

Allosteric modulation of transcription factor complexes

*Anna Mapp
University of Michigan
26 September 2016*

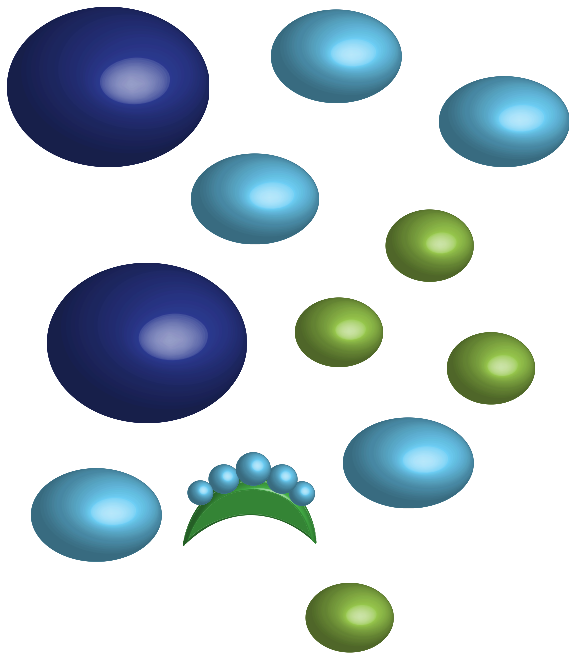


Everyday Machines

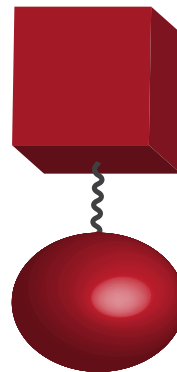
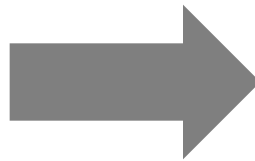


Transcription machine reads the DNA

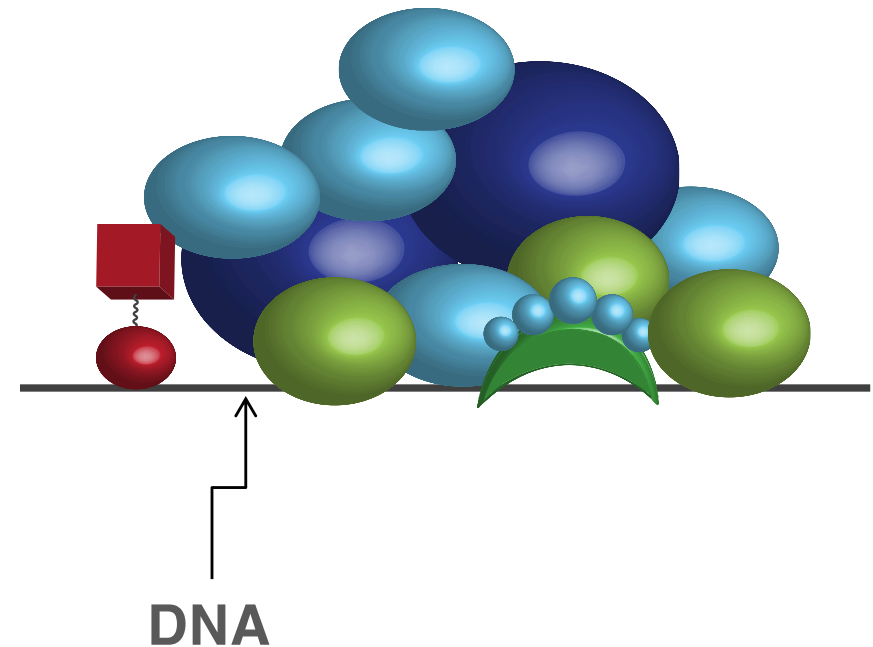
Building blocks
of the machine that
reads DNA



Activator
finds the gene
and
assembles
the machine

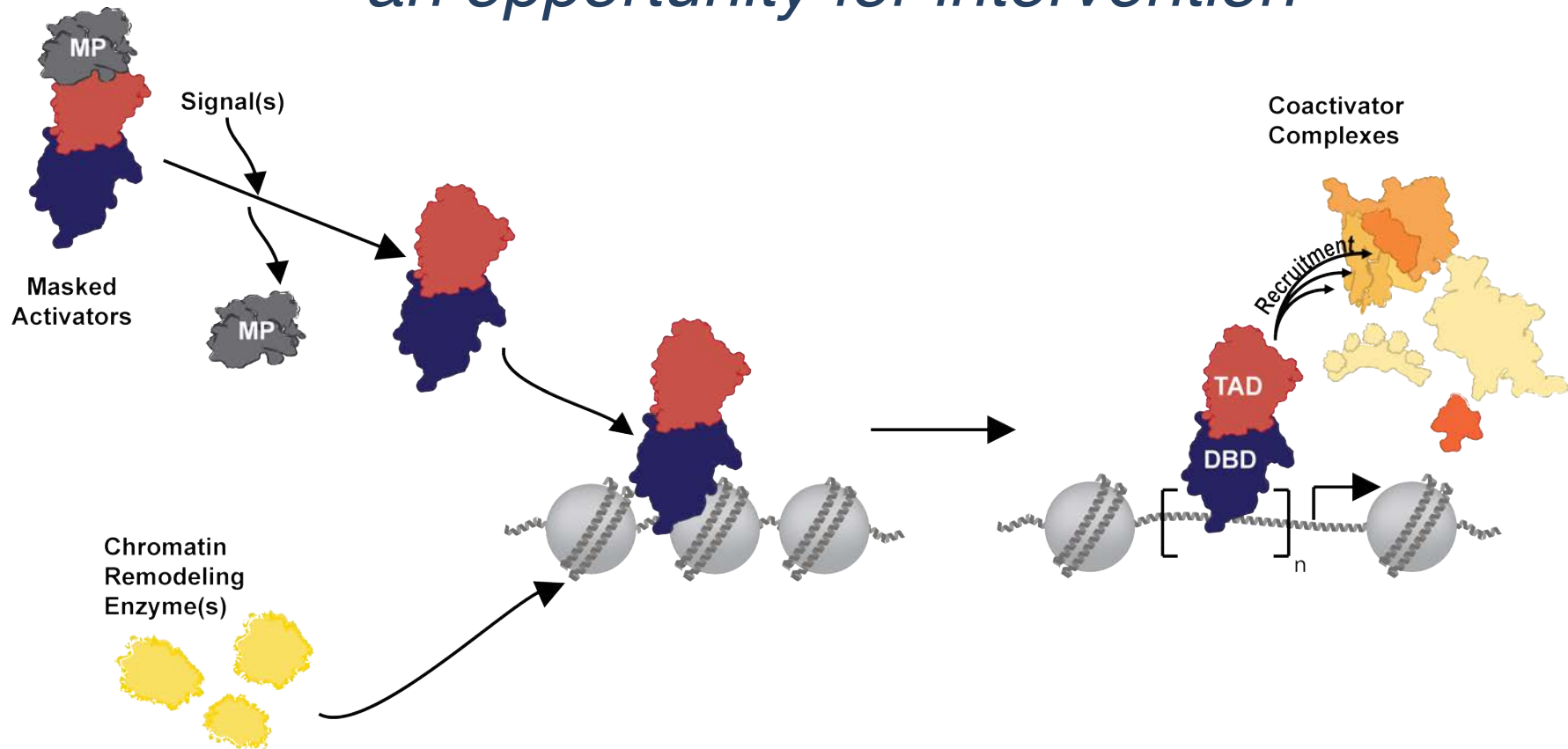


>50 different individual
building blocks



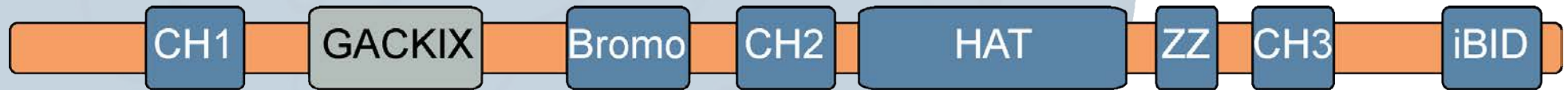
Activator•Coactivator Complexes

an opportunity for intervention



- Molecules that target the **coactivator** proteins offer the opportunity to inhibit or enhance activator function.
- Dynamic composition of activator-coactivator complexes offers a unique **specificity** opportunity.

cMyb-p300-MLL Network Dysregulated in Leukemogenesis



cMyb:

KEKRIKELELLLMSTENELKGQQAL

K_D 1 μ M

cMyb-KIX complex required for leukemogenesis

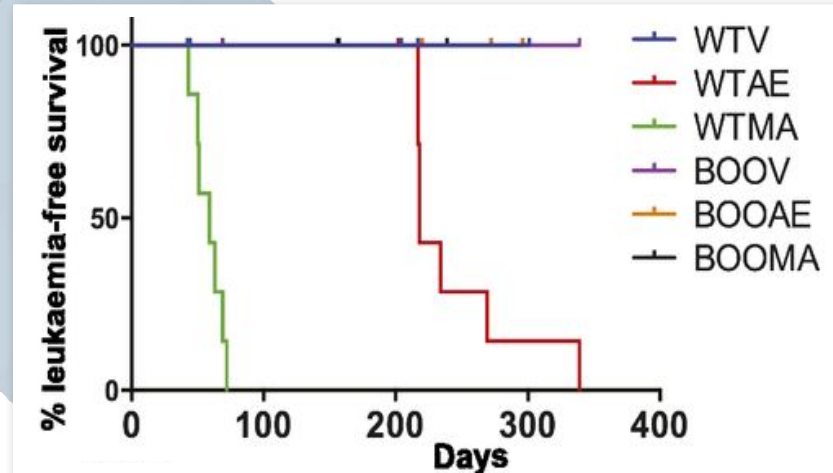
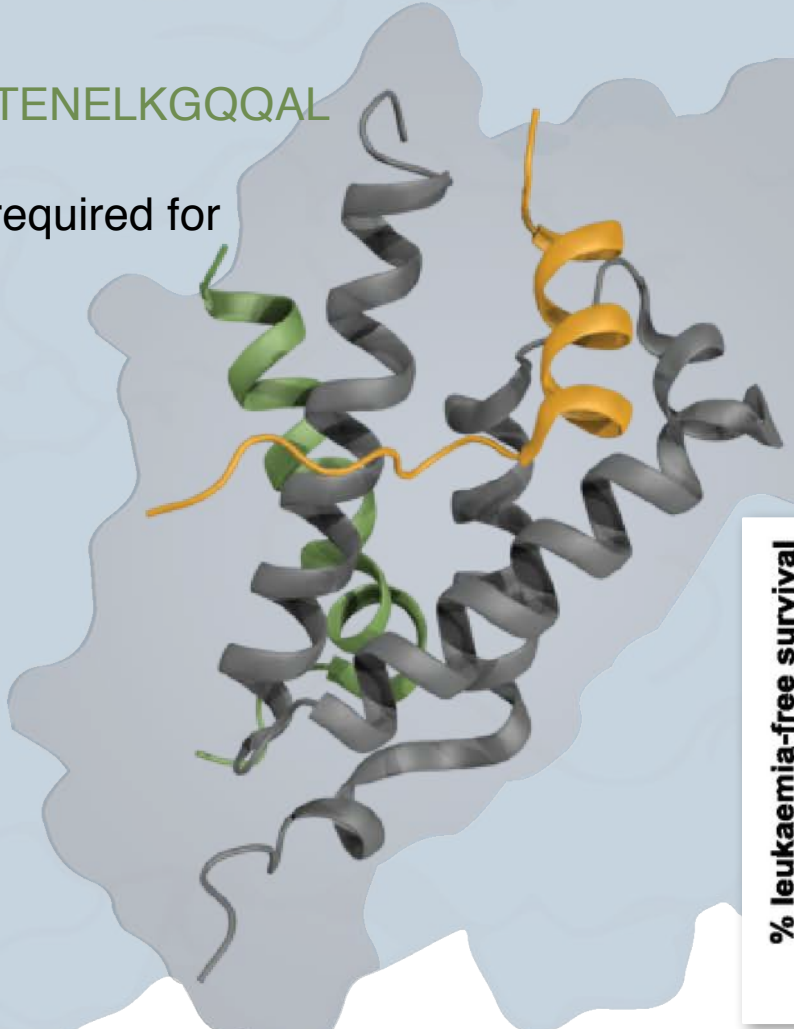
MLL: WDDAGNILPSPDIMDFVLKNT

K_D 3 μ M

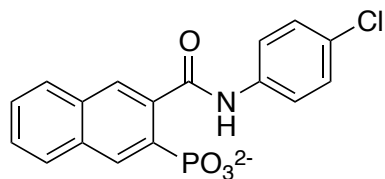
Translocations in AML:

MLL-AF9; MLL-ENL

Loss of KIX binding

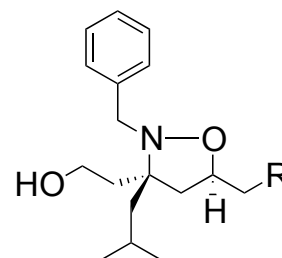


Small Molecule Modulators of KIX in CBP/p300



K_D 115 μM

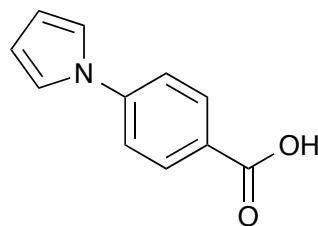
PNAS **2004**, 101, 17622;
Bioorg Med Chem Lett
2012, 20, 6811



& many
derivatives

K_D >200 μM

Mol Cancer Ther. **2013**, 12(8), 1515-25;
ACS Med Chem. Lett. **2012**, 3, 30-34;
ACS Chem. Biol. **2009**, 4, 335-44;
J. Am. Chem. Soc. **2007**, 129, 10654.



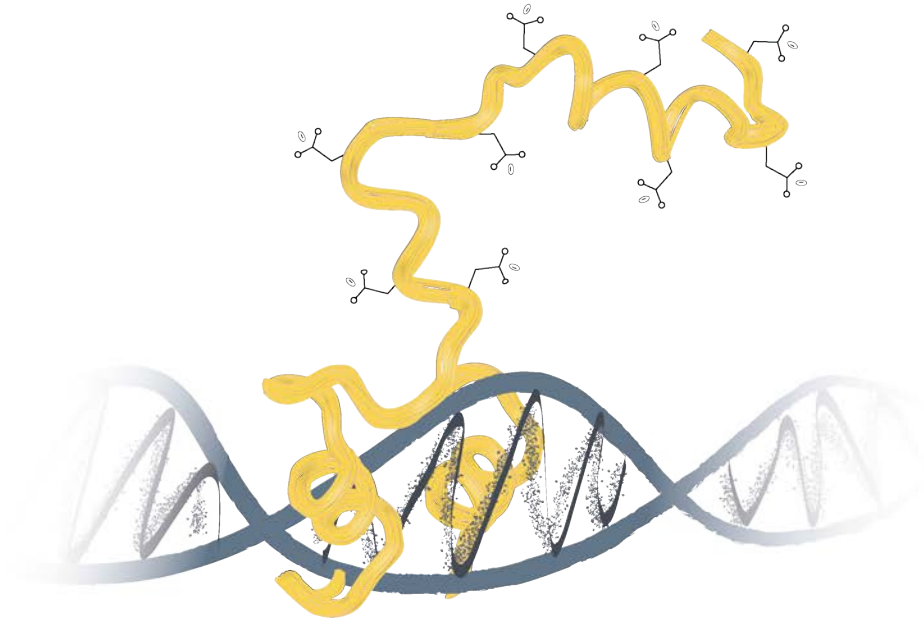
K_D 2 mM

ACS Chem Biol. **2012**, 7, 1345

Small molecule KIX modulators:

mimic natural activators, modest affinity & specificity
limited utility as tools , therapeutic agents

Acid Blobs and Negative Noodles

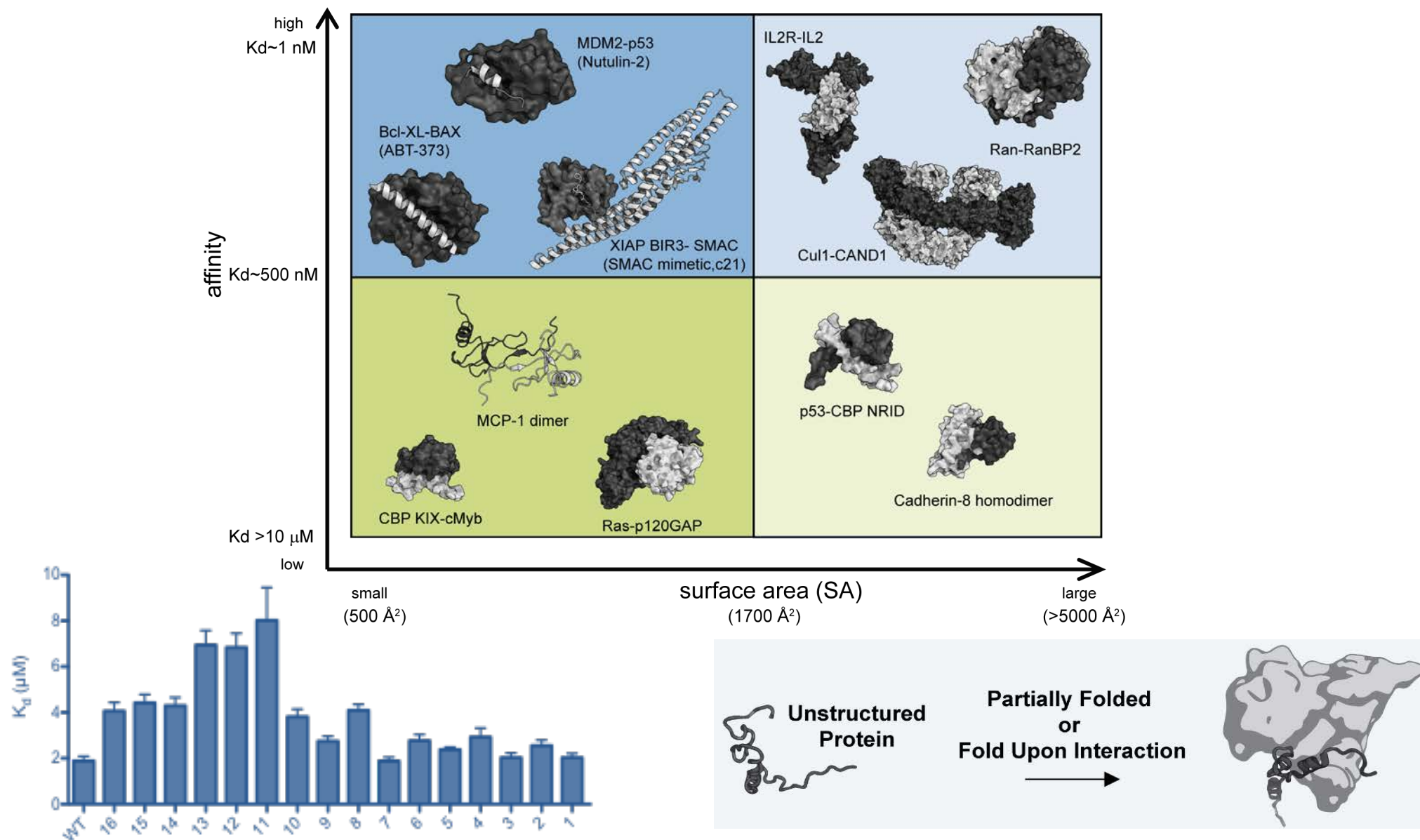


THE more that is known about the amino-acid sequences of proteins that participate in transcriptional activation, the clearer it becomes that many of the critical events cannot depend upon the precise geometrical complementarity that we associate with the interactions of globular proteins during molecular assembly, and the binding of substrates, cofactors and haptens.

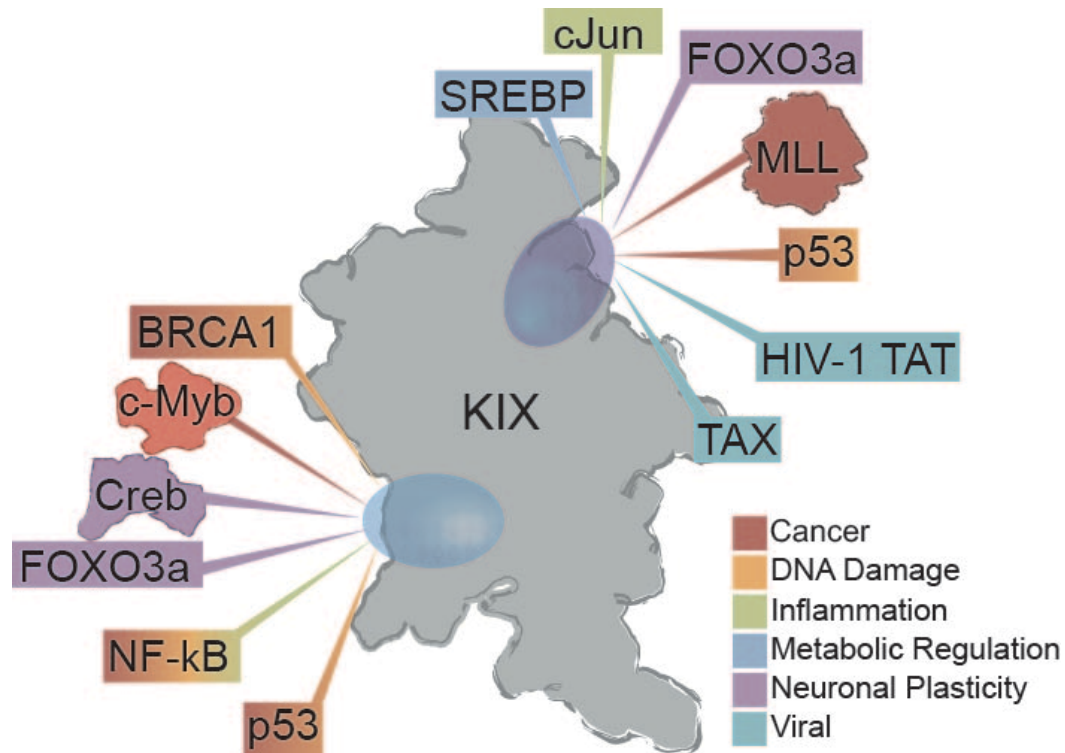
Quote from

P. Sigler *Nature* **1988** 333, 210-212

Activator•Coactivator PPIs are in the 'Untargetable' Space



CBP/p300 KIX Conformational Plasticity



Cooperativity values (α)

MLL + CREB: 3.1

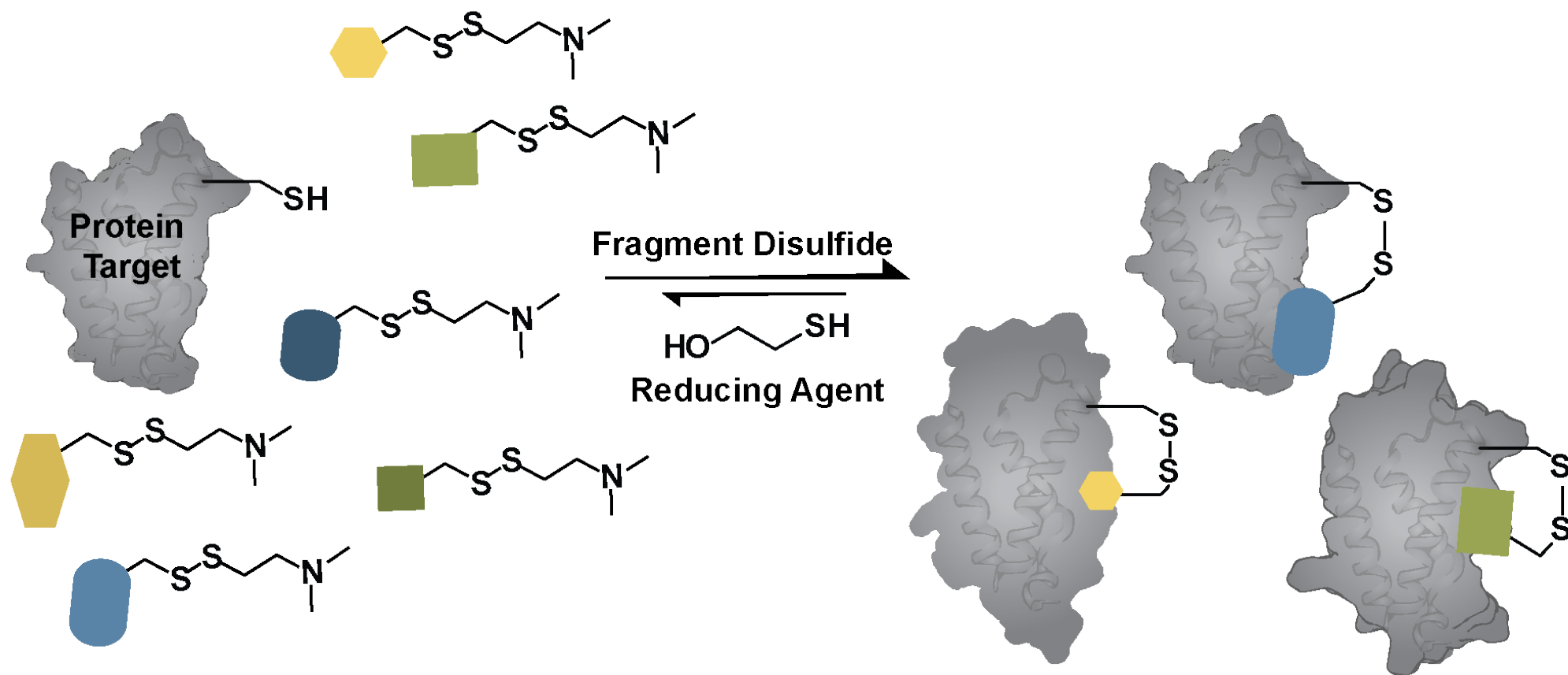
MLL + cMyb: 1.5

HBZ + cMyb: 19

from Shammass & Clarke PNAS 2014, 111, 12055.

- Conformational plasticity enables binding to many partners
- Cooperative binding enforces specificity

Covalent Co-Chaperone Discovery via Tethering

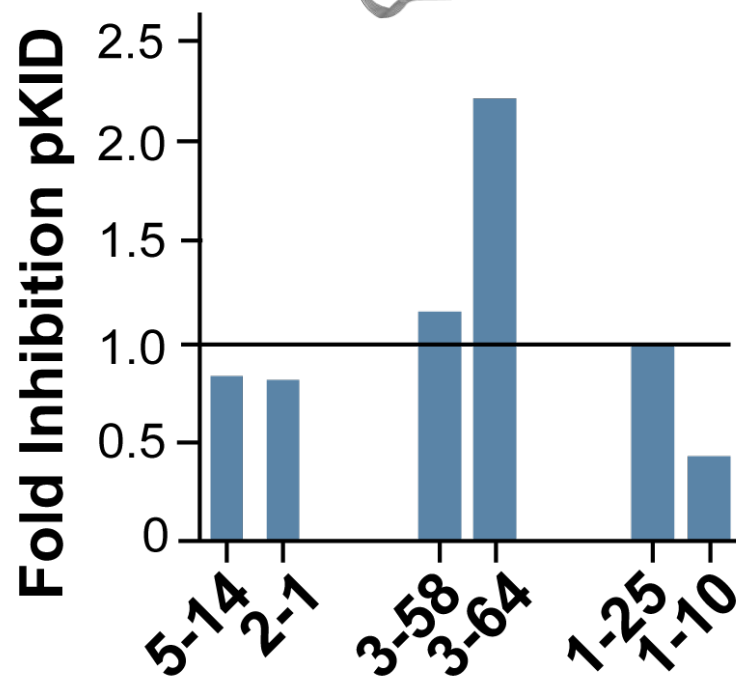
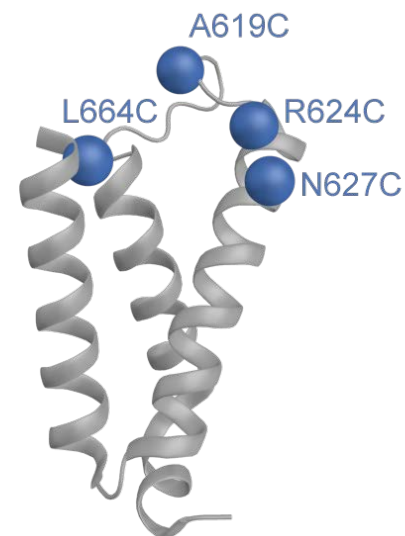
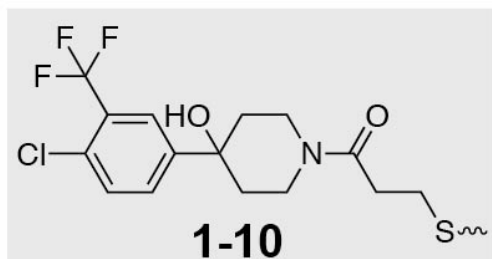
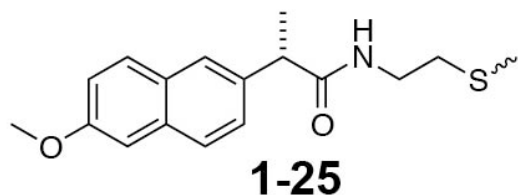
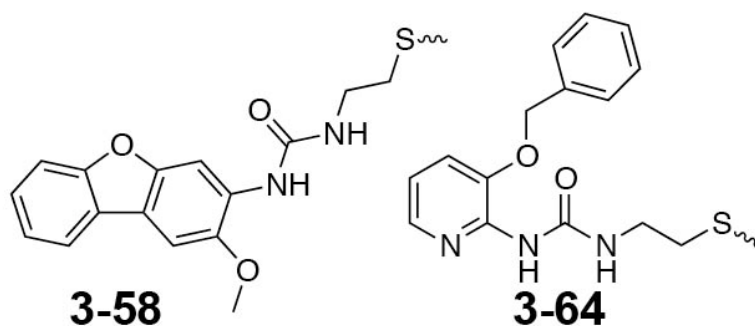
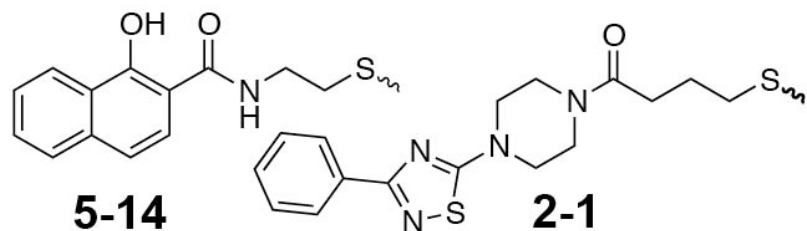


Tethering: Erlanson, D.A.; Wells, J.A.; Braisted, A.C. *Annu Rev Biophys Biomol Struct.* **2004**, 33, 199-223.

Wilson, C. G.; Arkin M.R. *Drug Disc Today* **2013**, 10, e501-e508.

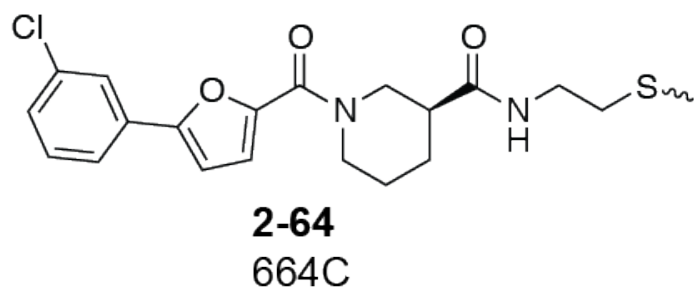
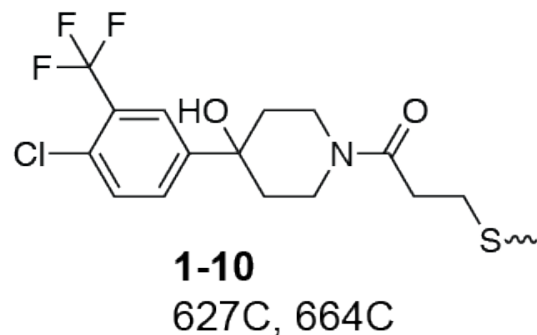
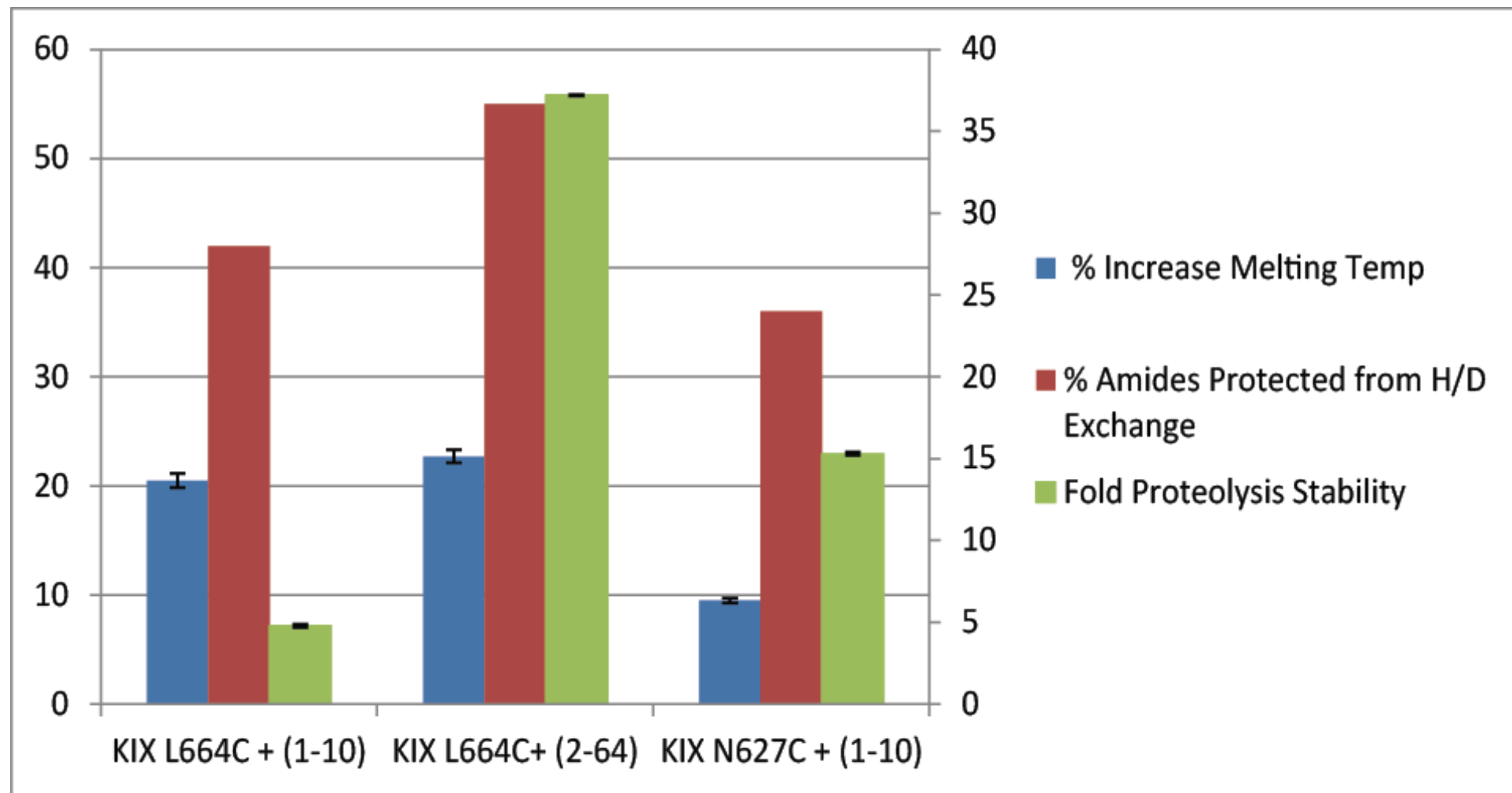
Sadowsky J D et al. *PNAS* **2011**, 108, 6056-6061.

Tethered Ligands Capture Unique Conformations



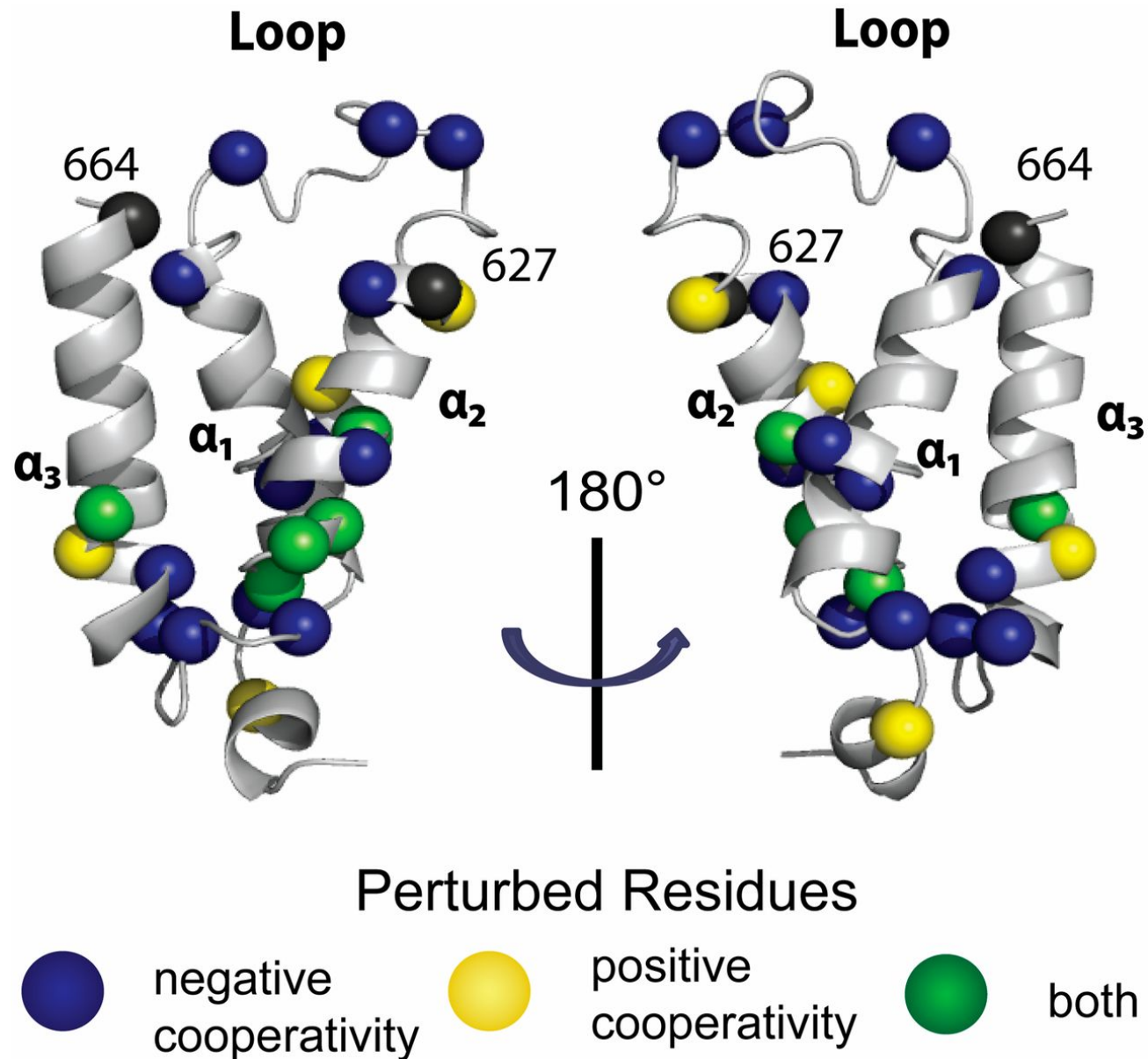
Tethering screen details: Wang et al. *J. Am. Chem. Soc.* **2013**, *135*, 3363

Tethered Fragments Stabilize Distinct KIX Conformations

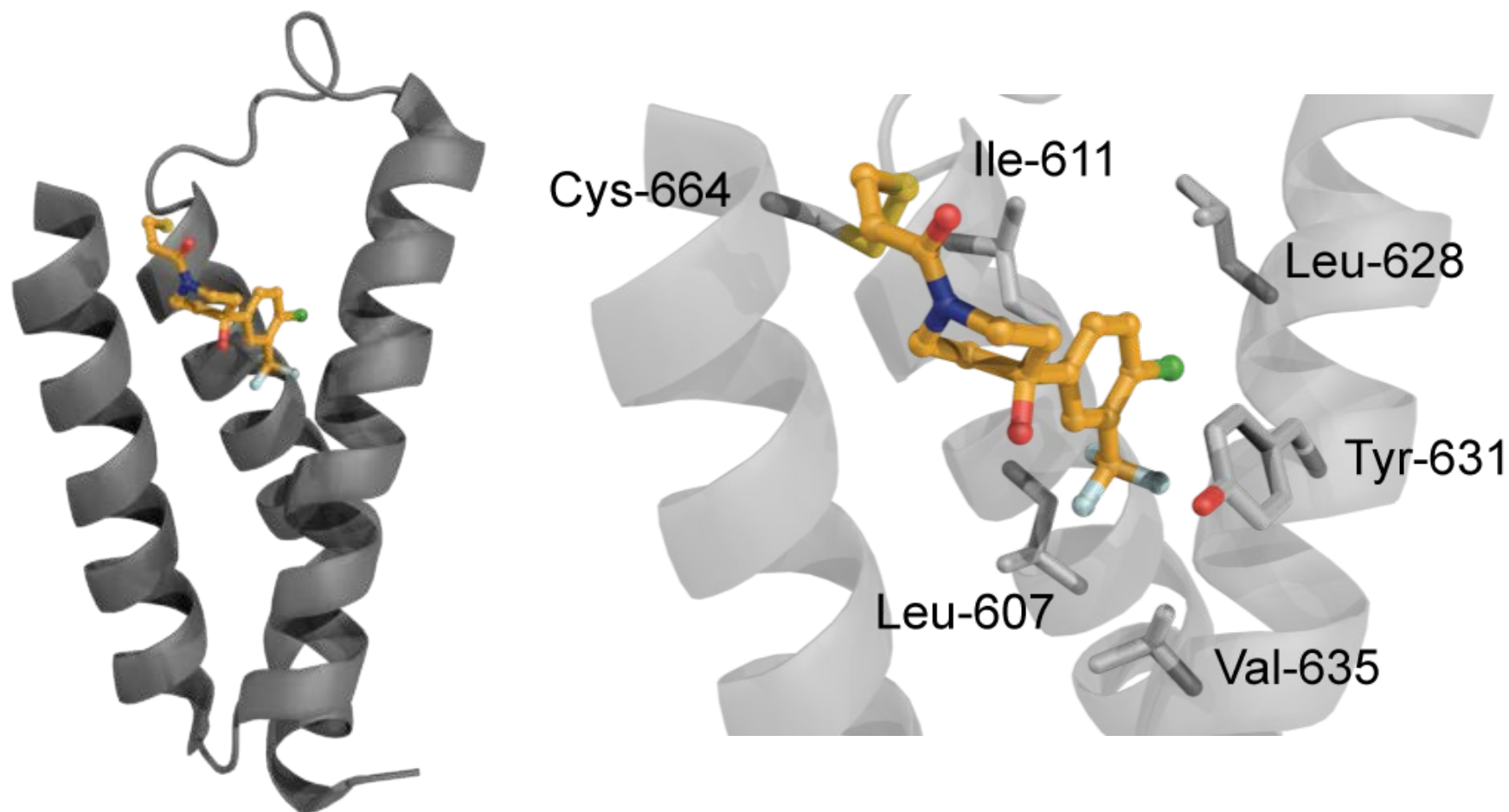


(Prof.) Dr. Will Pomerantz
Dr. Chinmay Majmudar
Dr. Ningkun Wang

Positive and Negative Cooperativity



Flexible Loop Communicates Allostery



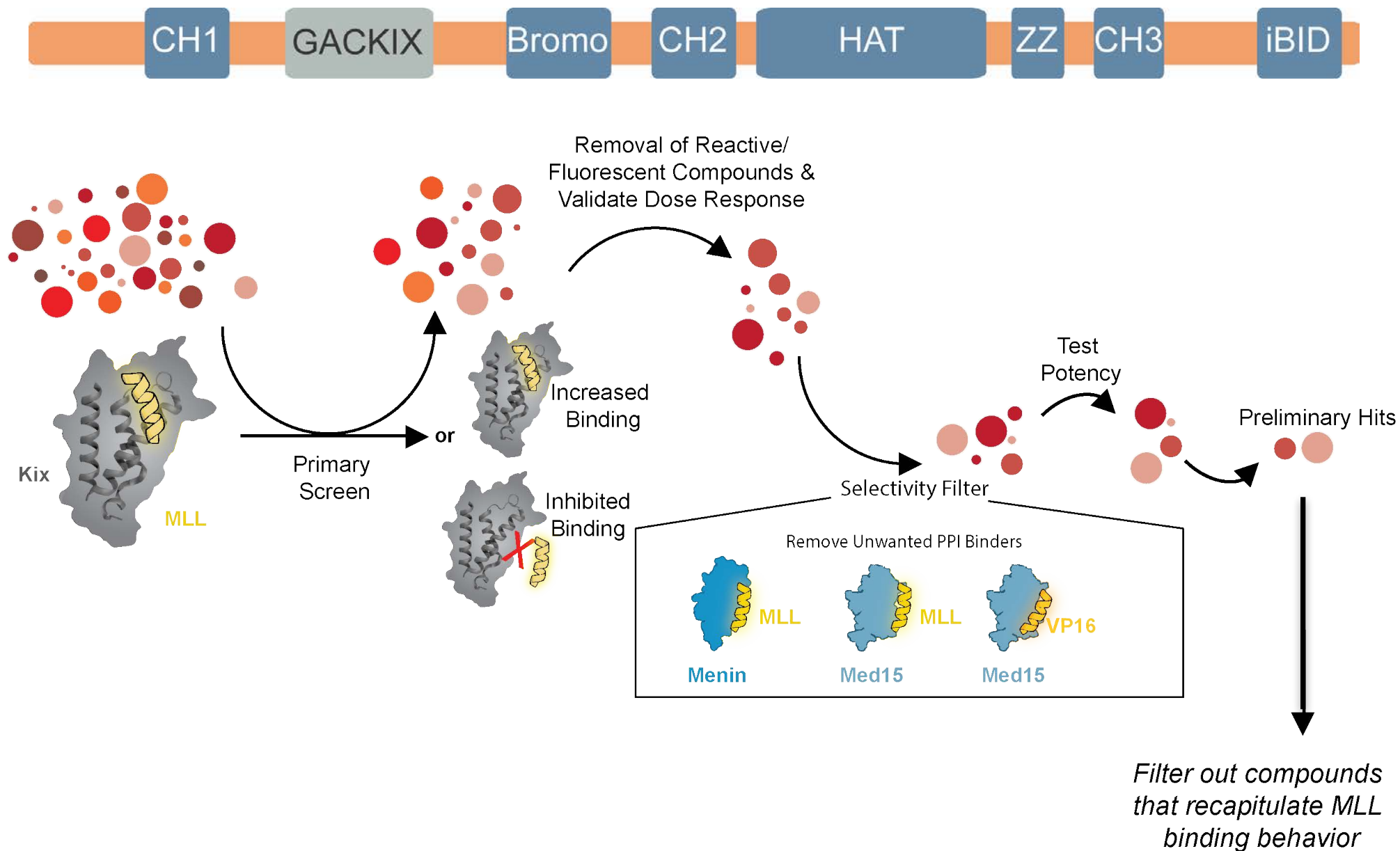
Wang *et al* *J. Am. Chem. Soc.* **2013**, 135, 3363.

Experimental & computational dissection of positive and negative allostery:

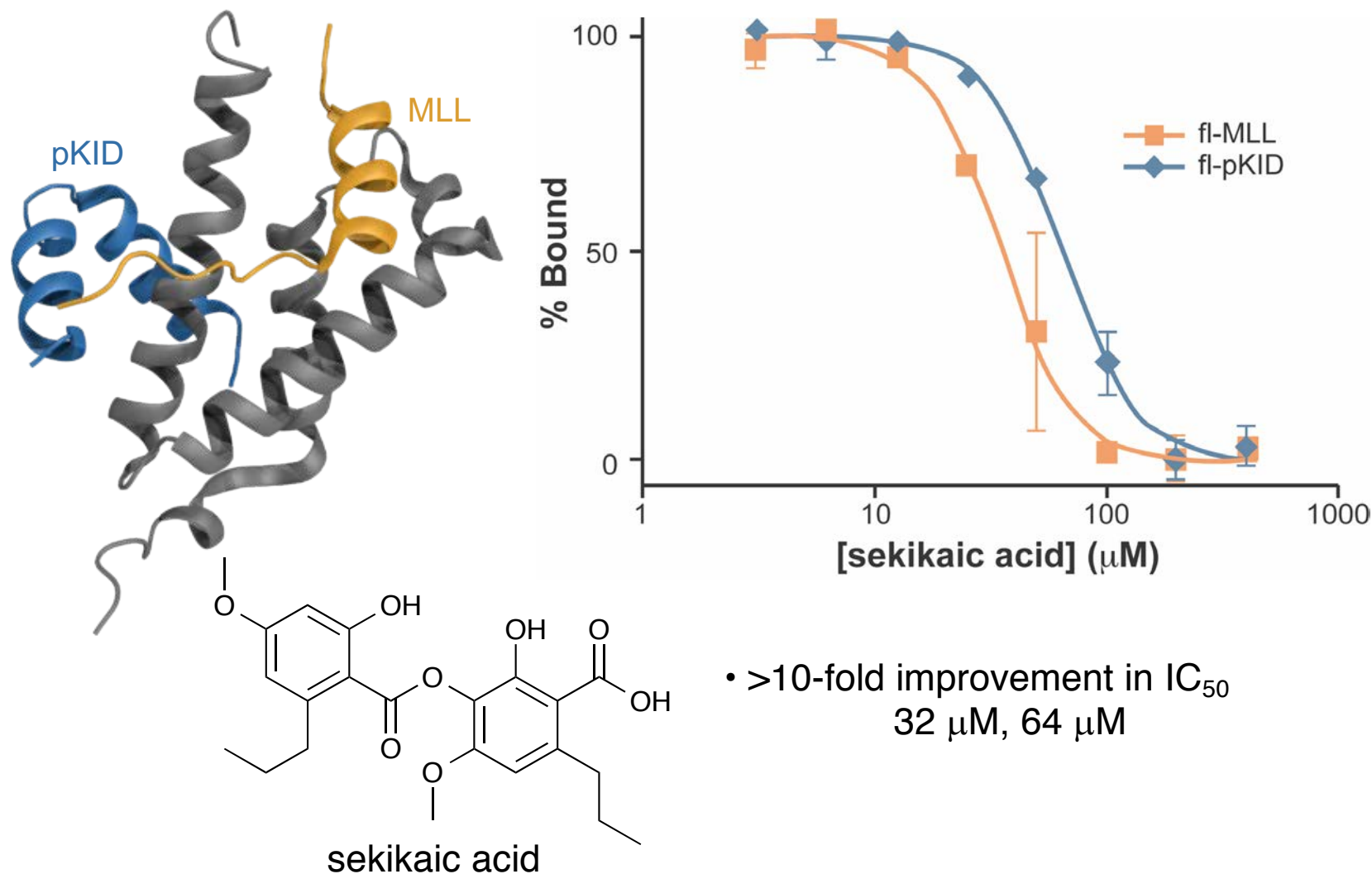
Wang N, Lodge, J.M., Fierke, C.A., Mapp A.K. *PNAS* **2014**, 111, 12061-12066

Law, Gagnon, Mapp, Brooks *PNAS* **2014**, 111, 12067-72

A Screening Strategy for Allosteric Modulators

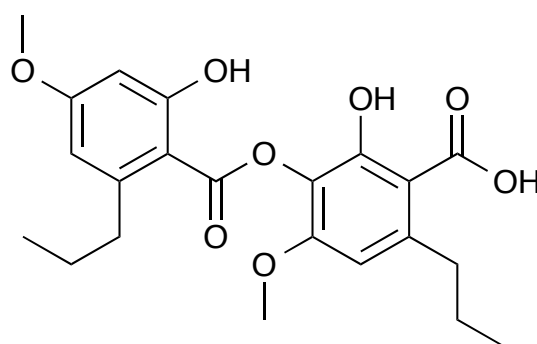
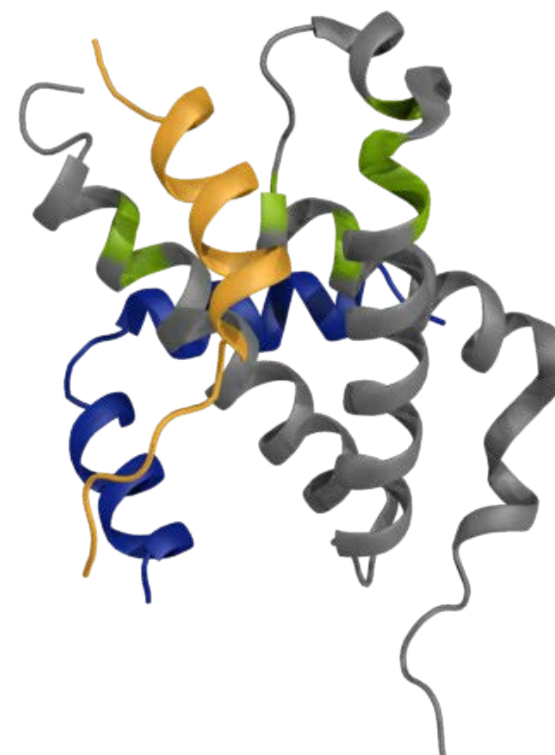
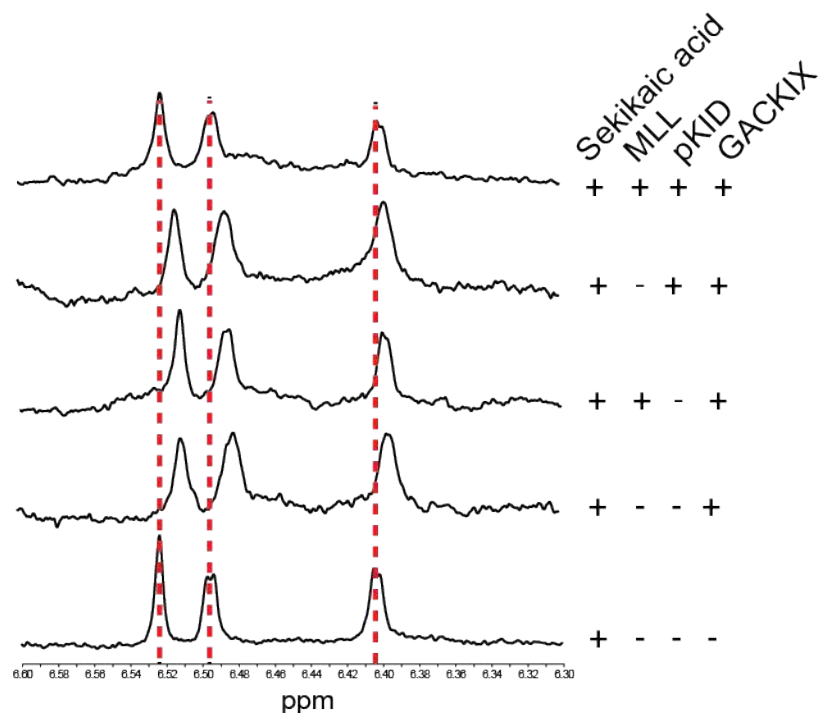


Sekikaic Acid Inhibits KIX PPIs



C.Y. Majmudar, J.W. Hojfeldt, C.J. Arevang, W. C. Pomerantz, J.K. Gagnon, P.J. Schultz, L.C. Cesa, C.H. Doss, S.P. Rowe, V. Vasquez, G. Tamayo, T. Cierpicki, C.L. Brooks, D.H. Sherman, A.K. Mapp. *Angew Chem Int Ed Engl* **2012**, 51, 11258-62.

Orthosteric and Allosteric Inhibition

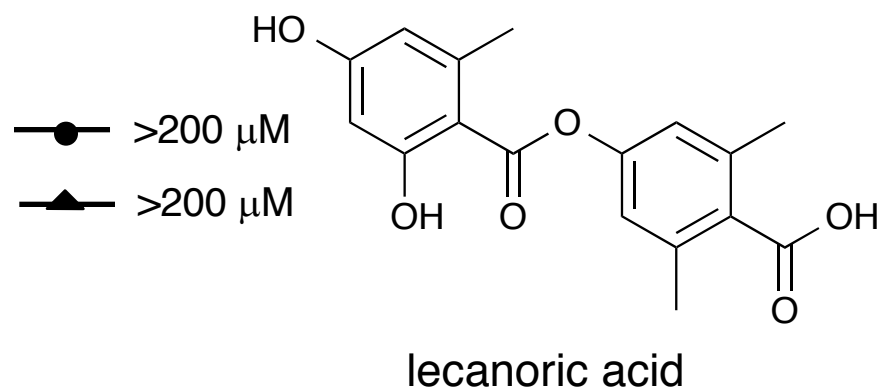
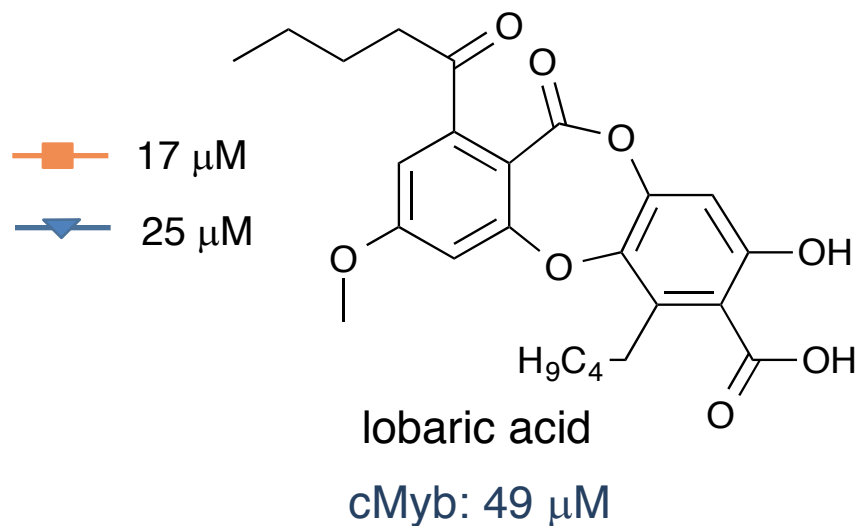
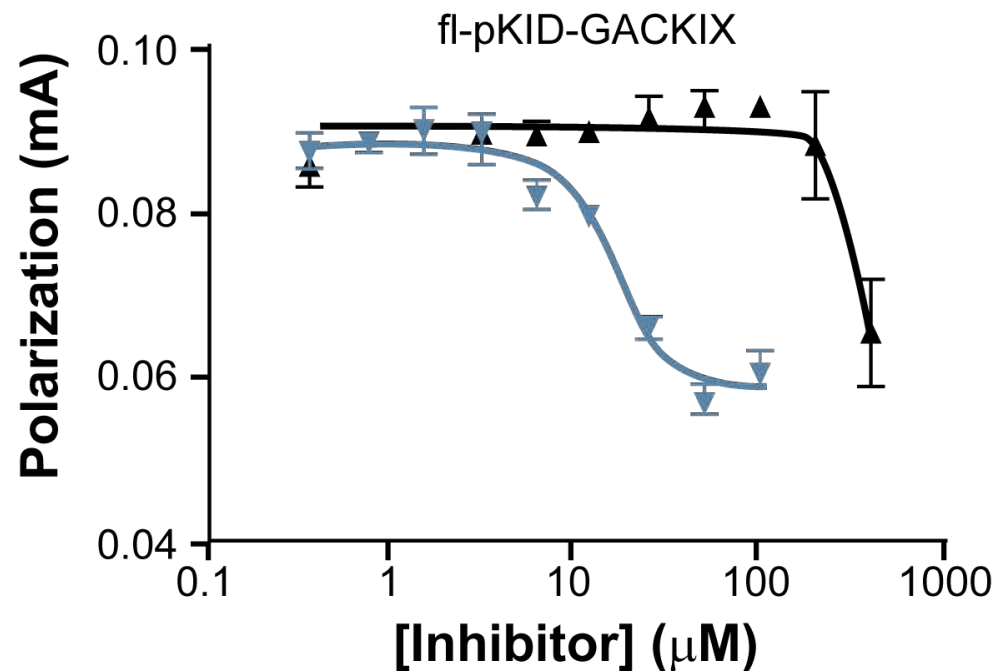
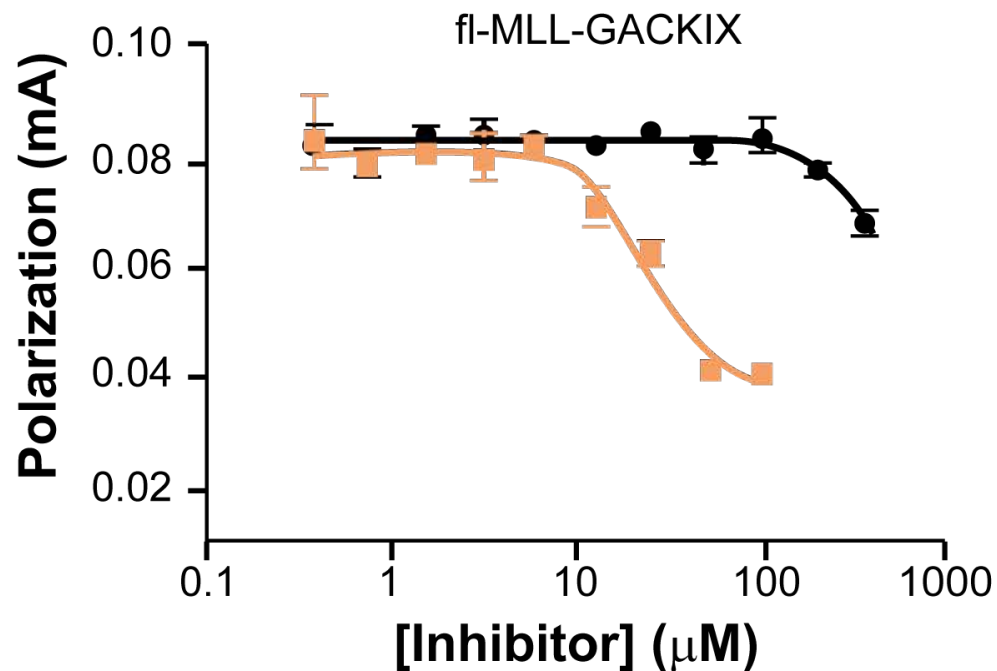


sekikaic acid

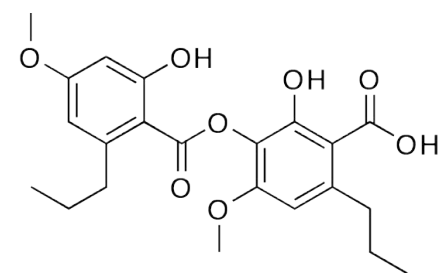
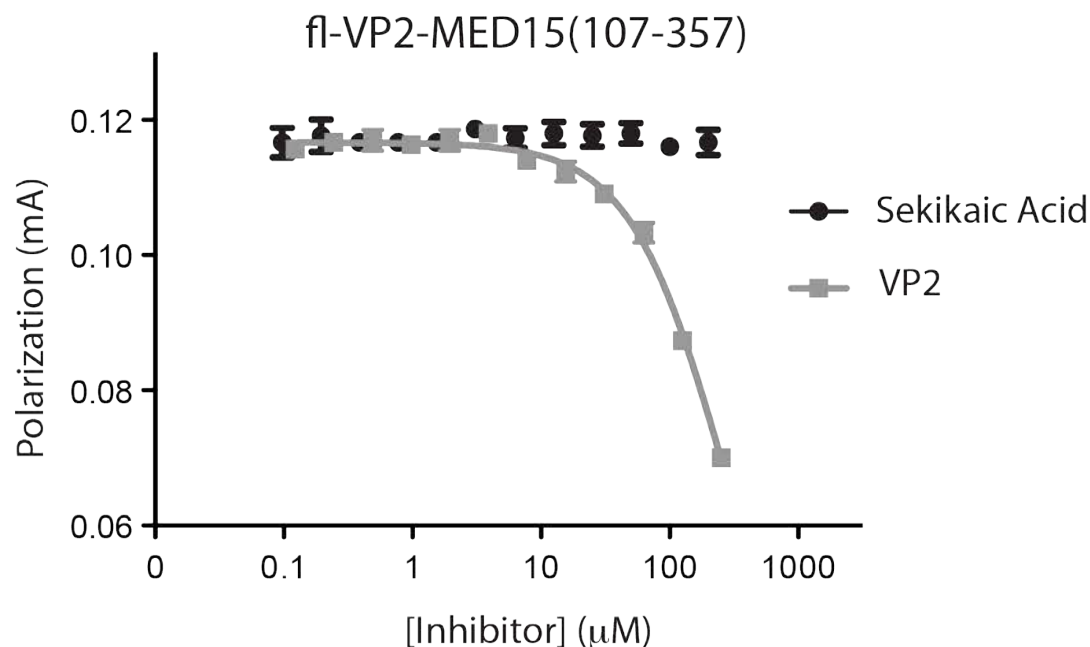
- Combined orthosteric and allosteric inhibition

Dr. Will Pomerantz
 Prof. Tomek Cierpicki
 Dr. Chinmay Majmudar

Lobaric Acid Inhibits KIX PPIs



Uniquely Specific Inhibitors



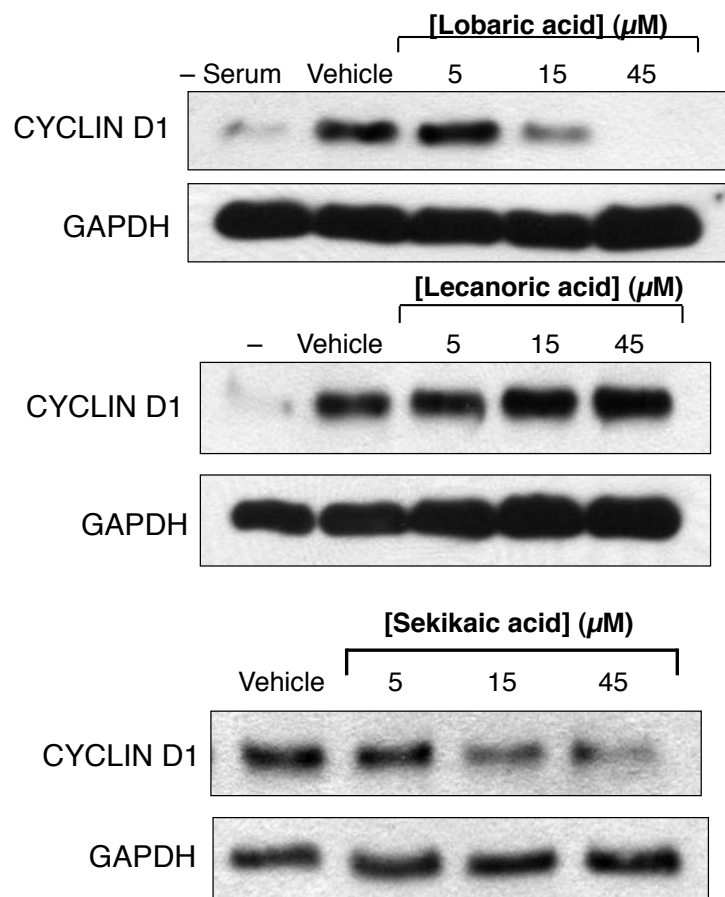
Sekikaic Acid

VP2: (AspPheAspLeuAspMetLeuGly)₂

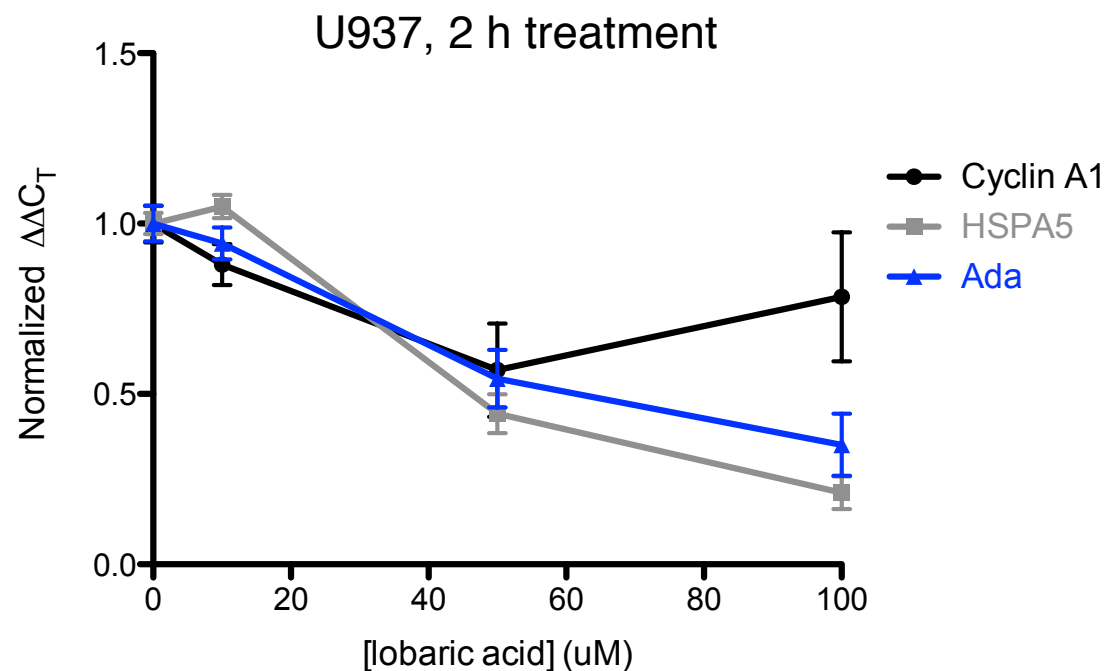
- VP16 activation domains: interact with the KIX domain, the B-Box activator binding motif, AcID motif of Med25
- Sekikaic acid does not competitively inhibit binding to B-Box or to Med25

C.Y. Majmudar, J.W. Hojfeldt, C.J. Arevang, W. C. Pomerantz, J.K. Gagnon, P.J. Schultz, L.C. Cesa, C.H. Doss, S.P. Rowe, V. Vasquez, G. Tamayo, T. Cierpicki, C.L. Brooks, D.H. Sherman, A.K. Mapp. *Angew Chem Int Ed Engl* **2012**, 51, 11258-62.

Cellular Activity Parallels Binding Ability



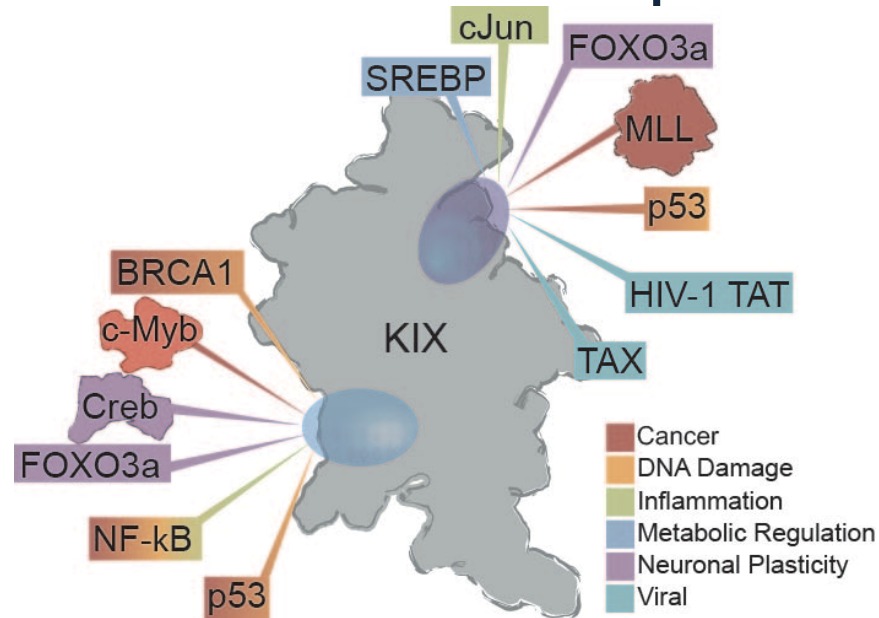
Dr. Conor Doss



Dr. Stephen Joy

C.Y. Majmudar, J.W. Højfeldt, C.J. Arevang, W. C. Pomerantz, J.K. Gagnon, P.J. Schultz, L.C. Cesa, C.H. Doss, S.P. Rowe, V. Vasquez, G. Tamayo, T. Cierpicki, C.L. Brooks, D.H. Sherman, A.K. Mapp. *Angew Chem Int Ed Engl* **2012**, 51, 11258-62.

Lessons from CBP/p300 KIX



- Covalent co-chaperones can capture distinct conformations
 - *valuable mechanistic probes (highly specific, confer stability)*
 - *support computational modeling (C. Brooks III)*
- HTS for allosteric modulators provides modulators with enhanced specificity & efficacy profile
 - *current secondary screen against cMyb*
- Tools to connect allostery in KIX with CBP/p300 PPI network

Wang N, Lodge, J.M., Fierke, C.A., Mapp A.K. *PNAS* **2014**, *111*, 12061-12066

Law, Gagnon, Mapp, Brooks *PNAS* **2014**, *111*, 12067-72

Sturlis, Pricer, Mapp *Nat Chem Biol* **2015** *11* 891-4

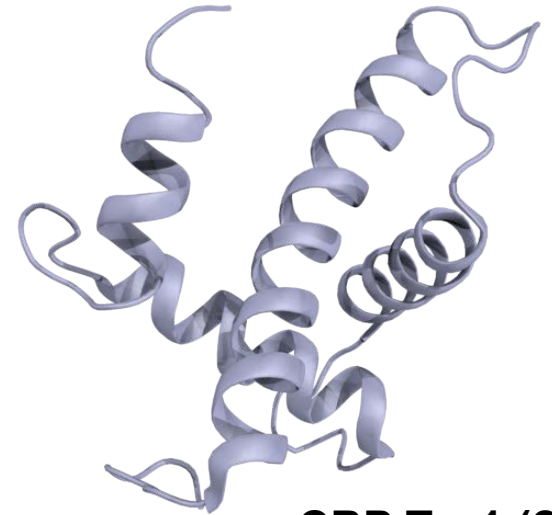
Activator-Binding Motifs Across Coactivators



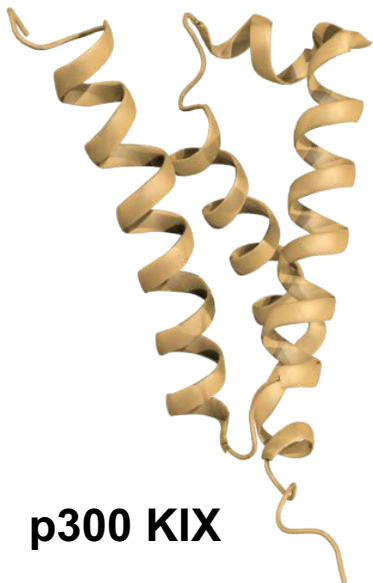
Arc105 KIX (??)



Med25 AcID(??)



CBP Taz1 (??)



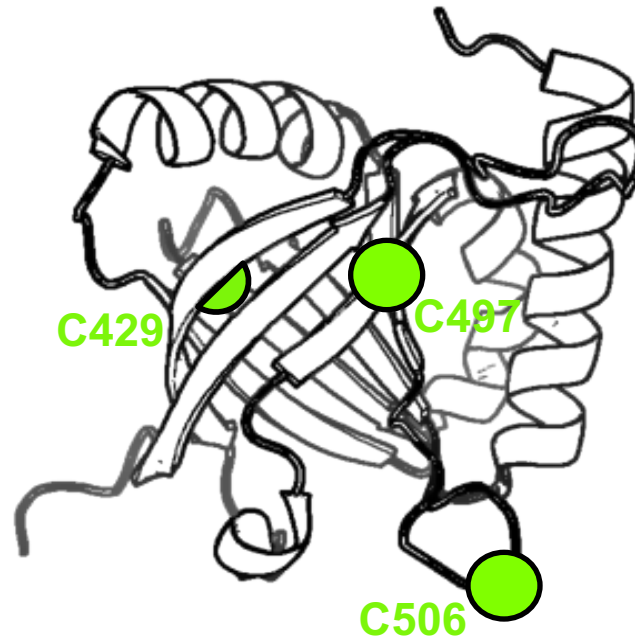
p300 KIX



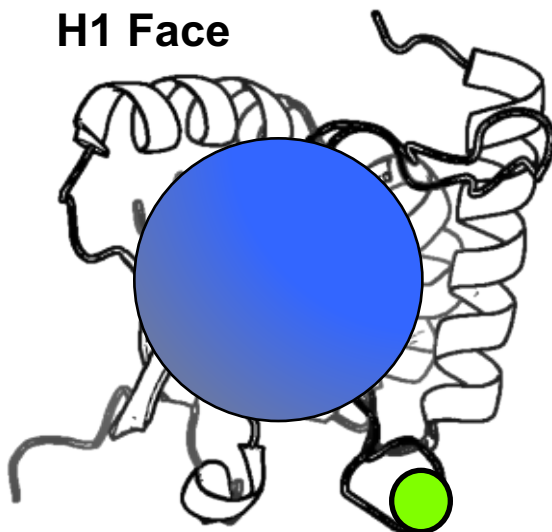
CBP KIX

Activator-Interaction Domain (AcID)

a unique motif

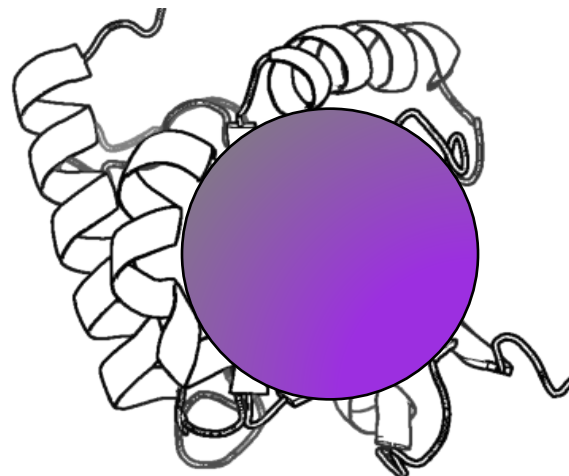


H1 Face



H2 Face

180°
↻



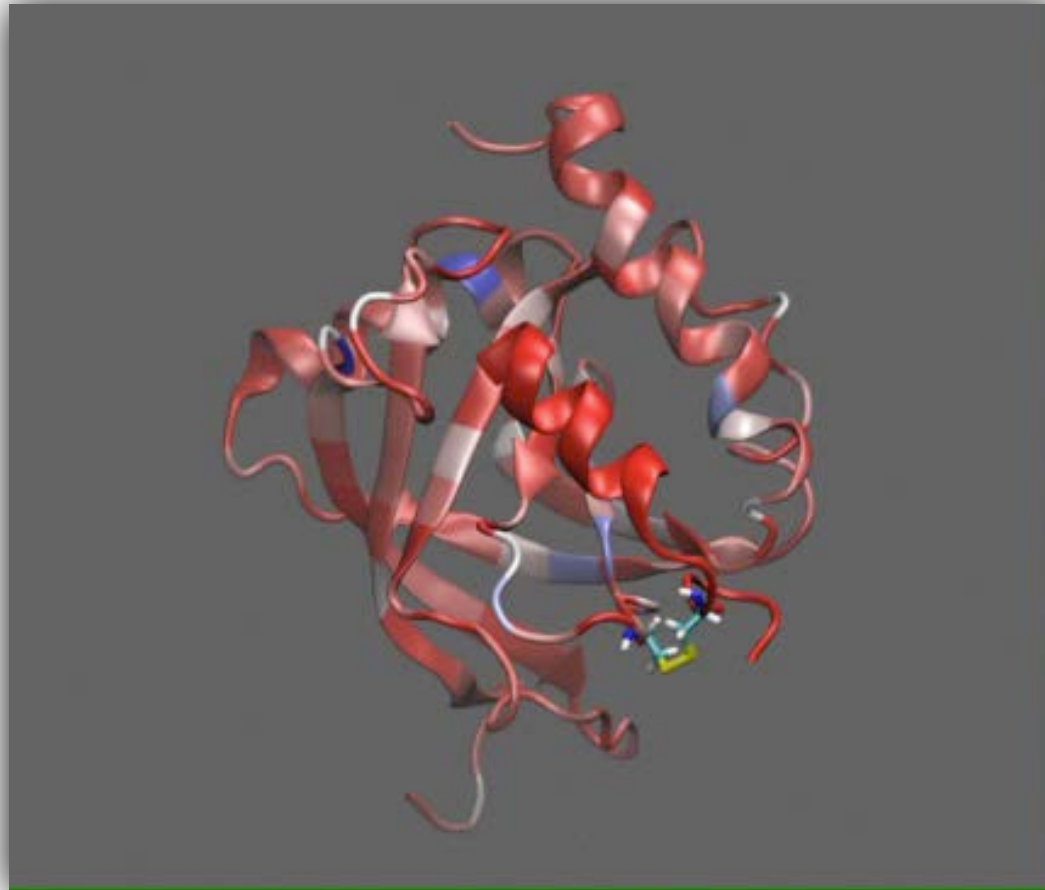
VP16 simultaneously
binds both surfaces with
ETV6, E1A, and TAD5

VP16 N'   C'
ATF-8 α : stress response

NMR-derived model from:
CBP/p300: ???

Vojnic et al. *Nat Struct, Mol. Biol.*, **2011**, 18, 404
Bontems et al. *J. Struct. Biol.*, **2011**, 174, 245
Milbradt et al. *Nat Struct, Mol. Biol.*, **2011**, 18, 410

Predicted Allosteric Network Connecting Binding Sites



- Transcriptional activator is solid red
- red → lowest chemical shift perturbation
- white → medium chemical shift perturbation
- blue → highest chemical shift perturbation

Nick Foster
Prof. Charles Brooks III

Depsidone Inhibitors

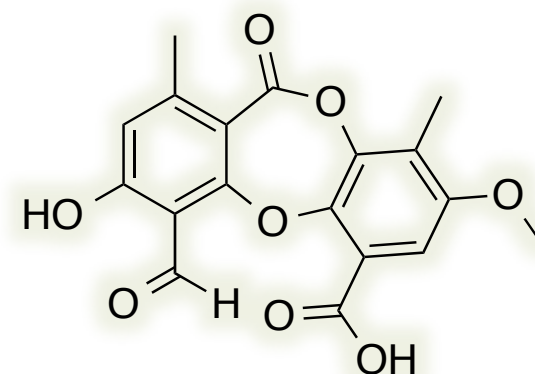
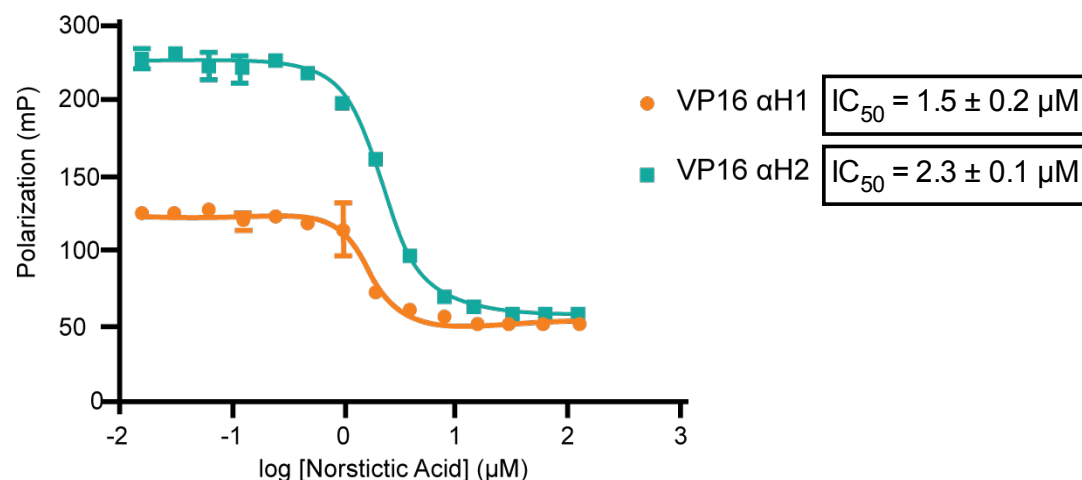
VP16 H1₄₁₃₋₄₅₁

VP16 H2₄₅₂₋₄₉₀

413 APPTDVSLGDELHLDGEDVAMAHADALDDFDLDM LGDGDS PGP GFTPHDSAPY GALDMADFEFEQMFTDALGIDEYGG 490

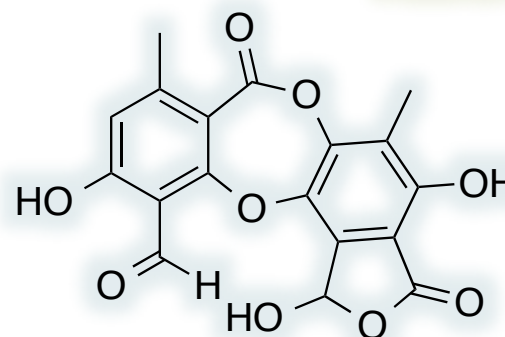
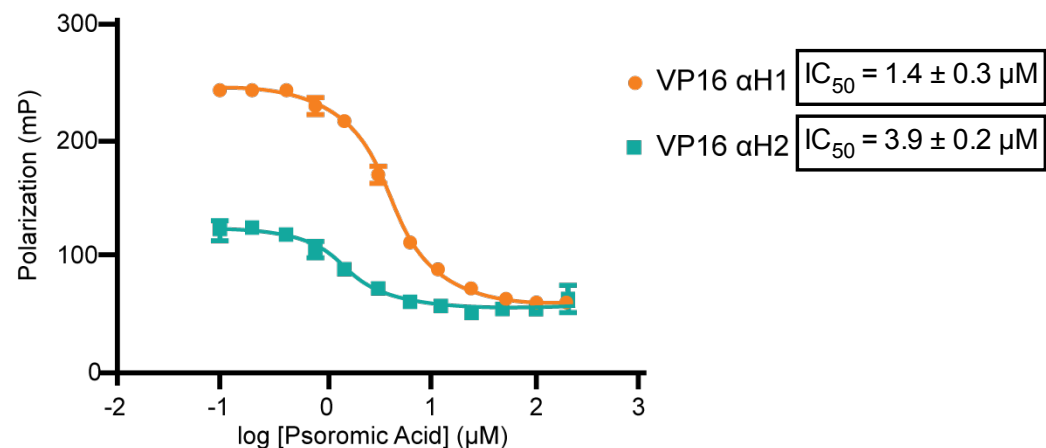


Norstictic Acid Med25 Inhibition



Psoromic Acid

Psoromic Acid Med25 Inhibition

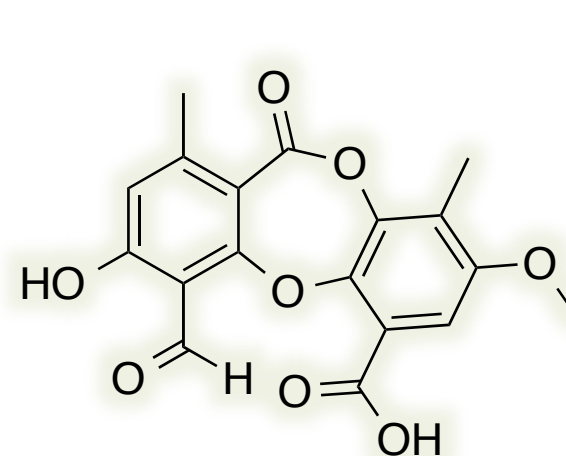
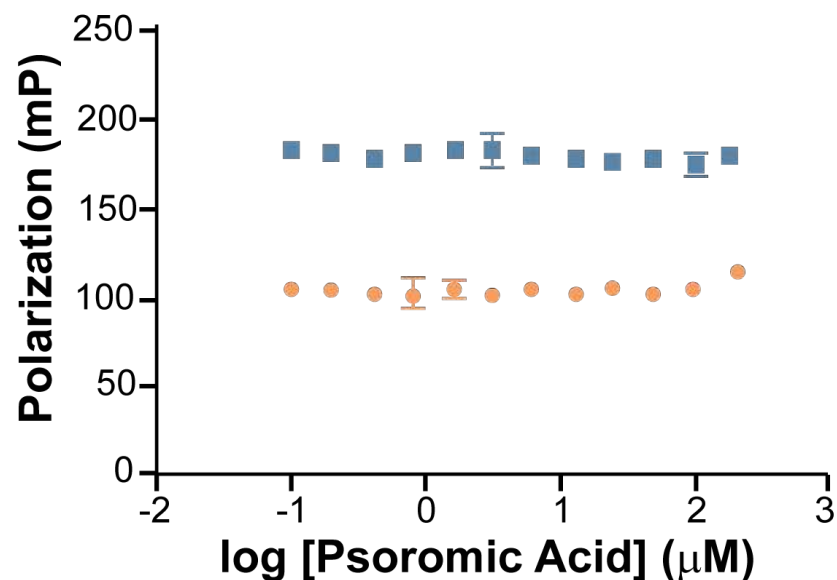


Norstictic Acid

*Steve Sturlis
Paul Bruno*

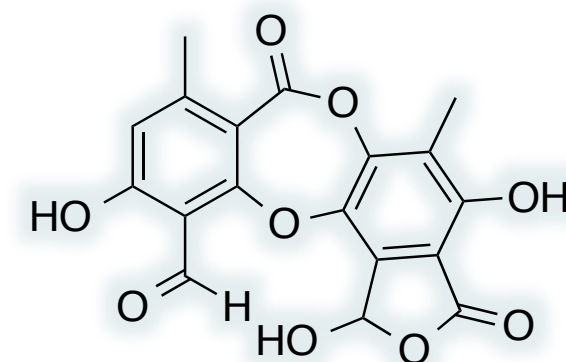
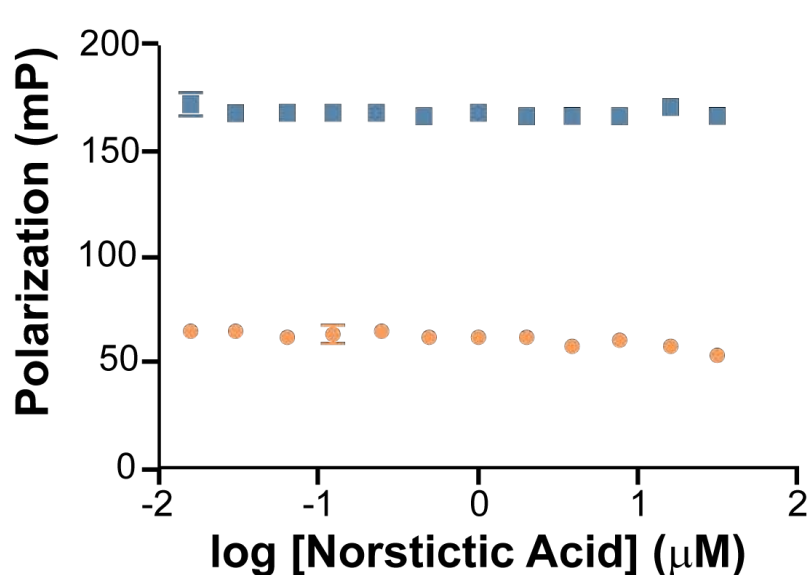
Depsidones Do Not Inhibit KIX Interactions

Psoromic Acid KIX Inhibition



Psoromic Acid

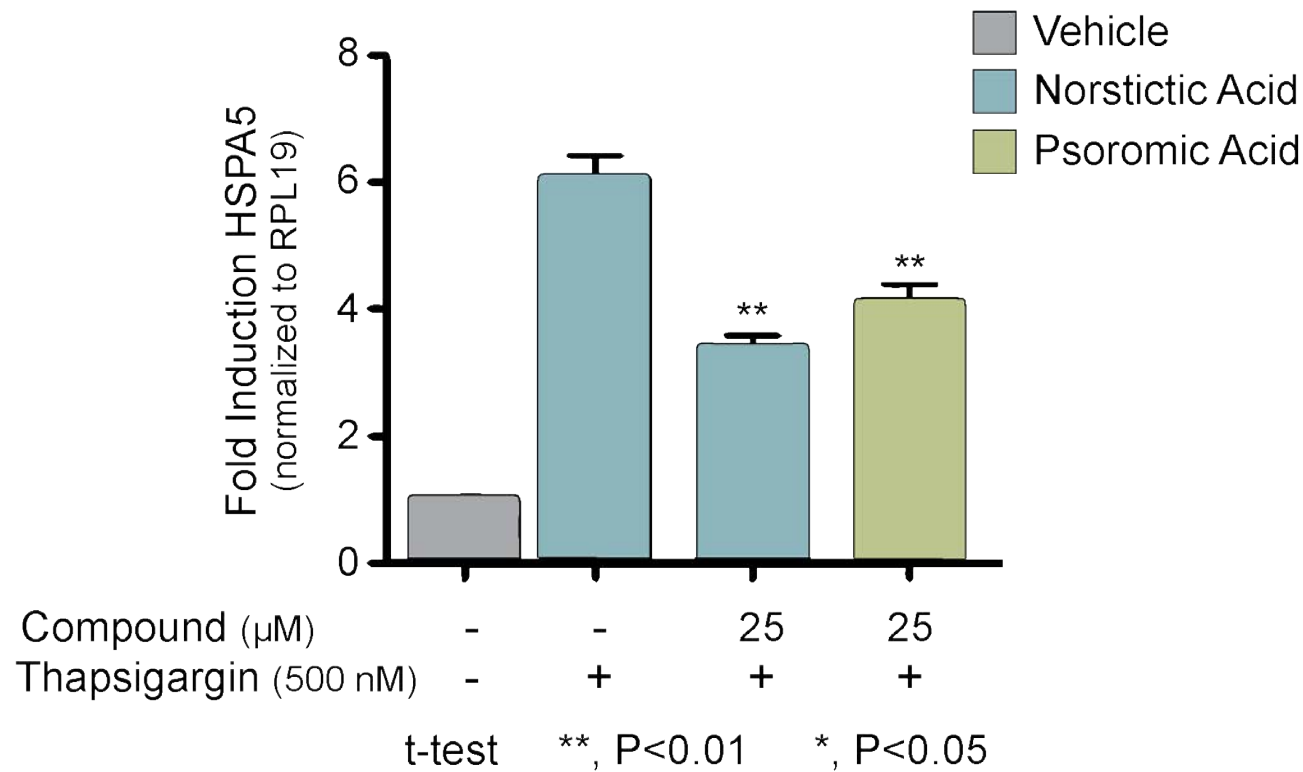
Norstictic Acid KIX Inhibition



Norstictic Acid

Steve Sturlis
Paul Bruno

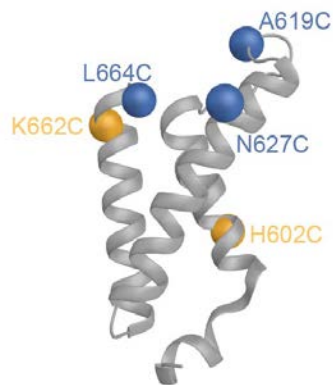
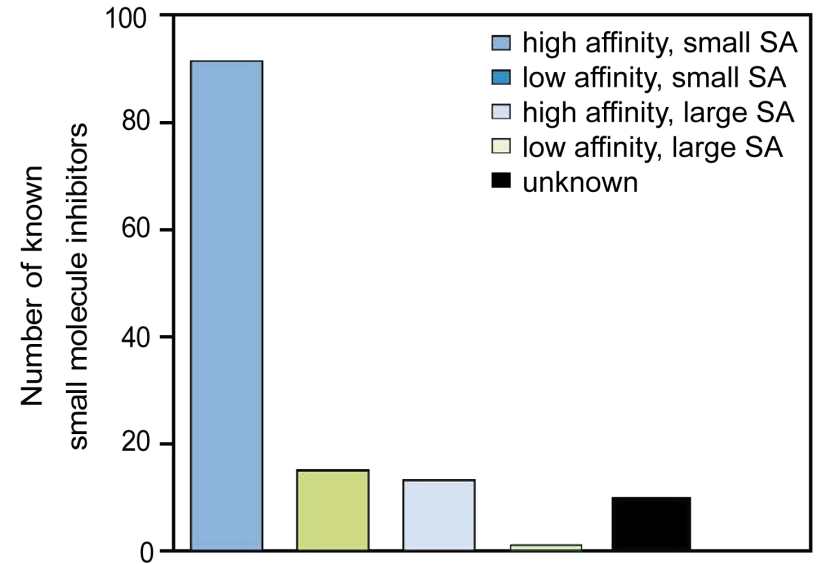
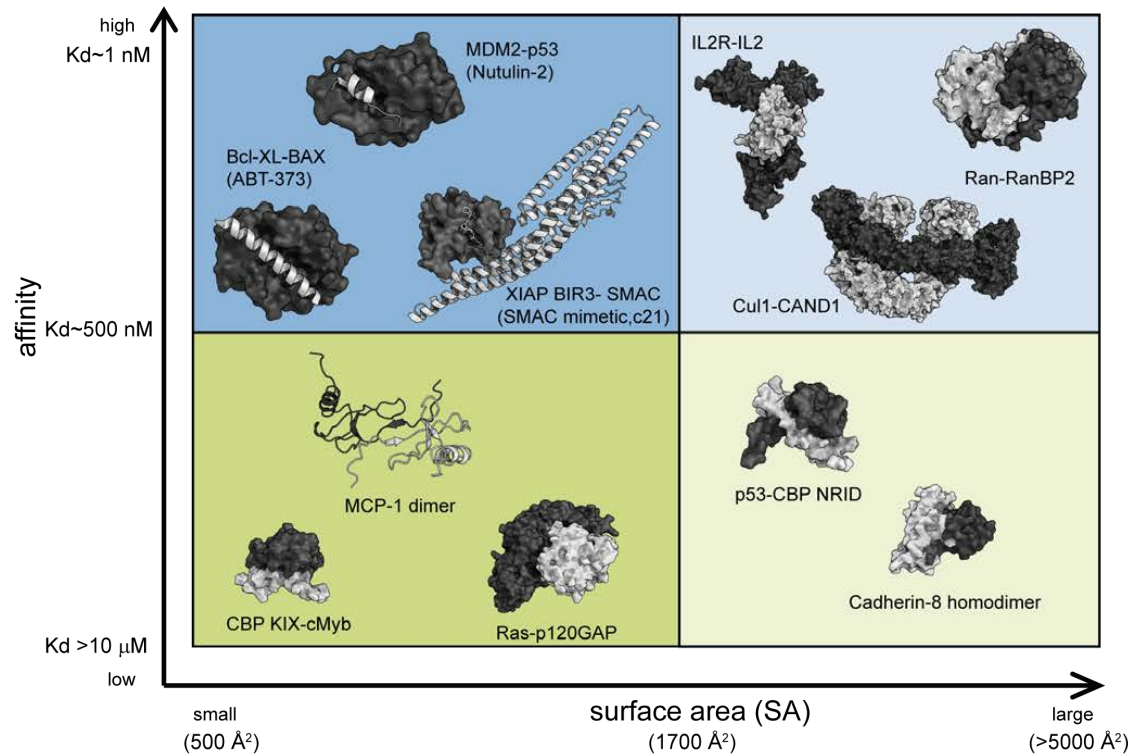
Depsidones Block Med25-Dependent Transcription



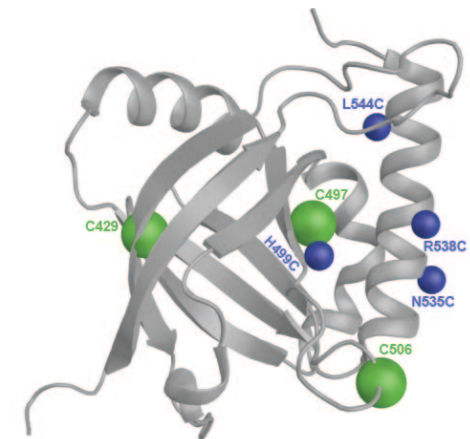
Assay from: Conaway and coworkers *J. Biol. Chem.* **2013**, 288, 16789

Dr. Steve Sturlis
Dr. Paul Bruno

Filling the 'Untargetable' Space



- transcription complex(es)
- protein folding
- signaling complexes



Acknowledgements



Current

Dr. Meg Breen
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Rachel Pricer
Cassie Joiner
Omari Baruti
Laura Cesa
Nick Foster
Matt Henley
Kevon Stanford

Gone but not forgotten

Prof. Will Pomerantz (UMinn)
Dr. Ningkun Wang (UC Berkeley)
Dr. Chinmay Majmudar (Mumbai)

\$\$

NIH: 3GM65530, 2CA140667
NSF: CHE 1412759
DOD: PC101214
ACCRF

Collaborators

Prof. C. Fierke (UM)
Prof. C. Brooks (UM)
Prof. J. Wells (UCSF)
Prof. D. Sherman (UM)
Prof. G. Tamayo (INBIO)
Prof. S. Merajver (UM)
Prof. B. Cravatt (Scripps)
Prof. T. Cierpicki (UM)
Dr. J. Bradner (DFCI/Novartis)
Dr. J. Qi (DFCI/HMS)