Kinetic capillary electrophoresis separates compounds in a bind

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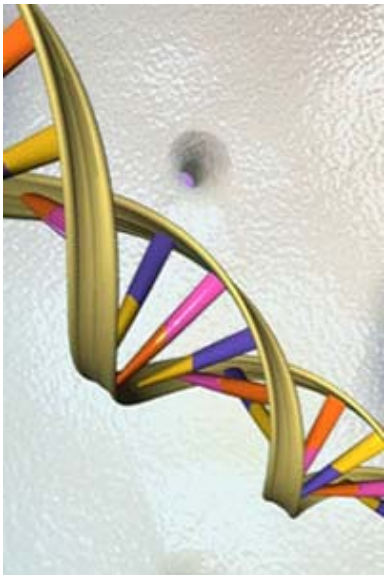
A team of Canadian chemists has come up with a new way of using capillary electrophoresis (CE) to study biomolecular interactions, such as the binding of proteins to DNA. By allowing scientists to study these important interactions in more detail, their work should help in the development of new drug candidates and novel medical diagnostic devices.



KCE can be used to study biomolecular interactions, such as the binding of proteins to DNA. (Image: NHGRI)

The chemists from York University, Toronto, led by Sergey Krylov, discovered their new electrophoresis variant, which they have termed Kinetic CE (KCE), by accident, when they realised that two newly-developed CE methods operated on the same principles. Krylov and his team created the methods to study the binding of proteins to DNA. Specifically, they were investigating the level of attraction between an individual protein and a strand of DNA, which is determined by the rates at which the protein binds with and releases the DNA strand (known as k_{on} and k_{off})

They were therefore trying to design CE methods that could measure k_{on} and k_{off} for different proteins and DNA strands, because simple and robust methods for doing this were lacking. They knew that proteins, DNA strands and their bound complexes migrated at different rates through a CE tube, and that these migration rates could reveal information about the binding rates. All they needed to do was set up the initial separation conditions in just the right way.

In 2002, they developed a method termed "nonequilibrium CE of equilibrium mixtures" (NECEEM) for measuring k_{off}. This involves inserting a short plug of an equilibrium mixture of protein, DNA and the bound complex into one end of a CE tube and then subjecting it to electrophoresis. The three components of the mixture migrate through the tube at different rates, during which time some of the complexes break apart. The precise shape of the three peaks in the subsequent chromatogram allows the researchers to calculate a value for k_{off}.

Then, in 2004, the researchers developed a method termed "sweeping CE" for measuring k_{on}. This involved filling the CE tube with a solution of DNA and then allowing a protein solution to migrate through it via electrophoresis. Some of the protein and DNA react together to form complexes, and the three compounds then migrate through the tube at different speeds. As with k_{off}, the shape of the

peaks in the chromatogram allowed the researchers to calculate a value for k_{on}.

Despite their differences, both these methods involve separating compounds that interact during electrophoresis, and Kylov and his team realised that they were both based on the same underlying concept, hence the development of KCE. Furthermore, Kylov and his team realised they could develop a number of related CE methods based on the concept, and that these could reveal further information about the interaction between different biological compounds.

They have since developed four additional KCE methods, which each involve the electrophoresis of different combinations of protein, DNA and bound complexes. For instance, ppKCE involves inserting one protein plug and one DNA plug into one end of a CE tube, while sSweepCEEM involves inserting a protein plug into a buffer consisting of an equilibrium mixture.

Using all these methods, the researchers explored the interaction between a DNA-binding protein and single-stranded DNA. They first developed a mathematical model of the likely interaction between the protein and DNA, based on estimates for k_{on} and k_{off}. They then compared the model's predictions with experimental results from the six different KCE methods, altering the model until it agreed with the experiments.

This allowed them to discover that the protein and DNA were interacting in two different ways - specific and non-specific - each of which had different values for k_{on} and k_{off}. Specific binding was highly stable and required the protein and DNA to be oriented in a certain way, while non-specific binding was less stable and happened independently of orientation.

Krylov and his team are now working to develop other KCE methods and are using them to study various biomolecular interactions. "In addition, one of our major gaols is to develop simple mathematical considerations for KCE methods, so that people who are not proficient in mathematical modelling could easily use the methods," Krylov told ***SeparationsNOW.com.***

Related Links:

- ***J. Am. Chem. Soc.*, 2002, 124, 13674** : "Nonequilibrium Capillary Electrophoresis of Equilibrium Mixtures - A Single Experiment Reveals Equilibrium and Kinetic Parameters of Protein-DNA Interactions"
- ***J. Am. Chem. Soc.*, 2004, 126, 7166** : "Sweeping Capillary Electrophoresis: A Non-Stopped-Flow Method for Measuring Bimolecular Rate Constant of Complex Formation between Protein and DNA"
- **J. Am. Chem. Soc., 2005, 127, 17104** : "Kinetic Capillary Electrophoresis (KCE): A Conceptual Platform for Kinetic Homogeneous Affinity Methods"

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