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Ligand-functionalized CdSe quantum dots and nanorods, and horse spleen ferritin bionanoparticles, were co-assembled at an oil-water interface, then used in polymerization at the interface, effectively cross-linking the assembled mixtures of nanoparticles into robust structures. Both ring-opening metathesis polymerization (ROMP), and imine formation, proved suitable for preparation of the desired ultra-thin films in the form of capsules and sheets. The nanoparticle-based films prepared by ROMP exhibit chemical stability, while those prepared by aldehyde-amine coupling could be disrupted by addition of acid. Characterization of these hybrid nanoparticle-based materials, using transmission electron microscopy (TEM) and fluorescence confocal microscopy, confirmed the presence of both synthetic and naturally derived nanoparticles in the hybrid materials.

Introduction

Nanoparticles of synthetic and biological origin, while typically considered for disparate applications, can also be applied in concert for the preparation of novel hybrid materials. While bionanoparticles and inorganic nanoparticles are distinct compositionally, their size-similarity opens opportunities to use self-assembly as a precursor to bio-nano hybrid materials. Nanoparticles of many varieties, both synthetic and biologically derived, can be tailored to segregate to interfaces, including fluid-fluid,^{1–4} fluid-air,^{5,6} and fluid-substrate² interfaces. However, aside from the localization of inorganic nanoparticles in bionanoparticle cavities, and on the exterior of bionanoparticles,^{7–15} little has been done to construct hybrid materials of both synthetic and biologically derived nanoparticle building blocks. Controlling surface functionality on various types of nanoparticles is critically important for doing so.

Here we report the preparation of robust thin films and capsules composed of CdSe semiconductor quantum dots and horse spleen ferritin nanoparticles. Quantum dots are of interest for their optoelectronic properties, especially their tunable and narrow photoluminescence emission in solution and in the solid state, making them attractive biosensors and components of light emitting and photovoltaic devices.^{16–21} Bionanoparticles are of exactly defined size, as prepared by nature, and possess surface available amino acid functionality amenable to conventional protein modification chemistry, provided the protein assembly is stable to the chosen reaction conditions. Decorating bionanoparticles with functionality integral to polymer synthesis enables their use in polymer grafting chemistry,^{22,23} and in conjunction

with synthetic nanoparticles containing similar, or complimentary, reactive functionality. In addition, bionanoparticles can be readily assembled at an oil-water interface, and robust structures can be generated by the inclusion of a cross-linker such as glutaraldehyde.⁴ As described below, we exploit the combination of synthetically modified bionanoparticles with functional quantum dots for the preparation of novel hybrid membranes and capsules. Co-assembly of the synthetic and bionanoparticles at the fluid-fluid interface, a consequence of the reduction of interfacial tension upon nanoparticle segregation,^{4,24–27} is the key prerequisite that enabled the preparation of the desired hybrid materials described here.

Results and discussion

Ferritin-quantum dot capsules and ultra-thin films by reductive amination

Ferritin nanoparticles are composed of 24 protein subunits, with ~3–4 surface-available lysine residues per subunit,^{28,29} to give ~80 amines for potential reaction with complimentary functionality on other particles. The ligand chemistry of CdSe quantum dots can be tailored to exploit these lysine residues. Benzaldehyde-functionalized quantum dots were prepared as shown in Scheme 1. Oxidative cleavage of di-*n*-octylstyrenyl phosphine oxide,³⁰ carried out in a 3:1 THF:water solution of OsO₄ and NaIO₄,^{31,32} gave benzaldehyde **1** in 74% yield as a white solid after purification by column chromatography on silica gel (eluting with CHCl₃/MeOH mixtures). Aldehyde-functionalized CdSe nanoparticles were prepared by ligand exchange with TOPO-covered nanoparticles,³⁰ first by heating the TOPO-covered particles in pyridine then stirring the pyridine-covered particles in chloroform with ligand **1** for 12 hours at 50 °C. Repeated dissolution in chloroform, and precipitation in methanol, removed excess ligand. The benzaldehyde-covered quantum dots are readily soluble in toluene and other organic solvents.

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Scheme 1 Synthesis of benzaldehyde ligand 1

In accordance with our prior studies of TOPO-covered CdSe quantum dots,^{3,25} the benzaldehyde-functionalized quantum dots segregate readily to the toluene-water interface, following vigorous shaking of a toluene solution of the particles with water, to give water-in-oil capsules. When 1,4-phenylenediamine was present in the aqueous phase (10 mg/mL), the assemblies generated upon shaking became robust, a result of imine formation at the interface that effectively cross-links the assembly. Performing the cross-linking from the minor aqueous phase (*i.e.*, inside the droplets) was preferred, as this minimized inter-droplet connections in the continuous oil phase.³³ Successful cross-linking was confirmed by confocal fluorescence microscopy imaging of the droplets, after washing the system with methanol to remove the interface that promoted the initial nanoparticle segregation. This imine formation imparts structural integrity to the capsules, and the capsular structure is maintained following the washing step. Without diamine in the aqueous phase, robust structures did not form, and washing with methanol completely disrupted the assemblies. Control experiments confirmed that TOPO-covered quantum dots used in this process did not lead to cross-linking, which might conceivably occur by ligand exchange between TOPO and the difunctional aromatic amine.

Quantum dot-ferritin hybrid materials

We find that nanoparticle interfacial assembly, while known for co-assemblies of different sizes and types of inorganic particles,^{3,34} is also amenable to co-assembly of ferritin and CdSe quantum dots. This was done (1) at a flat interface, by bringing an aqueous solution of ferritin into contact with an organic solution of quantum dots, and (2) as oil-in-water and water-in-oil droplets. In droplets consisting of mixed assemblies of ferritin and quantum dots at the interface, the fluorescence from the quantum dots is seen by fluorescence confocal microscopy. In co-assemblies of TOPO-covered quantum dots with ferritin, removal of the interface by washing destabilized the droplets and dispersed the particles. However, when the benzaldehyde-covered quantum dots were co-assembled with ferritin, robust structures formed, which when removed from the interface by solvent washing gave collapsed, but intact, structures. This is illustrated in Fig. 1. Both types of particles represent multifunctional materials, contributing ~80 lysine amines from each ferritin and ~20 benzaldehydes from each quantum dot. These ferritin-quantum dot networks were dried on a TEM grid, and imaged to show that the droplet structure, as a result of the interfacial chemistry, was maintained (Fig. 1c). The folds and ridges in the collapsed droplets are apparent. Fig. 1d shows a higher magnification TEM image of the dried structure containing the quantum dots and ferritin particles. Control

Fig. 1 (a) Collapsed cross-linked ferritin/CdSe-CHO mixed-assembly capsules made from CHCl_3 -in-water droplets, upon addition of THF; (b) cross-linked sheet made by mixed assembly of ferritin and CdSe-CHO nanoparticles at a flat toluene-water interface; (c) TEM image (100 keV) of cross-linked mixed-assembly capsules dried on a carbon-coated TEM grid; (d) higher magnification TEM (100 keV) image.

experiments performed using ferritin and TOPO-covered quantum dots failed to give robust capsules, confirming the need for functional ligands on the quantum dots, and that any coordination of the amine functionality of ferritin to the quantum dots is not sufficient to give robust materials.

This co-interfacial assembly and cross-linking of quantum dots and ferritin particles can also be carried out at a flat interface, by gently applying a toluene solution of benzaldehyde-covered CdSe nanoparticles on top of an aqueous solution of ferritin. The film formed at the interface was transferred to a concave microscope slide, and imaged by fluorescence confocal microscopy. Fig. 1b shows the free-floating sheet obtained when the cross-linked film is dispersed in toluene. The film was lifted from the interface with a carbon-coated copper grid, washed with toluene and water, and imaged by TEM to reveal individual nanoparticles (Fig. 1d). Analysis of the cross-linked films using X-ray photoelectron spectroscopy (XPS) revealed the presence of both types of nanoparticles (ESI^+). The measured 20 nm thickness of these cross-linked films, using scanning force microscopy (ESI^+), confirmed the approximately monolayer nature of the interfacial assembly, as expected from the interfacial energies associated with the fluid-fluid nanoparticle segregation.

The presence of ferritin particles on a water droplet suspended in toluene reduces the toluene-water interfacial tension from ~ 35 mN/m to ~ 13 mN/m, as we measured by pendant drop tensiometry. When TOPO-covered CdSe nanoparticles were included in the toluene phase, the reduction in interfacial tension was identical, in accord with the expected dominance of the larger, biologically derived nanoparticles on the interfacial energy (TOPO-covered CdSe nanoparticles reduces the toluene-water interfacial tension to ~ 19 mN/m²⁴). When the benzaldehyde-covered quantum dots were present in the toluene phase, and ferritin in the aqueous phase, the interfacial tension dropped to 16 mN/m and remained constant (ESI†). The network of nanoparticles formed on the droplet surface during the course of the pendant drop experiment arrests the dynamic nature of the nanoparticle assembly, preventing a fully equilibrated arrangement of particles, and leaving the interface inaccessible to the entry of new particles from the bulk solution.

In order to confirm the presence of both synthetic and bio-derived particles in the cross-linked films, benzaldehyde-covered CdSe nanorods (30×6 nm) were prepared by ligand exchange from alkane-covered nanorods (prepared by literature methods³⁵), and used in place of the quantum dots for interfacial assembly. Co-assembly and cross-linking of these nanorods with ferritin particles was then performed at a flat CHCl_3 -water interface to give films that were lifted with a carbon-coated copper TEM grid, washed with toluene then water, dried, and then imaged. As shown in Fig. 2, both ferritin particles and CdSe nanorods are present in the cross-linked film.

Fig. 2 TEM images of a cross-linked sheet made by co-assembly of ferritin and CdSe nanorods at a CHCl_3 -water interface (a) low magnification image, and (b) high magnification image showing individual CdSe nanorods (red ovals) and ferritin particles (blue circles).

Fig. 3 Confocal micrographs of CdSe/ferritin cross-linked sheets formed by co-assembly at a flat toluene-water interface (a) without addition of NaBH_3CN , (b) with addition of NaBH_3CN .

The imine cross-linked films and capsules made by this co-assembly process are susceptible to network degradation by acid-induced hydrolysis. Fig. 3a shows a confocal micrograph of a ferritin-quantum dot hybrid sheet dispersed in toluene, consisting of numerous small fragments. However, the conversion of the imine linkages to amines greatly reduced the susceptibility of the cross-linked film to hydrolysis. Addition of sodium cyanoborohydride³⁶ to the aqueous phase either during or after the co-assembly process gave cross-linked hybrid nanoparticle sheets that appeared as larger structures (>1 mm across), less prone to fragmentation (Fig. 3b).

The susceptibility of the imine-cross-linked films to hydrolysis can be exploited towards pH-induced degradable capsules and films. *para*-Toluenesulfonic acid (*p*TSA) was chosen as the source of acid for testing the CdSe/ferritin cross-linked films, while observing them using confocal microscopy. Upon addition of a methanolic solution of *p*TSA (50 mg/mL), the cross-linked capsules were disrupted. When the capsules contained amine linkages (by addition of NaBH_3CN to the imine conjugated capsules), the cross-linking is maintained upon addition of acid. The stability of the cross-linked films was tested by adding a drop of aqueous sulforhodamine-B to a vial containing a co-assembly of ferritin and benzaldehyde-covered CdSe nanoparticles at a flat interface (Fig. 4a,b). The ultra-thin film formed by cross-linking at the flat interface easily supported the drop. However, upon addition of aqueous or methanolic *p*TSA, the cross-linking was disrupted, and the sulforhodamine-B mixed with the underlying aqueous phase.

Ferritin-quantum dot hybrid materials by ROMP

Additional synthetic routes to prepare hybrid quantum dot-bionanoparticle capsules and films were considered to expand the scope of available polymerization chemistries amenable to hybrid film formation. Ring-opening metathesis polymerization (ROMP) has been applied to quantum dots,^{30,33} but not to

Fig. 4 (a) A drop of aqueous sulforhodamine-B is supported by a cross-linked CdSe/ferritin film at a flat toluene-water interface; (b) upon addition of acid, the cross-linking is disrupted and the dye mixes with the aqueous phase below.

bionanoparticle structures, or hybrids of the two. For this, both ferritin particles and quantum dots were functionalized with norbornene, then co-assembled at an oil-water interface in the presence of a ruthenium benzylidene catalyst to perform ROMP at the interface. To confirm the presence of both types of particles, the ferritin particles were also labeled with a fluorescent dye, fluorescein isothiocyanate isomer I (FITC), to distinguish them from the fluorescence emission of the quantum dots.

Ferritin nanoparticle functionalization with both FITC and norbornene is depicted in Scheme 2. FITC was stirred with 5-norbornene-2-carbonyl chloride and diisopropylethylamine in anhydrous THF for 12 hours under subdued light to give FITC/norbornene **2** in 55% yield as a yellow solid. The fluorescence of compound **2** in DMSO is similar to that of FITC, with peak emission at 550 nm. Ferritin was functionalized with FITC/norbornene **2** by reaction with the isothiocyanate moiety, in pH 9 buffer with 20% DMSO, for 3 hours at room temperature. The norbornene-functionalized ferritin particles thus obtained were purified by centrifugation through P-100 resin (BioRad) at 5000G. The FITC-labeled ferritin particles were characterized by high performance liquid chromatography (HPLC) through a size-exclusion column (BioSep-SEC-S 3000), detecting at 280 nm (ferritin absorption) and 495 nm (FITC absorption). Signals corresponding to both the modified and unmodified ferritin particles were seen at 5.2 mL elution volume, while the absorbance at 495 nm for the modified ferritin confirms the FITC attachment. The attachment of FITC to the ferritin particles was also confirmed by its solution fluorescence ($\lambda_{\max}$ 550 nm).

Cross-linked capsules and sheets of ferritin by ROMP

FITC/norbornene-functionalized ferritin particles were found to segregate readily to an oil-water interface upon shaking aqueous dispersions of the particles with organic solvents such as chloroform, toluene, or trichlorobenzene. Following interfacial assembly, the ferritin particles on the oil droplets were cross-linked by ROMP, using Grubbs Generation II catalyst in the oil phase. The presence of FITC on the ferritin particles enables facile visualization of the capsules by fluorescence confocal microscopy (excitation 488 nm; detection 550 nm), as shown in Fig. 5a. Upon removal of the interface, intact capsular structures are observed, providing evidence for interfacial cross-linking (Fig. 5b). TEM images of the cross-linked ferritin capsules after

Fig. 5 Fluorescence confocal microscope images of (a) cross-linked FITC/norbornene-functionalized capsules in toluene and (b) collapsed nanoparticle capsules obtained by removal of the interface upon addition of methanol; (c) TEM images of cross-linked FITC/norbornene-functionalized ferritin-based capsules, (d) higher magnification TEM image showing individual particles.

drying on carbon-coated copper grids show that the droplet structure is maintained, and the collapsed capsules, while nanoscopic in nature, resemble crumpled macroscopic sheets (Fig. 5c). At higher magnification (Fig. 5d), individual nanoparticles are seen, likely mirroring an interfacial liquid-like assembly of nanoparticles.³³ Whether the bionanoparticle density or packing structure, as well as the cross-link density, can be controlled finely is the topic of ongoing studies using scattering methods.

Cross-linked ultra-thin sheets of ferritin nanoparticles were prepared by bringing into contact a toluene solution of Grubbs Generation II catalyst with an aqueous dispersion of **2**-functionalized ferritin nanoparticles at a planar interface. The cross-linked film formed at the interface was lifted with an uncoated copper grid, and dried for imaging. As seen in Fig. 6a, the desired ultra-thin sheets were formed by cross-linking at a planar interface. Even without a carbon coating on the grid, the cross-linked

Scheme 2 Synthesis of FITC-norbornene compound **2**, and functionalization of ferritin nanoparticles

Fig. 6 (a) TEM images of cross-linked FITC/norbornene-functionalized ferritin-based cross-linked sheets; (b) higher magnification image showing individual particles.

film supports its own weight and stretches across the grid. These ultra-thin sheets possess surface areas of the order of 1 cm^2 ; much larger sheets are conceivable, controlled in principle by the surface area of the interface used in the experiment. Individual ferritin nanoparticles are observed at higher magnifications (Fig. 6b).

Ferritin-quantum dot capsules and sheets by ROMP

Mixed interfacial assemblies of norbornene-functionalized bionanoparticles and quantum dots were also prepared, using ZnS-coated quantum dots (5 nm diameter) and 2-functionalized ferritin. This was done most effectively when solutions of the two kinds of nanoparticles in toluene and water, respectively, were brought into contact at a flat interface. ROMP was conducted at the interface by the addition of Grubbs Generation II catalyst following interfacial assembly and rinsing the top phase. The CdSe/ZnS quantum dots used in these experiments have a fluorescence emission centered at 610 nm, significantly removed from the fluorescence arising from the FITC. Fig. 7a shows fluorescence confocal images of the resulting ultra-thin sheets dispersed in toluene, in which distinct quantum dot and FITC emissions (detection: 600–620 nm and 510–550 nm respectively) suggest a macroscopically uniform distribution of both kinds of nanoparticles in the cross-linked sheets. XPS analysis indicated similarly (ESI†). While wrinkling of the sheets occurs due to capillary forces,³⁷ the crumpled morphology results from shear stresses accumulated during manipulation of the sheets by a micropipette; pronounced ridges viewed by confocal microscopy are

similar to those seen in macroscopic crumpled elastic sheets.³⁸ TEM (Fig. 7b) also confirms the sheet-like morphology of the materials, while higher magnification TEM images (Fig. 7c) show liquid-like close-packing of the nanoparticles. As before, it is difficult to distinguish between the quantum dots and ferritin particles in the TEM images, owing to their comparable sizes and spherical shapes. Co-assembly and cross-linking using ROMP were therefore also carried out by replacing quantum dots with norbornene-functionalized CdSe nanorods, which by TEM confirm the presence of both the semiconductor and bionanoparticles in the cross-linked hybrid network. Cross-linked films containing norbornene-functionalized ferritin and quantum dots produced at a flat interface were also tested by adding a drop of aqueous sulforhodamine-B. The dye was easily supported by the cross-linked film at the interface. In this case, the film was not ruptured even upon addition of acid, thus demonstrating the irreversible (acid-stable) cross-linking given by the polyolefins obtained by ROMP.

Conclusions

In summary, we have shown two different routes to preparing hybrid assemblies and cross-linked thin film networks of ferritin nanoparticles and CdSe quantum dots or nanorods. By applying appropriate surface functionality to the nanoparticles, cross-linking was performed by either aldehyde-amine conjugation or ring-opening metathesis polymerization. Benzaldehyde-functionalized quantum dots and nanorods can be cross-linked by reacting with surface available lysines on the ferritin. Capsules and sheets containing both irreversible and pH-sensitive linkages can also be fabricated by this method. In addition, both ferritin and CdSe nanoparticles can be functionalized with norbornene moieties and cross-linked by ROMP, using metathesis polymerization catalysts such as Grubbs Generation II ruthenium benzylidene catalyst.

Experimental

General

5-Norbornene-2-carboxylic acid (endo/exo mixture) (98%), fluorescein isothiocyanate isomer I (90%), thionyl chloride (97%), diisopropylethylamine (99%), di(ethylene glycol) vinyl ether (98%), horse spleen ferritin (85 mg/mL stock solution),

Fig. 7 (a) Two-channel confocal fluorescence microscope images of ultra-thin cross-linked sheets containing norbornene-functionalized CdSe quantum dots (channel 1, green), and FITC/norbornene-functionalized ferritin bioNPs (channel 2, red, inset); (b) TEM image showing the same cross-linked sheet, (c) higher magnification TEM image showing individual nanoparticles.

sodium periodate (98%) and *p*-toluenesulfonic acid were purchased from Aldrich. Osmium tetroxide (2% in water) was purchased from Electron Microscopy Sciences. Dichloromethane was distilled following drying over calcium hydride, and tetrahydrofuran was freshly distilled over sodium/benzophenone ketyl before use. ^1H and ^{13}C NMR spectra were recorded on a Bruker-Spectrospin 400. Laser scanning confocal microscopy (LSCM) images were performed on a Leica TCS SP2 LSCM with excitation by an Ar ion laser (excitation: 488 nm, 514 nm; detection: 520–550 nm, 580–610 nm). TEM grids were purchased from Ted Pella, Inc and TEM images were recorded on JEOL 100CX and 2000FX microscopes. Scanning force microscopy was performed on a Digital Instruments Dimension 3100 microscope operating in tapping mode. Pendant drop tensiometry was performed on an OCA-20 tensiometer. HPLC was performed on Waters 2695 separation modules and a BioSep-SEC-S 3000 separation column. 0.05 M phosphate buffer (pH 7) was used for elution at 1 mL/min flow rate, and a UV detector was used at 280 and 495 nm.

Benzaldehyde ligand 1

p-Vinylbenzene-DOPO³⁰ (7.57 g, 19.4 mmol) was dissolved in tetrahydrofuran (THF, 60 mL), and to this solution was added osmium tetroxide (2 mL 2% w/w in H₂O), NaIO₄ (13.1 g, 61.4 mmol) and water (20 mL). Within a few minutes of addition of NaIO₄, the reaction mixture became cloudy. The mixture was stirred at room temperature for 6 hours, then poured into a mixture of methylene chloride (100 mL) and water (50 mL). The organic phase was separated and washed with water and saturated NaHSO₃ to give a dark green solution, which was dried over MgSO₄, treated with carbon-black, filtered through Celite and concentrated to get a light green viscous liquid. The product was purified by column chromatography eluting with CHCl₃/MeOH mixtures to get an off-white solid (3.94 g, 52%): ^1H NMR (300 MHz, CDCl₃) δ 9.99 (s, aldehyde), 7.84 (d, 2H, aromatic), 7.43 (d, 2H, aromatic), 3.18 (d, 2H, benzyl CH₂), 1.62 (br, 4 H, CH₂-P of *n*-octylchains), 1.34 (br, 24 H), 0.87 (t, 6H, CH₃) ppm; ^{13}C NMR (75 MHz, CDCl₃) δ 130.2, 31.9, 129.6, 126.5, 113.7, 36.3, 31.7, 30.9, 28.9, 27.7, 26.8, 22.6, 21.5, 14.0 ppm; ^{31}P (121 MHz, CDCl₃) δ 46.1 ppm

Synthesis of aldehyde-functionalized CdSe nanoparticles and nanorods

TOPO-covered CdSe quantum dots (2.5 nm diameter) and nanorods (40 nm in length, 6 nm in diameter) were synthesized according to procedures in the literature,^{30,39} and stirred in refluxing pyridine under an inert atmosphere for 6 h. Most of the pyridine was removed under reduced pressure to give a viscous solution. The nanoparticles were precipitated by addition of hexane, and this suspension was subjected to centrifugation. The pyridine-covered nanoparticles thus obtained (*ca.* 20 mg) were then stirred as a suspension in 5 mL chloroform. 150 mg of ligand **1** was added, and the suspension was stirred at 50 °C overnight. Most of the chloroform was removed under reduced pressure and the nanoparticles were precipitated by adding anhydrous methanol and centrifuged. The nanoparticles were then repeatedly washed by dissolving in a small amount of chloroform and

precipitating in methanol to remove excess ligand, and finally redissolved in toluene and used for further experiments.

FITC-norbornene 2

To a solution of 5-norbornene-2-carboxylic acid (69 mg, 0.5 mmol) (endo/exo mixture) was added thionyl chloride (1.64 g, 13.7 mmol). The mixture was stirred at room temperature for 2 hours under nitrogen. Unreacted SOCl₂ was removed by vacuum, and a solution of FITC (0.2 mmol, 78 mg) in anhydrous THF (10 mL) was added, followed by dropwise addition of diisopropylethylamine (74 mg, 0.6 mmol). The reaction was covered with aluminum foil, and stirred overnight under nitrogen at room temperature. The crude product was filtered, concentrated in vacuo, and then further purified by dissolution in CH₂Cl₂ and precipitation in hexanes (to remove DIPEA and 5-norbornene-2-carboxylic acid), and then redissolution in a CH₂Cl₂/ether mixture (to remove unreacted FITC) to give a yellow solid in 55% yield. FTIR: 2924 (s), 2854 (m), 2033 (m), 1743 (s), 1712 (s), 1604 (s), 1427 (s), 1360 (s), 1209 (m), 1107 (m), 859.8 (s) and 735 (s); UV: 460 nm (maximum absorbance in DMSO); fluorescence: 550 nm (maximum emission in DMSO). 400 MHz ^1H in D-DMSO is δ 8.2 (1H), 7.91 (1H), 7.52 (1H), 7.22 (2H), 6.94 (4H), 6.36 (2H), 6.14 (2H), 3.51 (1H), 2.94 (2H), 1.99 (2H), 1.39 (2H), 1.24 (2H) and 1.18 (2H) ppm. ^{13}C in D-DMSO is 174, 158, 142, 136, 129, 126, 121, 112, 109, 69, 66, 52, 49, 46, 43, 42, 40.7, 29.6, and 29 ppm. ESI (MASS): 629.8 g/mol (found) and 629.68 g/mole.

Functionalization of ferritin with norbornene 2

Horse spleen ferritin (10.0 mg, 0.545 μmol in protein unit, 1 eq.) was diluted to get 0.8 mL ferritin solution in 0.1 M sodium carbonate buffer (pH 9.0). 0.02 mmol of **2** was added to the buffer solution four times over one hour. Finally, 0.2 mL DMSO was added to the above solution, which was incubated for three hours in the dark. The excess small molecule reagents were then removed by centrifugation using a Bio-spin P-100 gel column (3 min at 800g). The product solution was then rinsed 10 times by adding Milli-Q water and centrifuging through centricon 30000 (MWCO) until no free FITC was detected. SDS-PAGE and HPLC were used to characterize the functionalized ferritin bionanoparticles. The main peak in HPLC was observed at 5.2 mL retention volume, which is consistent with ferritin nanoparticles, and confirms the integrity of the functionalized particles. SDS-PAGE shows a band of functionalized ferritin subunits shifts to a higher molecular weight region compared to ferritin subunits.

General procedure for preparing ferritin/CdSe-CHO cross-linked films and sheets

Oil-in-water droplets containing a mixed assembly of ferritin and CdSe nanoparticles were prepared by vigorously shaking 0.1 mL of a 2 mg/mL solution of CdSe nanoparticles (TOPO- or benzaldehyde-covered) in chloroform along with 0.9 mL of a 2 mg/mL solution of horse spleen ferritin in aqueous potassium phosphate buffer (0.01 M). To make water-in-oil droplets, 0.1 mL of aqueous ferritin solution was shaken with 0.9 mL of CdSe nanoparticle solution in toluene. For additional rigidity of cross-linking, 50 μL of a 10 mg/mL aqueous solution of sodium

cyanoborohydride was added to the water phase. For making cross-linked sheets, a toluene solution of CdSe nanoparticles was applied to an aqueous solution of ferritin particles. In all cases, the concentration of the nanoparticle solutions was kept constant (2 mg/mL each). To destabilize the cross-linking by imine hydrolysis, 50 μ L of a 50 mg/mL solution of *p*TSA in methanol was added to the capsules/sheets.

General procedure for the preparation of cross-linked ferritin capsules/sheets

To 500 μ L of a toluene solution of Grubbs generation II catalyst (1 mg/mL) in an Eppendorf tube 50 μ L of an aqueous solution of 2-functionalized ferritin nanoparticles (2 mg/mL) was added and shaken vigorously. For preparing cross-linked sheets, a toluene solution of Grubbs generation II catalyst (1 mg/mL) was applied to a solution of 2-functionalized ferritin nanoparticles (0.4 mg/mL). After one hour, the organic phase was rinsed with fresh toluene, and 50 μ L of a toluene solution of diethylene glycol vinyl ether (1 mg/mL) was added.

Preparation of cross-linked capsules/sheets containing functionalized ferritin and norbornene-functionalized CdSe quantum dots

50 μ L of an aqueous solution of 1 and 2-functionalized ferritin nanoparticles (2 mg/mL) was added to 500 μ L of norbornene-functionalized CdSe quantum dots in toluene (1mg/mL) and vigorously shaken to form capsules. Alternatively the toluene phase was laid on top of the aqueous solution to generate a flat interface. After two hours, the organic phase was rinsed with fresh toluene to remove excess CdSe nanoparticles, and 500 μ L of a 1 mg/mL solution of Grubbs generation II catalyst in toluene was added to cross-link both types of nanoparticles at the interface. After one hour, the oil phase was once again rinsed with fresh toluene, and 50 μ L of di(ethylene glycol) vinyl ether (1 mg/mL in toluene) was added to stop the cross-linking.

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