How do proteins interact… in the Furman lab?

Ora Schueler-Furman

The Hebrew University, Jerusalem, Israel

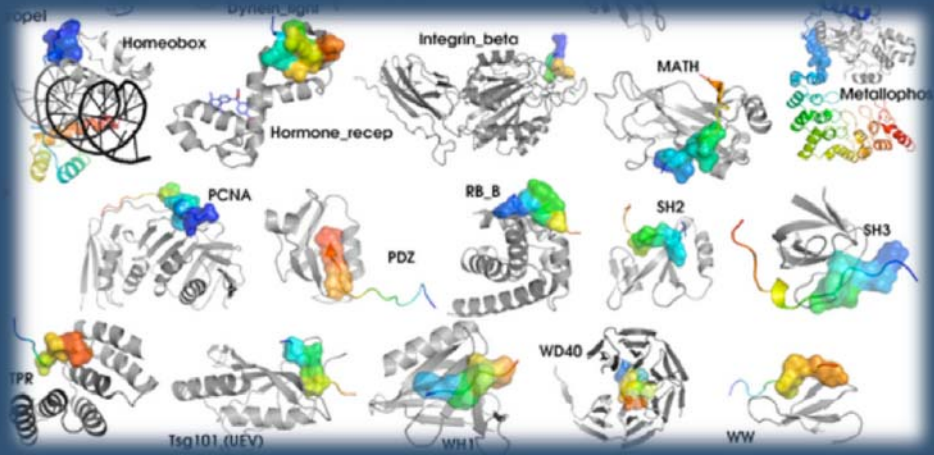
RosettaCon 2011 8/6/2011

People interact… so do proteins

Often with flexible, linear…. yes, **peptidic** regions

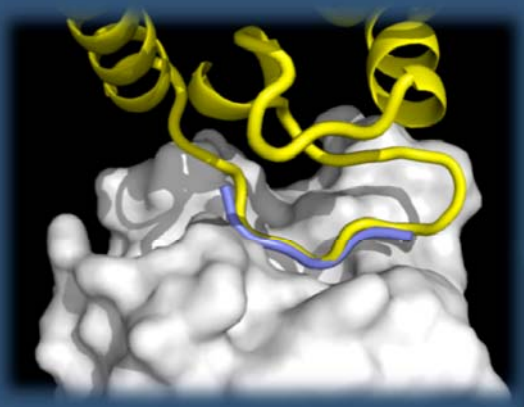
Peptide-mediated PPI's come in 3 flavors

1. Linear motifs within unstructured regions (signaling; modification)



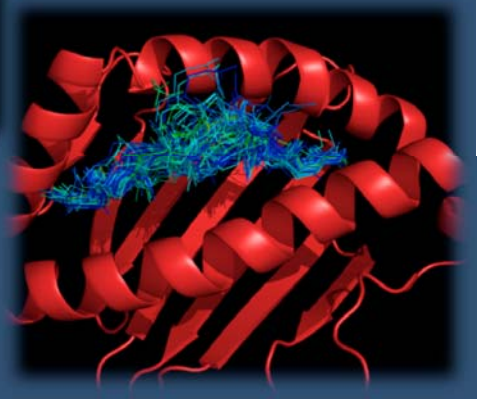
Aloy et al. Plos ONE (2008)

2. Continuous stretch at PPI interface. Contributes most of binding energy. Similar structure as free peptide



proline isomerase – HIV capsid protein (HAGPIA peptide)

3. Free peptide



MHC-peptide complex

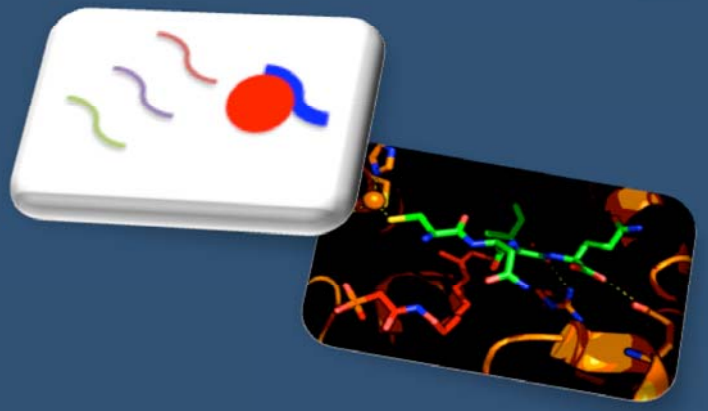
Some stories from the Furman lab

1. Model PepPI: *FlexPepDock*

1. Modulate PepPI: *FlexPepBind*

Binding and specificity prediction

- Farnesyltransferase peptide targets
- Apoptotic proteins Bcl-xL, Mcl-1 & Bcl-2



Some stories from the Furman lab

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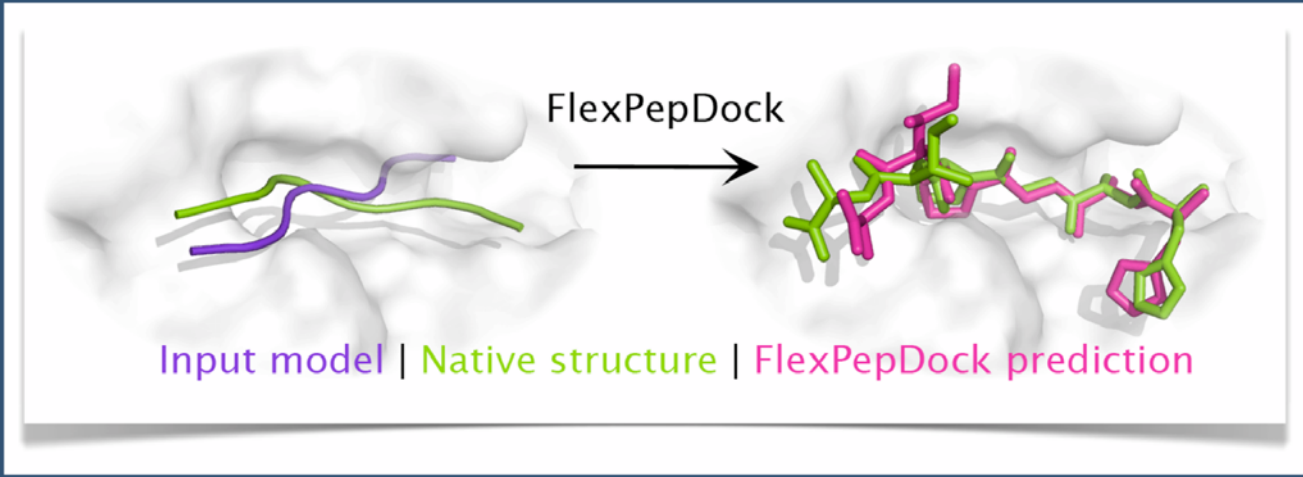


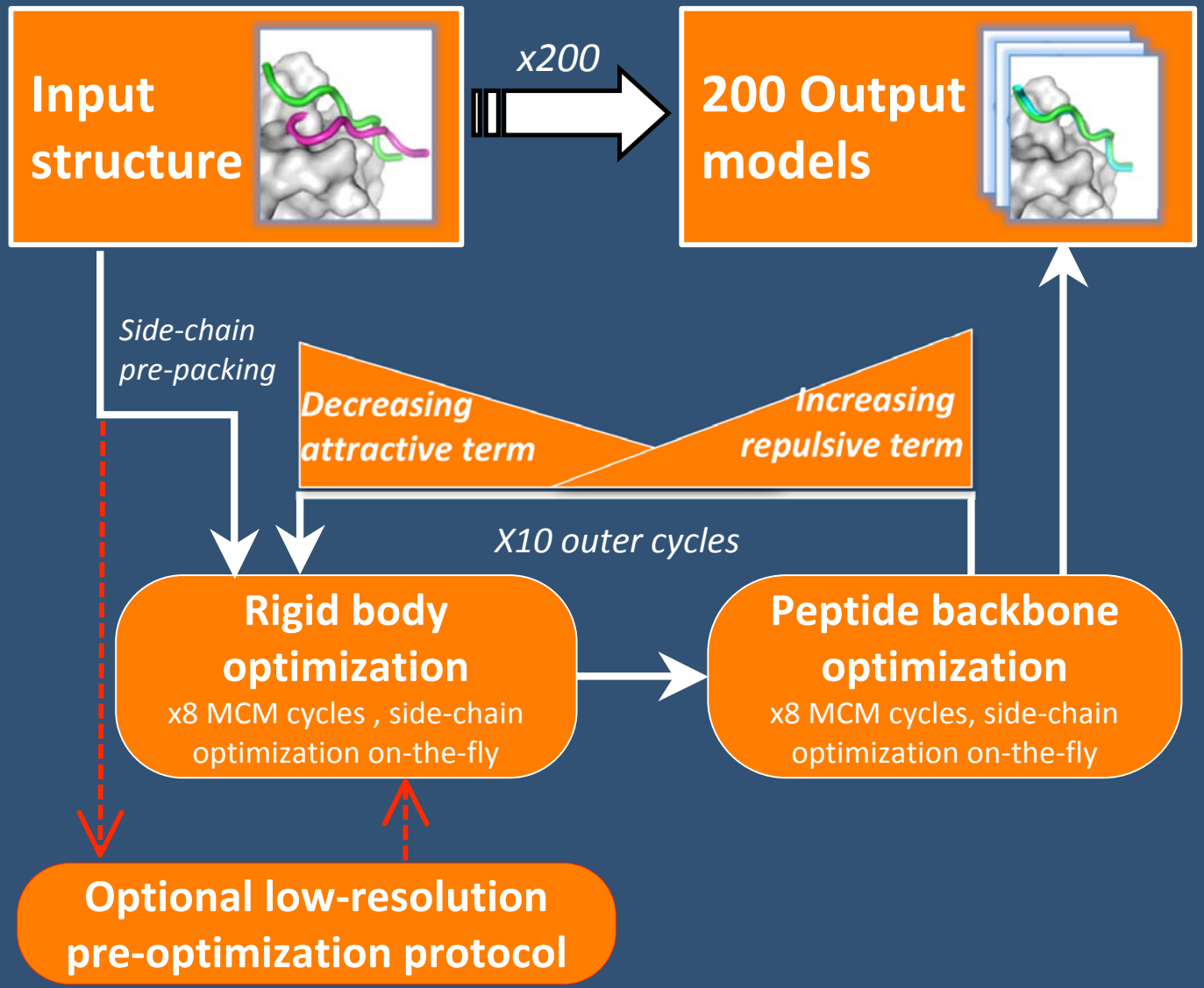
Barak Raveh, Nir London,

Lior Zimmerman,

Guy Fathy, Eyal Cohen

Raveh, London & Schueler-Furman. Proteins (2010)
Raveh, London, Zimmerman. PloS ONE Rosettacon Issue (2011)
London, Raveh, Cohen, Fathy & Schueler-Furman NAR (2011)

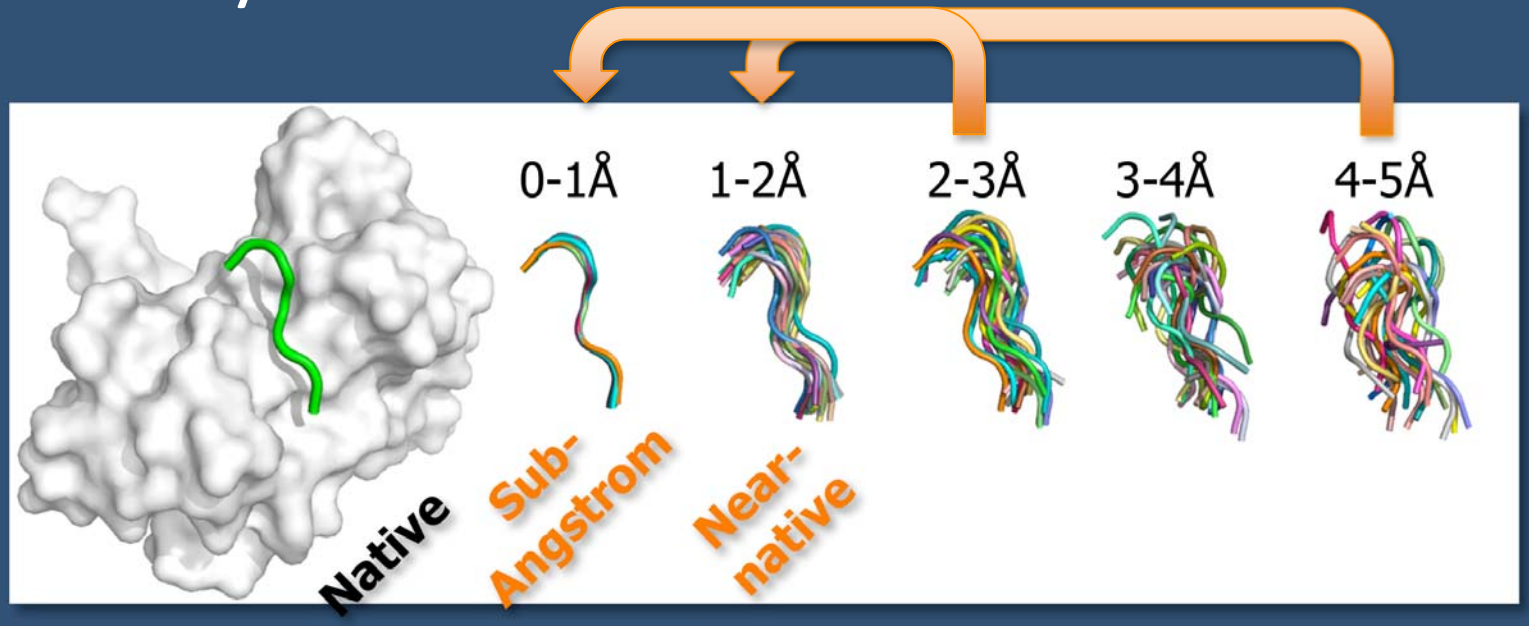
Outline of Rosetta *FlexPepDock*



Raveh, London & Schueler-Furman. Proteins (2010)

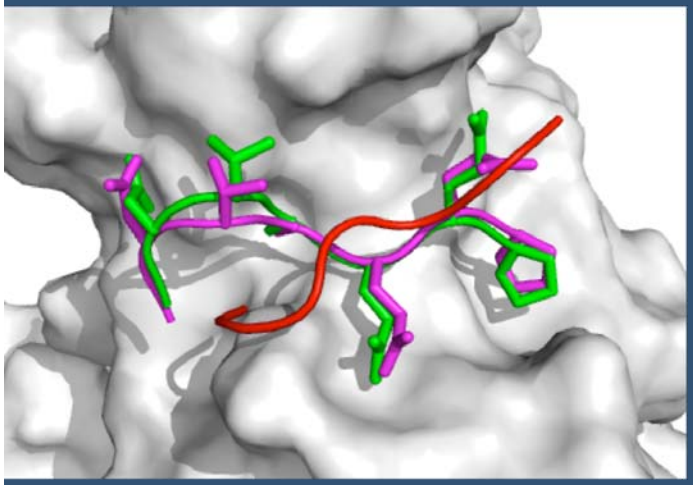
Scope & quality of ***FlexPepDock***

- General: tested on >50 complexes
- Intended for *refinement*
- Large effective range
- Very accurate

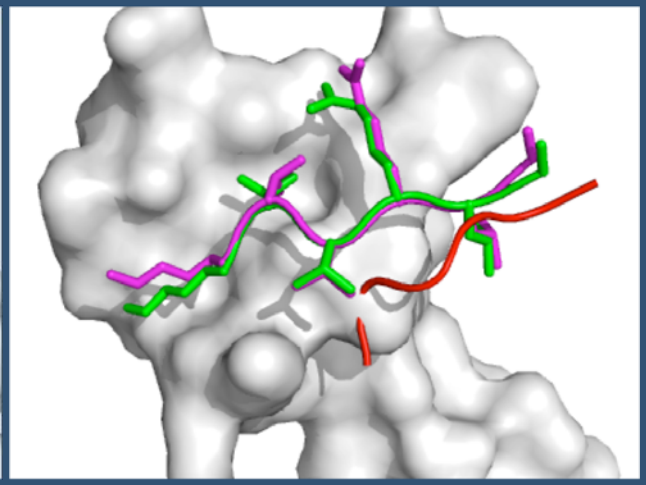


Raveh, London & Schueler-Furman. Proteins (2010)

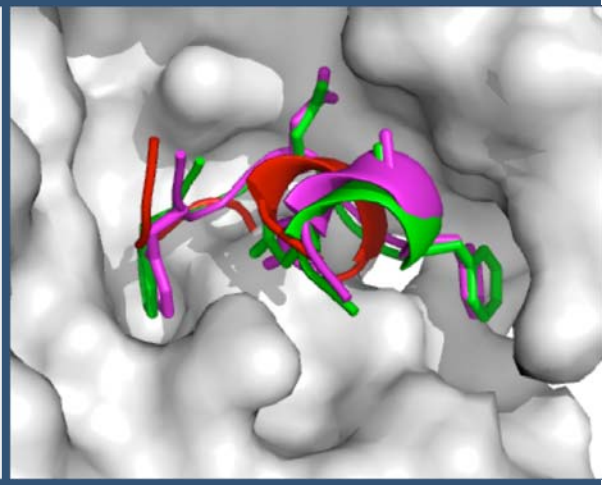
Examples



PDB: 1CZY
TRAF domain – LMP1 binding peptide



PDB: 2DS8
ClpX – SspB tail



PDB: 2IV9
AP2 – EPS15 peptide

native input prediction

➢Accurate prediction of motif residues

Raveh, London & Schueler-Furman. Proteins (2010)

More *FlexPepDock* …

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

- Rosetta ***FlexPepDock ab initio*** **:
  Extension towards longer range

➢See Barak's Poster!

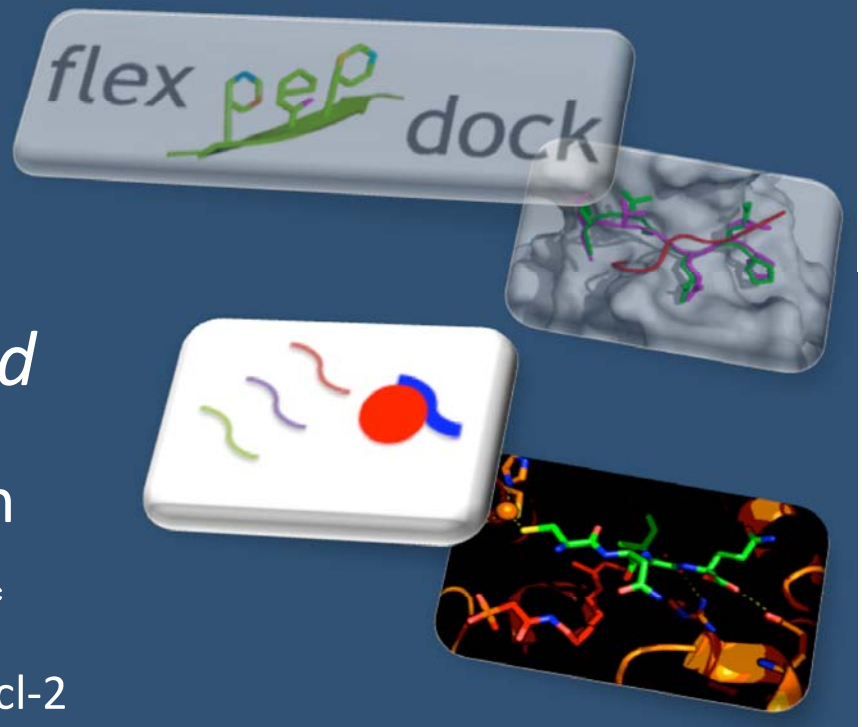
Current challenges:

- Identification of peptide binding sites on proteins
- Receptor backbone flexibility

** London, Raveh, Cohen, Fathy & Schueler-Furman NAR (2011)*
*** Raveh, London, Zimmerman. PloS ONE Rosettacon Issue (2011)*

Some stories from the Furman lab

1. Model PepPI: *FlexPepDock*



1. Modulate PepPI: *FlexPepBind*

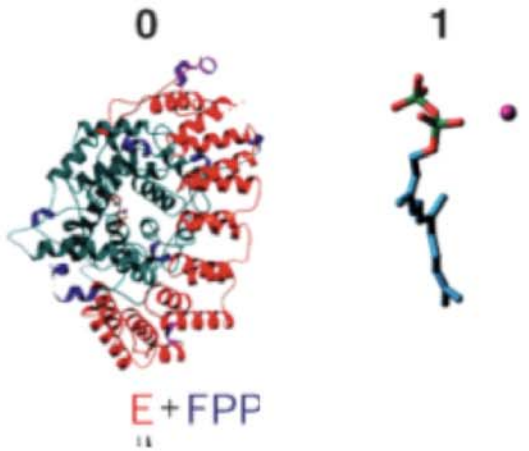
Binding and specificity prediction

- Farnesyltransferase peptide targets*
- Apoptotic proteins Bcl-xL, Mcl-1 & Bcl-2

Nir London, Michal Sperber

** London, Lamphear, Hougland, Fierke & Schueler-Furman, PLoS CB, in press*

(1) Protein Farnesylation

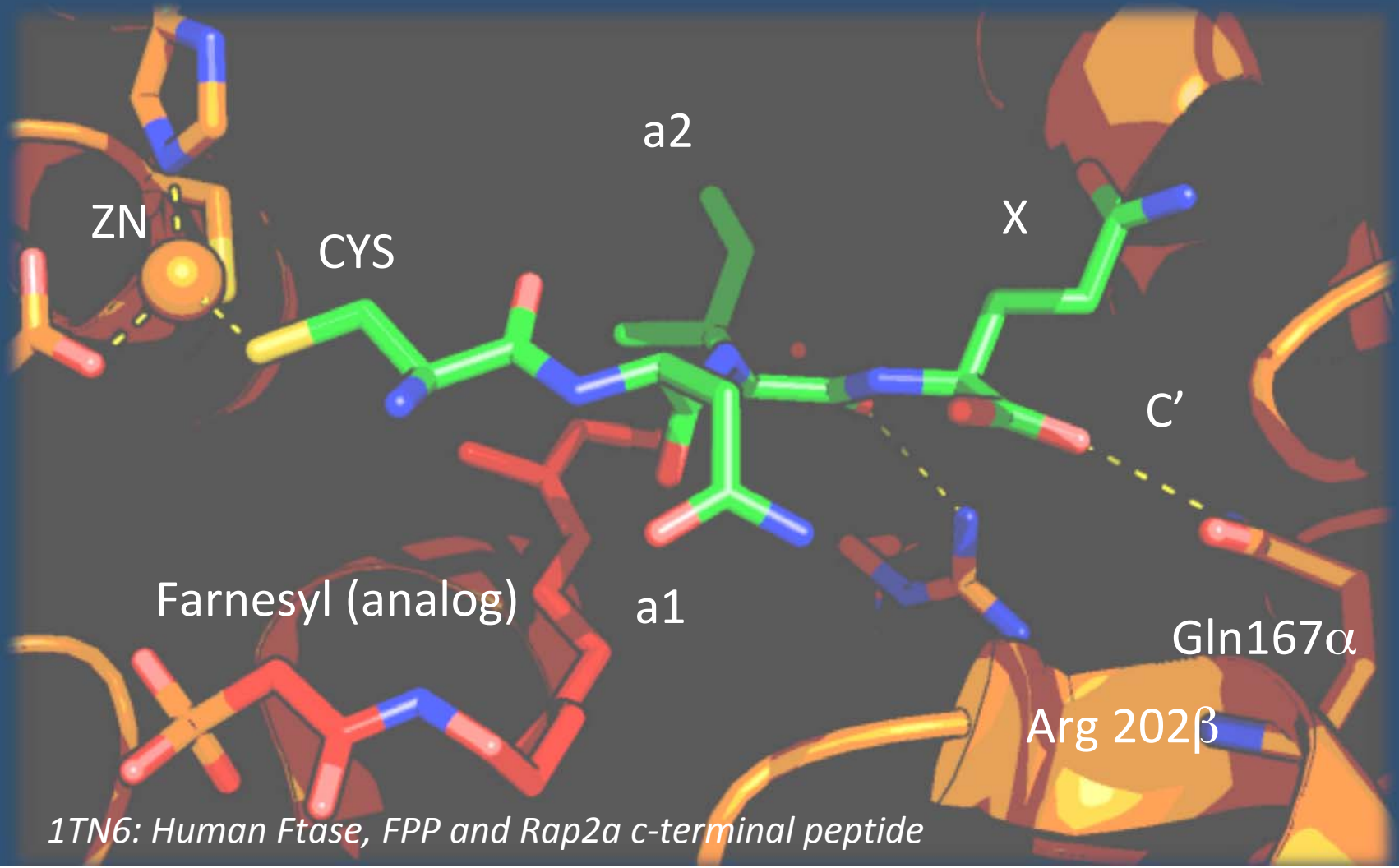


Assumption: binding ~ activity

Structure used in this study : peptide binds to E-FPP

Figure from Lane & Beese. J Lipid Res (2006)

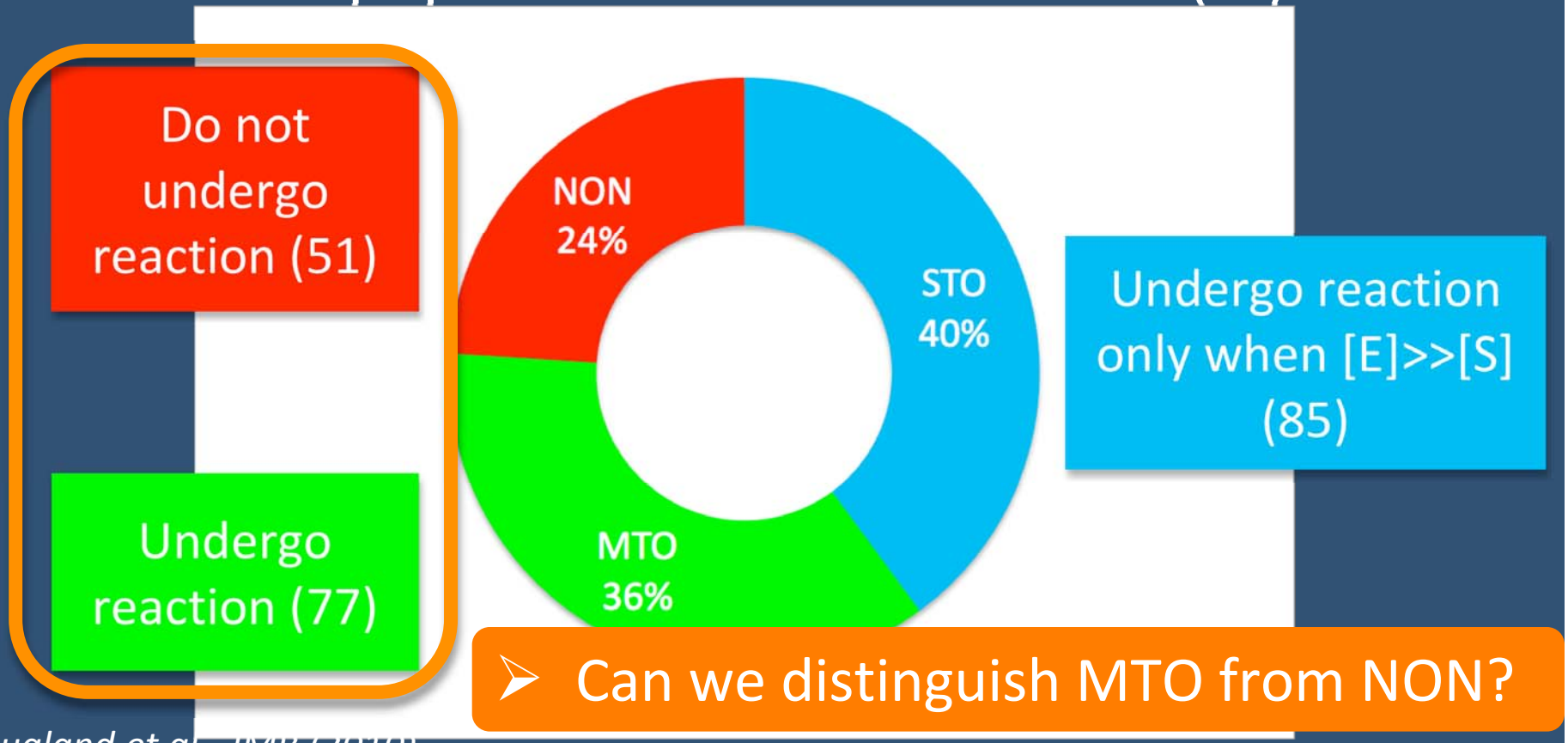
Overview of structure : FTase, FPP & peptide



1TN6: Human Ftase, FPP and Rap2a c-terminal peptide

Novel substrate peptides revealed

- Hougland *et al.** synthesized and characterized 213 hexapeptides of the form TKCxxx(C')

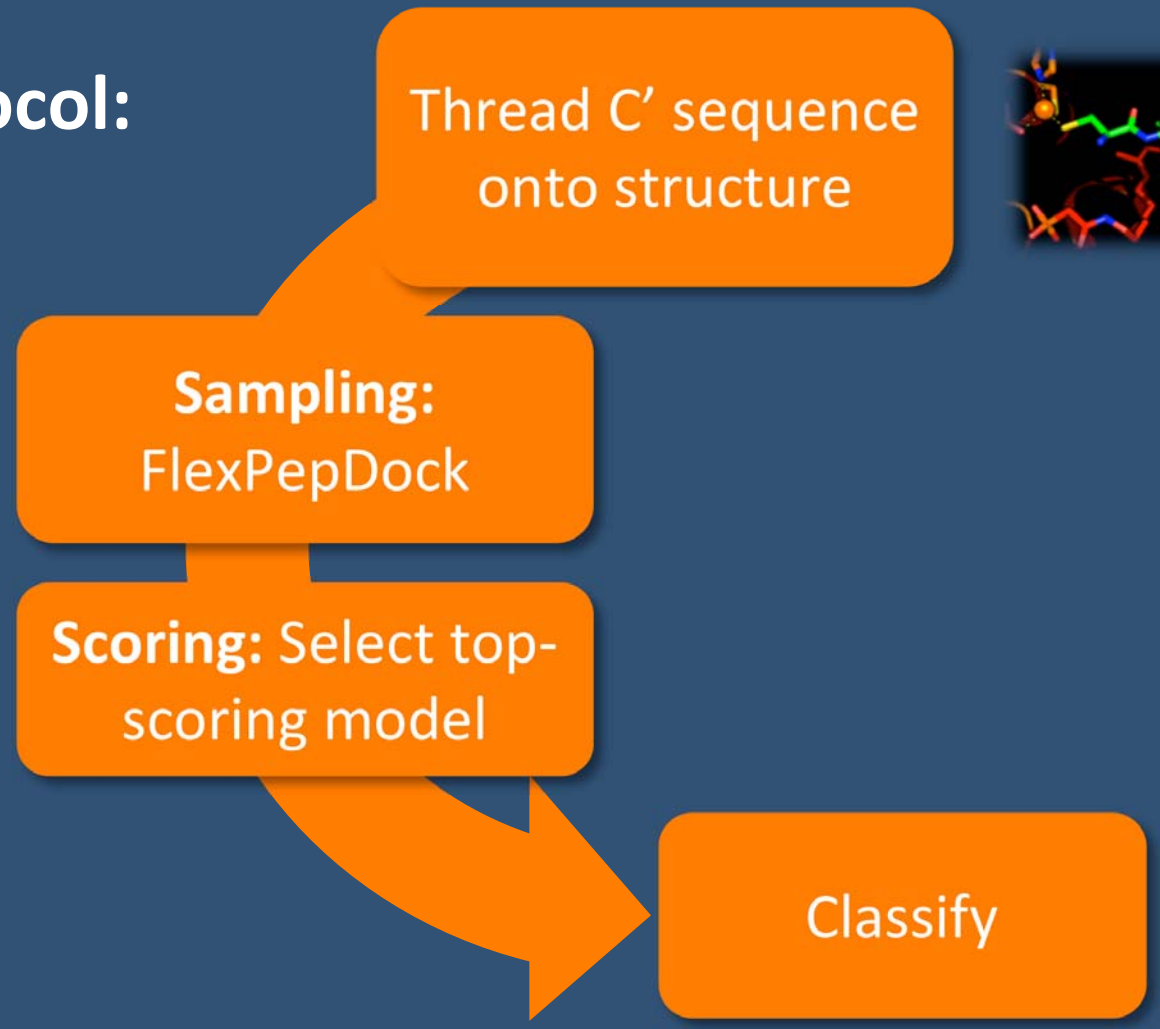


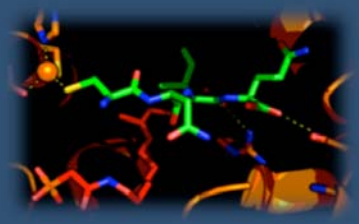
➢ Can we distinguish MTO from NON?

*Hougland et al., JMB (2010)

Can we distinguish MTO & NON?

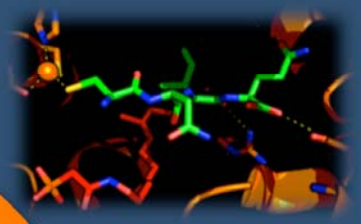
Protocol:





Can we distinguish MTO & NON?

Faster protocol :



Thread C' sequence onto structure

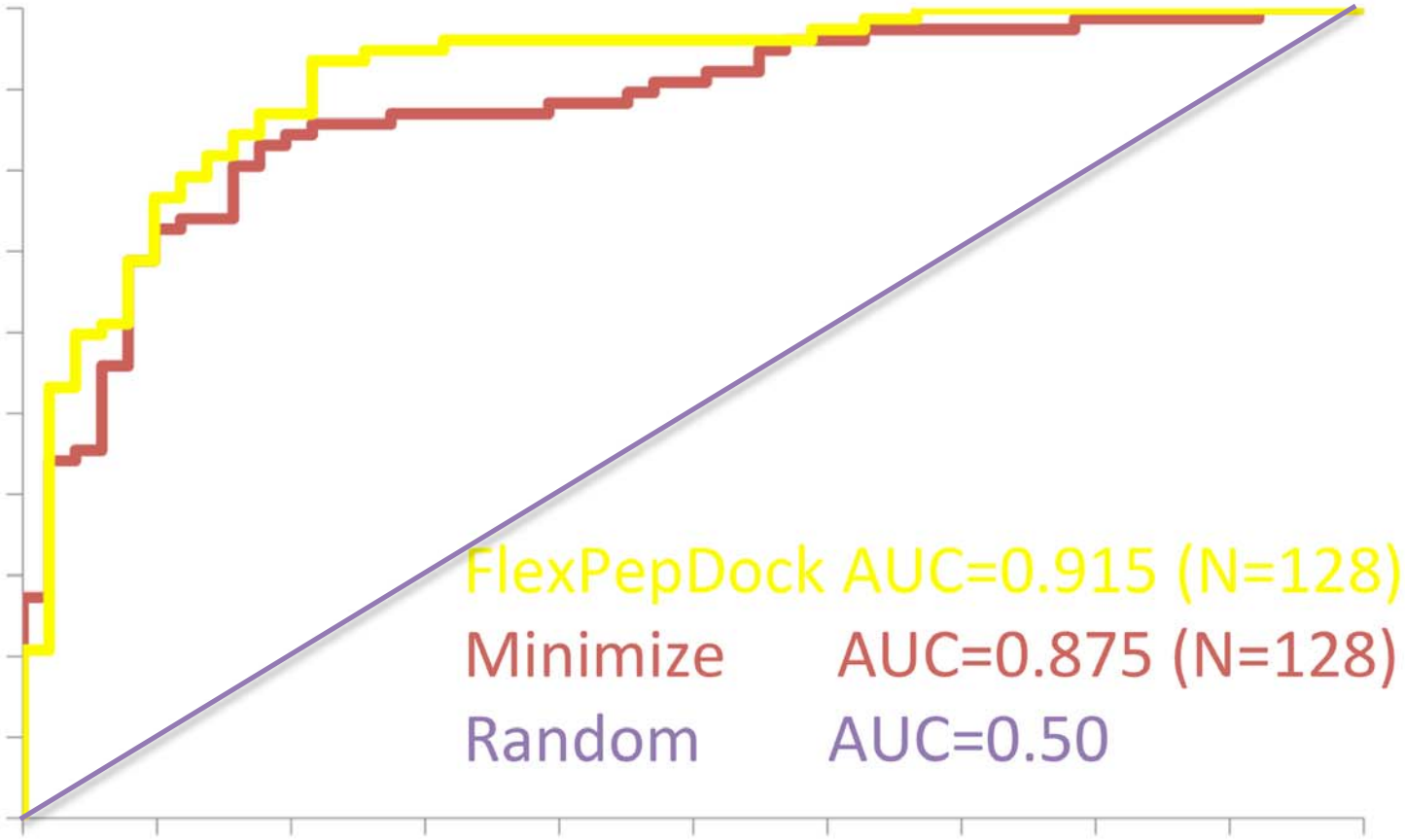
Sampling: Optimize side chains

Sampling: Minimize structure

Scoring: Classify

- Pack peptide side chains (extra $\chi 1$, $\chi 2$ rotamers)
- Minimize all interface side-chains and peptide's backbone

Good discrimination MTO/NON: Training set



Calibration of protocol for FTases

Sampling

Include constraints:

- Cys coordinated to Zn
- 2 Hydrogen bonds:

FlexPepDock vs minimization:

- Minimization ~ as good as FlexPepDock *(but 200x faster)*

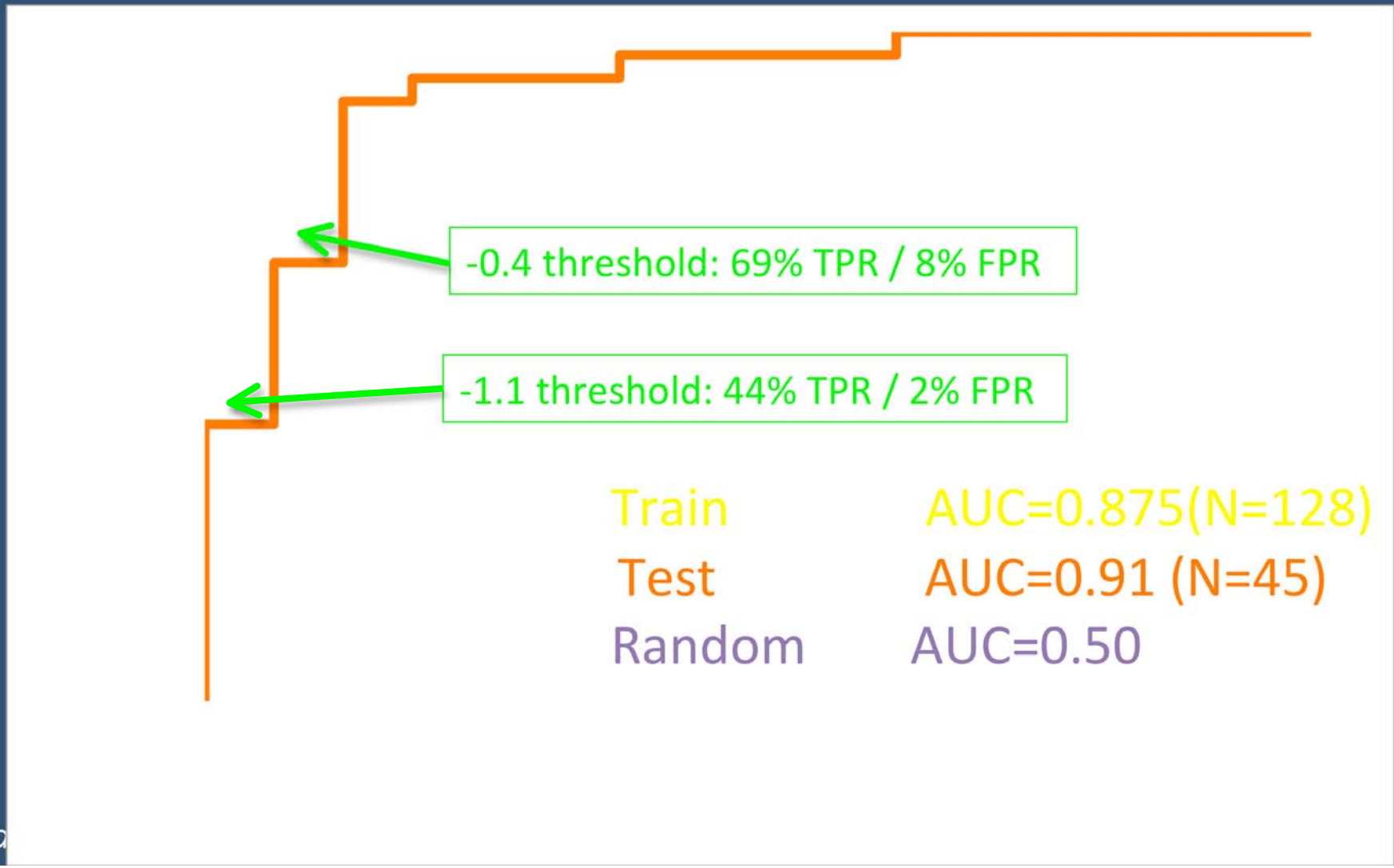
Scoring

Optimize rescoring function:

- Peptide score (internal + interface)
- No E_{aa} (amino acid reference energy)

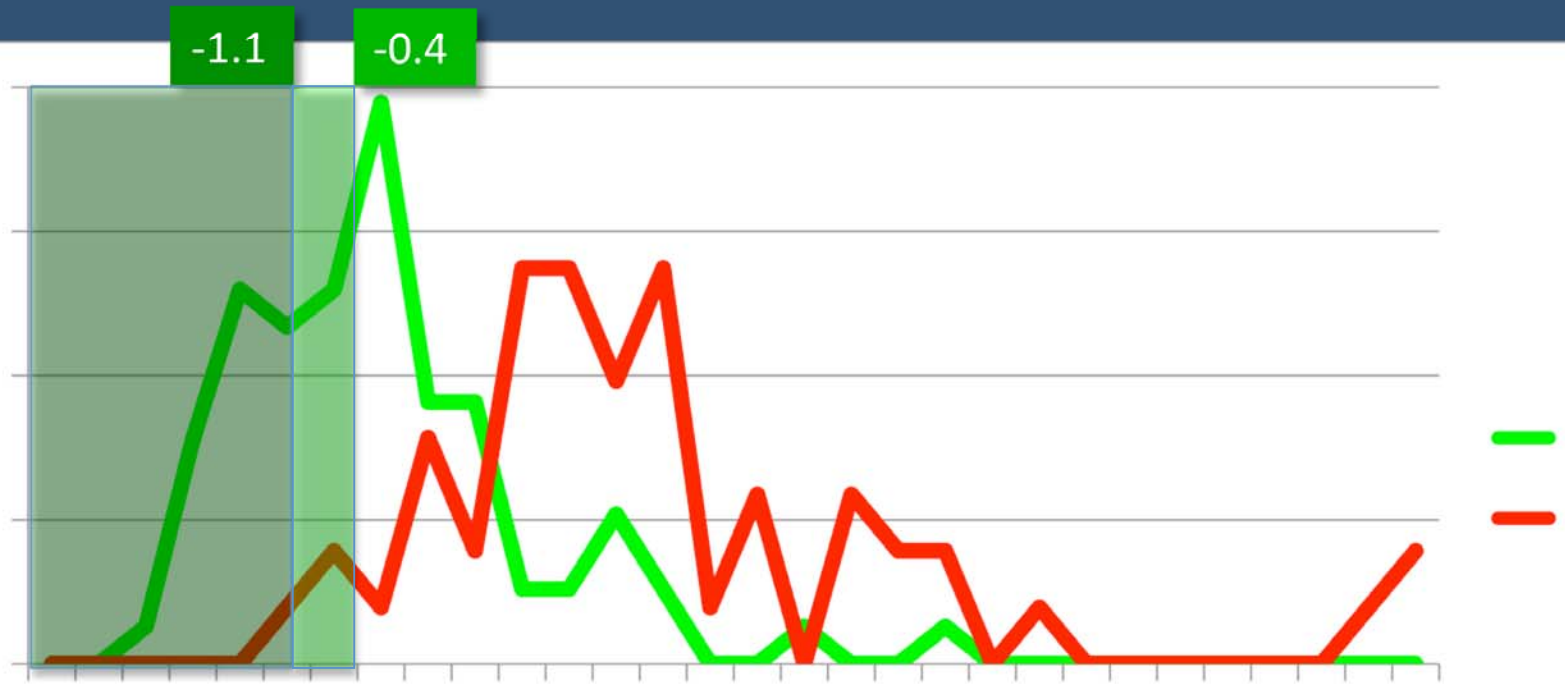
➢ Learn on training set only!

Good discrimination MTO/NON



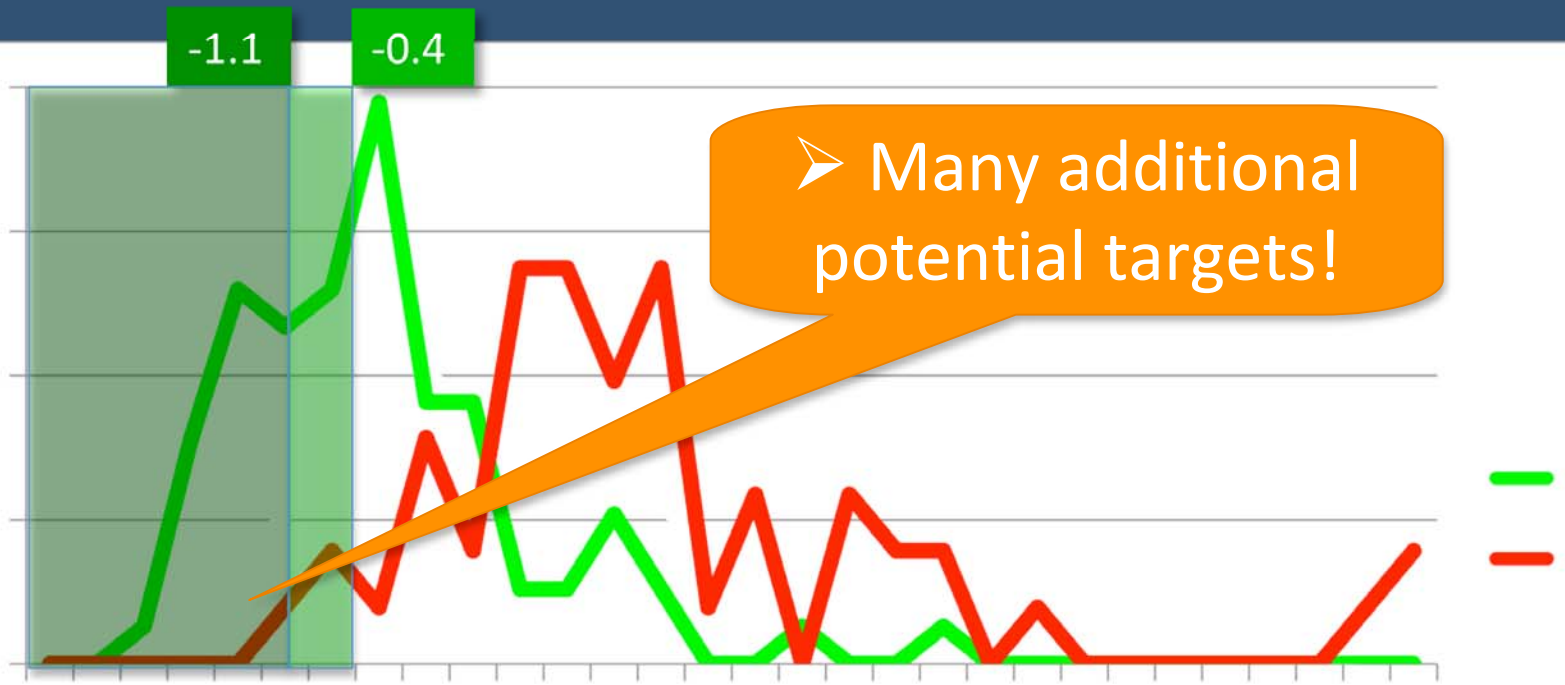
*ad

FTase – sequence mapping



FTase – sequence mapping

Calculate peptide scores for all possible 8000 Cxxx' combinations



Application I :
Scan genome for novel targets

Experimental validation

(Corissa Lamphear, Fierke Lab, U. Michigan)

MTO 9/16

STO 4/16

NON 3/16

Motif	Protein	PreP	Score	Result
CYVA	Q9NTW7-3	S	-2.88	MTO
CFLT	Q2UVF0	--	-2.74	MTO
CAFI	Q7Z2H8	--	-2.62	STO
CWLS	A6QL63-3	-	-2.46	MTO
CCLS	Q9NZM3-3	--	-2.37	MTO
CTTE	Q5T2R2-2	--	-2.14	STO
CHFH	Q8TCU3-2	---	-2.14	STO
CKLA	Q9BPZ7-6	-	-2.06	MTO
CWTC	Q8NFG4-3	-	-1.94	MTO
CSLI	Q14CB8-5	-	-1.90	MTO
CLFE	Q9UHP7-3	--	-1.77	None
CPFF	Q8N693	---	-1.69	STO
CGVG	A6NHS1	-	-1.65	MTO
CFDI	Q8NEB5	--	-1.59	None
CHCI	Q99988	--	-1.56	None
CVCV	O75391	-	-1.12	MTO

➢ Only 3/16 not farnesylated

MTO –multiple turnover conditions
STO – single turnover conditions

Application II : Novel inhibitors ?

Experimental validation of best-scoring peptides *

MTO 10/13

STO 3/13

NON 0/13

- All farnesylated
- A new class of substrates

Motif	PreP	Score	Result
CYLI	S	-3.96	MTO
CYLE	-	-3.82	STO
CYLV	-	-3.60	MTO
CFLV	-	-3.60	MTO
CLII	++	-3.51	MTO
CYLE	-	-3.43	MTO
CYLE	-	-3.40	MTO
CFLE	-	-3.34	STO
CLIV	++	-3.33	MTO
CYLL	-	-3.24	MTO
CYLD	-	-3.13	MTO
CWVI	-	-3.03	STO
CWLV	-	-3.01	MTO

** Corissa Lamphear, Fierke Lab, U. Michigan*

Application III : Pathogens use farnesylation

Gene Annotation	CaaX motif	FlexPep Bind	Pr	ent
(PelK) lpl2806*	CVIS	++	++	

Gene Annotation	CaaX motif	FlexPepBind	PrePS FTase	PrePS GGTase	Experiment
Vibrio cholerae					
VC_1703	CKQG	-	+	---	
VC_1840	CSDC	++	--	---	
Pseudomonas aeruginosa					
RL022	CQDE	+	--	---	
PA1388	CFDH	++	--	---	
PA1914	CNNT	+	--	---	
Legionella longbeachae NSW150					
llo0680	CVLL	+	++	++	known/sto/hplc
llo1854	CLLM	++	++	++	known/mto
llo2031	CLIL	++	++	+++	mto/hplc
llo2768	CIIL	+	++	+++	mto/hplc
llo3138	CILK	-	+	---	
pepO	CVIW	+	+	--	
Legionella pneumophila					
(AnkB) lpg2144*	CVLC	++	++	--	known
(PelA) lpg0254*	CVLM	-	++	+++	known
(PelB) lpg0770*	CLIK	-	+	--	known
(PelC) lpg1312	CTII	++	++	++	known/mto
(PelD) lpp1863*	CSLL	++	++	++	sto/hplc
(PelE) lpg1976*	CNLL	++	-	++	known
(PelF) lpg2375*	CSIL	++	++	+++	mto/hplc
(PelG) lpg2525*	CSIL	++	++	+++	mto/hplc
(PelH) lpg2541*	CTIM	+	+++	+++	known
(PelI) lpg2607*	CIIW	+	+	--	known
(PelJ) lpl2477*	CTIM	+	+++	+++	known
(PelK) lpl2806*	CVIS	++	+++	--	known

Bartonella henselae str Houston 1					
BH06270	CWFS	+	--	---	
Agrobacterium tumefaciens C58					
Atu0147	CTNC	++	-	---	
Atu0926	CGNA	+	-	---	
Atu3982	CWAV	+	--	---	
Atu5261	CADD	++	--	---	
Atu6147	CCSN	-	--	---	sto
Escherichia coli O157:H7 *str Sakai*					
ECs2028	CGLL	++	+	+	
ECs2616	CCLH	+	++	--	sto
ECs3681	CQLP	-	+	---	
Salmonella enterica subsp. *enterica* serovar Typhimurium str. LT2					
STM1055	CTIL	+	++	+++	hplc
STM1224 (SifA)*	CCFL	+	-	-	known
STM2601	CSPA	+	+	---	
Francisella tularensis subsp *tularensis* Schu S4					
FTT1693c	CFTR	-	-	---	sto
Mycobacterium tuberculosis H37Rv and CDC1551					
Rv1078	CVPI	-	+	--	
Rv1514c	CRTS	+	+	---	
Rv1927	CVVQ	++	+++	--	known
Rv2972c	CPTG	+	-	---	
MT0697	CSPT	+	-	---	sto
MT2165	CRQS	-		---	
Burkholderia pseudomallei K96243					
BPSS0407	CRLT	++	+	---	
BPSS0441	CWAV	+	--	---	
BPSS0542	CPTS	++	-	---	
BPSL1063	CFVA	++	+	--	
Yersinia pestis biovar *Microtus* str. 91001					
YP003848	CQLH	-	+	---	
Bordatella pertussis Tohama I					
BP1492	CYPG	++	-	---	
BP1507	CAAQ	+	-	---	
BP1896	CPAA	+	--	---	

Collaboration with Abu Kwaik Lab

Specific(ity) conclusions I

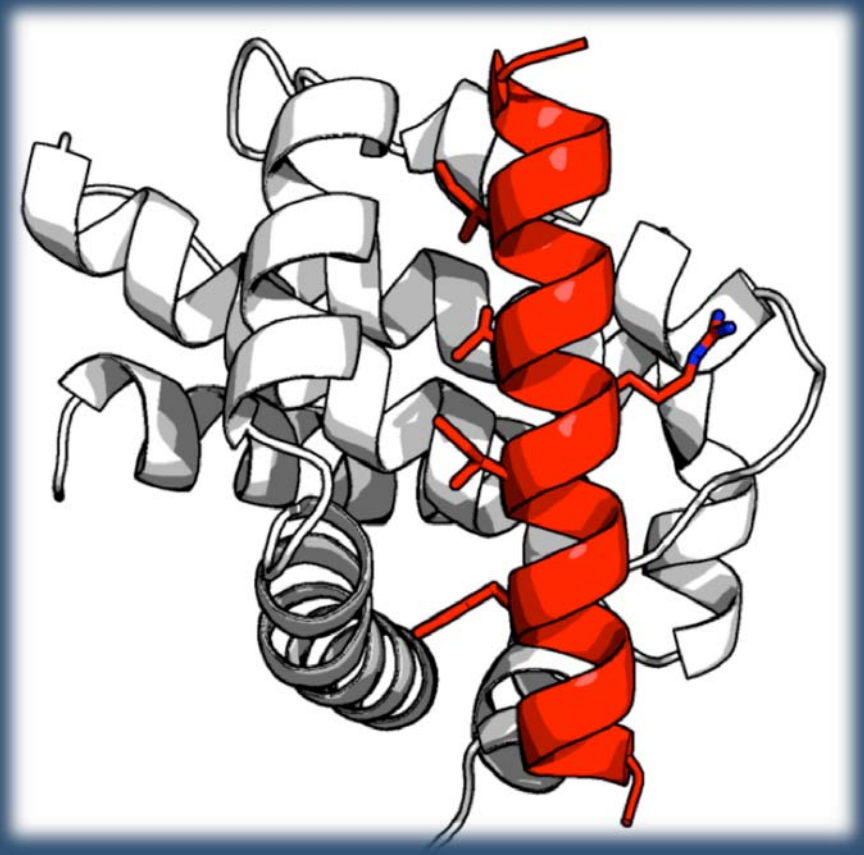
- ✓ *FTase FlexPepBind*: a robust model for FTase peptide binding specificity
- ✓ Binding is the bottleneck for farnesylation (*in vivo*….)
- ✓ Use of structure outperforms just sequence
- ✓ Detection of novel class of targets …. also in pathogens!

- ✓ Current focus: Extension to GGTases

➢See Michal's Poster!

(2) Extension to other systems: Modulation of apoptosis

Helical BH3 region in pro-apoptotic proteins binds to and inhibits pro-survival proteins



Goal:

understand binding specificity & modulate interactions:

BIM BH3 peptide –

Bcl-xL, Mcl-1, Bcl-2

** Dutta et al., JMB 2010*

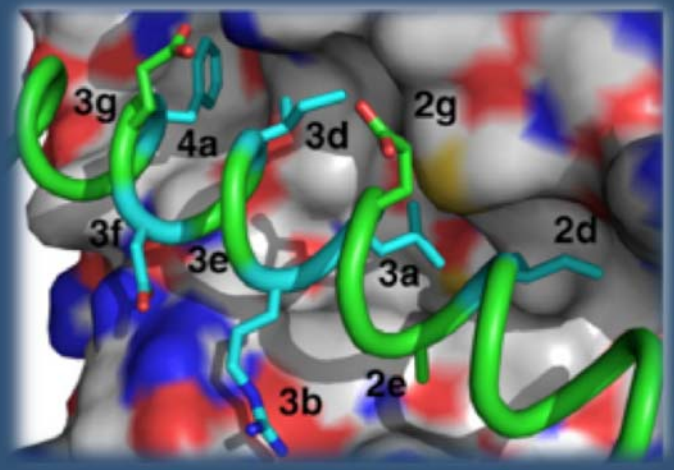
The data: SPOT arrays

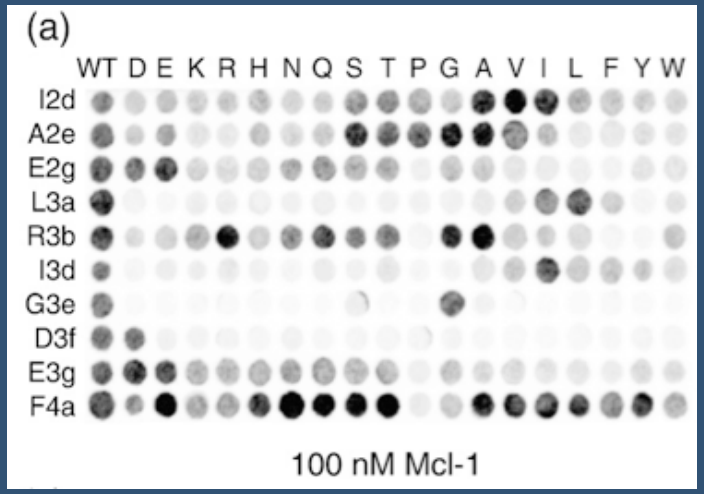
BIM BH3 peptide

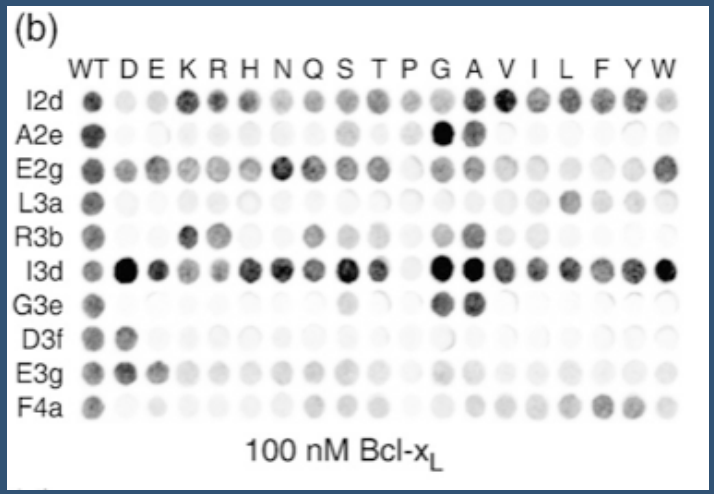
- binds to Mcl-1 & Bcl-xL
- **TRAIN 1**: combinatorial library

(n=360: 2d: IAF; 3a:LIFA; 3b:RD; 3d:IFDNA; 4a:FVN)

- **TRAIN 2**: single mutants (n=200)
- **SPOT** with Mcl-1 & Bcl-xL







** Dutta et al., JMB 2010*

Calibration of protocol for BIM BH3 - Bcl & Mcl interactions

Sampling

FlexPepDock

(simple minimization insufficient)

- *n=1000* models/sequence
- no constraints

Evaluate several templates

Scoring

Burial of polar atoms

- Up-weight penalty for burial of polar carbonyl atoms (-10.0 -> -13.5)
- Include coulomb energy (hack_elec))

Reweighted score (from *ab initio* FlexPepDock)

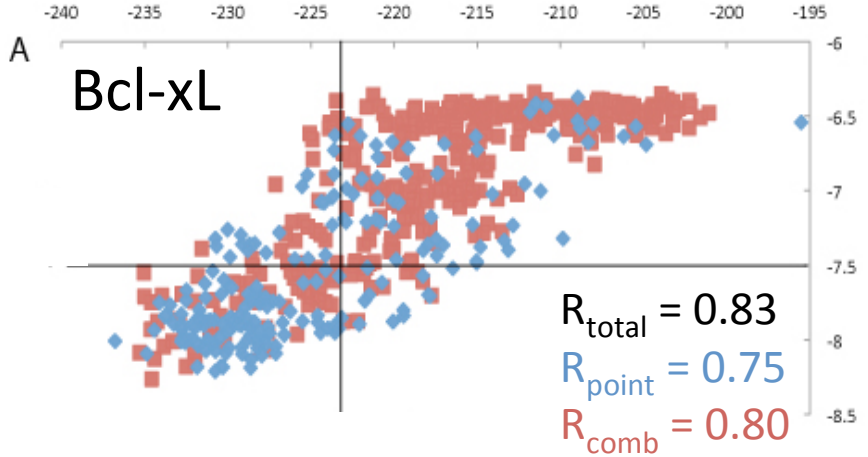
= (tot score + peptide score + interface score)/3

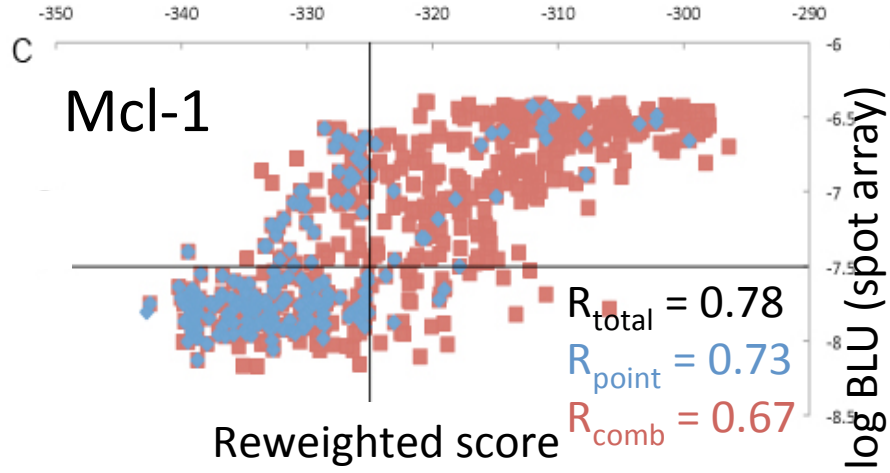
FTase revisited: → AUC=0.93

FlexPepdock with more decoys (1000 *vs* 200); Looser constraint (0.2 *vs* 0.1); using different templates

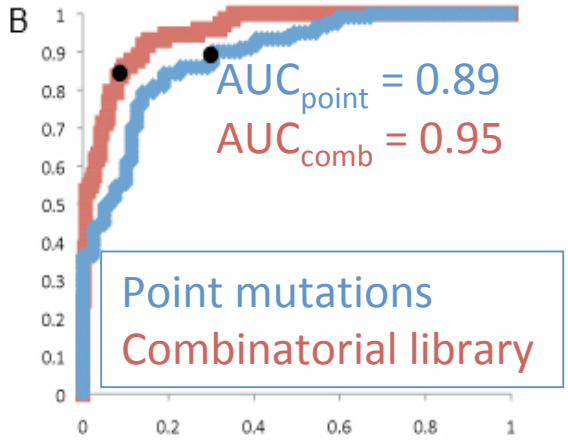
Bcl-xL & Mcl-1: Results

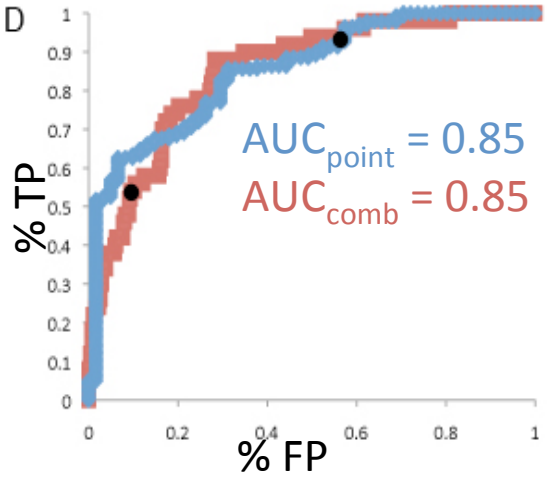
Correlation





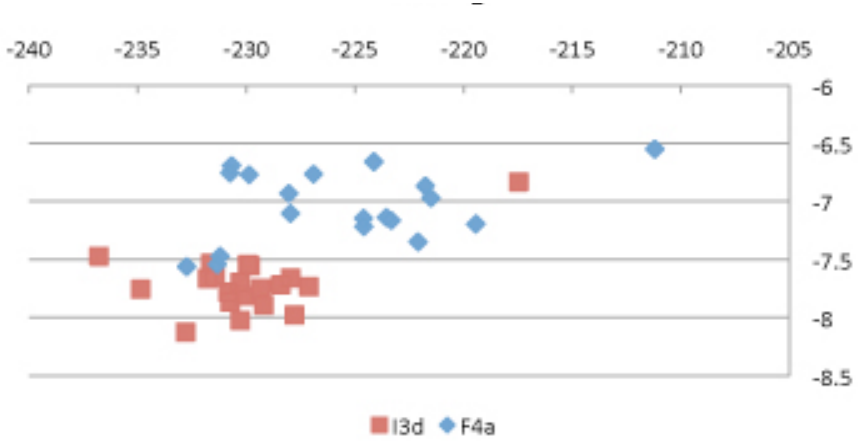
ROC





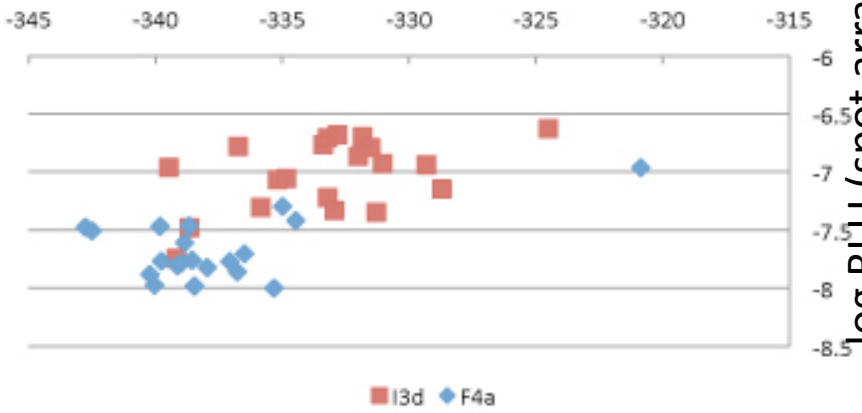
Bcl-xL & Mcl-1: Specificity positions

Bcl-xL



F4a: specific
I3d: promiscuous

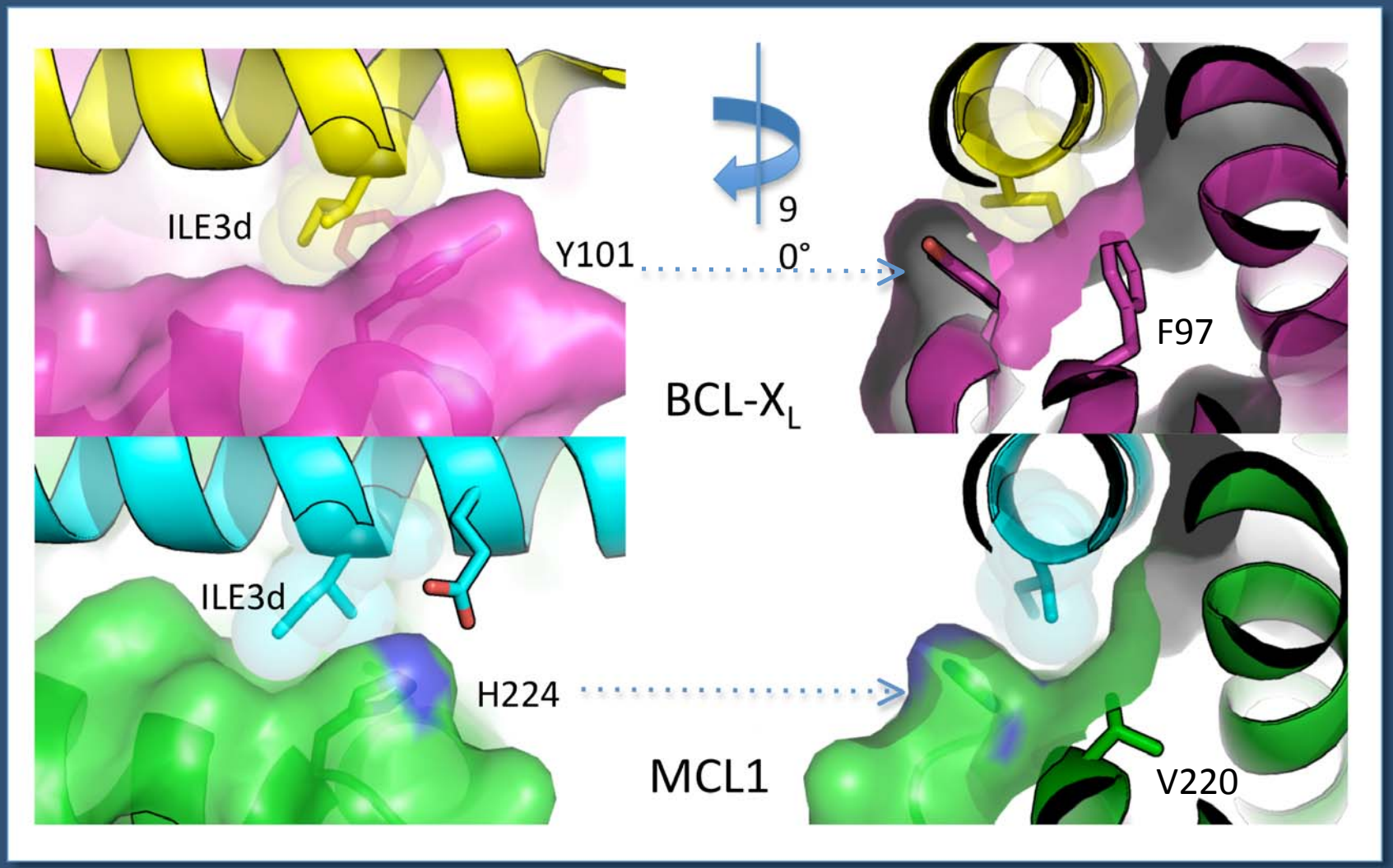
Mcl-1



log BLU (spot array)

F4a: promiscuous
I3d: specific

ILE 3d promiscuity/specificity



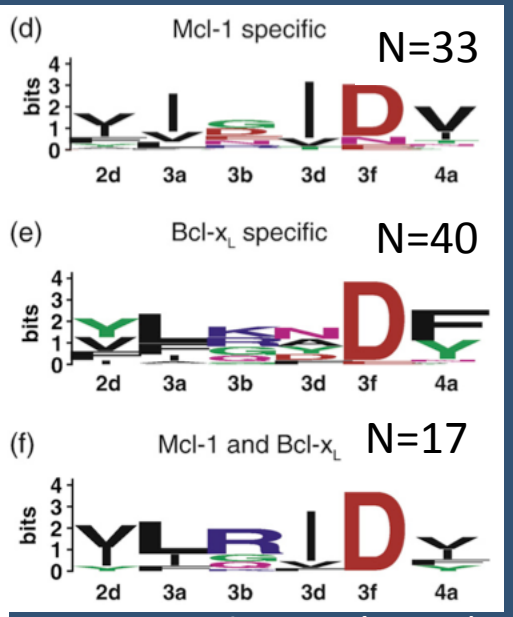
Test I: peptides isolated with yeast surface display

BIM BH3 peptide

- randomize 6 positions; express and select w yeast display
- apply positive / negative selections (FACS) to Bcl-xL and / or Mcl-1
- Measure binding affinity

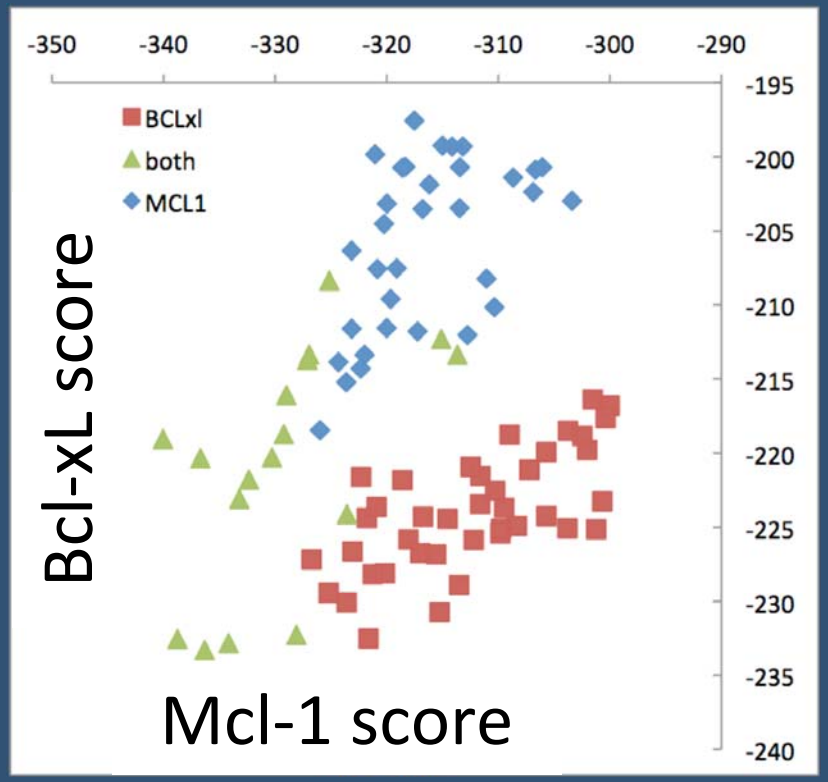
Peptide	Sequence	K_i (nM) Bcl-x_L	Bcl-w	Bcl-2	Mcl-1	Bfl-1
Bim	RPEIWIAQELRRIGDEFNAYYAR	1.3 ± 0.4	2.1 ± 0.3	1.4 ± 0.6	1.9 ± 0.3	2 ± 0.1
	Mcl-1 specific peptides					
MB1	RPEIWIAQEIDRIGDEVNAYYAR				4 ± 1.8	
MB2	RPEIWFAQEIDRIGDEVNAYYAR				20 ± 2	
MG1	RPEIWFAQEFSRIGDEVNAYYAR				30 ± 6	
MF11	RPEIWVAQELERIGEEVNAYYAR				192 ± 11	
MB7	RPEIWAAQEIRRIGDENNAYYAR				273 ± 37	
	Bcl-x_L specific peptides					
XH11	RPEIWVAQELKRNGDEFNAYYAR	2.9 ± 0.8	81 ± 11	73 ± 7		
XF8	RPEIWFAQELKRNGDEYNAYYAR	9 ± 2	40 ± 26	132 ± 61		
XG10	RPEIWYAQEIRRFGDEFNAYYAR	13 ± 6	220 ± 44	255 ± 3		
XD5	RPEIWYAQELRRNGDEFNAYYAR	6 ± 1	30 ± 9	137 ± 5		
XG12	RPEIWYAQELGRAGDEFNAYYAR	19 ± 1	109 ± 23	725 ± 249		

K_i < 10 nM 10 nM < K_i < 100 nM 100 nM < K_i < 1000 nM K_i > 1000 nM

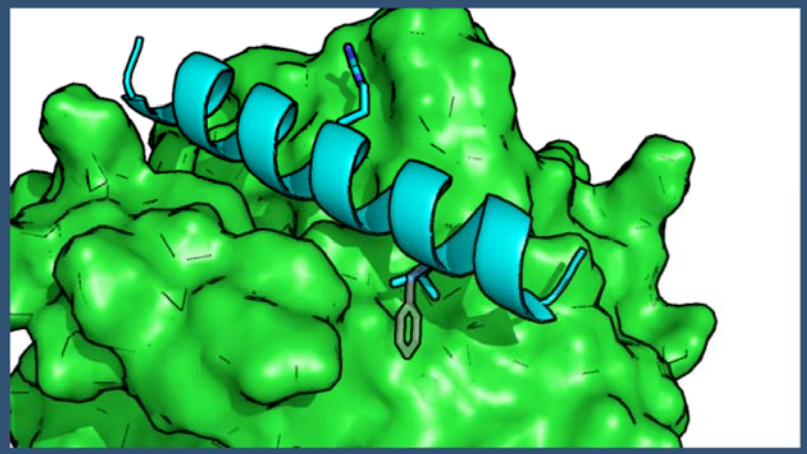


Dutta et al. JMB (2010)

Test I: peptides isolated with yeast surface display

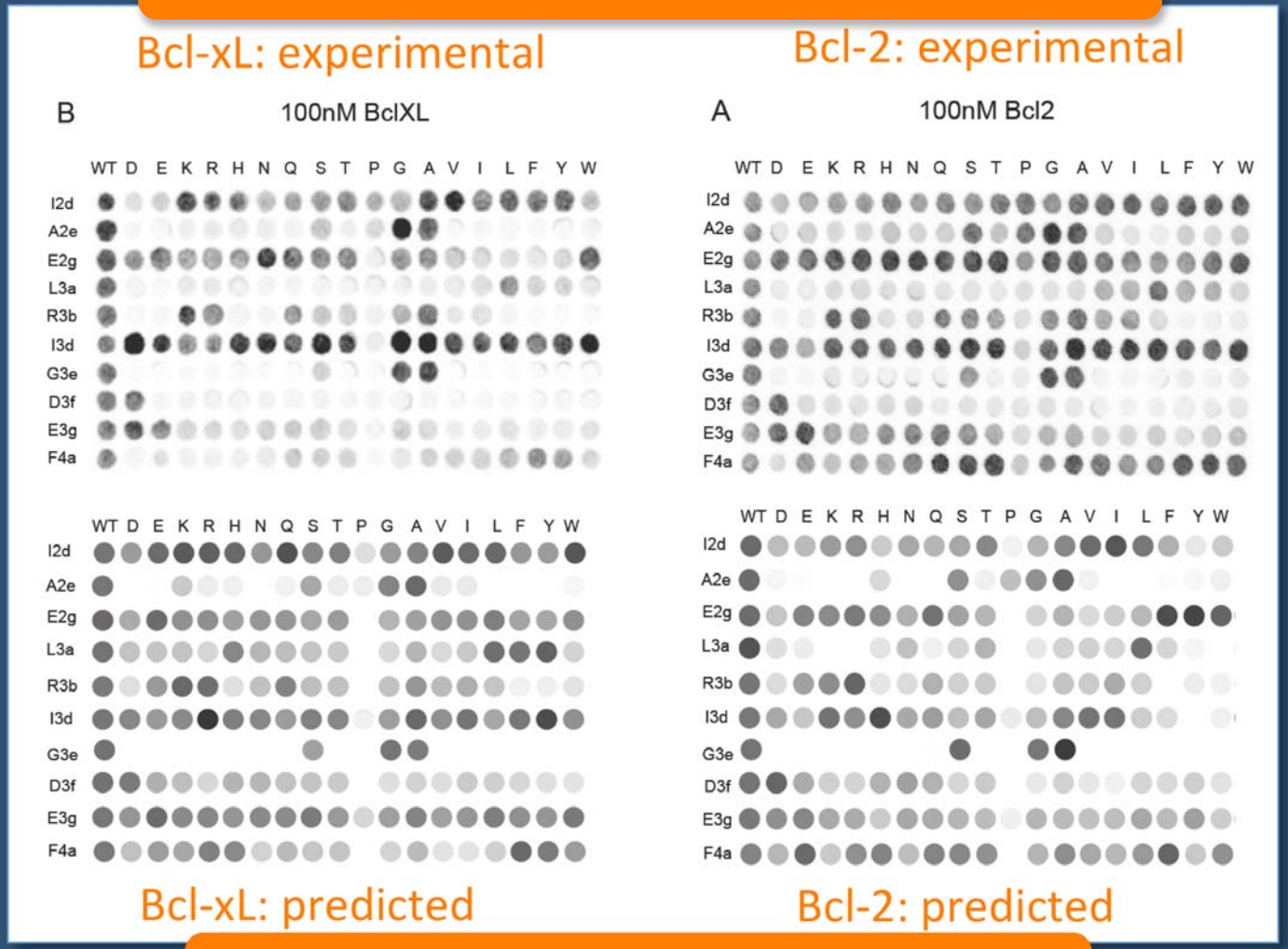


- ✓ Bcl-xL specific set
- ✓ Both promiscuous set
- Mcl-1 specific binders set (correlated with V4a)



Test II: application to Bcl-2

➢Accurate description of Bcl-2



➢Bcl-2 is very similar to Bcl-xL

In collaboration with Amy Keating, MIT

Specific(ity) conclusions II

- *Bcl-FlexPepBind*: Accurate description of binding specificities of (BIM-derived) BH3 peptides to pro-apoptotic proteins Bcl-xL, Mcl-I & Bcl-2
- FlexPepBind protocol can be adapted to new system with minor changes (e.g., calibration of burial of polar atoms)
- What we learned – good results are obtained with:
  - More FlexPepDock simulations (~1000)
  - Averaged & reweighted score
  - More available templates

Summary

Peptide-protein interactions are important

1. Rosetta FlexPepDock: Highly accurate modeling protocol that accounts for peptide flexibility
2. FlexPepBind: Prediction of binding specificity:
   - Identification of novel class of farnesylation targets
   - Accurate modeling of binding specificity of pro-apoptotic proteins Bcl-xL, Mcl-1 & Bcl-2

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Rosetta Commons

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Excited about this work?

- Want to study outside the US?
- Want to study near the GMEC on earth?
- We are looking for new PhD students and PostDocs

Furman Lab @ HU