

**P016** Characterisation of *Escherichia coli* NrfB: a deca-heme homodimer that transfers electrons to a deca-heme nitrite reductase  
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The deca-heme nitrite reductase NrfA is responsible for nitrite reduction to ammonium in *Escherichia coli*. While electron transfer to NrfA can be performed *in vitro* using sodium dithionite, the *in vivo* electron donor has not been determined. We have identified NrfB, a 20 kDa penta-heme c-type cytochrome, as a potential electron donor to NrfA. NrfB is encoded by the *nrfB* gene that follows the *nrfA* gene in the *E. coli nrf* operon, and can be overexpressed as a recombinant protein. Characterisation of purified NrfB by gel filtration indicates that NrfB is a deca-heme homodimer while UV-visible and MCD spectroscopy demonstrated that the 5 low spin ferric hemes are co-ordinated by two axial histidines. Furthermore, EPR studies revealed that, in two of the hemes, the planes of the histidine imidazole rings are near-parallel, while in the remaining three hemes the planes are perpendicular. Catalytically, UV-visible spectroscopy studies on reconstitution assays have shown reduced NrfB oxidises in the presence of both NrfA and nitrite, but remains reduced when either NrfA or nitrite is absent. These results demonstrate that NrfB is capable of electron transfer to NrfA, indicating the formation of a transient twenty-heme  $[\text{NrfB}]_2[\text{NrfA}]_2$  complex.