Supporting Information

Low affinity binding of plasma proteins to lipid-coated quantum dots as observed by in-situ fluorescence correlation spectroscopy

Yvonne Klapper, [a] *Pauline Maffre,* [a] *Li Shang,* [a] *Kristina N. Ekdahl,*[b,c] *Bo Nilsson,* [c] *Simon Hettler,* [d] *Manuel Dries,* [d] *Dagmar Gerthsen* [d] *and G. Ulrich Nienhaus,*[a,e,f,*]

[a] Institute of Applied Physics and Center for Functional Nanostructures, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany. E-mail: uli@illinois.edu

[b] Linnæus Center of Biomaterials Chemistry, Linnæus University, Kalmar, Sweden

[c] Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden

[d] Laboratory of Electron Microscopy (LEM), KIT, Karlsruhe, Germany

[e] Institute of Toxicology and Genetics, KIT, Eggenstein-Leopoldshafen, Germany

[f] Department of Physics, University of Illinois at Urbana-Champaign, Illinois, USA

Experimental Section

Chemicals: Ethanol (C_2H_6O; Roth, Karlsruhe, Germany), chloroform ($CHCl_3$; VWR, Darmstadt, Germany), methanol (CH_4O; Sigma-Aldrich, St. Louis, MO), acetic acid ($C_2H_4O_2$; Sigma-Aldrich), phosphate buffer (PBS; Dulbecco's Phosphate Buffered Saline with or without Ca^{2+} and Mg^{2+}, Life Technologies, Carlsbad, CA), potassium hydroxide (KOH; Sigma-Aldrich) were used as delivered. Water was filtered and demineralized with a Q-POD water delivery unit (Millipore Merck, Darmstadt, Germany). Myristoyl-hydroxyl-phosphatidylcholine (MHPC) was purchased from Avanti Polar Lipids (Avanti Polar Lipids, Alabaster, AL). Stock solutions of stearic acid (SA; Sigma-Aldrich, St. Louis, MO) in $CHCl_3$ (25 mg/ml) and MHPC (10 mg/ml) in $CHCl_3$:CH_3OH (20:1, v:v) were prepared and stored at –24 °C. Human plasma albumin (HSA, product number A8763) and apolipoprotein E3 (ApoE3, product number SRP4696) were purchased as lyophilized powder from Sigma-Aldrich. C3 was prepared from human plasma according to Hammer et al..[1] The concentration of the C3, HSA and ApoE3 protein stock solutions were determined by measuring the absorption using a UV\VIS-spectrometer. Extinction coefficients at 280 nm of 35,258 $M^{-1}cm^{-1}$ for HSA, 180,055 $M^{-1}cm^{-1}$ for C3 and 44,460 $M^{-1}cm^{-1}$ for ApoE3 were used, as calculated online with the program ProtParam (http://web.expasy.org/protparam/), based on the number of aromatic amino acids in the protein molecule.

Synthesis of DHLA- and lipid-QDs: Hydrophobic CdSe/ZnS core/shell QDs were purchased from Life Technologies (product number Q21701MP). DHLA-QDs were prepared by ligand-exchange with freshly prepared DHLA, as previously described.[2]

Lipid-wrapped QDs were prepared by procedures modified from previous reports.[3, 4] The total amount of lipids to encapsulate QDs was calculated by using the surface area of a bare QD and the footprints of MHPC and SA. A lipid excess of 50-fold to the calculated amount was used to ensure sufficient functionalization. All components were equilibrated before each preparation for 30 min at room temperature (RT). As an example, 150 µl QD solution (1 µM, in CH_3Cl), 574 µl MHPC (10 mg/ml) stock solution and 276 µl SA solution (5.2 mg/ml, in CH3Cl), and sonicated for 20 s each before being mixed together with a pipette. The solution was then added drop-wise (20 µl steps) to 2 ml water under vigorous magnetic stirring. The suspension was kept stirring for 1 min and then heated to 85 °C to evaporate the organic solvent from the suspension under gentle stirring. Then, the solution was sonicated at RT for 90 min and centrifuged in an Eppendorf vial for 3 min at 4,300 g. To remove excess lipids and empty micelles, 100 µl of the suspension were carefully added on top of 1 ml 30% (w/v) sucrose (Merck, Darmstadt, Germany) solution in H_2O and spun for 30 min at 57,300 g. A small fluorescent band from the purified lipid-QDs appearing under UV illumination (365 nm) was carefully extracted by using a syringe. Residual sucrose was then removed by repeated centrifugation (19,000 g, 15 min) using Nanosep filters (Pall Life Sciences, Ann Arbor, MI) with a molecular weight cut-off of 10 kDa. The brownish lipid-QDs were re-suspended in water and stored at 4 °C in the dark for later use.

Spectroscopic characterization of DHLA- and lipid-QDs: UV-Vis absorption spectra were recorded on a Cary 100 spectrophotometer (Varian, Palo Alto, CA); fluorescence emission spectra were taken on a Fluorolog-3 Spectrofluorometer (HORIBA Jobin Yvon, Edison, NJ, USA), using excitation at 470 nm. For the measurements, samples were kept in quartz cuvettes (Hellma GmbH & Co KG, Müllheim, Germany) with path length 3 mm.

Dynamic light scattering (DLS), ζ-potential and concentration determination: Both dynamic light scattering (DLS) and laser Doppler anemometry experiments were carried out on a Zetasizer Nano-ZS (Malvern Instruments, Malvern, UK) with a 633 nm He-Ne laser at 25 °C. For DLS, a disposable UV microcuvette (Brand, Wertheim, Germany) was used, and DLS data analysis was based on the number distributions. The measurements were repeated at least three times. ζ-potential measurements were carried out in a high concentration zeta-potential

cell (Malvern) at 20 °C. The concentration of both DHLA- and lipid-QDs, which were synthesized with inorganic cores from the same batch, was calculated from absorption measurements based on an extinction coefficient of 2.8×10^6 $M^{-1}cm^{-1}$ at 405 nm, as quoted by the supplier (Invitrogen, Application note, "Qdot ITK Carboxyl Quantum Dots").

Two-focus fluorescence correlation spectroscopy (2fFCS) measurements: 2fFCS measurements were performed as previously described,[5] with some small modifications. For excitation, we used 488 nm pulsed diode lasers (LDH-D-C-485, PicoQuant, Berlin, Germany), orthogonally polarized to each other. At this excitation wavelength, the distance between the two foci was evaluated at 330 nm, as measured with a reference sample (Atto488, ATTO-TEC, Siegen, Germany). A small pulse frequency (200 kHz) was chosen for the excitation because of the long fluorescence lifetime of the QDs. For good axial confinement of the detection volumes, a pinhole of 100 µm was used. Scattered excitation light was filtered out with a long-pass filter (545LP, AHF, Tübingen, Germany). Due to the strong fluorescence emission of QDs, care had to be taken to avoid optical saturation.[6] With careful calibration experiments, we noticed that optical saturation started to become noticeable for laser powers even below 1 µW, resulting in artificially large diffusion times of the QDs. Consequently, the power was set to 0.2 µW per laser for all 2fFCS measurements reported here. The diffusion coefficient of the QDs was extracted from the correlation functions as previously described,[5] based on a model proposed by Dertinger et al..[7] By using the Stokes-Einstein relation, the hydrodynamic radius, R_h, of the particles was calculated from the diffusion coefficient, D:

$$R_h = \frac{k_B T}{6\pi\eta D}\,, \tag{1}$$

with Boltzmann constant, k_B, temperature, T, and viscosity, η. At high protein concentrations, the viscosity η of the solution needs to be adjusted, based on the viscosity η_0 of water and on the intrinsic viscosity $[\eta]$ of the protein. We used a linear approximation,

$$\eta = \eta_0 \cdot (1 + [Pr] \cdot [\eta]), \tag{2}$$

with the protein concentration [Pr]. This correction was only applied for HSA, with $[\eta]$ = 4.2 $cm^3\ g^{-1}$ (as given by the supplier) because only small concentrations were used for the two other proteins, ApoE3 and C3. Before each measurement, proteins were incubated with QDs for 10 min at RT. For the measurement, the sample solution was injected into a home-built chamber consisting of a channel made of polyethylenglycol (PEG, 5 kDa) coated glass cover slides[8] glued together with double-sided adhesive tape.

Analysis of QD-protein interactions by FCS: Binding of protein molecules to the QDs was quantified by analyzing the size increase induced by the adsorbed protein on the QD surface as a function of protein concentration, as previously reported.[9] Briefly, the protein-coated QDs were taken as spherical objects, and the number of protein molecules bound was modeled by the Hill equation. Thus, the dependence of R_h on the average number N of bound proteins, $R_h(N)$, is

$$R_h(N) = R_h(0)\sqrt[3]{1 + cN}, \tag{3}$$

where $R_h(0)$ is he hydrodynamic radius of the bare QDs, c is a scaling factor equal to the ratio of the volume of one protein molecule to the volume of one bare QD, and N is the (average) number of adsorbed proteins,

$$N = N_{max}\frac{1}{1 + (K_D^{'}/[P])^n}. \tag{4}$$

Here, N_{max} is the maximum number of protein molecules that fit into the adsorption layer at saturation, K'_D is the apparent dissociation coefficient, *i.e.*, the free protein concentration at which, on average, $N_{max}/2$ protein molecules are bound on each QD. n is the Hill parameter which describes the cooperativity of the binding process, and $[P]$ is the free protein concentration. We worked at very low QD concentration (typically 0.1 to 1 nM) to ensure that the protein was in large excess in all our measurements, so that we could assume in our analysis that the known overall concentration of protein molecules provides a good approximation to the concentration of protein free in solution, which is needed in the analysis.[10]

Calculation of free protein concentration: ApoE3 and C3 have such a high binding affinity to DHLA-QDs that it is difficult to work with large protein excess in the QD-protein binding experiments. To ensure that the free protein concentration $[P]$ as used in equation (2) can still be set approximately equal to the known total protein concentration, we performed the following control calculations. First, for each protein concentration measured, we estimated the maximum and minimum number of proteins bound per QD based on the two (volume/surface) models as explained in the main text. The effective number of proteins bound per QD was taken as the average of these two extremes. Based on the known QD concentration, we then calculated the free protein concentration, *i.e.*, total protein minus

bound ones. Plots of the measured hydrodynamic radius as a function of free and total protein concentrations are shown in **Figure S3**. For C3, a slight shift of the binding curve toward lower concentration is visible when considering the "real" free protein concentration. This leads to a slightly different apparent dissociation coefficient of 0.015 ± 0.001 μM instead of 0.012 ± 0.001 μM. The Hill coefficient showed a minimal change (within the error) from 2.1 ± 0.2 to 2.2 ± 0.2. For apoE3, changes were completely negligible. Thus, our assumption that the concentration of total and free proteins is identical within the error is entirely valid.

Estimation of the number of protein molecules adsorbed onto QDs: In the experiment, we measure the diffusion coefficient, which is related to the variable, R_h, by the Stokes-Einstein relation (Equation 1). At the level of analysis used here, diffusion is taken as isotropic, although it is clear that neither the bare QDs nor the QDs with adsorbed proteins are strictly spherical. However, even for spherical QDs and only a few proteins bound, this is still an excellent approximation, especially if the protein molecules are smaller than the QDs, because diffusion is rather insensitive to deviations from sphericity. For example, let us take QDs and proteins as spheres of the same hydrodynamic radius, *r*. The very anisotropic complex with the configuration of a linear chain consisting of 1 protein/1 QD/1 protein would have a hydrodynamic radius of 1.71 *r*, as calculated theoretically,[11] while our model (Equation 3) would yield 1.44 *r*. Thus, the relative error would be 16%. In a less anisotropic configuration, with the two proteins bind to the QD to form a triangle, the theoretical hydrodynamic radius would be 1.61 *r*, corresponding to 10% error of our model. Note that these are extreme configurations, the more proteins bound, the less anisotropic the QD-protein complex is.

The parameter N_{max}, however, as introduced in Equation 3, is a derived quantity, equivalent to the number of protein molecules that fit into the volume attributed to the adsorption layer. Therefore, this number is an upper limit because the space may not be completely filled by protein molecules because their shapes may be incommensurate with tight packing. Also, hydration water is likely to accompany the proteins and fill voids. Alternatively, a lower bound on N_{max} may be obtained by dividing the available surface on the NP by the "footprint" of the protein molecule, i.e., the face with which it interacts. Naturally, this estimation requires knowledge (or at least educated guesses) regarding structural details of the interaction. In table 1, estimations of N_{max} are given, based on these two models. In a previous

study,[9] we presented yet another estimate by relating the surface of a sphere with radius $R_h(0) + \Delta R_h/2$ to the size of the protein.

The properties of the three proteins were obtained as follows. For HSA, a volume of 96.2 nm^3 was used, describing the protein by a equilateral triangular prism, with a side of 8.4 nm and a height of 3.2 nm.[12] For ApoE3, a protein volume of 125 (= 6.5 × 2.5 × 7.7) nm^3 was calculated. 6.5 nm and 3.8 nm are the dimensions of the four-helix bundle as determined from the structural model (pdb accession code 2kc3) with the program Pymol (The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC), and 7.7 nm is the dimension perpendicular to the helix bundle which we take equal to the thickness of the measured ApoE3 corona minus 0.5 nm to account for the loose tertiary structure of the two C-terminal helices. For C3, a volume of 510 (= 4 × 15 × 8.5) nm^3 was used, as obtained from the x-ray structure (pdb accession code 2a73) using Pymol.[13]

Figures

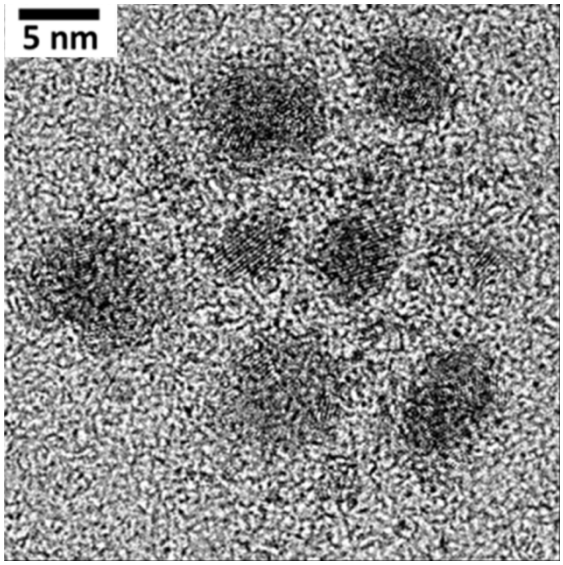

Figure S1 Typical TEM images of osmium stained lipid-QDs. The average diameter of the QDs determined from the image equals 6.2 ± 1.0 nm.

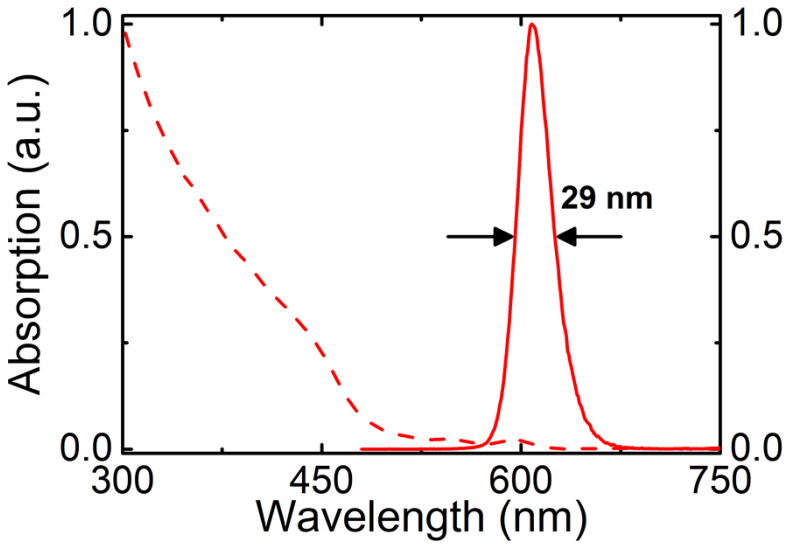

Figure S2 Absorption (dashed line) and emission (solid line) spectrum of DHLA-QDs in PBS. The emission spectrum was measured by exciting the QDs at 470 nm, the full width a half maximum of the emission band is also shown.

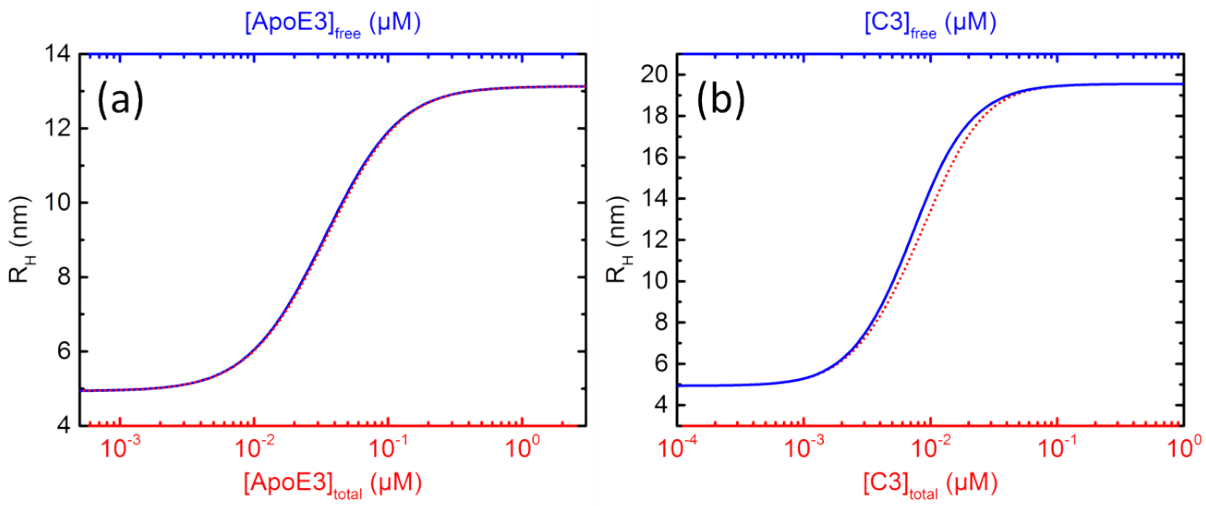

Figure S3 Hydrodynamic radius of DHLA-QDs measured as a function of the (a) apoE3 concentration and (b) C3 concentration. The data are those of the fits shown in Figure 2. The red dot lines represent the QD radius plotted as a function of the total protein concentration, the blue line as a function of the free protein concentration.

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