Session III - Membrane Protein Crystallization II

Sunday, July 3 - late afternoon

S3-L1

NEW ADVANCES IN STRUCTURAL DISCOVERY OF HUMAN G PROTEIN-COUPLED RECEPTORS: THE 826 PROJECT AND IMPORTANCE OF LIGAND STABILIZATION

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G protein-coupled receptors (GPCRs) constitute the largest membrane protein family in the human genome, mediating over 80% human cell signaling. Dysregulation of GPCRs could lead to numerous human diseases, including cancer, immune diseases, diabetes, neurodegeneration, cardiovascular disorder, and so on. Structural determination of the receptor in complex with its native or synthetic ligands is the key to understand the ligand recognition and signaling mechanism for GPCRs, and to guide rational drug design. Yet, despite their importance as therapeutic targets, detailed molecular structures of only 30 GPCRs have been determined to date. One of the key challenges to their structure determination is adequate protein expression. On the other hand, recent GPCR structure–function breakthroughs have all required ligand stabilization of the receptor in some manner, highlighting the natural instability of GPCRs. Here we report the quantification of protein expression for all 826 human GPCRs using two different fusion construct sets. Analysis of these data can be used to identify trends related to GPCR expression between different fusion frames and between different GPCR families, and to prioritize lead candidates for future structure determination feasibility. We also initiated a new era of discovery that highlights the importance of ligand–receptor interactions beyond the traditional mindset. We propose that receptor stability is related to receptor folding and residence in the cell membrane, affording a new dimension that should be considered when studying receptor function. Combining the new advances in receptor expression and ligand stabilization, we show some successful case studies at the end.

S3-L2

MECHANISM OF INHIBITION OF hGLUT1 IS CONSERVED BETWEEN CYTOCHALASIN B AND PHENYLALANINE AMIDES

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Human glucose transport (hGLUT1) belongs to a family of homologous sugar transporters or co-transporters found in both prokaryotes and eukaryotes [1]. It is a uniporter that transports glucose from the extracellular matrix into cells [2]. Cancerous cells have an acutely increased demand for energy leading to increased levels of hGLUT1 [3]. This highlights hGLUT1 as an important prognostic indicator in several cancer types [4, 5], as well as a potential new target for therapeutic inhibitors. Here we present inhibitor-bound inward-open structures of wild-type hGLUT1 crystallized with three different inhibitors: cytochalasin B, a nine-membered bicyclic ring fused to a 14-membered macrocycle, which has been described extensively in the literature of hGLUTs [6] and two novel phenylalanine amide

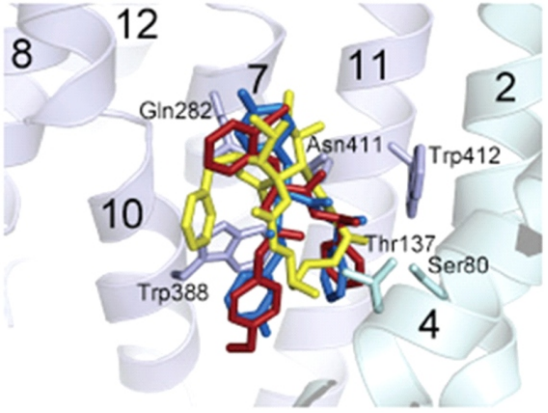

Figure 1. Overlapping inhibitor-binding sites in hGLUT1.

derived inhibitors. Despite very different chemical backbones, all three compounds bind in the central cavity of the inward-open state of hGLUT1 and all binding sites overlap the glucose-binding site (Figure 1). The inhibitory action of the compounds was determined for hGLUT family members, hGLUT1-4, using cell-based assays, and compared to homology models for these hGLUT members. This uncovered a probable basis for the observed differences in inhibition between family members.

We pinpoint regions of the hGLUT proteins that can be targeted to achieve isoform selectivity, and show that these same regions are used for inhibitors with very distinct structural backbones. These structures provide an important structural insight for the design of more selective inhibitors for hGLUTs and hGLUT1 in particular. Thus our results emphasize that modulation of glucose import by hGLUTs should focus on making good interaction points for compounds and that the actual chemical backbone of the inhibitor is of less importance.

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S3-L3

PURIFICATION AND CRYSTALLIZATION OF AN ANTIGENIC OUTER-MEMBRANE PROTEIN FROM *SALMONELLA TYPHI*

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ST50, an outer-membrane component of the multi-drug efflux system from *Salmonella enterica* serovar Typhi, is an obligatory diagnostic antigen for typhoid fever. ST50 is an excellent and unique diagnostic antigen with 95% specificity and 90% sensitivity and is used in the commercial diagnosis test kit (TYPHIDOT^{TM}). The crystal structure of ST50 at a resolution of 2.98 Å reveals a trimer that forms an -helical tunnel and a -barrel transmembrane channel traversing the periplasmic space and outer membrane. Structural investigations suggest significant conformational variations in the extracellular loop regions, especially extracellular loop 2. This is the location of the most plausible antibody-binding domain that could be used to target the design of new antigenic epitopes for the development of better diagnostics or drugs for the treatment of typhoid fever. A molecule of the detergent n-octyl- -D-glucoside is observed in the D-cage, which comprises three sets of Asp361 and Asp371 residues at the periplasmic entrance. These structural insights suggest a possible substrate transport mechanism in which the substrate first binds at the periplasmic entrance of ST50 and subsequently, via iris-like structural movements to open the periplasmic end, penetrates the periplasmic domain for efflux pumping of molecules, including poisonous metabolites or xenobiotics, for excretion outside the pathogen. The rational designs and strategies of purification and crystallization of membrane protein ST50 for the structural study will be presented and discussed.

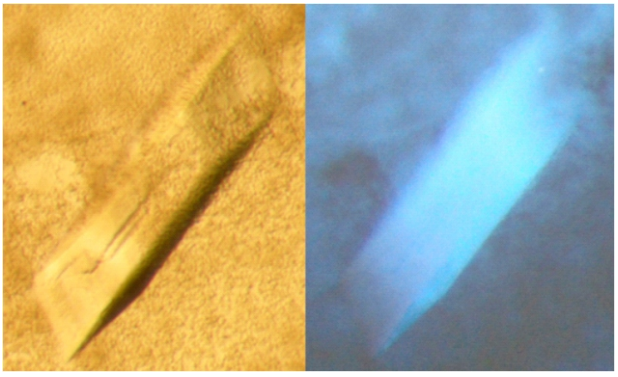

Figure 1. The single crystal of ST50 was illuminated under the white light (left) and the UV light (right).

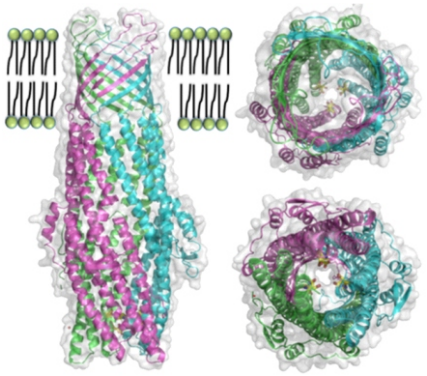

Figure 2. The structure of ST50 is viewed from the side (left), top (upper right) and bottom (bottom right)

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S3-L4

MICROSEED MATRIX-SCREENING (RMMS): INTRODUCTION, THEORY, PRACTICE AND A NEW TECHNIQUE FOR MEMBRANE PROTEIN CRYSTALLIZATION IN LCP

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Random Microseed Matrix-Screening (rMMS), where seed crystals are added automatically to random crystallization screens, is a significant recent breakthrough in protein crystallization [1]. During the eight years since the method was published, theoretical understanding of the method has increased [2 - 4], and several important practical variations of the basic method have emerged [5, 6]. We will briefly describe some of these variations, including cross-seeding, and introduce a novel method of making LCP seed stocks by scaling up LCP crystallization conditions. We will also describe a method of generating seed gradients across a plate so that the number of crystals in each LCP bolus can be varied, with a practical example.

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