Quantifying the Surface Coverage of Conjugate Molecules on Functionalized Nanoparticles

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Here we present a method to determine the surface coverage or surface density of biological molecules conjugated to nanoparticle surfaces. Electrospray-differential mobility analysis (ES-DMA) is used to determine a coating thickness by measuring the change in the size of gold nanoparticles before and after modification with thiol-derivatized single-stranded DNA. The DNA surface coverage is then obtained from the coating thickness through the use of a simple random coil model. The method requires neither fluorescent tagging nor calibration curves. We believe ES-DMA to be a broadly applicable nanometrology tool for the characterization of biologically conjugated nanoparticles.

This letter addresses a key problem in nanoscience and nanotechnology: how to determine the molecular surface coverage of nanoparticles derivatized with organic or biological molecules, particularly those without a fluorescent tag. Many approaches to functionalize engineered particles have been developed, but characterizing the number of moieties dotting the surface of a nanoparticle remains a critical technical challenge and a major barrier to commercial development. For example, measuring surface coverage will be key to the development, manufacturing, quality control, and regulatory approval of nanobiomaterials for therapeutic use. Additionally, surface coverage measurements will aid quantitative understanding of the results from nanoparticle sensors, enable engineering of particles for energy applications, and facilitate nanoparticle toxicology studies. Here we demonstrate the use of electrospray-differential mobility analysis (ES-DMA) to determine the surface coverage of semiflexible molecules on nanoparticles by measuring changes in particle size.

To demonstrate our approach, we select gold nanoparticles coated with single-stranded DNA (ssDNA), which hold potential for a variety of uses ranging from cancer treatments to genetic toxicology studies. We then develop a simple random coil model to approximate the projected area and volume occupied by an individual strand. The surface coverage then follows from the molecular footprint.

In differential mobility analysis, also referred to as gas-phase electrophoretic mobility molecular analysis (GEMMA), a particle suspension must be conveyed first into the gas phase. Electrospray ionization suits this purpose well, producing a narrow distribution of droplet diameters. The droplets pass through a neutralizing chamber where collisions with charged ions reduce the charge to a modified Boltzmann distribution. Consequently, the particles analyzed in the DMA carry predominantly a single net positive charge. As the droplets containing a gold nanoparticle dry, any salts or other nonvolatile impurities encrust the surface. As only a small fraction of droplets contain gold particles, most particles entering the DMA are simply dried salts. Within the analysis chamber, charged particles are attracted to a center electrode while being dragged along by a carrier gas. Particles for which the radial electrical force balances the radial drag force pass through a collection slit in the center electrode. The drag force in the free molecular regime depends on the charged particle counter enumerates the number of particles passing through the detector per cubic centimeter of gas flow. Stepping through the voltage yields a particle size distribution. In this manner, particles with diameters greater than 3 nm may be sized with high precision. For example, for nominally 10 nm Au nanoparticles, the standard deviation of the number-average diameter is ± 0.1 nm.

Figure 1 displays two ion mobility spectra: one (red) acquired from a solution containing bare Au nanoparticles nominally 20 nm in diameter and the other (blue) from a solution of 20 nm Au nanoparticles coated with thiol-modified ssDNA [(dT)$_{20}$-SH], where dT represents deoxythymine and the subscript denotes the number of bases per strand. Typical ion mobility spectra display two peaks. The first set of peaks (<10 nm) corresponds to salt particles that result from the drying of droplets not containing Au particles. Their location depends on the concentration of nonvolatile salts present in the droplets prior to drying, which can vary modestly with sample preparation. The second set of peaks (>15 nm) represents Au particles, one bare and the other modified with (dT)$_{20}$-SH, both encrusted with any nonvolatile salts. Because droplets with and without a gold particle have the same initial size, the thickness contributed by
nucleotides per strand, \(N\), similar to the work of Kaufman.9 The narrow distribution in the volume of the salt particle from that of the nanoparticle the shells of dry nonvolatile salts can be removed by subtracting \(H\) represents the number of bases per strand, \(N_b\), and the salt concentration, \(n_s\). The dependence of the coating thickness on the number of bases per strand is related to the spatial configuration of the bases within the strand in the dry state. If the strands pack together tightly in a brush structure, similar to alkanethiol self-assembled monolayers, then the bases will extend into configurations that minimize interstrand repulsion. Accordingly, the coating thickness should be proportional to the linear end-to-end distance, \(<x^2>^{1/2}\), of a strand. For freely jointed Gaussian chains, \(<x^2>^{1/2} = cN_b^{1/2}l_b^{1/2}\), where \(N_b\) represents the number of bases per Kuhn length \((l_b = N_b/(6c))\), \(l_b\) describes the length of a base, and \(c = 0.62\) for end-tethered strands on a hard sphere with minimal surface attraction [appropriate for oligo(dT) strands on gold],10 though a variety of prefactors remain available to account for surface—strand interactions.11 Accordingly, we expect \(H \sim N_b^{1/2}\), and indeed Figure 2 shows square root scaling to fit the data well. We thus conclude that the strands adopt a random coil configuration on the nanoparticle surface.11–13 Consequently, the coating does not meet the formal requirements for a polymer brush, though the DNA community often refers informally to these surface coatings as brushes.14

Knowing the configuration of the strands enables estimation of the surface coverage. Here we note that while the structure of the strands may change upon drying, the surface coverage does not. The drag force experienced by the coated particle in the DMA depends on the diameter of the particle, \(D\), the projected area of the coiled strands characterized by \(<x^2>^{1/2}\), and the surface coverage, \(\sigma\). Knowing \(D\) and \(<x^2>^{1/2}\) allows inference of \(\sigma\) from the data with a suitable model. We derive an analytical expression for the drag force for a “lumpy sphere” model in which the ssDNA occupies \(N_m\) hemispherical caps of radius \(<x^2>^{1/2}\), where \(N_m\) represents the number of caps around the two dimensional projection of the particle’s circumference (see Supporting Information).15 The apparent coating thickness, \(H\), determines \(N_m\), which gives a measure of a strand’s footprint. The surface coverage, consequently, may be approximated as \(\sigma = ((D + H)^2 - D^2)/[2\pi D <x^2>^2]^{1/2}\). Using this model, we find \(\sigma\) ranges between 2.0 \(\times\) \(10^{12}\) and 6.9 \(\times\) \(10^{13}\) strands/cm\(^2\) as displayed in Figure 3, assuming \(l_b = 0.59\) nm and \(N_b = 3\) \((l_b = 1.8\) nm) for \(N_b = 10 – 30\) bases.16,17 By comparison, “brushes” prepared under similar conditions have reported coverages from 1.0 \(\times\) \(10^{13}\) to 2.0 \(\times\) \(10^{13}\) strands/cm\(^2\) for 12 mers on nanoparticles and 4.0 \(\times\) \(10^{13}\) to 2.5 \(\times\) \(10^{13}\) strands/cm\(^2\) for 25 mers on planar substrates in reasonable agreement with the values derived from our model.3–5,18

An alternative approach approximates the surface coverage if the bulk density is known. Here the number of strands on the particle equals the ratio of the volume of the shell containing the DNA, \(\pi[(D + H)^3 - D^3]/6\), to the volume of an individual strand, \(m_bN_b + m_b]/(N_b\rho_{av})\), where \(\rho_{av}\) is Avogadro’s number, \(\rho_{av}\) is the average bulk density from DNA on planar surfaces, \(m_b\) is the molecular weight of a base, and \(m_b\) is the molecular weight of the thiol linkage. Dividing the number of strands by the surface area yields \(\sigma = N_b\rho_{av}/[(D + H)^3 - D^3]/[6D^2(m_bN_b + m_b)]\). With \(\rho_{av}\) of 0.89 to 1.3 g/cm\(^3\) reported for (dT)\(_{32}\)SH on a planar gold film by Petrovykh et al., \(m_b = 304\) g/mol, \(m_b = 134\) g/mol for the six carbon thiol linked, and \(N_b = 20\) or 30 bases, we find \(\sigma = 9.0 \times 10^{12}\) to 6.4 \(\times\) \(10^{13}\) strands as compared to \(\sigma = 2.0 \times\) \(10^{12}\) to 4.0 \(\times\) \(10^{13}\) strands/cm\(^2\) for the same
conditions with the previous method. Thus, where the bulk density on similar surfaces prepared at similar salt concentrations is known this alternative approach may find utility, while the previous method may be more useful where Kuhn or persistence lengths are known.

The relationship between the surface coverage and the salt concentration, \( n_s \), of the solutions used for ssDNA adsorption is shown in Figure 3. Scaling the surface coverage with the molecular footprint of a nonoverlapping strand collapses the data onto a single curve in Figure 4 and emphasizes the importance of the Debye length, \( \lambda_D \), to the Kuhn length, \( \lambda_k \), where \( \sigma^* \) is inversely proportional to the molecular footprint of an individual, nonoverlapping strand, \(
\langle x^2 \rangle^{-1} \). The line represents a best fit of \( \alpha/\sigma^* = 0.82(\alpha_D/\lambda_k)^{-1.7} \).

![Figure 4](image)

**Figure 4.** Scaled surface coverage, \( \sigma/\sigma^* \), versus the ratio of the Debye length, \( \lambda_D \), to the Kuhn length, \( \lambda_k \), where \( \sigma^* \) is inversely proportional to the molecular footprint of an individual, nonoverlapping strand. The line represents a best fit of \( \alpha/\sigma^* = 0.82(\alpha_D/\lambda_k)^{-1.7} \).

Kaufman adequately accounts for the presence of the salt, despite “crusts” ranging from approximately 0.1 to 1.2 nm in thickness. However, we cannot distinguish with this technique whether the crust lies on the top of the strands, at the base of the strands, or uniformly coats the strands, though the latter seems more likely.

In summary, these results indicate the potential of ES-DMA to quantify the coverage and configuration of biological molecules and organic coatings on nanoparticles. We considered the specific example of thiol-modified ssDNA and find it adopts a random coil configuration. We believe the analytical model to be valid generally for sufficiently long and flexible molecules (i.e., where \( N_0 \gg N_N \)).

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**Supporting Information Available:** Details of the sample preparation and data analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

**References and Notes**