SMS in protein-protein communication

How **S**hort **M**otif **S**equences are read and understood

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Albert Szent-Györgyi Lecture, Eötvös Loránd University, Budapest 31/5/2016

Albert Szent-Györgyi

- So what the school has to do, in the first place, is to make us **learn how to learn**, to whet our appetites for knowledge, to teach us the delight of doing a job well and the excitement of creativity, to teach us to love what we do, and to help us to find what we love to do.

From: Teaching and expanding the knowledge (1964) Science ***3649****:*1278

Communication by SMS

- Short Message Service
- Short Motif Sequences = peptides
- enhance communication

Communication by SMS

Communication may be :

- short
- regulated

Herbie (ERBB4) 1/3/2016 15:00
Hey Chap, R U free?
Give me a ride? ✋

Herbie (ERBB4) 1/3/2016 20:00
… I'm w8ing … 🙌

Chap (YAP1) 1/3/2016 21:00 🚗
Yep, I got a space 💺

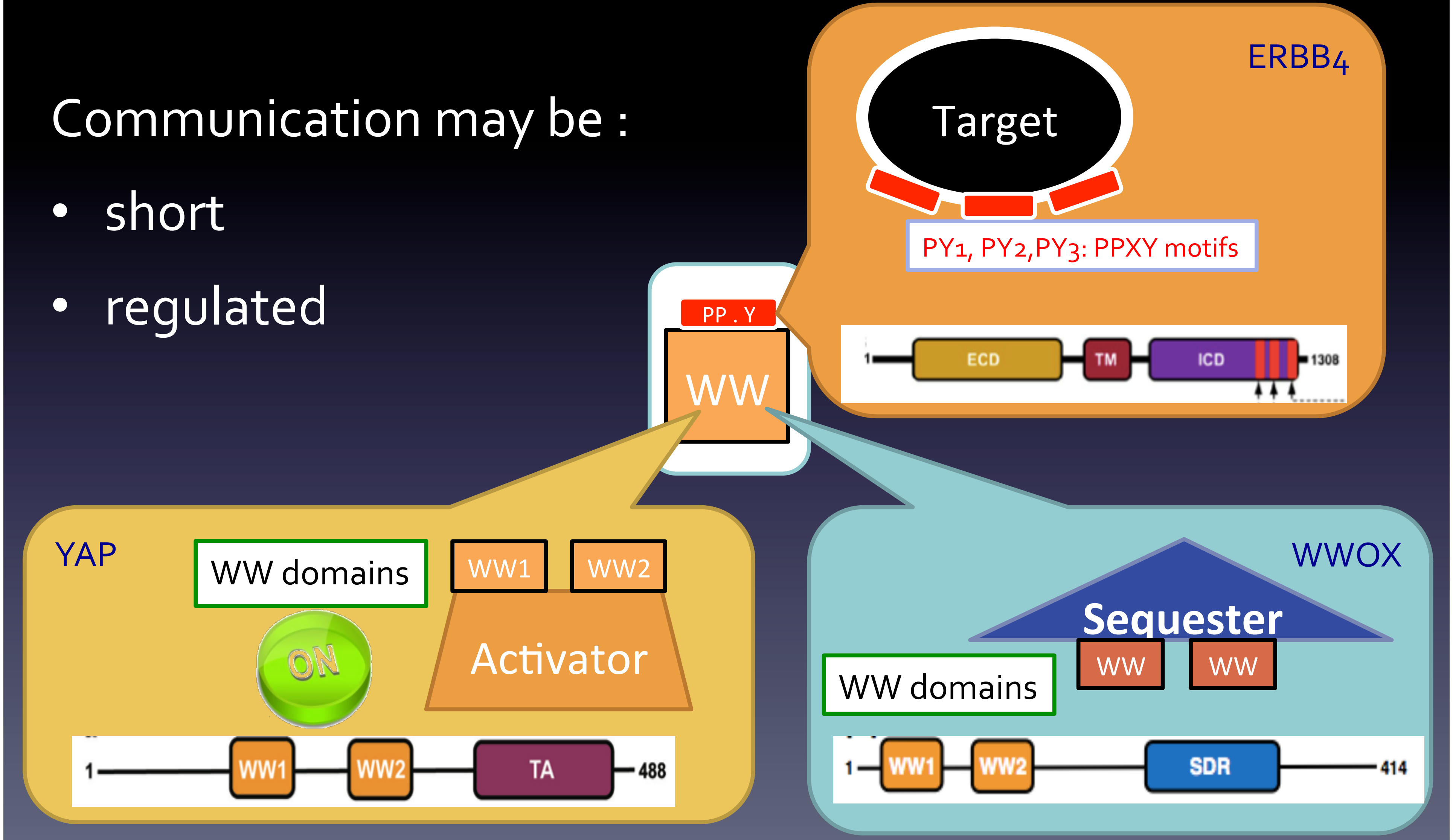
WOW (WWOX) 💺🍵
1/3/2016 21:00
Herbie, dinner!

Herbie (ERBB4) 1/3/2016 21:05
Sorry Chap - 2 l8 😟

Communication by SMS

Communication may be :

- short
- regulated



ERBB4

Target

PY1, PY2,PY3: PPXY motifs

PP . Y

WW

YAP

WW domains

ON

WW1

WW2

Activator

WWOX

Sequester

WW domains

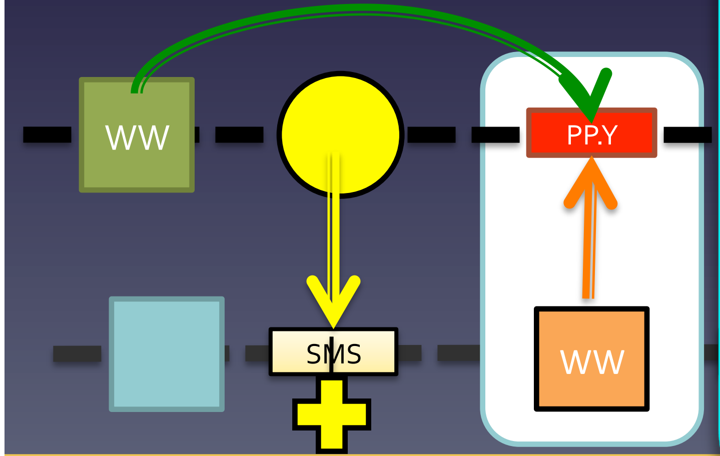
WW

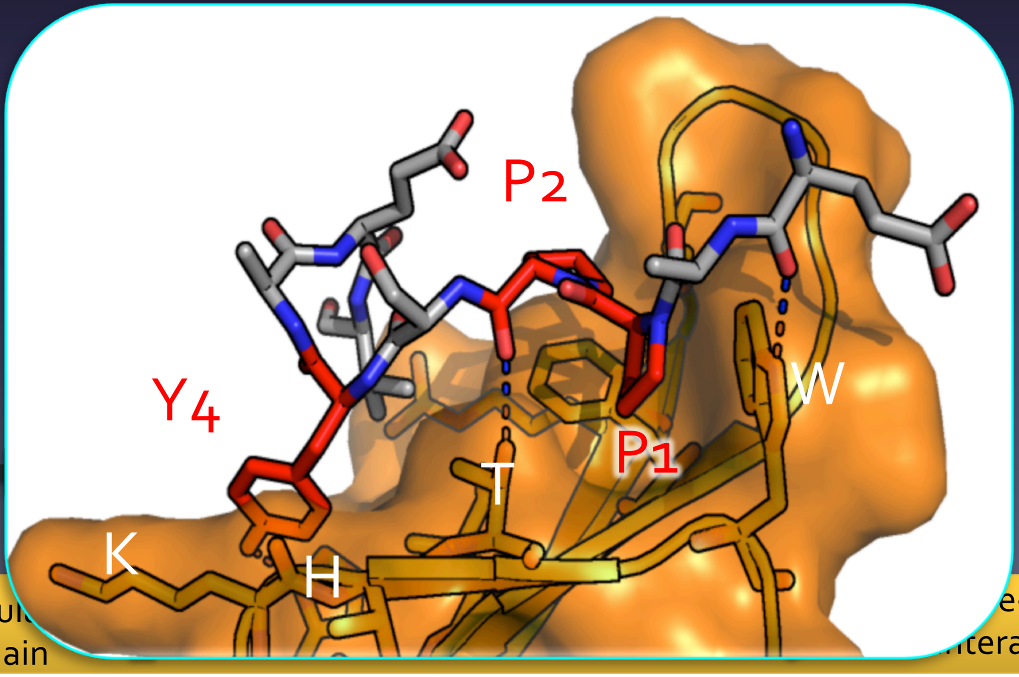
WW

SMS mediate many interactions

- transient
- regulation
- trans / cis
- PTM

- **sequence-motifs (SMS)**
- **structure :**
  - details of binding
  - drug design



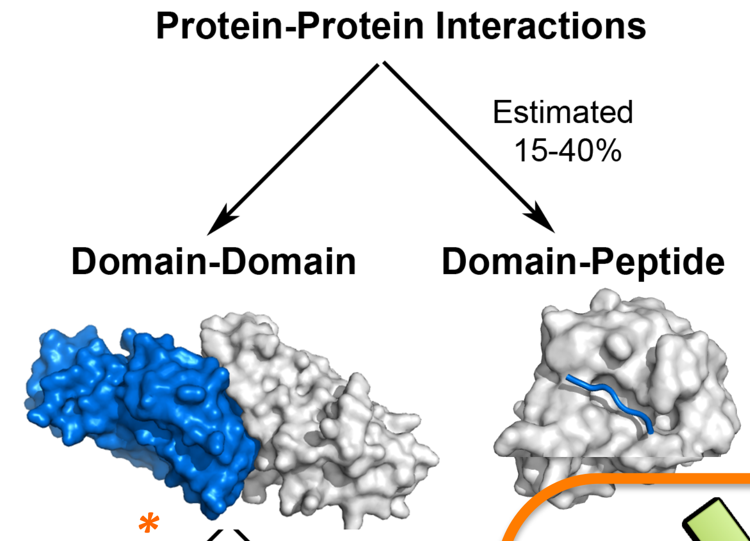


▬ ▬ unstructured region

☐ globul domain

e-protein nteraction

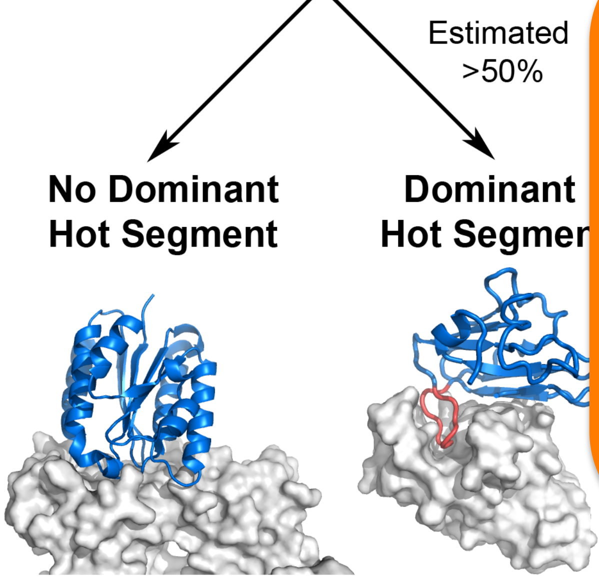
SMS mediate many interactions



Protein-Protein Interactions

Estimated 15-40%

Domain-Domain **Domain-Peptide**

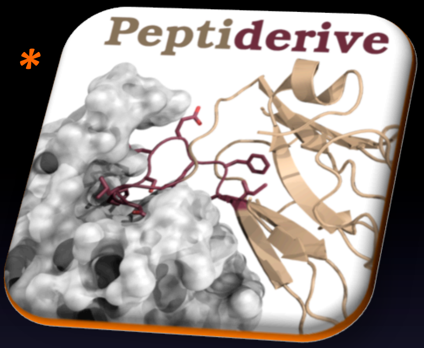


*

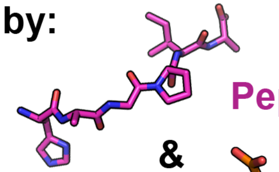
Estimated >50%

No Dominant Hot Segment

Dominant Hot Segmen

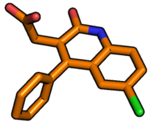


Amenable to inhibition by:

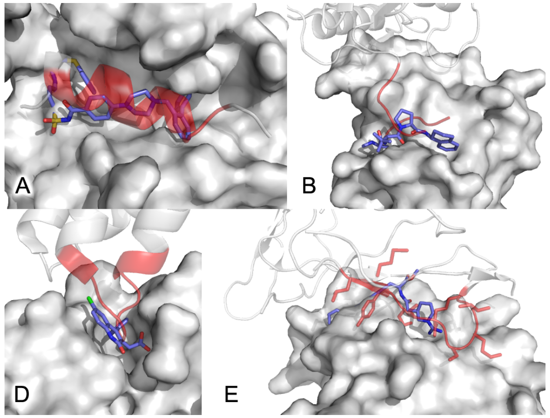


Peptides

&



Small molecules



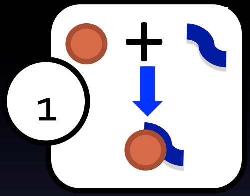
** London, Raveh, Movshovitz-Attias, Schueler-Furman. Proteins (2010); http://rosie.rosettacommons.org/peptiderive, Sedan & Marcu, NAR (2016)*

London, Barak and Schueler-Furman. Current Opinion Chemical Biology (2013)

Peptide-protein interactions - Aims

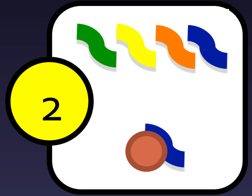
1. define structure of peptide-protein interaction

   Rosetta FlexPepDock & Cluspro PeptiDock

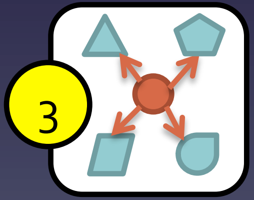


2. predict binding affinity & specificity

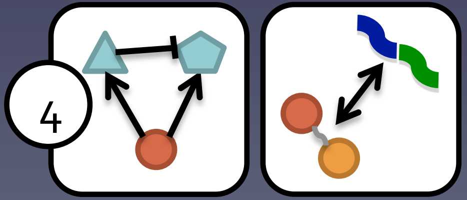
   Rosetta FlexPepBind



3. detect new interactions



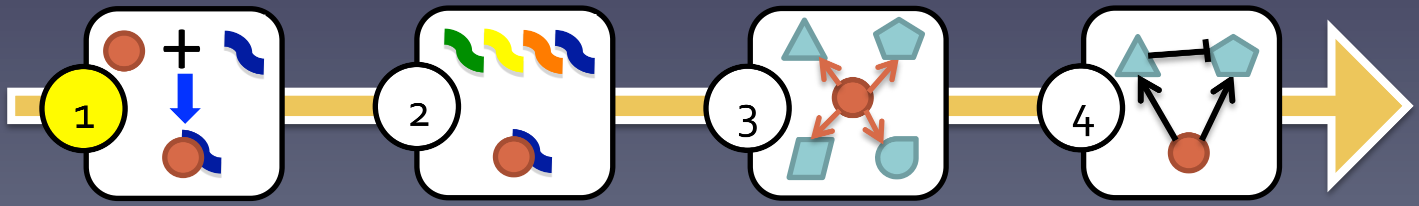
4. put things into context



Model structure: FlexPepdock

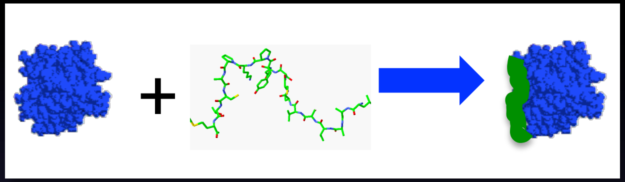
Nawsad Alam
Orly Marcu

Nir London
Barak Raveh
Lior Zimmerman



The challenge of peptide binding

Nature's challenge



$$\Delta G_{BIND} = \Delta H - T\Delta S$$

✧ **small** interface

✧ **flexible** peptide

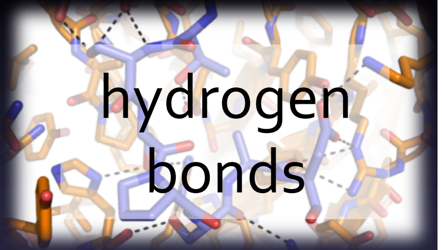
➢ how do peptides do it?

peptiDB

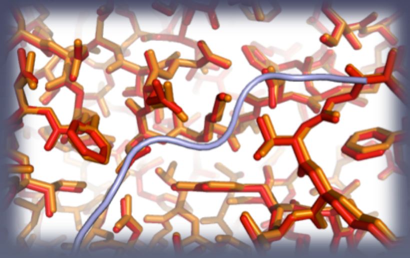
London, Movshovitz-Attias, Schueler-Furman (2010) Structure

Nature's solutions

① increase ΔH



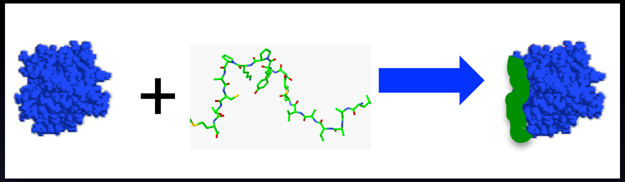
② decrease ΔS



prearranged receptor

The challenge of peptide binding

Our challenge



$\Delta G_{BIND} = \Delta H - T\Delta S$

✧ **small** interface

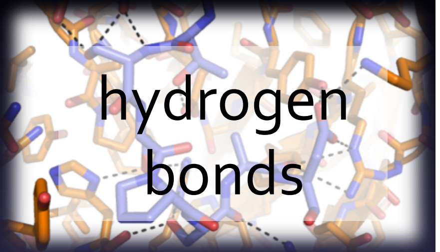
✧ **flexible** peptide

➢ how do we model these interactions?

Nature's solutions

① increase ΔH

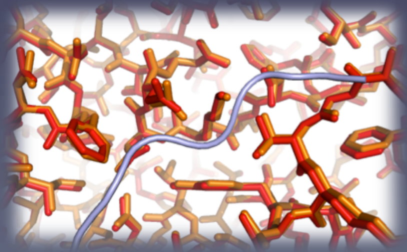

packing


hydrogen bonds


salt bridges

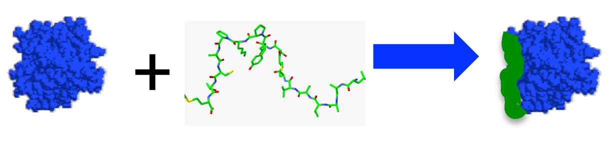

hot spots

② decrease ΔS


prearranged receptor

The challenge of peptide binding

Our challenge



$\Delta G_{BIND} = \Delta H - T\Delta S$

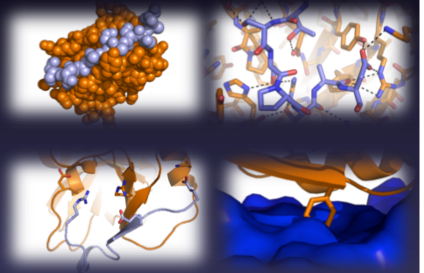
✧ **small** interface

✧ **flexible** peptide

➢ how do we model these interactions?

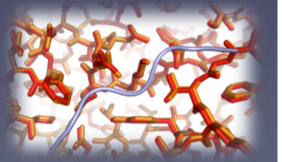
Our solution

① Extensive optimization of interface



② Restrict sampling to relevant space

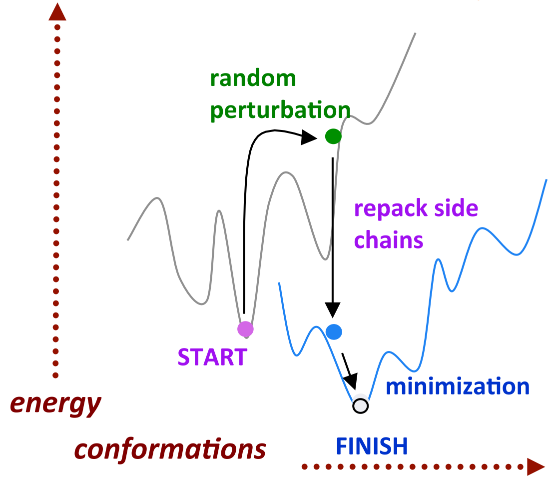
➢ focus on peptide



Rosetta infrastructure for structural modeling

Sampling and optimization

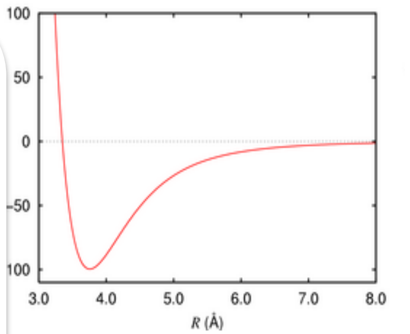
“**M**onte-**C**arlo search with energy **M**inimization” **MCM** (Liu *et al.*)



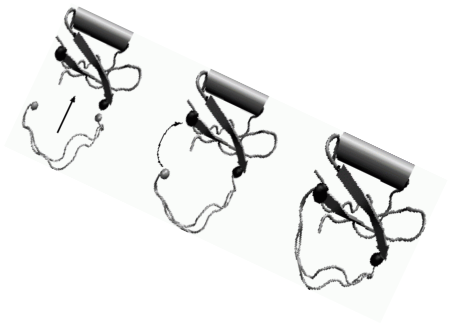
FlexPepDock protocol for flexible peptide docking and redesign

Energy function

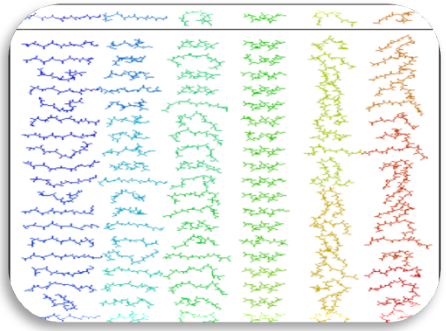
(full-atom/centroid side-chains)



Loop modeling

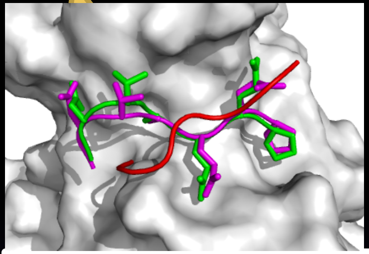


Fragment libraries



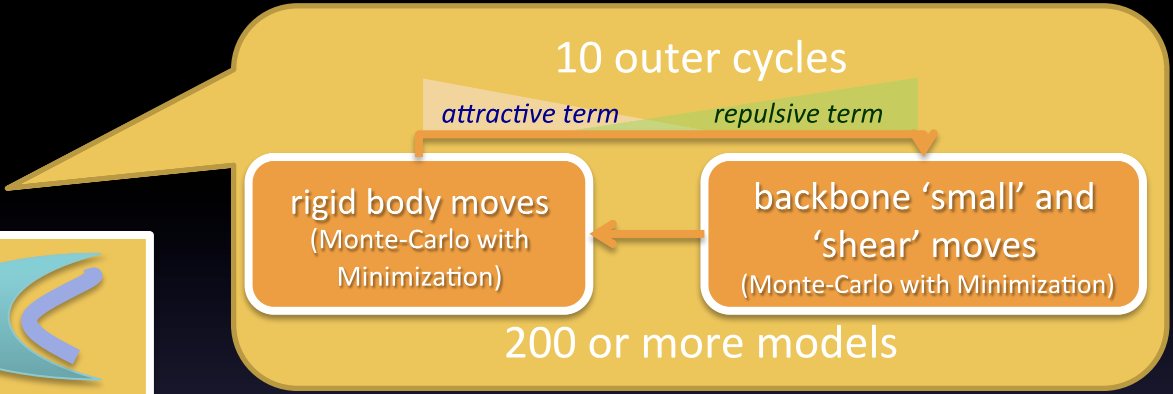
Raveh, et al. Proteins (2010)
Raveh et al. PLosOne (2011)
London et al. Current Opinion in Structural Biology (2013)

Rosetta FlexPepDock protocol



FlexPepDock

http://flexpepdock.furmanlab.cs.huji.ac.il



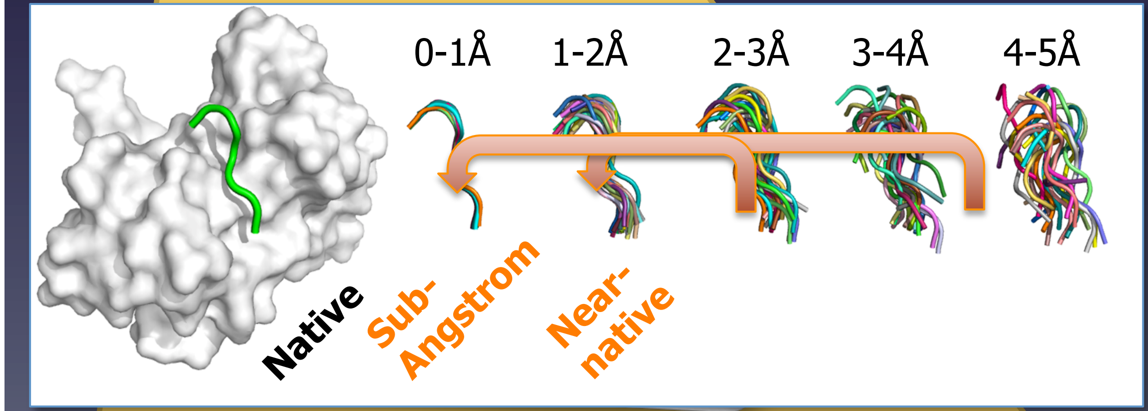
10 outer cycles

attractive term *repulsive term*

rigid body moves
(Monte-Carlo with Minimization)

backbone ‘small’ and ‘shear’ moves
(Monte-Carlo with Minimization)

200 or more models



General:

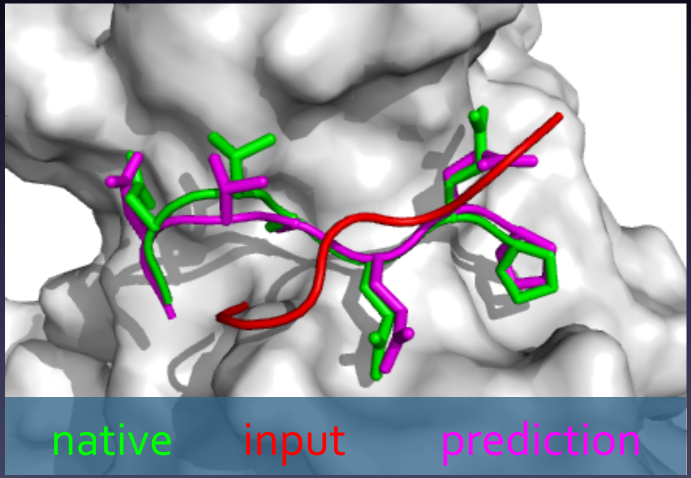
tested on large benchmark

peptiDB

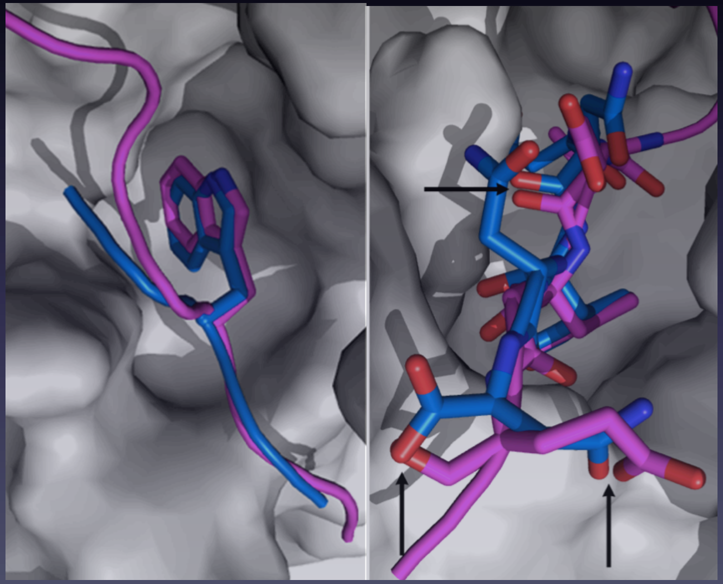
- Short peptides 5-15 aa
- High resolution < 2Å
- Non redundant < 70% seq id

Rosetta FlexPepDock

accurate prediction of motif residues



backbone rearrangements preserve critical interactions



- try our server :
http://flexpepdock.furmanlab.cs.huji.ac.il/

flex pep dock

London, Raveh et al. NAR (2011)

Impact of FlexPepDock

➜ first dedicated peptide-docking protocol

➜ motivated development of many new approaches

➜ CAPRI (docking competition) looks at peptides

➜ to appear soon: THE book on peptide docking

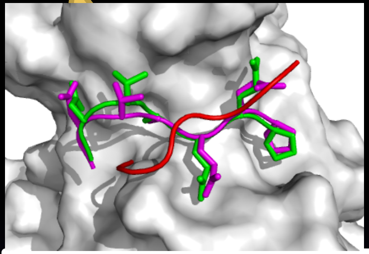
Springer Protocols

Methods in Molecular Biology

Humana Press

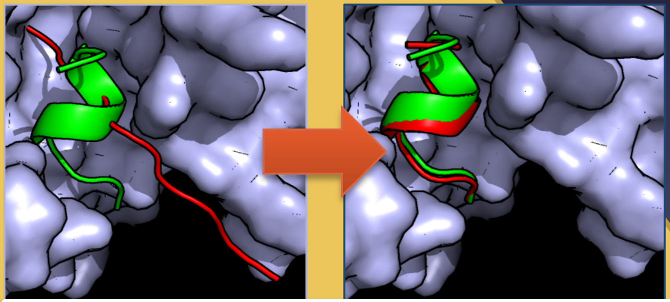
edited with Nir London

Extension of Rosetta FlexPepDock

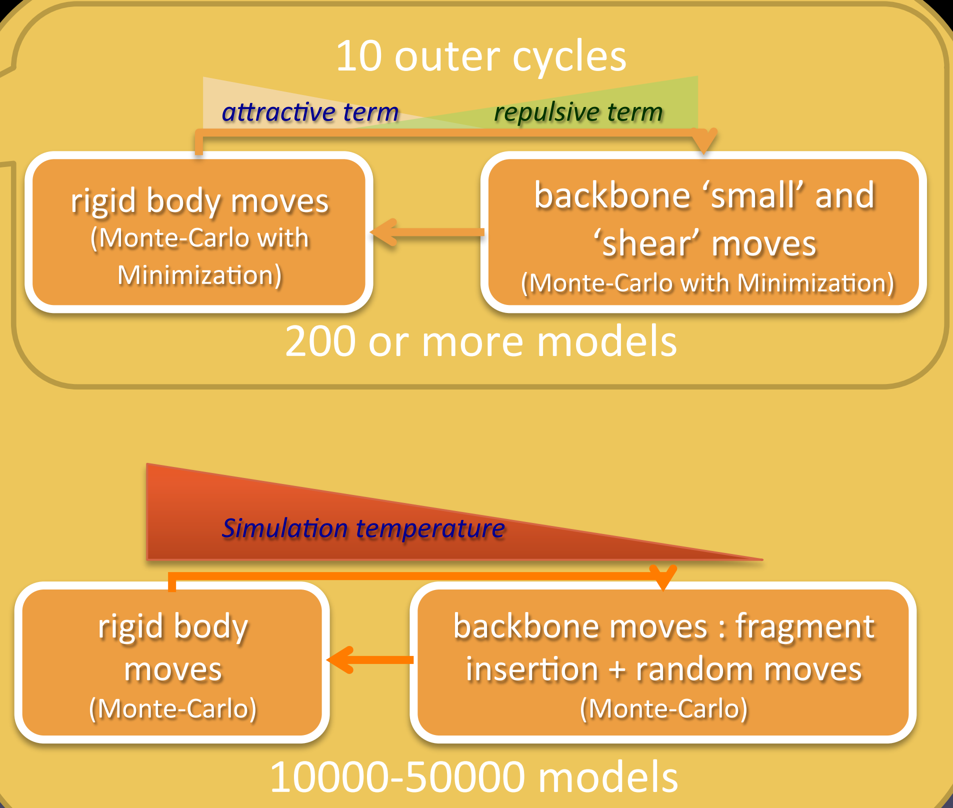


FlexPepDock

http://flexpepdock.furmanlab.cs.huji.ac.il



Ab initio FlexPepDock



10 outer cycles

attractive term *repulsive term*

rigid body moves
(Monte-Carlo with Minimization)

backbone 'small' and 'shear' moves
(Monte-Carlo with Minimization)

200 or more models

Simulation temperature

rigid body moves
(Monte-Carlo)

backbone moves : fragment insertion + random moves
(Monte-Carlo)

10000-50000 models

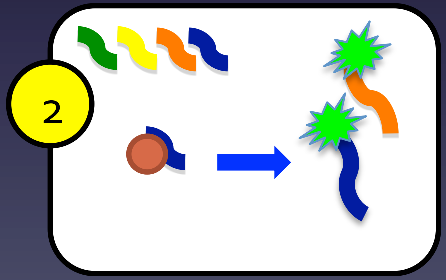
- **Full *ab initio***

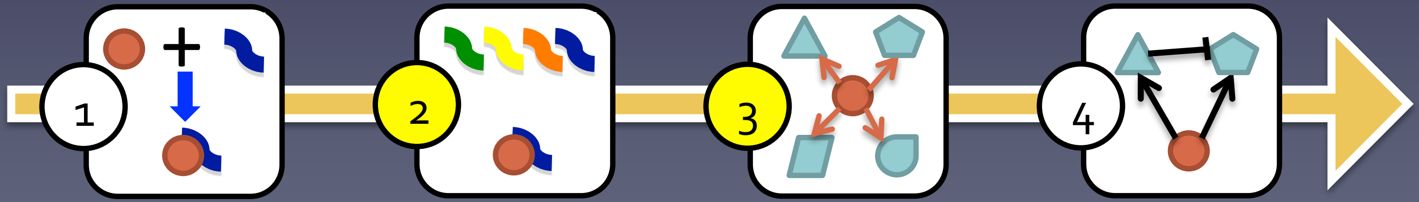
Model binding partners: FlexPepBind

Nawsad Alam

Nir London
Lior Zimmerman
Michal Sperber

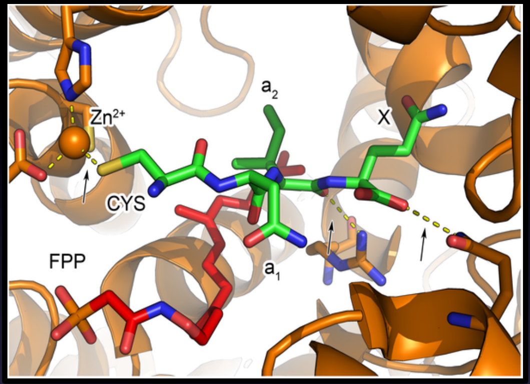
Experiments:
Noah Wolfson
Fierke lab, U Michigan





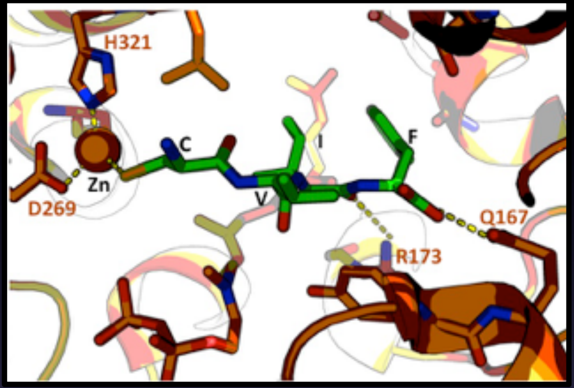
FlexPepBind applications

Farnesyl transferase



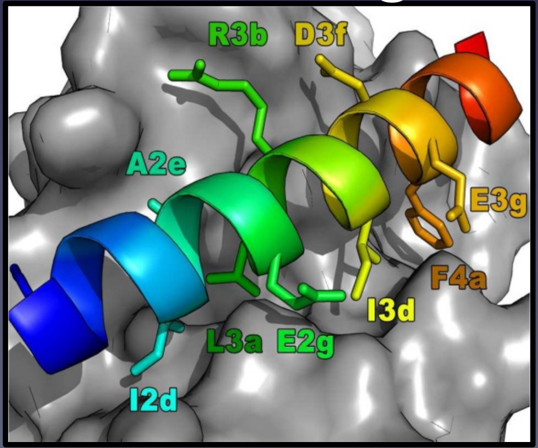
London *et al. PLoS Comput Biol* (2011)

Geranyl-geranyl transferase



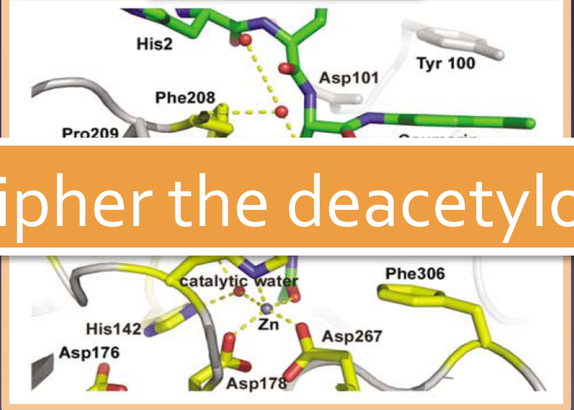
Sperber *et al., in preparation*

Bcl2-BH3



London *et al. Biochemistry* (2012)

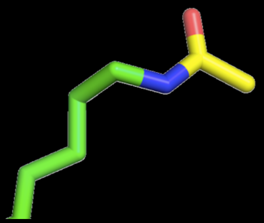
HDAC8



Decipher the deacetylome

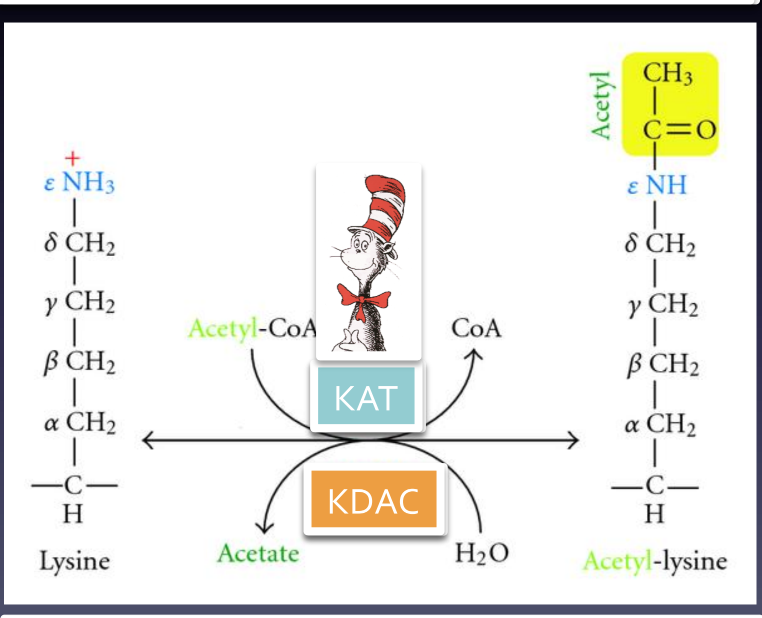
Alam *et al. Structure* (2016)

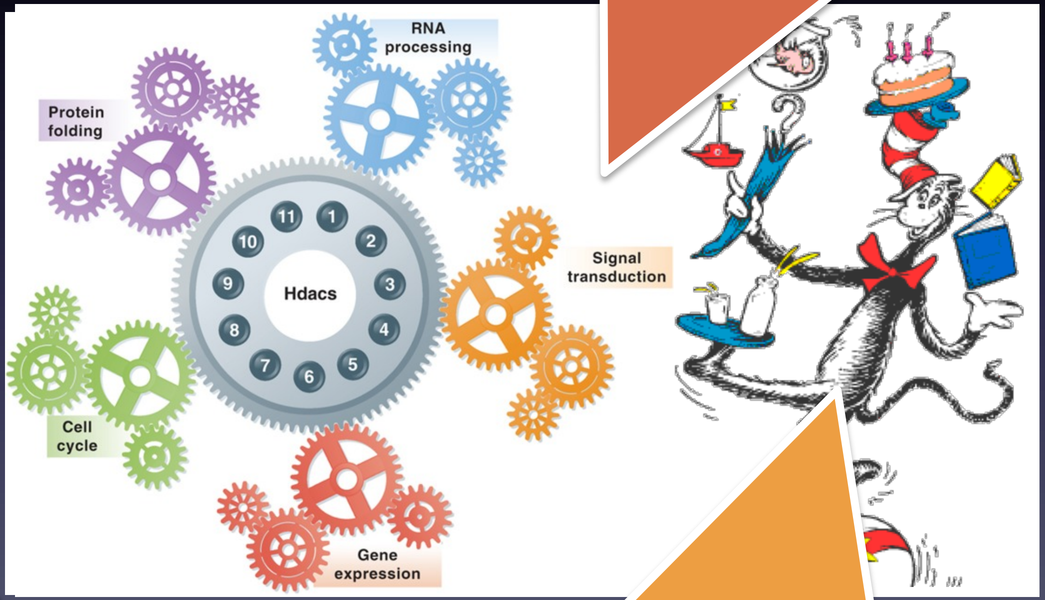
Lysine (de)acetylation



Lysine acetyl transferase

Can we find more substrates?

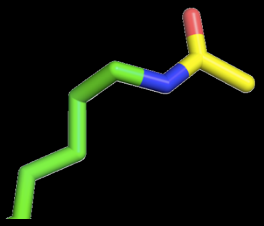


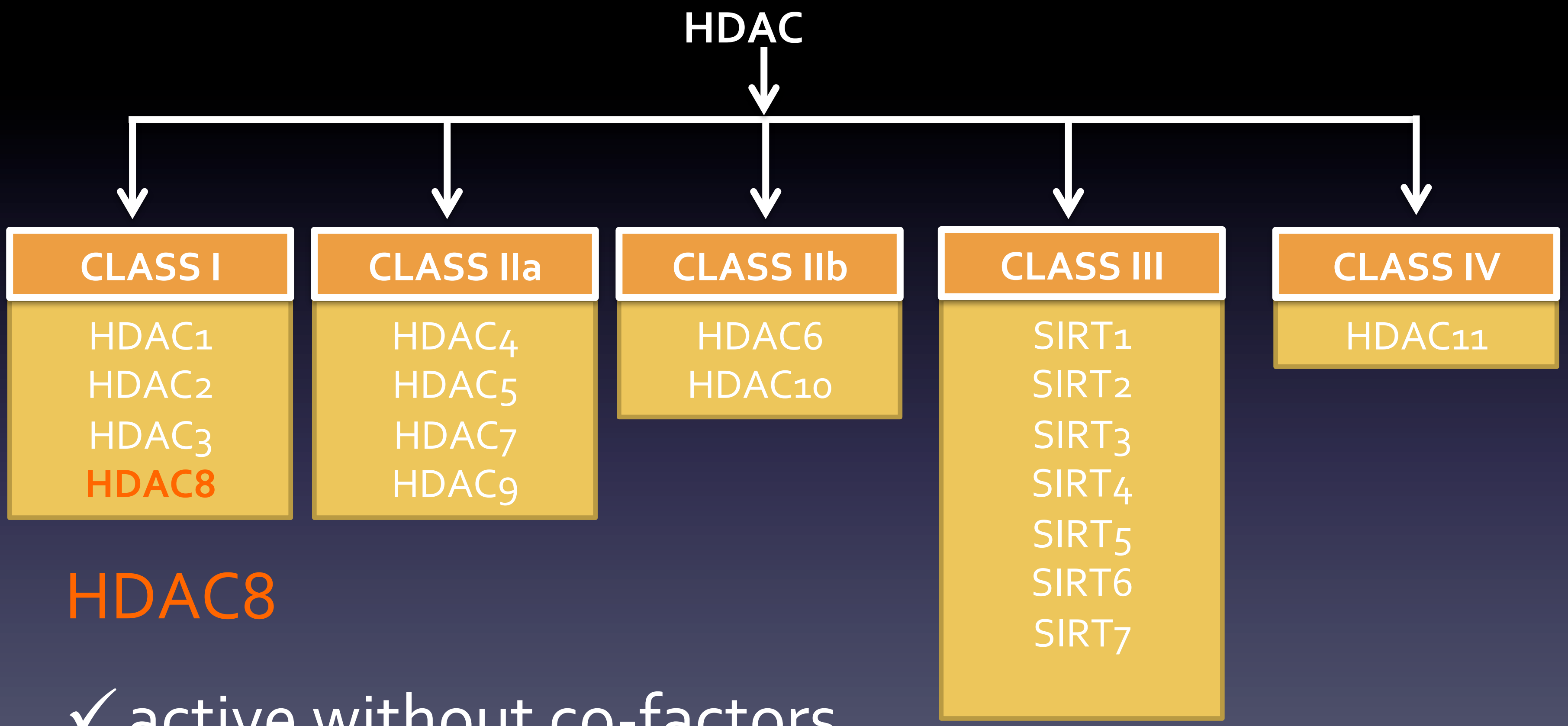


Lysine deacetylase

“Oh no, that is not all I can do” said the Kat in the Hat…

Lysine deacetylation





HDAC

CLASS I	CLASS IIa	CLASS IIb	CLASS III	CLASS IV
HDAC1	HDAC4	HDAC6	SIRT1	HDAC11
HDAC2	HDAC5	HDAC10	SIRT2	
HDAC3	HDAC7		SIRT3	
HDAC8	HDAC9		SIRT4	
			SIRT5	
			SIRT6	
			SIRT7	

HDAC8

✓ active without co-factors

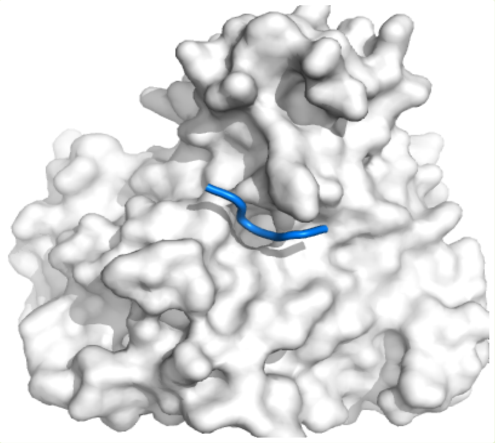
✓ related to important diseases

Known substrates:
P53, SMC3, ERRα, ARID1A, Cortactin 9, Inv(16)

FlexPepBind Framework

1 INPUT

- peptide-protein complex structure

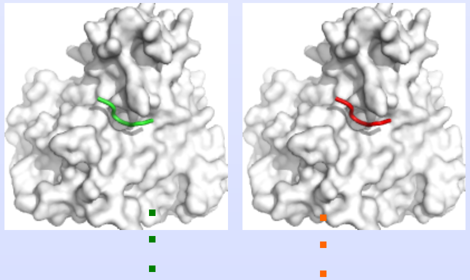


- training set

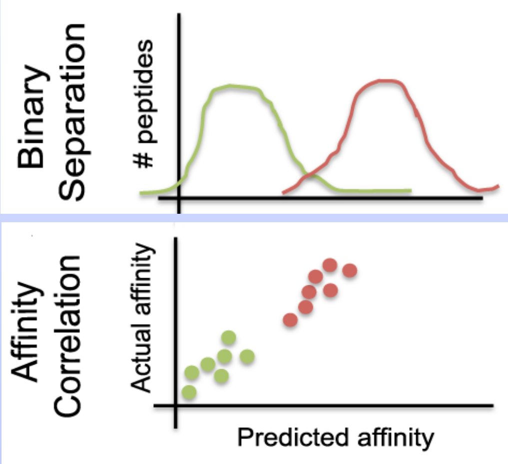
Binders	Non-Binders
GYKacFGC	GWKacMGC
GIKacWGC	GMKacGGC
GRKacFGC	GKKacKGC
⋮	⋮

2 CALIBRATION

- model set
- adjust protocol



- optimize for:



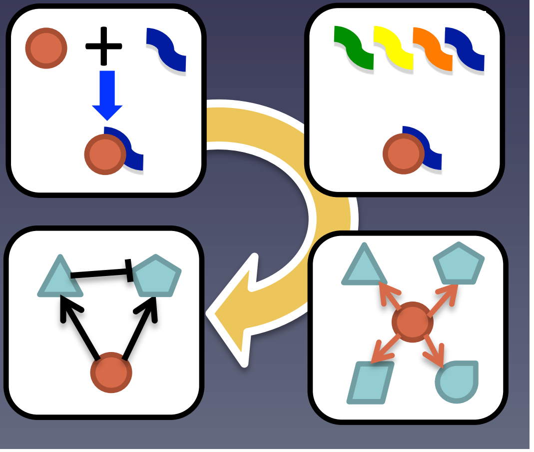
3 PREDICTION

- large-scale application

RESULTS

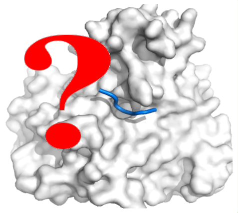
- identify new protein targets from discovered peptide substrates

**...ERFEMFRELNEALELKDAQ
AGKEPGGSRAHSSHLKSKKG
QSTSRHKKLMFKTEGP...**



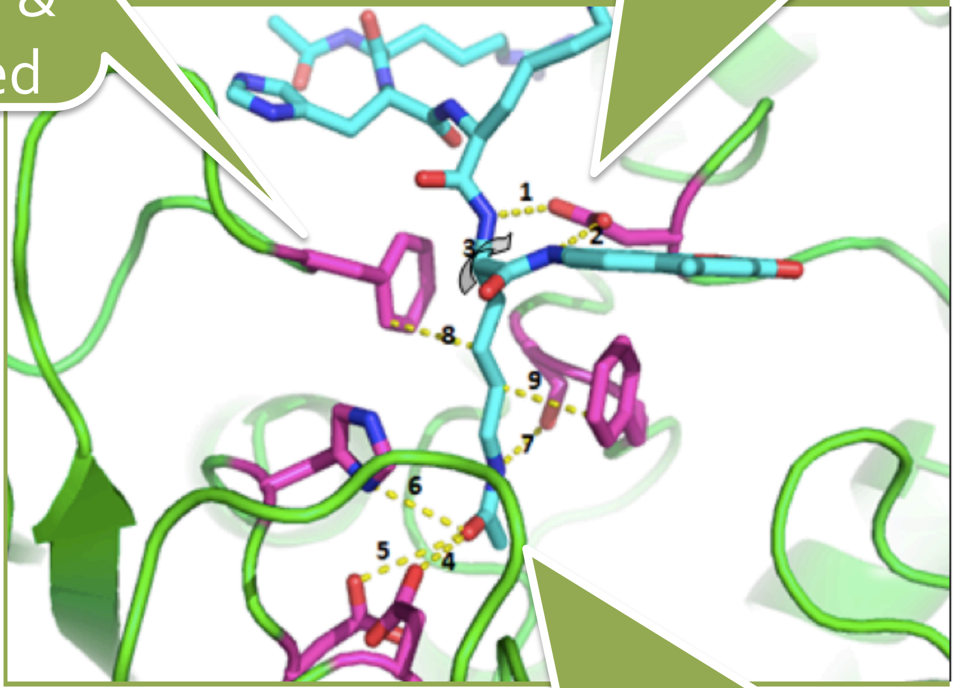
HDAC8 substrate hunt: input

1 **INPUT**



K_{Ac} side chain planar & stacked

K_{Ac} backbone fixed by 2 hydrogen bonds to D101



K_{Ac} acetyl coordinated by Zn ion

2v5w: HDAC8 - K-acetylated p53-derived peptide with coumarine tail

binding site surrounded by loops

HDAC8 substrate hunt: input (II)

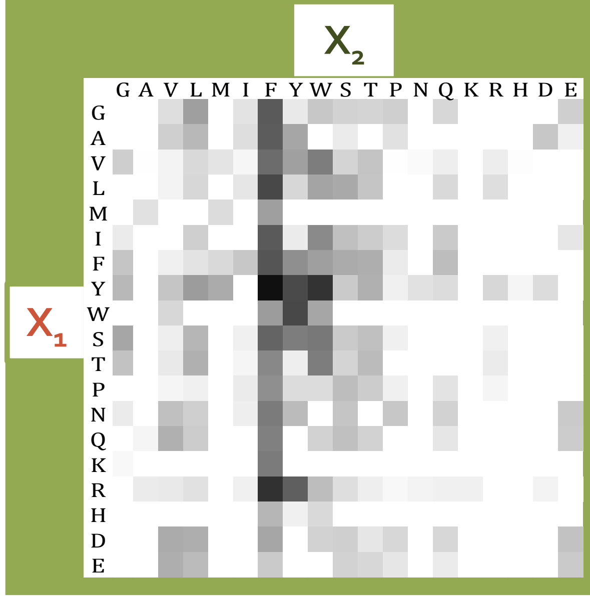
1

INPUT

Binders	?	Non-Binders
GYKacFGC		GWKacMGC
GIKacWGC		GMKacGGC
GRKacFGC		GKKacKGC

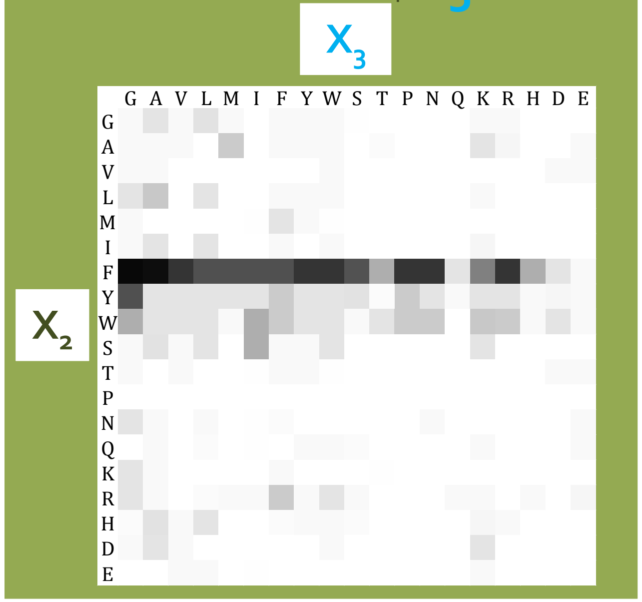
training set

GX_1KacX_2GC



test set

$GRKacX_2X_3GC$



SAMDI*

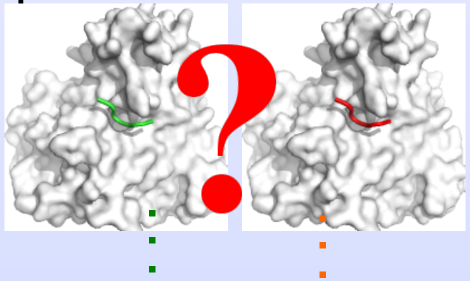
* **S**elf-**A**ssembled monolayers for **M**atrix assisted laser **D**esorption / **I**onization time-of-flight mass spectrometry

Gurard-Levin *et al. ACS Chem Biol.* (2010).

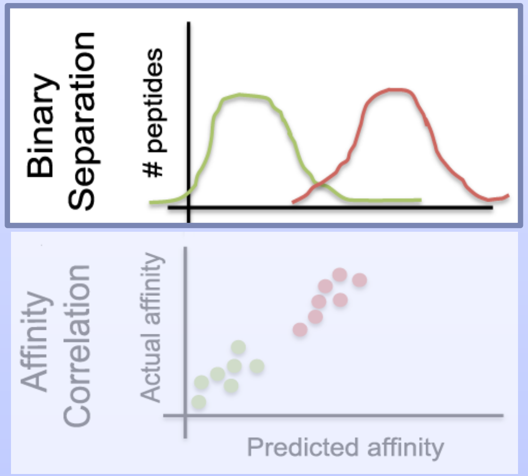
HDAC8 substrates: critical features

2 CALIBRATION

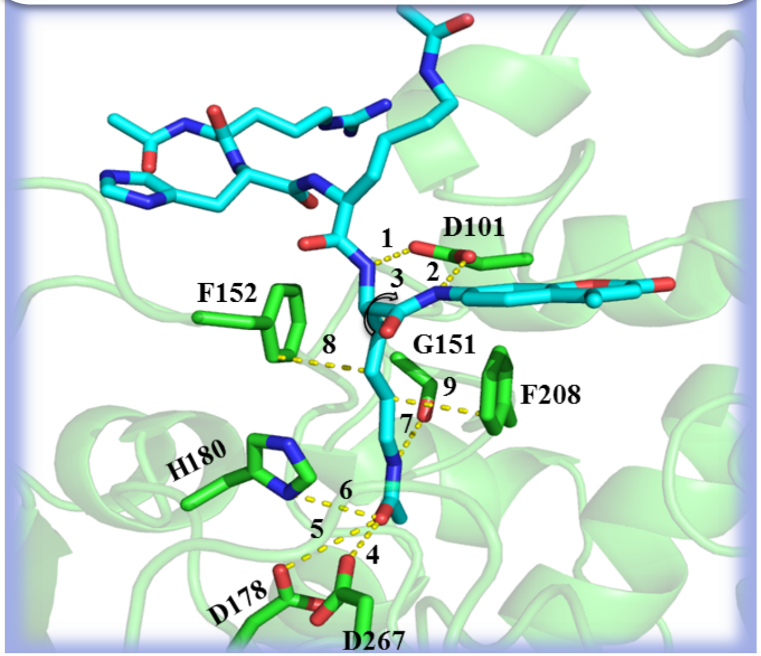
- model training set
- adjust protocol
- predict test set



optimize for:



reinforce critical features of interaction

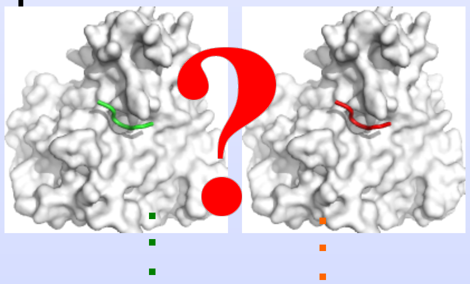


favorable binding ⇧ == ⇧ favorable catalysis

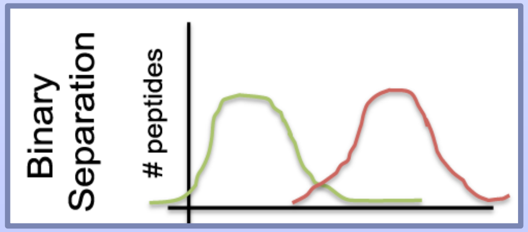
HDAC8 substrate hunt: optimize

② **CALIBRATION**

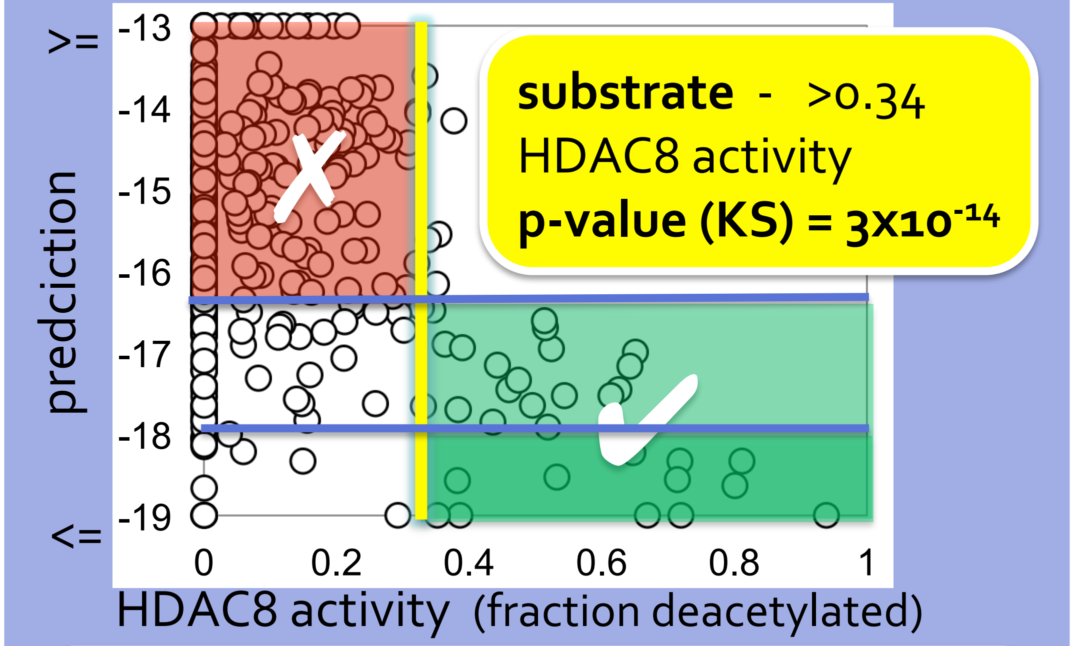
- model training set
- adjust protocol
- predict test set



optimize for binary separation



training set GX$_1$KacX$_2$GC



substrate - >0.34
HDAC8 activity
p-value (KS) = 3x10^{-14}

thresholds:

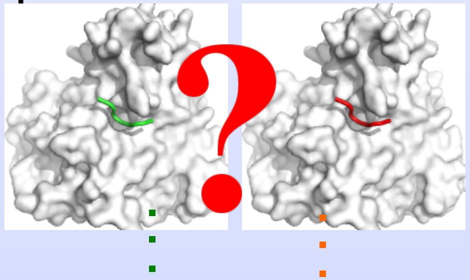
stringent <-18.0 **TP: 35% FP: 4%**

lenient <-16.5 **TP: 91% FP: 19%**

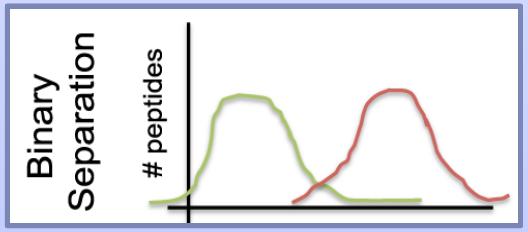
HDAC8 substrate hunt: validate

2 CALIBRATION

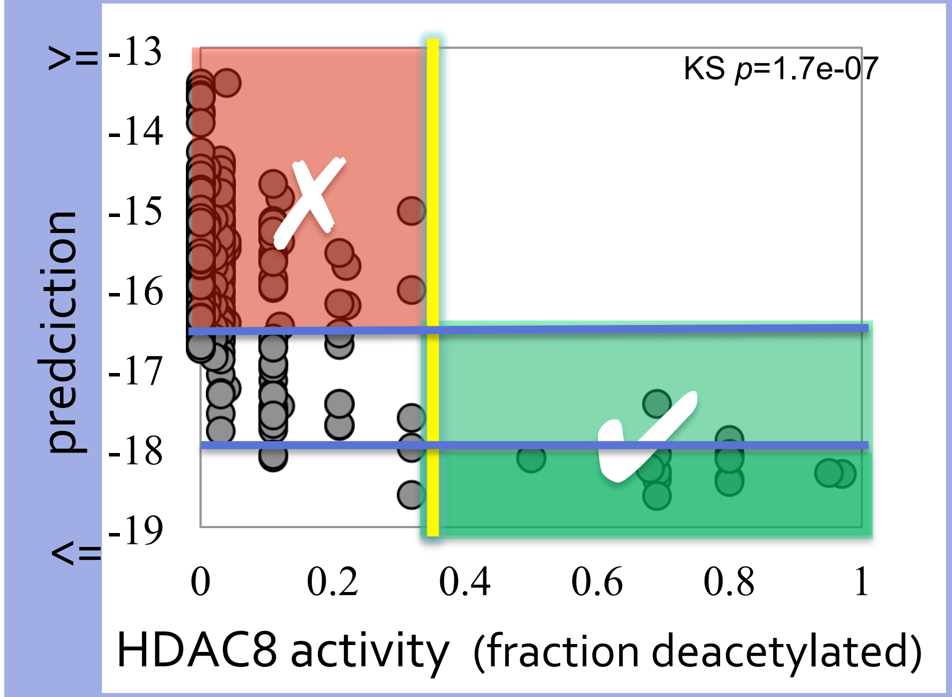
- model training set
- adjust protocol
- predict test set



optimize for binary separation



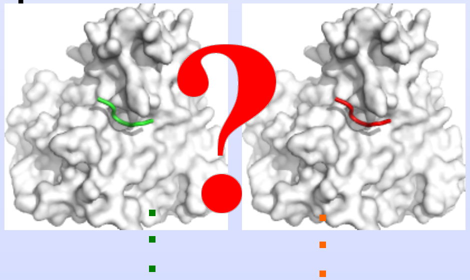
Test set GRKacX_2X_3GC



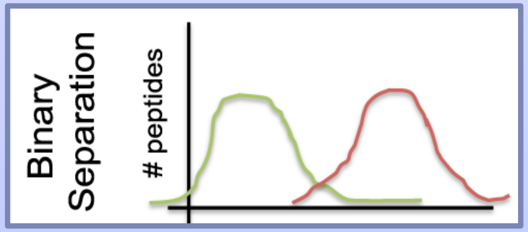
Summary of validation

2 CALIBRATION

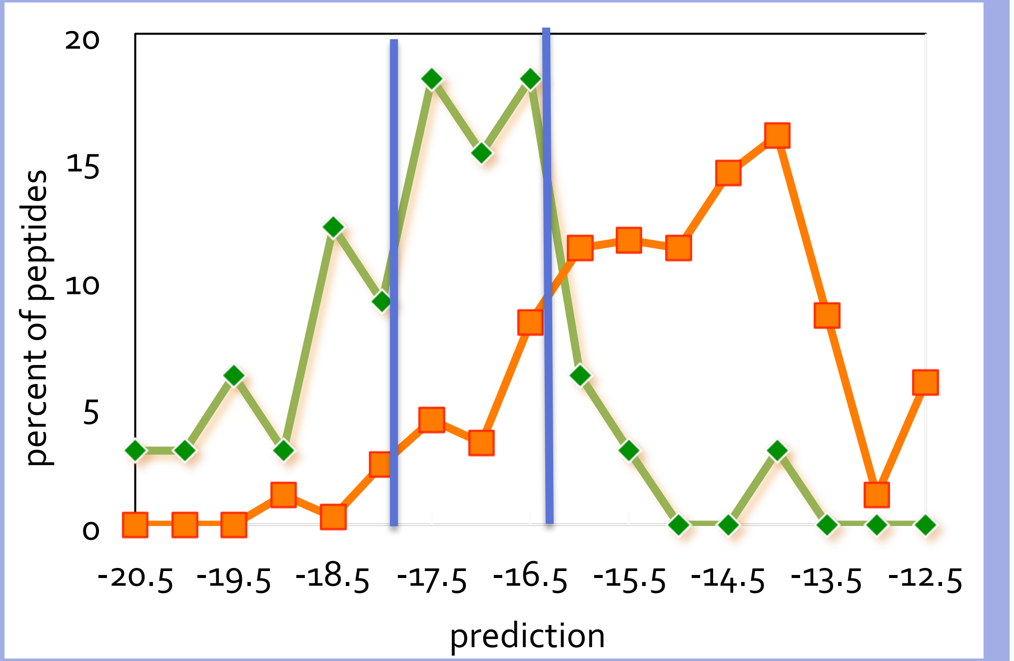
- model training set
- adjust protocol
- predict test set



optimize for binary separation



same, as histogram



Prediction of new HDAC8 substrates

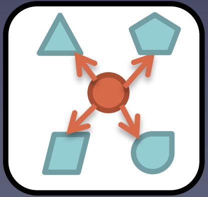
3 PREDICTION

- apply protocol to set of candidate sequences

RESULTS

- identify new protein targets from discovered peptide substrates

…ERFEMFRELNEALELKDAQ AGKEPGGSRAHSSHLKSKKG QSTSRHKKLMFKTEGP…



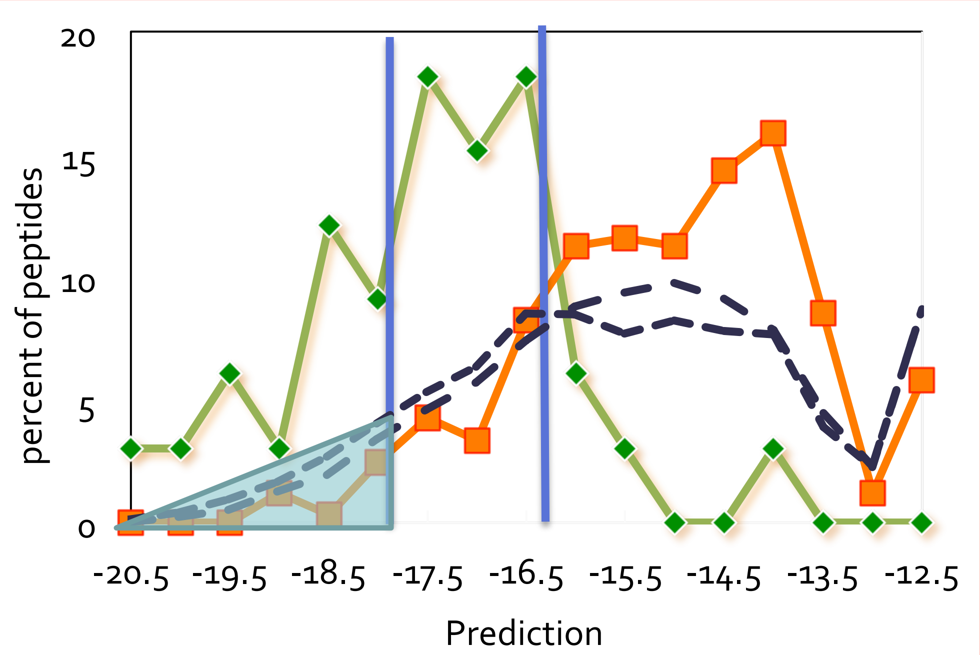
PhosphoSitePlus® (PSP) is an online systems biology resource providing comprehensive information and tools for the study of protein post-translational modifications (PTMs) including phosphorylation, ubiquitination, acetylation and methylation. See About PhosphoSite above for more information.

Please cite the following reference for this resource: Hornbeck PV, et al (2015) *PhosphoSitePlus, 2014: mutations, PTMs and recalibrations*. Nucleic Acids Res. 43:D512-20. [reprint]

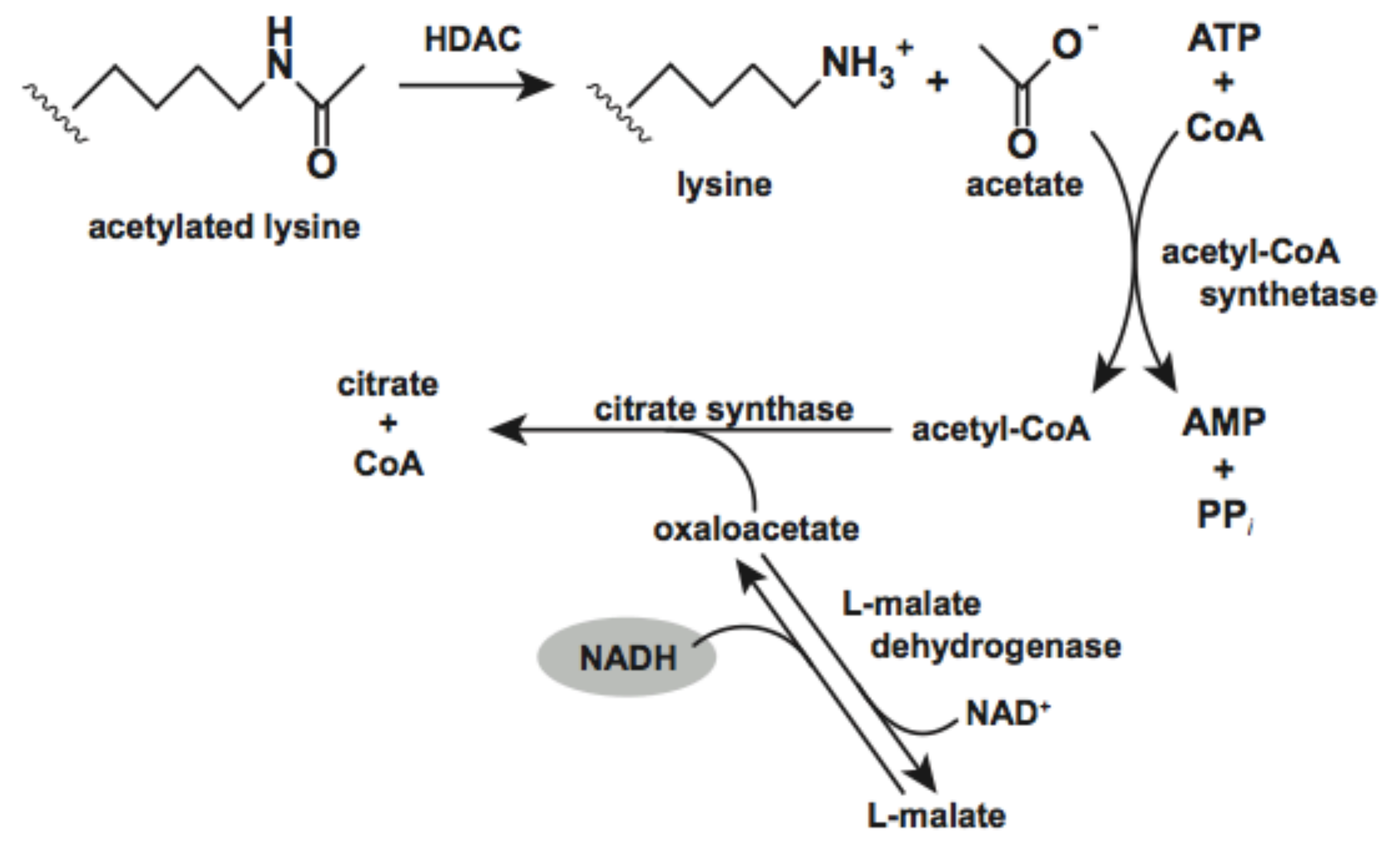
A PROTEIN MODIFICATION RESOURCE

Hornbeck *et al.* Proteomics. (2004)

PhosphoSite scanning: ~20'000 reported acetylated sites

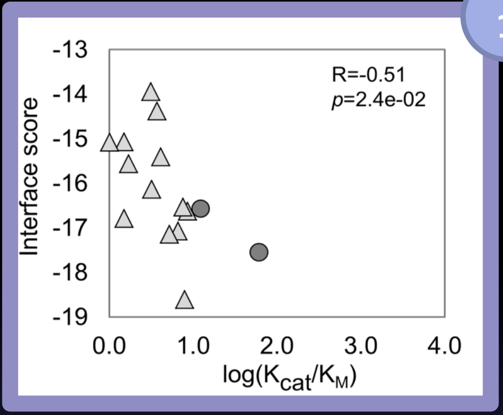


In vitro HDAC8 activity assay



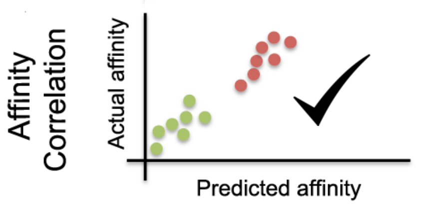
Wolfson et al., Analytical Biochemistry (2014)

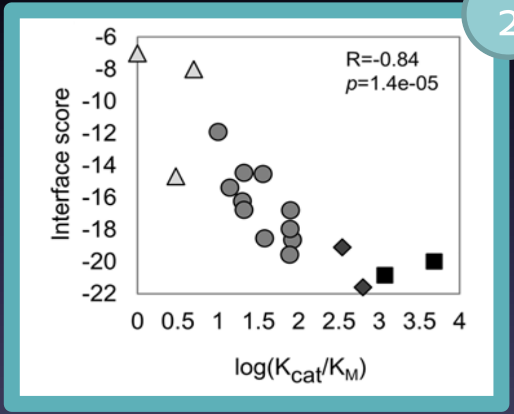
Prediction & experiment correlate



1

validation set 1
1st PhosphoSite screen
(n=20)





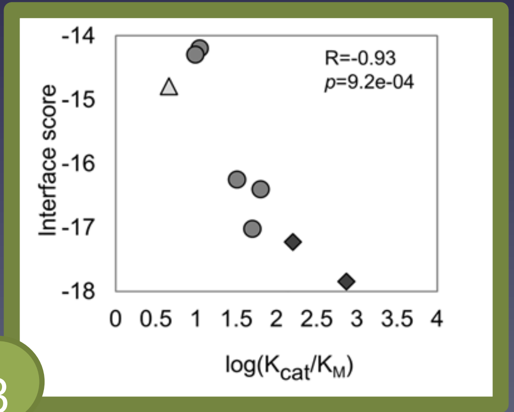
2

validation set 2
2nd PhosphoSite screen
(n=18)

prediction

reactive (k_{cat}/K_M >1000 $M^{-1}s^{-1}$) ■
reasonable (> 100 $M^{-1}s^{-1}$) ◆
mild (> 10 $M^{-1}s^{-1}$) ●
poor (> 1 $M^{-1}s^{-1}$) △

validation set 3
in vivo proteomic
assay* (n=8)

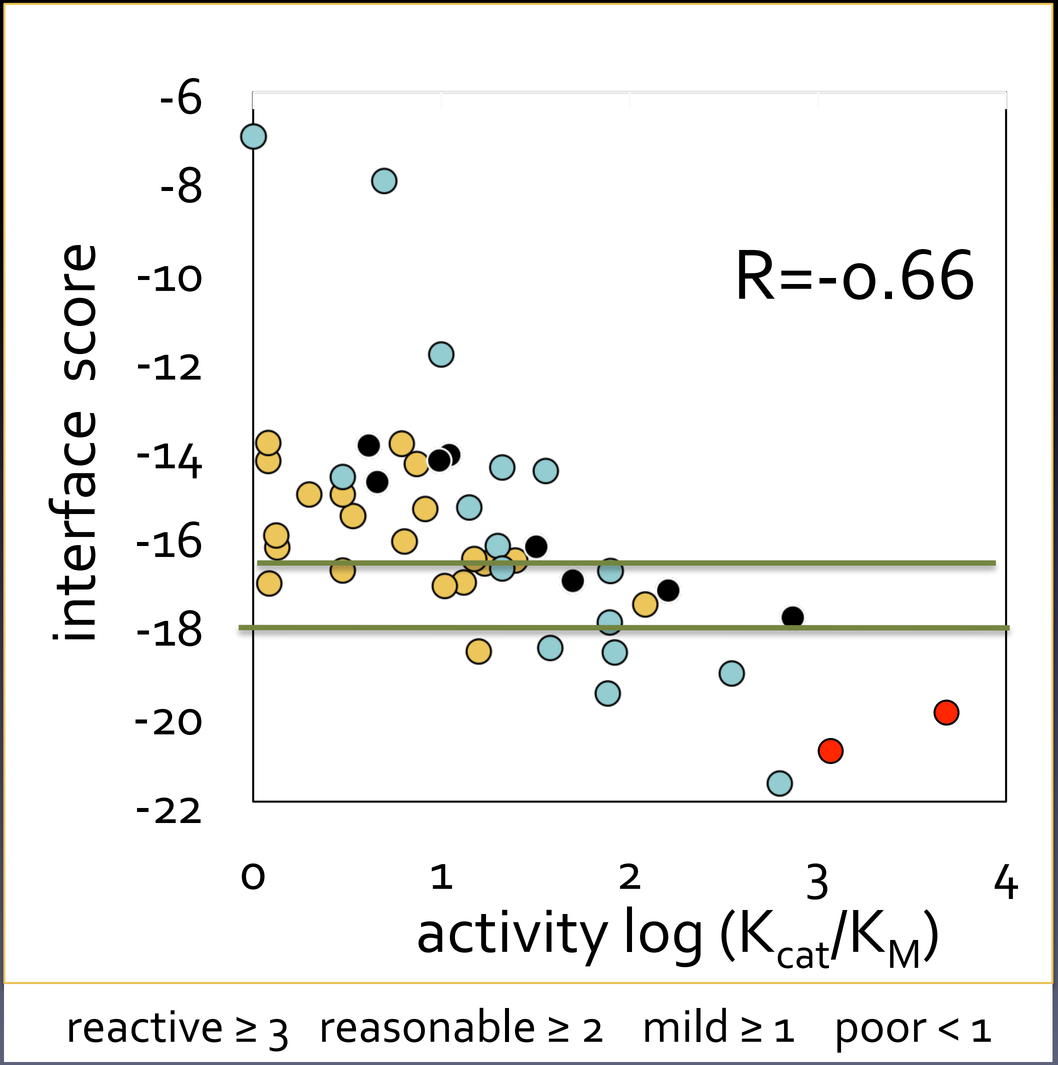


3

experiment

**Olson et al. ACS Chemical Biology 2014*

Summary of validation



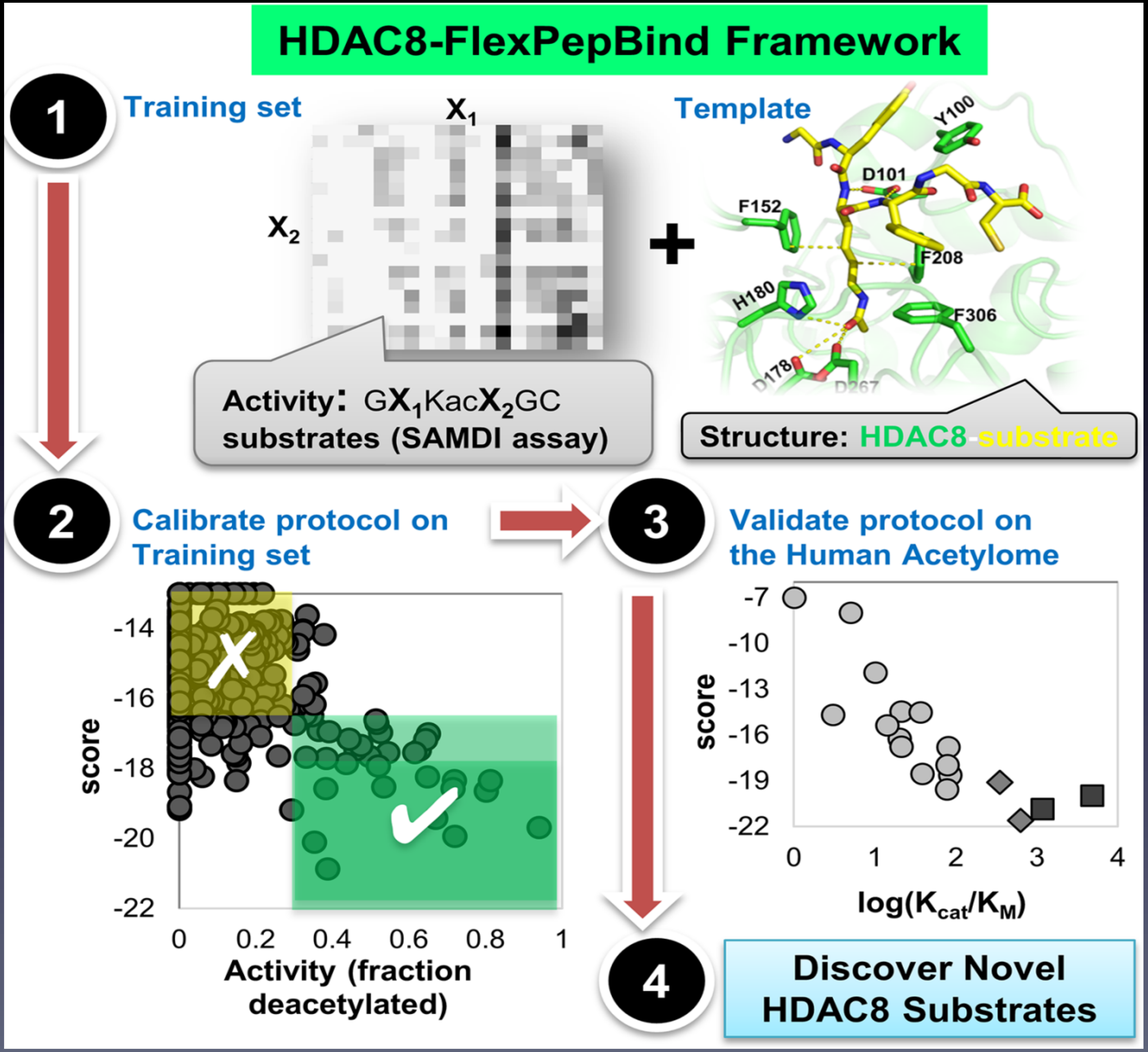
✓ good correlation *across* experiments

✓ stable thresholds

✓strongest measured HDAC8 substrates

- peptide reactive (>1000 K_{cat}/K_M)
- expected protein reactivity: x1000

Summary



Who are these substrates?

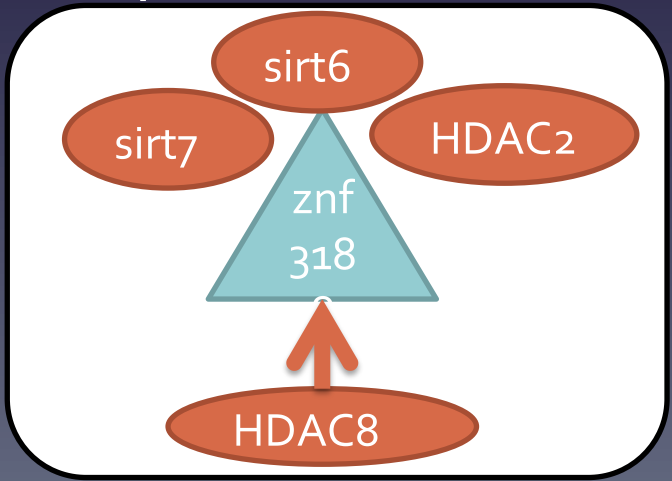
1

Strongest HDAC8 target: ZNF318

peptide	log (Kcat/K_M)	min. I_sc	protein
FG K1275 FSW	3.68	-20.0	Zinc finger protein 318

K1275: unstructured & accessible
- G1274R in breast cancer
- S1277 phosphorylated

ZNF318 represses Androgen Receptor
- related partners:
  - sirt6, sirt7 & HDAC2



reactive ≥ 3 **reasonable ≥ 2** mild ≥ 1 poor < 1

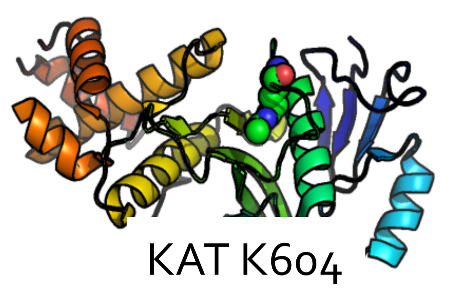
②

Targets: good old neighbors!

peptide	log (K_{cat}/K_M)	min. I_sc	protein
STPV K292 FISR	2.20	-17.23	CSRP2BP (HAT)
VS K350 GPF	2.09	-17.5	KAT6A (MYST3)
DH K604 TLY	1.18	-16.5	KAT6A
DS K1583 NAK	1.02	-17.1	CREB Binding Protein

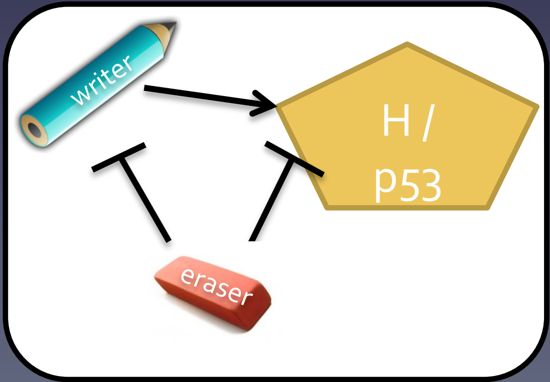
KAT acetylated for activation (@604)

- HDAC8 -> KAT6A
- sirt1 -> hMOF



KAT350 : in unstructured regions

- fine tuning?

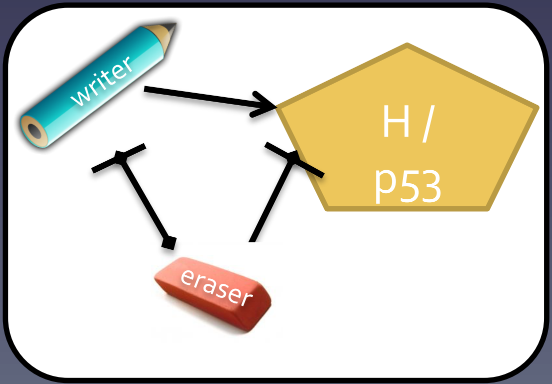


reactive ≥ 3 **reasonable ≥ 2** **mild ≥ 1** poor < 1

3 More chromatin remodelers …

peptide	log(Kcat/ Km)	min. I_sc	protein
KLIS **K1808 FDKL**	2.87	-17.85	ARID1A (chromatin remodeling)
SK**IQ K3579 QL**DQ	1.51	-16.25	MLL2 (methyltransferase)

- cross-talk between different modifications ?

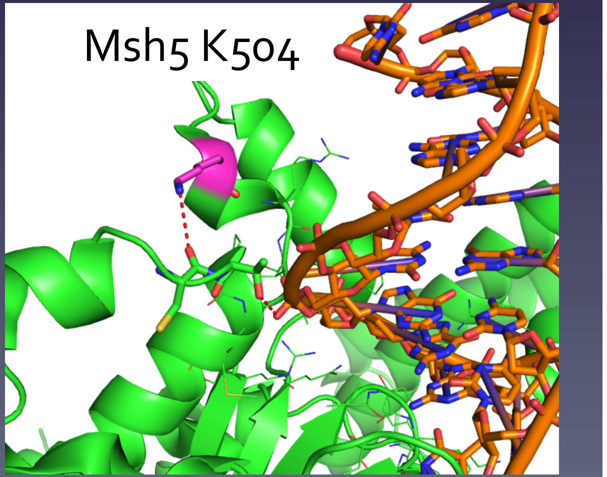


reactive ≥ 3 **reasonable ≥ 2** mild ≥ 1 poor < 1

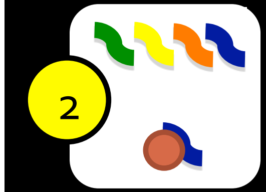
More cross-talk

peptide	log (K_{cat}/K_M)	min. I_sc	protein
SF K55 YAW	3.07	-20.8	EF1 α 1
LG K1017 FRR	2.54	-19.1	La-related protein 1
IS K504 YDR	1.92	-18.6	Msh6

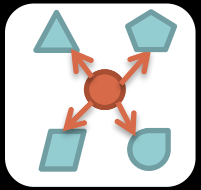
- EF1α1 - transcription/translation
- Larp1 - binds top5′ RNA
  - K1017 *adjacent* to RNA BD
- Msh6 - DNA mismatch repair
  - K504 *in periphery* of DNA



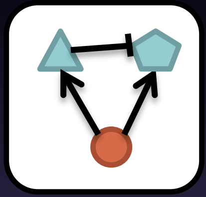
reactive ≥ 3 **reasonable ≥ 2** **mild ≥ 1** poor < 1



New targets - summary

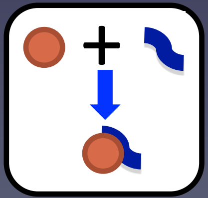


✓ found strongest known (peptide) HDAC8 substrates



✓ role of HDAC8 modifications
  - ✧*peripheral* fine-tuning
  - ✧extensive potential for crossregulation

✓ structure-based identification of substrates
  - ✧binding is the bottleneck for catalysis
  - ✧protocol works for **flexible & internal** sites



Why a structure-based approach ?

peptide	log (Kcat/Km)	min. I_sc
KLIS K1808 FDKL	2.87	-17.85
STPV K292 FISR	2.20	-17.23
KRILH K687 LLQN	1.70	-17.02
RVIGAK K106 DQY	1.80	-16.4
SKIQ K3579 QLDQ	1.51	-16.25
LGDG K387 MKS	0.66	-14.79
KLSG K167 EING	0.99	-14.29
KLGG K1087 QRAA	1.04	-14.19
TEIG K54 TLAEK	0.61	-13.96

structure and proteomic information complement each other

Proteomic identification of HDAC8 substrates

✓ *in vivo* data

✧ many known targets missed

✧ weak targets (GKac preferred) -> bias?

Why a structure-based approach ?

peptide	log (Kcat/ Km)	min. I_sc
DI K42 YPL	**1.20**	**-18.6**
VS K350 GPF	**2.09**	-17.5
DS K1583 NAK	1.02	-17.1
SG K256 YDL	1.12	-17.1
VS K93 GTL	1.23	-16.6
SF K769 SDQ	1.39	-16.6
DH K604 TLY	1.18	-16.5
LS K747 FLR	2.80	-21.6
SF K55 YAW	3.07	-20.8
FG K1275 FSW	**3.68**	**-20.0**
TW K78 ANF	1.89	-19.6
LG K1017 FRR	2.54	-19.1
IS K504 YDR	1.92	-18.6
KI K31 RLR	1.58	-18.5
SL K269 EFY	1.89	-18.0
RL K713 YSQ	1.90	-16.8
SG K517 YYY	1.32	-16.8
SG K8 YFA	1.30	-16.2
TF K292 GVD	1.15	-15.4
KG K878 DAE	1.56	-14.5
GG K149 AFG	1.32	-14.5
RK K1970 GEP	1.00	-11.9

KLIS K1808 FDKL	2.87	-17.85
STPV K292 FISR	2.20	-17.23
KRILH K687 LLQN	1.70	-17.02
RVIGAK K106 DQY	1.80	-16.4
SKIQ K3579 QLDQ	1.51	-16.25
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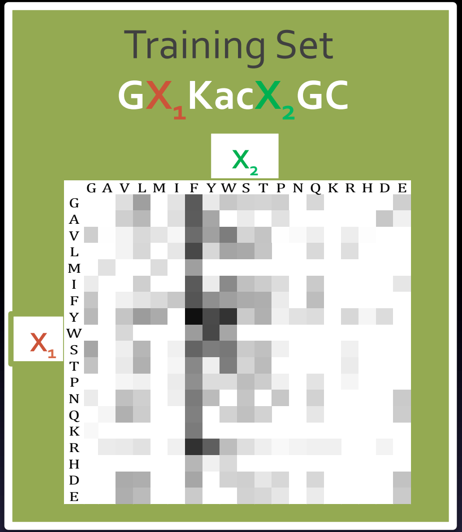

Training Set

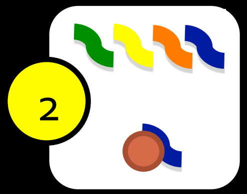
GX_1KacX_2GC

sequence based motif Kac[FY]

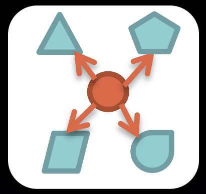
✓ identifies 6/7 strong substrates

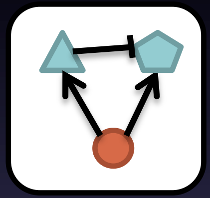
✧ many false positives

✧ weak substrates missed



What next ?





- ✓ found strongest known (peptide) HDAC8 substrates

- ➢ biology of new targets & functional impact
- ➢ differences and similarities among HDAC substrate specificities
    - ❖ targeted drugs ?

- ➢ extend to new systems (lipids, sugars, nucleotides…)

Back to the context

Jamal Fahoum

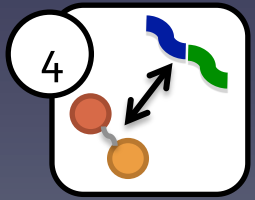
Emma-Joy Dodson

Shahar Rotem

Jordan Chill
Keren Adamsky
NMR Bar Ilan

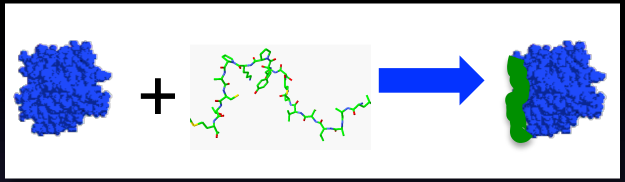
Rami Aqeilan
Saleh Khawaled

Reuven Wiener



Back to the challenge

Nature’s challenge



$\Delta G_{BIND} = \Delta H - T\Delta S$

✧ **small** interface

✧ **flexible** peptide

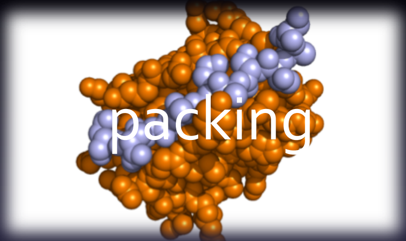
➢ how do peptides do it?

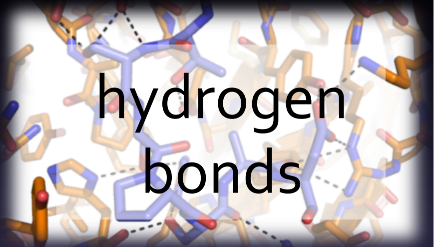
peptiDB

London, Movshovitz-Attias, Schueler-Furman (2010) Structure

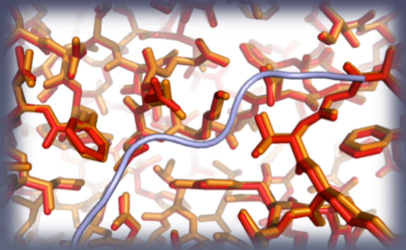
Nature’s solutions

① increase ΔH





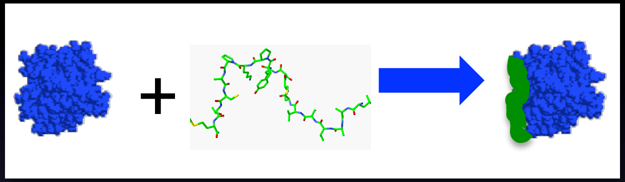
② decrease ΔS



prearranged
receptor
& peptide

Back to the challenge

Nature's challenge



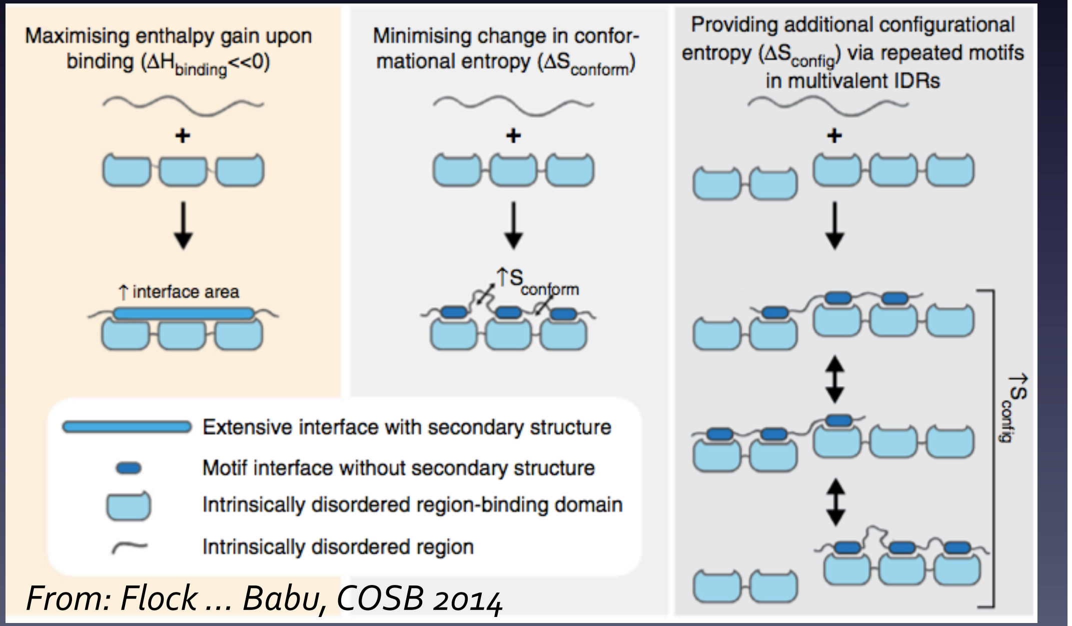
$$\Delta G_{BIND} = \Delta H - T\Delta S$$

✧ **small** interface

✧ **flexible** peptide

Nature's solutions

③ multiplicity



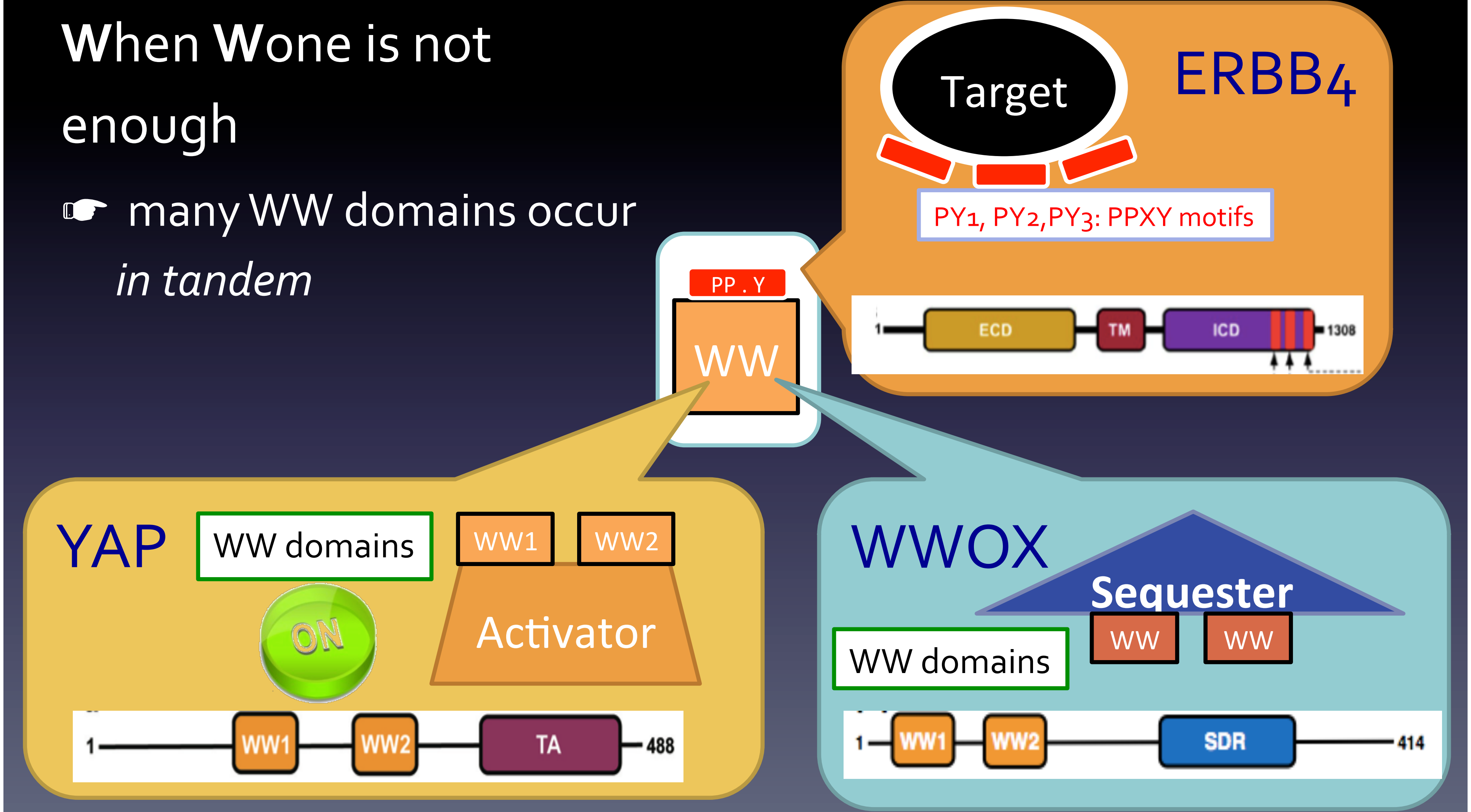
From: Flock … Babu, COSB 2014

➢ opportunities for regulation by context!

Communication by SMS

When Wone is not enough

☛ many WW domains occur *in tandem*



Target

ERBB4

PY1, PY2,PY3: PPXY motifs

PP . Y

WW

YAP

WW domains

ON

WW1

WW2

Activator

WWOX

Sequester

WW domains

WW

WW

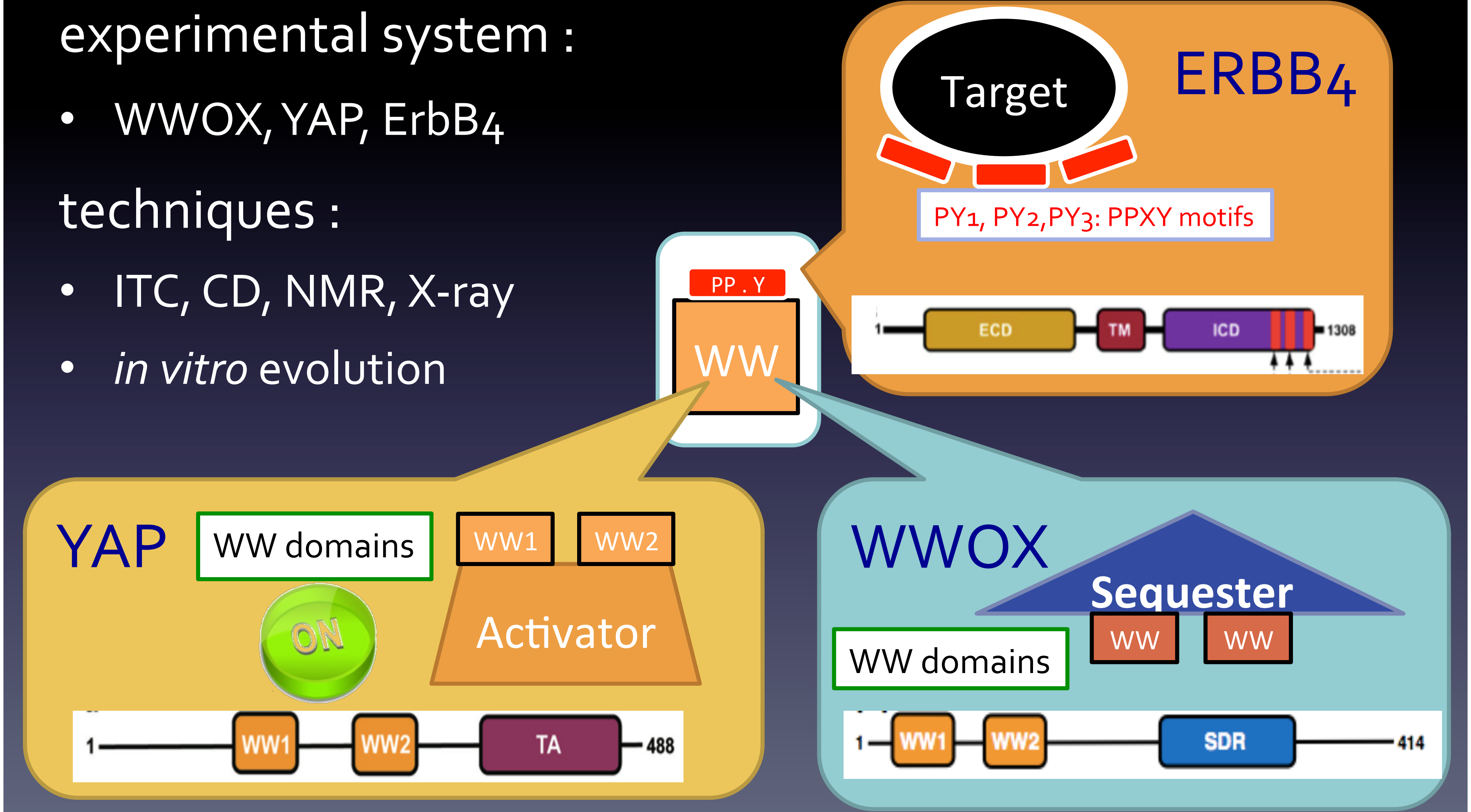
Communication by SMS

experimental system :

- WWOX, YAP, ErbB4

techniques :

- ITC, CD, NMR, X-ray
- *in vitro* evolution



ERBB4

Target

PY1, PY2,PY3: PPXY motifs

PP . Y

WW

YAP

WW domains

WW1

WW2

ON

Activator

WWOX

Sequester

WW domains

WW

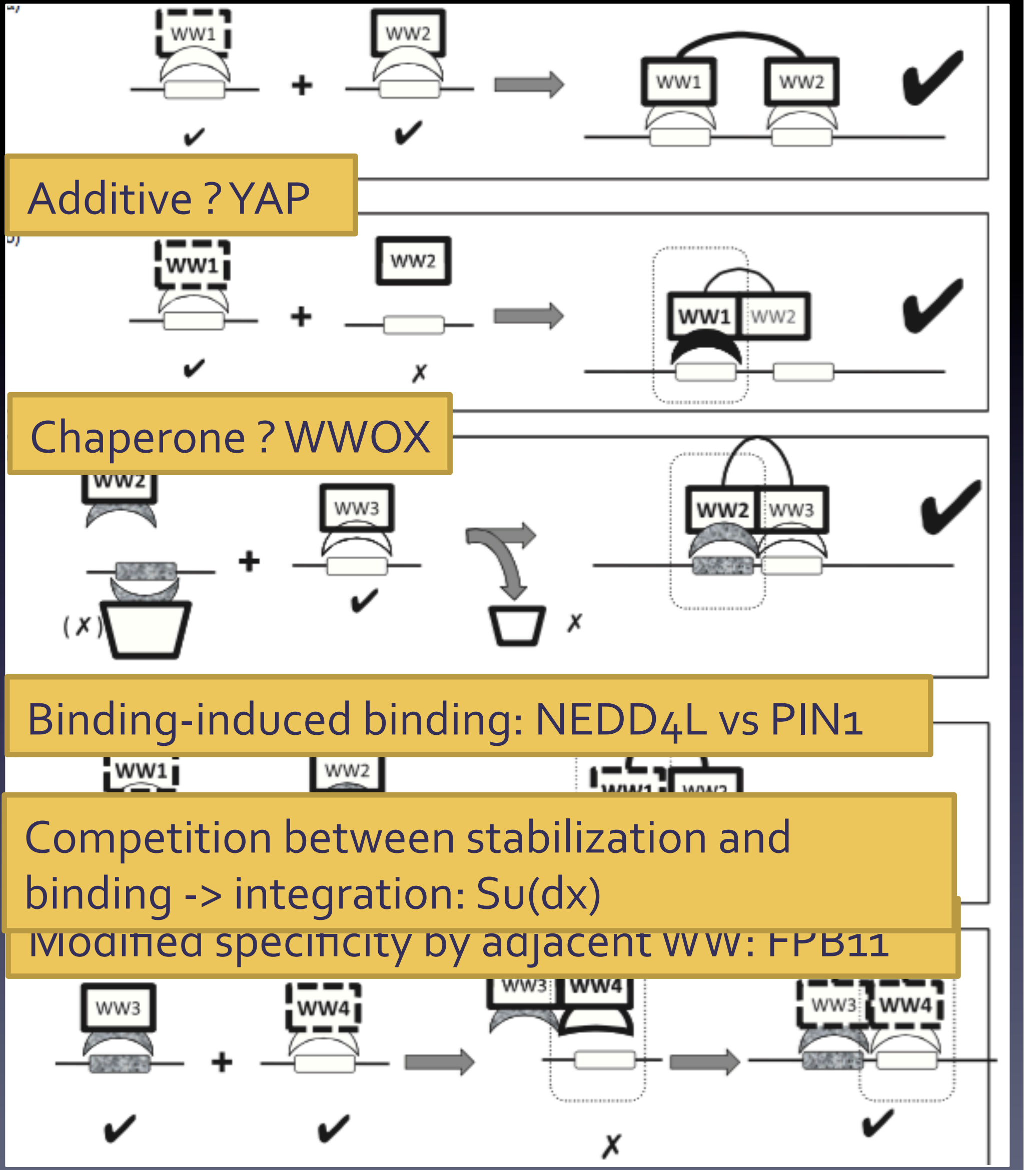
WW

When Wone is not enough

50% of WW domains occur *in tandem*

- ☛ *context* crucial for complex regulation
- ☛ variable integration strategies

Dodson,...., Schueler-Furman (2015). Versatile communication strategies among tandem WW domain repeats. Experimental Biology and Medicine, 240:351



Additive ? YAP

Chaperone ? WWOX

Binding-induced binding: NEDD4L vs PIN1

Competition between stabilization and binding -> integration: Su(dx)

Modified specificity by adjacent WW: FPB11

THANKS!

Fierke Lab U Michigan

Noah Wolfson
Caleb Joseph
Carol Fierke

Current lab members:

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Previous lab members

Nir London (Weizmann Institute), Barak Raveh (UCSF), Dan Reshef
Lavanya Pushpam, Michal Sperber, Vered Fishbain, Lior Zimmerman, Assaf Lavi, Dana Movshovitz-Attias (CMU)

NMR Jordan Chill, Keren Adamsky, Bar Ilan University

ITC & CD Assaf Friedler, Daniel Harries

Crystallography Reuven Wiener

***In vivo* assays** Rami Aqeilan, Saleh Khawaled

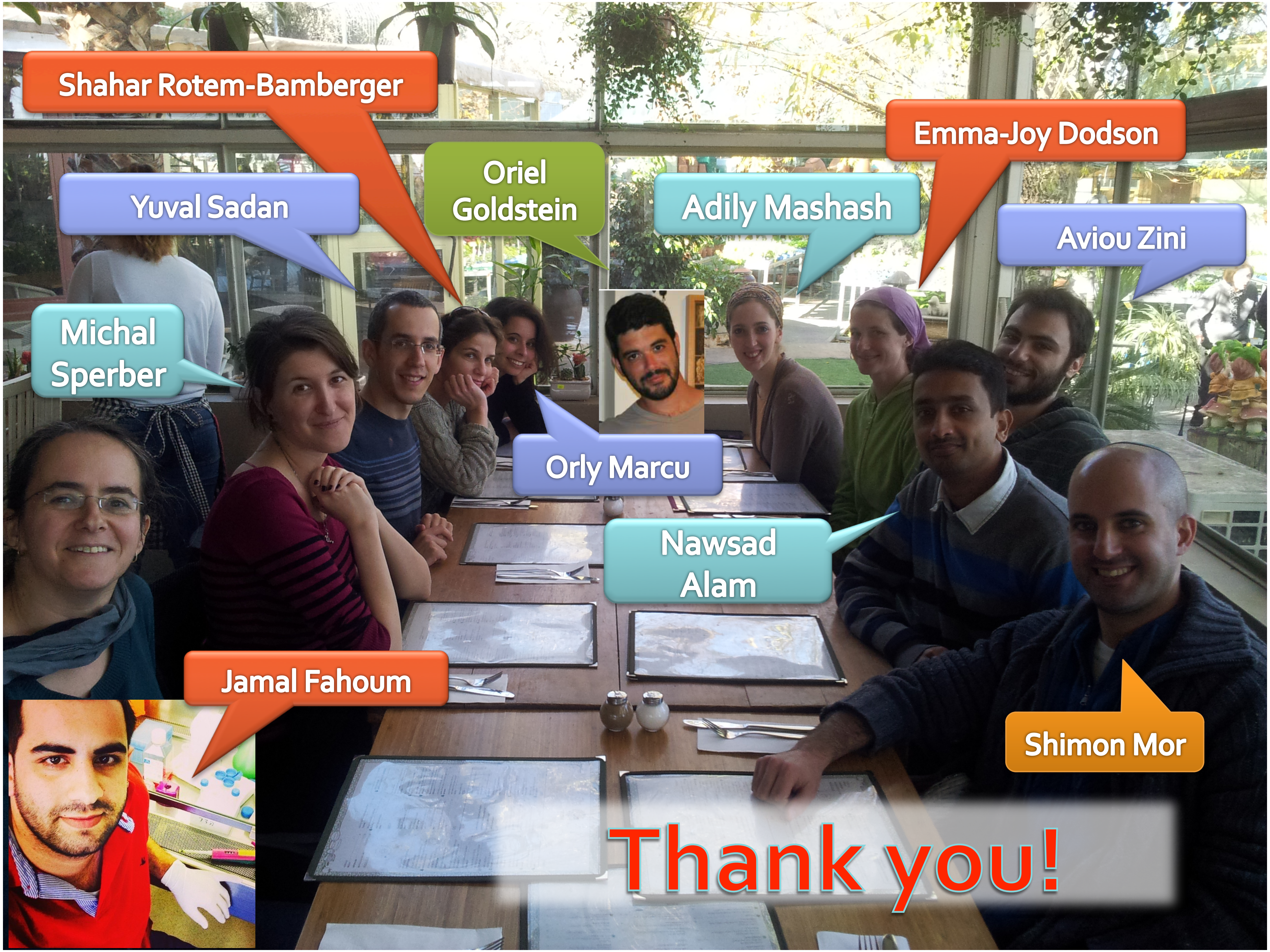
Dima Kozakov

Boston U & SUNY Stony Brook

Rosetta Community

BSF erc

Furman Lab @ HU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Thank you!