MS09-2-3 The detailed structure of a flavodoxin from Bacillus cereus revealed at ultrahigh resolution combined with in situ single-crystal spectroscopy
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Abstract
To obtain the correct structure of metallo- and redox proteins complementary *in situ* spectroscopic methods are needed to prove the redox state of these proteins. We have previously with single-crystal UV-vis and Raman spectroscopy followed the radiation-induced reduction of different haem- and flavoproteins [1,2]. For flavoproteins a bending of the flavin group due to radiation damage have been observed [2,3]. For a flavodoxin from *Bacillus cereus* previously solved to 1.3 Å, some negative electron density difference on the flavin group was observed [3]. The structural implication and whether this was due to radiation damage could not be explained. New crystallographic data to 0.8 Å presented here gives a more detailed explanation indicating some sort of flavin disorder. This study shows the added value of combining protein crystallography with *in situ* spectroscopy and ultrahigh resolution in the study of cofactor proteins.

References
[1] Hersleth, H.-P. & Andersson, K.K. How different oxidation states of crystalline myoglobin are influenced by X-rays. *Biochim. Biophys. Acta, Proteins Proteomics* (2011), 1814 , 785-796.

[2] Røhr, Å.K., Hersleth, H.-P. & Andersson, K.K. Tracking Flavin Conformations in Protein Crystal Structures with Raman Spectroscopy and QM/MM Calculations. *Angew. Chem. Int. Ed.* (2010), 49, 2324-2327.

[3] Gudim, I., Lofstad, M., van Beek, W. & Hersleth, H.-P. High-resolution crystal structures reveal a mixture of conformers of the Gly61-Asp62 peptide bond in an oxidised flavodoxin from *Bacillus cereus*. *Protein Sci*. (2018), 27, 1439-1449.

The previously unexplained electron density