

Multiple sequence alignment of USP Zf-UBD proteins

Using Molsoft ICM-Pro the sequences of USP Zf-UBDs and the structure of HDAC6 Zf-UBD ([5KH3](#), [5B8D](#)) were added to a new project file. The sequence of HDAC6 Zf-UBD was extracted. Sequences were selected in the workspace and a multiple sequence alignment was performed. The HDAC6 Zf-UBD sequence was linked to the structure and the residues lining the receptor pockets were manually selected using the 'select neighbor' tool around each ligand. The free software (ICM browser) to view the structural data can be downloaded from [Molsoft](#).

Table 1. USP Zf-UBD sequences

	Zf-UBD sequence
USP3	CSVCRSNKSPWVCLTCSSVHCGRYVNGHAKKHYEDAQVPLTNHKKSEKQDKVQHTVC MDCSSYSTCYRCDDFVNDT
USP5	CSKCDMRENLWLNLTGSLCGRRYFDGSGGNNHAVEHYRETGYPLAVKLGITITPDGAD VYSYDEDDMVLDPS
USP13	CARCDLRENLWLNLTGSLVLCGKWFFDSSGGNGHALEHYRDMGYPLAVKLGITITPDGA DVYSFQEEEPVLDPH
USP16	CQDCKTDNKVKDKAEETEETEEKPSVWLCLKCGHQGGRNSQEQHALKHYLTPRSEPHCLV LSLDNWSVWCYVCDNEVQY
USP20	CQSCGVTGPNLWACLQVACPVVGCGESFADHSTIHAQAKKHNLTVNLTTFRLWCYACE KEVF
USP22	CHVCGVHLNRLHSLCYCVFFGCFKKHIEHAKAKRHNLAIDLMYGGIYCFQCQDIYDKD
USP33	CQDCKVQGPNLWACLENRCSYVVGCGESQVDHSTIHSQETKHYLTVNLTLRVWCYACSK EVF
USP39	SHINAYACLVCGRKYFQGRGLKSHAYIHSVQFSHHVFLNLHTLKFYCLPDNYEIIDSS
USP44	CVDNNTTESIWACLSCSHVACGRYIEEHALKHFQESSHPVALEVNEMYVFCYLCDDYVLN DN
USP45	CSECLKERRFYDQQLVLTSDIWLCLKCGFQCGKNSQHSCLKHFKSSRTEPHCIINLSTW IIWCYECDEKLSTHC
USP49	CLECATTESVWACLKCSHVACGRYIEDHALKHFEETGHPLAMEVRDLYVFCYLCKDYVLN DN
USP51	CHVCSTHMNRLHSLSCVFFGCFTEKHHKHAETKQHHLAVDLYHGVIYCFMCKDYVYD KD

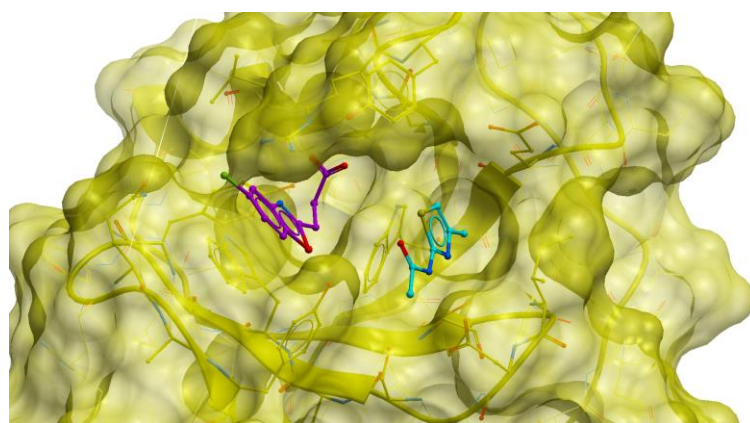


Figure 1. Structures of different ligands binding HDAC6 Zf-UBD at ubiquitin binding pocket (magenta: [5KH3](#)) and adjacent secondary pocket (cyan: [5B8D](#))

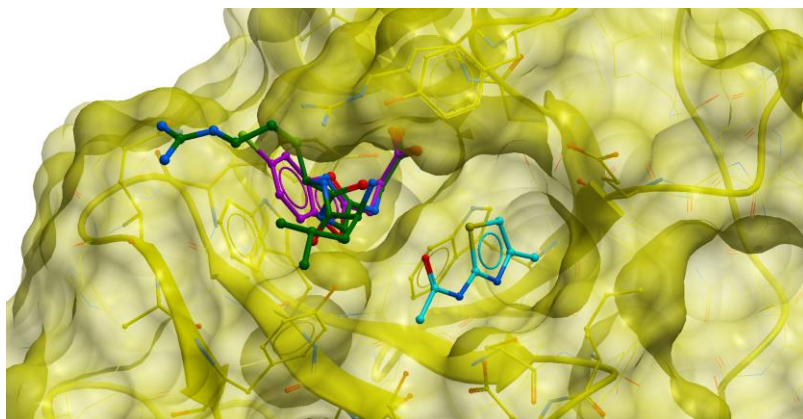


Figure 2. Structure of ubiquitin peptide RLRGG (green: [3GV4](#)) binding in primary binding pocket similarly to [5KH3](#)

		secondary binding pocket residues	primary binding pocket residues	
6% [14,139]		C..C.....	...#.CL.CG.....H.....###.....	..#...##C#.....#....
HDAC6	1109	PLPWCPLVAVCPIPAAGLDVTQPCGDCGTIQE	IVCLSL-CYQVYCGRI-NGHMIDHHGNSGHPLV	LSYIDLSPACIYCCAVVHHQALLDVKNIAHQNKFG
USP49	1	CLECATTES	IVACLK-CSHVACGRVI-EDHALKHFEETGHPLA	MEVVDLYVCEILCKDVLNDN
USP44	1	CVDCNTTES	IVACLK-CSHVACGRVI-EEHALKHFEESHPLA	LEVNMIVYVCEILCKDVLNDN
USP3	1	CSVCRSNKS	IVCLTL-CSSVHCGRVI-NGHAKKHVEDAQVPLTNHKKSEKQDKVQHTVCMDCSSYSTVYCRDIPVINDI	
USP13	1	CARCDLREN	IVNLTL-DGSVLCGRVFFDSSGGNGHAEHYRDMGYPLAVKLGTITPDGAD	VSPQEEEPVLDPH
USP5	1	CSKCDMREN	IVNLTL-DGSILCGRIYFDSSGGNNHAEHYRETGYPLAVKLGTITPDGAD	VSPQEDIDVLDPS
USP39	1	SHIN	IVACLK-VCGKVF-QSGRLKSHATLHVSQFSHHVF	LNHLTLKFCVLPDNIIDSS
USP45	1	CSECLKERRFYDGGQLVLTSD	IVNLTLK-CGFQGGGNS-ESQSLKHEKSSRTEPHCI	LNLSWLIVCELELSTHC
USP16	1	QDCKTDRKVKDAEETEEKFSPVNLCLK-CGHQGGGNS-QEQHALKHILTPRSEPHCLV		LSLDNWSVCHVQINAVQI
USP33	1	QDCKVQS	FNIVACLLENRCSPVCGGSSQ--VDHSTIH--SQETKHILT	VNLTTLKVMCHACSPVF
USP20	1	QSCGVVG	FNIVACLQVACPFVCGGSSQ--ADHSTIH--AQAKKHILT	VNLTTLKVMCHACSPVF
USP51	1	CHVCSHMIN	RVLSCLS-CVFFGC--FTKKHIEHAKAKRHILA	VDLYHGVINCHVQINAVYDKD
USP22	1	CHVCGVHLN	RVLSCLY-CVFFGC--FTKKHIEHAKAKRHILA	IDLMYGGIIVELQVLYDKD

Figure 3. Multiple sequence alignment of USP Zf-UBDs against HDAC6 Zf-UBD. Residues highlighted in magenta are more conserved and lining the primary pocket. Residues highlighted in cyan are less conserved and lining the secondary pocket.