SUPPLEMENTARY INFORMATION

Genetically Engineered Protein in Hydrogels Tailors Stimuli-Responsive Characteristics
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Parametric Studies

Hydrogels with 0.1, 0.5, 1, and 2% (w/w) quantities MBAA crosslinker relative to AAm were synthesized and examined. As expected, hydrogel swelling increased by reducing the crosslinker percentage (Table S1). It should be noted that the hydrogel with 0.1% crosslinker was quite fragile providing very low mechanical stability for the experiment. Varying the quantity of calmodulin (CaM) immobilized in the hydrogels was also investigated with hydrogels prepared with 1% (w/w) crosslinker relative to monomer. Hydrogels with CaM quantities of 1, 3, 6, and 9 nmol/100 mg monomer (keeping a 1:1 ratio of CaM: ligand) each produced a swelling response. The data suggested that there was a minimum protein quantity of 3 nmol/100 mg monomer needed to achieve maximal displacement, and additional quantities of CaM over that threshold had little effect on the overall swelling response of the hydrogel.

CaM-Containing Hydrogel (no phenothiazine derivative)

To investigate whether the CaM hydrophobicity influences the extent of hydrogel swelling, a stimuli-responsive hydrogel was prepared containing C-terminal allylamine moiety-modified CaM as the only covalently immobilized component within the acrylamide network (3-[2-(trifluoromethyl)-10H-phenothiazin-10-yl]propan-1-amine was not included in this formulation). Experiments showed that the hydrogel produced an initial minimal swelling response (≤20 μm) in the presence of free CPZ. This trend was expected because of the absence of the immobilized 3-[2-(trifluoromethyl)-10H-phenothiazin-10-yl]propan-1-amine binding ligand. Thus, the associated relaxation of the hydrogel network shown in Fig. 1d is not available. As expected, this hydrogel did swell reversibly when a conformational change was induced in CaM by removing Ca²⁺ from its binding sites (Fig. S1; black vs
hatched bars). In the presence of EGTA, the CaM underwent a conformational change to a more extended structure. This conformational change modifies the hydrophobic surface of CaM, lowering its hydrophobicity. The hydrophobic surface percentages (generated using QuaSAR molecular descriptions by MOE v2003.020 were calculated to be 45.4%, 47.2%, and 45.8% for Fig. 1a, b, and c, respectively. As a result, the water uptake of the polymer is altered, producing the observed hydrogel displacement. Addition of Ca\textsuperscript{2+} reversed the swelling causing an average displacement of $178 \pm 60 \, \mu m$ (n=5). The swelling attributed to the conformational change of CaM was consistent with the swelling difference observed between the CPZ-induced swelling stage (Fig. 2b) and the EGTA-induced swelling stage (Fig. 2a). We were able to dictate the amount of swelling produced by the stimuli-responsive hydrogel according to the degree of conformational change undergone by CaM transitioning from its bound conformation (Fig. 1b) to its native conformation (Fig. 1c).