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Towards structural determination of human membrane proteins.

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The results of various genome projects have shown that up to 30% of human proteins occur in cell membranes. Membrane proteins play crucial roles in many biological functions and are of key importance for medicine. Over 50% of commercially available drugs target membrane proteins. In spite of the abundance and importance of membrane proteins there are only 100 unique membrane protein structures in the Protein Data Bank. To address the technical bottlenecks preventing the structure determination of membrane proteins, we have recently started ''ERATO human receptor crystallography project'' supported by the Japanese Science and Technology Agency. We have also obtained a support from the Wellcome trust to establish an outstation of Imperial College London at the new UK synchrotron Diamond. I will discuss our strategy how to establish the structure determination method of human membrane proteins using these new facilities and its impact on biological sciences, pharmacology and medicine. I will also present our some recent results on membrane protein crystallography.

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Crystal structure of the membrane-bound complex cytochrome *c* nitrite reductase

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Cytochrome *c* nitrite reductase complex, NrfHA, from *D. vulgaris* Hildenborough catalyses nitrite conversion to ammonia by the oxidation of menaquinol [1]. The complex is bound to the membrane through the NrfH subunit, a tetraheme cytochrome *c* of 18 kDa that belongs to the NapC/NirT family of membrane-anchored quinol dehydrogenases. The catalytic subunit, NrfA, is a pentaheme cytochrome *c* of about 60 kDa that has been shown to form a biological dimer. No structural information is yet available for NrfH or for any member of the Napc/NirT protein family.

The native complex has been crystallized by the vapor diffusion method using PEG 4K as precipitant. Crystals diffract to ~2.4 Å resolution and belong to the orthorhombic space group $P2_12_12_1$, with unit cell parameters of a=79.5, b=256.7 and c=578.2 Å. The structure was solved by a combination of molecular replacement and MAD methods, showing that six copies of the complex are present in the asymmetric unit [2]. The current model has R and Rfree values of 21% and 24%, respectively.

The NrfH coordinates were submitted to DALI and no structural homologous structure were found. This novel fold should also be adopted by other members of the NapC/NirT family. The NrfH structure also reveals other interesting features, such as a new heme binding motif. The stoichiometry of the complex was shown to be one NrfA dimer bound to one NrfH, which is very stable, due the extensive protein-protein interactions observed within the interface. The attachment of the complex to the membrane and the possible multi-electron transport pathways will also be discussed.

[1] Pereira *et al.* (2000) Characterization of a heme *c* nitrite reductase from a non-ammonifying microorganism, *Desulfovibrio vulgaris* Hildenborough. *Biochim. Biophys. Acta,* 1481, 119-30.

[2] Rodrigues *at al.* (2006) Crystallization and preliminary structure determination of the membrane-bound complex cytochrome *c* nitrite reductase from *Desulfovibrio vulgaris* Hildenborough. *In preparation.*