established for a range of phenothiazine derivatives. Subsequently a miniaturised assay with robust performance measures has been developed for high throughput screening of small molecule inhibitors [3]. A focused screen of 11,000 compounds from a collection of 750,000 (Biofocus-DPI) has been undertaken and from these about 0.3% hits (active at <25 μM) have been identified. Dose response curves of hits show activity (IC_{50}) against Ndh at nM levels with corresponding sterilisation activity under both aerobic and anaerobic conditions. A number of structurally diverse templates have been prioritised and will be pursued using traditional medicinal chemistry QSAR.

Funded by The LeverhulmeTrust and National Institute for Health Research.

References

[1] Weinstein EA *et al.* (2005) *Proc. Natl. Acad. Sci. USA* **102:** 4548-4553.
[2] Boshoff HIM *et al.* (2004) *J. Biol. Chem.* **279:** 40174-40184.
[3] Fisher N *et al.* (2009) *Methods Enzymol.* **456:** 303-320.

doi:10.1016/j.bbabio.2010.04.352

14P.18 The distal pterin of *Escherichia coli* nitrate reductase A (NarGHI) participates in a charge transfer relay that modulates enzyme activity

Gregory J. Workun[1], Matthew Solomonson[1], Thomas Spreter[2], Maryam Zarapour[1], Nasim Bouromand[1], Natalie C.J. Strynadka[2], Joel H. Weiner[1]
[1]*Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7*
[2]*Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z3*
E-mail: joel.weiner@ualberta.cajoel.weiner@ualberta.ca

The current view of molybdoenzyme function is that the Mo atom plays the primary role in catalysis. We will present evidence that the pterin ring system can also play critical redox roles distinct from the redox reactions of the Mo. The bis-pterin molybdenum cofactor of *Escherichia coli* nitrate reductase A (NarGHI) contains tricyclic and bicyclic pterins that are proximal and distal to the FS0 [4Fe–4S] cluster, respectively. Site-directed mutagenesis, EPR spectroscopy, redox potentiometry, and protein crystallography were used to examine the assembly of a novel bicyclic distal pterin into the catalytic subunit (NarG). Inspection of available NarG protein structures reveals that the open pyran ring of the distal pterin is stabilized by hydrogen bonds between its hydroxyl oxygen and two conserved residues, NarG-S719 and NarG-H1163. The latter residue is also paired with a second conserved residue, NarG-H1184, forming a charge transfer relay between the pyran hydroxyl and three structurally-conserved water molecules. We have demonstrated that in a double mutant enzyme, NarG-S719A-H1163A, the pyran ring of the distal pterin is closed, rendering it similar to those of other bis-pterin molybdoenzymes of known structure. The NarG-S719A mutation has a less deleterious effect on enzyme function and molybdenum redox chemistry than either the NarG-H1163A mutant or the double mutant, indicating that the charge transfer relay plays a critical role in enzyme function. EPR spectroscopy in combination with potentiometric titrations indicates that NarG-H1163 and the charge transfer relay in which it participates play a critical role in defining redox chemistry and catalysis at the Mo atom. These results demonstrate the importance of residues contacting the organic component of the bis-pterin cofactor in controlling catalysis.

doi:10.1016/j.bbabio.2010.04.353