Department of Chemistry
University of Central Florida
Department of Chemistry Seminar Series – Fall 2021
In-Person Seminar
Monday, October 11th, HEC 101 from 10:00 to 11:30 AM

Addressing Analytical Challenges of “DNA-Encoded Library” Technology

Most therapeutic targets are regulatory or catalytic proteins, and modern drugs are developed to form stable complexes with them. Early-stage drug development aims to obtain and validate a large number of small-molecule hits capable of reversibly binding the target protein with acceptably low and known values of both the rate constant \( k_{\text{off}} \) and the equilibrium constant \( K_d = k_{\text{off}}/k_{\text{on}} \) in reaction:

\[
\text{hit} + \text{protein} \stackrel{k_{\text{on}}}{\rightleftharpoons} \ complexes
\]

During later stages of drug development, validated hits are gradually reduced to pre-clinical leads, clinical candidates, and eventually, a drug.

Developing a single approved drug requires as many as \( 10^9 \) validated hits. Such a large number of them can only be reliably obtained from enormously-diverse combinatorial libraries of small molecules. The most diverse libraries, providing a means of reliable identification of hits, are DNA-encoded libraries (DELs), which are mixtures of up to \( 10^{12} \) compounds each with a DNA tag encoding its chemical structure.

Validated hits are obtained from a DEL in five steps shown in the schematic: 1) synthesis of DEL from building blocks, 2) enriching hits in DEL, 3) sequencing the DNA tags to decode chemical structures of the hits, 4) synthesis/purification of DNA-free hits, and 5) measuring accurate \( K_d \) and \( k_{\text{off}} \) for hit-target complexes.

Steps 1, 2, 4, and 5 currently rely on non-robust processes, which preclude streamlined production of validated hits even if these processes are put on robotic platforms. The non-robustness of these four steps creates delays in early-stage drug development. To address this challenge, we are developing four robust processes intended for eventual integration into streamlined manufacturing of validated hits. The 1st process is solid-nonaqueous synthesis of DELs from a great diversity of building blocks. The 2nd process is 1-round DEL enrichment with hits of desirably-low \( k_{\text{off}} \) by capillary electrophoresis. The 3rd process is continuous-flow synthesis/purification of DNA-free molecules. The 4th process is continuous-flow microreactor with non-aqueous continuous-flow electrophoresis. The 4th process is accurate measurements of \( K_d \) and \( k_{\text{off}} \) for hit-target complexes by combining kinetic chromatography with mass-spectrometry.

In this lecture, I will explain our progress in this ambitious research program. The focus will be made on our recent achievements including: 1-round selection of protein binders from DNA libraries \([1–2]\), non-aqueous continuous-flow electrophoresis \([3–5]\), and accurate measurements of \( K_d \) of protein–drug complexes \([6–8]\).


