Small Molecule Modulators of Hsp70 as Potential Anti-Cancer Drug Leads

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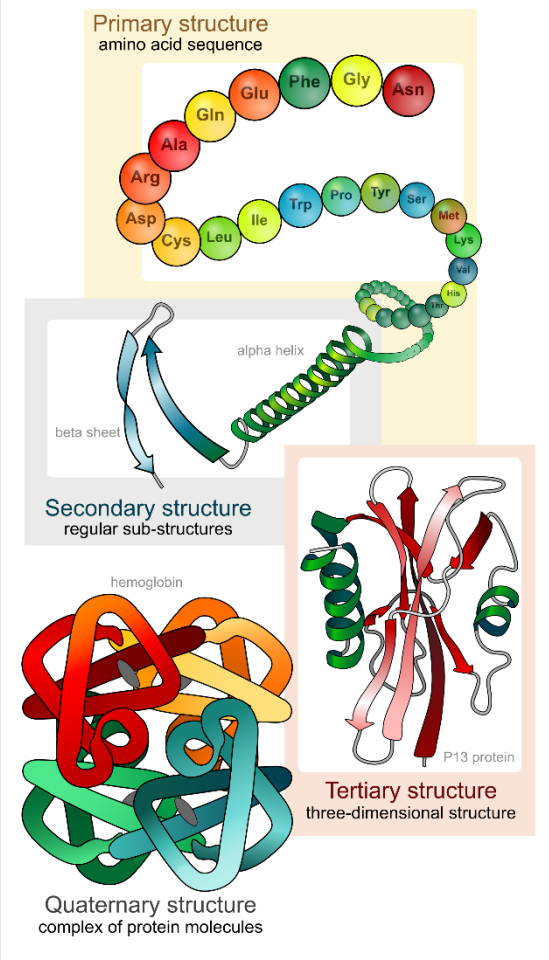
A. Manos-Turvey,

Wipf Group Topic Seminar

Feb. 1st, 2014

Protein Regulation

- ~24 000 protein encoding genes
  - mRNA splicing, post-translational modifications
- Normal cellular function relies upon the correct expression, folding and localisation of these proteins
- Cellular stresses can impact upon these processes
  - Heat shock
  - Nutrient deprivation
  - Cellular growth
  - Cell differentiation



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C. T. Walsh, S. Garneau-Tsodikova, G.J. Gatto, *Angew. Chem. Int. Ed.*, **2005**, *44*, 7342-7372
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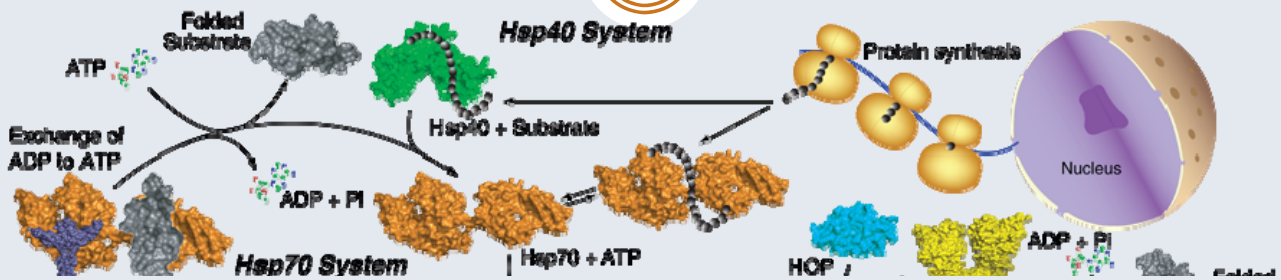
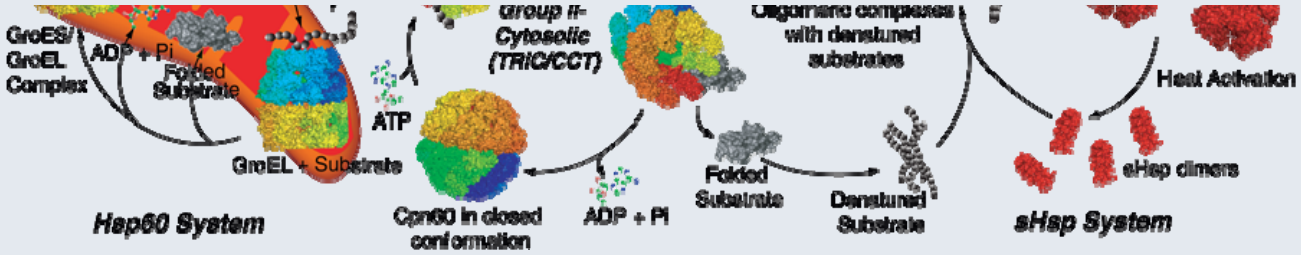
Molecular Chaperones: Heat Shock Proteins



- Molecular chaperones monitor proteins and intervene during cellular processes
  - HSPs are induced upon cellular stress, oxidative stress, in the presence of metal ions
  - are grouped according to molecular weight
- HSPs act to protect cells from apoptosis and autophagy
  - makes them interesting for anti-cancer research



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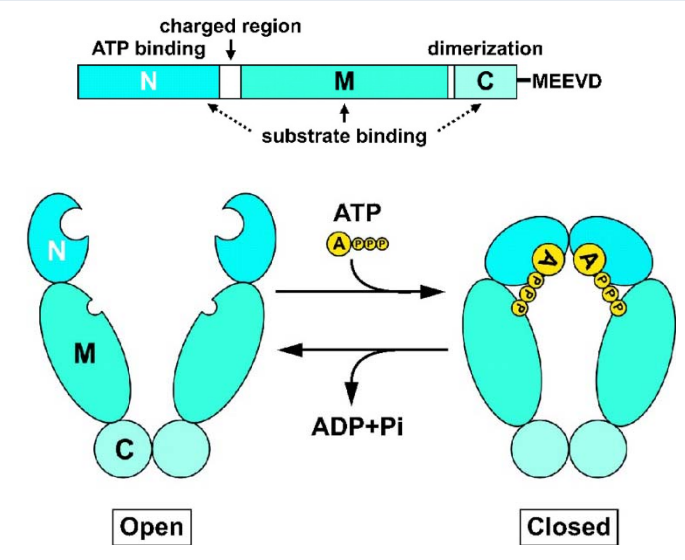
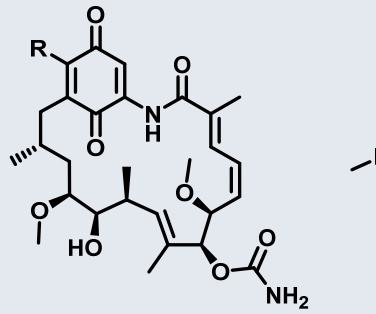
S. Kaushik, U. Bandyopadhyay, S. Sridhar *et al.*, *J. Cell Sci.*, **2011**, *124*, 495-499
Pic: http://pdslab.biochem.iisc.ernet.in/hspir/chaperone.php

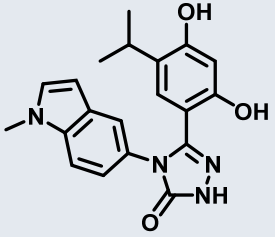
An Example of Anti-Cancer Potential: Hsp90

- Associated with oncogenic protein kinases and growth factors
  - cancer cell lines show upregulated Hsp90 levels and dependence
- Has a unique ATP-binding pocket, targeted by small molecule inhibitors
  - homodimeric structure

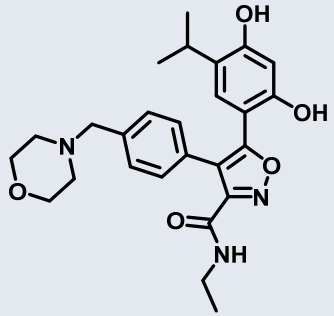




Geldanamycin: R = OCH3
17-AAG: R = $NHCH_2CHCH_2$
17-DMAG: R = $NH(CH_2)_2N(CH_3)_2$



STA-9090
(Ganetespib)



AUY922

- However as Hsp90 is inhibited, Hsp70 can be upregulated

H.J. Patel, S. Modi, G. Chiosis, T. Taldone, *Expert Opin. Drug Discov.*, **2011**, *6*, 559-587
L.H. Pearl, C. Prodromou, *Annu. Rev. Biochem.*, **2006**, *75*, 271-294
L. Whitesell, N.U. Lin, *BBA-Mol. Cell Res.*, **2012**, *1823*, 756-766
S. Pacey, R.H. Wilson, M. Walton *et al.*, *Clin. Cancer Res.*, **2011**, *17*, 1561-1570

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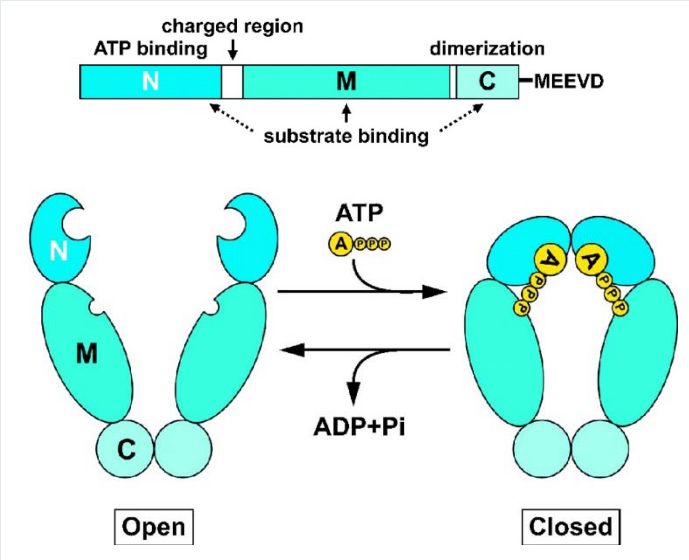

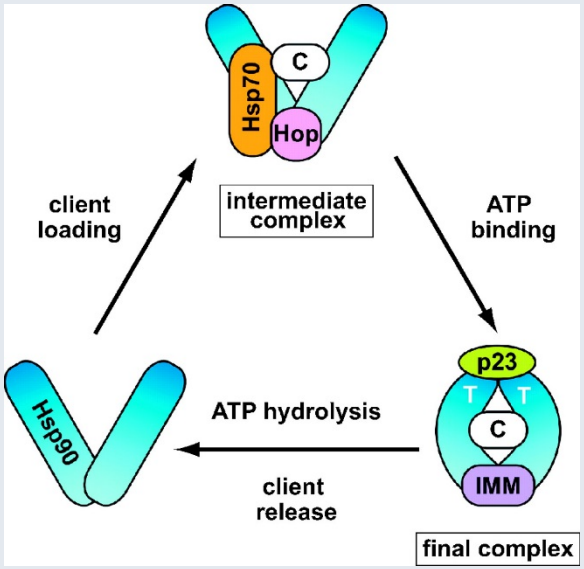

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The Importance of Hsp70

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- Hsp70 is expressed in all organisms and the activity is highly conserved
- Two major classes of Hsp70s: heat shock inducible + constitutively expressed (Hsc70)
  - humans have 8 confirmed isoforms
- Activity
  - faciliates DNA replication
  - stabilises membranes
  - corrects protein folding defects*
  - stabilises substrates*
  - helps form multiprotein complexes
  - identifies proteins for ubiquitination and subsequent degradation*

** indicates overlap with Hsp90*

S.A. Rensing, U.G. Maier, *J. Mol. Evol.*, **1994**, *39*, 80-86
L. Brocchieri, E.C. de Macario, A.J.L. Macario, *BMC Evol. Biol.*, **2008**, *8*
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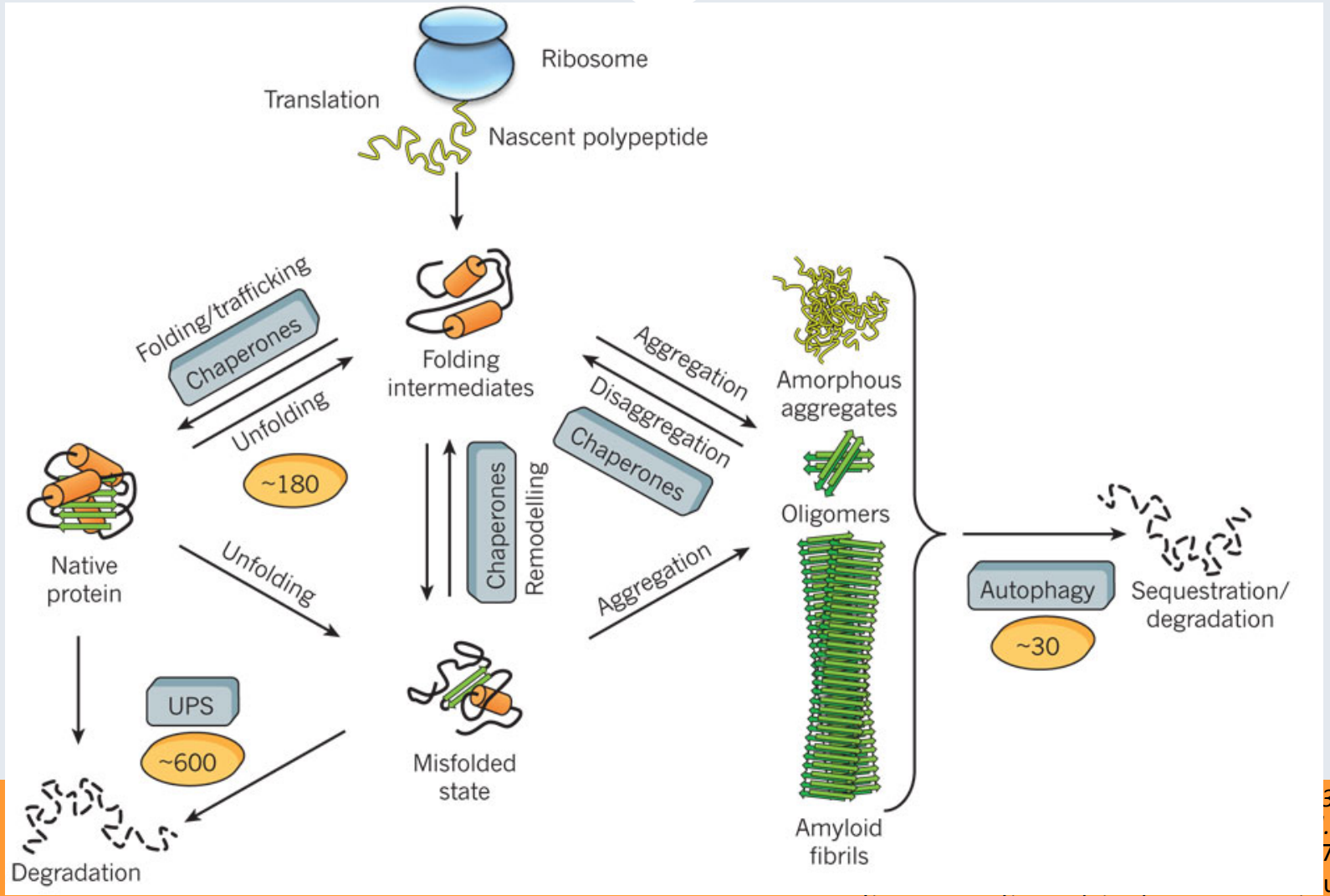
F.U. Hartl, A. Bracher, M. Hayer-Hartl, *Nature*, **2011**, *475*, 324-332

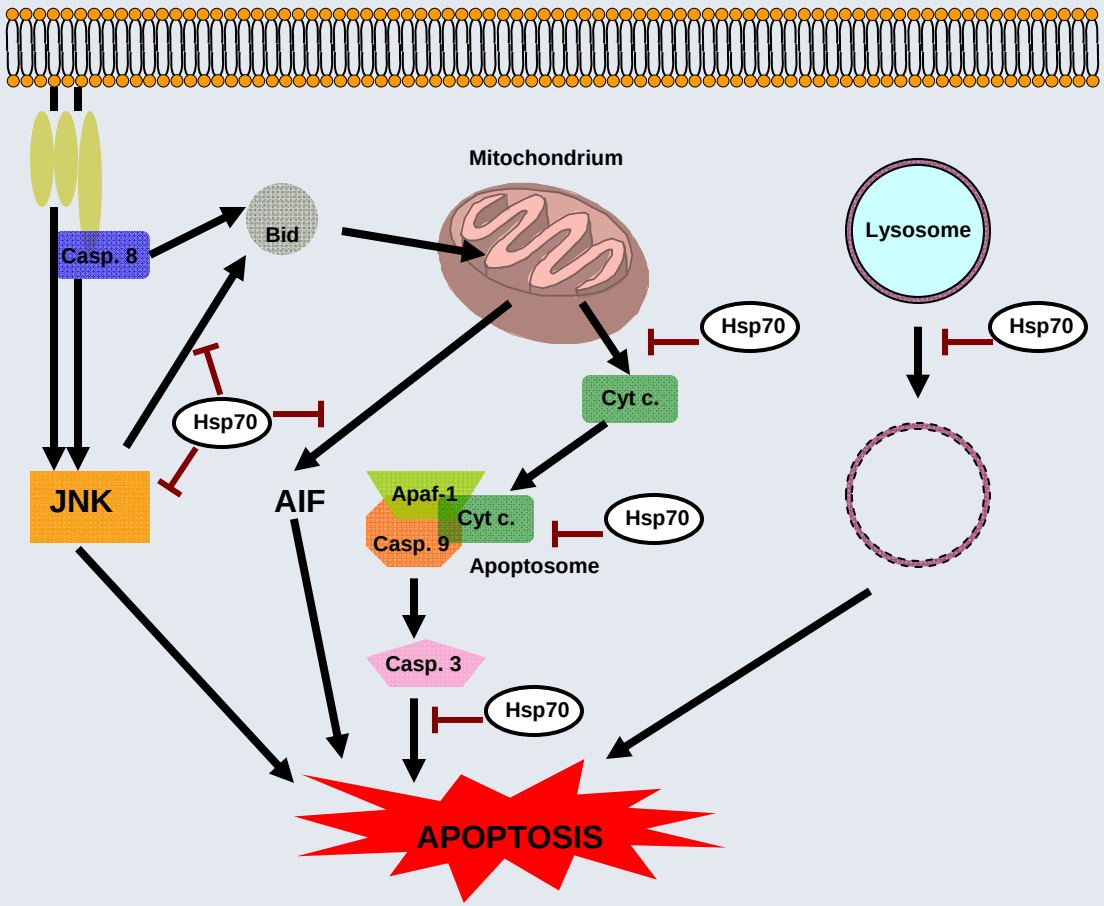
The Importance of Hsp70



39, 80-86
., **2008**, *8*
702-3710
ubmitted
F.U. Hartl, A. Bracher, M. Hayer-Hartl, *Nature*, **2011**, *475*, 324-332

The Importance of Hsp70: Apoptosis

- Activity
  - can inhibit apoptotic functions



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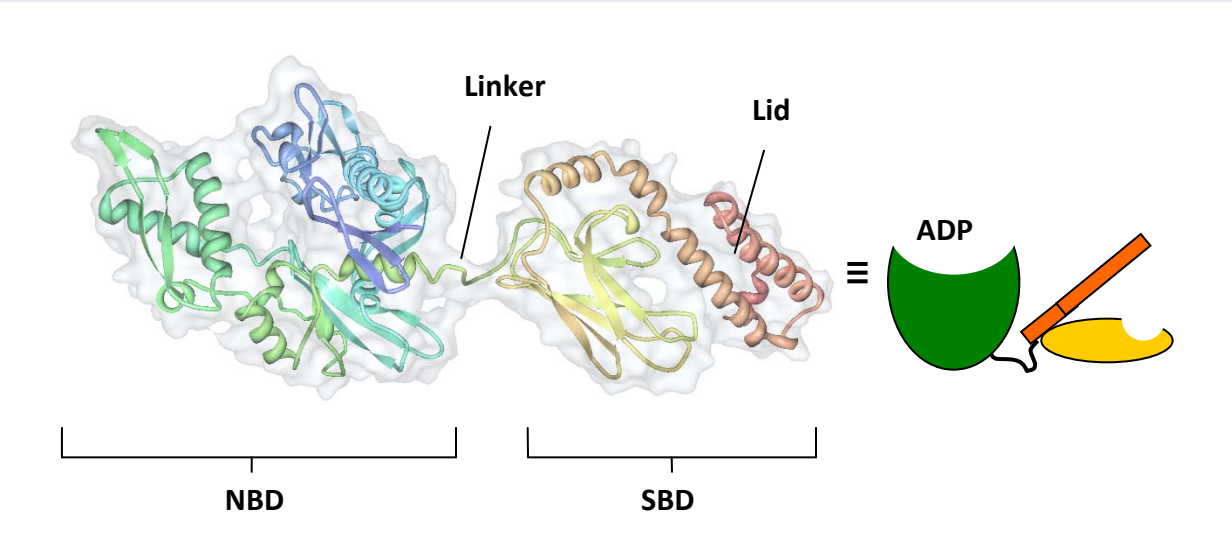
Structure of Hsp70

- High levels of sequence homology between prokaryotic and eukaryotic Hsp70
- Structure contains a nucleotide binding domain (NBD) and substrate binding domain (SBD)



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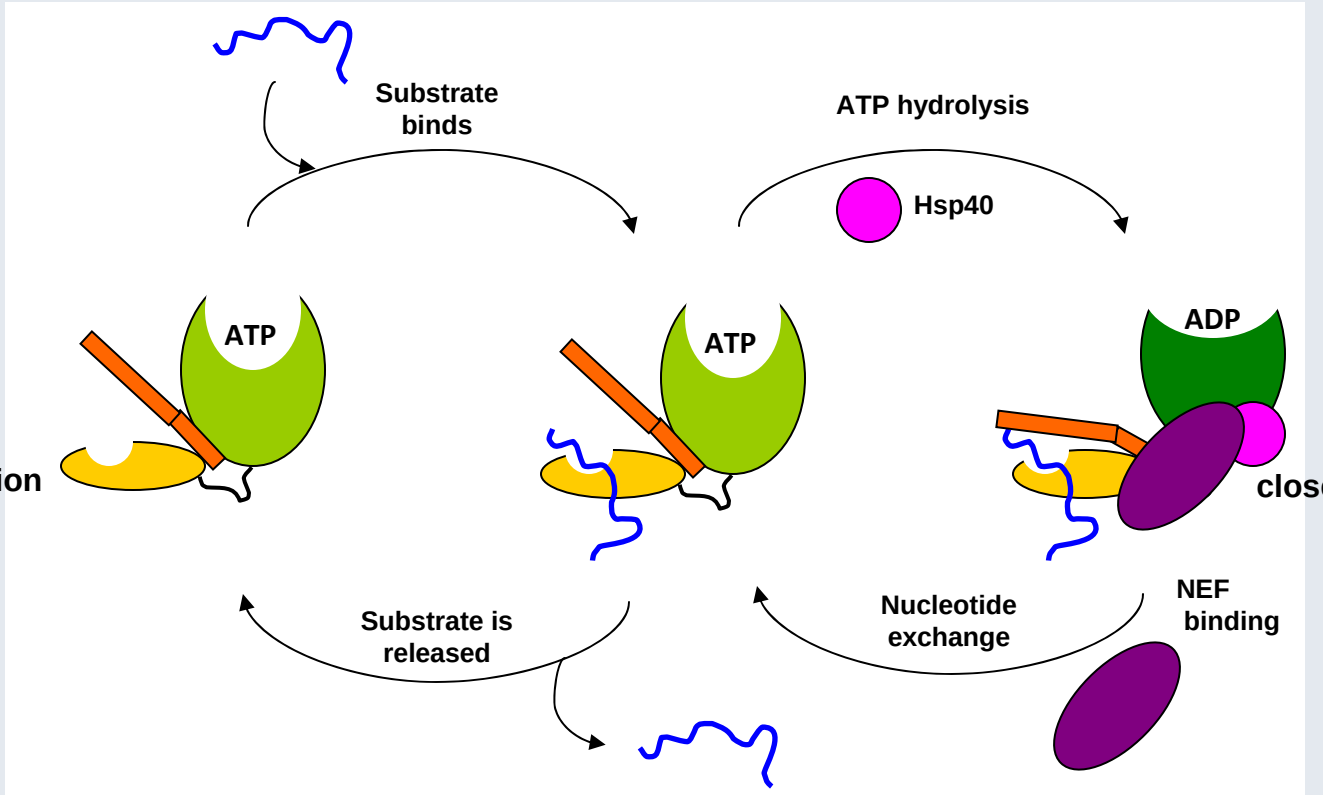
B.C. Freeman, M.P. Myers, R. Schumacher, R.I. Morimoto, *EMBO J.*, **1995**, *14*, 2281-2292

Activity of Hsp70

- Goes through catalytic cycle dependant upon the nucleotide binding



  - higher affinity for ATP than ADP helps continue the cycle
  - Hsp40s and NEFs help increase the poor activity of the "enzyme"

A. Zhuravleva, E.M. Clerico, L.M. Gierasch, *Cell*, **2012**, *151*, 1296-1307
H.H. Kampinga, E.A. Craig, *Mol. Cell Biol.*, **2010**, *11*, 579-592
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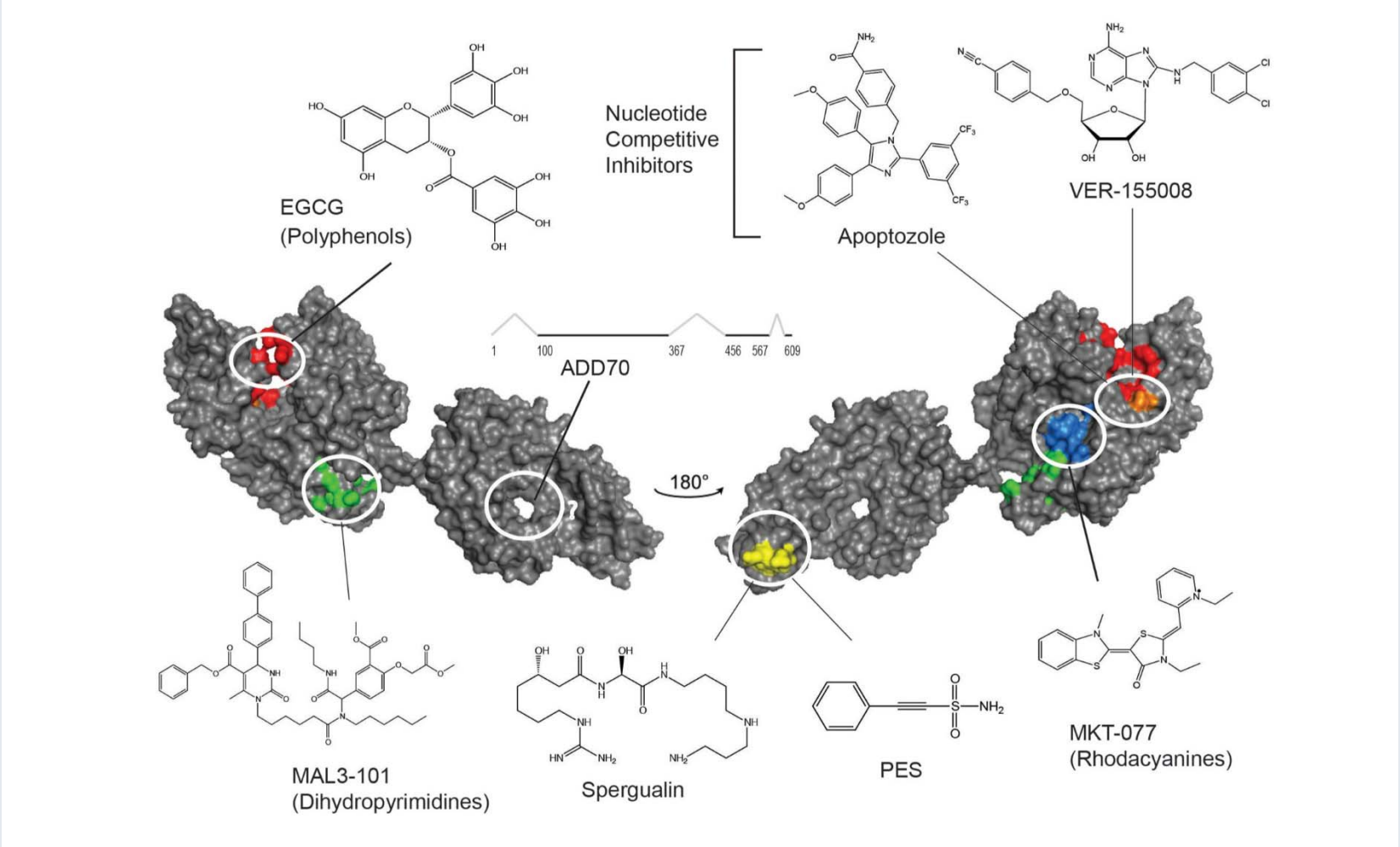
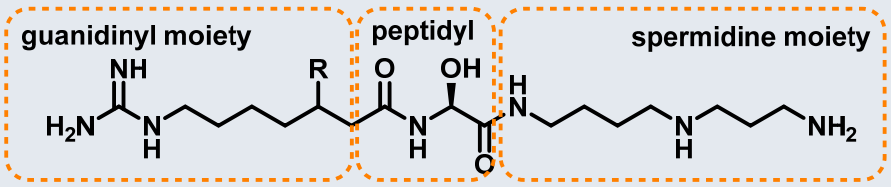
Known Hsp70 Inhibitors



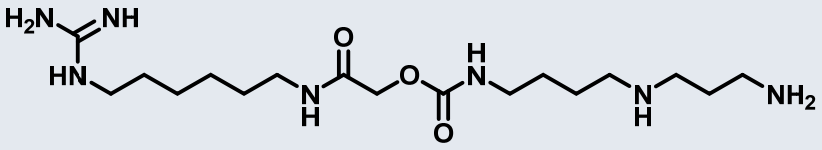
S.R. Srinivasan, X. Li and J.E. Gestwicki, *Trends Med Chem.* (in press).

Known Hsp70 Modulators: 15-Deoxyspergualin

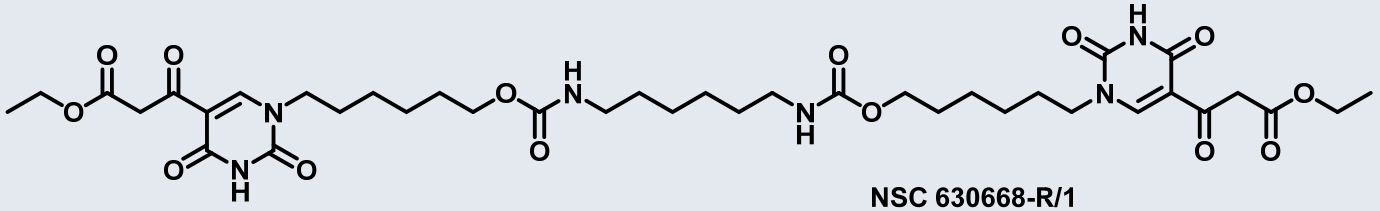
- 1982: 15-DSG was synthesised
  - derivative of spergualin
  - anti-tumour activity in mice
  - 1992: identified to target Hsp70
  - K_D values of 4 µM against Hsc70 and 5 µM against Hsp90
- 1999: tresperimus
  - greater stability, equal activity
- 2001: NSC 6030668-R/1



15-Deoxyspergualin (15-DSG): R = H
Spergualin: R = OH (*S*)



tresperimus



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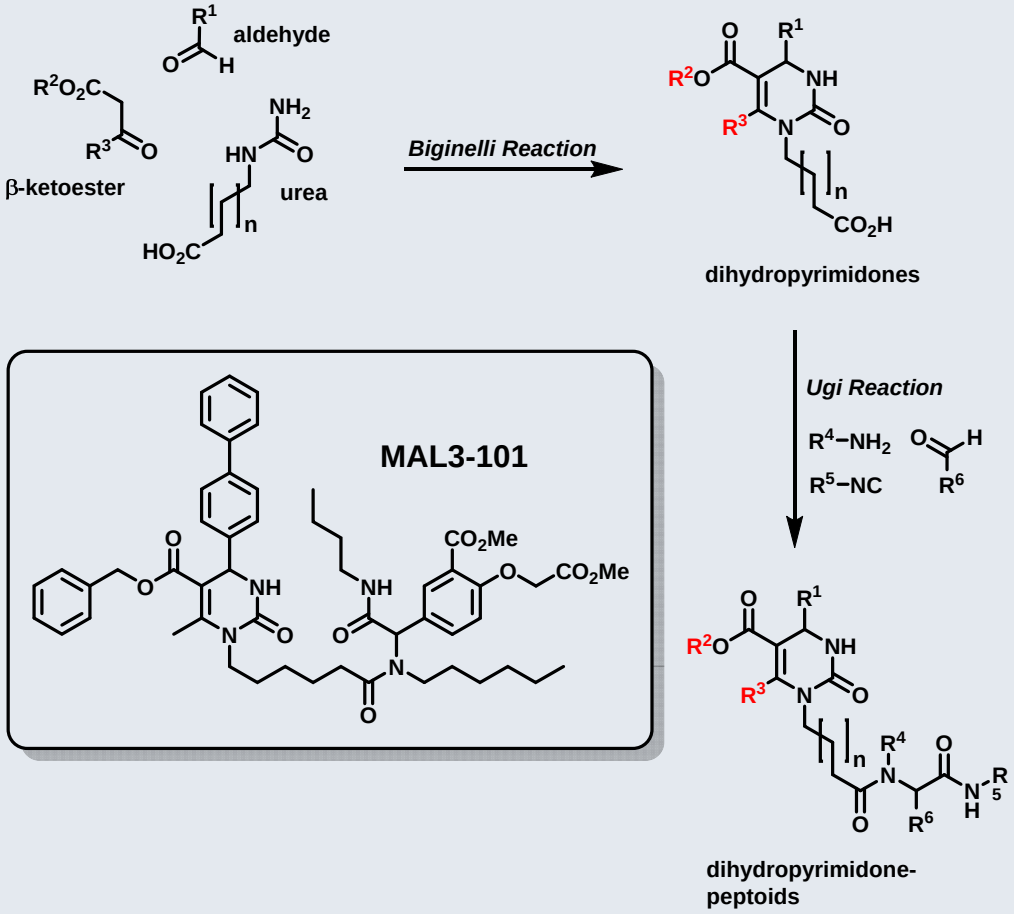
S.W. Fewell, B.W. Day, J.L. Brodsky, J. Biol. Chem., **2001**, *276*, 910-914

The Discovery of MAL3-101 and its Derivatives

- Using multi-component reactions ~500 compounds have been synthesised
  - maintain the pyrimidone core, while varying substituents
- MAL3-101 reduced Hsp40 ATPase activity stimulation
  - inhibits multiple myeloma and breast cancer cell proliferation
- Compounds show varied Hsp70 activity
  - With/without an Hsp40



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S. Werner, D.M. Turner, M.A. Lyon, D.M. Huryn, P. Wipf, *Synlett.*, **2006**, *14*, 2334-2338
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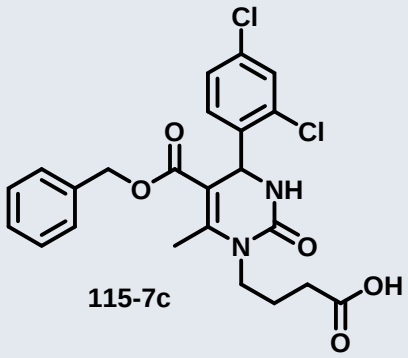
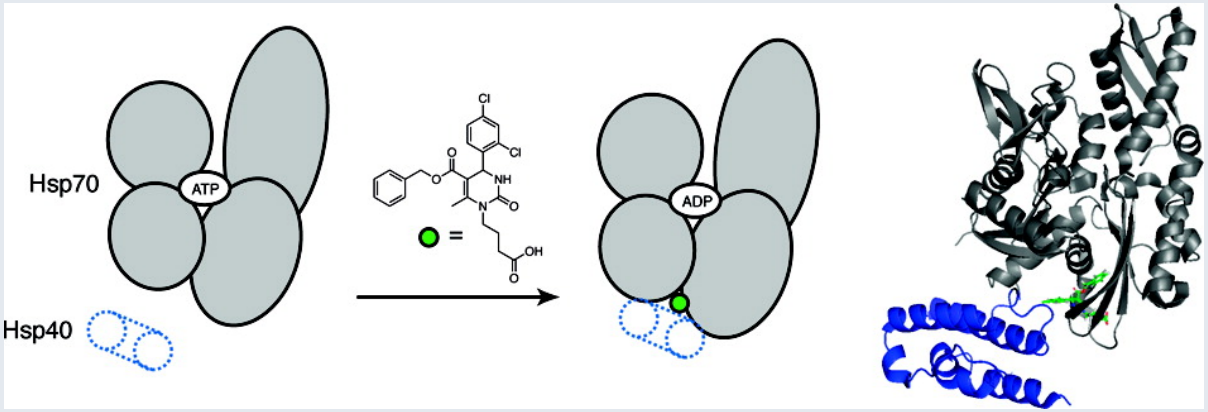
C.M. Wright, R.J. Chovatiya, N.E. Jameson *et al.*, *Bioorg. Med. Chem.*, **2008**, *16*, 3291-3301

MAL3-101 Derivatives of Interest

- Studies identified the interaction of model dihydropyrimidone 115-7c with the NBD of Hsp70
  - 115-7c is an agonist of Hsp70-Hsp40 activity





S. Wisén, E.B. Bertelsen, A.D. Thompson *et al.*, *Chem. Biol.*, **2010**, *5*, 611-622
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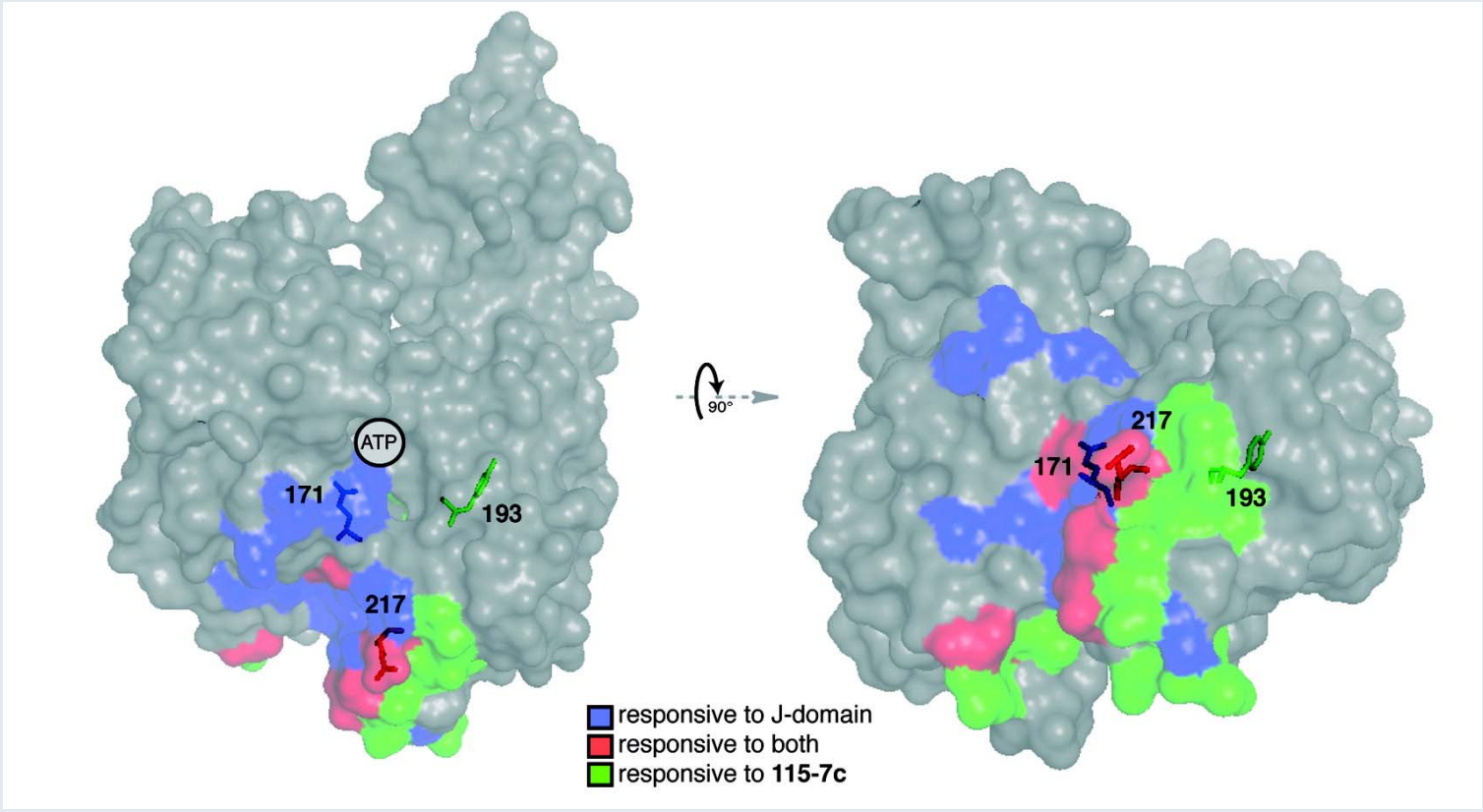
  - 

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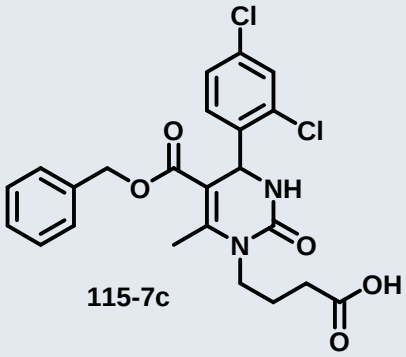
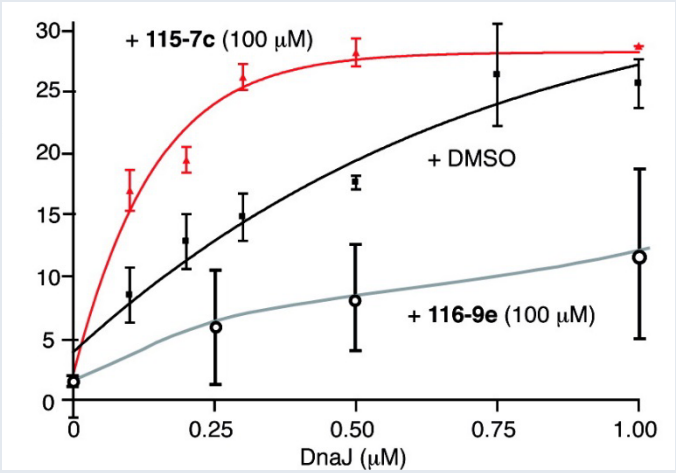
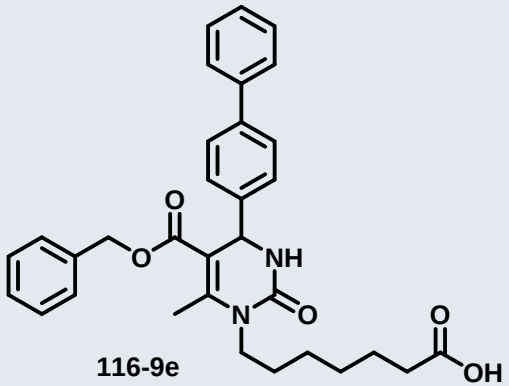


115-7c





116-9e

  - Compound 116-9e is an antagonist in the presence of Hsp40s
  - small changes can cause different Hsp70 modulation

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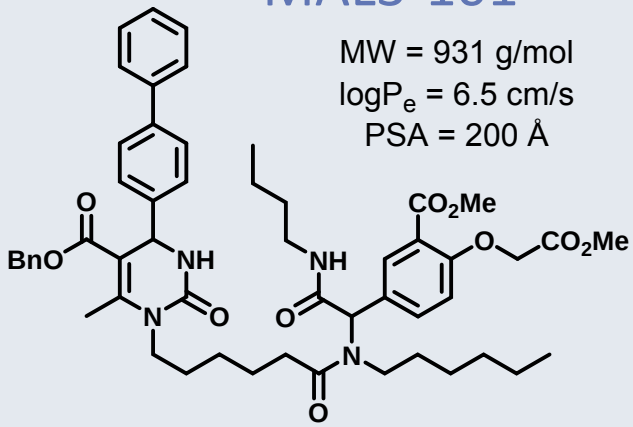
Improving MAL3-101

- Optimisation of a valuable lead:
  - Lipinski's Rule of 5:
    - Molecular weight (MW) less than 500 g/mol
    - No more than 10 Hydrogen Bond Acceptors
    - No more than 5 Hydrogen Bond Donors
    - A logP value ≤ 5
  - Veber Rules
    - Polar Surface Area ≤ 140 Å
    - Number of rotatable bonds ≤ 10

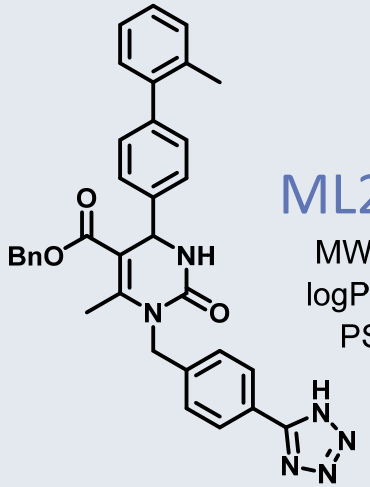
MAL3-101

MW = 931 g/mol
$\log P_e$ = 6.5 cm/s
PSA = 200 Å



ML282-86

MW = 571 g/mol
$\log P_e$ = 6.02 cm/s
PSA = 131 Å



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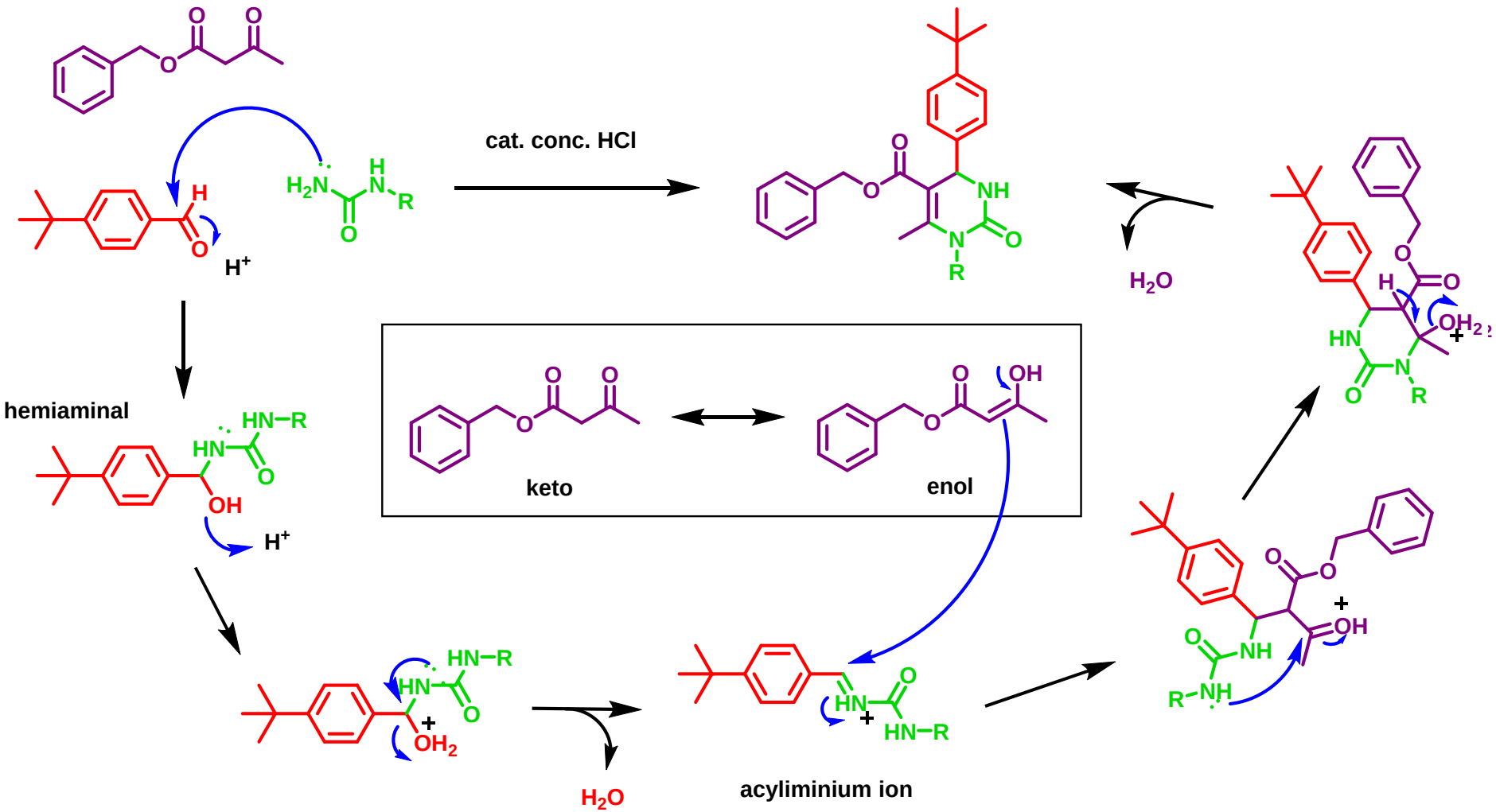
D.F. Veber, S.R. Johnson, H.-Y. Cheng, B.R. Smith, K.W. Ward, K.D. Kopple, *J. Med. Chem*, **2002**, *45*, 2615-2623

The Biginelli Reaction



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C. O. Kappe, *J. Org. Chem.*, **1997**, *62*, 7201-7204

Precursors Required for the Biginelli Reactions

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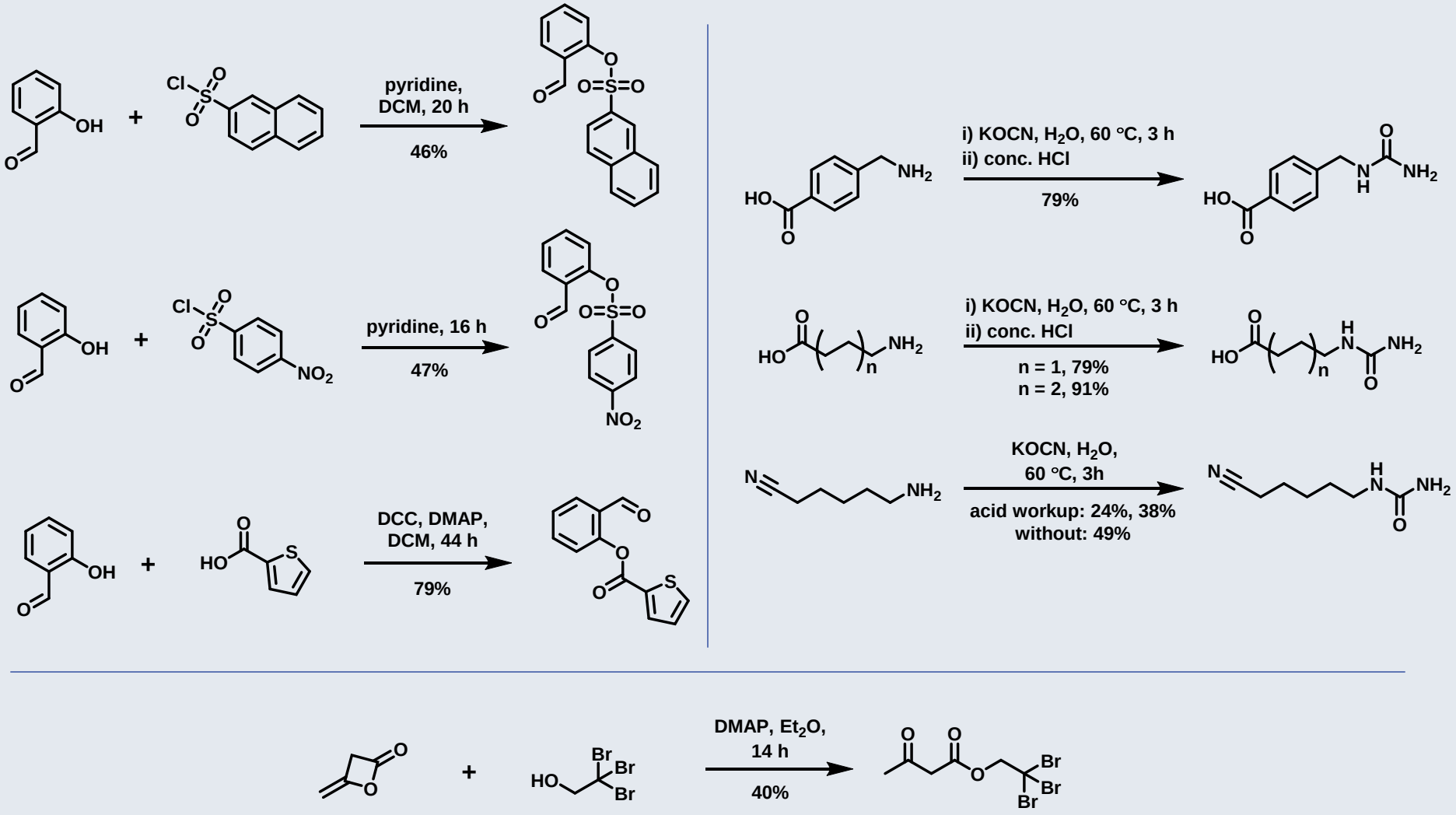
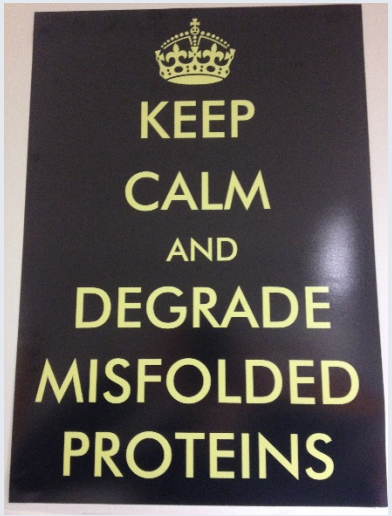


Acknowledgements

- Dr Peter Wipf
- Dr Jeff Brodsky
- Pete Chambers and Pitt NMR Facility
- Wipf and Brodsky Group Members (past and present)
- Funding: HHMI, AAAF



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