EMSL Research and Capability Development Proposals

The Affinity and Structure of Biomolecules Immobilized on a 3-D Substrate: Time-resolved EPR Detection During Mobile Phase Gradients

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The parameters governing the interaction between chemical binding partners are of fundamental interest to chemists, biochemists, and chemical engineers. Techniques (nuclear magnetic resonance (NMR), circular dichroism (CD), fluorescence) that can determine the affinity, kinetics, structural change, and chemical reactions due to interactions usually require the analyte and the ligand to be in solution. Two notable exceptions are surface plasmon resonance (SPR) and single-molecule fluorescence, where the ligand is attached to a surface and the analyte is suspended in a solution above. Although the solid support is necessary for technical reasons, the tethering of the ligand also provides experimental opportunities. For example, the analyte solution can be changed, by either steps or gradients, in composition (analyte or competitor concentration, pH, cosmotropes/chaotropes, allosteric effectors, etc.) while gathering time-resolved data from the ligand.

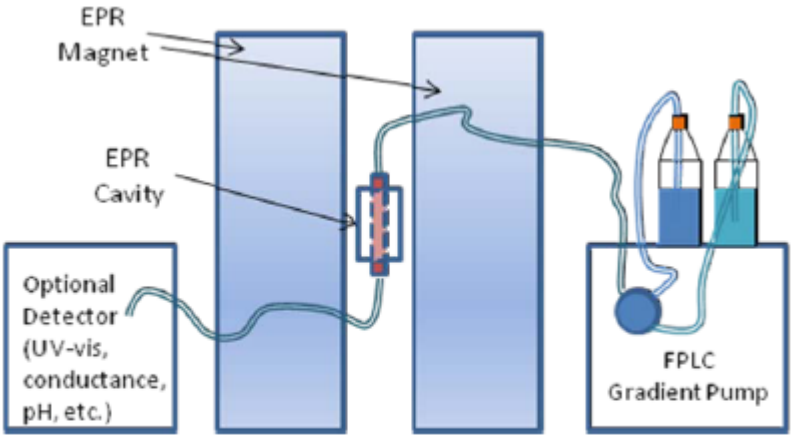

Figure 1. Schematic of proposed instrument.

This project will develop a new technique to remove the constraints for a two-dimensional surface and optical clarity by using affinity beads (or other column materials) as the solid phase and electron paramagnetic resonance (EPR) as the detection spectroscopy. Mechanically, this concept can be realized by replacing the normal EPR aqueous sample cell with a fritted column of the same dimensions. The sample column contains the solid phase with the tethered ligand, and a mobile phase containing the

analyte solution is pumped through the column using a typical fast protein liquid chromatography (FPLC) (see Figure 1).

Equilibrium Under Physiological Conditions

In vitro measurements are commonly made using the highly concentrated levels of protein dictated by instrument sensitivity. *In vivo*, biomolecules are exposed to an environment of low, but highly regulated, levels of many species. To determine if one of these species participates in a stoichiometric fashion with a protein as part of a binding event, it would be necessary to use a concentration many times the physiological level. However, this higher concentration may promote weak, non-physiologically relevant interactions or even unwanted chemistry. In a solution, maintaining a constant level requires a buffer system for each species. However, with a flow technique, the mobile phase can act as a large reservoir or bath to maintain the constant concentration of many species simultaneously.

Research

Though presented here in general terms, this technique was developed to answer some specific questions regarding amyloidogenic proteins. First, we will determine the affinity of the prion protein for copper in an environment with levels of potential competitors matching physiological conditions—the protein tethered at the C-terminus and bathed in the components of cerebral spinal fluid. Next, we will determine whether copper stabilizes or destabilizes the protein. Preliminary CD results indicate destabilization. However, aggregation of the partially unfolded protein precluded a check for reversibility. Here, the folding state of the protein will be monitored via the spectrum of an attached spin-label as a gradient of denaturant is flowed over the tethered protein. These results will have important implications for the entire field of neurodegenerative proteopathies. Several metal chelators that have been identified *in vitro* as possible drug candidates for prion diseases (Alzheimer's and Parkinson's) have had poor or even inverse effects *in vivo*, highlighting the complex relationship between metal ions and disease.

Products and Output

New Capability for EMSL Users

When offered as a capability for EMSL users, this technique should appeal to a variety of disciplines:

- This technique is will be useful for studying the interactions of metals with ligands and a powerful tool for Biogeochemistry.
- The stability and affinity of biocatalysts and biosensors can be studied in their working environments irrespective of the optical properties of the solvent or solid phase support. A select number of non-biocatalysts (i.e., zeolites) will also be amenable, increasing the relevance to studies of Interfacial Phenomena.
- The information derived from this technique is of broad interest within the field of Biological Interactions and Dynamics. The systems that can be studied include all classes of biomolecules, and the information is of fundamental importance.