Combinatorial Nano-Bio Interfaces

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ABSTRACT

Nano-bio interfaces are emerging from the explosive convergence of engineered nanomaterials and biological entities. Despite the rapid growth, the clinical translation of biomedical nanomaterials is heavily compromised, in the lack of comprehensive understanding of biophysicochemical interactions at nano-bio interfaces. In the past decade, a few investigations have adopted the combinatorial approach to decode nano-bio interfaces. Combinatorial nano-bio interfaces comprise the design of nanocombinatorial libraries and high-throughput bio-evaluation. In this perspective, challenges of the rising concept are addressed, calling for the multi-parametric nanocombinatorics (composition, morphology, mechanics, surface chemistry), the multi-scale bio-evaluation (biomolecules, organelles, cells, tissues/organs), and the recruitment of computational modelling and artificial intelligence. The leveraging of combinatorial nano-bio interfaces will shed a light on precision nanomedicine and their potential applications.

Revisiting Nano-bio Interfaces

Nano-bio interfaces, emerging at the convergence of nanotechnology and biomedicine, have been extensively programmed and implemented in such biomedical applications as nano-diagnosis, nanotherapy, and regenerative nanomedicine.[1] Recent advances in nano-bio interfaces have further promised various functional bio-integrated systems, including wearable and implantable nanobioelectronics and smart bionic prosthetics. Despite the explosive development, the bench-to-bedside translation of biomedical nanomaterials is frustrating. Taking targeted nanoparticles for drug delivery as an example, only 0.7% (median) of the administrated dose turned out to accumulate in the targeted solid tumor.[2]

This stems from the limited understanding of biophysicochemical interactions at nano-bio interfaces, and the lack of a comprehensive and standardized screening of comparable biomedical nanomaterials and their physicochemical interactions with the biological systems. This situation could lead to conflicting conclusions even on similar or identical nanomedicine formulations.[3] What's worse, many of current studies on nano-bio interfaces are single-parametric, rendering the drawn conclusions less predictive in elsewhere scenarios. In fact, different physicochemical parameters of the nanomaterials alone could exert a significant impact on the fate and biological performance of nanomaterials, including their shape, size, chemical composition, crystal structure, surface charge, surface functionalization, hydrophilicity, rigidity, topography. In the meanwhile, these parameters are interdependent and the interrelated effects define a narrow yet sophisticated window for the optimization of nanomaterials to be interfaced with biological entities. The variation in one of the physicochemical parameters could inevitably introduce discrepancies in other parameters, which further increases the complex interdependency of the physiochemical parameters of nanomaterials. For instance, different modification molecules with varying charges were utilized to investigate the effect of surface charging of nanoparticles on their cellular uptake,[4] whereas the herein introduced discrepancy in surface functionalization and protein absorption could also make a difference in their biological fate.[5] Additionally, different fabrication and analysis techniques are not intrinsically comparable, such as various nanoparticle sizing techniques (*e.g.*, dynamic light scattering, Brunauer-Emmett-Teller, transmission electron microscopy).[3] In addition, the biological effect of identical nanosystem formulations can vary greatly when exposed to different *in vitro* (patho-)physiological models and diverse *in vivo* organs or hosts.

These pitfalls in current research methodology could make the generality of many studies on the nano-bio interfaces in dispute, while frustrating the clinical translations of

bench achievements. Last but not least, multiple cycles of optimization are usually needed to gradually improve the performance of designed nano-bio interfaces, suffering from time-intensity, limited diversity and high reliance on prior knowledge.

The First-decade Journey of Combinatorial Nano-bio Interfaces

In the past decade, researchers have turned to the combinatorial methodology in a revisit of the nano-bio interfaces by developing a nanocombinatorial library and high-throughput screening of the massive library. The combinatorial methodology has been employed, to investigate the correlation of physicochemical properties of nanomaterials with their biological performance and other potential applications at an explicit and consistent context, which is here profiled as combinatorial nano-bio interfaces (Figure 1). The concept consists of two elementary yet multi-factorial aspects, namely the nanocombinatorial library and the bio-evaluation. On one hand, the nanocombinatorial library of nanomaterials can be achieved by programming diverse physicochemical parameters, including their morphology, composition, surface properties and mechanics. On the other hand, the biophysicochemical interactions and biological performance of the library can induce biological responses at multiple levels, ranging from biomolecular (*e.g.*, protein absorption, complex disassembly, enzyme inhibition) and subcellular level (*e.g.*, membrane disruption, functional loss of organelles) to cellular (*e.g.*, division, differentiation, migration, death) and tissue level (*e.g.*, inflammation, fibrosis, carcinogenesis). Numerous permutations of the nano-bio interactions are possible at the interface of nanocombinatorics and bio-evaluation. In another sense, the combinatorial nano-bio interface depicts the complex crosstalk of nanomaterials' intrinsic and emerging properties, where the biophysicochemical interactions of the nanomaterials with bio-entities can be regarded as their emerging properties while the physicochemical parameters of nanomaterials are their intrinsic properties.

Nanotoxicity and bioactivity of engineered nanomaterials have been raised as the major concern prior to the functionality of the nanomaterials, with the explosive introduction of nanomaterials into biological entities. Surface functionalization is one of the most accessible parameter to tune the nanotoxicity and biocompatibility of nanomaterials. A nanocombinatorial library, consisting of 80 surface-functionalized multi-walled carbon nanotubes (f-MWCNT) through the combination of pre-selected amines and acylators, was developed.[5] The library was validated by scoring multiple biological screenings of the library, including the protein binding, cytotoxicity, and immune responses, through which one acylator was identified as the preferred ligand for reducing protein-binding and enhancing biocompatibility of f-MWCNT. Subsequently, this nanocombinatorial library also identified formulations steering MWCNT from mannose receptor to scavenger receptor recognition, with correspondingly alleviated NFκB activation and reduced immunotoxicity.[6] Furthermore, this nanocombinatorial library of f-MWCNT was able to tune the magnitude of autophagy induction through the differentially activated signaling pathways, which could be employed for developing potential pharmaceutical autophagy modulators and biocompatible nanomaterials.[7] These studies are elegant paradigms of the combinatorial synthesis and nanotoxicity screening for their potential nanomedicine applications. A thorough and combinatorial strategy to investigate the relationship of nanomaterials' surface functionalization with their nanotoxicity and bio-activity allow researchers to efficiently identify nanomaterials with reduced nanotoxicity and optimized biological performance simultaneously.

Targeted drug delivery and targeted nanoparticles (TNP) have been spotlighted for decades, due to the promising capability of minimizing toxicity, bypassing immune clearance and particle extravasation, while achieving tissue penetration and specific cellular uptake.[8] However, a key challenge that frustrates the clinical translation of TNP is to define optimal

physicochemical parameters that simultaneously confer molecular targeting, NP trafficking, and controlled drug release. Although a considerable amount of information is available regarding individual factors that improve the biological fate of TNP, the key obstacle in the development of clinically effective TNPs is to resolve the complex interdependence between physicochemical properties (composition, morphology, surface properties, mechanics, *etc.*) and physiological trafficking (the sequential presentation of diverse biological barriers) of TNP. In 2005, a NPs library of 146 members was developed through multivalent attachment of small molecules, to identify specific binding affinity through the mediated multivalent binding to cell-surface receptors, without prior knowledge.[9] After screening against distinct cell lines or different physiological states of one cell type, derivative NPs with high specificity to endothelial cells or pancreatic cancer cells and those capable of macrophage activation were identified. Years later, pre-clinically effective targeted polymeric nanoparticle were successfully developed, encapsulating chemotherapeutic docetaxel (DTXL) for the treatment of prostate tumours.[10] To investigate the optimization of TNP for efficient drug delivery and release, the researchers developed a combinatorial library of over 100 formulations varying systematically with respect to NP size, surface hydrophilicity, targeting ligand density, drug load and drug release properties. The *in vitro* and *in vivo* performance of these formulations were evaluated, including their pharmacokinetics, biodistribution, tolerability, efficacy of drug release and tumour accumulation, through which the optimized DTXL-TNP was finally identified.

The promising utilization of nanoparticles as non-viral vehicles for delivering genetic materials (*e.g.*, siRNA, pDNA) can suffer from the relatively low transfection efficiency. Herein, a nanocombinatorial library of 648 supramolecular nanoparticles with a broad diversity in NPs size, surface functionalization, and DNA loading capacity, through the ratiometric combination of five molecular building blocks.[11] The five building blocks were

chosen to confer DNA complexation, water solubility, structural stability, protective passivation and cell-specificity, respectively. The validation of the nanocombinatorial library in a collection of fibroblast and cancer cell lines revealed a highly efficient gene delivery formulation, with supreme performance compared to commercial reagents. Similarly, a nanocombinatorial library of 1536 chemically diverse core-shell nanoparticles was constructed to elucidate the optimal parameters for intracellular delivery of genetic materials siRNA, and accordingly revealed such beneficial design as incorporating thin hydrophilic shells, a higher reactive block weight fraction, and stoichiometric equivalence between epoxides and amines.[12] In addition to delivering genetic molecules, combinatorial probes based on nanostructured microelectrodes were developed as high-throughput electrochemical sensors to detect mutations of the circulating tumour nucleic acids (*e.g.*, EGFR gene). The combinatorial approach could accurately detect mutant sequences, with more than 40 clinically relevant alterations directly in patient serum.[13]

Furthermore, the field of tissue engineering has been programming the physicochemical properties of nanomaterials and therefore biophysicochemical interactions at the interface to effectively modulate tissue physiology. Topography has been recognized as one potent regulator since 1990s. Recently, a combinatorial library with a tuneable gradient of feature sizes over customized patterns, ranging from nano- to microscale, was generated using the polymer pen lithography.[14] By tilting the polymer pen arrays, the researchers massively wrote gridded fibronectin patterns of varying feature size and spacing, and investigated the differential expression of osteogenic markers in the mesenchymal stem cells cultured on these fibronectin patterns. Given the potential of printing multiplexed biomolecules, combinatorial libraries with further variations in substrate composition could be further established.

In 2017, computational modelling has been employed to take advantage of the big

data sets of nanocombinatorial libraries, to further improve the efficiency of optimal nanomaterials identification and predictive power of combinatorial nano-bio interfaces.[15] On the basis of a synthetic nanocombinatorial library with 47 members of surface functionalized gold nanoparticles (f-GNP), robust quantitative structure-property relationship (QSPR) models were generated, to interrogate the enzyme-GNP interactions. The experimental screening and computational modeling, using a proof-of-concept enzyme acetylcholinesterase, both revealed the molecular basis for specific / non-specific enzyme binding and inhibition. Despite the high performance of the QSPR models in predicting protein-NP interactions, the library diversity is however limited. The lack of suitable descriptors for various physicochemical properties of nanomaterials (*e.g.*, size, shape, rigidity) and the corresponding biological effects (*e.g.*, cellular uptake) can heavily comprise the applicability of QSPR models. Accordingly, the alternative quantitative nanostructure activity relationship (QNAR) models were developed.[16] A nanocombinatorial library of f-GNP with diversity in surface functionalization and NPs size was developed and their cellular uptake was experimentally evaluated. The acquired data sets were subsequently utilized to construct a virtual GNP library and derive corresponding nanodescriptors through precise simulation, and finally to develop the predictive QNAR models. Given the improved diversity and predicivity, the herein predicted and designed GNPs can be experimentally validated and used as guidelines for nanomaterials design.

Further Extension of Multi-parametric Nanocombinatorial Libraries

The first decade of exploring combinatorial nano-bio interfaces has clearly demonstrated the advantage of the combinatorial methodology over the conventional "one-at-a-time" experimental practice in the field of nano-bio interfaces. However, many of current nanocombinatorial libraries consist of limited or even single variables (*e.g.*, surface

functionalization[7,15,17]), which unfortunately compromises the predictive power of combinatorial methodology.

A multi-parametric nanocombinatorial library would allow an improved versatility towards programming interactions at the nano-bio interfaces for precision nanomedicine. Given the complex interdependent multi-parametric nature of nanocombinatorics, an extensive combination of these physicochemical properties is necessary for the optimizing nano-bio interfaces and successful delivery of clinically-effective biomedical nanomaterials. For example, while surface decoration of targeting ligand has been proven efficient in promoting specific cellular uptake, physical parameters (*e.g.*, size, shape, rigidity) can also potently bias the cellular uptake of nanomaterials. Depending on the size of nanoparticles, cells can internalize the nanoparticles by different pathways, such as caveloin-dependent and clatharin-mediated endocytosis, receptor-mediated endocytosis, or simple translocation for smaller NP. Also, the shape, aspect ratio and docking orientation of nanomaterials have been reported to influence dynamic cell membrane wrapping and thereby the endocytosis process. Meanwhile, positively charged nanomaterials are found to be more readily internalized than neutral or negatively charged nanomaterials,[18] though higher toxicity could be incurred.[4] In addition, stiff nanomaterials (*e.g.*, metallic and semiconductor NP) are relatively more accessible to cellular endocytosis than soft nanomaterials (*e.g.*, liposome, micelles, polymeric NP), due to the differential cell membrane deformation and energy distribution.

In addition, current advances in combinatorial nano-bio interfaces are confined within nanotoxicity, targeted delivery of therapeutics and genetic materials, as well as tissues engineering. The advantageous combinatorial methodology should be further extended to other scenarios, such as the emerging field of nanobioelectronics. The past decade has also witnessed the burst of flexible and stretchable nanobioelectronic devices, owing to the nanotechnological strategies for introducing flexibility and stretchability to match the robust

mechanical properties of biological tissues, such as the softness, curvilinear topography and dynamic stretch of biological tissues.[19] So far, no studies utilized the combinatorial methodology to optimize and design flexible nanobioelectronic devices, to our best knowledge. However, there is an immediate demand to implement the combinatorial methodology in flexible nanobioelectronics, given the consideration of electronic properties and the herein increased complexity for device optimization. To establish a rational combinatorial library for flexible nanobioelectronics, the physicochemical properties to be optimized include the elastic rigidity, stretchability and flexibility, sensitivity and stability, surface modification and biocompatibility, adhesion (*e.g.*, between devices and tissues, between the functional modules and supporting modules), as well as the integration of multifunctionality.[20] A systematic understanding of the flexible and stretchable nanobioelectronics in a combinatorial approach will reciprocally contribute to the translation of conceptual smart healthcare and novel electroceuticals into clinical products.

Multi-scale Bio-evaluation Towards Clinical Translation

Nanomaterials interact with the biological systems at multiple scales, ranging from molecules (proteins, DNA, cytoskeletons, *etc.*), subcellular organelles (membranes, mitochondria, nuclei, *etc.*), to single cells, tissues and organs. Current studies on biological responses to nanomaterials, including those adopting the combinatorial methodology, however focus on a few or even one single selected aspects of the biological responses (*e.g.,* cellular uptake[9] and protein binding[15]). Such limited bio-evaluation can prohibitively influence the translation of promising nanomedicine formulations. For instance, the drug delivery efficiency and therapy effect could be significantly distinct among varying cell types, cancer types and tumor models, as well as the mouse and human test.[2] By contrast, a less comprehensive screening may fail to shed a light on multifaceted nano-bio interactions. For

instance, while NPs uptake showed little effect on the growth of primary tumor cells, they could however significantly retard their collective migration.[21] This discrepancy could rise from the differentially complex microenvironments of diverse bio-entities and unclear physiochemical interactions at the multiscale nano-bio interfaces.

Hence, a multi-scale bio-evaluation of the incurred biological responses would be necessary to obtain a full picture of nanomaterials' biological fate and performance. The robust biophysicochemical interactions at the nano-bio interface not only determines biological fate of the nanomaterials, but also causes widely-ranged biological responses, including protein absorption, cytoskeleton disassembly, membrane disruption, functional loss, cell migration, cell division, cell differentiation, cell death, wound healing, tissue fibrosis, inflammation, *etc*. At the bio-evaluation stage, a comprehensive evaluation of biological performance of the nanomaterials should be conducted in a bio-combinatorial (multi-scale) way, including the disassembly or adsorption of biomolecules, the disruption or functional loss of cellular organelles, such basic cell physiology as division, morphogenesis, migration, differentiation, and death, as well as the tissue level responses. Bio-responses should also be assessed on demand for specific scenarios (*e.g.*, the induction of apoptosis for cancer therapeutics, the activation of immune responses for implantable nanobioelectronic devices, cell membrane disruption and genotoxicity for gene therapeutics, microbe elimination efficiency for infection management[22]). A systematic evaluation and optimization of the nanomaterials at multiple scales can offer a full picture of their biological fate and performance, therefore improving the predictive power of the suggested optimizations for specific biomedical applications.

Recruitment of Computational Simulation and Artificial Intelligence

Although multi-parametric nanocombinatorics and multi-scale screening are on

demand in next decade, the space size of nano-bio interfaces is too immense, therefore physical experimental practice alone is challenging for the design and identification of optimal nano-bio interfaces. The space size could reach 10^{100} when simply counting possible compositional combinations and can be incredibly and inaccessibly huge when considering the diversity in other physicochemical properties.[23] There is an urgent demand on developing the appropriate models to link the physicochemically diverse nanomaterials and the complex biological responses, with appropriate descriptors for a good identification of relationships between biological systems and the physicochemical properties of nanoparticles, especially since the launch of the Materials Genome Initiative.[16,26]

Computational simulation of nanomaterials for biomedical applications can efficiently boost the design and optimization of biomedical nanomaterials. The emergence of combinatorial nano-bio interfaces makes it promising for computational modelling to comprehensively understand physicochemical properties of nanomaterials and the complex nano-bio interactions, thereby to quantitatively derive robust nanocombinatorial models with systematically diverse properties. In computational simulation, molecular dynamics and density functional theory are commonly employed to explore the microscopic or macroscopic properties of nanomaterials by computer programs, such as quantitative structure−activity relationship[15] and quantitative nanostructure activity relationship.[16] The advantage of these approaches is that they can precisely simulate molecular structures, due to the basis of the existing physical and chemical theories. However, they might suffer from huge computing burden and require extensive prior expertise knowledge, which may not be suitable for the predictions for big datasets and end points with complex mechanisms.

Recent advances in artificial intelligence and machine learning have endowed computers with the capability to learn from empirical material data and find hidden patterns without being explicitly programmed. It offers an alternative solution for predicting the

nanomaterials properties with improved time efficiency and prediction accuracy. Various machine learning algorithms, such as regression, decision tree, clustering, artificial neural network, evolutionary algorithms, can be explored in nanomaterials studies. For instance, random forest regression is favorable in data-mining and knowledge-extraction from prior data sets, to understand the inter-correlation of variables in physicochemical properties of biomedical nanomaterials and their corresponding influence in biological performace.[27] In evolutionary algorithms (also called as genetic algorithms), nanomaterials are mathematically represented as genomes and a population of promising candidates is generated by altering (mutating) the initial population (random or prior knowledge), which can be employed for rapid and rational design and evaluation of potentially useful nano-bio interfaces.[23] Artificial neural network (ANN) has discrete layers, interconnections, and directions of data propagation. The weighted interconnections of ANN can be trained through a learning process for predicting properties of nanomaterials (*e.g.*, grain size[28]). Recently, deep learning has also been widely invloved in image recognition, biomedical diagnosis and other application scenarios of artificial intelligence. It can even bypass training a machine with existing nanomaterials data for predictive modelling. In 2018, researchers have already employed deep learning to perform chemical synthesis route planning.[29] It is promising that deep learning would be implemented in decoding nano-bio interfaces in the near future. It should also be stressed that different algorithms possess their own scope of applications, including the discovery of new nanomaterials and the prediction of their properties.

The improved computational simulation and artificial intelligence can profoundly facilitate the design and identification of optimal nano-bio interfaces and effectively narrow down the nanocombinatorial library for the subsequent experimental validation. Identifying virtual nanocombinatorial variations and combination patterns out of synthetic high-quality libraries, is capable of rationally designing an on-demand library (predictive modelling) and

systematically recognizing promising candidates for further experimental validation by multi-scale bio-evaluation. This will increase the efficiency and accuracy of identifying clinically effective nanomedicine formulations through pre-filtering less promising candidates, given the massive nature of nano-bio interfaces when considering multi-parametric nanocombinatorics and multi-scale bio-evaluation.

Conclusion

In conclusion, the combinatorial methodology for optimizing nano-bio interfaces is still in its infancy. There is a rising call of the combinatorial nano-bio interfaces towards a comprehensive and comparable evaluation of the biological performance of the interfaced nanomaterials, towards a promising translation of the nanomaterials formulations into clinical practice. We are optimistic that, with the incorporation of extended multi-parametric nanocombinatorial libraries, high-throughput multi-scale bio-evaluation, and robust computational modelling and artificial intelligence, the combinatorial nano-bio interfaces and the accordingly-optimized nanomaterials formulations will eventually be delivered to clinical patients in a safe, efficient, and ubiquitous way.

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Author Contributions

The manuscript was conceptualized by X.C. and P.C., and written through contributions of P.C., X. Z., M. W., Y.-L. W., X. C. All authors have given approval to the final version of the manuscript.

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REFERENCES

(1) Cai, P.; Leow, W. R.; Wang, X.; Wu, Y. L.; Chen, X. Programmable Nano-Bio Interfaces for Functional Biointegrated Devices. *Adv. Mater.* **2017,** *29*, 1605529.

(2) Wilhelm, S.; Tavares, A. J.; Dai, Q.; Ohta, S.; Audet, J.; Dvorak, H. F.; Chan, W. C. W. Analysis of Nanoparticle Delivery to Tumours. *Nat. Rev. Mater.* **2016,** *1*, 16014.

(3) Azhdarzadeh, M.; Saei, A. A.; Sharifi, S.; Hajipour, M. J.; Alkilany, A. M.; Sharifzadeh, M.; Ramazani, F.; Laurent, S.; Mashaghi, A.; Mahmoudi, M. Nanotoxicology: Advances and Pitfalls in Research Methodology. *Nanomedicine* **2015,** *10*, 2931-2952.

(4) Li, R.; Wang, X.; Ji, Z.; Sun, B.; Zhang, H.; Chang, C. H.; Lin, S.; Meng, H.; Liao, Y. P.; Wang, M.; Li, Z.; Hwang, A. A.; Song, T. B.; Xu, R.; Yang, Y.; Zink, J. I.; Nel, A. E.; Xia, T. Surface Charge and Cellular Processing of Covalently Functionalized Multiwall Carbon Nanotubes Determine Pulmonary Toxicity. *ACS Nano* **2013,** *7*, 2352-2368.

(5) Mu, Q. X.; Liu, W.; Xing, Y. H.; Zhou, H. Y.; Li, Z. W.; Zhang, Y.; Ji, L. H.; Wang, F.; Si, Z. K.; Zhang, B.; Yan, B. Protein Binding by Functionalized Multiwalled Carbon Nanotubes Is Governed by the Surface Chemistry of Both Parties and the Nanotube Diameter. *J. Phys. Chem. C* **2008,** *112*, 3300-3307.

(6) Gao, N.; Zhang, Q.; Mu, Q.; Bai, Y.; Li, L.; Zhou, H.; Butch, E. R.; Powell, T. B.; Snyder, S. E.; Jiang, G.; Yan, B. Steering Carbon Nanotubes to Scavenger Receptor Recognition by Nanotube Surface Chemistry Modification Partially Alleviates Nfκb Activation and Reduces Its Immunotoxicity. *ACS Nano* **2011,** *5*, 4581-4591.

(7) Wu, L.; Zhang, Y.; Zhang, C.; Cui, X.; Zhai, S.; Liu, Y.; Li, C.; Zhu, H.; Qu, G.; Jiang, G.; Yan, B. Tuning Cell Autophagy by Diversifying Carbon Nanotube Surface Chemistry. *ACS Nano* **2014,** *8*, 2087-2099.

(8) Farokhzad, O. C.; Langer, R. Impact of Nanotechnology on Drug Delivery. *ACS Nano* **2009,** *3*, 16-20.

(9) Weissleder, R.; Kelly, K.; Sun, E. Y.; Shtatland, T.; Josephson, L. Cell-Specific Targeting of Nanoparticles by Multivalent Attachment of Small Molecules. *Nat. Biotechnol.* **2005,** *23*, 1418-1423.

(10) Hrkach, J.; Von Hoff, D.; Mukkaram Ali, M.; Andrianova, E.; Auer, J.; Campbell, T.; De Witt, D.; Figa, M.; Figueiredo, M.; Horhota, A.; Low, S.; McDonnell, K.; Peeke, E.; Retnarajan, B.; Sabnis, A.; Schnipper, E.; Song, J. J.; Song, Y. H.; Summa, J.; Tompsett, D.; Troiano, G.; Van Geen Hoven, T.; Wright, J.; LoRusso, P.; Kantoff, P. W.; Bander, N. H.; Sweeney, C.; Farokhzad, O. C.; Langer, R.; Zale, S. Preclinical Development and Clinical Translation of a Psma-Targeted Docetaxel Nanoparticle with a Differentiated Pharmacological Profile. *Sci. Transl. Med.* **2012,** *4*, 128ra139.

(11) Wang, H.; Liu, K.; Chen, K. J.; Lu, Y.; Wang, S.; Lin, W. Y.; Guo, F.; Kamei, K.; Chen, Y. C.; Ohashi, M.; Wang, M.; Garcia, M. A.; Zhao, X. Z.; Shen, C. K.; Tseng, H. R. A Rapid Pathway toward a Superb Gene Delivery System: Programming

Structural and Functional Diversity into a Supramolecular Nanoparticle Library. *ACS Nano* **2010,** *4*, 6235-6243.
(12) Siegwart, D. J.; Whitehead, K. A.; Nuhn, L.; Sahay, G.; Cheng, H.; Jiang, S.; Ma, M.; Lytton-Jean, A.; Vegas, A.; Fenton, P.; Levins, C. G.; Love, K. T.; Lee, H.; Cortez, C.; Collins, S. P.; Li, Y. F.; Jang, J.; Querbes, W.; Zurenko, C.; Novobrantseva, T.; Langer, R.; Anderson, D. G. Combinatorial Synthesis of Chemically Diverse Core-Shell Nanoparticles for Intracellular Delivery. *Proc. Natl. Acad. Sci. USA* **2011,** *108*, 12996-13001.
(13) Das, J.; Ivanov, I.; Safaei, T. S.; Sargent, E. H.; Kelley, S. O. Combinatorial Probes for High-Throughput Electrochemical Analysis of Circulating Nucleic Acids in Clinical Samples. *Angew. Chem. Int. Ed.* **2018,** *57*, 3711-3716.
(14) Giam, L. R.; Massich, M. D.; Hao, L.; Shin Wong, L.; Mader, C. C.; Mirkin, C. A. Scanning Probe-Enabled Nanocombinatorics Define the Relationship between Fibronectin Feature Size and Stem Cell Fate. *Proc. Natl. Acad. Sci. USA* **2012,** *109*, 4377-4382.
(15) Liu, Y.; Winkler, D. A.; Epa, V. C.; Zhang, B.; Yan, B. Probing Enzyme-Nanoparticle Interactions Using Combinatorial Gold Nanoparticle Libraries. *Nano Res.* **2015,** *8*, 1293-1308.
(16) Wang, W.; Sedykh, A.; Sun, H.; Zhao, L.; Russo, D. P.; Zhou, H.; Yan, B.; Zhu, H. Predicting Nano-Bio Interactions by Integrating Nanoparticle Libraries and Quantitative Nanostructure Activity Relationship Modeling. *ACS Nano* **2017,** *11*, 12641-12649.
(17) Zhou, H.; Mu, Q.; Gao, N.; Liu, A.; Xing, Y.; Gao, S.; Zhang, Q.; Qu, G.; Chen, Y.; Liu, G.; Zhang, B.; Yan, B. A Nano-Combinatorial Library Strategy for the Discovery of Nanotubes with Reduced Protein-Binding, Cytotoxicity, and Immune Response. *Nano Lett.* **2008,** *8*, 859-865.
(18) Wu, Y. L.; Putcha, N.; Ng, K. W.; Leong, D. T.; Lim, C. T.; Loo, S. C.; Chen, X. Biophysical Responses Upon the Interaction of Nanomaterials with Cellular Interfaces. *Acc. Chem. Res.* **2013,** *46*, 782-791.
(19) Cai, P.; Hu, B.; Leow, W. R.; Wang, X.; Loh, X. J.; Wu, Y.-L.; Chen, X. Biomechano-Interactive Materials and Interfaces. *Adv. Mater.* **2018,** *30*, 1800572.
(20) Chen, X. Making Electrodes Stretchable. *Small Methods* **2017,** *1*, 1600029.
(21) Tay, C. Y.; Cai, P.; Setyawati, M. I.; Fang, W.; Tan, L. P.; Hong, C. H.; Chen, X.; Leong, D. T. Nanoparticles Strengthen Intracellular Tension and Retard Cellular Migration. *Nano Lett.* **2014,** *14*, 83-88.
(22) Hu, B.; Owh, C.; Chee, P. L.; Leow, W. R.; Liu, X.; Wu, Y. L.; Guo, P.; Loh, X. J.; Chen, X. Supramolecular Hydrogels for Antimicrobial Therapy. *Chem. Soc. Rev.* **2018**, 10.1039/c8cs00128f.
(23) Le, T. C.; Winkler, D. A. Discovery and Optimization of Materials Using Evolutionary Approaches. *Chem. Rev.* **2016,** *116*, 6107-6132.
(24) Winkler, D. A. Recent Advances, and Unresolved Issues, in the Application of Computational Modelling to the Prediction of the Biological Effects of Nanomaterials. *Toxicol. Appl. Pharmacol.* **2016,** *299*, 96-100.
(25) Da Silva, C. G.; Rueda, F.; Lowik, C. W.; Ossendorp, F.; Cruz, L. J. Combinatorial Prospects of Nano-Targeted Chemoimmunotherapy. *Biomaterials* **2016,** *83*, 308-320.
(26) Epa, V. C.; Burden, F. R.; Tassa, C.; Weissleder, R.; Shaw, S.; Winkler, D. A. Modeling Biological Activities of Nanoparticles. *Nano Lett.* **2012,** *12*, 5808-5812.
(27) Oh, E.; Liu, R.; Nel, A.; Gemill, K. B.; Bilal, M.; Cohen, Y.; Medintz, I. L. Meta-Analysis of Cellular Toxicity for Cadmium-Containing Quantum Dots. *Nat. Nanotechnol.* **2016,** *11*, 479-486.

(28) Rashidi, A. M.; Eivani, A. R.; Amadeh, A. Application of Artificial Neural Networks to Predict the Grain Size of Nano-Crystalline Nickel Coatings. *Comput. Mater. Sci.* **2009,** *45*, 499-504.
(29) Segler, M. H. S.; Preuss, M.; Waller, M. P. Planning Chemical Syntheses with Deep Neural Networks and Symbolic AI. *Nature* **2018,** *555*, 604-610.

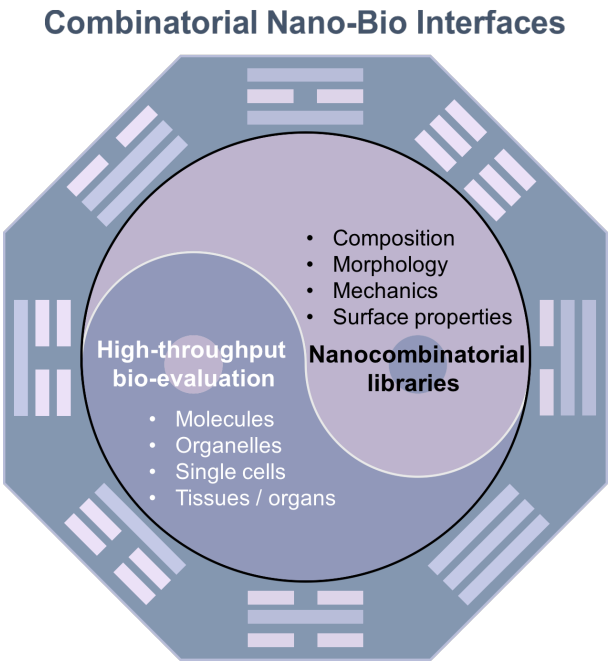

Figure 1. The concept of combinatorial nano-bio interfaces emerging at the convergence of nanocombinatorics and high-throughput bio-evaluation.

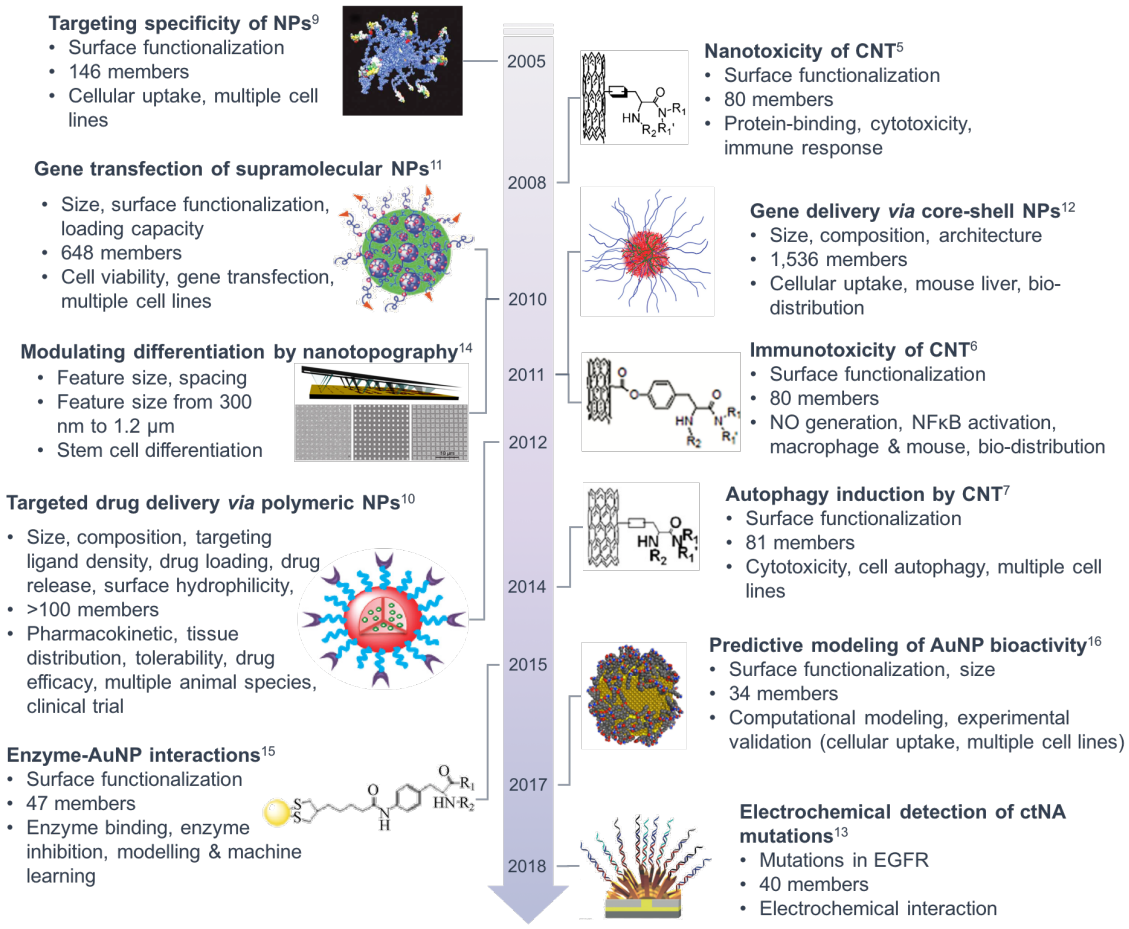

Figure 2. The first-decade journey of exploring combinatorial nano-bio interfaces.

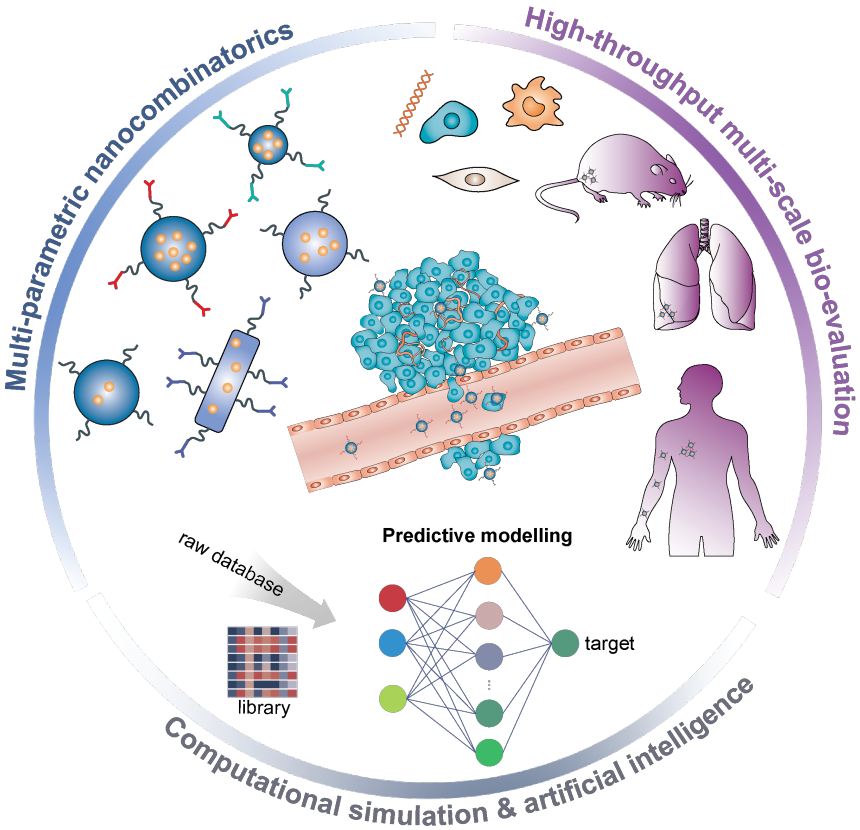

Figure 3. The pending challenges for the future advances of combinatorial nano-bio interfaces, including the integration of multi-parametric nanocombinatorics, high-throughput multi-scale bio-evaluation, as well as computational simulation and artificial intelligence.

Combinatorial Nano-bio Interfaces
ToC Figure