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# Functional Ligand-Enabled Particle Assembly for Bio–Nano Interactions

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## CONSPECTUS

Functional ligands consist of a wide range of small or large molecules that exhibit a spectrum of physical, chemical, and biological properties. A suite of small molecules (e.g., peptides) or macromolecular ligands (e.g., antibodies and polymers) have been conjugated to particle surfaces for specific applications. However, postfunctionalization of ligands often presents challenges in controlling the surface density and may require the chemical modification of ligands. As an alternative option to postfunctionalization, our work has focused on using functional ligands as building blocks to assemble particles while maintaining the intrinsic (functional) properties of the building blocks. Through self-assembly or template-mediated assembly strategies, we have

developed a range of protein-, peptide-, DNA-, polyphenol-, glycogen- and polymer-based nanoparticles. This Account discusses the assembly of such nanoengineered particles, which includes self-assembled nanoparticles, hollow capsules, replica particles, and core–shell particles, according to three categories of functional ligands (i.e., small molecules, polymers, and biomacromolecules) that are used as building blocks for their formation. We discuss a range of covalent and noncovalent interactions among ligand molecules that have been explored to facilitate the assembly of nanoparticles. The physicochemical properties of particles, including size, shape, surface charge, permeability, stability, thickness, stiffness, and stimuli-responsiveness, can be readily controlled by varying the ligand building block or by tuning the assembly method. By selecting specific ligands as building blocks, the bio–nano interactions (i.e., stealth, targeting, and cell trafficking) can be modulated. For instance, particles composed mainly of low-fouling polymers (i.e., poly(ethylene glycol)) exhibit an extended blood circulation time (half-life >12 h), while antibody-based nanoparticles demonstrate that a trade-off between stealth and targeting may be required when designing targeting nanoparticle systems. Small molecular ligands, such as polyphenols, have been used as building blocks for particle assembly as they can interact with various biomacromolecules through multiple noncovalent interactions, retain the function of biomacromolecules within the assembly, enable pH-responsive disassembly when coordinating with metal ions, and facilitate endosomal escape of nanoparticles. A perspective is provided on the current challenges associated with the clinical translation of ligand-based nanoparticles. This Account is also expected to serve as a reference to guide the fundamental research and development of functional particle systems assembled from various ligands for diverse applications.

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## 1. INTRODUCTION

Functional ligands have been widely used as building blocks for particle engineering.[5–8] The selection of ligands is broad, ranging from small molecules (e.g., aromatic molecules, polyphenols, amino acids, and peptides) to polymers (e.g., functional- and (multi)block-(*co*)polymers) and biomacromolecules (e.g., oligonucleotides and proteins).[6,8,9] Furthermore, different particle preparation methods, such as self-assembly and template-assisted assembly, have enabled the preparation of functional particles.[8,10] Combining the wide choice of ligand building blocks with different particle engineering methods, functional particles with diverse morphologies (e.g., size, shape, and shell), structures (e.g., core–shell, capsule, and replica particle (low/high density)), and compositions (e.g., polyphenol, polymer, oligonucleotide, protein, and glycogen) have been prepared.

Understanding and controlling the interactions between engineered particles and biological environments is an essential requirement for developing efficient and effective therapeutic delivery systems.[11] Engineering particle properties through the selection of a range of building blocks and assembly methods enables the optimization of particle systems for specific biomedical applications. For example: stealth and targeting properties can be balanced by tuning the ligand ratios on the particle surface;[12] cell internalization is influenced by the stiffness of particles, which can be tuned by the assembly method;[13,14] cell trafficking routes can be controlled by incorporating specific ligands to facilitate endosomal escape;[4] and the controlled release of cargos can be achieved by incorporating stimuli-responsive ligands.[1,15,16]

This Account focuses on our recent research in controlling bio–nano interactions through engineering particle properties using functional ligands as building blocks (Figure 1). Specifically, we present (i) the assembly of particles using functional ligand molecules ranging from small molecules to polymers and biomacromolecules and different preparation methods, (ii) the

physicochemical properties (e.g., size, aspect ratio (shape), charge, permeability, stability, thickness, stiffness, and stimuli-responsiveness) of particles derived from a selection of ligands, and (iii) bio–nano interactions influenced by the particle properties (e.g., stealth, targeting, and cell trafficking) (Figure 1). Further, we provide our perspectives on particle engineering using functional ligands as building blocks for controlling bio–nano interactions.

## 2. FUNCTIONAL LIGANDS AS BUILDING BLOCKS

Particle nanoengineering, which enables the synthesis of a range of nanostructured particles such as hollow capsules,[17] core–shell particles,[18,19] and self-assembled polymeric assemblies,[8,20] has gained widespread interest owing to the potential of such nanostructured particles in biomedical applications.[21,22] Through self-assembly and template-mediated assembly, various ligands have been exploited for engineering functional particles based on their noncovalent and covalent interactions. In this section, particle assembly is discussed according to three categories of functional ligands (i.e., small molecules, polymers, and biomacromolecules) that are used as building blocks for the synthesis of the particles.

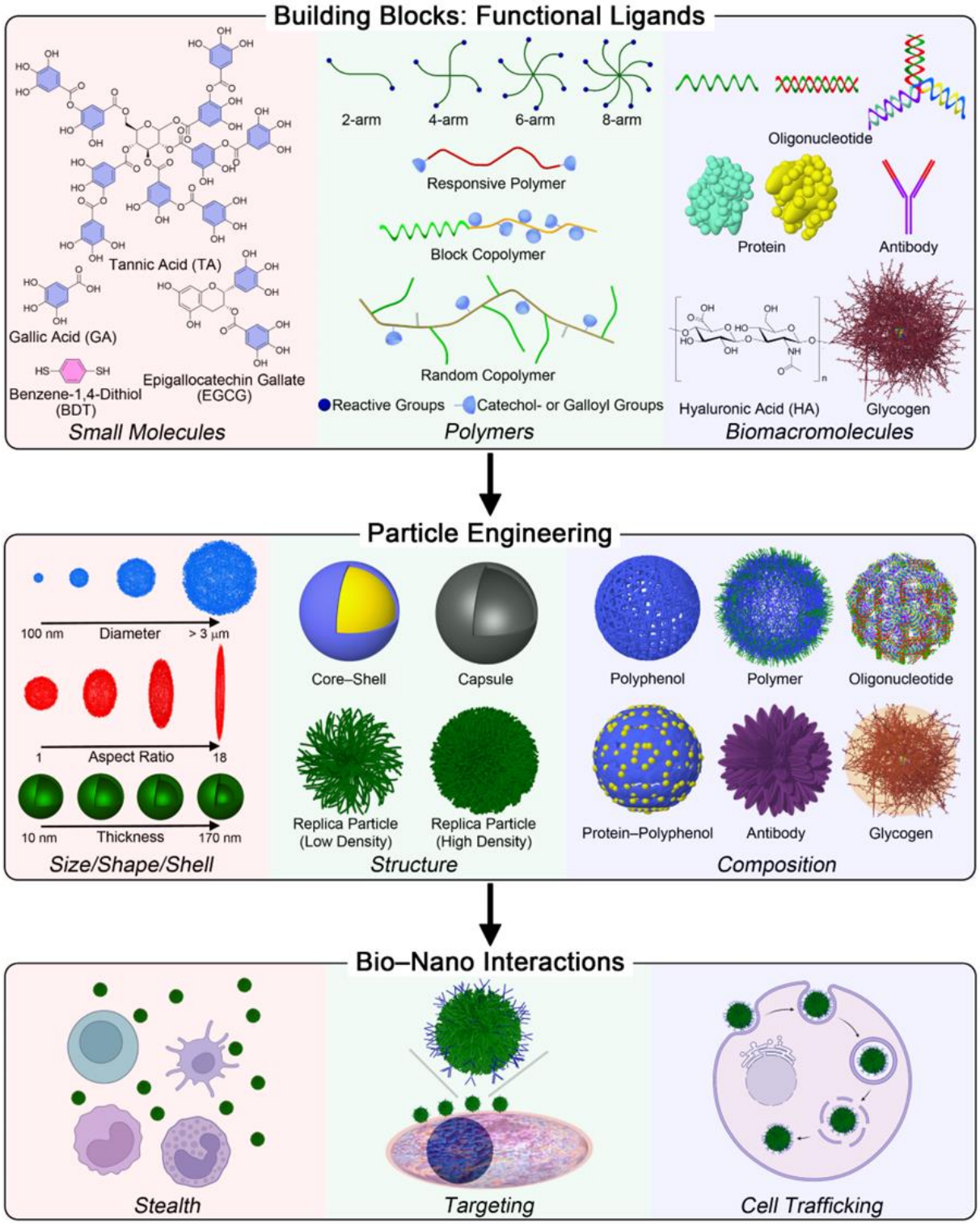


**Figure 1.** Schematic illustration of representative categories of functional ligand molecules (top), ligand-based particle engineering (middle), and bio–nano interactions (bottom). Created with BioRender.com.

### 2.1. Small Molecules

Small ligand molecules that have been used for particle assembly include polyphenols (e.g., tannic acid (TA), (−)-epigallocatechin gallate (EGCG), and gallic acid (GA)),[6] a self-polymerizable aromatic dithiol (i.e., benzene-1,4-dithiol (BDT)),[18] aromatic ligands (e.g., 4-methylbenzenethiol and 4-mercaptophenol),[23] monophenol compounds (e.g., phenol and L-tyrosine),[24] and bioactive peptides (e.g., KLAK peptides).[25] These small molecules have been exploited to fabricate functional particle systems based on different interactions, including metal coordination, π–π interactions, and self-polymerization. In 2013, we reported the assembly of metal–phenolic networks (MPNs) for depositing thin films and fabricating particles based on the pH-dependent coordination networks formed between TA (a polyphenol) and metal ions (Figure 2a).[17] In subsequent work, various MPN particles were fabricated through the introduction of different metal ions,[26] site-selective coordination of quercetin (QUE) containing three potential binding sites to metal ions,[27] coordination networks in a nonaqueous solvent,[28] heat-induced transformation processes to alter network formation in assembled structures,[29,30] sacrificial double cubic network polymer cubosomes to synthesize mesoporous particles,[31] conjugation of cyclodextrins and a polyphenol to integrate host–guest chemistry,[32] and supramolecular assembly-induced fluorescence labeling.[33]

Complexed particles with diverse physical and chemical properties can be engineered through the coexistence of noncovalent and covalent interactions in the supramolecular assembly process. For example, aromatic dithiol molecules (i.e., BDT) self-polymerized upon disulfide bond formation and subsequently assembled with TA through π–π interactions, resulting in the formation of uniform supramolecular particles (Figure 2a).[18] Moreover, the adherent nature of phenolic molecules on the BDT particle surface facilitated the engineering of a range of

monodisperse particles with well-defined structures (e.g., core–shell, hollow, and yolk–shell). To broaden the selection of building blocks for supramolecular assembly, in situ enzyme-mediated conversion (i.e., tyrosinase) of monophenols into catechols was exploited in the assembly process (Figure 2a).[24] The robust and functional coatings were deposited onto particle surfaces through covalent and coordination cross-linking from converted catechol moieties and metal ions.

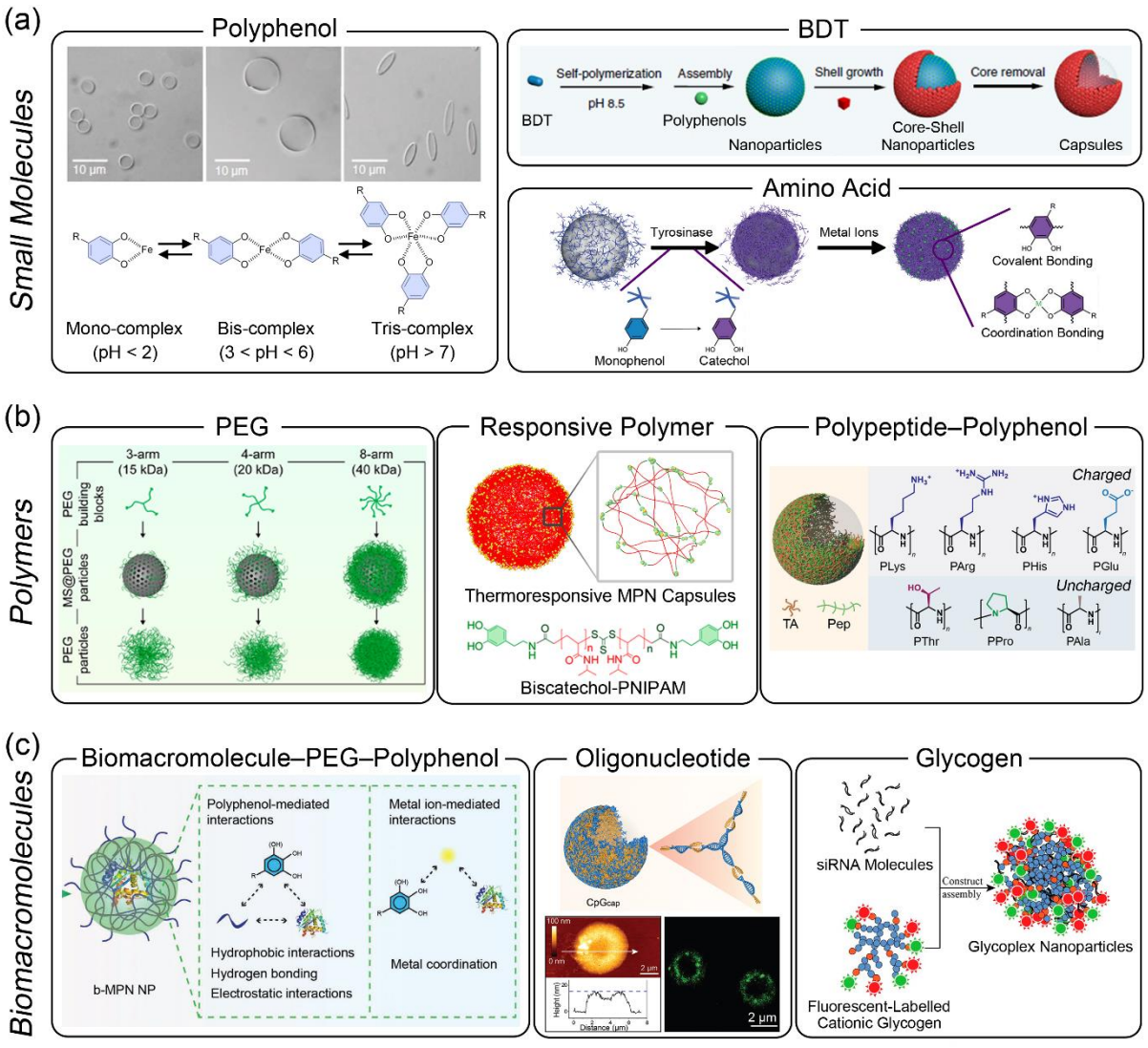


**Figure 2.** Particle assembly using functional ligands. (a) **Small molecules.** Differential interference contrast microscopy images of $Fe^{III}$–TA MPN capsules prepared using polystyrene template particles with different sizes and shapes based on pH-dependent metal coordination

networks (Polyphenol). Particle engineering through the supramolecular assembly of polymerized BDT and polyphenols via π–π interactions (BDT). Enzyme-mediated particle engineering through the tyrosinase-catalyzed conversion of a monophenol into a catechol (Amino Acid). Adapted with permission from refs 17, 18, 24. Copyright 2013 American Association for the Advancement of Science. Copyright 2020 The Authors, Published by Springer Nature. Copyright 2020 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (b) **Polymers.** PEG particle preparation using PEG building blocks of varying architectures and molecular weights (PEG). Synthesis of thermoresponsive MPN capsules using biscatechol–PNIPAM and metal ions (Responsive Polymer). Polypeptide–TA (PPep–TA) capsules formed by noncovalent interactions between TA and different types of polypeptides (PLys, polylysine; PArg, polyarginine; PHis, polyhistidine; PGlu, polyglutamic acid; PThr, polythreonine; PPro, polyproline; PAla, polyalanine) (Polypeptide–Polyphenol). Adapted with permission from refs 34, 1, 2. Copyright 2021, 2022, and 2022 American Chemical Society. (c) **Biomacromolecules.** Synthesis of b-MPN NPs via noncovalent interactions (Biomacromolecule–PEG–Polyphenol). Synthesis of CpG capsules through the hybridization of complementary DNA strands (Oligonucleotide). Self-assembly of glycoplex nanoparticles based on electrostatic interactions (Glycogen). Adapted with permission from refs 35–37. Copyright 2022 and 2020 Wiley-VCH GmbH. Copyright 2019 American Chemical Society.

### 2.2. Polymers

Synthetic macromolecules are suitable building block candidates for particle engineering owing to the modular properties of polymer strands such as architecture (e.g., 2-, 4-, 6-, and 8-arm) and charge (e.g., cationic, anionic, and neutral), controllable molecular weight, and diverse functionality (e.g., low fouling and thermoresponsiveness). For example, poly(ethylene glycol)

(PEG) with different molecular weights and molecular architectures was used as a low-fouling polymer building block to prepare PEG replica particles through a mesoporous silica (MS) templating method (Figure 2b).[3,38,39] The use of phenolic-functionalized PEG polymers enabled diverse particle systems to be fabricated by exploiting the coordination networks between phenolic groups and metal ions.[16] Conjugating phenolic groups with each terminus of an 8-arm PEG enabled the fabrication of low-fouling and pH-sensitive MPN capsules,[16] self-assembled PEGylated nanoparticles containing prodrugs,[40] and MPN-coated emulsions.[41] To precisely control the properties of assembled MPN capsules, PEG-based phenolic ligands were synthesized with different architectures (i.e., 2-, 4-, and 8-arm) and molecular weights (2.5–20 kDa).[42] In addition, we developed thermoresponsive MPN capsules by using a thermoresponsive polymer ligand (i.e., poly(*N*-isopropylacrylamide) (PNIPAM)) for MPN assembly (Figure 2b).[1] Biscatechol-functionalized PNIPAM polymers were designed and synthesized and the resulting PNIPAM chains were coordinated to 18 different metal ions, yielding a range of thermoresponsive MPN capsules. In another study, catechol-containing PEG brush copolymers were used to prepare thermoresponsive MPN capsules, which exhibited controllable thermal transition points depending on the catechol content in the copolymer ligands.[43] We also designed a DNA block copolymer composed of oligonucleotides, hydrophobic, and catechols as an MPN building block to fabricate programmable particles and capsules.[44] Nanoparticles were prepared using synthetic polymers and different types of polypeptide-based capsules were assembled from the supramolecular interactions between polyphenols and side groups in the polypeptides (Figure 2b).[2] Antimicrobial polymer ligands, as therapeutic agents, were also explored as building blocks using TA-mediated complexation with or without metal ions to assemble biofunctional microcapsules.[45]

### 2.3. Biomacromolecules

Owing to their intrinsic properties such as therapeutic modality, bio-catalytic activity, programmability, biodegradability, and biocompatibility, biomacromolecules have gained interest in the preparation of nanoparticles for materials science and biomedical applications.[46] Polyphenols (e.g., TA) have been exploited (owing to the availability of multiple functional sites for forming noncovalent interactions) for assembling functional biomacromolecules into particles. The one-pot metal–phenolic assembly of various synthetic and natural building blocks—that is PEG, polyphenols, metal ions, and bioactive macromolecules (e.g., proteins and siRNA)—resulted in a range of bioactive metal–phenolic nanoparticles (b-MPN NPs) based on the coordination and hydrophobic interactions of metal–phenolic complexes (Figure 2c).[35,47] Polyphenol-mediated assembly enabled the assembly of proteins and oligonucleotides into particle systems through noncovalent bonding including hydrogen bonding, hydrophobic, and ionic interactions without compromising the structure or functionality of the proteins and oligonucleotides.[48,49] The enzyme-mediated conversion of monophenols into catechols was developed to further expand the toolbox of functional proteins for MPN particle assembly.[24] Capsules mainly consisting of DNA were prepared through the specific binding interactions between Y-shaped DNA building blocks and duplex DNA linkers containing vaccine adjuvants cytosine–phosphate–guanosine oligodeoxynucleotides (CpG ODNs), which exhibited enhanced stability in serum and improved immunostimulatory effects of CpG (Figure 2c).[36] Template-mediated assembly was also used to fabricate antibody nanoparticles via cross-linking antibodies with PEG linkers.[50] In addition, glycogen was modified to bear cationic charges, thereby enabling complexation with therapeutic oligonucleotides through self-assembly and endosomal escape for siRNA delivery (Figure 2c).[37]

## 3. PARTICLE ENGINEERING

Controlling the physicochemical properties of particles is important as they can directly influence their biological behavior, including pharmacokinetics, particle–cell interactions, and drug loading and release profiles.[51,52] Physicochemical properties including size (100 nm to >3 μm), shape (aspect ratio = 1–18), surface chemistry (e.g., ζ-potential), permeability (impermeable and permeable), stability (or degradability), shell thickness (10–170 nm), stiffness (11–871 mN $m^{-1}$), and stimuli-responsiveness (pH-, redox-, enzyme-, light-, or temperature-responsive) can be controlled by the choice of ligand molecules and assembly method.

### 3.1. Size, Shape, and Surface Charge

Template-assisted assembly methods can be used to assemble particles with various sizes (from nano- to micrometer) and morphologies (e.g., spheres, rods, and ellipsoids) depending on the template particle. Four types of PEG capsules with different sizes (0.5 and 1.1 μm) and shapes (aspect ratios of 1, 7, and 18) were fabricated through atom transfer radical polymerization-mediated assembly of 8-arm PEG acrylate ligands using MS templates (Figure 3a).[39] Alternatively, the size and morphology of self-assembled particles (prepared in the absence of templates) could be tuned by controlling the concentration of the building ligand blocks. For example, particles fabricated through supramolecular complexation of TA with polymerized BDT were made larger by increasing the concentration of BDT while fixing the concentration of TA.[18] Additionally, the type of ligand molecules employed in particle assembly influences the surface charge of the particles. Polypeptide–polyphenol capsules with different ζ-potentials were obtained by using different polypeptides (Figure 3a).[2]

### 3.2. Permeability

Engineering capsule permeability has potential for various applications, including therapeutic delivery, biomimetics, synthetic biology, sensing, separations, and nano/microreactors.[1,53] We

have controlled permeability by modulating the assembly method,[54] selecting different structural building blocks,[42] and employing functional ligands.[1,44] For instance, the oxidation-mediated coordination (OMC) assembly method enabled the fabrication of films with significantly reduced permeability, unlike the discrete assembly, due to self-correction of the coordinating building blocks in the OMC assembly (Figure 3b).[54] Furthermore, the permeability of capsules is influenced by the chemical structure and molecular weight of the ligand molecules. Using 10 kDa 8-arm PEG resulted in less permeable capsules than the counterparts prepared from 5 kDa 2-arm PEG, probably because of their different shell thicknesses and cross-linking densities.[42]

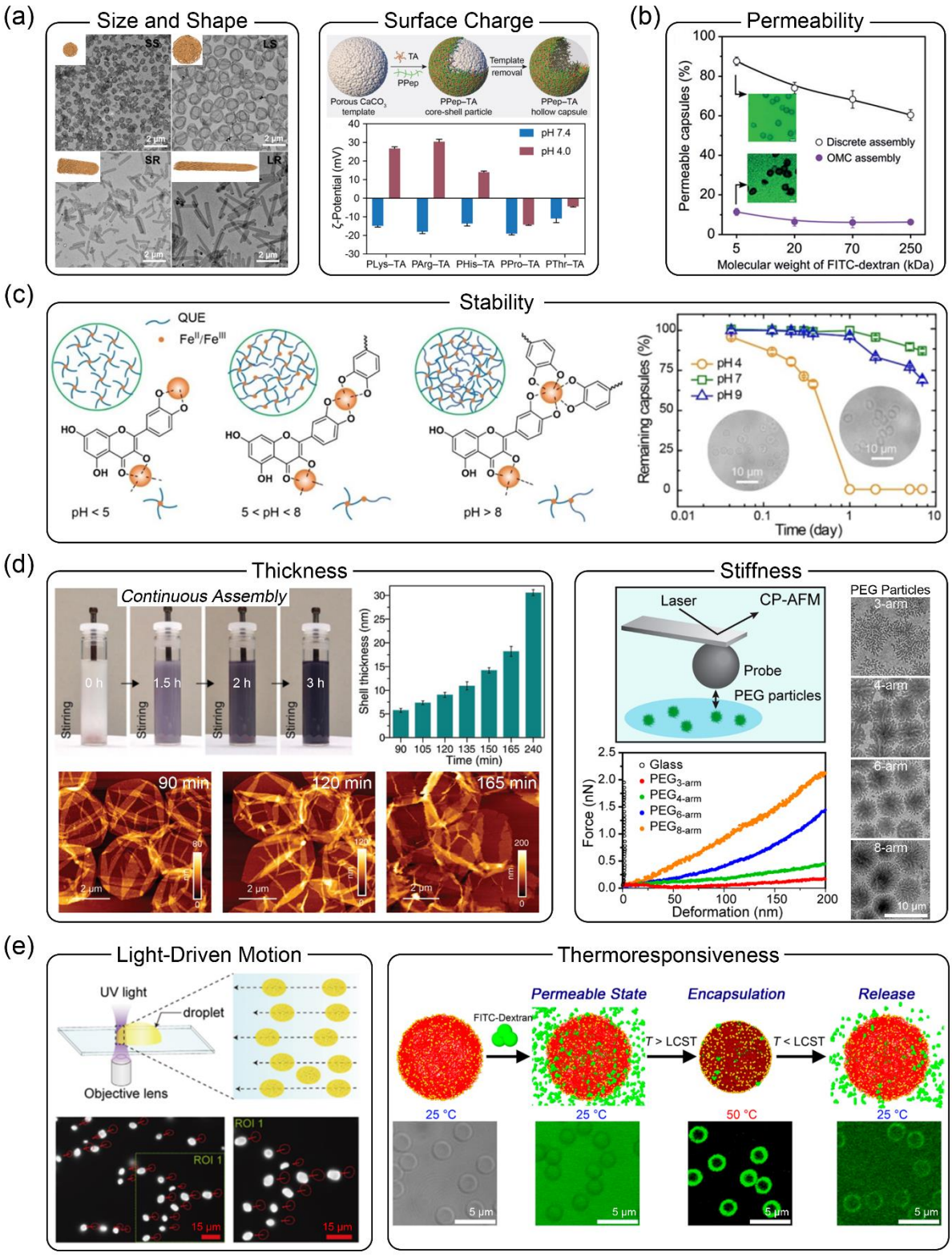


**Figure 3.** Engineering particle properties. (a) Transmission electron microscopy images of PEG capsules with different sizes and shapes (Size and Shape). ζ-Potentials of various Ppep–TA

capsules at different pH (Surface Charge). Adapted with permission from refs 39, 2. Copyright 2019 and 2022 American Chemical Society. (b) Permeability of MPN capsules prepared using discrete or OMC assembly methods. FITC, fluorescein isothiocyanate. Reproduced with permission from ref 54. Copyright 2019 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (c) Schematic illustration of the tunable binding sites of QUE toward metal ions ($Fe^{II}$ or $Fe^{III}$) at different pH. Stability of QUE MPN capsules assembled at different pH in Dulbecco's phosphate-buffered saline over time. Adapted with permission from ref 27. Copyright 2022 The Authors, Published by Wiley-VCH GmbH. (d) Continuous film thickness growth as a function of time using solid-state reactants (i.e., rusted iron objects) (Thickness). Force (F)–deformation curve measurements of PEG particles prepared using different structures (Stiffness). Adapted with permission from refs 55, 34. Copyright 2017 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. Copyright 2021 American Chemical Society. (e) Light-driven motion of microswimmers under UV illumination (Light-Driven Motion). Thermally induced encapsulation and release of cargo (500 kDa FITC-Dextran) from thermoresponsive MPN capsules; LCST, lower critical solution temperature (Thermoresponsiveness). Reproduced with permission from refs 56, 1. Copyright 2021 Wiley-VCH GmbH. Copyright 2022 American Chemical Society.

### 3.3. Stability

The stability of particles and capsules is determined by the interactions between ligand molecules: noncovalent (e.g., metal coordination, hydrogen bonding, π–π, ionic, and hydrophobic interactions) and covalent (e.g., amide and disulfide bonds) bonds. Cross-linking between PEG-amine and PEG-*N*-hydroxysuccinimide (NHS) through amide bond formation facilitated the preparation of rigid and stable PEG particles, which were used for biodistribution and biological interaction studies.[34,38] The coexistence of covalent and noncovalent interactions from π–π

interactions between TA and BDT resulted in versatile and robust coatings on particles that were highly stable in various harsh aqueous environments.[57] We demonstrated that the stability of MPN capsules could also be tuned by the assembly pH. When the assembly pH increased from 4 to 7 or 9, the dominant coordination changed from mono- to bis- or tris-state, leading to a higher cross-linking density of the network within the capsule shells (Figure 3c).[27] For protein–TA-based particles, the selection of proteins determined the type of noncovalent interactions in the assemblies.[48] Hydrophobic interactions were dominant in the assembly of lysozyme–TA and cytochrome C–TA, whereas hydrogen bonding and hydrophobic interactions were major interactions in the assembly of immunoglobulin G (IgG)–TA.

### 3.4. Thickness and Stiffness

The shell thickness of capsules is important to control as it can influence the permeability, stiffness, and cell association of capsules.[2,13,14,42,55] Our study demonstrated that the aerodynamic diameter of capsules could be controlled by tuning their shell thickness, which influenced the deposition of the capsules in a human lung model upon nebulization for pulmonary delivery.[13] The selection of PEG building blocks with different structures (i.e., 2-, 4-, and 8-arm) and molecular weights (2.5–20 kDa) and template particles (i.e., polystyrene and $CaCO_3$) resulted in capsules with various shell thickness (9–169 nm).[42] The continuous MPN assembly from solid-state reactants (i.e., rusted iron objects) allowed control over film thickness by adjusting the reaction time (Figure 3d).[55] The capsule thickness increased (from 5 to 31 nm) over time upon continuous etching of $Fe^{III}$ ions from the rust layer by phenolic ligands. Consequently, the stiffness of the capsules increased from 56 to 470 mN $m^{-1}$. The stiffness of PEG particles was controlled by using different PEG architectures or tuning the concentration of PEG cross-linkers.[34,58] PEG particles

prepared with increasing arm PEG building blocks (3 to 4, 6, and 8 arms) displayed increasing particle stiffness owing to higher cross-linking density between ligands (Figure 3d).[34]

### 3.5. Stimuli-Responsiveness

Ligand molecules with various (bio)chemical functionalities can provide particles with stimuli-responsiveness. For example, MPN capsules showed pH-responsive disassembly owing to the pH-dependent coordination between phenolic groups and metal ions.[17] The disassembly profiles of MPN capsules could also be controlled by selecting different metal ions.[26] PEG-based MPN and $Al^{III}$–TA particles readily disassembled at pH 5, enabling the release of cargos (e.g., proteins, dextran, and doxorubicin) in the intracellular compartments.[15,16,41] Redox-responsive peptide nanoparticles were assembled through disulfide cross-linking between therapeutic peptides (i.e., KLAK) and 8-arm PEG-SH, which allowed the release of free peptides upon cleavage of the disulfide-based cross-linkers.[25] Light-driven conformational transformation of metal–phenolic complex (i.e., coordination between ellagic acid and $Zn^{2+}$) allowed the preparation of particle microswimmers, which were capable of autonomously sensing and swimming toward an external light source (Figure 3e).[56] The movement of particles was regulated by the properties of the light source (i.e., direction, wavelength, and intensity). Additionally, thermoresponsive MPN capsules were fabricated through coordination between biscatechol–PNIPAM building blocks and metal ions. The capsules demonstrated reversible changes in capsule size, shell thickness, and permeability in response to temperature changes, thus allowing temperature-controlled encapsulation and release of cargos (Figure 3e).[1]

## 4. BIO–NANO INTERACTIONS

Understanding and controlling the interactions between biological systems and nanostructured materials (bio–nano interactions) is key to unlocking the full potential of nanomaterials for

biomedical applications. We have used functional ligands as building blocks to engineer particles with specific properties, including stealth to reduce nonspecific biological interactions, targeting to improve particle delivery to specific cells or tissues, and cell trafficking to achieve intracellular drug delivery.

### 4.1. Stealth

PEG is widely considered as a gold standard in nanomedicine and bioconjugation to maintain colloidal stability, prolong in vivo circulation, and improve drug delivery efficiency. For example, the clinically used SARS-CoV-2 mRNA lipid nanoparticle (LNP) vaccines (mRNA-1273 and BNTT162b2), small-interfering RNA LNP drugs (Onpattro), and anti-cancer liposomal doxorubicin agents (Doxil) contain PEG-conjugated lipids to maintain colloidal stability.[59] We demonstrated that 150 nm PEG nanoparticles composed mainly of PEG exhibited an extended blood circulation time (half-life >12 h) compared with PEGylated MS nanoparticles (Figure 4a).[38] Following this study, PEG–MPN capsules were prepared using PEG–polyphenol (catechol- or galloyl-functionalized PEG) as building blocks; the capsules showed reduced nonspecific protein adsorption and cell association.[16] The functionalization of PEG with phenolic groups allowed the coating of PEG on diverse templates, including calcium carbonate,[12,16] polystyrene,[42] water-in-oil emulsions,[40] and oil-in-water emulsions,[41] and enabled the encapsulation of small molecule drugs (e.g., doxorubicin and Pt prodrug).[12,40,41] PEG–MPN capsules (50, 100, and 150 nm) were prepared using BDT nanoparticles as templates.[60] Following removal of BDT templates, the obtained PEG nanocapsules demonstrated size-dependent blood circulation in vivo in mice and particle–leukocyte association ex vivo in human blood (Figure 4a).[60] Zwitterionic peptides and low-fouling proteins was also used as surface functionalization ligands or particle building blocks to enable stealth properties.[61] For example, we designed a recombinant protein with superior hydrophilic

properties provided by repeated amino acids (e.g., proline, alanine, and serine) as a building block to prepare particles through MS template-assisted assembly.[62] The obtained protein particles showed low-fouling behavior with a prolonged circulation time compared to albumin particles.[62] In addition to using proteins as stealth building blocks, we reported a protein precoating strategy by incubating nanoparticles with fetal bovine serum to modulate the biomolecular corona formation and reduce leukocyte association in human blood.[63]

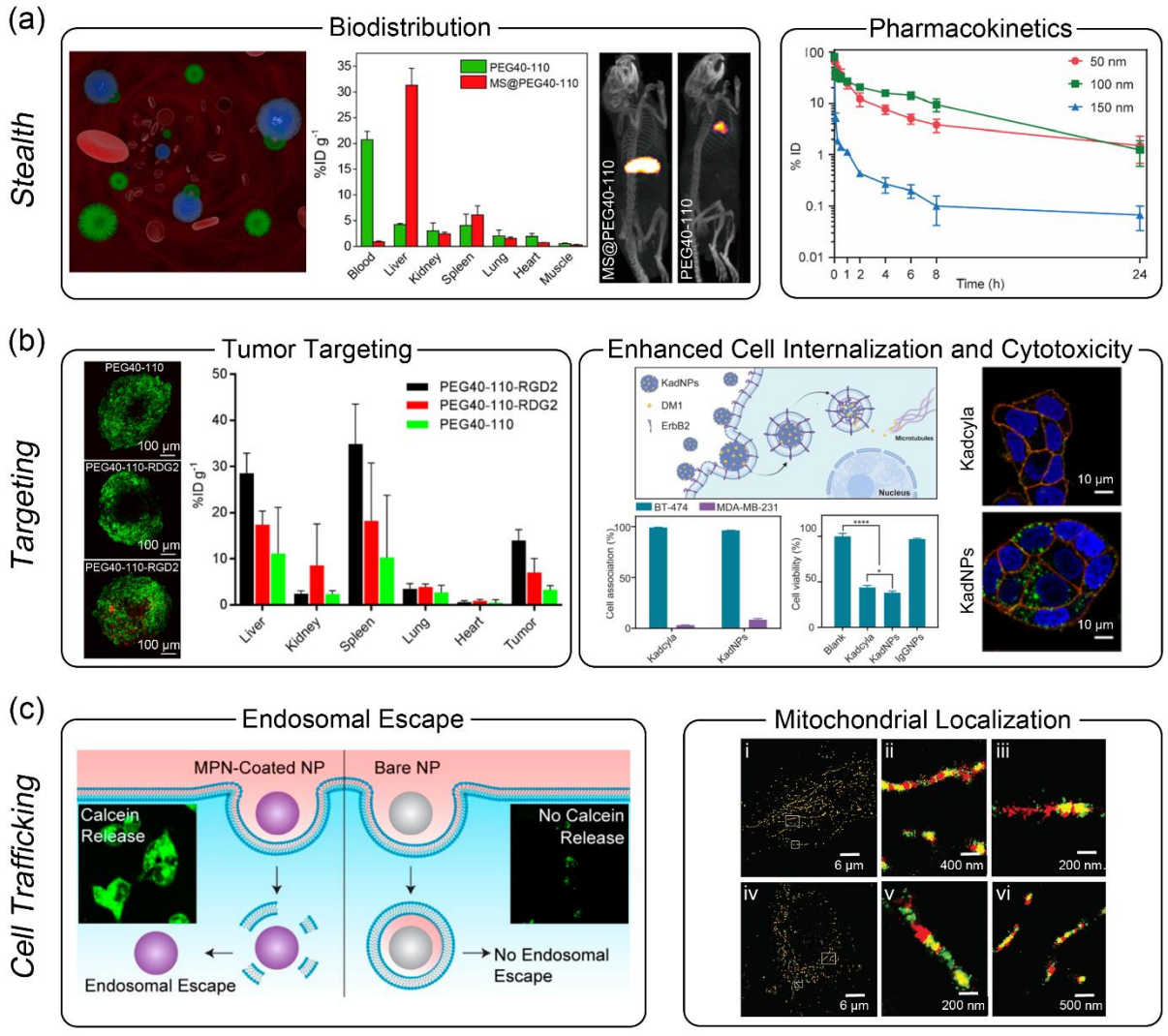


**Figure 4.** Bio–nano interactions. (a) Stealth. Schematic illustration of the extended blood circulation of PEG particles (green) owing to their lower association with phagocytic cells (blue).

Biodistribution of PEG nanoparticles with (MS@PEG40-110) and without (PEG40-110) MS templates and positron emission tomography–computed tomography images of mice at 12 h after intravenous injection. (Biodistribution). Blood clearance of PEG–MPN nanocapsules of different sizes after intravenous injection in rat (Pharmacokinetics). Adapted with permission from ref 38. Reproduced with permission from ref 60. Copyright 2015 and 2021 American Chemical Society. Copyright (b) Targeting. Multicellular spheroid association and biodistribution of PEG (PEG40-110), RGD-functionalized PEG (PEG40-110-RGD2), and RDG-functionalized PEG (PEG40-110-RDG2) particles in mice at 48 h after intravenous injection (Tumor Targeting). Schematic illustration of the internalization of Kadcyla nanoparticles (KadNPs) via ErbB2-mediated endocytosis, followed by release of emtansine (DM1) from KadNPs. Comparison of cell association and cytotoxicity of KadNPs, free antibody–drug conjugate (Kadcyla), and IgG nanoparticles (IgGNPs). Confocal microscopy images showing enhanced internalization of KadNPs in BT-474 cells in comparison to free Kadcyla. (Enhanced Cell Internalization and Cytotoxicity). Adapted with permission from refs 64, 50. Copyright 2019 and 2022 American Chemical Society. (c) Cell trafficking. MPN-enabled endosomal escape via the “proton-sponge” effect (Endosomal Escape). Super-resolution microscopy images of MDA-MB-231 cells showing the colocalization of doxorubicin-derived nanoparticles (green) with mitochondrial tubular nanostructures (red) after 24 h incubation at 37 °C: i, iv) large view of representative cell; ii, iii, v, vi) magnified images of the square sections in (i) and (iv). (Mitochondrial Localization). Reproduced with permission from ref 4. Adapted with permission from 65. Copyright 2019 American Chemical Society. Copyright 2022 The Authors, Published by Wiley-VCH GmbH.

### 4.2. Targeting

The conjugation of targeting ligands (e.g., antibodies and cell receptor ligands) can direct nanoparticles to specific cells and tissues to maximize their biological effect. However, there is a balance between stealth and targeting to consider when functionalizing targeting ligands.[66] Using catechol-functionalized hyaluronic acid (HA) and PEG as building blocks, we prepared MPN capsules to target CD44-overexpressed breast cancer cell lines.[12] By tuning the amount of HA and PEG, we found an optimized ratio of HA and PEG to enhance specific targeting while maintaining the stealth property of the capsules.[12] Furthermore, we demonstrated that the targeting specificity of HA–MPN capsules could be improved in the presence of protein coronas derived from human serum.[67] In subsequent studies, we used bispecific antibodies (one end specific to PEG and the other end specific to epidermal growth factor) or cyclic peptides containing Arg–Gly–Asp (RGD) to functionalize PEG particles; a balance in the targeting and stealth properties of the nanoparticles was achieved by tuning the amount of the targeting ligands.[64,68] The RGD-functionalized particles improved tumor accumulation but also increased the liver and spleen accumulation compared to the bare PEG particles, demonstrating a trade-off between stealth and targeting properties (Figure 4b).[64] We also explored the interactions between polyphenols (e.g., TA) and proteins to prepare protein-based capsules through template-assisted assembly.[48] More than 10 types of proteins (including enzymes, antibodies, and fluorescent and transport proteins) were used as building blocks for assembly onto templates and cross-linking with TA or other polyphenols (e.g., GA or EGCG). The proteins retained their structure and function after assembly into capsules. For example, anti-CD44 antibody–TA-based capsules exhibited specific targeting to CD44-overexpressing cells but had negligible interactions with CD44 minimal expression cells.[48] In another study, we developed a versatile template-mediated assembly technique to prepare antibody-based nanoparticles.[50] Antibodies or antibody–drug conjugates, such as Herceptin or

Kadcyla, were used as building blocks and infiltrated into MS nanoparticles, followed by cross-linking with PEG–NHS and template removal. The obtained Herceptin nanoparticles retained the targeting specificity of the monoclonal antibodies and demonstrated improved cell internalization through receptor-mediated endocytosis. Compared to the free antibody–drug conjugates, Kadcyla nanoparticles showed higher cytotoxicity to the targeted cell lines, probably due to the enhanced cellular uptake (Figure 4b). Similar to the targeting ligand-functionalized PEG particles, the Herceptin nanoparticles showed increased liver and spleen accumulation in mice compared to free Herceptin,[50] which is likely mediated by the formation of biomolecular coronas in blood.

### 4.3. Cell Trafficking

The intracellular delivery of nanoparticles and subsequent release of cargos at the target cellular compartments are essential to efficient and effective drug or gene delivery. Nanoparticles with a size range of 50–500 nm are typically internalized through endocytosis and can transport from early endosomes (pH ~6.3), late endosomes (pH ~5.5) to lysosomes (pH ~4.7).[4] The bioactive cargos (e.g., proteins, DNA, and RNA) can be degraded in the endosomes and lysosomes upon exposure to various degradation enzymes and acidic environment. Therefore, endosomal escape is important for efficient drug/gene delivery. We introduced the use of MPNs as versatile coatings to promote the escape of organic or inorganic nanoparticles from endosomes or lysosomes through the "proton-sponge" effect arising from the buffering capacity of MPNs (Figure 4c).[4] Using this knowledge, we developed protein–polyphenol,[69] polypeptide–polyphenol,[2] and DNA–polyphenol[49] particles that exhibited endosomal escape property. Protein–polyphenol particles were assembled based on the diverse supramolecular interactions between proteins and TA.[69] Three types of bioactive proteins, including cytochrome C, β-galactosidase, and IgG, were used as building blocks and loaded into MS templates and cross-linked with TA. Following template

removal, the resultant protein–TA nanoparticles showed pH-responsive surface charge reversal, transitioning from negative to positive charges when the pH was switched from neural (pH 7.4) to acidic (pH 4.5–5). This charge reversal property enabled the protein–TA nanoparticles to escape from the acidic intracellular compartments owing to their positively charged surface at pH 4.5–5. Furthermore, the proteins could be released from the nanoparticles in the cytosol after endosomal escape owing to the presence of endogenous peptides and amino acids (e.g., glutathione), triggering the disassembly of nanoparticles through competitive supramolecular interactions.[69] Similarly, polypeptide–polyphenol particles were assembled based on the supramolecular interactions (e.g., electrostatic, hydrogen, and/or hydrophobic interactions) between the polypeptide and TA.[2] The polypeptide–polyphenol particles assembled from positively charged polypeptides (e.g., PArg, PLys, and PHis) exhibited surface charge reversal from negative to positive when pH switched from 7.4 to 4. Confocal microscopy analysis confirmed that the PArg–TA-coated polystyrene particles exhibited endosomal escape behavior, likely due to the pH-dependent charge reversal of PArg–TA complex and/or the cell-penetrating property of PArg. As a result, doxorubicin-loaded PArg–TA particles showed enhanced drug delivery and cytotoxicity in vitro when compared with doxorubicin-loaded PPro–TA counterparts, which did not show pH-responsive charge reversal or endosomal escape properties.[2] DNA–polyphenol particles were also engineered through the supramolecular assembly of DNA and TA. The DNA–TA particles were stabilized primarily via their hydrogen bonding and π–π stacking interactions.[49] Following cellular internalization, the particles rapidly moved from early endosomes to late endosomes and lysosomes and underwent partial degradation upon exposure to the endo/lysosomal enzyme and subsequently translated to the cytosol, which was likely facilitated by the buffering capacity of TA.[49] In addition, we reported the localization of a chemically modified doxorubicin-based

nanodrug particle with tubular mitochondrial nanostructures (Figure 4c); this localization is likely due to the preferential affinity of the drug molecules for the membrane lipid raft domains through hydrophobic interactions.[65]

## 5. SUMMARY AND OUTLOOK

This Account focuses on our recent advances in particle assembly using functional ligands for controlled physicochemical properties and bio–nano interactions, including stealth, targeting, and cell trafficking. The functional ligands, small molecules (e.g., TA, EGCG), polymers (e.g., PEG, polypeptides), and biomacromolecules (e.g., proteins and DNA), are used as building blocks for particle assembly, which facilitate the engineering of particles with specific properties. Despite significant progress made on using functional ligands for controlled bio–nano interactions, only a limited number of particle systems have resulted in successful clinical translation. A key reason for this lack of translation success is the rapid recognition and deactivation of nanoparticles by the immune system. For example, the biomolecular coronas formed in human plasma are known to opsonize foreign materials, leading to the sequestration of nanoparticles by the immune cells. Our study has shown that a biomolecular corona formed in human plasma is person-specific,[3] which can potentially lead to varied responses to nanomedicines. Understanding the impact of person-specific biomolecular coronas will guide the design of nanoparticles for personalized therapies. There is also a desire to expand the toolbox of stealth ligands. PEGylation has been considered as a gold standard in bioconjugation and nanomedicine to maintain colloidal stability and extend blood circulation time. However, recent studies have shown that anti-PEG antibodies can be boosted in humans following vaccination with SARS-CoV-2 mRNA LNP vaccines that incorporate PEG to stabilize the particles.[70,71] Understanding the mechanism and clinical impact of vaccine-induced anti-PEG antibodies is important for future development of PEG-based

nanomedicines, which will require collaboration between immunologists, materials scientists, and clinicians. It will also be critical to develop and assess PEG alternatives, such as zwitterionic polymers (e.g., poly(carboxybetaine methacrylate)[72]) or peptides (e.g., glutamic acid/lysine alternating sequence[61,73]), that mimic the stealth properties of PEG. In addition to the precise control of particle properties, large scale and reproducible synthesis of nanoparticles is important to achieve successful clinical translation. The physicochemical properties, especially size, can be influenced by the scaling up process.[74] The design of particle preparation methods should be compatible with upscaling instruments, and video recording of detailed protocols may facilitate the reproducibility (in terms of synthesis and performance) and translation of nanoparticles.[10,11,74] A main goal of bio–nano interaction studies is to design and understand how particle systems can overcome biological barriers, thus improving their delivery efficiency. Overall, improving our understanding of particle design, fabrication, and bio–nano interactions will lead to new opportunities and innovative design of nanoparticle systems for biomedical applications.

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### Notes

The authors declare no competing financial interest.

### Biographies

**Yi Ju** received his Ph.D. degree from The University of Melbourne in 2017 under the supervision of Frank Caruso. Following his Ph.D. completion, he held a Research Fellow position in the same group (2017–2021) and moved to RMIT University in 2021 to take up a three-year Vice-Chancellor's Postdoctoral Fellowship. In 2022, he received an ARC Discovery Early Career Researcher Award (DECRA) to study the interactions between nanomaterials and the immune system.

**Chan-Jin Kim** completed his Ph.D. in 2014 at Yonsei University developing protease assay materials. He then carried out postdoctoral research on the synthesis of functional DNA block copolymer assemblies at Ewha Womans University (2014–2018) and on bacteria targeting at Nanyang Technological University (2018–2020). Since 2020, he has been working with Frank Caruso as a postdoctoral researcher. His research focuses on the engineering of particles for materials science and biomedical applications.

**Frank Caruso** received his Ph.D. in 1994 from The University of Melbourne and thereafter conducted postdoctoral research at the CSIRO Division of Chemicals and Polymers. He was an Alexander von Humboldt Research Fellow and a group leader at the Max Planck Institute of Colloids and Interfaces from 1997 to 2002. Since 2003, he has been a professor, and is currently a Melbourne Laureate Professor at The University of Melbourne. His research focuses on developing advanced nano- and biomaterials for biotechnology and medicine.

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