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Lanthanide-Based NMR: A Tool To Investigate Component Distribution in Mixed-Monolayer-Protected Nanoparticles

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Supporting Information

ABSTRACT: Gd^{3+} ions, once bound to the monolayer of organic molecules coating the surface of gold nanoparticles, produce a paramagnetic relaxation enhancement (PRE) that broadens and eventually cancels the signals of the nuclear spins located nearby (within 1.6 nm distance). In the case of nanoparticles coated with mixed monolayers, the signals arising from the different coating molecules experience different PRE, depending on their distance from the binding site. As a consequence, observation of the signal broadening patterns provides direct information on the monolayer organization.

The huge interest attracted by nanoparticles (NPs) stems not only from their size-related properties but also from their highly self-organized nature.[1] Indeed, the synthesis of a NP is just the spontaneous self-assembly of very simple precursors or building blocks to form highly complex structures, where the reaction conditions and/or the properties of the building blocks govern their structural features. Increasing evidence indicates that, in the case of NPs protected by a monolayer of organic molecules, control of the system organization can be extended from the nanocrystal core to the monolayer itself. Defined motifs formed by the coating molecules such as stripes, patches, or large domains have been observed.[2] In principle, upon acquiring the ability to control such monolayer organization, it will be possible to design sophisticated arrays and patterns of functional groups on the surface of the NPs. The potential of such topologically controlled monolayers in every field where NPs are applied, from nanomedicine[3] to catalysis[4] and materials development,[5] is virtually endless.[6]

Controlling the assembly of molecules on the surface of a NP requires suitable and accessible methods to study the monolayer organization. Obviously, to place the coating molecules where we want them to be, we must be first able to determine where they actually are. So far, this has proven to be not an easy task. Early observation of stripes by Stellacci and co-workers relied upon sophisticated scanning tunneling microscopy measurements,[7] which however are restricted to particular combinations of coating molecules with considerably different lengths.[8] Other methods based on radical probes and electronic paramagnetic resonance,[9] NPs' reactivity,[10] or analysis of multivalent substrate binding[11] or catalysis[11,12] have been proposed. Most such approaches, however, provide indirect information that must be interpreted on the basis of some model.

NMR spectroscopy is a powerful tool for the investigation of the structure and morphology of organic molecules and proteins. In the case of NPs, however, it has been seldom applied to structural investigations,[13] being mainly relegated to qualitative characterization and purity assessment.[14] In this paper we report a simple and direct method to investigate monolayer structure by the combined use of NMR spectroscopy and lanthanide ion probes.

Paramagnetic lanthanide ions are largely used in the context of NMR for their ability to induce paramagnetic relaxation enhancement (PRE).[15] This effect causes the signals of the resonant spins surrounding the metal ion to broaden and eventually disappear from the spectrum. On this basis, PRE has been exploited in structural studies of metal-binding proteins, basically by determining which signals (or nuclear Overhauser effects) are canceled in the NMR spectrum of the protein upon addition of paramagnetic metal ions.[16]

Similarly, binding a lanthanide ion to the surface-coating monolayer of a NP should cause the signals arising from the nearby molecules to disappear from the NMR spectrum. As such, different morphologies of the coating monolayer would result in different broadening patterns of the spectra, thus providing direct information on the monolayer organization. To investigate such a hypothesis, we prepared 1.8-nm-core diameter gold NPs ($Au_{180}SR_{70}$, see SI) coated with different relative amounts of the thiols depicted in Figure 1, and studied the broadening of their ^1H NMR signals upon addition of Gd^{3+} ions.

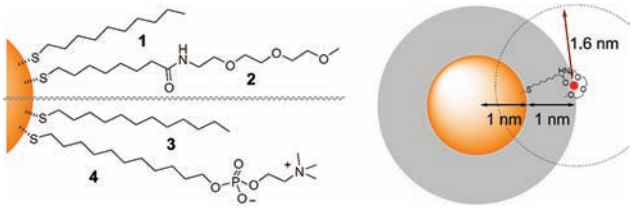

Figure 1. (Left) Nanoparticle-bound coating thiols used in this work. (Right) Schematic Gd^{3+} (red dot) binding site for thiol **2**-coated NPs (only one thiol is represented for simplicity, but cooperative binding by multiple thiols is likely to occur), and metal ion "quenching radius" (dashed line).

Thiol combinations **1**-**2** and **3**-**4** (Figure 1) were selected on the basis of the following considerations: (i) they are standard

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molecules used in many studies on monolayer-protected AuNPs; (ii) they have substantially different affinities for Gd^{3+} ions, with PEG derivative **2** and phosphorylcholine derivative **4** providing binding sites at the carbonyl, ether, or phosphoryl oxygen atoms, while alkyl thiols **1** and **3** are unable to interact with Gd^{3+}; and (iii) they provide clearly distinguishable NMR signals.

It is known that the binding affinity of carbonyl and ether oxygens (see thiol **2**) toward metal ions is quite low. However, the use of non-coordinating organic solvents as $CDCl_3$ amplifies the interaction to such an extent that, when Gd^{3+} ions are added as a triflate salt to the solution of NPs coated with thiol **2** (at 5 mM concentration of **2** units), the signals of **2** broaden from the very first addition of Gd^{3+}, and they eventually disappear when the concentration of metal ions is ~0.5 mM (Figure 2). In contrast, the signals of the residual solvents and TMS are not significantly broadened up to millimolar Gd^{3+} addition. PRE can result from both an *inter*molecular and an *intra*molecular mechanism, the first being long-ranged (with $1/r$ dependence) and the second being short-ranged (with $1/r^6$ dependence).[17] In this respect, signal broadening only for the NP-bound species indicates that the metal ions bind to the monolayer strongly enough not to induce *inter*molecular PRE on the free molecules in the solution.[18] Likely, this is a consequence of the crowding of functional groups on the particle surface which provide self-organized multidentate binding sites for the metal ions.[19,20] The data reported in Figure 2 also confirm that the intramolecular PRE driven by the lanthanide ion is strong enough to cancel not only the signals of the protons close to the binding site but also those of the inner alkyl portion of **2** ($-CH_2-$ signal at 1.2 ppm) and even of **2** units not bound to the metal ion. In fact, the complete broadening of the monolayer signals occurs when the concentration of the metal ion is only one-tenth that of **2**, indicating that the Gd^{3+} ions bound to the particle surface exert their PRE effect on a large number of surrounding coating molecules. Extrapolation of the linear part of the plot of the signal intensity[21] as a function of the Gd^{3+} ions/particles ratio (Figure 2B) reveals that as few as six metal ions are sufficient to quench the signals of the whole NP.[22] Thus, each surface-bound Gd^{3+} induces the broadening of ~12 surrounding thiols in the monolayer. Given that the radius of the NP, including the monolayer, is ~2 nm (Figure 1),[23] it is possible to estimate that a single Gd^{3+} ion bound to a **2**-coated particle has a "quenching radius", i.e., the distance below which the signals are broadened enough to become undetectable, of ~1.6 nm. Such a value is in accordance with that of 1.5 nm calculated for an 80-Hz line broadening (see SI) in the case of intramolecular relaxation (dipolar and Curie), where the relevant correlation times are 4.4 ns for the NP rotation (in $CDCl_3$ at 298 K) and 10.0 ns for the Gd electron relaxation.[17b] Similar results were obtained by titrating **2**-coated NPs in CD_3OD (but not in D_2O, where no Gd^{3+} binding occurs) or phosphorylcholine derivative **4**-coated NPs in both CD_3OD and D_2O (see SI).[24] Investigation of larger NPs (see SI) coated with **2** indicates that the method can be applied up to 3-nm core diameter, since at larger sizes broadening of the NMR signals prevents their identification.

When an equimolar mixture of NPs coated respectively with 100% **1** or 100% **2** (Figure 3) is studied, signals due to thiol **2** quickly disappear from the spectrum, but contrary to the previous case, not only the residual solvent signals (see SI) but also the signals arising from **1**-coated NPs are unaffected up to high Gd^{3+} concentrations, confirming the strong binding of the metal ion to the monolayer made of thiol **2**. In summary, the above experiments show that signal broadening induced by PRE follows quite different patterns depending on whether the NPs are passivated with thiols capable (**2**) or incapable (**1**) of binding Gd^{3+}, and that PRE is strong enough to allow the exploration of a substantial portion of the surrounding space.

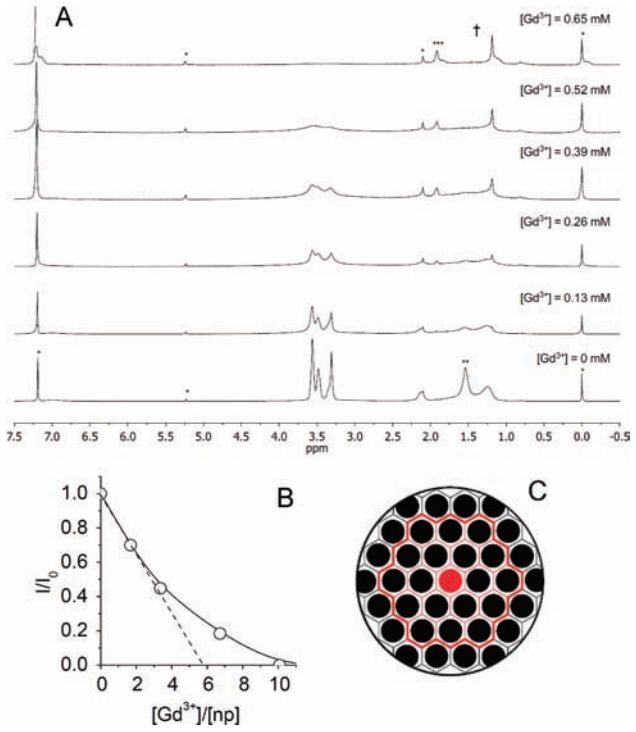

Figure 2. (A) ^1H NMR spectra of **2**-coated gold nanoparticles in $CDCl_3$ ([**2**] = 5 mM, [NP] = 0.07 mM) recorded upon addition of increasing amounts of $Gd(CF_3SO_3)_3$ in CD_3CN solution (*, residual solvents and TMS; **, water; ***, CH_3CN; †, Gd^{3+} impurity). (B) Relative intensity of the thiol **2** signal at 2.1 ppm as a function of the Gd^{3+} ions/particles ratio (solid line, data trend; dashed line, linear extrapolation of the first points). (C) Schematic interpretation of the experiment: filled hexagons indicate the thiols **2** in the monolayer, the red circle the Gd^{3+} ion, and the bold red profile its approximate "quenching area" where the signals are canceled.

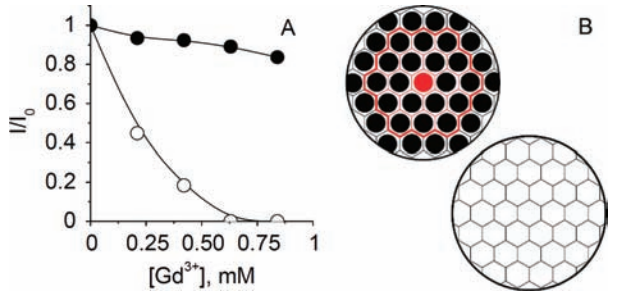

Figure 3. (A) Relative intensity of signals from thiols **1** (●, peak at 0.8 ppm) and **2** (○, peak at 2.1 ppm) in an equimolar mixture of **1**-coated and **2**-coated gold nanoparticles in $CDCl_3$ ([**1**] = [**2**] = 5 mM, [NP] = 0.07 mM each) as a function of the Gd^{3+} concentration (solid lines, data trend). (B) Schematic interpretation of the experiment: filled and empty hexagons indicate respectively the thiols **2** and **1**; Gd^{3+} ion binds only to the NPs coated with thiol **2**, canceling their signals.

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We are hence in the process of using this tool to map the distribution of thiols in NPs with mixed monolayers.

Thus, we titrated with Gd^{3+} a $CDCl_3$ solution containing NPs coated with a *mixed* monolayer made by thiols **1** and **2** in a 50:50 ratio (Figure 4). Here, the effect of Gd^{3+} addition is

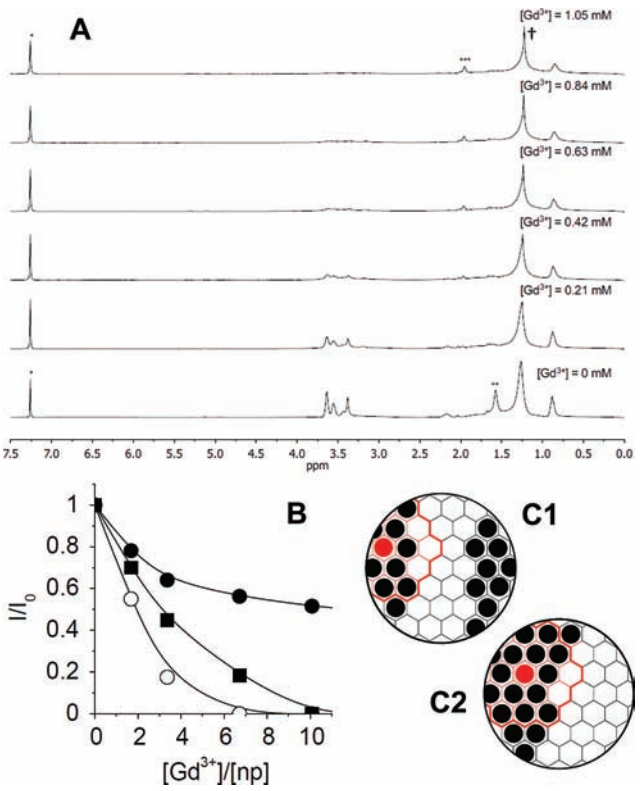

Figure 4. (A) ^1H NMR spectra of a $CDCl_3$ solution of gold nanoparticles coated with a mixed monolayer of thiols **1** and **2** in a 50:50 ratio ([**1**] = [**2**] = 5 mM, [NP] = 0.14 mM) recorded upon addition of increasing amounts of a CD_3CN solution of $Gd(CF_3SO_3)_3$ (*, residual solvents; **, water; ***, CH_3CN; †, Gd^{3+} impurity). (B) Relative intensity of signals from thiols **1** (●, peak at 0.8 ppm) and **2** (○, peak at 2.1 ppm) as a function of the Gd^{3+} ions/particles ratio (lines, data trend; ■, data from Figure 2B for 100% **2**-coated NPs). (C) Schematic interpretation of the experiment: Gd^{3+} ions (red circles) bind to thiols **2** (filled hexagons), which are grouped in patches on the surface of the NPs; as a consequence, only signals from thiols **2** are canceled, while thiols **1** are marginally affected.

completely different from the previous case. Signals of both thiols **1** and **2** experience considerable broadening; signals due to the PEG-thiol **2** eventually disappear from the spectrum, while signals due to alkyl thiol **1** (diagnostic is the one arising from the terminal methyl at 0.8 ppm) are still visible at the highest lanthanide ion concentration. This proves, on one hand, that thiols **1** and **2** are bound to the same NP (otherwise signals of thiol **1** would behave as in Figure 3A), but it also indicates that ~50% of thiols **1** are unaffected by the presence of Gd^{3+} bound to **2**. The initial decrease of the intensity of signals related to **2** is such that they would be completely canceled with just four metal ions bound to each NP (○, Figure 4B), compared to the six ions required for a NP coated with 100% thiol **2** (■, Figure 4B). Such a trend is confirmed when the experiment is repeated on a NP coated with a 75:25 mixture of thiols **1** and **2** (see SI): in this case the number of Gd^{3+} ions per NP required to cancel the signals of all thiols **2** further reduces to two. Similar results are obtained in CD_3OD (see SI).

Such evidence indicates that, in the mixed monolayer, the two thiols cannot be randomly dispersed. In such a case, the signals of both would broaden with similar trends and eventually disappear upon addition of ~6 Gd^{3+} ions per NP, as in the case of the NPs coated with 100% **2**. The need of only 4 Gd^{3+} ions per NP to fully broaden the signals of thiol **2** in the 50% NPs indicates that the most likely arrangement is one in which up to four large patches of **2** are formed on the monolayer (Figure 4C). The partial broadening of the signals of thiol **1** is due to the fact that the quenching radius of **2**-bound Gd^{3+} encompasses thiols **1** located at the boundaries of the patches. It is important to note that, even at high Gd^{3+} concentration, the **1** signals are not completely canceled. Geometrical calculations were carried out to assess the surface area covered by Gd^{3+} ions when saturating the patches of **2**, and hence affecting the maximum amount possible of **1** signals. Results obtained indicate that in the "four patches" scenario (Figure 4C1) all the **1** signals would be canceled at the end. Hence, the only thiols arrangement compatible with the results obtained is the "single cluster" or "janus" distribution (Figure 4C2).[25]

The different features of alkyl thiol **3** and phosphorylcholine thiol **4** with respect to **1** and **2** provided a negative control for the above clustering results. Indeed the titration with Gd^{3+} of NPs coated with a mixed monolayer of **3** and **4** in a 50:50 ratio (Figure 5 and SI) reveals that signals arising from both thiols

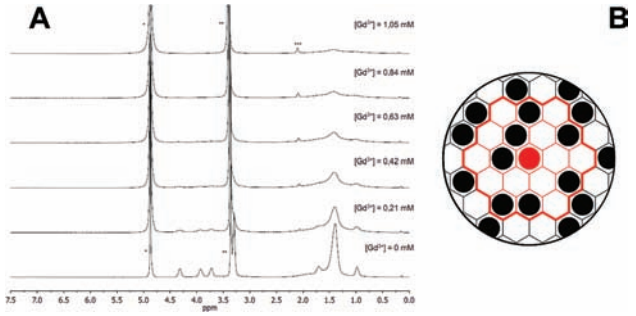

Figure 5. (A) ^1H NMR spectra of a CD_3OD solution of gold nanoparticles coated with a 1:1 mixture of thiols **3** and **4** ([**3**] = [**4**] = 5 mM, [NP] = 0.14 mM) recorded upon addition of increasing amounts of a CD_3CN solution of $Gd(CF_3SO_3)_3$ (*, methanol −OH; **, methanol $-CHD_2$; ***, CH_3CN). (B) Schematic interpretation of the experiment: Gd^{3+} ions (red circles) bind to thiols **4** (filled hexagons) on the surface of the NPs, canceling the signals of both thiols **3** and **4** that are randomly distributed.

are canceled from the spectrum upon Gd^{3+} addition. Hence, the distribution of the thiols on the surface of these NPs is such that all thiols **3** are close enough to thiols **4** to experience the broadening effect of a **4**-bound Gd^{3+} ion. This is the behavior expected for a random (Figure 5B), stripes, or very small patches distribution (all such motifs are indistinguishable with this method). Such results also confirm that the binding of the lanthanide probe does not cause the reorganization of the monolayer, at least on the time scale of the NMR experiments.

Remarkably, this finding of a different monolayer organization is also independently confirmed by the results of nuclear Overhauser effect (NOE) experiments (see SI). In fact, a weak negative NOE between the two coating molecules was detected in the NPs coated with thiols **3** and **4**, but none was observed in

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the NPs coated with **1** and **2**. This is in full agreement with the random distribution suggested by the Gd^{3+} experiment for NPs coated with **3** and **4**. In fact, such an organization brings a larger amount of different thiols close to each other (Figure 5B), and this enhances the probability to detect interchain NOE. The situation is reversed in a large patches distribution, where the different thiols come in close proximity only on the patches' boundaries (Figure 4B).

In conclusion, we have demonstrated how a paramagnetic lanthanide ion can act as a probe for the direct determination of the local organization of small nanoparticles coated by organic monolayers, provided that proper binding sites are present on the particle surface. Our data lend further support to the idea that the monomers that passivate gold nanoparticles can, under certain conditions, self-organize to form defined patterns. Whether such organization is controlled by the chemical structure of the thiols, their mutual interaction, the synthetic procedure, or the NPs size is a very intriguing question[8,12b,26] that the method here reported will contribute to answer, once a larger collection of data are analyzed. We are currently working to improve this approach with the aim of obtaining a detailed map of the monolayer and possibly detecting sophisticated surface patterns.

■ ASSOCIATED CONTENT

Supporting Information

Synthesis and characterization of the organic compounds and nanoparticles; additional NMR experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(21) All the signals arising from **2** follow a similar intensity decrease trend (see SI).

(22) Upon the assumption that each metal ion cancels all the signals arising from species within its "quenching radius", the signal intensity of the NP-grafted species must decrease, after each metal ion binding, by a quantity proportional to the fraction of the NP surface covered by the quenching area. The deviation from the linear trend at high Gd^{3+} concentrations is due to the partial overlapping of the quenching areas.

(23) Such value is obtained by adding 1 nm for the metal core and 1 nm for the distance of the binding site from the particle surface.

(24) For NPs coated with **2** in CD_3OD and with **4** in CD_3OD and D_2O, only 4.5 Gd^{3+} ions are needed to cancel the NMR signals. This indicates a larger quenching radius of Gd^{3+} (1.9 nm), likely because of its different relaxivity in these solvents. See ref 17.

(25) With the term "janus" here we mean two phase-segregated domains on each particle, which can be of the same size on each particle (limiting situation) or different sizes form particle to particle.

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