# IRBP protects retinoids from photodecomposition and effect of glycation – Pump-probe paradigm using an integrated cavity light toxicity chamber and rapid scanning spectrophotometry

#4456

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## PURPOSE AND BACKGROUND

Interphotoreceptor retinoid-binding protein (IRBP, RBP3) is the most abundant protein in the subretinal space. Decreased [IRBP] is associated with DR and increased [IRBP] is protective. IRBP has free radical scavenging activity and protects visual cycle retinoids from photodegradation [2-4]. However, little is known regarding the mechanism(s). Analyzing the process is challenging because light must be used to simultaneously cause and monitor photodecomposition. Furthermore, the low sensitivity of traditional spectrophotometers with 1 cm pathlength cuvettes, and the common problem of light scatter are obstacles.

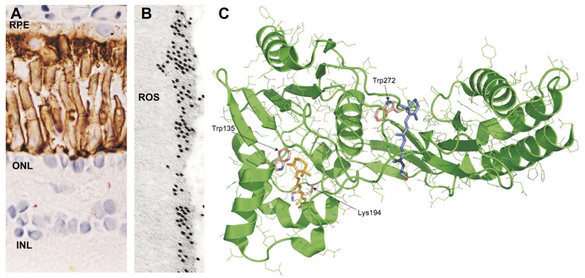


**Fig 1.** (**A**) IRBP in the albino Xenopus retina, ABC-immunochem.; adapted from Hessler *et al*, 1996. J Comp Neurol). (**B**) Immuno-EM localization of IRBP to matrix coating the rod outer segment (10 nm colloidal goal; compare with Gonzalez-Fernandez et al, 1998. Curr Eye Res). (**C**) Structure of X2IRBP docked with AT-retinol. [1]

## METHODS

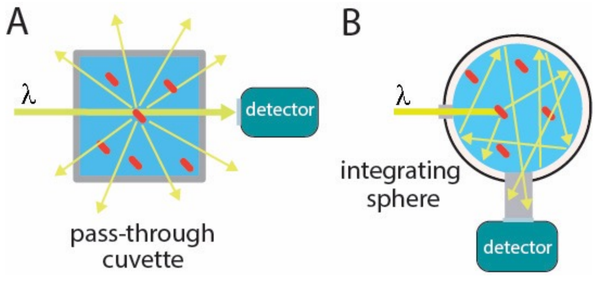


**Fig 2.** Comparison of standard cuvette (**A**) with integrating sphere (**B**) [4]. In rectangular cuvettes suspended material scatters the incoming light. In contrast, the integrating chamber is spherical and coated with a reflective material. The incoming light beam (λ) enters at left and instantly becomes diffuse. The diffuse light is detected at one port attached to the detector. This diffuse light is not influenced by particulates – only absorbance of light by the sample can affect the output to the detector.

Advantages of the integrating sphere:

1) Entire contents are exposed to the light beam (useful for photodecomposition studies).
2) Increased sensitivity due to increased pathlength.
3) The effect of light scatter is mitigated.

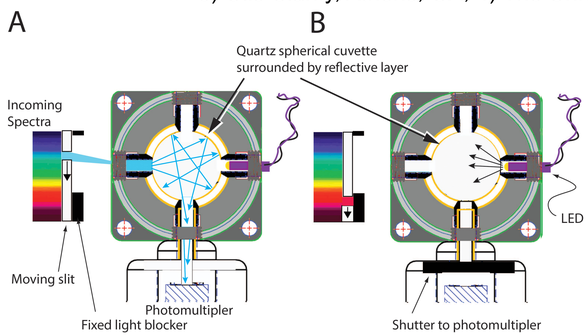


**Fig 3.** Rapid Scanning Monochromator (RSM) with integrating cavity absorption meter and actinic LED source [5]. **A**: Scan phase: The moving slit from the RSM creates 100 spectral scans /sec. The spherical quartz cuvette is surrounded by four light ports. Incoming light enters through the port opposite to the LED. The cuvette is surrounded by a reflective material scattering the measurement beam as well as the light from the LED. **B**: Actinic exposure pulse: A fixed light block creates a dark period within each scan. During this period, the LED is triggered to flash, and the photomultiplier shutter closes. By interleafing scans with LED flashes, light-induced chemical changes can be followed spectroscopically in real time.

## RESULTS

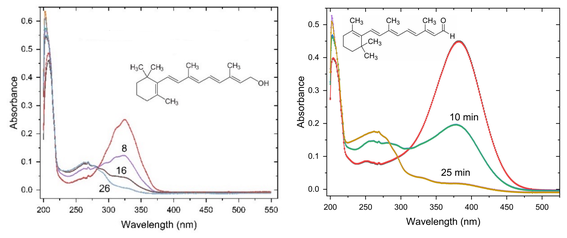


**Fig 4.** All-*trans* retinol and retinal readily photodecompose in presence of light. Here rectangular cuvettes containing the retinoid were exposed to direct sunlight for the minutes shown.

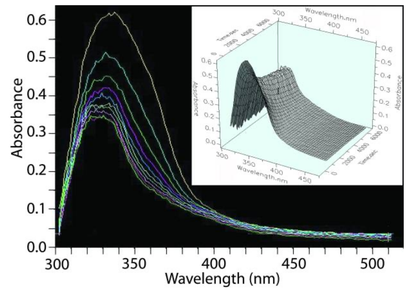


**Fig 5.** Photodecomposition of all-*trans* retinol (10 μM in PBS) monitored through rapid spectral scanning (100 scans/sec over 5,000 sec). Scans were interleafed between actinic 366 nm LED flashes driven at 100 flashes/sec. Retinol likely existed as a combination of monomers, multimers, and micelles, which may create light scattering. Light scatter is mitigated by the integrating spherical cuvette. Scans were averaged to 2 scans/sec. Ten such averaged scans are shown (one scan / min) [5].

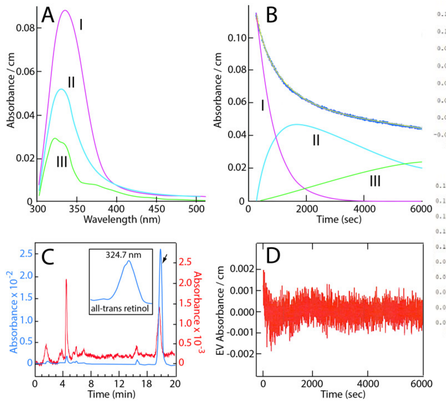


**Fig 6.** Kinetic and HPLC analysis of retinol photodecomposition [5].

**A**) 3-D data from the previous figure corrected for the increased path length and subjected to singular value decomposition. 3 spectral intermediates are (I, II, III).

**B**) The analysis favors a reaction mechanism: I -> II -> III.

**C**) HPLC analysis of the reactants at the beginning (blue) and the conclusion of the light exposure (red; note that red chromatogram is displayed at tenfold higher gain compared to the blue profile). In line spectral analysis of the peak, with λmax = 324.7 nm (arrow, prelight exposure) is consistent with all-trans retinol. The 16 min peak = 13-cis retinol.

**D**) Residual plot confirms the fit accuracy.

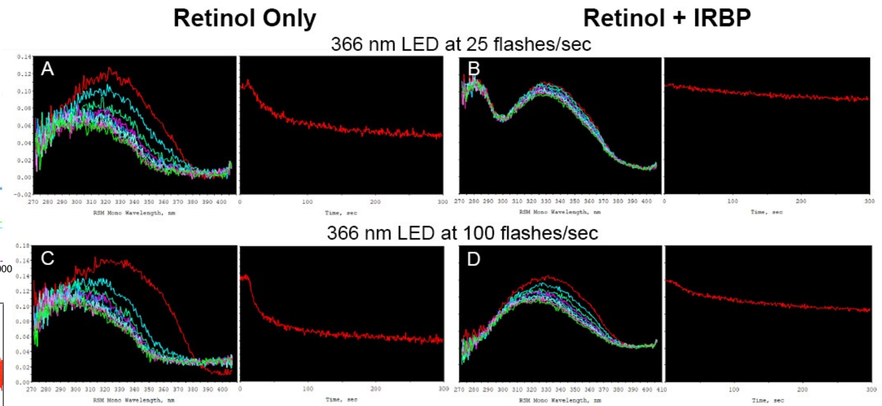


**Fig 7.** IRBP protects all-*trans* retinol from photodecomposition. 2 μM all-*trans* retinol in PBS, or in presence of 7 μM IRBP in PBS was exposed to 366 nm LED flashes (25 flashes/sec in **A,B** or 100 flashes/sec in **C,D**). All-*trans* retinol rapidly decomposes in PBS alone (**A,C**). The decomposition is faster at the higher LED-flash rate (compare the kinetic curves monitoring at 330 nm). IRBP inhibits photodecomposition at both LED-flash rates (**B,D**).

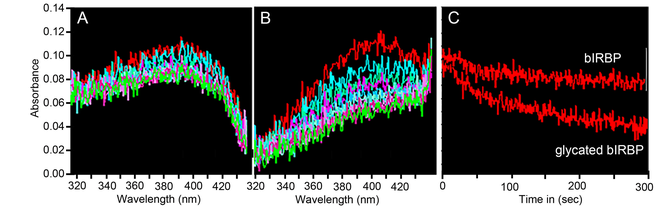


**Fig 8**. Nonenzymatic glycation retards bIRBP's ability to protect all-*trans* retinal from photodecompostion. **A**) 2 μM all-*trans* retinol in PBS. **B**) 2 μM all-*trans* retinal + 7 μM IRBP in PBS. **C**) Rate of decomposition monitored at 390 nm. bIRBP was nonezymatically glycated by incubating purified bIRBP in the prescence of glucose for 10 days at 37°C. Control IRBP was subjected to identical conditions without glucose.

## TECHNICAL SUMMARY

- Sensitivity is increased as the spherical cuvette is multipass.
- Important for photodecomposition studies, the spherical cuvette allows the actinic LED light to reach the entire sample.
- Flashes can be placed between scans allowing photodecomposition to be followed in real time.
- The system is immune to light scatter, allowing measurements to be made in turbid solutions.

## CONCLUSIONS

- IRBP can protect retinoids from photodecomposition.
- Nonenzymatic glycation inhibits IRBP's protective activity against retinoid photodecomposition.
- IRBP's activity in preventing retinoid photodecomposition may be important as the cytotoxicity of all-*trans* retinal increases upon photodecomposition [6].
- Relevance: The absorption spectrum of all-*trans* retinal extends into the violet-visible region, which reaches the retina. Photo-excitation of all-*trans* retinal with blue light in the presence of oxygen leads to the generation of singlet oxygen species [2,3,6]

## REFERENCES

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## ACKNOWLEGEMENTS

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