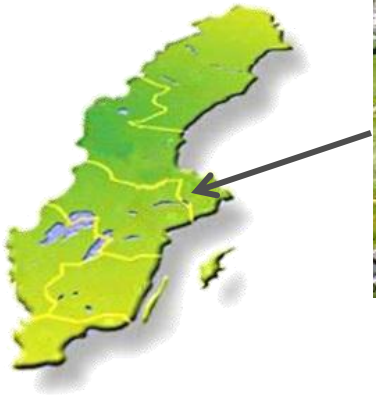
BIACTICA™

Interactions understood. Leads improved.

Exploiting interaction kinetic analysis for lead discovery and optimization

U. Helena Danielson

Who are we?



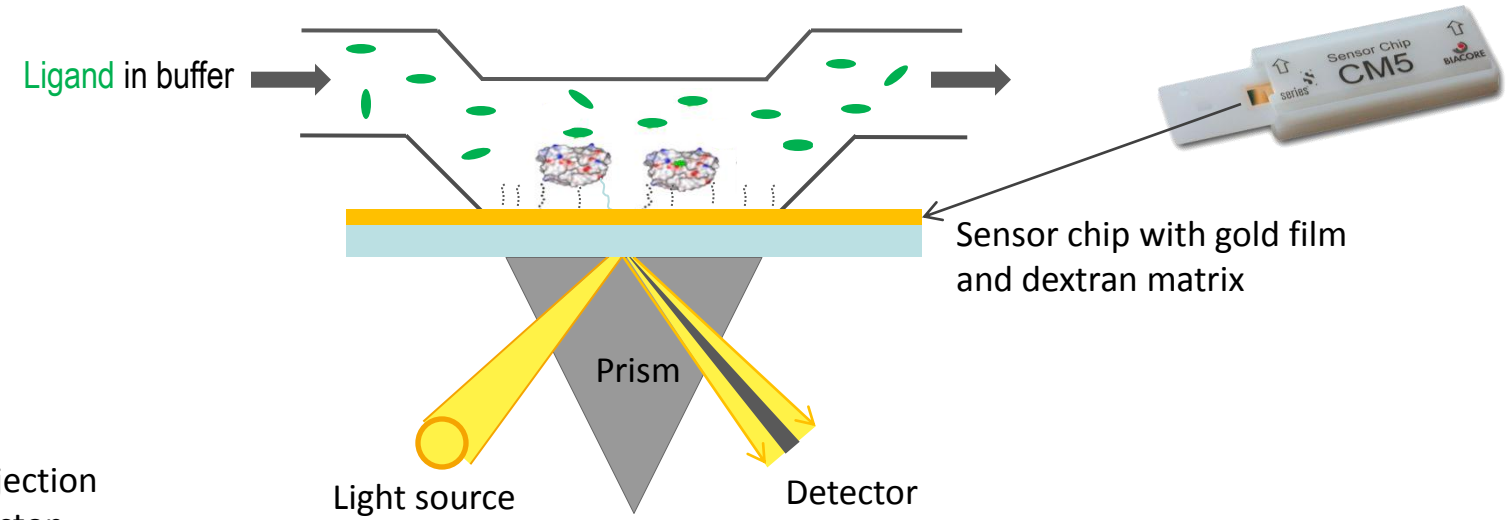
Medivir

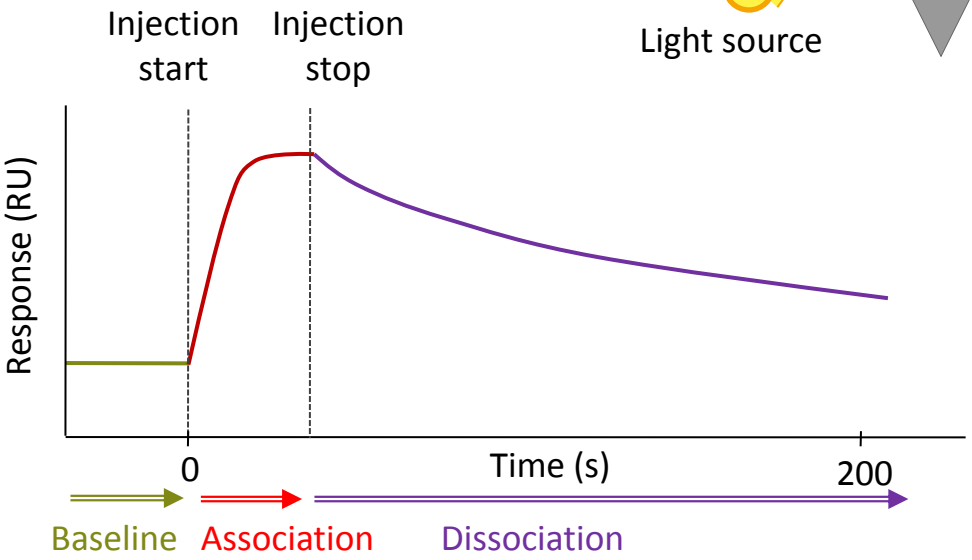
UPPSALA UNIVERSITET

SPR biosensor technology 1996 + Fragment-based drug discovery 2005 = Beactica 2006

BIACORE

Surface plasmon resonance (SPR) biosensor technology





Biacore™ T200, 4000 etc

Exploiting SPR biosensor analysis for drug discovery today

+ The technology is well established

+ The high information content is recognized

- Implementation is sometimes problematic

The user friendliness of commercial SPR biosensors is deceptive:

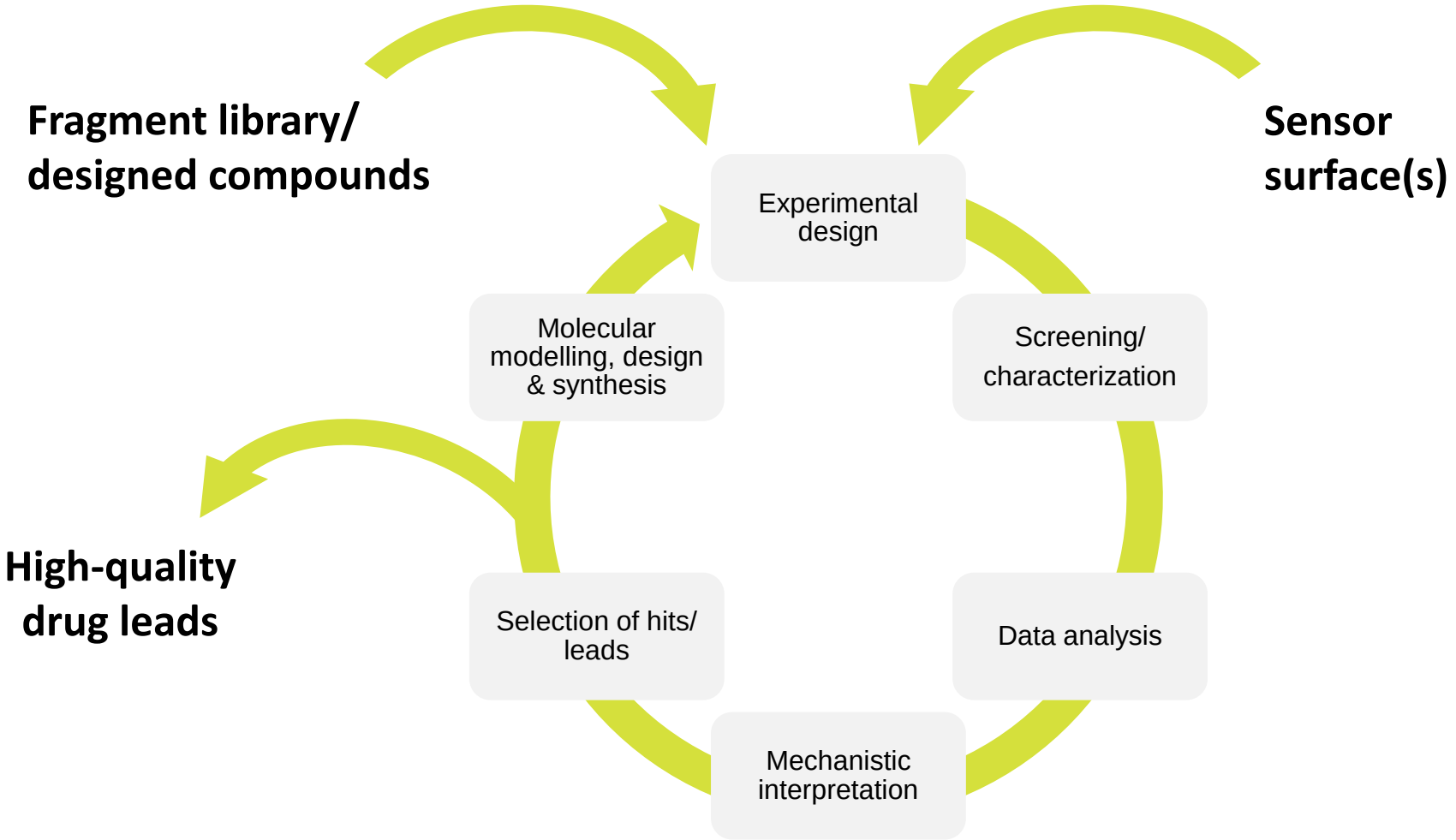
- Ease of use is not the same thing as ease of implementing the technology for actual projects
- Challenges in all steps from experimental design to interpretation of data

From fragment to lead

Identification and validation of fragment hits –

combining creative experimental design and data analysis with a SAR by catalogue strategy

From fragment to lead – an iterative process

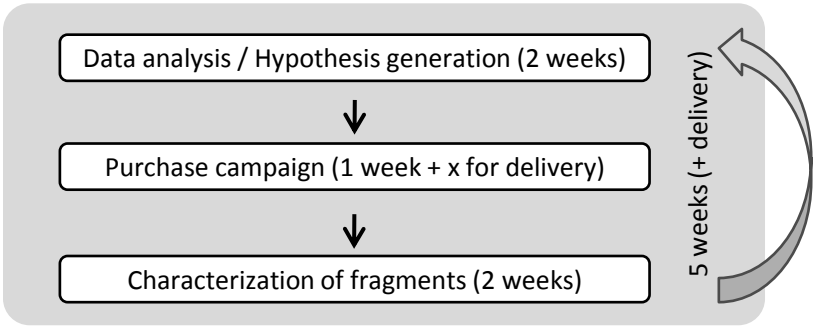


Outline of typical fragment-based lead identification project

- Fragment library screening
  - Provides the necessary starting points

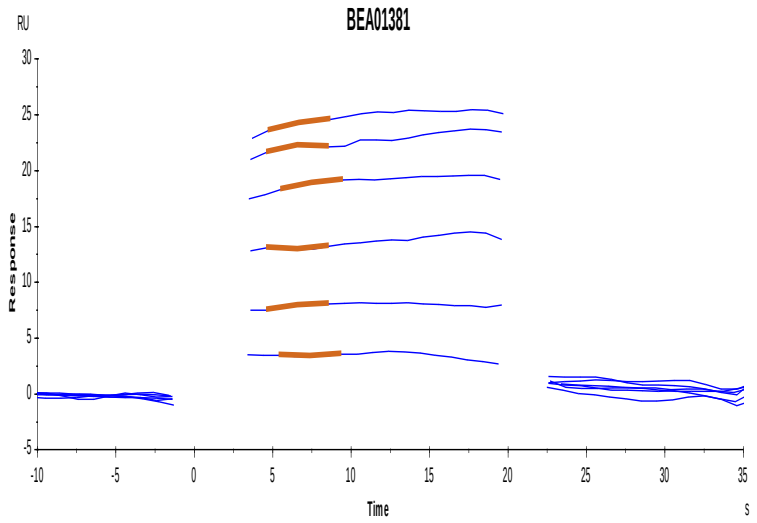
- SAR by catalogue – 1st iteration
  - Explores the scaffold space
  - Identifies new scaffolds
  - Discovers improved scaffolds

- SAR by catalogue – 2nd iteration
  - Defines the SAR around each scaffold
  - Identifies directions for chemistry
  - Improves affinity

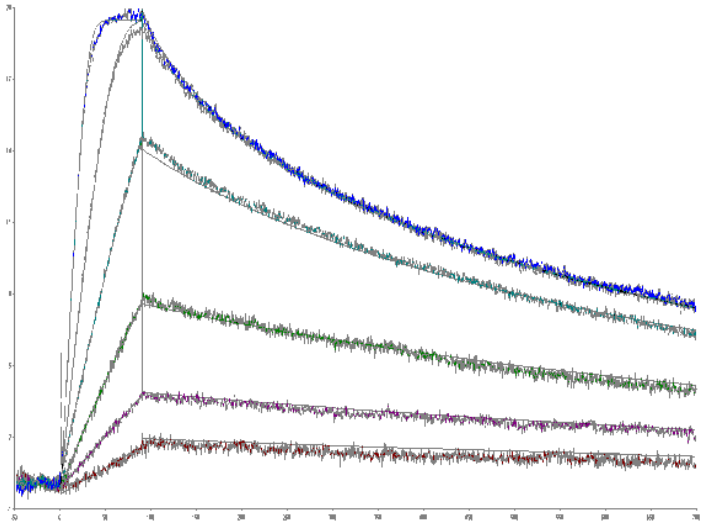


Challenges for detection of weakly interacting ligands

- Fragments provide little kinetic information

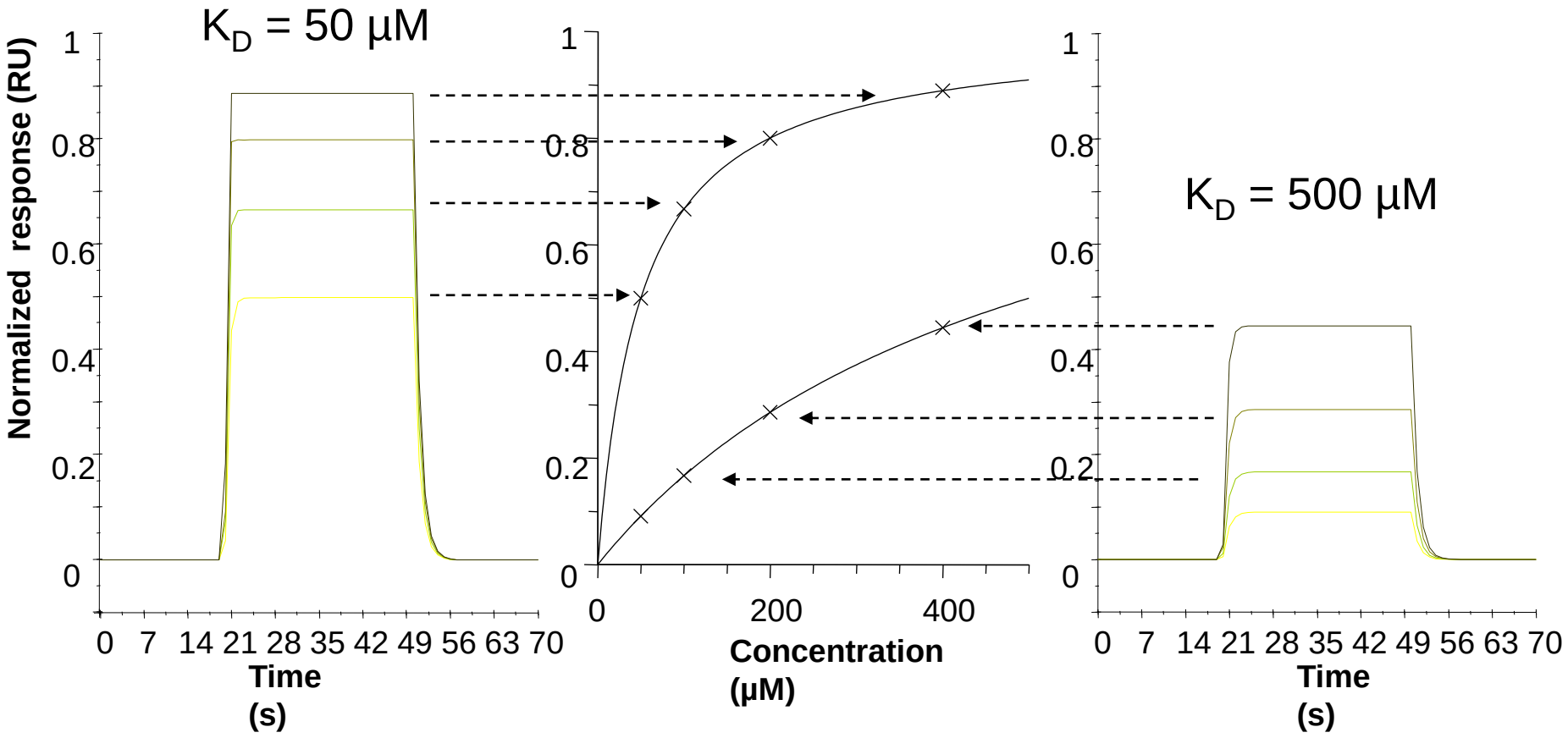


Typical fragment

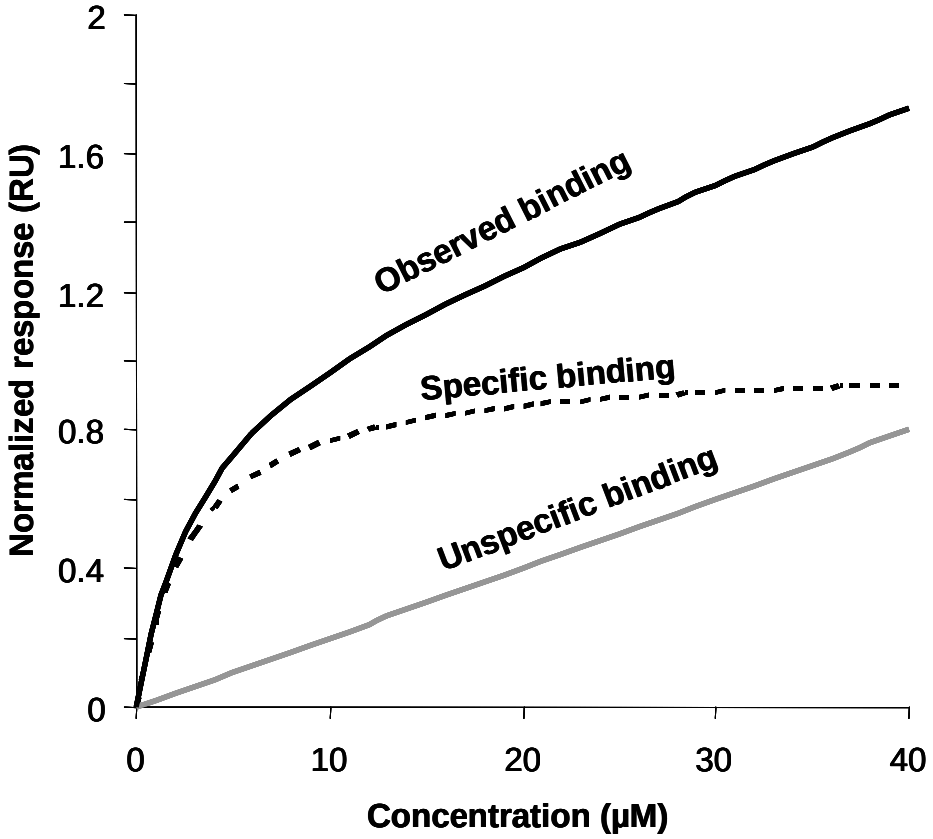


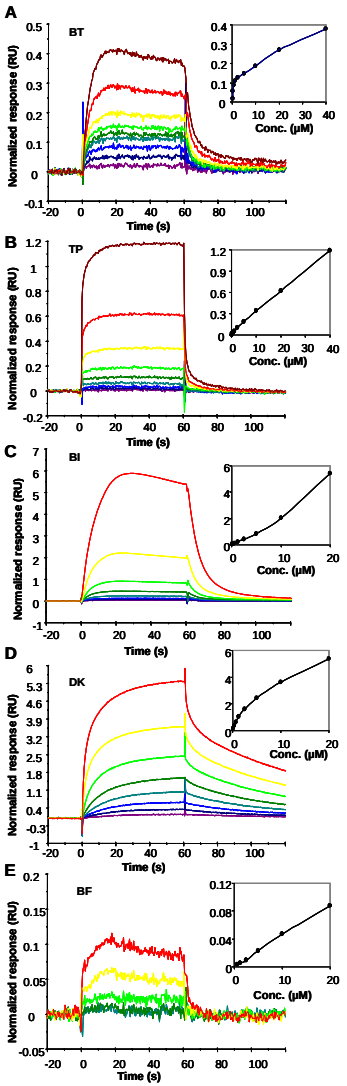
Typical lead

Steady-state analysis of sensorgrams



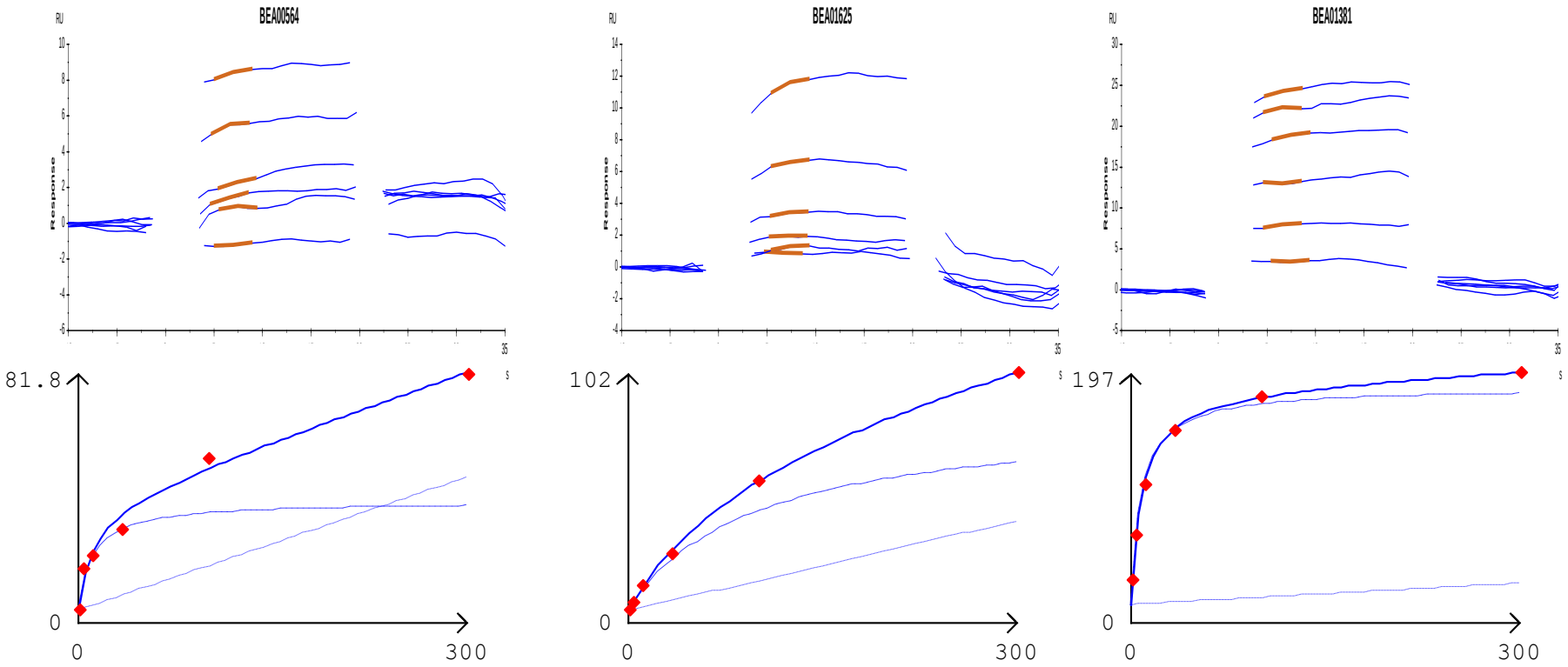
Distinguishing low affinity fragment hits from non-specific compounds





Examples of lead compounds with poor quality

Overcoming challenges by clever data analysis and selection filters

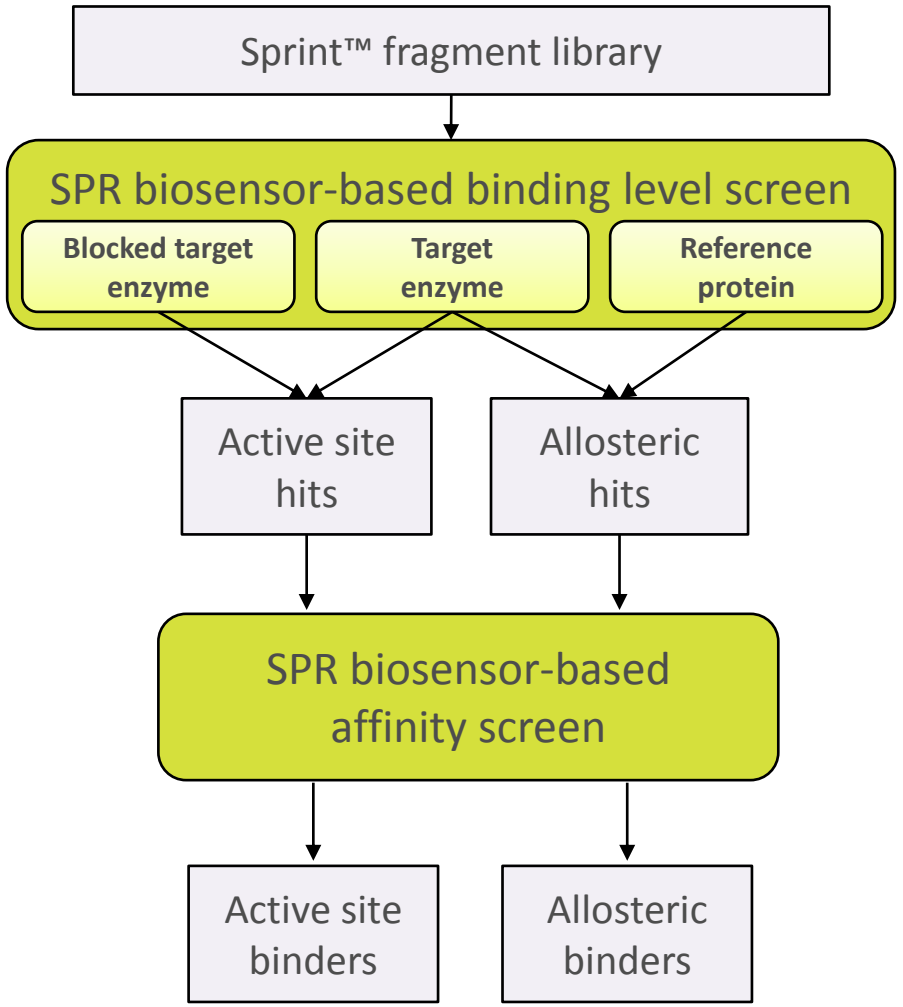


Which fragment should we pick? Possible selection criteria:

- Highest signal at highest concentration (simplistic end-point strategy)
- Relevant signal at saturation or apparent saturation (removes “sticky” hits)
- Low degree of un-specific interaction

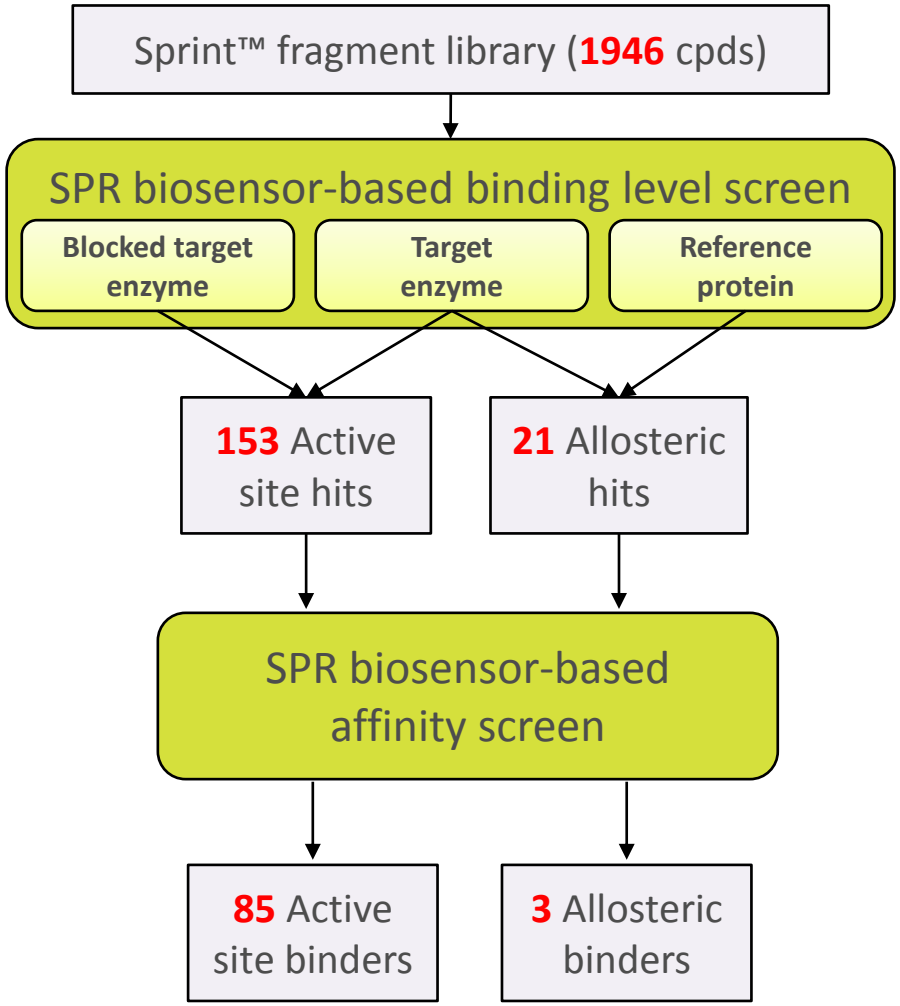
Example of experimental design for fragment library screening

- Screening at single concentration
  - Target protein identifies binders
  - Competition mode distinguishes active-site and allosteric binders
  - Reference protein excludes non-specific ligands
- Hits confirmed by analysis of concentration series



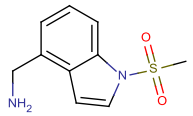
Step 1: Fragment library screening

- 85 active site binders identified
  - K_D values: 0.5–500 µM
  - LE values: 0.25–0.84 kcal mol^{-1}
  - ~25 chemical scaffolds identified amenable for medicinal chemistry exploration
- 3 allosteric binders identified
  - 3 unique scaffolds targeting a non-precedented allosteric binding site
- Assay development, screening and affinity determinations completed in less than 3 months

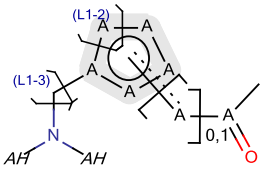


SAR by catalogue 1st iteration

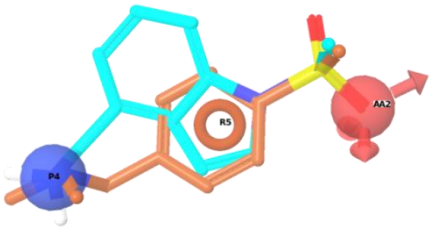
- Fragments of interest selected



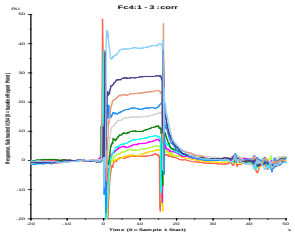
- Build an 'active substructure' hypothesis to search for analogues

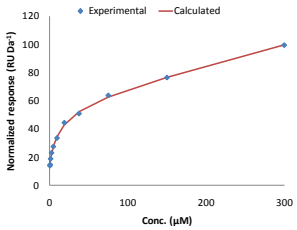


- Use identified analogues for constructing pharmacophore hypotheses to prioritize among commercial analogues



- Refine hypotheses by testing Sprint™ analogues and purchased fragments





→ 83 fragments purchased

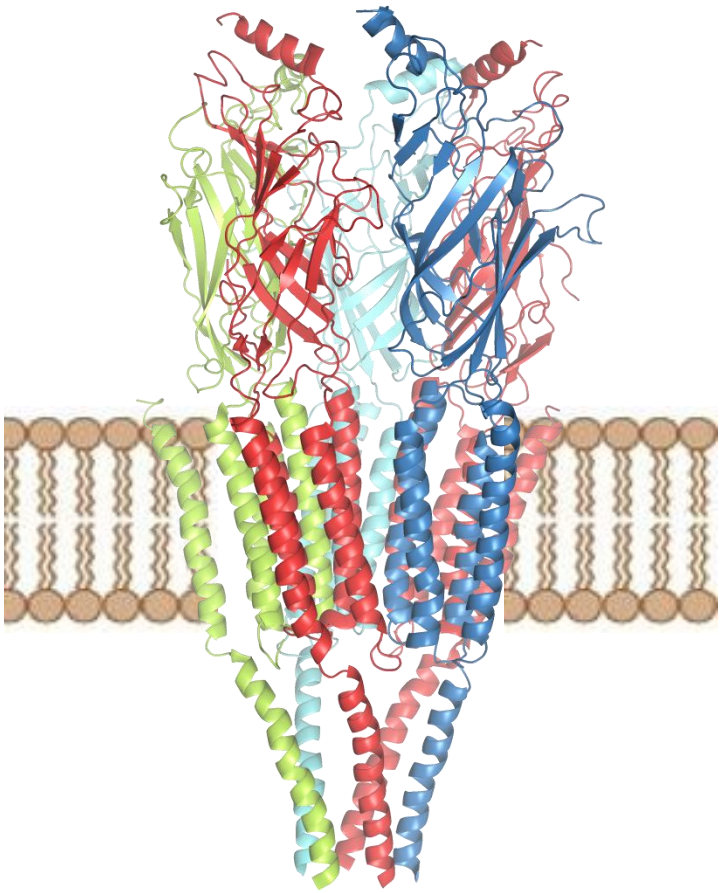
Step 2: SAR by catalogue 1st iteration

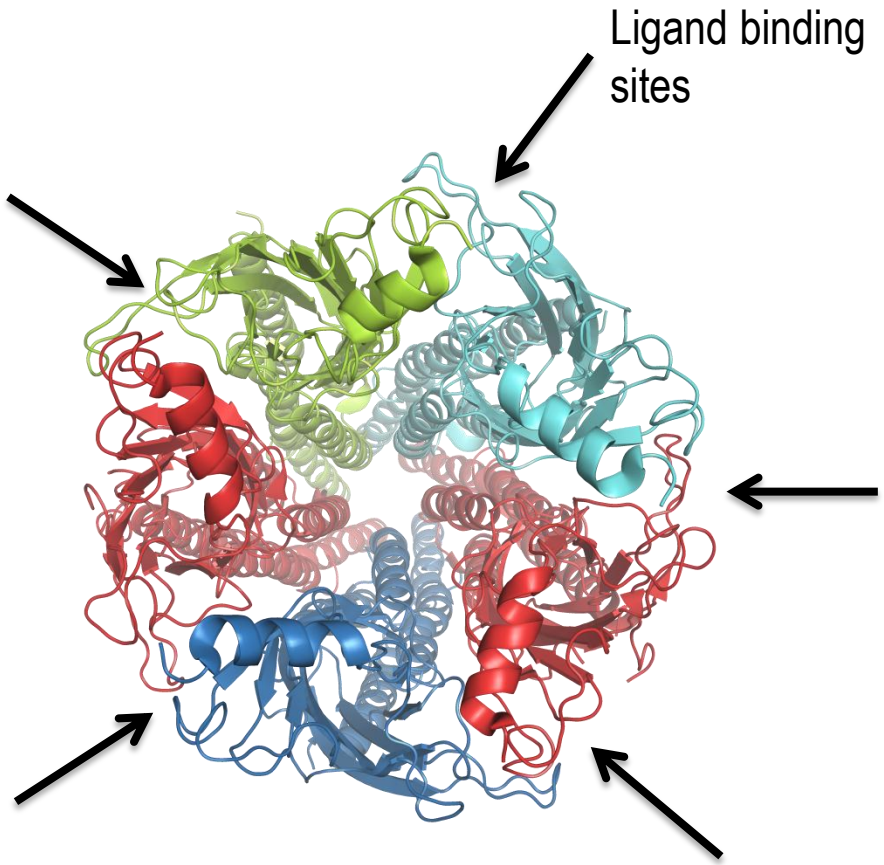
- 31 new fragment hits identified
  - K_D values: 0.2–400 µM
  - LE values: 0.37–0.85 kcal mol^{-1}
  - Series specific SAR starting to emerge
- 18 new chemical scaffolds identified
  - Structural diversity significantly improved
- First iteration completed in 6 FTE weeks
  - Molecular modelling, purchase campaign, and interaction analysis

From fragment to lead

Identification and validation of fragment hits – membrane bound targets

Cys-loop receptors – pentameric ligand gated ion channels

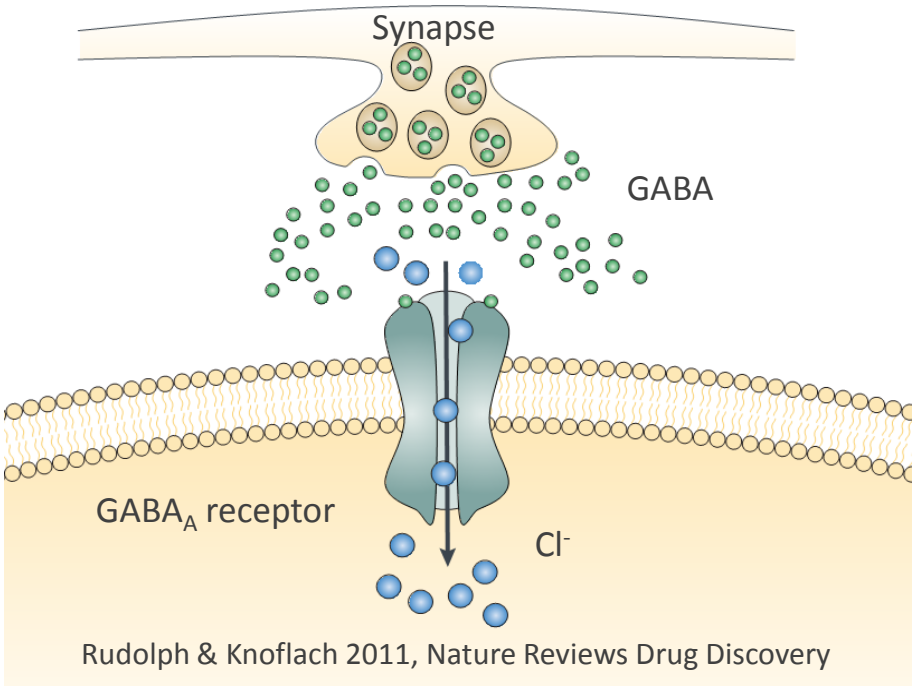




Nicotinic acetylcholine receptor (2BG9.pdb)

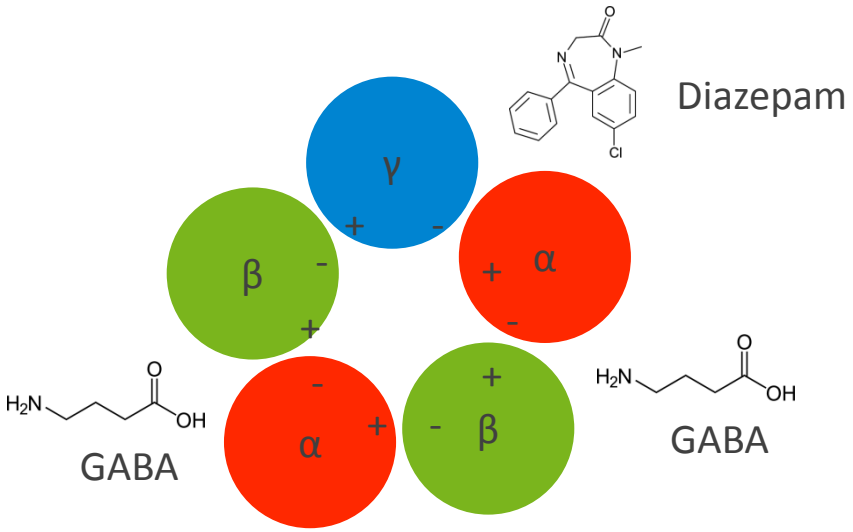
$GABA_A$ receptor

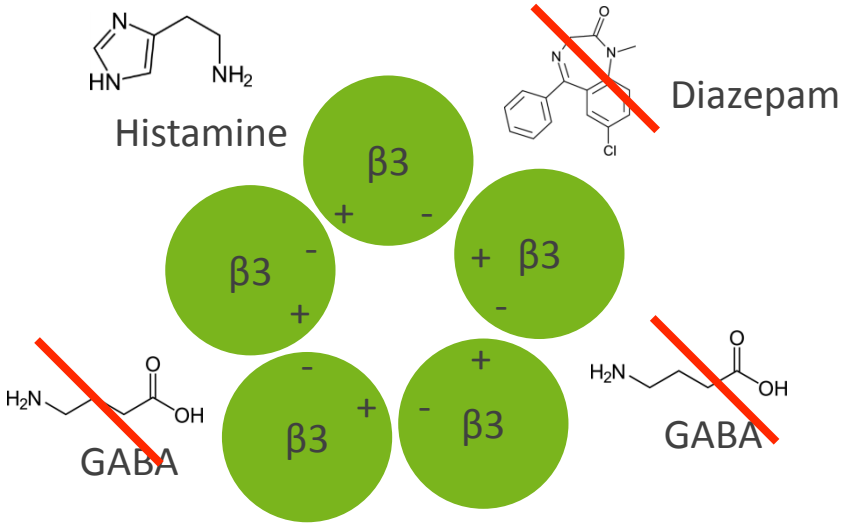
- Gated by GABA, major inhibitory neurotransmitter in CNS
- Involved in neurological disorders, like anxiety and depression
- Modulated by clinically relevant drugs
  - Benzodiazepines
  - Anaesthetics



Rudolph & Knoflach 2011, Nature Reviews Drug Discovery

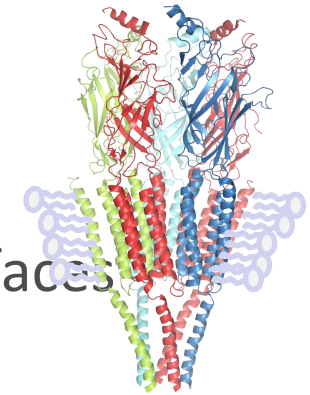
Modular pentameric structure provides variable specificity

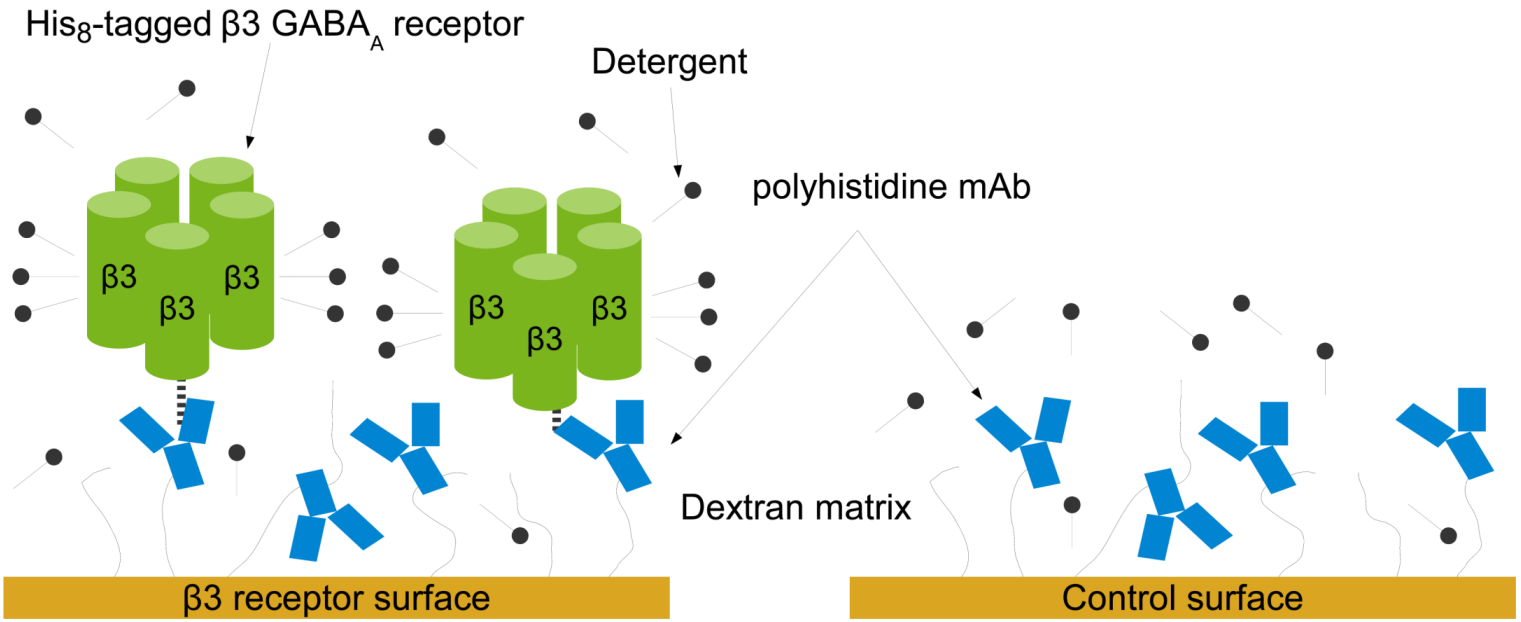




Capture of solubilized $GABA_A$ receptor via His-Ab

- Homo-oligomeric β3 $GABA_A$ receptors with His8-tag expressed in baculovirus infected Sf9 cells
- Solubilized receptor membranes captured to Ab-surfaces





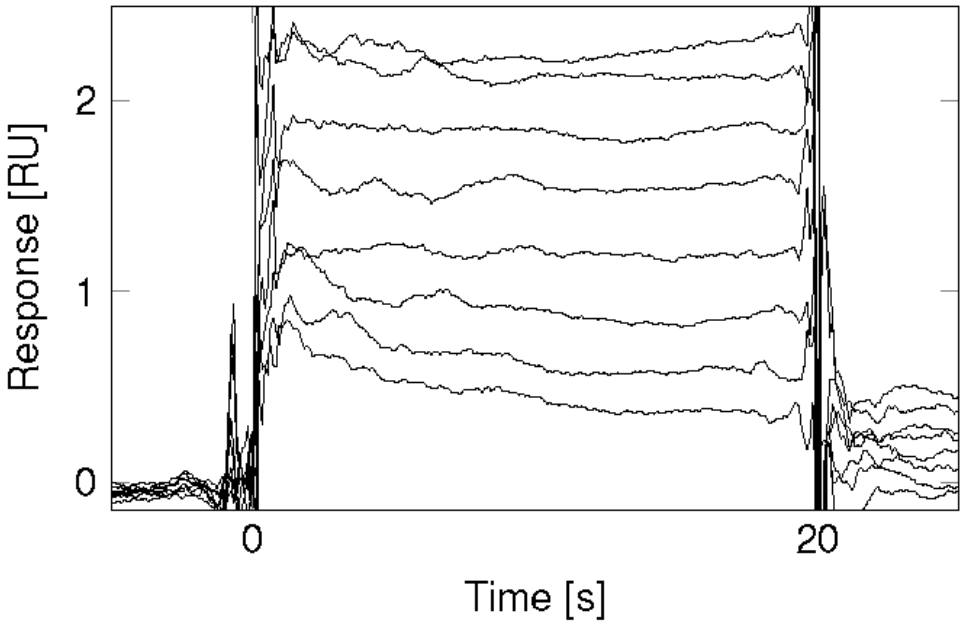
Screened 15 GABAergic and 51 histaminergic compounds

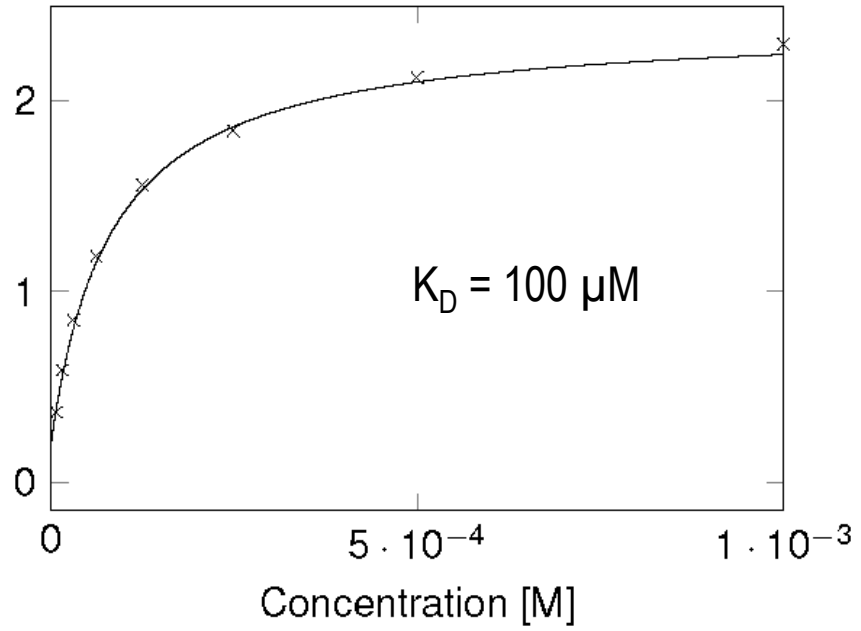
Ligand	M [g mol^{-1}]
4-Piperidine-sulfonic acid	165.2
Alfaxalone	332.5
Baclofen	213.7
Etazolate	289.3
Etomidate	244.3
Flumazenil	303.3
Flurazepam	387.9
GABA	103.1
Muscimol	114.1
Pentobarbital	225.3
PK 11195*	352.9
Propofol	178.3
Ro5-4864*	319.2
SR 95531	287.3
THIP	140.1

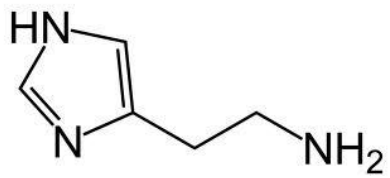
GABAergic and histaminergic modulators are fragment-like

Ligand	M [g mol^{-1}]	Ligand	M [g mol^{-1}]
(R)-α-Methylhistamine	125.2	Imetit	170.2
(S)-α-Methylhistamine	125.2	Immepip	165.2
(S)-Dimethindene	292.4	Immethridine	159.2
2-Pyridylethylamine	122.2	Impentamine	153.2
4-Methylhistamine	125.2	Iodophenpropit	414.3
A-943931	295.4	JNJ 10181457	312.4
A-987306	327.4	JNJ10191584	278.7
Aminopotentidine	477.6	JNJ7777120	277.7
Amthamine	157.2	Ketotifen	309.4
Astemizole	458.6	Loratidine	382.9
BF 2649	295.8	Mepyramine	285.4
Burimamide	212.3	Methimepip	179.3
Carcinine	182.2	Mirtazepine	265.4
Cetirizine	388.9	N^{α}-Methylhistamine	125.2
Cimetidine	252.3	Proxyfan	216.3
Clemastine	343.9	Ranitidine	314.4
Clobenpropit	308.8	ROS 234	241.3
Conessine	356.6	Terfenadine	471.7
Dimaprit	161.3	Thioperamide	292.4
Diphenhydramine	255.4	Tiotidine	312.4
Doxepin	279.4	Triprolidine	278.4
Famotidine	337.4	VUF 5681	193.3
Fexofenadine	501.7	VUF 8430	161.2
Histamine	111.1	Zolantidine	381.5
HTMT	382.4	Zotepine	331.9
ICI 162,846	306.3		

Interaction with histamine

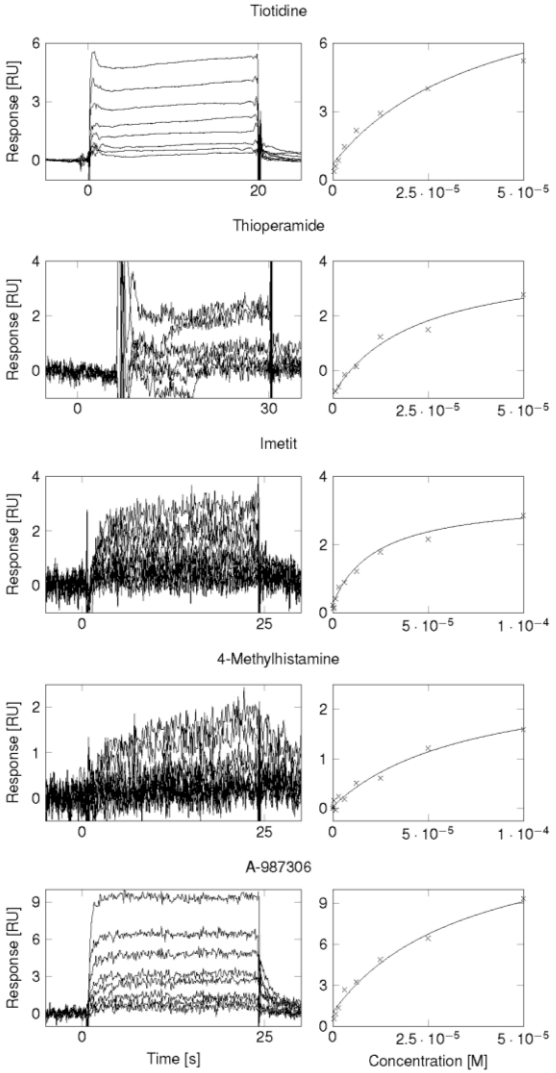


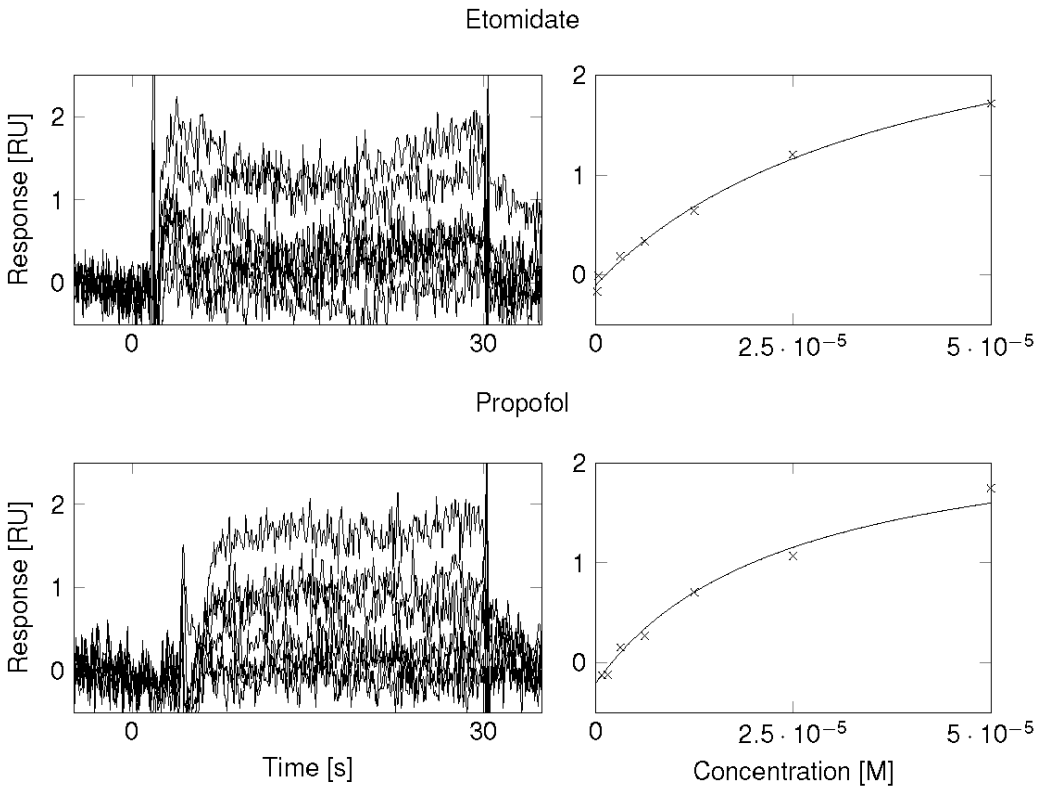




MW 111.1

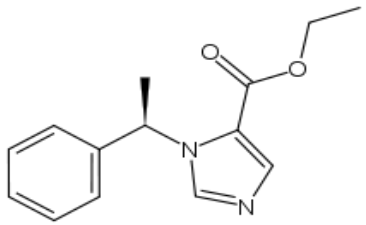
Sensorgrams for selected histaminergic ligands



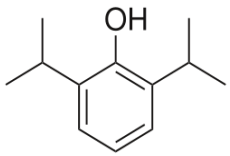


Affinities for GABAergic ligands

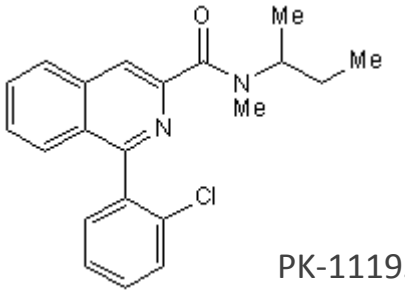
Ligand	K_D [µM]
Etomidate	38
Propofol	42
PK-11195	61
Ro5-4864	69
Etazolate	79



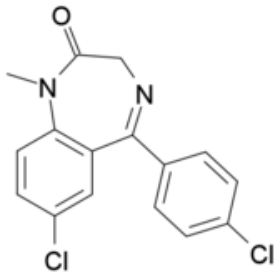
Etomidate



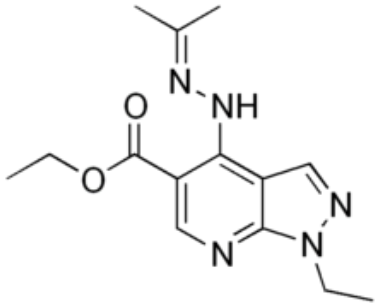
Propofol



PK-11195



Ro5-4864

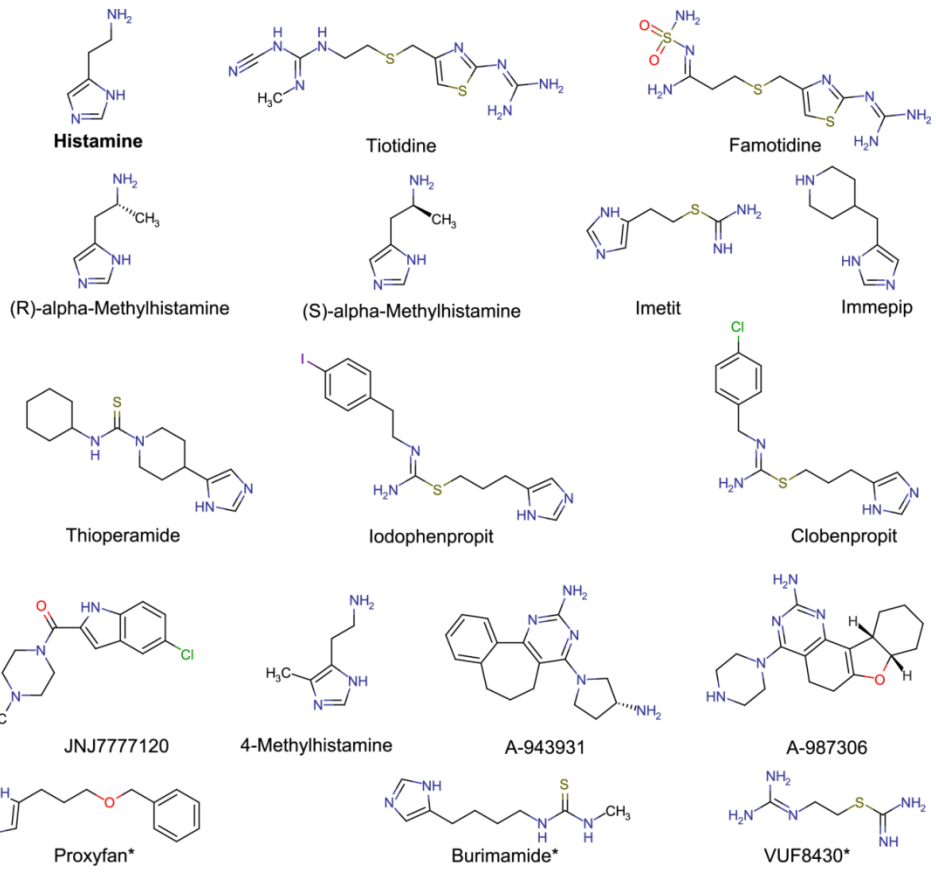


Etazolate

Assay useful for affinity determination and ranking

Affinities for histaminergic ligands

Ligand	K_D [μM]	Histamine receptor activity
Thioperamide	13	H3/H4 antagonist
JNJ7777120	28	H4 antagonist
4-Methylhistamine	32	H4 agonist
Tiotidine	33	H2 antagonist
Burimamide	33	H2/H3 antagonist, H4 agonist
A-987306	46	H4 antagonist
Imetit	51	H3/H4 agonist
(S)-α-Methylhistamine	51	H3/H4 agonist
VUF 8430	54	H4 agonist
Clobenpropit	57	H3 antagonist, H4 agonist
Immepip	69	H3/H4 agonist
Famotidine	81	H2 antagonist
Histamine	98	endogenous H1/H2/H3/H4 agonist
Proxyfan	110	H3/H4 agonist
A-943931	120	H4 antagonist
(R)-α-Methylhistamine	180	H3/H4 agonist
Iodophenpropit	300	H3/H4 antagonist



Conclusions

- Histaminergic compounds may extert effect via $GABA_A$ receptors

- Proof-of-principle demonstrated for ion channels
- Information provided
  - Binding (yes/no)
  - Affinity (M)
  - Competition with reference ligand (yes/no)
  - Induced conformational changes

The advantage of biosensor technology for membrane proteins

- Immobilization of solubilized or membrane bound target (targets do not have to be free in solution)
- Enrichment and purification of target on chip via capture (overcomes low expression levels)
- Increased stability upon target immobilization

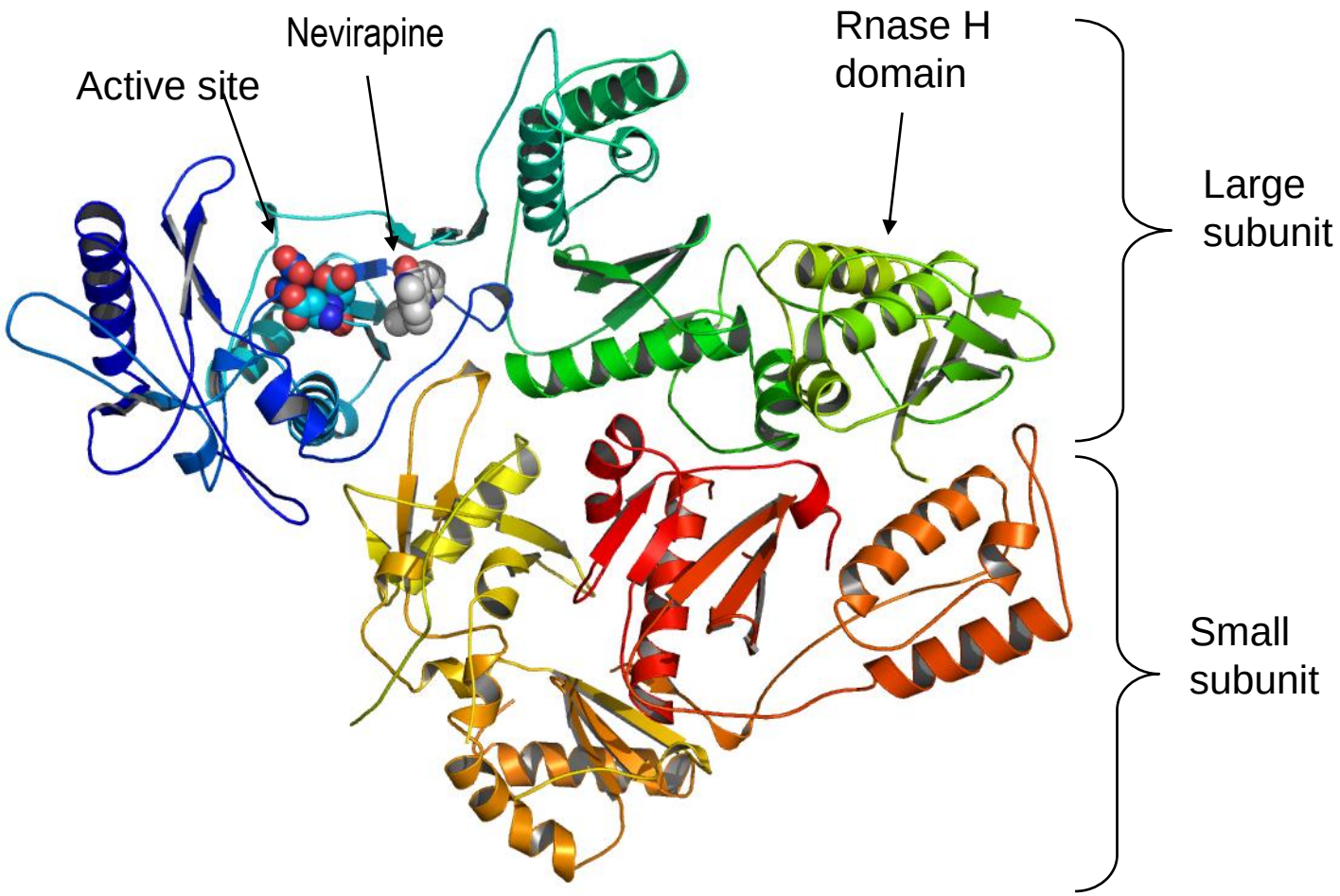
- Direct measurement of binding between ligand and target (overcomes disadvantage of coupled assay)
- Real time analysis

From hit to lead

Characterization of hits/leads –

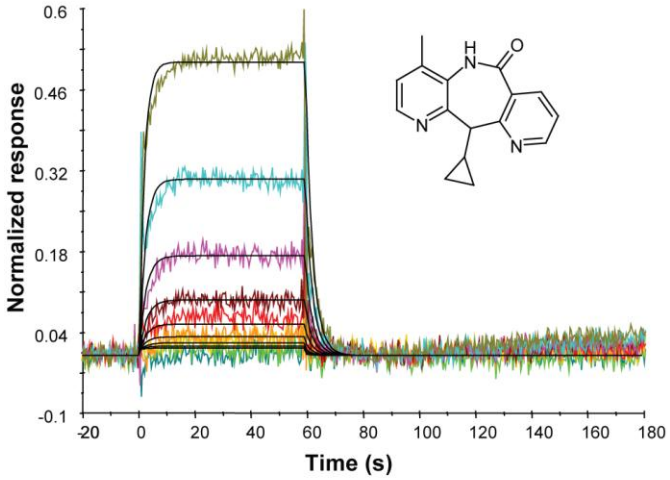
the use of mechanistic and kinetic information for selection and prioritization of leads

HIV reverse transcriptase

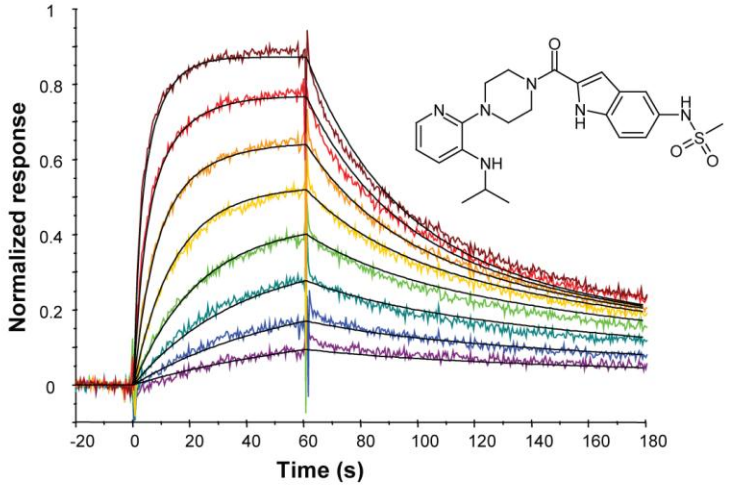


Mechanistic studies - HIV RT K103N mutant

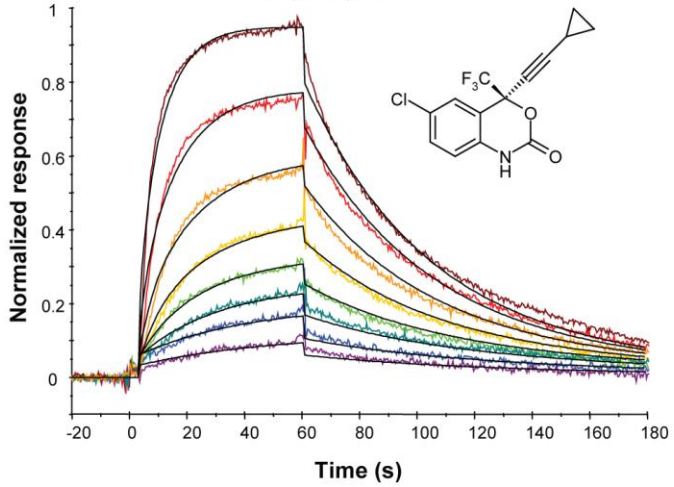
Nevirapine



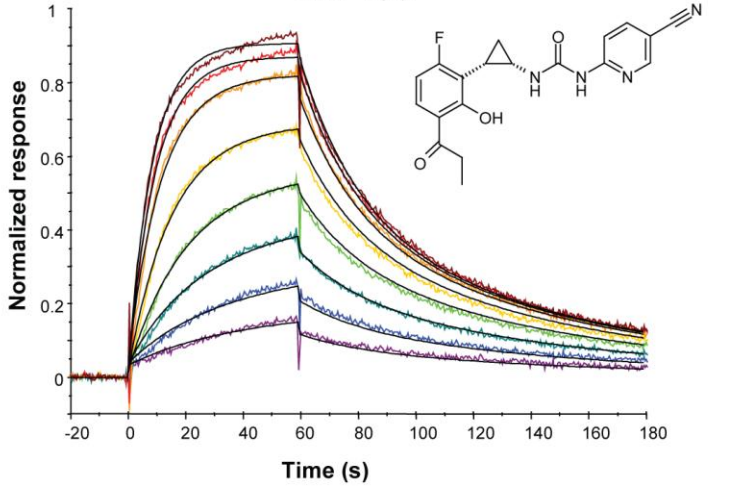
Delavirdine



Efavirenz



MIV-150



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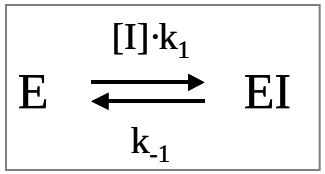
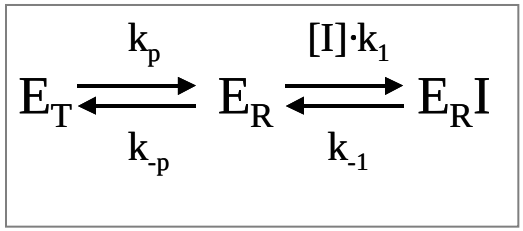
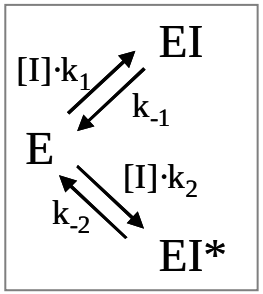
Determination of the interaction kinetic model

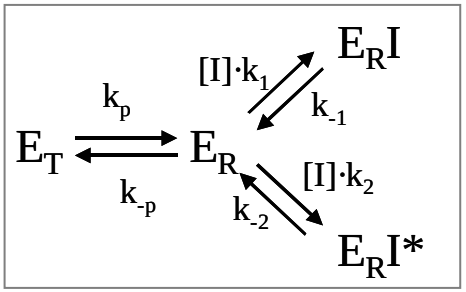
Which model?

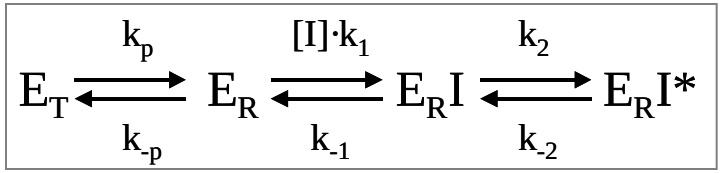


Is the reaction reversible?

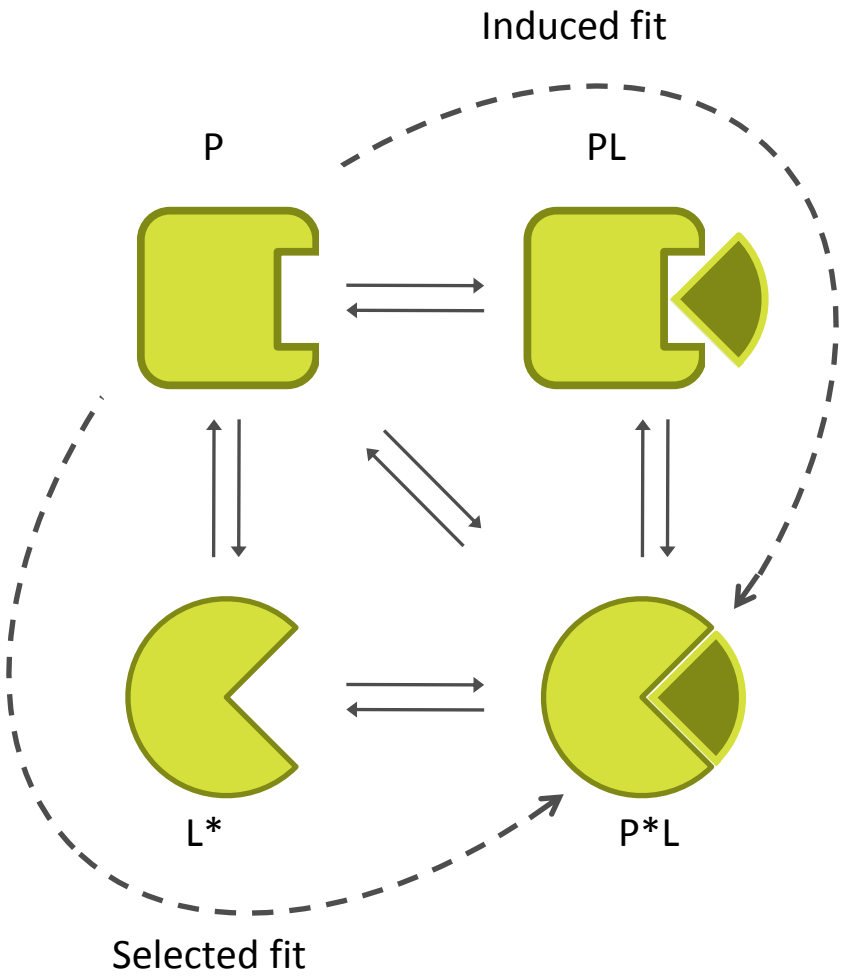








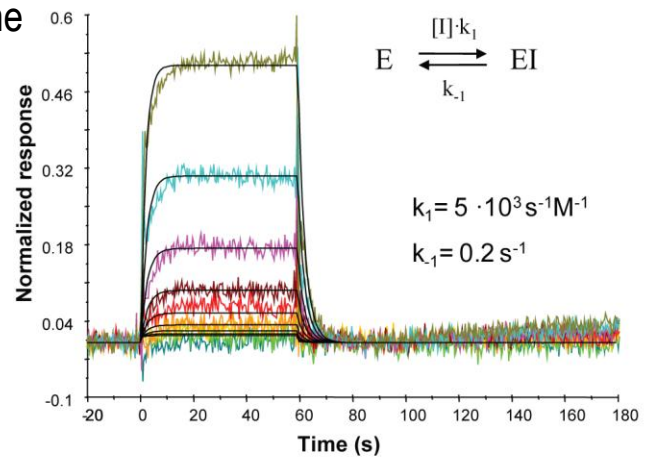
The general model



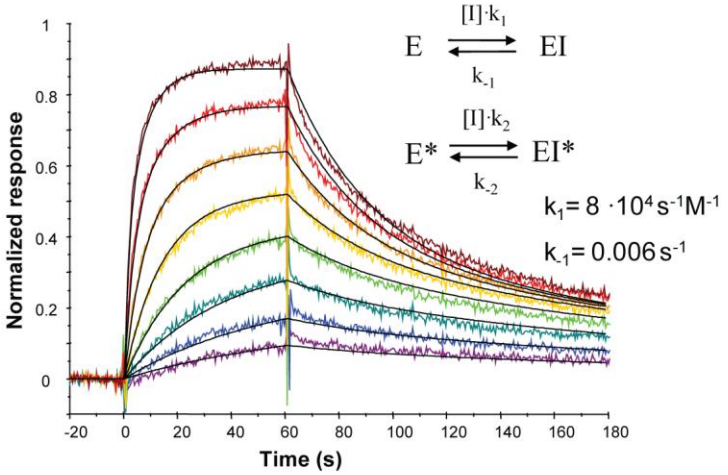
Adapted from Weikl & von Deuster, Proteins 2009; 75:104–110.

Mechanistic studies - HIV RT mutant

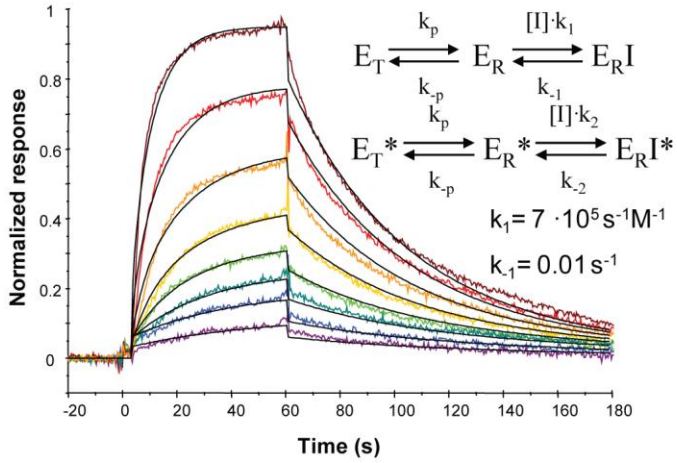
Nevirapine



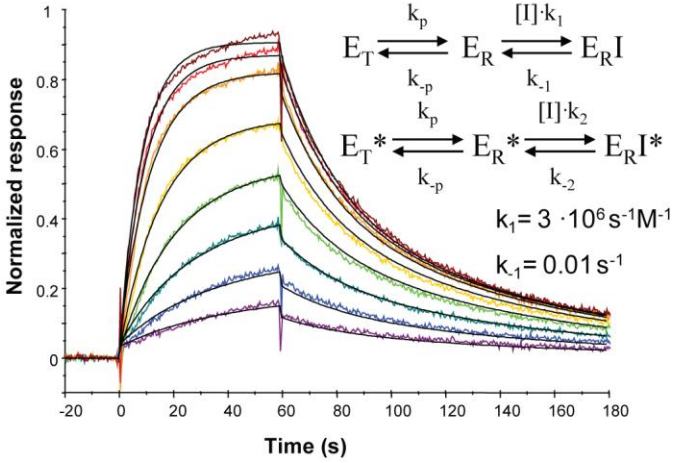
Delavirdine



Efavirenz

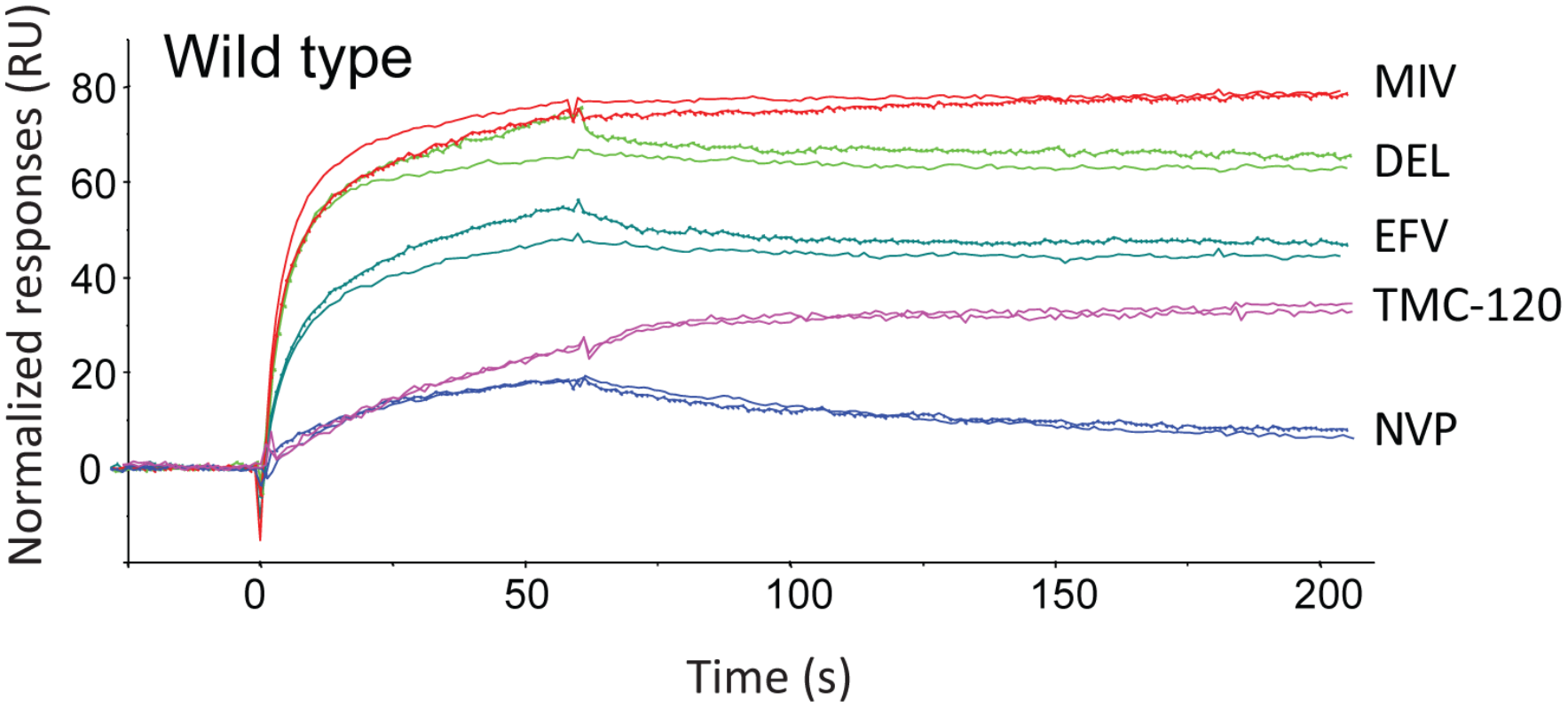


MIV-150



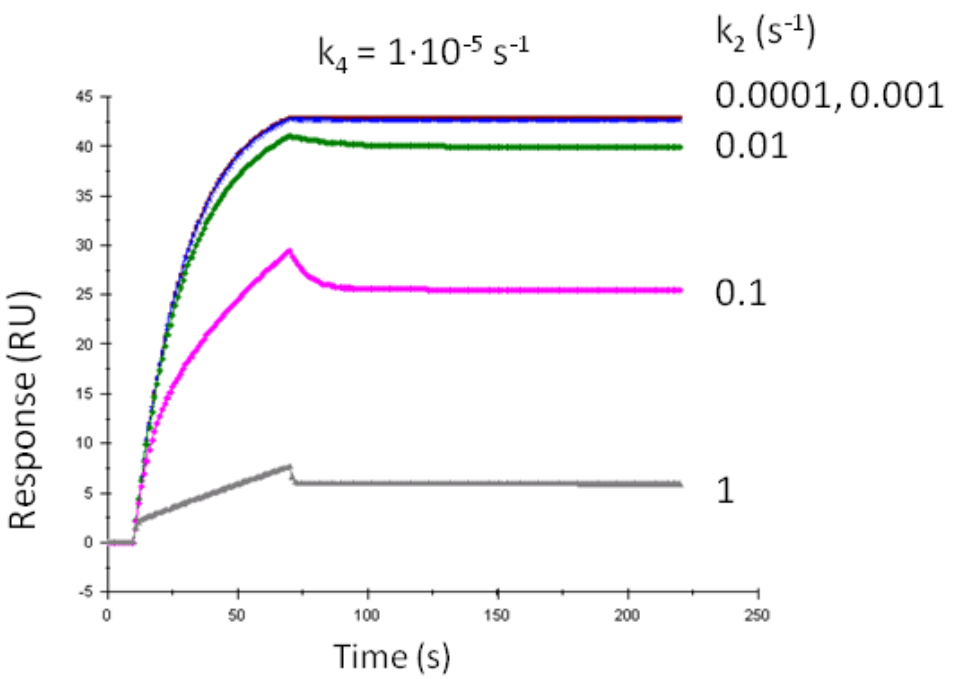
Mechanistic studies – wild type HIV RT

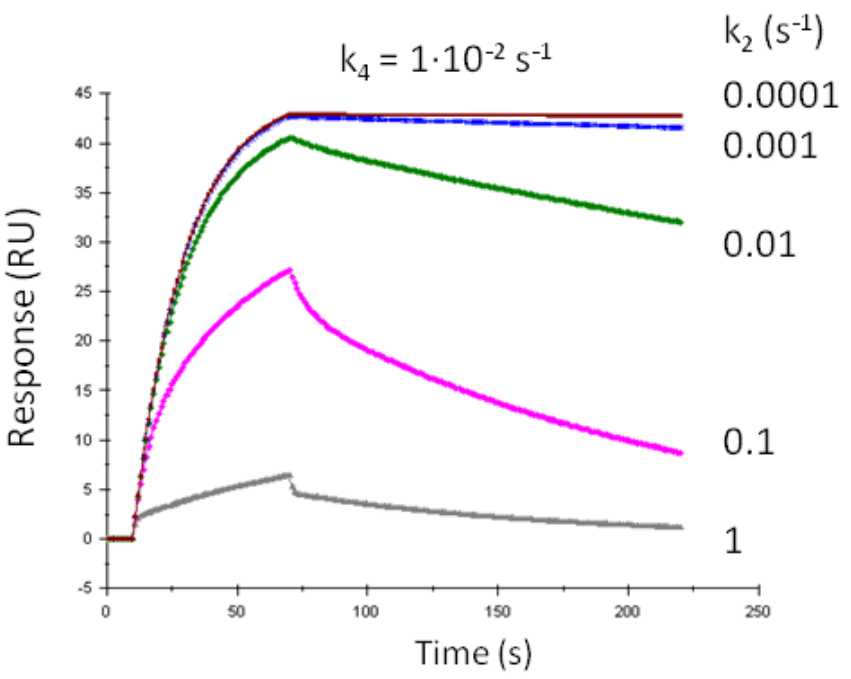
- Same concentration of different ligands show ”same” mechanism but different interaction profile



”Quantifying” kinetics of induced fit interaction by simulation

$$E_1 + I \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E_1I \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E_2I$$





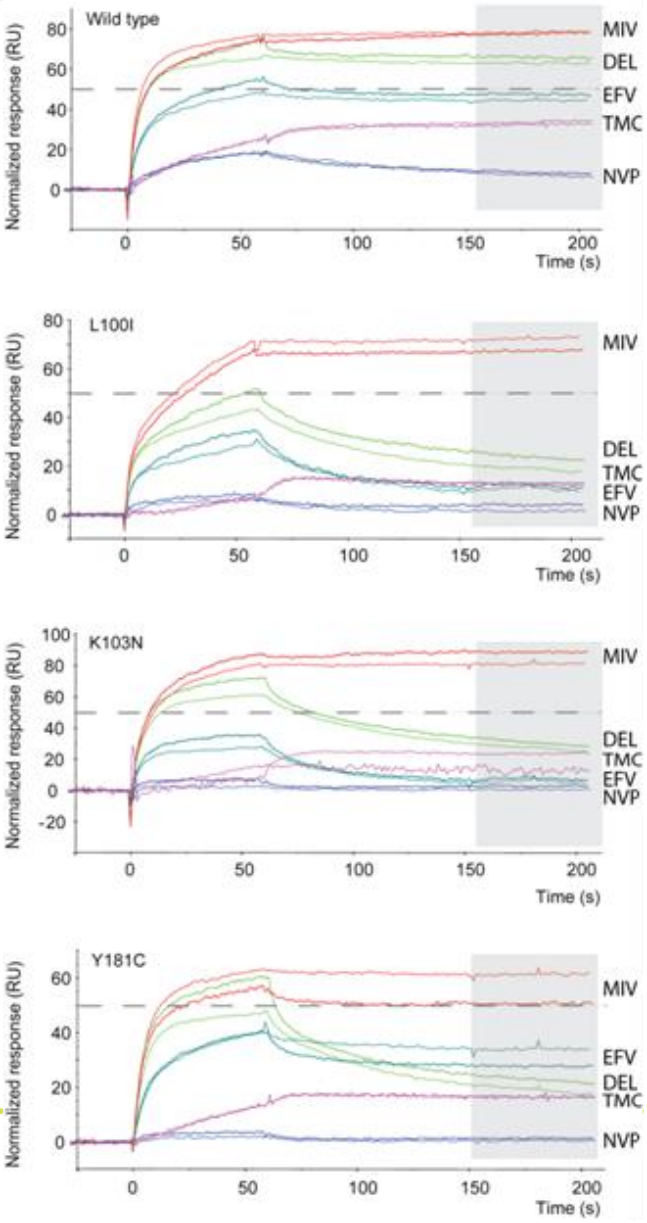
Relevance of detailed mechanistic analysis for drug design

What is the ideal profile of NNRTIs?

- Efficiency of drugs correlates with high concentrations of complex at steady state
- Slow dissociation

	EC_{50} (nM)				
Strain[a]	MIV-170	Delavirdine	Efavirenz	TMC-120	Nevirapine
Wt	0.97	110	1.6	1.2	170
L100I	9	7 900	88	40	1 200
K103N	3.2	6 400	20	5.5	>10 000
Y181C	5.3	5 000	3.9	16	>10 000

	EC_{50} (nM) (95% CI)[a]				
Strain[b]	MIV-170	Nevirapine	Delavirdine	Efavirenz	TMC-120
Wt HIV-1	2.1	370	190	4.9	3.8



Parameters for optimization of complex interactions

- High affinity?
- Dissociation rate constants?
- Residence time?

Biosensor-Based Kinetic Characterization of the Interaction between HIV-1 RT and Non-nucleoside Inhibitors. Geitmann, et al *J. Med. Chem.*, 2006; 49(8); 2367-2374.
Inhibition of HIV-1 by non-nucleoside reverse transcriptase inhibitors via an induced fit mechanism—Importance of slow dissociation and relaxation rates for antiviral efficacy.
Elinder, et al *Pharmacol. Biochem.* 2010; 80; 1133–1140.

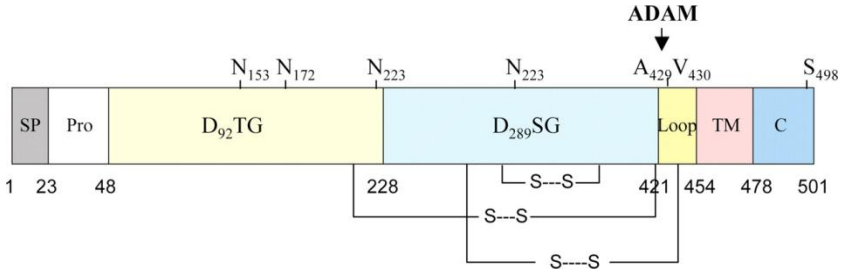
Advanced characterization of leads

Influence of model systems and conditions –

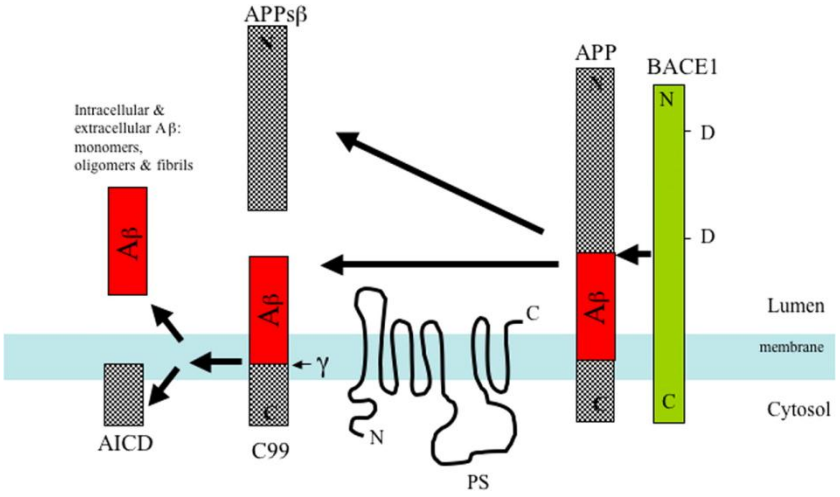
from mechanisms and kinetics to thermodynamics and chemodynamics

BACE1: β-secretase, β-site amyloid precursor protein cleaving enzyme

BACE1 (alias Asp2 or memapsin 2)



Amyloidogenic APP processing



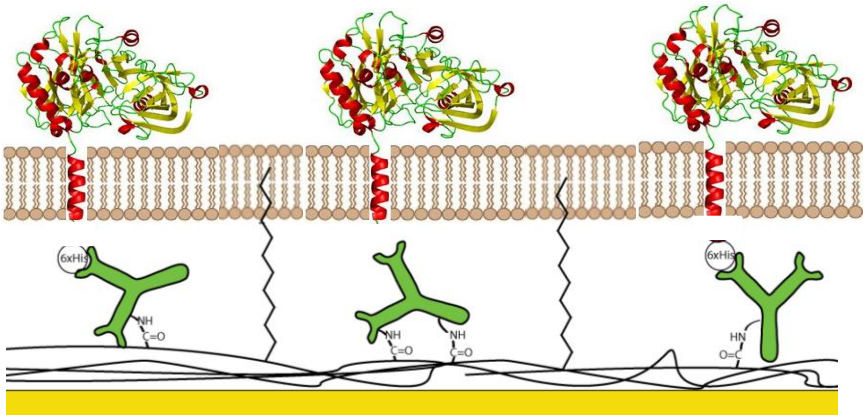
Poor correlation between inhibition of ectodomain BACE and APP cleavage in cells

- No discernible trend
- Why different?
  - Ectodomain vs. full length enzyme?
  - Conditions (pH 4.5 vs. 7.4)
  - Ca^{2+}?

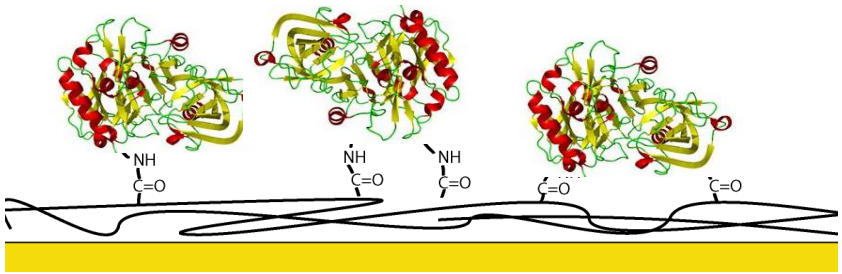
	IC_{50} (nM)	
Compound	Enzyme assay[a]	Cell assay[b]
1	8	0.1
2	29	18
3	9	86
4	13	3
5	5	900
6	30	?

Interaction analysis of inhibitors with BACE1

Full length BACE1 immobilized in a lipid membrane via antibody capture

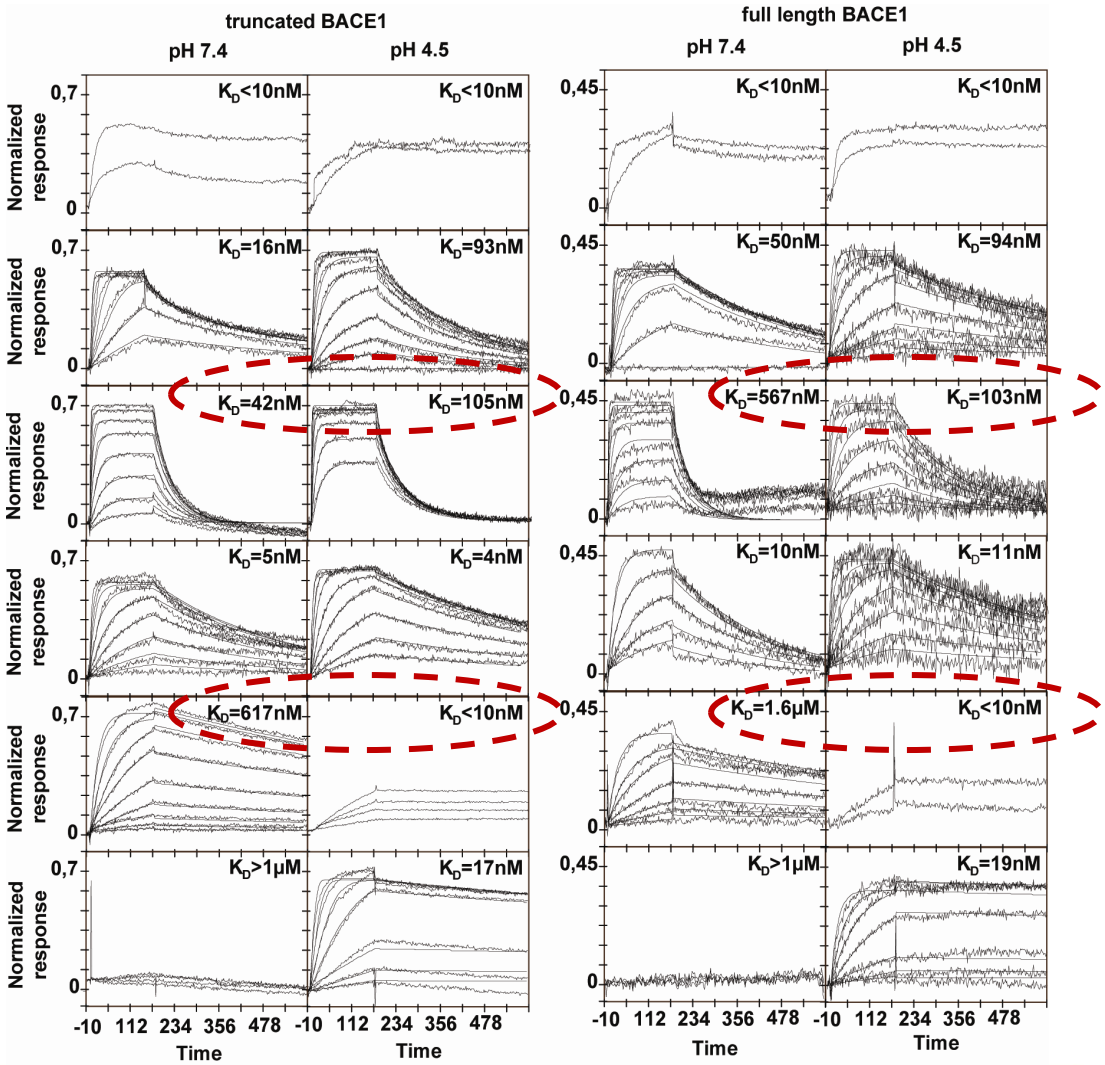


Ectodomain BACE1 immobilized directly (no transmembrane region)



Inhibitor interactions with BACE1 and inhibitory effects

	IC_{50} (nM)	
	Enzyme assay[a]	Cell assay[b]
	8	0.1
MV073921	29	18
	9	86
	13	3
OM99-2	5	900
	30	?



Better correlation between IC_{50} from cell assay and interaction data at pH 7.4 than 4.5, or with enzyme assay – why?

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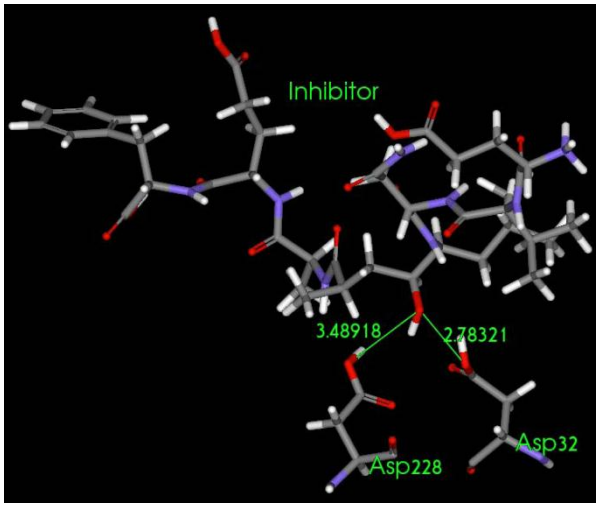
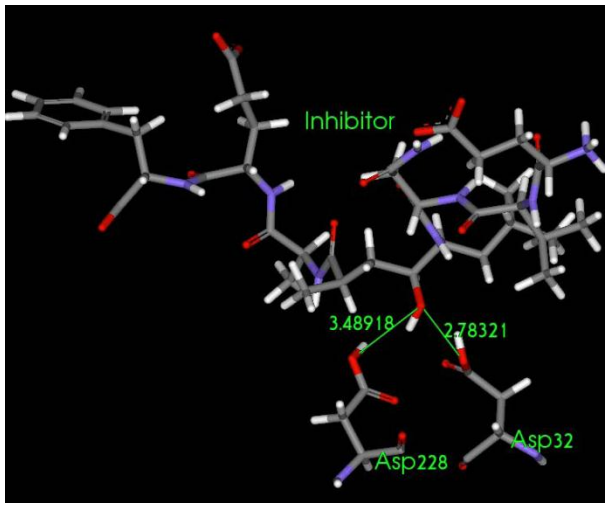
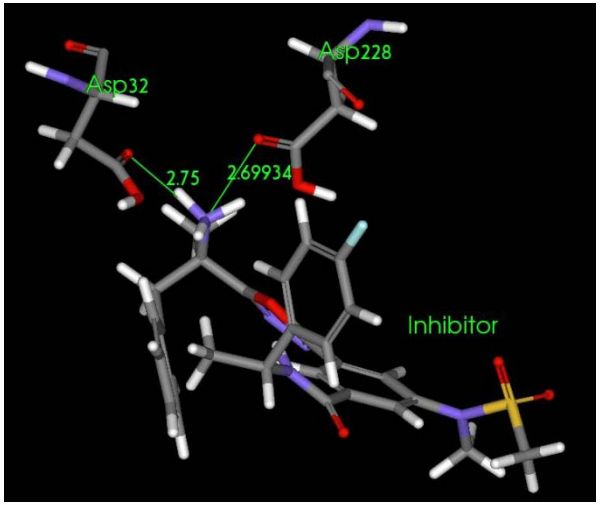
Modelling of inhibitor bound to catalytic aspartates of BACE1

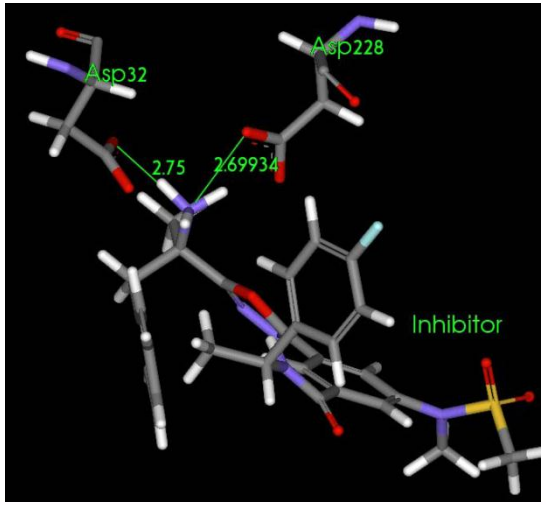
pH: 4.5



pH: 7.4



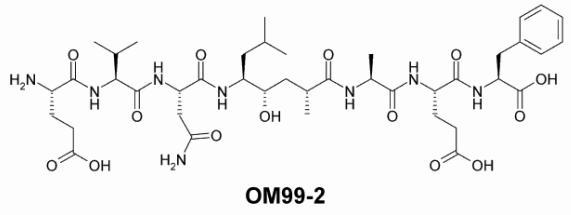




pKa evaluations of the Asp dyad

OM99-2

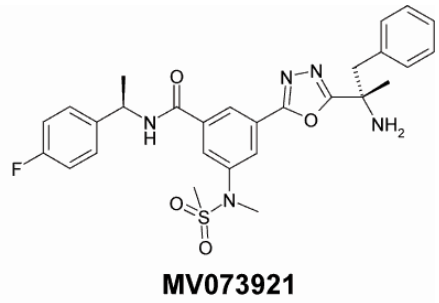
Diprotonated state at both pH values.



MV073921-1

Diprotonated state at pH=4.5

Doubly charged state at pH=7.4.



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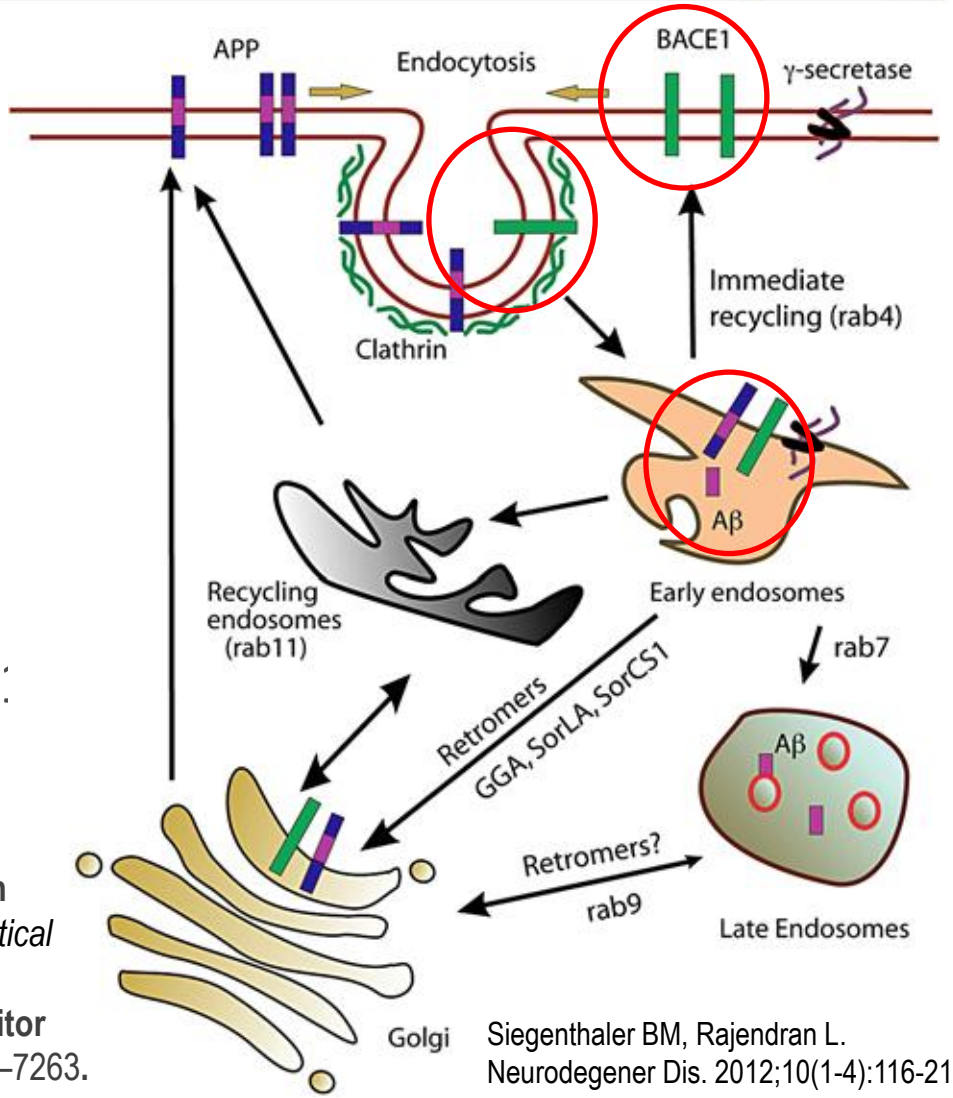
Why is an interaction assay better than an inhibition assay for prediction of cell effect?

- Inhibitors bind BACE1 at the cell surface (neutral pH)
- BACE1 is internalized into endosomes for cleavage (acidic pH)
- Inhibitors need to bind BACE1 at neutral and acidic pH!



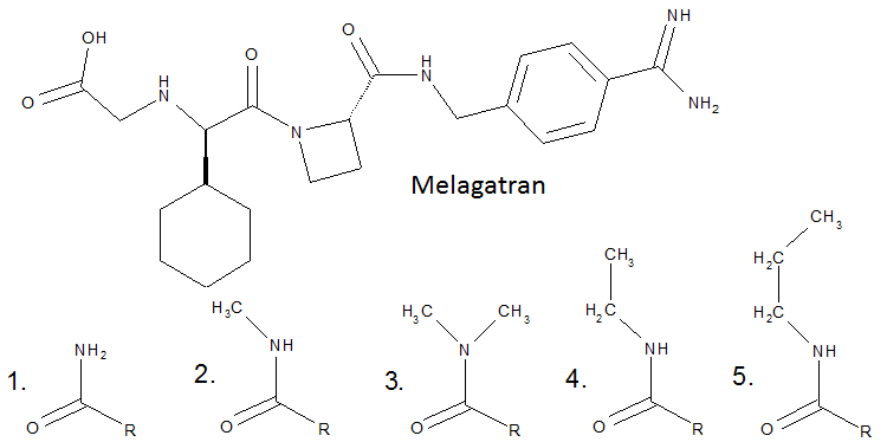
Siegenthaler BM, Rajendran L. Neurodegener Dis. 2012;10(1-4):116-21.

A surface plasmon resonance based biosensor with full-length BACE1 in a reconstituted membrane. Christopeit, T., et al. *Analytical Biochemistry* 2011, 414 pp. 14-22.

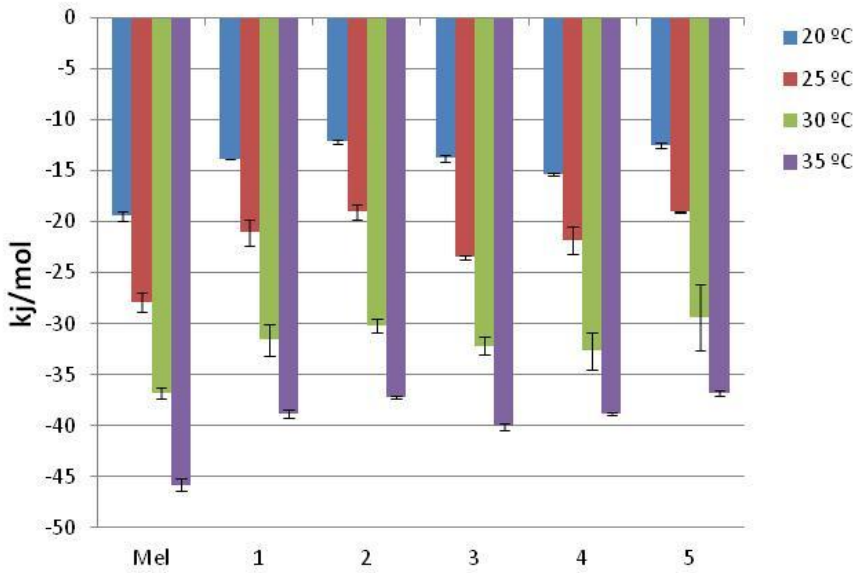
Effect of protonation state of the titrable residues on the inhibitor affinity to BACE1. Domínguez, et al *Biochemistry,* 2010; 49, 7255–7263.

Thermodynamic analysis of interactions using SPR biosensors

- Profiling of melagatran analogues interacting with thrombin

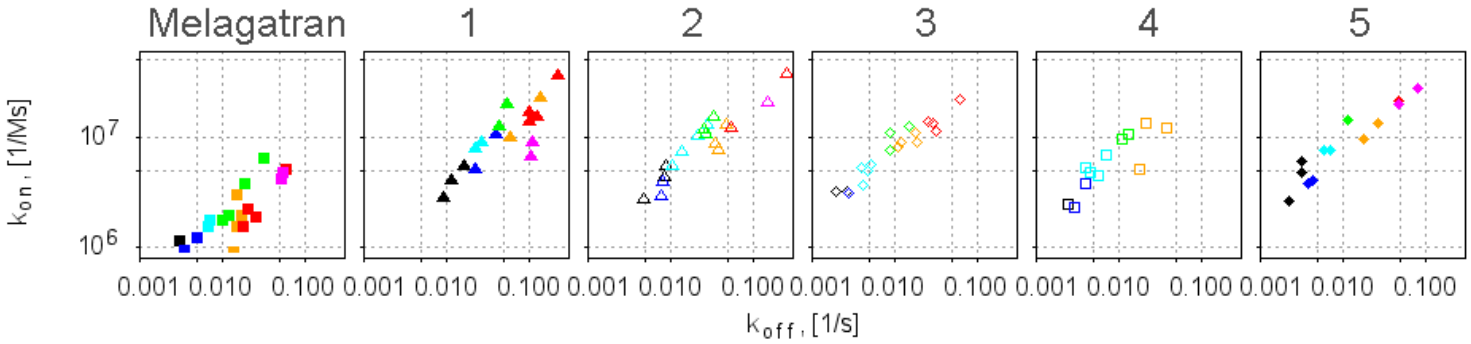


1. Determination of enthalpic contributions to binding by ITC at multiple temperatures



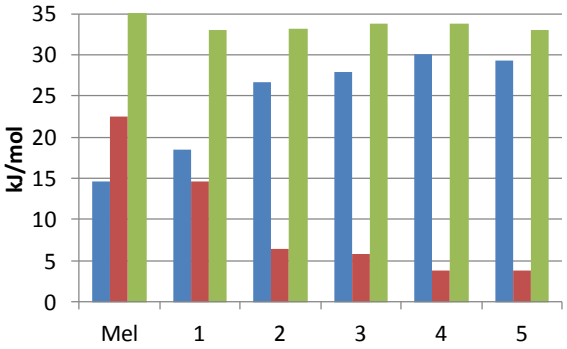
Thermodynamic analysis of interactions using SPR biosensors

Relationships between k_{on} and k_{off} over a range of temperatures

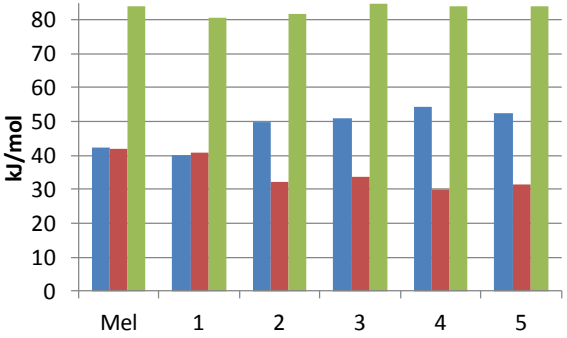


Thermodynamic profiles from SPR at 25 °C

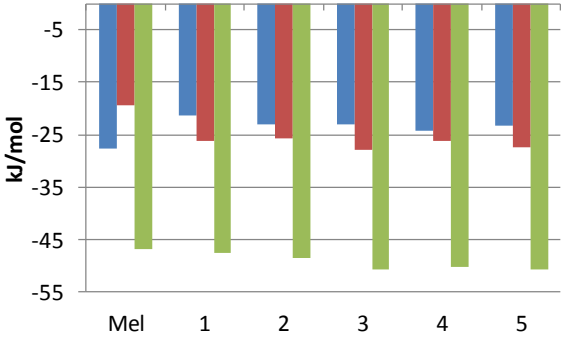
Association



Dissociation



Equilibrium



ΔH (blue), -TΔS (red), and ΔG (green)

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Interactions understood. Leads improved.