Novel tools for DUB inhibitor specificity profiling in cancer

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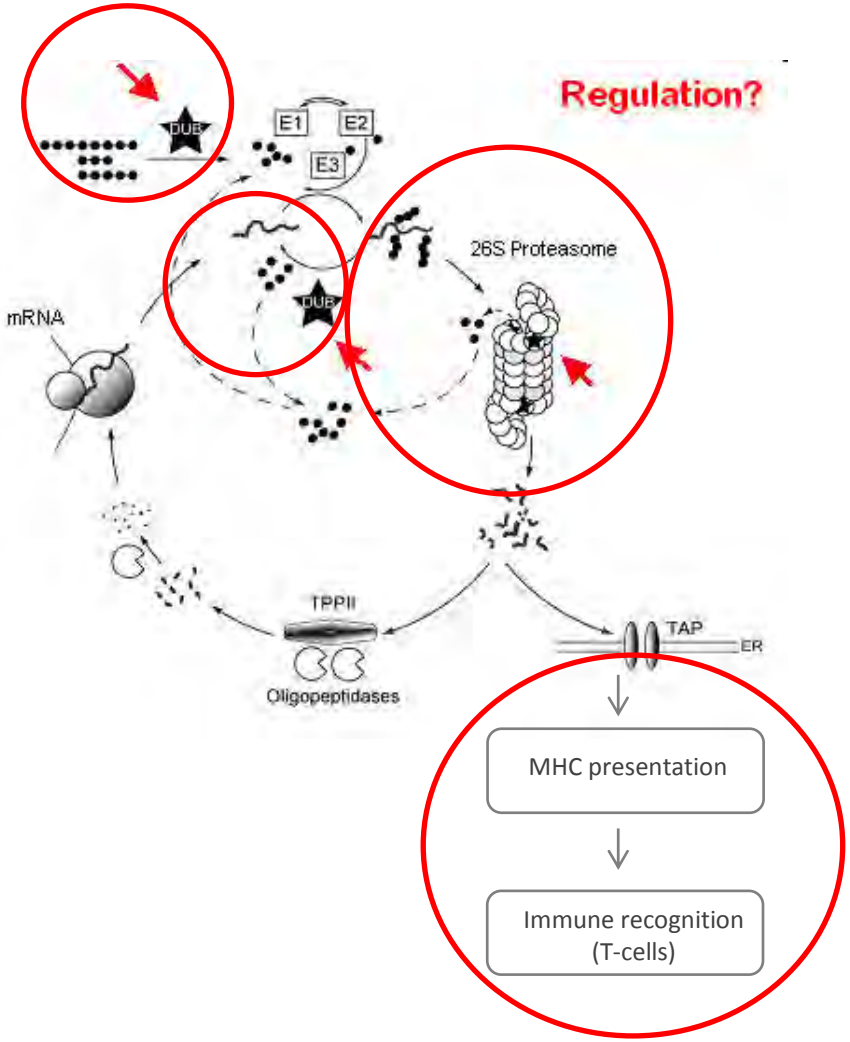
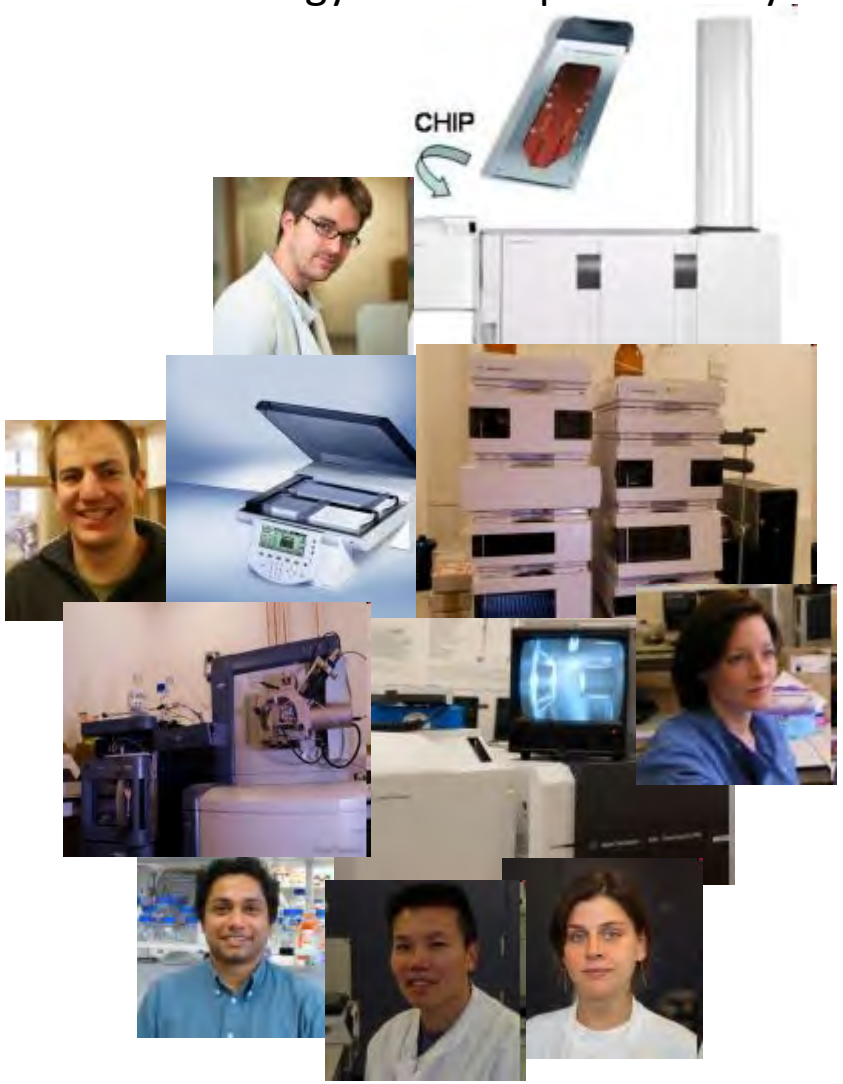
Group and Interests

Ubiquitin-Proteasome System

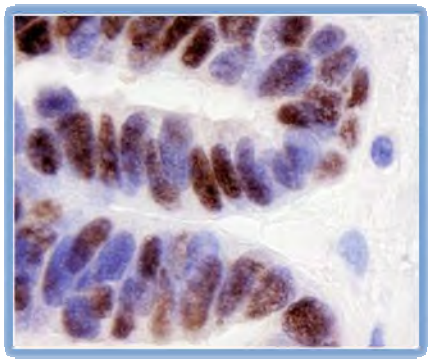


Chemical Biology & Mass Spectrometry

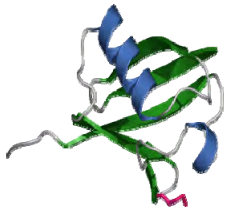


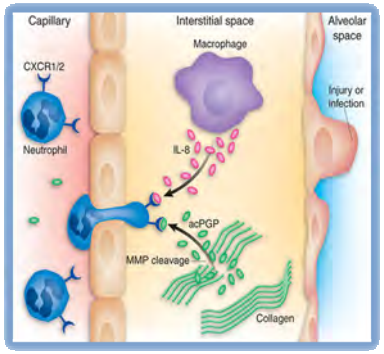
Ubiquitin System in Human Diseases

Neurodegenerative disease

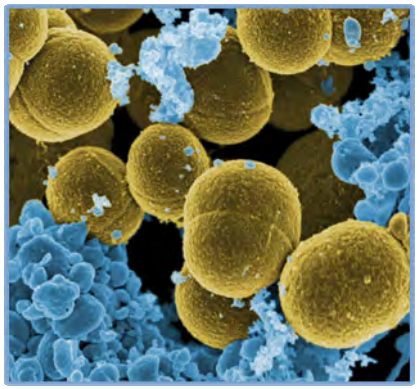


Cancer

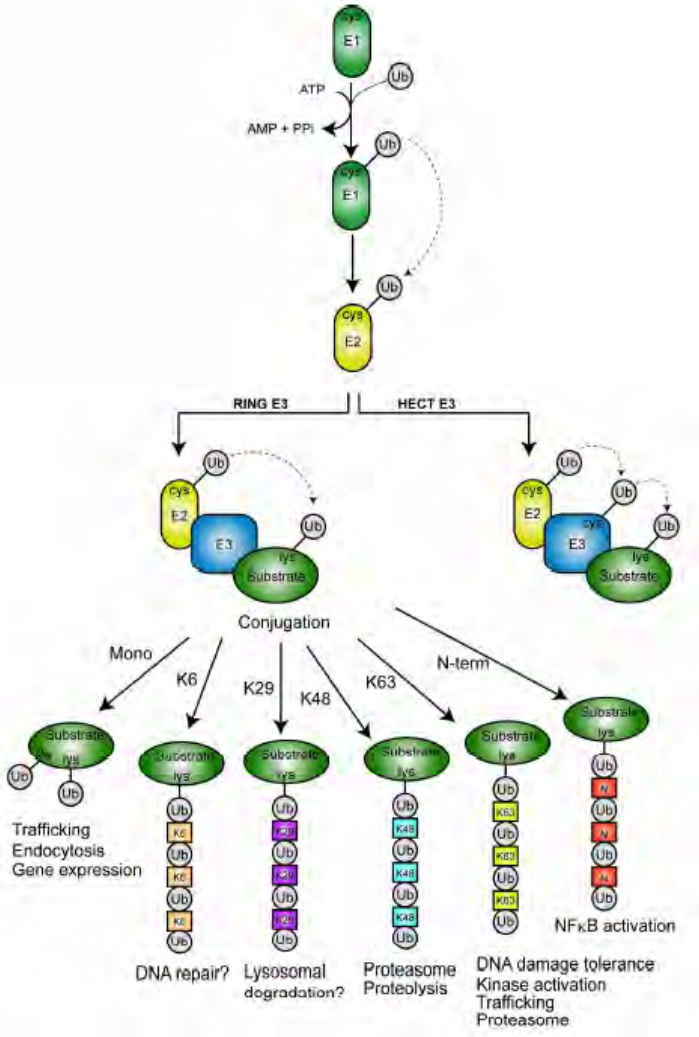




Inflammation



Bacterial and Viral Infections



Ub linkage type adds to biological complexity

DUBs in Disease (1)

Cancer
Neurodegeneration
Pathogens

Target discovery & Identification

"Learn" how to use them for disease intervention

DUB enzyme	Biology	Human Disease
UCH-L1	AKT – β-Catenin – NF-κB	Parkinson's disease (mutation/OE)
		B-cell malignancies, pancreas, colorectal, breast (OE)
USP6 (Tre2)		Oncogene
USP8	RTK, Wnt and HH pathways	Oncogene
USP14	26S editing	Ataxia (mouse)
CYLD	Wnt, NF-κB	Cylindromatosis (mutations)
VDU1, 2	bind VHL	Renal carcinoma
USP2	Mdm2/4, FASN, NF-κB, c-Myc	Prostate / breast cancer (OE)
USP7 / 7S*	Mdm2/4, PTEN, FOXO4 (p53)	Diverse cancers (OE)
USP15	TGF-β-R1, β-Catenin, SMADS	Glioblastoma, breast & ovarian (OE)
Cezanne 1	EGRF turnover	Breast cancer (amplification, OE)
OTUB1	UBC13/RNF 168, p53, RhoA	DNA damage, prostate cancer
USP1/UAF1	Chk1 & ID1-3 (CSC)	Melanoma, colon, lung, osteosarcome (OE, activation)
USP9X	Mcl-1, β-catenin, TGF-β	Colorectal, breast, lung, lymphoma (OE)
USP10	p53, AR, autophagy	Melanoma (OE)
USP13	MITF oncogene	10-20% of melanomas
USP22	p53, MYC	Aggressive cancers (OE)
USP4	TGF-β-R1, β-Catenin	
USP17 (Dub3)	Cdc25A turnover – GTPases	Breast, lung, colon hematopoietic cancers (OE)
USP33	Met signaling - apoptosis	Breast cancer

Khoronenkova Mol.Cell. 2012

DUBs in Disease (2)

DUBs in infection:	DUB-like enzyme	Pathogen
	UL36^{USP}	HSV
	CoV PLpro	SARS
	L protein	Hemorrhagic fever virus
	Avp	Adenovirus
	YopJ	*Yersinia*
	*Chla*Dub1, *Chla*Dub2	*Chlamydia*
	PFDub1	*Plasmodium Falciparum*

Problems, Challenges & Opportunities:

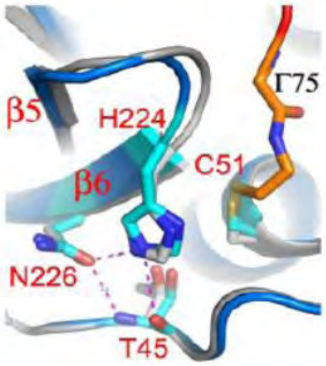
- How to discover & choose the right target(s) relevant for disease ?
- Best way to manipulate these targets for effective intervention ?

- Good knowledge about molecular target and pathway
- Substrate identity and function are unknown for most DUBs

- Understand how your target functions
- Inhibitor development and substrate ID *in vitro*
- Need to explore them in a cellular environment

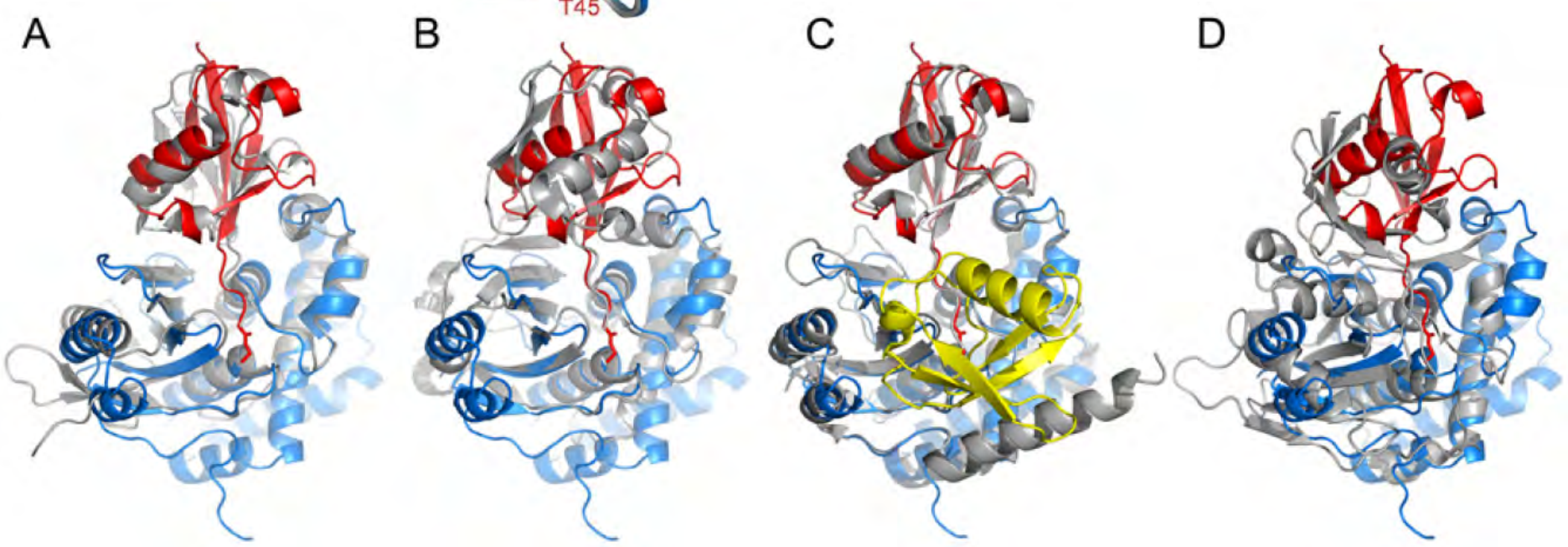
Know How Your DUB Works:
Structural Information for Inhibitor targeting & design

Altun M, 2013



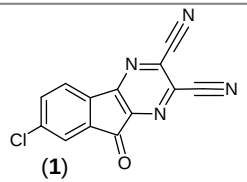
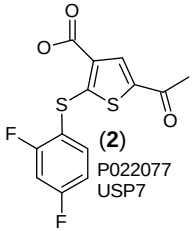
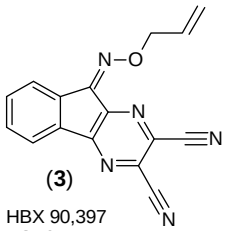
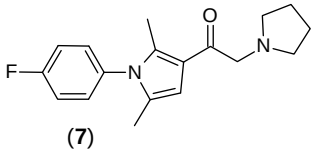
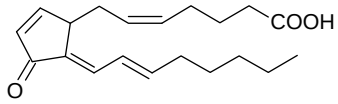
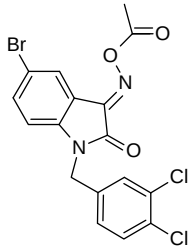
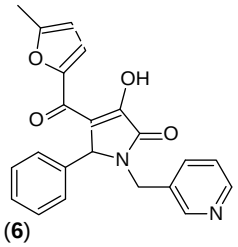
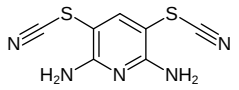
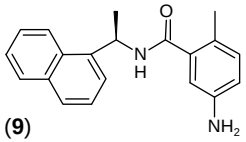
Otubain-2 catalytic centre

- Specific features of DUB cysteine proteases:
- Unusual triade
- Often in an "unproductive conformation" in apo form



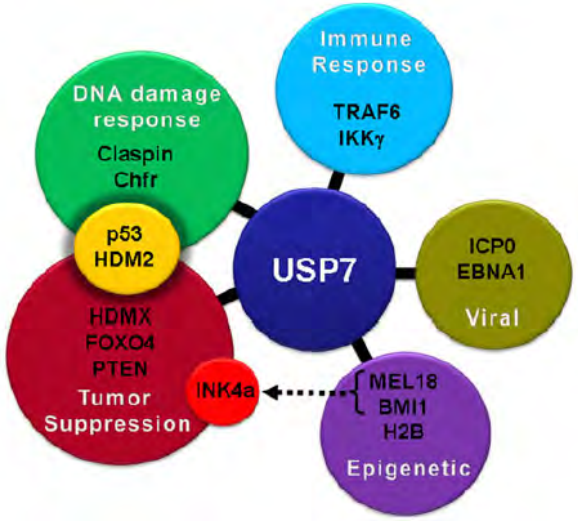
A	B	C	D
OTUB2-UbBr2	OTUB2-UbBr2	OTUB2-UbBr2	OTUB2-UbBr2
yOTU1-UbBr3	vOTU-Ub	OTUB1-Ubal-UBC13-Ub	DEN1-NEDD8

Small molecular DUB inhibitors in the UPS
Novel Therapeutics and Research Tools

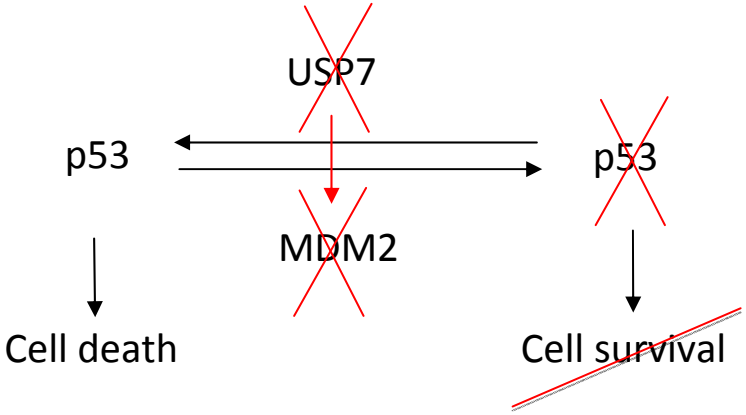
DUB	Inhibitor	Target/Disease association/ Therapeutic potential
USP Inhibitors	 **(1)** HBX 41,108 USP7	**USP7**: prostate cancer, non-small cell lung adenocarcinoma,
	 **(2)** P022077 USP7	**USP8**: Sensitivity to glioblastoma
	 **(3)** HBX 90,397 USP8	**USP14**: Neurodegeneration, ataxia
	 **(7)** UI1 USP14	
UCH Inhibitors	 **(4)** 15d-PGJ2 UCH-L3 UCH-L1	**UCH-L1**: Parkinson's disease
	 **(5)** Isatin O-acyl oximes UCH-L1	
	 **(6)** Isatin derivative UCH-L3	
Others	 **(8)** PR-619 Broad specificity DUB inhibitor	**Plpro**: SARS corona virus
	 **(9)** GRL0617 Plpro	

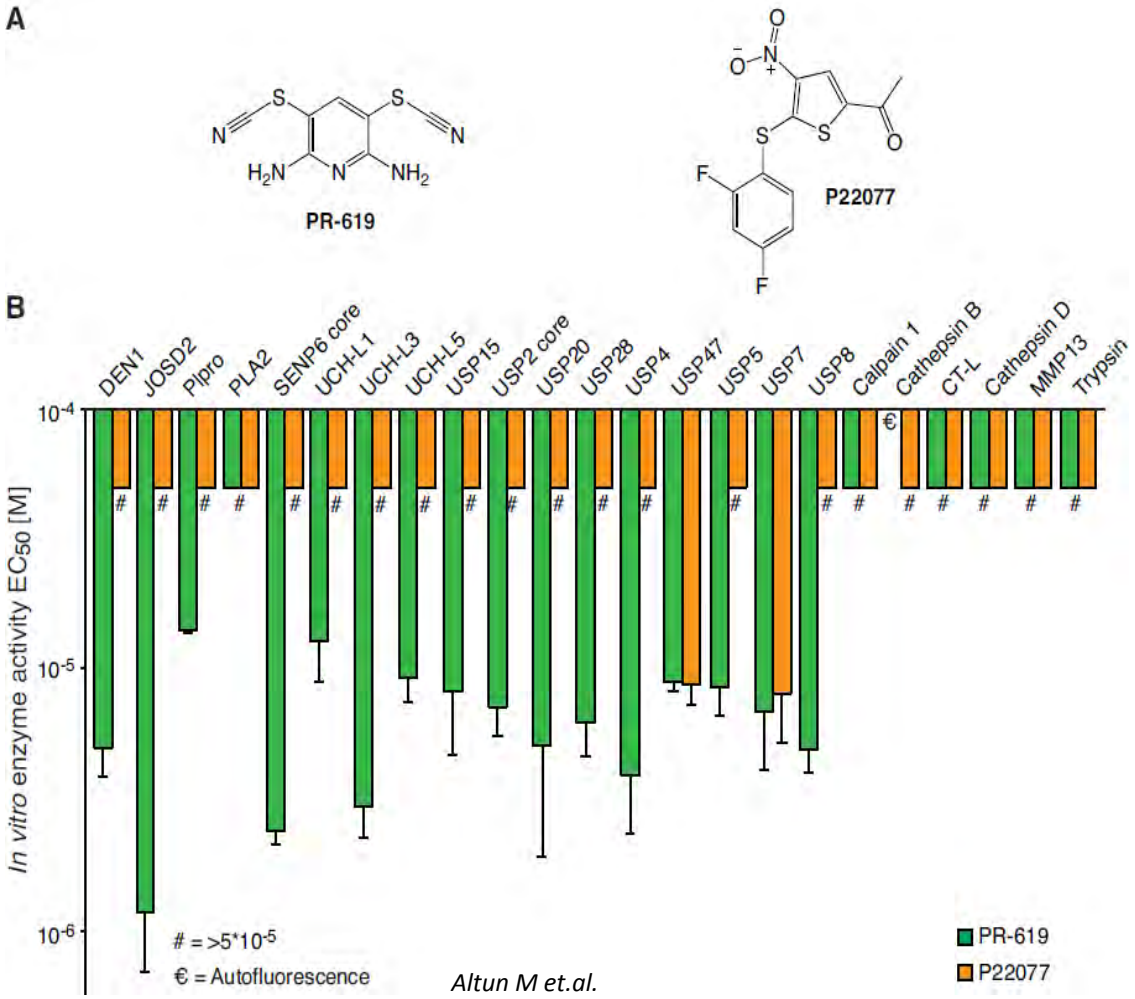
Edelmann MJ, Nicholson B, Kessler BM. Exp. Rev. Mol. Med. 2011

Structures of DUB inhibitors PR-619 and P22077 and in vitro DUB inhibition profiles



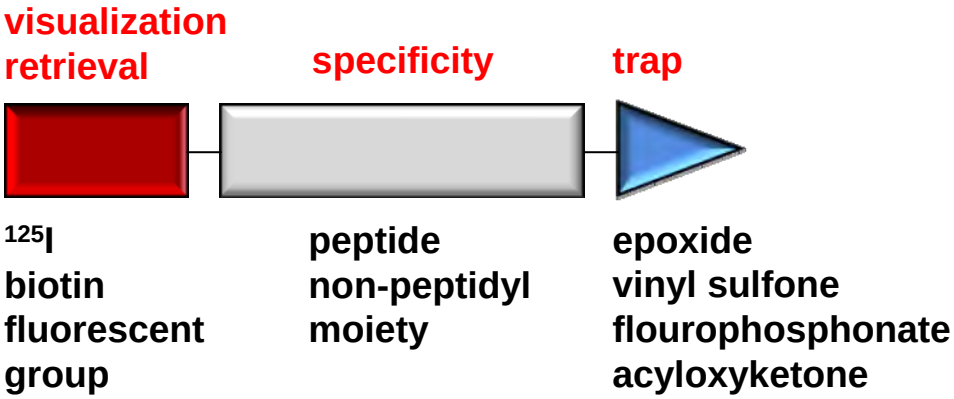
Nicholson B et.al.
Cell Biochem Biophys 2011

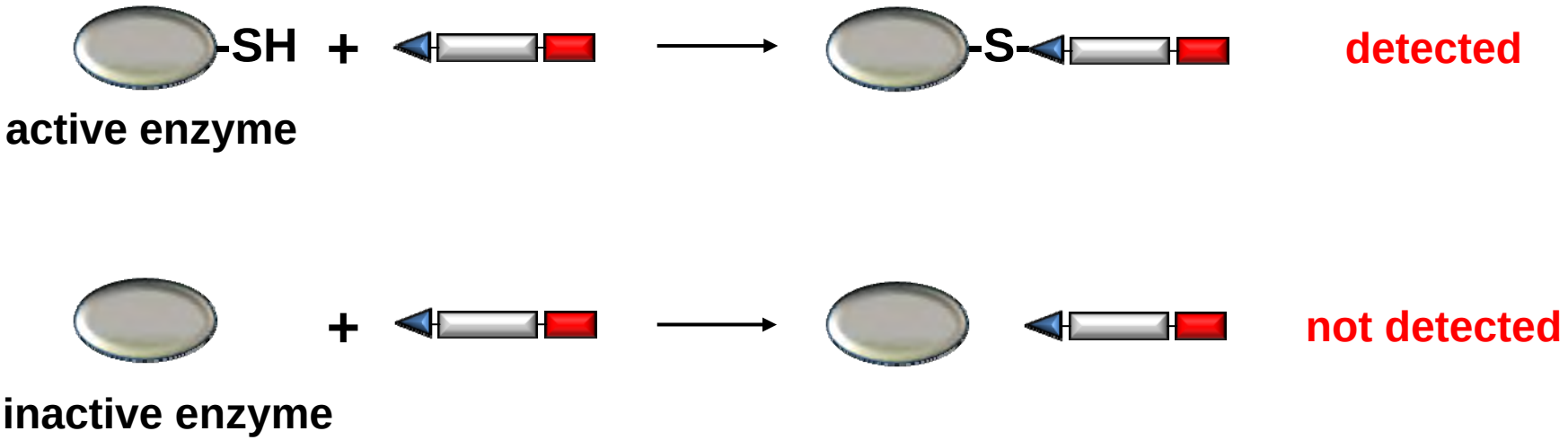




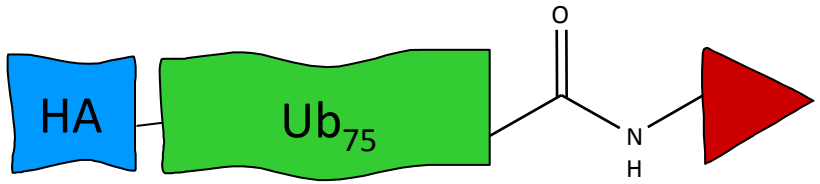
Altun M et.al.
Chem&Biol 2011

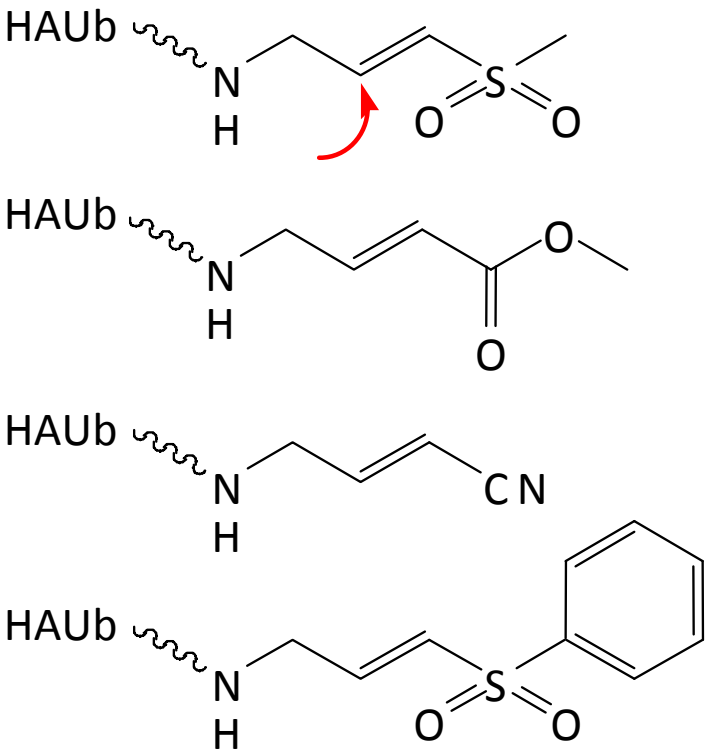
Chemoproteomics
Looking at DUBs in Cells: Activity-Based Probes



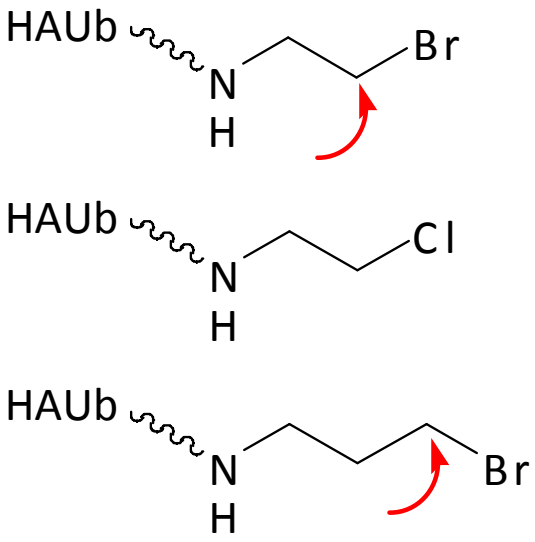


HAUb-derived probes





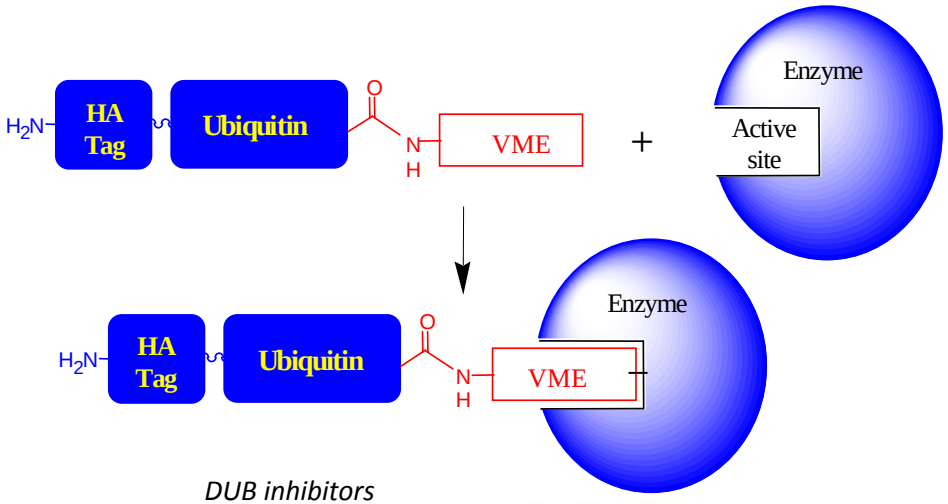
Michael acceptors



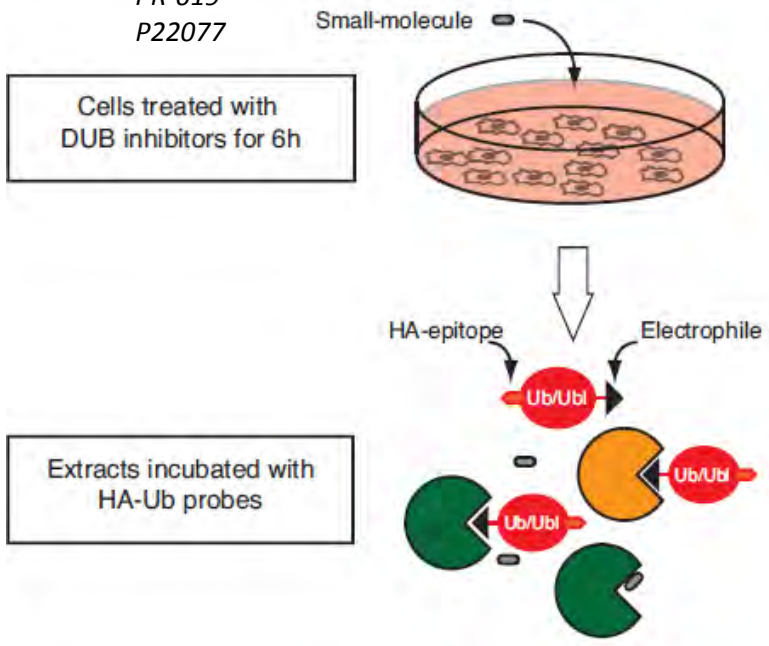
Alkyl halides

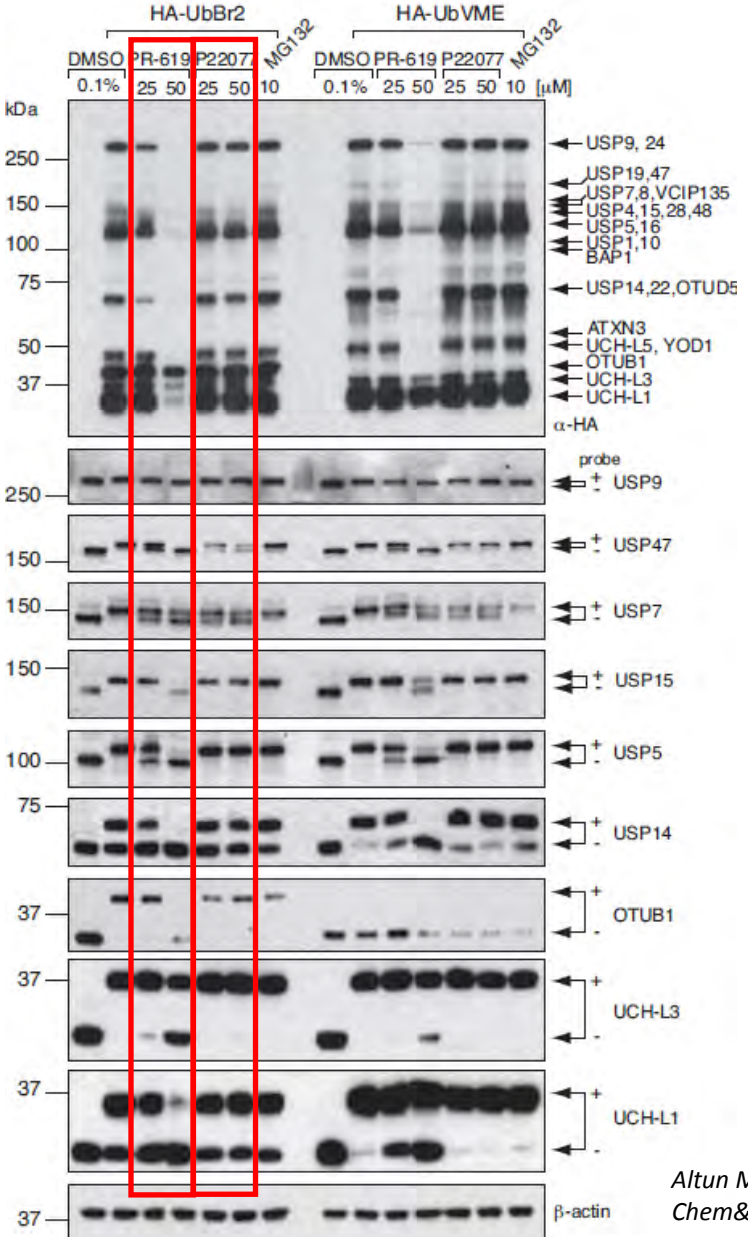
Borodovsky A, Ovaa H, Nagamallesawari K, Tudeviin G-E, Wilkinson KD, Ploegh HL and Kessler BM. Chemistry & Biology. 2002, 9 (10), 1149-1159

Biochemical Validation of DUB Inhibition in Cells



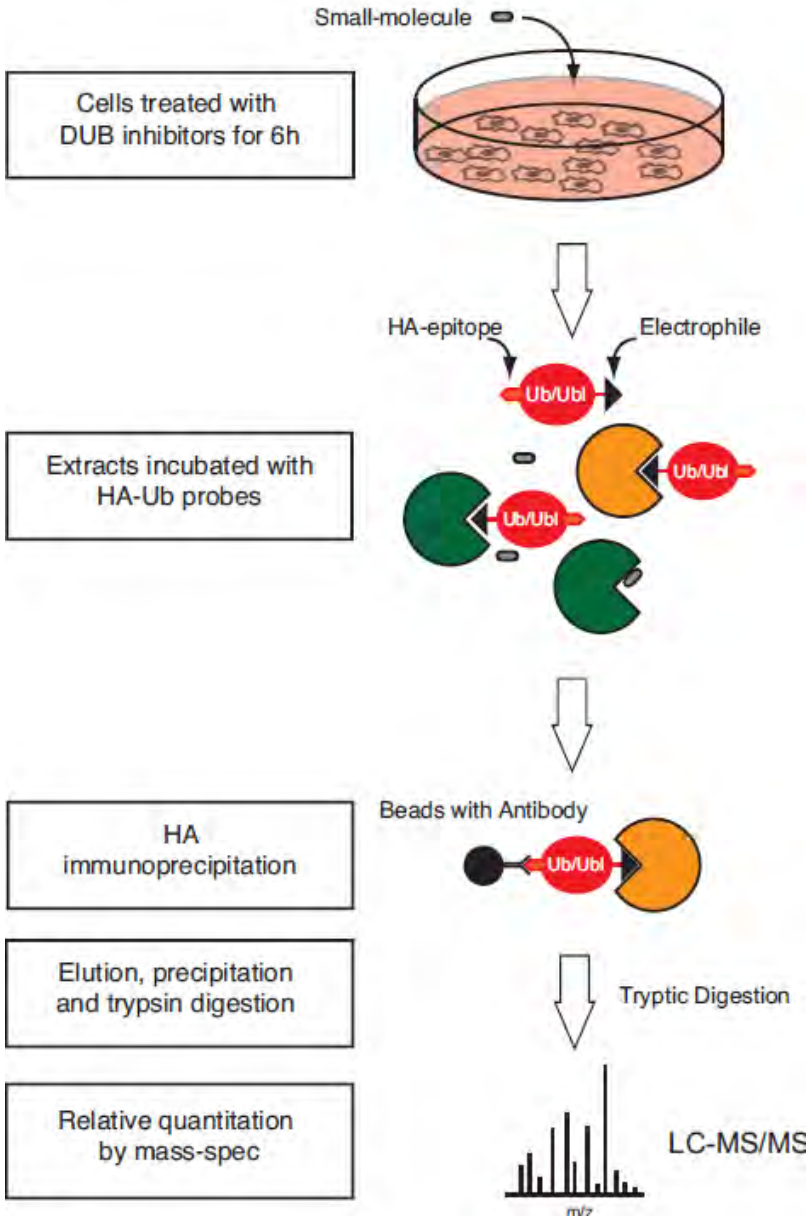
DUB inhibitors
PR-619
P22077





Altun M et.al.
Chem&Biol 2011

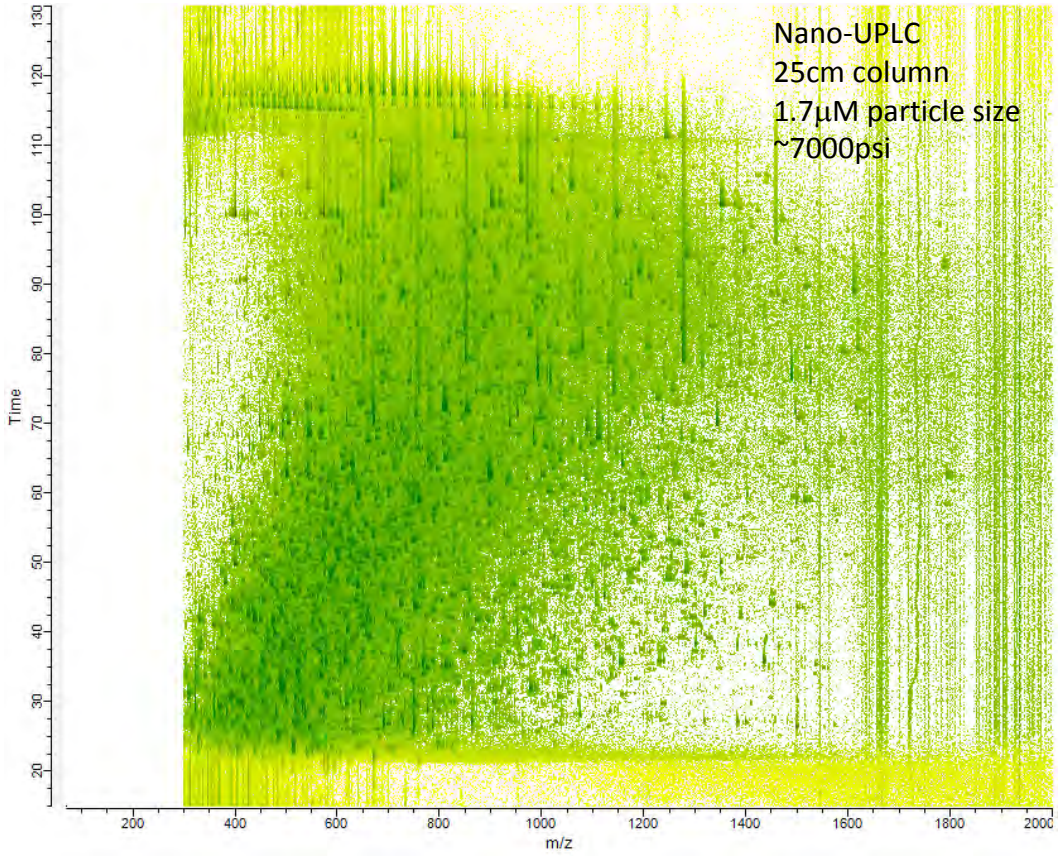
DUB Inhibitor Profiling in Cells using a Mass Spectrometry Approach

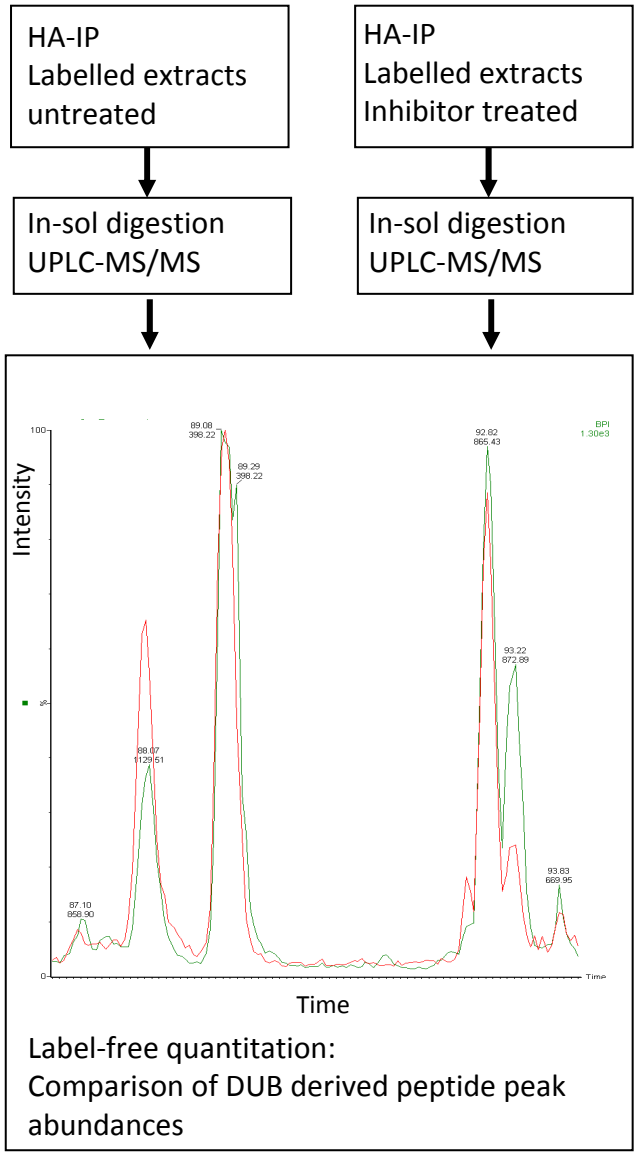


Altun et al., BBA 2012

Label-Free / SILAC Quantitation MS

1. Label-Free Quantitation - UPLC-MS^E
2. Label-Free Quantitation / SILAC – UPLC-Orbitrap Velos – LC-Progenesis / MaxQuant
1. Normalisation based on Ubiquitin derived peptides



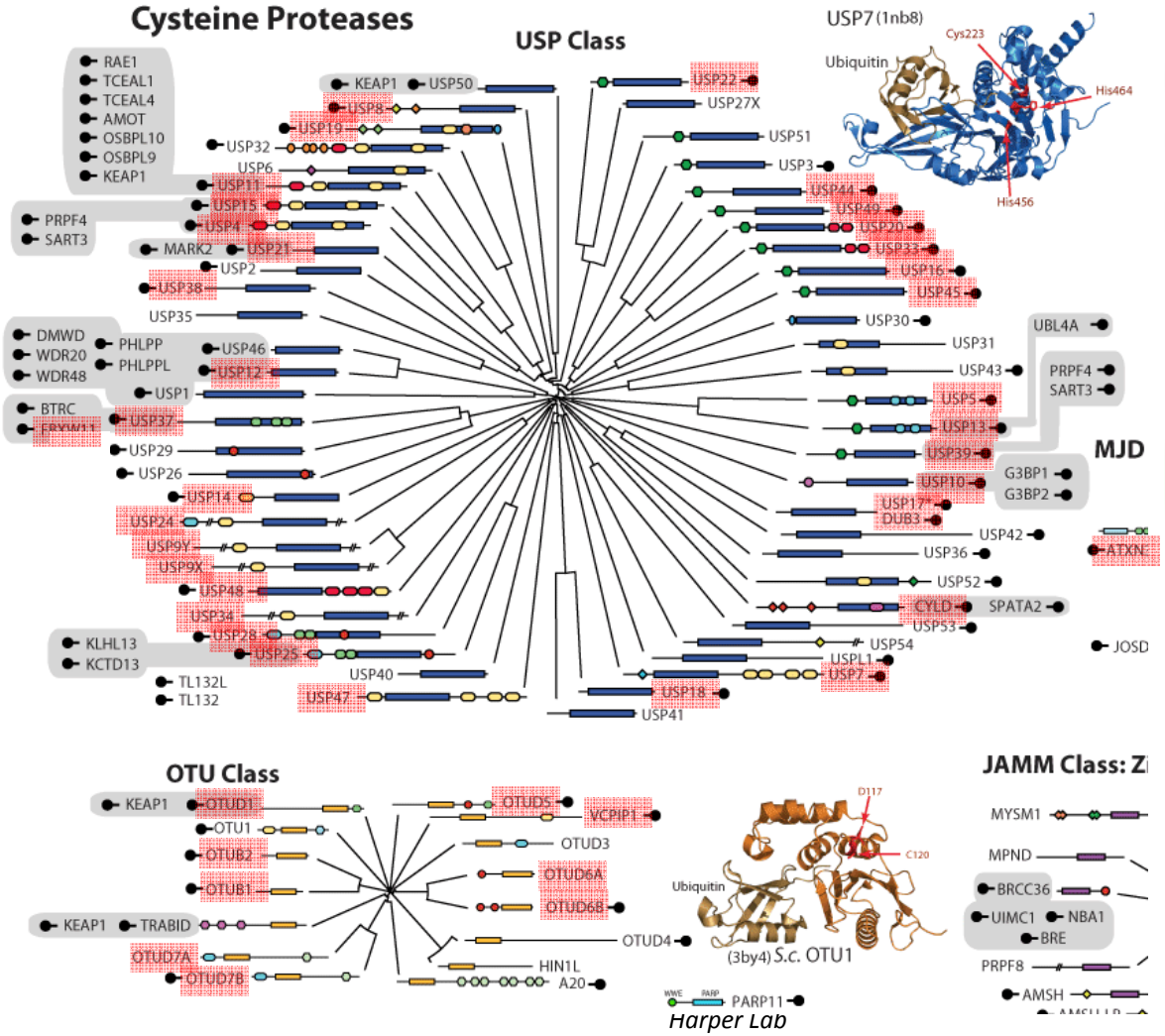


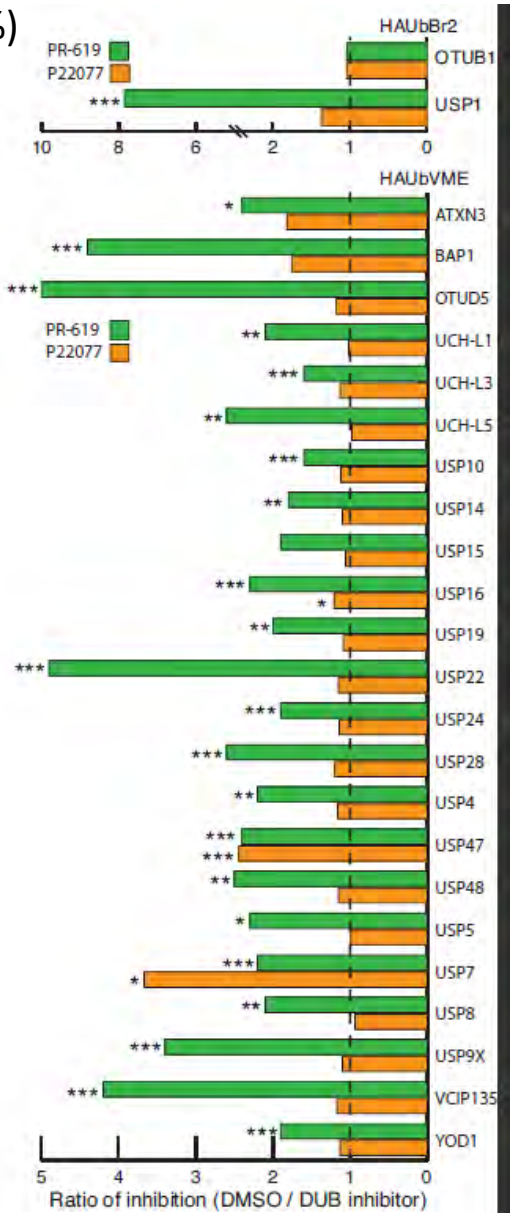
Label-free quantitation:
Comparison of DUB derived peptide peak abundances

Activity-Based Proteomics for DUB Inhibitor Profiling in Cells

49 DUBs covered (out of 71 known human Cysteine protease DUBs = 69%)

Expanding to NEDD8, SUMO, etc...





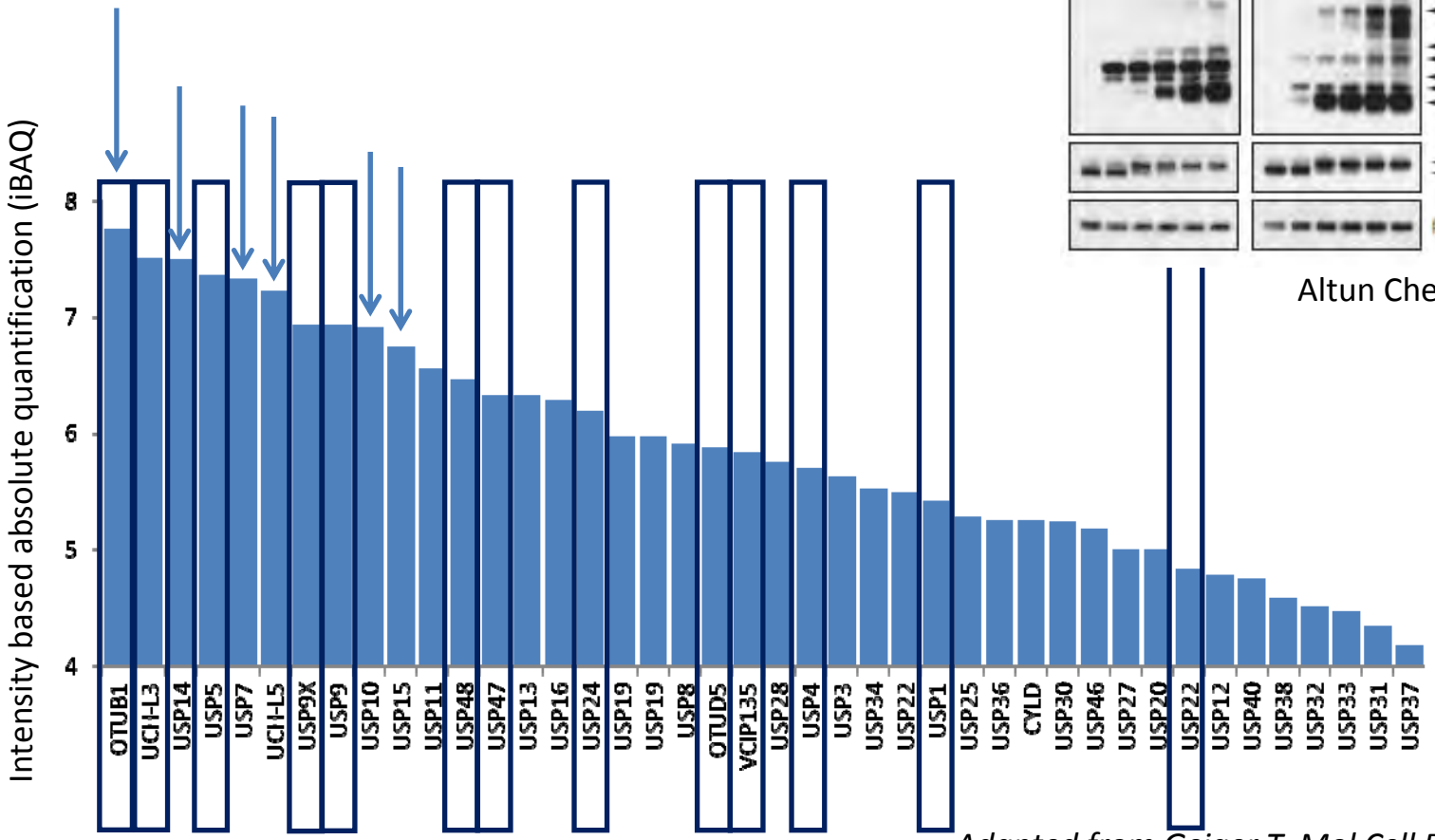
Altun M et.al. Chem&Biol 2011

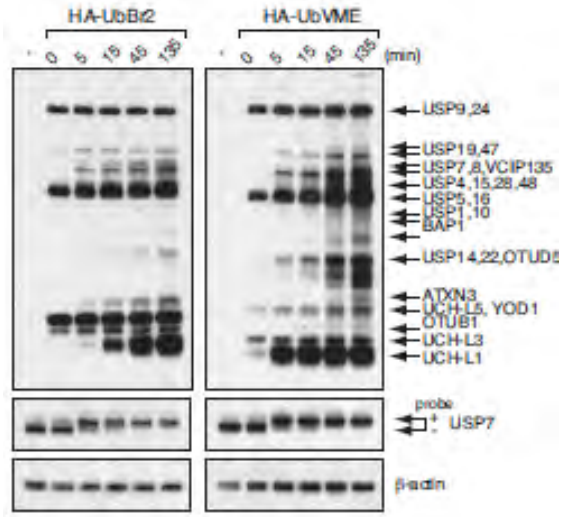
DUB Activity Versus Abundance

DUBs in HEKs

Correlation between
Abundance and labelling (activity)

+ -



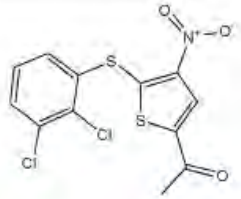


Altun Chem&Biol 2011

Adapted from Geiger T Mol Cell P roteomics2012
Kessler BM Curr. Opin Chem Biol 2013

P5091 is a USP7 Selective Inhibitor

A



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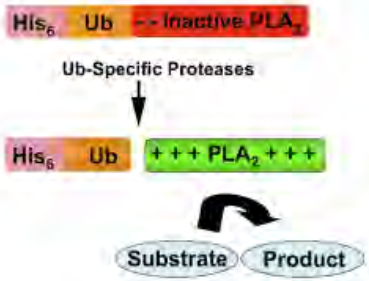
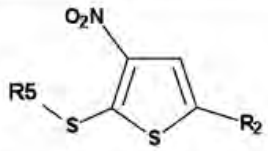


C

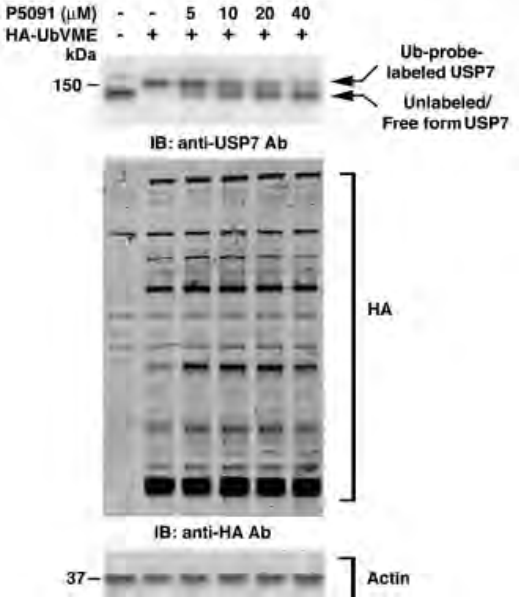


Compound #	R2	R5	USP7 inhibition EC_{50} (μM)
1	Acetyl	phenyl	>50
2	Acetyl	2-chlorophenyl	12.9 ± 4.3
3	Acetyl	3-chlorophenyl	7.0 ± 2.1
4	Acetyl	4-chlorophenyl	11.8 ± 3.0
5	Acetyl	2,3-dichlorophenyl	4.2 ± 0.9
6	Acetyl	3,5-dichlorophenyl	6.4 ± 1.4
7	Acetyl	2,4-dichlorophenyl	6.5 ± 2.6
8	Acetyl	2,4-difluorophenyl	8.0 ± 2.8
9	1-hydroxyethyl	2,4-dichlorophenyl	20.1 ± 4.3
10	Ethyl	2,3-dichlorophenyl	>50
11	n-butyl	2,4-dichlorophenyl	13.9 ± 4.2

D

Enzyme	EC_{50} (μM)	
	P5091	P22074
USP2c	>100	>100
USP5	>100	>100
USP7	4.2±0.9	>100
USP8c	>100	>100
USP15	>100	>100
USP20	>100	>100
USP21	>100	>100
USP28	>100	>100
UCH-L1	>100	>100
Caspase 3	>100	>100
Cathepsin K	>100	>100
Calpain 1	>100	>100
MMP13	>100	>100
Cathepsin D	>100	>100
EK_L	>100	>100
PLA_2	>100	>100

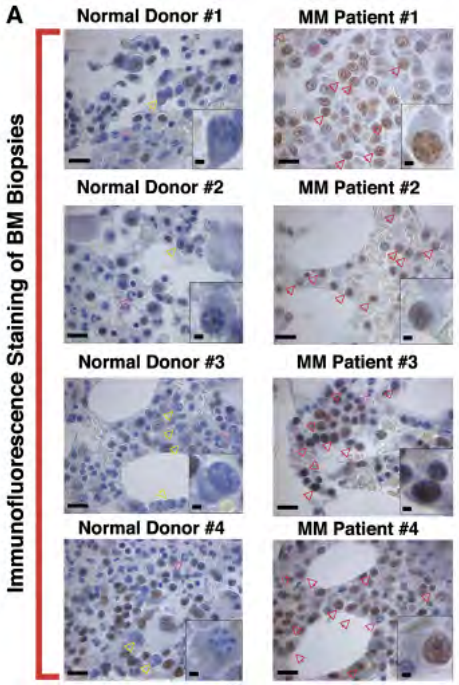
E

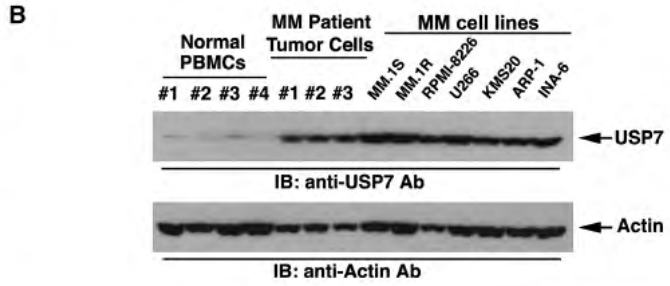


Chauhan D. et.al.
Mol Cell 2012

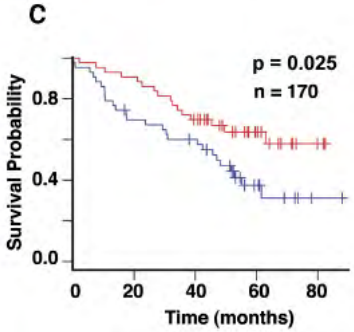
USP7 : A Therapeutic Target in Multiple Myeloma (MM)

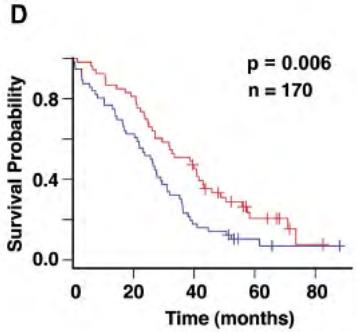
USP7 Expression And Prognostic Relevance in MM Cells

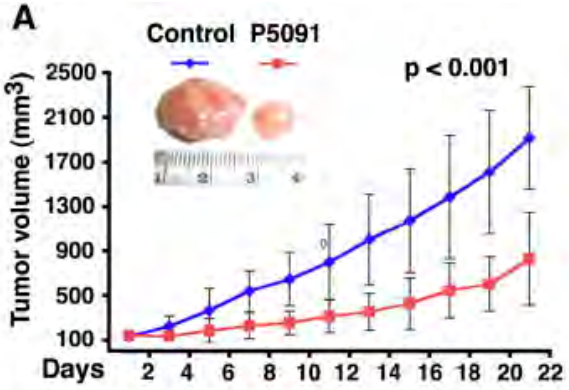


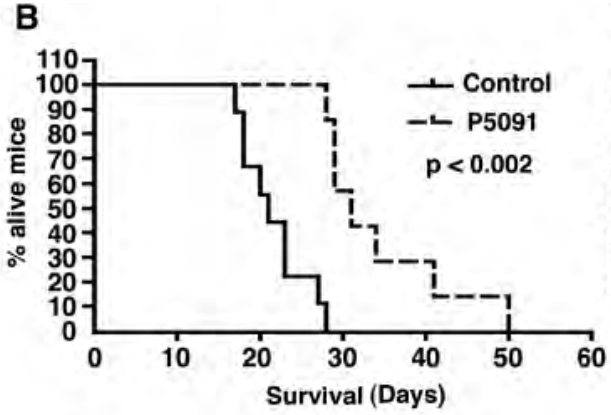


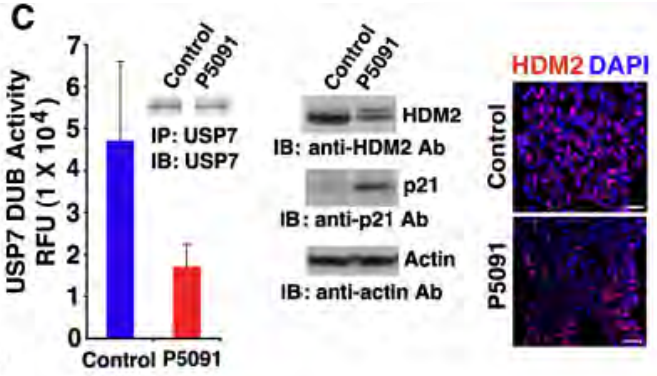
Chauhan D. et.al. Mol Cell 2012







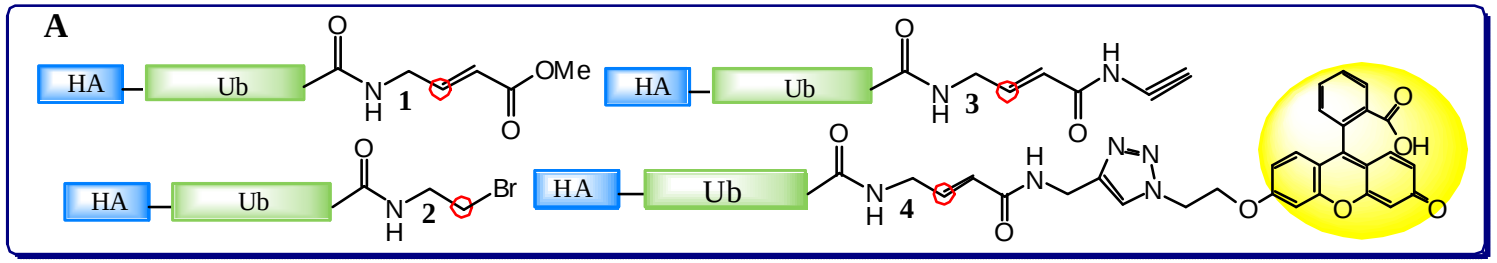




P5091 Overcomes Bortezomib-Resistance

Combination of P5091 and Lenalidomide, SAHA, or Dex Trigger Synergistic Anti-MM Activity

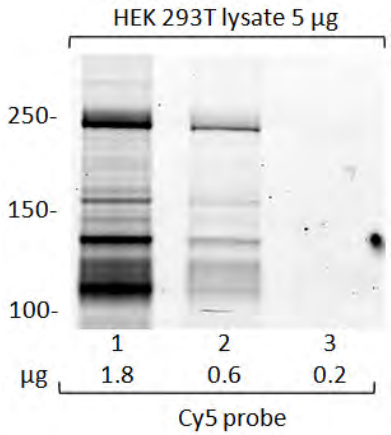
Development of Fluorescent Ub probes

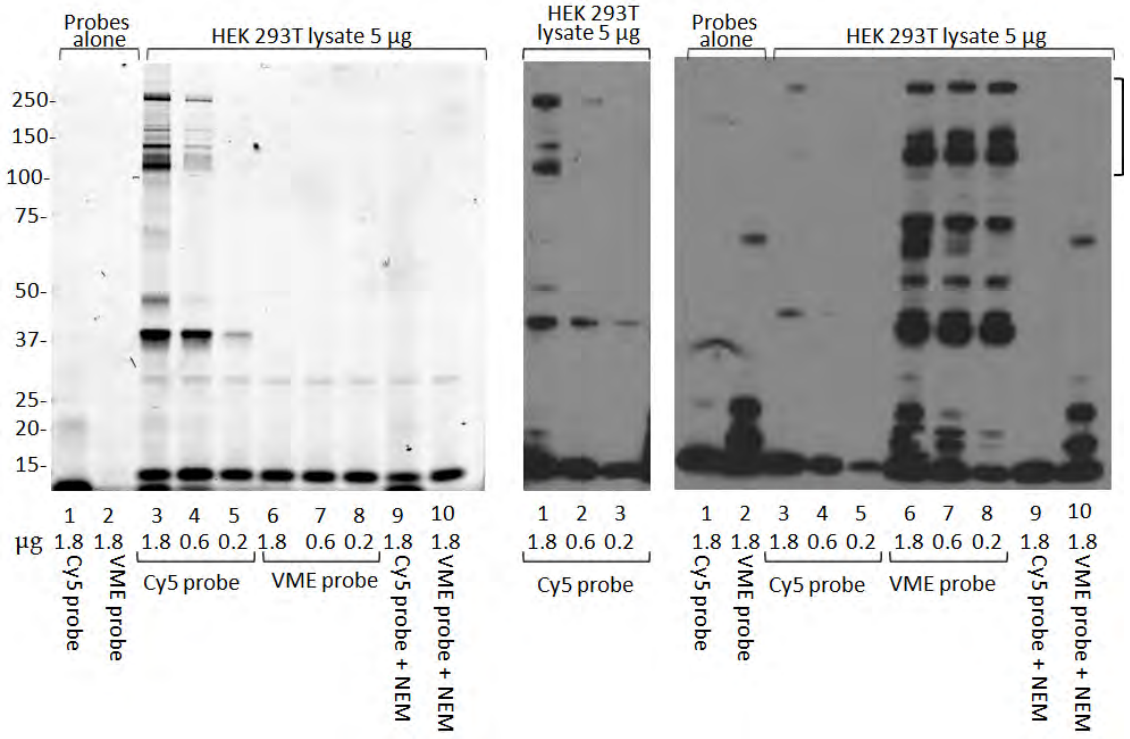


Patent **P38458GB**

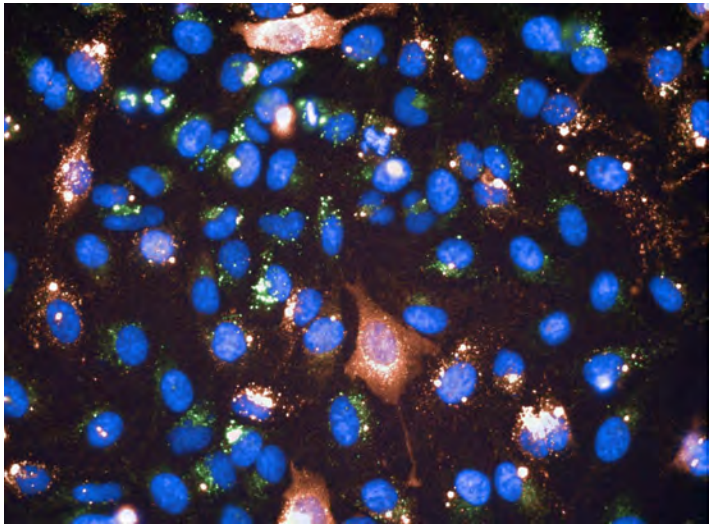
"Click-Chemistry"

N_3-CH_2-CH_2-CH_2-Cy3





Active DUB profiles in cells



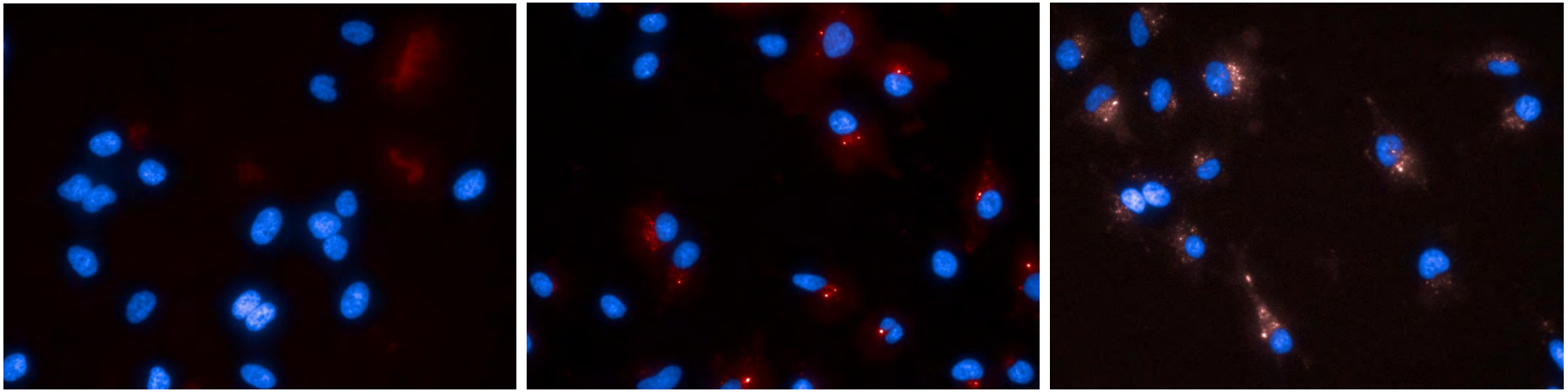
Blue: DAPI; green: lyso-tracker; red/yellow: DUBs

McGouran et al., OBC 2012

Active DUB Profiles in Cells

"Chariot" protein transfection reagent
HEK293T cells

CTRL (dye alone)	HA-Ub-VA-Cy3	HA-Ub-VA-Cy5

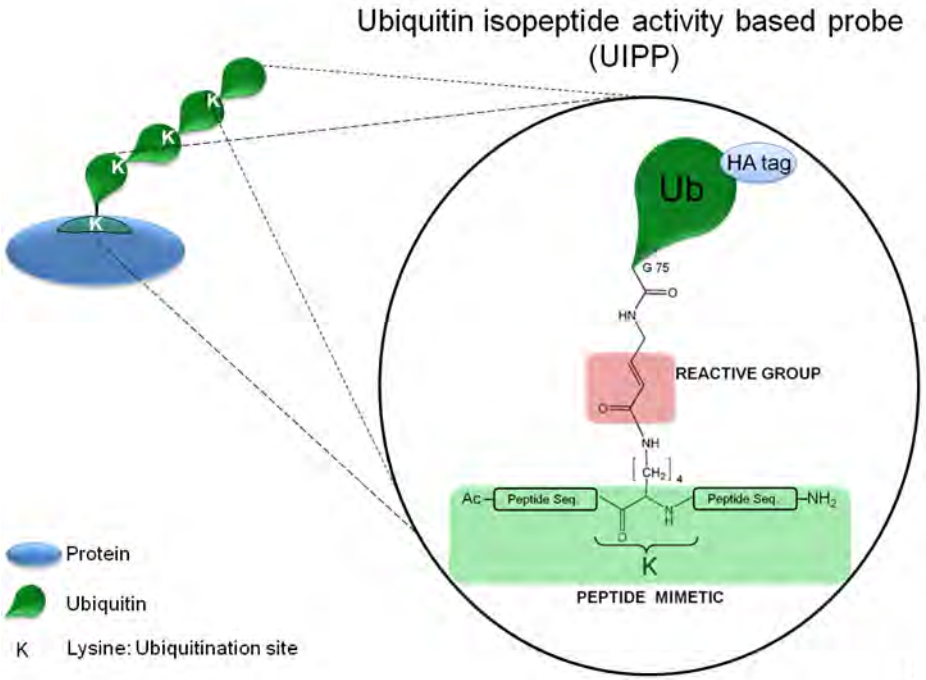


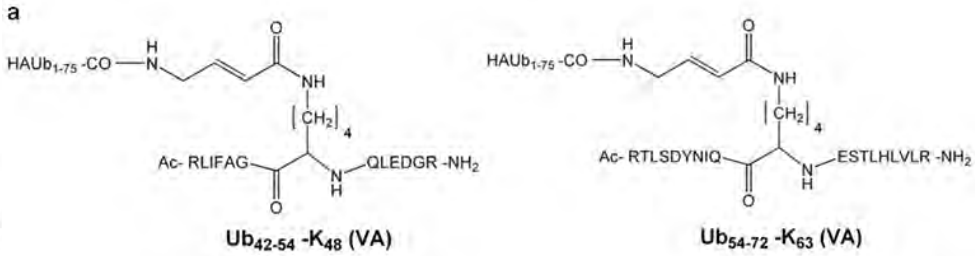
Patent **P38458GB**

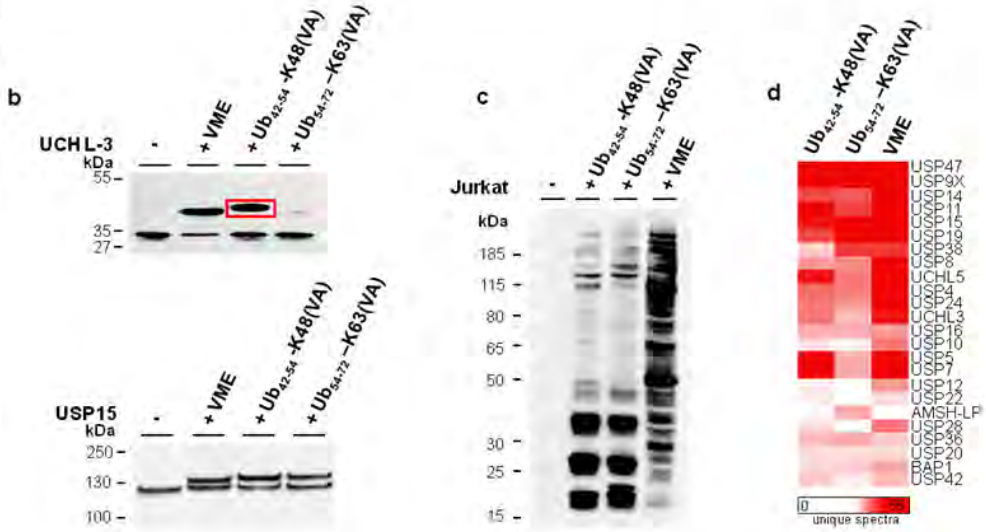
⇒*Information about localized DUB activity & inhibition in cells*

McGouran, 2012

"Branched"Ub Probes to Profile DUB Linkage Specificity





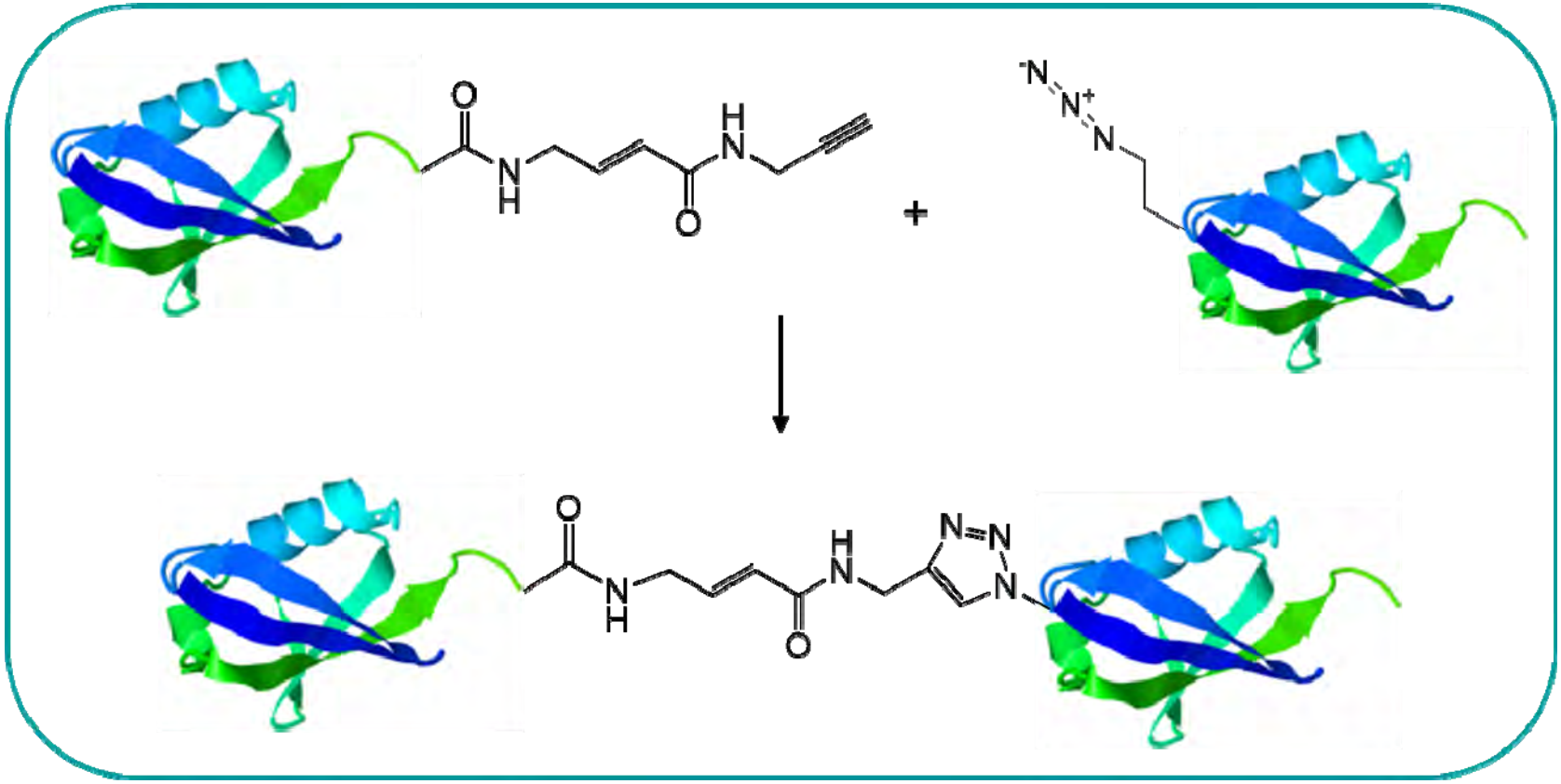


Differential profiling by IB Mass Spec

Iphöfer, ChemBiochem 2012

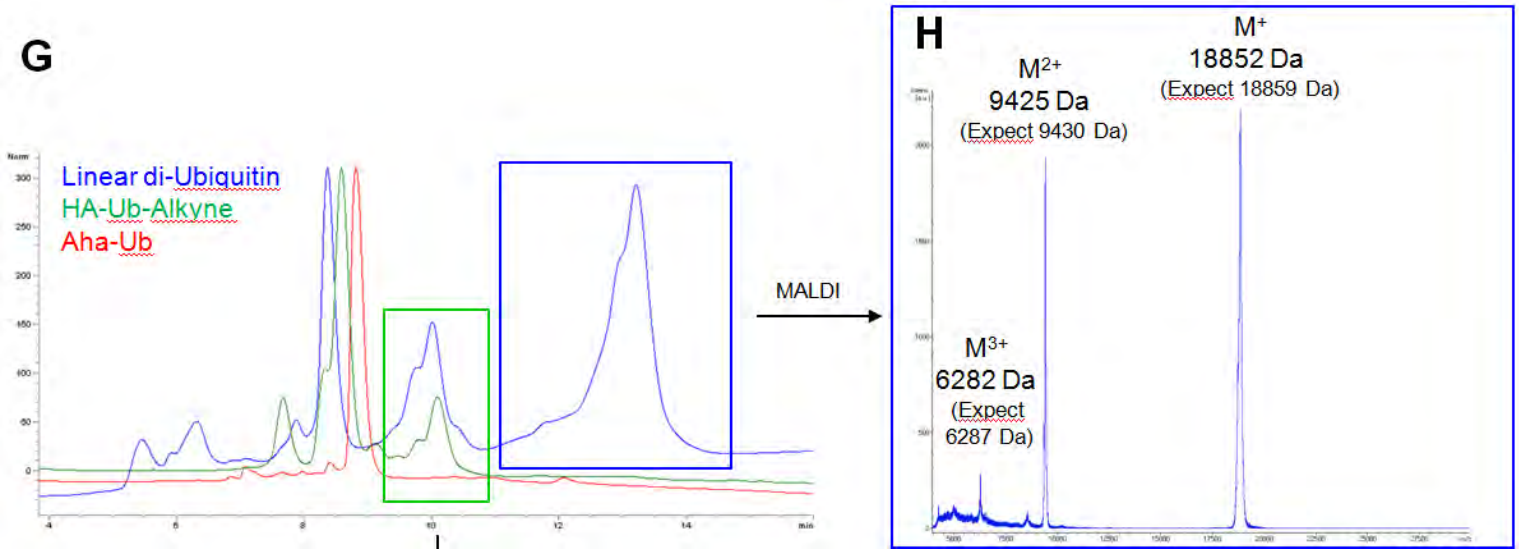
⇒*Information about DUB inhibition & Ub linkage specificity in a cellular environment*

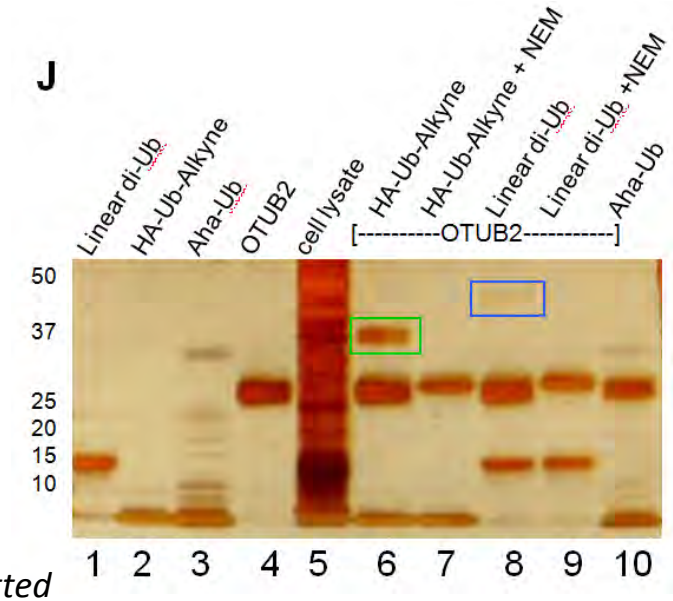
Novel di-Ubiquitin Probes Coupled by „Click“Chemistry



Joanna McGouran et al., 2013. Submitted

Purification and Characterisation of di-Ubiquitin active site probes





Joanna McGouran et al., 2013. Submitted

Conclusions & Perspectives

Challenges for future UPS / DUB drug development

- Obtaining specificity in targeting DUBs – demonstrated with selective USP7 inhibitors
- Chemoproteomics for DUB (inhibition) mechanism of action in cells – DUBs as drug targets
- USP7 inhibition has anti-tumour activity in vivo – synergistic with other drugs
- Novel di-Ub probes begin to address DUB Ub-linkage specificity in cells

- Opportunities for
  - Defining DUB subsets for different Ub-linkages
  - DUB Ub-linkage inhibitor screening
  - Deconvoluting DUB function:
    DUB – substrate probes to capture DUB(s) for a given substrate in cells

Thank You !

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