Accurate Constant, K_d, via Transient Incomplete Separation (ACTIS)

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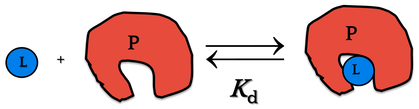
1. Motivation

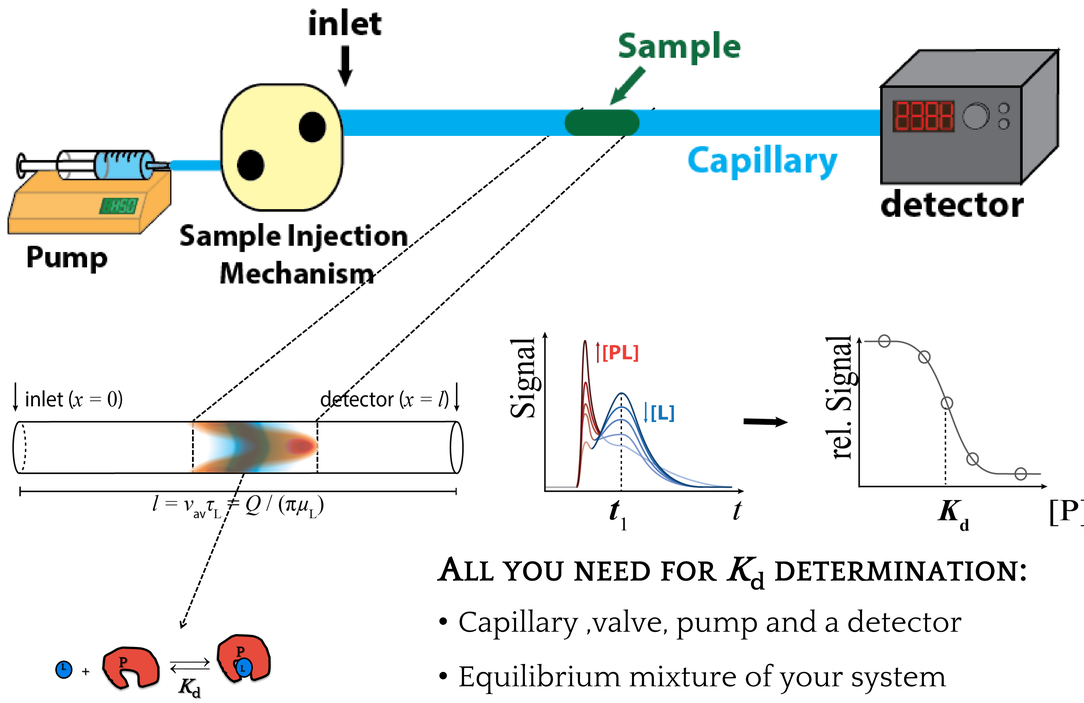
- Hit ranking for later stages of drug development is based on values of K_d of complexes between a small drug molecule and a target (protein).
- Currently mainly calorimetric and biosensoric approaches are used to find K_d of such complexes, but they suffer from inaccuracies as large as multiple orders of magnitude.
- We recently introduced "Accurate Constant via Transient Incomplete Separation" (ACTIS), a non-biosensoric and non-calorimetric approach for finding K_d, which appears to be free of inherent sources of inaccuracy

2. Model

Reversible binding of proteins (P) to small-molecule ligands (L) to form a complex (PL) was taken as our model



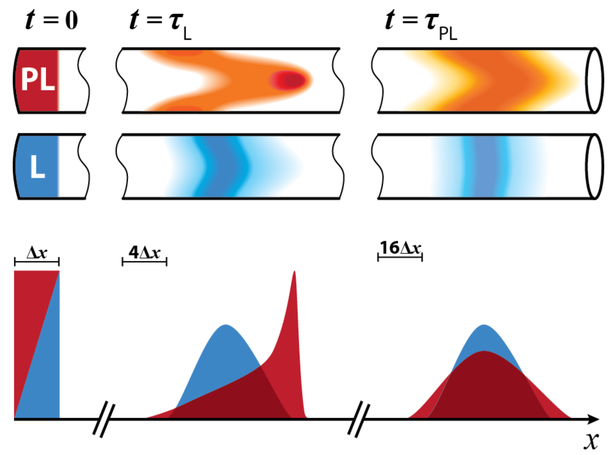
As simple as TIS



All you need for K_d determination:

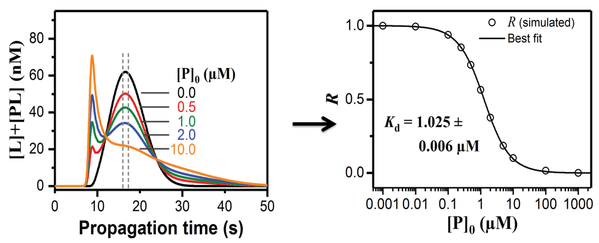
- Capillary ,valve, pump and a detector
- Equilibrium mixture of your system
- 60 to 90 seconds for a run

3. Transient Incomplete Separation (TIS)



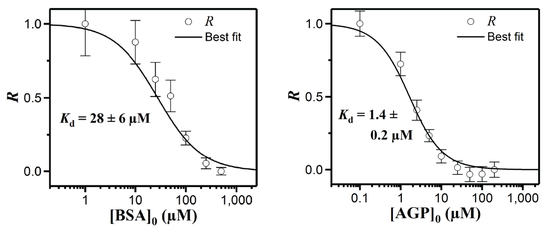
- τ_x is a^2 / μ_x where a is the capillary radius and μ_x the diffusion coefficient of specie x
- At $t = \tau_L$ L is fully diffused and PL is not fully diffused
- The separation is **incomplete** i.e. there is an overlap in distributions and **transient** i.e. no separation at $t = \tau_{PL}$

4. Simulations



Accuracy of less than 3 % with numerical simulations including non idealities of a real experiment

5. Experiments



BSA- Fluorescein
✓ K_d= 28 μM

AGP- Alprenolol
✓ K_d= 1.4 μM

Experimental results are in agreement with literature values

6. Advantages and limitations

- Fast enough: Not prone to protein absorption
- Label-free and immobilization-free
- Compatible with most detectors
- Simple instrumentation

- Interacting species should have at least 2 times difference in diffusion coefficient
- Upper K_d value limited by protein solubility
- Lower K_d value limited by limit of detection of the ligand

7. Conclusion and further studies

ACTIS been successfully used to measure the K_d of Protein – Small molecule. The next steps are measuring K_d for :

- Protein – Aptamer interactions
- Protein – Protein interactions

Reference

[1] Sisavath, N.; Rukundo, J. L.; Le Blanc, J. C. Y.; Galievsky, V. A.; Bao, J.; Kochmann, S.; Stasheuski, A. S.; Krylov, S. N. Angewandte Chemie 2019, 131 (20), 6707–6711.

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