Kritzer, J.A. Stapled peptides: Magic bullets in nature's arsenal. *Nat. Chem. Biol.* **2010** *6*(8), 566-567. doi:10.1038/nchembio.407.

Magic bullets from nature's stockpile

Joshua A Kritzer

Selectivity is a key obstacle in drug development. A new study describes how "peptide stapling", a technique for making peptide alpha-helices more potent and cell-permeable, allows the design of MCL-1 inhibitors with extraordinary selectivity.

The field of medicinal chemistry was ignited by Ehrlich's concepts of cellular receptors and the "magic bullets" that could distinguish among them at the molecular level.[1] The current age of molecular medicine has brought the need for selectivity into atomic resolution.[2] Many of the most tantalizing drug targets are members of large families of proteins that share high structural and functional homology, making selectivity a difficult problem at the molecular level. In addition, selectivity becomes a fresh obstacle at advanced stages of the drug development pipeline because each advancement into a new animal models or human trials brings new possibilities for off-target interactions. Unanticipated problems with drug selectivity underlie many failures of drugs in clinical trials, making high selectivity a critical goal of preclinical drug research. In this issue, Walensky and co-workers report that the "peptide stapling" approach allows the design of highly selective compounds that inhibit the BCL-2 family protein MCL-1 with unprecedented selectivity.[3] These compounds will be particularly important in exploring new therapies for some intractable and chemoresistant cancers that are driven by MCL-1 overexpression.

The "peptide stapling" strategy for stabilizing peptide alpha-helices involves using a ring-closing metathesis reaction to make an all-hydrocarbon "staple" between successive turns of a peptide alpha-helix.[4-5] Helix stabilization by cross-linking had been shown previously to dramatically increase the structure and potency of alpha-helical peptides,[6] but installing an all-hydrocarbon cross-linker resulted in profound improvements in structure, potency, protease resistance, and (most surprisingly of all) cell permeability.[7] This strategy has been applied to targeting BCL-2 family proteins, a group of dominant activators and inhibitors of apoptosis that have high homology but different binding selectivities and often antagonistic functions.[8] The resulting stapled helices, called Stabilized Alpha-Helix of BCL-2 domains or SAHBs, bind BCL-2 domains and activate apoptosis *in vivo*,[7] dramatically demonstrating that stapled helices are indeed viable drug candidates for a wide variety of human cancers.

Reporting in this issue of Nature Chemical Biology, Stewart *et al.* describe the development of SAHBs that recapitulate the extraordinary binding selectivity of BCL-2 family members.[3] They were spurred by the observation that current small-molecule BCL-2 inhibitors are not sufficiently selective to target MCL-1, a BCL-2 protein linked to several classes of difficult-to-treat cancers. In order to develop SAHBs that selectively target MCL-1, the authors synthesized a panel of stapled alpha-helices corresponding to the helical interaction domains from a variety of BCL-2 family proteins. *In vitro* binding assays revealed that stapling the alpha-helix from MCL-1 itself provided an exquisitely selective inhibitor for MCL-1, with no detectable binding

affinity for several homologous BCL-2 family members. By testing a series of peptides in which the identities of peptide side chains and the location of the staple along the helix were varied, the authors demonstrated how the MCL-1-derived SAHBs are able to recognize MCL-1 so selectively and drew parallels to other BCL-2 family interactions.

Investigation of MCL-1 SAHB selectivity was then brought to the atomic level through the crystallization and structural determination of the complex between the MCL-1 binding pocket and their optimized SAHB, MCL-1 $SAHB_D$. The structure justifies the conclusions from the binding assays regarding how the MCL-1 SAHBs recognize MCL-1. In addition, the structure highlights that the careful optimization of the MCL-1 SAHBs led to unanticipated interactions between the staple itself and the surface of MCL-1, highlighting that alteration of the staple itself should be used in future iterations of the strategy to enhance potency and selectivity of stapled alpha-helices.

Finally, the authors demonstrate that the MCL-1 SAHBs function as MCL-1 antagonists in biological assays and in cell culture. They show that MCL-1 $SAHB_D$ displaces the normal MCL-1 binding partner *in vitro* and in cultured cells, sensitizes isolated mitochondria to cytochrome *c* release in an MCL-1-dependent manner, and sensitizes cultured cancer cells to MCL-1-dependent inducers of apoptosis. Doubtlessly, the next step is testing in mouse xenografts and other organism-based cancer models. These studies will complement continuing evaluations of other SAHBs as well as small molecules targeting the BCL-2 family for the treatment of cancer.[9]

Peptides are universally recognized as excellent biological probes but are often judged to be poor drug leads. However, many factors are converging to change this prejudice, including the emergence of biological therapeutics, new technologies for discovering potent bioactive peptides, cost-effective synthetic methods, and promising chemical methods for enhancing peptides such as through all-hydrocarbon stapling. The overall result is the sudden ability to target proteins like MCL-1 that were previously thought to be “undruggable”. As the present work demonstrates, the helix itself was magic enough – it just needed to be made into a bullet.

1 Ehrlich, P. *Beiträge zur Theorie und Praxis der histologischen Färbung*, Univ. Leipzig, (1878).

2 Strebhardt, K. & Ullrich, A. Paul Ehrlich's magic bullet concept: 100 years of progress. *Nat Rev Cancer* **8**, 473-480 (2008).

3 Stewart, M. L., Fire, E., Keating, A. E. & Walensky, L. D. *Nature Chemical Biology* **6**, xxx-yyy (2010).

4 Blackwell, H. E. & Grubbs, R. H. Highly efficient synthesis of covalently cross-linked peptide helices by ring-closing metathesis. *Angewandte Chemie-International Edition* **37**, 3281-3284 (1998).

5 Schafmeister, C. E., Po, J. & Verdine, G. L. An all-hydrocarbon cross-linking system for enhancing the helicity and metabolic stability of peptides. *Journal of the American Chemical Society* **122**, 5891-5892 (2000).

6 Taylor, J. W. The synthesis and study of side-chain lactam-bridged peptides. *Peptide Science* **66**, 49-75 (2002).

7 Walensky, L. D. *et al.* Activation of apoptosis in vivo by a hydrocarbon-stapled BH3 helix. *Science* **305**, 1466-1470 (2004).

8 Youle, R. J. & Strasser, A. The BCL-2 protein family: opposing activities that mediate cell death. *Nature Reviews Molecular Cell Biology* **9**, 47-59 (2008).
9 Lessene, G., Czabotar, P. E. & Colman, P. M. BCL-2 family antagonists for cancer therapy. *Nat Rev Drug Discov* **7**, 989-1000 (2008).

Figure legend: A primary goal of drug development is to design "magic bullets," compounds that selectively target only one of a closely related family of proteins. The BH3 helix from MCL-1 (brown helix) is naturally selective for the MCL-1 helix-binding domain (tan surface). The authors used helix stapling to create a cell-permeable Stabilized Alpha Helix of BCL-2 (SAHB) molecule that targets the MCL-1 protein with magic bullet-like selectivity.

Joshua A. Kritzer is in the Department of Chemistry, Tufts University, Medford, Massachusetts, USA. E-mail: joshua.kritzer@tufts.edu