Supplementary information

for

Enzyme-containing double layer polymersomes coated by erythrocytes as a biomimetic nanoscavengers for in vivo protection from toxicants

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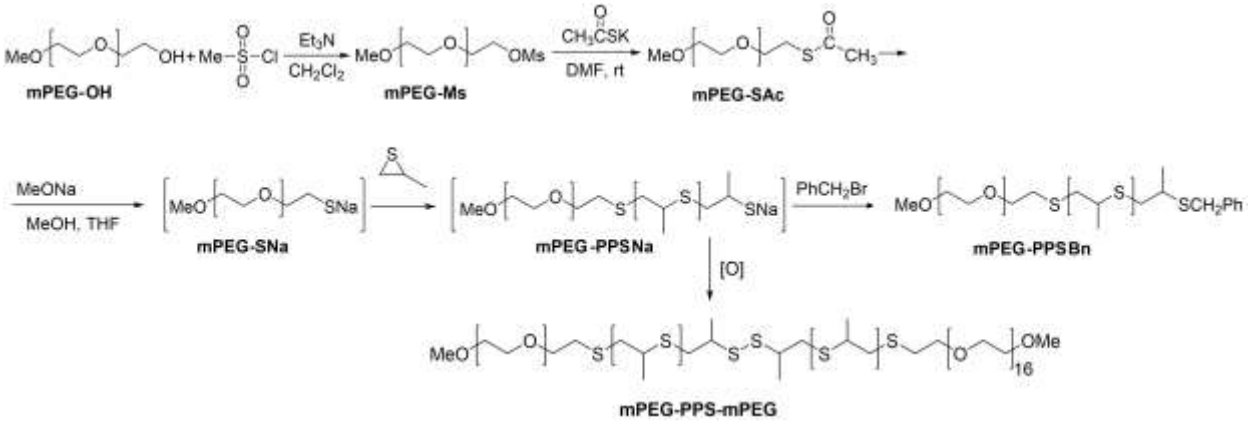

Scheme S1. General scheme of synthesis of block-copolymers

1. Calculation the amount of blood needed to coat PTE-polymersomes

Amount of erythrocytes needed to coat PTE-polymersomes were calculated according to [1]

Amount of erythrocytes in 1 ml of blood: $\mathbf{N_{eryt}} = 10^{10}$ cells/mL

Thickness of erythrocyte membrane $\mathbf{th_{eryt}} = 7.5$ nm

$\mathbf{S_{eryt}}$ (Surface area of 1 erythrocyte) = 75 mkm^2

$\mathbf{S_{total\text{-}eryt}}$ (Surface area of all erythrocytes in 1 ml of blood) = $\mathbf{N_{eryt}}$* $\mathbf{S_{eryt}}$ = 10^{10} cells/ml * 75 mkm^2 = 0.75 m^2

Radius of PTE-polymersomes $\mathbf{r_{NP}} = 80$ nm

Radius of erythrocyte membrane-camouflaged PTE-polymersomes $\mathbf{r_{NP\text{-}eryt}} = \mathbf{r_{NP}} + \mathbf{th_{eryt}}$ = 80 nm + 7.5 nm = 87.5 nm

Amount of PTE-polymersomes in 1 ml: $\mathbf{N_{NR}} = 1.78 * 10^{13}$ particles/ml [2]

$\mathbf{S_{NP\text{-}eryt}}$ (surface area of 1 NP-eryt) = $4*\pi*(\mathbf{r_{NP\text{-}eryt}})^2 = 4*3.14*(87.5\text{ nm})^2 = 0.96*10^{-13}$ m^2

$\mathbf{S_{total\text{-}NP\text{-}eryt}}$ (surface area of all NP-eryt in 1 ml) = $\mathbf{N_{NR}}$ * $\mathbf{S_{NP\text{-}eryt}}$ = $1.78 * 10^{13}$ particles/ml * 0.96 m^2 = 1.71 m^2

Volume of blood needed to coat 1 ml of PTE-polymersomes:

$\mathbf{S_{total\text{-}NP\text{-}eryt}} / \mathbf{S_{total\text{-}eryt}}$ = 2.28

2.28 ml of blood to coat 1 ml of PTE-polymersomes by erythrocytes is needed

Ratio PEG-PPS : erythrocytes	Amount of PEG-PPS, mg	Amount of Blood, ml
1:0.1	1	0.046
1:0.2	1	0.091
1:0.3	1	0.137
1:0.5	1	0.228
1:1	1	0.456

Ref.

[1] B.T. Luk, Ch.-M. J. Hu, R. H. Fang, D. Dehaini, C. Carpenter, W. Gao, Liangfang Zhang. Interfacial Interactions between Natural RBC Membranes and Synthetic Polymeric Nanoparticles, Nanoscale, 2014,6, 2730-2737.

[2] Pashirova T.N. et al., Colloid journal submitted.

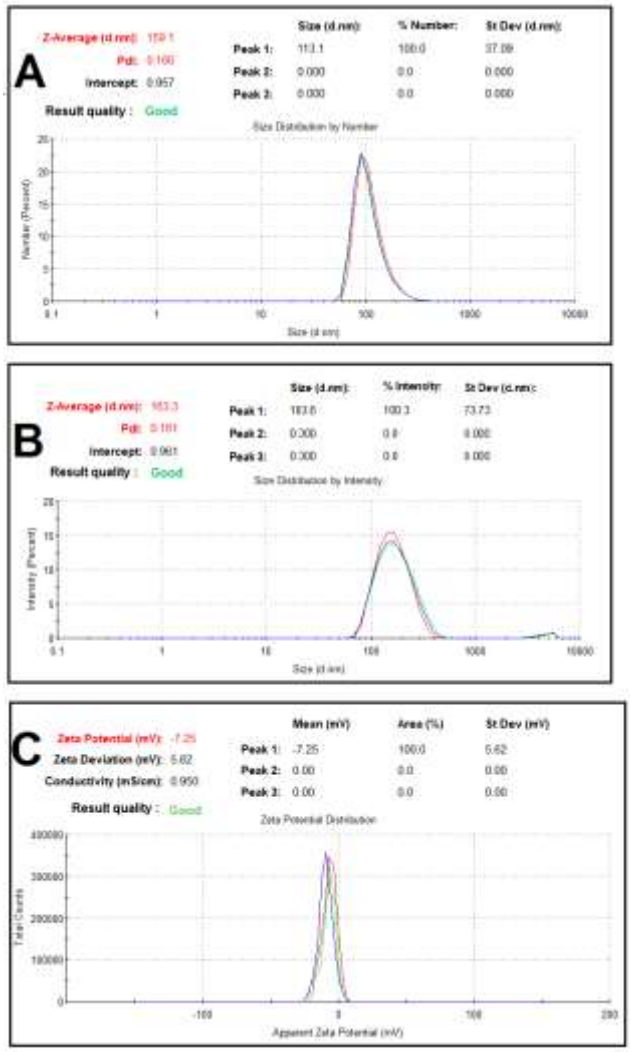

Figure S1. Screen shot of size distribution using the number (A) and intensity parameters (B) and dzeta-potential (C) of PTE-loaded polymersomes in 10 mM Tris buffer, pH = 7.4, C_1 = 5 mg/mL, 25 °C.

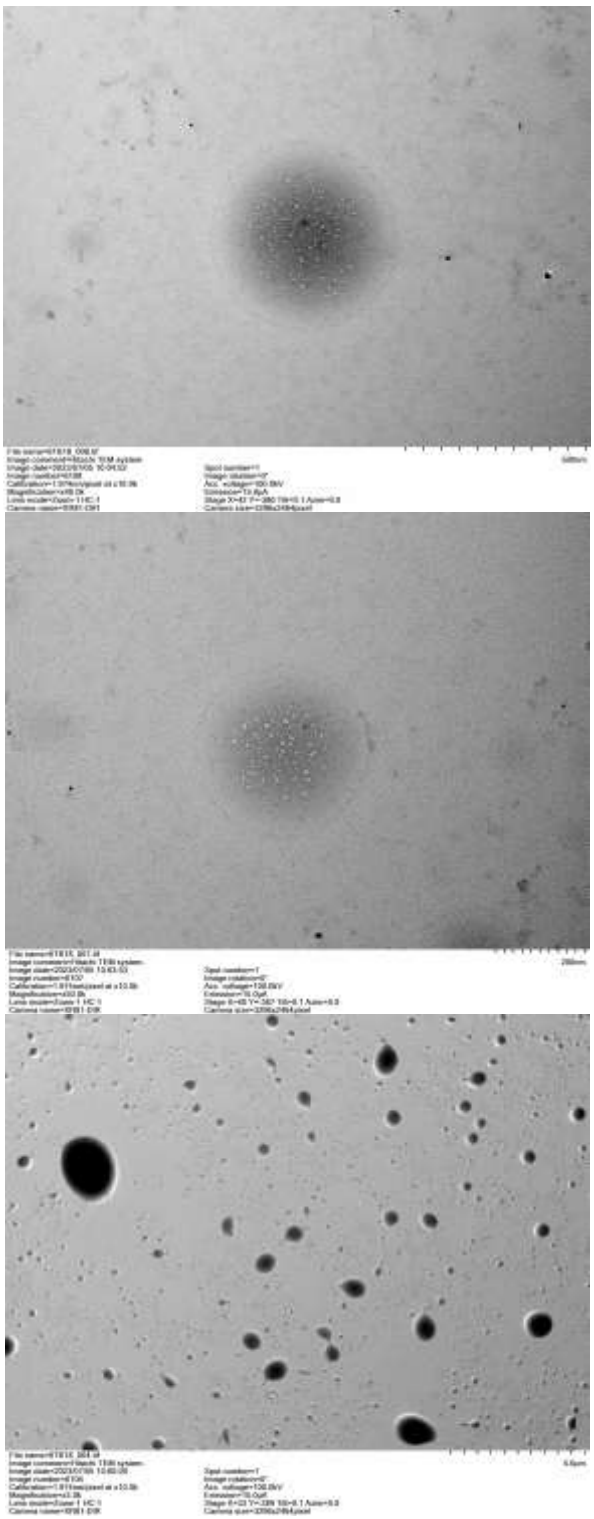

Figure S2. TEM imaging of PTE-loaded polymersomes in 10 mM Tris buffer, pH = 7.4, C_1 = 0.05 μg/mL, 25 °C.

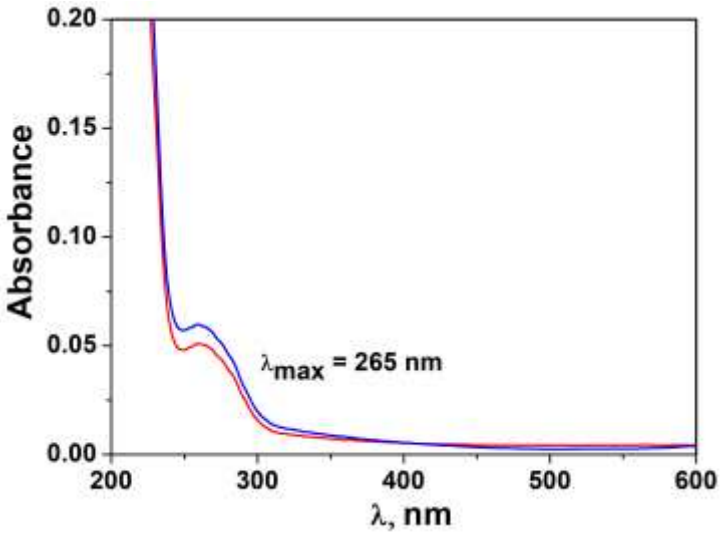

Figure S3. UV Absorbance spectra of PTE after ultracentrifugation of PTE-loaded polymersomes in 10mM Tris buffer, pH=7.4, C_1 = 5 mg/mL, 25°C.

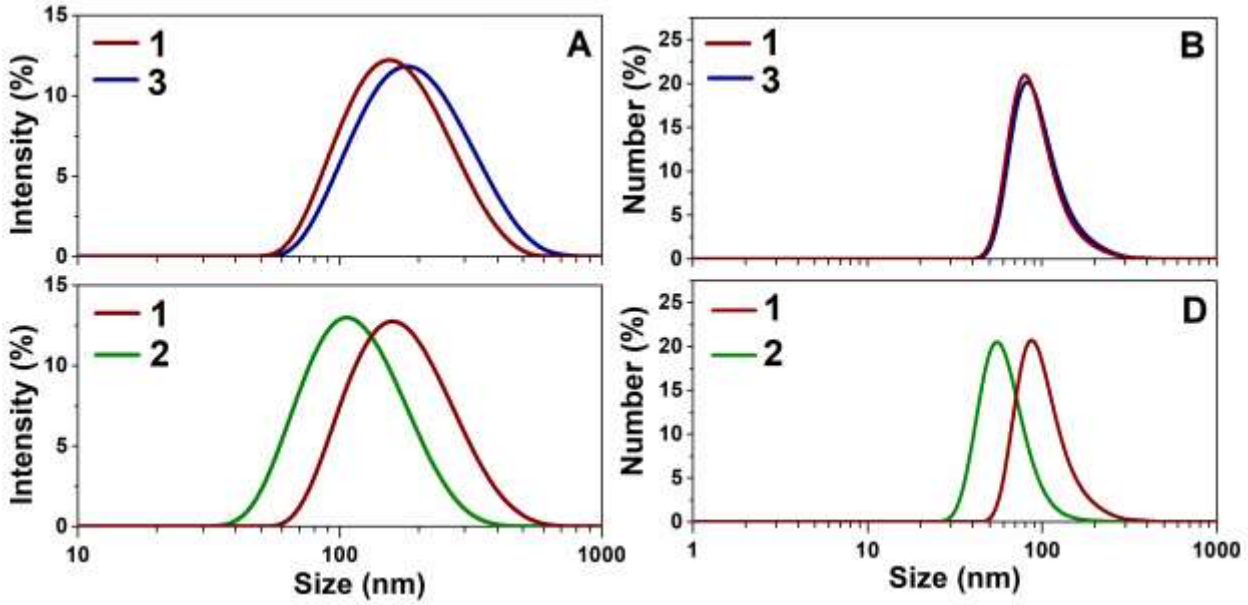

Figure S4. Size distribution of PTE-loaded polymersomes based on di- (1, PEG_{750}; 2, PEG_{2000}) and tri- (3, PEG_{750}) block copolymers using intensity (A, C) and number (B, D) parameters, $C_{polymer}$ = 10 mg/mL (A, B) and 5 mg/mL (C, D), C_{PTE} = 25 μM (A, B) and 12.5 μM (C, D), 25°C.

Table S1. Dynamic light scattering data for erythrocyte membrane-camouflaged polymersomes in 0.9% NaCl, pH = 7.4, 25 °C, size is hydrodynamic diameter, Z-average is the mean size, PDI is polydispersity index, ξ is zeta potential.

Ratio PEG-PPS **1**: erythrocytes	Diameter (nm)		PDI	ξ (mV)
	Int	Num		
1:0.1	142 ± 14	51 ± 9	0.25 ± 0.02	-14.1± 0.4
1:0.3	122 ± 13	51 ± 10	0.22 ± 0.01	-13.7± 0.7
1:0.5	122 ± 12	51 ± 11	0.27 ± 0.01	-17.8± 0.7
1:1	122 ± 13	51 ± 11	0.22 ± 0.02	-21.4± 1

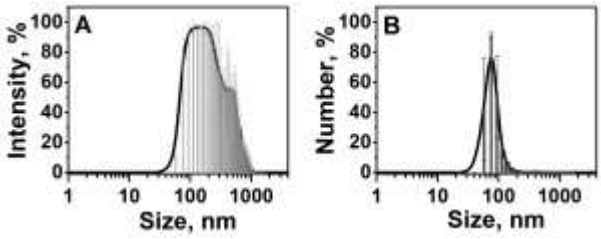

Figure S5. Size distribution of PTE-loaded polymersomes using intensity (A) and number (B) parameters in 10 mM Tris/NaCl (0.9%) buffer, pH = 7.4, 25 pH = 7.4, C_1 = 5 mg/mL, 25 °C.

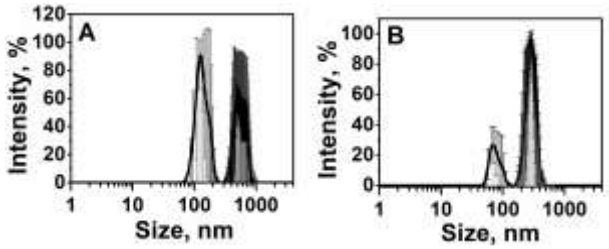

Figure S6. Size distribution using intensity parameter for empty (A) and PTE-loaded (B) erythrocyte membrane-camouflaged polymersomes in 10 mM Tris/NaCl (0.9%) buffer, pH = 7.4, C_1 = 5 mg/mL, 25 °C.

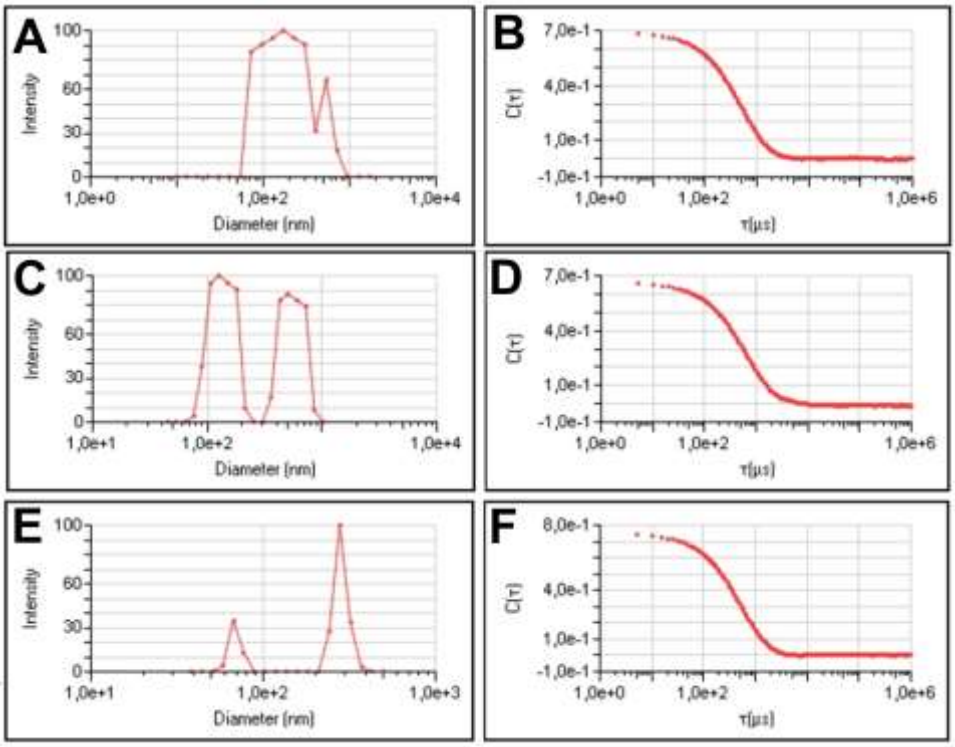

Figure S7. Screen shot for size distribution using intensity parameter (A,C,E) and correlation curves (B,D,F) for PTE-loaded polymersomes (A,B), empty (C,D) and PTE-loaded (E,F) erythrocyte membrane-camouflaged polymersomes in 10 mM Tris/NaCl (0.9%) buffer, C_1 = 5 mg/mL, pH = 7.4, 25 °C.

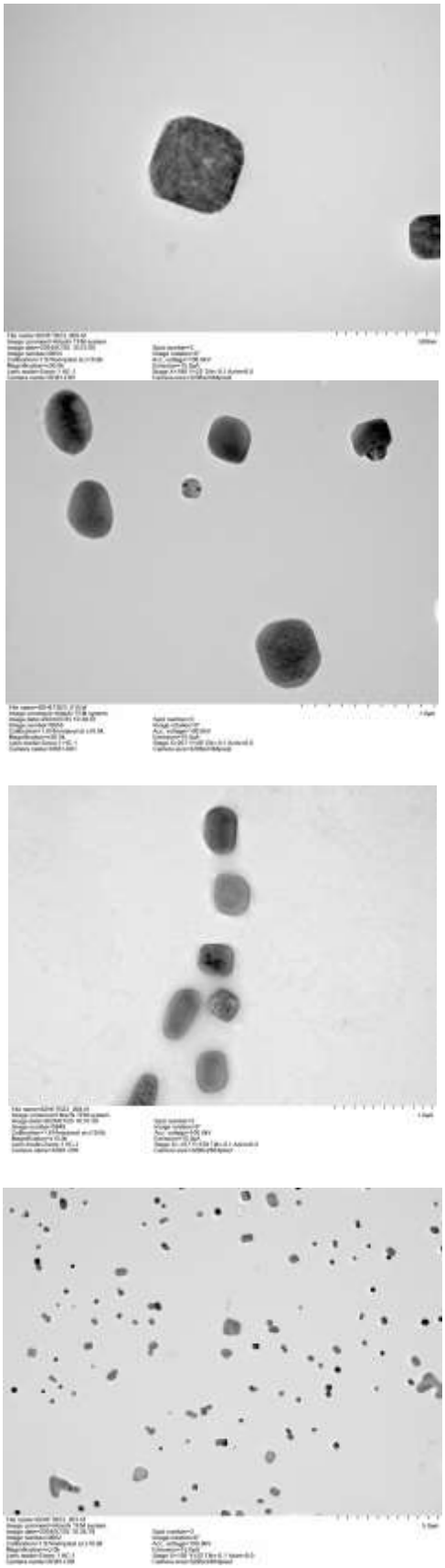

Figure S8. TEM imaging of erythrocyte membrane-camouflaged PTE-polymersomes in 0.9% NaCl, C_1 = 0.05 μg/mL, 25 °C

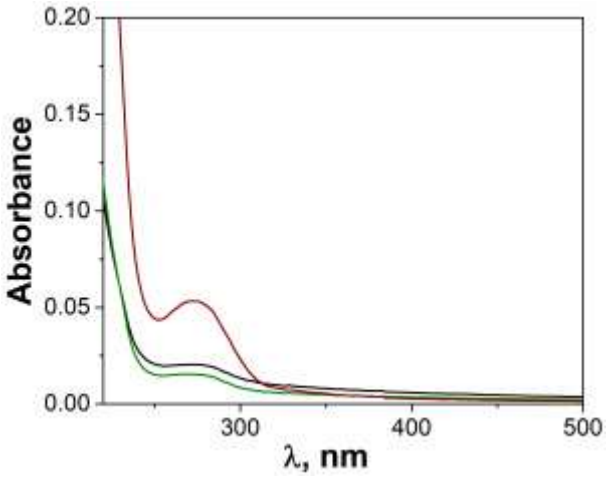

Figure S9. UV Absorbance spectra of PTE after ultracentrifugation of erythrocyte-modified PTE-polymersomes, 10 mM Tris/NaCl (0.9%) buffer, pH = 7.4, C_1 = 5 mg/mL, 25 °C.

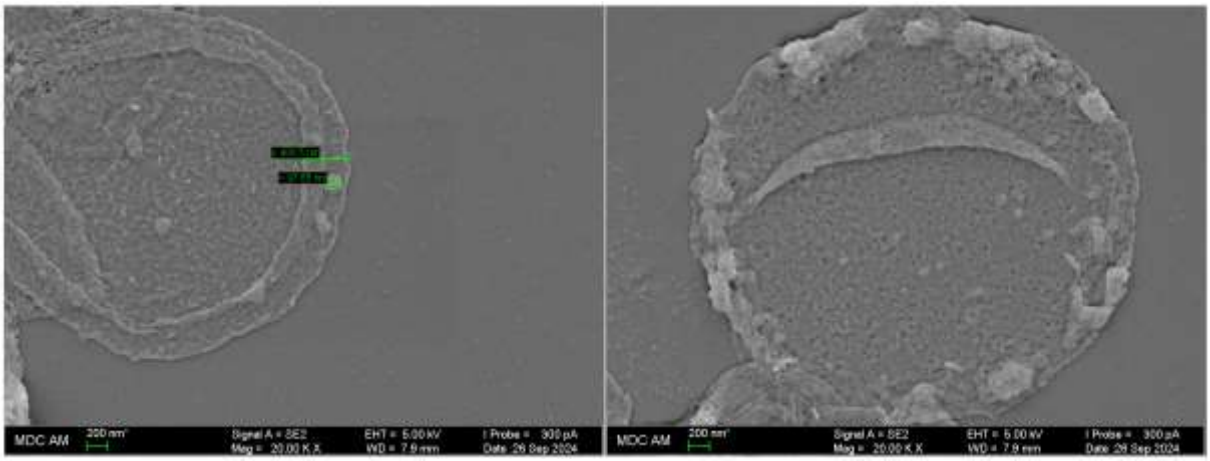

Figure S10. SEM imaging of erythrocyte coated PTE-polymersome microreactors in 0.9% NaCl, C_1 = 0.05 μg/mL, 25 °C.

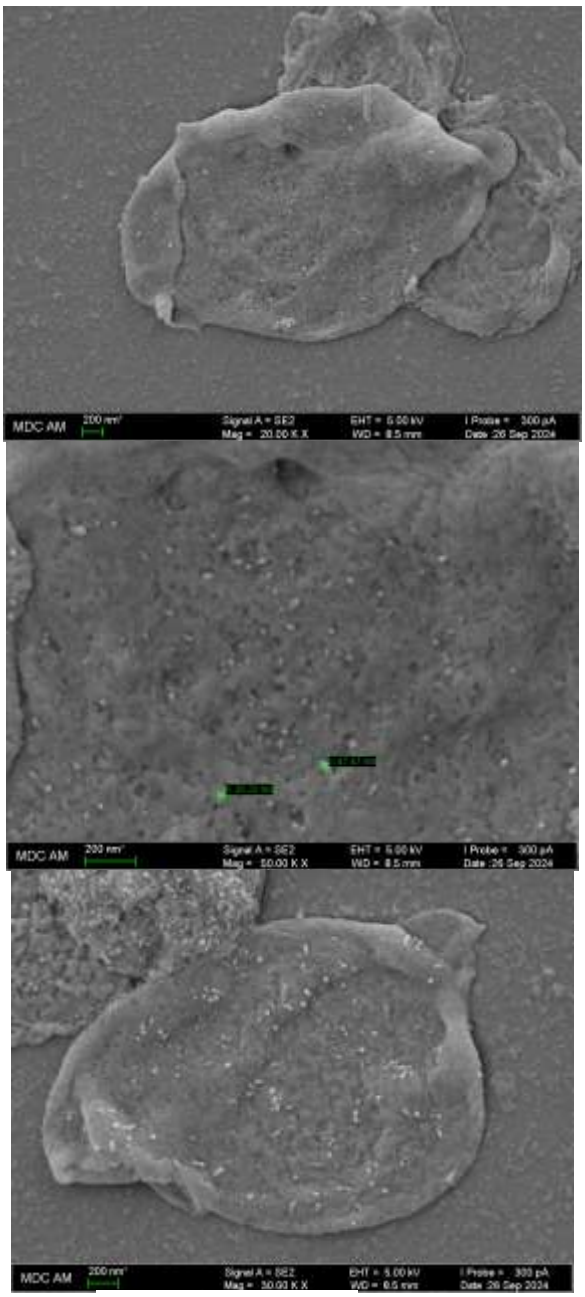

Figure S11. SEM imaging of erythrocyte coated PTE-polymersome microreactors in 0.9% NaCl, C_1 = 0.05 μg/mL, 25 °C.

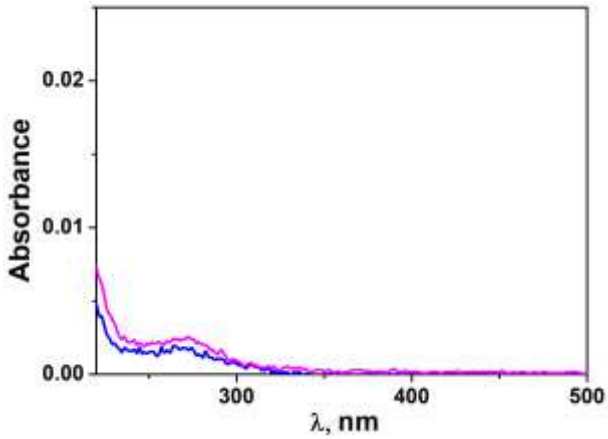

Figure S12. UV Absorbance spectra of PTE after ultracentrifugation of erythrocyte coated PTE-polymersome microreactor, 10mM Tris/NaCl (0.9%) buffer, pH = 7.4, C_1 = 5 mg/mL, 25 °C.

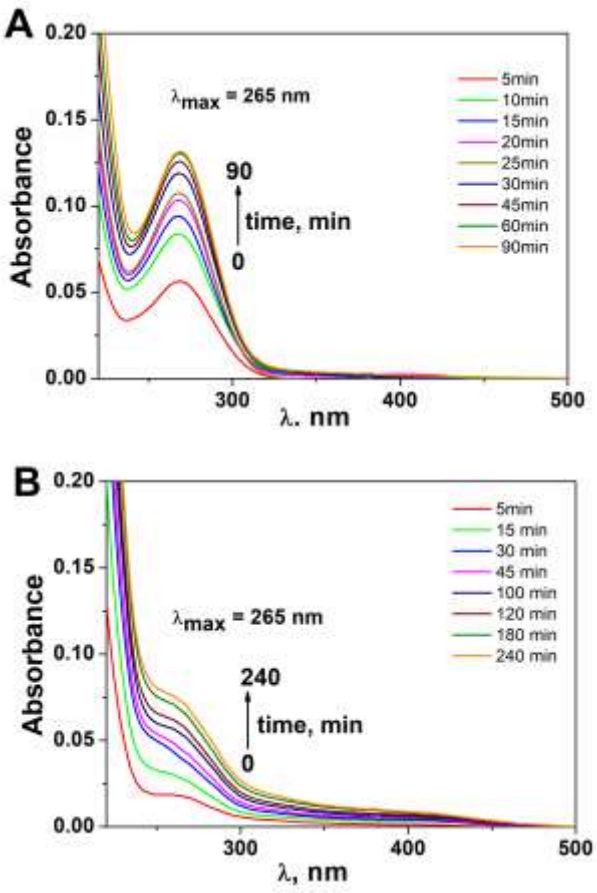

Figure S13. UV Absorbance spectra of PTE (A) control (none capsulated); (B) released from erythrocyte coated PTE-polymersome microreactor (B), monitored by dialysis method during time, 10mM Tris buffer, pH=7.4, 25°C.

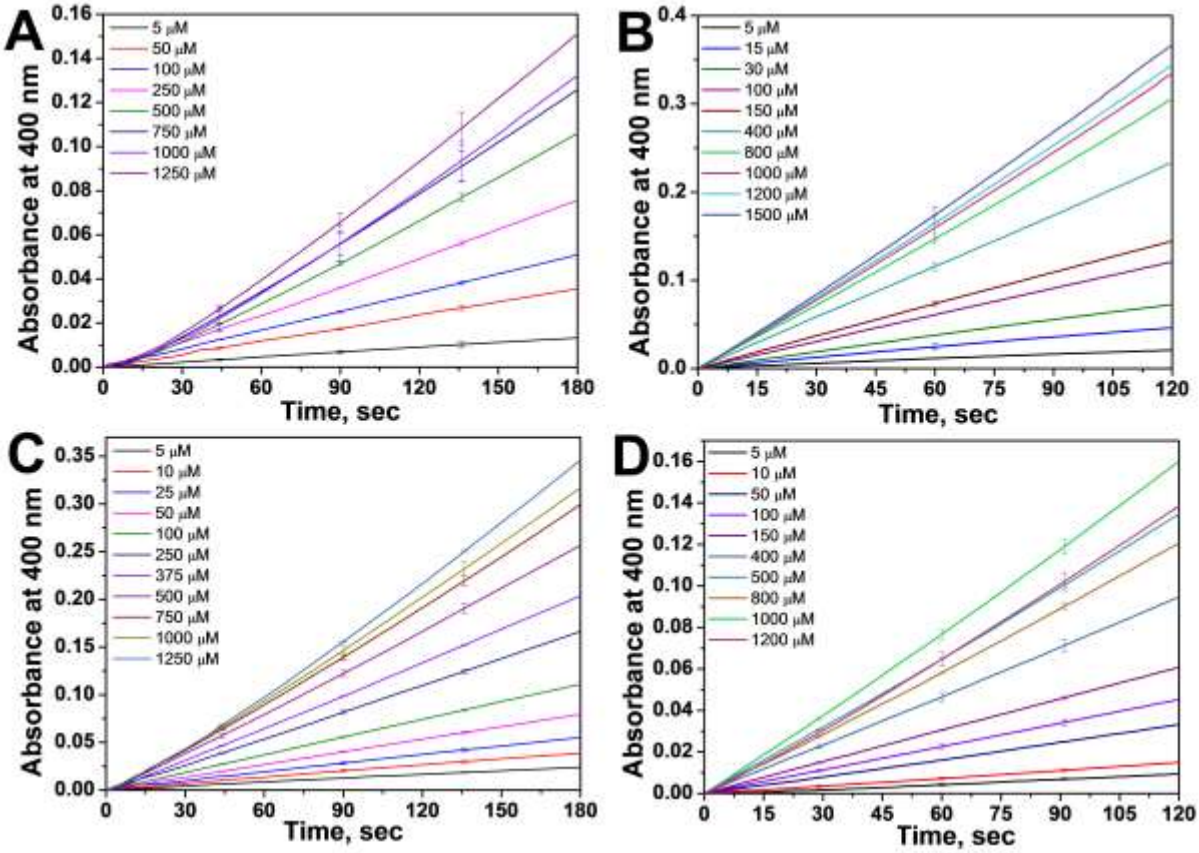

Figure S14. Kinetic curves of POX hydrolysis by free PTE (A), PTE-loaded in polymersomes (B), erythrocyte-modiffied PTE-polymersomes (C), erythrocyte-coated PTE-polymersome microreactors, C_{PTE} = 3.125 nM in 10 mM Tris buffer containing $CoCl_2$ (C=0.0002 M) and ethanol 1.5% (vol.), pH = 7.4, 25 °C.

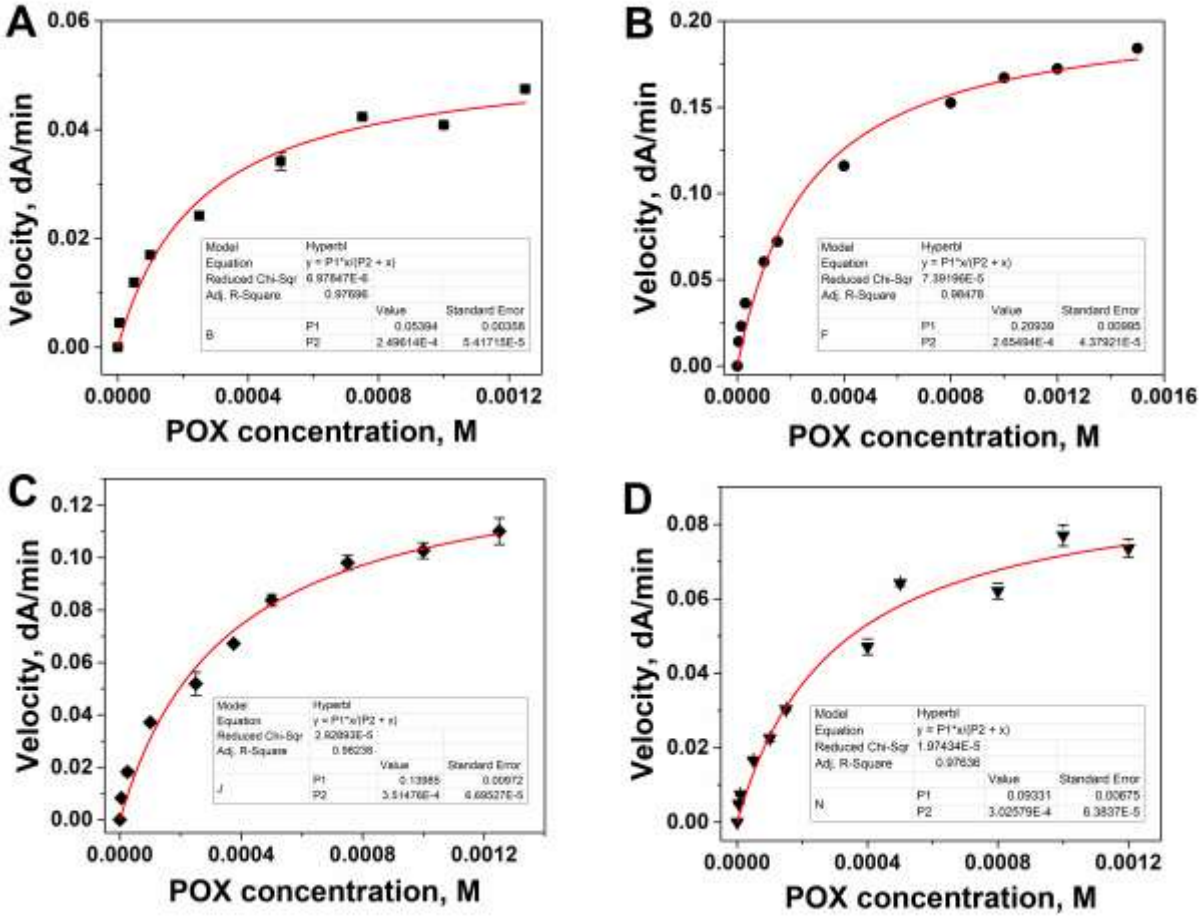

Figure S15. Dependences of POX hydrolysis by PTE (A), PTE-loaded polymersomes (B), erythrocyte-modified PTE-polymersomes (C), erythrocyte coated PTE-polymersome microreactors (D) on POX concentration in 10 mM Tris buffer containing $CoCl_2$ (C=0.0002 M) and ethanol 1.5% (vol.), pH = 7.4, 25 °C.

Table S2. Prophylaxis and post-exposure treatment of paraoxon *s.c.* acute toxicity by *i.v.* administration of PTE-loaded nanoreactors in mice.

Dose, mg/kg	Animals dead / total
Paraoxon acute toxicity study	
0.25	0/3
0.5	1/3
0.65	1/3
0.7	3/3
PTE-loaded polymersomes prophylactic *i.v.* administration	
5	0/3
7.5	2/3
10	2/3
15	3/3
PTE-loaded polymersomes *i.v.* treatment	
2.5	0/3
5	2/3
10	3/3
Erythrocyte-PTE-microreactors prophylactic *i.v.* administration	
1.5	0/3
2.5	0/3
5	1/5
7	1/3
10	3/3
Erythrocyte-PTE-microreactors *i.v.* treatment	
1.5	0/3
2.5	0/3
5	3/5
7	2/3

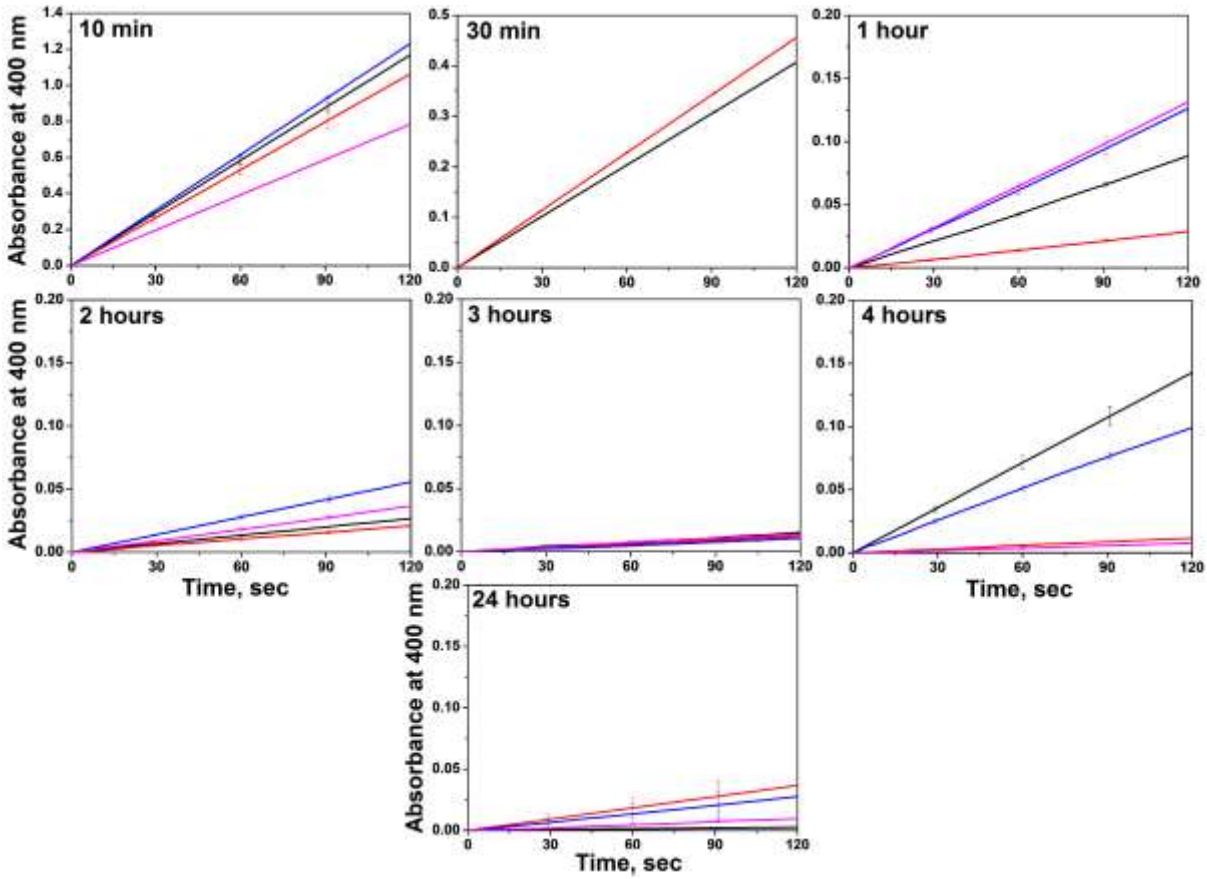

Figure S16. Kinetics of the paraoxon hydrolysis by erythrocyte coated PTE-polymersomes microreactors in mouse plasma in the pharmacokinetic study. 10 mM Tris buffer containing $CoCl_2$ (C=0.0002 M), pH = 7.4, $C_{Paraoxon}$ = 1 mM, 25 °C.

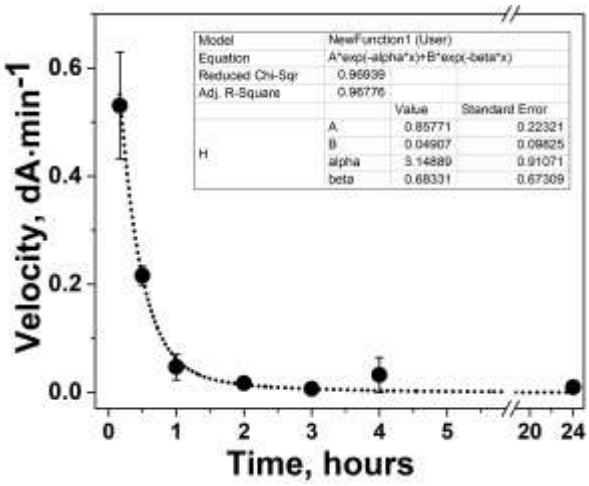

Figure S17. Fitting to the two-compartment model the pharmacokinetic plot of PTE in mouse plasma after intravenous injection of erythrocyte coated PTE-polymersome microreactors. PTE dose 3.7 mg/kg, block-copolymer dose 25 mg/kg. Each point represents the mean ± SD in four mice.