Broader subject: **Peptide libraries**

Title: **Combining biological and chemical diversity**

Author: Christian Heinis

Standfirst: Phage display enables screening of billions of peptides comprised mainly of natural amino acids. Now, a method to attach and encode a range of structurally diverse compounds has been reported. This method can expand the chemical space covered by phage display peptide libraries.

Developed in the 1980s and 1990s, biological display techniques such as phage display and mRNA display have revolutionized the extent, speed and ease in which peptide- and protein-based ligands can be generated[1–3]. In these techniques, biological systems are exploited to physically couple peptides or proteins to their encoding DNA, such that affinity-selected binders can be identified by sequencing the encoding DNA. This coupling enables the affinity-screening of billions of different peptide or protein variants in a single reaction tube. Since their development, these techniques have been expanded by strategies that incorporate non-standard amino acids[4] or that chemically transform displayed peptides into cyclic or bicyclic peptides[5]. However, incorporation of non-natural building blocks is typically limited to a handful of non-standard amino acids, cyclization linkers and chemical appendages. From a chemical diversity point of view, these "unnatural" display libraries are therefore still dominated by canonical amino acids, meaning that the chemical diversity of the screened libraries is rather small.

Writing in *Journal of the American Chemical Society* a team led by Ratmir Derda has now reported an approach to mitigate these limitations[6]. The strategy they developed appends chemicals with a high structural diversity to phage-displayed peptides in a convenient and straightforward fashion. The team achieved this by first cyclizing peptides on phage with a diketone linker that serves as a unique handle for attaching hydrazine-functionalized compounds via the bioorthogonal Knorr pyrazole reaction (Figure 1). Importantly, these hydrazines are commercially available in large numbers and varieties, allowing easy access to hundreds of diverse chemical building blocks that can be attached in parallel. In contrast to ribosomal incorporation of chemical fragments — where the synthetic molecules need to

resemble amino acids — this late-stage modification allows any type of chemical structure to be appended.

Next, Derda and co-workers had to overcome the issue of encoding the chemical modifications to provide an easy method to read the identity of the high affinity compounds. A key feature of biological display techniques is that all building blocks of the displayed molecules are genetically encoded, enabling deconvolution of the binding compounds that are enriched through affinity selections. In phage display, the amino acids of displayed peptides are encoded by DNA that is enclosed inside viral particles. However, if synthetic compounds are conjugated to the peptides, (as described by Derda and co-workers), they are of course not encoded. To expand the coding to include the late-stage chemical additions, the team used a strategy based on "silent DNA barcodes" that they had previously reported[7]. These are unique combinations of redundant codons coding for the same amino acids, such as a region of a phage protein. Derda and co-workers prepared phage peptide sub-libraries that contained different silent DNA barcodes and modified each sub-library with a defined, single chemical compound. After a round of phage selection, the identity of the appended chemical fragment could be identified by sequencing both the silent barcode and the encoded peptide sequence.

In their report, the team describe how they established the cyclization of peptides by dichloropentanedione and then how they subsequently appended the diverse hydrazine compounds to the diketone linker. All of this was done in aqueous solutions compatible with phage display. They show that the reactions, first performed on synthetic model peptides and then on phage, were efficient for many different alkyl- and aryl-hydrazines and gave impressively clean products. The strategy was then tested by panning a cyclic peptide library diversified using three distinct chemical compounds: a biotin-hydrazine, an *n*-decyl-hydrazine, and a benzenesulfonamide-hydrazine. Affinity selections with streptavidin isolated nearly exclusively peptides of the biotin-modified sub-library, and selections with carbonic anhydrase enriched peptides carrying sulfonamide, as anticipated based on the ligand binding capacities of these target proteins. An in depth analysis of peptides isolated against carbonic anhydrase showed that the best binders had good affinities in the nanomolar range and that both the peptide moiety and the chemical fragment jointly contributed to the binding. These proof-of-concept selections at a library level and characterizations showed that the late-stage chemical diversification and the encoding by silent codons work efficiently.

A particularly beautiful part of this new strategy is the integration of the appended chemical into the cyclic peptides. The reaction of the diketone linker with the hydrazine compounds forms a compact pyrazole ring structure that serves as a core to hold the peptide moiety and the non-natural compound, and this core could potentially form direct contacts with targets. The hydrazine can in principle react in two orientations with the diketone linker, which can slightly complicate the deconvolution, but also generates a larger structural diversity. A current limitation with the "silent DNA barcode" strategy is that only one round of selection can be performed, because phage propagated for a second round would not be separated into sub-libraries of identical silent DNA barcodes and thus could not be modified with chemicals in separate reactions. The Derda lab have overcome this limitation using deep sequencing, which allowed identification of enriched peptides after a single round of phage selection due to the large number of phage clones sequenced.

Given the massive expansion of the chemical diversity, the attractive format of the molecules with pyrazole cores, and the efficient and selective reactions, I am optimistic that the new approach, and the principles behind it, will be applied by many groups. In a next step, I expect that larger numbers of chemical fragments will be used in parallel to take full advantage of the convenient chemical diversification strategy. Due to the speed of biological affinity panning combined with the structural diversification of new chemical building blocks, I am optimistic that this technique will soon provide first ligands to important therapeutic targets.

Christian Heinis is at the Institute of Chemical Sciences and Engineering, Ecole Polytechnique Fédéral de Lausanne, BCH 5305, 1015 Lausanne, Switzerland.

E-mail: christian.heinis@epfl.ch

References

1. Smith, G. Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. *Science (80-.).* **228**, 1315–1317 (1985).
2. McCafferty, J., Griffiths, A. D., Winter, G. & Chiswell, D. J. Phage antibodies: filamentous phage displaying antibody variable domains. *Nature* **348**, 552–554 (1990).
3. Roberts, R. W. & Szostak, J. W. RNA-peptide fusions for the in vitro selection of peptides and proteins. *Proc. Natl. Acad. Sci.* **94**, 12297–12302 (1997).
4. Murakami, H., Ohta, A., Ashigai, H. & Suga, H. A highly flexible tRNA acylation

method for non-natural polypeptide synthesis. *Nat. Methods* **3**, 357–359 (2006).

5. Heinis, C., Rutherford, T., Freund, S. & Winter, G. Phage-encoded combinatorial chemical libraries based on bicyclic peptides. *Nat. Chem. Biol.* **5**, 502–507 (2009).

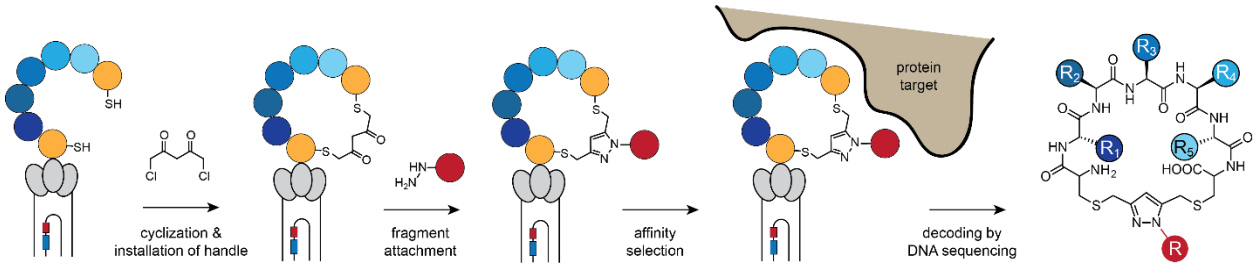
6. Ekanayake, A. I. *et al.* Genetically Encoded Fragment-Based Discovery from Phage-Displayed Macrocyclic Libraries with Genetically Encoded Unnatural Pharmacophores. *J. Am. Chem. Soc.* jacs.1c01186 (2021). doi:10.1021/jacs.1c01186

7. Tjhung, K. F. *et al.* Silent Encoding of Chemical Post-Translational Modifications in Phage-Displayed Libraries. *J. Am. Chem. Soc.* **138**, 32–35 (2016).

Figure caption

Fig. 1. Illustration of the approach to diversify phage-encoded peptide libraries by appending chemical compounds. Biological diversity (peptide) is shown in blue and chemical diversity in red (compound). DNA encoding the two components are shown with the same color code.

Figure



Competing interests statement

The author declares no competing interests.