Peptoids: Bio-Inspired Polymers as Potential Pharmaceuticals

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Abstract: Peptoids are a developing class of peptide-like oligomers originally invented for drug discovery in the early 1990s. While peptides hold great promise for therapeutic applications, current development of peptide-based pharmaceuticals is hindered by their potential for misfolding and aggregation, and particularly, for rapid *in vivo* degradation post-administration. Researchers have investigated alternative peptide-like constructs that may be able to circumvent such complications. Peptoids comprise a peptide-based backbone and *N*-substituted glycines for side chain residues, resulting in complete protease-resistance. Synthesis of peptoid sequences up to 50 units in length allows for controlled sequence composition and incorporation of diverse side chain chemistries. Though the landscape of peptoid structure is not clearly defined, secondary, tertiary, loop, turn, and random structures have been identified. As protease-resistant isomers of peptides, peptoids are being developed as versatile molecular tools in biochemistry and biophysics, and are becoming attractive candidates for therapeutic and diagnostic applications. Peptoids have thus far demonstrated bioactivity as protein mimics and as replacements for small molecule drugs. In this review, we discuss the most recent advances in peptoid research on the therapeutic front in the last few years, including *in vitro* and *in vivo* studies in the fields of lung surfactant therapy, antimicrobial agents, diagnostics, and cancer. We particularly focus on the biophysical activity of lipid-associated peptoids and their potential therapeutic applications.

Keywords: Peptoids, peptide, mimic, peptidomimetic, amphipathic, lung surfactant, antimicrobial peptides, host defense peptides.

INTRODUCTION

Since oligomers of *N*-substituted glycines, or peptoids, were first reported by Bartlett, Zuckermann, and co-workers in 1992 [1-2], researchers have explored the synthetic, conformational, and application-oriented aspects of the "peptoid landscape" with significant success. Peptoids are discrete oligomers with a peptidic backbone, where the side chains are appended to the backbone amide nitrogen rather than the α-carbon. They are essentially peptide isomers, but the seemingly simple shifting of the side chains to the nitrogen significantly changes the properties of the isomer peptoid relative to the peptide. The most biologically relevant consequence of this shift is protease insusceptibility, which arguably increases their therapeutic applicability as pharmaceuticals relative to peptides. Furthermore, a straightforward solid-phase synthesis of peptoids was developed using peptide synthesis equipment, making them amenable to library synthesis and a wide range of side chain functionalities. As a result, peptoids have since become attractive therapeutic candidates in biochemistry, molecular biophysics, diagnostics, and medicine.

Peptoids may be able to replace or supplement a protein or peptide required for a particular biological application. Peptides and proteins are very promising as therapeutics and account for 2% of marketed drugs and 50% of drugs in the pipeline for approval. However, these compounds have failed to deliver as initially expected due to multiple complications. Firstly, peptides and proteins are costly to chemically synthesize and purify in sufficient quantity for systemic or localized treatments; and while recombinant protein expression is an efficient and inexpensive method for synthesizing proteins, it remains a poor alternative for complex protein sequences or proteins with specific post-translationally ally modified functionalities. Extremely hydrophobic or structurally unstable peptides and proteins tend to easily aggregate or misfold, thus reducing the shelf life, bioavailability, or biostability to become manufactured drugs. The latter complication of biostability is particularly cumbersome, as almost all peptides are rapidly degraded *in vivo* by proteases upon administration. This leads to manufacturing problems with delivery, dosage, efficacy, and formulation. The last major concern for peptide-based pharmaceuticals is potential for toxicity and immune response, as the body is likely to view administered peptides and proteins as foreign invaders (this possibility exists for peptoids as well).

Peptoids can match or exceed peptide capabilities on these fronts, but sequence design is a crucial factor for success. Peptoids are completely sequence-specific and, due to a lack of backbone hydrogen bonding, can easily be engineered for minimum aggregation, appropriate solubility, yet high hydrophobicity. Peptoids are structurally stable even in the presence of denaturants [3], and as mentioned, are protease-invulnerable [4]. Even though peptoids should be viewed as invaders by the body, a recent study by Kodadek and co-workers showed that, when injected into the mouse, peptoids did not produce an anti-peptoid immune response [5]. In addition, the extent of immune response can be lowered through sequence design by including non-amino-acid-based side chains [6]. Finally, combination peptoid-peptide hybrids can also be synthesized to modulate the properties of peptide therapeutics.

It would be unreasonable to say that peptoids do not have any disadvantages. Peptoids lack backbone hydrogen bonding due to the absence of backbone hydrogen bond donors, and hence, cannot form β-sheets. However, a lack of β-sheet formation is in a way advantageous because the resulting peptoids do not aggregate as easily as peptides. To develop peptoids as therapeutics, it is important to understand their fates in the body, such as: (1) how fast are the peptoids eliminated from the body? (2) where do they concentrate or localize, if anywhere? (3) are peptoids xenobiotics? and (4) do peptoids produce hepatotoxicity? Unfortunately, not much has been published to address these concerns, and such studies are critical for the development of peptoids as potential pharmaceuticals.

In the realm of protein-protein interactions, peptoids could play a major role in replacing traditional small molecules that align with Lipinski's rules but are too small to sufficiently cover the surface area of the targeted protein binding site. Protein-protein interactions are integral for normal biological functioning, and protein complexes have become therapeutic targets in virus inhibition, cancer, cellular signaling, gene expression activation, and other types of disease-related processes [7]. Investigators have created peptides

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that emulate the sequence and structure of a native binding partner of the complex, but undesirable pharmokinetic side effects (formation of aggregates, proteolysis, and immunogenicity) can occur. Peptoids could eventually be designed to replicate or assist in the peptide-based activities that contribute to protein-protein interactions without these undesirable side effects. Peptide-based cell permeation as well as the disruption, modification, activation, or inhibition of protein targets are all possibilities to explore with peptoids [7]. Peptoids are also quite amenable to the creation of combinatorial libraries and high-throughput screening, allowing for quick identification of binding among hundreds of thousands of peptoid candidates [7]. Multivalent peptoid ligands containing diverse functionalities can also be synthesized to enhance binding to the desired protein target [8].

There is also growing interest in using peptoids as diagnostic agents and for use in the medical device industry. Peptoids have already been used to improve DNA sequencing separation by increasing friction [9, 10] and as anti-fouling polymers to coat capillaries, medical devices, and medical implants [11]. Peptoids are also in the initial stages of development as multivalent scaffolds for MRI contrast agents [12]. Therefore, peptoids have great potential to fill the void between the number of existing small molecule drugs and the countless number of biological targets and possible diagnostic applications.

Several reviews of peptoids have emerged in the past few years, and in light of this, our review will cover only the most recent peptoid research (~ 2008-2010) in the areas of *in vivo* or *in vitro* therapeutic progress. Peptoid structure and function was reviewed in 2008 by Yoo and Kirshenbaum [13], and peptoid structure-activity relationships for biological applications were summarized in 2009 by Fowler and Blackwell [8]. A comprehensive history of peptoid development and one-bead one-compound screening was also presented in 2009 by Zuckermann and Kodadek [7], and in 2010, a synopsis of peptoid-peptide hybrid compounds was provided by Olsen [14]. A very recent review by Culf and Ouellette extensively details peptoid synthesis and side chain structure, and lists current applications and instruments amenable to peptoids [15]. In this review, we hone in on peptoid-based mimics of lipid-associated, bioactive peptides, such as lung surfactant proteins and antimicrobial peptides, with discussion of the most recent *in vitro* and *in vivo* work and potential applications. A smaller portion of the review covers recent developments in the areas of peptoids as diagnostic agents, anti-cancer agents, and inhibitors.

PEPTOIDS

An attractive reason to select a peptoid-based design scaffold for therapeutic applications is the fairly straightforward synthesis of peptoids, which of course depends on both sequence and structural design. Peptoids are readily synthesized in high yield up to 50 residues in length on solid-phase support, usually Rink amide resin, using the submonomer approach developed by Zuckermann *et al.* (Fig. **1**) [1]. Synthesis proceeds from the *C*- to the *N*-terminus in an iterative building block-type synthesis method that differs from solid-phase peptide synthesis; however, peptoid synthesis can and is usually completed on an automated peptide synthesizer. A two-step process is repeated to desired chain length: initially, a haloacetic acid, usually bromo- or chloro-, is acylated using a diimide such as *N*,*N*-diisopropylcarbodiimide (DIC), followed by an S_N2 displacement of the halogen by a primary amine. Therefore, virtually any soluble, sterically favorable, and nucleophilic primary amine can easily be incorporated into the peptoid sequence as an *N*-substituted side chain.

Synthesized peptoid sequences are typically cleaved from the Rink amide linker of the resin using trifluoroacetic acid (TFA) with appropriate scavenging agents, leaving the peptoid with a carboxyamide terminus, and then purified by reversed-phase high performance liquid chromatography (RP-HPLC). Though peptoids may also be synthesized using microwave-assisted chemistry, α-peptide synthesis methods, or in solution-phase, the aforementioned method has the most widespread use.

Peptoid side chain and sequence nomenclature typically includes the "*N*" designation followed by an abbreviation of the chemical name, including stereochemistry, or if sufficiently similar, the closest amino acid name. Hence, *N*-(*S*)-(1-phenylethyl)glycine is *N*spe, and *N*-(4-aminobutyl)glycine is *N*Lys (for side chains, see Fig. (**2**)). Most amines can either be commercially purchased at low cost or feasibly synthesized in large-scale (hundreds of mg's) quantities. As in peptide synthesis, charged or labile side chains must have protecting groups during synthesis. Also similar to peptides, peptoid sequences are listed in X-Y-Z order, *e.g.*, (*N*Lys-*N*spe-*N*spe)$_4$.

As mentioned previously, the *N*-substitution of peptoid side chains renders the sequence backbone achiral and hydrogen bond donor deficient, lending conformational flexibility and insusceptibility to protease-induced degradation of the sequences. These features combined also result in a structure that is more biostable and less prone to irreversible aggregation than that of peptides. Furthermore, if chiral, bulky side chains are incorporated into the sequence, peptoids can adopt very stable helices with intrinsic handedness through steric and/or electronic repulsions of adjacent residues [16-19]. Circular dichroism (CD) spectroscopy is the technique of choice for gross assessment of peptoid helical intensities and structure. The helical structure of peptoids differs from the α-helices traditionally observed in peptides, where peptoids form polyproline type I-like helices with ~ 3 residues per turn and a ~ 6-6.7 Å pitch (a typical peptoid helix is depicted in Fig. (**3**)) [16, 18, 19]. The latter provides an excellent repeating scaffold of "three faces" for designing amphipathic or otherwise "facially segregated" protein mimics (Fig. (**3**)). This review will largely focus on achiral, chiral aliphatic, and chiral aromatic peptoid helices; for extensive discussion of peptoid structural motifs, please see recent reviews [8, 13].

PEPTOID-BASED, LIPID-ASSOCIATED PROTEIN AND PEPTIDE MIMICS

In many biological systems, a well-connected relationship exists between form and function. Research efforts often intensely

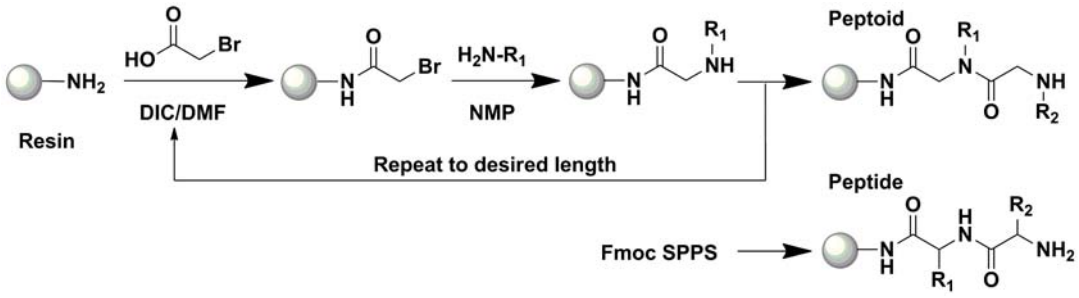

Fig. (1). Peptoid synthesis scheme and chemical structures of an α-peptide and an α-peptoid.

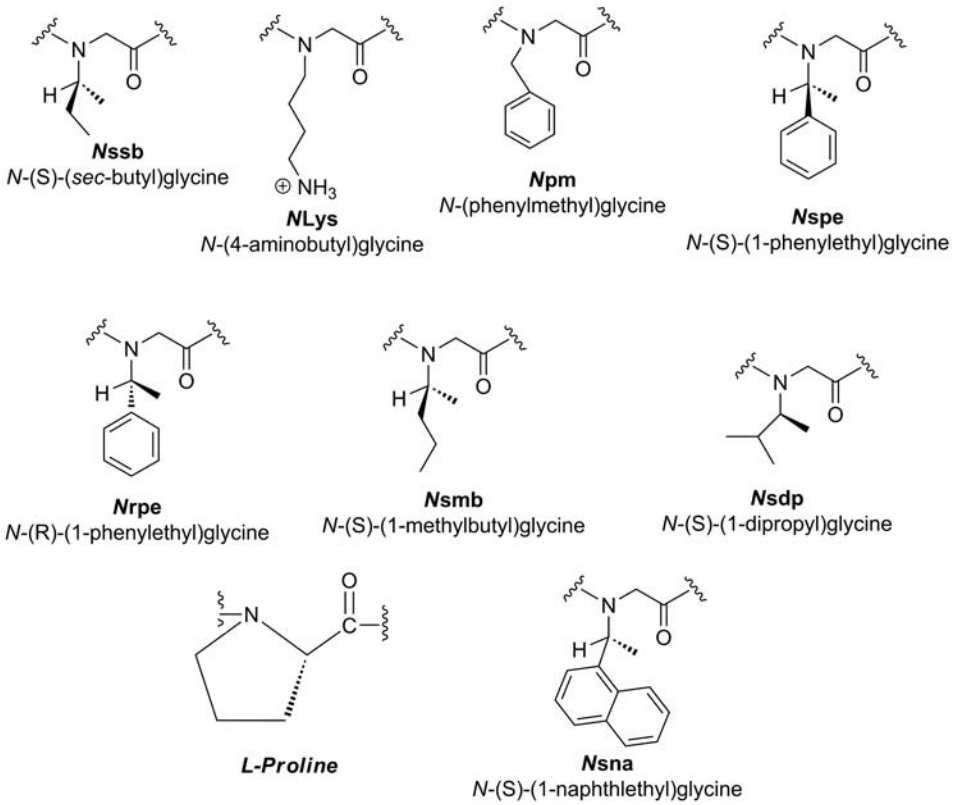

Fig. (2). Peptoid side chain chemical structures.

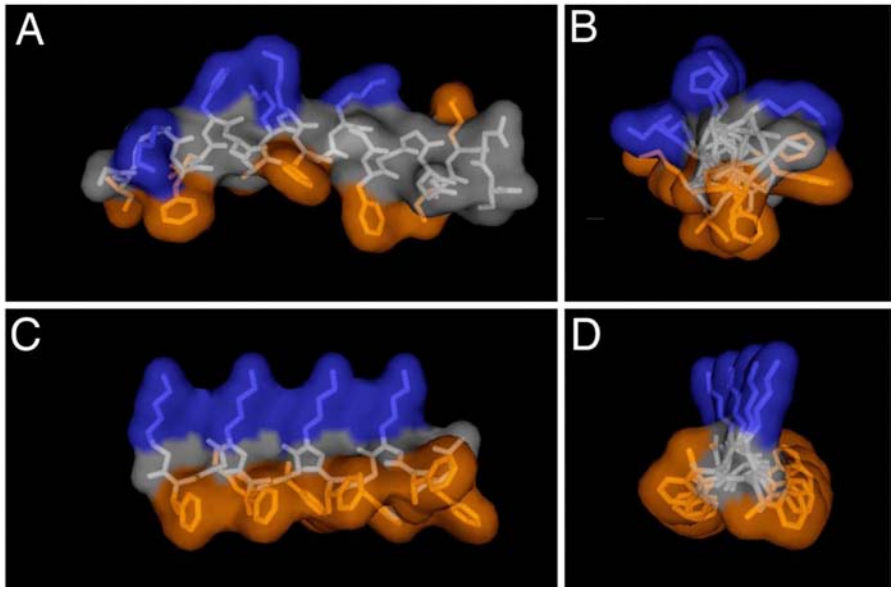

Fig. (3). Similarity between the model structures of peptoid **1** with NMR structure of magainin-2 in a DPPC micelle. (**A** and **C**) are parallel and (**B** and **D**) are perpendicular structures to the helical axis of magainin and peptoid **1**, respectively.

focus on structure-activity relationships of proteins and peptides, and by extrapolation, it can be hypothesized that an appropriately designed molecule that mimics the structure of a native protein will inevitably mimic its function [20-22]. To avoid the "protein folding" problem frequently encountered with designing peptide-based sequences, simpler, non-natural scaffolds offer an attractive alternative for designing novel therapeutic agents. These "foldamers," as they are often termed [23], are typically oligomeric or polymeric sequences that are able to adopt simple structural elements of proteins, such as basic secondary structure; and due to their non-natural origin, often possess improved bioavailability and biostability relative to their natural counterparts [6]. In addition, the parameters of the scaffold template can often be precisely controlled, which is a great advantage when pursuing structure-activity relationship investigations. Moreover, the mechanistic insight gained in these parallel studies of non-natural mimics may also apply to the natural system, resulting in a general increase in the applicable knowledge of both peptidomimetic and peptide- or protein-based systems.

Lipids are integral components of countless biological structures and processes, and consequently, protein-lipid interactions

constitute a large area of biochemistry and biophysics research. The driving forces behind lipid-protein interactions, namely charge and hydrophobicity resulting from intrinsic amphipathicity, contribute significantly to the membrane structure and function that protect and sustain the cells, vesicles, and liposomes that exist in the body. Unfortunately, many endogenously lipid-associated proteins and membrane-active peptides contain structural features that are difficult or impractical to replicate through chemical peptide synthesis or recombinant protein expression. As stated, the research and clinical development of peptide- and protein-based pharmaceuticals have been somewhat hindered by the body's innate ability to protect itself, as most of these molecules are rapidly degraded by proteases *in vivo*, and suffer significant complications from low bioavailability and biostability, leading to increased costs with diminished efficacy.

But, are the attributes of proteins and peptides needed for the body's biological processes easily replicated or "mimicked" by peptoids? Proteins and peptides often have sequences of amino acids that are evolutionarily conserved; this primary sequence also determines their secondary and (if present) tertiary structures. An ingenious avenue of research that circumvents these problems has been to create non-natural variants of peptides and proteins that mimic identifiable structural attributes, with the anticipation that form will inevitably lead to function. Two specific classes of molecules particularly amenable to biomimicry by non-natural mimics (peptoids) are lung surfactant proteins and antimicrobial peptides. Both classes have specific, lipid-associated biophysical activities that remain integral to their functions, and are thus complementary to each other in certain respects – they both contain helical, linear, cationic, and amphipathic sequences that can be difficult to obtain in properly folded form through biological or chemical synthesis.

In this section, a brief background is provided for both the lung surfactant and antimicrobial peptide biological systems, followed by a history of peptide-based mimicry in the two fields, and finally, advancements made by peptoids in the field. Proof-of-concept therapeutic *in vivo* studies are summarized, and additional potential therapeutic applications are suggested.

LUNG SURFACTANT PROTEIN MIMICS

Lung Surfactant

Pulmonary surfactant, or lung surfactant, is a lipid-protein mixture synthesized by the alveolar epithelium and secreted by alveolar type II cells. The surfactant coats the interior surface of air-breathing vertebrate lungs as a film, thereby minimizing the work in maintaining the largest surface area of the human body exposed to the environment, the lung, open to air [24-29]. Lung surfactant is also responsible for providing a primary innate pathogen-resistant barrier to protect the body against infection [28]. The molecular mechanisms through which lung surfactant enacts its biophysical and defensive activities have been extensively studied in past decades, and continue to be under active investigation [28, 30].

Lung surfactant comprises ~ 90% lipids and ~ 10% surfactant proteins, with ~ 80% of the lipid mass constituting phospholipids, highly surface-active, amphiphilic molecules that render the interfacial film capable of surface tension (expressed as γ in milliNewtons per meter, or mN m^{-1}) reduction and regulation at the air-liquid interface [28, 31]. In particular, the alveolar air-liquid interface must reach near-zero surface tension at end-expiration (exhalation) in order to prevent atelectasis (lack of gas exchange) and collapse of the alveolar network. However, without the evolutionarily conserved surfactant proteins B and C (SP-B and SP-C), the phospholipids exhibit poor adsorptive and re-spreading characteristics [32-33]. In fact, a lack of these proteins at birth causes respiratory failure [26]. These two hydrophobic proteins are endogenously lipid-associated and responsible for sustaining the efficacy of the film, promoting (i) rapid adsorption to the air/liquid interface (to ~ 23 mN/m in < 1 min), (ii) attainment of near-zero γ at end-expiration (<1 mN/m), and (iii) excellent re-spreading of material at the interface throughout the continuous respiratory cycles of surface area expansion and compression (maximum γ of <35 mN/m) [27, 34]. The other two surfactant proteins A and D assemble large macromolecular structures that bind to pathogens and aid in clearance of the respiratory airways [28].

Dysfunction, deactivation, or deficiency of lung surfactant in the immature lungs of premature infants results in infant respiratory distress syndrome (IRDS) [35]. The introduction of exogenous surfactant replacement therapy administration to infants until they are capable of synthesizing lung surfactant has significantly reduced neonatal mortality and morbidity [36]. Current surfactant preparations are routinely obtained from animal-derived, usually porcine or bovine, extracts that constitute the hydrophobic fraction of surfactant with variable amounts of surfactant proteins and different phospholipids [36]. However, animal-derived therapies have several drawbacks associated with them, including potential for pathogenic trans-species transmission, expensive isolation, purification, and production costs, and batch-to-batch variability [37]. In addition, lung inflammation, trauma, infection, toxicity, or other damage that causes lung surfactant dysfunction often results in another ailment, acute respiratory distress syndrome (ARDS), also called acute lung injury (ALI) [38-39]. Although several trials have been undertaken to potentially treat ARDS/ALI with exogenous surfactant replacement therapy, success has been limited and only in the adult population (both children and adults can suffer from ARDS), largely due to the heterogeneous nature of disease onset and progression [40-42]. Specifically, improvements in oxygenation were reported without an improvement in overall mortality.

It can therefore be surmised that an unmet clinical need exists for a readily available surfactant formulation that could be produced in large enough quantities to treat both infantile and adult syndromes [37]. Therefore, a partial objective of lung surfactant research is to develop new synthetic exogenous surfactant therapies capable of treating both IRDS and ARDS, hopefully with the gained advantages of biosafety, bioavailability, and biostability [43-44]. From current research, it has become clear that the key components to a successful and efficacious formulation are inclusion of SP-B and SP-C [45]. Unfortunately, a major caveat to developing functional synthetic preparations is the limited availability of SP-B and SP-C, which has spurred research efforts into the development of biomimics of surfactant proteins [37].

Lung Surfactant Proteins and Their Peptide-Based Mimics

SP-C is a 35-residue, extremely hydrophobic protein with a *C*-terminal helical region that matches the length of a 1,2-diacyl-sn-glycero-3-phosphocholine (DPPC) lipid bilayer [46-47]. At the *N*-terminus, two adjacent cationic residues at positions 11 and 12 facilitate Coulombic interactions with anionic lipid head groups [48]; meanwhile, the Val-rich helix and palmitoylation points 5 and 6 securely anchor SP-C into the lipid layer [49-50]. SP-C contributes to interfacial film stability and viscosity regulation [51], keeping lipid bilayers and multilayers "attached" to the interfacial layer and creating a surfactant reservoir. SP-C therefore enhances the adsorptive properties and reduces the minimum surface tension reached at end-expiration [52-53]. Palmitoylated SP-C is known to facilitate multilayer formation over the de-palmitoylated version [52]. Synthesized [54-56] and recombinant protein [57-58] SP-C mimics have emulated the hydrophobic helical region and palmitoylated moieties with limited success (for a recent review, see [20]). Venticute, a surfactant formulation containing recombinant SP-C (rSP-C) was recently used in a clinical trial for ARDS with mixed outcomes, including improved gas exchange, but did not contribute to long-term survival [59].

Monomeric SP-B is a helical, cationic, and amphipathic 79-residue protein (8.7 kDa), containing three intramolecular disulfide bonds and one intermolecular disulfide bond that promotes ho-

modimerization *in vivo* [47, 60-62]. SP-B comprises ten cationic residues (net charge +9) but has an unresolved structure believed to contain four or five amphipathic helices and a 45% overall helical content [63]. SP-B is hypothesized to act in a hinge-like manner at the air-liquid interface, bridging together sub-monolayer lipid structures and the interfacial lipid monolayer. The cationic and amphipathic nature of the protein is also believed to promote lipid transport and organization by transiently inserting into lipid layers through Coulombic and hydrophobic interactions, further facilitating folding and re-spreading of interfacial material.

Peptide-based biomimics of SP-B are usually designed to emulate the surface-active, amphipathic, and helical *N*-terminus of the protein, SP-B_{1-25}, by either replicating or simplifying the side chain chemistry of this segment [64-70]. SP-B's more complex structure has been mimicked with dimerized versions of two amphipathic helices, such as dSP-B_{1-25} (single-disulfide-bonding of two SP-B_{1-25} helices) and "Mini-B" (dimerized *N*-/*C*-terminal construct of SP-B_{1-25} and SP-B_{53-78}) [71-72]. Though somewhat efficacious, these mimics fail to reproduce the full surface activity of SP-B, or are otherwise difficult to synthesize on a larger, therapeutically relevant scale. Another peptide-based mimic that adopts simple SP-B-like patterning, $(Lys\text{-}Leu\text{-}Leu\text{-}Leu)_4$-Lys or KL_4 [69, 73-74], is surface-active and has been clinically used in lucinactant, otherwise known as Surfaxin [75-77]. Recently, this formulation was FDA-approved for the treatment of cystic fibrosis (CF).

Therefore, although peptide-based mimics have been developed for both proteins, the dimerized, somewhat constrained structure of SP-B and the extremely hydrophobic sequence of SP-C are not easily replaced by chemically synthesized peptide sequences using the same amino acids. This is largely due to the difficulty in obtaining SP-B and SP-C inspired peptide-based stable structures that do not misfold or aggregate. Peptoid-based sequences would quickly alleviate the problems of misfolding and aggregation while imparting protease stability. Here we discuss *in vitro* results of peptoid-based mimics of SP-B and SP-C, followed by results of a pilot *in vivo* therapeutic study.

Peptoid-Based SP-C Mimics

The development of peptoid-based SP-C mimics up until 2008 were reviewed by Brown, Johansson, and Barron in 2008 [20], and also by Fowler and Blackwell in 2009 [8]. Briefly, Barron and co-workers optimized the length and side chain chemistry of peptoid-based, helical, and non-palmitoylated SP-C mimics spiked into an *in vitro* mixed "Tanaka" lipid film DPPC: 1-palmitoyl-2-oleoyl-sn-glycero-3-[phospho-rac-(1-glycerol)] (POPG): palmitic acid (PA) 68:22:9 [wt] (DPPC:POPG:PA) [78], discovering that a 14-residue aromatic-rich (*N*spe-containing, Fig. (**2**)) helical region provided the necessary length and side chain rigidity to more closely emulate the surface activity of the native SP-C sequence [79-80]. Most recently, Barron and Bernardino de la Sema investigated the *in vitro* biophysical effects of introducing palmitoyl-like functionalities to the aromatic peptoid sequence by alkylating the *N*-terminus with a C18 hydrocarbon chain (octadecylamine, *N*ocd) (Fig. (**4**)) [81]. Results in the lipid film show that alkylation improves film compressibility and reduces the maximum surface tension reached during dynamic surface tension measurements obtained with pulsating bubble surfactometry [82]. While maintaining a minimum surface

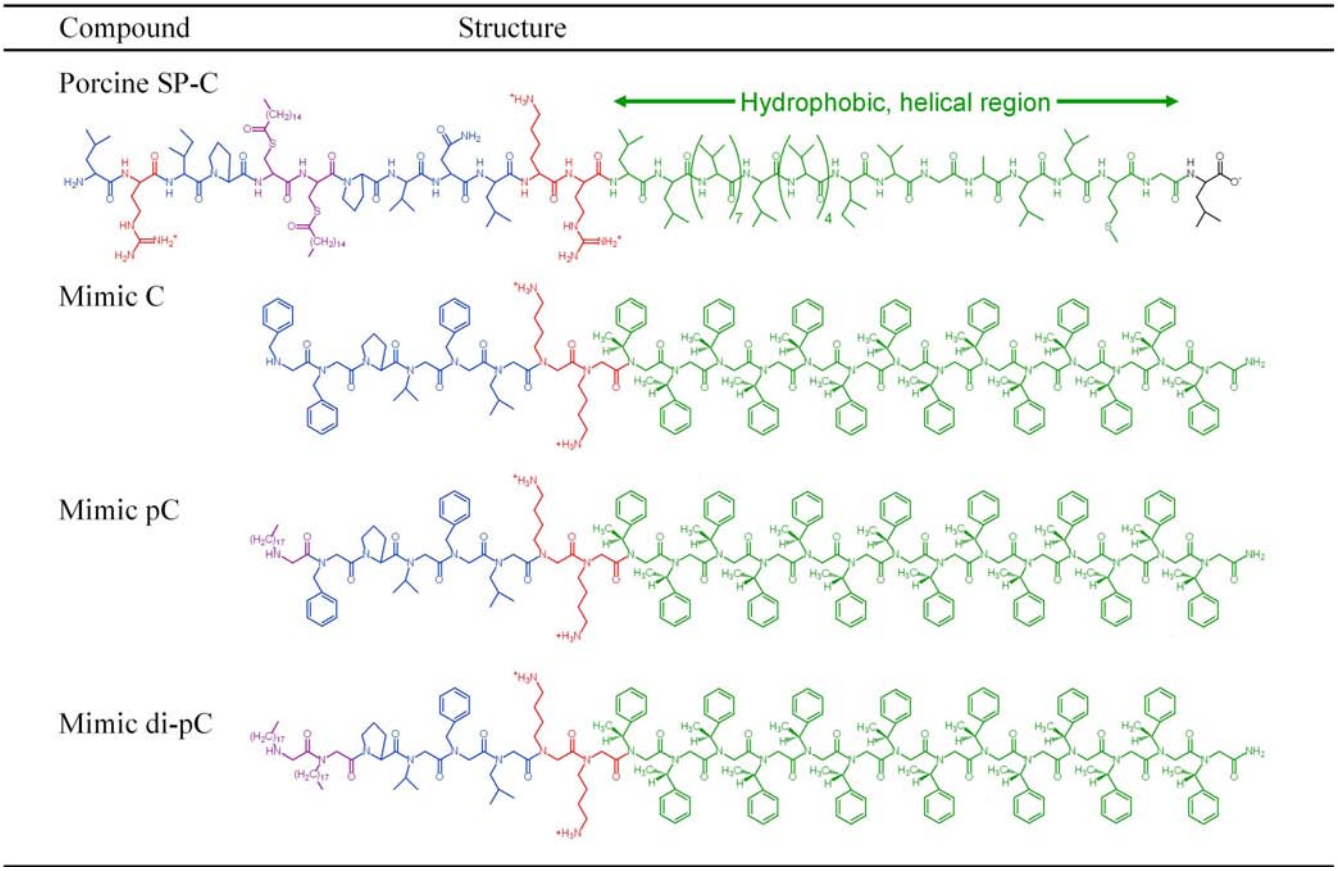

Fig. (4). SP-C and peptoid-based mimic sequences. Peptoids exhibit an aromatic-rich (Phe-like Nspe) helical region with an achiral N-terminus including two cationic Lys-like residues (NLys). Mimics pC and di-pC are N-terminally alkylated with C18-long octadecylamine (Nocd), which significantly improves *in vitro* surface activity. Peptoid pC in particular was found to be efficacious in treating lung lavage-induced acute lung injury (ALI) in an *in vivo* rat model. Adapted from ref [81].

tension near-zero, the di-alkylated mimic di-pC reached a maximum surface tension of 45 mN/m, much lower than the 64 mN/m obtained with the lipid-only formulation, and approaching that of the 36 mN/m obtained by porcine-derived SP-C [81].

In addition, atomic force microscopy (AFM) imaging confirmed that only the alkylated peptoids assist in a two- to three-dimensional structural transition in the fluid phase of the monolayer at high surface pressure (low surface tension) that maintains two segregated interfacial phases and seemingly promotes the formation of a sub-monolayer surfactant reservoir [81]. The increased ability of the peptoids to mimic SP-C, the relative ease of incorporating primary amine alkyl chains into the peptoid sequence, and the inability of these peptoids to aggregate or misfold all suggest that these peptoids have significant potential to act as therapeutic alternatives to SP-C.

Peptoid-Based SP-B Mimics

Like SP-C mimics, SP-B mimics have undergone several generations of development to optimize the sequence and structure, with the hope of thereby improving surface activity. Fowler and Blackwell reviewed the development of peptoid-based SP-B mimics until 2006 [8]. Briefly, in a similar vein to findings with SP-C mimics, aromatic-rich (*N*spe) and *N*Lys-containing peptoid-based SP-B mimics, such as B1 (Table **1**) were also found to improve surface activity moreso than aliphatic-rich (*N*ssb, Fig. (**2**)) SP-B mimics in a lipid film [70]. In a three-fold helical repeat, the design of these single-helix mimics are inspired by the sequences of SP-$B_{1\text{-}25}$ (amphipathic and cationic helix) and KL_4 (only two distinct amino acids), and so are facially amphipathic, with two *N*spe-containing faces and one entirely cationic face (Table **1**). In a subsequent study, it was discovered that including an eight-residue, helical, and aromatic-rich *N*-terminal insertion region (B_{ins}), meant to mimic the *N*-terminus of SP-$B_{1\text{-}25}$, substantially increased surfactant activity in the mixed lipid film (Table **1**) [83]. Though Langmuir-Wilhelmy surface balance, fluorescence microscopy, and pulsating bubble surfactometry results all suggested an increase in activity for the peptoids, the trend in decreasing adsorptive (γ_{ads}) and maximum (γ_{max}) surface tensions presented in Table **1** is a clear indicator of improving activity for these peptoids.

At this point, it was estimated that the developed peptide- or peptoid-based single-helix mimics of SP-B were probably unable to replicate all functional aspects of the native dimerized SP-B. On the basis of prior studies involving dimerization of peptide-based SP-B mimics [71-72] that afforded significant surface activity improvements relative to the monomers, the Barron and Zuckermann labs collaborated to illustrate the effects on *in vitro* surface activity of introducing a *C*-terminal achiral (*N*meg, *N*-(methoxyethyl)glycine) linker region to the basic peptoid-based SP-B "B1" sequence, followed by disulfide- or 'click'-mediated dimerization of two linear, cationic, and amphipathic helices (Table **1**) [84]. While disulfide-mediated dimerization led to little improvement in activity with the mixed lipid film (unlike peptide-based mimics), 'click'-mediated dimerization resulted in substantial reductions in interfacial surface tension and unique lipid phase morphologies relative to other mimics, including SP-$B_{1\text{-}25}$ and KL_4 (dB2c, Table **1**). It was hypothesized that the rigidity and polarity of the triazole moiety yielded conformational differences that increased favorable lipid-peptoid interactions in the film [84].

In practice, synthesizing large quantities of dimerized or 'clicked' peptides or peptoids for therapeutic use is impractical and costly for a lung surfactant therapy. To circumvent this complication, peptoid-based SP-C and SP-B mimics were "hybridized" by introducing SP-C-like *N*-terminus alkylation with *N*ocd to the insertion region of the amphipathic helix mimicking SP-B (C18-B_{ins} and diC18-B_{ins}, Table **1**) [85]. This small modification significantly increased lipid-peptoid interactions, likely by improving peptoid insertion into the lipid film, and resulted in *in vitro* surface activities that approached those of porcine-derived SP-B in the same lipid film (Table **1**). Employing the same AFM imaging technique used for SP-C mimics, it was shown that only alkylated SP-B mimics

Table 1. Pulsating Bubble Surfactometry (PBS) Static-Bubble Adsorption Data and Dynamic-Bubble mode Cycling Data for Lipid-Additive Films at 37 °C.

Name	Sequence (amino to carboxy)	γ_{eq}* (mN/m)	γ_{max}† (mN/m)	γ_{min}‡ (mN/m)	% Comp§
Lipids[84]	DPPC:POPG:PA (68:22:9 [wt])	54.5 ± 1.0	63.4 ± 0.7	10.1 ± 1.6	43.5 ± 1.1
B1[84]	*N*spe$_2$-(*N*Lys-*N*spe$_2$)$_5$	40.5 ± 5.0	53.5 ± 1.5	<1	36.0 ± 1.9
dB2c[84]	[*N*spe$_2$-(*N*Lys-*N*spe$_2$)$_5$-*N*meg$_4$-*N*'click']$_2$	22.1 ± 0.5	41.9 ± 2.2	<1	21.2 ± 3.8
B_{ins}[85]	*N*spe$_8$-*N*Lys-*N*spe$_2$-*N*Lys$_2$-*N*spe$_4$-*N*Lys-*N*spe$_2$	23.2 ± 0.9	46.1 ± 1.0	<1	26.4 ± 3.6
C18-B_{ins}[85]	*N*ocd-*N*spe$_7$-*N*Lys-*N*spe$_2$-*N*Lys$_2$-*N*spe$_4$-*N*Lys-*N*spe$_2$	25.3 ± 0.9	39.7 ± 2.8	<1	16.6 ± 3.5
diC18-B_{ins}[85]	*N*ocd$_2$-*N*spe$_6$-*N*Lys-*N*spe$_2$-*N*Lys$_2$-*N*spe$_4$-*N*Lys-*N*spe$_2$	25.6 ± 1.4	35.2 ± 2.0	<1	12.2 ± 3.8
B_{trunc}[87]	*N*spe$_2$-*N*Lys-*N*spe$_2$-*N*Lys$_2$-*N*spe$_4$-*N*Lys-*N*spe$_2$	41.8 ± 0.5	54.9 ± 1.2	1.7 ± 2.9	35.5 ± 4.2
$B_{ins\text{-}ach}$[87]	*N*pm$_8$-*N*Lys-*N*pm$_2$-*N*Lys$_2$-*N*pm$_4$-*N*Lys-*N*pm$_2$	26.8 ± 5.9	48.1 ± 0.9	<1	33.1 ± 1.8
Porcine SP-B[85]	Extracted protein (ref)	24.4 ± 1.1	35.8 ± 1.3	<1	21.0 ± 2.6
SP-$B_{1\text{-}25\ (C8,11\text{->}A)}$[84]	FPIPLPYAWLARALIKRIQAMIPKG	35.6 ± 1.3	49.9 ± 0.4	<1	33.2 ± 2.7
KL_4[84]	KLLLLKLLLLKLLLLKLLLLK	21.6 ± 0.8	48.7 ± 0.8	<1	25.0 ± 3.7

Dried lipid-peptoid films (lipids DPPC:POPG:PA 68:22:9 [wt] at ~ 1 mg/mL, additive at 2 mol%) were reconstituted in aqueous buffer (150 mM NaCl, 10 mM HEPES, 5 mM $CaCl_2$, pH 6.9) and allowed to adsorb to the air-liquid interface of a bubble for 20 min, and then pulsed at 20 cycles per minute (cpm) for 10 min. Data are average surface tension measurements (γ, mN/m) of a minimum of three replicates, and standard deviation of the mean (SD) is also reported. No SD values are available for "< 1" table entries. *Equilibrium adsorptive surface tension after 20 min. †Maximum and ‡minimum surface tensions reached during pulsation. §Percent bubble surface area compression to reach < 20 mN m^{-1}, where reduced compressibility is highly desired. *N*ocd is the octadecylamine side chain. Mimic C18-B_{ins} is also known as "pB," a peptoid that showed improvement over lipids in an *in vivo* lavage-induced rat model of acute lung injury (ALI). Data obtained from refs [70, 83-87].

sustained two segregated interfacial phases at high surface pressure, and indicated the formation of "nanosilos," ~ 5-8 nm in height pockets of attached yet excluded material that likely enhance re-spreading upon surface area expansion [85]. A hypothesized mode of lipid-peptoid surfactant interactions is presented in Fig. (**5**).

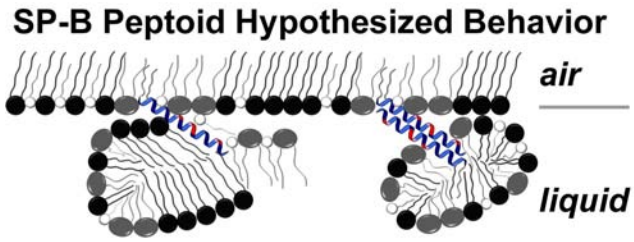

Fig. (5). A representation of the hypothesized mode of lipid-peptoid interactions for the di-alkylated peptoid-based SP-B mimic di-C18 Bins. In the cartoon, the DPPC lipid (zwitterionic) is represented by black circles, the POPG lipid (anionic) is represented by gray circles, and the lipid PA (anionic) is represented by white circles in the TL lipid mixture DPPC:POPG:PA 68:22:9 [wt]. The *N*-terminally diC18-alkylated (*N*ocd) *N*spe-containing 'insertion region' of the peptoid helix is thought to transiently insert into the air/liquid interfacial lipid monolayer, where the hydrocarbon alkyl chains would hydrophobically interact with the lipid acyl chains, and the amphipathic helix (red indicates cationic *N*Lys-containing regions) would associate with the anionic lipid headgroups. According to AFM images obtained for this system at high surface pressure (60 mN/m), it appears that peptoids sustain two separated interfacial regions that differentiate by their lipid packing and components. In addition, peptoids assist in the formation of sub-monolayer lipid structures, called "nanosilos" that are likely composed of excluded pockets of lipid-peptoid material. The alkylated peptoids therefore aid in the retaining and re-spreading of material at the air-liquid interface for the next expansion cycle. Adapted from ref [85].

Interested in further simplifying the peptoid-based SP-B mimic sequences, scientists began to investigate the necessity for a discrete sequence, as well as chirality, and hence, helicity, in non- and octadecanoylated (C18) sequence- and stereo-random nylon-3 β-polymer SP-B mimics [86]. These polymers demonstrated excellent *in vitro* surface activities, suggesting that a free-solution and chiral helical structure may not be necessary for lipid-associated activity, and that the *ability* to adopt an amphipathic albeit irregular structure could be sufficient for surface activity. This hypothesis is supported by recent work that returned to peptoid-based mimics to explore the *in vitro* effects on surface activity of cationic amphipathic patterning, the presence of an insertion region (B_{trunc}, Table **1**), and the presence of chirality ($B_{ins\text{-}ach}$, Table **1**), or *N*spe vs. *N*pm (Fig. (**2**)), in peptoid-based mimics of SP-B [87]. The results indicated that (i) an *N*-terminal insertion most positively affected surface-active behavior, (ii) an increased number of cationic residues improved adsorption, (iii) decreasing the amphipathic patterning in *chiral* and helical peptoids inhibited adsorption and increased the minimum dynamic surface tension, and (iv) removal of side chain chirality in insertion-region containing mimics had a minimal effect on surface activity (Table **1**).

In Vivo Efficacy of Peptoid-Enhanced Lung Surfactants

Once extensive *in vitro* work on peptoid-based mimics of SP-B and SP-C had been completed, a limited *in vivo* study was undertaken by the Barron, Veldhuizen, and Lewis labs to determine efficacy of peptoid-enhanced surfactants in a simple rat model of lung lavage-induced ARDS/ALI [88]. In brief, respiratory distress was induced in rats by repetitive lung lavage, after which a 50mg/kg dose was intratracheally administered as a surfactant bolus. Mono-alkylated SP-B and SP-C mimics "pB" (C18-B_{ins}) and "pC" were tested, individually and in combination, at 2 mol%, the same concentration used in *in vitro* studies, in the same lipid formulation (Tanaka lipids, DPPC:POPG:PA); these results were compared to those of bovine lipid extract surfactant (BLES) and the synthetic lipid-only formulation (Fig. (**6**)) [88]. A higher concentration of peptoid mimics were used, relative to the actual concentration of protein in lung surfactant (1-3% for SP-B and SP-C combined), for consistency with previous work and, in the case of SP-B, to accommodate the larger size of the protein (four to five helices rather than one). Results indicated that all peptoid-enhanced formulations showed improved outcomes relative to the synthetic lipids, but the pC mimic in particular most improved measured biochemical and physiological outcomes relative to pB and BLES (Fig. (**6**)). Interestingly, the pB/pC combination showed the best sustained response in certain physiological outcomes over the two hour recovery period. This unprecedented therapeutic study demonstrates the potential efficacy of peptoid-based lung surfactant protein mimics in the treatment of respiratory distress and other pulmonary-related diseases that inactivate lung surfactant. Furthermore, this study verifies that systematic and careful design of peptoids with specific structural attributes yields *in vitro* activity that can translate into *in vivo* efficacy.

ANTIMICROBIAL PEPTIDE MIMICS

Antimicrobial Peptides

Widespread use of antibiotics is progressively causing an inevitable emergence of drug-resistant bacterial strains, resulting in an increase number of acquired multi-drug resistant (MDR) infections [89-90]. The need for 6-12 months of prolonged antibiotic treatment to eradicate infections [91] and the recent increase in the number of MDR strains have led to a pressing need to develop new drug candidates that can improve current therapies. Antimicrobial peptides (AMPs), also known as host-defense peptides (HDP), are short bactericidal proteins with less than 40 amino acids that are integral components of the innate immune system [92]. AMPs are the first line of defense against a broad range of pathogens, including Gram-positive and Gram-negative bacteria, yeast, fungi, viruses, and protozoa [93]. These peptides are highly diverse in sequence but all adopt discrete amphipatic structures that segregate hydrophobic and cationic residues into distinct regions.

The antibiotic activity of AMPs is attributed to this amphipathic nature, Coulombically attracting them to and facilitating interactions with lipid membranes, subsequently disrupting membrane function [94]. Biological membranes contain numerous different lipid species, and cationic AMPs strongly bind to anionic lipid headgroups. This preferential binding explains the cell-type specific selectivity of some AMPs for bacterial cells (antimicrobial activity) over mammalian cells (cytotoxicity), since bacterial membranes have primarily anionic phospholipid bilayer membranes with charged headgroups oriented towards the exterior of the cell. In contrast, most mammalian cells possess an outer membrane that is largely zwitterionic [93]. Therefore, cationic AMPs are preferably attracted to bacteria over mammalian cells. This preferential attraction is a possible mechanism for selectivity of some AMPs for bacterial cells [93]**.** The mechanism of action of AMPs is thought to primarily involve interactions with the inner and outer membranes of bacteria, leading to membrane degradation and bacterial cell lysis [95, 96].

A hypothesis that AMP-induced channel formation occurs in membranes as part of the killing process has been asserted and verified in artificial membranes for two classes of AMPs, the magainins and defensins [95]; however, studies of AMPs such as indolicidin [97] and buforin II [98], previously presumed to act by disrupting cell membranes, have found no correlation between membrane disruption and antibacterial activity. The AMP indolicidin is known to permeabilize the outer and inner membranes of *E.coli*, but does not cause cell lysis [97]. In addition, indolicidin and other non-membrane-disruptive AMPs interact with intracellular targets like DNA, RNA, and mitochondria, as well as affect cellular ATP and

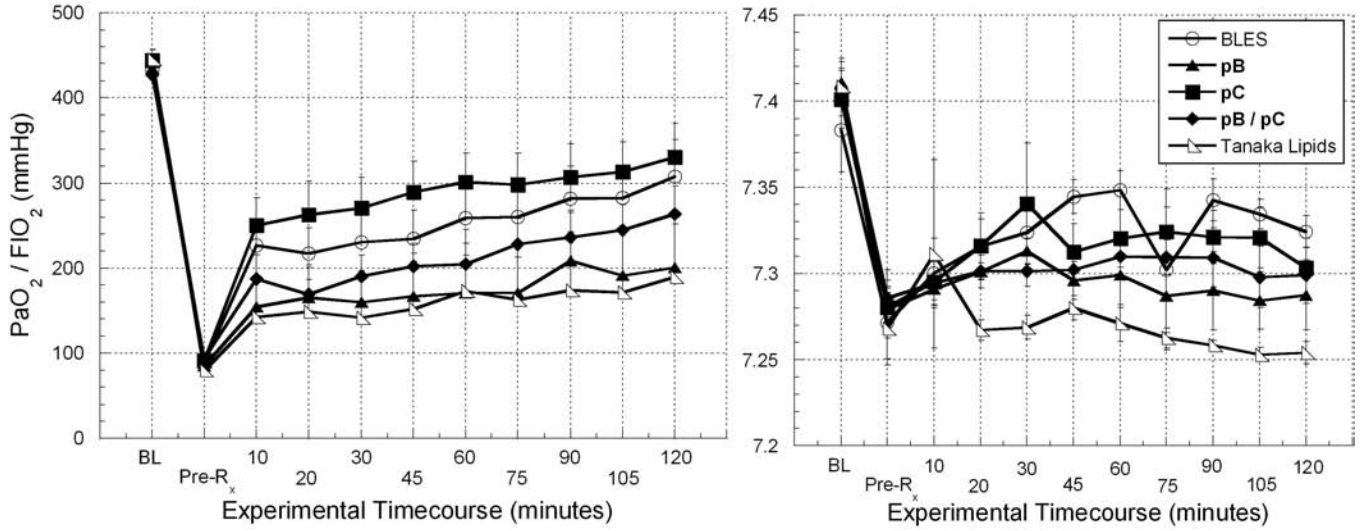

Fig. (6). Physiological indicators of pulmonary gas exchange function over time in a lung lavage-induced rat model of acute lung injury (ALI). (**A**) PaO_2/FIO_2 and (**B**) Blood pH over the time course of the experiment. PaO_2/FIO_2 gives an indication of increased gas exchange over time, while less acidic blood pH over time indicates improved pulmonary function. Error bars indicate the standard error of the mean (SEM). Statistical significance indicators: * indicates $p < 0.05$ between BLES treatment group and Tanaka Lipids; + indicates $p < 0.05$ between pC treatment group and Tanaka Lipids. Statistical analysis was performed using a one-way analysis of variance (ANOVA) with post-hoc multiple comparison testing using the Tukey-Kramer method. Adapted from ref [88].

protein synthesis [97, 99-102] . Regardless of their intended target(s), the attraction of AMPs toward the bacterial membrane is integral for their mechanism of action [93]. Rather than interacting with key specific bacterial enzymes, as most small-molecule antibiotics do, AMPs function by a non-specific, biophysical mechanism that makes bacterial resistance to AMPs rare and difficult [103].

Peptoid-Based Antimicrobial Peptide Mimics

Although AMPs have been studied for several decades as potential therapeutics, the short half life of peptides *in vivo* reduces their bioavailability, decreasing their attractiveness as drug candidates [92, 104]. Researchers have therefore been designing non-natural mimics of AMPs to provide better structural stability and bioavailability in compounds that are more advantageous to use as therapeutic candidates relative to peptides [3, 103, 105-108]. Some efforts to ameliorate disadvantageous peptide characteristics have relied upon the incorporation of D-amino acids, which is a useful strategy; however, D-amino acids can be ~ 10 times more expensive than L-amino acids. Peptaibols have been used as an alternative to conventional AMPs because they are resistant to proteolysis due to the presence of α-aminoisobutyric acid and hydroxylated C-terminal amino acids [109]. Azapeptides, and oligoureas have also been developed as peptide mimics [110-111], as well as various helical foldamers, including β- and γ-peptides, and poly-*N*-substituted glycines ("peptoids") [112-113]. We focus here on peptoid mimics of AMPs.

The *in vivo* structural stability of peptoids affords them a great potential for development as effective antimicrobial agents [114, 115]. Recently, Fowler and Blackwell reviewed the structure-activity relationships of antimicrobial peptoids or "ampetoids" [8] that were developed by Barron and co-workers in 2003. Here we will focus on more recent attempts to optimize peptoids as AMP mimics, with an emphasis on *N*-terminal alkylation, cyclization, and reduction in hydrophobicity of peptoids with the aim of improving activity while substantially reducing mammalian cytotoxicity. Strategies to incorporate a single peptoid residue into a peptide, creating a peptide-peptoid hybrid with improved therapeutic potential, are also presented. Finally, the *in vivo* biocompatibility and efficacy of antimicrobial peptoids to treat bacterial infections will also be discussed.

It has been previously shown that antimicrobial peptoids are both structural *and* functional mimics of AMPs [115-116]. Ampetoids are mimics of the magainins, a class of AMPs that are relatively short, cationic, and α-helical, and ampetoids have antimicrobial properties similar to the peptides they mimic [115]. A study in 2008 determined that the selectivity of peptoids towards bacterial cell lines (*E. coli* ATCC 35218 and *B. subtilis* ATCC 6633) over mammalian cells (lung epithelial A549 cell line) was enhanced by net cationicity and moderate hydrophobicity, whereas hemolytic activity (red blood cell lysis) increased with high hydrophobicity and amphipathicity [114] (Table **2**). In order for peptoids to be successful therapeutics, they must preferentially interact with bacterial over mammalian cells; when this criterion is met, peptoids are referred to as "selective". Mindful of net cationicity, hydrophobicity, and amphiphathicity, researchers subsequently designed and tested several families of ampetoids in order to fully understand the effects of sequence length, hydrophobicity, helicity, and charge-to-length ratio on the activity and selectivity of ampetoids (Table **2**) [117]. This effort resulted in the design and characterization of ~ 26 different analog derivatives of previously reported Peptoid **1,** a dodecamer peptoid that forms a three-sided polyproline type I-like helix, with one positively charged and two hydrophobic faces (Fig. (**3**)). Peptoid **1** has comparable activity to pexiganan, an optimized peptide-based analog of magainin-2 amide (Peptoid **1** $MIC_{E.coli}$ = 3.5 μM and $MIC_{B.subtilis}$ = 0.88 μM) but is more hemolytic (Peptoid **1** HD_{10} = 21 μM and pexiganan HD_{10} = 73 μM) (Table **2**) [114, 117]. Exactly 18 of the 26 variants matched the activity of Peptoid **1** and were less toxic to red blood cells [117]. Notably, a reduction in hydrophobicity by removal of a hydrophobic residue (*N*spe, **1**-11_{mer}), inclusion of Pro (**1**-Pro_6) or an achiral residue (*N*pm, **1**-$Npm_{2,5,8,11}$, Fig. (**2**)) in place of chiral Phe-like residues (*N*spe) made peptoids less hemolytic while retaining the antimicrobial activity (Table **2**) [117].

Since the antimicrobial assays performed in these studies involved relatively standard bacterial cell lines that were not highly pathogenic, it was necessary to determine the efficacy of antimicrobial peptoids against stronger, more resistant bacterial cell lines. Therefore, the most promising of the peptoids from these two studies were tested against clinical isolates and multi-drug resistant (MDR) strains of several Gram-positive and Gram-negative bacte-

Table 2. The Sequences and Activities of Antimicrobial Peptoids and Peptides, Including Antimicrobial Activity Against *E. coli* (ATCC 35218) and *B. subtilis* (ATCC 6633) Cell Lines, as well as Lytic Activity Against Red Blood Cells (RBCs, Hemolysis).

Class	Name	Sequence (amino to carboxy)	*E. coli* MIC (μM)	*B. subtilis* MIC (μM)	HD_{10}/HD_{50} (μM)
Basis [114]	**1**	(*N*Lys-*N*spe-*N*spe)$_4$	3.5	0.88	21 / 100
	1-*N*ssb	(*N*Lys-*N*ssb-*N*ssb)$_4$	31	3.9	>120/>120
Length [117]	$\mathbf{1}_{9mer}$	(*N*Lys-*N*spe-*N*spe)$_3$	9.1	1.2	>150/>150
	$\mathbf{1}_{11mer}$	(*N*Lys-*N*spe-*N*spe)$_3$- *N*Lys-*N*spe	6.3	0.78	>200/>200
Proline Variants [117]	**1**-Pro$_6$	*N*Lys-*N*spe-*N*spe- *N*Lys-*N*spe-*L*-Pro-(*N*Lys-*N*spe-*N*spe)$_2$	3.1	1.6	63/>110
	1-Pro$_9$	(*N*Lys-*N*spe-*N*spe)$_2$- *N*Lys-*N*spe-*L*-Pro- *N*Lys-*N*spe-*N*spe	12.5	1.6	165/>200
Achiral [117]	**1**-achiral	(*N*Lys-*N*pm-*N*pm)$_4$	12.5	0.78	183/>200
	1-*N*pm$_{2,5,8,11}$	(*N*Lys- *N*pm-*N*spe)$_4$	6.3	1.6	87/>200
Aliphatic [117]	**1**-*N*sdp$_{2,5,8,11}$	(*N*Lys- *N*sdp-*N*spe)$_4$	12.5	0.78	111/>200
Alkylation [120]	*N*tridec-$\mathbf{1}_{4mer}$	*N*tridec-*N*Lys-*N*spe-*N*spe-*N*Lys	12.5	1.6	65/>200
AMP [114]	Pexiganan	GIGKFLKKAKKFGKAFVKILKK	3.1	1.6	73/>200
	Melittin	GIGAVLKVLTTGLPALISWIKRKRQQ	1.6	0.78	2/6

The MIC is defined as the minimum inhibitory concentration of antimicrobial agent at which no bacterial growth is observed. HD_{10} is the hemolytic dose at which 10% of the red blood cells are killed. The *N*tridec side chain is tridecylamine, a C13 alkyl chain. The results are represented as an average of two parallel replicates in three independent trails. Data were obtained from refs [114], [117], and [120].

ria, and were found to be as active as pexiganan (Table **3**) [117]. Based on the peptoids' antimicrobial activities in this study, investigators designed and executed a computational model to predict the antimicrobial activities of peptoids based on their structures and sequences [118]. The activities of the peptoids when predicted by the model were consistent with their corresponding activities witnessed *in vitro* in cell-based assays, escalating the potential for computational studies to aid in the designing and testing of peptoids for this and other therapeutic applications.

Inspired by an earlier study in the Shai lab [119], the Barron and Mobashery lab then focused on synthesizing short, *N*-terminally alkylated peptoid-based sequences derivatized from Peptoid **1**. Peptoid *N*tridec-$\mathbf{1}_{4mer}$ (referred to as **1** C13$_{4mer}$), a short four-residue sequence with a C13 alkyl tail on the *N*-terminus, had nearly half of the molecular weight of Peptoid **1** but still exhibited comparable antibacterial activity ($MIC_{E.coli} \simeq$ 6.3 - 12.5 μM and $MIC_{B.subtilis}$ = 1.6 μM) and notably, significantly improved cell selectivity (HD_{10} = 65 μM) (Table **2**) [120]. Recently, Contag, Cirillo, and Barron labs also demonstrated that Peptoid **1** C13$_{4mer}$ is active at as low as ~ 6 - 7 μM against *Mycobacterium tuberculosis* (Mtb) and *Mycobacterium bovis* Bacille Calmete-Guérin (BCG), whereas the non-alkylated Peptoid $\mathbf{1}_{4mer}$ variant was inactive [121]. The presence of an alkyl tail is believed to enhance peptoid penetration into and transport through the bacterial membrane. Additionally, similar to the behavior observed for alkylated peptides [122], **1** C13$_{4mer}$ may posses the ability to micellize at its MIC, with the alkyl chains creating an internal hydrophobic core surrounded by aromatic- and cationic-rich segments that form the outer solvent-exposed portion. This micellization or other form of association based on hydrophobic chain-chain interactions could explain its their increased antimicrobial activity relative to the unalkylated variant. The *N*-terminal alkylation study also demonstrated that a C5 alkyl tail on the *N*-terminus of peptoid **1** did not increase the cytotoxicity of peptoid **1** toward red blood cells, whereas a C10 alkyl tail made peptoid **1** more toxic. Therefore, it is difficult to say if *N*-terminal peptoid alkylation would improve the therapeutic potential of peptoids; nevertheless, it is a promising technique to increase the therapeutic applicability of certain peptoids as drug candidates against certain bacterial strains.

Another strategy put forth to reduce hemolysis of red blood cells by peptoids was to design cyclized peptoids. Riccardis and coworkers recently attempted to cyclize linear peptoids to improve their antimicrobial properties and selectivity. Since cyclization tends to reduce conformational flexibility by constraining the residues, it is believed that structural rigidity would increase the ability of peptoids to permeabilize the bacterial membrane [123-124]. Successfully synthesized selective (no hemolysis up to 100 μM) α-cyclic peptoids were created with better antibacterial and antifungal activity than linear peptoids (MIC for linear peptoid ~ 90-360 μM, and cyclic ~ 90-200 μM). However, despite cyclization, the improvement in antibacterial activity was not comparable to the activity of previously reported linear peptoid-based mimics of magainin (MIC < 6 μM) [114-115, 120]. These results and unpublished work from the Barron lab (Kapoor and Barron) have indicated that upon cyclization, the selectivity of peptoids towards bacteria increases without a significant increase in antimicrobial potency. On the contrary, it has been shown that an unstructured and achiral peptoid, $\mathbf{1}_{achiral}$, is as equally active as structured and chiral Peptoid **1** ($MIC_{E.coli} \simeq$ 6.3 -12.5 μM) but is in fact more selective (HD_{10} = 183 μM). The circular dichorism studies of peptoid $1_{achiral}$ here demonstrated that the peptoid remained unstructured even on association with the bacterial and erythrocyte mimetic lipids, elucidating that structural rigidity and helicity may not be essential for activity [117].

Peptoid-Peptide Hybrid Mimics

Single peptoid residues or segments have also been used to improve the proteolytic stability, increase the antimicrobial activity, yet reduce the cytotoxicity of AMPs or their peptide-based mimics. Replacement of a single amino acid in a peptide by a peptoid resi-

Table 3. Broad-spectrum Antibacterial Activity of Ampetoids and Pexiganan Against Clinically Relevant Biosafety Level 2 (BSL2) and BSL3 Pathogenic Microbial Strains

Bacterial organism	Pexiganan	1	1-Pro_6	$1_{achiral}$	1-*N*$pm_{2,5,8,11}$	1-*N*$sdp_{2,5,8,11}$	*N*tridec-1_{4mer}
K. pneumoniae ATCC 33495	8	16	16	8	16	8	9.6
P. vulgaris ATCC 49132	32	32	32	32	64	32	38.3
E. aerogenes ATCC 35029	32	16	128	128	64	32	> 153.3
P. aeruginosa ATCC 27853*	4	8	32	16	64	16	9.6
E. coli ATCC 25922*	8	4	16	16	8	8	9.6
S. aureus VAN2§†	8	4	8	8	8	8	4.8
S. aureus NRS100 (COL)§	16	4	8	8	8	8	4.8
E. faecalis 99	128	8	64	64	64	64	19.2
E. faecium 106*	4	4	4	4	4	4	4.8

Minimum inhibitory concentrations (MICs) are represented in μg/mL. * indicates NCCLS recommended standard strain; § indicates methicillian-resistant *S. aureus* (MRSA) strain; and † indicates vancomycin-resistant strain [117].

due introduces some of the benefits of a peptoid into a peptide. Shin and co-workers demonstrated that substituting Trp_{19} in the bee venom AMP melittin with "*N*Trp," a Trp-based peptoid residue, significantly reduced the mammalian cytotoxicity of the melittin analogue while still retaining its antibacterial and antifungal properties [125]. In a followup study, it was also shown that substituting all Pro residues in Trp/Pro-rich AMPs such as indolicidin, tritrpticin-amide, and a symmetric tritrpticin analog (all Arg's substituted with Lys's) with *N*Lys residues afforded more activity against Gram-negative and Gram-positive bacteria (MIC ≃ 0.5 - 8 μM) and less hemolysis (minimum concentration that produces hemolysis (MHC) > 200 μM) [126].

In a similar study, Gennaro and co-workers replaced a single Arg or Leu residue with *N*Arg and *N*Leu, respectively, in apidacin Ib, an AMP found in insects [127]. Replacing cationic amino acids with cationic peptoid residues increased the proteolytic stability (no cleavage by trypsin after 24 h, whereas the natural peptide was cleaved in 30 min) and reduced the hemolysis (no hemolysis up to 300 μM), but with a sacrifice in antimicrobial activity (MIC ≃ 16 - > 128 μM).

Peptoids can also be used to increase the stability of inhibitor peptides. A very recent study focused on improving the proteolytic stability of peptides by introducing a single oligo-*N*-methoxyethylglycine (*N*meg) peptoid residue along with a spacer at the *N*-terminus of a previously identified peptide-based human respiratory syncytial virus (hRSV) fusion inhibitor, C_{20} peptide [128-129]. The solubility and serum stability increased by 80% and 25%, respectively, but the peptide retained its binding affinity (IC_{50} = 80.77 μM) [128]. These studies emphasize that incorporating a single peptoid residue or replacing positively charged amino acids by structurally similar peptoid residues improve the bioavailability and structural stability of peptides, thus increasing their attraction as therapeutic candidates.

In Vivo Biocompatibility and Efficacy of Antimicrobial Peptoids

In order to use peptoids as alternative therapeutic candidates to treat bacterial infections, *in vivo* biocompatibility must be ascertained. Recently, the Barron and Hancock labs focused on evaluating the toxicity of peptoids in a murine model [118]. The lead compound, potent Peptoid **1**, was instilled at a dose of 4 mg/kg (typical dose for AMPs is 1-24 mg/kg) in healthy mice intraperitoneally (i.p.), while the control group was injected with an equal volume of saline. Peptoid-injected mice initially exhibited hunched abdomens, but these symptoms disappeared 1 h post-injection. All mice were healthy and survived 24 h post-injection, showing that Peptoid **1** is not fatally toxic *in vivo* and therefore, has promising potential for further therapeutic studies.

In the late 90s, Winter and coworkers at Chiron showed that a single dose of 10 mg/kg, i.p.-injected peptoid trimer (CHIR29498) was able to treat *S. aureus* infection in CD-1 mice with a 100% survival rate, when treated at t = 0 [130]. Furthermore, 50% of the mice survived when dosed at 30 mg/kg 110 minutes post-infection. A more recent study also demonstrated the *in vivo* efficacy of peptoids to treat *S. aureus* infection in a murine model [118]. Peptoid **1** reduced mortality by 75% when 4 mg/kg was administered *via* i.p. injection after 4 h of infection, as compared to the saline-treated control. These preliminary studies demonstrate that peptoids are capable of treating infections caused by Gram-positive bacteria *in vivo* without being fatally toxic. Furthermore, detailed investigation on the optimum dose, efficacy against different Gram-positive and Gram-negative bacteria strains, mode of administration, and metabolic fate of peptoids need to be determined to develop them as therapeutics.

POTENTIAL APPLICATIONS OF LIPID-ASSOCIATED PEPTOID-BASED MIMICS

Antimicrobial peptoids' reduced bacterial resistance and stable structure offer significant advantages over conventional antibiotics, as well as AMPs and peptide-based mimics, to be developed as therapeutic compounds for a wide range of biologically related applications. Broad spectrum activity affords ampetoids the potential for development as anti- bacterial, fungal, viral, and cancer drugs. A few examples of promising applications for antimicrobial peptoids are discussed herein.

Contamination of medical devices such as stents, implants, and catheters leads to serious bacterial infections [131-133]. The attached bacteria in these devices can form biofilms on the surface that are more resistant to antibiotics than planktonic cells, due to the presence of an exopolysaccharide matrix [134]. Around 60% of all clinical infections are caused by bacteria that live in biofilms [135]. Recurrent Urinary Tract Infections (UTIs) and chronic Cystic Fibrosis (CF) are caused by *E.coli* and *Pseudomonas* biofilms, respectively [136-139]. In 2008, scientists demonstrated that peptoids are potent compounds in the antifouling of surfaces [11]. Surface-immobilized antimicrobial peptoids were able to attract anionic

bacteria and alter their membrane structure, a change that was hypothesized to prevent bacterial attachment to surfaces, thereby preventing biofilm formation.

More recently, it has been shown that at the minimum inhibitory concentration (MIC), select antimicrobial peptoids were able to prevent biofilm formation as well as reduce pre-existing biofilms better than two studied AMPs and three different conventional antibiotics [140]. Peptoid **1** was able to detach the pre-existing *P. aeruginosa* PA 14 biofilms by 60%, whereas alkylated Peptoid **1** $C13_{4mer}$ (*N*tridec-$\mathbf{1}_{4mer}$) was able to reduce the cell viability in established biofilms by 100-fold (~ 2-log decrease). These results suggest that ampetoids could be used to coat and sterilize endoscopes, which are routinely used in hospitals and other clinical settings. In addition, peptoids have the potential to be used prophylactically, as in the case of dental biofilms, and for treating existing infections caused by drug-resistant bacterial biofilms.

In combination with a lung surfactant formulation of natural or synthetic origin, ampetoids also have the potential to treat bacterial- or viral-borne pulmonary infections like tuberculosis, pneumonia, and cystic fibrosis (CF). The lung surfactant lipid carrier would offer an excellent delivery tool for efficient delivery and greater spreading of the antimicrobial peptoids in distal regions not easily accessible due to the presence of thick mucus in the diseased lung. Preliminary data in the Barron lab has also indicated that lung surfactant peptoids have little to no antimicrobial activity in free solution (Didwania and Barron), indicating that bactericidal action is largely controlled by the concentration of the antimicrobial peptoid in the peptoid "cocktail" mixture. However, a potential pitfall in using lung surfactant as a delivery method for ampetoids would be possible entrapment of ampetoids by the anionic lipid components of the surfactant, which could reduce antimicrobial potency. In order to use this method, the antimicrobial and interfacial activity of lipid-bound ampetoids would need to be thoroughly evaluated.

Moreover, lung surfactant peptoids with or without combination antimicrobial agents have the potential to treat multiple pulmonary syndromes aside from respiratory distress; exploring peptoid effects on infantile meconium aspiration, sepsis, lung damage, bronchopulmonary dysplasia, and asthma would all be of interest [28]. Lipid-peptoid surfactants may also function to reduce lung inflammation and the effects of lung injury. A crucial anionic lipid component of lung surfactant and of the lipid-peptoid formulations, POPG, has recently been shown to inhibit respiratory synctial virus (RSV) as well as lipopolysaccharide (LPS)-induced inflammatory responses [141]. This result suggests that an exogenously administered antimicrobial lipid-peptoid surfactant formulation may further assist in the inhibition of RSV, influenza, or other viral lung infections. Spiking extracted surfactant formulations with peptoids, or spiking recombinant SP-D into peptoid-enhanced surfactants, may also be viable treatment options to obtain necessary antibacterial and anti-inflammatory effects. The Eustachian tube of the ear also contains a form of surfactant, including SP-B and SP-C, with a slightly different composition from that located in the lung. Therefore, appropriately formulation lipid-peptoid surfactants may also have the potential to decrease inflammation in or treat severe ear infections.

THERAPEUTIC PEPTOIDS AS DIAGNOSTIC AND CANCER-FIGHTING AGENTS

An important aspect of drug discovery is identifying molecules that can inhibit protein-protein interactions. Small molecules may not be the ideal choice for such an application due to their inability to cover the entire protein surface and thereby inhibit protein-protein interactions [142]. Peptoids have been widely used to identify high affinity binding agents to therapeutic targets [143-145]. Here, we focus on the most recent studies in this area.

PEPTOIDS AS DIAGNOSTIC AGENTS

For approximately a decade, large libraries of peptoids have been used as diagnostics to identify targets for pharmaceutical applications [145-146]. Kodadek and co-workers were the first to identify the potential of peptoids to distinguish between immune and autoimmune cells [147]. They developed a one bead-one compound peptoid library as a tool for detecting specific binding agents to autoimmune T-cells in cases of Multiple Sclerosis (MS) [138]. MS is a nervous system disease in which the protective coating around the nerves is eaten off by the immune system, leading to demyelination and scarring. In this study, the $CD4_+$ T-cells were isolated from mice with Experimental Autoimmune Encephalomyelitis (EAE), and T-cells from the control mice were labeled with red and green quantum dots, respectively. The cells were mixed in a 1:1 ratio and incubated with an approximately 300,000 peptoid one-bead library. The beads that bound to only red-labeled cells contained peptoid binders to EAE $CD4_+$ T-cells with high specificity. Two peptoid hits (nonamers) were identified that bound to the $CD4_+$ T-cells from the EAE mice and not to the T-cells from the control mice. These results show that peptoid combinatorial libraries can effectively aid in identifying new synthetic molecules that can distinguish between immune and autoimmune cells.

Proteosome inhibitors have shown promise in anti-tumor therapy, and peptoid libraries have been used for identifying potential proteosome inhibitors. In a recent study, the Kodadek and co-workers used a one bead-one compound library of 30,000 peptoids to target ATPase and identify inhibitors of 19S Regulatory Particle (RP). The most common 26S proteosome has a 20S core and two 19S RP that contain ATPase active sites [148], and the inhibition of the 20S core particle present inside the 26S proteosome is an attractive target in cancer diseases. The 19S RP binds to the polyubiquitinated proteins, followed by protein unfolding, and steering the protein into the 20S core for degradation. Out of 30,000 peptoids used to target ATPase, Kodadek and co-workers identified one purine-capped peptoid heptamer as a proteosome binder, which inhibited the unfolding of the protein. Overall, these two studies clearly highlight that large peptoid libraries bear immense potential as tools for screening and binding to potential therapeutic targets for a wide range of diseases.

PEPTOIDS AS ANTI-CANCER DRUGS

In a separate study, Kodadek and co-workers have also shown that peptoid libraries bear promise in targeting vascular endothelial growth factor receptor 2 (VEGFR2) [149-151]. Over-expression of VEGF is a primary cause of many malignant and cancerous tumors, and subsequently, targeting tumor angiogenesis has become an important part of cancer therapy. Kodadek and co-workers identified peptoid GU40C4 (a dimer of a nonamer peptoid) that bound to the extracellular domain of the VEGFR2 with a binding affinity of 30 nM, and was a VEGFR2 antagonist *in vitro* and *in vivo*. A reduction in autophosphorylation of VEGFR2 in PAE-KDR and HUVEC cells was observed after peptoid treatment. Peptoid GU40C4 also reduced VEGF-induced proliferation of HUVEC cells [150].

To investigate the anti-cancer potential of peptoids in further detail, Kodadek and co-workers also developed GU81, a derivative of the GU40C4 peptoid, to improve the binding affinity of peptoid against VEGFR2 [152]. The potential of GU81 to reduce autophosphorylation was tested in PAE-KDR cells and its efficacy was further evaluated in an *in vivo* transgenic mouse breast cancer model. A dose of 260 μg/day of GU81 was administered in combination with doxorubicin (2 mg/kg, once a week) for 19 days, which resulted in a 1.6-fold reduced tumor volume compared to the control treatment with doxorubicin alone. However, GU81 administered alone did not show significant reduction compared to the control. Since most cancer-based treatments require combinations of drugs,

peptoids have the potential to be used in combination with conventional antibiotics to control tumor expression and development. Cancer is a huge burden to society, and along with existing cancer treatment therapies, these studies increase the possibility of peptoids as novel biological tools and therapeutics in identifying cancer targets.

CONCLUSIONS

We have highlighted the most recent contributions to peptoid research and development as potential pharmaceuticals in the fields of lung surfactant, antimicrobial peptides, diagnostics, and anti-cancer agents. In the lipid-associated lung surfactant and antimicrobial peptide fields, it is notable that successfully bioactive peptoid-based mimics displayed similar physiochemical properties that may indicate generalized characteristics needed for favorable lipid-peptoid interactions in other biological applications. The most active mimics in both classes were aromatic-rich rather than aliphatic, suggesting that sufficient hydrophobic and aromatic interactions, such as pi stacking or oligomerization, may be necessary as part of their mechanism of action. In addition, both sets of peptoid-based mimics needed a significant amount of cationic residues in order to be active, but for ampetoids, moderate hydrophobicity was needed for selectivity.

N-terminal alkylation of both lung surfactant and antimicrobial peptoids substantially increased activity. Primarily, alkylation serves to increase lipid affinity and insertion ability. In the case of lung surfactant peptoids, alkylation helps to anchor the peptoid and excluded lipid material to the interfacial layer; and in the case of antimicrobial peptoids, alkylation seems to aid in the penetration of particularly greasy biofilms and membranes, thereby increasing potency. Alkylation may also promote hydrophobic association between peptoids through chain-chain or helix-chain interactions, and also leaves open the possibility of micellization as a part of the mechanism of action.

A larger question that has arisen in recent years is whether a free-solution helical structure, or somewhat constrained amphipathicity, is necessary for activity. Through multiple studies, it has been shown that achiral peptoids or unstructured polymers are still able to emulate the surface activity and antimicrobial activity of proteins and peptides [86, 153-155]. For peptoid-based SP-C mimics, a rigid helix is probably necessary to span a lipid bilayer and sustain a multilayer of lipids. SP-B's disulfide bonds are also likely present to constrain the protein conformation in order to promote its behavior, but surprisingly, unstructured SP-B mimics are seemingly able to mimic aspects of its surface activity. In addition, the reduction in hydrophobicity resulting from achirality in Peptoid $\mathbf{1}_{\text{achiral}}$ relative to **1** retained antimicrobial activity and actually improved selectivity. In terms of lipid-peptoid interactions, it seems that a helix may not be strictly necessary for activity as long as the structure is flexible enough to adopt an amphipathic albeit irregular conformation once lipid-bound.

In the fields of diagnostics and anti-cancer agents, peptoids have demonstrated a significant potential to participate in binding and screening assays that identify binders to therapeutic targets. Peptoids are amenable to combinatorial library screenings due to their facile and stable synthesis. However, many more studies need to be conducted before peptoids would be used in diagnostic and binding assays in a clinic.

For peptoids to truly become therapeutic agents, several questions need to be addressed. Particularly, the fate and cytotoxicity of the peptoids are a significant concern. Though limited unpublished studies have been conducted on the cytotoxicity of lung surfactant peptoids (Czyzewski and Barron), there are indications in the literature that SP-B and SP-C cause cell lysis (due to their natural membrane activity), but the lipid-bound nature of the proteins significantly reduces this effect [156]. Some strategies that have been adopted to reduce the cytotoxicity of ampetoids are alkylating the peptoids, reducing hydrophobicity *via* incorporation of a proline residue in place of a hydrophobic *N*spe residue, introducing similarly charged or hydrophobic residues at *N*- and *C*-termini, and replacing chiral *N*spe monomers by achiral *N*pm. However, a complicating factor in reducing cytotoxicity is the translation from cell-based assays to *in vivo* experiments. In both lung surfactant peptoid and ampetoid *in vivo* experiments, many if not all peptoid-treated animals survived the experiment, even without an infection present. This is surprising given that Peptoid **1** was found to be moderately toxic *in vitro*. However, in the same ampetoid *in vivo* study, a peptoid $N\text{His}_{6,12}$, which was fairly non-toxic *in vitro*, killed all mice during the *in vivo* experiment [118]. The somewhat inconsistent nature between the results of *in vitro* and *in vivo* experiments can therefore hinder the therapeutic development of peptoids. Along with cytotoxic effects, peptoid researchers also need to investigate whether peptoids are affected by the xenobiotic metabolism, and whether they are are hepatotoxic.

Aside from toxicity, the metabolic fate of peptoids needs to be determined through biodistribution studies. Tse and co-workers in 1999 showed that a trimer peptoid had low oral absorption and was excreted intact into the feces in 2 h [157]. To understand the fate of the peptoids *in vivo*, similar detailed studies need to be conducted using structurally and sequentially different, and active and inactive peptoids. Though peptoid-based lung surfactant administration would most likely be localized, ampetoids theoretically have the potential to be used for both local and systemic treatments. In both cases, a careful judgment must be made on the mode of administration of the peptoids. Only non-hemolytic peptoids could be used systemically without being toxic to the red blood cells. Optimal dosing also needs to be determined to treat the infection or disorder without being toxic to humans. Studies using multiple types of infection models in different animal species would be needed. Fortunately, because peptoids are sequence-specific biopolymers, their activities and toxicities can be "tuned" or modulated for a particular biological application.

The potential for immunogenic response is another significant factor in the therapeutic development of peptoids. Though limited studies have shown peptoids to be non-immunogenic, the aforementioned "tunability" of peptoids may be beneficial, as non-natural (and therefore unrecognizable) side chains can be incorporated into the sequence. In addition, limited studies have indicated that proteolytic or "enzyme cleavage" peptide segments can be included in the peptide sequence to promote degradation and clearance post-treatment.

Given the tremendous increase in peptoid research in the last few years, there is a strong indication that peptoid-based biomimicry of proteins and peptides will evolve and eventually, the therapeutic potential of peptoids will be realized. As the number of *in vivo* studies increases, researchers will gain a better perspective of the limitations and possibilities of peptoids in a living body. Though the results described in this review are quite promising, there is a long road ahead in terms of peptoid-based pharmaceutical research and development. As more complicated secondary and tertiary peptoid structures are synthesized and explored, the number of possible therapeutic applications will only increase. In this way, peptoids will continue to be versatile and accessible compounds for exploration of the underlying mechanisms of biological processes that will lead to therapies for a significant number of diseases and syndromes.

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REFERENCES

[1] Zuckermann RN, Kerr JM, Kent SBH, Moos WH. Efficient method for the preparation of peptoids oligo(N-substituted glycines) by submonomer solid-phase synthesis. J Am Chem Soc 1992; 114: 10646-47.

[2] Simon RJ; Kania RS; Zuckermann RN; *et al*. Peptoids: a modular approach to drug discovery. Proc Natl Acad Sci USA 1992; 89: 9367-9371.

[3] Sanborn TJ, Wu CW, Zuckermann RN, Barron AE. Extreme stability of helices formed by water-soluble poly-N-substituted glycines (polypeptoids) with alpha-chiral side chains. Biopolymers 2002; 63: 12-20.

[4] Miller SM, Simon RJ, Ng S, Zuckermann RN, Kerr JM, Moos WH. Comparison of the proteolytic susceptibilities of homologous L-amino-acid, D-amino-acid, and N-substituted glycine peptide and peptoid oligomers. Drug Dev Res 1995; 35: 20-32.

[5] Astle JM, Udugamasooriya DG, Smallshaw JE, Kodadek T. A VEGFR2 antagonist and other peptoids evade immune recognition. International Journal of Peptide Research 2008; 14: 223-227.

[6] Gibbons JA, Hancock AA, Vitt CR, *et al*. Pharmacologic characterization of CHIR 2779, an N-substituted glycine peptoid with high-affinity binding for alpha 1-adrenoceptors. Pharmacology 1996; 277: 885-899.

[7] Zuckermann RN, Kodadek T. Peptoids as potential therapeutics. Curr Opin Mol Ther 2009; 11: 299-307.

[8] Fowler SA, Blackwell HE. Structure-function relationships in peptoids: recent advances toward deciphering the structural requirements for biological function. Org Biomol Chem 2009; 7: 1508-24.

[9] Haynes RD, Meagher RJ, Won JI, Bogdan FM, Barron AE. Comblike, monodisperse polypeptoid drag-tags for DNA separations by end-labeled free-solution electrophoresis (ELFSE). Bioconj Chem 2005; 16: 929-938.

[10] Meagher RJ, McCormick LC, Haynes RD, *et al*. Free-solution electrophoresis of DNA modified with drag-tags at both ends. Electrophoresis 2006; 27: 1702-1712.

[11] Statz AR, Park JP, Chongsiriwatana NP, Barron AE, Messersmith PB. Surface-immobilised antimicrobial peptoids. Biofouling 2008; 24: 439-48.

[12] Haynes RD. In Chemistry; Northwestern University: Evanston, 2008; Vol. Doctor of Philosophy.

[13] Yoo B, Kirshenbaum K. Peptoid architectures: elaboration, actuation, and application. Curr Opin Chem Biol 2008; 12: 714-721.

[14] Olsen CA. Peptoid–Peptide hybrid backbone architectures. ChemBioChem 2010; 11: 152-160.

[15] Culf AS, Ouellette RJ. Solid-Phase synthesis of N-substituted glycine oligomers (α-peptoids) and derivatives. Molecules 2010; 15: 5282-5335.

[16] Wu CW, Kirshenbaum K, Sanborn TJ, *et al.* Structural and spectroscopic studies of peptoid oligomers with alpha-chiral aliphatic side chains. J Am Chem Soc 2003; 125: 13525-13530.

[17] Wu CW, Sanborn TJ, Huang K, Zuckermann RN, Barron AE. Peptoid oligomers with alpha-chiral, aromatic side chains: sequence requirements for the formation of stable peptoid helices. J Am Chem Soc 2001; 123: 6778-6784.

[18] Wu CW, Sanborn TJ, Zuckermann RN, Barron AE. Peptoid oligomers with alpha-chiral, aromatic side chains: effects of chain length on secondary structure. J Am Chem Soc 2001; 123: 2958-2963.

[19] Armand P, Kirshenbaum K, Goldsmith RA, *et al.* NMR determination of the major solution conformation of a peptoid pentamer with chiral side chains. Proc Natl Acad Sci USA 1998; 95: 4309-4314.

[20] Brown NJ, Johansson J, Barron AE. Biomimicry of surfactant protein C. Acc Chem Res 2008; 41: 1409-1417.

[21] Czyzewski AM, Barron AE. Protein and peptide biomimicry: gold mining inspiration from nature's ingenuity. AlChE J 2008; 54: 1-7.

[22] Wu CW, Barron AE. In Biomimetic Materials and Design, A.K. Dillow, ed.; Marcel-Dekker: New York, 2002: pp. 565-633.

[23] Gellman SH. Foldamers: a manifesto. Acc Chem Res 1998; 31: 173-180.

[24] Creuwels L, vanGolde LMG, Haagsman HP. The pulmonary surfactant system: biochemical and clinical aspects. Lung 1997; 175: 1-39.

[25] Goerke J, Clements JA. In The Handbook of Physiology, Sec 3, The Respiratory System; The American Physiological Society: Bethesda, MD, 1986; Vol. 3: pp. 247-262.

[26] Notter RH. Lung surfactants: basic science and clinical applications, Marcel Dekker: New York 2000.

[27] Serrano AG, Perez-Gil J. Protein-lipid interactions and surface activity in the pulmonary surfactant system. Chem Phys Lipids 2006; 141: 105-118.

[28] Zuo YY, Veldhuizen RAW, Neumann AW, Petersen NO, Possmayer F. Current perspectives in pulmonary surfactant - inhibition, enhancement and evaluation. Biochim Biophys Acta 2008; 1778: 1947-1977.

[29] Orgeig S, Bernhard W, Biswas SC, *et al*. The anatomy, physics, and physiology of gas exchange surfaces: is there a universal function for pulmonary surfactant in animal respiratory structures? Integr Comp Biol 2007; 47: 610-627.

[30] Perez-Gil J, Weaver TE. Pulmonary surfactant pathophysiology: current models and open questions. Physiology 2010; 25: 132-141.

[31] Veldhuizen EJA, Haagsman HP. Role of pulmonary surfactant components in surface film formation and dynamics. Biochim Biophys Acta 2000; 1467: 255-270.

[32] Hawgood S, Schiffer K. Structures and properties of the surfactant-associated proteins. Annu Rev Physiol 1991; 53: 375-394.

[33] Johansson J, Curstedt T, Robertson B. The proteins of the surfactant system. Eur Respir J 1994; 7: 372-391.

[34] Perez-Gil J. Structure of pulmonary surfactant membranes and films: the role of proteins and lipid-protein interactions. Biochim Biophys Acta 2008; 1778: 1676-1695.

[35] Avery ME, Mead J. Surface properties in relation to atelectasis and hyaline membrane disease. Am J Dis Child 1959; 97: 517-523.

[36] Moya FR, Maturana A. Animal-derived surfactants versus past and current synthetic surfactants: current status. Clin Perinatol 2007; 34: 145-177.

[37] Mingarro I, Lukovic D, Vilar M, Perez-Gil J. Synthetic pulmonary surfactant preparations: new developments and future trends. Curr Med Chem 2008; 15: 393-403.

[38] Pison U, Seeger W, Buchhorn R, *et al.* Surfactant abnormalities in patients with respiratory-failure after multiple trauma. Am Rev Respir Dis 1989; 140: 1033-1039.

[39] Lewis JE, Jobe AH. Surfactant and the adult respiratory distress syndrome. Am Rev Respir Dis 1993; 147: 218-233.

[40] Bosma KJ, Taneja R, Lewis JF. Pharmacotherapy for prevention and treatment of acute respiratory distress syndrome. Drugs 2010; 70: 1255-1282.

[41] Playfor SD, Nootigattu VKT. Exogenous surfactant in paediatric acute lung injury and acute respiratory distress syndrome. Curr Drug Saf 2006; 10: 159-168.

[42] Lewis JF, Veldhuizen R. The role of exogenous surfactant in the treatment of acute lung injury. Annu Rev Physiol 2003; 65: 613-642.

[43] Robertson B, Johansson J, Curstedt T. Synthetic surfactants to treat neonatal lung disease. Mol Med Today 2000; 6: 119-124.

[44] Blanco O, Perez-Gil J. Biochemical and pharmacological differences between preparations of exogenous natural surfactant used to treat respiratory distress syndrome: role of the different components in an efficient pulmonary surfactant. Eur J Pharmacol 2007; 568: 1-15.

[45] Almlen A, Stichtenoth G, Linderholm B, *et al.* Surfactant proteins B and C are both necessary for alveolar stability at end expiration in premature rabbits with respiratory distress syndrome. J Appl Physiol 2008; 104: 1101-1108.

[46] Johansson J, Szyperski T, Curstedt T, Wuthrich K. The NMR structure of the pulmonary surfactant-associated polypeptide SP-C in an apolar solvent contains a valyl-rich α-helix. Biochemistry 1994; 33.

[47] Haagsman HP, Diemel RV. Surfactant-associated proteins: functions and structural variation. Comp Biochem Physiol A Mol Integr Physiol 2001; 129: 91-108.

[48] Creuwels LA, Boer EH, Demel RA, van Golde LMG, Haagsman HP. Neutralization of the positive charges of surfactant protein C: effects on structure and function. J Biol Chem 1995; 270: 16225-16229.

[49] Kramer A, Wintergalen A, Sieber M, Galla HJ, Amrein M, Guckenberger R. Distribution of the surfactant-associated protein C within a lung surfactant model film investigated by near-field optical microscopy. Biophys J 2000; 78: 458-465.

[50] Bi XH, Flach CR, Perez-Gil J, *et al*. Secondary structure and lipid interactions of the N-terminal segment of pulmonary surfactant SP-C in Langmuir films: IR reflection-absorption spectroscopy and surface pressure studies. Biochemistry 2002; 41: 8385-8395.

[51] Alonso C, Waring A, Zasadzinski JA. Keeping lung surfactant where it belongs: protein regulation of two-dimensional viscosity. Biophys J 2005; 89: 266-273.

[52] Nakorn PN, Meyer MC, Flach CR, Mendelsohn R, Galla HJ. Surfactant protein C and lung function: new insights into the role of alpha-helical length and palmitoylation. Eur Biophys J 2007; 36: 477-489.

[53] Plasencia I, Baumgart F, Andreu D, Marsh D, Perez-Gil J. Effect of acylation on the interaction of the N-terminal segment of pulmonary surfactant protein SP-C with phospholipid membranes. Biochim Biophys Acta 2008; 1778: 1274-1282.

[54] Takei T, Hashimoto Y, Aiba T, Sakai K, Fujiwara T. The surface properties of chemically synthesized peptides analogous to human pulmonary surfactant protein C. Biol Pharm Bull 1996; 19: 1247-1253.

[55] Ikegami M, Jobe AH. Surfactant protein-C in ventilated premature lamb lung. Pediatr Res 1998; 44: 860-864.

[56] Nilsson G, Gustafsson M, Vandenbussche G, *et al*. Synthetic peptide-containing surfactants - evaluation of transmembrane versus amphipathic helices and surfactant protein C poly-valyl to poly-leucyl substitution. Eur J Biochem 1998; 255: 116-124.

[57] Hawgood S, Ogawa A, Yukitake K, *et al.* Lung function in premature rabbits treated with recombinant human surfactant protein C. Am J Respir Crit Care Med 1996; 154: 484-490.

[58] Lukovic D, Plasencia I, Taberner FJ, *et al.* Production and characterisation of recombinant forms of human pulmonary surfactant protein C (SP-C): structure and surface activity. Biochim Biophys Acta 2006; 1758: 509-518.

[59] Taut FJH, Rippin G, Schenk P, *et al*. A search for subgroups of patients with ARDS who may benefit from surfactant replacement therapy: a pooled analysis of five studies with recombinant surfactant protein-C surfactant (Venticute). Chest 2008; 134: 724-732.

[60] Andersson M, Curstedt T, Jornvall H, Johansson J. An amphipathic helical motif common to tumourolytic polypeptide NK-Lysin and pulmonary surfactant polypeptide SP-B. FEBS Lett 1995; 362: 328-332.

[61] Hawgood S, Derrick M, Poulain F. Structure and properties of surfactant protein B. Biochim Biophys Acta 1998; 1408: 150-160.

[62] Zaltash S, Palmblad M, Curstedt T, Johansson J, Persson B. Pulmonary surfactant protein B: a structural model and a functional analogue. Biochim Biophys Acta 2000; 1466: 179-186.

[63] Vandenbussche G, Clercx A, Clercx M, *et al.* Secondary structure and orientation of the surfactant protein SP-B in a lipid environment - a Fourier-transform infrared-spectroscopy study. Biochemistry 1992; 31: 9169-9176.

[64] Waring A, Taeusch HW, Bruni R, Amirkhanian JD, Fan BR, Stevens R, Young J. Synthetic amphipathic sequences of surfactant protein-B mimic several physicochemical and *in vivo* properties of native pulmonary surfactant proteins. Pept Res 1989; 2: 308-313.

[65] Bruni R, Taeusch HW, Waring AJ. Surfactant protein-B - lipid interactions of synthetic peptides representing the amino-terminal amphipathic domain. Proc Natl Acad Sci USA 1991; 88: 7451-7455.

[66] Baatz JE, Sarin V, Absolom DR, Baxter C, Whitsett JA. Effects of surfactant-associated protein SP-B synthetic analogs on the structure and surface-activity of model membrane bilayers. Chem Phys Lipids 1991; 60: 163-178.

[67] Gordon LM, Horvath S, Longo ML, *et al.* Conformation and molecular topography of the N-terminal segment of surfactant protein B in structure-promoting environments. Protein Sci 1996; 5: 1662-1675.

[68] Gordon LM, Lee KYC, Lipp MM, Zasadzinski JA, Walther FJ, Sherman MA, Waring AJ. Conformational mapping of the N-terminal segment of surfactant protein B in lipid using C-13-enhanced Fourier transform infrared spectroscopy. J Pept Res 2000; 55: 330-347.

[69] Cochrane CG, Revak SD. Pulmonary surfactant protein-B (SP-B) - structure-function-relationships. Science 1991; 254: 566-568.

[70] Seurynck SL, Patch JA, Barron AE. Simple, helical peptoid analogs of lung surfactant protein B. Chem Biol 2005; 12: 77-88.

[71] Veldhuizen EJA, Waring AJ, Walther FJ, Batenburg JJ, van Golde LMG, Haagsman HP. Dimeric N-terminal segment of human surfactant protein B (dSP-B1-25) has enhanced surface properties compared to monomeric SP-B1-25. Biophys J 2000; 79: 377-384.

[72] Waring AJ, Walther F, Gordon LM, *et al.* The role of charged amphipathic helices in the structure and function of surfactant protein B. J Pept Res 2005; 66: 364-374.

[73] Cochrane CG. Surfactant protein B and mimic peptides in the function of pulmonary surfactant. FEBS Lett 1998; 430: 424-424.

[74] Cochrane CG, Revak SD, Merritt A, *et al.* The efficacy and safety of KL(4)-surfactant in infants with respiratory distress syndrome. Am J Respir Crit Care Med 1996; 153: 404-410.

[75] Moya FR, Gadzinowski J, Bancalari E, *et al.* A multicenter, randomized, masked, comparison trial of lucinactant, colfosceril palmitate, and beractant for the prevention of respiratory distress syndrome among very preterm infants. Pediatrics 2005; 115: 1018-1029.

[76] Sinha SK, Lacaze-Masmonteil T, Soler AVI, *et al*. A multicenter, randomized, controlled trial of lucinactant versus poractant alfa among very premature infants at high risk for respiratory distress syndrome. Pediatrics 2005; 115: 1030-1038.

[77] Wiswell TE, Knight GR, Finer NN, *et al*. A multicenter, randomized, controlled trial comparing surfaxin (lucinactant) lavage with standard care for treatment of meconium aspiration syndrome. Pediatrics 2002; 109: 1081-1087.

[78] Tanaka Y, Takei T, Aiba T, Masuda K, Kiuchi A, Fujiwara T. Development of synthetic lung surfactants. J Lipid Res 1986; 27: 475-485.

[79] Wu CW, Seurynck SL, Lee KYC, Barron AE. Helical peptoid mimics of lung surfactant protein C. Chem Biol 2003; 10: 1057-1063.

[80] Brown NJ, Wu CW, Seurynck-Servoss SL, Barron AE. Effects of hydrophobic helix length and side chain chemistry on biomimicry in peptoid analogues of SP-C. Biochemistry 2008; 47: 1808-1818.

[81] Brown NJ, Dohm MT, Bernardino de la Serna J, Barron AE. N-terminal alkylation improves biomimicry of a peptoid-based analogue of SP-C. Biophys J; accepted.

[82] Seurynck SL, Brown NJ, Wu CW, *et al*. Optical monitoring of bubble size and shape in a pulsating bubble surfactometer. J Appl Physiol 2005; 99: 624-633.

[83] Seurynck-Servoss SL, Dohm MT, Barron AE. Effects of including an N-terminal insertion region and arginine-mimetic side chains in helical peptoid analogues of lung surfactant protein B. Biochemistry 2006; 45: 11809-11818.

[84] Dohm MT, Seurynck-Servoss SL, Seo J, Zuckermann RN, Barron AE. Close mimicry of lung surfactant protein B by "clicked" dimers of helical, cationic peptoids. Biopolymers (Peptide Sci) 2009; 92: 538-553.

[85] Dohm MT, Brown NJ, Seurynck-Servoss SL, Bernardino de la Serna J, Barron AE. Mimicking SP-C palmitoylation on a peptoid-based SP-B analogue markedly improves surface activity. Biochim Biophys Acta- Biomem 2010; 1798: 1663-1678.

[86] Dohm MT, Mowery BP, Czyzewski AM, Stahl SS, Gellman SH, Barron AE. Biophysical mimicry of lung surfactant protein B by random nylon-3 copolymers. J Am Chem Soc 2010; 132: 7957-7967.

[87] Dohm MT, Barron AE. Surface activity determinants in peptoid-based biophysical mimicry of lung surfactant protein B. PLoS One; In Revision.

[88] Czyzewski AM, McCaig LM, Dohm MT, *et al.* The *in vivo* efficacy of biomimetic, peptoid-enhanced surfactants in treating acute lung injury. submitted.

[89] Kunin CM. Resistance to antimicrobial drugs--a worldwide calamity. Ann Intern Med 1993; 118: 557-61.

[90] Boucher HW, Talbot GH, Bradley JS, *et al.* Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis 2009; 48: 1-12.

[91] Glickman MS, Cahill SM, Jacobs WR. The mycobacterium tuberculosis cmaA2 gene encodes a mycolic acid trans-cyclopropane synthetase. J Biol Chem 2001; 276: 2228-2233.

[92] Hancock REW, Sahl HG. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol 2006; 24: 1551-1557.

[93] Zasloff M. Antimicrobial peptides of multicellular organisms. Nature 2002; 415: 389-395.

[94] Matsuzaki K. Why and how are peptide-lipid interactions utilized for self-defense? Magainins and tachyplesins as archetypes. Biochim Biophys Acta 1999; 1462: 1-10.

[95] Matsuzaki K, Murase O, Fujii N, Miyajima K. Translocation of a channel-forming antimicrobial peptide, magainin 2, across lipid bilayers by forming a pore. Biochemistry 1995; 34: 6521-6.

[96] Matsuzaki K, Sugishita K, Fujii N, Miyajima K. Molecular-Basis for membrane selectivity of an antimicrobial peptide, magainin-2. Biochemistry 1995; 34: 3423-3429.

[97] Subbalakshmi C, Sitaram N. Mechanism of antimicrobial action of indolicidin. FEMS Microbiol Lett 1998; 160: 91-6.

[98] Park CB, Kim HS, Kim SC. Mechanism of action of the antimicrobial peptide buforin II: Buforin II kills microorganisms by penetrating the cell membrane and inhibiting cellular functions. Biochem Biophys Res Commun 1998; 244: 253-257.

[99] Hale JD, Hancock REW. Alternative mechanisms of action of cationic antimicrobial peptides on bacteria. Expert Rev Anti Infect Ther 2007; 5: 951-959.

[100] Otvos Jr. L. Antibacterial peptides and proteins with multiple cellular targets. J Pept Sci 2005; 11: 697-706.

[101] Frantz MC, Wipf P. Mitochondria as a target in treatment. Environ Mol Mutag 2010; 51: 462-475.

[102] Hilpert K, McLeod B, Yu J, *et al.* Short cationic antimicrobial peptides interact with ATP. Antimicrob Agents Chemother 2010; 54: 4480-4483.

[103] Bessalle R, Kapitkovsky A, Gorea A, Shalit I, Fridkin M. All-D-magainin: chirality, antimicrobial activity and proteolytic resistance. FEBS Lett 1990; 274: 151-5.

[104] Brogden KA. Antimicrobial peptides: pore formers of metabolic inhibitors in bacteria? Nat Rev Microbiol 2005; 3: 238-250.

[105] Arnt L, Tew GN. New poly(phenyleneethynylene)s with cationic, facially amphipathic structures. J Am Chem Soc 2002; 124: 7664-7665.

[106] Porter EA, Wang X, Lee HS, Weisblum B, Gellman SH. Non-haemolytic beta-amino-acid oligomers. Nature 2000; 404: 565.

[107] Porter EA, Weisblum B, Gellman SH. Mimicry of host-defense peptides by unnatural oligomers: antimicrobial beta-peptides. J Am Chem Soc 2002; 124: 7324-30.

[108] Tew GN, Liu D, Chen B, *et al*. De novo design of biomimetic antimicrobial polymers. Proc Natl Acad Sci USA 2002; 99: 5110-4.

[109] Duclohier H. Antimicrobial peptides and peptaibols, substitutes for conventional antibiotics. Curr Pharm Des 2010; 16: 3212-3213.

[110] Ilker MF, Nusslein K, Tew GN, Coughlin EB. Tuning the hemolytic and antibacterial activities of amphiphilic polynorbornene derivatives. J Am Chem Soc 2004; 126: 15870-15875.

[111] Tang H, Doerksen RJ, Tew GN. Synthesis of urea oligomers and their antibacterial activity. Chemical Communications (Cambridge, England) 2005; 28: 1537-1539.

[112] Barron AE, Zuckermann RN. Bioinspired polymeric materials: in-between proteins and plastics. Curr Opin Struct Biol 1999; 3: 681-687.

[113] Kirshenbaum K, Zuckermann RN, Dill KA. Designing polymers that mimic biomolecules. Curr Opin Struct Biol 1999; 9: 530-535.

[114] Chongsiriwatana NP, Patch JA, Czyzewski AM, *et al.* Peptoids that mimic the structure, function, and mechanism of helical antimicrobial peptides. Proc Natl Acad Sci USA 2008; 105: 2794-2799.

[115] Patch JA, Barron AE. Helical peptoid mimics of magainin-2 amide. J Am Chem Soc 2003; 125: 12092-12093.

[116] Patch JA, Barron AE. Mimicry of bioactive peptides via non-natural, sequence-specific peptidomimetic oligomers. Curr Opin Chem Biol 2002; 6: 872-877.

[117] Czyzewski AM, Kapoor R, Chongsiriwatana NP, *et al.* Ampetoids as selective, broad-spectrum mimics of antimicrobial peptides. Antimicrob Agents Chemother; submitted.

[118] Czyzewski AM, Jenseen H, Dohm MT, *et al.* B. *In vitro* and *in vivo* antimicrobial activity of peptoids against multi-drug-resistant bacteria submitted.

[119] Makovitzki A, Avrahami D, Shai Y. Ultrashort antibacterial and antifungal lipopeptides. Proceedings of the National Academy of Sciences USA 2006; 103: 15997-16002.

[120] Chongsiriwatana NP, Miller TM, Wetzler M, *et al*. Short alkylated peptoid mimics of antimicrobial lipopeptides. Antimicrob Agents Chemother 2011; 55: 417-20.

[121] Kapoor R, Eimerman PE, Hardy HW, Cirillo J, Contag CH, Barron AE. Efficacy of antimicrobial peptoids against mycobacterium. Antimicrob Agents Chemother 2011; 55(6): 3054-7.

[122] Shai Y. Mode of action of membrane active antimicrobial peptides. Biopolymers 2002; 66: 236-48.

[123] Maulucci N, Izzo I, Bifulco G, *et al.* Synthesis, structures, and properties of nine-, twelve-, and eighteen-membered N-benzyloxyethyl cyclic alpha-peptoids. Chem Commun (Camb) 2008: 3927-9.

[124] Unciti-Broceta A, Diezmann F, Ou-Yang CY, Fara MA, Bradley M. Synthesis, penetrability and intracellular targeting of fluorescein-tagged peptoids and peptide-peptoid hybrids. Bioorg Med Chem 2009; 17: 959-66.

[125] Zhu WL, Park Y, Park IS, Park YS, Kim Y, Hahm KS, Shin SY. Improvement of bacterial cell selectivity of melittin by a single Trp mutation with a peptoid residue. Protein Pept Lett 2006; 13: 719-25.

[126] Zhu WL, Hahm KS, Shin SY. Cathelicidin-derived Trp/Pro-rich antimicrobial peptides with lysine peptoid residue (Nlys): therapeutic index and plausible mode of action. J Pept Sci 2007; 13: 529-35.

[127] Gobbo M, Benincasa M, Bertoloni G, *et al.* Substitution of the arginine/leucine residues in apidaecin Ib with peptoid residues: effect on antimicrobial activity, cellular uptake, and proteolytic degradation. J Med Chem 2009; 52: 5197-206.

[128] Park M, Jardetzky TS, Barron AE. NMEGylation as a potential method for enhancing the bioavailability of peptide-based therapeutics. Biopolymers (Peptide Sci) 2010; in revision.

[129] Park M, Matsuura H, Lamb RA, Barron AE, Jardetzky TS. A fluorescence polarization assay using an engineered human respiratory syncytial virus F protein as a direct screening platform. Anal Biochem In press.

[130] Goodson B, Ehrhardt A, Ng S, *et al.* Characterization of novel antimicrobial peptoids. Antimicrob Agents Chemother 1999; 43: 1429-34.

[131] Cuellar LE, Fernandez-Maldonado E, Rosenthal VD, *et al.* Device-associated infection rates and mortality in intensive care units of Peruvian hospitals: findings of the International Nosocomial Infection Control Consortium. Rev Panam Salud Publica 2008; 24: 16-24.

[132] Kola A, Schwab F, Barwolff S, *et al*. Is there an association between nosocomial infection rates and bacterial cross transmissions? Crit Care Med 2010; 38: 46-50.

[133] Kovaleva J, Meessen NE, Peters FT, *et al*. Is bacteriologic surveillance in endoscope reprocessing stringent enough? Endoscopy 2009; 41: 913-6.

[134] Mah TF, O'Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol 2001; 9: 34-9.

[135] Lewis K. Riddle of biofilm resistance. Antimicrob Agents Chemother 2001; 45: 999-1007.

[136] Aaron SD, Ferris W, Henry DA, Speert DP, MacDonald NE. Multiple combination bactericidal antibiotic testing for patients with cystic fibrosis infected with Burkholderia cepacia. Am J Respir Crit Care Med 2000; 161: 1206-1212.

[137] Anderson GG, Moreau-Marquis S, Stanton BA, O'Toole GA. *In vitro* analysis of tobramycin-treated Pseudomonas aeruginosa biofilms on cystic fibrosis-derived airway epithelial cells. Infect Immun 2008; 76: 1423-33.

[138] Killgore KM, March KL, Guglielmo BJ. Risk factors for community-acquired ciprofloxacin-resistant Escherichia coli urinary tract infection. Ann Pharmacother 2004; 38: 1148-52.

[139] Justice SS, Hung C, Theriot JA, *et al*. Differentiation and developmental pathways of uropathogenic Escherichia coli in urinary tract pathogenesis. Proc Natl Acad Sci USA 2004; 101: 1333-8.

[140] Kapoor R, Mayken WW, Czyzewski AM, Spormann AM, Barron AE. Antimicrobial peptoids are effective against *pseudomonas aeruginosa* biofilms. Antimicrob Agents Chemother 2011; 55(6): 3058-62.

[141] Numata M, Chu H, W. , Dakhama A, Voelker DR. Pulmonary surfactant phosphatidylglycerol inhibits respiratory syncytial virus-induced inflammation and infection. Proc Natl Acad Sci USA 2009; 107: 302-325.

[142] Lipinski CA. Drug-like properties and the causes of poor solubility and poor permeability. J Pharmacol Toxicol Methods 2000; 44: 235-49.

[143] Nguyen JT, Porter M, Amoui M, Miller WT, Zuckermann RN, Lim WA. Improving SH3 domain ligand selectivity using a non-natural scaffold. Chem Biol 2000; 7: 463-73.

[144] Nguyen JT, Turck CW, Cohen FE, Zuckermann RN, Lim WA. Exploiting the basis of proline recognition by SH3 and WW domains: design of N-substituted inhibitors. Science 1998; 282: 2088-92.

[145] Zuckermann RN, Martin EJ, Spellmeyer DC, *et al*. Discovery of nanomolar ligands for 7-transmembrane G-protein-coupled receptors from a diverse N-(substituted)glycine peptoid library. J Med Chem 1994; 37: 2678-85.

[146] Murphy JE, Uno T, Hamer JD, Cohen FE, Dwarki V, Zuckermann RN. A combinatorial approach to the discovery of efficient cationic peptoid reagents for gene delivery. Proc Natl Acad Sci USA 1998; 95: 1517-22.

[147] Gocke AR, Udugamasooriya DG, Archer CT, Lee J, Kodadek T. Isolation of antagonists of antigen-specific autoimmune T cell proliferation. Chem Biol 2009; 16: 1133-9.

[148] Lim HS, Archer CT, Kodadek T. Identification of a peptoid inhibitor of the proteasome 19S regulatory particle. J Am Chem Soc 2007; 129: 7750-1.

[149] Udugamasooriya DG, Dineen SP, Brekken RA, Kodadek T. A peptoid "antibody surrogate" that antagonizes VEGF receptor 2 activity. J Am Chem Soc 2008; 130: 5744-52.

[150] Udugamasooriya DG, Dunham G, Ritchie C, Brekken RA, Kodadek T. The pharmacophore of a peptoid VEGF receptor 2 antagonist includes both side chain and main chain residues. Bioorg Med Chem Lett 2008; 18: 5892-4.

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[151] Udugamasooriya DG, Ritchie C, Brekken RA, Kodadek T. A peptoid antagonist of VEGF receptor 2 recognizes a 'hotspot' in the extracellular domain distinct from the hormone-binding site. Bioorg Med Chem 2008; 16: 6338-43.

[152] Lynn KD, Udugamasooriya DG, Roland CL, Castrillon DH, Kodadek TJ, Brekken RA. GU81, a VEGFR2 antagonist peptoid, enhances the anti-tumor activity of doxorubicin in the murine MMTV-PyMT transgenic model of breast cancer. BMC Cancer 2003; 10: 397.

[153] Mowery BP, Lee SE, Kissounko DA, *et al*. Mimicry of antimicrobial host-defense peptides by random copolymers. J Am Chem Soc 2007; 129: 15474-6.

[154] Epand RF, Mowery BP, Lee SE, Stahl SS, Lehrer RI, Gellman SH. Dual mechanism of bacterial lethality for a cationic sequence-random copolymer that mimics host-defense antimicrobial peptides. J Mol Biol 2008; 379: 38-50.

[155] Mowery BP, Lindner AH, Weisblum B, Stahl SS, Gellman SH. Structure−activity relationships among random nylon-3 copolymers that mimic antibacterial host-defense peptides. J Am Chem Soc 2009; 131: 9735-45.

[156] Ryan MA, Akinbi HT, Serrano AG, *et al*. Antimicrobial activity of native and synthetic surfactant protein B peptides. J Immunol 2006; 176: 416-25.

[157] Wang Y, Lin H, Tullman R, Jewell CF, Jr., Weetall ML, Tse FL. Absorption and disposition of a tripeptoid and a tetrapeptide in the rat. Biopharm Drug Dispos 1999; 20: 69-75.