Electronic Supplementary Material (ESI) for Journal of Materials Chemistry

SUPPORTING INFORMATION

Ultrathin, Bioresponsive and Drug-Functionalized Protein Capsules

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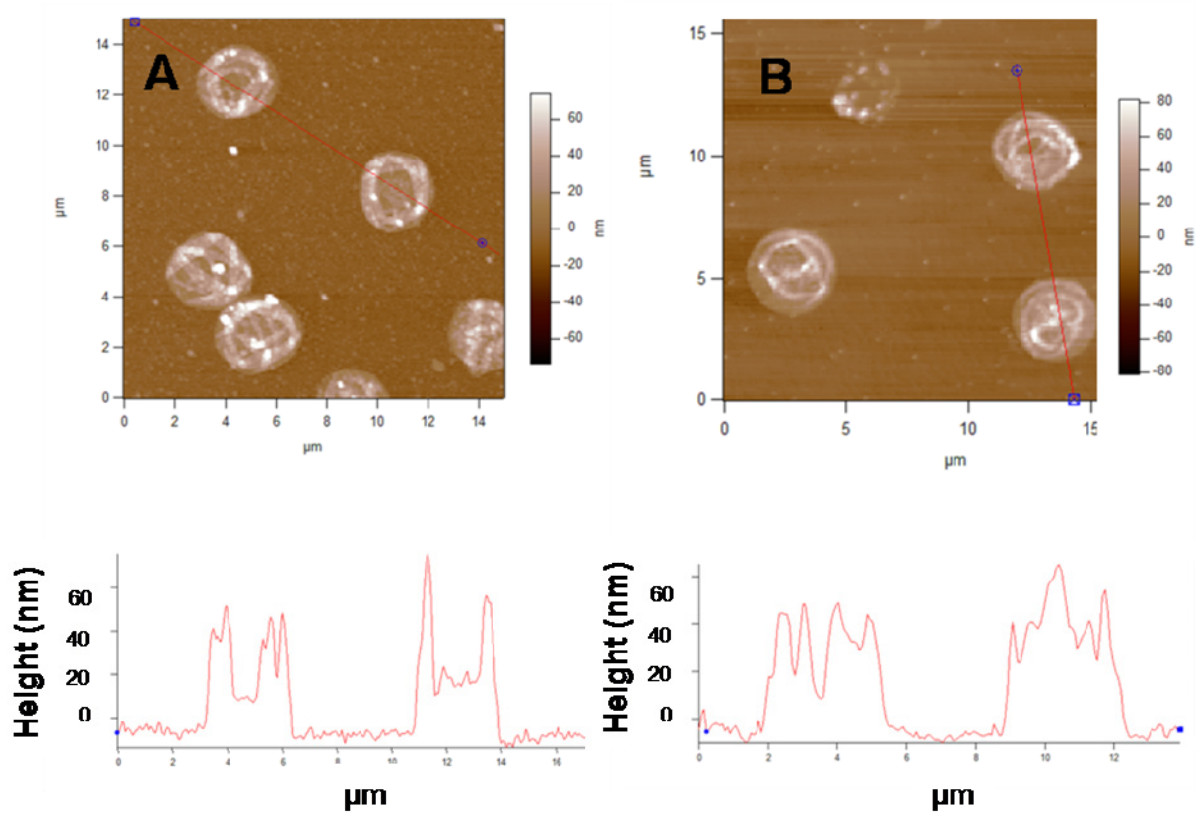


Fig. S1 Estimation of capsule wall thickness by AFM analysis. AFM images and associated z-profile of BrIBAM-AP capsules initially templated on SiO_2 particles (5 µm diameter) (A) without and (B) with DSP cross-linking. The capsule wall thickness was determined from z-profile analysis of 10 different capsules. The double wall thickness is estimated by taking the minimum of the z-profile of each capsule.

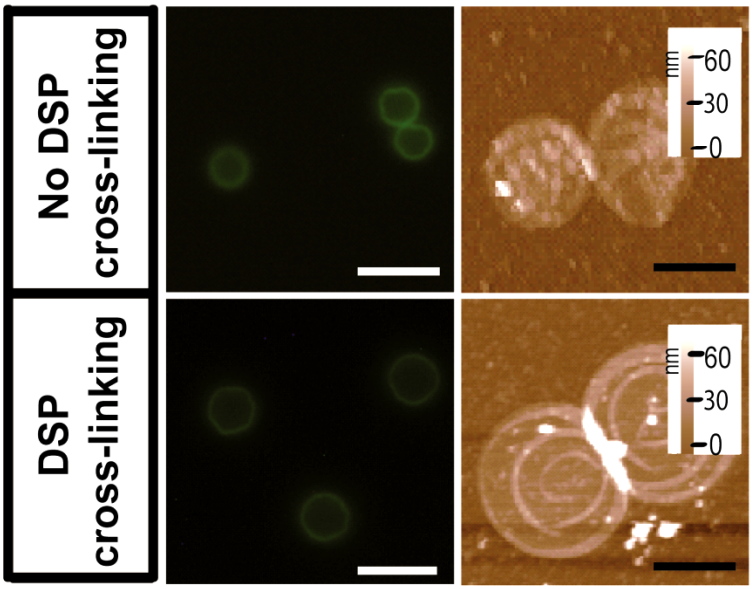


Fig. S2 Fluorescence microscopy and AFM images of BrIBAM-PLL capsules without and with DSP cross-linking. Scale bars are 5 μm for fluorescence microscopy and 2 μm for AFM images, respectively.

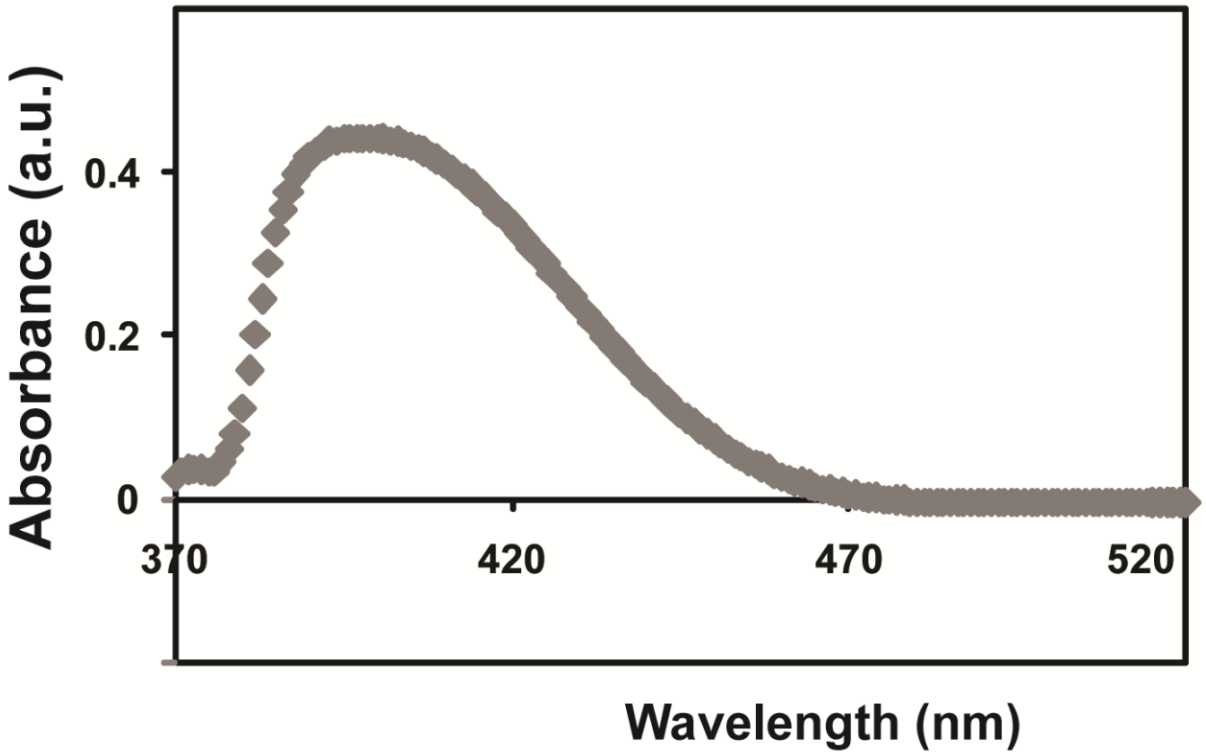


Fig. S3 Absorbance spectrum of PNP formation catalyzed by the DSP-BrIBAM-AP capsules and followed by UV/vis spectrophotometry after 96 h reaction. Baseline is for a solution of p-nitrophenylphosphate substrate.

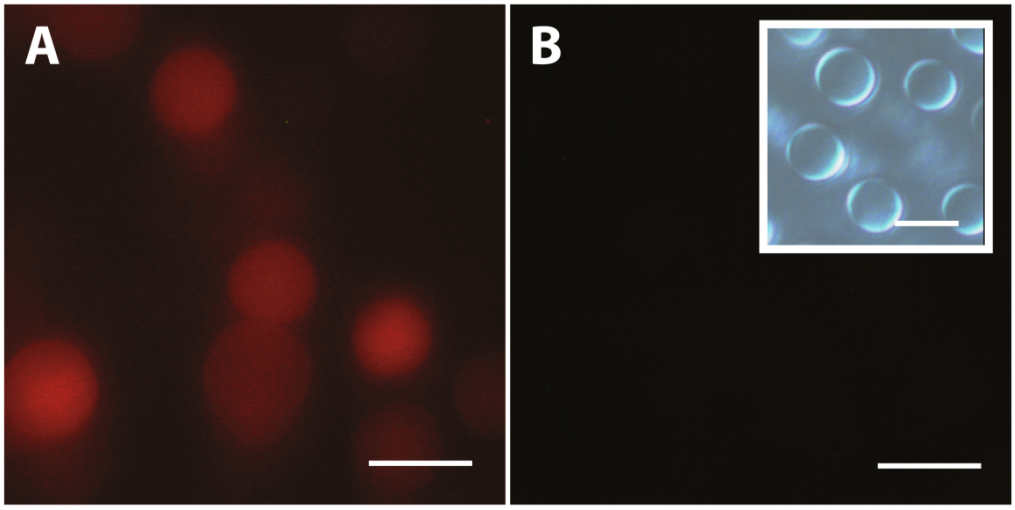


Fig. S4 Fluorescence microscopy images of DOX-incubated BrIBAM-HSA coated MS spheres (A) after DSP cross-linking and (B) without DSP cross-linking. Inset is a bright field microscopy image of BrIBAM-HSA coated MS spheres incubated with DOX. Scale bars are 5 μm for all images.

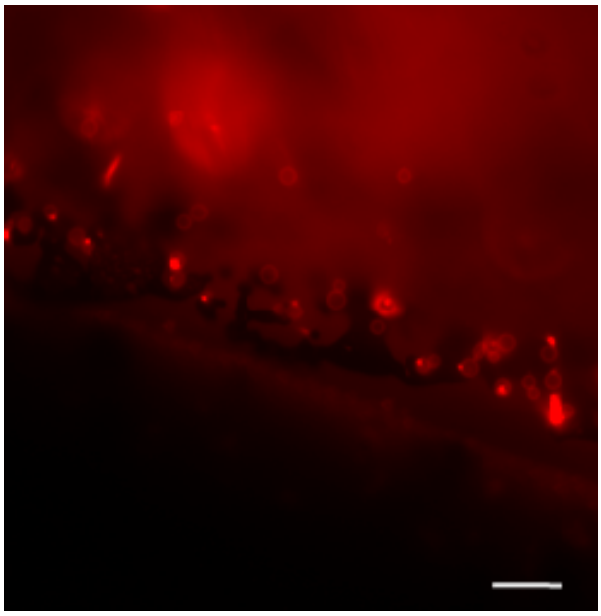


Fig. S5 Fluorescence microscopy image of DOX-functionalized BrIBAM-HSA capsules incubated with a GSH solution 5 mM during 6 h. The image shows the interface of the droplet containing the capsules. Scale bar is 10 μm.

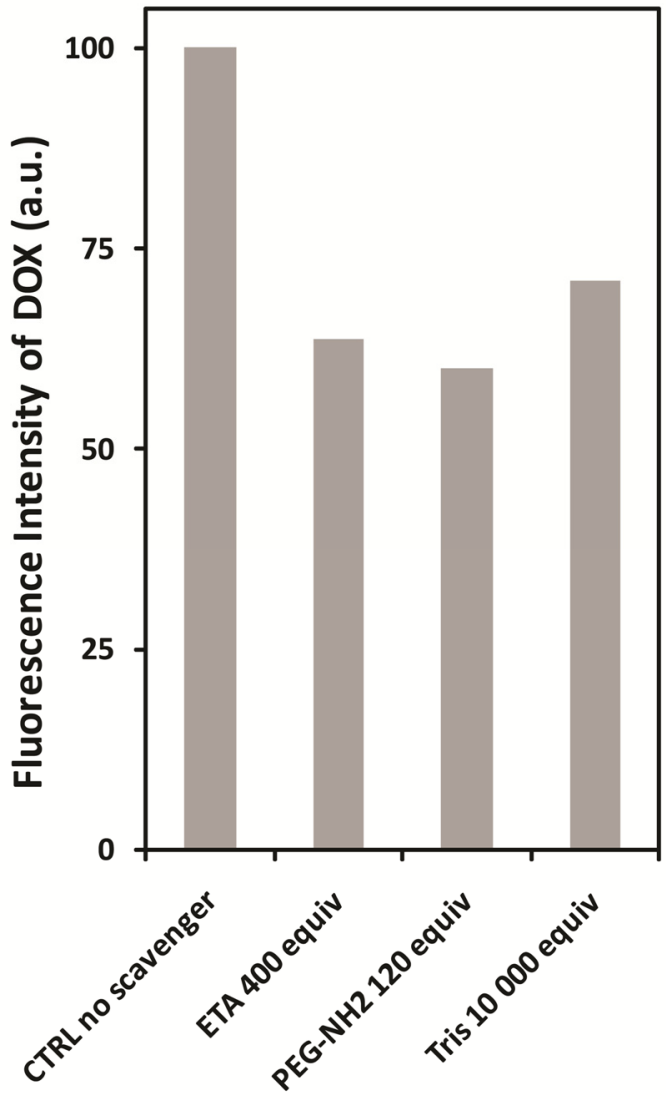


Fig. S6 Fluorescence intensities measured by flow cytometry of DSP cross-linked BrIBAM-HSA-coated silica particles incubated with DOX, after a pre-incubation period (1 h) in PBS buffer (control, no scavenger), with ETA (400 eq. in PBS buffer), with PEG-NH_2 (120 eq. in PBS buffer), with Tris (10 000 eq. in PBS buffer).

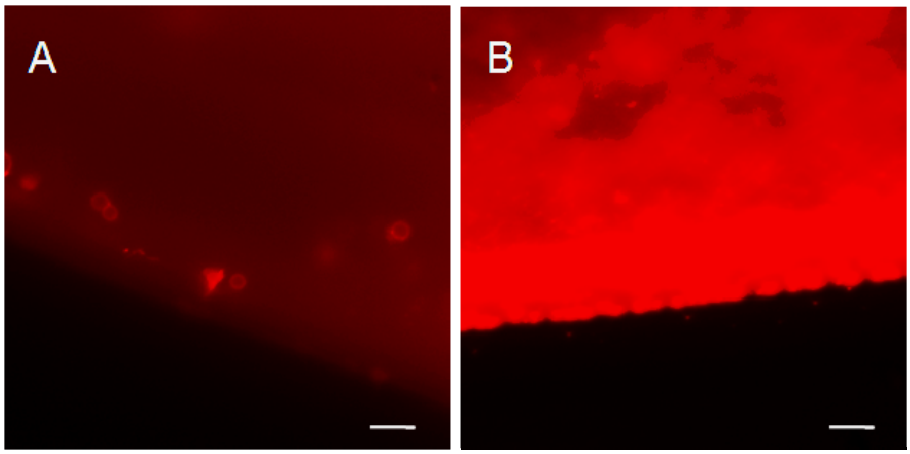


Fig. S7 Fluorescence microscopy images (taken with the same acquisition parameters – excitation and detector gain) of DOX-functionalized BrIBAM-HSA capsules incubated with a protease solution at 0.5 mg.mL^{-1} at 37 °C incubated (A) 3 h and (B) 34 h. The image shows the interface of the droplet containing the capsules. Scale bars are 10 μm.

Table S1 Molecular weight (MW) and isoelectric point (IEP) of proteins and adsorption conditions. All the data were obtained from suppliers (Sigma-Aldrich).

Protein	MW (kDa)	IEP	Adsorption Conditions
AP	57.1	5.7	Milli Q water (pH 5.8)
INS	5.8	5.3	Milli Q water (pH 5.8)
LYS	14.3	11.3	$NaHCO_3$ buffer (pH 10)
HSA	68	4.7	Milli Q water (pH 5.8)
HRP	44	3-9 (7 isosymes)	Milli Q water (pH 5.8)

Table S2 Diameters (μm) of the BrIBAM-protein capsules without and with DSP cross-linking, as assessed by microscopy analysis. For each capsule size measurement, the analysis was carried out on a minimum number of 30 capsules.

	AP	**INS**	**LYS**	**HSA**	**HRP**
No DSP cross-linking	2.8 ± 0.2	4.1 ± 0.4	2.9 ± 0.1	3.9 ± 0.2	3.3 ± 0.2
DSP cross-linking	3.4 ± 0.2	3.5 ± 0.3	3.5 ± 0.1	4.2 ± 0.4	4.2 ± 0.2

Table S3 Estimated wall thicknesses (nm) of the BrIBAM-protein capsules without and with DSP cross-linking obtained by AFM z-profile analysis. For each capsule size measurement, the analysis was carried out on a minimum number of 10 capsules.

	AP	**INS**	**LYS**	**HSA**	**HRP**
No DSP cross-linking	9.3 ± 1.9	6.0 ± 0.6	5.0 ± 1.0	5.7 ± 0.9	7.8 ± 2.1
DSP cross-linking	12.9 ±1.4	7.7 ± 1.0	9.6 ± 1.1	6.8 ± 0.9	8.4 ± 1.1

Table S4 Diameters and estimated wall thicknesses of the BrIBAM-PLL capsules without and with DSP cross-linking.

	Size (μm)	**Wall thickness (nm)**
No DSP cross-linking	1.7 ± 0.1	7.7 ± 1.1
DSP cross-linking	2.5 ± 0.1	15.7 ± 1.1

Table S5 Morphology of the structures observed after silica template removal for DSP cross-linked proteins (HSA, HRP and AP) pre-adsorbed onto an amino-modified prelayer (APTS, PLL or PEI).

	APTS	**PLL**	**PEI**
HSA	Disassembled	Collapsed	Collapsed
HRP	Disassembled	Collapsed	Collapsed
AP	Disassembled	Collapsed	Collapsed

Table S6A AFM images and associated z-profiles of DSP cross-linked BrIBAM-INS capsules, respectively, templated on 1.11 μm, 585, 304 and 147 nm size SiO_2 particles. Average diameter and estimated thickness of the capsules were obtained from analysis of 10 capsules. Z-profiles represent height (nm) vs. distance (μm) traced in red lines.

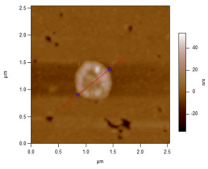
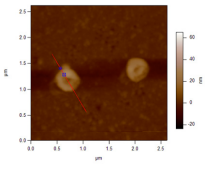
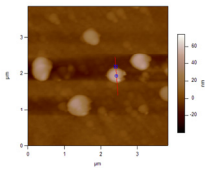
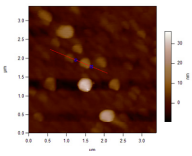
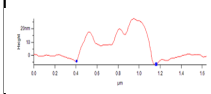
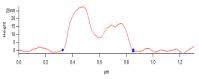
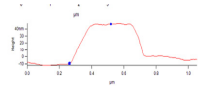
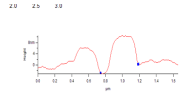
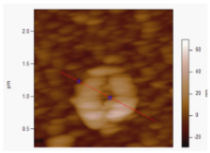
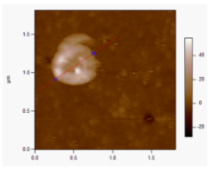
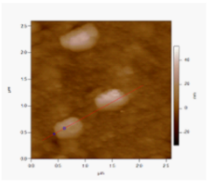
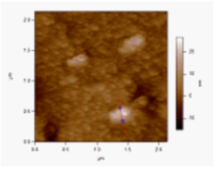
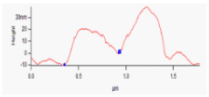
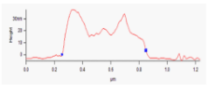
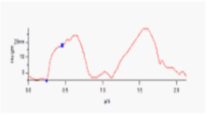
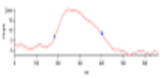
Template	1110 nm	585 nm	304 nm	147 nm
AFM image				
Z-Profile				
Average diameter	**856 ± 81 nm**	**542 ± 41 nm**	**367 ± 59 nm**	**324 ± 51 nm**

Table S6B AFM images and associated z-profiles of DSP cross-linked BrIBAM-HSA capsules, respectively, templated on 1.11 μm, 585, 304 and 147 nm size SiO_2 particles. Average diameter and estimated thickness of the capsules were obtained from analysis of 10 capsules. Z-profiles represent height (nm) vs. distance (μm) traced in red lines.

Template	1110 nm	585 nm	304 nm	147 nm
AFM image				
Z-Profile				
Average diameter	**1212 ± 79 nm**	**739 ± 134 nm**	**393 ± 11 nm**	**243 ± 50 nm**