Supplementary information for "Electrophoretic interpretation of PEGylated NP structure with and without peripheral charge"

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Materials, experimental methods and characterization

Materials

Chemicals from Sigma Aldrich include: tetraoctylammonium bromide (TOAB, 98%, Cat. No. 294136-5G), gold (III) chloride solution ($HAuCl_4$, 99.99%, trace metals basis, 30 wt.% in

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dilute HCl, Cat. No. 484385-10G), dodecylamine (DDA, 99+%, Cat. No. 325163-5ML), and sodium borohydride ($NaBH_4$, granular, 99.99%, trace metals basis, Cat. No. 480886-25G). TAE buffer (UltraPure, 10X, Cat. No. 15558042) and PBS buffer (Gibco, 10X, Cat. No. 70011-044) were from Life Technologies. Carboxymethyl poly(ethylene glycol) thiol (CM-PEG-HS, average MW 5000) was from Laysan Bio Inc. All chemicals were used without further purification.

Experimental methods

Synthesis of carboxymethyl PEGylated gold nanoparticles

All the glassware and magnetic stir bars were cleaned with aqua regia, rinsed with RO water, and dried in an oven (Lindberge/Blue) before use. $HAuCl_4$ solution (367 μl) was added to a mixture of toluene (5 ml) and TOAB (0.137 g) followed by addition of DDA (0.111 g) with moderate stirring. $NaBH_4$ solution (0.076 g in 1 ml of ice water) was slowly added dropwise to avoid violent bubbling, and this mixture was then stirred at room temperature for 30 minutes before centrifuging (Thermo Scientific Sorvall Biofuge Primo centrifuge) with ethanol twice at 5000 rpm for 10 min. The pellets in the centrifuge tube were collected and suspended in chloroform, forming a DDA-Au NP solution. To maximize the grafting density, an excess amount of CM-PEG-HS (mass calculated from the DDA-Au NP solution concentration for a fixed Au NP/CM-PEG-HS ratio 1:1000) was mixed with the DDA-Au NP solution (2.5 ml) and chloroform (2.5 ml), and stirred at room temperature for 2 days in a capped glass vial. This solution was dried overnight in a fume hood, and resuspended in RO water. Unreacted and loosely attached CM-PEG-HS was removed by centrifuging in a 30 kDa molecular weight cut-off membrane separated centrifuge tube (EMD Millipore Corporation) at 5000 rpm for 10 min. The wash in the lower compartment of the tube was discarded, and RO water was added to the top compartment to maintain the volume. This process was repeated 10 times. Note that the cut-off membrane did not exclude the Au NPs, as evidenced by the transparent color of the lower wash.

Drying and dispersion of DDA- and CM-PEG-capped Au NPs

Two methods were used to dry the Au NP solutions for subsequent characterization or redispersion. The stock CM-PEG-Au NP solution was diluted with RO water and then mixed with sodium chloride solid. After adding chloroform, the CM-PEG-Au NPs salted out from the aqueous phase (upper layer) to the organic phase (lower layer), which was later removed when the aqueous phase turned from red to colorless. Both this organic CM-PEG-Au NP solution and the stock DDA-Au NP solution were placed under the fume hood to evaporate chloroform, and the dry samples were collected by carefully scraping the glass vials with a spatula. Following Manson et al.[1], the stock CM-PEG-Au NP solution was oven dried overnight at 60°C, and then sonicated for 30 s (20% amplitude and 1 s pulse mode using a 1/8" microtip, QSonica Q500) to promote dispersion in buffers such as TAE or PBS with prescribed concentrations.

Characterization

Fourier transform infrared spectroscopy (FTIR)

Infrared spectra were obtained using a Nicolet FTIR 6700 spectrometer (Thermo Scientific) equipped with integrated diamond ATR (attenuated total reflectance) and DRIFT (diffuse reflectance infrared Fourier transform) accessories. Samples for DRIFT-FTIR were mixed with KBr, ground to a fine powder, and transferred to the sample well. For diamond ATR-FTIR, samples (dry powder without KBr) were placed directly on top of the diamond crystal and then pressed with a clamp. Sample holders were cleaned with isopropanol before and after each measurement. Transmittance and absorbance spectra are shown in figures 1 and 2.

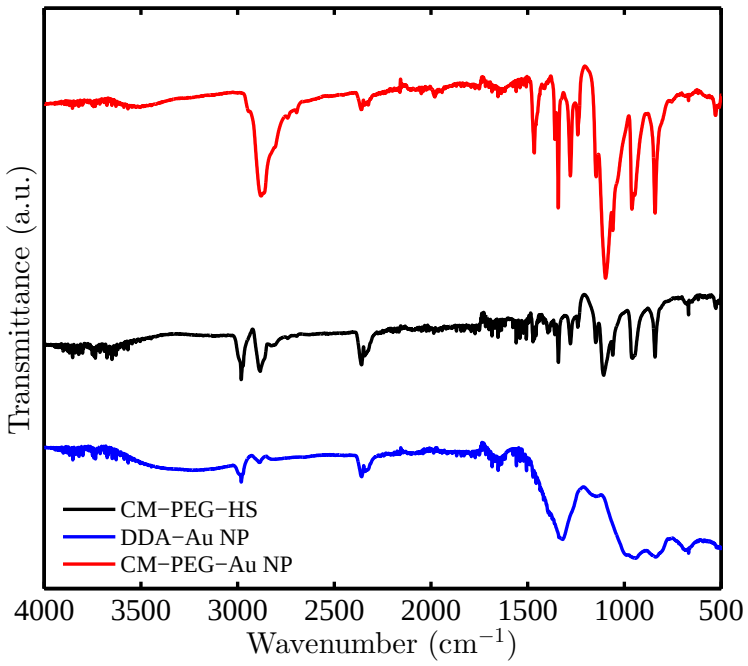


Figure 1: Diamond ATR-FTIR transmittance of CM-PEG-HS (black), DDA-Au NPs (blue), and CM-PEG-Au NPs (red).

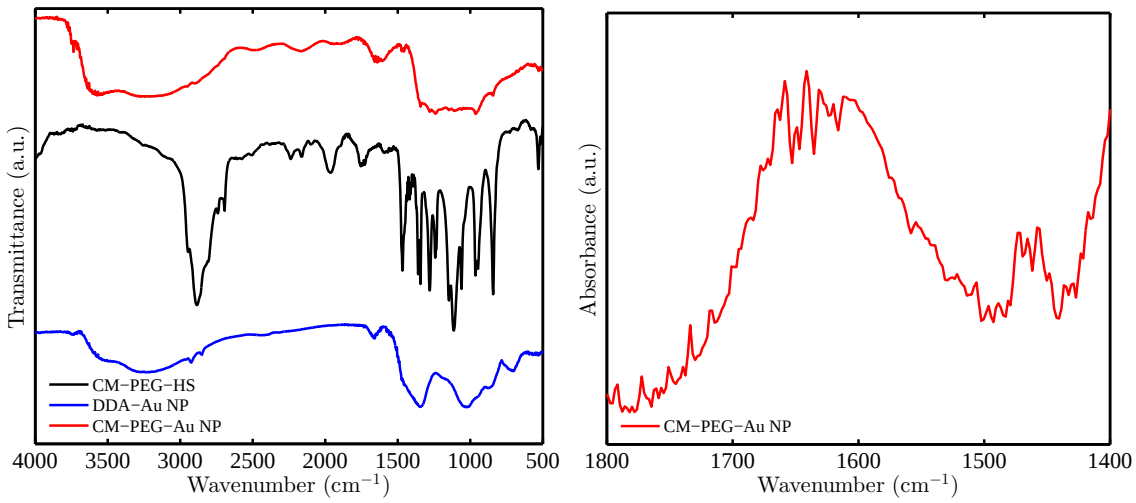
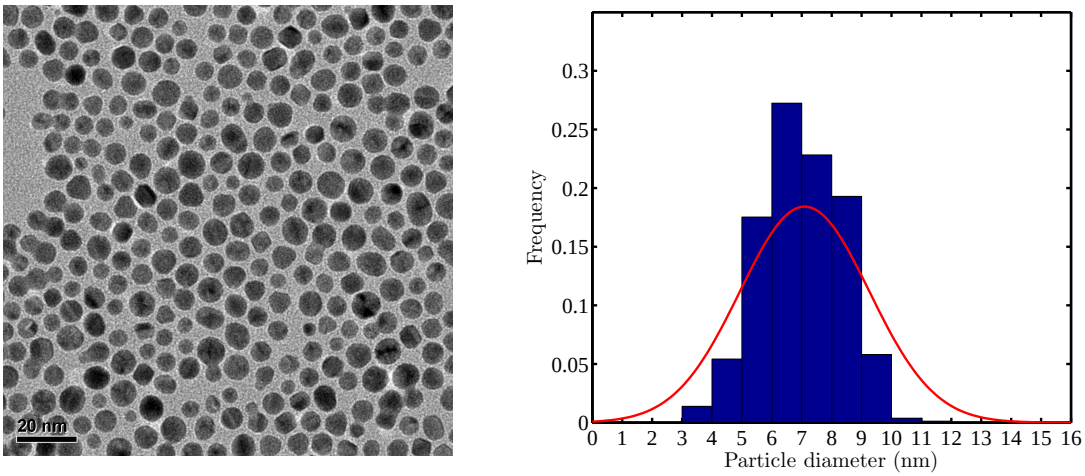


Figure 2: (a) DRIFT-FTIR transmittance of CM-PEG-HS (black), DDA-Au NPs (blue), and CM-PEG-Au NPs (red); (b) DRIFT-FTIR absorbance of CM-PEG-Au NPs.

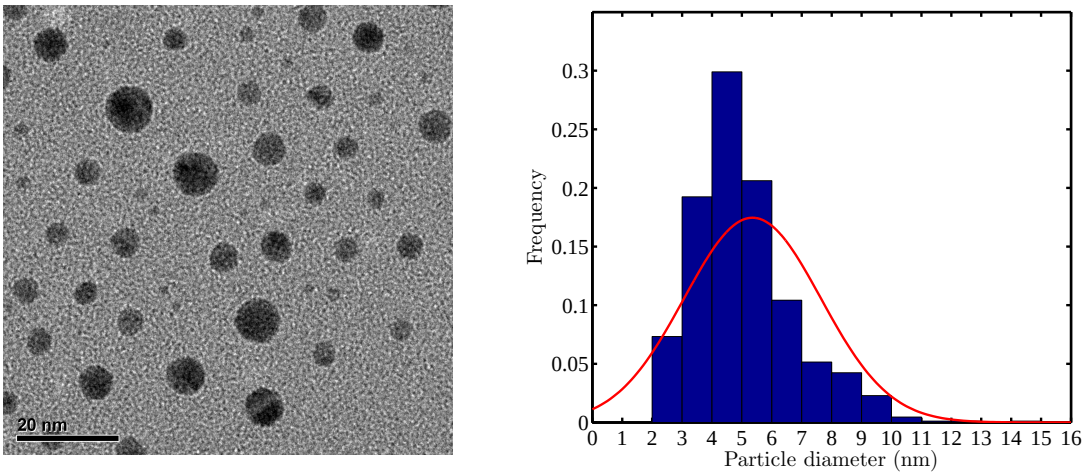
Transmission electron microscopy (TEM)

Solutions of DDA-Au NPs in chloroform and CM-PEG-Au NPs in RO water were dropped onto copper grids with carbon only film (200 mesh, SPI supplies), and dried under ambient conditions before being imaged using a Philips CM200 transmission electron microscope at 200 kV. TEM micrographs and accompanying size histograms for DDA-Au and CM-PEG-Au NPs are shown in figures 3 and 4, respectively.



(a) (b)

Figure 3: TEM micrograph (a) and size histogram (b) of DDA-Au NPs.



(a) (b)

Figure 4: TEM micrograph (a) and size histogram (b) of CM-PEG-Au NPs.

Thermogravimetric analysis (TGA)

Thermogravimetric analyses were performed using a TGA Q500 (TA Instruments). A dry sample ($\approx$ 6 mg) was transferred to the precleaned Pt pan, and heated from 20°C to 700°C at 20°C min^{-1} under nitrogen, which was changed to air after 550°C. The balance and flow gases were selected to be nitrogen with flow rates of 40 ml min^{-1} and 60 ml min^{-1}, respectively. The sample weights, reported as a percent, are shown in figure 5.

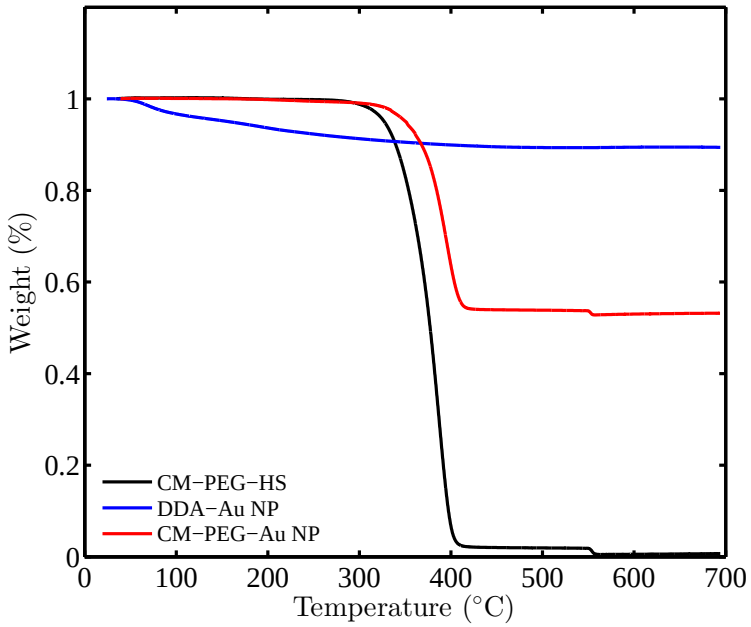
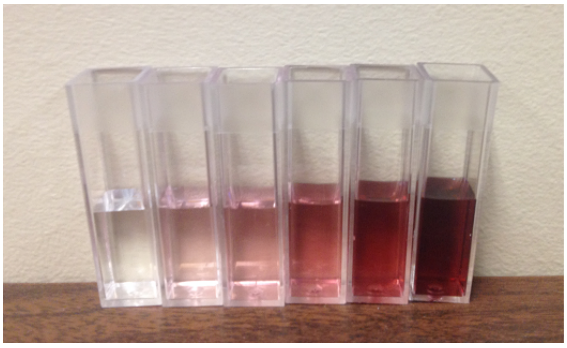


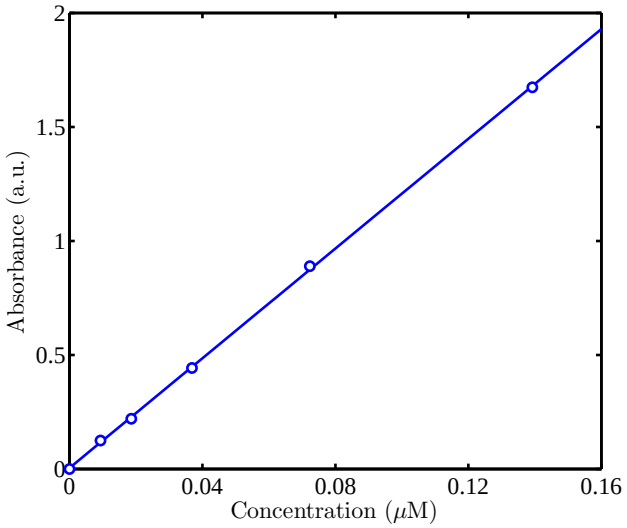
Figure 5: TGA of CM-PEG-HS (black), DDA-Au NPs (blue) and CM-PEG-Au NPs (red).

Ultraviolet-visible (UV-vis) spectroscopy

All UV-vis spectra were recorded using an Ultrospec 2100-pro UV-vis spectrophotometer (GE Healthcare Bio-Sciences) from 400 nm to 600 nm at 0.5 nm increments with 3500-μL quartz cuvettes for Au NPs in chloroform, and disposable plastic cuvettes for aqueous Au NP solutions (both with a through thickness of 1 cm). Diluted solutions and their peak absorbance (at 520 nm) are shown in figure 6.



(a)



(b)

Figure 6: CM-PEG-Au NP solutions (a) and their UV-vis absorbance at 520 nm (b).

Dynamic light scattering (DLS)

DLS measurements were conducted using a Nano ZetaSizer ZS series (Malvern Instruments). Before each measurement, samples were filtered with 0.22 μm syringe filters. Samples (2 ml) were placed in a disposable polystyrene cuvette for size measurement. To determine the ζ-potential, 1 ml was removed from the DLS cuvette into which a 'Universal Dip' cell was placed. Electrophoretic mobility was converted to the reported ζ-potential using the Smoluchowski formula.

Supplementary tables and figures

Table 1: Summary of PEG corona characteristics with layer thicknesses according to the model of Biver et al.[2] PEG characteristics from Russel et al.[3]

parameter	value
core radius, a	2.7 nm
aggregation number, N_a	146
PEG chain molecular weight	5 kDa
statistical segments per chain, N	83
statistical segment length, l	0.6 nm
statistical segment excluded volume, v/l^3	0.36
size of an ideal coil, $N^{1/2}l$	5.5 nm
chain contour length, $L_c = Nl$	50 nm
layer thickness on a flat substrate, L_{max}	18 nm
layer thickness on spherical core, L'	9.3 nm
grafting density, $\gamma l^2 = N_a l^2/(4\pi a^2)$	0.57
average statistical segment density, $n = N_a N/[4\pi(a+L')^3/3 - 4\pi a^3/3]$	2.8 M
average statistical segment volume fraction, nl^3	0.4
average fixed charge density, n/N	34 mM
Brinkman screening length, $\ell = 1/\sqrt{6\pi n l}$	0.23 nm
Debye screening length at $I = 1$ mM, κ^{-1}	9.6 nm

References

(1) Manson, J.; Kumar, D.; Meenan, B. J.; Dixon, D. *Gold Bull* **2011**, *44*, 99–105.

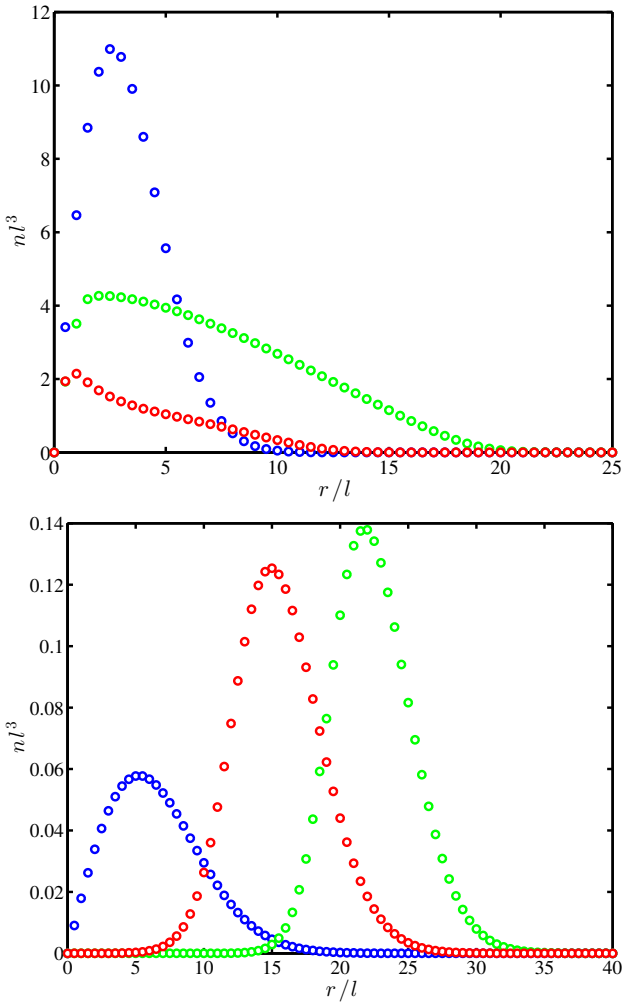


Figure 7: Self-consistent-field computations of the scaled segment density (top) and end-segment density (bottom): ideal terminally grafted chains (blue); excluded-volume ($v/l^3 = 0.25$) chains end-grafted to a nanoparticle (core radius $a = 2.7$ nm, statistical segment length $l = 0.6$ nm) (red) and a flat plate (green). Computations with dimensionless grafting density $\gamma l^2 = 0.57$ and chain length $N = 83$. Layer thicknesses are consistent with those predicted by the blob model of Biver et al.[2] (see table 1).

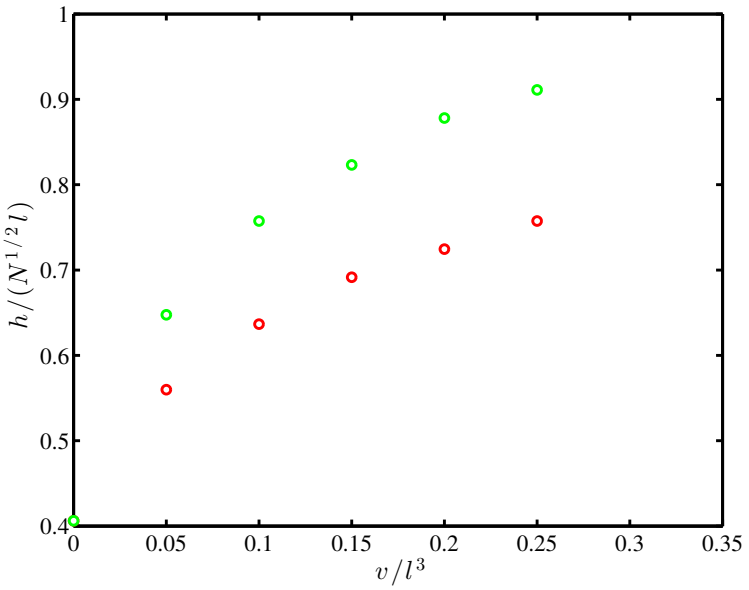


Figure 8: Self-consistent-field computation of the layer thickness $h/(N^{1/2}l)$ versus the dimensionless excluded volume parameter v/l^3 for uncharged excluded-volume chains end-grafted to a nanoparticle (core radius $a = 2.7$ nm) $l = 0.6$ nm) (red) and a flat plate (green). Computations with dimensionless grafting density $\gamma l^2 = 0.57$ and chain length $N = 83$ ($N^{1/2}l \approx 5.5$ nm).

(2) Biver, C.; Hariharan, R.; Mays, J.; Russel, W. B. *Macromolecules* **1997**, *30*, 1787–1792.

(3) Russel, W. B.; Saville, D. A.; Schowalter, W. R. *Colloidal Dispersions*; Cambridge University Press, 1989.

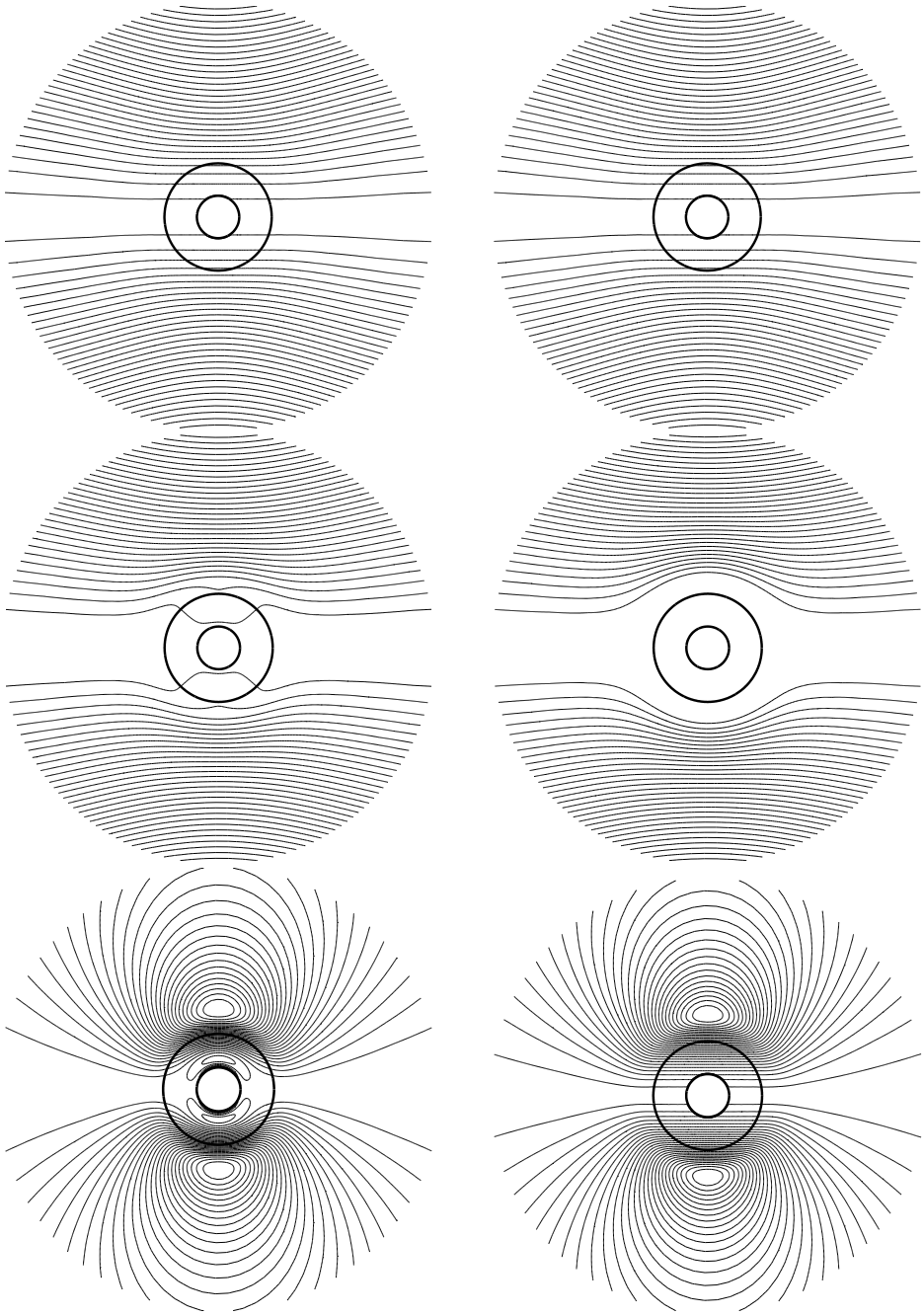


Figure 9: Streamlines for CM-5kPEG-Au NPs with $I = 100$ mM ($L \approx 5$ nm) for uncharged (left) and charged (right) coronas: translation in the absence of an electric field (diffusion, top); stationary particle in an electric field (electroosmosis, middle); translation under an electric field (force-free electrophoresis, bottom). Inner and outer circles identify the Au core and nominal coating periphery.

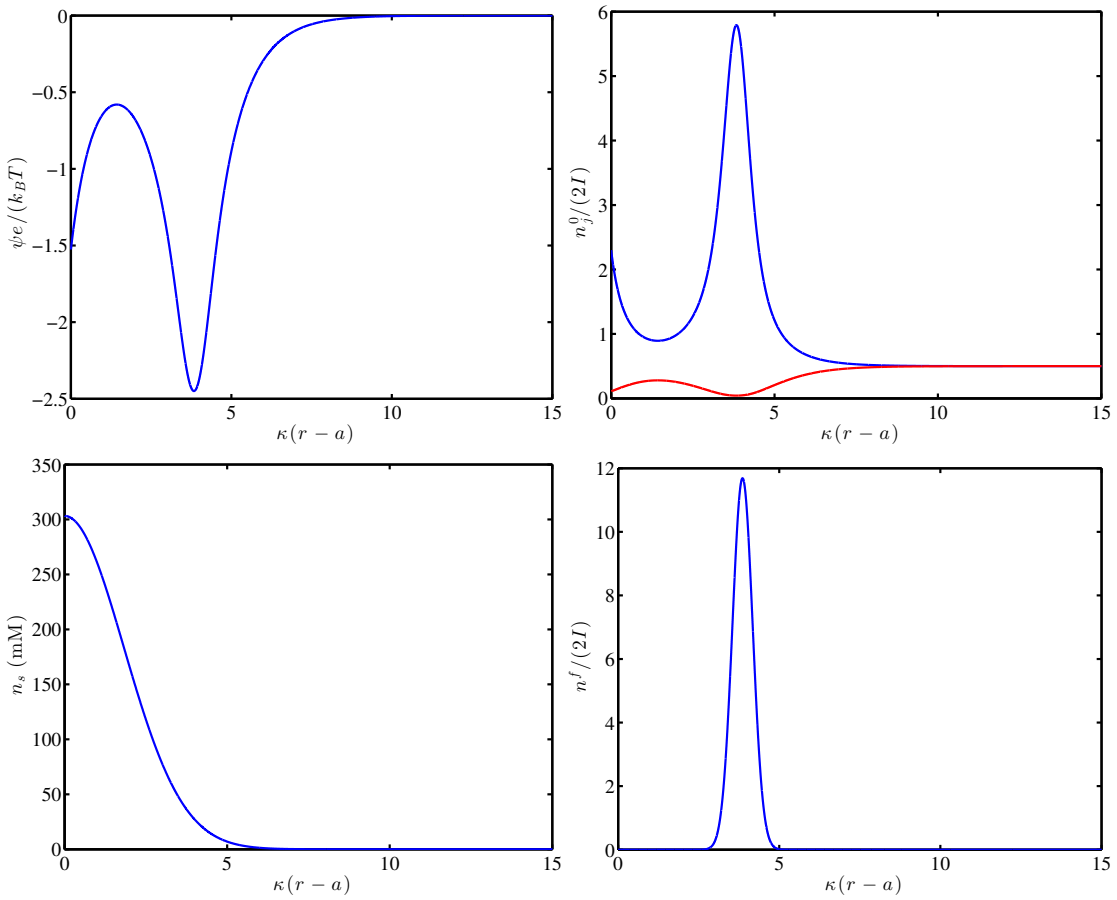


Figure 10: Radial profiles of electrostatic potential ψ (top left), equilibrium mobile ion densities n_j^0 (top right), segment (5kPEG-chain) density n_s (bottom left), and fixed charge density n_j^f (bottom right) for SH-PEG-COOH functionalized NPs ($a = 10$ nm, $\chi = 1$) at $I = 20$ mM.