Hsp70 chaperones: 
Mechanism 
Disease 
Inhibitors

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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,
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,

**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

No correspondence with crystal structures

Hsp70 – E. coli ADP.Pep tide 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

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

DnaK-substrate-binding domain,

Crystal-NRLLLTG bound vs Solution apo

The LID is not needed for allostery

**Fig. 1.** *In vitro* studies of DnaK(1–507) allostery 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 NRRRLTG, 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.

*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

ATP domain

Should affect substrate binding here

Thus ATP-domain induced changes here
Summary:
Model of Allosteric Communication in Hsp70

Also incorporating data from W. Hendrickson, B. Bukau, L. Gierasch
DnaJ – Hsp70 Interactions
DnaJ topologies

EMBO reports (2004) 5, 567
Hsp40 – DnaJ --- YDJ --- HDJ


Chemical shifts in DnaJ when binding DnaK

Mutagenesis-sensitive residues
Binding saturates with 1:1 stoichiometry and 16 uM KD
BUT THERE IS HARDLY ANY LINE BROADENING
When the 8 kDa DnaJ is bound to the 60 kDa DnaK!

The heavy line is a fit with $K_D = 16 \mu M$ 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 $\tau_c$ and $S^2$ of the 8 kDa DnaJ bound to the 60 kDa DnaK.

<table>
<thead>
<tr>
<th></th>
<th>$\tau_c$</th>
<th>$&lt;R&gt;_\text{exp}$</th>
<th>$&lt;R&gt;_\text{fit}$</th>
<th>$&lt;R_c&gt;_{\text{exp}}$</th>
<th>$&lt;R_c&gt;_{\text{fit}}$</th>
<th>$&lt;R_e&gt;_{\text{fit}}$</th>
<th>$&lt;S^2&gt;_{\text{fit}}$</th>
<th>$&lt;\tau&gt;_{\text{fit}}$</th>
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<tbody>
<tr>
<td>J-70 free</td>
<td>ns</td>
<td>(s$^{-1}$)</td>
<td>(s$^{-1}$)</td>
<td>(s$^{-1}$)</td>
<td>(s$^{-1}$)</td>
<td>(s$^{-1}$)</td>
<td>(ns)</td>
<td>-</td>
</tr>
<tr>
<td>J-70 73% bound</td>
<td>7.0$^a$</td>
<td>1.123</td>
<td>1.123</td>
<td>24.06</td>
<td>24.06</td>
<td>13.3</td>
<td>0.82</td>
<td>-</td>
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<tr>
<td>J-70 100% bound</td>
<td>8.0$^a$</td>
<td>1.02</td>
<td>1.02</td>
<td>28.98</td>
<td>28.98</td>
<td>16</td>
<td>0.85</td>
<td>-</td>
</tr>
<tr>
<td>J-70 100% bound</td>
<td>12.0$^b$</td>
<td>1.02</td>
<td>1.02</td>
<td>28.98</td>
<td>28.99</td>
<td>7.22</td>
<td>1.00</td>
<td>0.89</td>
</tr>
<tr>
<td>J-70 100% bound</td>
<td>16.0$^b$</td>
<td>1.02</td>
<td>1.02</td>
<td>28.98</td>
<td>28.99</td>
<td>7.44</td>
<td>0.71</td>
<td>2.86</td>
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<tr>
<td>J-70 100% bound</td>
<td>20.0$^b$</td>
<td>1.02</td>
<td>1.02</td>
<td>28.98</td>
<td>29.01</td>
<td>8.88</td>
<td>0.51</td>
<td>3.00</td>
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<tr>
<td>J-70 100% bound</td>
<td>24.0$^b$</td>
<td>1.02</td>
<td>1.02</td>
<td>28.98</td>
<td>28.98</td>
<td>7.08</td>
<td>0.45</td>
<td>3.53</td>
</tr>
<tr>
<td>J-70 100% bound</td>
<td>28.0$^b$</td>
<td>1.02</td>
<td>1.02</td>
<td>28.98</td>
<td>28.98</td>
<td>6.86</td>
<td>0.37</td>
<td>3.83</td>
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<tr>
<td>J-70 100% bound</td>
<td>32.0$^b$</td>
<td>1.02</td>
<td>1.02</td>
<td>28.98</td>
<td>28.98</td>
<td>5.66</td>
<td>0.28</td>
<td>6.68</td>
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<tr>
<td>J-70 100% bound</td>
<td>36.0$^b$</td>
<td>1.02</td>
<td>1.02</td>
<td>28.98</td>
<td>29.04</td>
<td>7.26</td>
<td>0.17</td>
<td>9.01</td>
</tr>
<tr>
<td>J-70 100% bound</td>
<td>40.0$^b$</td>
<td>1.02</td>
<td>1.01</td>
<td>28.98</td>
<td>29.05</td>
<td>9.08</td>
<td>0.12</td>
<td>8.68</td>
</tr>
<tr>
<td>J-70 100% bound</td>
<td>44.0$^b$</td>
<td>1.02</td>
<td>1.00</td>
<td>28.98</td>
<td>28.92</td>
<td>15.34</td>
<td>0.14</td>
<td>1.46</td>
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<tr>
<td>J-70 100% bound</td>
<td>50.0$^b$</td>
<td>1.02</td>
<td>1.02</td>
<td>28.98</td>
<td>28.92</td>
<td>13.26</td>
<td>0.16</td>
<td>0.75</td>
</tr>
</tbody>
</table>
**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 overall $\tau_c = 28$ ns, with the J-domain dynamically linked with $S^2 = 0.37$ and $\tau_{local} = 4$ns.

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.*
Ahmad A, Bhattacharya A, McDonald RA, Cordes M, Ellington B, Bertelsen EB, Zuiderweg ER.
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 Nitrooxide 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

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).

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


Hsp70 chaperones and disease

- Hsp70’s are inhibitors of apoptosis in cancer cells
- Hsp70’s aggravate Alzheimer’s; Huntington’s; Parkinson’s
http://www.ahaf.org/alzheimers/about/understanding/plaques-and-tangles.html
Inhibition of Hsp70 clears tau

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
- Amyotrophic 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


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?).
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. Step 1, tau is phosphorylated by GSK-3\(\beta\), Cdk5, and other kinases and is released from the microtubules. Step 2, hyperphosphorylated tau is ubiquitinated by the CHIP-Hsc70 complex for degradation in the proteosome (step 3) or the formation of aggregates (step 4). 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*
Hsp70 at the center of intra-cellular protein homeostasis

Apoptosome

NEF

HIP

HOP

Hsp90

proteasome

J

ATP/ADP

Client

CHIP

Client
Hsp70 at the center of intra-cellular protein homeostasis

- Apoptosome
- NEF
- HIP
- HOP
- J
- CHIP
- Hsp90
- proteasome

Client
Hsp70 at the center of intra-cellular protein homeostasis
Hsp70 at the center of intra-cellular protein homeostasis

MANY interference opportunities

Apoptosome

NEF

HIP

HOP

Hsp90

proteasome

CHIP

Client
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**

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

The anti-cancer drug MKT077 is an Hsp70 ALLOSTERIC effector

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
Table 2. MKT-077 binding enthalpy according to different computational methods

<table>
<thead>
<tr>
<th>Subfamily in Fig 10</th>
<th>AUTODOCK Energy (kCal/M)</th>
<th>MM GB Energy (kCal/M)</th>
<th>MM PBSA Energy (kCal/M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panel C</td>
<td>7.03</td>
<td>-16.6 ± 1.8</td>
<td>-7.5 ± 3.7</td>
</tr>
<tr>
<td>Panel D</td>
<td>6.32</td>
<td>-18.6 ± 1.5</td>
<td>-14.0 ± 2.1</td>
</tr>
<tr>
<td>Panel E</td>
<td>5.36</td>
<td>-18.1 ± 1.2</td>
<td>-10.8 ± 2.0</td>
</tr>
<tr>
<td>Panel F</td>
<td>5.25</td>
<td>-22.8 ± 1.3</td>
<td>-13.8 ± 2.6</td>
</tr>
</tbody>
</table>
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, Massachusetts
David McKay, Stanford
Jochen Reinstein, Dortmund
Alexander Joachimiak, Argonne
Matthias Mayer, Heidelberg
Jason Gestwicki, Michigan
RESEARCH FUNDING:
Structure, Dynamics and Function of Chaperone Domains

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\textdegree C.

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


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

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