Drug discovery application: Determination of dissociation constant (K_D) between a fragment and a protein

Introduction

Drug discovery is a long and rigorous process, and there are many approaches from different branches of science to find ideal drug candidates. Fragment-based drug discovery has been established as a strategy to identify small compounds that can be further developed into leads and clinical candidates (1, 2). Fragment compounds are typically < 300 Da in size (3), and they are typically screened against a target protein. The equilibrium dissociation constant (K_D) is one of the parameters sought as it indicates the extent of binding affinity of a fragment (or ligand, to be used interchangeably) to a protein that plays a key role in a target disease.

A combination of biophysical techniques, such as microscale thermophoresis (MST), nuclear magnetic resonance (NMR), and isothermal titration calorimetry (ITC) are often used to validate potential fragment screening hits for further development. MST requires fluorescent labels, and the hydrophobic fluorophores may interfere with the ligand-protein interaction through non-specific binding. ITC is relatively low-throughput and requires a large volume of sample, and it may take hours to run each sample. NMR is a label-free approach but is ideally suited for evaluating only weaker binders (mM to µM range).

An additional technique to assist with fragment validation can be surface plasmon resonance (SPR). Although SPR requires an immobilization step of the target protein, it uses a low amount of sample. It is also a label-free and fast method in which affinity information of the ligand-protein complex can be quickly determined in real-time. Affinité Instruments' P4SPR™, for example, can provide accurate results in less than 2 h. Furthermore, unlike most other commercial SPR instruments in the market, it is portable (small footprint) and user-friendly. There is no need to spend a long time to program the software setup as samples are injected manually (for steady-state measurements).

Furthermore, moderate throughput SPR instruments such as the P4SPR allows for more accurate determination of K_D of potential binders by taking advantage of the multichannel microfluidic cell. This application note will introduce the simplicity of using the P4SPR to determine the K_D of a potential fragment compound (ligand)-protein complex for drug discovery.

Experimental Procedures

A Ni-NTA coated sensor chip, dilutions of the ligand samples, protein solution, and other reagents were prepared ahead of time. Ni-NTA coated surfaces allow the immobilization of his-tagged proteins by chelation of histidine residues to the nickel ion. The ligand size was 368 Da and was derived from a follow-up optimization of an initial fragment screening hit. The sensor chip was inserted into a quad inlet model P4SPR (with 4 independent channels). Once the instrument was turned on and connected to the P4SPR Control software on a laptop, the baseline was stabilized by deionized (DI) water, followed by signal stabilization by the running buffer. His-tagged proteins at 10 µg/mL was injected into all 4 channels of the P4SPR and was left to react for 20 min. The sensor chip was then washed with DI water. Figure 1 depicts the experimental scheme and Figure 2 shows the microfluidic cell configuration that is placed on top of the sensor chip, allowing parallel investigation of samples and/replicates.

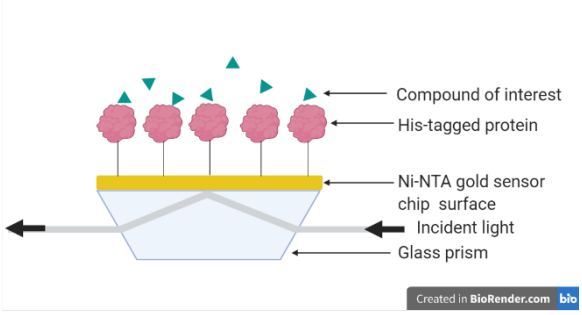

Figure 1 – Determination of K_D between a fragment and a his-tagged protein immobilized on a sensor chip in a quad inlet model P4SPR.

The lowest concentration of the ligand was injected into the channels of the P4SPR and was left to react for 10 min. The SPR shift was saved. Then, a higher concentration of the ligand was injected, and the sample injection steps were repeated until all 5 concentrations have been added.

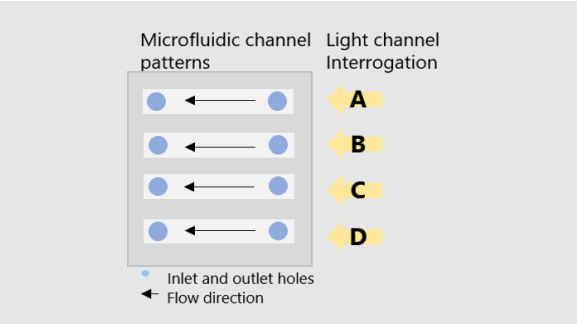

Figure 2 - Schematic of the microfluidic channel configuration in the quad inlet model.

The K_D of the binding interaction between the ligand and protein was determined by using the affinity curve fitting function in the P4SPR Control software.

Results and Discussion

The immobilization steps could be monitored in real-time. For example, the injection of the his-tagged protein led to an increase in SPR shift ~120 RU. After washing the channels with running buffer, the signal did not decrease. Therefore, the protein was effectively immobilized.

Figure 3 shows an example of a sensorgram of the ligand (40 µM) binding to the immobilized protein. The association phase between the fragment and protein is clearly visible. The SPR shift was already levelling off at about 5 min since the start of the injection. The total time it took to run the entire experiment involving 5 concentrations to determine the K_D of the ligand-protein complex was about 1.5 h.

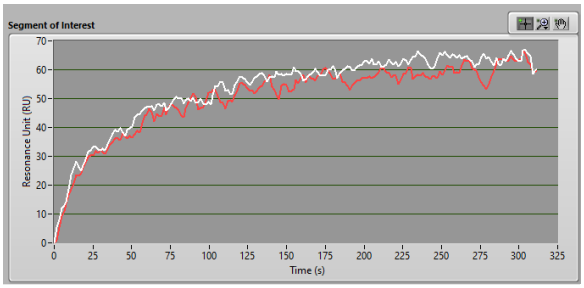

Figure 3 – Sensorgram showing the real-time binding data of the ligand at 40 µM to the immobilized protein in two of the channels (red and white curves).

The SPR shifts obtained at steady-state were plotted against the respective ligand concentration to obtain the K_D value using the P4SPR Control software, which uses the Langmuir binding isotherm to perform a non-linear curve fitting to find K_D. Figure 4 shows the graph and result. The K_D obtained by the P4SPR was in total agreement with the expected K_D.

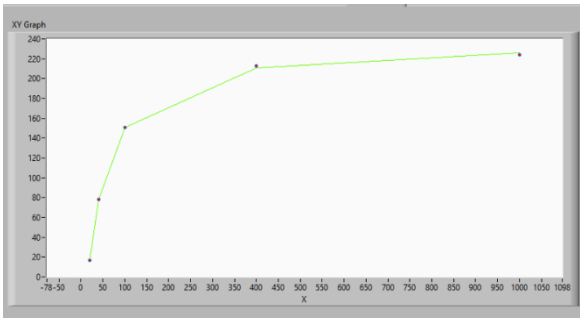

Figure 4 – Determination of K_D by plotting SPR shifts in RU vs. concentration in µM. The K_D was determined to be ~30 µM. χ^2 ~5.

Conclusions

The P4SPR was able to quickly evaluate the K_D between a fragment and a protein. With four microfluidic channels, an accurate K_D could be obtained by injecting at most four replicate samples at once. Injections of the same sample at increasing concentrations can be done on the same sensor chip, hence reducing consumable cost. Most importantly, these data show the value and convenience of having a simple, portable, and accessible SPR instrument to evaluate the K_D of biomolecular interactions in one's own research lab.

The P4SPR Advantage

Affinité instruments' P4SPR is lightweight, compact, and portable. The minimal requirements for setup space and signal stabilization are the P4SPR's key features. In the drug discovery field, it is advantageous to possess an instrument in one's lab to evaluate a binding interaction on the fly without waiting to book to access a shared SPR instrument. Coupling to our KNX2 pump module would provide kinetics data for determination of k_{on} and k_{off}, which are important parameters for drug design.

References

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About Affinité Instruments

Established in 2015 as a spin-off of the Université de Montréal, Affinité instruments' foundation is built deep knowledge accrued on 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.