Dynamic hydrogels based on changes in nanoscale protein assembly

William L. Murphy[1], Milan Mrksich[2].
University of Wisconsin-Madison[1], University of Chicago[2].

Statement of Purpose: Natural proteins perform a variety of functions in biological systems, including actuation, catalysis, structural support, and molecular sequestering. This variety of protein functions suggest that they could serve as valuable and versatile building blocks for synthesis of functional materials. Based on this premise, we have developed hydrogels composed of a functional, dynamic protein, calmodulin (CaM). Calmodulin has two distinct conformational states (Fig. 1). In the presence of calcium, CaM is in an extended conformation and is a dumbbell-shaped protein (PDB ID: 1CLL). Calcium-bound CaM undergoes a rapid transition from an extended dumbbell to a collapsed conformation in response to binding of ligands (PDB ID: 1CTR), which include the anti-psychotic drug trifluoperazine (TFP). We hypothesized that incorporation of CaM into a hydrogel network would enable its motion to be translated into a structural change in a polymer network, and consequently into a volume change in a dynamic hydrogel.

Methods: We prepared an engineered version of CaM that includes cysteine residues in place of tyrosine residues at the ends of the dumbbell-shaped protein (CaM Y34C, Y110C). The distance separating the two cysteine residues is approximately 50Å in the extended conformation but is reduced to approximately 15Å in the collapsed conformation. We incorporated these CaM building blocks into a hydrogel network using a photochemical approach. The engineered CaM was reacted with an excess of low molecular weight (575 Da) poly(ethylene glycol)-diacrylate (PEGDA) chains and purified to give linear, water soluble CaM molecules terminated on each end by PEG-acrylate moieties. These molecules were then cross-linked into a hydrogel network via exposure to UV irradiation (λ=365nm) in the presence of a photoinitiator (I2959, Ciba, Switzerland) to give a solid cylindrical hydrogel. Hydrogels were also prepared with varying ratios of PEGDA575 and CaM-PEG conjugates to create hydrogels with varying amounts of total % CaM incorporation, and the mass equilibrium swelling ratio of these gels was measured by characterizing their swollen vs. dry weight. These hydrogels were exposed to a solution of the CaM ligand TFP, and their mass and diameter were measured before and after ligand binding.

Results/Discussion: Our results indicate that protein-containing hydrogels can be formed via a photochemical crosslinking approach. The equilibrium swelling ratio of these gels is dependent on the amount of protein incorporated (Fig. 2b), and up to a 5mM total protein concentration has been included into these materials. The hydrogels undergo a pronounced volume change upon exposure to the TFP ligand, which binds to CaM and induces a change from an extended CaM conformation to a collapsed conformation (Fig. 2a,b). The magnitude of this conformational change is dependent on the total amount of protein incorporated into the hydrogel, as expected (Fig. 2d).

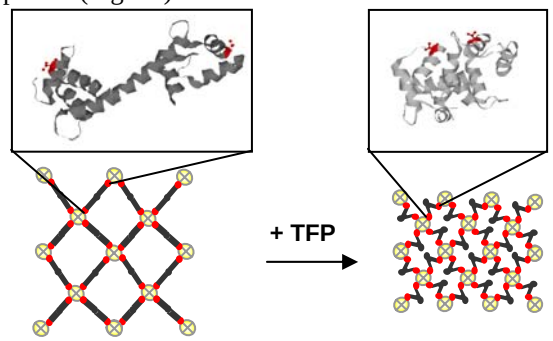

Figure 1. Schematic representation of the CaM conformational change, and the corresponding change in hydrogel network properties.

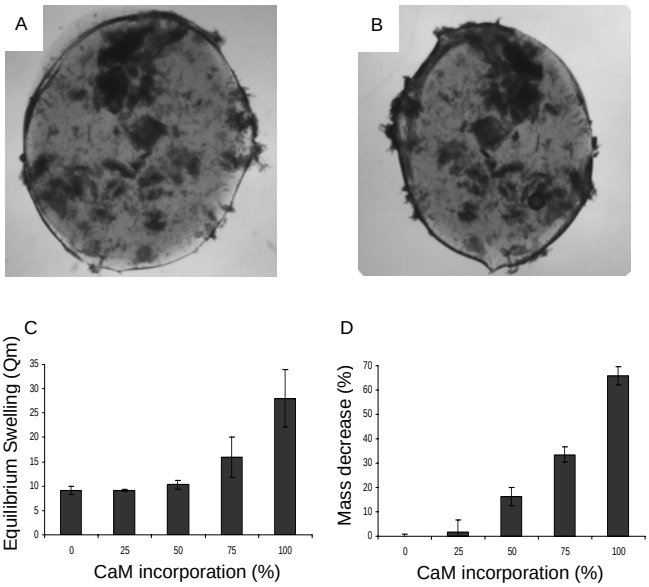

Figure 2. Hydrogels with 75% CaM included undergo a pronounced volume difference upon transfer from a ligand-free environment (a) to an environment loaded with the TFP ligand (b). c) Hydrogels can be prepared with a broad range of total protein incorporated, and d) the magnitude of the volume change is dependent upon the total amount of protein included into the hydrogel (% incorporation indicates mass % of CaM-PEG conjugate).

Conclusions: Our results indicate that it is possible to scale nanometer-scale protein conformational changes into macroscopic effects to generate dynamic, protein-based hydrogels. Ongoing studies are exploring the influence of hydrogel network assembly on response characteristics, and engineering these gels to respond to other biologically-relevant stimuli.