

# Anisotropic Self-Assembly of Hairy Inorganic Nanoparticles

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**CONSPECTUS:** Current interest in functional assemblies of inorganic nanoparticles (NPs) stems from their collective properties and diverse applications ranging from nanomedicines to optically active metamaterials. Coating the surface of NPs with polymers allows for tailoring of the interactions between NPs to assemble them into hybrid nanocomposites with targeted architectures. This class of building blocks is termed "hairy" inorganic NPs (HINPs). Regiospecific attachment of polymers has been used to achieve directional interactions for HINP assembly. However, to date anisotropic surface functionalization of NPs still remains a challenge.

This Account provides a review of the recent progress in the self-assembly of isotropically functionalized HINPs in both the condensed state and aqueous solution as well as the applications of assembled structures in such areas as biomedical imaging and therapy. It aims to provide fundamental mechanistic insights into the correlation between structural characteristics and self-assembly behaviors of HINPs, with an emphasis on HINPs made



from NPs grafted with linear block copolymer (BCP) brushes. The key to the anisotropic self-assembly of these HINPs is the generation of directional interactions between HINPs by designing the surrounding medium (e.g., polymer matrix) or engineering the surface chemistry of the HINPs. First, HINPs can self-assemble into a variety of 1D, 2D, or 3D nanostructures with a nonisotropic local arrangement of NPs in films. Although a template is not always required, a polymer matrix (BCPs or supramolecules) can be used to assist the assembly of HINPs to form hybrid architectures. The interactions between brushes of neighboring HINPs or between HINPs and the polymer matrix can be modulated by varying the grafting density and length of one or multiple types of polymers on the surface of the NPs. Second, the rational design of deformable brushes of BCP or mixed homopolymer tethers on HINPs enables the anisotropic assembly of HINPs (in analogy to molecular self-assembly) into complex functional structures in selective solvents. It is evidenced that the directional interactions between BCP-grafted NPs arise from the redistribution and conformation change of the long, flexible polymer tethers, while the lateral phase separation of brushes on NP surfaces is responsible for the assembly of HINPs carrying binary immiscible homopolymers. For HINPs decorated with amphiphilic BCP brushes, their self-assembly can produce a variety of hybrid structures, such as vesicles with a monolayer of densely packed NPs in the membranes and with controlled sizes, shapes (e.g., spherical, hemispherical, disklike), and morphologies (e.g., patchy, Janus-like). This strategy allows fine-tuning of the NP organization and collective properties of HINP assemblies, thus facilitating their application in effective cancer imaging, therapy, and drug delivery. We expect that the design and assembly of such HINPs with isotropic functionalization is likely to open up new avenues for the fabrication of new functional nanocomposites and devices because of its simplicity, low cost, and ease of scale-up.

# 1. INTRODUCTION

Current interest in assembling inorganic nanoparticles (NPs) stems from their collective properties, which may vastly differ from those of discrete NPs and the corresponding bulk materials. These interesting properties mainly arise from coupling interactions between NPs.<sup>1</sup> Despite recent breakthroughs in NP synthesis, controlled assembly of NPs into architectures with desired complexity and functionality remains a challenge. Generally, this challenge might be met through surface modification of NPs with organic coatings (e.g., small molecules, biomolecules, or polymers) to tailor the interaction between NPs.<sup>2-4</sup> Among these coatings, polymers feature diverse chemical structures and functionalities. The anchoring of polymers onto inorganic NP surfaces generates a new class of hybrid building blocks, called "hairy" inorganic nanoparticles (HINPs), which naturally combine the unique features of both inorganic NP cores and tethered polymers.<sup>3</sup>

Organization of HINPs into complex structures can often draw inspiration from well-established atomic and molecular self-assemblies.<sup>6,7</sup> Recent advances in this frontier have brought recognition that anisotropic interactions through chemical "patches" are crucial to the self-assembly of HINPs into targeted structures.<sup>6,7</sup> Regiospecific surface modifications are usually used to decorate inorganic NPs with surface patches of polymers or other molecules. Common strategies include selective attachment of polymers to certain crystal facets of NPs due to differential binding strength<sup>8,9</sup> and localized grafting of polymers by masking one side of the NP surface.<sup>10</sup> Nevertheless, anisotropic functionalization and surface characterization of the resulting NPs remain a challenge.

Interestingly, recent compelling evidence suggests that anisotropic functionalization may not be necessary for the

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anisotropic self-assembly of HINPs and that directional interactions can arise between NPs isotropically decorated with polymers.<sup>11</sup> The relative simplicity of isotropic functionalization makes this class of HINPs particularly intriguing from the perspectives of cost, scale-up, and processing. To date there exist few reviews summarizing the development of HINPs from different perspectives.<sup>4,5,12,13</sup> An overview of the state of the art and recent advancements will be beneficial to this burgeoning field. This Account provides a review of the anisotropic self-assembly of HINPs with isotropic functionalization and the biomedical applications of HINP assemblies.

## 2. TAILORED SYNTHESIS OF HINPS

The surface functionality and interparticle interactions of HINPs are largely dependent on the conformation of the polymer brushes on the NP surfaces, which is affected by the grafting density ( $\sigma$ ), polymer length and composition, and particle size and shape.<sup>5</sup> For spherical HINPs dispersed in good solvents, the brush conformation can be categorized into three regimes (Figure 1).<sup>5,14</sup> At low  $\sigma$  ( $\sigma < R_g^{-2}$ , where  $R_g$  is the



Figure 1. Schematic illustration of three regimes of polymeric brush conformation on NP surfaces. Reprinted with permission from ref 5. Copyright 2013 Material Research Society.

radius of gyration of the chains), the polymer chains adopt a random coil conformation and fall into the so-called "mushroom" regime. As  $\sigma$  exceeds the overlap threshold of chains, polymers are stretched from anchor points toward the solvent medium, entering the semidilute polymer brush (SDPB) regime. At even higher  $\sigma$ , the concentrated polymer brush (CPB) regime is accessed, which is featured by non-Gaussian chain characteristics and more extended chain conformations. The graft density and nature of the polymer dictate both the assembly behavior and ensemble properties of HINPs.<sup>15,16</sup>

Current strategies for the preparation of HINPs can be summarized into three categories: one-pot synthesis and "grafting from" and "grafting to" methods. In one-pot synthesis, the NP growth and surface modification occur simultaneously in one reaction system in which the polymer is used as a stabilizer and shape-directing agent. Moreover, block copolymer (BCP) micelles<sup>17</sup> or unimolecular micelles<sup>18,19</sup> can serve as nanoreactors to synthesize monodisperse HINPs with tunable size. However, this method shows limited control over the surface chemistry of HINPs. Both "grafting from" and "grafting to" methods use surface postmodification of NPs to produce HINPs. The "grafting from" method usually uses surfaceinitiated controlled/"living" polymerization to grow polymers from NP surfaces.<sup>20</sup> The resulting HINPs feature high  $\sigma$  (up to 4-5 chains/nm<sup>2</sup>) due to the higher diffusivity of the initiators/ monomers compared with the polymers. However, to obtain high-quality HINPs, several possible problems need to be

solved, including NP stability during polymerization and crosslinking of growing polymers.<sup>21</sup> In the "grafting to" method, assynthesized polymers are grafted onto NP surfaces through chemical reaction or ligand exchange. Polymers used for this purpose must contain one or more functional groups that can be immobilized either directly to the NP surface or to the original capping ligands on the surface.<sup>22–25</sup> Despite its simplicity and robustness, this method usually generates HINPs with a relatively low  $\sigma$  because of the steric hindrance of polymers on the NP surface.<sup>26</sup> Nevertheless, each of the above-mentioned strategies has its inherent uniqueness that can meet the requirements for specific applications of the end product.

### 3. SELF-ASSEMBLY OF HINPS

Although HINPs resemble organic molecules in self-assembly, their mobility and relaxation are significantly slower than those of organic molecules because of their relatively large dimensions.<sup>27</sup> This vastly changes the kinetics and thermodynamics of the HINP assembly.

### 3.1. Self-Assembly of HINPs in the Condensed State

Polymer matrices can be used to induce the anisotropic selfassembly of HINPs to produce hybrid nanocomposites or discrete structures.<sup>28,29</sup> The wettability of HINPs in the surrounding matrix can determine the dispersion (or segregation) and position of HINPs within the matrix.<sup>30</sup> For example, Tao and co-workers controlled the organization and orientation (i.e., face-to-face or edge-to-edge) of hydrophilic-homopolymer-modified Ag nanocubes in chains embedded in incompatible hydrophobic polystyrene (PS) matrices.<sup>31</sup> When HINPs are dispersed in compatible matrices (or neat HINPs), the entropy penalty arising from the compression and extension of brushes may play an important role in the selfassembly of HINPs.<sup>33,34</sup> This entropic effect can be dominant in assembly and result in the segregation of HINPs in spite of their compatibility with the matrix, particularly when  $\sigma$  is relatively high (usually in the SDPB or CPB regime). Such significant depletion of the attraction between HINPs is known as the autophobic dewetting effect.<sup>35</sup> This effect decreases with increasing surface curvature of the NPs and decreasing density of the grafted chains. Conversely, HINPs with a low  $\sigma$  may experience anisotropic interactions when incorporated within a polymer matrix. For example, PS-grafted SiO<sub>2</sub> NPs can assemble into strings, sheets, and platelet-like structures in a PS matrix as a result of phase separation between the matrix and the ungrafted NP surface.<sup>11</sup>

Supramolecular copolymers can be used to guide the selfassembly of HINPs. One typical system is constructed via hydrogen bonding between 3-pentadecylphenol (PDP) and pyridine groups of polystyrene-*b*-poly(4-vinylpyridine) (PS-*b*-P4VP) (PS-*b*-P4VP(PDP)) (Figure 2).<sup>36–38</sup> For example, Zhu and co-workers reported the uniform dispersion of PS-coated gold nanoparticles (AuNPs) within cylindrical PS phases of PS*b*-P4VP(PDP) assemblies and release of linear NP arrays from films by breakdown of hydrogen bonds in ethanol (Figure 2a).<sup>36</sup> When PS-tethered gold nanorods (AuNRs) were used, strong depletion of attractions arising from autophobic dewetting drove the hairy AuNRs to assemble side-by-side to form an ordered smectic B phase.<sup>39</sup> We found that grafting PS brushes with different lengths on AuNRs suppressed the autophobic dewetting effect,<sup>40</sup> thus improving the wettability and dispersion of NRs within PS domains. The orientation of



Figure 2. Self-assembly of HINPs templated by supramolecular copolymer assemblies and subsequent release of PS-tethered NP arrays from the template. (a) Generation of discrete AuNP arrays using PS-*b*-P4VP(PDP) assemblies. (b–d) TEM images of discrete AuNR arrays with different spatial arrangements using PS-*b*-P4VP(PDP) assemblies. (e, f) Assembly of PS-grafted AuNRs into superlattices using P4VP(PDP) assemblies. Reprinted with permission from refs 36, 39, and 41. Copyright 2011 Wiley-VCH, 2013 American Chemical Society, and 2015 Wiley-VCH, respectively.

the AuNRs can be tuned to be parallel or perpendicular to the PS cylinders by controlling the length and volume fraction of the AuNRs as well as the diameter of the cylindrical domains (Figure 2b–d). Our group further utilized lamellar structures of P4VP(PDP) assemblies to template the organization of PS-capped AuNPs with different shapes (e.g., rods and cubes) into free-standing 2D and 3D superlattices with controllable spacing (Figure 2e,f).<sup>41</sup>

In addition to the use of BCP templates, anisotropic selfassembly of neat HINPs has emerged as an attractive strategy to fabricate hybrid composites that may exhibit unique properties.<sup>16,42–44</sup> As an example, Vaia and co-workers reported the assembly of PS-grafted silica NPs into composite materials with a nonisotropic local arrangement of NPs.<sup>16</sup> The organization of NPs within the composites is strongly dependent on the grafting density of PS on the NPs. It is remarkable that the composites exhibit viscoelastic behavior during elongation, resembling that of semicrystalline elastomers. Moreover, phase separation of immiscible polymer brushes on HINPs can also drive the anisotropic assembly of neat HINPs into ordered morphology in films. Matsushita and co-workers reported AuNPs grafted with a binary mixture of immiscible polyisoprene (PI) and PS assembled into lamellar structures with NPs located at the PI-PS interface.<sup>42</sup> Despite the promise of HINP assembly, relatively small HINPs (usually with the size of the inorganic core <10 nm) are often used in current studies. This is largely due to the low mobility of larger HINPs and the difficulty in achieving equilibrium of assembled

structures. The field is in need of new assembly strategies to expand the size range of HINPs that can be assembled.

#### 3.2. Self-Assembly of HINPs in Solution

In the anisotropic assembly of HINPs, directional interactions arise from conformational changes of the polymer brushes and/ or phase separation of multiple immiscible polymer brushes on the NP surfaces.<sup>12,42,45</sup> It is often believed that mixed hydrophilic and hydrophobic molecules extended directly from the NP surface are required to induce NP amphiphilicity. This concept is frequently adopted for NP self-assembly such as in systems based on "V-shaped" BCPs<sup>25</sup> or binary polymer brushes.<sup>21,22</sup> We recently showed that the conformation change of linear BCP tethers can lead to controllable assembly of HINPs into various nanostructures in solution.<sup>23,26,46-48</sup>

**3.2.1. Self-Assembly by Film Rehydration.** Our group demonstrated that HINPs consisting of AuNPs grafted with PS*b*-PEO can self-assemble into hollow gold vesicles (GVs) and tubules via film rehydration (Figure 3).<sup>23</sup> Both nanostructures were composed of a monolayer of hexagonally packed AuNPs in the membrane (Figure 3b). The assembly morphologies are predominantly dependent on the hydrophilicity of the HINPs, which is determined by the ratio of the average root-mean-square end-to-end distance ( $R_0$ ) of the PS block to the size of the AuNPs ( $d_{Au}$ ). As shown in the phaselike diagram in Figure 3d, HINPs favor vesicle formation when  $R_0/d_{Au} < 0.5$  and tubule formation when  $R_0/d_{Au} \approx 0.5$  and cannot be rehydrated when  $R_0/d_{Au} > 0.5$ . The morphological transition is attributed to the surface-curvature-dependent hydrophilicity of the



**Figure 3.** Self-assembly of BCP-tethered AuNPs into hollow structures. (a–c) Assembly mechanism (a) and SEM images of the resulting vesicles (b) and tubules (c). (d) Product diagram of the self-assembly of HINPs into different structures:  $\triangle$ , vesicles;  $\bigcirc$ , tubules;  $\times$ , precipitates. (e) Plot of the interparticle spacing  $D_{Au}$  ( $\triangle$ ) and  $D_{Au}/2R_0$  ( $\bigcirc$ ) of vesicular assemblies as functions of PS length. Reprinted from ref 23. Copyright 2012 American Chemical Society.

HINPs, which is determined by the density of hydrophilic segments on the NP surfaces,  $\rho$  (defined as  $\rho = \sigma/[4(0.5 + R_0/d_{Au})^2]$ ). It is worth noting that a higher  $\sigma$  of polymer tethers can compensate for the reduction of hydrophilicity at elevated  $R_0/d_{Au}$ , thus triggering the assembly of HINPs.

The spacing between adjacent NPs in GVs  $(D_{Au})$  can be tuned by controlling the length of PS. Figure 3e shows that the average  $D_{Au}$  approximately linearly increases from 5.7  $\pm$  0.9 to 10.9  $\pm$  2.9 nm as the PS molar mass increases from 11.9 to 47.3 kg/mol. Such precise control over  $D_{Au}$  enables tuning of the coupling interactions between NPs and thus their plasmonic properties. It was found that  $D_{Au}$  decreased from 19.3 to 7.89 nm after the formation of vesicles, indicating rearrangement of the polymer segments from the gap between pairs of NPs toward both sides of vesicular membranes. This suggests that the conformational change of BCP brushes due to minimization of the interfacial energy contributes to the generation of anisotropic interactions between HINPs.

The assembly of HINPs by film rehydration can be affected by various factors, including the properties of the substrates for HINP films, physical features of the films (e.g., thickness), and the rehydration conditions (e.g., sonication power). As a result, the size, size distribution, and morphology of the assembled structures may vary from batch to batch. In addition to further understanding of the assembly mechanism, future research should improve the robustness and scalability of this assembly technique.

3.2.2. Self-Assembly in Selective Solvents. We explored the assembly thermodynamics of HINPs made from PS-b-PEOgrafted AuNPs in selective solvents (i.e., THF/H<sub>2</sub>O).<sup>46</sup> During the self-assembly, hydrophobic PS blocks on HINPs collapse to minimize the overall free energy of the system, leading to the formation of various nanostructures. The dimensions and morphologies of the assemblies are strongly dependent on the NP size and PS length. The assembly morphology transitions from hollow vesicles to small clusters to unimolecular micelles as the PS length decreases. Our dissipative particle dynamics (DPD) simulations based on coarse-grained potentials suggest that the morphological transition of assemblies is largely determined by the reorganization of brushes on the NP surfaces and the deformability of the polymer "shell". When the PS block is short, the PS segments cannot stretch and compact sufficiently to generate an anisotropic surface on the HINPs; thus, the HINPs preferentially aggregate into small clusters or unimolecular micelles. Once the PS block is sufficiently long, the HINPs can be effectively deformed as a result of



Figure 4. (a–c) Self-assembly of PS-*b*-PEO-grafted AuNPs (a) into chain vesicles (b) and nonchain vesicles (c). (d, e) SEM images of chain vesicles (d) and nonchain vesicles (e). Reprinted with permission from ref 26. Copyright 2015 Wiley-VCH.

redistribution of the long, flexible polymer tethers. The resulting HINPs behave as colloidal analogues of ABA tri-BCPs consisting of a middle hydrophobic section and two hydrophilic side blocks in self-assembly.

The  $\sigma$  of BCPs on NPs is crucial to the directional interactions between HINPs and their assembly paths toward different architectures. We demonstrated that depending on  $\sigma_{i}$ such HINPs assembled into either chain vesicles composed of linear NP strings or nonchain vesicles with a uniform distribution of NPs in the membrane (Figure 4).<sup>26</sup> The stepwise organization of HINPs into NP strings and further into chain vesicles occurs only at a relatively low  $\sigma$  (~0.03  $chain/nm^2$  for HINPs made from 13 nm AuNPs) (Figure 4d). The interparticle distance within chain vesicles is ~0.8 nm, which is much smaller than the value of  $\sim$ 9.0 nm for nonchain vesicles. As a result, chain vesicles exhibited a drastic red shift from  $\sim$ 540 to  $\sim$ 760 nm in the localized surface plasmon resonance (LSPR) peak due to strong plasmonic coupling between NPs, while a red shift of only ~40 nm was observed for nonchain vesicles.

The  $\sigma$ -dependent stepwise assembly of HINPs constitutes a novel assembly mechanism. In the initial stage, the formation of NP strings is a consequence of many-body interactions between HINPs.<sup>11</sup> When two HINPs with a low  $\sigma$  interact because of solvophobic attraction, the redistribution and deformation of hairy polymers creates a high-density polymer region near the point of contact. Consequently, a third particle preferentially approaches from the ends because of attractive van der Waals (vdW) forces rather than from the sides of the cluster because of increased steric hindrance of the high-density hairs. This is evidenced by the larger separation distance between strings (~12 nm) in vesicular membranes compared with the spacing between neighboring AuNPs in each string (~0.8 nm).

Conversely, interactions among HINPs with high  $\sigma$  are mediated by solvophobic forces rather than vdW forces. These many-body interactions lead to 2D planar monolayers that better shield the hydrophobic PS from the solvent medium and thus eventually the formation of nonchain vesicles.

In the future, more systematic studies should be performed to further understand the assembly mechanism of HINPs. Current experimental studies are centered on AuNPs tethered with polymers through Au–S boding. The assembly system might be more complicated than it looks, as polymers grafted by this approach are believed to be mobile on the surface of the NPs. Moreover, when shaped nanocrystals are used as HINP cores, the difference in the affinity of polymers to different crystal facets may increase the complexity of the system.

3.2.3. Coassembly of HINPs. Concurrent self-assembly of multiple building blocks has emerged as a versatile strategy for fabricating complex nanostructures.<sup>49</sup> Our group showed that amphiphilic HINPs composed of PS-b-PEO-tethered AuNPs can coassemble with free PS-b-PEO to generate a variety of hybrid vesicles with well-defined shapes and surface patterns in selective solvents (Figure 5).<sup>47</sup> They include patchy vesicles (PyVs) with multiple small HINP domains, Janus-like vesicles (JVs) with two distinct halves, and heterogeneous vesicles (HVs) with uniform distribution of HINPs. The interplay of bending modulus and line tension between the BCP and HINP domains gives rise to hybrid IVs with intriguing shapes, including spherical, hemispherical, and disklike shapes (Figure 5b-e). Compared with BCP domains, the Young's modulus of HINP domains can be readily tuned over a larger range by controlling the relative dimensions of the soft polymer shell and rigid inorganic core.

Our DPD simulations suggest that the lateral phase separation of BCPs and HINPs in vesicular membranes is



Figure 5. Coassembly of PS-*b*-PEO-tethered AuNPs and free PS-*b*-PEO into hybrid vesicles. (a) Schematic illustration of the building blocks. (b-g) SEM images of Janus vesicles (b-e), patchy vesicles (f), and heterogeneous vesicles (g). (h) Proposed phase separation mechanism of binary building blocks. Reprinted from ref 47. Copyright 2014 American Chemical Society.

driven by the conformational entropy gain of flexible polymer tethers, arising from the mismatch in the effective membrane thicknesses of the BCP and HINP domains (Figure 5h). Morphological transitions of assemblies from PyVs to JVs and to HVs can be tuned by varying the PS length. When the PS block is short, the HINPs constantly fluctuate toward the inner or outer leaflets of the BCP membrane, leading to the formation of PyVs. When the PS length is appropriate, complete phase separation between HINPs and BCPs drives the formation of JVs with two distinct halves. However, when the polymer tethers are too long, entanglement of the polymer chains significantly reduces the mobility of the HINPs, leading to the formation of HVs. This result manifests the similarities and differences between the assembly behaviors of amphiphilic HINPs and molecules.

We recently extended the coassembly strategy to the fabrication of multifunctional hybrid vesicles with controlled distribution of multiple types of NPs in the vesicular membrane by coassembling a mixture of hydrophobic magnetic NPs, free polystyrene-*b*-poly(acrylic acid), and PS-*b*-PEO-grafted AuNPs.<sup>50</sup> However, to date there has been no report on the coassembly of multiple types of HINPs in selective solvents.<sup>47,49–52</sup> Future research on this topic will provide guidance for the design of new materials with more complex architectures and richer functionalities.

**3.2.4. Kinetically Controlled Self-Assembly in Micro-fluidics.** The mobility and relaxation of HINPs are significantly slower than those of molecules, suggesting a critical role of

kinetics in HINP self-assembly. We developed a microfluidic strategy to control the assembly kinetics of HINPs to produce various architectures.<sup>48,53,54</sup> HINP assembly was triggered by forcing a solution of HINPs in THF between two water streams to form a laminar flow in microfluidic devices. The diffusive mixing between laminar fluids creates a well-defined solvent gradient to trigger the assembly of HINPs. By variation of the flow hydrodynamics, the assembly process generated various nonequilibrium structures. In the case of PS-*b*-PEO-tethered AuNRs, increasing the volumetric flow rate ratio of THF to water ( $Q_{\rm THF}/Q_{\rm H_2O}$ ) led to the morphological transition of assemblies from small micelles to giant vesicles and to disklike monolayers of AuNRs.<sup>48</sup> The formation of different assemblies was attributed to the difference in the quenching rate of the assembly process at different  $Q_{\rm THF}/Q_{\rm H_2O}$ .

The microfluidic strategy allows control of the size and morphology of assembled structures simply by manipulating fluidic flows.<sup>54</sup> In the case of vesicular assemblies, the vesicle size increased with increasing  $Q_{\rm THF}/Q_{\rm H_2O}$ . Under the same flow conditions, larger HINPs produced larger vesicles because of the reduced diffusion rate. Moreover, our group fabricated hybrid JVs with distinct halves by coassembly of PS-*b*-PEO tethered AuNRs and PtNPs together with free PS-*b*-PEO in MFFDs.<sup>53</sup> The overall size and NP distribution of these JVs can be controlled by varying  $Q_{\rm THF}/Q_{\rm H2O}$  and the relative concentration of free PS-*b*-PEO with respect to HINPs. Such JVs were demonstrated to be multifunctional nanoscale motors



**Figure 6.** (a) Schematic illustration of the use of NIR-responsive GVs for cancer imaging. (b) Photothermal imaging of tumors in mice after different irradiation times. (c) Photoacoustic imaging of tumors in mice before and after injection of GVs. (d) Comparison of in vivo photoacoustic signals for GV and BGV systems. (e) Photoacoustic imaging of mouse tissue before and after injection of chain vesicles or nonchain vesicles. Reprinted with permission from refs 24, 26, and 56. Copyright 2013 and 2015 Wiley-VCH and 2013 American Chemical Society, respectively.

capable of autonomous propulsion fueled by hydrogen peroxide and NIR-light-triggered release of payload.

# 4. **BIOMEDICAL APPLICATIONS**

Our group and others have been actively exploring the design of GVs for cancer imaging, cancer therapy, and imaging-guided drug delivery.<sup>13,24,26,46,55,56</sup> As mentioned above, the LSPR of GVs can be adjusted to the NIR range, which is more ideal than spherical AuNPs for in vivo applications because of its tissue transparency. The intense photothermal heating of GVs upon NIR irradiation enables their use in effective cancer imaging and therapy. Our group demonstrated the use of GVs assembled from 26 nm PS-b-PEO-tethered AuNPs for in vitro and in vivo photothermal and photoacoustic imaging of tumors (Figure 6a-c). After intratumoral injection of GVs into mice with subcutaneous MDA-MB-435 breast cancer, a 3.8-fold increase in the photoacoustic signal in the tumor region was observed (Figure 6c).<sup>56</sup> To further increase the imaging performance of GVs, we designed biodegradable GVs (BGVs) assembled from 26 nm poly(ethylene glycol)-bpoly(caprolactone) (PEG-b-PCL)-grafted AuNPs.<sup>24</sup> The NIR absorption and photothermal conversion efficiency of the BGVs was drastically improved as a result of the reduced distance and enhanced coupling between AuNPs. Preliminary in vivo studies showed that the photoacoustic signal of BGVs was doubled compared with that of GVs (Figure 6d).

It is usually challenging to tune the absorption of assemblies of small AuNPs to the NIR window, as the coupling strength between NPs decreases exponentially with decreasing NP size. The manipulation of NP organization within vesicular membranes offers a new route to enhance the NIR absorption of assemblies and hence their imaging performance. For example, we demonstrated the stepwise-assembly of PS-*b*-PEO-grafted AuNPs into strings and further into vesicles composed of NP strings in the membranes.<sup>26</sup> The NIR absorption of the resulting chain vesicles is much higher than that of nonchain vesicles because of the short interparticle distance in the strings. In in vivo studies, we demonstrated an 8-fold photoacoustic signal enhancement for chain vesicles was only 1.1-fold, compared with control groups (Figure 6e).

The large interior space of hollow GVs allows for efficient loading of therapeutic agents that can then be released remotely using NIR light. Our group demonstrated the NIR-lighttriggered release of model drugs from nanosized and microsized GVs.<sup>48,55</sup> The imaging and delivery capability of GVs enables their use in imaging-guided cancer therapy, in which the location of nanocarriers, release profile of drugs, and outcomes of treatment can be potentially monitored in vivo, thus maximizing the therapeutic efficacy. We demonstrated that GVs loaded with the photosensitizer Ce6 can be used for efficient multimodal-imaging-guided combination therapy of cancers (Figure 7).<sup>56</sup> The Ce6-loaded GVs were evaluated for trimodal (fluorescence, photothermal, and photoacoustic) imaging as well as for photothermal and photodynamic therapy both in vitro and in vivo. Upon 671 nm laser irradiation, the membranes of GVs were disrupted to release the payload, leading to the generation of fluorescence emission for imaging and singlet oxygen species for killing cancer cells (Figure 7a). At the same time, the rise in local temperature allowed for



Figure 7. (a) Schematic illustration of the use of Ce6-loaded GVs for imaging-guided photothermal and photodynamic cancer therapy. (b, c) MDA-MB-435 cell viability (b) and tumor growth curves for different groups treated with GVs, Ce6, and Ce6-loaded GVs with or without NIR laser irradiation (c). Reprinted from ref 56. Copyright 2013 American Chemical Society.

photothermal ablation of tumors as well as photothermal and photoacoustic imaging. In an in vitro study using MDA-MB-435 cells, this platform showed a 45–70% higher therapeutic efficiency than the sum effect of pristine GVs and Ce6 (Figure 7b). A drastically enhanced therapeutic efficacy arising from the same synergistic effect of combined therapies was also observed in our in vivo studies using a breast-tumor-bearing mouse model. The tumor progression was remarkably delayed for groups administrated with Ce6-loaded GVs and irradiated with a laser compared with control groups including free Ce-6 and unloaded GVs with or without laser irradiation on day 14 (Figure 7c).

## 5. CONCLUSIONS AND PERSPECTIVE

Despite promising potential, challenges still remain at this frontier. To achieve a predictive framework for the selfassembly of HINPs into target architectures, the underlying mechanisms of HINP assembly on multiple length scales need to be understood better. This requires elegantly designed experiments complemented by theoretical modeling and simulations. To meet the increasing demand for the construction of more complex architectures, concurrent selfassembly of multiple types of HINPs might be a good choice.<sup>49</sup> However, this will also pose new challenges due to the complexity and multiple-length-scale features of the forces involved in the assembly. In order to tackle this issue, new advances in computational modeling (e.g., newer models and algorithms) are needed.

Current studies on anisotropic self-assembly of HINPs are still mostly centered on manipulating fairly simple NP interactions (e.g., hydrophobic–hydrophobic). With appropriate polymer design, more complex interactions such as electrostatic interactions, dipole–dipole interactions, host–guest interactions, hydrogen-bonding interactions, metal chelating, etc. could conceivably be integrated into HINP systems to achieve higher complexity or new functionality of assembled materials. Moreover, depending on the specific shape and compositional features of the inorganic NP cores, external fields (e.g., shear field, electric and magnetic fields) or templates can be applied to achieve more exquisite control over anisotropic assembly of HINPs.<sup>57,58</sup> Additionally, many materials in nature (e.g., mineralized bone) exhibit several levels of structural hierarchy, which has not been seen in assembled structures of HINPs to date. More efforts should be devoted to the development of creative strategies for stepwise self-assembly of HINPs into a wealth of functional multi-hierarchical nanocomposites.<sup>27</sup>

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