Engineering heterogeneity of precision nanoparticles for biomedical delivery and therapy

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Abstract
Nanoparticle (NP)-based vehicles are attractive for biomedical delivery and therapy, owing to improved therapeutic efficiency and reduced adverse effects compared to free drugs. Uncontrolled NP heterogeneity at the suspension level or single particle level poses grand challenges in the effective use of NPs for biology and medicines. Precision nanoparticles (PNPs) are a class of discrete nanostructures exhibiting elaborately tailored surface or structural heterogeneity, but minimal variations between nanostructures in one solution. Benefiting from their precisely controlled NP heterogeneity, PNPs have emerged as promising platforms for modulating various biological processes to improve the biomedical performance of NP-based vehicles. This review summarizes recent advances in the development of precisely tailored PNPs for biomedical applications, with an emphasis on the nano-bio interactions, and drug delivery and therapy. We start with a brief overview of the major categories of PNPs and their fabrication strategies (section 2). We then focus on reviewing the use of PNPs for enhancing biomolecular recognition or detection, regulating the NP-protein interactions, modulating the NP-biomembrane interactions, and improving the biological delivery and therapy (section 3). Finally, we discuss the challenges and perspectives on the design and use of PNPs for biomedical applications (section 4).

KEYWORDS
assemblies, drug delivery, nano-bio interaction, nanoparticles, therapy

1 INTRODUCTION

Nanoparticle (NP)-based medicines are extensively explored for a variety of applications, including cancer therapy1, diabetes treatment,2 anti-infections,3 and anti-inflammation,4 owing to their improved therapeutic efficiency, and reduced adverse effects compared to free drugs. However, there remains challenging for sufficient accumulation of nanomedicines in targeted sites: Only about 0.7% (median) of intravenously administrated nanomedicines could be delivered to their destination.5 Intravenously-injected nanomedicines undergo the so-called circulation, accumulation, penetration, internalization, and release cascade process before reaching diseased...
sites. During this process, NPs may encounter a series of biological barriers (e.g., reticuloendothelial system clearance, dense extracellular matrix, and tightly packed tumor cells), leading to reduced delivery efficiency. The in vivo distribution and fate (e.g., formation of protein corona, cellular internalization, blood circulation, extravasation into perivascular microenvironment, tissue penetration, metabolism, and clearance) of the NP vehicles are governed by their physicochemical parameters of the NPs including size, shape, stiffness, porosity, elasticity, surface chemistry, and targeting ligands. In the past decades, tremendous efforts have been devoted to modulating the interactions between NPs and biological milieu (i.e., biofluids, biostructures, or microenvironment) to improve the biosafety, targeting efficiency and therapeutic performance of nanomedicines. However, our current understanding of nano-bio interactions is still limited largely because of the complexity and dynamicity of biological environment, as well as a usually overlooked aspect—the heterogeneity—of synthetic NPs.

NPs synthesized in the laboratory are usually heterogeneous either at the suspension level or at the single particle level. First of all, organic or inorganic NPs dispersed in solutions are usually different from each other. They display interparticle variations in their size, shape, morphology, etc. The particle-to-particle difference between NPs makes it difficult to determine which of the many factors in a single dispersion takes effect in a specific pathway, especially when the NP heterogeneity is not aware or not well characterized. Moreover, at the single particle level, NPs are not completely homogeneous in terms of their surface and structure. They exhibit intraparticle variations in such as, the distribution of targeting motifs, surface charges, ligand densities, ligand types (e.g., hydrophilic and hydrophobic microdomains), and topological features. Such NP heterogeneity at the single particle level can alter the interactions between NPs and proteins or cell membranes in vivo, thus influencing the pharmacokinetics, biodistribution, and fate of nanomaterials. In addition, the NP heterogeneity can even cause cytotoxicity or unwanted immune activation in biological systems. Nevertheless, uncontrolled NP heterogeneity poses grand challenges in the effective use of NPs for biomedical delivery and therapy, and in understanding the biological behaviors of NPs in vivo, which has become the barriers to the development of personalized medicines.

Over the past decades, there have been numerous efforts to address issues relevant to heterogeneity of NPs used in medicines. In particular, precision NPs (PNPs) (or nanoassemblies) have emerged as promising platforms for manipulation of their bioprocesses. Here PNPs refer to discrete uniform nanostructures exhibiting elaborately tailored surface or structural heterogeneity, but minimal variations among different nanostructures. One typical example is highly uniform DNA origami nanostructures with precisely engineered binding sites for species, such as targeting motifs, drug molecules, inorganic NPs, etc. Monodispersed PNPs with precisely tailored intraparticle variations show the following advantages over conventional nanoplateforms for medicine: 1. They enable us to establish quantitative correlations between characteristic parameters of NPs and their biological performances. 2. The elaborately designed surface heterogeneity of PNPs can accurately regulate the interactions of NPs with proteins and biomembranes, thus enhancing the accumulation of NPs at diseased sites. 3. The precise control over the content and position of therapeutic agents in each PNP can maximize the therapeutic efficiency and reduce adverse effects. 4. They can carry multiple types of therapeutic compounds with predetermined ratios and release them at one site simultaneously or multiple sites sequentially for synergistic therapy. 5. Multiple functional components (e.g., fluorescent dyes, drugs, targeting motifs) can be integrated in one PNP with controlled amount and site for each to perform multiple tasks of imaging, diagnosis, and therapy in vivo. In recent years, reasonable progress has been made in the development of PNPs to study their nano-bio interactions and enhance their performance in nanomedicines, such as efficacy of cancer diagnosis and therapy. For instance, Ding and coworkers designed a chemically well-defined DNA nanorobot to specifically deliver thrombin to tumor-associated blood vessels. The thrombin loaded in nanorobots could be inhibited during blood circulation and activated to coagulate at the tumor site to inhibit tumor growth via intravascular thrombosis.

This review summarizes recent advances in the development of precisely tailored PNPs for biomedical applications, with an emphasis on the biomolecular recognition, nano-bio interactions, and biomedical delivery and therapy (Figure 1). Our discussions focus on NPs with precisely designed surface and structural heterogeneity, rather than as-synthesized NPs, for medicines. We do not intend to provide a full literature survey and apologize if any important research work has not been cited or discussed here. In this review, we first briefly summarize the major categories of PNPs and their fabrication strategies (section 2). We then focus on discussing the use of heterogeneous PNPs to enhance biomolecular recognition or detection, regulate the NP-protein interactions, modulate the NP-biomembrane interactions, and improve the biological delivery and therapy through several representative examples (section 3). Finally, we discuss the challenges and perspectives on the design and use of PNPs for nanomedicine (Section 4).
FIGURE 1  Schematic representation of design and development of PNPs with precisely tailored surface and structural heterogeneity for biomedical delivery and therapy.

2  DESIGN AND FABRICATION OF PNPs

This section briefly summarizes two typical classes of PNPs, that is, highly uniform patchy NPs with tailored surface heterogeneity and discrete NP assemblies with defined spatial arrangement and surface of inorganic NPs. Specifically, here patchy NPs generally refer to those single nanoparticles with a well-defined number of heterogeneous decorations, while discrete NP assemblies are composed of two or more nanoparticle building blocks. Because the relevant field is broad, we only selectively highlight a few representative types of PNPs used in biological studies. The characteristic features of some representative PNP examples are summarized in Table 1. Readers who are interested in this topic are recommended to refer to more comprehensive review articles.25

2.1  Patchy nanoparticles

Patchy NPs can be tentatively divided into two major categories: 1. Nanostructures site specifically decorated with a well-defined number of molecules, such as polymers,20 DNAs,26 and proteins.27 The site-specific molecular tethers can be originated from post-functionalization of as-synthesized NPs or predesign of binding motifs on DNA building blocks of precise nanoassemblies (e.g., DNA origami).28 2. Nanostructures with topographically or chemically distinctive surface patches. They include such as inorganic NPs modified with ligand patches,25d,29 multicompartment polymer micelles obtained from phase separation,22,30 etc.

Precisely controlling the number and site of ligands on as-synthesized NPs is nontrivial. During surface functionalization, the attachment of a molecular ligand on an NP via a binding group does not limit the binding of a second molecule on another site of the NPs. As a result, the number of ligands on NPs is inevitably different from particle to particle in one batch (i.e., interparticle variation in ligand number). A number of strategies have been developed to fabricate NPs grafted with a discrete number of ligands at specific sites.26,31 One common approach relies on the binding of NPs to a surface (or a large particle) with a monolayer of molecules composed of segments to be attached onto the NPs.31a,32 As shown in an exemplary scheme in Figure 2A, the disulfide groups of 3′-amino-5′-disulfide DNA linkers adhered (via amino groups) on a carboxyl group-rich silica particle are reduced to thiol groups.31a AuNPs are attached onto the silica particle through thiol groups and released from the silica substrate upon neutralization of the carboxylic groups. The number of DNA strands picked up by an AuNP from the silica surfaces each time is governed by the size of AuNPs and the DNA grafting density on the silica surfaces. (This sentence is in the wrong place. Additionally, 26b and 26c need to be cited to support this sentence) This process can be repeated to obtain NPs decorated with precisely controlled number (up to six) of DNA molecules. During the surface modification, steric and electrostatic repulsion are used to confine the anchoring sites and orientation of newly added DNA strands on the NPs. To improve the purity of products, gel
### Table 1: Characteristic features of some representative types of PNP s

| Subclass                              | Components or building blocks                                                                 | Surface patterns or Structures                                                                 | Ref. |
|---------------------------------------|-----------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|------|
| Patchy nanoparticles                  | Nanostructure site-specifically decorated with precise numbers of motifs                     | Stoichiometric DNA tethered Au NP \((n = 1\sim6)\)                                              | (31a) |
|                                       | ※Au NP                                                                                         |                                                                                                 |      |
|                                       | ※Stiff DNA                                                                                     |                                                                                                 |      |
|                                       | ※Au NP                                                                                         |                                                                                                 |      |
|                                       | ※Single strand DNA                                                                           |                                                                                                 | (26b) |
| Inorganic NP with distinctive surface patches | ※Au NP                                                                                         | Patchy nanospheres \((n = 1\sim4)\)                                                           | (34) |
|                                       | ※Thiol ended polystyrene                                                                      |                                                                                                 |      |
| Multicompartment polymer micelles     | ※polystyrene-block-polybutadiene-block-poly(methacrylic acid)                                 | Football/Clover/Hamburger                                                                      | (30b) |
| Discrete precise assemblies of patchy NPs | ※Au NP                                                                                         | Core-satellite \((Satellites/core = 2, 8, 16, 24)\)                                          | (45c) |
|                                       | ※Single strand DNA                                                                           |                                                                                                 |      |
| DNA origami-based assemblies of NPs   | ※Au nanorod                                                                                   | Tripod \((trimer of Au nanorods)\)                                                            | (41b) |
|                                       | ※DNA origami                                                                                  |                                                                                                 |      |
|                                       | ※Au triangle                                                                                  | Bowtie \((dimer of Au triangle)\)                                                             | (41a) |
|                                       | ※DNA origami                                                                                  |                                                                                                 |      |
| Polymer-guided assembly of NPs        | ※Au@\((\text{acrylic acid-r-styrene})-\text{b-poly(ethylene oxide)}\)                       | AB\(_x\) \((x = 1\sim6)\) colloidal molecule                                               | (42)  |
|                                       | ※Au@\((\text{N,N-dimethylamino ethylmethylacrylate-r-styrene})-\text{b-poly(ethylene oxide)}\) |                                                                                                 |      |

Electrophoresis is usually required to separate desired NPs from unwanted byproducts (e.g., NPs with other numbers of molecules).\(^{26b,c}\) In addition to direct grafting method, unimolecular micelles as a template can be used to prepare patchy NPs with discrete number of molecular ligands.\(^{33}\) For instance, Yang and coworkers prepared unimolecular micelles with one or two extended arms at high yield via intramolecular cross-linking of single polymer chains and subsequently deposited metal (or metal oxide) in the micellar core rich in carboxyl (or tertiary amino) group to form PNP s tethered with one or two polymer chain(s).

The wrapping of long chain on NPs can also be used to introduce discrete number of binding sites on NPs. Single-strand DNA \((ssDNA)\) is programmed to have alternating sticky polyadenine domains and non-sticky binding domains. The polyadenine segments of ssDNA are strongly coordinated with the surface of AuNPs, leaving the binding domains extending into solution for specific hybridization reactions \((\text{Figure 2B})\).\(^{26b}\) The number and type of binding domains can be controlled by designing ssDNAs with different order, length, sequence of these sticky, and nonsticky segments. Similarly, the generation of products with high purity necessitates the separation of different fractions of the NPs using gel electrophoresis.

DNA origami is an emerging technology of folding long scaffold DNA strands into complex nanostructures using multiple staple binding strands. The DNA origami nanostructures can be tailored to have precisely controlled size and geometry (e.g., tetrahedron, cube, octahedron, icosahedron, etc.) by programming the DNA chains.\(^{35}\) Importantly, the constituent ssDNA can be designed to extend from any specific sites (e.g., vertexes or edges) of the nanostructures to produce specific motifs for binding with different species (e.g., NPs tethered with complementary oligonucleotides). The encaging of as-synthesized NPs within predesigned DNA origami nanostructures offers a powerful strategy to fabricate site-specifically functionalized patchy NPs. As shown in an example in Figure 2C, 3’-thiolated oligonucleotides tethered AuNPs are embedded into a planar square-like frame of DNA origami nanostructures via hybridization of complementary ssDNAs.\(^{28a}\) The stoichiometry, spatial distribution, and type of functional motifs on the AuNPs are precisely tuned to enable the precise direction interactions between the NPs.

DNA-based strategy allows for fabricating patchy NPs with high precision and complexity, but the method suffers from its high cost, low yield, and poor scalability. Phase-segregation of end-grafted polymers on NPs provides a simple yet inexpensive approach to the preparation of NPs with surface patterns of polymers.\(^{29a-c,36}\) For instance, when water was added into polystyrene-grafted AuNPs in DMF, the end-grafted polymer chains collapsed and segregated to form pinned micelles on the surface of NPs and hence defined polymeric patches \((\text{Figure 2D})\). The
segregation of end-grafted polymers is governed by polymer length, core diameter, polymer grafting density, and solvent quality. This strategy allows for effective control over the dimensions, spatial distribution and surface density of patches on NPs. When anisotropic NPs with different shapes are used, the formation of patches on NPs strongly depends on the shapes of inorganic NPs. For Au nanocubes, polymeric patches are preferentially formed on the high-curvature regions (e.g., vertices and edges) of the NPs.

In addition to patchy NPs with a rigid inorganic core, soft patchy NPs are attractive for biomedical applications owing to their good biocompatibility and degradability. Self-assembly of block copolymers offers an efficient tool to fabricate soft patchy NPs with physically or chemically distinct domains via phase separation. As shown in a representative example in Figure 2E, multicompartiment micelles can be produced by step-wise phase separation of tri-block copolymers. ABC triblock copolymers first form core-corona micelles with B block as the core and A/C
as the corona when non-solvent for B block is introduced. Subsequent addition of non-solvent for A block triggered the further phase-segregation of the blocks to form multi-compartment micelles with controllable surface patterns. Moreover, after the crosslinking of B patches, each multi-compartment micelle can be further dissociated into multiple Janus particles that are well dispersed in a good solvent for all the blocks.

2.2 Discrete precise assemblies of NPs

NP assembly-based PNPs show topological features determined by the shape and spatial arrangement of NP building blocks. Naturally, the surface of constituent NP subunits in the assembly can be tailored to show precisely controlled surface heterogeneity of the resulting PNPs. Discrete yet precise NP assemblies can be prepared by assembling defined patchy NPs (including NPs site-specifically modified with a defined number of ligands), template- (e.g., DNA origami) assisted assembly of NPs, polymer-guided assembly of NPs, etc. A variety of PNPs of this category have been assembled from inorganic NPs with different sizes, shapes (e.g., spheres, cubes, rods, etc.), and compositions (e.g., gold, silver, CdSe, FeOx etc.). Representative examples of such PNPs include such as chiral arrays of NPs, precise NP clusters (i.e., colloidal molecules), and even highly complex NP assemblies with a human shape (Figure 3).28a,40

As aforementioned, DNA origami technology has enabled the design of precise nanostructures with astonishing accuracy and complexity. More importantly, binding motifs for species (e.g., NPs, proteins) can be precisely programmed to locate at specific sites of the nanostructures. The origami structures served as ideal templates for precise position of NPs in space through hybridization of single DNA strands extended from DNA origami structure and complementary oligonucleotides decorated on NPs. Figure 3A shows a list of representative precise two- and three-dimensional NP assemblies based on DNA origami nanostructures. They include bowtie nanostructures of Au triangles, tripod of AuNRs, triangular double cone of AuNRs, spiral toroid of AuNRs, accurately oriented dimers of AuNRs, Au Vitruvian Man.28a,41 This method allows for precise control over the spatial distribution, interparticle, spacing and orientation of bounded NPs on the templates, as well as the stoichiometry of surface motifs or the proportion of functional multiple components.

The chemical or topological surface patches of patchy NPs can direct the attractive interactions between NPs and guide the precise assembly of NPs. Metal NPs (e.g., gold) tethered with ssDNA are the most widely used building blocks for assembling discrete-defined NP clusters (e.g., NP dimers, trimers, tetramers, etc.) via hybridization of complementary DNA strands. As shown in an example in Figure 3B, AuNPs (~10 nm in diameter) were modified with four ssDNAs containing different sequences (r’, p’, b’, and g’) via gold-thiol bonds to serve as A, and four kinds of smaller NPs (~6 nm in diameter) were modified with corresponding DNA linker with complementary sticky ends to serve as r-B, p-B, b-B, and g-B.26c The hybridization of DNA strands on NPs triggered the assembly of A and B to form various well-defined AB, AB2, AB3, and AB4 clusters, when specific types of B particles were introduced. The yield of ABx falls into the range of 57%~88%. Further purification is required to improve the yield of desired products.

Although assembly of patchy NPs is powerful for materials fabrication, the preparation of high-quality patchy NPs, specifically from as-synthesized inorganic NPs, at high yield remains challenging. The adaptive conformation change of polymer ligands on NPs offers a simple yet effective route to induce directional interactions between NPs and guide the assembly of NPs to form precisely controlled nanostructures.39c,39d,42,46 For instance, Nie and coworkers demonstrated the directional bonding of binary NPs into precise NP clusters with molecular-like configurations at high yield (Figure 3C).42 One of the binary NPs (A) was functionalized with poly(ethylene oxide)-block-(acrylic acid-random-styrene) [PEO-b-(AA-r-St)] and another (B) was modified with poly(ethylene oxide)-block-poly(N,N-dimethylaminoethyl methacrylate-random-styrene) [PEO-b-P(DMAEMA-r-St)]. When the two types of NPs were mixed in solution, the neutralization reaction between weak acid and base groups on NPs triggered the precise assembly of NPs into ABx nanostructures (x is the coordination number of B onto A) with high symmetry. The value of x is governed by the reaction stoichiometry of complementary reactive groups on single A and B, and the symmetry of the assemblies is determined by the steric and Coulombic repulsion between A-B colloidal bonds. In addition to high yield, the method does not require asymmetric surface functionalization of NPs, thus enabling simple, scalable, and efficient production of PNPs from different inorganic NPs for biological applications.

3 BIOLOGICAL APPLICATIONS OF PNPs

3.1 Enhancement of molecular recognition and detection

Molecular recognition through specific ligand-receptor interaction is ubiquitous in biological systems and plays a pivotal role in the function of living organisms. The receptors on cell membranes are complex with respects to
their spatial organization and accurate conformation. As a result, the binding affinity between NPs and cell membranes mainly depends on the stoichiometry and spatial distribution of ligands on the surface of NPs. Precise control over ligand distributions on NPs offers a route to regulate and enhance the recognition interactions between NPs and receptors on cell membranes. For instance, Zhu and co-workers prepared highly monodispersed \( n \)-simplexes \((n = 0, 1, 2, 3)\) of DNA scaffolds with accurate algebraic topologies via hybridization of complementary DNA strands and conjugated aptamers with precise stoichiometric number on the vertices of the DNA nanostructures. The precision nanostructures were used to investigate their binding interaction with EpCAM receptors on the membrane of EpCAM positive MCF-7 cells, EpCAM negative Ramos cells, and Hela cells. Among all the simplexes, 2-simplexes (i.e., DNA triangles) showed a highest \( \sim 18.6 \)-fold increase in binding affinity but poor endocytosis by cells, as compared with free aptamer ligand. The improved cell adhesion could be attributed to the stronger position constraining and reduced cellular internalization of 2-simplex, as confirmed by dissipative particle dynamics simulation.

Inspired by the enhanced binging affinity between cells and PNPs carrying spatially organized binding sites, Zhu and co-workers further developed a family of tetrahedral...
DNA frameworks (TDFs) which exhibited effective binding control for cell patterning and binding strength control of cells for cell sorting. The TDFs could be used as building blocks to topologically control the stoichiometry and spatial arrangement of ligands, thus leading to well-controlled binding strength of the elaborately designed nanoarchitectures. TDF was used as scaffold to construct 0-simplex, 1-simplex, 2-simplex by anchoring one, two, and three aptamers on the vertices of TDF, respectively. It was found that 2-simplex exhibited the highest binding affinity to EpCAM positive MCF-7 cells within the range of 33 pN–43 pN, whereas the aptamer ligand-EpCAM interaction lies mostly in the range of 4.7–9.6 pN. This enhanced binding strength of TDFs could be ascribed to the topological ligands-induced EpCAM rearrangement.

Discrete assemblies of inorganic NPs show collective properties owing to the coupling interactions between surface plasmons, excitons, and magnetic moments. The synergistic properties of the NP assemblies are sensitive to the spatial organization of NPs, which makes the nanomaterials attractive for molecular detection. Various precision assemblies of inorganic NPs (e.g., Au NP/Au NPs, Au nanorods/upconversion NPs) have been developed for the ultrasensitive detection of disease-related biomarkers. For instance, Xu and coworkers assembled Au nanorods and Yb/Er-doped NaGdF₄ upconversion NPs (UCNPs) into uniform planar tetramers composed of one UCNP surrounded by three Au nanorods via hairpin-like DNA linkers. The precision nanoassemblies were used to detect oligonucleotides (Hepatitis A virus Vall7 polyprotein gene) through interparticle distance-dependent variations of chiroptical and luminescent signals (Figure 4B). When targeting oligonucleotides were introduced, the hairpin-like DNA linkers opened to extend the interparticle distance between gold nanorods and central UCNP, resulting in a drastic decrease in both CD and luminescence signals. The limit of detection (LOD) was 20.3 aM for luminescence-based sensors and 13.2 aM for CD-based sensors, which is enhanced by about 21-fold in luminescence than the conventional UCNP-based sensors.
with pre-designed recognition sequences and fluorescence labels to simultaneously quantify two distinct miRNAs (miR-21 and miR-200b) as tumor markers (Figure 4C). When miR-21 was present, the hybridization of miR-21 with recognition sequences on AuNRs triggered the dissociation of UCNPs from AuNRs to recover the upconversion luminescent (UCL). Similarly, the presence of miR-200b induced the detachment of Cy5.5 labeled UCNPs from AuNRs to recover its UCL. The precision assemblies-based bimodality strategy exhibited an LOD of 3.2 zmol/ng miR-21 and an LOD of 10.3 zmol/ng miR-200b in living cells, which is thousands of orders of magnitude lower than other UCNP or plasmonic nanoparticle-based sensors.

### 3.2 Regulation of specific/nonspecific interaction of NPs with proteins

Understanding the nano-bio interactions in the dynamic and complex physiological environment is crucial to the development of safe and efficient nanomedicines. The nonspecific formation of protein corona is one of the typical examples of protein-nanoparticle interactions. In biological fluids such as serum, urine, synovial, and cerebrospinal fluid, nanomaterials can adsorb various proteins to form a protein corona with multilayers of proteins on the surface (Figure 5A). The protein corona plays a crucial role in determining the blood circulation, clearance, biodistribution, biodegradation, and delivery efficiency of NPs. For example, preferential adsorption of opsonins on NPs could enhance the phagocytosis and removal of NPs from circulation. In stark contrast, absorption of serum albumin and apolipoproteins on NPs was found to extend the blood circulation time of NPs. Protein corona formed on NPs could mask targeting groups (e.g., transferrin) conjugated on NPs and reduce the delivery efficiency of NPs in tumors. In another case, the delivery of NPs with well-tailored surface or structures can protect the cargoes from specific enzymes with degradation function. Cationic polymers on NPs could shield nucleic acid drugs loaded on NPs from being degraded by endonucleases in serum.

Engineering the chemical patterns of ligands on NPs can be used to manipulate the interactions between proteins and NPs. For example, PEGylation and zwitterionic modification of NPs have been widely used to reduce the nonspecific interactions between proteins and NPs. As a result, tailored surface heterogeneity of NPs can help prevent the nonspecific absorption of proteins, which is crucial to extend the circulation time of NPs in vivo and to enhance the accumulation of NPs at targeted sites. For instance, Stellacci and coworkers studied the interactions of heterogeneous Au NPs with stripe-like patterns of ligands with proteins. A mixture of mercaptopropionic acid (MPA) and 1-octanethiol (OT) ligands were attached

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**Figure 5** (A) Protein corona formation on 60 nm AuNPs in serum. Copyright 2020, American Chemical Society. (B) Simulation of the lowest-energy binding conformations of Cyt C on NPs with heterogeneous surface. Gray tubes represent protein backbone; CG model of HEME is shown using large, red spheres; residues which interact significantly with the NP are shown as spheres: Q, F, K, P represent glutamine, phenylalanine, lysine, and proline respectively. Copyright 2011, American Chemical Society. (C) Controlled degradation of five types of precision nano-assemblies with different structures and DNA linkers in serum. Copyright 2016, National Academy of Sciences.
onto the surface of Au NPs. The binary ligands spontaneously assembled into parallel ripples with alternating hydrophilic and hydrophobic regions on the NPs. The patterned NPs effectively prevented the nonspecific adsorption of proteins (e.g., lysozyme, fibrinogen, and protein cytochrome C).61 On the other hand, the organization or conformation of absorbed on NPs can be controlled by precisely engineering the surface heterogeneity of NPs. For instance, simulation result in Figure 5B shows that the lowest-energy binding conformations of Cyt C on NPs are strongly dependent on the surface heterogeneity of NPs.62 The result indicates the feasibility of manipulating the interaction between proteins and NPs by tailoring the heterogeneous patterning of ligands on NPs. In another example, the orientation of bovine serum albumin (BSA) adsorbed on Au NPs was found to be dependent on the surface patterns, rather than the chemical composition, of small molecular ligands on the NPs. Binary ligands of negatively charged 11-mercapto-1-undecanesulfonate (or MPA) and neutral OT were capped on AuNPs to have stripe-like patterns or random distribution of ligands, while possessing a similar chemical composition of ligands. BSA proteins absorbed on the NPs primary through electrostatic interactions. The absorbed proteins seemly adopted a “side-on” conformation on NPs with randomly distributed ligands, but an “end-on” conformation on NPs with stripe-like ligand patterns. PNPs with heterogeneous distribution of one or more binding ligands on the surface can be obtained by site specific modification of Janus or patchy NPs.64

Other than conditions in vitro, control over the interactions between proteins and PNPs not only determines the in vivo circulation of the NPs, but also offers a route to regulate the stability of the nanoarchitectures in physiological fluids with abundant enzymes (e.g., serum and whole blood).65 Chan and co-workers systematically investigated the degradation mechanism of precisely assembled clusters of DNA/PEG-modified Au NPs in serum.21 The degradation process of the NP clusters was governed by the physicochemical parameters (e.g., grafting density, ligands length, parity of DNA) of the assemblies and displayed three distinctive models, namely exonuclease, endonuclease, and desorption. For NPs carrying a high DNA density, short DNA strands, and thick PEG coatings, the steric shielding and stealth effect of PEG coatings protected the NPs from endogenous enzymes and prevented the exonuclease cleavage, thus enhancing the stability of NP clusters in serum. It was found that a relatively low PEG density of 0.64 chains/nm² was sufficient to protect the NPs from enzymatic degradation. In addition, blocking exposed 3’ ends of oligonucleotides or tethering 3’ ends onto NPs could also improve the stability of building blocks in serum. Transmission electron microscope (TEM) images in Figure 5C show the time-dependent degradation of five types of assemblies with different structures and surface features in serum. Thus, tailoring the degradation behavior of precision assemblies offers a promising route for multistage release of payloads from the nanocarriers.

The selective interaction with specific proteins in complicated biological fluids can be manipulated by precisely tailoring the organization of surface motifs on NPs. The NPs with precisely engineered surface can selectively interact with pathogenic proteins or function proteins, thus achieving the effective disease treatment. Shi and coworkers developed an interesting type of chaperone-like polymeric micelles with heterogeneous hydrophobic surface domains to prevent Alzheimer’s disease (AD) induced by the aggregation of amyloid beta peptides (Aβ) into amyloid plaques.66 The chaperone-like micelles were fabricated by co-assembling poly(β-amino ester)-block poly(c-caprolactone) (PAE-b-PCL), and poly(ethylene oxide)block-poly(c-caprolactone) (PEG-b-PCL) in an acidic aqueous solution into micellar structures with a hydrophobic PCL core and a mixed PAE/PEG shell, followed by deprotonation to collapse PAE segments into microdomains between outstretched PEG segments. The micelles with precisely tailored surface micro-domains could selectively capture Aβ via hydrophobic interactions and suppress Aβ aggregation, thus alleviating early symptoms of AD. Apart from the prevention of AD, artificial chaperones were also utilized to enhance the stability of insulin under hyperthermia/enzymatic degradation conditions67 and to refold the denatured lysozyme into bioactive states by elaborately designed surface micro-domains.68

3.3 Regulation of the interactions of NPs with bio-membrane and cells

The interactions between NPs and biomembranes are essential to the biological performance of NPs in vivo, such as enhanced NP accumulation in desired sites by suppressing their clearance through mononuclear phagocyte system,53c,69 the accurate delivery of therapeutic agents to cellular sub-organelle at a low cytotoxicity,70 and the effective activation of immune cells in vivo.71 NPs can interact with components of the plasma membrane or extracellular matrix to enter the cell via endocytosis, which leads to the engulfment of NPs in membranes, followed by their budding to form endocytic vesicles for intracellular sorting/trafficking. Depending on the cell type and biomolecules (e.g. proteins and lipids) involved in the process, endocytosis could be classified as phagocytosis, clathrin-mediated endocytosis, caveolin-mediated endocytosis, clathrin/caveole-independent endocytosis, and
The spatial organization of surface ligands on NPs plays an important role in the penetration of NPs through cell membranes. Therefore, PNs with precise control over ligand organization have been proposed to enhance the endocytosis of NPs and provide guidance for designing better nanocarriers for the delivery of imaging and therapeutic compounds. The hydrophilic/hydrophobic ligand distribution had a profound effect on the cellular internalization and sub-organelle location of NPs with different surface structures (Figure 6A). Hydrophilic 11-mercapto-1-undecanesulphonate (MUS) and hydrophobic OT mixture ligands were capped on the surface of Au NPs (∼6 nm in diameter) to produce: 1. structured NPs (s-NPs) with stripe-like patterns of binary ligands (molar ratio: MUS:OT = 34:66), 2. unstructured NPs (u-NPs) with random distribution of ligands (molar ratio: MUS:OT = 34:66), and 3. NPs homogeneously coated with MUS ligands (h-NPs). Compared with the h-NPs and r-NPs, the s-NPs with stripe-like ligand microdomains showed an over three-fold increase in cellular uptake by mouse dendritic cell clone DC2.4. Furthermore, at the subcellular level, the s-NPs preferred to accumulate in cytosol, whereas r-NPs and h-NPs were largely located in the endosomal compartments. The results indicate that the cellular uptake and subcellular location of PNPs could be well controlled by tuning the properties of surface patterns of the NPs.

In addition to enhanced transmembrane transportation, NPs with precisely tailored surfaces can directly disrupt cell membranes for potential biomedical applications (e.g., anti-bacteria/tumor with drug resistance). The spatial distribution of charges on NPs also has a profound effect on the interactions of NPs and biomembranes. Robert and coworkers compared the interactions between bacterial cell membranes and five types of positively charged diamond NPs (∼5 nm in diameter) with similar zeta potential but different arrangements of charges. The NPs were covalently linked with cationic poly(allylamine) (PAH) or short cationic ligands (i.e., C3 primary amine, C3 quaternary amine, C11 quaternary amine, and tetaethylenepentamine). When the concentration of cationic groups on the NPs kept constant, PAH-modified NPs induced the most notable membrane distortion and strongest toxicity to *S. oneidensis* among all the groups. This observation was attributed to the insertion of the charged long polymeric loops and tails into the layers of phospholipids (Figure 6B). In contrast, the charged short molecular...
ligands can only interact with the outermost surface of the biomembranes.

Control over the combination of ligand hydrophobicity and charge on NPs can be used to regulate the NP-biomembrane interactions. For instance, Yan and coworkers systematically investigated the effect of Janus-like surface structures of NPs on the interactions between NPs and phospholipid bilayers (Figure 6C). Silica particles (~100 nm in diameter) were asymmetrically functionalized with distinctive molecular ligands of different combinations. The resulting Janus-like NPs had: 1. cationic/hydrophobic halves (+/pho JP) 2. anionic/hydrophobic halves (-/pho JP) 3. uniformly modified with amine groups (+UPs), 4. carboxylic acid groups (−UPs), 5. mixed amine and hydrophobic groups (+pho UP), and 6. mixed carboxylic acid and hydrophobic groups (−pho UP). Cationic charges on NPs were found to promote the initial adhesion of the NPs onto the lipid membranes, and surface hydrophobicity helped the NPs to disrupt the bilayer to form holes, thus benefiting the cellular uptake of the NPs. Therefore, the Janus-like NPs with spatially separated cationic/hydrophobic halves showed a stronger membrane disruption than all the other amphiphilic NPs. Moreover, the hydrophilic–lipophilic balance of ligands on NPs is also critical to the disruption of lipid bilayers. A larger ratio of hydrophobic-to-hydrophilic surface area on +/-pho Janus-like particles induced a more profound membrane disruption and more defects in the lipid bilayers.

Unlike individual NPs, discrete assemblies of NPs can be designed to readily change their size, morphology, and surface features, thus achieving more complex interactions with cell membranes. In an interesting example, Chan and coworkers designed high-precision complex assemblies composed of two core NPs (~13 nm and ~6 nm in diameter) surrounded by many small satellite NPs (~3 nm in diameter) via programmed Watson–Crick base pairing of DNA linkers tethered on Au NPs. The satellite NPs can reversibly attach/detach to the two central cores to achieve configuration transformation in response to specific DNA sequences (Figure 7A). The introduction of pre-designed attaching strands (A1) triggered the attachment between small satellites that were initially bonded on the large core and the medium core to form an intermediate structure. Subsequently, when detaching strands (L1comp) were added, the small satellites relocated from the large core to the medium core. When the satellites, medium core, and large core were labeled with fluorescent molecules of Cy3, Cy5 and FAM respectively, the morphology transformation switched the Förster resonance energy transfer (FRET) models from FAM/Cy3 to Cy3/Cy5. Furthermore, the relocation of the satellite NPs in the assemblies could regulate the cellular internalization of the assemblies through controllable shielding and exposure of targeting moieties (folic acid, FA) on the large core in vitro. The satellites on the large core (morphology 1) impeded the targeting of the assemblies to U87-MG cells. When A1 and L1comp triggered the attachment of satellites onto the medium core (morphology 2), the cellular uptake of the assemblies (morphology 2) by U87-MG cells increased by 2.5-fold, owing to the exposure and binding of FA to the receptors on the cells (Figures 7B and 7C).

PNPs could provide a rigid scaffold for well-controlled spatial organization of biomolecules (e.g., peptides, proteins, nucleic acids), thus triggering unique immune/inflammatory responses, such as B-cell activation. For instance, Mark and coworkers fabricated icosahedral DNA origami with eOD-GT8 antigens and studied the activation of B-cell responses by the nanostructures. When the distance between antigens bonded on the DNA scaffold increased from 3 nm to 22 nm, the total calcium signal in glVRC01 B cells increased monotonically from ~0.45 to ~0.95 (normalized AUC), indicating that increasing the inter-antigen spacing can enhance presentation of antigens and activation of B cells (Figures 7D and 7E). This work further confirms the crucial role of spatial distribution of ligands in interactions between cells and PNPs.

3.4 Biological delivery and therapy in vivo

Control over the biodistribution, especially the accumulation of nanomaterials in target sites in vivo is critical for achieving efficient and safe imaging and therapy. Ideal nanocarriers of therapeutics agents should release the payloads at desired time and location to maximize therapeutic efficiency. Meanwhile, the nanocarriers are expected to be cleared out of the body immediately after the drug release to reduce potential toxicity caused by the nanocarriers themselves in vivo. Compared with conventional nanocarriers, PNP-based delivery vehicles could offer merits from the following aspects: 1. PNP could exhibit prolonged in vivo circulation via elaborately designed surface patterns and the resultant well controlled NP-protein interactions; 2. tunable sizes of PNPs and corresponding nanoassemblies ensure extended circulation before drug delivery and rapid clearance of the delivery vehicles after release of payloads; 3. PNPs can exhibit higher tumor accumulation owing to their elaborately tailored anisotropic surfaces and structures, thus leading to higher imaging and therapeutic efficiency than other delivery platforms; and 4. PNPs can carry multiple functional components, such as fluorescent dyes, drugs, targeting motifs, in different counterparts of one system to perform multiple tasks.
As discussed in section 3.3, PNPs (or nanoassemblies) exhibit highly regulated NP-protein interactions in biofluids or cell membranes. Thus, tailoring the surface of PNPs can prolong the circulation of delivery vehicles and enhance their accumulation in diseased sites (e.g., tumors). For instance, Tanja and coworkers designed spherical polyphenylene dendrimers with precisely controlled hydrophilic (sulfonic acid groups)/hydrophobic (n-propyl groups) patches on the surface (Figure 8A). The optimal spatial arrangement of negative charges and hydrophobic groups promoted the binding of the patchy dendrimers onto human adenovirus 5 (Ad5, a viral gene transfer vector) to form a “dendrimer corona”. The resulting Ad5-dendrimer complexes (PPD3/Ad5) had a composition different from protein corona in serum and showed an improved transfection performance in cells that are deficient of the primary Ad5 receptors. Moreover, the patchy dendrimers protected Ad5 from being neutralized by natural antibodies in human blood and hence altered their distribution in vivo. As a result, the expression of EGFP mediated by PPD3/Ad5 was reduced by \( \sim 40\% \) in spleen, liver, and lung, but enhanced by over 40% in heart, compared with unbonded Ad5 (Figure 8B).

Sufficiently large size of NPs (>6 nm) represents a critical requirement for reduced renal clearance, enhanced accumulation, and prolonged retention within tumors. However, the non-biodegradable NPs (e.g., most of metal NPs) with large sizes remain in body for a long time after i.v. administration, whereas ultrasmall NPs can be...
Figure 8: (A) Schematic illustration of gene transfection mediated by PPDs binding to Ad5. (B) Summary of the impact of PPD3 corona on cell Ad5 uptake processes in vitro and biodistribution in vivo. Copyright 2019, American Chemical Society. (C) Schematic illustration of the preparation of core-satellite assemblies. (D) TEM images of assemblies with different satellite-to-core ratio. (E) Relative macrophage uptake efficiency of 13 nm core NPs, 5 nm satellite NPs and their corresponding core-satellite assemblies. (F) Tumor accumulation of core-satellite assemblies, mixtures of unassembled components and core NPs alone. Copyright 2014, Springer-Nature.

Efficiently eliminated from the body without causing any chronic toxicity. Responsive assemblies of NPs with precise spatial arrangements can meet the contradictory size requirement of nanocarriers for efficient delivery and elimination in vivo. For instance, Chan and coworkers assembled DNA-grafted Au NP with complementary linker DNA strands into discrete core-satellite clusters (core: ∼13 nm in diameter; satellite: ∼3 or 5 nm in diameter) with precisely tuned satellite-to-core ratio (r = 2, 8, 16, 24) for enhanced delivery and rapid elimination of NPs in vivo (Figures 8C and 8D). Although the precision assemblies had a diameter about 2.5-times larger than the core NP, they exhibited a two-fold lower cellular uptake by the J774A.1 murine macrophages than individual cores, indicating that the unique surface features of the nanoassemblies inhibited their uptake by the RES system. As a result, the nanoassemblies showed over two-fold higher tumor accumulation than the non-assembled NPs in vivo (Figures 8E and 8F). Moreover, the disassembly of the clusters into small NPs facilitated the escape of NPs from phagosomes and eventually facilitate their rapid elimination from the body through the renal system. It was observed that the urinary excretion efficiency of superstructures closely mirrored the clearance behavior of their building blocks, suggesting the rapid elimination of the nanoassemblies from the body.

PNPs have shown great potentials in targeted delivery of imaging and therapy to diseased sites due to their unique properties arising from the topographically or chemically distinct surface domains. Compared with conventional drug delivery nanoplatforms, PNPs can exhibit higher tumor accumulation owing to their elaborately tailored surfaces and structures, thus leading to higher imaging or therapeutic efficiency than other delivery platforms. For instance, Du and coworkers assembled highly precise DNA origami nanostructures with different shapes (e.g., triangles, squares, and tubes) for targeted delivery of doxorubicin in cancer therapy. The DNA nanostructures showed enhanced passive tumor targeting that is dependent on the shape of nanostructures, owing to the enhanced permeability and retention effect. The triangular DNA nanostructures accumulated in tumors ∼2 times more than square-like and tubular DNA nanostructures and ∼4 times more than unassembled DNA strands in vivo. As a result, the efficient delivery of doxorubicin mediated by triangle DNA origami showed an antitumor rate of ∼67% without observable systemic toxicity in a subcutaneous breast tumor (MDA-MB-231) model. In contrast, the antitumor rate of free-DOX treated group was ∼50%.

PNPs can be integrated with multiple functional components, such as fluorescent dyes, drugs, targeting motifs,
in one system to perform multiple tasks. PNPs allow for accurate control over the ratio and specific location of the functional species which can be elaborately tailored to enhance the efficiency of imaging and therapy. Ding and coworkers constructed double-bundle DNA tetrahedrons (TETs) intercalated with platinum drug (56MESS) in DNA duplexes and hybridized with a precise number of EGFR antibody (Nb) at the edges (TET-Nb-56MESS) for targeted cancer therapy.\(^8^1\) The precisely engineered NPs showed a stronger affinity for cell lines with a higher level of EGFR expression. As a result, a relatively high concentration of 56MESS was detected in tumors overexpressing EGFR even 12 h post-injection of the antibody-conjugated DNA tetrahedrons. Owing to the efficient delivery and relatively long retention of 56MESS in tumors, the DNA-based nanocarriers exhibited noticeable therapeutic outcomes in A431 tumor-bearing mice with relatively low systemic toxicity. Compared with single-modal therapy mediated by TET-56MESS or TET-Nb, dual-modal therapy mediated by TET-Nb-56MESS elicited a significant enhanced proliferation-inhibition activity.

PNPs can be used to load multiple cargos or functional motifs with precisely controlled ratio of each component and to deliver the payload to a single site simultaneously or to multiple sites sequentially in vivo in a quantitative manner, which is crucial to customizing personalized treatment plan. For example, Stenzel and coworkers reported a strategy to segregate two chemically distinct molecules in either the patches or core compartment of patchy nanoparticles that bear a (bioactive) sugar corona (Figures 9A and 9B).\(^2^2\) The stepwise loading of doxorubicin (DOX) in the non-polar compartment and cyanine5 free acid (cy5) within the polar compartment allows for real-time monitoring of release of the co-loaded fluorophores in cells via FRET. In another example, triangle DNA origami were also used to co-deliver DOX and tumor therapeutic gene (p53) for chemo/gene complementary therapy of multidrug resistant tumor (MCF-7R).\(^8^3\) Kite-like nanostructures composed of a triangular DNA origami, and two DNA strands tails were loaded with DOX on the surface of DNA triangles, which were modified with MUC1 groups on the edges for active targeting, and capped with linear tumor suppressor gene p53 on two tails. The resultant nanostructures with precisely controlled arrangement of multiple functional parts exhibited effective inhibition of tumor growth in vitro/vivo without significant systemic toxicity. Furthermore, triangle DNA origami were conjugated with DOX/shPgp(silencing P-glycoprotein)/shSur...
PNPs based on precise NP assemblies are attractive for cancer theranostics (i.e., imaging, diagnosis, and therapy in one system), owing to their unique collective physical properties that are strongly dependent on the precise spatial arrangement of NPs. For instance, chiral core-satellite assemblies of AuNPs were fabricated by DNA-driven assembly and demonstrated for effective multimodality imaging-guided photodynamic therapy (Figure 10A). The chiral assemblies served as contrast agents for X-ray computed tomography (CT)/photoacoustic (PA) bimodal imaging and as chiral photosensitizers to generate reactive oxygen species efficiently under circular polarized light illumination. Tumors in mice treated with the assemblies and right circular polarized light were effectively eliminated, whereas in the group treated with the assemblies and left circular polarized light, tumors exhibited rapid growth of over five-fold increase in the relative tumor volume. In another example, Xu and coworkers assembled plasmonic AuNR dimers and Ce6-labeled NaGdF4 UCNPs into core–satellite PNPs (NR dimer-UCNP-Ce6) via DNA hybridization for imaging-guided photothermal therapy of tumor (Figure 10B). The core–satellite assemblies can be activated by 808 nm laser for photothermal therapy and 980 nm laser for photodynamic therapy of tumor in vivo. The combination therapy was guided by tetra-modal imaging, including PA, CT, UCL, and T$_{1}$-weighted magnetic resonance (MR) imaging. The core-satellite assemblies with AuNR dimer as core exhibited a two-fold increase in the heat transfer efficiency, compared with assemblies with single AuNR as core. This observation was attributed to the intense plasmonic coupling in the hot spot and the matching of plasmonic adsorption peak and laser wavelength.

4 | CONCLUSIONS AND PERSPECTIVES

PNPs have emerged as a promising nanoplatform for various biological applications. First, elaborate design of receptor distribution on the PNPs has been proved to enhance the binding affinity between receptors on PNPs and target analytes in biofluids. Second, engineering the chemical patterns of ligands on the NPs has been demonstrated for modulating the interactions between proteins and PNPs to extend the blood circulation of the NPs. Third, tuning the surface properties of NPs has been used to control the binding strength between PNPs and cell membranes and enhance the cellular uptake of the NPs.

Numerous advances on PNPs had been achieved for nanomedicines, but challenges still remain. First of all, there is still a lack of simple yet cost-effective strategies for the fabrication of PNPs with designed arbitrary surface, shape, and property. The most widely adopted strategy for
constructing PNPs relies on programmable DNA-based interactions. However, the inevitable destabilization and degradation of DNA linkers makes the nanoarchitectures difficult to retain their surface and structure intact in vivo, due to insufficient cations for DNA stabilization and abundant nucleases in biological fluids. Furthermore, because oligonucleotides can trigger potent immunostimulatory effects, unwanted immune response may be induced by the DNA building blocks or their degraded fragments. In contrast, polymer-enabled PNPs can be readily designed to be mechanically robust, biologically stable and non-immune stimulatory. However, the conformations of polymer chains are difficult to control, and the common interactions (e.g., Coulomb interaction, hydrophilic/hydrophobic interaction, hydrogen bond) between synthetic polymer chains are usually lack of specificity and controllability as biomolecules (e.g., DNAs) do. As a result, PNPs fabricated by polymer approach usually do not have the same level of precision, accuracy, and complexity as those made by DNA-based strategies. More efforts should be devoted to increase our ability to assemble synthetic polymers into uniform, precise, hierarchically complex nanostructures as observed in biological systems, and to precisely attach polymers.
onto NPs for guiding the organization of NPs with high precision.

The dynamic and complex nature of disease tissues and individual differences between patients remain major issues in clinical treatment. Multifunctional PNP s have demonstrated their great potential in personalized nanomedicines. However, as of now, there is no sufficient database or predictive model available for tailoring PNP design for efficient diagnosis and therapy. Current research is still largely based on the trial-and-error method, which is not time-efficient and practical from a long-term perspective. Limited understanding of how PNP s can benefit delivery and therapeutic efficiency has become barriers for the design and application of PNP s in nanomedicine, especially cancer therapy. Future research should focus on revealing the functioning mechanism of PNP s and establishing correlation between the traits of PNP s and their biological performance. This expectation, again, calls for advances in the effective fabrication of high-quality PNP s.

As mentioned earlier, PNP-based delivery vehicles inevitably experience various cascade processes or biological barriers with distinctive characteristics before reaching diseased sites. The design criteria of PNP s for overcoming individual barriers can be controversial, with respect to the surface or structure of the PNP s. One promising strategy to probe this challenge is to design PNPs with adaptive surface features (e.g., charge reversal) or transformable structures (e.g., switch of stealthy/exposed targeting ligands), so that the accumulation of the PNPs in specific sites can be enhanced or the metabolism, and clearance of the PNPs can be accelerated. Despite recent advances in engineering PNPs with precise architectures, there is an urgent need of new concepts or strategies for the fabrication of PNPs with spatiotemporally tunable physical or chemical traits. Moreover, multiple therapeutic or diagnostic compounds are often used to achieve imaging-guided or synergistic therapy, but the action location of these agents in biological systems can be drastically different. The realization of optimal therapeutic and/or diagnostic outcomes necessitates smart vehicles capable of sequentially delivering multiple cargoes to one or multiple sites. Thus, PNPs for this purpose should also possess adaptable and temporospatial features, in addition to precise architectures.

To enable clinical translation of the nanomaterials, ideal PNP s should: 1. possess highly uniform yet precisely tailored surface and structural heterogeneity for improving the blood circulation and tumor accumulation of materials; 2. exhibit good stability, biocompatibility, and rapid clearance after their biological use; and 3. carry various functional components in one particle with controlled stoichiometry and location as a multifunctional nanoplatform. PNPs loaded with multiple cargoes are anticipated to: 1. integrate imaging and therapy in one system; 2. monitor drug release and therapeutic outcomes in real time; and 3. realize synergistically enhanced therapy through simultaneous or sequential delivery of several therapeutic compounds to one or multiple sites.

**CONFLICT OF INTEREST**

The authors declare no competing financial interests.

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**REFERENCES**

1. a) R.vanderMeel, E. Sulheim, Y. Shi, F. Kiessling, W. J. M. Mulder, T. Lammers, Nat. Nanotechnol. 2019, 14, 1007. b) D. J. Irvine, E. L. Dane, Nat. Rev. Immunol. 2020, 20, 321.
2. Z. Chen, Z. Wang, Z. Gu, Acc. Chem. Res. 2019, 52, 1255.
3. X. Pang, X. Lui, Y. Cheng, C. Zhang, E. Ren, C. Liu, Y. Zhang, J. Zhu, X. Chen, G. Liu, Adv. Mater. 2019, 31, 1902530.
4. Y. Lee, K. Sugihara, M. G. Gilliland 3rd, S. Jon, N. Kamada, J. J. Moon, Nat. Mater. 2020, 19, 118.
5. B. Ouyang, W. Poon, Y. N. Zhang, Z. P. Lin, B. R. Kingston, A. J. Tavares, Y. Zhang, J. Chen, M. S. Valic, A. M. Syed, P. MacMillan, J. Couture-Senecal, G. Zheng, W. C. W. Chan, Nat. Mater. 2020, 19, 1362.
6. Q. Sun, Z. Zhou, N. Qiu, Y. Shen, Adv. Mater. 2017, 29, 1606628.
7. a) J. Shi, P. W. Kantoff, R. Wooster, O. C. Farokhzad, Nat. Rev. Cancer 2017, 17, 20. b) J. Nam, N. Won, J. Bang, H. Jin, J. Park, S. Jung, S. Jung, Y. Park, S. Kim, Adv. Drug Delivery Rev. 2013, 65, 622. c) S. E. A. Gratton, P. A. Ropp, P. D. Pohlhaus, J. C. Luft, V. J. Maddern, M. E. Napier, J. M. DeSimone, Proc. Natl. Acad. Sci. USA 2008, 105, 11613. d) A. Albanese, P. S. Tang, W. C. W. Chan, Annu. Rev. Biomed. Eng. 2012, 14, 1. e) D. H. Jo, J. H. Kim, T. G. Lee, J. H. Kim, Nanomedicine 2015, 11, 1603. f) J. S. Suk, Q. Xu, N. Kim, J. Hanes, L. M. Ensign, Adv. Drug Delivery Rev. 2016, 99, 28.
8. J. M. Rabanel, V. Adibnia, S. F. Tehrani, S. Sanche, P. Hildgen, X. Banquy, C. Ramassamy, Nanoscale 2019, 11, 383.
9. I. M. Adjei, C. Peetla, V. Labhasetwar, Nanomedicine (Lond) 2014, 9, 267.
10. Z. Poon, S. Chen, A. C. Engler, H. I. Lee, E. Atas, G. von Maltzahn, S. N. Bhatia, P. T. Hammond, Angew. Chem. Int. Ed. 2010, 49, 7266.
11. P. P. Pillai, S. Huda, B. Kowalczyk, B. A. Grzybowski, J. Am. Chem. Soc. 2013, 135, 6392.
12. L. Sun, F. Shen, J. Xu, X. Han, C. Fan, Z. Liu, Angew. Chem. Int. Ed. 2020, 59, 14842.
13. H. Gao, J. Xiong, T. Cheng, J. Liu, L. Chu, J. Liu, R. Ma, L. Shi, Biomacromolecules 2013, 14, 460.
14. H. Song, M. Yu, Y. Lu, Z. Gu, Y. Yang, M. Zhang, J. Fu, C. Yu, J. Am. Chem. Soc. 2017, 139, 18247.
42. C. Yi, H. Liu, S. Zhang, Y. Yang, Y. Zhang, Z. Lu, E. Kumacheva, Z. Nie, *Science* **2020**, *369*, 1369.

43. a) J. Sharma, R. Chhabra, A. Cheng, J. Brownell, Y. Liu, H. Yan, *Science* **2009**, *323*, 112. b) A. Kuzyk, R. Schreiber, Z. Fan, G. Par-datscher, E. M. Roller, A. Hogele, F. C.immel, A. O.戈vorov, T. Liedl, *Nature* **2012**, *483*, 311. c) D. H. Schaffert, A. H. Ohkomo, R. S. Sorensen, J. S. Nielsen, T. Torring, C. B. Rosen, A. L. Kodal, M. R. Mortensen, K. V. Gothel, J. Kjems, *Small* **2016**, *12*, 2634. d) T. A. Meyer, C. Zhang, G. Bao, Y. Ke, *Nano Lett.* **2020**, *20*, 2799.

45. a) S. Ohta, D. Glancy, W. C. Chan, *Science* **2016**, *351*, 841. b) A. Qu, M. Sun, L. Xu, C. Hao, X. Wu, C. Xu, N. A. Kotov, H. Kuang, *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 3391. c) L. Y. Chou, K. Zagarovskiy, W. C. Chan, *Nat. Nanotechnol.* **2014**, *9*, 148. d) P. K. Harimech, S. A. Gurrrard, A. H. El-Sagheer, T. Brown, A. G. Kanaras, *J. Am. Chem. Soc.* **2015**, *137*, 9242. e) L. Xu, Y. Gao, H. Kuang, L. M. Liz-Marzan, C. Xu, *Angew. Chem. Int. Ed.* **2018**, *57*, 10544.

46. a) M. Grzelczak, A. Sanchez-Iglesias, H. H. Mezerji, S. Bals, J. Perez-Juste, L. M. Liz-Marzan, *Nano Lett.* **2012**, *12*, 4380. b) Y. Liu, Y. Li, J. He, K. J. Duelge, Z. Lu, Z. Nie, *J. Am. Chem. Soc.* **2014**, *136*, 2602.

47. M. J. Taylor, K. Husain, Z. J. Garber, S. Mayor, R. D. Vale, *Cell* **2017**, *169*, 108.

48. M. Li, H. Ding, M. Lin, F. Yin, L. Song, X. Mao, F. Li, Z. Ge, L. Wang, X. Zuo, Y. Ma, C. Fan, *J. Am. Chem. Soc.* **2019**, *141*, 18910.

49. F. Yin, M. Li, X. Mao, F. Li, X. Xiang, Q. Li, L. Wang, X. Zuo, C. Fan, Y. Zhu, *Angew. Chem. Int. Ed.* **2020**, *59*, 10406.

50. X. Wu, L. Xu, W. Ma, L. Liu, H. Kuang, N. A. Kotov, C. Xu, *Adv. Mater.* **2016**, *28*, 5907.

51. J. Cai, C. Hao, M. Sun, W. Ma, C. Xu, H. Kuang, *Small* **2018**, *14*, 1703931.

52. W. Ma, P. Fu, M. Sun, L. Xu, H. Kuang, C. Xu, *J. Am. Chem. Soc.* **2017**, *139*, 11752.

53. Y. Wang, R. Cai, C. Chen, *Acc. Chem. Res.* **2019**, *52*, 1507.

54. J. Martel, D. Young, A. Young, C. Y. Wu, C. D. Chen, J. S. Yu, J. D. Young, *Anal. Biochem.* **2011**, *418*, 111.

55. Y. Zhang, J. L. Yu, W. J. Lazaro-vits, W. C. W. Chan, *J. Am. Chem. Soc.* **2020**, *142*, 8827.

56. R. Garcia-Alvarez, M. Hadijdemetriou, A. Sanchez-Iglesias, L. M. Liz-Marzan, K. Kostarelos, *Nanoscale* **2018**, *10*, 1256.

57. A. Salvati, A. S. Pitek, M. P. Monopoli, K. Prapainop, F. B. Bombelli, D. R. Hristov, P. M. Kelly, C. Abeg, E. Mahon, K. A. Dawson, *Nat. Nanotechnol.* **2013**, *8*, 137.

58. J. J. Nie, B. Qiao, S. Duan, C. Xu, B. Chen, W. Hao, B. Yu, Y. Li, J. Du, F. J. Xu, *Adv. Mater.* **2018**, *30*, 1801570.

59. a) Y. Min, K. C. Roche, S. Tian, M. J. Eblan, K. P. McKinnon, J. M. Caster, S. Chai, L. E. Herring, L. Zhang, T. Zhang, J. M. DeSimone, J. E. Teeter, B. G. Vincent, J. S. Serody, A. Z. Wang, *Nat. Nanotechnol.* **2017**, *12*, 877. b) S. Li, M. Li, S. Huo, Q. Wang, J. Chen, S. Ding, Z. Zeng, W. Zhou, Y. Zhang, J. Wang, *Adv. Mater.* **2020**, *33*, 2006160.

60. K. Pombo-Garcia, C. L. Rühl, R. Lam, J. A. Barreto, C.-S. Ang, P. J. Scammells, P. Comba, L. Spiccia, B. Graham, T. Joshi, H. Stephan, *ChemPlusChem* **2017**, *82*, 638.
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