Biophysical ligand screening for soluble and membrane proteins

September 2010

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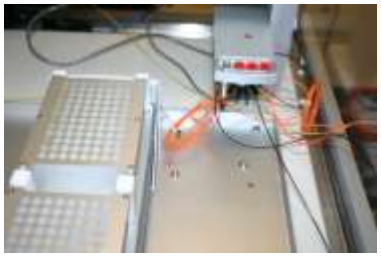

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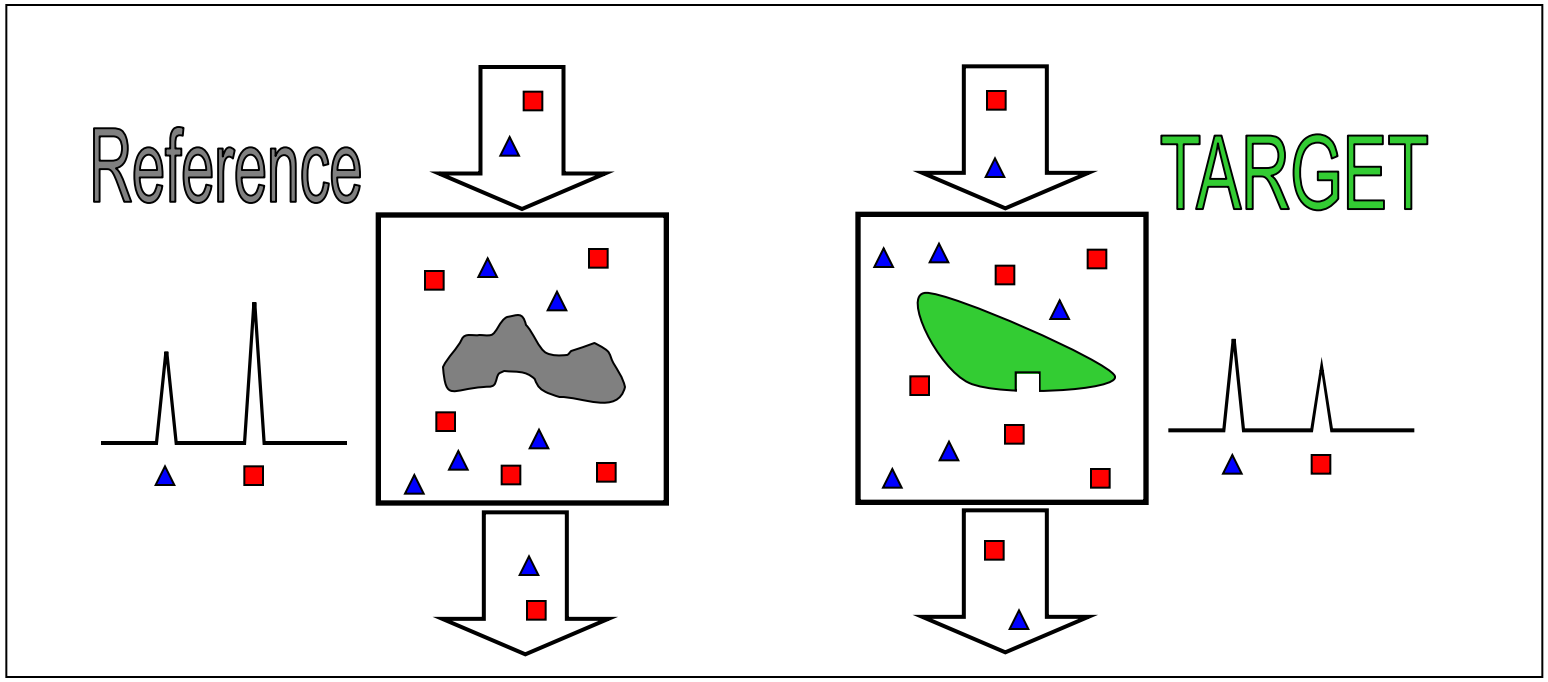
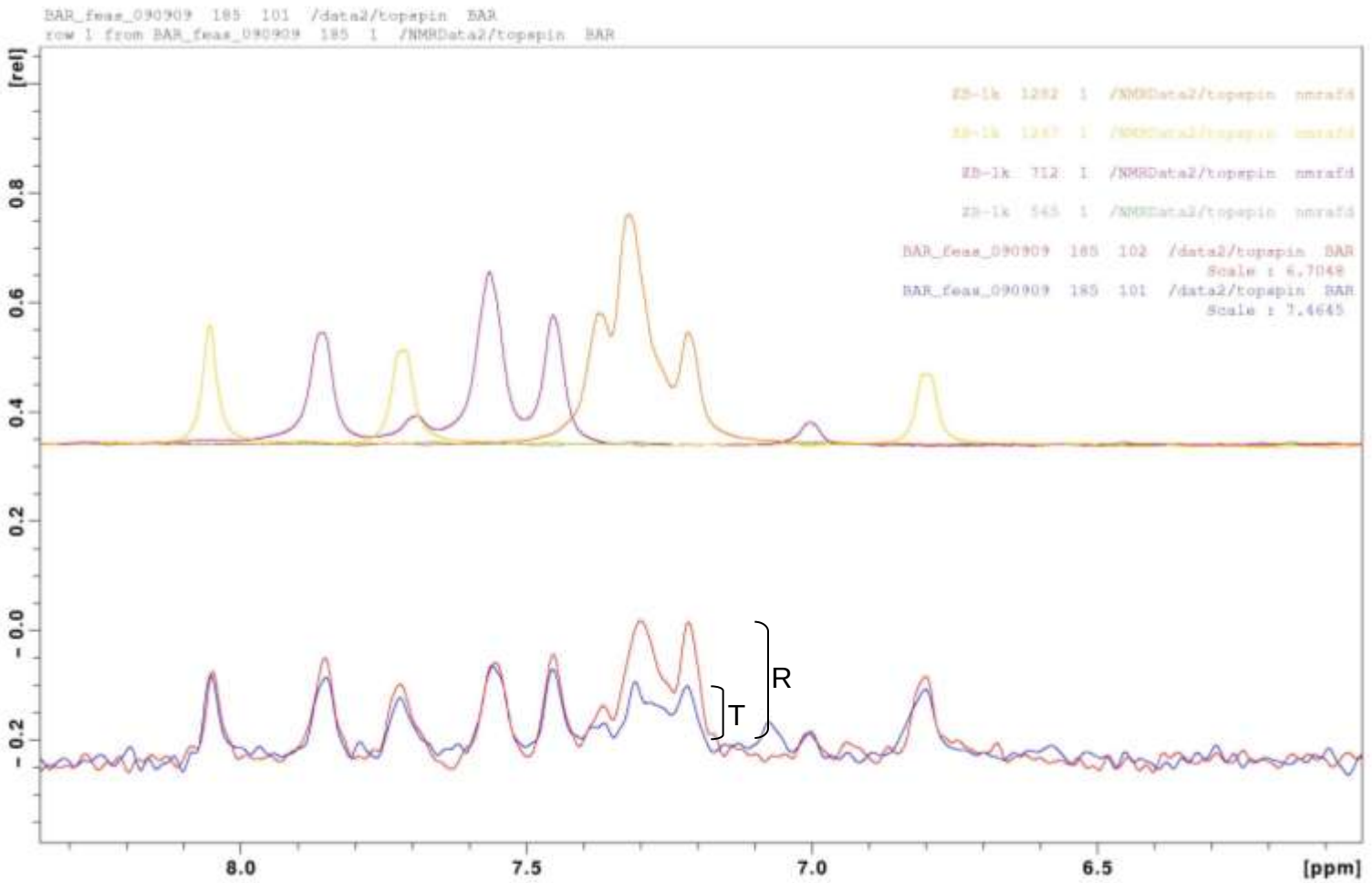
The TINS method for finding hits

TINS = Target Immobilized NMR Screening



Vanwetswinkel et al., 2005,12, p.207

An example of raw data



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Summary of Selected Immobilized Targets

Protein	Size	Functional	TINS hits
Protease	44 kDa	LB	5.9%
HSP90	24 kDa	LB	6.5%
Small GTPase	20 kDa	LB/BA	9% apo form 3% NDP form
Viral enzyme	67 kDa	LB/BA	9.5%
DsbB (Bacterial mem. Prot.)	14 kDa	BA	7.3%
Various kinases (pY,pS/T)	30-35 kDa	LB/BA	3.8-5.1%
KcsA (Ion channel)	57 kDa	LB	Feasibility only (95 cmpds, 7%)
Metalloproteins	105 kDa homotrimer	LB/BA	5-8.5%
Prot-Prot Interaction (5)	14-100 kDa	LB	3-6%

LB ligand binding
BA biochemical assay

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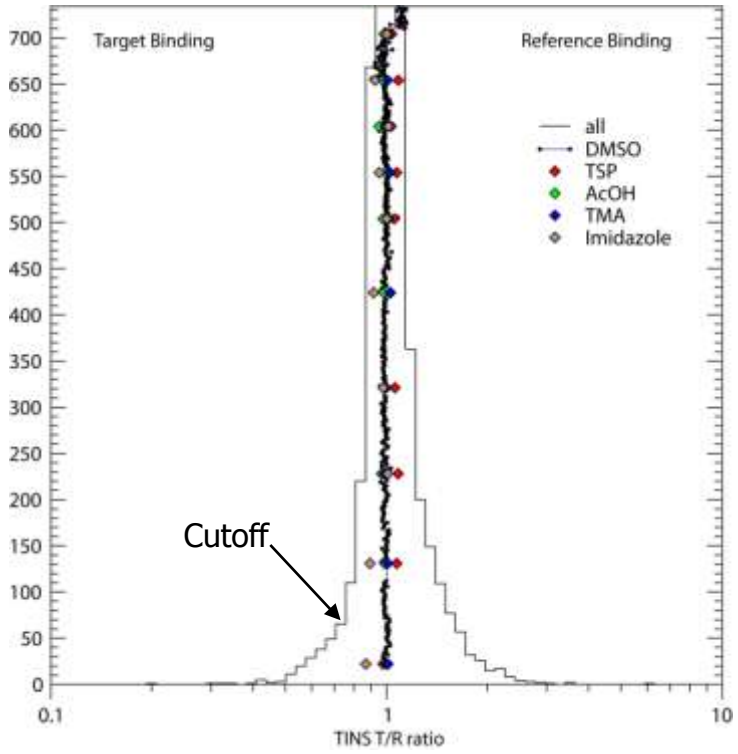
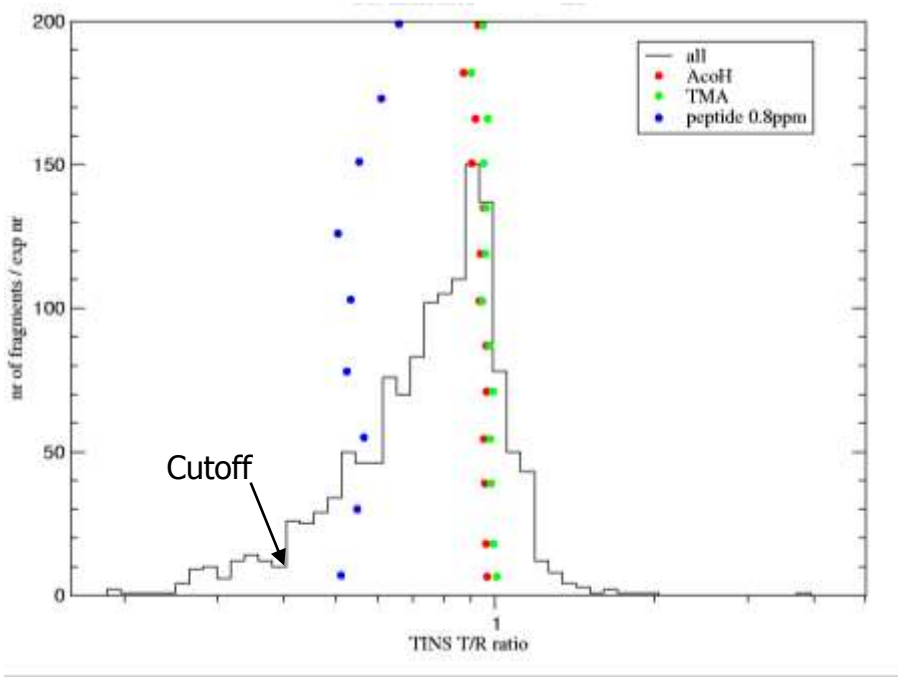
Fragment screening with TINS



Undruggable Target
11 hits



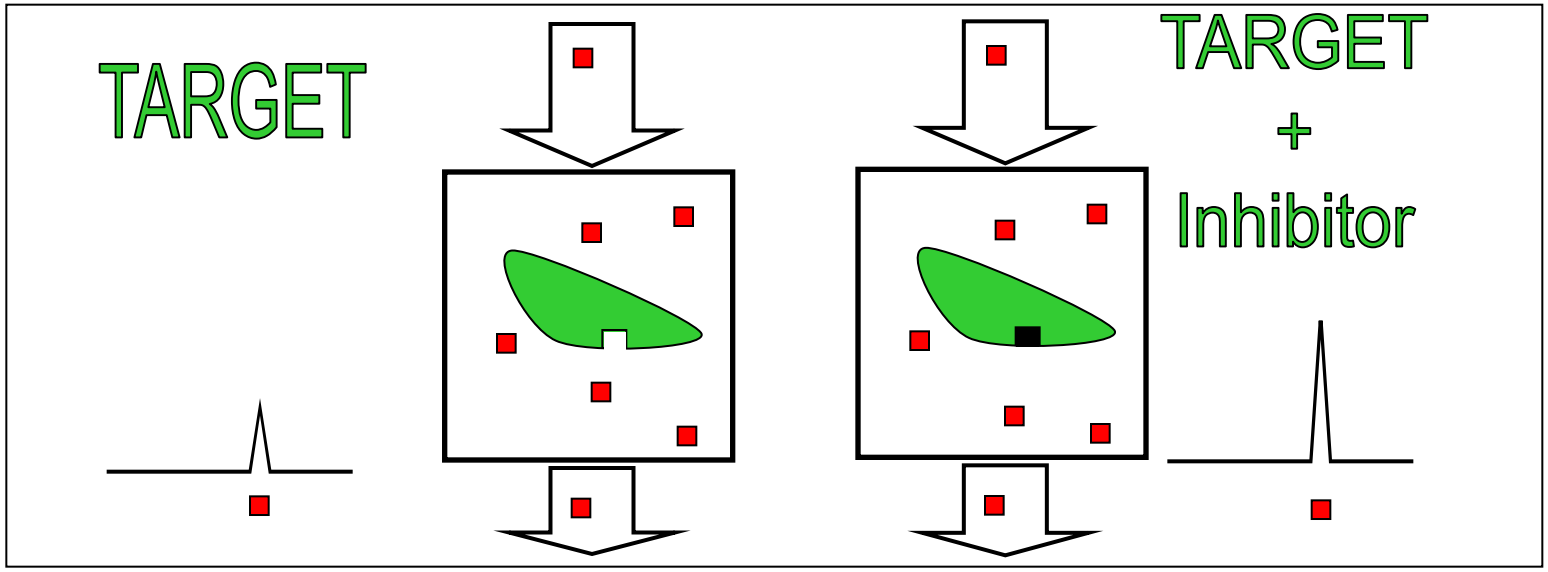
Druggable Target
89 hits

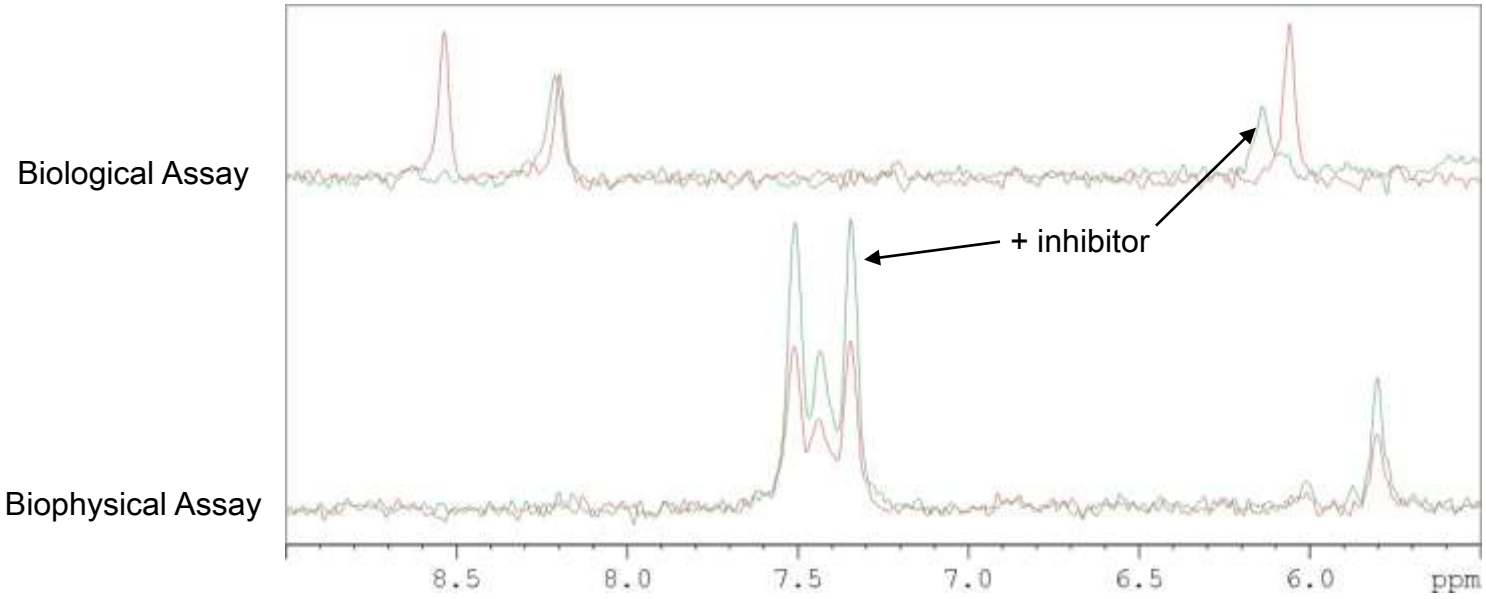
Methods for Hit Validation

- Affinity ranking

- Competition Binding
- SPR
- HSQC Binding Site Determination

Hit Validation: Competition Binding in TINS





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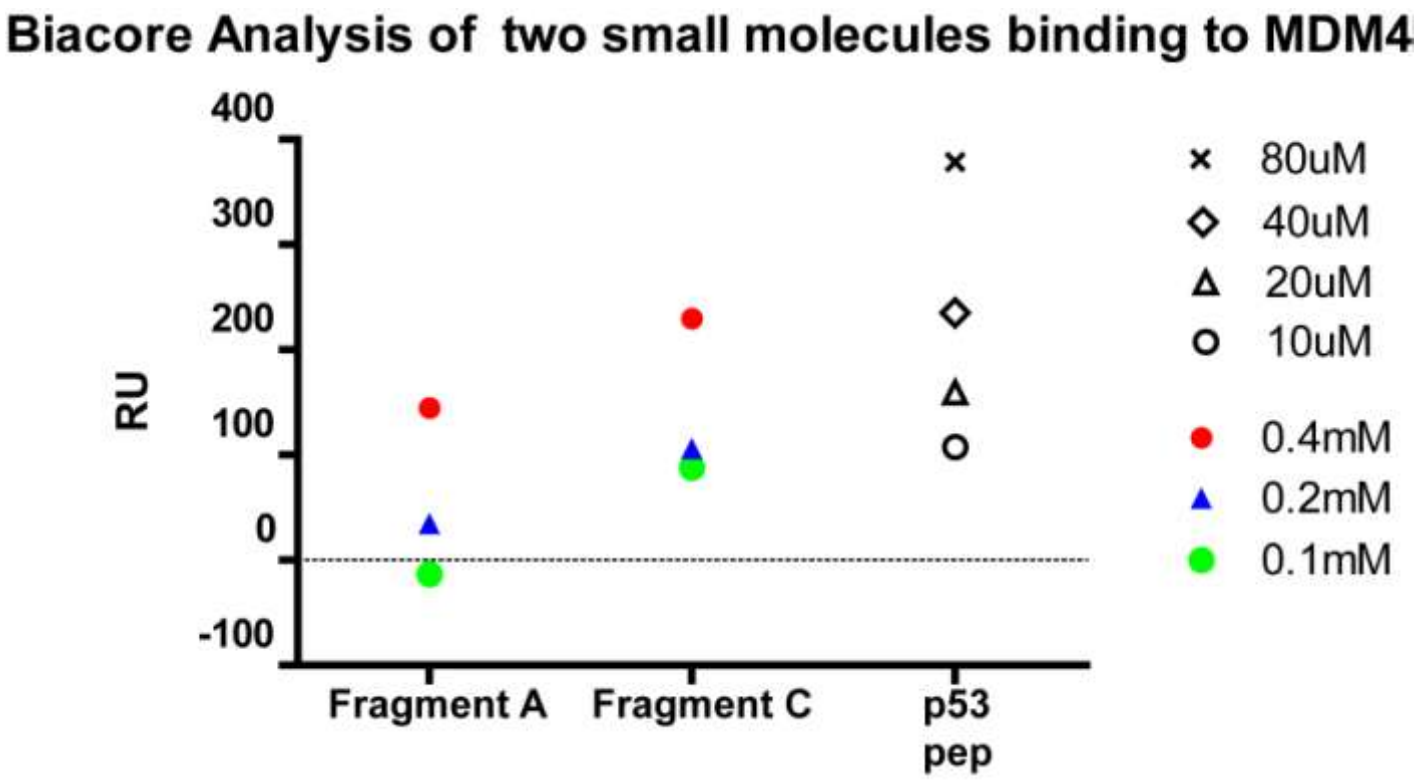
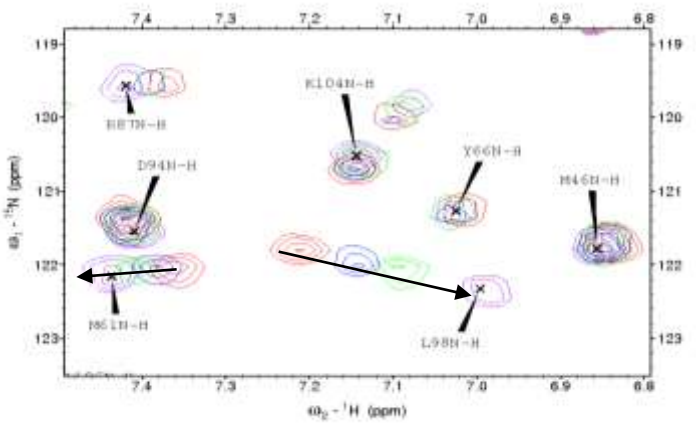
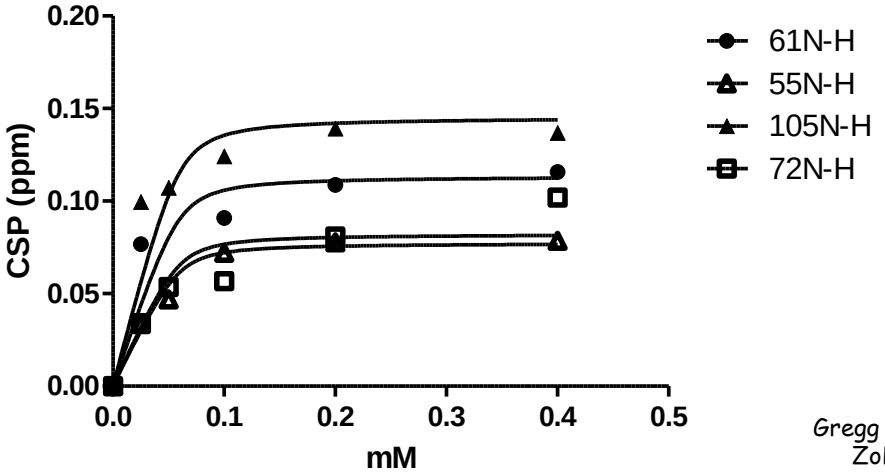
Hit Validation: SPR (Biacore T200)



Hit Validation: K_D and Binding Site Characterization by HSQC

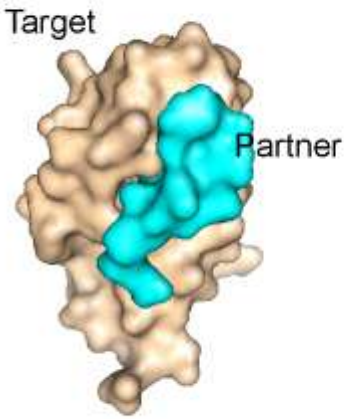
- Chemical shift perturbation for each HN
  – magnitude of resonance shift due to compound binding
- CSP = sqrt [$\Delta H_{ppm}^2 + (\Delta N_{ppm}/6.5)^2$]

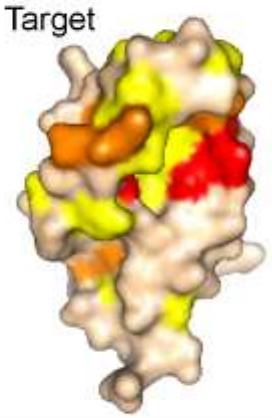




CSP observation on 80 TINS Hits

Low resolution binding site detemination





CSP: ■ > ■ > ■

Hits bind in the vicinity of PPI site

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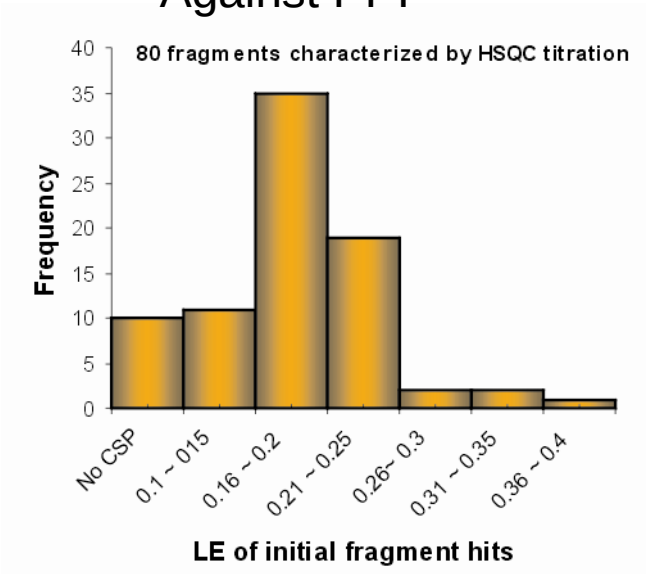
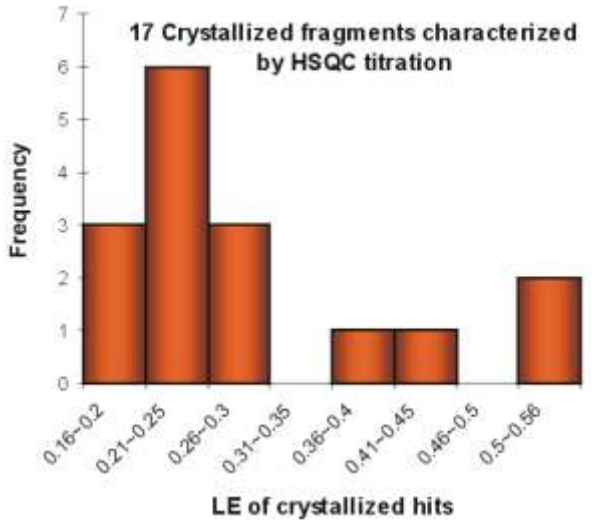
Characteristics of Fragment Hits from TINS

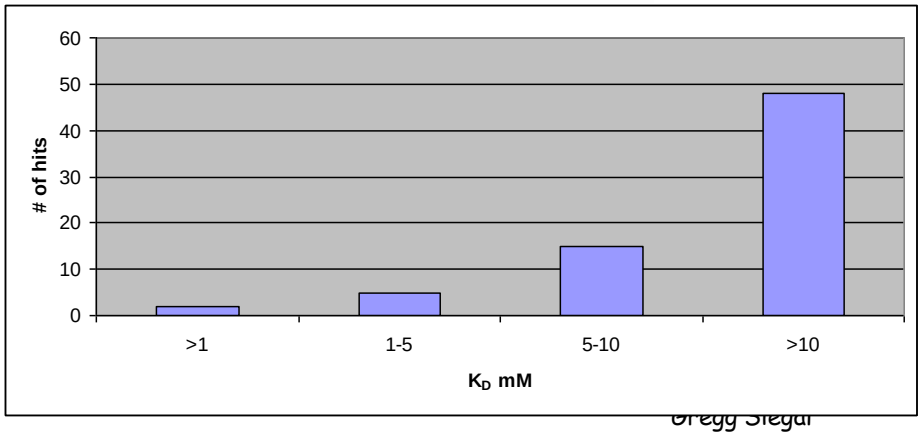
All TINS hits were assessed by [^{15}N,^{1}H]-HSQC titration

Against PPI



Against ATPase (HSP90)*





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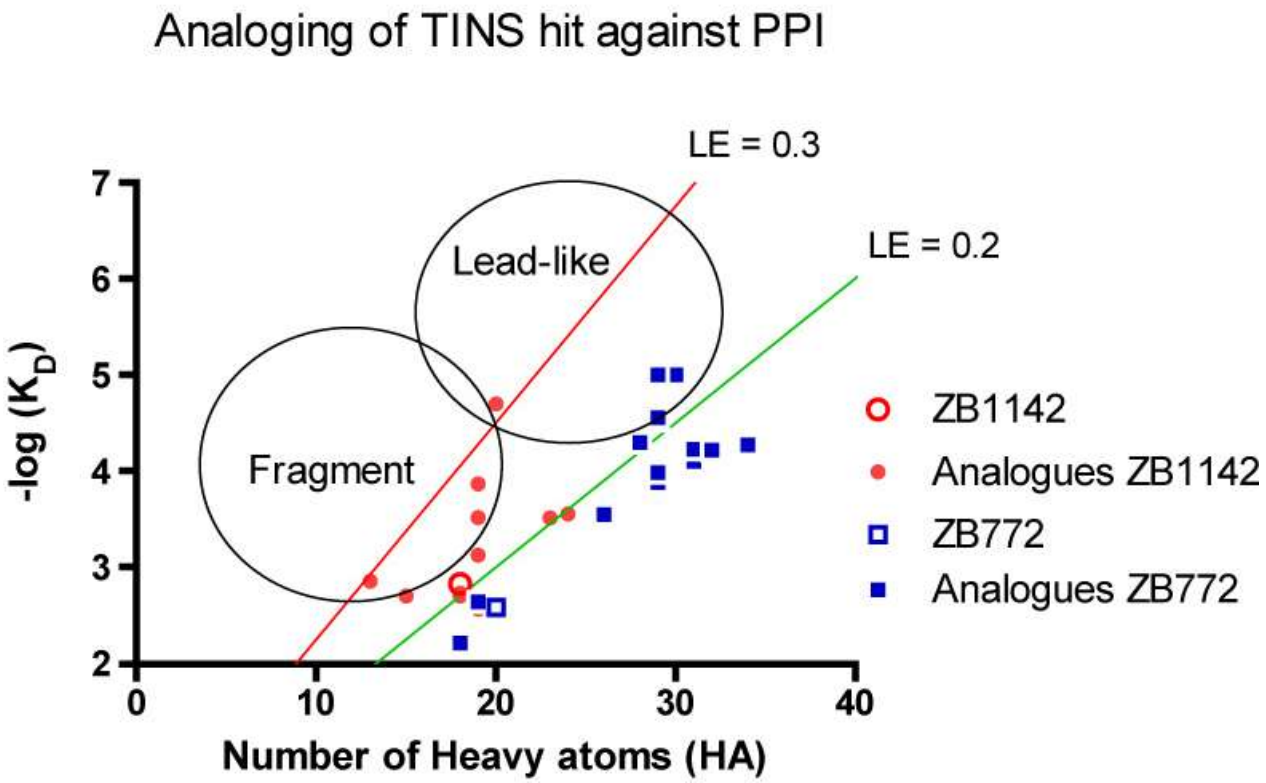
Elaboration of TINS hits guided by K_D from NMR titration

- X- ray crystallography failed to obtain protein – fragment complex
- Binding site and K_D determined by HSQC titration supported ligand-target docking



Good starting point as a Lead-like molecule

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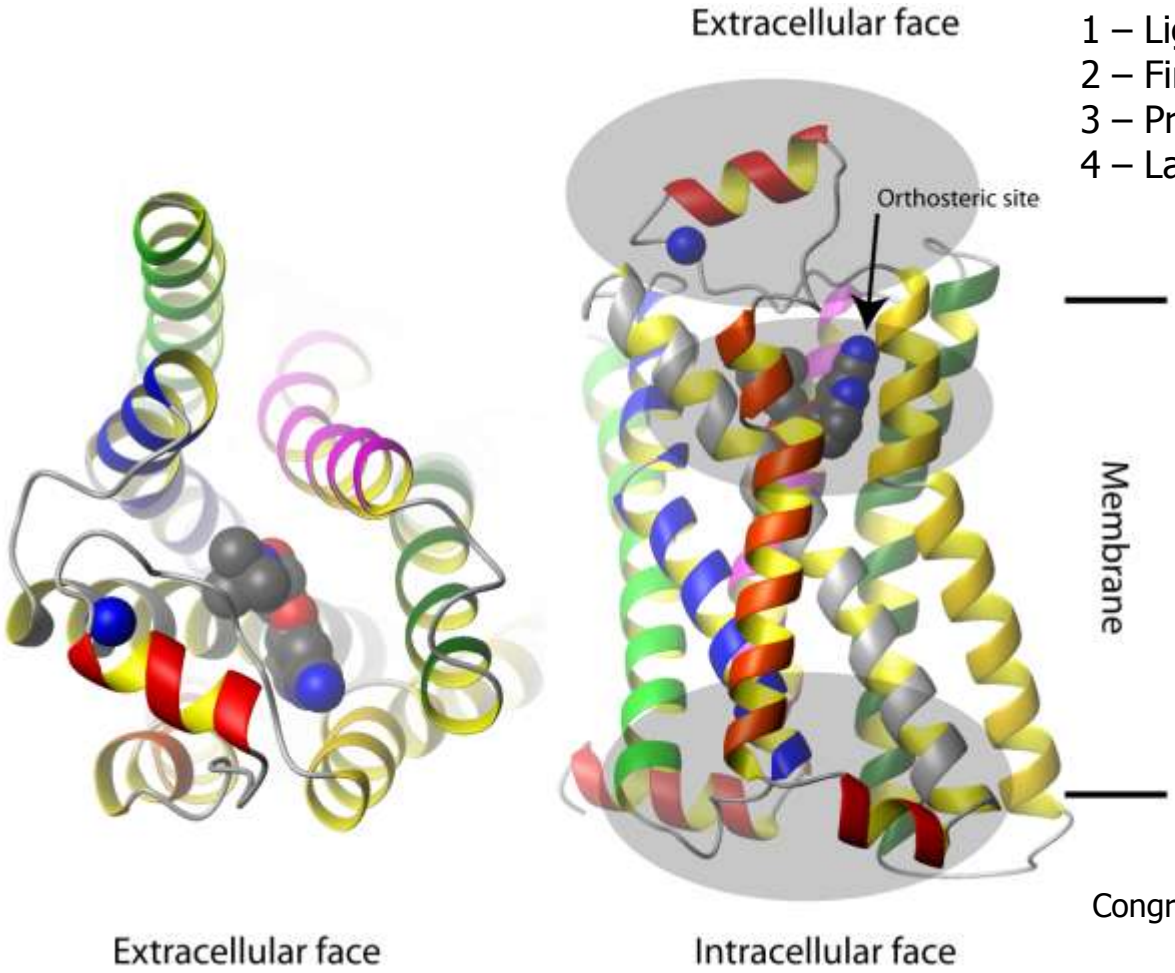
The Challenges of Fragment Discovery on MPs

- Protein production/solubilization/stability
- Non-specific binding
- Slow kinetics
- Fragment size

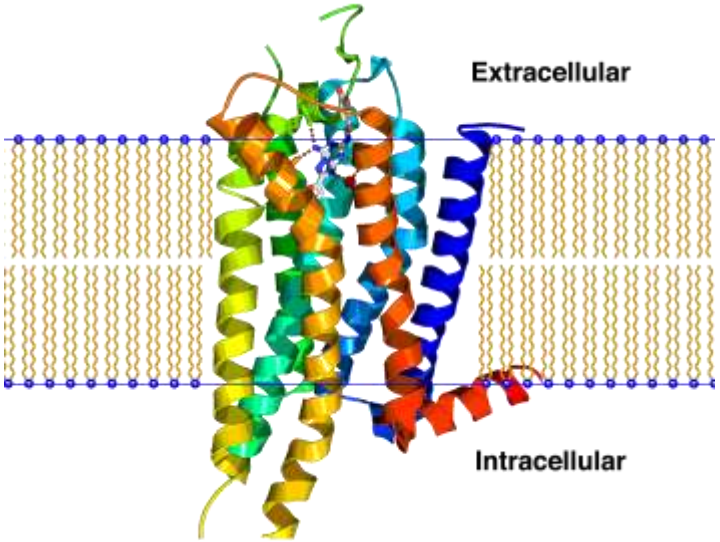
Why biophysical fragment screening for GPCRs?

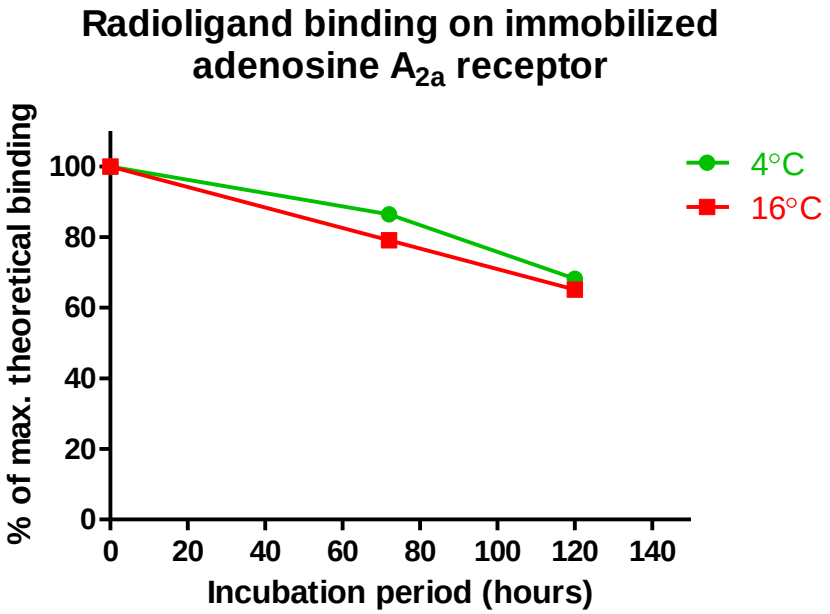


1 – Ligands for peptide activated GPCRs
2 – Find sites with novel biological function
3 – Provide structural information
4 – Label free

Congreve et al., Methods in Enzymology, in press

Fragment Screening of GPCRs: Adenosine 2a & β1 Adrenergic receptors





$A_{2a}R$ agonists – anti-inflammatory therapeutic potential

$A_{2a}R$ antagonists – used to treat Parkinson's disease as $A_{2a}R$ dimerises with dopamine D_2 receptor

Immobilized hA2aR maintained 60% the activity in five days.

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Profile of Ligand Binding in the Screen

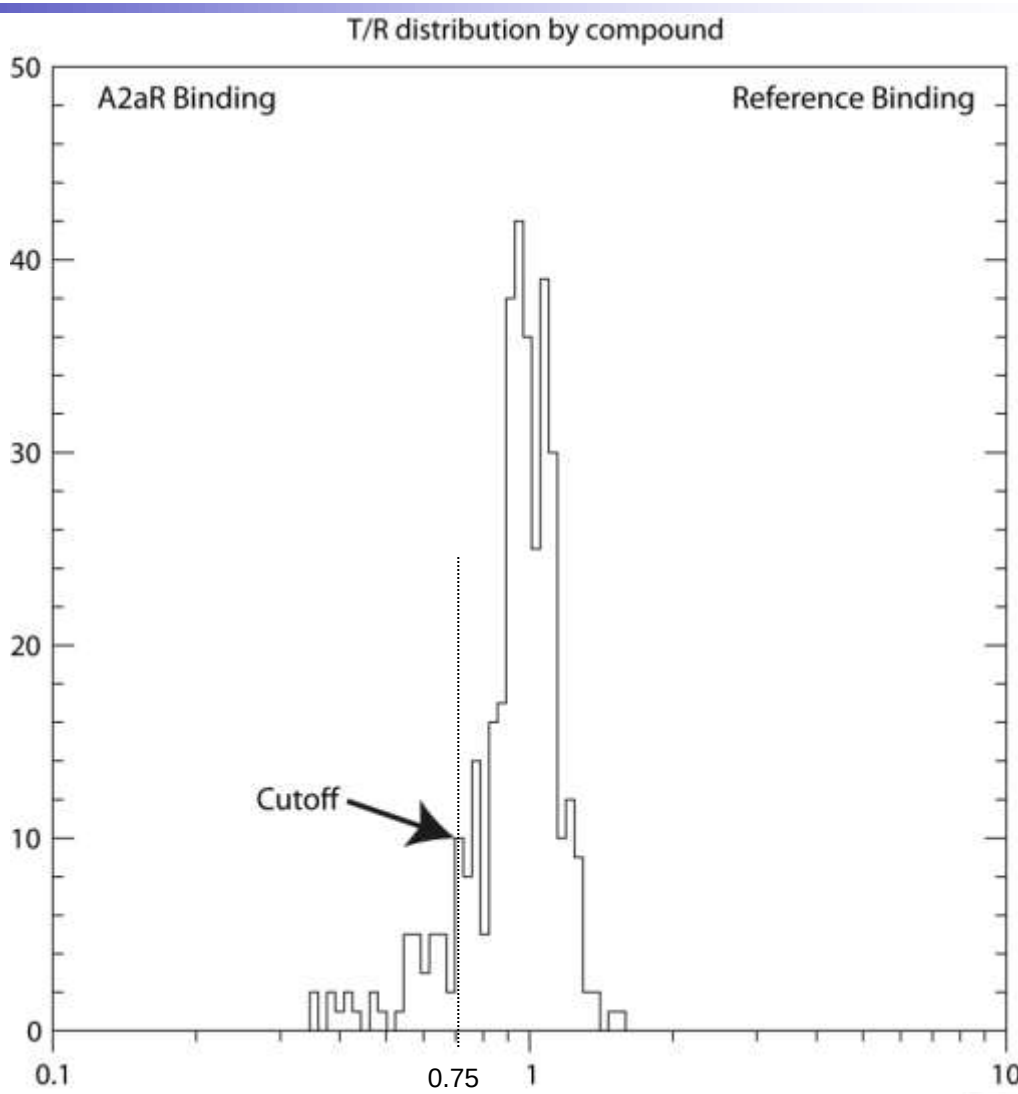
531 fragments assayed against **apo** A2aR

94 hits with very loose selection parameters

β1AR:
579 fragments screened
73 hits with a cut-off at T/R = 0.65
Congreve *et al*, MiE, in press

A2aR:
531 fragments screened
94 hits with a cut-off at T/R = 0.75

Number in common: 20 hits



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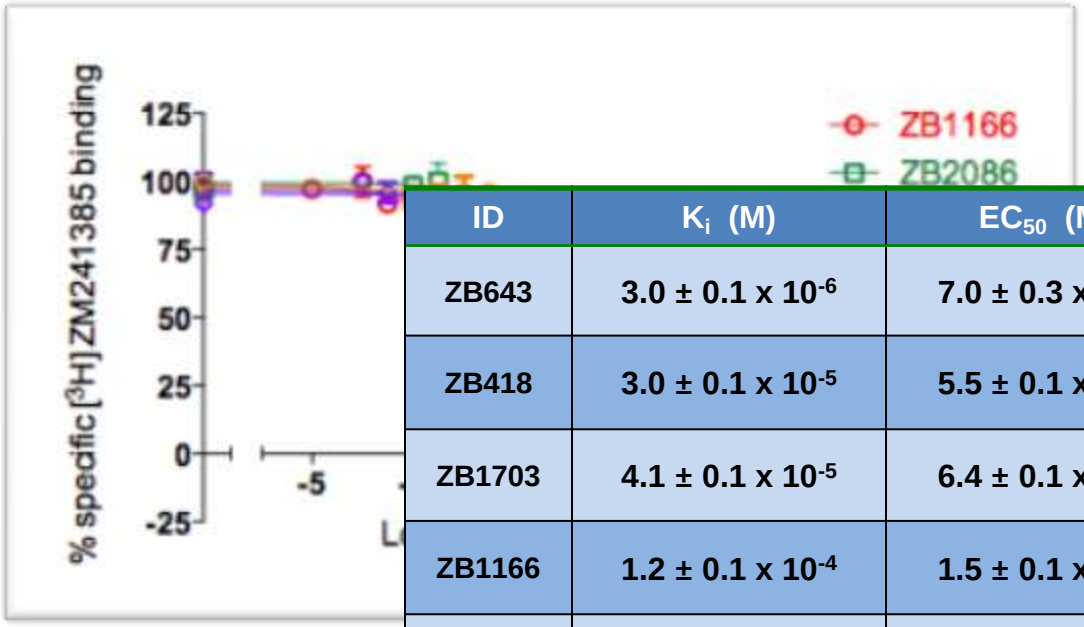
Hit validation by equilibrium radioligand displacement



Each hit assayed a

ID	K_i (M)	EC_{50} (M)	Hill Slope	T/R ratio	BEI
ZB643	$3.0 \pm 0.1 \times 10^{-6}$	$7.0 \pm 0.3 \times 10^{-6}$	-0.5 ± 0.1	0.65	26.3
ZB418	$3.0 \pm 0.1 \times 10^{-5}$	$5.5 \pm 0.1 \times 10^{-4}$	-2.0 ± 0.5	0.44	17.6
ZB1703	$4.1 \pm 0.1 \times 10^{-5}$	$6.4 \pm 0.1 \times 10^{-4}$	-1.1 ± 0.4	0.60	12.5
ZB1166	$1.2 \pm 0.1 \times 10^{-4}$	$1.5 \pm 0.1 \times 10^{-3}$	-0.9 ± 0.1	0.61	11.6
ZB2086	$1.2 \pm 0.2 \times 10^{-4}$	$2.4 \pm 0.3 \times 10^{-3}$	-0.9 ± 0.3	0.74	16.3
ZB114	$8.2 \pm 0.3 \times 10^{-5}$	$1.2 \pm 0.1 \times 10^{-3}$	-2.2 ± 5.4	0.59	13.5
ZB1605	$8.7 \pm 0.3 \times 10^{-5}$	$1.4 \pm 0.3 \times 10^{-3}$	-1.1 ± 0.5	0.37	12.8
ZB1967	$3.2 \pm 0.2 \times 10^{-4}$	$6.1 \pm 0.1 \times 10^{-3}$	-0.8 ± 1.3	0.61	10.8

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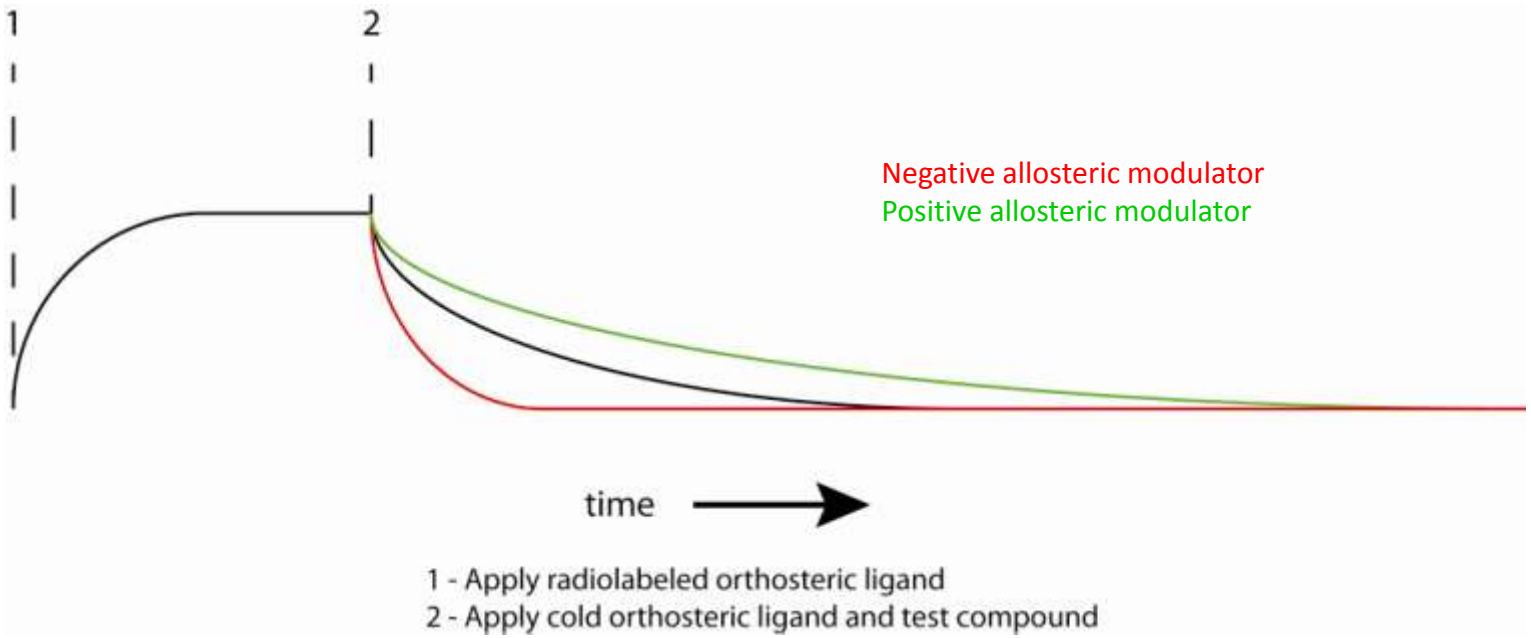
Mode of action: Orthosteric vs Allosteric modulators



Screen all hits at t = 50% ZM bound.

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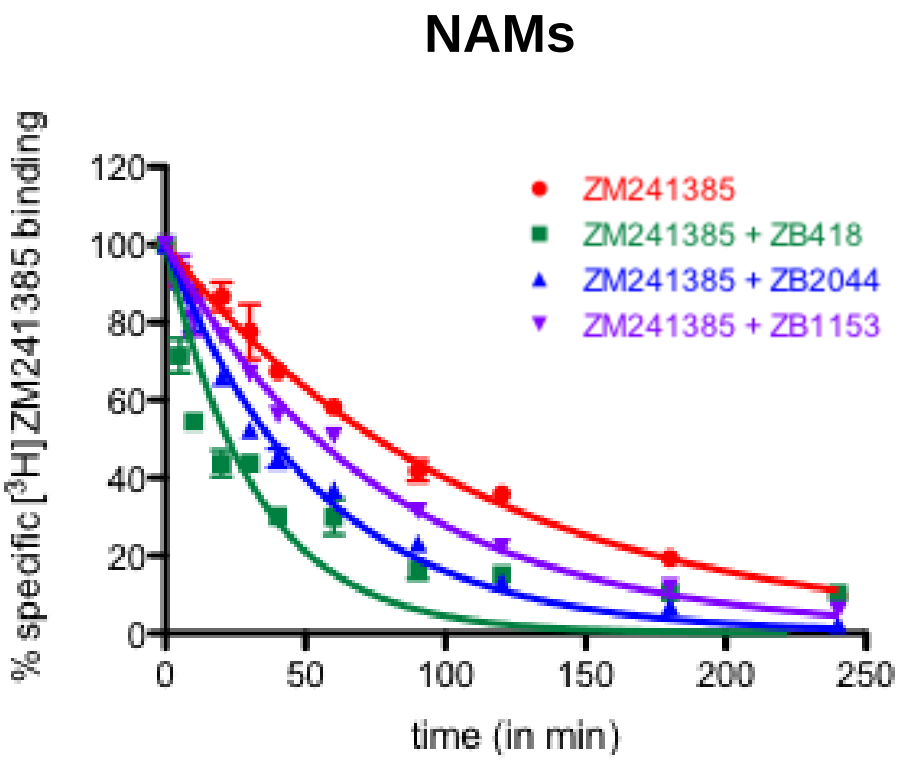
TINS hits as negative allosteric modulators (NAMs) of A2aR

Fragment	k_{off} of [^{3}H]ZM241385 (min^{-1}) [a]
Control ZM241385	0.011 + 0.002
+ 2.5mM ZB418	0.040 0.007
+ 2.5mM ZB2044	0.023 0.006
+ 2.5mM ZB1153	0.016 0.005



[a] k_{off} ± SEM (n=3).

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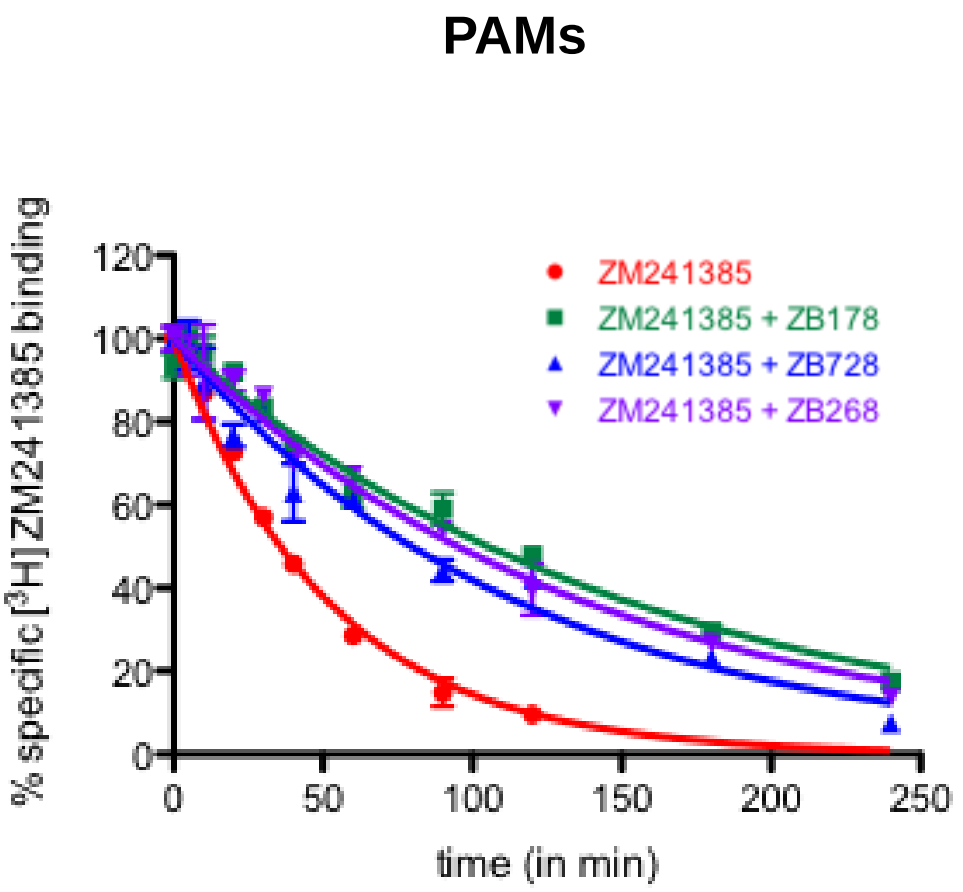
TINS hits as positive allosteric modulators (PAMs) of A2aR

Fragment	k_{off} of [^{3}H]ZM241385 (min^{-1}) [a]
Control ZM241385	0.0109 + 0.0018
+ 2.5mM ZB178	0.0057 0.0011
+ 2.5mM ZB728	0.0069 0.0018
+ 2.5mM ZB268	0.0066 0.0007



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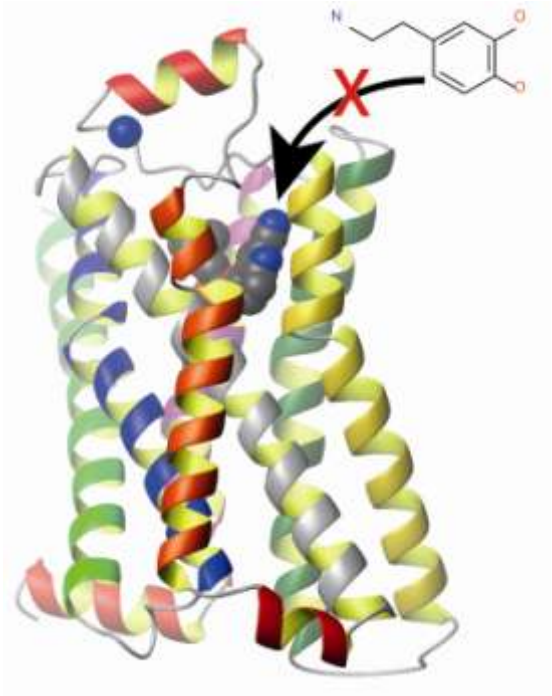
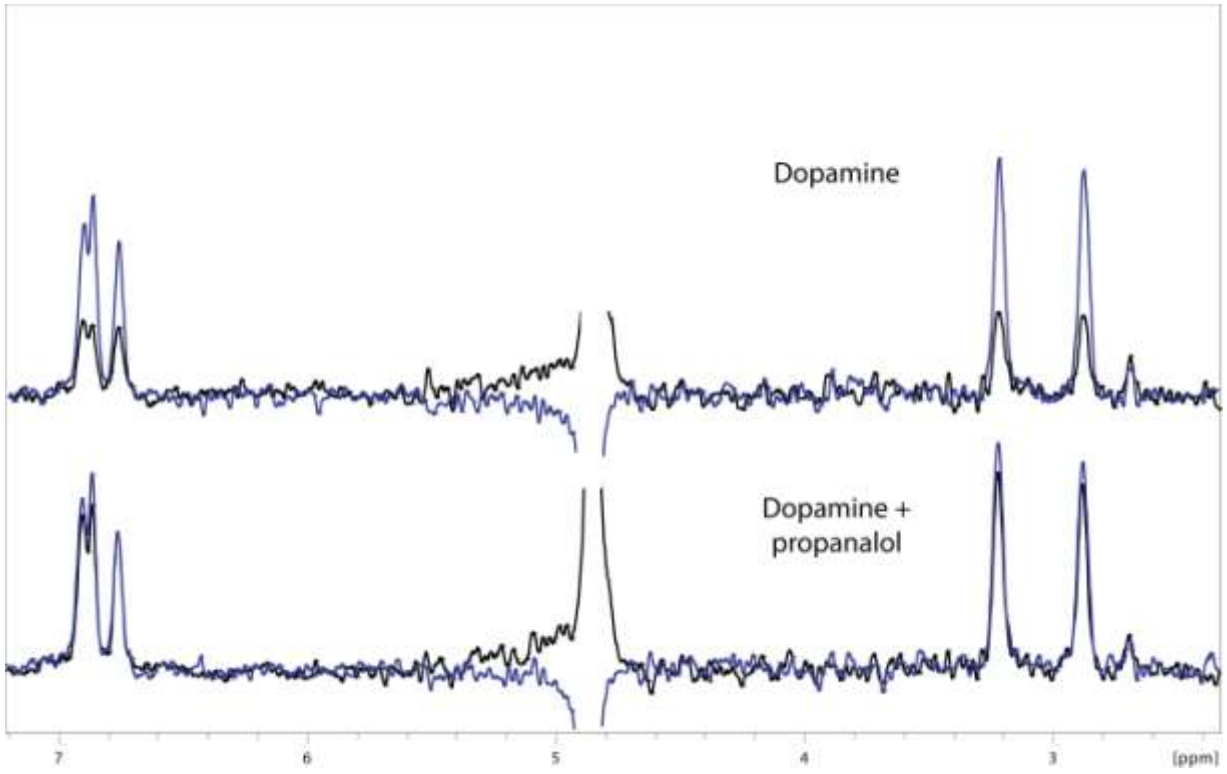
Structural information from TINS?



β1AR



Publications

- Comparison of fragment screening methods – Kobayashi et al., J. Biomol. Screen, 2010, **15**, p.978
- Fragment screening of memrbane proteins in detergents and nanodiscs – Früh et al., 2010, Chem & Biol., **17**, p.881

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