Rapid screening of protein-protein interactions

Introduction

The central dogma of molecular biology stipulates that DNA is transcribed to RNA, and RNA is subsequently translated into protein [1] . While the central dogma adequately encapsulates the field of molecular biology, protein-protein interactions are at the heart of virtually every biological process. While many are familiar with the term genome, the comprehensive genes of organism, or the term proteome, the comprehensive proteins expressed in an organism, fewer are familiar with an organism's interactome. In short, the interactome typically refers to the entire set of protein-protein interactions (PPIs) in an organism. The importance of protein-protein interactions, and its central nature in biological processes, is highlighted by its role in human diseases. More specifically, when a protein-protein interaction analysis was carried out for the autoimmune disease multiple sclerosis, dozens of interactions were revealed (Figure 1) [2].

Current experimental techniques make the mapping of an organism's interactome an exceptionally daunting task. The available high-throughput techniques to assess PPIs are either laborious or generate false positives (e.g., modifying/tagging the proteins) at high frequency. However, access to an intermediate throughput technique, that is both fast and accurate, would be amenable to mapping the PPIs of a specific biological pathway. In this application note, we will demonstrate how our portable, quad-channel, surface plasmon resonance (SPR) device called P4SPR™ can be used to map the PPIs involved in enterobactin biosynthesis in *Escherichia coli*. The biosynthetic pathway of enterobactin, a small molecule iron chelator, is comprised of six proteins, EntA-F. Since it has been determined that EntB and EntE in this biosynthetic pathway interact, there are very likely other PPIs occurring [3]. Furthermore, the simple design of P4SPR with manual injection will be highlighted as it allows for a quicker assessment and troubleshooting strategy to improve the assay protocol.

Experimental procedures

A quad inlet P4SPR™ that is equipped with 4 separate microfluidic channels was used for this study (see Figure 2). Briefly, to reduce non-specific binding, a gold sensor surface modified with our proprietary Afficoat™ was used, and all the following protein samples and reagents were manually injected into the P4SPR. To activate the sensor surface for protein immobilization, it was treated with EDC/NHS and subsequently washed with sodium acetate. The protein EntF (10 µg/mL), known to play a central role in the biosynthesis of enterobactin, was immobilized onto the sensor surface over 20 minutes. A repeat wash using sodium acetate was performed. To reduce non-specific adsorption from subsequent protein injections, the active sites on the sensor surface was blocked by injecting 1 M ethanolamine, pH 8.5, over 10 minutes. All channels were equilibrated with running buffer. To screen concurrently for interacting partners of EntF in the pathway, multiple proteins, EntA-D for example, were injected over the sensor surface in each channel, aptly labelled A-D.

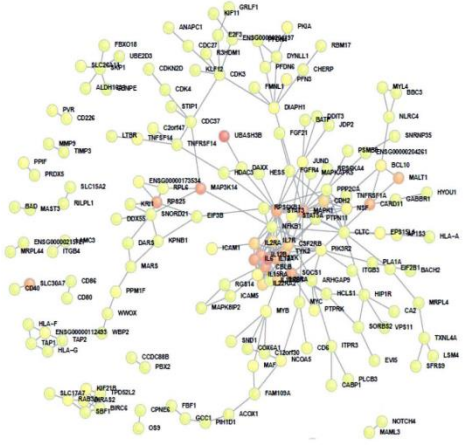

Figure 1 – A subset of the protein-protein interactions implicated in the pathogenesis of the autoimmune disease multiple sclerosis [2].

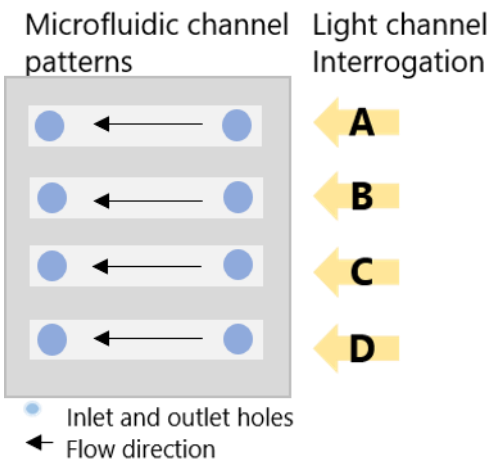

Figure 2 – Microfluidic channel design in the quad inlet P4SPR™.

Initially, injection of ligand over the immobilized protein resulted in sharp increases in the sensorgrams (Figure 3). It was determined that these sharp increases were the result of the presence of glycerol in the ligand samples due to its higher refractive index compared to that of standard buffers. Thus, prior to subsequent experiments, a buffer exchange using a filter column was performed in order to remove the excess glycerol.

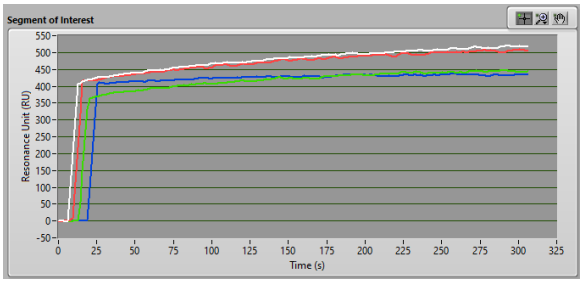

Figure 3 – Sharp increases in refractive index across all four channels due to the presence of glycerol in the ligand injections.

Results and Discussion

Following the method outlined, if an interaction were to occur with the EntF protein immobilized on the surface, one should be able to clearly observe an SPR signal indicating binding. An example of such an SPR signal is highlighted in Figure 4. The red and white time traces display a clear association phase, indicating an interaction, whereas the blue and green traces do not and are virtually indistinguishable from a baseline injection (not shown).

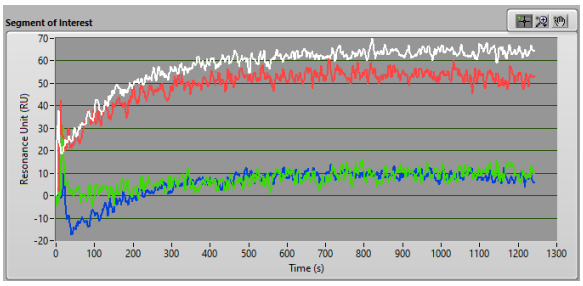

Figure 4 – Sensorgram displaying an example of a protein-protein interaction occurring between the immobilized protein and injected ligand (red and white), or the absence of an interaction (green and blue).

Conclusions

This study demonstrates the feasibility of the P4SPR™ to conduct interactome studies by focusing on the interactions between the immobilized EntF protein and a subset of proteins from the enterobactin biosynthesis pathway in *E. coli*. In addition, assay protocols can be quickly changed and improved due to the simple design of the P4SPR with manual injection.

The P4SPR™ advantage

Affinité instruments' P4SPR™ is a very user-friendly instrument to perform PPI screening. As our knowledge advances, it is becoming increasingly clear that identifying PPIs is of fundamental importance in understanding virtually any biological process or pathway. Their central importance to human health and disease is highlighted by the increasing interest in PPIs as therapeutic targets for drug discovery. As such, the ability to quickly, accurately, screen for PPIs is advantageous for both researchers attempting to understand biological processes, and researchers attempting to improve human health. The P4SPR™ from Affinité instruments allows one to do just that.

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About Affinité Instruments

Established in 2015 as a spin-off of the Université de Montréal, Affinité instruments' foundation is built on deep knowledge accrued over a decade of research in SPR. The commercialization of promising innovations is spearheaded by a diverse leadership experienced in business, science, and engineering.

About the Author

Dr. April Wong is an analytical chemist and has research experience in the biosensor area for over a decade. She obtained her Ph.D. at the University of Toronto.

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