Novel Protein Binding Kinetics Measurements in Complex Biological Samples using AGILE

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Abstract
AGILE Research is a graphene based electronic binding assay that is an extension of recent advancements in the material science community. Protein binding kinetics measurements are increasingly common as tools allowing for effective binding measurements to gain wider adoption. However, use of these tools is often limited to concentrations and sample types outside of what is practical in a research environment. For example, many kinetic measurement techniques require an optically clear, homogeneous sample, leading to significant purification work and loss of material during sample processing. Electronic binding measurements represent a novel approach to obtain kinetics, which leverages advances in nanoelectronic sensors. In the past, electronic protein binding tools would require non-ionic diluents, severely limiting biological sample compatibility. We demonstrate that new commercially available tools using graphene sensors overcome this challenge. Data from AGILE Research demonstrates protein-protein binding kinetics measurements directly in complex media such as plasma and cell culture supernatant.

Protocol
1. Functionalize sensor with EDC and sulfo-NHS. Then apply capture molecule.
2. Block and quench with Quench 1 and Quench 2.
3. Apply background buffer and target analyte solution.

Cell Lysate
- 100 ng/ml of crude cell lysate in 1X PBS with 1% BSA. Surface functionalized with monoclonal antibodies to bacteria target.

Protein Extract
- 50 µg crude protein extract from cultured mammalian cells. 6 wells from a 12 well plate. Prepared with antibodies to target protein.

Results

Cell Lysate
- 100 ng/ml of crude cell lysate in 1X PBS with 1% BSA. Surface functionalized with monoclonal antibodies to bacteria target.

Protein Extract
- 50 µg crude protein extract from cultured mammalian cells. 6 wells from a 12 well plate. Prepared with antibodies to target protein.

Growth Media
- 10 mg/mL beta-galactosidase in undiluted cell growth media. Surface prepared with monoclonal antibody to beta-gal.

Tissue Lysate
- 50 µg of protein from tissue lysate, each measurement from an individual embryo. Average (solid) and st. dev. (dotted).

Unstable Target
- Detection of an unstable protein (lifetime ~ 15 minutes) in buffer. Surface functionalized with synthetic peptide.

Assay Description
Our assay is a type of label free bioFET (biological field-effect-transistor). A current is run through the graphene and monitored. A potential varying between +/- 100 mV is applied to the liquid on top of the graphene. This varying potential changes the current through the graphene as in a field-effect-transistor. A capture molecule, such as an antibody or aptamer, is attached to the graphene surface. This capture molecule plays the role of “gate dielectric” found in a traditional field effect transistor. The capture molecule attached to the graphene changes how the potential applied to the liquid effects the graphene. Changes in any exposed charge groups, or to the configuration of the capture molecule, alters the overall electrical properties of the circuit.

The sensor chip above contains 40 graphene transistors in a single measurement “well.” Each of the transistors is functionalized with the same capture molecule. The sensitivity and specificity of the resulting bio-electronic device comes from the the attached capture molecule.

Conclusion
- The AGILE binding assay can be used in a wide variety of sample types from buffers to blood fractions.
- A variety of targets and capture molecules can be used with the AGILE assay, including antibodies, peptides, and cells.
- Kinetic data can be gathered using this assay either through generation of a standard curve or through real time kinetics.