Molekulární chaperony a jejich úloha v patogenezi lidských chorob

Petr Muller

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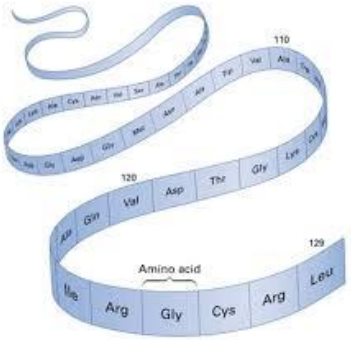
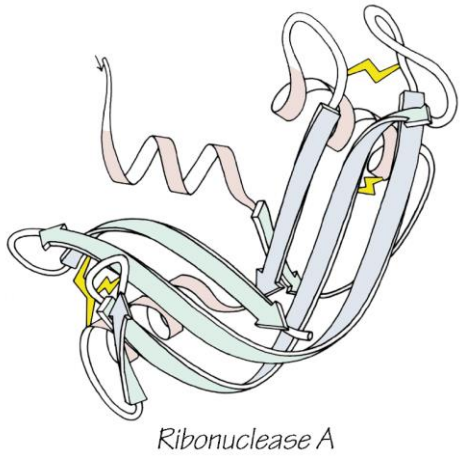
for Applied Molecular
Oncology

MASARYKŮV ONKOLOGICKÝ ÚSTAV

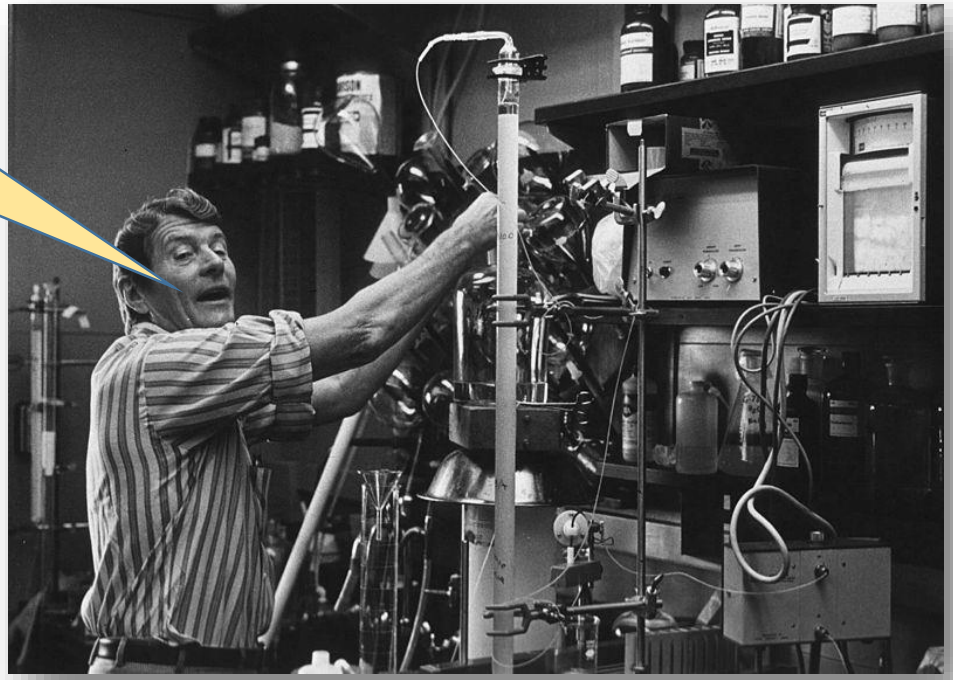
At the environmental conditions (temperature, solvent concentration and composition, etc.) at which folding occurs, the native structure is a unique, stable and kinetically accessible minimum of the free energy





Ribonuclease A

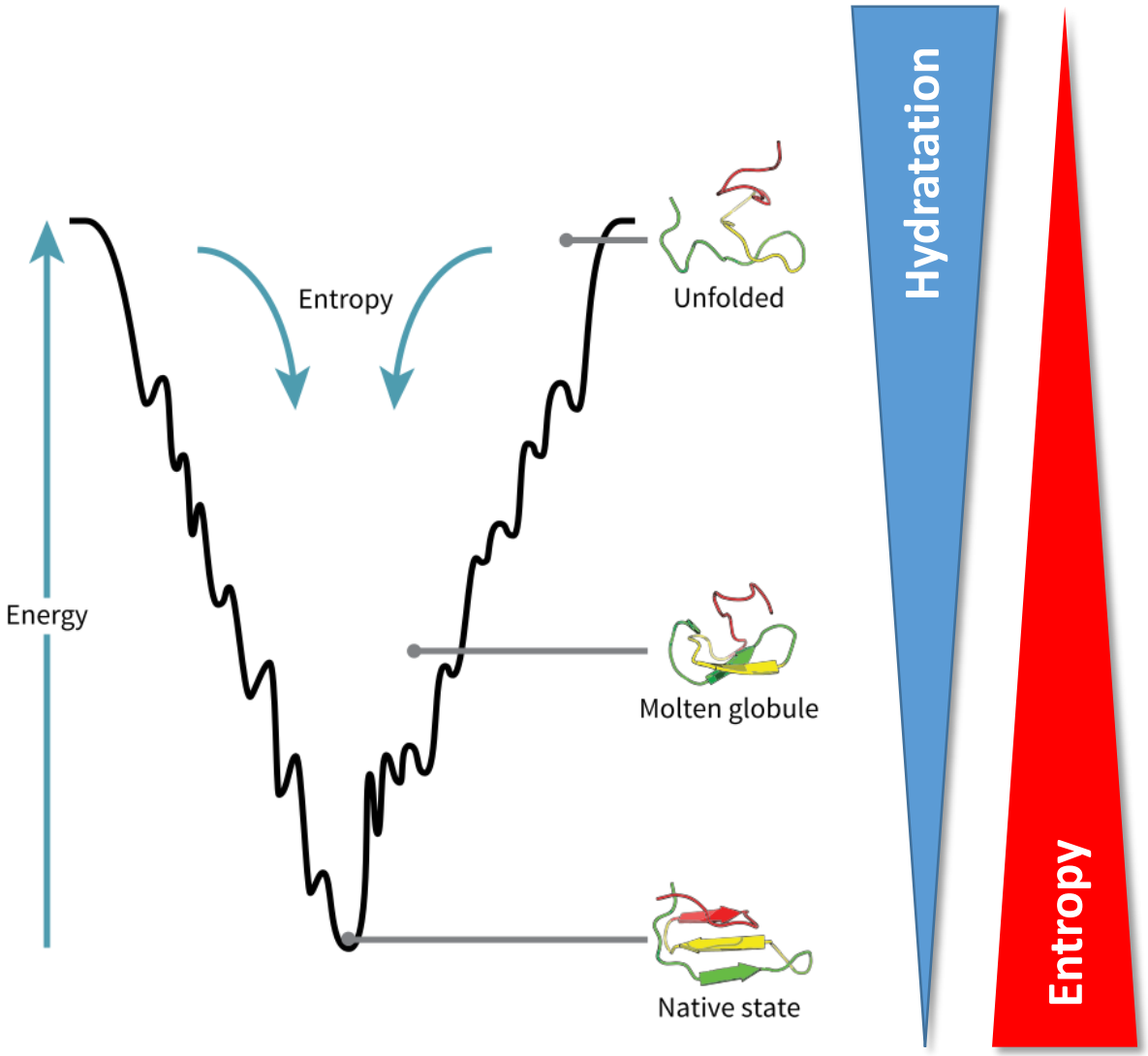
The native structure is determined only by the protein's amino acid sequence

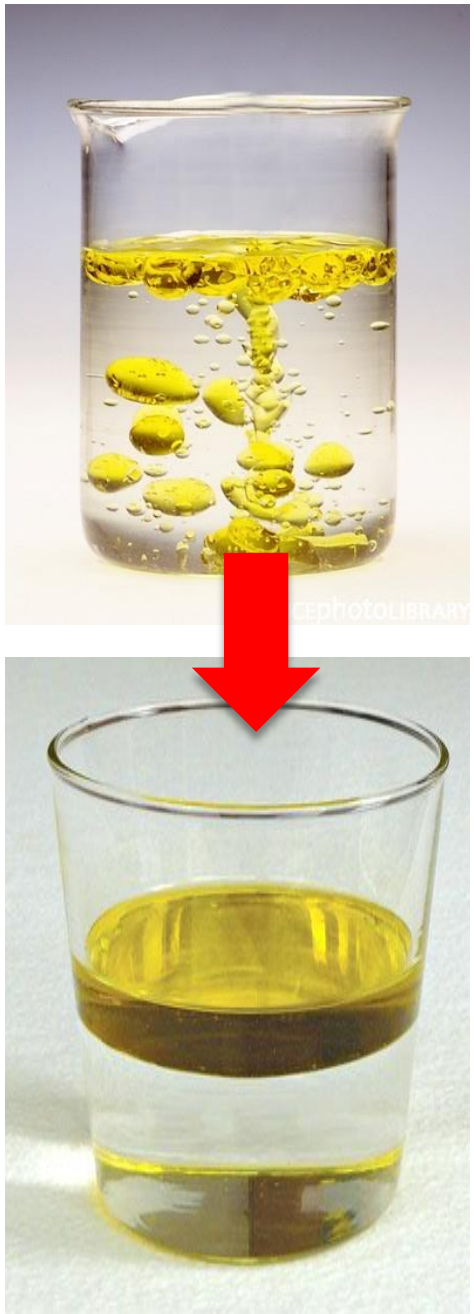


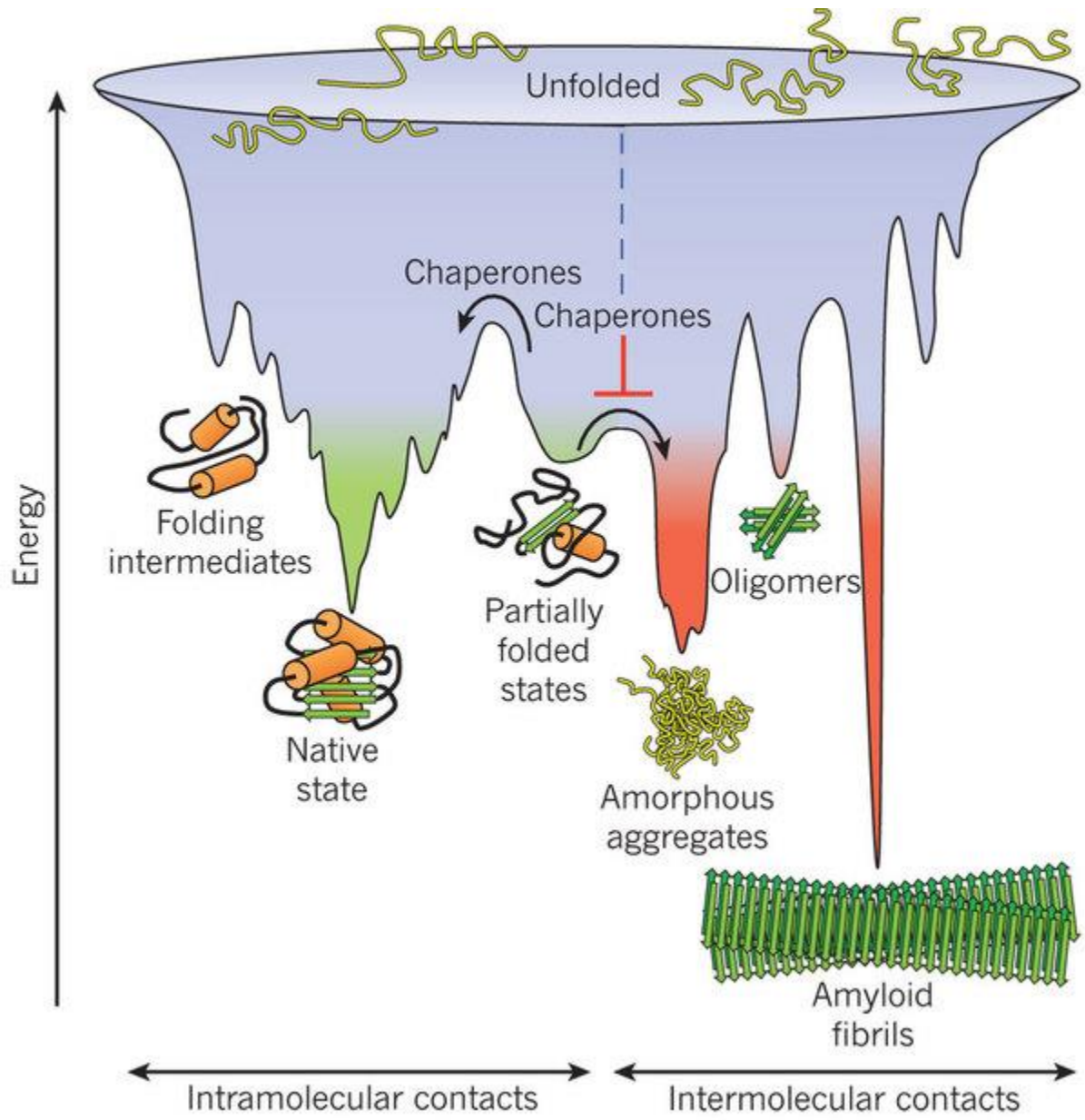
Christian Boehmer Anfinsen, Jr.
(March 26, 1916 – May 14, 1995)

Nobel Prize in Chemistry (1972)

Folding is entropy driven process

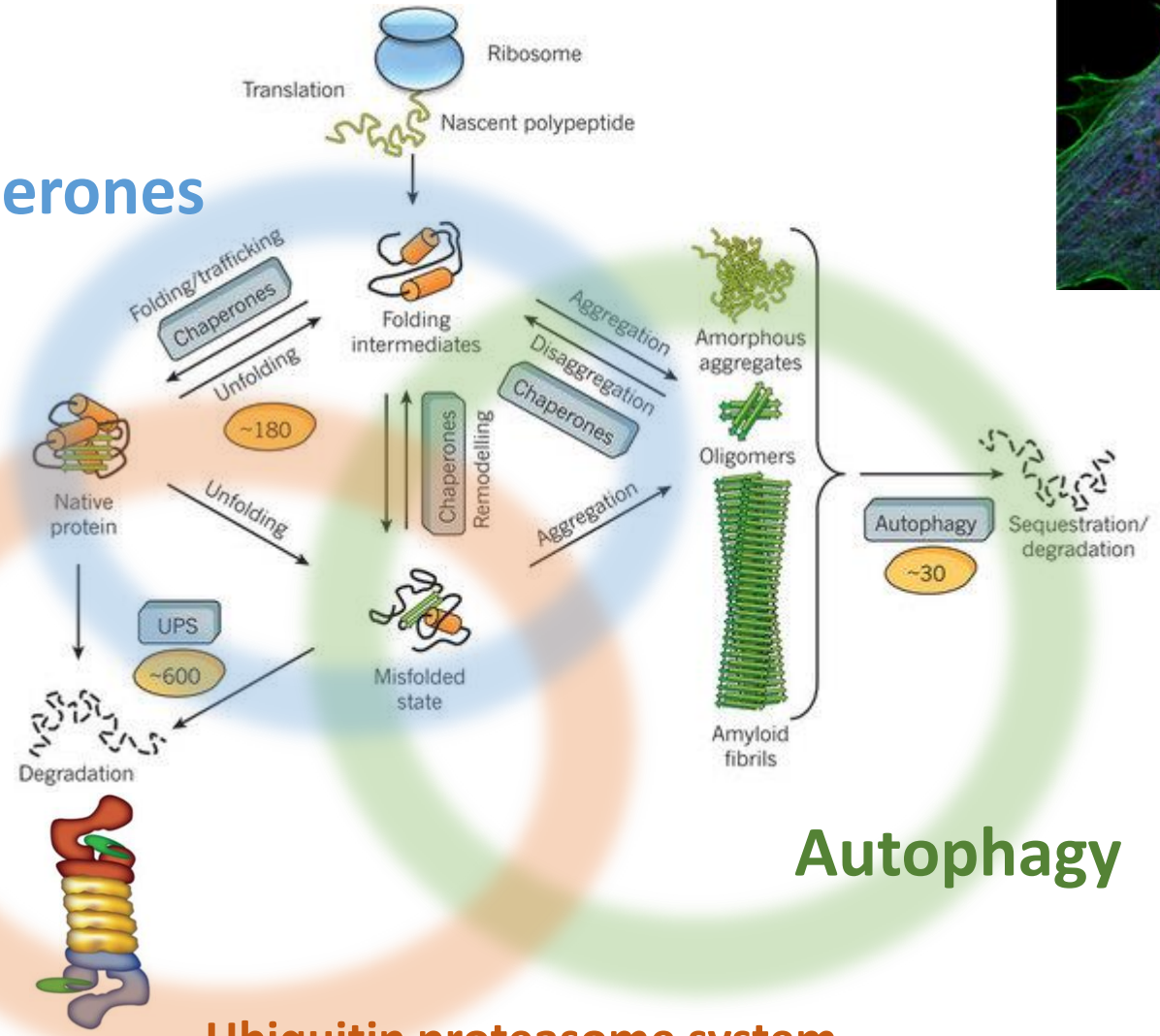





Unfolded
Chaperones
Chaperones
Folding intermediates
Native state
Partially folded states
Oligomers
Amorphous aggregates
Amyloid fibrils
Energy
Intramolecular contacts
Intermolecular contacts

Protein homeostasis / proteostasis

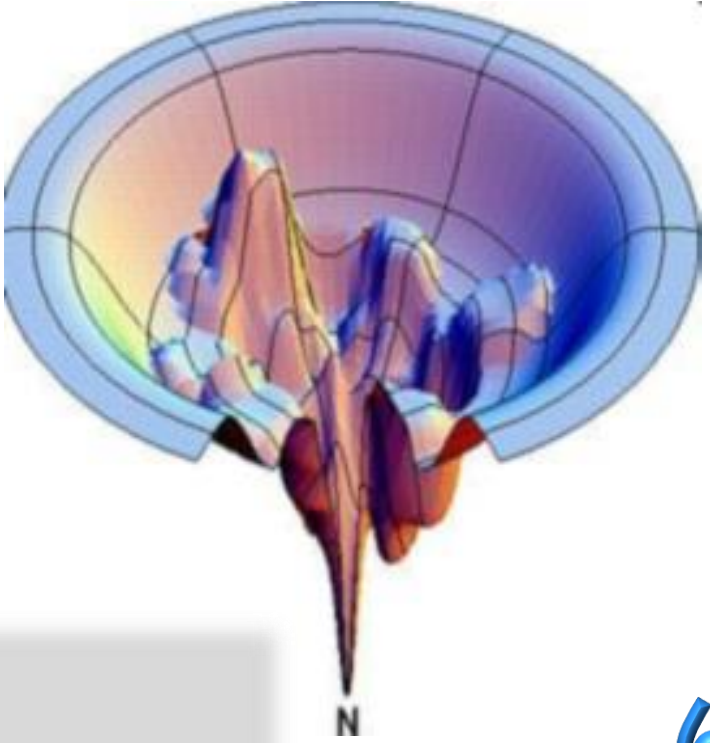
Chaperones



Autophagy

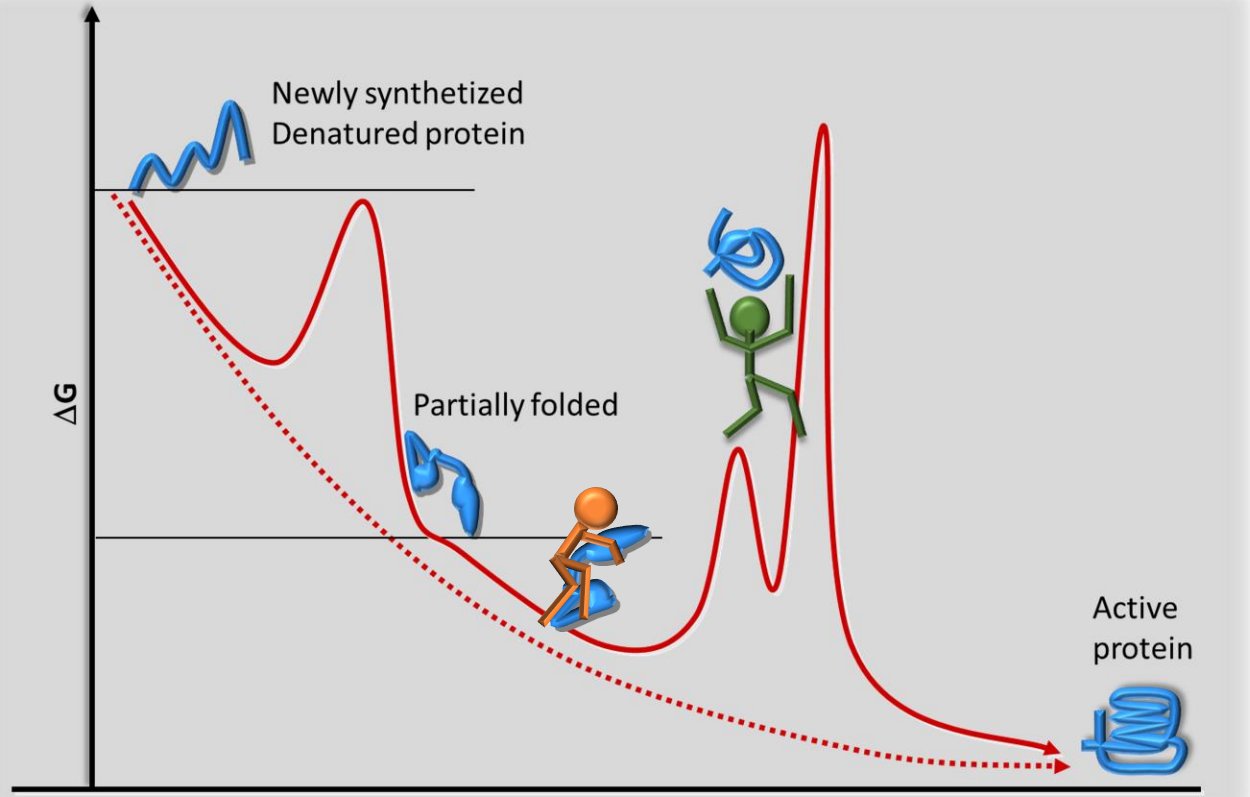
Ubiquitin proteasome system

Stress proteins / Chaperones



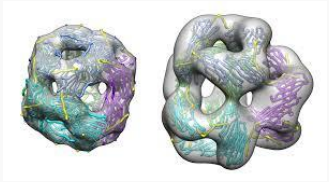
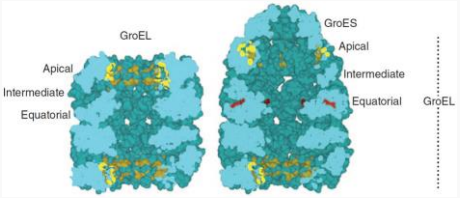
Holdases bind folding intermediates to prevent their aggregation

Crystalins, p23, Hsp40…

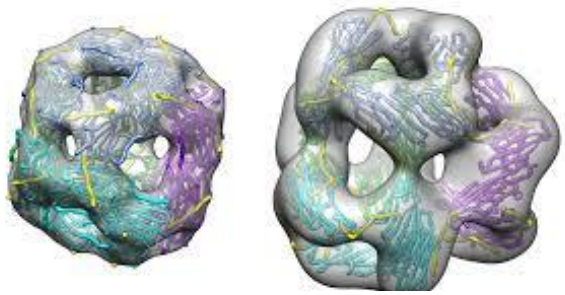


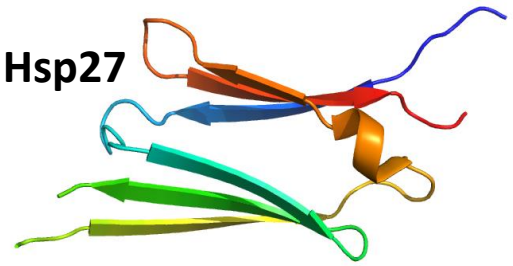
Foldases are chaperones that accompany other proteins to help them to overcome the energy barriers during folding to native conformation
(ATP dependent)

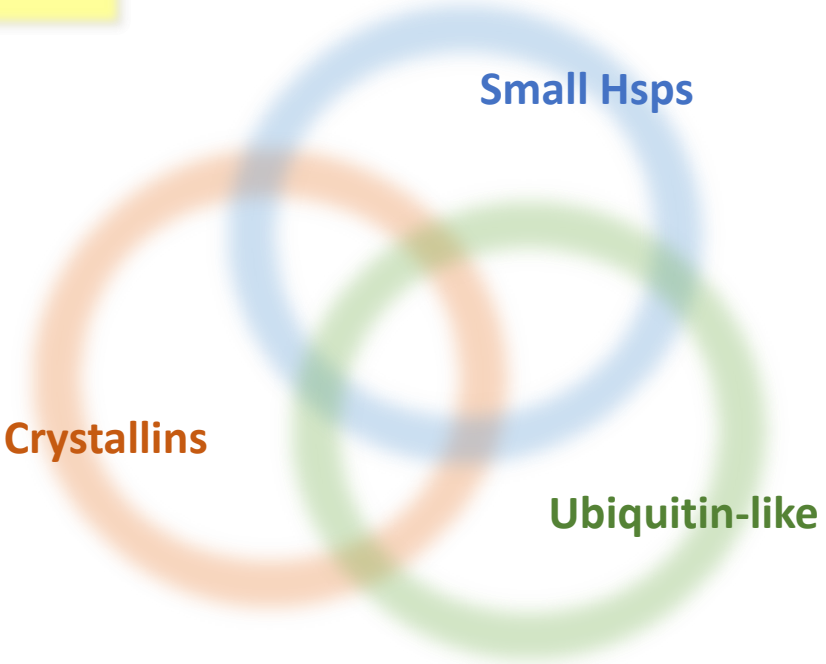
Hsp70, Hsp90, GroEL…

Approximate molecular weight(kDa)	Prokaryotic proteins	Eukaryotic proteins	Function
10 kDa	GroES	Hsp10	
20-30 kDa	GrpE	The HspB group of Hsp. Eleven members in mammals including Hsp27, HSPB6 or HspB1[28]	
40 kDa	DnaJ	Hsp40	Co-factor of Hsp70
60 kDa	GroEL, 60kDa antigen	Hsp60 	Involved in protein folding after its post-translational import to the mitochondrion/chloroplast
70 kDa	DnaK	The HspA group of Hsp including Hsp71,Hsp70, Hsp72, Grp78 (BiP), Hsx70 found only in primates	Protein folding and unfolding, provides thermotolerance to cell on exposure to heat stress. Also prevents protein folding during post-translational import into the mitochondria/chloroplast.
90 kDa	HtpG, C62.5	The HspC group of Hsp including Hsp90, Grp94	Maintenance of steroid receptors and transcription factors
100 kDa	ClpB, ClpA, ClpX	Hsp104, Hsp110	Tolerance of extreme temperature

HspB group/ small chaperones



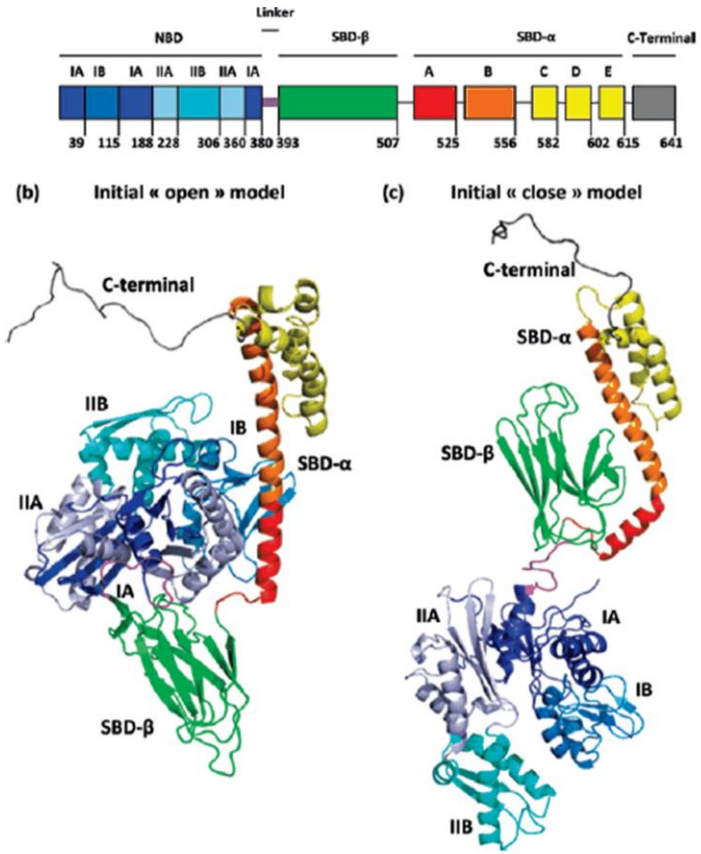




Prevent aggregation
Thermotolerance

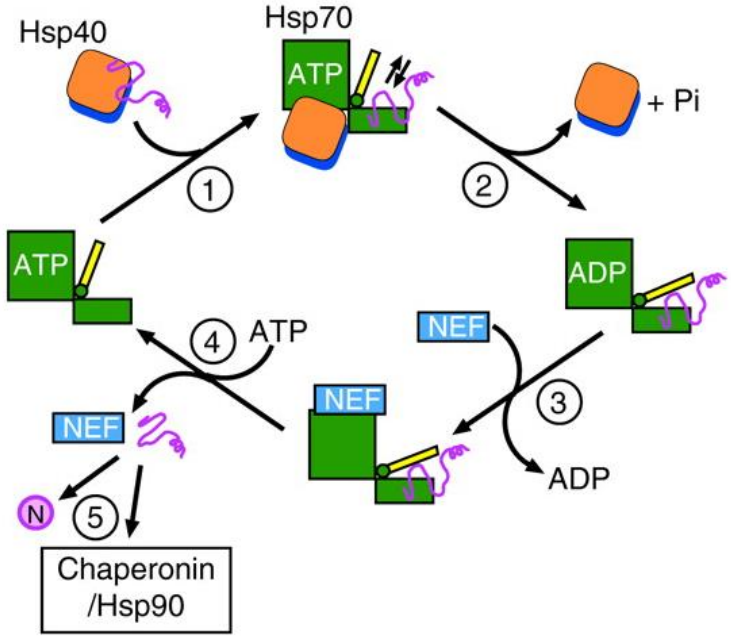
	Gene name	Protein name	Old names	Human gene ID	Mouse ortholog ID
1	*HSPB1*	HSPB1	CMT2F; HMN2B; HSP27; HSP28; HSP25; HS.76067; DKFZp586P1322	3315	15507
2	*HSPB2*	HSPB2	MKBP; HSP27; Hs.78846; LOH11CR1K; MGC133245	3316	69253
3	*HSPB3*	HSPB3	HSPL27	8988	56534
4	*HSPB4*[a]	HSPB4	crystallin alpha A; CRYAA, CRYA1	1409	12954
5	*HSPB5*[a]	HSPB5	crystallin alpha B, CRYAB; CRYA2	1410	12955
6	*HSPB6*	HSPB6	HSP20; FLJ32389	126393	243912
7	*HSPB7*	HSPB7	cvHSP; FLJ32733; DKFZp779D0968	27129	29818
8	*HSPB8*	HSPB8	H11; HMN2; CMT2L; DHMN2; E2IG1; HMN2A; HSP22	26353	80888
9	*HSPB9*	HSPB9	FLJ27437	94086	75482
10	*HSPB10*[a]	HSPB10	ODF1; ODF; RT7; ODF2; ODFP; SODF; ODF27; ODFPG; ODFPGA; ODFPGB; MGC129928; MGC129929	4956	18285
11	*HSPB11*	HSPB11	HSP16.2; C1orf41; PP25	51668	72938

Hsp70 (DnaK, Grp78,..) chaperone machinery



ATP

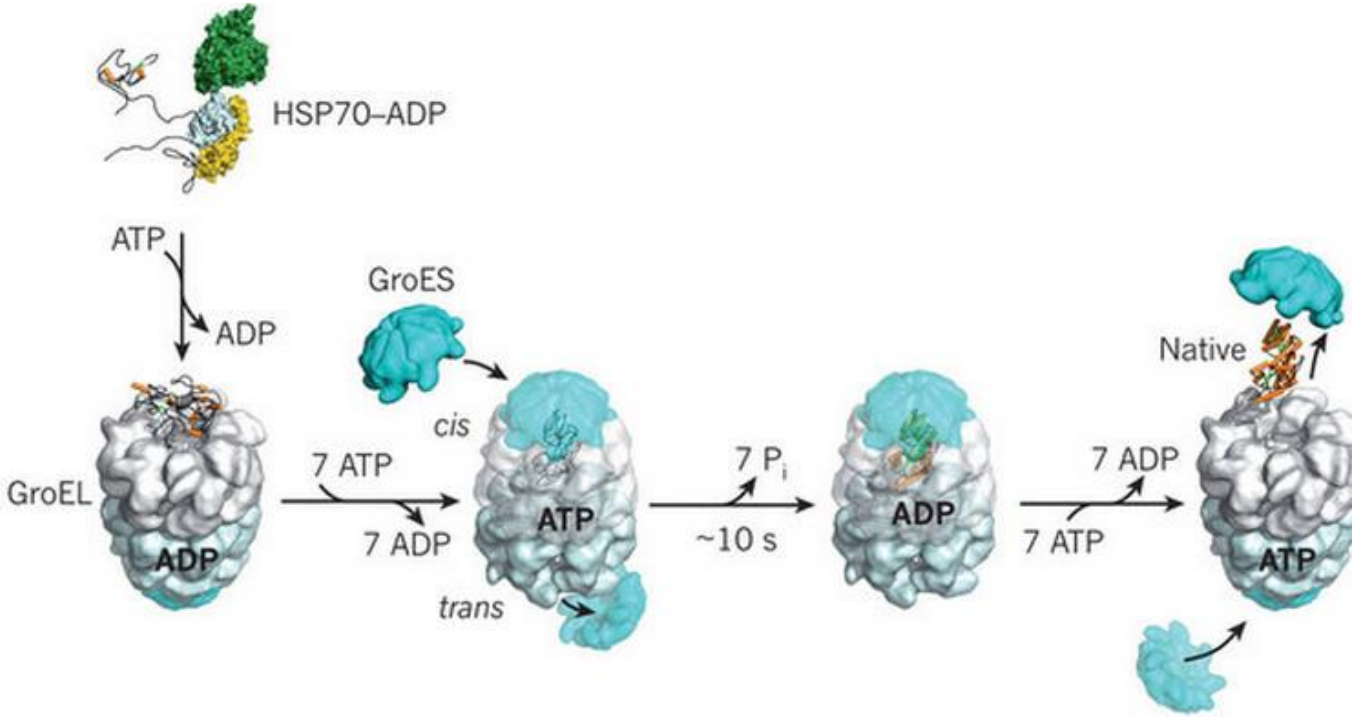
ADP



BAG NEF-Nucleotide exchange factor

Hsp40 DnaJ J-proteins

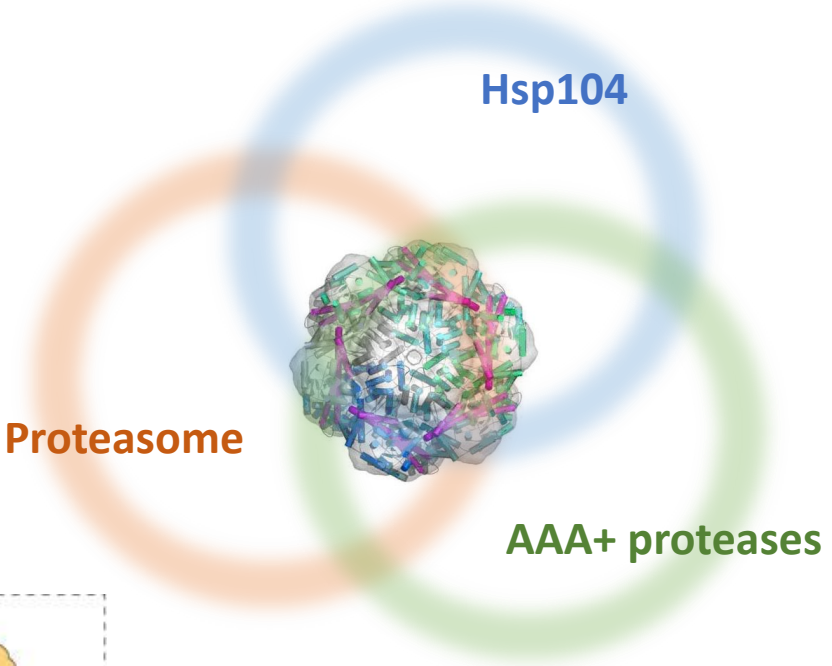
Chaperonins
(GroEL-GroES, Hsp60, CCT-TRiC)

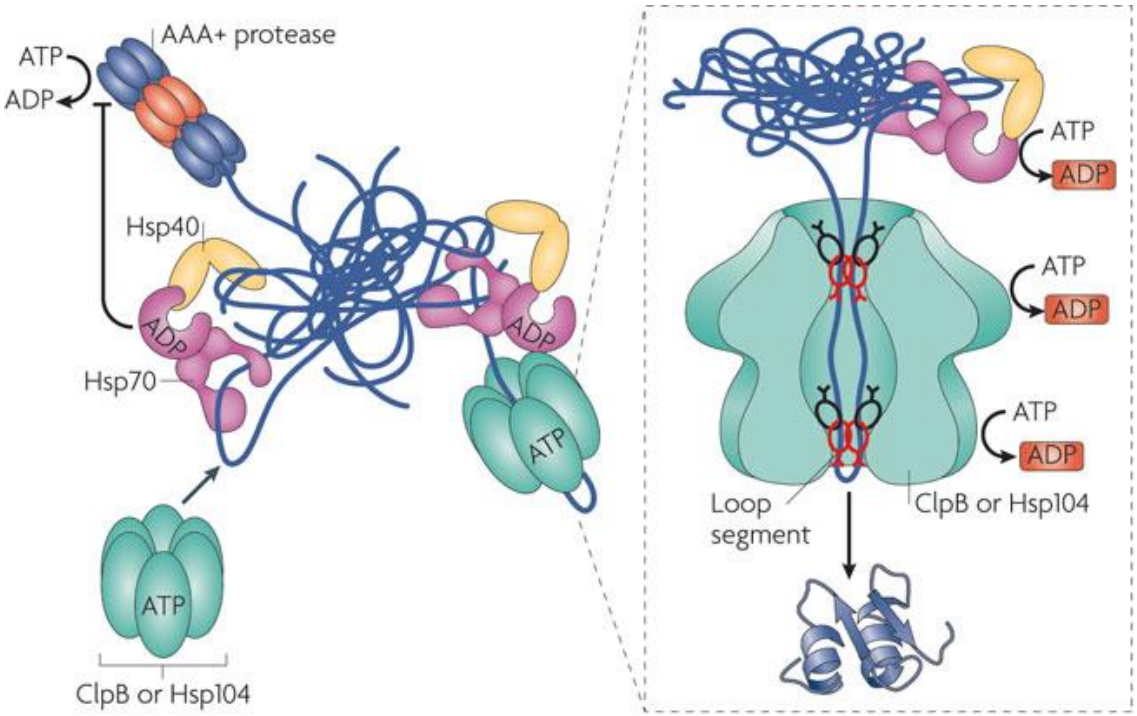


Folding of cytoskeletal proteins (tubulin)
Protein transport

Hsp104 (ClpB, ClpX,..)

Thermotolerance
Aggregate refolding
Prion folding (yeast Psi+/-)





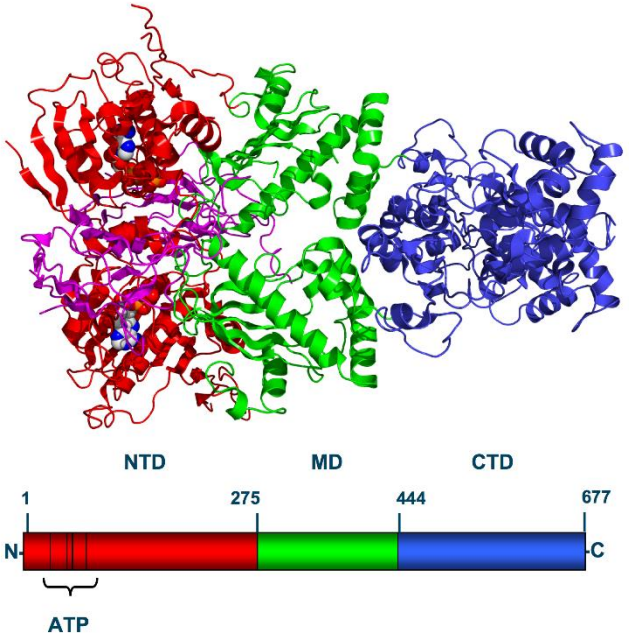
Nature Reviews | Molecular Cell Biology

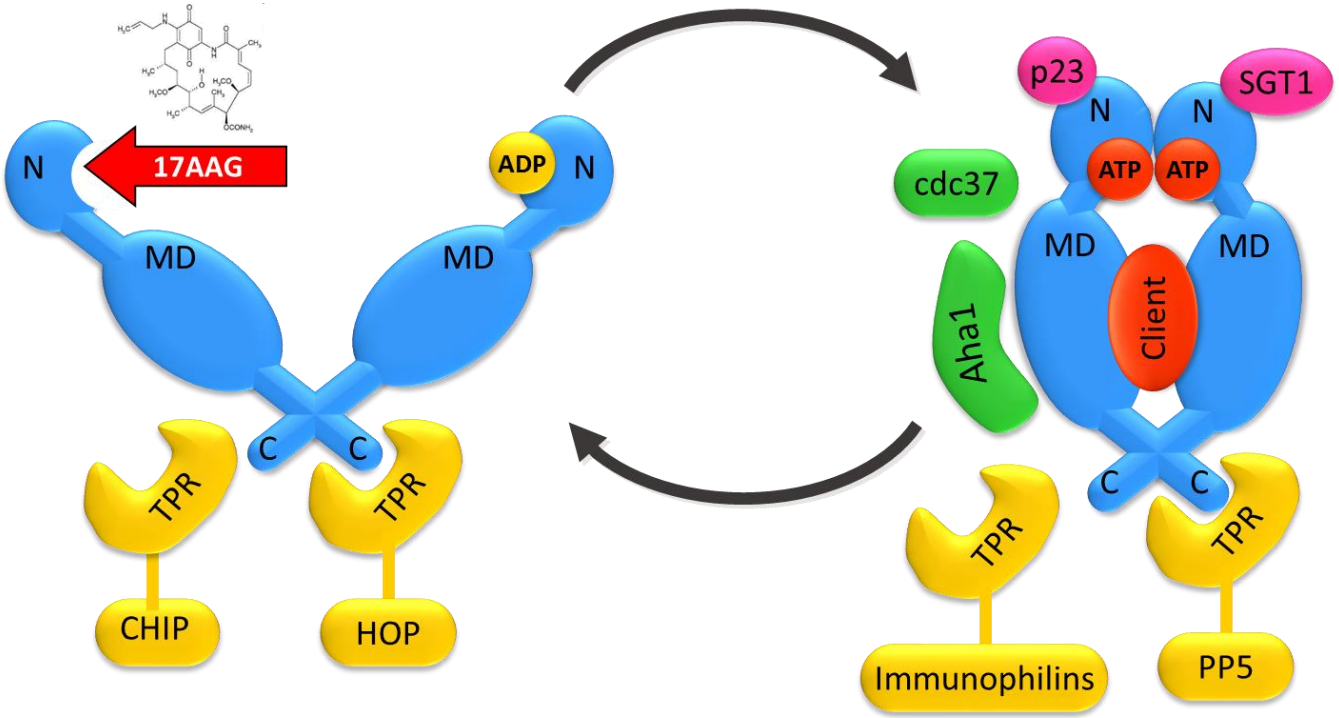
AAA+ ATPases

Converts ATP to "mechanical" energy (molecular motors)

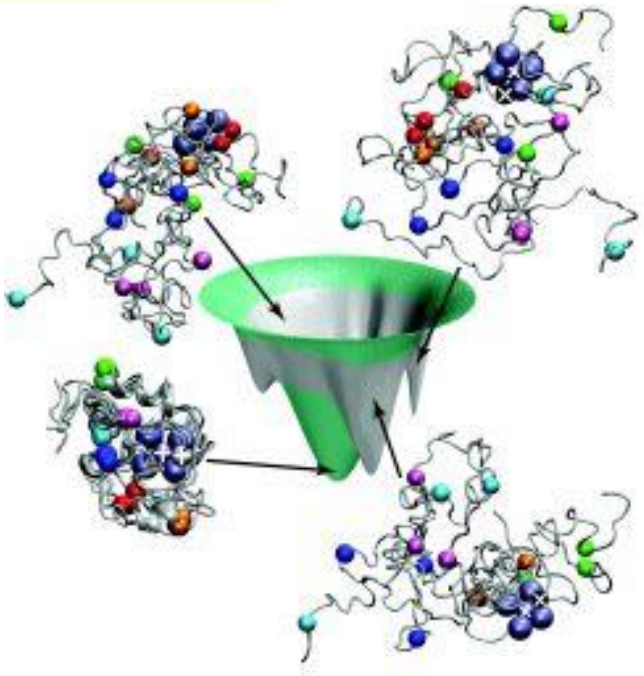
Hsp90
chaperone machinery

- Conserved from procarytes to mammals
- ATPase aktivity (like gyrase)
- Mitochndrial, ER, cytoplasmic
- Redundant isoformes





Stress proteins/ Chaperones/Hsp90



Hsp90 as a capacitor for morphological evolution

Suzanne L. Rutherford*† & Susan Lindquist*

*Howard Hughes Medical Institute, University of Chicago, 5841 South Maryland Avenue MC1028, Chicago, Illinois 60637, USA

NATURE | VOL 396 | 26 NOVEMBER 1998 | www.nature.com

FOLIA MENDELIANA 49/2
Supplementum ad Acta Musei Moraviae *XCVIII, 2013*

CHAPERONES AND EVOLUTION

FILIP TRCKA, BORIVOJ VOJTESEK, PETR MULLER
Regional Centre for Applied Molecular Oncology, Masaryk Memorial Cancer Institute,
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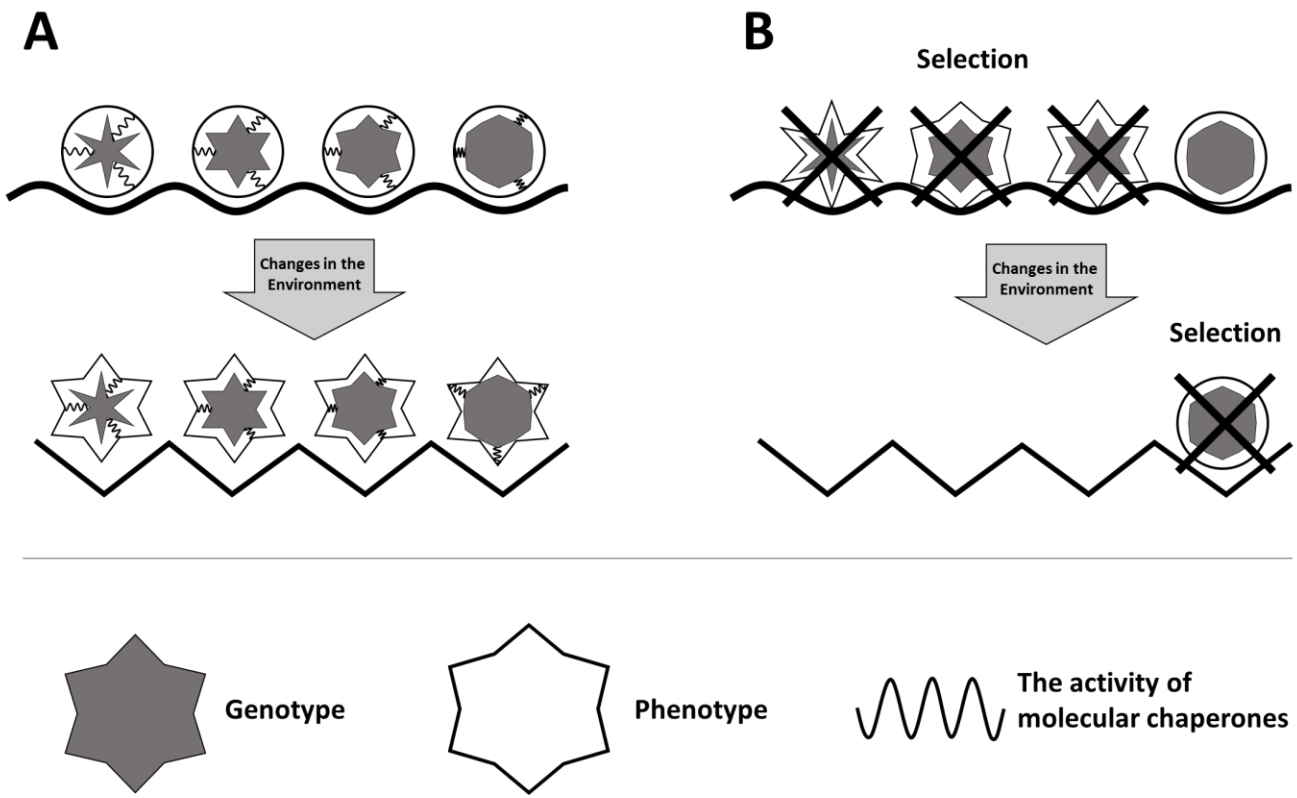
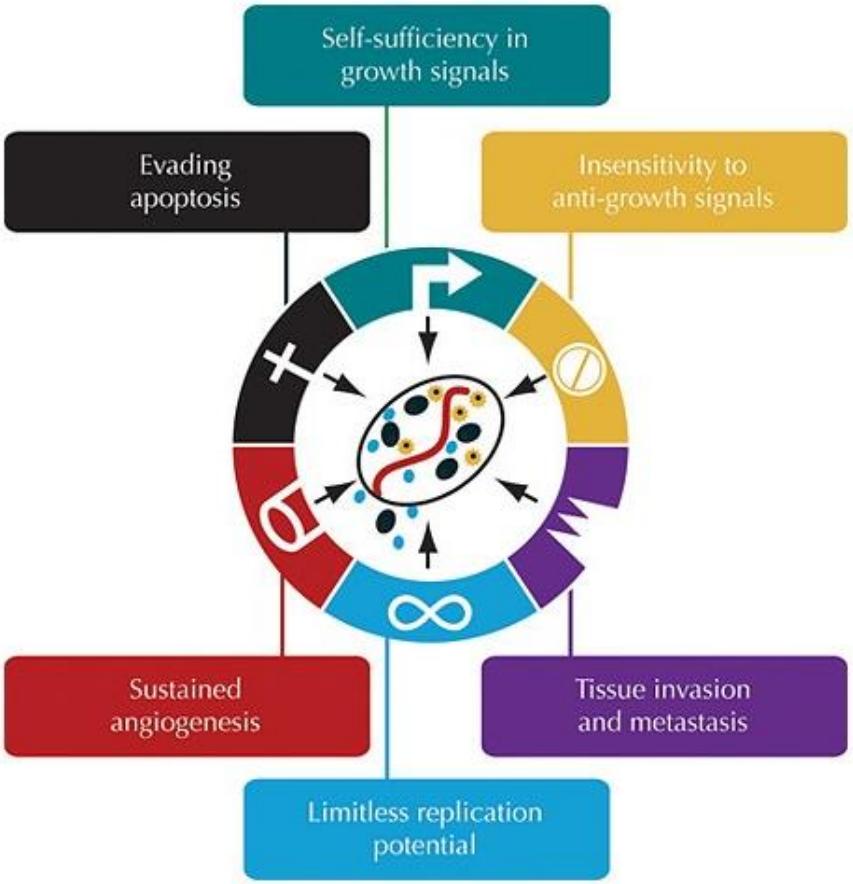
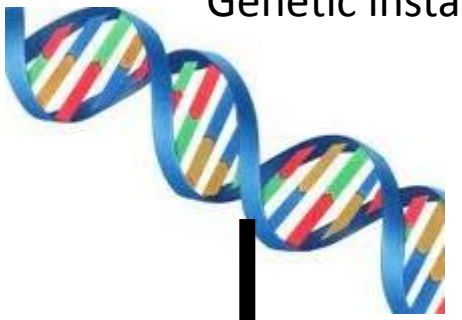


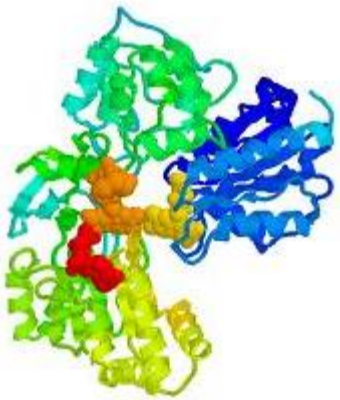
The tumor cells demand high quality and amount of protein



Genetic instability

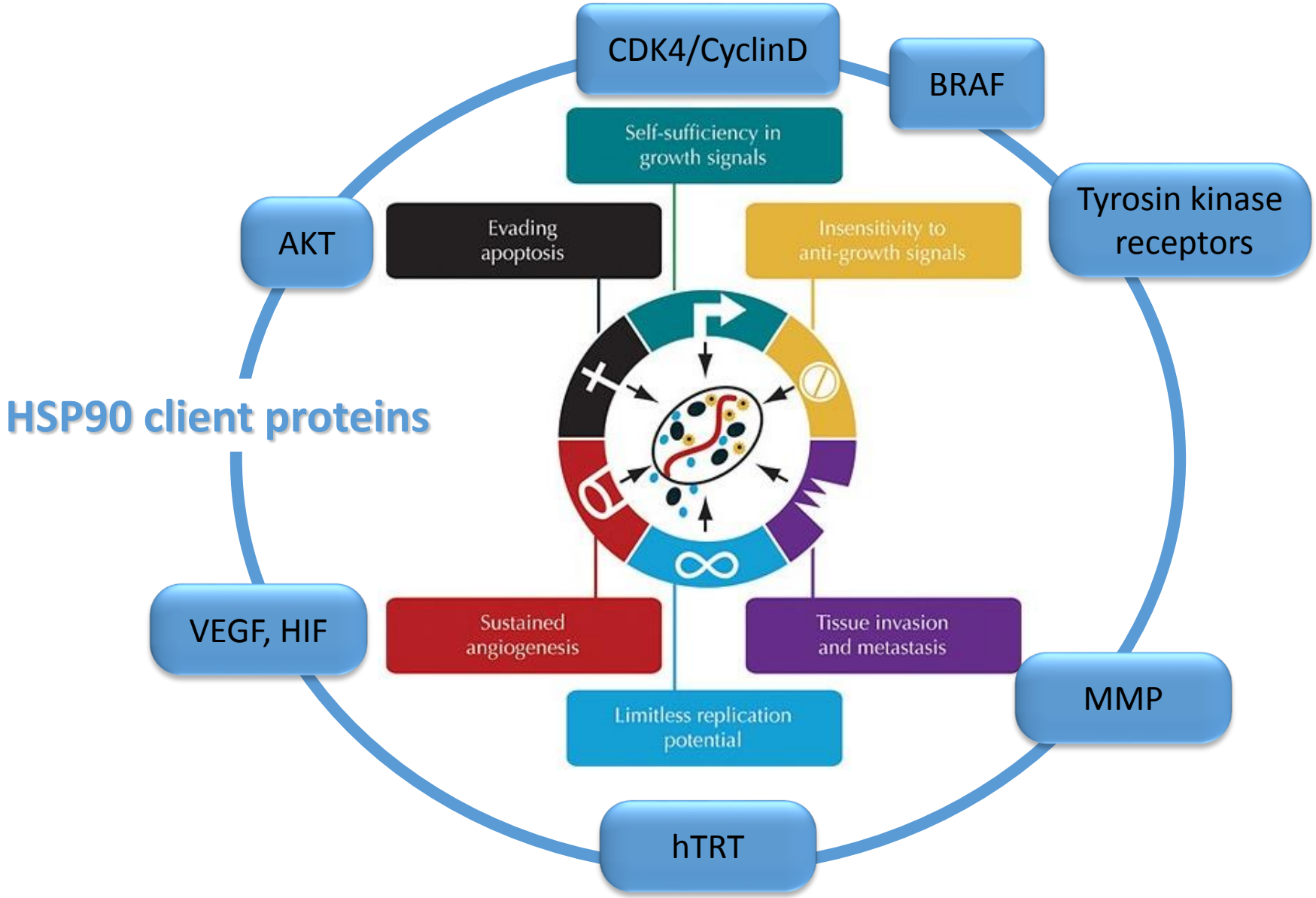


Enhanced proteosynthesis
Production of mutated, conformational instable protins



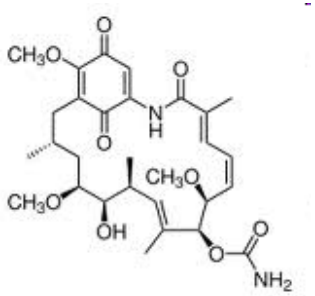
Hanahan D, Weinberg RA.: Cell. 2000 Jan 7;100(1):57-70.

Activity of Hsp90 is essential for expression of cancer phenotype

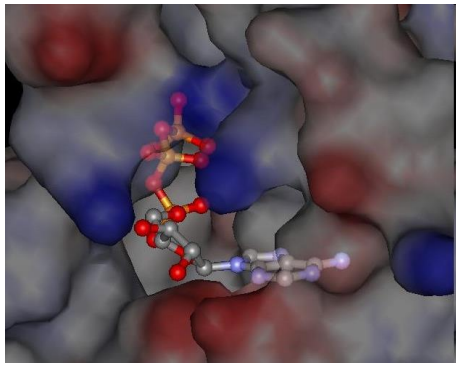


Specific inhibitors Hsp90

Isolation of Geldanamycin (1970)



Geldanamycin binds ATP cavity of Hsp90 (1997)

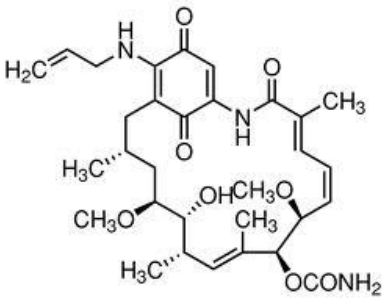


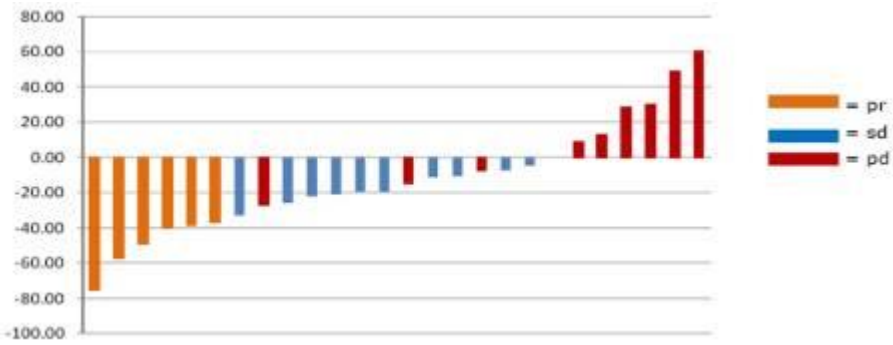
Clinical trials with Geldanamycin(2000)

	inhibitor	No of studies	phase	Company
1	tanespimycin (17AAAG)	36	III	Bristol-Myers Squibb, Kosan
2	retaspimycin (IPI-504)	11	II/III*	Infinity Pharmaceuticals
3	alvespimycin (17DMAG)	7	II	Bristol-Myers Squibb, Kosan
4	STA-9090	14	II	Synta Pharmaceuticals Corp.
5	AUY922	11	II	Novartis Pharmaceuticals
6	CNF2024 (BIIB021)	7	II	Biogen Idec
7	SNX-5422	4	I	Pfizer, Serenex, Inc.
8	AT13387	3	I	Astex Therapeutics
9	KW-2478	2	I/II	Kyowa Hakko Kirin Pharma, Inc.
10	IPI-493	2	I	Infinity Pharmaceuticals
11	HSP990	2	I	Novartis Pharmaceuticals
12	MPC-3100	1	I	Myrexis Inc.
13	Debio 0932	1	I	Debiopharm S.A.
15	BIIB028	1	I	Biogen Idec

Hsp90 is unique therapeutic target for anti-cancer therapy

more than 17 different molecules in clinical trials





Variable response
need for predictive markers

What does kill the cells:

- apoptosis, aggregation, ….

Different assembly of Hsp90 machinery ?

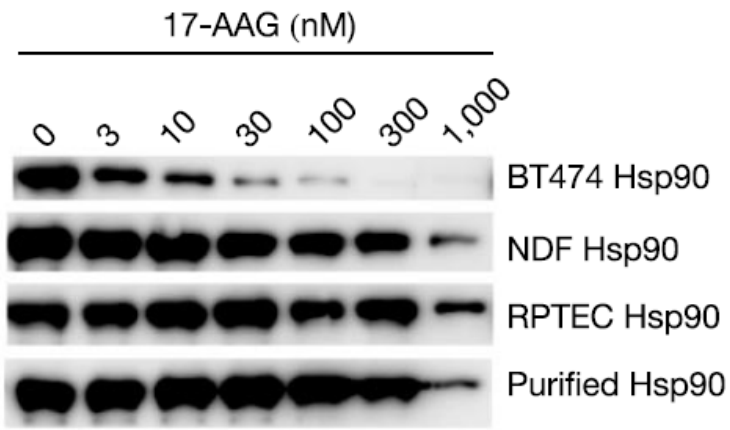
- posttranlational modifications
- expression pattern of co-chaperones

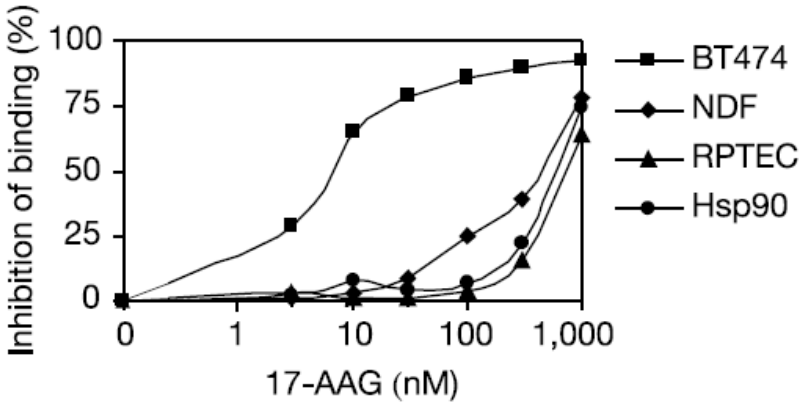
Client spectrum ?

letters to nature

A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors

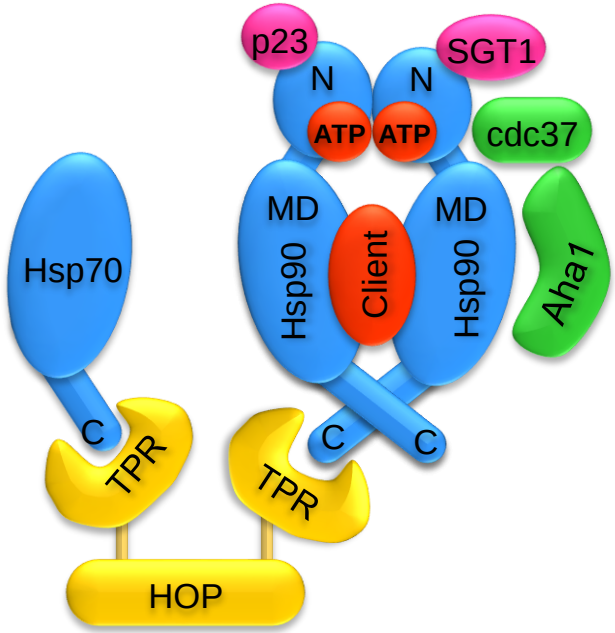
Adeela Kamal, Lia Thao, John Sensintaffar, Lin Zhang, Marcus F. Boehm, Lawrence C. Fritz & Francis J. Burrows





Multichaperone complex

- Hsp90+Hsp70
- cochaperones



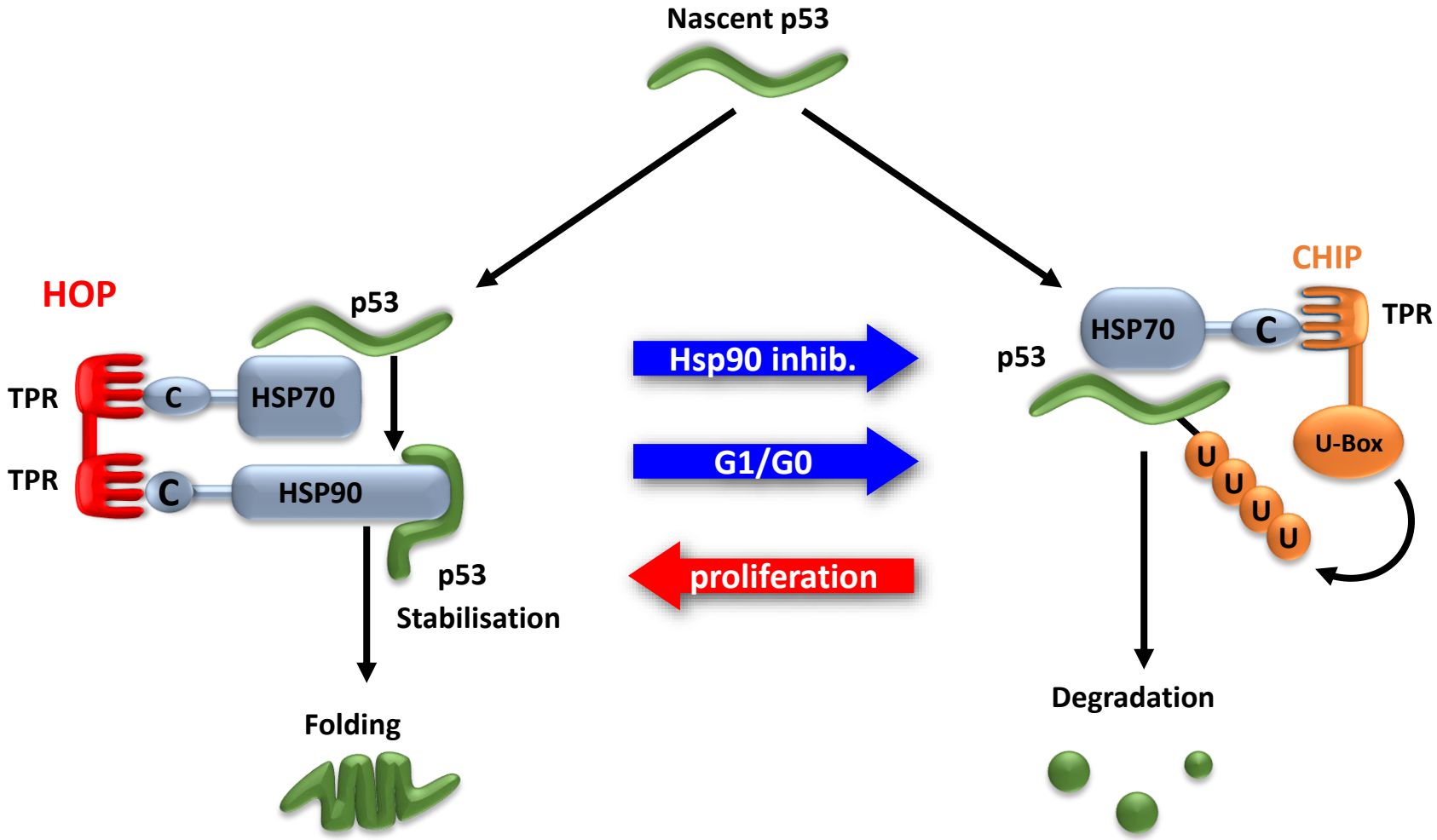
Oncogene (2008) 27, 3371–3383

www.nature.com/onc

ORIGINAL ARTICLE

Chaperone-dependent stabilization and degradation of p53 mutants

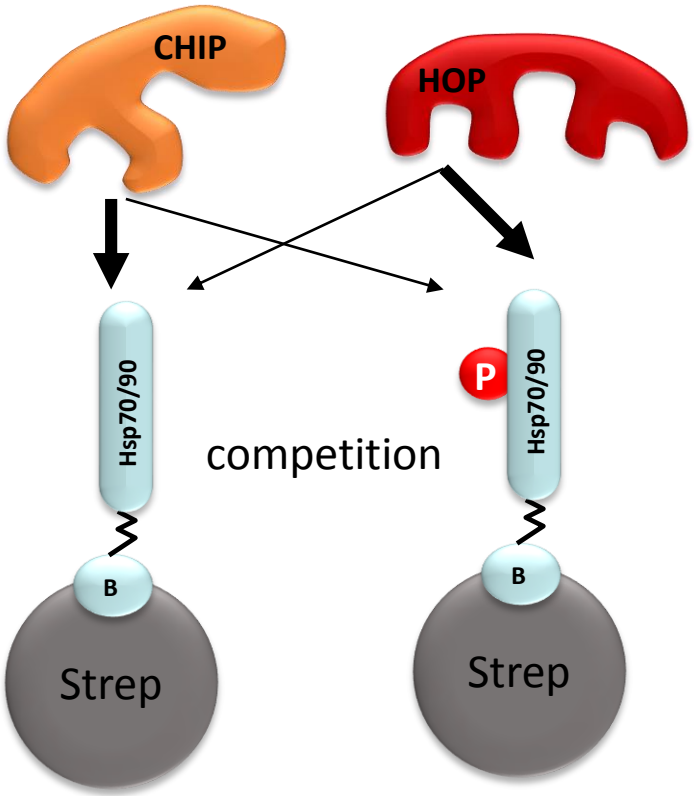
P Muller[1,2], R Hrstka[1], D Coomber[2], DP Lane[2] and B Vojtesek[1]

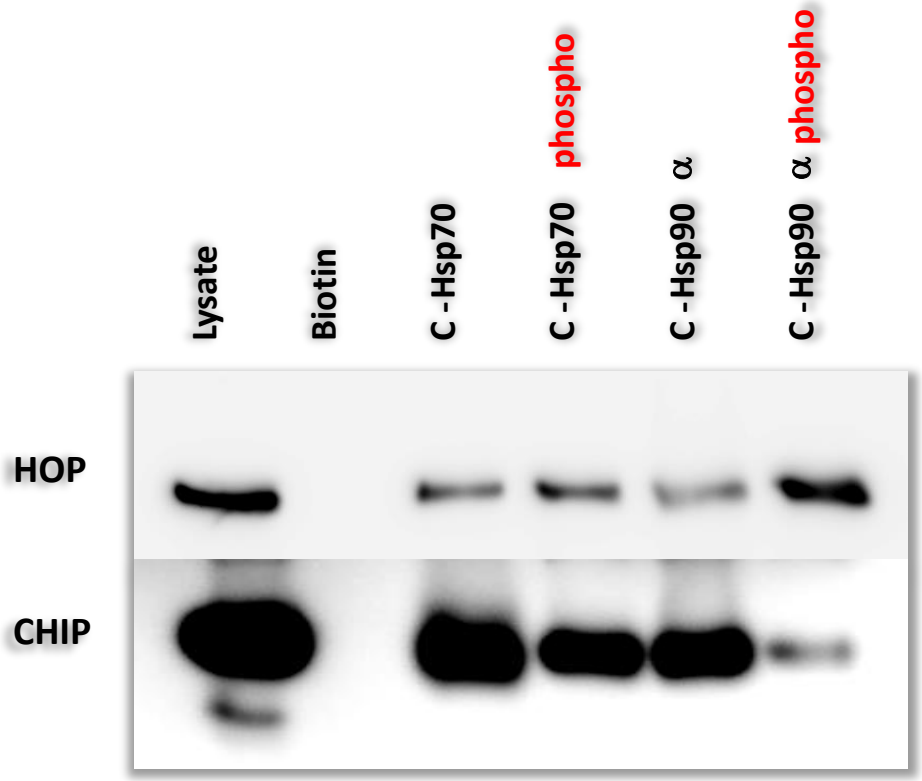


What is the mechanism regulating folding degradation balance ?

Cell lysate pulldown of HOP and CHIP

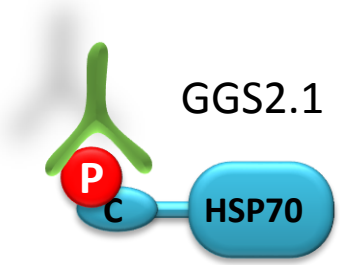
- **Biotinylated phospho/non phospho peptides of Hsp70/Hsp90**

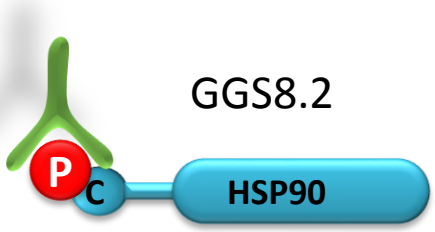


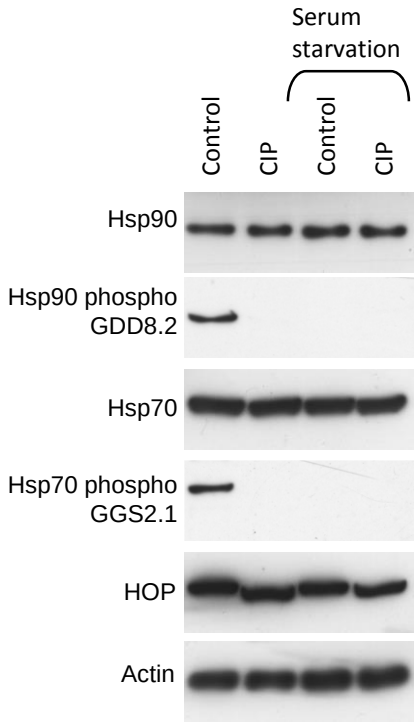


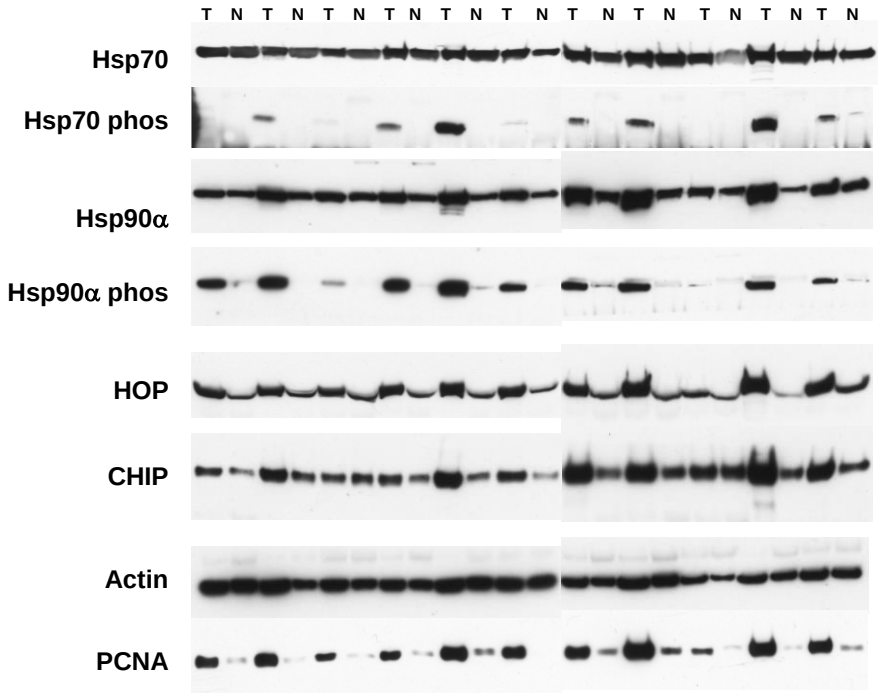
Detection of phosphorylated Hsp70 and Hsp90

phospho-specific monoclonal antibodies antibodies

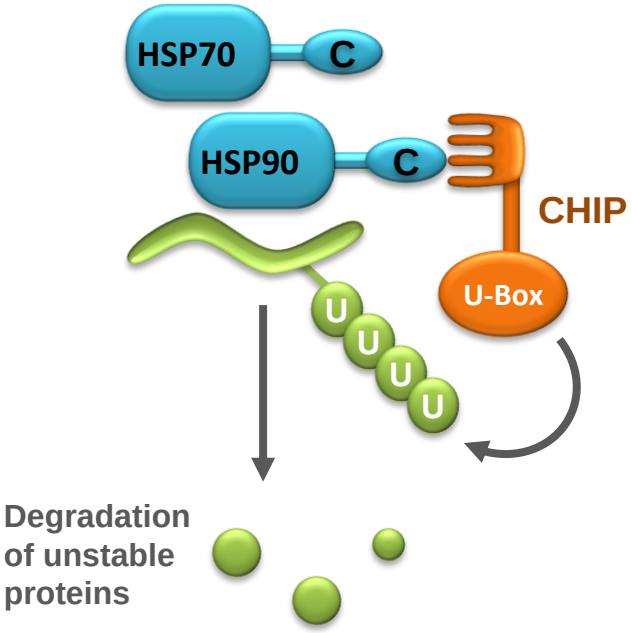




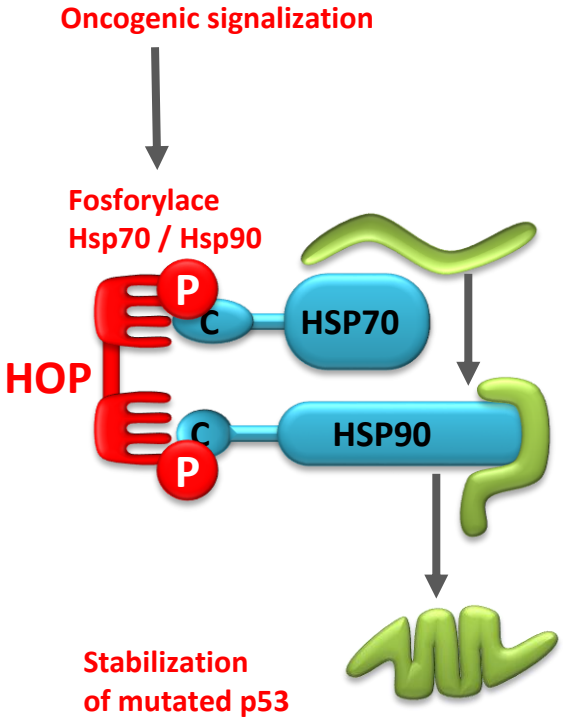




Normal differentiated cell



Cancer cell



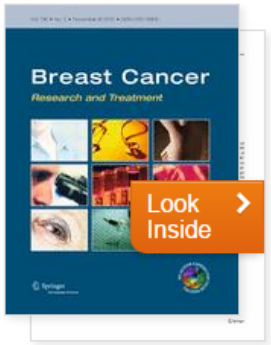
Normal differentiated cell	Cancer cell
C-terminus Hsp70/90 non phosphorylated	Phosphorylated Hsp90 Hsp70
Hsp bind preferentially CHIP	Hsps bind preferentially HOP
Designed to degrade unfolded protein	High folding capacity of Hsp90
Higher expression of CHIP	Increased level of HOP
Lower sensitivity ti Hsp90 inhibitors	High sensitivity to Hsp90 inhibitors

Breast Cancer Research and Treatment
November 2012, Volume 136, Issue 2, pp 419-427

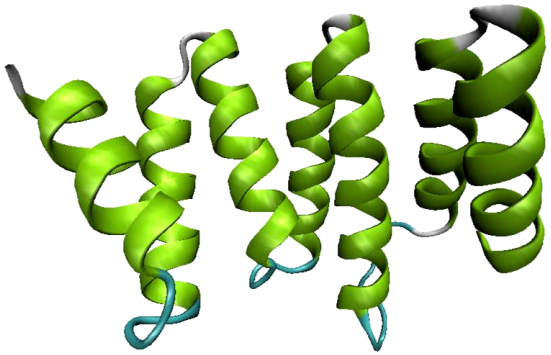
Date: 04 Oct 2012

TOMM34 expression in early invasive breast cancer: a biomarker associated with poor outcome

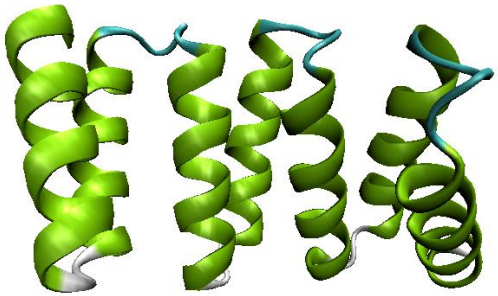
Mohammed A. Aleskandarany, Ola H. Negm, Emad A. Rakha, Mohamed A. H. Ahmed, Christopher C. Nolan, Graham R. Ball, Carlos Caldas, Andrew R. Green, Patrick J. Tighe, Ian O. Ellis



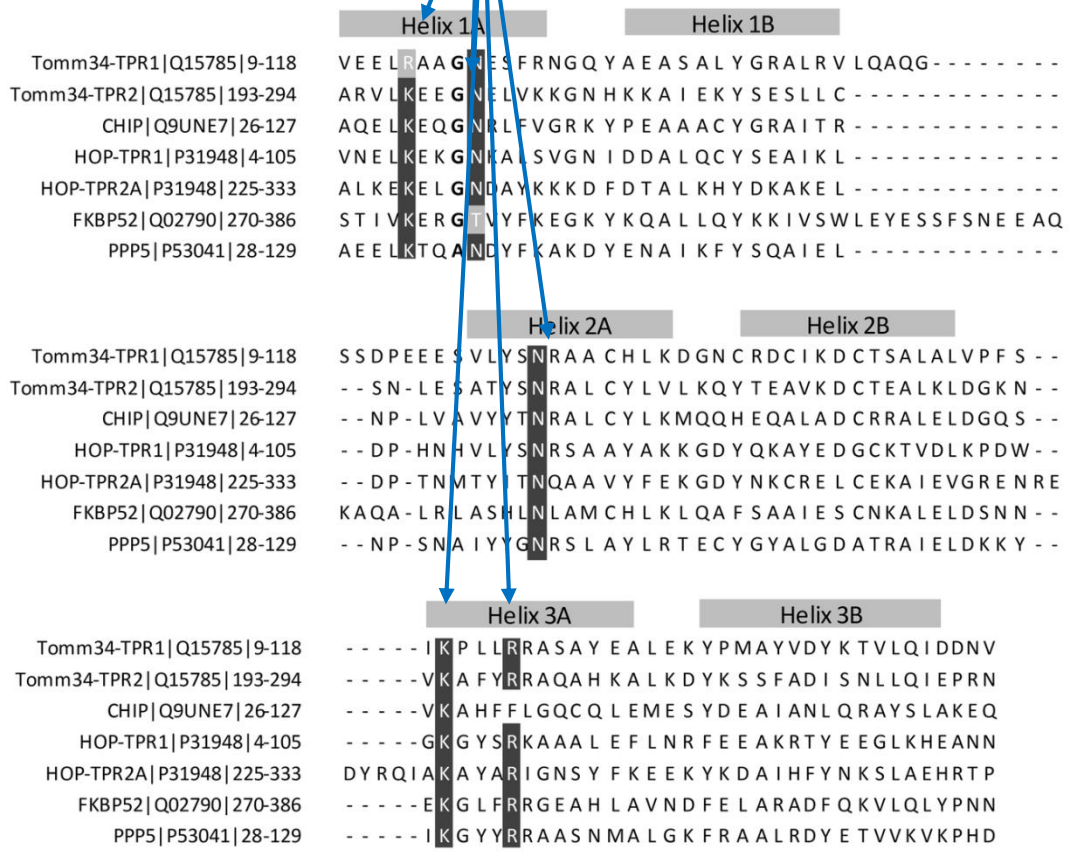
TPR1



TPR2



Positively charged clamp



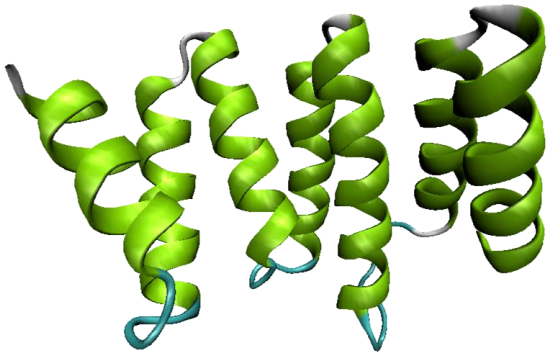
TOMM34 protein – co-chaperone

Tetratricopeptide repeat (TPR) domain

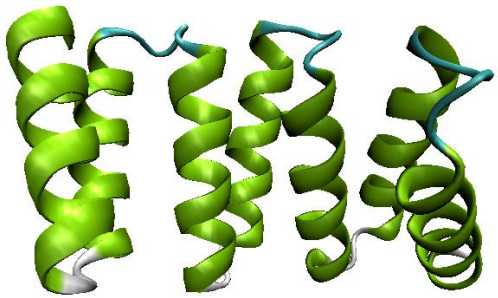
C-terminus Hsp70/Hsp90

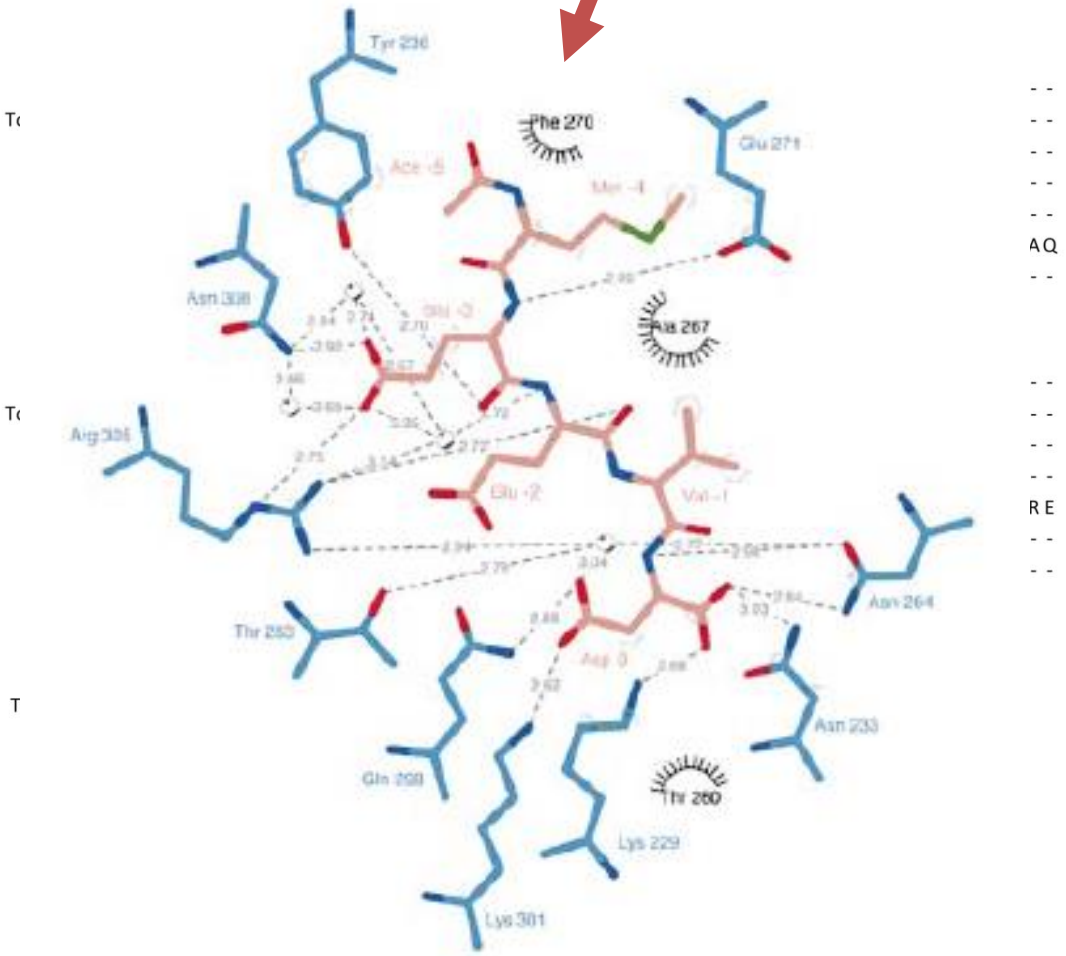
EEVD

TPR1



TPR2



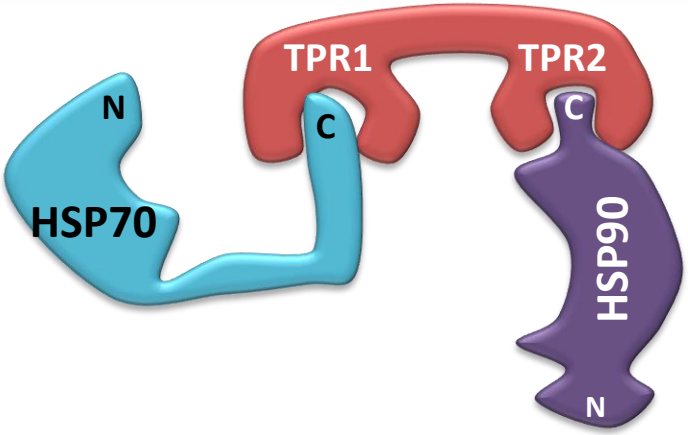


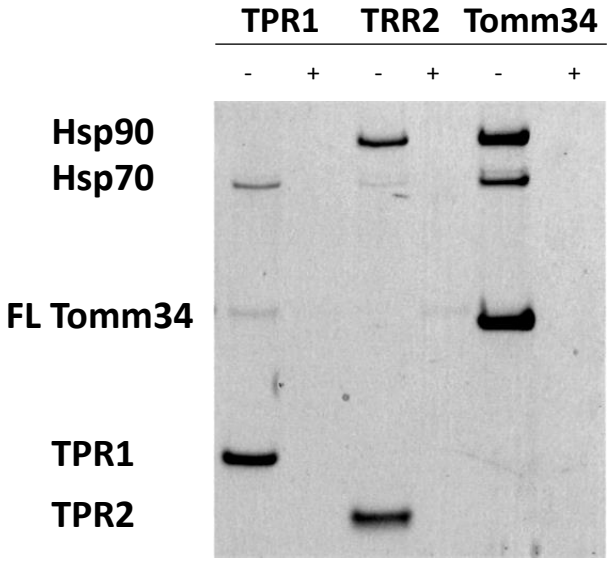
De novo modeled structure of **TOMM34 domains**

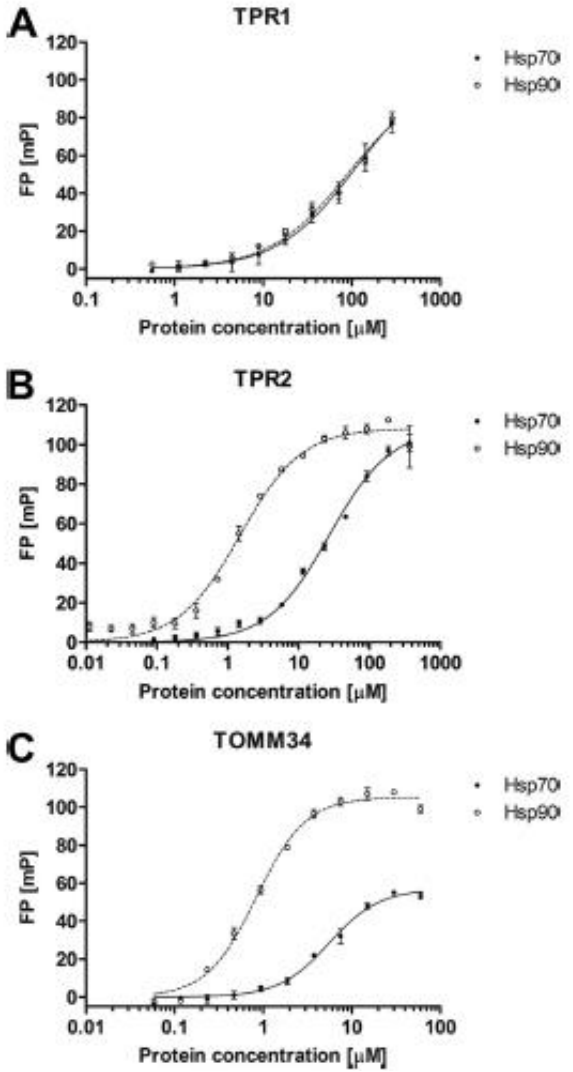
The Assembly and Intermolecular Properties of the Hsp70-Tomm34-Hsp90 Molecular Chaperone Complex*

Received for publication, October 11, 2013, and in revised form, February 19, 2014 Published, JBC Papers in Press, February 24, 2014, DOI 10.1074/jbc.M113.526046

Filip Trcka‡, Michal Durech‡, Petr Man§¶, Lenka Hernychova‡, Petr Muller‡1,2, and Borivoj Vojtesek‡1,3



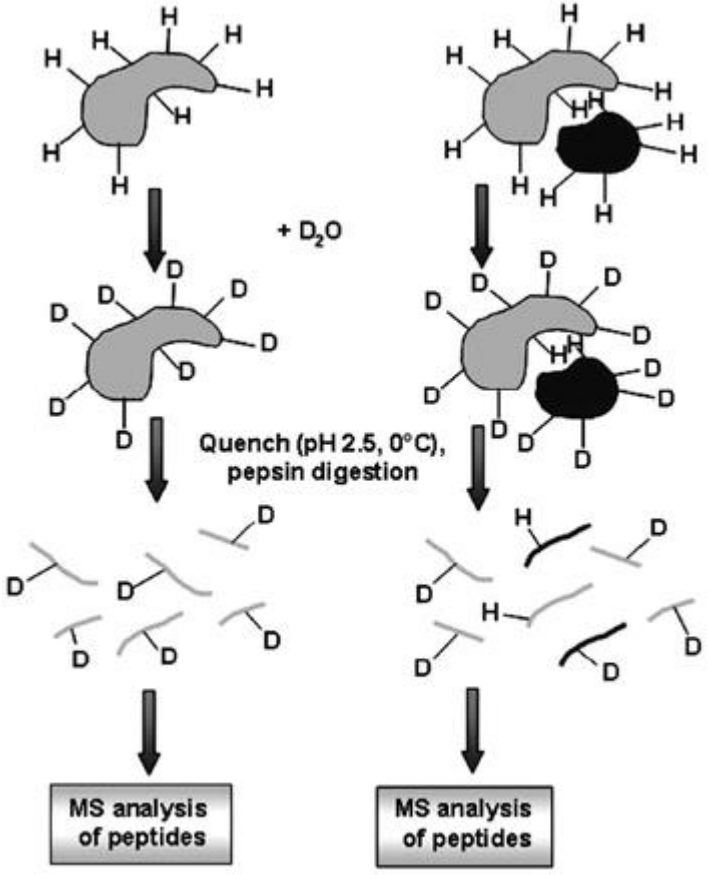


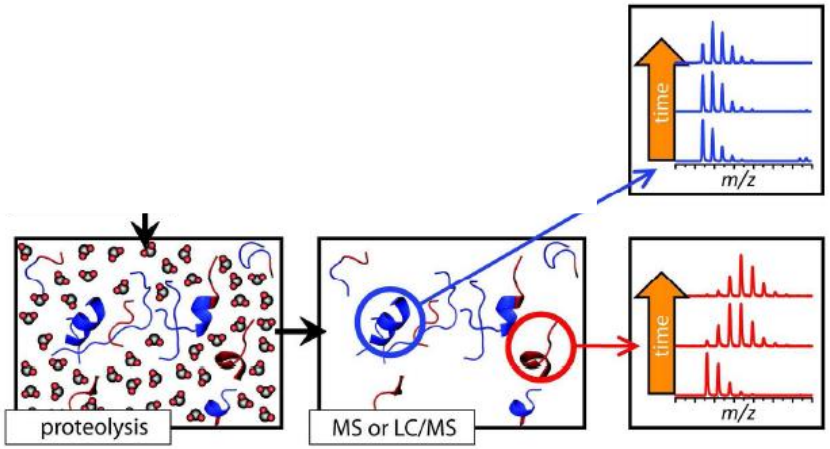


HDX – basic basics

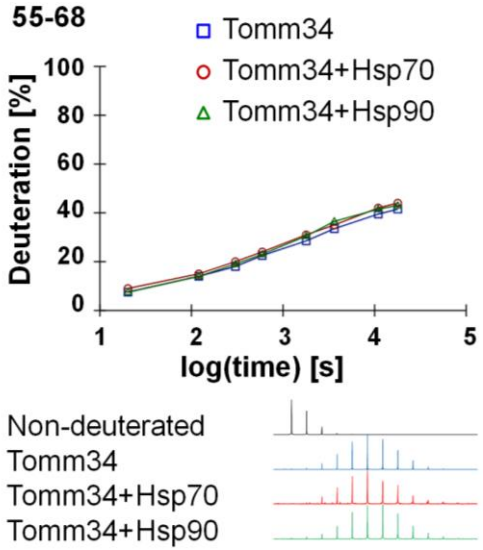
Exchangeable hydrogens:

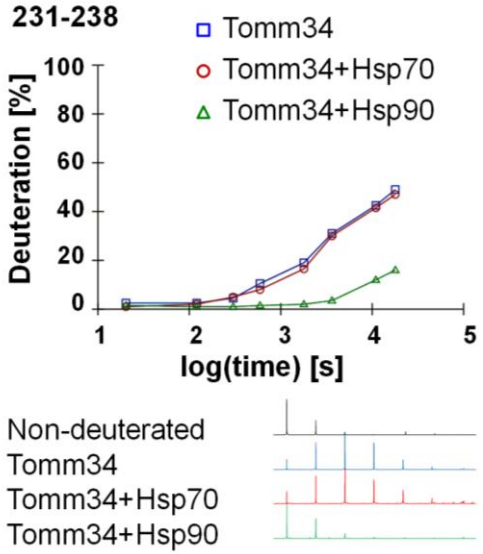
1) side chains containing –OH, –SH, –NH2, –COOH and –CONH2 groups and hydrogens from the amino and carboxy termini
2) carbon-bound aliphatic and aromatic hydrogens
3) hydrogens arising from the amide linkages between amino acids of the protein polypeptide chain

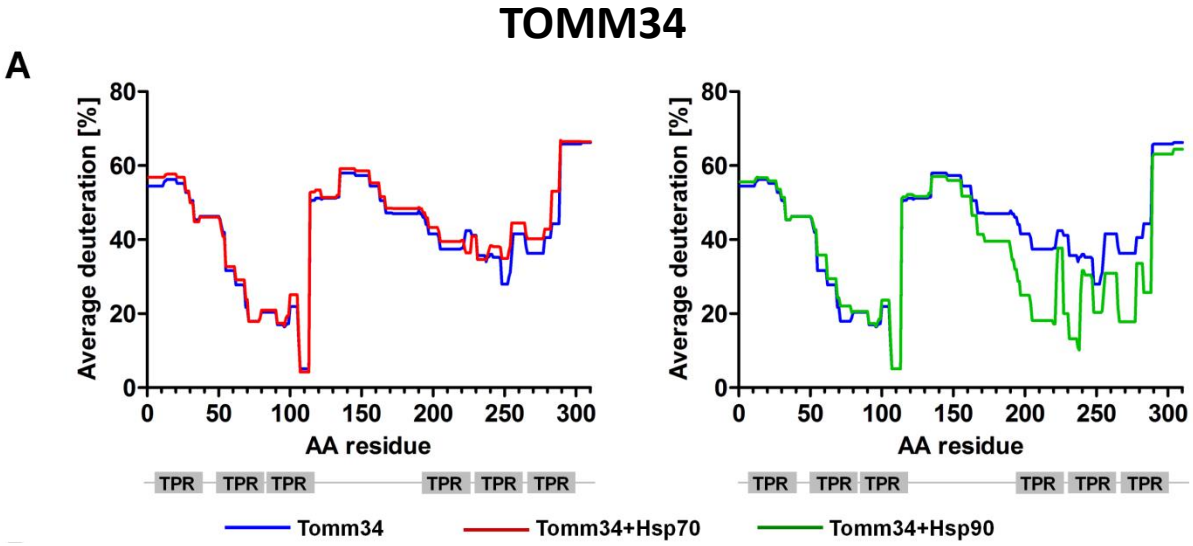


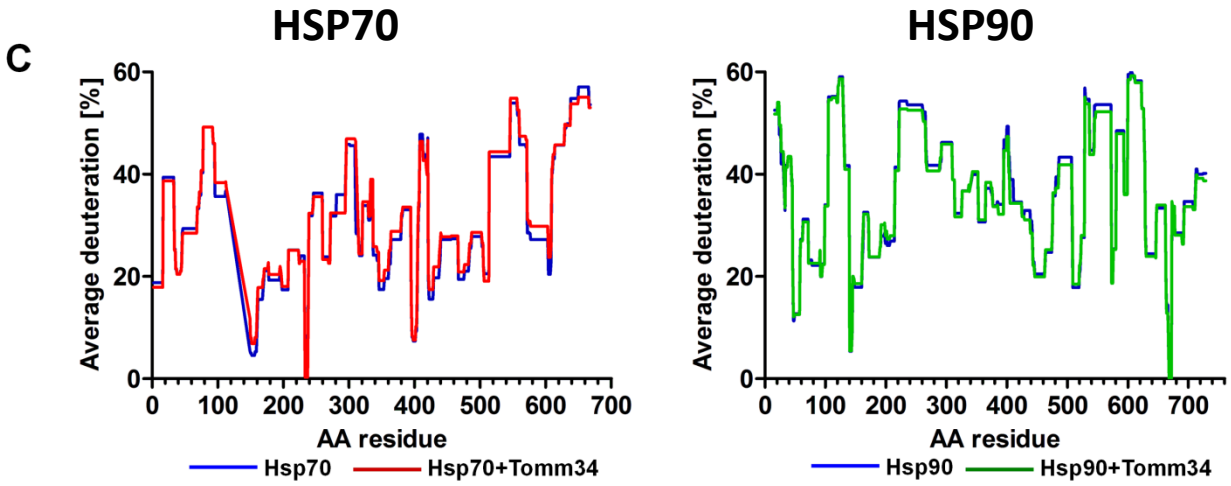


HDX – HSP70/90-TOMM34 interaction without ATP



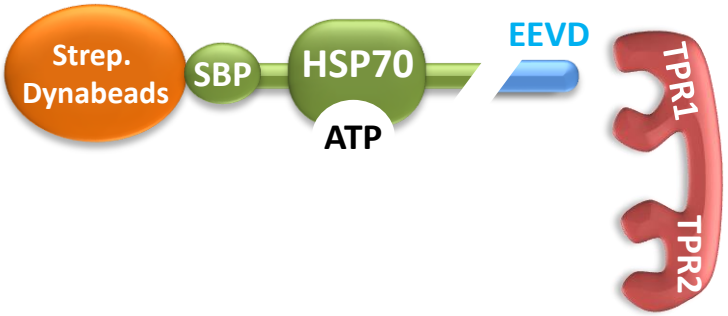


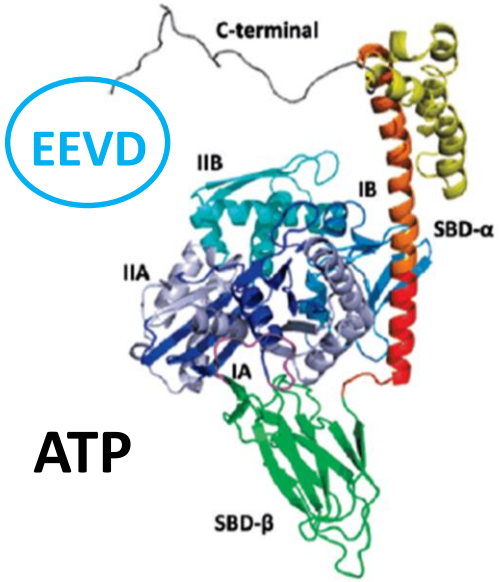


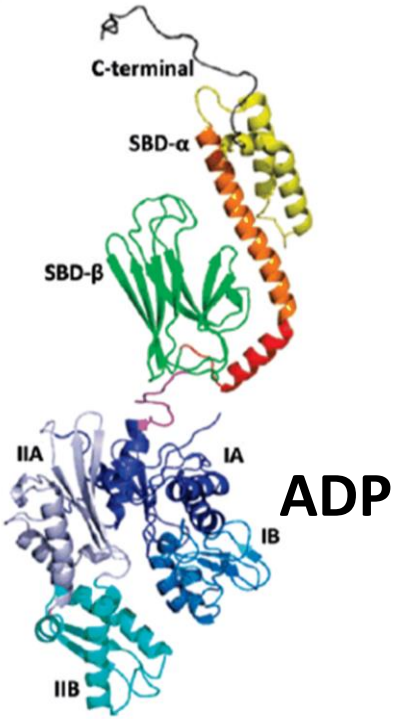


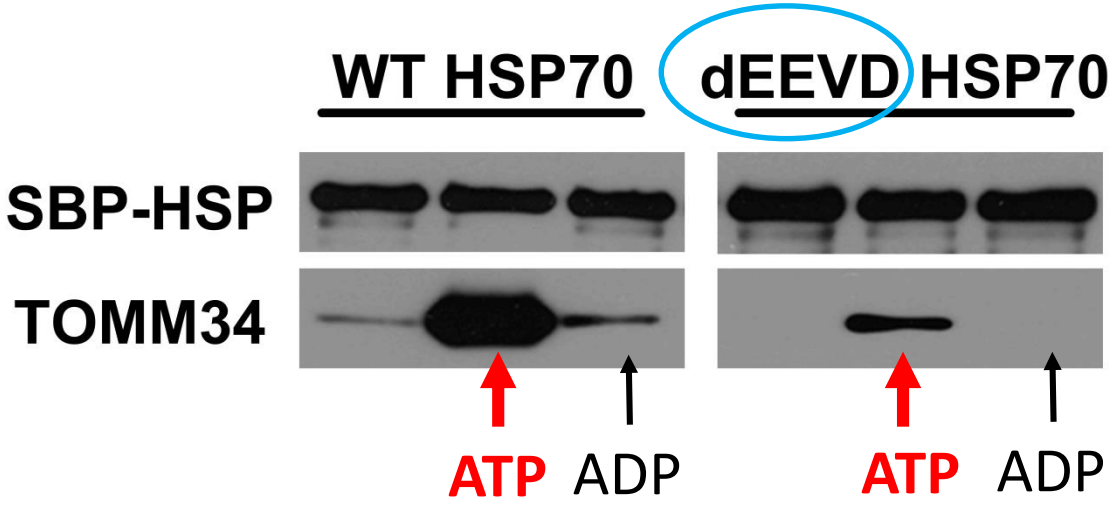
The effect of ATP

on HSP70 – Tomm34 interaction

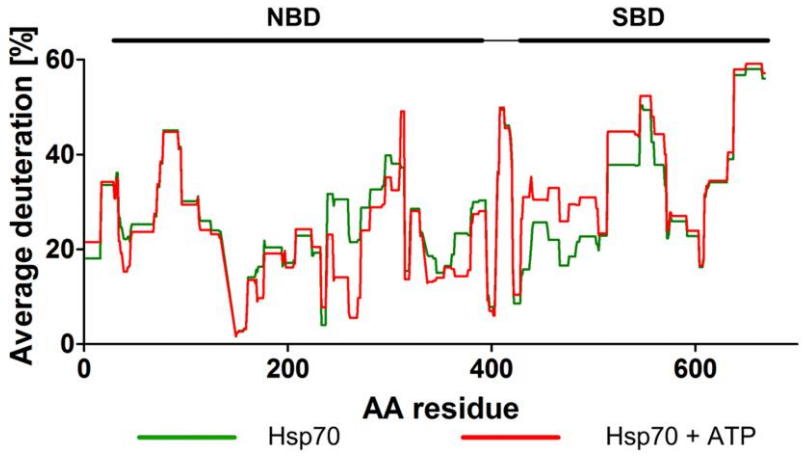


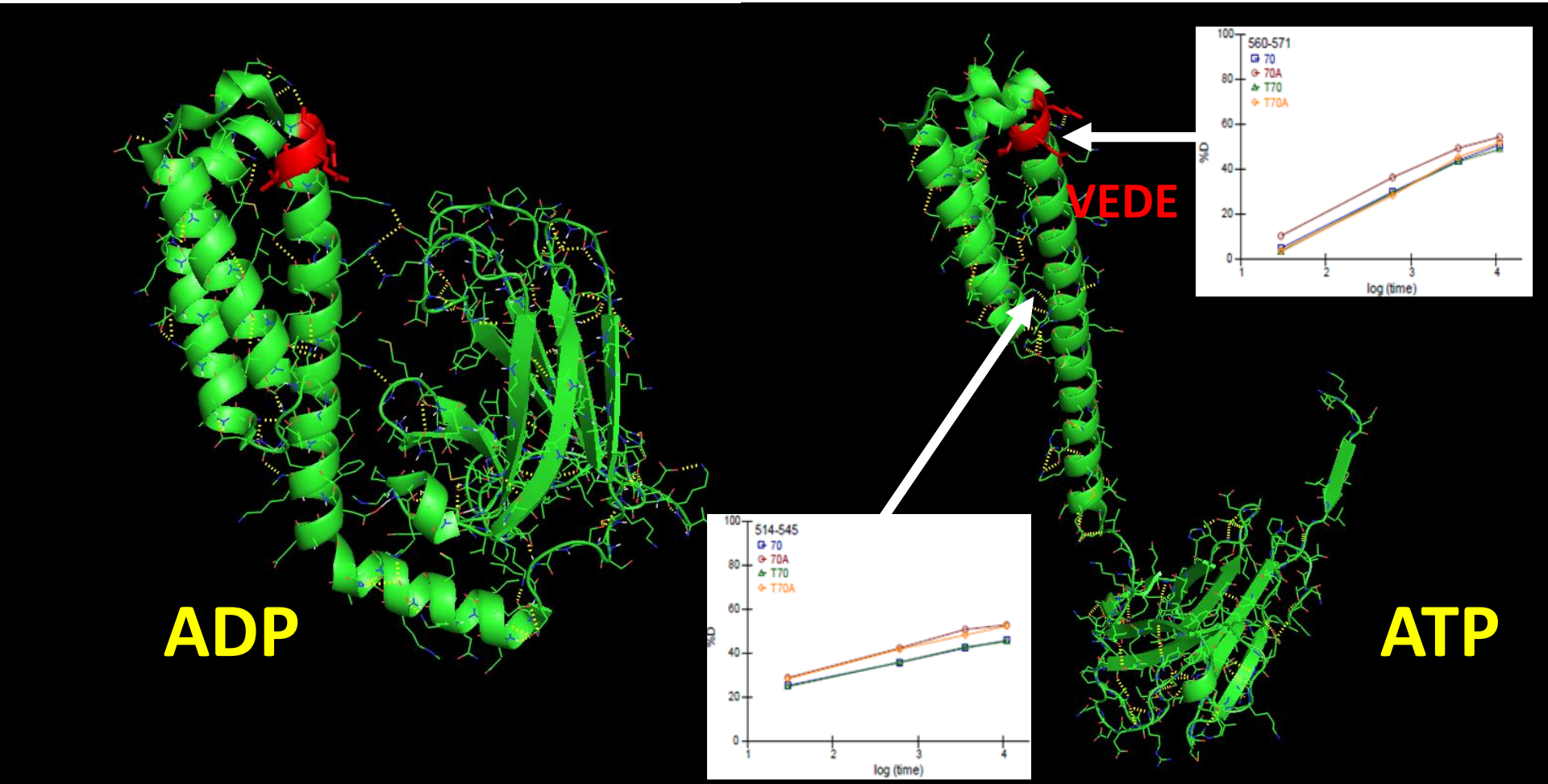




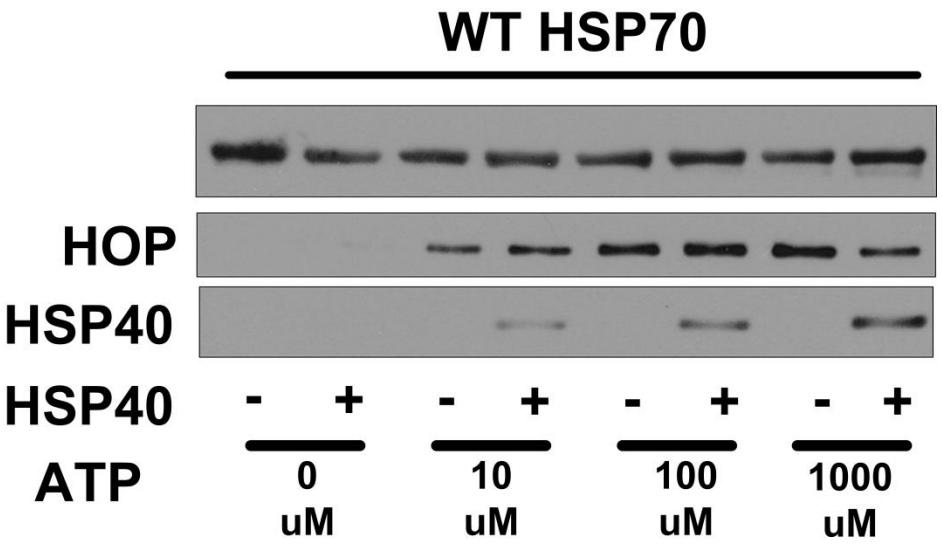


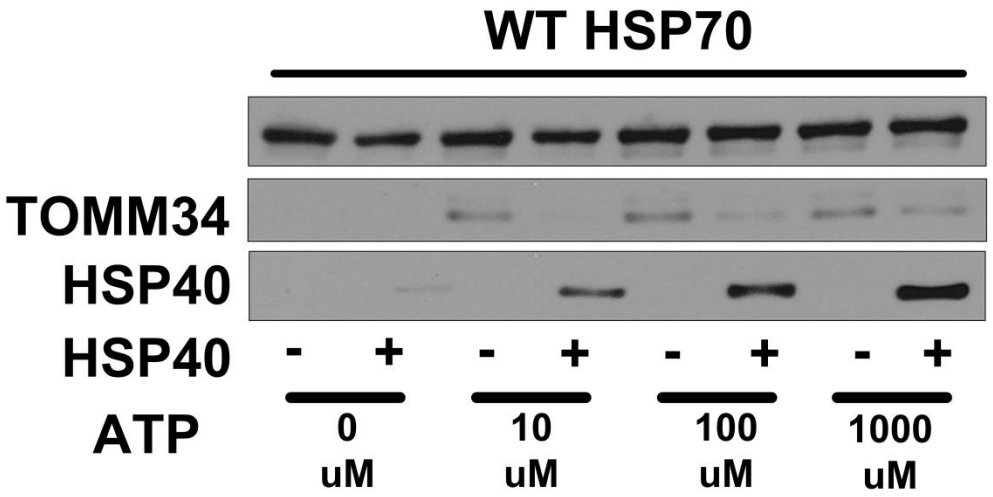
HDX – HSP70-TOMM34 interaction with ATP

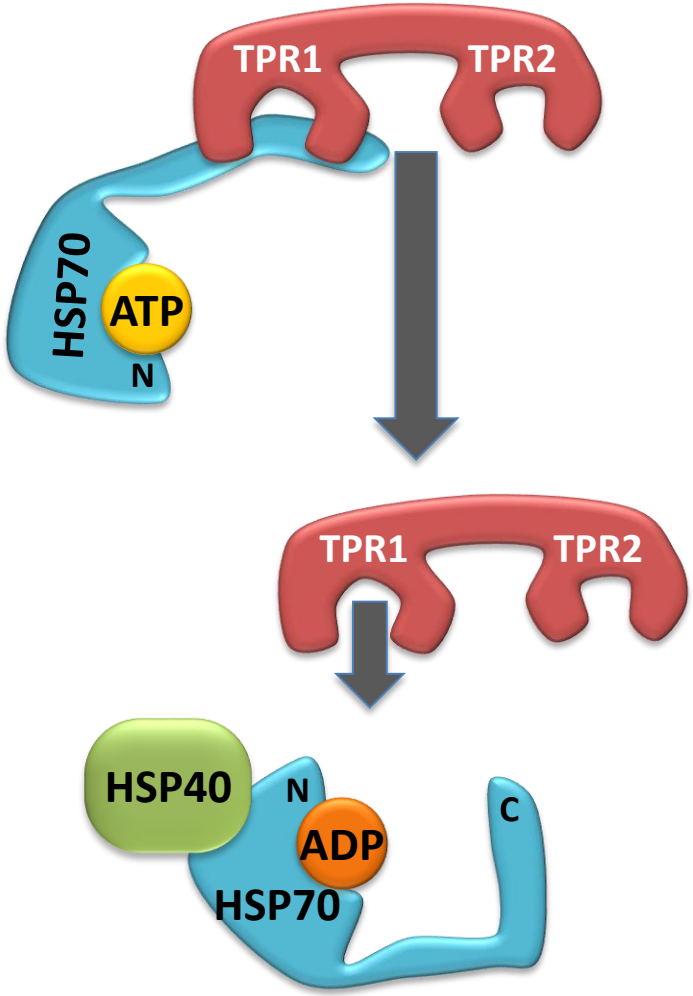




HSP70-TOMM34 interaction with ATP, the role of HSP40

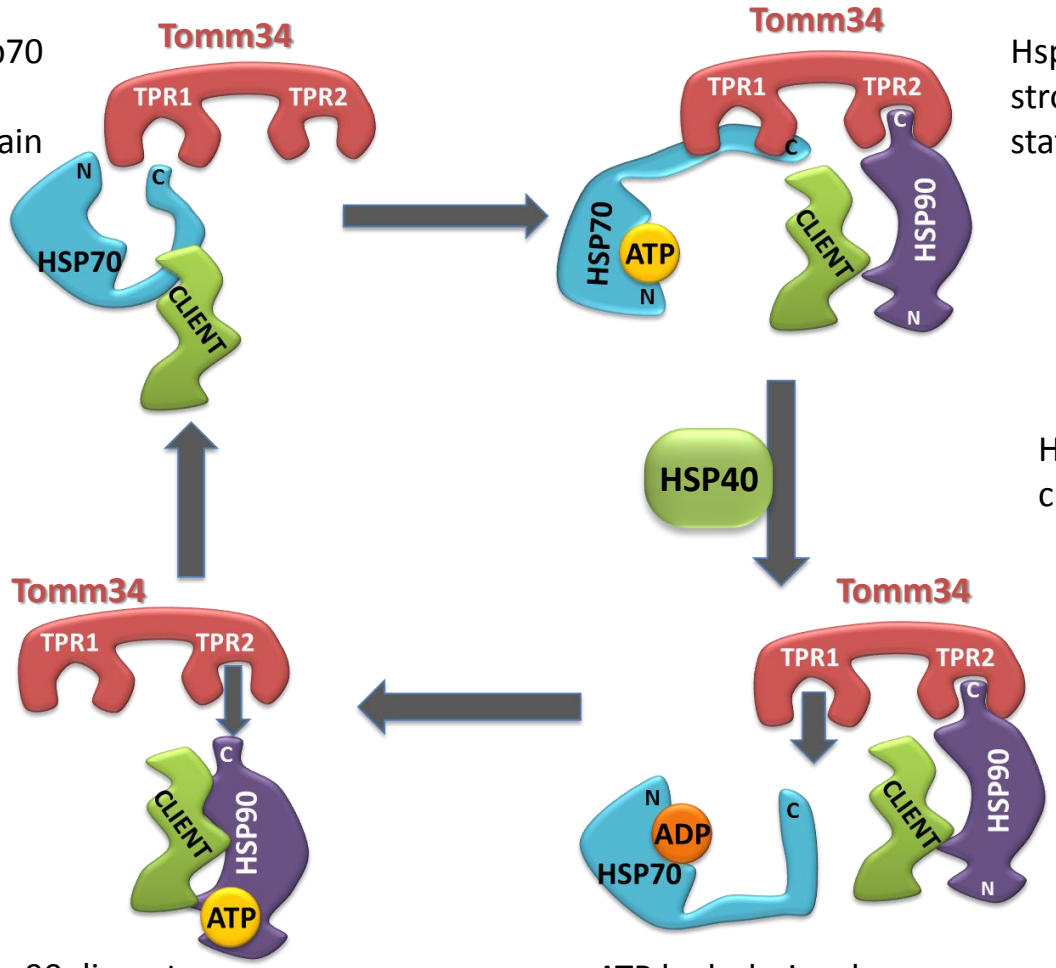






Cooperation of Hsp70/Hsp90 folding by Tomm34

nucleotide free Hsp70 binds TPR1 weakly through EEVD domain



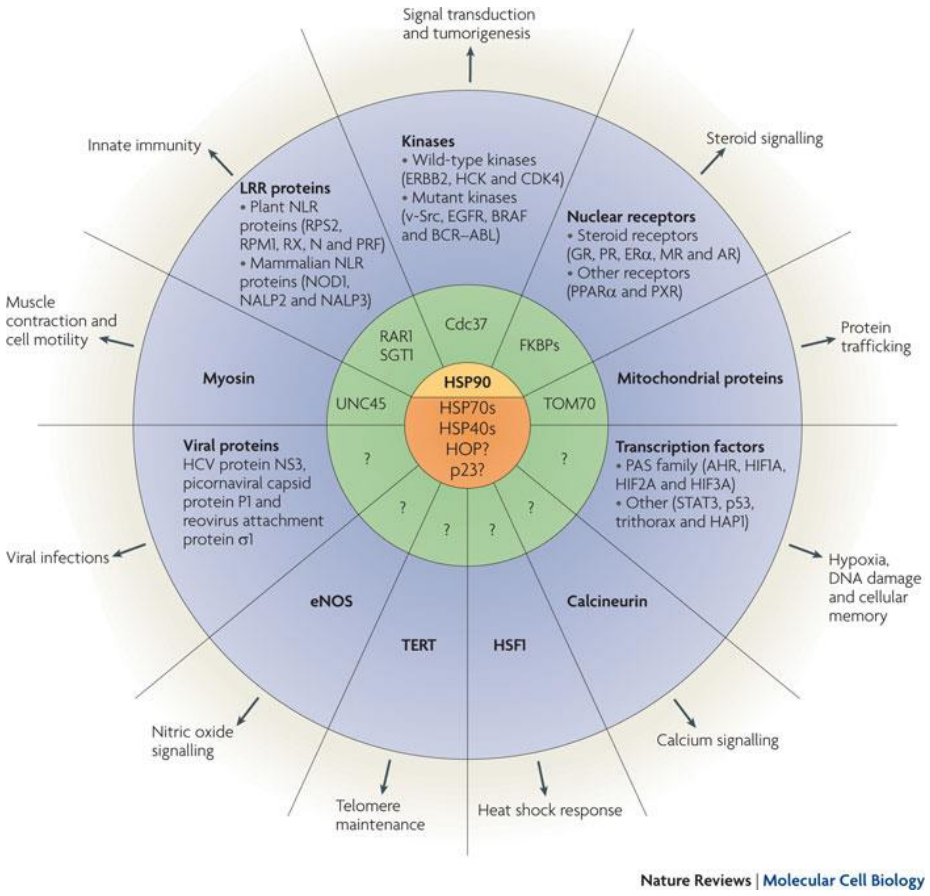
Hsp70 binds TPR1 strongly at ATP bound state, client is released

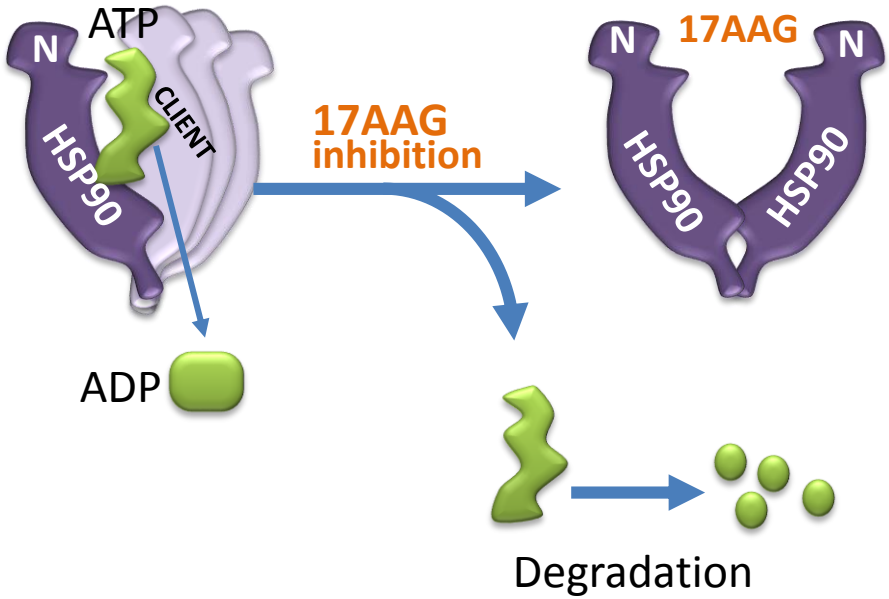
Hsp90 binds TPR2 at client free state

Client binding to Hsp90 disrupt its interaction with Tomm34

ATP hydrolysis releases Hsp70 from Tomm34

Hsp90 client proteins





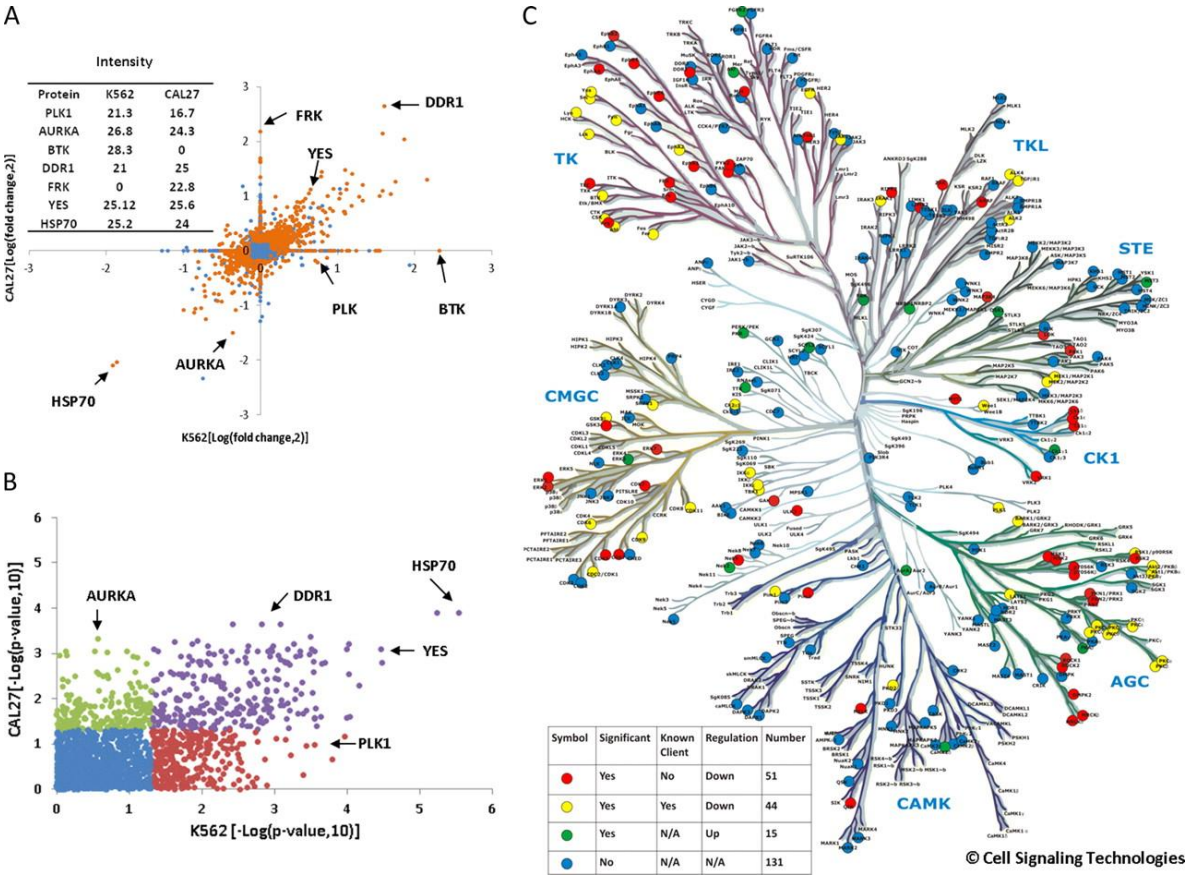
Is there any structural/motif motif recognized by Hsp90 ?

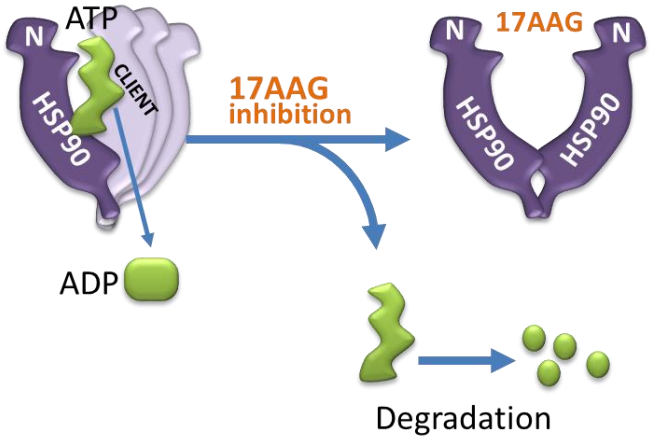
Research

This paper is available on line at http://www.mcponline.org

Systematic Identification of the HSP90 Regulated Proteome

Zhixiang Wu‡, Amin Moghaddas Gholami‡, and Bernhard Kuster‡§¶

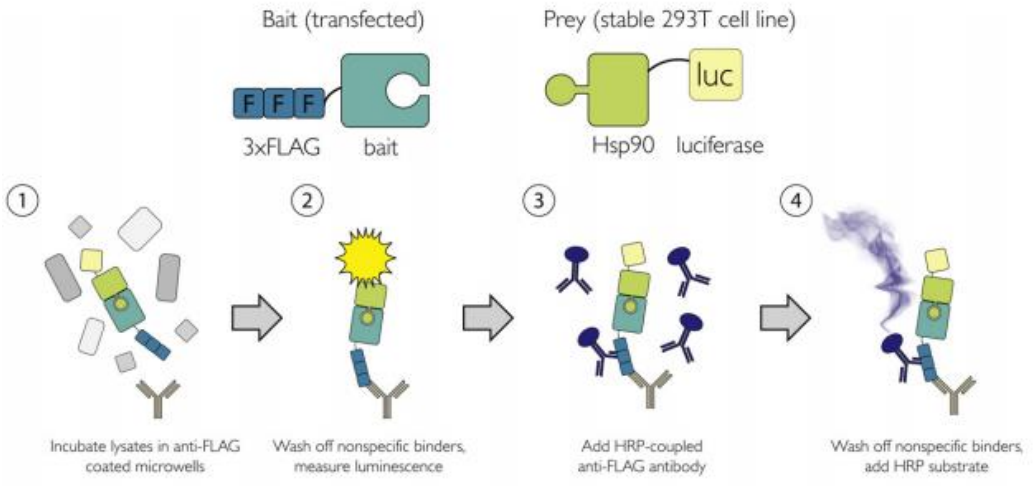


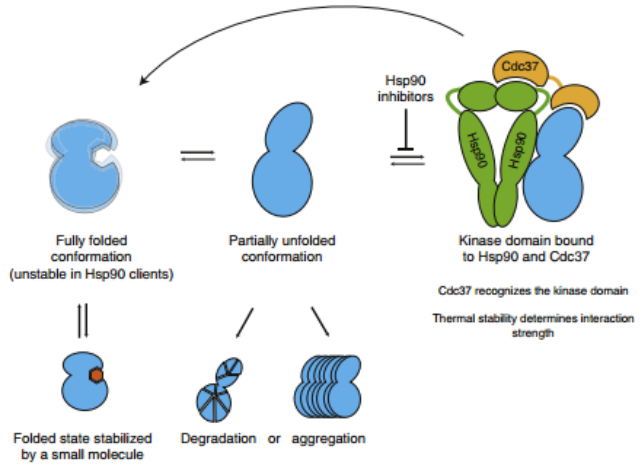


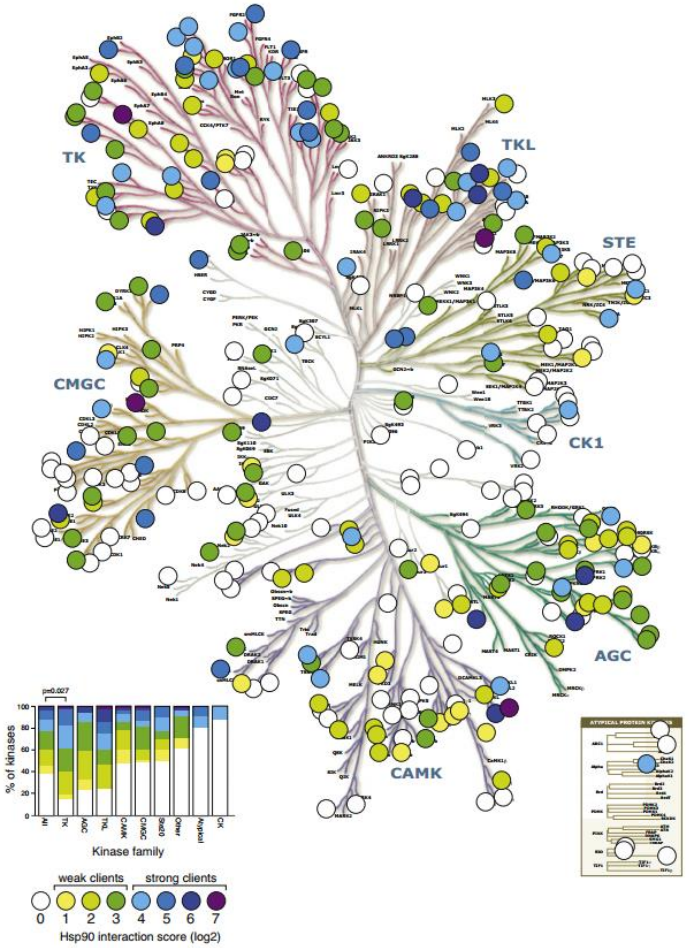
Quantitative Analysis of Hsp90-Client Interactions Reveals Principles of Substrate Recognition

Cell

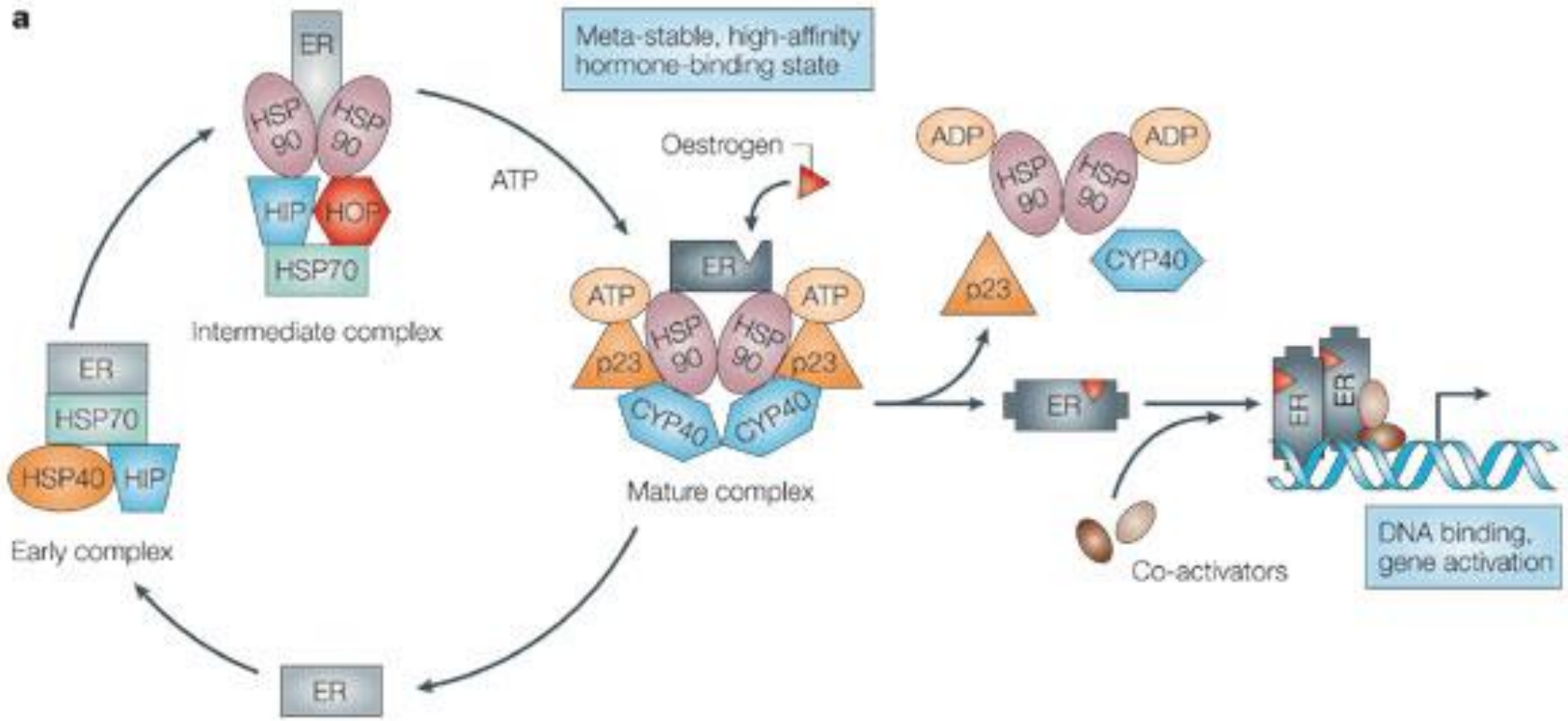
Mikko Taipale,[1] Irina Krykbaeva,[1] Martina Koeva,[1] Can Kayatekin,[1] Kenneth D. Westover,[2] Georgios I. Karras,[1] and Susan Lindquist[1,3,4,*]





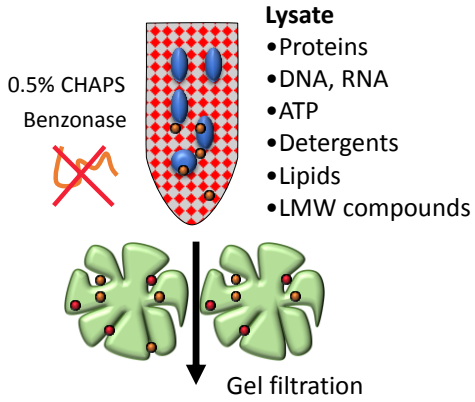


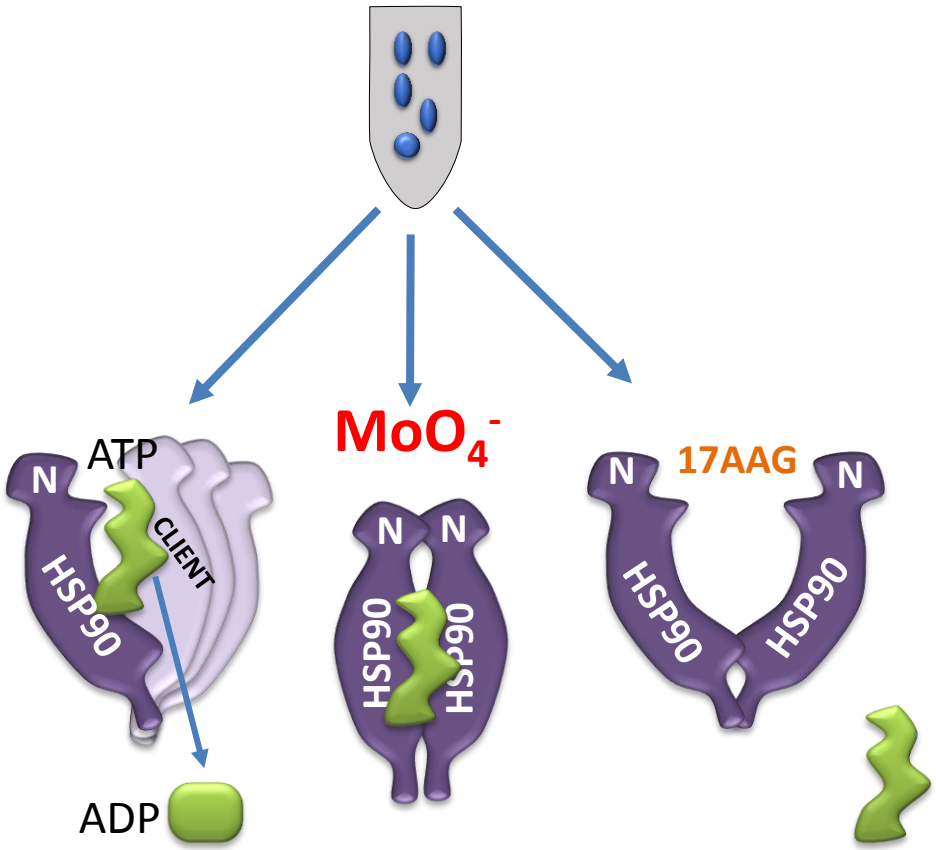
No consensus sequence of Hsp90 clients

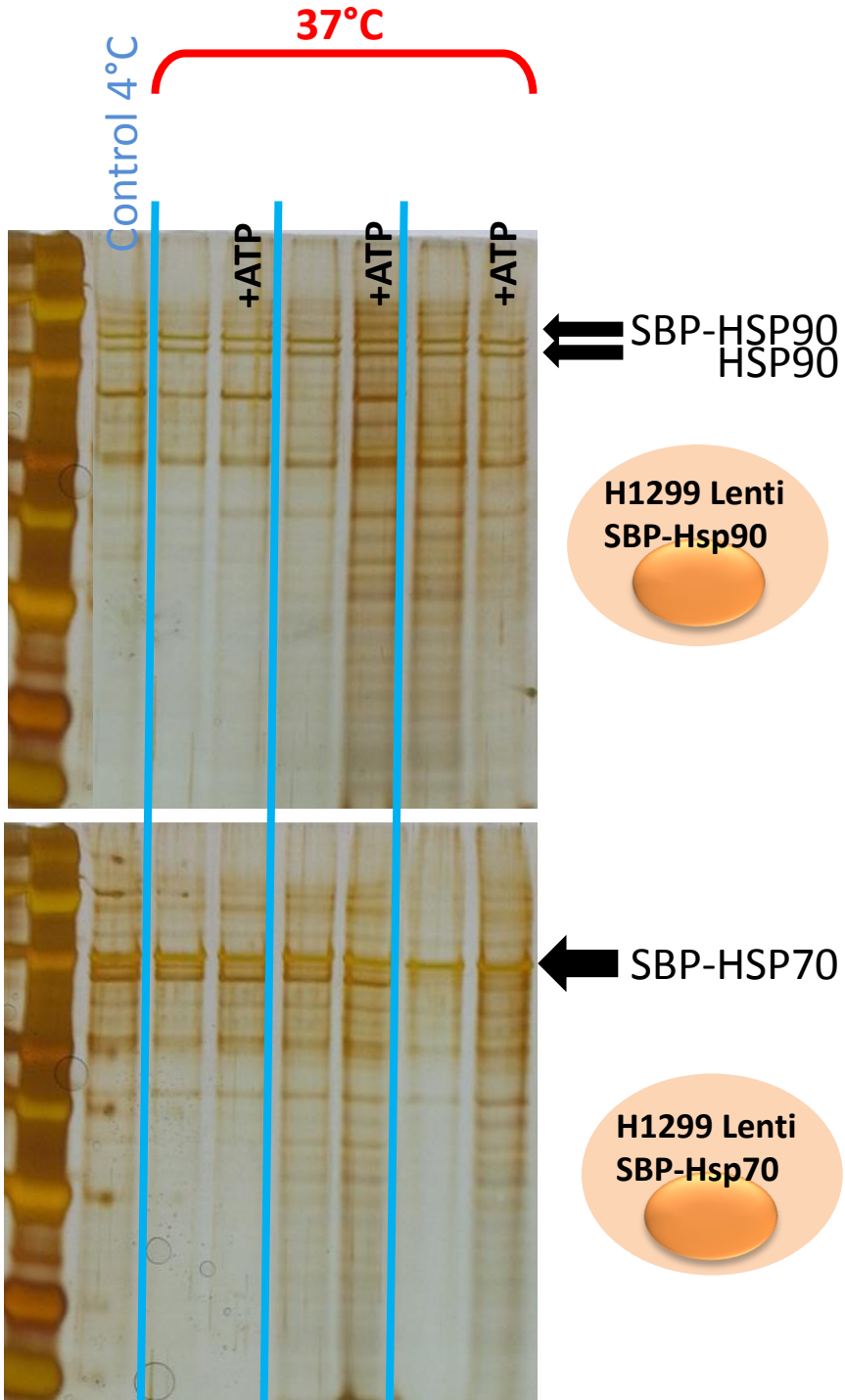


Ligand promotes conformational stabilization of steroid receptors

Functional proteomic to study Hsp90 complexes







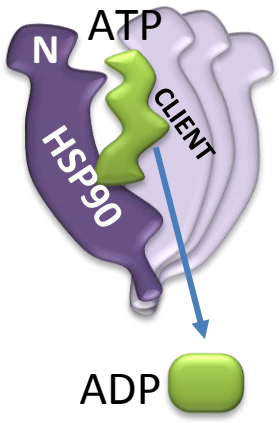
SWATH Acquisition

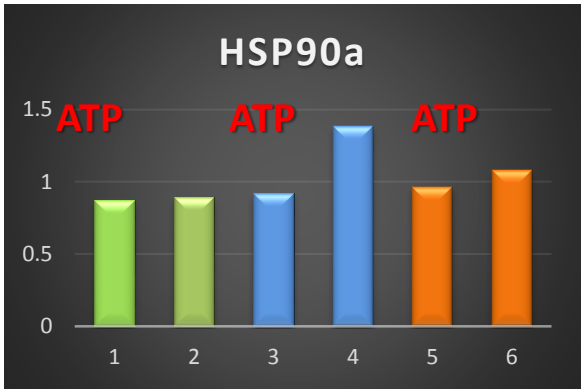
SBP Hsp90

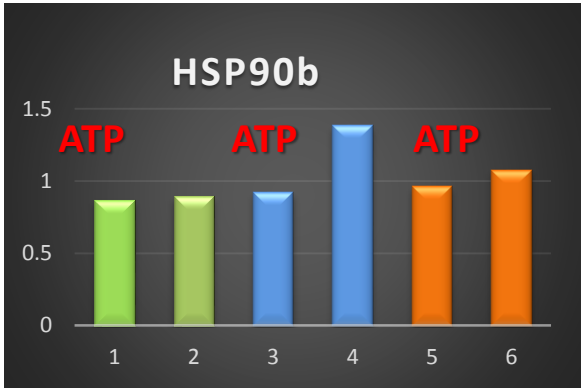
Control

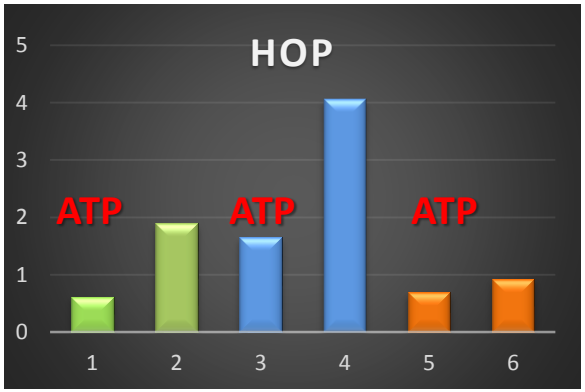
Molybdate

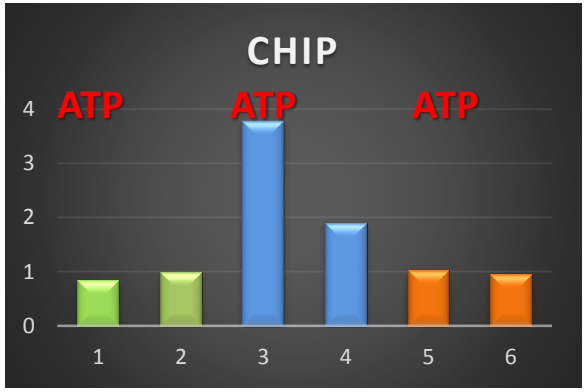
Hsp90 inh.

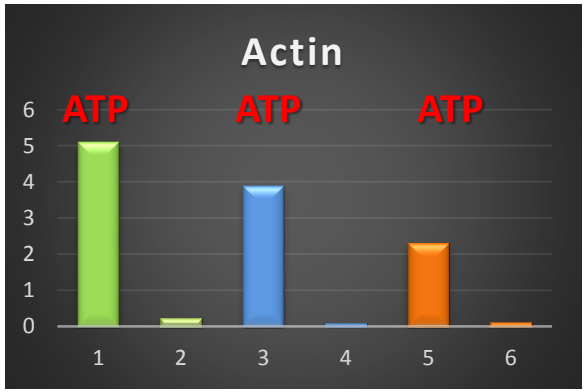


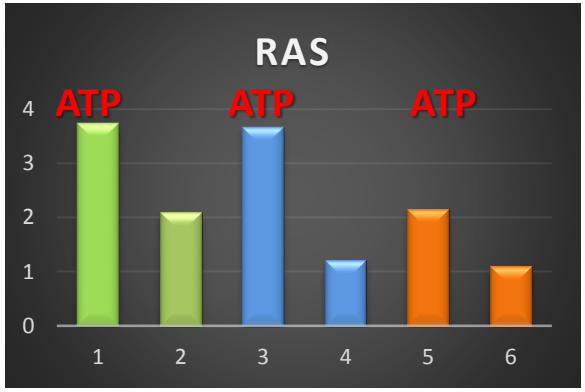






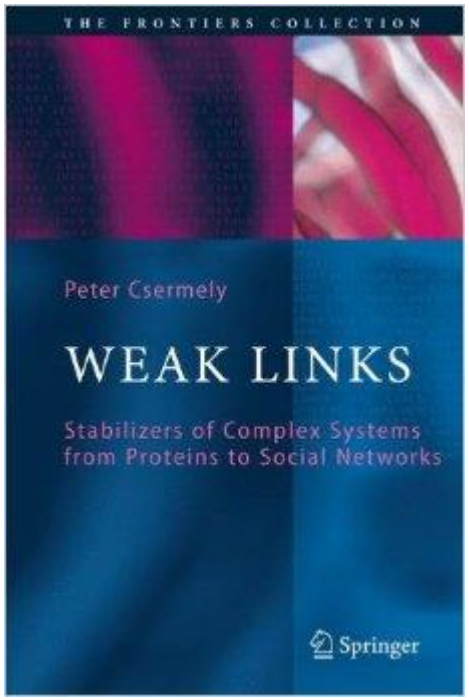






Any protein can be client of Hsp90

Both water and chaperones provide a diffuse set of rapidly fluctuating weak links (low affinity and low probability interactions), which allow the generalization of all these statements to a multitude of networks.



Weak Links

The Universal Key to the Stability of Networks and Complex Systems

Děkuji za pozornost

- Bořivoj Vojtěšek
- Filip Trčka
- Eva Růčková
- Michal Ďurech
- Kateřina Křivánková

Ted R. Hupp

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