Exploring the amphiphilicity of PEGylated gold nanorods: mechanical phase transfer and self-assembly†

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We introduce a mechanical approach to phase transfer of PEGylated gold nanorods. Amphiphilic PEG ligands can be switched between hydrophilic and hydrophobic states by applying mechanical force. PEG-GNRs in their hydrophobic state self-assemble into rings, a phenomenon previously observed only for GNRs capped with hydrophobic ligands.

The dispersibility of uncharged nanomaterials in liquids is determined by the solvation behaviour of the molecules attached to their surface.1,2 PEGylation is widely used in nanomedical research to induce hydrophilicity and reduce uptake of nanomaterials by the reticuloendothelial system (RES). However, the hydrophobic behaviour of PEGylated nanomaterials has been much less explored. Conjugation of PEG to nanomaterials3–7 has been exploited in studies of enzymatic activity in a non-aqueous environment.8,9 Here, we applied centrifugal force to PEGylated gold nanorod (PEG-GNR) dispersions to study their bi-directional phase transfer. In addition, we demonstrated self-assembly of PEG-GNRs in their hydrophobic state into ring shapes, a phenomenon previously reported only for GNRs grafted with a hydrophobic polymer.10

Freeze-drying has been used to transfer PEGylated proteins into a non-aqueous environment for studies of enzymatic activity.9,10 Similarly, we found that PEG-GNRs vacuum-dried from water were easily dispersed in chloroform. Thus, removal of water appears to be a necessary condition for phase transfer. GNR synthesis and ligand exchange followed previous reports.11–13 PEGylation was confirmed by TGA and 1H NMR (Fig. S1 and S2, ESI†). To mimic removal of water by vacuum drying, we concentrated the PEG-GNR dispersion by centrifugation. After removal of the supernatant, addition of chloroform, and vigorous shaking, a greenish blue dispersion was obtained, indicating that the PEG-GNRs were dispersed in chloroform (Fig. 1C). When water was added, some GNRs returned to the aqueous phase, but a substantial fraction remained in the chloroform phase (Fig. 1D).

This encouraging result led us to attempt direct phase transfer by application of mechanical (centrifugal) force. We added aqueous PEG-GNRs and chloroform to a centrifuge tube and then centrifuged the two-phase system at various speeds (83, 330, 1.3 K, 2.9 K, 5.3 K, 8.3 K, 12 K, and 14 KG, see the ESI† for the relationship between KG and rpm) for 15 minutes. For an aqueous PEG-GNR dispersion with an initial optical density (O.D.) of 3.4, small pellets of PEG-GNR could be found at the bottom of the tube (in the chloroform phase) after centrifugation at speeds ≥2.9 KG (Fig. 2, inset A). The amount of GNR at the bottom of the tube increased with increasing centrifugal speed. The transfer efficiency, $E_t$, obtained by measuring...
the optical density (O.D.) of the aqueous phase, reached ~56% for centrifugation at 14 KG (Fig. 2, inset B). Upon gentle shaking, the PEG-GNR pellet dispersed homogeneously in chloroform (Fig. 2, inset C). Dispersions of varying initial O.D. were tested, and $E_t$ was found to be concentration-dependent. However, the concentration of PEG-GNRs remaining in water, as indicated by the residual O.D. in Fig. 2, was nearly independent of the initial O.D. Aqueous samples with initial O.D. $\leq$ 1.0 produced no visible phase transfer. No reverse phase transfer from chloroform to water was observed over periods of as long as several months. Thus, this phase transfer does not represent a partitioning of GNRs between the two phases, as would be obtained for a substance with moderate solubility in each phase. Rather, forcing the GNRs into the chloroform phase via centrifugal force changes the state of the GNRs such that they do not return to the aqueous phase.

Previous research on molecular behaviour of crystalline PEG showed that the helical structure of PEG was retained by coordination of water molecules to each monomer unit via hydrogen bonding.\textsuperscript{14} Similarly, oriented water is strongly absorbed on the PEG layer of aqueous PEG-GNRs. Energy input is required to displace this hydration layer. Fig. 1D shows that, in the absence of a liquid–liquid interface, a small portion of PEG-GNRs went into the aqueous phase after the addition of water to the chloroform dispersion. This is attributed to an incomplete removal of water as shown in Fig. 1A. In this case, we believe that the mechanism of disruption of the hydration layer is simply the mechanical force bringing the PEG-GNRs into close contact and “squeezing” the hydration layer out from between the PEG layers. For direct phase transfer via centrifugation, although such close-contact is also occurring (Fig. 2), we speculate that a minimum amount of GNRs must be present in the aqueous phase for them to pile up at the liquid–liquid interface and provide the force required to “push” the PEG-GNRs through the interface into the chloroform phase, stripping off the hydration layer in the process. Such a mechanism is consistent with the fact that the phase transfer stopped when the amount of PEG-GNRs in the aqueous phase reached a critical value, so that the pressure at the bottom of the stack of PEG-GNR layers was no longer enough to disrupt the hydration layer. That would also explain the results in Fig. 2 showing that the residual O.D. was almost independent of the initial O.D. Scheme 1A and B illustrate these processes.

To further explore the role of hydration layer disruption in phase transfer, we added a small amount of NaCl to partially disrupt hydrogen bonding.\textsuperscript{15,16} Consistent with the proposed mechanism, this facilitated phase transfer, $E_t \geq 99\%$ was achieved at a centrifugal speed of 2.9 KG (Fig. 3A1–A3). Similarly, alcohols can destabilize the hydration layer.\textsuperscript{17} Although the PEG-GNRs are dispersible in ethanol, addition of a small amount of ethanol facilitated the phase transfer out of the water-ethanol phase into chloroform (Fig. 3B, $E_t = 90\%$). Indeed, when enough salt or alcohol was added, centrifugation was not necessary, and simple shaking was sufficient to achieve phase transfer (ESI†). From a thermodynamic perspective, the addition of a solute decreases the chemical potential of the bulk water in which the solute is dissolved, and with which the water in the hydration layer is in equilibrium. If the hydration layer excludes the solute, then a driving force for transfer of water molecules from the hydration layer into the bulk, analogous to osmosis, exists. Even when salt or alcohol was used to promote phase transfer, aggregation was not observed in either phase. The GNR size remained unchanged after phase transfer (Fig. 3C, insets). The peak of the longitudinal localized surface plasmonic resonance (LLSPR) absorption shifts by 20 nm after phase transfer, due to the higher refractive index of chloroform (Fig. 3C), but the absorption spectrum shows no evidence of aggregation.\textsuperscript{18} Aggregation could be induced at much higher salt concentration. Fig. 3C also shows the position of the LLSPR absorbance peak in common solvents, exhibiting the expected shift with changing refractive index. Other salts and alcohols were also effective in promoting phase transfer (Fig. S3, ESI†).

Water-to-chloroform phase transfer via hydration layer disruption implies that hydration layer re-formation (and CHCl$_3$ solvation layer disruption) should facilitate reverse phase transfer. Although PEG-GNRs can be re-dispersed in water by CHCl$_3$ evaporation and water addition, demonstration of reverse phase transfer via centrifugation supports the proposed mechanism of phase transfer. PEG-GNRs dispersed in a 1:1.5 (v/v) chloroform : toluene mixture (initial O.D. 2.9) was used. This solvent mixture allows good dispersion of PEG-GNRs and provides a system with a lower density than water (Fig. 4A). Again, no transfer is observed upon shaking. However, after centrifugation at 4 KG for 15 minutes, GNRs were transferred to...
the aqueous phase with $E_t \geq 99\%$. Compared with the water-to-chloroform case, reverse transfer required lower force. This could be because (1) the solvation layer in chloroform is less strongly bound than the hydration layer in water, and (2) although PEG-GNRs are well-dispersed in the mixed solvent, toluene itself is not a good solvent for PEG. Just as the addition of salt or alcohol in water serves to dehydrate the PEG, toluene may also serve to de-solvate the PEG in chloroform.

Volatile hydrophobic organic solvents are ideal for concentrating nanoparticles and can sometimes be used in evaporation-driven self-assembly processes. We observed that PEG in either CH$_2$Cl$_2$ or CHCl$_3$ could orient randomly upon drop-casting, as seen in Fig. 3C. However, we also found that a fraction of the PEG-GNRs self-assembled into rings on the TEM grid, during solvent evaporation. This was never observed for PEG-GNRs cast from water. Although the radii of the rings varied greatly, as shown in Fig. 5, most of the rings contain multiple layers of gold nanorods, while some small circles may have a single layer. Rings with both opened (Fig. 5B2, Fig. 5S, ESI†) and closed structures (Fig. 5 except B2, Fig. S4 and S6, ESI†) were observed. The second and subsequent layers may grow around the first layer, which serves as a template. This is most obvious in Fig. 5B1. More evidence of this ring shape can be found in the ESI† (Fig. S4-S6, ESI†).

Zubarev's group demonstrated that polystyrene-coated GNRs dispersed in dichloromethane can form rings when dropped onto a TEM grid. They proposed a water-droplet-assisted mechanism in which a tiny drop of water, condensed from the air as a result of fast cooling from the evaporation of CH$_2$Cl$_2$, templates the GNRs into a circular shape. They also demonstrated that PEG-GNRs were unable to form ring-like structures due to their hydrophilicity. However, PEG is amphiphilic and, as shown above, PEG-GNRs can adopt both hydrophilic and hydrophobic states that allow them to be well dispersed in aqueous and non-aqueous environments. We propose that in the droplet-assisted mechanism described by Zubarev, the solvation layer of the organic solvent stops PEG-GNRs from reeding into the water phase, so that PEG-GNRs behave like the polystyrene-coated GNRs and assemble into rings. The difference in assembly between CH$_2$Cl$_2$ and CHCl$_3$ may be related to the volatility difference of the two solvents (vapour pressures at 20 °C are 46 kPa for CH$_2$Cl$_2$ and 20 kPa for CHCl$_3$). More rapid evaporation of CH$_2$Cl$_2$ could produce a greater temperature decrease, creating larger water droplets and therefore larger rings. Similarly, slower evaporation of CHCl$_3$ provides more opportunity for end-to-end alignment of the GNR at the drop periphery.

Beyond phase transfer itself, the experiments reported here imply that mechanical force can be used to change the solvation behaviour of nanoscale objects. In addition to centrifugal force, other forces could be used, such as magnetic force (for PE Gylated magnetic particles) or direct mechanical force applied using an AFM tip. PEG-GNR was chosen as a model here, based upon the practical need to transfer GNRs to organic solvents for applications such as thin film coating, non-aqueous catalysis of chemical reactions, and plasmonic enhancement of light absorption in photovoltaics, as well as the interest in self-assembly of GNRs into organized structures for applications such as surface enhanced Raman spectroscopy (SERS).\textsuperscript{19-23}

In summary, the phase transfer and self-assembly demonstrated here illustrate the importance of manipulation of the solvation layer surrounding nanoparticles in solution. This solvation behaviour controls not only dispersibility, but also self-assembly of nanoparticles. The wide use of both PEG and gold nanorods makes the specific case investigated here a particularly important one, but the results are general. Future studies will address the applicability of this approach to other polymers as well as different nanomaterials and self-assembly strategies.

Notes and references
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