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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.**

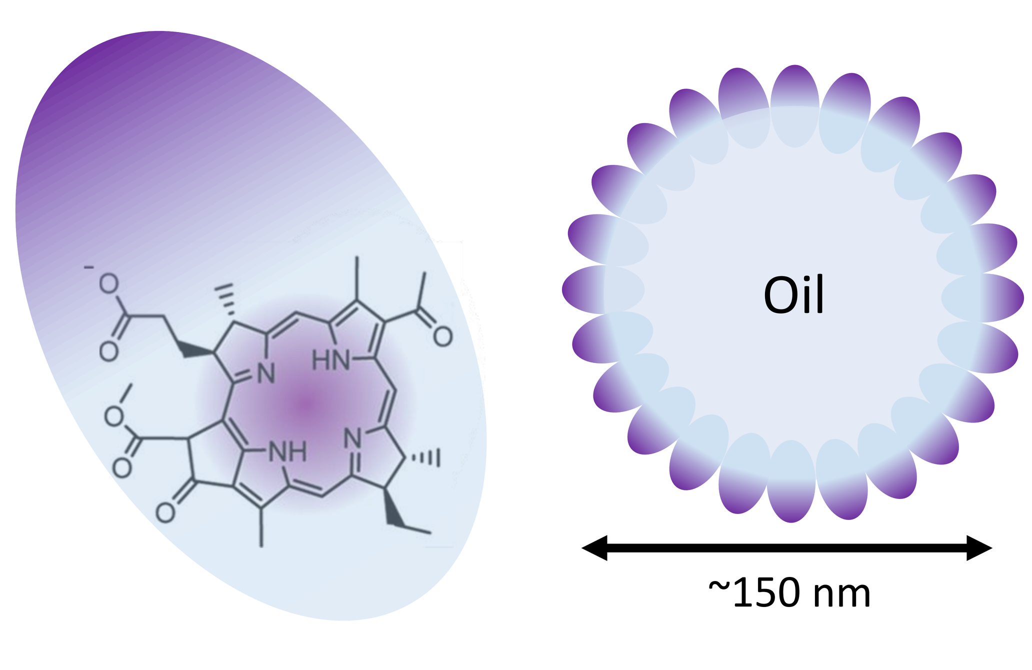

Fig. 1: Schematic representation of BPheo and nanodroplets.

Objective

Develop BPheo nanoemulsions and characterize their colloidal and photophysical properties in solution.

Methods

Characterization:

- **Dynamic light scattering (DLS)**
- **Transmission electron microscopy (TEM)**
- **Fluorescence spectroscopy**
- **UV-Vis spectroscopy**
- **Singlet oxygen sensor green assay**

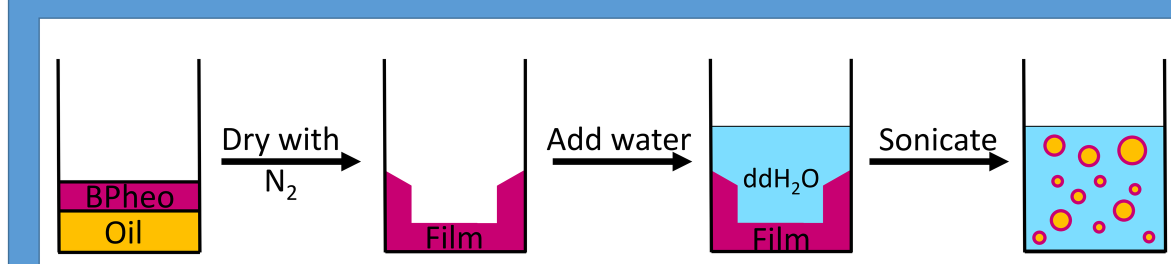

Fig. 2: BPheo nanoemulsion formation method.

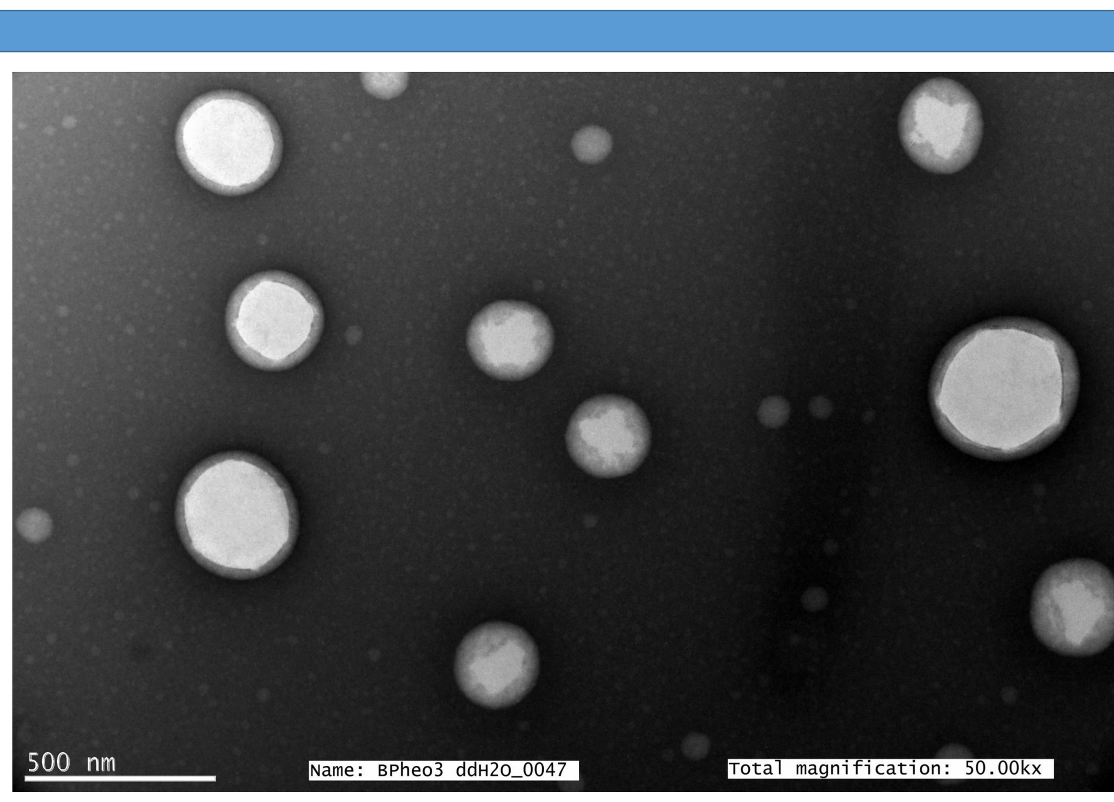

Fig. 3: TEM imaging of BPheo nanodroplets in ddH_2O

Results

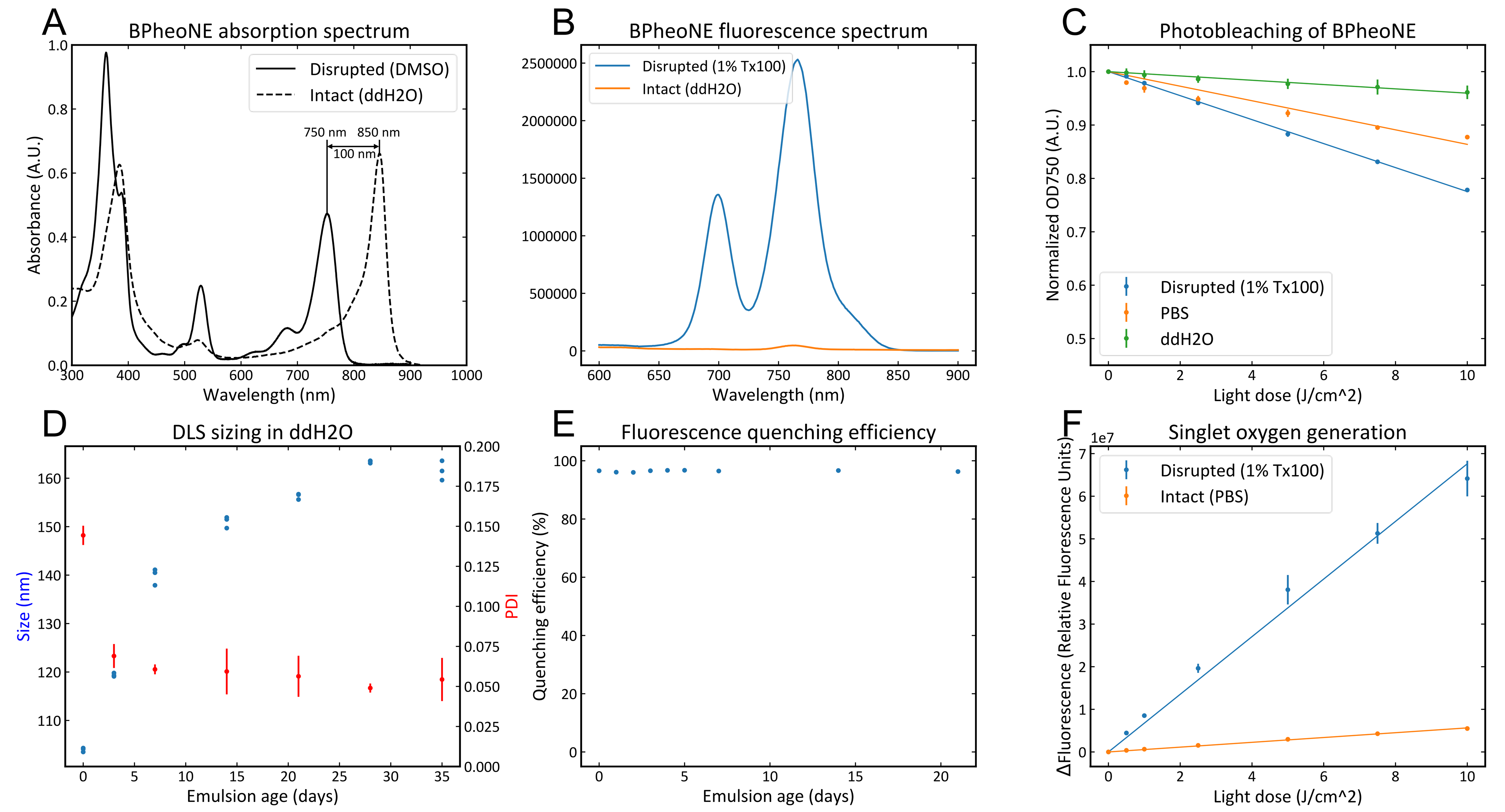

Fig. 4: A) Absorption spectrum of BPheoNE is red-shifted by 100 nm in ddH2O. B) BPheoNE fluorescence is 95% quenched in the intact state. C) BPheoNE is significantly more resistant to photobleaching than BPheo monomers. D) BPheoNE is colloidally stable in ddH2O. E) Fluorescence quenching of BPheoNE is stable over time. F) Singlet oxygen generation is reduced 10-fold in the intact state.

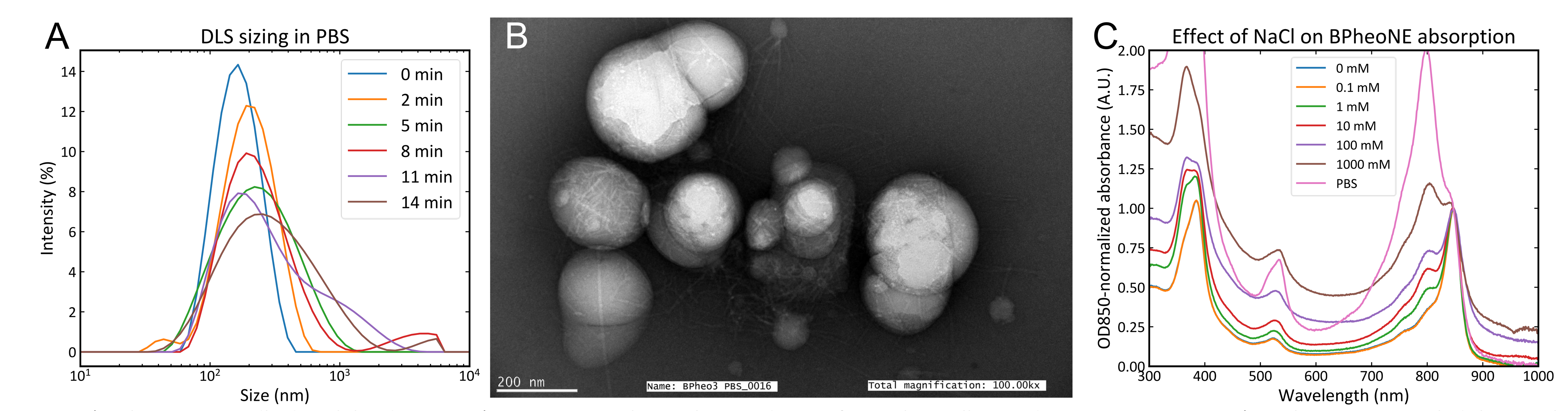

Fig. 5: A) BPheoNE is rapidly destabilized in PBS. B) TEM imaging shows clear evidence of particle swelling and aggregation in PBS. C) NaCl titration revealed that NaCl concentrations as low as 1 mM can induce spectral changes in BPheoNE, however NaCl alone does not account for all the changes seen in PBS.

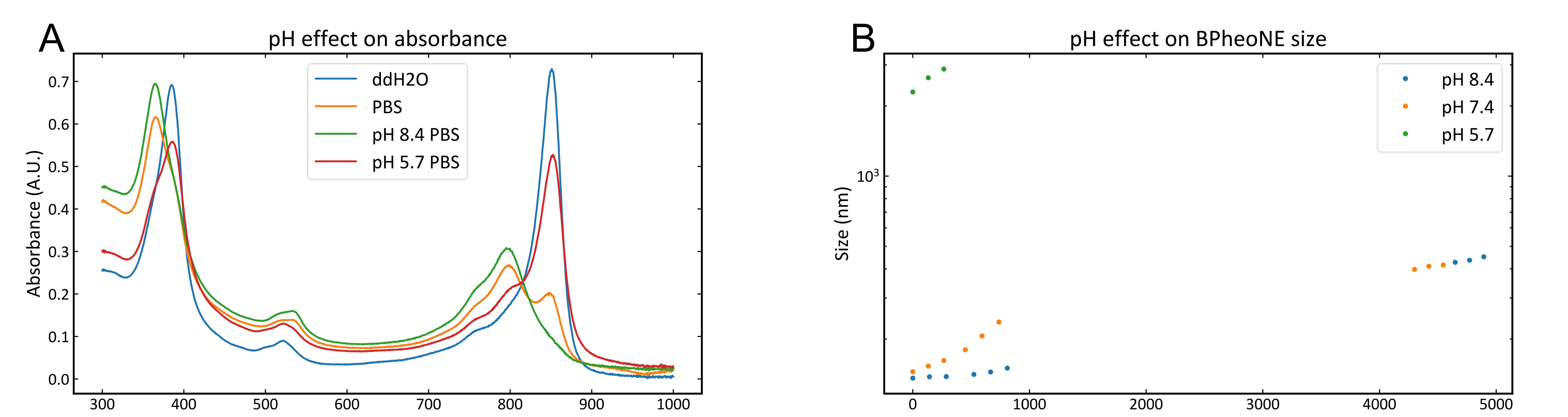

Fig. 6: A) In mildly acidic pH, BPheoNE spectrum is dominated by a peak at 850 nm. In alkaline conditions, the spectrum is broader and dominated by a peak at 800 nm. At neutral pH, the spectrum is mixed. B) BPheoNE is unstable in PBS, regardless of pH. However, BPheoNE is especially unstable in acidified PBS.

Conclusions and future work

- **Fluorescence of BPheoNE is 95% quenched in the intact state.**
- **Photobleaching and singlet oxygen generation are heavily quenched.**
- **BPheo nanoemulsions are stable in ddH_2O, but they are rapidly destabilised in PBS.**
- **Destabilization is largely caused by increases in ion concentrations, due to the charge-screening effect.**
- **Changes in pH induce spectral changes in the emulsion.**
- **Future efforts are focused on improving the nanoemulsion stability in PBS by surface optimizations, such as adjusting zeta potential, lipid modification and PEGylation, before moving on to biological applications.**

Acknowledgements

Nano Med Fab
ZHENG LAB Molecular Imaging and Nanomedicine
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NMIN
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[1] Y. Vakrat-Haglili et al., J. Am. Chem. Soc., 2005.