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*MEETING ABSTRACT*

**A2.1**

## Unnatural amino acids as a novel tool to study the folding of the serotonin transporter

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**Background:** The serotonin transporter (SERT) is a membrane protein, comprising cytosolic N- and C-termini, 12 transmembrane domains (TMDs) and a large extracellular loop between TMDs 3 and 4. SERT is responsible for the rapid reuptake of serotonin from the synaptic cleft, thus terminating neurotransmission. Mutations in the first cytoplasmic loop and the C-tail region of SERT lead to misfolding and ER retention of the transporter. The folding-defective mutants can be rescued by treatment with noribogaine and heat shock protein (HSP) inhibitors [1, 2].

**Methods:** Amber codons (TAG) were introduced into 23 locations of human SERT. HEK 293 T cells were co-transfected with plasmids encoding these constructs, the orthogonal (suppressor) tRNA and the evolved amino acyl-tRNA synthase pair. This allows for incorporation of *p*-benzoyl-L-phenylalanine at the amber codon. This specific unnatural amino acid (UAA) is also suitable for UV-induced photo-crosslinking, performed by irradiating the cells (365-nm UV light source) and identifying the cross-linked products by gel electrophoresis using a SERT-specific antibody.

**Results:** A series of residues located in the N- and C-termini of SERT, as well as within cytoplasmic loop and TMD regions, were replaced by the amber codon. All 23 mutants were functionally screened by measuring specific [$^3$H]5-HT uptake. Upon incorporation of the UAA, the functional activity of the mutants ranged from 10 to 80% of the wild-type uptake levels. However, some mutants were not recovered by adding UAA to the culturing media, even though they could be functionally rescued by the pharmacochaperone noribogaine. Moreover, UV-induced cross-linking experiments produced high molecular weight species, indicating an association of the mutants with partner proteins. Interestingly, the detected cross-linked species were not identical among the mutants we examined. This suggests that specific partners are coupled to SERT proteins trapped at distinct stages along the folding trajectory. On the other hand, no cross-linked products were found for amber codons introduced at locations known to face the lipid bilayer, although the same mutations exhibited specific [$^3$H]5-HT uptake comparable to wild-type SERT.

**Discussion:** Understanding the folding trajectory of SERT and other solute carrier 6 family members is of key physiological relevance, since point mutations in these proteins result in misfolding and cause clinically relevant phenotypes in people. Pharmacochaperoning may become a useful therapeutic option in the treatment of these diseases.

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