SUPPORTING INFORMATION

Membrane protein channels equipped with a cleavable linker for inducing catalysis inside nanocompartments

Luisa Zartner\textsuperscript{a}, Viviana Maffeis\textsuperscript{a,b}, Cora-Ann Schoenenberger\textsuperscript{a,b}, Ionel Adrian Dinu\textsuperscript{a,b}, Cornelia G. Palivan\textsuperscript{a,b,*}

\textsuperscript{a}Department of Chemistry, University of Basel, BPR1096, Mattenstrasse 24a, 4058 Basel, Switzerland

\textsuperscript{b}NCCR-Molecular Systems Engineering, BPR1095, Mattenstrasse 24a, 4058 Basel, Switzerland
**Figure S1.** $^1$H-NMR spectrum of Compound 2 in CDCl$_3$ at 500 MHz.
Figure S2. $^{13}$C-NMR spectrum of Compound 2 in CDCl$_3$ at 126 MHz.
Figure S3. $^1$H-NMR spectrum of the bismaleimide linker in CDCl$_3$ at 500 MHz.
Figure S4. $^{13}$C-NMR spectrum of the bismaleimide linker in CDCl$_3$ at 126 MHz and the image of the corresponding dissolved linker in the NMR tube.
**Figure S5.** SDS PAGE of OmpF-M (*left*, fluorogram; *right*, Coomassie-stained): L: Protein ladder; 1, 2: unlabeled OmpF-M, at 1/1 (1) and 2/1 (2) ratio (v/v) with loading buffer; 3, 4: OmpF-M labelled with the linker comprising fluorescent cyanine3 ($\lambda_{ex} = 550$ nm / $\lambda_{em} = 580$ nm). $M_W$ of OmpF around 40 kDa.

**Table S1.** Fluorescence correlation parameters of the free fluorophore cyanine3 maleimide, free linker, CNCs with OmpF-M-linker inserted in the membrane, stand-alone OmpF-M-linker, and OmpF-M-linker added to empty polymersomes before and after staining the polymersomes with BODIPY 630/650-X.

|                  | Counts per molecule (kHz) | Diffusion time (µs) |
|------------------|---------------------------|---------------------|
| Cyanine3 maleimide | 1.7                       | 61                  |
| Linker           | 2.3                       | 70                  |
| OmpF-M-Linker CNCs | 28.3                     | 4530                |
| Linker-OmpF-M    | 3.1                       | 452                 |
| CNCs AND linker-OmpF-M without BODIPY | 7.0 | 452 |
| CNCs AND linker-OmpF-M with BODIPY    | 138.3                     | 6000                |
**Figure S6.** $^1$H-NMR spectrum of poly(2-methyl-2-oxazoline)-$b$-poly(dimethylsiloxane)-$b$-poly(2-methyl-2-oxazoline) triblock copolymer (PMOXA$_{11}$-$b$-PDMS$_{104}$-$b$-PMOXA$_{11}$) in CDCl$_3$ at 500 MHz.

**Figure S7.** Elugram (GPC) of PMOXA$_{11}$-$b$-PDMS$_{104}$-$b$-PMOXA$_{11}$ in DMF.
Table S2. Data from NTA measurements of CNC-noOmpF, CNC-linker-OmpF-M, CNC-OmpF-M, and CNC-OmpF-WT samples diluted 1:1000 in PBS.

|                | CNC-noOmpF | CNC-linker-OmpF-M | CNC-OmpF-M    | CNC-OmpF-WT  |
|----------------|------------|-------------------|---------------|--------------|
| diameter (nm)  | 202 ± 47   | 192 ± 42          | 182 ± 42      | 215 ± 49     |
| concentration  | 2.6 × 10^8 ± 1.6 × 10^7 | 1.8 × 10^8 ± 4.5 × 10^6 | 3.1 × 10^8 ± 1.7 × 10^7 | 3.8 × 10^8 ± 2.4 × 10^7 |

Figure S8. Laccase activity of CNCs in response to NaIO₄. Measurements were carried out in triplicate at pH 7.4, over 9 h at RT: ABTS in PBS with CNC-OmpF-WT (black), ABTS in PBS with CNC-OmpF-M (red), ABTS in PBS with CNC-linker-OmpF-M in the presence (green) and absence of NaIO₄ (blue), CNC-noOmpF without (purple) and with NaIO₄ (yellow), and ATBS in PBS (turquoise). (A-C) represent 3 independent CNC preparations (each with standard deviation).
**Figure S9.** Cryo-TEM micrographs of polymersomes self-assembled from PMOXA$_{11}$-b-PDMS$_{104}$-b-PMOXA$_{11}$. Scale bars: 100 nm

**Figure S10.** FCS autocorrelation curves (solid line) and raw data (dots) of PBS solutions of cyanine3 maleimide (pink), OmpF-M-linker in 1% OG added to empty polymersomes without BODIPY 630/650-X (blue) and stained with BODIPY 630/650-X (red).
**Figure S11.** SDS PAGE of fungal laccase from Agaricus bisporus: L: Protein ladder; Lac1: 10 µg laccase; Lac2: 5 µg laccase.

**Figure S12.** Calibration curve for BCA assay performed according to the supplier’s protocol (Thermo Fisher Scientific, U.S.A.)