Cohesin-dockerin binding: Surprises in the study of a "simple" protein interaction

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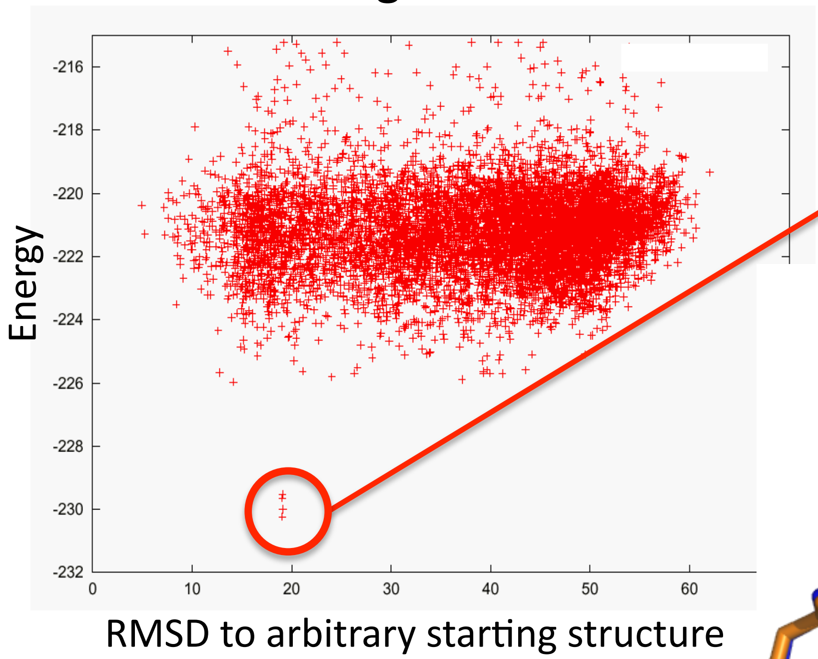
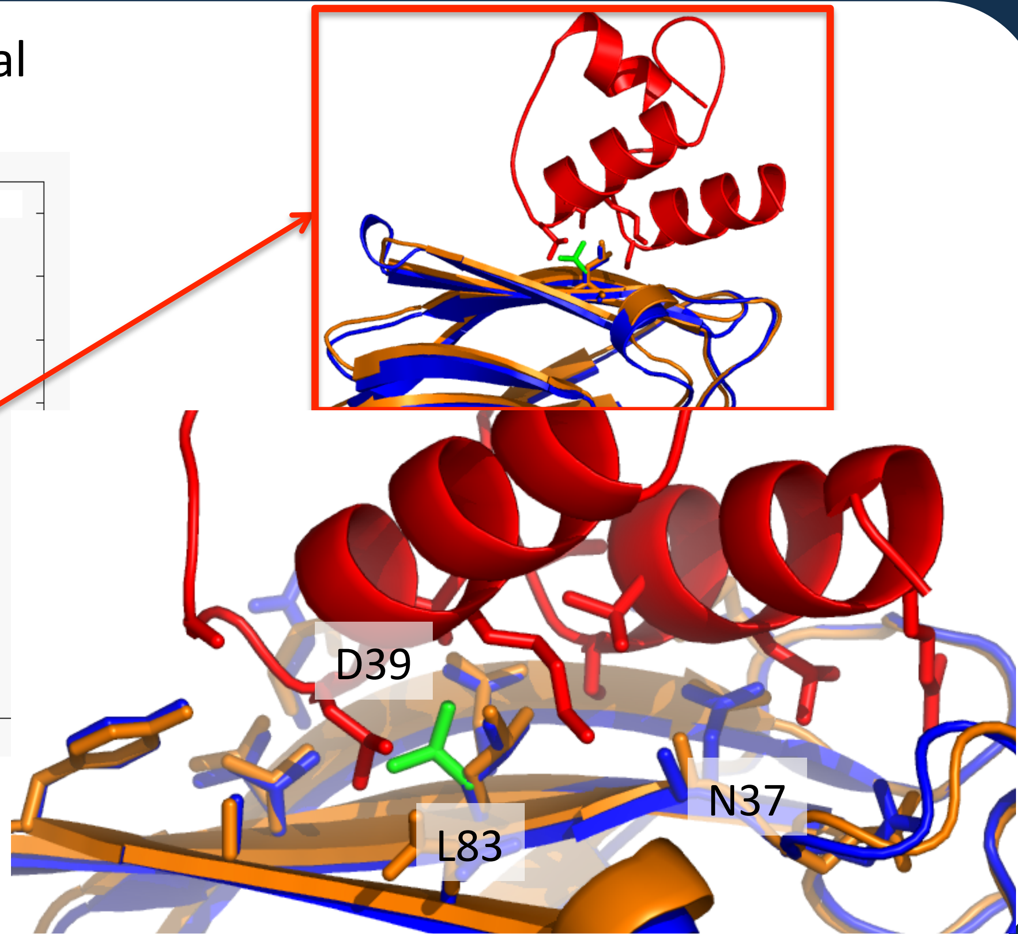
RosettaCon 2012, 7/31/2012

A couple of years ago ….

- CAPRI target 12, cohesin-dockerin interaction: high resolution prediction of protein complex

My very most successful global docking run ever …



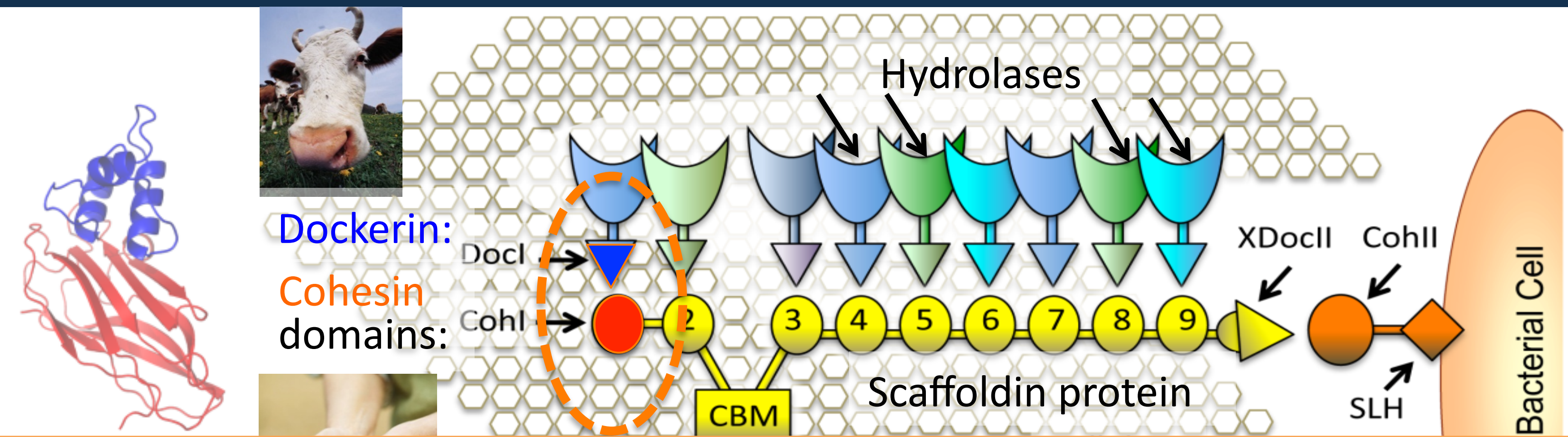


red, orange– xray; blue – model; green – unbound

The cellulosome

- Multi-protein complex that degrades cellulose
- Diversity achieved by:
  - Various hydrolases connected to dockerin
  - Promiscuous cohesin-dockerin binding
  - 2 different dockerin orientations
  - Different # of cohesin repeats on scaffoldin

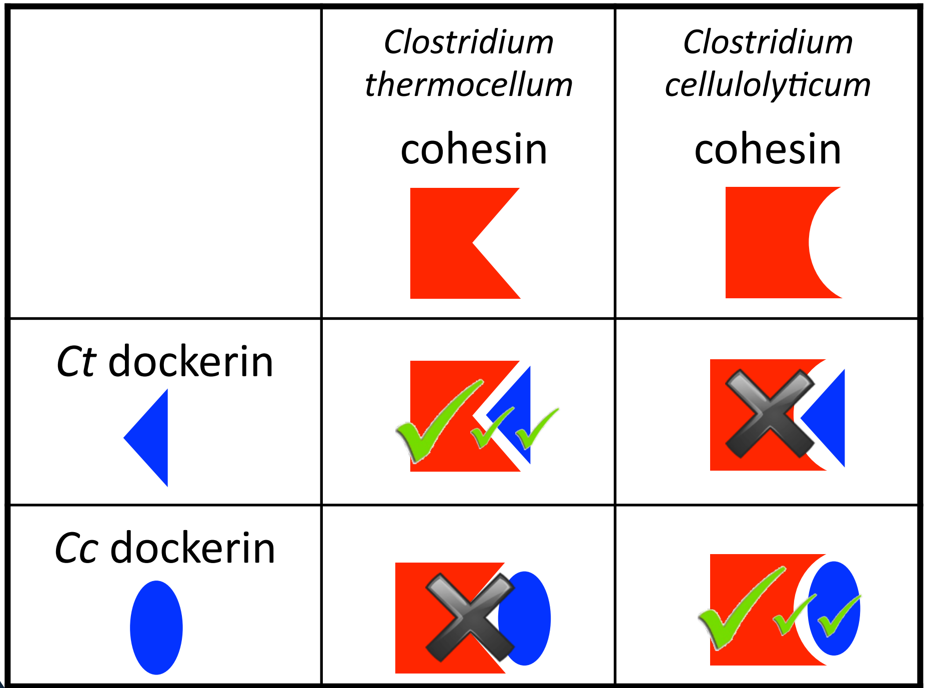
Ed Bayer
Weizmann Institute



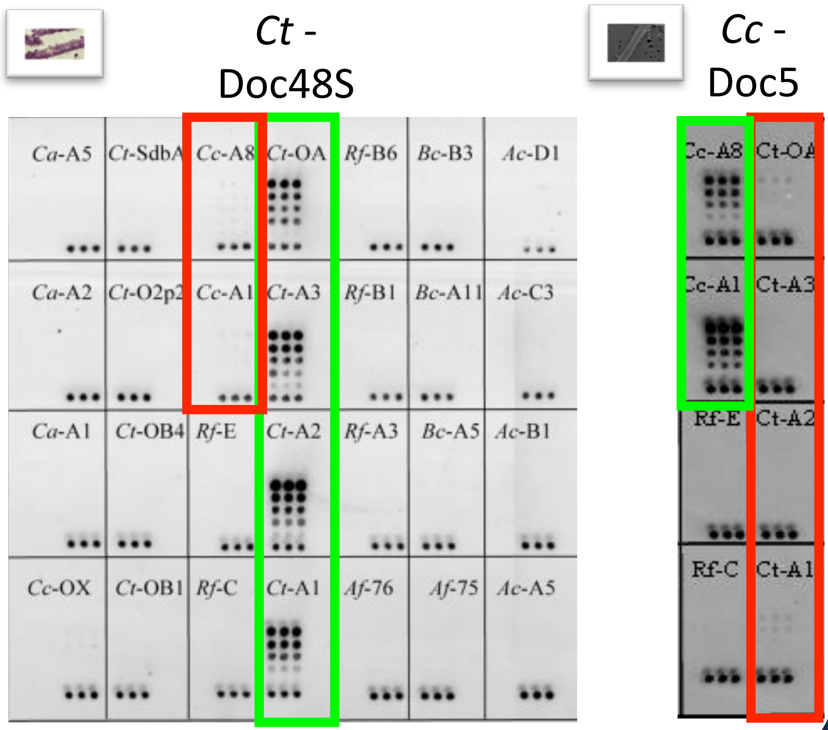
Cohesin-dockerin interactions define character of cellulosome

Affinity, promiscuity and specificity in the cohesin-dockerin interaction

- **Affinity**: Very high (10^{-12}M)
- **Promiscuity**: *Intra*-species
- **Specificity**: *Cross*-species

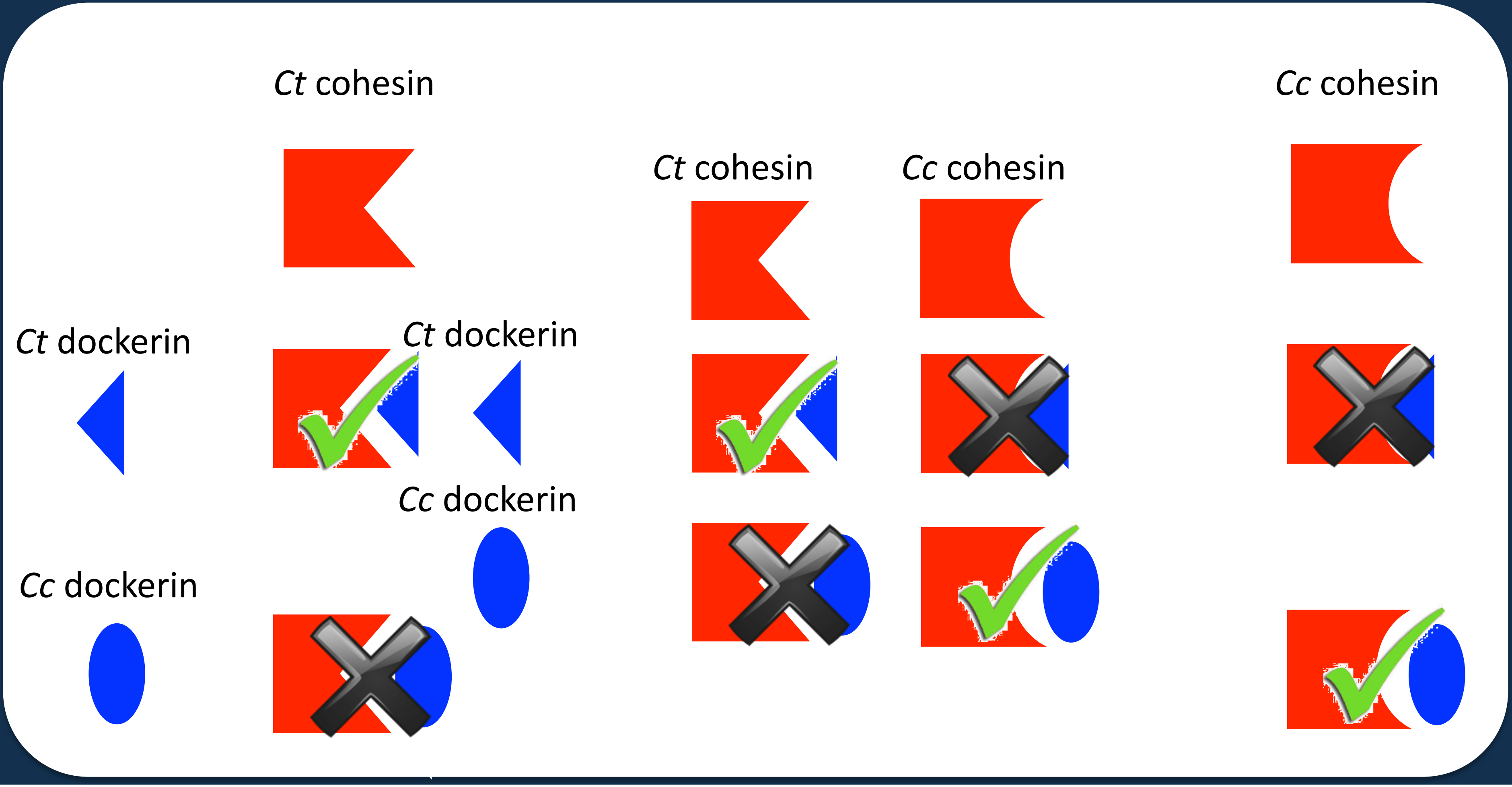


Cellulose membrane assay



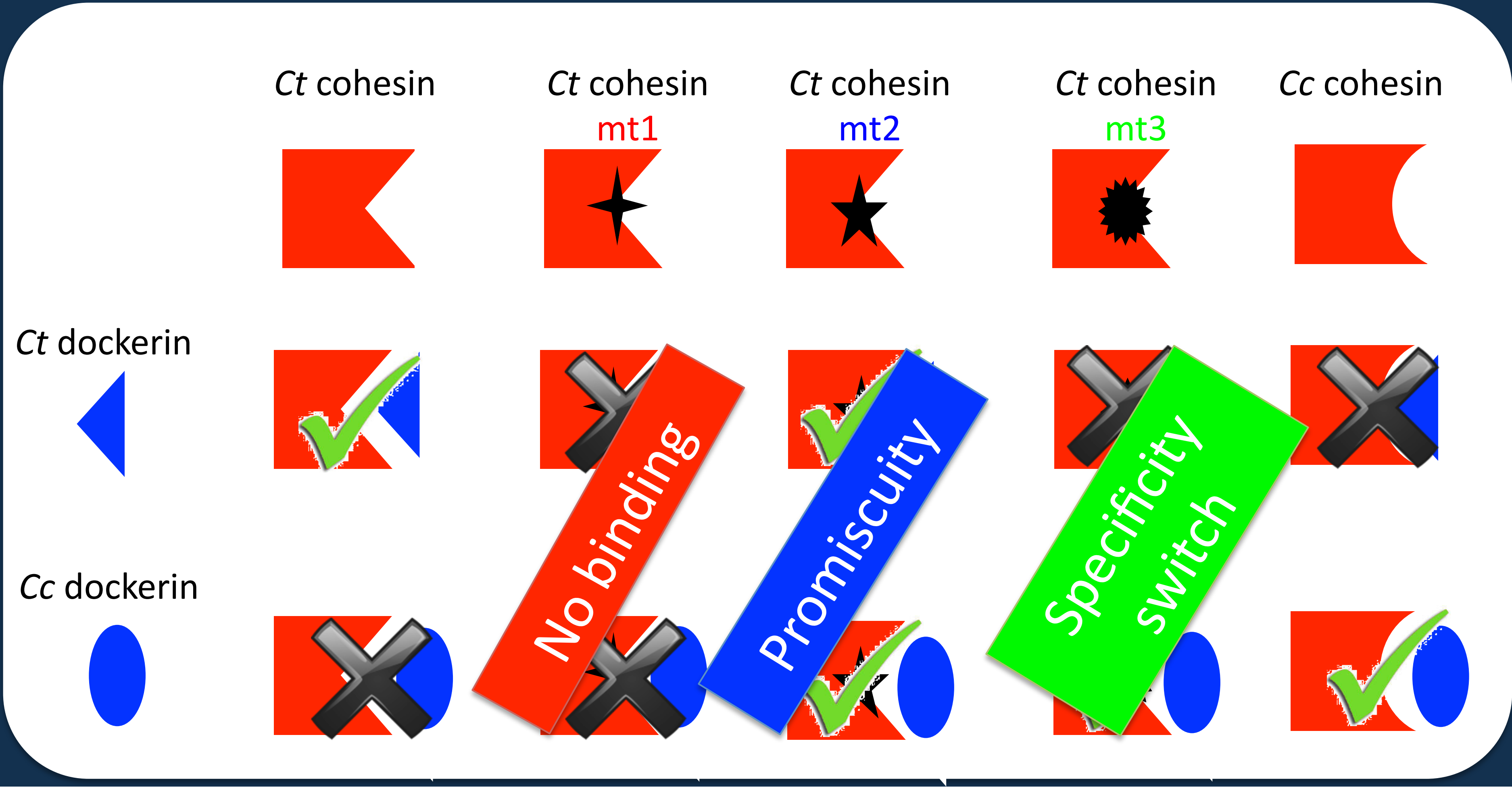
Haimovitz *et al.* (2008) Proteomics Barak Y, Bayer E

Affinity, promiscuity and specificity in the cohesin-dockerin interaction

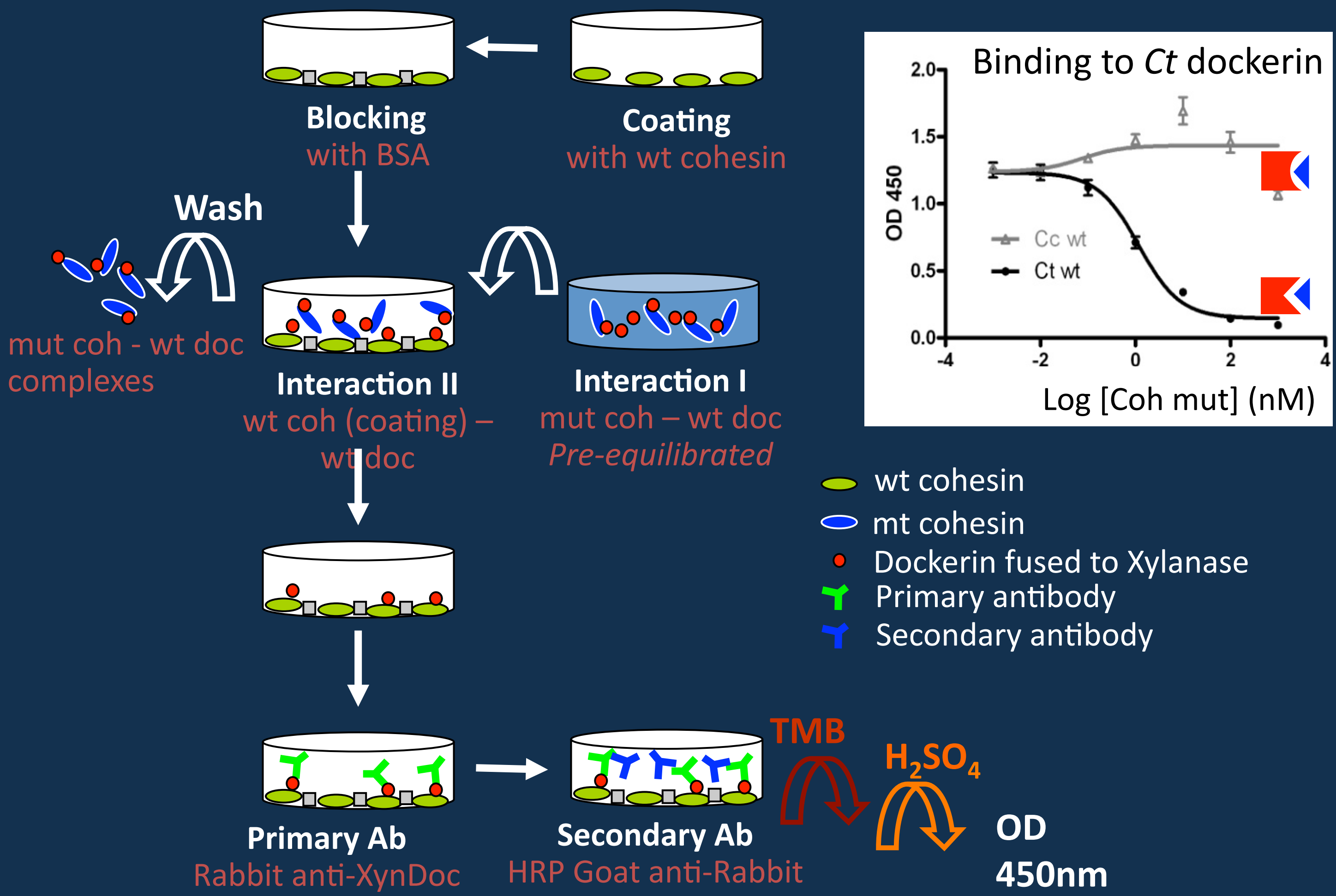


Affinity, promiscuity and specificity in the cohesin-dockerin interaction

Can we modify the specificity of this interaction?



Experimental Approach: Indirect ELISA (iELISA)



Slutzki, Barak, Reshef, Schueler-Furman, Lamed & Bayer. Methods in Enzymology 2012

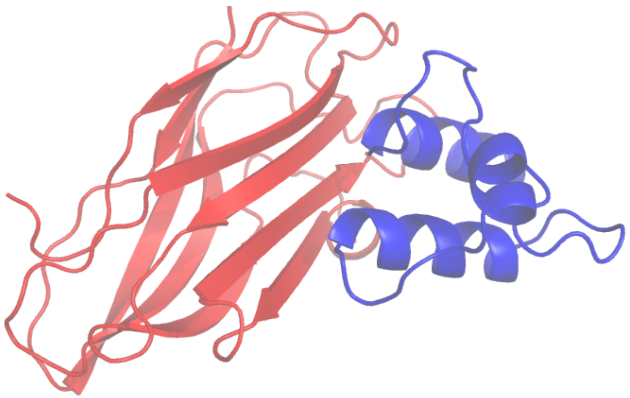
Computational Approach: Alanine Scanning, Docking and Design

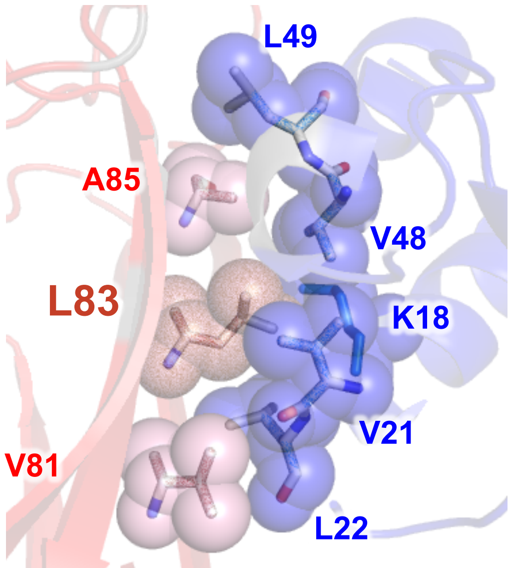
- Templates: complex structures of *Ct* and *Cc* coh-doc
- Alanine scanning -> interface hotspot residues
  - Rosetta 2.3
  - Rosetta 3.0 (different versions of Liz's protocol)
  - Also: FoldX, Hunter, Orbit, PBSA
- Docking -> model cohesin-dockerin complexes
- Design -> design of changes in binding affinity and specificity

Overview

1. The interface of the cohesin-dockerin interaction
   – Characterize interface patches and hotspots
2. Targeted manipulation of cohesin-dockerin binding specificity
   – Single mutations with dramatic effects
3. Outlook

Coh-doc interface:
(I) hydrophobic conserved patch





cohesin

	83
Ct_Repeat1	VFLFA
Ct_Repeat2	***VFLFA***
Ct_Repeat3	VFLFA
Ct_Repeat4	VFLFA
Ct_Repeat5	VFLFA
Ct_Repeat6	VFLFA
Ct_Repeat7	VFLFA
Ct_Repeat8	VFLFA
Ct_Repeat9	VFLFA
Cc_Repeat1	SFLFL
Cc_Repeat2	SFLFL
Cc_Repeat3	SFLFL
Cc_Repeat4	SFLFL
Cc_Repeat5	SFLFL
Cc_Repeat6	SFLFL
Cc_Repeat7	SFLFL
Cc_Repeat8	SILFL

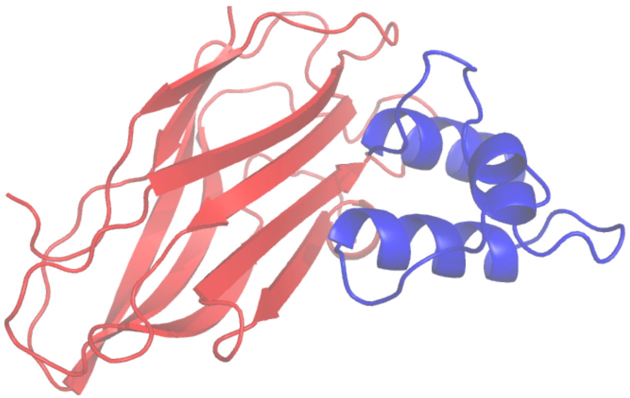
Ct

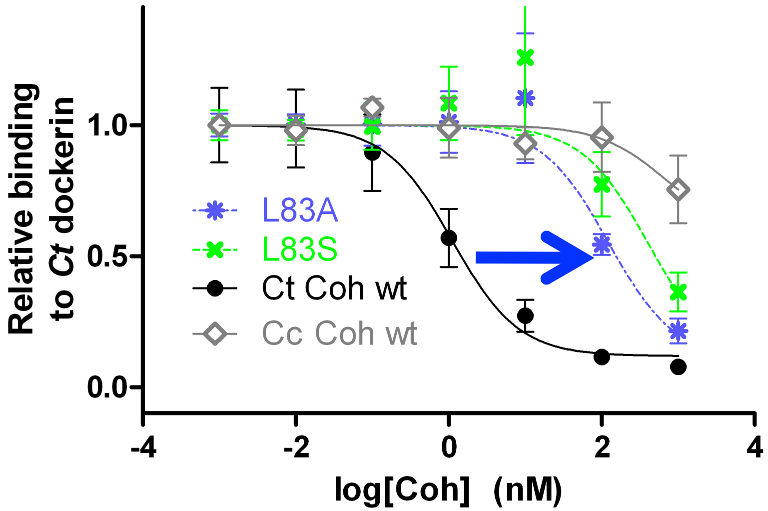
Cc

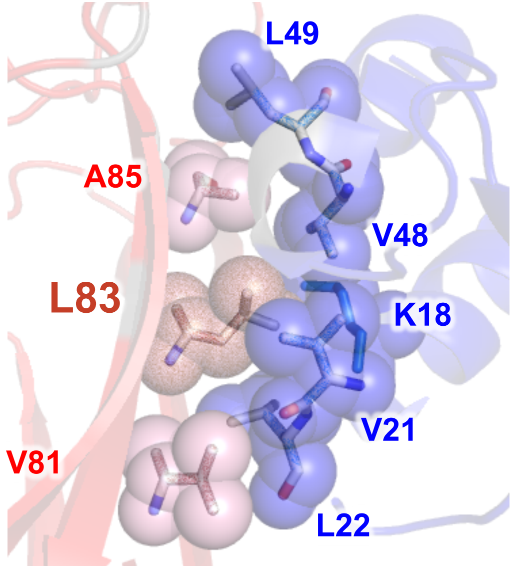
dockerin

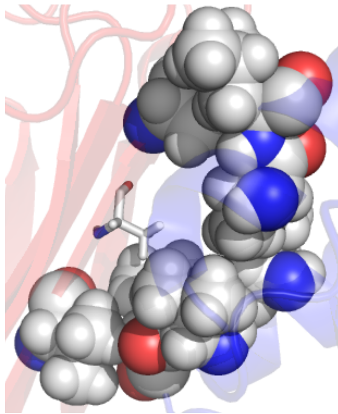
	18		21		48
Ct_XynY	***K***	//	***VL***	//	***VL***
Ct_GunA	K	//	LL	//	MT
Ct_GunD	K	//	VL	//	VT
Ct_GunF	K	//	VI	//	LY
Ct_XynZ	K	//	LL	//	YS
Cc_GunA	K	//	IM	//	LA
Cc_GunG	K	//	LL	//	MA
Cc_GunD	K	//	LL	//	FA
Cc_GunF	K	//	LL	//	YA
Cc_GunC	K	//	IL	//	FA

(I) hydrophobic conserved patch: L83 is important for binding







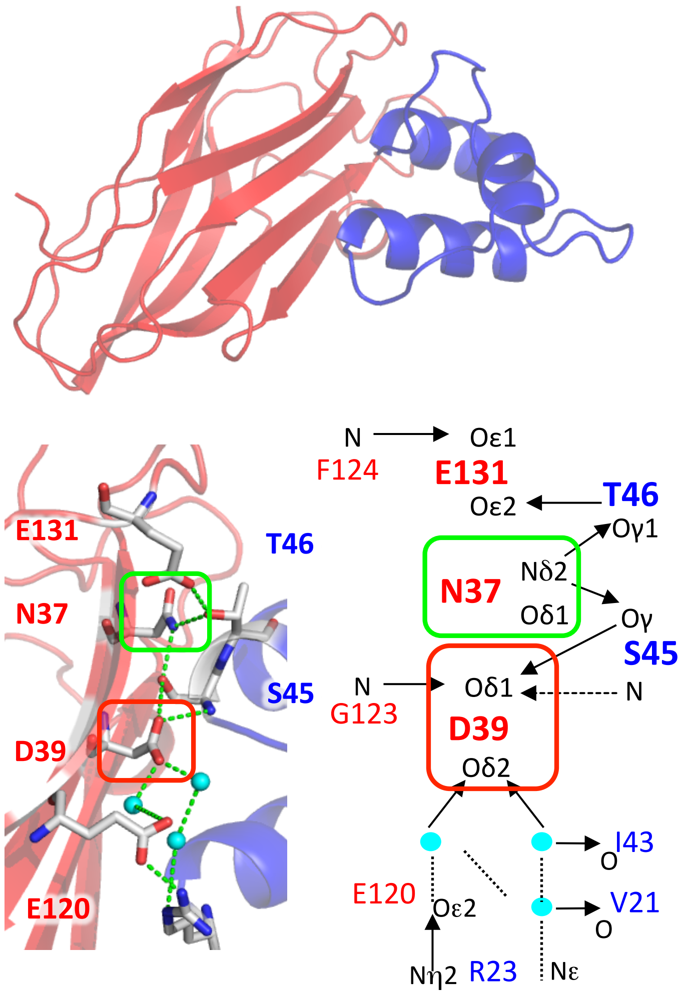


L83A

Disturbs packing

$\Delta\Delta G_{pred}$= +2.2

Coh-doc interface: (II) hydrophilic patch in *Ct*



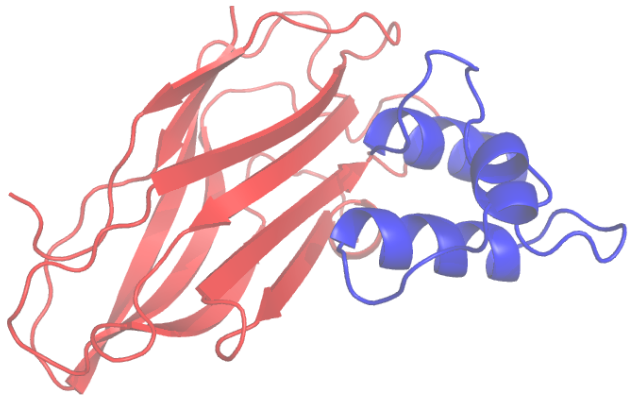
	37		120		131
Ct_1	NCD	//	E	//	E
Ct_2	***NCD***	***//***	***E***	***//***	***E***
Ct_3	NCD	//	E	//	E
Ct_4	NCD	//	E	//	E
Ct_5	NCD	//	E	//	E
Ct_6	[illegible]	//	E	//	E
Ct_7	[illegible]	//	E	//	E
Ct_8	NCD	//	E	//	E
Ct_9	SGD	//	E	//	E
Cc_1	TCN	//	D	//	K
Cc_2	TCN	//	D	//	K
Cc_3	TCN	//	D	//	K
Cc_4	TC[illegible]	//	D	//	K
Cc_5	[illegible]	//	D	//	K
Cc_6	[illegible]CN	//	D	//	K
Cc_7	TCN	//	D	//	K
Cc_8	TCN	//	D	//	K

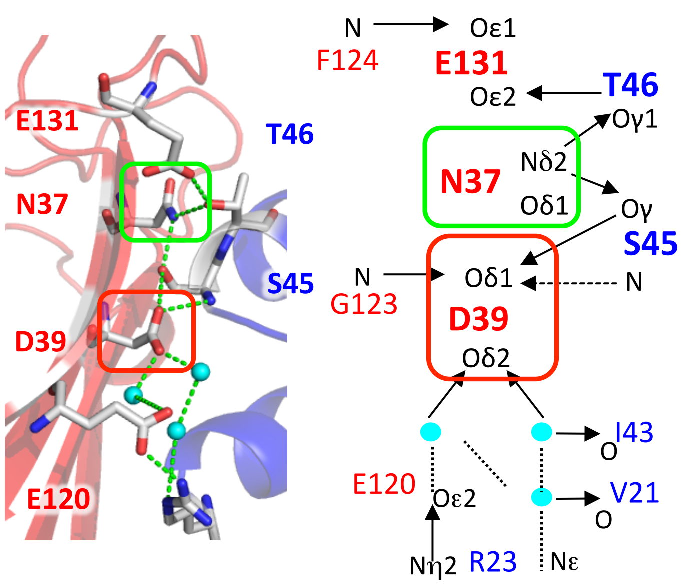
Ct

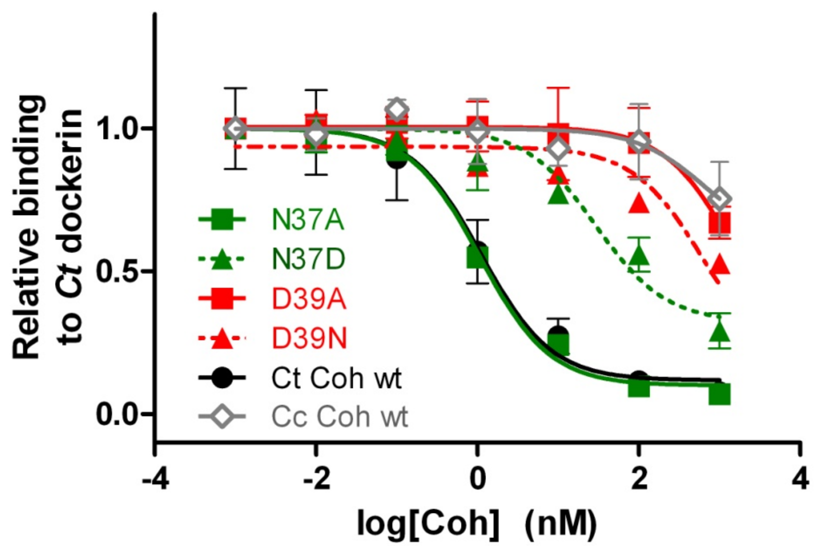
Cc

	23		45
Ct_XynY	**R**	**//**	**ST**
Ct_GunA	K	//	SS
Ct_GunD	K	//	SS
Ct_GunF	K	//	ST
Ct_XynZ	G	//	ST
Cc_GunA	A	//	AF
Cc_GunG	G	//	AI
Cc_GunD	D	//	AI
Cc_GunF	N	//	AI
Cc_GunC	T	//	AI

(II) hydrophilic patch in *Ct*: polar effects are more difficult to predict

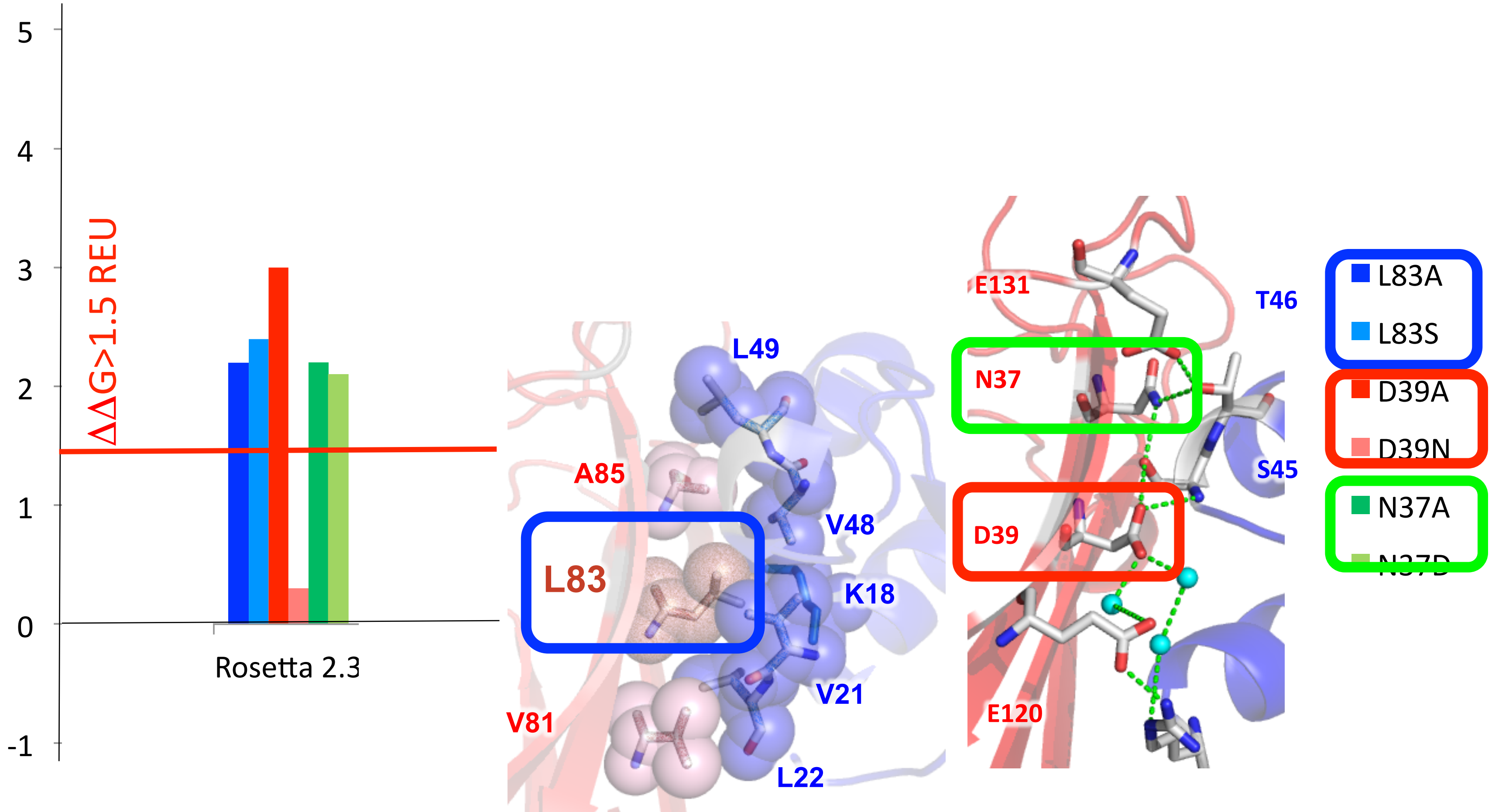




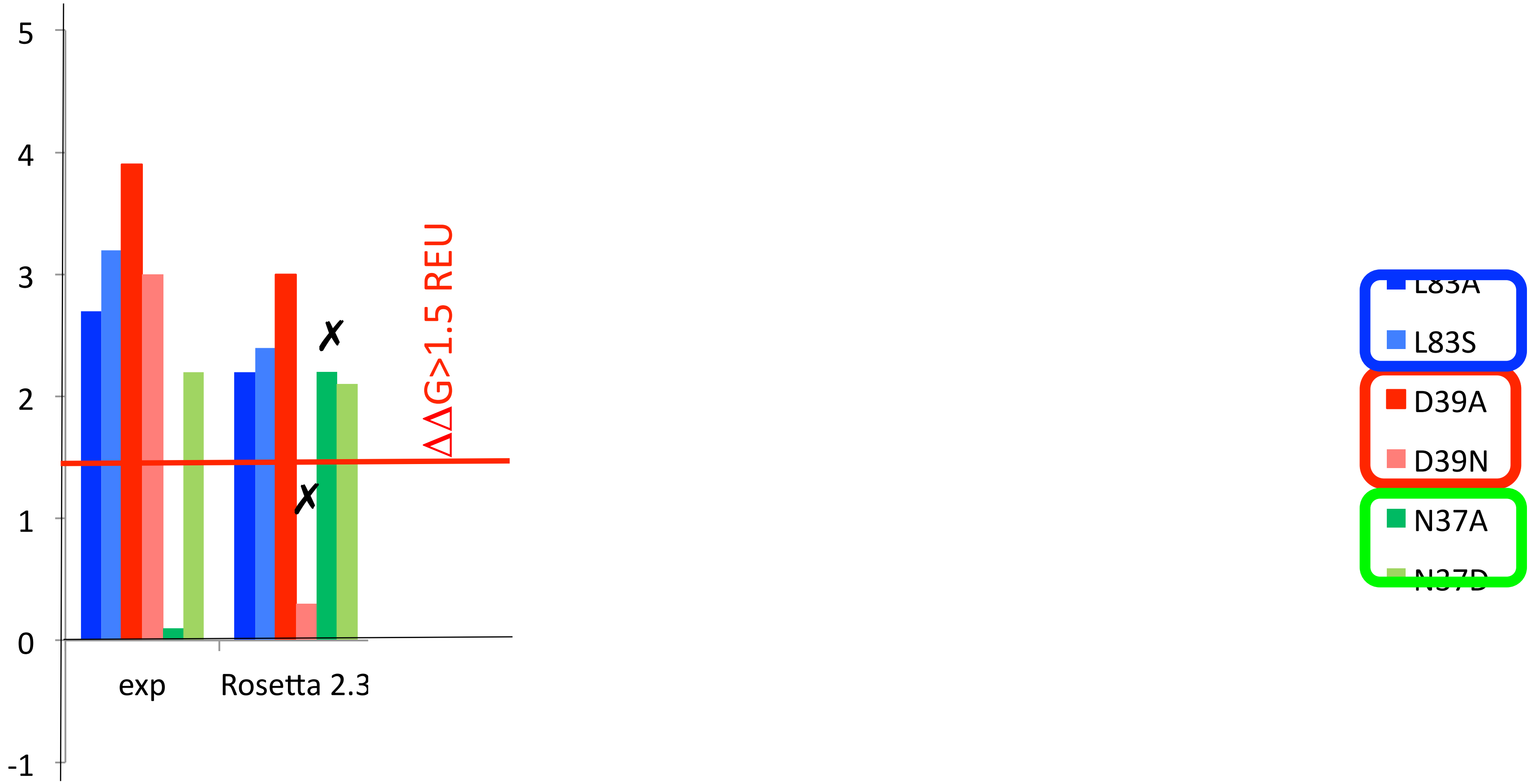


	$\Delta\Delta G_{pred}$	$\Delta\Delta G_{exp}$	Predicted	Measured
D39A	3	3.9	Lose HB	No binding
D39N	0.3	3	-	No binding
N37A	2.2	0	Lose HB	No change
N37D	2.1	2	HB changed	Reduced binding

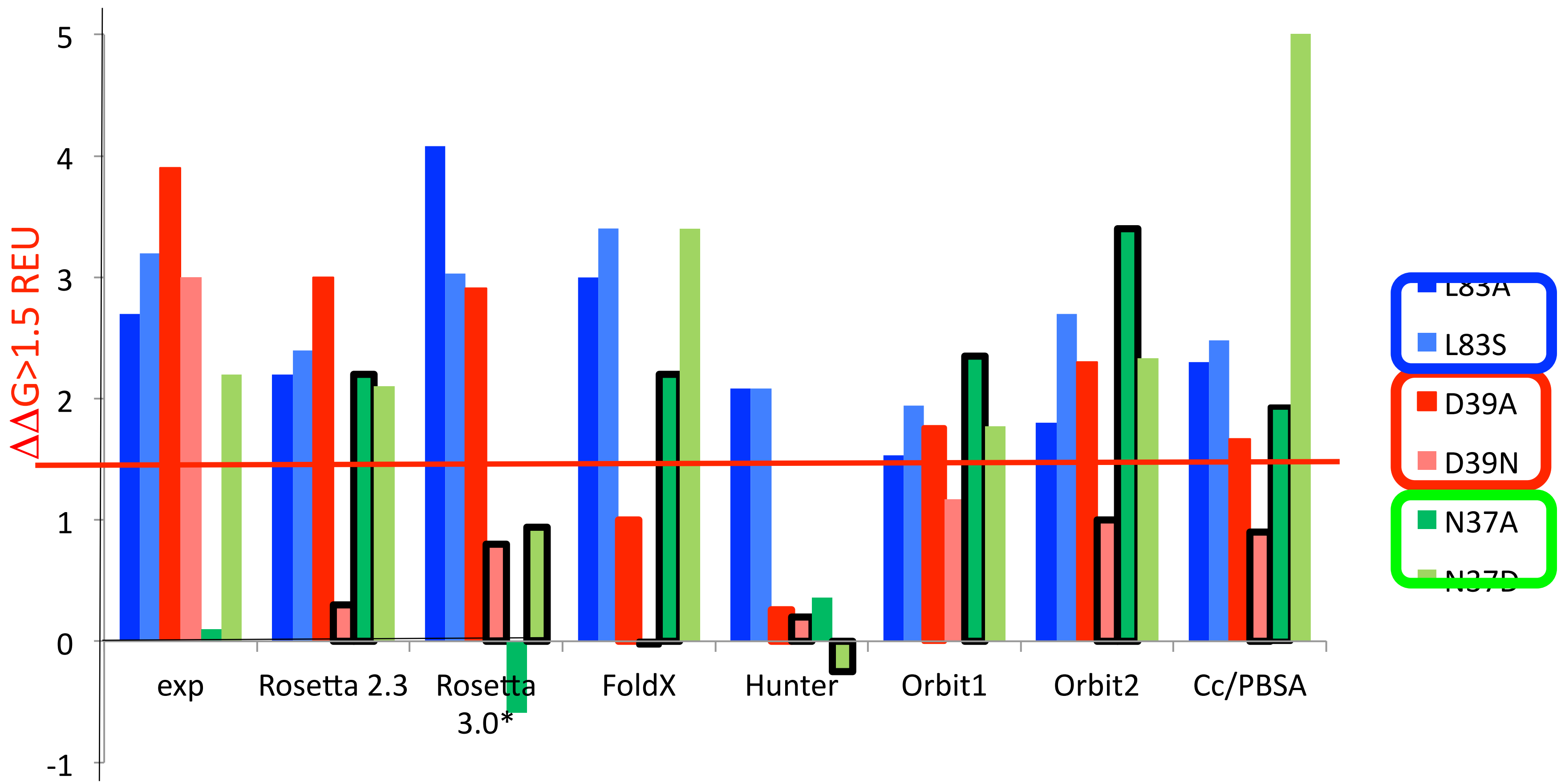
Coh-doc interface: both patches contain putative interface hotspot residues …



.. but not all are actually hotspots



No approach works ☹

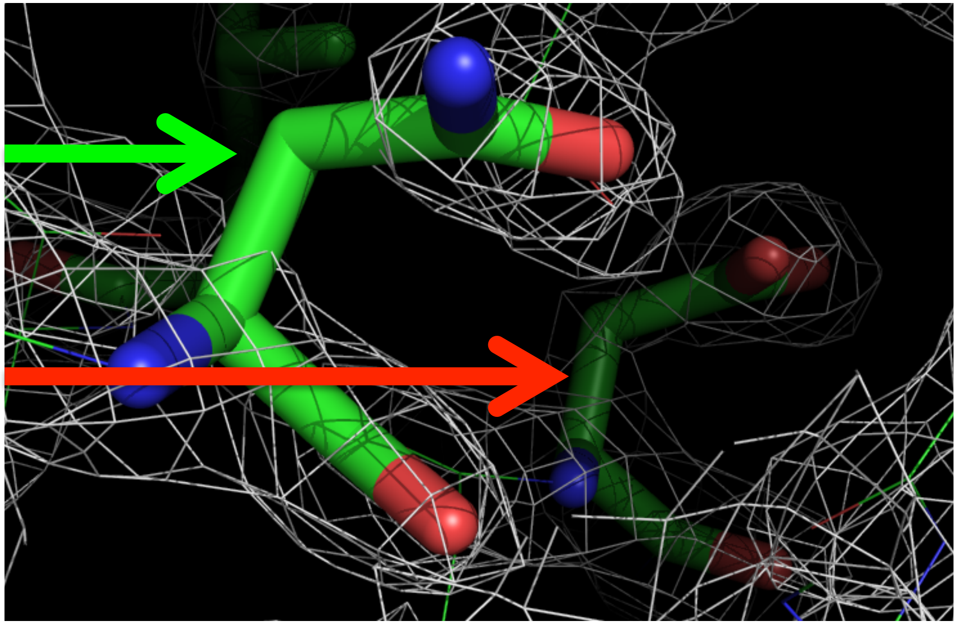


What went wrong? N37A *vs.* D39A

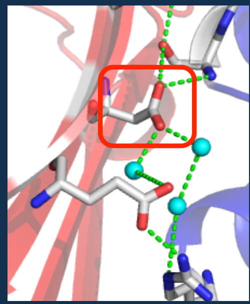
Possible reasons:

- N37 at rim of interface – flexible (flip sc)

- N37C_β not defined (2σ)
- D39 defined

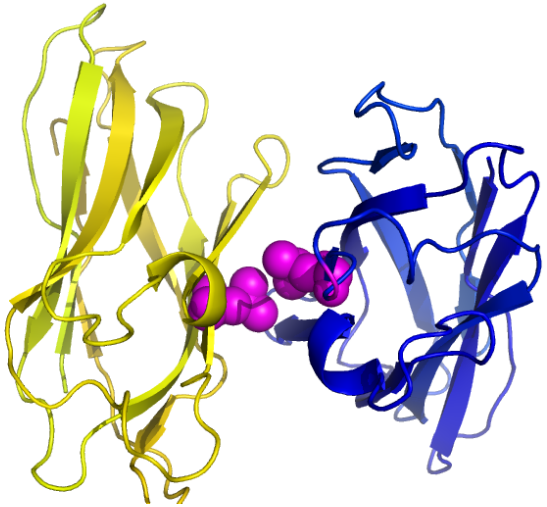


What went wrong? D39N

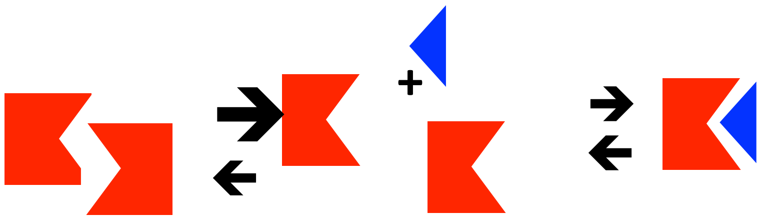


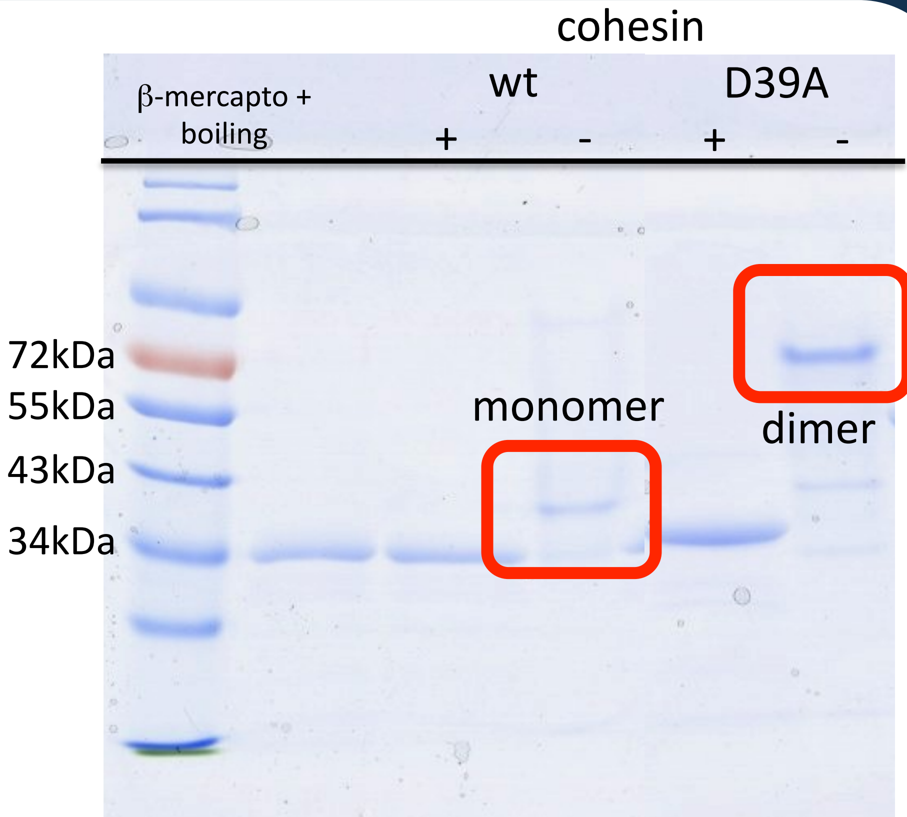
Possible reason:

D39 prevents homo-dimerization of *Ct* cohesin



Orientation as in wt ***Cc*** cohesin structure





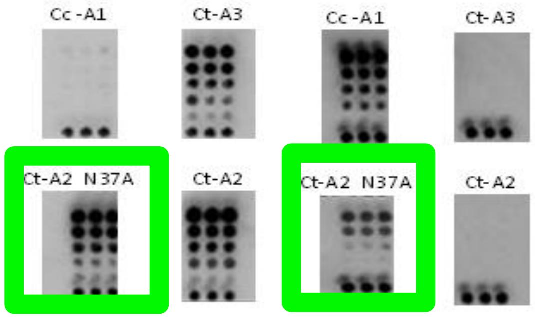
Overview

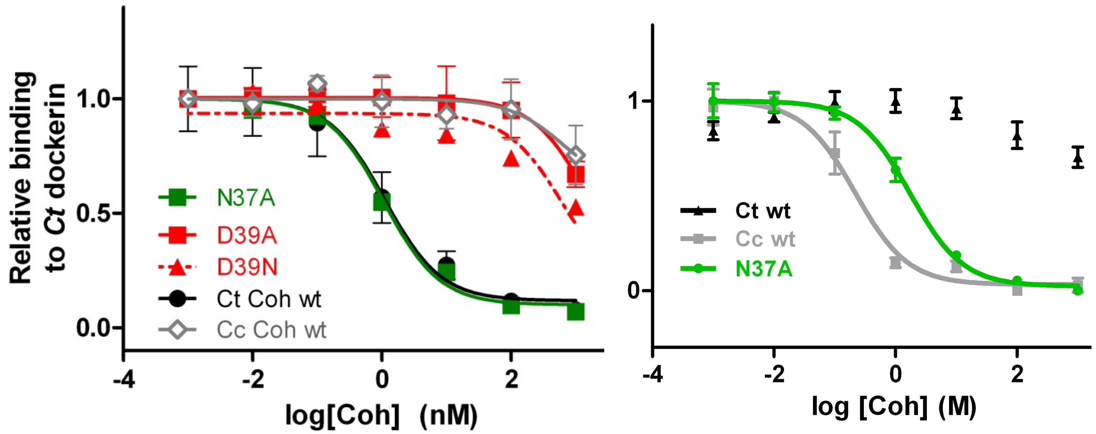
1. The interface of the cohesin-dockerin interaction
   – Not as trivial as you would have thought.....
2. Targeted manipulation of cohesin-dockerin binding specificity
   – Single mutations with dramatic effects
3. Outlook

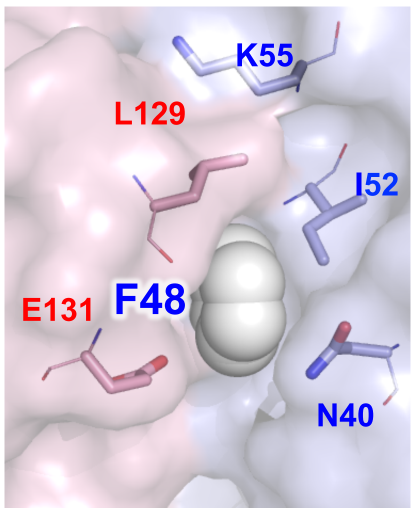
Coh N37A: promiscuous

Cellulose membrane assay

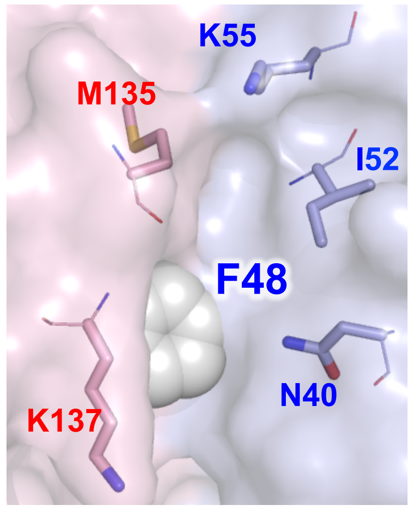
Ct dockerin *Cc* dockerin





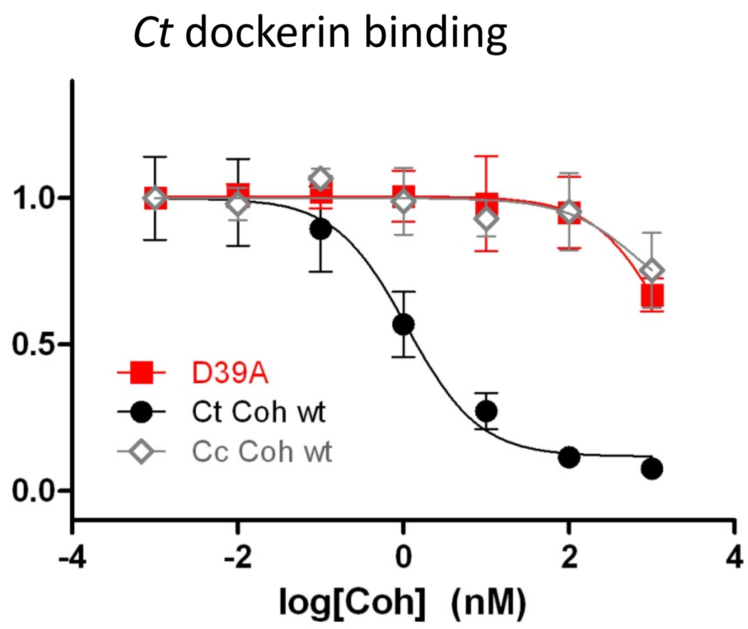


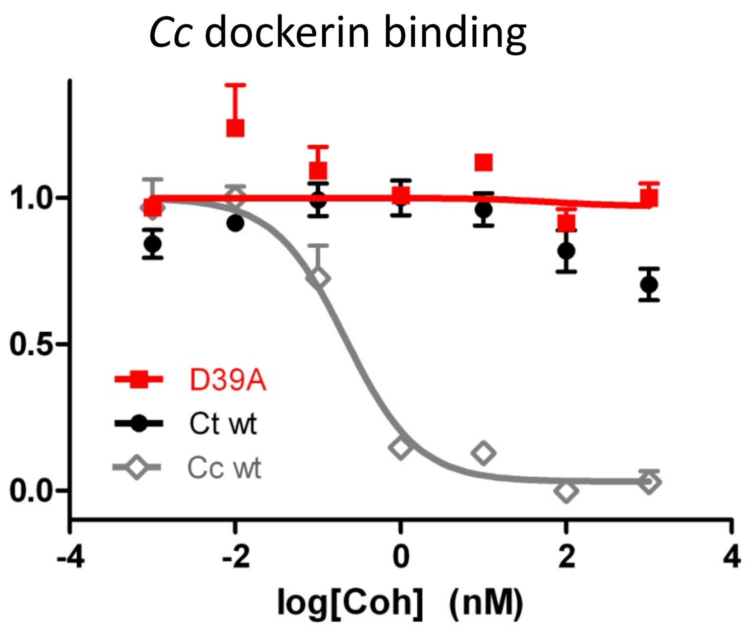
Model of *Ct* N37A cohesin-*Cc* dockerin



Crystal structure of *Cc* cohesin-*Cc* dockerin

Coh D39A: does not bind at all

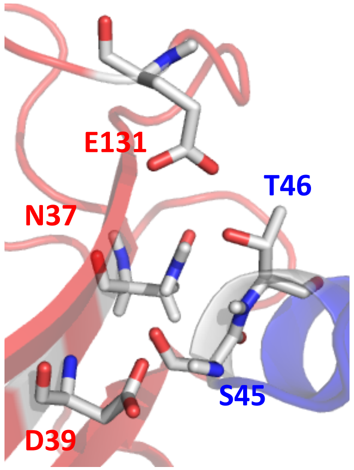




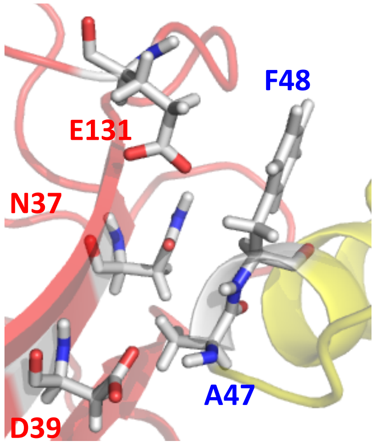
D39A binds neither to *Ct* dockerin… .. nor to *Cc* dockerin

Coh N37L: (partial) specificity switch

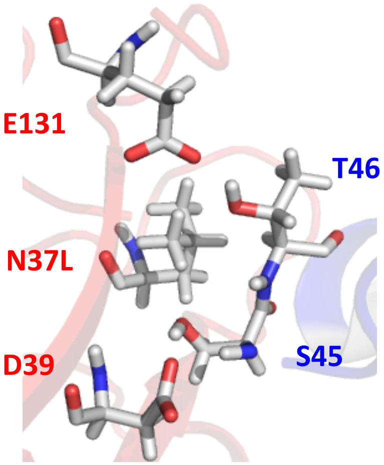
Ct - *Ct*



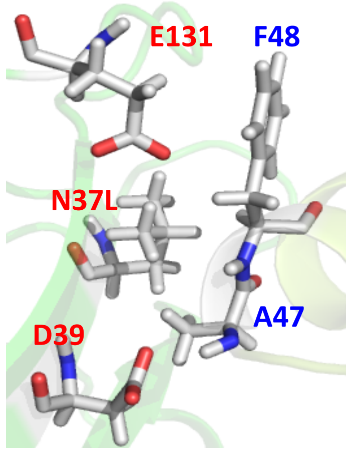
Ct - *Cc*



Ct(N37L) - *Ct*



Ct(N37L) - *Cc*



N37 fits in *Ct* ST pocket…

.. but not in *Cc* AF pocket

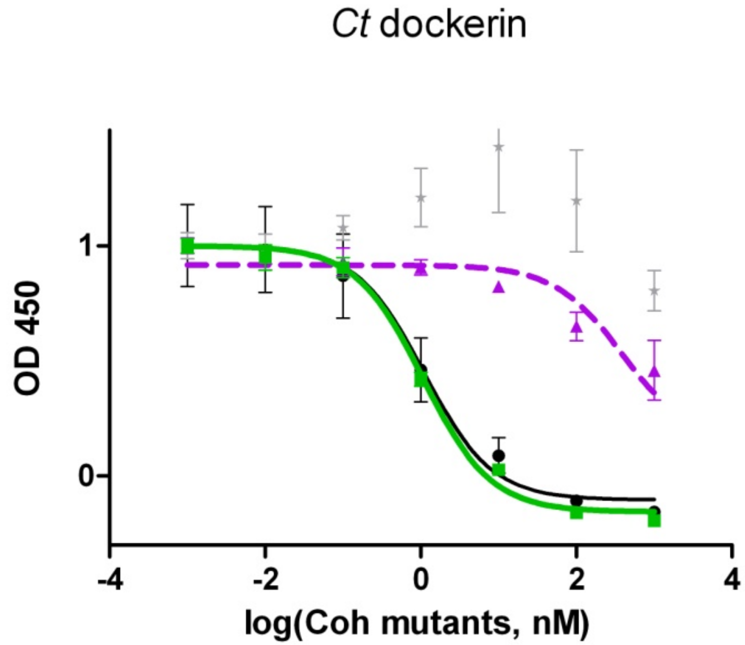
.. but not the ST motif

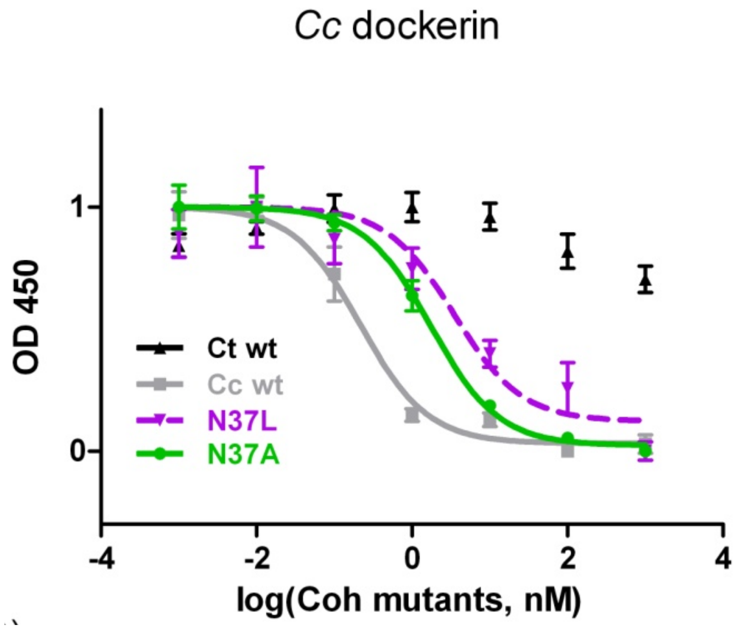
N37L fits in AF pocket…

$\Delta\Delta G_{pred}$=1.4

$\Delta\Delta G_{pred}$=-1.2

Coh N37L: (partial) specificity switch



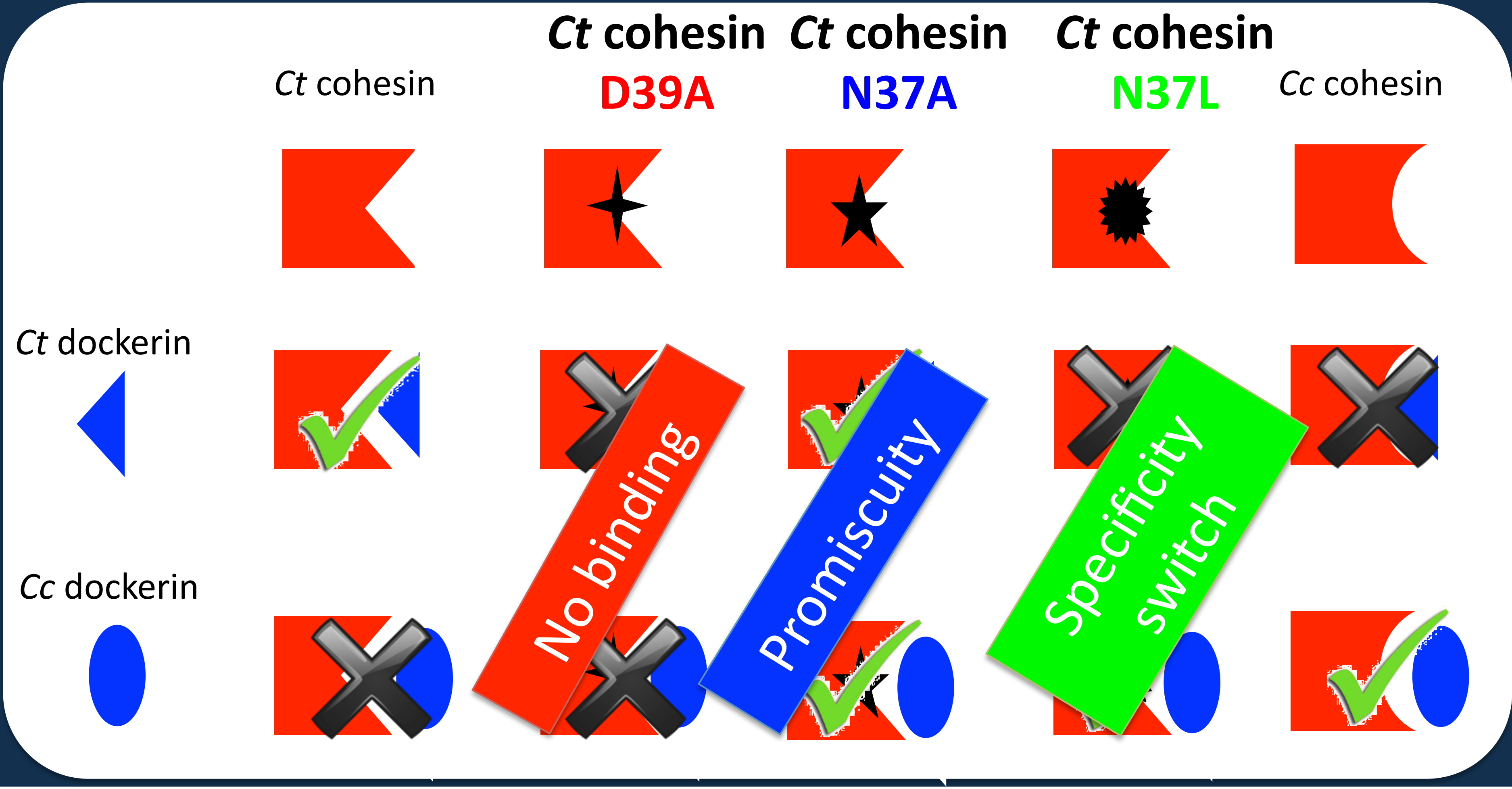


N37L does not bind to its cognate *Ct* dockerin anymore…

.. but to the non-cognate *Cc* dockerin

Can we modify the specificity of this interaction? Yes we can!

… with single mutations



Conclusions

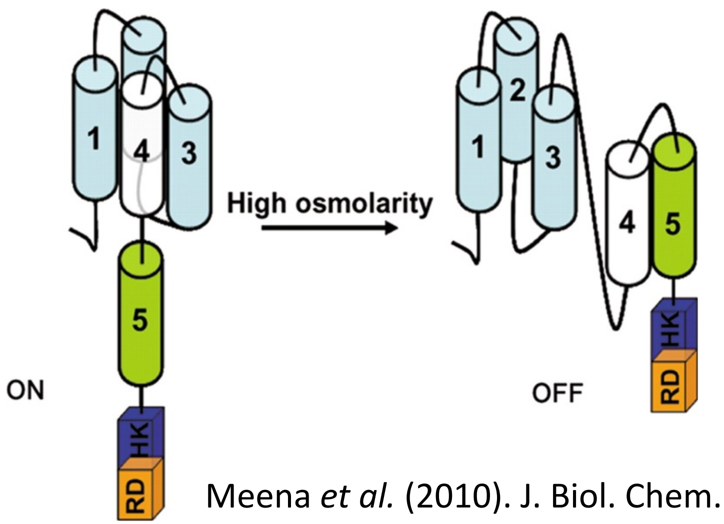
- Cohesin-dockerin interaction as model system for interaction affinity, specificity and promiscuity
  – Single mutations with significant effect
- Hotspot prediction: easy for hydrophobic residues, very difficult for polar residues
- Additional parameters might be important:
    - Inaccuracies in solved structures
    - Competition between Homo-dimers and heterodimers

➜ Outlook: study **context** of interaction

Outlook: context of interactions

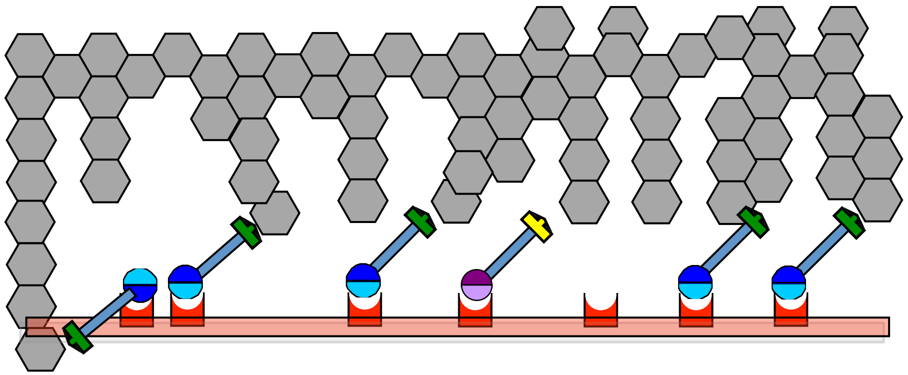
Interactions do not occur in isolation…

Example 1: Regulation by dimerization of HAMP domains



Meena *et al.* (2010). J. Biol. Chem.

Example 2: Regulation of substrate binding affinity by multiple cohesin repeats+ several ways to interact



… Science does not either!

Acknowledgements

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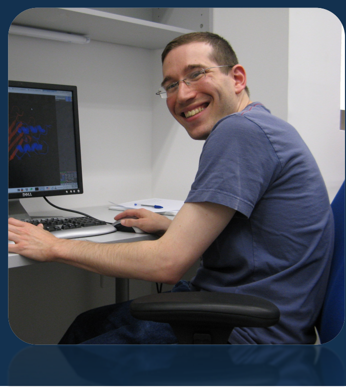
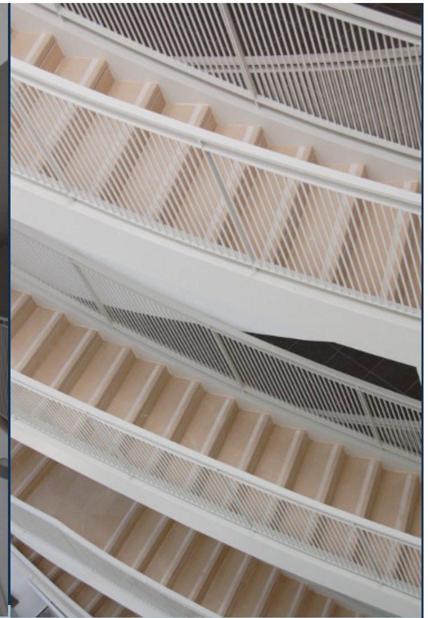
Bayer lab: **Michal Slutzki** & Yoav Barak





Funding

Excited about this work?

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

Furman Lab @ HU