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## Chromatography Method Measures Protein-Small Molecule Interactions

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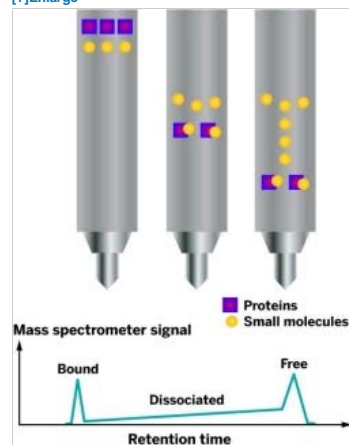
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## COMPLEX SEPARATION

A small molecule and a protein are separately injected into a size-exclusion column (left). The protein moves faster on the column than the small molecule. The protein binds some of the molecules as it travels past (middle). This protein-molecule complex dissociates as it continues through the column (right). Sample exiting the column flows into a tandem mass spectrometer tuned to detect the small molecule. Researchers plot the appearance of the small molecule over time (bottom) to determine the relative amount of bound and free molecules.

Credit: Adapted from *Anal. Chem.*

A new method allows chemists to measure how fast a small molecule binds to and unbinds from a protein without modifying or labeling either molecule (*Anal. Chem.* 2014, DOI: [10.1021/ac503391c](#)). Researchers could use the approach to rank potential drug candidates by their ability to form stable complexes with a target protein, the method's developers say.

The stability of a protein-small molecule complex is determined by how quickly the two molecules associate and dissociate. When pharmaceutical chemists look for stable complexes between a drug candidate and an enzyme or receptor, they want to find small molecules that quickly bind to the protein but fall off slowly. Some common methods for measuring the rate constants of association and dissociation require attaching either molecule to a surface and monitoring the other partner's binding. This modification can alter how the two molecules interact, leading to an inaccurate picture of the complex's stability.

To measure rate constants without modifying the protein or small molecule, [Sergey N. Krylov of York University](#), in Toronto, and his colleagues turned to size-exclusion chromatography, a technique that separates molecules simply by their size. Big molecules such as proteins move quickly through the chromatography column, while small molecules run more slowly. In the new method, the team tracked the breakup of a protein-small molecule complex over time by separating the complex from any dissociated, free molecule.

They tested their method using the enzyme carbonic anhydrase and its small-molecule inhibitor acetazolamide. The researchers injected acetazolamide into a size-exclusion column, followed by the protein. Because of its size, the enzyme raced past the band of acetazolamide, forming complexes with some of the small molecules as it flowed by. These complexes continued to run quickly down the column, but some started to dissociate, leaving a trail of slower-moving free acetazolamide. Molecules exited the column and flowed into a tandem mass spectrometer tuned to detect the small molecule.

The researchers plotted the mass signal for acetazolamide over time. Bound inhibitor came off the column first as a distinct peak, followed by a long streak of dissociated molecules. Small molecules that never formed a complex exited the column in a final sharp peak. With a mathematical model the team developed, the researchers used the observed pattern of free and bound acetazolamide to calculate a dissociation constant of about 120 nM, which is close to the value found using isothermal titration calorimetry, another solution-based, label-free approach. That indicates the new method provides reasonable data, Krylov says.

[Alexander Scheeline](#), who studies protein kinetics at the [University of Illinois, Urbana-Champaign](#), calls the work a powerful experiment to get enzyme kinetics. He thinks the approach also could be easily tuned to look at the rates of product formation by enzymes. Instead of first injecting an inhibitor, researchers would inject a molecule the enzymes could transform. The mass spectrometer would then detect the appearance of the product over time, data that researchers could use to calculate rate constants.

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