



AVVISO DI SEMINARIO

Il giorno 6 Maggio alle ore 10.30
nell'aula Seminari

Il Prof. Sergey N. Krylov

Center for Research on Biomolecular Interactions, York
University, Toronto, Canada

terrà un seminario dal titolo

**“Overlooked Causes and Costly Consequences of Gross
Inaccuracies in Binding Studies: Time to Act”**

Proponente: Prof. Francesco Ricci

Overlooked Causes and Costly Consequences of Gross Inaccuracies in Binding Studies: Time to Act

Sergey N. Krylov

Center for Research on Biomolecular Interactions, York University, Toronto, Canada

Reliable decision-making in drug discovery, chemical biology, and diagnostic development depends on accurate measures of molecular recognition and function. Yet binding studies often treat imprecision, that is, random error, as the sole marker of data quality, while overlooking inaccuracy, that is, systematic bias. In this seminar, I will explain why standard assays for binding affinity (K_d) can hide systematic deviations of orders of magnitude, even when curve fits look acceptable and standard errors are small.

Drawing on our recent theoretical and experimental studies [1-9], I will show how amplified propagation of concentration errors in nonlinear regression models can greatly change apparent K_d values and distort decisions in hit triage and candidate ranking. I will also demonstrate accessible browser-based tools for estimating the accuracy of K_d from individual binding curves. The discussion will remain at the level of general principles while also illustrating their relevance to aptamers and aptasensors [10-12].

Finally, I will outline the goals of an emerging consortium that brings together academic laboratories with instrumentation and high-throughput screening developers to establish reliable experimental practices and consensus reporting standards.

Relevant Publications

1. Wang, T.Y.; Chan, T.; Krylova, S.M.; Krylov, S.N. A Fundamental Source of Misclassification in Single-Concentration Binding Screens. *ChemRxiv* **2026**. <https://chemrxiv.org/doi/full/10.26434/chemrxiv.15002218/v1>
2. Wang, T.Y.; Dhillon, P.; Matsunaga, K.; Tan, H.P.; Kimoto, M.; Hirao, I.; Krylov, S.N. Deterministic Error Propagation in Kinetic K_d Determination: General Theory with Application to Surface-Based Assays. *ACS Sensors*, accepted. <https://chemrxiv.org/doi/full/10.26434/chemrxiv.15002022/v1>
3. Wang, T.Y.; Dhillon, P.; Schreiber, S.; Krylova, S.M.; Golemi-Kotra D.; Jose, J.; Krylov, S.N. Introducing Quantitative Assessment of Michaelis Constant (K_m) Accuracy. *ChemBioChem* **2025**, 26(24), e202500660. <https://doi.org/10.1002/cbic.202500660>
4. Wang, T.Y.; Bijlani, A.; Kaiyum, Y.A.; Krylova, S.M.; Johnson, P.E.; Krylov, S.N. A Browser-Based Tool for Assessing Accuracy of ITC-Derived Parameters: K_d , ΔH° , and n . *ChemBioChem* **2025**, 26(15), e202500194. <https://doi.org/10.1002/cbic.202500194>
5. Wang, T.Y.; Krylov, S.N. Deterministic Error Propagation in ITC: Revealing Multi-Fold Errors in K_d Values Under Standard Conditions. *Biophysical Chemistry* **2025**, 323, 107455. <https://doi.org/10.1016/j.bpc.2025.107455>
6. Wang, T.Y.; Latimer, J.; Rukundo, J.-L.; Kogan, I.; Krylova, S.M.; Schreiber, S.; Kohlmann, P.; Jose, J.; Krylov, S.N. A Practical Approach to Quantitatively Assessing Equilibrium-Constant Accuracy from a Single Binding Isotherm. *Precision Chemistry* **2025**, 3(2), 89–104. <https://doi.org/10.1021/prechem.4c00085>
7. Wang, T.Y.; Rukundo, J.-L.; Mao, Z.; Krylov, S.N. Maximizing the Accuracy of Equilibrium Dissociation Constants for Affinity Complexes: From Theory to Practical Recommendations. *ACS Chemical Biology* **2024**, 19(9), 1852–1867. <https://doi.org/10.1021/acscchembio.4c00259>
8. Krylov, S.N. Underestimation of the Complexity of K_d Determination: Causes, Implications, and Ways to Improve. *ACS Measurement Science Au* **2024**, 4(3), 231–232. <https://doi.org/10.1021/acsmesuresci.4c00023>
9. Wang, T.Y.; Ji, W.; Evetron, D.; Le, A.T.H.; Krylova, S.M.; Fournier, R.; Krylov, S.N. Fundamental Determinants of the Accuracy of Equilibrium Constants for Affinity Complexes. *Analytical Chemistry* **2023**, 95(42), 15826–15832. <https://doi.org/10.1021/acs.analchem.3c03557>
10. Le, A.T.H.; Krylova, S.M.; Krylov, S.N. A Roadmap for Reliable Determination of Aptamer–Target Equilibrium Dissociation Constants (K_d). *ACS Sensors* **2026**, 11(2), 779–791. <https://doi.org/10.1021/acssensors.6c00130>
11. Krylov, S.N.; Krylova, S.M.; Le, A.T.H.; Johnson, P.E.; Hili, R.; Wilson, D.J.; Kim, S.B.; Stovall, G.M.; Xu, G.; Dzantiev, B.B.; DeRosa, M.; Bueno, P.R.; Dauphin-Ducharme, P.; Tanner, J.A.; Whitehouse, W.L.; Zhao, Q.; Zhu, J.-J.; Crous, A.; Ferapontova, E. Widespread Omission of Aptamer–Target Binding Verification in Aptasensor Development: Consequences for Sensor Performance and the Need for a " K_d Gate". *Biosensors and Bioelectronics*, under revision. <https://chemrxiv.org/doi/full/10.26434/chemrxiv.15001564/v1>

12. Le, Q.D.; Le, A.T.H.; Wang, T.Y.; Krylova, S.M.; Clark, N.A.; Seghetti-Hebble, D.; Matsunaga, K.-I.; Kimoto, M.; Hirao, I.; Krylov, S.N. A widely used C-Reactive Protein aptamer does not bind C-Reactive Protein, suggesting potential streptavidin-driven artifacts in biosensors. *Biosensors and Bioelectronics*, submitted. <https://chemrxiv.org/doi/full/10.26434/chemrxiv.15001875/v1>