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MEETING ABSTRACT Open Access

Luminescence resonance energy transfer-based intramolecular distance measurements in leucine transporter from *Aquifex aeolicus*

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Background

Solute carrier 6 (SLC6) membrane proteins are integral membrane proteins and of particular pharmacological interest because they are targets of many clinically important drugs. These SLC6 proteins play crucial roles ranging from nutrient uptake to neurotransmitter clearance. A leucine transporter $LeuT_{Aa}$ from *Aquifex aeolicus* has been recognized as a bacterial orthologue of mammalian SLC6 family proteins. $LeuT_{Aa}$ has been crystallized and its structure was resolved to high resolution. With respect to its kinship to other SLC6 transporters, though with low sequence identity (~20–25%), there are crucial regions in transmembrane segments 1, 3, 6 and 8 where conservation reaches ~50%. For this very reason $LeuT_{Aa}$ provides a good structural paradigm to study homology models of SLC6 family members and learn more about the structure/function relationship in mammalian transporters.

Methods and results

In order to test proposed models, we initiated a study to measure intramolecular distance changes associated with the dynamic process of substrate transport. We employed luminescence resonance energy transfer (LRET) to measure the changes in intramolecular distances. For LRET-based measurements we have introduced LBT (lanthanide binding tags) to accommodate terbium, as the donor element, along with cysteines, where acceptor fluorophores are attached, at selected positions in $LeuT_{Aa}$. After expression and purification of these mutants, we obtained the first distances at atomic resolution.

Conclusions

Taken together our LRET measurements can help us to validate or propose a dynamic substrate transport model for $LeuT_{Aa}$. Our future plan focusses on the establishment of functionality assays for screening of functional $LeuT_{Aa}$ mutants along with their LRET measurements.

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