Published in final edited form as:

Wiley Interdiscip Rev Syst Biol Med. 2015 ; 7(5): 253–265. doi:10.1002/wsbm.1304.

Systems approaches to design of targeted therapeutic delivery

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Abstract

Targeted drug delivery aims to improve therapeutic effects and enable mechanisms that are not feasible for untargeted agents (e.g., due to impermeable biological barriers). To achieve targeting, a drug or its carrier should possess properties providing specific accumulation from circulation at the desired site. There are several examples of systems-inspired approaches that have been applied to achieve this goal. First, proteomics analysis of plasma membrane fraction of the vascular endothelium has identified a series of target molecules and their ligands (e.g., antibodies) that deliver conjugated cargoes to well-defined vascular cells and subcellular compartments. Second, selection of ligands binding to cells of interest using phage display libraries *in vitro* and *in vivo* has provided peptides and polypeptides that bind to normal and pathologically altered cells. Finally, large-scale high-throughput combinatorial synthesis and selection of lipid- and polymer-based nanocarriers varying their chemical components has yielded a series of carriers accumulating in diverse organs and delivering RNA interference agents to diverse cells. Together, these approaches offer a basis for systems-based design and selection of targets, targeting molecules, and targeting vehicles. Current studies focus on expanding the arsenal of these and alternative targeting strategies, devising drug delivery systems capitalizing on these strategies and evaluation of their benefit/risk ratio in adequate animal models of human diseases. These efforts, combined with better understanding of mechanisms and unintended consequences of these targeted interventions, need to be ultimately translated into industrial development and the clinical domain.

INTRODUCTION

Localization of a drug in the body and the fraction of administered dose that accumulates at the intended site of action dictate both its beneficial and deleterious effects. Some agents—in particular, biological molecules such as recombinant enzymes, nucleic acids, and peptides—have no efficacy unless they reach specific cells or subcellular compartments, where they can interact with endogenous partners critical to their mechanism of action.

Unfortunately, most drugs do not accumulate naturally at their intended targets. To achieve this aim, they must be joined to affinity ligands that bind specific molecules on the surface of target cells or, in some cases, loaded in carriers that protect them from inactivation and allow preferential accumulation at the site of interest using these ligands or other

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Conflict of interest: The authors have declared no conflicts of interest for this article.

mechanisms. These straightforward concepts, formulated by Paul Ehrlich a century ago, all rely on the identification of target molecules or mechanisms for homing drugs and carriers to the organs, cells, and intracellular compartments of interest.

The field of drug delivery (AKA drug targeting, nanomedicine, and targeted therapeutics) pursues this challenging objective via a multitude of approaches, including the definition of target molecules, synthesis of affinity ligands, and engineering of targeted carriers. This brief review will focus on high-throughput chemical and biological systems approaches, which have contributed to the growth of this field over the past several decades.

Among a plethora of target cells and tissues, this review focuses primarily on delivery to the vascular endothelium.¹ These cells, which line the lumen of blood vessels, represent an important target—and barrier—for drug delivery in a wide variety of human diseases. While vascular endothelial cells (ECs) would seem to be an easily accessible target, in that they form a huge surface area directly accessible to the bloodstream, untargeted drugs and drug carriers do not bind to the endothelium effectively, and only a tiny fraction of administered dose is typically retained at the vascular margin.

The first section of this review focuses on ligand-based targeting strategies and the use of systems approaches to identify novel targets allowing selective delivery of agents to particular organs or regions within the vasculature. In the second portion of this review, we discuss the use of combinatorial chemistry to define the biophysical characteristics (or combinations thereof) capable of directing nanocarrier-mediated drug delivery to specific regions within an organism, tissue, or cell. In combination, the two sections show how systems approaches can lead to selection of targeting strategies and inform design of targeting vehicles.

SYSTEMS-BASED APPROACHES IN THE IDENTIFICATION OF NOVEL VASCULAR ENDOTHELIAL TARGETS

Perhaps the single most important parameter of targeted vascular drug delivery is the choice of luminal surface target and corresponding affinity ligand. Antibodies, antibody fragments, peptides, and other ligands specifically binding to surface determinants are used (often as carrier components) to achieve targeting objectives. As reviewed below, candidate determinants can be selected based on high-throughput analytical approaches of the functional genomics and proteomics of the tissue of interest.

The best-characterized targets for vascular endothelium are transmembrane glycoproteins originally identified because of their involvement in cell–cell interactions, either between ECs (e.g., VE-cadherin and PECAM-1) or the endothelial monolayer and other cell types (e.g., ICAM-1, E-selectin, and P-selectin).^{1–4} Most efforts to target these molecular determinants have been accomplished using monoclonal antibodies or molecules derived from their endogenous ligands (e.g., ICAM-1 binding peptides from fibrinogen or LFA-1).^{5–7} While these early, hypothesis-driven studies laid the groundwork for the field of vascular drug delivery, the relatively modest repertoire of available targets has limited

efforts to selectively direct drugs, nanoparticles (NPs), and imaging agents to specific organs and/or diseased tissue.

In particular, agents targeted to pan-endothelial surface determinants tend to accumulate in the lungs after intravenous injection, both because of anatomic factors (the pulmonary circulation is the ‘first pass’, receiving half the cardiac output) and because the lungs contain the major fraction of endothelial surface in the body. Our group and others have demonstrated the utility of delivering therapeutics to angiotensin-converting enzyme (ACE), PECAM-1, ICAM-1, and a short list of other endothelial targets. These interventions generate effects unrivaled by non-targeted drugs and have the potential to revolutionize the treatment of pulmonary vascular pathologies, including acute respiratory distress syndrome (ARDS), pulmonary arterial hypertension (PAH), and pulmonary embolism.⁸

In order to extend these early successes to the full spectrum of human disease, novel surface targets and affinity ligands are needed. Apart from enabling selective delivery to tissues, novel ligands may facilitate targeting of inflamed or pathologic vessels (e.g., tumor endothelium), as well as transcytosis of drugs and drug carriers across the endothelial monolayer into organ parenchyma. In this section, we review the application of two contemporary high-throughput techniques, proteomics and phage display, to these aims, and their contribution to defining the molecular heterogeneity of the vascular endothelium.

Proteomics Tools for Identifying Epitopes Useful for Targeting

Proteomics is the large-scale study of proteins, focused on identifying a proteome, the entire set of proteins found within an organism, cell type, or other compartment. The techniques classically associated with proteomics include methods to isolate proteins from a cellular compartment, and then analyze all purified proteins in parallel, rather than focusing on one protein at a time. To identify molecular determinants for targeted drug delivery, the proteome of obvious interest is the entire complement of proteins localized on the surface of cells of interest. The cell surface proteome (often called the ‘membranome’) has been interrogated for numerous healthy and pathological cell types (reviewed in Josic and Clifton⁹). In order to illustrate the power of cell surface proteomics for targeted drug delivery and imaging, we will focus on the pulmonary ECs. We will review the recent development of analytical techniques to elucidate the EC proteome and touch on targeting-relevant discoveries in preliminary implementation of these techniques.

Strategies for Isolation of Endothelial Surface Proteins—There are several challenges in defining the endothelial proteome. The first problem is that integral membrane proteins (IMPs; those traversing the membrane) are difficult to purify and analyze using mass spectrometry (MS). IMPs are intrinsically very hydrophobic and thus do not purify with the majority of cellular proteins which are much more soluble in aqueous buffers. MS signal of low-abundance IMPs is difficult to detect. The second challenge of EC proteomics is that it is difficult to purify ECs from the surrounding parenchyma. ECs constitute a small fraction of cells in most organs, even in the highly vascular lungs.

Arterial perfusion with small molecule tags to label the EC surface proteins of the lungs provided a first solution to the problem of isolating IMPs. De La Fuente et al. employed a

small molecule (sulfosuccinimidyl 6-biotin-amido hexanoate (NHS-LC-biotin)) that biotinylates surface proteins but is membrane-impermeable, and therefore should not significantly label cytosolic proteins.¹⁰ The biotinylated protein fraction was separated via interaction with streptavidin-coated beads. However, NHS-LC-biotin and other small molecules can diffuse between endothelial cell–cell junctions, thereby also labeling the membranome of non-EC lung cells.

Schnitzer et al. avoided the problem of tag extravasation by replacing NHS-LC-biotin with larger polycationic colloidal silica NP (protocol reviewed in Griffin and Schnitzer¹¹). These particles bind to the negatively charged pulmonary EC cell surface via electrostatic interactions. After centrifugal separation of the plasma membranes with bound silica NPs, Oh et al. showed 20-fold enrichment of known EC markers (e.g., ACE, VE-cadherin) and 20-fold depletion of non-EC proteins¹² in NP-membrane fractions (Figure 1).

Refinement of Proteomics Data for Selection of Endothelial Targets—The Schnitzer lab used the purified EC membrane protein fraction in two ways: (1) They created an antibody library using the EC membrane protein fraction as the antigenic stimulus and (2) They analyzed the EC membrane protein fraction using MS to identify all component proteins. These two proteomics approaches each led to the identification of targeting determinants with impressive results in assays of lung-targeted delivery of drugs and imaging agents.

In their antibody library, Schnitzer et al. identified TX3.833, which targets a 90-kDa protein whose expression appears to be limited to pulmonary ECs.¹³ Ultimately, the targeted protein was shown to be aminopeptidase P (APP). APP was also later identified using the MS approach.

The initial MS analysis of the pulmonary EC membrane protein fraction identified ~450 proteins.¹³ However, only ~15% were IMPs (a smaller fraction than prior estimates) and numerous known EC membrane proteins were not detected with this method. Therefore, a variety of additional processing and analysis steps were used to improve detection and annotation.

The first improvement of the MS-generated protein list involved optimizing the technique for fractionating the membrane protein fraction prior to MS. Among four separation methods, the best was SDS-PAGE pre-fractionation followed by trypsin digestion of gel slices to generate peptides for two-dimensional (2D) LCMS analysis.¹⁴ The optimized separation technique expanded the pulmonary EC membranome to 1833 proteins, including > 500 IMPs.

The second technique to improve the MS-generated protein list utilized bioinformatics tools to categorize each protein into a specific biological process. The categorizations were primarily based on prior datasets that annotated protein function based on large-scale experimental datasets in the literature. About 30% of the proteins were automatically classified in terms of biological function. Thus, proteins identified through MS are unlikely to have known function, which increases the risk of using them as targeting determinants.

The informatics approach also identified many proteins in the pulmonary EC membranome that had previously been reported to be restricted to neurons. The large-scale, unbiased approach of MS therefore identified potential targeting determinants that would not have been candidates simply based on the prior literature.

A third method to improve the utility of the MS-generated protein list entailed more accurate determination of protein abundance.¹⁵ Protein abundance may be a useful factor in choosing which of the 1800 proteins in the membranome would be useful targeting determinants. A metric based on three MS abundance features (peptide count, spectral count, and fragmentation intensity) assigned an absolute abundance to the proteins identified by MS. In addition to creating a list of the abundances of the EC membranome, the quantitative MS studies of the membrane protein fraction produced two important findings with ramifications for target identification and evaluation. First, they quantitated how much the EC membranome changes when ECs are changed from the *in vivo* to *in vitro* environment. Of 450 proteins identified *in vivo*, only 263 (58%) were present *in vitro*^{12,16}. The second major scientific finding was that each organ's ECs express a unique complement of cell surface proteins. The EC membrane protein fraction from five organs (brain, heart, lung, liver, and kidney) was subjected to 2D gel electrophoresis and later MS.¹² Numerous protein spots on the 2D gels were common to each organ, but several spots were found only in one organ but not in others. This suggests that targeting determinants can be produced that select the endothelium of one organ but not others.

Targeting to Proteomics-Derived Endothelial Markers—These early membranome discoveries permitted the development of antibodies with tremendous promise as targeting determinants for drug and imaging agent delivery. Schnitzer et al. first sought to create an antibody against a protein expressed only on pulmonary ECs and not ECs from other organs. In order to enrich for lung EC-specific proteins, the team applied two more filters. First, they compared the MS results of *in vivo* lung ECs versus lung ECs grown *in vitro*.¹² They hypothesized that the *in vitro* environment would not provide lung-specific cues to the ECs and would stop expressing lung EC-specific proteins. Second, they used bioinformatics algorithms that predicted structure and glycosylation to enrich for those proteins predicted to have a domain exposed on the cell surface. Western blots were used to search the EC membranes of 4 organs for 11 proteins selected via these techniques. Two of these proteins, OX-45/CD48 and APP, were expressed solely on ECs in the lung. Antibodies against OX-45 did not markedly accumulate in the lung [only 2% of injected dose/gram of tissue (ID/g)], because CD-45 is also expressed on leukocytes and thus the antibody remained in the blood pool. However, anti-APP monoclonal antibodies (mAPP) rapidly accumulated in the lung. I¹²⁵-labeled mAPP was found at 65–90% ID/g in the lungs within 60 min. Further characterization of the anti-APP antibody showed (1) it is enriched in caveolae; (2) it extravasates into lung parenchyma in a caveolin-1-dependent manner; (3) its lung accumulation is stronger than a large panel of other endothelial antibodies (PECAM, ACE, etc.); (4) its lung accumulation persists > 48 h; and (5) conjugation of a small-molecule toxin to the antibody increased the toxin's lung concentration 172-fold.^{12,13,17}

Schnitzer et al. used the same subtractive MS approach to compare normal lungs with lungs containing breast cancer metastases. They identified 15 proteins present in tumor-associated

EC membranes but not in the ECs of healthy lungs. One of these proteins, Annexin A1 (AnnA1), was found only on the tumor EC membranes but not in other assayed organs.^{12,18} anti-AnnA1 has > 100-fold greater accumulation in tumors than a panel of other antibodies tested, and thus has huge potential for delivery of drugs or imaging agents.

In summary, proteomics has added much to the field of targeted delivery of drugs and imaging agents, especially delivery to ECs. Proteomics has produced at least three major scientific findings that inform the search for targeting determinants: (1) Each organ's ECs express a unique complement of cell surface proteins, potentially allowing for 'organ addressing'. (2) The EC membranome changes dramatically when cells are changed from the *in vivo* to *in vitro* environments, suggesting that *in vitro* studies can be very misleading. (3) Some targeting determinants (e.g., anti-APP antibodies) can affect transcytosis across the endothelium, thus allowing intraparenchymal deliver of cargo. In addition to the scientific findings, these proteomics studies have produced two antibodies with potential clinical promise, anti-APP and anti-AnnA1. Further testing is necessary for these antibodies, especially as determinants of the maximal size of drug carriers (the 50-nm caveolar neck severely limits nanocarrier choice) and for possible side effects of antibody targeting (e.g., APP is a key regulator of bradykinin in the lung, and blocking it could have deleterious consequences).

Several follow-up experiments are now feasible with existing proteomics tools. Most importantly, these proteomics tools can be used to determine which proteins change abundance in disease states of the lungs. Many pulmonary EC surface proteins are known to change levels dramatically in response to pathology. For example, in animal models of ARDS, VCAM-1 levels increase at least 10-fold from a baseline that is often reported as undetectable.¹⁹ Therefore, based solely on protein abundance from a proteomics analysis of healthy animals, VCAM-1 would appear as a poor targeting determinant and imaging target. However, the fact that VCAM-1 levels are high in portions of lung affected by ARDS makes VCAM-1 an attractive target for both drug delivery and for imaging agents used to detect ARDS. Proteomics can find proteins with similar properties, which may improve the panel of targeting determinants available for lung disease.

Using Phage Display for Endothelial Targeting

Phage display, first introduced by George Smith in 1985²⁰, is a method in which combinatorial peptides or proteins are expressed on the surface of bacteriophage, with each individual virion containing DNA encoding the particular protein displayed on its exterior. Molecular cloning allows generation of large phage libraries containing up to 10¹⁰ variants, which can then be screened to identify phage with desired properties—for instance, binding to a known surface determinant, cell type, or vascular bed. By linking surface-displayed protein with its complementary DNA, phage display allows rapid recovery and identification of the affinity ligand (i.e., by DNA sequencing), as well as recursive rounds of amplification and positive (or negative) selection.

The technical details of phage display and recent innovations in this technology have been reviewed extensively elsewhere.^{21,22} However, the current discussion provides a brief overview, focusing on several key features, which have allowed its application to vascular

drug delivery. The most common phage used in display technology is the filamentous M13 strain, which contains a single-stranded DNA genome encoding, among others, the pIII coat protein. Several copies of this protein, which mediates attachment of the virus to Gram-negative bacteria, are expressed in a cluster on the tip of the phage. Insertion of complementary DNA into the M13 genome just upstream of the pIII gene results in display of variant peptides as N-terminal fusions of this protein. Luckily, not all copies of the coat protein are produced as fusions, allowing the phage to retain its infectivity and enabling easy amplification and recovery of genetic material for sequencing and manipulation.

Design of Phage Display Libraries—While phage have traditionally been used as a means of screening combinatorial peptides, recent technology enabling the expression of larger and more complex proteins in *Escherichia coli* has allowed the display of a variety of different immune-derived affinity molecules, including single chain (scFv) and bivalent (e.g., Fab) antibody fragments.²³ Beyond simply adding new molecules to the existing paradigm, this capability has expanded the potential application of phage display, allowing the creation of libraries encompassing the immunologic repertoire of whole animals. Heavy and light chain variable genes can be amplified from peripheral blood lymphocytes, bone marrow, or spleen cells taken from naïve or immunized animals or even human donors.²⁴ These immune-derived libraries allow identification of high affinity human antibodies, ‘humanization’ of antibodies derived from other species, and even characterization of the serologic responses of patients – with infections, cancer, or autoimmune disorders.^{24–26} Entirely synthetic antibody phage libraries have also been created, in which VH and VL genes have been assembled into consensus frameworks, with subsequent randomization of the CDR3 encoding regions.^{27,28}

Of particular interest to the field of vascular drug delivery is the development of libraries of highly stable, single-domain antibodies (sdAb) derived from camelids, which are half the size of standard mammalian immunoglobulins, can be produced recombinantly in bacteria, and tend to have remarkably stable folds, resistant to heat, gastric acid, and proteases.²⁹ As an example, a phage display library derived from an unimmunized llama was used to identify a sdAb capable of binding the cerebrovasculature and transmigration across the blood–brain barrier (BBB).³⁰ Fc fusion proteins of this sdAb accumulated in the brain following intravenous injection in rats, as indicated by a significant rise in CSF levels. Likewise, conjugation of these fusion proteins to BBB-impermeable neuroactive peptides allowed their delivery to the CNS, resulting in a significant increase in analgesic effect compared to untargeted peptide.³¹

Phage Selection Methods—Apart from the design of the library, the key feature of any phage display experiment is the choice of method of screening, also known as ‘panning’. Both *in vitro* and *in vivo* screening methods have been developed and tailored toward a variety of applications.

Panning on immobilized immunoglobulin was the method of selection utilized in the first phage display experiments, in which peptide libraries were used to map antibody epitopes.³² Indeed, purified protein is still considered by many to be the standard means of screening phage libraries. Within the context of vascular drug delivery, however, this technique is

limited to situations in which a particular endothelial surface target has already been identified and cloned. In this case, phage display is essentially an alternative to standard hybridoma-based techniques, allowing identification of new protein binders and affinity maturation of existing ligands.³³ The molecule of interest is typically synthesized recombinantly, purified extensively, and immobilized or adsorbed onto a solid surface, such as an affinity column, allowing rapid separation of specifically bound phage from weak and non-specific binders. With each successive round of panning, the stringency of selection may be increased to isolate binders with higher affinity. This technique has been used to identify peptides or proteins specific for a variety of different endothelial surface targets, including – integrins, selectins, and cell adhesion molecules.^{34–36} One limitation of this approach is the potential for loss of native and/or functional conformation of the target protein, either during production or immobilization. In the worst-case scenario, this can result in selection of affinity ligands unable to bind to the desired vascular determinant *in vivo*. In contrast, peptides found by panning against proteins presented in their natural configuration tend to share sequence homology or even biological activity with endogenous ligands.³⁷ For example, panning of a phage library on immobilized human ICAM-1 resulted in the isolation of a peptide with homology to one ICAM-1 ligand, the rhinovirus coat protein 14, as well as the ability to inhibit binding of a second ligand, the β 2-integrin LFA-1.³⁶

Another commonly used technique, which avoids some issues arising with the use of purified protein, is the screening of phage libraries against the surface of cells. In most cases, cells express target antigens with appropriate post-translational modifications and in native conformation. Membrane proteins are also free to complex with surface partners, revealing potential binding sites which may not present when panning on purified protein. Phage may be panned on ECs or on transfected cells overexpressing a protein of interest. The latter approach, for example, was used to identify a peptide specific for the urokinase receptor and has the advantage of allowing negative selection on untransfected cells, a useful means of depleting both non-specific and irrelevant binders.³⁸ In other cases, investigators have focused their peptide selection by exploiting the differences in protein expression between ECs of different organs. A VCAM-1-binding peptide, for example, was identified via positive selection of a phage library on mouse cardiac ECs, which constitutively express this surface molecule, alternated with negative selection on mouse lung ECs, which have essentially no baseline expression of VCAM-1.³⁹ Screening of phage libraries on ECs can also be used as a means of identifying novel targets for vascular drug delivery, as well as to probe differences between endothelial vascular beds, vessel types (e.g., arterioles, capillaries, venules, etc), or phenotypic states (e.g., following inflammatory activation).⁴⁰ This approach has been utilized by several groups to identify peptide and protein ligands that bind to brain endothelium, a site which typically receives only a fraction of a percent of the injected dose of therapeutics targeted to pan-endothelial markers.^{30,41}

In 1996, Pasqualini and Ruoslahti reported the isolation of phage-displayed peptides capable of homing to distinct vascular beds following intravenous injection in a live animal.⁴² So-called ‘biopanning in mice’ has a number of advantages, including the built-in removal of many weak binders, which are depleted by plasma proteins and blood cells, and the inherent

stringency of screening under physiologic flow conditions. Perhaps the most attractive feature of *in vivo* phage display, however, is the ability to screen affinity ligands in a variety of disease states, with the hope of identifying ligands capable of selectively delivering imaging agents and/or therapeutics to affected tissue.⁴⁰ This technology has led to the discovery of several novel ligand/receptor pairs, including a lung-homing peptide, which binds an unidentified surface target on lung ECs, and a adipose endothelial binding peptide identified by *in vivo* selection in leptin-deficient, obese mice.^{43,44} Phage library has even been infused in several terminally ill human cancer patients, with subsequent recovery of phage from various organs.⁴⁵ Indeed, this group has proposed the use of *in vivo* phage display as a means for defining the molecular heterogeneity of the human vasculature, using combinations of affinity ligands to define unique 'vascular ZIP codes', each identifying a given organ, vessel type, or pathologic state.^{40,45,46}

Phage Display and Vascular Drug Delivery: Challenges and Future Directions

—As with any technology, certain challenges arise in the application of phage display to vascular drug delivery. In particular, phage-derived peptides are typically fairly low affinity, and while their binding may be improved via synthesis of multimeric complexes, they may still not match the affinity of monoclonal antibodies (mAbs) or their derivatives.⁴⁷ This is particularly problematic when significant time and resources are invested in conducting an unbiased search for ligand/receptor pairs, only to have the selection converge on a peptide specific for a previously known protein to which high affinity mAbs already exist.⁴⁸ Likewise, unbiased screening does not exclude endothelial surface targets (e.g., ACE or thrombomodulin) with critical biologic functions. As such, phage display may result in the isolation of ligands which cause undesired side effects upon binding the endothelial surface.⁴⁹ Finally, when delivering biotherapeutic cargoes like fibrinolytic enzymes or siRNA, retention on the luminal surface membrane or delivery to a specific subcellular compartment may be critical for achieving efficacy.^{50,51} While additional technology may help to address these issues, existing screening methods provide only rudimentary means of biasing selection toward ligand/receptor pairs appropriate for these applications.

CHEMICAL LIBRARY-BASED APPROACHES TO DESIGN OF TARGETED NANOCARRIERS

Among a variety of strategies for drug delivery to targeting determinants, NPs have a wealth of advantages, including high loading capacity, multivalent presentation of targeting molecules, and capacity for imaging contrast. While the general use of targeting molecules (including those derived from phage display and proteomics) with NPs has been described elsewhere^{52–55}, this portion of the review will focus on the design of NPs themselves via a systems pharmacology approach. This approach broadly considers the full range of properties available to NPs and their components and how those properties interact with biological systems.

General Principles for Systems Design of Targeted NPs

Variation in several key design elements can have profound effects on the behavior of NP drug carriers *in vivo*, including homing to different tissues, cells, and subcellular

compartments following intravenous injection.^{53,54} Several decades of research have suggested the importance of size, shape, plasticity, surface charge, functionality, and chemical properties for biomolecular systems targeting. Combinatorial analysis of properties optimal for specific target sites, however, has only recently received attention.^{56–58}

In 2010, Decuzzi et al. proposed a general framework for optimizing targeted NP design via integrated modeling and arrayed characterization. They described mathematical models for predicting *in vivo* behavior based on transport properties, cellular internalization, and target affinity and proposed an integrated approach for validating these models and identifying optimal combinations of biophysical characteristics.⁵⁹ Their framework aims to take advantage of the multitude of NP properties accessible via self-assembly chemistry techniques and the vast options for characterization of NPs and their interactions with *in vitro* and *in vivo* environments (electron microscopy, dynamic light scattering, optical contrast etc.) to optimize the design of NPs for biomedical applications.

Fundamentally, Decuzzi et al. proposed treating NPs as systems unto themselves, capable of interacting with biological systems in a manner responsive to component properties. As reviewed below, several studies have pursued precisely these aims, i.e., systematic study of NP components, their role in carrier localization, and their relevance to targeted biomedical applications.

A driving force behind the idea of systematic variation of NP properties has been the Anderson group at MIT. In work focused on the delivery of small interfering RNA (siRNA) for gene interference, Anderson's group has developed libraries of chemical components for polymeric, lipid, or lipid-mimetic NPs. In a growing body of work, these components have been methodically combined to form particles that can be screened for capacity to deliver therapeutics to specific organs, cell compartments, or cell types.

Combinatorial Chemistry for Organ-Specific NPs—In Akinc et al., lipid-like materials, termed 'lipidoids', were developed as building blocks of NPs designed for hepatic siRNA delivery.⁶⁰ siRNA therapies face tremendous challenges of systemic effects and ineffective nuclear translocation, so effective targeted delivery vehicles for siRNA are highly sought to mediate toxicity and facilitate therapeutic effects.

Previously, lipid formulations had facilitated delivery of siRNA to livers in mice in work building on a body of evidence aimed at direct siRNA complexing and delivery via cationic lipids.⁶¹ Nonetheless, a comprehensive study of safe and effective NP-based siRNA delivery techniques was first accomplished in Akinc et al. From a library of over 1200 lipidoids, Akinc et al. identified chemical properties (e.g., length, charge, head and tail group size) conducive to siRNA and miRNA delivery in HeLA cells. Selected successful lipidoids were applied further at several doses in HeLA cells, hepatocellular carcinoma cells, and bone marrow murine macrophages.⁶⁰

Top performing lipidoids were selected for *in vivo* application based on *in vitro* results. Two plasma proteins produced in the liver, Factor VII and ApoB, were used as test targets. Protein knockdown specific to the targeted sequences and organs was observed for seven

formulations in mice and rats. Further, nasal administration permitted lung targeting of siRNA vehicles and peritoneal administration allowed suppression of peritoneal macrophage activation. Library-derived lipidoid vehicles also showed capacity for miRNA delivery. Including tests in initial primate models, lipidoid siRNA vehicles showed no observed toxicity. Thus, synthetic generation of a library of lipid-like materials allowed synthesis of a diverse collection of NP delivery constructs readily evaluated *in vitro* and *in vivo* as tools for lung and liver specific siRNA therapy.⁶⁰

Combinatorial Chemistry for Cell Compartment Specificity—In further work emphasizing siRNA delivery, analogous combinatorial NP synthesis was used to explore NP access to specific cellular components. With respect to varied particle parameters, Siegwart et al. were able to explore trends in complexation of siRNA, internalization of particles in cells, and delivery of siRNA to HeLA cell cytoplasm using combinations of component properties in core-shell polymeric NPs. High throughput syntheses were conducted on 96-well plates, followed by analogous high-throughput characterization of particle properties and screening in cells.⁶²

The core-shell polymer NP systems explored enabled a great range of NP size control with a variety of component polymer groups. siRNA was able to complex to or encapsulate within a cationic particle core, protected by the polymer shell. Optimal general properties for core-shell epoxide-amine polymeric NPs were identified *in vitro* and deployed for *in vivo* liver hepatocyte transfection. For *in vivo* application, no gross toxicity was observed and sequence-specific Factor VII silencing was accomplished.⁶²

Library approaches were also employed for intracellular siRNA delivery via lipidoid particles⁶⁰ incorporating cell-penetrating peptides (CPPs). CPPs have generally promising ability to facilitate access to cytoplasm for drug delivery, either through fusion with cell membranes in endocytosis or through membrane pore formation. With common features of positive charge and amphiphilicity, CPPs were hypothesized as being suited to the task of complexing siRNAs for targeted delivery.⁶³

CPPs selected from a newly synthesized library were covalently conjugated to surfaces of lipidoid particles with demonstrated capacity⁶⁰ for siRNA encapsulation but not transfection. Transfection capacity was conferred on the NPs by addition of various CPPs in the library, with macropinocytosis indicated as an internalization mechanism. Successful transfection CPPs were examined for relationships between structure and transfection function. Positively charged residues with charge distribution conferring amphiphilicity were isolated as common characteristics after Poisson-Boltzmann analysis.⁶³

In vivo application of the CPP-lipidoid NPs was noteworthy for implementation of NPs with intrinsic imaging contrast capacity, allowing assessment of organ specificity *in vivo* after assessment of cellular internalization *in vitro*. Incorporation of near-infrared fluorophores or X-ray contrast agents permitted identification of NP accumulation focused on the liver and lungs. Likewise, with encapsulated Factor VII targeted siRNA, liver-specific protein silencing was observed. In general, no gross toxicity was observed and localized siRNA efficacy scaled with contrast-derived biodistribution data.⁶³

Combinatorial Approaches to Design of NPs for Endothelial Targeting—The Anderson group's most recent explorations of library techniques for siRNA delivery with nanocarriers have turned to endothelial targeting. Dahlman et al. applied combinatorial polymeric NP development techniques to endothelial siRNA targeting.⁶⁴ Previous work in endothelial delivery of siRNA had required relatively large doses of cationic lipid vehicles to achieve noteworthy silencing.⁶⁵ With a focus on polyethyleneimine (PEI) polymer-lipidoid hybrid NPs, a new component library was screened for siRNA delivery capacity in HeLa cells. Formulations successful in HeLa cells were subsequently assayed in bEnd3 and HMVEC ECs. A formulation was chosen based on the sum of these screening results, with subsequent characterization of surface charge indicating pH responsive behavior optimized for ECs, rather than hepatocytes. Indeed, silencing of multiple endothelial genes with lipidoid-PEI siRNA particles was observed *in vivo* with minimal relative effect in hepatocytes and peritoneal immune cells.⁶⁴

Additional lung targeting work with the same class of particle component compounds explored miRNA and siRNA delivery specifically for lung cancer. Lung endothelium specific NPs were targeted to lung adenocarcinoma cells *in vitro* and to lung tumors *in vivo*. siRNA targeted to oncogenes inhibited tumor growth and improved therapeutic response.⁶⁶

In summary, a diverse array of NPs has been developed for biomedical applications, but techniques for optimization of base particle properties for drug delivery are needed. Anderson et al. have explored combinatorial approaches to polymer delivery vehicle synthesis analogous to those used in small molecule drug discovery. High-throughput robotic techniques have been employed to enable the application of an array of functional groups to synthesis of an array of polymer and lipidic NPs. In these studies, libraries of components have generally determined NP architecture, delivery functionality, and capacity for interaction with biological systems. As a synthetic method, the library approach in these studies enables a holistic characterization of design and functional criteria during particle formulation (Figure 2).

CONCLUSION

Systems strategies for targeted drug delivery can consider selection and design of targeting determinants and targeting vehicles. Systems approaches focused on selection of specific ligands binding to defined surface components of the cells and tissues of interest—proteomics analysis of plasma membranes and phage display libraries—provide specific targeting mechanisms to deliver conjugated cargoes to desired cells and subcellular compartments. Knowing the identity of target molecules hints to what consequences of their engagement can be expected and therefore excludes targeting directed to functionally 'untouchable' molecules and focuses on more appropriate molecules (although different formulations targeted to the same molecule may induce different effects).

Toward the design of drug delivery vehicles, unbiased selection of formulations, obtained by chemical combinatorial carrier synthesis, has accorded readouts of genetic interference *in vivo*. This approach to carrier design is attractive for many reasons, including the criterion of screening, i.e., localization of the desirable effect in the target cell, tissue, or organ. This

outcome dictates the necessity of retrospect study and modeling of distribution and mechanism of action of the selected carriers (i.e., which specific features mediate the targeting? to which specific cells and subcellular compartments? what are potential adverse effects?).

Current studies focus on expanding the arsenal of these and alternative targeting strategies, devising drug delivery systems capitalizing on these strategies and evaluation of their benefit/risk ratio in adequate animal models of human diseases. These efforts include deciphering mechanisms of targeting and evaluation of therapeutic efficacy and unintended consequences of these targeted interventions in appropriate animal models of human diseases. These parameters, as well as practical and financial considerations, will decide acceptability and feasibility of the ultimate translation of promising strategies into the industrial development and clinical domain.

REFERENCES

1. Muzykantov VR. Biomedical aspects of targeted delivery of drugs to pulmonary endothelium. *Expert Opin Drug Deliv.* 2005; 2:909–926. [PubMed: 16296786]
2. Albelda SM, Muller WA, Buck CA, Newman PJ. Molecular and cellular properties of PECAM-1 (endo-CAM/CD31): a novel vascular cell-cell adhesion molecule. *J Cell Biol.* 1991; 114:1059–1068. [PubMed: 1874786]
3. Marlin SD, Springer TA. Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen 1 (LFA-1). *Cell.* 1987; 51:813–819. [PubMed: 3315233]
4. Jubeli E, Moine L, Vergnaud-Gauduchon J, Barratt G. E-selectin as a target for drug delivery and molecular imaging. *J Control Release.* 2012; 158:194–206. [PubMed: 21983284]
5. Muzykantov VR. Targeted drug delivery to endothelial adhesion molecules. *ISRN Vasc Med.* 2013; 2013:1–27.
6. Altieri DC, Duperray A, Plescia J, Thornton GB, Languino LR. Structural recognition of a novel fibrinogen chain sequence (117133) by intercellular adhesion molecule-1 mediates leukocyte-endothelium interaction. *J Biol Chem.* 1995; 270:696–699. [PubMed: 7822297]
7. Anderson ME, Siahaan TJ. Targeting ICAM-1/LFA-1 interaction for controlling autoimmune diseases: designing peptide and small molecule inhibitors. *Peptides.* 2003; 24:487–501. [PubMed: 12732350]
8. Brenner JS, Greineder C, Shuvaev V, Muzykantov V. Endothelial nanomedicine for the treatment of pulmonary disease. *Expert Opin Drug Deliv.* 2014; 12:239–261. [PubMed: 25394760]
9. Josic D, Clifton JG. Mammalian plasma membrane proteomics. *Proteomics.* 2007; 7:3010–3029. [PubMed: 17654460]
10. De La Fuente EK, Dawson CA, Nelin LD, Bongard RD, McAuliffe TL, Merker MP. Biotinylation of membrane proteins accessible via the pulmonary circulation in normal and hyperoxic rats. *Am J Physiol.* 1997; 272:L461–L470. [PubMed: 9124603]
11. Griffin NM, Schnitzer JE. Proteomic mapping of the vascular endothelium in vivo for vascular targeting. *Methods Enzymol.* 2008; 445:177–208. [PubMed: 19022060]
12. Oh P, Li Y, Yu J, Durr E, Krasinska KM, Carver LA, Testa JE, Schnitzer JE. Subtractive proteomic mapping of the endothelial surface in lung and solid tumours for tissue-specific therapy. *Nature.* 2004; 429:629–635. [PubMed: 15190345]
13. McIntosh DP, Tan XY, Oh P, Schnitzer JE. Targeting endothelium and its dynamic caveolae for tissue-specific transcytosis in vivo: a pathway to overcome cell barriers to drug and gene delivery. *Proc Natl Acad Sci USA.* 2002; 99:1996–2001. [PubMed: 11854497]
14. Li Y, Yu J, Wang Y, Griffin NM, Long F, Shore S, Oh P, Schnitzer JE. Enhancing identifications of lipid-embedded proteins by mass spectrometry for improved mapping of endothelial plasma membranes in vivo. *Mol Cell Proteomics.* 2009; 8:1219–1235. [PubMed: 19155209]

15. Griffin NM, Yu J, Long F, Oh P, Shore S, Li Y, Koziol JA, Schnitzer JE. Label-free, normalized quantification of complex mass spectrometry data for proteomic analysis. *Nat Biotechnol.* 2010; 28:83–89. [PubMed: 20010810]
16. Durr E, Yu J, Krasinska KM, Carver LA, Yates JR, Testa JE, Oh P, Schnitzer JE. Direct proteomic mapping of the lung microvascular endothelial cell surface in vivo and in cell culture. *Nat Biotechnol.* 2004; 22:985–992. [PubMed: 15258593]
17. Chrastina A, Valadon P, Massey KA, Schnitzer JE. Lung vascular targeting using antibody to aminopeptidase P: CT-SPECT imaging, biodistribution and pharmacokinetic analysis. *J Vasc Res.* 2010; 47:531–543. [PubMed: 20431301]
18. Oh P, Testa JE, Borgstrom P, Witkiewicz H, Li Y, Schnitzer JE. In vivo proteomic imaging analysis of caveolae reveals pumping system to penetrate solid tumors. *Nat Med.* 2014; 20:1062–1068. [PubMed: 25129480]
19. Reutershan J, Ley K. Bench-to-bedside review: acute respiratory distress syndrome – how neutrophils migrate into the lung. *Crit Care.* 2004; 8:453–461. [PubMed: 15566616]
20. Smith GP. Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. *Science.* 1985; 228:1315–1317. [PubMed: 4001944]
21. Azzazy HME, Highsmith WE Jr. Phage display technology: clinical applications and recent innovations. *Clin Biochem.* 2002; 35:425–445. [PubMed: 12413604]
22. Bratkovic T. Progress in phage display: evolution of the technique and its application. *Cell Mol Life Sci.* 2010; 67:749–767. [PubMed: 20196239]
23. Better M, Chang CP, Robinson RR, Horwitz AH. *Escherichia coli* secretion of an active chimeric antibody fragment. *Science.* 1988; 240:1041–1043. [PubMed: 3285471]
24. Vaughan TJ, Williams AJ, Pritchard K, Osbourn JK, Pope AR, Earnshaw JC, McCafferty J, Hodits RA, Wilton J, Johnson KS. Human antibodies with sub-nanomolar affinities isolated from a large non-immunized phage display library. *Nat Biotechnol.* 1996; 14:309–314. [PubMed: 9630891]
25. Nishibori N, Horiuchi H, Furusawa S, Matsuda H. Humanization of chicken monoclonal antibody using phage-display system. *Mol Immunol.* 2006; 43:634–642. [PubMed: 16360012]
26. Siegel DL. Translational applications of antibody phage display. *Immunol Res.* 2008; 42:118–131. [PubMed: 18773308]
27. Knappik A, Ge L, Honegger A, Pack P, Fischer M, Wellenhofer G, Hoess A, Wölle J, Plückthun A, Virnekäs B. Fully synthetic human combinatorial antibody libraries (HuCAL) based on modular consensus frameworks and CDRs randomized with trinucleotides. *J Mol Biol.* 2000; 296:57–86. [PubMed: 10656818]
28. Benhar I. Design of synthetic antibody libraries. *Expert Opin Biol Ther.* 2007; 7:763–779. [PubMed: 17477812]
29. Harmsen MM, De Haard HJ. Properties, production, and applications of camelid single-domain antibody fragments. *Appl Microbiol Biotechnol.* 2007; 77:13–22. [PubMed: 17704915]
30. Muruganandam A, Tanha J, Narang S, Stanimirovic D. Selection of phage-displayed llama single-domain antibodies that transmute across human blood–brain barrier endothelium. *FASEB J.* 2002; 16:240–242. [PubMed: 11772942]
31. Farrington GK, Caram-Salas N, Haqqani AS, Brunette E, Eldredge J, Pepinsky B, Antognetti G, Baumann E, Ding W, Garber E, et al. A novel platform for engineering blood–brain barrier-crossing bispecific biologics. *FASEB J.* 2014; 28:4764–4778. [PubMed: 25070367]
32. Scott JK, Smith GP. Searching for peptide ligands with an epitope library. *Science.* 1990; 249:386–390. [PubMed: 1696028]
33. McCafferty J, Griffiths AD, Winter G, Chiswell DJ. Phage antibodies: filamentous phage displaying antibody variable domains. *Nature.* 1991; 348:552–554. [PubMed: 2247164]
34. Koivunen E, Wang B, Ruoslahti E. Isolation of a highly specific ligand for the alpha 5 beta 1 integrin from a phage display library. *J Cell Biol.* 1994; 124:373–380. [PubMed: 7507494]
35. Molenaar TJM, Appeldoorn CCM, de Haas SAM, Michon IN, Bonnefoy A, Hoylaerts MF, Pannekoek H, van Berkel TJ, Kuiper J, Biessen EA. Specific inhibition of P-selectin-mediated cell adhesion by phage display-derived peptide antagonists. *Blood.* 2002; 100:3570–3577. [PubMed: 12393589]

36. Welply JK, Steininger CN, Caparon M, Michener ML, Howard SC, Pegg LE, Meyer DM, De Ciecchi PA, Devine CS, Casperson GF. A peptide isolated by phage display binds to ICAM-1 and inhibits binding to LFA-1. *Proteins*. 1996; 26:262–270. [PubMed: 8953648]
37. Staquicini FI, Dias-Neto E, Li J, Snyder EY, Sidman RL, Pasqualini R, Arap W. Discovery of a functional protein complex of netrin-4, laminin gamma1 chain, and integrin alpha6beta1 in mouse neural stem cells. *Proc Natl Acad Sci USA*. 2009; 106:2903–2908. [PubMed: 19193855]
38. Goodson RJ, Doyle MV, Kaufman SE, Rosenberg S. High-affinity urokinase receptor antagonists identified with bacteriophage peptide display. *Proc Natl Acad Sci USA*. 1994; 91:7129–7133. [PubMed: 8041758]
39. Kelly KA, Allport JR, Tsourkas A, Shinde-Patil VR, Josephson L, Weissleder R. Detection of vascular adhesion molecule-1 expression using a novel multimodal nanoparticle. *Circ Res*. 2005; 96:327–336. [PubMed: 15653572]
40. Pasqualini R, Moeller BJ, Arap W. Leveraging molecular heterogeneity of the vascular endothelium for targeted drug delivery and imaging. *Semin Thromb Hemost*. 2010; 36:343–351. [PubMed: 20490984]
41. Van Rooy I, Cakir-Tascioglu S, Couraud P-O, Romero IA, Weksler B, Storm G, Hennink WE, Schiffelers RM, Mastrobattista E. Identification of peptide ligands for targeting to the blood–brain barrier. *Pharm Res*. 2010; 27:673–682. [PubMed: 20162339]
42. Pasqualini R, Ruoslahti E. Organ targeting in vivo using phage display peptide libraries. *Nature*. 1996; 380:364–366. [PubMed: 8598934]
43. Giordano RJ, Lahdenranta J, Zhen L, Chukwueke U, Petrache I, Langley RR, Fidler IJ, Pasqualini R, Tuder RM, Arap W. Targeted induction of lung endothelial cell apoptosis causes emphysema-like changes in the mouse. *J Biol Chem*. 2008; 283:29447–29460. [PubMed: 18718906]
44. Kolonin MG, Saha PK, Chan L, Pasqualini R, Arap W. Reversal of obesity by targeted ablation of adipose tissue. *Nat Med*. 2004; 10:625–632. [PubMed: 15133506]
45. Arap W, Kolonin MG, Trepel M, Lahdenranta J, Cardó-Vila M, Giordano RJ, Mintz PJ, Ardelt PU, Yao VJ, Vidal CI, et al. Steps toward mapping the human vasculature by phage display. *Nat Med*. 2002; 8:121–127. [PubMed: 11821895]
46. Rajotte D, Arap W, Hagedorn M, Koivunen E, Pasqualini R, Ruoslahti E. Molecular heterogeneity of the vascular endothelium revealed by in vivo phage display. *J Clin Invest*. 1998; 102:430–437. [PubMed: 9664085]
47. Gray BP, Li S, Brown KC. From phage display to nanoparticle delivery: functionalizing liposomes with multivalent peptides improves targeting to a cancer biomarker. *Bioconjug Chem*. 2013; 24:85–96. [PubMed: 23186007]
48. Staquicini FI, Ozawa MG, Moya CA, Driessen WHP, Barbu EM, Nishimori H, Soghomonyan S, Flores LG 2nd, Liang X, Paolillo V, et al. Systemic combinatorial peptide selection yields a non-canonical iron-mimicry mechanism for targeting tumors in a mouse model of human glioblastoma. *J Clin Invest*. 2011; 121:161–173. [PubMed: 21183793]
49. Christofidou-Solomidou M, Kennel S, Scherpereel A, Wiewrodt R, Solomides CC, Pietra GG, Murciano JC, Shah SA, Ischiropoulos H, Albelda SM, et al. Vascular immunotargeting of glucose oxidase to the endothelial antigens induces distinct forms of oxidant acute lung injury. *Am J Pathol*. 2002; 160:1155–1169. [PubMed: 11891211]
50. Greineder CF, Howard MD, Carnemolla R, Cines DB, Muzykantov VR. Advanced drug delivery systems for antithrombotic agents. *Blood*. 2013; 122:1565–1575. [PubMed: 23798715]
51. Wang J, Lu Z, Wientjes MG, Au JL-S. Delivery of siRNA therapeutics: barriers and carriers. *AAPS J*. 2010; 12:492–503. [PubMed: 20544328]
52. Chacko AM, Hood ED, Zern BJ, Muzykantov VR. Targeted nanocarriers for imaging and therapy of vascular inflammation. *Curr Opin Colloid Interface Sci*. 2011; 16:215–227. [PubMed: 21709761]
53. Howard M, Zern BJ, Anselmo AC, Shuvaev VV, Mitragotri S, Muzykantov V. Vascular targeting of nanocarriers: perplexing aspects of the seemingly straightforward paradigm. *ACS Nano*. 2014; 8:4100–4132. [PubMed: 24787360]
54. Liu Y, Tan J, Thomas A, Ou-Yang D, Muzykantov VR. The shape of things to come: importance of design in nanotechnology for drug delivery. *Ther Deliv*. 2012; 3:181–194. [PubMed: 22834196]

55. Cutler CS, Chanda N, Shukla R, Sisay N, Cantorias M, Zambre A, McLaughlin M, Kelsey J, Upenandran A, Robertson D, et al. Nanoparticles and phage display selected peptides for imaging and therapy of cancer. *Recent Results Cancer Res.* 2013; 194:133–147. [PubMed: 22918758]
56. Valencia PM, Pridgen EM, Rhee M, Langer R, Farokhzad OC, Karnik R. Microfluidic platform for combinatorial synthesis and optimization of targeted nanoparticles for cancer therapy. *ACS Nano.* 2013; 7:10671–10680. [PubMed: 24215426]
57. Wang AZ, Gu F, Zhang L, Chan JM, Radovic-Moreno A, Shaikh MR, Farokhzad OC. Biofunctionalized targeted nanoparticles for therapeutic applications. *Expert Opin Biol Ther.* 2008; 8:1063–1070. [PubMed: 18613759]
58. Ferrari M. Cancer nanotechnology: opportunities and challenges. *Nat Rev Cancer.* 2005; 5:161–171. [PubMed: 15738981]
59. Godin B, Driessen WH, Proneth B, Lee SY, Srinivasan S, Rumbaut R, Arap W, Pasqualini R, Ferrari M, Decuzzi P. An integrated approach for the rational design of nanovectors for biomedical imaging and therapy. *Adv Genet.* 2010; 69:31–64. [PubMed: 20807601]
60. Akinc A, Zumbuehl A, Goldberg M, Leshchiner ES, Busini V, Hossain N, Bacallado SA, Nguyen DN, Fuller J, Alvarez R, et al. A combinatorial library of lipid-like materials for delivery of RNAi therapeutics. *Nat Biotechnol.* 2008; 26:561–569. [PubMed: 18438401]
61. Zimmermann TS, Lee ACH, Akinc A, Bramlage B, Bumcrot D, Fedoruk MN, Harborth J, Heyes JA, Jeffs LB, John M, et al. RNAi-mediated gene silencing in non-human primates. *Nature.* 2006; 441:111–114. [PubMed: 16565705]
62. Siegwart DJ, Whitehead KA, Nuhn L, Sahay G, Cheng H, Jiang S, Ma M, Lytton-Jean A, Vegas A, Fenton P, et al. Combinatorial synthesis of chemically diverse core-shell nanoparticles for intracellular delivery. *Proc Natl Acad Sci USA.* 2011; 108:12996–13001. [PubMed: 21784981]
63. Karagiannis ED, Urbanska AM, Sahay G, Pelet JM, Jhunjhunwala S, Langer R, Anderson DG. Rational design of a biomimetic cell penetrating peptide library. *ACS Nano.* 2013; 7:8616–8626. [PubMed: 24047542]
64. Dahlman JE, Barnes C, Khan OF, Thiriot A, Jhunjhunwala S, Shaw TE, Xing Y, Sager HB, Sahay G, Speciner L, et al. In vivo endothelial siRNA delivery using polymeric nanoparticles with low molecular weight. *Nat Nanotechnol.* 2014; 9:648–654. [PubMed: 24813696]
65. Polach KJ, Matar M, Rice J, Slobodkin G, Sparks J, Congo R, Rea-Ramsey A, McClure D, Brunhoeber E, Krampert M, et al. Delivery of siRNA to the mouse lung via a functionalized lipopolyamine. *Mol Ther.* 2012; 20:91–100. [PubMed: 21988874]
66. Xue W, Dahlman JE, Tammela T, Khan OF, Sood S, Dave A, Cai W, Chirino LM, Yang GR, Bronson R, et al. Small RNA combination therapy for lung cancer. *Proc Natl Acad Sci USA.* 2014; 111:3553–3561.

FIGURE 1.

Method of isolating the ‘membranome’ of pulmonary endothelial cells. The pulmonary vasculature is accessed by cannulating the right ventricle (top left) and then injecting a solution of polycationic silica nanoparticles (circles with ‘+’ symbols in the center). The positively charged nanoparticles bind to the negatively charged glycocalyx of the pulmonary endothelial cells (top right). The lungs are then minced and homogenized. The homogenate is then subjected to multiple rounds of centrifugation, after which the nanoparticles are pelleted, along with the endothelial membranes they are bound to (bottom left). Electron micrographs have confirmed the beads are associated with sheets of plasma membranes, studded with membrane-associated proteins (blue shapes, bottom right). The resulting protein mixture can then be cleared of lipid and nanoparticles, and subjected to gel separation, liquid chromatography, mass spectrometry, or directly used as antigens for antibody generation.

FIGURE 2.

Schematic of work flow for development of nanoparticles via chemical library techniques as employed by Anderson et al. Libraries of chemical components with targeted variation of properties (e.g., lipid-like molecules, cell-penetrating peptides, epoxy-containing polymers, and amines) (a) are combined in arrays under conditions conducive to nanoparticle formation (b). Nanoparticles are evaluated in physical and analytical techniques (e.g., gel-permeation chromatography, dynamic light scattering, and atomic force microscopy) to assess stability, size, and surface chemistry as a function of component properties. With

consideration for capacity to carry therapeutic agents, nanoparticles evaluated in the initial array are applied in analogous arrays *in vitro* via automated cell culture (c). For the case of siRNA delivery, two-color luciferase assays evaluated gene knockdown in arrayed assays 62. Nanoparticles selected via success in cell and biophysical assays are applied in animal models, where, in addition to therapeutic readout, image-based tracking (e.g., via bioluminescence, CT, and fluorescence) enables evaluation of *in vivo* performance and distribution (d).

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