Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information:

Title: Structurally Colored Protease Responsive Nanoparticle Hydrogels with Degradation-Directed Assembly

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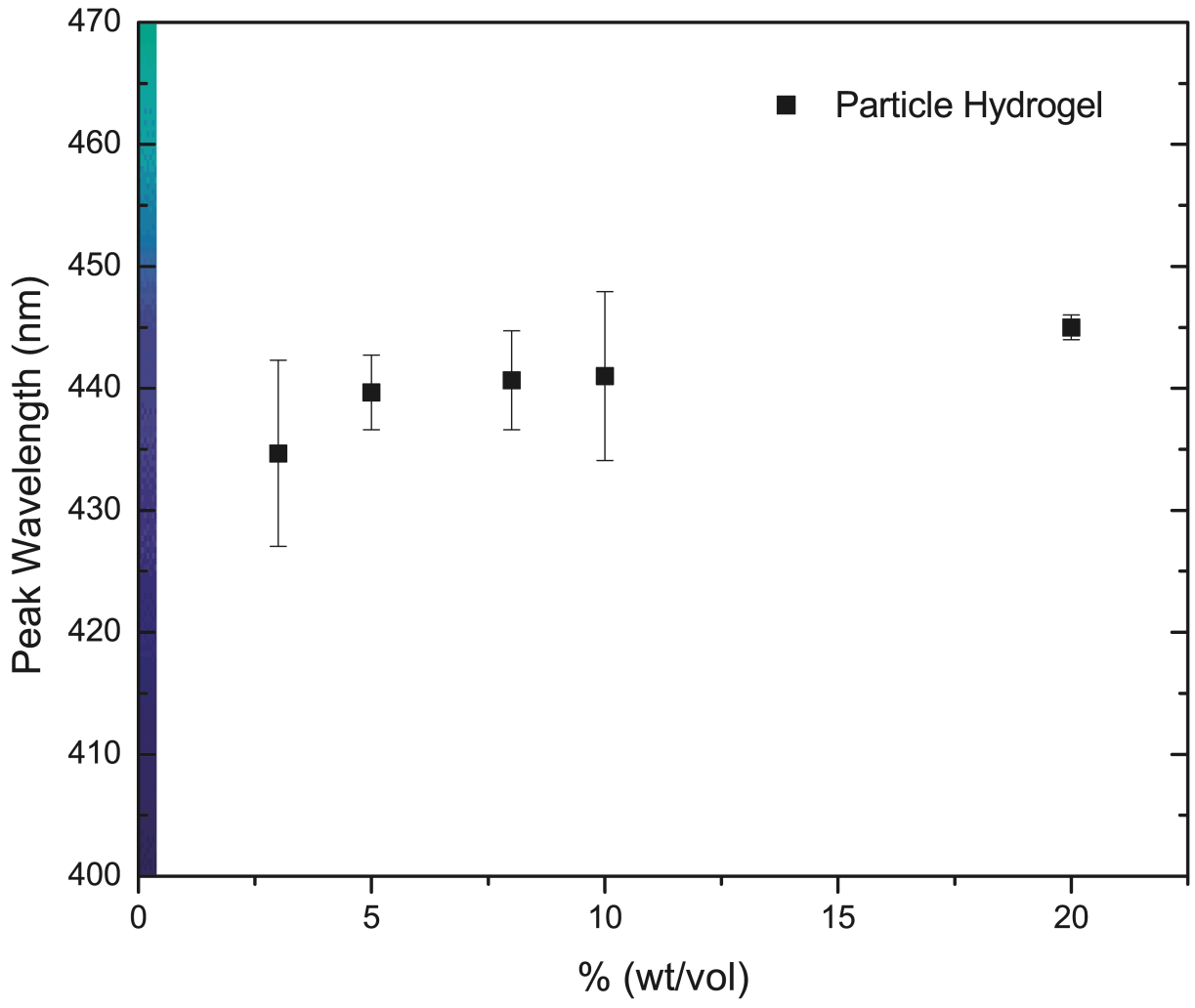
How does particle concentration affect structural color of centrifuged silica pellets:

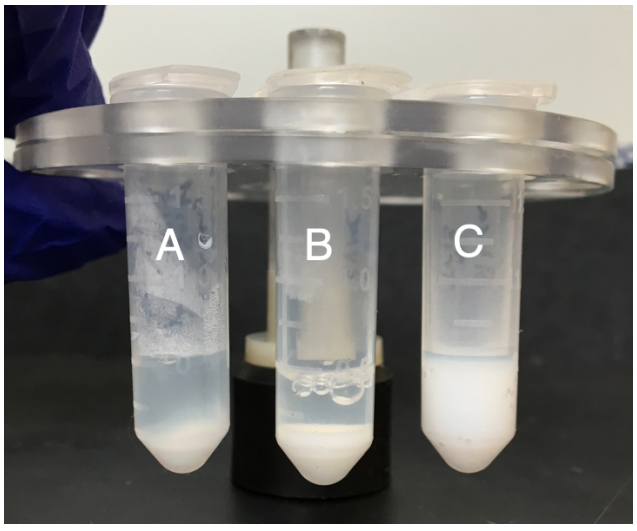
Particles were suspended at concentrations between 3 – 20 % w/v in 100 µL of a 4PEGN and CYKC solution (15 % and 1.5 w/v, respectively). The suspensions were centrifuged with 19318 G for 2.5 minutes to produce structural color. The pellets were compressed between two glass slides and polymerized for 20 minutes in a UV oven (KAIS). The reflectance spectrum was recorded for each PRNH using reflectance probe spectroscopy (Figure S1). The peak wavelength for all PRNH fabricated did not statistically vary for the initial particle concentrations investigated. By introducing 4PEGN and CYKC peptide, water becomes less available to the particles because the polymer and peptide must be solvated. In turn, this concentrates the particles and allows for particles to compact at similar spacing during centrifugation due to less available volume for them to assemble into.



Supplemental Fig. 1 Particle concentration does not affect the PRNH structural color exhibited after centrifugation. PRNH were fabricated with 15 % 4PEGN and 1.5 % CYKC and centrifuged for 2.5 minutes with 19318 G.

Silica particle confinement determines assembly into structurally colored composite:

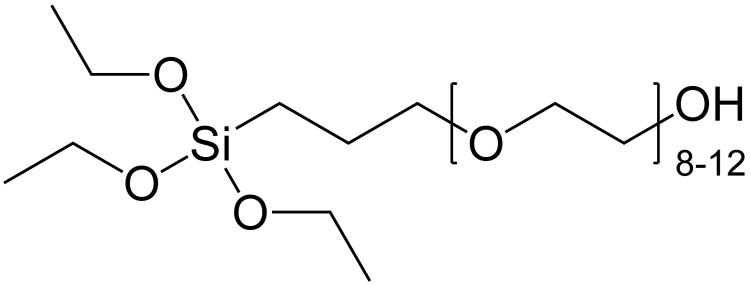
To determine whether the degradation-directed assembly could produce structurally colored composite (SCC) without confining silica particles to a close-packed structure, 205 nm THOPS-SiO_2 was mixed with a degraded 4PEGN-CY↓KC hydrogel, degraded CY↓KC, or Tris buffer (**Figure S2**). A 100 µL hydrogel was made with 15 mg of 4PEGN and 1.5 mg of CYKC, 0.1 mg of 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone, and deionized water via UV polymerization in a 2.0 mL centrifuge tube (VWR). The hydrogel or 1.5 mg CYKC peptide was degraded completely with 100 µL solution of 10 µg/ml chymotrypsin, 100 mM Tris-HCl and 10 mM $CaCl_2$ at 37 °C for 1 hour. This length of time allowed for complete degradation of the hydrogel, and CY↓KC, as no visible hydrogel remained in the vial. THOPS-SiO_2 (10% w/v) was suspended in either the degraded gel or CY↓KC solution and allowed to assemble. In 1 hour, the particles aggregated and sedimented to the bottom of the vial (Figure S2A and S2B) with no structural color observed. The particles were mixed with 100 µL Tris-HCl (100 mM) and $CaCl_2$ (10 mM) as a control and remained suspended for more than 24 hours. This suggests that for the degradation-directed assembly to produce SCCs, the particles should be confined in an arrangement that already shows structural color.

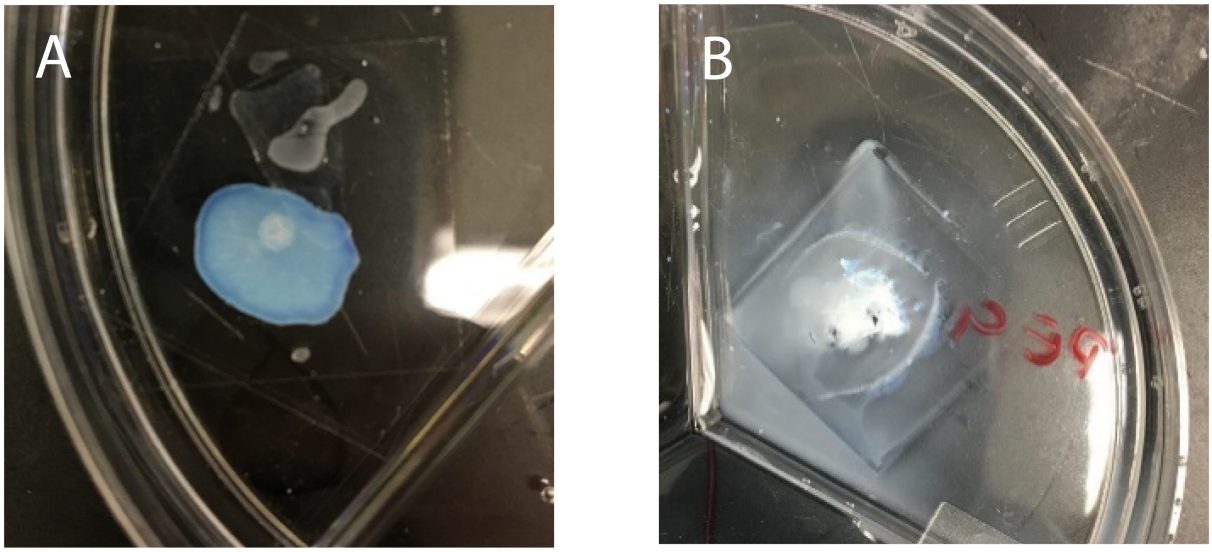


Supplemental Fig 2 Confinement of nanoparticles is required for degradation-directed assembly into a SCC. 205 nm THOPS-SiO_2 were mixed with (A) degraded 4PEGN-CY↓KC hydrogel, (B) degraded CY↓KC, or (C) tris buffer as a control. Images were taken 12 hours after the particles were suspended in solution. In A and B, particle aggregation is observed, while in C the particles are suspended.

Physical Interactions Required for Degradation-Directed Assembly into SCC:

To determine whether physical adsorption and electrostatic interactions on the particle surface are required for the formation of SCCs, the entire particle surface of 200 nm SiO_2 was functionalized with a [hydroxy(polyethyleneoxy)propyl]triethoxysilane, 8-12 ethylene glycol repeat units (PEG-silane), using a previous reported protocol.[1,2] The linear PEG on the surface of the particle would prevent interactions between the surrounding polymer matrix and particle surface during degradation. Protease-responsive nanoparticle hydrogels (PRNH) were fabricated with PEG-SiO_2, 15 mg 4PEGN, and 1.5 mg CY↓KC in a centrifuge with a centrifugal force of 19,318 G for 2.5 minutes. The resulting pellet was compressed between glass slides, polymerized for 20 minutes, and placed in deionized water (Figure S3A). After one hour of swelling in the deionized water, the PRNH was placed in a 1 µg/mL chymotrypsin, 100 mM Tris-HCl and 10 mM $CaCl_2$ at 37 °C. After 30 minutes, the PRNH began to show signs of bulk degradation as evidence by particles diffusing into the surrounding solution (Figure S3B). After 1 hour the PRNH completely degraded, and the particles suspended fully into the solution. Under the same chymotrypsin concentration, the PRNH with THOPS-SiO_2 would color change from red to blue.

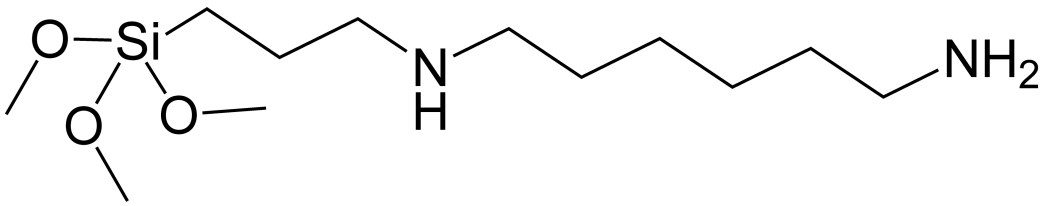


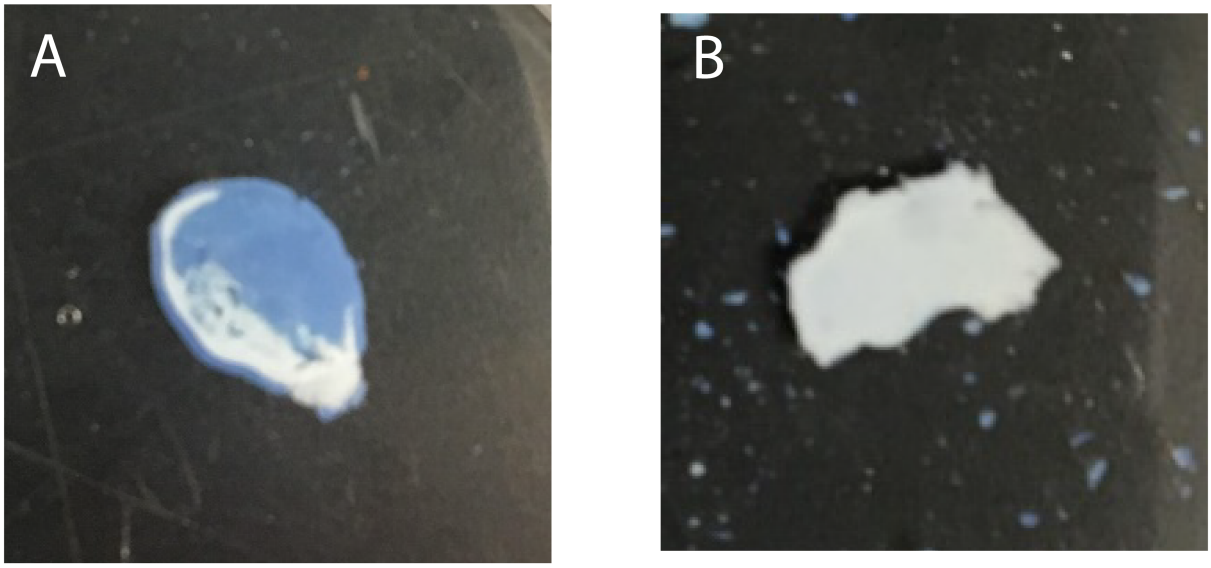


Supplementary Fig. 3 Optical images of PRNH with PEG-SiO_2 before and after degradation. (A) The PRNH with PEG-SiO_2 after initially fabricated. (B) the PRNH with PEG-SiO_2 after 1 hour of chymotrypsin degradation. The increase in turbidity around the original hydrogel is an indicator that the particles are resuspending into the surrounding solution. The particles could be suspended and are stable in the surrounding solution without sedimenting.

Degradation of the PRNH$^+$ Does Not Yield a SCC:

PRNH fabricated with N-(6 aminohexyl)aminopropyltrimethoxysilane functionalized SiO_2, 15 mg 4PEGN, and 1.5 mg CY↓KC in a centrifuge with a centrifugal force of 19,318 G for 2.5 minutes. The pellet was compressed between two glass slides, polymerized, and placed in deionized water (Figure S4A). After one hour of swelling in the deionized water, the PRNH was placed in a 1 µg/mL chymotrypsin, 100 mM Tris-HCl and 10 mM $CaCl_2$ at 37 °C. After 30 minutes the color had disappeared and the PRNH appeared white due to destructive scattering of incident light (Figure S4B).





Supplementary Fig. 4 Optical images of the PRNH$^+$ before and after degradation. (A) The PRNH$^+$ after initially fabricated. (B) the PRNH$^+$ after 1 hour of chymotrypsin degradation showing no color due to aggregated AHAPS-SiO_2 causing destructive interference of incident light.

References

1 T. G. Waddell, D. E. Leyden and M. T. DeBello, The nature of organosilane to silica-surface bonding, *Journal of the American Chemical Society*, 1981, **103**, 5303–5307.

2 C. Graf, Q. Gao, I. Schütz, C. N. Noufele, W. Ruan, U. Posselt, E. Korotianskiy, D. Nordmeyer, F. Rancan, S. Hadam, A. Vogt, J. Lademann, V. Haucke and E. Rühl, Surface Functionalization of Silica Nanoparticles Supports Colloidal Stability in Physiological Media and Facilitates Internalization in Cells, *Langmuir*, 2012, **28**, 7598–7613.