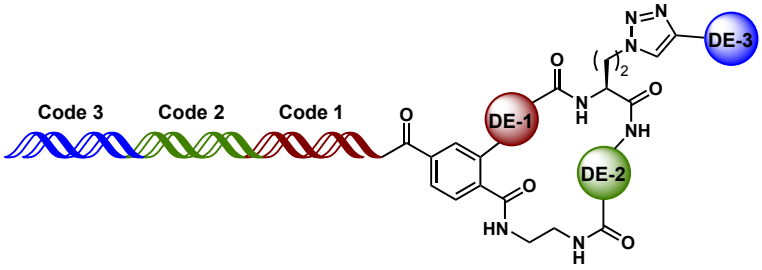
Development of a Natural Product-Like DNA-Encoded Macrocycle Library for Screening against Biologically Relevant Protein Targets

Cedric Stress, Dennis G. Gillingham

University of Basel, St. Johanns-Ring 19, 4056 Basel, Switzerland
cedric.stress@unibas.ch

DNA-encoded chemical libraries (DECLs) were first proposed by Brenner and Lerner in 1992.[1] This technology uses the advantages of combining genetic sequencing and combinatorial chemical synthesis. Due to this, large collections of molecules (up to billions) can quickly be synthesized and tested all at once. Potential protein binders are identified by the attached unique DNA code. Therefore, screening assays become much faster, more cost effective and less storage space for the library is needed.
Macrocycles, however, still are an underdeveloped class of compounds, which show unique properties and have a big potential for medicinal chemistry research. There are a couple of very effective macrocycles on the drug market as well as many very important natural products contain a macrocyclic scaffold.[2]



We therefore synthesized a DECL comprising of natural product-like macrocycles (approx. 1.5 million members) with very diverse macrocyclic scaffolds (>2000). The diversity was introduced using three building blocks that were incorporated by amide bond formation and click reaction. The individual building blocks were subsequently encoded by enzymatic DNA elongation reactions. The first building blocks (DE-1) were designed to bear typical natural product elements such as polyenes, alkyl chains, substituted aromatic rings or heteroaromatic moieties. Diversity element 2 (DE-2) consists of a set of natural and non-natural amino acids. The third building block (DE-3) gives the major diversity using copper-catalyzed click reactions. We tried to cover as much chemical space as possible by the selection of a diverse set of chemical functionalities and sterics.
The obtained library was then tested against biologically relevant proteins to find potential binders. PCR amplification, followed by next generation sequencing revealed the binding structures. Chemical resynthesis and protein binding affinity measurements of the elucidated hits gave an idea of the efficiency of our macrocycles in binding to our protein targets.

[1] S. Brenner, R. A. Lerner, *Proc. Natl. Acad. Sci. USA*. **1992**, *89*, 5381-5383.
[2] A. Whitty *et al*. *Drug Discovery Today*, **2016**, *21*, 712-717.