STRUCTURAL BIOLOGY
COMMUNICATIONS

**Volume 73 (2017)**

**Supporting information for article:**

# Structural studies of domain movement in active-site mutants of porphobilinogen deaminase from *Bacillus megaterium*

**Jingxu Guo, Peter Erskine, Alun R. Coker, Steve P. Wood and Jonathan B, Cooper**

### S1. Analysis of PBGD mutants by use of Ehrlich's reagent

The free *α*-position of the dipyrromethane (DPM) cofactor of PBGD can react with Ehrlich's reagent which initially gives a dark purple colour to the solution with a $\lambda_{max}$ of 565 nm. This is due to formation of a double bond between the *para*-dimethylaminobenzaldehyde (DMAB) and the free *α*-position of the DPM cofactor, as shown in Fig. 1 (below). The colour changes to orange after 15 min which gives a $\lambda_{max}$ at 495 nm due to the formation of conjugated bonds (Jordan and Warren, 1987, *FEBS Lett*. **225**, 87-92).

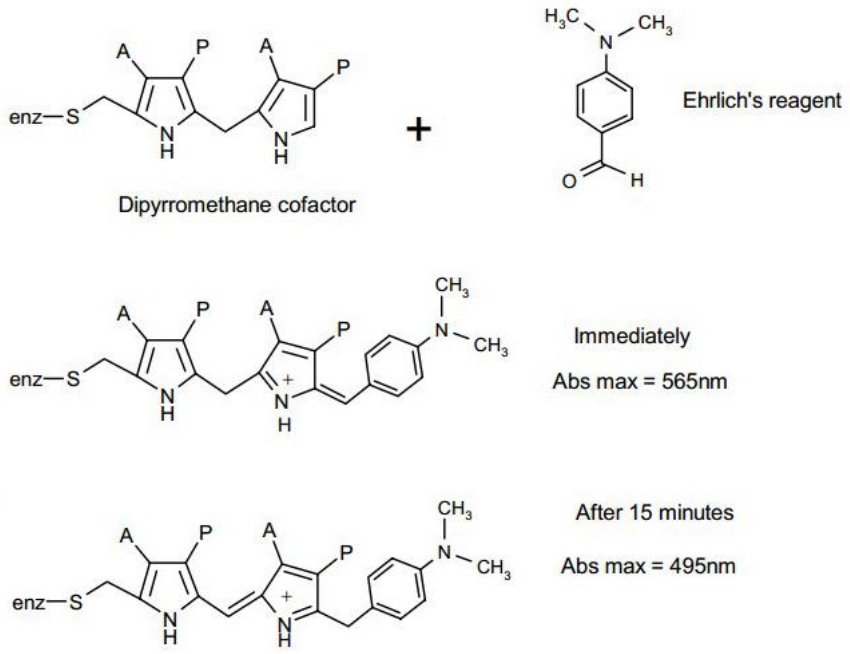


**Figure S1** The reaction of the DPM cofactor with Ehrlich's reagent.

Ehrlich's reagent was prepared according to the following scheme: 1 g of 4-dimethylaminobenzaldehyde, 42 ml of glacial acetic acid and 8 ml of 70 % perchloric acid. The reagent was made fresh every time and was kept in the dark. An aliquot (500 µl) of enzyme solution (1 mg/ml) in 50 mM Tris-HCl pH 7.3, 100 mM NaCl was mixed with 500 µl of the reagent at room temperature and any precipitate was removed by centrifugation. The absorbance for each sample was monitored by a wave scan between 380 nm and 600 nm after 1 and 15 min using an Ultrospec 3000 UV/Visible Spectrophotometer (GE Healthcare, Buckinghamshire, UK). 500 µl of protein buffer (50 mM Tris, 100 mM NaCl, pH 7.3) was mixed with an equal volume of the reagent as a blank. Observation of a peak at λ-max = 565 nm which subsequently shifted to a λ-max = 495 nm after 15 min was taken as confirmation of pyrrole moieties bound to the enzyme.

As shown in Fig. 2, the WT-PBGD had a distinct peak at 565 nm at 1 min which shifted to 495 nm after 15 min, confirming the presence of the cofactor. The D82E mutant protein had the same peaks which also indicated the presence of the cofactor. In contrast, the peaks were much lower for the D82A mutant, which indicated that the occupancy of the cofactor was probably quite low in this mutant. The apparent loss of the C2 ring in the D82N mutant structure has apparently not affected the reaction with DMAB which can also bind to the C1 ring of the partially assembled cofactor, as indicated by the peak at around 565 nm. However,

the lower peak at 495 nm may indicate that a less conjugated product is being formed due to the lower occupancy of the C2 ring.

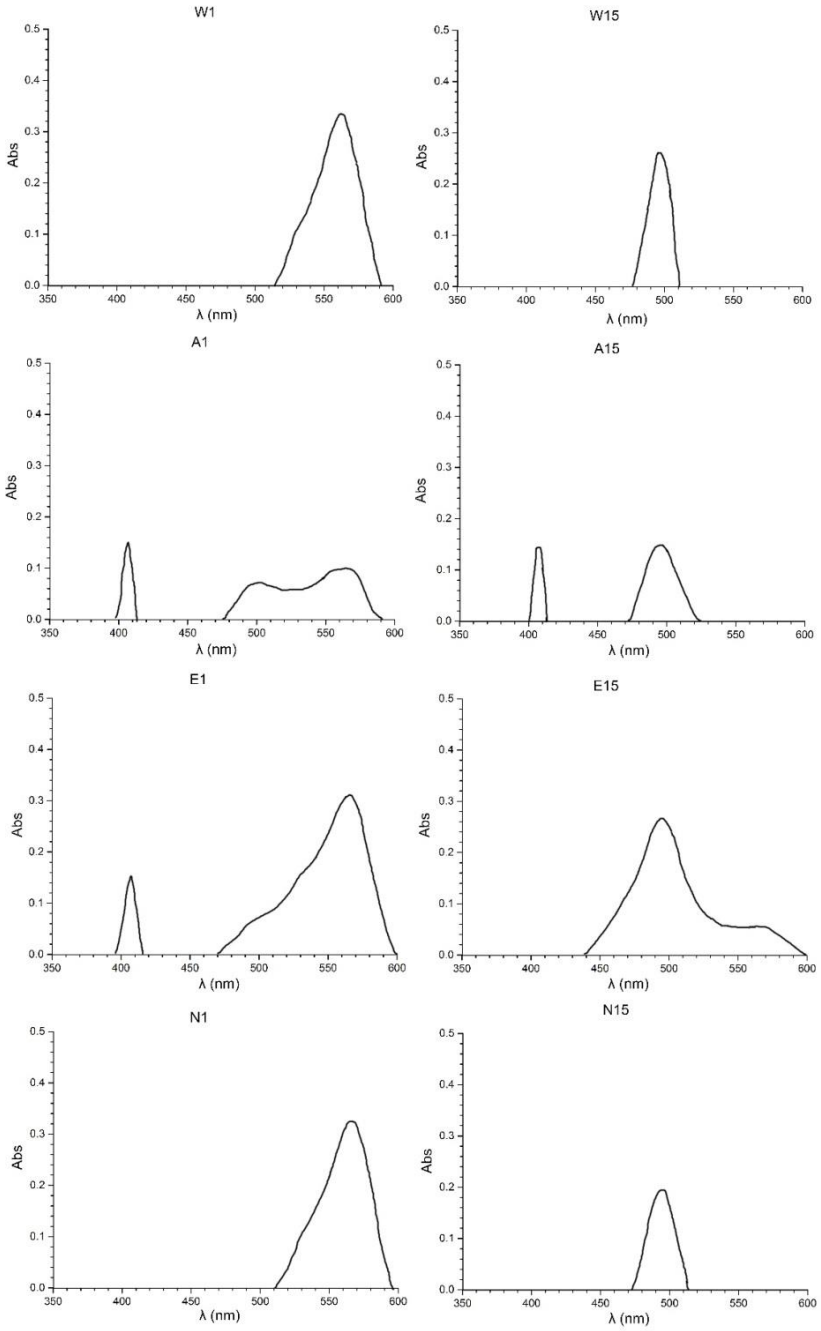


**Figure S2** Absorbance spectra of the mutants following reaction with Ehrlich's reagent. The column on the left indicates the spectrum after 1 minute and the column on the right corresponds to the spectrum after 15 minutes. The letters W, E, N and A stand for the WT, D82E, D82N and D82A mutants with the number following representing 1 min or 15 min of reaction time.