The impact on protein and enzyme encapsulation in self-assembled nanoparticles synthesized via photo-polymerization

*Irma G. Vermeijlen, Will Anderson, Gayathri R. Ediriweera, Nicholas L. Fletcher, Craig A. Bell, and Kristofer J. Thurecht**

Building 57, University Dr. / Building 75, Cnr College Rd & Cooper Rd
Centre for Advanced Imaging, Australian Institute for Bioengineering and Nanotechnology
St. Lucia, Queensland, Australia
i.vermeijlen@uq.edu.au, k.thurecht@uq.edu.au

Biological therapeutics are a promising approach for a range of diseases, however delivery of active biologics to the site of action remains challenging. Previously, PEGylated conjugates of biological therapeutics have been synthesized to improve *in vivo* stability. Alternatively, encapsulated therapeutics show great potential for targeted drug delivery without the need for covalent binding or chemical modifications.[1] This project aims to engineer benign production methods to reproducibly construct semi-permeable nanoparticles (NPs) for protein and enzyme encapsulation, with the ultimate goal to improve biocompatibility, biological half-life, and stability while maintaining function and activity.

Photo polymerisation-induced self-assembly (photo-PISA) has gained attention as a versatile one-pot self-assembly method to combine synthetic amphiphilic block copolymers and biomolecules for various applications. The most significant advantage of photo-PISA is synthesizing polymeric nanostructures of higher-order morphologies under benign conditions (i.e. aqueous, room temperature) without compromising the function of biological therapeutics. We tuned reaction conditions within a straightforward one-step photo-PISA, using PEG-based starting material, a water-soluble photoinitiator, and custom-built LED photoreactor.

Analysis of the results showed that self-assembly can be controlled to form micelles, worms, and polymersomes, even in the presence of additives, such as Bovine Serum Albumin or *L*-Asparaginase (**Fig. 1**). We then expanded this approach to encapsulate radiolabelled biologics to probe the *in vivo* behaviour and delivery of payloads, showing the versatility and robustness of this photo-PISA system, enabled by the mild assembly conditions.

We have shown that it is important to use photo-PISA in combination with controlled reaction conditions, achieved through our custom photoreactor, as a critical tool to reproducibly synthesize protein loaded NPs and effectively deliver this protein cargo to areas of therapeutic or enzymatic benefit. Future research will focus on understanding the impact of encapsulation on enzyme activity and stability and continue to study the synthesized NPs biodistribution.

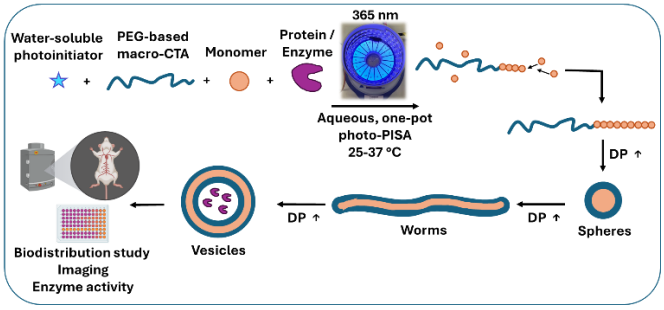

Figure 1: Schematic image of morphological transition during photo-PISA. Starting from the hydrophilic PPEGMA macro-CTA and free HPMA monomer in solution to the growth of the hydrophobic HPMA block and self-assembly into micelles, worms, and polymersomes with encapsulated proteins/enzymes.

References:

[1] Blackman, L.D.; et al. *ACS Cent Sci.* **2018**, *4 (6),* 718-723.
[2] Tan, J.; et al. *ACS Macro Lett.* **2015**, *4 (11),* 1249-1253.