# Neurotransmitter transporter homolog –Structural basis for substrate recognition and transport inhibition–

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In the central nervous system of vertebrates, synaptic transmission is terminated by uptake of neurotransmitters from the synaptic cleft to the cytoplasm of neurons and glia catalyzed by specific transporters. $Na^+/Cl^-$-dependent neurotransmitter transporters (also referred to as neurotransmitter sodium symporters; NSS) employ sodium and chloride electrochemical gradients to drive transport of a wide array or substrates, including the biogenic amines (serotonin, dopamine, etc.) and amino acids (GABA, glycine, etc.). Dysfunction of these transporters causes various psychiatric disorders, including depression. NSS members are the targets of therapeutic agents for these diseases, such as antidepressants. Although it has been highly required to understand the functional mechanisms of NSS, structural analysis by X-ray crystallography has so far been hampered by the difficulties generally encountered in membrane protein crystallization. To circumvent the problems, we exploited the presence of bacterial homologs, because they were assumed to be more stable and amenable to large-scale expression than the eukaryotic counterparts. The homologs have strict conservation of residues essential to the function of NSS as well as clusters of highly conserved sequences throughout the molecules, implying that they share similar architectures as well as functional mechanisms with NSS. Among several bacterial homologs we cloned, we found a highly-stable protein from *Aquifex aeolicus*, LeuT. LeuT is amenable to crystallographic structure analysis and resulted atomic structural information of the state binding its substrates, L-leucine and sodium ions, at 1.65 Å resolution [1]. Moreover, we also found that a tricyclic antidepressant clomipramine inhibits substrate uptake by LeuT, and characterized its mechanism of action by flux, binding, and crystallographic analyses [2]. In the symposium, we will discuss about mechanisms of substrate recognition and transport inhibition of LeuT based on our recent structural and functional studies.

[1] Yamashita, A., Singh, S. K., Kawate, T., Jin, Y., and Gouaux., E., Crystal structure of a bacterial homologue of $Na^+/Cl^-$-dependent neurotransmitter transporters. (2005) *Nature* **437**, 215-223.

[2] Singh, S. K., Yamashita, A., and Gouaux, E., Antidepressant binding site in a bacterial homologue of neurotransmitter transporters. (2007) *Nature* **448**, 952-956.