Organic Nanoflowers From a Wide Variety of Molecules Templated By A Hierarchical Supramolecular Scaffold

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A. General experimental procedures

All reagents from commercial sources were used without further purification. Rhodamine B (RhB) and Doxorubicin (Dox) were purchased from Sigma-Aldrich, Inc. Dextran Texas Red 3 kDa (DTR-3), 10 kDa (DTR-10) and 70 kDa (DTR-70) were purchased from Bioanalytical Instruments Inc. The synthesis of ImAG was an adaptation of our previously reported procedures[1,2] The polystyrene microspheres described here as polystyrene beads were purchased from Phosphorex, Inc. with 1.5 μm in diameter and carboxylic acids on the surface.

The rDsRed2 (DsR) protein was purchased from Clontech Laboratories, Inc. with a concentration of 1 mg/mL. This protein was used diluted to obtain a solution with final concentration of 0.04 mM. These were stored at -20 °C until used. Ovalbumin (Ova) and Cytochrome c (Cyt) were purchased from Sigma-Aldrich, Inc. and solutions were prepared for a final concentration of 0.04 mM. The mCherry (mCh) protein was donated by Professor Abel Baerga's Lab from UPR Medical Sciences Campus. The mCh protein was obtained from the transfection of pRSET A (2.9 kb) from Invitrogen-Life Technologies. The encapsulated plasmids pIRES2-EGFP (pGFP) and pE2-Crimson-C1 (pCri) were purchased from Clontech Laboratories, Inc.

B. Preparation of *f*-SHS

Samples of supramolecular G-quadruplexes (SGQs) made from the 8ArG termed **ImAG** were prepared using 1X PBS (pH 7.4) from Fisher Scientific, total volume 650 μL, 5 mM ImAG monomer and 2 M of KI. These samples were stored overnight (~12 h) in the refrigerator (-10 °C) without further treatment. Values of the concentrations of the resulting assemblies of SGQ

and fixed Supramolecular Hacky Sacks (*f*-SHS) are reported based on the concentration of the ImAG monomer. For *f*-SHS concentrations, we diluted 0.1 mL of the SHS colloid at 40 °C formed by LCST in 1.57 mL of PBS (1X at pH 7.4) to obtain a resulting concentration of 0.303 mM *f*-SHS and 121 mM KI. By using this method, we were able to obtain *f*-SHS by diluting from molar to millimolar range while preserving their integrity and shape without the need of covalent crosslinking agents or further modifications to increase the stability.

C. Nanoflower construction

Once the *f*-SHS solution is prepared, we transfer 200 μL of *f*-SHS (0.303 mM of ImAG) to an Eppendorf tube followed by the addition in equivalents of the guest that will be encapsulated by the *f*-SHS (Fig. S1). For example, to add 5 equiv. of each dextran Texas Red (DTR) polymer solution we transferred 37 μL of 0.0685 mM of stock solution to the corresponding Eppendorf tube. Then all samples were incubated for 1 h at 35 ˚C in a shaker. After this time, the nanoflower was prepared by drop-casting method in copper SEM grids by placing a drop on each grid using a Pasteur pipette. Each grid was held by non-magnetic tweezers located near the heat source (hot plate) for air-dry solvent evaporation. The resulting nanoflower was analyzed in ultrathin carbon film/holey carbon 400 mesh cooper grids without coating the solids (e.g. nanoflowers). We represent encapsulated cargos with an "@", for example, *f*-SHS with encapsulated DTR 3 kDa is represented as *f*-SHS@DTR-3 where "3" is the molecular weight of the DTR in kDa. We use the same nomenclature for other encapsulated cargos in the following studies.

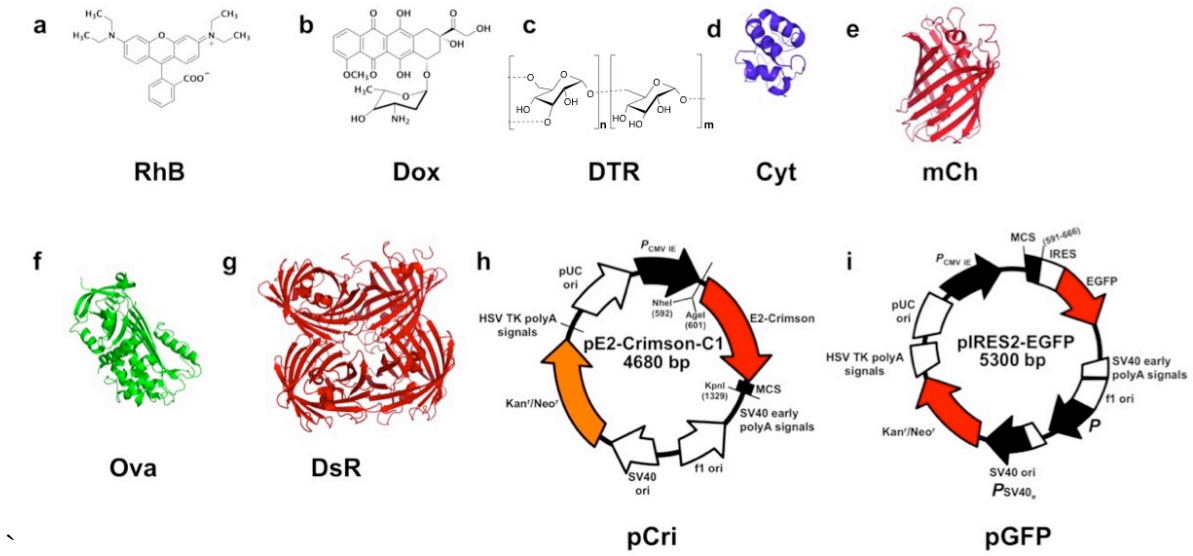

Figure S1. Guests used during the encapsulation process with *f*-SHS: (a) rhodamine b (RhB), (b) doxorubicin (Dox), (c) dextran (n = 18.5 for 3 kDa; n = 61.7 for 10 kDa; n = 431.7 for 70 kDa) labeled with Texas Red (not shown), (d) cytochrome c (Cyt), (e) mCherry (mCh), (f) ovalbumin (Ova), (g) DsRed2 (DsR), (h) pCrimson (pCri) and (i) pGFP. The PDBs of Cyt, mCh, Ova and DsR are 1OCD, 2H5Q, 1UHG and 1G7K respectively.

D. Scanning Electron Microscopy (SEM)

For the SEM experiments we used a high-resolution field emission JEOL JSM-7500F SEM as previously described in section A. The sample of *f*-SHS was described in sections B and C. The same parameters described in sections A were used to obtain the SEM imaged for the controls of *f*-SHS, beads and cargo.

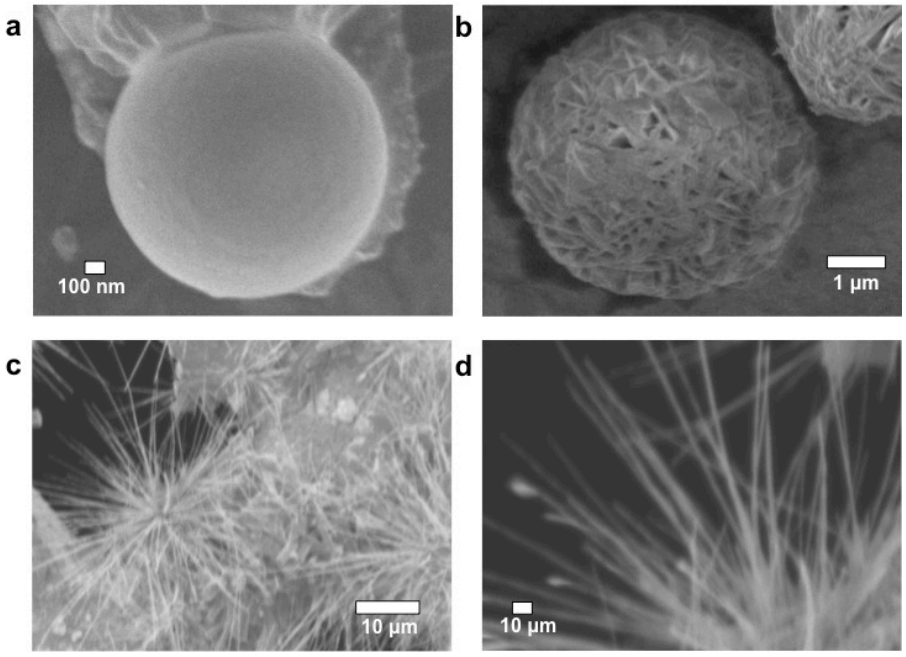

Figure S2. SEM images of *f*-SHS (0.303 mM ImAG, 121 mM KI, in 1X PBS, pH 7.4): (a) *f*-SHS air-dried at 36 °C (zoom X50000), (b) lyophilized *f*-SHS (zoom X17000), (c) *f*-SHS air-dried at 65 °C (zoom X1800) and (d) zoom of image (c) (zoom X1500).

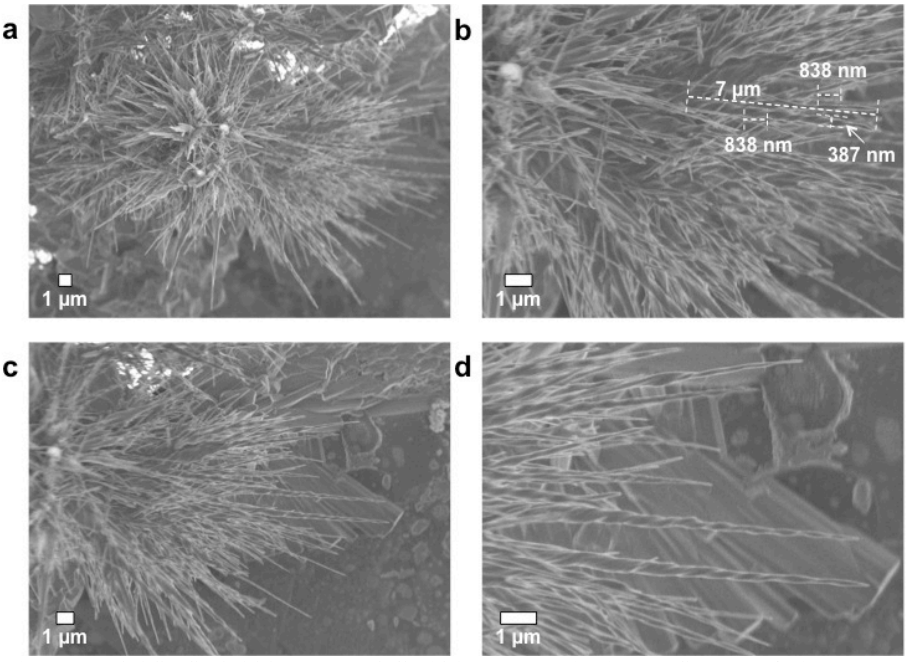

Figure S3. SEM images of *f*-SHS (0.303 mM ImAG, 121 mM KI, in 1X PBS, pH 7.4) incubated with Dox (100 equiv., 0.30 mM). In image S3b the length of the twisted spike is ~7 µm, helical pitch 838 nm and width of 387 nm. The sample was air-dried at 36 °C after 1 h of incubation. Zoom: (a) X3300, (b) X7500, (c) X4500 and (d) X10000.

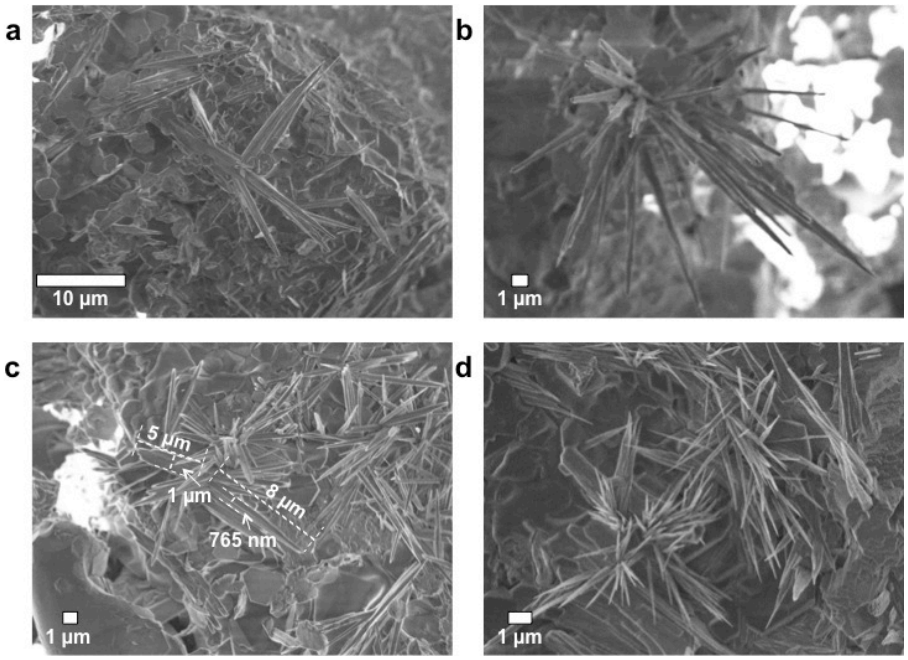

Figure S4. SEM images of *f*-SHS (0.303 mM ImAG, 121 mM KI, in 1X PBS, pH 7.4) incubated with Dox (100 equiv., 0.30 mM). The sample was air-dried at 65 °C after 1 h of incubation. Image S4c shows spike length values of 5-8 µm and width values of 1 µm to 765 nm respectively. Zoom: (a) X2500, (b) X4300, (c) X4000 and (d) X6500.

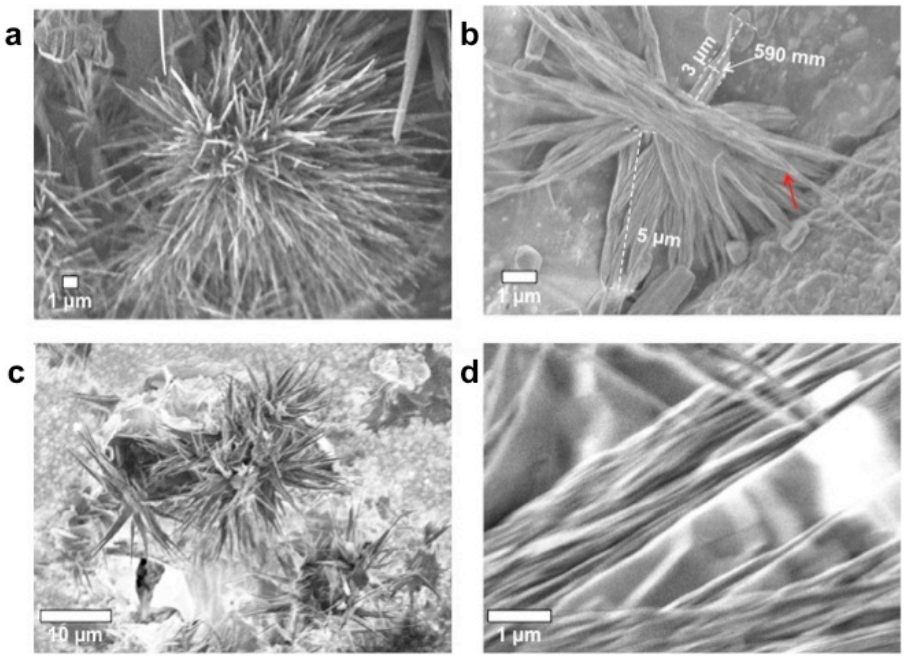

Figure S5. SEM images of *f*-SHS (0.303 mM ImAG, 121 mM KI, in 1X PBS, pH 7.4) incubated with RhB, (10 equiv., 3.8 mM). The sample was air-dried at 36 °C after 1 h of incubation. Image S5b shows spike length values between 3-5 µm with a width around 590 nm. Zoom: (a) X4300, (b) X9500, (c) X2000 and (d) X18000.

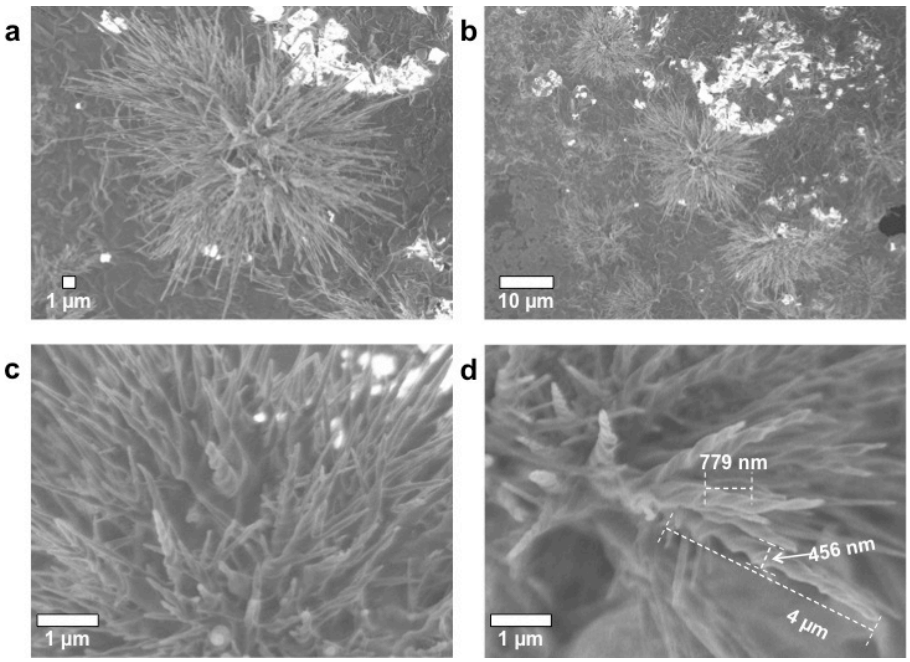

Figure S6. SEM images of *f*-SHS (0.303 mM ImAG, 121 mM KI, in 1X PBS, pH 7.4) incubated with RhB, (10 equiv., 3.8 mM). The sample was air-dried at 65 °C after 1 h of incubation. Image d shows spikes with length values around 4 µm, 456 nm of width and helical pitch of 779 nm. Zoom: (a) X3500, (b) X1500, (c) 17000 and (d) X17000.

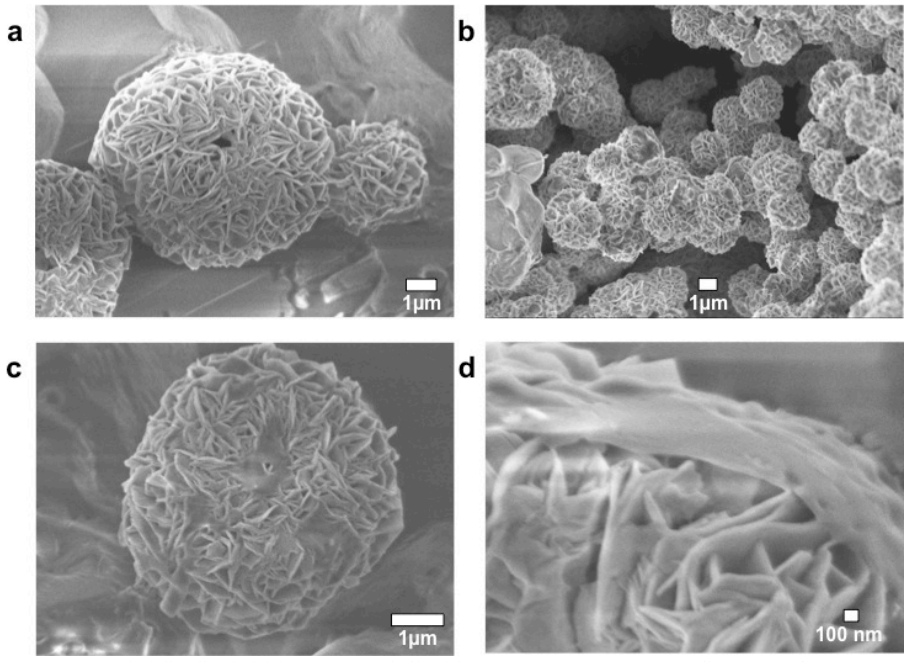

Figure S7. SEM images of *f*-SHS (0.303 mM ImAG, 121 mM KI, in 1X PBS, pH 7.4) incubated with DTR-3 conjugate (5 equiv., 0.07 mM). The sample was air-dried at 36 °C after 1 h of incubation. Zoom: (a) X8500, (b) X5000, (c) X15000 and (d) X40000.

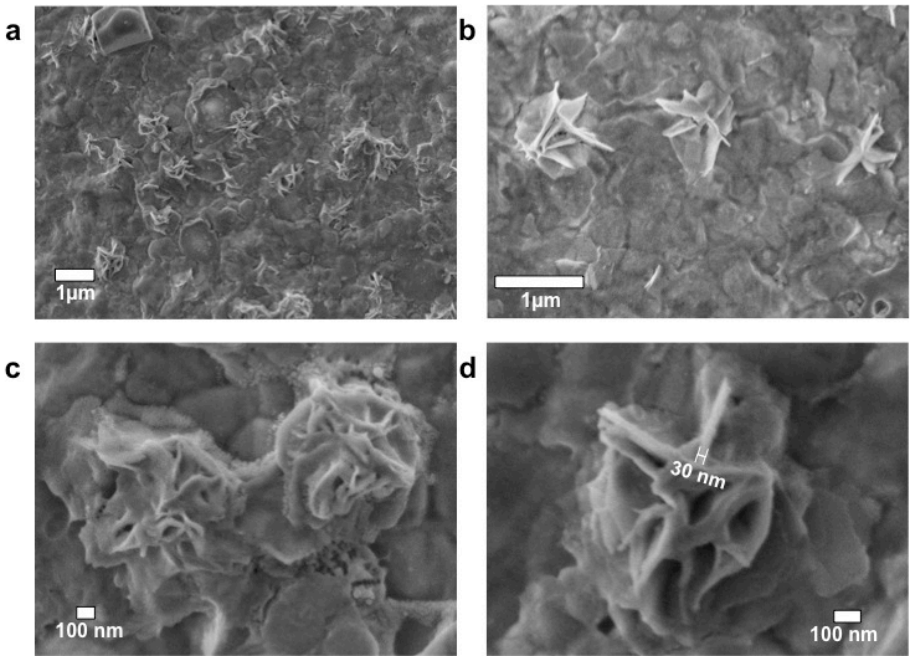

Figure S8. SEM images of *f*-SHS (0.303 mM ImAG, 121 mM KI, in 1X PBS, pH 7.4) incubated with DTR-3 conjugate (5 equiv., 0.07 mM). The sample was air-dried at 65 °C after 1 h of incubation. Image S8d shows a petal thickness of 30 nm. Zoom: (a) X11000, (b) X25000, (c) X50000 and (d) X75000.

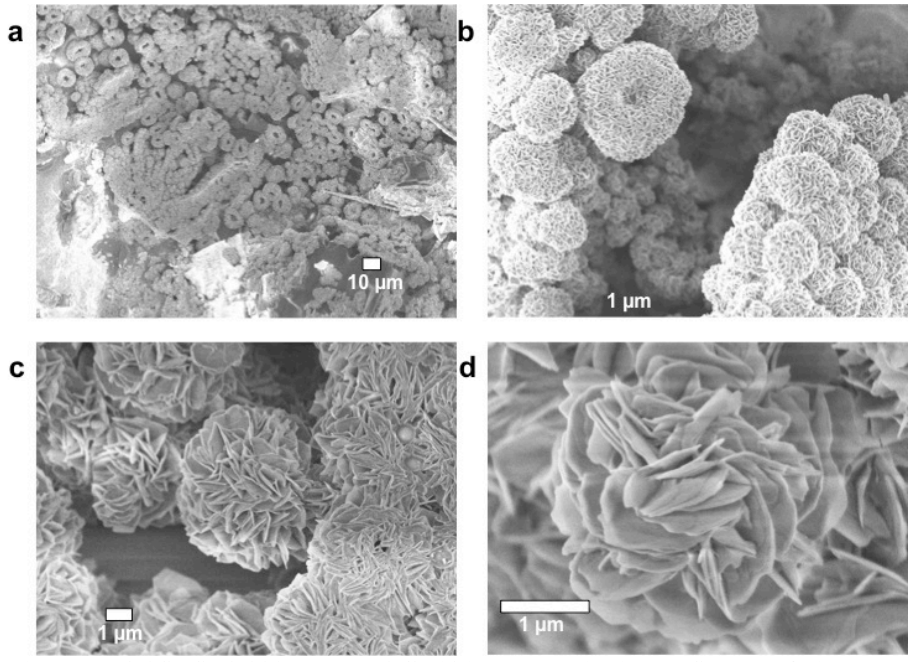

Figure S9. SEM images of *f*-SHS (0.303 mM ImAG, 121 mM KI, in 1X PBS, pH 7.4) incubated with DTR-10 conjugate, (5 equiv., 0.07 mM). The sample was air-dried at 36 °C after 1 h of incubation. Zoom: (a) X500, (b) X4500, (c) X7500 and (d) X25000.

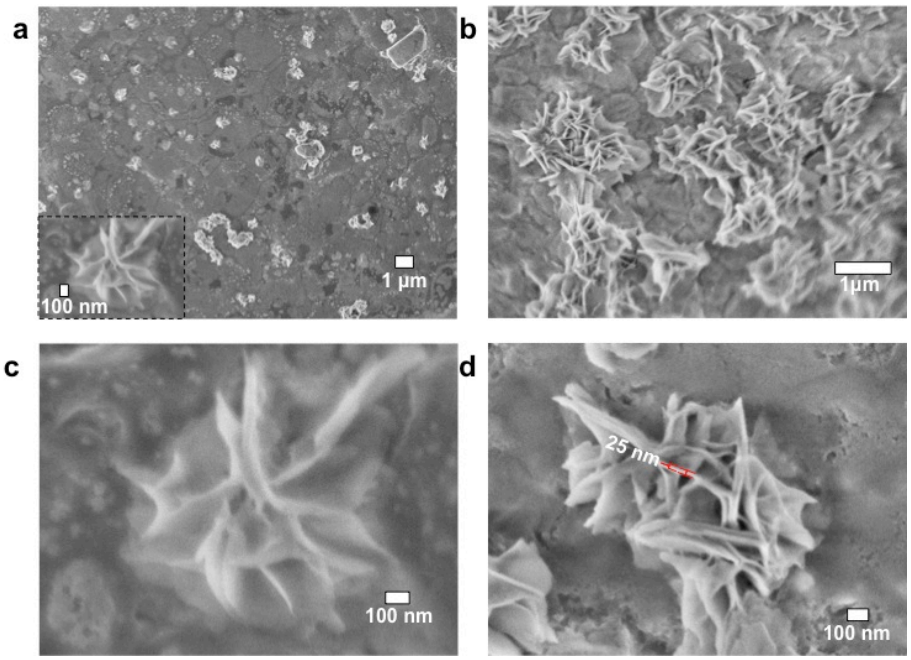

Figure S10. SEM images of *f*-SHS (0.303 mM ImAG, 121 mM KI, in 1X PBS, pH 7.4) incubated with DTR-10 conjugate, (5 equiv., 0.07 mM). The sample was air-dried at 65 °C after 1 h of incubation. The sample was air-dried at 65 °C after 1 h of incubation. Image S9d shows a petal thickness of 25 nm. Zoom: (a) X5000 (inner box X65000), (b) X16000, (c) X19000 and (d) X60000.

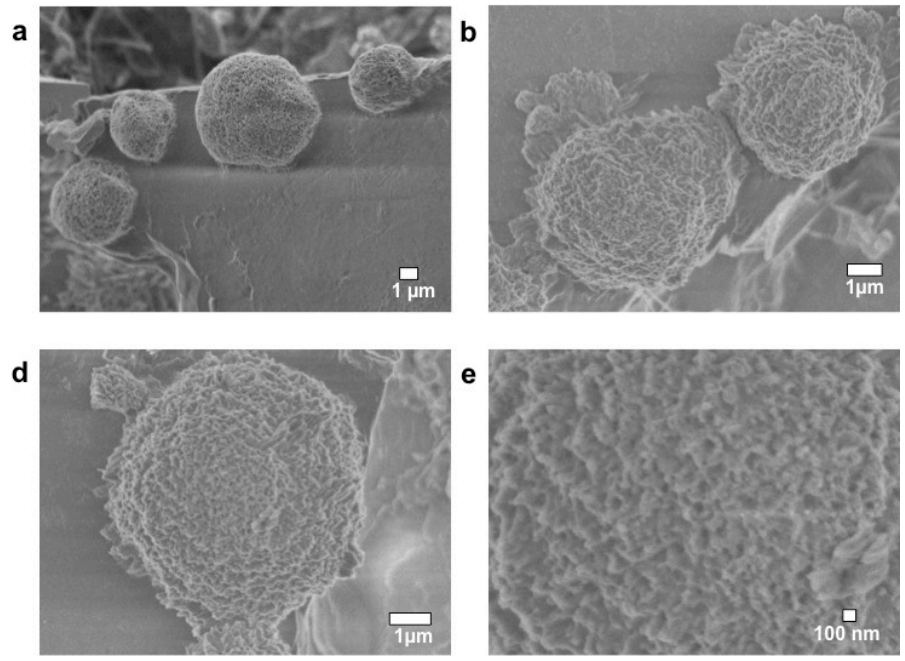

Figure S11. SEM images of *f*-SHS (0.303 mM ImAG, 121 mM KI, in 1X PBS, pH 7.4) incubated with DTR-70 conjugate, (5 equiv., 0.07 mM). The sample was air-dried at 36 °C after 1 h of incubation. Zoom: (a) X5000, (b) X10000, (c) X12000 and (d) X35000.

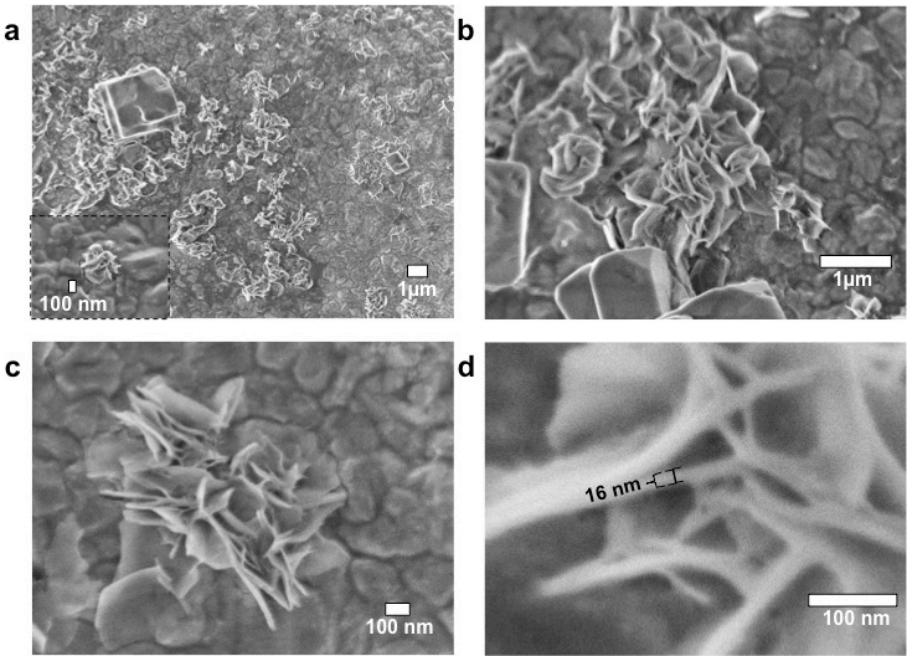

Figure S12. SEM images of *f*-SHS (0.303 mM ImAG, 121 mM KI, in 1X PBS, pH 7.4) incubated with DTR-70 conjugate, (5 equiv., 0.07 mM). The sample was air-dried at 65 °C after 1 h of incubation. Image S12d shows a petal thickness of 16 nm. Zoom: (a) X5500 (inner box X65000), (b) X20000, (c) X100000 and (d) X250000.

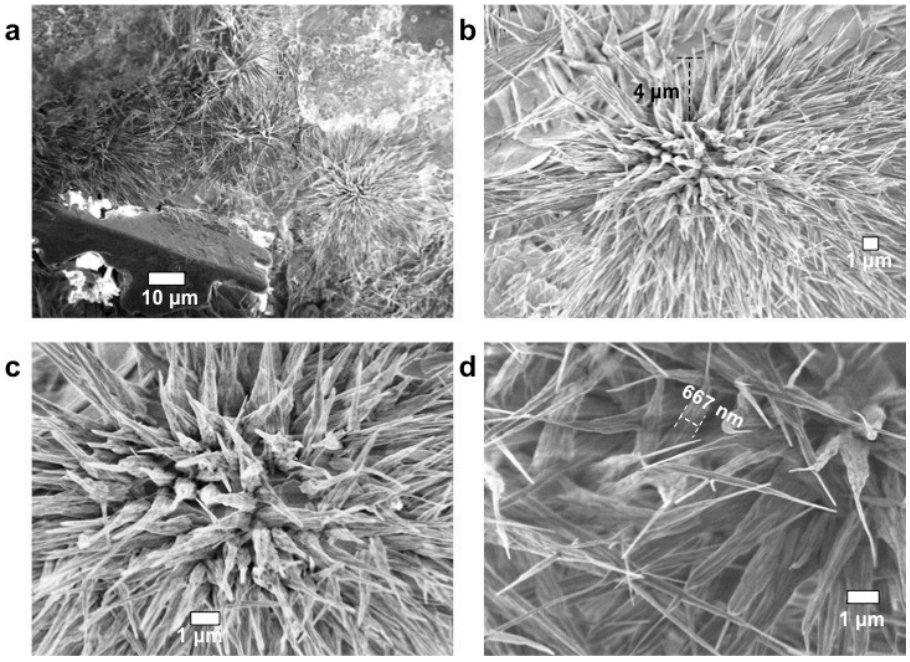

Figure S13. SEM images of *f*-SHS (0.303 mM ImAG, 121 mM KI, in 1X PBS, pH 7.4) incubated with Cyt (0.001 equiv., 0.04 mM). The sample was air-dried at 36 °C after 1 h of incubation. Image S13b shows spikes with length values around 4 µm, while image S13d shows spikes with 667 nm of width. Zoom: (a) X1000, (b) X4000, (c) X8000 and (d) X9000.

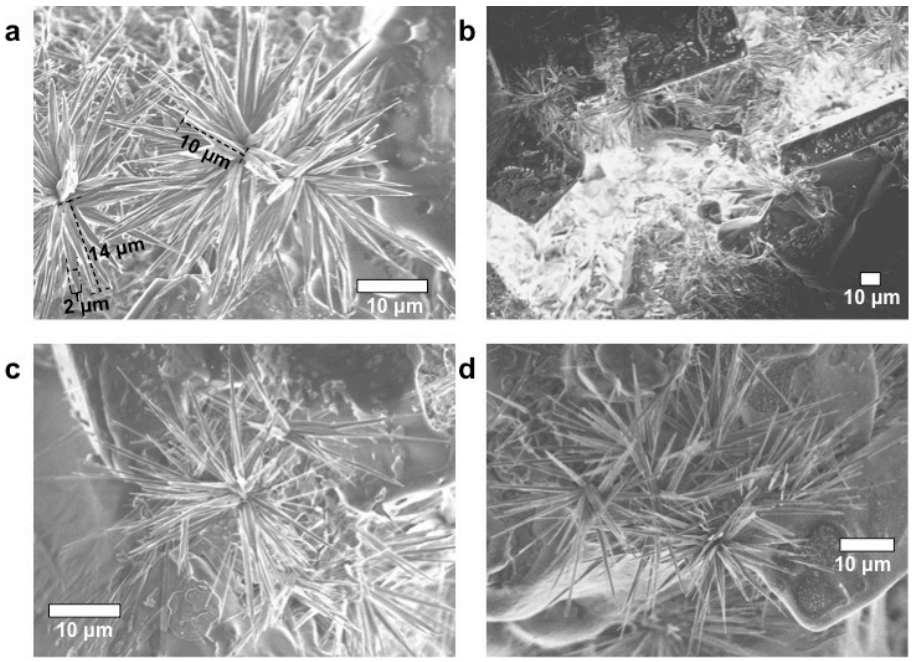

Figure S14. SEM images of *f*-SHS (0.303 mM ImAG, 121 mM KI, in 1X PBS, pH 7.4) incubated with Cyt (0.001 equiv., 0.04 mM). The sample was air-dried at 65 °C after 1 h of incubation. Image S14a shows spikes with length values around 10 µm and 14 µm, including 2 µm of width. Zoom: (a) X2000, (b) X550, (c) X2000 and (d) X1500.

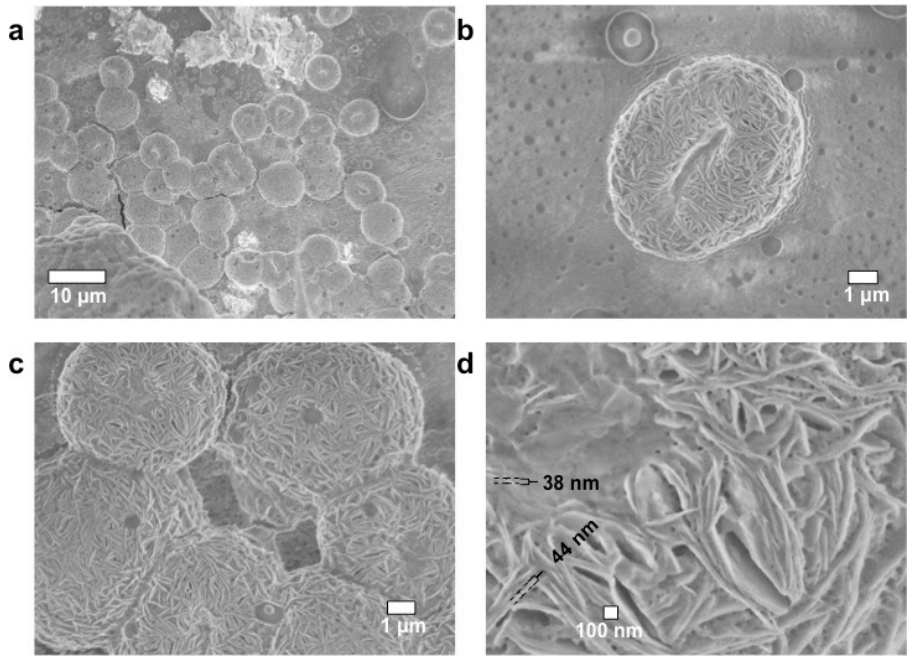

Figure S15. SEM images of *f*-SHS (0.303 mM ImAG, 121 mM KI, in 1X PBS, pH 7.4) incubated with mCh (0.004 equiv., 0.1 mM). The sample was air-dried at 36 °C after 30 min of incubation. Image S15d shows petals with 44 nm of thickness and pores with diameters of 38 nm. Zoom: (a) X1600, (b) X8000. (c) X7500 and (d) X37000.

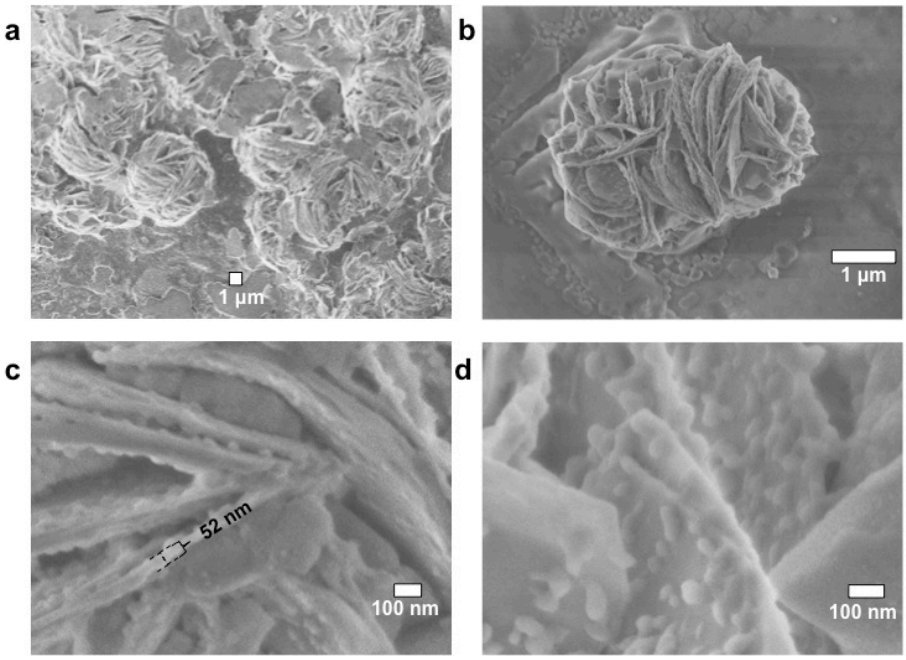

Figure S16. SEM images of *f*-SHS (0.303 mM ImAG, 121 mM KI, in 1X PBS, pH 7.4) incubated with mCh (0.004 equiv., 0.1 mM). The sample was air-dried at 65 °C after 30 min of incubation. Image S16c shows petals with 52 nm of thickness. Zoom: (a) X3500, (b) X18000, (c) X75000 and (d) X100000.

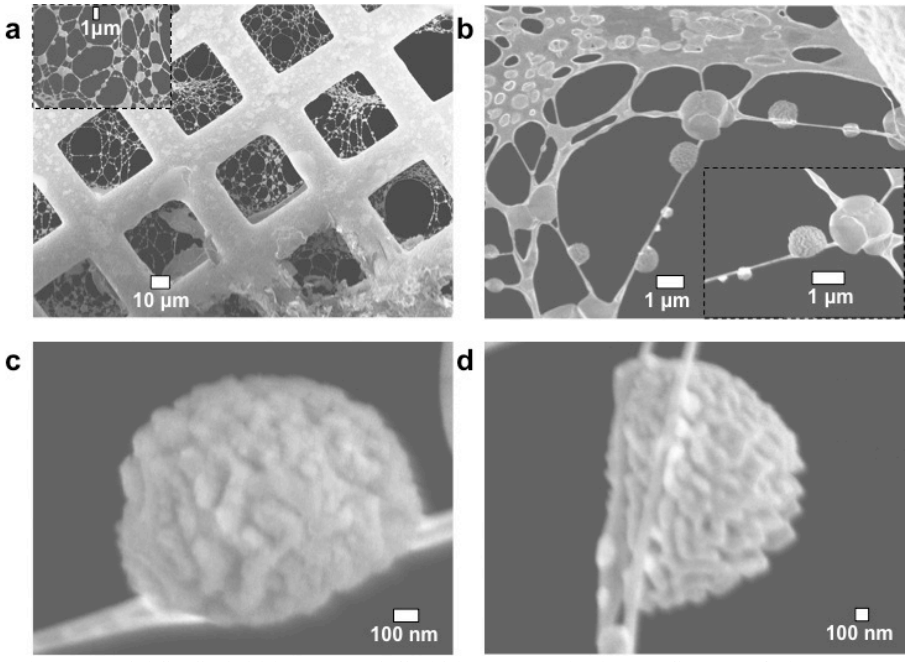

Figure S17. SEM images of *f*-SHS (0.303 mM ImAG, 121 mM KI, in 1X PBS, pH 7.4) incubated with Ova (0.001 equiv., 0.04 mM). The sample was air-dried at 36 °C after 1 h of incubation. Zoom: (a) X500 (inner box X3700), (b) X7500 (inner box X19000), (c) X70000 and (d) X37,000.

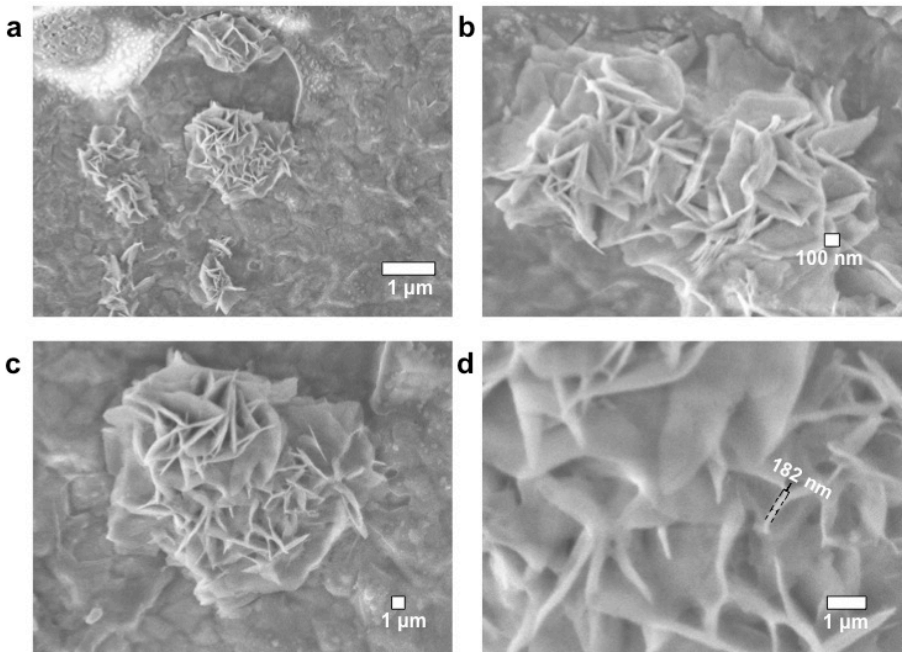

Figure S18. SEM images of *f*-SHS (0.303 mM ImAG, 121 mM KI, in 1X PBS, pH 7.4) incubated with Ova (0.001 equiv., 0.04 mM). Image S18d shows petals with 182 nm of thickness. The sample was air-dried at 65 °C after 1 h of incubation. Zoom: (a) X15000, (b) X40000, (c) X37000 and (d) X110000.

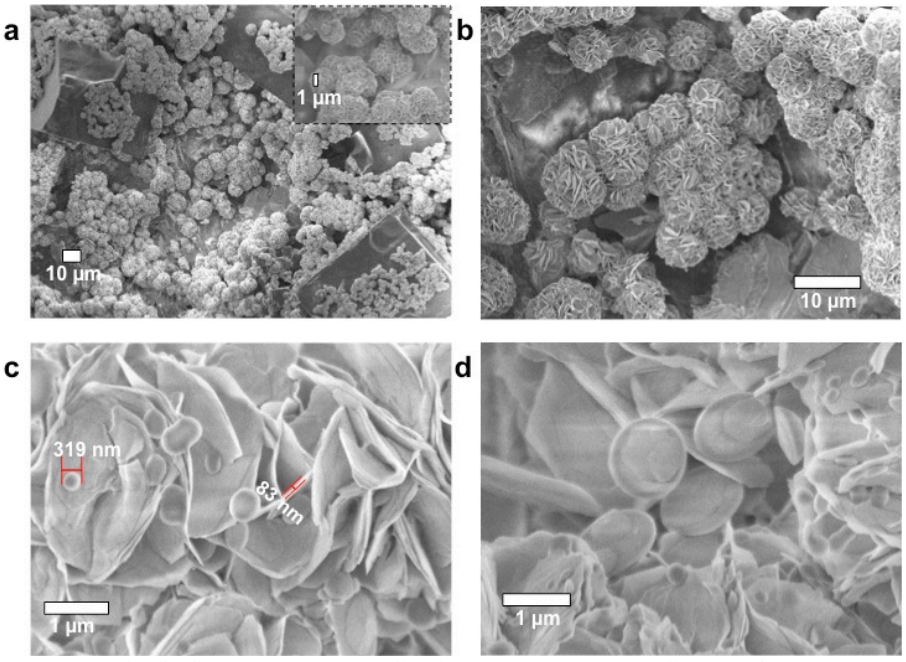

Figure S19. SEM images of *f*-SHS (0.303 mM ImAG, 121 mM KI, in 1X PBS, pH 7.4) incubated with DsR (0.004 equiv., 0.04 mM). The sample was air-dried at 36 °C after 1 h of incubation. Image S19c shows petals with 83 nm of thickness and disc-shaped structures from 319 nm to 1 µm. Zoom: (a) X500 (inner box X3300), (b) X1700, (c) X18000 and (d) X19000.

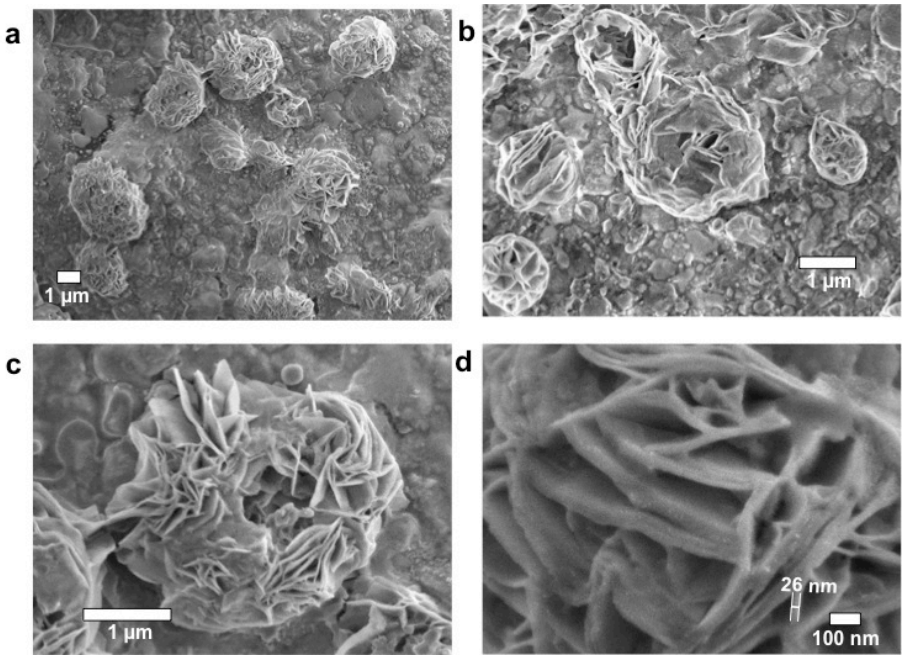

Figure S20. SEM images of *f*-SHS (0.303 mM ImAG, 121 mM KI, in 1X PBS, pH 7.4) incubated with DsR (0.004 equiv., 0.04 mM). The sample was air-dried at 65 °C after 1 h of incubation. Image S20d shows petals with 26 nm of thickness. Zoom: (a) X1600, (b) X16000, (c) X18000 and (d) X27000.

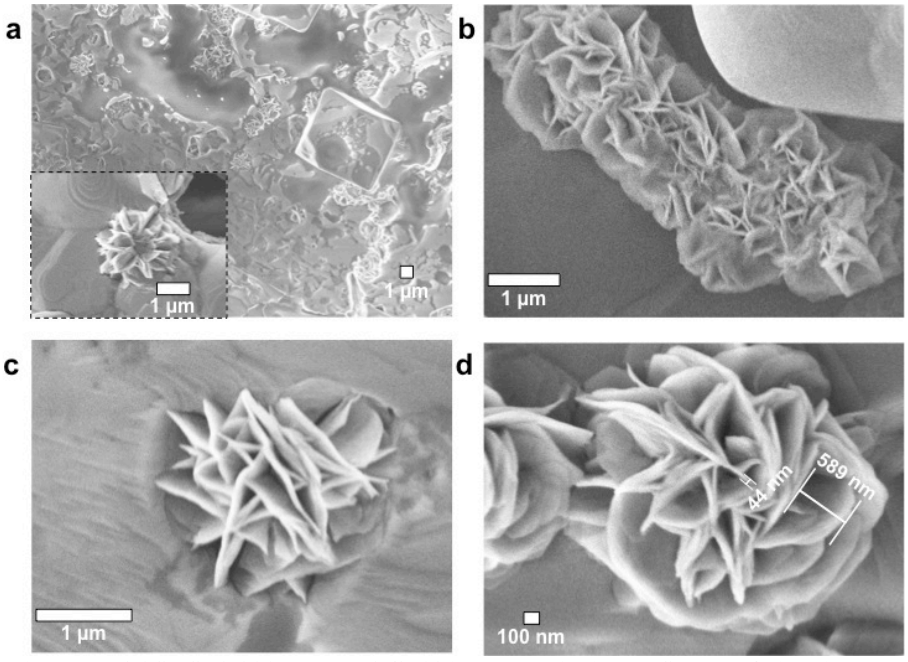

Figure S21. SEM images of *f*-SHS (0.303 mM ImAG, 121 mM KI, in 1X PBS, pH 7.4) incubated with pCri (0.001 equiv., 0.003 mM). The sample was air-dried at 36 °C after 1 h of incubation. Image S21d shows petals with 589 nm of length and 44 nm of thickness. Zoom: (a) X3500 (inner box X20000), (b) X20000, (c) X27000 and (d) X43000.

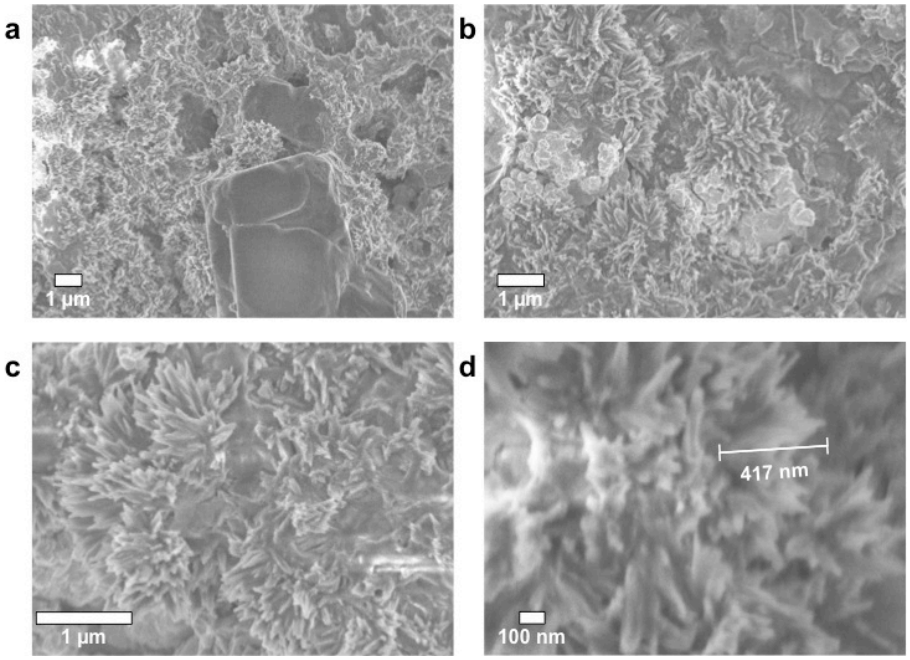

Figure S22. SEM images of *f*-SHS (0.303 mM ImAG, 121 mM KI, in 1X PBS, pH 7.4) incubated with pCri (0.001 equiv., 0.003 mM). The sample was air-dried at 65 °C after 1 h of incubation. Image S22d shows petals with 417 nm of length. Zoom: (a) X7500, (b) X13000, (c) X27000 and (d) X70000.

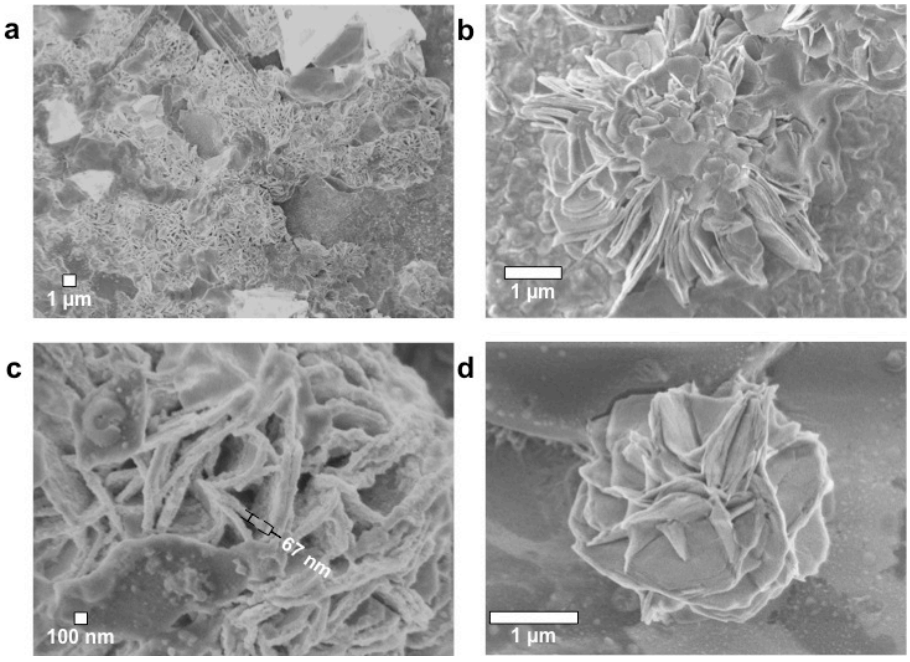

Figure S23. SEM images of *f*-SHS (0.303 mM ImAG, 121 mM KI, in 1X PBS, pH 7.4) incubated with pGFP (0.003 equiv., 0.003 mM). The sample was air-dried at 36 °C after 1 h of incubation. Image S23c shows petals with 67 nm of thickness. Zoom: (a) X3300, (b) X16000, (c) X33000 and (d) X25000.

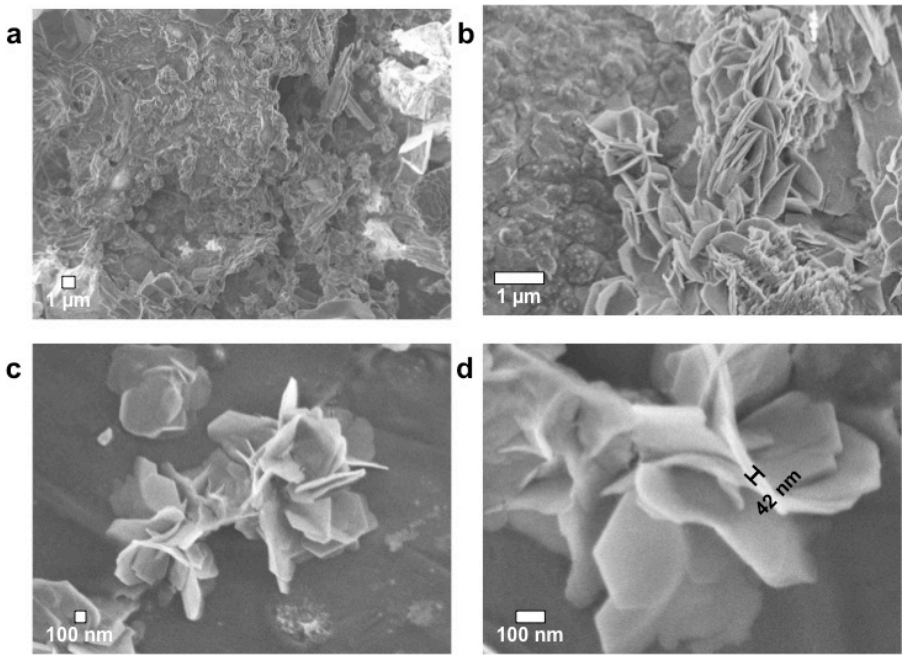

Figure S24. SEM images of *f*-SHS (0.303 mM ImAG, 121 mM KI, in 1X PBS, pH 7.4) incubated with pGFP (0.003 equiv., 0.003 mM). The sample was air-dried at 65 °C after 1 h of incubation. Image S24d shows petals with 42 nm of thickness. Zoom: (a) X3500, (b) X14000, (c) X30000, and (d) 80000.

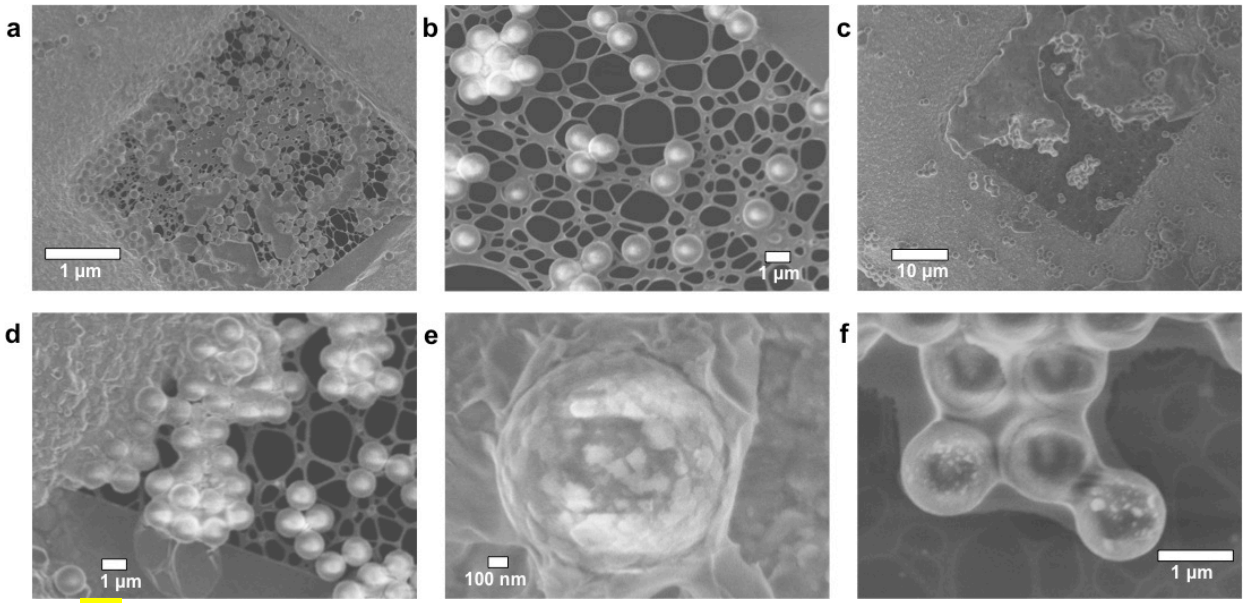

Figure S25. SEM images of 0.303 mM polystyrene beads: (a) polystyrene beads (control), X2300, (b) polystyrene beads incubated with DTR-3 (5 equiv., 0.07 mM), X7500, (c) polystyrene beads incubated with Rh B (10 equiv., 3.4 mM), X1700 (d) X7500, (e) X60000, and (f) 23,000 are zooms of a, b, and c, respectively. The samples were air-dried at 36 °C after 1 h of incubation.

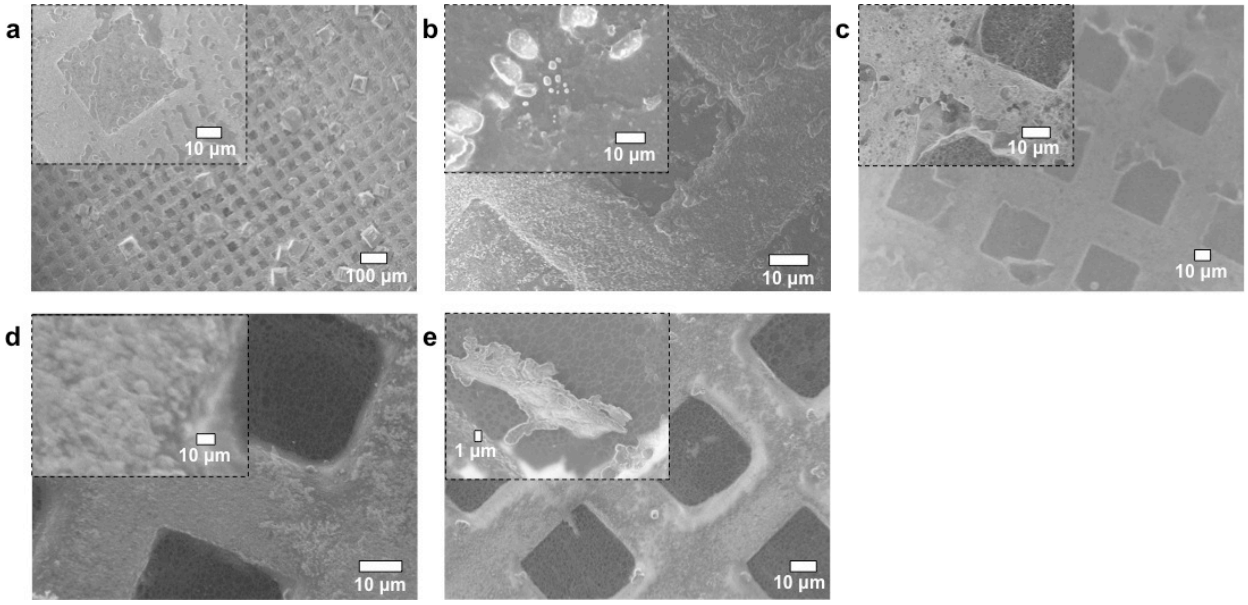

Figure S26. SEM images of the cargos used for encapsulation alone without *f*-SHS: (a) 0.3 mM Dox, X80, (b) 3.8 mM Rh B, X1200, (c) 0.07 mM DTR-3, X500, (d) 0.003 mM pGFP, X1300, and (e) 0.003 mM pCri, X800. The samples were air-dried at 36 °C.

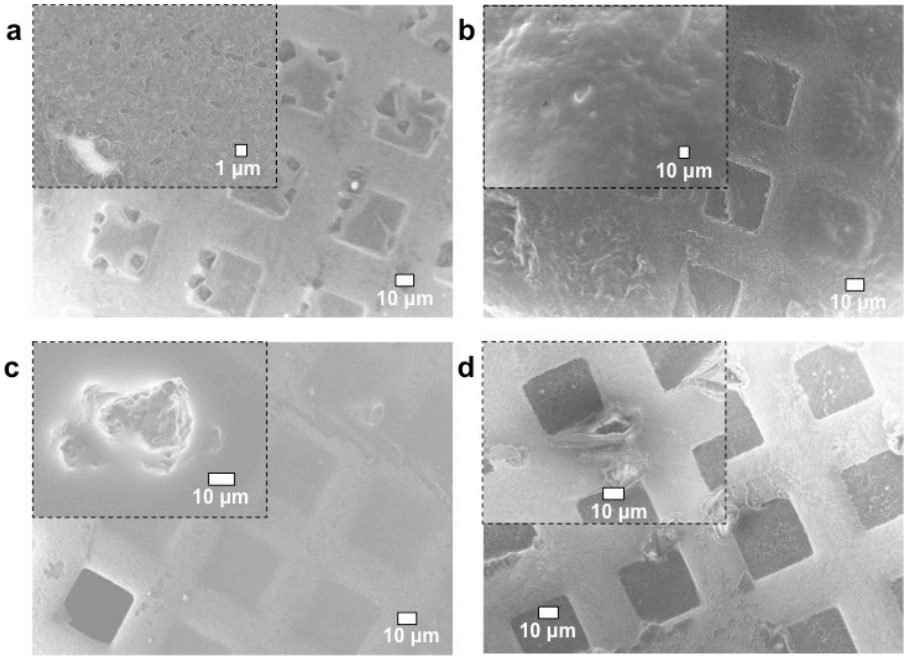

Figure S27. SEM images of the cargos used for encapsulation alone without *f*-SHS: (a) 0.04 mM Ova, X500, (b) 0.1 mM mCh, X500, (c) 0.04 mM DsR, X500, and (d) 0.04 mM Cyt, X550. The samples were air-dried at 36 °C.

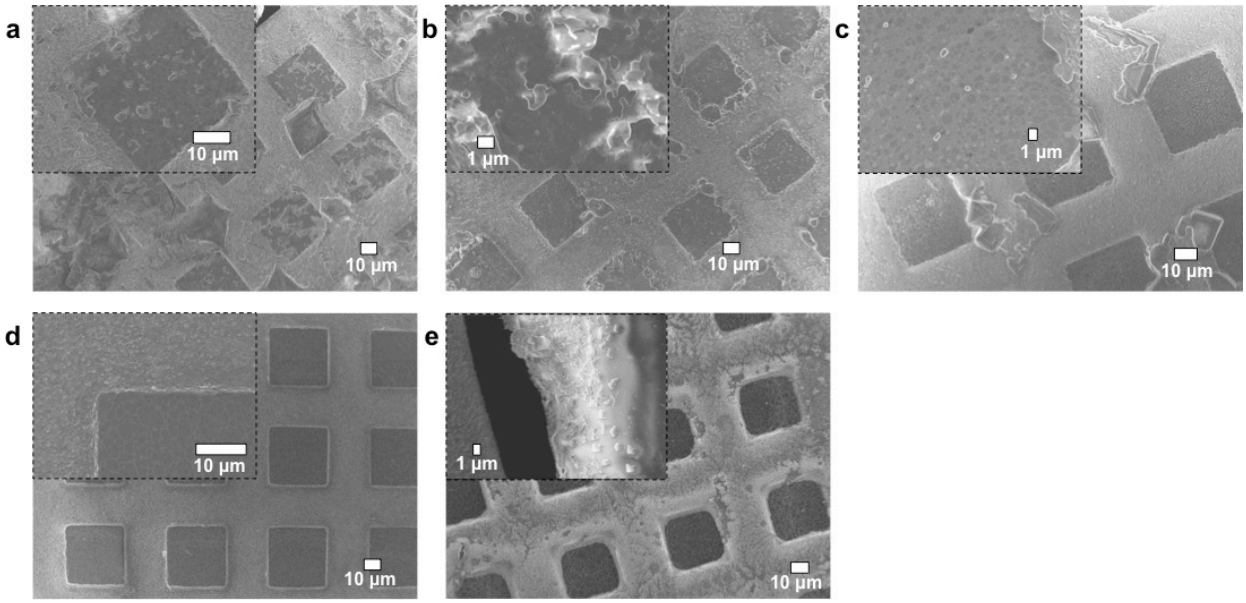

Figure S28. SEM images of the cargos used for encapsulation alone without *f*-SHS: (a) 0.3 mM Dox, X500, (b) 3.8 mM Rh B, X500, (c) 0.07 mM DTR-3, X700, (d) 0.003 mM pGFP, X500, and (e) 0.003 mM pCri, X550. The samples were air-dried at 65 °C.

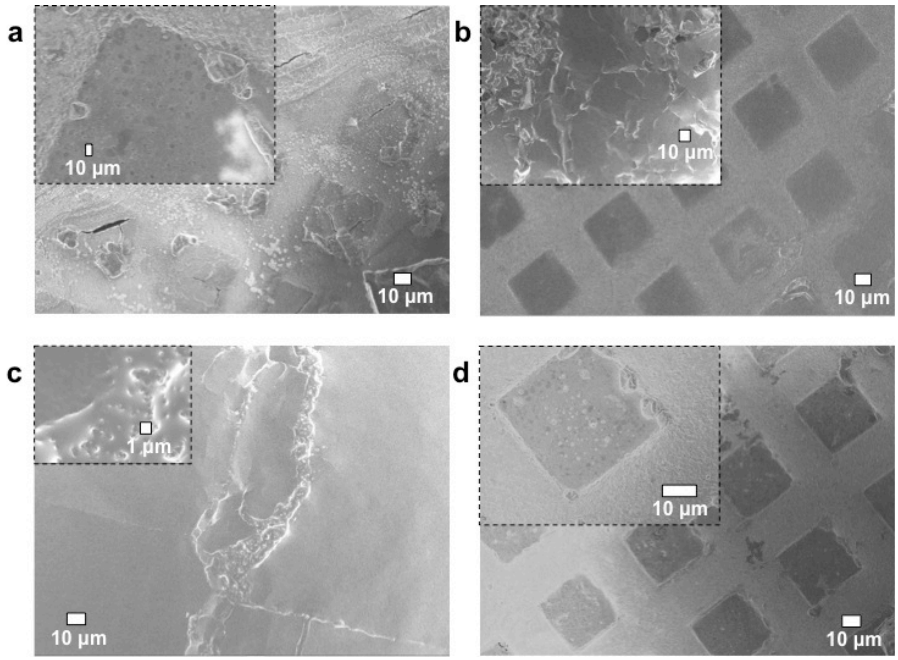

Figure S29. SEM images of the cargos used for encapsulation alone without *f*-SHS: (a) 0.04 mM Ova, X500, (b) 0.1 mM mCh, X450, (c) 0.04 mM DsR, X500, and (d) 0.04 mM Cyt, X500. The samples were air-dried at 65 °C.

Table S1. Average measurements approximation in triplicate of some morphological features of the resulting NF air-dried at 35 °C. The measurements were taken manually with the scale bars of the SEM images.

Sample	Average Length (nm)	Average Width (nm)	Average Thickness (nm)	Average Pitch (nm)
f-SHS	n/a	n/a	n/a	n/a
f-SHS@RhB	4,000 ± 1	496 ± 82	n/a	n/a
f-SHS@Dox	6,000 ± 1	387 ± 1	n/a	903 ± 57
f-SHS@Cyt	5,000 ± 1	519 ± 189	n/a	n/a
f-SHS@mCh	113 ± 11	n/a	58 ± 13	n/a
f-SHS@Ova	n/a	n/a	91 ± 16	n/a
f-SHS@DsR	403 ± 100	n/a	93 ± 16	n/a
f-SHS@DTR-3	n/a	n/a	98 ± 6	n/a
f-SHS@DTR-10	n/a	n/a	67 ± 7	n/a
f-SHS@DTR-70	75 ± 16	n/a	n/a	n/a
f-SHS@pCri	331 ± 224	n/a	54 ± 8	n/a
f-SHS@pGFP	n/a	n/a	80 ± 13	n/a

Table S2. Average measurements approximation in triplicate of some morphological features of the resulting NF air-dried at 65 °C. The measurements were taken manually with the scale bars of the SEM images.

Sample	Average Length (nm)	Average Width (nm)	Average Thickness (nm)	Average Pitch (nm)
f-SHS	49 ± 19	261 ± 114	n/a	n/a
f-SHS@RhB	3 ± 1	363 ± 90	n/a	n/a
f-SHS@Dox	6 ± 2	955 ± 172	n/a	701 ± 140
f-SHS@Cyt	14 ± 5	2 ± 0	n/a	n/a
f-SHS@mCh	n/a	n/a	80 ± 30	n/a
f-SHS@Ova	n/a	n/a	265 ± 73	n/a
f-SHS@DsR	n/a	n/a	39 ± 13	n/a
f-SHS@DTR-3	n/a	n/a	35 ± 29	n/a
f-SHS@DTR-10	n/a	n/a	36 ± 13	n/a
f-SHS@DTR-70	n/a	n/a	18 ± 3	n/a
f-SHS@pCri	383 ± 48	n/a	n/a	n/a
f-SHS@pGFP	n/a	n/a	38 ± 7	n/a

E. Confocal Laser Scanning Microscopy

For encapsulation experiments we used a Zeiss LSM 510 Meta Confocal Microscope with an excitation range of 405 nm, 458 nm, 477 nm, 488 nm, 514 nm, 561 nm, 633 nm and emission range of 400-730 nm. After sample incubation (1 h at 35 ˚C) process for encapsulated guests in

f-SHS described in section E, we mixed 2.5 µL of sample with 2.5 µL of 90% glycerol and 10% 1X PBS in a microscope slide (purchased from Fisher Scientific, 3" x 1" x 1.00 mm). All the fluorescence confocal microscopy images and movie were processed in Zeiss LSM Image Browser software. In the case of the movie, the sample was observed in 2 well-cubed coverslip chambers filled with PBS (1X at pH 7.4).

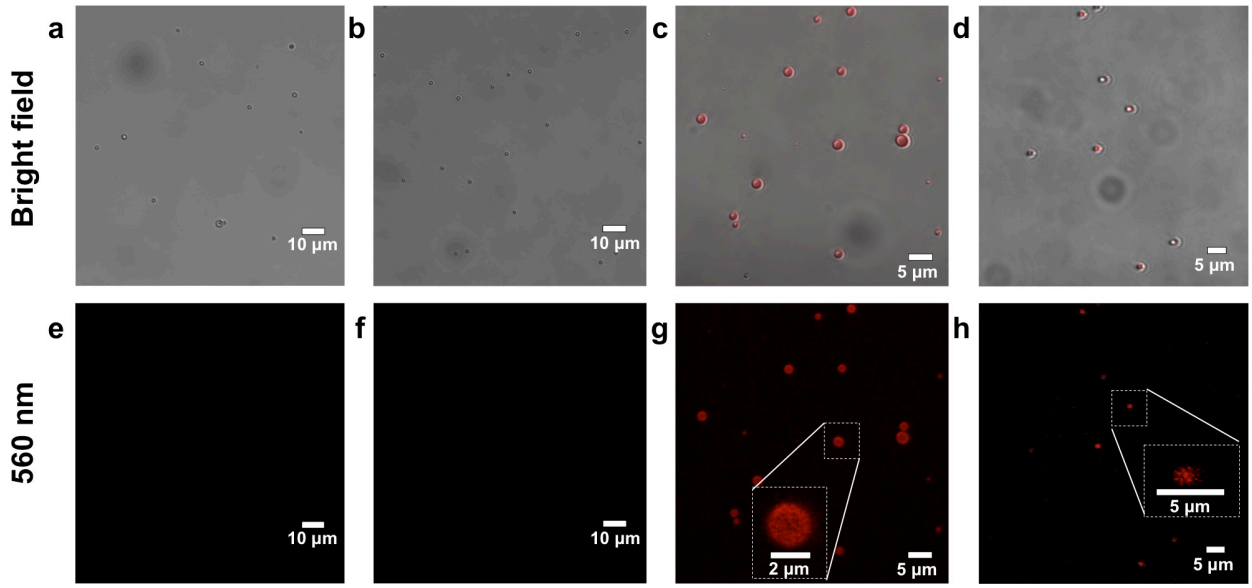

Figure S29. CLSM images of: (a) *f*-SHS (0.303 mM ImAG, 121 mM KI, (b) 0.303 mM polystyrene beads, (c) *f*-SHS (0.303 mM ImAG, 121 mM KI) incubated with DTR-3 (5 equiv., 0.07 mM), and (d) 0.303 mM polystyrene beads incubated with DTR-3 (5 equiv., 0.07 mM). Excitation wavelength used was 561 nm with a LP 575 emission filter (1X PBS, pH 7.4, 25 ˚C).

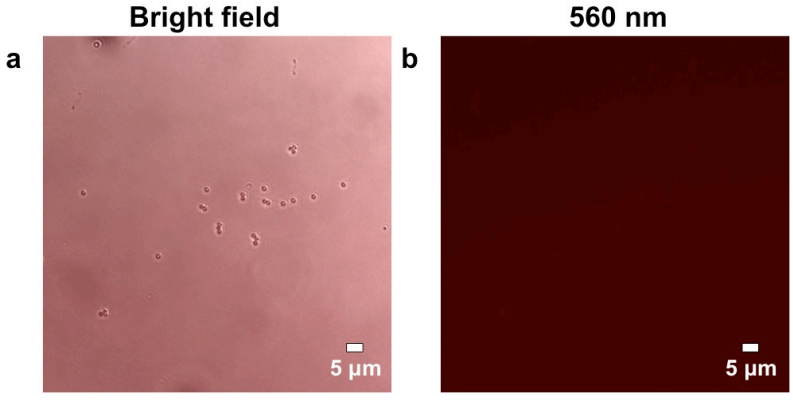

Figure S30. CLSM images of 0.303 mM polystyrene beads incubated with RhB (10 equiv., 3.8 mM). Excitation wavelength used was 561 nm with a BP 575-615 IR emission filter (1X PBS, pH 7.4, 25 ˚C).

F. Dynamic Light-Scattering (DLS) Experiments

A Zetasizer Nano ZS (model ZEN3600) from Malvern with a 4 mW laser of 632.8 nm wavelength and a backscatter angle of 173˚ was used to measure the hydrodynamic size of *f*-SHS, *f*-SHS with encapsulated guest and the controls at 25 ˚C. The parameters in size mode performed for the DLS experiments are described in section C. The results were extracted from Malvern Zetasizer Software version 7.10 to construct the graphs using Excel and Graph Pad PRISM version 5.0.

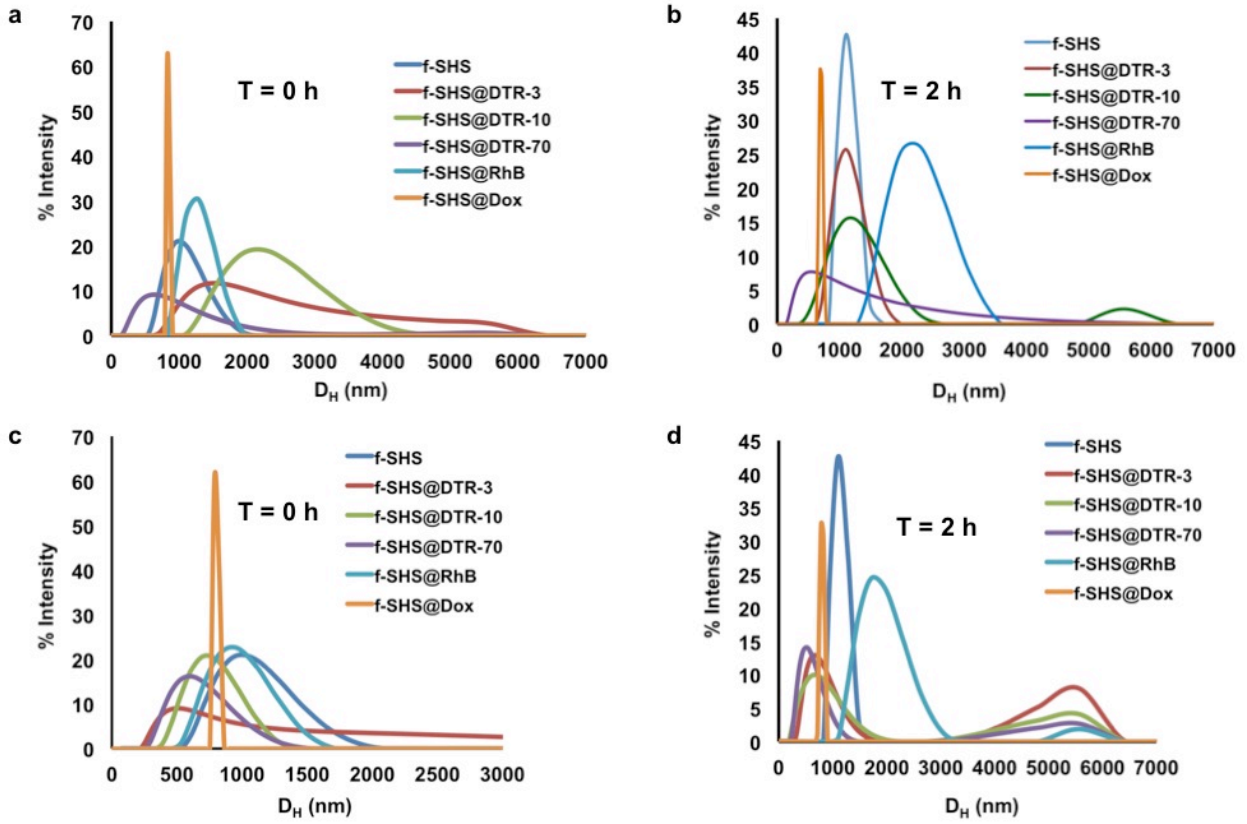

Figure S31. DLS studies of *f*-SHS (0.303 mM ImAG, 121 mM KI, 1X PBS, pH 7.4, 25 ˚C) incubated with: (a) 5 equiv. of different cargos at 0 h, (b) 5 equiv. of different cargos after 2 h, (c) 10 equiv. of different cargos at 0 h and (d) 10 equiv. of different cargos after 2 h. The cargos used during the incubation with *f*-SHS are 0.07 mM DTR-3, 0.07 mM DTR-10, 0.07 mM DTR-70, 3.8 mM RhB and 0.3 mM Dox.

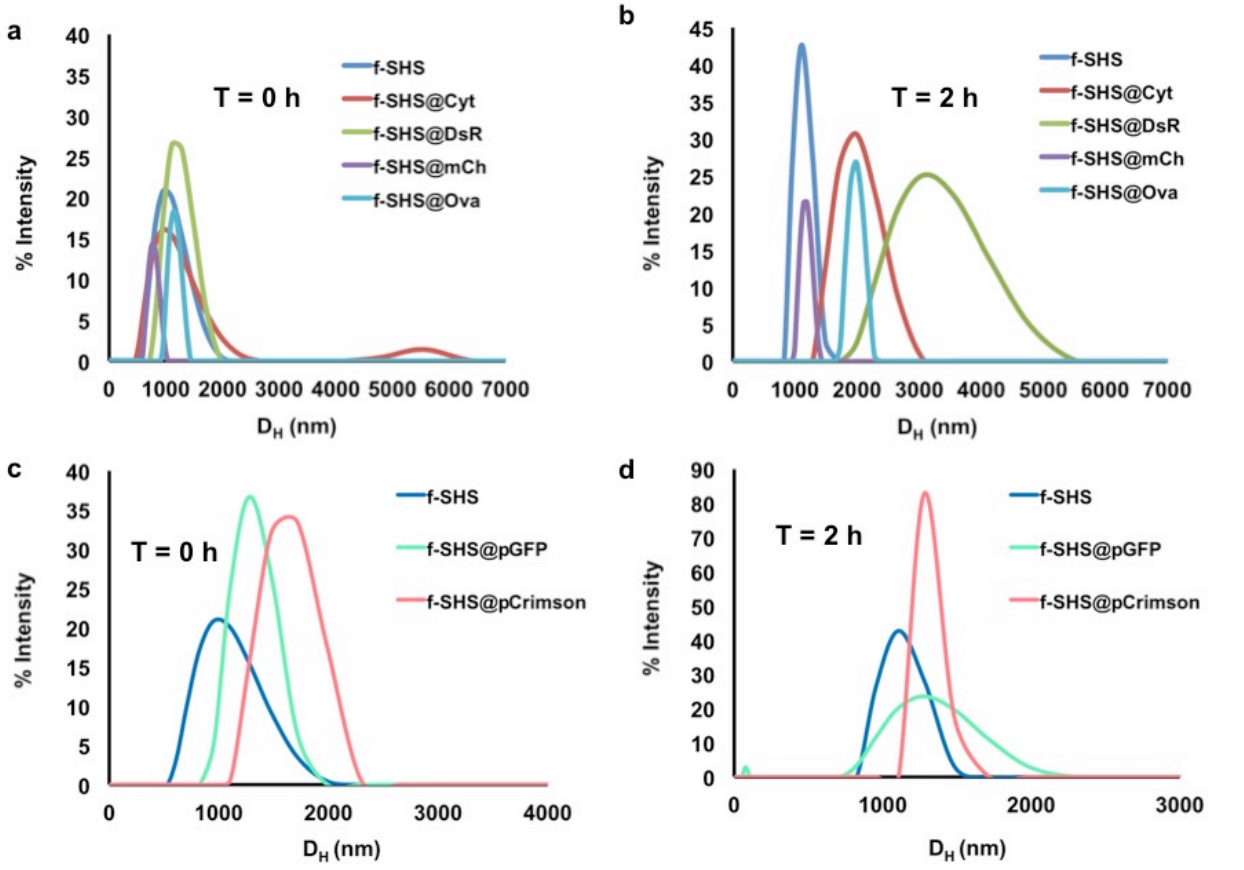

Figure S32. DLS studies of *f*-SHS (0.303 mM ImAG, 121 mM KI, 1X PBS, pH 7.4, 25 ˚C) incubated with: (a) different proteins at 0 h, and (b) after 2 h. The proteins (0.04 mM) used for S34a and S34b are 0.001 equiv. Cyt, 0.004 equiv. DsR, 0.004 equiv. mCh and 0.001 equiv. Ova. Incubation of different plasmids (0.003 mM) at (c) 0 h and (d) after 2 h was performed with 0.003 equiv. pGFP and 0.001 equiv. pCri.

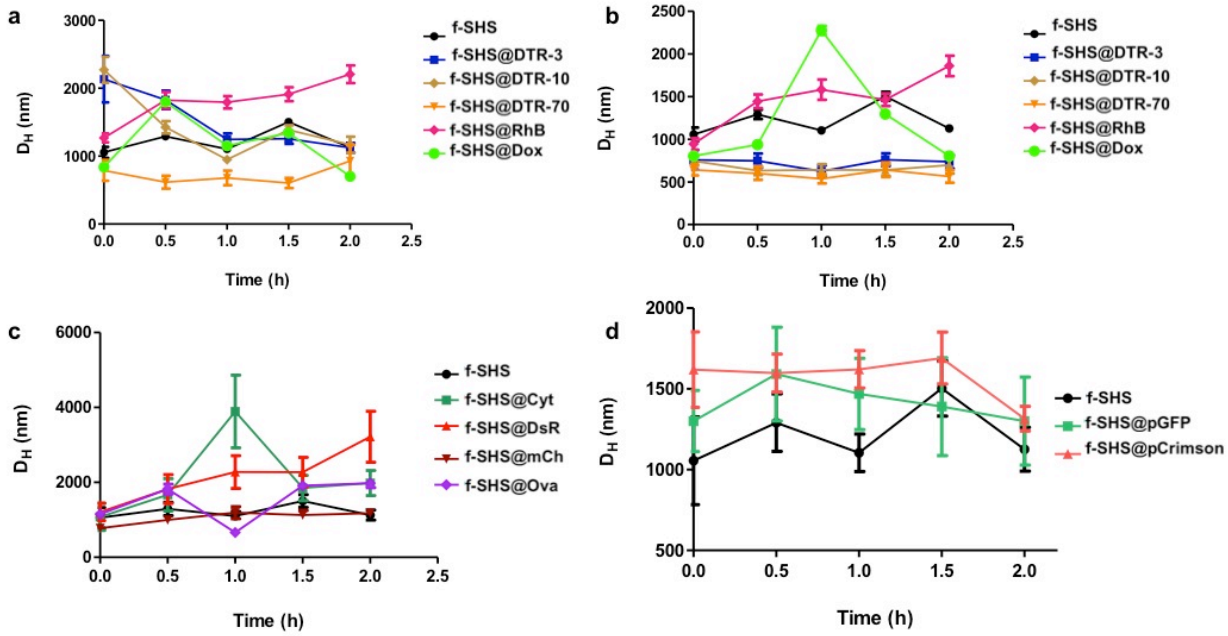

Figure S33. DLS studies of D_H as a function of time of *f*-SHS (0.303 mM ImAG, 121 mM KI, 1X PBS, pH 7.4, 25 ˚C) incubated with: (a) 5 equiv. DTR-3, DTR-10, DTR-70, RhB and Dox. (b) 10 equiv. DTR-3, DTR-10, DTR-70, RhB and Dox. (c) 0.001 equiv. Cyt, 0.004 equiv. DsR, 0.004 equiv. mCh and 0.001 equiv. Ova. (d) 0.003 equiv. pGFP and 0.001 Equiv. pCri. The concentration values appear in Supplementary Figures S34 and S35.

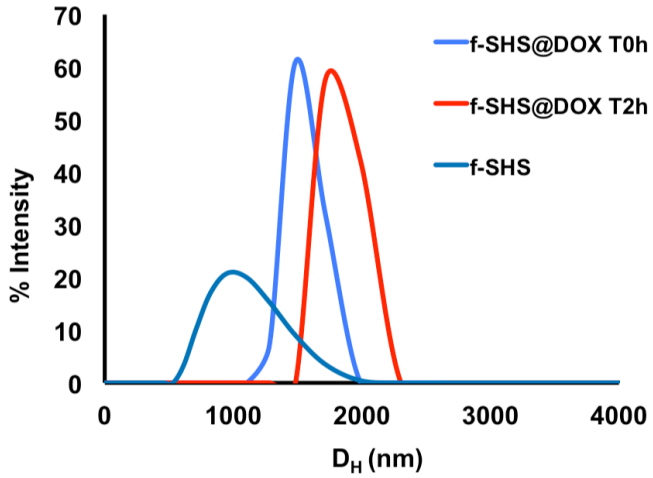

Figure S34. DLS studies of *f*-SHS (0.303 mM ImAG, 121 mM KI, 1X PBS, pH 7.4, 25 ˚C) incubated with 100 equiv. of 0.3 mM Dox.

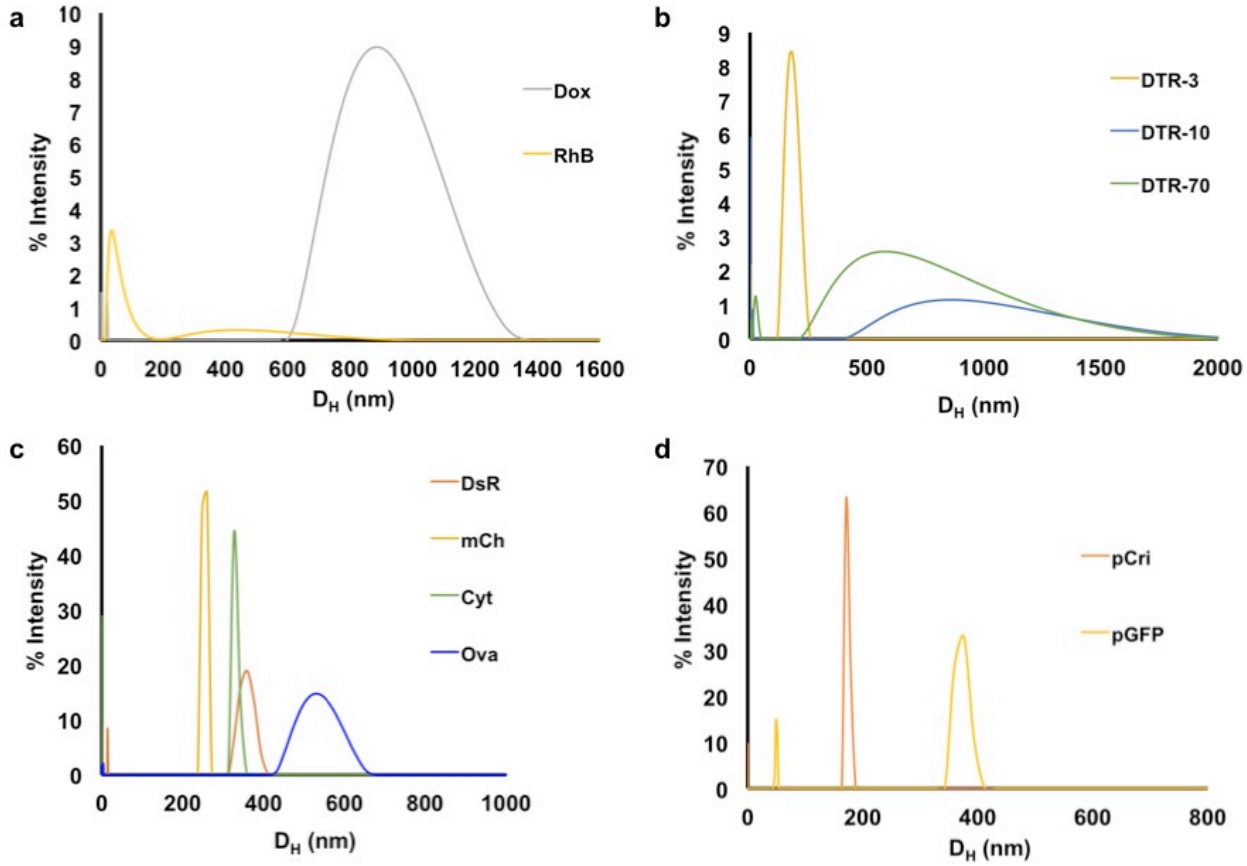

Figure S35. DLS control studies without *f*-SHS of: (a) 0.3 mM Dox 3.8 mM and RhB. (b) 0.07 mM of DTR-3, DTR-10 and DTR-70. (c) 0.04 mM of DsR, mCh, Cyt and Ova. (d) 0.003 mM of pCri and pGFP. Experiments were performed in 1X PBS at pH 7.4 and 25 ˚C.

G. Zeta Potential

Zeta potential experiments were performed also with Zetasizer Nano ZS (model ZEN3600) from Malvern Instruments Ltd. with the parameters described in section C. The samples were measured in disposable folded capillary cells (DTS1070) from Malvern Instruments Ltd. The results were extracted from Malvern Zetasizer Software version 7.10 to construct the graphs using Excel and Graph Pad PRISM version 5.0.

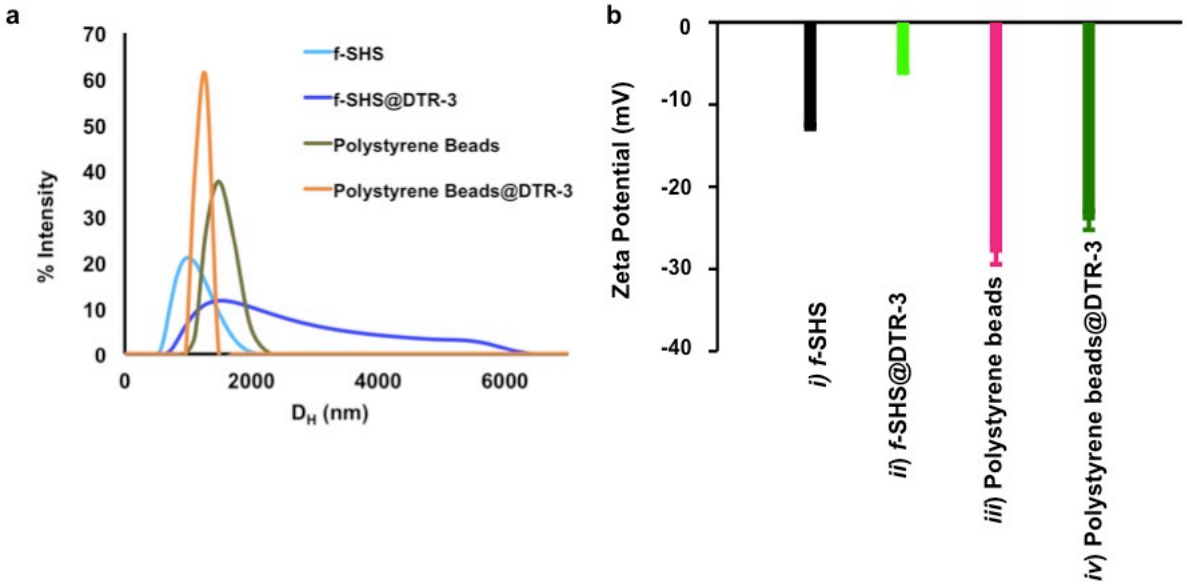

Figure S36. (a) DLS and (b) ZP studies of *f*-SHS (0.303 mM ImAG, 121 mM KI); 0.303 mM polystyrene beads; *f*-SHS incubated with DTR-3 (5 equiv., 0.07 mM); and polystyrene beads incubated with DTR-3 (5 equiv., 0.07 mM). Samples were incubated at 30 min at 35 ˚C. Experiments were performed in 1X PBS at pH 7.4 and 25 ˚C.

Table S3. Dynamic light scattering (DLS) and zeta potential (ZP) values of the *f*-SHS@Guest complexes after 1 h of incubation and before air-drying to form the organic NF (0.303 mM ImAG, 121 mM KI, in 1X PBS, pH 7.4).

Sample	DLS (nm)	Zeta potential (mV)
***f*-SHS**	1104 ± 116	-12.7 ± 0.3
***f*-SHS@RhB**	1580 ± 393	-8.8 ± 0.6
***f*-SHS@Dox**	2006 ± 122	6.9 ± 0.7
***f*-SHS@Cyt**	3892 ± 969	-8.2 ± 1.1
***f*-SHS@mCh**	1191 ± 162	-11.9 ± 1.4
***f*-SHS@Ova**	664 ± 14	-8.2 ± 0.5
***f*-SHS@DsR**	2275 ± 439	-8.1 ± 0.5
***f*-SHS@DTR-3**	1246 ± 291	-5.9 ± 0.2
***f*-SHS@DTR-10**	949 ± 101	-1.2 ± 0.2
***f*-SHS@DTR-70**	679 ± 356	0.4 ± 0.2
***f*-SHS@pCri**	1621 ± 116	-9.4 ± 0.3
***f*-SHS@pGFP**	1469 ± 221	-8.5 ± 0.4

Table S4. Zeta Potential (ZP) control studies without *f*-SHS of 3.8 mM RhB, 0.3 mM Dox, 0.04 mM Cyt, 0.04 mM mCh, 0.04 mM Ova, 0.04 mM DsR, 0.07 mM DTR-3, 0.07 mM DTR-10, 0.07 mM DTR-70, 0.003 mM pCri and 0.003 mM pGFP. The table also includes the ZP values of 0.303 mM polystyrene beads (beads) and polystyrene beads incubated with DTR-3 (5 equiv., 0.07 mM). Experiments were performed in 1X PBS at pH 7.4 and 25 ˚C.

Sample	Zeta potential (mV)
RhB	-25.3 ± 1.8
Dox	9.5 ± 0.3
Cyt	-5.0 ± 1.7
mCh	-9.6 ± 0.8
Ova	-9.8 ± 1.9
DsR	-10.3 ± 1.4
DTR-3	-6.5 ± 1.6
DTR-10	-2.3 ± 1.7
DTR-70	0.4 ± 0.1
pCri	-32.9 ± 2.1
pGFP	-34.6 ± 2.4
Polystyrene Beads	-28.0 ± 1.4
Polystyrene Beads@DTR-3	-24.1 ± 1.1

H. Molecular Models

The molecular model representations were minimized using: OPLS_2005 *(MacroModel)*, Version Maestro 9.3.5; Schrödinger, LLC: New York, 2007, representing water as a continuum solvent. The SGQ model presented was built upon previously published models in which the SGQ was minimized by freezing the three K^+ ions and the guanine core up to the 1' carbon of the sugar, allowing free movement of the side chains.

References

1. Betancourt, J. E.; Rivera, J. M. Tuning Thermoresponsive Supramolecular G-Quadruplexes. *Langmuir* **2015,** *31*, 2095-2103.
2. Betancourt, J. E.; Rivera, J. M. Drug encapsulation within self-assembled microglobules formed by thermoresponsive supramolecules. *J. Am. Chem. Soc.* **2009,** *131*, 16666-16668.