Targeting therapeutics to the plasma membrane: opportunities for nanoparticle-mediated delivery abound

“...it is the combined function of the ensemble construct (NP–biological–drug/cargo) that mediates the delivery of the therapeutic cargo to the plasma membrane with fine control.”

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Why target therapeutics to the plasma membrane?

The plasma membrane of mammalian cells has become an attractive target in the development of therapeutics as it serves a multitude of functions that are vital for cellular health and homeostasis including: the separation and protection of the cytosol from the extracellular environment; the mediation of signaling processes from outside the cell to the cell interior; and the maintenance of ion homeostasis and membrane potential. A variety of current therapeutics are active at the plasma membrane, such as membrane-disrupting peptides, membrane receptor modulators and mediators of lipid peroxidation. Membrane-disrupting peptides vary in their structure and sequence, but most are amphiphilic and act by accumulating on and disrupting the membrane by forming pores through a variety of mechanisms [1]. Membrane receptor modulators are a diverse class of therapeutics that can either antagonize or agonize a targeted receptor to regulate its signal transduction pathways (e.g., protein kinase cascades, gene transcription, metabolism alterations and ion channel activity), which subsequently modulate cellular behavior. Lipid peroxidation is typically achieved via the production of reactive oxygen species or singlet oxygen through photodynamic therapy (PDT) [2]. Here, a photoexcited PDT drug (e.g., zinc phthalocyanine [ZnPC]) reacts with triplet

oxygen in tissue to generate reactive oxygen species [3]. However, many membrane-targeting therapeutics are often hindered by their inherent physicochemical properties that can limit their overall efficacy. These attributes include their hydrophobicity, poor solubility, decreased circulation time and nonspecific uptake by nontargeted sites. Additionally, many therapeutic agents function optimally when they are targeted to the membrane bilayer. For example, many photosensitizers used in PDT must exist in monomeric form for optimal function and this is directly facilitated by the hydrophobic environment of the membrane bilayer. The use of nanoparticle (NP) materials to target and deliver therapeutic drug cargos to the membrane offers tremendous promise in helping to overcome these limitations [4].

The role of NPs in improving targeting of therapeutics to the plasma membrane

So what advantages can NPs offer in improving the targeted delivery and overall efficacy of therapeutics intended to function at or near the plasma membrane? A first hint is provided by considering the innate physicochemical properties of NPs which include their small size, cargo-loading capacity, biocompatibility and ease of bioconjugation. First, their small size is often on the same size scale as the 5 nm expanse of the plasma membrane bilayer allowing for minimally

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invasive interactions with the membrane as well as availing deeper tissue penetration. NPs also possess large surface area:volume ratios (in the case of ‘hard’ NPs) and significant core-loading capacity (for ‘soft’ NPs) for augmented delivery of on-board therapeutic cargos [5,6]. Examples of hard/compact NPs amenable to surface loading include metals, metal oxides and semiconductor quantum dots while soft or porous NP platforms encompass liposomes, dendrimers and polymers that can accommodate attachment of therapeutics on their surface or encapsulation in their interior. Additionally, surface coatings and customized ligands facilitate NP stability and biocompatibility. Furthermore, NPs have proven to be easily amenable to conjugation with a broad array of biomolecules, including proteins, peptides and drugs. Finally, a variety of actuation modalities can be employed for the release of NP-associated cargos, such as: passive actuation where cargo merely effluxes from the carrier; active/extracellular actuation which utilizes an applied external stimulus; or active/intracellular actuation which relies on innate conditions (e.g., pH, enzymes) in the cytosolic environment [5,7].

The targeting of NPs to the plasma membrane has typically depended on the use of receptor-specific moieties, such as antibodies, peptides, and small molecules. Although, these targeting moieties bind to their membrane receptor with high affinity, their sustained membrane retention is hampered by constitutive endocytic uptake. Furthermore, the reliance on endosomal pathways for cellular internalization exposes cargo to hydrolytic and enzymatic lysosomal degradation, which can decrease therapeutic efficiency. Therefore, two of the most important challenges facing the NP-mediated delivery of therapeutics to the plasma membrane remain: achieving the persistent residence of the NP–drug cargo on/at the plasma membrane while also minimizing the internalization of the NP–cargo complex via any of the cell’s multiple endocytosis pathways [8–10]. Recent examples point to how NP–bioconjugate systems can help circumvent these issues. The identification of membrane-tethering biologicals, such as lipophilic moieties [11,12] and lipophilic moiety–peptide conjugates [13,14] offer promising routes to localizing therapeutic NPs to the plasma membrane. In some instances, persistent membrane residence coupled with concomitant negligible cellular internalization of the NP–peptide bioconjugates has been observed [13].

Current approaches to NP-mediated drug/cargo delivery to the plasma membrane

Work from numerous groups has shown exciting promise for NP-mediated drug/cargo delivery

directly to the plasma membrane. Our group, using a liquid crystal NP (LCNP) platform, demonstrated the ability to modulate both the membrane residence time and cytotoxicity of a carbocyanine-based potentiometric membrane-labeling dye (3,3-diocadecyloxacarbocyanine perchlorate [DiO]) by loading the hydrophobic dye into the core of cholesterol-decorated LCNPs. The membrane-tethered LCNPs localized in lipid raft microdomains with sustained retention, which in turn facilitated enhanced partitioning of DiO into the membrane bilayer. Compared with free DiO delivered from bulk solution, the half-life of the membrane residence time for DiO delivered from the NP core was doubled while its toxicity was reduced by approximately 40% [5]. Similarly, Kim *et al.* employed membrane fusogenic liposomes for the specific delivery of ZnPC to the plasma membrane bilayer of various cancer cell lines (H460 human non-small-cell lung cancer, CT26 mouse colon cancer and U87MG human glioblastoma) [15]. The fusogenic liposomes facilitated augmented cytotoxicity (up to 40% increased cell killing) compared with control (nonfusogenic) liposomes, which delivered payload to the cytoplasm via endocytosis. Recently, Yang *et al.* promoted membrane fusion between live cells and liposomes by utilizing complementary coiled-coil lipopeptides and demonstrated cargo release at the plasma membrane, as well as significant IC_{50} enhancement of doxorubicin over free drug in HeLa cells [16]. In all of these examples, it is the combined function of the ensemble construct (NP–biological–drug/cargo) that mediates the delivery of the therapeutic cargo to the plasma membrane with fine control.

The future of NP-facilitated membrane delivery of therapeutics

So what can we expect going forward in the realm of membrane-targeted therapeutic NPs? Clearly, the brief examples described above point to significant advances in the fundamental understanding of how to elicit fine control over NPs to maximize the efficacy of the on-board drug/cargo in the context of the plasma membrane. We anticipate that such basic studies will continue along with further investments in the identification of novel membrane-targeting moieties (e.g., peptides, aptamers, small molecules) including peptidyl motifs comprised of non-natural amino acids, which expands the structural diversity of currently available recognition elements for membrane targeting and sustained residence while evading the endolysosomal system. We foresee that these investigations will lead to the development of specific biomarker/ligand pairs for the targeting of drug-

loaded NPs preferentially to diseased tissues in order to reduce therapeutic toxicity profiles. We also expect refinement of cargo-release using more complex NP designs that take advantage of the full range of NP actuation modalities (passive, active/extracellular and active/intracellular) to affect the release of the NP-associated cargo [5]. Ultimately, we envision advancements in combinatorial delivery of multiple cargos, such as synergistic therapeutic drug payloads or theranostic agents on a single NP platform coupled with their controlled release in a spatiotemporal manner.

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