

Molekulární chaperony a jejich úloha v patogenezi lidských chorob

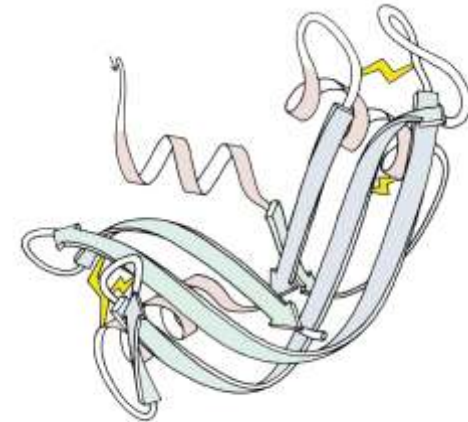
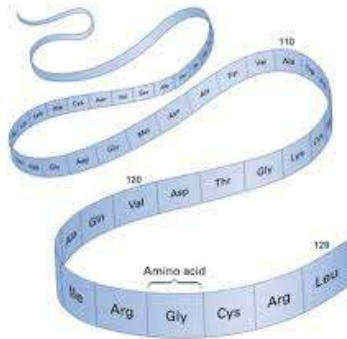
Petr Muller

RECAM 

Regional Centre
for Applied Molecular
Oncology

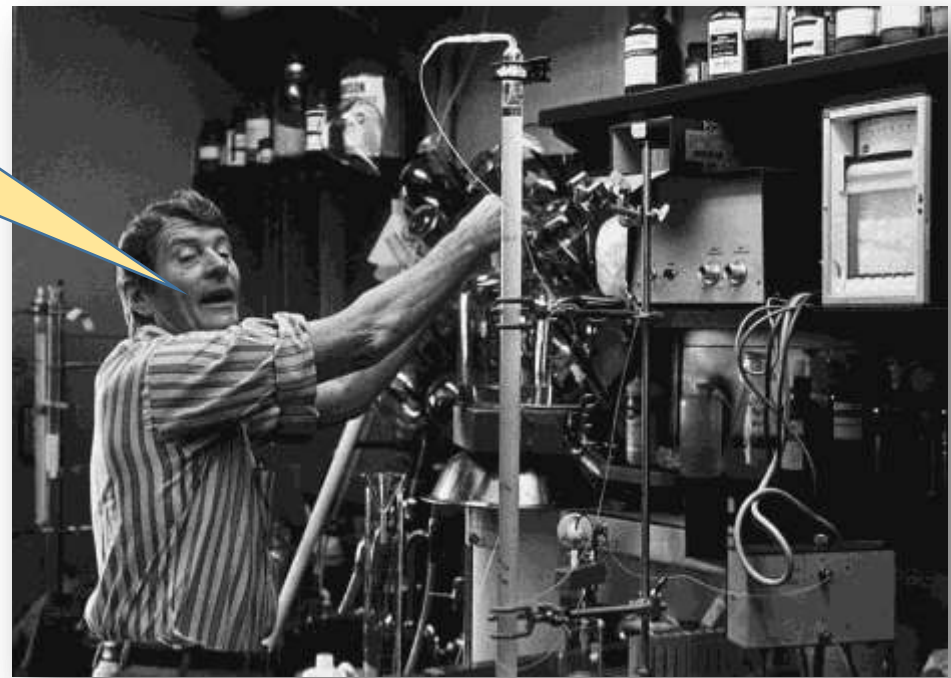


At the environmental conditions (temperature, solvent concentration and composition, etc.) at which folding occurs, the native structure is a unique, stable and kinetically accessible minimum of the free energy



Ribonuclease A

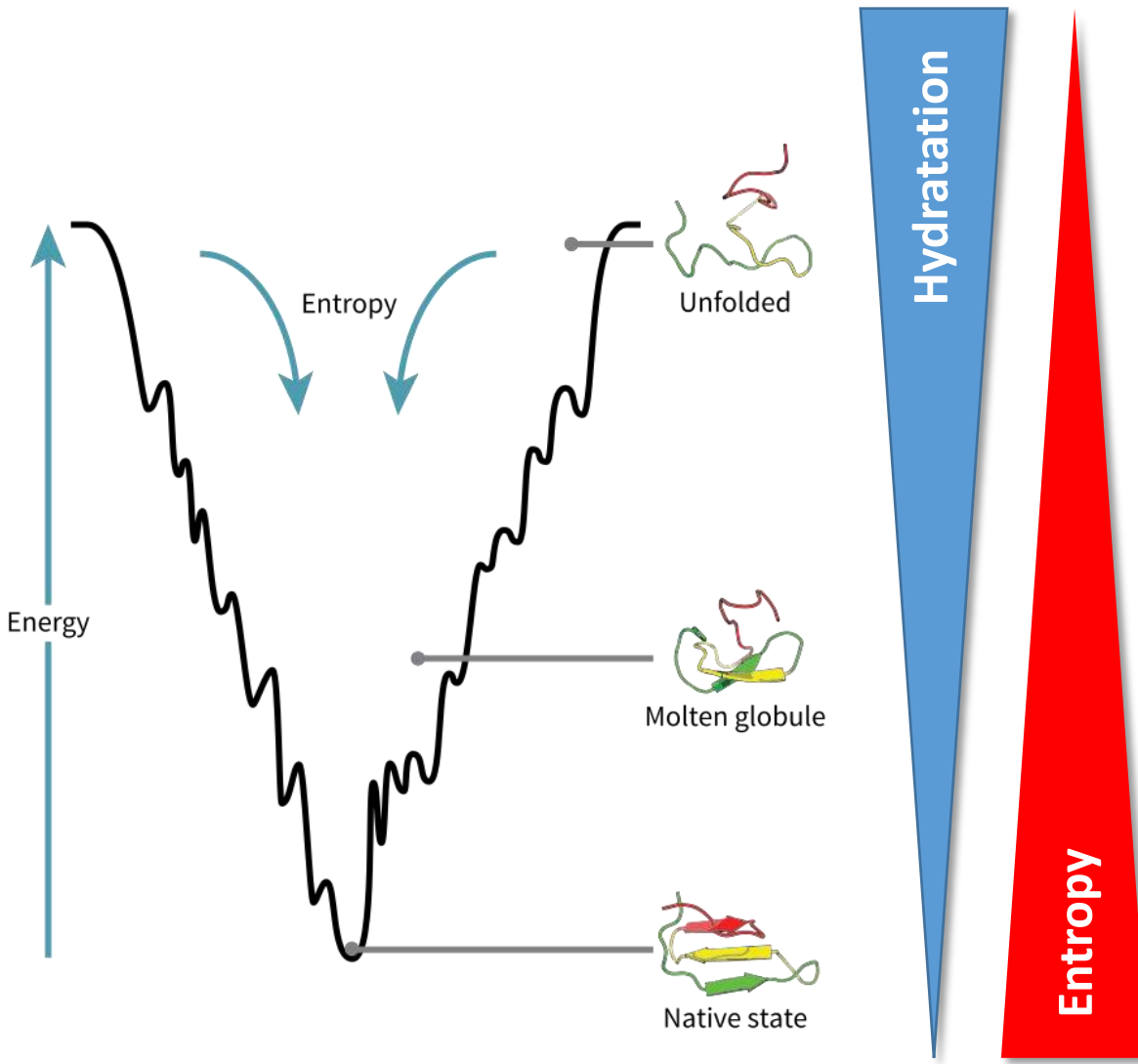
The native structure is determined only by the protein's amino acid sequence

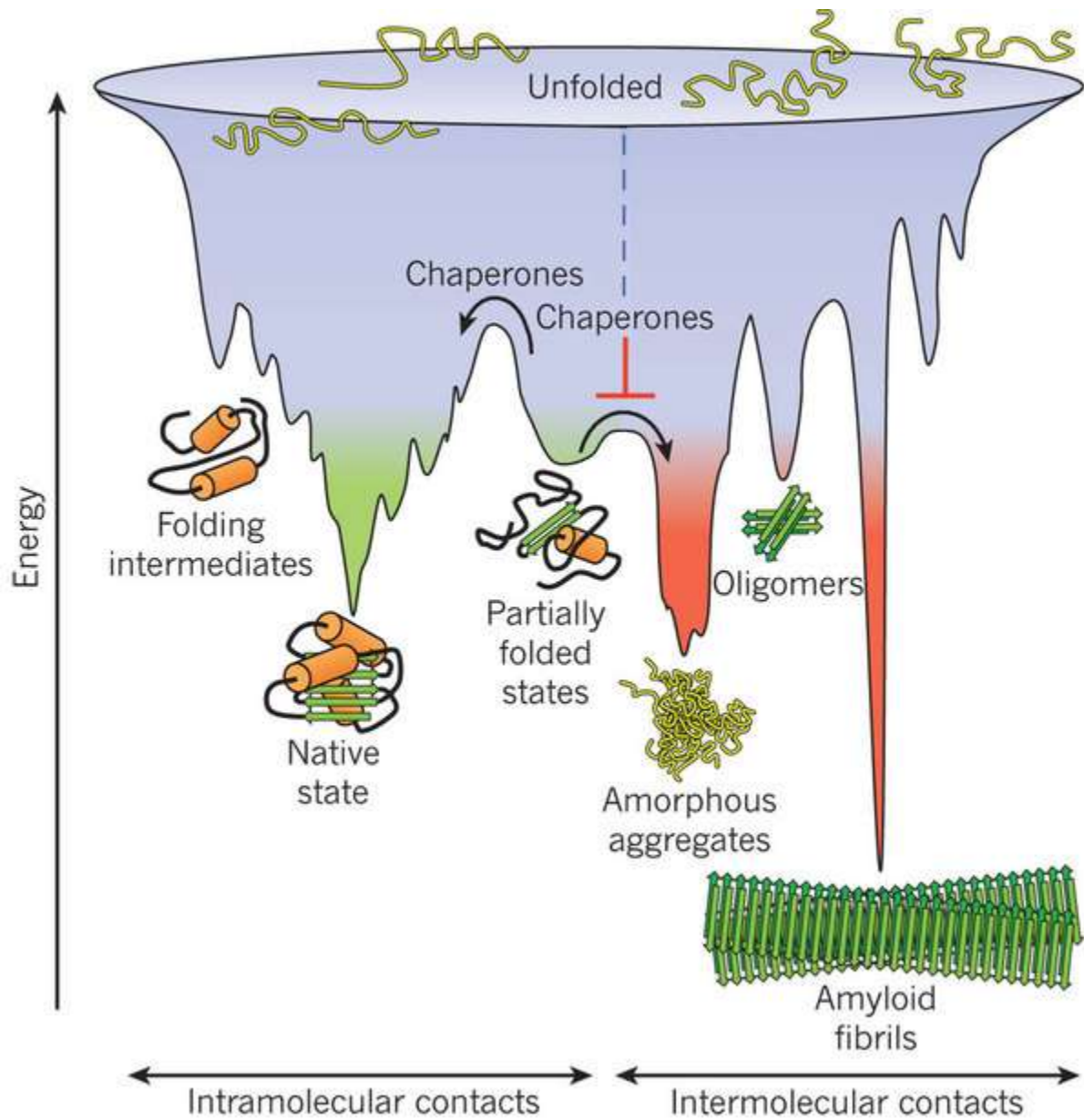


Christian Boehmer Anfinsen, Jr.
(March 26, 1916 – May 14, 1995)

Nobel Prize in Chemistry (1972)

Folding is entropy driven process

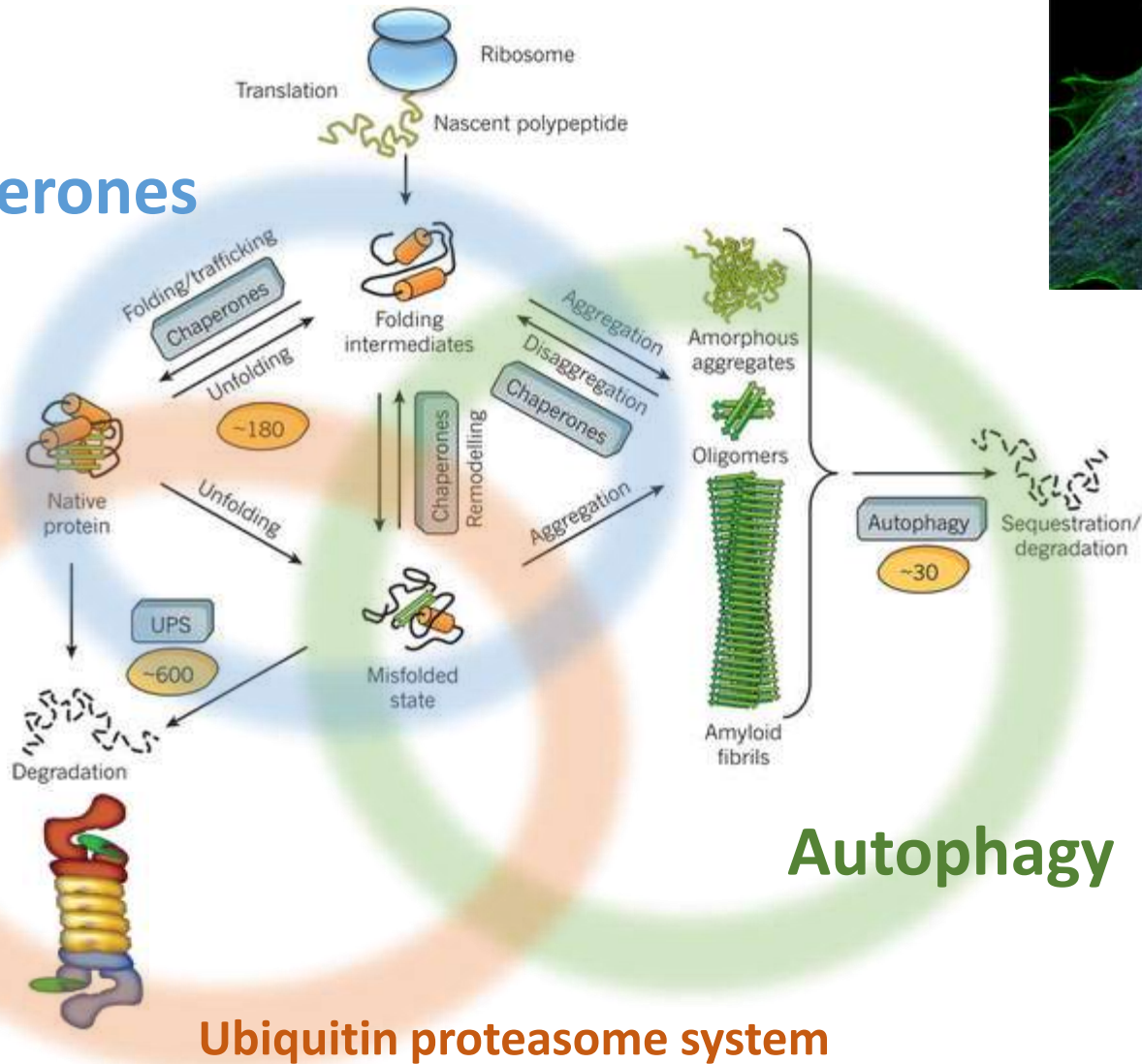




Protein homeostasis / proteostasis



Chaperones



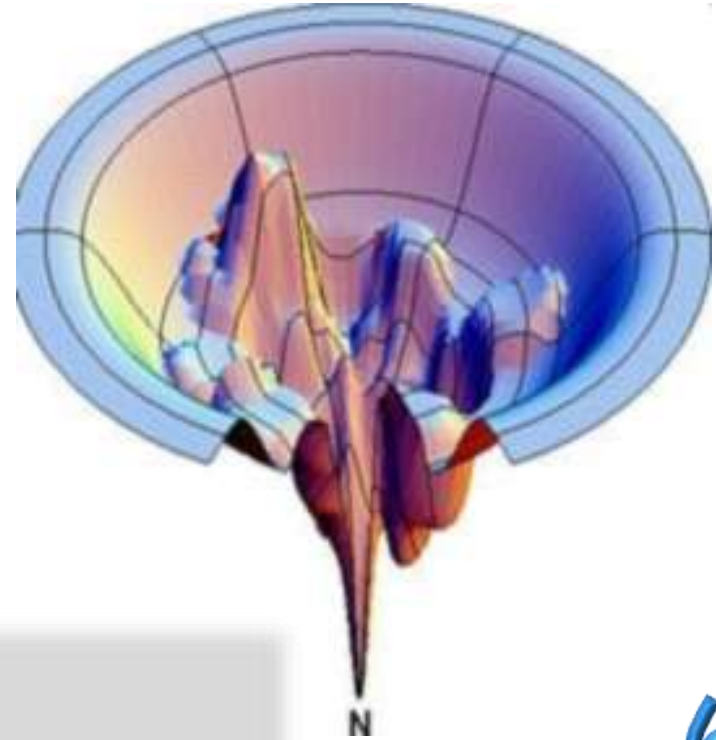
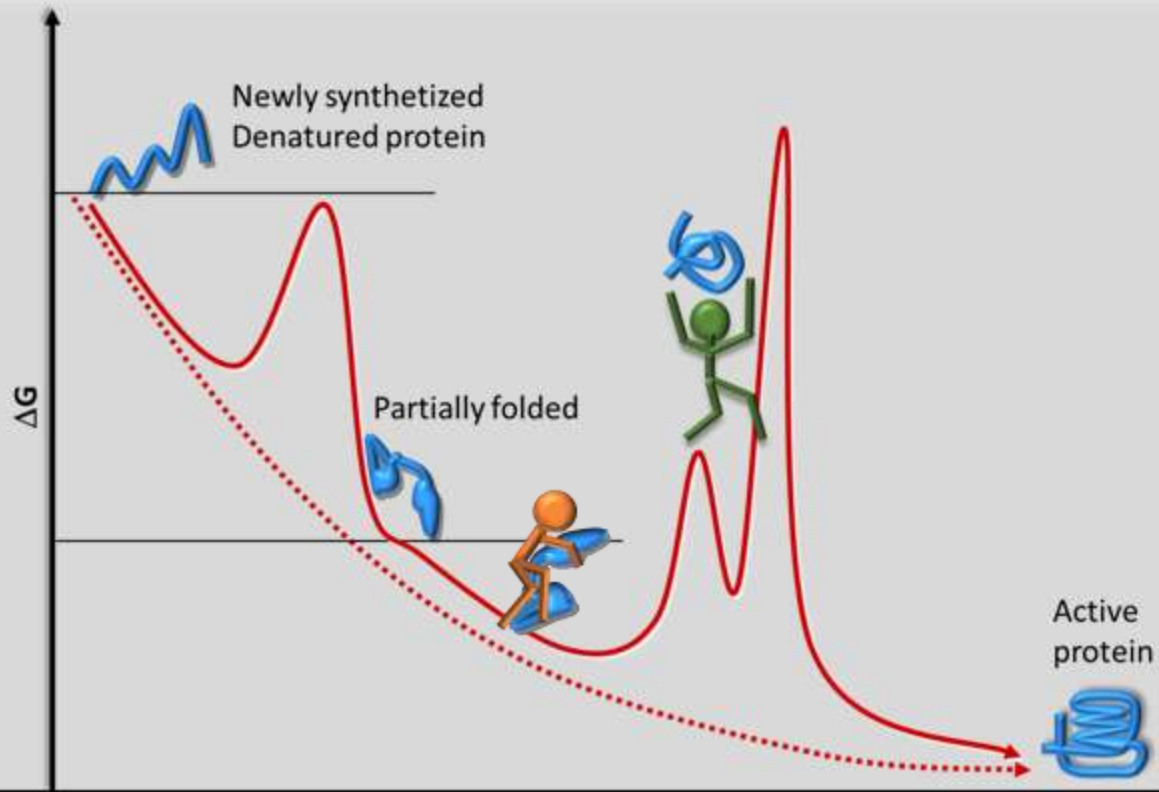
Autophagy

Stress proteins / Chaperones

Holdases bind folding intermediates to prevent their aggregation

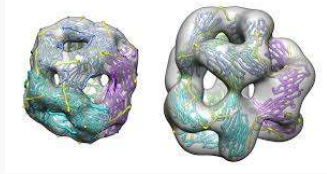
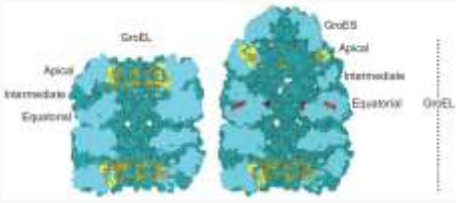


Crystalins, p23, Hsp40...

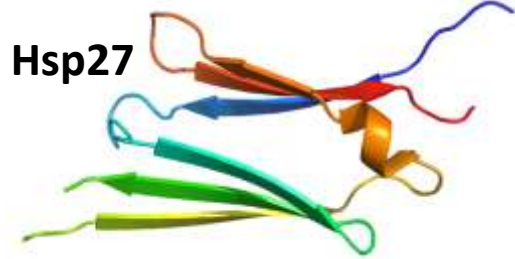
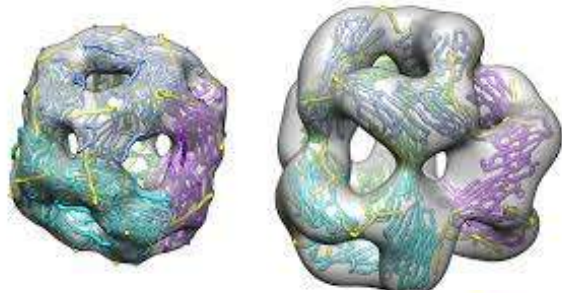


Foldases are chaperones that accompany other proteins to help them to overcome the energy barriers during folding to native conformation (ATP dependent)

Hsp70, Hsp90, GroEL...

Approximate molecular weight(kDa)	<u>Prokaryotic</u> proteins	<u>Eukaryotic</u> proteins	Function
<u>10 kDa</u>	GroES	Hsp10	
20-30 kDa	GrpE	The HspB group of Hsp. Eleven members in mammals including Hsp27 , HSPB6 or HspB1 ^[28]	
<u>40 kDa</u>	DnaJ	Hsp40	Co-factor of Hsp70
<u>60 kDa</u>	GroEL, 60kDa antigen	Hsp60 	Involved in protein folding after its post-translational import to the mitochondrion/chloroplast
<u>70 kDa</u>	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.
<u>90 kDa</u>	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



Hsp27

Crystallins

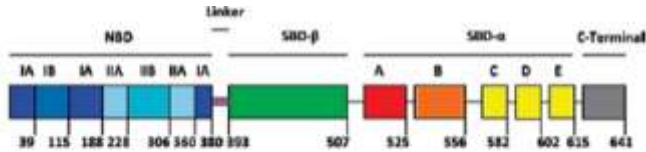
Small Hsps

Ubiquitin-like

Prevent aggregation
Thermotolerance

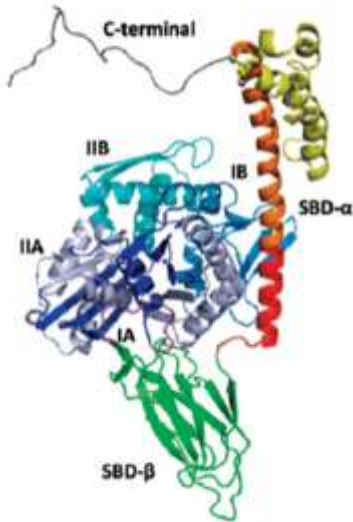
	Gene name	Protein name	Old names	Human gene ID	Mouse ortholog ID
1	<i>HSPB1</i>	HSPB1	CMT2F; HMN2B; HSP27; HSP28; HSP25; HS.76067; DKFZp586P1322	3315	15507
2	<i>HSPB2</i>	HSPB2	MKBP; HSP27; Hs.78846; LOH11CR1K; MGC133245	3316	69253
3	<i>HSPB3</i>	HSPB3	HSPL27	8988	56534
4	<i>HSPB4^a</i>	HSPB4	crystallin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

Hsp70 (DnaK, Grp78,..) chaperone machinery

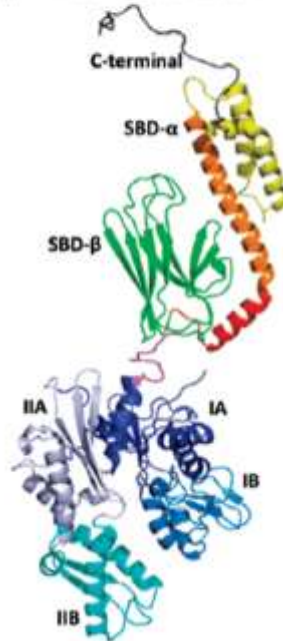


ATP

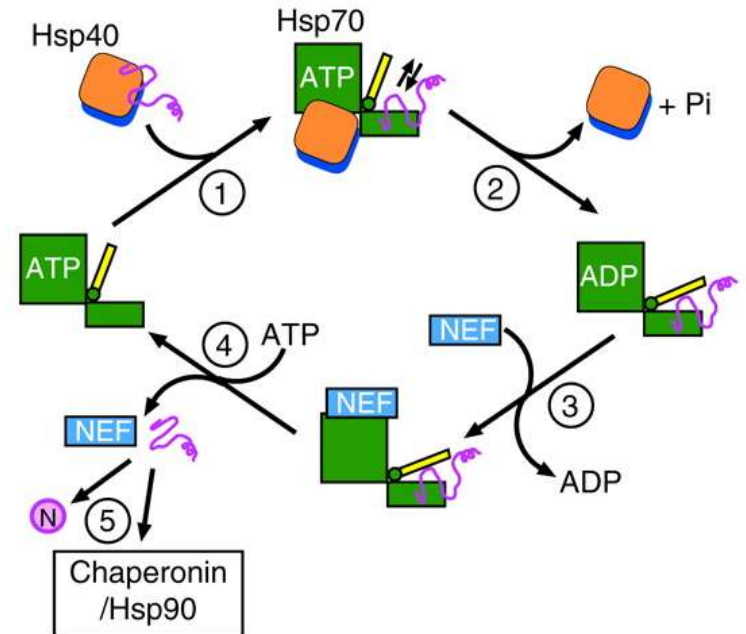
(b) Initial « open » model



(c) Initial « close » model



ADP



BAG NEF-Nucleotide exchange factor

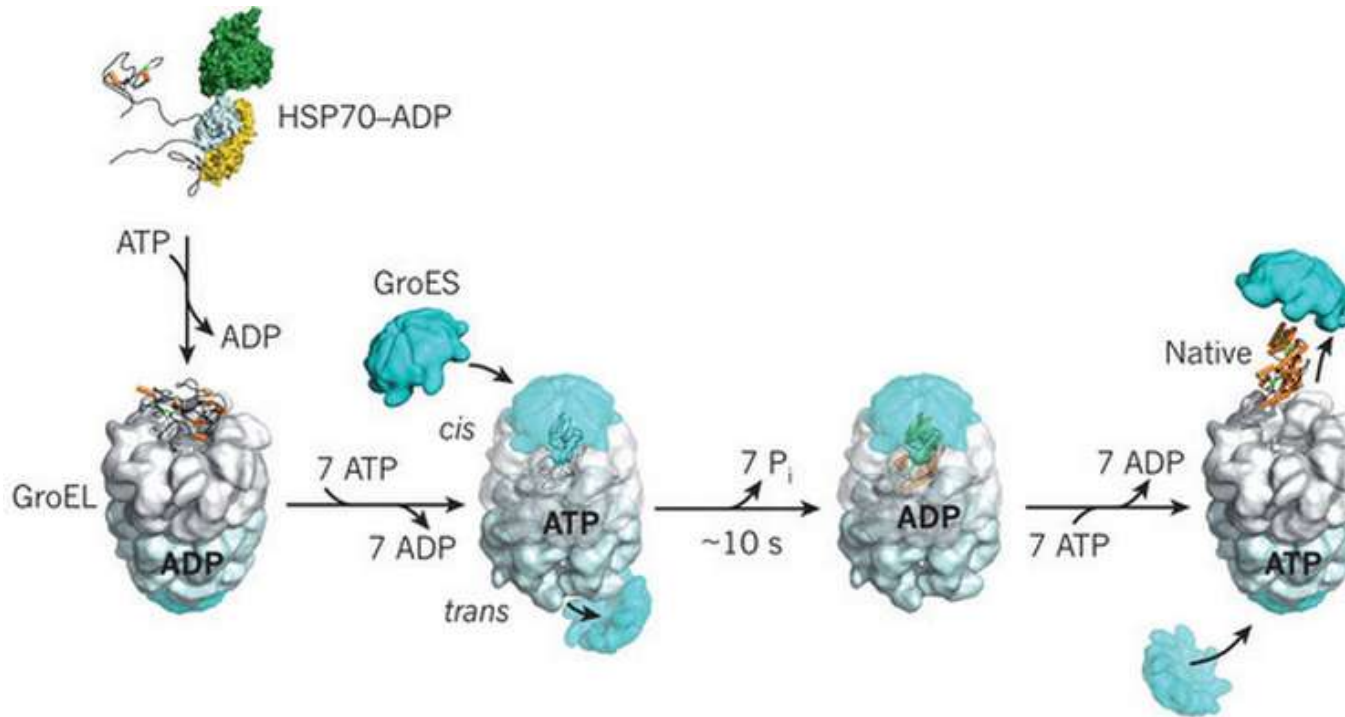
Hsp40

DnaJ

J-proteins

Chaperonins

(GroEL-GroES, Hsp60, CCT-TRiC)

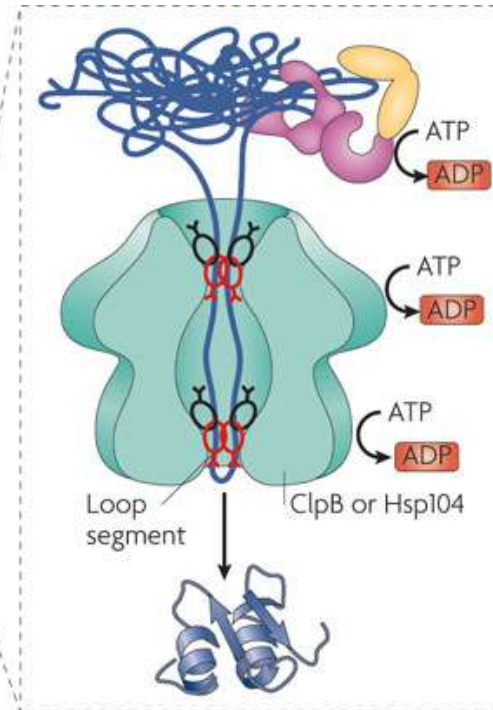
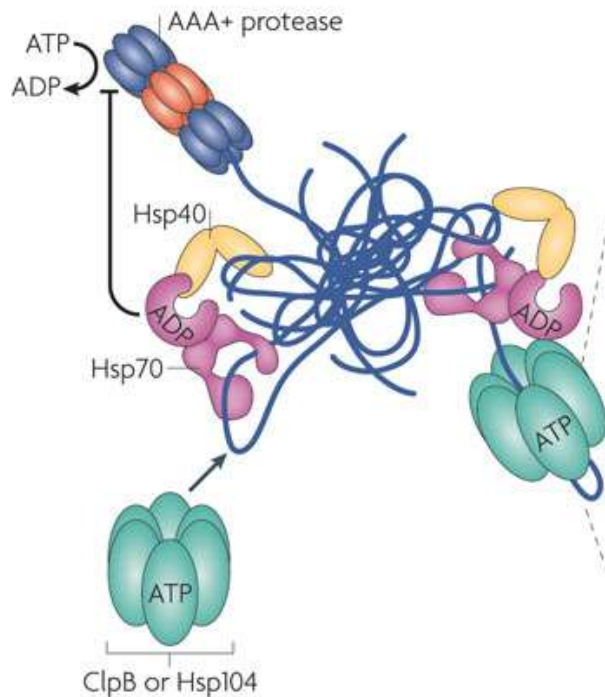
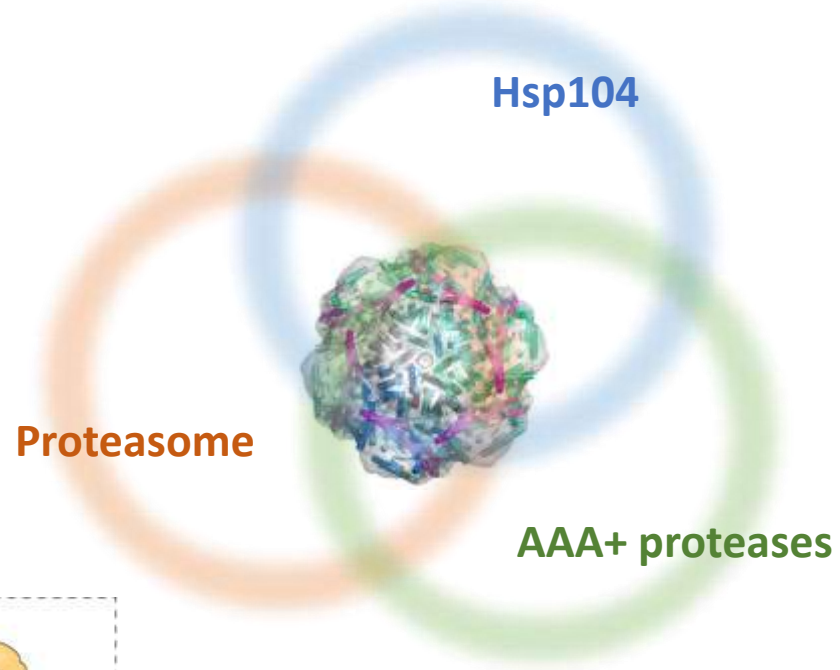


Folding of cytoskeletal proteins (tubulin)

Protein transport

Hsp104 (ClpB, ClpX,..)

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

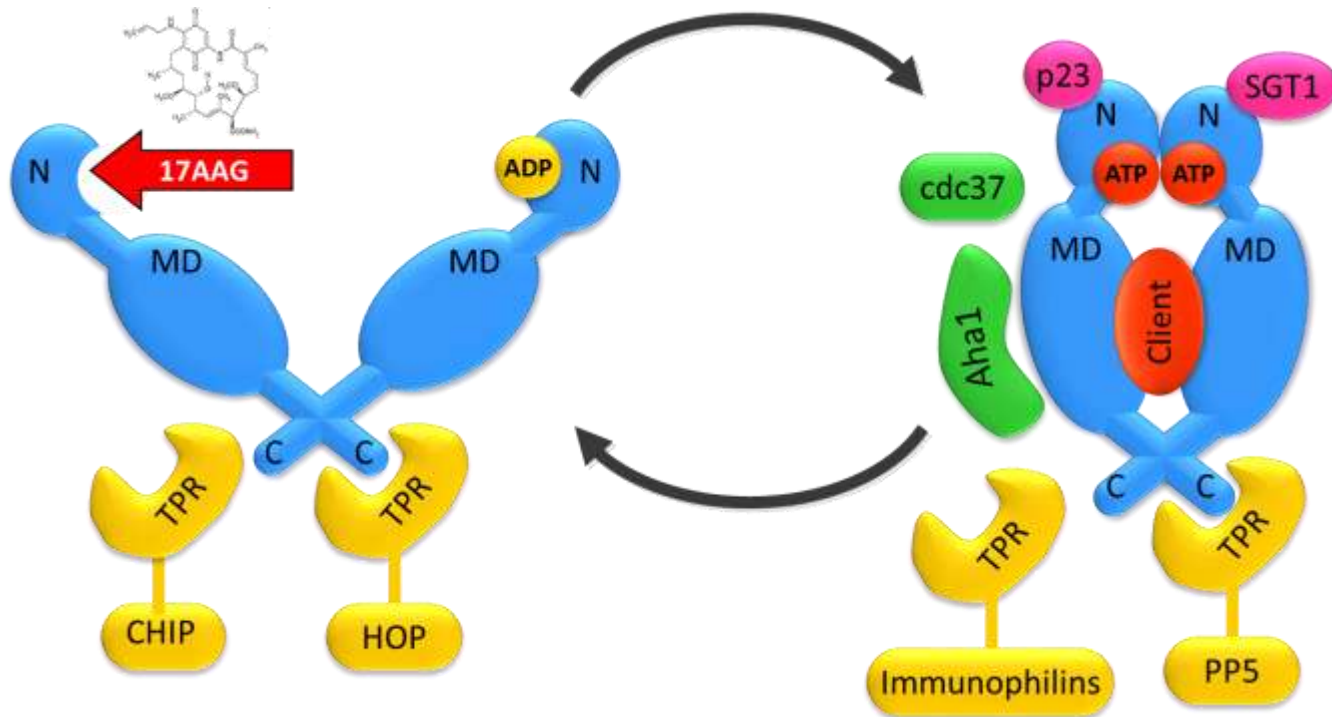
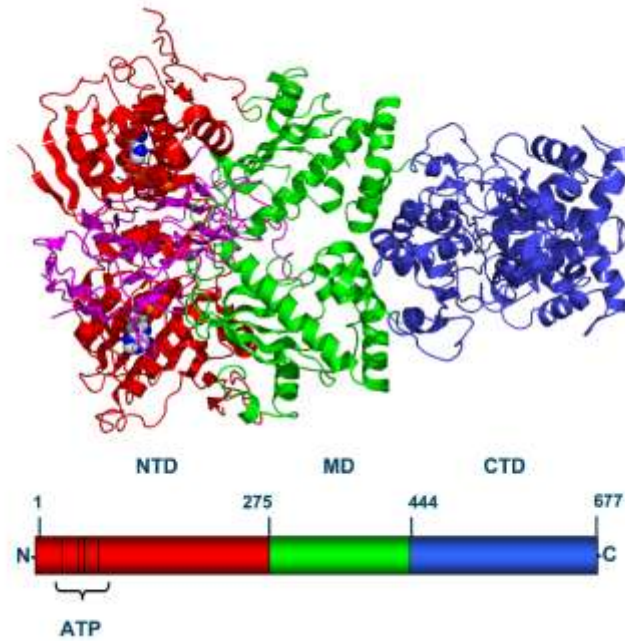


AAA+ ATPases

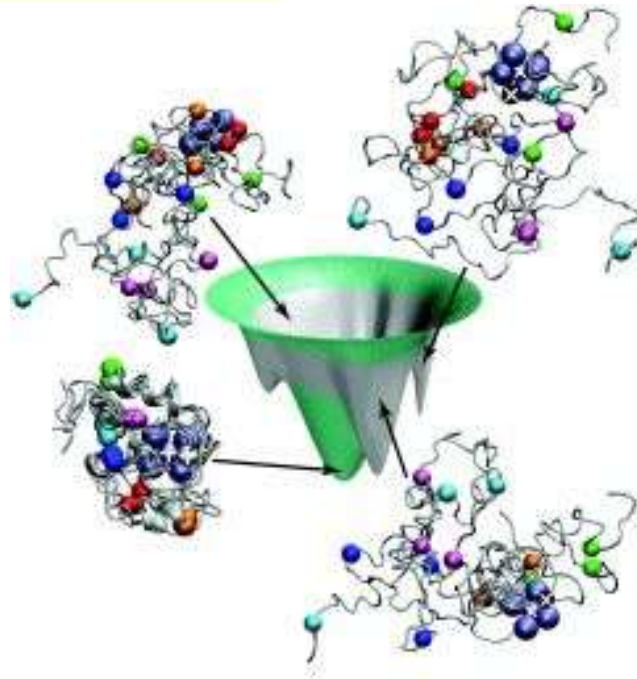
Converts ATP to “mechanical” energy (molecular motors)

Hsp90 chaperone machinery

- Conserved from procaryotes to mammals
- ATPase aktivty (like gyrase)
- Mitochondrial, ER, cytoplasmic
- Redundant isoformes



Stress proteins/ Chaperones/Hsp90



Hsp90 as a capacitor for morphological evolution

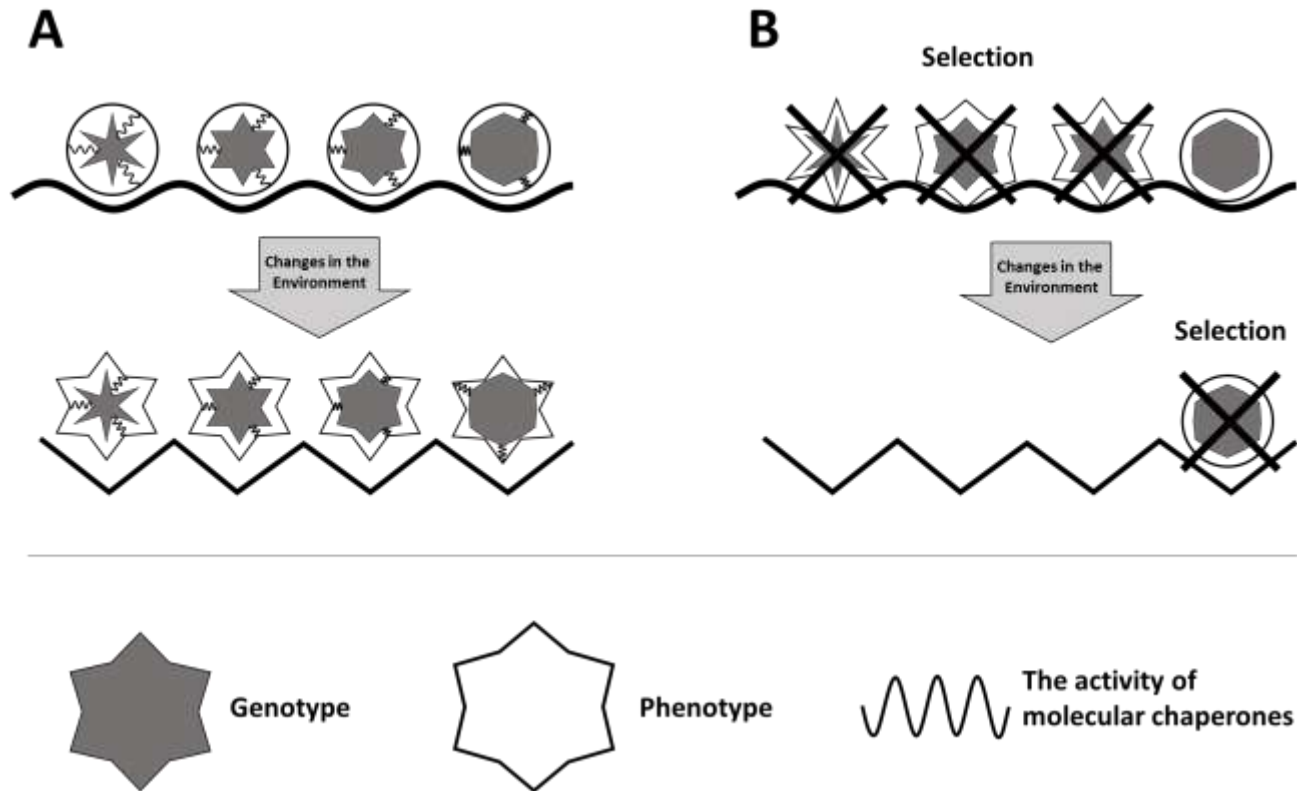
Suzanne L. Rutherford¹* & Susan Lindquist¹

¹Howard Hughes Medical Institute, University of Chicago, 5841 South Maryland Avenue MC3026, Chicago, Illinois 60637, USA

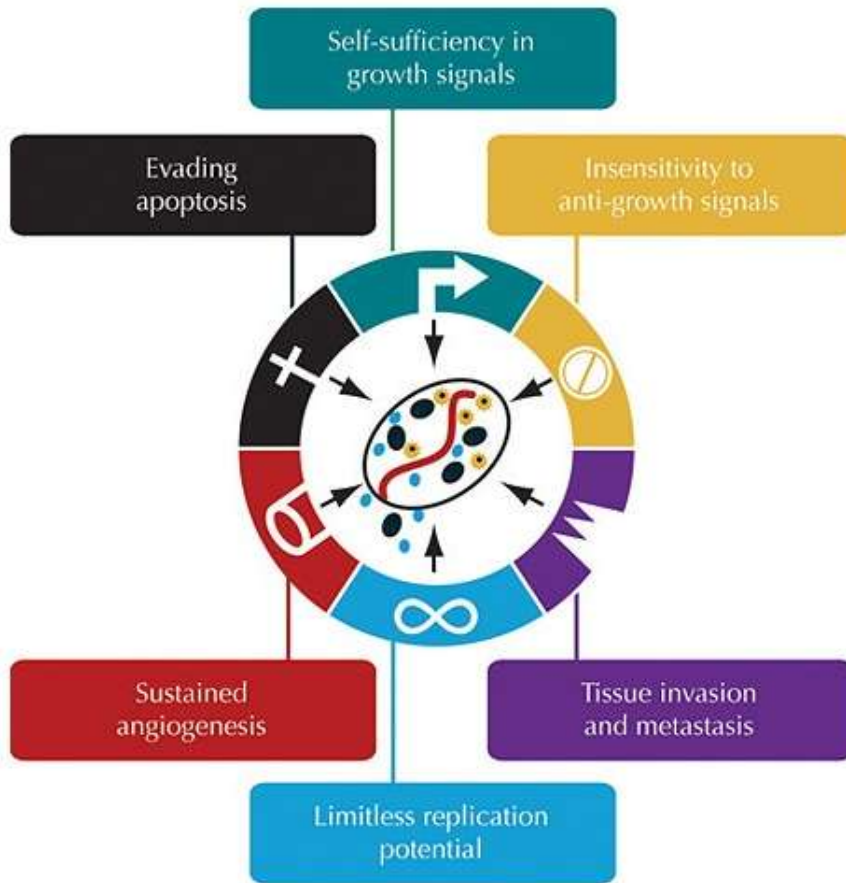
NATURE | VOL 396 | 26 NOVEMBER 1998 | www.nature.com

CHAPERONES AND EVOLUTION

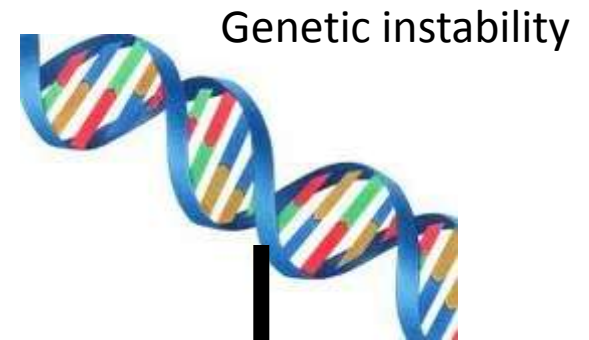
FILIP TRČKA, BORIVOJ VOJTESEK, PETR MULLER
 Regional Centre for Applied Molecular Oncology, Masaryk Memorial Cancer Institute,
 Zlutý kopec 7, 656 53 Brno



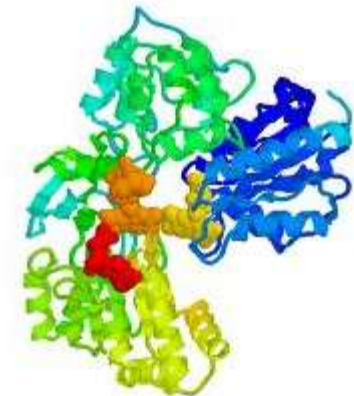
The tumor cells demand high quality and amount of protein



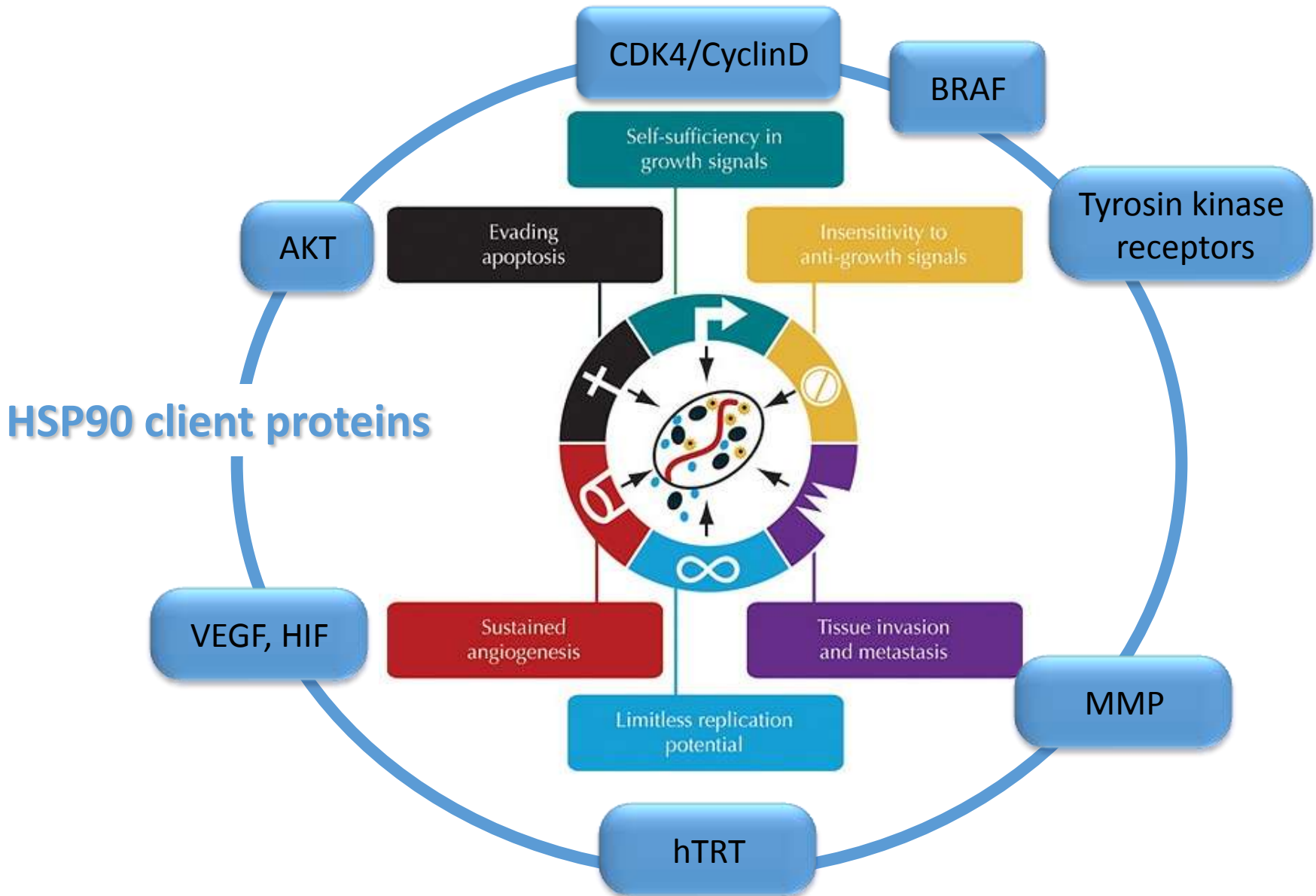
Hanahan D, Weinberg RA.: Cell. 2000 Jan 7;100(1):57-70.



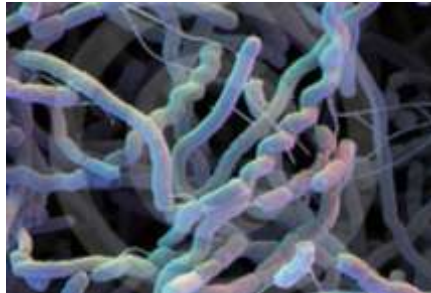
Enhanced proteosynthesis
Production of mutated,
conformational instable protins



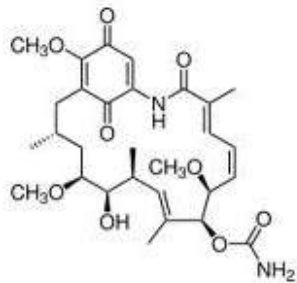
Activity of Hsp90 is essential for expression of cancer phenotype



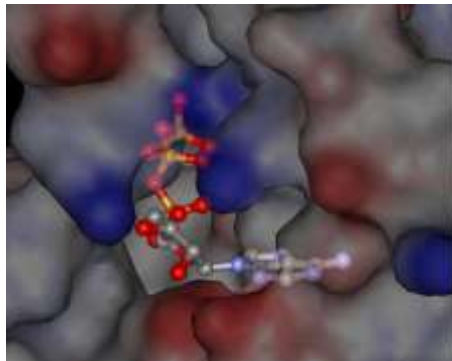
Specific inhibitors Hsp90



Isolation of Geldanamycin (1970)



Geldanamycin binds ATP cavity of Hsp90 (1997)



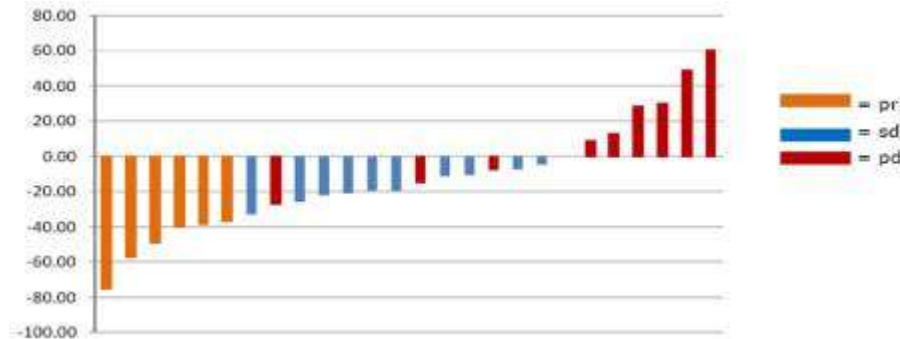
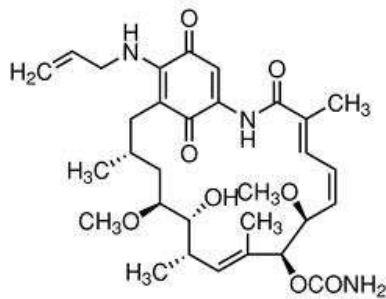
Clinical trials with Geldanamycin(2000)

	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	Myrexix 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 ?

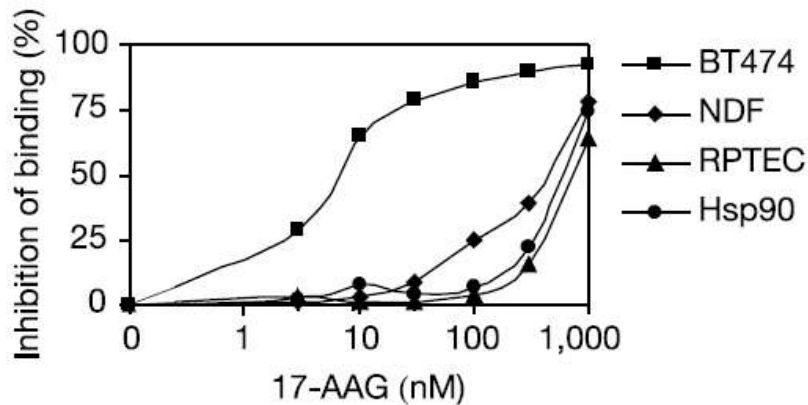
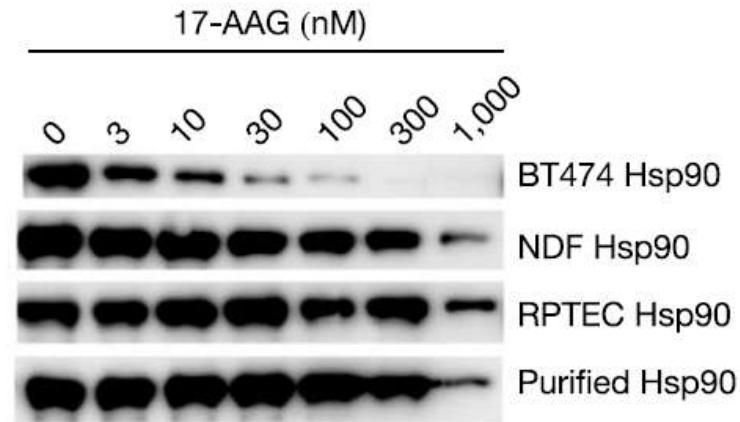
- posttranslational modifications
- expression pattern of co-chaperones

Client spectrum ?

letters to nature

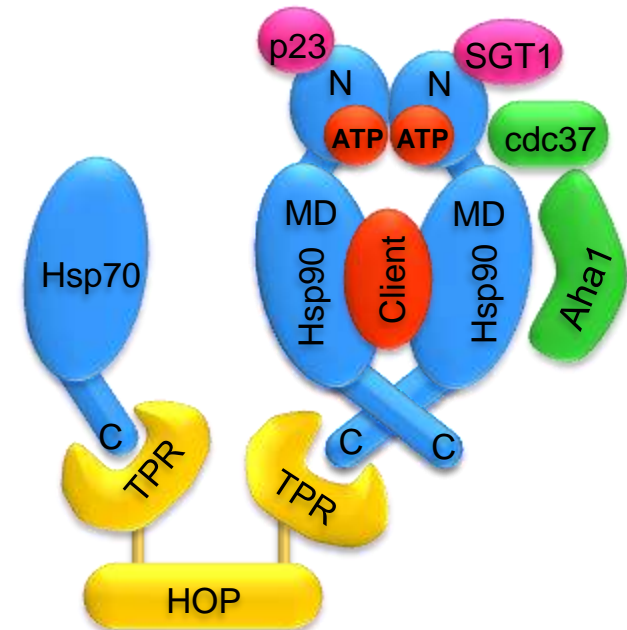
A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors

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



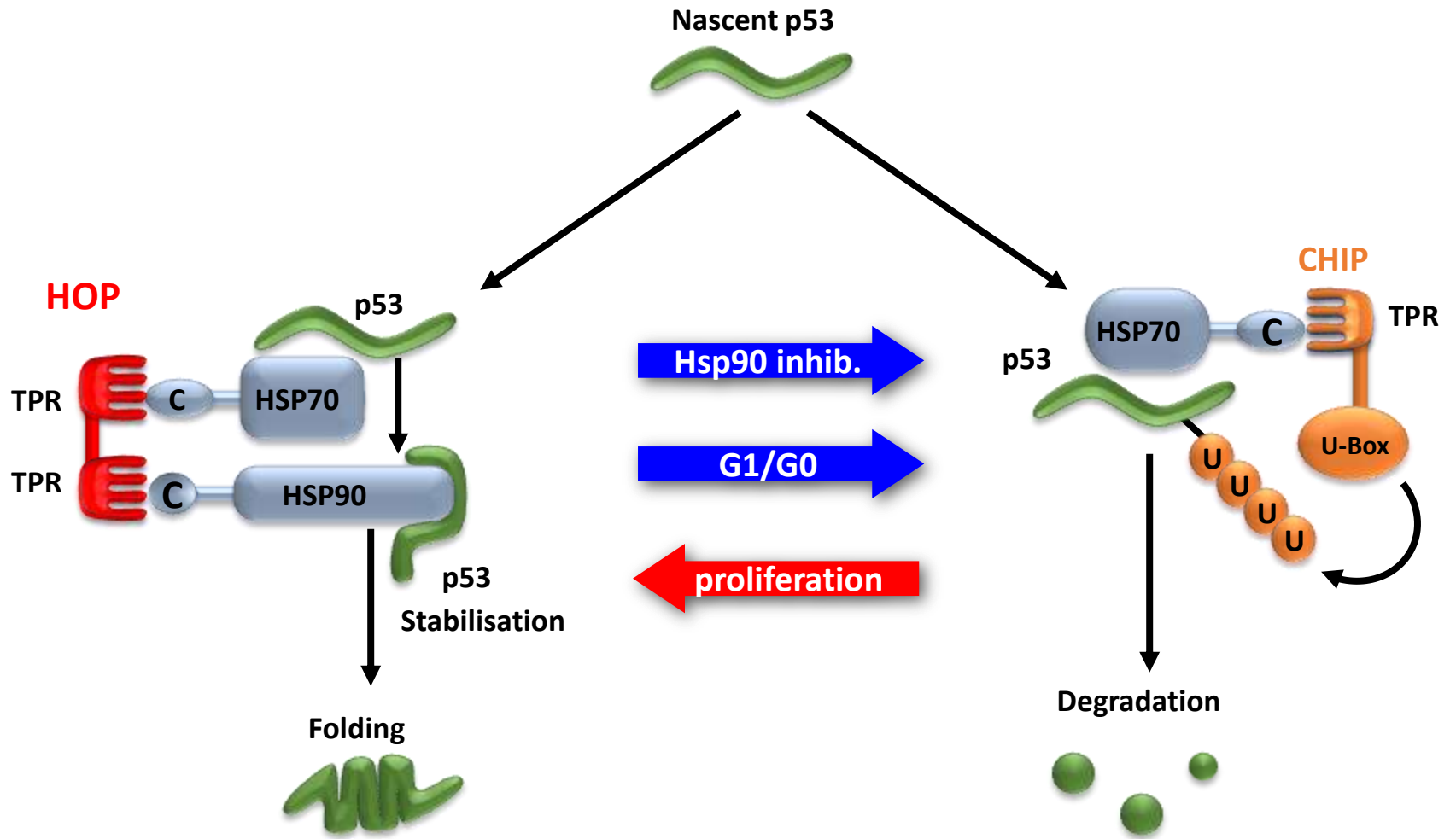
Multichaperone complex

- Hsp90+Hsp70
- cochaperones



Chaperone-dependent stabilization and degradation of p53 mutants

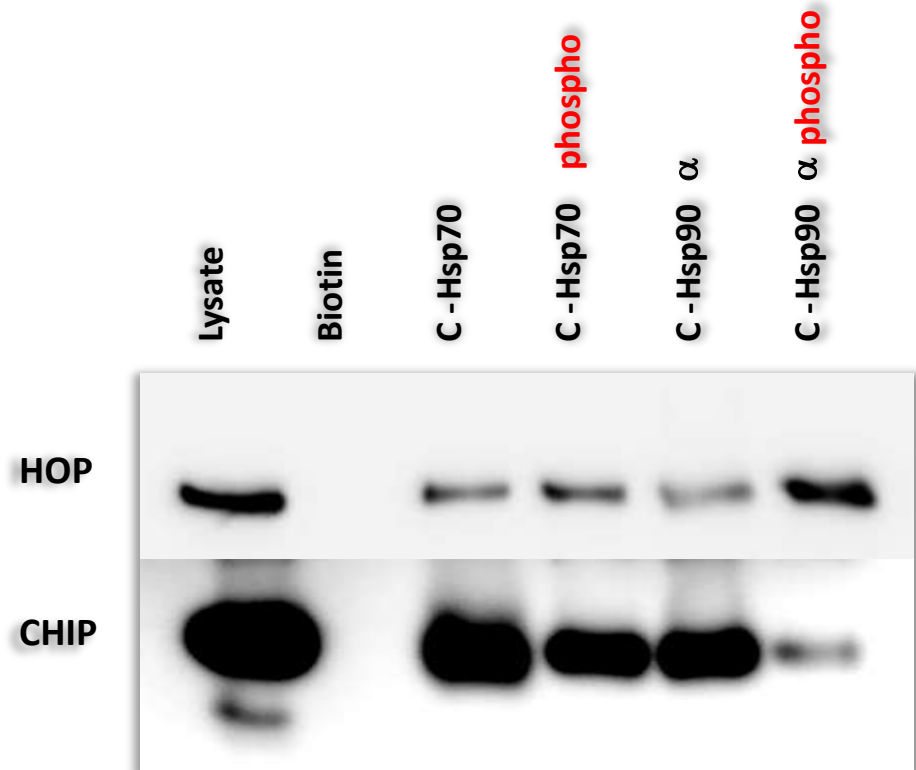
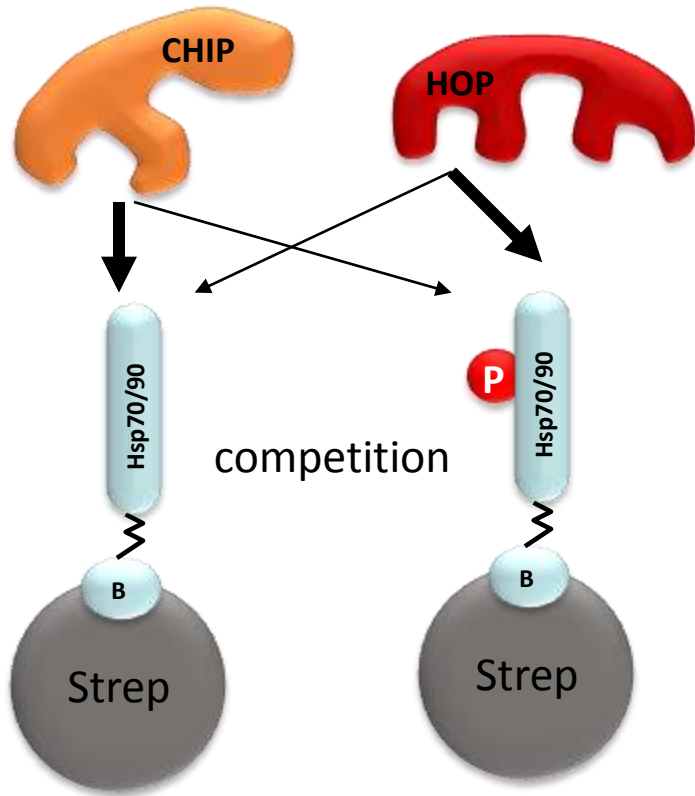
P Müller^{1,2}, R Hrstka¹, D Coomber², DP Lane² and B Vojtesek¹



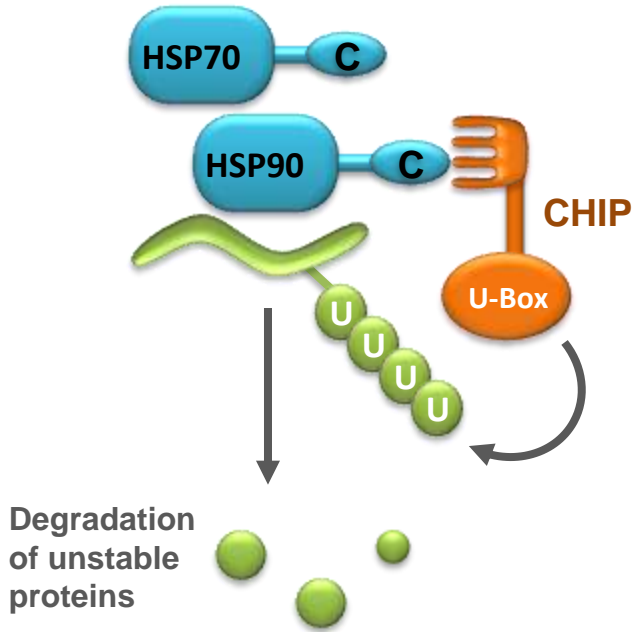
What is the mechanism regulating folding degradation balance ?

Cell lysate pulldown of HOP and CHIP

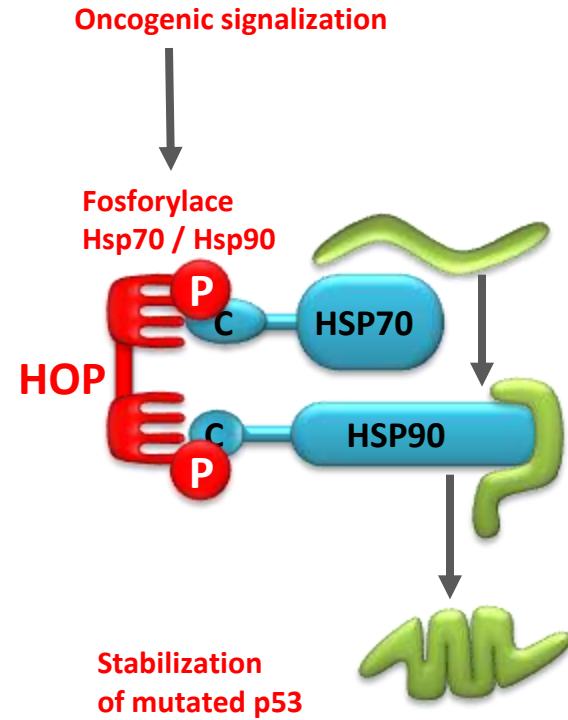
- Biotinylated phospho/non phospho peptides of Hsp70/Hsp90



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

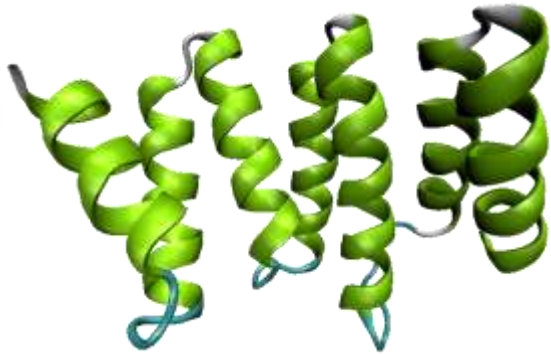
TOMM34 expression in early invasive breast cancer: a biomarker associated with poor outcome

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

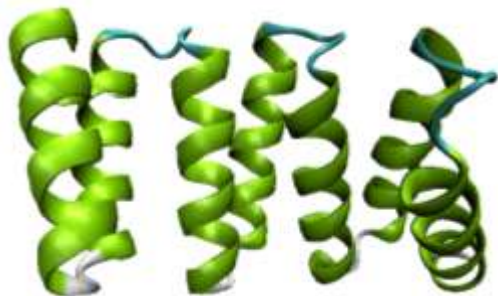


Positively charged clamp

TPR1



TPR2

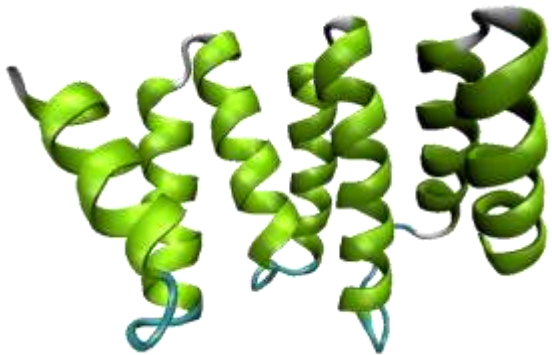


	Helix 1A	Helix 1B
Tom34-TPR1 Q15785 9-118	VEELRAAG	ESFRNGQYAEA
Tom34-TPR2 Q15785 193-294	ARVLKEEG	NELVVKGNHKA
CHIP Q9UNE7 26-127	AQELKEQG	NRLFVGRKYPEAA
HOP-TPR1 P31948 4-105	VNELKEKG	NKASVGNIDDA
HOP-TPR2A P31948 225-333	ALKEKELG	NDAYKKKDFDT
FKBP52 Q02790 270-386	STIVKERG	IVYFKEGKYKQ
PPP5 P53041 28-129	AEEELITQA	MDYFKAKDYEN
	Helix 2A	Helix 2B
Tom34-TPR1 Q15785 9-118	SSDPEEE	SVLYSNRAACH
Tom34-TPR2 Q15785 193-294	--SNLE	GSATYSNRAL
CHIP Q9UNE7 26-127	--NP-LV	AVVYTNRAL
HOP-TPR1 P31948 4-105	--DP-HN	HVLYSNRSAA
HOP-TPR2A P31948 225-333	--DP-TN	MTYITNQAA
FKBP52 Q02790 270-386	KAQA-LR	LASHLNLAM
PPP5 P53041 28-129	--NP-SN	AIYYGNRS
	Helix 3A	Helix 3B
Tom34-TPR1 Q15785 9-118	-----	IRPLLRASAY
Tom34-TPR2 Q15785 193-294	-----	VKA FYBRAQAH
CHIP Q9UNE7 26-127	-----	VKA HFFLGQCQ
HOP-TPR1 P31948 4-105	-----	GKGYSRKAAA
HOP-TPR2A P31948 225-333	DYRQIA	KAYARIGNSY
FKBP52 Q02790 270-386	-----	EKG LFRRGEAH
PPP5 P53041 28-129	-----	IKGYRRAAS

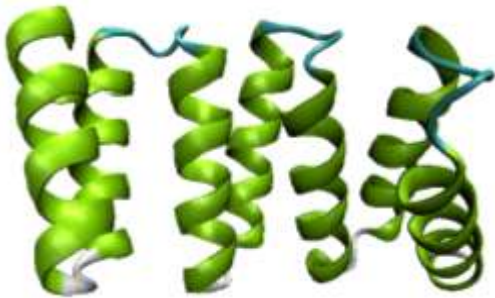
TOMM34 protein – co-chaperone

Tetratricopeptide repeat (TPR) domain

TPR1

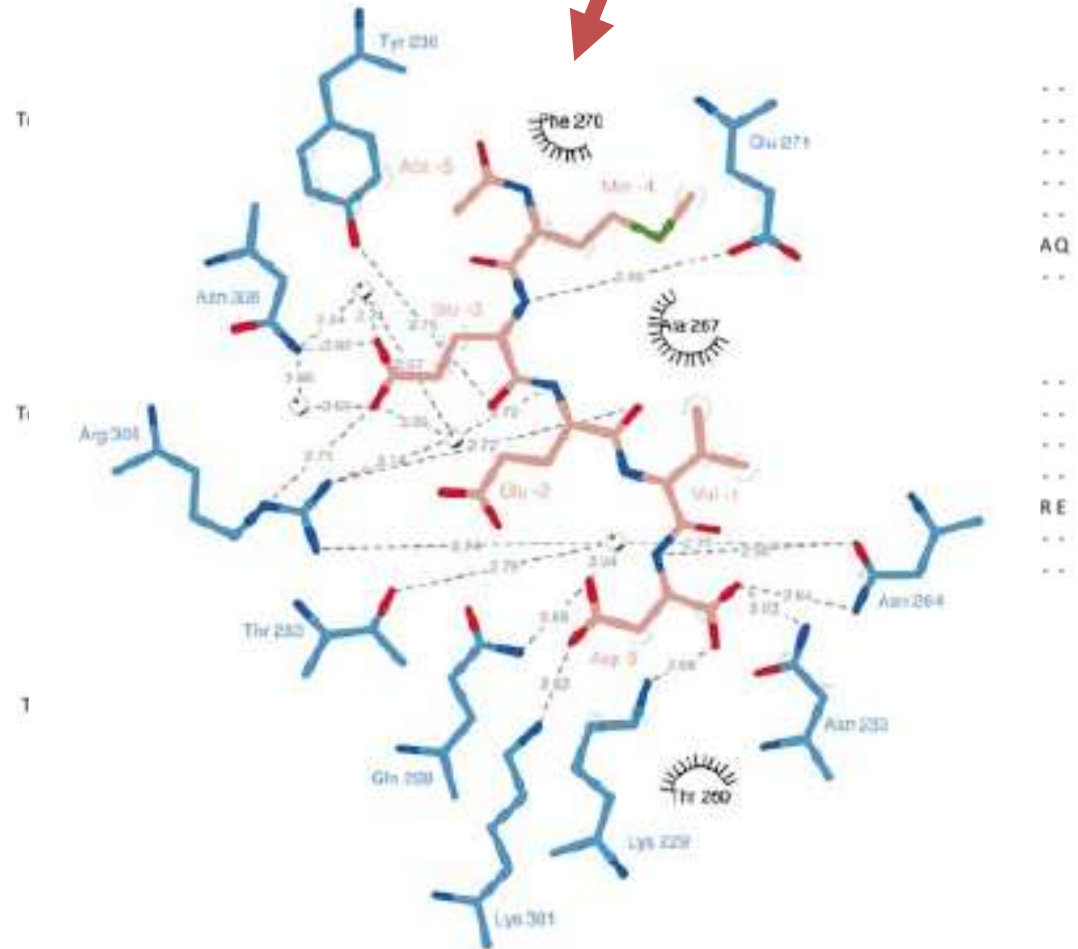


TPR2



C-terminus Hsp70/Hsp90

EEVD

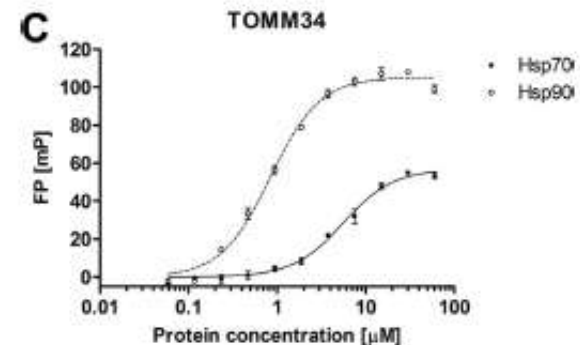
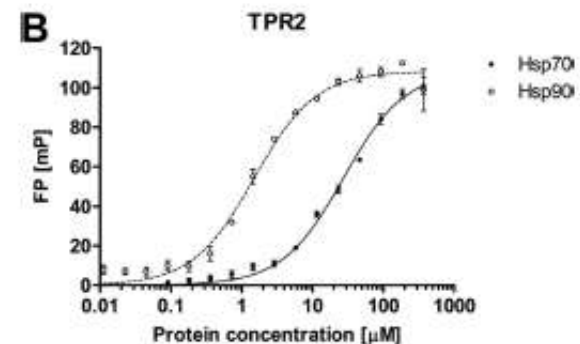
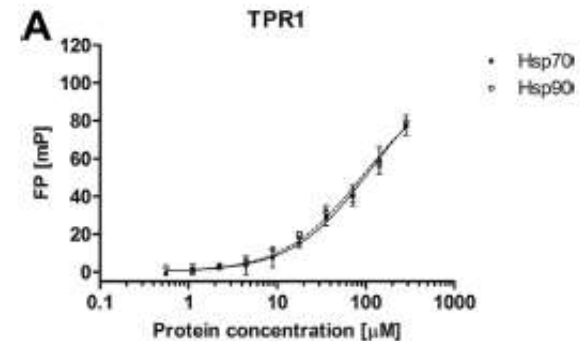
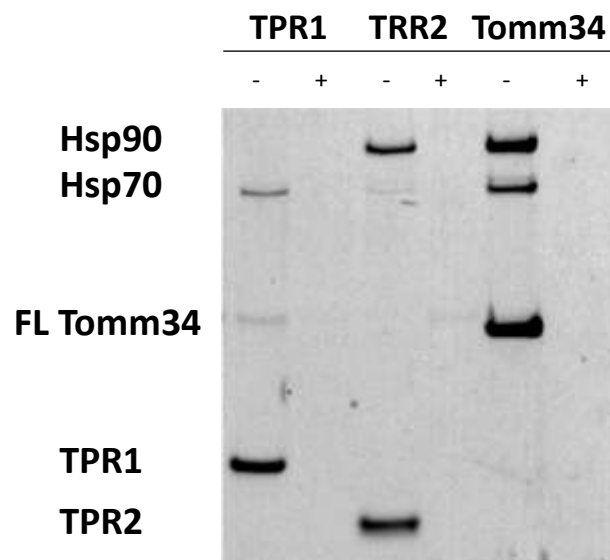
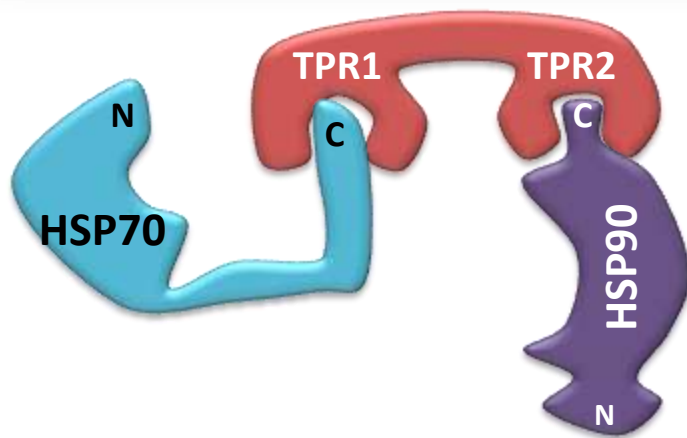


De novo modeled structure of TOMM34 domains

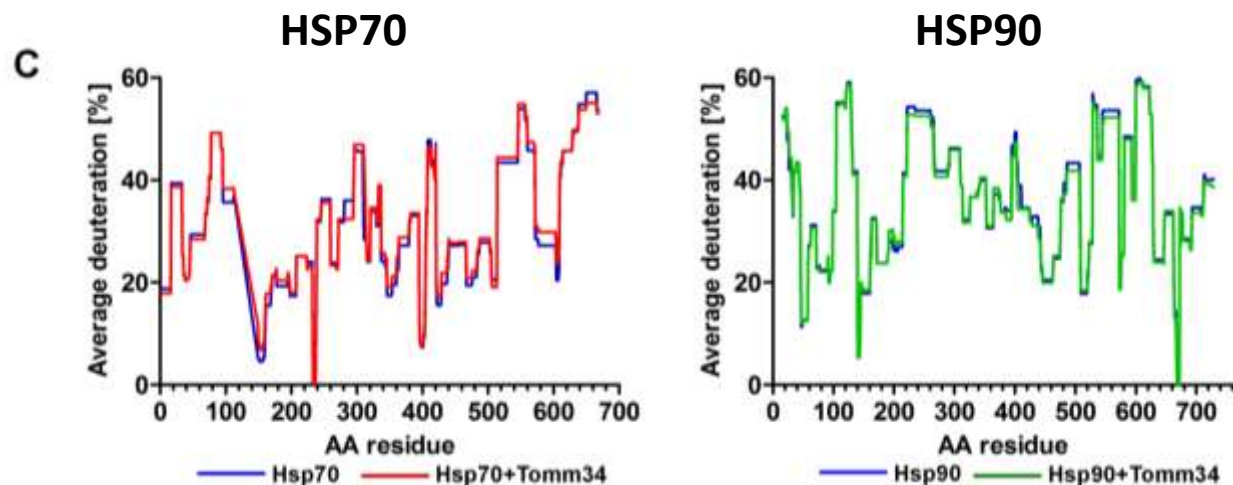
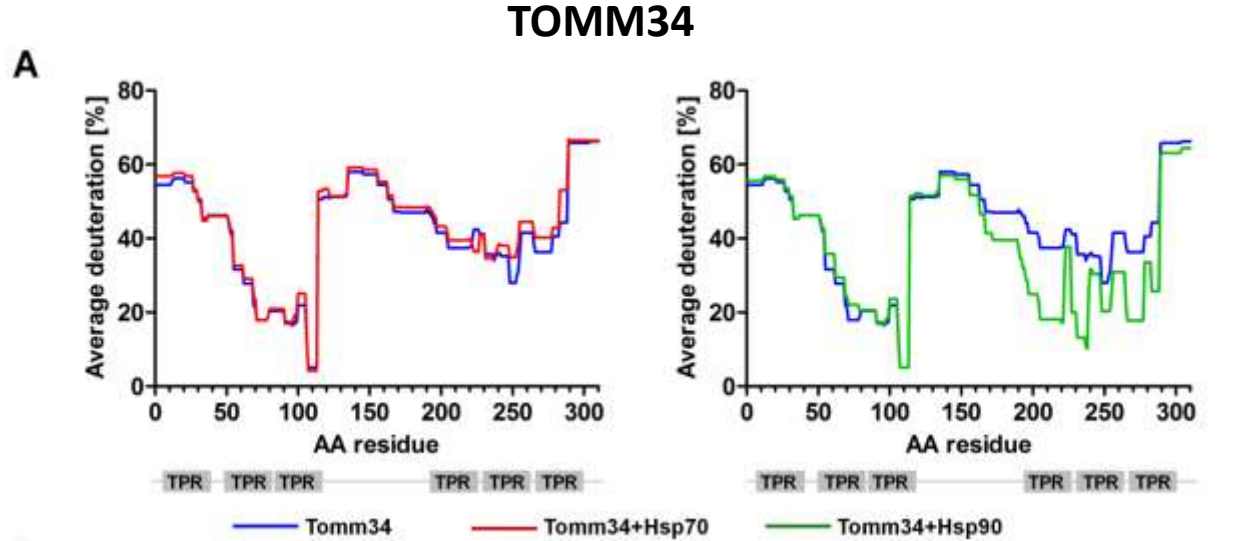
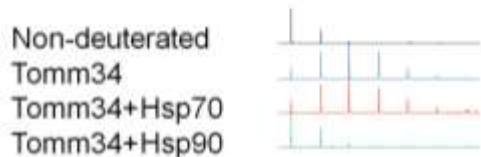
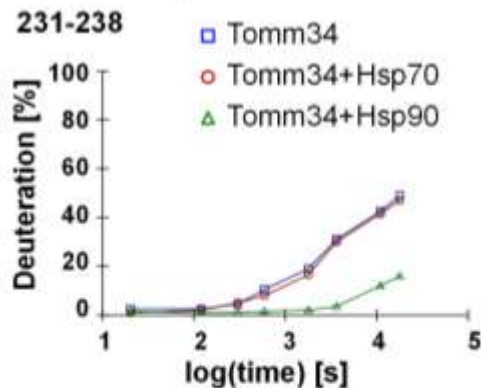
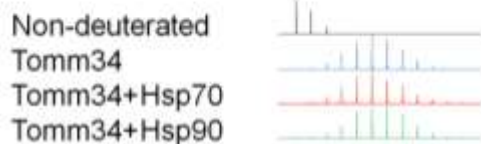
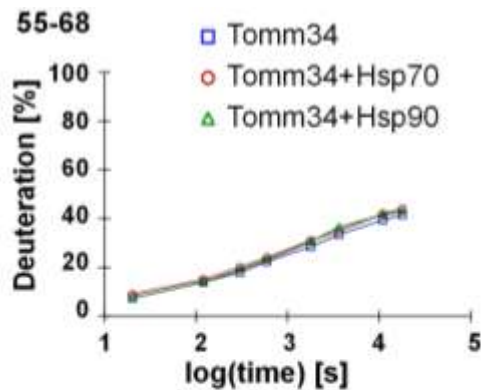
The Assembly and Intermolecular Properties of the Hsp70-Tomm34-Hsp90 Molecular Chaperone Complex*

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Fillip Trcka[‡], Michal Durech[‡], Petr Man^{§¶}, Lenka Hernychova[‡], Petr Muller^{†1,2}, and Borivoj Vojtesek^{†1,3}

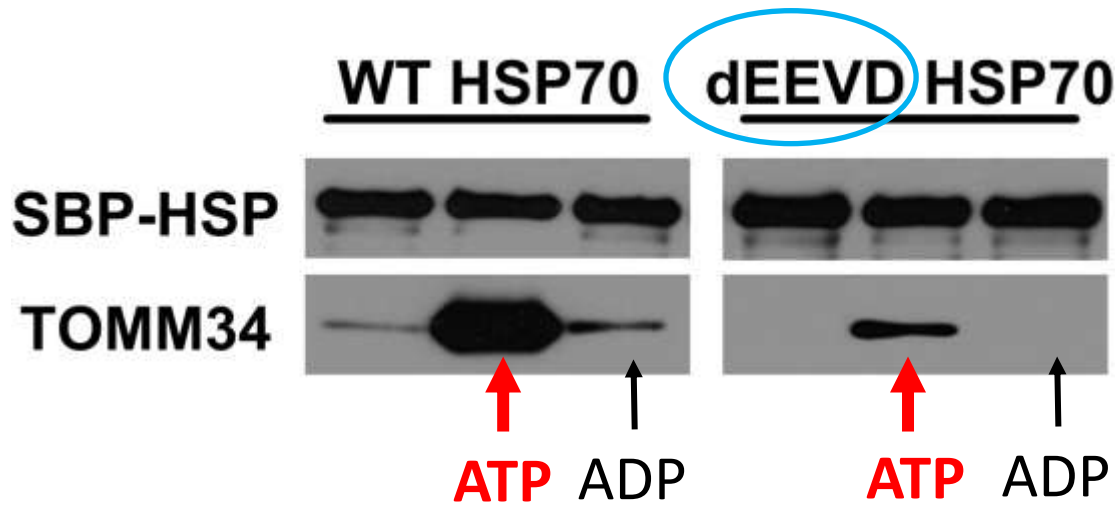
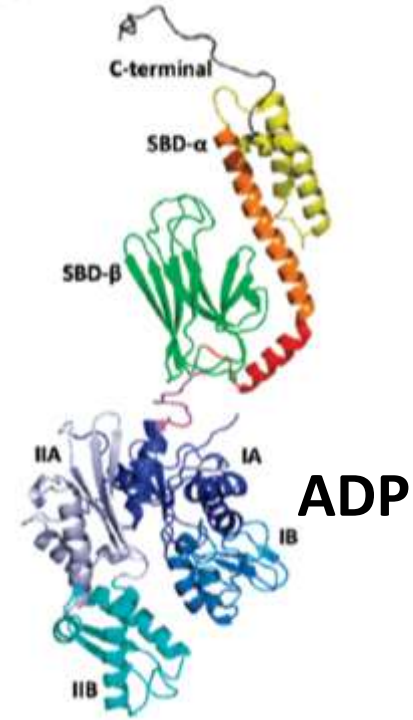
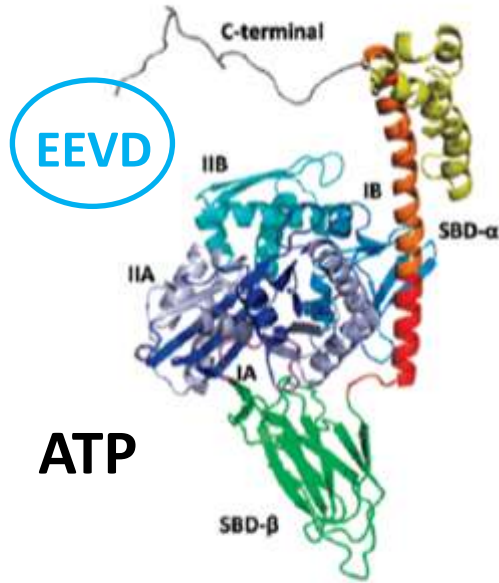
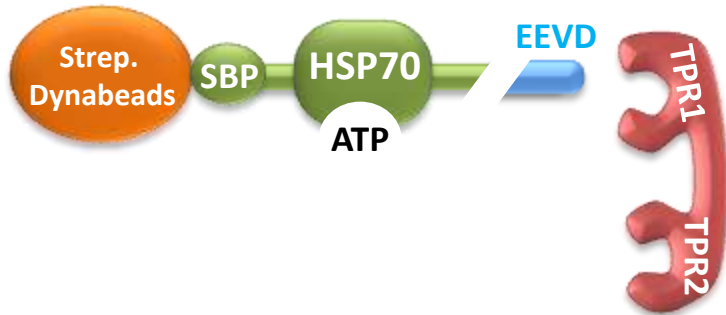


HDX – HSP70/90-TOMM34 interaction without ATP

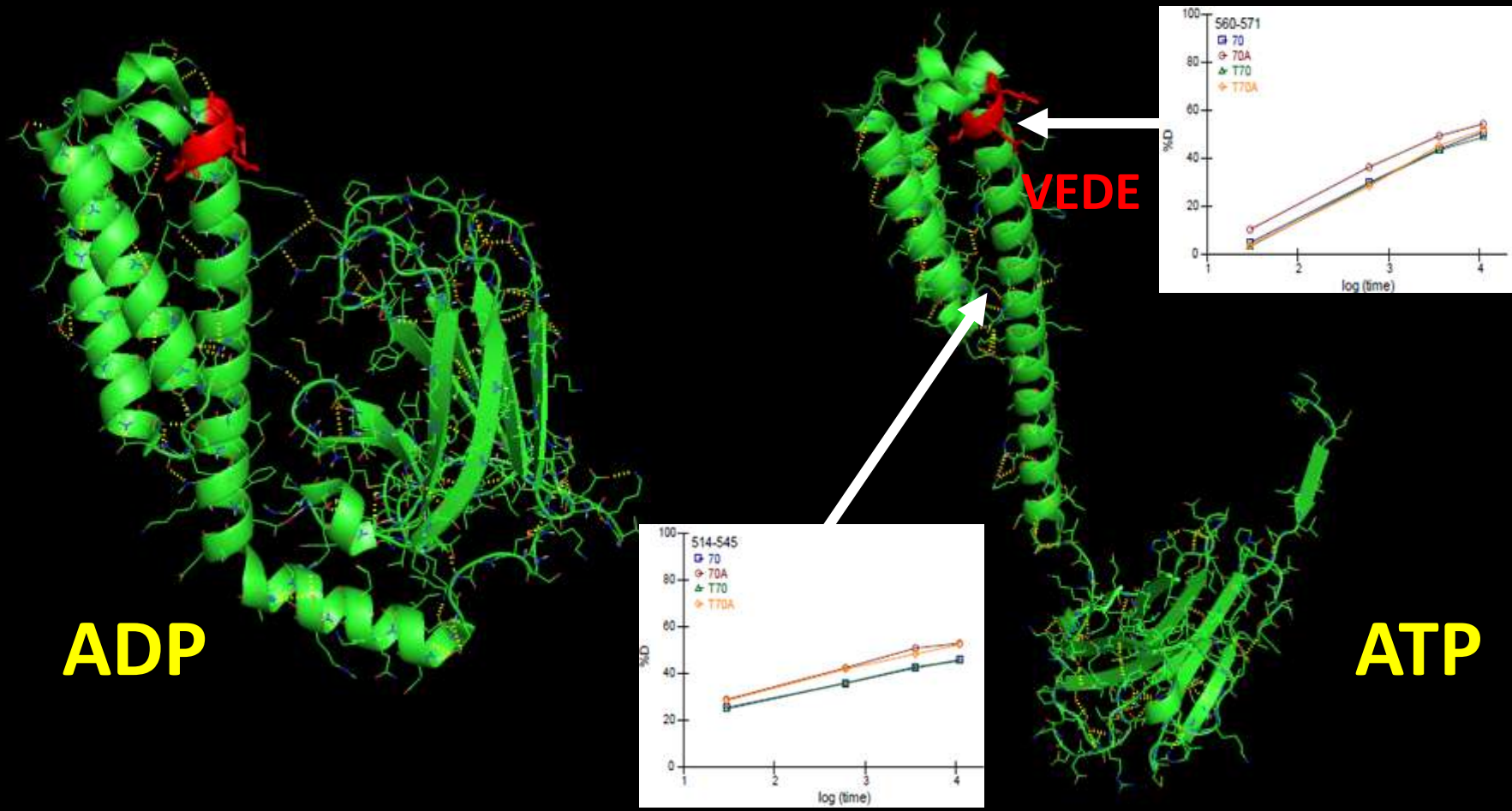
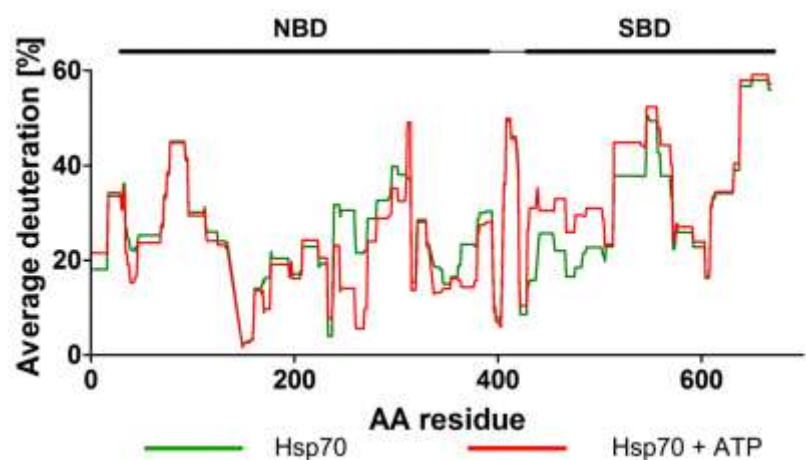


The effect of ATP

on HSP70 – Tomm34 interaction

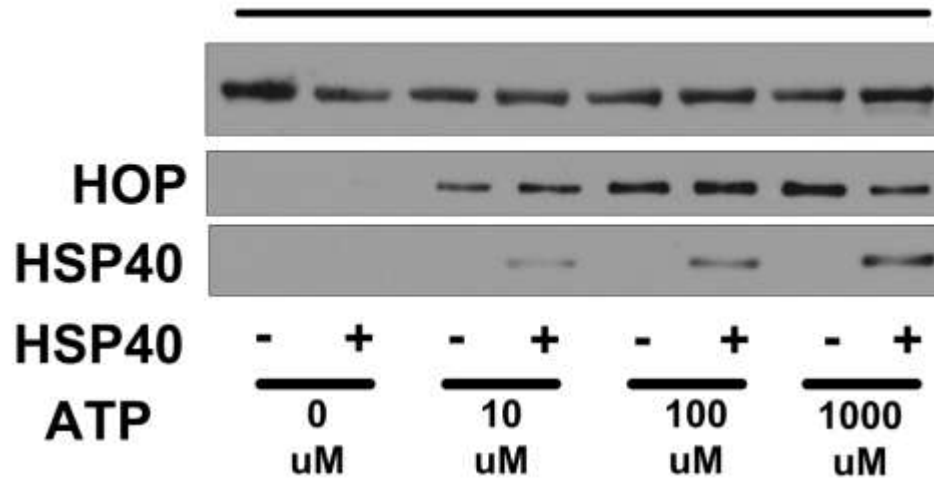


HDX – HSP70-TOMM34 interaction with ATP

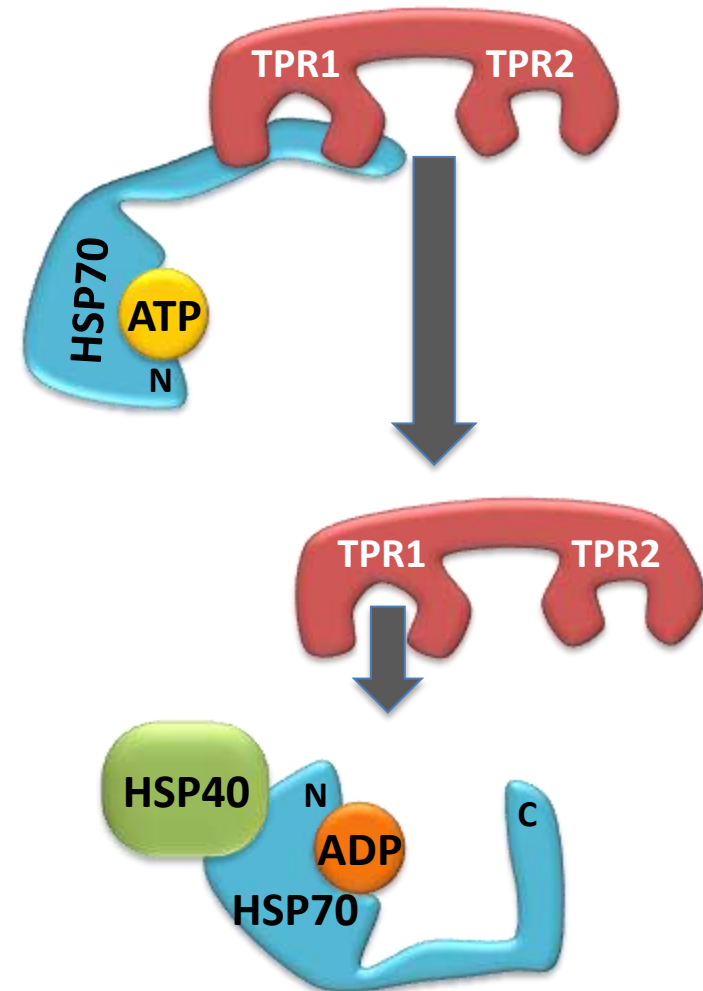
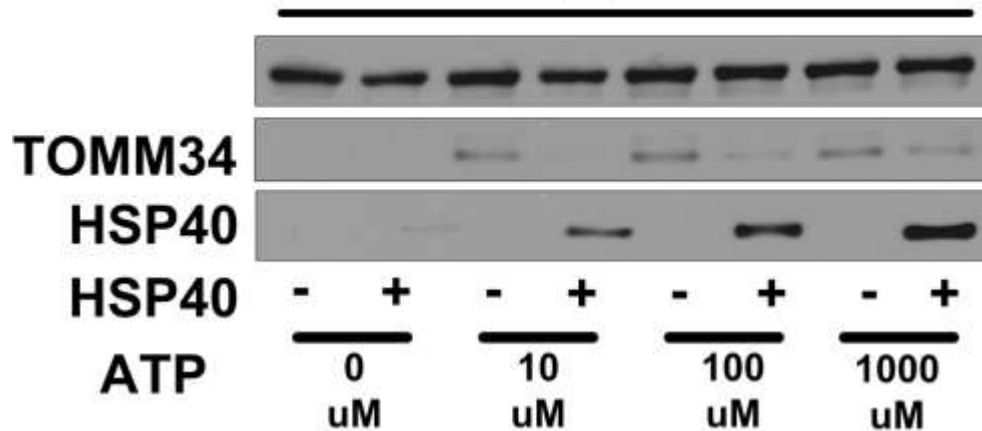


HSP70-TOMM34 interaction with ATP, the role of HSP40

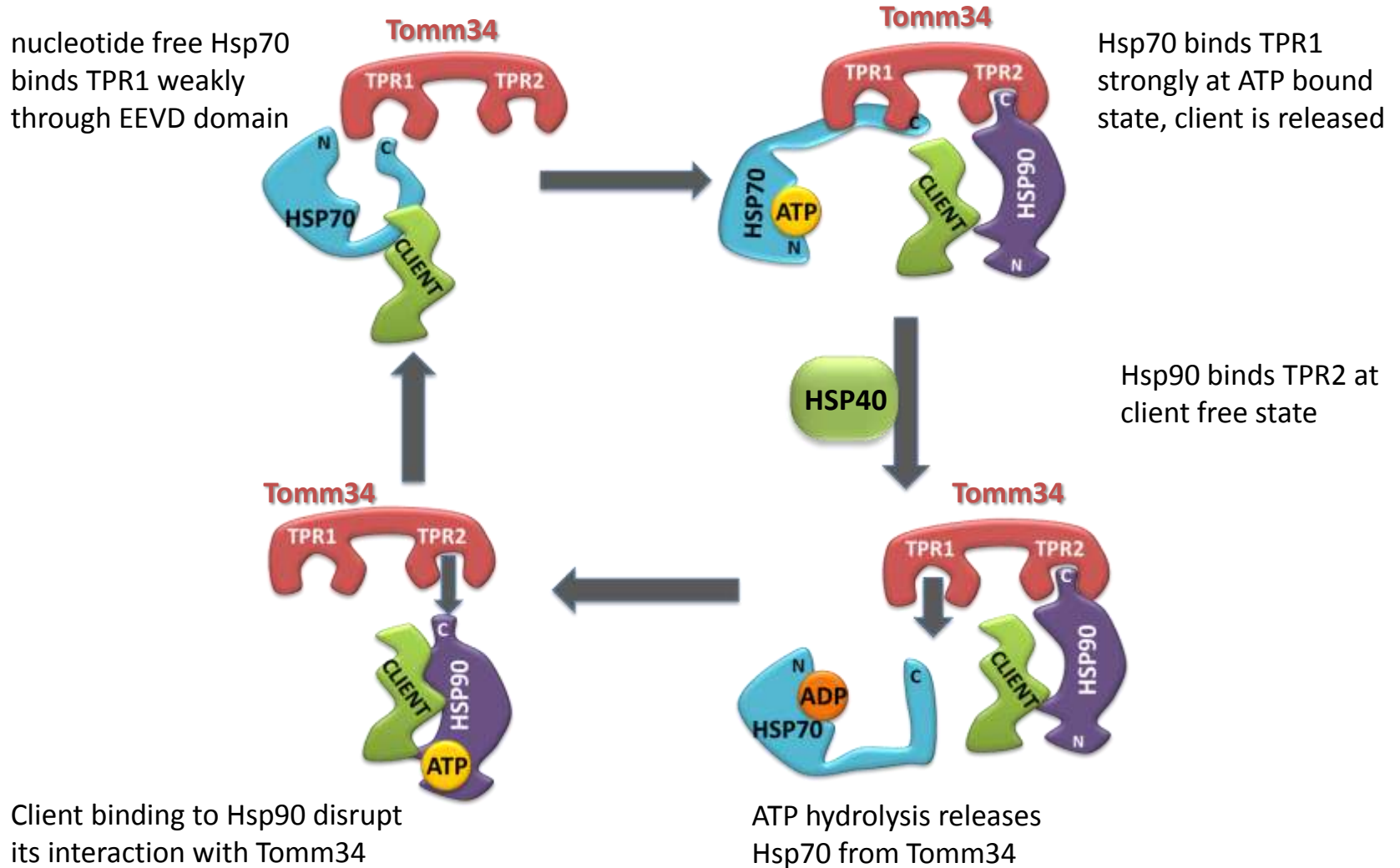
WT HSP70



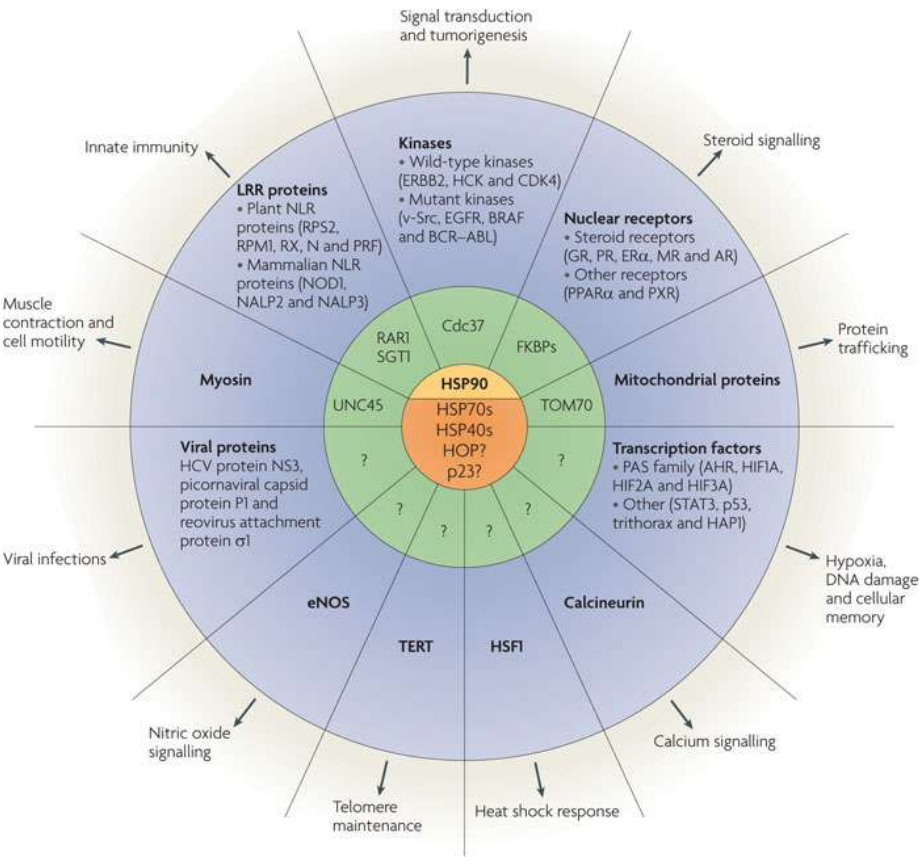
WT HSP70



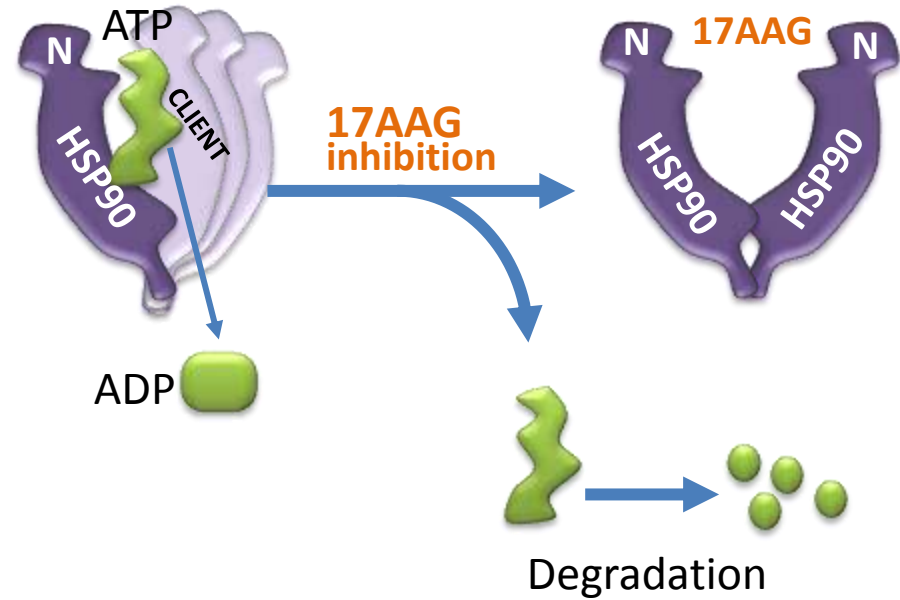
Cooperation of Hsp70/Hsp90 folding by Tomm34



Hsp90 client proteins



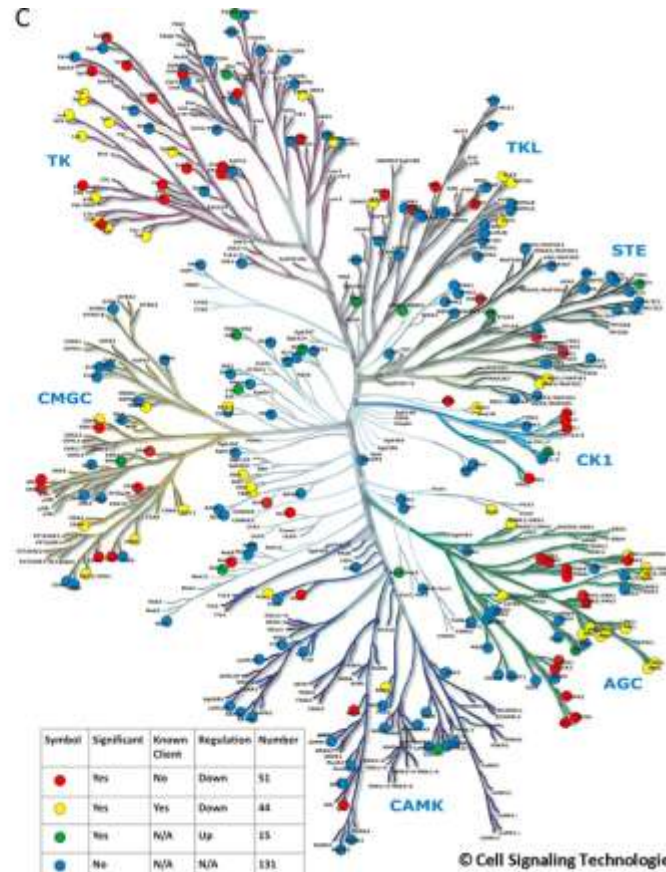
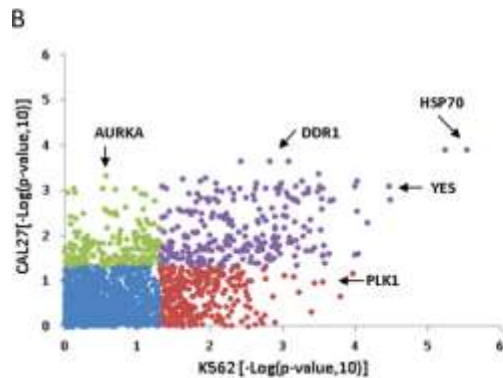
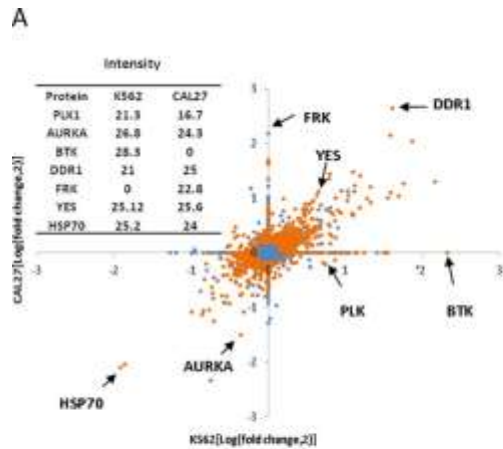
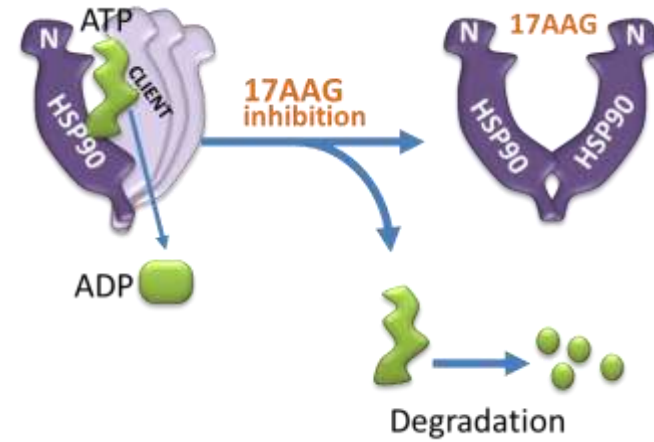
Nature Reviews | Molecular Cell Biology



Is there any structural/motif motif recognized by Hsp90 ?

Systematic Identification of the HSP90 Regulated Proteome[®]

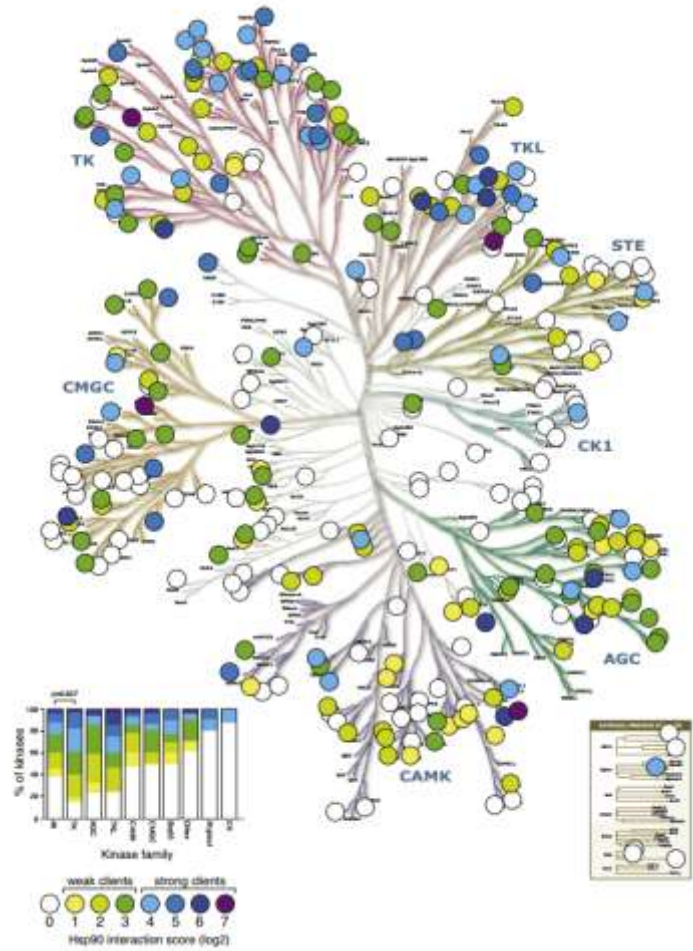
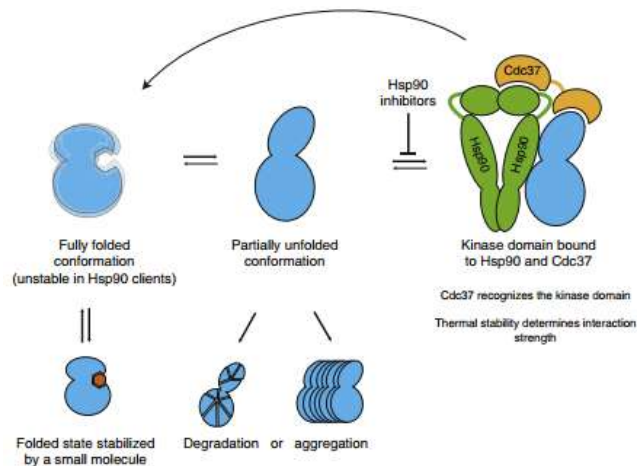
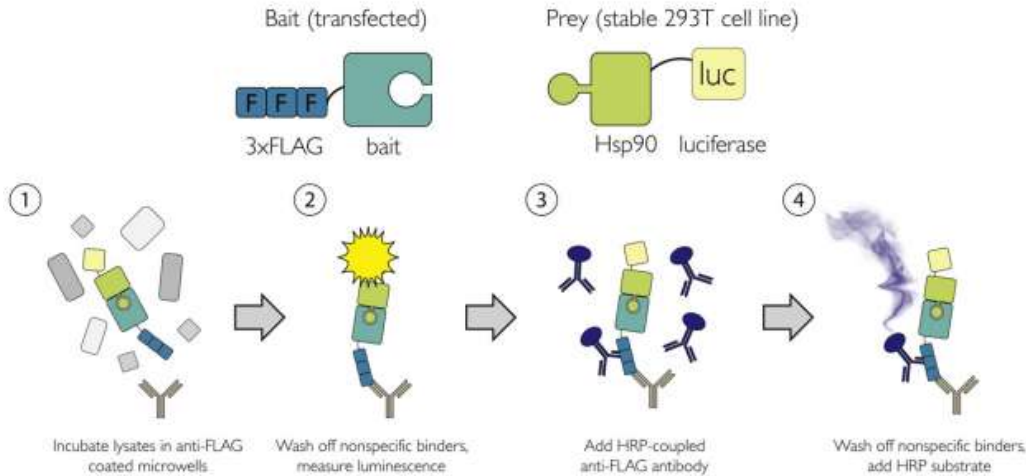
Zhixiang Wu[‡], Amin Moghaddas Gholami[‡], and Bernhard Kuster^{‡§¶}



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

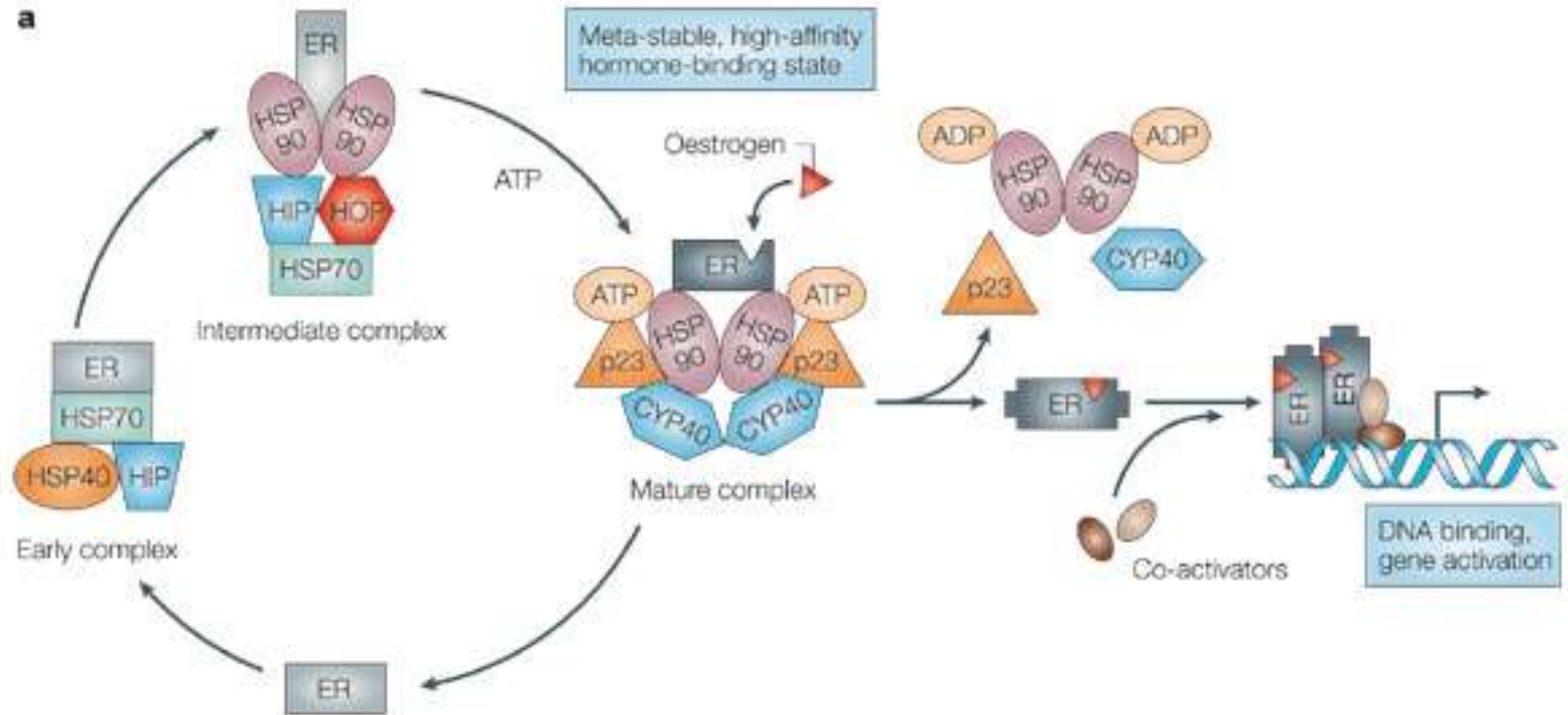
Cell

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,*}



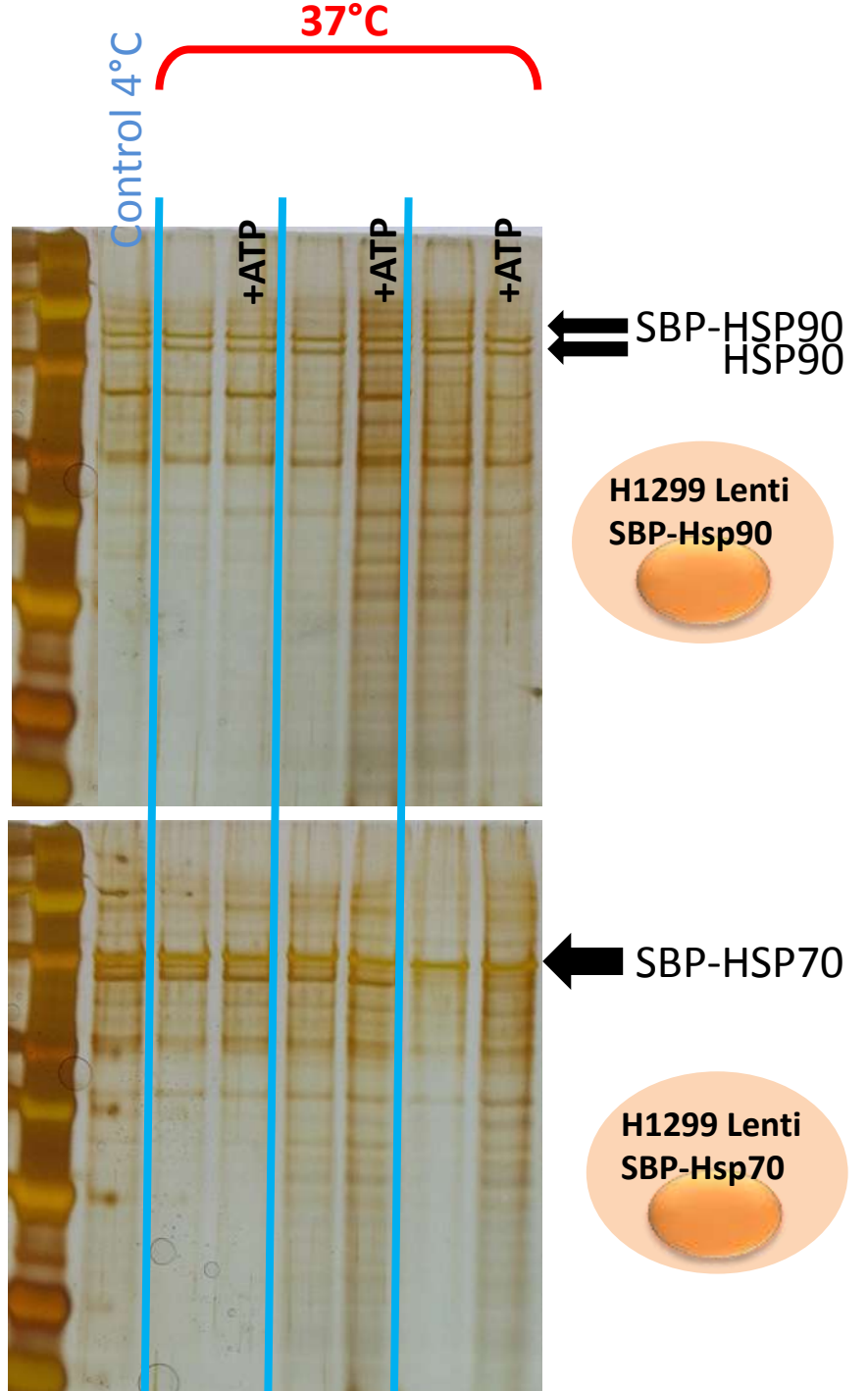
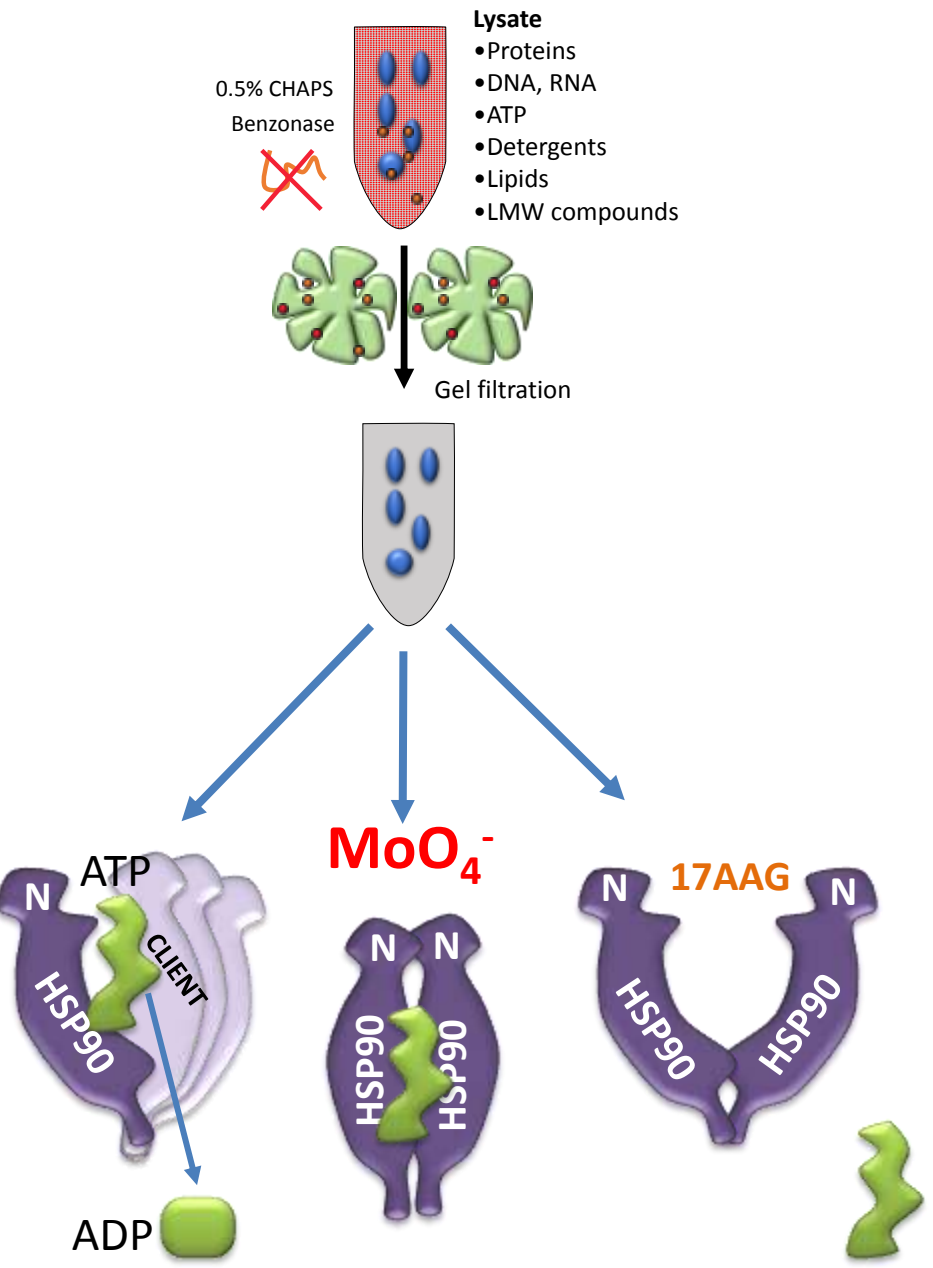
No consensus sequence of Hsp90 clients

a



Ligand promotes conformational stabilization of steroid receptors

Functional proteomic to study Hsp90 complexes

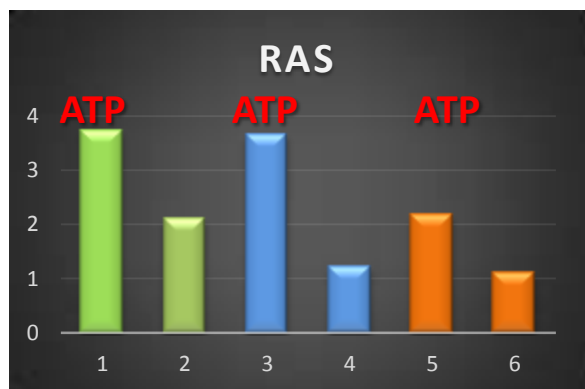
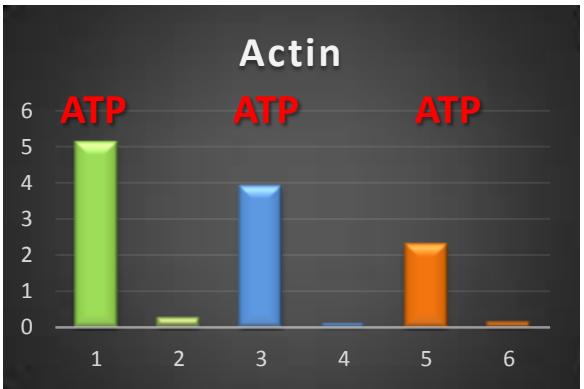
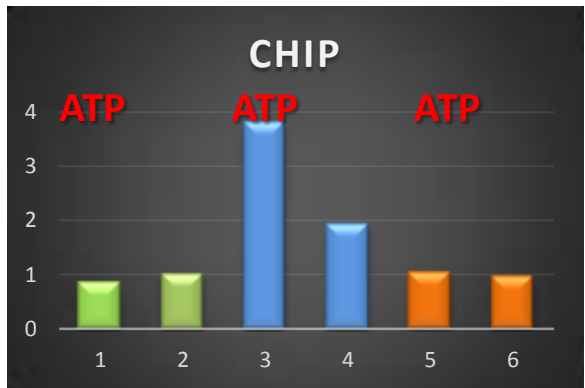
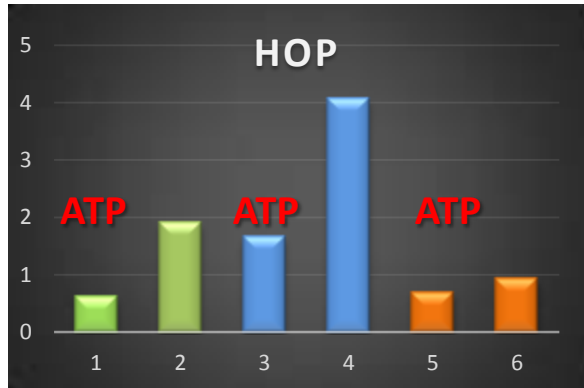
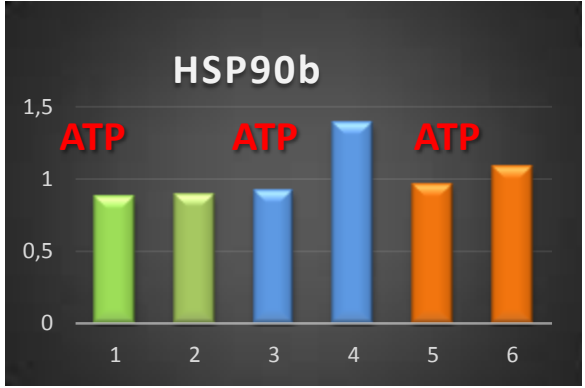
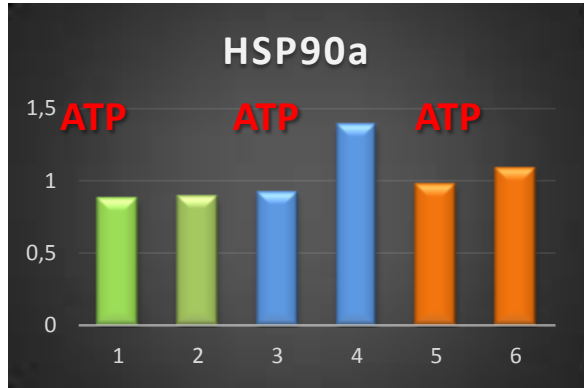
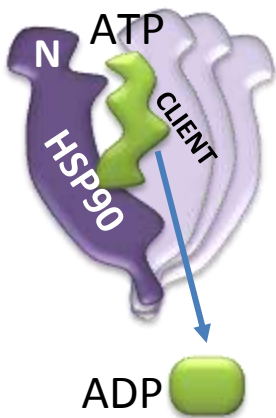


SBP Hsp90

Control

Molybdate

Hsp90 inh.

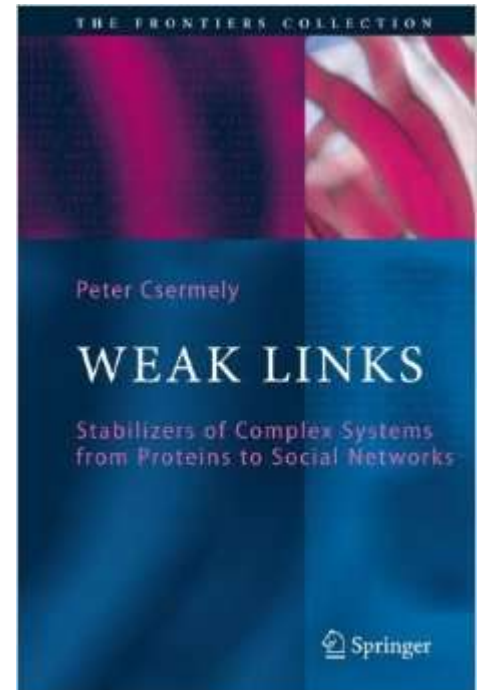


Any protein can be client of Hsp90

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



Edinburgh Cancer
Research Centre

Ted R. Hupp