Structural and Functional Insights into Retinol-Binding Protein 3: CryoEM and SAXS Reveal Ligand-Induced Conformational Dynamics Relevant to Retinal Disease

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1. Main Text

The vertebrate visual cycle relies on the continuous regeneration of 11-cis-retinal, the chromophore essential for phototransduction in photoreceptors. This process demands efficient intercellular transport of retinoids between photoreceptors and the retinal pigment epithelium (RPE). Retinol-binding protein 3 (RBP3), a 140 kDa glycoprotein localized in the interphotoreceptor matrix (IPM), is proposed to mediate these retinoids shuttling. Its unique architecture, comprising four homologous ligand-binding modules, allows it to bind both retinoids and fatty acids, orchestrating precise molecular exchange across cell types [1].

Despite its physiological relevance, the high-resolution structure of full-length native RBP3 has remained elusive. Prior structural work included isolated modules and a low-resolution cryoEM reconstruction of bovine RBP3 bound to a monoclonal antibody fragment [2]. Dysfunction of RBP3 has been associated with multiple retinal disorders, including diabetic retinopathy, retinitis pigmentosa, pan-retinal degeneration, and myopia [3-5], highlighting the need for detailed molecular characterization.

Here, we present an integrative structural biology study resolving the full-length porcine native RBP3 structure by single-particle cryo-electron microscopy (cryoEM) at 3.67 Å resolution. Complementary small-angle X-ray scattering (SAXS) experiments revealed conformational flexibility upon ligand binding, while molecular docking identified putative retinoid and fatty acid binding modes. Fluorescence-based binding assays confirmed RBP3's interaction with both retinoids (11-cis-retinal, all-trans-retinol) and fatty acids (oleic acid, DHA), suggesting dynamic regulation of ligand exchange within the IPM [6].

The structural model elucidates interdomain arrangements and provides a mechanistic basis for previously identified pathogenic mutations in RBP3 linked to retinitis pigmentosa and inherited myopia [4, 5]. Furthermore, observed ligand-dependent conformational shifts may underlie dynamic retinoid delivery under physiological conditions and support emerging two-photon-based functional imaging strategies targeting RBP3 as a potential biomarker [1, 7, 8]. These results advance our understanding of RBP3's role at the interface of structural biology and functional imaging, with translational implications for retinal disease diagnostics and therapeutics.

2. Methods and Results

- Purification of native porcine RBP3 from interphotoreceptor matrix
- CryoEM single-particle data collection and structure refinement at 3.67 Å
- Fluorescence-based ligand binding assays for retinoids and fatty acids
- SAXS experiments to probe conformational flexibility upon ligand binding
- Molecular docking to identify putative ligand-binding sites

CryoEM analysis resolved the full-length RBP3 in apo state (Fig. 1A), revealing domain arrangements and intrinsic flexibility [6]. SAXS titration experiments demonstrated ligand-induced conformational changes shifting between compact and extended states (Fig. 1C). Binding assays confirmed preferential binding to 11-cis-retinal, while molecular docking localized distinct hydrophobic binding pockets for retinoids and fatty acids.

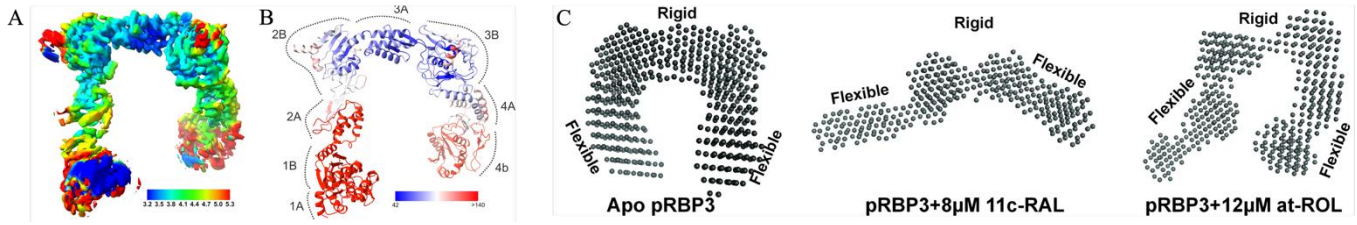

Fig. 1. Porcine RBP3 structure. A) CryoEM-density map filtered and coloured according to local resolution. B) Cartoon representation of the refined atomic model of pRBP3 coloured by residue-averaged atomic B factor. C) DAMMIF-averaged ab initio models of apo pRBP3, pRBP3 with 8 μM 11c-RAL, and pRBP3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

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4. References

1. Palczewski, K. and P.D. Kiser, *Shedding new light on the generation of the visual chromophore.* Proc Natl Acad Sci U S A, 2020. **117**(33): p. 19629–19638.
2. Sears, A.E., et al., *Single particle cryo-EM of the complex between interphotoreceptor retinoid-binding protein and a monoclonal antibody.* FASEB J, 2020. **34**(10): p. 13918–13934.
3. Garcia-Ramirez, M., et al., *Interphotoreceptor retinoid-binding protein (IRBP) is downregulated at early stages of diabetic retinopathy.* Diabetologia, 2009. **52**(12): p. 2633–41.
4. Li, S., et al., *Secretory defect and cytotoxicity: the potential disease mechanisms for the retinitis pigmentosa (RP)-associated interphotoreceptor retinoid-binding protein (IRBP).* J Biol Chem, 2013. **288**(16): p. 11395–406.
5. Georgiou, M., et al., *RBP3-Retinopathy-Inherited High Myopia and Retinal Dystrophy: Genetic Characterization, Natural History, and Deep Phenotyping.* Am J Ophthalmol, 2024. **258**: p. 119–129.
6. Kaushik, V., et al., *CryoEM structure and small-angle X-ray scattering analyses of porcine retinol-binding protein 3.* Open Biol, 2025. **15**(1): p. 240180.
7. Kaushik, V., et al., *Towards a New Biomarker for Diabetic Retinopathy: Exploring RBP3 Structure and Retinoids Binding for Functional Imaging of Eyes In Vivo.* Int J Mol Sci, 2023. **24**(5).
8. Yokomizo, H., et al., *Retinol binding protein 3 is increased in the retina of patients with diabetes resistant to diabetic retinopathy.* Sci Transl Med, 2019. **11**(499).