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Supporting Information

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# Labelling Bacterial Nanocages with Photo-switchable Fluorophores

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## S1. Sequence and characterization of *B. linens* encapsulin and spiropyran-labeled encapsulin

The encapsulin of B. linens was expressed in *E. coli* and purified based on the procedure reported in literatures.[1, 2] The sequence of encapsulin monomer is given below based on the DNA database: UniProtKB - Q45296 (LIN18_BRELN). Lysine residues are highlighted in red.

MNNLYRELAPIPGPAWAEIEEEARRTFKRNIAGRRIVDVAGPTGFETSAVTTGHIRDVQS

ETSGLQVKQRIVQEYIELRTPFTVTRQAIDDVARGSGDSDWQPVKDAATTIAMAEDRAIL

HGLDAAGIGGIVPGSSNAAVAIPDAVEDFADAVAQALSVLRTVGVDGPYSLLLSSAEYTK

VSESTDHGYPIREHLSRQLGAGEIIWAPALEGALLVSTRGGDYELHLGQDLSIGYYSHDS

ETVELYLQETFGFLALTDESSVPLSL

Since there are 4 lysines for each monomer, an encapsulin nanocage thus contains 240 lysines, which are further used as the modification sites for spiropyran attachment. We characterize the purified encapsulin using size-exclusion chromatography, UV-Vis spectrophotometry and denaturing gel electrophoresis/SDS-PAGE (Fig. S1). The size-exclusion chromatogram (Fig. S1a) displays the characteristic encapsulin peak at V = 12 mL. The small peak at V = 8 mL is from bacterial ribosomes that co-elute with the encapsulin due to size similarity (~2 MDa).The UV-visible absorption spectrum of native encapsulin (without spiropyran modification) is shown in Fig. S1b.There is no absorption at λ = 350 nm of the encapsulin when the spiropyran is not present in the system. Therefore, we are able to use the absorbance at λ = 350 nm of the hybrid system to determine the spiropyran concentration in the hybrid system (ε = 7,994 $M^{-1}$ $cm^{-1}$), which result in the value of 1.16 mM of spiropyran.

On the other hand, the encapsulin monomer is shown in SDS-PAGE gel, corresponding to the band at around 28 kDa (lanes 2, 3, 4 in Fig. S1c). Importantly, lane number 3 confirms the present of the encapsulin component in the hybrid of encapsulin-spiropyran. By comparing the intensity of the encapsulin band in lane 3 and the intensity of the encapsulin standard in lane 4 (known concentration = 0.14 mM), we could roughly estimate the concentration of the encapsulin in the hybrid system that is 0.01 mM. Furthermore, we could estimate the number of spiropyran molecules per one encapsulin particle that is ~ (1.16 mM/0.01 mM) = 116.

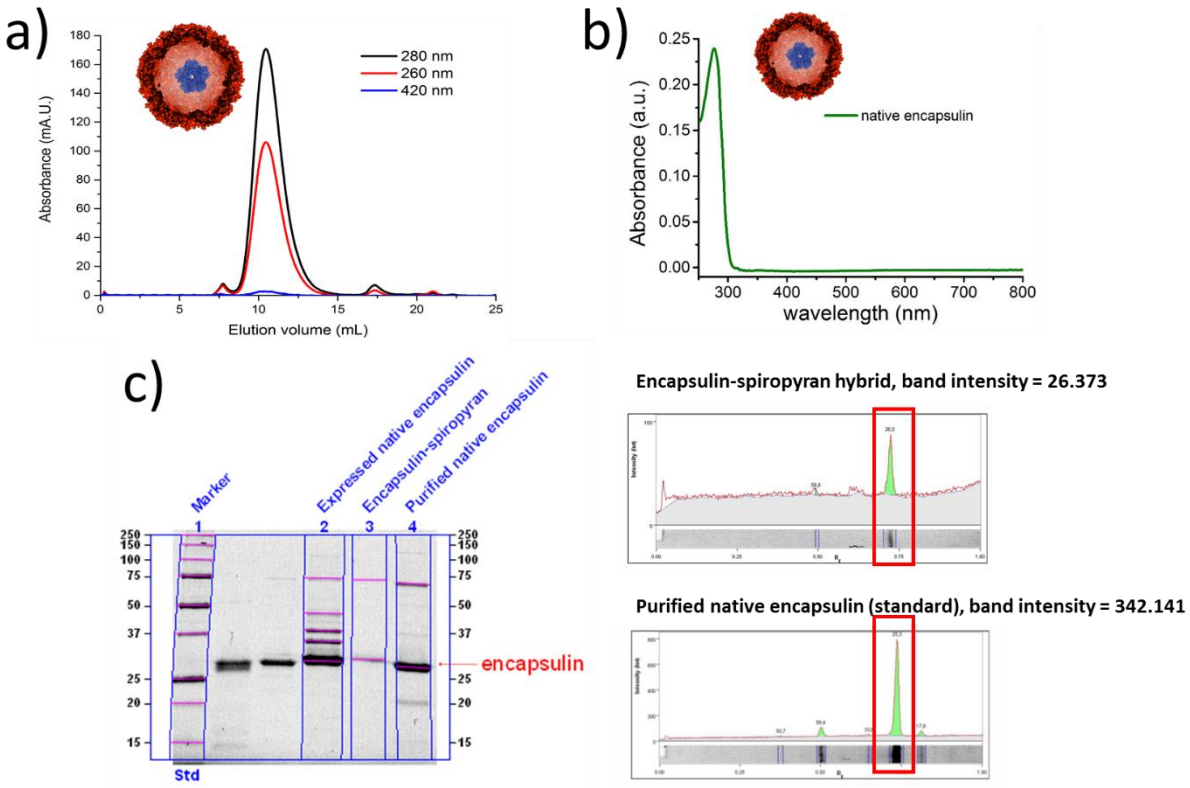


**Figure S1.** Characterization of encapsulin. a) Size-exclusion chromatogram showing the characteristic encapsulin elution at V = 12 mL. b) UV-visible spectrum of purified native encapsulin particles, indicating that the absorption of encapsulin alone at λ = 350 nm is negligible**.** c) Denaturing gel electrophoresis confirms the presence of encapsulin in the hybrid sample (lane 3) based on the characteristic encapsulin band at around 28 kDa (the monomer of the encapsulin cage), which is comparable to the native encapsulin (lane 2 and lane 4). The intensity of the encapsulin band in lane 4 is used as standard (known concentration = 0.14 mM) to estimate the concentration of the encapsulin in the hybrid system (lane 3). The systematic comparison of band intensity (gel densitometry) is performed with *Bio-Rad Image Lab* software.

## S2. Synthesis and characterization of succinimide-bearing nitrospiropyran

The synthesis of product **1** to **4** was carried out according to literatures.[3, 4]

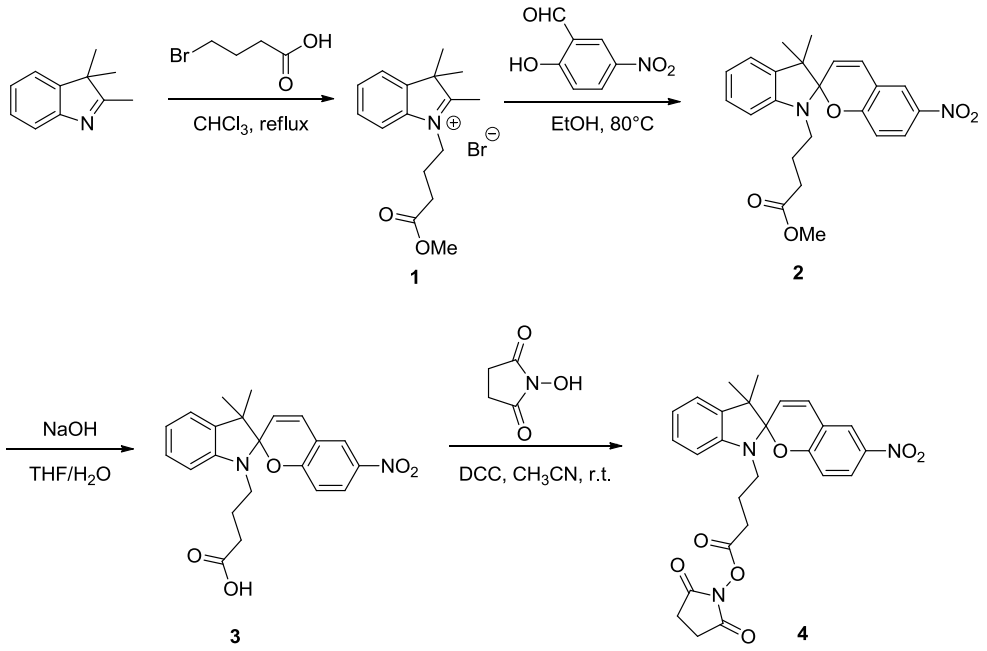


**Figure S2.** Synthesis of succnimide-bearing nitrospiropyran.

**1-(3-Carbomethoxypropyl)-3,3-dimethyl-2-methyleneindoline (1).** To a solution of 2,3,3-trimethyl-3H-indole (500 mg, 3.14 mmol, 1 eq) in 5 mL of chloroform, methyl 4-bromobutyrate (600 mg, 3.30 mmol, 1.05 eq) was added. The reaction mixture was stirred under $N_2$ for 24 hours at 70 °C. The chloroform was evaporated and diethylether was added to obtain a precipitate. The solid was extensively washed with diethylether. 490 mg was obtained as a purple solid.

$^1$H NMR (400 MHz, $CD_3CN$) δ7.92 – 7.86 (m, 1H), 7.74 – 7.69 (m, 1H), 7.66 – 7.61 (m, 2H), 4.49 – 4.41 (m, 2H), 3.63 (s, 3H), 2.78 (s, 3H), 2.61 (dd, J = 8.6, 5.0 Hz, 2H), 2.23 – 2.13 (m, 3H), 1.55 (s, 6H).

$^{13}$C NMR (101 MHz, $CD_3CN$) δ 197.8, 173.9, 143.0, 130.9, 130.2, 124.4, 116.3, 55.6, 52.4, 48.3, 30.9, 23.4, 22.6, 14.9.

ESI MS (*m/z*) 260.02, calculated for $[C_{16}H_{22}NO_2]^+$ = 260.16.

**1'-(3-Carbomethoxypropyl)-3',3'-dimethyl-6-nitrospiro[2H-1]-benzopyran-2,2'-indoline 3 (2).** To a solution of 5-nitrosalicylaldehyde (68 mg, 0.41 mmol, 1 eq) in 3 mL of ethanol, the compound **1** (140 mg, 0.41 mmol, 1 eq) dissolved in 2 mL of ethanol was added slowly. The reaction mixture was stirred at 80 °C for 5 hours. The solvent was evaporated and the residue was purified by chromatography on silica gel to afford the product as a purple solid (58 mg, 56%)

$^{1}$H NMR (400 MHz, $CD_3CN$) δ 8.07 (d, *J* = 2.8 Hz, 1H), 8.00 (d, *J* = 2.8 Hz, 1H), 7.98 (d, *J* = 2.8 Hz, 1H), 7.15 (td, *J* = 7.7, 1.3 Hz, 1H), 7.11 (dd, *J* = 7.3, 0.8 Hz, 1H), 7.02 (m, 1H), 6.87 – 6.81 (m, 1H), 6.71 (m, 1H), 6.66 (d, *J* = 7.8 Hz, 1H), 5.95 (d, *J* = 10.4 Hz, 1H), 3.57 (s, 3H), 3.26 – 3.10 (m, 2H), 2.32 (tt, *J* = 8.1, 4.1 Hz, 2H), 1.92 – 1.75 (m, 2H), 1.26 (s, 3H), 1.15 (s, 3H).

$^{13}$C NMR (101 MHz, $CD_3CN$) δ 174.44, 160.39, 148.13, 142.08, 137.11, 129.11, 128.72, 126.60, 123.73, 122.81, 122.74, 120.41, 119.97, 116.27, 107.93, 107.81, 53.36, 52.00, 43.58, 31.81, 26.27, 24.77, 20.00.

ESI MS (*m/z*) 409.28, calculated for $[C_{23}H_{24}N_2O_5H]^+$ = 409.17.

**1'-(3-Carboxypropyl)-3',3'-dimethyl-6-nitrospiro[2H-1]benzopyran-2,2'-indoline (3).** To a solution of **2** (150 mg) in THF (3 mL), 1 mL of NaOH 10% was added. The reaction mixture was stirred at room temperature for 18 hours. Afterwards, it was acidified with a solution of HCl (1 M). The aqueous phase was extracted with ethylacetate and concentrated to afford the product as a yellow solid. (124 mg, 86%).

$^{1}$H NMR (400 MHz, $CD_3CN$) δ 10.06 (d, *J* = 0.4 Hz, 1H), 8.70 (dd, *J* = 11.1, 2.9 Hz, 1H), 8.42 (dd, *J* = 9.2, 2.8 Hz, 1H), 8.10 (d, *J* = 2.8 Hz, 2H), 8.02 (dd, *J* = 9.0, 2.8 Hz, 2H), 7.21 – 7.12 (m, 5H), 7.05 (dd, *J* = 10.2, 4.3 Hz, 2H), 6.86 (td, *J* = 7.5, 0.9 Hz, 2H), 6.74 (d, *J* = 9.0 Hz, 2H), 6.70 (d, *J* = 7.8 Hz, 2H), 3.31 – 3.13 (m, 4H), 2.38 – 2.28 (m, 5H), 1.85 (dddd, *J* = 15.4, 13.8, 10.7, 6.2 Hz, 6H), 1.27 (s, 8H), 1.18 (d, *J* = 6.1 Hz, 3H).

$^{13}$C NMR (101 MHz, $CD_3CN$) δ 197.6, 174.7, 166.8, 160.4, 148.2, 142.1, 137.1, 132.5, 130.9, 129.1, 128.7, 126.6, 123.7, 122.8, 122.7, 120.4, 120, 119.5, 116.3, 107.9, 107.8, 53.4, 43.64, 31.5, 26.3, 24.7, 20.0.

ESI MS (*m/z*) 395.09, calculated for $[C_{22}H_{22}N_2O_5H]^+$ = 395.16.

**Succinimide spiropyran (4).** Compound **3** (50 mg, 0.13 mmol, 1 eq) and N-hydroxysuccinimide (15 mg, 0.13 mmol, 1 eq) were dissolved in 5 mL in dried acetonitrile. DCC (26 mg, 0.13 mmol, 1 eq) was added and the reaction mixture was stirred at room temperature for 18 hours under nitrogen. The solvent was evaporated and the residue was purified by chromatography over silica gel with hexane/ethylacetate to afford the product as a purple solid (20 mg, 32%).

$^{1}$H NMR (400 MHz, $CD_3CN$) δ 8.07 (d, *J* = 2.7 Hz, 1H), 7.99 (dd, *J* = 9.0, 2.7 Hz, 1H), 7.17 – 7.07 (m, 2H), 7.03 (dd, *J* = 10.4, 6.2 Hz, 1H), 6.88 – 6.81 (m, 1H), 6.74 – 6.63 (m, 2H), 5.98 (dd, *J* = 10.4, 7.0 Hz, 1H), 3.32 – 3.11 (m, 2H), 2.76 (s, 2H), 2.66 (m, 2H), 2.59 – 2.56 (m, 2H), 2.36 – 2.26 (m, 2H), 1.25 (s, 3H), 1.15 (s, 3H).

$^{13}$C NMR (101 MHz, $CD_3CN$) δ 169.7, 168.2, 159.1, 146.8, 140.8, 136.0, 128.08, 127.8, 125.3, 122.4, 121.5, 121.4, 119.3, 118.7, 114.7, 106.5, 52.0, 42.3, 41.8, 27.9, 25.0, 23.4, 18.7

ESI MS (*m/z*) 491.94, calculated for $[C_{26}H_{25}N_3O_7H]^+$ = 492.17.

## S3. Thermal relaxation of merocyanine to spiropyran

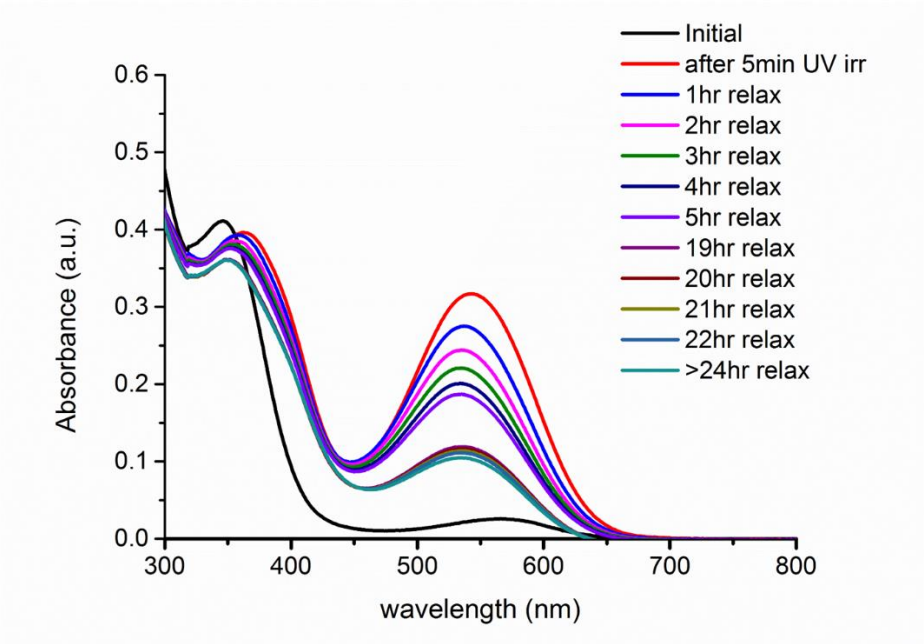


**Figure S3.** Thermal relaxation of merocyanine into spiropyran in aqueous system (the switch is attached to a model protein, human serum albumin). The relaxation is indicated by the disappearance of absorption band at λ = 550 nm, which is much slower (within hours) in comparison to the light-triggered conversion (within 7 min).

## S4. Effect of irradiation to encapsulin structural integrity

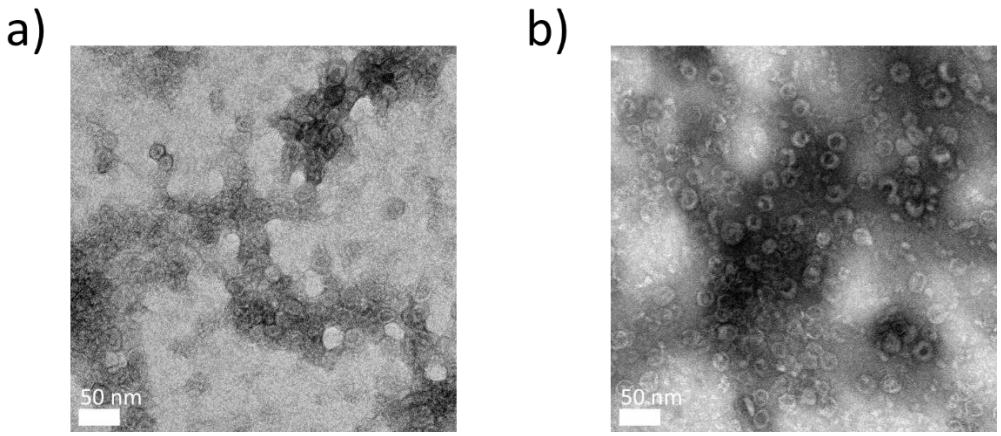


**Figure S4.** TEM images of encapsulin particles (diameter = 20-24 nm) a) before irradiation and b) after irradiation with UV-light (for 2 min), confirming that the majority of the particles are still intact upon irradiation.

## S5. Effect of prolonged irradiation to encapsulin structural integrity

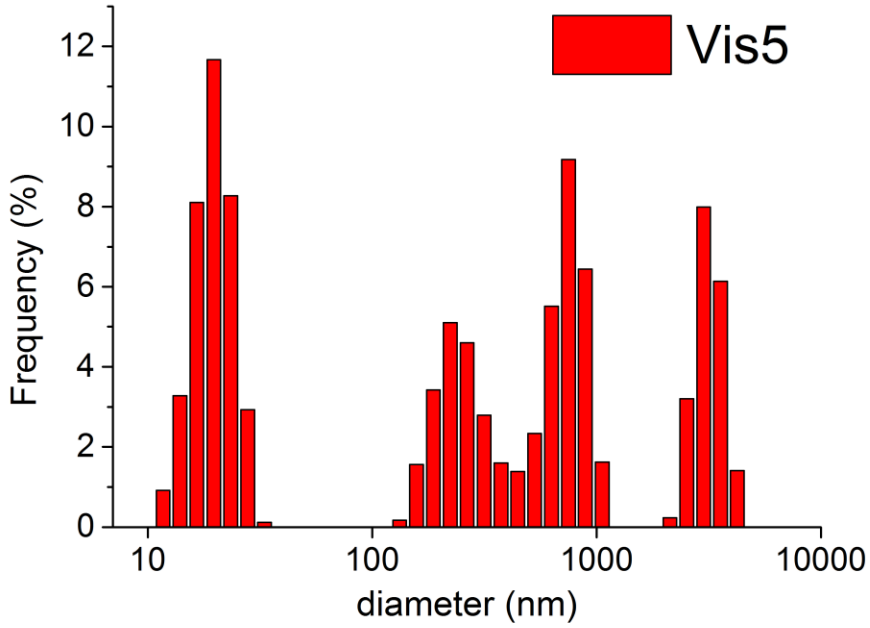


**Figure S5.** Size distribution of encapsulin particles (diameter = 20 nm) after 5 cycles of irradiation showing that the intact encapsulin particles are present but larger structures also form (diameter >100 nm).

## References


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