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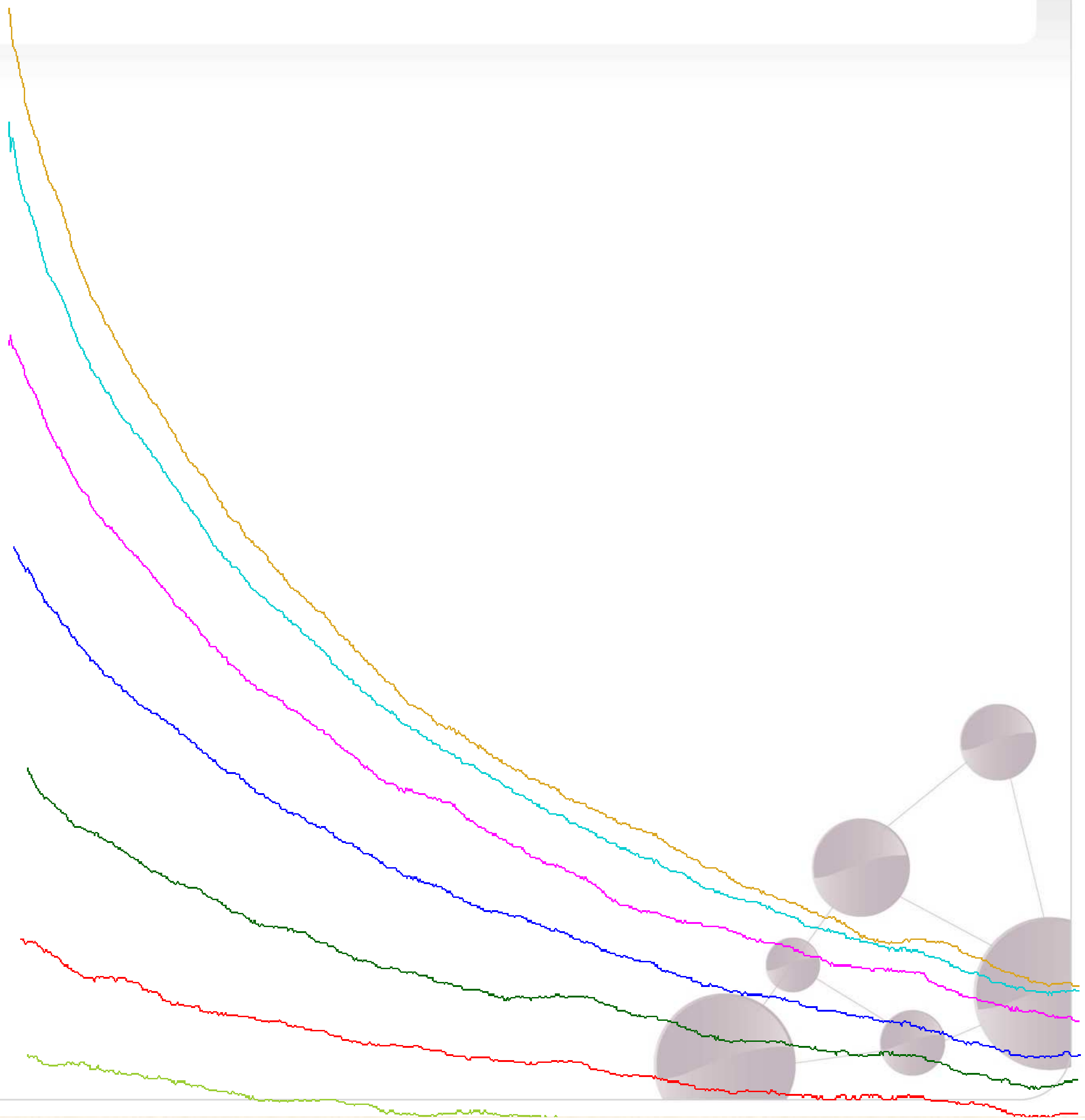
From the k_{off}

Stephen Roughley

Vernalis (R&D) Ltd

s.roughley@vernalis.com



Outline

- Simple binding
  - Rate constants
- Exploiting the dissociation rate constant
  - Screening mixtures
  - Retrospective example: Hsp90
- Lead generation
  - PDHK1
  - TNKS1/2
- Summary

Simple binding

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- In fragment to lead chemistry the primary goal is to improve compound affinity

$$P_F + L_F \underset{k_a}{\overset{k_d}{\leftrightharpoons}} PL$$

$$K_D = \frac{k_d}{k_a}$$

Dis(As)sociation rate constants

- Dissociation rates vary from the very fast >> 1 s^{-1} to infinite!
  - eg stable covalent
- Association rates above 10^7 $M^{-1}s^{-1}$ are unlikely to be therapeutically useful
  - diffusion likely to be rate limiting
  - Typically a narrow distribution range

Target	Number of Series	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})
HSP90	3	$10^7 - 10^5$	$>1 - 10^{-5}$
Kinase 1	2	$10^7 - 10^6$	$>1 - 10^{-5}$
Kinase 2	2	$10^7 - 10^6$	$>1 - 10^{-4}$
Bcl-2	2	$10^6 - 10^5$	$>1 - 10^{-5}$
PPI-1	1	$10^7 - 10^6$	$>1 - 10^{-5}$

Exploiting the dissociation rate constant

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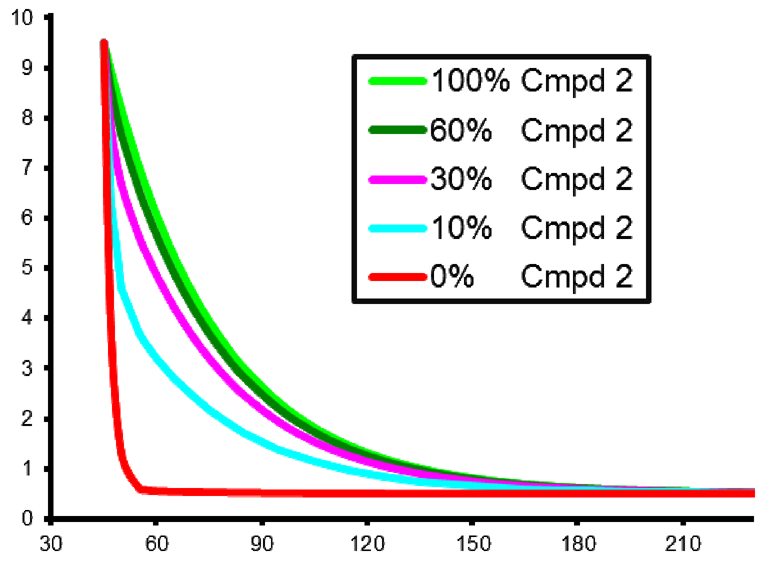
- We have seen that it is the key driver of potency
  - As have many others (see review Copeland, Future Med. Chem. 3(12), 2011)
- IMPORTANTLY: Independent of concentration
  - Can we exploit this to assess crude single reactions by SPR?

  - **aka Off-Rate Screening (ORS)**

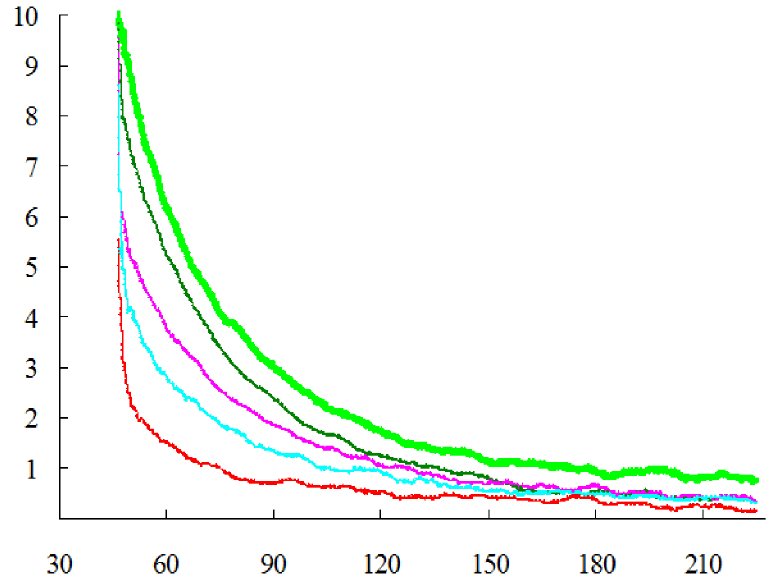
$$K_D = \frac{k_d\ (s^{-1})}{k_a (M^{-1}s^{-1})}$$

Example 1: Model 2 component system

- Two component system, only 10-fold difference in K_D and k_d
- Component 1 K_D 2 uM, k_d 0.5 s^{-1}
  Component 2 K_D 0.36 uM, k_d 0.032 s^{-1}
- Which dominates dissociation phase of the sensorgram?



Prediction: slowest off



Experiment: slowest off

Example 2. HSP90 retrospective proof of concept

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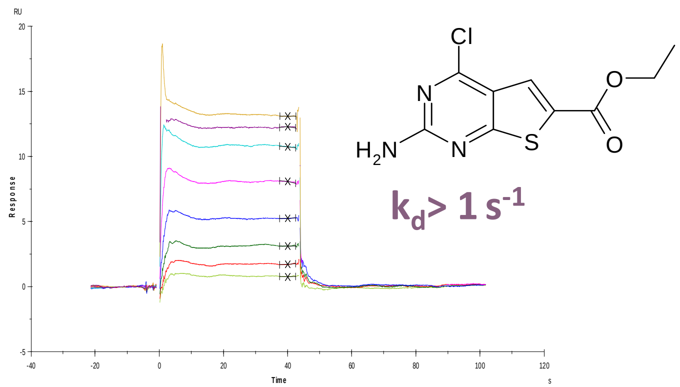
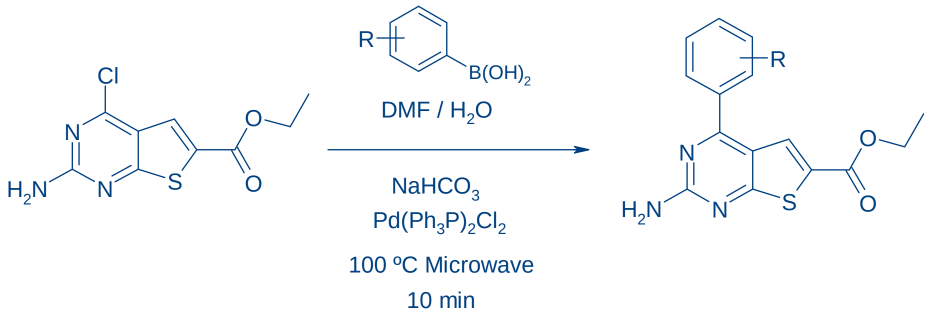
- A set of thienopyrimidines were re-prepared by Suzuki reaction



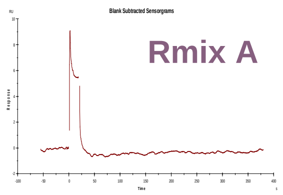
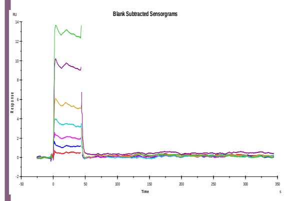
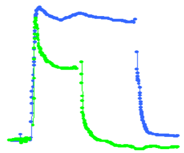
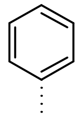
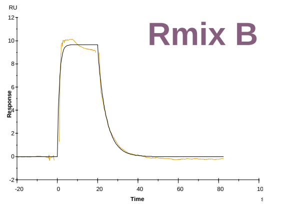
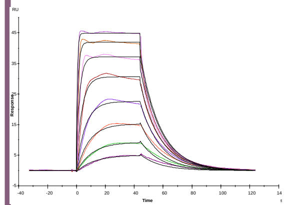
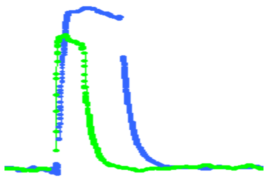
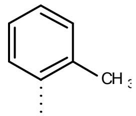
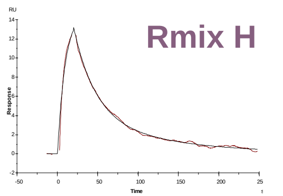
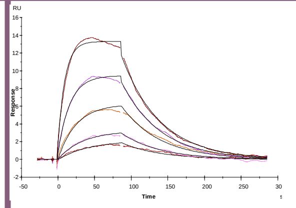
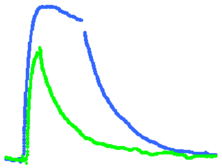
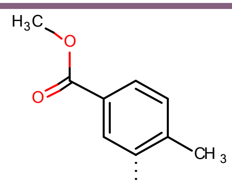
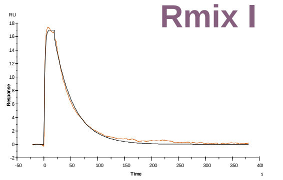
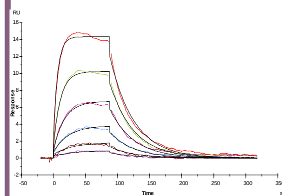
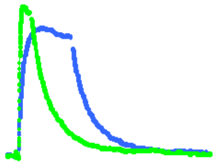
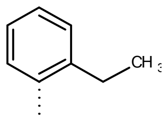
$k_d > 1\ s^{-1}$



- Minimal work-up
  - Evaporate
  - Add DMSO
  - Purity ~50 % (LCMS)/~30% NMR

- **What affect will carry over contaminants have on the observed off-rate?**

Example 2. HSP90 retrospective proof of concept

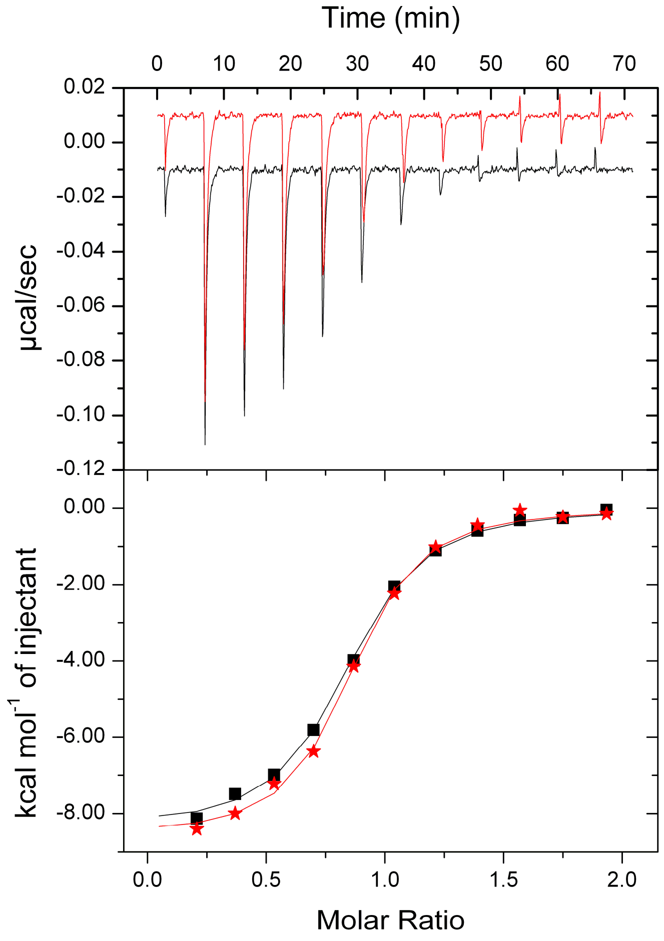
Crude 1:1000	Pure	Crude / Pure	R	Crude kd s-1	Pure kd s-1
			 VER-00053833	fast	fast
			 VER-00054353	0.18 $t_{1/2}$ 4s	0.15 $t_{1/2}$ 4.6s
			 VER-00055133	0.021 $t_{1/2}$ 32s	0.019 $t_{1/2}$ 36s
			 VER-00082099	0.028 $t_{1/2}$ 24s	0.027 $t_{1/2}$ 25s

- NONE !

Example 2: HSP90 retrospective

- **And if you really need to know the affinity of the active component**
- Use ITC!
  - In ITC only one component is needed to be known accurately
  - Titrate protein onto crude reaction
    - 1% DMSO
    - Dilution corrected for purity

- Black: pure compound VER-0082099
- Red: reaction mixture R-mixI
  - K_D: 360 vs 300 nM



Model systems: Summary

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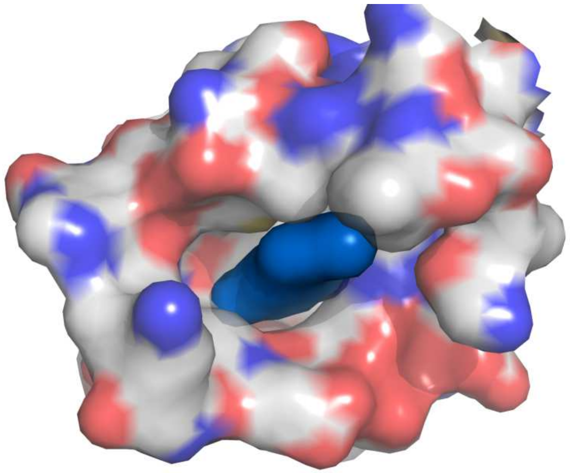
- ✓ Theory matches prediction - good start
- ✓ Focus on dissociation rate recapitulates bulk of SAR
- ✓ Excellent agreement between crude and pure samples
- ✓ Affinity can also be determined from crude samples

BUT

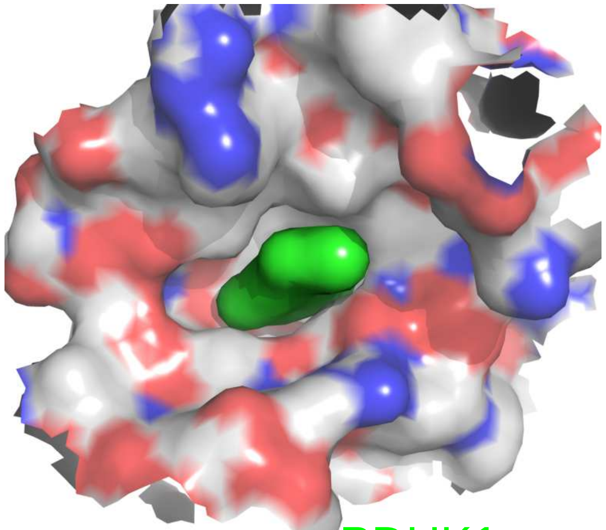
Can it be used prospectively for drug discovery?

Example 3: PDHK1 Lead Generation

- GHKL family protein, a kinase essential for PDC regulation
  - Multiple sites, ATP pocket most druggable
  - NMR based fragment screen
- Promising fragment also binds to HSP90
  - Selectivity a big question
- ORS challenge: to discover potent selective series for LO



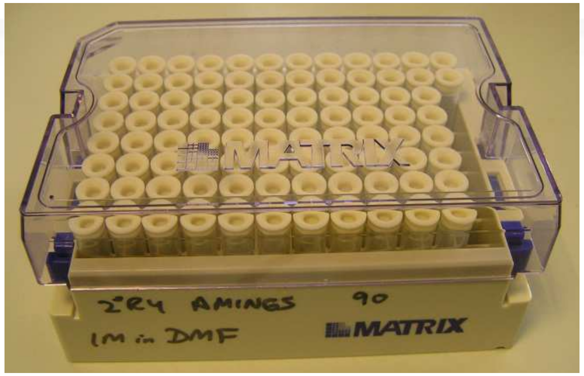
HSP90

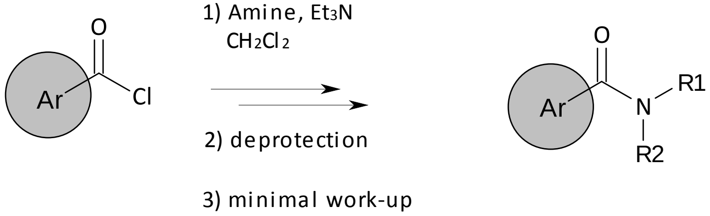


PDHK1

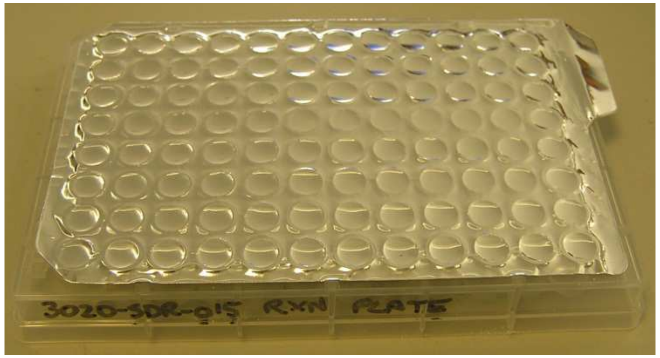
Example 3: PDHK1 Lead Generation

- Method development enabled synthesis of 90 compounds on 1 μmol scale from ~35 mg of parent acid
  - Starting fragment 500 μM
  - Substituents from in-house or external libraries
- Crude compounds tested by ORS
- Simultaneously screened against both targets
  - PDHK1
  - HSP90
- 1 chemist, 4 weeks, 90 compounds
  - Route redevelopment
- LCMS purity ranges from < 5% to > 95%





Standard screening plate



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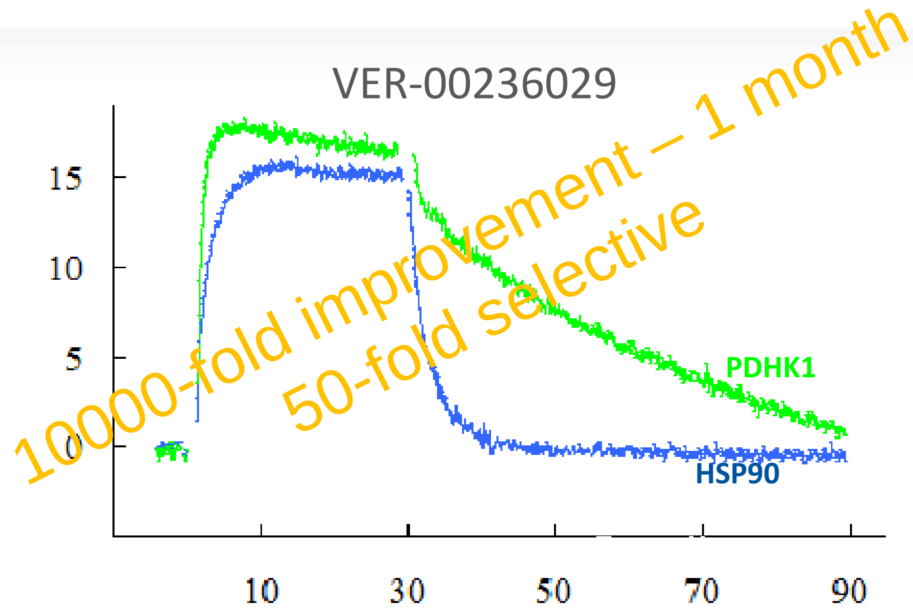
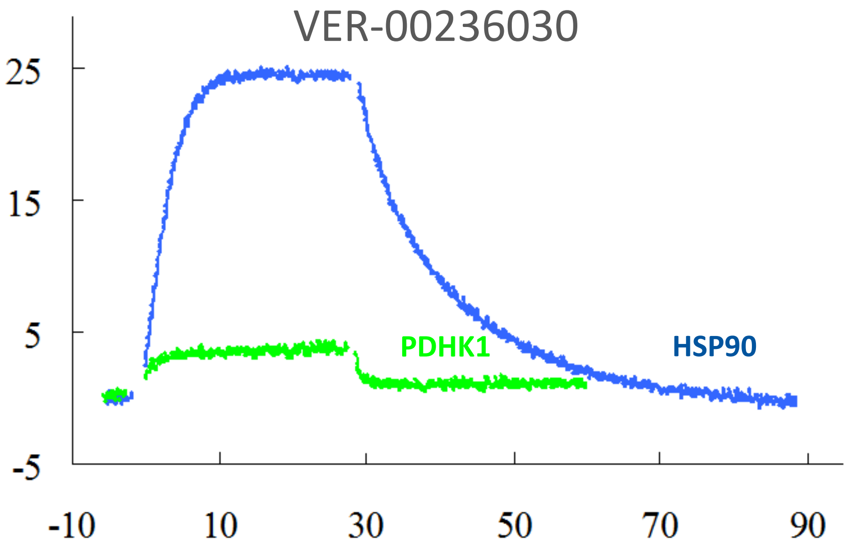
Example 3: PDHK1 Lead Generation

- Good results: selective PDHK1 compounds eg VER-00236029
    - Pure K_D 50 nM (PDHK1)
    - (Starting fragment 500 μM)
- Other results: selective HSP90 compounds eg VER-00236030

Compound	Batch	k_d (s^{-1}) PDHK1	k_d (s^{-1}) HSP90
VER-00236029	1 (crude)	0.021	0.34
VER-00236029	2 (pure)	0.019	0.29
VER-00236030	1 (crude)	1.1	0.07





Example 3: PDHK1 beyond Lead Generation

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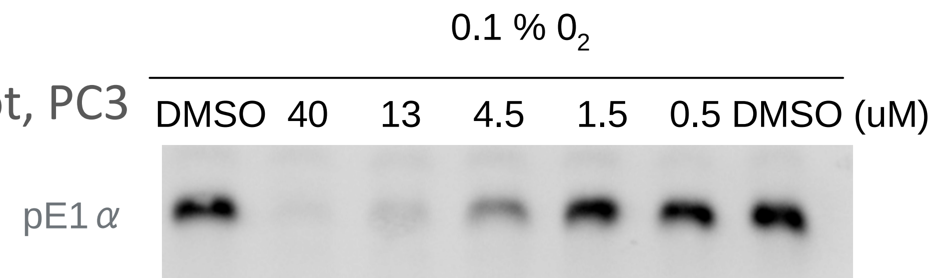
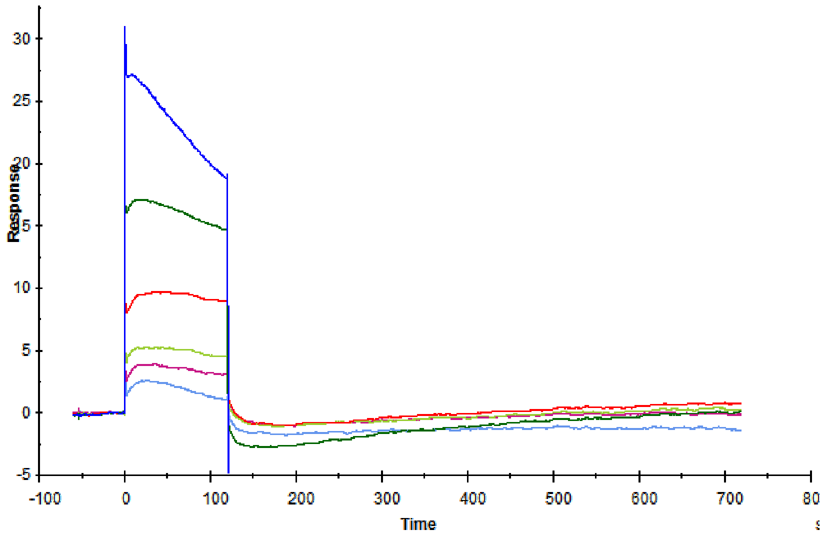
- Lead series identified by **ORS** (VER-00236029)
  - K_D 50 / 45 / 80 nM (SPR/ITC/ cK_I)
- Further evolution by combination of SBDD and ORS
  - Decent LE 0.37
- Selectivity over Hsp90 > 100-fold (FP and SPR)
  - confirmed in cells; no Hsp70 induction in cells
- PD marker effected changes in several cell lines.
  - IC_{50} <100 nM for best compounds (Elisa assay)
  - IC_{50} <20nM in cell free functional assay (Delfia)
- Compounds effective under hypoxic conditions *in vitro*

Western Blot, PC3



Example 4: TNKS1/2 Lead Generation

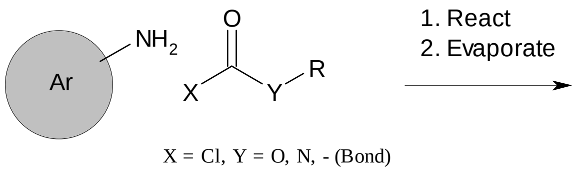
- PARP-Family
- Fragment screen targeting the NAD^+ binding-site
- Crystallographic fragment screen
- An interesting fragment was not suitable for rapid chemistry
  - Designed an isosteric fragment



K_D: 700µM; $k_d >> 1\ s^{-1}$

Example 4: TNKS1/2 Lead Generation

- Library – amine-bearing fragment
  - Amides, carbamates, ureas
  - 1 chemist 2 days; 80 compounds

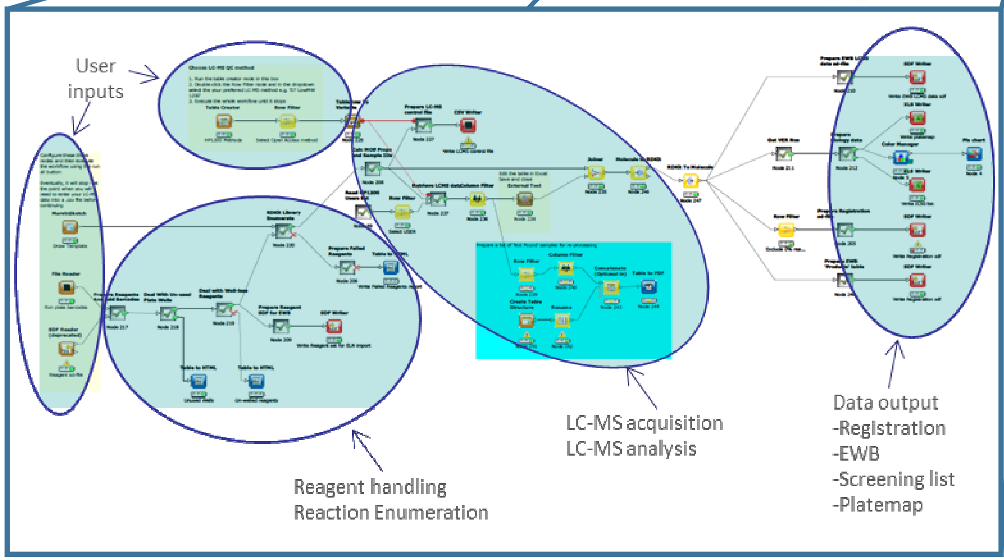


X = Cl, Y = O, N, - (Bond)
X = OH, Y = - (Bond)

ORS

SAR heat map

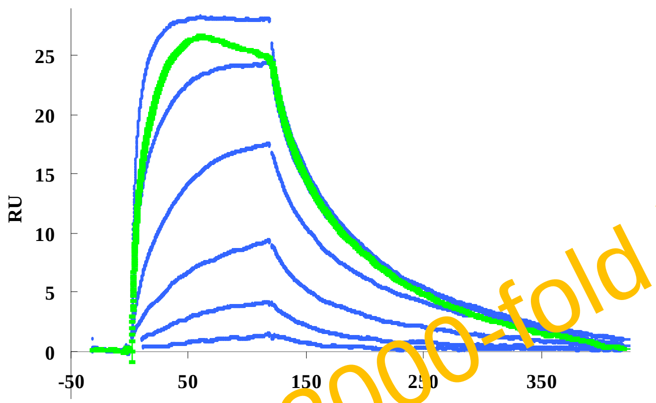
1.0	##	##	1.0	##	0.3	1.0	##
##	0.4	0.2	1.0	0.0	0.1	##	##
0.1	0.1	##	0.1	##	##	##	0.1
0.0	0.1	##	##	0.1	0.2	##	0.9
##	##	##	##	##	1.0	##	1.0
0.2	0.3	##	##	##	##	0.0	##
##	##	1.0	1.0	0.9	1.0	##	##
0.0	##	##	##	##	##	0.3	##
##	##	##	##	##	##	##	0.0
##	1.0	1.0	##	##	##	##	0.1



Example 4: TNKS1/2 Lead Generation

- Crystals soaked directly with reaction mixtures
  - Pure K_D 350 nM (TNKS2)

2000-fold improvement – 2days



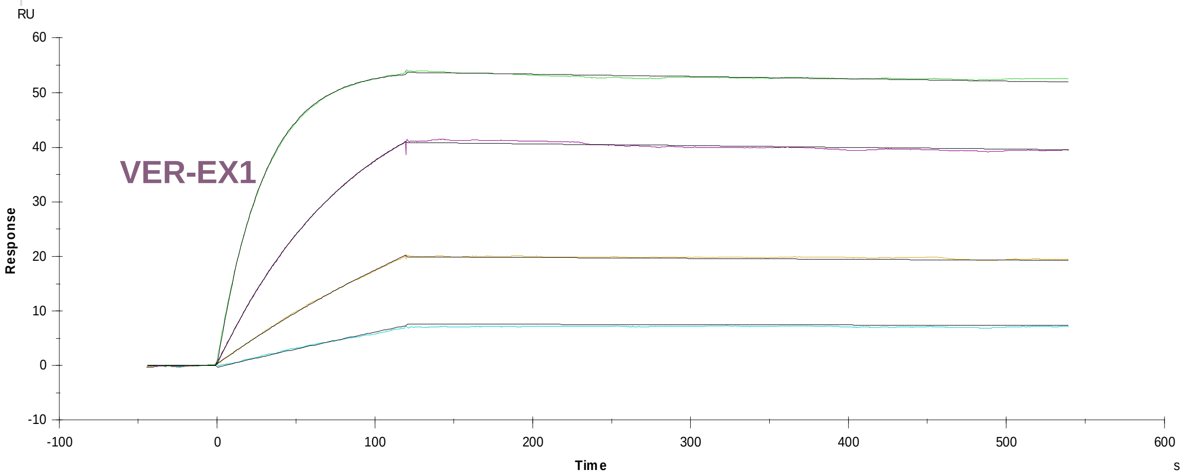
Crude 30% ; $k_d = 0.02 s^{-1}$

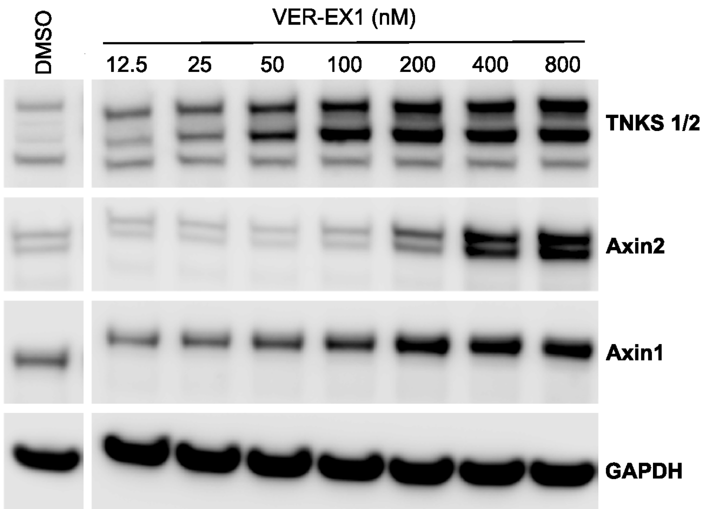
K_D: 350nM; $k_d = 0.02 s^{-1}$

(Fragment K_D: 700µM; $k_d >> 1\ s^{-1}$)

Example 4. TNKS1/2 Lead Generation

- Subsequent series evolution driven using SBDD
  - 800 pM vs TNKS2, high LE (0.60)
  - Cellular PD markers respond appropriately
  - **TNKS1/2 and Axin 1/2** stabilised
  - > 100 fold selective over PARP1





k_a 1.8×10^{-5} $M^{-1}s^{-1}$
k_d 1.5×10^{-4} s^{-1}
K_D 822 pM

Top 10 Reactions

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Reaction	% of Total Reactions	ORS?
N-Acylation → Amide	16.0	✓
N-Heterocycle formation	7.4	✓
N-Arylation	6.3	
RCO_2H Deprotection	5.4	✓
N-Alkylation with R-X	5.3	
Reductive Amination	5.3	
N-Boc Deprotection	4.9	✓
Suzuki Coupling	4.6	✓
O-Substitution	4.4	✓
Other NH Deprotection	2.9	✓
TOTAL	**62.4**	-

Careful attention to reagents, Protecting Groups and conditions may be required!

Roughley/Jordan *J. Med. Chem.*, 2011, **54**, 3451-3479

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Summary: Off Rate Screening (ORS)

- Excellent kinetic agreement between pure and crude samples
  - Low reagent consumption, cost effective
- Significant productivity improvement
  - Rapid vector assessment
- Applicable to wide range of targets
  - Kinases, ATPases , PARPs, PPIs, Proteases…
- Applicable to wide range of chemistries
  - Number steps key determinate for success

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Credits

- ORS
- SPR: Natalia Matassova
-   James Murray
- ITC: James Murray
- Chemistry: Stephen Roughley
  Paul Brough

- Many more at Vernalis
  - James Davidson
  - Ben Davis
  - Rod Hubbard

- Tankyrase: Alba Macias, Chris Graham and team

- PDHK1: Paul Brough, Jon Moore and Team it never hurts to stick your ORS in