Tryptophan and tryptophan analogs as spectroscopic probes to characterize the dimeric mannitol transporter from *E. coli*.

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We study the structure and dynamics of the membrane-embedded mannitol transporter, EII^{mtl}, from *E. coli* using fluorescence and phosphorescence spectroscopy methodologies. Twenty functional single Trp-containing EII^{mtl} mutants have been constructed and labeled with Trp analogs like 5-fluorotryptophan and 7-azatryptophan. These analogs can be efficiently biosynthetically incorporated using an *E.coli* Trp auxotroph and this procedure will be discussed.
EII^{mtl} is functional as a dimer and each dimer harbors one high affinity mannitol binding site. By using a FRET approach we were able to localize the position of this binding site.
Tryptophan phosphorescence spectroscopy provided information about the local viscosity of the protein matrix surrounding the tryptophan residues. Site specific information about the impact of mannitol binding on the transporter structure was collected with this very sensitive technique.
The lecture will be concluded by presenting a recently developed method to incorporate tryptophan analogs with more bulky substituents in recombinant proteins.

Bibliographic sketch Dr. Jaap Broos

Jaap Broos got his Ph.D. degree in 1994 at the University of Twente with Prof. Reinhoudt. The title of his thesis was "Catalysis of enzyme-catalyzed reaction in organic solutions by macrocyclic polyethers". In the same year he was appointed at a tenure track position in enzymology at the University of Groningen, joining the group of Prof. Robillard. In 1997 he became assistant professor and in 2009 associate professor at the University of Groningen. He went for sabbatical stays (2-4 months) to the group of Dr. Strambini, CNR, Pisa, Italy in 2002 (Trp phosphorescence spectroscopy), to the group of prof. Jackson, University of Birmingham University, UK, in 2008 (transhydrogenase domain 1 fluorescence) and in 2009 to the group of Prof. Haas, Bar-Ilan University, Israel (dynamic FRET with 5-fluorotryptophan as donor).

Research highlights:

A major research topic in the group is studying membrane proteins by taking advantage of their intrinsic tryptophan fluorescence properties. The group pioneered exploring the use of tryptophan analogues in membrane protein research. The buildup expertise in Trp (analog) fluorescence spectroscopy has been applied in particular to characterize the mannitol transporter from *E. coli*. In a more fundamental program, the impact of the protein matrix on Trp (analog) fluorescent properties is investigated. Highlights of his research include:

- Development of protocols to biosynthetically incorporate tryptophan (Trp) analogs in proteins with very high efficiency using *E. coli* or *L. lactis* as expression host (*Biochemistry* 1999, *38*, 9798-9803; *Protein Sci.* 2003, *12*, 1991-2000; *Biochem. J.* 2008, *409*, 193-198)
- .The finding that 5-fluorotryptophan in proteins decays monoexponentially and the rationalization of this phenomenon (*J. Am. Chem. Soc.* 2004, *126*, 22-23; *J. Am. Chem. Soc.* 2005, *127*, 4104-4113). This property makes possible to extract much more information using fluorescence spectroscopy. For example, the homogeneous decay of 5-fluorotryptophan was key to localize the single binding site in the dimeric mannitol transporter. With 19 single 5-fluorotryptophan mutants as donor and a chromophoric substrate analog as acceptor (*J. Am. Chem. Soc.* 2002, *124*, 6812-6813*7*), the position of the binding site in the dimer could be established with high precision as time-resolved FRET experiments informed about the FRET efficiency of each 5-fluorotryptophan containing protomer (*J. Biol. Chem.* 2010, *285*, 25324-25331).
- First report of a Trp-containing protein showing ^1L_b emission (*Angew. Chem., Int. Ed.* 2007, *46*, 5137-5139; *Biophys J.* 2008, 95, 3419-3428). This protein, a mutant of domain 1 of the transhydrogenase protein from *Rhodospirillum rubrum*, also features the bluest emission maximum reported for Trp in a protein (λ^{max} = 304 nm). Quantum mechanical/ molecular mechanical calculations indicated the ^1L_a state is minimally stabilized by the rigid protein matrix, surrounding the Trp, making ^1L_b the emitting state.