Review

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Intelligent nanomaterials for cancer therapy: recent progresses and future possibilities

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Abstract: Intelligent nanomedicine is currently one of the most active frontiers in cancer therapy development. Empowered by the recent progresses of nanobiotechnology, a new generation of multifunctional nanotherapeutics and imaging platforms has remarkably improved our capability to cope with the highly heterogeneous and complicated nature of cancer. With rationally designed multifunctionality and programmable assembly of functional subunits, the *in vivo* behaviors of intelligent nanosystems have become increasingly tunable, making them more efficient in performing sophisticated actions in physiological and pathological microenvironments. In recent years, intelligent nanomaterial-based theranostic platforms have showed great potential in tumor-targeted delivery, biological barrier circumvention, multi-responsive tumor sensing and drug release, as well as convergence with precise medication approaches such as personalized tumor vaccines. On the other hand, the increasing system complexity of anti-cancer nanomedicines also pose significant challenges in characterization, monitoring and clinical use, requesting a more comprehensive and dynamic understanding of nano-bio interactions. This review aims to briefly summarize the recent progresses achieved by intelligent nanomaterials in tumor-targeted drug delivery, tumor immunotherapy and temporospatially specific tumor imaging, as well as important advances of our knowledge on their interaction with biological systems. In the perspective of clinical translation, we have further discussed the major possibilities provided by disease-oriented development of anti-cancer nanomaterials, highlighting the critical importance clinically-oriented system design.

Keywords: intelligent nanomedicine; anti-cancer nanomaterials; tumor microenvironment; tumor vaccines; immunotherapy; personalized medication

Introduction

The rapid advances of nanobiotechnology have been introducing revolutionary changes in cancer medicine. Compared with traditional formulations, nanomaterial-based therapeutics provide a much wider range of biological functionalities as well as their combinations, allowing them to cope with the complexity of living systems more efficiently [1–4]. In anti-cancer drug development, nanocarriers have long been used to attain tumor-targeted delivery, controlled drug release, optimized pharmacokinetics, or to deliver multiple drugs at the same time [1–3, 5–7]. However, the highly heterogeneous and complicated nature of tumor microenvironment (TME), and the presence of diverse biological barriers against drug delivery, are still major challenges for the development of nanomedicine [2, 8–11]. Intelligent nanomaterials, namely nanostructures that can be precisely controlled to perform a complex series of actions (e.g., guided navigation, assembly or disassembly, topological change, surface charge reversal) *in vivo*, either in response to specific biological conditions (e.g., pH, oxidative stress, inflammation, cell phenotype abnormalities), or modulated by external input signals (e.g., radiation, ultrasound, magnetic field), are therefore attracting much research interest and

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opening new possibilities for nanotechnology-enabled cancer treatment [1, 3, 12–15].

Early study on anticancer nanomaterials mainly focused on their application as drug carrier to enhance the efficacy of traditional therapeutics. In the recent years, the immense multifunctional capacity of new-generation intelligent nanomaterials has allowed them to be exploited in an increasingly diversified manner. Intelligent nanosystems have demonstrated great potential in emerging fields such as immunotherapy [4, 16–18] and real-time imaging [3, 19, 20], and provided excellent platforms for personalized medication [21–27]. Significant effort has also been made to promote our understanding of the *in vivo* behavior of complex nanosystems, which helped to pave the way for the rational design of intelligent nanotherapeutic as well as their effective clinical translation [8, 28–31]. This review would like to provide a brief summary on the recent advances in using intelligent nanomaterials for cancer imaging and treatment, and on our knowledge regarding their interaction with the living system (Figure 1).

Novel strategies for tumor-specific drug delivery

Tissue-targeted drug delivery remains one of the most important medical applications of nanotechnology. Nanomaterial-based vehicles could be designed to specifically recognize diseased tissues or cell types, protect the encapsulated cargo from macrophage or enzyme clearance, actively penetrate biological barriers, and "intelligently" release drug in response to particular microenvironmental signals [8, 9, 32]. While molecular recognition and stimuli-triggered drug release are still the most commonly used strategies for nanomaterial-based tumor targeting, new-generation intelligent nanocarriers have sought to integrate these simple activities rationally and hierarchically, in order to enable a more precise temporospatial control over their actions [33–35].

Assembly and disassembly of composite nanostructure plays a critical role in the cargo encapsulation and release behavior of intelligent drug carriers. Developing biomaterials with programmable supramolecular behavior would therefore greatly facilitate the quality control of nanotherapeutics as well as reduce non-specific drug release *in vivo*. Compared to other self-assembly platforms such as peptides, polymers, or liposomes, nucleic acid-based nanomaterials showed particular promise in this aspect, since their self-assembly process could be precisely manipulated via base pairing. It has long been reported that by rationally programming the nucleotide sequences of building blocks, DNA and RNA molecules could be organized into an infinite variety of 2D and 3D structures, with rigorous control over their size, shape and surface functionality [15, 35–43]. Using such bottom-up approach, therapeutic agents, targeting ligands and stimuli-sensitive switches can be integrated into the nanosystem with striking spatial and stoichiometric accuracy. Previous study has already demonstrated that DNA self-assembled nanostructures were able to safely deliver thrombin, a potent blood coagulator, through intravenous administration to the tumor site without causing non-specific peripheral thrombosis [44]. Notably, the application of DNA origami technique allows thrombin molecules to be precisely arranged at a series of fixed binding sites inside the tubular vehicle, therefore significantly minimizing

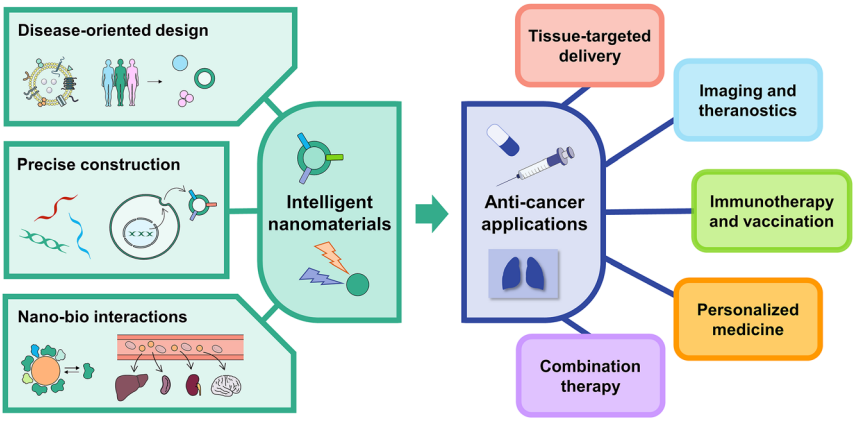


Figure 1: Schematic overview of the development of intelligent nanomaterials for anti-cancer applications. Anti-cancer nanomaterials should be rationally designed according to the specific needs of different diseases and patients, while their multifunctionality and biomedical properties should be precisely manipulated during construction. To address the current challenges in clinical translation, deep understanding of their interactions with biological systems is also necessary.

non-specific exposure in bloodstream (Figure 2A). This advantage of programmable drug loading has been further exploited to deliver multiple agents at the same time. For example, a DNA-origami-delivered tumor nanovaccine was constructed by loading three active ingredients (a tumor antigen peptide plus two different nucleic acid adjuvants) together into a similar tubular "nanorobot" (Figure 2B) [45]. Being equipped with a set of pH-sensitive DNA locking strands, the nanostructure maintained its "closed" tubular form in neutral environment, but can be triggered to "open up" by the acidic pH in endosomes of dendritic cells (DCs) following cellular internalization. The two nucleic acid adjuvants, both toll-like receptor (TLR) agonists, simultaneously activated TLR3 and TLR9 pathways, respectively, thus promoting DC maturation and antigen presentation via a synergic mechanism. Although the potential of polymeric or liposomal nanoparticles in multidrug delivery has already been elaborately described, DNA self-assembled carriers hold unique advantage in that different drugs could be delivered with exact stoichiometric ratio, since the number/position of drug loading sites and conjugation linkers were accurately programmed via sequence encoding. Particularly, small nucleic acid drugs could be efficiently loaded into nucleic acid nanocarriers via hybridization and safely delivered avoiding enzyme degradation.

In vivo self-assembly of nanomaterials represents another emerging trend for intelligent drug delivery. While nanoparticle formulations may improve targeted delivery of antitumor therapeutic, their ability to penetrate into tumor tissue is limited compared to free drugs [8, 46]. This could be particularly problematic in solid tumors with highly fibrotic and dense stroma. Small-sized building blocks that can be administered in their free form but selectively aggregate/assemble into nanostructures at the tumor site were thus developed to address this challenge. So far, existing *in vivo* self-assembly platforms were mainly constructed with functional short peptides, which were hydrophilic in their original form yet became largely hydrophobic in response to tumor-related stimuli, therefore ending up in spontaneous aggregation [19, 47–49]. Cong et al. reported a polymer–peptide conjugate that underwent a sharp hydrophobicity increase when pH changed from 7.4 to 6.5, allowing the monomer to specifically self-assemble in the weakly acidic

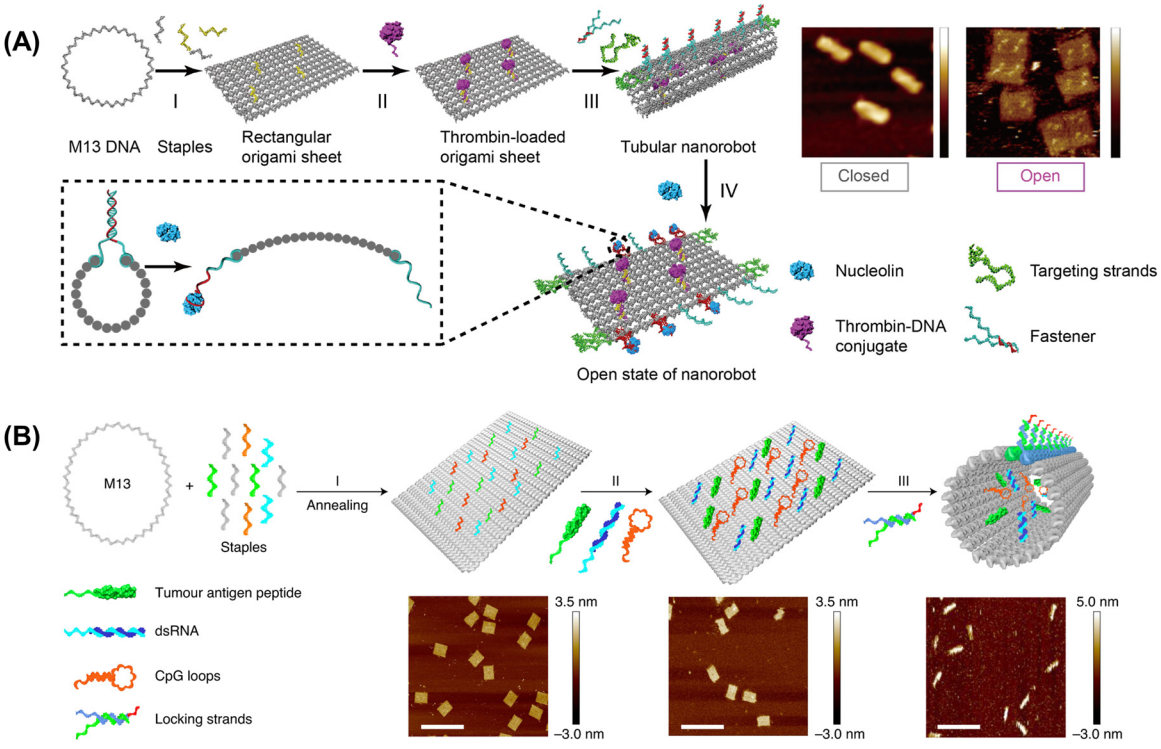


Figure 2: DNA nanotechnology-based intelligent nanomedicines for cancer therapy. (A) Schematic illustration and atomic force microscopy (AFM) characterization of a DNA origami nanorobot for tumor-specific delivery of thrombin. The drug-loaded tubular structure could selectively recognize nucleolin, a tumor blood vessel-specific biomarker. Reproduced with permission [44]. Copyright © 2018 Springer Nature. (B) Schematic illustration and AFM characterization of a pH-sensitive DNA origami nanorobot for tumor vaccination. Reproduced with permission [45]. Copyright © 2020 Springer Nature.

TME [46]. Through the incorporation of a cell penetrating peptide motif, the monomer was able to deeply penetrate into the tumor, facilitating the delivery of a conjugated cytotoxic peptide. Since such stimuli-triggered assembly typically occurs in a deeper region of tumor tissue, *in situ* constructed self-assembly often show remarkably prolonged tumor retention compared to intravenously administered nanoparticles, while off-target monomers could be quickly degraded to minimize side effects [49]. For example, a photosensitizer-conjugated peptide that can be specifically cleaved by the TME-overexpressed enzyme gelatinase has been employed for targeted photothermal therapy, with the enzyme-induced peptide aggregation significantly improving the intratumoral half-life of photosensitizer [50]. An et al. conjugated a caspase 3/7 cleavable peptide with a molecular recognition motif targeting X-linked inhibitor of apoptosis protein (XIAP), characteristically upregulated in many types of tumor cells [51]. The specific binding between XIAP and the peptide monomer induced cell uptake and activated intracellular caspase 3/7 enzyme, leading to the cleavage of the monomer, which subsequently self-assembled into fiber-like superstructures. When the peptide was further linked with doxorubicin, this *in situ* cascade activatable fibril formation significantly improve the accumulation and detention of the chemotherapeutic drug. Recently, it has also been demonstrated that certain peptide sequences acquire tendency of aggregation only after binding with proteins. An amyloid-like peptide GNNNQNY conjugated with integrin-targeted motif RGD was reported to show "intelligent" self-assembly behavior that was strictly integrin-dependent, leading to highly efficient targeted delivery based on tumor-related integrin overexpression [52].

Besides bottom-up approaches aiming at rigorously manipulate the intelligent behavior of nanosystem from the construction level, the use of naturally derived materials as functional elements has also witnessed notable progress. It has been suggested that naturally formed supramolecular systems, such as protein coronas [29, 53], plasma membranes [54–56], cell-secreted vesicles [13, 57, 58], often retain unique biomimetic activity that cannot be readily simulated by artificial nanomaterials. For instance, Zeng et al. reported that cisplatin, a platinum-containing first-line chemotherapeutic, spontaneously form Pt nanoparticles with diameter of 6–8 nm in human blood due to serum albumin-induced nucleation [59]. The product nanoparticles were coated with a serum protein corona and could selectively accumulate in tumor tissues via albumin-mediated tumor targeting. Particularly, the intratumoral half-life of Pt nanoparticles resulted from blood-triggered synthesis was remarkably longer compared to chemically synthesized Pt nanoparticles coated with albumin. The authors suggested that while single-composition albumin coating was vulnerable to hydrolyzation *in vivo*, while the complex corona formed in human blood could effectively protect the albumin molecules from rapid degradation.

Cell-membrane modified nanomaterials have attracted enormous attention in these years. Coating nanoparticles with plasma membrane isolated from living cells may allow us to exploit the complex membrane protein profile for tumor-targeting, biological barrier penetration, or immunotherapy, while avoiding complicated chemical modification [56, 60–62]. A biomimetic nanoparticle coated with tumor-associated macrophage (TAM) membrane has recently been developed to improve the strongly immunosuppressive TME after photothermal therapy treatment [63]. When injected intravenously, the nanoparticles showed chemotactic behavior similar to living TAMs, and actively targeted the post-treatment tumor tissue rich of inflammatory cytokines. Platelet membrane coated nanomaterials, on the other hand, were expected to show platelet-like wound-binding activity and tumor cell adhesion [64–66], thus have been exploited for targeted delivery to post-surgical tumor sites [67]. Loaded with an immune checkpoint inhibitor and an anti-angiogenesis drug, this wound-targeted nanosystem effectively suppressed the relapse rate after surgery in a hepatocellular carcinoma mouse model.

Nanovaccines and enhanced immunotherapy

In cancer nanomedicine, immunotherapy aims at stimulating the immune system to "automatically" monitor and deplete malignant cells, and is currently regarded as one of the most potent and promising therapeutic modalities against cancer [68, 69]. While impressive clinical success has already been achieved by various immunotherapeutic approaches, including immune checkpoint inhibition and adoptive T cell therapy, their response rates among patients are still largely unsatisfactory [16, 70]. Much effort has been dedicated to the development of novel strategies to enhance anti-tumor immunity, in which nanomaterial-based platforms are playing an increasingly crucial role [16–18, 71].

Similar to vaccines against infectious diseases, tumor vaccines present tumor-related biological information (e.g., antigens) to the immune system and elicit antigen-specific immune recognition and response [72]. Tumor

vaccination is able to provoke long-term systematic immune memory against metastases and recurrence, and allows highly individualized treatment [21–23, 73]. Traditionally, peptides containing tumor antigen information were delivered to antigen presenting cells (APCs), especially DCs, whose maturation subsequently activated antigen-specific CD8+ T cell immunity [72]. However, the immunogenicity of such antigen peptides is typically weak, requiring co-delivery of adjuvants. Nanovaccines that are capable of delivering multiple components simultaneously into the same APC are therefore of great interest, and multifunctional strategies to actively target APCs, promote antigen cross-presentation, or facilitate customized treatment have been accordingly developed [23, 72, 74, 75].

Qin et al. proposed a two-step strategy for the targeted delivery of nanovaccines to lymph nodes (LNs) [76]. Compared to peripheral DCs, which are the conventional targets of subcutaneously or intramuscularly injected vaccines, LN-resident DCs can be more attractive targets for tumor antigen delivery, due to their dense local population and close proximity to T cells. A major obstacle of LN-targeted delivery, however, is the lack of convenient molecular target for ligand-receptor recognition. In this work, a PEGylated phospholipid precursor containing a terminal azide group was first injected subcutaneously. Through a previously known mechanism called albumin hitchhiking [77], the precursor efficiently accumulated in LNs after binding to intrinsic albumin. The lipid moiety could then readily insert into the plasma membrane of lymphatic endothelial cells, "labeling" LNs with the azide group. Tumor antigen and adjuvants were then delivered by a liposome-coated nanoparticle modified with dibenzocyclooctyne (DBCO) groups, which selectively react with azide in physiological conditions via bioorthogonal chemistry [78]. The authors stated that targeting LN-resident cells helped to circumvent the limited efficiency of DC migration from peripheral tissues, and significantly enhanced the antigen presentation process.

Yin et al. presented another strategy to improve the APC-mediated immune response of subcutaneous vaccines [79]. An intelligent transformable hydrogel was constructed with graphene oxide and low molecular weight polyethylenimine via electrostatic interaction. Once injected into living body, this hydrogel tended to slowly transform into small nanoparticles on the nano-bio interface. Encapsulated with an mRNA antigen and an adjuvant, the hydrogel could hence gradually produce antigen- and adjuvant-loaded nanoparticles *in situ* for over 30 days after subcutaneous administration, providing a much more persistent immunostimulatory effect compared to conventional nanovaccines. By continuously presenting the same antigen to the immune system, this transformable platform also effectively enhanced antigen-specific T cell response.

One of the predominant limitations of traditional tumor vaccines lies in the fact that tumor cells are highly heterogeneous among patients and it is impossible for a prescribed vaccine formulation to effectively treat all the cases [21, 22, 73, 80]. Developing nanoplatforms that can be easily customized case-by-case to deliver patient-specific information may thus help to solve this problem. A "plug-and-display" platform based on bacteria outer membrane vesicles (OMVs) has been developed to deliver customizable combination of multiple tumor antigens [81]. As bacteria-derived nanosized membrane vesicles, OMVs contain various pathogen-associated molecular patterns (PAMPs) and have intrinsic immunostimulatory effects [82–84]. Previously, OMVs have been used as anti-tumor immunotherapeutic [85] and to deliver checkpoint antibodies [86]. By genetically manipulate the source bacteria, two different protein catchers were expressed on the OMV surface, which could respectively bind specific protein tags through the peptide superglue technology, allowing different antigens linked to these tags to be rapidly and flexibly displayed (Figure 3) [81]. The OMV platform served as both vehicle and adjuvant. Such versatile platforms represent an emerging trend in tumor vaccine development and may facilitate the clinical translation of nanovaccines against cancer.

In addition to synthetic validated tumor antigens (e.g., peptides and mRNAs), the use of tumor cell-derived components (e.g., whole cells, cell lysates, cell-derived vesicles) as antigenic material has also been proposed [73, 87–89]. Tumor cell membranes, for example, contain a great variety of antigenic motifs, especially neoantigens and provide an excellent platform for personalized antigen delivery. Diverse strategies have demonstrated that co-delivery of tumor cell membrane fragments and adjuvants could elicit strong tumor-specific immunity in animal models [90–94]. Recently, Chen et al. reported a hybrid membrane nanovaccine formulation, constructed by fusing autologous tumor cell membrane with bacterial cytoplasmic membrane into a hybrid coating layer, which was further decorated on the surface of polymeric nanoparticles (Figure 4A) [25]. Similar to OMVs, bacterial cytoplasmic membrane could be used as naturally-derived adjuvant, with favorable biosafety due to the separation from toxic cell wall components such as lipopolysaccharide (LPS). In the hybrid product, the immunostimulatory properties of the bacteria-derived components enhanced APC uptake, and subsequently the processing and presentation of antigens on the tumor cell membrane surface. Such strategy was able to elicit anti-tumor immunity and immune memory without the need of antigen isolation and validation, while the use

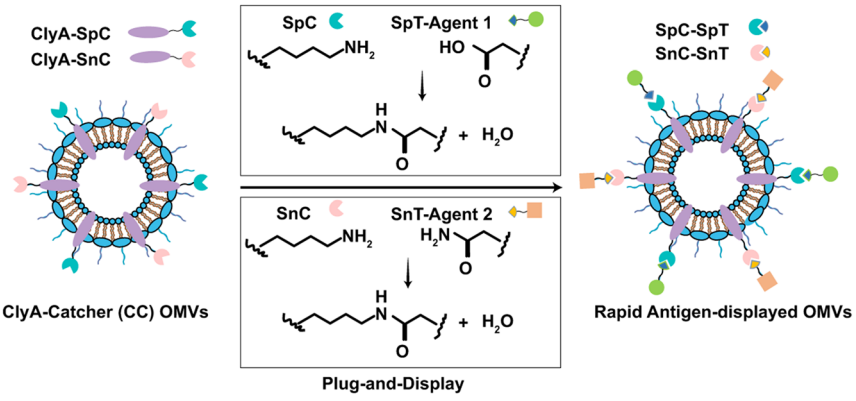
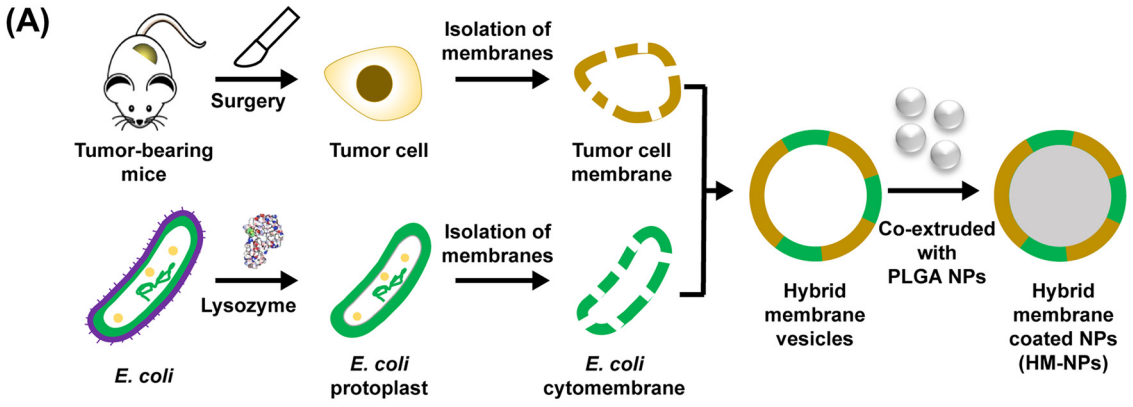


Figure 3: Schematic illustration of a customizable tumor antigen delivery platform enabled by biologically engineered bacteria outer membrane vesicles. Reproduced under the terms of the CC-BY Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/) [81].



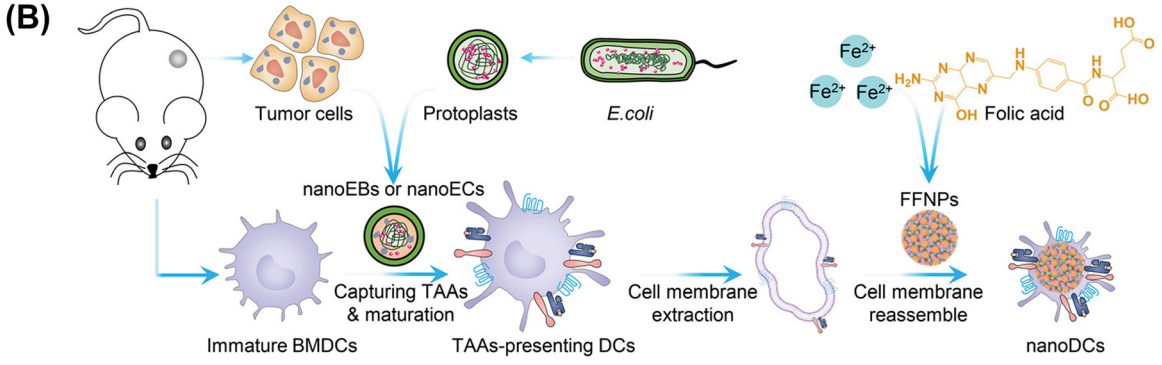


Figure 4: Engineering autologous tumor cell membranes for enhanced and personalized tumor vaccination. (A) Schematic illustration of a hybrid membrane nanovaccine using bacterial cytoplasmic membranes as biomimetic adjuvant. Reproduced with permission [25]. Copyright © 2021 The American Association for the Advancement of Science. (B) Schematic illustration of a DC membrane nanovaccine presenting autologous tumor antigens. Reproduced with permission [95]. Copyright © 2022 Wiley-VCH GmbH. NPs, nanoparticles; nanoEBs, *E. coli*-B16-OVA hybrid nanostructures; nanoECs, *E. coli*-CT26 hybrid nanostructures; TAAs, tumor-associated antigens; BMDCs, bone-marrow-derived cells; DCs, dendritic cells; FFNPs, folic acid and ferrous ion self-assembled nanostructures.

of autologous tumor cell membranes provided a possibility to dynamically represent the biological information of heterogeneous tumors on individual level. In addition, Zhang et al. used nanoparticles co-assembled by tumor cell membrane and *E. coli*-derived membrane to stimulate bone-marrow-derived cells (BMDCs). The pulsed BMDCs expressed tumor-specific molecules class I (MHC-I) antigen complexes, costimulatory molecules and lymphocyte homing

molecules on their membrane, which could be extracted and decorated on dendritic nanostructures resulting in a dendritic cell-mimicking nanovaccine system (Figure 4B) [95].

Compared with other administration routes, oral administration of therapeutics has many advantages in terms of safety, patient compliance and cost [96, 97]. As the largest immune organ in human body, intestine contains over 70 % of the body's immune cells, making orally administered tumor vaccines particularly attractive [98]. In order to circumvent the challenges posed by the complex gastrointestinal environment to vaccine delivery, Yue et al. developed a genetically engineered *E. coli* bacteria, which could be readily retained in the intestine after oral administration [99]. The bacteria started to produce tumor antigen-containing OMVs *in situ* only when induced by another orally taken substance, arabinose. These OMVs could effectively penetrate the intestinal epithelial barrier [100] to encounter the DCs in the lamina propria, leading to enhanced antigen presentation and DC maturation (Figure 5).

While current nanovaccines have been demonstrated as encouragingly powerful in eliciting anti-tumor immune response [72, 74], there are still many other factors that affect their effectiveness in clinic. In many solid tumors, a highly immunosuppressive microenvironment prevents the infiltration and activity of cytotoxic immune cells, resulting in reduced response rate and resistance [21, 70, 101]. A great number of intelligent nanomaterial-based strategies have been developed to remodel the TME and to sensitize such "cold" tumors to anti-tumor immunity [70, 102], most commonly via enhanced immune checkpoint inhibition [86, 103–105] and macrophage repolarization [106–109]. For example, Lin et al. reported that the immunosuppressive TME could be effectively relieved by restoring the local expression of phosphatase and tensin homolog deleted on chromosome 10 (PTEN), a crucial tumor suppressor gene mutated frequently in a wide range of tumors. A nanocarrier encapsulating PTEN-encoding mRNA significantly improved the therapeutic efficacy of immune checkpoint inhibition treatment [110]. On the other hand, Feng et al. constructed an OMV-based intelligent nanosystem to re-educate tumor-associated macrophages (TAMs), the most abundant immune suppressive cells in TME [106]. A CD47-neutralizing nanobody was expressed on the OMV surface, which could block the CD47-dependent anti-phagocytosis signal on tumor cells, making them vulnerable to macrophage-mediated immunity. The whole system thus acted as a two-way adapter that simultaneously bound tumor cells via the nanobody and TLRs on TAMs via the immunogenic OMV vehicle, repolarizing TAMs and sensitizing tumor cells at the same time. The nanoplatform was further coated with a polymeric later containing diselenide bonds, which shielded its

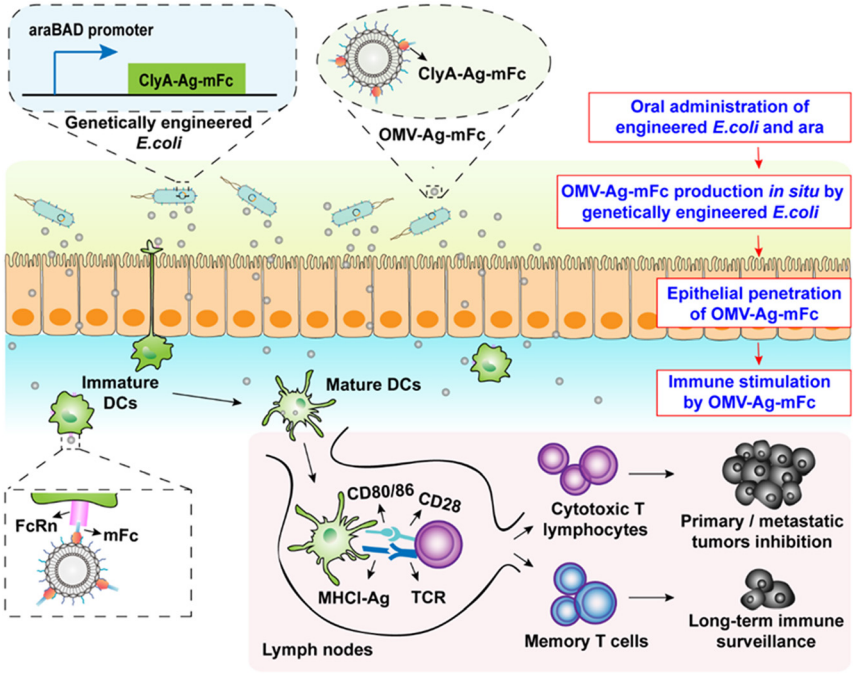


Figure 5: Schematic illustration of an orally administered tumor vaccine *in situ* produced by engineered intestinal bacteria. Reproduced under the terms of the CC-BY Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/) [63]. DCs, dendritic cells; TCR, T cell receptor.

immunogenicity during intravenous delivery, but could selectively release the active OMV-nanobody composite upon localized radiation treatment.

Nanomaterial platforms for temporospatially specific tumor imaging

Techniques for high-resolution and real-time tissue imaging are of tremendous importance in cancer diagnosis and treatment, and have always been among the most active fields in nanobiotechnology [20]. Advanced imaging techniques allow clinicians to diagnose and stratify tumors in their early stage, perform image-guided surgery, monitor disease progression or patient response to treatment. Adequate bioanalytical methods are also critical in studying the molecular mechanisms involved in tumor development or the interactions between tumor cells and anti-cancer nanotherapeutics. In recent years, many intelligent strategies have been developed to improve the sensitivity, selectivity, and time or space resolution of nanomaterial-based imaging platforms, with notable progress being made in this aspect [3, 111].

In early-generation nanomaterial-based imaging strategies, small molecule dyes or nanoparticle probes were encapsulated in nanocarrier and delivered to the tumor site via molecular targeting or stimuli-triggered activation. Nonetheless, conventional nanocarriers underwent degradation in the tumor tissue in a relatively short time, and the small-sized imaging agents encapsulated were also rapidly cleared from the tumor. Novel delivery strategies based on assembly/aggregation-induced retention (AIR) effect of fluorescent probes have been reported to significantly amplify imaging signals by allowing the imaging agent to be stably retained in the target tissue for a longer time [112, 113]. Zhao et al. designed a functional peptide-conjugated near-infrared (NIR) dye that was responsive to fibroblast activation protein-α (FAP-α), therefore forming fiber-like β-sheet nanostructures selectively in the presence of cancer-associated fibroblasts, a major cellular component of TME (Figure 6A) [114]. Such TME-responsive aggregation behavior led to a 5.5-fold signal amplification 48 h post-injection. To improve the tumor specificity, this platform has been further modified to address the challenge of small-sized tumor monitoring. A tumor-targeting motif and a long-circulation motif were incorporated into the peptide-NIR dye conjugate, enabling it to actively target small orthotropic pancreatic tumors. The AIR effect further extended the imaging window to as long as 96 h post-injection. Since the fibroblast-triggered nanofiber formation selectively occurred at the boundary of pancreatic tumors, this AIR-based strategy may greatly facilitate boundary imaging and identification of small tumors during surgery. Zhou et al., on the other hand, constructed an iron oxide nanoparticle-based *in situ* self-assembly platform for the imaging of hypoxic tumors [115]. Ultrasmall iron oxide nanoparticles were modified with a thiol-containing crosslinking ligand and a nitroimidazole-based hypoxia-sensitive ligand, which remained inert in normal tissues but could irreversibly crosslink with thiol groups under hypoxia conditions in the presence of NADPH and reductase. When intravenously injected *in vivo*, this intelligent nanoprobe selectively aggregated in response to hypoxia regions of tumor, thus significantly amplifying the local T2-weighted magnetic resonance signal in a hypoxia-dependent manner (Figure 6B). Since hypoxia condition played an important role in solid tumor's resistance to chemotherapy and radiotherapy, the author suggested that such stimuli-responsive assembly strategy might be potentially exploited for hypoxia-targeted theranostics.

Among existing stimuli-triggered imaging nanoplatforms, DNA nanodevices have showed noteworthy promise since their high programmability and sequence-specific recognition potential enabled extraordinary flexibility and precision in terms of stimuli-responsive design [111, 116, 117]. Particularly, a variety of DNA-based fluorescence nanoprobes have been constructed to achieve precise temporal and spatial control of signal activation, especially on the cellular and subcellular level. Shao et al. reported a DNA nanosensor that could selectively visualize enzyme activity in a specific subcellular organelle [118]. The nanosystem was consisted of an ultraviolet (UV) light-activatable DNA nanoprobe, an upconversion nanoparticle (NIR-to-UV transducer) and a mitochondria- or nucleus-targeting ligand. The conjugated targeting ligand helped to deliver the nanoprobe into interested organelles, and under light radiation, the otherwise inert DNA nanoprobe transformed to a molecular beacon structure that could be specifically cleaved by apurinic/apyrimidinic endonuclease 1 (APE1), an enzyme with important roles in tumor occurrence and metastasis. The coupling with upconversion nanoparticles allowed the nanoprobe to respond to NIR light that had superior tissue penetration capability. Another organelle-targeted nanosystem was similarly constructed for the detection of mitochondrial microRNAs (miRNAs) [119]. After mitochondria-targeted localization mediated by triphenylphosphonium (TPP), the nanosensor could be

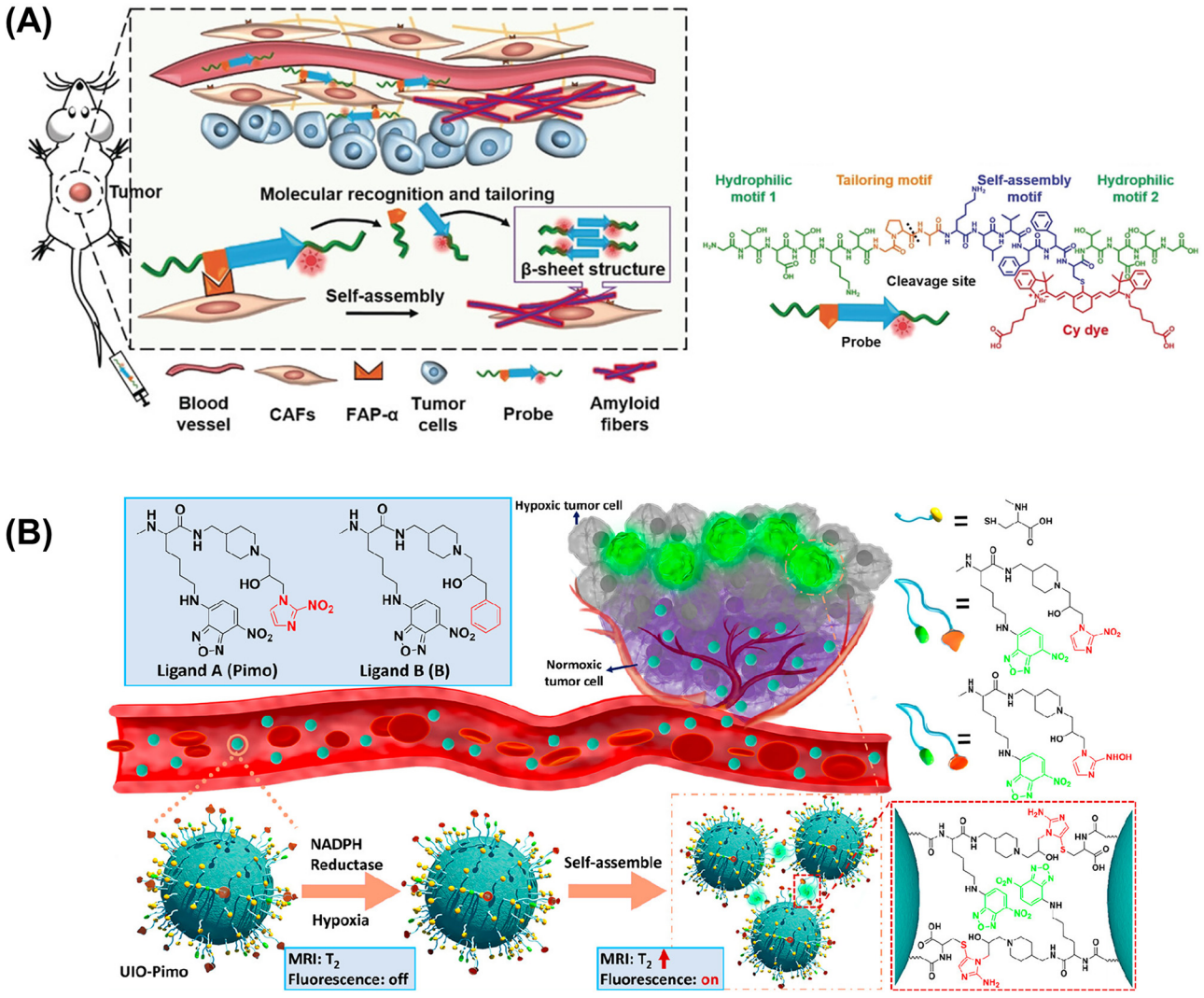


Figure 6: *In situ* self-assembled nanosystems for intelligent tumor imaging. (A) Schematic illustration of tumor associated fibroblast-responsive self-assembly of rationally designed peptide probes. Reproduced with permission [114]. Copyright © 2019 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (B) Schematic illustration of hypoxia-sensitive *in situ* assembly of ultrasmall iron oxide nanoparticles for enhanced magnetic resonance imaging. Reproduced with permission [115]. CFAs, cancer associated fibroblasts; FAP-α, fibroblast activation protein-α.

selectively "switched on" by NIR light signal, and react with specific local miRNA molecules via toehold-mediated strand displacement reactions to activate strong fluorescence (Figure 7A). Notably, by carefully programming the original DNA sequence, this system can be set to only respond the simultaneous presence of two different miRNAs, enabling logic-gated imaging. Xiang et al. also reported a multivariate-gated nanoprobe that responded to dual targets, but relying on an interesting peptide nucleic acid (PNA)-enabled system [120]. It has been demonstrated that by incorporating PNA/peptide copolymers into DNA nanostructures, the biological activity of functional DNA sequences could be manipulated via peptide-targeted approaches such as enzyme cleavage, significantly expanding the potential of stimuli-responsiveness. This nanosystem was constructed with a matrix metalloproteinase (MMP)-responsive PNA/peptide/PNA copolymer, the cleavage of which by MMP2/9 in TME consequently activated an ATP-sensitive DNA fluorescence nanoprobe. The whole system was further coupled with a tumor-targeting aptamer, leading to spatially specific correlated imaging of MMP2/9 and ATP, both characteristic biomarkers of tumor invasiveness (Figure 7B). By providing temporospatially specific methods to monitor the production, activity, subcellular localization of key proteins/signaling molecules involved in tumor progression, such new-generation intelligent nanoprobes might eventually promote our understanding of the heterogeneous and dynamic pathology of cancer.

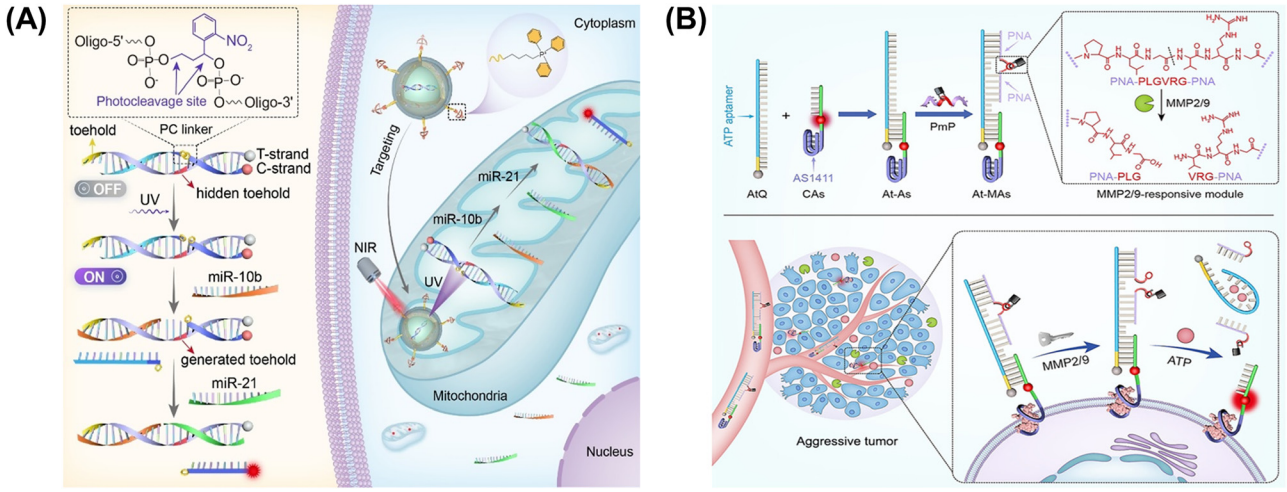


Figure 7: Intelligent DNA nanobiosensors for multi-target tumor imaging. (A) Schematic illustration of temporospatially specific detection of multiple mitochondrial microRNAs via DNA technology. Reproduced with permission [119]. Copyright © 2021 Wiley-VCH GmbH. (B) Schematic illustration of correlated imaging of two TME biomarkers by a peptide nucleic acid nanosystem. Reproduced with permission [120]. Copyright © 2021 Wiley-VCH GmbH.

Understanding nano-bio interactions

Despite of the continuous emergence of novel intelligent nanomaterials that were supposed to hold great promise in medical applications, the clinical translation of nanomaterial-based treatments remained notoriously troublesome [2, 69, 121, 122]. Among the primary obstacles hindering the translation of anti-cancer nanomaterials from benchside to bedside, it has long been noted that the additional complexity compared with traditional drug forms has made it much more difficult to accurately characterize and predict their *in vivo* behaviors [2, 8, 123, 124]. With the increased research interest dedicated to highly sophisticated, robot-like nanosystems that are expected to perform multiple *in vivo* actions, the complexity in their interactions with biological systems is becoming particularly problematic. Integrated methods and tools to systematically evaluate nano-bio interactions are urgently needed, and deep understanding of the *in vivo* fate of intelligent nanomaterials will be essential for the development of next-generation nanomedicines. Below we would like to briefly summarize several representative advances in this aspect.

Formation of protein corona at the nano-bio interface inevitably occurs to all the nanosystems working in biological environment, and is seen as an essential event for nanomaterials to acquire biological identity [29, 53]. Real-time, *in situ* methods to characterize the dynamic evolution of adsorbed proteins would be highly necessary for accurate prediction of nanomaterial behavior in complex application scenarios and have attracted much research interest [30, 125–128]. In particular, the so-called "soft" corona, consisting of a superficial layer of relatively loosely attached proteins, is posing specific challenges for bioanalytical study, both due to their critical roles in nano-bio interaction as the outmost layer of nanomaterial-protein complexes, and their highly dynamic and unstable identity [126–129]. Sanchez-Guzman et al. demonstrated that using cryo-transmission electron microscopy (cryoTEM) and *in situ* techniques such as synchrotron-radiation circular dichroism (SRCD), it was possible to trace the conformation changes of soft corona components caused by nanosurface-driven unfolding [127]. The authors suggested that such changes could not be detected using traditional methods that require nanomaterials being separated from the biological medium, since soft coronas were largely lost during centrifugation (Figure 8). In order to address this separation challenge, Baimanov et al. have recently reported a bio-layer interferometry (BLI)-based strategy for centrifugation-free analysis of soft corona proteins [129]. Immobilizing Cu_2S nanoparticles on BLI biosensors enabled time-resolved *in situ* monitoring of corona formation, and the soft corona components could be selectively eluted for tandem mass spectrometry profiling (Figure 9).

Synchrotron radiation-based analytical techniques have undergone enormous advancement in recent years and have provided critical tools for study of nano-bio interactions on the cellular and tissue levels. Synchrotron light sources are able to produce radiation with wavelength tunable from infrared to X-ray region with extremely high brilliance,

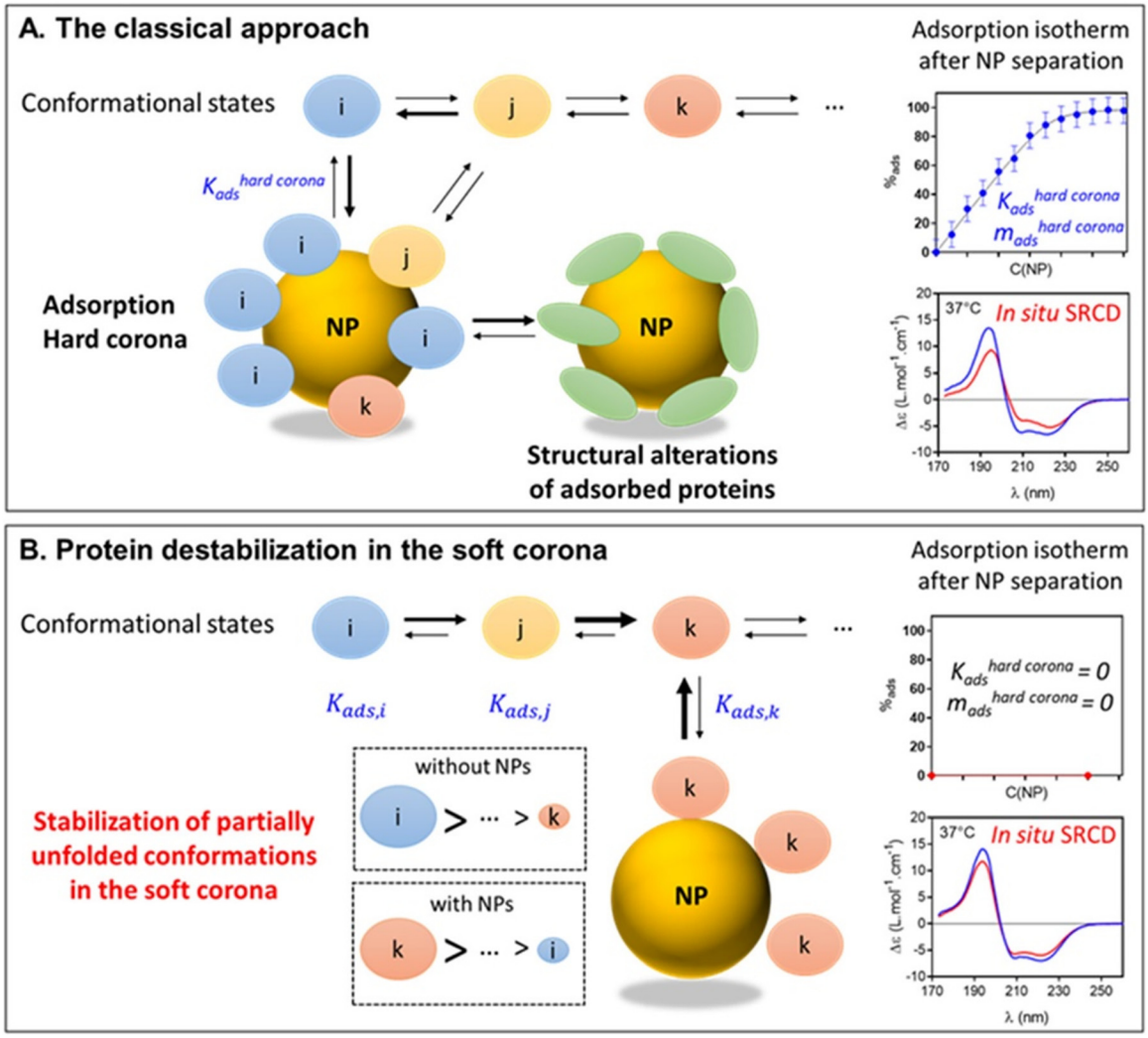


Figure 8: Detection of soft coronas on nanoparticle surface. Classical isolation approaches typically resulted in the loss of soft coronas and conformation changes of corona proteins compared to their *in situ* states (upper panel). It is therefore important to develop analytical methods to capture the subtle conformation alterations of weakly bound proteins induced by the nano-bio interface (bottom panel). Reproduced with permission [127]. Copyright © 2020 American Chemical Society.

stability and level of polarization, compared to conventional sources. Coupling synchrotron radiation sources with X-ray based analytical techniques enabled non-invasive and *in situ* mapping of nanomaterials in living cells or tissues with ultrahigh spatial resolution and excellent penetration depth, and could be used to trace the dynamic change of nanomaterials during nano-bio interactions according to properties such as elemental composition, oxidation state, chemical coordination, crystalline phase, etc., as reviewed elsewhere [130–132]. Synchrotron X-ray based methods are particularly powerful for detection of interactions between nanomaterials and living cells due to their subcellular resolution and living cell imaging ability, and have been applied to characterize the intracellular fate and cell-level biological effects of a wide range of nanomaterials, including inorganic nanostructures, lipid-based nanoparticles [133] and metal-organic frameworks [134]. For example, the cell uptake process and subcellular localization of nanovaccine containing manganese nanoadjuvant during its interaction with DCs has been characterized using synchrotron radiation hard X-ray nano-computed tomography (nano-CT) [135]. In an integrated study [136], diverse synchrotron radiation-based tools were combined to trace the *in vivo* course of MoS_2 nanomaterials on both cellular and tissue level. Soft X-ray nano-CT revealed the subcellular distribution of MoS_2 nanodots in various blood cells, with synchrotron radiation microbeam X-ray fluorescence (SRXRF) being applied to map the precise distribution of molybdenum

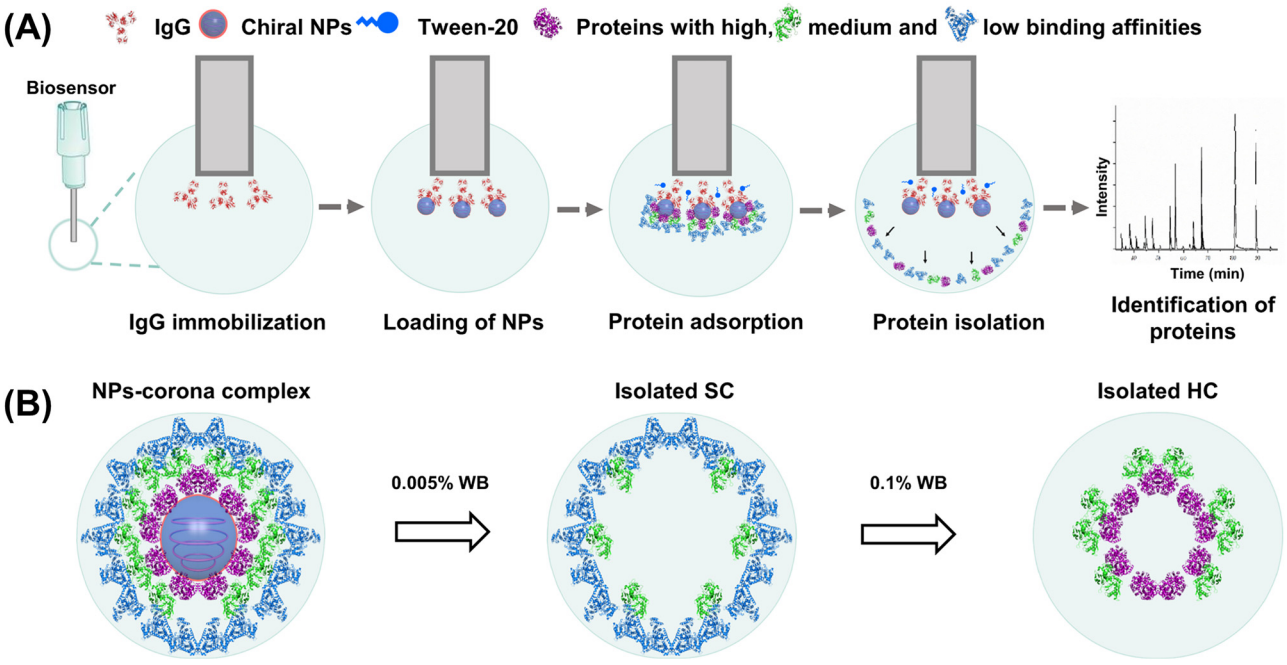


Figure 9: Schematic illustration of bio-layer interferometry (BLI)-based separation of hard and soft coronas. (A) Using BLI biosensors to monitor the dynamic protein adsorption on the surface of immobilized nanoparticles. (B) Centrifuge-free separation of hard and soft coronas through washing buffer (WB). Reproduced under the terms of the CC-BY Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/) [129]. NPs, nanoparticles; SC, soft corona; HC, hard corona.

and other elements in liver and spleen. X-ray absorption near-edge spectroscopy (XANES) was further employed to study the metabolism of nanomaterial in liver according to the chemical form changes of molybdenum. It was demonstrated that via the mediation of surface adsorbed serum proteins, principally apolipoprotein E (ApoE), intravenously injected MoS_2 nanomaterials significantly accumulated in liver sinusoid and splenic red pulp. Interestingly, biotransformation data indicated that hepatic metabolism of MoS_2 transformed the nanomaterial into bioavailable molybdenum, which could be incorporated into molybdenum-dependent metabolic enzymes in liver, up-regulating their activity. The authors suggested that this study indicated the possibility of nanomaterials being actively reused by organisms and in turn having a long-term impact on liver function, implying a more complicated picture regarding the *in vivo* fate of nanomaterials compared to the traditional biodistribution-metabolism-excretion paradigm (Figure 10).

Multi-omics research is another analytical approach that have been demonstrated as particularly useful in nano-bio interface characterization, especially in systematically exploring the underlying molecular mechanisms of nanomaterial-associated biological effects [137, 138]. For example, through integrative application of proteomic, metabonomic and lipidomic methods, Cai et al. analyzed the impact of composition changes in gold nanoparticles' protein corona during nanoparticle-cell interaction on cellular metabolism [139]. Proteomic profiling suggested that the protein composition of corona dramatically evolved across the different stages of nanoparticle-cell interactions (in the bloodstream, in the lysosomal compartment, and in the cytoplasm after lysosome escape), with a notable abundance of intracellular proteins, particularly pyruvate kinase M2 (PKM2) and chaperones, subsequently displacing the initial serum proteins after cellular uptake. Metabonomic and lipidomic data further indicated that this dynamic change in nanomaterial surface protein coating had significant influences on the intracellular metabolism, likely by interfering with corona protein-dependent processes such as glycolysis (PKM2-dependent) and chaperone-mediated autophagy.

Understanding the complex events at nano-bio interfaces as well as their principles also facilitate the development of novel anti-cancer treatments. For example, it has been reported that the composition of adsorbed serum proteins could be manipulated through nanomaterial surface properties and be exploited for tissue-targeted delivery [29, 53, 140]. Tuning the surface charge of liposomes could lead to differences in their reticuloendothelial system clearance profile and selectively increase their liver or spleen accumulation to facilitate organ-specific

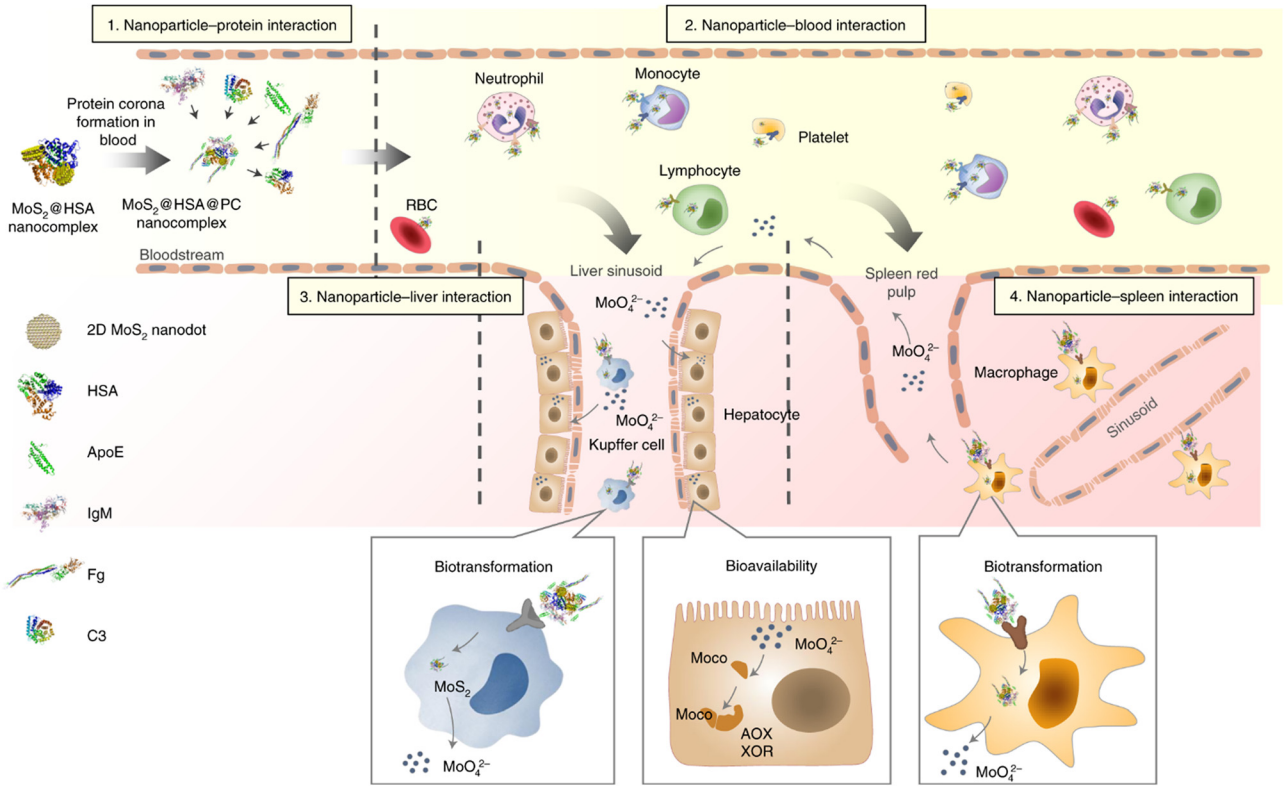


Figure 10: Precise understanding of nano-bio interactions enabled by advanced analytical techniques. Integrated application of synchrotron radiation-based methods revealed the complicated *in vivo* fate of MoS_2 nanomaterials on cell, tissue and whole body levels. Reproduced with permission [136].

drug delivery [141]. During the interaction with single cells, it was demonstrated that the morphology of nanomaterials significantly affects their tendency to enter the cell or adhere to cell surface, allowing researchers to design nanocarriers with specific size/shape to maximize their extravasation or cell uptake [8, 124]. Liang et al. reported that surface rigidity of nanoparticles also influenced their tumor-targeting efficiency, presumably by modulating their contact with plasma membrane of endothelial cells and tumor cells [142].

It has long been demonstrated that certain nanomaterials have specific binding capability with functional proteins and could regulate their biological activity through specific molecular interactions. Proteomic analysis indicated that metallofullerenol nanoparticle $Gd@C_{82}(OH)_{22}$ had a strong binding affinity with complement 1q (C1q), a protein component of complement system [143]. Such binding spontaneously altered the protein's secondary structure, making metallofullerenol-binding C1q capable of eliciting innate immune response, promoting the secretion of pro-inflammatory cytokines and activating macrophages. C1q-coated $Gd@C_{82}(OH)_{22}$ nanoparticles could therefore be potentially exploited for immunomodulation. In another study, it was reported that graphdiyne oxide (GDYO) nanosheets specifically interacted with signal transducer and activator of transcription 3 (STAT3), a key signaling molecule in the polarization of macrophages [144]. Molecular simulation suggested that the GDYO-STAT3 affinity was driven by multiple factors, including structure matching, hydrogen bonding and salt bridges. Importantly, when GDYO was administered to tumor-bearing mice and internalized by macrophages in the TME, this strong interaction between GDYO and STAT3 eventually resulted in the inhibition of immunosuppressive macrophage phenotype (M2) and the re-polarization of TAMs towards the anti-tumor phenotype (M1). These results implied that besides biological effects related to general nano properties such as shape, size, softness, surface charge, nanomaterials could also regulate cellular activity via specific molecular mechanisms. Exploring such mechanisms, especially those closely related to tumor-associated pathways and proteins, may thus provide invaluable information for drug discovery in the future.

So far, complicated biological effects remained one of the major obstacles in nanomedicine development and it is expected that further technical and methodological advances would be required to confront this challenge.

Regarding characterization methods, current super-resolution microscopic techniques such as structured illumination microscopy (SIM) or photo-activated localization microscopy (PALM) allowed fluorescence imaging of cellular and subcellular structures with spatial resolution of less than 100 nm [145], and have already been combined with electron microscopic methods to study nanoscale targets (including fluorescent nanostructures) in an ultrastructural context [146]. Further integration with techniques capable of 3D scanning and reconstruction of cells or organoids such as lightsheet microscopy [147], serial block-face electron microscopy (SBEM) [148] and micro-computed tomography (micro-CT)/nano-CT [136, 148], and those for dynamic imaging of living tissues such as multiphoton microscopy [149], would allow us to correlate biological information over a wide range of scales (from molecular level to whole animal level), and in both spatially resolved and time-resolved manner. One of such multi-scale imaging platforms has already been successfully constructed to map the comprehensive mitochondrial and metabolic network in lung cancer mouse models [148], and these techniques could be extremely helpful in characterizing interactions between nanomaterials and living body via a holistic approach.

While proteome analysis has always played a critical role in nano-bio interface study, its potential has been limited by the fact that protein samples must be extracted from isolated and lysed cells. Recent advances in labeling and profiling techniques, for example proximity labeling, have enabled proteomic profiling of living cells, resulting in the possibility to perform proteome analysis in a highly spatially specific fashion [150, 151]. Xie et al., for instance, conducted a proximity labeling-based, centrosome-specific proteome study to identify the key centrosomal proteins in cancer cell mitosis, implying the potential application in mapping nanomaterial-induced changes on the organelle level. Moreover, Zhang et al. exploited this method for *in situ* labeling of nanoparticle protein coronas, which has enabled dynamic proteomic study of the rapidly formed corona at different stages of nanoparticle-cell interactions [152].

To further deepen our knowledge of nano-bio interactions, efficient experimental and theoretical models to predict nanomaterials' *in vivo* behavior would be necessary. Animal-free tissue models, including microfluidic organ-on-chips and organoids, have showed great potential for high-throughput analysis of nano-bio interface in complex tissue microenvironment [153, 154]. In current nanomedicine, the application of these models was mainly limited to toxicity assessment, and how to construct animal-free organ models to study different types of nano-bio interactions remained largely unclear. Nonetheless, it has already been reported that vascular-on-chips could be used to mimic normal and tumor blood vessels, and to evaluate the extravasation ability of nanoparticles under different conditions [155, 156]. Similarly, blood-brain-barrier-on-a-chip models have been used to study the principles of nanoparticle transportation into brain [157]. Recently, an emerging type of animal-free tumor model, patient-derived tumor-like cell clusters (PTCs) have drawn much interest. Constructed with patient-derived tumor samples retaining much of the microenvironmental components, PTCs showed attractive performance in prediction of individual chemosensitivity. However, whether PTCs are also suitable for studying nanomaterial-tumor interactions, including targeting, stroma penetration, and stimuli-triggered drug release is still to be verified.

Statistical and computational tools have long been employed to predict bioactivity and biological effects of nanomaterials. For several types of nanomaterials, especially metal and metal oxide nanostructures, structure-activity relationship models (nano-SARs) have already been extensively constructed to predict toxicological properties [158]. Computational techniques like molecular dynamics have been proved particularly useful in simulating interactions involving biological components, for example biomaterial self-assembly, protein binding, cellular uptake, and penetration across endothelial barriers [159, 160]. Predictive models for highly complicated nanostructures, however, are still much needed, especially for those containing "soft" building blocks such as biopolymers, lipids, peptides and nucleic acids. In addition, while a considerable number of models have been developed to predict biomedical properties of nanomaterials, such as size, surface charge, drug encapsulation efficiency, or cellular uptake [160, 161], it is often unclear how should these properties be correlated to the *in vivo* delivery and therapeutic efficiency of the nanosystem. In recent years, many have suggested that the rapid development of machine learning algorithms could remarkably improve our capability of mass data interpretation and exploitation, and eventually lead to the accurate understanding of complicated nano-bio interfaces [160, 162]. Sengottiyan et al., for instance, demonstrated that through the application of genetic algorithm-based machine learning techniques, it has become possible to include protein corona fingerprint information into the descriptors of structure-property relationship models, which could be an attractive progress in bridging the gap between properties of nanomaterials and their eventual biological effects [163].

Clinically-oriented development of intelligent nanomedicine

Since the approval of the first nanomaterial-based anti-cancer drug, liposomal doxorubicin (Doxil) in United States, 1995, many nanomaterials have been approved for clinical usage or entered clinical trials [6, 7, 121, 164]. The majority of these early-generation nanotherapeutics, however, were based on relatively simple re-formulation of existing anti-cancer drugs and were therefore difficult to achieve significantly improved efficacy. In the recent years, it has been recognized that to facilitate the translation of nanotherapeutics, intelligent nanosystems should be rationally designed in a clinically-oriented manner [69, 165]. This implied developing therapies not according to currently available drugs, but to the specific needs presented by individual cases of disease and patient, in order to optimize the therapeutic response and minimize non-specific effects.

With the increased understanding of cancer pathology and the continuous advancement of diagnostic and prognostic methods, the convergence with precise patient stratification is regarded as one of the golden opportunities for nanomedicine [3, 69]. In clinical trials and bedside application, patients that are more likely to respond to a certain nanotherapy could be pre-selected, so that the therapeutic potential of treatment could be fully demonstrated and exploited. Moreover, due to the great heterogeneity of cancer, it is also necessary to develop specific nanotherapies for each distinct subgroup of patients. For example, in patients with pancreatic cancer, approximately 5 % carry mutation in genes encoding the BRCA proteins, which regularly participate in cellular DNA repair via the homologous recombination pathway [166]. Pancreatic cancer with *BRCA* mutations is therefore vulnerable to poly-ADP-ribose polymerase (PARP) inhibitors, since they could cause DNA double-strand breaks and lead to synthetic lethality in the absence of BRCA-mediated repair mechanism. Du et al. showed that a targeted nanoparticle that co-deliver PARP inhibitor olaparib, and a first-line chemotherapeutic for pancreatic cancer, gemcitabine, could significantly improve the therapeutic efficacy of both drugs in *BRCA*-mutated pancreatic tumors and effectively suppressed tumor growth [166]. Similarly, Ebeid et al. reported that in cancer cells with loss-of-function p53 mutation, the combination of paclitaxel and tyrosine kinase inhibitor delivered by nanoparticles specifically induce synthetic lethality due to the abrogation of G2/M checkpoint [167]. By carefully selecting the targeted cell genotype, such “customized” nanotherapies also had the potential advantage of improved tumor specificity since normal cells without the targeted gene mutations would not be affected by the treatment.

While patient pre-selection has facilitated the development of subgroup-specific treatments, fully individualized, patient-specific nanotherapy is still the ultimate goal of precision nanomedicine. In this aspect, immunotherapeutic approaches have probably made the most encouraging progress, given that many types of tumor vaccines could be personally prepared using autologous material, including tumor tissue sample and tumor cell-derived extracellular vesicles, as previously discussed [21, 73]. Another example of therapeutic nanoplatform with potential of individualized medicine was the use of tumor cell membrane-decorated nanosystems [168–171] for drug delivery via the so-called homotypic targeting effect [56, 172]. In a previous study, Ren et al. suggested that patient-derived proteins could also be exploited as individualized surface modification of therapeutic nanomaterials [143]. This conceptual strategy was inspired by the fact that certain disease-related proteins could be specifically enriched in the “corona” formed at the nano-bio interface and this enrichment might lead to particular biological response mediated by nanomaterial-protein interactions. On the other hand, versatile platforms to encapsulate and deliver personalized therapeutic agents with high efficiency are also of great importance. For example, nucleic acid-based therapeutics have huge potential in precision medicine since their sequence could be easily altered to encode patient-specific biological information [173, 174]. An OMV-based anti-tumor nanovaccine was hence developed for rapid and universal loading of mRNA antigens [27]. OMVs were engineered to express an RNA binding protein and a lysosomal escape protein on the membrane, enabling the membrane surface to quickly adsorb any mRNA antigen labeled with a binding sequence that could be specifically recognized by the RNA binding element.

In clinical practice, cancer patients are typically treated with a combination of multiple drugs or therapeutic modalities. Although early-generation nanotherapeutics were mainly single-drug formulations, there is an increasing consensus that improved combination therapy represents a major advantage of nanomedicine and should be exploited maximally [4, 69, 175]. Nanomaterial carriers are able to deliver multiple drugs ratiometrically to the same tissue/cell at the same time, optimizing their synergistic effect. Furthermore, the multifunctional potential of nanocarriers also enable them to release the loaded drug in a temporospatially controlled manner, delivering different drugs to diverse targets or sequentially at a fixed order. Intelligent nanoplatforms should therefore be specifically developed to assist the rational design of new combined treatments

that could synthetically deal with the heterogeneous and complicated tumor biology. For instance, although nanomaterial-based delivery has facilitated the application of procoagulant agents in tumor vasculature occlusion therapy, their efficacy could be limited by possible recurrence due to the uneven distribution of blood vessels within the tumor tissue. Co-delivery with chemotherapeutic drug was reported to effectively reduce the recurrence rate in animal models after such blood vessel occlusion and might be preferable to single-modality treatment [176]. In another study, a tumor-targeted nanocarrier was constructed for combined administration of olaparib and JQ1, a drug that could disrupt homologous recombination pathway-dependent DNA repair, against non *BRCA*-mutant pancreatic cancer [177]. While pancreatic tumors without *BRCA* mutations were insensitive to PARP inhibitors, the co-application of JQ1 could create DNA repair deficiency in tumor cells to sensitize them towards olaparib. The use of multi-drug loaded nanocarrier significantly enhanced their synergistic effect and reduced the toxicity, which were difficult to be achieved by simultaneous administration of free drugs due to their different pharmacokinetic profiles and non-coordinated biodistribution.

In summary, disease-oriented rational design of nanomedicine for patient-specific and synergistic combinatory therapy has given rise to the major opportunities of next-generation anticancer nanomaterials. Many challenges, however, are still lying ahead on the road of their eventual clinical translation. The enormous design flexibility of intelligent nanomaterials inevitably complicated their industrial manufacturing (cost, scalability, reproducibility, processability) and pharmaceutical properties (*in vivo* dynamic transformation, interactions with serum proteins, pharmacokinetics, immunogenity, metabolism and clearance). Moreover, while the multifunctionality potential of nanoformulations has rendered them particularly attractive for precision healthcare, such customizability also made it extremely difficult to develop standardized quality control and evaluation protocols (both preclinical and clinical) for highly complex nanosystems. To properly address these challenges, a deeply interdisciplinary endeavor is probably needed, so that both industrial and clinical concerns, including scalable and biosafe fabrication, transportation and storage, quality control procedures, as well as real world scenarios of usage, could be fully incorporated into nanomaterial rational design. On the other hand, since intelligent nanomaterials are often designed to have a more sophisticated therapeutic mechanism compared to conventional drugs, it is also important to develop specific evaluation methods and clinical trial formulas in accord with the system design. In current cancer nanomedicine, patient stratification techniques are mainly applied to identify cases that are more sensitive to the delivered therapeutic agents. It has already been suggested that developing biomarkers to predict the patient-specific functions of nanocarriers, such as extravasation and tumor penetration, would greatly facilitate clinical trials and real-world application of nanoformulations [2].

Perspectives

Intelligent nanomaterials have demonstrated important potential in a wide range of theranostic anti-cancer applications. Through the rapid advancement of material and biological engineering technologies, nanosystems could be designed to perform complex actions in a temporospatially controlled manner, and to encode personalized biological information for patient-specific treatment. Based on the recent advances and emerging trends discussed in previous sections, we suggest that further research endeavor directed at rational design of combination therapies, convergence with patient stratification and individualized drug delivery, as well as precise knowledge of nanomaterials' *in vivo* behavior will be highly desirable for the future of cancer nanomedicine. In the end of this review, we would also like to suggest several issues and prospects that can be particularly important for the field of cancer nanomedicine in the next few years.

Programmable synthesis of nanomaterials

Many of the existing problems in clinical translation of nanomedicines derived from our limited ability to construct a complicated nanosystem that precisely correspond to the conceptual design. Developing protocols and techniques to synthesis and fabricate nanomaterials in a programmable and reproducible fashion would greatly facilitate their quality control, characterization and biosafety management, as already preliminarily demonstrated by the promising anti-cancer activities of DNA origami nanostructures. At present, it has become possible to synthesize many simple nanostructures with tunable properties, yet for most kinds of nanomaterials the programmable assembly of multiple functional units into ordered 2D or 3D structure with high spatial addressability remained a challenge. Full control over the synthesis and assembly of nanostructures, moreover, would also benefit the incorporation of many stimuli-responsive functions such as *in situ* aggregation and time-resolved drug release. In these years, encouraging progresses have been made in nanomaterial synthesis

guided by computational simulation and artificial intelligence. Machine learning techniques have been applied to screen optimal protocols for nanomaterial preparation, while molecular simulation tools have enabled us to predict assembly behaviors of macrobiomolecules [159, 162]. These methods can also be used to predict important biomedical properties of new types of nanomaterials with different design and composition [159, 161, 162, 178]. A more profound convergence with *in silico* techniques would be very helpful in identifying novel strategies to manipulate the synthesis of complex nanosystems.

Nanovaccines

As we have discussed in previous sections, nanovaccines represented one of the most important opportunities for anti-cancer nanomaterials. While we believe that clinically-oriented development should be the universal principle of future nanomedicine, it seems to be particularly relevant in the case of nanovaccines since they are currently among the most promising nanoplatforms in terms of clinical translation [69]. We suggest that in the next few years, research effort should be specifically dedicated to the translational issues of nanomaterials for tumor vaccination, e.g., evaluation methods (preclinical models, characterization standards, patient stratification strategies) particularly suitable for nanomaterial-based vaccines and adjuvants; efficient and safe protocols for acquirement, processing and storage of patient-derived materials; therapeutic regimens for combination with other clinically available immunotherapies or other therapeutic modalities. The success of mRNA vaccines against epidemic diseases could provide important reference and insight on the manufacturing and real-world application of nanoplatform-delivered vaccines.

Regulations and ethical considerations

It has now been vastly recognized that the challenges created by the increasing complexity of nanomaterials are not restricted to scientific contexts. Notably, the expansion of intelligent nanomedicine has introduced an unprecedented number of novel drug types and forms, resulting in an unprecedented need of specific regulations (e.g., industry guidelines, standardized assays, data requirements, and specific approval pathways) that so far has remained largely unmet. Multiple reports have indicated that regulatory uncertainties strongly affected the innovation process in nanomedicine research and development [179, 180]. Particularly, it has been suggested that the lack of data collection protocols, safety and ethical guidelines, and appropriate approval pathways caused researchers and industrial sponsors to heavily rely on clinically approved drugs and technologies, which in turn limited the possibility of disease-oriented innovation [180]. To address this challenge, it seems to us that a collaborative participation of both innovators and regulatory bodies should be firmly pursued. While more scientific effort should be dedicated to the establishment of practical assays and techniques for preclinical and clinical evaluation of anti-cancer nanomaterials, the regulatory bodies need to collaborate with the scientific community and industry to convert the frontier knowledge into detailed and definite protocols or guidelines.

In summary, despite of many encouraging progresses made by state-of-the-art nanotechnology in cancer theranostics, the highly heterogeneous nature of cancers and the complicated nano-bio interactions would always remain extremely demanding challenges for nanoscientists. Only a profound cooperation with clinical, industrial and regulatory forces could further expand the boundaries of nanomedicine, and eventually translate the bright promise of intelligent nanomaterials into concrete clinical success against cancer.

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