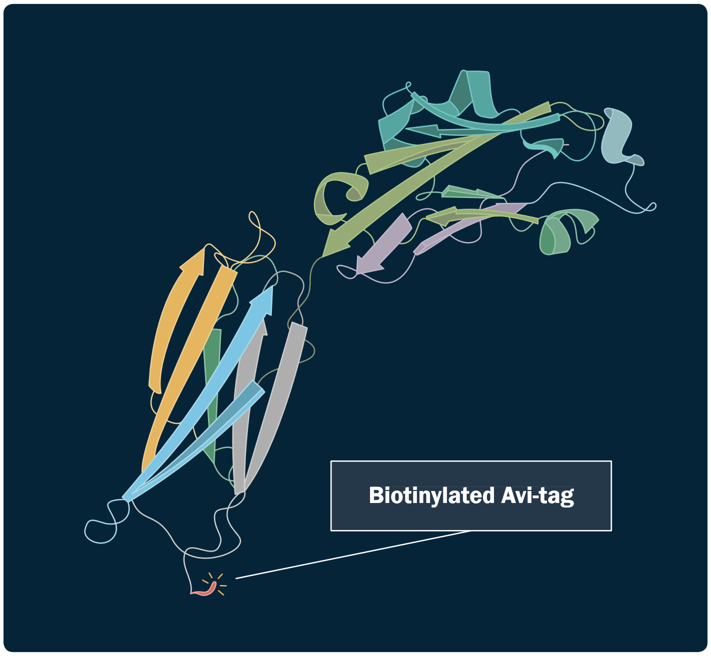
APPLICATION NOTE

Using Avi-tag Biotinylated Proteins to Determine Protein Interaction Kinetics and Affinity with Surface Plasmon Resonance

INTRODUCTION

Surface plasmon resonance (SPR) is a powerful, accurate, and direct way to measure protein interactions in real-time without the need for antibodies or detection reagents. Typically, a protein is covalently immobilized or non-covalently captured on a sensor chip, then an analyte is passed over the surface at varying concentrations and allowed to associate with the protein ligand. These binding events are detected by measuring changes in the refractive index caused by an increase of mass near the sensor chip surface. Biacore (GE Healthcare) is the most widely used SPR system and is currently used for all SPR experiments at R&D Systems. In a typical Biacore experiment, the covalently immobilized protein is often coupled to the sensor chip in a random orientation by amine or thiol coupling. This works well in many cases, however these random thiol or amine immobilization methods can produce unsatisfactory results, especially with small proteins or proteins with reactive groups in the binding interface resulting in valuable time spent troubleshooting. In this study, we employ R&D Systems Avi-tag Biotinylated Proteins along with streptavidin Series S chips to specifically and uniformly capture proteins in a Biacore experiment, as an alternative to chemical or amine coupling.

R&D Systems Avi-tag Biotinylated Proteins feature biotinylation at a single lysine residue that resides within the Avi-tag, a unique 15 amino acid peptide. The Avi-tag is enzymatically biotinylated by the E. coli biotin ligase BirA. All R&D Systems Avi-tag Biotinylated Proteins undergo rigorous QC testing to ensure high bioactivity and lot-to-lot consistency.



MATERIALS AND METHODS:

Binding measurements were performed by surface plasmon resonance (SPR) on a Biacore T200 instrument (GE Healthcare). R&D Systems Avi-tag biotinylated proteins, either Recombinant Human CD155/PVR Fc Chimera Avi-tag protein (Catalog # AVI9174) or Recombinant Human PD-L1/B7-H1 His-tag Avi-tag protein (Catalog # AVI9049) were captured at a low coupling density on Streptavidin Series S sensor chip SA (GE Healthcare), and increasing concentrations of the corresponding analytes, Recombinant Human TIGIT-Fc protein (Catalog # 9464-TG) or Recombinant Human PD-1 His-tag protein (Catalog # 8986-PD), respectively, were passed over both active and reference flow cells. Double-referenced sensorgrams were analyzed using the Biacore evaluation software to determine the binding kinetics and affinity.

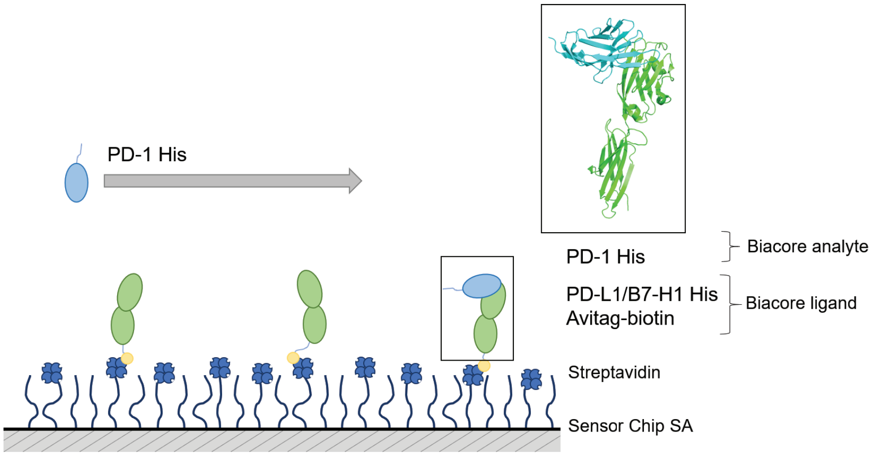
RESULTS:

We have chosen to highlight the use of our Avi-tag proteins in SPR experimentation by using the following Avi-tag products: Recombinant Human PD-L1/B7-H1 His-tag Avi-tag Protein (Cat# AVI9049) and Recombinant Human CD155/PVR Fc Chimera Avi-tag Protein (Cat# AVI9174).

The binding interaction between Recombinant Human PD-L1/B7-H1 His-tag Avi-tag Protein and Recombinant PD-1 His-tag is shown in Figure 1. We determined the interaction affinity using binding kinetics (B) as well as steady state affinity (C). In this experiment, recombinant human PD-L1/B7-H1 His-tag Avi-tag biotinylated protein was captured at a low coupling density to the active flow cell via the Avi-tag biotin. Then, recombinant PD-1 His-tag protein at a concentration range between 3.2 nM and 13.2 uM was passed over both active and uncoupled reference flow cells at each concentration. The association phase at each concentration was 60 seconds followed by a 40 second dissociation. Double-referenced sensorgrams of captured Recombinant Human PD-L1/B7-H1 His-tag Avi-tag Protein binding to Recombinant PD-1 His-tag and the corresponding overlaid kinetic fits are shown in Figure 1B. Kinetic sensorgrams were fit to a 1:1 binding model and the interaction affinity was calculated at KD=1.122 μM. The steady state affinity calculation is in line with the kinetic data producing an affinity of KD = 1.528 uM Figure 1C.

Figure 1:

A. PD-1:PD-L1 Schematic



B. PD-1:PD-L1 Sensorgrams

C. PD-1:PD-L1 Steady State Affinity Fit

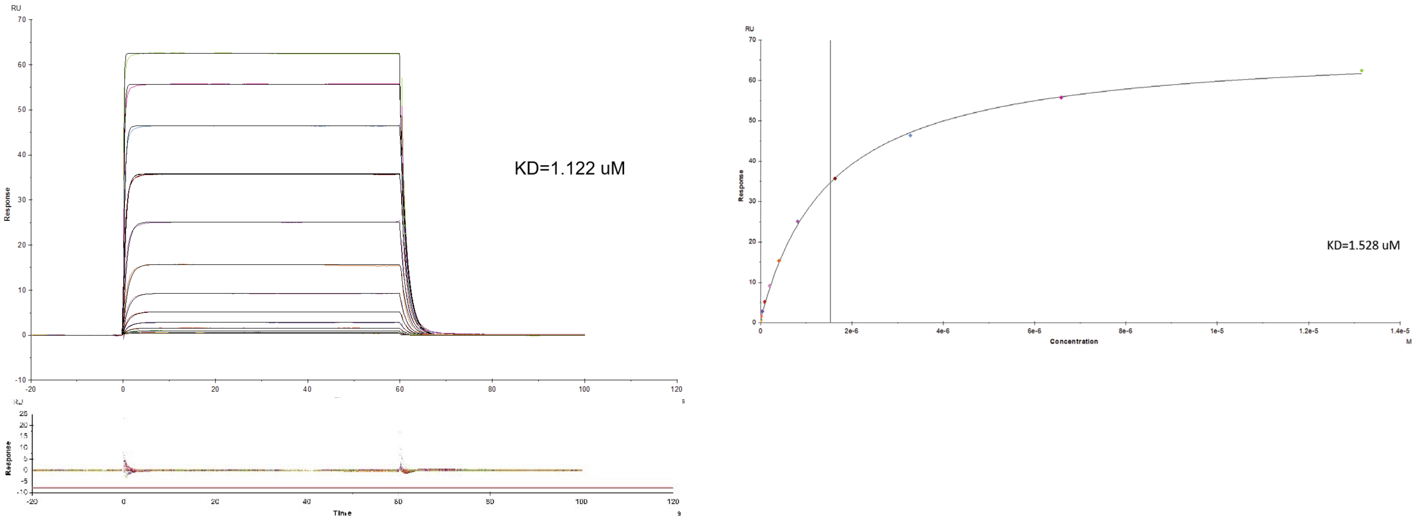
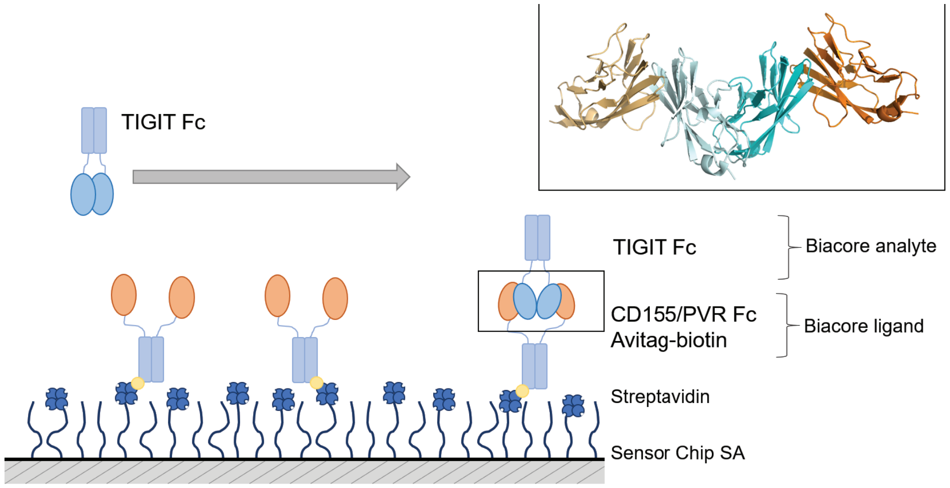
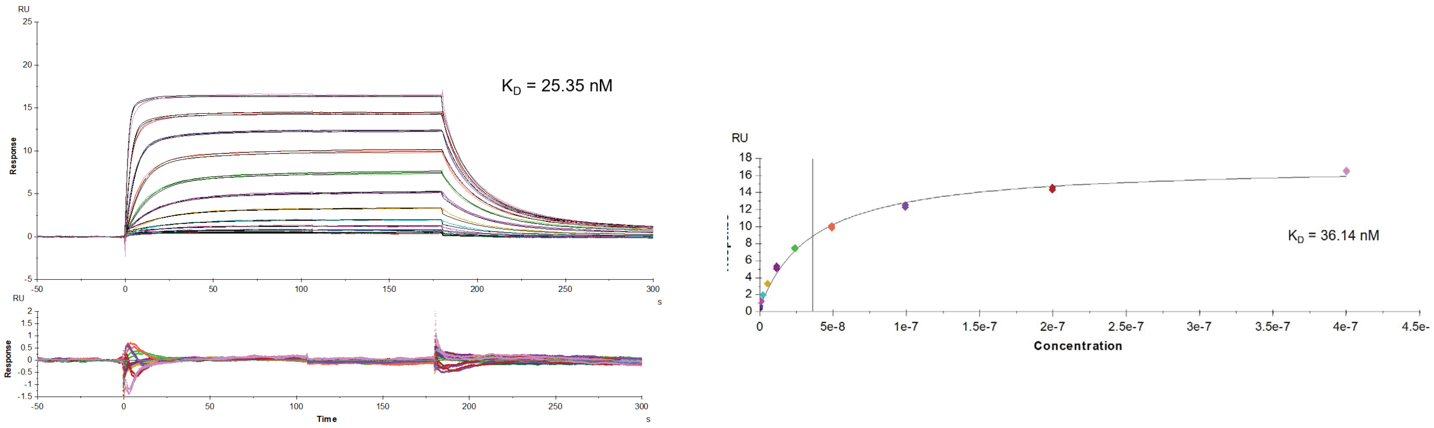


Figure 1. Affinity measurements and binding kinetics of the PD-1:PD-L1 interaction by SPR. A. Schematic cartoon of the SPR experiment. Ribbon structure from https://www.rcsb.org/structure/3BIK. B. and the corresponding overlaid kinetic fits with the residual plot shown Sensorgram data of captured Recombinant Human PD-L1/B7-H1 His-tag Avi-tag binding to Recombinant Human PD-1 His-tag below. The concentration of PD-1 His-tag ranged from 3.2 nM to 13.2 uM. C. The corresponding steady state affinity fit.

Next, we analyzed the CD155/PVR:TIGIT binding interaction using this same method. The results of this SPR experiment also performed on the Biacore T200 are shown in Figure 2. Here, we captured recombinant human CD155/PVR Fc Chimera Avi-tag at a low coupling density to the active flow cell via the biotin moiety residing within the Avi-tag. Recombinant human TIGIT-Fc was passed over both active and uncoupled reference flow cells in duplicate at different concentrations ranging from 0.2 nM to 400 nM. The association phase at each concentration was 180 seconds followed by a 120 second dissociation. Double-referenced sensorgrams were fit to a 1:1 binding model to determine the binding kinetics and affinity, and the calculated interaction affinity was KD = 25.35 nM Figure 2B. Steady state affinity is plotted in Figure 2C and the determined affinity of KD = 36.14 nM falls in line with the kinetic data. These affinity results are consistent with the TIGIT Fc Protein interacting with CD155/PVR Fc chimera (non-biotinylated) covalently immobilized using amine chemistry (data not shown).

A. CD155/PVR:TIGIT Schematic





B. CD155/PVR:TIGIT Sensorgram

C. CD155/PVR:TIGIT Steady State Affinity Fit

Figure 2. Affinity measurements and binding kinetics of the CD155/PVR:TIGIT interaction by SPR. A. Schematic cartoon of the SPR experiment. Ribbon structure from http://www.rcsb.org/structure/3UDW. B. Sensorgram data of captured Recombinant Human CD155/PVR Fc Avi-tag binding to Recombinant Human TIGIT His-tag and the corresponding overlaid kinetic fits with the residual plot shown below. The concentration of TIGIT His-tag ranged from 0.2 nM to 400 nM. C. The corresponding steady state affinity fit.

CONCLUSIONS

In this study we demonstrate how R&D Systems Avi-tag Biotinylated Proteins can be used in conjunction with Streptavidin Series S Sensor Chips to generate useful kinetic and affinity measurements by surface plasmon resonance using Biacore.

ORDERING INFORMATION

Complete list of Avi-tag Biotinylated Proteins

PROTEIN	SPECIES	SOURCE	TAG	CATALOG #	ACTIVITY
B7-1/CD80	Human	CHO	His, Avi-tag	AVI9050	Binds CTLA-4
5T4	Human	CHO	His, Avi-tag	AVI10290	Binds 5T4 antibody MAB49751
B7-1/CD80	Human	CHO	Fc, Avi-tag	AVI10107	Binds CTLA-4
	Human	CHO	His, Avi-tag	AVI9050	
B7-2/CD86	Human	CHO	Fc, Avi-tag	AVI7625	Binds CTLA-4
BCMA/TNFRSF17	Human	HEK293	Fc, Avi-tag	AVI193	Binds APRIL/TNFSF13
BTN1A1/Butyrophilin	Human	HEK293	His, Avi-tag	AVI8467	Binds BTN1A1 antibody MAB84671
BTN3A2	Human	HEK293	His, Avi-tag	AVI9514	Binds BTN3A1/2 antibody AF7136
CD19	Human	CHO	Fc, Avi-tag	AVI9269	Binds CD19 antibody
CD30/TNFRSF8	Human	HEK293	Fc, Avi-tag	AVI10240	Binds CD30 Ligand/TNFSF8
	Human	HEK293	His, Avi-tag	AVI10239	
CD47	Human	CHO	Fc, Avi-tag	AVI4670	Binds SIRP alpha/CD172a
CD155/PVR	Human	HEK293	Fc, Avi-tag	AVI9174	Binds CD96v2
	Human	HEK293	His, Avi-tag	AVI2530	Binds TIGIT
CD200	Human	HEK293	Fc, Avi-tag	AVI2724	Binds CD200R1
CTLA-4	Human	HEK293	Fc, Avi-tag	AVI7268	Binds B7-1/CD80
DNAM-1/CD226	Human	HEK293	His, Avi-tag	AVI9298	Binds CD155/PVR
	Human	CHO	Fc, Avi-tag	AVI666	
ErbB2/Her2	Human	HEK293	Fc, Avi-tag	AVI1129	Binds Trastuzumab
Fc gamma RIII	Human	HEK293	His, Avi-tag	AVI8894	Binds human IgG
IgG1	Human	HEK293	Fc, Avi-tag	AVI110	Binds C1q
IL-7R alpha/CD127	Human	HEK293	Fc, Avi-tag	AVI10317	Binds IL-7
LAIR1	Human	HEK293	His, Avi-tag	AVI2664	Binds bovine Collagen
LAIR2	Human	HEK293	His, Avi-tag	AVI2665	
Lymphotoxin alpha1/beta2	Human	HEK293	His, Avi-tag	AVI8884	Binds Lymphotoxin beta R
PD-1	Human	CHO	Fc, Avi-tag	AVI1086	Binds PD-L1/B7-H1
	Human	HEK293	His, Avi-tag	AVI8986	
PD-L1/B7-H1	Human	HEK293	His, Avi-tag	AVI9049	Binds PD-1
	Human	CHO	Fc, Avi-tag	AVI156	
PD-L2/B7-DC	Human	HEK293	Fc, Avi-tag	AVI1224	Binds PD-1
	Human	HEK293	His, Avi-tag	AVI9075	
TIM-3	Human	CHO	His, Avi-tag	AVI10241	Binds Galectin-9
uPAR	Human	HEK293	His, Avi-tag	AVI807	Binds uPA/Urokinase
VEGF 165	Human	HEK293	Avi-tag	AVI293	Binds VEGFR1/Flt-1
VISTA/B7-H5/PD-1H	Human	HEK293	Fc, Avi-tag	AVI7126	Binds VSIG3

NOTES

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