## 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 | CSVCRSNKSPWVCLTCSSVHCGRYVNGHAKKHYEDAQVPLTNHKKSEKQDKVQHTVCMDCSSYSTYCYRCDDFVVNDT |
| USP5 | CSKCDMRENLWLNLTDGSILCGRRYFDGSGGNNHAVEHYRETGYPLAVKLGTITPDGADVYSYDEDDMVLDPS |
| USP13 | CARCDLRENLWLNLTDGSVLCGKWFFDSSGGNGHALEHYRDMGYPLAVKLGTITPDGADVYSFQEEEPVLDPH |
| USP16 | CQDCKTDNKVKDKAEEETEEKPSVWLCLKCGHQGCGRNSQEQHALKHYLTPRSEPHCLVLSLDNWSVWCYVCDNEVQY |
| USP20 | CQSCGVTGPNLWACLQVACPYVGCGESFADHSTIHAQAKKHNLTVNLTTFRLWCYACEKEVF |
| USP22 | CHVCGVHLNRLHSCLYCVFFGCFTKKHIHEHAKAKRHNLAIDLMYGGIYCFLCQDYIYDKD |
| USP33 | CQDCKVQGPNLWACLENRCSYVGCGESQVDHSTIHSQETKHYLTVNLTTLRVWCYACSKEVF |
| USP39 | SHINAYACLVCGKYFQGRGLKSHAYIHSVQFSHHVFLNLHTLKFYCLPDNYEIIDSS |
| USP44 | CVDCNTTESIWACLSCSHVACGRYIEEHALKHFQESSHPVALEVNEMYVFCYLCDDYVLNDN |
| USP45 | CSECLKERRFYDGQLVLTSDIWLCLKCGFQGCGKNSESQHSLKHFKSSRTEPHCIIINLSTWIIWCYECDEKLSTHC |
| USP49 | CLECATTESVWACLKCSHVACGRYIEDHALKHFEETGHPLAMEVRDLYVFCYLCKDYVLNDN |
| USP51 | CHVCSTHMNRLHSCLSCVFFGCFTEKHIHKHAETKQHHLAVDLYHGVIYCFMCKDYVYDKD |

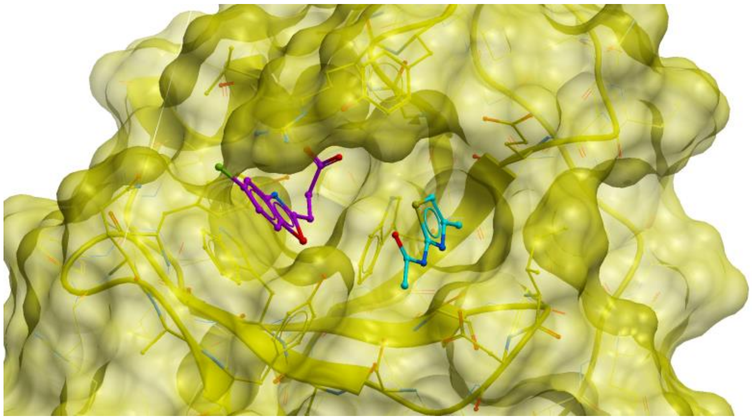


**Figure 1.** Structures of different ligands binding HDAC6 Zf-UBD at ubiquitin binding pocket (magenta: 5KH3) and adjacent secondary pocket (cyan: 5B8D)

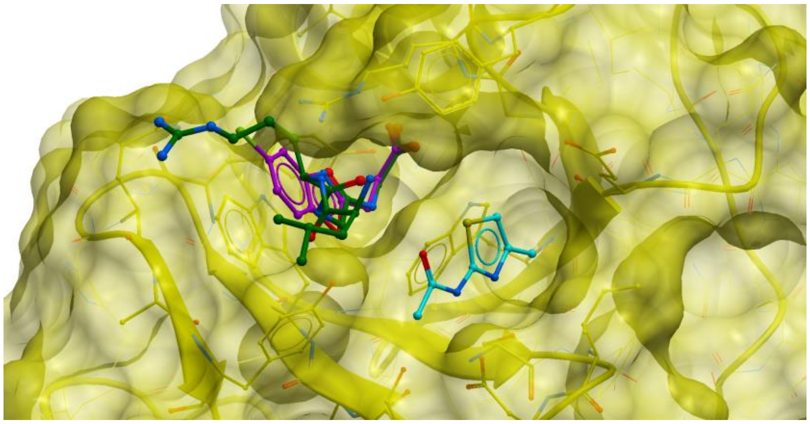
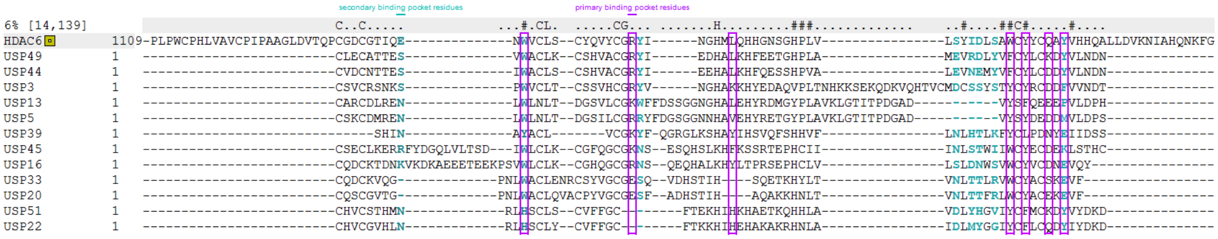


**Figure 2**. Structure of ubiquitin peptide RLRGG (green: 3GV4) binding in primary binding pocket similarly to 5KH3



secondary binding pocket residues    primary binding pocket residues

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6% [14,139]                              C..C.....            ...#.CL.  .....CG... .......H.........###.............    ..#....##C#.....#....
HDAC6       1109-PLPWCPHLVAVCPIPAAGLDVTQPCGDCGTIQE--------------NWVCLS--CYQVYCGRYI------NGHMLQHHGNSGHPLV----------------LSYIDLSAWCYYCQAYVHHQALLDVKNIAHQNKFG
USP49       1   -------------------------CLECATTES--------------VWACLK--CSHVACGRYI------EDHALKHFEETGHPLA----------------MEVRDLYVFCYLCKDYVLNDN--------------
USP44       1   -------------------------CVDCNTTES--------------IWACLS--CSHVACGRYI------EEHALKHFQESSHPVA----------------LEVNEMYVFCYLCDDYVLNDN--------------
USP3        1   -------------------------CSVCRSNKS--------------PWVCLT--CSSVHCGRYV------NGHAKKHYEDAQVPLTNHKKSEKQDKVQHTVCMDCSSYSTYCYRCDDFVVNDT--------------
USP13       1   -------------------------CARCDLREN--------------LWLNLT--DGSVLCGKWFFDSSGGNGHALEHYRDMGYPLAVKLGTITPDGAD-----------VYSFQEEEFVLDPH--------------
USP5        1   -------------------------CSKCDMREN--------------LWLNLT--DGSILCGRRYFDGSGGNNHAVEHYRETGYPLAVKLGTITPDGAD-----------VYSYDEDDMVLDPS--------------
USP39       1   ------------------------------SHIN--------------AYACL-------VCGKYF-QGRGLKSHAYIHSVQFSHHVF----------------LNLHTLKFYCLPDNYEIIDSS--------------
USP45       1   -------------------------CSECLKERRFYDGQLVLTSD---IWLCLK--CGFQGCGKNS--ESQHSLKHFKSSRTEPHCII----------------INLSTWIIWCYECDEKLSTHC--------------
USP16       1   -------------------------CQDCKTDNKVKDKAEEETEEKPSVWLCLK--CGHQGCGRNS--QEQHALKHYLTPRSEPHCLV----------------LSLDNWSVWCYVCDNEVQY----------------
USP33       1   -------------------------CQDCKVQG-------------PNLWACLENRCSYVGCGESQ--VDHSTIH----SQETKHYLT----------------VNLTTLRVWCYACSKEVF-----------------
USP20       1   -------------------------CQSCGVTG-------------PNLWACLQVACPYVGCGESF--ADHSTIH----AQAKKHNLT----------------VNLTTFRLWCYACEKEVF-----------------
USP51       1   -------------------------CHVCSTHMN-------------RLHSCLS--CVFFGC--------FTEKHIHKHAETKQHHLA----------------VDLYHGVIYCFMCKDYVYDKD--------------
USP22       1   -------------------------CHVCGVHLN-------------RLHSCLY--CVFFGC--------FTKKHIHEHAKAKRHNLA----------------IDLMYGGIYCFLCQDYIYDKD--------------
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**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.