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Optically Controlled Reversible Protein Hydrogels Based on Photoswitchable Fluorescent Protein Dronpa

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Electronic Supplementary Information

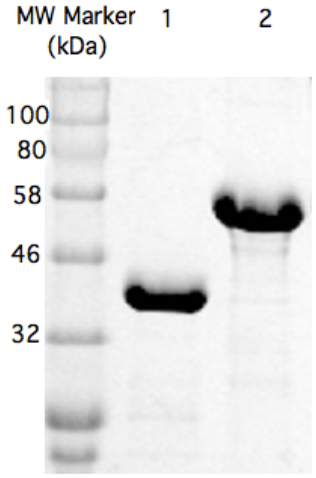

Figure S1. SDS-PAGE of engineered proteins, Lane 1: G2-St-D, Lane 2: (G-Sc)3.

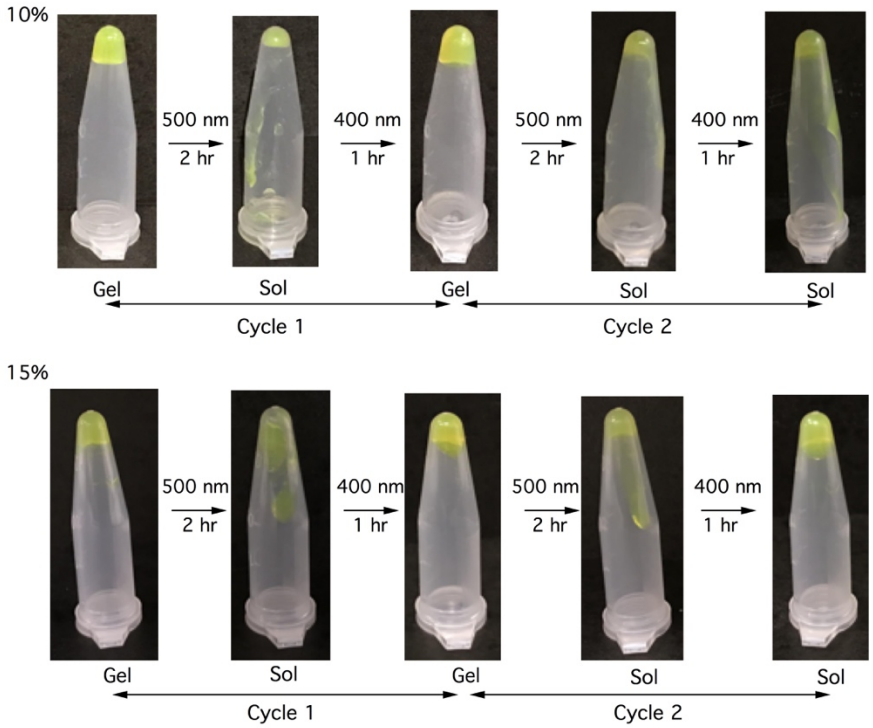

Figure S2. Reversibility of the optically switching of the Dronpa145N-based hydrogels. Cyan light illumination induced gel-sol transition, and violet illumination induced sol-gel transition. After 1.5 cycles of optical switching, violet illumination can no longer induce the sol-gel transition, and the protein solution remained as a viscous liquid even after 3 hrs of violet illumination.

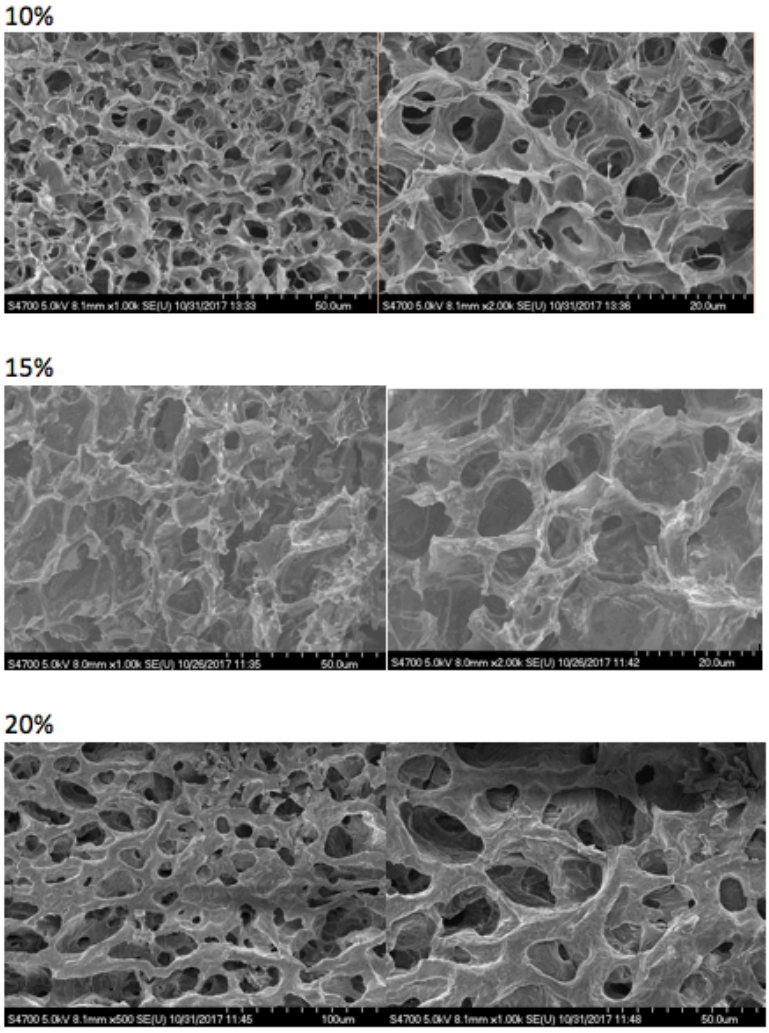

Figure S3. SEM photographs of Dronpa145N-containng protein hydrogels.

The possibility of engineering optically switchable protein hydrogels using Dronpa145N-containing comb polymers

If multiple Dronpa145N monomer (in its dark state) can be incorporated into a single protein polymer chain to form a Dronpa145N-containing comb-like polymer (in which Dronpa145N is the pendant group), it should be feasible to use violet illumination to optically induce the tetramerization of the pendant Dronpa145N domains from different protein polymer chains and causes the physical crosslinking and the formation of the protein hydrogel.

Fig. S4 shows a preliminary attempt in the regard. We first illuminated G_2-St-D with cyan light (~500 nm) to switch Dronpa145N to its monomeric dark state, and then reacted G_2-St-D with $(G\text{-}Sc)_3$ at a Sc:St molar ratio of 1:1. After 1 hr of reaction at 37 °C, a mixture of products formed, corresponding to $(G\text{-}Sc)_3\text{-}(G_2\text{-}St\text{-}D)_x$, where x equals to 1 to 3, respectively. Instead of purifying comb-like protein polymer, we directly used the reaction mixture to construct the hydrogel. The aqueous solution of the reaction mixture remains a liquid within all protein concentrations we tested (5-30%), and no gelation was observed at ambient condition in dark even after 24 hrs. However, illumination of the solution (>10%) with 400 nm violet light for 10 minutes induced the hydrogel formation and resulted in a transparent fluorescent hydrogel, which is evident from the inverse tube test (Fig. S4). Although systematic efforts will be required to optimize the reaction condition and produce mono-dispersed Dronpa145N containing comb polymer, this preliminary result clearly demonstrates the feasibility of engineering optically switchable protein hydrogels using Dronpa145N-containing comb polymers.

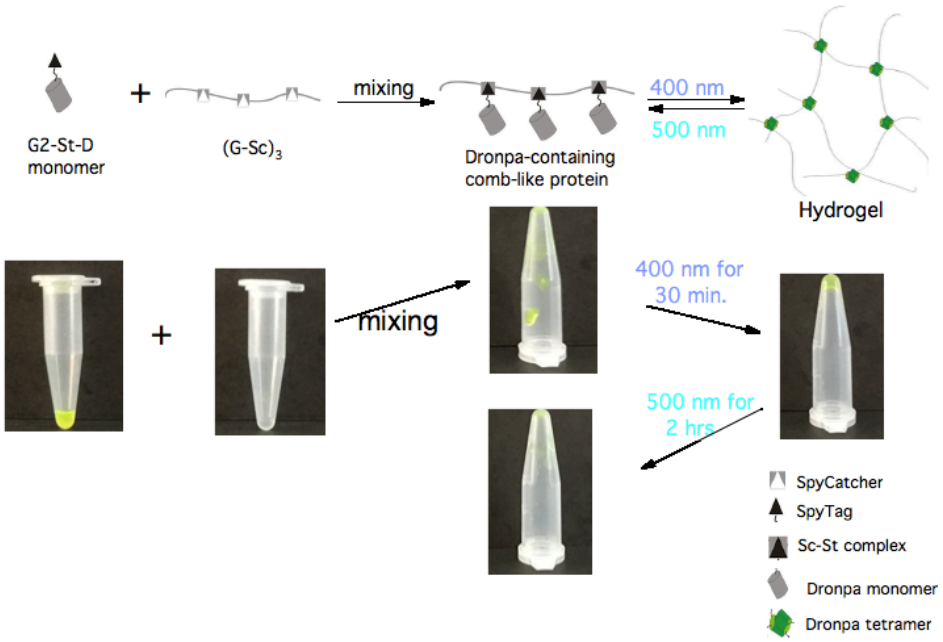

Figure S4. Optically controlled reversible protein hydrogels based on Dronpa145N-containing comb-like protein polymers. Top panel shows the schematics of optically controlled sol-gel and gel-sol transition. Bottom panel shows the photographs of this optically controlled hydrogelation and degradation reaction.

Materials and Methods

Protein engineering: The gene encoding Dronpa145N, which contained a 5' BamHI and 3' BglII, KpnI restriction site, was purchased from Genscript (Piscataway, NJ, US). The gene encoding G2-St-D was constructed in the cloning vector pUC19 using standard molecular biology techniques following a well-established stepwise construction scheme.[1] This method uses the identical sticky ends generated by BamHI and BglII restriction enzymes to build genes encoding multidomain proteins in a stepwise fashion. Then, genes were subcloned from pUC19 vector into the expression vector pET29b, which carries a C-terminal His-tag sequence. The expression vector was transformed into the Escherichia coli (E. coli) strain BL21. The bacteria culture was grown at 37 °C in a 2.5% lysogeny broth (LB) containing 100 mg/L ampicillin. Protein overexpression was induced with 1 mM isopropyl-1-β-D-thiogalactoside (IPTG) when the OD600 reached ~0.8. Protein expression continued for 4 hrs. The bacterial cells were harvested by centrifugation at 4000 rpm for 10 min, and lysed using a 2.5 mg mL-1 lysozyme solution. Proteins were harvested from the soluble fraction via Co2+-affinity chromatography; and the protein solutions were dialyzed against deionized water (changed every 7 h) for 72 h to remove salts. After dialysis, proteins were lyophilized. Molecular weights of proteins were determined using sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). Proteins (G-Sc)3 and (G-Sc)2 were engineered as previously described.[2]

UV-Vis and Fluorescence Spectroscopy: Absorption spectra were recorded with a Varian Cary 4000 UV/VIS Spectrometer. A Varian Cary Eclipse fluorescence spectrometer with excitation at 457 nm (10 nm slit) was used for measuring emission spectra.[3]

Hydrogel Construction: Lyophilized $(G\text{-}Sc)_3$ and G_2-St-D proteins were weighted and dissolved in PBS to obtain protein solutions at desired concentrations. For "physical" hydrogelation, G_2-St-D solution was illuminated with 500 nm light source for 2 hrs to bring Dronpa145N to its dark monomeric state, and then mixed with $(G\text{-}Sc)_3$ solution at a Sc:St stoichiometry of 1:1. Hydrogelation was initiated by illuminating the corresponding protein solution with 400 nm light for 10 minutes. For "chemical" hydrogelation, the G_2-St-D solution in its initial bright state was mixed with $(G\text{-}Sc)_3$ solution at a Sc:St stoichiometry of 1:1. Hydrogelation proceeded at room temperature for 10 minutes. For photo-degradation of the Dronpa145N-containing hydrogels, the hydrogel was illuminated with cyan light (500 nm) for 2 hrs.

Rheology Measurements: Frequency-dependent viscoelastic moduli (storage modulus G' and loss modulus G") were measured using a TA Instruments Discovery Hybrid Rheometer equipped with an 8 mm flat plate. The mechanical properties of $(G\text{-}Sc)_3$ and G_2-St-D-based hydrogels were measured in frequency modes at room temperature at a 200 μm gap with a strain of 2% by a frequency-dependent sweep from 0.1 to 100 rad s^{-1}.

Optical Switching: Photoswitching experiments were performed by using custom-built light sources equipped with nine 3 W cyan (500 nm) or violet (400 nm) LED beads (Getian Group, Guangdong, China). To check the photoswitching property of G_2-St-D, G_2-St-Dronpa solution in a quartz cuvette was illuminated. During illumination, absorption and fluorescent spectra of the G_2-St-Dronpa solution were recorded at certain time interval. Hydrogels (15-30 wt%) prepared in Eppendorf tubes were illuminated using the same light sources to examine the light dependent hydrogelation or degradation behaviors.

Scanning electron microscopy (SEM) imaging of hydrogel SEM images of hydrogels were taken using a Hitachi S-3000 N scanning electron microscope with an energy-dispersive X-ray light element. Hydrogel samples were shock-frozen in liquid nitrogen, quickly transferred to a freeze drier and lyophilized for 12 h. Dry samples were carefully fractured in liquid nitrogen and fixed on aluminum stubs. The surface of the samples was coated by gold before SEM observation.

References

1. Y. Cao and H. Li, *Nature Materials*, 2007, **6**, 109.
2. X. Gao, J. Fang, B. Xue, L. Fu and H. Li, *Biomacromolecules*, 2016, **17**, 2812.
3. R. Ando, H. Mizuno and A. Miyawaki, *Science*, 2004, **306**, 1370.