our strategy has a promising potential for clinical application in HCC therapy.

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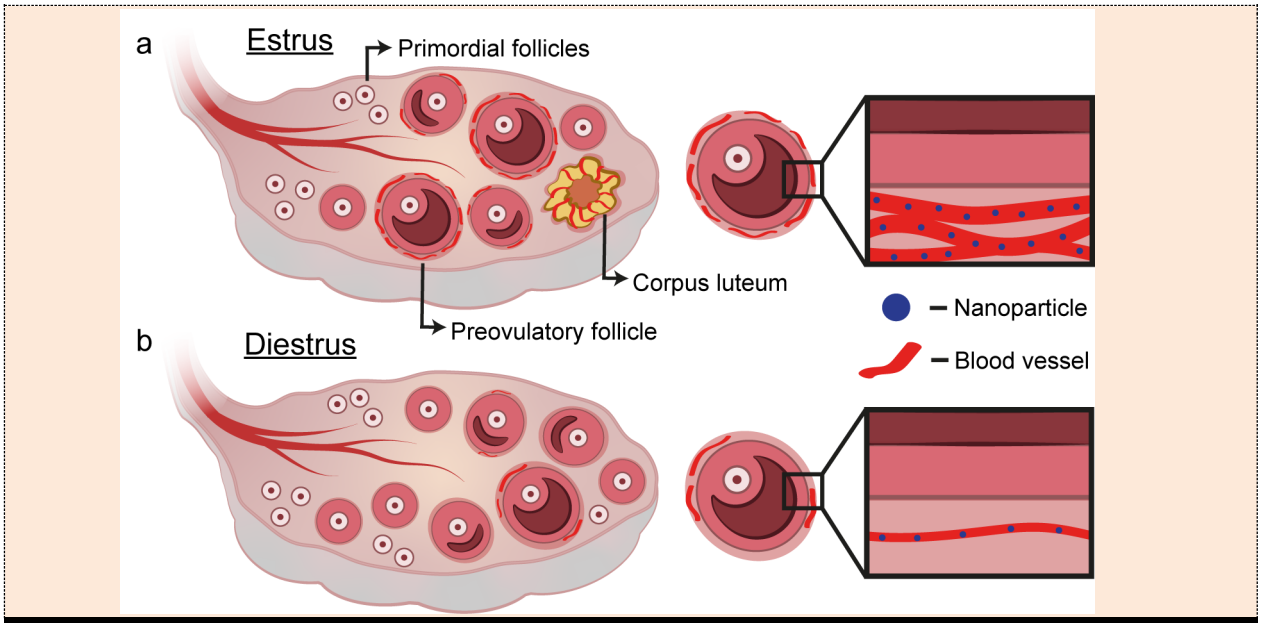


# Chemotherapeutic Nanoparticles Accumulate in the Female Reproductive System during Ovulation Affecting Fertility and Anticancer Activity

Maria Poley[1], Yael Shammai[1], Maya Kaduri[1], Lilach Koren[1], Omer Adir[1,2], Jeny Shklover[1], Janna Shainsky[1], Irit Ben-Aharon[3,4],Assaf Zinger[5,6,*] and Avi Schroeder[1,*]

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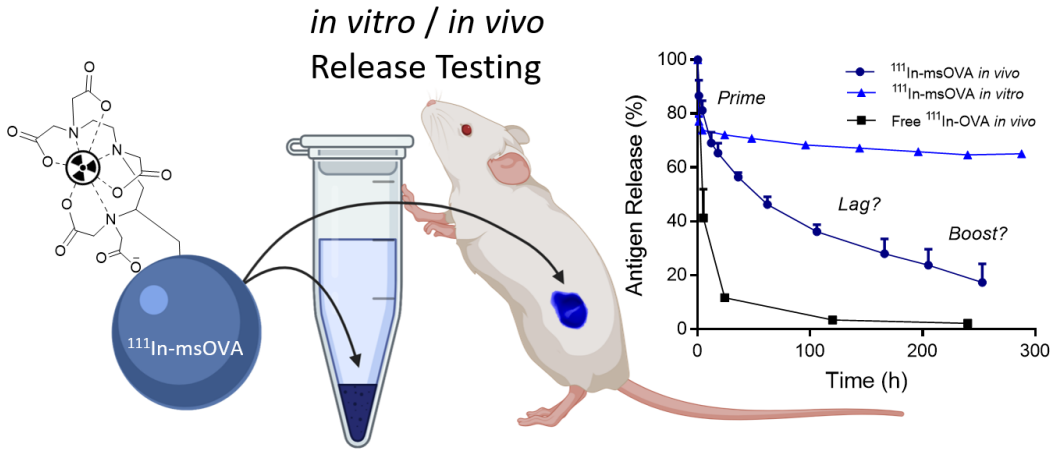
Throughout the female menstrual cycle, physiological changes occur that affect the biodistribution of nanoparticles within the reproductive system. This can have positive or negative effects. We demonstrate a 2-fold increase in nanoparticle accumulation in the ovaries during female mouse ovulation (Fig. 1a) compared to the non-ovulatory stage (Fig. 1b) following intravenous administration. Accumulation in the reproductive system is favored by nanoparticles smaller than 100 nm. Chemotherapeutic nanoparticles administered during ovulation increased ovarian toxicity and decreased short-term and long-term fertility when compared to the free drug. Breast cancer treated with nanomedicines during ovulation results in higher drug accumulation in the reproductive system rather than at the site of the tumor, reducing treatment efficacy. Conversely, ovarian cancer treatment was improved by enhanced nanoparticle accumulation in the ovaries during ovulation. Our findings suggest that the menstrual cycle should be considered when designing and implementing nanotherapeutics for females.




# Correlation Between *In Vitro* and *In Vivo* Release of Radiolabeled Antigen from PLGA Microspheres Using SPECT/CT Imaging

Tullio V. F. Esposito[1,2,#,*], Jacqueline C. Y. Lai[3,#], **Marta Bergamo**[1,‡], Colin Blackadar[1], Cristina Rodríguez-Rodríguez[1,4], Katayoun Saatchi[1,*], Jan P. Dutz[3] and Urs O. Häfeli[1,2,*]

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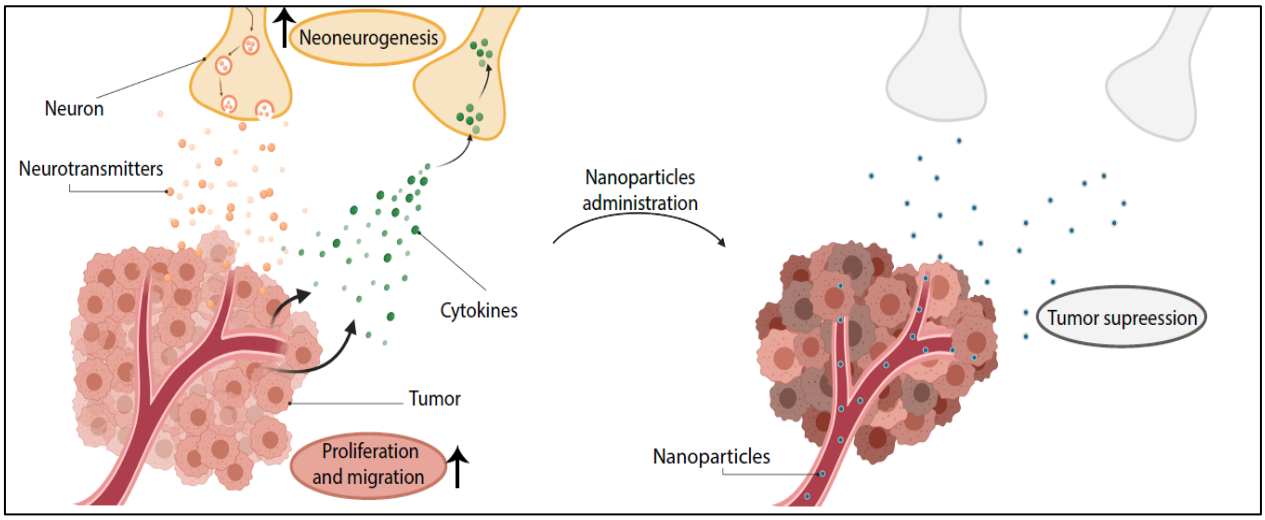
Typically, for inactivated subunit vaccines, multiple doses have to be administered to generate a protective response: an initial priming injection followed by one or more booster shots. To improve patient compliance with such vaccination schedules, drug delivery systems have been developed to release antigen over a tightly controlled series of burst and lag phases from a single injection. Biodegradable poly(lactide-co-glycolic acid) (PLGA) have received considerable attention for this application due to their peculiar 'prime-lag-boost' release profile. Although the release of antigens from PLGA microspheres is well characterized on the bench in buffers and physiological media, the *in vivo* performance of these particles remains largely unknown.

To shed a light on the latter, the model antigen ovalbumin (OVA) was radiolabeled with the long-lived gamma-emitter $^{111}$In and encapsulated into PLGA microspheres using a W/O/W emulsion technique; these particles are termed $^{111}$In-msOVA. The morphology of the particles was assessed using scanning electron microscopy (SEM) and dynamic light scattering (DLS; Z-Ave 4.6 ± 0.9 µm). Benchtop release curves were obtained in 1x PBS and diluted mouse serum using a gamma counter over a period of 3.5 weeks. The same microspheres were also injected into the subcutaneous fat of healthy female C57Bl/6 mice, and using a preclinical SPECT/CT imager, the activity remaining at the site of administration was measured over a 2 week period. A prime phase of release was observed both *in vitro* and *in vivo*, consisting of ~30-40% of the antigen load over the first 24 hours. However, while the *in vitro* microspheres entered a prolonged lag phase for the next 2 weeks, antigen was continually released from the *in vivo* injection site. No apparent boost phase was observed for the *in vivo* microspheres either. However, $^{111}$In-msOVA did prolong antigen retention compared to free OVA. The timing of antigen administration is crucial in producing the desired immune response from a vaccine. Our data shows that the *in vitro* release profile of antigen from PLGA microspheres does not always reflect what occurs in the body. In order to design and develop better vaccine delivery systems, a particularly important topic in the time of the COVID-19 pandemic, more attention needs to be addressed to their pharmacokinetics.




# Cancer and the Nervous System: Using nanotechnology to target cancer associated neurons as a tool for treating breast cancer

Maya Kaduri[1], Mor Sela[1], Maria Poley[1], Janna Shainsky-Roitman[1], Jeny Shklover[1], and Avi Schroeder[1]

[1]Laboratory for Targeted Drug Delivery and Personalized Medicine Technologies, Department of Chemical Engineering, Technion – Israel Institute of Technology, Haifa 32000, Israel

It has been discovered that there is a connection between the nervous system and cancer progression. Cancer cells can grow and invade the nerves in the tumor microenvironment, and use it as a mean for metastatic spread. Moreover, nerves and their axons actively infiltrate the tumor tissue and stimulating cancer-cell growth, proliferation, invasion and migration. These processes are promoted by cancer cells through the secretion of neurotrophic factors, but also by the nervous system through the secretion of chemokines and neurotransmitters [1,2]. In my research, I study the collaborative interactions between cancer and nerves and develop a new nanotechnology to treat cancer as a single or combined therapy. Nanotechnologies are becoming impactful therapeutic tools, granting tissue-targeting and cellular precision that cannot be attained using systems of larger scale. **We hypothesize that by reducing nerve ↔ cancer interactions via nanotechnology we will inhibit tumor growth and metastasis**. Our preliminary results show that cancer cells stimulate neuronal growth and that, in turn, neurons stimulate cancer cell prolifer ation and survival. Moreover, our nanoparticles are taken up by neurons efficiently. I aim to utilize this novel approach as a mean of targeting medicine to neurons, possibly also for treating other disease of the nervous system.

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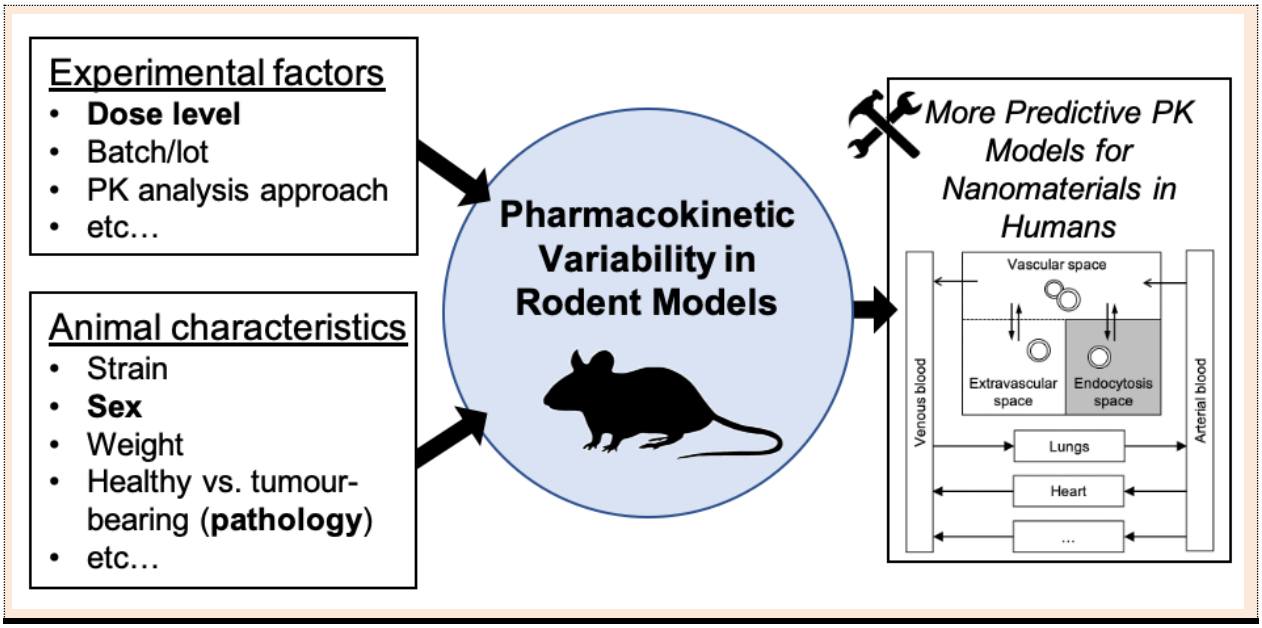
2. Zahalka, A. H., & Frenette, P. S. (2020). **Nerves in cancer**. *Nature Reviews Cancer*, 1-15.




# Pharmacokinetic Variability of Long-circulating Nanomaterials: Insights into its Origins and Neglected Importance in Rodent Models

Michael S Valic [1], Wenlei Jiang [1], Lili Ding [1], Michael Halim [1], Pamela Schimmer [1], Chris J Zhang [1], Tina Ye [1], Juan Chen [1], Marcus Q Bernardini [1], Brian C Wilson [1], Gang Zheng [1]*

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Rodent models fulfill several roles in the preclinical testing of novel nanomaterial-based drug delivery systems, including screening for drug pharmacokinetics, efficacy, toxicity, etc. Since the success of nanomaterial formulations are often judged on their pharmacokinetic uniformity, experimental reproducibility in rodent models is critical. However, in the context of many clinical nanomaterials, significant interpatient pharmacokinetic variability is routinely observed and is believed to significantly contribute to the failure of some nanomedicines in the early clinical phase [1]. Thus, a paradox exists wherein the rodent models and benchmarks/expectations used for evaluating the success of nanomaterials—i.e., consistent and long-circulating pharmacokinetics—are unrepresentative and potentially misleading of behaviors observed in patients.

To address this paradox, our team investigated the origins of pharmacokinetic variability of long-circulating nanomaterials in rodent models from among experimental factors (dose level) and animal model characteristics (sex, weight, strain, pathology). We analysed post hoc the plasma pharmacokinetic profiles of porphyrin-lipid based nanomaterials (Porphysomes) from over 200+ rodent models. Our findings revealed the greatest influences on Porphysome pharmacokinetic variability were dose level, model pathology (e.g., healthy vs tumour-bearing) and sex. Although our analysis was limited from identifying the mechanisms underlying the observed variability, our findings highlight the importance of its consideration for accurately assessing nanomaterial pharmacokinetics and for developing models to predict profiles in patients using rodent datasets.

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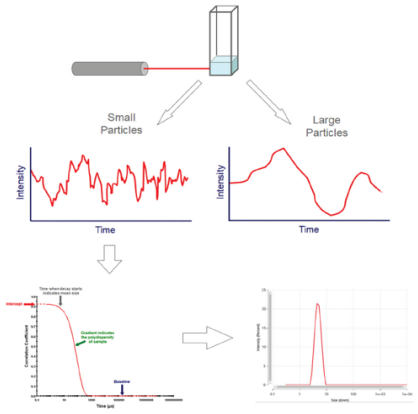
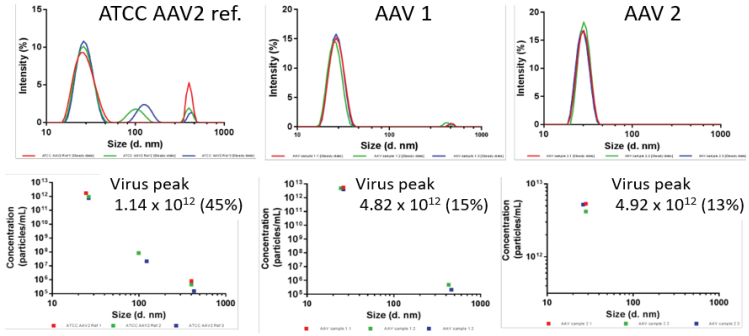



**Dynamic Light Scattering is a useful biophysical tool to measure particle hydrodynamic diameter in solution.**




**Using Multi-Angle Dynamic Light Scattering (MADLS) to Measure Size, Aggregation State and Particle Concentration.** Particle size distributions for three AAV samples were measured in triplicate. Using the new Particle Concentration measurement, alongside the MADLS measurement, the concentration of AAV and each aggregated species was also measured.

# MEASURING THE CONCENTRATION OF ADENO-ASSOCIATED VIRUS (AAV) WITH MULTI-ANGLE DYNAMIC LIGHT SCATTERING (MADLS) USING THE NEW ZETASIZER™ ULTRA

Muneera Beach [1*], Hanna Jankevics Jones[2], Natalia Markova[2], Ian Anderson[3] and Kirsty McManus[3]

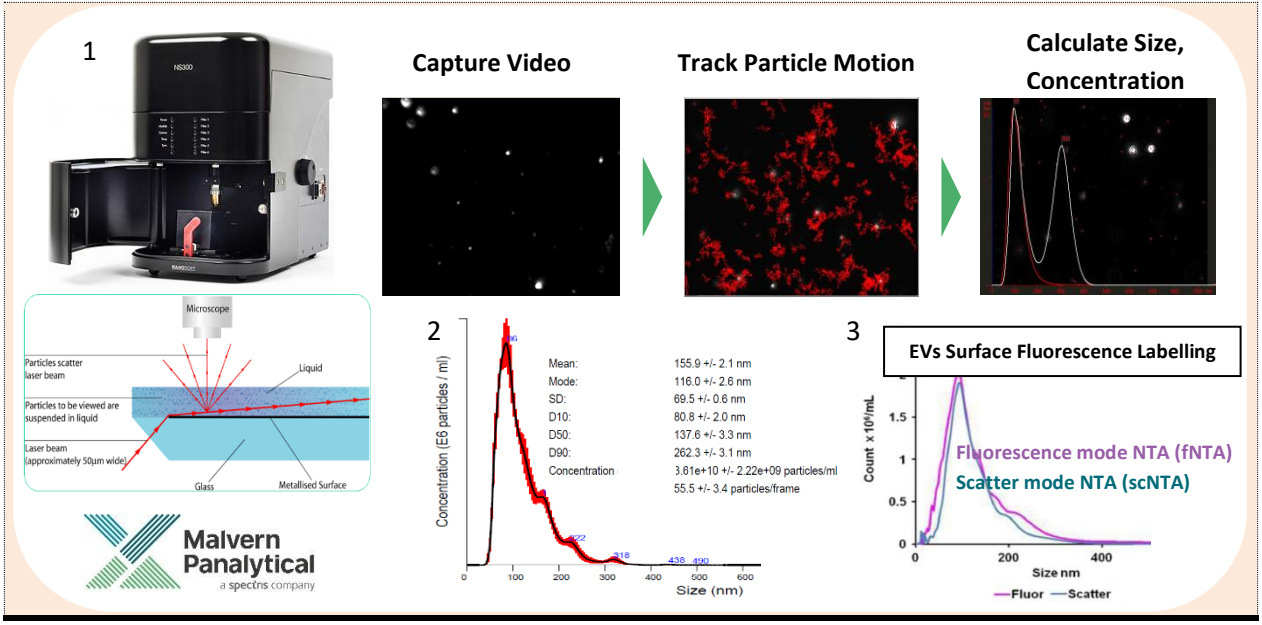
[1]Malvern Panalytical, Westborough, MA, Muneera.Beach@malvern.com; [2]Malvern Panalytical, Malvern UK; [3]Allergan Biologics Ltd., Chester, United Kingdom

Recombinant Adeno-Associated Viruses (rAAV) are a well-studied class of viral vector that is being investigated intensively in the development of gene therapies. rAAVs are typically produced in engineered cell lines in large bioreactors. Once produced inside the cell, the rAAV particles are released by lysis, and then isolated from the bulk harvest through multiple rounds of isolation; for example, via density gradient centrifugation, ion-exchange chromatography, and tangential flow filtration. Throughout the downstream purification process, multiple assays are performed to determine key analytical attributes for the determination of yield, efficacy, and safety, as well as to enable process understanding and control. These parameters are typically: capsid or particle count, genome count, % genome-containing or % full rAAV particles, serotype characterization, particle size, aggregation level and the presence of unwanted host-cell proteins and nucleotides.

Multi-angle Dynamic Light Scattering (MADLS) is a novel technique capable of simultaneously measuring multiple rAAV parameters, including aggregation, capsid charge, and particle concentration. It is well-suited as a complementary assay that can be utilized in existing analytical workflows to provide rapid, label-free, non-destructive, low volume determination of the total rAAV particle concentration.

In this poster, we will describe the MADLS measurement principle and the application of this novel method to the characterization of rAAV model samples, as well as rAAV therapies in development. Resulting data on the viral concentration and size of rAAV particles will be discussed, along with the effects of sample properties, such as material refractive index and viscosity. There are also real world-examples of multi-serotype rAAV analysis case studies of drug substance and process development samples.(1)

1. Measuring the concentration of Adeno-Associated Virus (AAV) with multi-angle dynamic light scattering (MADLS), Malvern Panalytical application note 2020 https://www.malvernpanalytical.com/en/learn/knowledge-center/application-notes/AN180608AdenoVirusConcentrationMADLS




# Using Nanoparticle Tracking Analysis to Characterize Size and Concentration of Nanoparticles in Drug Delivery Systems

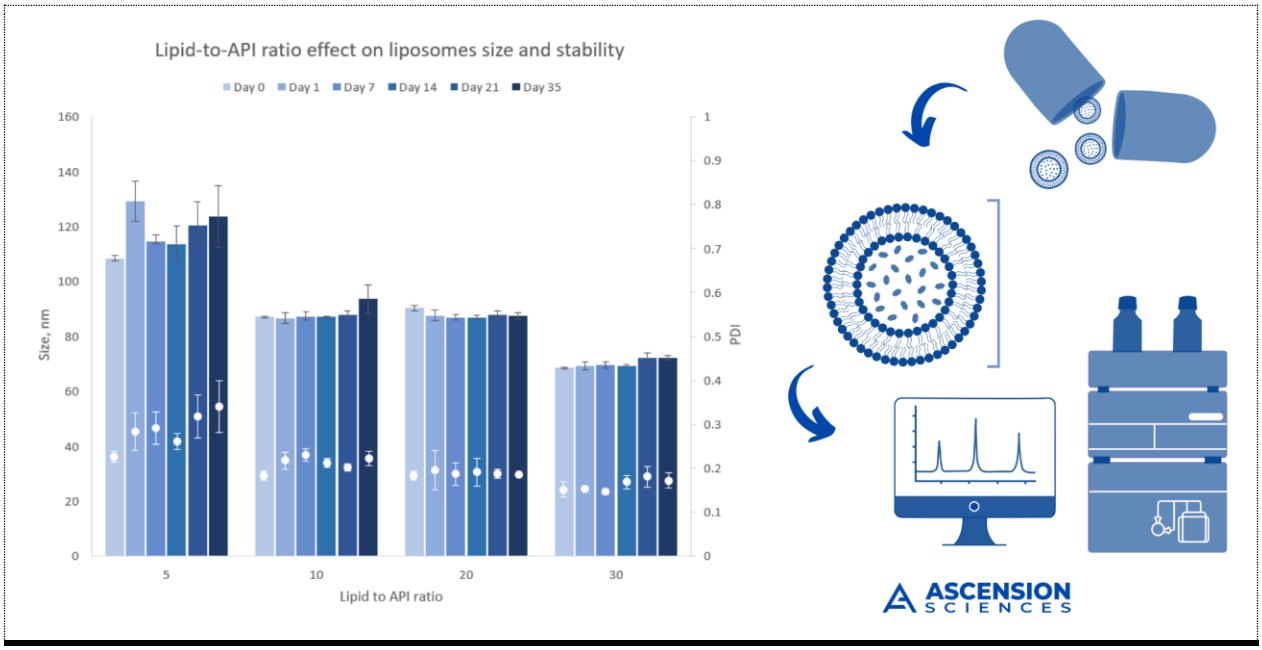
Muneera Beach[1], Arianna Biesso[1],
Ronald Soriano[1], Verna Frasca[2], Agnieszka Siupa[3]

[1] Application Scientists, Malvern Panalytical, Food and Pharmaceuticals, Westborough Massachusetts, USA, muneera.beach@malvern.com, [2] Sector Development Manager, Food and Pharmaceuticals, Malvern Panalytical, Westborough, Massachusetts, USA, [3] Product Manager, Nanomaterials, agnieszka.siupa@malvern.com, Malvern Panalytical, Malvern, United Kingdom

Malvern Panalytical's NanoSight instrument is capable of delivering high resolution particle sizing between 40 nm to 1 micron and determining particle concentration calibration free utilizing nanoparticle tracking analysis or NTA. This makes NTA a great companion for studies in viral vaccine research, nanotoxicology and biomarker detection, as well as the characterization of extracellular vesicles for disease state studies. Applications covered in this poster presentation include characterizing extracellular vessicle (EV) preparation purity, use in the clinical diagnostic space, and viral vector quantification strategies. Finally we will describe how NTA fluorescence capability can be utilized for purity assessment, enrichment monitoring and phenotyping based on surface epitopes and cargo of nanoparticles such as EVs and viruses.

**Figure 1**. NanoSight instrument and schematic for optical array, virus particle video capture, particle tracking and size and concentration analysis. Overlaid particle size distribution plots of a virus preparation before (white) and after (red) a final purification step.
**Figure 2.** Exosome preparation particle count and concentration determination.
**Figure 3.** EV sample labeled with CellMask™ Orange Plasma membrane dye. EVs characterized using both fNTA (EVs membrane specific) and scNTA (total particle) modes confirming purity of the prep [1].

1. Dragovic, R., Gardiner C., Brooks A., Tannetta D., Ferguson D., Hole P., Carr B., Redman C.W.G., Harris A., Dobson P., Harrison P., Sargent I. (2011). **Sizing and phenotyping of cellular vesicles using Nanoparticle Tracking Analysis**. Nanomedicine. Dec;7(6):780-8.




# Impact of Lipid Composition on Liposome Stability and Cannabinoid Drug Encapsulation Efficiency

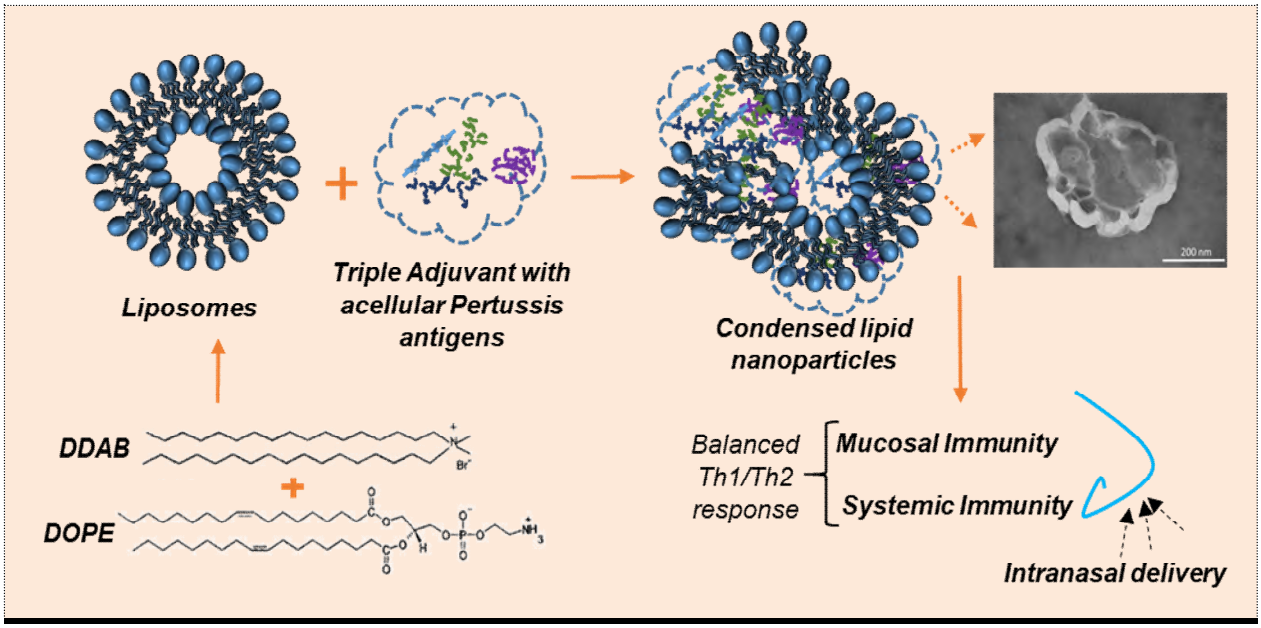
Natalia Sannikova [1], Melanie Law[1], Andrea Olaizola[1], Melody Lin[1], Tomas Skrinskas[1]

[1]Ascension Sciences, Vancouver, V5Y 1K6, Canada; tskrinskas@ascensionsciences.com

Recent literature has been increasingly supporting the therapeutic use of cannabinoids in the development of novel medicines. Cannabinoids have been shown to be beneficial in the treatment of various diseases such as Alzheimer's disease and multiple sclerosis. However, such benefits are hindered by their poor aqueous solubility, limiting bioavailability. Lipid nanoparticles offer an effective alternative to improve pharmacokinetic and biodistribution profiles of drug payloads. Crucial considerations for these systems pertain to size and uniformity, which impact liposome circulation time and tissue penetration.

Here, the impact of phospholipid type (synthetic vs. organic, unsaturated vs saturated) and lipid components ratio on liposome drug retention, size, and stability was investigated. Microfluidic techniques were utilized in the formulation of liposomes which varied in cholesterol content, with either SoyPC, POPC, or DSPC as the primary bilayer constituent, and THC as the API.

Increases in the lipid-to-API ratio demonstrated decreasing trends in liposome size ranging from 120 nm to 60 nm and increasing EE from 40 to 100 %. Varying cholesterol concentration captured different size and stability trends depending on phospholipid selection, whereas THC encapsulation efficiency remained unimpacted. Our approach validates the significance of the type of lipid synthesis and cholesterol percentage as important parameters in fine-tuning liposome formulation for controlled cannabinoid delivery.




# Rethinking the Pertussis vaccine: Formulation of lipid nanoparticles with vaccine adjuvants to achieve enhanced immunity.

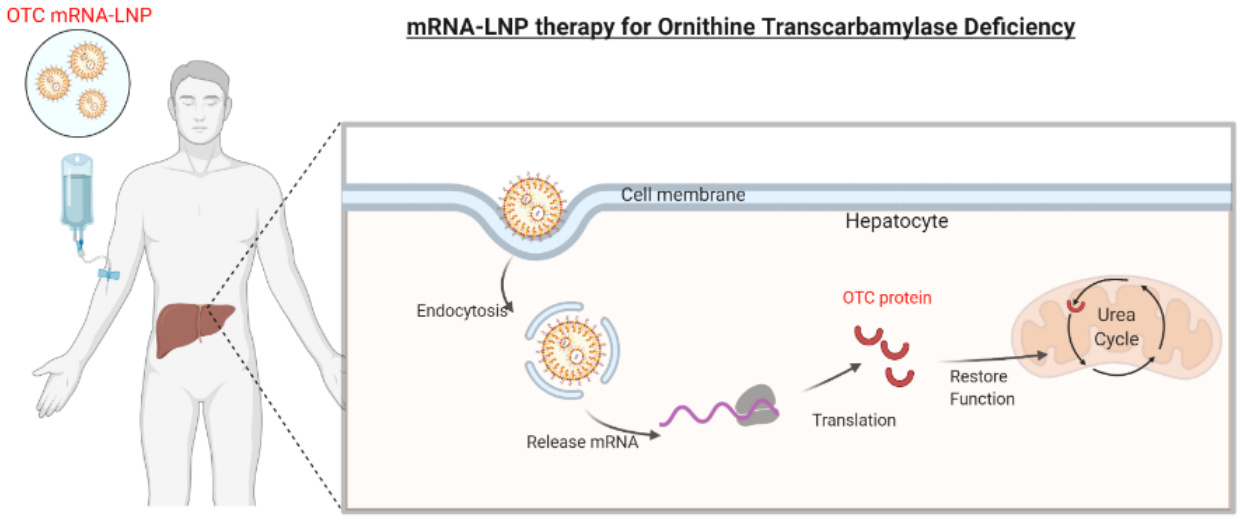
Noorjahan Aibani[1,2], Volker Gerdts [3], Robert E.W. Hancock[4], Kishor Wasan [2,5], Ellen Wasan [1,2]*

[1]College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK. [2]Nanomedicine Innovation Network, University of British Columbia, Vancouver, BC. [3]Vaccine and Infectious Disease Organization – International Vaccine Centre (VIDO-InterVac), University of Saskatchewan, Saskatoon, SK, [4]Dept. of Microbiology and Immunology University of British Columbia, Vancouver, BC, [5]Dept. of Urological Sciences, Faculty of Medicine, University of British Columbia, Vancouver, BC.

Pertussis is an infection of the lungs and pulmonary airways. This potentially fatal disease is globally endemic. Recent outbreaks of pertussis have revealed that effectiveness of the current acellular vaccine is suboptimal, hence it is essential to make improved vaccines. A triple adjuvant consisting of a TLR agonist [poly(I:C)] , an immunostimulant host defense peptide (IDR-1002) and polyphosphazene has achieved stronger, faster and long-lasting immune responses [1]. Formulation of this triple adjuvant into cationic lipid nanoparticles for intranasal delivery of pertussis vaccines may provide efficient mucosal adhesion and induce enhanced mucosal and systemic immune response [2]. The triple adjuvant (Triadj) was prepared by mixing the three components in an experimentally optimized ratio (1:2:1) and followed by complexation to cationic liposomes to form L-Triadj. Addition of acellular pertussis antigens namely Pertussis Toxin Mutant (PTM), Pertactin (PRN) and Fimbriae 2/3 (Fim 2/3) at the TriAdj assembling stage allowed for complete incorporation of antigens into the L-TriAdj system as cationic lipid nanoparticles. Characterization included particle sizing (254±51nm), zeta potential (+55±3.5) and transmission electron microscopy (TEM), showing discrete amorphous particles. In vivo assessment is planned. This lipid-based triple adjuvant formulation can have broad applications for various therapeutic and vaccine formulations.

1. Garg R, Babiuk L, van Drunen Littel-van den Hurk S, Gerdts V (2017). **A novel combination adjuvant platform for human and animal vaccines**. Vaccine 35, 4486-4489.

2. Wasan EK, Syeda J, Strom S, Cawthray J, Hancock RE, Wasan K, Gerdts V (2019). **A lipidic delivery system of a triple vaccine adjuvant enhances mucosal immunity following nasal administration in mice.** Vaccine 37(11),1503-1515.



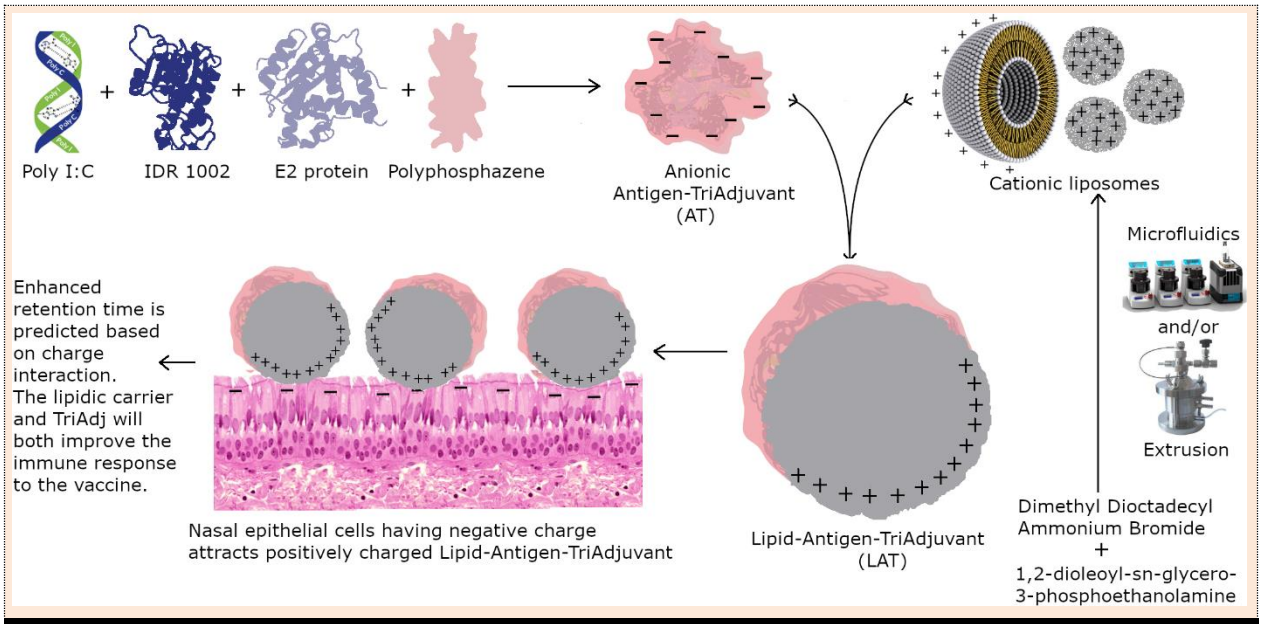

# PRECLINICAL EVALUATION OF A MODIFIED MRNA FOR THE TREATMENT OF OTC DEFICIENCY

Daly, O.,[1] Lam, K.,[1] Meffen, T.,[1] Reid, S.,[1] Yaworski, E.,[1] Tyler, S.,[1] Vlatkovic, I.,[2] Mahiny, A.J.,[2] Reinholz, J.,[2] Besold , K.,[2] Fesser, S.,[2] Lepper, M.,[2] Berte, N.,[2] Lindemann, C.,[2] Marlot, P.T.,[2] Kuhn, A.N.,[2] Karikó, K.,[3] Lutwyche, P.,[1] Esau, C.,[1]

[1] Genevant Sciences Corporation, 155-887 Great Northern Way, Vancouver, BC V5T 4T5. owen.daly@genevant.com
[2] BioNTech RNA Pharmaceuticals GmbH, An der Goldgrube 12, 55131 Mainz, Germany

Ornithine transcarbamylase (OTC) deficiency is a rare X-linked genetic disorder characterized by complete or partial lack of the OTC enzyme. OTC plays a key role in the urea cycle; its absence leads to inability to metabolize ammonia and is associated with permanent brain damage and death. Genevant and BioNTech are collaborating to co-develop an mRNA therapy enabled by Genevant's industry-leading nucleic acid delivery capabilities to treat OTC deficiency.

Codon-optimized, nucleoside-modified human OTC mRNA was encapsulated in lipid nanoparticles before being delivered to both wild-type (WT) and OTC-deficient mice (OTCspf-ash). OTCspf-ash mice are a widely accepted model for the study of OTC deficiency with residual OTC expression levels of ~5% compared to WT, and a low tolerance to a high protein diet. Here, we have used the challenge of a high protein diet in OTCspf-ash mice to demonstrate the efficacy of our OTC mRNA-LNP in a diseased state. We subsequently tested the lead mRNA-LNP in a multi-dose NHP study leading to robust OTC protein expression and no changes in liver parameters at low dose levels.




# A subunit vaccine for bovine viral diarrhea: lipid-based formulation containing bovine viral diarrheas virus E2 protein in combination with adjuvants to improve immunogenicity

Parth Patel[1], Sylvia Van Drunen Littel-van den Hurk[2], Grace Cuddihy[1], Volker Gerdts[2], Kishor Wasan[3], Ellen Wasan[1]*

[1]College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK; [2]Vaccine and Infectious Disease Organization – International Vaccine Centre (VIDO-InterVac), Saskatoon, SK; [3]Faculty of Medicine, University of British Columbia, Vancouver, BC

Bovine viral diarrhea virus (BVDV) contributes to a respiratory disease complex in cattle and is an important pathogen in the cattle industry. The objective of the research is to fabricate a cationic lipid-based delivery system for intranasal administration of the E2 protein as a BVDV vaccine and to determine its in vivo efficacy. We hypothesize that attraction between lipidic carrier and nasal epithelia improves its retention time and will enhance the immunogenicity of vaccine.

TriAdj [Poly (I:C), Innate defense regulator protein (IDR -1002) and Polyphosphazene] forms an electrostatic complex with DDAB:DOPE (1:1 mol:mol) cationic liposomes creating lipidic particle (L-TriAdj; <150+/-13.5 nm). L-TriAdj particles lyophilized with 5% w/v dextrose then reconstituted showed consistent mean diameters (373.6+/-11.1 nm) L-TriAdj was mixed with E2 protein to prepare whole vaccine particles. Vaccines made with lyophilized L-TriAdj had a greater mean diameter than those prepared fresh (378.8+/-25.20 nm vs. 232.7+/-38.86 nm). As an alternative formulation, the E2 protein antigen was incorporated during TriAdj preparation then combined with the cationic liposomes to prepare Lipid-Antigen-TriAdjuvant (LAT; mean diameter 452.7+/-317.6 nm, zeta potential: +41.5+/-0.9 mV). An in vivo efficacy study comparing the lipidic vaccine formulations is planned.

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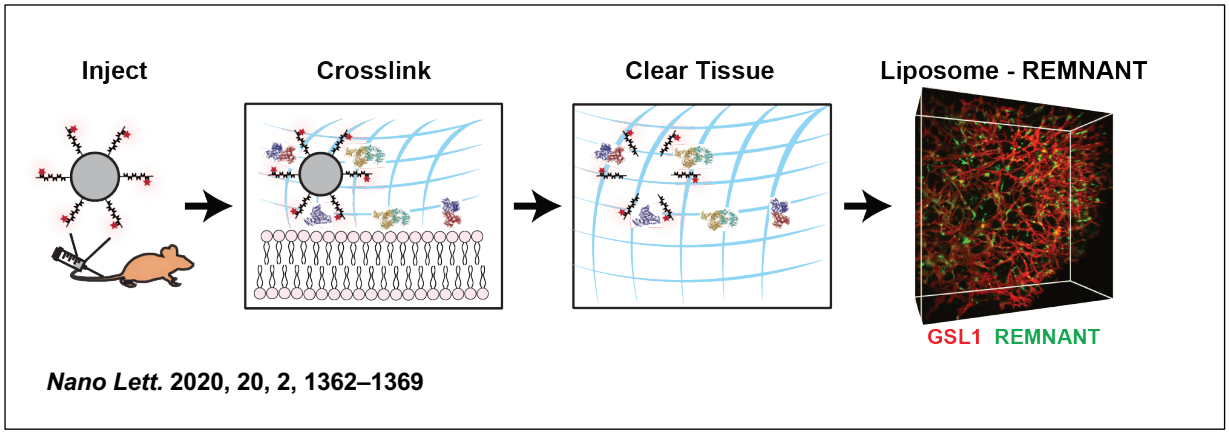


## Liposome Imaging in Optically Cleared Tissues

Abdullah Muhammad Syed ‡, **Presley MacMillan**‡, Jessica Ngai‡, Stefan Wilhelm, Shrey Sindhwani, Benjamin R. Kingston, Jamie L. Y. Wu, Pablo Llano-Suárez, Zachary Pengju Lin, Ben Ouyang, Zaina Kahiel, Suresh Gadde, Warren C. W. Chan*
‡ authors contributed equally.

Three-dimensional (3D) optical microscopy is a useful tool for studying nanoparticle delivery in biological tissues. Unfortunately, most of the methods required to render tissues transparent for 3D microscopy destroy or degrade clinically relevant nanoparticles. As a result, it is not possible to study the distribution of nanoparticles such as liposomes using 3D microscopy. Here, we have developed a nanoparticle tag termed REMANT, which is capable of surviving tissue clearing and enables the liposome distribution in optically clear tissues to obtained. We also show that using REMNANT, the release rate of liposome encapsulated therapeutic agents can be determined. Using this method, we found that liposomes release their cargo >100 fold faster in tissues *in vivo* when compared to *in vitro* assays. This allowed us to design liposome formulations with optimized drug release rates resulting in an enhanced ability to kill tumour associated macrophages. Our tag opens up new avenues for studying the chemical properties and pharmacodynamics of administered organic materials in an intact biological environment. Our approach provides insight into the *in vivo* behaviour of degradable nanomaterials which can used to improve future generations of therapeutic agents.

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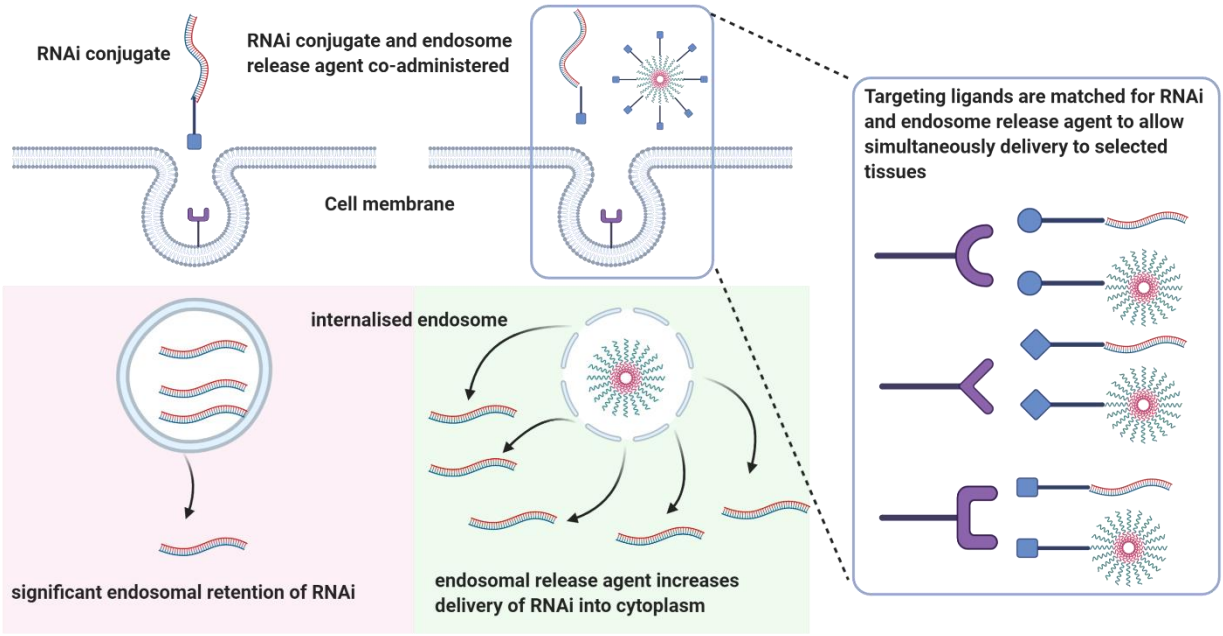


# PH-responsive endosomal release agents to enhance RNAi conjugate activity across multiple cell types and receptors

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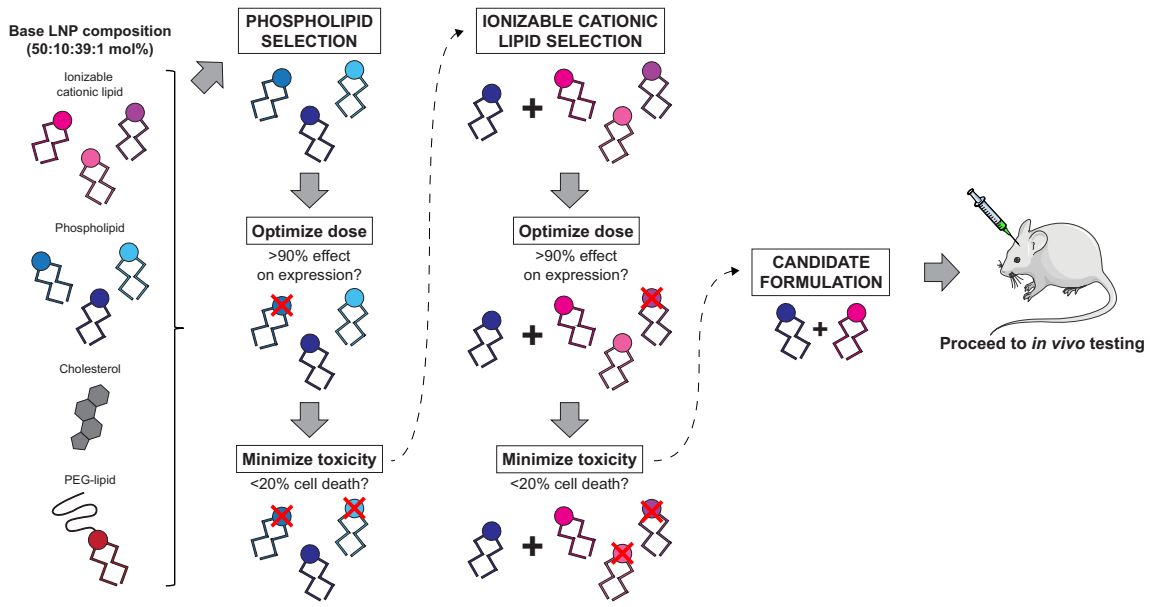
The great promise of ligand targeted RNA interference (RNAi) therapeutics was further realized in 2019 with the regulatory approval of Givosiran (Givlaari®), a GalNAc-targeted siRNA for treatment of acute hepatic porphyria AHP. Adaptation of this therapeutic modality for cell types beyond hepatocytes has been slow, and, for reasons that are not fully understood, GalNAc mediated delivery appears to be a unique case. Even though conjugates can be targeted to other cell types readily with appropriate ligand selection, little if any of the internalized conjugate escapes the endosome before cellular degradation occurs. This significantly curtails biological activity, in most cases completely.

Genevant has a proprietary, pH-responsive endosomal release agent which can be simultaneously delivered with an RNAi conjugate to cells and tissues of choice by matching the targeting ligand in both entities. Subcutaneous co-administration in nonhuman primate studies has been shown to speed onset of activity and substantially improve both potency and duration of effect of GalNAc conjugates. Moreover, we have a expanding portfolio of extra-hepatocyte ligands with demonstrated biological activity across different cell types positioned to benefit from this unique approach. The modular design of this platform could further enable the realization of RNAi conjugates as therapeutic agents far beyond hepatic diseases.




# Design of lipid nanoparticle systems for brain gene therapy

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The majority of genetic neurological diseases are caused by toxic gain-of-function of a mutant protein or loss-of-function of a wild-type protein. The treatment of these disorders, either by knockdown of gene products or by gene replacement therapy, is a viable strategy provided gene therapy agents can be delivered to the affected cells and regions of the central nervous system. Many current approaches to brain gene therapy utilize the direct injection of drugs into the cerebrospinal fluid or brain parenchyma, which has facilitated the delivery of both antisense oligonucleotide (ASO) and adeno-associated viral (AAV) therapies. These modalities are limited by functionality, potency, and safety, leaving ample opportunity for innovation of gene therapy drugs and delivery methods.

Lipid nanoparticle (LNP)-enabled gene therapy is a promising alternative approach for the treatment of genetic brain diseases. The safety of LNP systems is well-established[1], and we and others have demonstrated that neurons, the primary cells of interest in the brain, are highly amenable to transfection by LNPs carrying gene therapy agents.[2]

We have developed a systematic screening strategy for the evaluation of LNP formulations carrying nucleic acid payloads using neurons *ex vivo*. This iterative approach utilizes a reporter system to identify optimal formulation parameters while minimizing the disruption of endogenous gene expression programs. Here, we demonstrate that LNP-mediated delivery of siRNA targeting eGFP induces knockdown in eGFP-expressing primary cortical neurons and show that efficacy and toxicity vary with lipid composition and dose. We will also apply this strategy to optimize LNP-mRNA formulations, and all *ex vivo* results will be validated using direct brain injection *in vivo*. This work will enable the comparison of LNP-based gene therapy methods to existing approaches and will support the future development of target-specific treatments for genetic brain disease.

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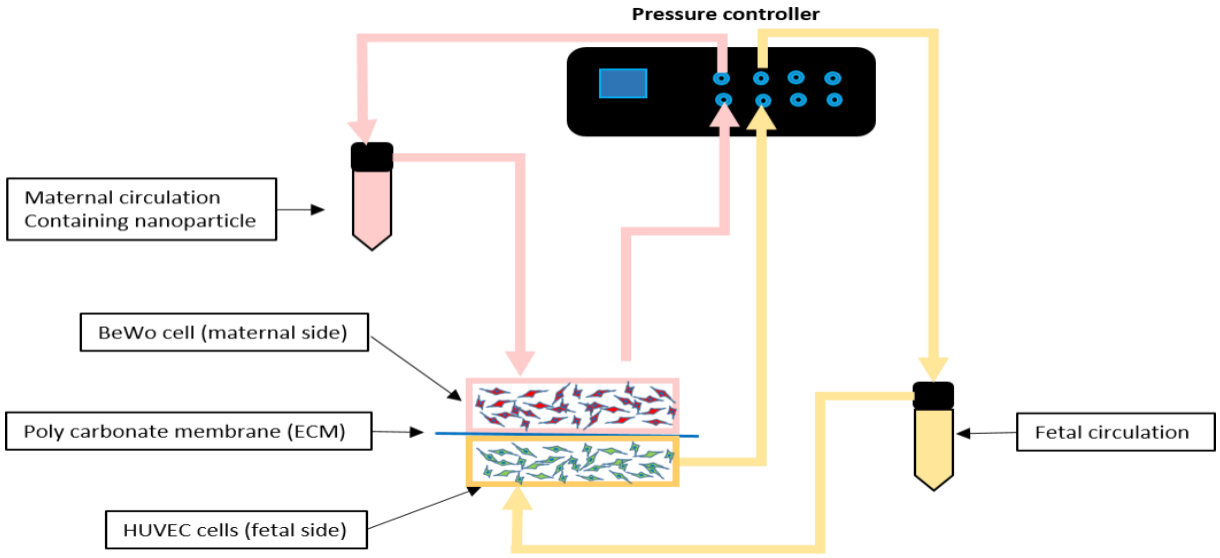


# A dynamic microphysiological model of placenta to study the effect of gold nanoparticles

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Placenta is formed during pregnancy to separate maternal blood from fetal circulation. It is composed of the fetal capillary endothelium and maternal trophoblasts that regulate the trafficking of material to provide a healthy environment for the development of the fetus. Nanotechnology revolutionized medicine by increasing efficiency and decreasing off-target effects of the drugs. Gold nanoparticles (AuNPs) have attracted extensive attention due to their application as both therapeutic and diagnostic material however their effect on susceptible populations like embryos is not defined yet.

Development of a dynamic placenta-on-chip model help to study the effect of AuNPs on fetus as flow-related factors impact the interaction of the nanoparticles with the placental barrier. A two-channel microfluidic chip in a three-layer with co-culturing of human trophoblasts and human fetal endothelial cells separated by a layer of membrane was developed to mimic the placenta. Formation of syncytiotrophoblast and microvilli is assessed by immunostaining of markers of tight junction and actin cytoskeleton. AuNPs are introduced to the maternal side and their effects on the placental and fetal cells under different flow rate and shear stress conditions are assessed using viability assay. The dynamic model of the placenta simulates the in vivo situation and is suitable to screen the effect of a wide spectrum of nanoparticles like AuNPs under different flow rate conditions on the placental structure and function.

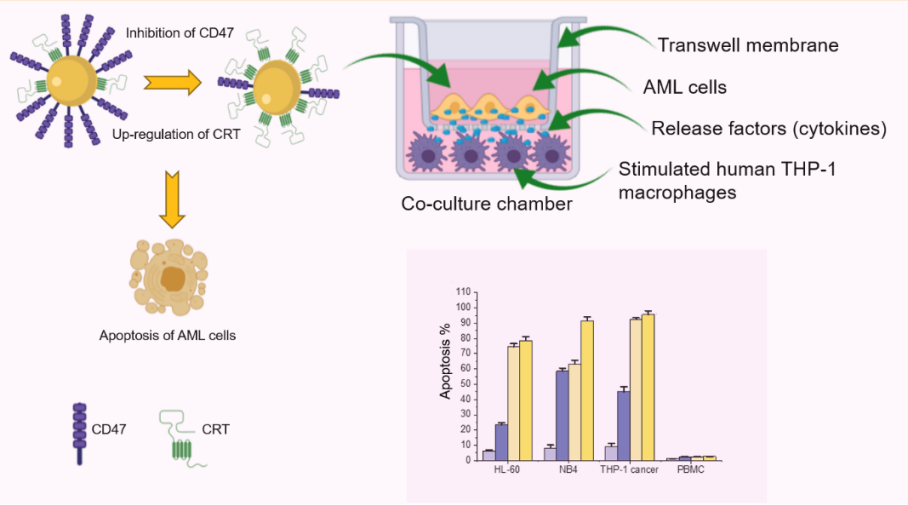


Figure 1. Stimulated human macrophages inhibit CD47 and up-regulate CRT in AML cells leading to their elimination. This is selective to only AML cells and not normal blood cells.

# Anti-Leukemia Effect by Stimulated-Macrophages in Co-Culture

Eman M. Hassan[1], Gilbert C. Walker[2], Chen Wang[3], and Shan Zou[1*]

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CD47 is over-expressed in Acute Myeloid Leukemia (AML) and functions as inhibitory signal, suppressing phagocytosis by binding to signal regulatory protein α (SIRPα) on the surface of macrophages. Inhibition of CD47 restores the immune surveillance of AML cells. However, the inhibition of CD47 in AML by activated macrophages and the subsequent effects on different immune response parameters are not fully understood. Here, we demonstrate the use of a distinct co-culture method to inhibit CD47 and therefore eliminate AML cells by macrophages *in vitro*, shown in Figure 1. Human chemical induced THP-1 macrophages were activated by using different concentrations of lipopolysaccharide (LPS) and co-culturing with three AML cancer cell lines (HL-60, NB4, and THP-1), respectively, as well as normal cells. CD47 inhibition was successful and selective in AML but not normal cells. Additionally, calreticulin (CRT) levels were elevated in the same cell lines simultaneously, after co-culturing with activated human macrophages, but not in normal cells. We also show that activated macrophages secreted high levels of cytokines including, IL-12p70, IL-6 and TNF-α, consistent with the elimination of AML by macrophages. [1] Our study reveals the potential of this model for screening new drugs against AML and the possibility to use human macrophages in AML treatment in the future.

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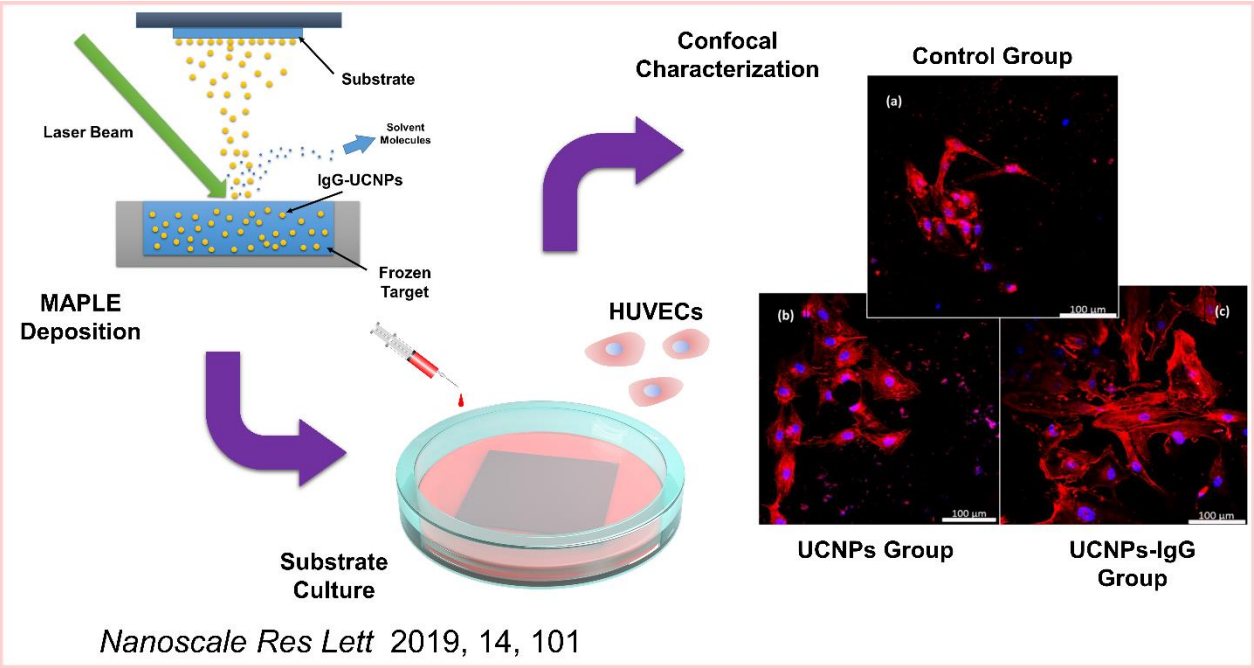


# Development of A Suitable Surface by Coating with Antibody Modified Up-conversion Nanoparticles for Improvement of Cell Culture Performance

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A suitable surface is vital for maintaining or even promoting cells' function and communication. Researches indicated that nanostructured coatings could have a potential in improving cell adhesion [1]. However, it hardly minimizes the contamination by using traditional solution-coating technology. Matrix assisted pulsed laser evaporation (MAPLE) technique is a contamination-free process that meets the requirements of an efficient process to deposit biopolymer without damaging their backbone on the surface of various substrates [2].

As shown in **Figure 1** up-conversion nanoparticles ($NaGdF_4$: $Yb^{3+}$, $Er^{3+}$, UCNPs) with/without immunoglobulin G (IgG) modification were produced by a one-pot synthesis method. MAPLE system equipped with Nd:YAG laser (λ = 532 nm, ν = 10 Hz) is applied to deposit UCNPs with/without IgG modification on the glass bottom of the culture dish. The results show different behaviors of human umbilical vein endothelial cells (HUVECs) cultured on the culture dishes coated with UCNPs with/without IgG, compared to the control sample (glass coated with gelatin). No toxic effect is imposed on cells. The results of this work indicate that the nanostructured coatings enhance the adhesion and proliferation of cells and MAPLE is an efficient method in the fabrication of nanostructured biomaterials coating.

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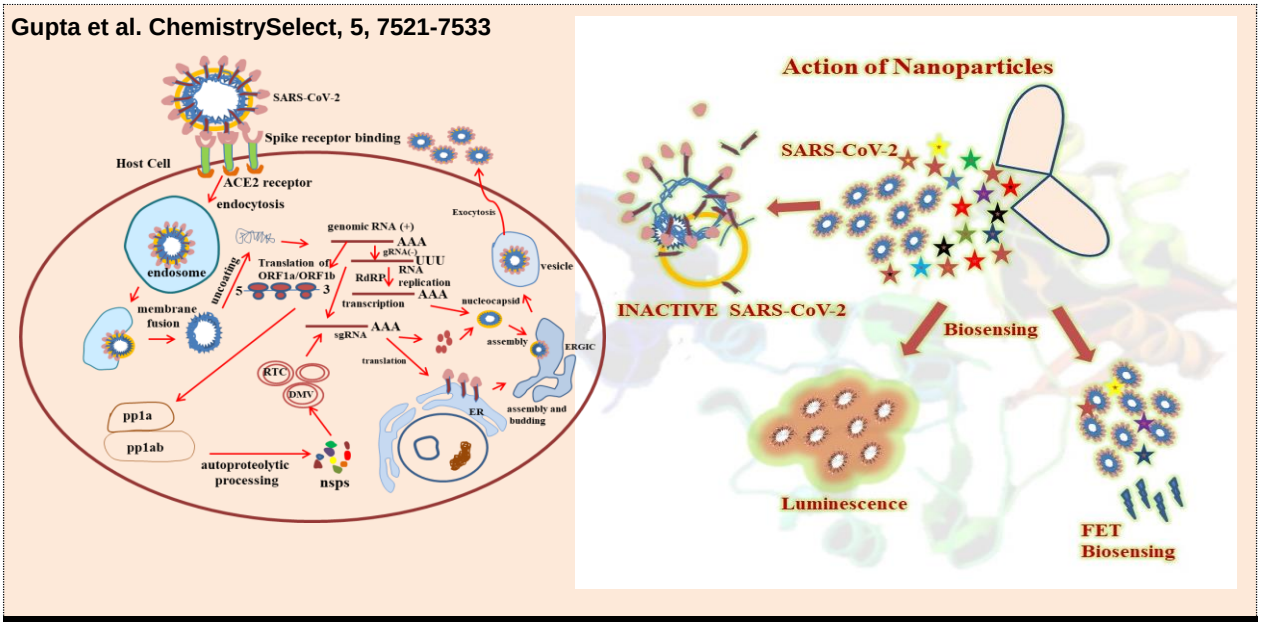


# Coronavirus Outbreaks: Nanomedicine and Future Perspectives

Akanksha Gupta[1], Sanjay Kumar[2], Ashish Kumar Choudhary[3], Vinod Kumar*[4]

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COVID19 has become a serious public health challenge for all the countries and established public health emergency. This infectious disease has dragged down the economy of the all top developed and developing nations. The coronaviruses caused several epidemics such as SARS (2002-2003), MERS (2005) and followed by COVID-19 (2019-2020). The zoonotic origin and their crossover to humans forewarn the world about the consequences of perturbing ecological niches of viruses. According to WHO as of August 16, 2020, COVID19 has been spread in 216 countries with 21.5 million confirmed cases, 7.66 million confirmed deaths. The historical background of viral infection encountered with present day challenges and futuristic approaches with the help of nanotechnology to minimize the spread of infectious viruses. Nanotechnology has improvised therapeutic advancements in recent years and is advanced combating tool in drug designing as well as drug targeting. Scientists could be encouraged toward use of nanomaterials for targeting viral structures and depraving the impact of such novel viral infections (1).

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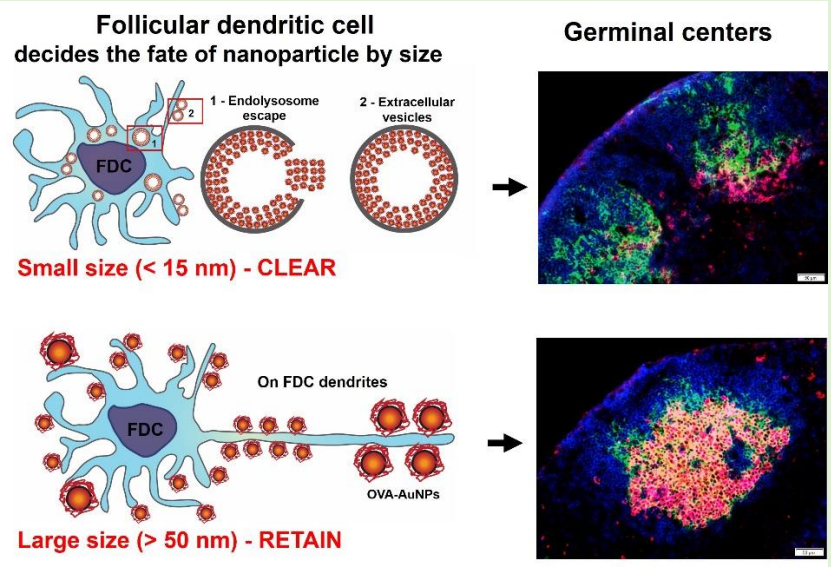


**Figure 1**: Nanoparticle size impacts humoral immune response in a lymph node

# Nanoparticle size influences antigen retention and presentation in lymph node follicles for humoral immunity

Yi-Nan Zhang[1,2], James Lazarovits*[1,2], Wilson Poon*[1,2], Ben Ouyang*[1,2,3], Luan N.M. Nguyen[1,2], Benjamin R. Kingston[1,2], Warren C.W. Chan**[1,2,4,5,6]

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Lymph node follicles capture and retain antigens to induce germinal centers and long-lived humoral immunity. However, control over antigen retention has been limited. Here we discovered that antigen conjugated to nanoparticle carriers of different sizes impacts the intralymph node transport and specific cell interaction. We found that follicular dendritic cell (FDC) networks determine the intralymph node follicle fate of these nanoparticles by clearing smaller ones (5-15 nm) within 48 h and retaining larger ones (50-100 nm) for over 5 weeks. The 50-100 nm-sized nanoparticles had 175-fold more delivery of antigen at the FDC dendrites, 5-fold enhanced humoral immune responses of germinal center B cell formation, and 5-fold more antigen-specific antibody production over 5-15 nm nanoparticles, shown in **Figure 1**. Our results show that we can tune humoral immunity by simply manipulating the carrier size design to produce effectiveness of vaccines [1].

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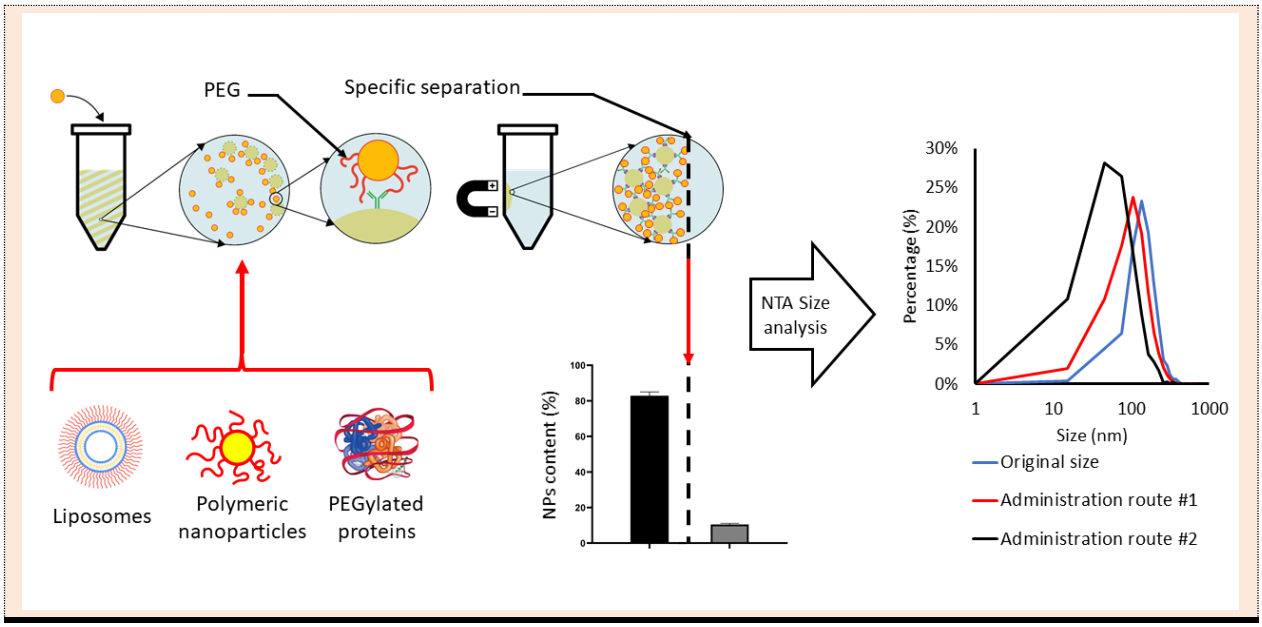


# Extraction of PEGylated nanoparticles by immunoprecipitation

Youcef Chakib Hacene [1], Nicolas Bertrand [1]*

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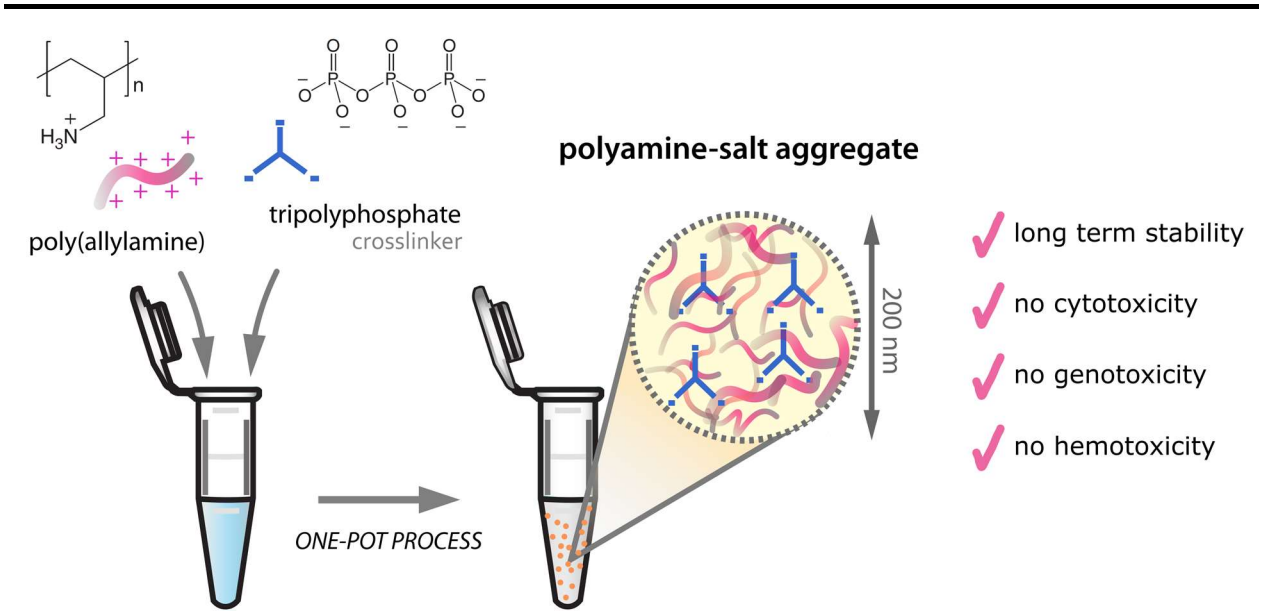
Polyethylene glycol (PEG) is considered as the gold standard polymer for the preparation of long circulating nanoparticles (NPs) as it shields NPs from opsonization and prevents rapid blood clearance. Upon administration in vivo, characterization of PEGylated NPs requires their separation from the rest of plasma components. In this study, we describe an immunoprecipitation method, using antiPEG antibodies crosslinked to magnetic beads, for the specific extraction of three types of radiolabeled PEGylated systems: polymeric, liposomes, and therapeutic proteins. The extraction protocol is characterized in terms of extraction capacity and kinetics. We show that this extraction is possible for NPs after their administration in vivo. Using Nanoparticle Tracking Analysis (NTA), we show that this extraction technique can be used to determine changes in size of NPs after intravenous and intraperitoneal administration.




# Poly(allylamine)-tripolyphosphate self-assemblies: Towards robust and biocompatible drug nanocarriers

Agustín S. Picco,[1] Santiago Herrera,[1] Maximiliano Agazzi,[1] Gisel Padula,[2,3] Analía Seoane,[2] Maria Lis Alomar,[4] Franco Cabrerizo,[4] Lorena Cortez,[1] Omar Azzaroni[1]

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Nano-sized self-assemblies produced through ionic crosslinking of polyelectrolytes with multivalent ions are attractive platforms for delivery of drugs, genes, and imaging agents. They are formed under mild conditions and can be easily loaded with cargo molecules. Moreover, owing to the wide spectrum of available building blocks (polyelectrolytes and crosslinkers), they can be designed for responding to different external stimuli such as pH, temperature, light, redox, among others.[1,2,3] However, lack of stability during storage or when dispersed in relevant biological fluids combined with insufficient toxicological characterization, limits their ultimate application.[4]

Here we present the advantages of poly(allylamine) (PAH) – tripolyphosphate (TPP) self-assemblies. Monodisperse PAH-TPP colloidal complexes of ca. 200 nm (as seen by TEM and DLS) were prepared by a simple one-pot procedure, showing long-time stability (> 6 months) and capacity to be dispersed in complex media (DMEM + FBS) without aggregation. Moreover, they did not exhibit cytotoxicity in A549 cells, neither genotoxicity (as confirmed by comet and micronucleus assays), nor hemolytic activity. Owing to these remarkable features, PAH-TPP self-assemblies are promising candidates for using as stable and safe drug nanocarriers.

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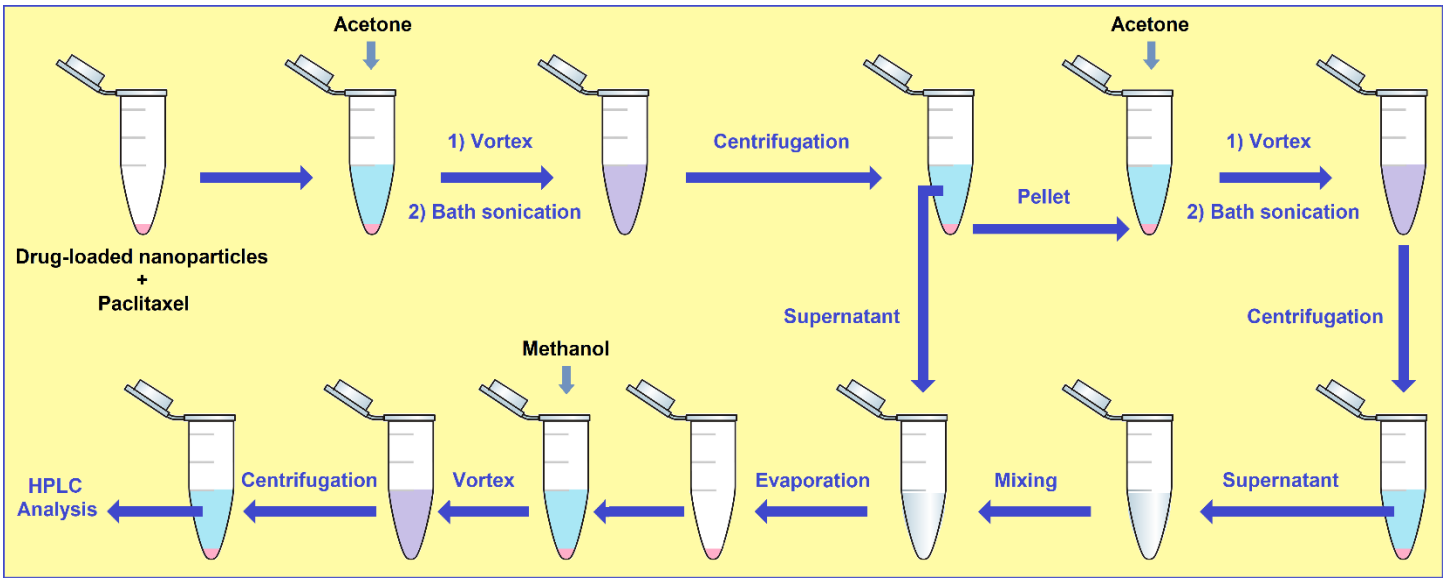


# Development of an extraction method for the quantification of docetaxel loaded in PLGA nanoparticles

Amir Khajavinia[1], Pedram Rafiei[2], Azita Haddadi[1]*

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A critically essential step in the development of any drug-loaded nanoparticle formulation is the determination of the loading parameters [1]. Quantification might become feasible by the application of an extraction method. The purpose of this study is to develop an efficient extraction method to be used for the quantification of docetaxel in PLGA nanoparticles.

PLGA nanoparticles were prepared using a solvent evaporation technique [2]. The extraction process was initiated by adding paclitaxel as the internal standard, and subsequent addition of acetone to the drug-loaded or drug-spiked nanoparticles to dissolve both the polymer and the drug. The obtained mixture was vigorously shaken and subsequently subjected to bath sonication. After centrifugation, the supernatant was separated and transferred to new Eppendorf tubes. The same procedure was repeated by dissolving the precipitate in acetone. The collected supernatants from the first and the second centrifugation steps were mixed and evaporated. Methanol was added to the residue and vortexed for one minute followed by another centrifugation step. The supernatant was transferred to HPLC vials for quantification. Encapsulation efficiency, drug loading percentage, extraction efficiency, and extraction recovery percentage were determined to assess the effectiveness of the developed method. The results showed that the extraction process was able to meet the criteria of the Food and Drug Administration (FDA) guidelines. Moreover, the developed method was able to quantify docetaxel in the PLGA nanoparticle (mean size 170.2±4.2 nm) matrix at a concentration as low as 15.6 ng/ml.

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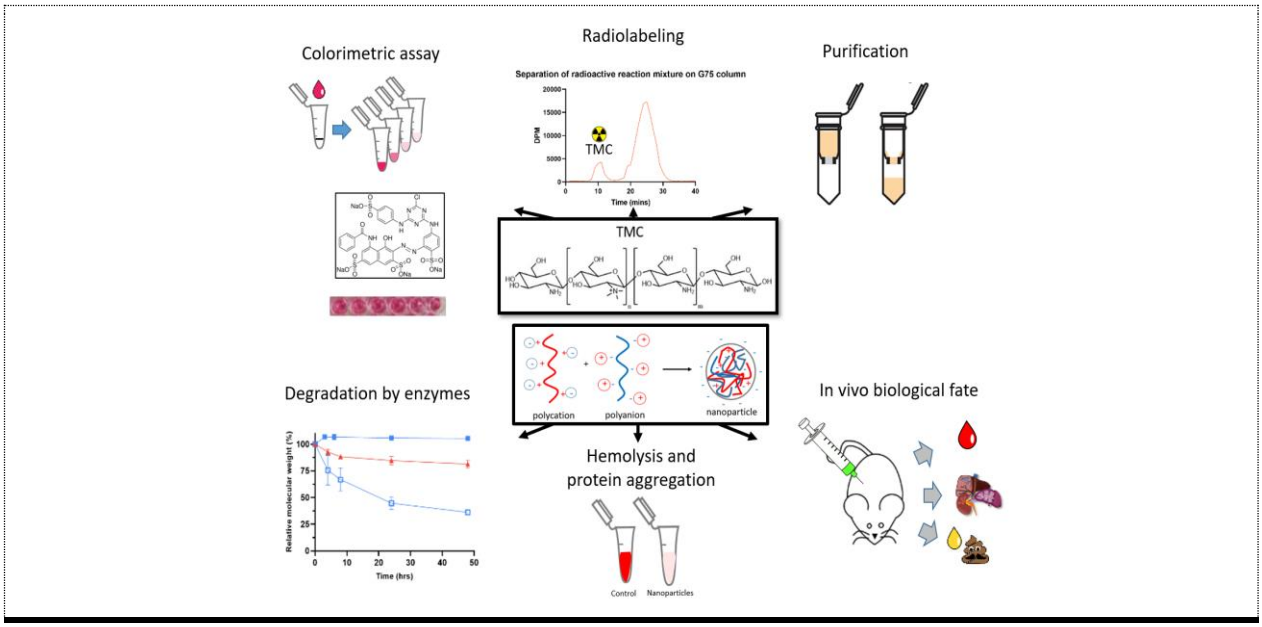


# Physico-chemical characterization and biodegradation of a natural polymer and nanoparticles for applications in drug delivery.

Amrita Dikpati and Nicolas Bertrand

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Trimethyl chitosan (TMC), is a quaternized derivative of chitosan, soluble over a wider pH range which shows potential as a bio-adhesive excipient.[1] The physicochemical properties and the biological fate of novel biopolymers destined to be used in parenteral drug delivery systems must be thoroughly characterized. The objective of this study is to develop suitable characterization methods to establish how TMC can be used in the preparation of nanoparticles.

Herein, quantification and purification methods were developed to study the in vitro degradation of the polymer, to monitor the self-assembly of TMC nanoparticles, and to study their fate in vivo.

Cibacron red dye test was developed to quantify TMC in aqueous solutions. In vitro chemical degradation was performed using concentrated acid and base. In vitro enzyme degradation studies were carried out for TMC in the presence of different enzymes. Sampling was done at different time points and the molecular weights were determined using GPC. Degradation was ascertained by measuring viscosities post treatment with chemicals and enzymes. TMC nanoparticles were prepared using a negatively charged poly methacrylic-PEG polymer and the size and charge were characterized. Nanoparticles were purified from free TMC via cation exchange gel chromatography. Radiolabeled TMC was used to prepare nanoparticles to track them in vivo. The physico-chemical characterization of polymer and nanoparticles, and sensitivity to different degrading agents provides an insight into possible biodegradation pathways that could help in its integration in different formulations as a parenteral drug delivery excipient.

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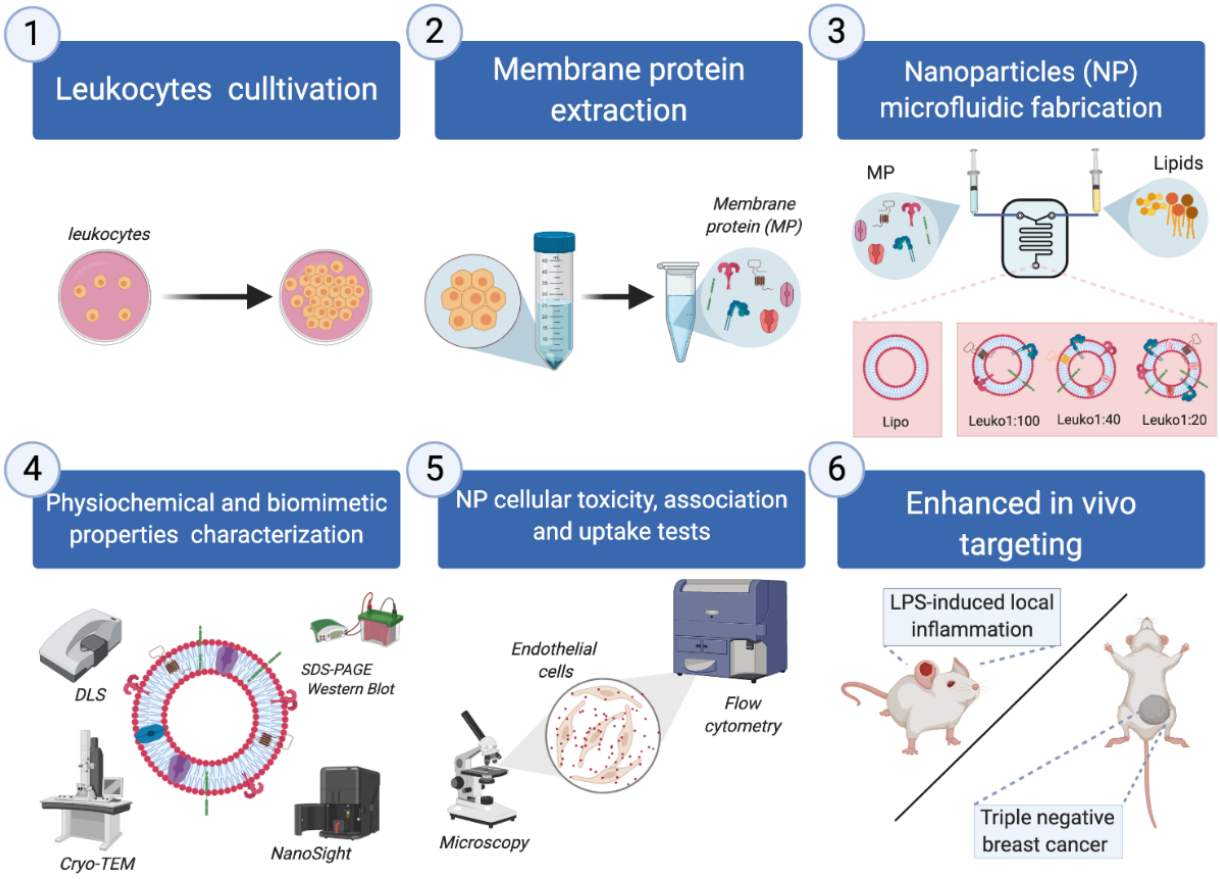


# Enhancing Inflammation Targeting Using Tunable Leukocyte-based Biomimetic Nanoparticles

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Biomimetic nanoparticles aim to effectively emulate the behavior of either cells or exosomes. [1]Leukocyte-based biomimetic nanoparticles, for instance, incorporate cell membrane proteins to transfer the natural tropism of leukocytes to the final delivery platform. However, tuning the protein integration can affect the *in vivo* behavior of these nanoparticles and alter their efficacy. Here we show that, while increasing the protein:lipid ratio to a maximum of 1:20 (w/w) maintained the nanoparticle's structural properties, increasing protein content resulted in improved targeting of inflamed endothelium in two different animal models. Our combined use of a microfluidic, bottom-up approach and tuning of key synthesis parameter enabled the synthesis of reproducible, enhanced biomimetic nanoparticles that have the potential to improve treatment of inflammatory-based conditions through targeted nanodelivery **(Fig. 1)**.

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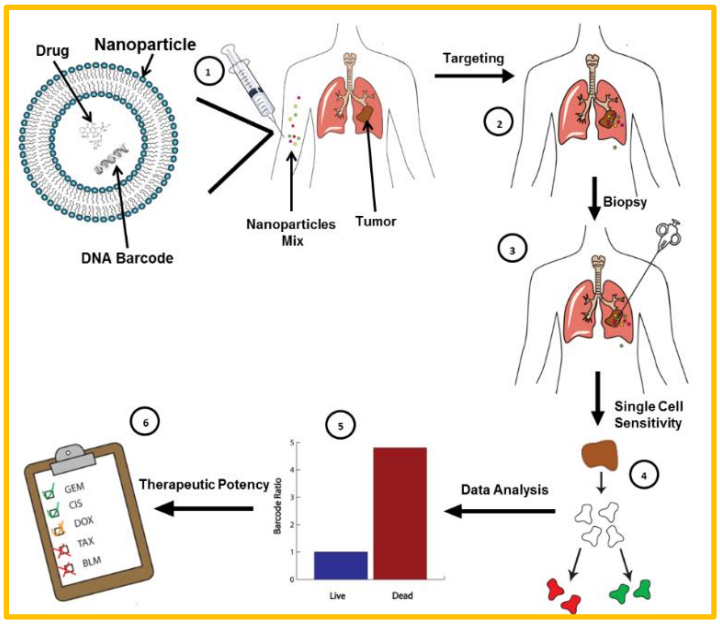


# Barcoded Nanoparticles for Precision Cancer Medicine: Targeting pancreatic tumors with nanomedicine

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Medicine is taking its first steps towards patient-specific cancer care. Nanoparticles have many benefits for treating cancer, including the ability to transport complex molecular cargoes including siRNA and protein, as well as targeting to specific cell populations.

The talk will discuss 'barcoded nanoparticles' that target sites of cancer where they perform a programmed therapeutic task [1]. Specifically, liposomes that diagnose the tumor and metastasis for their sensitivity to different medications, providing patient-specific drug activity information that can be used to improve the medication choice, shown in **Figure 1**.

The talk will describe how the liposomal lipid composition can be used as multi-functional systems for degrading the pancreatic stroma to allow subsequent drug penetration into pancreatic adenocarcinoma, and how the nanoparticle configuration can be leveraged to induce an anti-tumor immune response.

The evolution of drug delivery systems into *synthetic cells*, programmed nanoparticles that have an autonomous capacity to synthesize diagnostic and therapeutic proteins inside the body, and their promise for treating cancer and immunotherapy, will be discussed.

1. Theranostic barcoded nanoparticles for personalized cancer medicine, Yaari et al. ***Nature Communications***, 2016, 7, 13325
2. Collagenase nanoparticles enhance the penetration of drugs into pancreatic tumors, Zinger et al., ***ACS Nano,*** 2019

# Time Course Study of Blood-pool and Liver Targeting Gold Nanoparticle Contrast Agents

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## Introduction

Computed tomography (CT) is a common non-invasive medical imaging technique that provides 3-dimensional images of organs and tissues using contrast agents (CA). Gold nanoparticle contrast agents (AuNP CA) have gained research interest as a CT CA for their modifiable surface, long circulation time, biocompatibility, and high X-ray attenuation.[1] MVivo Au, an AuNP CA, alone behaves as a blood pool CA but when the surface of AuNP CA is modified with a moiety it can be used to target an organ or tissue type.

## Methods

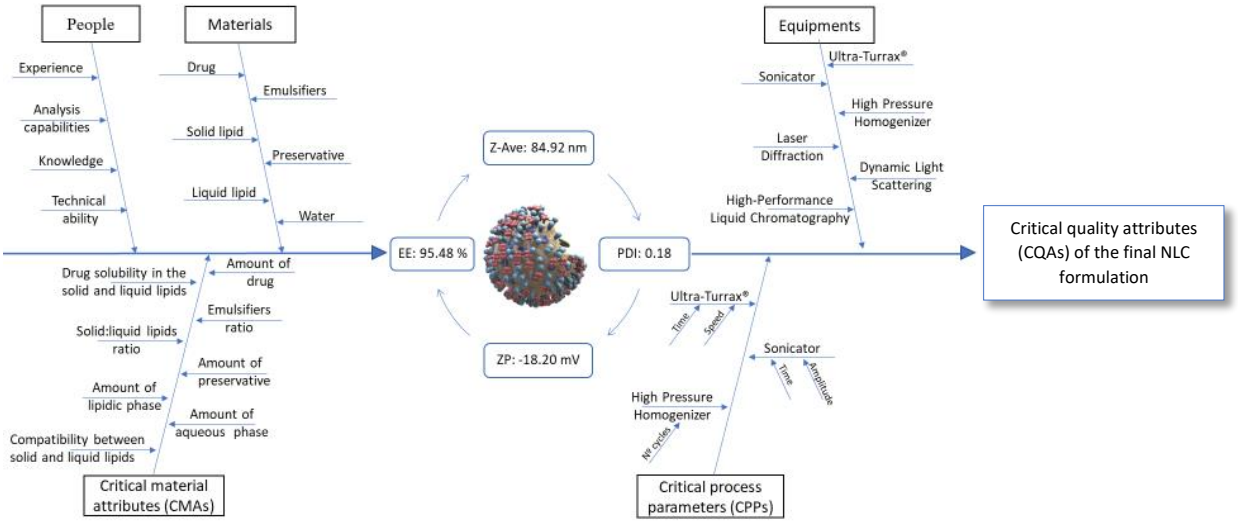
A time-course experiment is used to investigate and compare the contrast enhancement and behavior of MVivo Au a blood pool agent and MVivo $AuNH_2$ a liver targeting agent. Each contrast agent is injected into five healthy C57Bl6 mice via tail vein injection at 0.1mL per mouse following anesthesia. Mice are anesthetized using 5% isoflurane in $O_2$ in the induction box then transferred to a nose-cone at 2% isoflurane in $O_2$ in the GE eXplore CT 120. Each *in vivo* scan is obtained using the continuous rotation technique at the following time points: pre-contrast, post-contrast 0, 0.5, and 24 hours. The contrast enhancement is measured using MicroView for three groups of organs: non-enhancing regions (air and left leg muscle), vascular system organs (right ventricle and vena cava) and clearance organs (liver, kidney, spleen and bladder).[2]

## Results

The micro-CT images at the same time points for MVivo Au and MVivo $AuNH_2$ are compared. As expected the raw data confirms the increase in contrast enhancement for both CA from pre-contrast to post-contrast 0.5 hours for both the non-enhancing and enhanced vascular system organs. In comparison, the clearance organs continued to increase in contrast enhancement from post-contrast 0.5 hours to 24 hours while the other organs displayed contrast enhancement decrease. The liver targeting MVivo $AuNH_2$ was expected to accumulate in the liver causing a significant increase in contrast enhancement measurements but the results of MVivo $AuNH_2$ were similar to MVivo Au.

## Conclusions

These results indicate that MVivo $AuNH_2$ did not perform as expected. Contrast enhancement in the liver for mice injected with MVivo $AuNH_2$ were similar to mice injected with MVivo Au. But both CA travelled through the circulation slower as seen by the increased contrast enhancement in the three groups of organs up to 0.5 hours post-contrast. Overall the modification and further testing of MVivo $AuNH_2$ is required to ensure the liver targeting CA accumulates in the liver at a significant amount.




# Optimization of diazepam-loaded nanostructured lipid carriers (NLC) for nose-to-brain delivery using the quality by design (QbD) approach

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Epilepsy requires fast and effective treatment, targeting the brain. Herein, intranasal administration of nanostructured lipid carriers (NLC) has been suggested as a promising strategy [1]. In addition, the quality-by-design (QbD) approach is a useful tool for the optimization of manufacturing variables, resulting in effective and safe pharmaceutical products [2].

The aim of this work was to use the QbD approach to optimize a NLC formulation for the nose-to-brain delivery of diazepam, improving the emergency therapy of epilepsy. The studies began with screening of excipients and assessing lipid-drug compatibility. The central composite design was used to evaluate the effects of critical material attributes (CMAs) (ratio of solid and liquid lipids and amount of emulsifiers) on the critical quality attributes (CQAs) of the NLC formulation (particle size, polydispersity index (PDI), zeta potential (ZP) and encapsulation efficiency (EE)).

The results showed that the most adequate ratios of lipids and emulsifiers were 6.65:2.85 and 4.2:0.3 (%, w/w), with values of 84.92 nm, 0.18, -18.20 mV and 95.48% for particle size, PDI, ZP and EE, respectively. This formulation was selected for further studies on the optimization of critical process parameters (CPPs).

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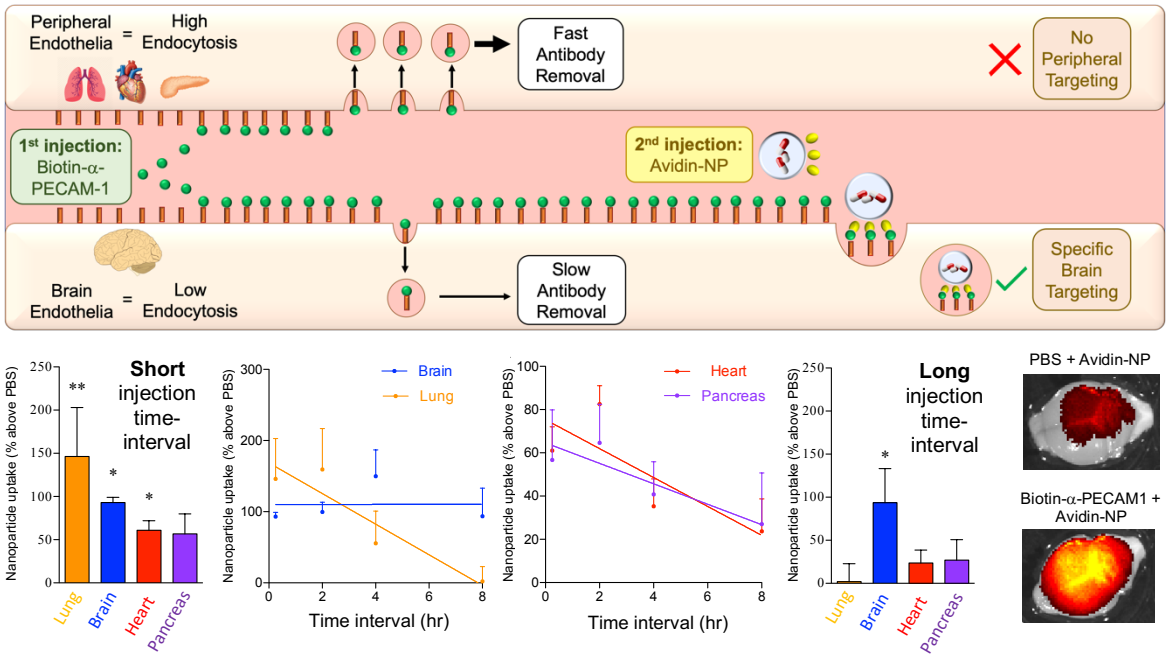
2. Cunha, S., et al., Double Optimization of Rivastigmine-Loaded Nanostructured Lipid Carriers (NLC) for Nose-to-Brain Delivery Using the Quality by Design (QbD) Approach: Formulation Variables and Instrumental Parameters. Pharmaceutics, 2020. 12(7), 599.

Scheme 1.




# Targeting nanoparticles to the brain by exploiting the blood–brain barrier impermeability to selectively label the brain endothelium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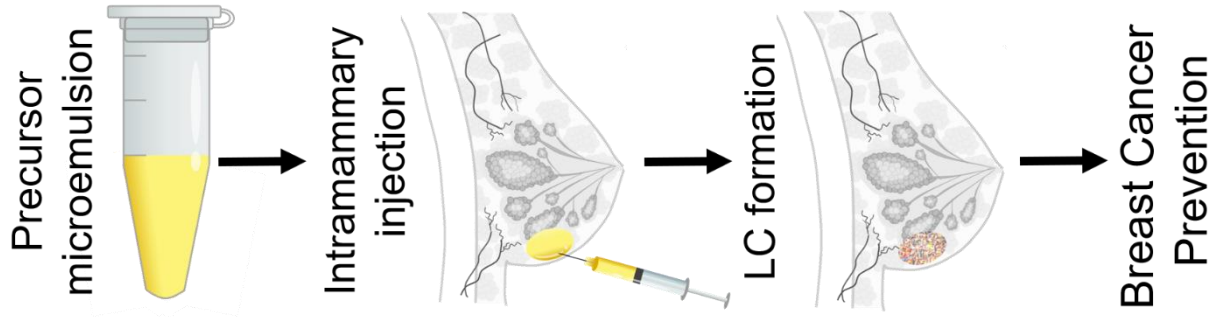
Nanoparticle (NP) brain-delivery strategies targeting proteins overexpressed at the brain micro-vasculature (*e.g.* TfR1, Glut1) have substantial specificity limitations due to significant protein expression in peripheral organs [1]. We have developed a new strategy [2] to target NPs to the brain by instead selectively labelling the brain microvasculature. We exploit the lower endocytic rate of brain endothelial cells (BEC)[3] to promote retention of free ligands (*i.e.* labels) selectively on the surface of BEC. NPs capable of recognizing the endothelial label are subsequently targeted to the brain without peripheral targeting (scheme 1). We demonstrate the *in vivo* feasibility of this strategy by injecting biotinylated α-PECAM1 antibodies (to label endothelial cell surfaces) followed by injection of avidin-functionalized nanoparticles (Avidin-NP) at increasing time-intervals. While short time-intervals result in avidin-NP targeting to the lung, brain, heart and pancreas, long time-intervals result in avidin-NP targeting only to the brain.

The present work therefore provides the basis for a new targeting strategy which exploits the physiology of BEC to generate the required NP targeting specificity.


**References**: [1] Fillebeen, et al. (2019). Blood, 133 (4): 344-355; [2] Gonzalez-Carter, et al. (2020) PNAS doi: 10.1073/pnas.2002016117 (online ahead of print); [3] Ben-Zvi, et al. (2014). Nature, 509 (7501): 507-511.




# BIORESPONSIVE MICROEMULSION FOR PREVENTION OF BREAST CANCER DEVELOPMENT

Giovanna Cassone Salata[1]; Isabella Drazsesski Malagó[1]; Márcia Carvalho de Abreu Fantini[2]; Luciana Biagini Lopes[1].

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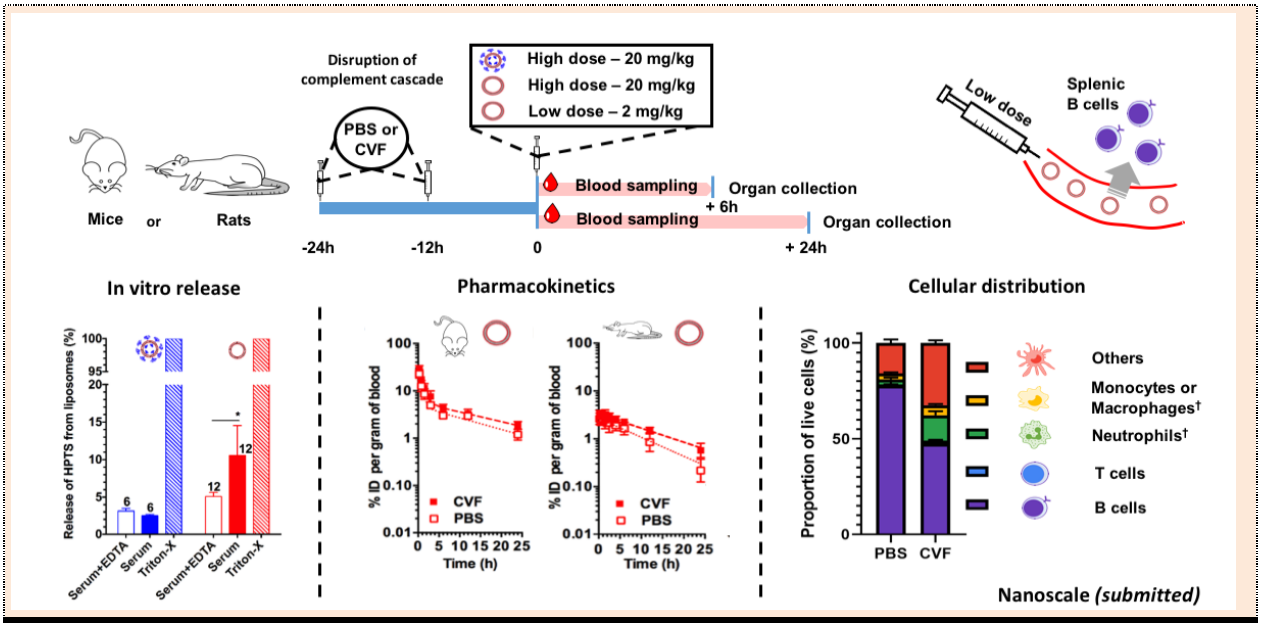
**Introduction.** Although breast cancer is the most common type of neoplasm in woman worldwide, few pharmacological strategies for its chemoprevention are available and well-accepted by high-risk patients, especially due to the serious adverse effects that current strategies present, highlighting the need for new and safe forms of prevention therapies. Fenretinide is a retinoid, considered a promising candidate for chemoprevention due to its ability to regulate cell growth and differentiation and accumulate preferentially in the breast tissue. However, the serious systemic adverse effects and low bioavailability limits its systemic use. To overcome these limitations, we developed a bioresponsive, phosphatidylcholine-based microemulsion, capable of transitioning into a liquid-crystalline system upon intramammary administration for sustained release of fenretinide locally in the breast. **Methods.** Selected microemulsions and liquid crystalline phases formed upon water uptake were characterized using dynamic light scattering, polarized light microscopy and small angle X-ray scattering (SAXS). Subsequently, we evaluated the ability of the system to (i) sustain the *in vitro* fenretinide release, (ii) decrease cellular viability and migration in 2D and 3D models using T47D cells, and (iii) reduce breast cancer incidence *in vivo* in a chemically induced breast cancer model. **Results.** The precursor microemulsion displayed size of 175.3 nm (PDI=0.106), and was capable to form lamellar liquid crystalline gels when water was added at 5% and up. The system released 30% of its fenretinide content at 9 days, highlighting its ability of sustained release. Migration of T47D cells was inhibited by the formulation at $IC_{15}$ (concentration necessary to reduce cell viability by 15%) compared to the unloaded formulation and control solution in approximately 4-fold. Treatment for 4 days at a dose equivalent to $IC_{30}$ reduced the viability of T47D spheroids compared to untreated or unloaded ME-treated cells (1.4-fold), while fenretinide in solution precipitated in the culture medium. Using an *in vivo* model of n-methyl-n-nitrosourea-induced carcinogenesis, intramammary administration of the fenretinide-loaded ME every 3 weeks for 3 months reduced the incidence of breast tumors by 4.5-fold compared to untreated animals. **Conclusion.** These results demonstrate the strong potential of the formulation to provide an effective and much needed platform to inhibit breast cancer development.




# Complement cascade and the biological fate of liposomes in rodents

Iara Maíra de Oliveira Viana [1,2], Philippe Grenier [2], Joan Defrêne [3], Frédéric Barabé [3], Eliana Martins Lima [1]*, Nicolas Bertrand [2]*

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Liposomes are used in the clinic since the beginning of nanomedicine and remain one of the main strategies for drug delivery. Evidences that liposomes activate the complement cascade have been described in animal models and patients. Conversely, we have shown in previous work that complement cascade cannot fully explain the blood clearance of polymeric nanoparticles [1]. Here, we aim to evaluate the role of complement in the fate of liposomes in mice and rats. For this, the complement was depleted by administration of cobra venom factor, 12 and 24 hours before the injection of PEGylated or non-PEGylated liposomes, at doses of 2 and 20 mg/kg. In vitro, complement proteins failed to destabilize liposomes. At 20 mg/kg, the elimination of liposomes, with or without PEG, was not affected by complement cascade. At 2 mg/kg, the complement proteins appear to modestly impact elimination, especially 12 and 24 hours after liposomes administration. Complement proteins seem to favor the distribution of non-PEGylated liposomes to splenic B cells. Despite that PEGylated and non-PEGylated liposomes can activate complement, the impact of this cascade on their circulation times is minor and mostly perceivable at later phase of distribution. This work enlightens on the biological pathways responsible for the in vivo clearance of liposomes and will help orienting future research elucidating the nano-bio interface.

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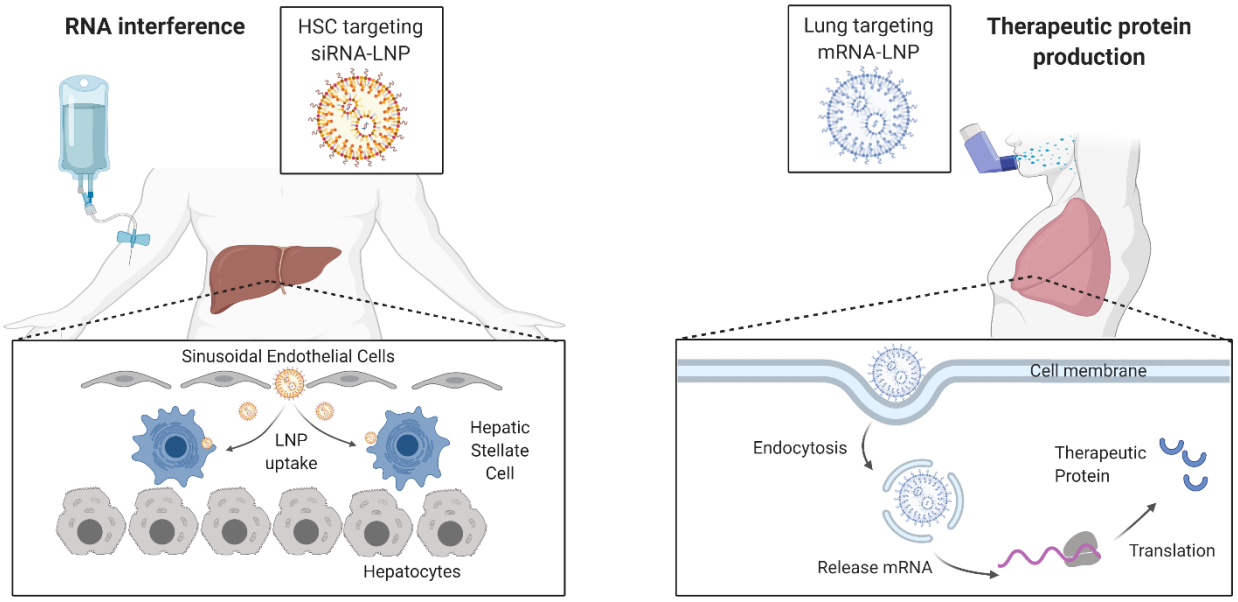


# Lipid nanoparticles enabling specific and functional extrahepatic delivery of nucleic acids

Kieu Lam, Lorne Palmer, Petra Schreiner, Richard Holland, Wenxuan Zhang, James Heyes

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Despite clinical successes with RNAi applications and advances in mRNA therapies, the efficient delivery of nucleic acids for non-liver targets remains elusive. Lipid nanoparticles (LNPs) can be engineered to maximize cellular uptake and provide efficient endosomal escape; however, they have a particularly high affinity for hepatocytes. Applying our knowledge of structure-activity relationships and design principles, we have extended our LNP technology for extrahepatic delivery.

Hepatic stellate cells (HSCs) play a significant role in regulation of liver fibrosis but constitute a minor cell population in the liver (~5-8%). Without the use of an exogenous targeting ligand, we have identified a highly specific formulation that effectively silenced HSC-specific gene targets (e.g., RELN, SERPHINH1) *in vivo*, with no TTR silencing observed in hepatocytes. At 0.025 mg/kg, we achieved >70% knockdown in HSCs. Furthermore, effective gene silencing translated to activated HSCs in a carbon tetrachloride-induced mouse model.

Delivery to the airway is complicated by the mucosal barrier of the respiratory epithelium, which facilitates ciliary clearance of foreign particulates. Novel formulations have been optimized to impart stability and specificity, thereby facilitating functional delivery of nucleic acids (e.g., luciferase mRNA) via aerosolization. Luciferase expression is localized to lung tissue, with no broad distribution in other organs such as the liver. Due to their biodegradable functionality, these LNPs were rapidly cleared from the lung within 24h post-dose and were well tolerated.

The ability to rationally design LNPs to target specific tissues selectively unlocks a wide array of therapeutic opportunities and potential to address serious unmet medical needs.

Acknowledgment: Graphic illustration created with Biorender.com

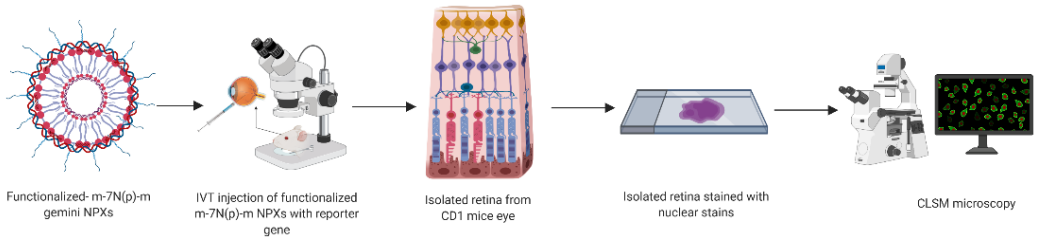


Figure:1 Schematic of m-7N(p)-m NPXs with reporter gene treatment and processing of treated retinas. Figure created by Lokesh Narsineni using BioRender.com

## Development of peptide-modified gemini nanoplexes for non-viral gene delivery to the retina

Lokesh Narsineni[1,2*] Ding-wen (Roger) Chen[1,3] and Marianna Foldvari[1,4]

[1]School of Pharmacy, University of Waterloo, Waterloo, Canada, [2]Department of Biochemistry and Molecular Biology, The University of British Columbia, Vancouver, Canada, lokeshn@mail.ubc.ca; [3]Children's Hospital of Philadelphia, Philadelphia, PA, USA, [4]Waterloo Institute of Nanotechnology, University of Waterloo, Canada

The absence of targeting and cell recognition moieties to deliver therapeutic gene to specific cell population is one major factor that can be associated with low transfection efficiencies with non-viral gene delivery systems[1]. Neural-Cell Adhesion Molecule (NCAM) is identified to promote the cell adhesion in the retina [2], the binding of an IgCAM molecule will not only allow for an adhesive interaction but also the functional status and structure of the cell and its components can be influenced by these CAMs. The main objective was the development of a new generation of nanoplex (NPX) system based on Ig-superfamily (IgSF) peptide modified di-cationic amphiphiles (m-s(p)-m) for targeted delivery of neurotrophic factor genes to the retina. Five IgSF-Peptide ($p_{1\text{-}5}$) modified m-s($p_{1\text{-}5}$)-m gemini surfactants (*m-alkyl tail [12-18 C], s-spacer 7C with imino-peptide substitution*) were synthesized and purified using prep-HPLC and analyzed by ESI-MS, $^1$H- NMR and HPLC. NPXs constructed from m-7N($p_{1\text{-}5}$)-m and gWIZ-GFP plasmid were optimized at various ratios and assessed for size, zeta potential, transfection efficiency (TE) and toxicity in A7 astrocytes using flow cytometry and confocal microscopy. Penetration studies were performed in an Epicorneal® tissue equivalent model. Molecular docking studies were performed using Biovia discovery studio to assess the bonding interaction (BI) of m-7N($p_{1\text{-}5}$)-m compounds with integrin receptors. *In-vivo* studies were performed in 6-week old CD1 mice (n=4 eyes/group); m-7N($p_1$ and $p_3$)-m NPXs and 18-7NH-18 NPXs were injected intravitreally containing 0.5 µg tdTomato plasmid/2µL dose, Lipo 3000 and normal saline as controls. Whole retinas were isolated and gene expression was evaluated by CLSM.
m-7N($p_1$ and $p_3$)-m NPXs with 5:1 charge ratio, 220.96±2.5nm and 257.4±3.2 nm size and +30 ± 0.2 mV and +37.13± 0.5 mV zeta potential, respectively, were selected for *in vivo* studies. m-7N($p_3$)-m NPXs showed improved TE compared to m-7NH-m NPXs and Lipo 3000, 15.36±1.5%, 10.97±0.4% and 12.07±1% (n=6), respectively, and >90% viability. NPX penetration studies in the Epicorneal model showed 90±20µM penetration of the m-7N($p_1$ and $p_3$)-m NPXs compared to 30±10µM for m-7NH-m NPXs. *In-silico* docking studies showed superior BI of m-s($p_1$)-m and m-s($p_3$)-m with $\alpha_5\beta_3$ integrin receptors, -83.02 and -89.48, respectively, versus -27.95 kcal/mol with m-7NH-m. Intravitreal m-s($p_3$)-m-NPXs treated mice showed higher reporter gene expression pattern in the retinal layers compared to m-s($p_1$)-m, 18-7NH-18-NPXs and controls. IgSF-m-s($p_3$)-m NPXs show potential for targeted non-viral gene delivery to the retina to treat ocular neurodegenerative conditions.

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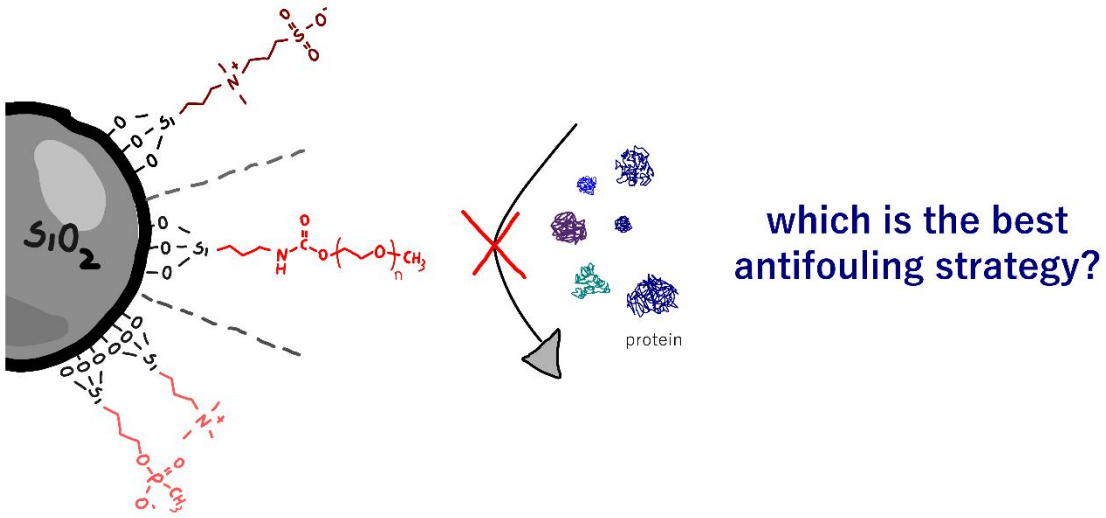


# Comparing Antifouling Strategies on Silica Nanoparticles

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Protein adsorption onto nanoparticles immersed in biological fluids alters fundamental surface properties and ultimately modifies the interaction of nanomaterials with biological systems.[1] Thus, finding antifouling strategies has been an active subfield of research within nanomedicine since its very origin.[2] In this context, developing theoretical tools for rapid screening of potential antifouling candidates is of paramount importance.

In this work, we present a theoretical study of various antifouling strategies to prevent protein adsorption on silica nanoparticles using a *molecular theory* approach.[3] We evaluated surface modifications with short zwitterions, with PEG and with mixed silanes (producing pseudo zwitterionic surfaces) against the adsorption of lysozyme. This cationic protein is strongly adsorbed onto negatively born surfaces and represents a big challenge for antifouling coatings.[4] The influence of grafting density, remnant non-reacted silica silanols, and media pH and ionic strength was assessed to understand the mechanism underlying the prevention of protein adsorption provided by the above mentioned functionalizations. Theoretical results were compared with experimental characterizations using DLS, SAXS, ITC and UV-Vis spectroscopy.

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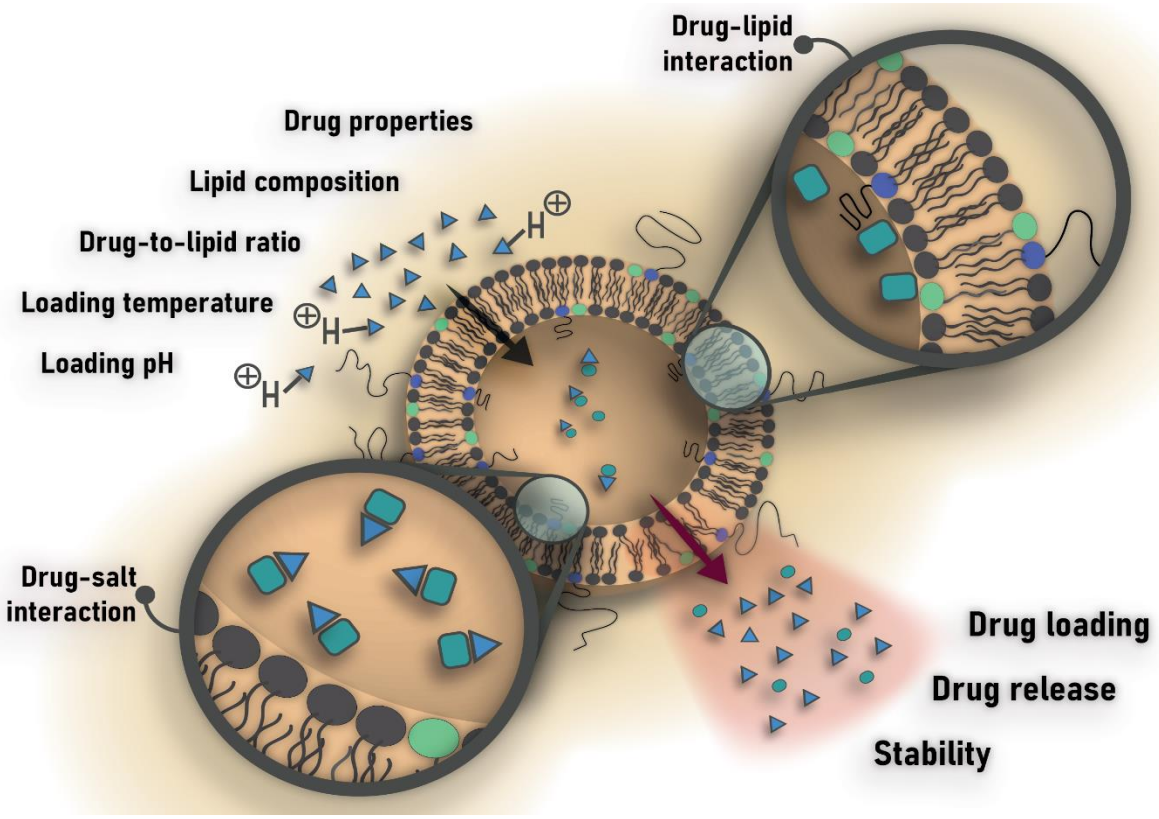


## Drug loading levels affect *in vitro* release of vinorelbine from thermosensitive liposomes

Maximilian Regenold[1], Elisa Siniscalchi[1,2], Jessica Steigenberger[1,3], Heiko Heerklotz[1,3], Christine Allen[1]

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Triggered drug release from thermosensitive liposomes (TSL) has been proposed as a strategy to address limitations associated with conventional liposomal drug delivery: heterogenous drug uptake and distribution as well as limited drug release at the target site. Ongoing clinical studies with a TSL formulation of doxorubicin are highlighting the potential of this treatment and delivery approach. Many other drugs would benefit from the same localized and targeted delivery strategy as it can result in significant improvements in therapeutic index. Our lab has developed TSLs loaded with cisplatin, alvespimicyn and the vinca alkaloid vinorelbine (VRL) [1,2]. Here we aimed to understand how formulation parameters (e.g. drug loading level, internal/external buffer) influence the performance (e.g. drug release, stability) of a TSL formulation encapsulating VRL (**Figure 1**).

Lyso-lipid containing TSLs were loaded with varied amounts of drug and heat-triggered release over a temperature range of 37 to 42°C was measured in protein containing release media. Differential scanning calorimetry (DSC) was used to evaluate the effect of formulation preparation and drug loading on the formulation's thermal properties to predict their *in vitro* release behaviour. Cryogenic transmission electron microscopy (cryo-TEM) images were obtained and image gray values were determined to assess the degree of drug precipitation (if any) within the liposomes. The *in vitro* release behaviour is assessed in the absence and presence of protein.

The current studies demonstrate the importance of thorough *in vitro* characterization during development of a TSL formulation. The results highlight that previously determined formulation characteristics and their influence on a formulation's performance cannot be easily translated from non-thermosensitive liposomes to their thermosensitive counterparts.

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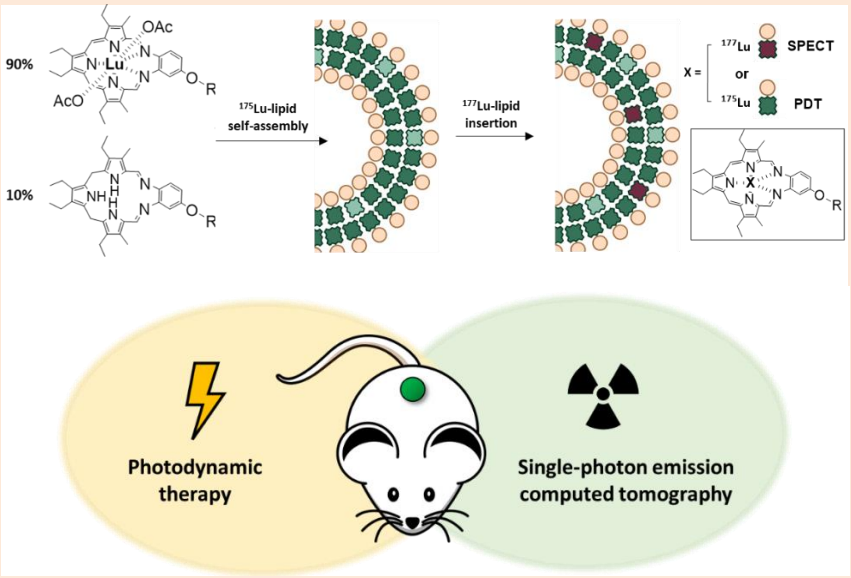


# A single metal multifunctional nanotexaphyrin as a radiotheranostics agent for cancer imaging and therapy

Miffy H. Y. Cheng[1], Marta Overchuk[1,2], Maneesha Rajora[1,2], Juan Chen[1], and Gang Zheng[1,2]*

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Oligometastatic prostate cancer is potentially curable with surgery and external beam radiation therapy if diagnosed and treated appropriately. However, the lack of precision imaging and low treatment efficacy in ablative therapies had lead to poor prognosis for patients and cancer recurrence. To overcome these challenges, we propose a non-invasive multifunctional nanoparticle using the "one-for-all" approach[1] for radionuclide imaging and focal photodynamic therapy.

We developed a theranostics liposomal nanoparticle known as Lu-nanotexaphyrin that self-assembled from a texaphyrin-phospholipid building block[2] chelated with a Lu metal ion. We quantitively chelated the 'cold' $^{175}$Lu and the 'hot' $^{177}$Lu and the formulation was optimized for both structural and optical stability. Lu-nanotexaphyrin becomes activated as it disrupts, producing high levels of singlet oxygen under light irradiation. Lu-nanotexaphyrin demonstrated cellular uptake and potent *in vitro* PDT effects in PC3-luc-6 cells at 24h. *In vivo* fluorescence imaging revealed tumour accumulations and activation of Lu-nanotexaphyrin in subcutaneous PC3 tumour mice at 24 h post-*i.v.* injection. Further studies investigated the biodistribution and pharmacokinetic profiles through gamma counting. This work aims to develop the metallo-nanotexaphyrins as a customizable and multifunctional nanomedicine platform for non-invasive SPECT imaging and image-guided photodynamic therapy.

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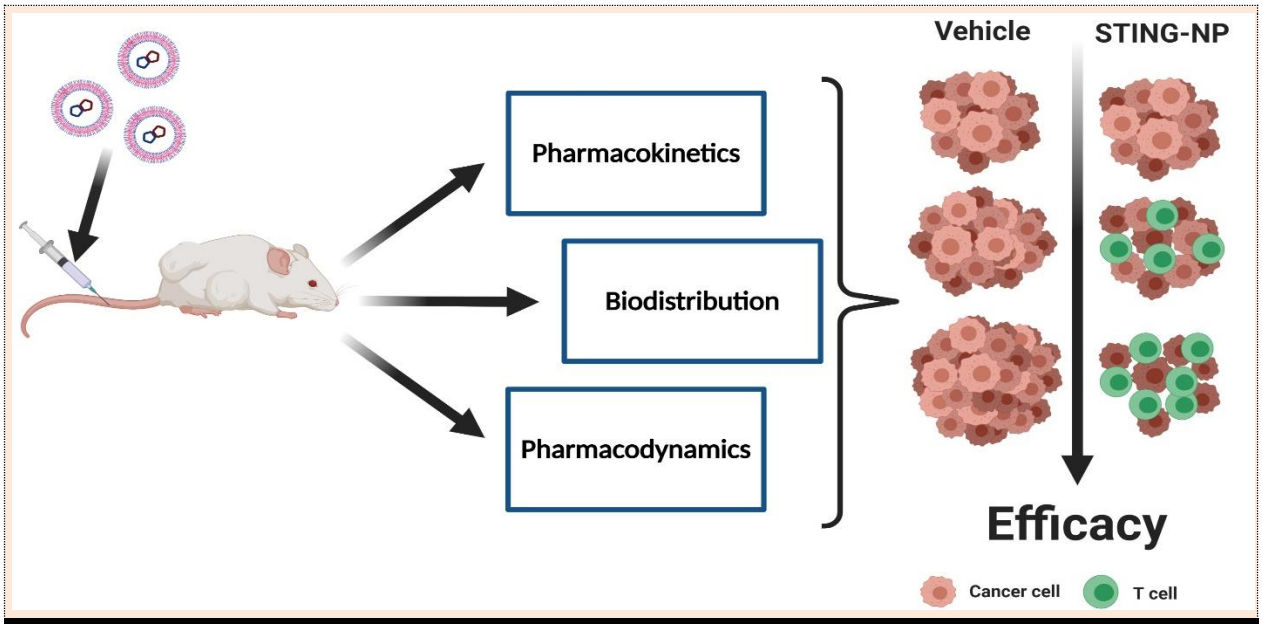


# Investigating the PK-PD relationship governing STING agonist nanoparticle efficacy upon systemic administration

Mohamed Wehbe [1,2], Lihong-Wang Bishop[1], Kyle W. Becker[1], Dan Shae[1], Jessalyn Baljon[2], John. T. Wilson[1,2]

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Immunotherapeutics have revolutionized cancer medicine but the physiochemical properties of many of these treatments cause difficulty in the formulation, administration and efficacy of these agents. Nanotechnology has made great strides in drug delivery improving the pharmacokinetic, pharmacodynamic and distribution properties of therapeutics including chemotherapies. In recent years, many have utilized drug delivery technology to improve the delivery of immunotherapeutics successfully. Yet, to date, little is known and understood about what constitutes an "optimized" drug delivery platform for immunotherapeutic delivery. The design criteria and approaches used for the development of successful chemotherapies will differ from immunotherapies do to the differences in both cellular targets and mechanism of action. Thus, this work aims to understand the pharmacokinetic-pharmacodynamic (PK-PD) relationship of STING-activating nanoparticles (STING-NPs)[(1)], the most potent and only polymeric STING agonist delivery platform. These studies will reveal new insight into how nanocarriers influence the safety and efficacy of intravenously administered STING agonists with potential to inform the rationale design of 2$^{nd}$ generation STING agonist technologies for systemic delivery.

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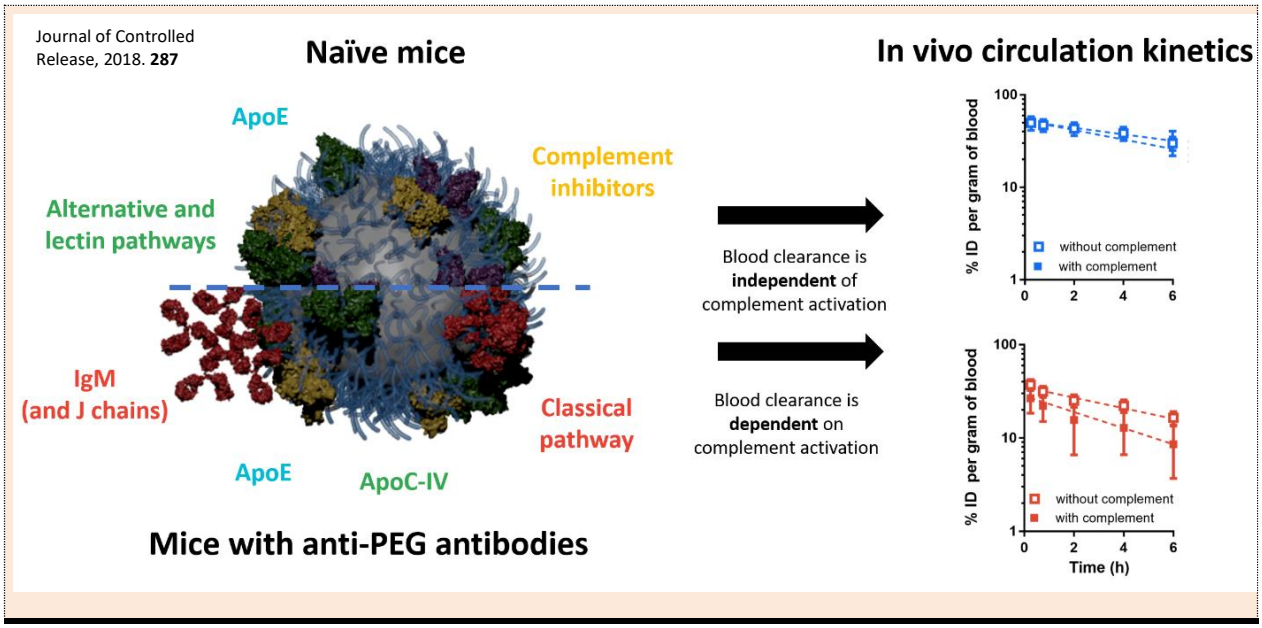


# Anti-polyethylene Glycol antibodies alter the protein corona on nanoparticles and their fate *in vivo*

**Philippe Grenier**[1], Iara Maíra de Oliveira Viana[1,2], Eliana Martins Lima[2], Nicolas Bertrand[1]
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Some nanomedicines and therapeutic proteins employ poly(ethylene glycol) (PEG) to enhance circulation times. However, some papers reveal that anti-PEG antibodies could be highly prevalent in the general population. These antibodies can reduce efficacy of PEGylated nanomedicines. In some case, adverse immune reactions are also observed with the administration of PEGylated drugs.

We developed nanoparticles using PLGA-PEG polymers to assess the importance of anti-PEG antibodies on biological performances. Here, we show that an injection of PEGylated nanoparticles can trigger the production of anti-PEG immunoglobulin M (IgM). IgM anti-PEG in circulation have significant neutralizing effects on subsequent doses of PEGylated nanosystems. The circulation times of PEGylated nanoparticles and liposomes were strongly reduced in animals with IgM anti-PEG, irrespectively of the PEG density or the surface properties. The circulation time of free methoxy PEG and PEGylated bovine serum albumin (BSA) was unchanged with IgM anti-PEG in circulation, even though IgM anti-PEG can bind free methoxy terminated PEG and PEGylated BSA.

The binding of IgM anti-PEG on the surface of PEGylated protein change the proteins absorbed in the corona. These changes are responsible for the observed differences in circulation times. These results inform on how anti-PEG antibodies can affect the fate of PEGylated nanomaterials and highlight how the architecture of nanoparticles impacts the deposition of the protein corona. [1]

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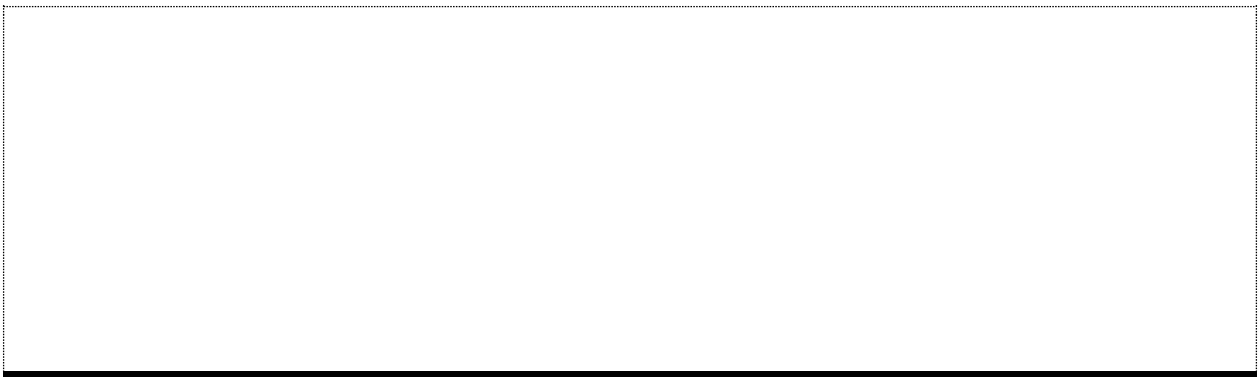

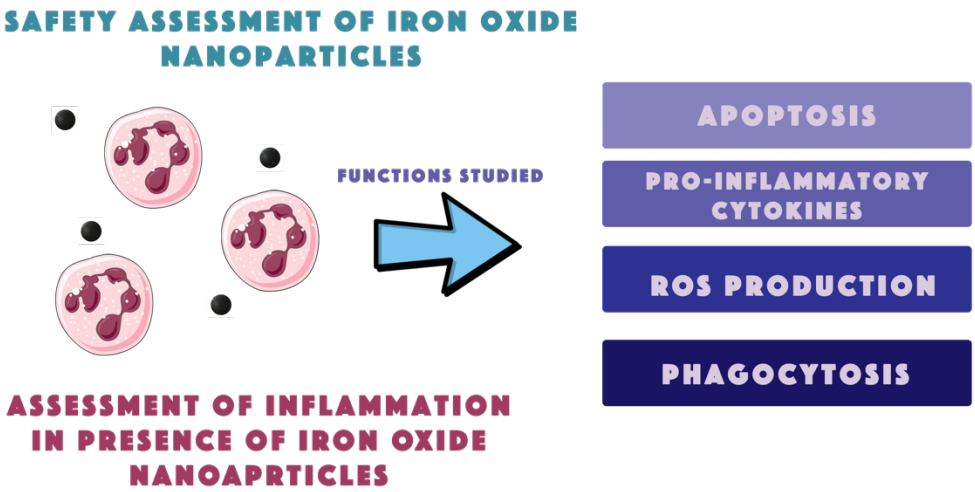


# Iron oxide nanoparticles: Question of nanosafety for nanomedicine applications

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Iron oxide nanoparticles ($Fe_3O_4$ NPs) are probably the NPs that have received the most increasing attention in nanomedicine. These NPs have been found to possess some properties related with their superparamagnetic behaviour. In fact, this characteristic offers them a great potential to develop a variety of applications in medicine, including the treatment of iron deficiency, thermotherapy, drug delivery and so on. However, many safety concerns are rising, mostly regarding their interactions with innate immune cells. For example, their capacity to induce inflammation, which is one of the most undesired side effects associated with NP exposures, needs to be studied more in depth.

The aim of this project is to understand the effects of $Fe_3O_4$ NPs on the biology of human neutrophils, key player cells in inflammation and the most important leukocyte population present in the circulation.

The iron oxide nanoparticles ($Fe_3O_4$ NPs) were purchased from Sigma. According to the manufacturer, the particle size is 9-11 nm as assessed by transmission electronic microscopy (TEM). The solution is at 5 mg/ml in de-ionized water and a fraction was further diluted to obtain a stock solution at 1000 X to work with and was used as is. The endotoxin level of the NPs suspension was determined by the classical Limulus amebocyte lysate (LAL) assay using the ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit and were under the detection limit of 0.01 EU/mL.

Before performing any experiments, we previously determined that $Fe_3O_4$ NPs do not induce cell necrosis in our experimental conditions used as assessed by the trypan blue exclusion assay.

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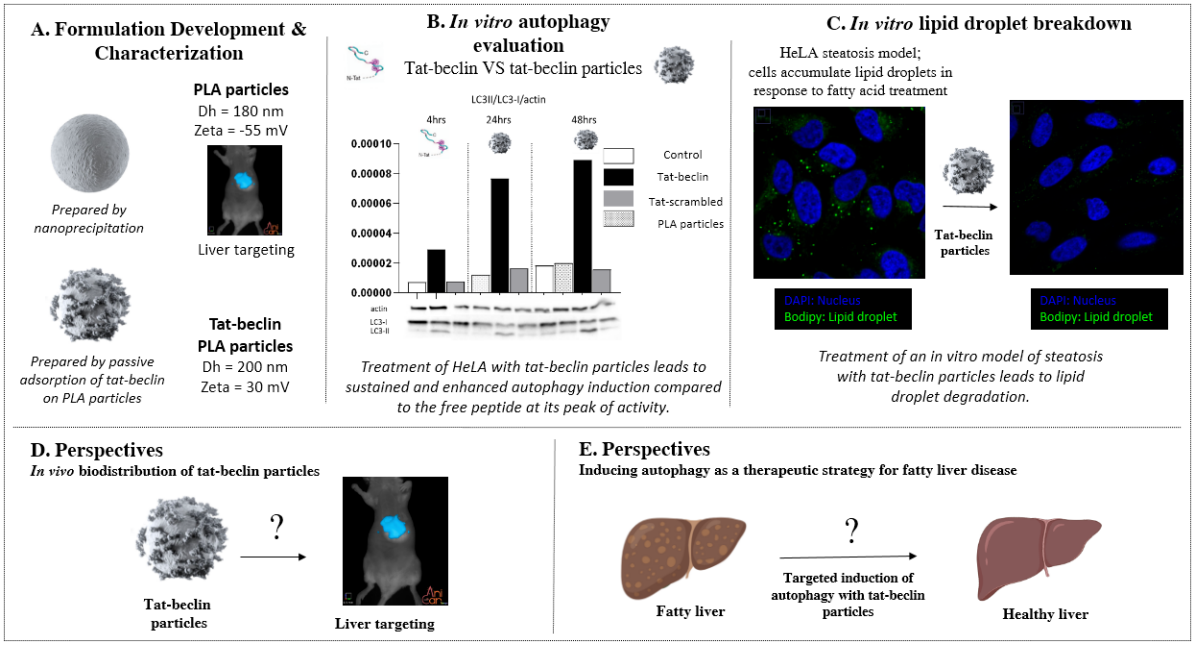


# Breaking Down Fat with Autophagy-Inducing Particles

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Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease of the developed world and it can often manifest to steatohepatitis (NASH) and hepatocellular carcinoma. NAFLD is characterized by an efflux of free fatty acids in the liver, where steatosis and lipotoxicity contribute to disease progression [1]. Recently, a novel role of autophagy in the degradation of lipid droplets has come to light [2]. Various molecules have shown great promise in preclinical studies in the context of NAFLD or NASH, partially owing to their autophagy-inducing properties [3-5]. Targeted autophagy induction in the liver may be beneficial for the treatment of NAFLD or NASH. In this study, we aimed to improve the bioavailability and targeting of a specific autophagy inducer [6], the tat-beclin peptide, using polymeric particles for targeted delivery in the liver. A reproducible and stable formulation of tat-beclin was developed by adsorbing it on biodegradable, biocompatible particles, made of poly-(lactic acid). Tat-beclin autophagy inducing and tat-scrambled control particles were produced and characterized. Autophagy modulation by tat-beclin or tat-scrambled, either in free form or in particles, was examined by western blot in HeLa and by microscopy in HeLA GFP-LC3-RFP cells. Tat-beclin particles were more efficient in inducing autophagy compared to the peptide and autophagy induction was longer-lasting. When tested in an *in vitro* model of steatosis, tat-beclin particles were more efficient in inducing lipid droplet breakdown compared to the free peptide. Overall, we have successfully developed and characterized tat-beclin particles, which are potent inducers of autophagy and can degrade lipid droplets. Further studies will focus on elucidating if these particles can target the liver *in vivo* and evaluating their therapeutic efficacy in an animal model of NAFLD.

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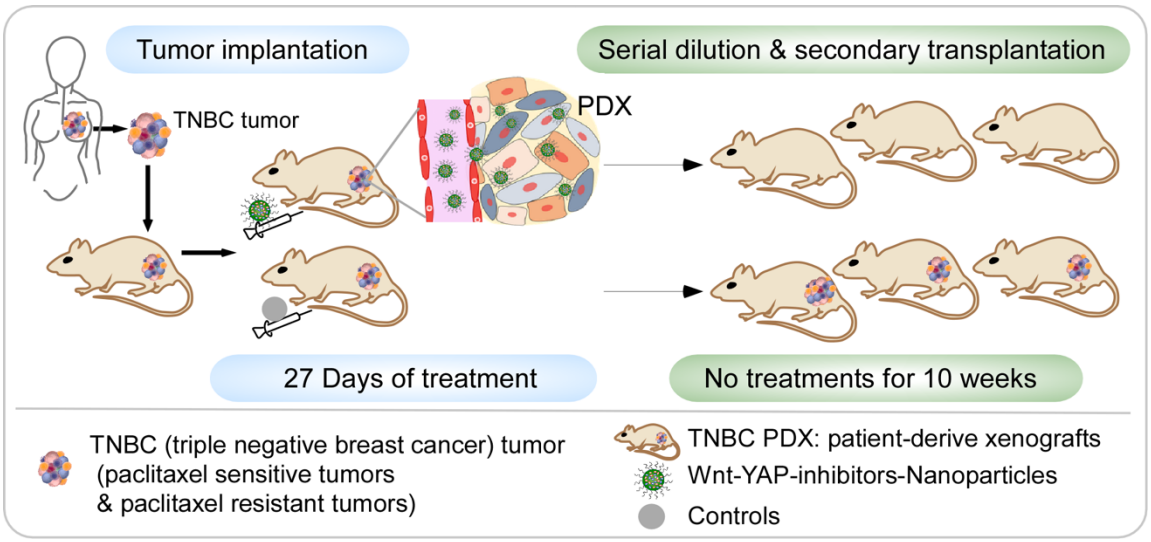


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# Translational Nanomedicines for the Treatment of Triple Negative Breast Cancer

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Triple negative breast cancer (TNBC) is the most refractory subtype of breast cancer, and it disproportionately accounts for the majority of breast cancer-related deaths. This is largely attributed to the lack of specific therapies capable of targeting both bulk tumor mass and cancer stem cells (CSC); appropriate animal models to accurately evaluate treatment efficacy for the clinical translation [1]. Thus, the development of effective and clinically translatable targeted therapies for TNBC is an unmet medical need. The growing interest in cancer nanotechnology is attributed to its uniquely appealing features for drug delivery, diagnosis, and imaging. In this context, we developed nanotherapeutic strategies capable of targeting both bulk tumor mass and CSC; and studied their effects in clinically relevant patient-derived xenograft models. PDX models retain the patient's tumour heterogeneity, vasculature, and three-dimensional architecture, and showed a strong correlation between the PDX and actual patient response [1, 2]. Our studies showed that nanoparticles selectively accumulated in TNBC PDX tumors, retarded tumor growth, inhibited chemotherapy-induced cancer stem cell enrichment, and tumorigenicity [1, 2]. These studies highlight the clinical potential of our nanotherapies for TNBC.

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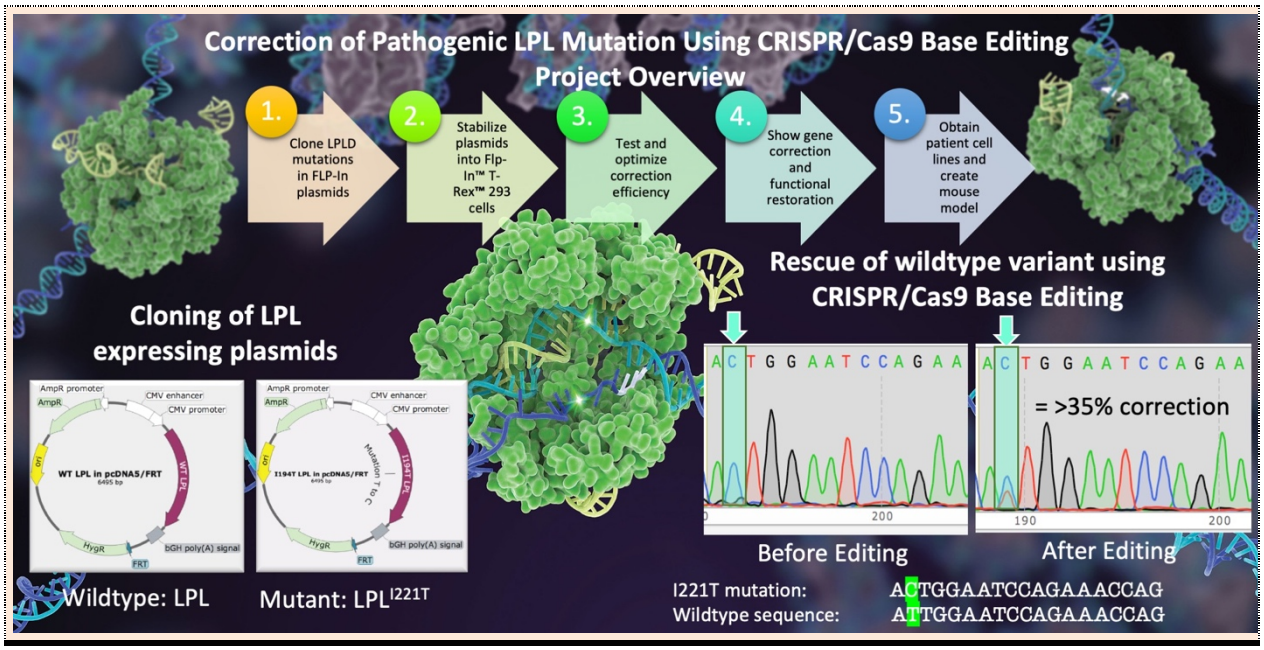


# Successful Correction of a Pathogenic Mutation of the *LPL* gene Causing Lipoprotein Lipase Deficiency, Using a CRISPR/Cas9 Base Editing Therapeutic Approach

Tiffany Carlaw[1], Yuejun Guo[2], Siyue Yu[2], Linhua Zhang[2] and Colin Ross[2]
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Lipoprotein lipase deficiency (LPLD) is a Mendelian disease caused by mutations in *LPL*, which hydrolyses triglycerides in blood serum. Patients present extreme hypertriglyceridemia, chronic abdominal pain, hepatosplenomegaly and pancreatitis. Previously, Dr. Ross developed an innovative gene therapy to treat LPLD, using AAV delivery of the *LPL* gene. This treatment became the first gene-augmentation therapy to receive regulatory approval (Glybera®)[1,2]. Due to the high cost of the AAV production, the drug was priced over $1 million per treatment and was withdrawn from the market[2].

**We hypothesize that CRISPR/Cas9 base editing can successfully repair mutant *LPL* and demonstrate superior therapeutic benefit over the previous approach**. CRISPR/Cas9 base editing approaches use a fusion of a deactivated Cas9 (dCas9) protein coupled with a cytidine deaminase to directly convert C-G to T-A at a target locus[3].

We successfully established a new human cell model of LPLD, possessing one of the most common mutations, *I221T*. We cloned *LPL*$^{I221T}$ and wildtype *LPL* into pcDNA™5/FRT and transfected each into a Flp-In™ T-REx™ 293 cell line. In collaboration with Dr. David Liu, we have successfully demonstrated more than 35% correction of the LPL$^{I221T}$ mutation, using the SakkhBE3 base editor[3]. Successfully edited cells will be analyzed using an LPL expression assay and we expect phenotypic rescue to be observed. We are currently optimizing our approach using stabilized guide RNAs and by adopting new base editor constructs. We are generating a humanized mouse model of *LPL*$^{I221T}$. We will optimize a novel nanoparticle delivery strategy (in collaboration with Dr. Cullis at UBC). In the future, this strategy could provide a better and more cost-effective treatment for genetic diseases such as LPLD.

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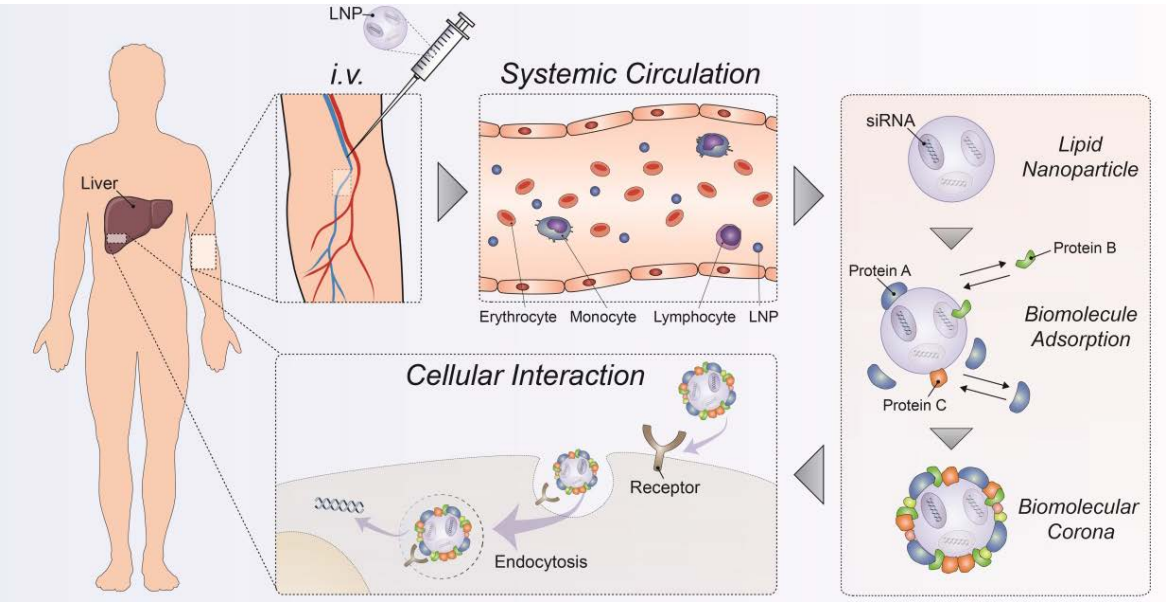


# Exploring the Potential of a Personalized Corona in Lipid-based Nanoparticles for siRNA delivery

Valentina Francia[1,2,*], Dominik Witzigmann[1], Raymond Schiffelers[2], Pieter Cullis[1]

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Gene therapy represents a potential solution for the treatment of several diseases, through the delivery of nucleic acids to target cells. These nucleic acids can be encapsulated into carriers such as lipid nanoparticles (LNPs), with the aim of improving their circulation times, reducing undesirable side effects, and, overall, better controlling their targeting [1]. Recently, it has been observed that LNP's targeting is strictly dependent on the interactions with its surroundings: following intravenous administration, blood biomolecules adsorb on LNPs' surface forming a protein layer called "corona", able to confer LNPs new surface and targeting properties [2] (**Figure 1**). For example, the hepatic gene silencing efficacy of Onpattro®, the first LNP-encapsulated siRNA medicine, relies on the presence of Apo E in this protein layer [3]. Despite the widely recognized contribution of the corona to the targeting capabilities of LNPs, so far, a clear picture of the correlation between corona composition and cell interactions is still missing.

Therefore, my aim is to understand and exploit the corona of clinically-relevant nanomedicines, such as LNPs for siRNA delivery. Since the corona is species- and patient-specific, LNPs are incubated, *ex vivo*, with blood samples derived from patients with different conditions and diseases. This knowledge will allow us to improve the design of targeted LNPs, control their localization, uptake, and contribute to further improve their success *in vivo*. The results indicate that a personalized corona is formed on the surface of selected LNPs and that different corona biomolecules have a key role in triggering a specific cellular recognition.

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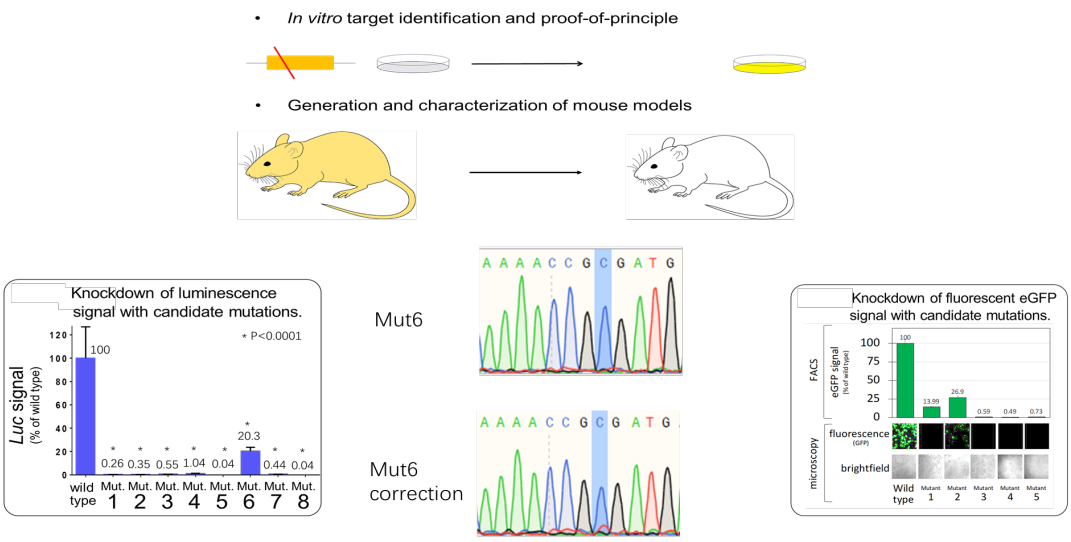


**DEVELOPMENT OF I*N VIVO* MUTANT REPORTER MOUSE MODELS TO OPTIMIZE AND EVALUATE CRISPR/CAS9 THERAPEUTIC BASE EDITING**

Siyue Yu[1], Tiffany Carlaw[2], Linhua Zhang[1], Colin Ross[1]
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Of the over 6,250 known monogenic genetic diseases, <5% have effective treatments[1]. New advances in genome engineering, such as CRISPR/Cas9, have provided a new therapeutic opportunity to directly repair disease-causing mutations in patients. We are especially interested in the use of CRISPR/Cas9 base editors to precisely and accurately repair specific nucleotides in the DNA. It has been shown the base editors can achieve up to 15-75% editing efficiency *in vitro*[2,3]. However, there is still a great need to fully understand base editing *in vivo* in order to optimize and evaluate this approach before therapeutic applications. **We aim to develop two mutant reporter mouse models which allow us to easily and precisely quantify gene editing efficiency and analyze off-target effects.**

The point mutations identified in both reporter genes abolish up to 99.96% of the activity *in vitro*. Upon base editing, ~27% mutant nucleotides were corrected to wildtype restoring luminescence/fluorescence which could be quantified. Currently, desired mutations are successfully installed in the founder mice which carry the non-functional reporter genes. Live animal whole body imaging has also confirmed the loss of reporter gene signals in the mice.

We have generated two mutant reporter mouse models. These animal models can be used to monitor *in vivo* genome editing, to optimize delivery of genome-editing components into a variety of target tissues to aid many gene therapy applications, and to compare new generations of base editors.

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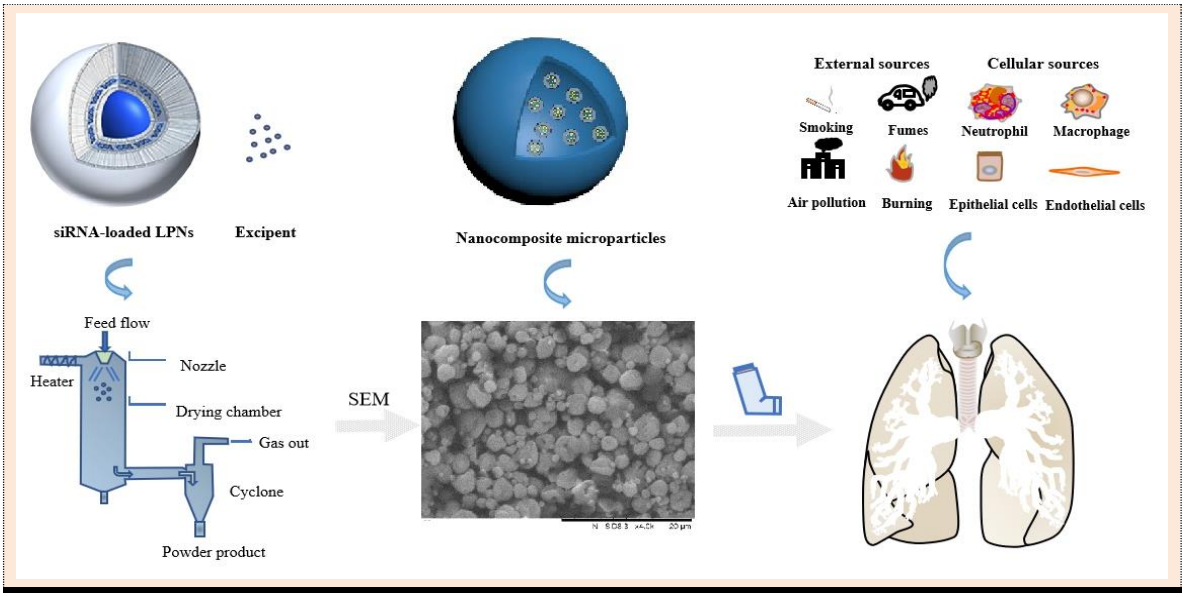


# Design of spray-dried TNF-α siRNA-loaded LPNs with high aerosol performance for treatment of COPD

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Therapeutics based on siRNA are highly target-specific and promising for the treatment of chronic obstructive pulmonary disease (COPD)[1,2]. Local delivery of siRNA to the lungs constitutes a promising new area in drug delivery. The aim of the present study was to design inhalable formulations of siRNA-loaded lipidoid-polymer hybrid nanoparticles (LPNs) powder by spray drying (SD) using the sugars trehalose (tre) and dextran (dex) as stabilizing excipients and the amino acid leucine (leu) as dispersion enhancer. LPNs were prepared by using the double emulsion solvent evaporation method[1]. First, the effect of the ratio between the disaccharide tre and the polysaccharide dex was investigated in detail to identify the optimal combination. Tre and dex at a weight ratio of 10:90 showed promising result. To further optimize the powder properties, different weight ratios of leu (5% to 100%) was included. With increasing leu content, the yield was not affected, whereas the mean molecular aerodynamic diameter (MMAD) and moisture content were decreased. Morphological analysis showed that powders containing less than 20% of leu displayed a slightly corrugated surface, powders with a leu content of 20-50% had a rough and dimpled surface, and the surface was broken at a leu content higher than 60%. Aerosol testing using the preciseinhale equipment showed that the flowability of the powder was enhanced with increasing content of leu up to 70%, however, the yield was maximal after 30%. In addition, reconstituted LPNs displayed almost the same size and zeta potential as before SD at low leu ratios, whereas the size increased when the leu ratio reached 70%. LPNs containing 40% leu and 60% of a mixture of tre and dex (10:90) displayed a high yield (84%), a low moisture content (2.7%), a suitable MMAD (3.5 µm), and almost the same size and zeta potential as the original sample. Importantly, this formulation had high aerosol performance, which is promising for in vivo lung deposition. Hence, the combination of sugars and leu is promising for producing nanocomposite microparticles comprising siRNA-containing LPNs for COPD in inhalation therapy.

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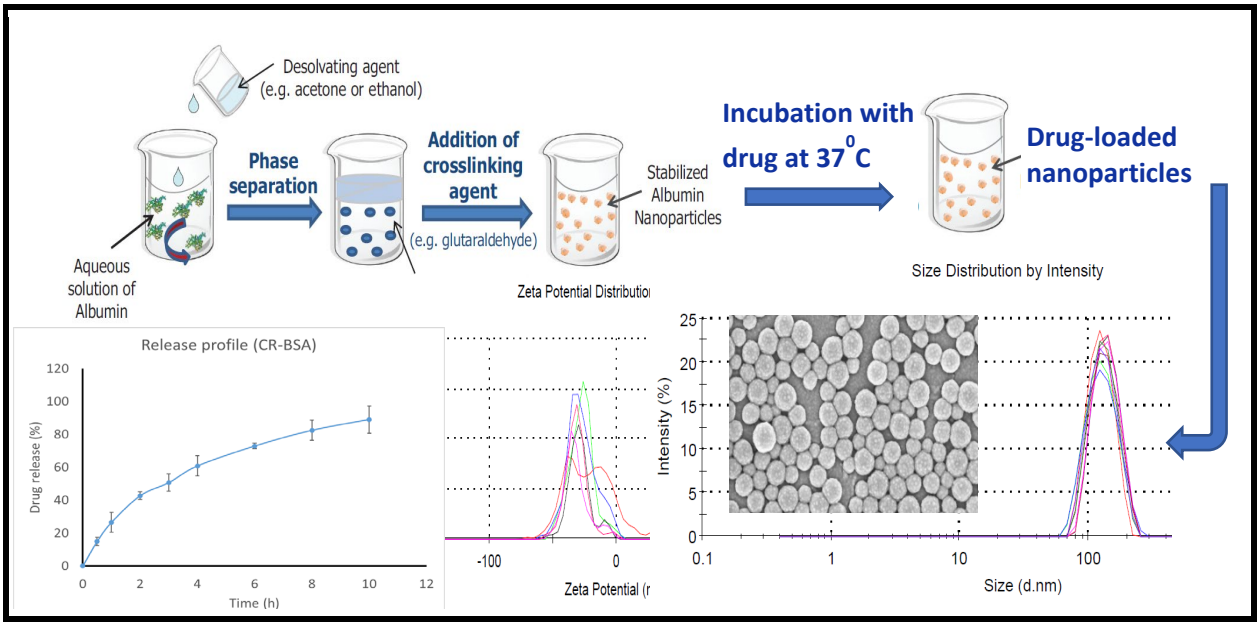


# Design and evaluation of albumin nanoparticles for the delivery of a β-tubulin polymerization inhibitor

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Albumin, a nontoxic, non-immunogenic, biocompatible, biodegradable protein, strongly interacting with both hydrophilic and hydrophobic drugs is an ideal and versatile substance to fabricate nanoparticles for drug delivery. The albumin carrier could specifically target the desired site due to the albumin receptors, gp60 and SPARC overexpressed in tumors [1]. CR-42-024, a tubulin polymerization inhibitor, recently synthesized and patented at University of Alberta, has shown potent anti-cancer activity in various cancer cell lines and has particularly been effective in cancer cells resistant to paclitaxel. However, its poor water solubility, poor uptake into the tumors, nonselective distribution and uptake by normal cells hinder its efficacy upon *in vivo* administration. CR-42-024 was loaded into serum albumin nanoparticles (CR–SA NPs) using a modified desolvation method [2]. The CR–SA NPs were assessed for particle size, zeta potential, drug loading, drug release, morphology and cell toxicity against PANC-1 pancreatic cancer cell line. The spherical nanoparticles obtained were negatively charged (~-30 mV) and had an average diameter of ~ 130 nm. The *in vitro* release of CR-42-024 showed a sustained release pattern over 8-10 hours. The drug loading was around 7 µg of the drug per mg albumin. Cellular toxicity studies of the free drug showed an $IC_{50}$ of 4 nM in PANC-1 cancer cell line and CR–SA NPs exhibited greater activities compared to the positive control free CR-42-024. Although the results of this study indicate the potential use of CR–SA NPs in biological systems, the formulation of such nanoparticles should be further modified to provide higher loaded drug levels for *in vivo* evaluations.

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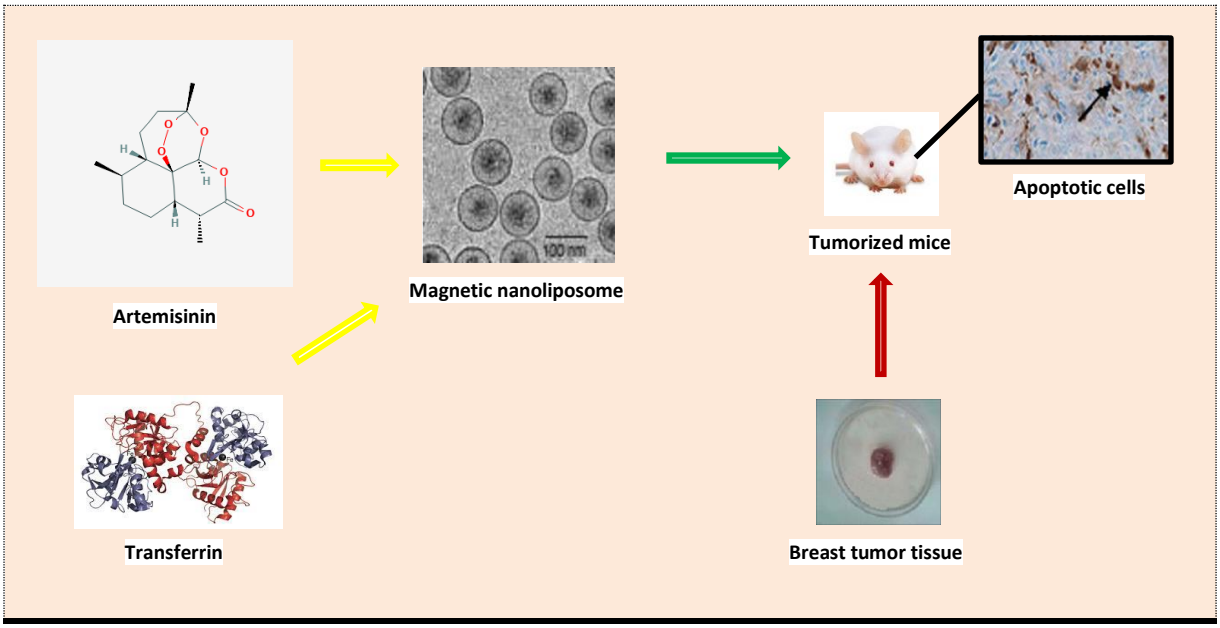


# Experimental treatment of breast cancer-bearing BALB/c mice by artemisinin and transferrin-loaded magnetic nanoliposomes

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The combination of artemisinin and transferrin exhibits versatile anticancer activities. In previous, we successfully prepared artemisinin and transferrin-loaded magnetic nanoliposomes and evaluated their anti-proliferative activity against MCF-7 and MDA-MB-231 cell lines *in vitro* [1]. In this study, we investigate the *in vivo* anti-breast cancer activity of artemisinin and transferrin-loaded magnetic nanoliposome against breast transplanted tumors in BALB/c mice model. Artemisinin and transferrin-loaded magnetic nanoliposomes as shown in Figure 1 were prepared and characterized for some physiochemical properties. Pieces of tumor tissue from the breast cancer-bearing BALB/c mice were transplanted subcutaneously to the syngeneic female BALB/c mice. In the presence of the external magnet that placed at the breast tumor site, the tissue distribution and tumor-suppressing effects of prepared nanoliposomes on tumor growth was evaluated. The prepared nanoliposomes have fine spherical shape, rough surface, nano-sized diameter and magnetic properties. At 2h after treatment, the intravenous administration of artemisinin and transferrin-loaded magnetic nanoliposomes followed using the magnetic field approximately produced 10-fold higher levels of artemisinin and transferrin in the tumors, respectively, compared with free artemisinin and transferrin. Moreover, in the presence of an external magnetic field, the prepared nanoliposomes could significantly induce apoptosis in the mice breast cancer cells as well as could reduce tumor volume in tumorized mice at 15 days after treatment.

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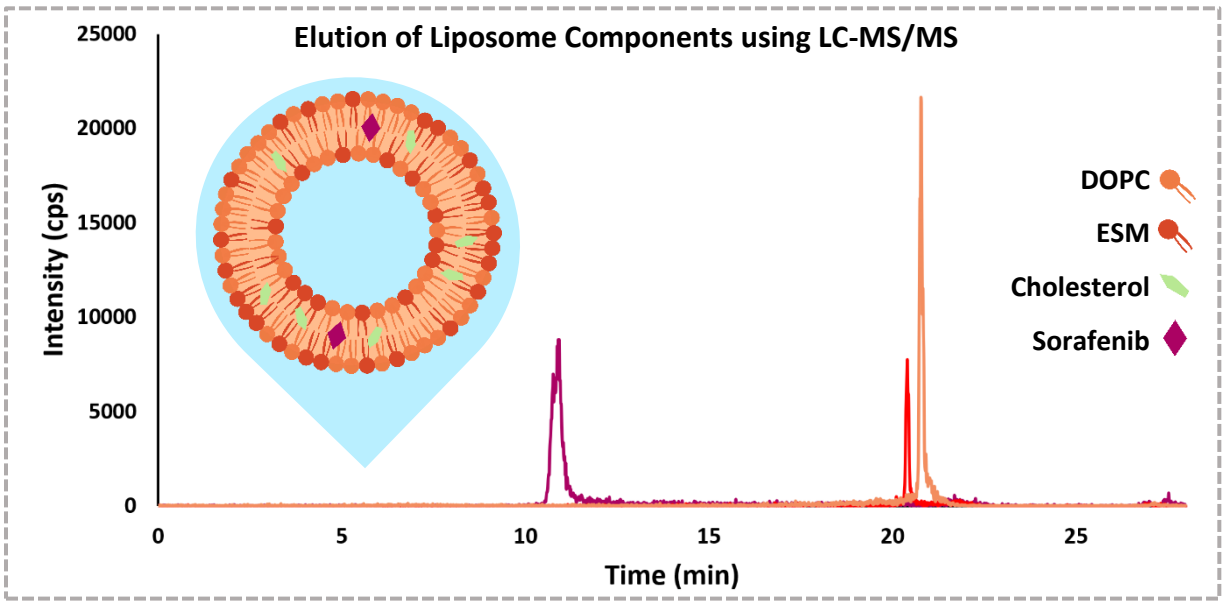


# Development of an LC-MS/MS Method for the Characterization of Liposomes used in Drug Delivery

Antonella Ibarguen Mejia[a], Loryn Arnett[a], Mitchell Winnik[a] and Gilbert Walker[a,b]

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Liposomes are ideal candidates for drug delivery due to the variety of properties they can access based on structure, surface charge, lipid composition, and size.[1] To ensure these vesicles behave as intended, it is imperative to characterize if their structure after preparation agrees with the expected formulation. Thus, an LC-MS/MS protocol able to simultaneously measure lipids and a drug was developed. From a chromatography protocol adjusted solely for lipid quantitation, adjustments were made until sorafenib, an anti-cancer hydrophobic drug, was well-resolved without affecting the elution of lipids, DOPC and ESM. These changes included decreasing the initial mobile phase solvent strength, then increasing it slightly for the elution of lipids, and finally elongating the total run time to allow for the full elution of all components. The optimization of cholesterol, the remaining constituent in DEC221 liposomes, was challenging due to its incompatibility with the ionization source used. A derivative was thus synthesized for its quantitation and a potential ion pair for MS/MS analysis was identified. After the incorporation of this derivative, the protocol developed will be used to evaluate how these liposomes vary from the original proposed design by comparing unloaded and drug-loaded vesicles, as this could have undesirable consequences on the delivery of the drug. Additionally, this method will be used for investigations regarding encapsulation efficiency as well as drug delivery kinetics.

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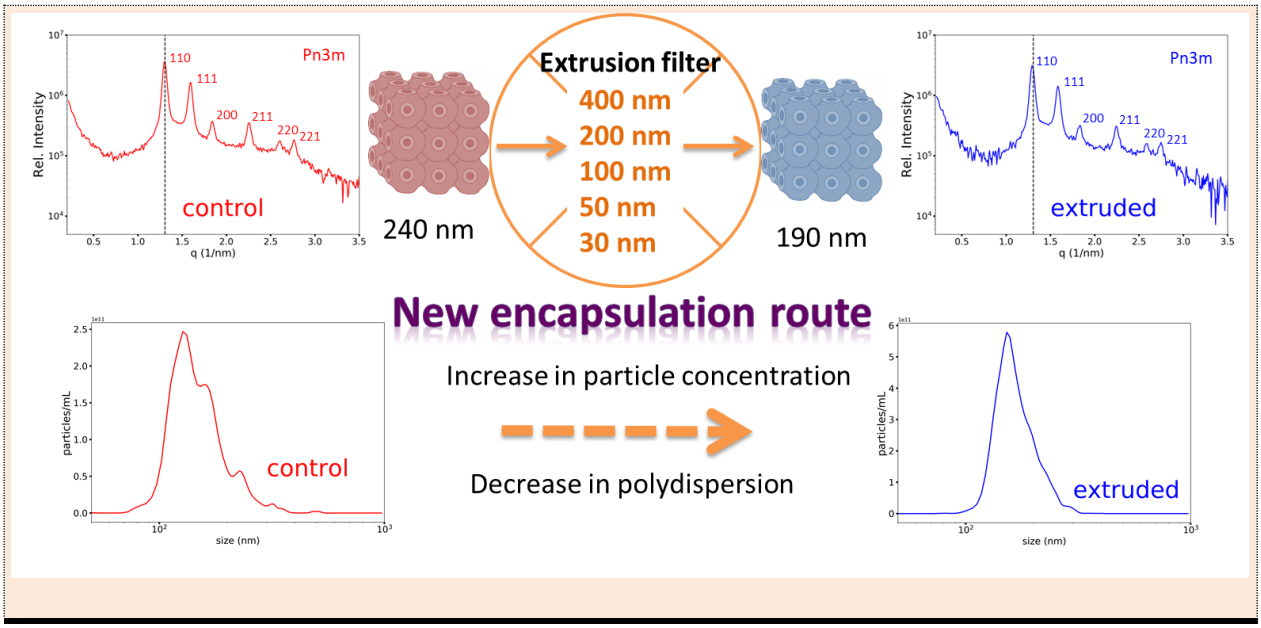


# Phytantriol cubosomes flexibility and malleability evidenced by extrusion: a new method for drug encapsulation

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The use of nanoparticles is intended to improve bioavailability of drugs while decreasing undesired side effects, therefore, nanoparticles offer both a protection for the active molecules and drugs as a carrying vehicle. Cubosomes are capable of storing both hydrophilic, hydrophobic and amphiphilic molecules within its structure[1]. They have approximately 50% hydrophobic area, being able to carry more molecules than other nanoparticles. Particularly, cubosomes are quite easy to produce in which lipids (monoolein, phytantriol (PHY), etc) self-assembly in water medium[2]. In the present study, we investigated the malleability of PHY-cubosomes under extrusion, by SAXS, DLS, NTA and electron microscopy. Our observations show that after being extruded the nanoparticles do not lose their morphology and particle size is not affected by the pore size of the extrusion filter. On this ground, cubosomes show a large malleability even when undergoing extrusion in a 50 nm pore size filter, presenting average size of 185±2 nm compared to 237±5 nm of the control sample in ultrapure water, similar results are found for PBS buffer medium. We believe these results open a new way for encapsulating drugs into cubosomes. Polydispersion is slightly decreased. Regarding concentration, for both systems, there is an increase from $4.09\pm0.66\text{x}10^{12}$ particles/mL to $7.88\pm0.66\text{x}10^{12}$ particles/mL in ultrapure water, indicating that larger particles are broken into smaller ones, in a rearranging process.

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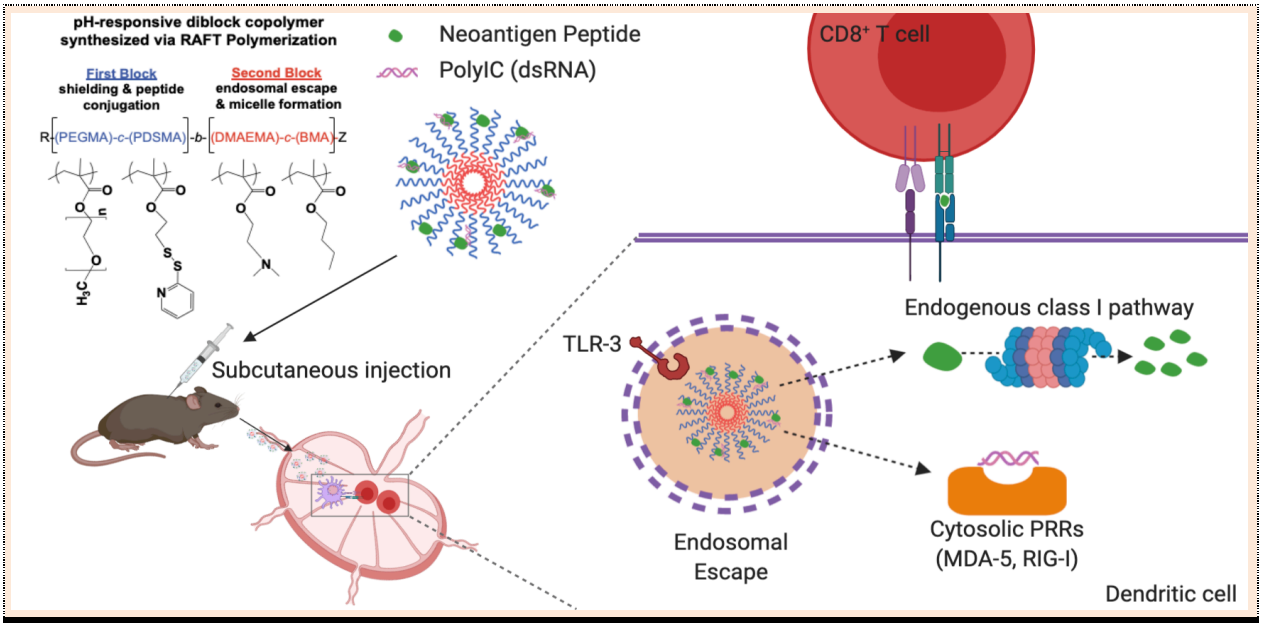


# Engineering Nanotechnologies to Improve Immune Responses to Personalized Cancer Vaccines

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Efficient cancer vaccines readily induce an MHC-I-restricted cytotoxic T lymphocyte (CTL) response by delivering tumor neo-antigens to the cytosol of dendritic cells (DCs). Nucleic acid adjuvants have been shown to further enhance CTL responses by triggering intracellular signaling pathways in DCs that are associated with activation, maturation and migration to the lymph nodes where priming of CTLs takes place. Studies show that DCs are present in high numbers in LNs relative to peripheral tissues such as the skin, suggesting that delivery of antigen and adjuvant to the LN might enhance vaccine efficiency[1]. Studies also show that to effectively prime antigen-specific CTLs, both the antigen and adjuvant need to be co-delivered to the same DC[2].

Our optimized NP vaccine enables neo-antigens and adjuvants to be loaded on one delivery vehicle and co-delivered to DCs. Since particle size plays a major role in the efficiency and route by which these vaccines reach LNs, we strategically optimized the NPs to be ~50 nm in size with a slight cationic surface charge to improve accumulation in LNs from the injection site. Thereby, allowing the NP vaccine to reach the majority of DCs in the LN. Upon uptake by LN-resident DCs, the pH-responsive NP will respond to the decreased pH within endosomal compartments and disassemble allowing cargo to be released into the cytosol. Cytosolically delivered neo-peptides are subsequently loaded on MHC-I molecules and presented to CTLs. Thus, the optimized NP vaccine can potentially improve targeting of LNs, activation of LN-resident DCs, and enhanced induction of antigen-specific CTLs.

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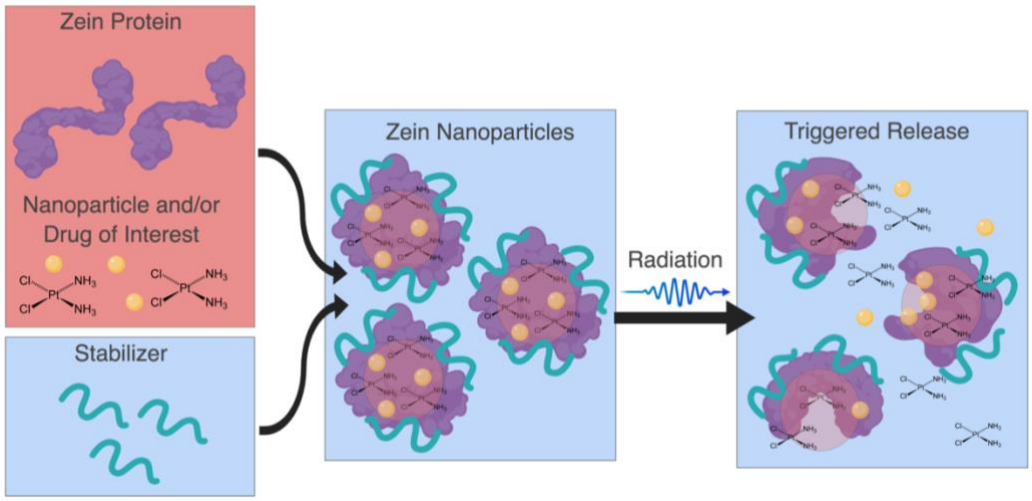


**Figure 1:** Schematic Representing the Formation and Radiation Induced Triggered Release of Zein NPs

# Microfluidic Synthesis of Protein-Gold Nanoparticle Hybrids: Potential for X-Ray triggered drug release

Courtney van Ballegooie[1,4*], Alice Man[1,4], Alessia Pallaoro[2,4], Byron Gates[2], Cornelia Hoehr[5], Donald Yapp[3,4]

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Zein is a class of prolamin proteins found in the endosperm of corn and is an attractive drug carrier for several reasons: its (1) biocompatibility, (2) renewable and abundant source, and (3) its amphiphilic nature. The amphiphilic nature of Zein allows the encapsulation of hydrophobic drugs and formation of nanoparticles (NPs) [1]. Our group has previously used microfluidics to produce these NPs in a robust and reproducible manner [2]. We are now examining the use of X-rays as an external trigger to release drugs from Zein NPs. The radiolysis of water by X-rays generates reactive oxygen species (ROS) which, under certain conditions, is enhanced by the presence of gold NPs. ROS are known to react with surrounding materials, such as DNA, proteins, and lipids, to degrade and destabilize their structure [3]. The destabilization of Zein NPs in this way could potentially be used to release drug on-demand with the application of X-rays. We present here, the design of Zein NPs that are destabilised by exposure to X-rays. We also show data indicating how the total X-ray dose, dosing schedule, and presence of gold NPs, impact the structure of Zein NPs. Overall, the presence of gold NPs and increase in applied total dose lead to a greater degree of protein modification in the Zein NPs. Interestingly, the dosing schedule, whether the dose was applied at a single time point or as smaller doses at multiple time points, was found to have no impact on the degree of modification of Zein NPs.

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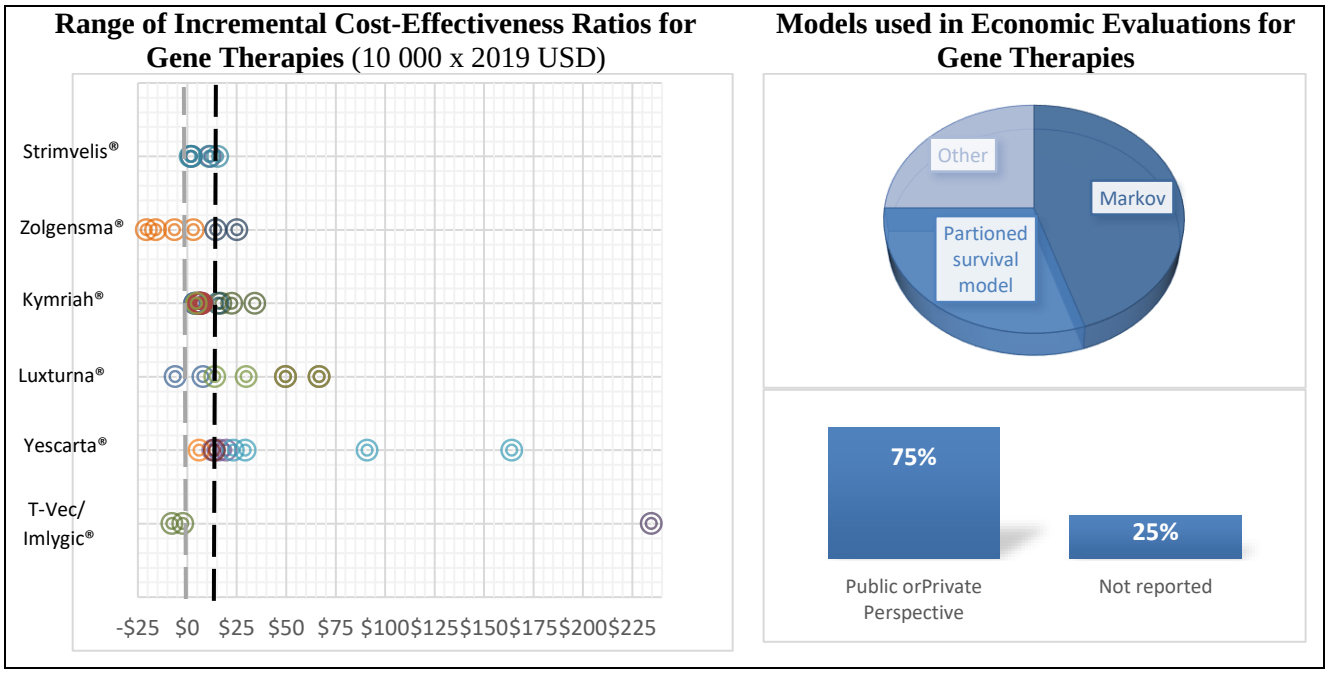


# Economic Evidence on Potentially Curative Gene Therapy Products: A Systematic Review

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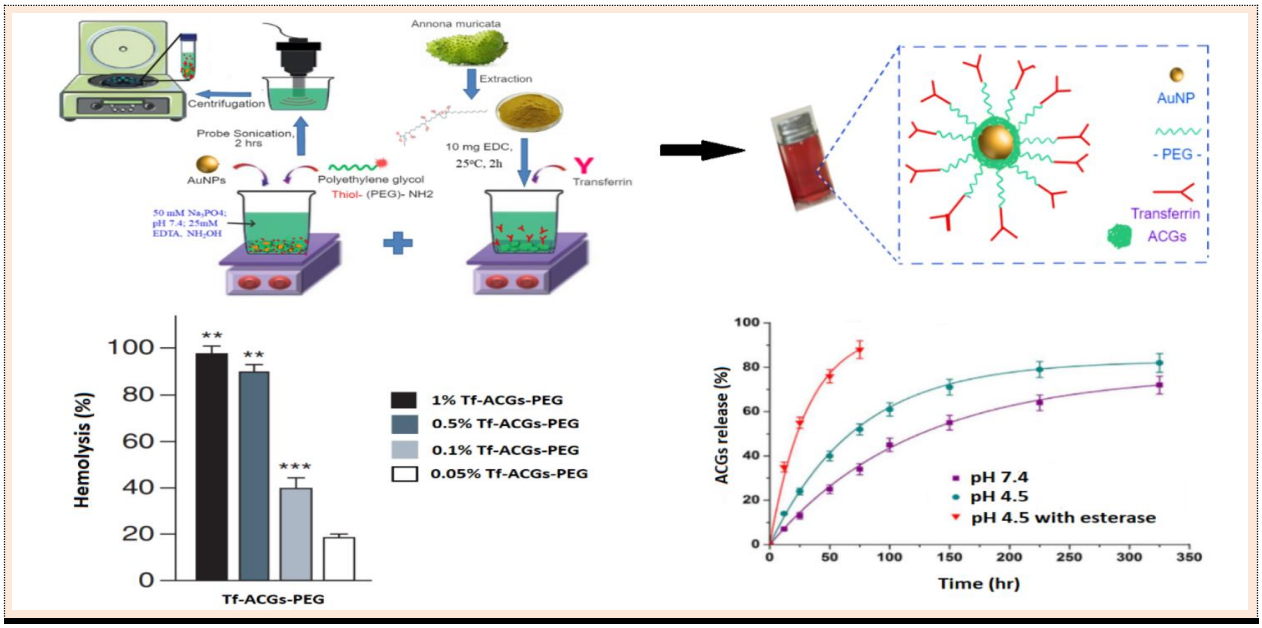
Gene therapy is an emerging class of treatment based on altering gene expression to treat or cure severe medical conditions. Curative gene therapies offer the potential for lifelong benefits from a single treatment, which may result in these therapies being more cost-effective over the long term relative to current therapies, even at significantly higher costs. Given the high cost, uncertainty about long-term efficacy, and increases in eligible patient populations as new treatments are approved, concerns have emerged around the feasibility and financial impacts of gene therapies for patients and healthcare systems. The objective of this study was to conduct a systematic review of existing cost-effectiveness studies on curative gene therapies, and identify potential challenges that economic evaluations face in this area.

All gene therapies were deemed effective relative to their comparators; however, due to high costs, most were not deemed cost-effective. The range in cost effectiveness ratios was substantial, both between and within specific gene therapy products, ranging from dominant to over 2 million USD per Quality-Adjusted Life Year (QALY), far exceeding accepted thresholds for cost-effectiveness. Some of the model parameters with the greatest impact on cost-effectiveness included assumptions about the efficacy and duration of the therapy, alternative treatment used as comparator, and the inclusion of indirect costs.




# Transferrin-Targeted Nanoparticle Delivery of ACGs: Release Kinetics and *In-vitro* Stability Studies

Avan Erhunmwunsee Dalton. [*1,2], Okolie Ngozi Paulinus.[1]

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The therapeutic potential of Annonaceous acetogenins (ACGs), fractions isolated from the Annona muricata plant are known for their antitumor efficacy and broad antineoplastic spectrum but for their low solubility, bioavailability and dissolution rate. A liposome nanoparticle capable of self-assembly will not only carry these cytotoxic small molecules but will also ensure a precise delivery to the target site.

To this end, we encapsulated ACGs in polyethylene glycol decorated with transferrin. The synthesized and physiochemically characterized nanoparticles [1] were observed for entrapment/loading efficiency and release kinetics. Also, stability with synthetic biological fluids and interaction with endosome-mimicking membranes [2] was determined.

The nanosytem showed accelerated release under weakly acidic conditions (pH 4.5) and the release kinetics followed a first order mechanism. The nanosystem showed no aggregation in the synthetic biological fluid and the PSD was not affected. The endosomal escape properties as determined by the interaction of nanosystem with erythrocyte membranes showed a significantly ($p < 0.01$) higher hemolysis at 1% Tf-ACGs-PEG.

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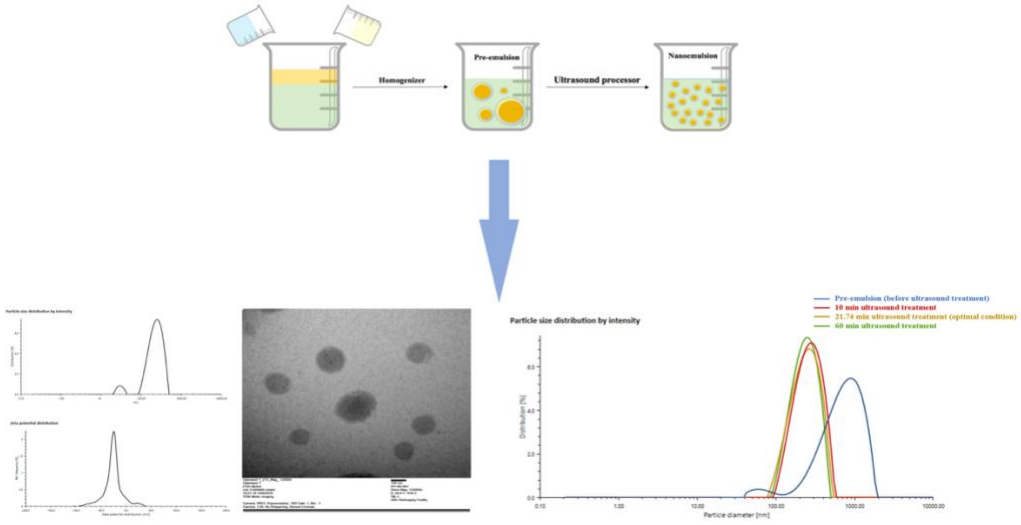


# Towards an understanding of optimum ultrasonication process time on size reduction of hempseed oil nanoemulsions

Farahnaz Fathordoobady [1], Yigong Guo [1], Natalia Natalia Sannikova [2], Anubhav Pratap Singh [1]*

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Nanoemulsions have been used in medical applications over the past few decades due to their high surface area, long shelf life, transparent appearance and desired rheology properties [1]. An essential feature of a nanoemulsion system is the small size of dispersed droplets. Ultrasonication is a widely used high energy method which efficiently reduces the particle size in small scale production systems. The energy introduced by soundwaves (>20 kHz) creates cavities and sinusoidal pressure variation at a liquid-liquid interphase in the emulsion system. This process leads to microjet and shock-wave impacts and collisions between particles, resulting in particle-size reduction [2,3].

Based on a previous study, the droplet size first decreased exponentially with increasing ultrasonication time, then tended to be stationary after certain minutes [4]. Thus, it is not necessary to keep increasing the ultrasound process time to get the smallest particle size. For the purpose of saving time and energy, this study focuses on introducing the optimum ultrasound process time for different formulations of oil in water (O/W) nanoemulsions. To produce O/W nanoemulsions, hempseed oil derived from *Cannabis sativa* L. was selected as the oil phase and emulsified with two types of surfactants. Based on the results of this study, it can be theorized that a nanoemulaion formulation with the ideal level of size-reduction can be developed by ultrasonication process and potentially used for drug and pharmaceuticals delivery applications.

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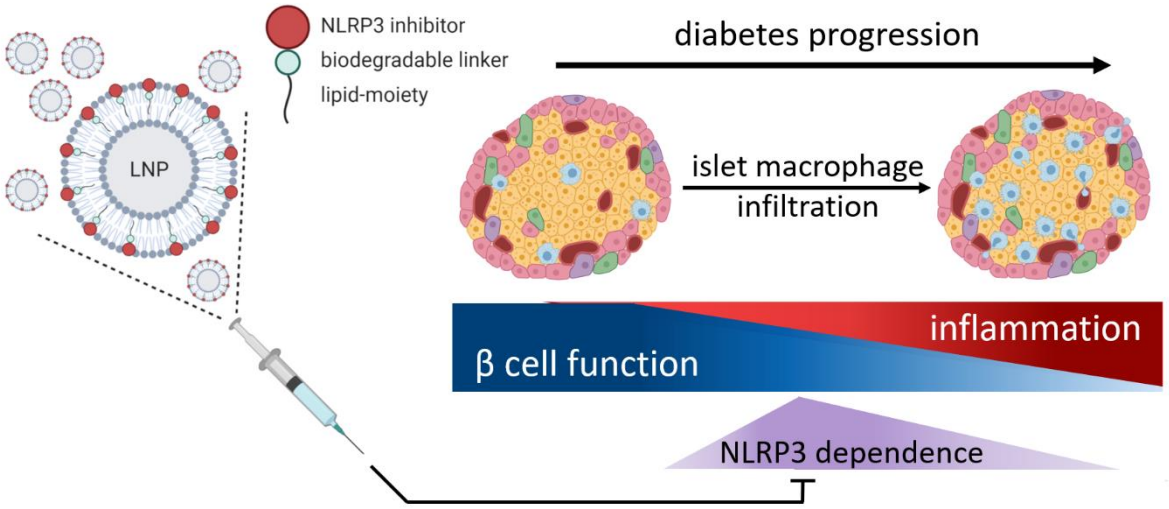


# A nanomedicine approach for diabetes: targeting the NLRP3 inflammasome in tissue-resident macrophages.

Heather Denroche[1,4], Nahae Kim[1,4], Alex Hsu[1,4], Sam Chen[4], Joshua Zaifman[2,4], Ying Tam[4], Chris Tam[4], Marco Ciufolini[2,4], Pieter R. Cullis[3,4], C. Bruce Verchere*[1,4]

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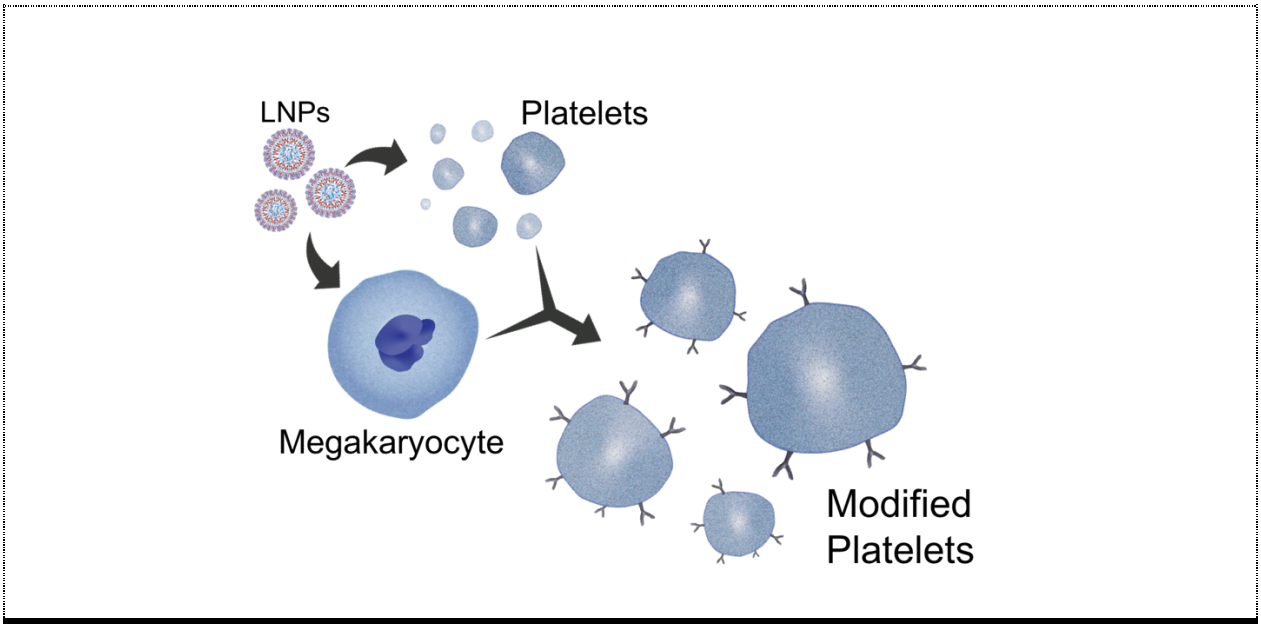
Type 2 diabetes (T2D) is a devastating disease with an enormous economic burden, caused by progressive loss of functional insulin-producing beta cells in the pancreas together with insulin resistance. Inflammation is a driver of T2D pathogenesis, in which macrophages in adipose tissue and pancreatic islets produce IL-1β by a mechanism involving activation of the NLRP3 inflammasome. Clinical use of anti-inflammatory drugs in T2D is hampered by lack of cell specificity and off-target side-effects, including liver toxicity. We are developing a nanomedicine therapeutic for T2D that will target the NLRP3 inflammasome specifically in macrophages, inhibiting local production of IL-1β and thus enhancing insulin sensitivity and beta-cell function. We have developed several effective pro-drug inhibitors of the NLRP3 inflammasome encapsulated in a lipid nanoparticle (LNP) formulation that preferentially targets macrophages. Ex vivo, our lead pro-drug inhibits IL-1β secretion by bone-marrow derived macrophages stimulated by canonical NLRP3 inflammasome activators. In vivo, our LNP formulation is selective for tissue-resident macrophages (including macrophages in pancreatic islets and in adipose tissue) compared to other cell types, and efficient (>95% of islet macrophages targeted). Administration of our LNP formulation reduced *Il1b* expression in pancreatic islets of non-diabetic mice, indicating effective drug delivery to islet macrophages. Furthermore, long-term administration (15 weeks) in mice did not alter circulating liver enzymes or show other signs of liver toxicity compared to controls. In future studies, we aim to test whether this LNP formulation prevents diabetes in mouse models of T2D.




# Enhancing Platelets with Gene Therapy for More Effective Transfusions

Jerry Leung[1,2], Andrew Hagner[3], Dominik Witzigmann[1], Lih Jiin Juang[1,2], Peter Zandstra[2,3], Pieter Cullis[1], Christian Kastrup[1,2]

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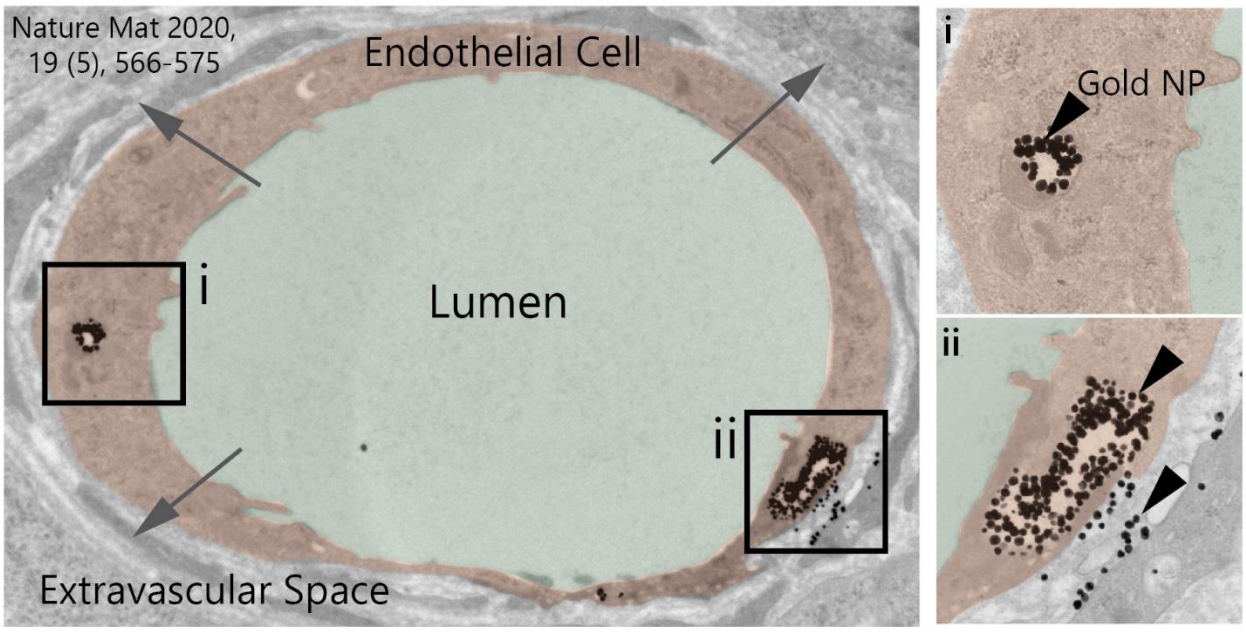
Platelets are small circulating blood cells that act as natural delivery vehicles for numerous biomolecules, and regulate many important processes such as blood clotting, immune function, and even cancer metastasis. Platelets are regularly transfused to help stop severe bleeding, but can be ineffective in the most severe cases of hemorrhage. In addition to hemostasis, platelets are a potential cell therapy in other applications, but development has been hindered by inadequate methods to control which proteins are expressed by platelets. In this proposal, we therefore aim to develop novel approaches to directly engineer transfusable platelets by using lipid nanoparticles (LNPs) to deliver messenger RNA (mRNA) and alter the protein composition of platelets. Once successful, this strategy will create a platform technology to produce transfusable platelets with new and modified functions that current transfused platelets do not possess, and in the long-term yield more effective platelet products, increase the efficacy of transfusions, and decrease the number of transfusion-related adverse effects. The technology will also enable transfused platelets to locally deliver therapeutics to injured tissues, such as sites of trauma, or potentially to diseased vasculature associated with atherosclerosis and Alzheimer's disease in the future. By controlling the expression of proteins in platelets, this technology will be an important tool for the field of platelet research.




# The entry of nanoparticles into solid tumours

Shrey Sindhwani,[1,†] Abdullah Muhammad Syed,[1,†] **Jessica Ngai,[1, 2, *]** Benjamin R. Kingston,[1, *] Laura Maiorino, [3,4,*]Jeremy Rothschild, [5] Presley MacMillan, [6] Yuwei Zhang, [6,] Netra Unni Rajesh, [7,] Tran Hoang, [1] Jamie L.Y. Wu, [1] Stefan Wilhelm, [8] Anton Zilman, [5] Suresh Gadde, [9] Andrew Sulaiman, [9] Ben Ouyang,[1] Zachary Lin,[1] Lisheng Wang [9] Mikala Egeblad, [3]and Warren C. W. Chan[1,2,6,7,10,11] warren.chan@utoronto.ca

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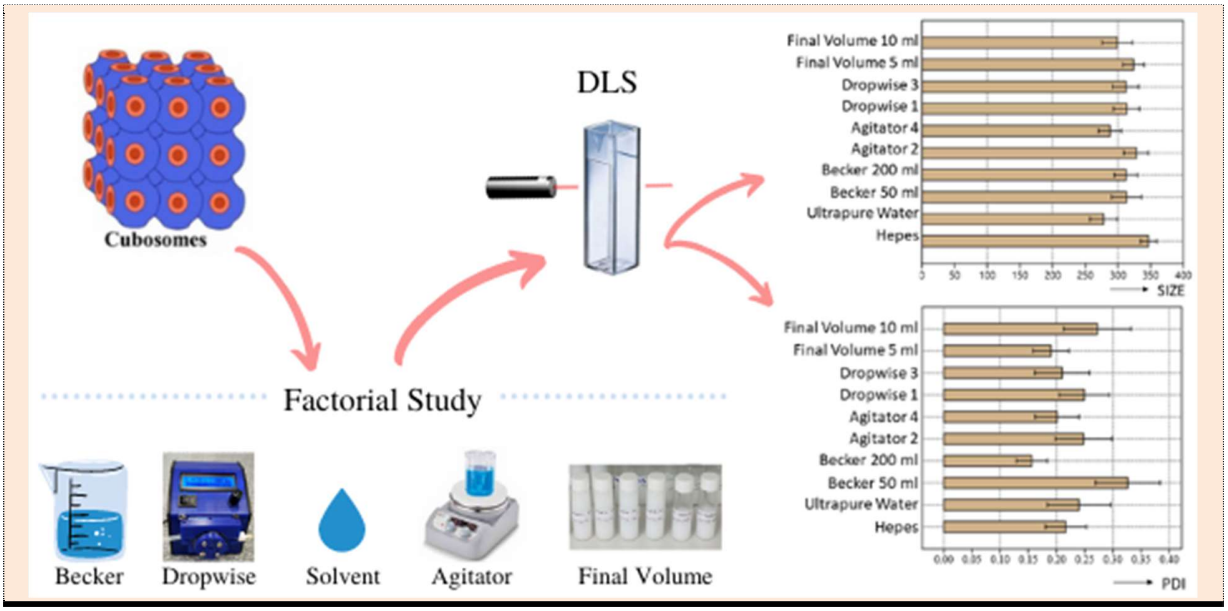
The concept of nanoparticle transport through gaps between endothelial cells (inter-endothelial gaps) in the tumour blood vessel is a central paradigm in cancer nanomedicine. The size of these gaps was found to be up to 2000 nm. This justified the development of nanoparticles for treating solid tumours as their size is small enough to extravasate and access the tumour microenvironment. Here, we show that these inter-endothelial gaps are not responsible for transport of nanoparticles into solid tumours. Instead, we found that up to 97% of nanoparticles enter tumours through an active process through endothelial cells. This result is derived from analysis of 4 different mouse models, 3 different types of human tumours, 2 different types of imaging techniques and a new "Zombie model" that preserves the architecture of vessels to isolate the role of passive pathways. These results challenge our current rationale for developing cancer nanomedicine and suggest that understanding these active pathways will unlock strategies to enhance tumour accumulation.




# Factorial Study of Cubic Nanostructures Formulations and Structural Characterization

Letícia F. Casotti[1], Mayra C. G. Lotierzo[2], Bruna R. Casadei[1], and Leandro R. S. Barbosa[1]

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Cubosomes are nanostructured particles composed of a specific combination of some types of lipids, such as monolein and phyanthriol, in the presence of a non-ionic polymer used as a stabilizer for the cubic phase [1]. Cubosomes are structured in a sort of continuous and highly curved lipid bilayer. They stand out, in relation to the others, by the great structural advantage of encapsulating both highly hydrophobic molecules and hydrophilic molecules. They are potential carriers to be used in nanomedicine to increase the effectiveness of diagnostic agents and drugs, including anticancer, antimicrobial and antiviral agents [2]. A challenge for the production of this nanoparticle is the lack of certainty about its quality, therefore, a factorial study was carried out to establish the best conditions for the production of samples, through structural characterization of cubosomes using the Dynamic Light Scattering (DLS). The production process directly interferes with the final results of size and polydispersity index. However, when we standardize the protocol, to obtain a smaller size and more homogeneous samples, it is necessary to produce samples in a Becker with a volume much larger than the final sample volume, or work with reduced sample volumes. In addition, higher agitation speeds associated with slow dripping and ultrapure water solvent are great assistants in the process.

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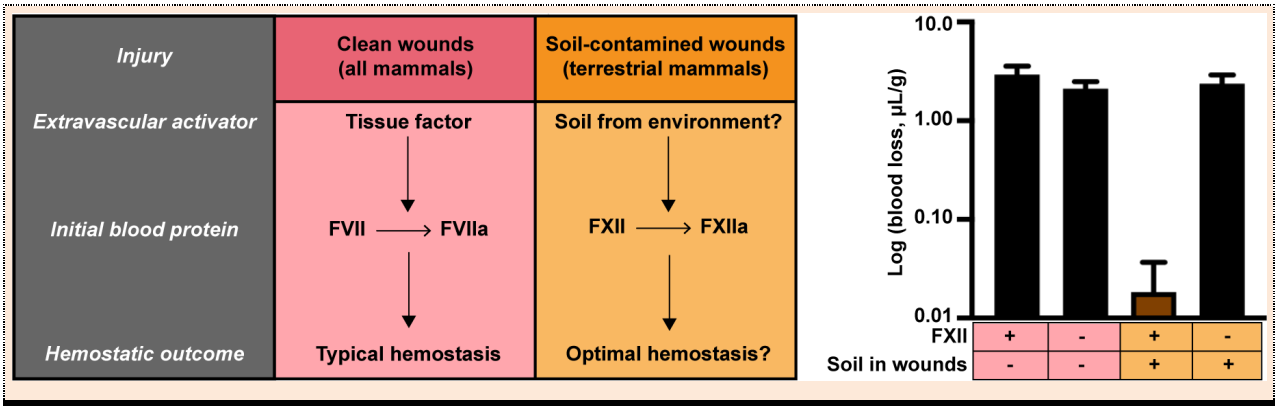


# Coagulation factor XII contributes to hemostasis when activated by soil in wounds

Lih Jiin Juang[1], Nima Mazinani[1], Stefanie K. Novakowski[1], Emily N. P. Prowse[1], Martin Haulena[2], David Gailani[3], Leslie Lavkulich[4], Christian J. Kastrup[1*]

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FXII is a plasma protein found in the blood of most vertebrates, first appearing in evolution in amphibians. The findings that FXII deficiency in humans and mice do not result in a bleeding phenotype[1, 2] (observed in relatively soil-free environments) led the coagulation community to believe that FXII does not participate in clotting in response to blood vessel damage, to seal the wound and prevent blood loss. Instead, FXII has only been implicated in thrombosis,[3] the pathological process that results in formation of a clot that obstructs the blood vessel. Thus, it is still not clear whether FXII can contribute to hemostasis following injury and if so, what is the agent responsible for its activation.

Silicates such as clay and glass are among the non-biologic substances that trigger FXII activation,[4] they are natural nanoparticles ubiquitous in the environment as major components of soil. In this study, we discovered that soil is a potent activator of FXII and promotes faster clotting in the plasma of humans and wild type mice, but not in the plasmas of birds and dolphins. This may explain why expression of the FXII protein was lost in the evolution of these species – because they had ancestors that were not in regular contact with soil. It also explains why FXII-deficient people do not appear to have a bleeding phenotype – because modern homes and clothing separate us from soil. In summary, we discovered a novel mechanism that terrestrial mammals use to sense injury, demonstrating that proteins in the blood of terrestrial mammals are activated by soil-based silicates to prevent blood loss. In doing so, it also clarifies one of the oldest questions in hemostasis: Is there a role for 'coagulation factor' XII (FXII) in hemostasis after injury?

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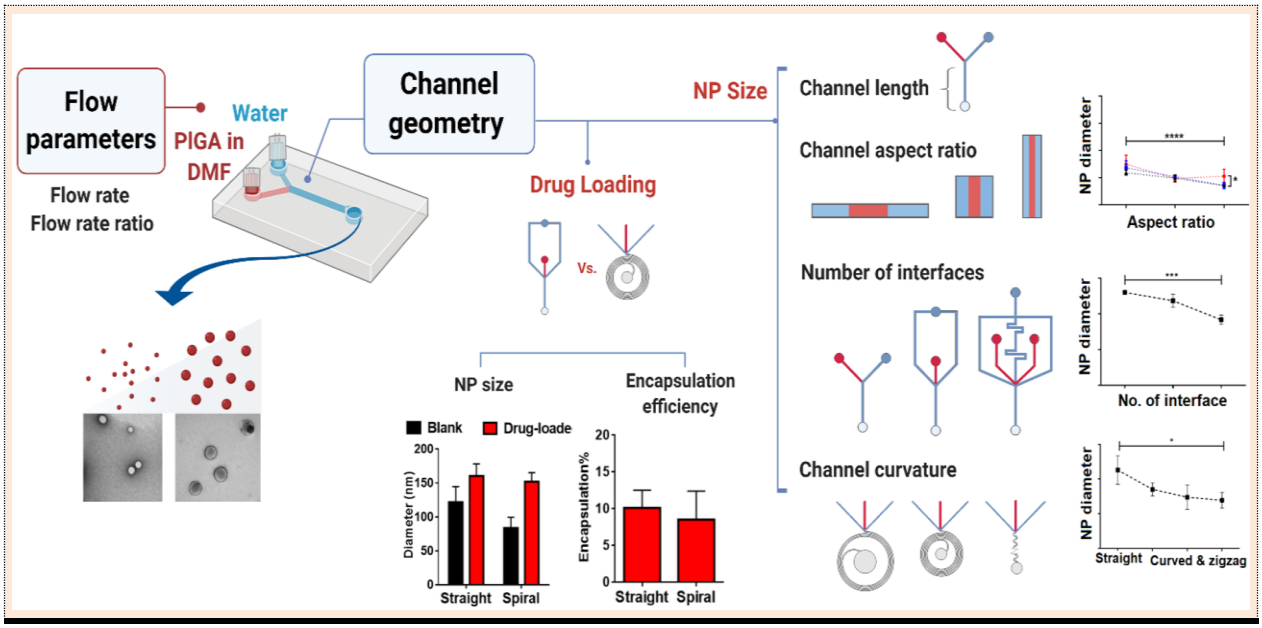


# Tuning Nanoparticles Size via Microchannel Geometry

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Nanoparticles (NPs) are recognized as an important and versatile tool for different therapeutic and diagnostic applications, in particular, polymeric NPs prepared from biocompatible and biodegradable materials such as poly (lactic-co-glycolic acid) (PLGA)[1]. Nanoprecipitation is a popular method for formation of polymeric NPs; however, it suffers from size-heterogeneity and polydispersity due to the slow and uncontrolled mixing time. Microfluidics allow flexible tuning of the fluid mixing time; thus, providing a great control over NP formation[2-3]. However, minimum attention has been given to the effect of channel geometry on nanoprecipitation process.

While different flow parameters continue to be the main approach for adjusting NPs properties, modification of channel geometry enabled tuning of NPs' size using simple designs that can be easily adapted. Here, microchannel aspect ratio, curvature, and number of solvent/anti-solvent interfaces were examined for controlling the mixing time, and consequently, the particle size of PLGA NPs. These parameters can be adapted instead of flow parameters (flow rate and flow rate ratio) in situations where the latter cannot be altered. Moreover, we examined how drug loading affects properties of NPs prepared in microchannels of different designs, where rifampicin (RIF) was used as a model drug. RIF loading increased particle size for NPs prepared in both straight and spiral channels, but RIF encapsulation efficiency was not affected by channel shape.

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**[Poster]**

**Characterization of small extracellular vesicles produced by human mesenchymal stromal cells in an improved extracellular vesicle-free medium**

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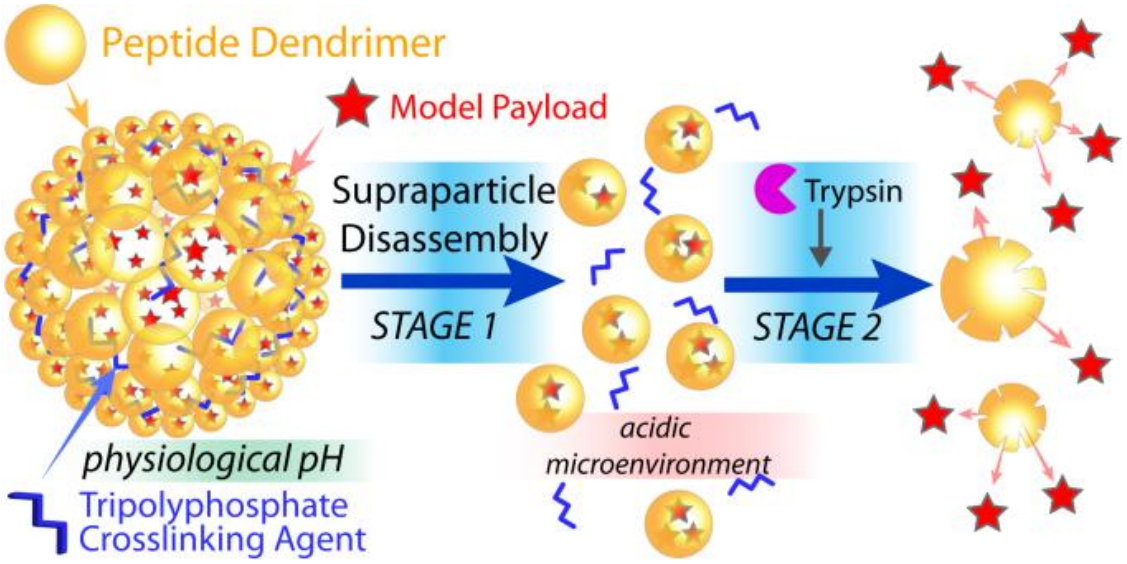
Small extracellular vesicles (sEVs) produced by mesenchymal stromal cells (MSC-sEVs) may be useful for cell-free therapies in immunomodulation and tissue regeneration. To characterize MSC-sEVs produced ex vivo, human bone marrow MSCs were cultured in MesenCult-ACF Plus (MACFP), an improved EV-free and animal component-free culture medium for 3 days after reaching cell confluence. The 'spent' medium was then collected to isolate and characterize sEVs. Analysis of fresh MACFP by nanoparticle-tracking analysis (NTA) and Western blotting confirmed the absence of sEVs. MSC-sEVs isolated from spent MACFP by ultracentrifugation ranged from 80-150 nm in size and were positive for CD63, CD9, and CD81 proteins. These sEVs could be stored -80°C for 4 months in solution with minimal loss based on NTA analysis. The MSC-sEVs were found to contain the MSC-associated microRNAs (miRNAs) let7a, miR21, and miR26a by qPCR. The biological function of ex vivo isolated MSC-sEVs was assessed using a human umbilical vein endothelial cell (HUVEC) tube formation assay. HUVECs treated with MSC-sEV generated tubes as early as 6 h after seeding, which were not observed in control HUVEC cultures until 15 h. Moreover, the number of branch points present in such tube structures was >4-fold higher in HUVEC cultures (n=3) supplemented with MSC-sEVs versus control, with the former lasting >60 hours and the latter lasting <50 h in culture. Direct comparison of the performance of MACFP medium to media containing non-depleted or EV-depleted fetal bovine serum (FBS) demonstrated that only MSCs cultured in MACFP (n=3) were able to expand robustly with a doubling time of 1.1 days, 2.1 and 8.9 days in these different media, respectively. Lastly, methods for isolating sEVs using newly developed EasySep™-EV magnetic separation kits will be presented. Taken together, these data demonstrate that MSC-sEVs can be produced in high yield in MACFP medium and that these possess similar physical, phenotypic and functional characteristics as sEVs in vivo.




# Self-Assembled Peptide Dendrigraft Supraparticles with Potential Application in pH/enzyme-Triggered Multistage Drug Release

M. A. Agazzi[1], S. E. Herrera[1], M. L. Cortez[1], W. A. Marmisollé[1], O. Azzaroni[1].

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Nanocarriers with a size of approximately 100-200 nm show good tumor accumulation but have limited tissue penetration capability. In contrast a size smaller than 10 nm is capable of deep tumor penetration but will be quickly cleared by the systemic circulation. To surmount this dilemma, multistage delivery systems with size reduction capacity have been proposed.[1]

Here we developed a simple and fast supramolecular approach to construct size-shrinkable polyamine-salt aggregates by ionic cross-linking of biodegradable poly-L-lysine dendrigraft with tripolyphosphate anion.[2] The use of a peptide dendrimer as a nanobuilding block (7 nm in diameter) allows the formation of supraparticles (SPs) with well-defined dimensions (200 nm in diameter), narrow size distribution and great capacity to encapsulate different molecules, including chemotherapeutic agents as Curcumin and Doxorubicin. When exposed to slightly acidic environments, the crosslinked matrix is instantaneously disassembled to free dendrimer units. Subsequently, model cargo molecules entrapped in the dendrimer architecture can be released by the action of trypsin enzyme through peptide biodegradation. Therefore, these SPs with proved sequential pH and enzyme-responsiveness could be exploited as nanocarriers in multistage drug delivery systems.

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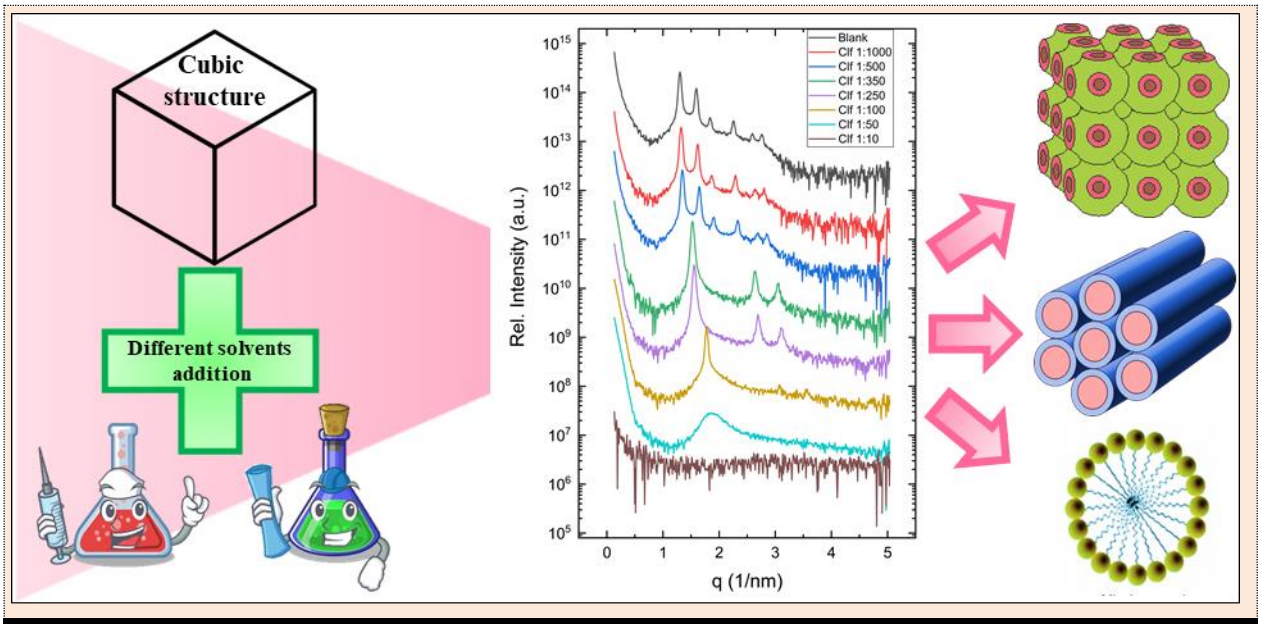


# Micellar to cubic transition in phytanthriol based nanoparticles

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Cubosomes are composed of a mixture of specific lipids with the ability to self-associate, such as phytanthriol (PHY), and polymers that act as a stabilizer, such as poloxamer (F127). [1] These nanoparticles have a high hydrophobic volume, approximately 50%, which makes them promising vehicles for drug delivery of hydrophobic molecules. A challenge for incorporating molecules into nanoparticles is the use of organic solvents in the process. [2] In this study, we investigated the structural influence of four different solvents (acetone, ethanol, chloroform and octane), using low-angle X-ray scattering and cryogenic electron microscopy techniques, aiming to help choose the most appropriate solvent to charge the drug in the cubosome. In the presence of chloroform and acetone, transitions from cubic to micellar phase were observed. Chloroform and octane have different effects on PHY-based cubosomes compared to acetone and ethanol, both of which induced a hexagonal phase transition. These effects are associated with the interaction of the solvent in the hydrophobic phase of the cubosomes, increasing their volume. After the 24-hour incubation period, interesting structural changes were observed in the samples, compared to the freshly prepared ones.

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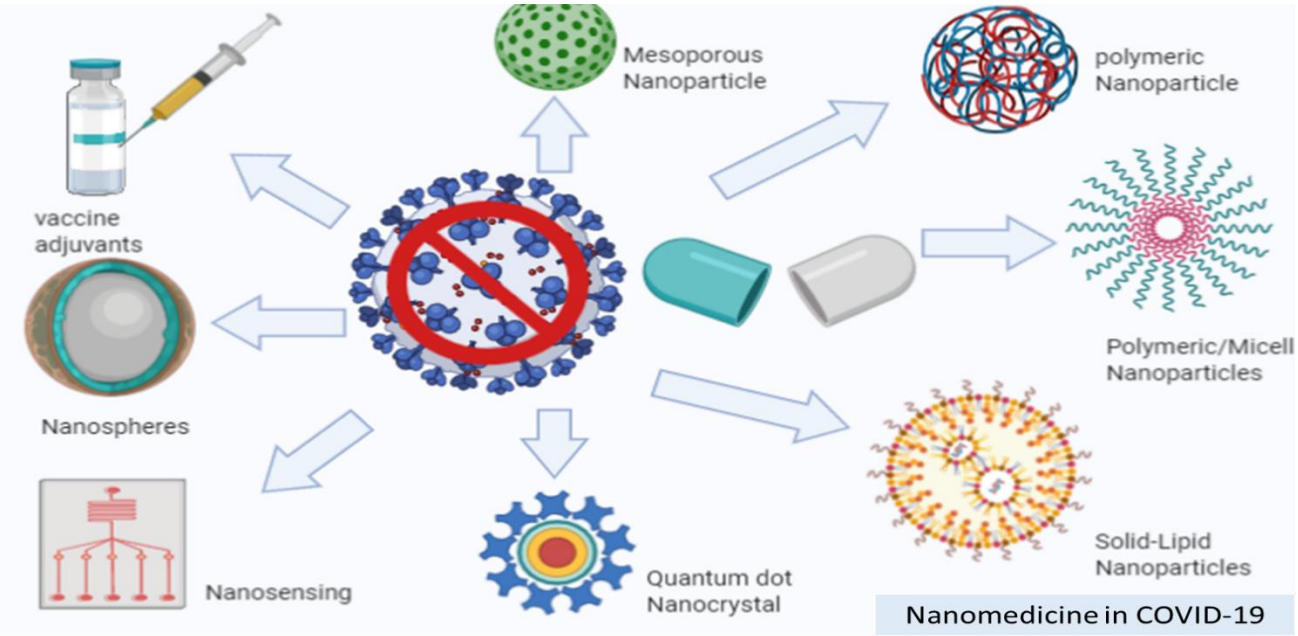


## NANOMEDICINE-AN UNEXPLORED THERAPEUTIC REALM AGAINST COVID-19.

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**Abstract:** Nanomedicine influences almost each and every fields of medicine, and has been considered an important tool for novel diagnostics, medical imaging, nanotherapeutics, vaccines and to develop biomaterials for regenerative medicine. Drug-based nanoparticles have been developed for decades, and several are under clinical trials for cancer, neurodegenerative, inflammatory, cardiovascular and infectious diseases, although only few of them are approved for human use. The occurrence of novel viruses such as SARS-CoV-2, Ebola etc. and their heterogeneity currently demands innovative and fail-safe therapies. This way, considering specific targeting, nanotechnology opens a new avenue for antiviral therapy. The strategy of using nanoparticles to combat SARS-CoV-2 could involve mechanisms that effect the entry of the virus into the host cell until their inactivation. To date, there are view specific approved drugs for treating SARS-CoV-2, and vaccines are under clinical trials. All efforts are welcome to combat the virus, and nanotech-based approaches would bring a new perspective to conventional medicine for the inhibition of virus internalization or treatment. So far, no treatment for COVID-19 has been considered effective and several strategies are being tested. Although it is well-established that nanotech-based drug-delivery systems improve existing therapeutics in medicine, its application in viral diseases is underexplored and underused, as observed in the SARS-CoV-2 pandemic. Nanostructured systems can impact diagnosis, since they can improve the detection, sensitivity and increase the signal amplification specificity in polymerase chain reaction analysis; and prophylaxis as adjuvants for vaccines, as well as therapeutics for COVID-19 through the targeting of antiviral drugs. So, Nanotechnology could represent a convenient strategy in addition to other approaches to provide positive outcomes for COVID-19 treatment.

**Keywords:**COVID-19, nanotechnology, nanomaterials, nanoparticles, nanodiagnostics, nanomedicine.

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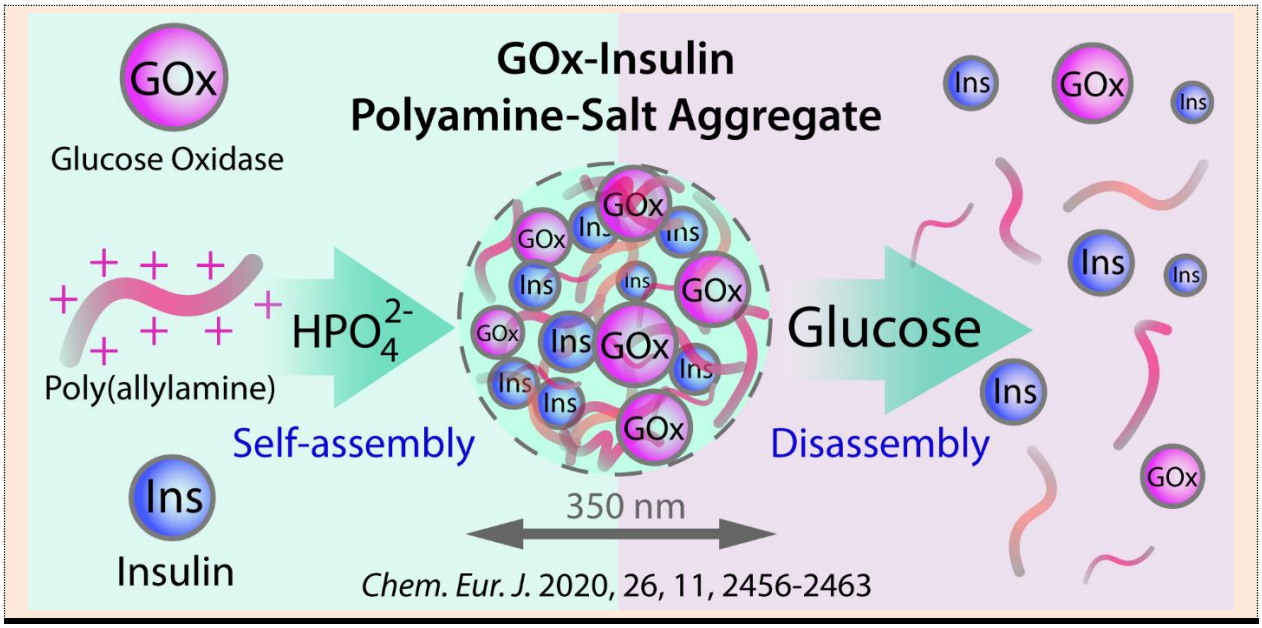


# Insulin-Delivery from Glucose-Responsive Polyamine-Salt Aggregates: Smart "Sense-and-Treat" Nanocarriers Made Easy

Santiago E. Herrera [1], Maximiliano L. Agazzi [1], M. Lorena Cortez [1], Waldemar A. Marmisollé [1], Mario Tagliazucchi [2], Omar Azzaroni [1]*

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Polyamine-salt aggregates (PSA) are biomimetic soft-materials that have attracted great attention due to their straightforward fabrication methods, high drug-loading efficiencies and attractive properties for pH-triggered release [1,2]. In this work, we constructed poly(allylamine hydrochloride)/phosphate PSAs through one-pot ionic gelation [3] containing glucose oxidase as a glucose-responsive element, and human recombinant insulin as therapeutic drug for diabetes mellitus treatment (GI-PSA). The self-assembly process is depicted in **Figure 1** (left side).

The addition of increasing glucose concentrations promotes the release of insulin due to the disassembly of GI-PSA, triggered by catalytic in-situ formation of gluconic acid (**Figure 1**, right side). While under normoglycemia, the carrier integrity remained intact for at least 24 h without losing Insulin, hyperglycemic conditions produced a 100% of cargo releasing after 4 h of glucose addition. This entirely supramolecular strategy presents great potential for the construction of smart glucose-responsive delivery nanocarriers.

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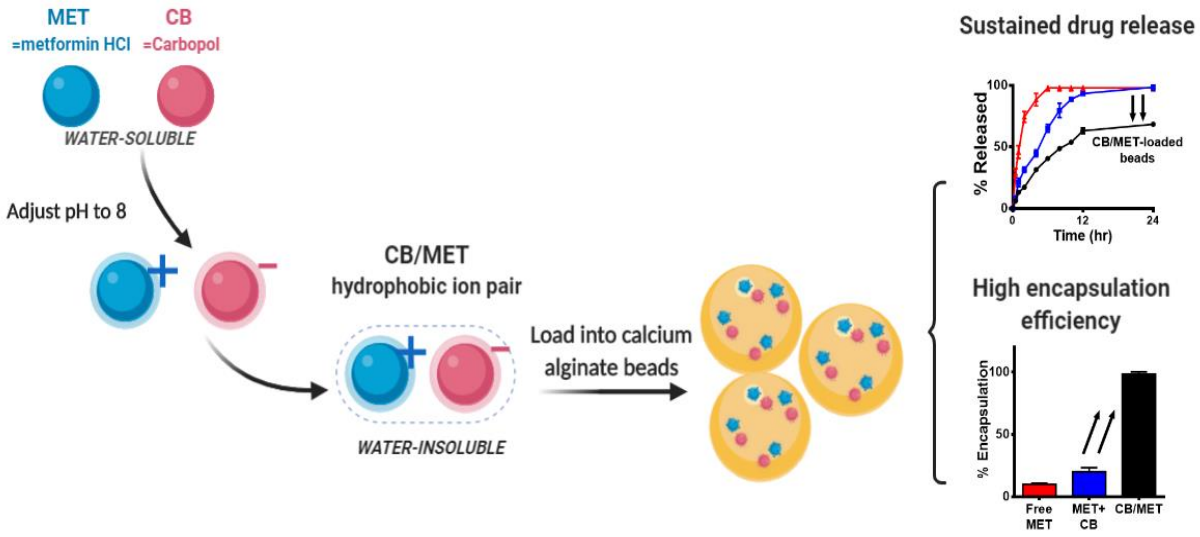


# Modulation of metformin hydrochloride water solubility via hydrophobic ion pairing approach to sustain its release from calcium alginate beads

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Type II diabetes patients receiving metformin hydrochloride (MET) are challenged by its frequent dosing which is attributed to its high water solubility and consequent rapid renal elimination ($t_{1/2}$=2 to 6 hr). In this work, the water solubility of MET was modulated via hydrophobic ion-pairing (HIP) technique [1], where cationic MET was paired with an anionic polymer to form a more hydrophobic MET nanocomplex that can be efficiently loaded into calcium alginate beads for sustained and less frequent oral delivery of MET.

Different anionic polymers where screened and Carbopol (CB) was determined to be optimum ligand for MET HIP in the form of Carbopol/MET complex (CB/MET). Complexation efficiency of CB/MET complex was determined to be dependent on used charge ratio of the drug and polymer reaching 55.65 % at 2/1 CB/MET. Fourier transform infra-red (FTIR) and differential scanning calorimetry (DSC) were used to understand the nature of interaction in formed complex. CB/MET complex appeared as crosslinked aggregates under transmission electron microscope with an average particle size of 1670 nm.

When loaded into calcium alginate beads, ~98.6% encapsulation efficiency was achieved in comparison to only 10% achieved with free MET. Importantly, a sustained MET release profile was achieved in intestinal pH (6.8) reaching 69% after 24 hours, while beads loaded with free MET or MET+ CB physical mixture completely released the drug over the same period of time. In summary, HIP of MET was shown to be a successful strategy for efficient loading of water-soluble MET into polymeric carriers, and dramatically improved MET release profile as formed complexes acted as additional limiting step for its release from alginate beads.

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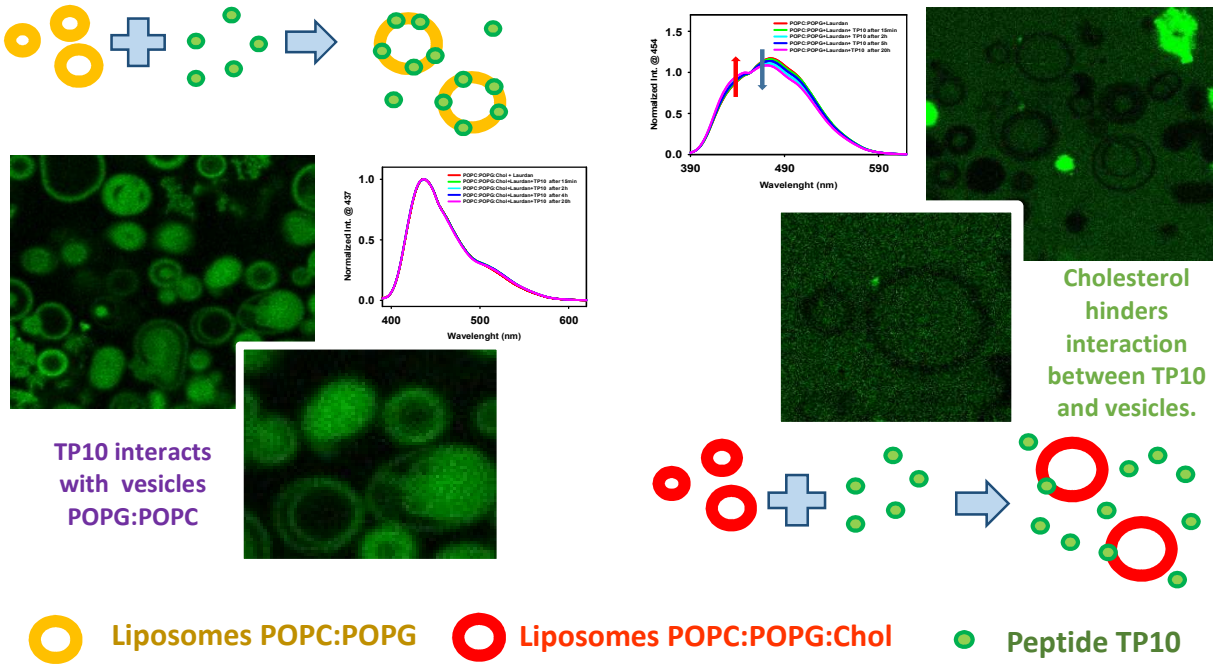


# Effect of cholesterol on the interaction between amphyphylic peptides and liposomes

Sara Anselmo[1], Giuseppe Sancataldo[1] and Hanne Mørck Nielsen[2], Vito Foderà[2] and Valeria Vetri[1]

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With the rise of antibiotic resistance, antimicrobial peptides (AMPs) have been proposed as an alternative novel class of therapeutic agents. They are polycationic, with a net positive charge of more than +2, and they are characterized by amphipathic structures, with both a hydrophobic and a hydrophilic domain. These characteristics allow them to selectively bind to negatively charged lipids (largely present in bacteria, not in mammalian cells), via hydrophobic and electrostatic interactions. Moreover, mammalian cells are characterized by a high content of cholesterol [1].
For this reason, here we present an experimental study on the effect of the presence of cholesterol on the capability of amphyphylic peptide Trasportant 10 (TP10) to interact with model membranes with selected composition. The study was performed by means of fluorescence spectroscopy and fluorescence confocal microscopy measurements also exploiting the advantages of phasor plot analysis of Fluorescence Lifetime Imaging (FLIM) measurements.
Our results show that the presence of cholesterol inhibits TP-10 interaction with lipid vesicles, the extent of the observed effect being dependent on the cholesterol concentration in the membrane.

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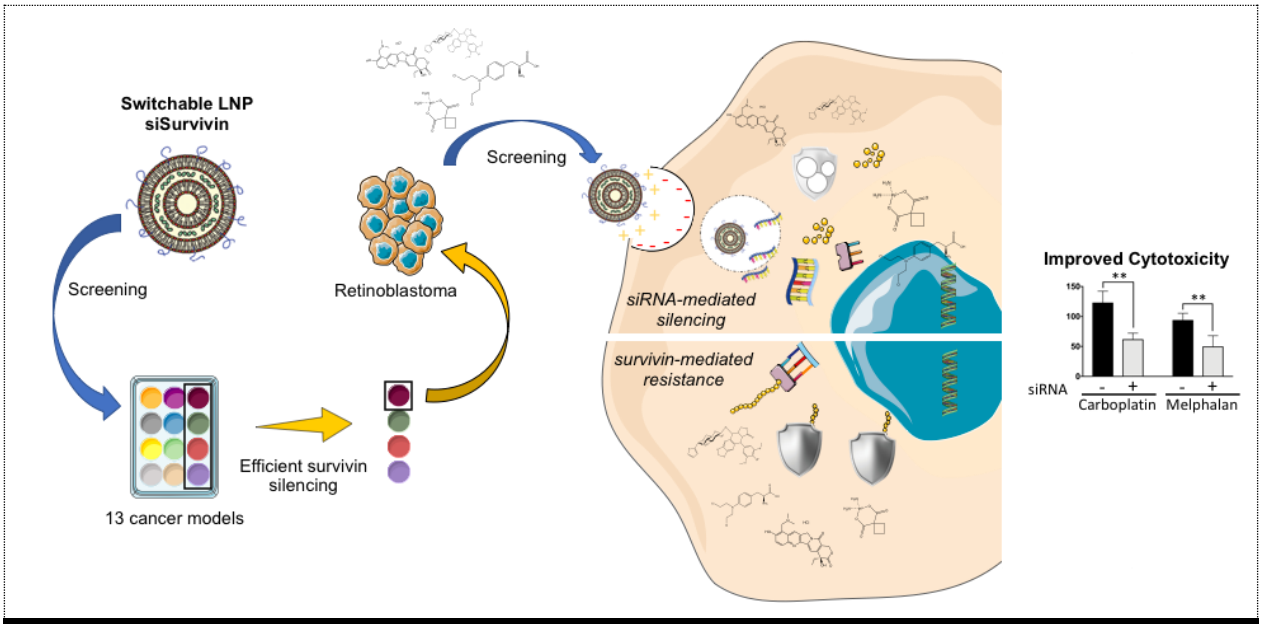


# *Survivin-targeted siRNA sensitizes retinoblastoma primary cells to Melphalan and Carboplatin*

*Victor Passos Gibson* [1], Rabeb Mouna Derbali[1], Houda Tahiri [2], Pierre Hardy[2], Jeanne Leblond Chain*[3]

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Survivin is one of the most cancer-specific targets discovered (1). Clinical trials based solely on survivin inhibitors failed in reaching their primary endpoints. Therefore, efforts have been made to use survivin inhibition as an adjuvant strategy to improve the cytotoxicity of drugs and decrease survivin-induced cancer resistance. **Purpose**: We aim to deliver a survivin-targeted siRNA (siSurv, 20 nM) using cationic switchable lipid nanoparticle (LNP), and evaluate its efficacy as a pre-treatment of chemotherapies. Once taken up by cells, the switchable LNP undergoes a fast pH-triggered conformational change, enabling membrane destabilization and cytosolic delivery of siRNA (2) (**Figure 1**). **Results**: siSuvr-loaded switchable LNP Survivin efficiently downregulated survivin mRNA and protein levels to an extent compared to Lipofectamine RNAiMAX® 48 hours after transfection in human retinoblastoma cells (Y79). Survivin silencing, followed by drug treatment synergistically improved the cytotoxicity of Carboplatin (CI = 0.87) and Melphalan (CI = 0.75), but not Topotecan, in Y79 and primary RB cells. **Conclusion**: Taken together, our investigation unveils Switchable LNP as an efficient siSurv delivery carrier to immortalized and primary RB cells.

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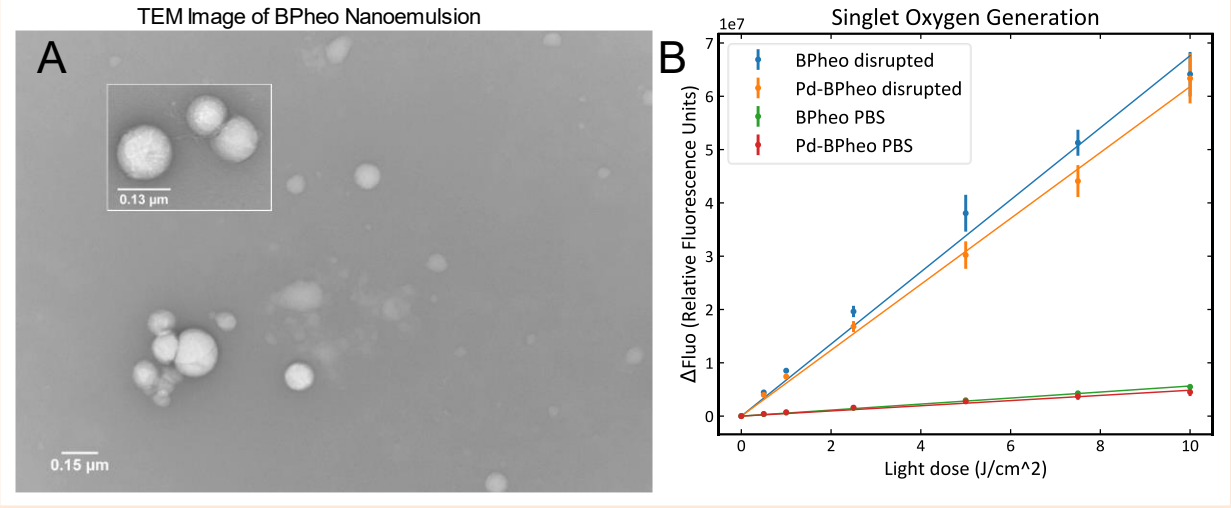


# Bacteriopheophorbide nanoemulsions as photodynamic therapy agents

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Bacteriopheophorbides (BPheo) make up a family of compounds which exhibit ideal photophysical properties for use in photodynamic therapy. They boast exceptionally high extinction coefficients, near-infrared absorption peaks, and high singlet oxygen quantum yields.[1] However, these compounds have poor water solubility, causing them to aggregate and rapidly clear from circulation. For example, Pd-BPheo, the most heavily investigated BPheo, has a 20-minute circulation half-life in humans and is thus unable to extravasate and accumulate in tissues of interest.[2] We aim to create novel nanoemulsions of these agents, exploiting the amphiphilic nature of BPheo to accumulate at the oil-water interface of nanodroplets. In doing so, we hope to improve the circulation kinetics of the agents and allow for tumor uptake and accumulation.

The dense loading of BPheo at the oil-water interface also allows us to exploit the phenomenon known as self-quenching, whereby photosensitizer molecules in close proximity to one another become quenched. In their quenched state, the typically photoactive BPheo molecules generate 10-fold less singlet oxygen, as measured in an assay (**Figure 1B**). This renders the BPheo nanoemulsions "off-on" probes, whereby their therapeutic effect is silenced in circulation before becoming activated upon cellular uptake.

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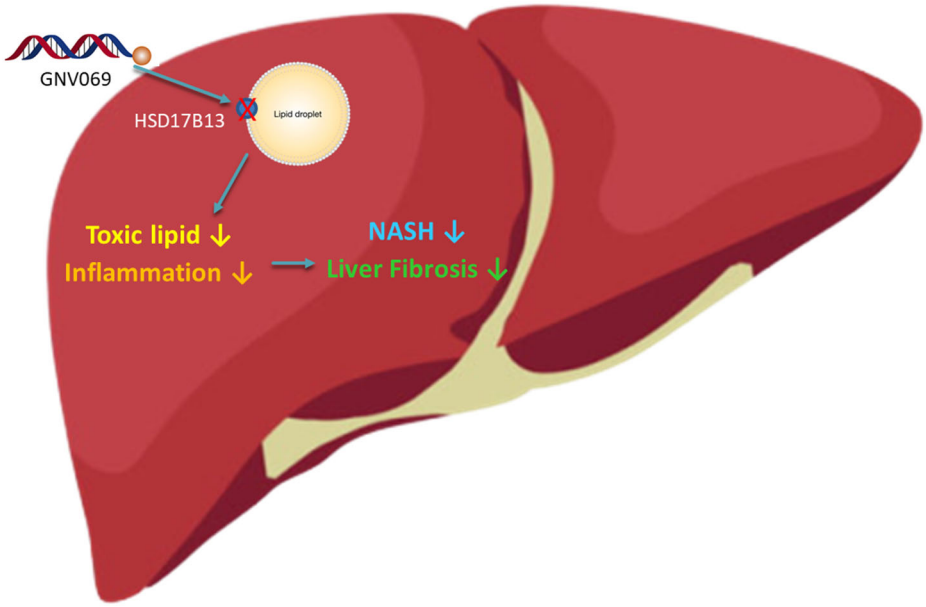



# Development of HSD17B13 Targeting siRNA-GalNAc as a Potential Therapy for Fibrosis in NASH

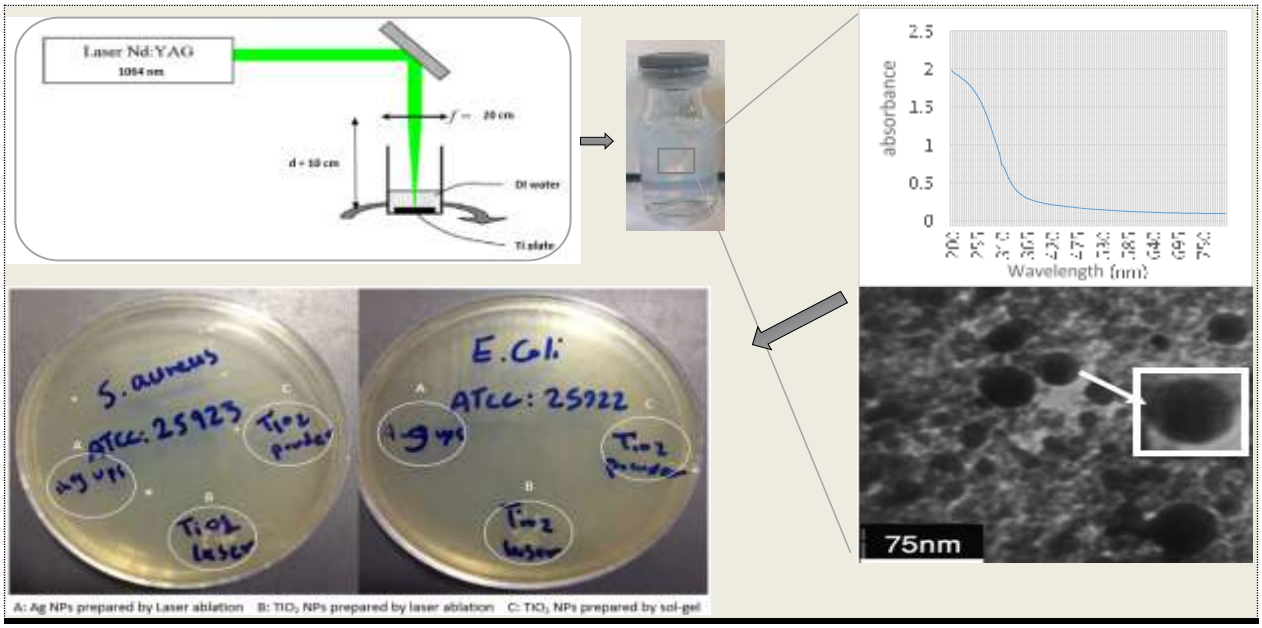
Xin Ye, Steven Tyler, Alice Li, Chris Pasetka, David Le, Mark Wood, Richard Holland, Christy Esau, James Heyes

Genevant Sciences Corporation, Vancouver, BC, V5T4T5, Canada

Nonalcoholic steatohepatitis (NASH) is one of the most common chronic liver diseases globally. It is characterized by steatosis (hepatic lipid accumulation), hepatocyte ballooning, lobular inflammation with increased risk of liver fibrosis. The progression of liver fibrosis to cirrhosis, hepatocellular carcinoma and end-stage liver disease is a key pathogenic factor for morbidity and mortality. Currently, no drug therapy has been approved for NASH management.

HSD17B13 is a lipid droplet associated protein that was recently identified as a potential therapeutic target for fibrosis in NASH. Human genetic studies have demonstrated that loss of function HSD17B13 mutants provide a protective effect against the development of liver fibrosis in the disease. We have therefore developed a siRNA-GalNAc conjugate, GNV069, to knockdown HSD17B13 as a potential therapy for liver fibrosis in NASH.

GNV069 comprises a potent siRNA that specifically recognizes and triggers the cleavage of the HSD17B13 mRNA and a proprietary GalNAc ligand that enables the effective delivery of this conjugate to hepatocytes through the ASGPR receptor. In vitro assessment demonstrated effective inhibition of HSD17B13 expression by GNV069 in both 2- and 3-dimensional culture of primary hepatocytes. An *in vivo* study in nonhuman primates (NHP) demonstrated 70% inhibition of HSD17B13 in the liver at Day 14 post a single 3 mg/kg dose. GNV069 was also well tolerated in both rat and NHP, providing a promising treatment for fibrosis in NASH.




# Comparison of the Antibacterial Effect of $Tio_2$ and Ag Nanoparticles Prepared by Laser Ablation and Sol-Gel on Burn Related Bacteria

Zahra Eivazi Zadeh [1], Atefeh Solouk [1], Asma Motamedi [2]

[1]Faculty of Biomedical Engineering, Amirkabir University of Technology (Tehran Polytechnic), Tehran, Iran [2]Photonic and Quantum Technologies Research School, Nuclear Science and Technology Research Institute, Tehran, Iran

Burn is one of the major types of injury, and their infections are the most damaging effects. One of the most common ways to inhibit the growth of pathogens and their entrance to the wound site is antibiotics. Overuse of antibiotics caused drug resistance and because of the high rate of mortality related to the low efficiency of antibiotics, it is among the major worldwide clinical problems (1). The use of smart wound dressing which is included antibacterial nanoparticles (NPs) is the newest and most promising method in burn infection control. Antibacterial NPs not only increase the efficiency of antibiotics, in some species affects the intracellular activity of pathogens and can control infection by this mechanism without any side effect (2).

In this study, a homogeneous solution of $TiO_2$ NPs with a diameter of 20-30 nm in deionized water was prepared by Nd:YAG laser. Its antibacterial properties, on Staphylococcus aureus and E.coli, was compared with rutile-$TiO_2$ NPs prepared by sol-gel purchased from Nano Pars Lima Co. Ltd Tehran, Iran, and Ag NPs prepared by laser ablation with the same diameter. Based on the results of antibiogram tests, laser-ablated $TiO_2$ NPs could not inhibit the growth of E.coli and Staphylococcus aureus, while the others greatly inhibit the growth of these bacteria colony. The main reason attributed to the amorphous structure of laser-ablated NPs, since antibacterial properties of $TiO_2$ is a photocatalytic procedure that only acts in their rutile structure. To conclude, although laser ablation is a simple method to prepare a homogeneous solution of NPs with small diameter, because of the fast preparation in the case of $TiO_2$, its crystalline structure is not good enough for antibacterial applications.

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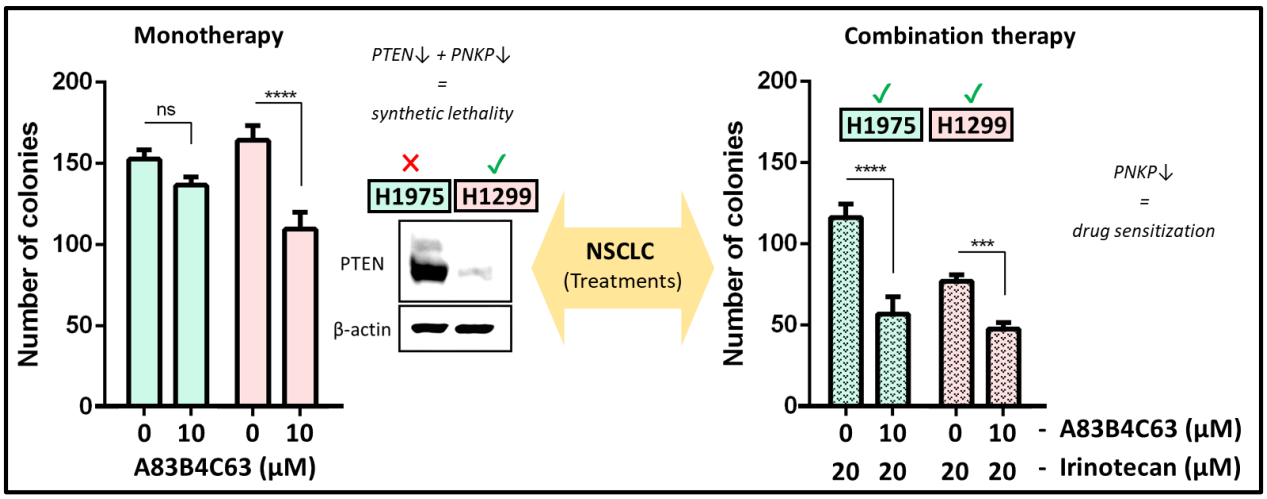


# Polymeric micelles of a novel inhibitor of DNA repair enzyme, polynucleotide kinase/phosphatase (PNKP), for targeted treatment of non-small cell lung cancer as monotherapy or in combination with Irinotecan

Igor M. Paiva[1], Sams Sadat[1], Mohammad R. Vakili[1], Feridoun K. Busheri[2], Marco Paladino[3], Dennis G. Hall[3], Michael Weinfeld[2], Afsaneh Lavasanifar[1].

*[1]Faculty of Pharmacy and Pharmaceutical Sciences; [2]Department of Oncology, Faculty of Medicine and Dentistry; [3]Department of Chemistry, Faculty of Science, University of Alberta, Edmonton, AB, Canada.*

Our research team has previously identified that the concomitant disruption of polynucleotide kinase/phosphatase (PNKP), an enzyme involved in DNA repair, and the tumor suppressor protein phosphatase and tensin homologue (PTEN), lead to synthetic lethality in different cancer models. Downregulation of PTEN is a frequent observation in non-small lung cancers (NSCLC), thus, pharmacological disruption of PNKP is expected to provide a viable and targeted mono-therapeutic strategy in NSCLC. Inhibition of PNKP can also make NSCLC more susceptible to cytotoxic effects of topoisomerase I inhibitors. Recently, the development of a potent PNKP inhibitor, namely A83B4C63, and its polymeric micellar formulations has been reported by our group [1]. The aim of the present study was to develop polymeric micelles of A83B4C63 modified on their surface with peptides targeting NSCLC cells. We also assessed the activity of A83B4C63 in NSCLC cell lines expressing different levels of PTEN expression as monotherapy or in combination with irinotecan. Polymeric micellar formulations modified on their surface with H2009 peptide have shown specific interaction with NSCLC cells overexpressing $\alpha_v\beta_6$-integrin. The PTEN positive NSCLC cells, i.e., H1975 cells, did not show loss of viability upon treatment with A83B4C63 monotherapy as shown by MTS and colony-forming assays. The PTEN-deficient H1299 cells, on the other hand, showed less growth and colony formation following treatment with A83B4C63 monotherapy. Treatment with A83B4C63 made both cells, more sensitive to Irinotecan. The sensitization effect of A83B4C63 upon combination therapy with irinotecan was significantly enhanced for H1299 over H1975 cells. The results imply a potential for polymeric micellar formulations of A83B4C63 as mono-therapeutics in PTEN deficient NSCLC cells and/or as targeted sensitizers to topoisomerase I inhibitors in NSCLC models.

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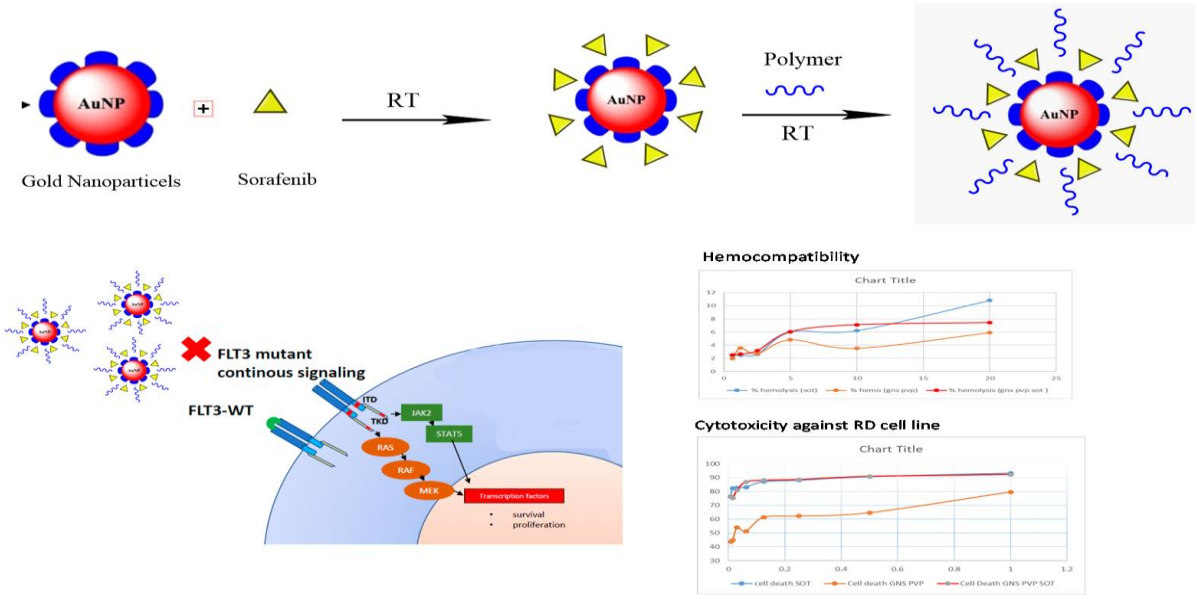


# Development of Polymer Capped Sorafenib Loaded Gold Nanoparticles for Treatment of FLT3 Positive Acute Myeloid Leukemia

[1,2]Nashmia Zia, [1]Logan Zettle, [1]Gilbert Walker*

[1]Department of Chemistry | University of Toronto, 80 St. George Street | Toronto, ON | M5S 3H6; *gilbert.walker@utoronto.ca; [2]Departemnt of Pharmacy university of Peshawar, Pakistan.

Acute myeloid leukemia (AML) is a heterogenous hematopoietic stem cell (HSC) neoplasm with poor prognosis especially in a subset of AML patients having activating mutation in the Fms-like tyrosine-3 (FLT3) gene. Sorafenib, a multikinase/FLT3 inhibitor, has shown its efficacy in AML+FLT3. But it's not very well tolerated in some patients and off-site side effects are the major limitation in continuous treatment [1]. Moreover, the dose needs to be adjusted to check for the tolerability of the patients towards the therapeutic agent [2, 3]. Nanoparticles have the ability to concentrate in the tumor cells. Loading of sorafenib on polymer capped gold nanoparticle allows for delivery of large amount of drug to the cancer cells and hence can prevent side effects produced due to exposure of normal tissues to high concentration of drug for prolonged periods of time.

Here we explored the therapeutic potential of polymer capped sorafenib loaded gold nanoparticles (GNS-Sot) in a panel of AML cancer cell lines that differ in their FLT3-mutation status and sensitivity to routine chemotherapeutic approaches. GNS-Sot was prepared by using bottom-up chemical synthesis method with high loading efficiency of 13.3 μg/mg of GNP's. The loading efficiency was confirmed by both HPLC and $^{19}$F NMR. Our preliminary results showed that GNS-Sot has a significant therapeutic effect in both AML cell lines as compared to free sorafenib or gold nanoparticles. Further stability studies and drug release studies also showed that GNS-Sot is a potential candidate for future in-vivo studies.

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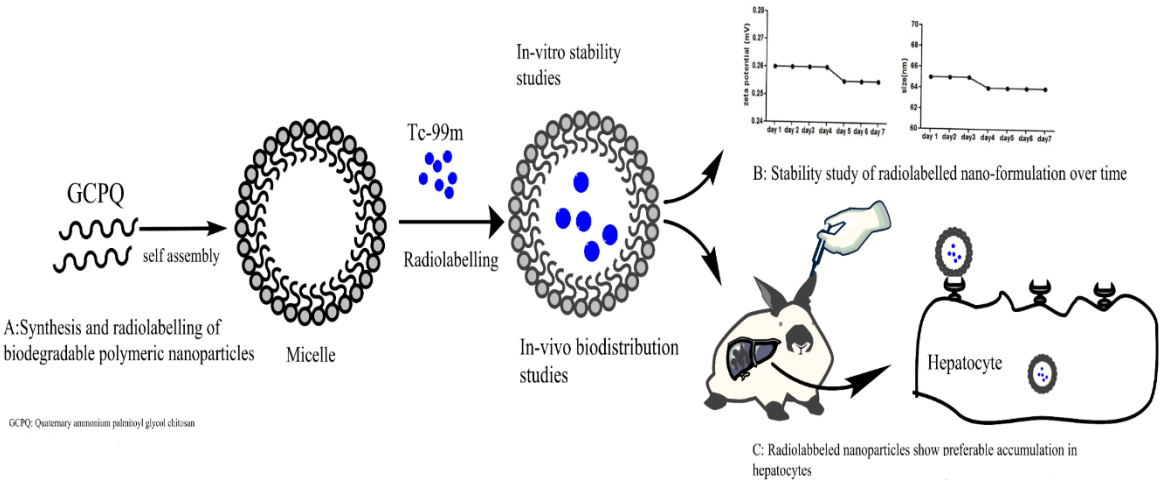


# In-vivo study of self-assembled glycol chitosan nano-radiopharmaceutical for liver imaging

Nashmia Zia [1,3], Aadarash Zia [2], Gilbert Walker [1] *

[1]Department of Chemistry | University of Toronto, 80 St. George Street | Toronto, ON | M5S 3H6; *gilbert.walker@utoronto.ca ; [2]Faculty of Pharmacy and Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville VIC 3052, Australia; [3]Departemnt of Pharmacy university of Peshawar, Pakistan.

Hepatocytes are the most abundant type of cells preset in liver. Major functions of liver are carried out by hepatocytes. The liver function can be estimated by evaluating the by-products, substrates and end products of these processes [1]. Single-photon emission computed tomography (SPECT) nuclear imaging is of the techniques that can be employed to quantify the spatial functional reserve of liver. There are SPECT tracers available which employ the use of human serum albumin-based compounds to quantify the functional status of hepatocytes, but they have their own limitations [2]. Use of nanoparticles as a SPECT tracer for liver imaging with biocompatible properties is a significant target in this field.

Here we have explored the development of a modified chitosan-based technetium labelled nanoparticle that can be used as SPECT radiotracer for liver imaging. The prepared nano-formulation has radiolabeling yield of more than 99%. It showed biocompatibility and high stability in normal saline and in human serum. The in-vivo biodistribution in rabbits showed optimum characteristics for liver imaging and images showed very good morphological resolution of liver. The radiotracer uptake profile was consistent with reversible binding kinetics and both modelling methods of analysis i.e. 2T and Logan were found to fit the data. Further confocal studies were done to verify the uptake of prepared radiopharmaceutical within hepatocytes.

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2. Yang, W., et al., *Copolymer-based hepatocyte asialoglycoprotein receptor targeting agent for SPECT.* Journal of Nuclear Medicine, 2011. **52**(6): p. 978-985.

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# FAST FACTS


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**Dr. Pieter Cullis**
Professor, Biochemistry & Molecular Biology, University of British Columbia

***Associate Scientific Director***

**Dr. Gilbert Walker**
Professor, Chemistry, University of Toronto

***Executive Director***

**Dr. Diana Royce**
Executive Director, NMIN


## VISION:

to establish and mobilize a **network** drawn from academia, industry, and other not-for-profit research enterprises to maintain and improve Canada's position as a **global leader in developing next generation nanomedicines**

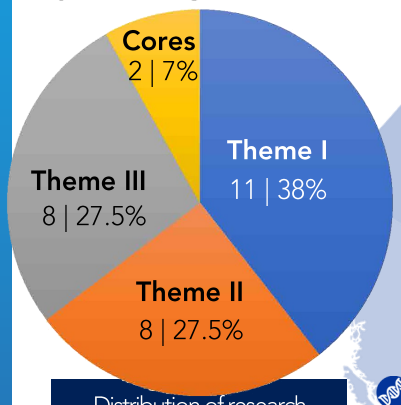
## MISSION:

to develop **novel therapeutics** to cure high-burden human diseases and new diagnostics to detect disease more precisely; to commercialize these products to **bring health and economic benefits to Canadians**; and to train the skilled workforce required by the growing nanomedicines industry


## FUNDING:

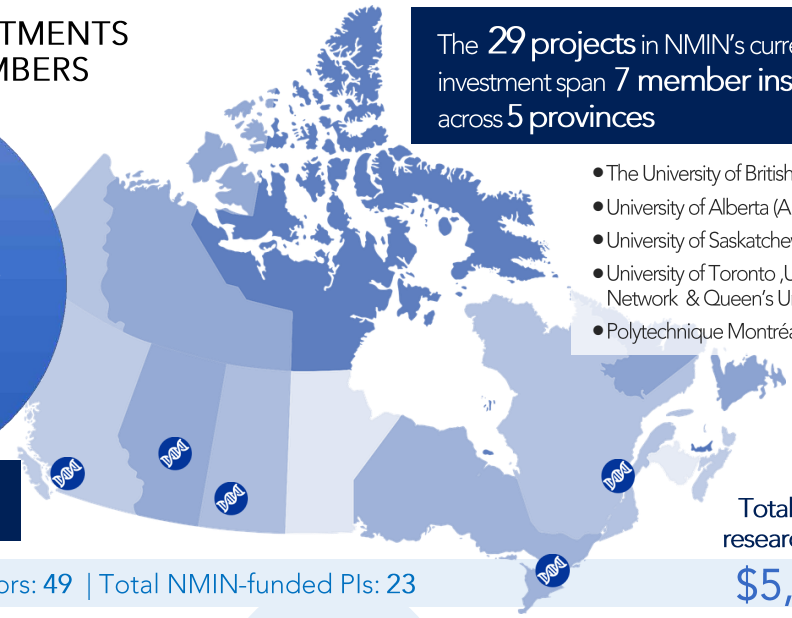
NMIN was awarded **$18,532,000** in funding over **6 years** (2019-2025) by the Government of Canada through the Networks of Centres of Excellence (NCE) Program.


## RESEARCH INVESTMENTS & NETWORK MEMBERS




Distribution of research investments ($) by Theme

The **29** projects in NMIN's current research investment span **7** member institutions across **5** provinces



- The University of British Columbia (BC)
- University of Alberta (AB)
- University of Saskatchewan (SK)
- University of Toronto ,University Health Network & Queen's University (ON)
- Polytechnique Montréal (QC)

Total current NMIN research investments

$5,662,561

Total NMIN investigators: 49 | Total NMIN-funded PIs: 23

## RESEARCH THEMES:

### Targeted Drug Delivery (Theme I)

Leaders: **Dr. Marcel Bally**, University of British Columbia
**Dr. Shyh-Dar Li,** University of British Columbia

### Enabling Gene Therapies (Theme II)

Leaders: **Dr. Pieter Cullis,** University of British Columbia
**Dr. Christian Kastrup**, University of British Columbia

### Diagnostics (Theme III)

Leaders: **Dr. Shana Kelley**, University of Toronto
**Dr. Gilbert Walker**, University of Toronto

## CORE FACILITIES:

### NANOCORE

*Nanomedicines Formulation and Characterization Core Facility*

Leader: **Dr. Pieter Cullis**
University of British Columbia

Co-leader: **Dr. Christian Kastrup**
University of British Columbia

### PHARMACORE

*Pharmacology/Toxicology and Scale-up Core Facility*

Leader: **Dr. Marcel Bally**
University of British Columbia

Co-leader: **Dr. Shyh-Dar Li**
University of British Columbia


**NMIN**

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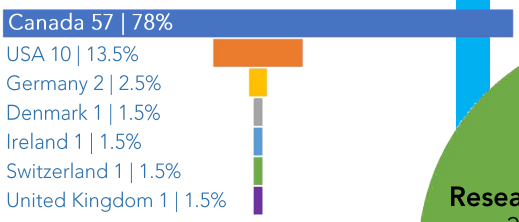
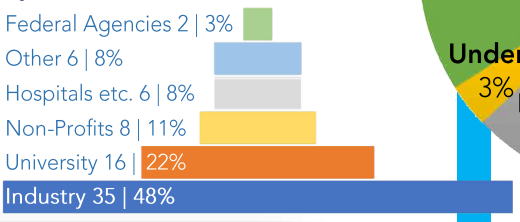
## PARTNERS

Total partner organizations: 73

### By country



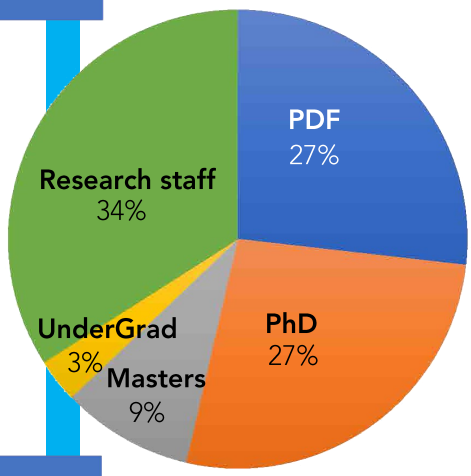

### By sector



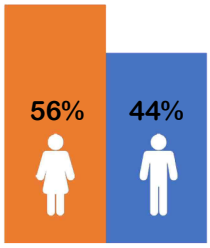

## HQP (working on NMIN projects)

Total NMIN HQP: 97

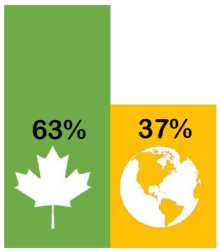
### By level




### By gender




### By nationality




HQP = Highly Qualified Personnel

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## ABOUT NMIN's CORE FACILITIES

### NANOCORE

*Nanomedicines Formulation and Characterization Core Facility*

MISSION: To develop high-quality, state-of-the-art lipid nanoparticles encapsulating small molecule or nucleic acid drugs that enable proof-of-concept (POC) animal studies

To standardize the physicochemical characterization in order to identify critical parameters

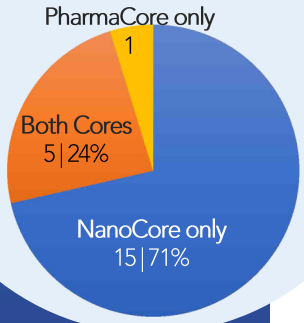
Formulation: High-quality, state-of-the-art nanoparticle formulations encapsulating small molecule, peptide or nucleic acid drugs that enable proof-of-concept (POC) animal studies.

Physiochemical characterization: Comprehensive portfolio of characterization assays including sizing & structure analyses that guarantee reliable interpretation of in vitro & in vivo studies & further optimization.

*No nanoparticle formulation will enter animal studies in NMIN without being rigorously characterized.*

**NMIN projects working with Core Facilities**

21 out of 29 projects | 78%




### PHARMACORE

*Pharmacology/Toxicology and Scale-up Core Facility*

MISSION: To help research partners develop promising nanomedicines and provide capabilities to advance new treatments from the bench to the clinic.

Capabilities: Pre-clinical in vitro, pre-clinical pharmacology, GLP-guiding safety, manufacturing


Contacts

NanoCore Dominik Witzigmann | Admin Lead | dominik.witzigmann@ubc.ca

PharmaCore Nancy Dos Santos | Admin Lead | ndossantos@bccrc.ca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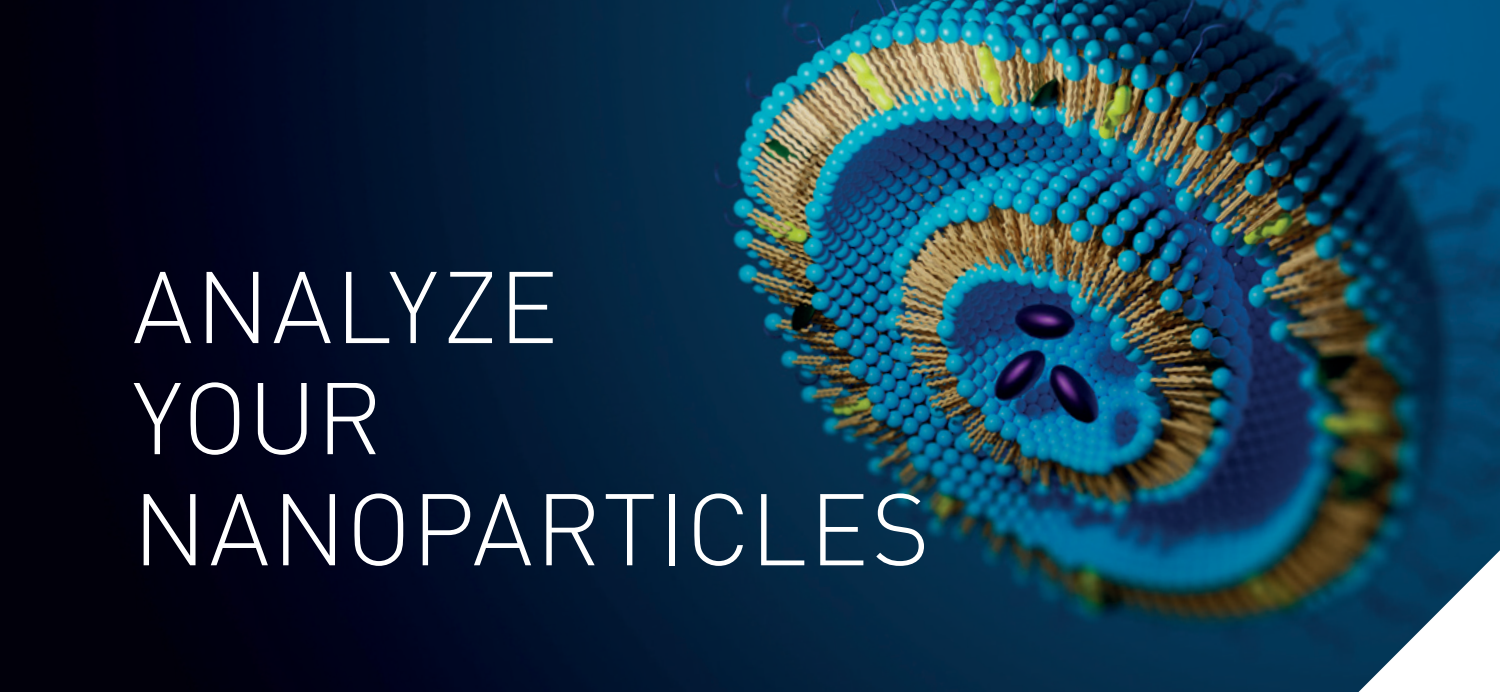

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- Urinary excretion kinetics

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- *In vivo* oral bioavailability / PK

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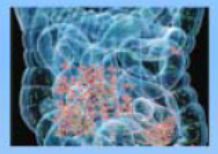

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