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Nature Chemical Biology Grand Challenges

Beyond Discovery: Developing Molecules that See, Grab, and Poke

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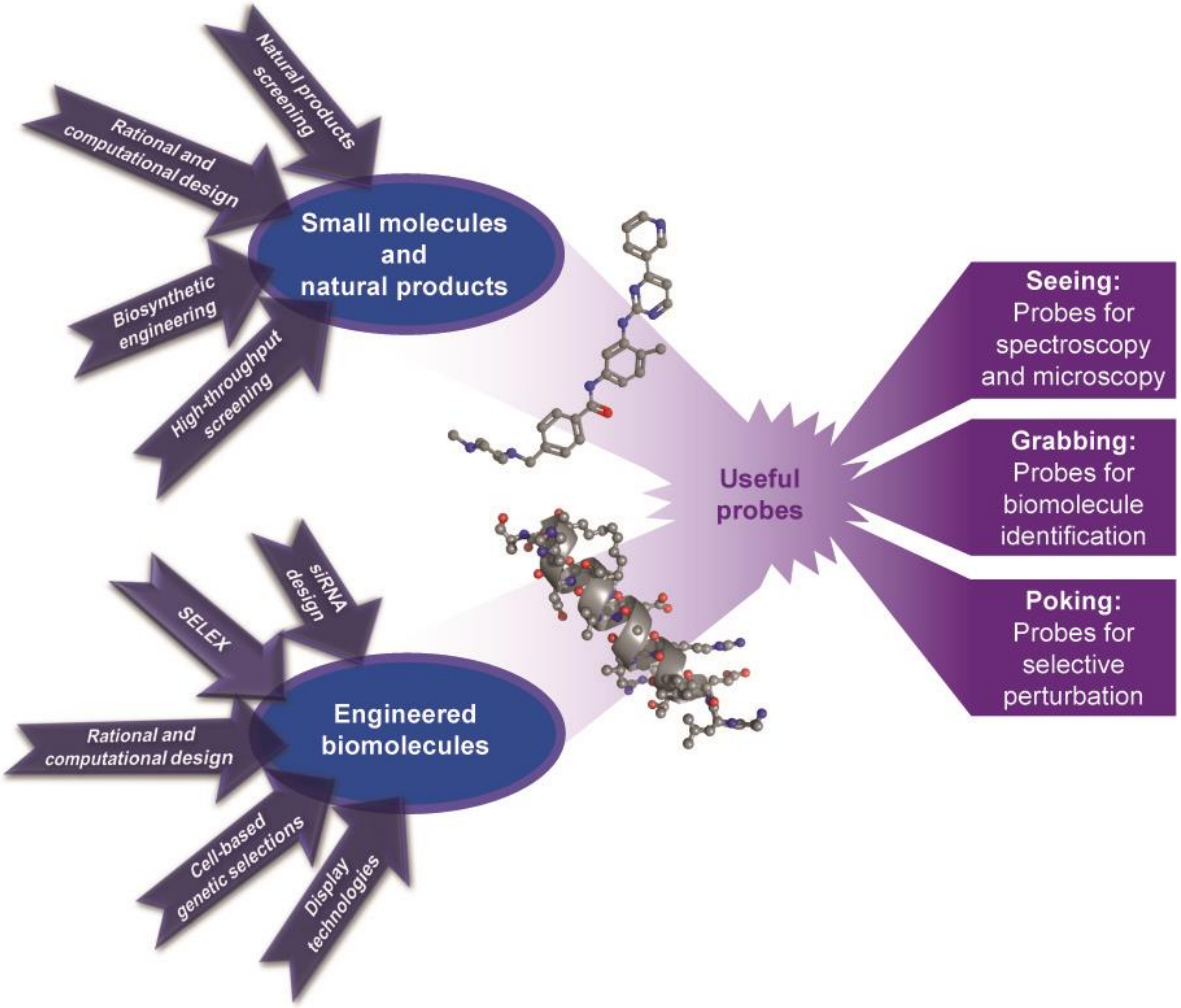

Figure 1. The probe development challenge. Chemical biologists have diverse methods of discovering new small molecules, natural products, and engineered biomolecules. However, there are comparatively few general methods for developing those compounds into useful probes that can provide concrete answers to mechanistic questions in biology. General development strategies are needed to more rapidly apply new molecules for "seeing," "grabbing," and "poking" inside the cell.

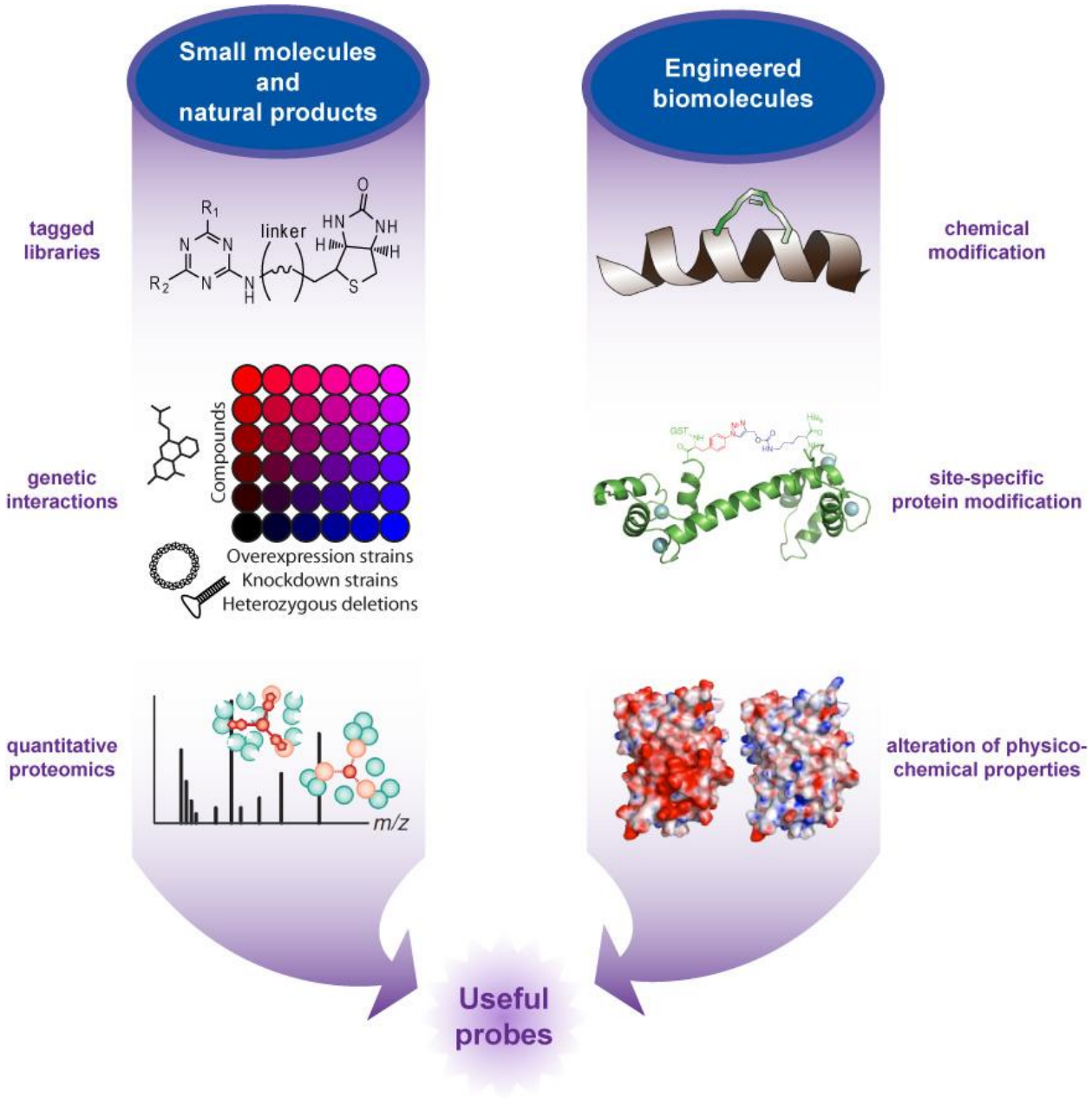

Figure 2. A few general methods for probe development. The modes of action and global specificities of small molecules and natural products must be well-understood before they can be used to dissect biological pathways. Many methods are emerging as wider solutions to this problem, including tagged screening libraries, high-throughput genetics and chemical genetics assays, and quantitative proteomics techniques. Engineered biomolecules, on the other hand, must be reliably engineered to be stable and properly compartmentalized before they can be used to probe biological systems. Some clever solutions to this problem are shown here, and involve direct chemical modification, selective incorporation of non-natural functional groups, or modification of overall physico-chemical properties.

Chemical biology is now able to discover molecules that target almost any biological target or process. It remains a Grand Challenge to leverage these molecules into useful probes that can be used to address unsolved problems in biology.

It happened quite suddenly. I was following an interest in protein folding, and one day I looked up and realized that I was the only chemist in a genetics lab. This led to many patient conversations wherein I would learn the difference between epistasis and complementation, or I would explain the difference between a lactam and a lactone. However, once we overcame the language barrier, it became clear that my chemical biology approach was seen as a valuable and complementary method for exploring protein folding in the cell. Others in the lab might generate novel mutant strains to analyze protein function, while I would devise a screen to isolate an inhibitor – but we'd both reach the same ultimate goal. Many other young chemists have had similar opportunities to apply chemical approaches in biomedical research environments, and our experiences show how widely accepted chemical biology has become as an independent approach to studying living systems. This acceptance is reassuring, but it also raises the pressure to deliver on one of chemical biology's central promises: to generate truly useful chemical probes for any cellular target or pathway. For chemical biologists, this represents a Grand Challenge to consistently reach beyond discovery into the development and application of probes, to ensure that new molecules can be used to answer mechanistic biological questions.

Bioactive molecules are the currency of chemical biology, and the field has exploited many ways to find them: natural products screening, peptidomimetics, computational design, phage display, SELEX, protein engineering, siRNA design, genetic screening, and high-throughput screening, to name a few. These techniques demonstrate that, from algorithms to virology, there seems to be no technology chemists can't use to design or screen for interesting molecules (Fig. 1). However, none of these technologies are universally applicable or without difficult bottlenecks. For instance, phage and RNA display allow the screening of billions or even trillions of molecules, but generally yield peptides or proteins that require additional modification to be useful *in vivo* probes. One such modification, attachment of highly cationic transducing sequences, has allowed some applications of peptides and proteins as exogenously applied probes.[1] Still, we lack truly general methods for turning potent peptides into cellular probes. By contrast, small molecules from high-throughput screens can sometimes be used without modification. However, most approaches for small-molecule screening cannot readily determine the specificity of the resulting molecules or identify their molecular mechanisms. This leads to unanticipated off-target effects or a frustrating inability to unambiguously identify the relevant cellular targets.[2-3] Because they have complementary strengths and drawbacks, different molecule discovery techniques have proven optimal for targeting different proteins or cellular processes. Taken together, they represent a comprehensive solution to molecule discovery. In this patchwork manner, chemical biology has largely conquered the problem of discovering molecules that affect nearly any biological target or pathway of interest.

However, it turns out that molecule discovery is only the first step. There is a second step to this process, one that is often implicit but nonetheless essential: development of novel molecules into useful probes. For the purposes of this discussion, development encompasses all experiments and controls required to apply novel molecules to living systems and to interpret their effects. These include understanding the structure-activity relationships and global specificity of small molecules, or engineering biomolecules to be more cell-permeable and resistant to enzymatic degradation. These studies are often not as glamorous as new molecule discovery, but they are every bit as important, for without them the molecule is useless as a probe. The ultimate test of a probe's utility is whether its application can definitively answer mechanistic biological questions such as: "How does Aurora kinase B control mitotic spindle function?" "Which glycans mediate neuron-neuron recognition?" or "Do intracellular protein aggregates cause Parkinson's disease?"

Answering these mechanistic questions using a chemical approach generally means developing molecules into one of three classes of probes: probes for "seeing" through spectroscopy and microscopy, probes for "grabbing" through detection of intermolecular interactions, and probes for "poking" through highly specific perturbation of a cellular target or functional pathway (Fig. 1). "Seeing probes" include MRI contrast agents and fluorescence-based sensors.[4-5] "Grabbing probes" include activity-based covalent probes and other small molecules that help isolate and identify their targets.[6] Often, grabbing probes can be further developed for modular control of intermolecular interactions, for instance as chemical inducers of dimerization or antibody recruitment.[7-8] "Poking probes" include a wide variety of molecules that perturb specific proteins and pathways, but also molecules that can control cellular function such as artificial transcription factors.[9] Going forward, if your newly discovered molecule can't be developed into a probe for seeing, grabbing, or poking a living system, it won't be useful for answering mechanistic biological questions. This is why chemical biology faces a renewed challenge to develop reliable strategies for turning newly discovered molecules into useful probes. Such strategies will be key to maintaining and increasing the relevance of chemical biology as a valuable approach to understanding biology and disease.

For a time, small-molecule-based approaches seemed to have this problem licked. High-throughput screening technologies made the development of useful probes seem like a problem of scale: screen enough small molecules in enough *in vitro* and *in vivo* screens, and you will find that you have plenty of ready-to-use probes already in your freezer. But it has become apparent that development of screening hits into genuinely useful probes requires additional steps. Diversity and complexity of screening libraries must be balanced to discover truly novel probes.[10] After screening, hits must be sorted, false positives weeded out, and compounds (whose screening stocks might be from long ago or far away) re-synthesized, re-tested and derivatized. In most cases knowledge of mechanisms of action, global interaction specificities, and off-target effects are required before molecules can be used with confidence to address biological questions. New solutions to these bottlenecks are emerging, including incorporation of chemical handles within libraries prior to screening,[11] systems biology approaches to determine all biological effects of small molecules,[12] and microarray and proteomics techniques that help define targets and specificities.[13-14] Going forward, these and other solutions will accelerate the rates with which small molecules move out of the chemistry lab and into the biology lab.

Engineered biomolecules are also a challenge to develop into useful probes, but for different reasons. Modified proteins and peptides can be wondrously potent and selective, and can also bind targets or catalyze reactions that small molecules cannot. Genetic expression is an elegant solution in some cases, as in the expression of cyclic peptides in the dopaminergic neurons of live nematodes,[15] but it is not a workable solution for polypeptides with more extensive modifications. Other classes of biomolecules, including modified sugars, amino acids and lipids, can be reliably incorporated into living cells or even whole organisms, allowing direct applications such as the covalent tagging of modified sugars in live mice,[16] For all these modified biomolecules, this functional flexibility is a blessing and a curse, because molecules cannot be used for seeing, grabbing or poking if they are metabolized or compartmentalized by the living system. Chemical modifications are the most common answer to this problem, exemplified by progress in 2' sugar modifications for delivery of synthetic siRNA and side chain cross-linking for stabilization of α-helical peptides.[17-18] Other promising approaches for the development of biomolecules as useful probes include efficient site-specific protein modification,[19] and even "super-charging" proteins to allow cellular transduction.[20] These are all clever chemical solutions to the probe development challenge, and we can expect these and other strategies to expand in scope and applications in the next few years.

While it is appealing to envision general solutions to the probe development challenge, the truth is that, as with molecule discovery, many and diverse solutions will be required to conquer it. Until now, the glamour has been largely reserved for those who discover molecules with novel activities, and those who apply probes to answer important biological questions. But the development work in between is so critical to the success of the overall endeavor that more effort should be applied to probe development, and more acknowledgment should be paid to clever solutions. Luckily, the newest wave of chemical biologists is particularly suited to the task. Interdisciplinary training has become more common, and being the lone chemist in a genetics lab is no longer an anomaly. This frees young scientists to focus their training on the *questions they want to answer*, rather than the tools they choose to employ. This inquiry-based viewpoint means that many of us are less interested in proof-of-principle variations of molecule discovery and more eager for proof-in-the-pudding applications. We will not be satisfied until we have used our molecules to yield conclusive answers to important mechanistic questions. This is a positive development, because young chemists motivated to solve biological problems will be optimally suited to carry new molecules through development and into meaningful application. No matter the molecule or model system, the relevance of chemical biology as a field stems from a continuous and profound impact on biology. This is why, looking to the future, it remains a Grand Challenge of chemical biology to streamline the development of probes for seeing, grabbing, and poking biological systems.

References

1 Stewart, KM, Horton, KL & Kelley, SO. Cell-penetrating peptides as delivery vehicles for biology and medicine. *Org Biomol Chem* **6**, 2242-2255, (2008).

2 Walsh, DP & Chang, YT. Chemical genetics. *Chem Rev* **106**, 2476-2530, (2006).

3 Burdine, L & Kodadek, T. Target identification in chemical genetics: The (often) missing link. *Chem Biol* **11**, 593-597, (2004).
4 Terreno, E, Castelli, DD, Viale, A & Aime, S. Challenges for molecular magnetic resonance imaging. *Chem Rev* **110**, 3019-3042, (2010).
5 Rothman, DM, Shults, MD & Imperiali, B. Chemical approaches for investigating phosphorylation in signal transduction networks. *Trends Cell Biol* **15**, 502-510, (2005).
6 Cravatt, BF, Wright, AT & Kozarich, JW. Activity-based protein profiling: From enzyme chemistry. *Annu Rev Biochem* **77**, 383-414, (2008).
7 Pollock, R & Clackson, T. Dimerizer-regulated gene expression. *Curr Opin Biotechnol* **13**, 459-467, (2002).
8 Raina, K & Crews, CM. Chemical inducers of targeted protein degradation. *J Biol Chem* **285**, 11057-11060, (2010).
9 Lee, LW & Mapp, AK. Transcriptional switches: Chemical approaches to gene regulation. *J Biol Chem* **285**, 11033-11038, (2010).
10 Nielsen, Thomas E & Schreiber, Stuart L. Towards the optimal screening collection: A synthesis strategy13. *Angew Chem Int Ed* **47**, 48-56, (2008).
11 Ahn, YH & Chang, YT. Tagged small molecule library approach for facilitated chemical genetics. *Accounts of Chemical Research* **40**, 1025-1033, (2007).
12 Oprea, TI, Tropsha, A, Faulon, JL & Rintoul, MD. Systems chemical biology. *Nat Chem Biol* **3**, 447-450, (2007).
13 Vegas, AJ, Fuller, JH & Koehler, AN. Small-molecule microarrays as tools in ligand discovery. *Chem Soc Rev* **37**, 1385-1394, (2008).
14 Rix, U & Superti-Furga, G. Target profiling of small molecules by chemical proteomics. *Nat Chem Biol* **5**, 616-624, (2009).
15 Kritzer, JA, Hamamichi, S *et al.* Rapid selection of cyclic peptides that reduce alpha-synuclein toxicity in yeast and animal models. *Nat Chem Biol* **5**, 655-663, (2009).
16 Chang, PV, Prescher, JA *et al.* Copper-free click chemistry in living animals. *Proc Natl Acad Sci USA* **107**, 1821-1826, (2010).
17 Walensky, LD, Kung, AL *et al.* Activation of apoptosis in vivo by a hydrocarbon-stapled bh3 helix. *Science* **305**, 1466-1470, (2004).
18 Behlke, MA. Progress towards in vivo use of sirnas. *Mol Ther* **13**, 644-670, (2006).
19 Neumann, H, Wang, K, Davis, L, Garcia-Alai, M & Chin, JW. Encoding multiple unnatural amino acids via evolution of a quadruplet-decoding ribosome. *Nature* **464**, 441-444, (2010).
20 McNaughton, BR, Cronican, JJ, Thompson, DB & Liu, DR. Mammalian cell penetration, sirna transfection, and DNA transfection by supercharged proteins. *Proc Natl Acad Sci USA* **106**, 6111-6116, (2009).