Published in final edited form as:

Methods Mol Biol. 2019 ; 1860: 199–210. doi:10.1007/978-1-4939-8760-3_12.

Use of Surface Plasmon Resonance (SPR) to Determine Binding Affinities and Kinetic Parameters Between Components Important in Fusion Machinery

Robert P Sparks¹, Jermaine L Jenkins², Rutilio Fratti¹

¹Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL, USA.

²Structural Biology and Biophysics Facility, University of Rochester, Rochester, NY, USA.

Abstract

Surface plasmon resonance (SPR) can be used to analyze both binding affinities and kinetic parameters between a ligand and an analyte. SPR can be performed by either cross-linking a given ligand to a sensor chip covalently or utilizing high-affinity non-covalent interactions to secure a ligand in a particular conformation to a chip, both of which have their potential advantages. SPR measurements are mass based and reflect the proportional amount of analyte bound to a given ligand at a given concentration when flowed at a set rate in order to determine the binding parameters of a given biochemical interaction. The resultant sensorgram can indicate different types of binding events as well as provide both k_a and k_d , which can be used to determine an equilibrium dissociation constant K_D . SPR can be used to measure binding affinity of proteins involved in fusion such as between SNAREs, SNAREs, and proteins that interact with them such as Sec18 (NSF) or Sec17 (alpha-SNAP), or to measure the binding of any fusion-related protein to a specific lipid or other small molecules; however, K_D s are determined by SPR using a titration of concentrations of analyte and a maximum point on the sensorgram signifying saturation of the protein in order to determine a steady-state K_D .

Keywords

Phosphatidic acid; Sec18; NSF; Sec17; α -SNAP; Nanodisc; SNARE

1 Introduction

Surface plasmon resonance (SPR) is a technique that can be utilized to study diverse types of biophysical interactions in order to determine both steady-state and kinetic binding affinities

4.1 **Buffer Selection** Choosing appropriate buffers in SPR often depends on two main factors: (1) *pH effects* for a given interaction for covalent attachment of ligand, regeneration, and binding measurements, and (2) *buffer matching* to remove artifacts from signal measured. pH of buffer must allow for the ligand to interact with the surface in a manner conducive to covalent attachment, and for optimal capture efficiency a pH difference of 3 units below the P_I is recommended. Furthermore, for pH to allow regeneration of the chip, it must be sufficiently low or high to perturb the interaction of ligand with analyte based on the pK_A of the residues involved in binding (generally 1–2 pH units above or below). Additionally, binding measurements are often pH dependent so it is important to pick a buffer pH that is suitable or physiologically relevant to the interaction you are measuring. Buffer matching is critical in order to remove noise from a measurement. For example, a 1% DMSO mismatch can lead to a 1000 RU signal measured. It is important to keep all buffer concentrations constant with the only changes in buffer from running buffer to injection being as to the amount of analyte being analyzed.

between relevant binding partners [1]. SPR measurements are label free and are based on quantitative binding of an analyte to a ligand either covalently or non-covalently attached to a microfluidic chip. SPR measurements are based on changes in the refractive index near the sensor surface where analytes are continuously flowed and measured along the sensor surface. Generally, SPR was designed for determining relative affinities of protein-protein interactions such as antibodies to an antigen of interest, in which the analyte and the ligand have approximately similar sizes [2]. However, SPR is now frequently used to determine affinity between relatively smaller analytes (<1 kDa) and macromolecular ligands (>10 kDa) and routinely for fragment-based drug discovery screening in the pharmaceutical industry [3].

Resolution for SPR is determined by the relative mass ratio between the analyte and the ligand. Generally, for kinetic measurements a maximum response of ~100 RU is preferred [2]. A simple calculation can be used to approximate how much ligand to attach to the surface based on the size of both the ligand and analyte where $[\text{Response}_{\text{max}} = (\text{Response}_{\text{Ligand}} \times \text{Mass}_{\text{Analyte}}) / \text{Mass}_{\text{Ligand}}]$. From this equation one can determine the amount of ligand to immobilize to obtain a maximum response generated when the ligand is saturated with a given analyte. Further modifications of this formula may be necessary if it is predetermined that the ligand has multiple binding sites $[\text{Response}_{\text{max}} = (\text{Response}_{\text{Ligand}} \times \text{Mass}_{\text{analyte}} \times \text{Valency}_{\text{Ligand}}) / \text{Mass}_{\text{Ligand}}]$. Additionally, the method used to immobilize the ligand could affect the activity of said ligand with capture techniques usually resulting in a higher chance of immobilizing a ligand that retains its binding activity. To illustrate difficulties in obtaining quality SPR data when the mass differential of a ligand and analyte is large, imagine a ligand such as protein of 100 kDa and a small molecule of the size of 100 Da. To have an R_{max} of 1 RU, you would need to have 1000 RU of ligand attached to an SPR chip, and to have an R_{max} of 100 RU you would need to have 100,000 RU of ligand attached to the SPR chip. However, currently that exceeds the amount of RU of ligand that can be attached to GE SPR chips such as CM5 or Ni-NTA (~5000–10,000 RU without crowding) and even CM7 chips designed to allow greater capture of ligand to address small-molecule binding. Therefore, when measuring small-molecule binding, if kinetics is desired it may be necessary to use fragments of larger ligands that encapsulate a given binding site in order to increase resolution of resultant sensorgrams.

In order to design a sensible SPR experiment taking mass considerations into account, it is next necessary to determine the type of chemical interaction for immobilization of ligand to the chip surface. There are many different types of chip surfaces available based on different immobilization techniques, either covalent or non-covalent attachment. We will restrict our discussion to the popular GE Healthcare's Biacore line of instruments and chips because that is what we have the most experience with but we recognize that there are many excellent alternative instruments and chips available. A standard covalent chip is the CM5 chip that has a surface comprised of carboxymethylated dextran allowing immobilization via NHS/EDC amine chemistry of proteinaceous ligands taking advantage of amide bonds. However, this coupling technology has the disadvantage that it can create heterogeneous attachment configurations.

Oftentimes, heterogeneous cross-linking of ligands to a chip still yields quality data; however, protein tags such as 6X-His, biotin, or an inherent Fc portion of an antibody can be used to enable a ligand to adopt an oriented uniform conformation across a specific chip surface (Ni-NTA, streptavidin, or protein A, respectively). For each of these pre-immobilized sensor chips that are ready to use out of the package can be purchased requiring only the first step of capturing the ligand, followed by a cross-linking step to create a more stable and robust chip capable of handling harsher regeneration conditions.

In setting up given experiment, it will then be necessary to assess thoroughly preliminary conditions before purchasing the correct chip and attaching the correct ligand to the chip such as the type of protein to be measured, where a given tag is placed such that it can be utilized to simulate natural conditions of the protein, and mass of protein versus analyte to get a quality signal. It is important to understand that there are additional considerations to take into account in setting up a quality SPR experiment that will not be covered herein but have been covered in past publication reviews.

Running buffer:

SPR is amenable to several different variations of standard running buffers. It is important to select a running buffer with an appropriate pH for the given interaction such that protonation states match natural conditions such that the measured K_D is biologically relevant. Include ions whose presence plays a role under natural conditions of keeping a protein in an appropriate conformation. Furthermore, for proteins that dimerize or hexamerize such as Sec18 (NSF), it may be important to add additional small molecules to the buffer such as ATP along with the ion magnesium in order to ensure the desired macromolecular structure.

Regeneration buffer:

For experiments utilizing ligands cross-linked to the chip surface, oftentimes it is time prohibitive to allow slowly disassociating analytes to naturally dissociate. This problem could be solved by cross-linking multiple chips to obtain a fresh sensorgram; however, this can be cost prohibitive because SPR chips are costly. Therefore, regeneration conditions ranging from mild (less risk of damaging ligand) to harsh (more risk of damage to ligand) can be utilized to remove a given analyte from a ligand and speed up a given SPR experiment. It is important to understand that some regeneration conditions that may be satisfactory for one type of ligand may cause irreversible damage to another ligand, so often a trial-and-error procedure for selecting the appropriate regeneration buffer may still require multiple SPR chips in order to achieve satisfactory conditions that can be used across multiple injections for a given experiment.

Association and disassociation times:

Binding of analyte to ligand (association) results in higher RU as determined by continuously flowing analyte over both a ligand-attached flow cell and a ligand-free flow cell subtracting the ligand-free flow cell from the ligand-attached flow cell (Fig. 1a). However, removal of analyte from ligand resulting from continuous washing with buffer results in less signal (Fig. 1c). Assuming a high enough concentration of analyte is flowed for the given ligand concentration present on the chip, saturation or equilibrium can be

reached and $\text{Response}_{\text{max}}$ achieved (Fig. 1b). However, for a given chemical interaction, it is important to allow enough association and disassociation time to achieve equilibrium for a given interaction, and this is relative to the given ligand and analyte. Reasonable association times and disassociation times vary greatly and may depend on the type of experiment and/or data that one would want to obtain.

Buffer selection:

Many traditional buffers can be used for SPR such as HEPES, Tris, or PBS. One important consideration when choosing a running buffer is matching potential solvent used for chemicals such as active molecules in pharmaceutical formulations because these molecules are often dissolved in organic solvents such as DMSO. Importantly, DMSO can create significant distortions in response or buffer mismatch. Therefore, it is important to determine firstly what percentage of DMSO is required to dissolve a particular organic molecule at its highest concentration and to match the DMSO for all titrations as well as for the running buffer so that all solutions contain the same percentage of DMSO.

In this protocol, we describe the binding of the SNARE-activating protein Sec18 to phosphatidic acid (PA)-containing nanodiscs. We have previously reported that PA sequesters Sec18 away from SNARE complexes to prevent priming [4, 5]. These studies were carried out using purified vacuoles or artificial liposomes, neither of which can be used to determine the specific binding constant for Sec18-PA interactions.

2 Materials

1. Phosphatidylcholine (PC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine); phosphatidylethanolamine (PE, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine); phosphatidic acid (PA, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphate) (Avanti Polar Lipids).
2. Sec18: His₆-Sec18 can be expressed as a fusion protein in *E. coli* and purified using Ni-NTA and size-exclusion chromatography [5]. Hexamer and monomer pools are separated by size-exclusion chromatography.
3. Membrane scaffold protein (MSP1D1 nanodiscs prepared with 3.023 μmol dipalmitoyl phosphatidylcholine (PC), 0.098 μmol diC16-PA, and 0.78 μmol 1-palmitoyl, 2-oleoyl phosphatidylethanolamine (PE) as described [6, 7].
4. Biobeads (Bio-beads SM-2, Biorad or amberlite XAD-2, Sigma Aldrich).
5. Running buffer: 10 mM HEPES pH 7.4, 150 mM NaCl.
6. Mild regeneration solution: 2 M NaCl.
7. Acidic regeneration, solution: 10 mM Glycine pH 2.
8. Nickel solution: 150 mM NiCl₂.
9. EDTA stripping solution: 350 mM EDTA.

10. Coupling buffer: Optimally select buffer approximately 3 pH units below that of the isoelectric point of ligand to be immobilized. Assuming pI of approximately 8 for Sec18: 10 mM acetic acid pH 5 (see **Note 1**).
11. Nickel running buffer: 10 mM HEPES pH 7.4, 150 mM NaCl, 50 μ M EDTA.
12. ATP running buffer: 10 mM HEPES pH 7.4, 150 mM NaCl, 50 μ M EDTA, 3 mM ATP, 3 mM MgCl.
13. TBS: 50 mM Tris-HCl, 150 mM NaCl, pH 7.4.
14. His6-Sec17p expressed as a fusion protein in *E. coli* and prepared using NTA and size-exclusion chromatography [8].

3 Methods

Three hypothetical SPR experimental protocols are given below according to a Standard Protocol developed by Nico J. de Mol and Marcel J.E. Fischer [9].

3.1 Assembly of 5% PA Nanodiscs (20% PE, 75% POPC, and 5% PA) Adapted from the Sligar Lab Protocol Available at sligarlab.life.uiuc.edu/nanodisc/protocols.html

1. Total concentration of lipid is 3.9 μ M so for PC 2.925 μ M, PE 0.78 μ M, and PA.195 μ M lipid is required in order to make 2.9 μ L of 1 M 5% PA nanodiscs.
2. Determine the volume of lipid required to make concentrations determined in **step 1**. Assume all solutions of lipid are in a stock concentration of 25 mg/mL. For example, to make 75% PC liposomes, determine concentration required by multiplying $0.75 \times 3.9 \mu\text{M}$ to get 2.925 μ M PC. POPC has a molecular weight of approximately 760 Da, so to determine the amount of POPC needed multiply $760 \times 2.925 \mu\text{M}$ to get 0.022 mg of POPC. Since the stock solution has 25 mg/mL POPC, this will require 88.9 μ L of 25 mg/mL POPC added to a glass tube using a Hamilton syringe. Do this for PE and PA as well. For additional examples of these calculations, refer to the Morrissey Lab Protocol for Preparing SUV available at <https://tf7.org/suv.pdf>.
3. Once all volumes of lipids are added to the same glass tube, dry overnight in vacuum-sealed desiccator after evaporating solvent using inert gas such as nitrogen.
4. Add deoxycholate solution at 2:1 deoxycholate:phospholipid concentration. Thus, $2 \times 22.4 \text{ mM}$ deoxycholate out of 100 mM stock ($44.8 \times 174 \mu\text{L}/100 \text{ mM}$) is 78 μ L.
5. Bring volume of lipid solution to 174 μ L with TBS ($3.9 \mu\text{mol}/x = 22.4 \text{ mM}$, 174 μ L), adding 96 μ L of TBS.
6. Cover solution with parafilm and sonicate 4 cycles of 5 min until the solution is clear.
7. Add MSP1D1 using a concentration of phospholipid greater than 5 mM (5.2 mM) and a volume greater than 0.3 mL (0.5 mL) to calculate appropriate

concentration. For example, an MSP1D1 nanodisc can be assumed to have 70 phospholipids per layer of bilayer such that $5.2 \text{ mM}/70 = 74 \text{ } \mu\text{M}$. Volume of MSP1D1 is calculated using $74 \text{ } \mu\text{M} \times 500 \text{ } \mu\text{L}/[\text{MSP1D1}]$. MSP1D1 concentration can be determined by measuring A_{280} , $E_{280} = 21000 \text{ M}^{-1} \text{ cm}^{-1}$.

8. Determine appropriate lipid volume to add, $5.2 \text{ mM} \times 500 \text{ } \mu\text{L}/22.4 \text{ mM} = 116 \text{ } \mu\text{L}$ phospholipid/detergent.
9. Add MSP1D1 volume determined from **step 8** and 116 μL of detergent/lipid solution bringing total volume to 500 μL with TBS.
10. Add a half volume of Biobeads to solution and allow equilibration for at least 1.5 h to allow deoxycholate solution to be taken out of nanodisc/lipid preparation.
11. Run on an S200 size exclusion column equilibrated with 50 mM Tris HCl, 150 mM NaCl, pH 7.4, and collect earliest fractions with a uniform peak at around 10–12 mL and immediately store at 4 °C.

3.2 Sec18 to PA Using Heterogeneous Cross-Linking of Ligand to Chip

Assume use of GE CM7 chip to maximize RU for small analyte and large ligand. Assume NHS/EDC and ethanolamine provided by GE. For optimal signal (~100 RU), determine the first appropriate amount of ligand to attach to SPR chip assuming one binding site on ligand Sec18 monomer (Sec18_{mon}) 84,056 Da for one molecule analyte di-C8 phosphatidic acid (PA) MW 446.453 Da. Using formula: $[\text{Response}_{\text{max}} = (\text{Response}_{\text{Ligand}} \times \text{Mass}_{\text{Analyte}})/\text{Mass}_{\text{Ligand}}]$ you would need 18,827.5 RU of Sec18 cross-linked to dextran surface to yield $\text{Response}_{\text{max}}$ 100 assuming 1:1 binding of PA to Sec18_{mon}.

1. Attach and flow running buffer over both flow cells 1 and 2 or flow cells 3 and 4 and assuming use of newly opened CM7 chip.
2. Run Prime command preparing flow cells 1 and 2 choosing 2–1 for reference subtraction.
3. Set flow rate to 10 $\mu\text{L}/\text{s}$.
4. Wash flow cells 1 and 2 with coupling buffer by injecting 30 μL coupling buffer a couple times.
5. Mix EDC/NHS mixture and inject 70 μL (420 s).
6. Wash with 30 μL ethanolamine.
7. Wash with coupling buffer 1.
8. Switch to flow cell 2.
9. Inject ligand using either auto response on Biacore 300 or manually in short bursts attempting to achieve slightly higher RU than 18,827.5 as not all ligand is cross-linked.
10. Switch to flow cells 1 and 2.
11. Inject 70 μL ethanolamine (420 s) to complete cross-linking.

12. Set flow rate to 30 $\mu\text{L/s}$.
13. Inject 60 μL running buffer a couple times to establish a horizontal baseline and note the amount of RU of ligand cross-linked to chip as viewing sample (flow cell 2) is set to subtract from reference (flow cell 1) and new baseline represents the amount of RU of ligand cross-linked to chip.
14. Kinject (kinetic inject) 90 μL of analyte with 180-s dissociation to obtain sensorgram.
15. Inject 30 μL acidic regeneration solution multiple times until dissociation reaches original baseline prior to Kinject. If needed, try mild regeneration solution to return to baseline indicating that all analyte has dissociated from ligand.
16. Once all analyte is washed off ligand run additional Kinject using a titration of analyte to obtain steady-state K_D .
17. Analyze kinetic parameters using BiaEvaluate; see Note 4.2.

3.3 PA Binding to Sec18 Analyte Using Non-covalent Coordination of Ligand to Chip

Assume the use of GE Ni-NTA chip. Assume each ligand comprised of 2 MSP1D1 nanodiscs MW 24,661.9 Da (49,323.8 Da) of 140 lipid groups. Assume lipid composition of 70% POPC phosphatidylcholine (PC) MW 760 (74,480 Da), 10% POPE phosphatidylethanolamine (PE) MW 746.6 (10,452.4), and 10% POPA phosphatidic acid (PA) MW 697 (9758). As in Subheading 2, **item 1**, assume Sec18 monomer of MW 84,056 Da; however, now serving as analyte therefore optimal ligand on chip is 177.33 RU nanodisc and it might be safe to shoot for 200 RU nanodisc on chip. Assume priming procedure as in Subheading 2, **item 1**.

1. Attach and flow nickel running buffer over both flow cells 1 and 2 or flow cells 3 and 4 setting flow rate to 30 $\mu\text{L/s}$.
2. Flow EDTA stripping solution over both flow cells 1 and 2 or flow cells 3 and 4 using 60 μL EDTA stripping solution.
3. Switch to a single flow cell on Ni-NTA chip either flow cell 2 or flow cell 4, and assume use of flow cell 2 for newly opened Ni-NTA.
4. Set flow rate to 5 $\mu\text{L/s}$.
5. Flow 10 μL nickel solution (60s).
6. Set flow rate to 30 $\mu\text{L/s}$.
7. Inject 60 μL nickel running buffer to flow off extraneous nickel solution.

4.2 Modeling Selection Picking the appropriate model for a kinetic fit requires that the user have a firm biochemical understanding of the interaction being measured. For instance, the kinetic fit chosen in Fig. 1 utilizes a two-state binding model indicating that there are two superimposed curves and two separate k_s (k_{a1} and k_{a2}) as well as two separate k_d (k_{d1} and k_{d2}). Though this kinetic data fits the overlay plot of sensorgrams well, it may not be able to be utilized unless the researcher has good reason to select this particular kinetic model. For instance, in the case of PA binding of the N-domain of Sec18, it might be that a researcher might have data indicating that PA-binding sites generally go through two phases of binding, an initial searching phase for the PA head group and then a locking phase where the PA locks into a particular conformation within a specific PA-binding site, which would have separate kinetics. The researcher can then use software to separate out two separate kinetic binding models [10].

8. Set flow rate to 5 $\mu\text{L/s}$; lower flow rate often allows better equilibration of non-covalent 6X-His of nanodisc to Ni-NTA chip surface.
9. Inject PA nanodiscs in short bursts to get on approximately 200 RU; it may be necessary to lower flow rate to help ligand to stick. Take note of baseline and ensure that ligand remains on after injection.
10. Once 200 RU on switch to flow cells 2–1.
11. Set flow rate to 30 $\mu\text{L/s}$.
12. Inject 90 μL Sec18 with 120 s dissociation time; this can potentially run multiple injections, if no regeneration solution is required; however, advantage of Ni-NTA chip is that a fresh run can be achieved by following the rest of the procedure.
13. Wash off both ligand and analyte by injecting 60 μL EDTA stripping solution.
14. Repeat **steps 3–11**.
15. Analyze kinetic parameters using BiaEvaluate; *see* Note 4.2.

3.4 Sec18 to Sec17 Analyte Using Covalent Attachment of Ligand to Chip

Assume that each ligand comprised of 6 Sec18 monomers of MW 84,056 Da monomers and 12 ATP MW 507.18 Da (510,422.2 Da). Assume yeast Sec17 MW 32792.1 Da. Using calculation from Subheading 2, **item 1**, RU on chip should be 1556.54 or approximately 1600 RU. Assume priming procedure as in Subheading 2, **item 1**.

1. Attach and flow ATP running buffer over both flow cells 1 and 2 or flow cells 3 and 4 setting flow rate to 30 $\mu\text{L/s}$.
2. Set flow rate to 10 $\mu\text{L/s}$.
3. Switch to flow cells 2–1.
4. Mix EDC/NHS mixture and inject 70 μL (420 s).
5. Wash with coupling buffer 1.
6. Switch to flow cell 2.
7. Inject Sec18 Hexamer in short bursts to get on approximately 1600 RU as in Subheading 2, **item 2**.
8. Switch to flow cell 2–1.
9. Wash with coupling buffer 1.
10. Inject 70 μL ethanolamine (420 s) to complete cross-linking.
11. Set flow rate to 30 $\mu\text{L/s}$.
12. Inject 60 μL ATP running buffer a couple times to establish a horizontal baseline and note the amount of RU of ligand cross-linked to chip as viewing sample (flow cell 2) is set to subtract from reference (flow cell 1) and new baseline represents the amount of RU of ligand cross-linked to chip.

13. Kinject (kinetic inject) 90 μL of analyte Sec17 with 180 s dissociation to obtain sensorgram.
14. Inject 30 μL acidic regeneration solution multiple times until dissociation reaches original baseline prior to Kinject. If needed, try mild regeneration solution to return to baseline indicating that all analyte has dissociated from ligand.
15. Once all analyte is washed off ligand run additional Kinject using a titration of analyte to obtain steady-state K_D .
16. Analyze kinetic parameters using BiaEvaluate; see **Note 2**.

3.5 Analysis of Sensorgram Data

1. Overlay plots are generated using BiaEvaluate software, where multiple sensorgrams are overlaid on a single plot in order for analysis. These overlay plots can be used to generate both kinetic and steady-state data so long as the flow time for both association and disassociation matches. BiaEvaluate for 2000/3000 series BiaCore instruments requires manual matching of sensorgrams, whereas BiaEvaluate for 200/300 series BiaCore instruments presets parameters for aligning multiple sensorgrams in a given run.
2. Kinetic: GE BiaEvaluate software offers both preset kinetic fit models and the ability to edit kinetic models depending on the version of software you are licensed. Generally, the BiaCore 200/300 series does not allow the editing of models and the 2000/3000 series does. Kinetic parameters generally give both an on rate k_a (Fig. 1a) and an off rate k_d (Fig. 1c), from which the equilibrium dissociation constant K_D can be calculated $K_D = k_d/k_a$.
3. Steady state: GE BiaEvaluate software offers the ability to take RU measured from baseline to point on y-axis (Fig. 2a) at a given time (Fig. 2b) across multiple sensorgrams using an overlay plot. The user can set the time where this point is taken manually, and generally it is taken from a point where the maximum concentration of analyte flowed has definitively reached equilibrium as in Fig. 2.

Acknowledgments

This work was supported in part by NIH grant GM101132 to RAF.

References

1. Bakhtiar R (2012) Surface Plasmon resonance spectroscopy: a versatile technique in a Biochemist's toolbox. *J Chem Ed* 90:203–209
2. Karlsson R, Michaelsson A, Mattsson L (1991) Kinetic analysis of monoclonal antibody-antigen interactions with a new biosensor based analytical system. *J Immunol Methods* 145:229–240 [PubMed: 1765656]
3. Neumann T, Junker HD, Schmidt K, Sekul R (2007) SPR-based fragment screening: advantages and applications. *Curr Top Med Chem* 7:1630–1642 [PubMed: 17979772]

4. Sasser T, Qiu QS, Karunakaran S, Padolina M, Reyes A, Flood Bet al. (2012) Yeast lipin 1 orthologue pah1p regulates vacuole homeostasis and membrane fusion. *J Biol Chem* 287:2221–2236 [PubMed: 22121197]
5. Starr ML, Hurst LR, Fratti RA (2016) Phosphatidic acid sequesters Sec18p from cis-SNARE complexes to inhibit priming. *Traffic* 17:1091–1109 [PubMed: 27364524]
6. Bayburt TH, Sligar SG (2002) Single-molecule height measurements on microsomal cytochrome P450 in nanometer-scale phospholipid bilayer disks. *Proc Natl Acad Sci U S A* 99:6725–6730 [PubMed: 11997441]
7. Denisov IG, Grinkova YV, Lazarides AA, Sligar SG (2004) Directed self-assembly of monodisperse phospholipid bilayer Nanodiscs with controlled size. *J Am Chem Soc* 126:3477–3487 [PubMed: 15025475]
8. Haas A, Wickner W (1996) Homotypic vacuole fusion requires Sec17p (yeast alpha-SNAP) and Sec18p (yeast NSF). *EMBO J* 15:3296–3305 [PubMed: 8670830]
9. de Mol NJ, Fischer MJ (2010) Surface plasmon resonance: a general introduction. *Methods Mol Biol* 627:1–14 [PubMed: 20217611]
10. Futamura M, Dhanasekaran P, Handa T, Phillips MC, Lund-Katz S, Saito H (2005) Two-step mechanism of binding of apolipoprotein E to heparin: implications for the kinetics of apolipoprotein E-heparan sulfate proteoglycan complex formation on cell surfaces. *J Biol Chem* 280:5414–5422 [PubMed: 15583000]

Fig. 1.

Representative sensorgram of the Sec18 N-domain analyte-binding PA nanodisc ligand overlay plot with kinetic fit. This is a representative overlay plot of four sensorgrams taken from real data of the N-domain of Sec18-binding PA nanodiscs with response units given on y -axis and time given in seconds on x -axis. Concentrations titrated at a 1:1 ratio with red being the highest concentration and green the lowest concentration flowing at 25 $\mu\text{L/s}$ with a total association time of 90 s and disassociation of 180 s. The black kinetic fit is for a two-state model. (a) Association: Notice that the initial part of the graph is curved indicative of an exponential association formula of $R_T = (R_{\text{max}}[A]) / (K_D + [A]) \times [1 - e^{-(k_a[A] + k_d)t}]$. (b) Steady state: Curve levels out at maximum concentration of analyte indicating the number of association events equal to dissociation events representing equilibrium or R_{eq} . Equilibrium depends on the number of ligand-binding sites and analyte concentration flowed where $R_{\text{eq}} = [(R_{\text{max}}[A]) / ([A] + K_D)]$. (c) Dissociation: Only dependent on dissociation rate and should be long enough to begin to flatten out over time following what can be a sharp declining slope at injection stop according to $R_T = R_0 e^{-k_d t}$

Fig. 2. Representative sensorgram of the Sec18 N-domain analyte binding PA nanodisc ligand overlay plot with steady-state fit. (a) Baseline. (b) Steady-state time point. (c) Dose-dependent saturation binding of PA nanodiscs