Bacteriopheophorbide nanoemulsions as photodynamic therapy agents

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Intro

- **Bacteriopheophorbides (BPheo) exhibit ideal photosensitizer properties: exceptionally high molar extinction coefficient, near infrared excitation wavelength, and large singlet oxygen quantum yields.[1]**
- **However, poor water solubility causes BPheo to rapidly aggregate and clear from circulation.**
- **We aim to create novel BPheo nanoemulsions (BPheoNE), exploiting the amphiphilic nature of BPheo salts to accumulate at the oil-water interface.**
- **Dense loading of BPheo at the oil-water interface can lead to an off-on probe as BPheo molecules in close proximity are self-quenched.**

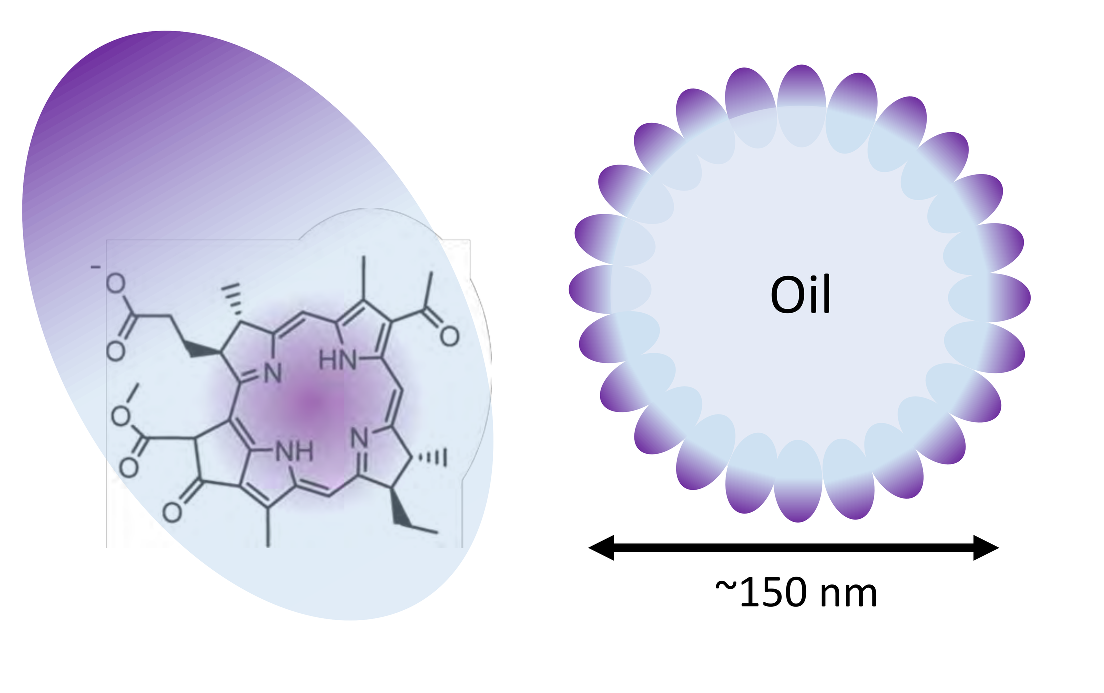


Fig. 1: Schematic representation of BPheo and nanodroplets.

Objective

Develop BPheo nanoemulsions in an attempt to improve the circulation kinetics of BPheo, while retaining the potent photoproperties of BPheo.

Methods

Characterization:

- **Dynamic light scattering (DLS)**
- **Transmission electron microscopy (TEM)**
- **Fluorescence spectroscopy/microscopy**
- **Singlet oxygen sensor green assay**
- **Blood sampling**

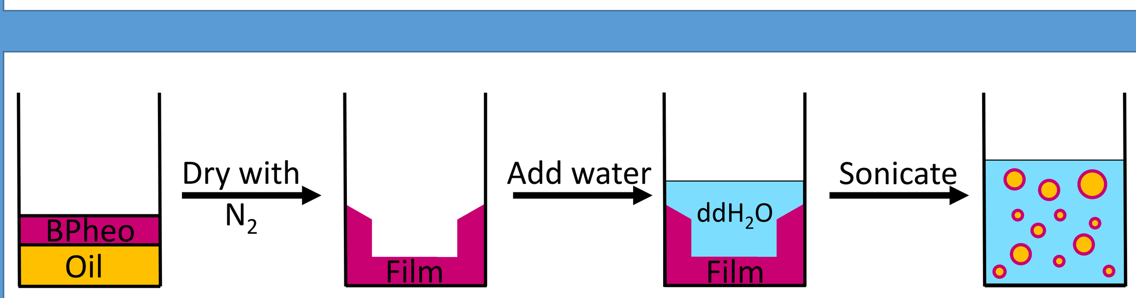


Fig. 2: BPheo nanoemulsion formation method.

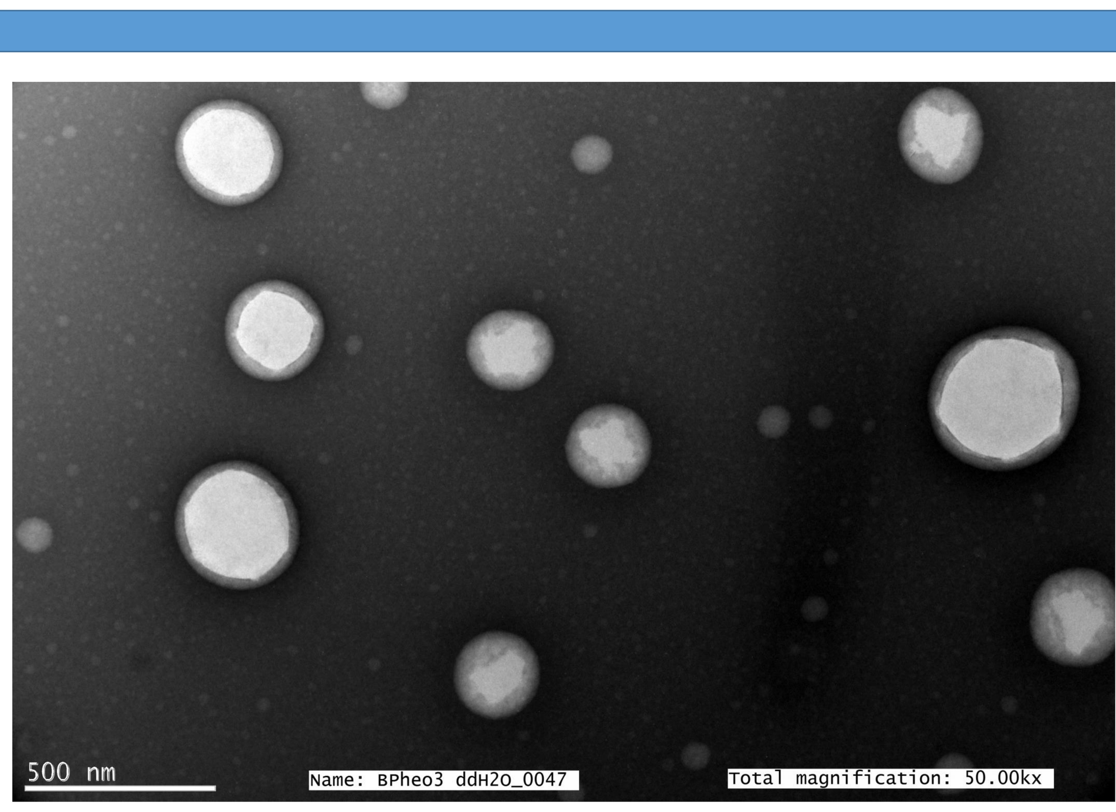


Fig. 3: TEM imaging of BPheo nanodroplets in ddH_2O

Results

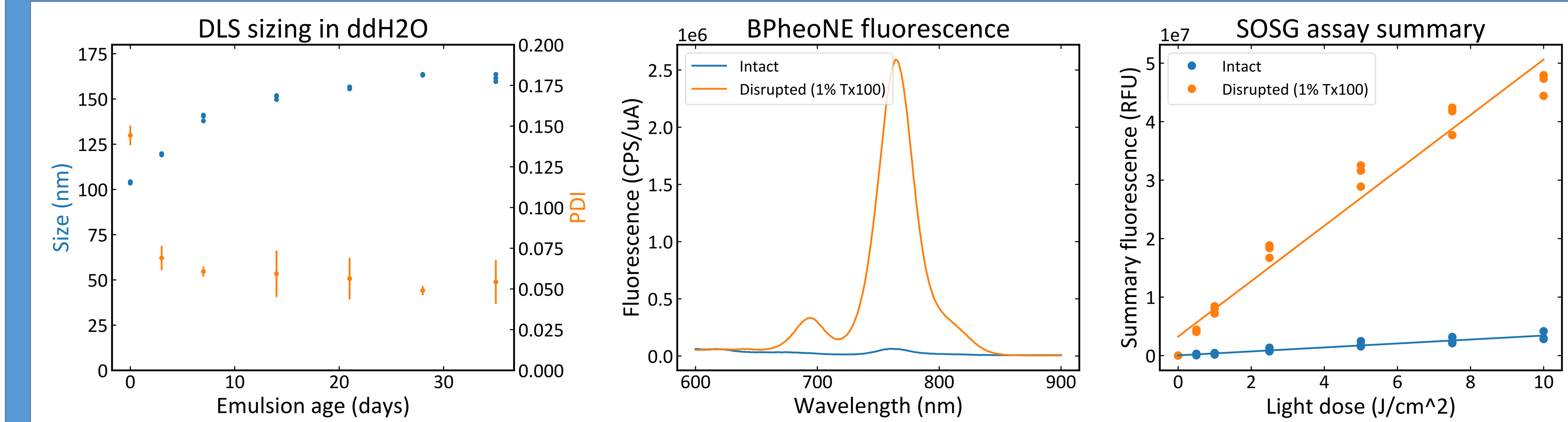


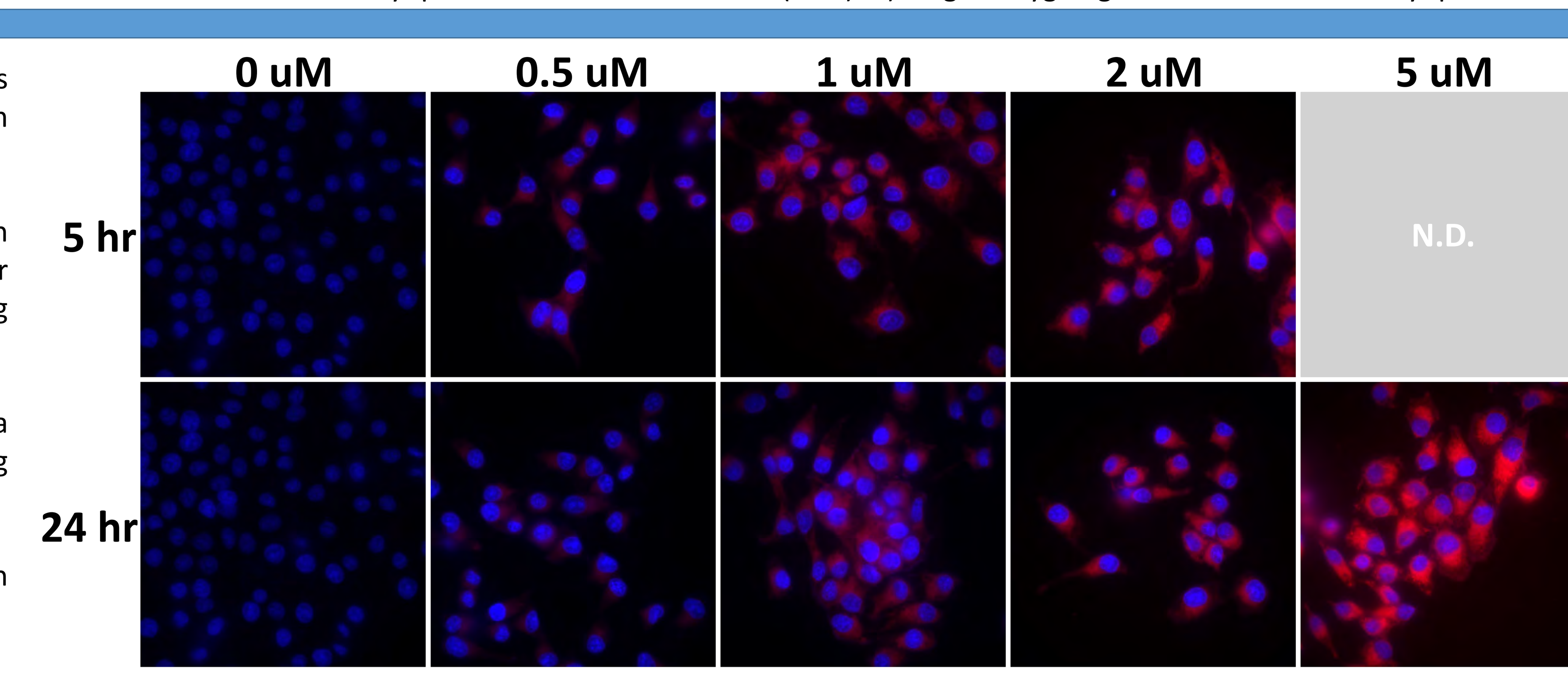
Fig. 4: A) Droplets are stable in storage. B) BPheoNE fluorescence is heavily quenched in the intact state (90%). C) Singlet oxygen generation is also heavily quenched.

Fig. 5: Fluorescence imaging of KB cells after incubation with BPheoNE (708/70 nm ex., 788/20 nm em.)

No significant differences were observed in fluorescence intensity when comparing 5 hr and 24 hr incubation periods, indicating rapid cellular uptake.

Fluorescence intensity increases as a function of drug concentration, indicating concentration-dependent cellular uptake.

Hoechst 33258 was used as nuclear stain (387/11 nm ex., 447/60 em.)



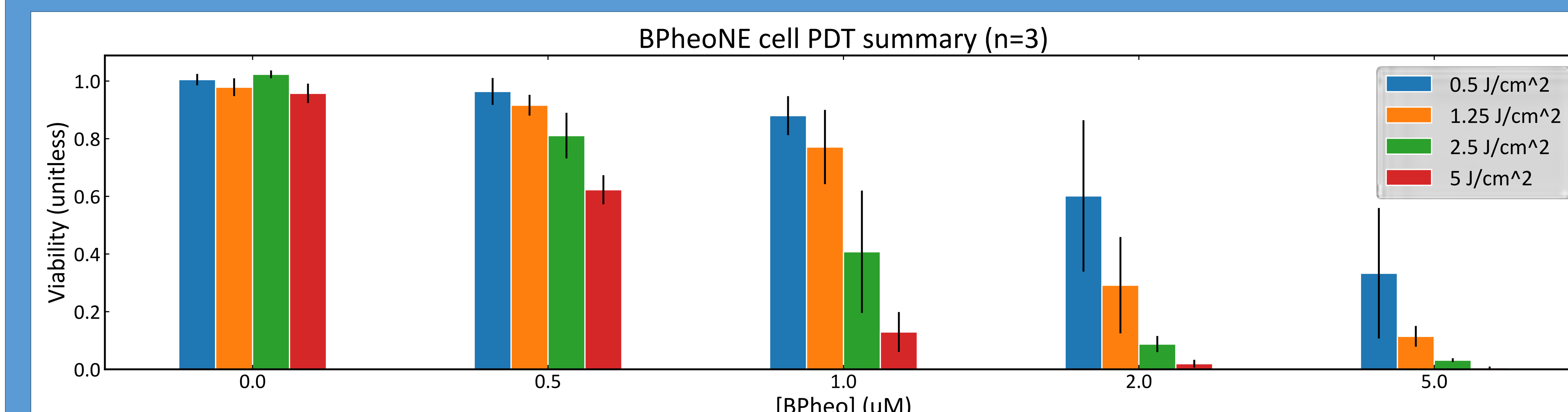


Fig. 6: *In vitro* PDT of KB cells after 24 hr incubation with BPheoNE. Drug and light dose-dependent behavior was observed. 750 nm laser, 50 mW/cm².

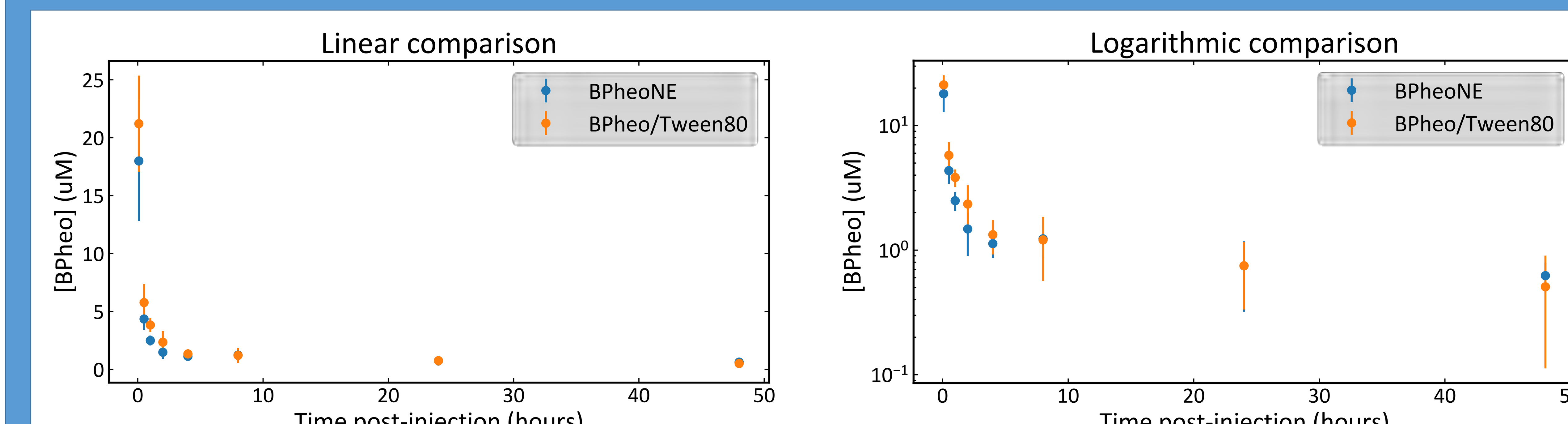


Fig. 7: Blood sampling studies in Balb/c mice showed that BPheo is rapidly cleared from circulation, with no significant difference in pharmacokinetics between BPheoNE and a micellar formulation (BPheo/Tween80).

Conclusions and future work

- **BPheoNE droplets are monodisperse and stable in storage.**
- **Fluorescence and singlet oxygen are both heavily quenched in the intact state.**
- **BPheoNE is rapidly taken up by cells.**
- **Treated cells are very sensitive to PDT.**
- **Circulation kinetics of BPheo are unimproved in the BPheoNE formulation.**
- **Future work will focus on improving the circulation kinetics of BPheoNE via surface modifications of the droplets.**

Acknowledgements

Nano Med Fab · ZHENG LAB Molecular Imaging and Nanomedicine http://zhenglab.utoronto.ca/ · Medical Biophysics UNIVERSITY OF TORONTO · UHN

[1] Y. Vakrat-Haglili et al., J. Am. Chem. Soc., 2005.