

Letters to the Editor

^{1}H, ^{15}N and ^{13}C resonance assignments of a protein involved in the autophagy process, At4g21980.1 from *Arabidopsis thaliana*

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APG8a (At4g21980.1) from *Arabidopsis thaliana* is a single-chain protein of 122 amino acids (13.7 kDa) involved in the autophagy process, which plays a key role in protein recycling during starvation and senescence (Doelling et al., 2002). We have initiated an NMR investigation of recombinant [U−^{13}C,^{15}N]-At4g21980.1. Solubilization of the protein required a specialized solvent consisting of 20 mM sodium phosphate, pH 7.0, with 1% glycerol, 0.5 M urea, and 300 mM NaCl (Chae et al., 2004). Dynamic disorder, most notably in 13 N-terminal residues, limited the overall backbone assignment completeness: $^{1}H^{N}$ (85%), $^{1}H^{\alpha}$ (79%), ^{15}N (86%), $^{13}C^{\alpha}$ (86%), $^{13}C^{\beta}$ (81%), and $^{13}C'$ (86%). Supported by the NIH Protein Structure Initiative (GM P50 GM64598). NMR data were collected at the National Magnetic Resonance Facility at Madison. BMRB deposit with accession number 6610.

References: Doelling et al. (2002) *J. Biol. Chem.*, **277**, 33105–33114; Chae et al. (2004) *Protein Prod. Purif.*, **34**, 280–283.

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Backbone NMR assignment of the C-terminal haemopexin-like domain (HPLD) of human matrix metalloproteinase MMP-13

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MMP-13 is a matrix metalloproteinase involved in the degradation of the extracellular matrix and is important in human breast cancer pathology and in arthritic processes. The specificity of MMP-13 to cleave peptide bonds of native triple-helical collagen is mediated by the C-terminal haemopexin-like domain (HPLD). To gain further insight into the interaction of the HPLD domain with collagen, an NMR investigation was initiated of the HPLD domain L265-C471, where a crystal structure is available (Gomis-Rüth et al., 1996). MMP13-HPLD expressed in *E. coli* was refolded from inclusion bodies. 3D heteronuclear NMR experiments with ^{2}H, ^{13}C, ^{15}N-labelled HPLD were used to assign the backbone and C_{β} atoms. Only a partial assignment was possible, since 160 signals out of 191 expected signals were observed in the HSQC spectrum (84%). The observed signals have been assigned almost completely (96%). BMRB deposit with Accession No. 6617.

Reference: Gomis-Rüth et al. (1996) *J. Mol. Biol.*, **264**, 556–566.

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