Nanoparticle Assemblies

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Hierarchical Self-Assembly for Nanomedicine

*Kyle J. M. Bishop**

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Medical imaging and therapies based on light require efficient probes that absorb strongly at near-infrared (NIR) wavelengths, for which biological tissue is most transparent.[1] Gold nanoparticles (GNPs) have been widely used in this context as their optical properties can be tuned over a broad range by controlling particle size and shape. Achieving strong absorption in the NIR typically requires larger particles with characteristic sizes of more than 50 nm; however, smaller GNPs—less than 6 nm—are often desirable in medical contexts as they are more easily cleared from the body.[2] The competing requirements for small particles and NIR adsorption can be reconciled by organizing individual GNPs within larger multiparticle assemblies.[3] When particles get together in close proximity, their collective electronic excitations—surface plasmons—interact to shift the resonant excitation from visible to infrared wavelengths.[4] For example, the peak absorbance of linear GNP chains can be tuned from around 520 nm to about 800 nm by controlling the length of the chain and the spacing between the particles.[5] Thus, the assembly of NPs into specific architectures provides an attractive route to achieving designer contrast agents for medical imaging (e.g., photothermal, photoacoustic, and surface enhanced Raman scattering (SERS) imaging).

But why stop there? Nanoparticle assemblies can serve additional functions. GNPs functionalized with amphiphilic block copolymers (BCPs) can assemble spontaneously to form vesicular structures that serve as microscopic containers for the transport and controlled-release of therapeutic agents.[6] These materials provide a platform for cancer theranostics that combines photoacousitc imaging with therapies based on light-induced drug release. However, owing to the relatively large interparticle spacing, vesicles of small BCP–GNPs adsorb most strongly in the visible spectrum as opposed to the desired NIR region. More generally, as we progress to materials with more functions, it becomes increasingly difficult to perform each function well.

The challenge of achieving multifunctional assemblies is often a problem of hierarchical assembly, in which structures at different scales impart different functions. Ideally, GNPs should assemble to form well-defined clusters that maximize NIR adsorption as well as larger vesicles that carry therapeutic payloads. While we are increasingly able to design nanoparticle assemblies at one scale (e.g., to assembly particle chains or vesicles), programing hierarchical structure into nanoscale components remains an outstanding challenge for nanoscience.

Recently, Nie, Chen, and co-workers showed how surface chemistry and assembly kinetics can be tuned to direct the assembly of BCP-GNPs into linear particle "strings" that subsequently wrap to form vesicular containers.[7] Owing to the ordering of the particles within the vesicle walls, these assemblies absorb most strongly at NIR wavelengths (ca. 760 nm). They also showed how this subtle change in hierarchical order leads to a nearly tenfold enhancement in the photoacoustic signal for in vivo imaging. Furthermore, the mechanism by which these multifunctional assemblies form offers general insights to engineering hierarchical order in other nanoparticle assemblies.

In their experiments, nanoparticle vesicles are assembled in solution from GNPs (13 nm diameter) functionalized with amphiphilic BCPs (polystyrene-b-polyethylene oxide; PS-b-PEO). Initially, the particles are well dispersed in tetrahydrofuran (THF), which acts as a good solvent for the hydrophobic PS blocks. Water is slowly introduced by dialysis to induce attractive solvophobic interactions between the particles. Ultimately, these interactions direct the formation of vesicles (up to 500 nm diameter) analogous to those formed by lipids or BCPs but with GNPs embedded in their walls. Importantly, the organization of particles within the final assembly depends sensitively on the grafting density of polymers on their surface (Figure 1).

For high grafting densities ($\sigma = 0.08\ \mathrm{nm}^{-2}$), GNPs form an open lattice with a characteristic spacing comparable to the thickness of the vesicle wall (Figure 1a). Plasmon coupling among the GNPs results in a red shift in the peak absorbance from 540 nm to around 580 nm but falls well short of the NIR window. When, however, the grafting density is reduced ($\sigma = 0.03\ \mathrm{nm}^{-2}$), the particles organize into linear chains with interparticle spacings of less than 1 nm (Figure 1b). Strong coupling within arrays of nearly touching GNPs results in a significant shift in the peak absorbance to about 760 nm—ideal for NIR-based bioimaging.

To understand how a rather subtle change in the grafting density can lead to such qualitatively different assemblies, consider that the size of the BCP ligands (e.g., the radius of gyration, $R_g \approx 6$ nm) is commensurate with their separation, σ^{-2}. This ratio is often expressed as a reduced grafting density, $\Sigma = \pi R_g^2 \sigma$, which varies from around 3 to 9 in the experiments.

[*] Prof. K. J. M. Bishop
Department of Chemical Engineering
The Pennsylvania State University
University Park, PA 16802 (USA)
E-mail: kjmbishop@engr.psu.edu

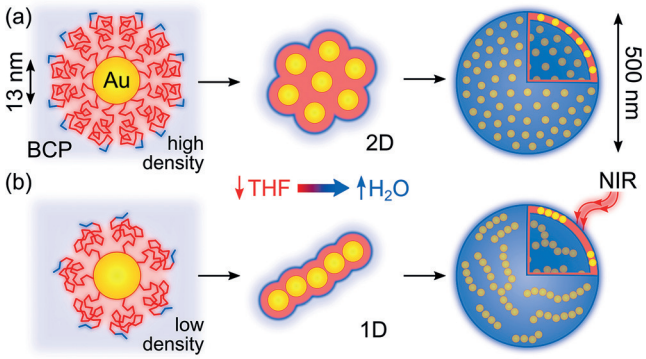

Figure 1. Gold nanoparticles (GNPs) functionalized with amphiphilic block copolymers (BCPs) assemble to form spherical vesicles. Depending on the grafting density of BCPs, GNPs form open 2D arrays (a) or close-packed 1D stings (b) within the vesicle wall. The 1D strings absorb strongly at near infrared (NIR) wavelengths.

Not coincidentally, these values correspond closely to the transition between isolated polymer chains ($\Sigma < 1$) and the stretched chains of a polymer brush ($\Sigma > 5$).[8] Reducing the grafting density in this transition region results in polymer coatings that are both thinner and more compressible.

Under these conditions, new interactions come into play: strong van der Waals (vdW) forces between proximal GNPs cause the collapse of the polymer coatings, resulting in small interparticle separations (or even particle fusion). Such interactions are negligible at high grafting densities owing to the thicker polymer layer, which forces larger separations between the particles (Figure 2a).

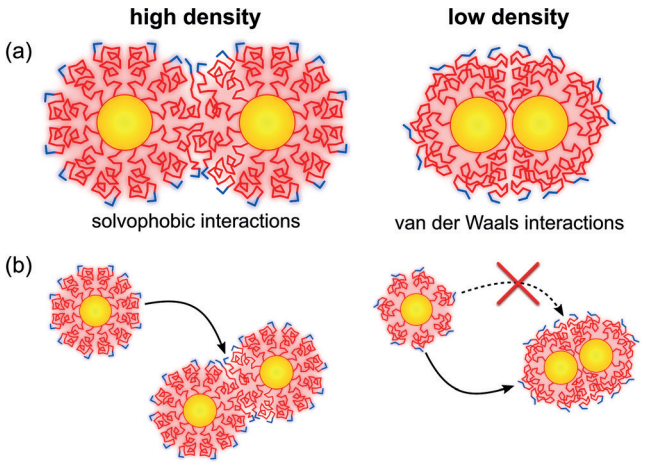

Figure 2. a) GNPs with high grafting density interact primarily through solvophobic interactions between polymer coatings; GNPs with low grafting density interact primarily through van der Waals (vdW) interactions between particle cores. b) Solvophobic interactions favor formation of planar assemblies while a combination of vdW attraction and steric repulsion favors formation of particle chains.

The formation of particle chains is a consequence of many-body interactions between polymer-stabilized particles (Figure 2b).[9] When two BCP–GNPs interact, their polymer coatings redistribute to create a high-density region near the point of contact. Consequently, a third particle prefers to approach from the ends where it can make close vdW contact and not from the sides where such contact is prohibited. By contrast, interactions among BCP–GNPs with high grafting densities are mediated by solvophobic forces between the polymers (not by vdW forces). These many-body interactions lead to planar assemblies that best conceal the hydrophobic polymer from the polar solvent.

The final ingredient to achieving these hierarchical structures is control over the assembly kinetics. As water is gradually introduced to the system, GNPs first assemble into linear chains that grow longer with time. Further increase in the water content leads to stronger solvophobic interactions between the chains and mediates their organization into planar assemblies and ultimately closed vesicles. Consequently, the characteristic length of the GNP chains within the final vesicles is influenced by the rate at which water is introduced relative to that of chain formation and growth. In this way it is likely possible to tune the characteristic length of the GNP chains (and thereby the peak absorbance) by tuning the assembly kinetics.

This specific example of hierarchical self-assembly offers general insights for designing other multiscale structures. First, organization at multiple length scales requires multiple pairs of competing forces. In this case, the small spacing between GNPs within the chains is set by a competition between vdWs attraction and steric repulsion; the larger spacing between chains is controlled by solvophobic interactions between the polymer coatings. Second, many-body forces offer an attractive route to complex directional interactions among spherically symmetric components.[9] Such forces often emerge between components with adaptive surfaces that reconfigure as they interact.[10] Despite their potential utility, the rational design of these interactions will require predictive models that abandon the common assumption of pairwise additive forces. Finally, tuning organization at multiple scales requires control over assembly kinetics such that successive stages of hierarchal ordering are activated at appropriate times. In this case, this control is achieved by tuning solvent polarity so as to increase the strength of solvophobic interactions at a prescribed rate.

From a practical perspective, these hierarchical assemblies show immediate promise for the realization of theranostic tools that combine imaging, chemical detection, phototherapy, and controlled release functionalities within a common platform. Nie, Chen, and co-workers show that their hierarchical assemblies can lead to significant enhancements in photoacoustic imaging, which uses pulsed optical excitation in the NIR to induce acoustic waves that are imaged by ultrasonic detectors. By injecting their "chain vesicles" into the tissue of nude mice, they demonstrate an eightfold enhancement in the photoacoustic signal; no such enhancement is observed for non-chain vesicles owing to their inferior absorbance. Beyond this initial demonstration, other applications based on NIR excitation are sure to follow: phototherapy treatments that require the localized heating of targeted tissues (e.g., tumors); light-induced release of therapeutic drugs encapsulated within the vesicle interiors; in vivo chemical detection based on surface enhanced Raman

scattering (SERS). Nanoscale self-assembly has much to offer and more to gain—by way of new and important challenges—from the burgeoning field of nanomedicine.

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[1] R. Weissleder, *Nat. Biotechnol.* **2001**, *19*, 316–317.
[2] T. L. Doane, C. Burda, *Chem. Soc. Rev.* **2012**, *41*, 2885–2911.
[3] J. M. Tam, J. O. Tam, A. Murthy, D. R. Ingram, L. L. Ma, K. Travis, K. P. Johnston, K. V. Sokolov, *ACS Nano* **2010**, *4*, 2178–2184.
[4] N. J. Halas, S. Lal, W. S. Chang, S. Link, P. Nordlander, *Chem. Rev.* **2011**, *111*, 3913–3961.
[5] T. Chen, M. Pourmand, A. Feizpour, B. Cushman, B. M. Reinhard, *J. Phys. Chem. Lett.* **2013**, *4*, 2147–2152.
[6] Y. Liu, J.-J. Yin, Z. Nie, *Nano Res.* **2014**, *7*, 1719–1730.
[7] Y. Liu, J. He, K. Yang, C. Yi, Y. Liu, L. Nie, N. M. Khashab, X. Chen, Z. Nie, *Angew. Chem. Int. Ed.* **2015**, *54*, 15809–15812; *Angew. Chem.* **2015**, *127*, 16035–16038.
[8] W. J. Brittain, S. Minko, *J. Polym. Sci. Part A* **2007**, *45*, 3505–3512.
[9] P. Akcora, H. Liu, S. K. Kumar, J. Moll, Y. Li, B. C. Benicewicz, L. S. Schadler, D. Acehan, A. Z. Panagiotopoulos, V. Pryamitsyn, et al., *Nat. Mater.* **2009**, *8*, 354–359.
[10] H.-Y. Lee, S. H. R. Shin, A. M. Drews, A. M. Chirsan, S. A. Lewis, K. J. M. Bishop, *ACS Nano* **2014**, *8*, 9979–9987.

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