From Molecular Motion to Clinical Insight: Protein Conformational Dynamics as Biomarkers in Ophthalmic Imaging

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1. Main Text

Vision is a complex process governed by an orchestrated interplay of proteins distributed across retinal tissues. Central to this are the phototransduction cascade and visual cycle, driven respectively by proteins such as phosphodiesterase 6 (PDE6) and retinol-binding protein 3 (RBP3). Phototransduction initiates with visual pigment activation, triggering G-protein signaling that activates PDE6, leading to cGMP hydrolysis, channel closure, and photoreceptor hyperpolarization. In parallel, the visual cycle regenerates 11-cis-retinal via enzymatic isomerization of all-trans-retinol, enabling continued photon capture by photoreceptors. The interphotoreceptor matrix, where RBP3 resides, supports retinoid transport between photoreceptors and the retinal pigment epithelium (RPE) to sustain this cycle.

Dysfunctions in these proteins contribute to retinal diseases [1], but their molecular transitions may also serve as functional biomarkers. Functional imaging techniques, including two-photon imaging and optoretinography (ORG), have emerged to noninvasively track biochemical and structural retinal changes. Two-photon imaging allows in vivo assessment of retinoid visual cycle intermediates [2, 3], while ORG captures nanometer-scale outer segment length modulations upon light stimulation within milliseconds [4, 5]. However, connecting these physical changes to precise molecular mechanisms is essential for their diagnostic application.

In our studies, we apply structural biology to define the molecular basis behind these imaging biomarkers. Recent improvements in cryoEM resolution enabled us to resolve porcine RBP3 structure at 3.67 Å, revealing its conformational flexibility upon ligand binding, with implications for its role in diabetic retinopathy diagnostics through two-photon-based quantification [6, 7]. Complementarily, we established that conformational changes in PDE6 drive nanometer-scale outer segment elongations measurable by flicker optoretinography (f-ORG), using spatiotemporal OCT combined with pharmacological PDE6 inhibition to confirm the direct link between phototransduction events and ORG signals [8]. These integrative approaches provide mechanistic insight into how conformational transitions of key visual proteins are translated into functional imaging biomarkers, opening new avenues for non-invasive retinal diagnostics and monitoring.

2. Methods and Results

- Protein purification and biophysical characterization
- CryoEM
- CryoET
- cryoSXT
- cryoFIB SEM
- TEM
- SAXS

We improved the global resolution of porcine RBP3 from the previous 7.4 Å to 3.67 Å by cryoEM [7, 9], revealing its modular architecture and ligand-induced conformational changes (Fig. 1A, B). SAXS experiments confirmed RBP3's ability to adopt distinct conformations upon retinoid binding [10], shifting from a compact to an extended conformation (Fig. 1C). These findings reinforce its role in retinoid trafficking and potential as a biomarker in diabetic retinopathy [6].

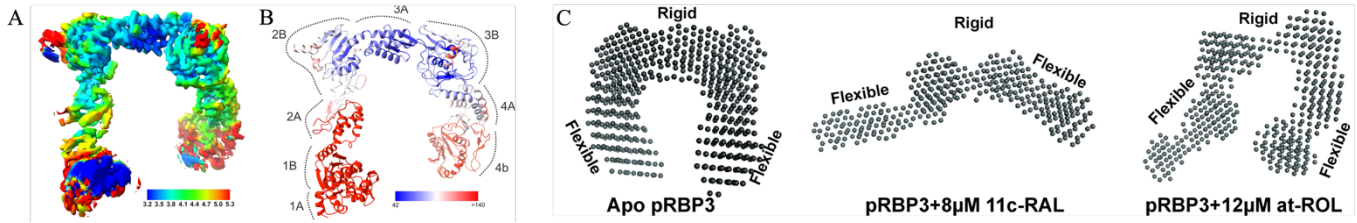

Fig. 1. Porcine RBP3 structure. A) CryoEM-density map filtered and coloured according to local resolution. B) Cartoon representation of the refined atomic model of pRBP3 coloured by residue-averaged atomic B factor. C) DAMMIF-averaged ab initio models of apo pRBP3, pRBP3 with 8 μM 11c-RAL, and pRBP3 with 12 μM at-ROL.

To elucidate the molecular basis of ORG signals, we employed spatiotemporal optical coherence tomography (STOC-T) and demonstrated that PDE6 conformational transitions propagate into nanometer-scale photoreceptor outer segment elongations,

synchronized with light flicker stimuli [8]. Using sildenafil as a PDE6 inhibitor in rodent models, we confirmed that blocking PDE6 activity significantly attenuates these light-induced elongations, directly linking phototransduction to mechanical optical signals captured in ORG [8]. Additionally, we developed protocols to isolate rod outer segments (ROS) and apply cryoET, cryoSXT, cryoFIB-SEM, and TEM imaging to extract disc-spacing parameters (Fig. 2A–D), while in vitro assays with purified porcine PDE6 further characterized its catalytic activity (Fig. 2E).

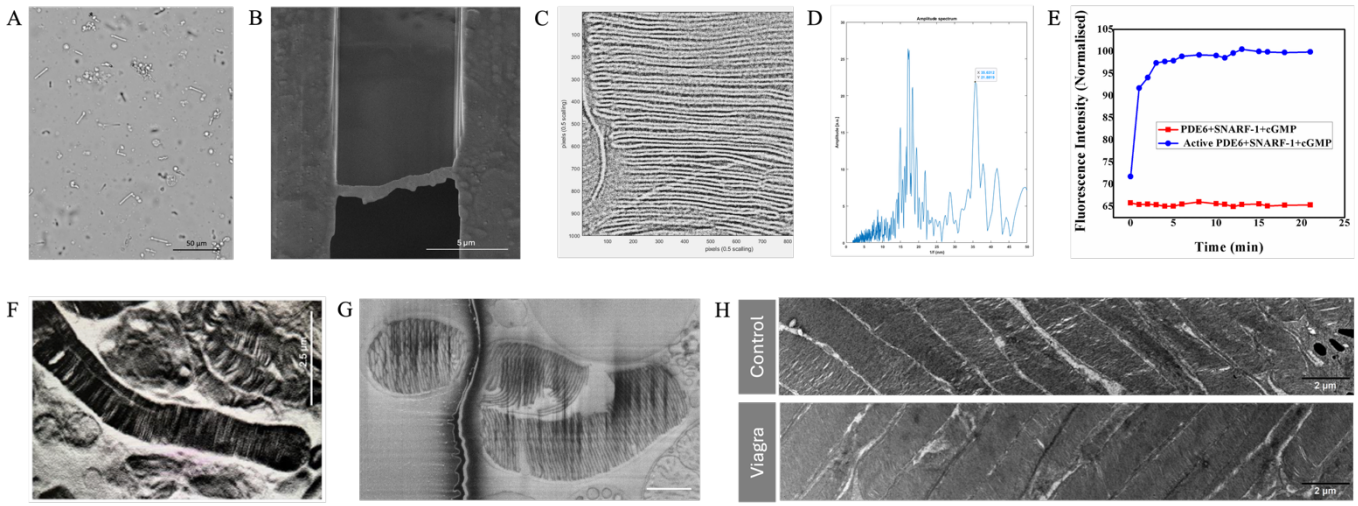

Fig. 2. Mouse ROS and isolation and characterization. A) Mouse ROS visualized under a light microscope. (B) FIB lamella preparation from ROS cryo grids. (C) CryoET ROS tomograms. (D) Distances between disc measurements. (E) PDE6 activity assay, measuring degradation of cGMP. (F) CryoSXT. (G) CryoFIB SEM. (H) TEM.

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