Biolayer Interferometry (BLI) Protocol for Nanodiscs

Materials

- Antibody
- Nanodisc
- Running buffer (Quantitation (Q) Buffer, Gator #120010, or similar)
- Protein A Probe (Gator #160001, or similar)
- Black 96-well plate (Greiner #655209, or similar)
- BLI instrument (e.g. Gator®, or similar)

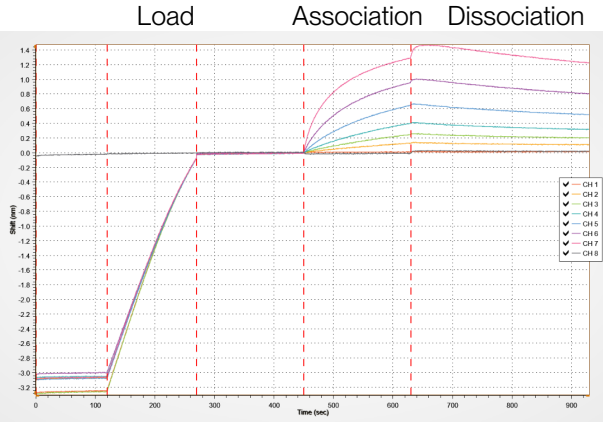
Protocol

1. Preparation:
   - Dilute antibody to 5µg/mL with running buffer and load onto Protein A Probe.
   - Prepare serial dilutions of the nanodisc ranging from 6.25 nM to 100 nM.
2. Probe Loading:
   - Load the diluted antibody onto the Protein A Probe.
   - Loading time: 150 seconds.
   - Loading level: Approximately 3.0nm shift.
3. Association Phase:
   - Add the diluted nanodisc samples into the wells of a black 96-well plate.
   - Immerse the loaded Protein A Probe into the wells containing the nanodisc at various concentrations.
   - Association Time: 180 seconds for each concentration.
4. Dissociation Phase:
   - Move the probes into wells containing buffer only (without nanodisc) to monitor dissociation.
   - Dissociation Time: 300 seconds.

Example Data

Human CCR8 Nanodisc (Cat No. CR8-HM1N29)

Q buffer was used as the running buffer throughout the experiment. The anti-CCR8 antibody was diluted to 5 µg/mL and loaded onto a Protein A probe, with a loading time of 150 sec and a loading level of approximately 3.0 nm. Diluted Human CCR8 Nanodisc was added to a black 96-well plate at concentrations ranging from 6.25nM to 100nm. The association phase lasted for 180 sec, followed by a dissociation phase of 300 sec.



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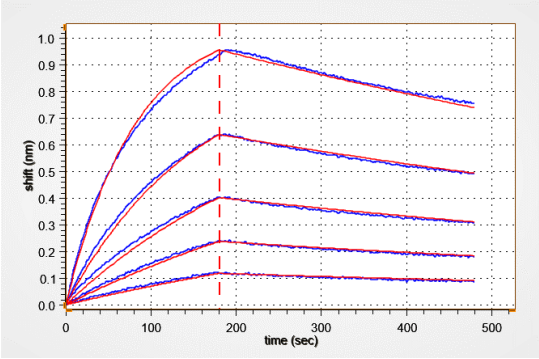
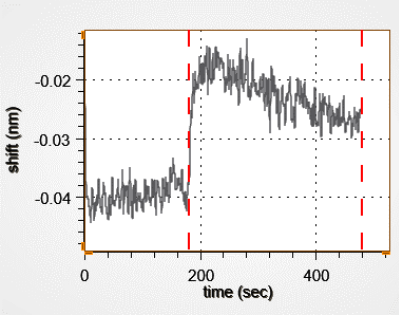
Example Data cont.

Human CCR8 Nanodisc (Cat No. CR8-HM1N29)

Kon (1/Ms)	Koff (1/s)	KD (M)
1.13E+05	0.000857	7.61E-09

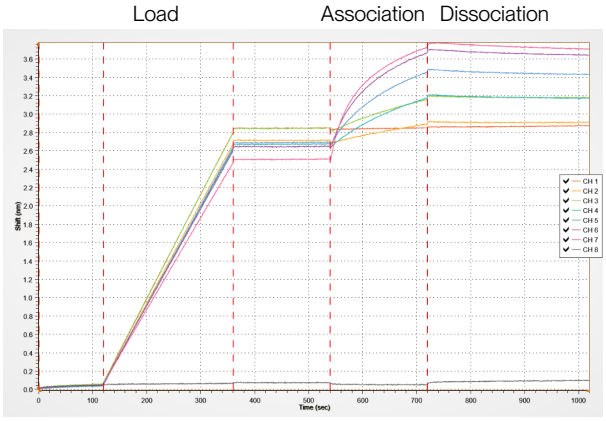


There is no non-specific binding of the CCR8 nanodisc to the Protein A Probe.

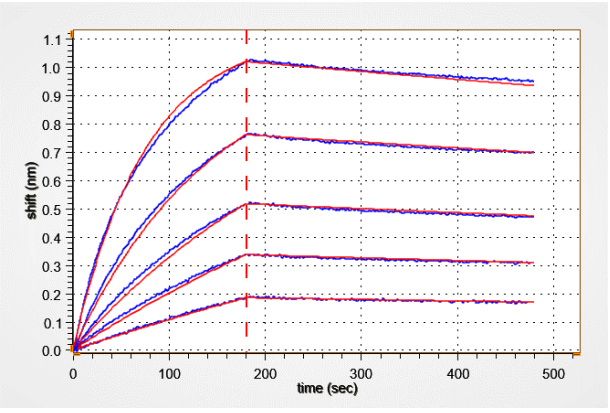


Human A2AR Nanodisc (Cat No. A2R-HM1N1)

Q buffer was used as the running buffer for the experiment. The anti-A2AR antibody was diluted to 2 µg/mL and loaded onto a Protein A probe, with a loading time of 240 sec and a loading level of approximately 2.6 nm. Diluted Human A2AR Nanodisc was added to a 96-well black plate at concentrations ranging from 200 nM to 12.5 nM. The association phase lasted for 180 sec, followed by a dissociation phase of 300 sec.



Kon (1/Ms)	Koff (1/s)	KD (M)
6.51E+04	0.000289	4.44E-09



There is no non-specific binding of the A2AR nanodisc to the Protein A Probe.