Chemical Probes for Epigenetic Targets

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SCI
Highlights in Medicinal Chemistry II
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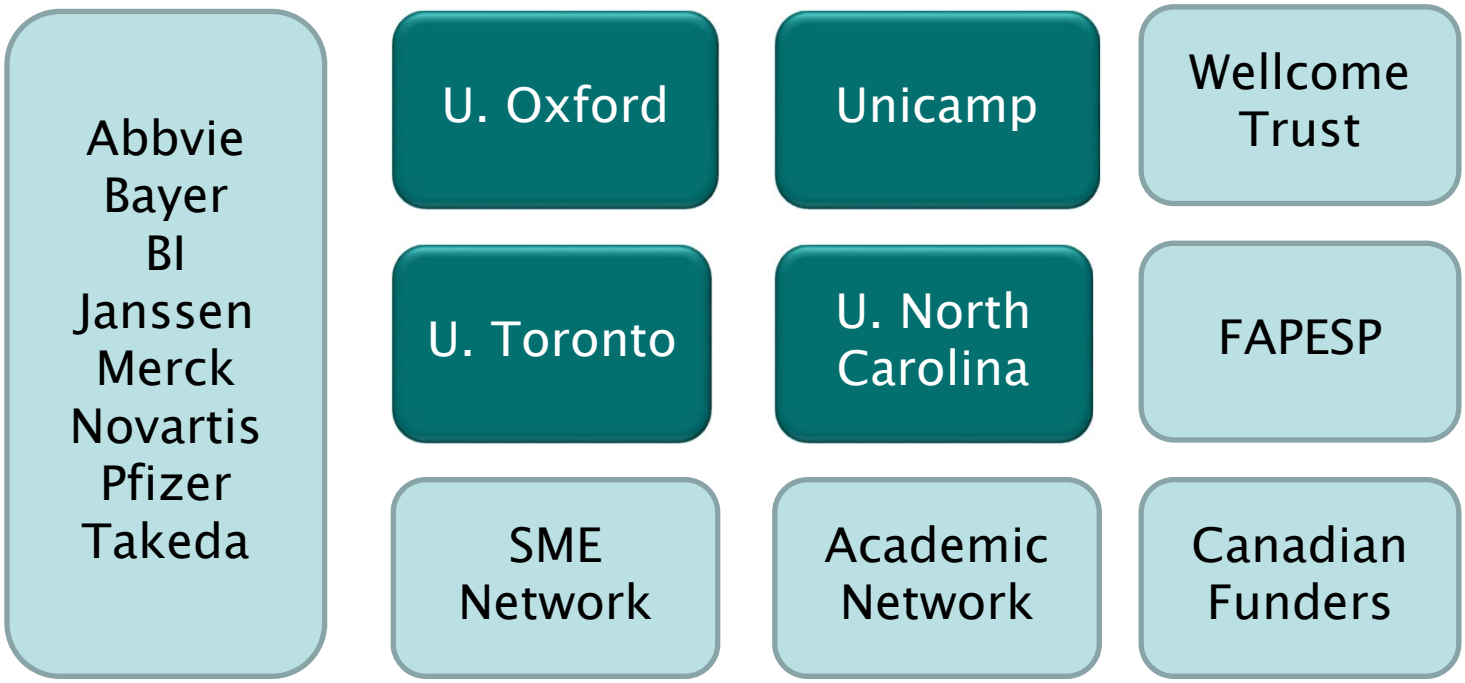
DRUG DISCOVERY INSTITUTE

INTRODUCING THE SGC

A model for open access public-private partnership

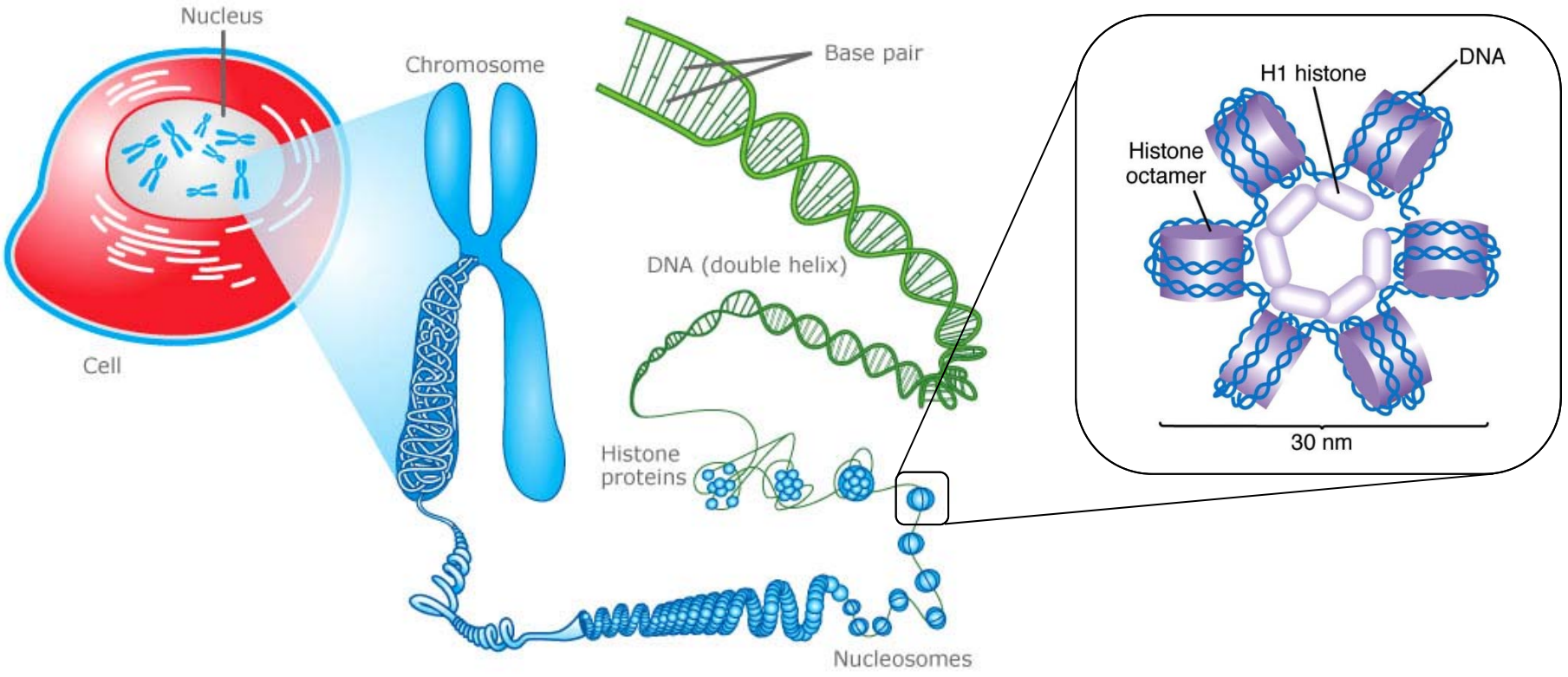


'Open Source' science

- All structures/results are made freely available promptly
- Funding partners receive no prior access or rights to data or progress information
- **No IP**

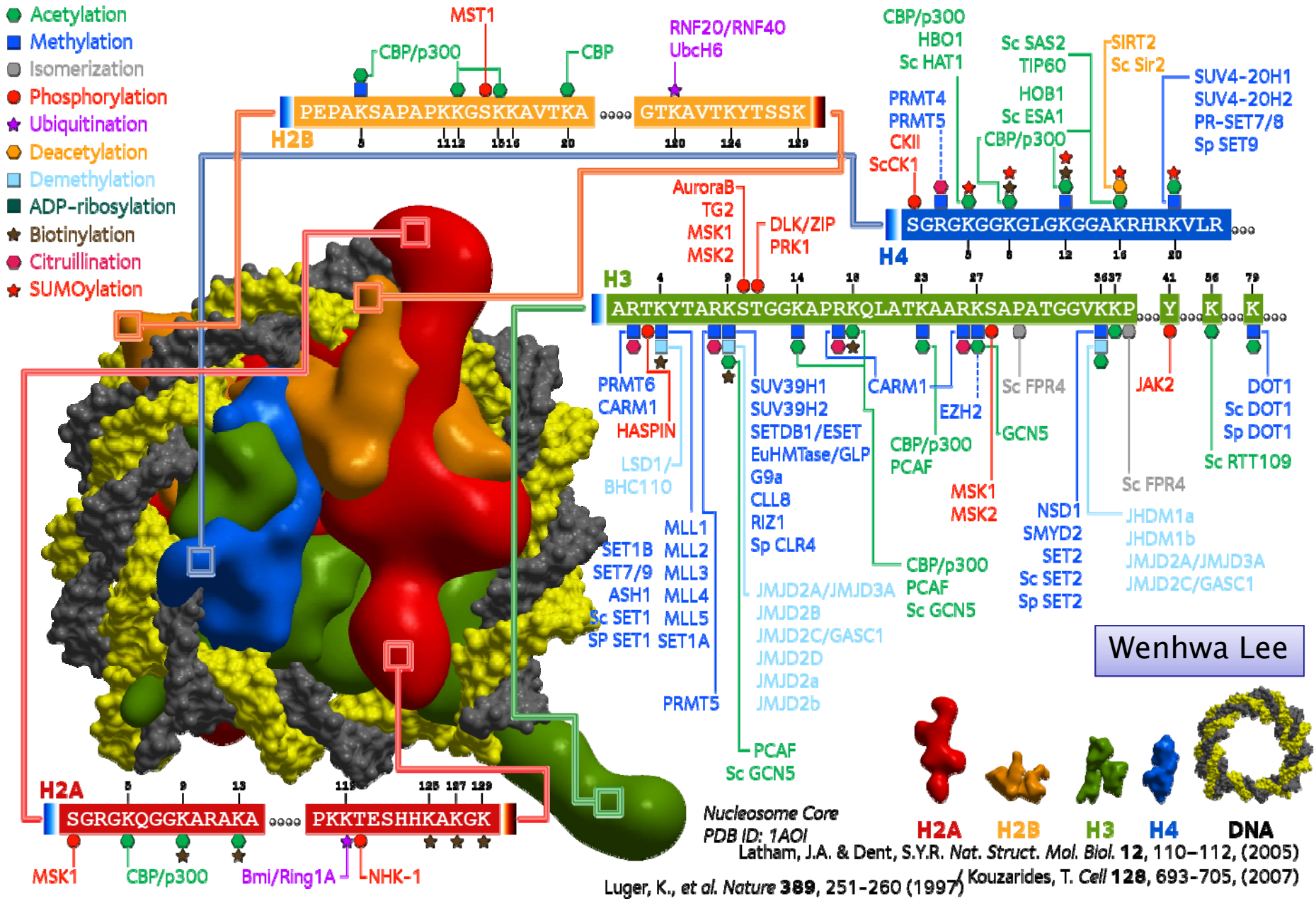
Chromatin and Nucleosomes

- The largest human cells are 0.1 µm (0.0000001 m) wide.
- There is 2 m of DNA in every cell.

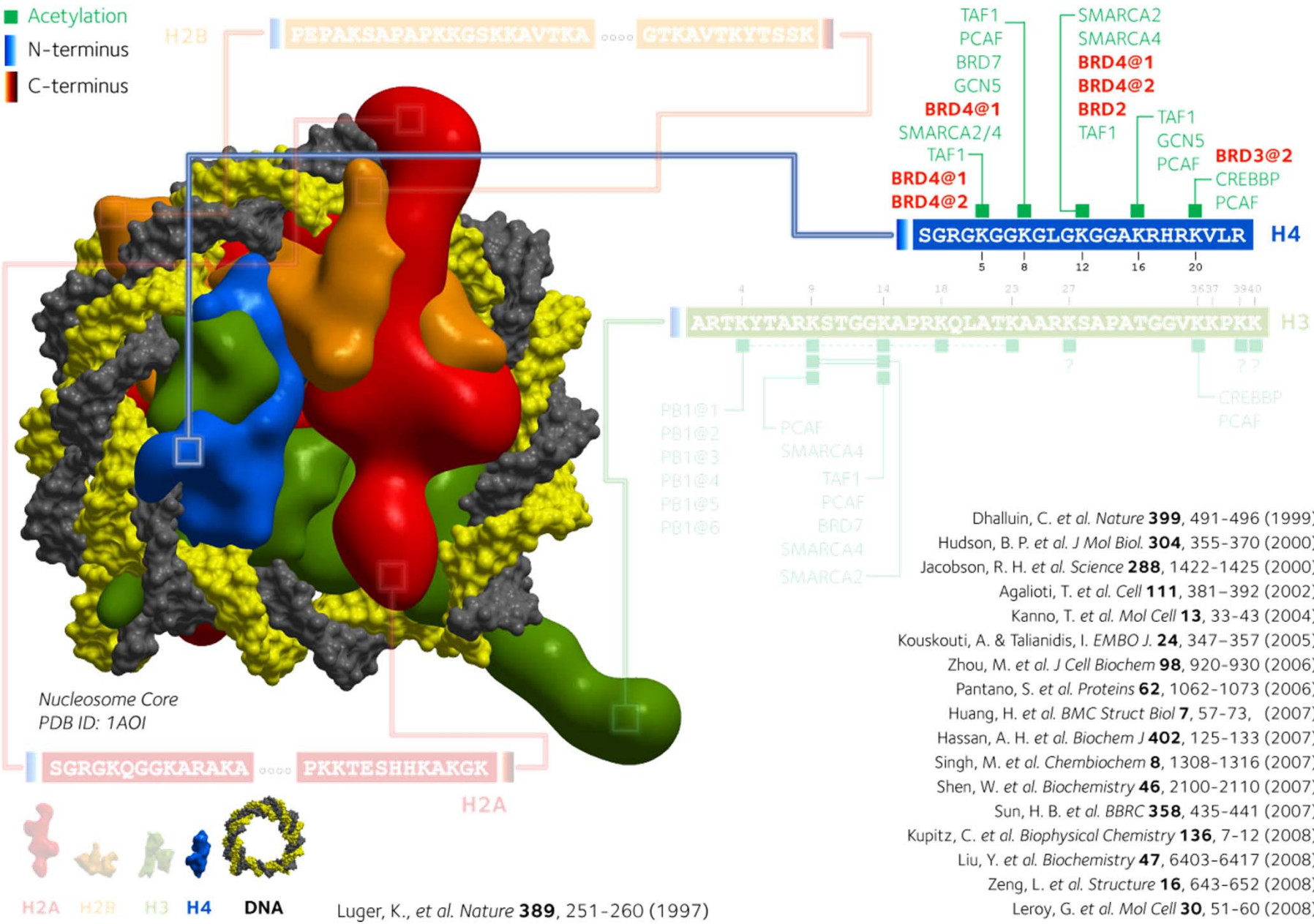


- Reversible DNA modification
- Reversible histone modification

Histone Code: Writers and Erasers



Histone Code: Reader



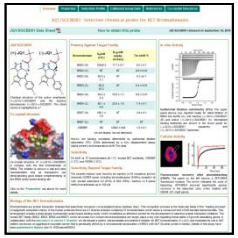
Luger, K., *et al. Nature* **389**, 251-260 (1997)

Dhalluin, C. *et al. Nature* **399**, 491-496 (1999)
Hudson, B. P. *et al. J Mol Biol.* **304**, 355-370 (2000)
Jacobson, R. H. *et al. Science* **288**, 1422-1425 (2000)
Agalioti, T. *et al. Cell* **111**, 381–392 (2002)
Kanno, T. *et al. Mol Cell* **13**, 33-43 (2004)
Kouskouti, A. & Talianidis, I. *EMBO J.* **24**, 347–357 (2005)
Zhou, M. *et al. J Cell Biochem* **98**, 920-930 (2006)
Pantano, S. *et al. Proteins* **62**, 1062-1073 (2006)
Huang, H. *et al. BMC Struct Biol* **7**, 57-73, (2007)
Hassan, A. H. *et al. Biochem J* **402**, 125-133 (2007)
Singh, M. *et al. Chembiochem* **8**, 1308-1316 (2007)
Shen, W. *et al. Biochemistry* **46**, 2100-2110 (2007)
Sun, H. B. *et al. BBRC* **358**, 435-441 (2007)
Kupitz, C. *et al. Biophysical Chemistry* **136**, 7-12 (2008)
Liu, Y. *et al. Biochemistry* **47**, 6403-6417 (2008)
Zeng, L. *et al. Structure* **16**, 643-652 (2008)
Leroy, G. *et al. Mol Cell* **30**, 51-60 (2008)

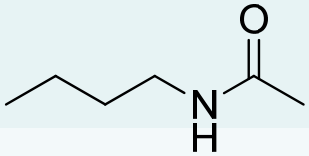
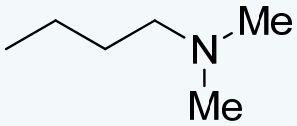
SGC Chemical Probe Discovery

Chemical Probe Criteria

- **In vitro activity:** IC_{50} or Kd 100 nM
- **Cellular activity:** IC_{50} 1 µM
- **In vitro selectivity:** 30-fold vs. other branches of phylogenetic tree

Assay develop-ment	Focused sets VLS Fragments HTS	Analogue purchase Synthesis SAR generation SAR analysis Complex structures Selectivity	Secondary assays Cellular assays Selectivity	

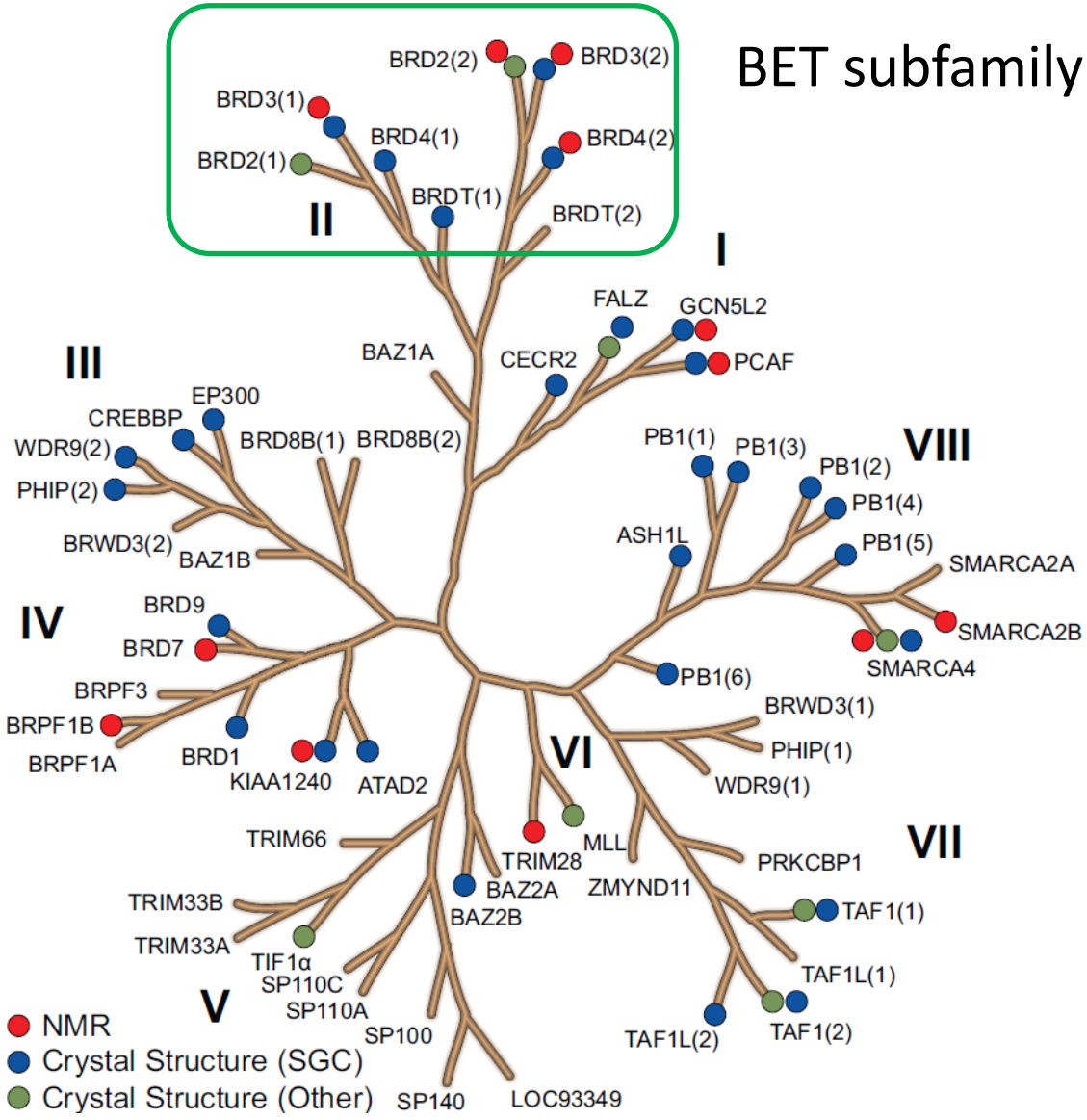
Histone Code

	Histone Modification	Writer	Reader	Eraser
				
	Acetyl	HAT	Bromo	HDAC
	Methyl	HMT	Chromo, PHD, Tudor, MBT	KDM

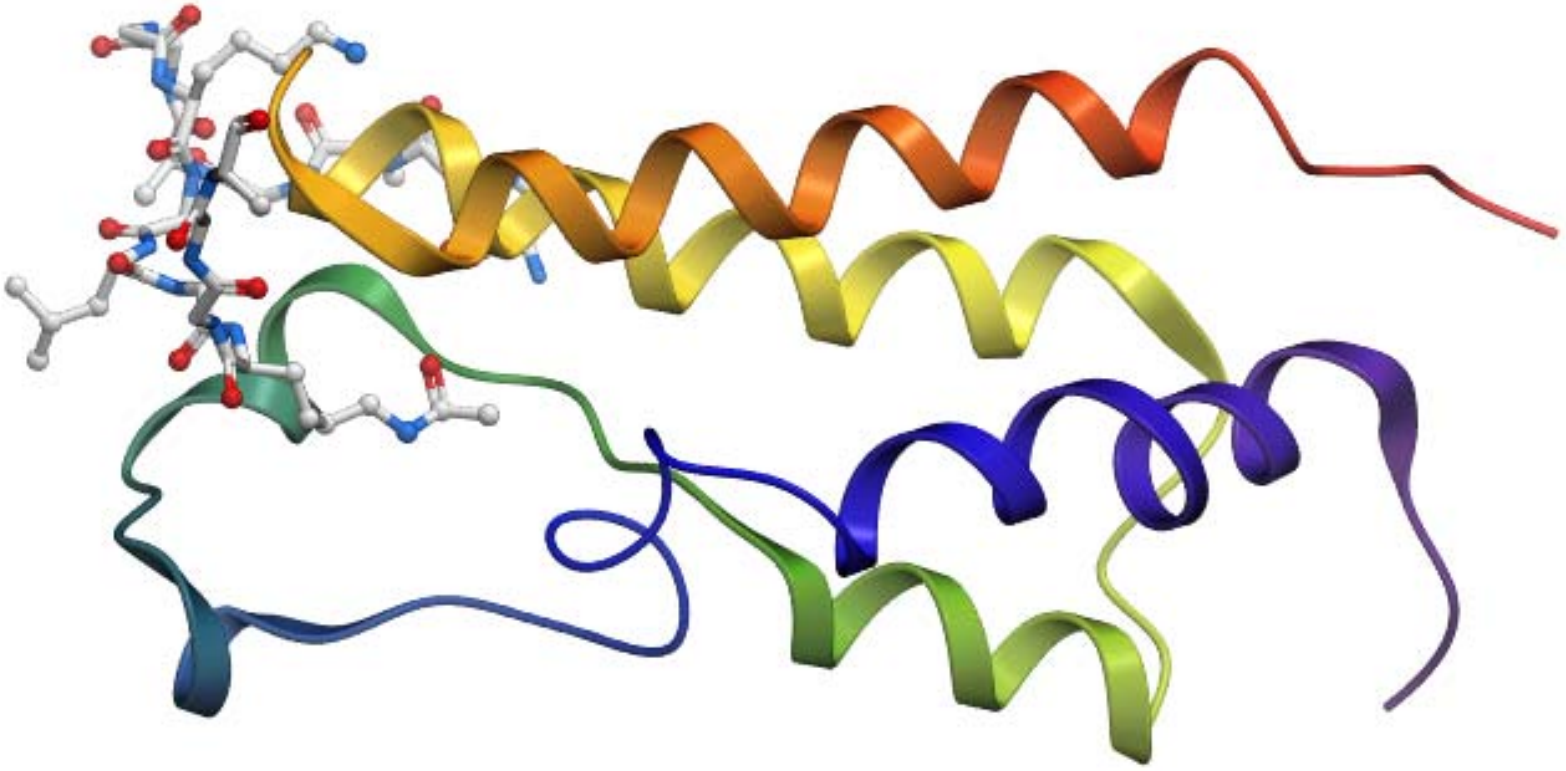
- Oxford + collaborators
- Toronto + collaborators
- Rest of world

Bromodomain Family Tree

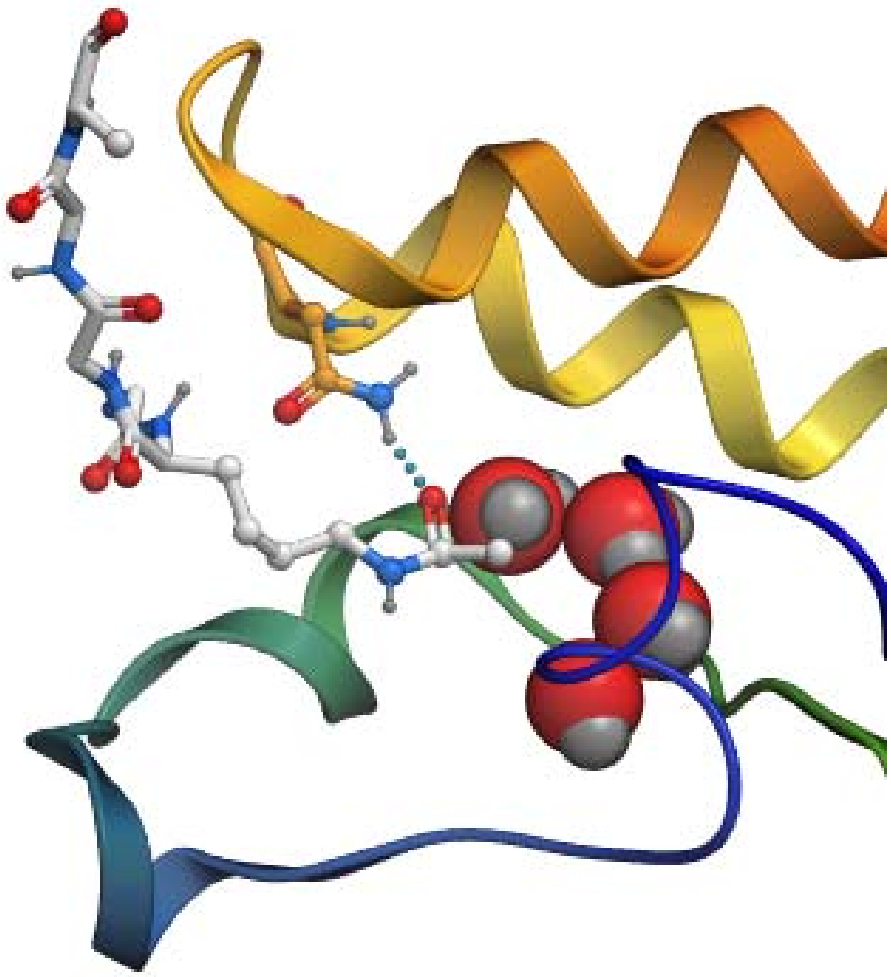
- 61 bromodomains in 41 proteins
- 8 subfamilies
- Acetyl lysine reader

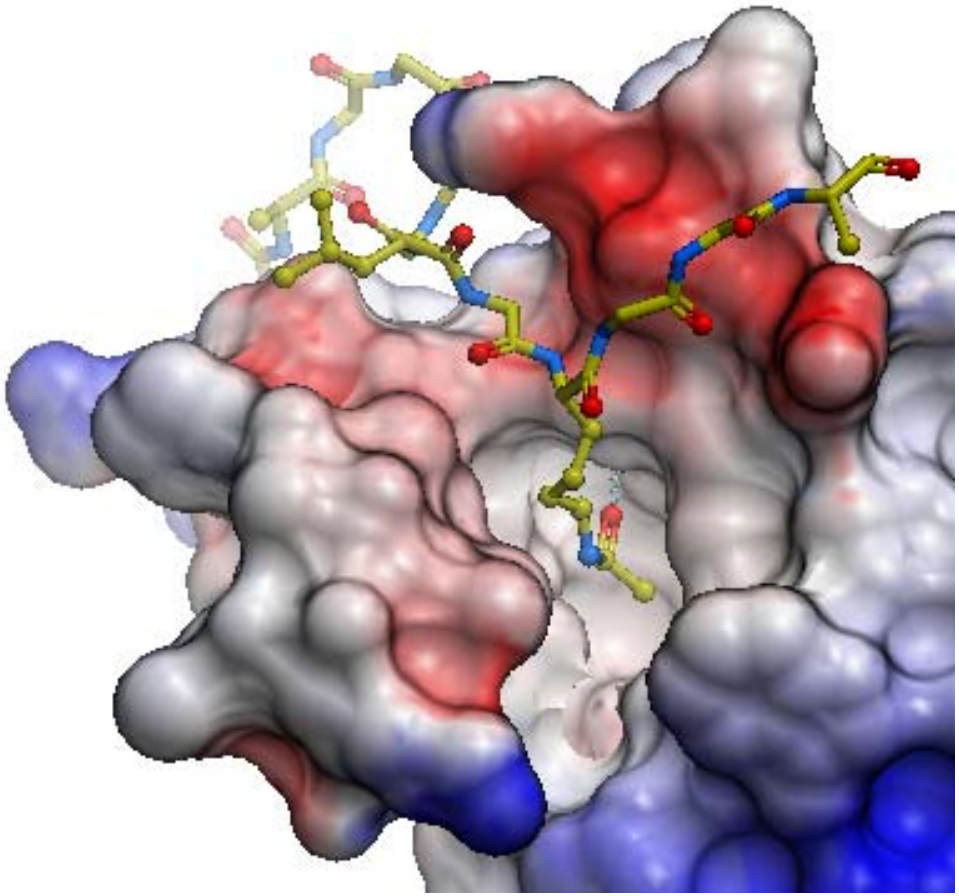


Intro to Bromodomains

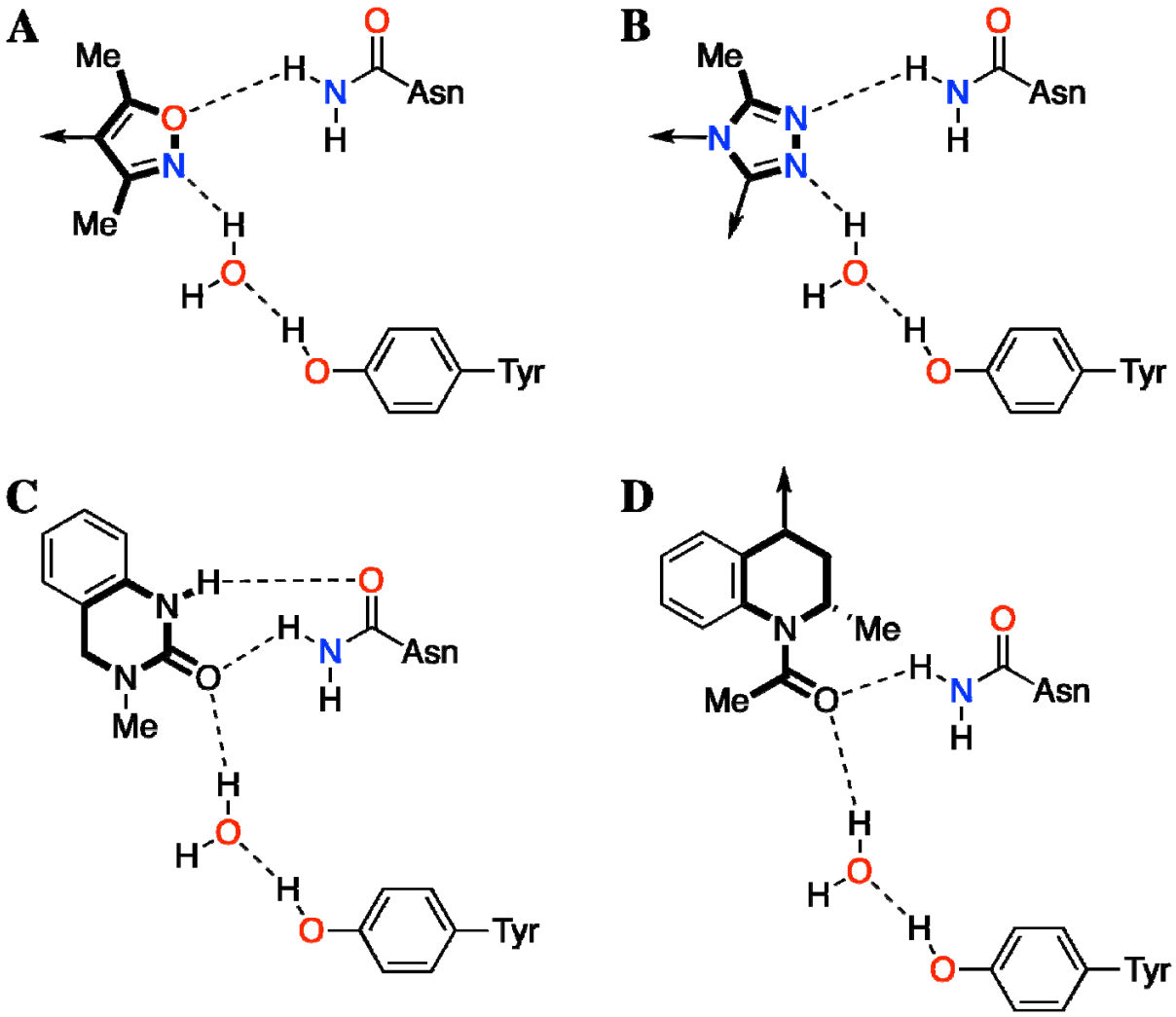


Intro to Bromodomains



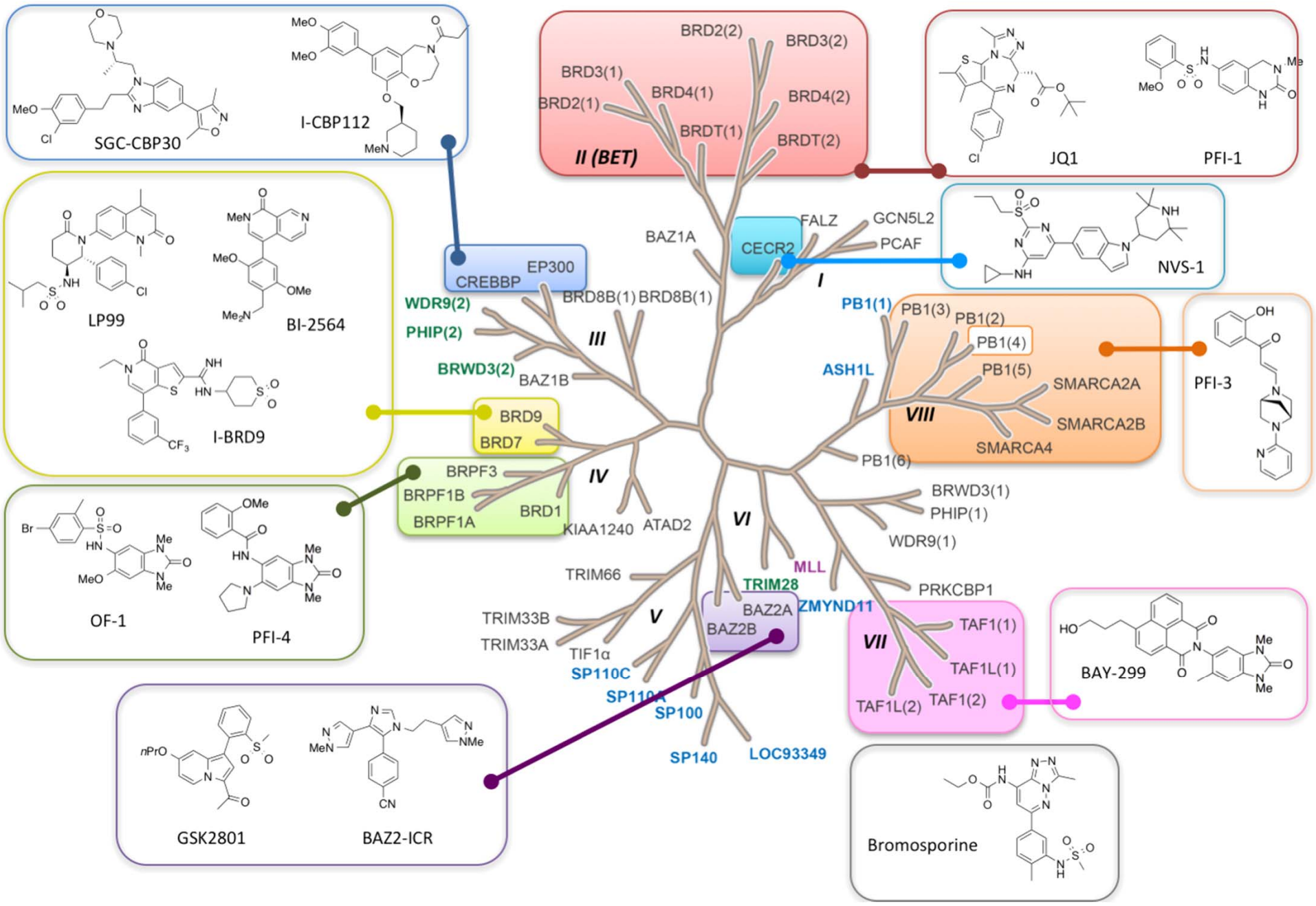


Bromodomain Inhibitor Chemotypes

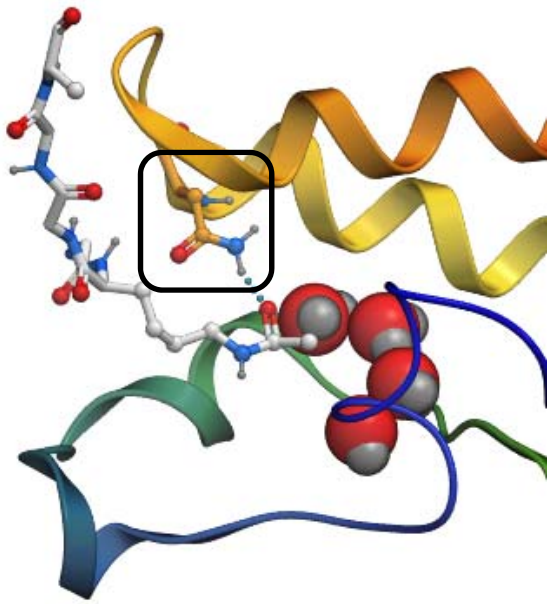


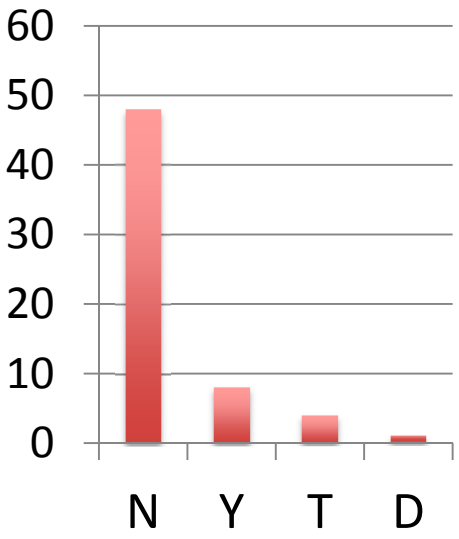
Hewings, et al, *Progress in the Development and Application of Small Molecule Inhibitors of Bromodomain–Acetyl-lysine Interactions,* J. Med. Chem. **2012** 55 (22) 9393–9413

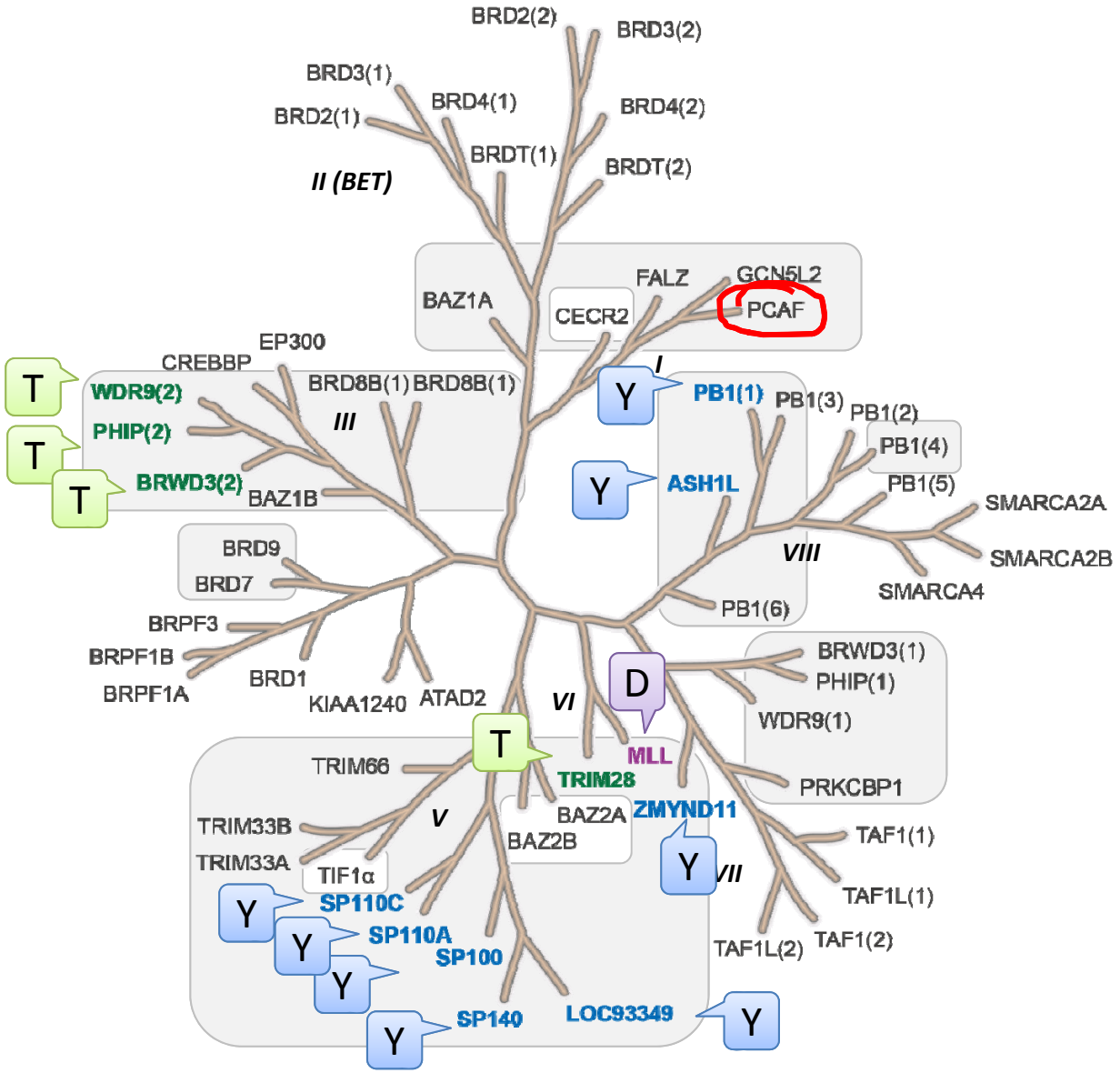
SGC Bromodomain Chemical Probes



Unprobed Bromodomains





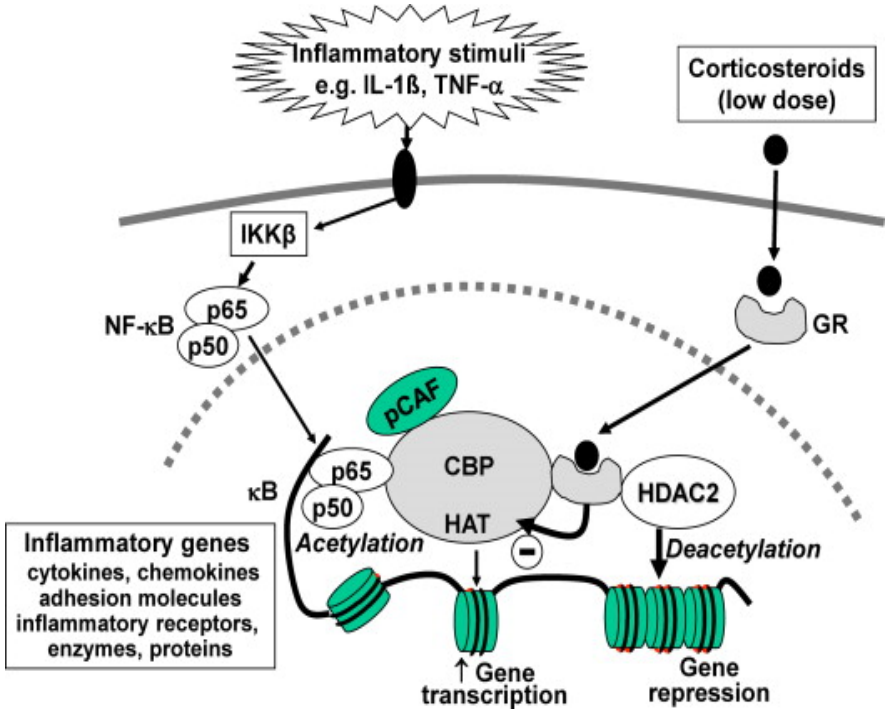


PCAF in Neuroinflammation

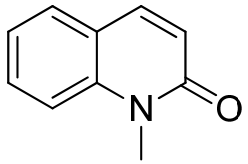
- Neuro-inflammation genetically implicated in Alzheimer's disease (AD)
- Nuclear factor-κB (NF-κB) is a master regulator of inflammatory signalling
- Evidence of dysregulation of NF-κB pathway in AD, as seen by upregulation of p65 subunit and increased NF-κB activity in the brains of AD patients in proximity of plaques:

Kaltschmidt B., PNAS, 1997
Boissiere F., Neuroreport, 1997
Lukiw WJ., J. Neurosci. Res, 1998

- NF-κB is activated by acetylation on Lys122 by the histone acetyl transferase (HAT) PCAF.
- 2 publications from Yoon H. group demonstrating reversal of AD cognitive deficits in in vivo mouse models by small molecule inhibitors of PCAF (HAT).



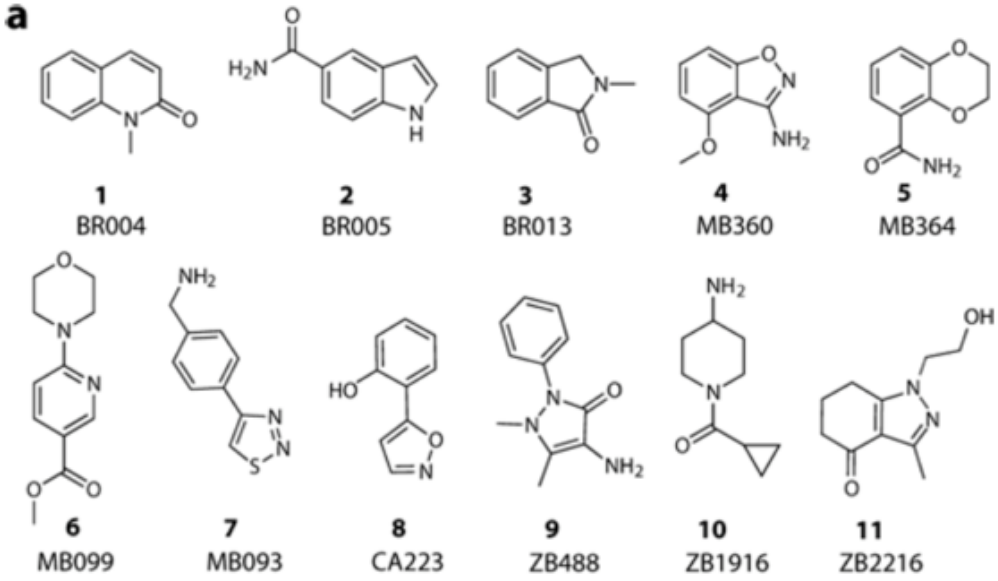
PCAF Fragments



BR004

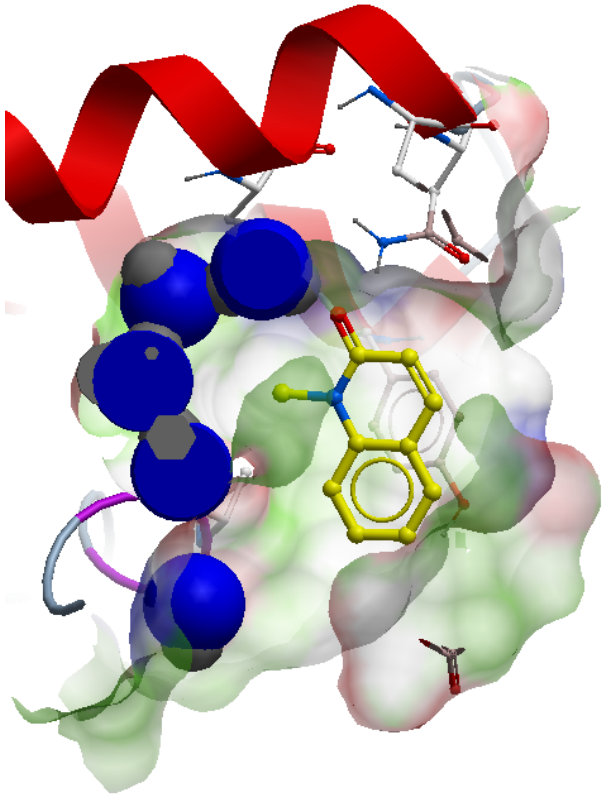
PCAF ITC Kd 320 µM

a



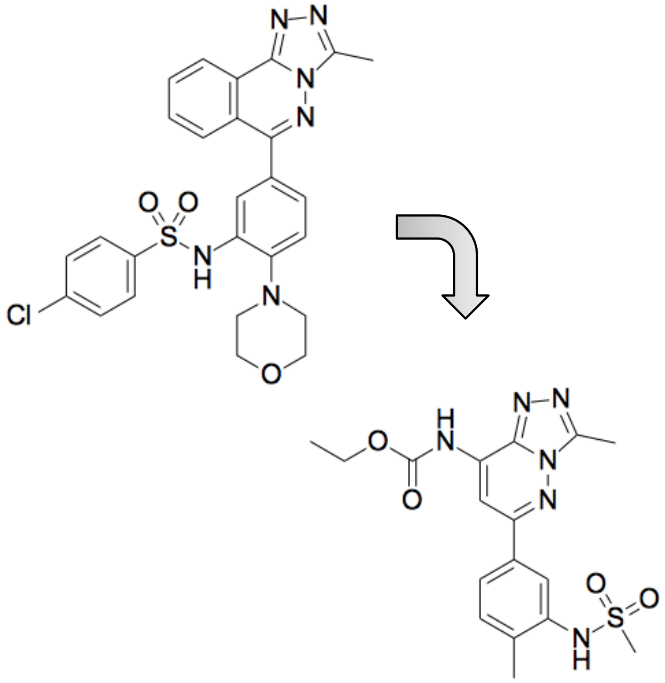
b

fragment	1	2	3	4	5	6	7	8	9	10	11
ΔTm (°C) at 1 mM (± SEM)	2.0 (0.5)	2.0 (0.4)	1.5 (0.5)	0.8 (0.4)	0.8 (0.3)	0.7 (0.3)	1.0 (0.4)	1.3 (0.8)	0.6 (0.4)	1.0 (0.4)	0.9 (0.3)

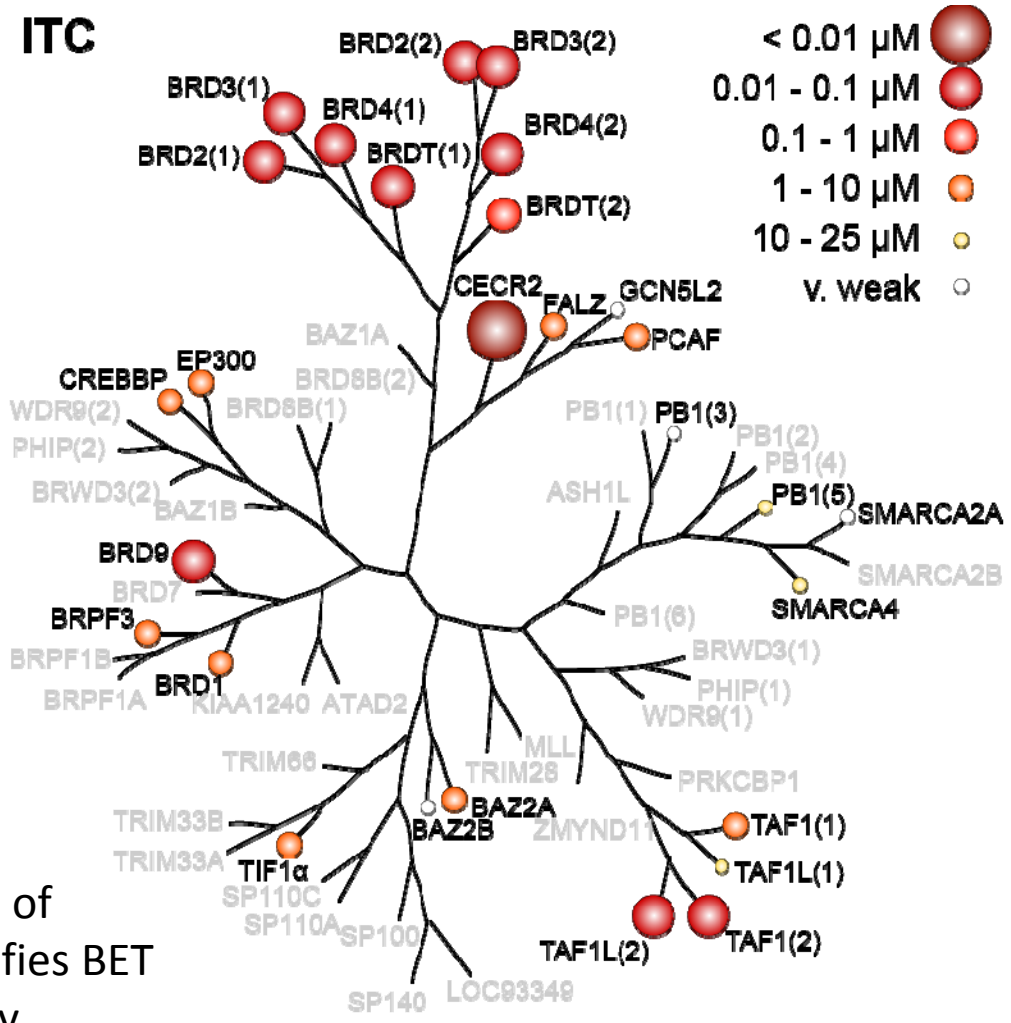


Chaikuad, A. et al. Structure-Based Identification of Inhibitory Fragments Targeting the p300/CBP-Associated Factor Bromodomain. J. Med. Chem. (2016). doi:10.1021/acs.jmedchem.5b01719

BROMOSPORINE

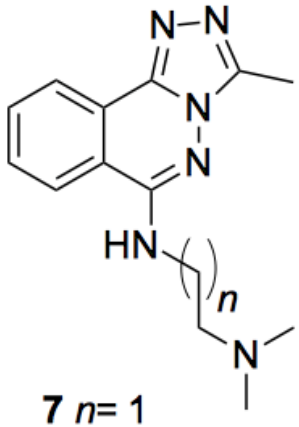


Bromosporine
PCAF ITC K_D 4.7 µM

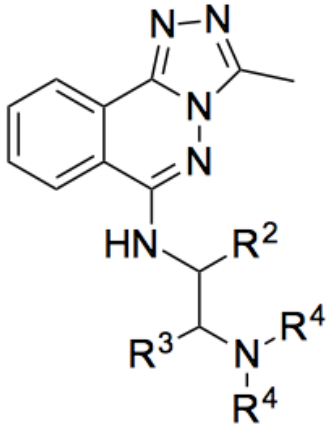


Picaud, S.; et al., Promiscuous targeting of bromodomains by bromosporine identifies BET proteins as master regulators of primary transcription response in leukemia. *Science Advances* **2016,** *2* (10)

PCAF SAR



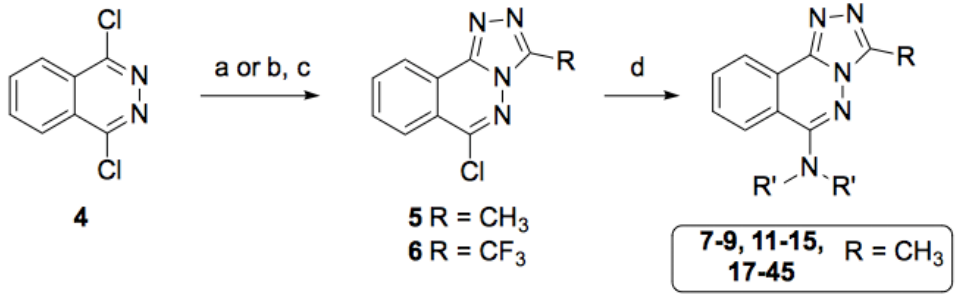
7 *n*= 1
8 *n*= 2
9 *n*= 3



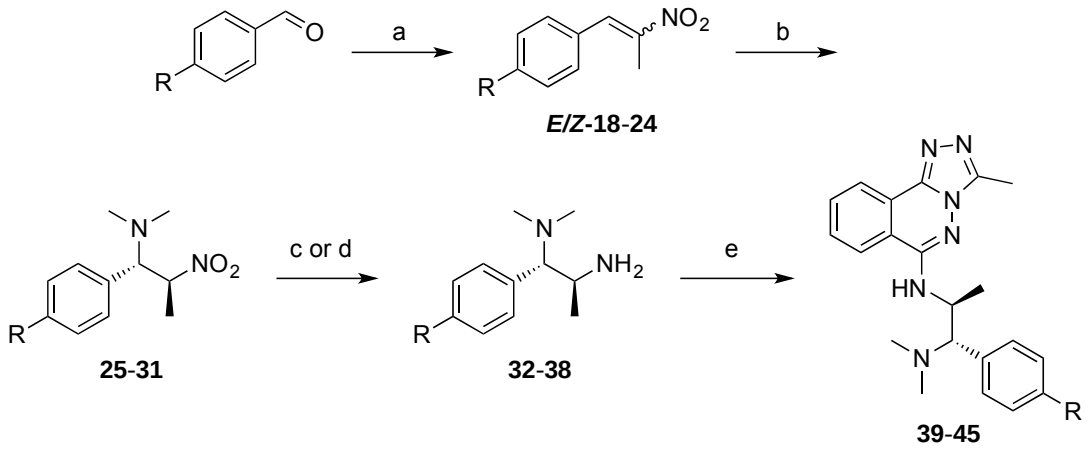
10-17

	R^2	R^3	R^4	n	ΔT_m°C[a]	K_D (μM)
7	H	H	Me	1	8.5[b]	8.0
8	H	H	Me	2	ND	>30
9	H	H	Me	3	ND	>30
10	H	Ph	Me	1	1.7	1.0
11	Me	H	Me	1	5.6	0.30
(S)-11	Me	H	Me	1	7.4	0.28
12	Et	H	Me	1	3.3	1.8
13	iBu	H	Me	1	0.85	>30
14	Me	H	Et	1	0.0	>30
15	H	Me	Me	1	4.6	7.3
16	H	Et	Me	1	ND	6.9

Synthesis of Triazolophthalazines

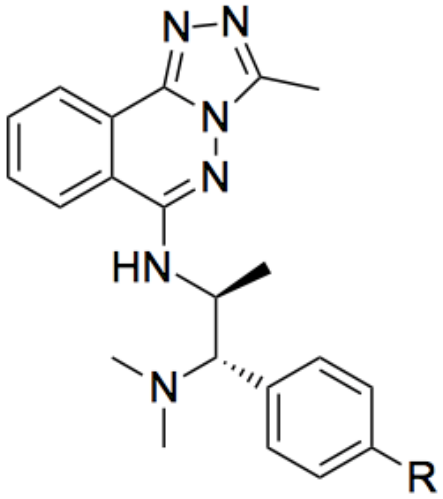


Reagents and conditions: a) Acetohydrazide, DMF 120°C 16 h, 62%; b) $N_2H_4.H_2O$, EtOH, 120°C, 10 mins, *quant*.; c) TFA, 100°C, 2 h, 43%; d) R'_2NH (1.5-2.0 eq) KI (0.1 eq), HCl (0.05 eq), EtOH or *i*PrOH, reflux, 3 days 8-94%.



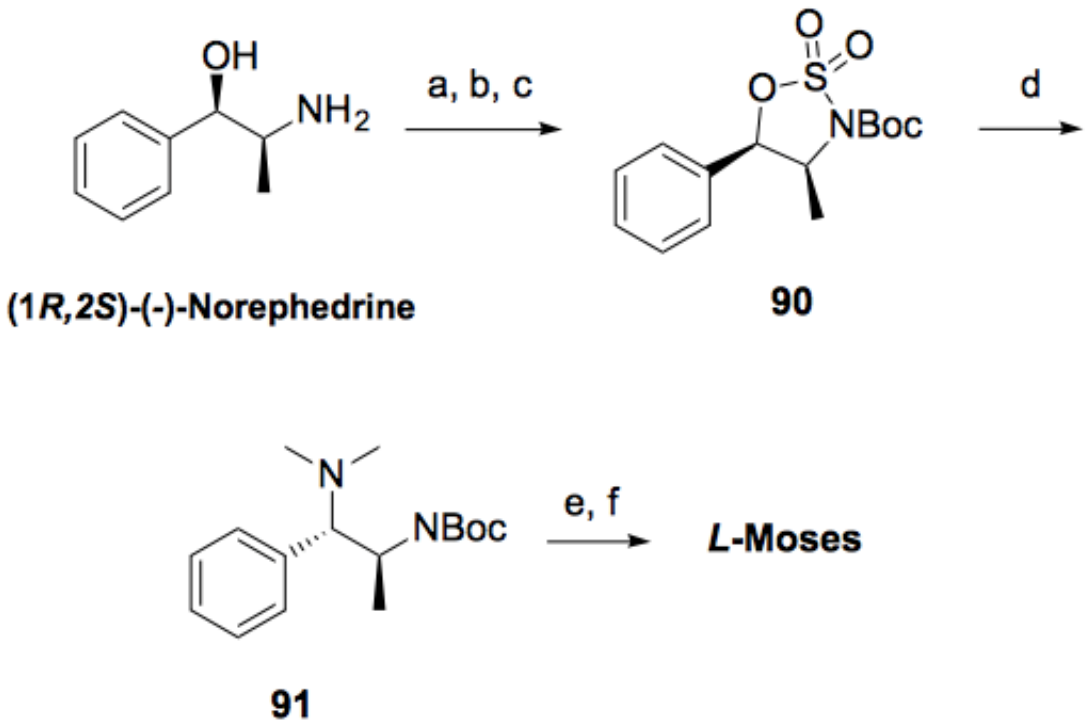
Reagents and conditions: a) NH_4OAc (0.2 eq), $EtNO_2$, reflux, 1:1 *E/Z, quant.*; b) Me_2NH (5 eq), THF, rt 16 h, *dr* 4.6:1 - 33:1; c) H_2 (1 atm), Pd/C (10%), MeOH, rt, 16 h, 11-15% over two steps, single diastereomer; d) H_2 (1 atm), Ra/Ni (0.3 eq), MeOH, rt, 16 h, 25-28%, over two steps, single diastereomer; e) **5** (0.8 eq) KI (0.1 eq), HCl (0.05 eq), EtOH or *i*PrOH, reflux, 3 days 16-79%.

PCAF SAR



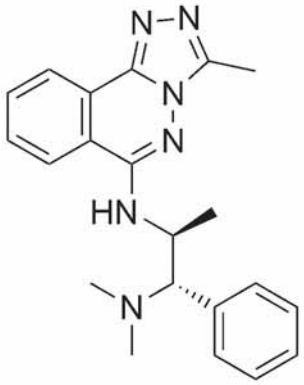
	R	Stereo	K_D (nM)
39	F	*rac-(1S*, 2S*)*	195
40	CO_2Me	*rac-(1S*, 2S*)*	133
41	Me	*rac-(1S*, 2S*)*	160
42	Cl	*rac-(1S*, 2S*)*	223
43	CF_3	*rac-(1S*, 2S*)*	163
44	OMe	*rac-(1S*, 2S*)*	179
45	H	*rac-(1S*, 2S*)*	168
***L*-45**	H	*(1S, 2S)*	126
***D*-45**	H	*(1R, 2R)*	Inactive

Asymmetric Synthesis of *L*-45



Reagents and conditions: a) Boc_2O, DIPEA, CH_2Cl_2, rt, 16 h, 51% b) $SOCl_2$, Pyridine, MeCN, 2 h, -40°C to 0 °C c) $NaIO_4$ (1.5 eq), $RuCl_3.3H_2O$ (0.05 eq), MeCN, 1 h, 0 °C, 48% (over two steps); d) Me_2NH (3 eq), THF, rt 16 h, 63%; e) TFA, CH_2Cl_2, *quant.*; f) **5** (0.8 eq) KI (0.1 eq), HCl (0.05 eq), iPrOH, reflux, 3 days, 30%.

L-Moses



***L*-45** (*L*-Moses)

PCAF ITC K_D 126 nM

HTRF K_i 47 nM

GCN5 ITC K_D 600 nM

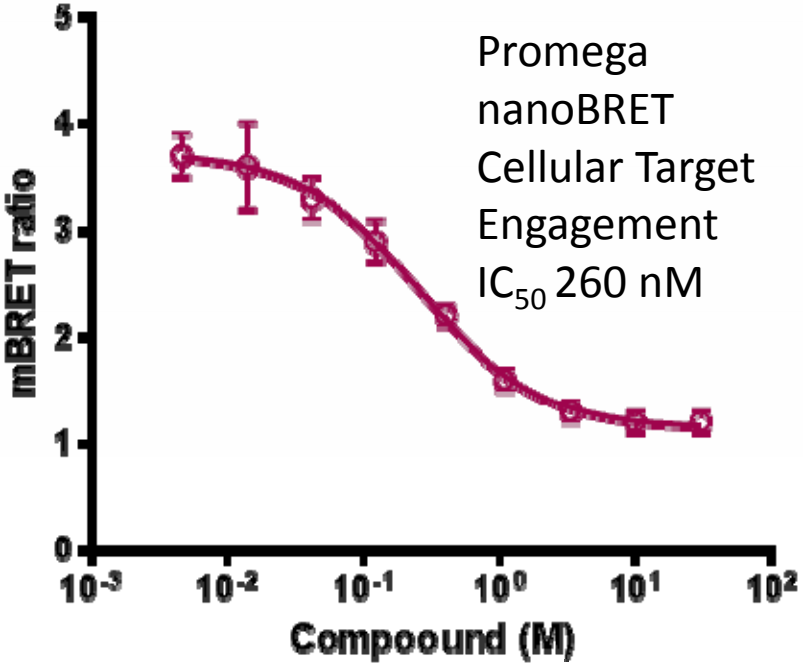
ΔTm BRD4(1) <0.4 °C

>4500-fold BRD4 selectivity

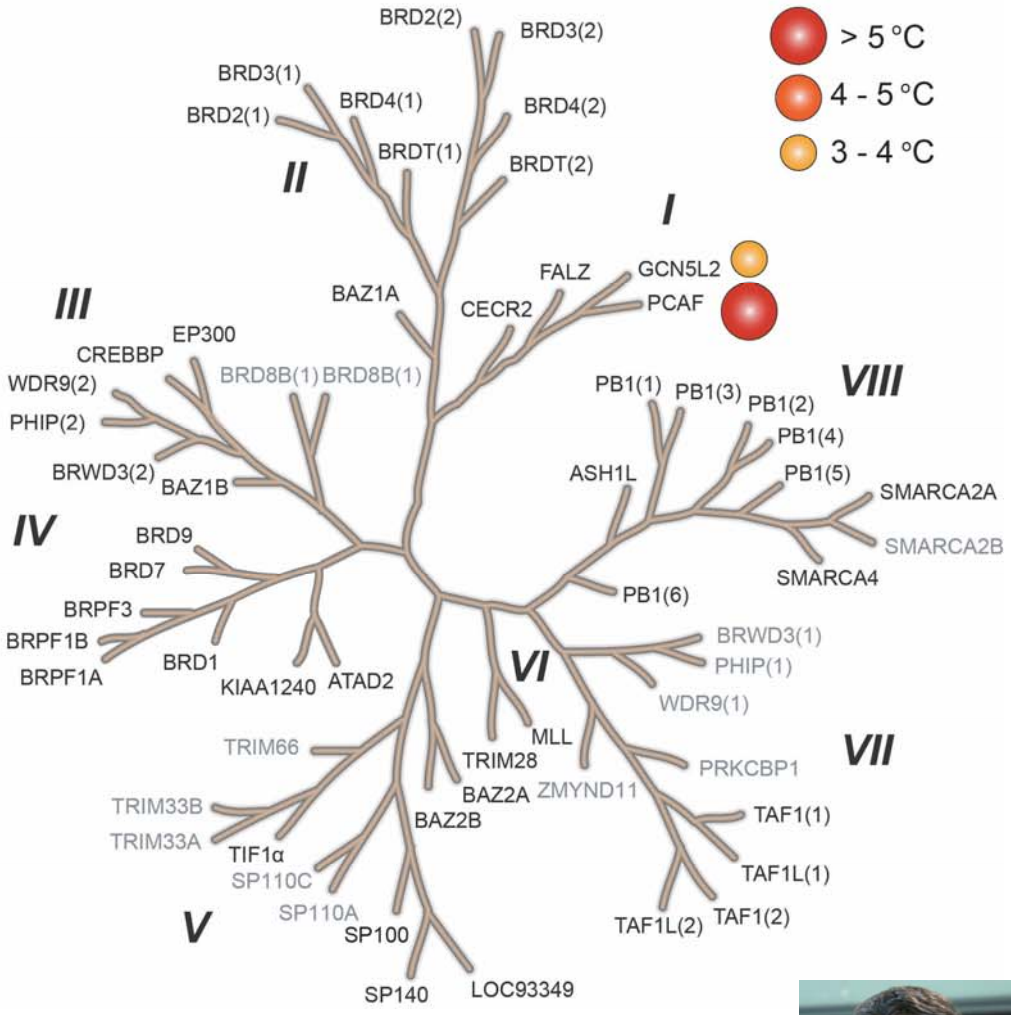
clogD[a] 1.0; LE[b] 0.35

HLM/MLM $t_{1/2}$ (min) 40/38

MDCK-MDR1 (10^{-6} cm/s) 59/70

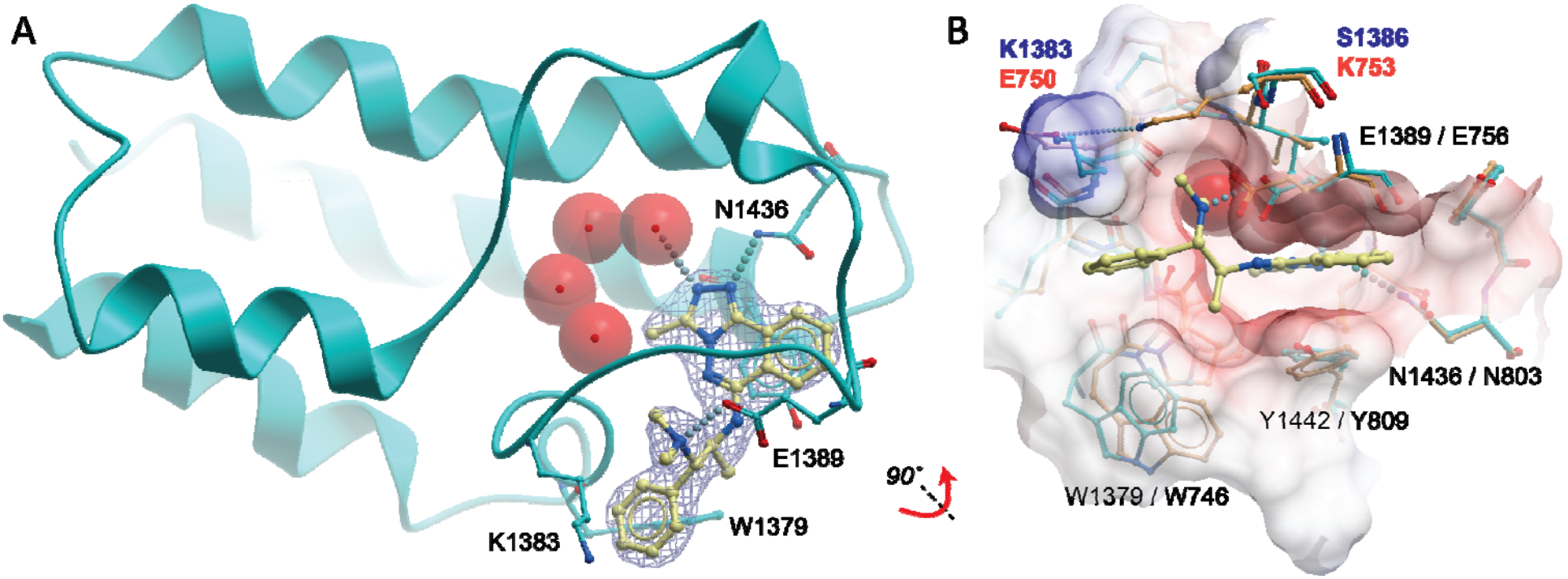


Promega nanoBRET Cellular Target Engagement IC_{50} 260 nM



Moses Moustakim
Darren Dixon
Angewandte Chemie *in press*

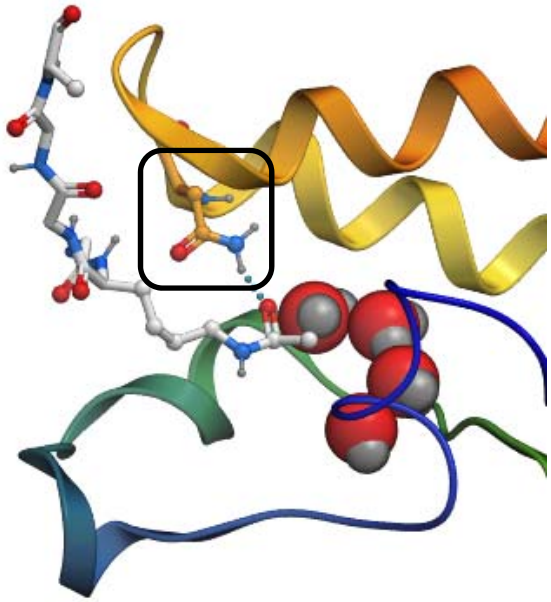
L-Moses in Pf-GCN5

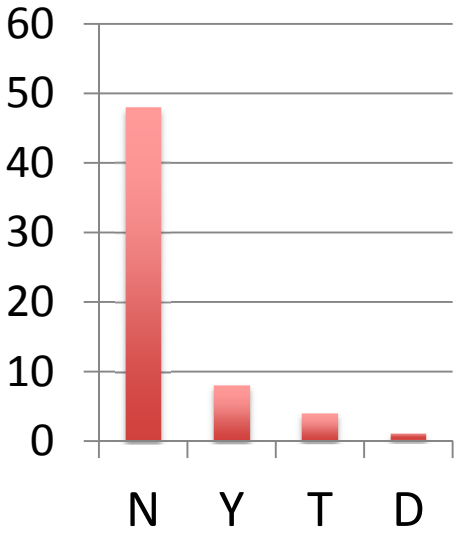


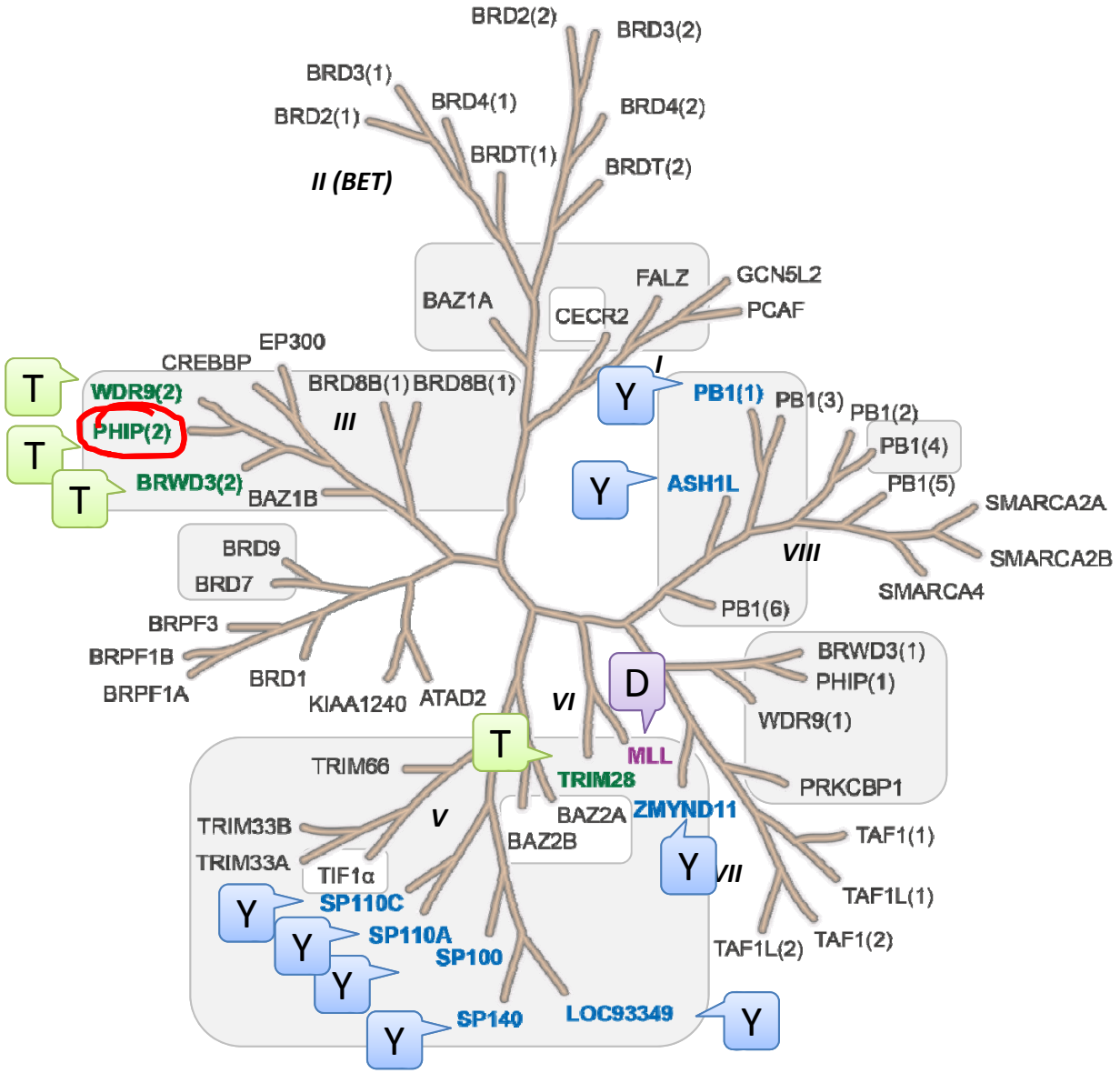
Human PCAF

Pf GCN5

Atypical Bromodomains







PHIP Background

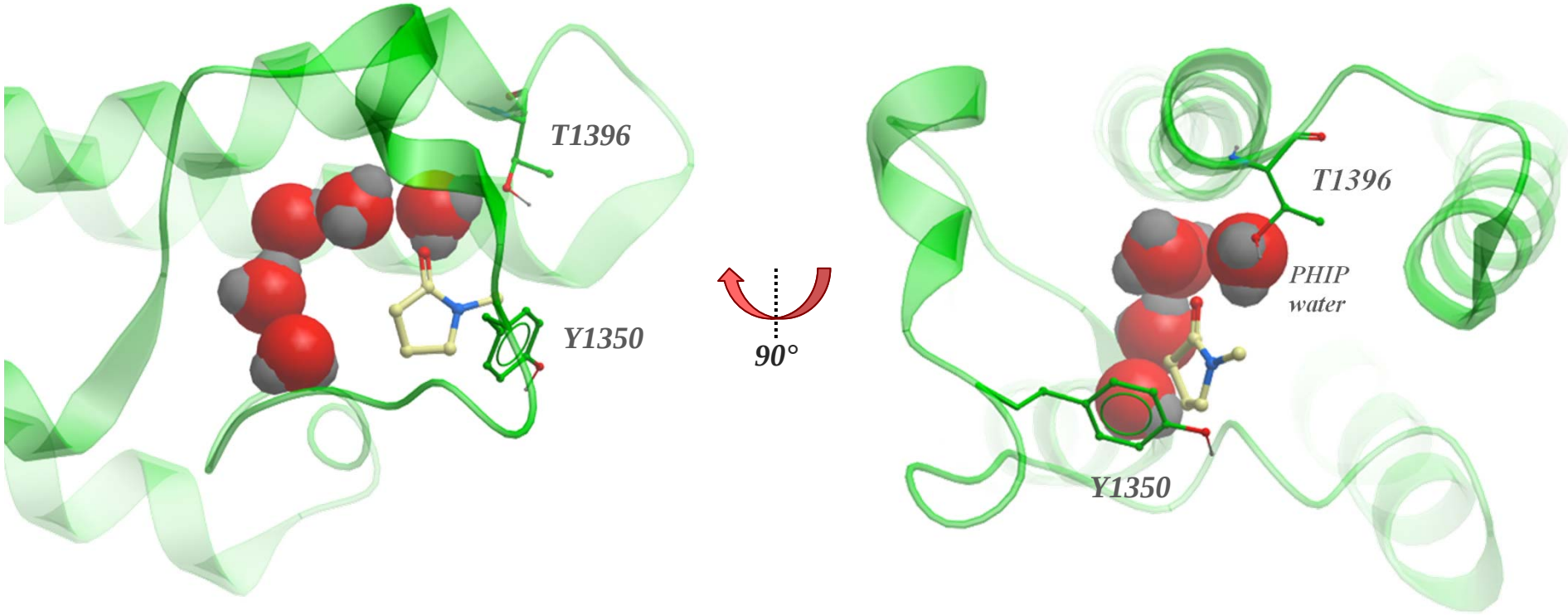
BRWD1-3 Family (Bromodomain and WD repeat-containing proteins)

PHIP (BRWD2) contains a WD40 repeat (Kme binder) and 2 Bromodomains (Kac binder)

"...identified PHIP as the gene most highly overexpressed in metastatic melanomas, compared with primary tumors…" [1]
"…activation of Pleckstrin homology domain-interacting protein (PHIP), promotes melanoma metastasis, can be used to classify a subset of primary melanomas, and is a prognostic biomarker for melanoma." [2]
"Elevated PHIP copy number was associated with significantly reduced distant metastasis–free survival (P = 0.01) and disease specific survival (P = 0.009) by Kaplan-Meier analyses"
"…important role for PHIP as a molecular marker of melanoma ulceration, metastasis and survival…" [3]

(1) Haqq, C.; et al. The gene expression signatures of melanoma progression. *PNAS*, **2005**, *102* (17), 6092–6097. (2) De Semir, et al., Pleckstrin homology domain-interacting protein (PHIP) as a marker and mediator of melanoma metastasis. *Proc. Natl. Acad. Sci. U. S. A.*, **2012**, *109* (18), 7067–7072. (3) Bezrookove, V.; et al. Prognostic impact of PHIP copy number in melanoma: linkage to ulceration. *J. Invest. Dermatol.*, **2014**, *134* (3), 783–790.

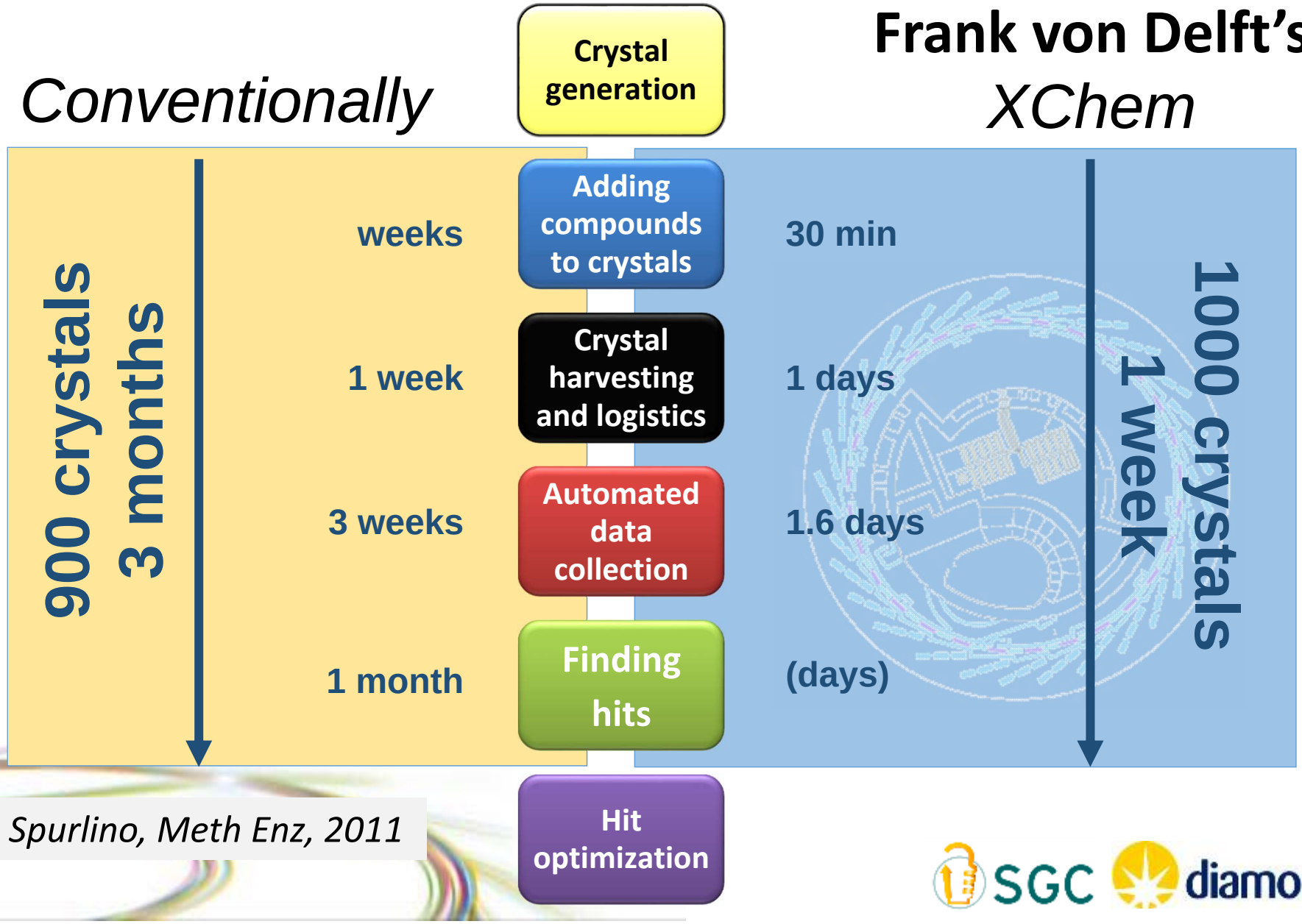
PHIP(2) is an Atypical BRD



PHIP(2) previously impervious to hit discovery, including screening Maybridge fragment library by AlphaScreen at 500 µM.

3MB3

XChem at I04-1 Beamline: Rapid Xtal Fragment Screening



XChem Advantages

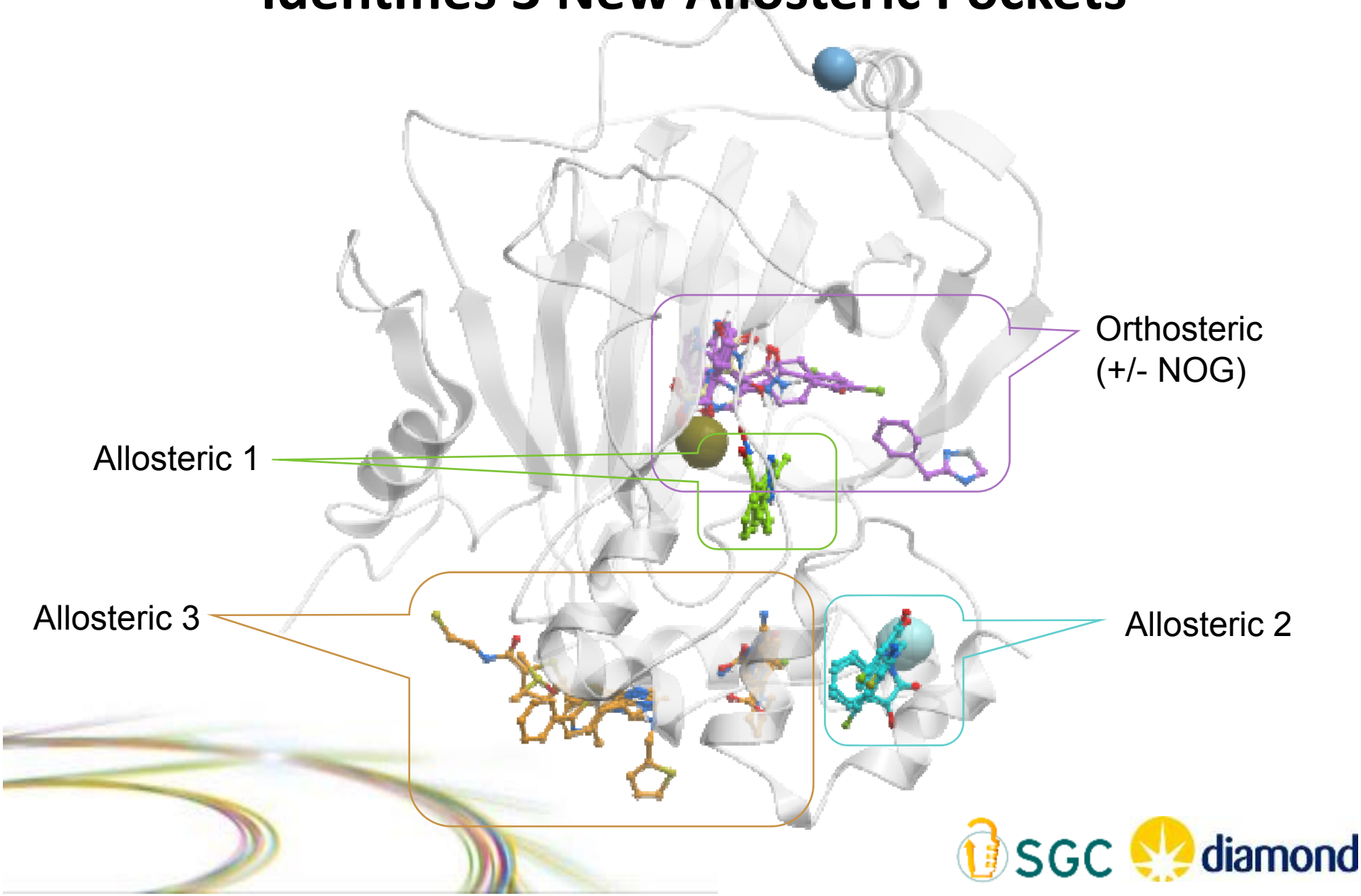
	Classical HTS	Biophysical Fragment Screen	XChem Fragment Screen
Concentration	1 - 10 µM	100-1,000 µM	1000 – 200,000 µM
Hit potency	Low – Med uM	High uM	Low - ? mM
Hit rate	Low	Medium	High
Structure	No	No*	Yes §

* 2D-NMR is the exception

§ Immediate identification of allosteric binders

SGC diamond

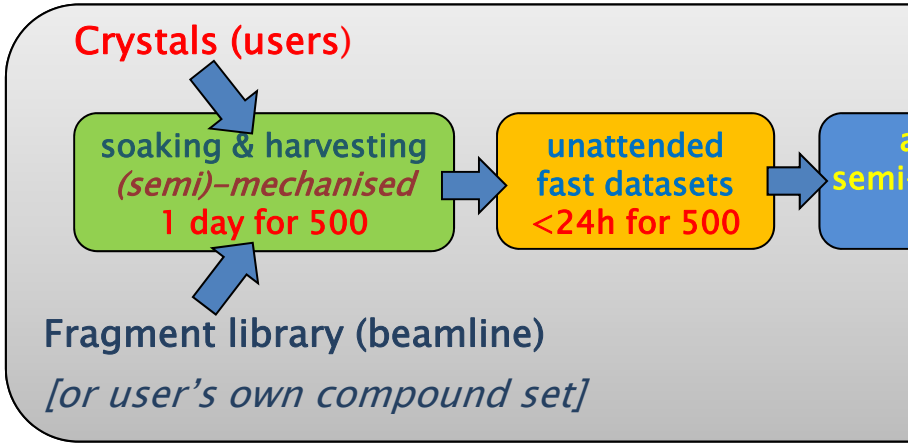
High Concentration Fragment Soaking in KDM4D Identifies 3 New Allosteric Pockets



SGC diamond

How XChem Facility Works

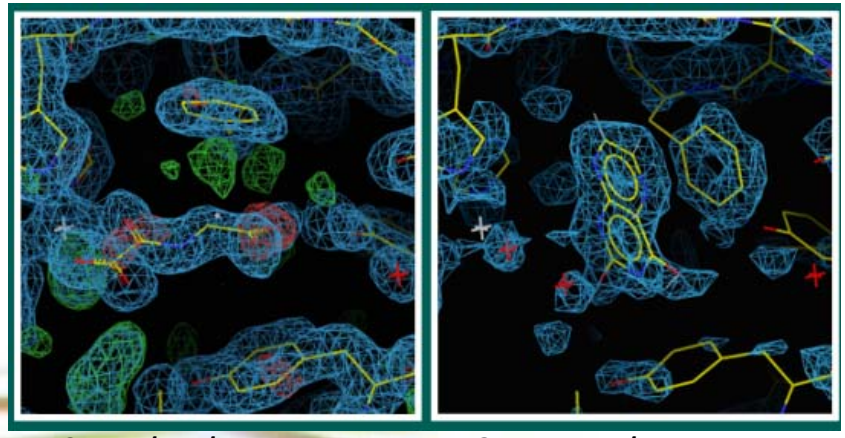
- Apply for time: Peer-reviewed
  - Science case
  - Technically feasible: good crystals
  - Can progress: assays
  - Can progress: chemistry support
- Industry: access at cost – Industrial Liaison Group
  - No IP for Diamond



XChem Highlights

- Regular users since September 2015
- Officially opened: November 2015 (media *etc*)
- Users to date: 12 academic, 7 industry
- Targets: >30. Crystals: >28,000. Hits: >>250

PANDDA: 3D background correction



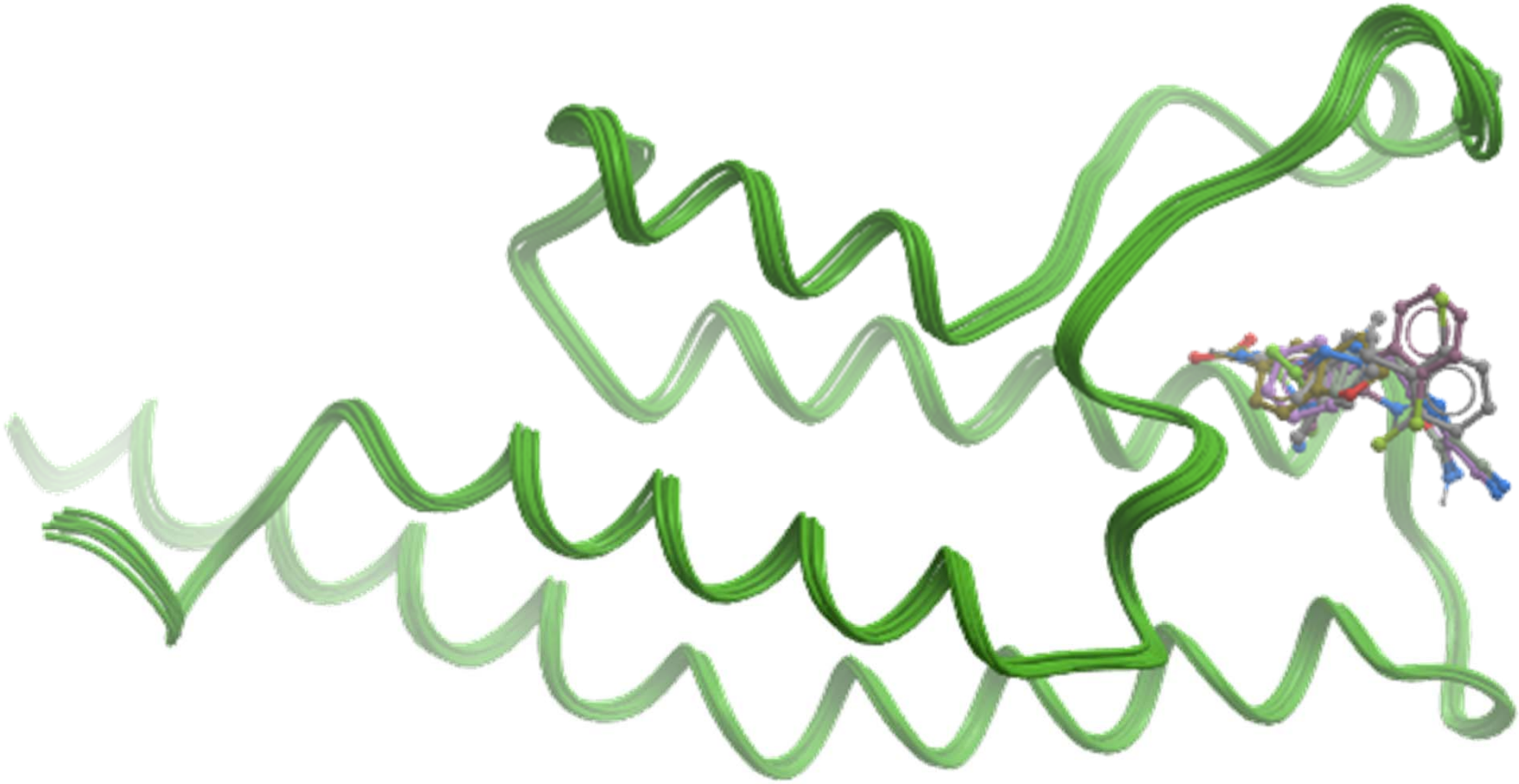
Standard maps *Corrected maps*

New paradigm in crystallography

Shifter: robot-assisted crystal harvesting

Up to 200 crystals / per hour
Fully recorded experiments

9 PHIP(2) Hits from XChem

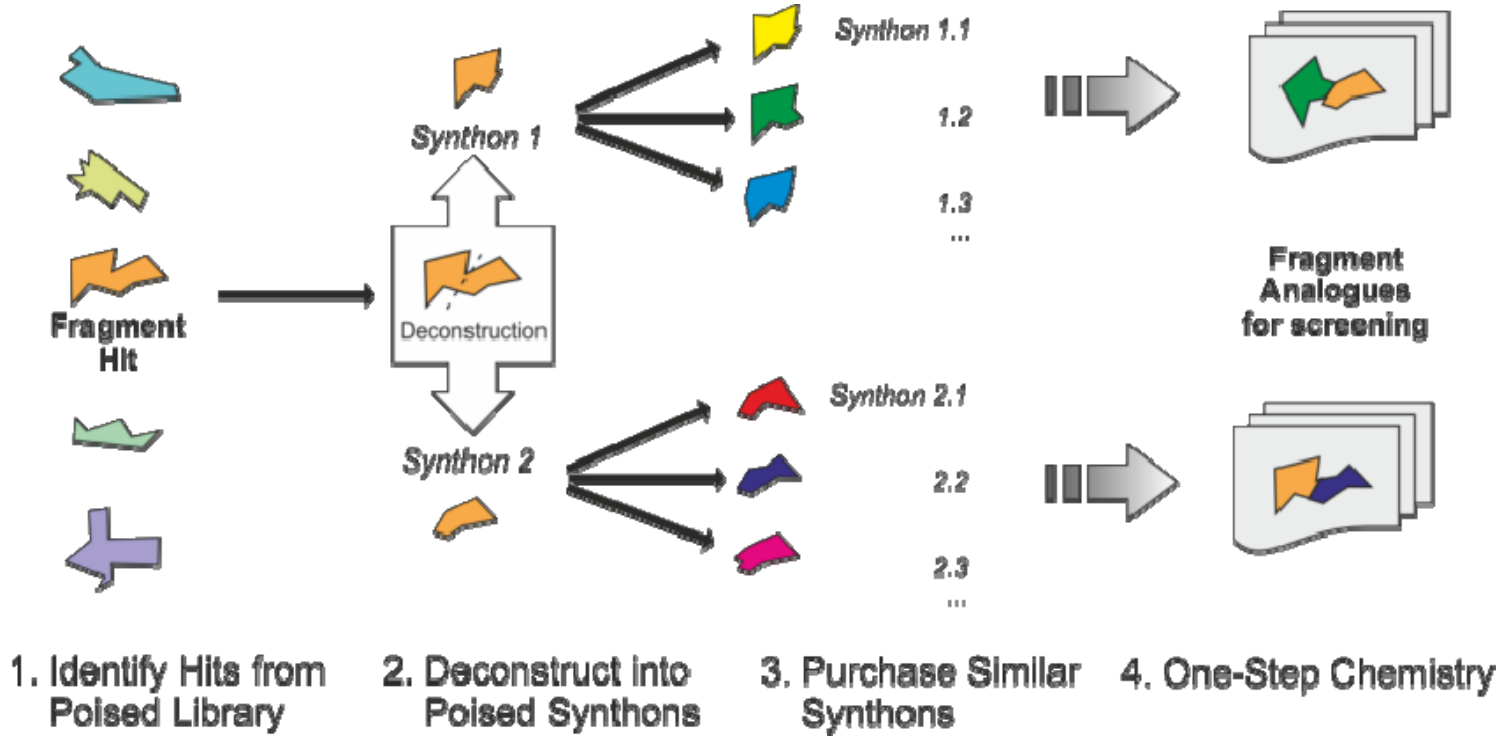


Now What?

- Typically: grow, merge or link fragment hits.
- But hits need to be on-scale in activity assay.
- XChem hits may be too weak for assays.

SGC diamond

Poised Fragments: med chem with weak XChem hits



Poised reactions are:

- robust and reliable
- make drug-like products
- be possible using commercially available starting points
- compatible with a range of substrates

Poised fragments:

- Allow optimization of weak XChem hits
- Relies on comp chem
- Provides scope for compound design algorithm testing

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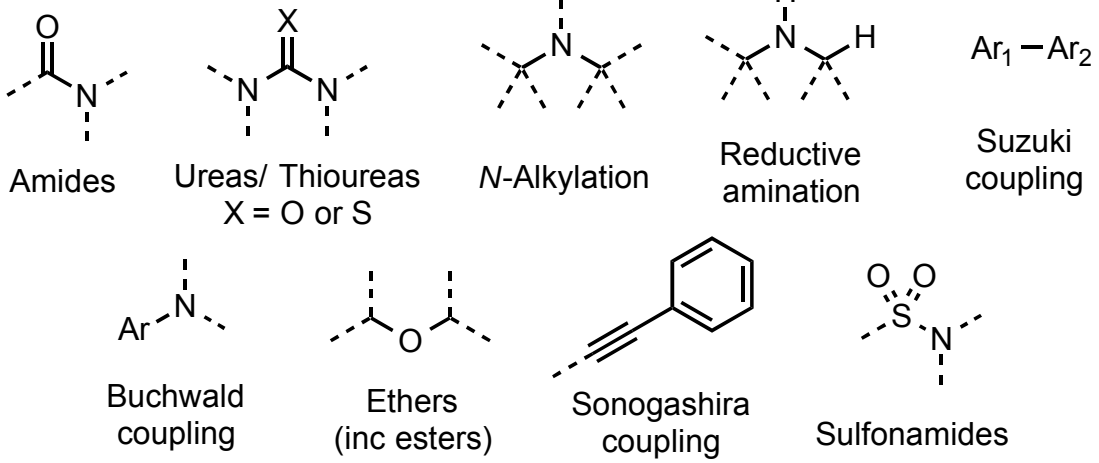
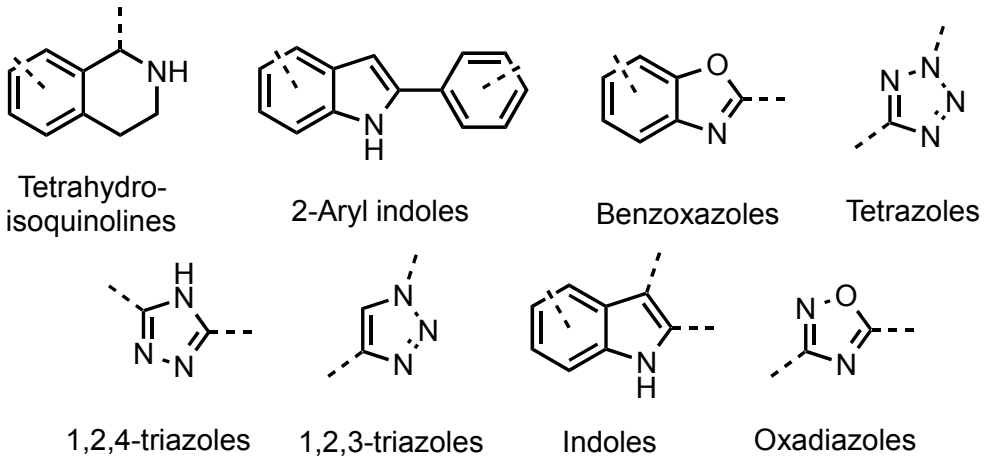
Poised Chemical Scaffolds

Most commonly used reactions in medicinal chemistry:
Roughley, S. D. *et al.*, *J. Med. Chem.,* **2011,** 54, 3451–3479



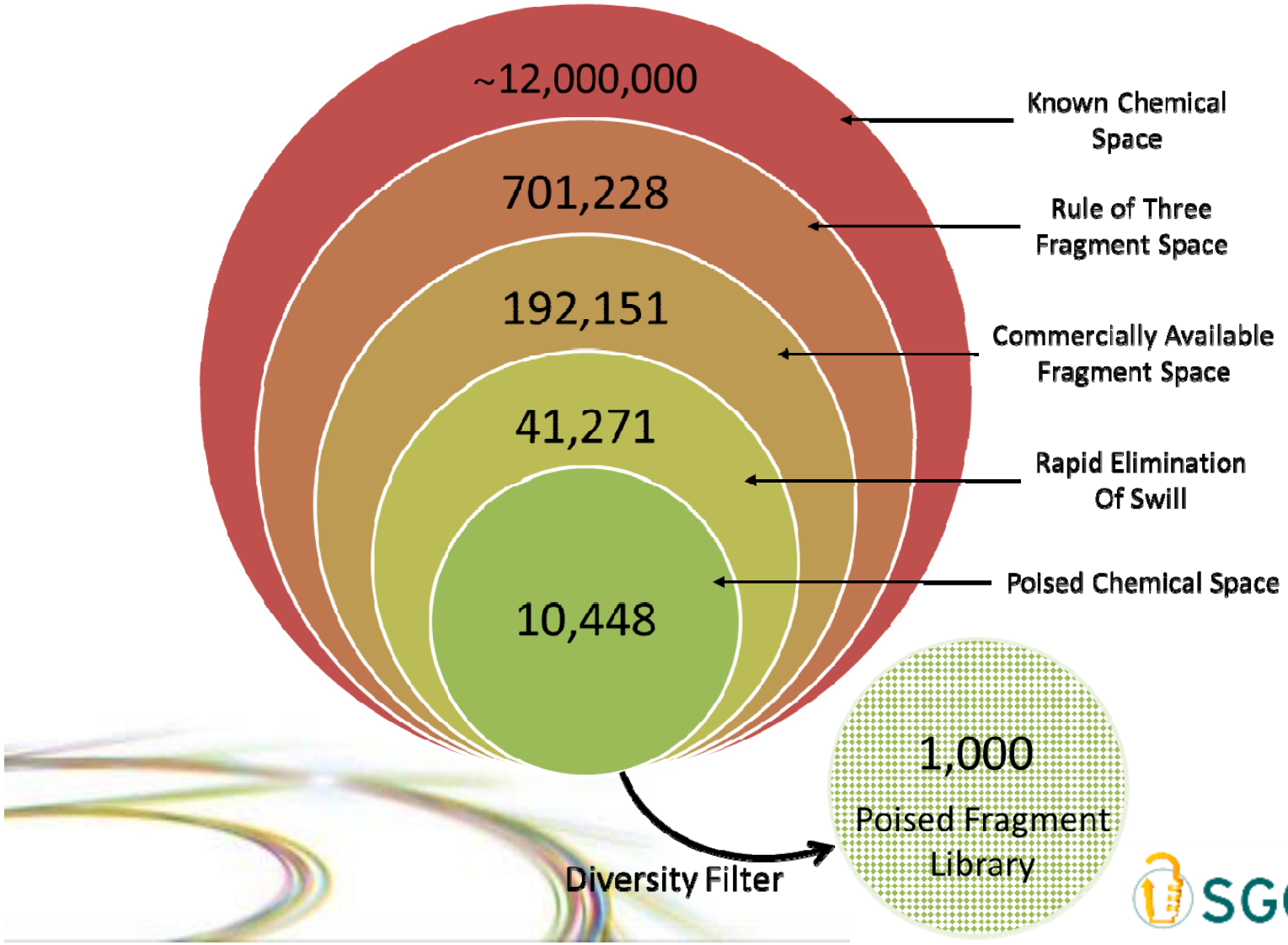
- Reliable reactions
- Tolerant of a range of substrates
- Examples of both synthons available

N–Containing Heterocycles:
Hartenfeller M. *et al.*, *J. Chem. Inf. Model.,* **2012,** 52, 1167–1178



DPSL: Diamond/SGC Poised Library

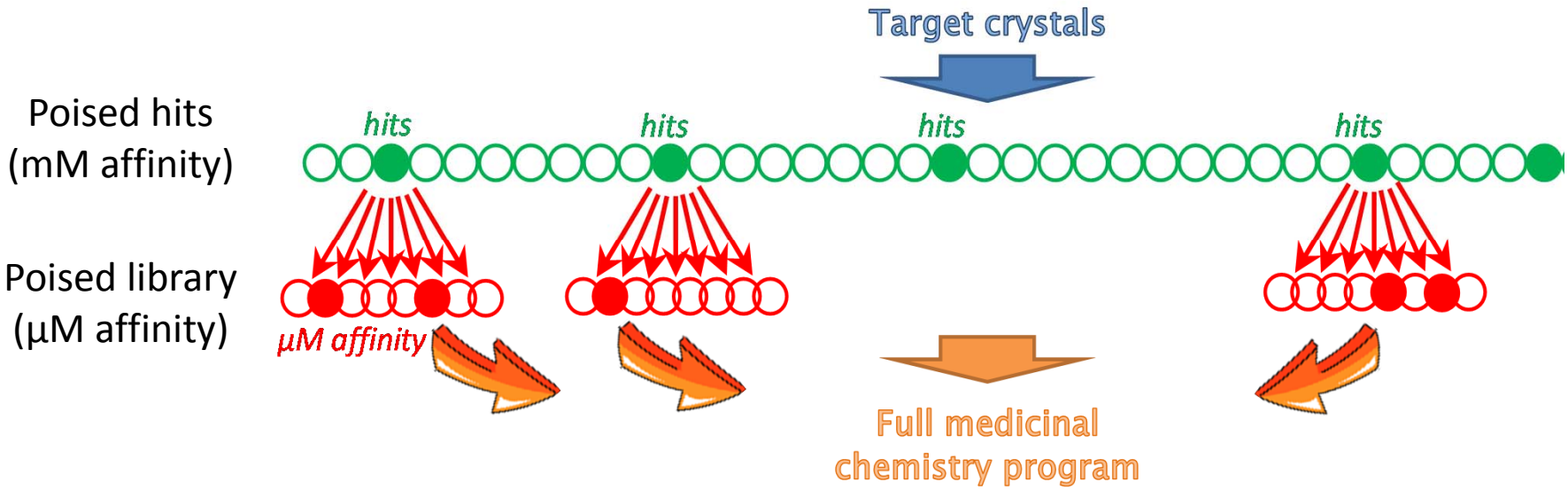
Properties: small, soluble, diverse



Oakley Cox

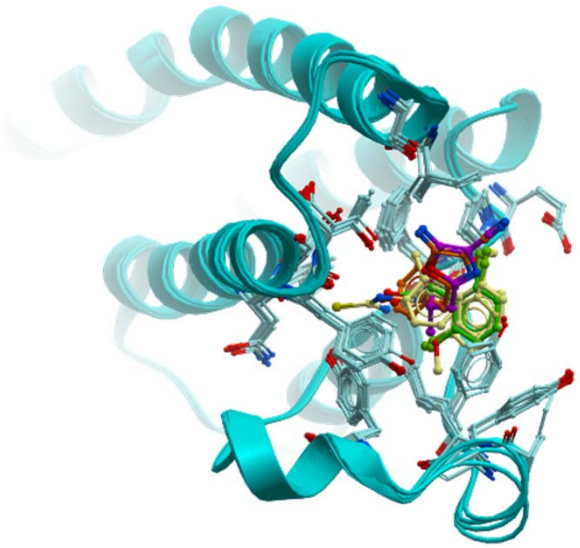
SGC diamond

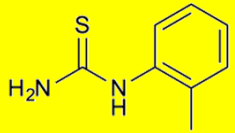
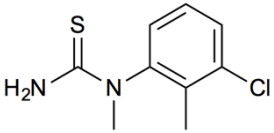
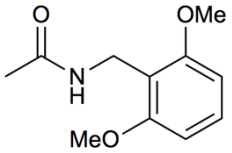
Philosophy of Hit Follow-Up



SGC diamond

PHIP(2) Hits



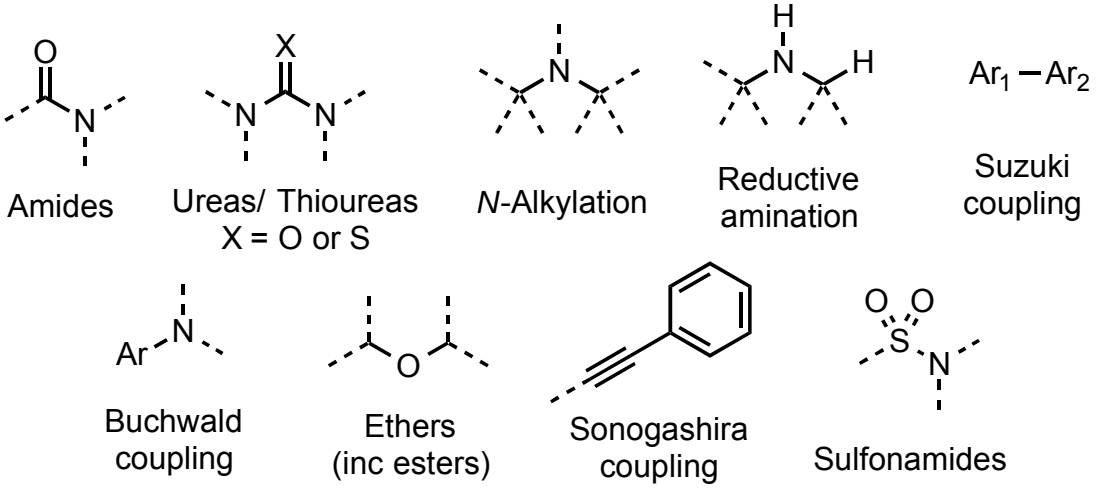
ID		IC_{50} (µM)
XST942		768
FMOOA463		68
FMOMB76b		>5000
FMOOA322a		190

Cox, O. B.; et al., *Chem. Sci.*, **2016**, 7, 2322-2330

SGC diamond

Poised Fragments are…

Most commonly used reactions in medicinal chemistry:
Roughley, S. D. *et al.*, *J. Med. Chem.,* **2011,** 54, 3451–3479



N–Containing Heterocycles:
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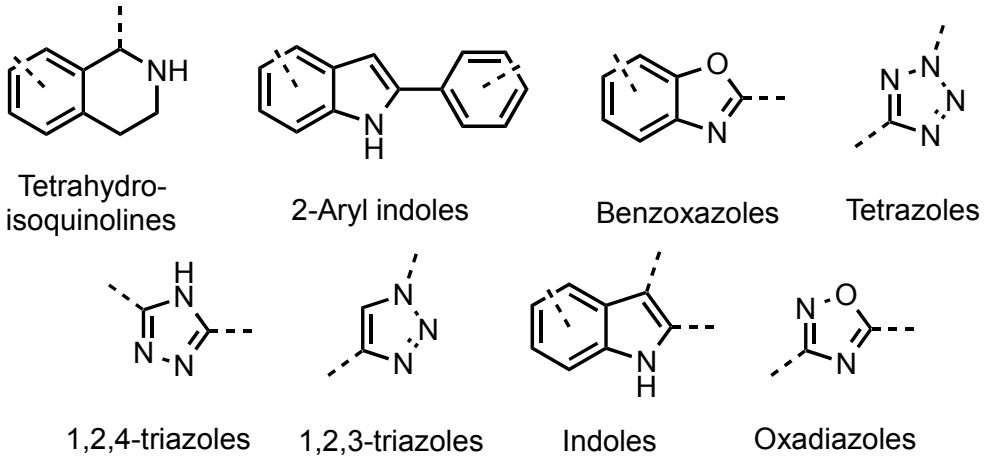

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Poised Fragments are…

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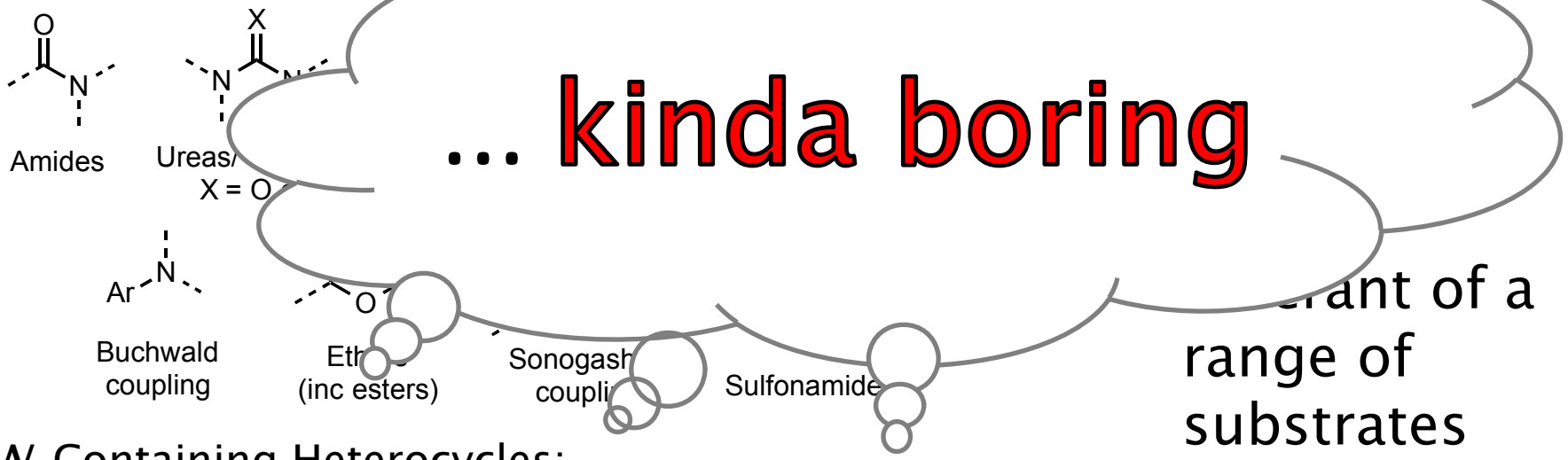
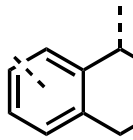
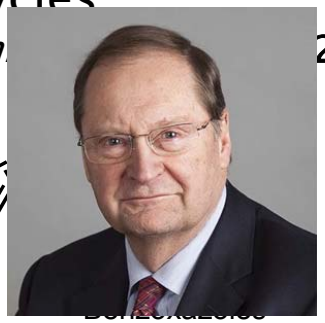
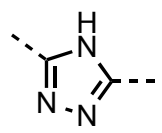


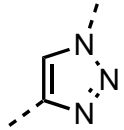
… kinda boring

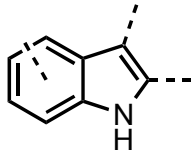
N–Containing Heterocycles:
Hartenfeller M *m* 2, 8

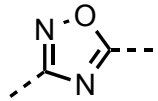

Tetrahydro-isoquinolines




1,2,4-triazoles


1,2,3-triazoles


Indoles

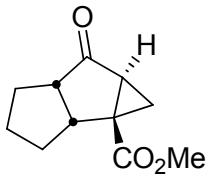

Oxadiazoles

…rant of a range of substrates

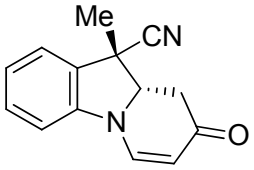
- Examples of both synthons available

Potential Martin D. Smith Fragments

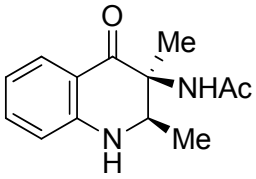
Fragments



14 HA



17 HA



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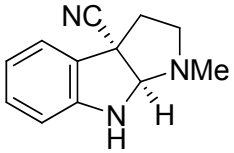

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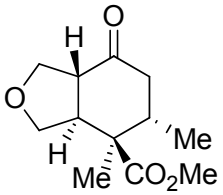

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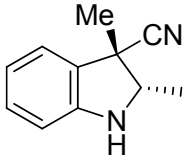
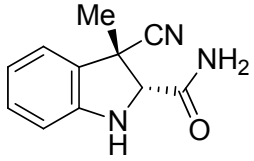


13 HA



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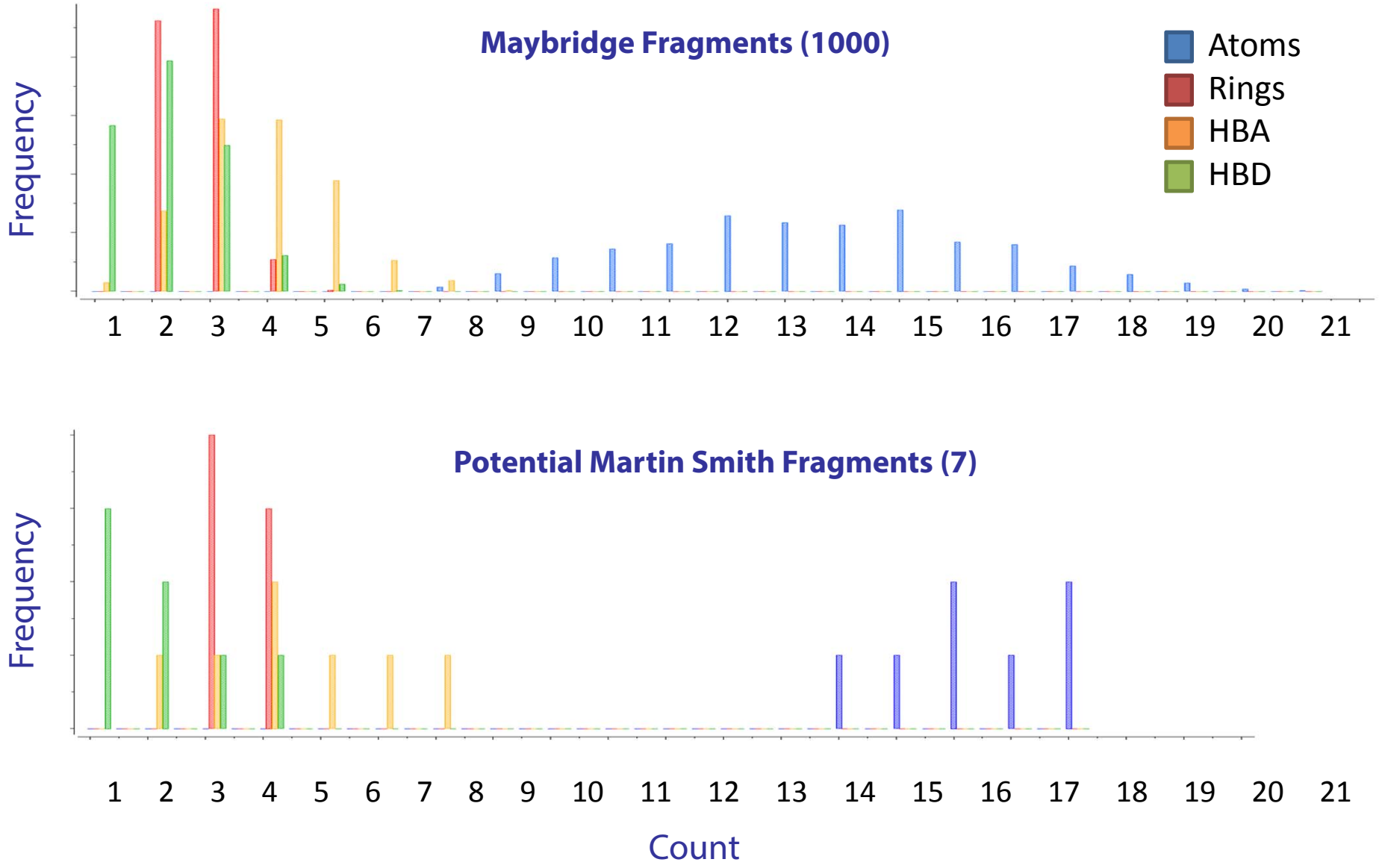
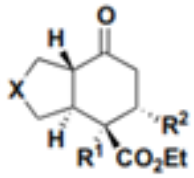
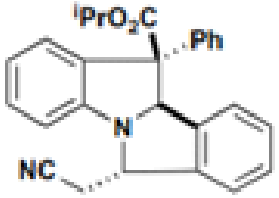
Physicochemical Properties

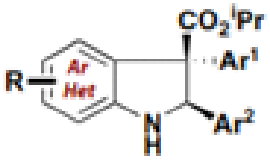


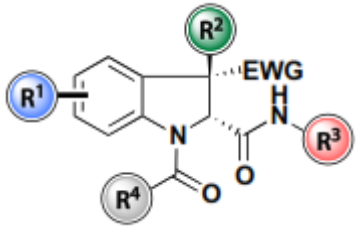
Expanding Fragment Hits

Tier2: Analogues

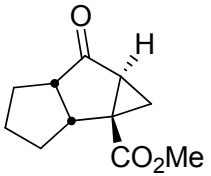








Tier 1: Fragments



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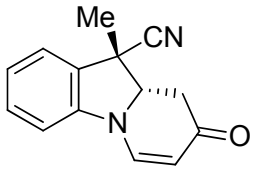

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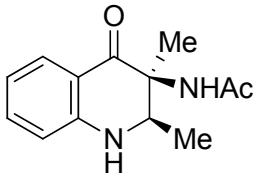

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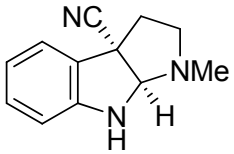

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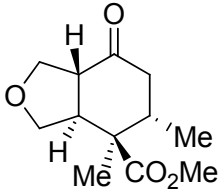

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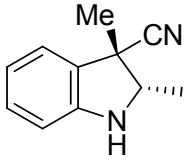

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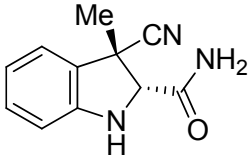

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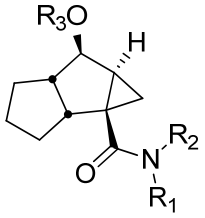
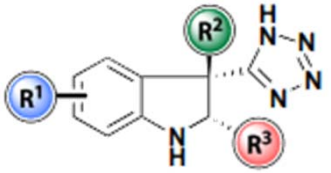
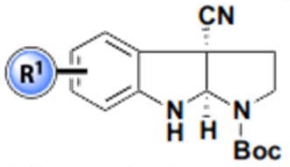
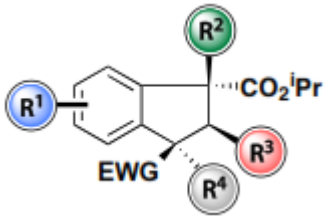
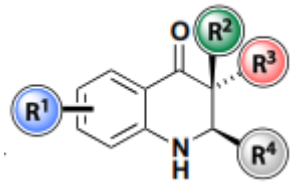
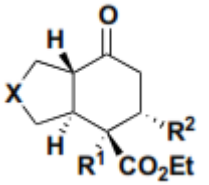




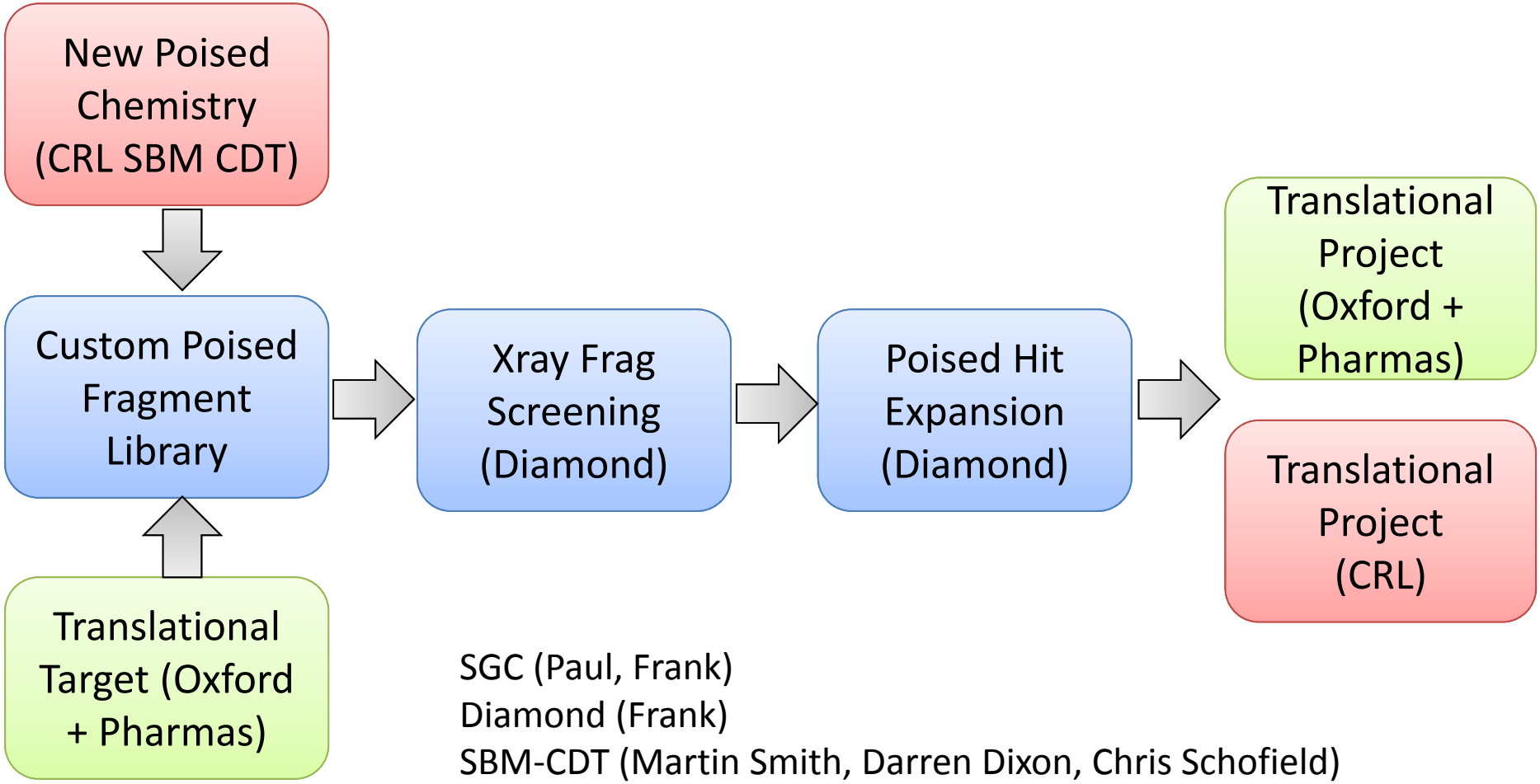








X-Chem Project
(John Fell Fund/Wellcome ISSF Grant)



SGC (Paul, Frank)
Diamond (Frank)
SBM-CDT (Martin Smith, Darren Dixon, Chris Schofield)

Symposium: “Future of Fragments”

- **28-29 November** (Mon-Tue) @ Diamond, UK
  - Noon-to-noon, evening for discussions

- Programme
  - Session 1: Fragment Screening & Assays
  - Session 2: Computational Methods
  - Session 3: New Synthetic Methods
  - Session 4: Automated Chemistry

SGC diamond

Acknowledgements

SGC Epigenetics Oxford

Chemistry
Oakley Cox
Moses Moustakim

Screening
Oleg Fedorov
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James Bennett
Catherine Rogers

Crystallography
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Tobias Krojer
Romain Talon
Nathan Wright
John Raynor
Catrine Johansson
Carina Gileadi
Kannan Velupilla
Anthony Bradley

Biotech
Nicola Burgess-Brown
Aleksandra Szykowska
Pavel Savitsky

Other

Tiger Frystone (Reigate Grammar)
Max Brennan (St Edwards Oxford)
Jag Heer (UCB)

Many more…

SGC Toronto

Ray Hui
Chun-Feng D. Hou
Yu-Hui Lin[
John R. Walker

Promega

Danette Daniels
Jacqui Mendez-Johnson

ARUK ODDI

Matthias Ehebauer
Emma Murphy
John Davis
Elena Di Daniel

Diamond Light Source

Frank von Delft
Patrick Collins
Jose Brandao-Neto
Alice Douangamath

Oxford Chemistry

Darren Dixon
Peter Clark
Laura Trulli
Martin Smith

U. Sussex

John Spencer
Jahangir Amin

SGC Oxford

Apirat Chaikuad
Sarah Picaud
Panagis Filippakopolous
Stefan Knapp
Brian Marsden
Chas Bountra
Kilian Huber

The SGC is a registered charity (number 109773 7) that receives funds from AbbVie, Bayer Pharma AG, Boehringer Ingelheim, Canada Foundation for Innovation, Eshelman Institute for Innovation, Genome Canada, Innovative Medicines Initiative (EU/EFPIA [ULTRA-DD grant no. 115766], Janssen, Merck & Co., Novartis Pharma AG, Ontario Ministry of Economic Development and Innovation, Pfizer, São Paulo Research Foundation-FAPESP, Takeda, and Wellcome Trust [092809/Z/10/Z]. M. M. is supported by the EPSRC Centre for Doctoral Training in Synthesis for Biology and Medicine (EP/L015838/1). P.

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