Protocells (Nanoporous Particle Supported Lipid Bilayers) For Targeted Drug Delivery

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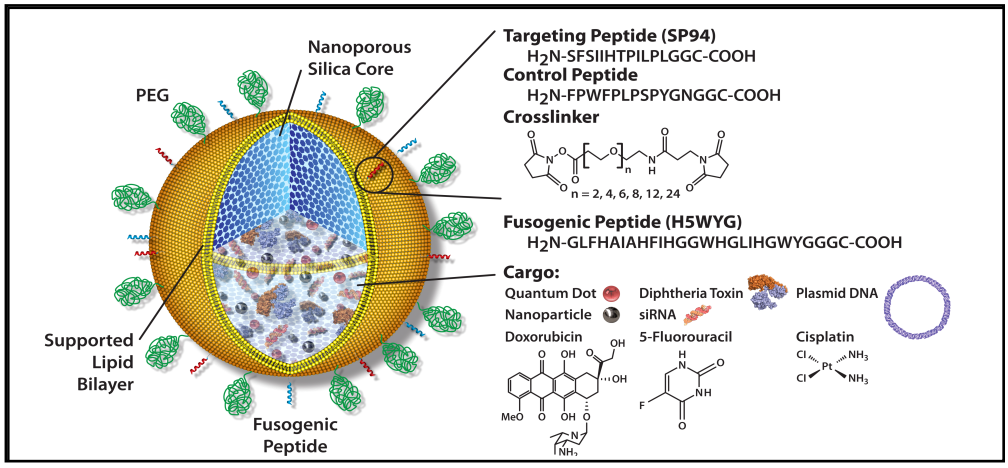

Figure - Protocell Design. Nanoporous silica cores are loaded with multiple drug cargos by adsorption to the silica matrix. The drug-loaded core is then enveloped by a single lipid bilayer, which is further functionalized with: i) polyethylene glycol (PEG) to reduce nonspecific interactions with its environment; ii) targeting peptides to direct protocell binding to specific cells; and iii) pH-responsive peptides which cause disruption of endosomes and the bilayer coating upon particle internalization into acidic intracellular compartments, allowing drug delivery into the cytosol of the target cell.

Stable nanoparticles capable of specifically binding to cancer cells and delivering high doses of therapeutic compounds could be transformational for cancer therapy by making drug delivery into cancer cells more efficient, while reducing toxic side effects in healthy cells and tissues. An ideal targeted nanoparticle drug carrier, or "nanocarrier" should have the: 1) capacity for carrying high levels of multiple diverse molecular cargos (small molecules, drugs with varying physiochemical properties, siRNAs, peptides, imaging agents); 2) ability to circulate in the blood *in vivo* for extended periods without elimination by the immune or excretory systems; 3) specificity for binding only to target disease cells, while avoiding normal, healthy cells; and 4) low immunogenicity and toxicity. A number of nanoparticle-based therapies are now in use in the clinic, many of which are derivatives of liposomes (vesicles comprising a lipid bilayer surrounding an aqueous core) or polymers. Liposomes and polymers have low immunogenicity, excellent safety profiles in humans, and established approaches for large-scale clinical manufacturing. However, despite these attractive features, both liposomes and polymer conjugates have significant limitations, which have impeded their use as targeted nanocarriers. For example, the physical properties of liposomes most favored for efficient drug delivery and stability *in vivo* require rigid gel-phase structures; however such rigid structures limit the ability of targeting ligands incorporated within the liposomal membrane to diffuse and engage in high-avidity multivalent binding needed to achieve specificity and induce internalization. While fluid liposomes may provide ligand mobility, fluid liposomal membranes are unstable *in vivo* and too permeable to efficiently deliver high concentrations of therapeutic drugs. Alternatively, whereas high copy numbers of targeting ligands incorporated in rigid liposomal membranes could increase on–target binding affinity, high ligand density increases non-specific, off-target binding

and the likelihood of an immune response. In fact *this trade-off between specific and non-specific binding and immunogenicity is inherent to all existing targeted nanocarrier platforms.*

To overcome the challenges of exisiting targeted nanocarriers, our team has recently devised a solution to this complex engineering problem through the creation of a composite nanocarrier termed a "protocell" (Figure). Targeted protocells, first reported in a 2011 article in *Nature Materials* and more recently in *ACS Nano,* are formed by fusion of liposomes on high surface area (>1000 m^2/g) porous silica nanoparticle cores (50-200 nm in diameter) followed by conjugation of the supported lipid bilayer with targeting and trafficking ligands and PEG. They synergistically combine the advantages of liposomes (low inherent toxicity, immunogenicity, and long circulation times) and porous nanoparticles (stability and an enormous capacity for multiple cargos and disparate cargo combinations). We have demonstrated that protocell-supported lipid bilayer membranes retain both high in-plane, two-dimensional mobility and high stability against destabilization on exposure to blood components and leakage of drug cargos from the silica core. This desirable combination of fluidity and stability arises from the adhesion energy between the lipid headgroups and surface silanols (≡Si-OH), which suppresses large scale membrane bilayer fluctuations responsible for leakage, along with surface nanopores that modulate lipid headgroup packing and enhance lateral fluidity. These factors allow protocells, incorporating very low densities of targeting peptide ligands or single chain antibodies as targeting moieties, to bind selectively to target cells via multivalent binding enabled by targeting ligand diffusivity and recruitment by cell surface receptors. This allows high affinity, cell-specific binding while minimizing off-target binding and immunogenicity. We have demonstrated that protocells have a 100-fold greater specificity in target-cell binding than equivalent fluid-phase liposomes. The design of the protocell has also overcome a second disadvantage of liposomal and most other nanocarrier drug delivery strategies, namely the inability to deliver high levels of multiple drug cargos, particularly where the drug combinations have different charges, polarities, and molecular weights. In contrast, protocells can simultaneously adsorb multiple diverse cargos (imaging agents, peptides, siRNAs, and drugs with different physicochemical properties) into their nanoporous silica cores, with reversible binding to silica providing the means to stably retain high levels of each cargo before envelopment of the particle into its protective lipid membrane shell. Adsorption-mediated drug loading into the silica cores leads to a 1,000-fold greater dose of doxorubicin on a per protocell particle basis, compared to FDA-approved liposomal doxorubicin. A third major advantage of the protocell is that combinatorial cargos are retained until they are efficiently delivered into intracellular compartments of the cytosol of the target cell "on cue" via pH-triggered destabilization of the protocell's supported lipid bilayer and endosomic swelling and disruption orchestrated by endosomolytic peptides incorporated within the lipid bilayer. Until now, "endosomal escape" has been a major obstacle of other targeted nanocarrier strategies. Inclusion of an octa-arginine peptide, which stimulates macropinocytosis, enables both selective targeting and intracellular delivery for cells where cell-specific receptors are not readily endocytosed. Using a model of hepatocellular carcinoma, we demonstrated that protocells carrying a cocktail of doxorubicin, 5-FU, and cisplatin were so potent that a single targeted protocell could kill a multidrug-resistant hepatocellular carcinoma cell *in vitro*, representing a 10^6-fold improvement over liposomes.

References

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2. C.E. Ashley, Brinker *et al.* Delivery of Small Interfering RNA by Peptide-Targeted Mesoporous Silica Nanoparticle-Supported Lipid Bilayers, **ACS Nano**, 6 2174-2188 (2012).