Electronic Supplementary Material (ESI) for Soft Matter.

Stable and Permeable Polyion Complex Vesicles Designed as Enzymatic Nanoreactors

Yuting Wan, Yuening Qiu, Jin Zhou, Jinbo Liu, Martien A. Cohen Stuart, Yangfeng Peng* and Junyou Wang*

State-Key Laboratory of Chemical Engineering, and Shanghai Key Laboratory of Multiphase Materials Chemical Engineering, East China University of Science and Technology, 130 Meilong Road, 200237, Shanghai, People's Republic of China

Corresponding author: junyouwang@ecust.edu.cn, yfpeng@ecust.edu.cn.

This PDF file includes:

1. ^{1}H NMR spectrum of PSS_{96}-*b*-PEO_{113} (Figure S1)
2. Size distribution of formed vesicles before and after remove of the salt. (Figure S2)
3. TEM image of PIC vesicles prepared at $f_{+/-}$ = 1.1 (Figure S3)
4. Average diameter and membrane thickness based on TEM image (Table S1)
5. Angular dependence of the diffusion coefficient D of PIC vesicles (Figure S4)
6. Static light scattering results (KC/R *vs* q^2) of PIC vesicles (Figure S5)
7. Light scattering intensity, R_h and PDI over time of PIC vesicles (Figure S6)
8. Mixing UOX and PEI at different salt concentrations at pH 6.0 (Figure S7)
9. The radius distribution of different mixtures prepared at $f_{+/-}$ = 1.1 (Figure S8)
10. TEM image of UOX-loaded vesicles prepared at $f_{+/-}$ = 1.1 (FigureS9)
11. Fluorescence emission of Dex solution and Dex-loaded vesicles after 24h dialysis (λ_{ex} = 550 nm) (FigureS10)
12. Normalized intensity and size distribution of Dex-loaded vesicles before and after dialysis. (FigureS11)

13. Encapsulation efficiency (EE), encapsulation amount (EA) and relative enzymatic activity of UOX-loaded vesicles prepared at different vesicle concentrations. (Figure S12)

14. TEM image of HRP-loaded vesicles prepared at $f_{+/-}$ = 1.1. (Figure S13)

Figure S1

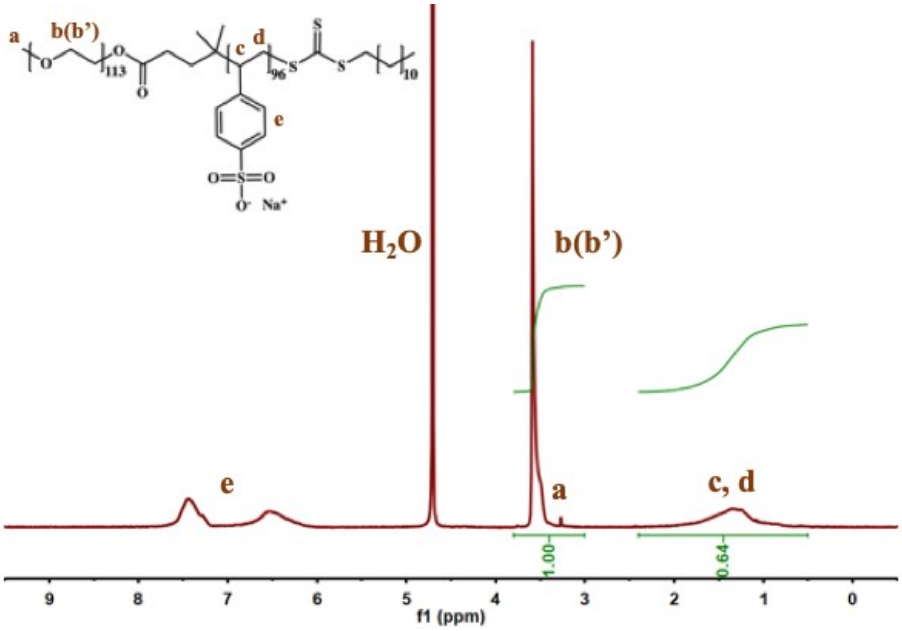
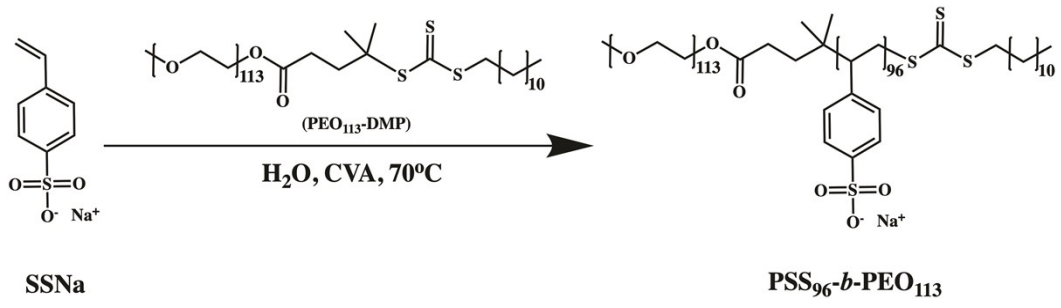


Figure S1. ^{1}H NMR spectrum of PSS_{96}-*b*-PEO_{113}.

It can be observed from Figure S1: δ 0.5-2.4 (3H, $CHCH_2$, c, d), δ 3.4 (3H, CH_2, a),δ 3.6 (4H, CH_2CH_2O, b, b'), δ 6-7.6 (5 h, phenyl, e). There are no other peaks in the figure except the predicted peak of product structure and the solvent, which can determine that the product synthesized is pure. Next, we take the group CH_2CH_2O in polyethylene glycol (PEO) as the reference, and set its characteristic peak integral as 1, which can obtain the characteristic peak integral of $CHCH_2$ in sodium polystyrenesulfonate (PSS) of 0.64, and calculate the polymerization degree of SS in our synthesized polymer of about 96.



Reaction formula was used for the preparation of block copolymer PSS_{96}-b-PEO_{113}

Figure S2

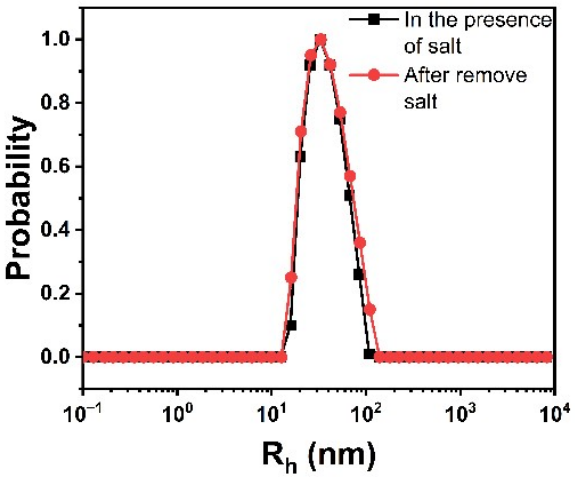


Figure S2. Size distribution of formed vesicles before and after remove of the salt.

Figure S3

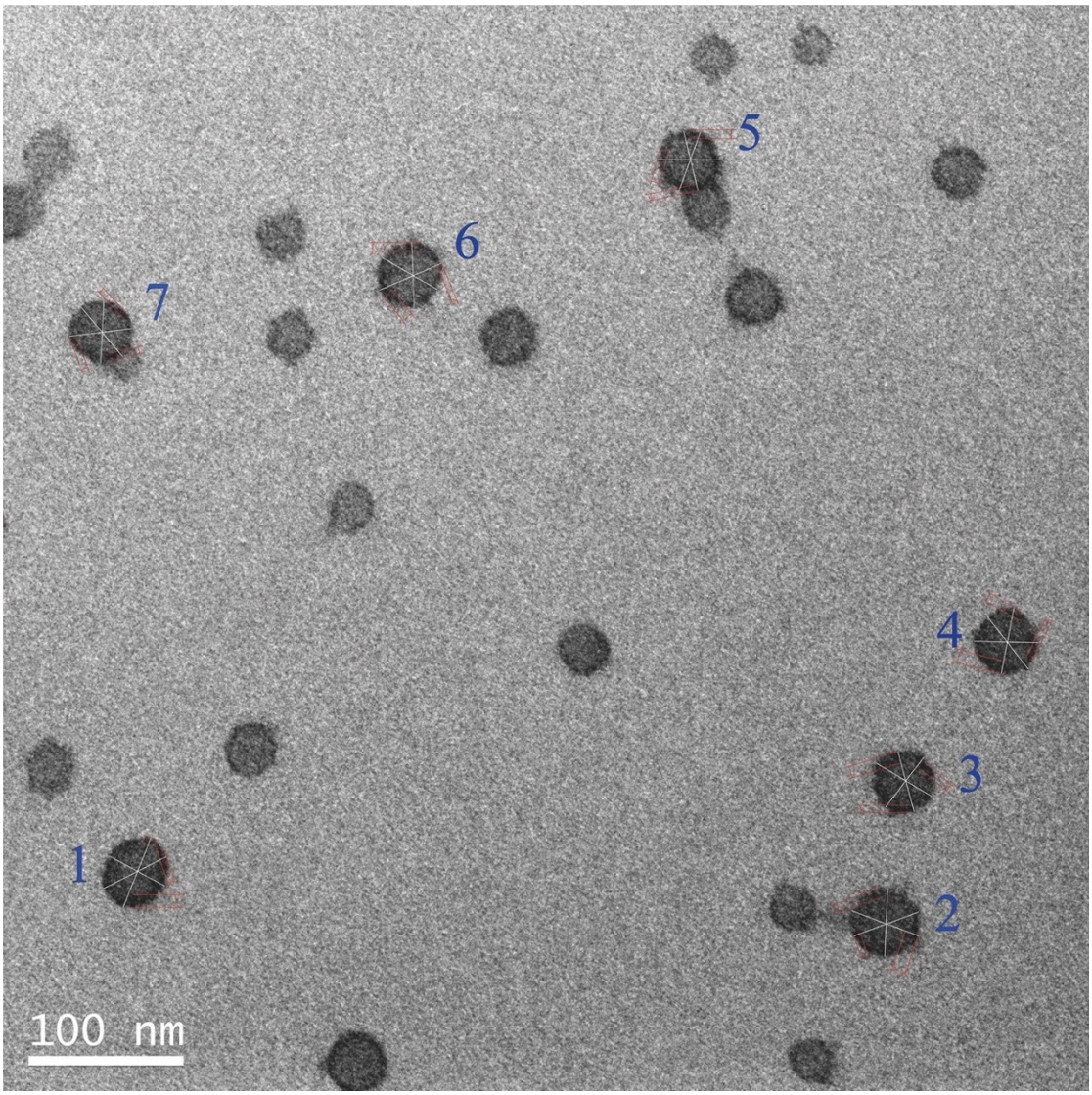


Figure S3. TEM image of PIC vesicles prepared at $f_{+/-} = 1.1$

Table S1. Average diameter and membrane thickness based on TEM image of PIC vesicles prepared at $f_{+/-} = 1.1$（**Figure S3**）

Number	Average diameter （nm）	Average membrane thickness （nm）
1	46.0	6.1
2	44.7	6.5
3	41.3	5.8
4	42.8	6.1
5	40.2	5.2
6	42.2	5.3
7	41.5	4.3
Average	**42.7**	**5.6**
Standard deviation	**2.0**	**0.7**

According to TEM image of PIC vesicles prepared at $f_{+/-} = 1.1$（Figure S3）, the diameter and membrane thickness of each vesicle were measured three times on average at different locations.

Figure S4

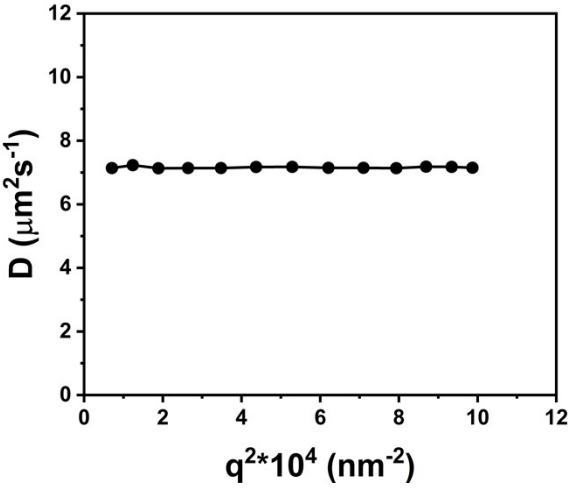


Figure S4. Angular dependence of the diffusion coefficient D of PIC vesicles prepared at $f_{+/-}$ = 1.1.

Figure S5

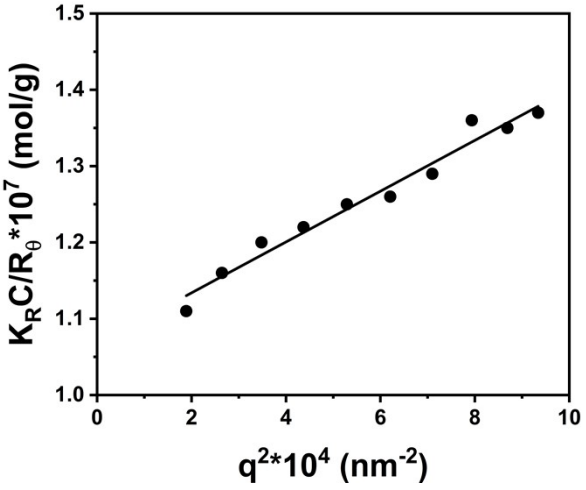


Figure S5. Static light scattering results (KC/R *vs* q^2) of PIC vesicles prepared at $f_{+/-}$ = 1.1.

The Zimm plots were recorded from 50° to 140° at intervals of 10°, while with one polymer concentration of about 0.164 g/L. The refractive index increment (dn/dc) was determined from the weight average of the component,[1-3] which is about 0.1576 mL/g. The estimated R_g is about 37.9 nm. The molecular weight of the PIC vesicles is obtained from the intercept, which is about 2.3×10^7 g/mol. According to the Mw and molecular weight of PEI and PSS_{96}-*b*-PEO_{113}, together with the charge mixing ratio +/- of 1.1/1, we can calculate the aggregation number of one PIC vesicle, which is formed by 2512 PEI and 745 PSS_{96}-*b*-PEO_{113}.

Figure S6

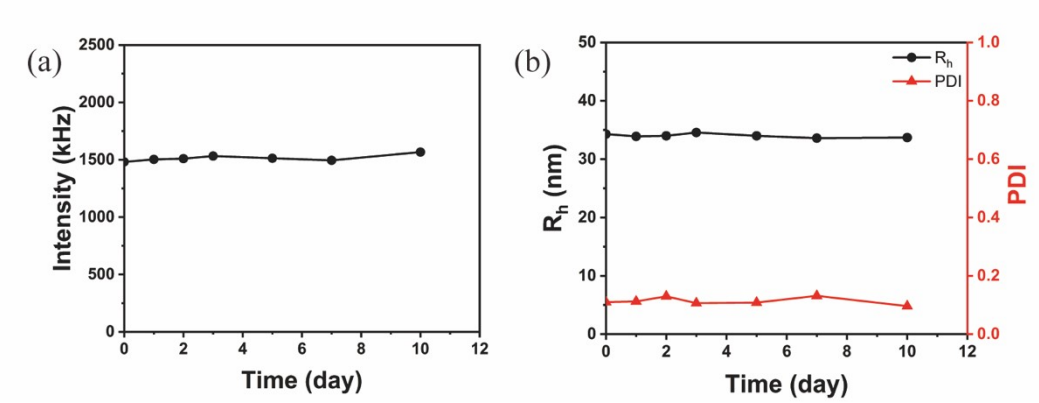


Figure S6. Light scattering intensity, R_h and PDI over time of PIC vesicles prepared at $f_{+/-} = 1.1$.

Figure S7

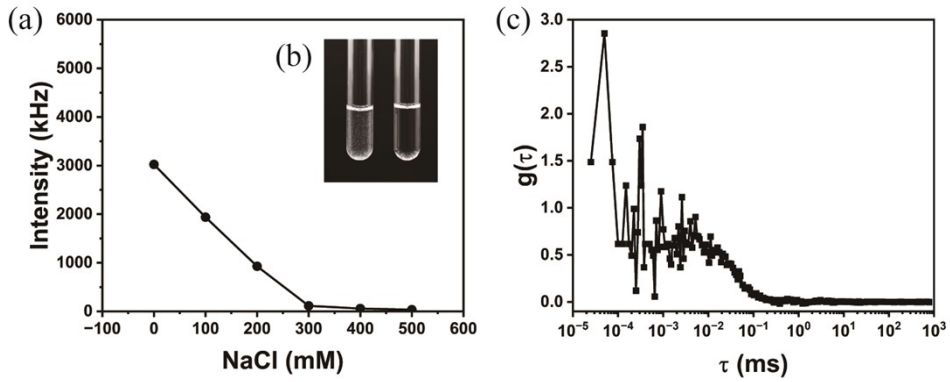


Figure S7. (a) The scattering intensity of mixing UOX and PEI at different NaCl concentrations. (b) Solution of mixing UOX and PEI with 0 mM (left) and 500 mM (right) NaCl. (c) Autocorrelation decay function of UOX and PEI at 500 mM NaCl. [UOX]=200 μg/mL, [+] from PEI = 0.55 mM, pH=6.0.

Figure S8

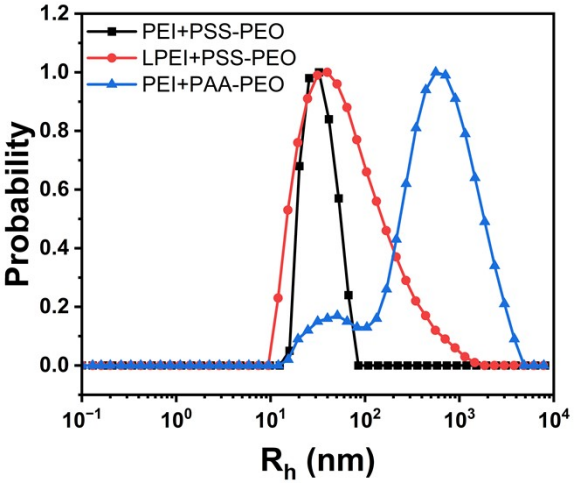


Figure S8. The radius distribution of different mixtures at pH 6.0 ($f_{+/-}$ = 1.1).

Figure S9

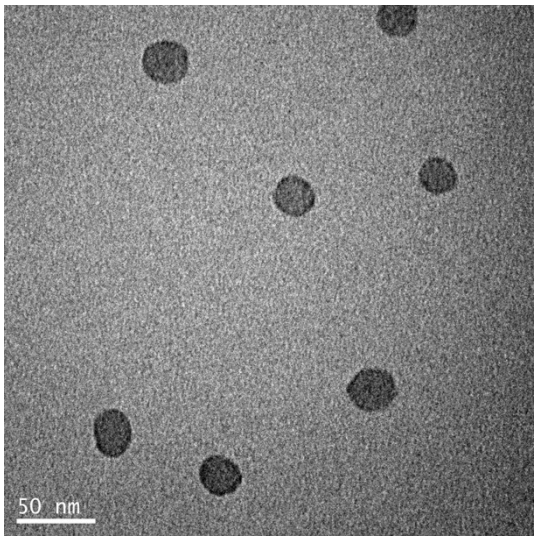


Figure S9. TEM image of UOX-loaded vesicles prepared at $f_{+/-}$ = 1.1.

Figure S10

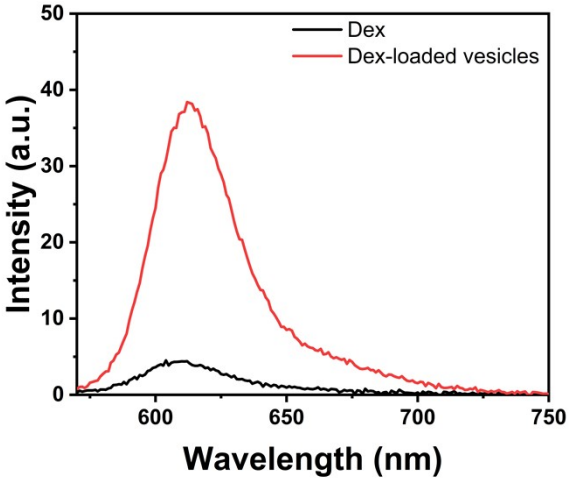


Figure S10. Fluorescence emission of free Dex and Dex-loaded vesicles after 24h dialysis (λ_{ex} = 550 nm).

Figure S10 shows after 24h dialysis, there was almost no fluorescence emission in the free Dex solution. This result confirms that, the first 24h dialysis shall be enough to remove the un-loaded Dex in the Dex-vesicle solution. In other words, the remained Dex (as evidenced by the fluorescence emission) shall be loaded in the PIC vesicles. The encapsulation efficiency of Dex is obtained by the ratio of residual fluorescence intensity to initial fluorescence intensity of the solution, which is about 7.5%. Then the following dialysis was implemented to monitor the release of the Dex.

Figure S11

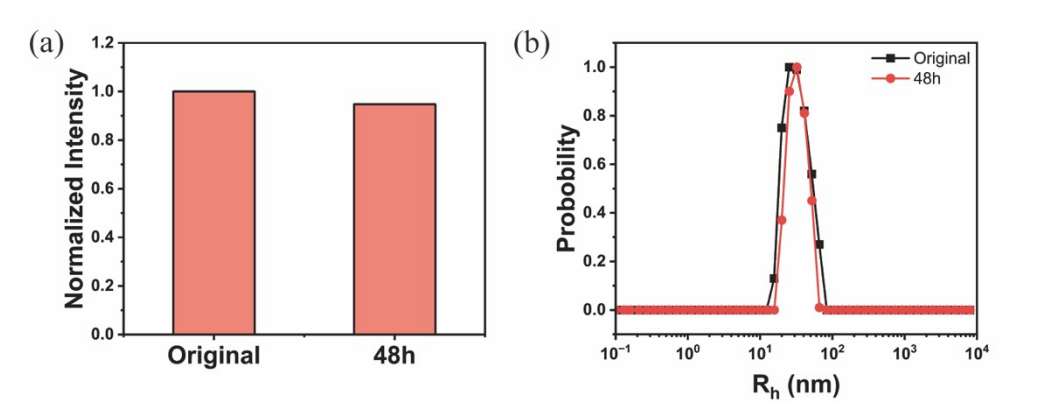


Figure S11. (a) Normalized intensity and (b) size distribution of Dex-loaded vesicles before and after dialysis.

Figure S12

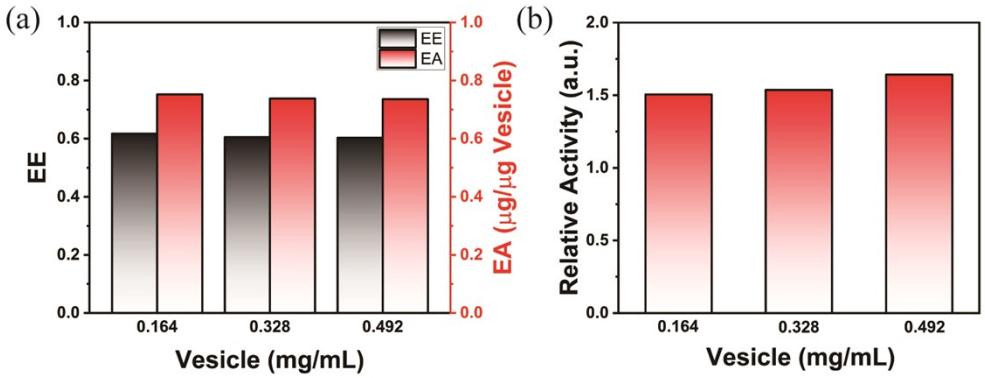


Figure S12. (a) Encapsulation efficiency (EE) and encapsulation amount (EA), (b) relative enzymatic activity of UOX-loaded vesicles prepared at different vesicle concentrations. The input enzyme loading was fixed at 200μg/164μg (UOX/vesicle).

Figure S13

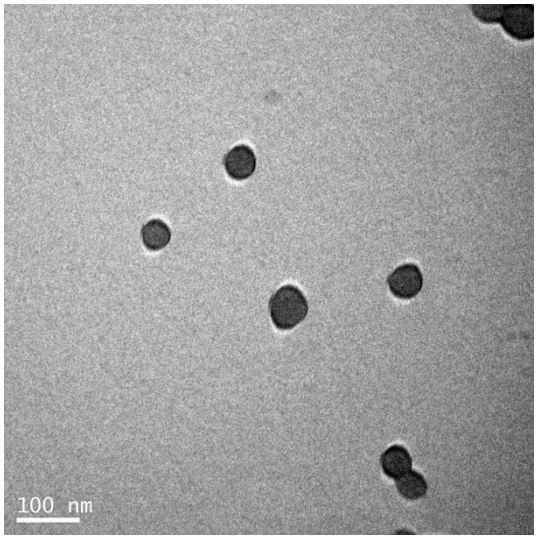


Figure S13. TEM image of HRP-loaded vesicles prepared at $f_{+/-}$ = 1.1.

Reference:

1. I. H. P. a. E.-J. Choi, *Polymer*, 1996, **37**, 313-319.
2. W. Zhang, M. L. Coughlin, J. M. Metzger, B. J. Hackel, F. S. Bates and T. P. Lodge, *Langmuir*, 2019, **35**, 7231-7241.
3. S. Prelesnik, S. Larin, V. Aseyev, H. Tenhu and K. Kogej, *J. Phys. Chem. B*, 2011, **115**, 3793-3803.