



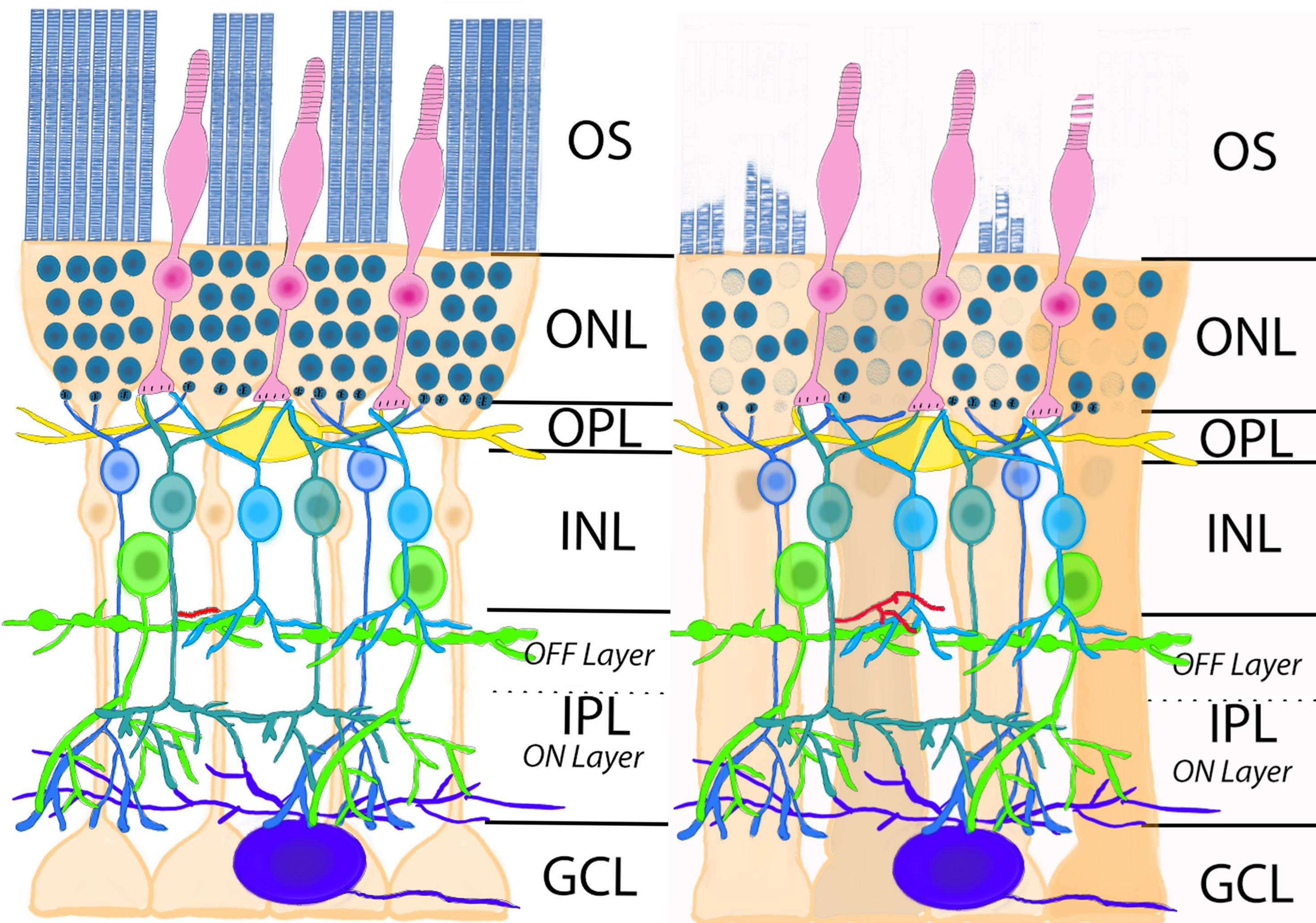
# OFF-layer Branches of ON Cone Bipolar Cells in Early Retinal Degeneration

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### Purpose:

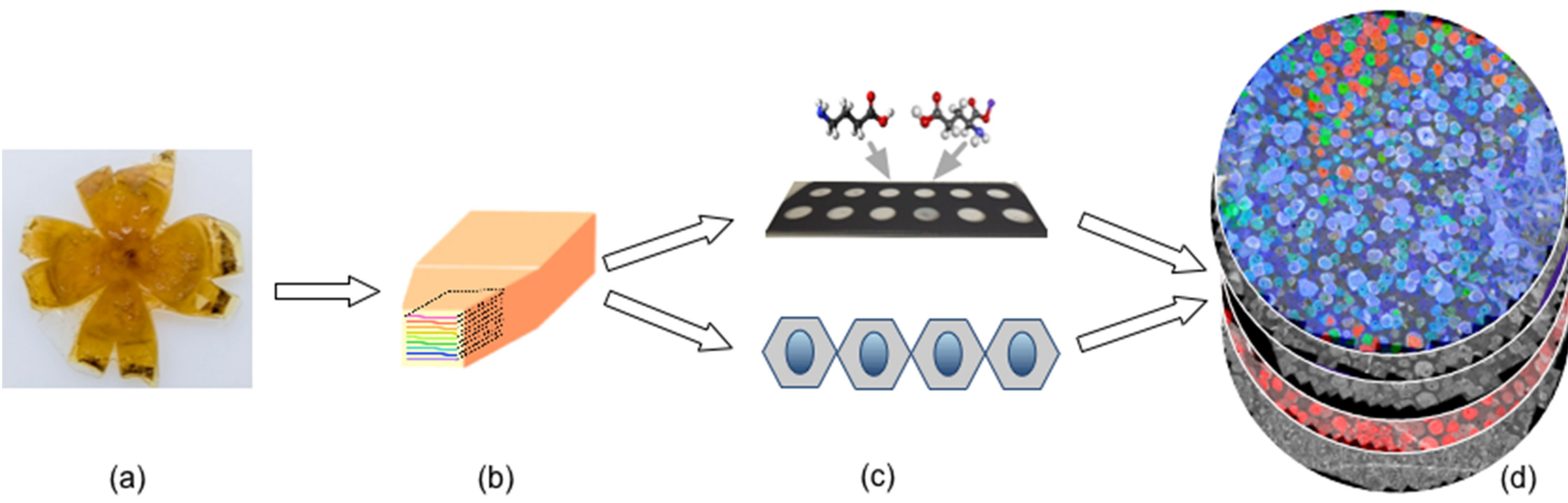
Cone bipolar cells are customarily classified into superclasses of ON (ON-BCs) and OFF (OFF-BCs). ON or OFF specialization is further segregated by stratification within the inner plexiform layer. Retinal degeneration induces negative plasticity termed remodeling, that includes aberrant neurite extension from multiple cell types (rewiring) and ON-BCs switching their glutamate receptors to match that of OFF-BCs (reprogramming). Previous analysis in healthy retina shows that ON-BCs can make small ribbon synapses in the descending axon or, less frequently, simple single branch projections in the OFF layer. What impact remodeling has on these OFF-layer branches is unknown. In this study, we compare OFF branches from ON-BCs in a connectome of early retinal degeneration (RPC1) to our healthy retinal connectome (RC1).



**Figure 1: Basic anatomy of the eye and retina layer.** Retinal layers in the healthy retina vs degenerative retina. Off layer branch of a ON-BC is represented in red.

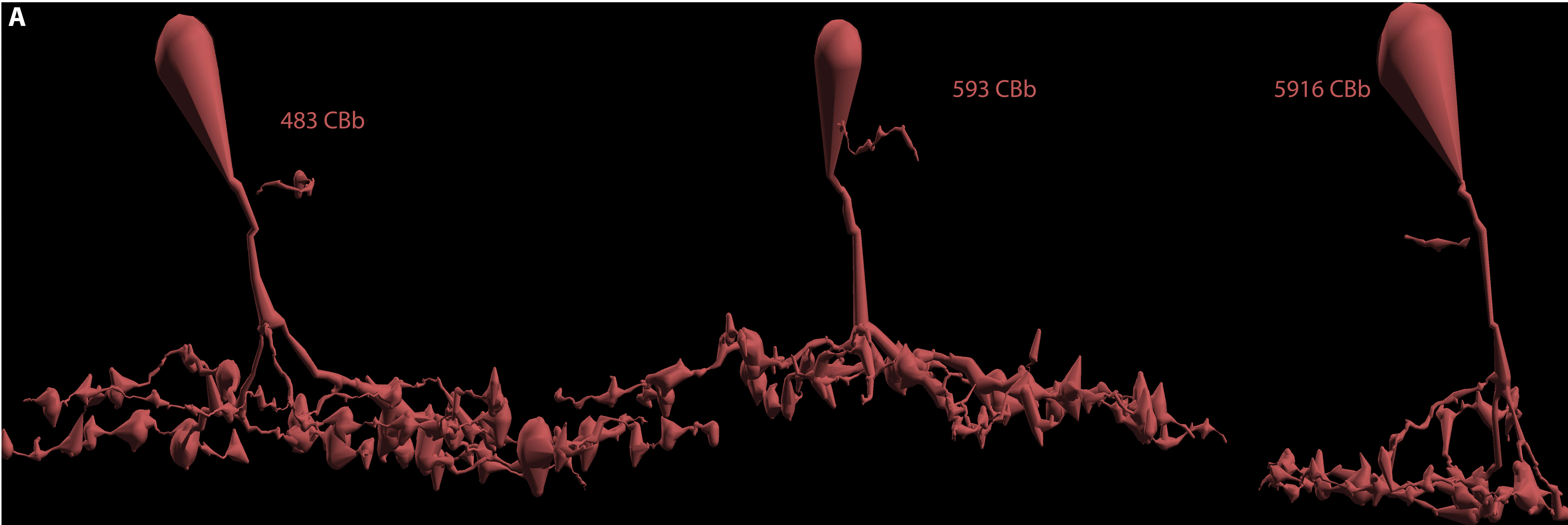
### Methods:

Retinal tissues selected for RC1 and RPC1 were collected post-mortem from a 13 month old Dutch-belted healthy female rabbit and a male 10 month old transgenic P347L rabbit model of autosomal dominant retinitis pigmentosa, respectively. RPC1 shows signs of remodeling including rod outer-segment degeneration and aberrant neurite extension. Tissues were fixed in mixed aldehydes and subsequently osmicated, dehydrated, resin embedded, and sectioned at 70 nm. Sections were placed on formvar grids, stained, and imaged at 2nm/px on a JEOL JEM-1400 TEM using SerialEM software. 1 section was reserved from every 30 for Computational Molecular Phenotyping, and probed for small molecules (Figure 2). Both volumes were evaluated using the Viking software suite.



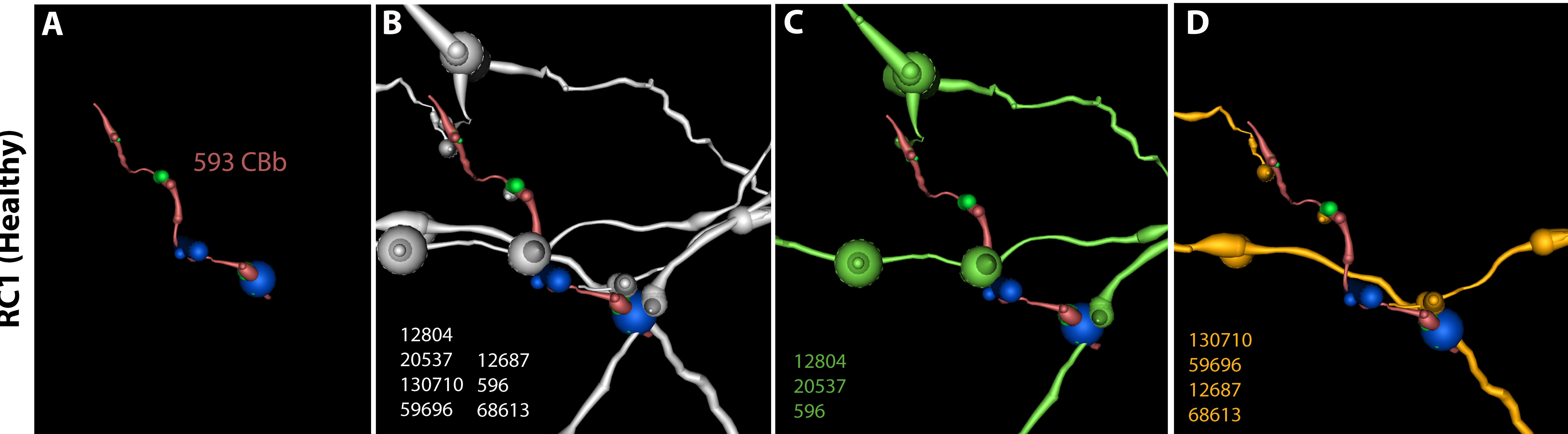
**Figure 2: Fixed retinal tissue process** (a) Dehydrated fixed tissue. (b) Resin-embedded and sectioned (c) Sections for TEM placed on formvar grids. 1 in 30 reserved for Computational Molecular Phenotyping (d) Serial TEM sections aligned and assembled into 946 section volume with 14 interleaved CMP sections.

**A**



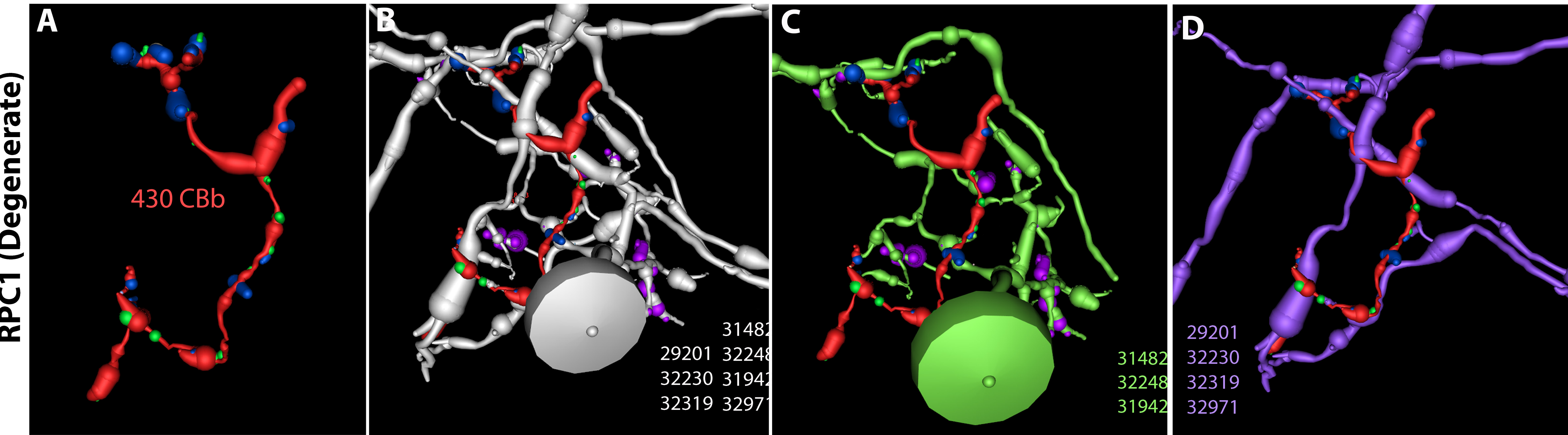
**Figure 3: ON Cone Bipolar Cells Morphology** Scaled 3D renderings of ON- Cone BCs from RC1 and RPC1 (A) Of 145 classified ON-Cone BC's in RC1, 7 have morphologically shown branching in the OFF layer. ON-Cone BC 593 is the most complex of the 7. (B) There are currently 28 identified ON-Cone BC's in RPC1. ON-Cone BC 430 demonstrates OFF layer branching significantly more complex than OFF branching found in healthy retina.

**A**



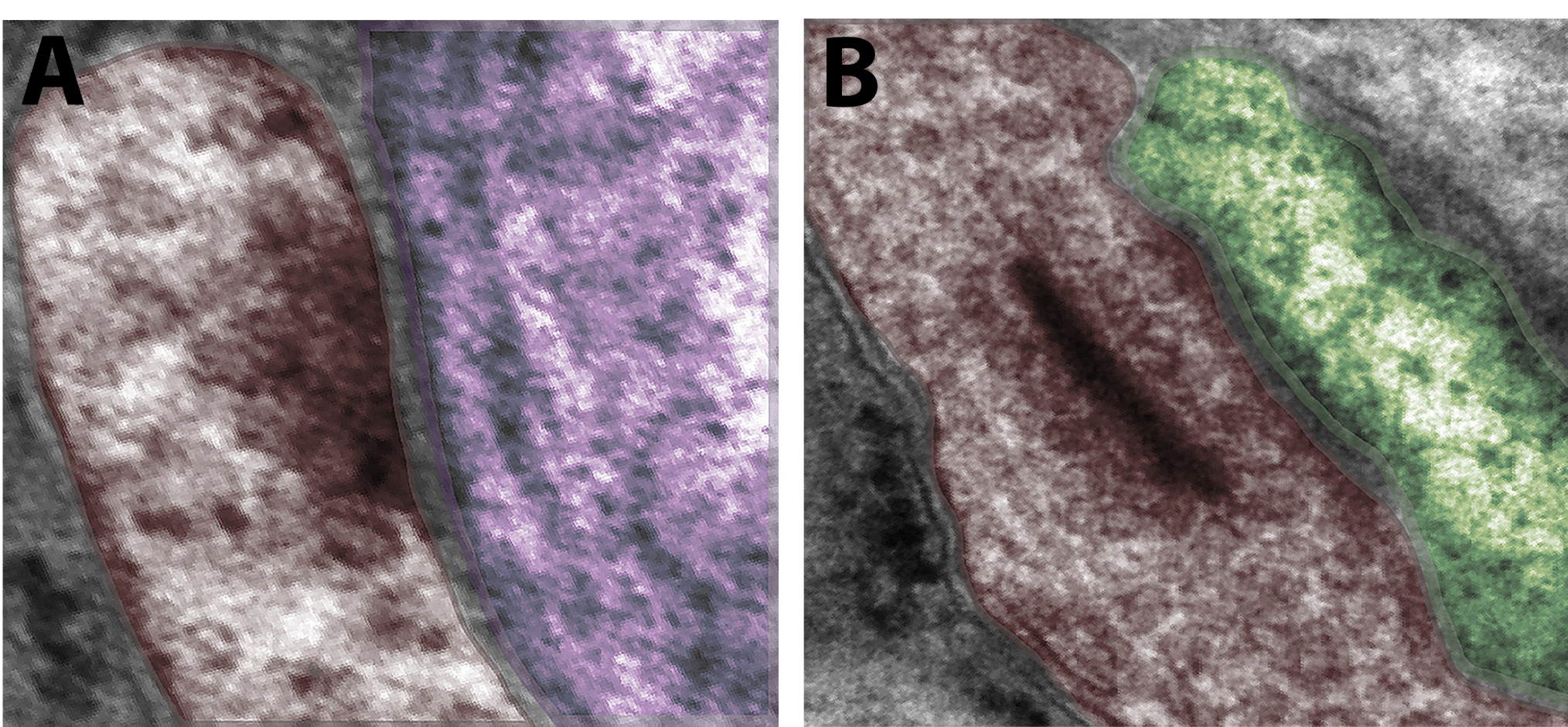
**Figure 4: ON-Cone BC 593. ON-Cone BC 593 OFF Layer Branch Synaptic Partners** (A)OFF-Layer branch showing Ribbon synapse and BCS. (B) 593 Ribbon and Bipolar Conventional Synaptic partner cells. (C) Amacrine synaptic partner cells. (D) Unknown synaptic partner cells.

**A**



**Figure 5: ON-Cone BC 430. ON Cone BC 430 OFF Layer Branch Synaptic Partners** (A) OFF- Layer branch showing Ribbon synapse and BCS (B) 430 Ribbon and BC Conventional Synaptic Partner Cells. (C) Amacrine Synaptic partner cells. (D) Ganglion Synaptic partner cell.

**A**

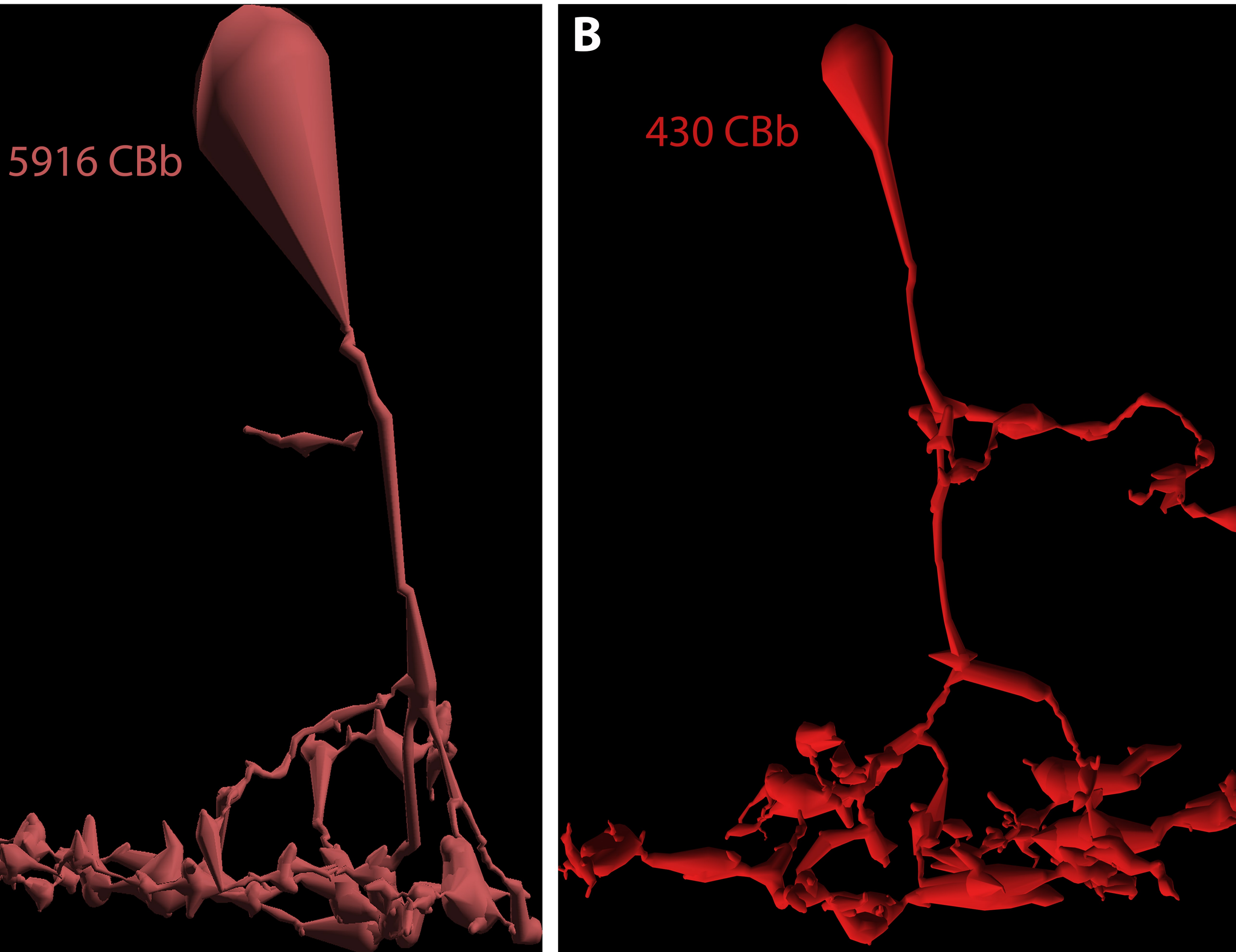


**Figure 6: Synapses** (A) Pre-Synaptic BC Conventional to post synaptic Ganglion Cell. (B) Pre-synaptic BC Ribbon to post-synaptic Amacrine cell.

593	Ribbon	BC Conventional	430	Ribbon	BC Conventional
Total	213	109	Total	177	71
OFF-Layer Branch	9	3	OFF-Layer Branch	20	13
% OFF Branch	4.22%	2.75%	% OFF Branch	11.30%	18.31%

**Table 1: Ribbon and BC Conventional Synapses.** (A) Table of synapses in cell 593 comparing the number of synapses in the OFF-Layer branch and entire cell. (B) Table of synapses in cell 430 comparing the number of synapses in the OFF-layer branch and entire cell.

**B**



**Figure 8: OFF layer branching synaptic partners 430 and 593.** (A) BC 593 web of synaptic partners in the OFF-Layer. 4 Unknown partners and 3 Amacrine partners (B) BC 430 web of synaptic partners in the OFF-Layer. 8 Amacrine Partners and 9 Ganglion partners.

### Conclusions:

- Morphological differences in the ON-Cone Bipolar Cells OFF layer branching: OFF-Layer branch of ON-Cone bipolar cell 430 in degenerate retina is more complex and the sprouts are longer.
- More Ribbon and BC synapses in OFF branch in degenerate retina vs Healthy retina. There is a higher percentage of OFF-layer synapses in comparison to the total number of Ribbon and BCS.
- GC 32230 is post synaptic to ON-BC 430 and OFF -BC 33285, indicating possible change in cell identity or a previously undescribed crossover motif.

### Related Projects:

- Crystal L. Sigulinsky**  
Coupling architecture of the Aii/ON cone bipolar cell network in degenerate retina  
536-6441  
11:15 am
- Selena Wirthlin**  
548 - B0010  
Comparative anatomy and connectivity of the Aii amacrine cell in mouse and rabbit retina
- Jeebika Dahal**  
551 - B0013  
Aii Amacrine Cell Connectivity in Degenerating Retina

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