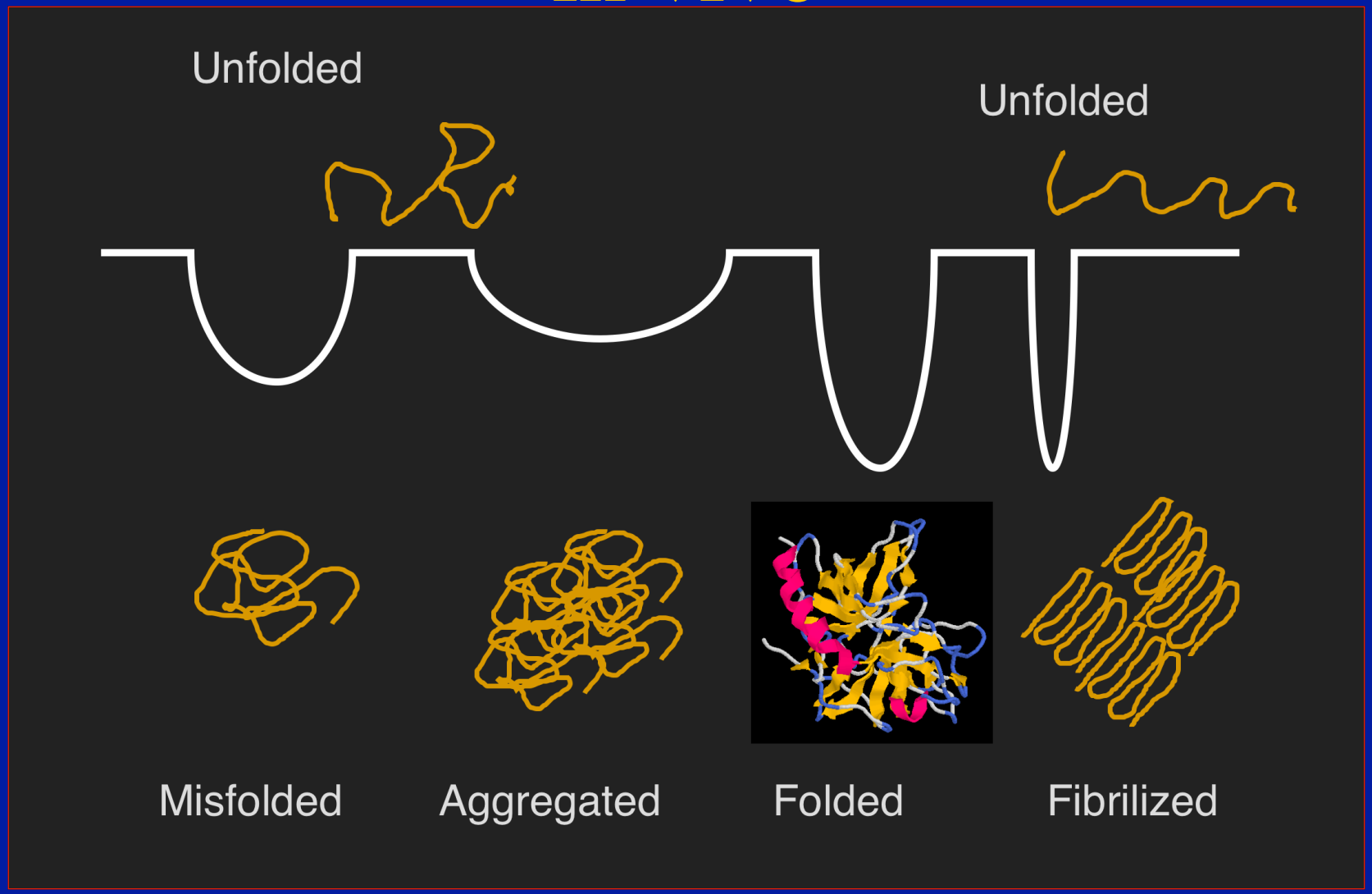
Hsp70 chaperones:
Mechanism
Disease
Inhibitors

Erik R.P. Zuiderweg

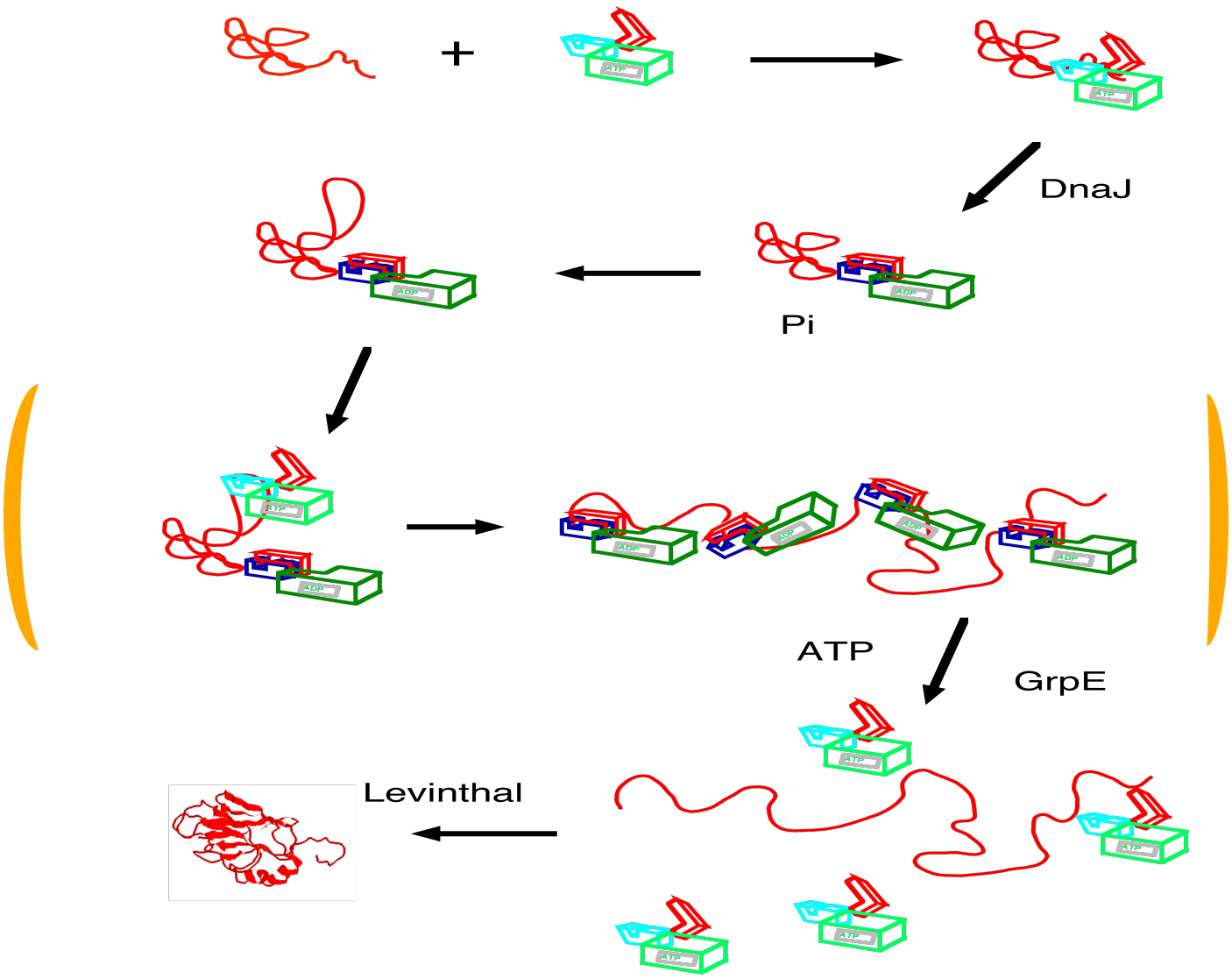
University of Michigan

NIH-funded NMR Research
1995-2011

Protein (mis)folding in vivo

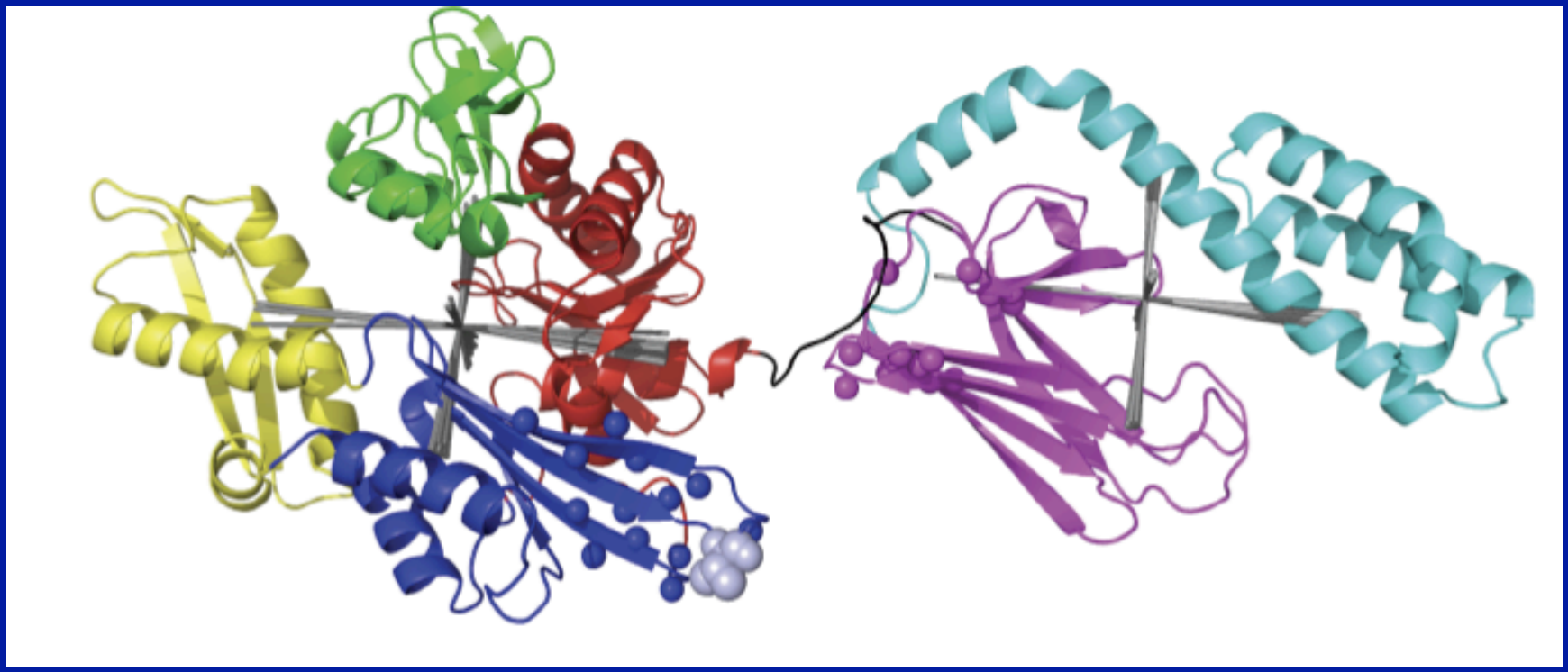


Annealing by the Hsp70 Chaperones



Solution– NMR structure

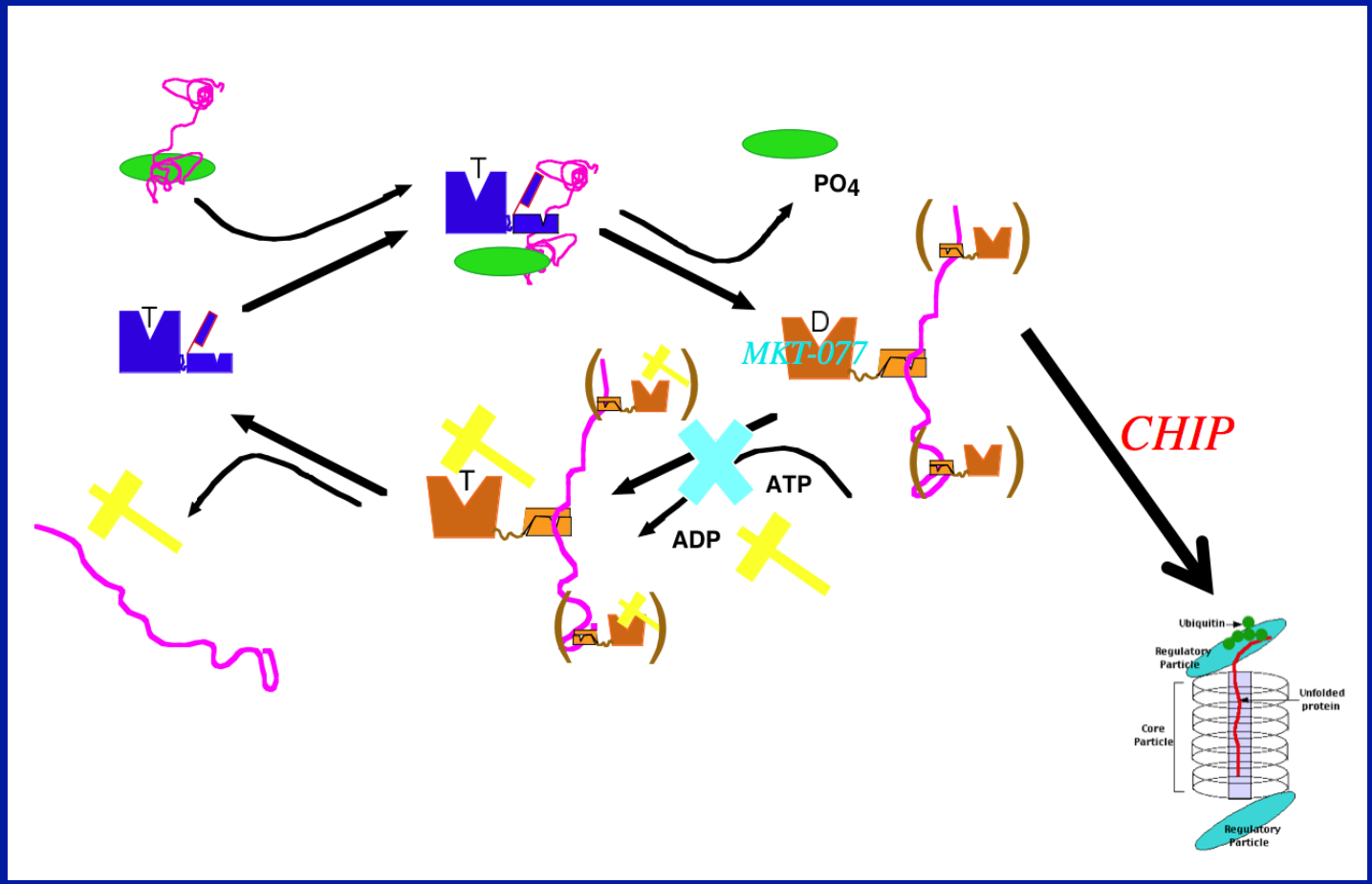
of Hsp70 E.coli in the ADP. Peptide state



RDC, spin-labeling, allignment computations

Bertelsen, .., Zuiderweg,

Proc. Natl. Acad. Sci. 106, 8471-8476 (2009)


T
PO4
T
D
MKT-077
CHIP
T
ATP
ADP
Ubiquitin
Regulatory Particle
Unfolded protein
Core Particle
Regulatory Particle

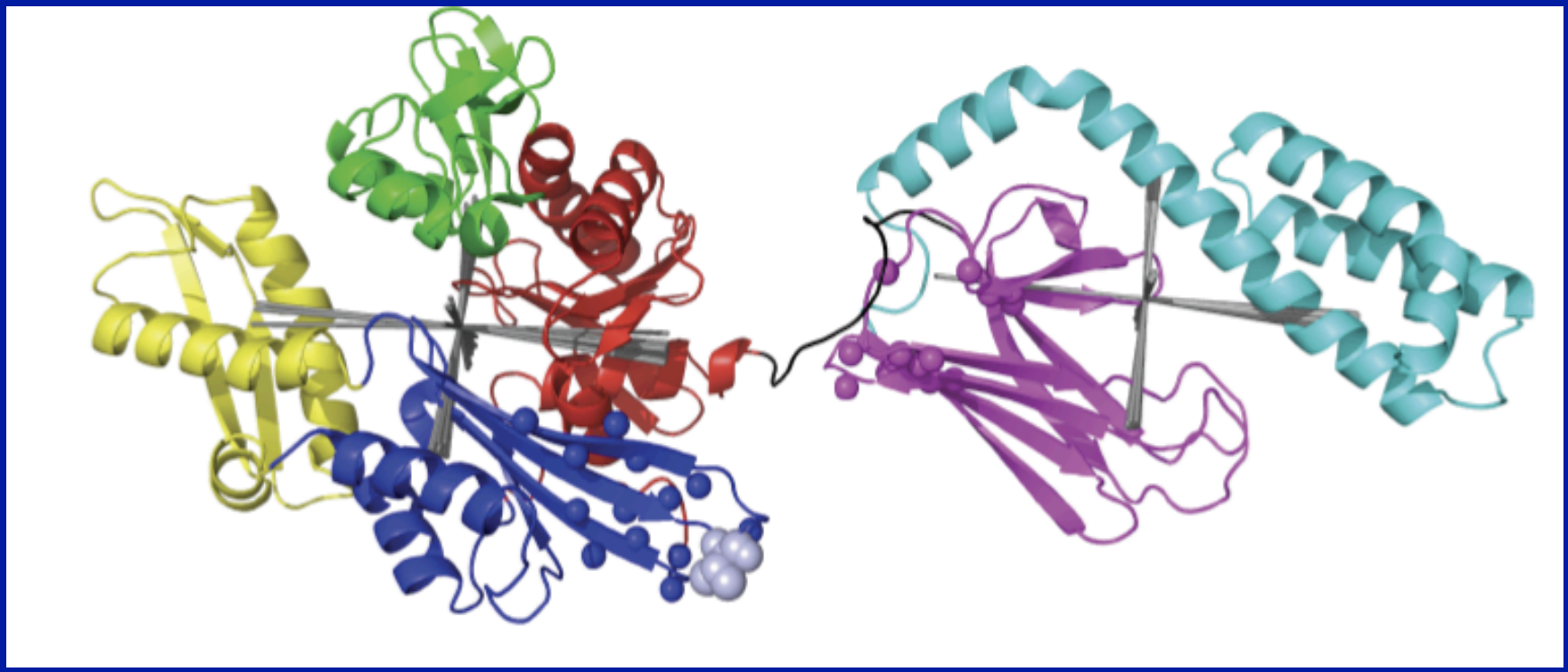
Hsp70 nomenclature

- Bacteria: DnaK (1 isoform)
- Yeast: SSA, SSB (4 isoforms)
- Human: HSPA1- 14, 11 isoforms

- HSPA8 (Hsc70); HSPA4(Bip);
  HSPA9 (mt) --- constitutive
- All others inducible with HSPA1 (Hsp71) most abundant

Solution– NMR structure

of Hsp70 E.coli in the ADP. Peptide state

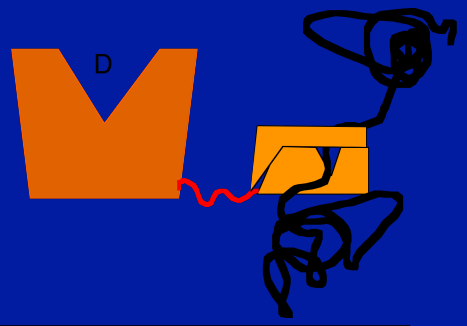


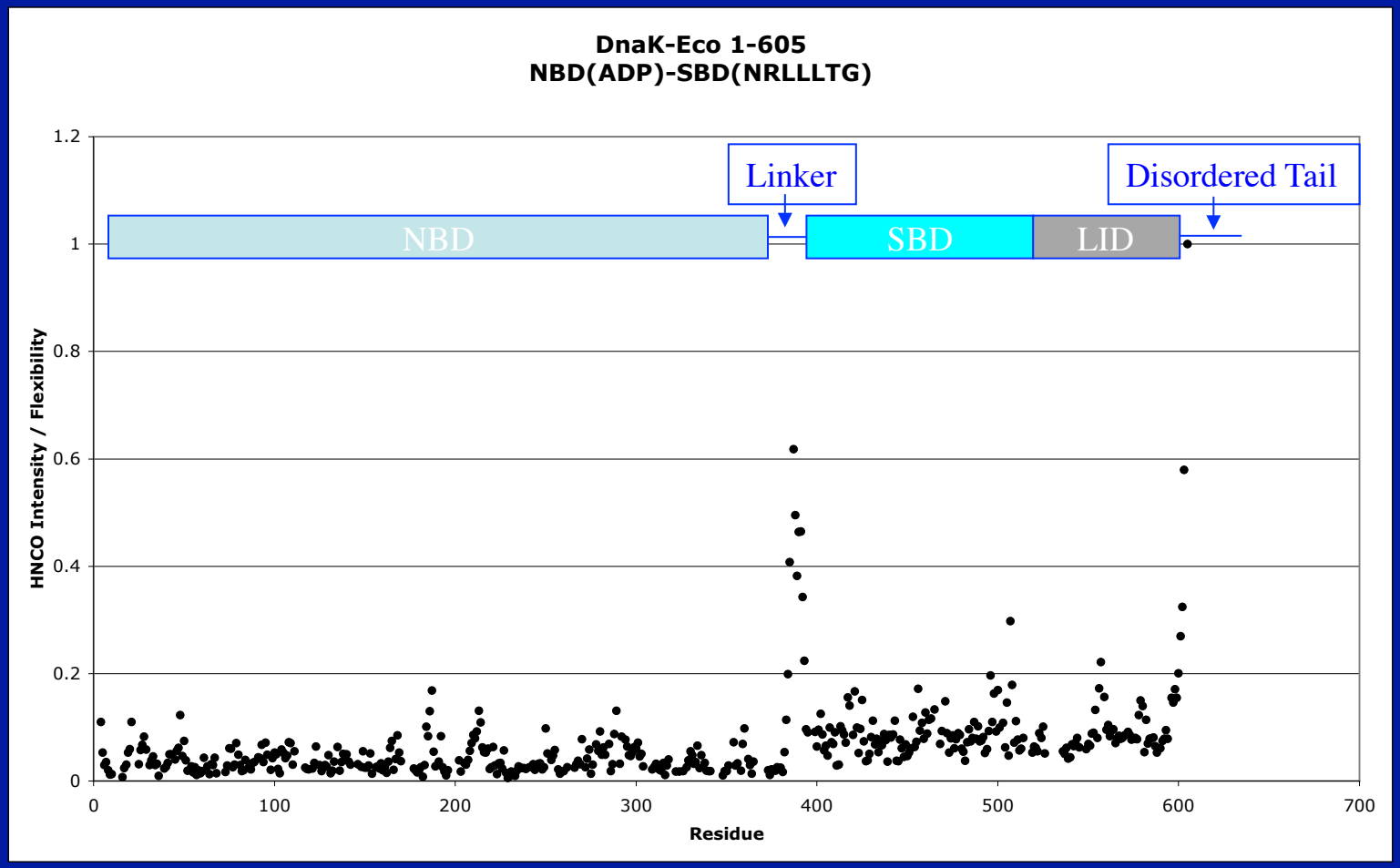
RDC, spin-labeling, allignment computations

Bertelsen, .., Zuiderweg,

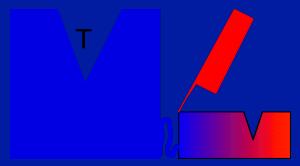
Proc. Natl. Acad. Sci. 106, 8471-8476 (2009)

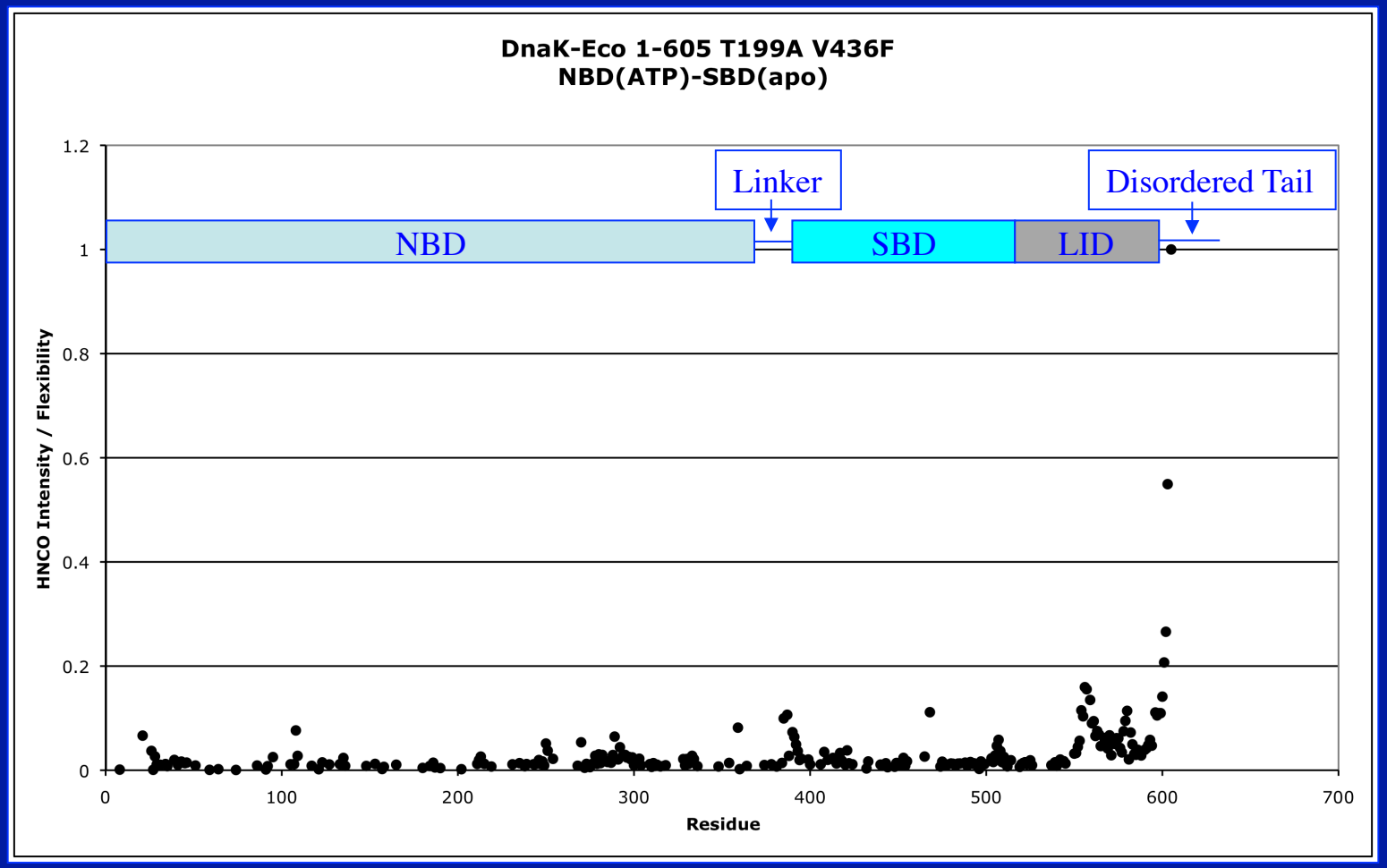
HNCO intensities show that in the ADP state, the linker is flexible, and that SBD and LID are docked, and that NBD and SBD are NOT docked.



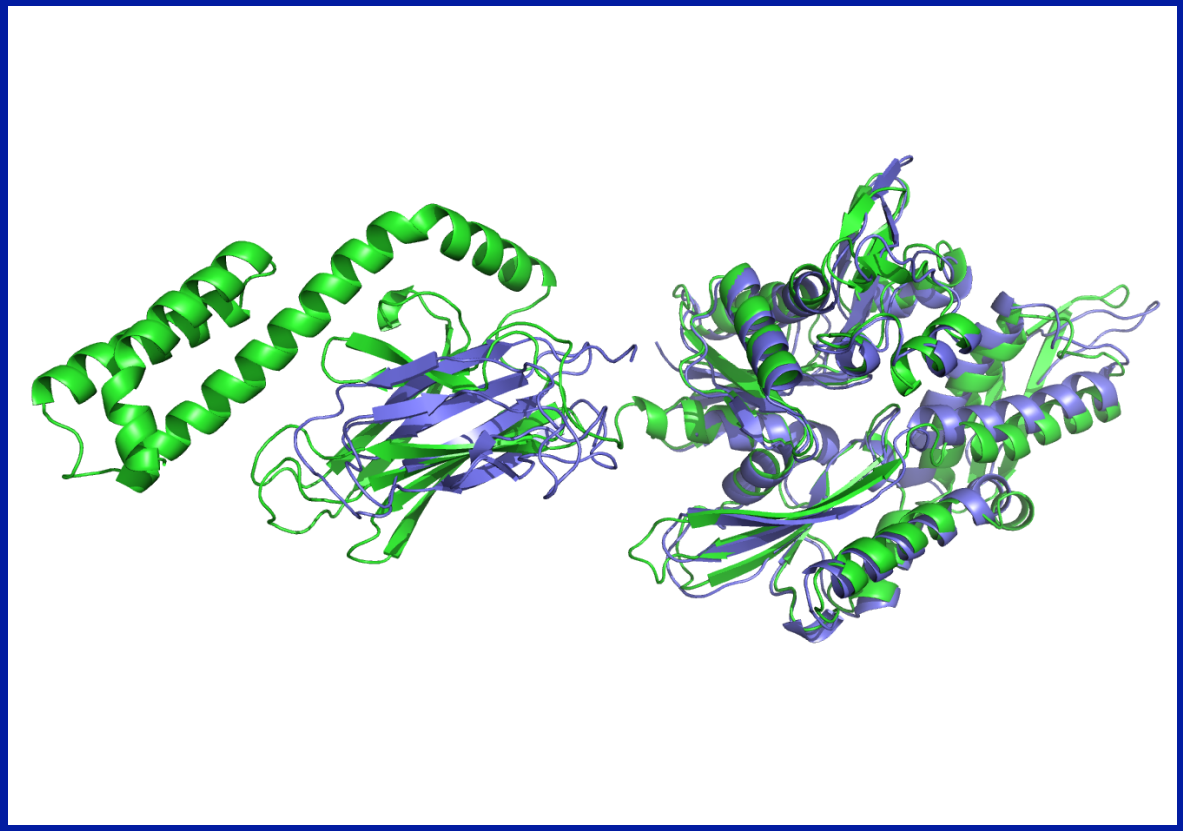


HNCO intensities show that in the ATP state, the linker is docked, and that SBD and LID are NOT docked, and that NBD and SBD are docked.





Correspondence:
Ecoli DnaK vs TTh DnaK in the ADP state (NMR)



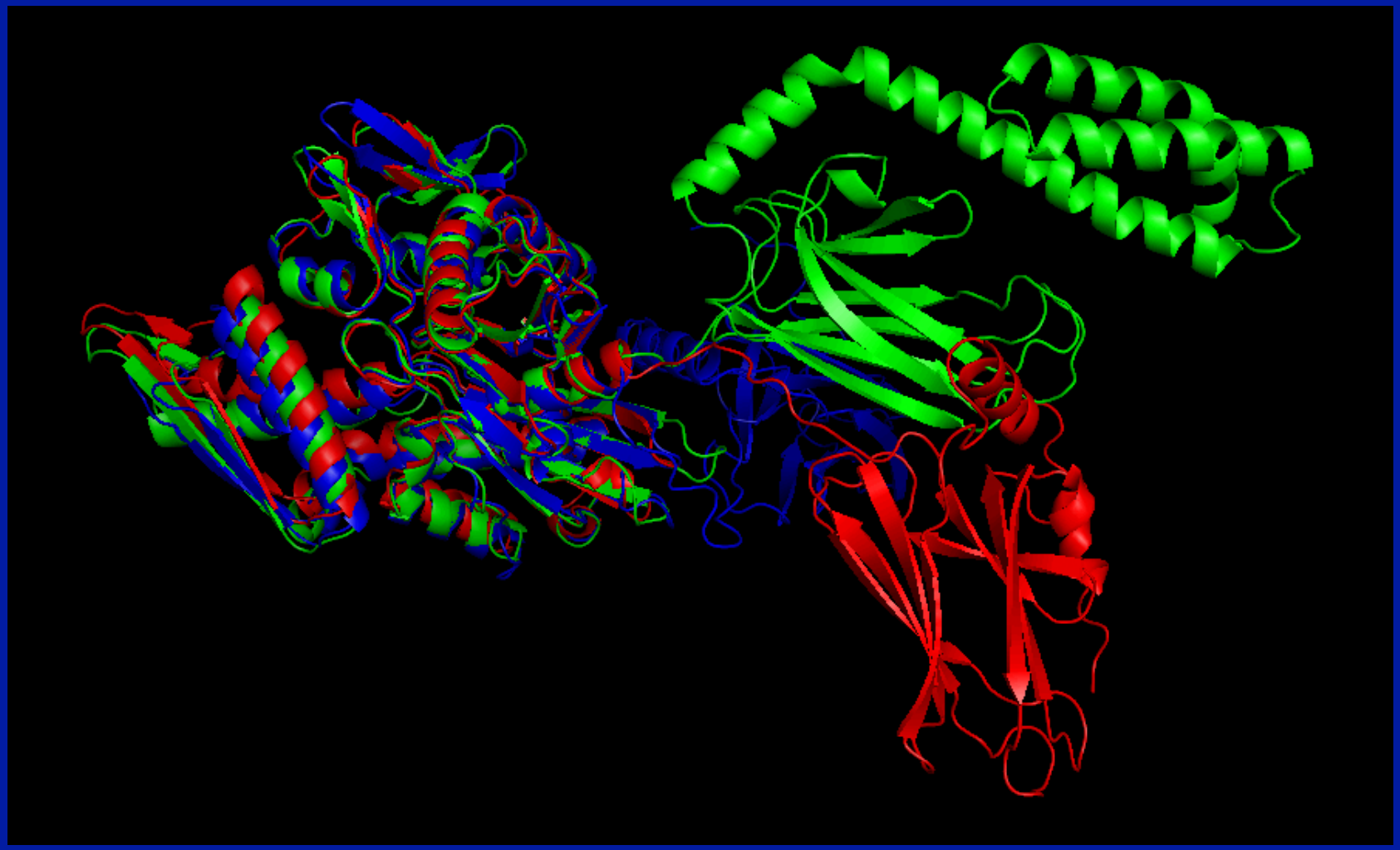
Revington, M., Zhang, Yip, G.N.B., Kurochkin, A.V. and Zuiderweg, E.R.P.

NMR investigations of allosteric processes in a two-domain Thermus thermophilus Hsp70 molecular chaperone

J. Mol. Biol. 349, 163-183 (2005)

No correpondence with crystal structures

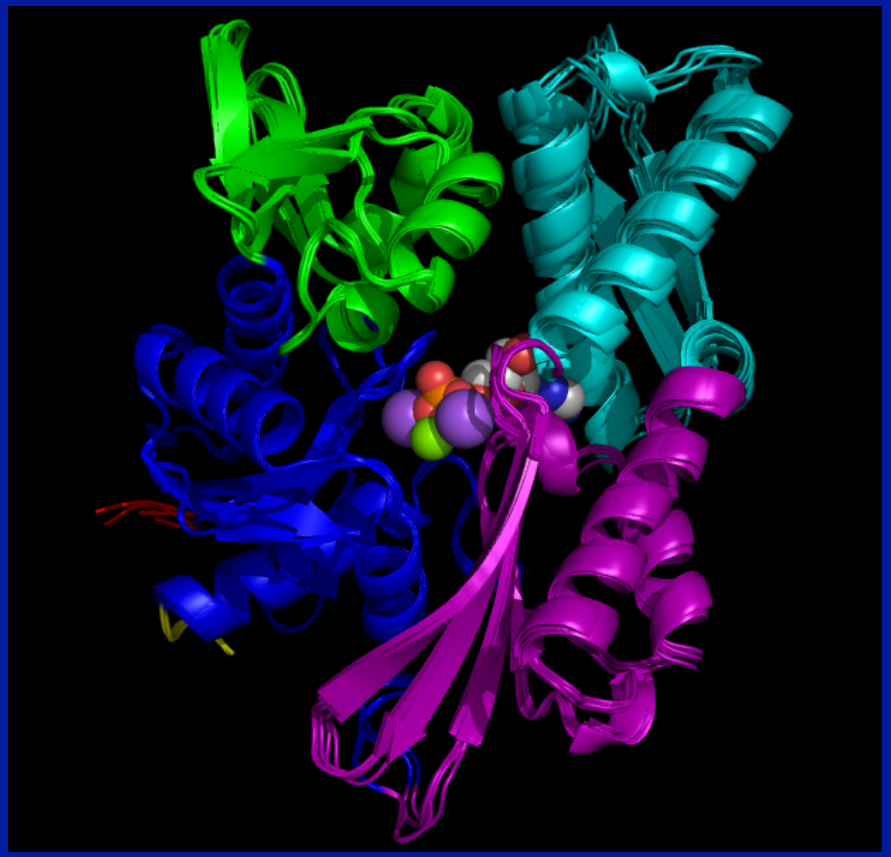
Hsp70 – E.coli ADP.Peptide in solution (PNAS 106, 8471)



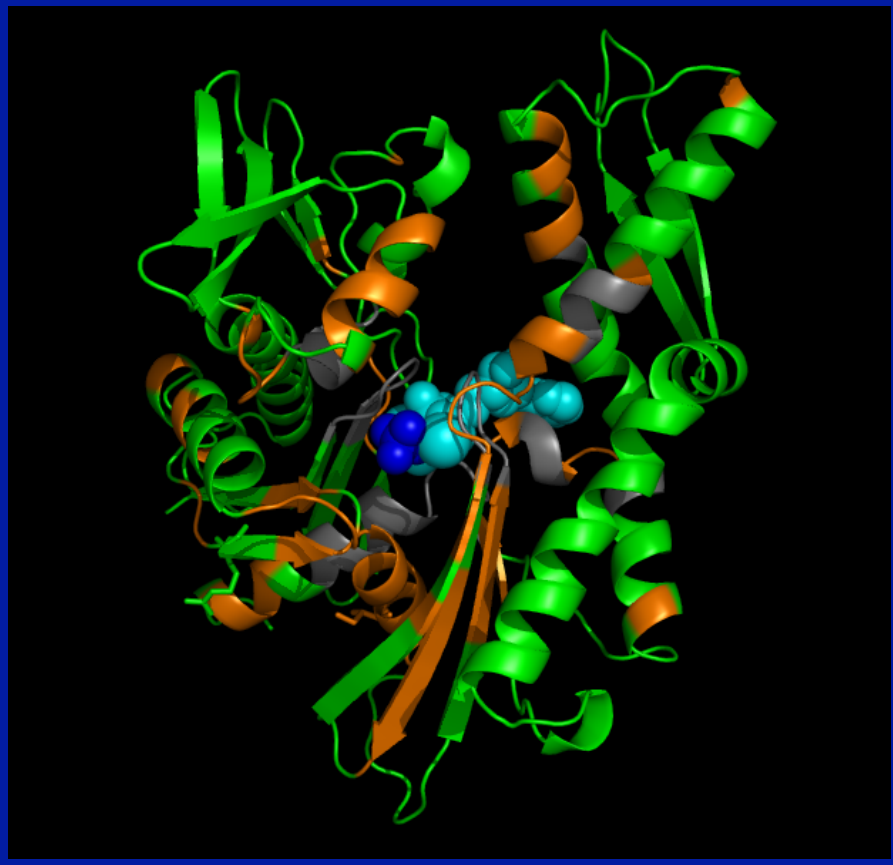
Hsp70 – G. kaustophilus in crystal (JBC 283, 15502)

Hsp70 – H. sapiens in crystal (MolCell 20, 513)

What about changes in the NBD between ADP and ATP state?



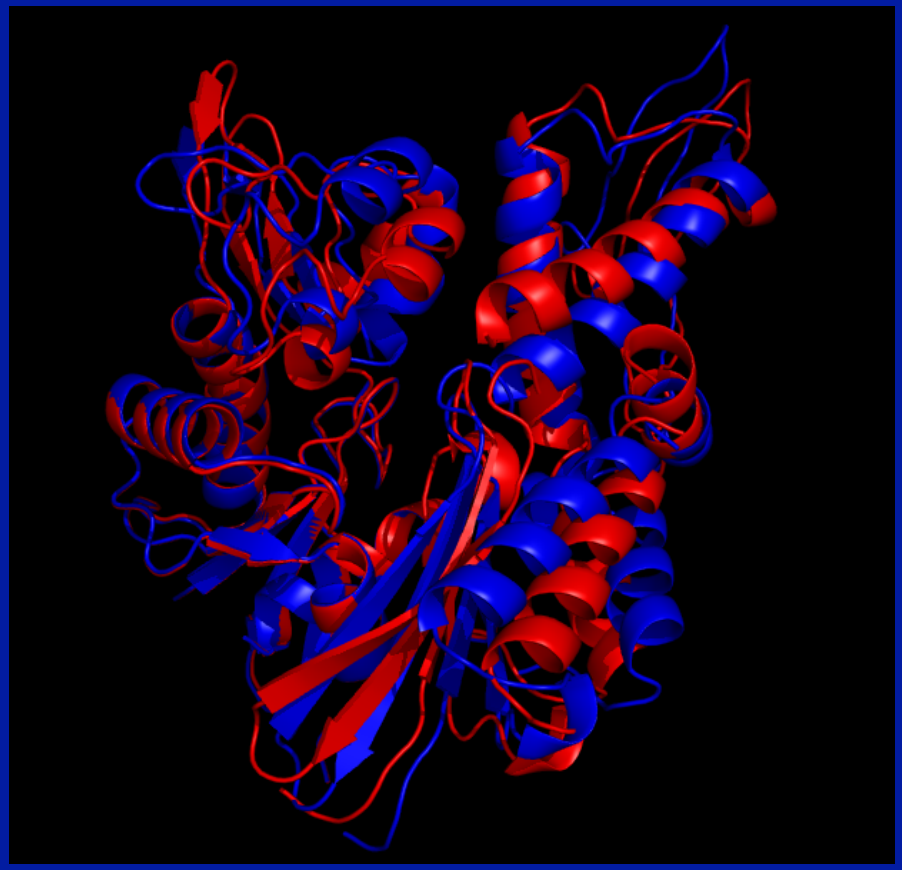
Hsc in crystal: no difference for ATP, ADP, AMPPNP (5 structures) (McKay, Sousa)



Hsc in solution ADP vs ATP: many shifts (Bhattacharya et al, JMB 2009)

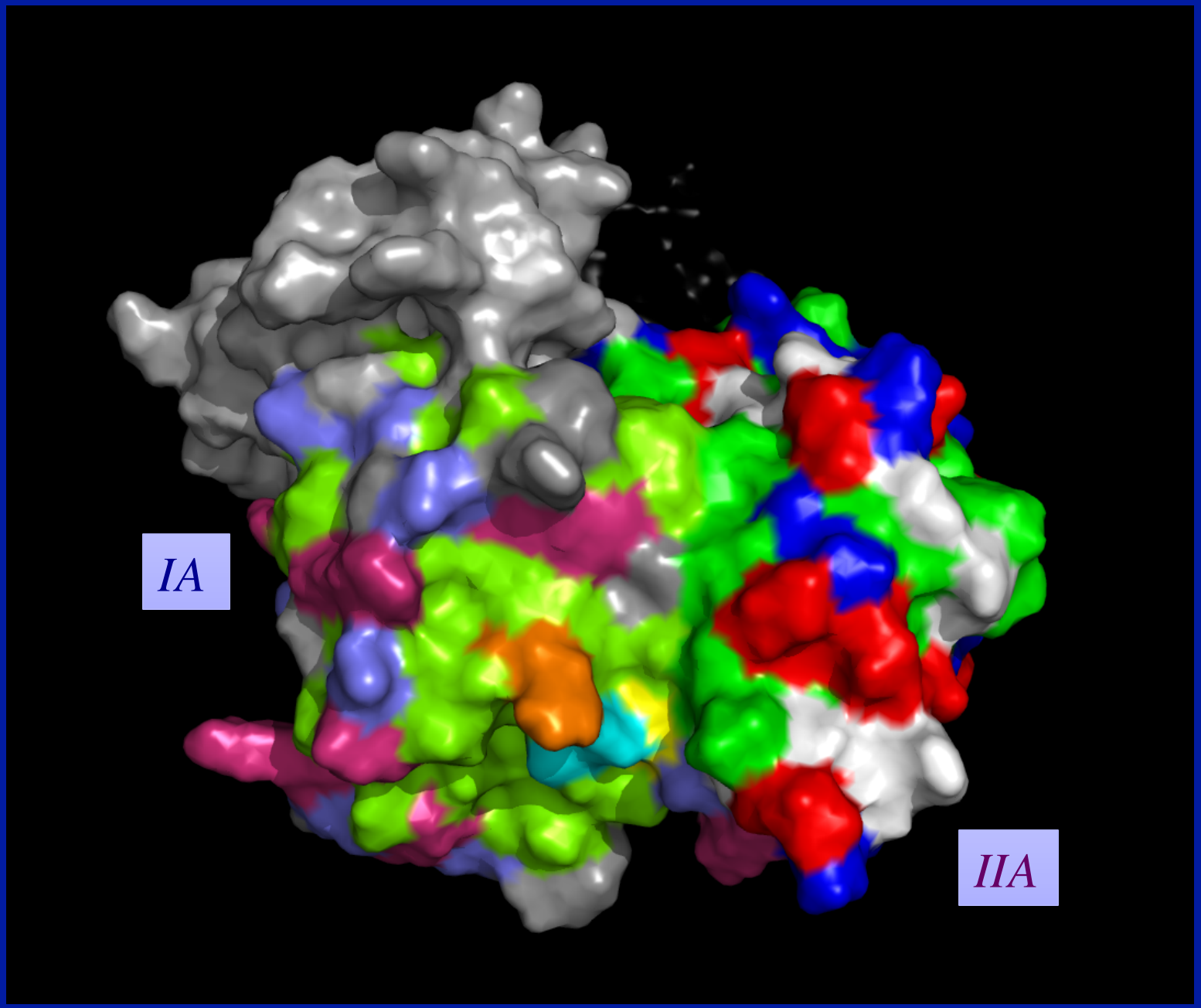
What about changes in the NBD between ADP and ATP state?

DnaK Tth in solution: many shifts

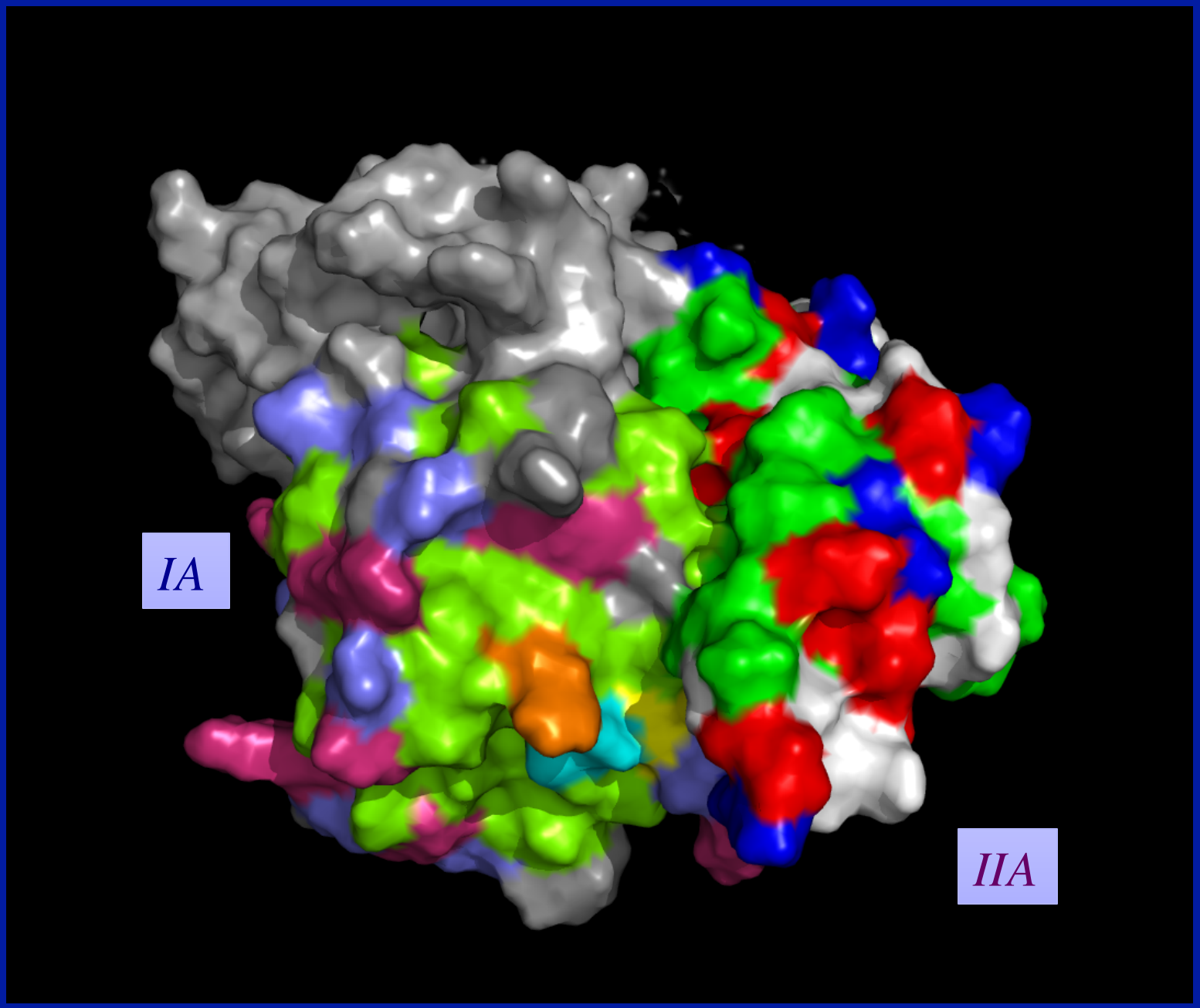


Conformational changes between ADP and AMPPNP (RDC NMR, Bhattacharya, A., …. , Zuiderweg, E. J. Mol. Biol. 388, 475-90 (2009).

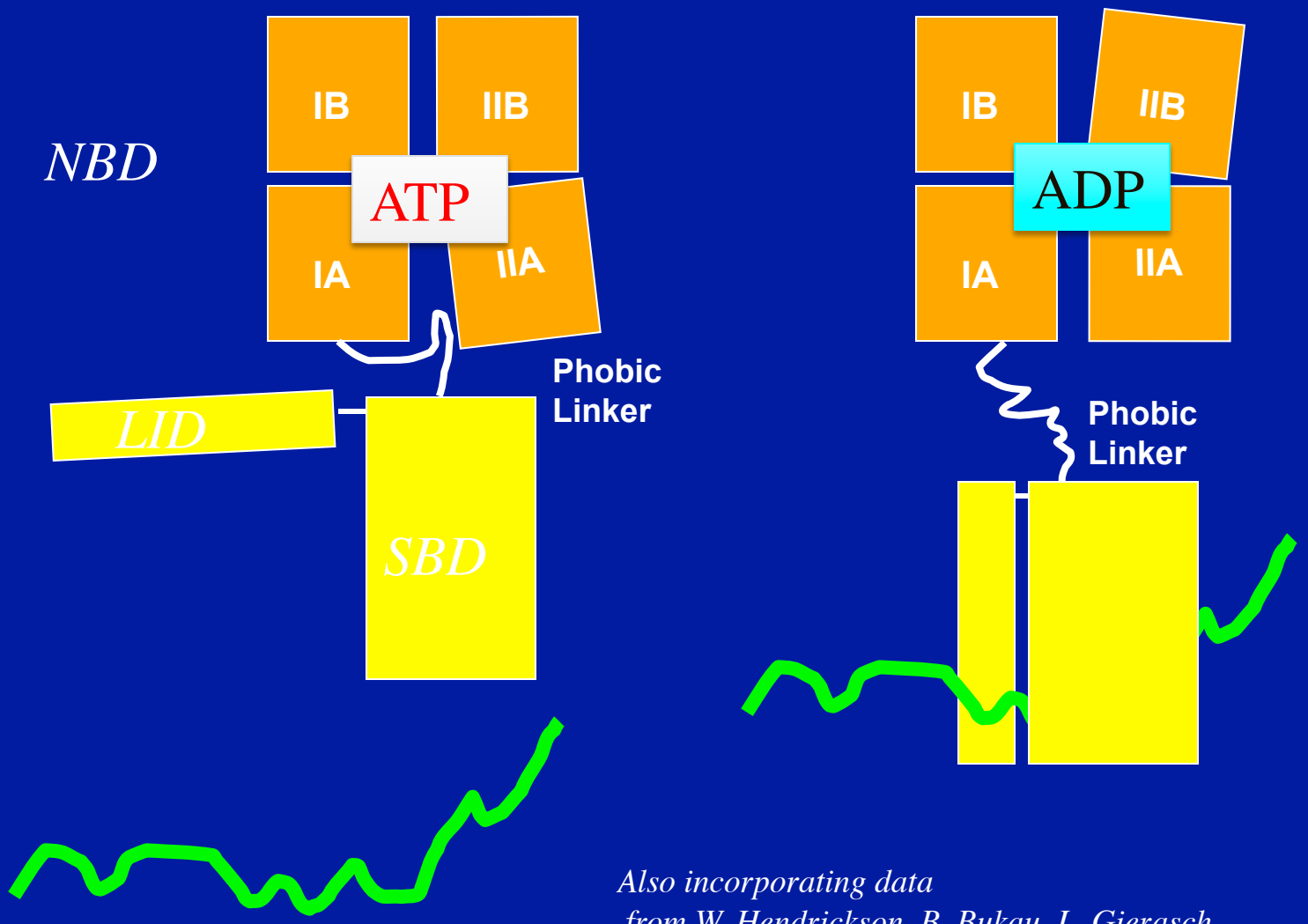
T. th.-DnaK bottom view showing IA/IIA interface



T. th.-DnaK bottom view showing IA/IIA interface



Summary:
Model of Allosteric Communication in Hsp70



Also incorporating data
from W. Hendrickson, B. Bukau, L. Gierasch

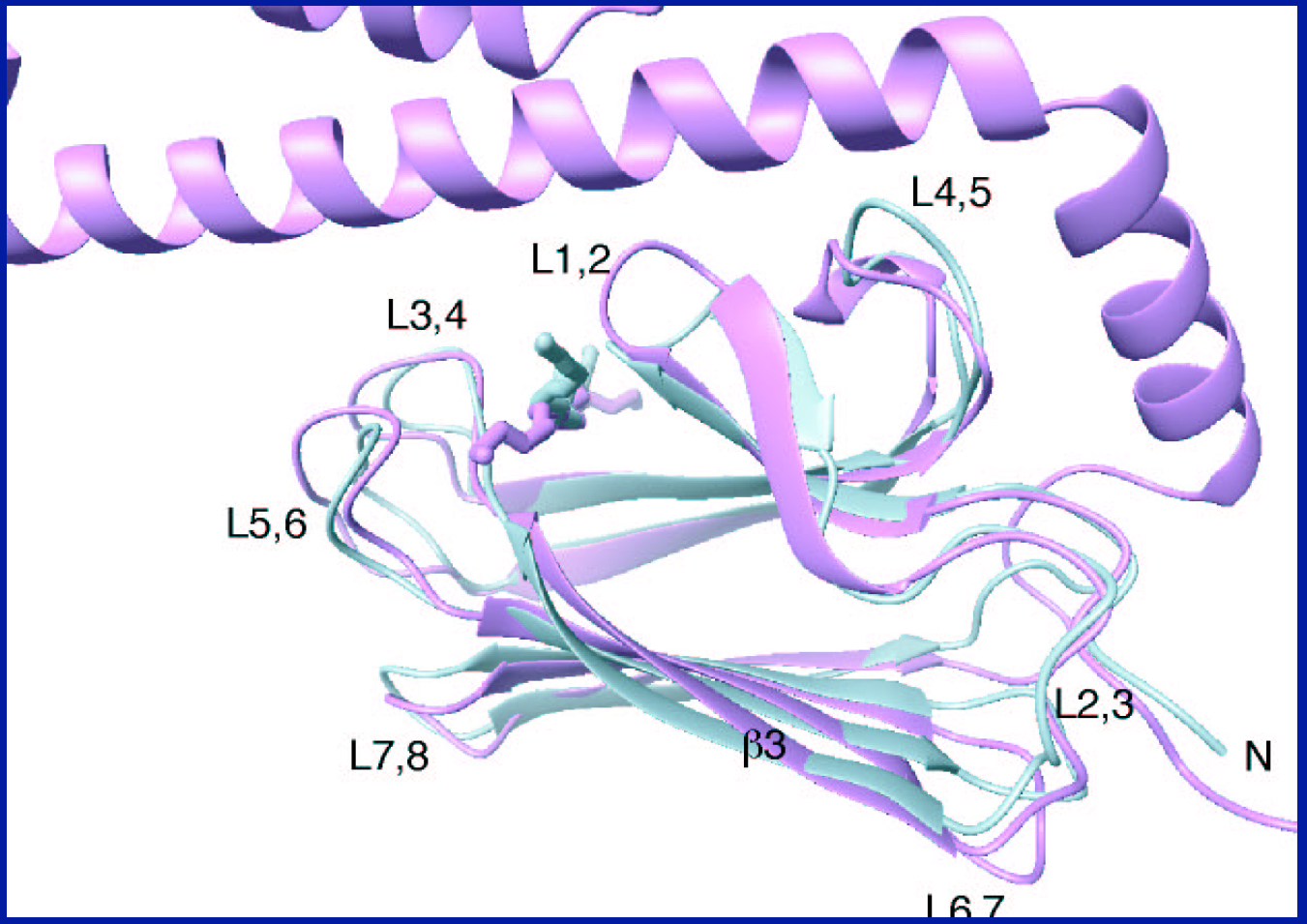
HSP70 CHAPERONES

ALLOSTERICS,

SBD side

DnaK-substrate-binding domain,

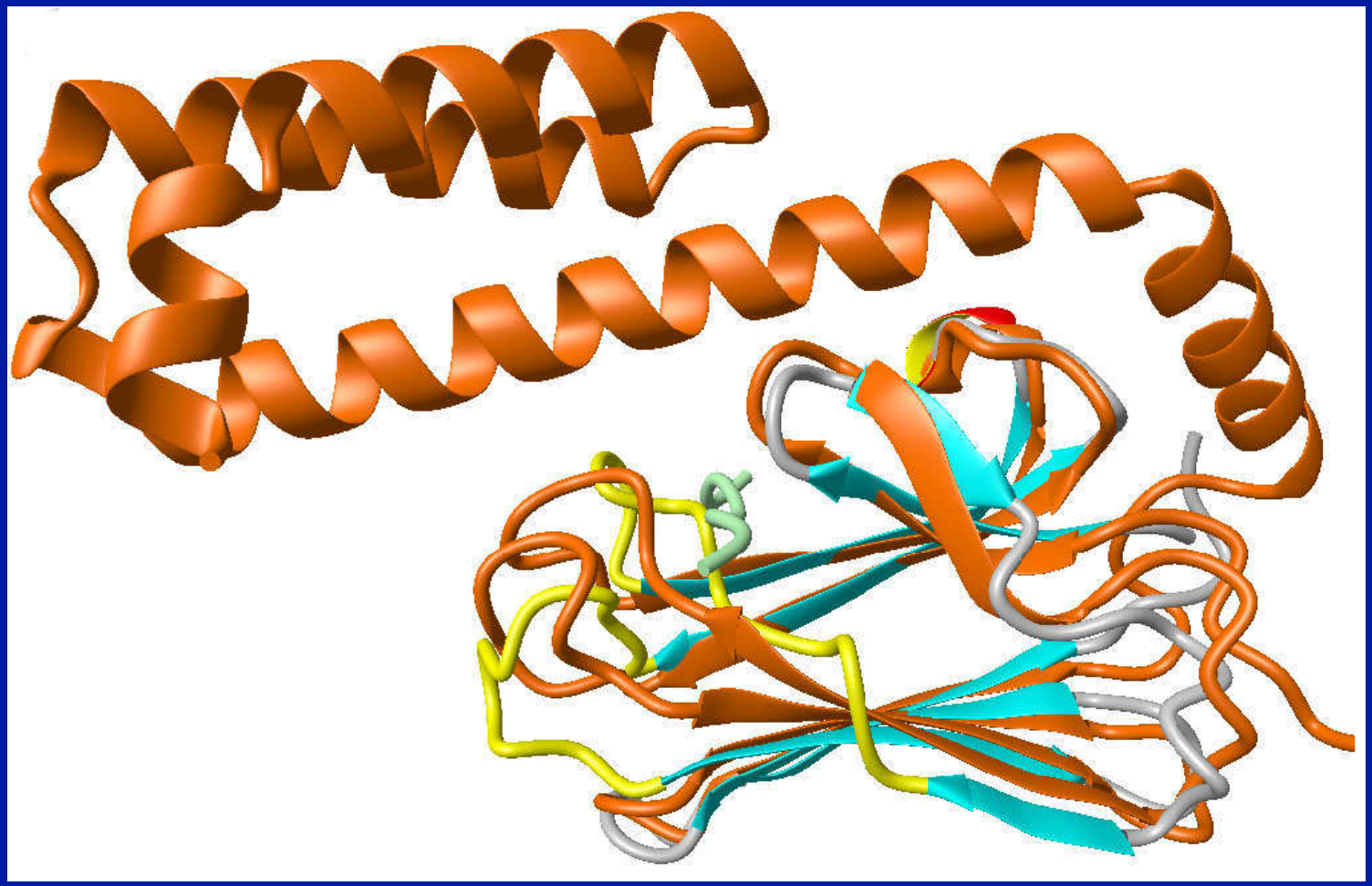
Crystal -NRLLLTG bound vs Solution -NRLLLTG bound



Stevens S.Y., Cai, S, Pellecchia, M. & Zuiderweg, E.R.P. (2003). The solution structure of the bacterial HSP70 chaperone protein domain DnaK(393-507) in complex with the peptide NRLLLTG. *Protein Sci*. **12**, 2588-2596.

DnaK-substrate-binding domain,

Crystal-NRLLLTG bound vs Solution apo



Pellecchia, M., Stevens, S.Y., Vander Kooi, C.W., Montgomery, D.H., Feng, E.H., Gierasch, L.M., and Zuiderweg, E.R.P.. Nature Structural Biology,7, 298- 303 (2000)

The LID is not needed for allostery

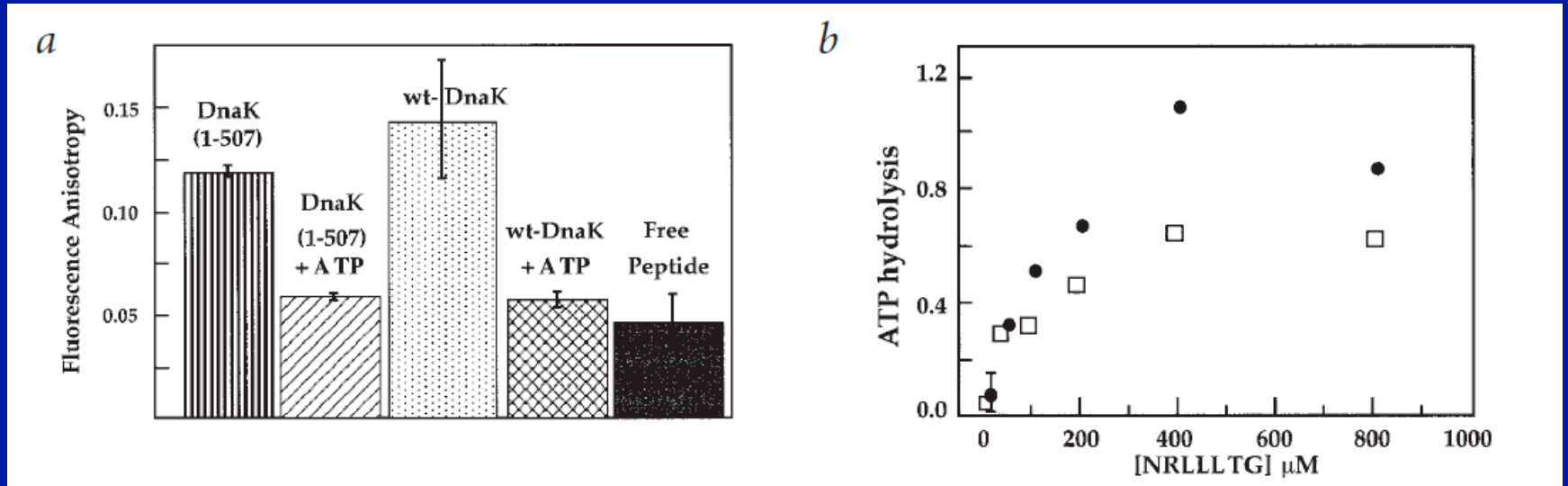


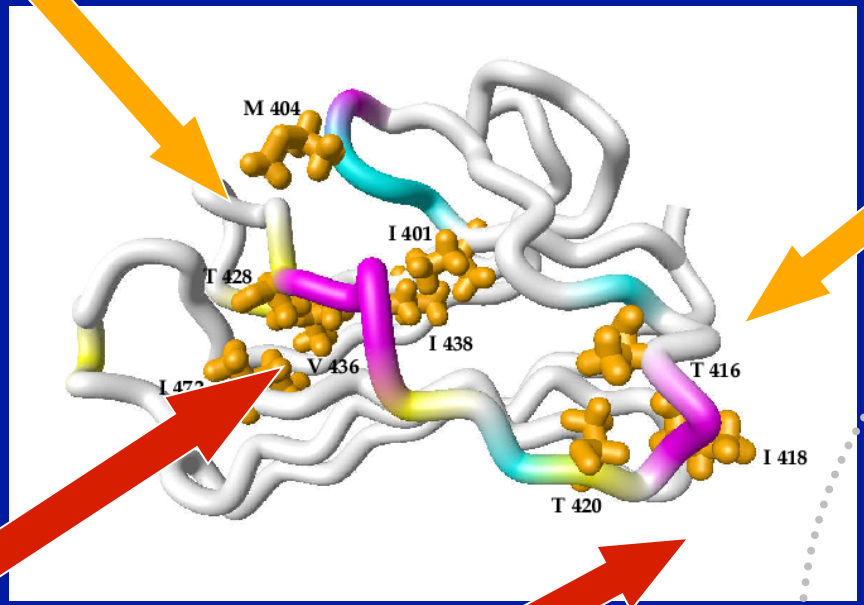
Fig. 1. *In vitro* studies of DnaK(1–507) allosteric function. ***a***, ATP-induced release of peptide F-APPY in DnaK(1–507) measured by fluorescence anisotropy. The first bar represents the anisotropy value for peptide bound to 1.1 μM DnaK(1–507). The second bar represents the anisotropy value 5 min after addition of 0.44 mM ATP. The third and fourth bars represent the values for wtDnaK under comparable conditions, and the last bar indicates the anisotropy value of free peptide. Error bars reflect the standard deviation from a mean of three measurements. ***b***, Peptide stimulation of ATPase activity of DnaK(1–507) (●) and wtDnaK (□). As DnaK(1–507) is titrated with the peptide NRLLLTG, the ATPase activity is stimulated in a manner similar to that of wtDnaK. The hydrolysis rate is reported as moles of ATP hydrolyzed per minute per mole of DnaK(1–507) or wtDnaK. The error bar on the first point reflects the standard deviation from a mean of three measurements and is valid for both assays.

Pellecchia, M., Stevens, S.Y., Vander Kooi, C.W., Montgomery, D.H., Feng, E.H., Gierasch, L.M., and Zuiderweg, E.R.P.
Structural insights into substrate binding by the molecular chaperone DnaK.
Nature Structural Biology,7, 298- 303 (2000)

Allosteric Lever

Binding substrate here

Affects conformation here

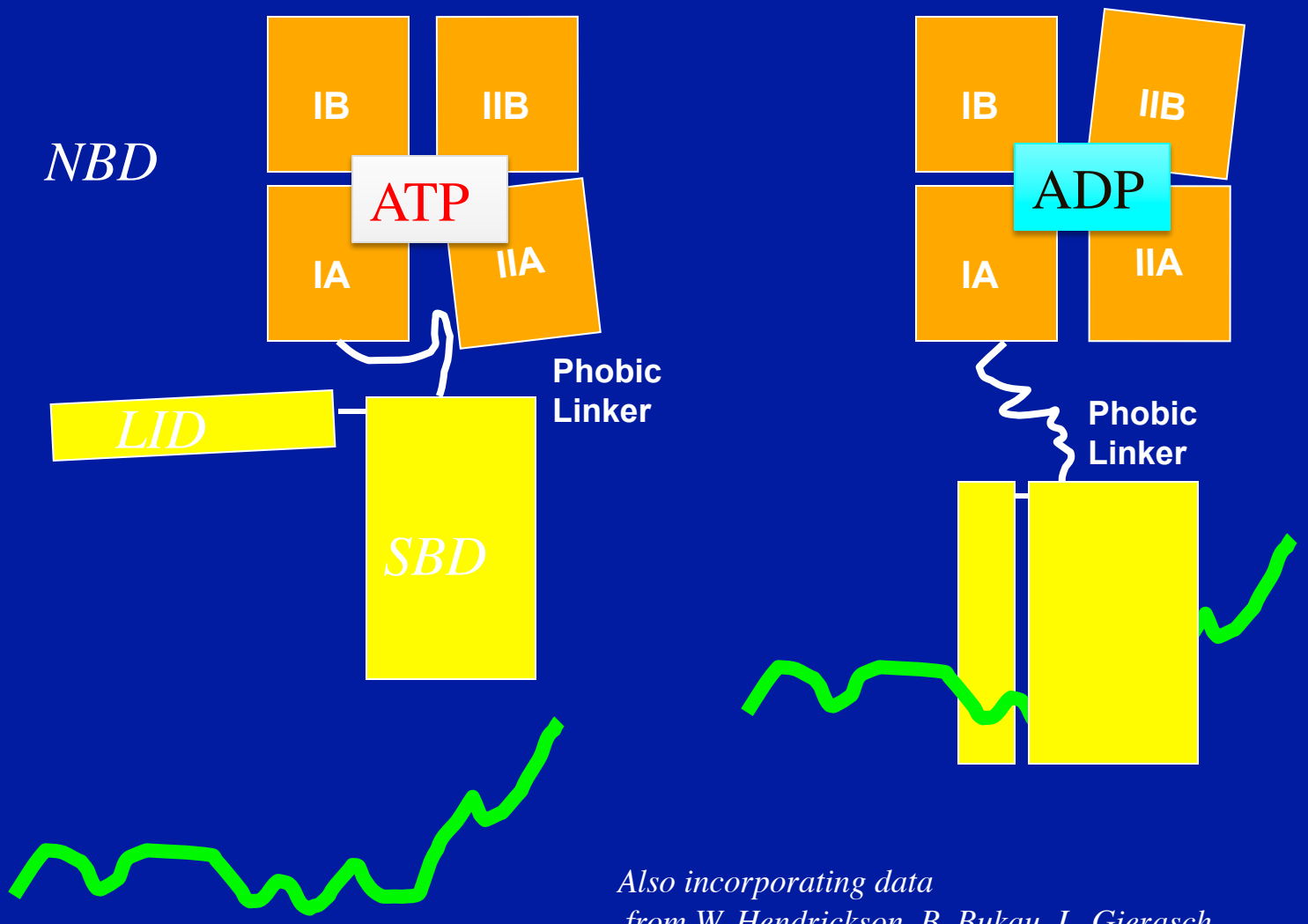


Should affect substrate binding here

Thus ATP-domain induced changes here

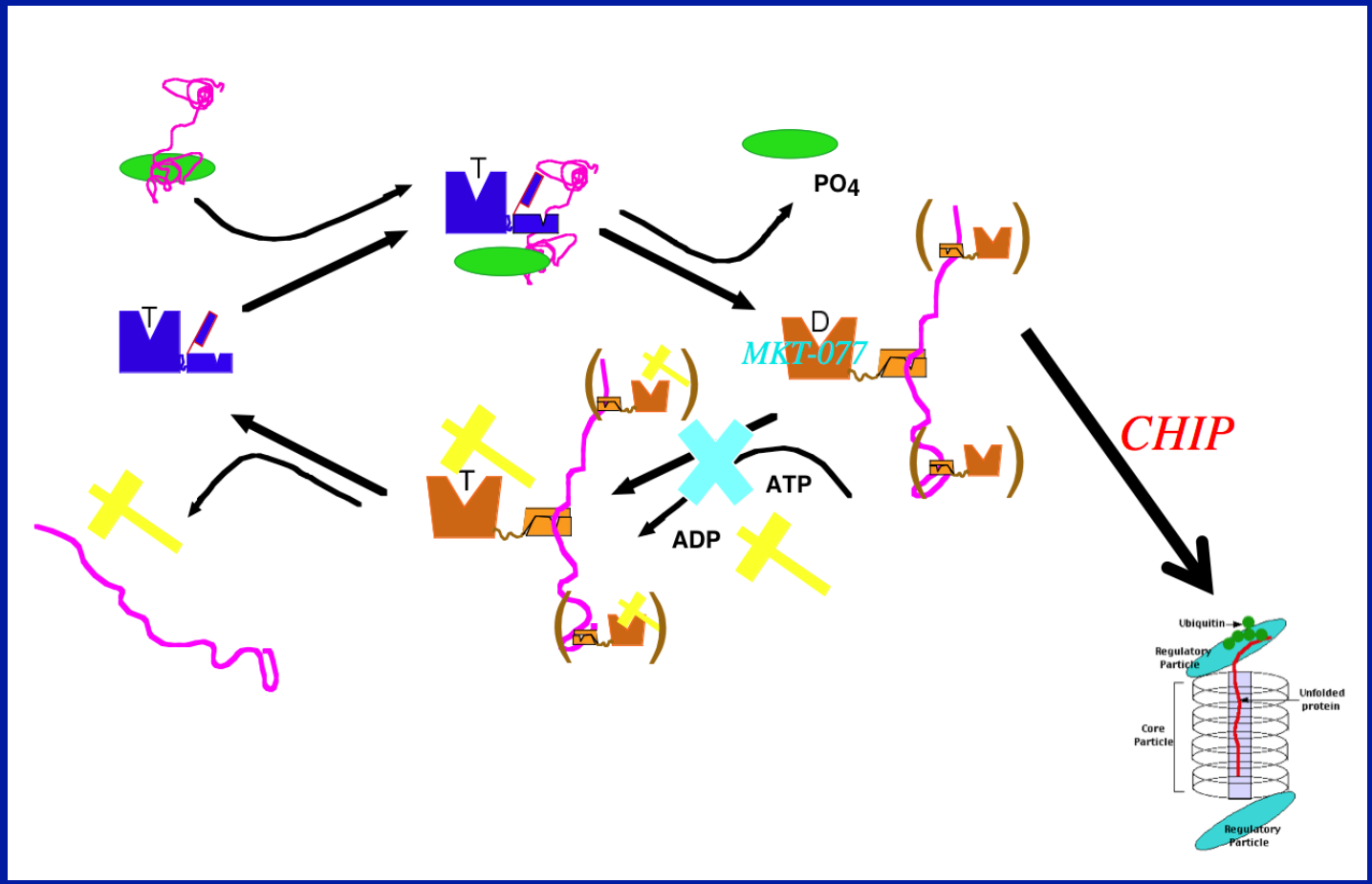
ATP domain

Summary:
Model of Allosteric Communication in Hsp70

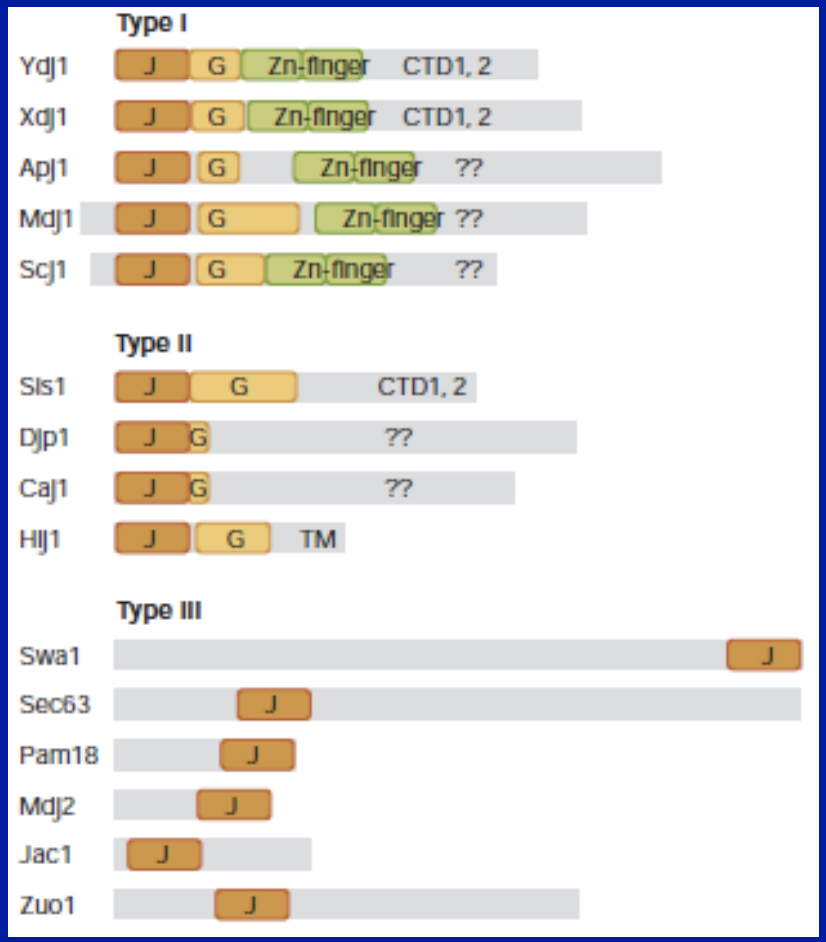


Also incorporating data
from W. Hendrickson, B. Bukau, L. Gierasch

DnaJ – Hsp70 Interactions

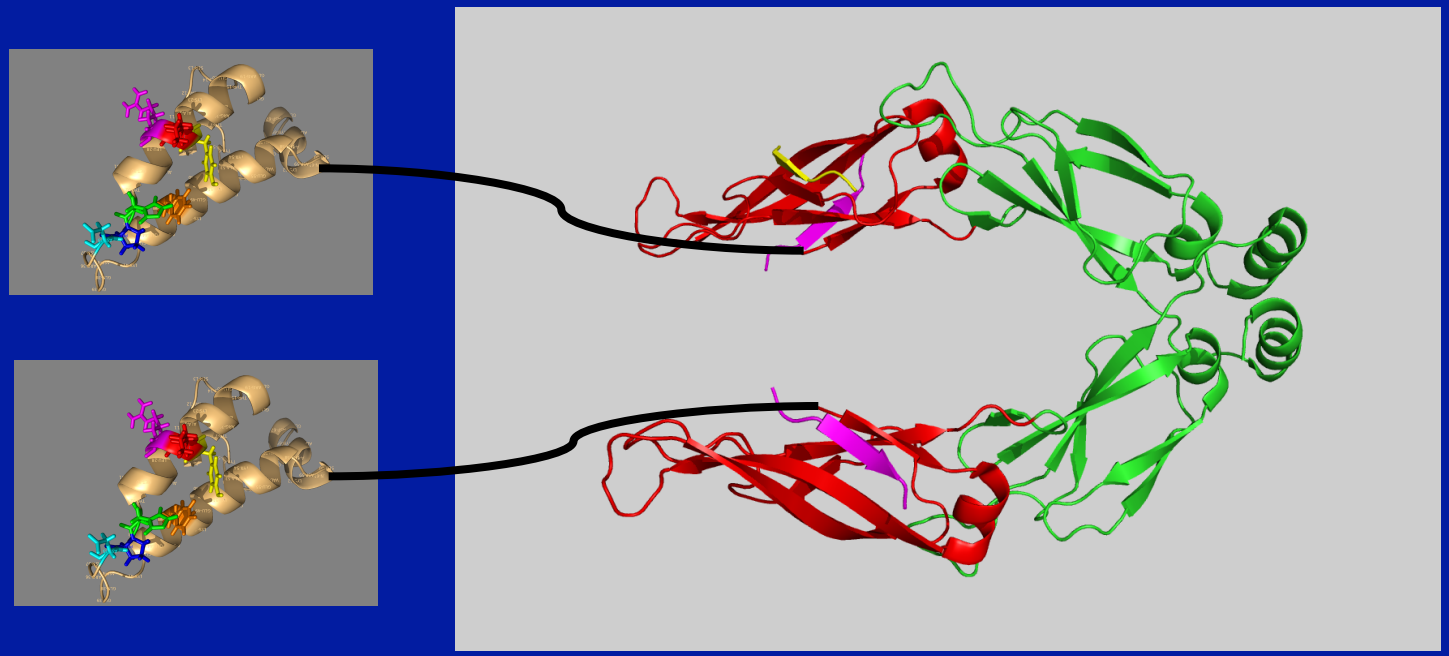

T
PO4
T
D
MKT-077
CHIP
T
ATP
ADP
Ubiquitin
Regulatory Particle
Unfolded protein
Core Particle
Regulatory Particle

DnaJ topologies



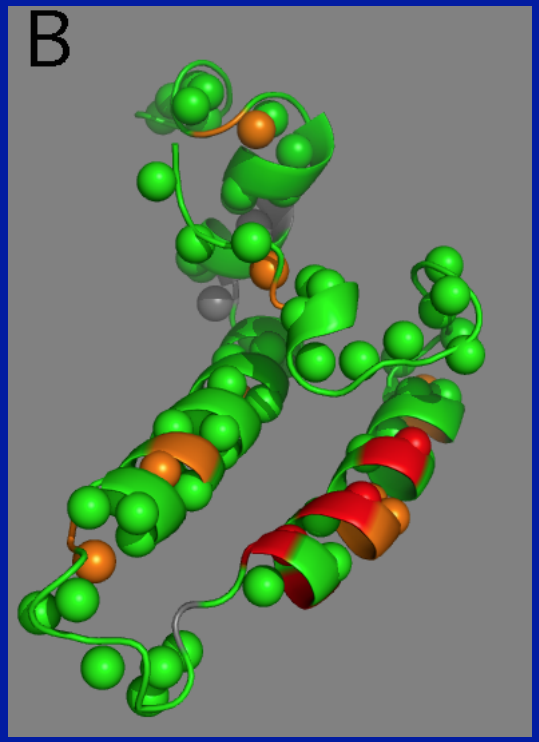
EMBO reports (2004) ***5,*** *567*

Hsp40 – DnaJ --- YDJ --- HDJ

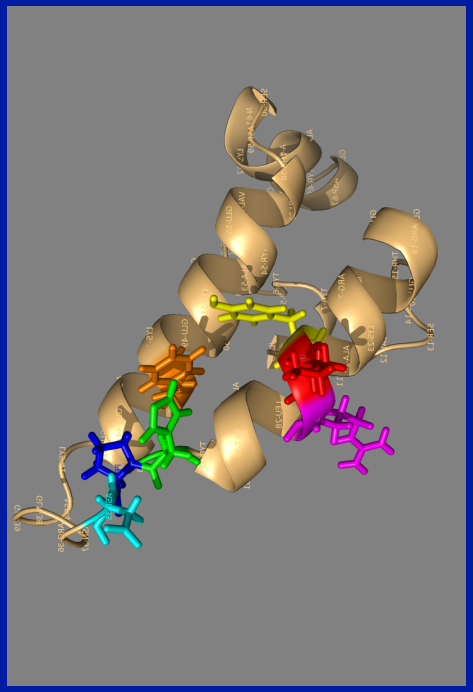


Pellecchia, M., Szyperski, T., Wall, D., Georgopoulos, C. & Wuthrich, K. (1996). NMR structure of the J-domain and the Gly/Phe-rich region of the Escherichia coli DnaJ chaperone. J. Mol. Biol. ***260****, 236-250.*

Suzuki, H., Noguchi, S., Arakawa, H., Tokida, T., Hashimoto, M. & Satow, Y. (2010). Peptide-Binding Sites As Revealed by the Crystal Structures of the Human Hsp40 Hdj1 C-Terminal Domain in Complex with the Octapeptide from Human Hsp70. Biochemistry ***49****, 8577-8584.*



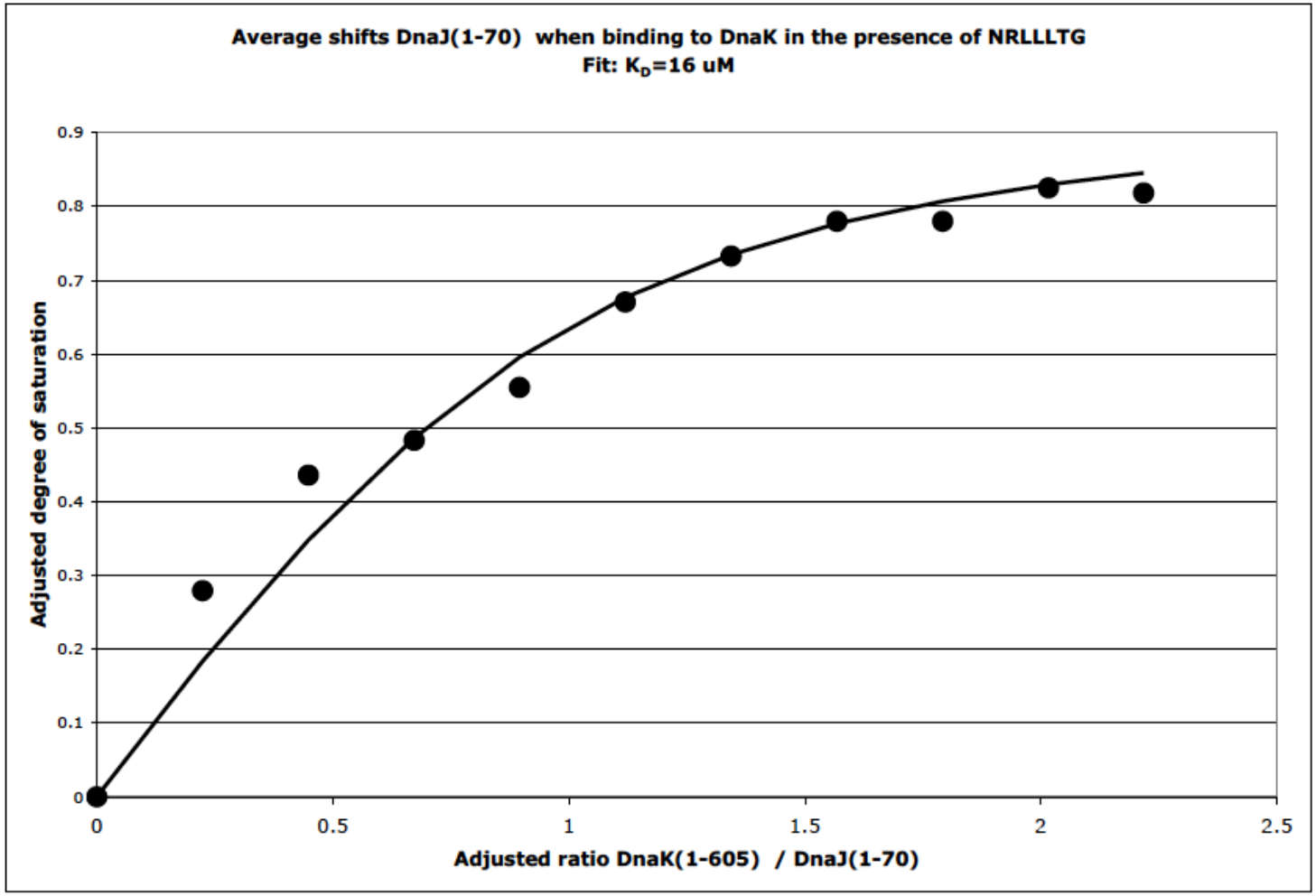
Chemical shifts in DnaJ when binding DnaK



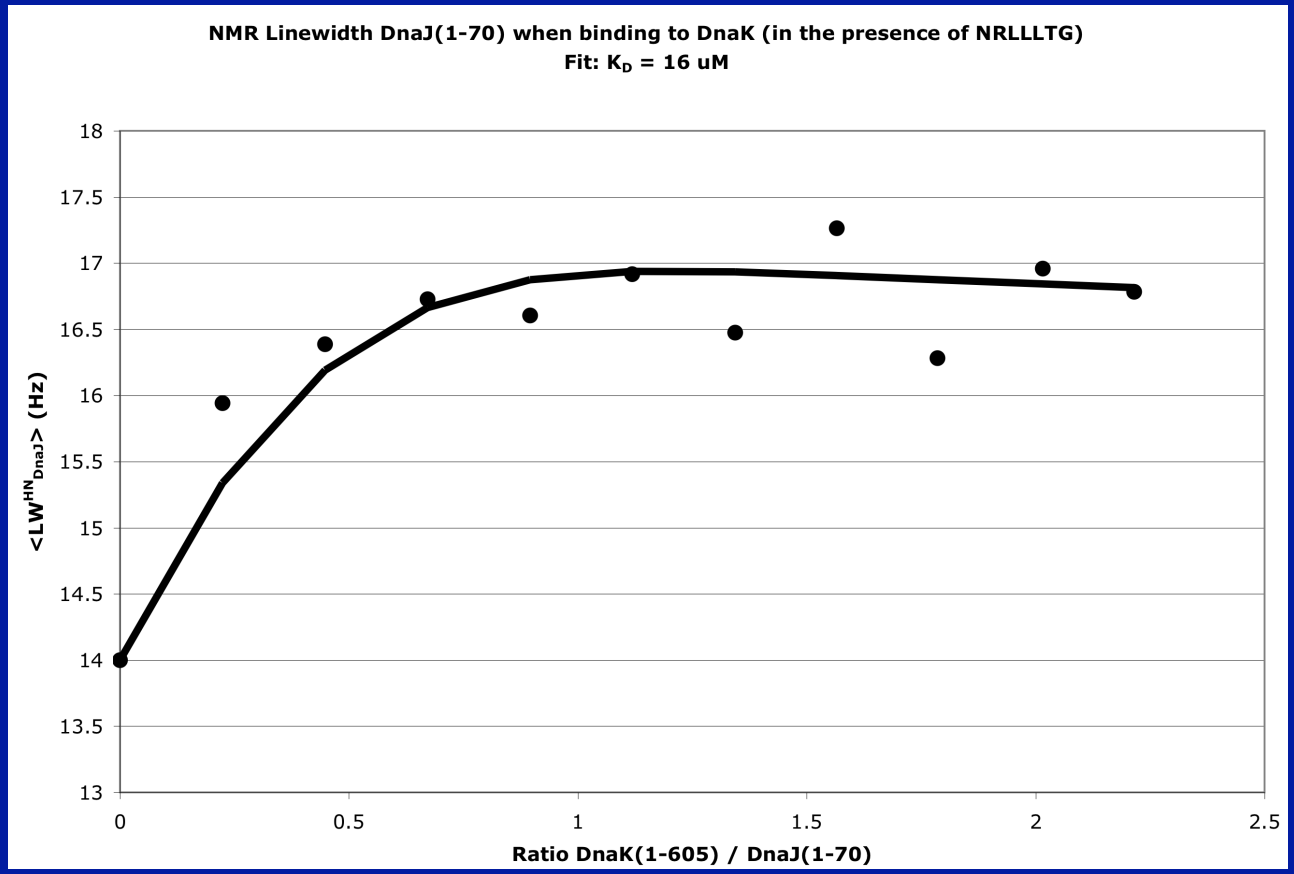
Mutagenesis-sensitive residues

Heat shock protein 70 kDa chaperone/DnaJ cochaperone complex employs an unusual dynamic interface.
Ahmad A, Bhattacharya A, McDonald RA, Cordes M, Ellington B, Bertelsen EB, ***Zuiderweg*** *ER.*
Proc Natl Acad Sci U S A. 108 18966-18971 (2011)

Binding saturates with 1:1 stoichiometry and 16 uM KD



BUT THERE IS HARDLY ANY LINE BOADENING
When the 8 kDa DnaJ is bound to the 60 kDa DnaK!



The heavy line is a fit with K_D = 16 uM and allowing for chemical exchange broadening in the fast regime due to a k_{off} of 14 s^{-1}.

Using ^{15}N relaxation data to find the τ_c and S^2 of the 8 kDa DnaJ bound to the 60 kDa DnaK.

Table 4. Fitting ^{15}N relaxation data of DnaJ(1-70) free and bound to DnaK

	τ_c	$\langle R_1 \rangle_{exp}$	$\langle R_1 \rangle_{fit}$	$\langle R_2 \rangle_{exp}$	$\langle R_2 \rangle_{fit}$	$\langle R_{ex} \rangle_{fit}$	$\langle S^2 \rangle_{fit}$	$\langle \tau_e \rangle_{fit}$
	ns	(s^{-1})	(s^{-1})	(s^{-1})	(s^{-1})	(s^{-1})		(ns)
J-70 free	5.2[a]	1.39	1.39	10.68	10.68	2.7	0.80	-
J-70 73 % bound	7.0[a]	1.123	1.123	24.06	24.06	13.3	0.82	-
J-70 100% bound	8.0[a]	1.02	1.02	28.98	28.98	16	0.85	-
J-70 100% bound	12.0[b]	1.02	1.02	28.98	28.98	7.22	1.00	0.89
J-70 100% bound	16.0[b]	1.02	1.02	28.98	28.99	7.44	0.71	2.86
J-70 100% bound	20.0[b]	1.02	1.02	28.98	29.01	8.88	0.51	3.00
J-70 100% bound	24.0[b]	1.02	1.02	28.98	28.98	7.08	0.45	3.53
J-70 100% bound	**28.0**[b]	**1.02**	**1.02**	**28.98**	**28.98**	**6.86**	**0.37**	**3.83**
J-70 100% bound	32.0[b]	1.02	1.02	28.98	28.98	5.66	0.28	6.68
J-70 100% bound	36.0[b]	1.02	1.02	28.98	29.04	7.26	0.17	9.01
J-70 100% bound	40.0[b]	1.02	1.01	28.98	29.05	9.08	0.12	8.68
J-70 100% bound	44.0[b]	1.02	1.00	28.98	28.92	15.34	0.14	1.46
J-70 100% bound	50.0[b]	1.02	1.02	28.98	28.92	13.26	0.16	0.75

Result:
The 8 kDa DnaJ is still moving when it is bound to the 60 kDa DnaK. We call this a "tethered" binding-mode.

The relaxation analysis yield a best fit for overal τ_c *= 28 ns, with the J-domain dynamically linked with* S^2*=0.37 and* τ_{local} *= 4ns*

This would correspond to motion in a cone with 90 degree opening angle

Heat shock protein 70 kDa chaperone/DnaJ cochaperone complex employs an unusual dynamic interface.
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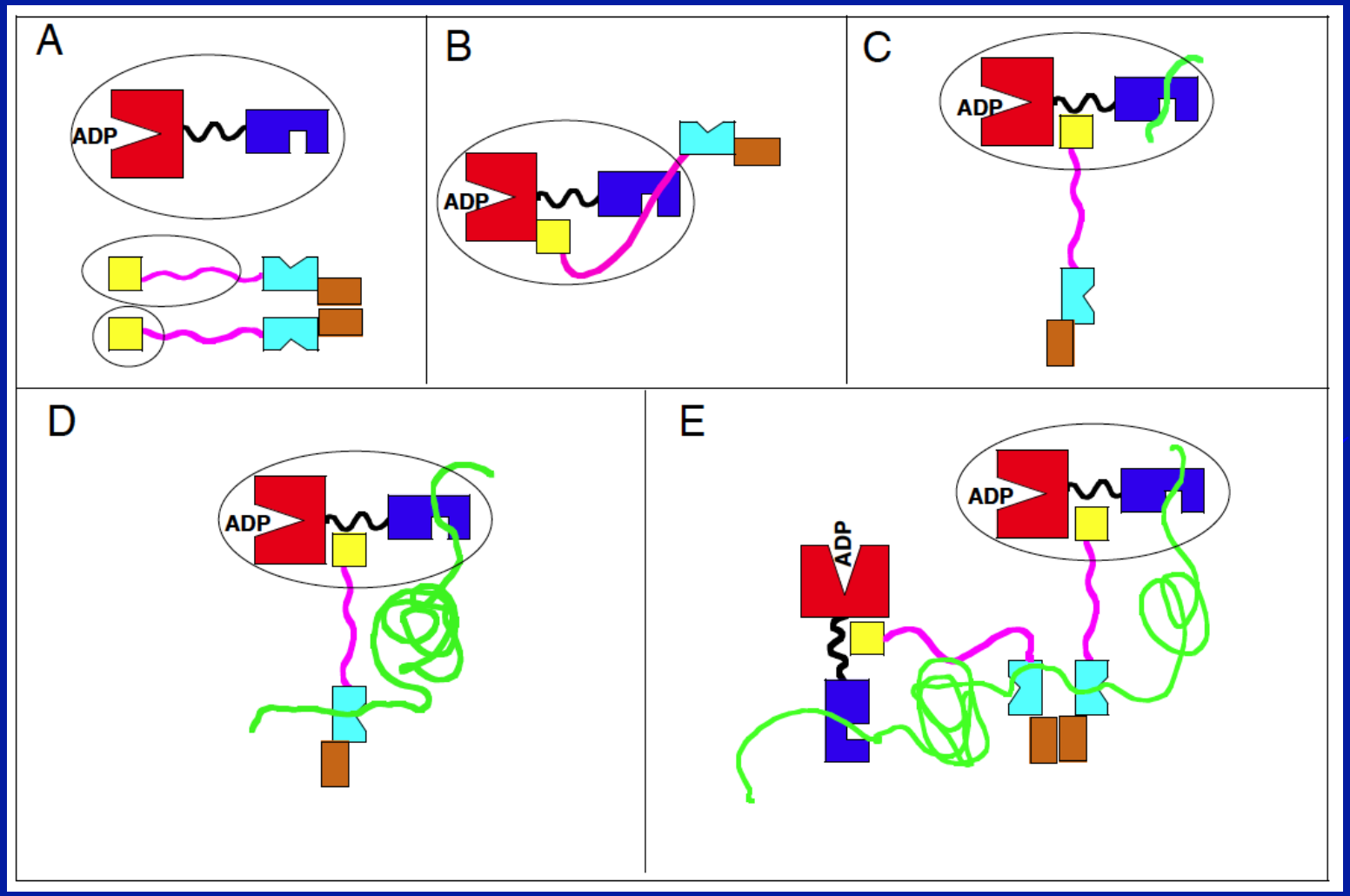
Proc Natl Acad Sci U S A. 108 18966-18971 (2011)

The chaperones are present at ~ 1 uM levels in the cells.

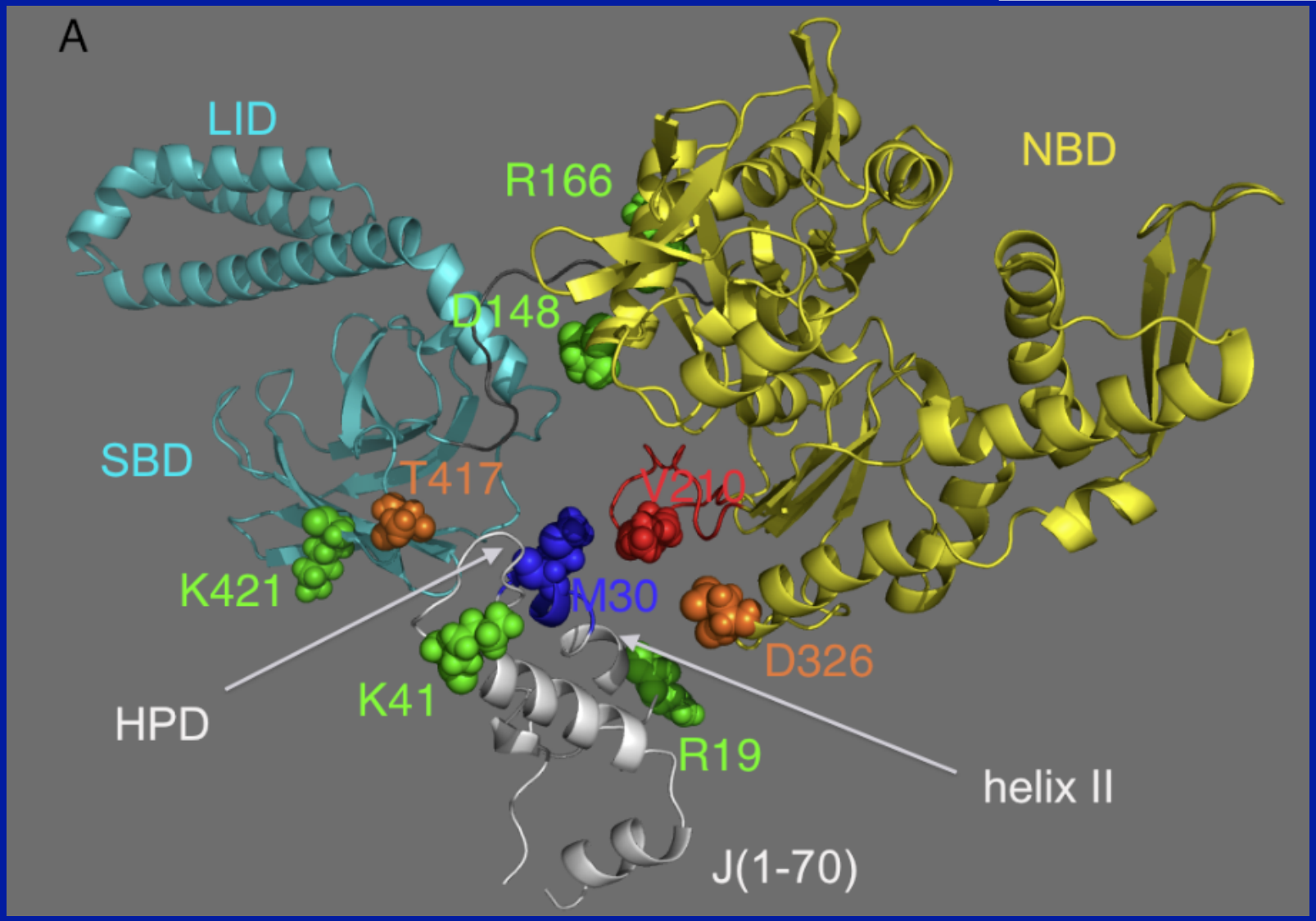
Can an interaction with a 16 uM K_D be relevant in these conditions?

We think so, since DnaK and DnaJ can interact in a multi-dentate fashion (see next slide), with a combine much higher affinity

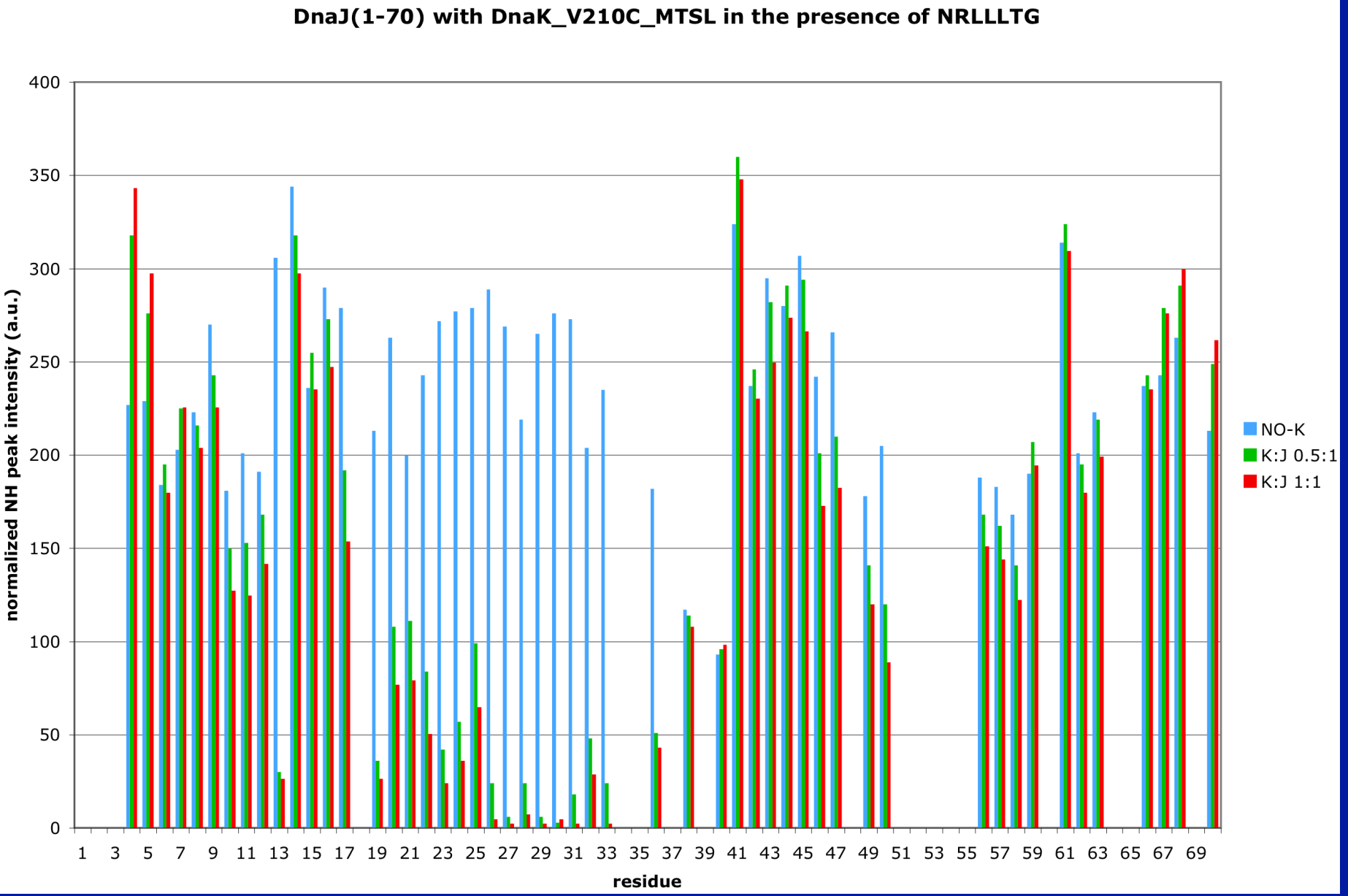
Possible scenarios of DnaK_DnaJ interactions (ADP)



Multi-site Nitroxide Spin-labeling
To obtain the binding site of DnaJ on DnaK

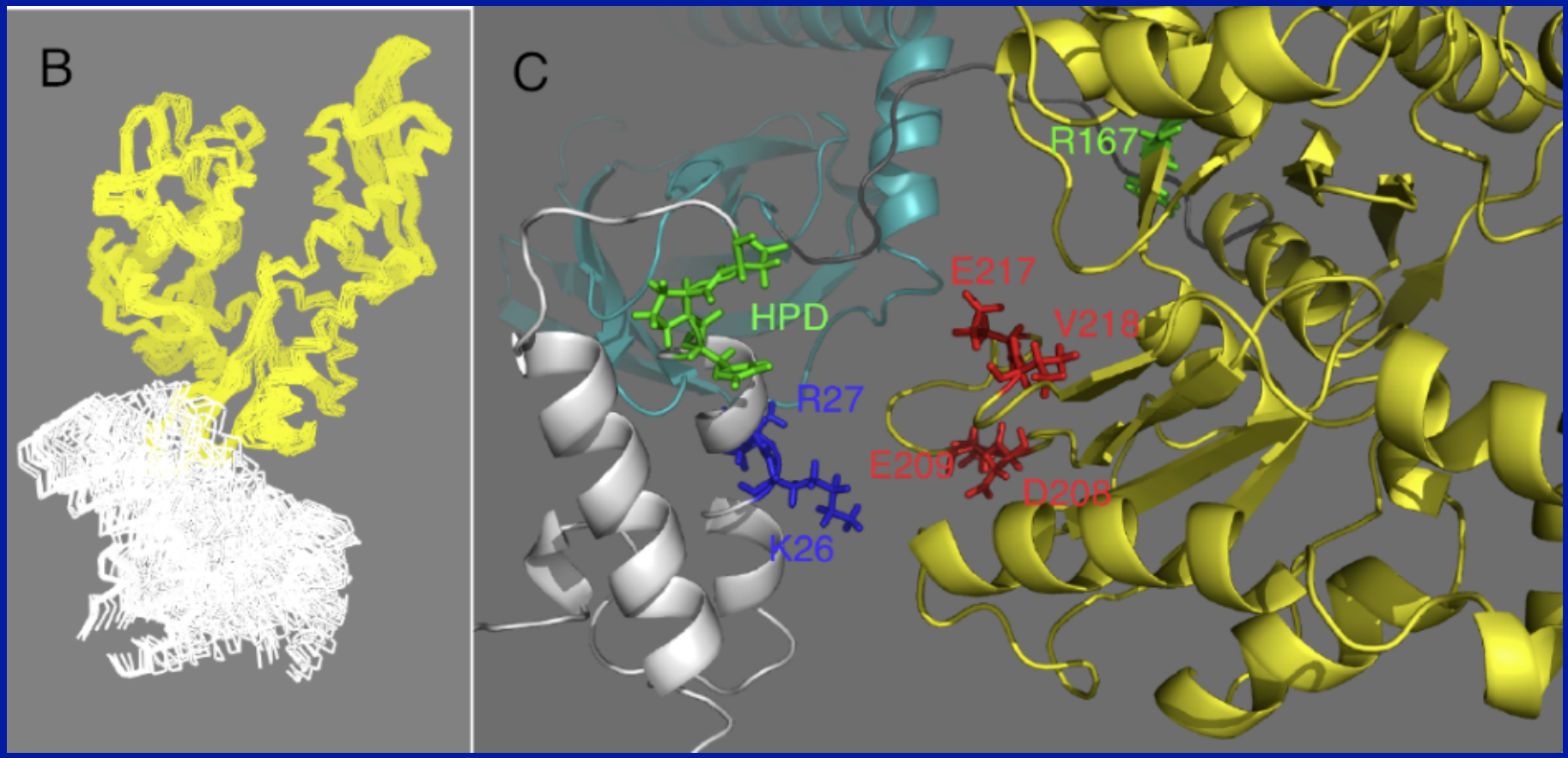


Analysis of MTSL data shows the region of DnaJ affected



➢ *Similarly we did many more of these DnaK-MTSL and DnaJ-MTSL studies*

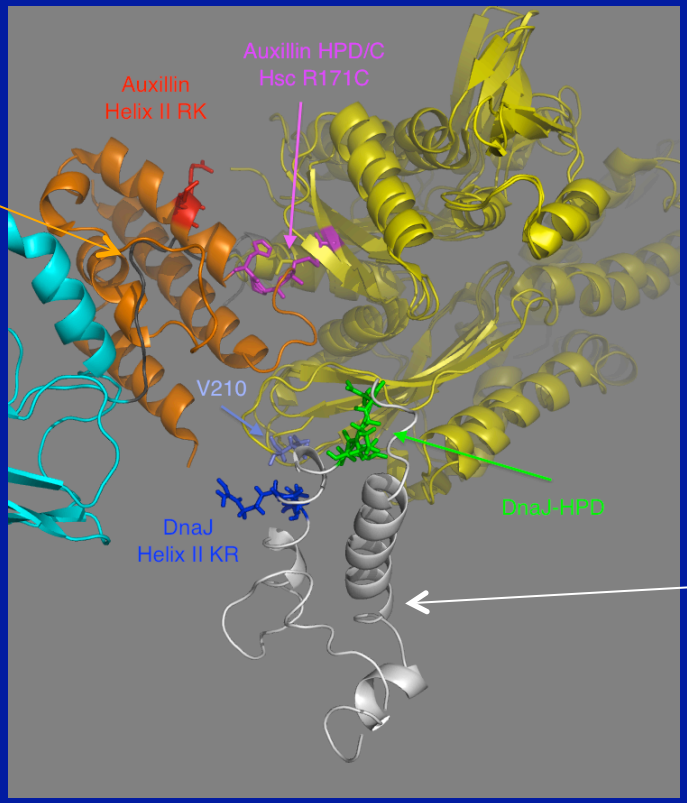
MD using mostly "repulsive" PRE's locate the J-domain surprisingly well



Heat shock protein 70 kDa chaperone/DnaJ cochaperone complex employs an unusual dynamic interface.
Ahmad A, Bhattacharya A, McDonald RA, Cordes M, Ellington B, Bertelsen EB, ***Zuiderweg*** *ER.*
Proc Natl Acad Sci U S A. 108 18966-18971 (2011)

Our solution complex is completely different from a crystal structure of an artificially di-sulfide-linked adduct of highly homologous human Hsp70 with highly homologous auxilin (1) .

"their" J-domain location



"our" J-domain location

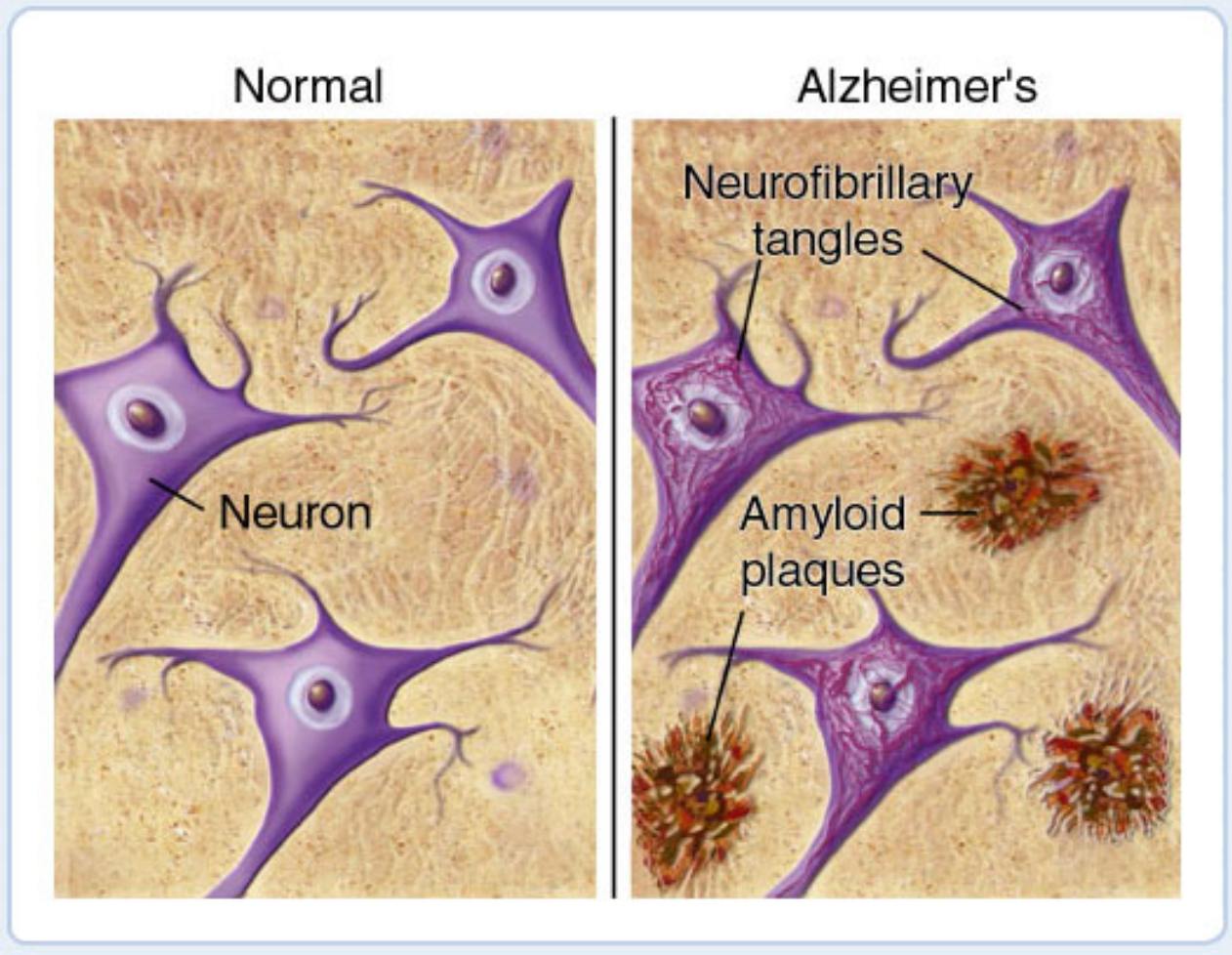
(1) Structural basis of J cochaperone binding and regulation of Hsp70.

Jiang J, Maes EG, Taylor AB, Wang L, Hinck AP, Lafer EM, Sousa R.

Mol Cell. 2007 , 28, 422-33.

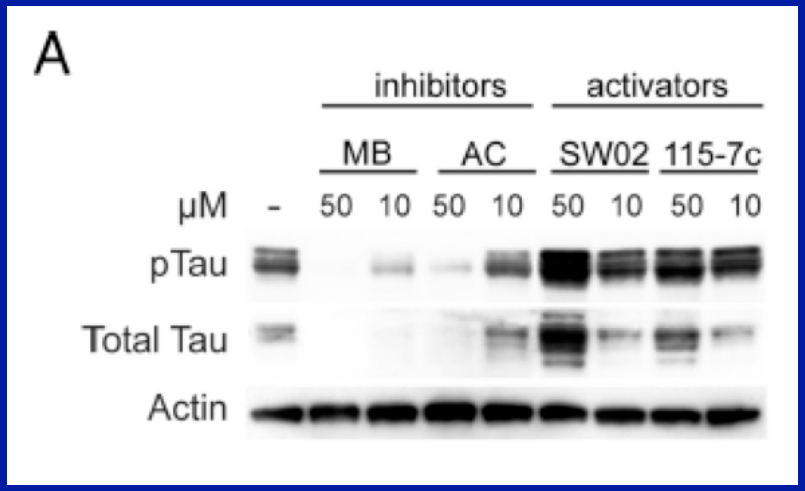
Hsp70 chaperones and disease

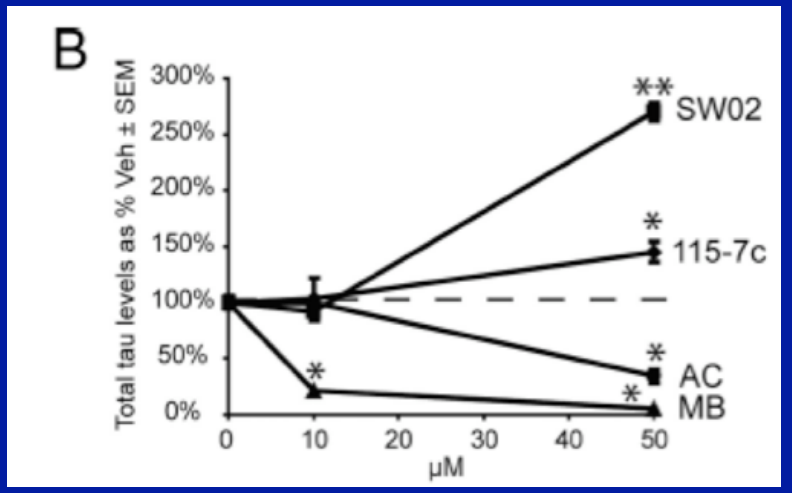
- Hsp70's are inhibitors of apoptosis in cancer cells
- Hsp70's aggrevate Alzheimer's; Huntington's; Parkinson's



http://www.ahaf.org/alzheimers/about/understanding/plaques-and-tangles.html

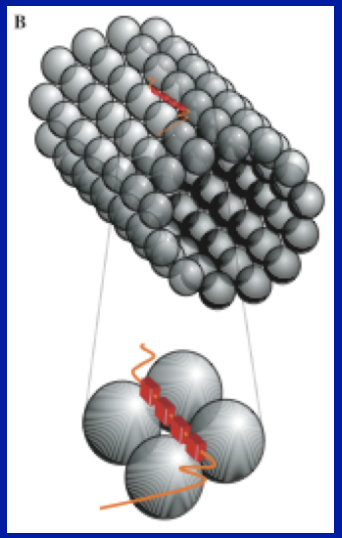
Inhibition of Hsp70 clears tau





Jinwal, U. K., Miyata, Y., Koren, J., Jones, J. R., Trotter, J. H., Chang, L., O'Leary, J., Morgan, D., Lee, D. C., Shults, C. L., Rousaki, A., Weeber, E. J., Zuiderweg, E. R. P., Gestwicki, J. E., and Dickey, C. A. (2009) Chemical Manipulation of Hsp70 ATPase Activity Regulates Tau Stability, Journal of Neuroscience 29, 12079-12088.

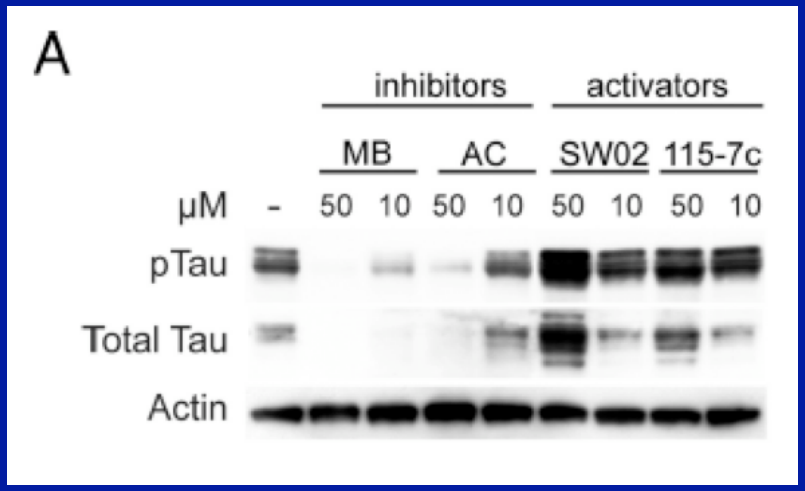
Tau
is a microtubule-associated protein expressed mainly in neurons where it has a role in the assembly and stability of the microtubule network. It localizes mainly in the axon.

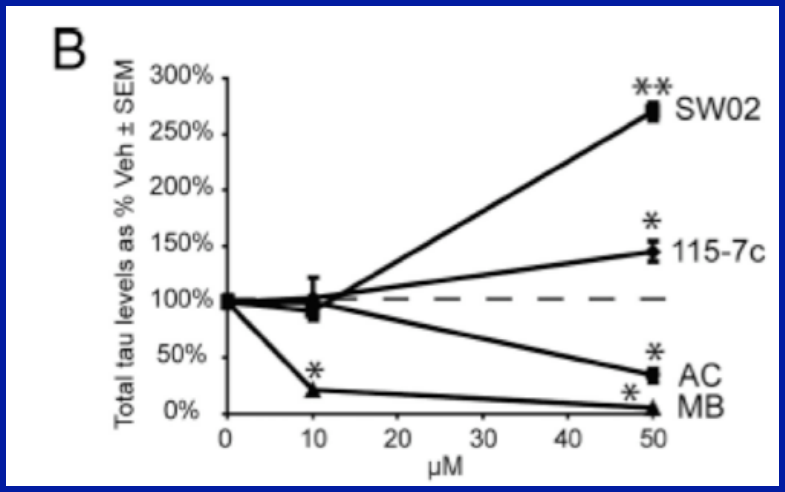


tauopathies

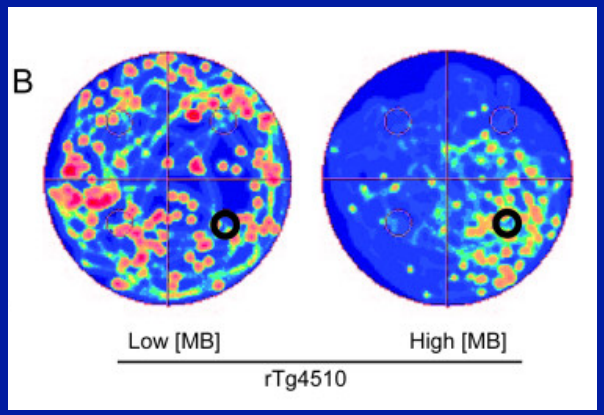
- AD
- Pick's
- Parkinsons
- Down's syndrome
- Argyrophilic grain disease
- Tangle only dementia
- Corticobasal degeneration
- Progressive supranuclear palsy
- Amyotropic lateral sclerosis
- Niemann-Pick disease type C
- Subacute sclerosing panencephalitis
- Postencephalitic parkinsonism
- Dementia pugilistica
- Myotonic dystrophy
- Gestmann-Straussler-Scheinker disease with tangles
- Prion protein amyloid angiopathy
- Presenile dementia with tangles and calcifications
- Hallervorden-Spatz disease
- Cancer

*Tolnay, M. and A. Probst, REVIEW: tau protein pathology in Alzheimer's disease and related disorders. Neuropathology and Applied Neurobiology, 1999., **25**, 171-187.*





Jinwal, U. K., Miyata, Y., Koren, J., Jones, J. R., Trotter, J. H., Chang, L., O'Leary, J., Morgan, D., Lee, D. C., Shults, C. L., Rousaki, A., Weeber, E. J., Zuiderweg, E. R. P., Gestwicki, J. E., and Dickey, C. A. (2009) Chemical Manipulation of Hsp70 ATPase Activity Regulates Tau Stability, Journal of Neuroscience 29, 12079-12088.



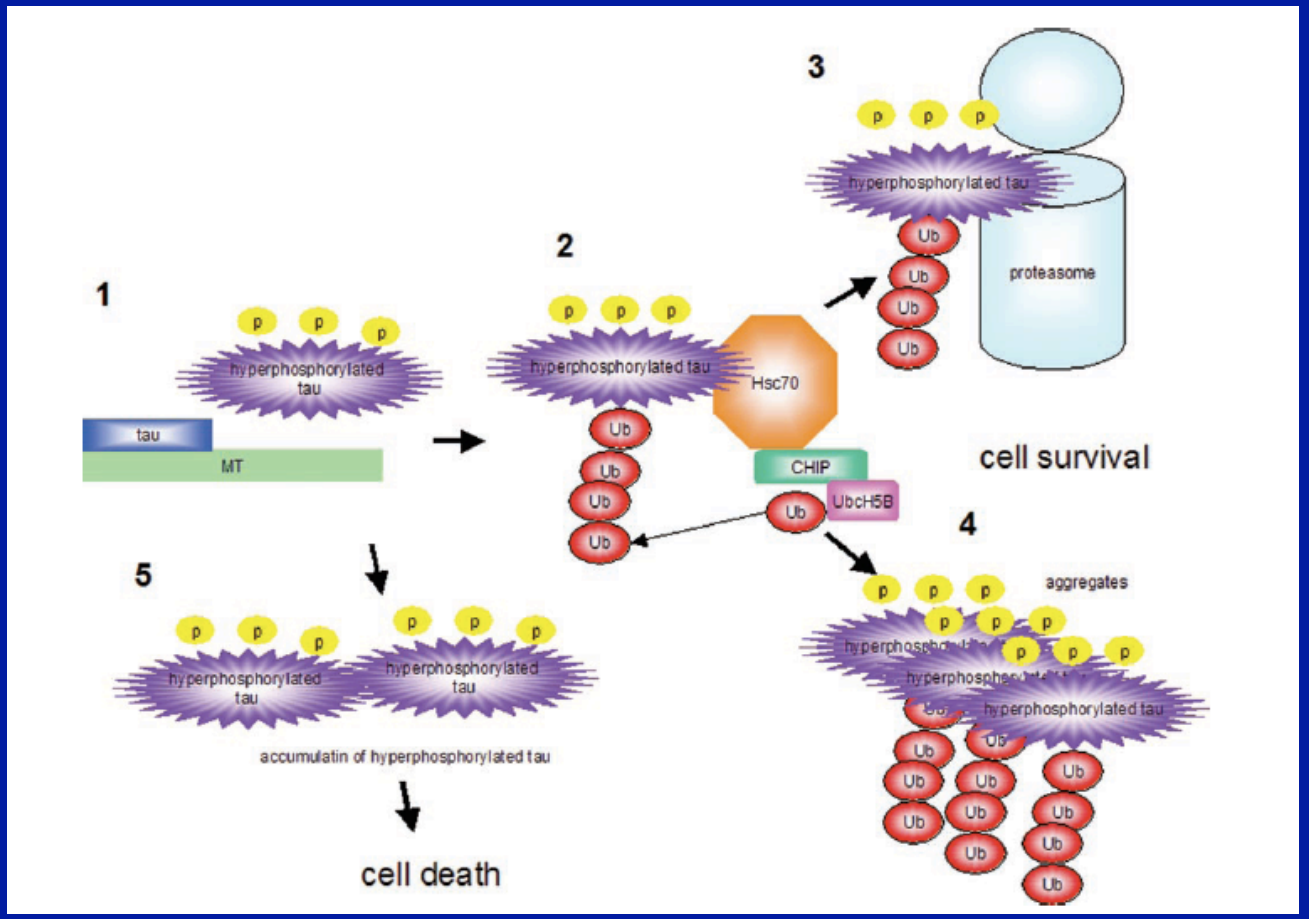
Mice ability to remember the location of a hidden target

Thus:

Inhibiting Hsp70 maybe a way to control tauopathies such as Alzheimer's

(and cancer as well?).

Possible Mechanism relating Hsp70 inhibition to tau clearance



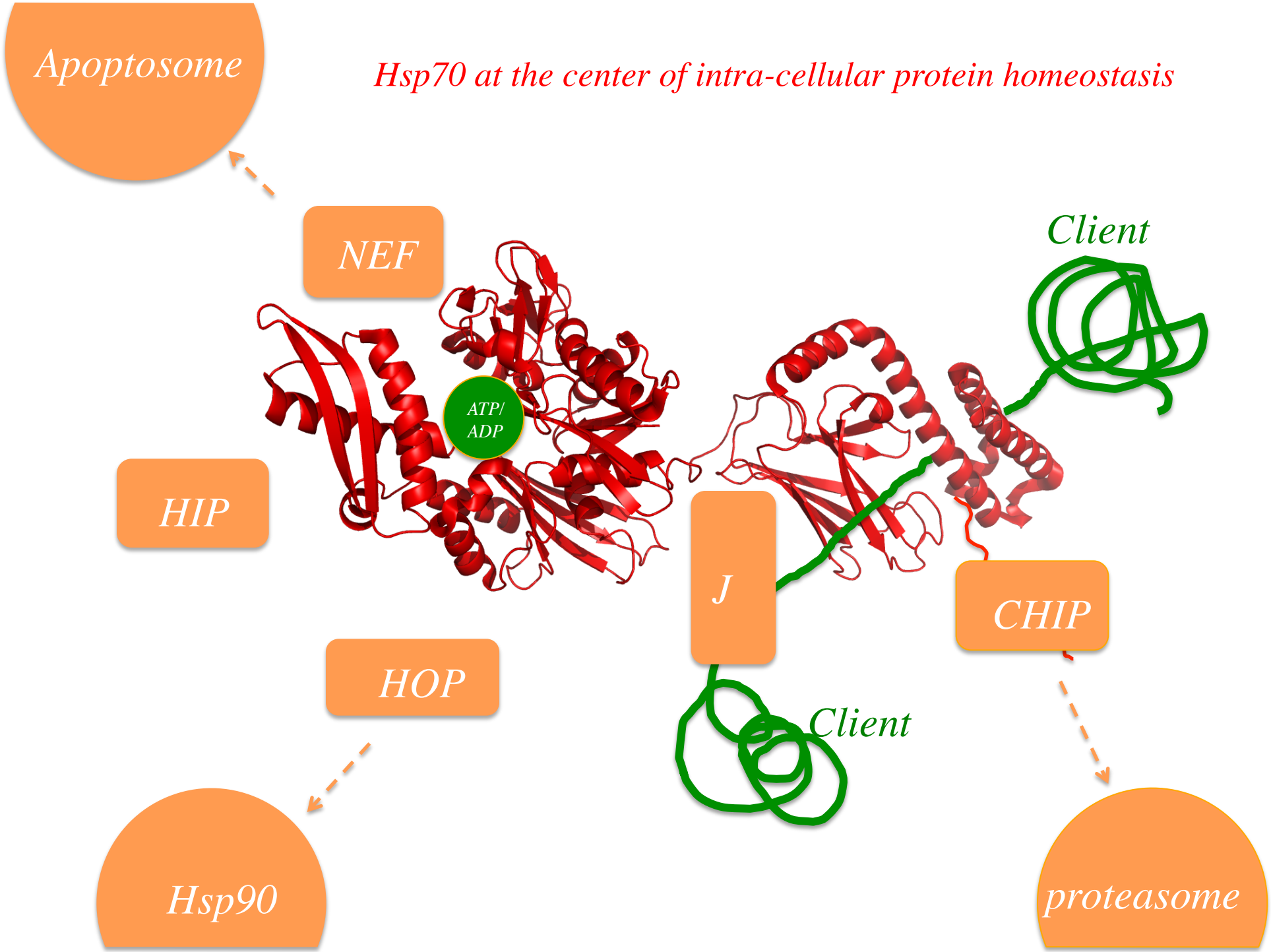
- Figure III.1.9 : Model according to which the CHIP-Hsc70 complex decreases the toxicity of hyperphosphorylated tau by either leading it to the proteosome for degradation or by the formation of non toxic tangles. Step1, tau is phosphorylated by GSK-3, Cdk5, and other kinases and is released from the microtubules Step2, hyperphosphorylated tau is ubiquitinated by the CHIP-Hsc70 complex for degradation in the proteosome (step 3) or the formation of aggregates (step4) Step 5, interference with step 2 leads to the accumulation of hyperphosphorylated tau [213]

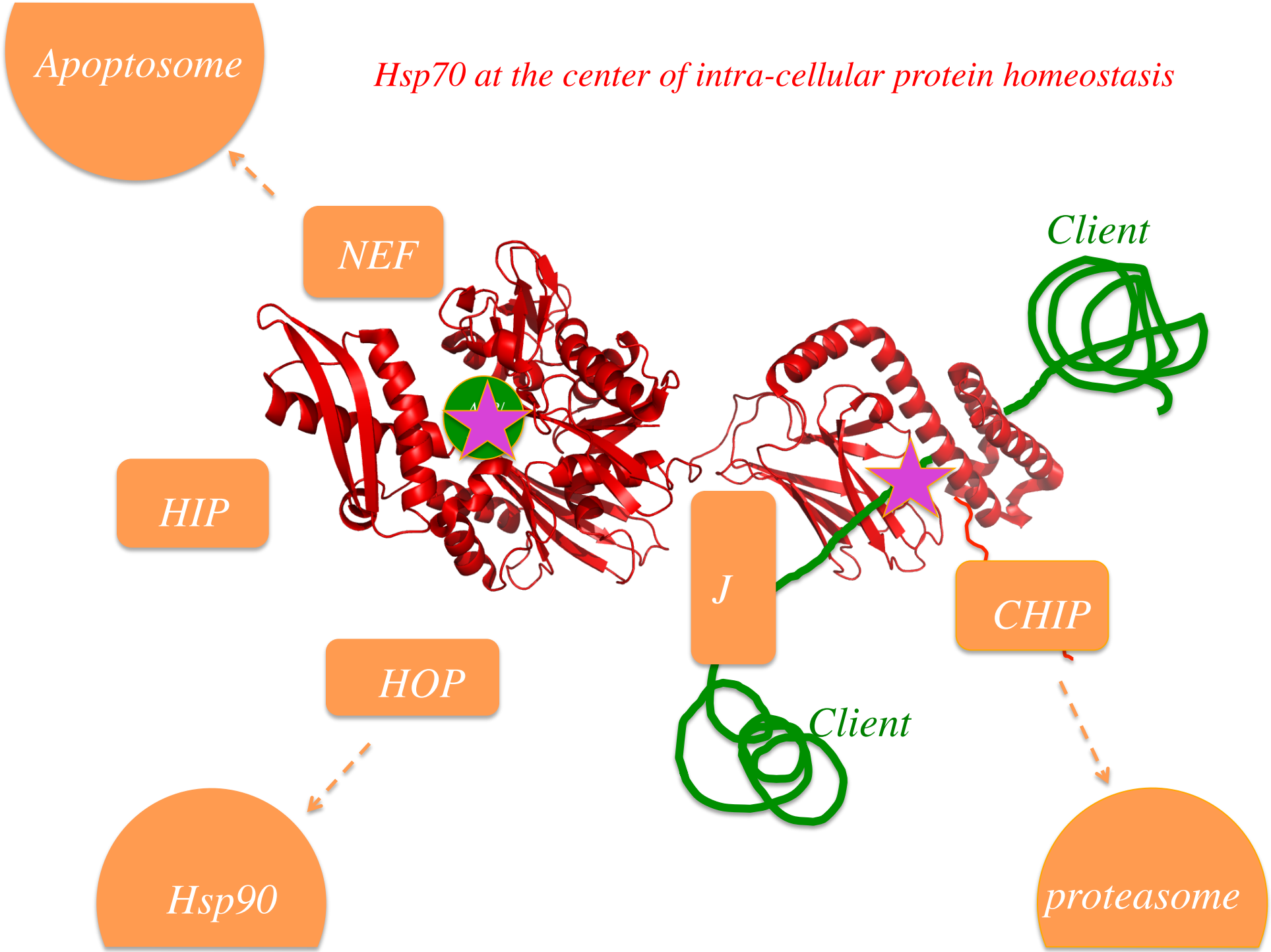
Thus:

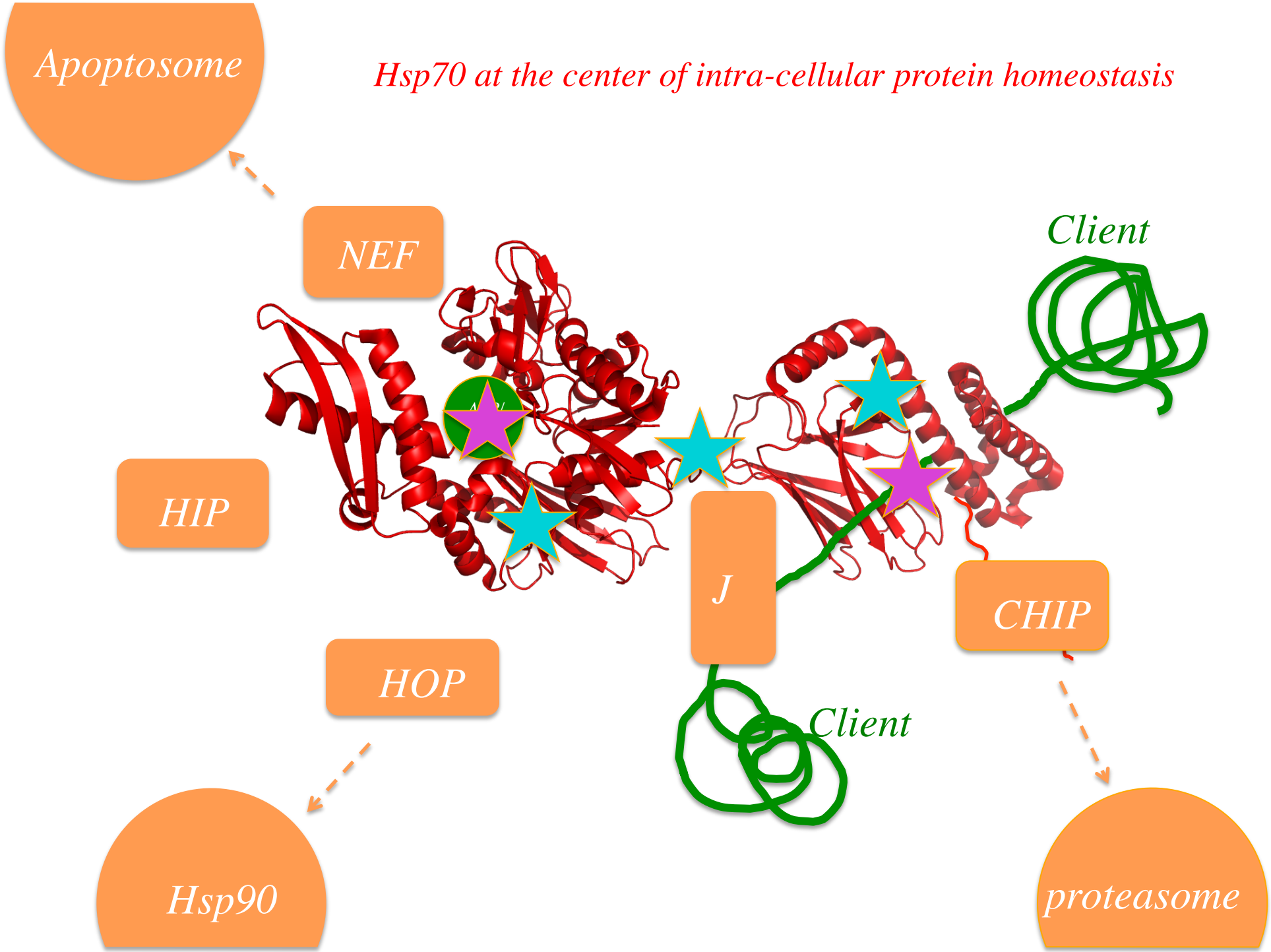
Inhibiting Hsp70 maybe a way to control tauopathies such as Alzheimer's

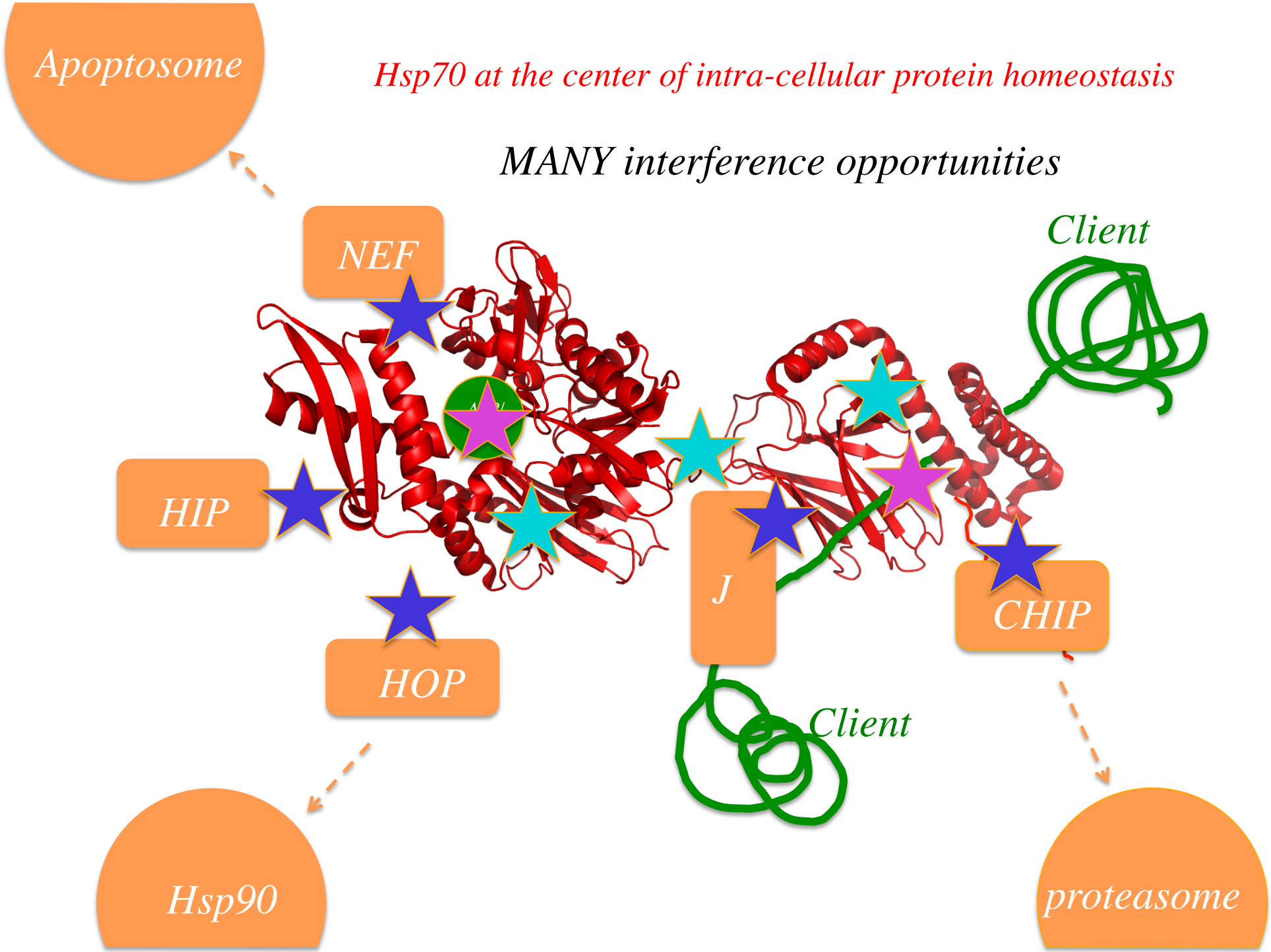
(and cancer as well?).

Hence:
Collaboration with Jason Gestwicki to find inhibitors


Apoptosome
Hsp70 at the center of intra-cellular protein homeostasis
NEF
Client
ATP/
ADP
HIP
J
CHIP
HOP
Client
Hsp90
proteasome


Apoptosome
Hsp70 at the center of intra-cellular protein homeostasis
NEF
Client
HIP
J
CHIP
HOP
Client
Hsp90
proteasome

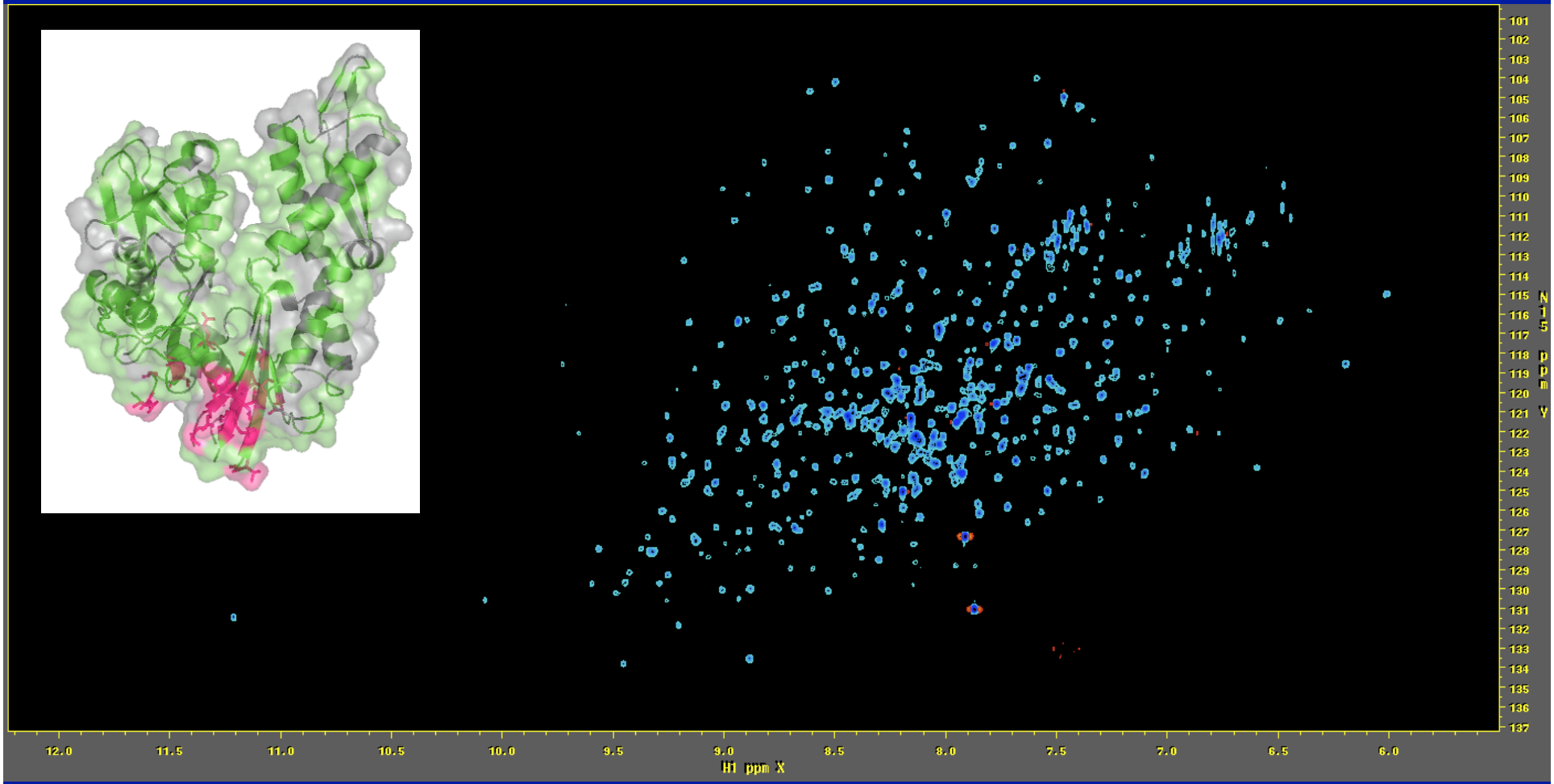

Apoptosome
Hsp70 at the center of intra-cellular protein homeostasis
NEF
Client
HIP
J
CHIP
HOP
Client
Hsp90
proteasome


Apoptosome
Hsp70 at the center of intra-cellular protein homeostasis
MANY interference opportunities
NEF
Client
HIP
J
CHIP
HOP
Client
Hsp90
proteasome

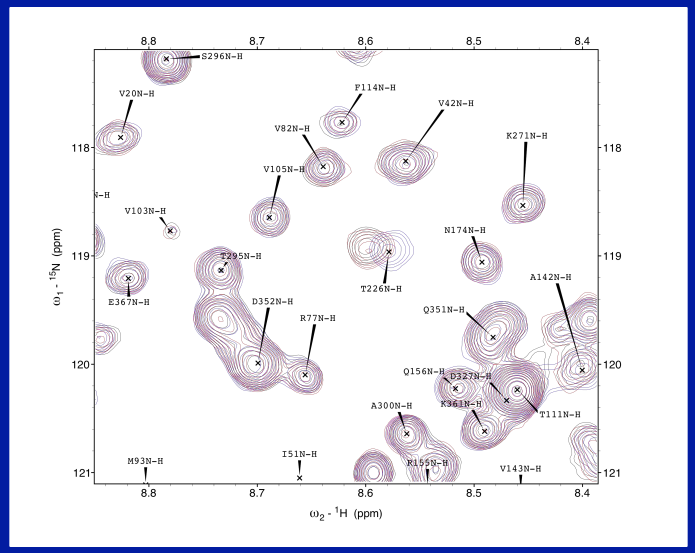
NMR for protein-drug interactions

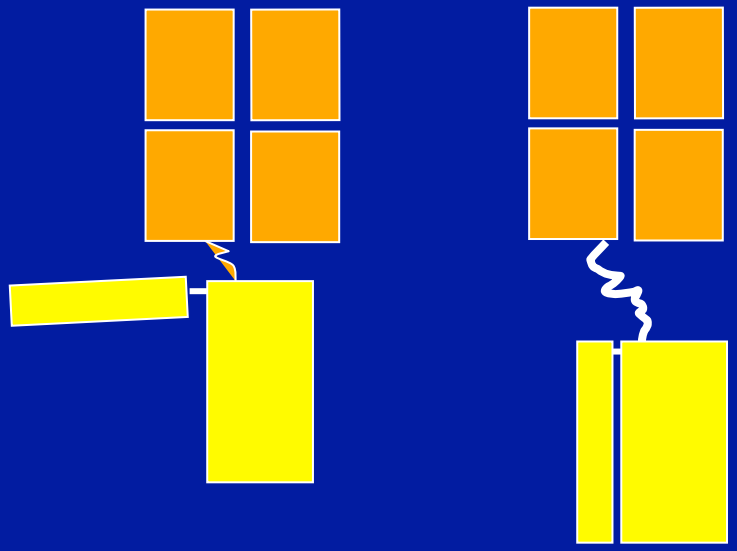
800 MHz TROSY ^{1}H-^{15}N Correlation Hsp70

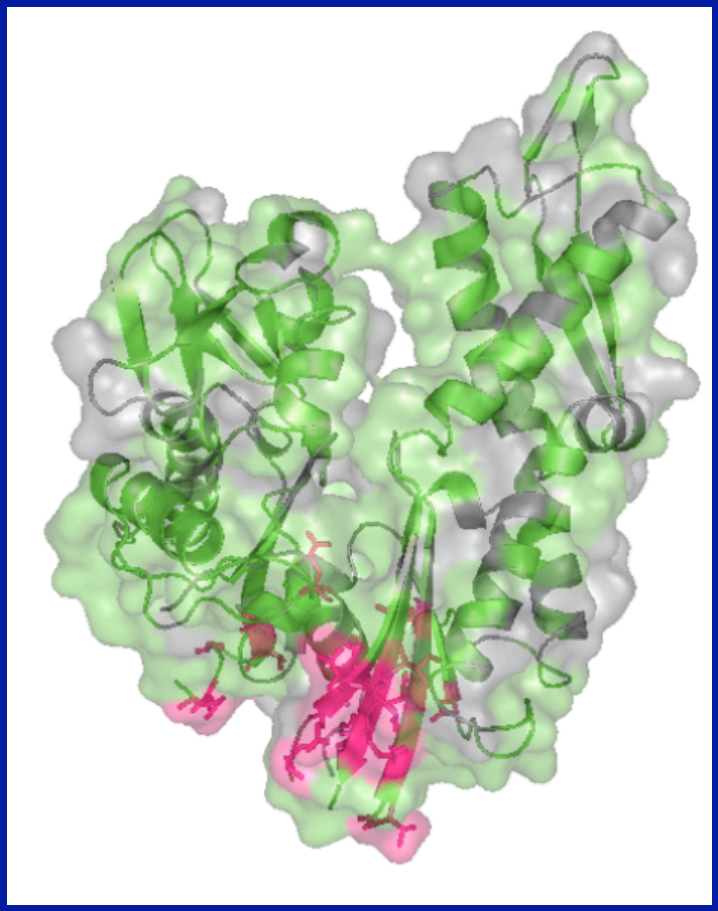
Key: we have most of these cross peaks assigned



Promising chemicals: Chemical Shift Perturbation



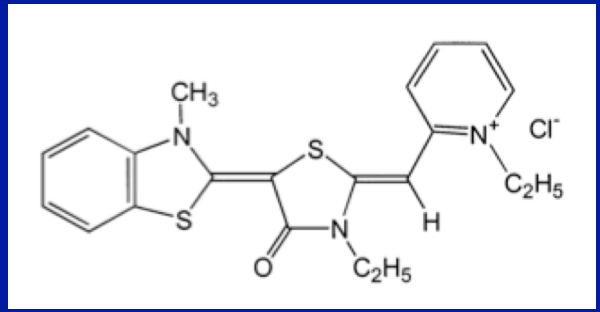


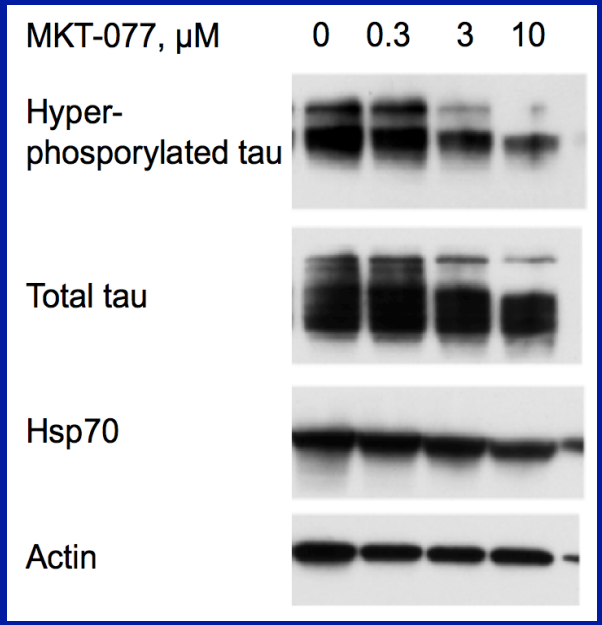


CT007 is a Hsp70 inhibitor affecting the allosteric interface

Wisen, S., Bertelsen, E.B., Thompson, A.D., Patury, S., Ung, P., **Chang, L.**, Evans, C.G., Walter, G.M., Wipf, P., Carlson, H.A., Zuiderweg, E. R. P. and Gestwicki, J. E. (2010). Binding of a small molecule at a protein-protein interface regulates the chaperone activity of hsp70-hsp40. *ACS Chemical Biology 5*, 611-622.

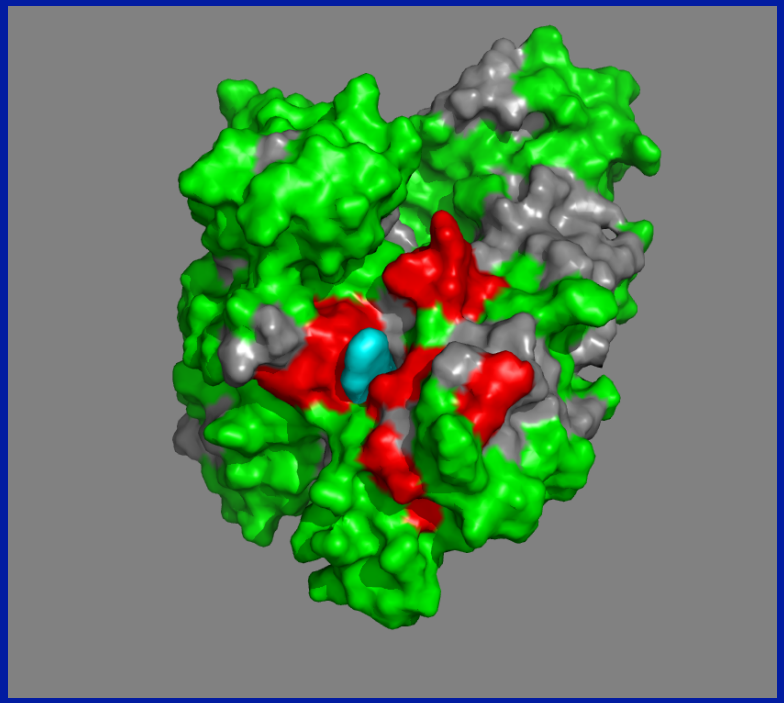
Anti-cancer drug MKT-077 binds to Hsp70's and also clears tau



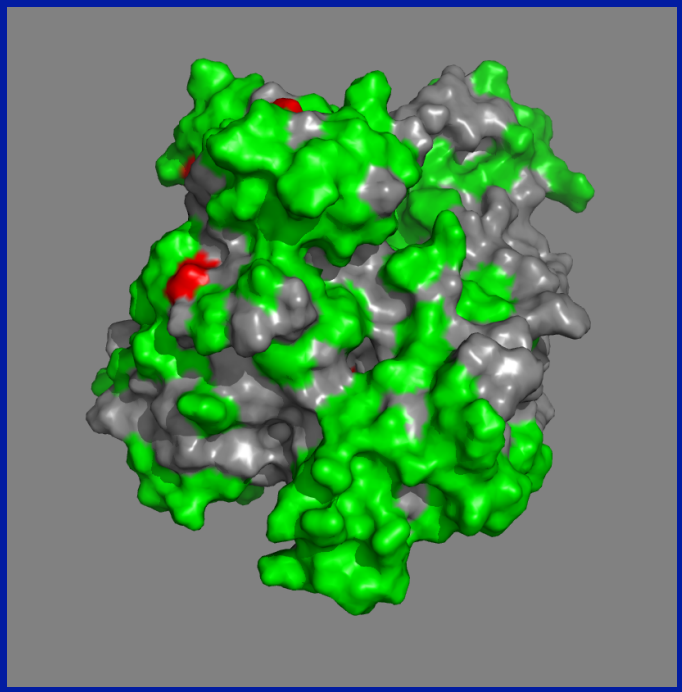


Rousaki A, Miyata Y, Jinwal UK, Dickey CA, Gestwicki JE, Zuiderweg ER.
Allosteric drugs: the interaction of antitumor compound MKT-077 with human Hsp70 chaperones.
J Mol Biol. 2011, 411, 614-32

ADP state

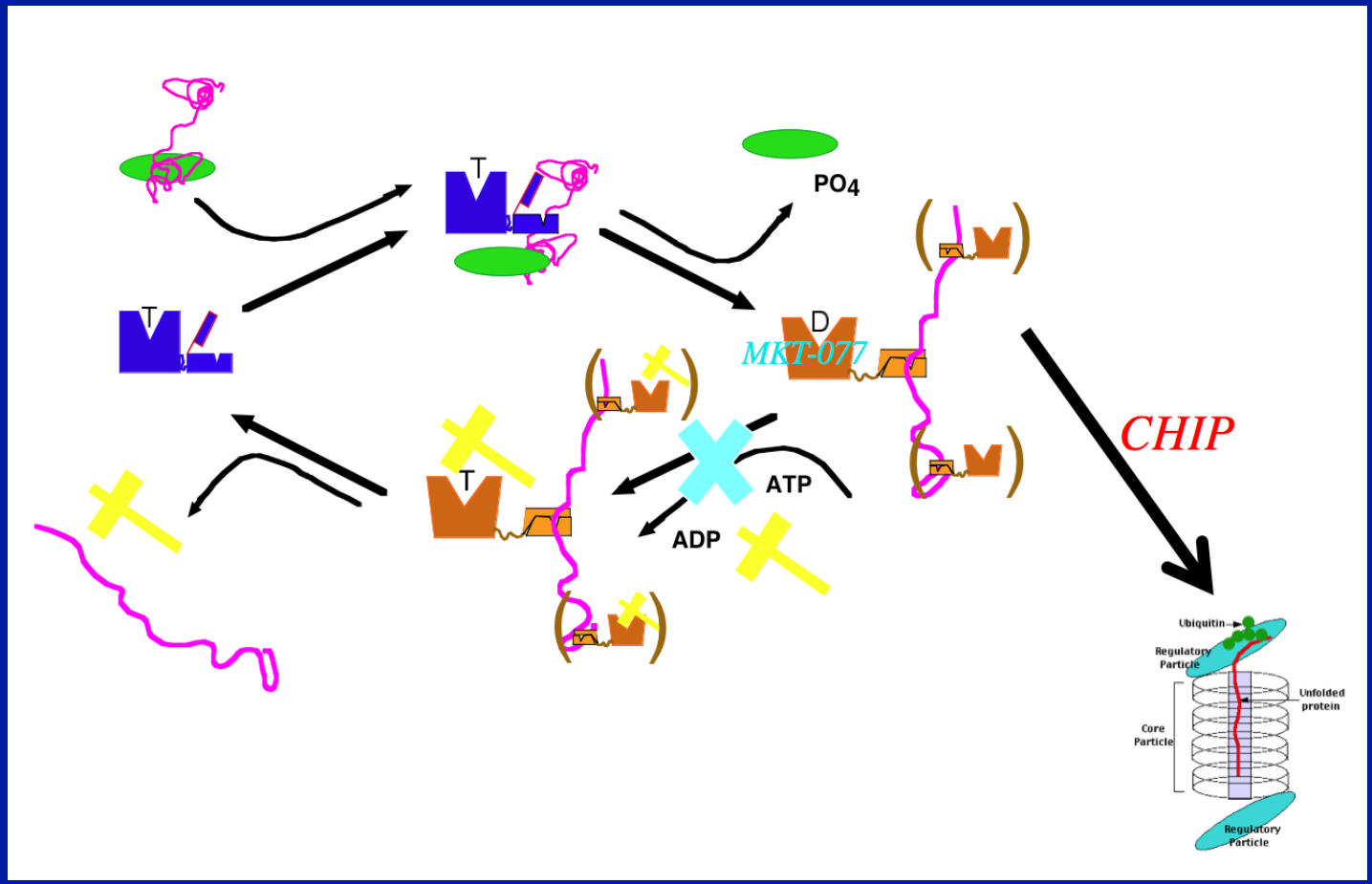


ATP state



The anti-cancer drug MKT077 is an Hsp70 ALLOSTERIC effector

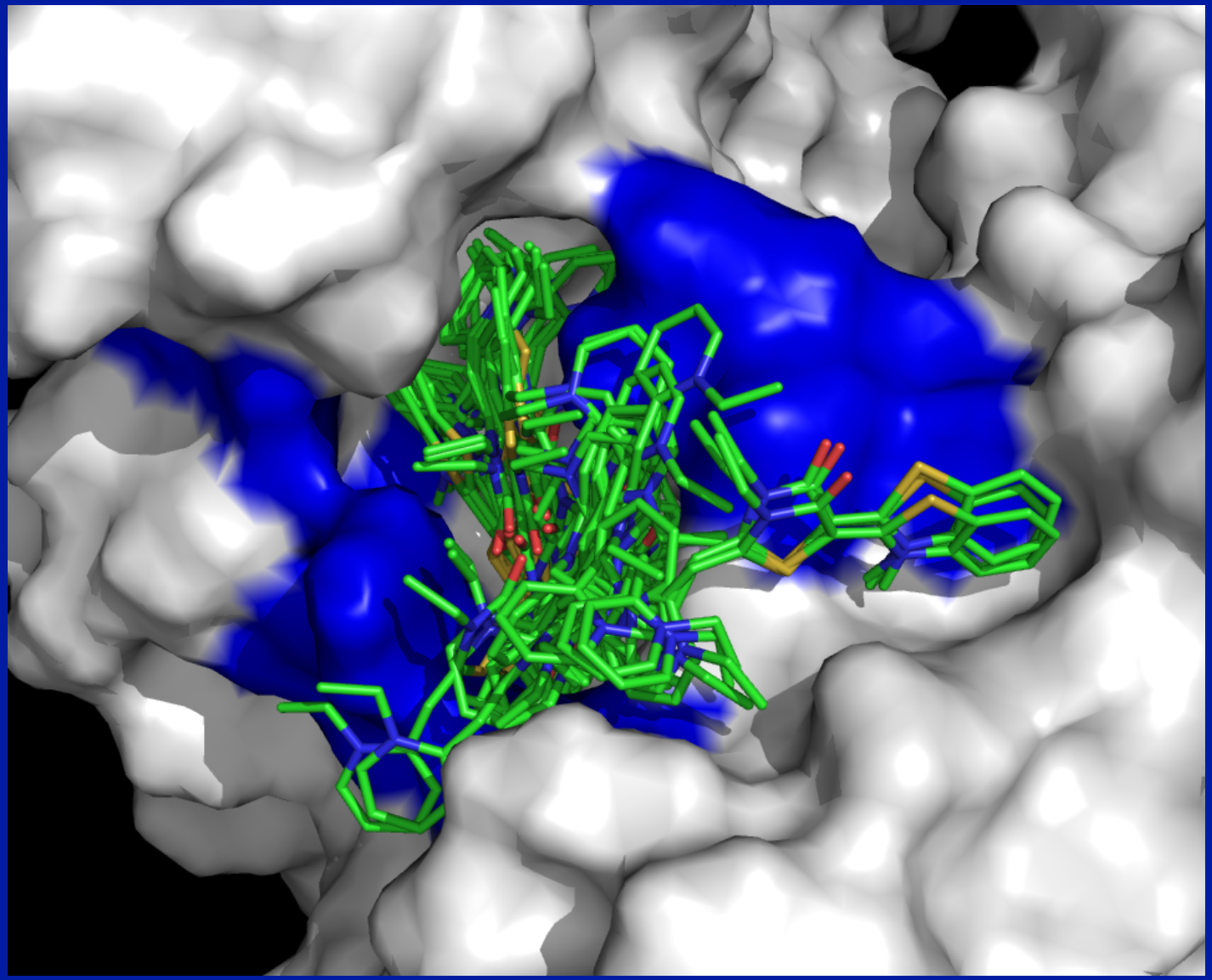
Rousaki A, Miyata Y, Jinwal UK, Dickey CA, Gestwicki JE, Zuiderweg ER.
Allosteric drugs: the interaction of antitumor compound MKT-077 with human Hsp70 chaperones.
J Mol Biol. 2011, 411, 614-32


T
PO4
T
D
MKT-077
CHIP
T
ATP
ADP
Ubiquitin
Regulatory Particle
Unfolded protein
Core Particle
Regulatory Particle

Protocol

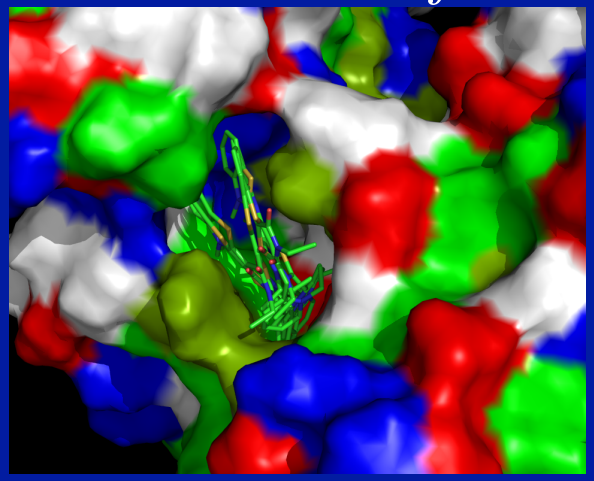
- NMR to obtain binding location
- NMR-restrained AUTODOCK to obtain binding poses
- Molecular dynamics to obtain dynamically averaged binding energies for pose selection
- (Amber – GB/PB)

NMR-restrained AUTODOCK poses

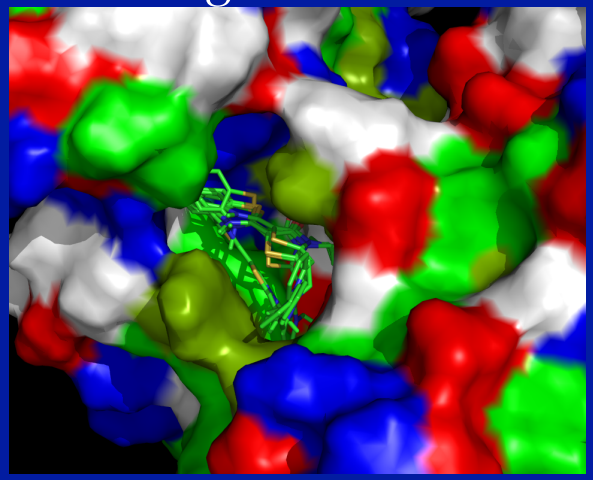


Pose families and Amber Scoring

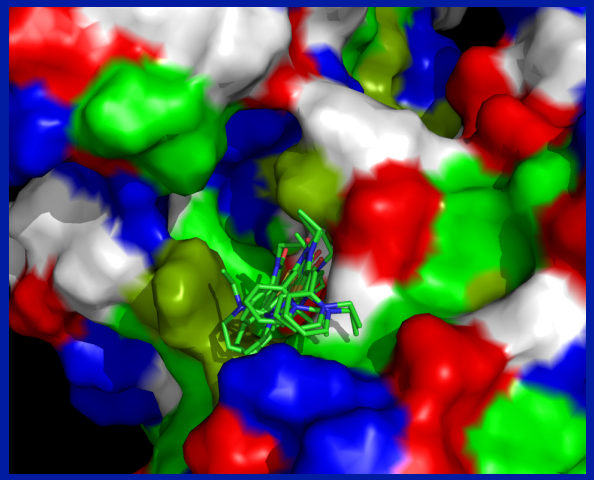
C



D



E



F

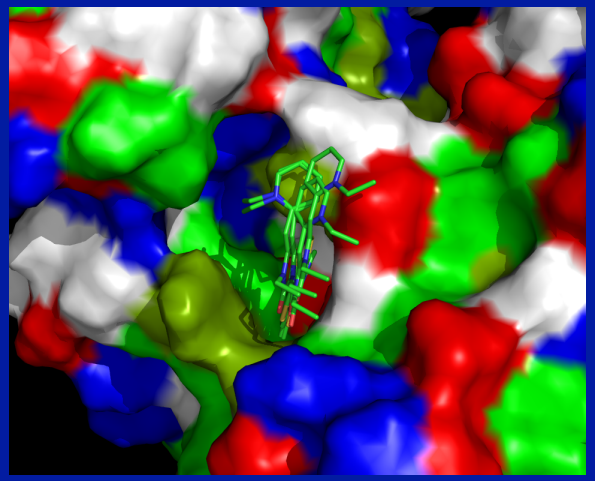
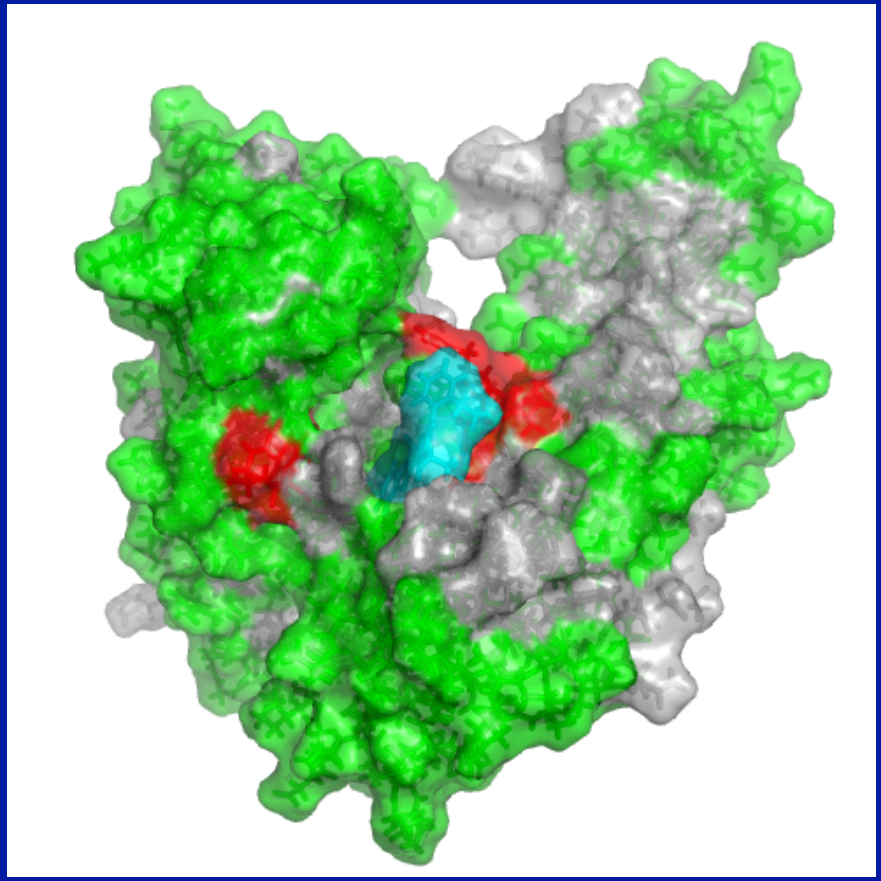


Table 2. MKT-077 binding enthalpy according to different computational methods			
Subfamily in Fig 10	AUTODOCK Energy	MM GB Energy	MM PBSA Energy
	kCal/M	kCal/M	kCal/M
Panel C	7.03	-16.6 ± 1.8	-7.5 ± 3.7
Panel D	6.32	-18.6 ± 1.5	-14.0 ± 2.1
Panel E	5.36	-18.1 ± 1.2	-10.8 ± 2.0
Panel F	5.25	-22.8 ± 1.3	-13.8 ± 2.6

Rousaki A, Miyata Y, Jinwal UK, Dickey CA, Gestwicki JE, Zuiderweg ER.
Allosteric drugs: the interaction of antitumor compound MKT-077 with human Hsp70 chaperones.
J Mol Biol. 2011, 411, 614-32

Final best pose obtained with NMR-restrained AUTODOCK, and refined with AMBER NMR-restrained simulated annealing



No shifts, *shifts*, *not known*, *MKT-077*

Key contributers to the Hsp70 chaperone research in the EZ lab

Hong Wang (GSRA 1991-1995 PhD)
Hong Wang, PhD (PostDoc 1995-1997)
Robert Morshauser (GSRA 1992-1998, PhD)
Maurizio Pellecchia, PhD (PostDoc 1998-1999)
Shawn Stevens, PhD (PostDoc 1998-2002)
Matt Revington, PhD (PostDoc 2001-2004)
Yongbo Zhang, PhD (PostDoc 2001-2004)
Mark Berjanskii, PhD (PostDoc 2002-2004)
Grover Yip (GSRA 2001-2006 PhD)
Eric Bertelsen, PhD (PostDoc 2005-2007)
Akash Bhattacharya (GSRA 2006-2009 PhD)
Akash Bhattacharya, PhD (Post-doc 2009-2010)
Aikatarini Rousaki (GSRA 2006-2011 PhD)
Atta Ahmad, PhD (PostDoc 2008-2011)
Ramsay McDonald (UGSRA) (2009-2011)
Alexander Kurochkin, PhD (NMR-maintenance 1992 -)

Collaborators providing plasmids

Greg Flynn, Oregon
Lila Gierasch, Massachussets
David McKay, Stanford
Jochen Reinstein, Dortmund
Alexander Joachimiak, Argonne
Matthias Mayer, Heidelberg
Jason Gestwicki, Michigan

RESEARCH FUNDING:
Structure, Dynamics and Function of Chaperone Domains
NIH-GMS (1995-2003)

Study of allosteric proteins by NMR
NIH-GMS (2001-2009)

Study of allosteric proteins by NMR
NIH-ARRA (2009-2011)

Molecular Chaperones and Small Molecules
NIH-NS (2008 -) (J. Gestwicki, PI)

KEY EQUIPMENT FUNDING:

NCCR Shared Instrumentation (NIH)
800 MHz NMR Instrument *1998*

NSF
800 MHz NMR Instrument *1998*

Keck Foundation
800 MHz NMR Instrument *1999*

NCCR Shared Instrumentation (NIH)
800 MHz NMR Cryoprobe *2004*

Technical Background
How to work with such large proteins ?
Concentrations are typically 400 uM and we work at 30^0C.

Divide and conquer:
We started with the SBD (20 kDa) when we had a 600 MHz instrument (around 1995).
Moved to the NBD (45 kDa) when we had a 800 MHz and TROSY became available (2000)
Moved to larger assemblies when the cryo-probe became available (2005)

The assignments were obtained with the full suite of 3D triple resonance TROSYs on the domains using a 800 MHz Varian instrument, using our own optimized sequences, and were transferred to the larger constructs using HNCA and HNCO TROSY only. Everything was perdeuterated.

The assignments were done "by hand" in Sparky, and later checked using SAGA

Crippen, G.M., Rousaki, A, Revington, M., Zhang, Y. and Zuiderweg, E.R.P.SAGA: Rapid automatic mainchain NMR assignment for large proteins J. Biomol. NMR 46 281-298. (2010))

The RDC's were obtained using 2D "shifted" TROSY's (Bhattacharya, A., Revington, M., and Zuiderweg, E.R.P. Measurement and interpretation of ^{15}N-^{1}H residual dipolar couplings in larger proteins J. Magn. Reson. 203 11–28, (2010)) in phage and in gels.

The spinlabel data was obtained with TROSYs and checked with HNCO.

The drug-binding data was obtained with TROSY and checked with HNCO.