Hierarchically Structured Protein Hydrogels for Controlled Nanoparticle Release
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**Statement of Purpose:** One of the overarching challenges in drug delivery is the ability to control and sustain cargo release. A number of strategies have been employed to address this issue, ranging from controlled degradation to engineered permeability (1). Here, we present a simple method with potential for controlling and sustaining the release of molecularly-loaded protein nanoparticles via the design of hierarchically structured elastin-like polypeptide (ELP) hydrogels. This strategy enables prolonged release of drug delivery vehicles that in turn can take advantage of the enhanced permeability and retention effect for treatment of solid tumors (2). These protein nanoparticles can also be genetically programmed to respond to various stimuli, which can be harnessed for downstream targeting using environmental triggers such as localized heating (3). The ability to precisely control the molecular makeup of the protein building blocks comprising these hierarchically structured hydrogels enables the design of advanced drug depots.

**Methods:** We designed several new ELPs capable of forming nanoparticles and crosslinking into hydrogel networks (Figure 1A and E). We created genes that encode for these proteins using recursive directional ligation by plasmid reconstruction. After expression in host E. coli cells and purification via inverse transition cycling, we triggered the formation of nanoparticles with temperature or conjugation of hydrophobic small molecules to cysteine residues. We verified nanoparticle formation with dynamic light scattering. Utilizing metal crosslinking sites displayed on the surface of the nanoparticles, we fabricated hydrogels via the addition of Zn$^{2+}$ ions and characterized their microstructure with small-angle x-ray scattering. Finally, we evaluated the ability of hybrid hydrogels to modulate the release of nanoparticles using spectrophotometry.

**Results:** We engineered diblock protein polymers that self-assemble into monodisperse nanoparticles with a hydrodynamic radius of $\approx 50$ nm upon heating (Figure 1B). When assembled, these nanoparticles display a large number of zinc-binding motifs (based on the catalytic domain found in matrix metalloproteinases) that we use to crosslink these nanoparticles to form hydrogels by addition of Zn$^{2+}$ ions and characterized their microstructure with small-angle x-ray scattering. Finally, we evaluated the ability of hybrid hydrogels to modulate the release of nanoparticles using spectrophotometry.

**Conclusions:** We describe a method for fabricating reversibly crosslinked hydrogels built of protein nanoparticles. We extend these hierarchically structured hydrogels to include hybrid, covalently crosslinked engineered proteins. By simply changing the Zn$^{2+}$ chemical potential between the hydrogel network and the surroundings, we show controlled and sustained nanoparticle release. We believe that nanoparticle release kinetics can be further tuned by controlling the concentration of covalently crosslinked engineered proteins, which effectively controls the covalent network mesh size.


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