Binding Kinetics of Protein-Lipid Interactions using OpenSPR

Summary

- Protein-lipid interactions were analyzed using OpenSPR™ instrument
- Liposomes were formed from two different phospholipids and immobilized onto LIP-1 Sensors
- Kinetic analysis was used to determine the on rate, off rate, and affinity constant of the interaction between protein and phospholipid liposomes

Overview

Surface plasmon resonance (SPR) is a commonly used technique for analyzing protein interactions. One interaction that is especially interesting to examine is the interaction between proteins and lipids. In this application note we provide an example of how these types of interactions can be examined using OpenSPR.

Materials and Equipment

- OpenSPR™ Instrument
- TraceDrawer Kinetic Analysis Software
- OpenSPR LIP-1 Sensor Chips
- HBS running buffer (20 mM HEPES, 150 mM NaCl, pH 7.4)
- Phospholipids #1 and #2
- 25 kDa protein

Procedure

1. Liposomes were formed by hydrating with HBS running buffer (20 mM HEPES, 150 mM NaCl, pH 7.4). The hydrated lipids were vortexed for 1 hr, then extruded using a syringe and a 100 nm polycarbonate filter.
2. Surface plasmon resonance (SPR) measurements were obtained using OpenSPR. Tests were run at 20 µL/min using HBS running buffer. LIP-1 Sensors were first cleaned with 40 mM Octyl ß-D-glucopyranoside and 20 mM CHAPS.
3. Liposomes (0.17 µg/mL) were run over the chip surface until 1 nm of immobilization was observed. An injection of 1% w/v bovine serum albumin (BSA) in running buffer was used as a blocking agent to prevent non-specific binding of protein to the chip surface.
4. Four samples of the protein were run over the immobilized liposomes at increasing concentrations (460nM, 1.37 µM, 4.12 µM, to 12.4 µM). A negative control test was also performed by injecting protein onto a blank sensor chip to check for non-specific binding.

Results and Discussion

SPR measurements were analyzed using TraceDrawer 1.6.1. One-to-one kinetic models of the interactions between phospholipids #1 and #2 and the protein are shown in *Figure 1* and *Figure 2*. The models closely fit the experimental data and show clear binding of protein to the lipsosome. Overall, signal noise levels remained under 10 pm and minimal bulk effects were observed. The negative control test confirmed that non-specific binding of the protein to the sensor chip was negligible.

Kinetic evaluations of the interactions are summarized in *Table 1*, with errors shown in brackets. For phospholipid #1, the equilibrium constant (K_D) was found to be 682 nM, the association rate (k_a) was 810 $(M{\cdot}s)^{-1}$, and the dissociation rate (k_d) was 5.53 x 10^{-4}. For phospholipid #2, the K_D was found to be 841 nM, the k_a was 1100 $(M{\cdot}s)^{-1}$, and the dissociation rate was 9.24 x 10^{-4}. The differences in binding kinetics are likely due to differences in the location of the phosphate group between lipid #1 and #2.

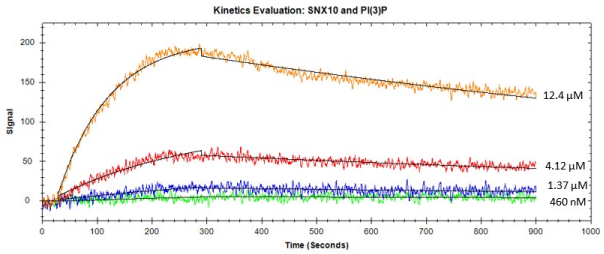

Figure 1. Protein binding to immobilized liposome #1 on OpenSPR

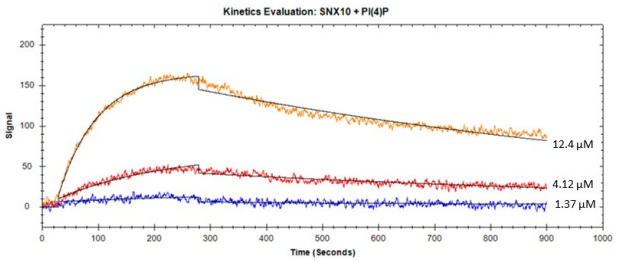

Figure 2. Protein binding to immobilized liposome #2 on OpenSPR

Table 1. Kinetic constants determined using SPR for protein-lipid interactions

Lipid	k_a (1/[M·s])	k_d (1/s)	K_D (nM)
#1	810 (±66.7)	5.53×10^{-4} ($\pm 2.19 \times 10^{-6}$)	682 (±59.2)
#2	1100 (±150)	9.24×10^{-4} ($\pm 1.20 \times 10^{-8}$)	841 (±11.5)

Conclusions

OpenSPR was used to measure the binding kinetics between a protein and two different lipids, identifying the impact of the phosphate group location on the kinetics. This illustrates the value of surface plasmon resonance analysis in such applications.