to activated carboxyl groups of QDs surface [3], as well as by specific interaction between ZnS and ZnS-specific protein tags [4]. Hybrid structures were characterised by several methods, including chromatography (for overall size and stability), spectrophotometry and fluorymetry (for luminescent properties and the enzyme activity) as well as microscopy (for surface topography, size and stability). Conjugation did not significantly change fluorescent properties of QDs. What is of high importance, FNR activity was preserved. Determined parameters of enzyme kinetics (Km, kcat) indicate that an active site is not altered, although a substrate binding may be partially hampered.

Our novel nanohybrids may serve in studies of *in vivo/in vitro* localization of FNR or its interaction with other proteins, as well as in examination of electron flow between QD and redox-active proteins.

Acknowledgement

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Structure of a novel octaheme cytochrome *c* from *Campylobacter concisus*

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The family of multiheme cytochromes *c* (MCC) comprises diverse electron carriers and redox enzymes playing key roles in several metabolic pathways. The few structurally characterized MCCs all contain conserved heme-packing motifs, albeit their primary structures are largely unrelated [1,2,3]. As prototype MCC enzyme serves NrfA [1], a pentaheme cytochrome *c* nitrite reductase with a wide substrate range [4]. Some other known MCCs belong to the subfamily of octaheme cytochromes *c* (OCC) [5], such as octaheme cytochrome *c* nitrite reductase (ONR) [3] or hydroxylamine oxidoreductase (HAO) [2]. Notably, the latter is the only OCC known to date to function as an oxidase. Remarkably, some ε-proteobacteria, including various *Campylobacter* species, are described as nitrite ammonifiers but lacking an NrfA homologue. Instead, these organisms all harbor an uncharacterized class of OCCs termed εHAOs, derived from their originally postulated function as hydroxylamine oxidoreductases. These εHAOs have been hypothesized to be the sought after candidates for catalyzing the full reduction of nitrite via hydroxyl-

amine to ammonium, thus functionally replacing NrfA [6]. We produced the εHAO from *Campylobacter consisus* recombinantly in *E. coli* with the aid of the pEC86 plasmid [7], and then solved the atomic structure at a resolution of 2.2 Å by means of single crystal X-ray diffraction. The structure reveals some unique features hitherto not observed in other OCCs. In particular the formation of the active site and the coordination of heme group 7 with methionine as distal ligand differ from all previously characterized OCCs. In addition we could identify nitrite and hydroxyl-amine as substrates for reduction, yet with activities considerably lower as the aforementioned nitrite reductases[1,3].

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Genetically-encoded ATP biosensor for low temperatures

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We have previously reported genetically-encoded FRET biosensors for ATP, named ATeam [1,2]. ATeam is a powerful tool to monitor ATP levels inside living mammalian cells with high temporal and spatial resolutions. One of the major drawbacks of the original biosensors is that their affinity to ATP is very sensitive to temperature changes. Although dissociation constant (K_d) of the original biosensor, AT1.03, at 37 °C is 3.3 mM, K_d at 24 °C is less than 0.6 mM, far below physiological ATP concentrations. This means at low temperatures (around 24 °C) the FRET signal of AT1.03 biosensor must be saturated with the physiological concentrations of ATP, making it difficult to detect a slight change of ATP levels. Because body temperatures of many model organisms, such as *Caenorhabditis elegans* and *Drosophila melanogaster*, are the same with ambient temperatures (20–25 °C), it does not seem that the use of AT1.03 biosensor is suitable for these organisms or cells from them.

In this study, we constructed mutants of AT1.03 by substituting amino acid residues at the ATP binding domain, the e subunit of F_oF_1-ATPase. We found that one of the mutants (AT1.03NL) showed much lower affinity to ATP than the original AT1.03; K_d was 2.1 mM at 24 °C and 1.4 mM at 20 °C. To examine if AT1.03NL is actually effective in ATP imaging at low temperatures, we expressed AT1.03NL and AT1.03 in S2 cell, which originated from *D. melanogaster* and was cultured at 25 °C. If the FRET signal is saturated, it will not respond quickly to a metabolic challenge. When AT1.03-expressing cells were treated with 2-deoxyglucose and oligomycin A, there was a lag before the FRET signal started to decrease. This suggests that the FRET signal of AT1.03 is mostly saturated in S2 cells. On the other hand, the FRET signal of AT1.03NL-expressing cells started to decrease immediately after addition of inhibitors. Thus, the FRET signal of AT1.03NL is not