Self-Assembled Monolayers

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Formation of Patches on 3D SAMs Driven by Thiols with Immiscible Chains Observed by ESR Spectroscopy**

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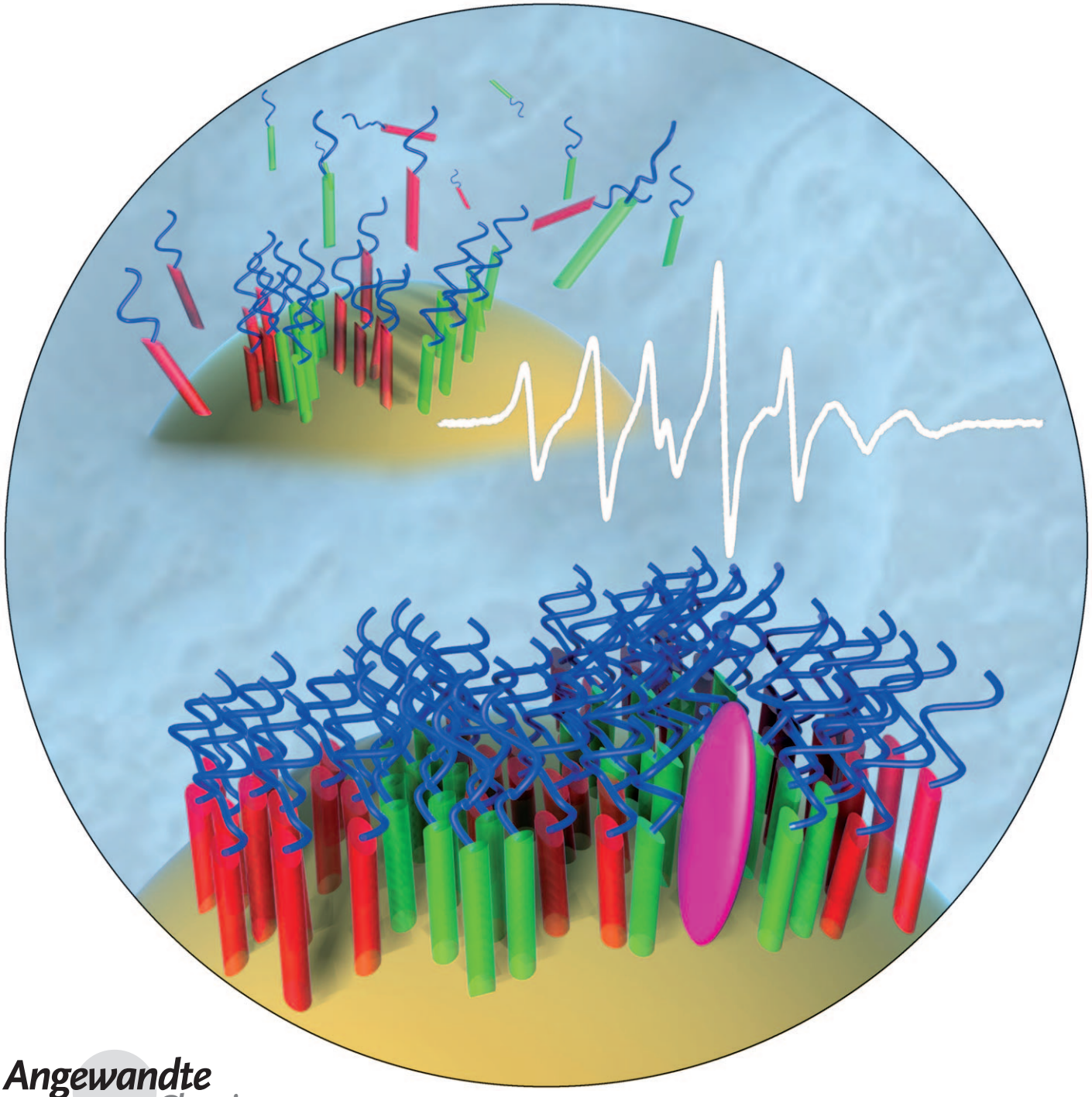

Ligands of different natures need "instructions" to self-assemble with specific morphology on the surface of metal nanoparticles. The disclosure of the principles behind the spontaneous formation of anisotropic monolayers extends the capacity to design and fabricate new nanoparticles and to use them as building blocks for innovative materials.[1] Self-assembled monolayers (SAMs) on gold nanoparticles are ideal models for these studies for several reasons: They are the most investigated examples of self-assembled monolayers in three dimensions (3D SAMs); they may be prepared with reproducible procedures in a variety of dimensions and shapes; and their properties can be compared with those reported for 2D SAMs made of thiols assembled on a flat gold surface.[2] Moreover, gold nanoparticles protected by thiolates are extremely interesting per se because they combine the properties of a metallic gold core in the nanoscale regime with stability greater than that of other metal nanoparticles as well as biological compatibility.[3–6]

Tailored procedures are available to build 3D SAMs composed of more than one type of ligand having different functional groups (heteroligand functional monolayers), and this enables diverse applications in many fields such as materials science,[7–9] biology,[10–12] and medicine.[13,14] In particular, spatially close functional groups may cooperate to catalyze a specific process[15] or for multivalent (or polyvalent) recognition,[16–19] and are essential for the preparation of new materials.[20–22] Functional thiols are generally present in monolayers without a specific topological organization. This, of course, calls attention to the need for a controlled spatial confinement of different ligands in mixed monolayers (monolayers composed of mixture of different ligands) wrapped around nanoparticles.[1]

Strategies for the segregation of different thiolates on 3D SAMs have been reported; these include the use of geometric restriction templates and the response to the solvating properties of the surrounding media.[23–25] Clustering of thiols by simple self-assembly of the ligands on the surface of the gold nanoparticle is a much more appealing approach because it is synthetically less demanding and the resulting nanoparticles are generally very stable. A very elegant investigation concerning the self-organization of different thiols on 3D surfaces has been recently reported by Stellacci and his group.[26] As a result of the competition between enthalpic losses and entropic gains at phase boundaries, stripe-like patterns are formed by thiols of different lengths coadsorbed on the surface of gold nanoparticles which have a core diameter ranging from 2.5 to 8.0 nm.[27] This specific type of organization determines new properties of the nanoparticles[28] and opens the way to new applications.[29]

However, many applications, as described before, require or prefer monolayers that are composed of islands where functional ligands are segregated from (inexpensive) "neutral" ligands (i.e. ligands that do not present functional groups like alkylthiols or thiols containing a poly(oxoethylene) chain) rather than homoligand functional monolayers (all the ligands present the functional moiety) or homogeneous mixed monolayers, which are a random mixture of functional and "neutral" ligands. An example in this direction has been recently reported for a mixture of CALNN pentapeptide and $<2.1\,\%$ of a 20 amino acid peptide containing biotin on colloidal gold particles 10 nm in diameter.[30]

To address this issue we reasoned that a blend of perfluoroalkyl and alkyl amphiphilic thiolates should form domains on the nanoparticle surface and self-organize in a way to minimize the contact surface between the two types of thiols because of the reciprocal phobicity; that is, patches should form. Indeed, the combination of the extreme hydrophobicity and lipophobicity of the perfluorinated chains promotes phase separation in molecular systems containing a mixture of fluorinated and hydrogenated surfactants. Examples include segregated micelles,[31] liposomes,[32] and Langmuir monolayers.[33]

Here we present an ESR spectroscopy study aimed to probe ligand organization of 3D mixed monolayers composed of the thiols HS-C8-TEG (**1**) and HS-F8-PEG (**2**; see Scheme 1); the radical probe **3** is used to "sense" the hydrophobicity of the monolayer. The thiols present a short polyethylene chain (3 oxoethylene units for **1** and 12 or 13 units for **2**) to guarantee solubility of the thiols and of the final nanoparticles in most organic solvents and in aqueous media. The synthesis of thiols **1** and **2** and of gold nanoparticles protected by MPC (monolayer-protected cluster) homoligands have been reported.[34–36] Gold nanoparticles with a core diameter in the range 1.6–2.5 nm and coated with mixtures of thiolates of **1** and **2** in different molar ratios (see Table 1) were synthesized following a procedure reported recently by some of us.[36] In particular, to increase the modest nucleophilic character of the sulfur in α position to the perfluorinated chain, in place of thiols **1** and **2**, the corresponding thiolates were reacted with Au^{III}.

In order to compare the organization of the mixed monolayers obtained by different synthetic procedures, a sample of nanoparticles protected by HS-F8-PEG/HS-C8-

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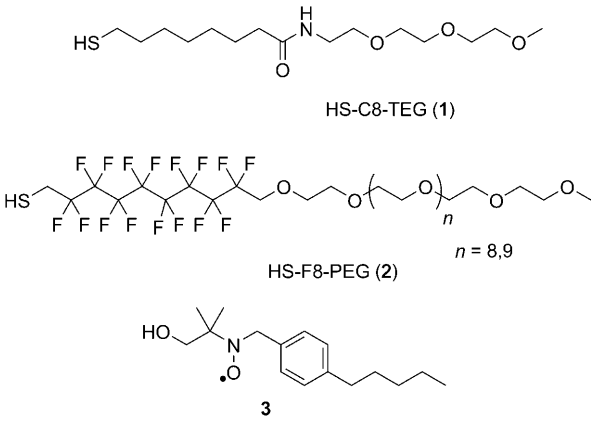
Supporting information for this article, including general procedures for the synthesis of MMPC-C8-TEG/F8-PEG and their characterization, is available on the WWW under http://dx.doi.org/10.1002/anie.200805321.



Scheme 1. Structures of thiols **1** and **2** and the radical probe **3**.

Table 1: Gold nanoparticles coated with mixtures of thiolates of **1** and **2**.

Sample	R_{SAM}[a]	Core diameter [nm][b]	MPC composition[c]
1	1	2.2 ± 0.4	$Au_{400}(S\text{-}C8\text{-}TEG)_{54}(S\text{-}F8\text{-}PEG)_{54}$
2[d]	1	1.6 ± 0.2	$Au_{150}(S\text{-}C8\text{-}TEG)_{33}(S\text{-}F8\text{-}PEG)_{33}$
3	2.5	2.5 ± 0.4	$Au_{540}(S\text{-}C8\text{-}TEG)_{108}(S\text{-}F8\text{-}PEG)_{43}$
4	4	1.9 ± 0.2	$Au_{230}(S\text{-}C8\text{-}TEG)_{66}(S\text{-}F8\text{-}PEG)_{16}$
5	20	1.9 ± 0.3	$Au_{240}(S\text{-}C8\text{-}TEG)_{68}(S\text{-}F8\text{-}PEG)_{3}$

[a] Ratio of the two thiols forming the monolayer determined by integration of ^{1}H NMR signals of thiol **1** and thiol **2** in the nanoparticles. [b] Average core diameter determined by transmission electron microscopy (TEM) measurements of at least 300 nanoparticles. [c] Estimated composition based on TEM and TGA and considering R_{SAM}. [d] Prepared from MPC-F8-PEG by place-exchange procedure.

TEG (1:1) was also prepared by exploiting the place-exchange protocol.[36,37] In particular, perfluorinated thiolates in MPC-F8-PEG were exchanged with one equivalent of HS-C8-TEG (**1**) in methanol solution.

We have already shown that nitroxide **3** located in MPC-F8-PEG and MPC-C8-TEG monolayers is characterized by sizeable different hyperfine splitting constants (hfsc; $a(N)_{F8\text{-}PEG} = 15.46$ G, $a(2H)_{F8\text{-}PEG} = 8.68$ G; $a(N)_{C8\text{-}TEG} = 15.67$ G, $a(2H)_{C8\text{-}TEG} = 8.97$ G).[36,38] This difference is the result of the diverse polarity of the environment experienced by the nitroxide radical when **3** is dissolved in the homoligand monolayers and gives rise to a notable variation in the corresponding apparent overall splitting of the spectral lines. Actually, the field separation (ΔG) between the low-field lines due to radical **3** partitioned in water (which resonates always at the same field value and can be considered as a field marker)[39] and in the monolayer decreases from 2.05 G when the spectrum is recorded in the presence of MPC-F8-PEG to 1.40 G in the presence of MPC-C8-TEG.

Figure 1 shows the variations in spectral shape observed as function of monolayer composition (R_{SAM} = [HS-C8-TEG]/[HS-F8-PEG]). The experiments carried out at intervals of 15 min show that after one hour the ESR spectral shape does not change; only the overall signal intensity decreases because of the decay of the radical probe.

The dependence of ΔG on R_{SAM} is reported in Figure 2. When R_{SAM} is less than 2.5 the value of ΔG is nearly

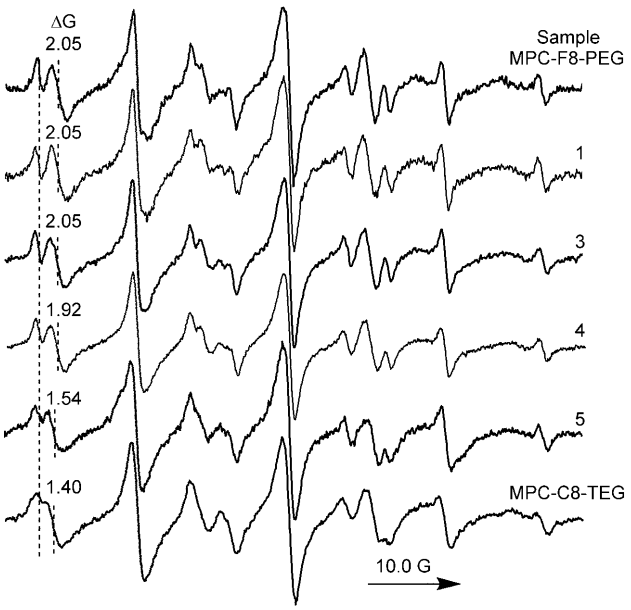


Figure 1. ESR spectra of radical **3** recorded at 298 K in the presence of MPCs of different composition (see Table 1). Dotted lines refer to the low-field lines of the radical partitioned in water and in the monolayer (ΔG, in gauss).

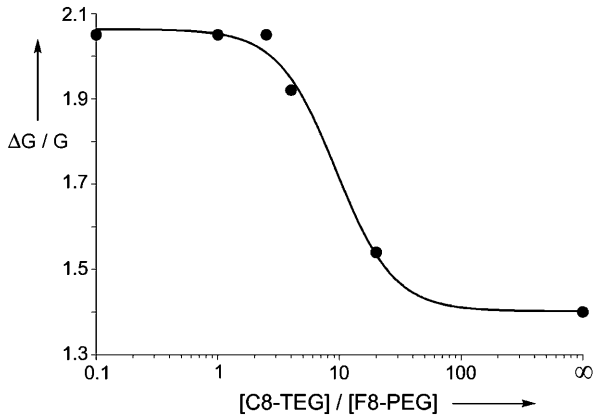


Figure 2. Dependence of ΔG (in gauss) as function of monolayer composition.

independent of R_{SAM} and equivalent to the value found in MPC-F8-PEG. The equivalence of spectroscopic parameters strongly suggests exposure of the probe mainly in islands having a polarity similar to that of a homogenous phase of perfluorinated chains. This behavior corresponds to that expected for a phase-separated system different from a homogeneous arrangement of the binary mixture of the ligands. When the two different ligands are macroscopically phase separated, the local structures of the separated phases are expected to be approximately the same as that of the pure phases. Islands of one ligand should thus behave very similarly to a homoligand monolayer until the islands become so small that disorder at their borders becomes significant in determining the polarity of the environment.

These data also indicate that the nitroxide probe is partitioned exclusively in the fluorinated islands, thus con-

firming the greater affinity of the probe for the perfluorinated phase relative to that for the nonfluorinated phase.[36]

In the presence of a larger amount of hydrocarbon chains ($R_{SAM} > 2.5$) the field separation between the lines of the radical partitioned in water and in the monolayer starts to decrease indicating that the probe experiences a monolayer containing also hydrocarbon ligands. This can be explained either by the simultaneous presence of radicals partitioned in C8-TEG and F8-PEG islands or as consequence of the smaller size of the fluorinated island when R_{SAM} becomes high. In the first assumption, the hfsc values are not sufficiently different to allow the spectral resolution of the ESR signals when radicals are simultaneously partitioned in the two types of monolayers. Under this condition, the variation in the relative amount of the radicals located in the C8-TEG and F8-PEG islands is revealed only by a change in the overall splitting. In the second case, the reduction of fluorinated chains gives rise to fluorinated islands so small that the presence of hydrocarbon chains at the borders becomes significant in determining the polarity of the environment; thus the overall splitting of the signal is changed as a result of different polarity experienced by the radical located in the monolayer. When $R_{SAM} = 20$ the spectrum of the probe closely resembles that one recorded in the MPC-C8-TEG, indicating that the radical probes are practically surrounded by hydrocarbon chains.

In principle the observed behavior for SAMS with $R_{SAM} < 2.5$ can also be justified by admitting the presence of mixtures of homoligand nanoparticles. To exclude this hypothesis, we recorded also the ESR spectra of **3** in the presence of nanoparticles with $R_{SAM} = 1$ prepared by the place-exchange reaction. The spectrum obtained (see Figure S6 in the Supporting Information) is completely superimposable with that obtained in the presence of nanoparticles prepared by direct synthesis. This strongly suggests that 3D mixed monolayers are formed with both synthetic procedures and that the macroscopic domains formed in the monolayer are thermodynamically stable.

In conclusion, we have shown by ESR spectroscopy that in heteroligand 3D SAMs phase separation occurs forming islands of homoligands triggered by the lipophobicity of perfluorocarbons. Considering that perfluorinated amphiphilic thiolates may be easily functionalized by simple chemical reactions, this strategy opens up a variety of new applications of nanoparticles as, for example, scaffolds for polyvalent interactions with biomolecules, thanks to the solubility of the systems in water, and also in the fabrication of new materials. Moreover, this is the first example of a multicompartment water-soluble 3D SAM composed of two hydrophobic but immiscible components and one hydrophilic poly(ethylene oxide) compartment; this SAM was obtained by spontaneous self-assembly with the potential for storage and release of two or more active but incompatible agents.

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