

Molekulární chaperony a jejich úloha v patogenezi lidských chorob

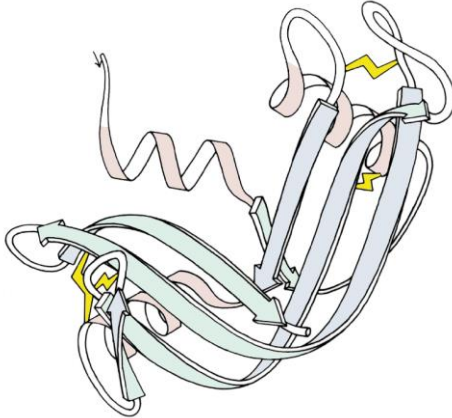
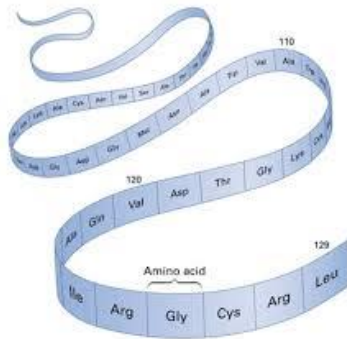
Petr Muller

RECAM 

Regional Centre
for Applied Molecular
Oncology

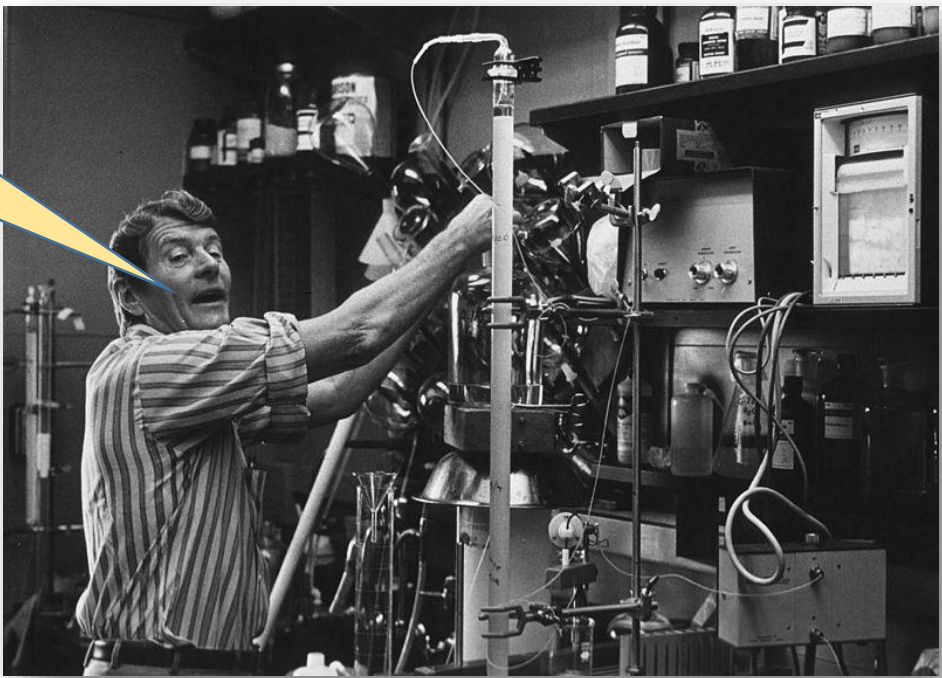


At the environmental conditions (temperature, solvent concentration and composition, etc.) at which folding occurs, the native structure is a unique, stable and kinetically accessible minimum of the free energy

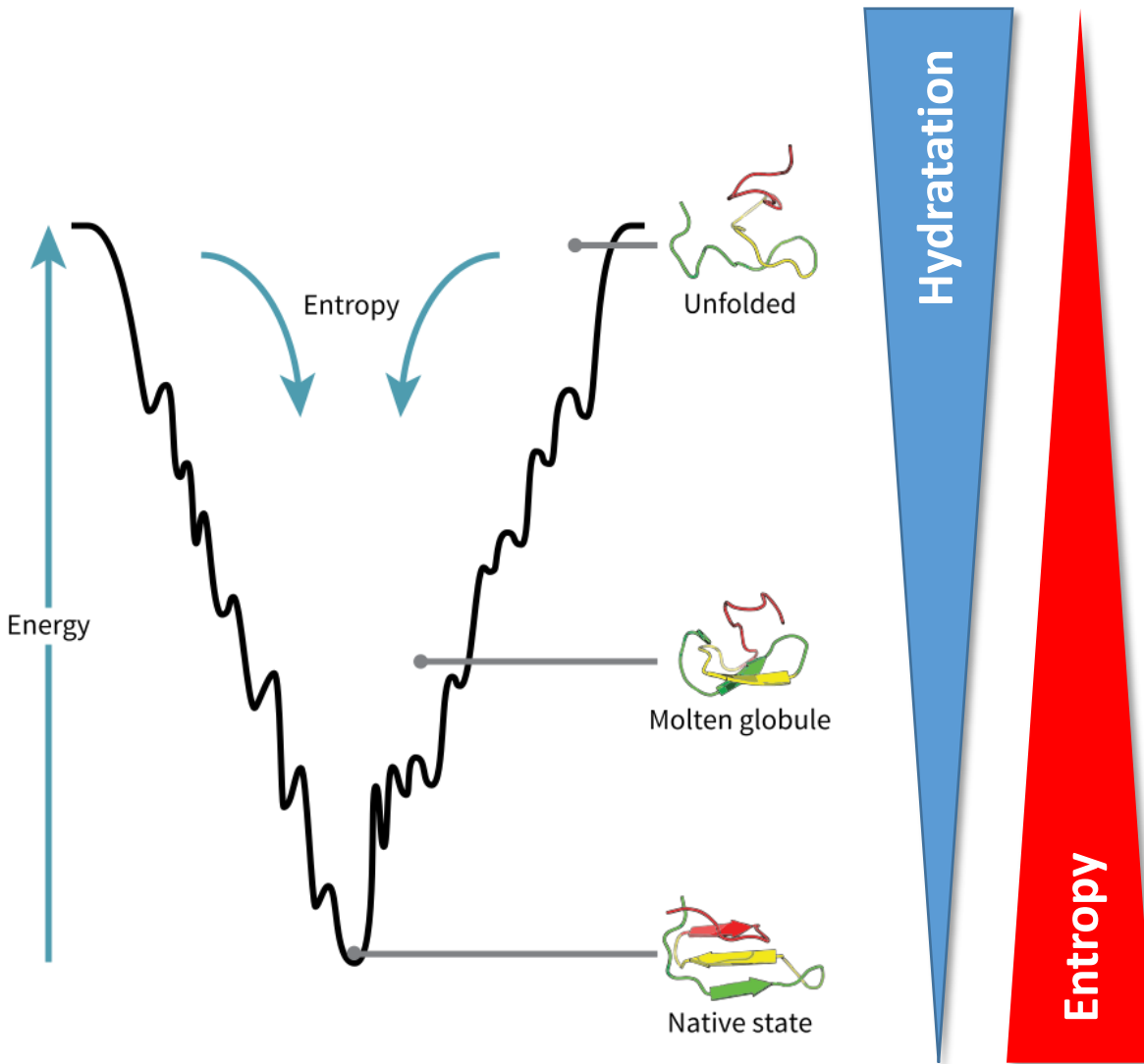


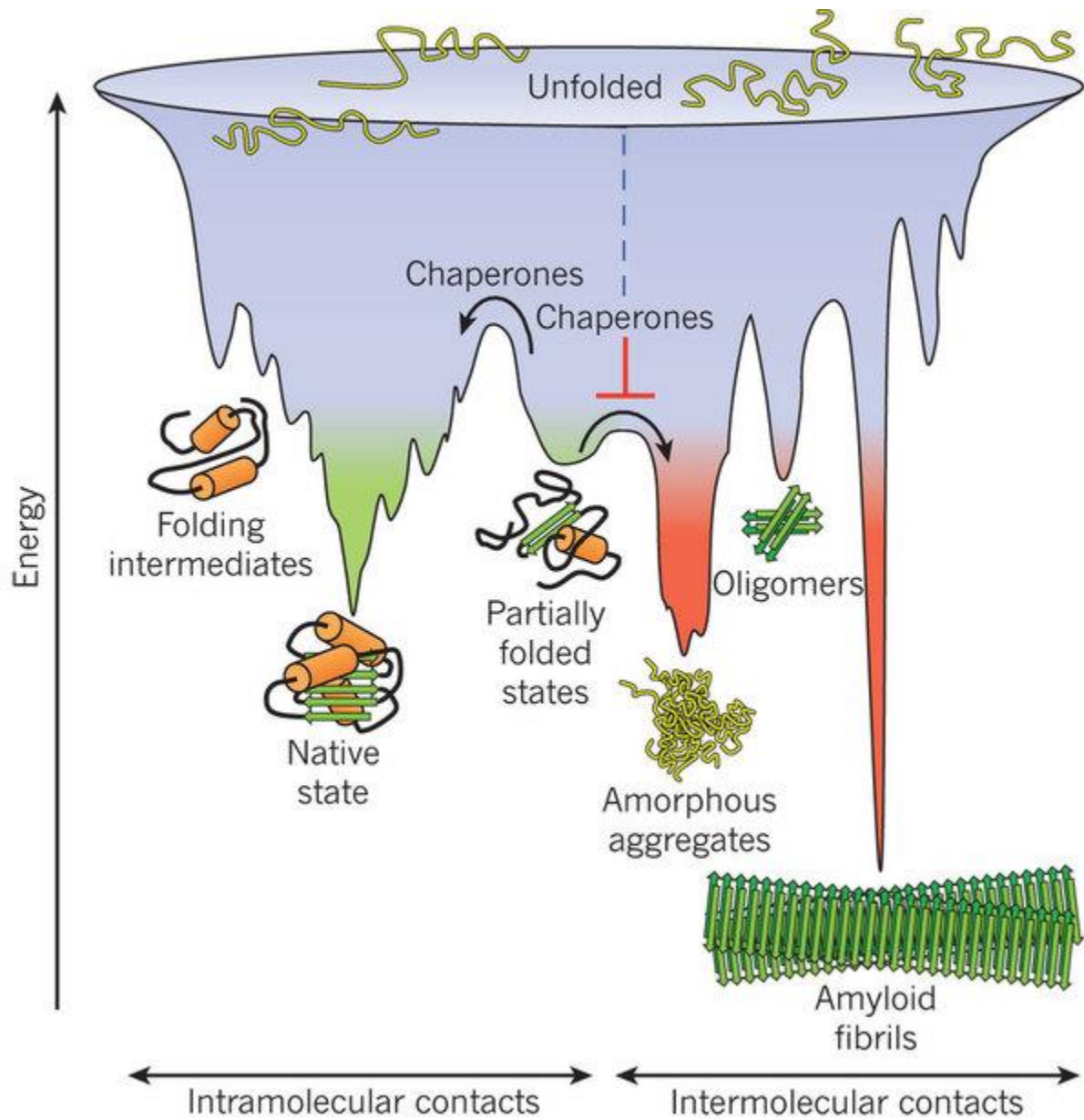
The native structure is determined only by the protein's amino acid sequence

Christian Boehmer Anfinsen, Jr.
(March 26, 1916 – May 14, 1995)
Nobel Prize in Chemistry (1972)



Folding is entropy driven process

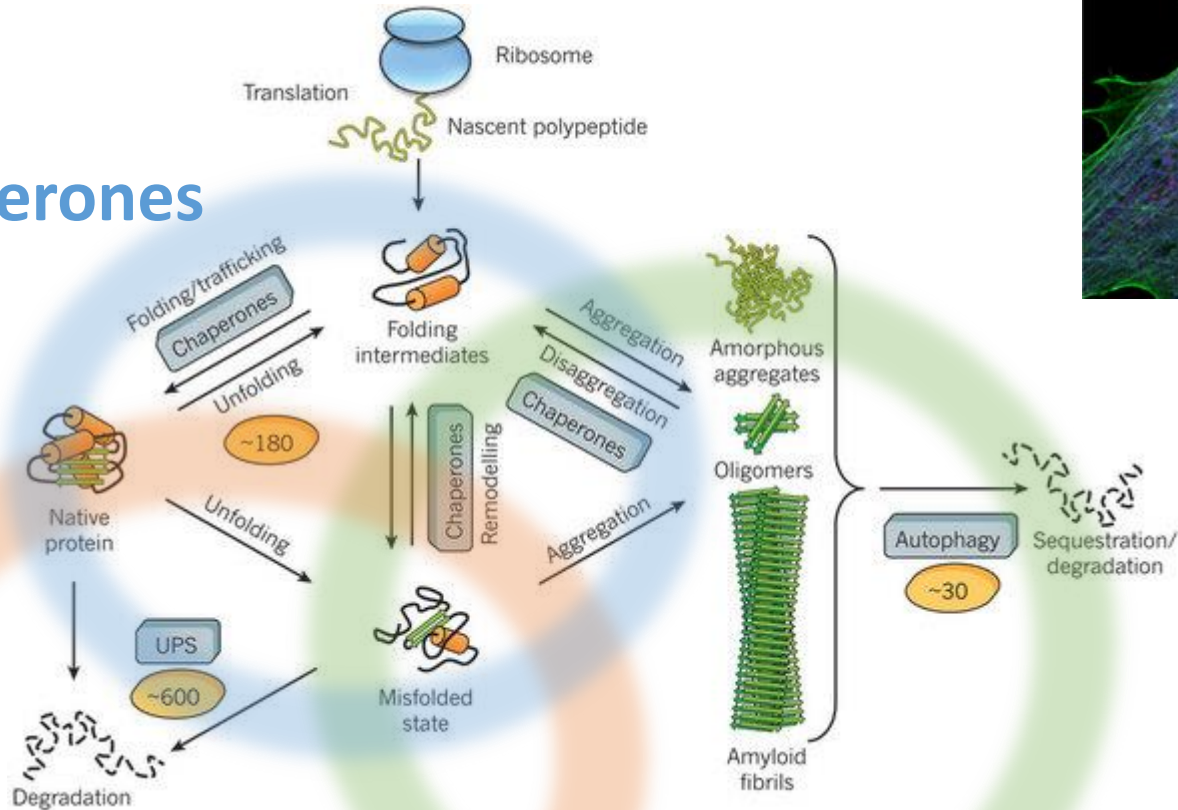




Protein homeostasis / proteostasis



Chaperones



Autophagy

Ubiquitin proteasome system

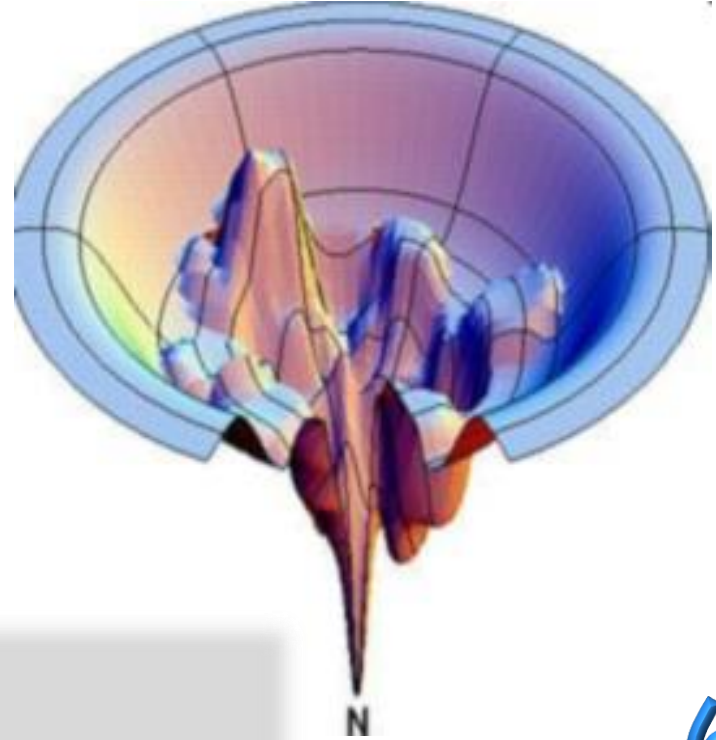
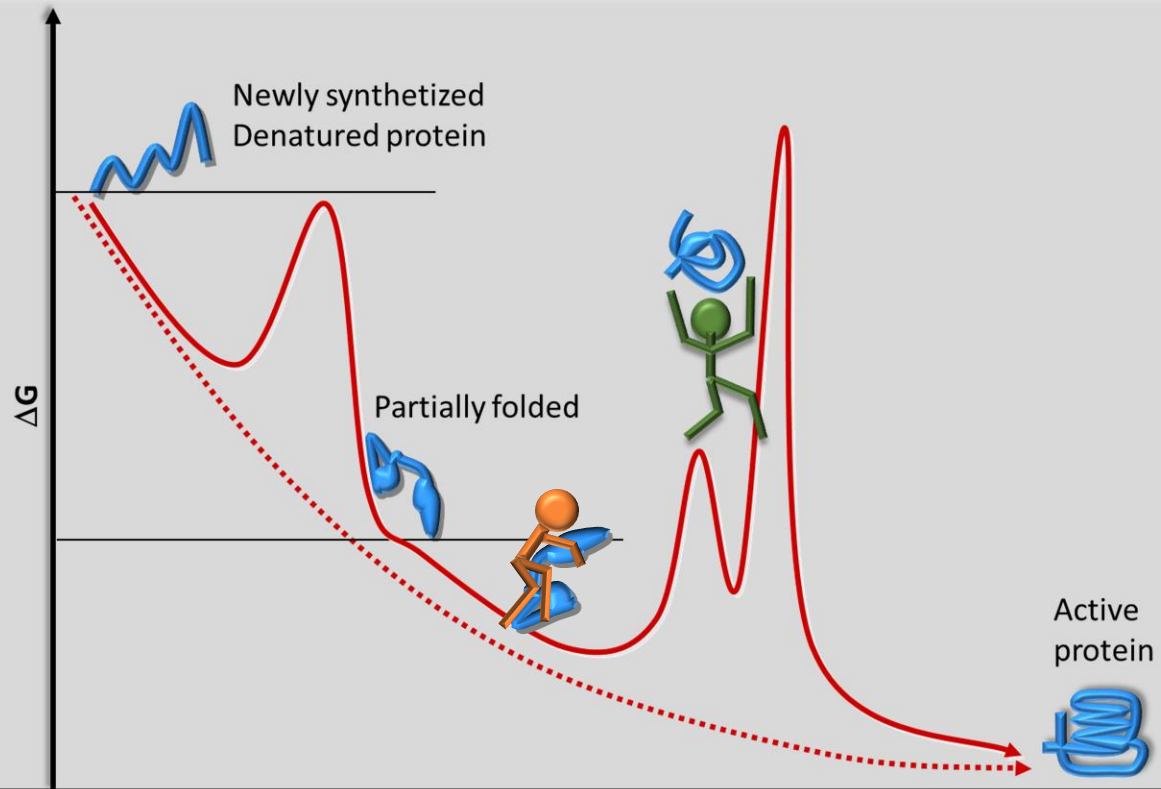


Stress proteins / Chaperones

Holdases bind folding intermediates to prevent their aggregation



Crystalins, p23, Hsp40...

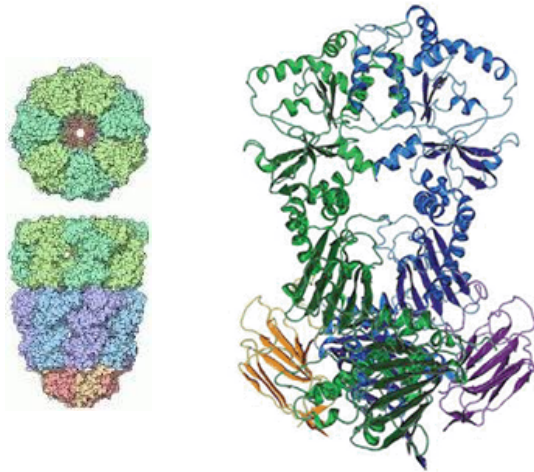


Foldases are chaperones that accompany other proteins to help them to overcome the energy barriers during folding to native conformation (ATP dependent)

Hsp70, Hsp90, GroEL...

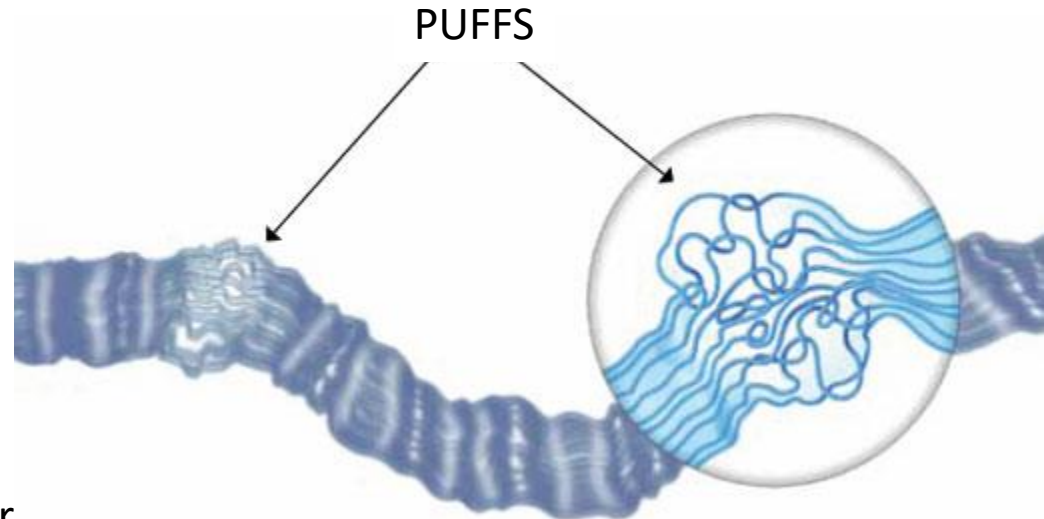
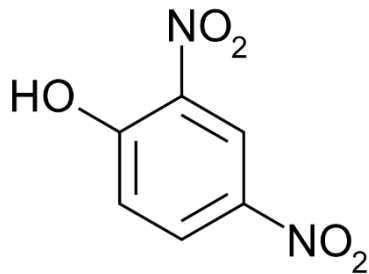
Stresové proteiny

Proteiny indukované tepelným stresem, převážně chaperony



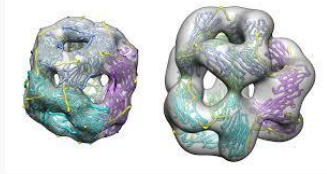
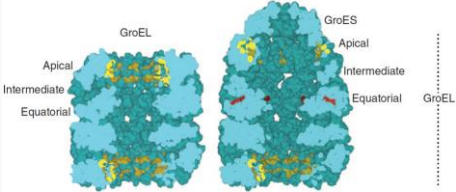
Hsp90
Hsp70, DnaK
Hsp60, GroEL
Hsp40 DnaJ
Hsp27, Crystalins,
Hsp10, chaperonins, GroES

Teplota

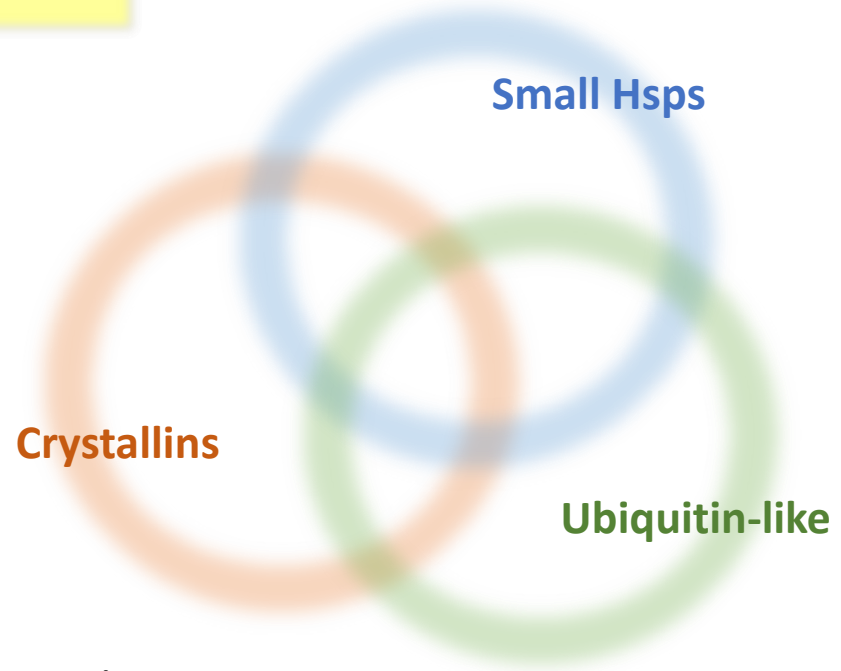
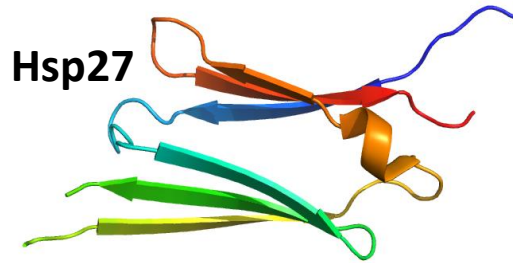
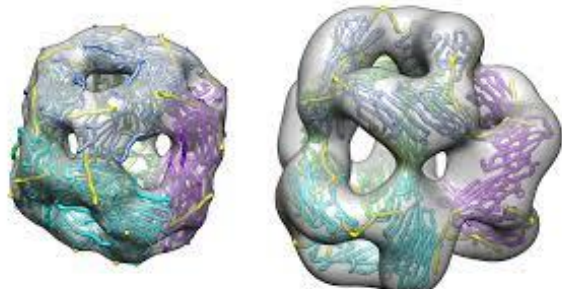


Dinitrophenol-mitochondriální uncoupler

1962 by Ferruccio Ritossa

Approximate molecular weight(kDa)	<u>Prokaryotic</u> proteins	<u>Eukaryotic</u> 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

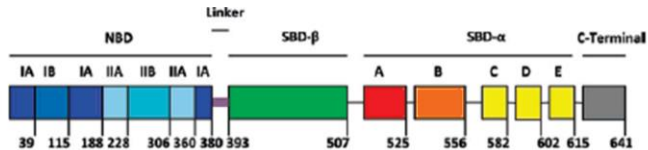
HspB group/ small chaperones



Prevent aggregation
Thermotolerance

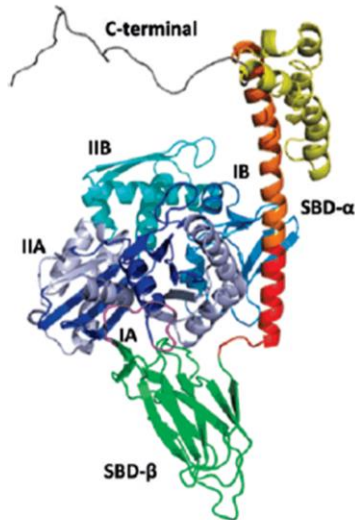
	Gene name	Protein name	Old names	Human gene ID	Mouse ortholog ID
1	<i>HSPB1</i>	HSPB1	CMT2F; HMN2B; HSP27; HSP28; HSP25; HS.76067; DKFZp586P1322	3315	15507
2	<i>HSPB2</i>	HSPB2	MKBP; HSP27; Hs.78846; LOH11CR1K; MGC133245	3316	69253
3	<i>HSPB3</i>	HSPB3	HSPL27	8988	56534
4	<i>HSPB4^a</i>	HSPB4	crystallin 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

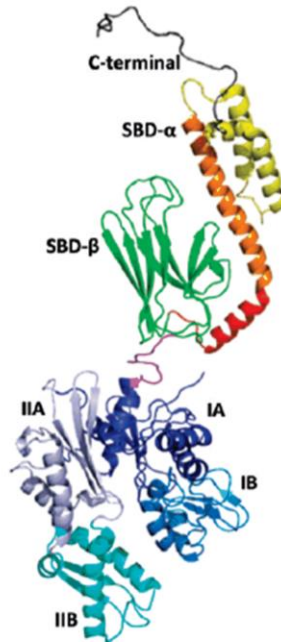


ATP

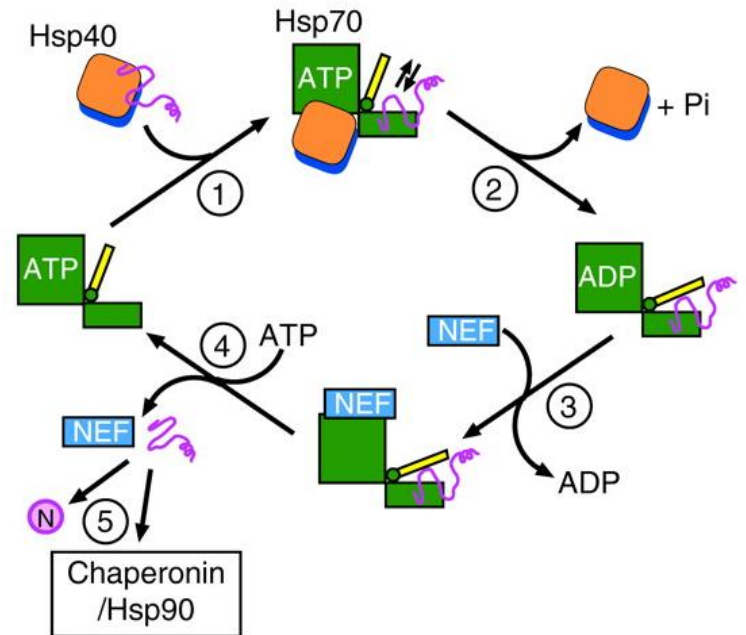
(b) Initial « open » model



(c) Initial « close » model



ADP



BAG NEF-Nucleotide exchange factor

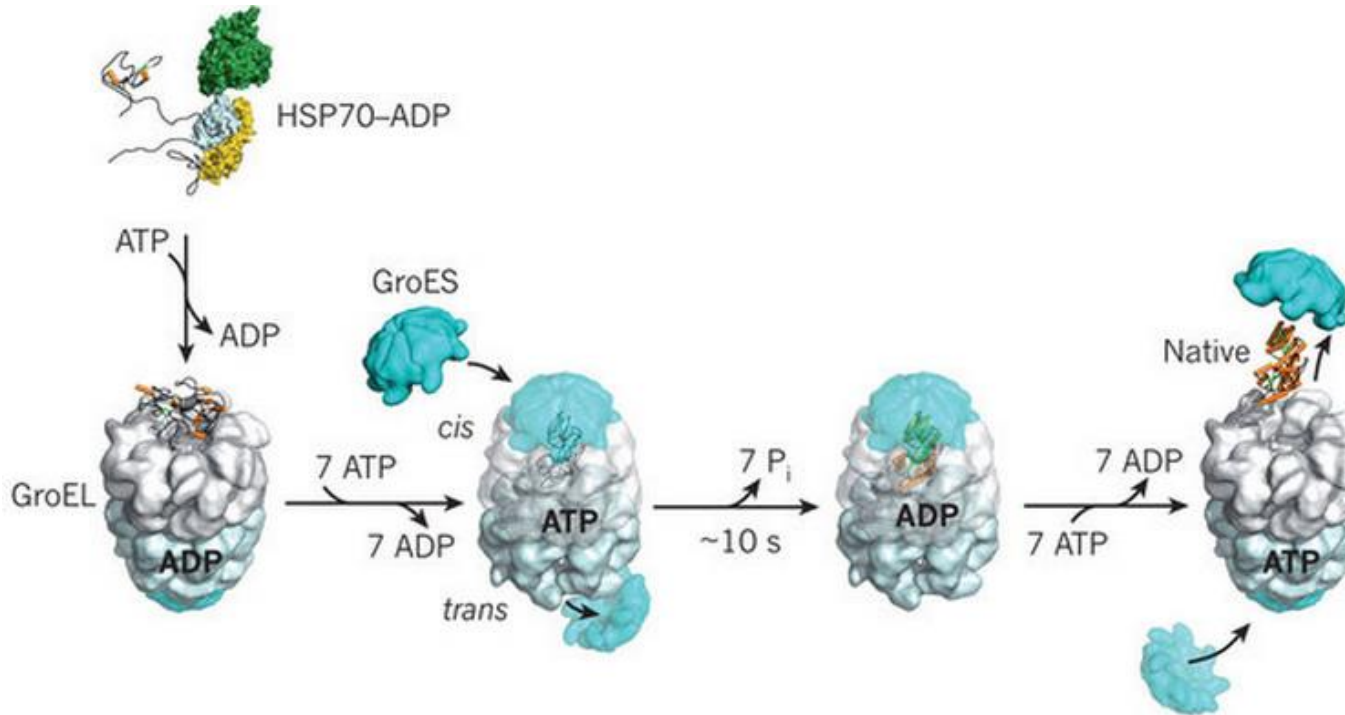
Hsp40

DnaJ

J-proteins

Chaperonins

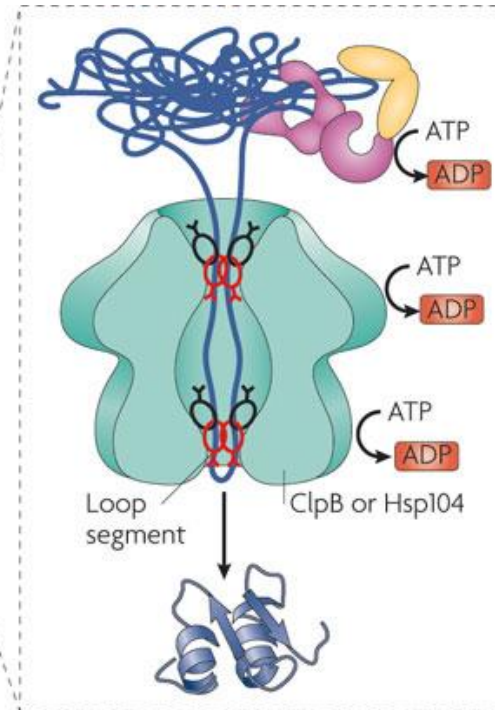
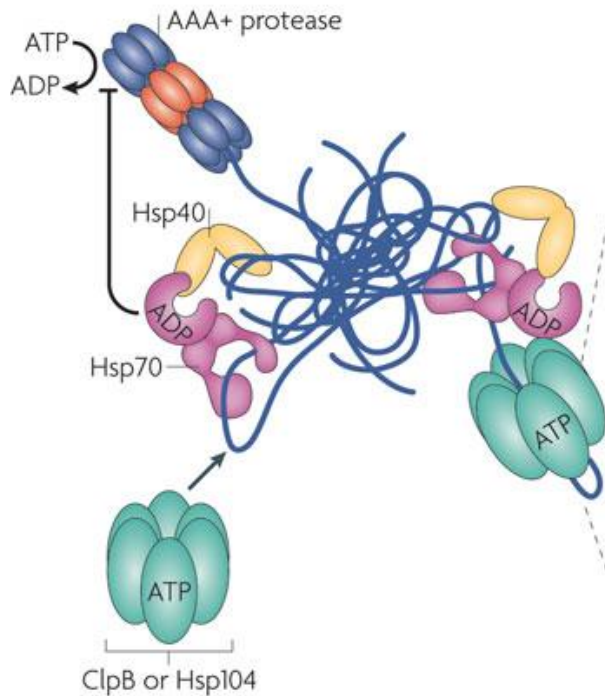
(GroEL-GroES, Hsp60, CCT-TRiC)



Folding of cytoskeletal proteins (tubulin)
Protein transport

Hsp104 (ClpB, ClpX,..)

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



Proteasome

Hsp104

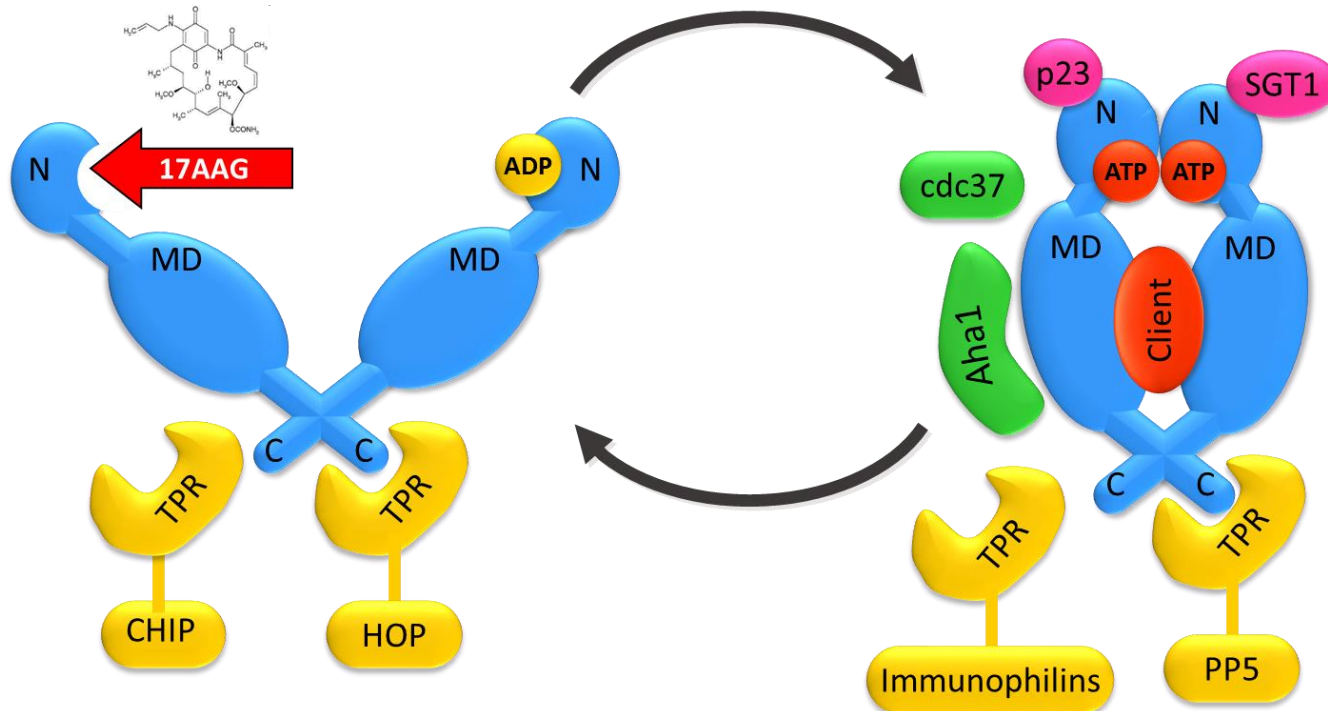
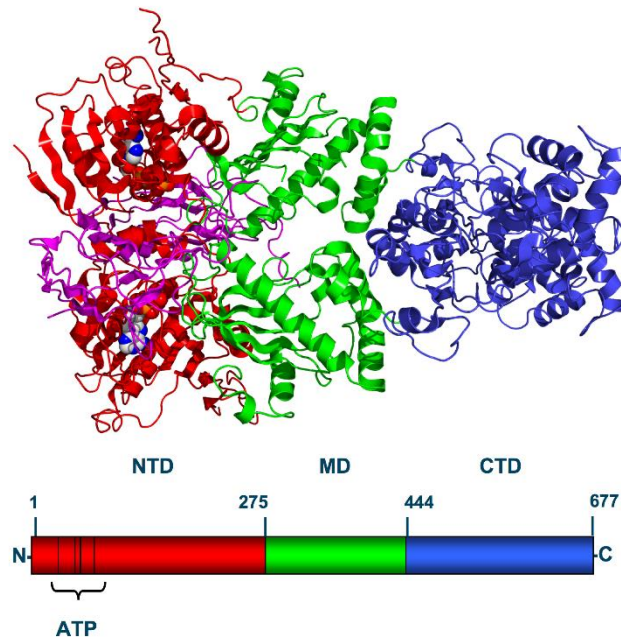
AAA+ proteases

AAA+ ATPases

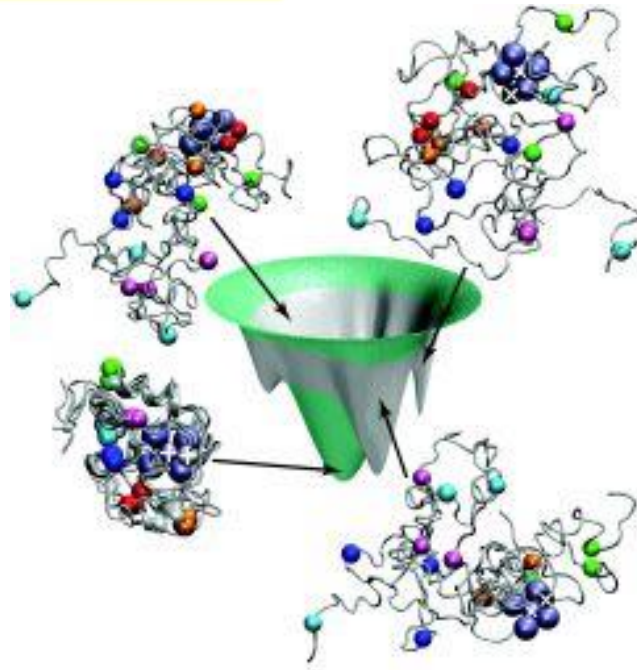
Converts ATP to "mechanical" energy (molecular motors)

Hsp90 chaperone machinery

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



Stress proteins/ Chaperones/Hsp90



Hsp90 as a capacitor for morphological evolution

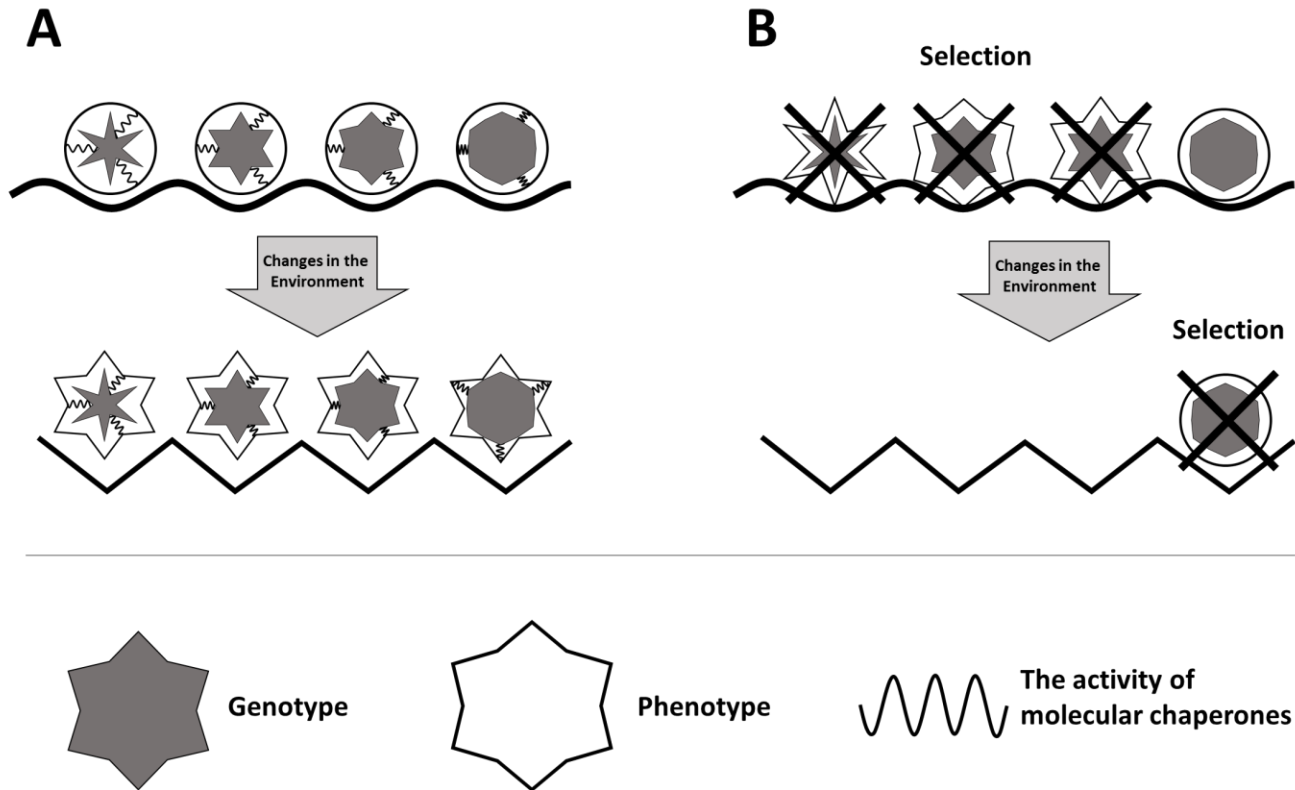
Suzanne L. Rutherford^{††} & Susan Lindquist^{*}

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

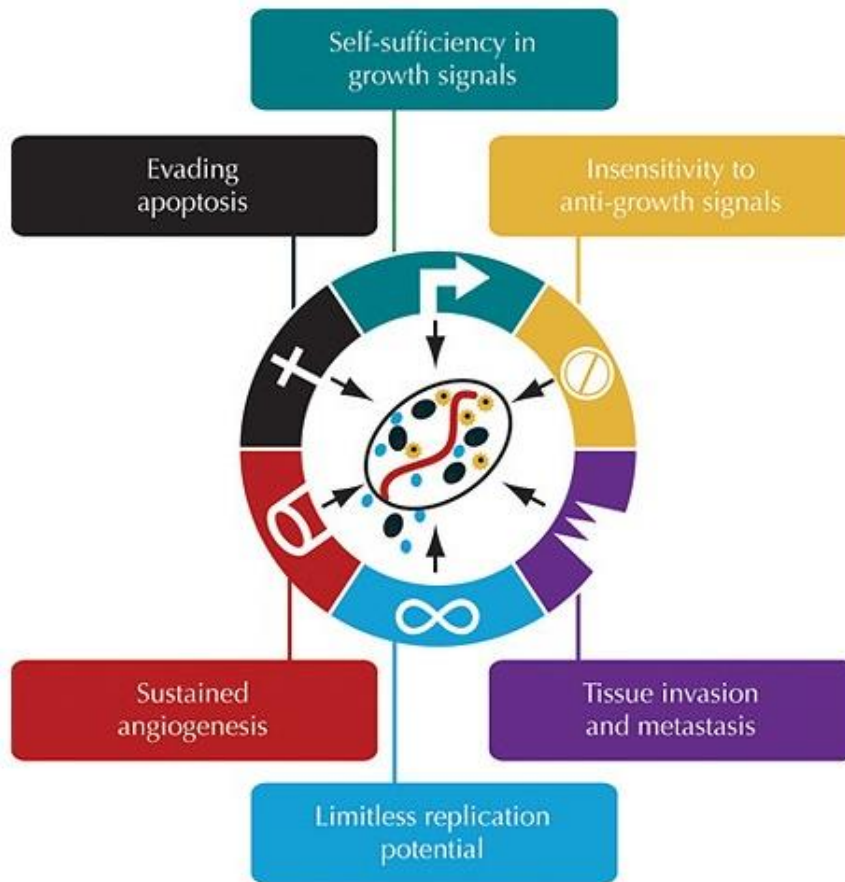
NATURE | VOL 396 | 26 NOVEMBER 1998 | www.nature.com

CHAPERONES AND EVOLUTION

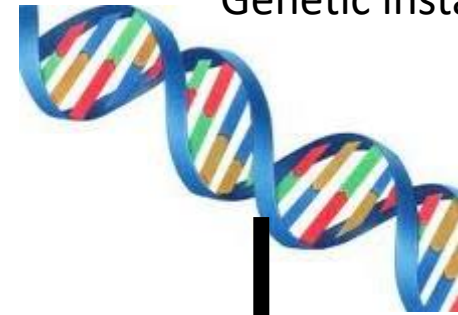
FILIP TRCKA, BORIVOJ VOJTESEK, PETR MULLER
 Regional Centre for Applied Molecular Oncology, Masaryk Memorial Cancer Institute,
 Zlutý kopec 7, 656 53 Brno



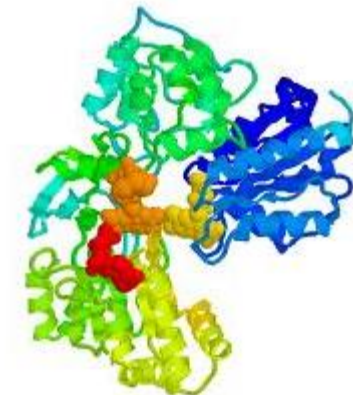
The tumor cells demand high quality and amount of protein



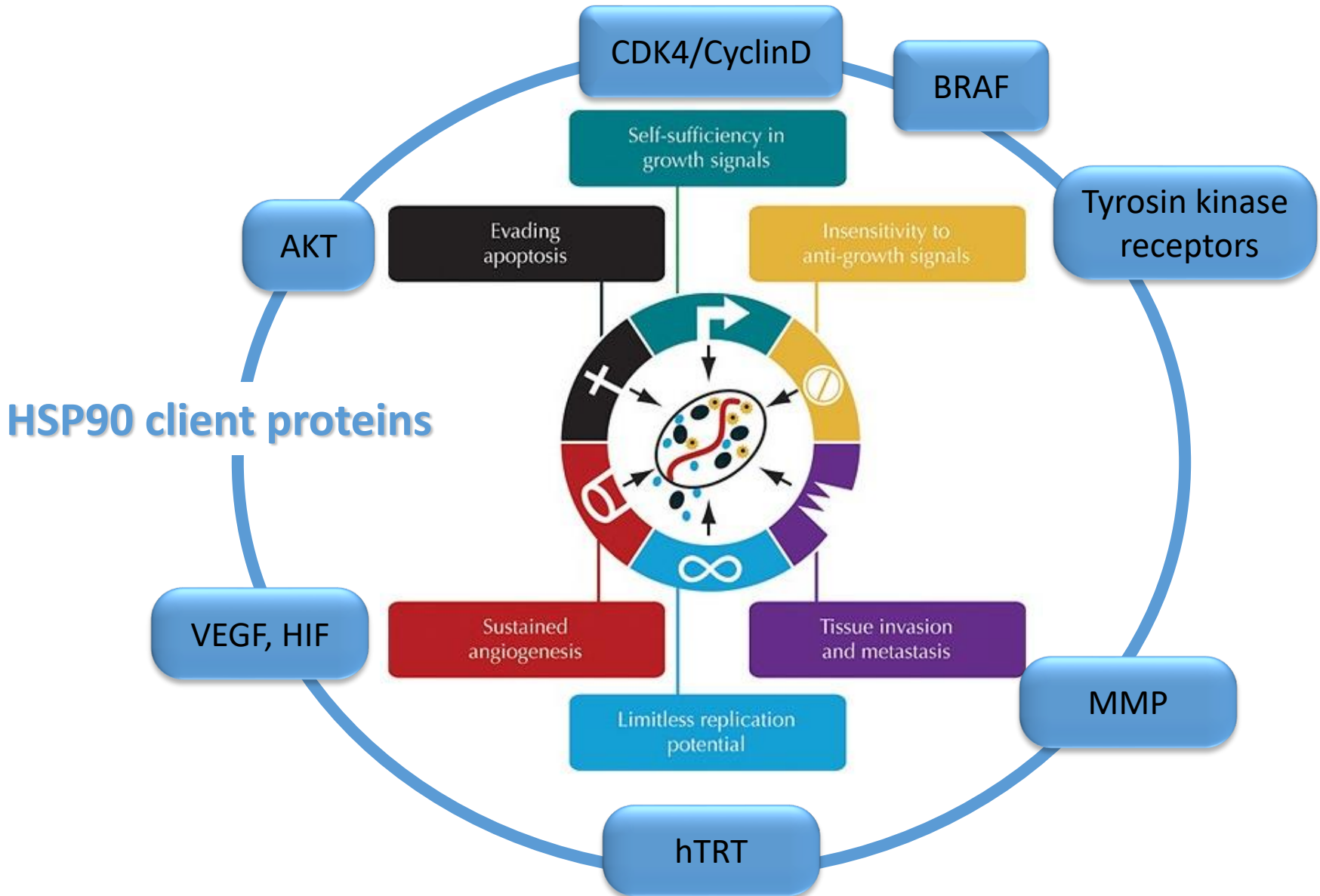
Genetic instability



Enhanced proteosynthesis
Production of mutated,
conformational instable protins



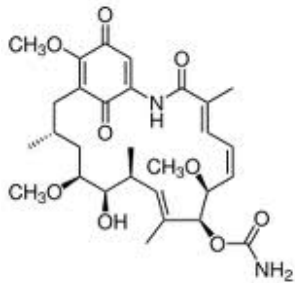
Activity of Hsp90 is essential for expression of cancer phenotype



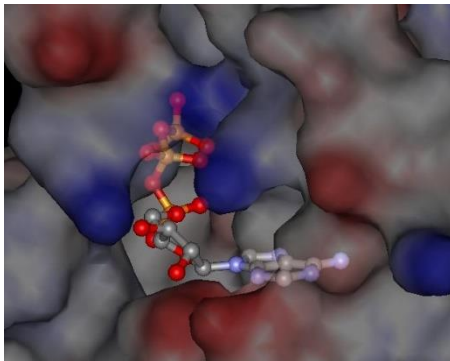
Specific inhibitors Hsp90



Isolation of Geldanamycin (1970)



Geldanamycin binds ATP cavity of Hsp90 (1997)



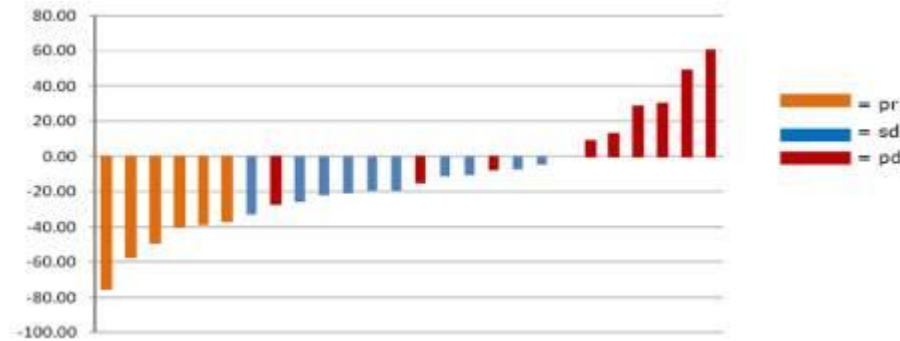
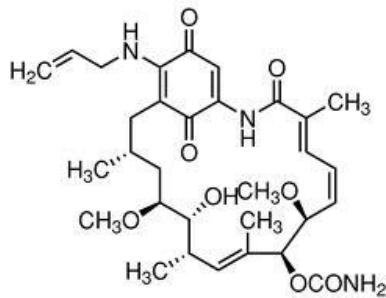
Clinical trials with Geldanamycin(2000)

	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	Myrex 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 ?

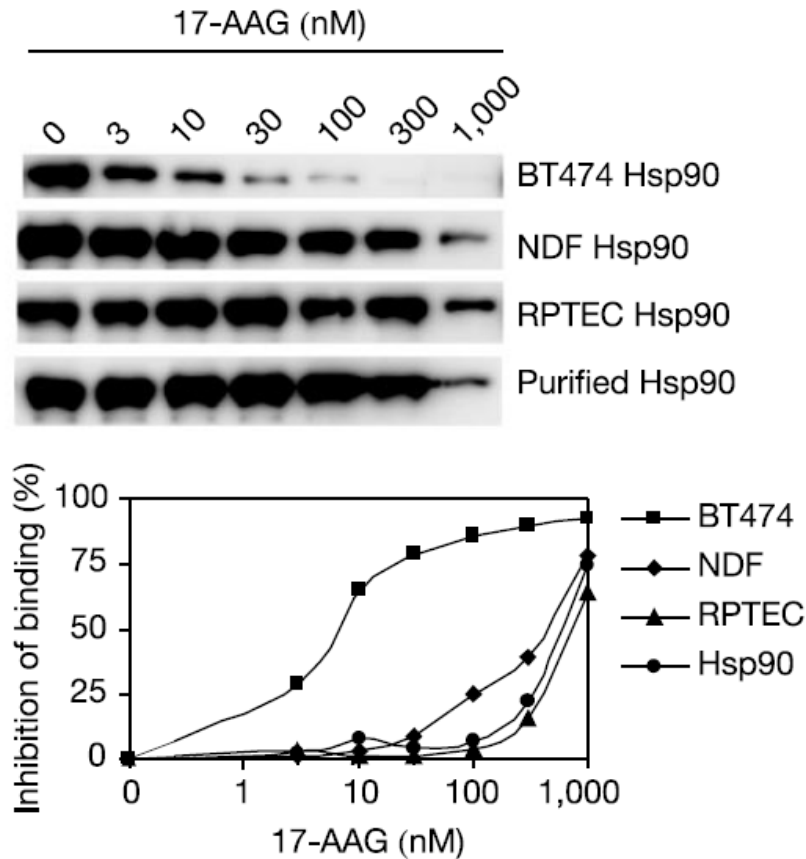
- posttranslational modifications
- expression pattern of co-chaperones

Client spectrum ?

letters to nature

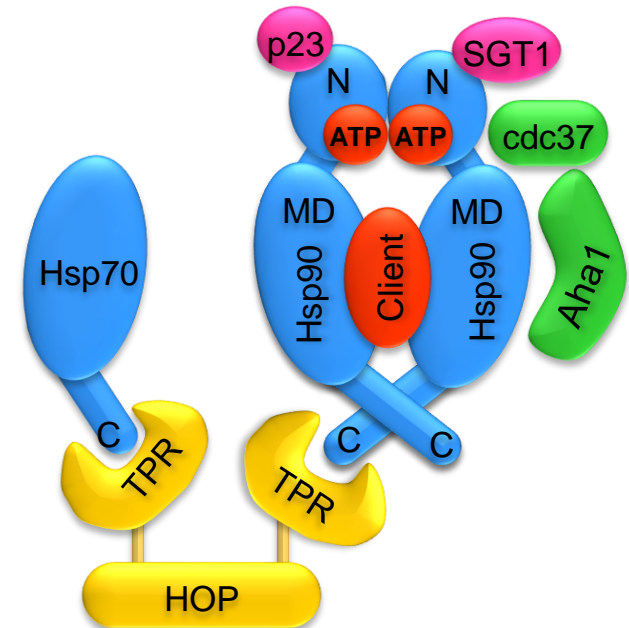
A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors

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



Multichaperone complex

- Hsp90+Hsp70
- cochaperones

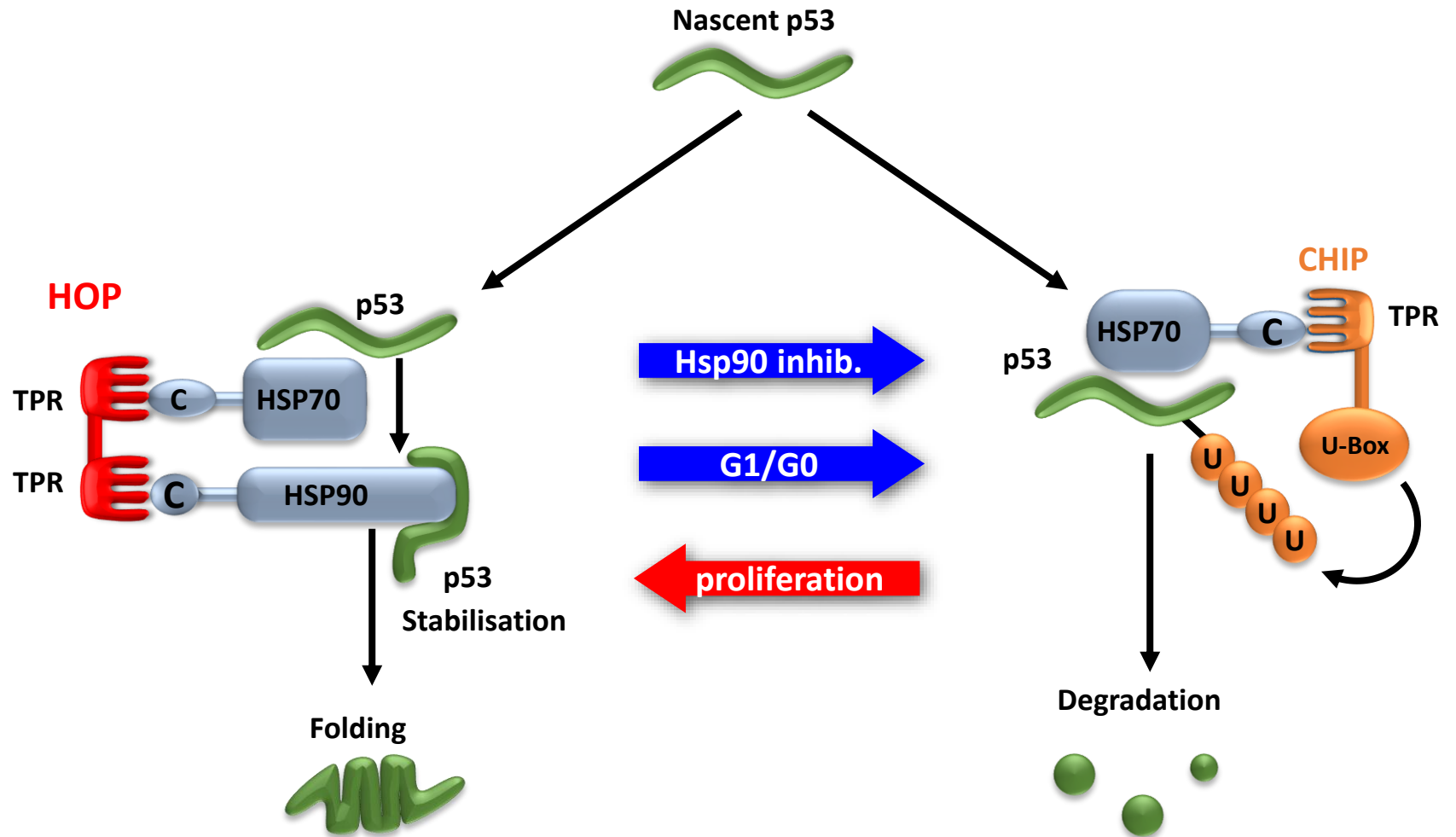




ORIGINAL ARTICLE

Chaperone-dependent stabilization and degradation of p53 mutants

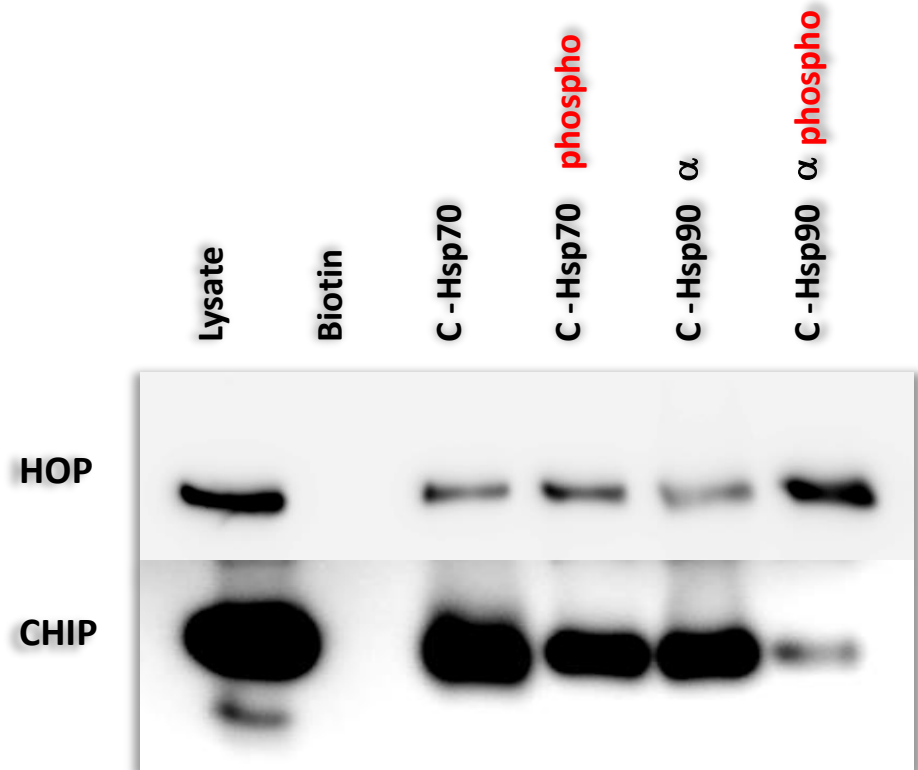
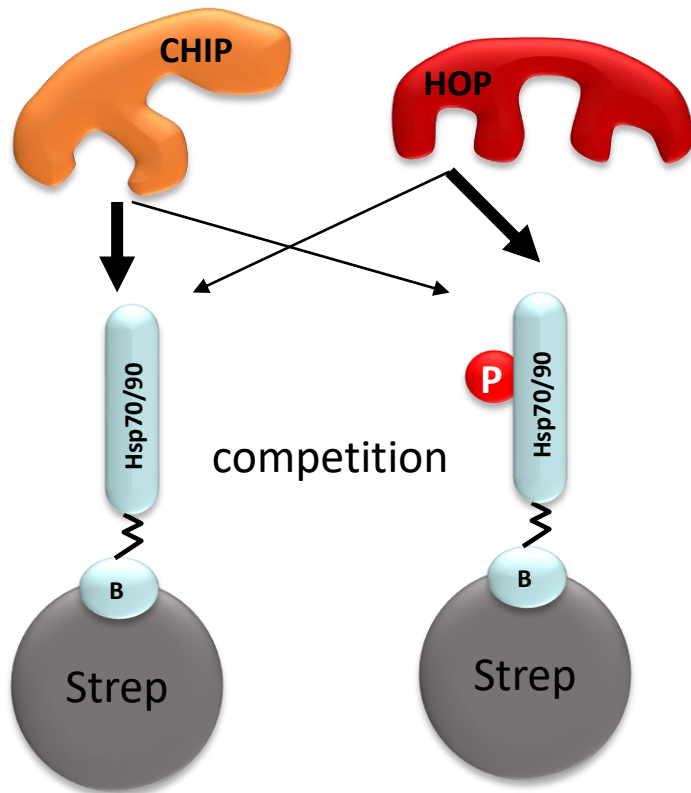
P Muller^{1,2}, R Hrstka¹, D Coomber², DP Lane² and B Vojtesek¹



What is the mechanism regulating folding degradation balance ?

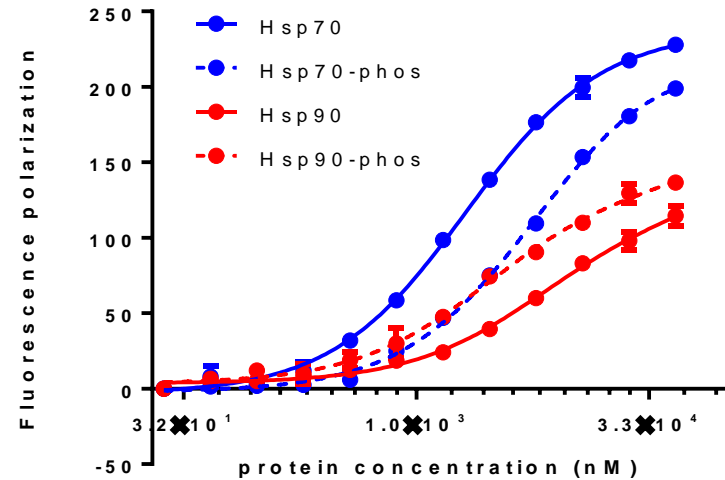
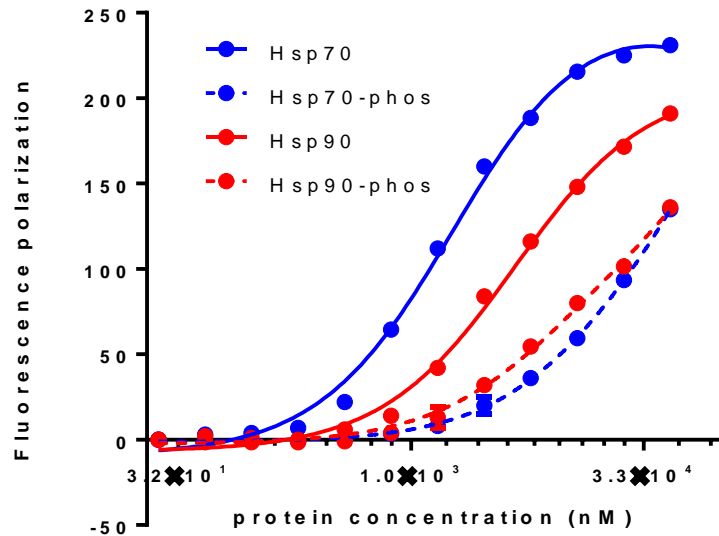
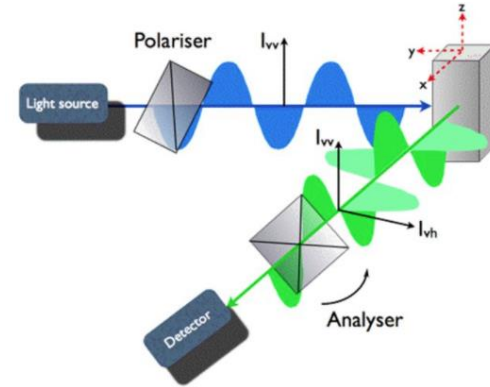
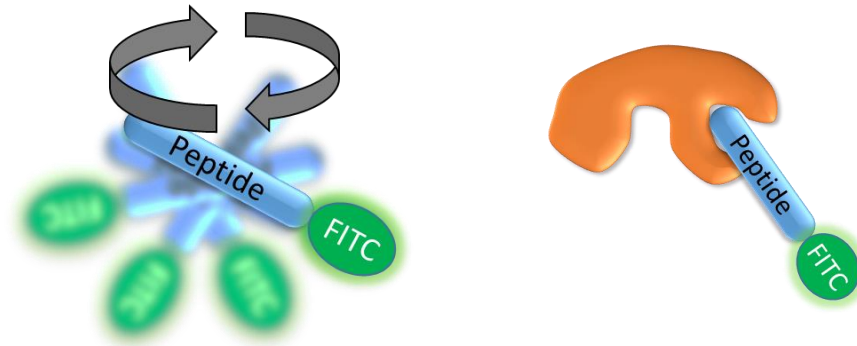
Cell lysate pulldown of HOP and CHIP

- Biotinylated phospho/non phospho peptides of Hsp70/Hsp90



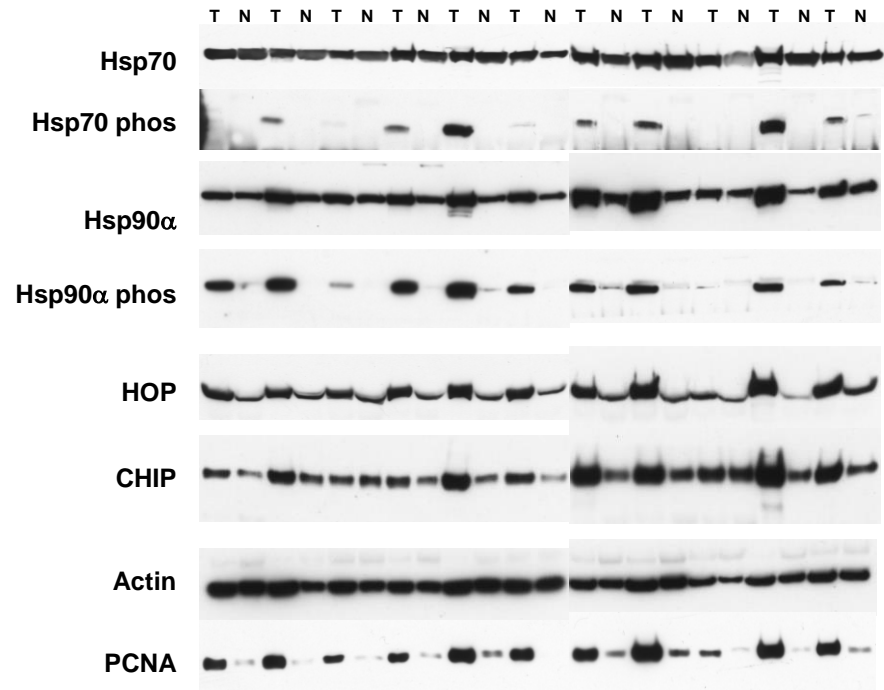
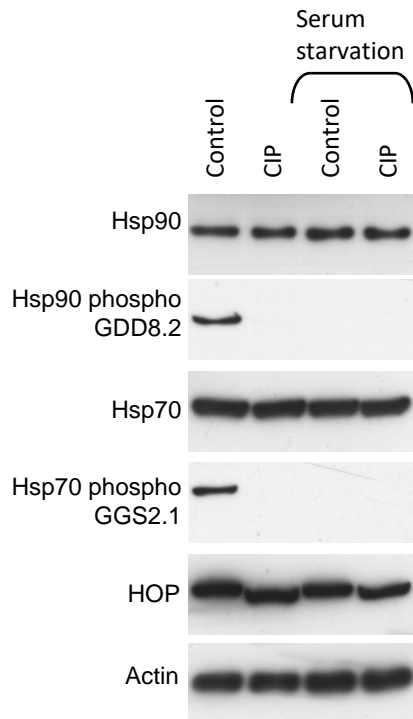
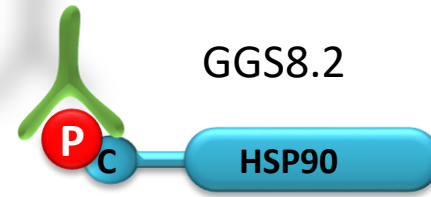
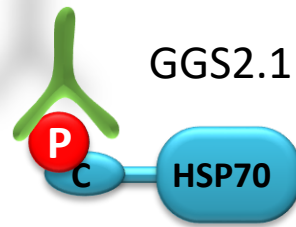
Detekce interakce proteinu s peptidem pomocí fluorescenční polarizace

Fluorescence lifetime – „doba mezi přijetím excitačního fotonu a vyzářením fotonu emitovaného“

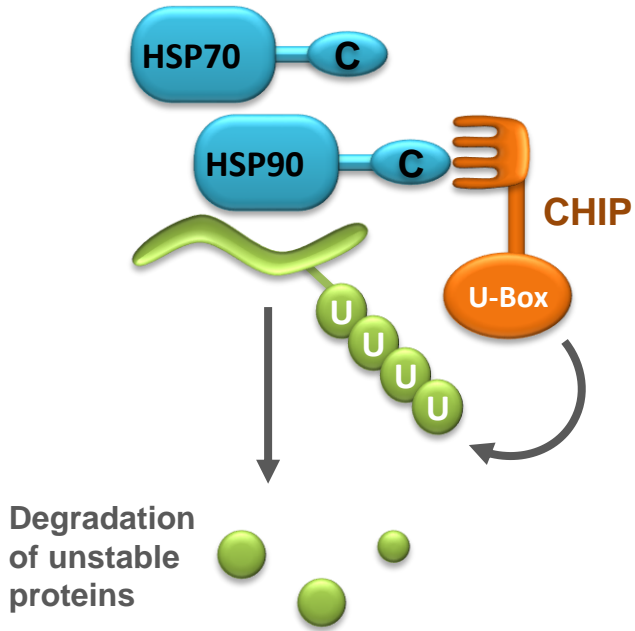


Detection of phosphorylated Hsp70 and Hsp90

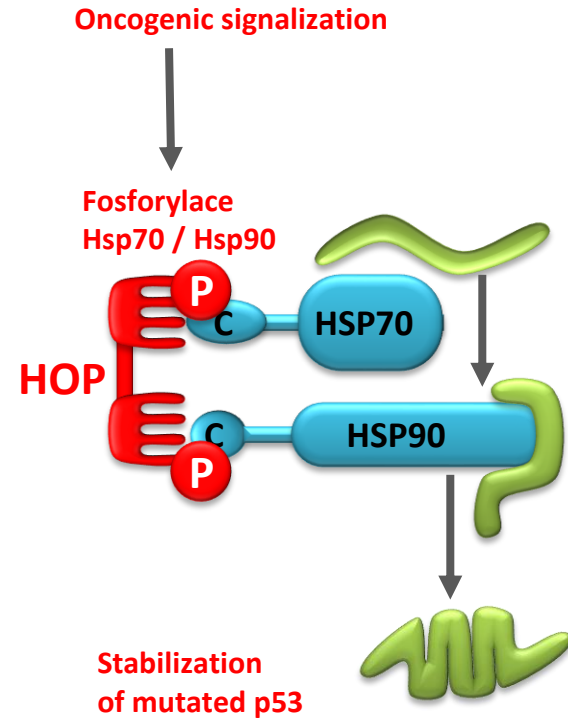
phospho-specific monoclonal antibodies



Normal differentiated cell



Cancer cell



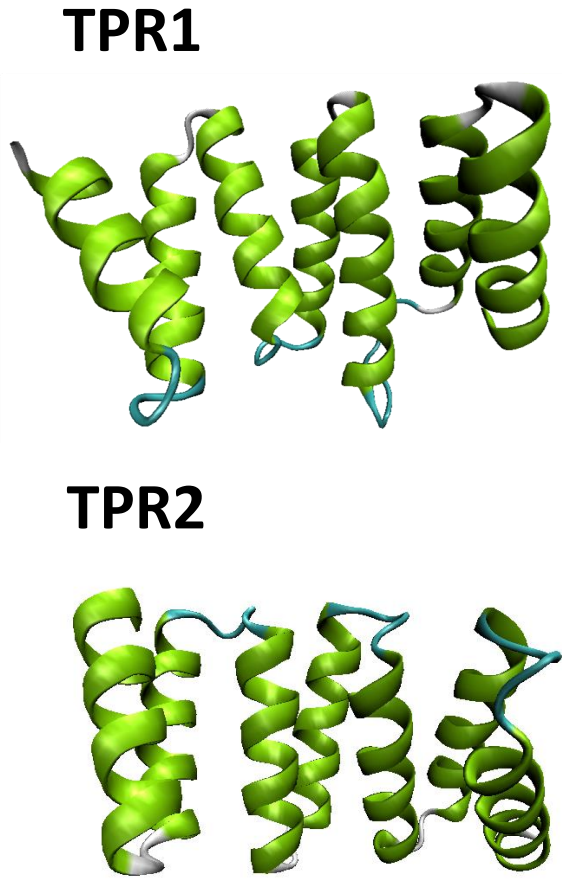
Normal differentiated cell	Cancer cell
C-terminus Hsp70/90 non phosphorylated	Phosphorylated Hsp90 Hsp70
Hsp bind preferentially CHIP	Hsps bind preferentially HOP
Designed to degrade unfolded protein	High folding capacity of Hsp90
Higher expression of CHIP	Increased level of HOP
Lower sensitivity ti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

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

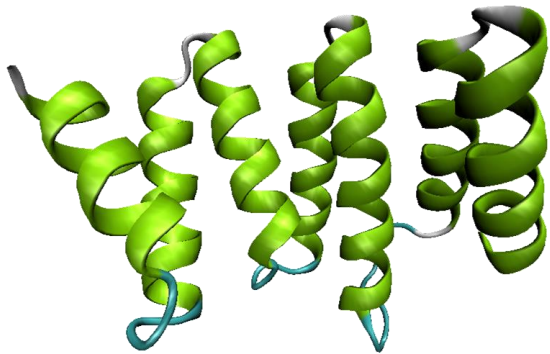


	Helix 1A	Helix 1B
Tom34-TPR1 Q15785 9-118	VEELRAAGNESFRNGQYAEASALYGRALRVLQAQG-----	
Tom34-TPR2 Q15785 193-294	ARVLKEEGVELVKKGNHKKAI EK YSESLLC-----	
CHIP Q9UNE7 26-127	AQELKEQG NRLFVGRKYPEAAACYGRAITR-----	
HOP-TPR1 P31948 4-105	VNELKEKGNKALSVGNIDDALQCYSEA I KL-----	
HOP-TPR2A P31948 225-333	ALKEKELGNDAYKKKDFDTALKH YDKAKEL-----	
FKBP52 Q02790 270-386	STIVKERGTVYFKEGKYKQALLQYKKIVSWLEYESSFSNEEAQ	
PPP5 P53041 28-129	AEELKTQANDYFKAKDYENAIKFYSQAIE L-----	
	Helix 2A	Helix 2B
Tom34-TPR1 Q15785 9-118	SSDPEEE SVLYSNRAA CHLKDGNCRDCIKDCT SALALV PFS--	
Tom34-TPR2 Q15785 193-294	--SN-LESATYSNRALCYLV LKQY TEAVKDCTEAL KLDGKN--	
CHIP Q9UNE7 26-127	--NP-LVAVYYTNRALCY LKMQQHEQALADCRRALELDGQS--	
HOP-TPR1 P31948 4-105	--DP-HNHVLYSNRSAAYAKKGDYQKAYEDGCKTVDLKPDW--	
HOP-TPR2A P31948 225-333	--DP-TNMTY TNQAAVYFEKGDY NKCRELCEKAI EVGRENRE	
FKBP52 Q02790 270-386	KAQA-LRLASHLNLAMCH LK LQAF SAAIESCNKALELDSNN--	
PPP5 P53041 28-129	--NP-SNAIYYGNRS LAYLRTECYGYALGDATRAIELDKKY--	
	Helix 3A	Helix 3B
Tom34-TPR1 Q15785 9-118	-----IKPLLRASAYEAL EK YPMAYVDYKTVLQIDDNV	
Tom34-TPR2 Q15785 193-294	-----VKA FYRRAQAH KALKDYKSS FADISNLLQIEPRN	
CHIP Q9UNE7 26-127	-----VKAHFFLGQCQL EMESYDEAIANLQRAYSLAKEQ	
HOP-TPR1 P31948 4-105	-----GKGYSRKAAAL EFLNRFEEAKRTYEEGLKHEANN	
HOP-TPR2A P31948 225-333	DYRQIAKAYARIGNSY FKEEKYKDAIH FYNKSLAEHRT P	
FKBP52 Q02790 270-386	-----EKG LFRGEAHLAVNDFELARAD FQKVLQLYPNN	
PPP5 P53041 28-129	-----IKGYRRAASNMA L GKFRALRDYETVVKV KPHD	

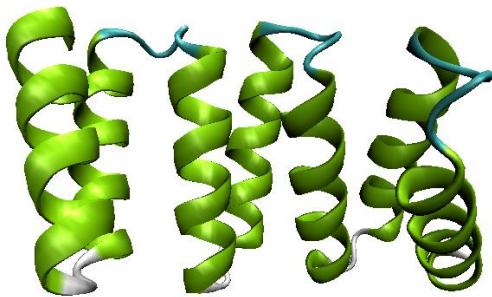
TOMM34 protein – co-chaperone

Tetratricopeptide repeat (TPR) domain

TPR1

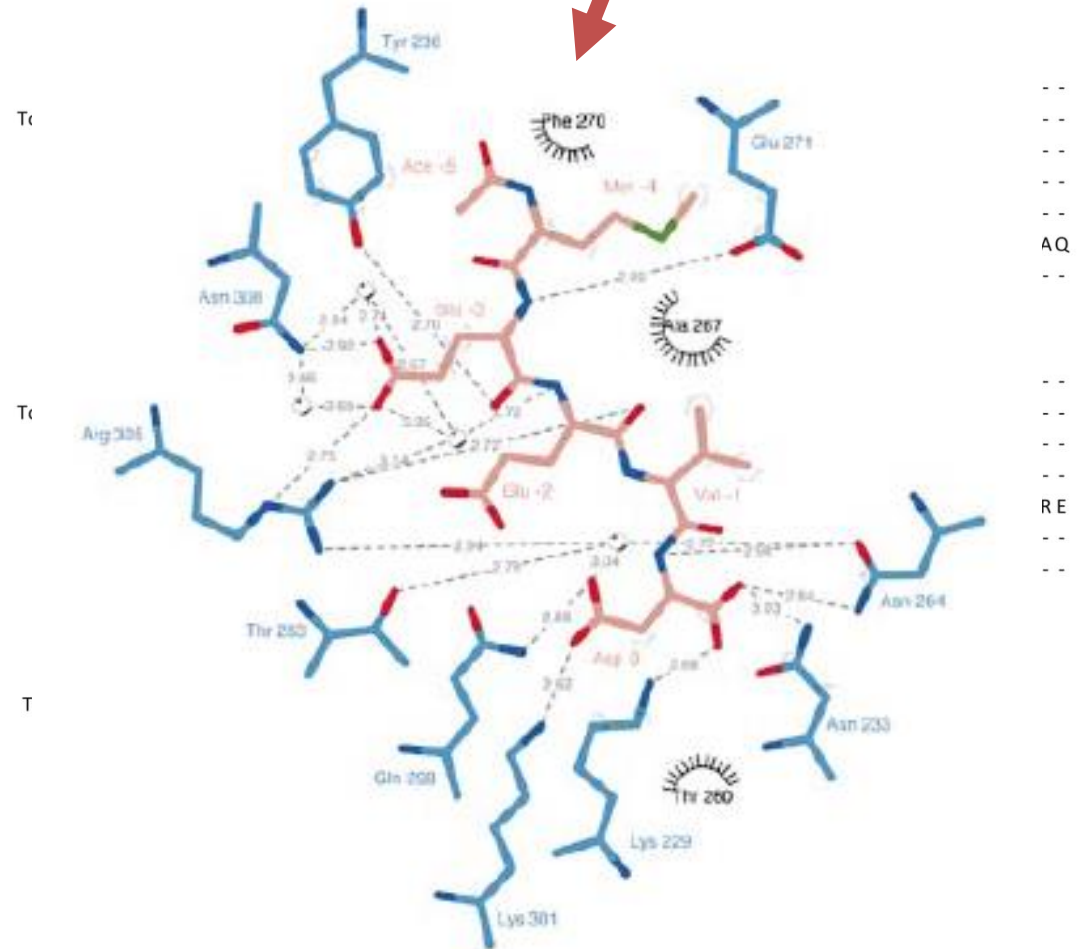


TPR2



C-terminus Hsp70/Hsp90

EEVD

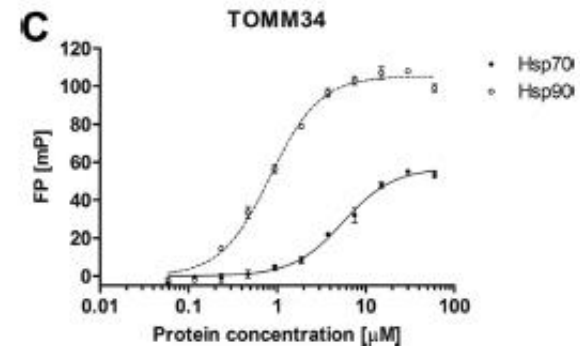
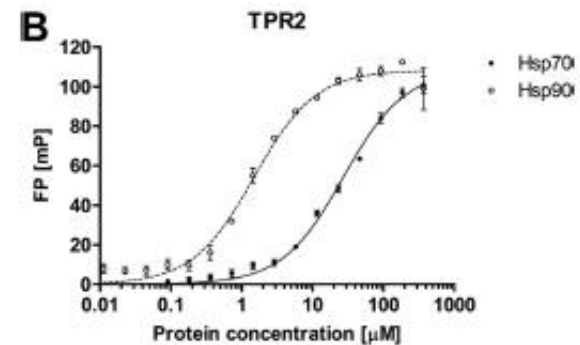
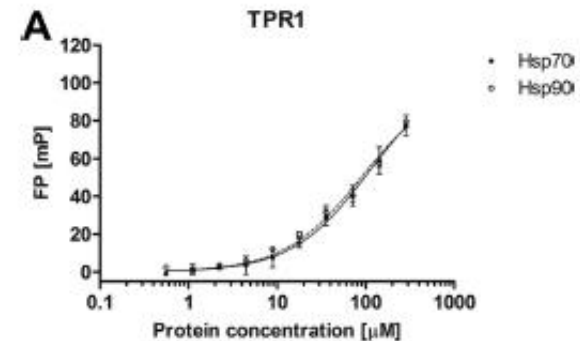
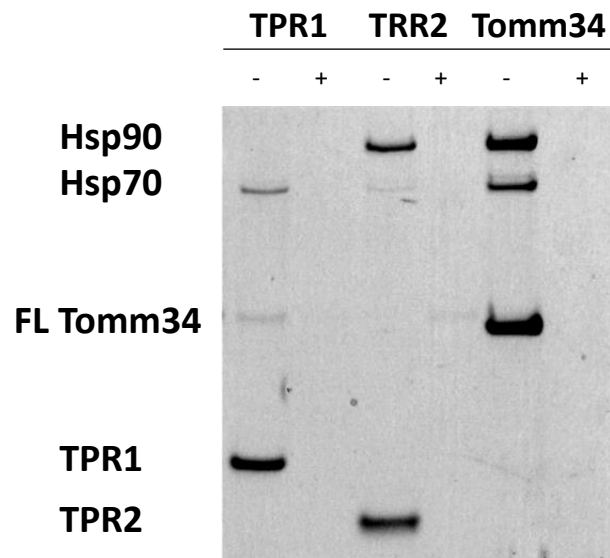
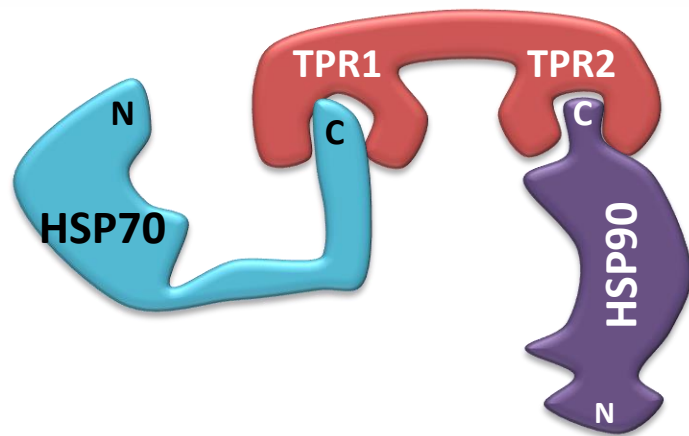


De novo modeled structure of TOMM34 domains

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

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

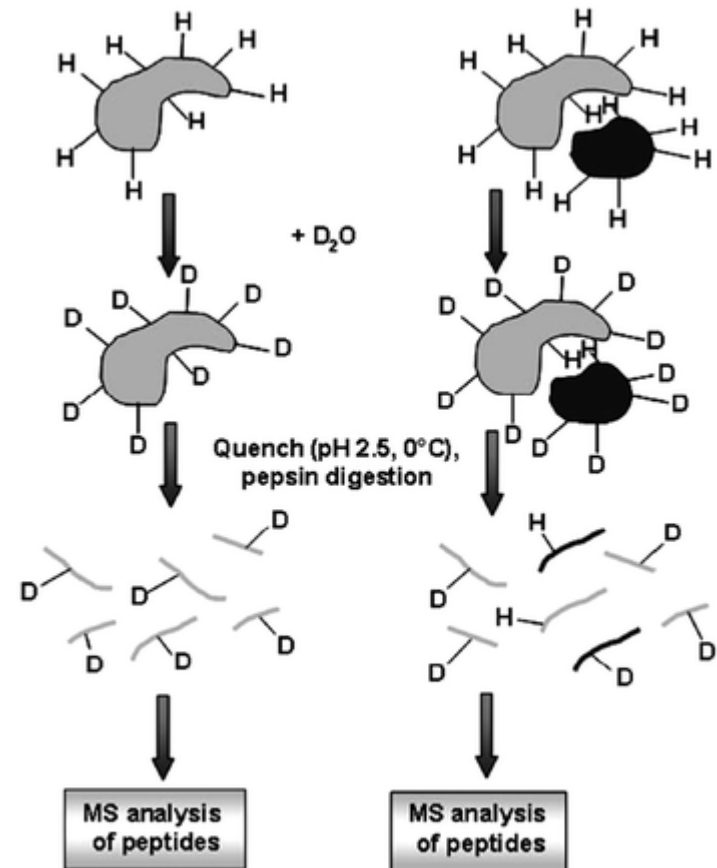
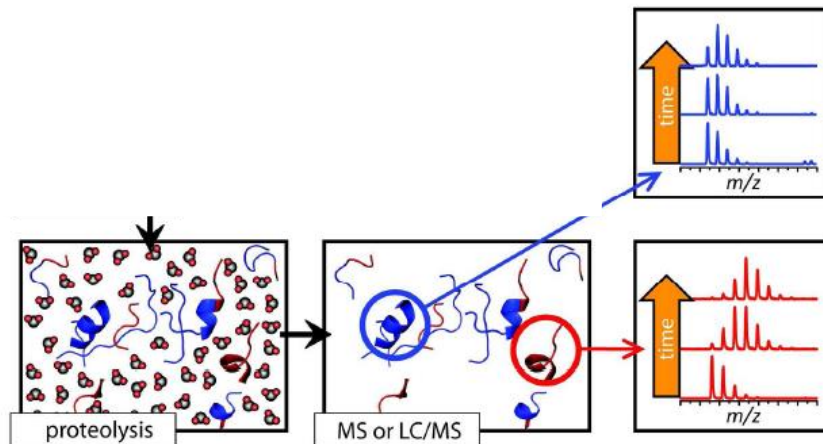
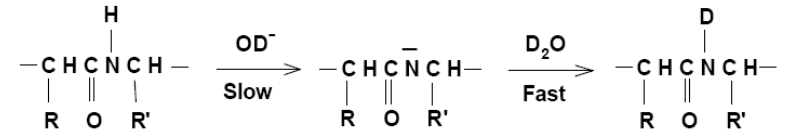
Fillip Trcka[‡], Michal Durech[‡], Petr Man^{§¶}, Lenka Hernychova[‡], Petr Muller^{¶1,2}, and Borivoj Vojtesek^{¶1,3}



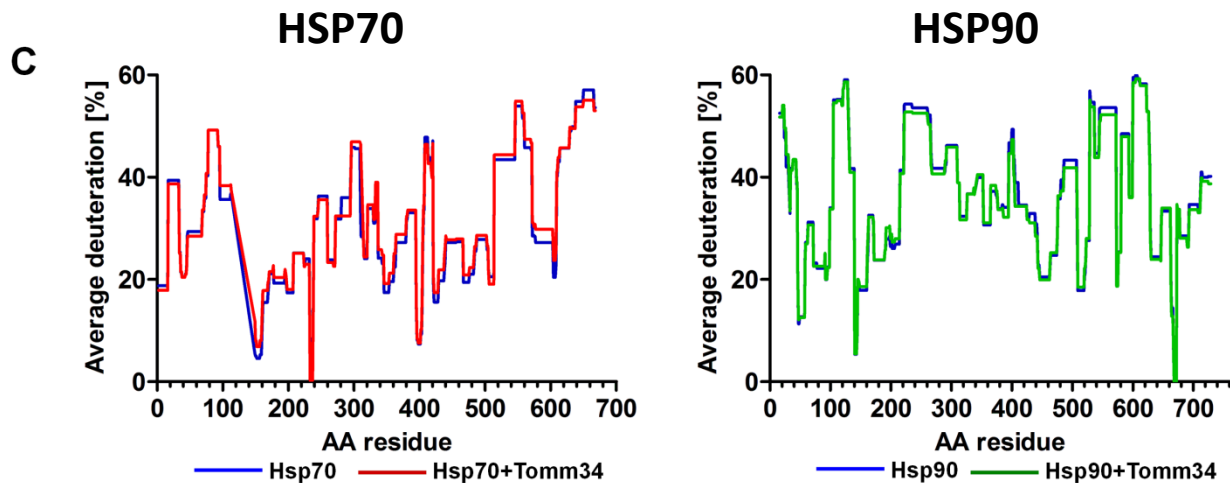
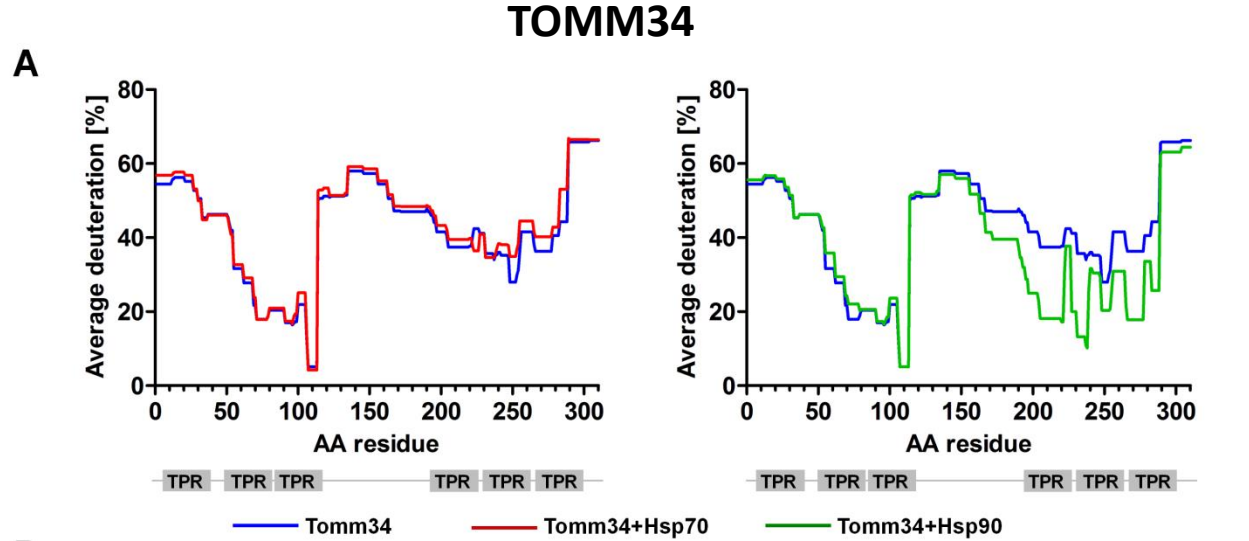
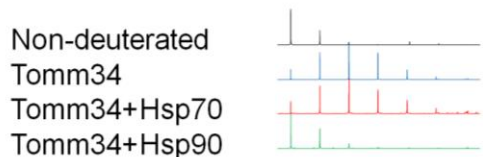
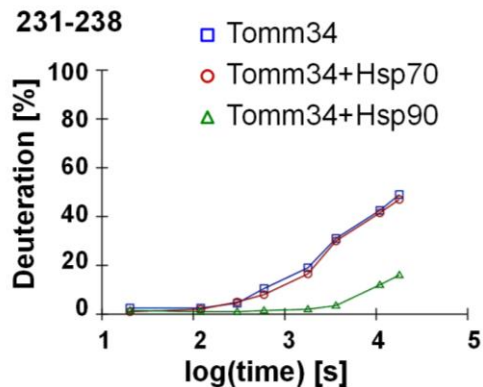
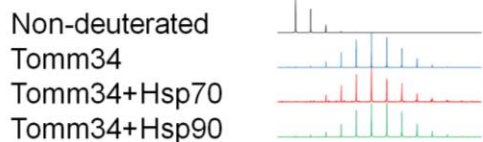
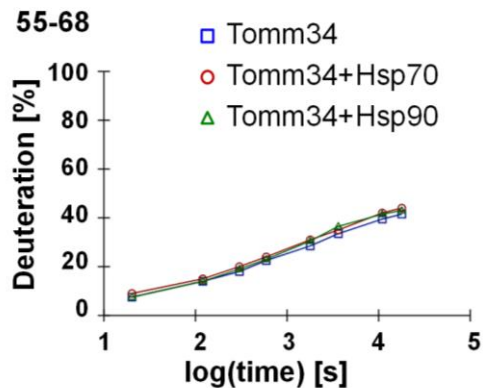
HDX – basic basics

Exchangeable hydrogens:

- 1) side chains containing $-OH$, $-SH$, $-NH_2$, $-COOH$ and $-CONH_2$ 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

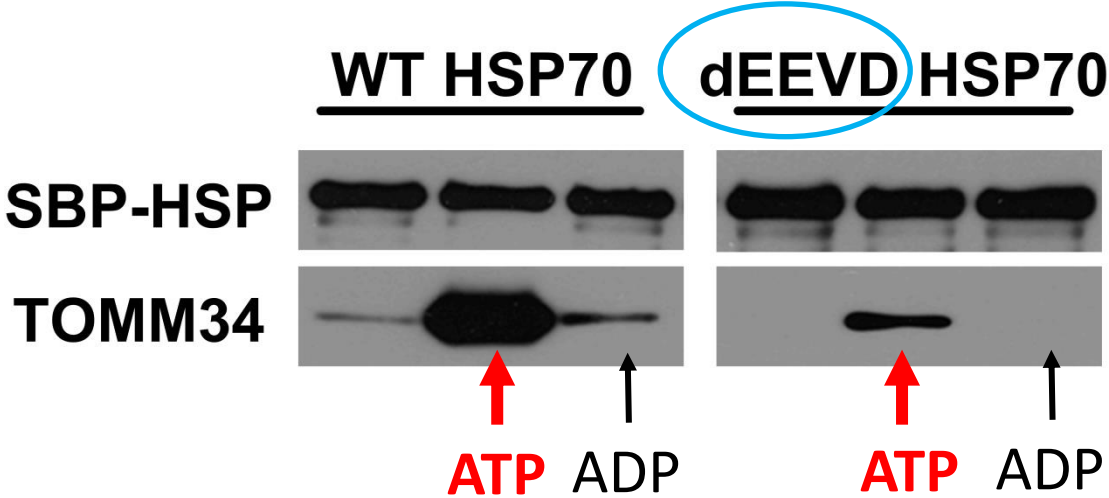
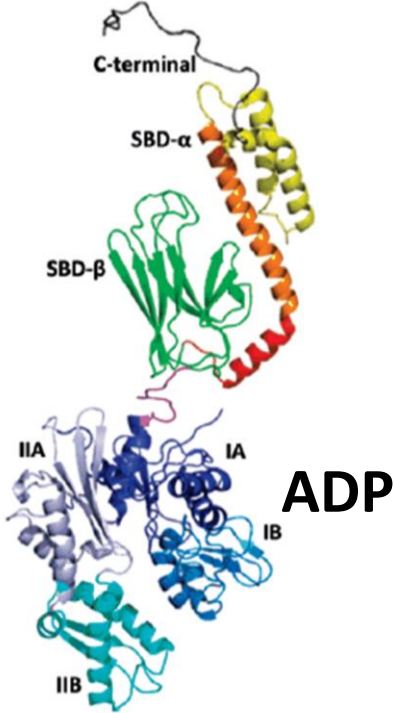
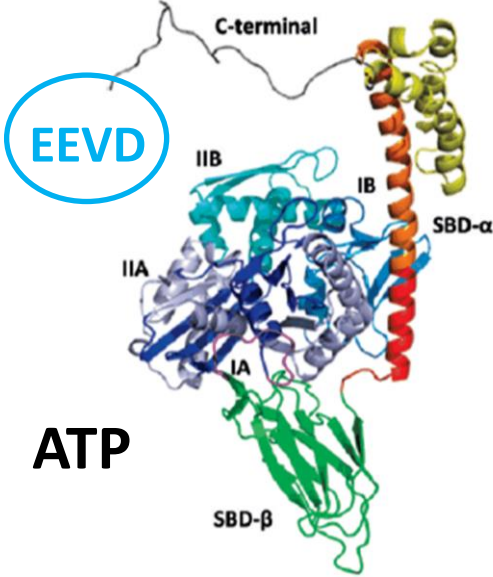
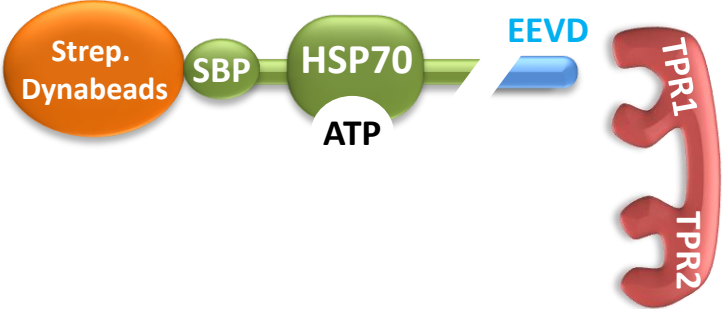


HDX – HSP70/90-TOMM34 interaction without ATP

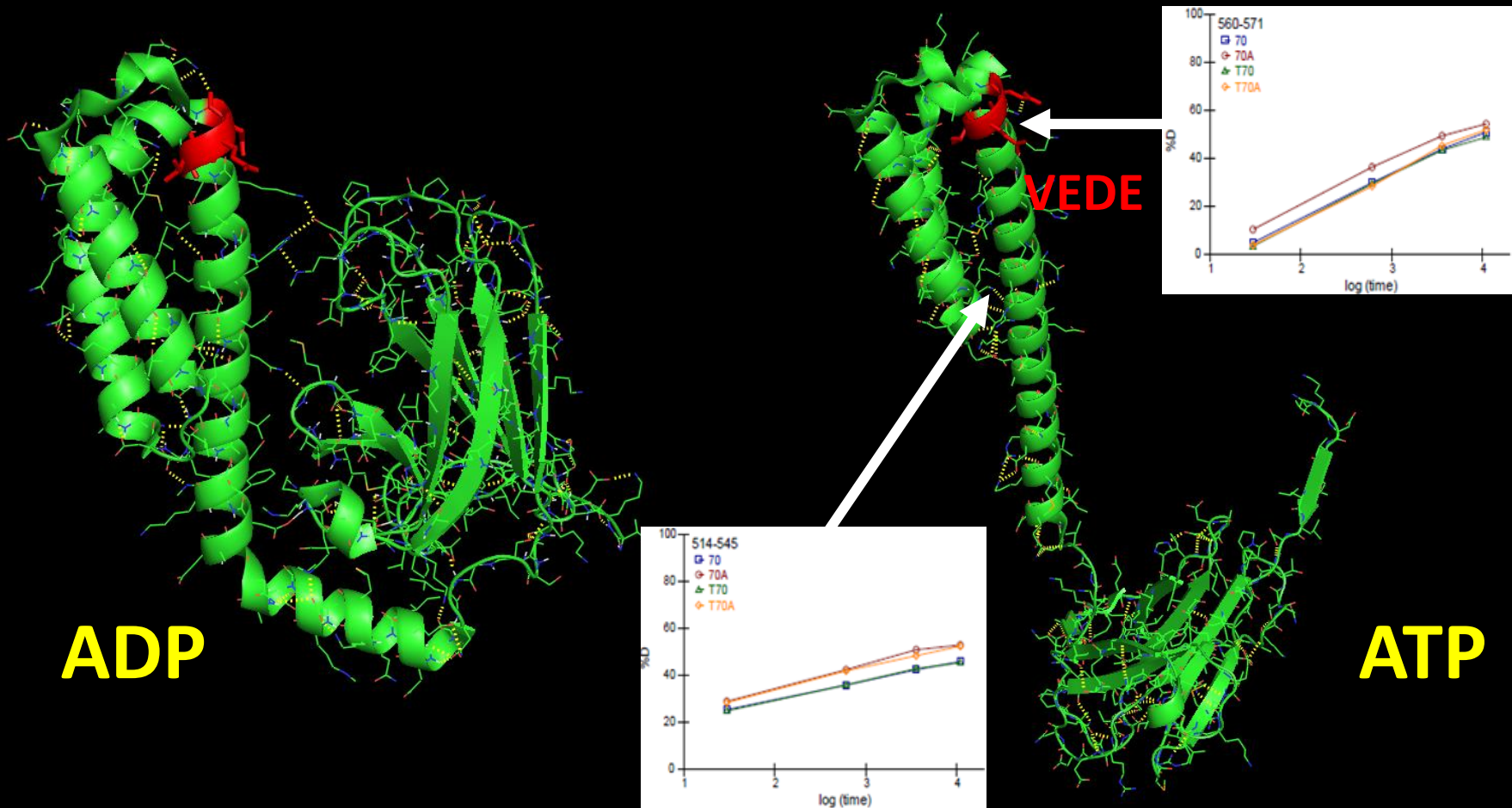
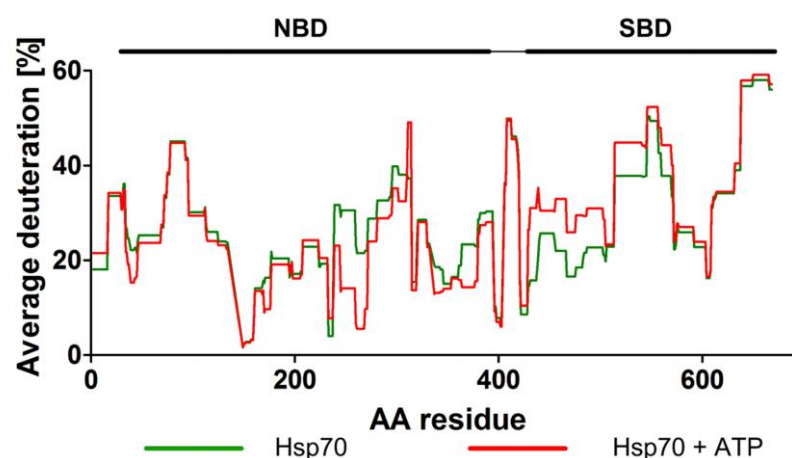


The effect of ATP

on HSP70 – Tomm34 interaction

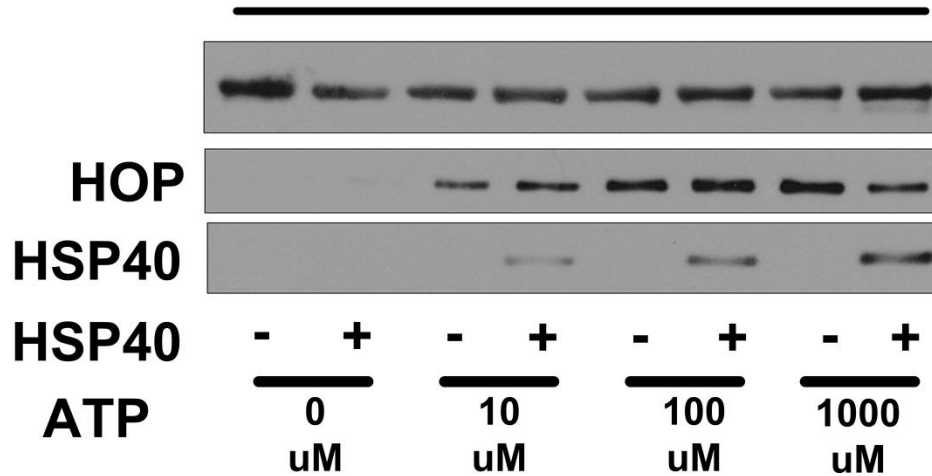


HDX – HSP70-TOMM34 interaction with ATP

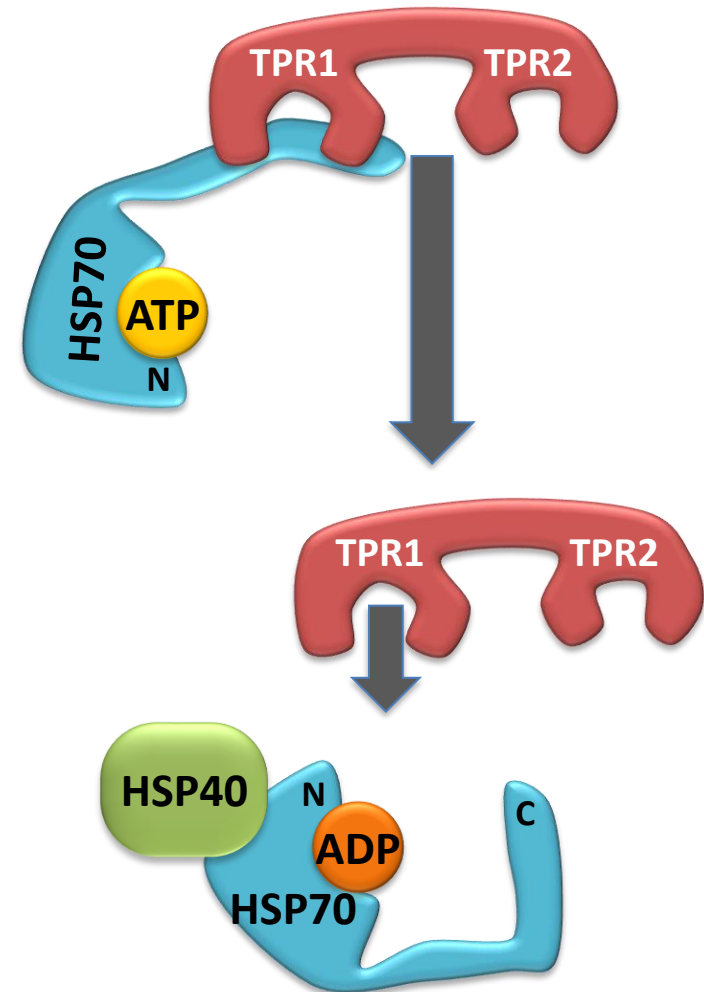
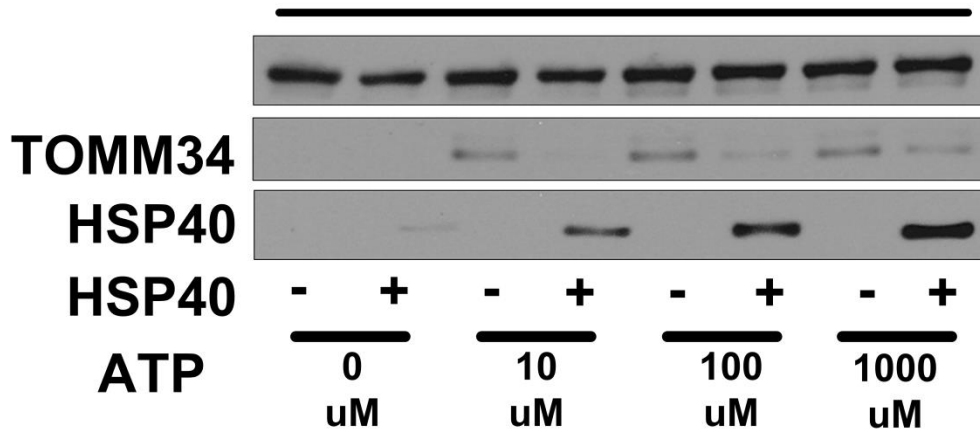


HSP70-TOMM34 interaction with ATP, the role of HSP40

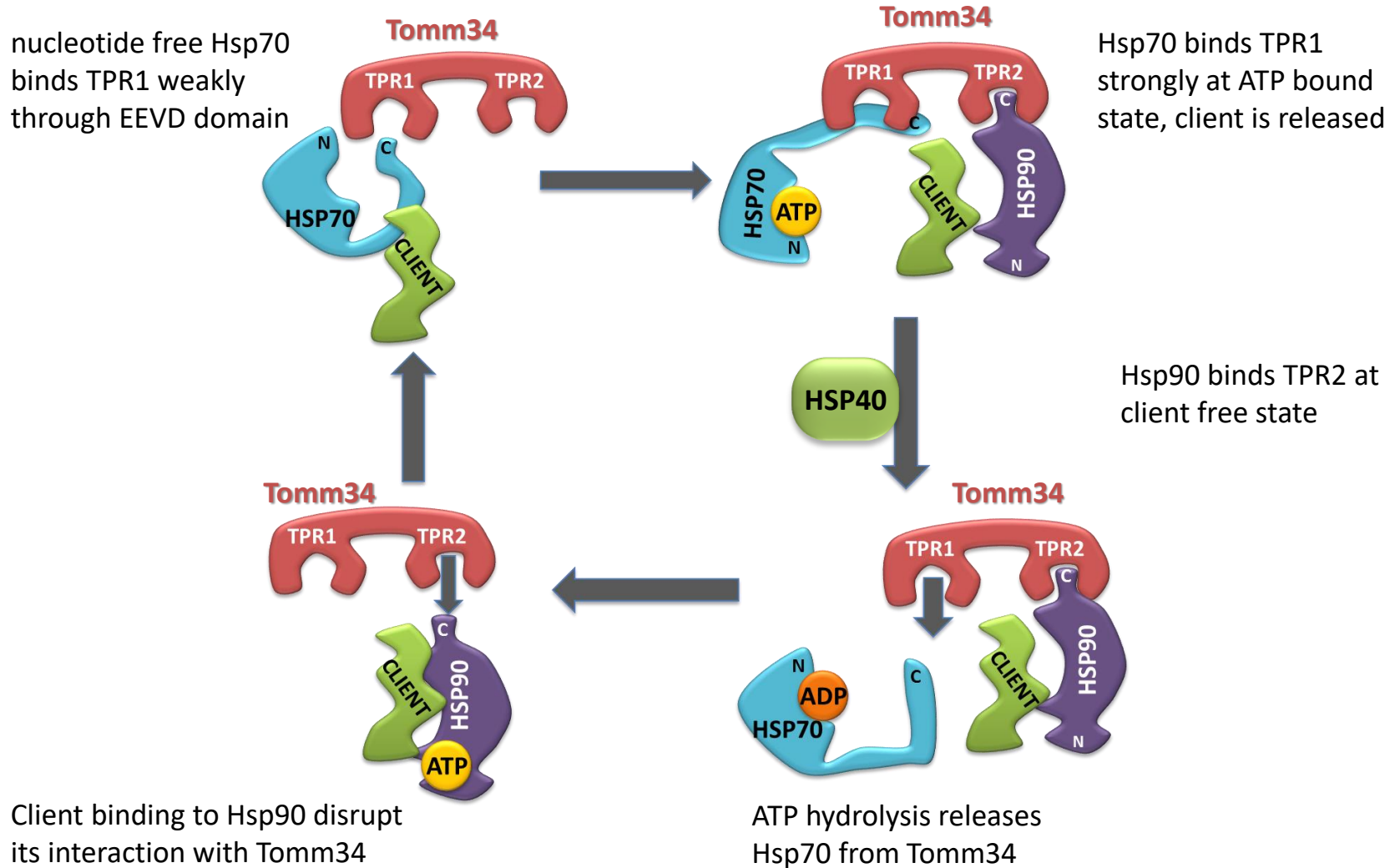
WT HSP70



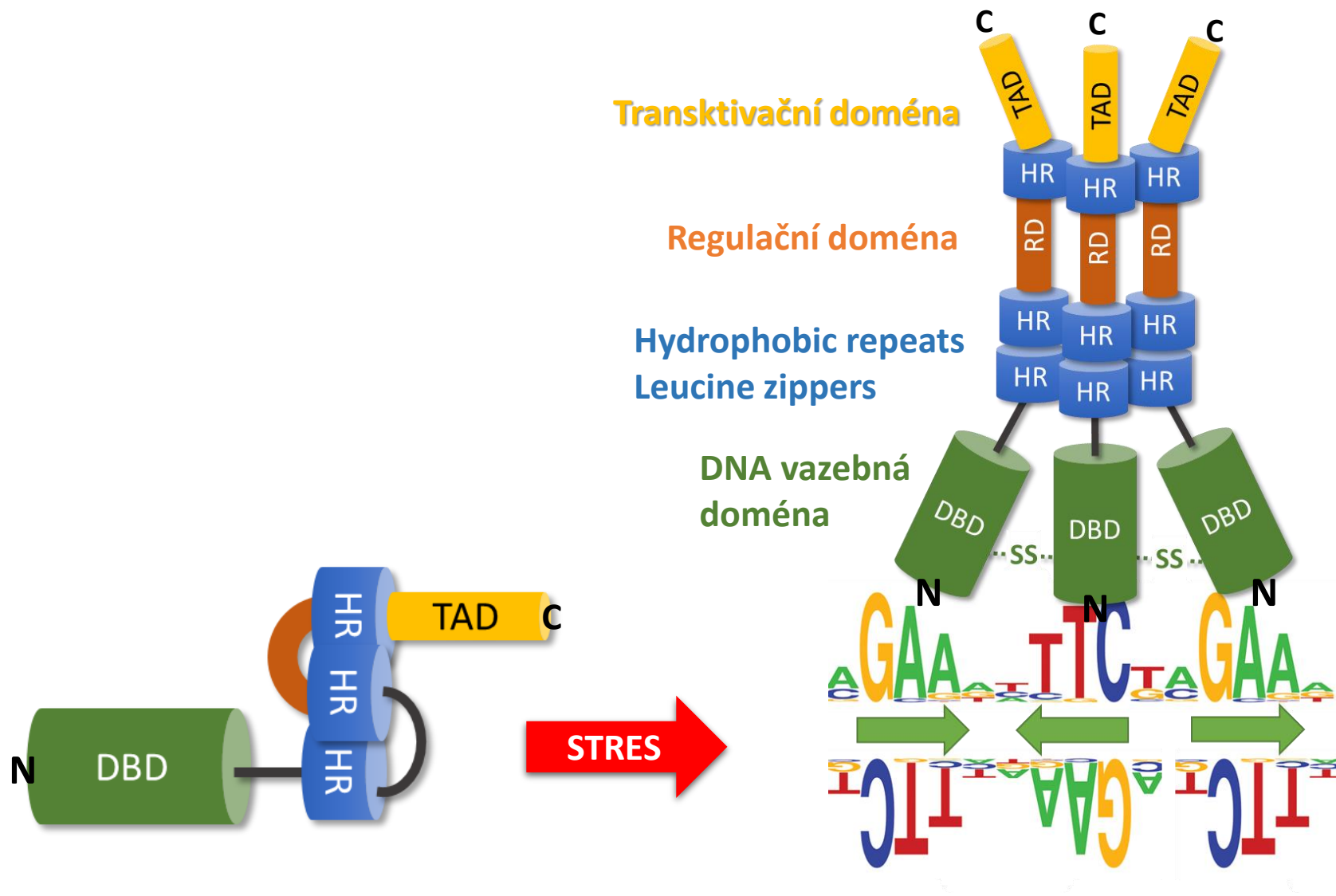
WT HSP70



Cooperation of Hsp70/Hsp90 folding by Tomm34

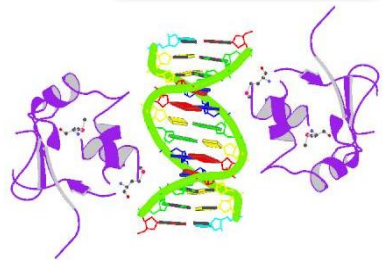
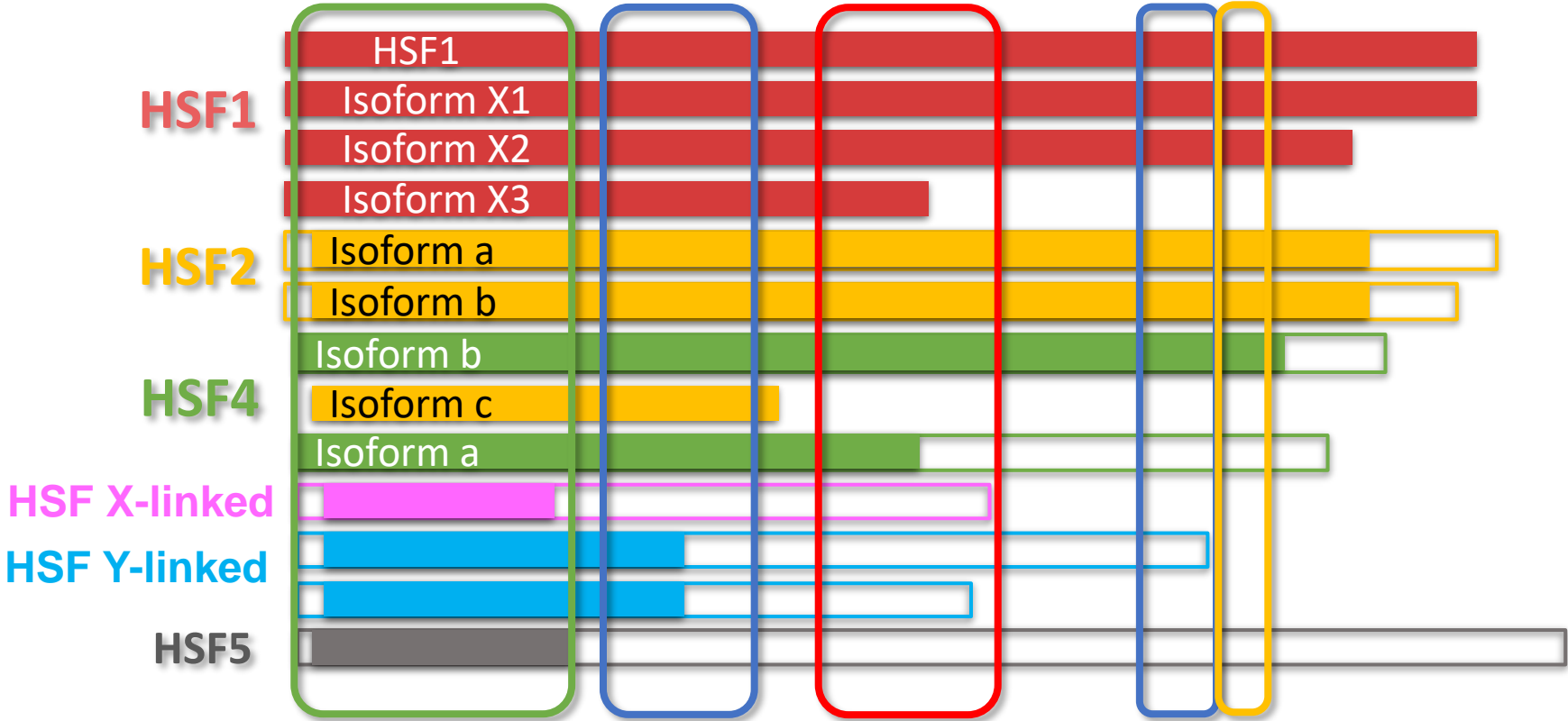


Regulace genové exprese chaperonů



Lidské HSF
Geny a isoformy

15-120 DNA binding
 130-203 Hydrophobic repeat HR-A/B
 221-310 Regulatory domain

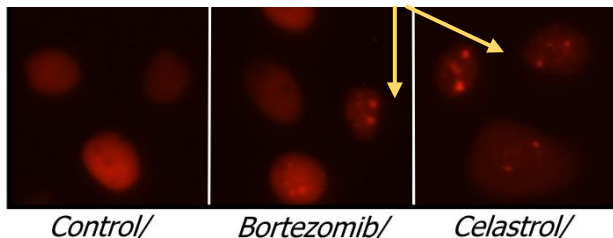


1 100 200 300 400 500

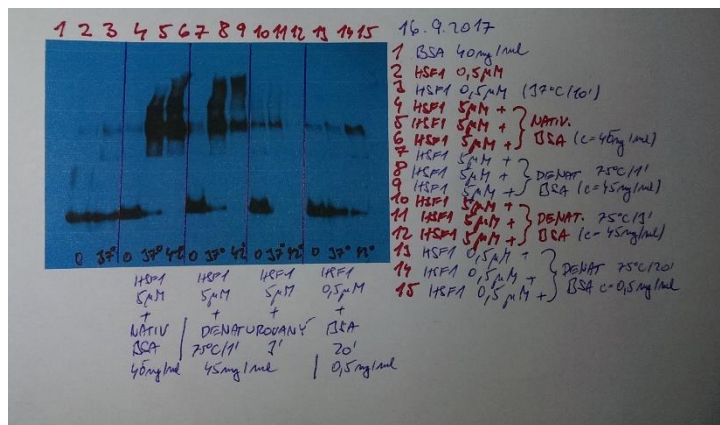
Analysis of HSF1 activation

HSF1-mCherry in A375 and H1299

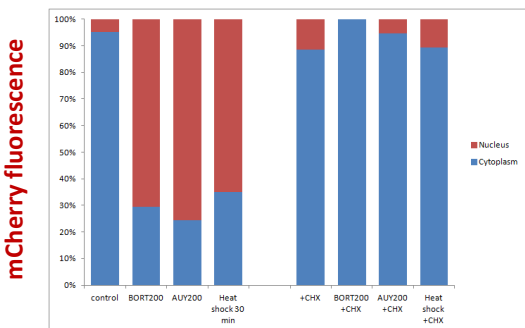
Nuclear stress bodies



Native gel, detection of trimers



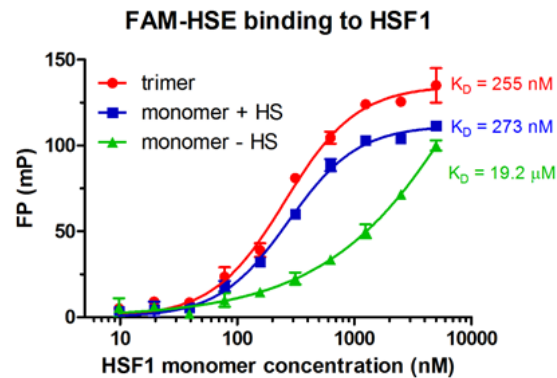
Cell fractionation



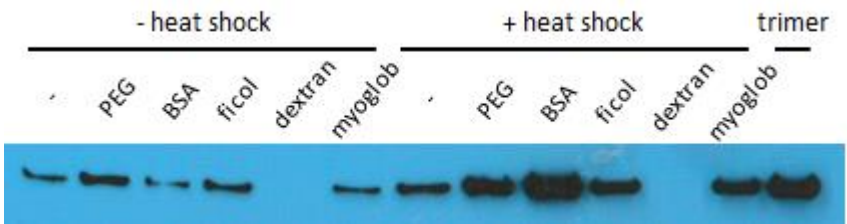
WB

- Crosslinkink
- Fractionation
- phosphorylation

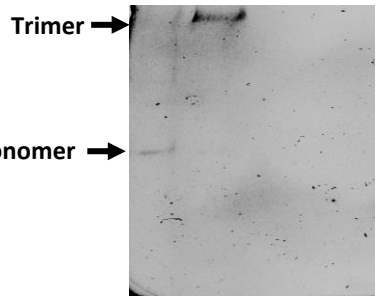
Measurement of DNA binding capacity



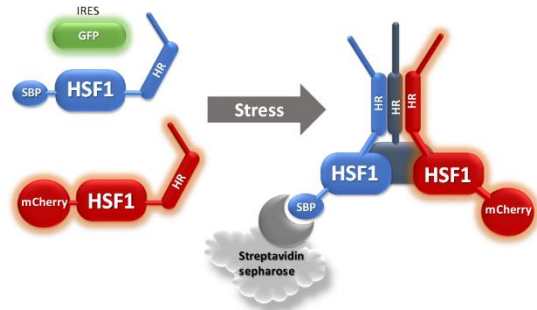
Fluorescence polarization



Native gel - mCherry

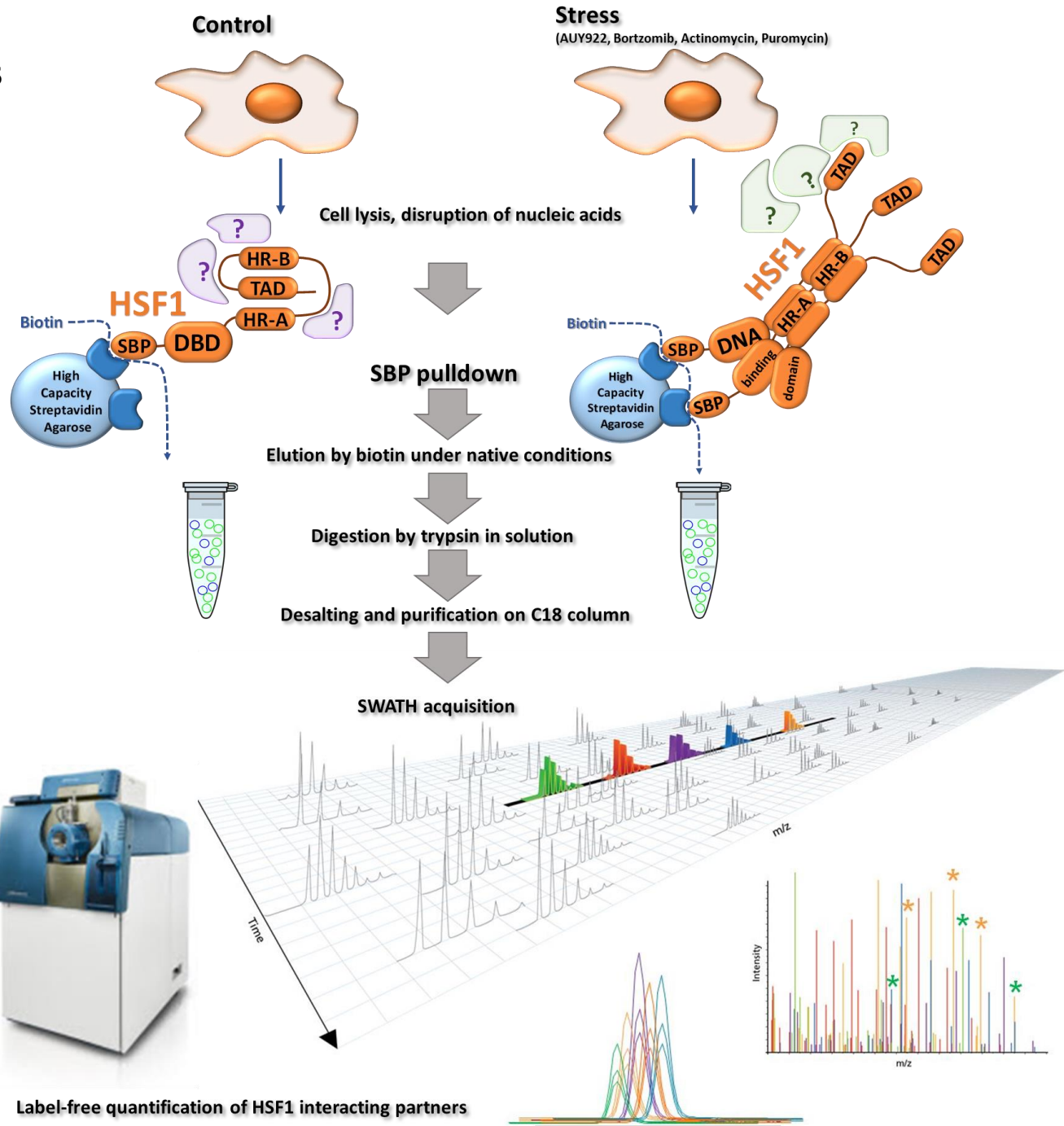


HSF1-mCherry + SBP-HSF1



Typhoon FLA 9500

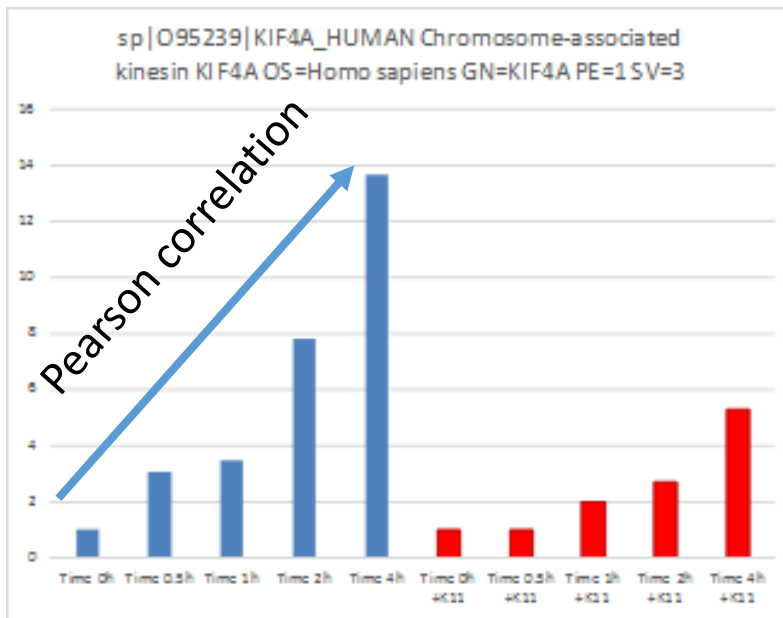
Analysis of HSF1 interaction partners Using functional proteomics



Label-free quantification of HSF1 interacting partners

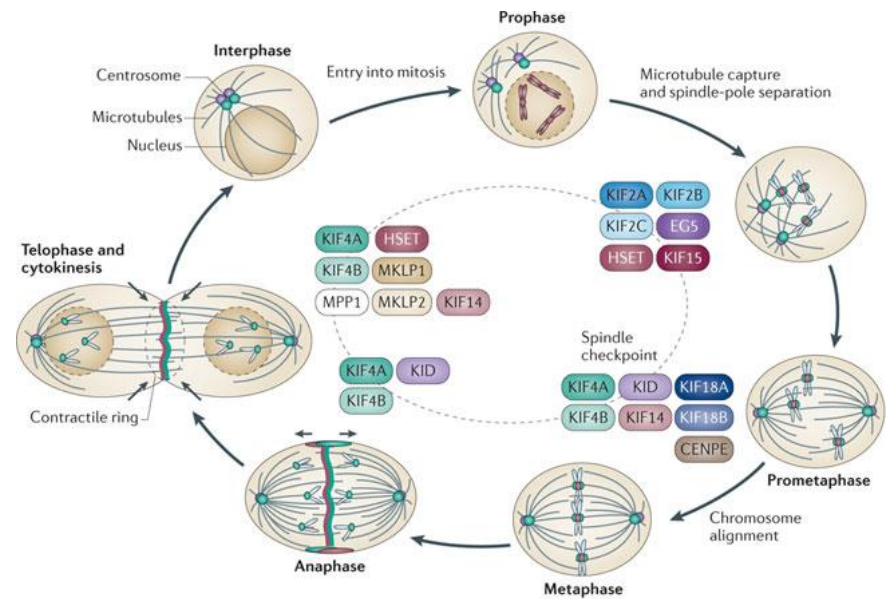
Time dependent interaction

KIF4A

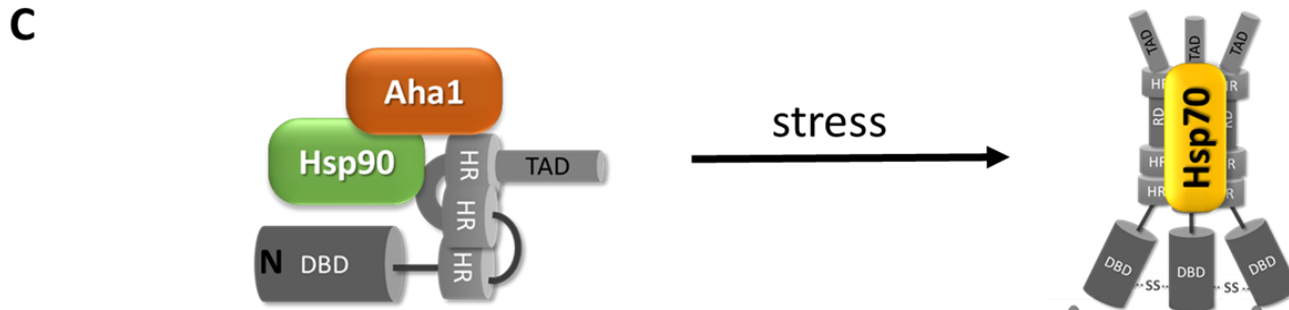
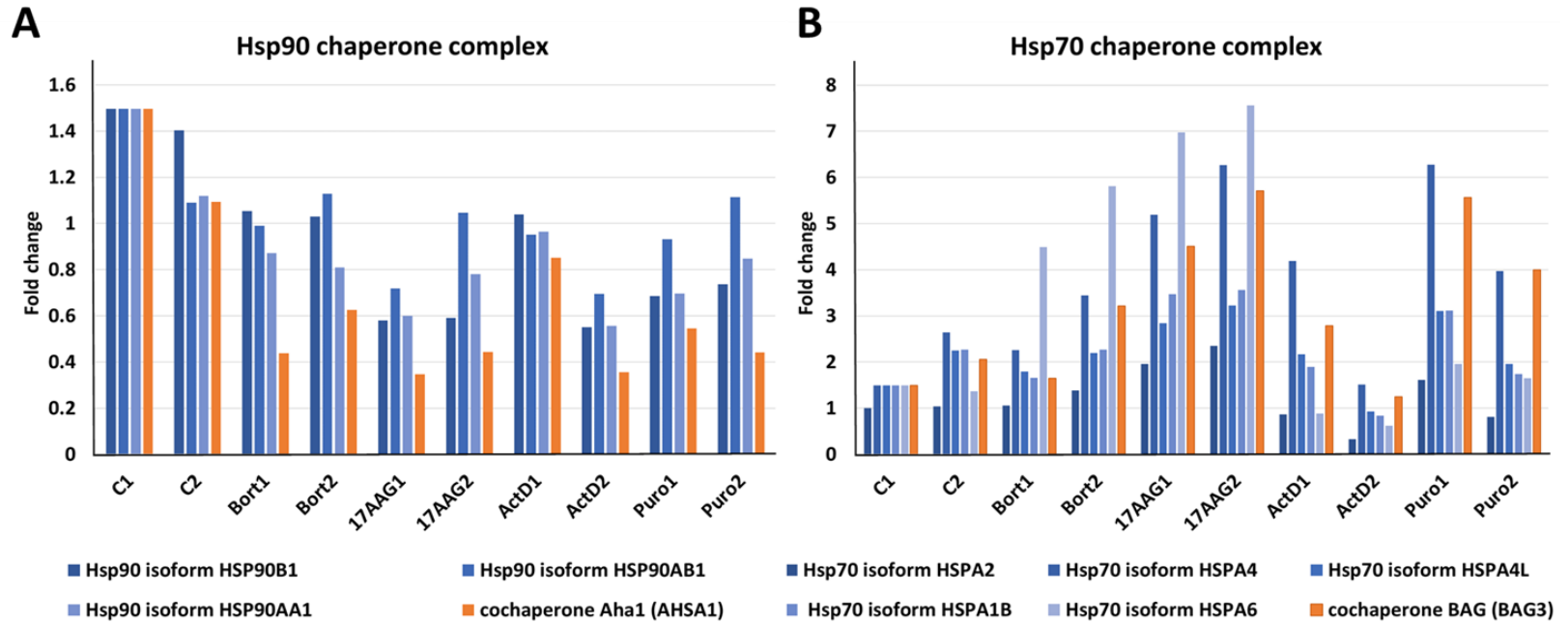


Bortezomib

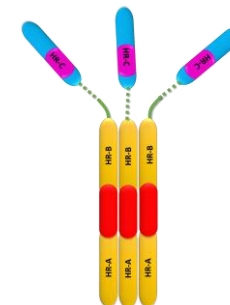
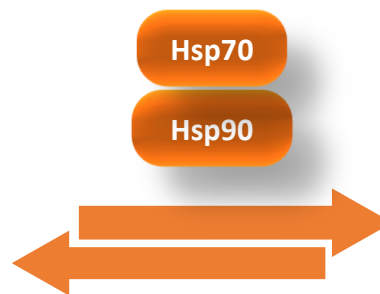
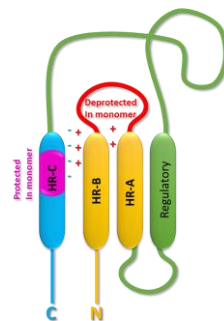
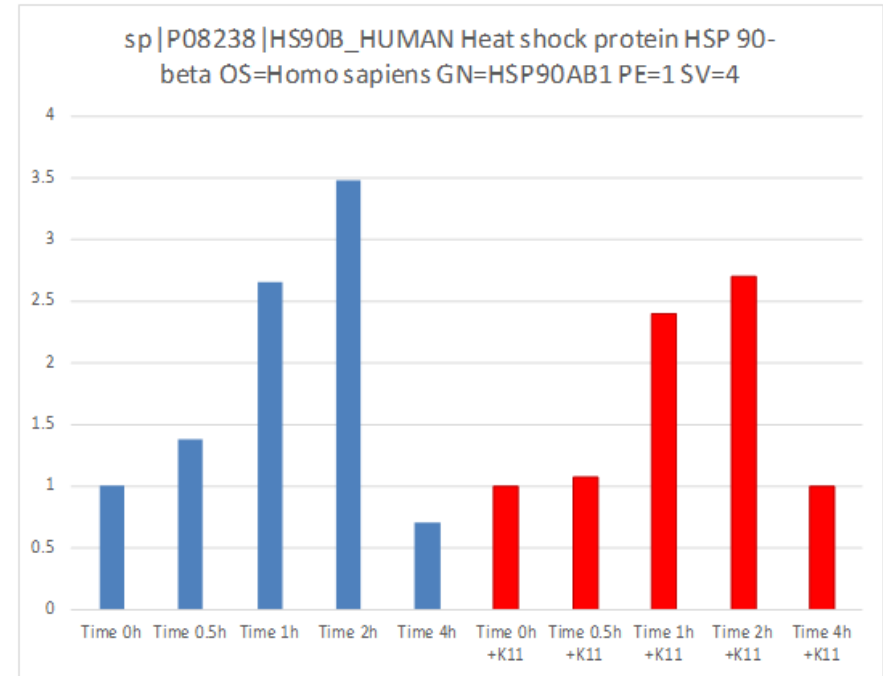
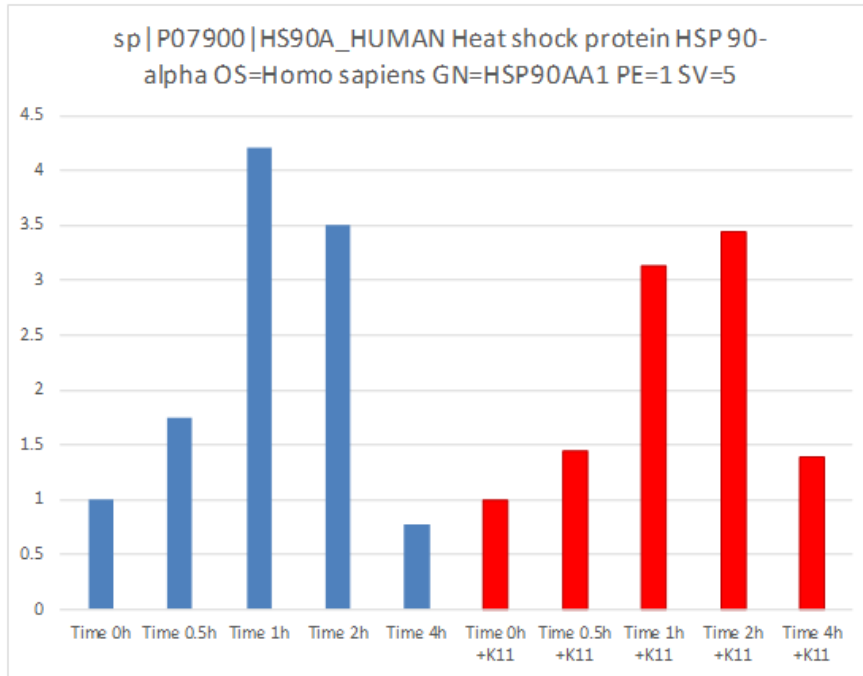
Bortezomib
+ KRIBB11



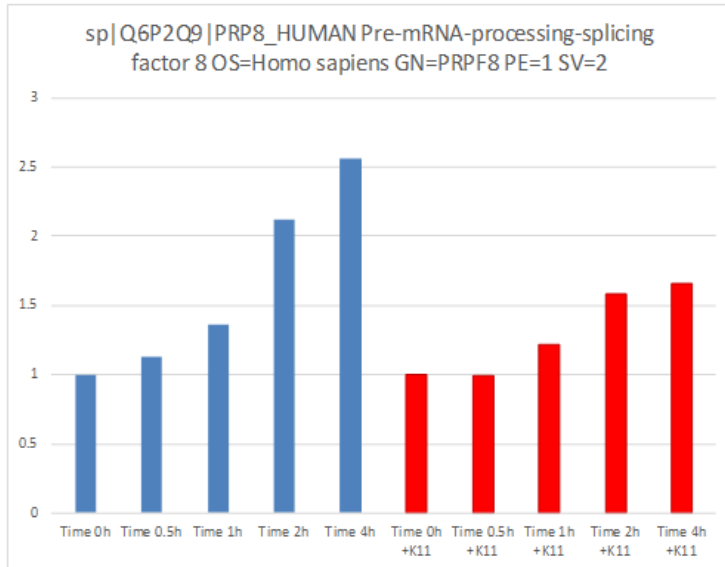
Hsp90alpha/Hsp90beta



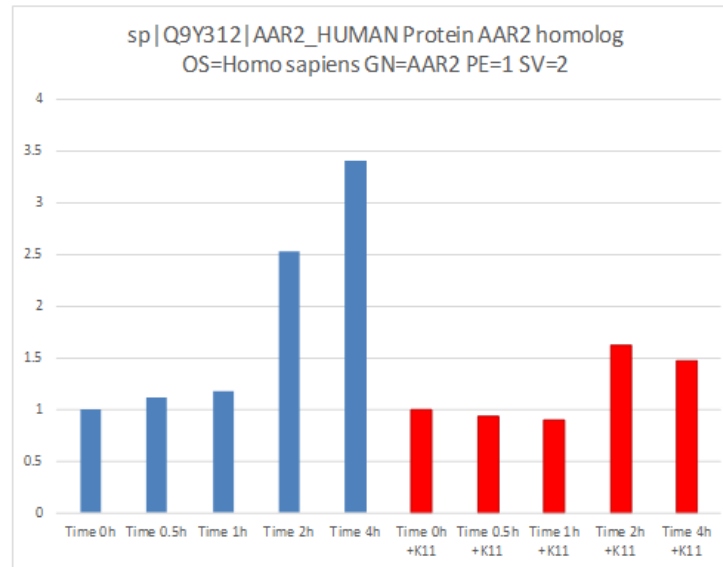
Interaction of HSF1 with Hsp90alpha/Hsp90beta after Bortezomib treatment



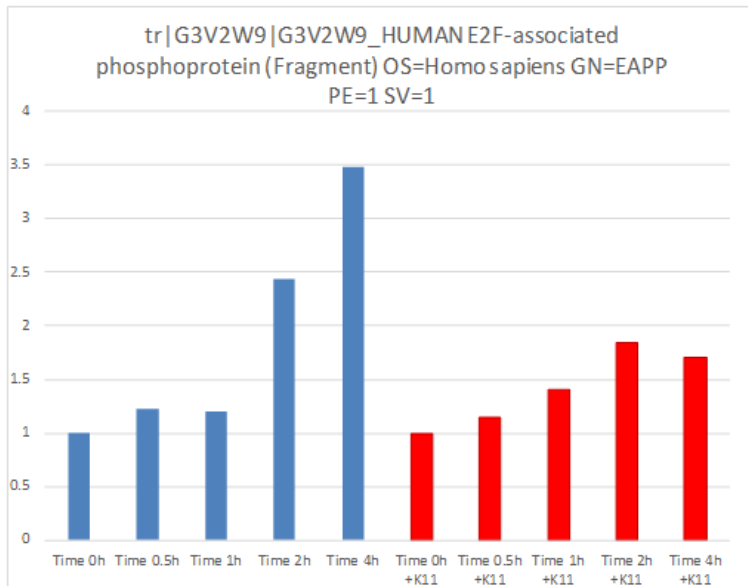
PRP8



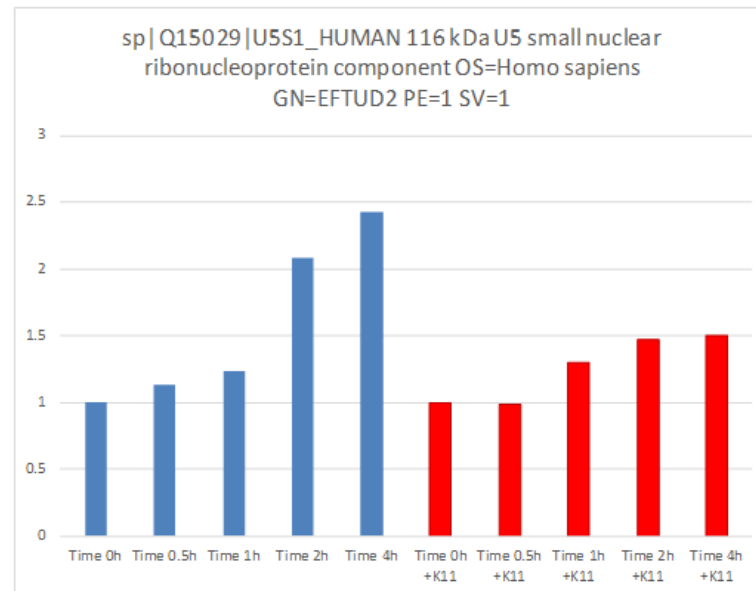
AAR2



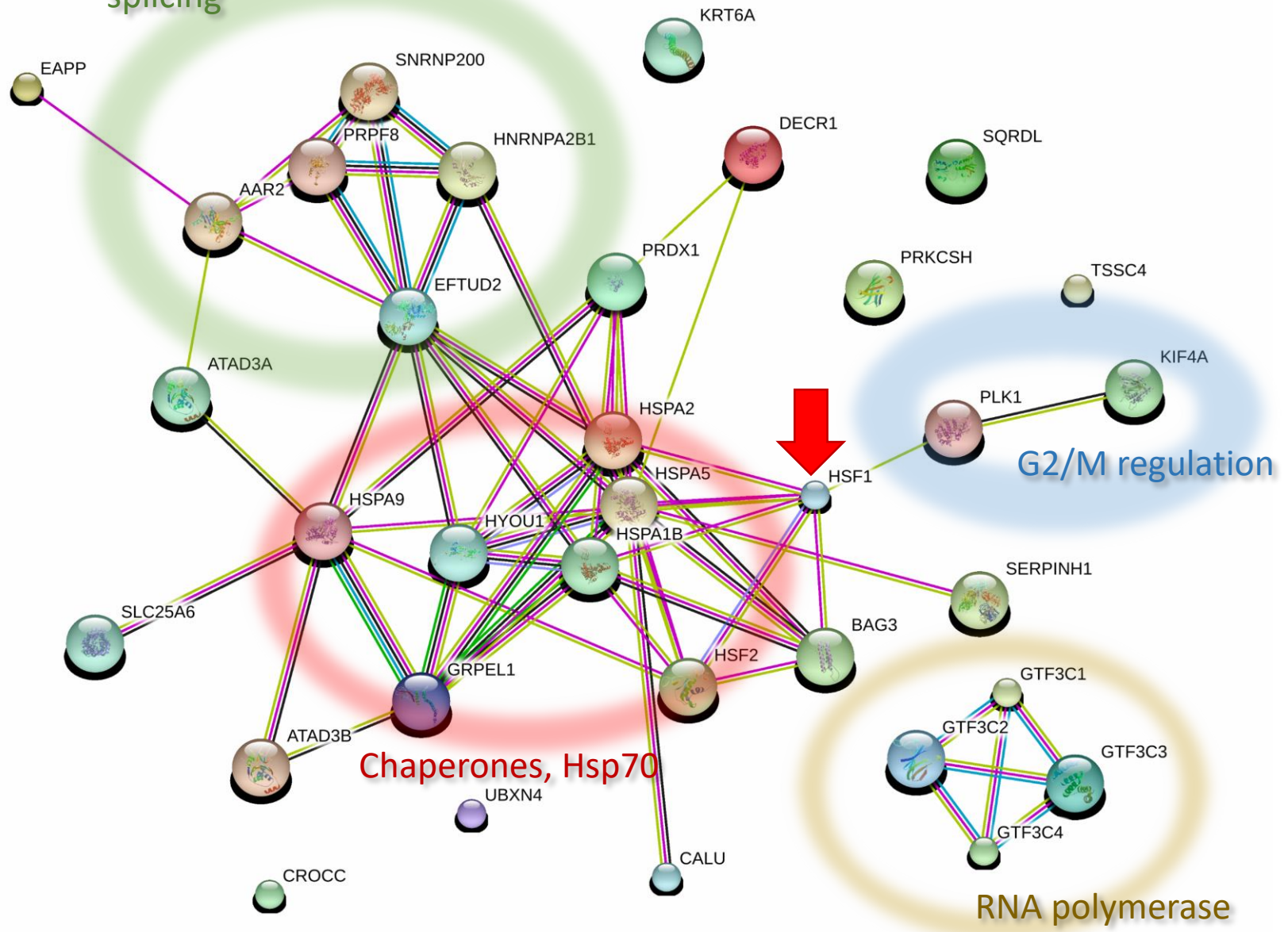
EAPP



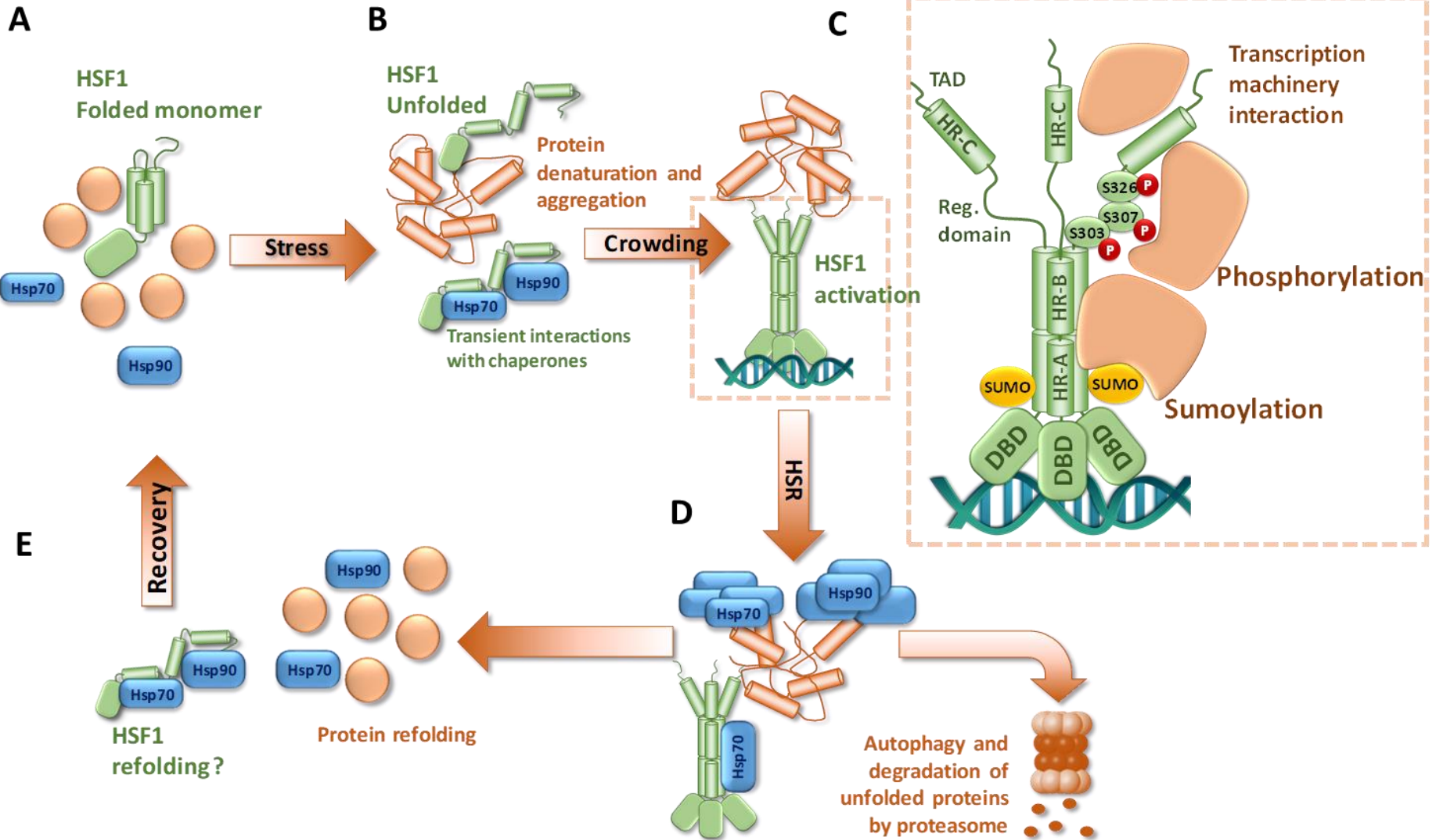
EFTUD2



Pre mRNA processing
splicing



Mechanisms of HSF1 activation



Děkuji za pozornost



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



Edinburgh Cancer
Research Centre

Ted R. Hupp