

DRUGGING THE “UNDRUGGABLE”

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I. INTRODUCTION

The stunning pace of advancement in uncovering the molecular basis for human disease has not been matched by a corresponding profusion of drugs that exploit these newly gained mechanistic insights. Though not one single factor is responsible for this lag in pharmaceutical innovation, there is good reason to believe that a major factor lies in the limited toolkit of molecules available to drug discoverers today. The great majority of approved drugs and investigational agents currently undergoing human clinical testing belong to two broad molecular classes, namely small molecules and biologicals. Small molecules typically possess fewer than a hundred atoms, with a molecular mass under 1000 daltons, whereas biologicals may possess thousands of atoms and may reach masses above 100,000 daltons. The limited size of small molecules can endow them with highly beneficial pharmaceutical properties, such as the ability to penetrate broadly and deeply into the tissue of living animals, and to diffuse passively across biological hindrances such as the outer cell membrane and the blood–brain barrier. But this economy of atoms also places significant limitations on the targeting ability of small molecules. With only a limited surface area available for engagement in energetically favorable contacts with a molecular target, small molecules require engulfment by their targets in order to maximize the total contact surface area. This simple biophysical imperative underlies the well-known principle that small molecules are, for the most part, capable of targeting only those proteins that possess a deep surface involution lined with hydrophobic

amino acid side chains, a feature commonly referred to as a “hydrophobic pocket.” How seriously does this restriction limit the utility of small molecules in drug discovery? While the answer to this question may never be known precisely, current estimates based on surface character analysis of proteins in the Protein Data Bank, and on empirical observations gained through high-throughput library screening, place the number of human proteins amenable to small molecule targeting at approximately 2000, a mere ~10% of the proteins encoded in the human genome (Hopkins and Groom, 2002; Russ and Lampel, 2005).

Unlike small molecules, biologicals possess large contact surface areas, and this largely frees them from restrictions on the types of target surfaces they can bind; protein:protein interaction surfaces vary widely in shape and chemical composition. The ability to select and evolve diversifiable protein scaffolds, initially monoclonal antibodies but now encompassing many different types of protein scaffolds (Binz et al., 2005; Skerra, 2007; Nuttall and Walsh, 2008; Stumpp et al., 2008), offers a virtually limitless palette of protein binders with which to target disease-relevant proteins. That said, protein therapeutics suffer from a targeting problem of their own, which imposes severe limitations on the scope of applicability: all but a few proteins lack the ability to traverse cell membranes, and therefore, the targeting range of biologics is essentially restricted to the subset of human proteins that are expressed on the cell surface or are secreted by cells. Current estimates, again imperfect but in the right ballpark, indicate that extracellular-accessible proteins comprise another ~10% of all human proteins (Verdine and Walensky, 2007).

Assuming generously that the ~10% of proteins with hydrophobic pockets is completely distinct from the ~10% that is accessible from outside the cell, one arrives at the shocking conclusion that only a meager ~20% of all human proteins are targetable by the two well-established targeting classes of molecules, and that an overwhelming majority of all prospective targets, greater than 80%, fall into the molecular limbo of targets currently considered “undruggable.” No area of therapy is left unscathed by the problem of undruggability. Cancer presents an especially compelling example, in part not only because of the urgency of the need for new treatments but also because of the vast and ever-accumulating body of human genetic and animal model data that together represent a smoking gun for the involvement of certain oncoproteins as indispensable drivers of tumor cell proliferation. The majority of these are

proteins that regulate transcription initiation and that participate in cell signaling via engagement of intracellular protein:protein interactions, two classes of proteins that are widely considered among the most intractable of undruggable targets. This intractability explains why K-Ras and cMyc, the two most widely activated oncoproteins in all of human cancer, today continue their lethal rampage with no impediment even remotely within sight. The enormous and ever-accumulating opportunity costs associated with the inability to target the 80% of intractable proteins continues to provide a powerful incentive for molecule discoverers to develop new ways to “drug the undruggable.”

The targeting deficiencies of small molecules and biologics result from factors that are fundamental to the chemical structures of these molecules. It thus stands to reason that new classes of molecules having fundamentally new targeting properties will be required to meet the challenge presented by intractable targets. It is worth noting that nucleic acid (NA) therapeutics such as antisense oligonucleotides and siRNAs offer considerable promise to access intractable targets by interfering with the activity not of the proteins themselves, but of the RNA encoding them. NA therapeutics have proven quite challenging to develop, primarily because they rarely show adequate levels of systemic bioavailability, but efforts to overcome this hurdle are both intensive and wide ranging.

Our own efforts toward drugging undruggable targets have focused on discovering new classes of molecules that we call “synthetic biologics” because they combine the versatile surface-recognition properties of biologics with the cell permeability and synthetic manipulability of small molecules. We further decided to focus our attention on the broad goal of creating molecules that could exert their actions directly at the level of intracellular protein:protein interactions. Numerous previous attempts have been made to target protein:protein interactions through structural mimicry (Hershberger et al., 2007) or fragment-based approaches (Bartoli et al., 2007; Hajduk and Greer, 2007); thus far, these efforts have met with limited success. We reasoned that nature had already spent billions of years to arrive at certain winning solutions to the problem of targeting proteins, and therefore, instead of attempting to mimic nature, we elected to borrow directly from it. Our synthetic biologics therefore represent stripped-down versions of proteins retaining primarily the structural elements that come into direct contact with a target. This exercise in protein

deconstruction is most straightforward to practice when most or all of the target contact region of a protein reside on one contiguous polypeptide chain because in such cases, the chain connectivity in the protein provides the connectivity in the peptide. Noncontiguous contact regions, on the other hand, require one to confront the difficult and largely unsolved problem of connecting the various fragmentary contact elements correctly and efficiently. The practical advantages of contiguous contact elements led us to focus on one particular element of protein secondary structure, the left-handed α -helix, a self-contained folding unit that is employed more widely than any other unit in intracellular protein:protein interactions (Henchey et al., 2008).

Past efforts at protein deconstruction have occasionally afforded the so-called “dominant negative” peptides useful for *in vitro* biochemical studies, but have rarely provided agents with robust activity in cellular assays or in animals. Why should the very same peptide sequence be potentially active when incorporated into a protein but devoid of activity when disincorporated from the protein? The answer to this is straightforward: the remainder of the protein provides a scaffold that stabilizes the bioactive conformation of the incorporated peptide, and consequently, disincorporation leads to unfolding of the peptide. Unfolding of the peptide typically renders it essentially devoid of biological activity, for several well-understood reasons. Firstly, whereas the folded contact region is typically pre-organized in its bioactive conformation, the unfolded peptide exists as an enormous mixture of rapidly interconverting species. Binding of the peptide comes at the significant entropic cost of ordering an inherently disordered molecule; hence, the disincorporated peptide binds the target much more weakly than the parent protein from which it was derived. Secondly, unfolding exposes the peptide to proteolytic inactivation. The large body of structural information available on proteases bound to substrates and inhibitors has revealed that, regardless of mechanistic class, all of these enzymes bind their substrates in an extended conformation, with the scissile amide bond fully engaged in the active site and disengaged from folding interactions. Folding thus protects polypeptides from proteolysis and unfolding exposes them to it. Thirdly, the amide bonds in a peptide each carry substantial dispersed charge, to which solvent water molecules are attracted and which pose a substantial impediment toward passive diffusion through the cell membrane. Folding of a peptide results in the shedding of amide-bound water molecules and

reduction of exposed charge in the backbone by internal charge pairing, both of which are expected to increase the probability of passive membrane diffusion. Any one of the problems caused by unfolding would be sufficient to render peptides unsuitable for most studies whose success hinges on activity in a biological setting. The combination of the three problems has had a devastating effect on the development of peptides as chemical genetic ligands and as drugs.

In considering potential solutions to the problems mentioned above, we were struck by the realization that all three – weak binding, high proteolytic susceptibility and poor cell penetration – were caused or exacerbated by unfolding, and thus, all might be addressed in one fell swoop by introducing a structural modification that restores the original bioactive conformation. We were not the first to make this realization; prior to our entry into the field, synthetic enforcement of α -helical structure had been demonstrated in several systems (Henchey et al., 2008), but to our knowledge, none of these had been demonstrated to provide the combination of high levels of helix induction, proteolytic stability and activity in cells. Some of these systems introduced functionality that seems to be incompatible, by design, with our ultimate objective of modulating intracellular protein:protein interactions; lactam bridges, for example, which would be expected to impede cell penetration, or disulfide linkages, which would be cleaved upon exposure to the reductive environment present inside cells.

Our design for an α -helix enforcement system (Schafmeister et al., 2000), now known as a helix “staple,” employs only membrane-compatible hydrocarbon atoms and combines two distinct and individually powerful modes of conformational bias toward a right-handed α -helical structure. One biasing element of the staple consists of an all-hydrocarbon cross-link attached to the peptide backbone so as to straddle either one or two successive turns of the helix (i and $i+4$ or $i+7$ positions, respectively); this provides a global macrocyclic constraint that extends over the entire cross-linked segment. The second biasing element of the staple consists of α -methylation at the same α -carbon atoms that serve as attachment points for the all-hydrocarbon cross-link; this introduces a local conformational bias on the peptide chain at the site of the α -methyl, α -cross-linked amino acid against non- α -helical torsion angles (Karle and Balaram, 1990; Marshall et al., 1990). The chemical composition of the stapling system was conceived and optimized in our lab by Chris

Schafmeister, who demonstrated that the staple could be introduced into peptides in a synthetically efficient and operationally simple manner by incorporating two α -methyl, α -n-alkenyl amino acids into the peptide at the i and either $i+4$ or $i+7$ positions, and then performing ruthenium-mediated olefin metathesis (Grubbs, 2004) on the resin-bound peptide to close the macrocyclic hydrocarbon cross-link (Schafmeister et al., 2000). We chose olefin metathesis for the ring-closing reaction because it is one of the very few C-C bond-forming reactions that can be performed efficiently and under mild conditions on highly functionalized molecules such as peptides (Miller et al., 1996), and it is the powerful combination of these attributes that earned the developers of the reaction, Grubbs, Schrock and Chauvin, the Nobel Prize in Chemistry in 2005. In studies on model peptides derived from RNase A, Schafmeister, and later Young Woo Kim, in our lab have found that the $i,i+4$ staple is formed most effectively and gives the greatest extent of helix stabilization when both amino acids bear the *S*-stereochemical configuration and the product contains an 8-carbon cross-link (formed using two units of *S*- α -methyl, α -n-pentenylglycine, designated **S5**); the $i,i+7$ staple is optimal when the N-terminal amino acid is *R*-configured and the C-terminal amino acid is *S*-configured, with an 11-carbon cross-link [usually formed using either one unit of *R*- α -methyl, α -n-octenylglycine (**R8**) and one of **S5**; or one each of **R5** and one of **S8**] (Schafmeister et al., 2000; Kim and Verdine, 2009). It should be noted that prior to our studies, Grubbs, O'Leary and coworkers had reported the use of olefin metathesis on peptides containing two O-allylated *L*-serine residues to introduce $i,i+4$ cross-links into peptides, but this particular system was found to enforce the $_{3,10}$ -helical conformation in solution, rather than the α -helical conformation (Blackwell et al., 2001; Boal et al., 2007).

The first application of our peptide stapling system to a biological problem was undertaken by Loren Walensky, an Oncology Fellow at the Dana-Farber Cancer Institute (DFCI) who created a vital bridge between our lab with that of Stan Korsmeyer at the Howard Hughes Medical Institute, DFCI and Harvard Medical School. The Korsmeyer lab had done seminal work on the mitochondrial events leading to the initiation of programmed cell death in human cells, work that had highlighted the importance of intracellular protein:protein interactions as mediators of apoptotic inhibition and activation (Korsmeyer, 1992; Chao and Korsmeyer, 1998; Gross et al., 1999; Danial and Korsmeyer, 2004). It occurred to us that stapled peptides might provide a unique opportunity

to study and modulate apoptotic signaling in human cells. Our focus was on proteins of the Bcl-2 family, members of which reside on the cytoplasmic face of the outer mitochondrial membrane. These structurally related proteins come in three distinct functional guises: anti-apoptotic members such as Bcl-2, Bcl-X_L and Mcl-1, which are required to activate apoptosis via permeabilization of the mitochondrial membrane; pro-apoptotic members such as Bax, Bak and Mcl-1 that serve to inhibit programmed cell death; and mediators such as Bid and Bim that act as receivers for apoptotic stimuli in the cell, and that transduce those signals through direct interactions with pro- and anti-apoptotic Bcl-2 family members (Walensky, 2006; Danial, 2007). These three families of protein constitute, in effect, a cell fate homeostat, the position of which determines whether a cell survives or undergoes programmed death. In the absence of apoptotic signals, the mediator proteins are primarily engaged with anti-apoptotic Bcl-2 family members, hence the cell survives; upon activation of apoptotic signals, the mediators then engage with pro-apoptotic Bcl-2 family members, and cell death ensues. The default position of the homeostat in normal cells is usually tipped toward survival, but a wide variety of developmental and environmental cues can reset the cell fate switch to favor death. For example, cells constantly monitor the state of their genomes, and under conditions in which genomic aberrations accumulate beyond a certain threshold level, apoptotic mediators become activated and cell death ensues. One of the most profound discoveries made by Stan Korsmeyer and his coworkers is that cancer cells can perturb the position of the cell death homeostat so as to acquire survivability despite having accumulated otherwise lethal levels of genomic aberrations. Specifically, it was found that follicular lymphoma cells contain a gene translocation results in constitutive overexpression of the *bcl-2* gene from the IgH promoter. These cells produce an abnormally large reservoir of the pro-survival Bcl-2 protein, which sequesters apoptotic mediators, thereby preventing them through mass action from productive engagement with pro-apoptotic family members (Korsmeyer, 1992). Thus, Bcl-2 overexpression causes derangement of cell fate homeostat so as to favoring survival in cells that should be undergoing apoptotic death. A large body of subsequent work has extended the generality of these initial observations, leading to the current view that most, if not all, transformed cells harbor derangements in apoptotic pathways that support tumor survival and growth by subverting programmed cell death (Hanahan and Weinberg, 2000).

We reasoned that it might be possible to reset the position of the apoptotic homeostat in cancer cells that overexpress Bcl-2 by interfering with the ability of Bcl-2 to bind apoptotic mediators such as Bid and Bim. A substantial body of structural work was available to guide this effort. All pro- and anti-apoptotic Bcl-2 family members contain three signature motifs, designated BH1, BH2 and BH3 (for Bcl-2 homology 1, 2 and 3). Apoptotic mediators, though structurally related to BH1,2,3 proteins, exhibit sequence similarity only in their BH3 motif (Petros et al., 2004; Walensky, 2006; Danial, 2007). The BH3 domain of these BH3-only apoptotic mediators present the majority of the contact surface responsible for binding to BH1,2,3 proteins, and peptides comprising just the BH3 domain of mediators bind weakly but specifically to BH1,2,3 proteins. Fesik and coworkers were the first to demonstrate that a BH3 peptide from BAK binds in a shallow cleft on Bcl-_{XL}, and that this binding event induces the folding of the BH3 peptide from a random coil into an extended α -helical structure (Sattler et al., 1997). Subsequent structural studies have shown that the BH3 domain indeed adopts an α -helical structure when incorporated into a BH3-only protein (Petros et al., 2004), which makes it virtually certain that the α -helix is the bioactive secondary structure for all BH3 domains in proteins and in peptides derived therefrom. Additional structures of BH3 domain peptides bound to anti-apoptotic BH1,2,3 proteins have revealed that these peptides binds in a structurally related cleft on the proteins (Petros et al., 2004). However, very recent studies have introduced a new wrinkle into this structural story by showing that the binding site employed by the Bid BH3 domain to interact with and thus activate Bax, a pro-apoptotic Bcl-2 family member, is completely distinct from the conserved cleft used by BH3 domains to contact anti-apoptotic Bcl-2 family members (Gavathiotis et al., 2008).

Taking all of the aforementioned factors into account, we set out to test the notion that stapling of a BH3 domain peptide might yield a stable, cell-permeable ligand capable of displacing BH3-only proteins from pro-survival Bcl-2 family members, thereby liberating the BH3-only protein to engage pro-death members of the family and consequently activate apoptosis. We initially selected the BH3 domain from Bid for stapling because Bid appeared to be able to interact with both pro- and anti-apoptotic receptors, and this increased the likelihood that notwithstanding the unknown complexities of apoptosis biology we would be

able to observe some sort of biological effect with the stapled peptide. To identify the optimal position in the 22-amino-acid Bid BH3 domain for incorporation of the staple, and the optimal type of staple, a small library of stapled peptides was constructed and individual members were assayed for various physical and functional properties such as percent helicity, solubility, Bcl-2 binding affinity, cell-permeability and so on. Based on the results from this screen, we selected the stapled peptide designated SAHB_A (stapled alpha helix of BH3, isomer A) for further investigation (Walensky et al., 2004).

Circular dichroism (CD) spectra of all α -helical proteins and peptides exhibit characteristic minima at wavelengths of 208 and 222 nm, and the intensity of these as a function of concentration can be used to determine the percent helicity at a given temperature. CD analysis of the unmodified Bid BH3 domain showed that it contained less than 20% helical character at room temperature. Introduction of a single $i,i+4$ staple into the center of this peptide, thus giving SAHB_A, increased the helicity to a value approaching 90% (Walensky et al., 2004). Though only a five-amino-acid stretch in SAHB_A comprises the stapled portion, the gain in helicity extends over nearly the entire 22 amino acids of the peptide, which provides strong evidence that the staple serves to nucleate the α -helical structure on both its N- and C-terminal flanking regions. Subsequently, studies on a diverse array of stapled peptides having different sequences and staple types have shown that the helix-nucleating influence of the staple can extend over stretches as long as ~ 20 amino acids, providing that they contain no helix-disrupting sequences. Resistance to thermal melting or guanidine denaturation provides another means to assess conformational stability in peptides and proteins. We found that whereas the unmodified Bid BH3 22-mer has a transition temperature for half melting (T_m) below 15°C, SAHB_A exhibited a T_m of 64°C. Introduction of the staple thus had the remarkable effect of conferring on a small BH3 peptide the extreme thermal stability of a thermophilic protein.

We next assayed the effect of stapling on the binding of the Bid peptides to Bcl-2 using fluorescence polarization spectroscopy. The unmodified Bid BH3 peptide bound Bcl-2 with an equilibrium dissociation constant (K_d) of ~ 270 nM, while SAHB_A bound with a K_d of ~ 40 nM, a ~ 7 -fold improvement (Walensky et al., 2004). In the design of SAHB_A, we had intentionally modified only the face of the BH3 helix that in the Bid protein were directed inward toward the packed protein core, and we

avoided modifying residues that projected outward from the protein surface. This being the case, the most likely source of the gain in binding affinity is from the entropic benefit of pre-organizing the peptide in its bioactive conformation. Nearly all of the stapled peptides studied in our lab to date show some gain in binding affinity, with the 7-fold effect seen with Bid on the lower end of the spectrum and the ~5000-fold gain seen with an hDM2-binding peptide being at the high end (Bernal et al., 2006). It could well be in the latter case that the staple not only pre-organizes the peptide in a helical conformation but also gains some affinity from direct contacts with hDM2.

If stapled peptides embody the target-binding domain of BH3-only proteins, then the peptides should recapitulate the binding specificity of the proteins from which they were derived. This was assessed by creating a panel of stapled BH3 peptides and measuring their binding affinities for a series of BH1,2,3 targets (Walensky et al., 2006). The stapled peptides indeed were found to exhibit specificities that closely mirrored those of their parent BH3-only proteins. For example, the stapled Bad BH3 peptide showed narrow specificity for only anti-apoptotic targets, much like the Bad protein, whereas the Bid and Bim stapled peptides exhibited the ability to target both pro- and anti-apoptotic Bcl-2 family members. Like the Bid and Bim proteins, the Bid stapled peptide did not target Mcl-1 effectively, whereas the Bim stapled peptide did. The observation that stapled Bid and Bim peptides could bind the pro-apoptotic Bax and Bax directly and specifically to activate apoptosis has helped to resolve a long-standing debate in the apoptosis community as to whether the corresponding BH3-only proteins act in a similar manner (Walensky et al., 2006). Recent structural studies by Walensky and coworkers have led to the remarkable observation that the binding site for the stapled Bid peptide – and by extension, the Bid protein – is on the opposite side of the Bad protein from the canonical BH3-binding pocket of anti-apoptotics, and this unexpected finding helps to resolve some of the confusion in the field concerning the mode of apoptosis induction by certain BH3-only proteins such as Bid and Bim (Gavathiotis et al., 2008).

Extensive work done by the Walensky lab has generated an invaluable panel of cell-permeable, bioactive stapled peptides representing virtually every known BH3 domain from a BH3-only protein, and these invaluable reagents promise to yield a treasure trove of information to elucidate the complexities of apoptosis biology (Bird et al., 2008; Pitter et al., 2008).

Stapled BH3 domain peptides may also hold promise as biological tools and human therapeutics outside of the apoptosis area. Nika Danial, in work begun while a Postdoctoral Fellow with Stan Korsmeyer and continued independently, has recently shown that the BH3-only protein Bad is a component of the mitochondrial glucokinase complex and is required to promote insulin secretion by pancreatic islet beta cells in response to exposure to elevated levels of glucose. This effect is stimulated by phosphorylation on the BH3 domain of Bad. Working in collaboration, the Danial and Walensky labs demonstrated that a phosphorylated, stapled version of the Bad BH3 domain exhibits the ability to recouple insulin secretion in response to glucose challenge in mouse $\text{Bad}^{-/-}$ islets (Danial et al., 2008). This work points to a potential therapeutic application of stapled Bad peptides in the treatment of type II diabetes.

The original Schafmeister design of stapled peptides aimed to increase the likelihood that these molecules would passively diffuse across the outer cell membrane by (1) utilizing only hydrophobic atoms in the staple, and (2) maximizing the internal hydrogen bonding of backbone amides (a consequence of maximizing α -helicity). When Loren Walensky performed our first analysis of cell permeability by a stapled peptide, namely SAHB_A, he found indeed that indeed the stapled peptide was cell permeable, whereas the corresponding unmodified Bid BH3 peptide was not (Walensky et al., 2004). He furthermore found that SAHB_A was localized to the mitochondrial membrane, in which the molecular targets of the stapled peptide reside; on the other hand, a control peptide bearing a point mutation that significantly weakened Bcl-2 binding also permeated cells but did not show mitochondrial localization. The fortunate surprise came when Walensky observed that the stapled peptide did not diffuse passively across the cell membrane but instead was actively imported into cells through an energy-dependent manner mediated by endocytic vesicles (Walensky et al., 2004). Also extremely important was the observation that the import vesicles do not seem to fuse with lysosomes, and they uncoat to disgorge their contents into the cytoplasm. Subsequent work in our labs, in the Walensky lab, at Aileron Therapeutics and in other labs has shown the active transport of stapled peptides to be remarkably general. Peptides having a wide variety of amino acid sequences, staple locations within the sequence and types of staple – $i,i+4$ and $i,i+7$ – utilize endocytic vesicle trafficking to gain entry into cells. Of course, not all of these are imported with equal efficiency, and in particular, basic stapled

peptides seem to be imported preferentially, while those having net negative charge are often taken up less efficiently (Bernal et al., 2006). For this reason, and also to promote aqueous solubility, it has become common practice in labs doing stapled peptide research to replace non-essential acidic residues with neutral or basic ones, and also to replace non-essential hydrophobic residues with polar or basic residues.

SAHB_A was found to induce apoptotic cell death in Jurkat cells and a panel of human leukemia cells at 2–10 mM concentrations, whereas the single-point mutant negative control peptide and the unmodified Bid BH3 peptide were completely inactive over this concentration range (Walensky et al., 2004). To assess the activity of SAHB_A in mouse tumor models, we teamed up with Andrew Kung, who engineered refractory RS4;1:1 human leukemia cells to express luciferase, and then transplanted these into γ -irradiated severe combined immunodeficient mice. The leukemia was allowed to engraft for 3 days, and then either SAHB_A or the inactive SAHB_A point mutant was administered by tail vein injection at 10 mg/kg/day for 3 days, and the tumor burden was assessed by whole body luminescence imaging using the Xenogen IVIS system (Caliper Life Sciences, Hopkinton, MA). A separate cohort of animals was treated with the Dimethylsulfoxide (DMSO) vehicle and imaged on the same schedule. The SAHB_A-treated animals showed a highly significant reduction in tumor burden and mean survival time as compared with the vehicle-treated animals, while the animals treated with the point mutant peptide fared little better than the vehicle-treated control (Walensky et al., 2004). These data provided compelling evidence that stapled peptides can exert a potent anti-tumor effect in a mouse model of cancer.

The pharmaceutical optimization of SAHB_A and related stapled BH3 domain peptides is ongoing, an effort being undertaken by Aileron Therapeutics. Among the numerous contributions made to stapled peptide development by scientists at Aileron is the discovery that relatively routine sequence optimization of these peptides can yield versions with half-lives of 12–24 hours in rats, with high-capacity but low-affinity binding to serum albumin, and having an *in vivo* clearance mechanism primarily consisting of hepatobiliary excretion rather than renal filtration, with no evidence of processing by cytochrome P450 oxidases (T. Sawyer and R. Kapeller, pers. comm.). Taken together, these pharmacological data paint a picture for stapled peptides that is completely distinct from the norm for peptides, one that bodes well for the emergence of this as an entirely new class of therapeutics for treatment of human disease.

If small molecules are applicable to ~10% of all human targets and biologicals are applicable to another 10%, what fraction of targets currently considered “undruggable” will become tractable with the advent of stapled peptides as drugs? The answer to this question, of course, remains to be determined, but it is not beyond the realm of possibility that stapled peptide technology could ultimately render another 10% of human targets druggable. Thus, the need to discover additional new classes of molecules beyond stapled peptides continues to remain an urgent one.

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