

## Recombinant Polypeptide Nanoparticles and Synthetic Polymer Brushes for Biomaterial Applications

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This lecture will cover developments in my laboratory over the past decade with relevance to the molecular design of Biomaterials. I will focus on two areas of my research: (1) the genetically encoded synthesis of stimulus-responsive recombinant biopolymers and their self-assembly into nanoparticles for drug delivery; and (2) the design of protein- and cell-resistant polymer brushes from macroscopic planar surfaces and from the surface of proteins.

In the first part of my talk, I will illustrate the capacity of stimulus-responsive elastin-like polypeptides (ELPs) to self-assemble into nanostructures in response to a range of stimuli. In one example of this behavior, we designed a chimeric polypeptide that consists of two segments: an ELP segment that consists of  $(VPGXG)_n$  repeats (where  $n$  ranges from 60-150) followed by a short  $(GGY)_8$  segment, and showed that attachment of multiple copies of a hydrophobic molecule at the Y position can impart sufficient amphiphilicity to the polypeptide and thereby drive its self-assembly into near-monodisperse nanoparticles with the attached hydrophobic small molecule embedded in the core of the nanoparticle. This is an interesting finding, because it appears that any molecule with a hydrophobicity that is greater than a threshold value appears to drive attachment-triggered self-assembly of the chimeric polypeptide into a nanoparticle. Because many cancer chemotherapeutics are insoluble hydrophobic small molecules with poor bioavailability, this approach of attachment-triggered encapsulation of small hydrophobic molecules into soluble nanoparticles has great utility to increase the solubility, plasma-half-life and tumor accumulation of cancer chemotherapeutics. As a specific example, I will show how conjugation of multiple copies of the cancer chemotherapeutic Doxorubicin (Dox) via a pH-sensitive linker to the end of an ELP spontaneously triggers ELP self-assembly into near-monodisperse micelles. These nanoparticles are ~40 nm in diameter, release drug at pH 5.0 (relevant to endolysosomal release), are taken up by cells, show subsequent localization of the drug to the nucleus, and are cytotoxic. Notably, these Dox-loaded nanoparticles have a four-fold higher maximum tolerated dose than free drug and induce near complete tumor regression in a murine cancer model following a single dose.

In a parallel line of investigation, we have also designed diblock ELPs that self-assemble into monodisperse micelles in response to a thermal trigger, other variants that can disassemble in response to a pH trigger, and others that are stabilized by metal ion chelation within the core of the micelle. This family of self-assembling ELPs provides rich opportunities for application in biotechnology and medicine.

In the second part of my talk, I will discuss the *in situ* synthesis of nanometer thick brushes of an oligoethyleneglycol-functionalized polymer, poly(oligo(ethylene glycol) methyl ether methacrylate) (poly(OEGMA)), by surface-initiated atom transfer radical polymerization (ATRP) from macroscopic, planar surfaces. These oligoethyleneglycol-functionalized polymer brushes are useful for the fabrication of protein microarrays, as their resistance to adventitious adsorption of proteins allows us to assay for analytes from serum and from whole blood down to the femtomolar concentration level.

I will then describe the consequences of scaling down the surface—from a macroscopic planar surface to the molecular surface of a protein—on which the same polymer is grown, and its consequences on the *in vivo* behavior of protein-POEGMA conjugates. In this context, I will discuss two new and general routes to grow a POEGMA chain solely from the N-terminus or C-terminus of a protein by *in situ* ATRP under aqueous conditions, to yield site-specific (N- or C-terminal) and stoichiometric conjugates (1:1) with low polydispersity and high yield. Notably, both a myoglobin-poly(OEGMA) conjugate (N-terminal conjugate) and a green fluorescent protein conjugate (C-terminal conjugate) showed a 40-50 fold increase in their blood exposure compared to the unmodified protein after intravenous administration to mice, thereby demonstrating that comb polymers that present short oligo(ethylene glycol) side-chains are a new class of PEG-like polymers that can significantly improve the pharmacological properties of proteins. We believe that this new approach for the synthesis of N/C-terminal protein conjugates of poly(OEGMA) may be applicable to a large subset of protein and peptide drugs, and thereby provide a general methodology for improvement of their pharmacological profiles.