Sohail *et al.: Intrinsic Activity*, 2015; **3**(Suppl.2):A2.15
http://www.intrinsicactivity.org/2015/3/S2/A2.15
published online: 9 September 2015

21st Scientific Symposium of the Austrian Pharmacological Society: Joint Meeting with the British Pharmacological Society and the Pharmacological Societies of Croatia, Serbia and Slovenia

Graz, 16–18 September 2015

MEETING ABSTRACT

A2.15

Monitoring the movement of helix 1a of LeuT$_{Aa}$ in micelles versus liposome system by using luminescence resonance energy transfer (LRET)

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Background: Solute carrier class 6 proteins (SLC6) have gained a great attention in terms of their pharmacological importance. Malfunctioning of SLC6 proteins results in numerous debilitating central and nervous system diseases. LeuT$_{Aa}$, a bacterial homologue of SLC6 protein, with various high-resolution crystal structures is serving to date as structural and functional paradigm to SLC6 proteins. Large-scale changes in helix 1a (TM1a) of LeuT$_{Aa}$ in solution have been investigated using single molecule FRET studies relating these movements to the substrate-releasing state of LeuT$_{Aa}$. We used LRET as a tool to study the movement of TM1a in micelles as well as in a more native lipid membrane environment.

Methods: Employing lanthanide-based resonance energy transfer (LRET) as a tool of trade, we measured the intramolecular distance changes in LeuT$_{Aa}$. Mutants were screened for their functional activity using scintillation proximity assay. These mutants were further characterized by accessing their uptake activity after successfully reconstituting them in POPC liposomes. LRET-based intramolecular distance measurements were done in DDM detergent micelles from purified pre-labeled proteins. In case of lipid membrane environment, pre-labeled protein was reconstituted into POPC liposomes. Ionic gradient was excluded during measurement in POPC proteoliposomes.

Results: The C-terminal LBT (R519-LBT-G520_LeuT) and its cysteine mutants (R519-LBT-G520_A9C_LeuT) showed substrate binding and transport activity comparable to the wild-type LeuT$_{Aa}$. Focusing TM1a movements in Na^+-bound (outward-open) and Na^+-free (inward-open) conformations of LeuT$_{Aa}$, LRET measurements were carried out. In case of DDM detergent micelles environment TM1a was quite flexible in inward-open *vs.* outward-open conformations. In contrast to detergent micelle environment, lipid environment posed a great constrain over the flexibility of TM1a. These experimental results were also supported strongly with our *in silico* studies. In addition, LeuT$_{Aa}$ was reconstituted into giant unilamellar vesicles (GUVs) of defined composition to gain a gradient over the plasma membrane.

Discussion: Lipid membranes pose a constrained environment for TM1a movement in substrate-releasing conformation. While the constraint of TM1a movement in lipid environment is released for relatively flexible movements of TM1a in detergent micelles system. In-house LeuT$_{Aa}$ GUVs are quite stable and will provide a nice gradient over the plasma membrane.

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