Integrating single-particle cryo-electron microscopy and hydrogen/ deuterium exchange mass spectrometry to elucidate the structure and dynamics of G-protein coupled receptors

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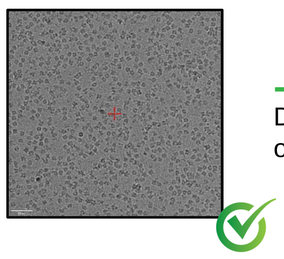
Abstract

Single-Particle Cryo-Electron Microscopy (cryo-EM) has revolutionized the structural biology field by allowing high-resolution imaging of challenging macromolecules, such as membrane proteins, in their native lipid environment. Among these membrane proteins, G protein-coupled receptors (GPCRs) play a key role in cell signaling pathways and thus represent a target for developing new drugs. TGR5, a member of the GPCR family, plays a pivotal role in bile acid signaling and has emerged as a promising therapeutic target for various metabolic and inflammatory diseases. We showcase how cryo-EM has enabled us to obtain atomic-level insights into the architecture of TGR5, shedding light on its ligand binding sites, conformational changes, and membrane interactions. Complementing cryo-EM, HDX-MS offers a unique perspective on protein dynamics by measuring the exchange rates of hydrogen and deuterium atoms in proteins. We present our findings on applying HDX-MS to probe the flexibility and conformational dynamics of TGR5. By mapping regions of TGR5 susceptible to deuterium exchange, we gain insights into the conformational changes associated with ligand binding, activation, and signal transduction. The integration of cryo-EM and HDX-MS represents a powerful approach to comprehensively characterize the structure-function relationship of TGR5 and membrane proteins in the broadest sense. We highlight the significance of combining cutting-edge structural and dynamic techniques in unraveling the mysteries of membrane proteins, with implications for drug discovery and precision medicine.

Methods

Sample

Grid preparation/ screening



Data collection on Glacios

Thermo Fischer Glacios
Low-medium resolution

Data collection on Krios

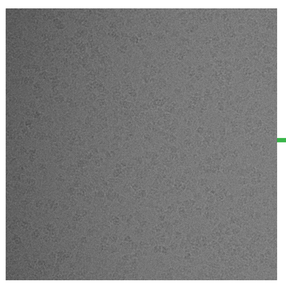
Thermo Fischer Titan Krios
High resolution

HDX-MS

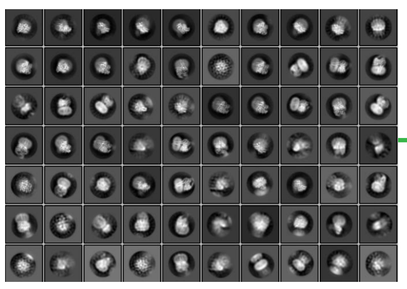
ESI-Q-TOF mass spectrometer (Xevo G2-XS)

Results

A TGR5 / Protein G / P395 / Nb35 complex

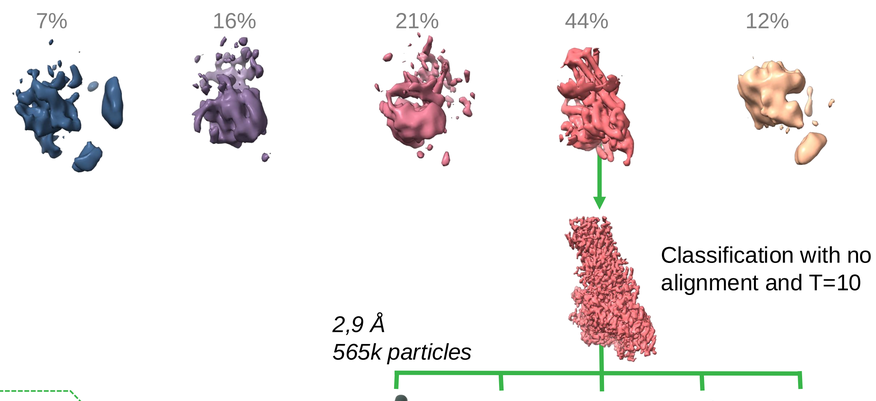


9700 movies collected on Titan Krios G4 equipped with Selectris X energy filter. Pixel size = 0,731Å



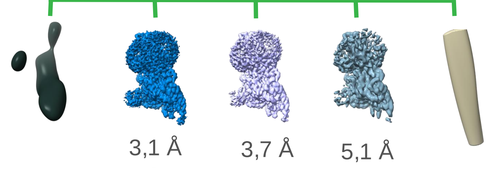
2D Classification:
1 269 000 particles selected

Classification with downscaled particles



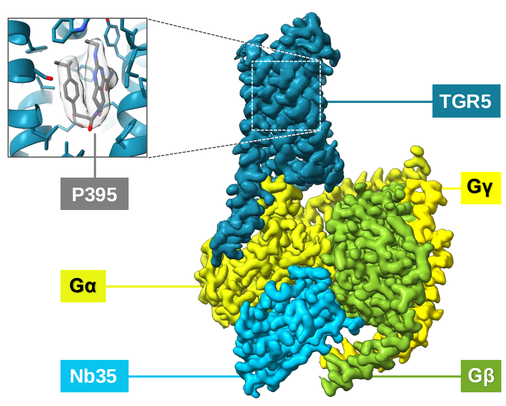
Classification with no alignment and T=10

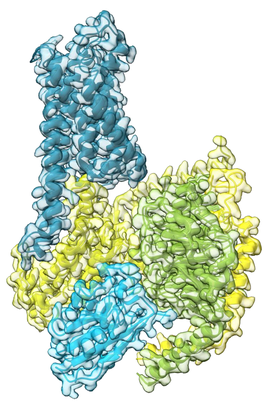
2,9 Å
565k particles

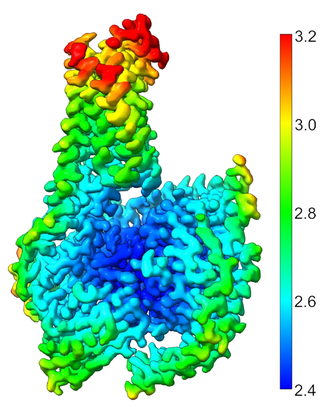


Final refinement after CTF optimization and polishing

2,5 Å
160k particles

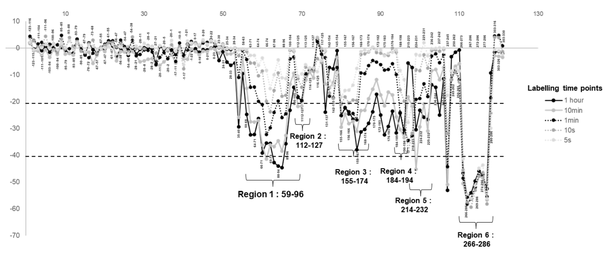






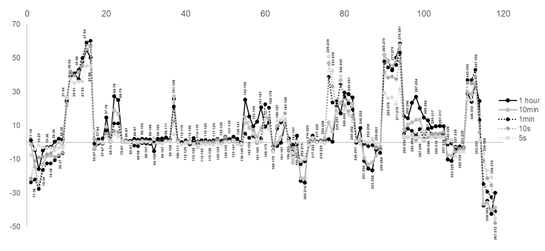
HDX-MS results: HoloTGR5 VS ApoTGR5

Differential relative uptake (%) : HoloTGR5 – ApoTGR5

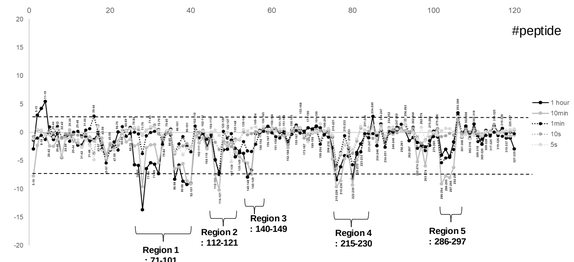


HDX-MS results: HoloGα VS ApoGα

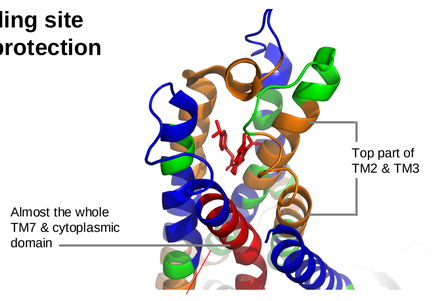
Differential relative uptake (%) : HoloGα–ApoGα

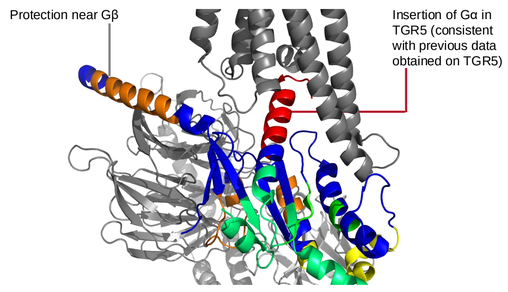


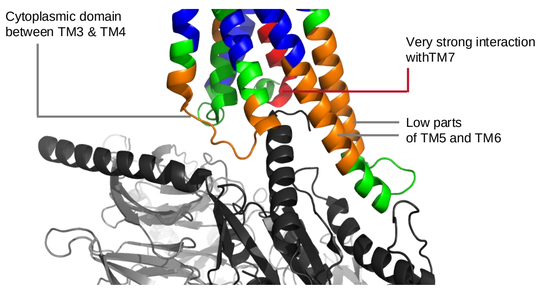
HDX-MS r esults: HoloGβ VS ApoGβ



P395 binding site induced protection effect







No HDX-MS data
Highly exposed area (+30%>X)
Exposed area (+30%>X>+10%X)
Covered regions with no HDX difference
Protected area (-10%<X<-30%)
Hot spot (-30%<X)

Summary

Here we demonstrated the powerful integrated CryoEM and HDX-MS workflow. The TGR5 structure has been successfully elucidated at an exceptionally high resolution, revealing the binding site of the ligand P395. Moreover, we managed to resolve a flexible domain that is visible for the very first time. This observation aligns with the high-quality HDX-MS data we have gathered. Collectively, these data provide invaluable insights into the dynamics of TGR5 and can potentially be extrapolated to shed light on various G protein-coupled receptors (GPCRs) and, more broadly, other proteins. Novalix has played a pivotal role in producing all these proteins, generating quantities and qualities compatible with our research methods.

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