Determination of the Equilibrium Constant and Rate Constant of Protein–DNA Complex Dissociation under the Conditions of Ideal-Filter Capillary Electrophoresis

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1. Introduction

Importance. Selection of protein binders from oligonucleotide libraries, e.g. random DNA libraries or DNA-encoded libraries, can provide diverse pools of molecules for development of diagnostic probes and drugs.[1] The conventional selection methods involve surface-based techniques, which suffer from low partitioning efficiency caused by non-specific binding of library onto the surface.[2] We recently developed Ideal-Filter Capillary Electrophoresis (IFCE) – a disruptive partitioning approach which facilitates binder selection in a single step of partitioning.[3] In IFCE, protein-binder complexes and nonbinders move inside the capillary in the opposite directions, and the efficiency of their partitioning reaches 10^9 (10^7 times higher than the efficiency of classical surface-based partitioning).

Problem. The movement of the unbound oligonucleotides away from the detection end of the capillary makes their detection impossible in IFCE. This creates a problem for measuring the equilibrium constant (K_d) and rate constant (k_{off}) of protein–DNA complex dissociation by IFCE.

Objective. Our objective is to develop a CE-based method to find K_d and k_{off} of protein–DNA complexes selected by IFCE .

References

1. Keefe, A. D. *et al. Nat. Rev. Drug. Discov.* 2010, 9, 537–550.

2. Irvine, D. *et al. J. Mol. Biol.* 1991, 222, 739–761.

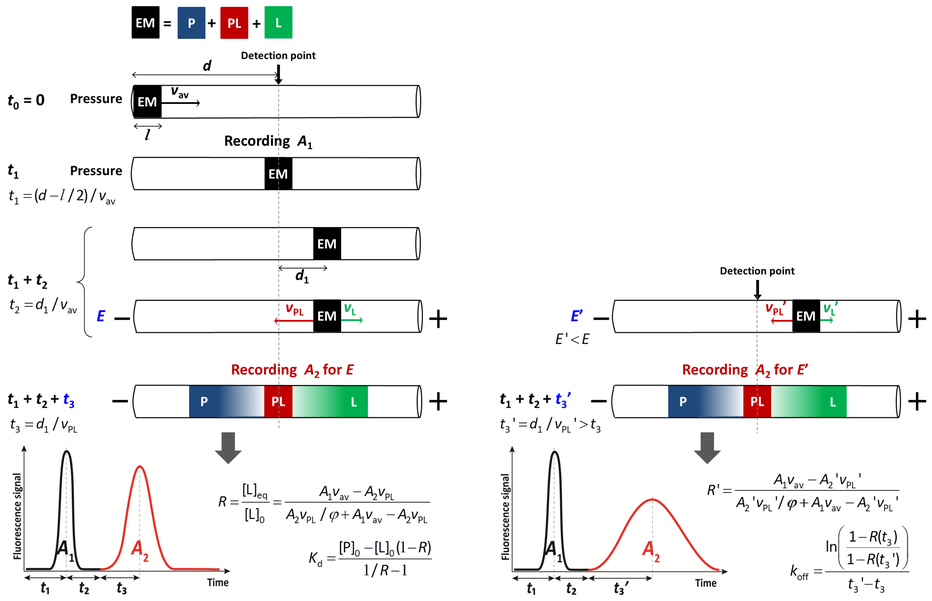
3. Le, A.T.H. *et al. Angew. Chem. Int. Ed.* 2019, 59, 2739–2743.

4. Kanoatov, M. *et al. Anal. Chem.* 2015, 87, 3099-3106.

5. Drabovich, A. P. *et al. Anal. Chem*. 2006, 78, 3171-3178.

Online version **Lab webpage**

2. General procedures for finding K_d and k_{off} via double-passage approach



Equilibrium mixture (EM) = protein (P) + ligand (L) + protein–ligand complex (PL)

1) 1st passage (record A_1): EM passes to the detector at pressure-driven velocity v_{av}

2) 2nd passage (record A_2): PL moves back to the detector at electrophoretic velocity v_{PL}

3) Velocity corrected peak areas ($A_1 v_{av}$ and $A_2 v_{PL}$) are used to calculate K_d and k_{off}

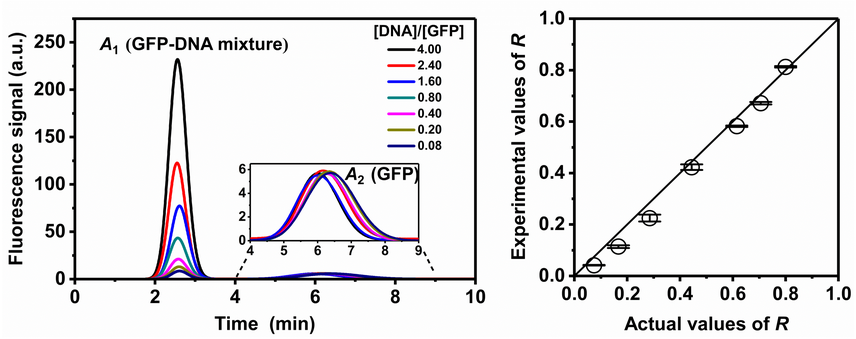
4. Recovery of *R*

Recovery of *R* (fraction of unbound ligand) was found experimentally by:

1) model equilibrium mixtures of a protein–DNA binding pair by mixing GFP and a fluorescently-labeled DNA at different known values of [DNA]/[GFP]

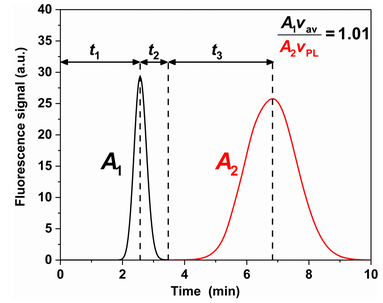
2) plot experimental values of R against actual values of *R*

The recovery of *R* proved to be satisfactory for the whole its range.



3. Recovery of peak areas

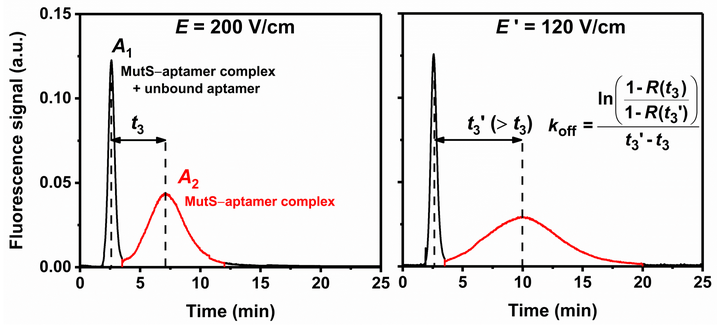
Recovery of peak areas was evaluated by sampling 1 μM green fluorescent protein (GFP) for the double-passage experiment (GFP as an analyte mimicking PL). 10 repetitions were done: $A_1 v_{av}/(A_2 v_{PL}) = 1.00 \pm 0.02$, confirming full recovery of velocity-corrected peak areas.



5. Experimental determination of K_d and k_{off} of protein–aptamer complex

The double-passage approach was utilized to find K_d and k_{off} values for an affinity complex between MutS protein and its DNA aptamer. The equilibrium mixture contained 0.5 nM MutS and 0.2 nM aptamer.

Kinetic parameter	Experimental value	Literature value [4, 5]
K_d	0.20±0.02 nM	0.1 nM
k_{off}	$(1.1\pm0.5)\times10^{-3}$ s^{-1}	0.4×10^{-3} s^{-1}



6. Conclusion

We developed the double passage approach for determination of K_d and k_{off} of protein-DNA complexes under IFCE conditions (near-physiological ionic strength and pH). This approach is needed for assessing stability of protein–DNA complexes selected by IFCE.