 

# Letters to the Editor

## Backbone NMR assignment of the human E2 ubiquitin conjugating enzyme UbcH5α (F72K,F82S) double mutant

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Protein degradation via the proteasome pathway requires specific ubiquination. This reaction is carried out by ubiquitin-conjugating enzymes (UBC or E2) which covalently bind ubiquitin and then transfer relate it to the activated target protein. The ubiquitin-conjugating enzyme UBCH5α has been pursued as an oncogenic drug target because it is involved in the degradation of the tumor suppressor p53 (Scheffner et al., 1994). Wild-type UBCH5α has a high tendency to aggregate and thus exhibits adverse NMR relaxation properties. Based on an analysis of the structure of human Ubc9 (Tong et al., 1997) we have therefore designed a double mutant UBCH5α (F72K,F82S) which behaves like a monomer. $^{2}H$, $^{13}C$, $^{15}N$ labelled UBCH5α (F72K,F82S) with an N-terminal His-tag (21 residues) was assigned using 3D heteronuclear NMR experiments. Backbone and $C_{\beta}$ shifts are essentially complete except for the His-Tag and residues 38–40. Residues H41, K72, F90, S126, Y166 and A167 could only be partially assigned. All assigned resonances have been deposited in the BMRB (Accession No. 6584).

References: Scheffner et al. (1994) *PNAS*, **91**, 8797–8801; Tong et al. (1997) *J. Biol. Chem.*, **272**, 21381–21387.

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**Supplementary material** to this paper is available in electronic format at http://dx.dio.org/10.1007/10858-005-0464-6.

## $^{1}H$, $^{15}N$ and $^{13}C$ resonance assignments of the heme-binding protein murine p22HBP

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p22HBP, a 22 kDa cytoplasmic heme binding protein from mouse liver cell extracts, was first purified by Taketani et al. (1998). Subsequently, Blackmon et al. (2002) determined that p22HBP is a generic tetrapyrrole-binding protein rather than a dedicated heme-binding protein. The amino acid sequence of p22HBP has 44% homology to SOUL an heme-binding protein expressed in retina and pineal gland. p22HBP expressed in *E. coli* is a monomer, has one binding site for heme and can bind tetrapyrroles in general. Thus, binding properties of a small heme-binding protein, p22 HBP, are unique when compared with those of other heme-binding proteins. No structural information exists for p22HBP, and sequence analysis has identified no obvious similarity to known protein folds. Therefore we initiated a NMR structure determination of p22HBP and recorded 2D/3D NMR spectra on $^{15}N$, $^{13}C$ and $^{2}H$ labeled protein. Excluding the disordered amino terminus (residues 1–17), ~ 95% of all $^{1}H$, $^{13}C$ and $^{15}N$ resonances in the protein have been assigned.

References: Taketani et al. (1998) *J. Biol. Chem.*, **273,** 31388–31394; Blackmon, B.J. et al. (2002) *Arch. Biochem. Biophys.*, **407**, 196–201

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