Inhibition of selectin binding by colloidal gold with functionalized shells

Supporting information

The characterization of the unsulfated colloids by ^{1}H-NMR-spectroscopy is very difficult because they cannot be properly re-dissolved after complete removal of the solvent. Instead we characterized the more stable sulfated colloids.

Synthesis of Au/DT. Dodecanethiol functionalized gold nanoparticles (Au/DT) were synthesized according to a procedure published by Stucky *et al.*[1] An amount of 0.435 g of *tert*-butylamin-borane complex was added to a solution of 0.247 g $AuPPh_3Cl$ (0.5 mmol, 1 eq) and 0.25 ml dodecanethiol (1.0 mmol, 2 eq) in 40 ml benzene. The mixture was stirred at 55 °C for 1 hour before cool ing to room temperature. Then 40 ml of ethanol were added and the precipitant separated by centrifugation. The black solid powder was washed with ethanol at least three times, dried under vacuum, and resolved in 25 ml of chloroform.

Synthesis of Au/MUDHSE. An amount of 95 mg of N-hydroxysuccinimid-11-mercapto-undecanoate (MUDHSE) (0.6 mmol) was solved in anhydrous N,N-dimethylformamide (DMF) and 1 ml of a 3 μM solution of dodecanethiol-protected gold nanoparticles in chloroform was added dropwise to the stirring solution. After 15 minutes the main part of the chloroform was removed under reduced pressure and the solution was stirred at r.t. for another 24 h. The nanoparticle solution was dialysed against 400 ml DMF three times, dried under vacuum and resolved in 15 ml of anhydrous DMF.

Synthesis of Au/MUDSulfate (7). An amount of 29.8 mg 11-mercatoundecanyl sulfate (0.1 mmol) was solved in a solution of 5 ml triple-distilled water and 50 μl tetramethylammonium hydroxide (TMAH). 1 mL of a 3 μM solution of dodecanethiol-protected gold nanoparticles in chloroform was added and the mixture stirred at room temperature for 2 hours, which led to a complete phase transfer of the colloids from the organic into the water phase. The organic phase was removed and, in order to remove the ligand's excess and to reduce the pH to approximately 8, the water phase was dialysed against 300 ml triple-distilled water three times. For storage the solution was deluted to a 0.03 μM concentration of nanoparticles.

^{1}H-NMR (D_2O, 400 MHz): δ = 1.34 (*bs*), 1.71 (*bs*), 2.73 (*bs*, CH_2-SH), 4.06 (*bs*, CH_2-OSO_3^-).

ATR-IR: ν = 3602 cm^{-1} (w), 3040 (*w*), 2916 and 2849 (*s*, ν(C-H)), 1490 - 1468 and 1220 (*s*, R-O-SO_2-OR'), 1066 – 950 (*s*), 783 (*s*), 623 (*s*), 577 (*s*).

Synthesis of AP-functionalized gold nanoparticles (2). A solution of 4.2 mg AP (0.02 mmol) in anhydrous DMF was added to 5 ml of a 0.2 μM solution of MUDHSE-

protected gold nanoparticles in DMF. After 30 minutes of stirring at r.t. 20 µl triethylamine (0.14 mmol) was added and the mixture stirred for another 24 h. The clear colloid solution was dialysed against 300 ml of DMF for three times, dried under vacuum and resolved in 5 ml of anhydrous DMF.

Sulfation of AP-functionalized gold nanoparticles (3). At 0 °C 15.3 mg SO_3·DMF-complex (0.1 mmol), solved in 1 ml of anhydrous DMF, was slowly dropped to 10 ml of a 0.1 µM solution of AP-functionalized gold nanoparticles in anhydrous DMF. After 24 h of stirring at r.t. the clear solution was dialysed against 300 ml of DMF for one time and 300 ml of triple-distilled water for three times. For storage the colloid solution was deluted to a 0.03 µM concentration of nanoparticles.

^{1}H-NMR (D_2O, 400 MHz): δ = 1.30 (*bs*), 1.32 (*s*), 1.47 (*s*), 1.59 (*m*), 1.68 (*m*), 2.30 (*bt*, *CH_2*-CONH), 2.39 (*q*), 2.76 (bs, *CH_2*-SH), 4.01 (*m*), 4.18 (*m*), 4.46 (*m*), 4.54 (*m*).

ATR-IR: ν = 3448 cm^{-1} (*w*, ν(O-H)), 3075 (*w*), 2923 and 2852 (*s*, ν(C-H)), 2484 (*w*), 1687 and 1647 (*s*, ν(C=O)), 1529 (*s*, δ(N-H)), 1467 and 1207 (*s*, R-O-SO_2-OR'), 1058 (*s*), 985 (*s*), 810 (*s*), 582 (*s*).

Synthesis of tromethamine-functionalized gold nanoparticles. A solution of 18.2 mg tromethamine (0.15 mmol) in anhydrous DMF was added to 5 ml of a 0.2 µM solution of MUDHSE-protected gold nanoparticles in DMF. After 30 minutes of stirring at r.t. 80 µl triethylamine (0.56 mmol) was added and the mixture stirred for another 24 h. The clear colloid solution was dialysed against 300 ml of DMF for three times, dried under vacuum and resolved in 5 ml of anhydrous DMF.

Sulfation of tromethamine-functionalized gold nanoparticles (4). At 0 °C 300 mg SO_3·DMF-complex (2.0 mmol), solved in 1 ml of anhydrous DMF, was slowly dropped to 10 ml of a 0.1 µM solution of tromethamine-functionalized gold nanoparticles in anhydrous DMF. After 24 h of stirring at r.t. the clear solution was dialysed against 300 ml of DMF for two times and 300 ml of triple-distilled water for three times. For storage the colloid solution was deluted to a 0.03 µM concentration of nanoparticles.

^{1}H-NMR (D_2O, 400 MHz, c_{NP} = 1 µM): δ = 1.29 (*bs*), 1.59 (*bs*), 2.24 (*bs*, *CH_2*-S), 2.90 (*bs*), 4.3 (*bs*, *CH_2*-O-SO_3^-).

ATR-IR: ν = 3291 cm^{-1} (*w*, ν(N-H)), 2920 and 2851 (*s*, ν(C-H)), 2465 (*w*, ν(S-H)), 1682 and 1633 (*s*, ν(C=O)), 1528 (*s*, δ(N-H)), 1466 - 1453 and 1210 (*s*, R-O-SO_2-OR'), 1002 (*s*), 801 – 774 (*s*), 578 (*s*).

Synthesis of serinol-functionalized gold nanoparticles. A solution of 9 mg serinol (0.1 mmol) in anhydrous DMF was added to 5 ml of a 0.2 µM solution of MUDHSE-protected gold nanoparticles in DMF. After 30 minutes of stirring at r.t. 20 µl triethylamine (0.14 mmol) was added and the mixture stirred for another 24 h. The clear colloid solution was dialysed against 300 ml of DMF for three times, dried under vacuum and resolved in 5 ml of anhydrous DMF.

Sulfation of serinol-functionalized gold nanoparticles (5). At 0 °C 154 mg SO_3·DMF-complex (1.0 mmol), solved in 1 ml of anhydrous DMF, was slowly dropped to 10 ml of a 0.1 µM solution of serinol-functionalized gold nanoparticles in anhydrous DMF. After 24 h of stirring at r.t. the clear solution was dialysed against 300 ml of DMF for two times and 300 ml of triple-distilled water for three times. For storage the colloid solution was deluted to a 0.03 µM concentration of nanoparticles.

^{1}H-NMR (D_2O, 400 MHz): δ = 1.32 (*bs*), 1.62 (*bs*), 2.30 (*bs*, *CH_2*-CONH), 2.89 (*bs*, *CH_2*-SH), 4.15 (*bs*, *CH_2*-OSO_3^-), 4.43 (*bs*, *CH*-($CH_2OSO_3^-$)$_3$).

ATR-IR: ν = 3482 und 3294 cm^{-1} (*s*, ν(O-H)), 3039 (*w*), 2924 and 2853 (*s*, ν(C-H)), 1683 and 1653 (*s*, ν(C=O)), 1539 (*s*, δ(N-H)), 1490 (*s*), 1465 - 1453 and 1218 (*s*, R-O-SO_2-OR'), 1004 (*s*), 950 (*s*), 788 (*s*), 577 (*s*).

Synthesis of ethanolamine-functionalized gold nanoparticles. A solution of 9 µl ethanolamine (0.15 mmol) in anhydrous DMF was added to 5 ml of a 0.2 µM solution of MUDHSE-protected gold nanoparticles in DMF. After 30 minutes of stirring at r.t. 20 µl triethylamine (0.14 mmol) was added and the mixture stirred for another 24 h. The clear colloid solution was dialysed against 300 ml of DMF for three times, dried under vacuum and resolved in 5 ml of anhydrous DMF.

Sulfation of ethanolamine-functionalized gold nanoparticles (6). At 0 °C 300 mg SO_3·DMF-complex (2.0 mmol), solved in 1 ml of anhydrous DMF, was slowly dropped to 10 ml of a 0.1 µM solution of serinol-functionalized gold nanoparticles in anhydrous DMF. After 24 h of stirring at r.t. the clear solution was dialysed against 300 ml of DMF for two times and 300 ml of triple-distilled water for three times. For storage the colloid solution was diluted to a 0.03 µM concentration of nanoparticles.

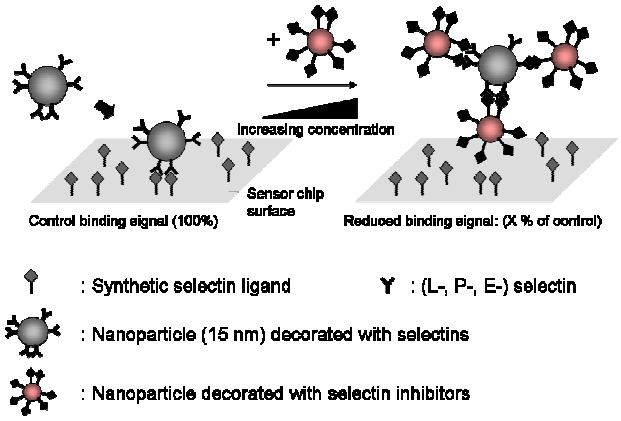
^{1}H-NMR (D_2O, 400 MHz, c_{NP} = 1 µM): δ = 1.25 (*bs*), 1.56 (*bs*), 2.22 (*bs*, *CH_2*-CONH), 2.69 (*bs*, *CH_2*-SH), 3.45 (*bs*, *CH_2*-NHCO), 4.06 (*bs*, *CH_2*-OSO_3^-).

ATR-IR: ν = 3302 cm^{-1} (*s*, ν(N-H)), 2918 and 2850 (*s*, ν(C-H)), 1686 and 1639 (*s*, ν(C=O)), 1532 (*s*, δ(N-H)), 1465 - 1452 and 1211 (*s*, R-O-SO_2-OR'), 1066 (*s*), 1020 (*s*), 959 (*w*), 719 (*s*), 579 (*s*).

Cytotoxicity assay. The Promega CellTiter 96® AQ_{ueous} Cell Proliferation Assay was applied. L-selectin expressing T-cells (Jurkat) cells were seeded in 96-well plated at a density of 5000 cells/well. Au-colloids with sulfated derivatives of 11-mercaptoundecanoic acid, serinol and AP were added at following concentrations: of 3 pM, 30 pM, 300 pM, 3 nM and 30 nM. In comparison to the untreated control cell proliferation was detected after 3 day incubation by measuring the conversion of the tetrazolium compound 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) and an electron coupling reagent, phenazine ethosulfat (PES) into a water-soluble formazan that absorbs light at 490 nm by cellular dehydrogenases. The assay was performed in triplicates at each concentration. The untreated control was set to 100 % proliferation.

Supporting information - Additional Information

SPR based competitive selectin-ligand binding assay



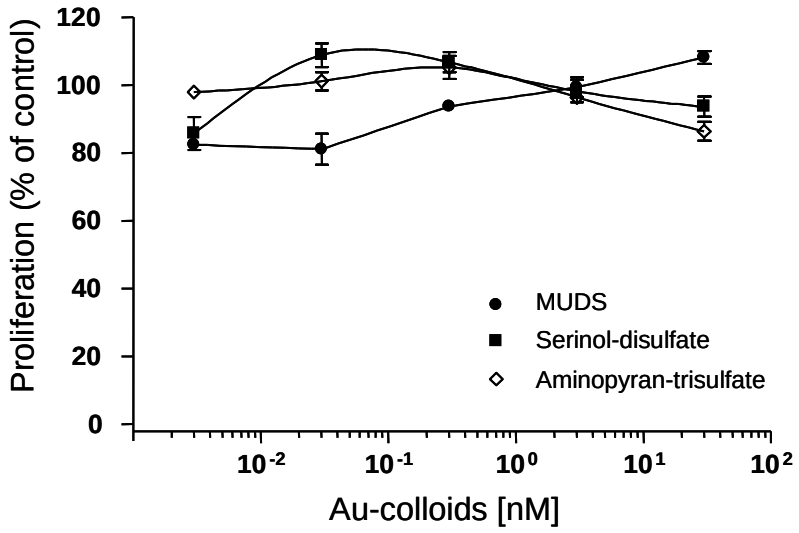
Selectin-ligand binding was analyzed by surface plasmon resonance (SPR). First, the binding (detected as resonance units) of selectin-coated nanoparticles to a selectin ligand, immobilized on a sensor chip, was tested, and the resulting signal was henceforth referred to as 100% binding. Preincubation of the selectin-coated particles with varying concentrations of a selectin inhibitor (here a gold colloid with a ligand shell) decreased the binding signal. The calculated IC_{50} values (half inhibitory concentration) is the molar concentration of the inhibitor needed to reduce the binding signal to 50% of the initial value.

Determination of the number of thiol ligands in the particle shell

The number of thiols in the ligand shell of a 6 nm Au particle was determined by thermogravimetric analysis of the isolated gold colloid and by EDX measurements of the Au:S ratio using subsequent analytical treatment of the combined intensity curves of Au:M and S:K lines. The average number of thiols as determined by thermogravimetric analysis was found to be 1190 +/- 120, EDX analysis gave an average number of thiols of 1411 +/- 135 molecules.

Gel electrophoresis of charged colloids. Gel electrophoresis was performed at room temperature with a Labnet Gel XL Ultra V-2 Electrophoresis System in a 1 % agarose gel in TRIS/HCl buffer (0.05 M, pH 7.2). The output voltage was held constant at 100 V. Electrophoresis was stopped when the bands began to broaden (after approximately 20-25 minutes).

Toxicity of Au colloids on T Cells (Jurkat)



References:

1. Zheng, N.; Fan, J.; Stucky, G. D. *J. Am. Chem. Soc.* **2006**, *128*, 6550-6551.