## FA1-MS10-T01

**Investigating the Oxygen Reactivity in Enzymes.**
Andrea Mattevi[a], Roberto Orrù[a], Riccardo Baron[b], Pimchai Chaiyen [c], Marco W. Fraaije[d]. [a]*Department of Genetics and Microbiology, University of Pavia, Pavia, Italy.*[b] *Department of Chemistry and Biochemistry, University of California, San Diego, USA.* [c]*Department of Biochemistry and Center for Excellence in Protein Structure and Function, Mahidol University, Bangkok, Thailand.* [d]*Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, The Netherlands.*
E-mail: andrea.mattevi@unipv.it

Our presentation will summarize our efforts towards a more in-depth understanding of the ability of flavoenzymes to differentially react with oxygen [1]. We have investigated two different types of monooxygenases, which are capable of activating molecular oxygen through the stabilization of a flavin-(hydro)peroxide intermediate. These enzymes exhibit a properly shaped cavity in front of the C4a atom of the flavin that promotes intermediate stabilization [2,3]. Most remarkably, in flavin-containing monooxygenases flavin-hydroperoxide formation directly involves the $NADP^+$ ligand, which, therefore, appears to play the dual function of reducing the flavin and stabilizing a critical catalytic intermediate. We are using a combination of site directed mutagenesis and molecular dynamics to investigate the role of residues surrounding the flavin in tuning oxygen reactivity.

[1] Mattevi, A. (2006) *Trends Biochem. Sci. 31*, 276-283. [2] Alfieri, A., Ferini, F., Ruangchan, N., Prongjit, M., Chaiyen, P., Mattevi, A. (2007) *Proc. Natl. Acad. Sci. USA 104*, 1177-1182. [3] Alfieri, A., Malito, E., Orru, R. Fraaije, M.W., Mattevi, A. (2008) *Proc. Natl. Acad. Sci. USA* 195, 6572-6577. [4] Baron, R., Riley, C., Chenprakhon, P., Thotsaporn, K., Winter, R., Alfieri, A., Forneris, F., van Berkel, W., Chaiyen, P., Fraaije, M.W., Mattevi, A., McCammon, J.A. (2009) *Proc. Natl. Acad. Sci. USA* 106, 10603-10608. [5] Baron, R, McCammon, J.A., Mattevi, A. (2009) *Curr. Opin. Struct. Biol.* 19, 672-679.

**Keywords: reactive oxygen species, binding, biocatalysis**

## FA1-MS10-T02

**Photosystem II at 2.9 Å resolution - Quinones, lipids, channels and chloride ion.**
Wolfram Saenger[a], Albert Guskov[a], Azat Gabdulkhakov[a], Matthias Broser[b], Jan Kern[b], Athina Zouni[b], [a]*Freie Universitaet Berlin, Institute for Chemistry and Biochemistry/ Crystallography, Berlin, Germany.* [b]*Technische Universitaet Berlin, Max Volmer Laboratory for Biophysical Chemistry,Berlin,Germany.*
E-mail: saenger@chemie.fu-berlin.de

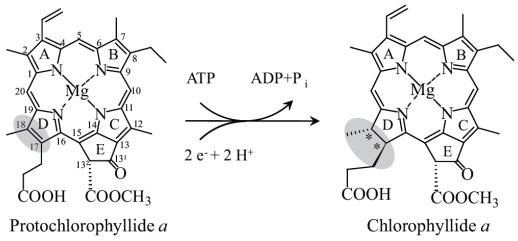
Photosystem II (PSII) is a homodimeric protein-cofactor complex acting as light-driven water:plastoquinone oxido-reductase and is located in the photosynthetic thylakoid membrane of plants, green algae and cyanobacteria. PSII oxidizes two water molecules at the unique $Mn_4Ca$ cluster to molecular (atmospheric) oxygen, 4 protons and 4 electrons. The protons drive ATP synthetase, and the electrons reduce plastoquinone ($Q_B$) to plastoquinol ($Q_BH_2$) that is exported and delivers the electrons (through the cytochrome $b_6f$ complex) to photosystem I. Here the electrons gain a high reducing potential and serve at NADP reductase to generate NADPH that together with ATP reduces $CO_2$ to carbohydrates in the Calvin cycle.
The crystal structure of PSII from *Thermosynecho-coccus elongatus* at 2.9-Å resolution allowed the unambiguous assignment of all 20 protein subunits and complete modeling of all 86 cofactors, among them 25 integral lipids, per PSII monomer [1]. The presence of a third plastoquinone $Q_C$ and a second plastoquinone-transfer channel, which were not observed before, suggest mechanisms for plastoquinol-plasto-quinone exchange, and we calculated possible water or dioxygen and proton channels. Putative oxygen positions obtained from Xenon derivative crystals indicate a role for lipids in oxygen diffusion to the cytoplasmic side of PSII. The chloride position suggests a role in proton-transfer reactions because it is bound through a putative water molecule to the $Mn_4Ca$ cluster and is close to two possible proton transfer channels.

[1] Guskov A., Gabdulkhakov A., Broser M., Zouni A., Saenger W. *Nature Struct. Mol. Biol.*, 2009, 16, 334.

**Keywords: photosynthesis, membrane protein, cofactors**

## FA1-MS10-T03

**Crystal Structure of of the Nitrogenase-like Dark Operative Protochlorophyllide Oxidoreductase Catalytic Complex,**
Wolf-Dieter Schubert[a,b], Markus Bröcker[c], Sebastian Schomburg[c], Dirk W. Heinz[b], Dieter Jahn[c], Jürgen Moser[c]. [a]*Department of Biotechnology, University of the Western Cape, Bellville, Cape Town, South Africa.* [b]*Structural Biology, Helmholtz-Centre for Infection Research, Braunschweig, Germany.* [c]*Institute of Microbiology, Technical University Braunschweig, Germany.*
E-mail: wschubert@uwc.ac.za




In the biosynthesis pathway of (bacterio)chlorophyll, two evolutionary distinct enzymes catalyze the two electron reduction of ring D of protochlorophyllide to chlorophyllide: In angiosperms monomeric, light-dependent protochloro-phyllide oxidoreductase (LPOR) catalyses the reaction, whereas anoxygenic, photosynthetic bacteria make use of an ATP-dependent process catalyzed by dark operative protochlorophyllide oxidoreductase (DPOR). DPOR is composed of three distinct subunits, ChlL, ChlN and ChlB. ChlL forms a homodimer $ChlL_2$ with an intersubunit [4Fe-4S] cluster. $ChlL_2$ is an ATP-dependent reductase transferring single electrons to the heterotetrameric complex of the other two proteins $(ChlN/ChlB)_2$. Each half of this tetramer bears an intersubunit [4Fe 4S]-cluster and has a protochlorophyllide binding site.