

Overall research theme:

Receptors and transporters for biogenic amines: structure, molecular function and cellular regulation

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Characteristics of the research group:

Biogenic amines, such as dopamine, norepinephrine and serotonin, are some of the most important chemical messengers in the central and peripheral nervous system playing key roles in regulation of cardiovascular function. Upon release from the presynaptic nerve terminal into the synaptic cleft the biogenic amines exert their effects by activating distinct *post- and presynaptic receptors*, which almost exclusively belong to the large superfamily of G protein coupled receptors. The effects exerted by the biogenic amines are rapidly terminated by *presynaptic transporter proteins* that mediate reuptake of the monoamines into the presynaptic nerve terminal.

Currently the research group consists of 13-15 people and the overall purpose our research is to achieve insight into the molecular and cellular function of the Na⁺/Cl⁻ coupled transporters and the G protein coupled receptors for biogenic amine transmitters. Specifically, it is our goals i) to obtain insight into the tertiary structure of the transporters and receptors, ii) to characterize the nature of the molecular processes involved in substrate translocation and receptor activation, iii) to determine the structural basis for how these processes can be blocked by different classes of drugs used for treatment of cardiovascular diseases, and iiiii) to define the mechanisms responsible for the cellular targeting, trafficking and regulation of the transporters and receptors, including identification and characterization of functionally associated proteins.

To achieve these goals we employ a broad range of molecular biological, pharmacological, biochemical and biophysical techniques including e.g. engineering of artificial Zn²⁺ binding site, fluorescence spectroscopy (e.g. FRET and FCS), confocal fluorescence microscopy, proteomics approaches, two-hybrid screens and transgenic models.

Running projects: Titles and abstracts:

Recent publications related to the projects described above:

Relevant publications 2000-2002:

A. D. Jensen and U. Gether: Assessing adrenergic receptor using chemically reactive fluorescent probes. In **Adrenergic Receptor Protocols**, pp. 345-361 (ed. C. Machida) in the **Methods for Molecular Biology** (series ed. J. Walker), Humana Press Inc., 2000

U. Gether: Uncovering Molecular Mechanisms Involved in Activation of G Protein Coupled Receptors. **Endocrine Reviews** (2000) 21, 90-113.

P. Ghanouni, H.T. Schambye, R. Seifert, T.W. Lee, U. Gether and B.K. Kobilka: Evidence for protonation in β_2 adrenergic receptor activation: The effect of pH on receptor function. **J. Biol. Chem** (2000) 275, 3121-3127.

L. Norregaard, Irache Visiers, Claus J. Loland, Juan Ballesteros, Harel Weinstein and U. Gether: Structural probing of a microdomain in the dopamine transporter by engineering of artificial Zn²⁺ binding sites.

Biochemistry (2000) 39:15836-46.

S.G.F. Rasmussen, A. D. Jensen, M.J. Maresch, I. Carroll, C. Tate, and U. Gether: Biophysical characterization of the cocaine binding pocket in the serotonin transporter using a fluorescent cocaine analogue as a molecular reporter.

J. Biol. Chem. (2001) 276, 4717-4723.

A. D. Jensen, F. Guarnieri, S.G.F. Rasmussen, F. Asmar, J. Ballesteros, and U. Gether: Mapping agonist-induced conformational changes at the cytoplasmic side of transmembrane segment 6 in the β_2 adrenergic receptor by site-selective fluorescent labeling.

J. Biol. Chem., (2001) 276, 9279-9290.

N. Boxenbaum, U. Gether, D. Klaerke and T. Zeuthen: Water transport by the glutamate EAAT-1 transporter.

J. Physiol. (2001) 530, 367-378.

J. A. Ballesteros, A. D. Jensen, G. Liapakis, S.G.F. Rasmussen, L. Shi, U. Gether, and J. Javitch: Activation of the β_2 adrenergic receptor involves disruption of an ionic lock between the cytoplasmic ends of transmembrane segments 3 and 6

J. Biol. Chem. (2001) 276,29171-29177.

U. Gether, C.J. Loland and L. Norregaard: Delineating Structure-Function Relationships in the Dopamine Transporter from Natural and Engineered Zn^{2+} Binding Sites. In Proceedings of the 3rd International Symposium on Membrane Receptors, Signal Transduction and Drug Action, Yokohama 2000.

Life Science (2001) 68, 2187-98.

R. Seifert, K. Wenzel-Seifert, U. Gether, B. K. Kobilka: Functional Differences between Full and Partial Agonists: Evidence for Ligand-Specific Receptor Conformations.

J. Pharmacol. Exp. Ther. (2001) 297,1218-26.

N. MacAulay, A. Bendahan, C. J. Loland, T. Zeuthen, B. Kanner, and U. Gether: Engineered Zn^{2+} Switches in the GABA Transporter-1: Differential Effects on GABA Uptake and Currents.

J. Biol. Chem. (2001) 276, 40476-85.

L. Norregaard and U. Gether: The monoamine neurotransmitter transporters: structure, conformational changes and molecular gating.

Curr. Opin. Drug Discov. Dev. (2001) 4, 591-601.

U. Gether and B. K. Kobilka: Use of fluorescence spectroscopy to study conformational changes in the 2-adrenoceptor.

Methods Enzymol. (2001) 343,170-82.

C.J. Loland, L. Norregaard and U. Gether: Generation of an activating Zn^{2+} switch in the dopamine transporter: Mutation of an intracellular tyrosine constitutively alters the conformational equilibrium of the transport cycle.

Proc. Natl. Acad. Sci. U S A (2002) 99, 1683-8.

P. Scholze, L. Norregaard, E.A. Singer, M. Freissmuth, U. Gether, H.H. Sitte: The role of zinc ions in reverse transport mediated by monoamine transporters.

J. Biol. Chem. (2002) 277, 21505-13.

S.G.F. Rasmussen and U. Gether: Structural basis for activation of G protein coupled receptors.

In **Structure and function of GPCRs in the nervous system** (eds. M.N. Pangalos and C.H. Davies) Oxford Press, 2002, in press.

K. Nørsgaard-Nielsen, L. Norregaard, H. Hastrup, J.A. Javitch and U. Gether: Zn^{2+} site engineering at the oligomeric interface of the dopamine transporter.

FEBS Letter (2002) 524, 87-91.

MacAulay N, Gether U, Klaerke DA, Zeuthen T.: Passive water and urea permeability of a human Na(+)-glutamate cotransporter expressed in *Xenopus* oocytes.

J. Physiol. (2002), 542,817-828

N. MacAulay, T. Zeuthen and U. Gether: Conformational basis for the Li⁺-induced leak current in the γ -aminobutyric acid (GABA) transporter-1.

J. Physiol. (2002), in press.

C. Granas, J. Ferrer, C.J. Loland, J.A. Javitch and U. Gether: N-terminal phosphorylation of the dopamine transporter occurs independently of transporter internalization.

J. Biol. Chem. (2002), in press.