Sohail *et al.*: *Intrinsic Activity*, 2014; **2**(Suppl.1):A1.1
published online: 22 September 2014
http://www.intrinsicactivity.org

20th Scientific Symposium of the Austrian Pharmacological Society APHAR

Innsbruck, Austria, 26–27 September 2014

MEETING ABSTRACT

A1.1

Decrypting the structure of LeuT$_{Aa}$ employing luminescence resonance energy transfer (LRET)

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Background: Neurotransmitter sodium symporters (NSS) are located in the brain and retrieve neurotransmitters from the synaptic cleft to end synaptic transmission. Solute carrier class 6 proteins (SCLC6) are of great pharmacological importance in terms of their localization and function. The crystal structures obtained from a bacterial homolog, the leucine transporter LeuT$_{Aa}$, in open-to-outward, occluded and open-to-inward conformations are present in frozen state with high resolution. Due to its close kinship with SLC6 proteins, LeuT$_{Aa}$ serves as a paradigm for these transporters.

Methods: In order to address the dynamics of the substrate transport cycle in LeuT$_{Aa}$, we use the lanthanide-based resonance energy transfer (LRET) technique. This method is a spin-off of the fluorescence resonance energy transfer using Förster resonance energy transfer. We employ LRET by introducing the genetically encoded lanthanide-binding tags (LBT) as donor elements. Exogenous cysteine residues labeled with cysteine-specific fluorophores are used as acceptor elements. This technique is an alternative to address the movement of helices with great resolution and has been employed successfully to examine potassium channels.

Results: We screened for the functional LBT mutants using the scintillation proximity assay. The LeuT_A335-LBT-G336 mutant displayed function in terms of its binding activity. Within this background, we generated cysteine mutants. To date, we have successfully measured the intramolecular distances in different LBT_LeuT_Cys mutants. Furthermore, we observed intramolecular distance changes from these purified proteins in detergent micelles. Along these lines we are successfully carrying out LRET measurements of LeuT$_{Aa}$ reconstituted in POPC liposomes, a neutrally charged lipid environment.

Discussion: Our LRET measurements will help us to understand the transport cycle and help to complete the missing steps in the substrate transport cycle of LeuT$_{Aa}$. Currently, we focus on the reconstitution of purified LeuT$_{Aa}$ into liposomes and have our LRET measurements in a reconstituted system that allows us to have a more physiological ionic gradient environment.

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