SciBX
Science-Business eXchange

Bringing macrocycles full circle

By Joanne Kotz, Senior Editor

The opportunity afforded by macrocycles and constrained peptides to open up vast new therapeutic real estate is rivaled only by the challenge of puzzling out the basic science underlying these molecules.

Now, a think tank convened by *SciBX* has outlined an agenda for how to shed light into this black box, potentially providing a path to systematically identify macrocycle- or constrained peptide–based medicines that would combine the cell permeability and oral bioavailability of small molecules with the potency and selectivity of mAbs.

Successfully implementing the agenda may allow drug developers to more readily modulate challenging targets such as intracellular protein-protein interactions, which would open up the pharmaceutical landscape to a wide range of new targets that are currently undruggable.

Solving for the unknowns also would keep the momentum behind the surge of efforts by biotech and pharma companies to create footholds in the macrocycle space and would maintain investor interest.

The *SciBX* think tank, comprising academic, biotech, pharma and VC stakeholders, discussed the state of the macrocycle space at the first *SciBX* Innovation in Drug Discovery and Development Summit in Boston in September, which was attended by key opinion leaders in the macrocycle and constrained peptide community (*see* **Box 1, "*SciBX* innovation summits launched"**).

During the summit, participants identified four areas of science in which work is needed to enable drug innovation. Three of them—pharmacokinetics, cell permeability and oral bioavailability—aim to create an understanding of the rules that govern the behavior of macrocycles and thus enable developers to better identify drug-like compounds. The fourth one tackles the issue of targeting.

First, improving pharmacokinetics, particularly serum half-life and tissue exposure, will be critical to effectively modulate extracellular and intracellular targets and make macrocycles commercially competitive.

Second, cracking the code for cell permeability was pegged as a critical advance needed to propel the field forward. Most of the 40 or so existing macrocycle drugs are naturally occurring or very close analogs of naturally occurring molecules. Many of these macrocycle drugs enter cells via passive diffusion, but how they do so is not well understood.

Even less is known about active transport via endocytosis, although this mode of cell permeability will almost certainly be required for larger members of the macrocycle class to get into cells.

Third, achieving oral bioavailability would broaden the range of indications and targets that macrocycles and constrained peptides could address.

The fourth area is the need for a more detailed understanding of how macrocycles engage their targets. This would enable the selection of targets best suited to the properties of macrocycles and constrained peptides.

Strategic target selection for the current generation of macrocycles—which differ from most existing macrocycle drugs in that they are identified from synthetic libraries or by rational design rather than derived from natural compounds—will be necessary to provide proof of principle for this new therapeutic modality, which would give venture and public equity investors incentives to fund second-generation drugs.

Summit participants noted that many of these essential insights do not exist because the basic biology has been in areas not prioritized by government funders and academic scientists. After identifying the translational hurdles, the *SciBX* think tank identified action items that would enable progress across the various macrocycle platforms.

To start, the group called for massive parallel screening combined with computational approaches to interrogate both existing macrocycle drugs and other macrocycles and constrained peptides to help discover the rules of passive cell permeability.

An additional benefit to understanding the rules would be the ability to bias screening libraries so they contain more molecules likely to be cell permeable or have other desired pharmacological properties.

In the absence of rules that ultimately will be revealed by systematic studies, phenotypic screening provides an empirical approach for discovering cell-permeable macrocycles.

Next, the think tank recommended focusing more basic research on understanding active cellular transport, which may most effectively open up intracellular target space for larger members of the macrocycle class.

Then, the working group recommended the creation and validation of benchmark molecules, which would make it easier for researchers to assess the quality and applicability of new research.

Finally, the group concluded that much of this research should be done in the precompetitive setting because most of the work does not fit neatly into either conventional academic research or the corporate world.

According to think tank member Spiros Liras, VP of cardiovascular, metabolic and endocrine medicinal chemistry at **Pfizer Inc.**, "The idea of promoting the concept of industry-academic collaboration to proliferate knowledge in this area is spot on. A single organization or entity will not be able to deliver this by itself. Partnerships between industry and academia, and finding ways for funding agencies to promote this, will be critical."

In addition to Liras, the summit task force consisted of Bruce Booth, Barry Morgan, Patrick Reid, Tomi Sawyer and Gregory Verdine.

Booth is a partner at **Atlas Venture** and sits on the board of phage display–based macrocycle company **Bicycle Therapeutics Ltd.**; Morgan is VP of molecular discovery research at **GlaxoSmithKline plc**; Reid is cofounder and CSO of the *in vitro* display–based macrocycle

company **PeptiDream Inc.**; Sawyer is CSO and SVP of drug discovery and innovative technologies at **Aileron Therapeutics Inc.**, which is developing stapled peptides, or α-helical peptides constrained by a hydrocarbon linker; and Verdine is a professor in the Department of Chemistry and Chemical Biology and the Department of Stem Cell and Regenerative Biology at **Harvard University**, a founder of Aileron and a venture partner at **Third Rock Ventures**.

The backdrop

Macrocycles, including cyclic small molecules or peptides and closely related chemically constrained peptides, are typically 500–2,000 Da in size and thus fall between small molecules and biologics.

Cyclosporine illustrates the potential of the macrocycle class. The immunosuppressant is a 1,200 Da cyclic peptide that is orally bioavailable and targets an intracellular protein. For small molecules, 500 Da typically is considered an upper limit for reliably achieving cell permeability and oral bioavailability.

However, in the 40 years since the discovery of cyclosporine, efforts to systematically identify cyclosporine-like molecules have had limited success.

The area has seen a surge in interest over the last five years based on the development of new approaches for synthesizing and screening macrocycles. This scientific progress has fueled a burst of business activity. There are at least 12 biotech companies in the space, of which almost half were founded in the last 5 years.

Collectively, these companies have entered into 27 discovery partnerships over the same period (*see* **Figure 1, "Recent company and partnership formation trends in the macrocycle space"**).

Thus far, the initial crop of next-generation macrocycles from these companies are nearly all directed against targets outside the cell. For more details on the platforms, companies and deals in the macrocycle space, see the linked *BioCentury* article, "Excited about cycling."

The buzz

If macrocycles are to live up to their promise, the real impact will be through the intracellular targets for which the transformational potential is greatest. Indeed, the excitement surrounding macrocycles rests on the hope that this class of molecules will hit targets that are inaccessible to small molecules and biologics.

"As a biotech sector, we've launched a bunch of companies over the last five years. The possibility that these platforms will open up novel targets is the many-million-dollar question that a lot of VCs are putting their hopes on," said Booth.

Added Morgan, "The hope is that larger molecules will have more traction against protein-protein interactions. That's the driver for all of this: it's a class of targets that traditionally have been undruggable, and the hope is that these targets have been transformed into druggable space by this class of molecules."

Verdine noted that although it has increasingly become feasible to identify relevant disease targets, the capacity to therapeutically modulate most of these targets has lagged.

"While the science of disease mechanisms has flourished, the science of targetability has not fundamentally changed in the last 30 or 40 years since the advent of monoclonal antibody therapeutics. Small molecules target only the 10% of proteins that have a discrete hydrophobic pocket, and biologics target only the 10% of targets that are integral to the membrane or outside of the cell. This leaves an 80% chance or better that even if you know what is driving a disease, it is not actionable," Verdine emphasized.

Liras agreed. "Over the last 10 years we have observed an erosion in pharma productivity that was driven primarily, at least in my opinion, by

Box 1. *SciBX* innovation summits launched.

This week, *SciBX* is publishing the first Roadmap for Innovation resulting from a *SciBX* Innovation in Drug Discovery and Development Summit. In keeping with the newsletter's focus on identifying scientific advances with translational potential and analyzing them in their scientific and commercial context, *SciBX* has launched this summit series to catalyze discussion in select areas of biomedical innovation.

The *SciBX* summits bring together key opinion leaders in the translational space and focus on areas poised to reach an inflection point. The goal is to lay out the technical challenges and strategic opportunities for basic science, VC, biotech and pharma to lift the field to the next level.

To bring together key players from a variety of backgrounds to discuss these issues in depth, the *SciBX* editorial team convenes a working group of four to eight key opinion leaders in advance of the summit to develop a picture of the state of the field and help frame the discussion.

The first *SciBX* summit focused on macrocycles and constrained peptides—drug platforms with the clear potential to unlock new target space but for which there are many unanswered basic science questions.

The summit was hosted as a special session by BioPharm America at its 2012 conference in Boston. At the September meeting, the six members of the working group were joined by an audience comprised of other leaders in the field, including CEOs and CSOs of several macrocycle companies, representatives from big pharmas working in the space and top academics from the Boston area and beyond.

This first *SciBX* summit was produced with support from the following sponsors: **Aileron Therapeutics Inc.**; **Amgen Inc.**; **BioDuro LLC**, a **PPD Inc.** company; **Ipsen Group**; **MedImmune LLC**; **PeptiDream Inc.**; **Sanofi**; **Sofinnova Ventures**; **Takeda Pharmaceutical Co. Ltd.**; and **Third Rock Ventures**.

Following the positive reception to the summit, the *SciBX* editorial team has begun considering new targets, pathways and therapeutic approaches to discuss in 2013.

—Gaspar Taroncher-Oldenburg, Managing Editor

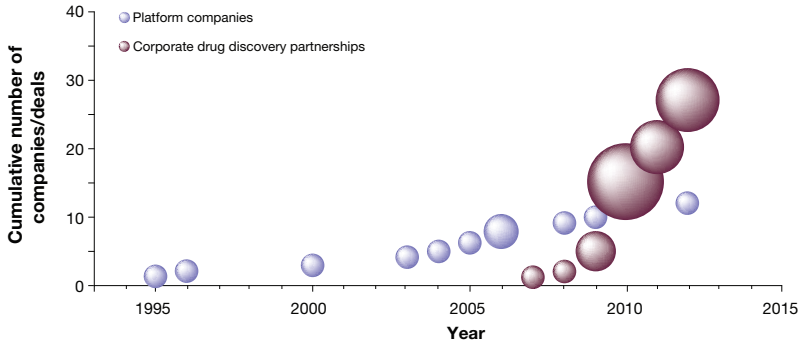

Figure 1. Recent company and partnership formation trends in the macrocycle space. Although company formation in the macrocycle space has been relatively steady since 1995, partnership activity for these companies has surged in the last five years.

For macrocycle company formation and the establishment of corporate drug discovery partnerships, the size of the dot represents the number of companies formed or partnerships established during that year, and the plotted value indicates the cumulative number of companies or partnerships in the space.

Data are taken from Cain, C. *BioCentury* **20**(38), A7–A13; Sept. 17, 2012.

our instinctive choice of targets on the basis of the technical feasibility of delivering clinical candidates," he said.

"There is now renewed interest in looking at more therapeutically relevant targets, and these, quite frankly, appear to be far more difficult, and they require an amount of innovation in medicinal chemistry that we haven't really engaged in previously," he added. "Our philosophy is to select some key yet difficult targets and try to explore any modality that is likely to impact the delivery of this target, and that is why we are here."

The rub

The need for unprecedented medicinal chemistry innovation is not an exaggeration, as there are significant hurdles to overcome before reaching the ultimate goal of orally bioavailable and cell-permeable macrocycle drugs against new targets.

Compared with small molecules, macrocycles that bind potently *in vitro* to challenging targets can be identified more readily because their larger size and conformational flexibility enables high-affinity interactions with the relatively flat protein surfaces that characterize these difficult targets. But these macrocycle hits are difficult to optimize into leads with improved pharmacological properties.

Liras noted that "the field has done well at producing technologies that may serve as hit-identification platforms. But it would be nice to try to identify lead-quality matter: ligands that have a certain affinity for a target and that also have desirable pharmacokinetic attributes for cell permeability and oral bioavailability."

"There's been a lot of buzz about macrocycles being really special molecules and some clearly are, but most of them are not. Most of them don't get into cells, many of them are poorly soluble and you have to fine-tune the properties case by case," added Nick Terrett, CSO of synthetic macrocycle company **Ensemble Therapeutics Corp.**

"Our ability to navigate through the pharmacological properties for small molecules is exceptionally high. The same ability doesn't exist for these larger molecules. We don't even know that the same small molecule physicochemical properties are relevant," added Liras.

Another part of the challenge in deciphering pharmacology rules is the chemical diversity contained within macrocycles—and the resulting variability within this class in terms of target interactions and pharmacology.

"We can't lump all these molecules into one class because they clearly cover a range of different structural classes and different molecular weights. A stapled peptide is very different from a large cyclic peptide, which is very different from a synthetic macrocycle, which is very different from a natural product macrocycle," said Terrett.

For example, he said, researchers may face greater challenges with pharmaceutical properties for larger macrocycles, but it may be relatively easier to achieve potency against difficult targets. In contrast, macrocycles on the smaller end of the spectrum might pose fewer pharmacology issues but struggle more to achieve potency.

Pharmacokinetics 101

Because of the myriad challenges in achieving cell permeability, the disclosed preclinical programs from macrocycle companies are predominantly directed against extracellular targets.

However, even attaining the pharmacokinetic properties necessary to effectively modulate an extracellular target may often not be straightforward.

"You need to make sure that when you have administered a drug it gets to tissues with the right level of exposure and the right half-life. A linear peptide disappears in minutes, and even cyclic, constrained peptides that might be more stable to proteolysis are often gone in minutes. In terms of getting early wins we're going to need to solve this, and it probably involves modifying these peptides in some way," noted Booth.

A lot will be riding on these molecules, as success could increase momentum in the field and failure could drive away pharma and investor interest.

"I think that clinical data will be the next critical step necessary to drive further interest in the approach," said Morgan.

Optimizing macrocycle pharmacokinetics is likely not amenable to a cookie-cutter approach.

"I think this is an area where there won't be a generalizable solution," Booth said, adding that conjugating lipid groups, tacking on albumin-binding domains or incorporating non-natural amino acids are all possible paths to pursue.

In addition to optimizing pharmacokinetics for drug distribution and exposure, Liras said that optimizing clearance routes of macrocycles to avoid toxicity will also be important. He added that lipophilicity, polarity and ionization states are all macrocycle properties likely to impact clearance mechanisms.

Booth said that if the pharmacokinetics can be optimized, macrocycles that hit extracellular targets could be differentiated from competing therapeutic approaches. He noted that unlike antibodies, macrocycles are chemically synthesized, which allows their structure to be more precisely controlled. Also, macrocycles are smaller in size than biologics, which may result in improved tissue penetration.

Because of these distinctions, he added, macrocycle-drug conjugates and bispecific macrocycles may offer advantages over the analogous antibody-based approaches.

Point of entry

Of the many pharmacological properties of macrocycles that are not well understood, cell permeability is the central issue for systematically unlocking intracellular targets. And much of what dictates cell permeability is completely unknown.

"We have found cyclic peptides that penetrate cells, but we have no logical understanding of why they go in. We've also had cell-penetrating cyclic peptides where we change just one amino acid and the resulting molecule no longer goes into a cell at all. It's completely dead. Understanding cell permeability is going to be challenging," said Reid.

The mechanisms by which macrocycles can be taken up into cells can be broadly grouped into two categories—passive diffusion and active transport.

Passive diffusion, the route of cell entry also used by small molecules, is conceptually straightforward. Molecules diffuse from the blood through the cell membrane and into the cell. But the properties that control passive diffusion for macrocycles differ from those that have previously been deciphered for small molecules.

A few general properties that influence macrocycle cell permeability are beginning to emerge.

Masking amide bonds, often by N-methylation, to facilitate macrocycles passing through the cell membrane appears to be one key piece of the puzzle. Indeed, N-methylation is almost certainly critical for the cell penetration of cyclosporin, in which 7 of the 11 amide bonds are N-methylated.

A Pfizer-academic team reported last fall that selectively N-methylating accessible amide sites in a cyclic peptide resulted in about a fivefold increase in passive membrane permeability compared with that of the unmethylated parent molecule. The best identified molecule was 30% orally bioavailable in rats.[1]

Liras thinks two of the other key factors affecting permeability are molecular volume and molecular shape—and that looking at macrocycles with 3D glasses will be critical for determining which parameters govern passive diffusion into cells.

"We recently published a paper where we did an analysis of our own file of molecules that violate the rule of five and established a correlation between hydrodynamic radius of gyration, 3D polar surface area and cell permeability," he said, referring to work published earlier this year.[2]

"But there is a huge caveat. The sample we interrogated to get to this correlation is limited and driven by looking predominantly at natural products. There is definitely a major opportunity for people to challenge these principles and to add to them," he noted.

Although there has been some early progress in understanding the rules of passive cell penetration, the factors that dictate active uptake are almost completely unknown. A key issue, said Verdine, is that active transport probably comprises a multitude of pathways—not just one.

In the first step of active transport, a molecule interacts with a receptor or transporter on the cell surface. A portion of the membrane then involutes and pinches off to form an endocytic vesicle that brings the molecular cargo from outside the cell to the inside. While contained within the vesicle, the molecule is inaccessible to the interior components of the cell. In the second step, the endocytic vesicle traffics through the cell and then breaks down to release the molecule into the cytosol.

"We need to understand everything—all the way from engagement at the cell surface to why that causes the formation of an endocytic vesicle—and we have no idea," said Verdine. "And knowing what triggers the formation of the endocytic vesicle is not good enough. The vesicle has to break up because if it doesn't break up you don't have a drug, and the unfortunate fact is that we understand little about this process of uncoating."

The lack of mechanistic understanding has a ripple effect throughout the drug discovery process.

For example, PeptiDream has looked at 30 closely related 10-mer to 15-mer cyclic peptides that are all active *in vitro* and are all getting into cells. Of these, only a few had cellular activity against the intended cytosolic target.

"If you have 30 peptides that are going into the cell, why are only two or three active?" asked Reid. He thinks the problem is that the molecules do not end up at the necessary cellular location.

"I think mislocalization or mistrafficking during endocytosis is going to be a huge task to try to figure out to get intracellularly active cyclic peptides," he said.

"You need to make sure that when you have administered a drug it gets to tissues with the right level of exposure and the right half-life."

—Bruce Booth, Atlas Venture

Oral arguments

Oral bioavailability is an even higher hurdle than cell permeability. It requires that a molecule is stable in the digestive track and passes through the endothelial cell barrier of the intestine before being exposed in the portal vein to the liver, where it must avoid metabolism or excretion to enter the bloodstream.

Despite the challenges, identifying macrocycles that are orally bioavailable is certainly possible. Adrian Whitty noted that about a third of the 40 approved macrocycle drugs are orally bioavailable. Whitty is associate professor of chemistry at **Boston University**. He was previously director of physical biochemistry at **Biogen Idec Inc.**

However, it is unclear whether orally bioavailable macrocycles exist across a broad swath of macrocycle chemical diversity or whether oral bioavailability exists only within small clusters of macrocycle structures. If researchers can identify specific macrocycle structures that lend themselves to oral bioavailability, Liras said there may be an opportunity to start with such scaffolds and then graft on the required potency.

Engaging targets

Even as the cell permeability and oral bioavailability issues are being addressed, it is unclear how best to select targets.

"We don't know much about how macrocycles as a class engage with proteins. Do they bind in the same kind of sites where conventional drugs

bind? And how do macrocycles engage those sites?" asked Whitty. "We would like to look at a target and say this would be a great target to choose, and a macrocycle is the right way to go or a conventional small molecule is the right way to go."

Terrett agreed. "It's almost been a given in this discussion that you can't address protein-protein interactions with small molecules, and therefore you need a macrocycle which is bigger, almost like a biologic," he said. "I don't think we fully understand the reason for that. For some targets we can screen libraries and find hits very easily, whereas other targets are totally intractable. What is the difference between such targets?"

One important advance would be to better define pockets where macrocycles might bind.

Whitty said that he and his collaborators have looked at about 24 published high-resolution crystal structures of distinct macrocycles binding to target proteins. "It turns out, for example, that peripheral atoms—those atoms that are one atom removed from the ring—play an unexpectedly large role in contacting the protein compared with the ring atoms themselves."

What this observation suggests as far as the types of pockets that might best be targeted with macrocycles is still not clear, noted Whitty.

Perhaps an even greater challenge will be to identify cryptic pockets—pockets that are not formed until after a ligand binds—that are potentially druggable with macrocycles.

These allosteric pockets outside of a direct protein-protein interface could be important to identify because the sites may open up a way to modulate an otherwise intractable protein target or may provide a unique mechanistic effect that cannot be achieved by directly blocking a protein-protein interaction.

The trick is to find these hidden ligand-binding sites.

"On the protein-protein interaction topic, I don't think that directly disrupting the interaction is the only way to go about it. We also need to identify cryptic allosteric sites, and that is a pretty sophisticated question," said Liras.

"We are beginning to use macrocycles that bind to shallow surfaces at protein-protein interaction interfaces as chemical biology tools to reveal cryptic pockets—conformational changes that we can see in real time in one of the protein partners that opens up new binding sites that can then be exploited," Liras added.

"With display technologies, you can certainly screen for cryptic binding sites by blocking hot spots where lots of peptides bind and then looking for new binding sites that emerge. At Bicycle, we've been able to identify cryptic sites in various screens we've done," noted Booth.

Indeed, researchers have been using stapled peptides to identify allosteric sites that have not been discovered using small molecules.

"What excites me the most is that stapled peptides are enabling us to discover new and functional interaction sites on protein targets. This speaks to the fact that they can engage binding surfaces that were previously not accessible to other modalities," said Loren Walensky, associate professor of pediatric oncology and chemical biology at the **Dana-Farber Cancer Institute** and a cofounder of Aileron.

"We can't lump all these molecules into one class because they clearly cover a range of different structural classes and different molecular weights. A stapled peptide is very different from a large cyclic peptide, which is very different from a synthetic macrocycle, which is very different from a natural product macrocycle."

—Nick Terrett, Ensemble Therapeutics Corp.

Passive aggressive

Having laid out the issues, participants at the *SciBX* summit proposed a variety of initiatives with the express goal of providing answers and solutions.

One solution identified at the summit would be an effort to decipher the rules of passive permeability by using computational approaches to analyze and model large-scale assay data across the varied types of macrocycles.

"The right investment in my opinion would be to galvanize a community of *in silico* masters to try to help us understand how macrocycles passively enter cells. I think that's a pretty big experiment that we should aim to do," said Liras.

"Computation is going to be key to addressing many of the questions about macrocycle passive cell permeability," said Whitty. "But because the kind of molecules we're looking at lie outside the molecules we have good data on, we are going to need significant parallel data acquisition in order to guide that computation."

He added, "The problem is that this is not a research problem that fits very well in either the academic or industrial mold. I don't know if there is a mechanism where I could approach a pharmaceutical company and say, 'We have these libraries, and if we could use your assays we could get a systematic picture for this set of macrocycle chemotypes—how different substitution patterns, different rigidities, different polarities and different ring sizes affect passive permeability.'"

The necessary assays to characterize macrocycle passive permeability are indeed available within pharma.

"We do have validated assays that are transporter free. I would be able to answer that question about passive permeability without any intrusion of any active transport mechanism, for example," said Liras.

But an individual pharma, despite having these assays, cannot by itself collect the necessary data to look systematically at the passive cell permeability of macrocycles. The obstacle is a lack of access to a sufficiently diverse range of molecules.

"The caveat is that there is probably not enough depth in corporate compound libraries. Where our gap appears to be right now is the diversity of macrocyclic structures that would allow us to get a little more comprehensive," noted Liras.

A wide range of macrocycles are available but are scattered across various pharma, biotech and academic groups. A precompetitive effort to match molecules and assays could therefore provide information that would broadly benefit the field.

"There is an opportunity here to galvanize the community to provide compounds for assays that we will all agree could inform this operation. The data would then be accessible to everybody. I think that would propel this field and would give us a go or no-go answer as to whether we can reliably achieve cell permeability, at least for certain parts of the macrocycle space," Liras continued.

Not everyone was convinced that these data—even if available—would make it possible to decipher passive permeability rules. For instance, Terrett questioned whether computational methods could be developed that would be truly predictive of passive cell permeability.

"I am confident we could identify some general principles, but it may be difficult to be really predictive for a particular compound," said Terrett, noting that "the conformational flexibility of macrocycles adds an additional level of complexity to molecular modeling" not seen in efforts directed at small molecules.

In the face of this complexity, one alternative could be to take an empirical approach to identifying functionally active, cell-permeable macrocycles. For instance, cell-based phenotypic screening can identify molecules that both enter a cell and induce the desired activity in the cell.

"This field seems like a natural match for phenotypic screening because then you're saying, 'We don't know which of these molecules get into cells, and we don't really know why, but we're only going to look at the ones that do'," said Derek Lowe, research fellow at **Vertex Pharmaceuticals Inc.**

Lowe did say that for most of the existing macrocycle libraries, which are either displayed on mRNA or phage or tethered to DNA, "phenotypic screening will be impossible."

Liras added that a phenotypic screening approach would only be effective if the initial library contains molecules that are cell permeable.

"I'm not sure that the necessary design principles have gone into informing the libraries," he said. This, said Liras, highlights again the importance of systematically understanding the drug-like space for macrocycles and how to bias macrocycle libraries toward better pharmacological properties.

Quicker on the (active) uptake

Another action item identified at the summit would be to target research funding toward gaining a mechanistic understanding of endocytosis.

"The idea of promoting the concept of industry-academic collaboration to proliferate knowledge in this area is spot on."

—Spiros Liras, Pfizer Inc.

"I could not find a single paper that showed a whole-genome small hairpin RNA screen of any endocytic import process for a drug anywhere in the entire literature. What that says is that the biology of endocytic vesicle trafficking is starved for investment. Absent that investment, I think we're all skating on very thin ice. We are exploiting a cell uptake pathway that is very, very poorly understood," said Verdine.

He said correcting this knowledge gap will require targeted research funding.

"We need to have a very focused effort on really understanding what exactly are the mechanisms of endocytic vesicle trafficking," according to Verdine. "This is going to require that the federal funding agencies stop thinking of endosomal biology as a backwater, just as 25–30 years ago RNA biology was a backwater. We have to stay ahead of the curve and say endosomal biology is the next big thing in biology."

Beyond understanding basic mechanisms, knowing certain pieces of the endocytosis puzzle would be particularly useful for efforts to hijack the pathway for drug delivery.

"We need to have a really comprehensive understanding of what are all of the human transporters—the energy-dependent tractor proteins that pull things into cells," said Verdine. "What is their tissue distribution? What are the address labels that allow you to bind to that transport protein? Does the transport protein get you into the cell to hit intracellular targets or across the cell to reach the portal vein so that you can get oral activity?"

This knowledge, he said, would enable researchers to develop assays for rationally optimizing the pharmacological properties of macrocycles using this cell-entry route.

As a first stop, focusing on transporters in the intestine may make sense because these could impact oral bioavailability.

Liras noted, "The idea of active transport is a prime opportunity once again for a bigger, more coordinated experiment. I'm thinking there may be an opportunity in the gut, for example, for a methodic and large-scale analysis of transporters and to try to understand species differences and prevalent polymorphisms. That's going to require a lot of work, but it's going to be high-value work in my opinion."

Even in the face of a focused effort, gaining traction on getting drugs into cells via endocytosis will not be easy.

Doug Treco, cofounder, president and CEO of **Ra Pharmaceuticals Inc.**, noted that "this area has stymied nucleic acid delivery for about 25 years. People have known since the late '80s that nucleic acids are a potentially potent and specific way to treat disease—and yet people still can't get them into cells. I do have some concerns that this problem has been around for a long time with incredible motivation to solve it, and yet people are still coming up against a brick wall in terms of getting nucleic acids into cells and then released from endosomes."

Treco continued, "My personal feeling is the most important place to focus may be on getting molecules released from endosomes. This seems to be a very focused aspect to look at that may help drive the field forward."

Although the difficulties the nucleic acid therapeutics field has faced in trying to capitalize on endocytosis highlights the significant challenges, this also means any progress in this area will serve to advance not only the macrocycle field but also drug discovery efforts in other new drug modalities.

"There is definitely a huge overlap here with oligonucleotide therapies, which also face similar challenges around obtaining, measuring and standardizing what 'productive' uptake looks like for getting out of endosomes and into cytosol. A joint effort focused on endocytic trafficking could be helpful here," said Booth.

In addition to nucleic acid–based therapies, getting protein-based therapeutics into cells or across cellular barriers requires making use of endocytic trafficking. For instance, Verdine pointed out that active transport will be required to get antibodies across the blood brain barrier.

Benchmarking progress

A final opportunity identified at the summit would be for companies and academics to contribute benchmark molecules that could be made openly available to the community through a repository. These macrocycles would span the diversity of chemical structures and have well-characterized and validated mechanisms of cell uptake and functional activity.

"What is lacking in this field is that there is never any head-to-head comparison of macrocycles for any kind of functional parameter," Verdine said. "We should think about how to create a repository of reference molecules, which would allow people to do head-to-head comparisons."

Looking at molecules that are taken up into cells by endocytosis does require particular rigor to ensure accurate conclusions.

"The artifacts that are seen in a huge number of science papers happen because the molecules are trapped inside of vesicles. When the investigator lyses the cells, all of a sudden the drug—whether it's an oligonucleotide or a peptide—engages its target. I think that is a common challenge of a lot of the published literature," Booth noted.

Avoiding these artifacts requires correlating target binding inside a cell with a functional readout.

Sawyer noted that a more rigorous approach, and where the field should be moving, would be to combine evidence of target engagement with imaging of fluorescently labeled molecules to correlate cellular localization with functional data.

"We need to have a very focused effort on really understanding what exactly are the mechanisms of endocytic vesicle trafficking. This is going to require that the federal funding agencies stop thinking of endosomal biology as a backwater."

—Gregory Verdine, Harvard University

Verdine agreed. "Functional readouts are where the field needs to go ASAP."

Precompetitive push

Much of the progress needed to push the macrocycle field forward may best be done precompetitively—and some of the actions suggested at the *SciBX* summit may only be achievable if academics and companies collaborate in the open.

"We are excited about this field but cautious about our ability to get to a standard solution with predictability. My view is it will require a community effort to solve it," Liras said.

The need to gather passive permeability data on a wide variety of macrocycles is a prime example. Accessing a sufficiently large and representative breadth of data to make meaningful conclusions on the rules governing passive cell permeability will require the combined efforts of multiple companies and academics—as well as the participation of funding agencies.

"Some kind of partnership is necessary to solve this in a systematic way with a wide enough range of compound structures to make it informative," said Whitty.

"I think there is an opportunity here for industry, academia and funding agencies to support this cause with certain assets like small libraries, validated assays and computational methods," said Liras. "The data, which will be such an important contributor to the knowledge that will allow us to design these molecules, would then be made accessible to everybody with no constraints."

Targeted research funding in the basic science of pharmacology also will be critical.

"One field that the academic community and funding agencies have really allowed to go fallow is pharmacology," said Verdine. "Any new therapeutic modality will come along with its own enormous pharmacology challenge. If the NIH invested in this area, academics and companies working in this space wouldn't need to discover this new pharmacology from scratch."

He argued that federal funding agencies should be taking a leading-edge position in the area of next-generation therapeutics rather than saying "this doesn't fit the historical paradigm of what a drug is."

Convincing macrocycle companies to participate in these efforts may not be out of reach.

"Some could argue that macrocycle companies are competing in the world of capital for resources, which they are. But fundamentally the competition here will be at the disease indication and target level, and so in a lot of ways all of us are collaborating to be able to advance a field to make great drugs," concluded Booth.

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COMPANIES AND INSTITUTIONS MENTIONED

Aileron Therapeutics Inc., Cambridge, Mass.
Amgen Inc. (NASDAQ:AMGN), Thousand Oaks, Calif.
Atlas Venture, Cambridge, Mass.
Bicycle Therapeutics Ltd., Cambridge, U.K.
BioDuro LLC, Beijing, China
Biogen Idec Inc. (NASDAQ:BIIB), Weston, Mass.
Boston University, Boston, Mass.
Dana-Farber Cancer Institute, Boston, Mass.
Ensemble Therapeutics Corp., Cambridge, Mass.
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
Harvard University, Cambridge, Mass.
Ipsen Group (Euronext:IPN; Pink:IPSEY), Boulogne-Billancourt, France
MedImmune LLC, Gaithersburg, Md.
PeptiDream Inc., Tokyo, Japan
Pfizer Inc. (NYSE:PFE), New York, N.Y.
PPD Inc. (NASDAQ:PPDI), Wilmington, N.C.
Ra Pharmaceuticals Inc., Cambridge, Mass.
Sanofi (Euronext:SAN; NYSE:SNY), Paris, France
Sofinnova Ventures, Menlo Park, Calif.
Takeda Pharmaceutical Co. Ltd. (Tokyo:4502), Osaka, Japan
Third Rock Ventures, Boston, Mass.
Vertex Pharmaceuticals Inc. (NASDAQ:VRTX), Cambridge, Mass.