Supporting information with:

Mapping of the binding site on pseudoazurin in the transient 152 kDa complex with nitrite reductase

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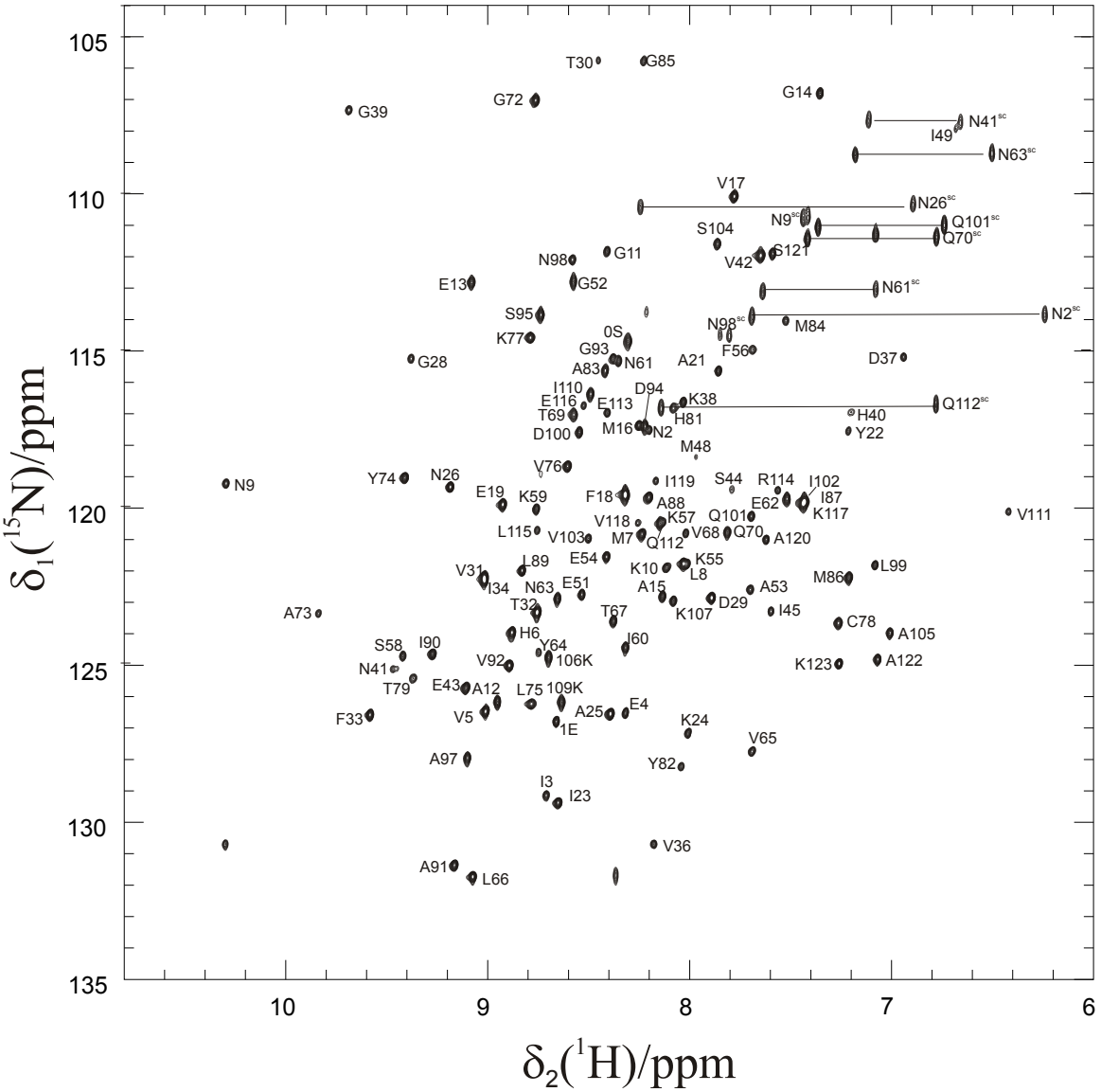
Protocol for the production of deuterium labelled PAZ in *Escherichia coli*.

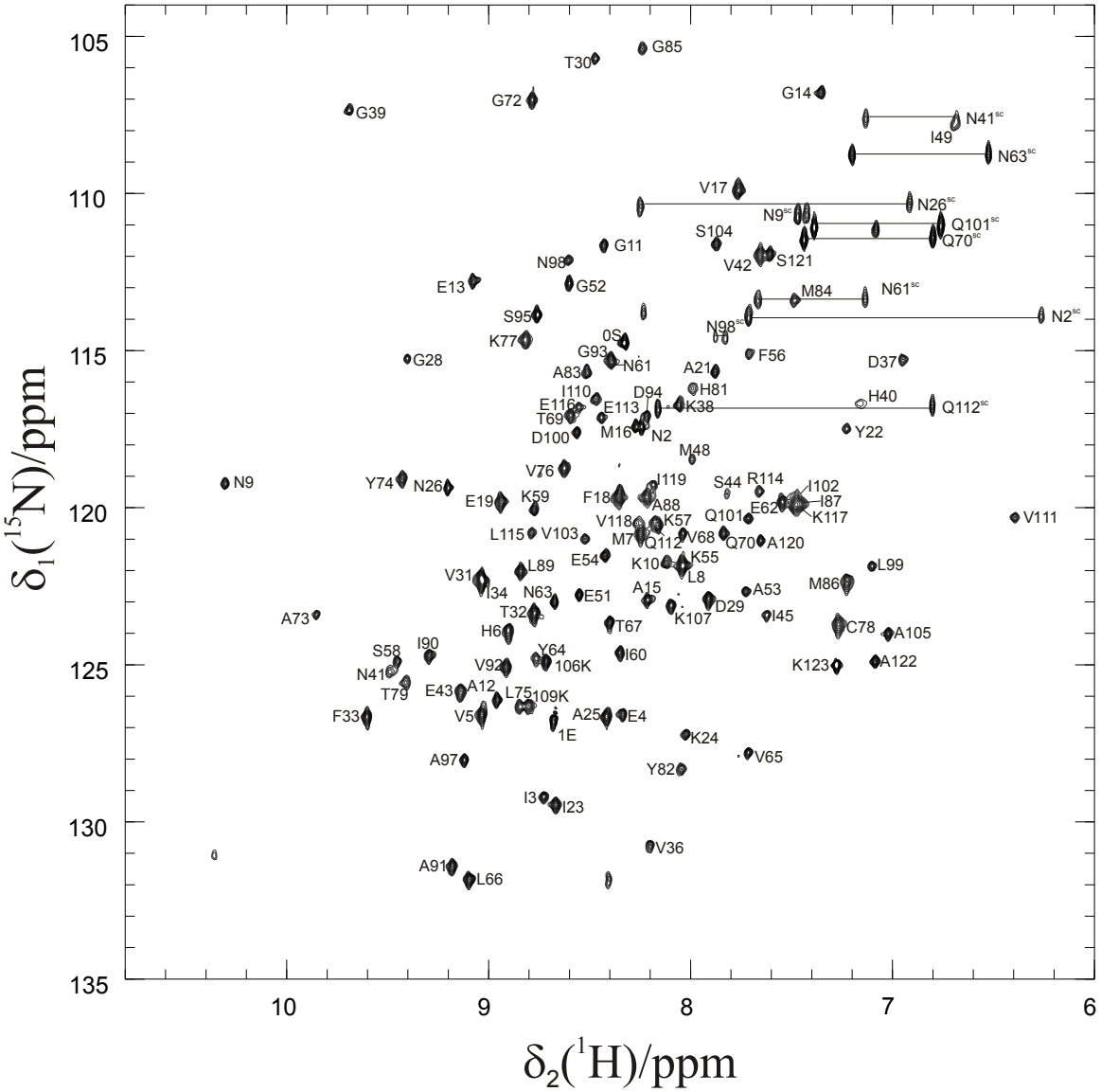
Part of the PAZ gene coding for the mature protein was subcloned in pET-28a(+), creating a plasmid for expression in the cytoplasm of *Escherichia coli*. Protein was produced in *E. coli* strain HMS174 in a M9 minimal medium containing 0.7 g/L $^{15}NH_4Cl$ and 5 g/L Na-acetate-d_3. Cultures were incubated at 37°C with a shaking speed of 250 rpm. *E. coli* was adapted for growth in D_2O by increasing the percentage of D_2O in consecutive 5 ml cultures from 0% to 50%, 80%, 95% and 99.9%. The cultures were used for inoculationa (at a ratio 1:5) when their OD_{600} was 0.4. In this way cells slowly adapted first to the deuterated carbon source and then to the deuterated medium. The final preculture was diluted several times to a ratio 1:5. Each dilution to a larger volume was done upon reaching OD_{600} = 0.4. The final volume was 1 litre. In this way, the bacteria remained in the exponential growth phase during the dilution steps. In the final culture, expression was induced at OD_{600} = 0.6 with 0.5 mM IPTG, and 100 μM copper citrate was added. Cultures were harvested at OD_{600} = 0.9 by centrifugation, 10 h after induction. From the first dilution step until harvest took 7 days. After centrifugation, the cell pellet was resuspended in 20 mM phosphate buffer pH 7.0 containing 500 mM NaCl, 1 mM PMSF, DNAse, 0.5 mM $CuCl_2$ and lysed using a French pressure cell (15.000 PSIG). After centrifugation for 15 min at 10.000 rpm supernatant was dialysed against 20mM phosphate buffer pH 7.0 and loaded onto a CM column equilibrated with the same buffer. PAZ eluted at circa 90 mM using a gradient of 0-250mM NaCl. The fractions containing PAZ were concentrated and purified further on a Superdex 75 FPLC gel filtration column. The 277/595 absorbance ratio of PAZ was 1.9 indicating a purity >95%. The yield was 7.5 mg/L and the deuteration level of the aliphatic protons was determined by mass spectrometry to be 93%.

NMR samples containing 100 μM ^{2}H-^{15}N-PAZ with or without 23 μM T2D-NiR were left for 8 h at room temperature and 2 days at 4 °C in a buffer solution of 90% D_2O / 10% H_2O to allow for equilibration of the amide exchange process.

^{1}H-^{15}N TROSY-SPECTRUM of ^{2}H-^{15}N PAZ Cu (I) 1mM
in 10%H_2O/90%$^{2}H_2O$ potassium phosphate buffer 20 mM pH 6.5



^{1}H-^{15}N TROSY-SPECTRUM without irradiation
of ^{2}H-^{15}N PAZ Cu (I) 1 mM in complex with 0.2 mM trimeric NiR Cu (I) T2D
in 10% H_2O/90% $^{2}H_2O$ potassium phosphate buffer 20 mM pH 6.5



^{1}H-^{15}N TROSY-SPECTRUM with irradiation of ^{2}H-^{15}N PAZ Cu (I) 1 mM in complex with 0.2 mM trimeric NiR Cu (I) T2D in 10% H_2O/90% $^{2}H_2O$ potassium phosphate buffer 20 mM pH 6.5
Strongly affected signals (see Fig. 1) are encircled.