Fragment-based screening for inhibitors of PDE4A using enthalpy arrays and X-ray crystallography

Michael Recht

October 12, 2010

ZENOBIA THERAPEUTICS

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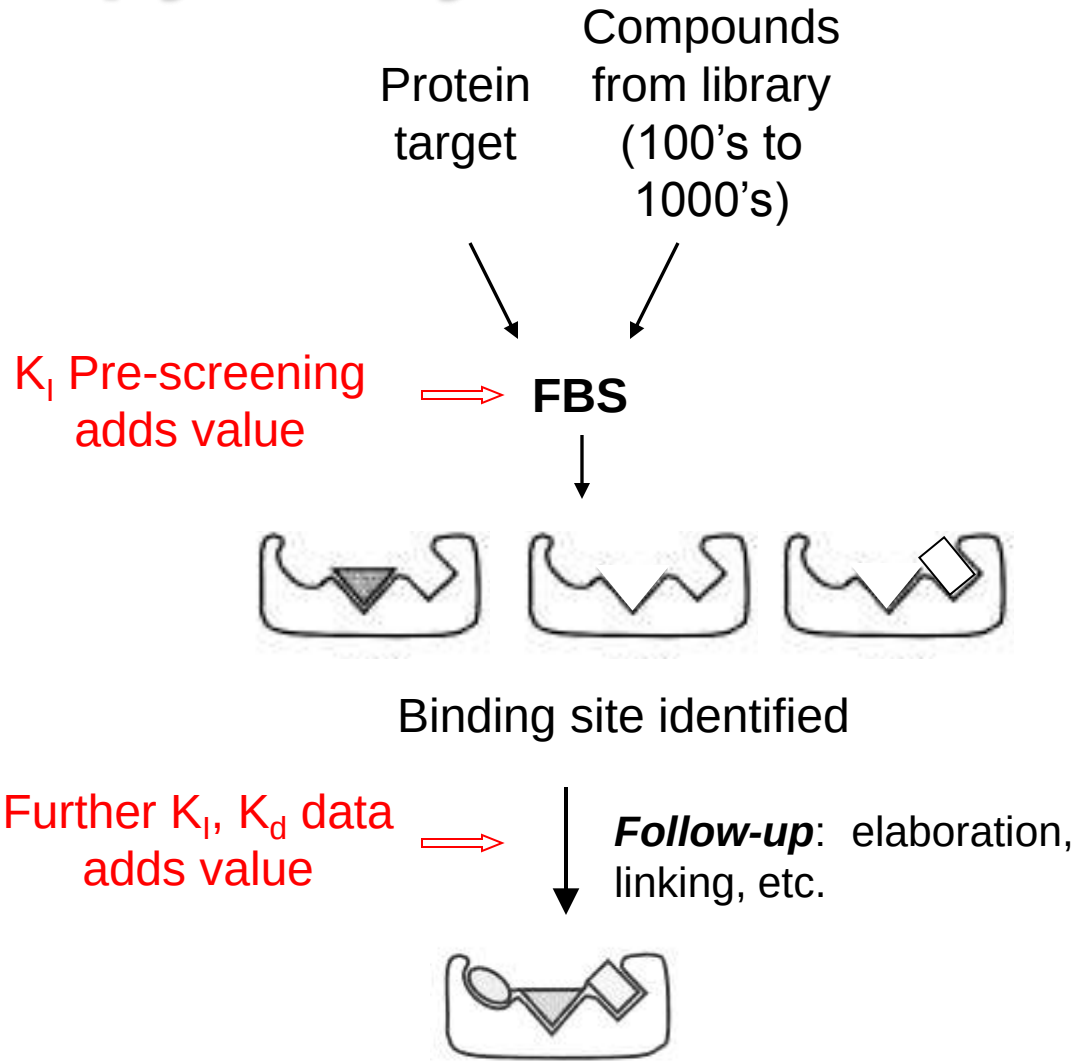
Activity pre-screening using Enthalpy Arrays



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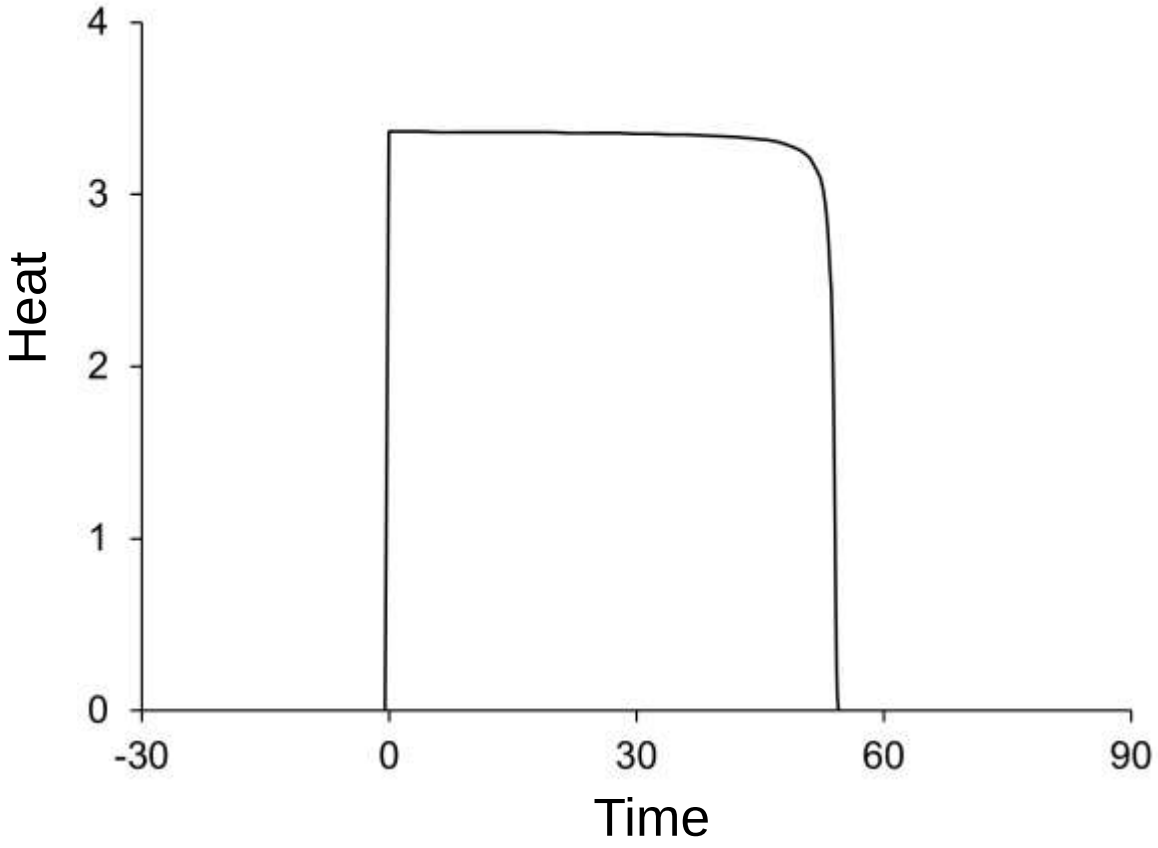
K_I from enthalpy

Measure heat as a function of time



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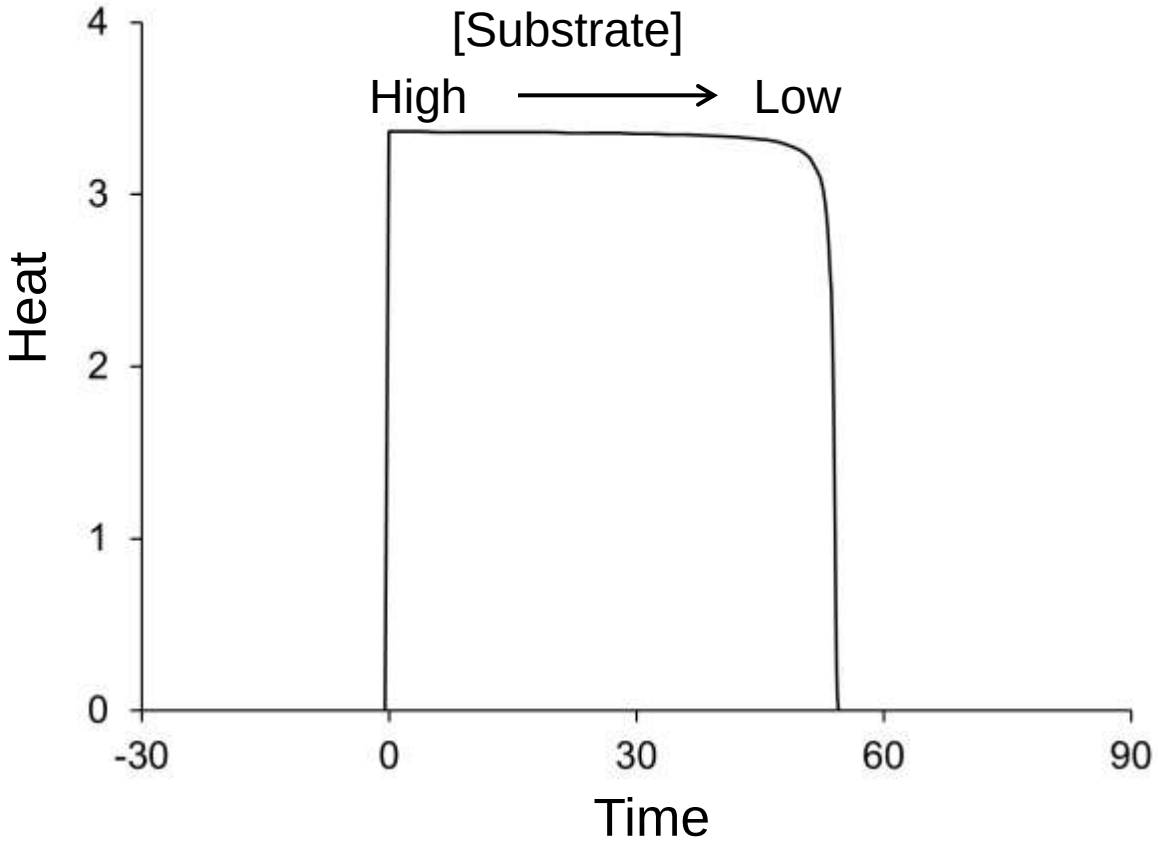
K_I from enthalpy

Measure heat evolution from a single reaction



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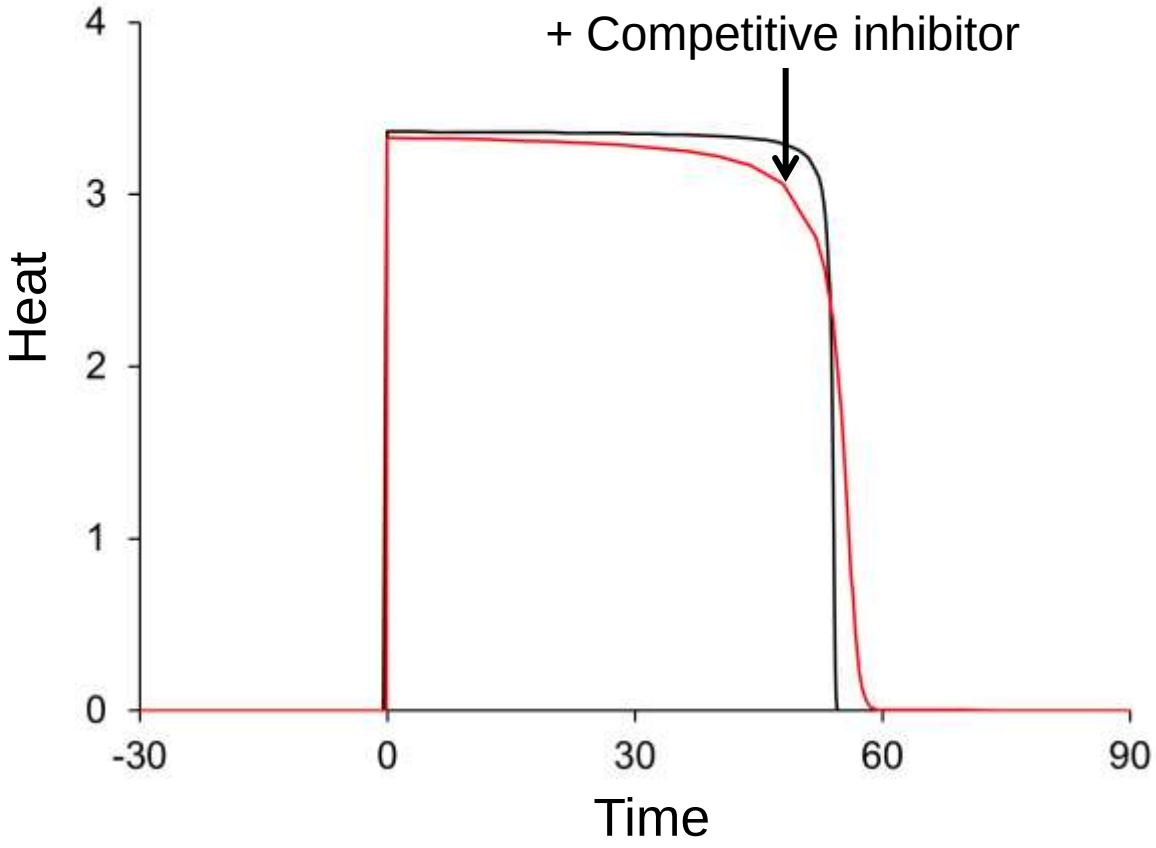
K_I from enthalpy

Rate at which heat evolves is function of K_I



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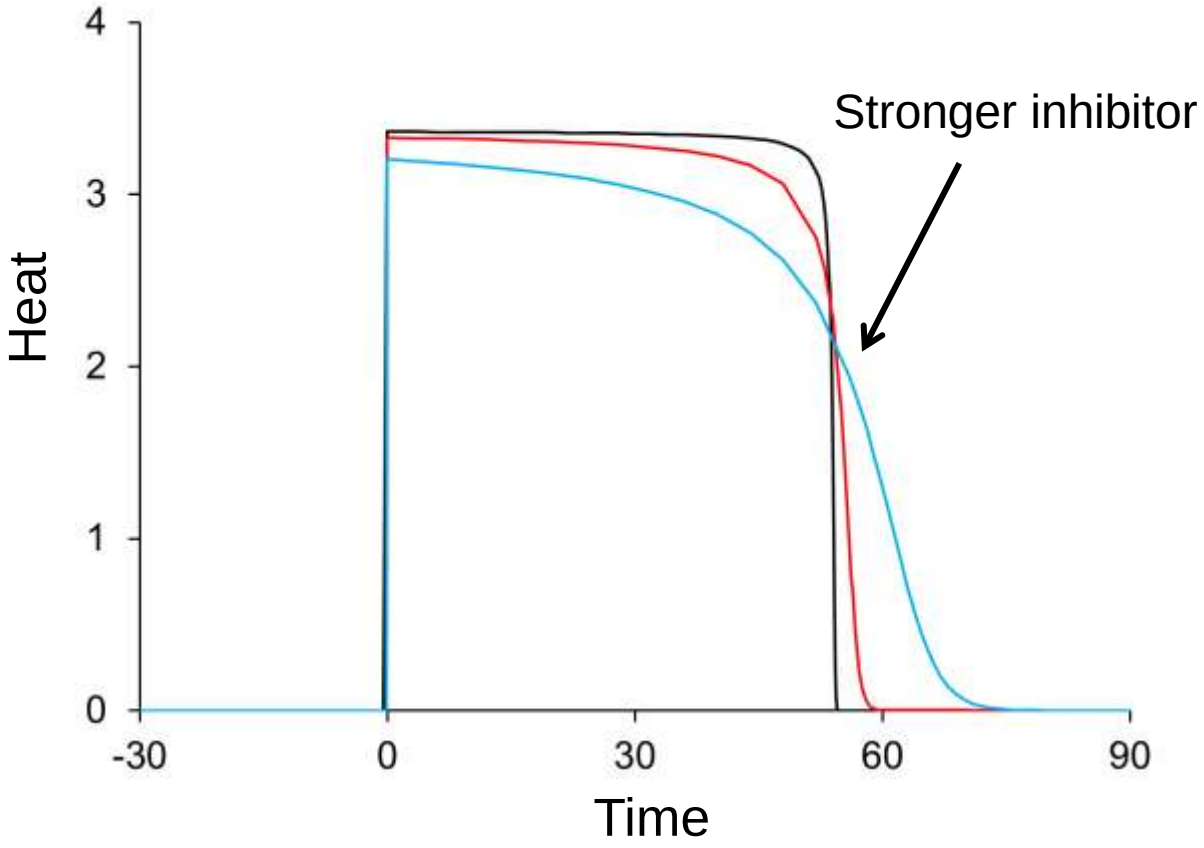
K_I from enthalpy

Rate at which heat evolves is function of K_I



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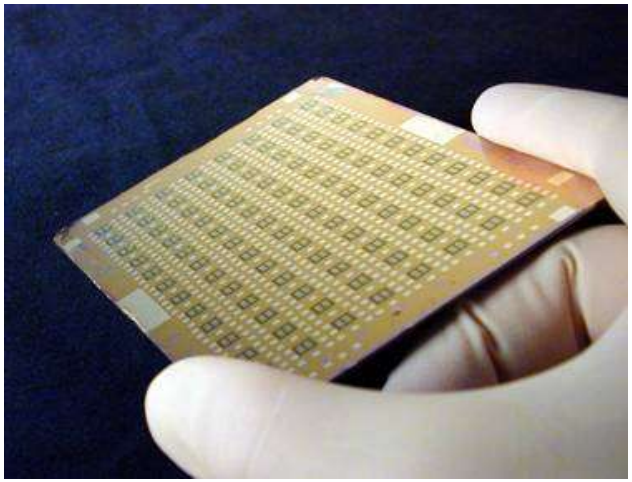
Enthalpy Arrays

- Miniaturized calorimetry
  - 250 nl drops, ~25 pmole reagent
  - 72 detector array



- Technology challenges
  - Detectors with <20 µK thermal noise
  - Minimizing environmental effects
  - Rapid mixing

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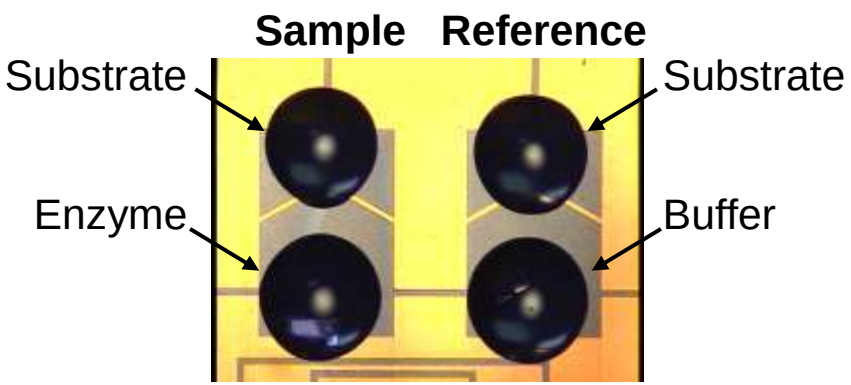
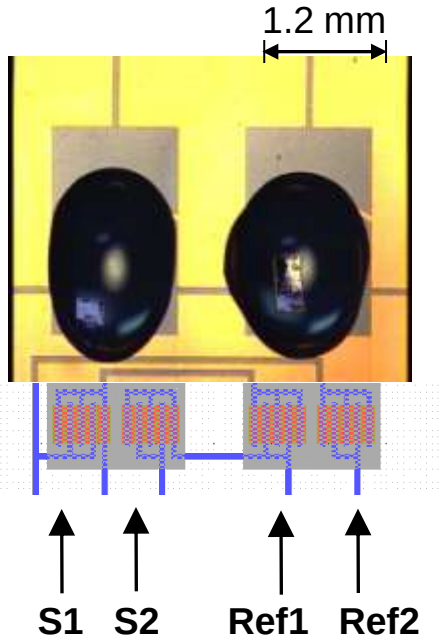
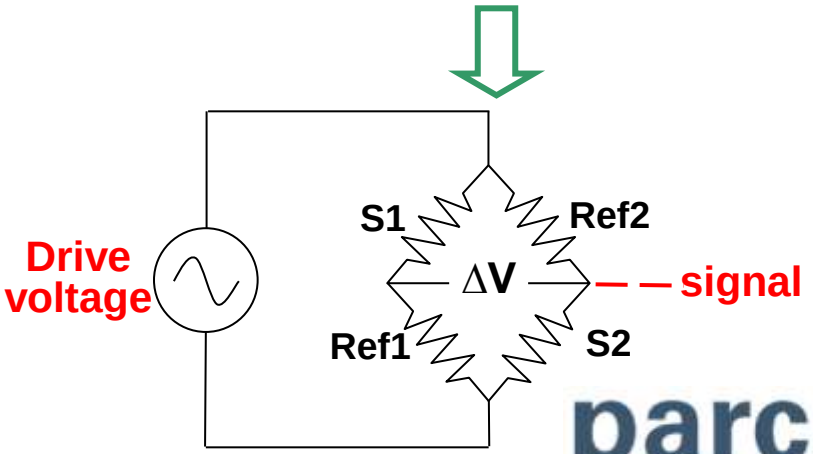
Reducing Environmental Effects



Merging



- Common Mode
  - Background drift
  - Thermal dissipation (evaporation and conduction)
- Bridge circuit and reference



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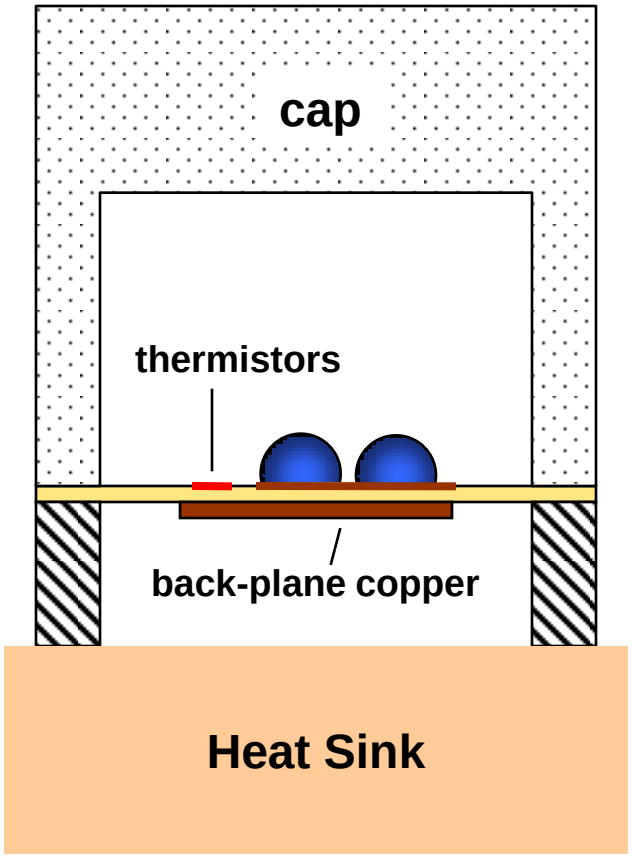
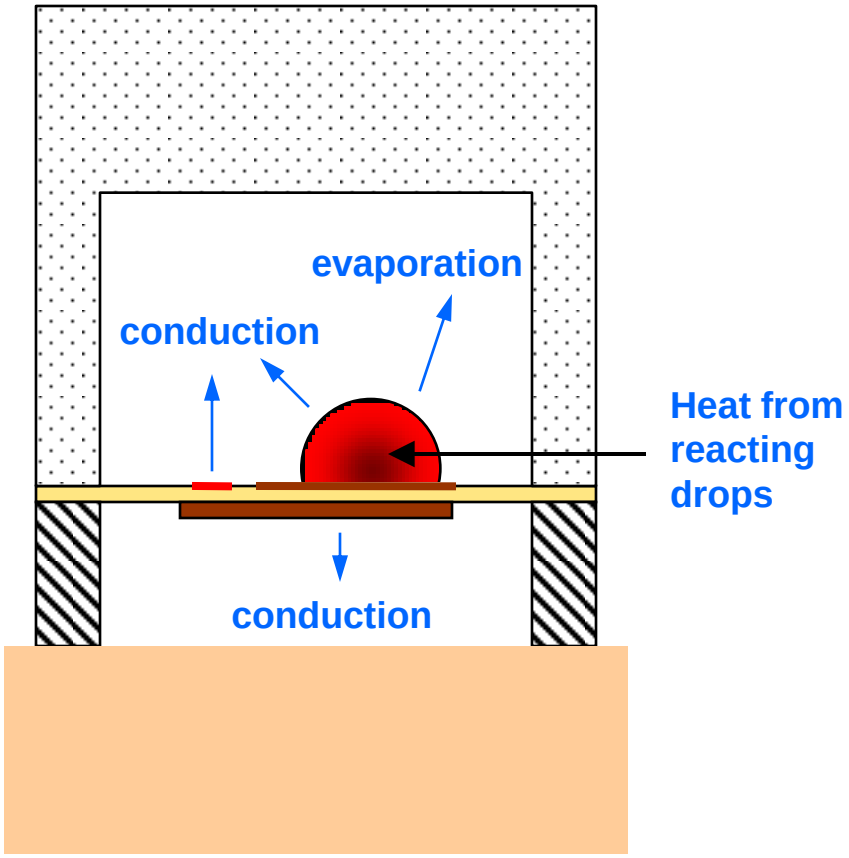
Heat Transport during a Measurement

Initial State



Reaction + Thermal Dissipation



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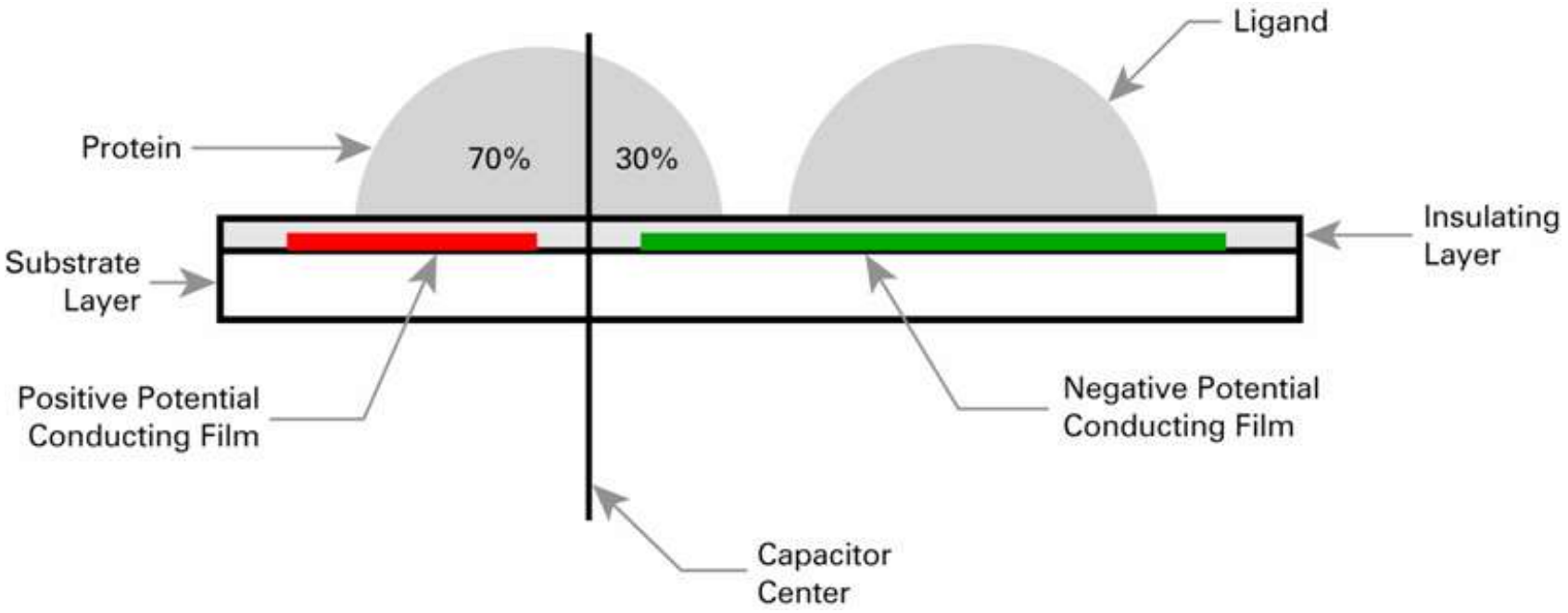
Technology challenges

Isothermal reaction initiation

- Electrostatics



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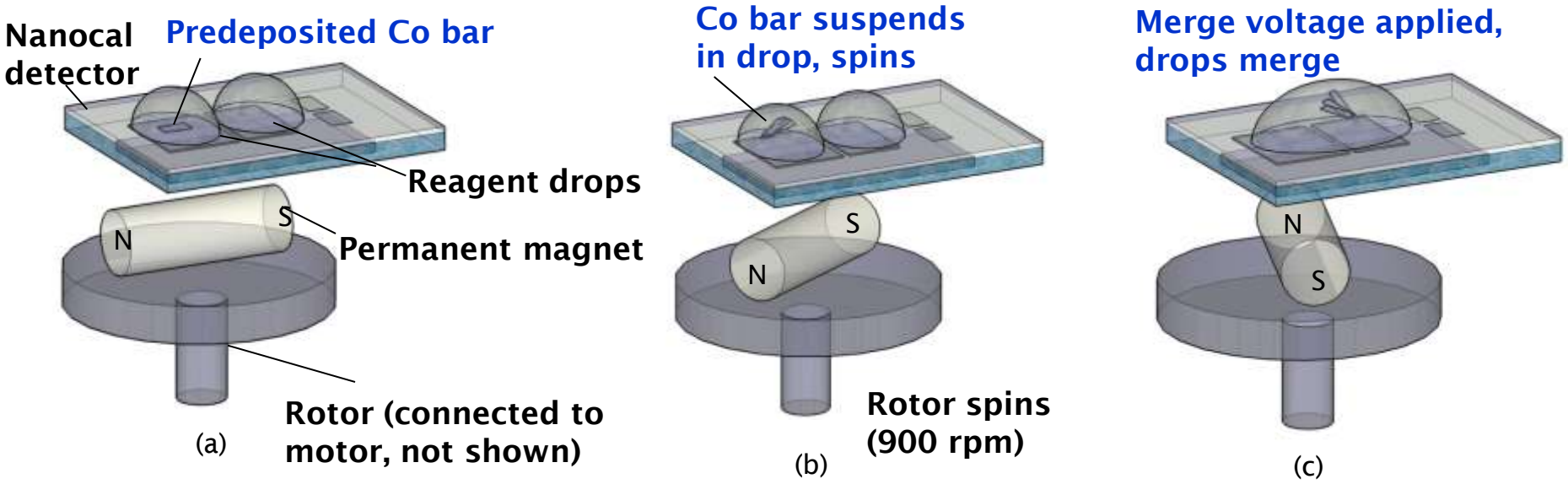
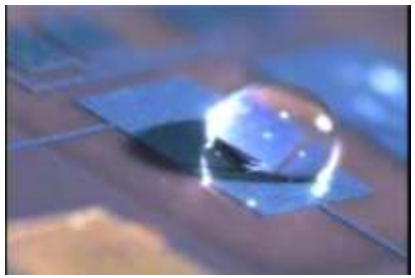
Magnetic mixing improves sensitivity





- Cobalt stir bar
- Fast mixing confirmed by FRET experiments, $BaCl_2$/18-crown-6 and enzyme reaction data
- Bars coated with SiON and PEGylated

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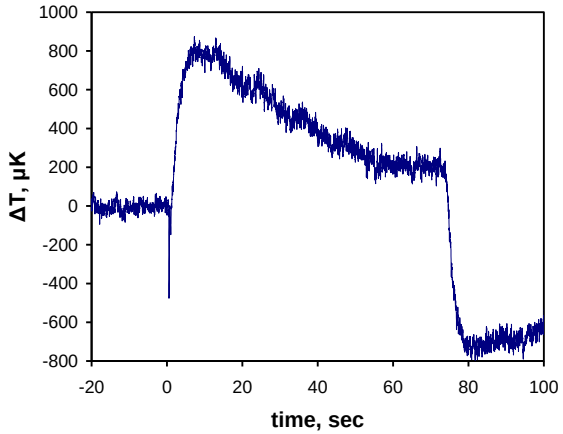
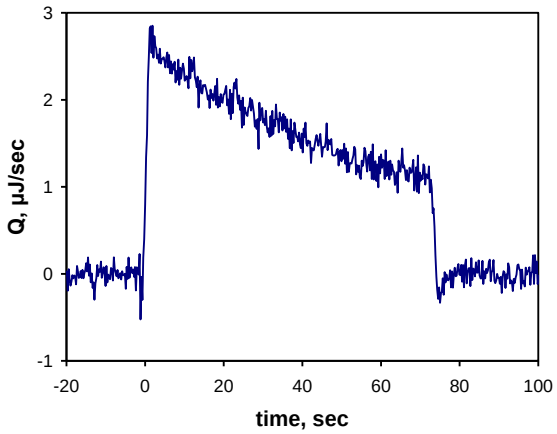
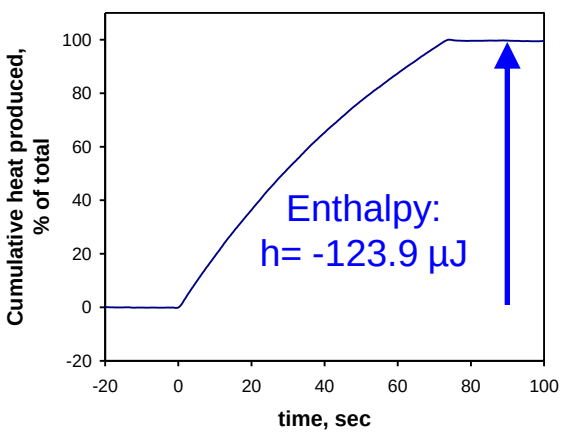
Technology challenges

Determining enthalpy and kinetic parameters from temperature data



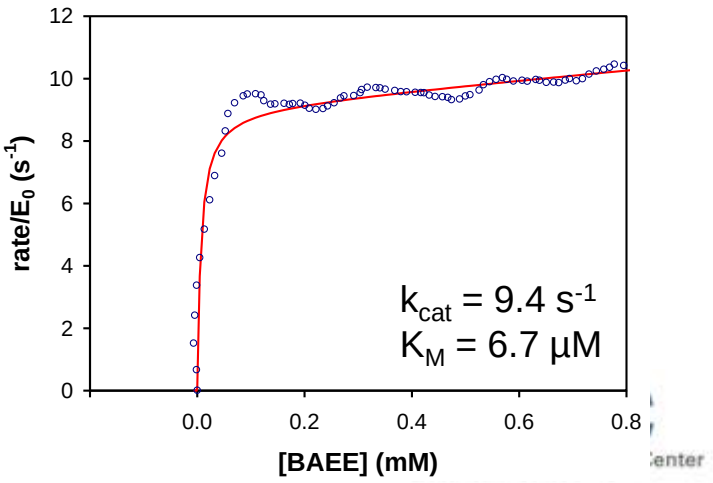




Differential temperature → Deconvolute → **Power** → Integrate → **Cumulative heat**

$$\Delta T(t) = \int_{merge}^{t} Q(\tau)\,\mathcal{J}(t-\tau)\,d\tau.$$

- Regression analysis yields enzyme kinetic parameters

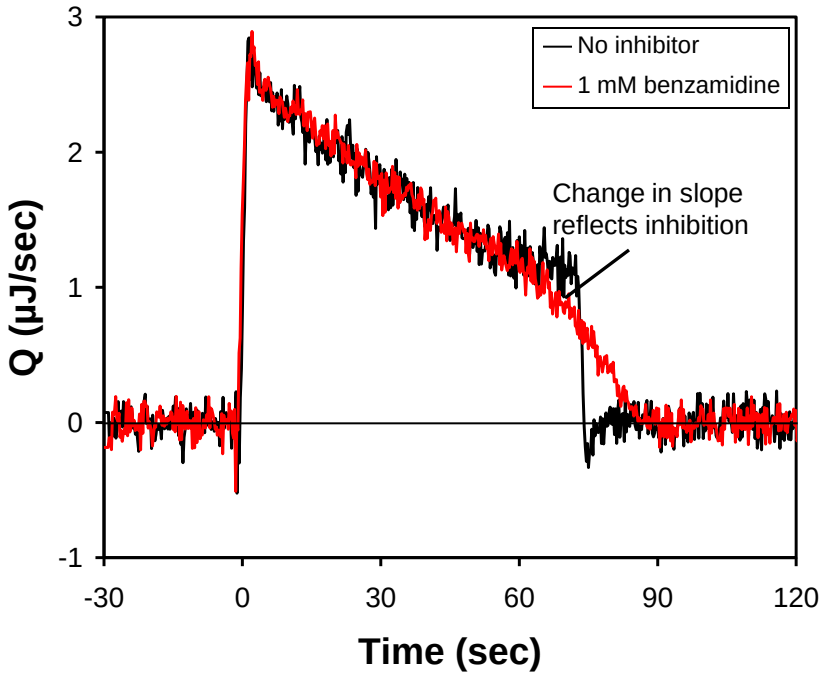


Measurements

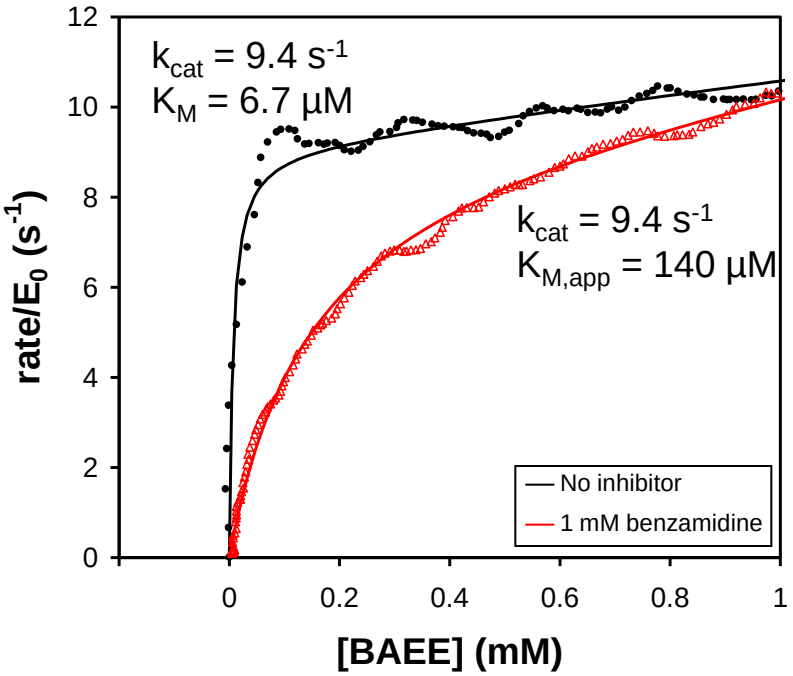
Enzyme kinetic parameters from individual samples

Trypsin hydrolysis of BAEE

Power data



Kinetics data



5 µM trypsin, 5 mM BAEE

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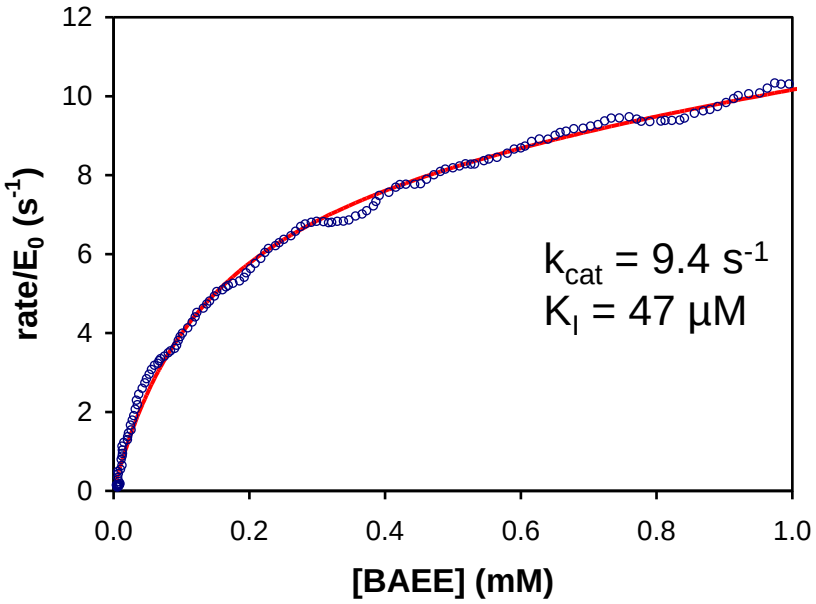
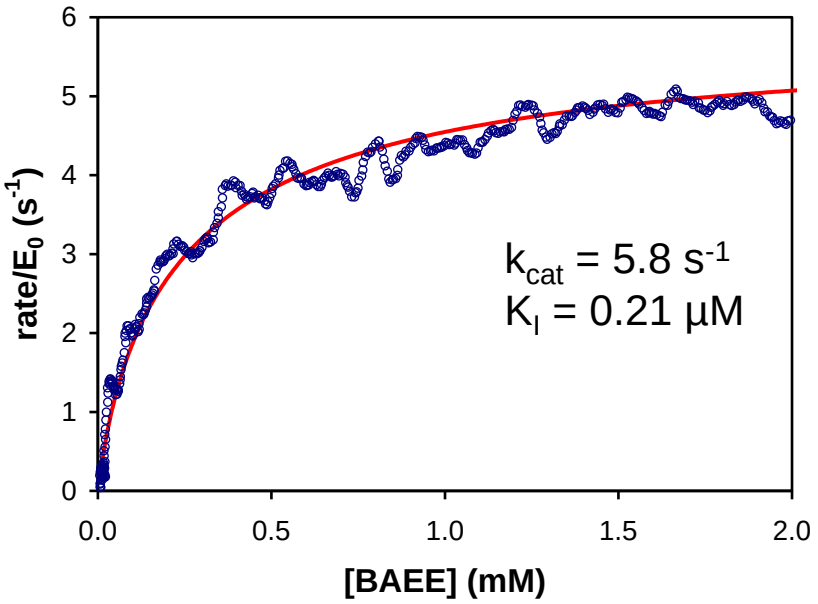
Measurements

K_I for competitive inhibitors

Trypsin + 1 mM benzamidine



Trypsin + 10 µM leupeptin



Inhibitor	K_I, µM measured	K_I, µM literature	k_{cat}, sec^{-1} measured	k_{cat}, sec^{-1} literature
benzamidine	43	18	8.3	15-22
leupeptin	0.13	0.13	6	-

Notes:
1. Determination of K_I assumes competitive inhibition and K_M=6.4 µM.
2. Calculation of k_{cat} assumes the enzyme concentration is 5 µM.

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Measurements

Fragment screening

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PDE4A

- One of four PDE4 enzyme family members (PDE4A-D)
- PDE4 inhibitors
  - Anti-inflammatory therapeutics
    - » Asthma, COPD
- cAMP-specific phosphodiesterase
- Expressed in many cell types, tissues
- Several splice variants produced

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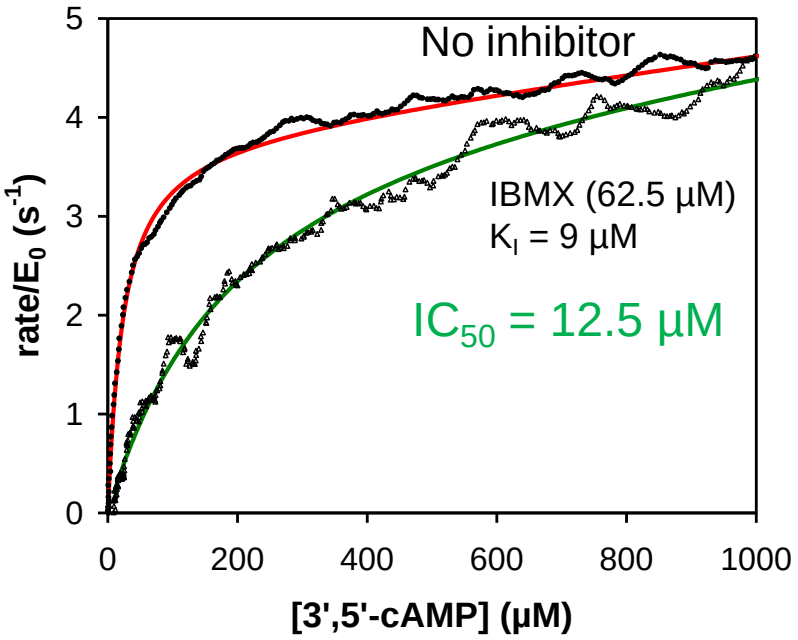
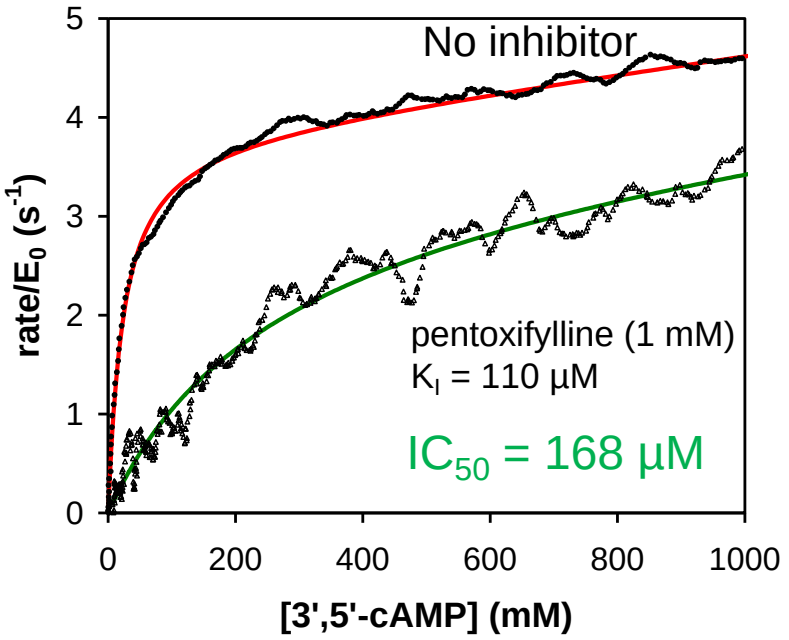
PDE4A known inhibitors

Good agreement with values in literature

IBMX



pentoxifylline



100 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 1 mM TCEP

1. Owens et al, Biochem. J. 1997, 2. Rao et al, Chem. Biol. 2005

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PDE4A activity based fragment screen

- Catalytic domain of PDE4A10
- 160-compound library
  - Average MW = 154 Da, # Heavy atoms = 10.4
- Obtained k_{cat} and K_M for every reaction in presence of each compound
- Competitive inhibitors produce an apparent increase in K_M
- Control reactions (no inhibitor) performed for every 5 compounds tested

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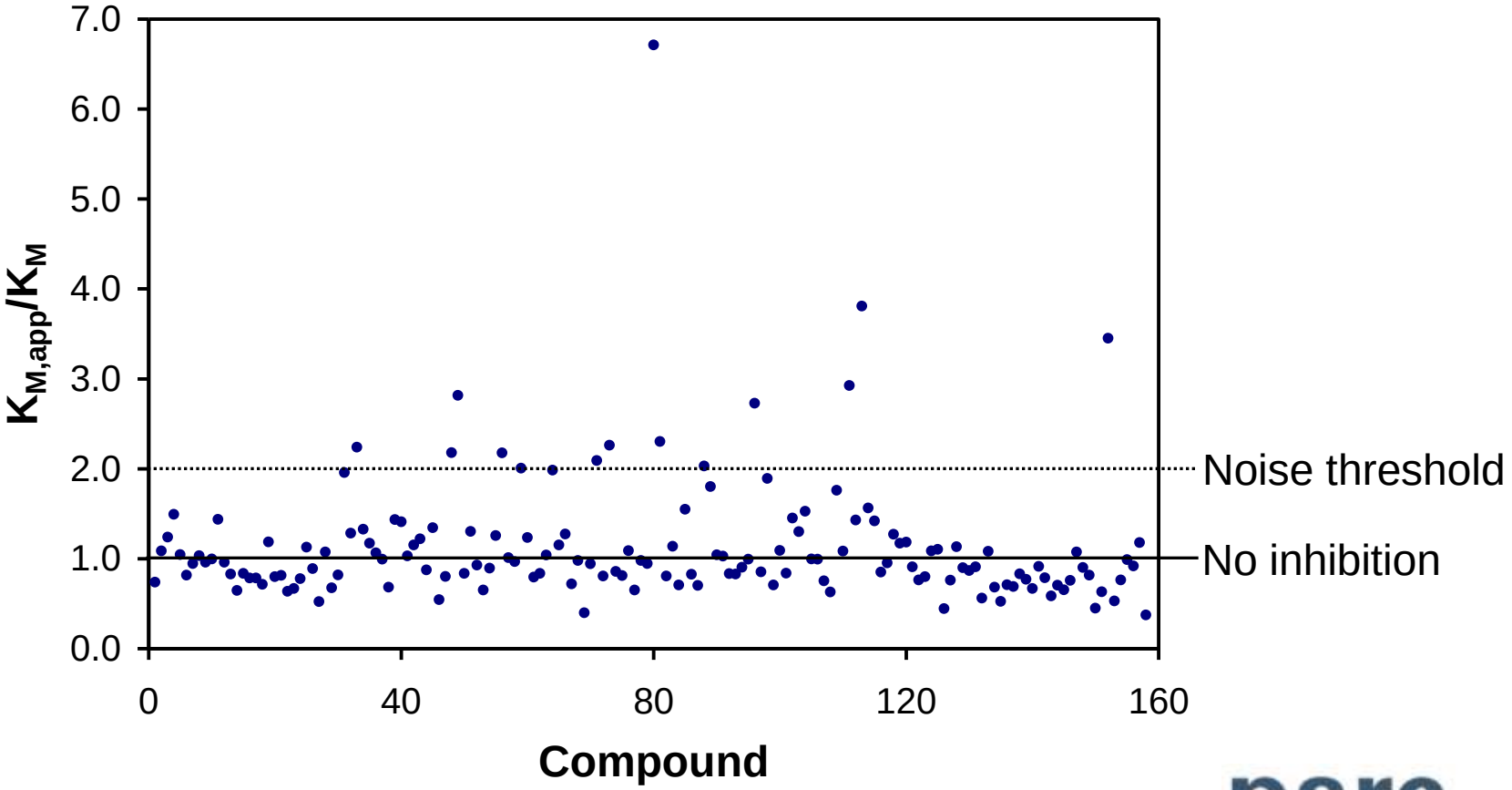
PDE4A activity based fragment screen

Compounds tested at 2 mM

Hit defined as $K_{M,app} \geq 2X\ K_{M,control}$



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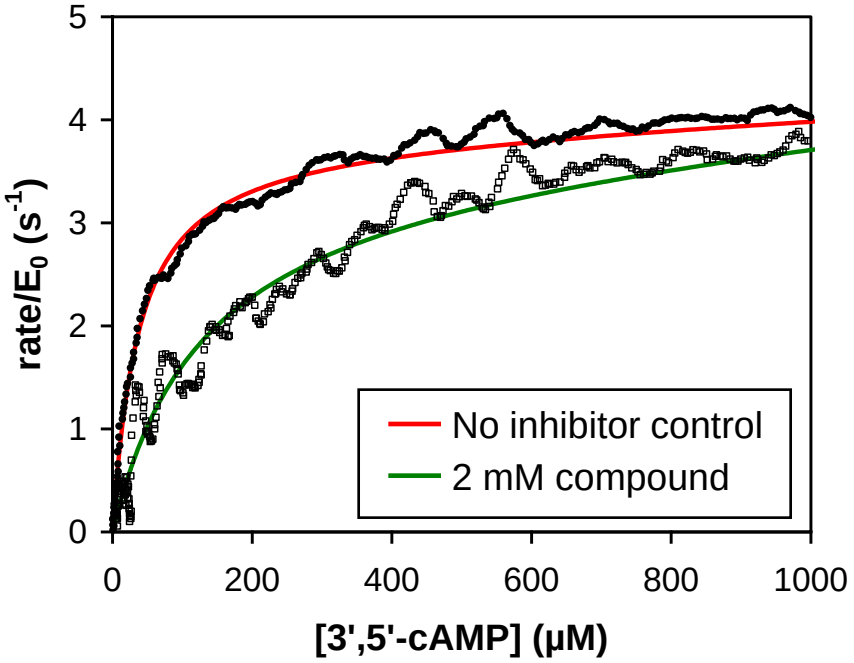
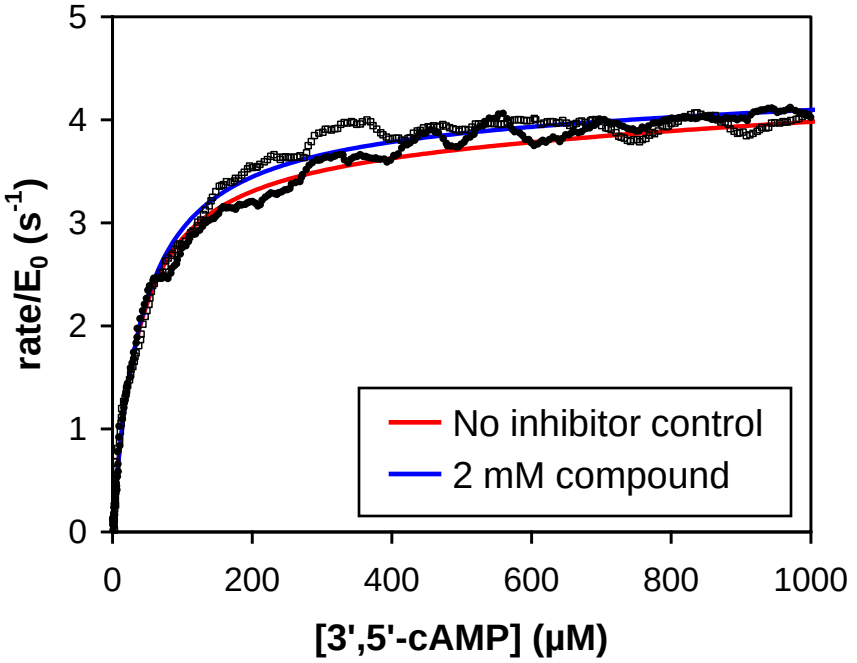
PDE4A inhibition – detection of hits

Compound 49, K_I = 1.4 mM



Compound 50, not a hit, K_I > 2mM



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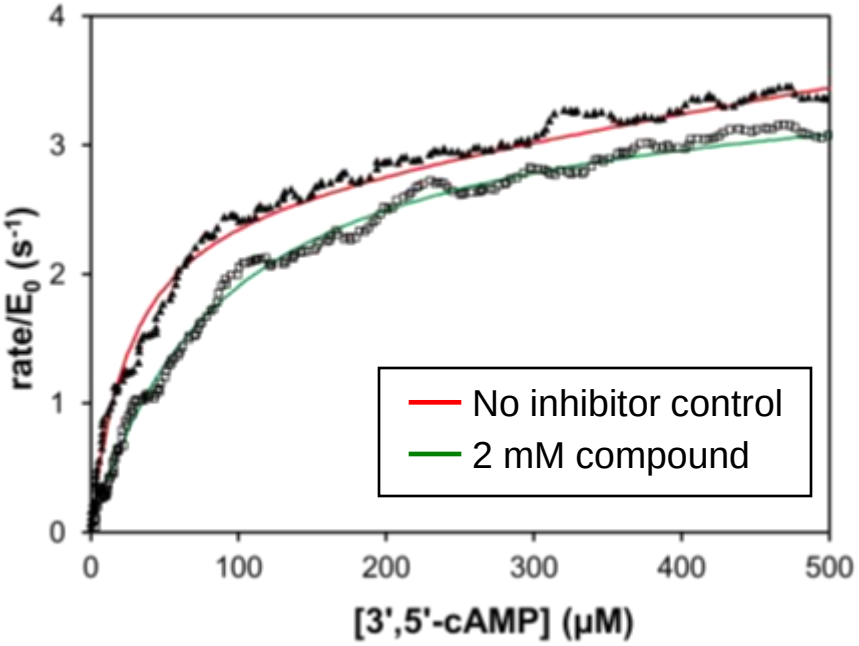
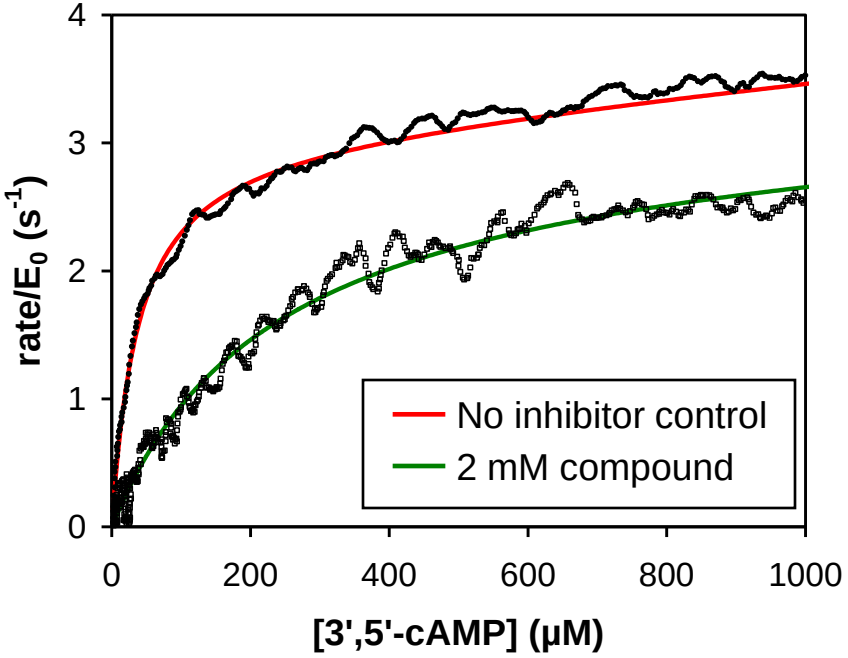
PDE4A inhibitors – range of K_I

Compound 48, K_I = 2 mM



Compound 80, K_I = 0.32 mM



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K_I pre-screening identified 11 compounds to follow-up by X-ray crystallography

Compound	K_I (mM)	Ligand Efficiency
113	0.81	0.32
81	0.58	0.40
80	0.32	0.39
88	0.56	0.36
96	0.71	0.35
109	1.34	0.39
33	1.10	0.36
48	2.00	0.40
49	1.40	0.43
73	0.37	0.38
111	0.46	0.35

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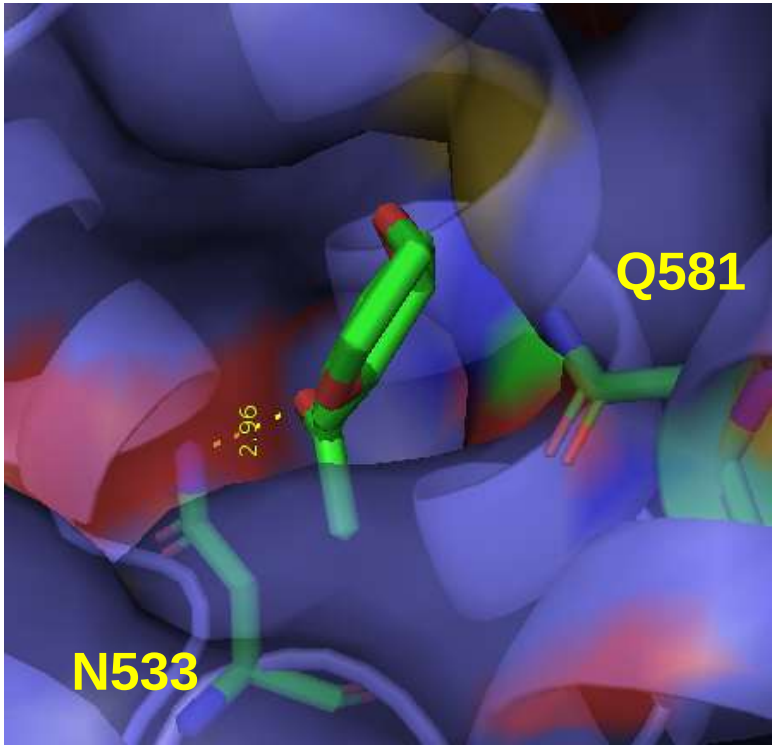
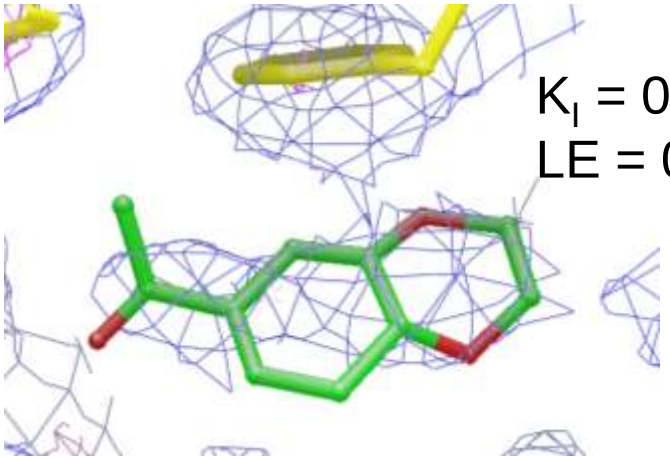
Preliminary structure of hit 113 shows hydrogen bond with N533





- Preliminary 3.0 Å structure of 113 with PDE4A10
- 113 ketone is hydrogen bonding with Asn 533 which is implicated in AMP binding
- Asn533 rotates to accommodate fragment

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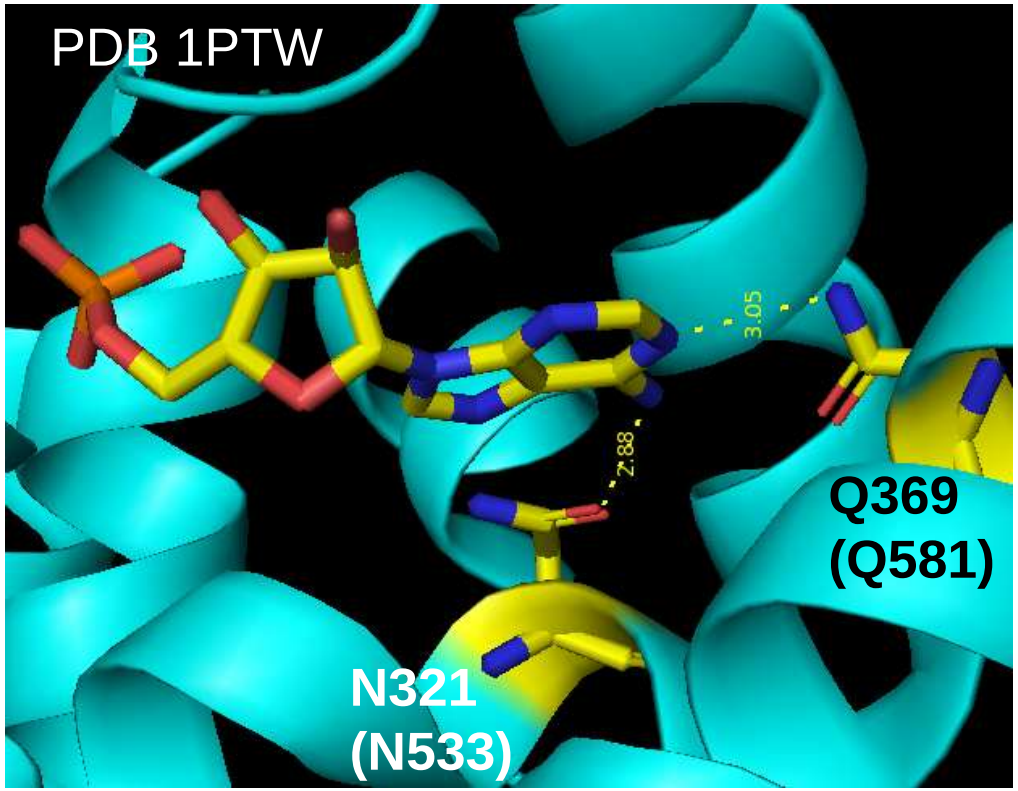
Binding of AMP to PDE4D



- Adenosine of AMP hydrogen bonds to both N321 and Q369 in PDE4D
  - Corresponding residues in PDE4A are N533 and Q581

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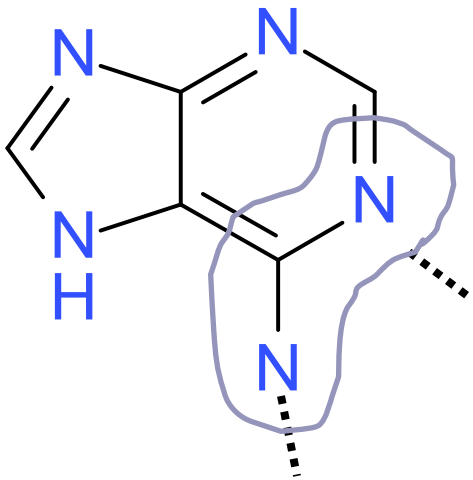
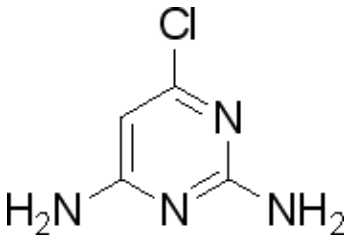
Fragment hits with adenine binding motif

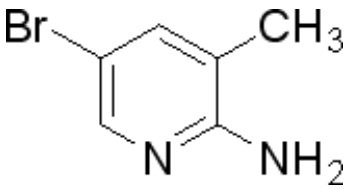


Gln581

Asn533



Compound 49
K_I =1.4 mM
LE=0.43



Compound 48
K_I =2.0 mM
LE=0.40

- Conserved in multiple fragment hits

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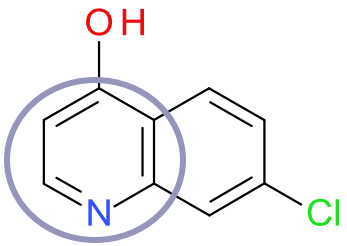
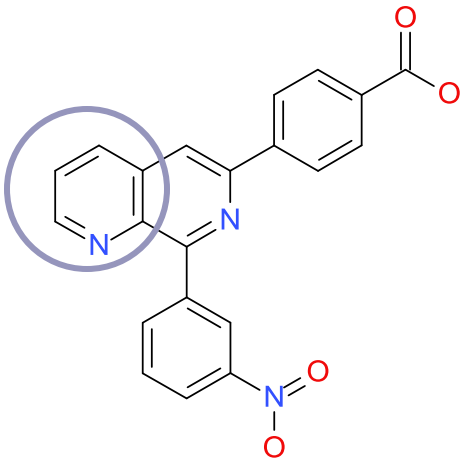
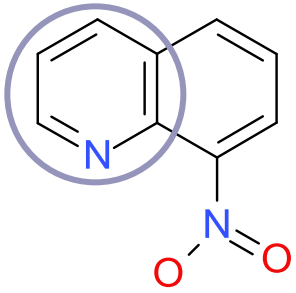
Quinoline fragment hits share binding motif with known PDE4 inhibitors



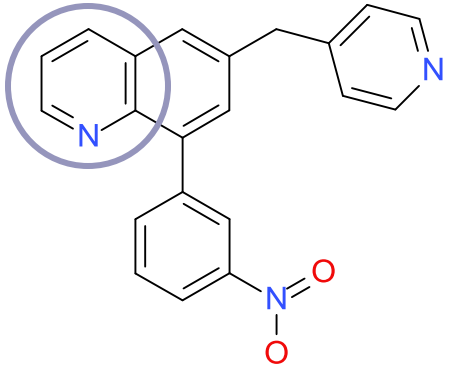
Compound 73
K_I =0.4 mM
LE=0.38



NVP
IC_{50} PDE4A = 3.3 µM



Compound 111
K_I =0.5 mM
LE=0.35



Quinoline series
IC_{50} PDE4A = 3.3 µM

Hersperger et al., J Med Chem 43, 675-682 J Med Chem. 43: *3820-3* (2000)

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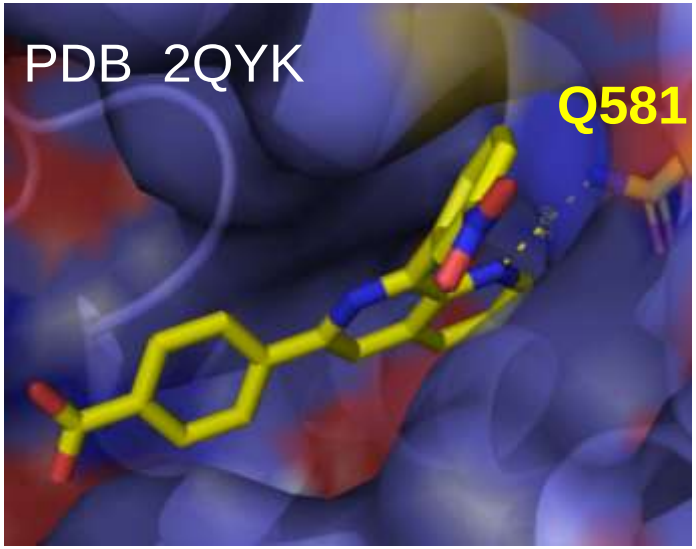
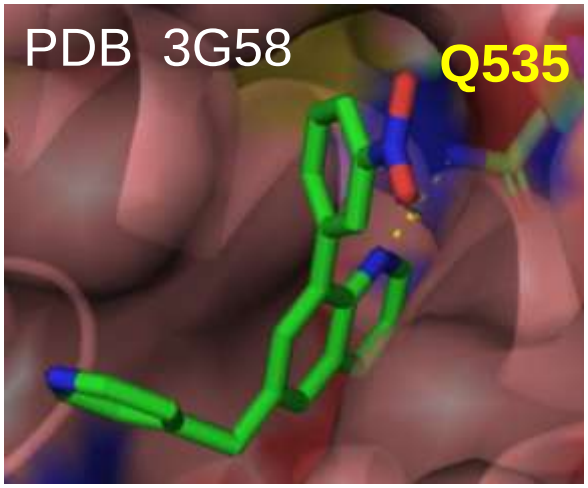
Quinoline (naphthyridine) motif hydrogen bonds with Q581



Co-crystal structure of PDE4A10 with NVP shows hydrogen bond between 1,7 naphthyridine nitrogen and Gln 581

Wang et al., Biochem Journal (2007) 408 ,193-201



Co-crystal structure of PDE4D shows hydrogen bond between quinoline nitrogen and structurally conserved Gln 535

Burgin et al., Nature Biotechnology (2010) 28 63-70

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Compounds with $K_I \leq 2$ mM

	Compound	K_I (mM)	Ligand Efficiency
Structure	113	0.81	0.32
	81	0.58	0.40
	80	0.32	0.39
	88	0.56	0.36
	96	0.71	0.35
Adenine motif	109	1.34	0.39
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	49	1.40	0.43
Quinoline	73	0.37	0.38
	111	0.46	0.35

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Summary

- Calorimetric enzyme activity based fragment screen
- Identified competitive inhibitors of PDE4A with LE > 0.35
- X-ray crystallographic follow-up in progress

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Acknowledgements

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Sorrento Technologies

Duncan McRee

Sridhar Prasad

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