HHS Public Access

Author manuscript

Methods Mol Biol. Author manuscript; available in PMC 2018 May 23.

Published in final edited form as:

Methods Mol Biol. 2016 ; 1376: 141–153. doi:10.1007/978-1-4939-3170-5_12.

Using Surface Plasmon Resonance to Quantitatively Assess Lipid-Protein Interactions

Kathryn Del Vecchio[1] and **Robert V. Stahelin**[1,2,*]

[1]Department of Chemistry & Biochemistry, University of Notre Dame, Notre Dame, IN 46556

[2]Department of Biochemistry & Molecular Biology, Indiana University School of Medicine-South Bend, South Bend, IN 46617

Summary

Surface Plasmon Resonance (SPR) is a quantitative, label-free method for determining molecular interactions in real time. The technology involves fixing a ligand onto a senor chip, measuring a baseline resonance angle, and flowing an analyte in bulk solution over the fixed ligand to measure the subsequent change in resonance angle. The mass of analyte bound to fixed ligand is directly proportional to the resonance angle change and the system is sensitive enough to detect as little as picomolar amounts of analyte in the bulk solution. SPR can be used to determine both the specificity of molecular interactions as well as the kinetics and affinity of an interaction. This technique has been especially useful in measuring the affinities of lipid-binding proteins to intact liposomes of varying lipid compositions.

Keywords

Binding affinity; Equilibrium binding; Kinetics; Lipid-protein interactions; Surface Plasmon Resonance

1. Introduction

A number of techniques have been developed to assess peripheral protein interactions with lipid membranes. Surface plasmon resonance (SPR) is one such technique that has emerged for quantifying protein affinity and specificity for different lipids [1,2]. Most SPR instruments are based upon the attenuated total reflectance configuration, which relies on the phenomenon of total internal reflection. Total internal reflection is observed when light travelling through an optically dense medium (e.g. glass) reaches an interface between this medium and a medium of lower optical density (e.g. air), and is reflected back. Detection of binding events is possible as an evanescent wave is a component of the incident light that is able to couple with free oscillating electrons (plasmons) in gold film at the interface. A specific angle of incidence (resonance angle) produces a SPR because of energy transfer between the evanescent wave and plasmons on a gold surface. Thus, the SPR signal is sensitive to the mass concentration on the gold surface and is expressed in resonance units

*To whom correspondence should be addressed: 143 Raclin-Carmichael Hall, 1234 Notre Dame Avenue, South Bend, IN 46617. TEL: 1-574-631-5054; FAX: 1-574-631-7821; rstaheli@iu.edu.

(RU). The mass change on the surface can be detected in a time dependent manner, which allows for real-time biomolecular interaction analysis.

SPR has been used extensively to observe protein-protein and small molecule-protein interactions, and more recently has been used to explore the interactions of proteins with other biomolecules such as lipids [2–4]. Lipid biochemistry, especially in eukaryotic systems is complex and not wholly understood; membranes may be comprised of over 1000 different lipid species [5], and many cell-signaling pathways are dependent on protein-lipid interactions [6]. As nearly half of proteins are located within or on membranes, it is imperative to characterize the specifics of lipid-protein interactions in order to discern the role these proteins and lipids play on a broader scale. Inherent advantages of SPR included interactions that can be monitored in real-time, neither the ligand nor the analyte require labeling, instruments have high sensitivity, and high throughput of samples can be performed.

Here we will discuss how SPR can be used to determine an apparent K_d after approximately eight hours of data collection. We have demonstrated sensitivity of this instrument to detect nanomolar quantities of protein in bulk solution [7]. Additionally, the method can be used to quantify both on- and off-rates and binding affinities of lipid-protein interactions. These applications allow a user to dig deeper into mechanisms regulating peripheral protein association and dissociation from lipid vesicles of varying compositions [8,3,4,9]. This guide details methods that can be used with a BiacoreX system and software.

2. Materials

Prepare all solutions using ultrapure water (18 MΩ resistivity at 25C) and analytical grade reagents. It is recommended that you use autoclaved, degassed buffers for both running the instrument as well as sample preparation. Diligently follow all waste disposal procedures. All solutions are kept at room temperature (25C) unless stated otherwise.

2.1 Buffer Preparation

1. SPR running buffer: in the most ideal experimental setup, the SPR running buffer should be the same buffer in which the analyte is stored. This will help to minimize any refractive index changes caused by small differences in buffer components (e.g., salt concentration). The running buffer should be free of all detergents as this would destabilize lipid vesicles (*see* Note [1]). In the case that there is a buffer incompatibility between the analyte storage buffer and the SPR running buffer, a common alternative SPR running buffer is HEPES-KCl (10 mM HEPES, 150 mM KCl, pH = 7.4) (*see* Note [2]).

[1]One drawback to the absence of detergents in SPR buffers is that the instrument should be cleaned more frequently (every 2-3 days) as protein will be lost to the inner tube walls of the SPR during experimentation. Additionally, it is recommended that an SPR instrument is cleaned with the desorb procedure approximately every two days when working with lipid vesicles to minimize any contamination effects on the lipid surface.

[2]It is best to make a one L solution of SPR running buffer, autoclave it, and degas immediately before use using a water bath sonicator or vacuum filter prior to use. Keep the SPR running buffer covered with parafilm or capped with a lid at all times.

2. 50 mM NaOH: Measure out 0.10 g of NaOH and add to ~ 25 mL of autoclaved ddH_2O to dissolve the NaOH pellets, followed by dilution to a final volume of 50 mL. Sterile filter this solution through a 0.2 μm filter. Store at room temperature.

3. 20 mM CHAPS detergent: Measure out 0.614 g CHAPS and add to ~25 mL of autoclaved ddH_2O to dissolve the detergent. Once the detergent is solubilized, dilute to a final volume of 50 mL with autoclaved ddH_2O. Sterile filter this solution through a 0.2 μm sterile syringe filter. Store at room temperature.

4. 40 mM Octyl-β-D-Glucopyranoside: Measure out 0.585 g of Octyl-β-Gluco-Pyranoside and add to ~25 mL of autoclaved ddH_2O to dissolve the detergent. Once the detergent is solubilized, dilute to a final volume of 50 mL with autoclaved ddH_2O. Sterile filter this solution through a 0.2μm filter. Store at room temperature.

5. GE L1 Sensor Chip: Choose a sensor chip that is appropriate for the SPR instrument model you are using. Two common chips are the Sensor Chip L1 and the Series S Sensor Chip L1. The HPA chip can also be used to create a supported bilayer (*see* Note [3]).

2.2 Lipids and Lipid Vesicle Preparation

It is customary to prepare two samples of lipid vesicles: a control vesicle that contains physiologically relevant compositions of lipids that minimally interact with your analyte, and a second variable component vesicle that contains the same lipids as control vesicles with a single, additional lipid species "spiked" in. Avanti Polar Lipids is the gold standard in terms of lipid purity. Additionally, this setup will help to assess any nonspecific binding of protein analyte to the L1 sensor chip surface.

1. 16:0-18:1 PC. 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine

2. 16:0-18:1 PE. 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine

3. 16:0-18:1 PS. 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine (sodium salt)

4. Other commonly used lipids: Lipid-protein SPR can be used to test affinities to lipids other than those listed above. Members of the phosphoinositide (PIP) family [9–11], as well as ceramide-1-phosphate [12,13] have been used in lipid SPR studies. Most other phospholipids should be amenable to study via this technique (*see* Note [4]).

[3]A variety of methods have been utilized to capture lipids on the sensor surface of SPR instrumentation. The most popular and standardized methods are the supported bilayer (HPA chip) or intact lipid vesicles (L1 chip). The HPA chip utilizes hydrophobic interactions between alkanethiol groups on the gold sensor surface, which will capture the hydrophobic tails of lipid molecules injected into the instrument. This forms a lipid monolayer on the alkanethiol referred to as a supported bilayer. The L1 chip captures intact lipid vesicles injected into the instrument using proprietary hydrophobic groups on the gold carboxymethyldextran sensor surface. In our experience both systems work well for coating and lipid-binding experiments with the L1 chip providing more reproducibility and a longer lifetime of the sensor surface. On the other hand, the HPA chip is better served for proteins that may or are known to cause vesicle fusion as these interactions can change the appearance of the vesicles on the L1 chip surface.

[4]For phosphoinositdes (PIP) it is recommended that concentrations in the 1-3 mol% range be used in a phosphatidylcholine (PC) vesicle. This way phosphatidylcholine can be used as a control to directly compare binding of the protein to PC or PC:PIP (97:3) vesicles.

5. Avanti Lipids Mini-Extruder: https://www.avantilipids.com/index.php?option=com_content&view=article&id=509&Itemid=292&catnumber=610023

6. Whatman Filter Membranes: Whatman Nuclepore Track-etch Membrane Filtration Product #800309. Specifications: 19 mm diameter, 0.1 μm pore size.

2.3 Protein (Analyte)

1. For the sake of this guide, we will discuss proteins as the primary SPR analyte. It is recommended that one follows an established protein purification protocol, keeping in mind that large or bulky tags may interfere with a true SPR signal. In our experience, hexahistitidine tags do not seem to cause much issue, but other, larger tags may pose a problem. If your protein is stored in glycerol for increased stability, it is recommended your running buffer contain 5% glycerol to minimize refractive index changes [14,15]. It is also advised that proteins remain on ice until just prior to an SPR run.

3. Methods

Carry out all procedures at room temperature unless otherwise specified.

3.1 Preparation of SPR Instrument

1. Cleaning and Maintenance: This procedure is recommended as routine maintenance and should be done before starting a new experiment if the SPR has been unused for some time. It is important that any buffers or solutions injected into the instrument are degassed and filtered sterilized. Running buffers should be freshly prepared and detergent-free. Run the following cleaning steps with a blank sensor chip (a “Maintenance” Chip) docked in the instrument to avoid permanently damaging a good Sensor Chip.

   Ensure the buffer intake for the SPR is placed in fresh, degassed, and detergent-free running buffer. Run **Desorb**, using BIAdesorb Solution 1 (0.5% w/v SDS in pure water) and BIAdesorb Solution 2 (50 mM glycine-NaOH pH 9.5) as per the instrument prompts. Follow the desorb procedure with the **Sanitize** (10% bleach solution) procedure according to the BIA instrument handbook and as per the instrument prompts. Allow the instrument to run on the **Continue** setting or at a low, continuous flow rate until it is time to run an experiment. It is recommended to dock a proper L1 Sensor Chip at least 12 hours prior to running an experiment so that the chip can become equilibrated with the running buffer.

3.2 Preparation of Lipids/LUVs

1. Control Vesicles: A standard ratio of lipids in control vesicles is 100 mol% POPC or 80:20 mole percent POPC:POPE. These lipid control compositions work well for protein analytes that bind anionic lipids. Prepare 0.5 mL of 0.5 mM lipid mixture. It will be necessary to calculate the proper volume of stock lipid (in organic solvent) to create the mixture. The formula is as follows:

Where M is Molecular Weight of Stock Lipid, in g/mol; TV is Target Volume, in mL; c is Target Concentration, in mM; P is Target Mole Percentage, as a decimal value of 1 (e.g. 60% noted as 0.6); C is Concentration of Stock Lipid, in mg/mL.

Measure out each volume of stock lipid precisely with a gastight Hamilton glass syringe. Then dry lipid mixtures under N_2 gas (*see* Note [5]). Alternatively, a rotary evaporator can be used. Re-suspend lipid mixture in the pre-determined amount of SPR Running Buffer (component TV in the equation above) by vortexing the sample for 10 seconds. Extrude this lipid mixture as per the protocol provided by Avanti Polar Lipids, Inc. (https://www.avantilipids.com/index.php?option=com_content&view=article&id=1600&Itemid=381). It is recommended to extrude lipids 41 times, i.e. so that the lipid mixture passes through the inner membrane filter 41 times. An odd number of extrusions are necessary so as to collect the lipid vesicles on the opposite side of filter membrane from which the extrusion is started. Take note that vesicles used in SPR are generally 0.1 μm, it is advised to use a proper sized filter accordingly. Store vesicles in a 1.5 mL tube at room temperature (*see* Note [6]). Vesicles of this size are stable for approximately 36-48 hours. Dynamic light scattering can be used to assess the mean vesicle diameter.

2. Variable Component Vesicles: A standard ratio of lipids in variable component vesicles will add in a physiologically relevant percentage of your new lipid species, and account for this mole percent addition by subtracting from the total mole percent of POPC. (Ex. Preparing 80:20 POPC:POPE control vesicles and comparing to 60:20:20 POPC:POPE:POPS vesicles). Prepare these vesicles as described in Step 1 (*see* Note [7]).

3.3 Preparation of the sensor surface

1. CHAPS & Octyl Glucoside washes: Begin a new sensorgram with access to both flow channels and a flow rate of 30 μL/minute. Inject 50 μL of 20 mM CHAPS. On the sensorgram, press the “inject” button, and input the injected volume as 25 μL. (*see* Note [8]). Follow this with an injection of 50 μL of 40 mM Octyl Glucoside (Octyl-β-D-Glucopyranoside). On the sensorgram inject window, input the injected volume as 25 μL. After both injections, exit the sensorgram and prime the system by selecting Tools Working Tools Prime. Set the SPR to continue or a low (5 μL/min), continuous flow rate until ready to coat the chip with lipids.

---

[5] Lipid solutions that are prepared in glass amber vials can be dried down under N_2 gas and stored at −20C for up to 6 months. It is recommended to wrap the junction between the cap and vial in Parafilm.

[6] An odd number of extrusions is necessary so as to collect the lipid vesicles on the opposite side of filter membrane from which the extrusion is started. Take note that vesicles used in SPR are generally 0.1 μm, it is advised to use a proper sized filter accordingly. Vesicles of this size are stable for approximately 36-48 hours and dynamic light scattering can be used to assess the mean vesicle diameter.

[7] The variable component vesicles should be extruded after control vesicles, so that there is no risk of contaminating the control lipids with any of the variable lipid species.

[8] It is always a good idea to inject a higher volume of solution into the SPR to minimize the accidental introduction of air bubbles into the system. The SPR will inject the volume that is input in the program and will divert any leftover solution into the waste—in this way, there is always more than enough liquid in the system and air introduction is minimized.

2. Coating the chip with variable-component liposomes: Begin a new sensorgram with access only to flow channel 2 (FC2) at a flow rate of 5 μL/minute (*see* Note [9]). Allow the baseline to equilibrate for 2-3 minutes. Set a baseline for both curves. Pipet up 105 μL of variable component vesicles (made in section 3.2.2). Then add 5 μL of air by dialing the pipet up to 110 μL, ensuring the tip is exposed to the air. Then add 5 μL of sample by dialing the pipet up to 115 μL, ensuring the tip is submerged in the sample tube. Then add one final 5 μL of air. Inject all 120 μL of volume into the SPR. On the sensorgram inject window, input the injected volume as 80 μL. After the lipid injection, change the flow rate to 50 μL/minute. Inject 50 μL of 50 mM NaOH. On the sensorgram inject window, input the injected volume as 10 μL. After the injection, change the flow rate back to 5 μL/minute and keep the sensorgram running. Make note of both the absolute response value as well as the relative response value in resonance units (*see* Note [10]).

3. Coating the chip with control liposomes: In the same sensorgram window, ensure the flow rate is 5 μL/minute. Change the flow channel from Flow Channel 2 (FC2) to Flow Channel 1 (FC1) by using Command Flow Cell Flow Cell 1(*see* Note [11]). Prepare the injection of control vesicles as above: 105 μL liposomes, 5 μL air, 5 μL liposomes, 5 μL air. Inject all 120 μL into the injection port. On the sensorgram inject window, switch the Injection Type dropdown to “Manual Mode” Continue. Input volume as 80 μL. Pause the injection when the relative response level of FC1 matches FC2, keeping in mind that some of the FC1 coating will come off with the NaOH wash, so erring slightly on overshooting is a good strategy. Once FC1 sufficiently matches FC2, exit the manual injection; any leftover liposomes will be diverted to waste. After the lipid injection, change the flow rate to 50 μL/minute. Inject 50 μL of 50 mM NaOH. On the sensorgram inject window, input the injected volume as 10 μL. Repeat this cycle of NaOH injections two more times. After the three NaOH injections, change the flow rate back to 5 μL/minute. Keep the sensorgram running. Make note of both the absolute response value as well as the relative response value in resonance units for both channels. (*see* Note [12]). Stop this sensorgram; it is recommended to save the sensorgram as “Lipid Coat,” for reference. Before starting a new sensorgram, prime the system twice by using Tools Working Tools Prime.

3.4 Collecting SPR data of protein-lipid Interactions

1. Initial blocking with BSA: It is often necessary to block any exposed surfaces of the chip with a stable but unreactive protein (see Note [13]). Bovine Serum Albumen (BSA) is often a good choice for this as it does not specifically bind to

[9]Flow rates faster than 5 μL/min will not robustly support sufficient and timely coating of liposomes on the L1 sensor chip surface.
[10]The relative response value is just the Δresponse unit (ΔRU) change in absolute response units.
[11]Preparing flow cell 1 as the control and flow cell 2 as the active surface will prevent migration and sample loss of some lipids from flow cell 1 to flow cell 2. In our experience, this is necessary to obtain reproducible data over the course of 1 or 2 days of experimentation with a lipid surface.
[12]It is best to have relative response levels be within 3-5% between the channels so as not to bias data collection one-way or the other. The closer the channels match, the better.

most lipids that would be used in the SPR. This is also a good test of assessing the coating efficiency of the L1 sensor chip. Begin a new sensorgram with access to both FC1 and FC2 at a flow rate of 5 μL/minute. Allow the baseline to equilibrate for 2-3 minutes before setting a baseline for both flow channels. Prepare a 150 μL sample of 0.1 mg/mL BSA. (*see* Note [14]) Pipet up 105 μL of protein, 5 μL of air, 5 μL of protein, and 5 μL of air. Inject all 120 μL into the SPR. On the sensorgram inject window, input the injected volume as 80 μL. After the lipid injection, change the flow rate to 50 μL/minute. Inject 50 μL of 50 mM NaOH. On the sensorgram inject window, input the injected volume as 10 μL. After the injection, change the flow rate back to 5 μL/minute. Repeat this process as necessary to get the relative response value as close to the baseline as possible. (*see* Note [15]). Make note of both the absolute response value as well as the relative response value in resonance units for both channels. This is the "new" baseline coating that will be used to collect all protein injection data.

2. Injections of protein over the sensor surface: For each protein injection, do not prepare the dilution until just prior to the injection. Collect a separate sensorgram for each protein injection to better organize the data sets. It is also advised to use fresh, active protein, and to prior to use, spin the stock sample of protein at 50,000 *g* for 20 minutes to remove any precipitated protein. Plan the protein dilutions that will be tested over the sensor surface. It is a good idea to go as low as 10-fold below the predicted K_d and 10-fold above the predicted K_d. A curve should have no fewer than 6 points, and 8 or more points usually comprise a good data set for curve fitting with twelve being an optimal number of data points for fitting. Measurements should be taken from the lowest concentration of protein to the highest concentration. (*see* Note [16]).

Begin a new sensorgram with access to both FC1 and FC2 at a flow rate of 5 μL/minute. Allow the baseline to equilibrate for 2-3 minutes before setting a baseline for both flow channels. Prepare a 150 μL sample of dilute protein (*see* Note [17]). Pipet up 105 μL of sample, 5 μL of air, 5 μL of sample, and 5 μL of air. Inject all 120 μL into the SPR. On the sensorgram inject window, input the injected volume as 80 μL and set a delay for washing of 200 seconds (*see* Note [18]). Make note of the absolute response value and the relative response value of each channel. After the lipid injection, change the flow rate to 50 μL/

[13] How the lipid vesicles form on the L1 surface is still under debate with most studies suggesting that vesicles are retained intact on the L1 chip surface. One studied suggested the vesicles fuse and form a lipid bilayer [16], while several others using imaging and dye leakage assessment have strong evidence that the lipid vesicles are intact on the sensor surface [4]. The type of surface that forms may be specific to the types and origins of the lipids and lipid mixtures employed as well as the pH and osmolarity of the running buffer. Either way vesicles anchored to the L1 chip adopt a structure that is relevant for examining lipid-protein interactions.

[14] The significance of lipid-coating can be verified by injecting 0.1 mg/mL BSA as less than 100 RU of BSA should bind to a well coated surface while > 1000 RU of BSA will bind to an uncoated or poorly coated lipid surface. We've demonstrated that BSA left on the sensor surface will not influence lipid-binding parameters and under some conditions can reduce nonspecific binding to the L1 chip should the protein of interest nonspecifically associate with the carboxylmethyldextran layer.

[15] If the relative response value goes down to a certain point but does not completely reach baseline, this is the BSA that has "blocked" the exposed hydrophobic portions of the chip. This often does not take more than 3-5 NaOH washes. Typically, BSA response will be less than 100 resonance units for a sufficiently lipid coated L1 sensor chip.

[16] Start with low protein concentration first in case protein binds or sticks to chip or is hard to remove from the lipid vesicles.

[17] Only prepare protein sample dilutions right before you are going to inject them into the SPR.

[18] It is advised to add a 200+ second delay so that washing of the injection port, which ensues immediately following an injection, does not significantly influence the SPR signal stability. Washing of the injection port can contribute to noise in the SPR signal. When performing saturation (equilibrium binding) measurements a short delay of 200 seconds or so is sufficient to avoid these issues.

minute. Inject 50 μL of 50 mM NaOH. On the sensorgram inject window, input the injected volume as 10 μL. After the injection, change the flow rate back to 5 μL/minute. Repeat this process as necessary to get the relative response value as close to the baseline as possible. Make note of both the absolute response value as well as the relative response value for both channels, noting especially if there is any minimal protein remaining on the chip. Proceed with a new sensorgram for each new injection. Once all injections are collected, continue with "Preparing the Sensor Chip for storage" (*see* Note [19]).

3.5 Preparing the Sensor Chip for storage

1. CHAPS and octyl glucoside washes: After all protein-lipid binding measurements have been made, the chip should have all liposomes removed before storage. In a sensorgram with access to both FC1 and FC2 at a flow rate of 30 μL/minute, inject 50 μL of 20 mM CHAPS. On the sensorgram, press the "inject" button, and input the injected volume as 25 μL. Follow this with an injection of 50 μL of 40 mM Octyl Glucoside (Octyl-β-D-Glucopyranoside). On the sensorgram inject window, input the injected volume as 25 μL. After both injections, exit the sensorgram.

2. Undock L1 chip and store at 4C: Undock the L1 chip by navigating to Command Undock. Take out the L1 chip and store in a 50 mL conical tube containing approximately 200 μL of running buffer at the bottom to ensure slightly damp storage conditions. To reduce oxidation, a stream of N_2 or argon gas can be used to displace the air in the conical tube prior to storage. Store the sealed tube at 4C. Place a maintenance chip in the SPR and dock it. Leave the instrument running on continue or at a low (5 μL/min), continuous flow rate until it is time to run another experiment. Ensure that the running buffer does not run out. Should the system not be needed for use in three days, perform a shutdown procedure can be run according to the manufacturer's instructions.

3.6 Data analysis

This guide will describe how to process the data collected using BIAevaluation and KaleidaGraph software.

1. Quantify Δresonance Unit (ΔRU) for each injection: In BIAevaluation software, open the first protein injection. This will open the data files for both FC1 and FC2. Select both of these curves and display them using the chart button. Right click and drag to select a small section prior to the time of injection and select Y-Transform Zero at Average of Selection Replace Original. Then select X-Transform Curve Alignment Next. Zoom in to the area just prior to the injection. Move the cross-hatches for each curve to the point of the injection. Select

---

Should a user wish to perform detailed kinetic analysis of the off-rate, it is advised to use a longer delay in washing so as to monitor the off-rate as long as possible. This will provide more data points for analysis without noise in signal that comes from the SPR wash step.

[19]SPR is also a technique that should receive dedication once a system is working and reproducible. The lifetime of a lipid surface on a L1 chip can last from 12-48 hours so we recommend dedication, organization, and experimental planning during these times for lab members to collect robust reproducible data over a period of one to two days.

Accept/OK and this will align the curves at the same X-position. From the curve: dropdown, select the second of the two curves being analyzed. In the Y-Transform window, select "Curve – Curve 2 (Blank Run Subtraction)" and select the first curve in the pair Replace Original. Delete off the NaOH washes by right clicking with the mouse and dragging to just before the NaOH washes begin and selecting Edit Cut. Note the response unit value at the point of saturation on the curve. Repeat this process for all remaining injections, making a table of protein concentration vs. ΔRU value at saturation. It is not necessary to keep the "odd" curves (the zeroed curves) in the analysis—one can plot all of the "even" curves together to obtain a saturation profile (*see* Note [20]).

2. KaleidaGraph curve analysis of SPR data: Open KaliedaGraph and plot protein concentration in Column A, ΔRU Responses in Column B. Select Gallery Linear Scatter. Select Protein Concentration as X and ΔRU as Y. Select Curve Fit General Fit1 Define (m0*m1)/(m0+m2);m1=1100;m2=1 Check the "ΔRU" box. The m2 value that appears on the graph is the apparent K_d of the interaction based on the data from Columns A and B. Other graphing programs can be used according to user familiarity and preference.

Acknowledgments

The NIH (AI081077) and NSF (1122068) have supported lipid-protein interaction work in the Stahelin lab using SPR. K.D. is funded by a CBBI NIH T32 Predoctoral fellowship (NIH T32GM075762).

References

1. Narayan K, Lemmon MA. Determining selectivity of phosphoinositide-binding domains. Methods. 2006; 39(2):122–133. DOI: 10.1016/j.ymeth.2006.05.006 [PubMed: 16829131]
2. Stahelin RV. Surface plasmon resonance: a useful technique for cell biologists to characterize biomolecular interactions. Molecular biology of the cell. 2013; 24(7):883–886. DOI: 10.1091/mbc.E12-10-0713 [PubMed: 23533209]
3. Stahelin RV, Cho W. Roles of calcium ions in the membrane binding of C2 domains. The Biochemical journal. 2001; 359(Pt 3):679–685. [PubMed: 11672443]
4. Stahelin RV, Cho W. Differential roles of ionic, aliphatic, and aromatic residues in membrane-protein interactions: a surface plasmon resonance study on phospholipases A2. Biochemistry. 2001; 40(15):4672–4678. [PubMed: 11294634]
5. van Meer G, Voelker DR, Feigenson GW. Membrane lipids: where they are and how they behave. Nature reviews Molecular cell biology. 2008; 9(2):112–124. DOI: 10.1038/nrm2330 [PubMed: 18216768]
6. Moravcevic K, Oxley CL, Lemmon MA. Conditional peripheral membrane proteins: facing up to limited specificity. Structure. 2012; 20(1):15–27. DOI: 10.1016/j.str.2011.11.012 [PubMed: 22193136]

[20]Kinetic analysis of SPR data can be quite cumbersome and requires careful consideration before publishing results. In brief, the rate of adsorption and desorption are dependent on intrinsic kinetics and mass transport of the system. Diffusion through the boundary layer is usually much slower than the intrinsic adsorption kinetics and is, therefore, the rate determining factor. The best method of detecting a mass transport limitation is to vary the flow rate of the system, and calculate rate constants under these varying conditions. If mass transport is not rate limiting then rate constants will be consistent over a broad range of flow rates. This holds true because diffusion kinetics are dependent on the flow rate while intrinsic kinetics are not. To eliminate potential mass transport effects, the rate of diffusion must be increased and the rate of binding reduced. Thus, increasing the flow rate and decreasing the ligand density so as to reduce the number of available binding sites are two ways of minimizing the mass transport limitations of a system.

7. Soni SP, Adu-Gyamfi E, Yong SS, Jee CS, Stahelin RV. The Ebola virus matrix protein deeply penetrates the plasma membrane: an important step in viral egress. Biophysical journal. 2013; 104(9):1940–1949. DOI: 10.1016/j.bpj.2013.03.021 [PubMed: 23663837]

8. Bittova L, Stahelin RV, Cho W. Roles of ionic residues of the C1 domain in protein kinase C-alpha activation and the origin of phosphatidylserine specificity. The Journal of biological chemistry. 2001; 276(6):4218–4226. DOI: 10.1074/jbc.M008491200 [PubMed: 11029472]

9. Stahelin RV, Long F, Diraviyam K, Bruzik KS, Murray D, Cho W. Phosphatidylinositol 3-phosphate induces the membrane penetration of the FYVE domains of Vps27p and Hrs. The Journal of biological chemistry. 2002; 277(29):26379–26388. DOI: 10.1074/jbc.M201106200 [PubMed: 12006563]

10. Blatner NR, Stahelin RV, Diraviyam K, Hawkins PT, Hong W, Murray D, Cho W. The molecular basis of the differential subcellular localization of FYVE domains. The Journal of biological chemistry. 2004; 279(51):53818–53827. DOI: 10.1074/jbc.M408408200 [PubMed: 15452113]

11. Stahelin RV, Karathanassis D, Murray D, Williams RL, Cho W. Structural and membrane binding analysis of the Phox homology domain of Bem1p: basis of phosphatidylinositol 4-phosphate specificity. The Journal of biological chemistry. 2007; 282(35):25737–25747. DOI: 10.1074/jbc.M702861200 [PubMed: 17581820]

12. Stahelin RV, Subramanian P, Vora M, Cho W, Chalfant CE. Ceramide-1-phosphate binds group IVA cytosolic phospholipase a2 via a novel site in the C2 domain. The Journal of biological chemistry. 2007; 282(28):20467–20474. DOI: 10.1074/jbc.M701396200 [PubMed: 17472963]

13. Ward KE, Bhardwaj N, Vora M, Chalfant CE, Lu H, Stahelin RV. The molecular basis of ceramide-1-phosphate recognition by C2 domains. Journal of lipid research. 2013; 54(3):636–648. DOI: 10.1194/jlr.M031088 [PubMed: 23277511]

14. Stahelin RV, Digman MA, Medkova M, Ananthanarayanan B, Rafter JD, Melowic HR, Cho W. Mechanism of diacylglycerol-induced membrane targeting and activation of protein kinase Cdelta. The Journal of biological chemistry. 2004; 279(28):29501–29512. DOI: 10.1074/jbc.M403191200 [PubMed: 15105418]

15. Stahelin RV, Digman MA, Medkova M, Ananthanarayanan B, Melowic HR, Rafter JD, Cho W. Diacylglycerol-induced membrane targeting and activation of protein kinase Cepsilon: mechanistic differences between protein kinases Cdelta and Cepsilon. The Journal of biological chemistry. 2005; 280(20):19784–19793. DOI: 10.1074/jbc.M411285200 [PubMed: 15769752]

16. Erb EM, Chen X, Allen S, Roberts CJ, Tendler SJ, Davies MC, Forsen S. Characterization of the surfaces generated by liposome binding to the modified dextran matrix of a surface plasmon resonance sensor chip. Analytical biochemistry. 2000; 280(1):29–35. DOI: 10.1006/abio.1999.4469 [PubMed: 10805517]