A Facile Approach to Functionalize Cell Membrane-Coated Nanoparticles

Hao Zhou[1], Zhiyuan Fan[1], Pelin K. Lemons[1] & Hao Cheng[1,2,*]

Supplementary data

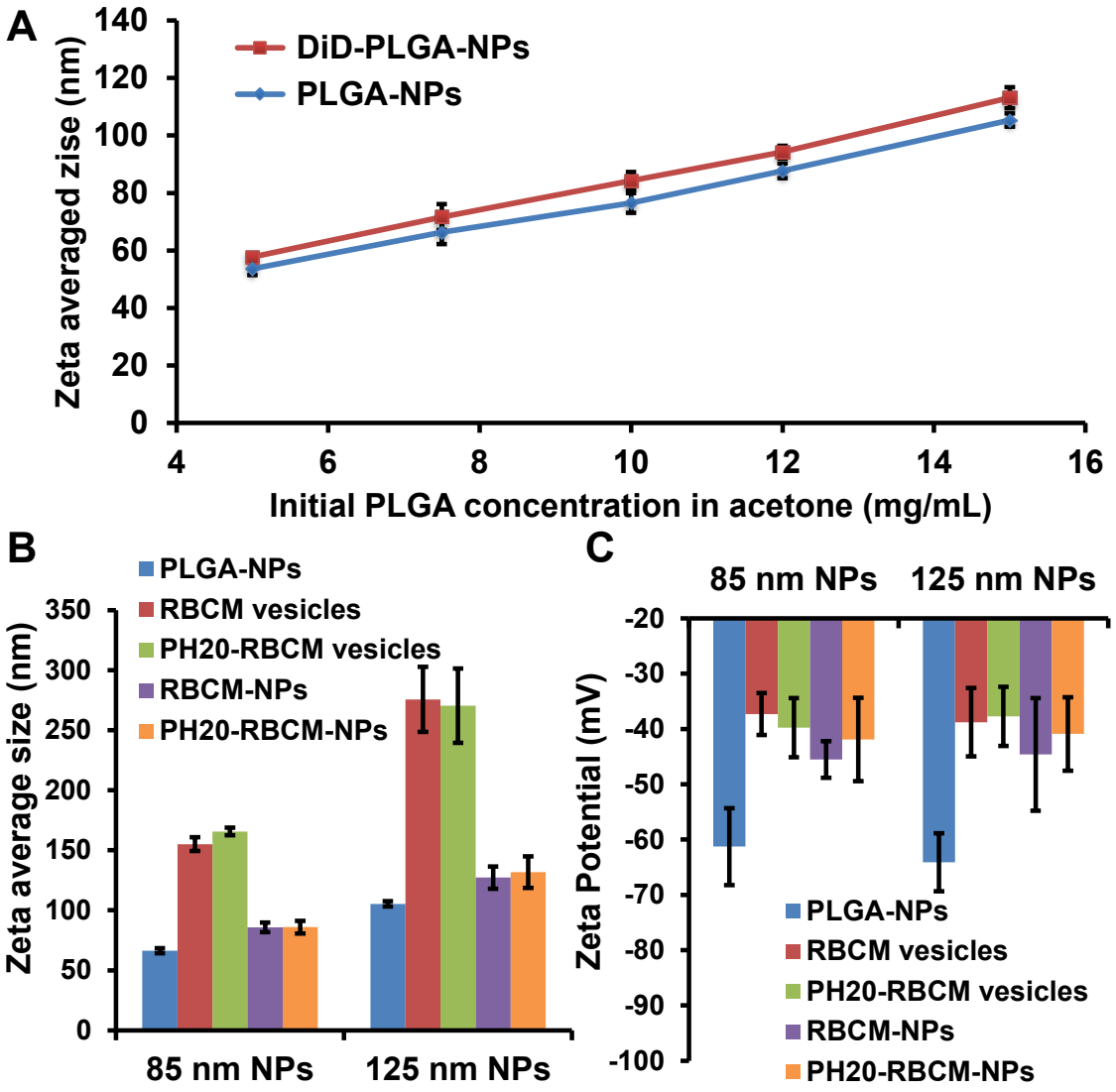


Figure S1. Characterization of NPs. A) Zeta-averaged sizes of PLGA-NPs and DiD-labeled PLGA-NPs prepared using PLGA acetone solutions at various concentrations. (5, 7.5, 10, 12, 15 mg/mL) Values are mean ± s.d., n=3. B) Zeta-averaged sizes of PLGA-NPs, RBCM-NPs and PH20-RBCM-NPs. Vesicles are membrane-formed particles without PLGA-NP core. Values are mean ± s.d., n=3. C) Zeta potential of PLGA-NPs, RBCM-NPs and PH20-RBCM-NPs. Values are mean ± s.d., n=3.

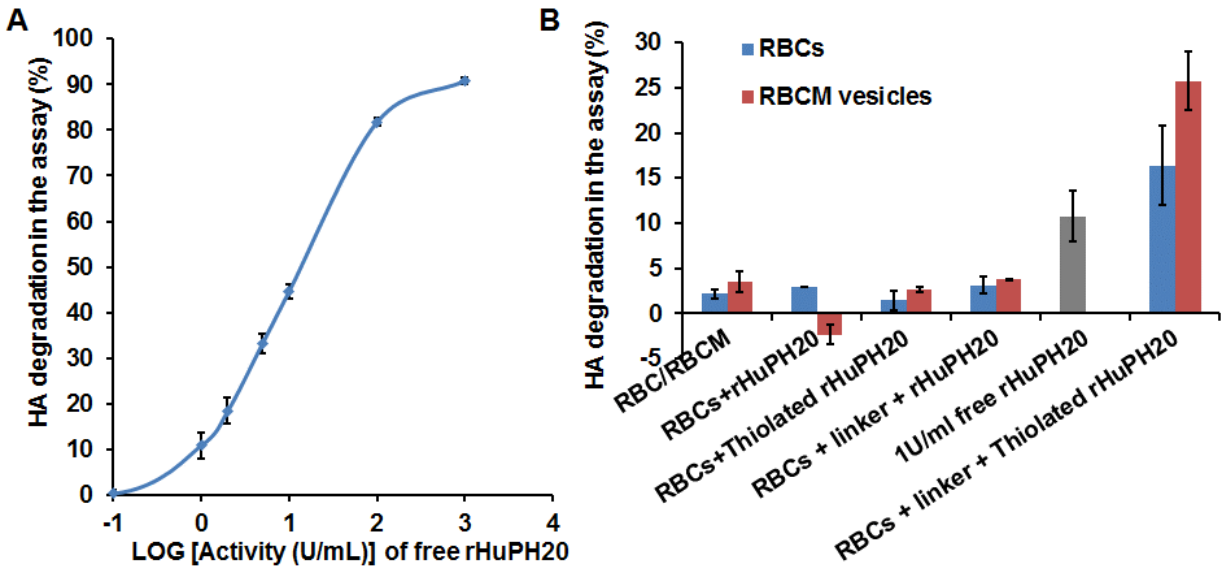


Figure S2. rHuPH20 activity assay demonstrating the covalent conjugation of rHuPH20 on the RBC membranes. (A) Standard curve of free rHuPH20 activity ranging from 0.1 U/mL to 1000 U/mL. Values are mean ± s.d., n=3. (B) rHuPH20 activity of RBCs or RBC membrane vesicles that are modified or prepared under conditions with or without linker and/or rHuPH20 thiolation. Free rHuPH20 served as a positive control. Vesicles refer to RBC membrane-formed particles without PLGA-NP core. The RBC membranes were isolated to form small vesicles (RBCM vesicles) in order to increase the access of conjugated rHuPH20 to HA matrix in the assay. Values are mean ± s.d., n=3.

# of Effective rHuPH20 per NP	Short linker	Long linker
Small NP	8.10 ± 1.43	8.34 ± 0.98
Large NP	11.5 ± 2.33	14.4 ± 2.12

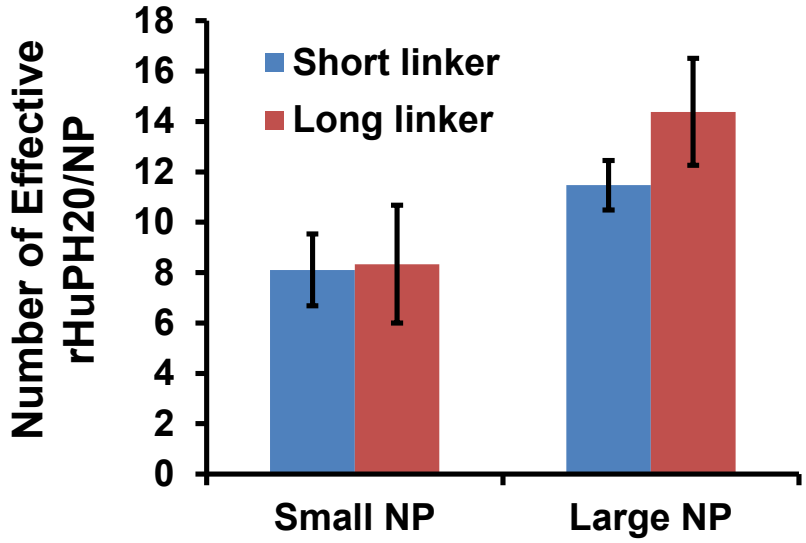
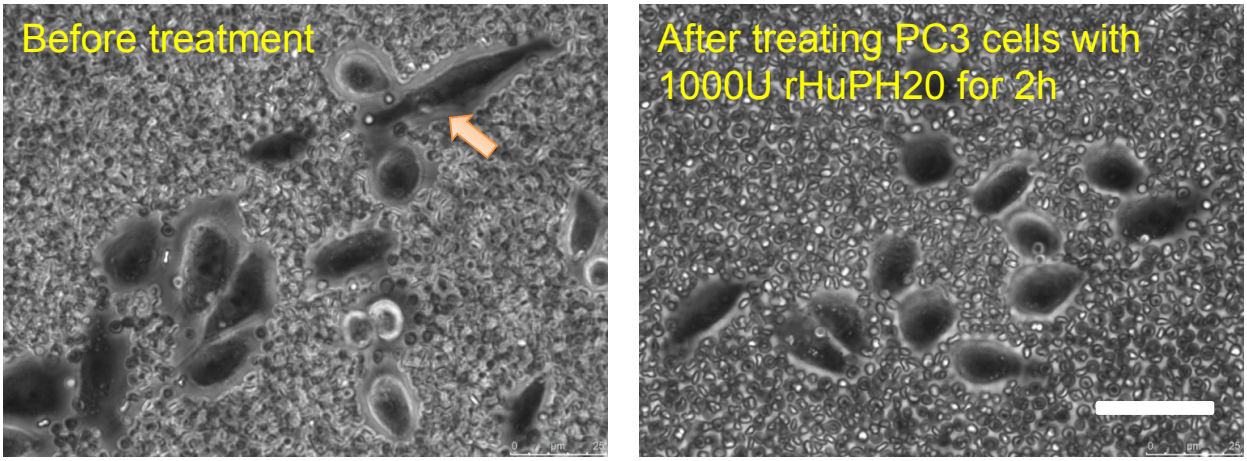


Figure S3. Number of effective rHuPH20/NP. Values indicate mean ± s.d. (n=3).



Exclusion area per cell

Before rHuPH20 treatment	After rHuPH20 treatment	Depleted Matrix area
128.3 ± 21.9 μm^2	40.2 ± 6.4 μm^2	88.1 μm^2

Figure S4. Measurement of the pericellular HA matrix around PC3 cells via a RBC exclusion assay (scale bar = 20 µm). PC3 cells were treated without and with 1000 U/mL of free rHuPH20 for 2 hours before adding fixed-RBCs. The arrow is pointing to the matrix. The images were analyzed through ImageJ, and the matrix area/cell was calculated. Values are mean ± s.d. (n=10).

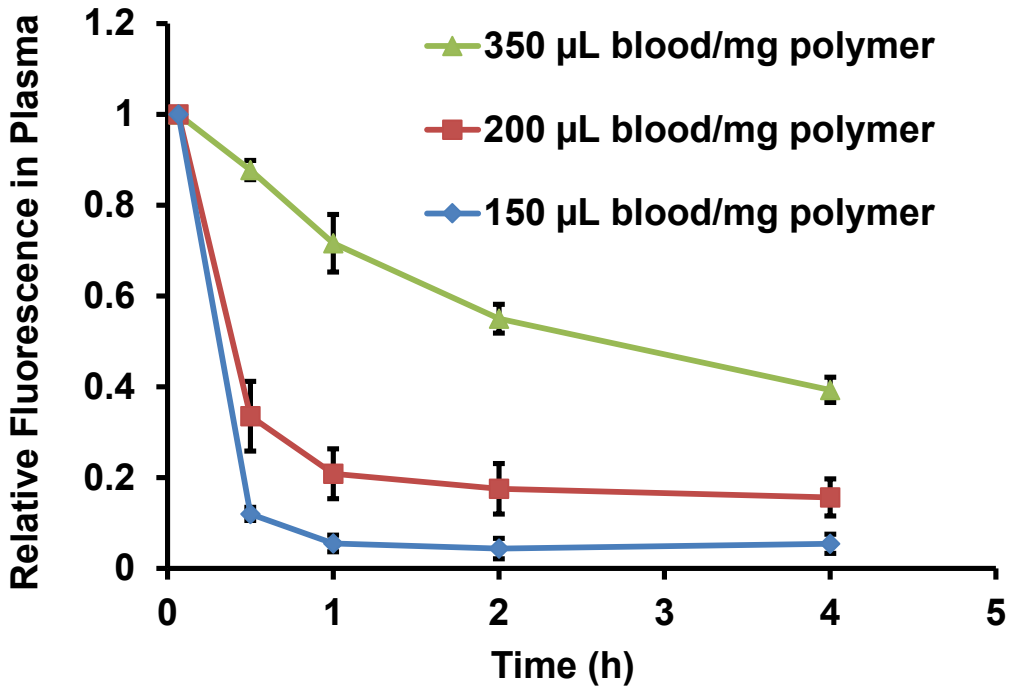


Figure S5. Effect of cell membrane/PLGA-NP surface area ratio on RBCM-NP blood circulation. For every mg of PLGA-NPs, a series amount of RBC membranes from 350, 200, 150 μL of blood were mixed and extruded together. The resulting RBCM-NPs were administered through tail vein injection at 100 μL of 8 mg/mL per mouse. At different time points, blood was collected and the fluorescence intensity in plasma was detected via TECAN at 600nm/655nm. Values indicate mean ± s.d. (n = 3-4).

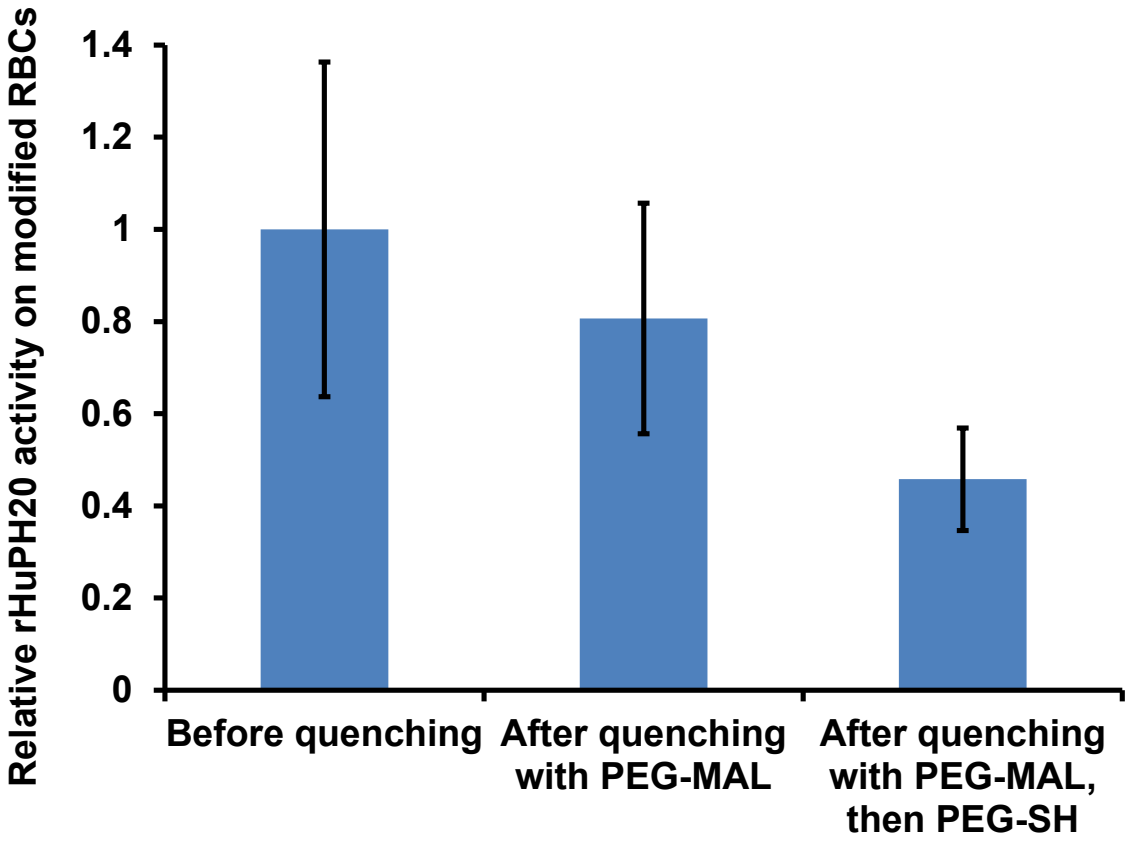


Figure S6. Relative rHuPH20 activity on modified RBCs before and after quenching unreacted thiol and maleimide groups with PEG_{2K}-maleimide and PEG_{2K}-thiol sequentially. Value indicates mean ± s.d. (n = 3).