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

Prof. Sergey N. Krylov, York University, Toronto

http://www.yorku.ca/skrylov/

Key areas of our research include:

Biomolecular interactions
Personalized cancer medicine
High-throughput drug screening
Continuous-flow chemistry
Ultra-sensitive chemical analysis

Host: Dmitry Kolpashchikov

Addressing Analytical Challenges of "DNA-Encoded Library" Technology

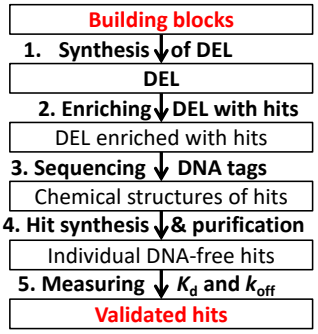
Most therapeutic targets are regulatory or catalytic proteins, and modern drugs are developed to form stable complexes with them. Early-stage drug development aims to obtain and validate a large number of small-molecule hits capable of reversibly binding the target protein with acceptably low and known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

$$\text{hit} + \text{protein} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \text{complex}$$

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 10^4 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 (**DEL**s), 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_{off} for hit-target complexes.



eps 1, 2, 4, and 5 currently rely on non-robust sses, which preclude streamlined production of ited hits even if these processes are put on robotic rms. The non-robustness of these four steps creates delays in early-stage drug development. To ss this challenge, we are developing four robust processes intended for eventual ation into streamlined manufacturing of validated hits. The 1st process is solid- non-aqueous synthesis of DELs from a great diversity of building blocks. The 2n ss is 1-round DEL enrichment with hits of desirably-low k_{off} by capillary ophoresis. The 3rd process is continuous-flow synthesis/ purification of DNA-free y integration of a continuous-flow microreactor with non-aqueous continuous-flo ophoresis. The 4th process is accurate measurements of K_d and k_{off} for hit–target lexes 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]**.

1. Le, A.T.H.; Krylova, S.M.; Kanoatov, M.; Desai, S.; Krylov, S.N. Ideal-filter capillary electrophoresis (IFCE) facilitates the one-step selection of aptamers. *Angew. Chem. Int. Ed.* **2019**, *58*, 2739–2743.
2. Le, A.T.H; Krylova, S.M.; Krylov, S.N. Determination of the equilibrium constant and rate constant of protein–oligonucleotide complex dissociation under the conditions of ideal-filter capillary electrophoresis. *Anal. Chem.* **2019**, *91*, 8532–8539.

3. Ivanov, N.A.; Lie, Y.; Kochmann, S.; Krylov, S.N. Non-aqueous continuous-flow electrophoresis (NACFE): separation complement for continuous-flow organic synthesis. *Lab Chip* **2019**, *19*, 2156–2160.
4. Ivanov, N.A.; Kochmann, S.; Krylov, S.N. Visualization of Streams of Small Organic Molecules in Continuous-Flow Electrophoresis. *Anal. Chem.* **2020**, *92*, 2907–2910.
5. Kochmann, S.; Ivanov, N.A.; Lucas, K.S.; Krylov, S.N. Topino: A Graphical Tool for Quantitative Assessment of Molecular Stream Separations. *Anal. Chem.* **2021**, *93*, 9980–9985.
6. Sisavath, N.; Rukundo, J.L.; J.C.Y. LeBlanc; Galievsky, V.; Bao, J.; Kochmann, S.; Stasheuski, A.S.; Krylov, S.N. Transient incomplete separation facilitates finding accurate equilibrium dissociation constant of protein–small molecule complex. *Angew. Chem. Int. Ed*. **2019**, *58*, 6635–6639.
7. Rukundo, J.-L.; Le Blanc, J.C.Y.; Kochmann, S.; Krylov, S.N. Assessing Accuracy of an Analytical Method *in silico*: Application to "Accurate Constant via Transient Incomplete Separation" (ACTIS). *Anal. Chem.* **2020**, *92*, 11973–11980.
8. Rukundo, J.-L.; Kochmann, S.; Wang, T.Y.; Ivanov, N.A.; Le Blanc, J.C.Y.; Gorin, B.I. Krylov, S.N. Template Instrumentation for "Accurate Constant *via* Transient Incomplete Separation" (ACTIS). *Anal. Chem.* **2021**, *93*, accepted