

Supporting Information

Protein-Mediated Colloidal Assembly

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1. General

Restriction enzymes and ligase were purchased from New England Biolabs (Beverly, NJ). PfuUltraII polymerase was purchased from Agilent Technologies (Santa Clara, CA). Nickel NTA was purchased from Qiagen (Hilden, Germany). N-Hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) were purchased from Life Technologies (Carlsbad, CA). 2-(2-Pyridinyldithio)ethaneamine hydrochloride (PDEA) was purchased from GE Healthcare (Piscataway, NJ). Fluorescent, carboxylated polystyrene particles were purchased from Sigma Aldrich (St. Louis, MO). Fluorescence images were obtained with a Zeiss LSM 800 laser scanning confocal microscope, using 20x/0.8 and 100x/1.46 Plan-Apochromat objectives and acquired with Zeiss's Zen software. Images were taken as 30-80 z-stacks with a spacing of 1.1 μm per slice for 20x images and 0.4 μm per slice for 100x images, and shown as maximum intensity projections unless otherwise stated.

2. Cloning of Recombinant Proteins

Recombinant fusion proteins were produced by standard recombinant DNA technology. DH10b or Mach1 strains of *Escherichia coli* were used for all cloning steps. A modified pQE-80L expression vector was used for expression. The *XhoI* site upstream of the T5 promoter was deleted and a C-terminal in-frame cysteine residue was added down-stream of the multiple cloning site by two rounds of site-directed mutagenesis. The modified pQE-80L expression vector was denoted pQE-80X-Cys.

The DNA sequence encoding the elastin-like polypeptide segment of each fusion protein was synthesized in two 250 bp segments denoted E3 and E3' (Genscript) with 5' *BamHI* and *SalI* sites and 3' *XhoI* and *HindIII* sites. These segments have identical amino acid sequences but different degenerate DNA sequences to decrease sequence similarity at the DNA level. A polyhistidine tag was added between the *XhoI* and *HindIII* sites. These DNA fragments were inserted into pQE-80X-Cys between the *BamHI* and *HindIII* sites to make plasmids pQE-80X-E₃-Cys and pQE-80X-E₃'-Cys and transformed into chemically competent *E. coli*. The full-length elastin-like polypeptide was synthesized via recursive directional ligation.⁴² Briefly, pQE-80X-E₃-Cys was digested with *XhoI* and *HindIII* to linearize the plasmid, while pQE-80X-E₃'-Cys was digested with *SalI* and *HindIII*. The resulting DNA fragments were ligated together to fuse the two elastin-like sequences. Because *SalI* and *XhoI* have complementary overhangs, the restriction sites are ablated, leaving a two-amino acid scar, but retaining the 5' *BamHI* and *SalI* sites and 3' *XhoI* and *HindIII* sites in the plasmid. This construct

	GAGVPGAGVPGEGVPGAGVPGAGVPGAGVPGAGVPGEGV PGAGVPGAGVPGAGVPGAGVPGEGVPGAGVPGAGLEHHH HHHKLC
SpyTag-E-Cys	MRGSHHHHHHGSVDGSGSGSGSGSAAHIVMVDAYKPTKG SGSGSGSGSGALDVPAGVPGAGVPGEGVPGAGVPGAGVPG GAGVPGAGVPGEGVPGAGVPGAGVPGAGVPGAGVPGEGV PGAGVPGAGLDVPAGVPGAGVPGEGVPGAGVPGAGVPG AGVPGAGVPGEGVPGAGVPGAGVPGAGVPGAGVPGAGVPG GAGVPGAGLEHHHHHHKLC
SpyCatcher-E-Cys	MRGSHHHHHHGSVDGSGSGSGSGSAAAMVDTLSGLSSEQG QSGDMTIEEDSATHIKFSKRDEDGKELAGATMELRDSSGKTI STWISDGQVKDFYLYPGKYTFVETAAPDGYEVATAITFTVN EQGQVTVNGKATKGDHIDGSGSGSGSGSGALDVPAGVPG GAGVPGEGVPGAGVPGAGVPGAGVPGAGVPGEGVPGAGV PGAGVPGAGVPGAGVPGEGVPGAGVPGAGLDVPAGVPG AGVPGEGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPG GAGVPGAGVPGAGVPGEGVPGAGVPGAGLEHHHHHHKLC

3. Protein Expression and Purification

Constructs were transformed into *E. coli* strain BL21 for expression. Expression was performed in Terrific Broth (12 g/L casein, 24 g/L yeast extract, 0.4% w/v glycerol, 0.017 M monobasic potassium phosphate, 0.072 M dibasic potassium phosphate). Cultures were induced at an optical density of 0.6-0.9 to a final concentration of 1 mM isopropyl β -D-1-thiogalactopyranoside (IPTG). Expression was allowed to proceed for 5 h, after which cells were harvested by centrifugation. For all proteins other than SpyCatcher-E-Cys, cultures were resuspended in denaturing lysis buffer (8 M urea, 0.1 M Na₂HPO₄, 10 mM imidazole; pH 8.0), and lysed by sonication. Lysates were cleared by centrifugation and incubated with NiNTA resin. The resin was washed with lysis buffer and wash buffer (8 M urea, 0.1 M Na₂HPO₄, 25 mM imidazole; pH 6.3). Protein was eluted with elution buffer (8 M urea, 0.1 M Na₂HPO₄, 250 mM imidazole; pH 3.5). Purity was confirmed with SDS-PAGE. Proteins were then extensively dialyzed against water and lyophilized for storage.

SpyCatcher-E-Cys was purified under native conditions. Cultures were resuspended in native lysis buffer (50 mM NaH₂PO₄, 300 mM NaCl, 10 mM imidazole, 1 mg/mL lysozyme; pH 8.0). Cells were lysed by sonication, and cleared lysates were incubated with NiNTA resin. The resin was washed with native wash buffer (50 mM NaH₂PO₄, 300 mM NaCl, 25 mM imidazole; pH 8.0), and protein was eluted with native elution buffer (50 mM NaH₂PO₄, 300 mM NaCl, 250 mM imidazole; pH 8.0). Purity was confirmed with SDS-PAGE, and purified SpyCatcher-E-Cys was dialyzed against water and lyophilized.

4. Control of Cluster Size

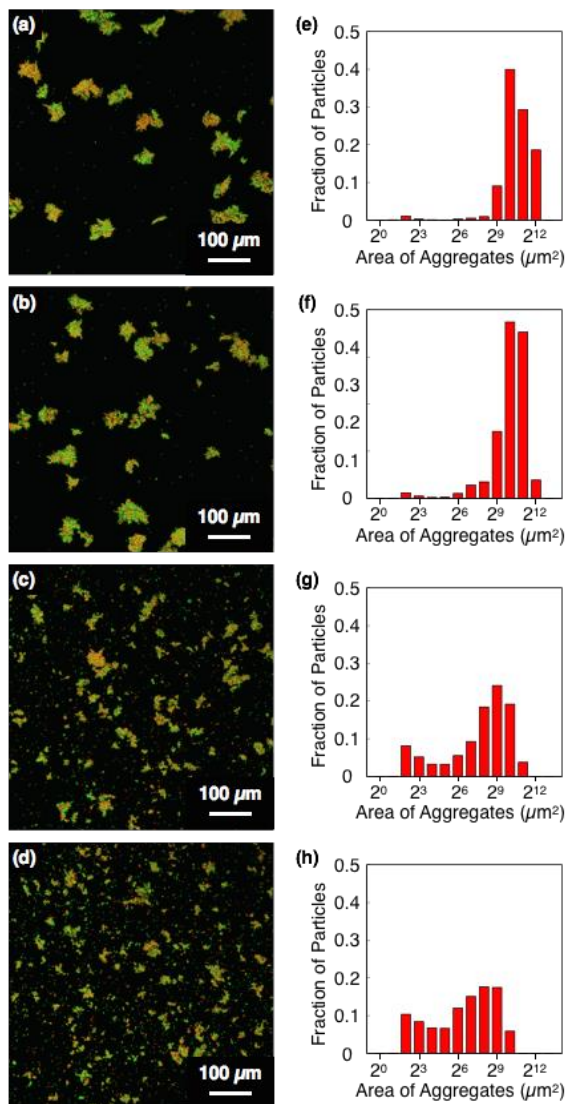


Figure S1. Assembly of particles functionalized with SpyCatcher and Z17 (green) and SpyTag-functionalized particles (red). For the preparation of green particles, 1 mg/mL solutions of SpyCatcher and Z17 were mixed at ratios of (a) 3:1, (b) 2:1, (c) 1:1 and (d) 0.5:1 (v/v), and proteins were immobilized via thiol exchange. Each population of green particles was mixed with red particles treated with SpyTag and suspensions were continuously mixed for 30 min at 25 °C in PBS (pH 7.5) with 0.005% tween 20 ([green particle] = [red particle] = 1×10^5 particle/μL). (e-h) Size distribution histograms of particle aggregates shown in (a-d).

5. Dissociation of Z17/Z18 Aggregates by Soluble Z17

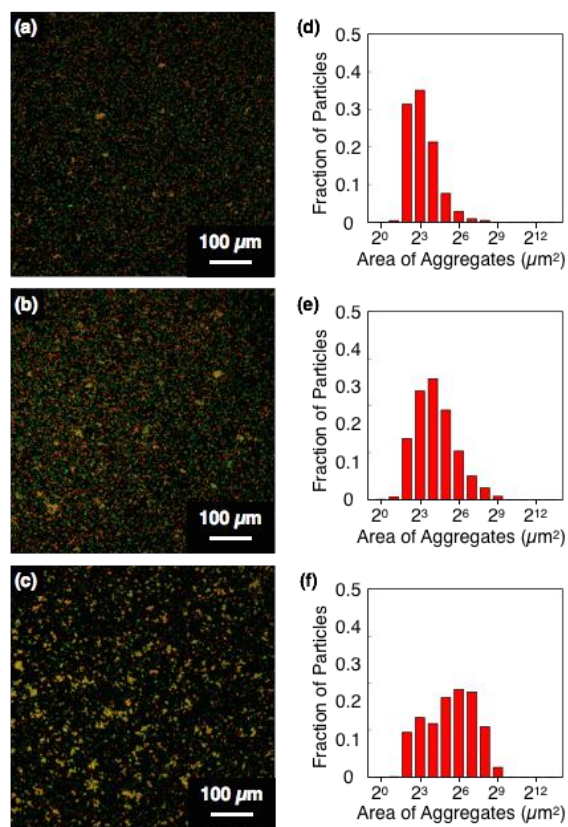


Figure S2. Dissociation of the aggregates of Z17- (green) and Z18- (red) functionalized particles by addition of soluble Z17. Particle aggregates were prepared by continuously mixing Z17- (green) and Z18- (red) functionalized particles for 40 min at 25 °C in PBS (pH 7.5) with 0.005% tween 20 ([Z17 (green) particle] = [Z18 (red) particle] = 1×10^5 particle/μL). Then, (a) 0.2 mg/mL (b) 0.04 mg/mL and (c) 0.008 mg/mL of free Z17 solution was added to the particle aggregates and mixed at 25 °C for 24 h. (d-f) Size distribution histograms of particle aggregates shown in (a-c).

6. Dissociation of Z17/Z18 Aggregates by GuHCl

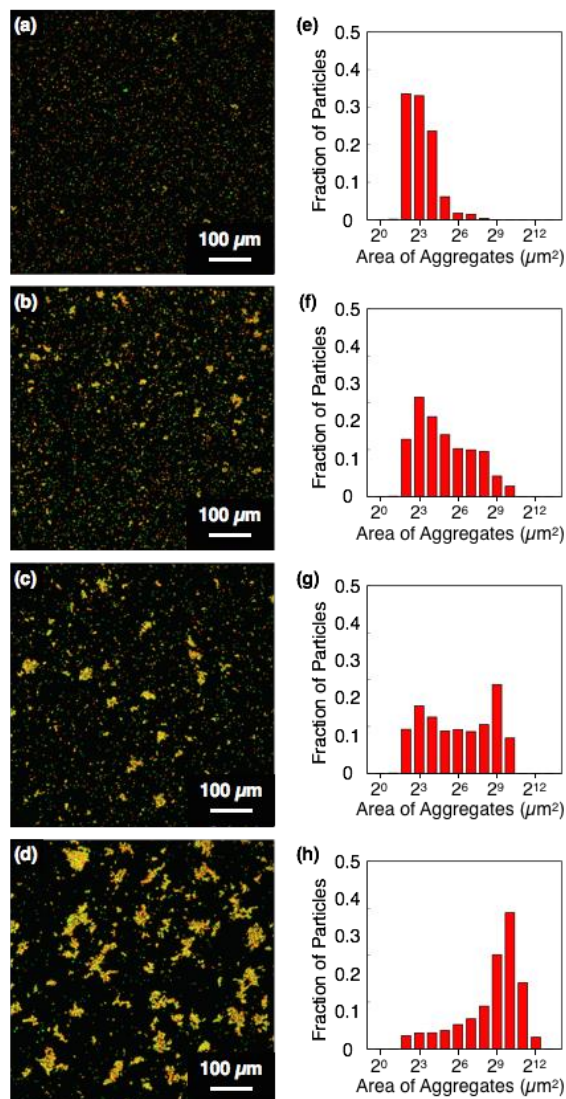


Figure S3. Dissociation of the aggregates of Z17- (green) and Z18- (red) functionalized particles by addition of GuHCl. Particle aggregates were prepared by mixing Z17- (green) and Z18- (red) functionalized particles for 40 min at 25 °C in PBS (pH 7.5) with 0.005% tween 20 ([Z17 (green) particle] = [Z18 (red) particle] = 1×10^5 particle/μL). Then, (a) 5 M (b) 1 M, (c) 0.2 M and (d) 0.04 M of GuHCl was added to the particle aggregates and mixed at 25 °C for 3 h. (e-h) Size distribution histograms of particle aggregates shown in (a-d).

7. Aggregates of SpyTag/SpyCatcher under Dissociative Conditions

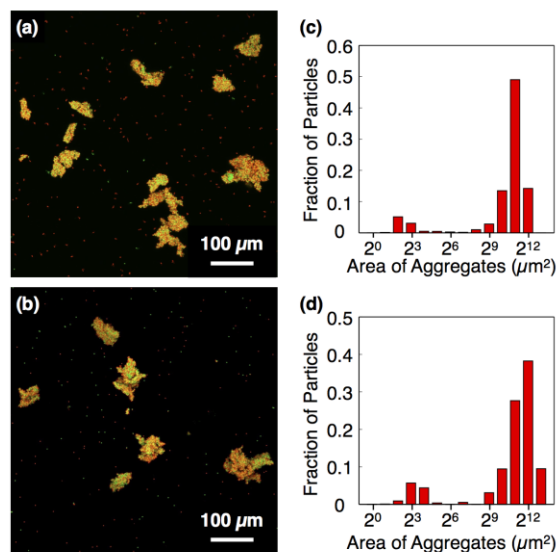


Figure S4. Aggregates of SpyCatcher- (green) and SpyTag- (red) functionalized particles are stable in the presence of GuHCl and soluble SpyTag. Particle aggregates were prepared by mixing SpyCatcher- (green) and SpyTag- (red) functionalized particles for 40 min at 25 °C in PBS (pH 7.5) with 0.005% tween 20 ([SpyCatcher (green) particle] = [SpyTag (red) particle] = 1×10^5 particle/μL). Then, (a) 5 M GuHCl and (b) 1 mg/mL soluble SpyTag were added the aggregate solution and mixed at 25 °C for 3 h and 24 h, respectively. (c, d) Size distribution histograms of (a, b).

8. Orthogonal Assembly and Selective Dissociation

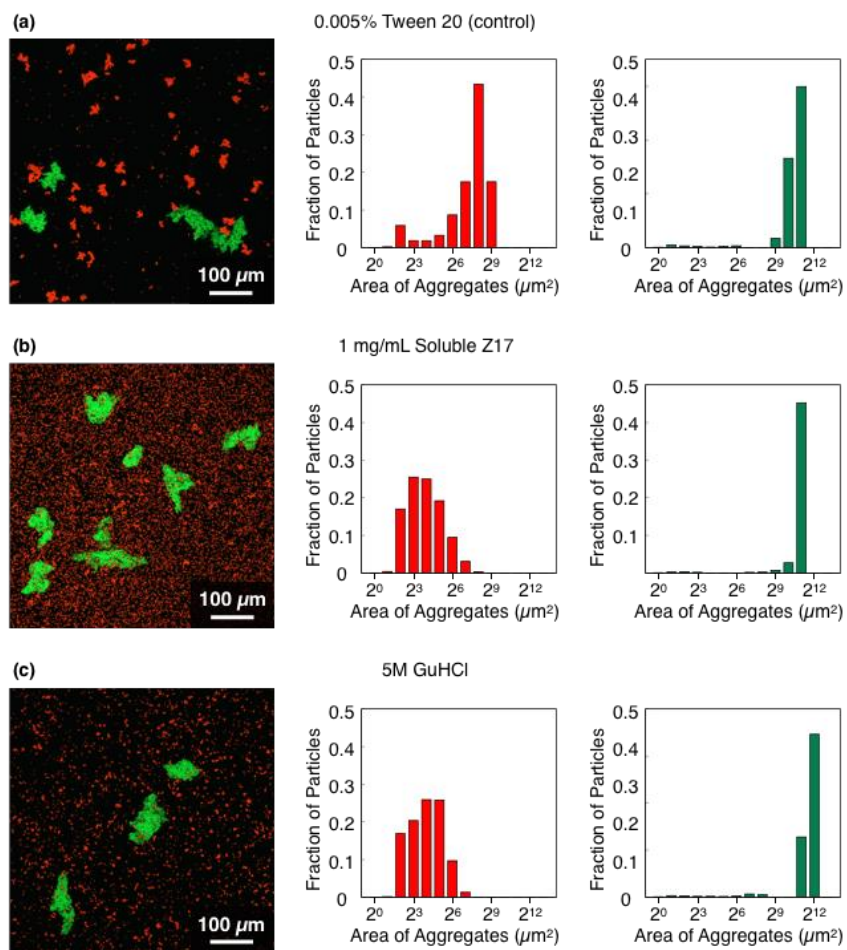


Figure S5. (a) A 1:1:1:1 particle mixture of Z17 (red), Z18 (red), SpyCatcher (green) and SpyTag(green) in PBS with 0.005% tween 20 at 25 °C ($[\text{Z17 (red) particle}] = [\text{Z18 (red) particle}] = [\text{SpyCatcher (green) particle}] = [\text{SpyTag (green) particle}] = 0.5 \times 10^5$ particle/ μL). (b) 0.1% Soluble Z17 or (c) 5 M GuHCl was added to the assembled particle mixture. Aggregates of Z17 and Z18 (red) were selectively dissociated while aggregates of SpyCatcher and SpyTag (green) were stable.

9. Core–Shell Formation

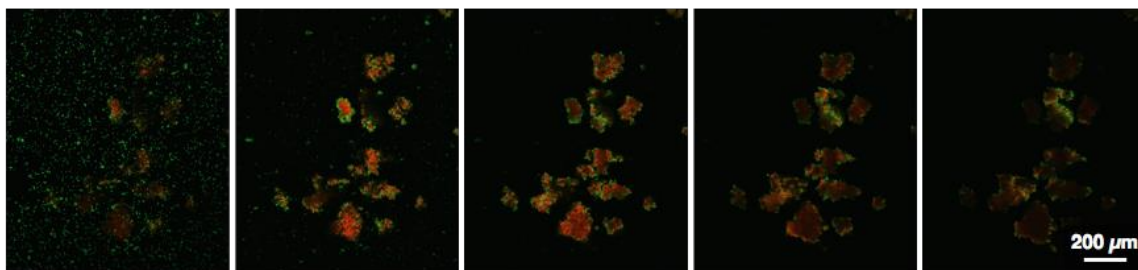


Figure S6. Z-stack images of core–shell structure formed by SpyTag- and SpyCatcher-functionalized particles related to Figure 5b. SpyTag- (red) and SpyCatcher- (red) functionalized particles were mixed at 25 °C for 30 min in PBS (pH 7.5) with 0.005% tween 20 to form core structures. Then, SpyCatcher-coated particles (green) were added to the aggregates and mixed at 25 °C for 1 h to form the shell. $[\text{SpyCatcher (red) particle}] = [\text{SpyTag (red) particle}] = [\text{SpyCatcher (green) particle}] = 0.7 \times 10^5 \text{ particle}/\mu\text{L}$. Images were shown with 3.42 μm slice spacing. Total thickness is 13.7 μm .

10. Surface Coverage Analysis

Surface coverage of core-shell aggregates was analyzed as follows. Three-dimensional z-stacks of core-shell aggregates were obtained at 100x magnification and with 0.39 μm z-spacing. Then, a maximum intensity projection was thresholded, and the projected area of the large cluster was identified. Starting from the top of the image, in each z-slice bright pixels in the projected area of the cluster were identified and classified as red or green. Locations of these bright pixels are then preserved for successive z-slices of the aggregate such that only pixels that are on the outside of the aggregate are counted. The ratio of “shell” pixels to the total number of bright pixels represents the surface coverage. This process is illustrated in Figures S7 and S8.

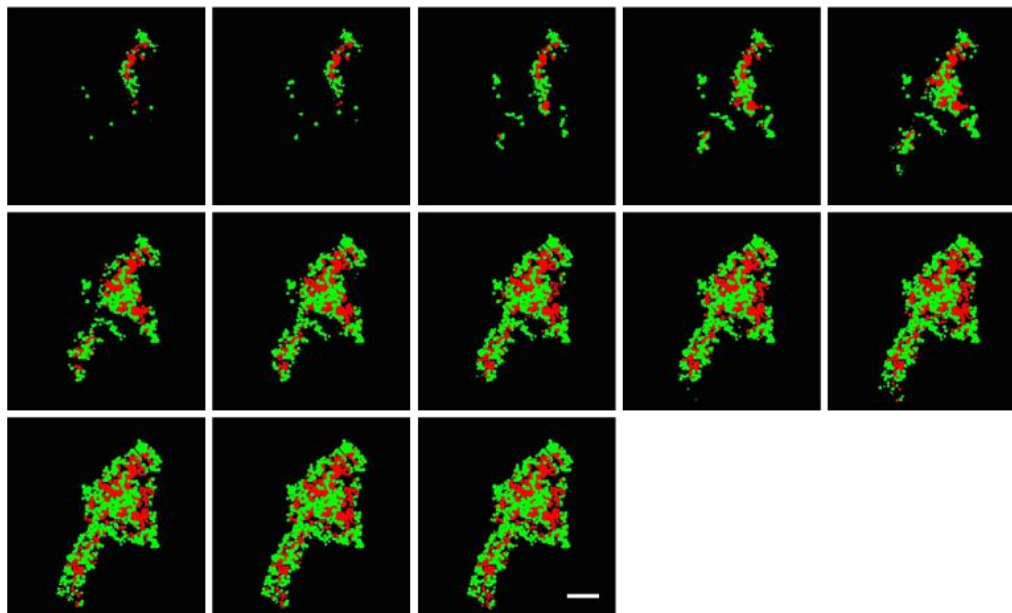


Figure S7. Successive steps of the surface projection algorithm of a core-shell structure formed by SpyTag- and SpyCatcher-functionalized particles. SpyTag- (red) and SpyCatcher- (red) functionalized particles were mixed at 25 °C for 30 min in PBS (pH 7.5) with 0.005% tween 20 to form core structures. Then, SpyCatcher-coated particles (green) were added to the aggregates and mixed at 25 °C for 1 h to form the shell. ([SpyCatcher (red) particle] = [SpyTag (red) particle] = [SpyCatcher (green) particle] = 0.7×10^5 particle/ μL). Successive z-slices from each step of the algorithm are shown with 2.34 μm of slice spacing. Total thickness: 28.1 μm . Scale bar: 20 μm .

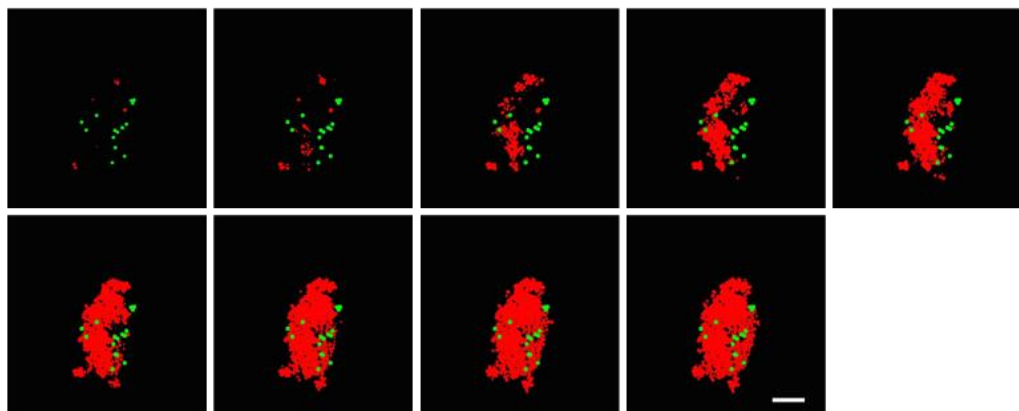


Figure S8. Successive steps of surface projection algorithm for control experiment for core-shell formation. E-functionalized particles (green) were added to the aggregates of SpyTag- (red) and SpyCatcher- (red) functionalized particles and mixed at 25 °C for 1 h in PBS (pH 7.5) with 0.005% tween 20 ([SpyCatcher (red) particle] = [SpyTag (red) particle] = [E (green) particle] = 0.7×10^5 particle/ μL). Successive z-slice images from each step of the algorithm are shown with 2.34 μm of slice spacing. Total thickness: 18.7 μm .

11. Supporting Reference

(42) McDaniel, J. R.; MacKay, J. A.; García Quiroz, F.; Chilkoti, A. *Biomacromolecules* **2010**, *11*, 944.