

## AILERON Therapeutics

*Profile:* AILERON Therapeutics is a privately held biopharmaceutical company located in Cambridge, Massachusetts, USA. AILERON Therapeutics is developing a revolutionary class of drugs, called Stapled Peptides, that have the capability to address thousands of currently 'undruggable' therapeutic targets and promising clinical applications for many human diseases.

*Website:* <http://www.aileronrx.com>

### About AILERON Therapeutics

AILERON Therapeutics was founded in 2005 to develop and advance a new therapeutic modality leveraging 'Stapled Peptides' as a revolutionary class of drugs. AILERON's novel Stapled Peptides have unique chemical, biological and structural properties to address both intracellular and extracellular protein–protein interactions that serve as critical control points in disease mechanisms, including cellular survival/death, signal transduction and gene regulation. Noteworthy, such protein–protein interactions have eluded small-molecule strategies, except for limited success requiring extraordinary tour de force campaigns. AILERON's proprietary technology platform converts peptides into their biologically competent  $\alpha$ -helical shape which endows the Stapled Peptide with inimitable properties, including efficient cell penetration, high affinity binding to large target protein surfaces, and remarkable metabolic stability and pharmacokinetic properties *in vivo*. AILERON's R&D programs are deep-rooted in disease mechanisms pioneered by its founding scientists from Harvard University and the Dana-Farber Cancer Institute, namely, Stanley Korsmeyer, Gregory Verdine and Loren Walensky (1–10). A robust, multi-targeted disease strategy has been established to expand on AILERON's lead R&D program that is well poised to advance the first-in-class Stapled Peptide clinical candidate for the treatment of cancer.

### Drug Discovery: Tackling 'Undruggable' Therapeutic Targets

Relative to existing drug discovery approaches, the pharmaceutical industry has created a vast chemical collection of small molecules and an emerging arsenal of 'biologics', including proteins (e.g. antibodies), peptides (e.g. hormones, growth factors, cytokines) and nucleic acids (e.g. siRNAs). Collectively, approximately 10–20% of known therapeutic targets (i.e. ~500 of about 5000–10 000 mapped from the human genome) have been addressed by such efforts. Beyond the identification of potential therapeutic targets, it is critically important to unravel the mechanisms of disease for drug

discovery. Importantly, a considerable portion of the genomic universe of therapeutic targets is yet viewed to be 'undruggable' by existing small molecule and biologic modalities. In particular, intracellular protein–protein interactions are an extraordinary challenge for existing small molecule and biologic modalities. In particular, the large binding surfaces for intracellular protein–protein interactions generally exclude small-molecule modulators from being effective. Furthermore, although traditional peptides and proteins may have the chemical size and functionality to effectively modulate intracellular protein–protein interactions, such biologics do not possess cell-penetrating properties and are, therefore, used primarily to modulate extracellular therapeutic targets (e.g. receptors). Thus, an almost unimaginable opportunity for tackling 'undruggable' therapeutic targets has remained quiescent throughout our modern time of drug discovery. Is there yet a solution? Yes, indeed, and given the fact that a vast number of 'undruggable' therapeutic targets are known to include protein–protein interactions that involve  $\alpha$ -helical type lock-and-key mechanisms, the solution has been astonishingly obvious: design  $\alpha$ -helical peptides having both structural and functional properties that enable them to penetrate into the cell, bind to the therapeutic target, and modulate the biological pathway in the desired way (Scheme 1). Several proof-of-concept studies showing that Stapled Peptides successfully modulate protein–protein interactions (intracellular and extracellular) have been described relative to Bcl-2 family of apoptotic proteins (1,4,7–9; for reviews: 3,5), glucokinase (2), p53 transcription factor (6), NOTCH (11), HIV capsid (12) and HIV gp41-cell fusion (13). It is predicted that about 1500–3000  $\alpha$ -helical type protein–protein interaction therapeutic targets exist and are intimately involved in a wide scope of human diseases.

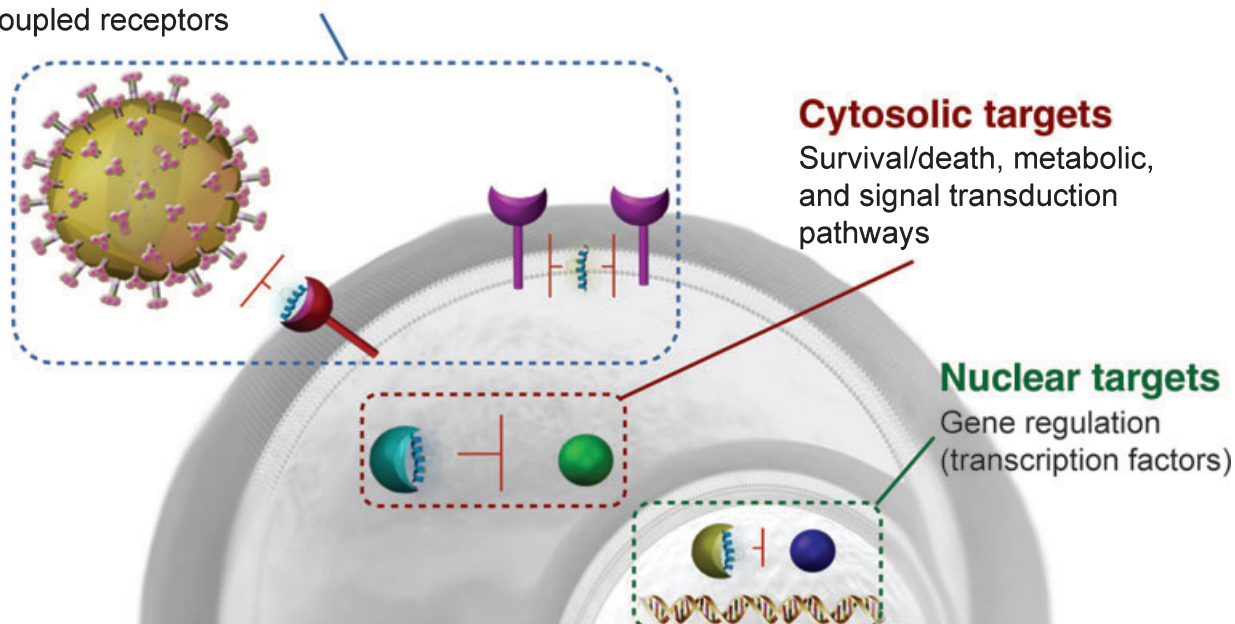
### Innovative Technologies: Designing Stapled Peptide Drugs

Peptides are a proven class of medicines, with more than 40 marketed drugs and about 300 molecules in clinical trials. Despite these achievements, peptide drugs have been limited to extracellular therapeutic targets (e.g. receptors), and only as a result of significant transformation of their chemical structures have second-generation peptidomimetic drugs been advanced for specific intracellular therapeutic targets (e.g. proteases) (14). Not surprisingly, a foggy distinction between peptidomimetics and small molecule drugs exists from a chemical perspective, especially in those cases where the peptide scaffold has undergone extensive modifications or replacement. In contrast, Stapled Peptide analogs of  $\alpha$ -helical motifs of 'protein keys' involved in specific intracellular protein–protein interactions are acknowledged as a revolutionary and promising class of peptide drugs endowed with a

A

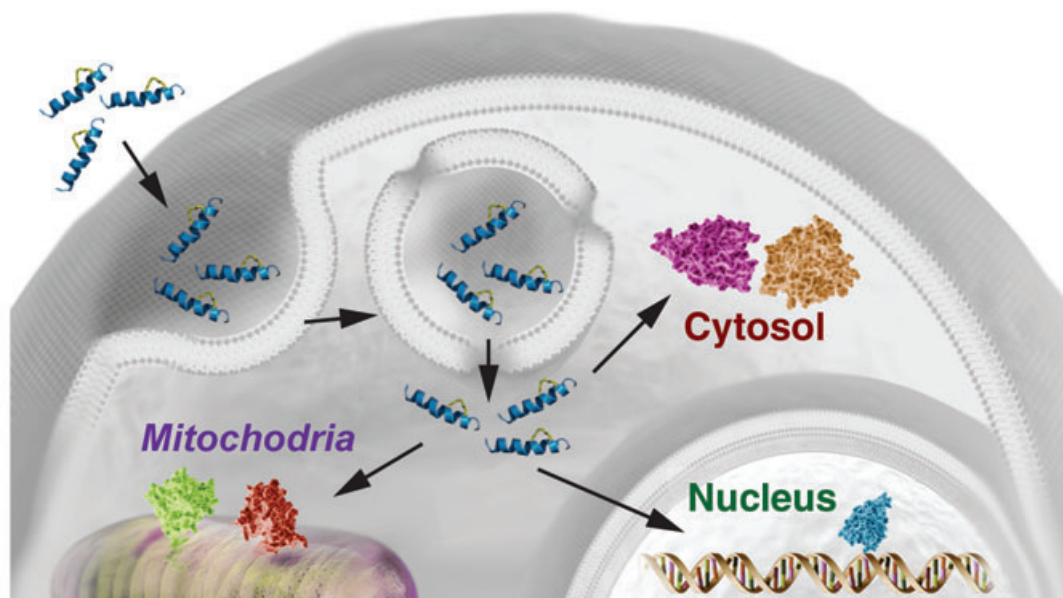
## Cell surface and plasma membrane targets

Viral, cytokine, chemokine, growth factor and G-protein coupled receptors



## Proposed model of cellular penetration by stapled peptides

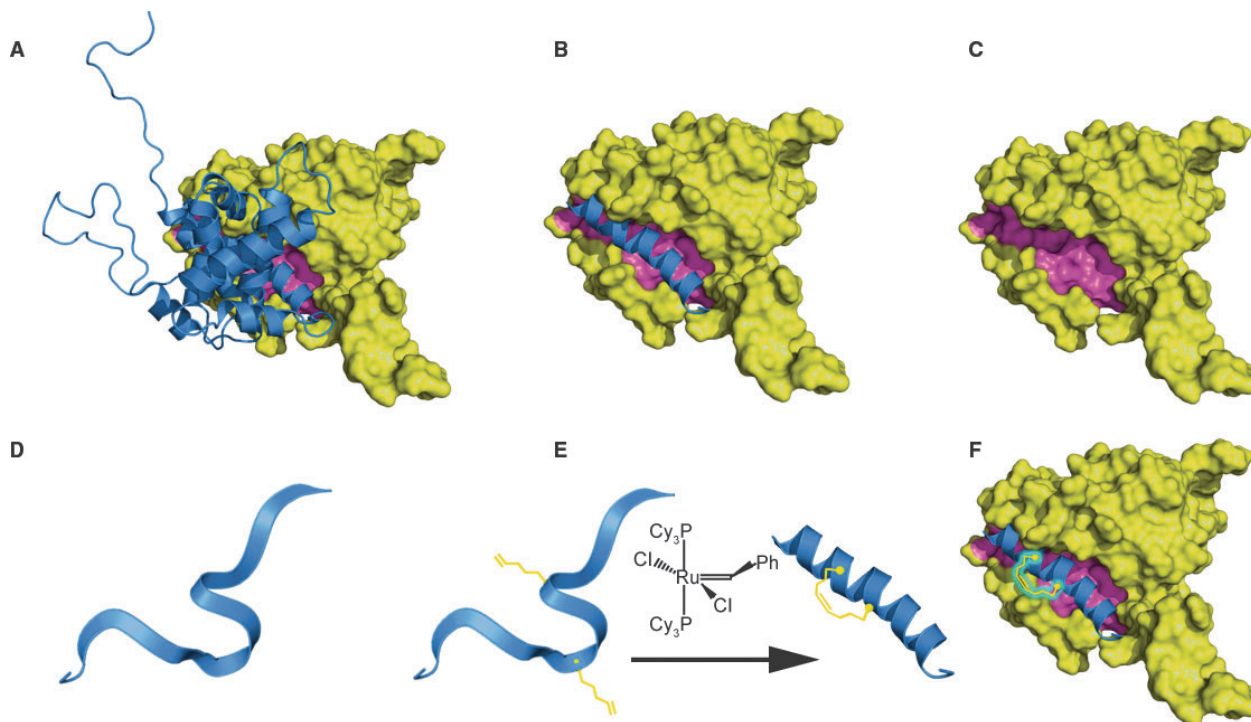
B



**Scheme 1:** (A) Examples of therapeutic targets and pathways for Stapled Peptides. (B) A proposed model of cellular penetration by Stapled Peptides.

unique combination of cell penetrating, metabolic stability and pharmacokinetic properties (15). Stapled  $\alpha$ -helical peptides (Figure 1) exploit chemical stabilization of secondary structure by way of ring-closing metathesis via side-chain or backbone functionalities (9,10–13,16–18,20–22 for reviews 4,19). By leveraging novel

building blocks and macrocyclization chemistries along with sophisticated drug design, molecular informatics and biophysical tools, AILERON's innovative technologies (23) are creating a molecular armamentarium of stapled  $\alpha$ -helical peptides for a plethora of therapeutic targets.



**Figure 1:** Structural model for helical protein–protein interaction and drug design strategies to generate novel Stapled Peptides. (A) Protein–protein interactions leverage  $\alpha$ -helical molecular recognition ('lock' and 'key'). (B) The  $\alpha$ -helical motif or 'key' exploits multiple hydrophobic, H-bonding and ionic interactions with a large and oftentimes flat binding surface or 'lock'. (C) The  $\alpha$ -helical binding surface, with limited exceptions, is viewed to be 'undruggable' by traditional small-molecule ligands. (D) The  $\alpha$ -helical motif as extracted from the cognate protein is not conformationally stabilized, but is susceptible to proteolytic cleavage and lacks cell penetrating properties. (E) The  $\alpha$ -helical motif as a synthetically modified macrocyclic peptide using ruthenium catalyzed ring-closing metathesis is conformationally and proteolytically stabilized. The Stapled Peptide is also endowed with remarkable cell-penetrating and pharmacokinetic properties. (F) The Stapled Peptide presents the  $\alpha$ -helical motif to effectively bind to its cognate target with high affinity and selectivity.

**Table 1:** Examples of promising therapeutic targets involving  $\alpha$ -helical protein–protein interactions that have been shown to be effectively modulated by Stapled Peptides *in vitro* and/or *in vivo*

Target class	Therapeutic target	Helical peptide	Biological mechanism(s)	Clinical indication(s)
Bcl-2 Family	BAX, BAK, Bcl-2, Mcl-1	BH3 $\alpha$ -helix (e.g. BIM, BID)	Activation of cell death	Cancer, autoimmunity
Kinase	Glucokinase	BAD BH3 $\alpha$ -helix (pSer form)	Activation of glucokinase	Diabetes
Transcription factor	MDM2-p53	P53 $\alpha$ -helix	Activation of cell death	Cancer
	NOTCH-CSL-MAML	MAML $\alpha$ -helix	Activation of cell death	Cancer (including cancer stem cells), cardiovascular disease
Viral protein	HIV-1 capsid assembly	Capsid $\alpha$ -helix	Inhibition of HIV particle assembly	HIV (AIDS)
	HIV-1 Gp41–CD4	Gp41 HR2 $\alpha$ -helix	Inhibition of HIV fusion	HIV (AIDS)

## Research and Development: from Concept to Clinic

AILERON's R&D programs embrace both scope in terms of its technology platform and depth with respect to advancing its preclinical research toward achieving the first-in-class Stapled Peptide clinical candidate for cancer therapy. In the latter case, a series of novel Stapled Peptides targeting the Bcl-2 family of apoptotic proteins have shown promising *in vivo* efficacy in hematologic cancer and solid tumor models (24,25). As

exemplified by initial proof-of-concept studies described from academic researchers on Stapled Peptides showing the potential application to multiple disease areas (e.g. cancer, diabetes, inflammation and infectious diseases) (Table 1), AILERON'S treasury of therapeutic targets for drug discovery is continuously growing. There is a worldwide paradigm shift in many pharmaceutical companies towards balancing their pipelines of traditional small molecule drugs with emerging biologic drugs, and AILERON'S Stapled Peptides provide tremendous opportunities for strategic partnerships in both research and development toward

## R&D Spotlight

realizing their promising clinical applications for many human diseases.

Tomi K. Sawyer

Chief Scientific Officer and Senior Vice-President, Drug Discovery and Innovative Technologies, AILERON Therapeutics, Cambridge, MA, USA

## References

1. Gavathiotis E., Suzuki M., Davis M.L., Pitter K., Bird G.H., Katz S.G., Tu H.-C., Kim H., Cheng E.H.Y., Tjandra N., Walensky L.D. (2008) BAX activation is initiated at a novel interaction site. *Nature*;455:1076–1081.
2. Danial N.N., Walensky L.D., Zhang C.Y., Choi C.S., Fisher J.K., Molina A.J., Datta S.R. *et al.* (2008) Dual role of proapoptotic BAD in insulin secretion and beta cell survival. *Nature Med*;14:144–153.
3. Pitter K., Bernal F., Labelle J., Walensky L.D. (2008) Dissection of the BCL-2 family signaling network with stabilized alpha-helices of BCL-2 domains. *Methods Enzymol*;446:387–408.
4. Bird G.H., Bernal F., Pitter K., Walensky L.D. (2008) Synthesis and biophysical characterization of stabilized alpha-helices of BCL-2 domains. *Methods Enzymol*;446:369–386.
5. Verdine G.L., Walensky L.D. (2007) The challenge of drugging undruggable targets in cancer: lessons learned from targeting BCL-2 family members. *Clin Cancer Res*;13:7264–7270.
6. Bernal F., Tyler A.F., Korsmeyer S.J., Walensky L.D., Verdine G.L. (2007) Reactivation of the p53 tumor suppressor pathway by a stapled p53 peptide. *J Am Chem Soc*;129:2456–2457.
7. Walensky L.D., Pitter K., Morash J., Oh K.J., Barbuto S., Fisher J., Smith E., Verdine G.L., Korsmeyer S.J. (2006) A stapled BID BH3 helix directly binds and activates BAX. *Mol Cell*;24:199–210.
8. Walensky L.D. (2006) BCL-2 in the crosshairs: tipping the balance of life and death. *Cell Death Differ*;13:1339–1350.
9. Walensky L.D., Kung A.L., Escher I., Malia T.J., Barbuto S., Wright R.D., Wagner G., Verdine G.L., Korsmeyer S.J. (2004) Activation of apoptosis in vivo by a hydrocarbon-stapled BH3 helix. *Science*;305:1466–1470.
10. Schafmeister C.E., Po J., Verdine G.L. (2000) An all-hydrocarbon cross-linking system for enhancing the helicity and metabolic stability of peptides. *J Am Chem Soc*;122:5891–5892.
11. Bradner J., Moellering R., Verdine G.L. (2008) Stabilized MAM Peptides and Uses Thereof. International Patent Application WO-2008/061192-A2. Geneva, Switzerland: World Intellectual Property Organisation.
12. Zhang H., Zhao Q., Bhattacharya S., Waheed A.A., Tong X., Hong A., Heck S., Curreli F., Goger M., Cowburn D., Freed E.O., Debnath A.K. (2008) A cell-penetrating helical peptide as a potential HIV-1 inhibitor. *J Mol Biol*;378:565–580.
13. Wang D., Lu M., Arora P.S. (2008) Inhibition of HIV-1 fusion by hydrogen-bond-surrogate-based alpha helices. *Angew Chem Int Ed Engl*;47:1879–1882.
14. Sawyer T.K. (2005) Synthetic peptides: chemistry, biology and drug design. In: Meyers R.A., editor. *Encyclopedia of Molecular Cell Biology and Molecular Medicine*, Vol. 14. New York: Wiley-VCH Publishers, Inc; p. 91–122.
15. Drahl C. (2008) Harnessing helices: chemical braces hold peptides in place, heralding a potential new class of therapeutics. *Chem Eng News*;86:18–23.
16. Patgiri A., Jochim A.L., Arora P.S. (2008) A hydrogen bond surrogate approach for stabilization of short peptide sequences in alpha-helical conformation. *Acc Chem Res*;41:1289–1300.
17. Liu J., Wang D., Zheng Q., Lu M., Arora P.S. (2008) Atomic structure of a short alpha-helix stabilized by a main chain hydrogen-bond surrogate. *J Am Chem Soc*;130:4334–4337.
18. Bhattacharya S., Zhang H., Debnath A.K., Cowburn D. (2008) Solution structure of a hydrocarbon stapled peptide inhibitor in complex with monomeric C-terminal domain of HIV-1 capsid. *J Biol Chem*;283:16274–16278.
19. Henchey L.K., Jochim A.L., Arora P.S. (2008) Contemporary strategies for the stabilization of peptides in the alpha-helical conformation. *Curr Opin Chem Biol* (in press).
20. Wang D., Chen K., Kulp J.L., III, Arora P.S. (2006) Evaluation of biologically relevant short alpha-helices stabilized by a main-chain hydrogen-bond surrogate. *J Am Chem Soc*;128:9248–9256.
21. Wang D., Chen K., Dimartino G., Arora P.S. (2006) Nucleation and stability of hydrogen-bond surrogate-based alpha-helices. *Org Biomol Chem*;4:4074–4081.
22. Chapman R.N., Dimartino G., Arora P.S. (2004) A highly stable short alpha-helix constrained by a main-chain hydrogen-bond surrogate. *J Am Chem Soc*;126:12252–12253.
23. Sawyer T.K. (2008) Stapled Peptides: A New Therapeutic Modality. Waltham, MA: New England Structural Biology Association's Second Annual Protein Science Meeting. Keynote Lecture.
24. Kapeller R., Nash H.M., Kung A.L., Sawyer T.K. (2008) BH3-Stapled Helical Peptides: A Novel Class of Biologic Drugs for Cancer Therapy. San Diego, CA: American Association of Cancer Research Annual Meeting. Poster Abstract No. LB-230.
25. Kapeller R., Han J., Sun K., Gangurde P., Kawahata N., Iadanza M., Guerlevais V., Horstick J., Noehre J., Annis A., Licklider L., Nash H.M., Kung A.L., Sawyer T.K. (2008) Stapled Peptides: Leveraging the BH3  $\alpha$ -Helix to Create a New Class of Drugs to Treat Hematological Malignancies. San Francisco, CA: American Society of Hematology 50th Annual Meeting. Poster Abstract No. 2929.