Predicting Electrophoretic Mobility of the Complex between Protein and DNA-Encoded Ligand in Kinetic Capillary Electrophoresis

Jiayin Bao,[1] Svetlana M. Krylova,[1] Leonid T. Cherney,[1] Robert L. Hale,[2] Svetlana L. Belyanskaya,[2] Cynthia H. Chiu,[2] Alex Shaginian,[2] Christopher C. Arico-Muendel,[2] and Sergey N. Krylov[1]

[1]*Department of Chemistry and Centre for Research on Biomolecular Interactions, York University, Toronto, Ontario M3J 1P3, Canada*
[2]*GlaxoSmithKline, 830 Winter St., Waltham, MA 02451-8714, USA*

Background

Selection of protein-binding ligands from highly-diverse combinatorial libraries of DNA-encoded small molecules is a rapidly developing approach in drug-lead discovery. The conventional selection methods involves surface-based techniques, which suffer from low partitioning efficiency caused by non-specific binding of the library molecules to the surface. Methods of kinetic capillary electrophoresis can facilitate highly efficient homogeneous selection required for such selection. However, KCE based selection methods require the precise prediction of electrophoretic mobility of protein-ligand complex. Here we present a theoretical approach for accurate estimate of electrophoretic mobility of such complexes.

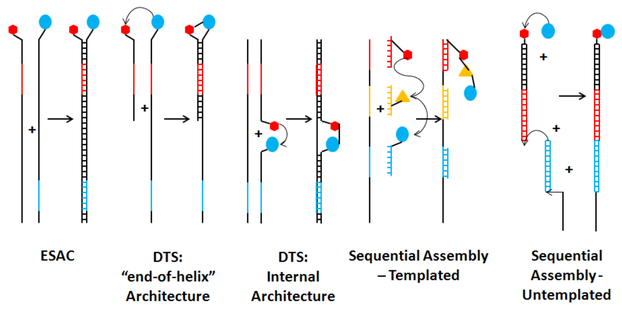

Figure 1. Schematic representation of assembly routes and corresponding structures of various DNA-encoded small molecules.

Methods

The model is based on a theory of the thin double layer and corresponding expressions used for the mobilities of globular protein connected, through a single point (small molecule), to a linear DNA tag. The DNA tag can be fully rod-like dsDNA as well as the combination of alternating dsDNA and ssDNA regions of varying lengths. The unknown electrophoretic mobility of protein-DNA complex can be calculated by using experimentally determined electrophoretic mobilities of the protein and DNA. Mobility prediction was initially tested by using streptavidin-biotin as the model system, and 18 biotinylated mock ligands with various DNA compositions have been constructed and tested. Finally, the prediction was tested for 2 proteins with 2 real DNA-encoded small molecules: streptavidin with biotin ligand and carbonic anhydrase II with GLCBS-L-Leucine ligand.

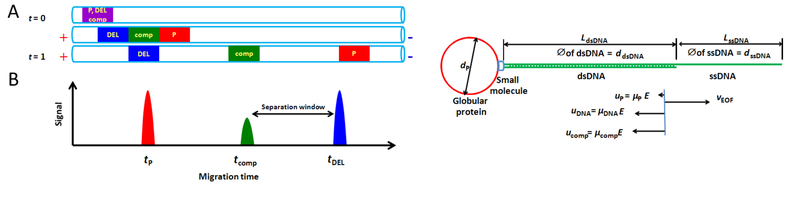

Figure 2. Conceptual depiction of migration patterns of DNA-encoded small molecule, protein, and complex in CE with strong electroosmotic flow (EOF).

Results

By testing the complexes between streptavidin with 18 biotinylated DNA different constructs, the model demonstrated the accuracy with error of less than 10% in prediction of electrophoretic mobilities, and with error less than 11% in prediction of migration times of complexes. Using two practical DNA-encoded small molecules from pharmaceutical company we assessed the final developed model. Two proteins were used in this test: streptavidin and carbonic anhydrase II. The model proved to be accurate in predicting complex mobility; the deviation of the predicted electrophoretic mobility from the experimental measured one did not exceed 5% for either of the proteins.

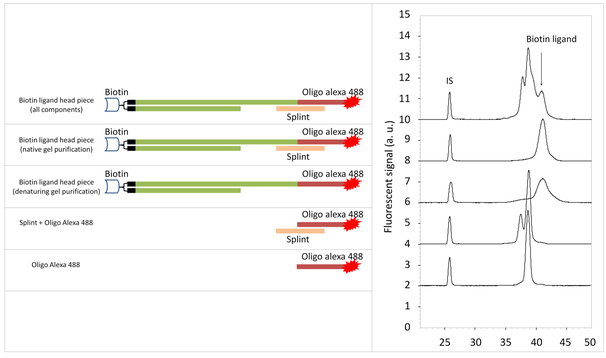

Figure 3. Peak identification of DNA-encoded small molecule.

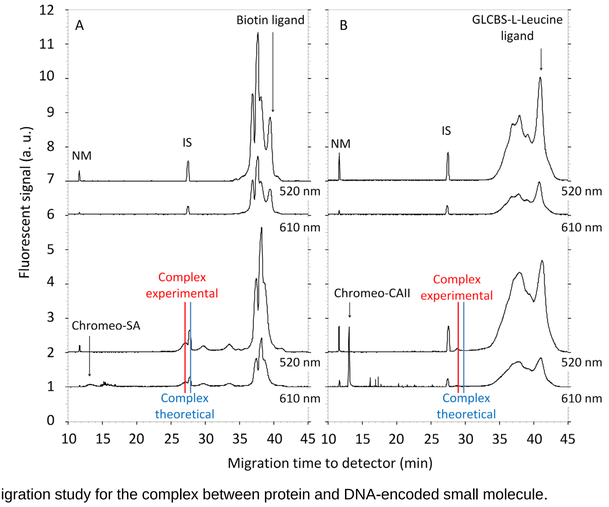

Figure 4. Migration study for the complex between protein and DNA-encoded small molecule.

References:

Bao, J.; Krylova, S.M., Cherney, L.T.; Hale, R.L.; Belyanskaya, S.L.; Chiu, C.H.; Shaginian, A.; Arico-Muendel, C.C.; Krylov, S.N. Predicting electrophoretic mobility of protein-ligand complexes for ligands from DNA-encoded librares of small molecules. *Analytical Chemistry, submitted*.

Bao, J.; Krylova, S.M., Cherney, L.T.; Hale, R.L.; Belyanskaya, S.L.; Chiu, C.H.; Arico-Muendel, C.C.; Krylov, S.N. Prediction of protein-DNA complex mobility in gel-free capillary electrophoresis. *Analytical Chemistry* 2015, 87, 2474-2479.

Table 1. Electrophoretic mobilities of complexes between streptavidin and biotin-ds-ssDNA chimeras of different structures.

ds-ssDNA name	Structures	Experimental complex mobility, mm^2/kVs	Theoretical complex mobility, mm^2/kVs	Deviation between theoretical and experimental mobilities*
20ds		13.39 ± 0.36	12.63 ± 0.39	6%
40ds		16.44 ± 0.08	16.70 ± 0.01	2%
80ds		20.07 ± 0.27	20.80 ± 0.13	4%
120ds		21.95 ± 0.08	22.81 ± 0.01	4%
40-0		15.15 ± 0.16	14.03 ± 0.70	7%
60-0		16.74 ± 0.08	17.48 ± 0.12	4%
80-0		17.76 ± 0.06	18.59 ± 0.07	5%
40-1		15.82 ± 0.02	16.37 ± 0.05	3%
60-1		17.05 ± 0.09	18.38 ± 0.11	8%
60-2		16.87 ± 0.09	18.42 ± 0.04	9%
80-1		17.64 ± 0.11	19.28 ± 0.07	9%
80-2		17.73 ± 0.04	19.30 ± 0.05	9%
80-3		17.63 ± 0.12	19.29 ± 0.07	9%
60-1-2		16.97 ± 0.03	18.66 ± 0.09	10%
80-1-2		17.75 ± 0.03	19.35 ± 0.11	9%
80-2-3		17.65 ± 0.07	19.36 ± 0.08	10%
80-1-3		17.70 ± 0.05	19.49 ± 0.06	10%
80-1-2-3		17.61 ± 0.14	19.50 ± 0.03	11%

* The deviation for each complex was calculated by using | theoretical mobility – experimental mobility| / experimental mobility

Conclusions

In conclusion, we have tested our model with various DNA structures of DNA encoded small molecules. The results confirm the accuracy and robustness of our model. This model will facilitate the reliable use of KCE methods in selecting drug leads from DNA-encoded small molecule libraries and diagnostic probes.

Acknowledgements:
Funding for this work was provided by the National Science and Engineering Research Council of Canada (NSERC)

Please scan the QR codes for online access of this poster and group webpage