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Chemiexcitation in preventing macular degeneration

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Quantum biology typically involves light exciting an electron to a higher energy state, or a magnetic field splitting a single state into several having different energies, or tunneling to flout the energy barrier between states. In chemiexcitation, electrons reach an excited state without light. A ground-state chemical reaction creates a reaction product born in the excited state, due to transient mixing between ground- and excited-state wavefunctions when the reaction intermediate molecule is twisted. We outline the chemiexcitation process and its biological triggers, describe the distinctive molecules susceptible to chemiexcitation, and review recent evidence that melanin in the human retina is chemiexcited as a strategy to prevent age-related macular degeneration.

KEYWORDS

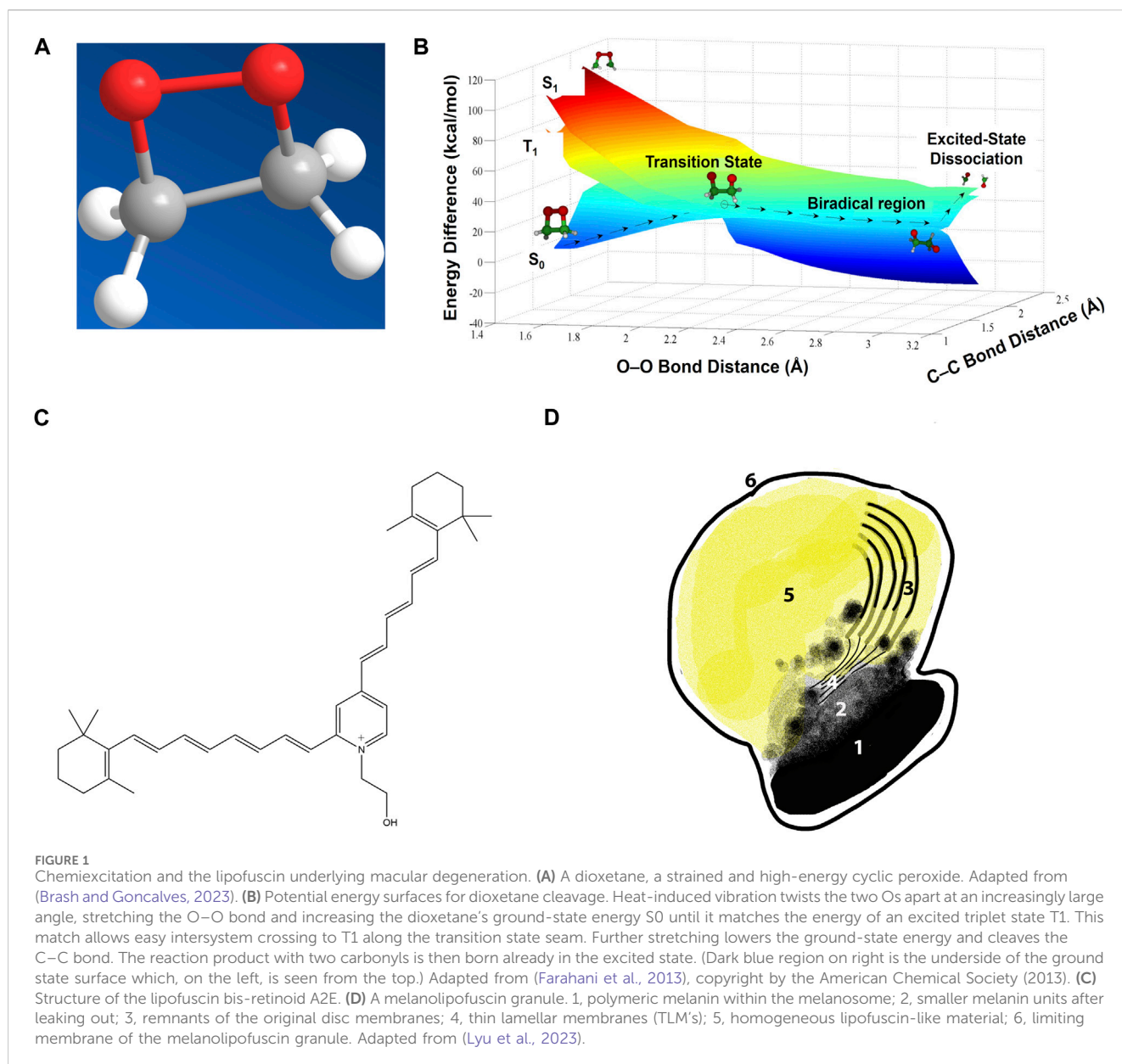
chemiexcitation, dioxetane, triplet state, photoreceptor, retinal pigment epithelium, melanin, lipofuscin, macular degeneration

1 Introduction

In ordinary chemistry, molecular collisions excite a molecule's vibrational or rotational states to an energy that overcomes an activation energy barrier and rearranges the sharing of bonded electron pairs in their electronic ground state. Even redox reactions remain in the ground state. Photosynthesis reaches 30-fold higher energies, using a photon to excite an electron from the ground state to a high-energy orbital. High energy levels also have altered orbital shapes. This combination allows reactions that are quantum forbidden in body-temperature biochemistry. A classic example occurs in DNA when UV excitation joins adjacent cytosines or thymines by concerted formation of two bonds—the cyclobutane pyrimidine dimer (CPD) that is lethal, mutagenic, and carcinogenic (Brash, 2015; Schreier et al., 2015).

If, in addition, the excited electron's spin flips, the typical singlet state is converted to a triplet. This spin flip is also forbidden, making it likely in only special molecules and functional groups. It is enabled by spin mixing between the two states (Turro and Kraeutler, 1980; Woodward, 2002). Decay back to the singlet ground state is also forbidden, resulting in the triplet state having a much longer lifetime than the excited singlet state (msec vs. nsec). This million fold longer lifetime allows a triplet excited state molecule to transfer its

Abbreviations: AMD, age-related macular degeneration; AMPPD, (3-(2'-spiroadamantyl)-4-methoxy-4-(3''-phosphoryloxy)-phenyl-1,2-dioxetane); A2E, (2-[2,6-dimethyl-8-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1E,3E,5E, 7Eoctatetraenyl]-1- (2-hydroxyethyl)-4-[4-methyl-6 (2,6,6-trimethyl-1-cyclohexen-1-yl)-1E,3E,5E,7E- hexatrienyl]-pyridinium); CPD, cyclobutane pyrimidine dimer; dCPD, dark cyclobutane pyrimidine dimer; HOMO, highest occupied molecular orbital; LUMO, lowest unoccupied molecular orbital; RPE, retinal pigment epithelium.



energy to an acceptor or react chemically. O_2 is interesting because its ground state electronic configuration is a triplet. Ambient O_2 therefore reacts with triplet excited states via energy transfer to form highly reactive singlet oxygen.

2 Chemiexcitation

Chemiexcitation is instead a chemical reaction, one in which a ground-state reactant creates a product molecule born in the excited state (>3 eV, 70 kcal/mol, 40,000 K) rather than being excited from the ground state. In biology, a prior reaction with oxygen creates a moiety termed a dioxetane (Figure 1A), a strained C–O–O–C–ring, or a version of it, that has unusual chemical and quantum mechanical properties (Adam and Cilento, 1982; Brash and Goncalves, 2023). When its host molecule is twisted by ambient thermal energy, its energy level surfaces assume unusual shapes

(Figure 1B). In the chemiexcitation reaction *per se*, twisting causes the ground state singlet energy level to increase and the excited state triplet energy level to decrease, with the two meeting at a conical intersection or, in this case, a seam that erases the energy difference (Farahani et al., 2013). This meeting of the energy surfaces facilitates crossing from the ground state singlet to the excited triplet. The O–O and C–C bonds break to form two carbonyls, one of which acquires most of the energy. Moreover, carbonyls are excited via an $n \rightarrow \pi^*$ transition, which favors crossing from singlet to triplet; the result is an excited triplet state carbonyl. Then as the 2 C’s separate, the singlet and triplet energies of the molecule again diverge. Ultimately, the high triplet energy came from the C–C bond, the O–O bond, and free radical or singlet oxygen that created the strained dioxetane. The archetype is bioluminescence in lower organisms (Wilson and Hastings, 2013). Chemiexcitation in mammals was sought for 50 years, but was not found until we discovered CPDs generated in the dark (“dark CPDs,” dCPD) by chemiexcitation of the pigment

melanin in skin melanocytes long after UV radiation ended (Premi et al., 2015; Delinasios et al., 2018; Lawrence et al., 2018; Fajuyigbe et al., 2021).

In melanocytes, the upstream process creating the dioxetane is that UV activates nitric oxide synthase and NADPH oxidase for hours (by unknown signaling pathways), generating the radicals $\cdot\text{NO}$ and $\text{O}_2^{\cdot-}$ which rapidly combine to form peroxynitrite (ONOO^-), which oxidizes melanin to a radical. The enzymes horseradish peroxidase and monoamine oxidase can substitute for peroxynitrite (Premi et al., 2015; Gonçalves et al., 2023). Melanin-like biomolecules like the neurotransmitters serotonin and dopamine are also subject to chemiexcitation (Gonçalves et al., 2023).

Evidence indicates that ambient O_2 forms a dioxetane ring on the melanin radical. In the chemiexcitation step, the ring cleaves, leaving one of the two excited carbonyls in the triplet-state, at a UV-like energy higher than in bioluminescence. The long-lived triplet state can generate phosphorescence, transfer its energy to DNA or ambient O_2 by Dexter electron exchange (exchange of a high energy electron for a low energy ground state one), or initiate a redox reaction such as electron or H abstraction (Epe et al., 1992; Brash and Gonçalves, 2023). Unlike photoexcitation, chemiexcitation does not rely on the absorption spectrum of the initial molecule, carbonyl-containing product, or acceptor, allowing a wider range of acceptors, analogous to a photosensitizer's energy transfer (Cantrell et al., 2001).

3 Melanin and neurotransmitters as chemiexcitation targets

Melanin's role as a protective pigment in the skin stems from its exotic structure (Meredith and Sarna, 2006; Hong and Simon, 2007; Simon and Peles, 2010; Mostert et al., 2018). Its variety of subunits have different oxidation states and thus a range of energy separation between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO). As a result, melanin absorbs light from UV to visible and possibly infrared (Kohl et al., 2019). Moreover, its energy is dissipated as heat, which in a solid is the quantized vibrations termed phonons. Melanin's electron \rightarrow phonon coupling is so strong that energy from excited electrons dissipates $\geq 10^6$ fold faster as heat than by emitting light (Crippa and Viappiani, 1990). It also has highly mobile pi electrons that are easily ejected, due to a high fraction of semiquinones—stable radicals capable of 1-electron transfer. New semiquinone radicals are generated by low energy visible light because excited quinones abstract an electron from nearby hydroquinones (Felix et al., 1979). Melanin's quinones have a very low energy LUMO, which therefore readily accepts electrons. The HOMO is only slightly lower than the LUMO, so little energy is needed for an electron to be excited (Pullman and Pullman, 1961) or ejected completely (Chedekel et al., 1978). The unpaired electrons of two nearby semiquinones can couple through the pi clouds (Blois, 1971); even ambient heat (approximately 0.01 eV) can flip the spin of one of the radicals so they have the same direction, putting the radical pair into a triplet state (Najder-Kozdrowska et al., 2010). The ease of electron excitation, a property shared with serotonin and dopamine, entails an ease of oxidation that facilitates the free radical or singlet oxygen reaction that creates the dioxetane, thereby facilitating chemiexcitation.

Melanin has served many useful purposes over the course of evolution. Yet this Swiss Army knife is double-edged because chemiexcitation can lead to CPDs and electron or H abstraction. In addition, melanin extensively binds Fe, Cu, and Zn ions (Hong and Simon, 2007), metals facilitating Fenton-like reactions in which H_2O_2 formed from $\text{O}_2^{\cdot-}$ abstracts an electron from the metal to make $\cdot\text{OH}$ radicals. Other ancient indoles and catecholamines with similar properties—like serotonin, melatonin, and dopamine—will face a similar duality. Why would brains rely on such hazardous molecules? Evolution apparently arrived at this point because the useful low excitation energies evolved in the long ago low- O_2 world (Azmitia, 2010). Yet, the switch to a high O_2 atmosphere 2 billion years ago, with accompanying risk of oxidation, was not followed by an evolutionary search for alternative solutions. This stasis follows from the principle of antagonistic pleiotropy, that evolution cannot select against events that are harmful after the age of reproduction (Williams, 1957).

Because the retina contains melanin, assumed to reduce light scatter in the eye, it seemed that its chemiexcitation by inflammation-induced $\cdot\text{NO}$ and $\text{O}_2^{\cdot-}$ might underlie the malady of macular degeneration (Brash et al., 2018). Yet the plot thickened.

4 Photoreceptor turnover and macular degeneration

The leading cause of vision loss in industrialized countries is age-related macular degeneration (AMD). This deterioration of central vision is concurrent with the accumulation of droplets of lipid and protein called lipofuscin in the retina, damaging cells. Effective treatments to reverse AMD are not available and it remains unclear how healthy eyes prevent this accumulation.

The starting point of this disease is the photoreceptor (Kaufmann and Han, 2024), a specialized cell in the eye that convert light into signals sent to the brain. The outer portion of these cells contain stacks of membranes called discs that are filled with rhodopsin, the molecule that captures light and converts *cis*-retinal to all-*trans*-retinal, resulting in an electrical signal. Once exposed to light, discs are shed and undergo recycling that restores all-*trans*-retinal to *cis* for reuse by newly formed discs. This process repeats daily throughout a person's life.

But a fraction of the recycling goes awry. Occasional reaction of all-*trans*-retinal with the disc membrane's phosphatidylethanolamine produces N-retinylidene-phosphatidylethanolamine, which then reacts with remaining all-*trans*-retinal to form bis-retinoids such as N-retinyl-N-retinylidene ethanolamine (usually abbreviated A2E) (Figure 1C). In A2E, the two retinoids are fused via a charged pyridinium ring (Parish et al., 1998). When photoreceptor discs are shed for recycling, they are phagocytosed by the underlying retinal pigment epithelium (RPE), a layer of cells that also contain melanosomes. Bis-retinoids cannot be digested by lysosomal enzymes in the RPE's phagosomes, resulting in membrane-limited melanolipofuscin granules in the RPE of aged human eyes, Stargardt disease patients, and Stargardt model mice (Figure 1D) (reviewed in (Lyu et al., 2023)). The accumulation of melanolipofuscin granules, sometimes termed "melanolysosomes," reflects onset and progression of AMD more closely than lipofuscin accumulation alone (Feeney-Burns et al., 1984).

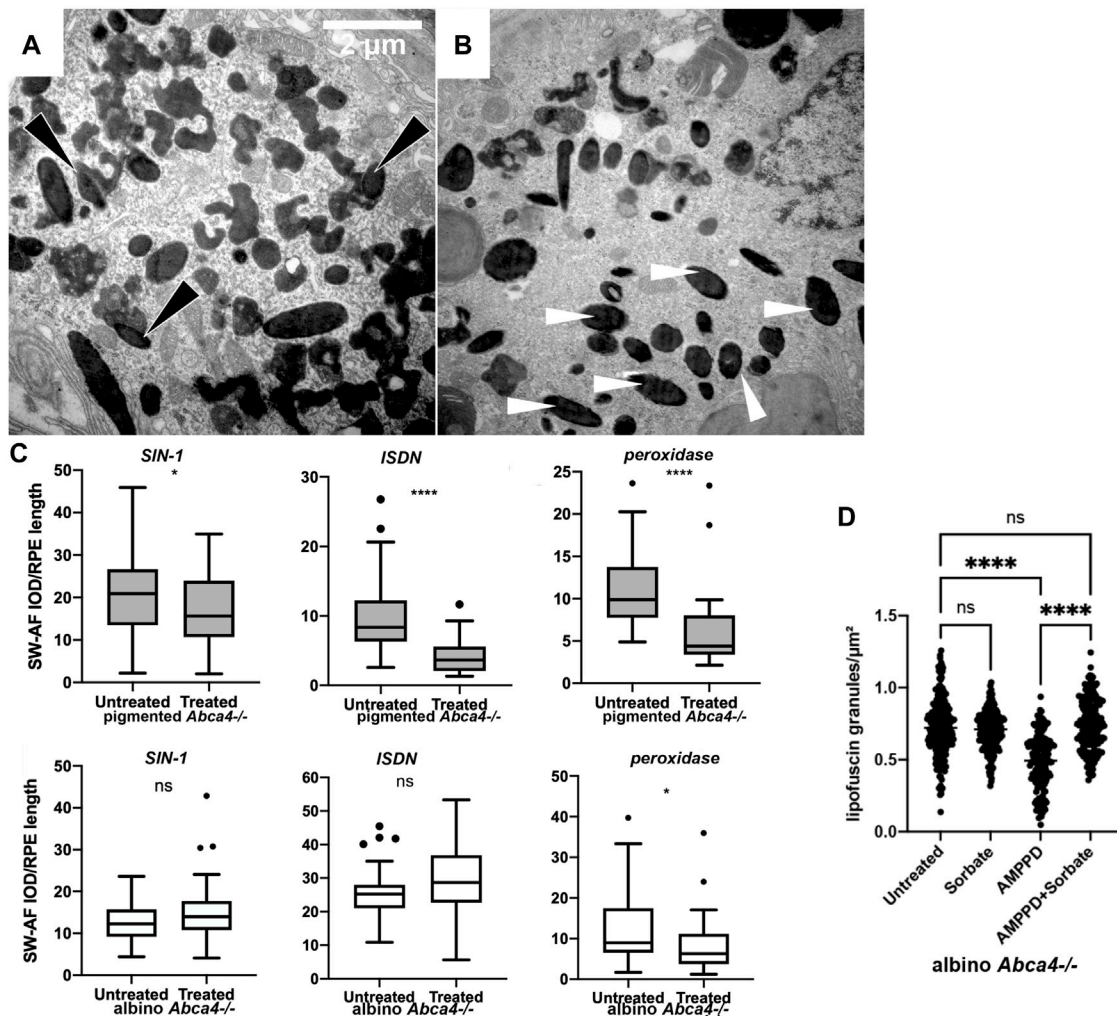


FIGURE 2 Removal of retinal lipofuscin by chemiexcitation and melanin. (A,B) Injecting a generator of superoxide plus nitric oxide stimulates lipofuscin removal from the RPE of pigmented *Abca4*^{-/-} Stargardt model mice but not in albino. SIN-1, a generator of superoxide O₂^{-•} and •NO, reduced the amount of lipofuscin present in the RPE cells of pigmented *Abca4*^{-/-} mice 48 h later. (A) Pigmented mouse, untreated. Melanin granules (dark opacity) fused with lipofuscin (light opacity), forming a melanolipofuscin granule (black arrowheads). (B) Pigmented mouse, contralateral eye treated with SIN-1. Melanin granules occur more frequently as single melanosomes (white arrowheads) that are not associated with lipofuscin. (C) Melanin-dependent lipofuscin removal by a generator of superoxide, nitric oxide, and peroxyntirite (SIN-1); by a generator of nitric oxide alone (ISDN); or by peroxidase. Murine eye flatmounts were treated with the indicated reagent and lipofuscin autofluorescence was scored. These reagents can each initiate chemiexcitation in tubo (Adam and Cilento, 1982; Premi et al., 2015). Y axis quantifies lipofuscin autofluorescence per RPE length. (D) Treating murine eye flatmounts with the dioxetane-containing molecule AMPPD, which generates excited electronic states autonomously, removes lipofuscin granules from the RPE even in the absence of melanin. Autofluorescent lipofuscin granules in the retinal pigment epithelium were reduced by half in 2 days. This reduction was inhibited by the triplet-state quencher sorbate.

5 Quantum chemistry in the eye

Albino Stargardt mice accumulate lipofuscin faster and develop retinal degeneration earlier than pigmented strains, suggesting that melanin is beneficial (Schraermeyer and Heimann, 1999; Wu et al., 2010; Radu et al., 2011; Taubitz et al., 2018). The known decrease in RPE melanin with age (Bonilha, 2008) would then diminish protection. This conclusion is supported by a gene therapy experiment injecting the gene for tyrosinase, the main synthetic enzyme for melanin, into the retina of albino mice. The treatment induced melanosomes and removed lipofuscin (Lyu et al., 2023).

Pharmacologically, the lipofuscin component can be degraded by intravitreal injection with generators of oxygen radicals such as superoxide (O₂^{-•}), peroxy (OO[•]), and hydroperoxy (HOO[•]), in both monkeys and Stargardt mice (Lyu et al., 2023). These treatments include: Remofuscin plus ambient or blue light (Fang et al., 2022); riboflavin (Schraermeyer et al., 2019); and Visudyne plus red light (Schraermeyer et al., 2019). Blue light plus lipofuscin generates superoxide and degrades lipofuscin, but is toxic (Gaillard et al., 2004; Ueda et al., 2016). The enzymes horseradish peroxidase and manganese peroxidase also degrade lipofuscin (Wu et al., 2011; Moody et al., 2018). Remofuscin rescues existing retinal

degeneration in the Stargardt mouse model. Melanilipofuscin granules are also degraded by the nitric oxide (NO) generators SIN-1 (Figures 2A,B) and isosorbide dinitrate (Lyu et al., 2023). These results are conventionally taken to indicate a direct free radical pathway for *bis*-retinoid degradation during phagocytosis of the disc outer segments, inasmuch as RPE phagocytosis generates oxygen radicals, hydrogen peroxide (H₂O₂), and the resulting hazardous Fenton-type reaction. However, these mechanisms should be operative in albinos lacking melanin; perhaps more efficiently because ocular melanin scavenges radicals (Sarna, 1992). An alternative comes from the fact that this same list of radicals and enzymes have another activity—chemiexcitation of melanin.

The possibility that lipofuscin removal was due to melanin chemiexcitation—biology's use of quantum medicine—was tested experimentally in three ways (Lyu et al., 2023). First, in albino mice the radical-generators and peroxidase were ineffective (Figure 2C). Second, a reagent that undergoes chemiexcitation on its own, without melanin, was effective (Figure 2D). That reagent, AMPPD (3-(2'-spiroadamantyl)-4-methoxy-4-(3''-phosphoryloxy)-phenyl-1,2-dioxetane), contains a dioxetane stabilized by a protecting group that prevents the molecular twisting (Bronstein et al., 1989). This protecting group is removed by alkaline phosphatase, an enzyme present in the RPE (Korte et al., 1991). Third, AMPPD's removal of lipofuscin was blocked by a specific quencher of excited triplet states, sorbate (Velosa et al., 2007) (Figure 2D). These results suggest that the combination of these events could be the eye's normal lipofuscin-disposal mechanism in youth. As a melanin-suicide mechanism, it is consistent with the known depletion of retinal melanin with age.

A direct *in vivo* test of the role of chemiexcitation in youthful AMD prevention or drug-induced remediation is to use triplet state quenchers that cross the blood-retina barrier. Lipofuscin would accumulate earlier and faster. Several biomolecules that are effective triplet state quenchers (Angelé-Martínez et al., 2022) do cross the blood-brain barrier; these or their analogs include resveratrol, lutein, vitamin E, and rapamycin (which contains a sorbate-like moiety) (Meng et al., 2021).

6 Converting physics to chemistry

A triplet-excited molecule eventually dissipates its energy in one of several ways (Brash and Goncalves, 2023). Light, heat, and *cis-trans* isomerization are relatively autonomous. Because an electronically excited molecule resembles a diradical, it can instead initiate chemistry: split between two Cs to make two radicals, add to a C=C double bond, or abstract an H from another molecule. In a purely physical reaction, it can transfer its energy to a ground-state singlet acceptor, rendering the acceptor a triplet; the acceptor now has the same options just described. Alternatively, the triplet-excited molecule can transfer its energy to ambient O₂, exciting it to very reactive singlet oxygen. Singlet oxygen can initiate chemistry, forming a peroxide, peroxy radical, or dioxetane (Di Mascio et al., 2019). Which of these pathways destroys the lipofuscin?

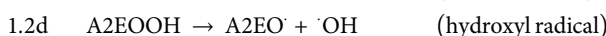
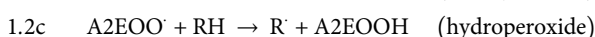
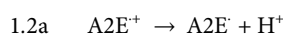
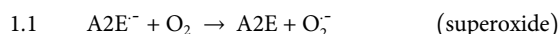
In the case of a *bis*-retinoid like A2E, these options provide a limited menu of potential routes depending on melanin. For clarity, we will use the * convention for each excited state and the superscript 1 or 3 to

denote a singlet or triplet state. The permanent positive charge on the A2E pyridinium ring will be omitted for simplicity.

6.1 Melanin chemiexcitation

The energy of chemiexcited ³melanin* is approximately 70 kcal/mol (Premi et al., 2015), so it could transfer its energy to molecules having lower energy levels. A2E has a singlet state ¹A2E* of approximately 66 kcal/mol, excitable by even blue light (Lamb et al., 2001). A2E undergoes negligible intersystem crossing to the triplet state when photoexcited (Cantrell et al., 2001), but it does accept triplet energy from a triplet-energy donor having energy higher than its own of <33 kcal/mol (Ragauskaite et al., 2001). Therefore chemiexciting melanin can access A2E's triplet state whereas photoexciting A2E cannot. Subsequent events can be:

- Energy in the excited A2E reversing the *bis*-linkage or otherwise cleaving A2E. Laboratory synthesis of *bis*-retinoids involves two sigmatropic rearrangements (Parish et al., 1998), which can be mediated by electron excitation and are sometimes reversible. The pyridinium ring is fairly stable, yet violet light is known to degrade A2E and degradation is blocked by vitamin E (Ueda et al., 2016), which is both a radical scavenger and a triplet-state quencher (Angelé-Martínez et al., 2022).
- Energy in the excited A2E isomerizing one of its double bonds, perhaps making it degradable or facilitating macrophage engulfment.
- ¹A2E* abstracting an electron or H from a ground state A2E, making A2E peroxide or peroxy radical, respectively:



R indicates the rest of the molecule. Steps 1.2c and 1.2d initiate chain reactions via R[·] and the hydroxyl radical. Because ³melanin* can abstract an electron from A2E, it could drive reaction 1.2a directly. If its hydroquinones donate H, ³melanin* would also drive reaction 2. The initial abstractions require electronic excitation because ground-state A2E does not readily donate electrons (its reduction potential is -700 to -900 mV) nor does it react with O₂⁻, hydroperoxide, or CO₃⁻ (Broniec et al., 2005).

The peroxide or peroxy radical would then add a single O to one or more double bonds in A2E, particularly adjacent to the β-ionone ring (Gaillard et al., 2004). Cyclization of the peroxy radical would create a dioxetane on a conjugated double bond, also leading to cleavage (Ukai et al., 1994).

- ³Melanin* can instead transfer its UVB-like energy to ambient O₂, creating singlet oxygen, ¹O₂* (Szewczyk et al., 2016). Photoexcited

A2E does not, due to its inefficient crossing to the triplet state (Kanofsky et al., 2003). $^1\text{O}_2^*$ can diffuse appreciable distances and cross membranes (Skovsen et al., 2005), which would allow it to reach lipofuscin. It can add to a conjugated double bond to create a new dioxetane and triplet carbonyl (Ukai et al., 1994), potentially propagating the process spatially in a $^1\text{O}_2^*$ -dioxetane chain reaction. Upon reaching a target:

The $^1\text{O}_2^*$ can split polyenes (such as in A2E), leaving a single O on each product (Stratton et al., 1993; Ukai et al., 1994). There are preferred cleavage sites. Depending on the cleavage site, this split can leave highly reactive and toxic aldehydes or less toxic ketones (Ukai et al., 1994; Ueda et al., 2016).

$^1\text{O}_2^*$ can isomerize the A2E polyene (Foote et al., 1970), perhaps making it degradable or facilitating macrophage engulfment.

6.2 Melanin as a scaffold

Melanin contains stable radicals and binds metals, riboflavin, and electron-donating amine drugs (Kozik et al., 1990; Meredith and Sarna, 2006; Karlsson and Lindquist, 2016)}. Therefore:

- A melanolipofuscin granule would juxtapose the reactants in the radical reactions above. For example, melanin binds A2E (Dontsov et al., 2013) and binds tightly to another pyridinium-containing molecule that induces Parkinson's (D'Amato et al., 1986).
- Unexcited melanin could enhance those radical reactions by its bound transition metals serving as the electron donor for making A2E^- , or its semiquinones serving as H donors to make A2EH^- .
- Melanin's bound metals can participate in Fenton-like electron transfer reactions.
- When riboflavin is excited by violet or blue light, the $\text{O}_2^{\cdot-}$ and $^1\text{O}_2^*$ it generates (Jung et al., 2007; Cardoso et al., 2012) would be adjacent to the lipofuscin target. Indeed, melanin itself generates $\text{O}_2^{\cdot-}$ upon light exposure (Meredith and Sarna, 2006).

7 Discussion—clinical implications

One route to prevention would be to restore melanin by intravitreal injections of melanin-nanoparticles (Kaufmann and Han, 2024), supplanting the current practice of intravitreal

injections to prevent blood vessel proliferation in advanced AMD. Yet the eye's immune privilege means that any infection during injection is serious. A permanent approach would be a single introduction of the tyrosinase gene to re-activate melanin synthesis (Lyu et al., 2023). An alternative circumventing the need for melanin would be a drug directly generating the high-energy excited states without melanin, in analogy to AMPPD. The ideal drug would traverse the blood-retina barrier, obviating intravitreal injection, and minimize triplet-state side-reactions by targeting melanolipofuscin granules. That drug would be the first example of quantum biology entering the clinic.

Author contributions

DB: Conceptualization, Funding acquisition, Writing—original draft, Writing—review and editing. EG: Funding acquisition, Writing—original draft, Writing—review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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