Biophysics Core

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Biophysics Core Instruments

Surface Plasmon Resonance (SPR)

- Binding affinity (K_D)
- Association rate (k_a)
- Dissociation rate (k_d)
- **Binding affinity:** pM – ~2 mM

Circular Dichroism (CD)

- Protein secondary and tertiary structure (α-helix, β-sheet, random)
- Conformational stability of a protein at varying conditions
- Comparing the structures of protein vs. mutants

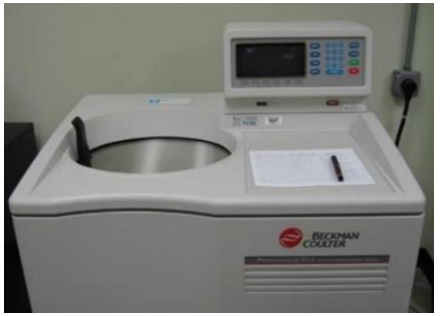
Dynamic Light Scattering (DLS)

- Estimate protein molecular weight
- Observe protein oligomerization
- Determine the quality of a protein sample for structural studies.

Isothermal Titration Calorimetry (ITC)

- Association constant (K_a)
- Reaction stoichiometry (n)
- Heat capacity (ΔCp) of the reaction
- Binding free energy (Δ*G*)
- entropy (ΔS) and enthalpy (ΔH)
- **Binding affinity:** low nM – ~5 mM

Analytical Ultracentrifugation (AUC)



- Determine the molar mass of proteins and complexes
- Determine the number of species in a sample
- Determine the stoichiometry of complexes
- Analysis of self- and hetero-association
- **Binding constants:** mid nM – 1 mM

Biophysics Core Instruments for Protein Production

Large-scale culture

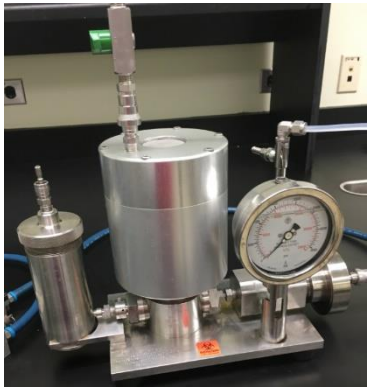
Temp. controlled Incubator Shaker

Harvest cells

Sorvall LYNX 4000 Centrifuge

Cell lysis

Emulsiflex C5

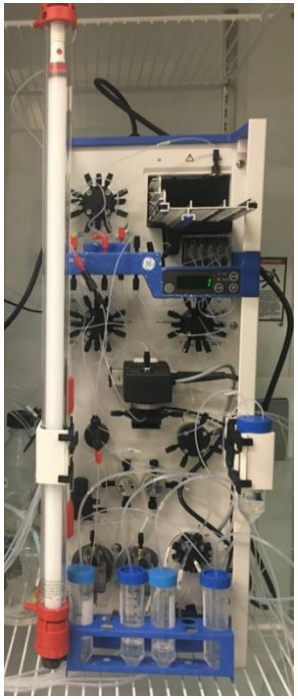


Sonicator

Protein Purification

AKTA Purifier FPLC

AKTAxpress FPLC



Protein concentration measurement

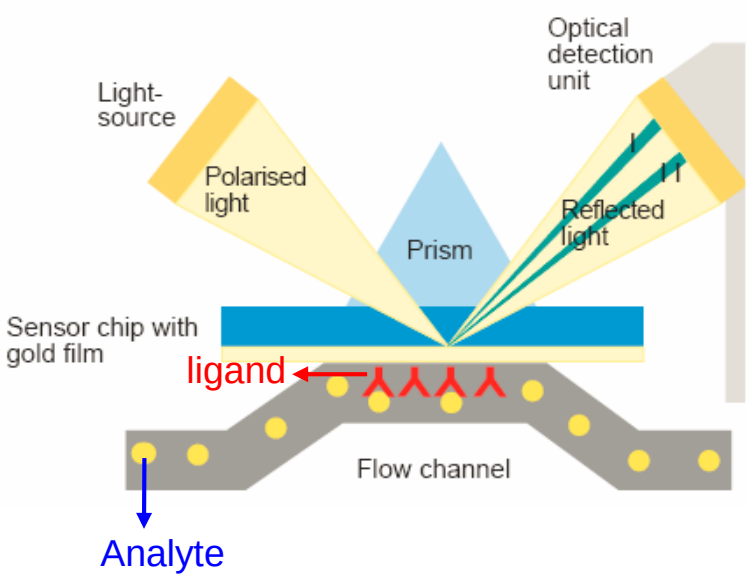
NanoDrop

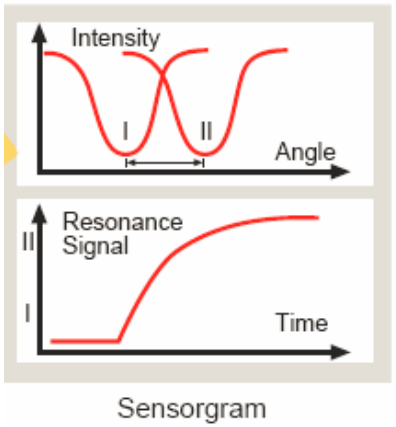
SPR Basic Principles

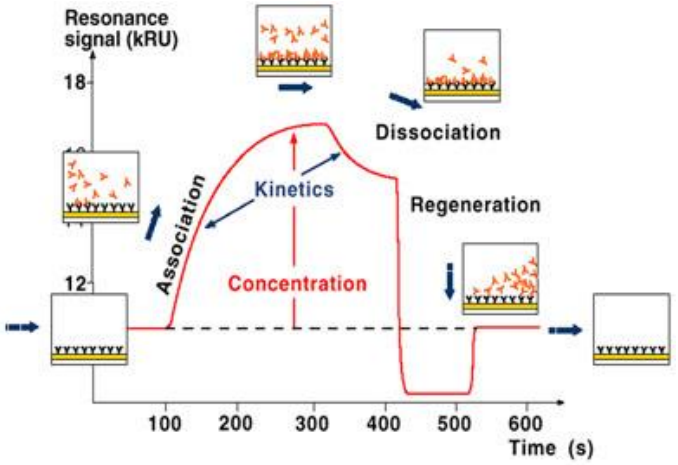
Surface Plasmon Resonance (SPR)

- One interacting partner ("ligand") is attached to the surface of a chip
- the passing of a sample containing the second interaction partner ("analyte") over the surface of the chip.
- Binding of molecules to the sensor surface generates a response that is proportional to the bound mass
- The changes in angle of the reflected light is measured in real time.

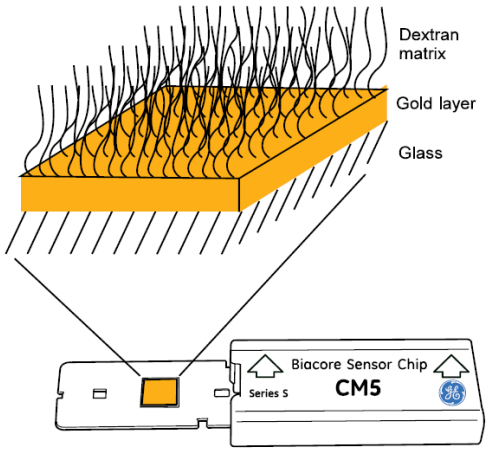
0.1˚ angle change = 1 ng/mm^2 = 1000 Response unit (RU)

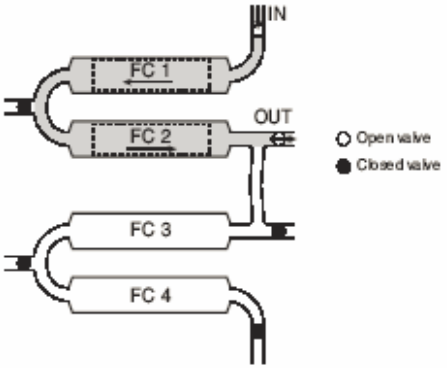




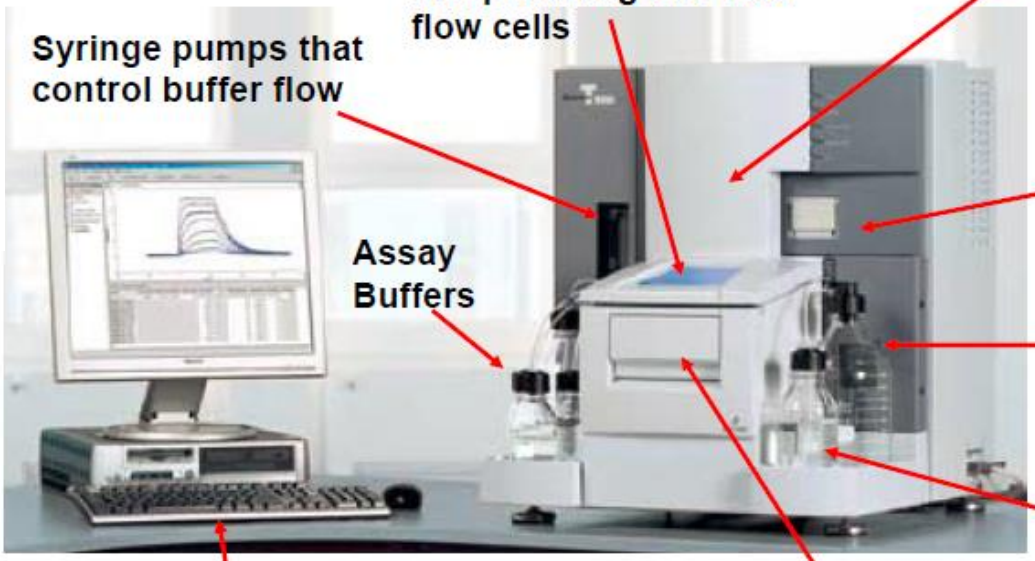


SPR Instruments: Biacore T200





Biacore T200



Optics and micro-channel flow system are enclosed in here

Needle which delivers samples/reagents into flow cells

Syringe pumps that control buffer flow

Chip in docked in here

Assay Buffers

Waste

Water to wash needle

"User friendly" control and evaluation software

Samples and reagents are inserted in this temperature-controlled compartment

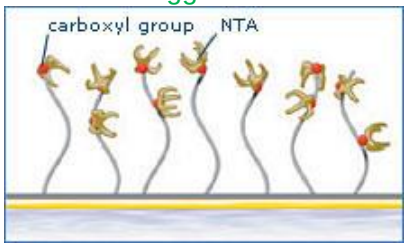
Sensor Chip types

Sensor Chip:	CM7	CM5	CM4	CM3	C1	SA	HPA	L1	NTA
Molecule to be immobilized									
Proteins	●	●	◒	◒	◒	●			
Tagged proteins		●							●
LMW molecules, typically <1000 Da	●	●	◒	◒					
Membrane–associated molecules							●	●	
Nucleic acids	●	●	◒	◒	◒	●			
Carbohydrates	●	●	◒	◒	◒	●			
Viruses or intact cells				●	●				

● Recommended choice ◒ Good alternative

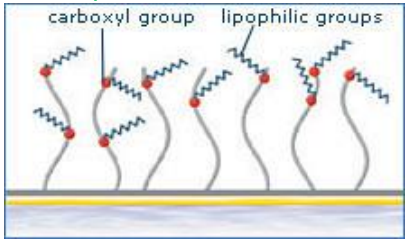
SPR core: CM5, SA, NTA

Load His-tagged molecules



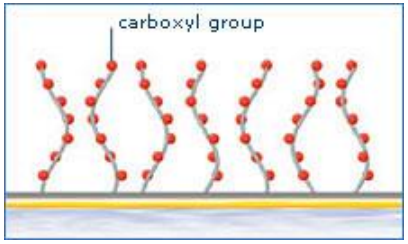
NTA (carboxymethylated dextran pre-immobilized with nitrilotriacetic acid (NTA))

Load lipid membrane vesicles



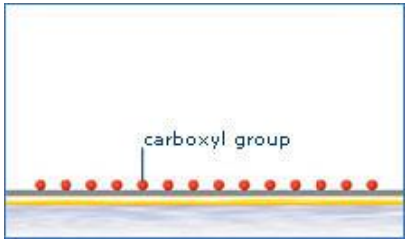
L1 (lipophilic groups are covalently attached to carboxymethylated dextran)

Covalently coupled via amine, thiol, aldehyde or carboxyl



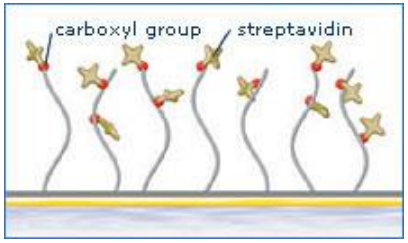
CM (carboxymethylated dextran covalently attached to a gold surface)

Load large molecules like cells



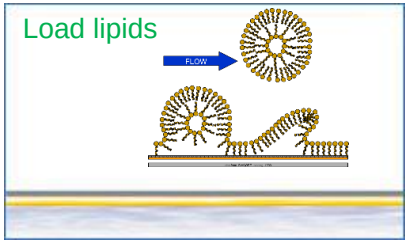
C1 (low carboxymethylated surface)

Load biotinylated molecules



SA (carboxymethylated dextran pre-immobilized with streptavidin)

Load lipids

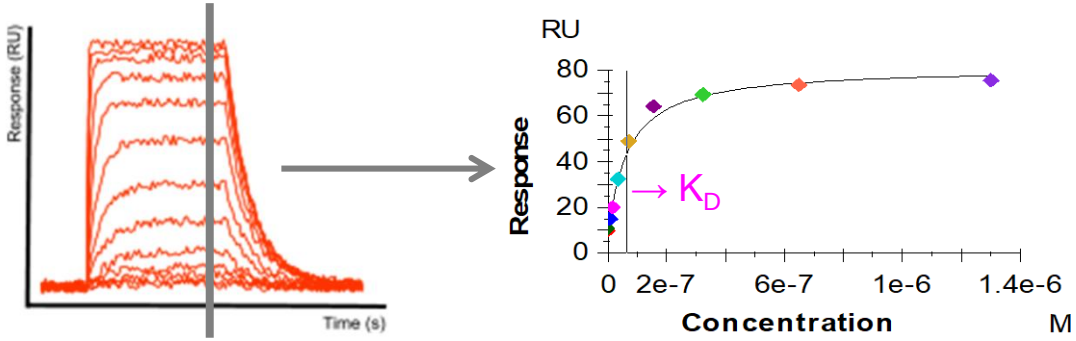


HPA (A flat hydrophobic surface consisting of long-chain alkanethiol molecules)

Dissociation equilibrium constant (K_D) & rate constants (k_{on} & k_{off})

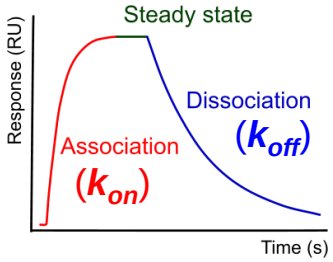
▼ Determination of dissociation equilibrium constant (K_D)

- immobilize a target protein on sensor surface (ex, CM5 chip)
- flow a series of increasing concentration of compounds (0 – 100 µM)
- fit the data for steady-state affinity
  - uses RU signals at the steady state
- reflects binding affinity of a compound



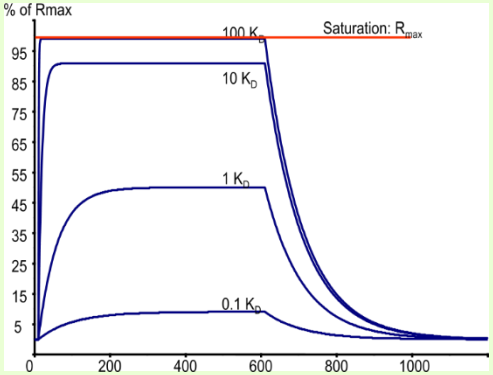
▼ Determination of rate constants (k_{on} & k_{off})

- fit the same data for kinetics
- usually fast on-rate and slow off-rate are preferred for good inhibitors



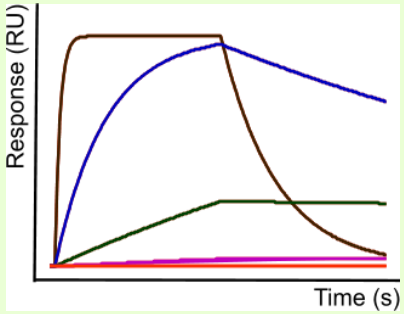
$$\rightarrow K_D = \frac{k_{off}}{k_{on}}$$

Testing concentration range



0.1 K_D ~ 10 K_D

Same binding affinity, but very different on and off rates



Various SPR Applications

▼ The real-time detection and monitoring of the biomolecule interactions

- protein & protein
- protein & peptide
- protein & DNA/RNA
- protein & Lipid
- protein & compound

▼ It can provide quantitative information on

- binding specificity: search for binding partners
  screen for inhibitors
- binding affinity (K_D): strength of binding
- kinetics (k_{on} & k_{off}): rates of reactions
  complex formation (k_{on})
  complex dissociation (k_{off})
- concentration: nanomolar concentration can be measured in both purified molecules and complex mixtures

▼ This technique can also be applied to every stage of drug-discovery process

- mid-throughput automated compound screening
- hit confirmation and validation
- hit characterization via kinetics
- mechanism of action (Competition SPR)

SPR Applications: Example 1

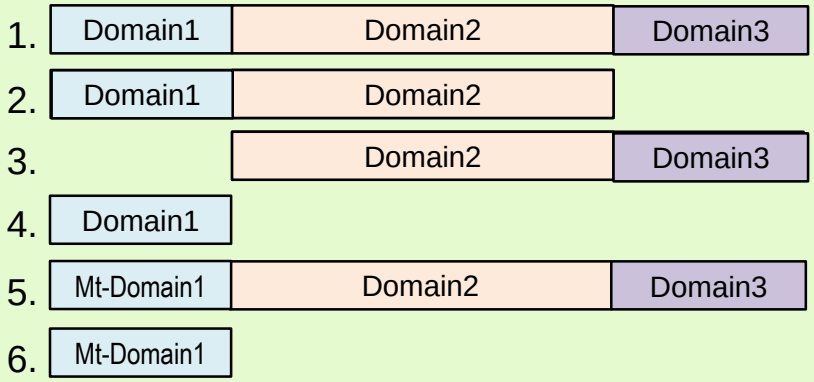
WT-Protein vs Mutant protein interaction

▼ A Protein binds to a compound

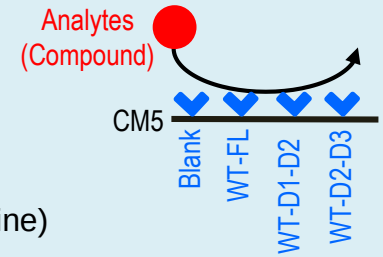
- 10,000 compounds were screened
- one very potent compound was identified
- target protein was identified as A protein

Questions:

1. Where does the compound bind?
2. How would a mutation affect the binding?

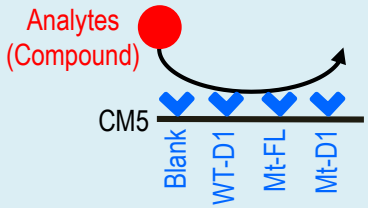


▼ SPR Experiment Set1



- **Sensor Chip: CM5**
- **Immobilized proteins:**
  - FC1: Reference (ethanolamine)
  - FC2: WT-FL-A protein
  - FC3: WT-D1-D2-A protein
  - FC4: WT-D2-D3-A protein
- **Analytes:** Compound

▼ SPR Experiment Set2



- **Sensor Chip: CM5**
- **Immobilized proteins:**
  - FC1: Reference (ethanolamine)
  - FC2: WT-D1-A protein
  - FC3: Mt-FL-A protein
  - FC4: Mt-D1-A protein
- **Analytes:** Compound

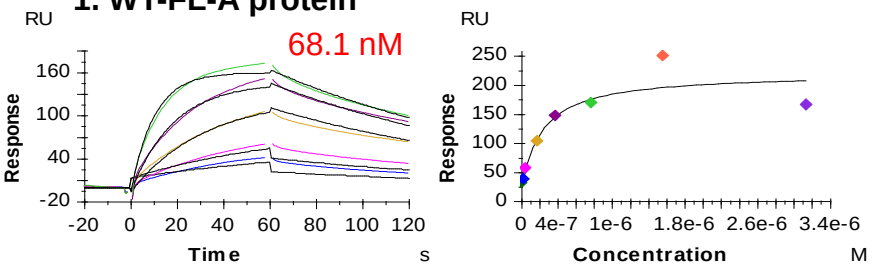
(ITR, Manuscript in preparation)

SPR Applications: Example 1 continued

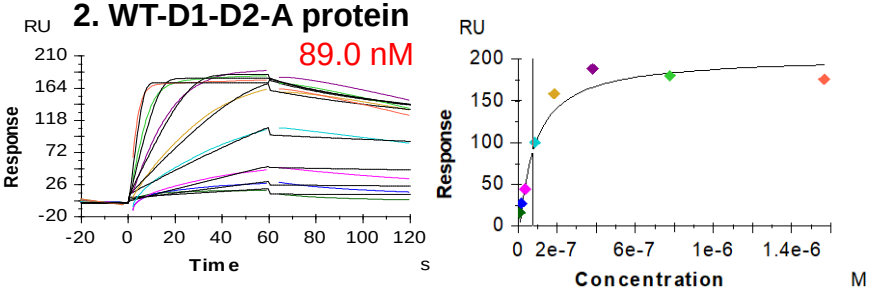
WT-Protein vs Mutant protein interaction

		K^D values
1.	Domain1 – Domain2 – Domain3	68.1 nM
2.	Domain1 – Domain2	89.0 nM
3.	Domain2 – Domain3	No binding
4.	Domain1	44.4 nM
5.	Mt-Domain1 – Domain2 – Domain3	1891 nM
6.	Mt-Domain1	3941 nM

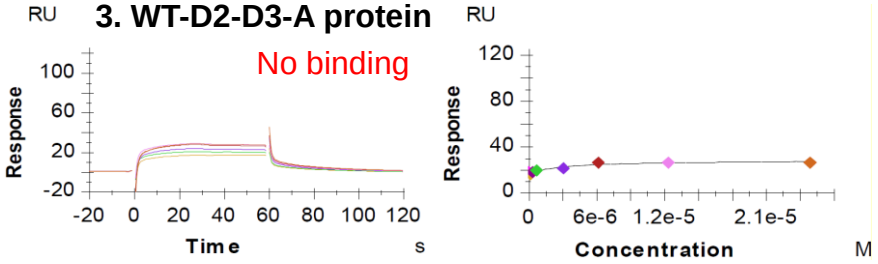
1. WT-FL-A protein — 68.1 nM



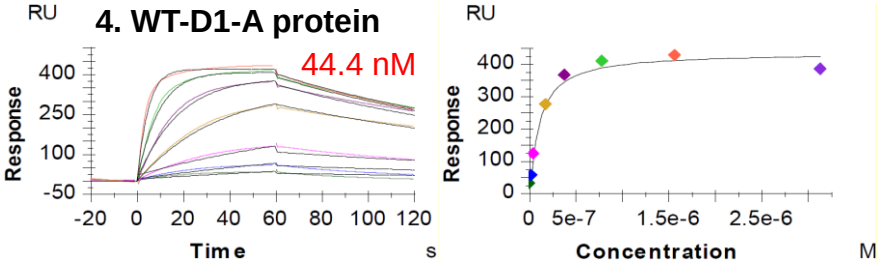
2. WT-D1-D2-A protein — 89.0 nM



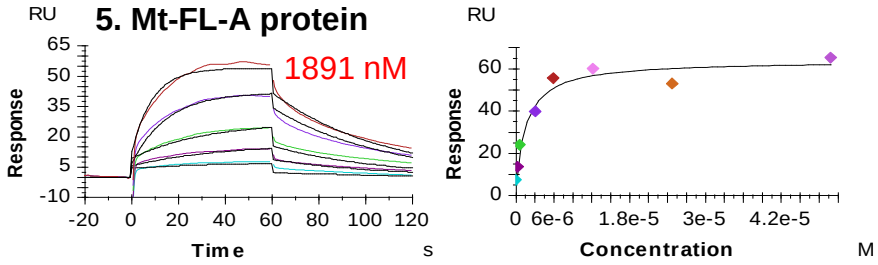
3. WT-D2-D3-A protein — No binding



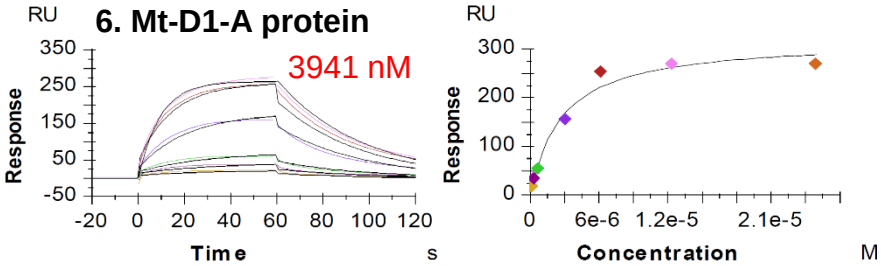
4. WT-D1-A protein — 44.4 nM



5. Mt-FL-A protein — 1891 nM



6. Mt-D1-A protein — 3941 nM



(ITR, Manuscript in preparation)

SPR Applications: Example 2

Two different targets binding to a Protein

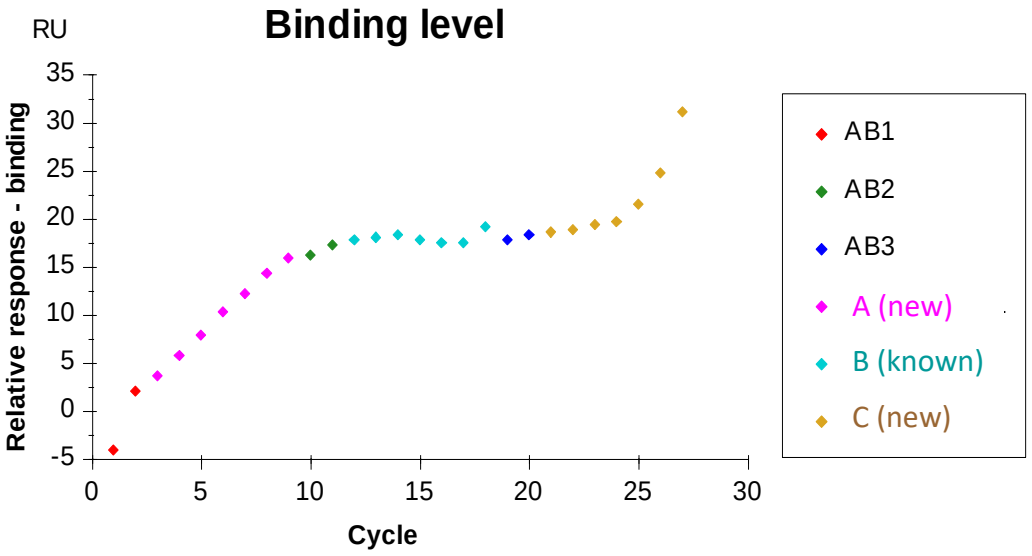
▼ Three protein interaction

- Apol1 (protein of interest)
- discovered two new binding partner proteins
- there is one already known binding partner

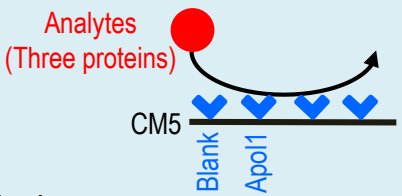
Questions:

1. Does a newly identified target directly bind to Apol1?

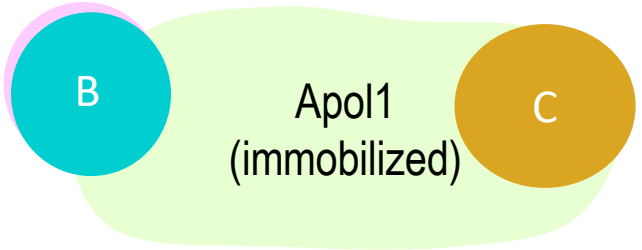
2. Will it bind to the same location as the two other known ones?



▼ SPR Experiment Set1



- **Sensor Chip: CM5**
- **Immobilized proteins:**
  FC1: Reference (ethanolamine)
  FC2: Apol1 (immobilized by amine coupling)
- **Analyzed proteins:** Proteins A, B, C



→ A new target A bind to the same location of the Apol1 as the known binding partner B.

→ Target C seems to bind a different location from where A and B bind.

(Hayek et al., Nature Medicine, 2017)

SPR Applications: Example 3

Competition SPR

▼ Mode of Inhibition studies

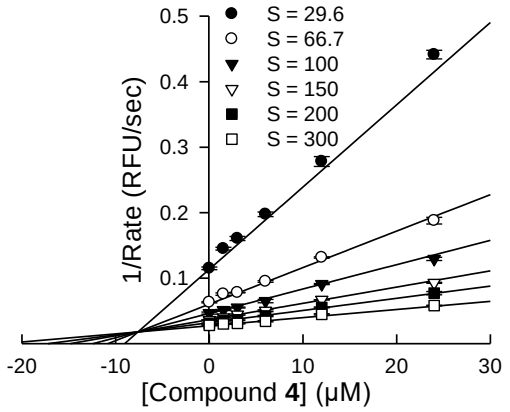
- Enzymatic assay results had ambiguity.
- needed an alternative method to clarify this.

Questions:

1. Will compound 4 bind to MERS-CoV PLpro in the active site?

SARS-PLpro	AICc
Mixed-type	-49 (α=2.6)
Noncompetitive	-42
Competitive	-10
Uncompetitive	85

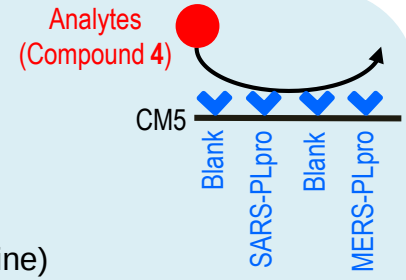
MERS-PLpro	AICc
Competitive	-72
Mixed-type	-71 (α=13.4)
Noncompetitive	-52
Uncompetitive	67



Compound **4**

Enzyme	IC_{50} (μM)	Kinetic mode (K_i, μM)
SARS-PLpro	10.9 ± 0.9	Mixed inhibition (11.5)
MERS-PLpro	6.2 ± 0.9	Competitive inhibition (7.6)

▼ SPR Experiment Set1

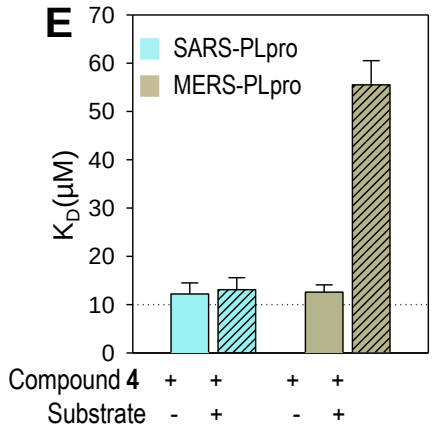


♦ **Sensor Chip: CM5**

♦ **Immobilized proteins:**

FC1: Reference (ethanolamine)
FC2: SARS-PLpro (immobilized by amine coupling)
FC3: Reference (ethanolamine)
FC4: SARS-PLpro (immobilized by amine coupling)

♦ **Analyzed proteins:** Compound **4**



→ Substrate does compete with compound **4** in the active site of the MERS-PLpro.

(Lee et al., ACS Chemical Biology, 2015)

SPR Applications: Example 4

Compound Screening for drug candidates

Fragment library screening

Hit validation

1st screening
SPR

2nd screening
STD-NMR

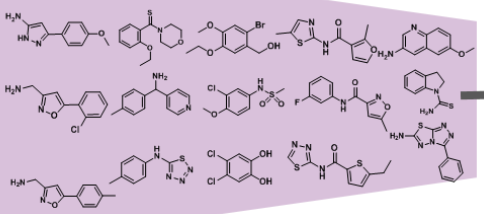
3rd screening
Competition STD-NMR

Competition SPR (find active site binders only)

(KD determination)
SPR / ITF

UV assay

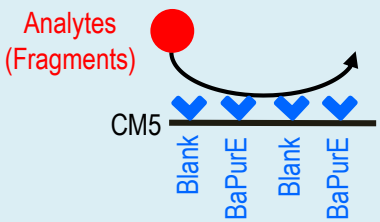
3,000 Filtered ChemBridge fragments → **750** Primary hits → **194** Secondary hits → **15** Final hits



Binding affinity → Enzyme inhibition

▼ SPR Experiments

- **Sensor Chip: CM5**
- **Immobilized proteins:**
  - FC1: Reference (unmodified surface)
  - FC2: BaPurE
  - FC3: Reference (unmodified surface)
  - FC4: BaPurE
- **Analytes:** Fragment compounds



→ produce duplicate data

(Lei et al., Bioorg. Med. Chem, 2015)

SPR Applications: Example 4 continued

SPR assay plate set up using liquid handler

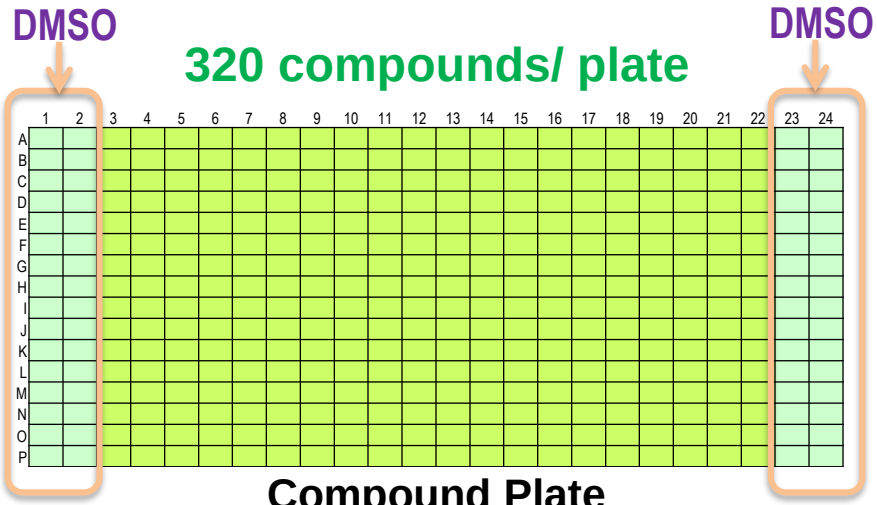
Distribute 100 µL each of 1.9% DMSO assay buffer to 384-well plates (wells 1 through 24)

↓

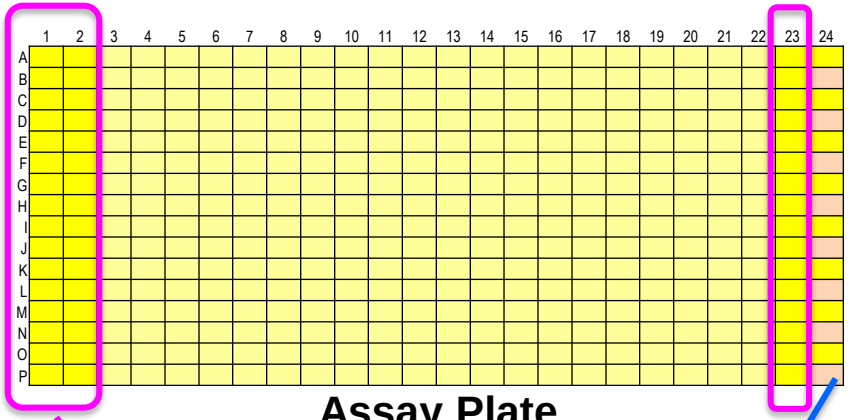
Add 0.1 µL of 200 mM compounds (200 µM final concentration) - wells 3 -22
Add 0.1 µL of DMSO to control wells- wells 1-2 and 23-24 (parts of 24 with a control compound)

↓

Prepare all 10 plates and apply to Biacore T200



Compound Plate
(10 plates- Chem. Frag. Lib.)



Assay Plate

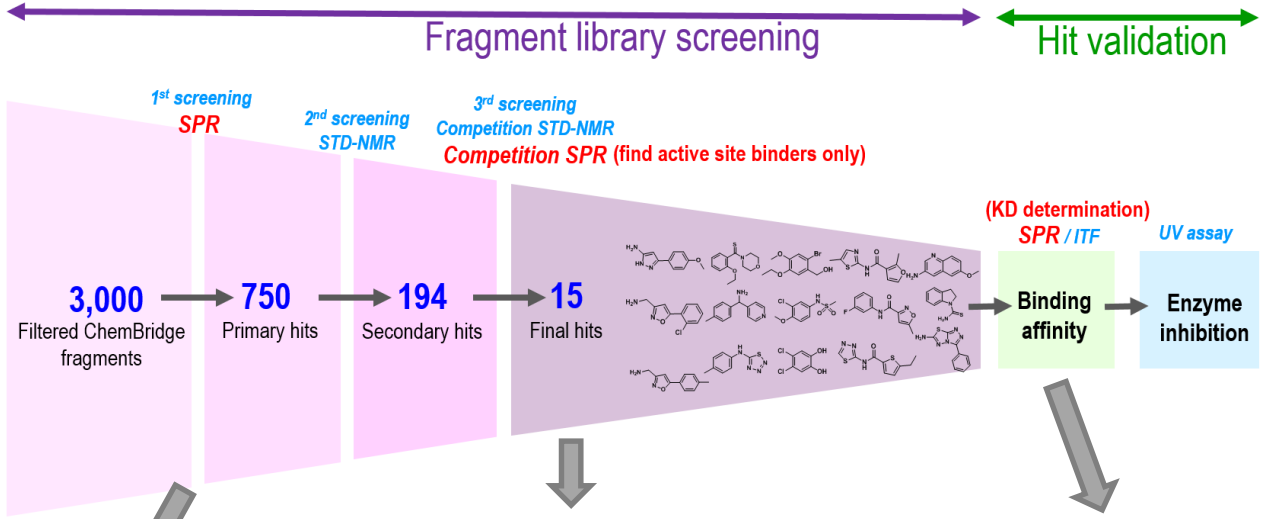
DMSO controls

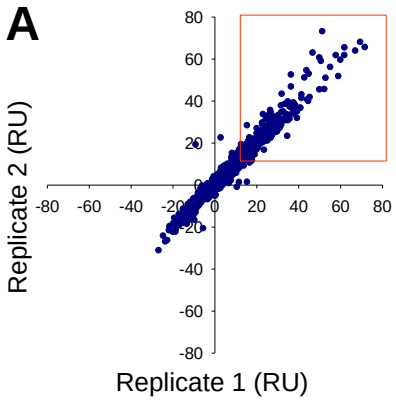
Control compounds

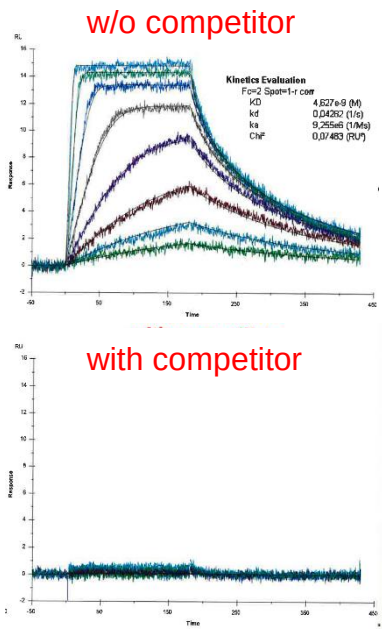
Research Resources Center

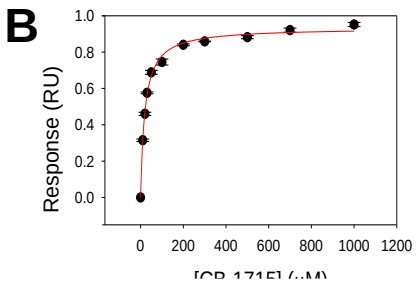
SPR Applications: Example 3 continued

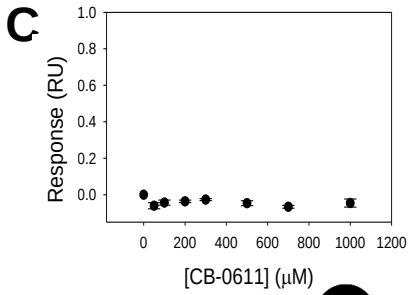
SPR result summary











(Lei et al., Bioorg. Med. Chem, 2015)

UIC Research Resources Center

SPR Applications: Example 4 continued

hit characterization via kinetics by SPR

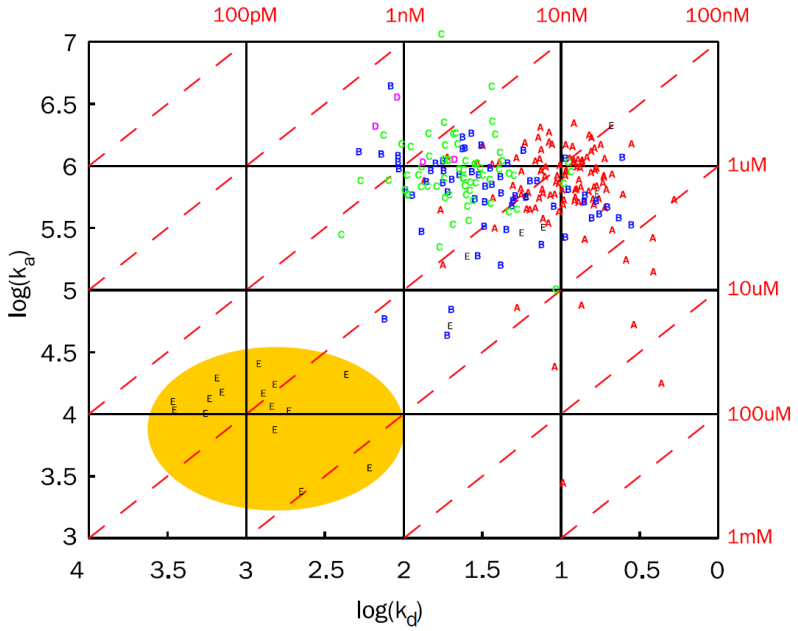


FIG. 4. A k_{on}/k_{off} **map of lead inhibitor compound** series binding to CD80.

- A total of 259 lead compounds belonging to five chemically related series (AÐE) were analyzed.
- Plots of log (*k*a) against log (*k*d) are shown.
- The dotted lines indicate isometric affinity diagonals.
- "Ideal" binding characteristics (most **rapid** association and **slowest** dissociation) would appear in the top left quadrant of the map.

(Stefan Lofas, ASSAY and Drug Development Technologies, 2004)

Biophysics Core services & charges

1. Surface Plasmon Resonance

SPR (Biacore T200)	Internal UIC	External Academic	Non-academic	Unit
Instrument_Biacore T200_self-run	$200.00	$250.00	$400.00	1 day
Instrument_Biacore T200_assisted-run	$400.00	$500.00	$800.00	1 day
Service_Trouble shooting/Experimental consults	$50.00	$62.50	$100.00	1h
Service_SPR Data Analysis	$50.00	$62.50	$100.00	1h
Service_Reagent/screening preparation	$50.00	$62.50	$100.00	1h
Service_Compound cherry picking	$30.00	$37.50	$60.00	0.5h
Training_Biacore T200_1 person	$200.00	$250.00	$400.00	1 person
Training_Biacore T200_2 people in a group	$150.00	$187.50	$300.00	1 person
Training_Biacore T200_3 people in a group	$130.00	$162.5	$260.00	1 person
Training_Biacore T200_4 people in a group	$115.00	$143.75	$230.00	1 person
Training_Biacore T200_5 people in a group	$100.00	$125.00	$200.00	1 person
Training_SPR Data Analysis_Basic	$50.00	$62.50	$100.00	1h
SPR Research project collaboration	contact Hyun Lee (danielhl@uic.edu)			

Biophysics Core services & charges

2. Other Biophysical Instruments

Other Instruments	Internal UIC	External Academic	Non-academic	Unit
Instrument_AUC	$390.00	$630.00	$787.5	per run
Instrument_CD	$25.00	$31.25	$50.00	1 h
Instrument_Emulsiflex	$11.25	$14.06	$22.50	30 min
Instrument_ITC	$20.00	$25.00	$45.00	1 h
Instrument_DLS	$20.00	$25.00	$45.00	1 h
Instrument_ PerkinElmer_Victor3V plate reader	$20.00	$25.00	$45.00	1 h
Instrument_Centrifuge_Sorvall LYNX 4000	$7.50	$9.40	$15.00	30 min
Training_CD, Emulsiflex, DLS, ITC	$50.00	$62.50	$100.00	1-2 hours
Training_AUC	$250.00	$312.50	$500.00	1 person
Training_ Data Analysis_CD, DLS, ITC	$50.00	$62.50	$100.00	1 h
Training_ Data Analysis_AUC	$100.00	$125.00	$200.00	1-2 hours
User Assistance	$50.00	$80.00	$120.00	1 h
Research project collaboration	contact Hyun Lee (danielhl@uic.edu)			

Biophysics Core services & charges

3. Protein over-expression and Purification

Protein Purification	Internal UIC	External Academic	Non-academic	Unit
Transformation into DH5a (or XL1) cells_Option	$50.00	$62.50	$100.00	1 plasmid
Transformation into Rosetta2(DE3) or BL21(DE3) cells_Option	$50.00	$62.50	$100.00	1 plasmid
2L culture_Protein purification_Affinity column (Ni-NTA_HisTrap) → Basic	**$1,000.00**	**$1,250.00**	**$2,000.00**	**1 purification**
Protein purification_tag-cleavage_reload (Ni-NTA) _Option	$250.00	$312.50	$500.00	1 purification
TEV_protease_Option	$50.00	$62.50	$100.00	1 purification
SUMO_protease_Option	$50.00	$62.50	$100.00	1 purification
Protein_purification_Extra_column_Ion-xchange_Option	$250.00	$312.50	$500.00	1 purification
Protein_purification_Extra_column_Size-Exclusion (Superdex 75 or Superdex 200) _Option	$350.00	$437.50	$600.00	1 purification

Biophysics Core services continued

4. Structural study collaboration by X-ray crystallography & Cryo-EM

→ Key step is protein purification for both

X-ray crystallography

Protein purification (~10 mg/mL)
↓
Set up Crystal Screen by Tecan liquid handling robot
↓
Set up Crystal with further optimized condition by hand
↓
Diffract crystals at Argonne
↓
Solve structures

- UICentre project is in process for X-ray
- ITR project is in process for X-ray

Cryo-EM

Protein purification (0.005 - 1 mg/mL, >100 nM)
↓
Optimize buffer condition
↓
Screen by Negative staining
↓
Get Cryo-EM images (U. of Chicago)
↓
Solve structures (U. of Chicago)

- ITR project is in process for Cryo-EM

South Korea (M.S.& work)	Ohio State University (Ph.D)	UIC (Postdoc & Res.Assi.Prof.)	UIC (Biophysics core director)
Protein NMR ➡	Protein NMR/SPR/X-ray ➡	HTS/SPR/X-ray ➡	SPR/X-ray/Cryo-EM
1 NMR structure	3 NMR structures	3 X-ray crystal structures	Present/ Future
(Lee et al., *JBC* **2000**)	(Lee et al., *Mol.Cell* **2008**) Featured Article (Mol. Cell); Editor's Choice (Science 321, 17); Editor's Choice (Sci. Signal. 1, ec230)	(Lei et al., *ACS Chem.Biol*. **2015**) (Rice et al., *Bioorg.&Med.Chem*. **2016**) (Lee et al., *Antiviral Res*. **2017**) - *Tecan EVOware/Liquid Handling Workshop* - *SPR Advanced data analysis* - *CCP4 Workshop for crystallography at Argonne* **(24 publications)**	(Keystone Cryo-EM symposium, Feb.5-8)

How do you start using Biophysics Core services?

1. SPR (Biacore T200)

1. Self-run:

- Get a training
- An assisted-run for the very first day (A staff can watch the whole time)
- Become an approved user

2. Assisted run :

(i) Assisted without collaboration

- Instrument charge: $400/day
- Staff time: $50/hour (research on the topic, sample preparation, trouble shooting and data analysis.)

(ii) Assisted with collaboration

- Instrument charge: $400/day

2. Other Biophysics core instruments: ITC, DLS, CD and AUC

3. Protein Purification

Contact Hyun Lee for consult and discussion (danielhl@uic.edu)

4. Structural study

Contact Hyun Lee for consult and discussion (danielhl@uic.edu)