

# Transient incomplete separation of species with close diffusivity to study the stability of affinity complexes

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Large molecules can be generically separated from small ones, though partially and temporarily, in a pressure-driven flow inside a capillary. This transient incomplete separation has been only applied to species with diffusion coefficients different by at least an order of magnitude.<sup>[1–5]</sup> Here, we demonstrate, for the first time, analytical utility of transient incomplete separation for species with close diffusion coefficients. First, we prove *in silico* that even a small difference in diffusivity can lead to detectable transient incomplete separation of species. Second, we use computer simulation to prove that such separation can be used for reliable determination of equilibrium dissociation constant ( $K_d$ ) of complexes composed of similar-size molecules. Finally, we demonstrate experimentally the use of this separation for accurate determination of  $K_d$  value for a protein-aptamer complex. We conclude that “accurate constant via transient incomplete separation” (ACTIS) can serve as a reference method for affinity characterization of protein–aptamer binding in solution.

## References

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