Electronic Supplementary Material (ESI) for ChemComm.

SUPPORTING INFORMATION FOR

Construction of Biological Hybrid Microcapsules with Defined Permeability towards Programmed Release of Biomacromolecules

1. Material

Dextran T-70 (ZhenJie Technology, China, analytically pure), albumin from bovine serum (BSA, Sigma, 98 %), lysozyme (Lys, Sigma, 98 %), N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC, Sigma-Aldrich, 98 %), PEG-bis (N-succinimidyl succinate) (Sigma, 98 %), 1,6-diaminohexane (Sigma, 98 %), FITC (Fluorescein isothiocyanate isomer I, Sigma 90 %), RBITC (Rhodamine B isothiocyanate, Sigma), trichloroethyl phosphate (TCEP, Sigma, 98 %), cystamine dihydrochloride (Energy, 98%), 2,4,6-trinitrobenzene sulfonic acid (TNBSA) solution (5 % (w/v) in H_2O, Sigma), protease (Sigma, 98%), lyticase (Sigma, 98 %), fluorescein isothiocyanate-labeled dextran (FITC-Dextran, MW 4 kDa, 10 kDa, 20 kDa, 40 kDa, 70 kDa, 150 kDa, 500 kDa, 2000 kDa, Sigma, 98 %), DNA (salmon testes, Sigma, 98 %), Milli-Q water was used to prepare all the solutions in this study. N-isopropylacrylamide (NIPAAm, Sigma, 98 %) was recrystallized twice in hexane and toluene prior to use.

2. Characterization methods

SEM images were obtained on a HITACHI UHR FE2SEM SU8000 with the samples coated by platinum with the thickness of 10 nm. Atomic force microscopy (AFM) images were performed on a 3D manipulation force microscope. Samples were prepared by adding one drop of microcapsule solution (0.1 mg/mL) onto a clean silica wafer and dried in vacuum for one day. Optical and fluorescence microscopy was performed on a Leica DMI8 manual inverted fluorescence microscope at 10x, 20x, 40x and 100x magnification. Fluorescence measurements were performed on a PerkinElmer spectrofluorometer (LS55, USA). UV-vis spectra were measured on a

PerkinElmer spectrophotometer (Lambda 750S, USA). The pH measurements were made with a Seven Compact meter (METTLER TOLEDO, SUI). Oscillator was employed by a VORTEX instrument (IKA, GER). Assimilated solutions were executed by pipettors (GILSON, FRA).

3. Experimental section

3.1 Synthesis of the Dextran-COOH and Lysozyme-COOH

A solution of succinic anhydride (1.85 g, 18.5 mmol) was added dropwise to a stirred solution of the Dextran (1.0 g, 14.3 μmol, dissolved in 150 ml of DMSO). The reaction lasted for 16 h at 60 °C. Then the solution was purified by using a centrifugal filter to remove any unreacted reactants and salts. After freeze-drying, the Dex-COOH powder was obtained.

A solution of succinic anhydride (0.8 g, 8.0 mmol) was added dropwise to a stirred solution of the lysozyme (0.1 g, 7.14 μmol). The reaction lasted for 16 h at RT. Then the solution was purified by using a centrifugal filter to remove any unreacted reactants and salts. After freeze-drying, the Lysozyme-COOH powder was obtained.

3.2 Synthesis of the cationized Dextran (Dex-NH_2), cationized Lysozyme (Lys-NH_2) and cationized bovine serum albumin (BSA-NH_2)

Cationized Dextran (Dex-NH_2) was synthesized by carbodiimide activated conjugation of 1, 6-diaminohexane to aspartic and glutamic acid residues on the external surface of the Dextran-COOH. For this, a solution of 1, 6-diaminohexane (3.45 g, 29.7 mmol) was adjusted to pH 6.5 using 5 M HCl and added dropwise to a stirred solution of the Dextran-COOH (115 mg, 1.64 μmol). The coupling reaction was initiated by adding N-ethyl-N'-(3-(dimethylamino) propyl) carbodiimide hydrochloride (EDAC, 100 mg) immediately and again 100 mg after 3 h and again 80 mg after 2 h. The pH value was maintained at 6.5 using dilute HCl, and the solution was stirred for a further 6 h. The solution was then centrifuged to remove any precipitate, and the supernatant was dialyzed (dialysis tubing 12-14 kDa MWCO) extensively against Milli-Q water.

Cationized Lysozyme (Lys-NH_2) was synthesized by carbodiimide activated

conjugation of cystamine dihydrochloride to aspartic and glutamic acid residues on the external surface of the Lys-COOH. For this, a solution of cystamine dihydrochloride (1.5 g, 6.7 mmol) was adjusted to pH 6.5 using 5 M HCl and added dropwise to a stirred solution of the Lys-COOH (200 mg, 14.3 μmol). The coupling reaction was initiated by adding N-ethyl-N'-(3-(dimethylamino) propyl) carbodiimide hydrochloride (EDAC, 100 mg) immediately and again (50 mg) after 5 h. The pH value was maintained at 6.5 using dilute NaOH, and the solution was stirred for a further 6 h. The solution was then centrifuged to remove any precipitate, and the supernatant was dialyzed (dialysis tubing 3 kDa MWCO) extensively against Milli-Q water.

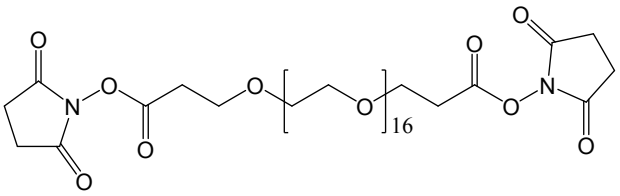
Cationized bovine serum albumin (BSA-NH_2) was synthesized by carbodiimide activated conjugation of 1, 6-diaminohexane to aspartic and glutamic acid residues on the external surface of the protein. For this, a solution of 1, 6-diaminohexane (1.5 g, 12.9 mmol) was adjusted to pH 6.5 using 5 M HCl and added dropwise to a stirred solution of the protein (200 mg, 2.98 μmol). The coupling reaction was initiated by adding N-ethyl-N'-(3-(dimethylamino) propyl) carbodiimide hydrochloride (EDAC, 100 mg) immediately and again 50 mg after 5 h. The pH value was maintained at 6.5 using dilute HCl, and the solution was stirred for a further 6 h. The solution was then centrifuged to remove any precipitate, and the supernatant was dialyzed (dialysis tubing 12-14 kDa MWCO) extensively against Milli-Q water.

3.3 Synthesis of Dex-NH_2-PNIPAAm, Lys-NH_2-PNIPAAm and BSA-NH_2-PNIPAAm nanoconjugates

End-capped mercaptothiazoline-activated PNIPAAm (Mn = 12000 g mol^{-1}, 10 mg in 5mL of water) was synthesized according to our previous reported method and added to a stirred solution of Dex-NH_2, Lys-NH_2, or BSA-NH_2 (10 mg in 5 mL of PBS buffer at pH 8.0). The mixed solution was stirred for 12 h, and then purified by using a centrifugal filter to remove any unreacted PNIPAAm and salts. After freeze-drying, the BSA-NH_2-PNIPAAm Lys-NH_2-PNIPAAm and BSA-NH_2-PNIPAAm nanoconjugate were obtained.

3.4 Preparation of microcapsule

The triple hybrid microcapsules were prepared by mixing an aqueous solution of Dex-NH_2–PNIPAAm, Lys-NH_2–PNIPAAm and BSA-NH_2–PNIPAAm with the concentration of 10 mg/ml in the ratio of 10 : 1 : 1 with 2-ethyl-1-hexanol at an aqueous/oil volume fraction of 0.05 followed by shaking the mixture by hand for 10 s. Typically, 20 μL of the mixed solution after adding 8 μL of sodium carbonate buffer (pH = 8.0) were mixed with 400 μL of the oil. The triple hybrid microcapsules were then cross-linked in the continuous oil phase by the addition of PEG-bis (N-succinimidyl succinate) (0.5 mg), which reacted with free primary amine groups in the conjugates. Transfer of the cross-linked triple hybrid microcapsule into water was achieved as follows. After 3 h sedimentation, the upper clear oil layer was discarded and 1 mL of 75 % ethanol was added. The triple hybrid microcapsule were washed three times by 75 % ethanol via centrifugation-disperse process, then washed by Milli-Q water to complete the phase transfer process. The structure of PEG-bis (N-succinimidyl succinate) (n=16, MW, 1061.14) is as follows:

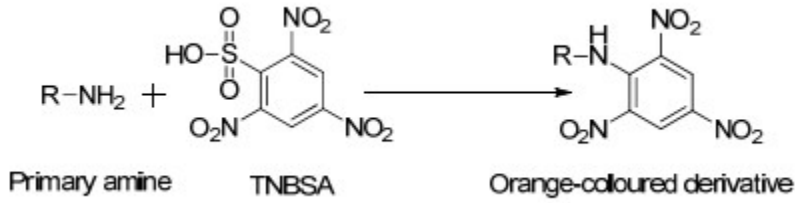


The procedure of in situ encapsulating different compound into the microcapsules was as follows: FITC-dextran (MW, 500 kDa) or FITC-Lysozyme (MW, 14 kDa) or RBITC-dextran (MW, 70 kDa) were added to the aqueous Dex-NH_2/PNIPAAm, Lys-NH_2/PNIPAAm and BSA-NH_2/PNIPAAm solution with the concentration of 10 mg/ml in the ratio of 10 : 1 : 1 and mixed uniformly, followed by mixing with the oil phase to obtain the triple hybrid microcapsules with aimed loadings. After cross-linking, the hybrid microcapsules with encapsulants were transferred to the water phase in the same way described above.

3.5 Determination of primary amine group on the surface of Dex-NH_2, Lys-NH_2 and BSA-NH_2 by TNBSA measurement

2,4,6-Trinitrobenzene sulfonic acid (TNBSA) is a rapid and sensitive assay reagent for the determination of free primary amine groups. Primary amines, upon reaction

with TNBSA, form highly chromogenic derivatives, which can be measured at 339 nm by UV-vis spectroscopy. Typically, sample solutions of Dex-NH_2 (0.025-1.000 mg/mL) were prepared in 0.1 M sodium bicarbonate buffer (pH 8.5). The supplied 5 % TNBSA solution was diluted 250-fold in 0.1 M sodium bicarbonate buffer (pH 8.5). Then the diluted TNBSA solution (0.5 mL) was added to 0.25 mL of sample solution, and incubated at 37 °C for 2 hours. Next, 0.2 M HCl (0.5 mL) was added to each sample to stop the reaction. The UV-vis spectra of the solutions were recorded. To determine the concentration of amine, a standard primary amine absorbance curve was performed based on the same procedure using glycine as a standard compound. In comparison with glycine, the number of primary amine groups per Dex-NH_2 was determined to be *ca.* 90 on average. The same method was used to measure the number of primary amine groups per lysozyme, lysozyme-NH_2, BSA and BSA-NH_2.



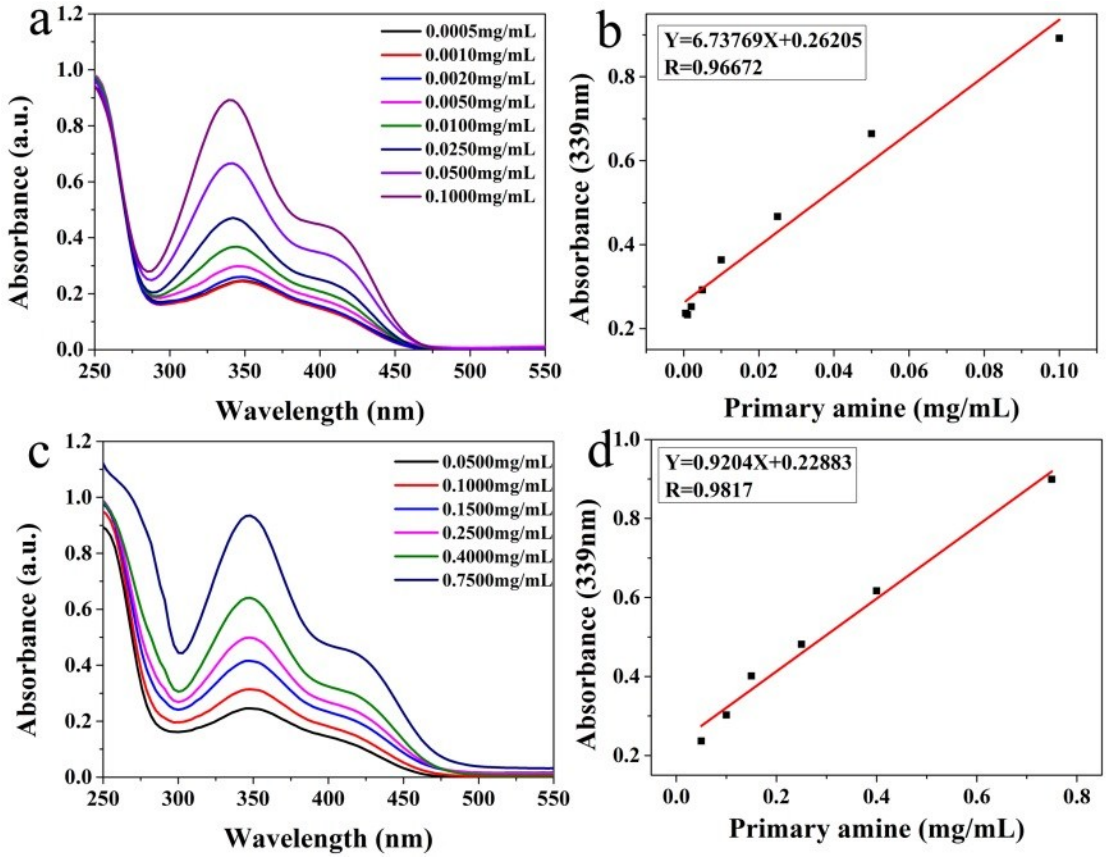


Figure S1. (a, c) UV-Vis spectra obtained for TNBSA/glycine control assay and Dex-NH_2, (b, d) corresponding calibration curve based on plotting the absorbance at 339 nm against the concentration of primary amine. Table S1 is the number of primary amine groups (*ca.* 122 on average) in Dex-NH_2.

Table S1: Number of primary amine groups in Dex-NH_2.

A(339nm)	Gly (μmol/mL)	Dex-NH_2 (μmol/mL)	Number(-NH_2)
0.39074	0.24349	0.00224	108.8
0.47911	0.43404	0.00340	127.6
0.57010	0.60203	0.00468	128.8

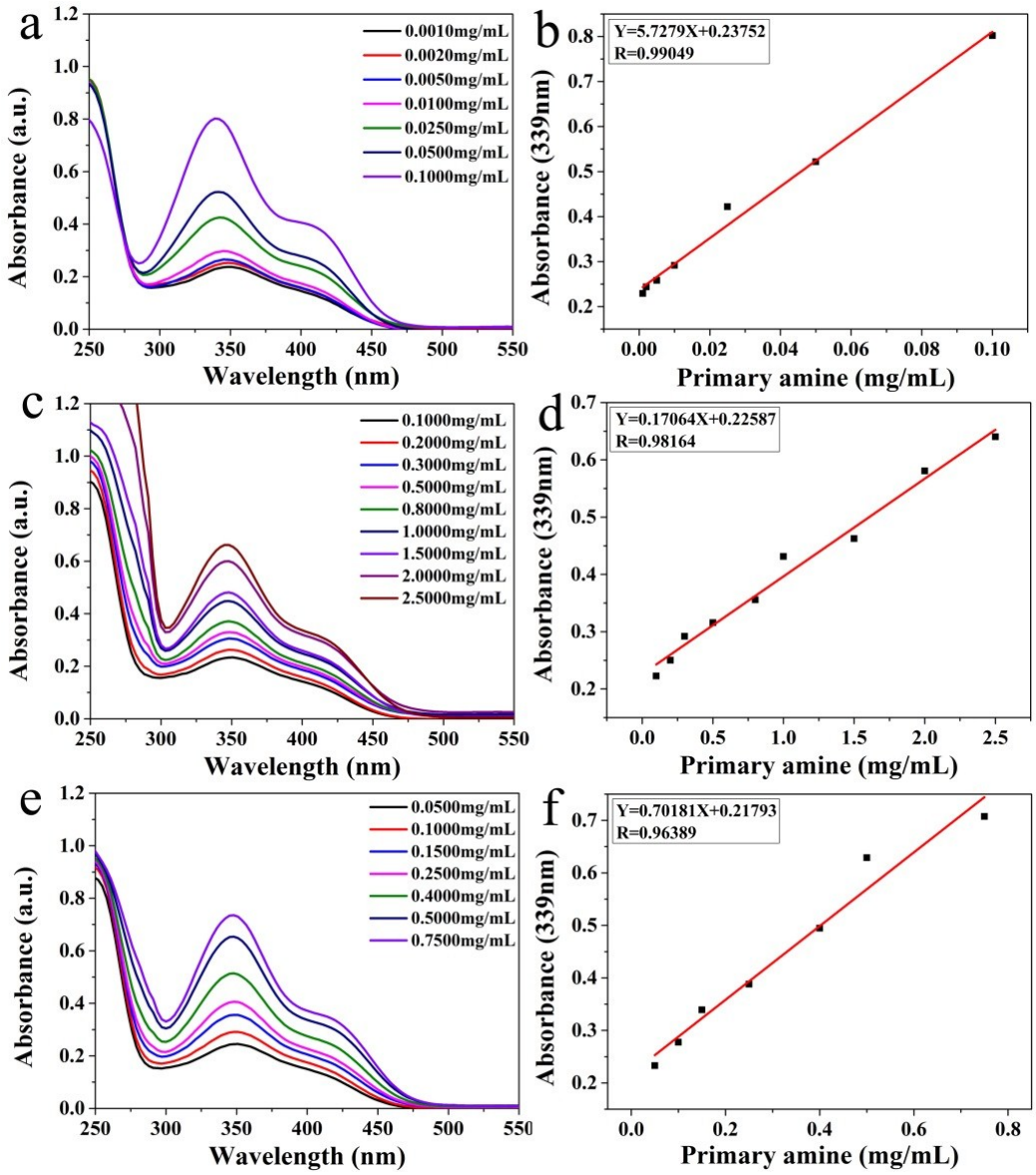


Figure S2. (a, c, e) UV-Vis spectra obtained for TNBSA/glycine control assay, Lys and Lys-NH_2, (b, d, f) corresponding calibration curve based on plotting the absorbance at 339 nm against the concentration of primary amine. Table S2 is the number of primary amine groups (*ca.* 5 and 27 on average) in Lys and Lys-NH_2.

Table S2: Number of primary amine groups in Lys and Lys-NH_2.

A(339nm)	Gly (μmol/mL)	Lys (μmol/mL)	Number (-NH_2)	Lys-NH_2 (μmol/mL)	Number (-NH_2)
0.56127	0.65329	0.14010	4.7	0.02689	24.3
0.58939	0.81209	0.15045	5.4	0.02919	27.8
0.64919	0.97089	0.17798	5.5	0.03452	28.1

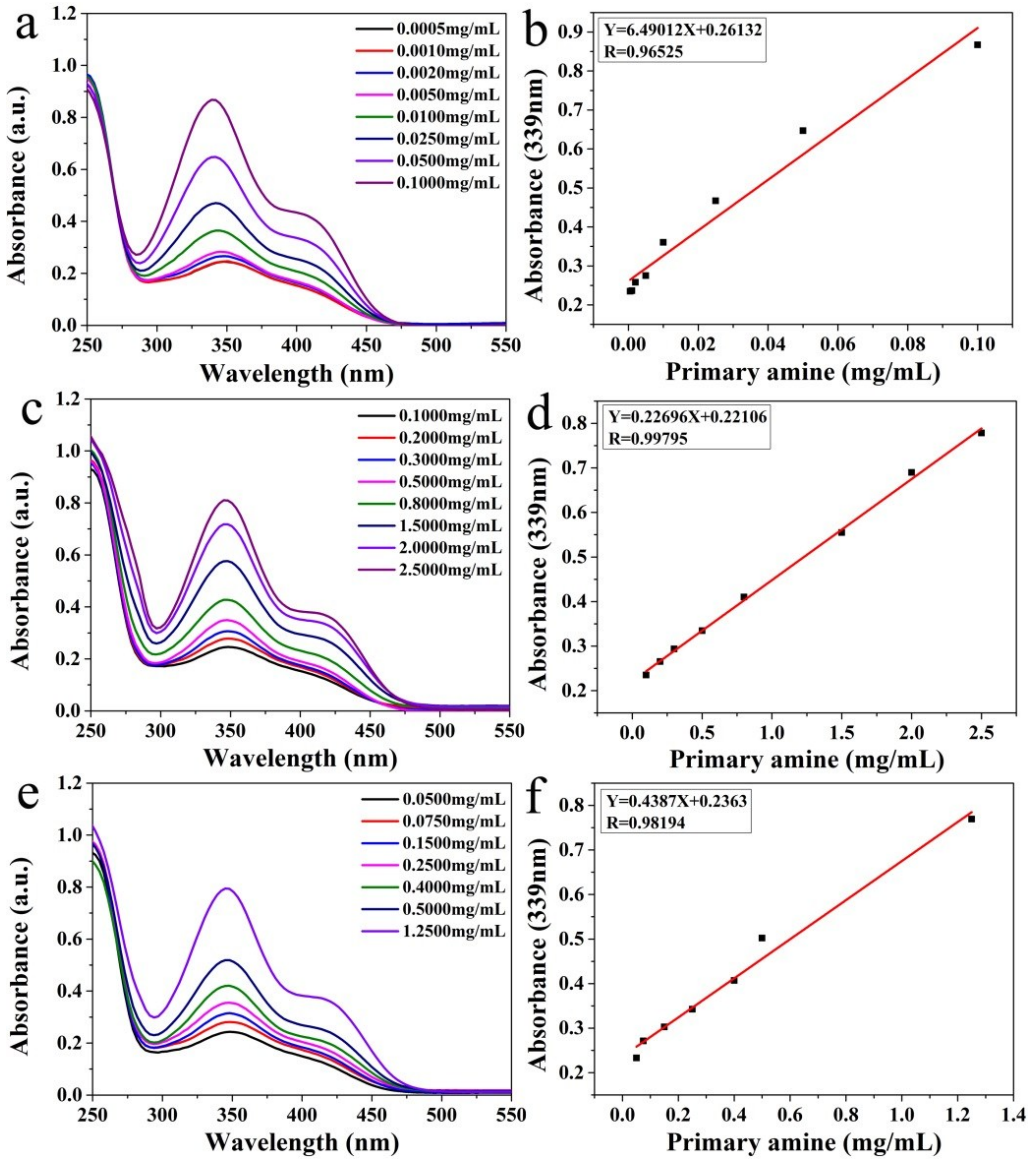


Figure S3. (a, c, e) UV-Vis spectra obtained for TNBSA/glycine control assay, BSA and BSA-NH_2, (b, d, f) corresponding calibration curve based on plotting the absorbance at 339 nm against the concentration of primary amine. Table S3 is the number of primary amine groups (*ca.* 28 and 60 on average) in BSA and BSA-NH_2.

Table S3: Number of primary amine groups in BSA and BSA-NH_2.

A(339nm)	Gly (μmol/mL)	BSA (μmol/mL)	Number (-NH_2)	BSA-NH_2 (μmol/mL)	Number (-NH_2)
0.6820595	0.89568	0.03044	29.0	0.01470	60.0
0.7182495	0.97089	0.03265	27.0	0.01591	60.1
0.7674768	1.05446	0.03612	28.8	0.01755	59.2

3.6 Synthesis of end-capped mercaptopyridine-activated PNIPAAm by RAFT polymerization

Mercaptopyridine-activated trithiol-RAFT agent (3 mg, 6.7 μmol) [1], tris(2,2'-bipyridine) dichlororuthenium(II) hexahydrate (0.1 mg, 0.13 μmol), NIPAAm (600 mg, 5.31 mmol) and acetonitrile (3 mL) were added to a 10 mL of round-bottom flask. The flask was then sealed and the solution was degassed by bubbling argon. The polymerization was carried out at UV-irradiation (395 nm) for 24 h (conversion 80 %), and purified by three times precipitation in diethyl ether/hexane (2 : 1 volume ratio). The obtained polymer was characterized by ^{1}H NMR spectroscopy in $CDCl_3$. The proton signal (δ, 2H. 4.6-4.7 ppm) from the mercaptothiazoline at the end of the PNIPAAm chain was clearly visible in the ^{1}H NMR spectrum. The molecular weight of the obtained PNIPAAm was determined by ^{1}H NMR by comparing the integral of the proton of the CH signal at δ = 4.6 ppm in mercaptothiazoline with that of the characteristic CH signal at δ = 4.0 ppm in the repeat unit of NIPAAm (Mn, 12000 g/mol).

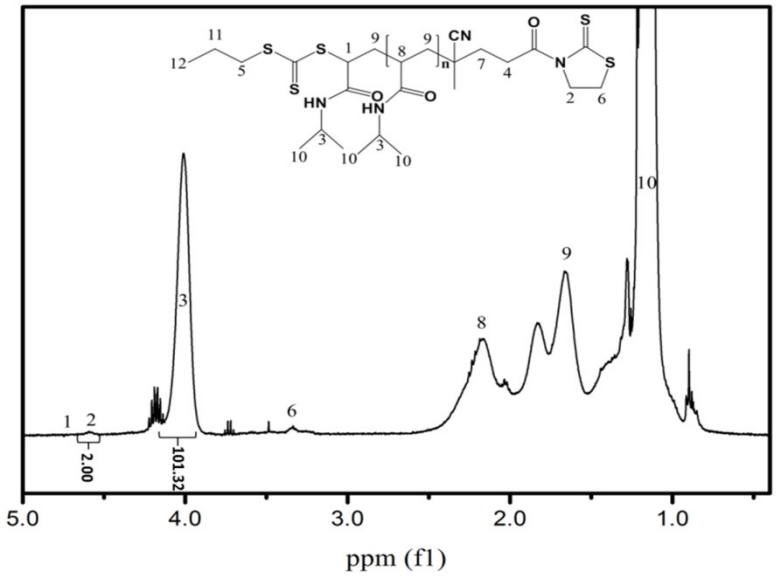


Figure S4. ^{1}H NMR spectrum of end-capped mercaptothiazoline activated PNIPAAm (Mn 12000 g/mol) in $CDCl_3$, 400 MHz.

3.7 Determination of the number of PNIPAAm on the surface of Dex-NH_2-PNIPAAm, Lys-NH_2-PNIPAAm and BSA-NH_2-PNIPAAm

The composition of the Dex-NH_2-PNIPAAm nanoconjugate was determined by UV

spectroscopy. Aqueous solutions of the PNIPAAm were prepared at a range of known concentrations (0.5, 1.0 and 1.5 mg/mL) and in each case the Dex-NH_2-PNIPAAm concentration was determined using calibration curves obtained by measuring the concentration-dependent characteristic UV absorbance of the Dex-NH_2-PNIPAAm at 308 nm (Figure S5). The PNIPAAm content in each case was then determined from the difference between the known PNIPAAm concentration and determined Dex-NH_2-PNIPAAm concentration. The average number of PNIPAAm chains per Dextran was 8.2 (Table S4).

The composition of the Lys-NH_2-PNIPAAm nanoconjugate was determined by UV spectroscopy. Aqueous solutions of the Lys-NH_2-PNIPAAm conjugate were prepared at a range of known concentrations (0.05, 0.20, 0.50 mg/mL) and in each case the Lys concentration was determined using calibration curves obtained by measuring the concentration-dependent characteristic UV absorbance of the native Lys at 280 nm (Figure S6). The PNIPAAm content in each case was then determined from the difference between the known conjugate concentration and determined Lys concentration. The average number of PNIPAAm chains per Lys was 5.0 (Table S5).

The composition of the BSA-NH_2-PNIPAAm conjugate was determined by UV spectroscopy. Aqueous solutions of the BSA-NH_2-PNIPAAm conjugate were prepared at a range of known concentrations (0.05, 0.50, 1.00 mg/mL) and in each case the BSA concentration was determined using calibration curves obtained by measuring the concentration-dependent characteristic UV absorbance of the native BSA at 280 nm (Figure S7). The PNIPAAm content in each case was then determined from the difference between the known conjugate concentration and determined BSA concentration. The average number of PNIPAAm chains per BSA was 3.2 (Table S6).

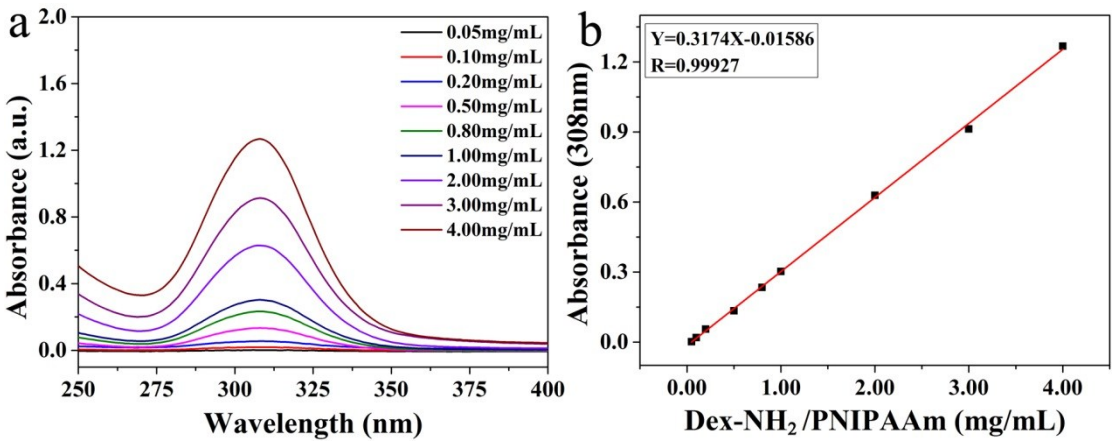


Figure S5. (a) UV-vis spectra of Dex-NH_2-PNIPAAm at different concentrations from 0.05 to 4.00 mg/mL in aqueous solution. (b) Calibration curve for Dex-NH_2-PNIPAAm determined by plotting the UV-vis absorbance at 308 nm against concentration. Table S4 is numbers (*ca*. 8.2) of PNIPAAm conjugated per Dextran molecule.

Table S5: Numbers of PNIPAAm conjugated per Dextran molecule:

A(308nm)	PNIPAAm (mg/ml)	Dex-NH_2-PNIPAAm (mg/ml)	Number of polymer
0.26565	0.5	0.88674	7.5
0.51730	1.0	1.67958	8.6
0.78395	1.5	2.51969	8.6

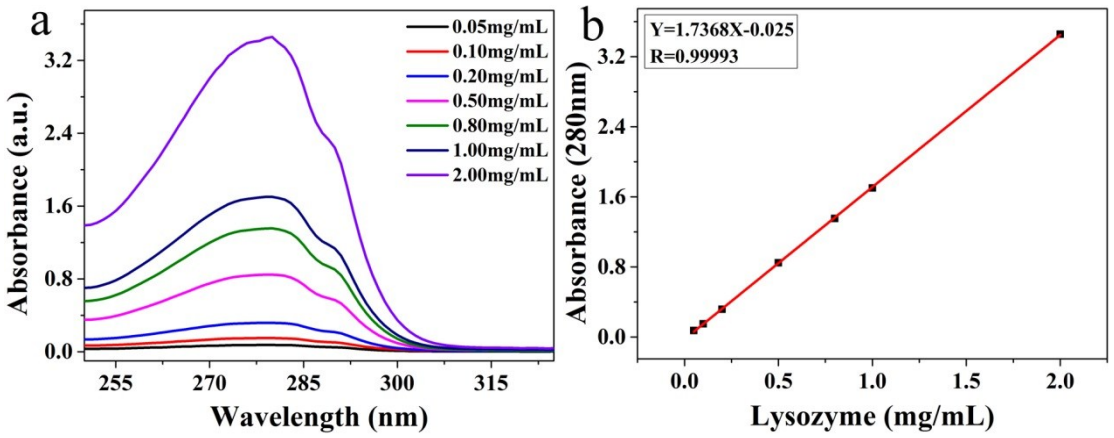


Figure S6. (a) UV-vis spectra of lysozyme at different concentrations from 0.05 to 2.00 mg/mL in aqueous solution. (b) Calibration curve for lysozyme determined by plotting the UV-vis absorbance at 280 nm against concentration. Table S5 is numbers (ca. 5.0) of PNIPAAm conjugated per Lysozyme molecule.

Table S6: Numbers of PNIPAAm conjugated per Lysozyme molecule:

A(280nm)	Lys-NH_2-PNIPAAm (mg/ml)	Lys (mg/ml)	Number of polymer
0.03643	0.05	0.03537	3.6
0.16629	0.2	0.11013	5.6
0.44587	0.5	0.27111	5.9

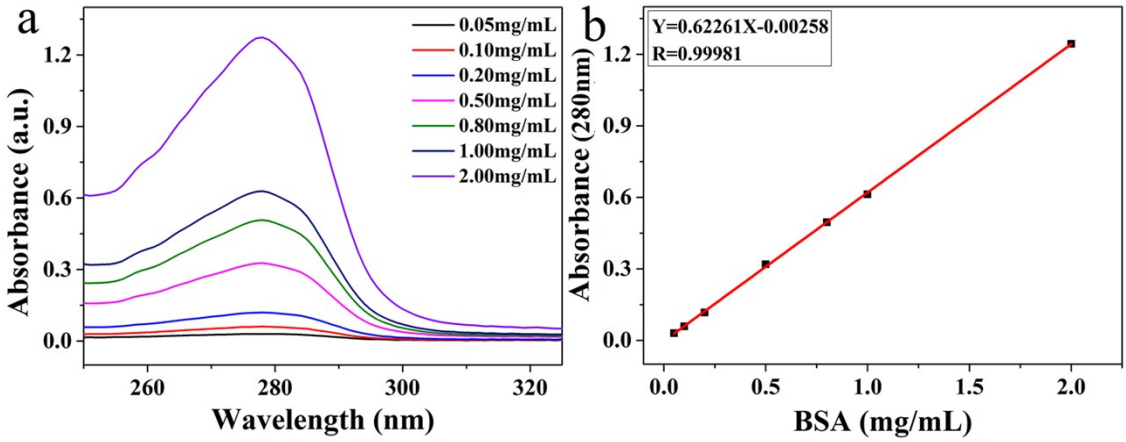


Figure S7. (a) UV-vis spectra of BSA at different concentrations from 0.05 to 2.00 mg/mL in aqueous solution. (b) Calibration curve for BSA determined by plotting the UV-vis absorbance at 280 nm against concentration. Table S6 is numbers (*ca*. 3.2) of PNIPAAm conjugated per BSA.

Table S7: Numbers of PNIPAAm conjugated per BSA molecule:

A(280nm)	BSA-NH_2-PNIPAAm (mg/ml)	BSA (mg/ml)	Number of polymer
0.01640	0.05	0.03048	3.5
0.19660	0.50	0.31991	3.1
0.40324	1.00	0.65180	2.9

3.8 Labelling experiments

BSA/NH_2-PNIPAAm, Lysozyme/NH_2-PNIPAAm, lysozyme, or dextran (10.0 mg)

was dissolved into 2.0 mL of pH 8.5 sodium carbonate buffer solution (100 mM). Then 50 μL of fluorescein isothiocyanate or rhodamine B isothiocyanate DMSO solution (1.0 mg/mL) was added dropwise. The solution was stirred at room temperature for 5 h, purified by dialyzing against Milli-Q water, and freeze-dried.

3.9 Atomic force microscope (AFM) measurements

In this study, we proposed an independent development AFM-based nanorobotics system for the cell force measurement.[2] The half-opening angle of the tip apex was 45°. The spring constant of the cantilevers was determined using the method proposed by Sader *et al*.[3] with values of 0.006 N/m. The relationship between force and deformation of the AFM probe is in accordance with Hooke's law.[4] That is, when the cantilever exerted a small force, a suitable deformation could be detected. Meanwhile, the probe stiffness needs to be close to the stiffness of the samples. When the deformation of the probe caused by the force is basically the same as the deformation of the sample itself, the surface topography and mechanical properties of the sample are most similar to the actual situation.[5] The cell deformation conformed to the modified Hertz model[6,7] because the loading rate was low and the cell deformation was less than half of the cell height. The apparent Young's modulus E of cells was related to the position of the contact point between cell surface, the tip, and the slope of the force–distance curve. The modified Hertz model for a stiff cone and a flat surface can be expressed as

$$F = \frac{2}{\pi} \cdot \frac{Etan\alpha}{(1-\upsilon^2)} \delta^2$$

Where F indicates the force of the probe, v represents Poisson's ratio of cells of 0.5 in this system and δ is the indentation depth of the tip. The point-to-point search method is used to find the contact point between the cell and the tip by Matlab. The force–distance curve behind the contact point can be used to calculate the slope, and finally, the Young's modulus can be obtained[8].

3.10 Biomacromolecules release experiments

FITC-lysozyme (20.0 mg/mL, 6 μL), RBITC-dextran (20.0 mg/mL, 6 μL) and DNA (2.0 mg/mL, 6 μL) were encapsulated in the hybrid microcapsules. The release

percentage of FITC-lysozyme (green) and RBITC-dextran (red) upon TCEP (4.0 mg/mL) and protease (2.0 mg/mL) with time were measured by fluorescence spectrometer under 529 nm and 580 nm of emission wavelength, respectively. Then the release percentage of DNA upon lyticase (3.0 mg/mL) was measured by UV-vis spectra.

To observe the release of DNA without being confused with that of FITC-lysozyme (green), we combined the SYBR green with the hybrid microcapsules loaded with DNA, then the disassembly of the dextran-based microcapsule and the release of SYBR green I-stained DNA (green) by lyticase with time were observed in the fluorescence microscope.

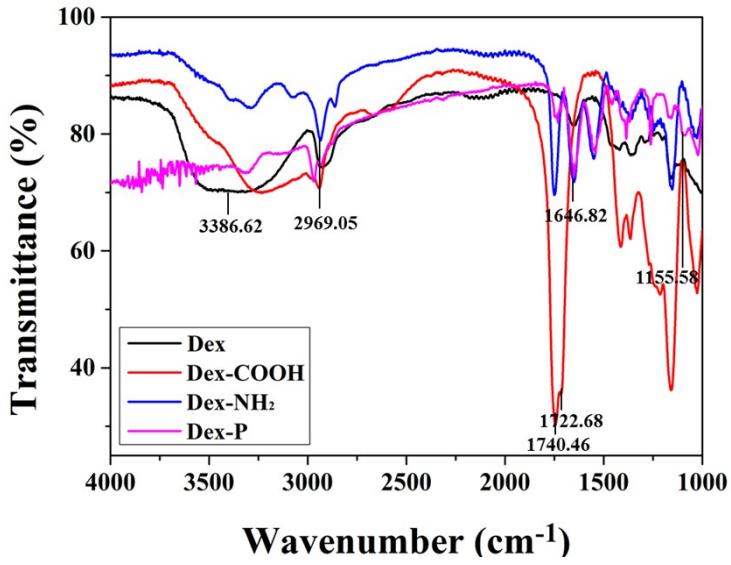


Figure S8. The FTIR spectra of Dextran (Dex), Dex-COOH, Dex-NH_2 and Dextran/NH_2-PNIPAAm (Dex-P). The disappearance of wavenumber 3386.62 cm^{-1} (Dex, black, -OH) compared with the Dex-COOH, and the appearance of the wavenumber 1740.46 cm^{-1}(-C=O-, ester) and the wavenumber 1722.68 cm^{-1} (-COOH) suggested the successful preparation of Dex-COOH; the wavenumber 1646.82 cm^{-1} (-CO-NH-) showed the achievement of dextran-NH_2; the wavenumber 1155.58 cm^{-1} and 2969.05 cm^{-1} belonging to the "-C=S-" bond and "-CH_3" group on PNIPAAm respectively, represented that the Dex/NH_2-PNIPAAm was synthesized successfully.

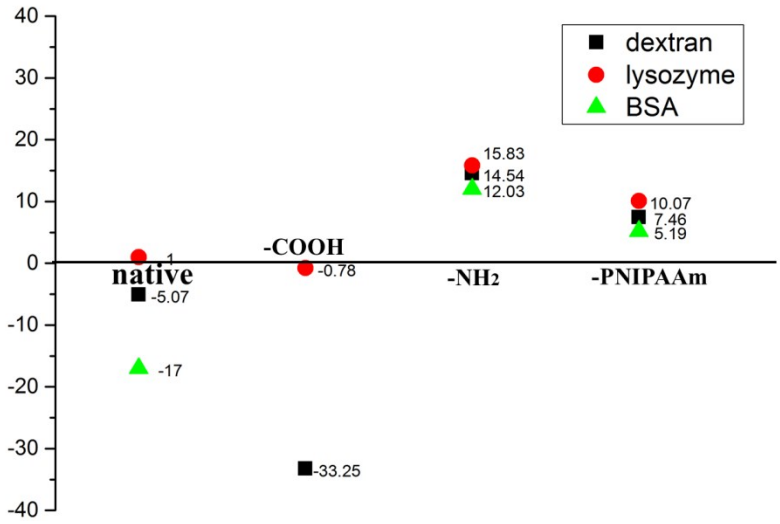


Figure S9. Zeta potential measurements for native dextran, lysozyme and BSA, dextran-COOH and lysozyme-COOH, dextran-NH_2, lysozyme-NH_2 and BSA-NH_2, dextran/NH_2-PNIPAAm, lysozyme/NH_2-PNIPAAm and BSA/NH_2-PNIPAAm in 10.0 mM of PBS (pH 8.0) buffer solution at room temperature, which shows the change of zeta potential for each pre-building blocks and proves their successful preparation. The more negative charge compared with the native ones showed the successful modification of "-COOH" for Dex and Lys; and then the sharp positive charge proved the attachments of "-NH_2" for Dex-COOH, Lys-COOH and BSA; finally, the obvious decreases of the positive charge for Dex-NH_2, Lys-NH_2 and BSA-NH_2 proved the conjugation of PNIPAAm for each building blocks.

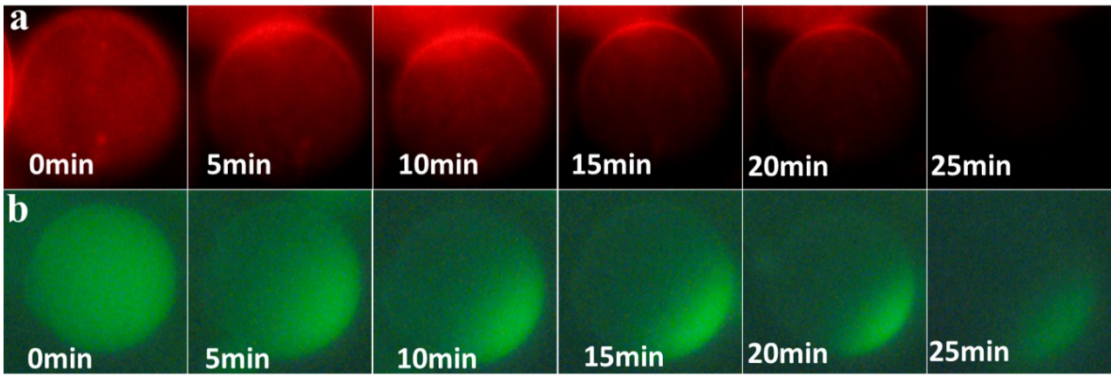
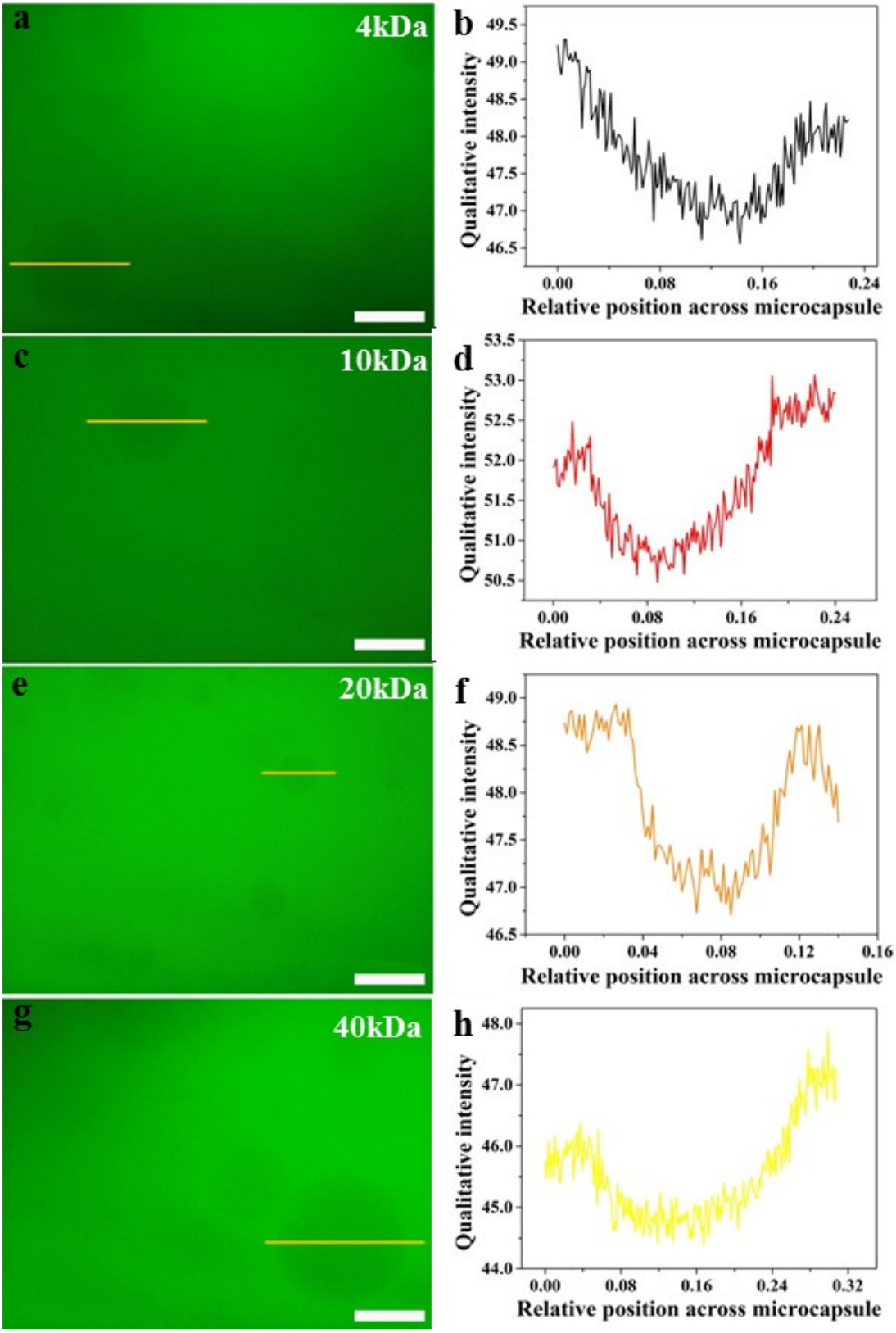


Figure S10. Fluorescence microscopy image showing the leaving of the (a) lysozyme and (b) BSA from the microcapsule upon adding TCEP and protease, respectively. Lysozyme was labelled by RBITC (red) and BSA was labelled by FITC (green).


a
4kDa
b
Qualitative intensity
Relative position across microcapsule
c
10kDa
d
Qualitative intensity
Relative position across microcapsule
e
20kDa
f
Qualitative intensity
Relative position across microcapsule
g
40kDa
h
Qualitative intensity
Relative position across microcapsule

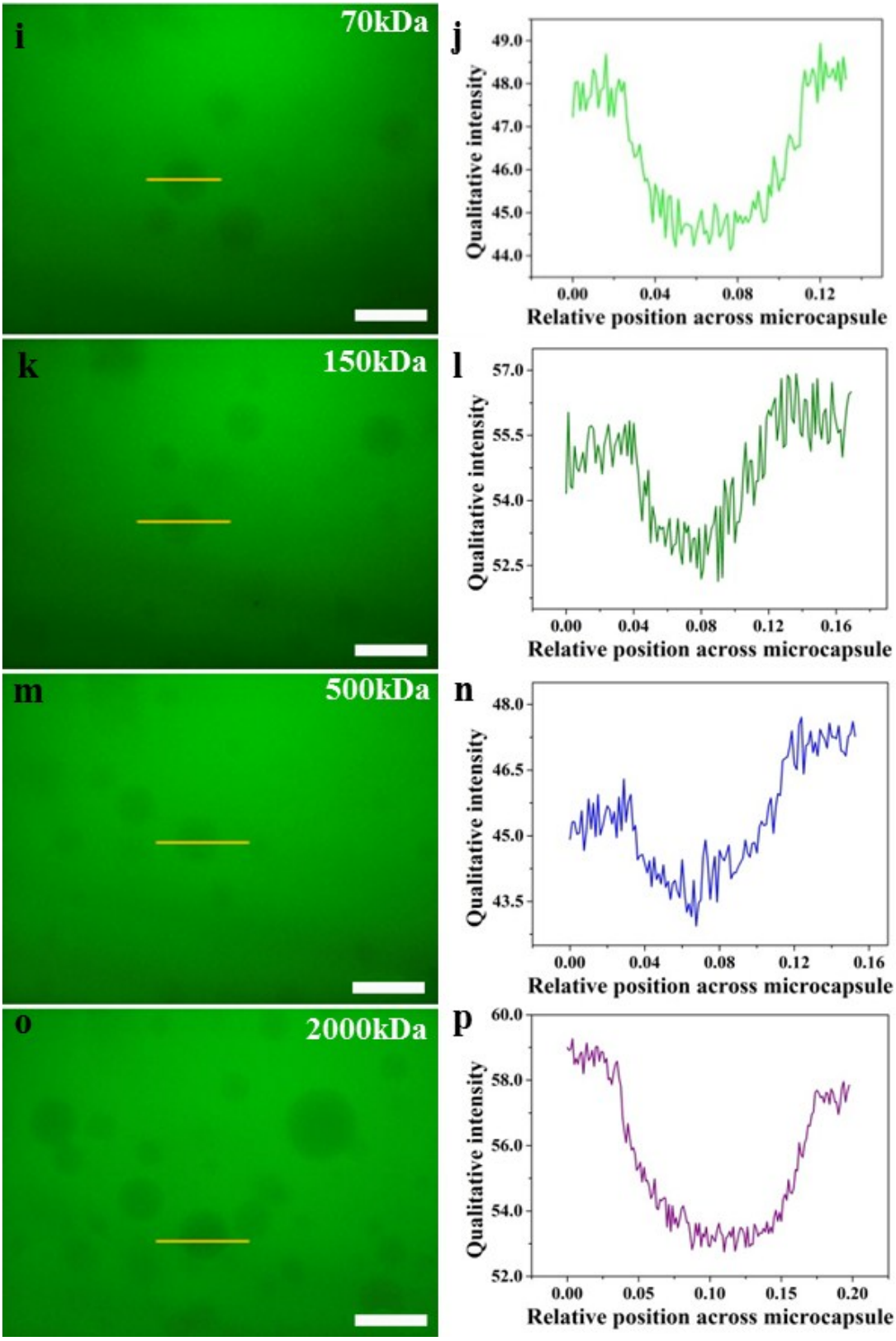
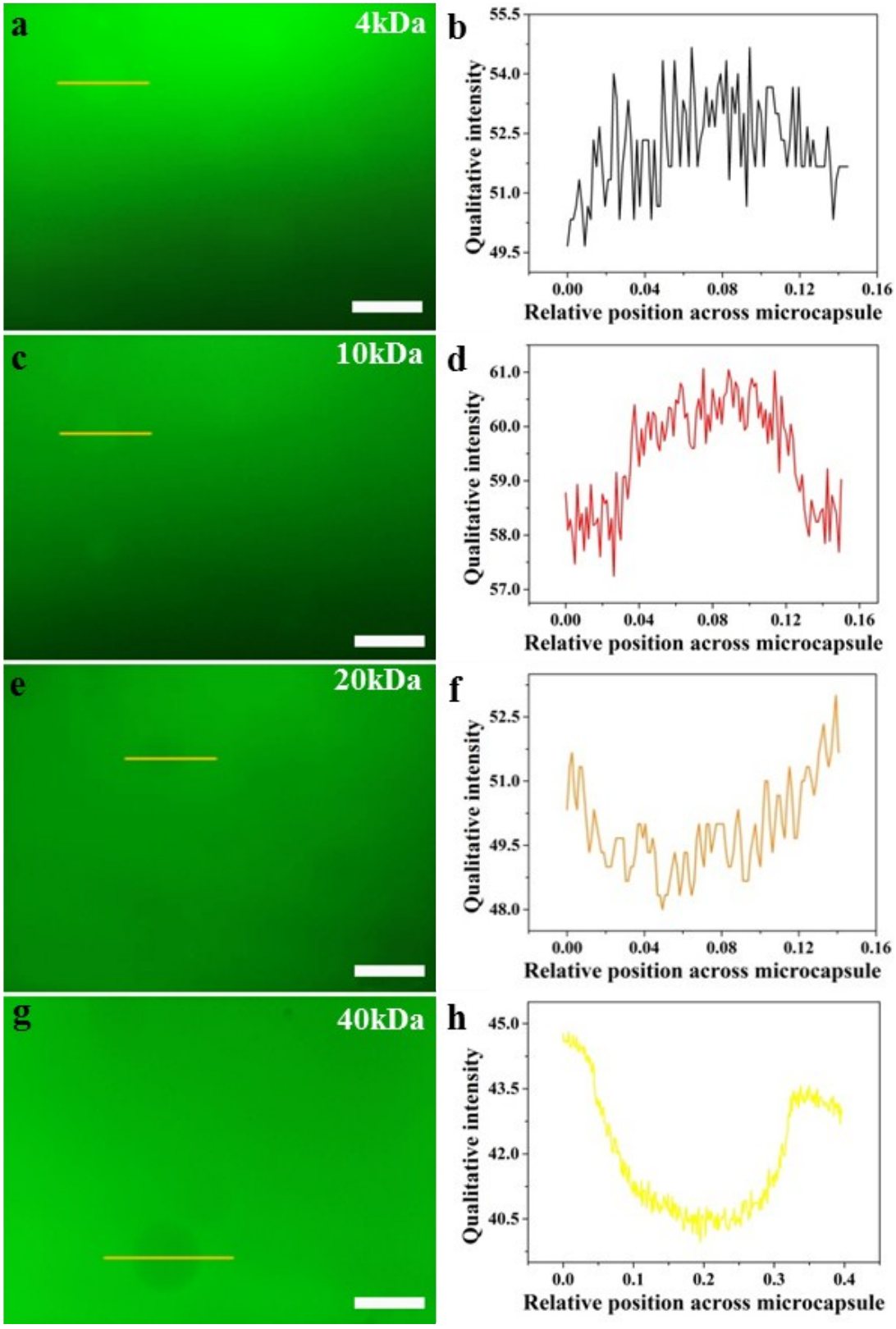


Figure S11. Study of permeability of the constructed triple hybrid microcapsules based on the diffusion of the fluorescent-labeled dextrans (FITC-dextran) with molecular weights from 4 to 2000 kDa. The experiments were performed by mixing 5 μL of 0.1 mg/mL FITC-dextran solution

with 50 μL of the microcapsule aqueous dispersion. After incubating the mixture at 25 °C for 30 min, the corresponding fluorescence microscopy images were captured under the same condition (a, c, e, g, i, k, m, o) in the presence of FITC-dextran with a molecular weight of 4, 10, 20, 40, 70, 150, 500 or 2000 kDa, respectively. (b, d, f, h, j, l, n, p) Corresponding fluorescence intensity line profiles of selected microcapsule are shown in the fluorescence images. Scale bars: 50 μm.


a
4kDa
b
Qualitative intensity
55.5
54.0
52.5
51.0
49.5
0.00
0.04
0.08
0.12
0.16
Relative position across microcapsule
c
10kDa
d
Qualitative intensity
61.0
60.0
59.0
58.0
57.0
0.00
0.04
0.08
0.12
0.16
Relative position across microcapsule
e
20kDa
f
Qualitative intensity
52.5
51.0
49.5
48.0
0.00
0.04
0.08
0.12
0.16
Relative position across microcapsule
g
40kDa
h
Qualitative intensity
45.0
43.5
42.0
40.5
0.0
0.1
0.2
0.3
0.4
Relative position across microcapsule

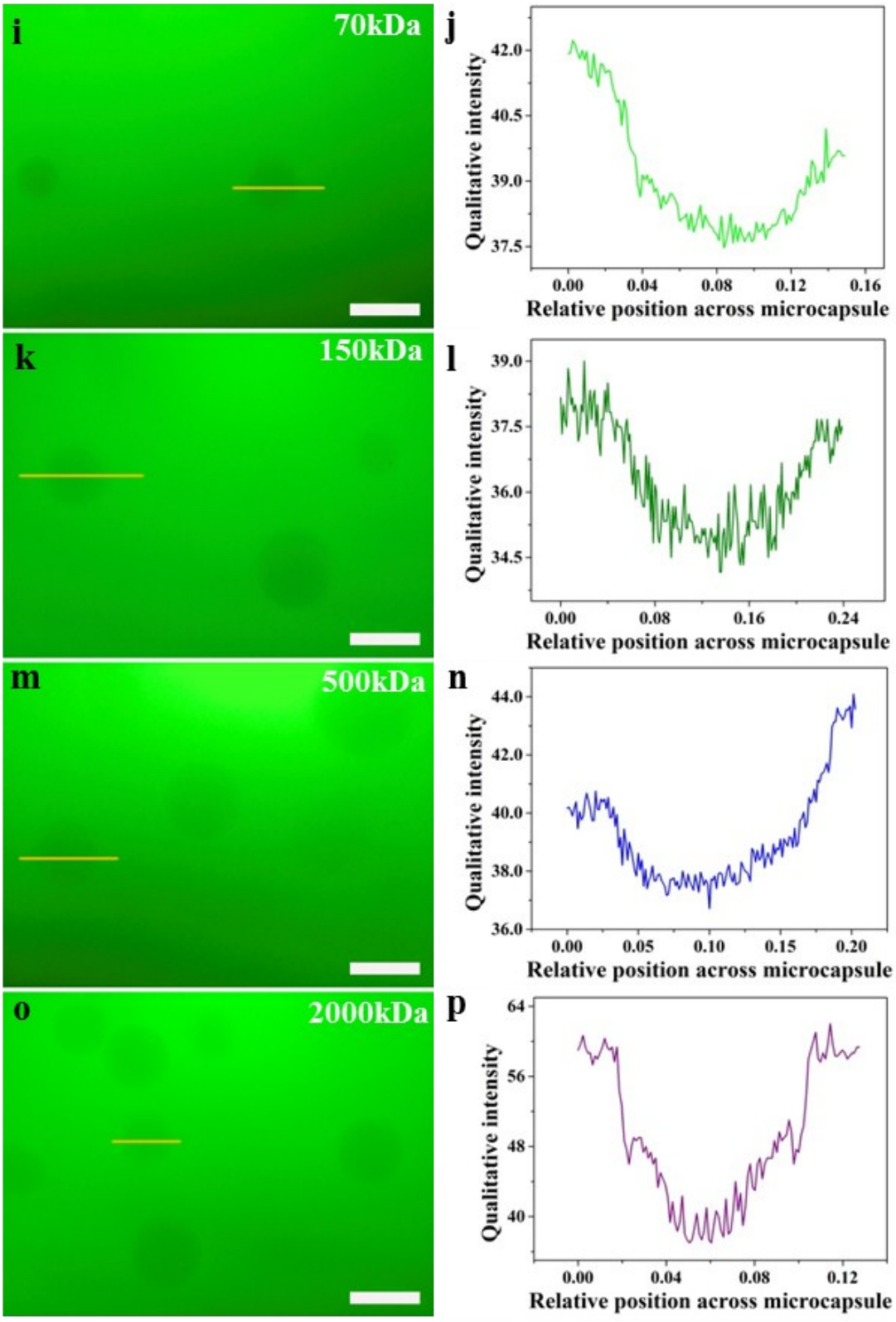
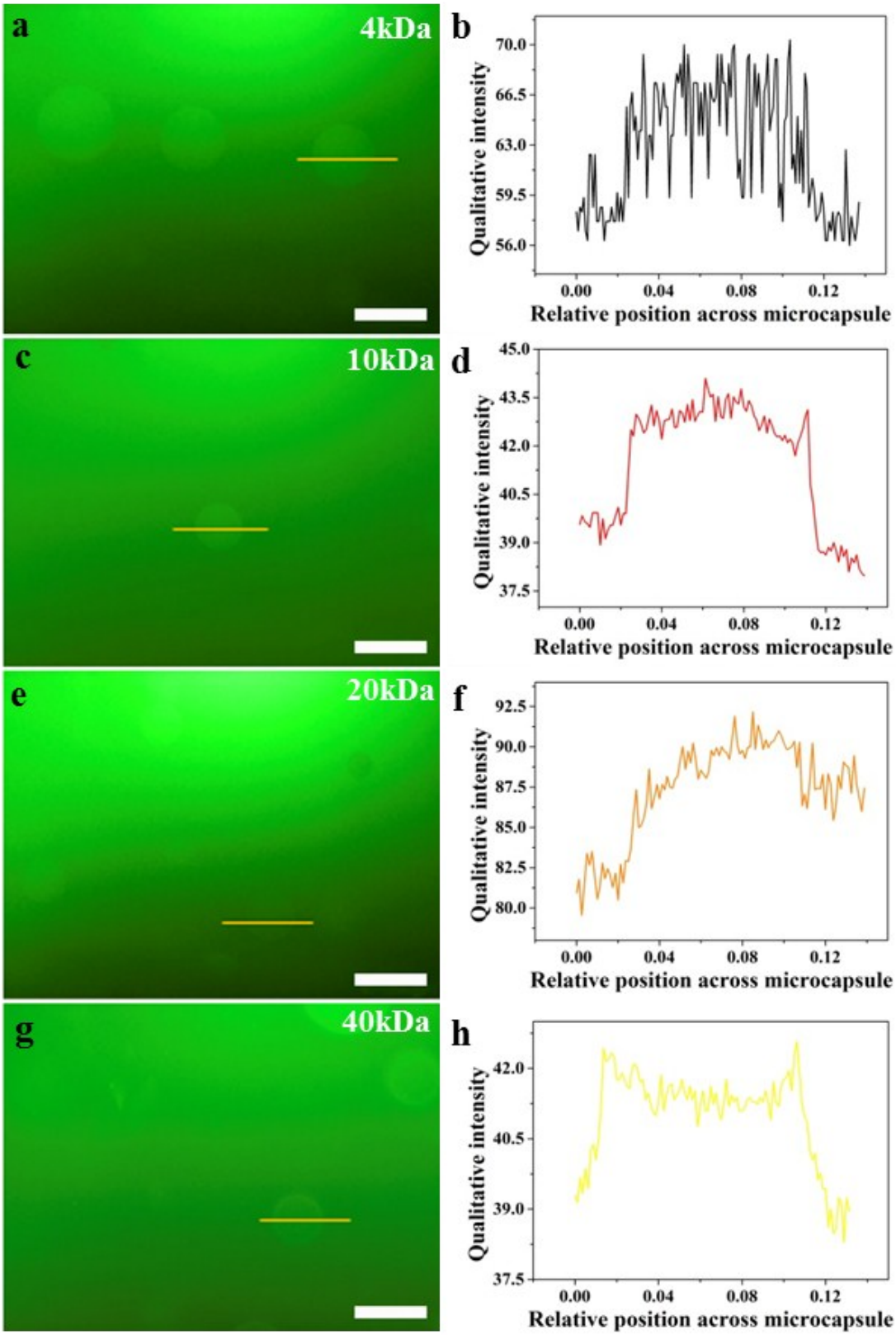


Figure S12. Study of permeability of the constructed triple hybrid microcapsules based on the diffusion of the fluorescent-labeled dextrans (FITC-dextran) with molecular weights from 4 to 2000 kDa in the presence of 4 mg/mL of TCEP. The experiments were performed by mixing 5 μL of 0.1 mg/mL FITC-dextran solution with 50 μL of the microcapsule aqueous dispersion. After

incubating the mixture at 25 °C for 30 min, the corresponding fluorescence microscopy images were captured under the same condition (a, c, e, g, i, k, m, o) in the presence of FITC-dextran with a molecular weight of 4, 10, 20, 40, 70, 150, 500 or 2000 kDa, respectively. (b, d, f, h, j, l, n, p) Corresponding fluorescence intensity line profiles of selected microcapsule are shown in the fluorescence images. Scale bars: 50 μm.


a
4kDa
b
Qualitative intensity
70.0
66.5
63.0
59.5
56.0
0.00
0.04
0.08
0.12
Relative position across microcapsule
c
10kDa
d
Qualitative intensity
45.0
43.5
42.0
40.5
39.0
37.5
0.00
0.04
0.08
0.12
Relative position across microcapsule
e
20kDa
f
Qualitative intensity
92.5
90.0
87.5
85.0
82.5
80.0
0.00
0.04
0.08
0.12
Relative position across microcapsule
g
40kDa
h
Qualitative intensity
42.0
40.5
39.0
37.5
0.00
0.04
0.08
0.12
Relative position across microcapsule

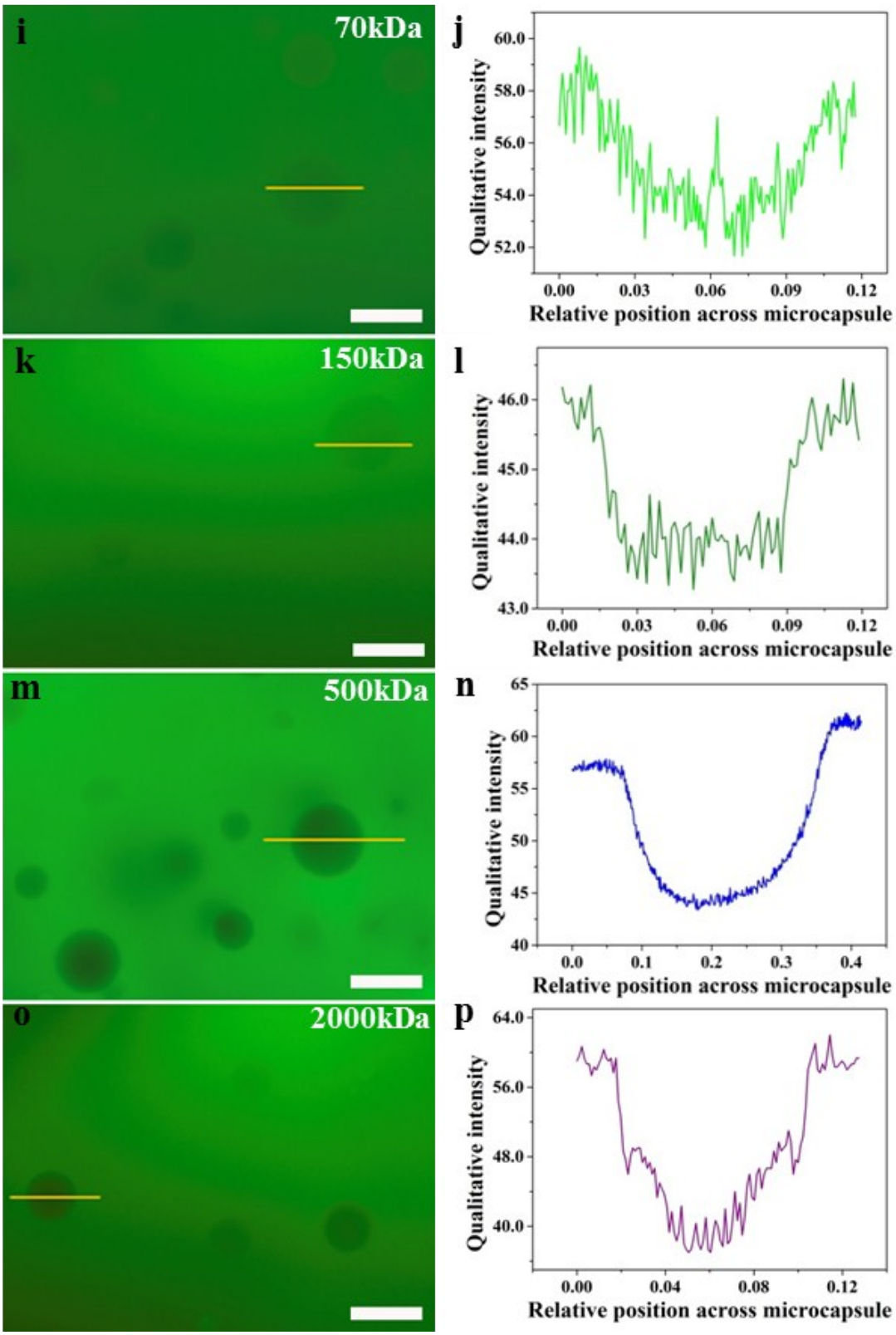


Figure S13. Study of permeability of the constructed triple hybrid microcapsules based on the diffusion of the fluorescent-labeled dextrans (FITC-dextran) with molecular weights from 4 to 2000 kDa in the presence of 2 mg/mL of protease. The experiments were performed by mixing 5 μL of 0.1 mg/mL FITC-dextran solution with 50 μL of the microcapsule aqueous dispersion. After

incubating the mixture at 25 °C for 30 min, the corresponding fluorescence microscopy images were captured under the same condition (a, c, e, g, i, k, m, o) in the presence of FITC-dextran with a molecular weight of 4, 10, 20, 40, 70, 150, 500 or 2000 kDa, respectively. (b, d, f, h, j, l, n, p) Corresponding fluorescence intensity line profiles of selected microcapsule are shown in the fluorescence images. Scale bars: 50 μm.

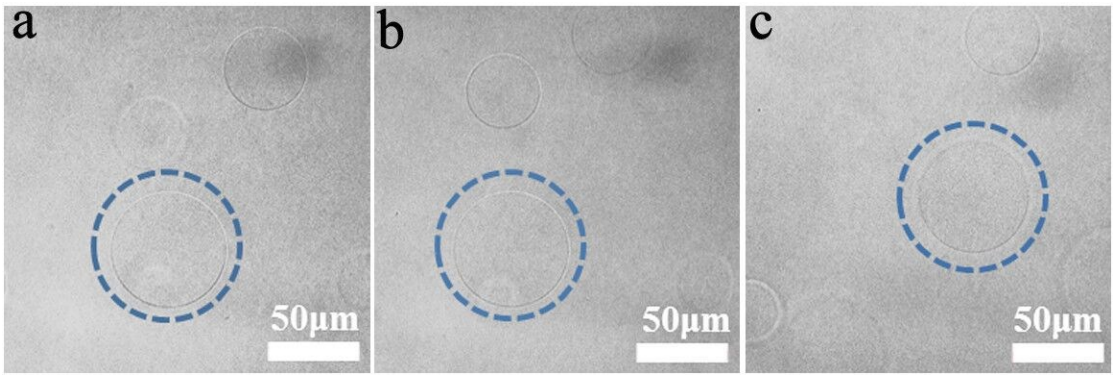


Figure S14. The optical microscopy image of (a) the original triple hybrid microcapsules, with the presence of (b) TCEP and (c) protease, which indicated that despite the removal of the two building blocks from the membrane, the spherical structure of the generated porous microcapsules could be well maintained.

Notes and reference

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