Electronic Supporting Information

Protein coronas suppress the hemolytic activity of hydrophilic and hydrophobic nanoparticles

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Nanoparticle synthesis and characterization

Gold nanoparticles cores (d~2nm) stabilized with a monolayer of 1-pentanethiol were synthesized following the Brust-Schiffrin methodology.[1] All the impurities were removed according to our own protocol to certify that the phase-transfer catalyst do no interfere in the biological tests.[2] To functionalize the cores with the desired ligands, a Murray place-exchange reaction[3] was performed by dissolving the thiolated ligand in dry DCM with the gold cores, stirring for 3 days at room temperature (5:1 w/w ratio of ligand/gold cores). DCM was then evaporated under reduced pressure and the residue was dissolved in distilled water. Dialysis was performed during 5 days (membrane MWCO = 10,000) to remove excess of ligand and salts remaining in the nanoparticle solution. After dialysis, the particles were lyophilized and dissolved in deionized MQ water. Nanoparticle concentration was obtained following the reported procedure.[4] The ligands were synthesized according to our previous reports.[5]

Table S1. Nanoparticles physicochemical properties.

	Size (nm) by DLS Number	Zeta Potential (mV)
NP1	12.96 ± 2.77	16.9 ± 13.6
NP2	9.20 ± 1.90	20.4 ± 11.1
NP3	12.01 ± 2.33	24.1 ± 8.7
NP4	14.19 ± 2.65	17.7 ± 9.6
NP5	12.72 ± 2.76	20.1 ± 6.0
NP6	14.06 ± 3.50	17.3 ± 6.7
NP7	12.61 ± 2.68	21.8 ± 9.4
NP8	16.00 ± 4.42	20.7 ± 7.0
NP9	10.17 ± 1.87	15.7 ± 9.9
R1	14.75 ± 4.08	-0.88 ± 7.65
R2	11.18 ± 2.23	-53.5 ± 11.0
R3	8.11 ± 1.76	0.98 ± 6.15

Hemolysis assay in the absence of plasma proteins

Citrate-stabilized human whole blood (pooled, mixed gender) was purchased from Bioreclamation LLC, NY and processed as soon as received. 10 mL of phosphate buffered saline (PBS) was added to the blood and centrifuged at 5000 r.pm. for 5 minutes. The supernant was carefully discarded and the red blood cells (RBCs) were dispersed in 10 mL of PBS. This step was repeated at least five times. The purified RBCs were diluted in 10 mL of PBS and kept on ice during the sample preparation. 0.1 mL of RBC solution was added to 0.4 mL of nanoparticle (NP) solution in PBS in 1.5 mL centrifuge tube (Fisher) and mixed gently by pipetting. RBCs incubated with PBS and water were used as negative and positive control, respectively. All NP samples as well as controls were prepared in triplicate. The mixture was incubated at 37 ˚C for 30 minutes while shaking at 150 r.p.m. After incubation period, the solution was centrifuged at 4000 r.p.m. for 5 minutes and 100 µL of supernatant was transferred to a 96-well plate. The absorbance value of the supernatant was measured at 570 nm using a microplate reader (SpectraMax M2, Molecular devices) with absorbance at 655 nm as a reference. The percent hemolysis was calculated using the following formula:

% Hemolysis = ((sample absorbance-negative control absorbance)) / ((positive control absorbance-negative control absorbance)) × 100.

Hemolysis assay in the presence of plasma proteins

Human plasma (pooled, mixed gender) were purchased from Biochemed Pharmaceuticals, VA and kept at -20 ˚C for further use. NPs were pre-incubated in 55% of plasma solution in PBS (v/v) for 30 minutes at 37 ˚C. After the pre-incubation period, 0.1 mL of washed RBCs were added to the solution and further incubated for 30 minutes or 24 hours. The same procedure was followed to determine the hemolysis in the presence of plasma (*vide supra*).

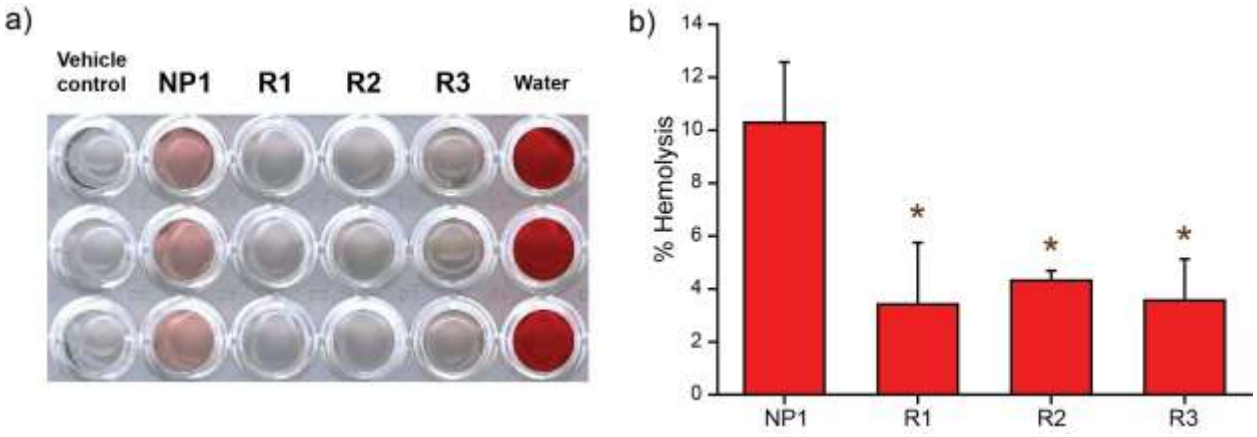

Figure S1. The effect of the nanoparticle surface charge on hemolysis. a) Physical observation of the supernatant after nanoparticle addition and centrifugation. b) Values of hemolysis relative to positive control. Experiments were performed by triplicate. Extreme left and right values are negative control (no nanoparticles) and positive control (water).

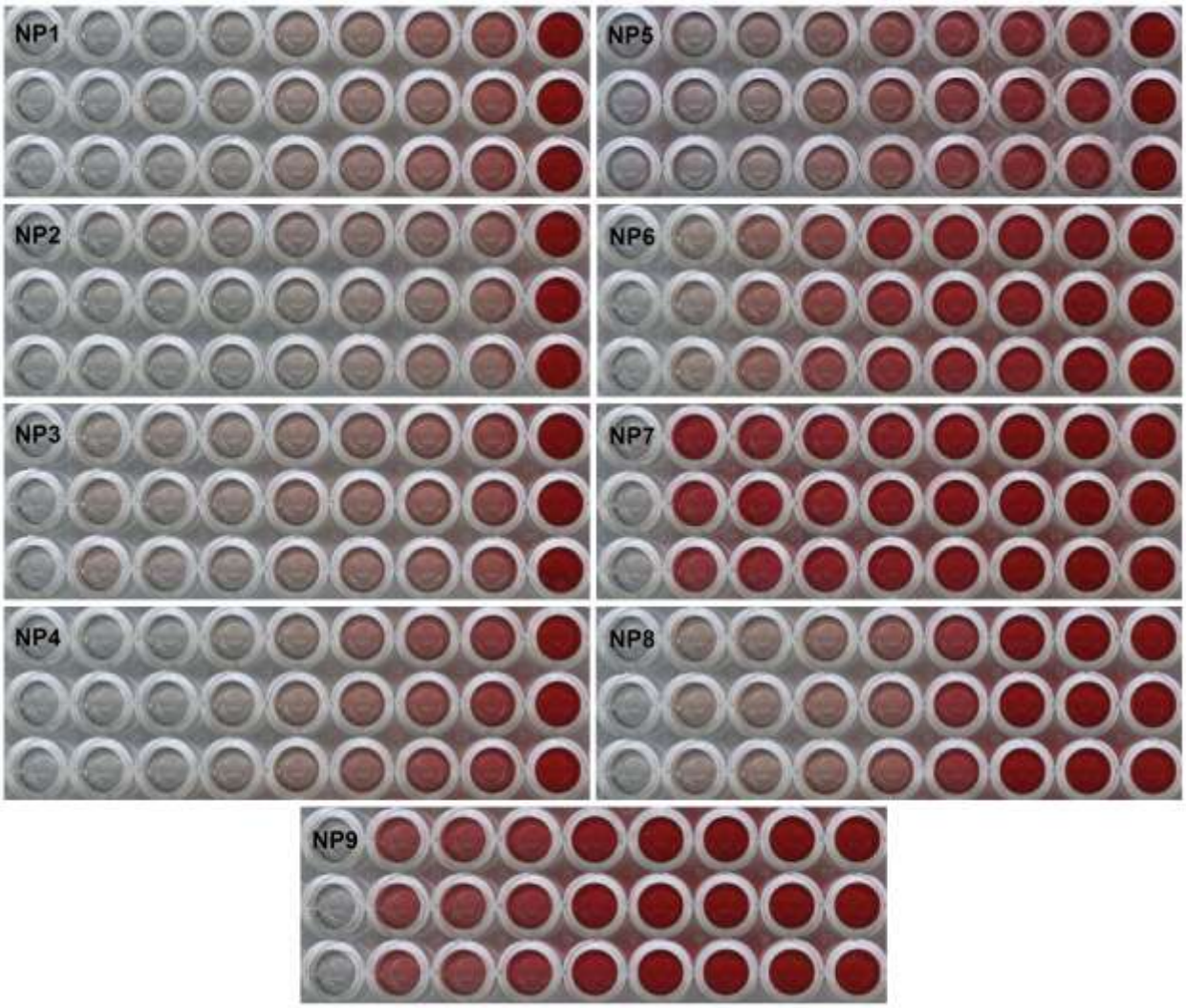

Figure S2. Dose dependence study for nanoparticles NP1-NP9. Experiments were performed by triplicate using concentrations of 8, 16, 32, 64, 125, 250, 500nM. Extreme left and right values are negative control (no nanoparticles) and positive control (water).

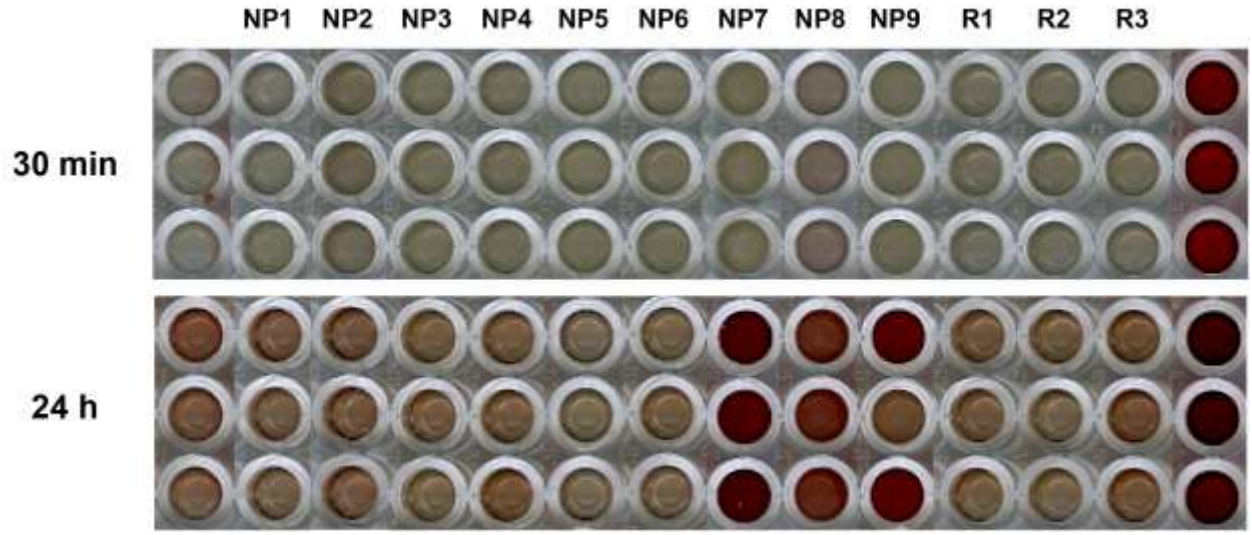

Figure S3. Hemolysis in the presence of plasma for NP1-NP9 at 30 min and 24 h. Extreme left and right values are negative control (no nanoparticles) and positive control (water).

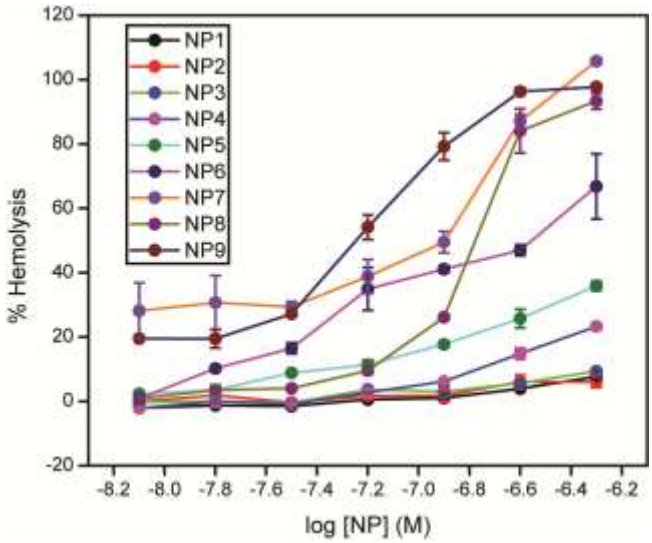

Figure S4. Dose-dependent hemolysis in log scale demonstrating the co-operative nature of the hemolytic process for more hydrophobic NPs.

References

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