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Secretion and reversible assembly of extracellular-like matrix by enzyme-active colloidosome-based protocells

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Supporting Information: Figures

Figure S1. Optical microscopy image showing intact ALP-containing silica nanoparticle-based colloidosomes after transfer from oil into water. Scale bar = 100 μm

Figure S2. Photographs of Hoechst 33258-stained Fmoc-TyrOH hydrogel/colloidosome composites observed under normal light (**a,c**) or UV irradiation (**b,d**). Samples were prepared by mixing 50 μL (**a,b**) or 200 μL (**c,d**) of an aqueous dispersion of ALP-containing colloidosomes with 25 μmol of Fmoc-TyrP in alkaline buffer solution and aging for 2 days. Samples are shown inverted in a plastic tube.

Figure S3. Time-dependent UV-vis spectra of the supernatant associated with the formation of a hydrogel/colloidosome composite. Spectra were recorded hourly for a period of 20 h. The decrease in absorbance at 288 and 299 nm with time corresponds to a reduction in the concentration of Fmoc-TyrP as the dephosphorylation reaction proceeds.

Figure S4. SEM image of Fmoc-TyrOH hydrogel prepared in bulk solution by ALP-mediated dephosphorylation of Fmoc-TyrP. Note the dense superstructure of bundled fibres. Scale bar = 5 μm .

Figure S5. Confocal fluorescence (a) and corresponding optical (b) microscopy images showing single colloidosome and associated Fmoc-TyrOH nanofilaments stained with the blue fluorescent dye, Hoechst 33258. Sample was aged for 20 h. Note the virtual absence of staining within the protocell after this time period. Scale bar = 30 μm .

Figure S6. TEM images of fractured unstained sample of a Fmoc-TyrOH hydrogel/colloidosome composite isolated after (a) 5 and (b) 20 h showing transformation from internal unstructured hydrogel to dense network of externally located nanofilaments. Scale bars = 0.25 and (b) 1 μm in (a) and (b), respectively.

Figure S7. DSC thermogram of Fmoc-TyrOH hydrogel prepared from ALP-mediated dephosphorylation of Fmoc-TyrP showing broad gel to sol transition centred at 53°C.

Figure S8. Fluorescence and corresponding bright field confocal microscopy images of single ALP-containing colloidosomes after addition of Fmoc-TyrP and aging at room temperature for 20 h, heating to 60°C for 2 h, and cooling to room temperature and left for **(a)** 0 **(b)** 3, **(c)** 4, and **(d)** 5 h; scale = 30 μm .

Figure S9. SEM image of fractured colloidosome showing densely packed reassembled bundles of Fmoc-TyrOH filaments produced by cooling a sample of the hydrogel/protocell composite from 60°C to room temperature and aging the mixture for 20 h. Scale bar = 2 μ m.

Figure S10. Fluorescence images of individual protocells showing (a) internalized reassembly of hydrogel matrix after disassembly at 60°C followed by cooling to room temperature, (b) same sample after reheating to 60°C showing disassembly of reassembled hydrogel, and (c) cooling to room temperature displaying next cycle of reassembly specifically in the colloidosome interior. Scale bar for all images, 40 μ m.

Figure S11. (a,b) Fluorescence and corresponding bright field images of a population of colloidosomes containing EDTA-deactivated ALP after addition of the protocell dispersion to a preformed Fmoc-TyrOH hydrogel produced in bulk solution, and the mixture heated above the gel-sol transition temperature and then cooled to room temperature overnight. No blue fluorescence corresponding to the reassembled hydrogel is observed within the colloidosome micro-compartments. Scale bar = 50 μm (c,d) Images from samples prepared as above but by addition of water-filled (no ALP) colloidosomes to the preformed hydrogel showing absence of Fmoc-TyrOH nanofilaments in the protocell interior. Scale bar = 20 μm .

Figure S12. (a) Photograph of sample tube containing hydrogel-filled ALP-containing colloidosomes after disassembly at 60°C followed by cooling to room temperature for 20 h. (b,c) Same sample after addition of *p*-nitrophenylphosphate (pNPP) and after 1 h (b) and 8 h (c) showing development of yellow colouration due to formation of the dephosphorylated product, *p*-nitrophenyl (pNP) within the colloidosomes and diffusion into the supernatant.

Figure S13. Fluorescence (left) and optical (right) microscopy images of a single ALP-free colloidosome incubated in an aqueous solution of Fmoc-TyrP and showing the absence of hydrogel formation. The background blue fluorescence originates from Hoechst 33258 molecules adventitiously absorbed onto the silica nanoparticles of the colloidosome membrane. Scale bar = 22 μm .