SciBX
Science-Business eXchange

FROM THE MAKERS OF BioCentury® AND nature

REPRINT FROM JANUARY 26, 2012

Macrocycles by the trillions

By Joanne Kotz, Senior Editor

Japanese researchers have developed a method for creating large libraries of N-methylated peptide macrocycles,[1] a class of molecules that are sized between small molecules and biologics and have the potential to combine the pharmacological versatility of the former and the therapeutic specificity of the latter. **PeptiDream Inc.** has exclusively licensed the technology and entered collaborations with six pharmas.

Small molecules are typically less than 500 Da in size, whereas biologics start at approximately 5,000 Da and cover a range of one to two orders of magnitude. Macrocycles, a structure frequently found in natural products, range in size from 500–2,000 Da.

Due to their structural rigidity and high target affinity, the hope has been for these compounds to be able to modulate challenging targets typically reserved for biologics, such as protein-protein interactions, while retaining the advantages of small molecules—cell permeability and oral bioavailability.[2]

One challenge has been generating sufficiently large and diverse libraries of synthetic macrocycles to identify drug leads. To overcome this hurdle, a team led by Hiroaki Suga has now merged two technologies—one developed by Suga in 2008 to incorporate non-natural amino acids into large libraries of peptide macrocycles[3] and the other developed independently in 1997 by Jack Szostak and Hiroshi Yanagawa for displaying natural peptide and protein libraries on mRNA.[4,5]

Suga is a professor in the Department of Chemistry at **The University of Tokyo**. Yanagawa is a professor at **Keio University**. Szostak is a professor at **Harvard Medical School** and **Massachusetts General Hospital**.

The integrated method, called RaPID, starts from a cDNA library encoding non-natural peptides of 8–15 residues. The peptides contain a random mixture of 12 natural amino acids and 4 N-methylated (non-natural) amino acids. Non-natural amino acids can increase the types of chemical groups in peptides beyond what is offered by natural amino acids. In particular, N-methylated amino acids increase a peptide's cell permeability.

The cDNA library is then transcribed to an mRNA library and linked to a second mRNA oligonucleotide that ends in a puromycin residue. The residue causes the growing peptide chain to be covalently connected to and displayed by its own mRNA template. *In vitro* translation of these mRNAs resulted in a library of about 1,012 N-methylated peptide macrocycles displayed on mRNA (*see* **Figure 1, "Making macrocycles RaPID-ly"**).

As proof of principle, the Japanese team used the library to select for macrocycles that bound the ligase domain of ubiquitin protein ligase E3A (UBE3A; E6AP). Identifying potent and selective inhibitors that interfere with the protein-protein interactions made by ubiquitin ligases has proven challenging. The team identified three distinct macrocycles that each bound to E6AP with low or subnanomolar affinity.

Results were published in *Chemistry & Biology*.

"This is a very clever technology. Synthetic tRNAs to introduce new amino acids in combination with mRNA allows you to create vast libraries of novel peptides," said Nick Terrett, CSO of macrocycle company **Ensemble Therapeutics Corp.**

To isolate drugs with more complex function, such as disrupting protein-protein interactions, researchers are turning to larger molecules like peptides. "The real value of [this] work is that extremely large and diverse libraries can be produced and used in affinity-based selections, which greatly increases the likelihood that highly potent protein-protein interaction inhibitors can be isolated. These arguments also apply to cases where highly specific drugs are needed, for example, when attempting to inhibit one member in a family of closely related proteins," said Douglas Treco, president, CEO and cofounder of **Ra Pharmaceuticals Inc.**

Ra Pharma is using *in vitro* display technologies to produce libraries of cyclic peptidomimetics to identify therapeutics in a variety of diseases. The company was cofounded by Szostak.

"The affinities described for the ubiquitin ligase are impressive. It will be key to demonstrate that high-affinity binders can be isolated for a broad range of targets," said Christian Heinis, assistant professor in the Institute of Chemical Sciences and Engineering at the **Swiss Federal Institute of Technology Lausanne**. "I like the RaPID approach very much since it allows the facile incorporation of non-natural amino acids."

Heinis is a cofounder of **Bicycle Therapeutics Ltd.**, a company developing cyclic peptide therapeutics using phage display.

However, Terrett said the molecules generated by RaPID could be too big to become drug leads. "The molecular weight is too high," as the macrocycles described in the paper were around 2,000 Da, he said. "My guess would be that cell membrane permeability and the *in vivo* bioavailability would be low."

Ensemble's macrocycles, which Terrett said are typically cell permeable, are in the range of 600–1,000 Da. For example, Ensemble's macrocyclic IL-17 antagonists are 700 Da and have low nanomolar potency. The compounds are in preclinical development for autoimmune and inflammatory diseases.

Ensemble synthesizes macrocycles using a process called DNA-programmed chemistry that relies on DNA as a template but generates

SciBX
Science-Business eXchange

EDITORIAL
Editor-in-Chief: Karen Bernstein, Ph.D.
Executive Editors: Gaspar Taroncher-Oldenburg, Ph.D.; Steve Edelson
Editors: Susan Schaeffer; Tracey Baas, Ph.D.
Writers: Jesse Beckstein; Aaron Bouchie; Michael Flanagan; Tim Fulmer, Ph.D.; Michael Haas; Stephen Hansen; Kai-Jye Lou; Lauren Martz; Brian Moy; Lev Osherovich, Ph.D.; Steve Usdin
Research Director: Walter Yang
Research Manager: Kevin Lehnbeuter
Managing Production Editor: Ingrid McNamara
Senior Production Editor: Brandy Cafarella
Production Editor: Amanda Crawford
Copy Editor: Nicole DeGennaro
Editorial Assistant: Mark Zipkin
Design: Claudia Bentley; Miles Davies
For inquiries, contact editorial@scibx.com

PUBLISHING
Publisher: Peter Collins, Ph.D.
Associate Publishers: Melanie Brazil, Ph.D.; Eric Pierce
Marketing: Sara Girard; Tim Tulloch
Technology: Anthony Barrera; Julia Kulikova
Sales: Diana Cabral; John McGuire; Geoff Worton

OFFICES
BioCentury Publications, Inc.
San Francisco
PO Box 1246
San Carlos, CA 94070-1246
T: +1 650 595 5333

Chadds Ford
223 Wilmington-West Chester Pike
Chadds Ford, PA 19317
T: +1 610 558 1873

Chicago
20 N. Wacker Drive, Suite 1465
Chicago, IL 60606-2902
T: +1 312 755 0798

Oxford
287 Banbury Road
Oxford OX4 7JA
United Kingdom
T: +44 (0)18 6551 2184

Washington, DC
2008 Q Street, NW, Suite 100
Washington, DC 20009
T: +1 202 462 9582

Nature Publishing Group
New York
75 Varick Street, 9th Floor
New York, NY 10013-1917
T: +1 212 726 9200

London
The Macmillan Building
4 Crinan Street
London N1 9XW
United Kingdom
T: +44 (0)20 7833 4000

Tokyo
Chiyoda Building 6F
2-37 Ichigayatamachi
Shinjuku-ku, Tokyo 162-0843
Japan
T: +81 3 3267 8751

SciBX is produced by BioCentury Publications, Inc. and Nature Publishing Group Joint Steering Committee: Karen Bernstein, Ph.D., Chairman & Editor-in-Chief, BioCentury; David Flores, President & CEO, BioCentury; Bennet Weintraub, Finance Director, BioCentury; Steven Inchcoombe, Managing Director, Nature Publishing Group; Peter Collins, Ph.D., Publishing Director, NPG; Richard Hartgill, Chief Financial Officer, NPG.

macrocycles using synthetic rather than peptide building blocks. "We are also using a purely synthetic process, so there is a lot of chemistry we can incorporate," noted Terrett.

According to Terrett, Ensemble has generated a cumulative library of about 4 million macrocycles. He said Ensemble frequently has been able to find compounds with "micromolar to nanomolar potency against previously undruggable targets."

Ensemble recently extended their partnership with **Bristol-Myers Squibb Co.** to develop macrocycles against undisclosed targets.[6]

Terrett suggested adapting the RaPID technology "to make compounds that are smaller from the get-go. If you could make smaller cyclic peptides, you'd be at a better starting point for drug discovery. The compromise would be lower structural diversity. It's a trade-off."

PeptiDreaming

PeptiDream, which was founded by Suga, is identifying macrocyclic drug leads using technology that is similar to RaPID but with a different,

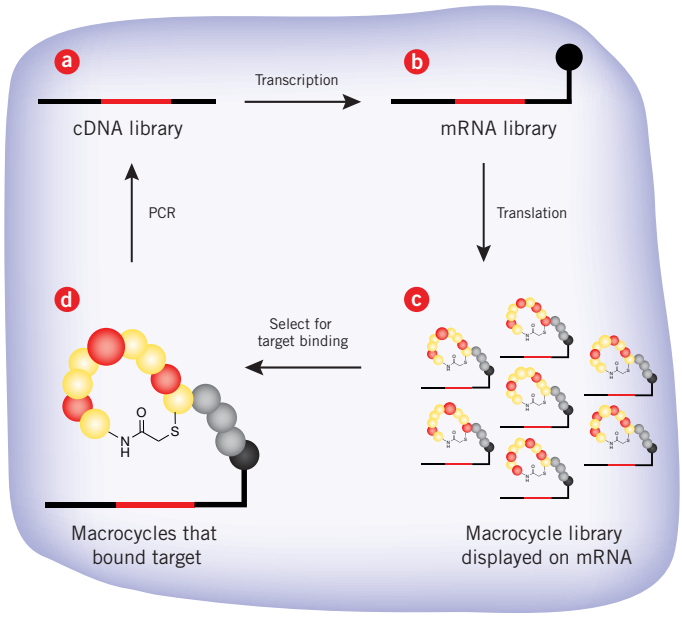

Figure 1. Making macrocycles RaPID-ly. A team from **The University of Tokyo** has developed a method called RaPID for generating libraries of trillions of N-methylated peptide macrocycles displayed on mRNA. First, a cDNA library is generated (red bar) that encodes 8–15 residue–long peptides containing a mixture of natural and non-natural amino acids [**a**]. The cDNA library is transcribed to an mRNA library and subsequently linked to a second mRNA oligonucleotide that terminates in a puromycin residue (black circle) [**b**]. When the mRNA library is translated, the puromycin causes the growing peptide chain to remain linked to its mRNA template. The displayed macrocyclic peptides contain a linker region (gray), natural amino acids (yellow) and N-methylated amino acids (red) [**c**]. After selecting for macrocycles that bind to a desired target, binding molecules can be identified and/or amplified for a second round of selection by PCR of the linked mRNA template [**d**].

undisclosed display method.

"We developed an alternative to mRNA display that is simplified," said CSO Patrick Reid.

He said PeptiDream has identified hits with nanomolar to subnanomolar potency against enzymes and protein-protein interactions. "Macrocycle peptides seem to be able to target most anything—β-sheets, α-helices, random coils—we have found peptides that have bound to them all."

Reid disagreed with Terrett that size was the critical factor governing cell permeability. He said PeptiDream's technology can make cell-permeable macrocycles of 800–2,000 Da. "In the studies that we have done, size doesn't seem to matter that much," he said.

Reid added that hydrophobicity and the 3D structure of the macrocycle seem to be more important than size in determining cell permeability.

PeptiDream's most advanced program is a macrocycle that blocks influenza A virus hemagglutinin, an extracellular target, and prevents the virus from penetrating cells. The project is being funded by the Japanese government.

Reid said about half of the company's targets are extracellular and the other half are intracellular. PeptiDream's most advanced program against an intracellular target is an undisclosed protein-protein interaction being pursued as part of a pharma collaboration. PeptiDream and its undisclosed partner hope to start mouse studies in the next few months, according to Reid.

The next steps for Suga include designing new libraries with different scaffolds and developing new selection techniques, with an eye toward increasing cell permeability. "We need to look at the structural requirements for cell permeability. That is really the major direction that we are taking right now," he said.

Suga told *SciBX* that the University of Tokyo has received a patent covering technology for generating the engineered tRNAs that introduce non-natural amino acids into peptides. The university has filed for patents covering both the use of the engineered tRNAs for *in vitro* translation and the integrated RaPID method. The university also has filed for patents covering technologies for introducing *N*-methyl peptides, cyclizing the peptides and for other modifications. All have been exclusively licensed to PeptiDream.

Reid said the company has collaborations with **AstraZeneca plc**'s **MedImmune LLC** subsidiary, two undisclosed Japanese pharmas and three undisclosed non-Japanese pharmas.

Kotz, J. *SciBX* 5(4); doi:10.1038/scibx.2012.87
Published online Jan. 26, 2012

REFERENCES

1. Yamagishi, Y. *et al. Chem. Biol.*; published online Dec. 23, 2011; doi:10.1016/j.chembiol.2011.09.013
   Contact: Hiroaki Suga, The University of Tokyo, Tokyo, Japan
   e-mail: hsuga@chem.s.u-tokyo.ac.jp
2. Driggers, E.M. *et al. Nat. Rev. Drug Discov.* **7**, 608–624 (2008)
3. Kawakami, T. *et al. Chem. Biol.* **15**, 32–42 (2008)
4. Nemoto, N. *et al. FEBS Lett.* **414**, 405–408 (1997)
5. Roberts, R.W. & Szostak, J.W. *Proc. Natl. Acad. Sci. USA* **94**, 12297–12302 (1997)
6. Edelson, S. *BioCentury* **19**(4), A7–A8; Jan. 17, 2011

COMPANIES AND INSTITUTIONS MENTIONED

AstraZeneca plc (LSE:AZN; NYSE:AZN), London, U.K.
Bicycle Therapeutics Ltd., Cambridge, U.K.
Bristol-Myers Squibb Co. (NYSE:BMY), New York, N.Y.
Ensemble Therapeutics Corp., Cambridge, Mass.
Harvard Medical School, Boston, Mass.
Keio University, Tsuruoka, Japan
Massachusetts General Hospital, Boston, Mass.
MedImmune LLC, Gaithersburg, Md.
PeptiDream Inc., Tokyo, Japan
Ra Pharmaceuticals Inc., Boston, Mass.
Swiss Federal Institute of Technology Lausanne, Lausanne, Switzerland
The University of Tokyo, Tokyo, Japan