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BOOK OF ABSTRACTS

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Capturing Experimental Dynamics of Human Glutamine Synthetase by Single-Particle Cryo-EM and Neural Ab Initio Reconstruction

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Human glutamine synthetase (hGS) toggles between catalytically active decamers and catalytically inert filaments, yet the structural pathway linking these assemblies remains poorly defined. Single-particle analysis cryo-EM (SPA) can, in principle, capture the full spectrum of conformers present in vitrified samples, but conventional consensus reconstruction averages these states into a single map, obscuring site-specific and assembly-level heterogeneity. We seek to combine high-throughput cryo-EM data collection with deep generative latent-variable modelling to resolve discrete particle subsets that represent the “experimental dynamics” of hGS. Starting from datasets collected in the presence of catalysis substrates or products, we first perform ab initio reconstructions enforcing or relaxing D5 symmetry. Machine learning-based clustering reveals particle classes in which active-site densities vary from fully occupied to vacant, indicating that substrate binding does not occur uniformly across the decameric ring. These partially occupied intermediates suggest cooperative or anti-cooperative communication between neighboring active sites, challenging the assumption of ten-site equivalence. Parallel datasets of filamentous hGS, obtained by nucleotide depletion and low ionic strength, are subjected to identical analysis. Filament particles are consistent with a conformational “lock” via a truly symmetric hydrophobic interface that excludes ligand binding and stabilizes the inactive state. Comparative principal-component analysis of latent vectors from decameric and filamentous reconstructions maps the trajectory of structural change, highlighting a progressive opening at the subunit interfaces that correlates with loss of catalytic competence. We seek to showcase how single-particle cryo-EM, augmented by data-driven neural modelling, can dissect dynamic equilibria that underlie enzyme regulation. Beyond providing a framework for hGS, the workflow is broadly applicable to other metabolic assemblies where functional asymmetry and assembly-state transitions modulate activity.