

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

GENE COMPANY PTY LTD,
Petitioner

v.

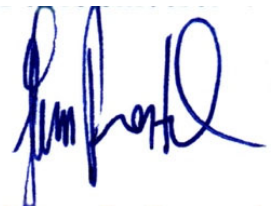
THE TRUSTEES OF COLUMBIA UNIVERSITY
IN THE CITY OF NEW YORK,
Patent Owner

Case No. IPR2024-00313
Patent No. RE47,045

DECLARATION OF DR. GLENN PRESTWICH, PH. D

I declare that all statements made herein of my knowledge are true, that all statements made herein on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

By:



Date: 22 September 2024

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I. Introduction

1. I, Glenn Prestwich, Ph.D. have been retained by Finnegan, Henderson, Farabow, Garrett & Dunner, LLP, counsel for Patent Owner The Trustees of Columbia University in the City of New York (“Columbia”).

2. I am being compensated for the time I spend on this matter, but my compensation is not contingent upon my opinions or the outcome of this or any other proceeding.

A. Summary of Qualifications

3. I have significant educational and professional experience in chemistry, biological chemistry, chemical biology, biochemistry, and bioengineering. Without limitation, this includes some four decades of experience in synthesis and biochemical/physiological studies of lipids, including without limitation sterol derivatives, cyclic and acyclic terpenoids, polyunsaturated lipids, phospholipids and lysophospholipids, and phosphoinositides. During these studies, my research team and I synthesized and evaluated many deuterium- and tritium-labeled lipids, and also deuterium-labeled hyaluronic acid. Many of my research publications address the synthesis and use of isotopically-labeled lipids for the elucidation of biochemical mechanisms in insect and mammalian processes *in vitro* and *in vivo*.

4. In addition, I have experience with chemical biology and synthesis related to ophthalmology. Specifically, I invented a disulfide-crosslinked hyaluronan composition that is employed for corneal injury repair, treatment of dry eye, and controlled release of antibiotics into the eye. See, for example: G. Yang, L. Espandar, N. Mamalis, G. D. Prestwich, “A Crosslinked Hyaluronan Derivative Accelerates Healing of Corneal Epithelial Abrasion and Alkali Burn Injuries in Rabbits,” *Vet. Ophthalmology*, 13, 144–150 (2010). The veterinary applications of this technology for use in dogs, cats, and horses and other species were licensed from the University of Utah by Sentrx Animal Care, Inc., which I co-founded in 2005. The best-selling ophthalmic product line led to acquisition of Sentrx by Dômes Pharma (France) in 2023. The rights to the human ophthalmic uses were licensed by University of Utah to startup Jade Therapeutics, Inc., which was acquired by the public company Eyegate Pharmaceuticals, Inc. Eyegate conducted Phase I and II human clinical studies with these medical device polymers in the 2015 to 2019 period.

5. At the University of Utah, scientists at the Moran Eye Center recruited me to create new chemical entities (NCEs) that inhibited the receptor TRPV4 in mammalian eyes in order to lower intraocular pressure without damage to the retinal ganglia cells. These NCEs were then evaluated in preclinical studies for treatment of glaucoma. I was a co-author on the initial paper describing this mechanism of action: D. Ryskamp, A. Frye, T. Phuong, Y. Oleg, A. O. Jo, Y. Xu, M. Lakk, A. Iuso,

P. Barabas, S. N. Redmon, B. K. Ambati, G. Hageman, T. Torrejon, G. D. Prestwich, and D. Krizaj, TRPV4 signals generate and transduce intraocular pressure in glaucoma, *Nature Scientific Reports* 11 (6) 30583 (2016). Moreover, this work led to a patent application describing the NCEs and their use: D. Krizaj, G. D. Prestwich, P. Barabas, Y. Xu, and D. Ryskamp, Compounds with TRPV4 activity compositions and associate methods thereof (US Provisional Patent Application, March 15, 2013; published US 2013/0303539 A1, November 14, 2013).

B. Education and Experience

6. I obtained my B.S. with Honors in Chemistry in 1970 from the California Institute of Technology in Pasadena, CA, and my Ph.D. in Organic Chemistry in 1974 from Stanford University in Palo Alto, CA.

7. In 1974, and again in 1977, I worked in the Department of Chemistry at Cornell University in Ithaca, NY, pursuant to an NIH postdoctoral fellowship. Between 1974 and 1977, I was a Research Scientist in the Chemistry Unit of the International Centre of Insect Physiology and Ecology (“ICIPE”) in Nairobi, Kenya.

8. I started my academic career in 1977 as an Assistant Professor of Chemistry at the State University of New York at Stony Brook (“SUNY, Stony Brook”). I continued working there until 1996, and during my tenure I became an Associate Professor and then a Full Professor of Chemistry. In 1992, I became Professor of Biochemistry and Cellular Biology. From 1992 to 1996, I was the

Director of the New York State Center for Advanced Technology in Biotechnology at Stony Brook, a.k.a., The Center for Biotechnology.

9. In 1996, I was recruited to the University of Utah in Salt Lake City, UT as Presidential Professor and Chair of Medicinal Chemistry, where I also held several adjunct or research positions, including: Research Professor of Biochemistry; Adjunct Professor of Chemistry, Adjunct Professor of Bioengineering, and Adjunct Professor of Surgery. From 2008 to 2018, I was also the Presidential Special Assistant for Faculty Entrepreneurship, and I founded and led the Entrepreneurial Faculty Scholar program at the University of Utah. I currently hold the rank of Emeritus Professor of Medicinal Chemistry and retain several of my adjunct and research positions at the University of Utah.

10. While at the University of Utah, I secured two competitive awards for technology commercialization through the State of Utah's Centers of Excellence program. From 1997 to 2002, I was the Director of the Center for Cell Signaling, and I led the spinout of Echelon Research Laboratories. Now known as Echelon Biosciences, Inc., this highly respected supplier of specialty synthetic lipids (and technical support) for biologists and other non-chemists, is in its 27th year of commercial activity.

11. From 2004 to 2008, I was Director of the Center for Therapeutic Biomaterials, and I led the spinout of Sentrax Surgical, Inc., which subsequently split

into three companies for different fields of use for the same hyaluronic acid (HA) modification technology: Carbylan Biosurgery, Inc. (medical devices & cell therapy co-exclusive), Glycosan Biosystems, Inc. (research use and cell therapy co-exclusive), and Sentrx Animal Care, Inc. (all veterinary uses). Later, I started GlycoMira Therapeutics, LLC (now, GlycoMira Therapeutics, Inc.) based on anti-inflammatory molecules discovered at the Center for Therapeutic Biomaterials.

12. From 2015 to 2018, I was the Chancellor's Distinguished Visiting Professor at Washington State University ("WSU"), and I co-founded the Entrepreneurial Faculty Ambassadors program. Beginning in 2018, and finishing at the end of 2021, I held the position of President's Distinguished Professor and worked primarily at Health Sciences Spokane in Spokane, WA. In this position, I was tasked with developing and growing an ecosystem of innovation and entrepreneurship in Spokane, including creation of Spinout Space in Spokane, a.k.a, "sp3nw," a life sciences innovation hub. I was the Founding Director of sp3nw, and I have facilitated the founding of over 18 new life science companies since January 2020. In addition, I was Principal Investigator on a grant from the United States Economic Development Administration to build a Flexible Infrastructure for Resilient Entrepreneurship ("FIRE"). I then assisted with securing an award for the Evergreen Bioscience Innovation Cluster ("EBIC") from Washington State's Department of Commerce. EBIC's goal is to build a membership organization to

create, grow, and nurture a robust pharmaceutical and medical device supply chain in the Mountain Northwest. I am a member of EBIC and hold the entrepreneur seat on the Board of Directors for EBIC. Since January 2022, I have been the President's Distinguished Professor (Emeritus) at WSU, and I consult for sp3nw, FIRE, and EBIC.

13. I became engaged as an academic-based entrepreneur in co-founding companies and working with start-up companies based on faculty technologies in 1992, and I have held various titles at several of these companies, including: co-founder of Clear Solutions Biotech, Inc.; co-founder and Chief Scientific Officer of Echelon Biosciences, Inc.; Science Advisor of Echelon Biosciences, Incorporated; co-founder and Chief Scientific Officer of Sentrx Surgical, Inc.; Senior Scientific Advisor of Carbylan BioSurgery, Inc.; co-founder and Chief Scientific Officer of Glycosan Biosystems, Inc.; Senior Scientific Advisor of Sentrx Animal Care, Inc.; founder and President, Clear Solutions Biomedical LLC; co-founder and Chief Scientific Officer of GlycoMira, LLC and GlycoMira Therapeutics, Inc.; Scientific Advisor of BioTime, Inc.; co-founder and Manager of Deuteria Agrochemicals; co-founder and Manager of Deuteria Biomaterials LLC; co-founder and Chief Executive Officer of Metallosensors, Inc.; co-founder and President of Matrix Diagnostics Biosystems, Inc.; co-founder and Vice President, Maana Discoveries, Inc.; Director of Brickell Biotech, Inc.; Scientific Advisor of BioTime, Inc. (now

Lineage Cell Therapeutics, Inc.); Scientific Advisory Board Member for Organovo; Scientific Advisory Board Member for Modern Meadow, Inc.; and former Scientific Advisory Board Member, Executive Vice President and Chief Scientific Officer of Chemistry at Symic Bio, Inc.; Scientific Advisor to Valitor, Inc.; Scientific Advisor to Rebel Medicine, Inc.; Scientific Advisor to Shasqi, Inc.; Scientific Advisor to Tambo, Inc., Scientific Advisor to Amasa Therapeutics, Inc; Scientific Advisor to IHP Therapeutics, Inc.; Scientific Advisor to Glycologix, Inc.; Scientific Advisor to EicOsis Human Health, Inc., Scientific Advisor to Endo Pharmaceuticals, Inc., and Science and Innovation Advisor to HTL Biotechnology.

14. I have also held several visiting and adjunct professorships, including: Visiting Professor at the Department of Chemistry at Harvard University in 1992; Adjunct Professor of Biology at Utah State University; and Chancellor's Distinguished Visiting Professor at Washington State University.

15. Over the course of my career, my research has focused on the areas of synthetic and structural organic chemistry, medicinal chemistry, chemical ecology, bioengineering, and targeted drug delivery. My research activities encompassed a number of diverse areas, including: (i) chemistry and biochemistry of natural compounds, pheromones, and hormones, including structure determinations using x-ray crystallography; (ii) purification, cloning, expression and mechanistic studies of polypeptides and proteins important in insect olfaction, insect and crustacean

development, vertebrate steroid metabolism, and vertebrate lipid signaling, (iii) discovery of cholesterol-lowering drugs by inhibition of key biosynthetic enzymes; (iv) development of chemical and photochemical cross-linking reagents and protocols for modification and purification of macromolecules, including polypeptides, proteins and glycosaminoglycans; (v) chemical synthesis and uses of affinity reagents for biological studies of phosphoinositides and lysophospholipids; (vi) development of new reagents for lipid signaling in cell biology and cancer treatment; (vii) chemical synthesis of chemically-modified and/or crosslinked hyaluronan and other glycosaminoglycan and protein-based biomaterials for wound repair, cartilage repair, tissue engineering, bioprinting, cell therapy, scar-free healing, and toxicology and tumor xenograft models; and (viii) invention and synthesis of sulfated glycosaminoglycan analogues as inflammation modulators for clinical utility in treatment of inflammation of skin, bladder, periodontal, and sinus tissues, and for treating oral mucositis due to radiation therapy in head and neck cancer.

16. I have published over 606 original peer-reviewed research publications as of April 2024, as well as four books and edited symposia, and 60 book chapters and symposium papers. I have given more than 700 invited lectures since 1975. I am also a named inventor on more than 93 patents and patent applications, of which at least 50 U.S. and foreign patents have issued. These inventions cover many areas,

including the control of insect pests, cholesterol lowering agents, a peptidase-resistant peptide thrombin receptor ligand, labeled phospholipids and phosphoinositides in drug discovery assays, anti-cancer and anti-angiogenic agents, signal transduction modifiers, mercury sensing chemicals, peptide-modified glycosaminoglycan therapeutics, wound-healing and regenerative medicine compositions, anti-inflammatory drugs, as well as the compositions and uses for a wide variety of chemically modified hyaluronan (also known as hyaluronic acid, or HA) derivatives.

17. I received peer and community recognition and numerous awards for my work, including the Alfred P. Sloan Research Award (1981–1985) and Camille and Henry Dreyfus Teacher-Scholar Award (1981–1986). In 2001, I was awarded the prestigious Silverstein-Simeone Award of the International Society of Chemical Ecology, for lifetime research achievements in the chemistry, biology, and biochemistry of insect chemical communication.

18. I received both the 1998 Paul Dawson Biotechnology Award and the 2008 Volwiler Research Award of the American Association of Colleges of Pharmacy. I was elected as a Fellow of the American Institute for Medical and Biological Engineering in 2005, named one of VSpring's Top 100 Entrepreneurs in 2005 and 2006, and selected as a recipient of a TIAA-CREF "Greater Good" award (2006), as a Utah Business Magazine "Health Care Hero" for 2006. I was awarded

the Utah Governor's Medal for Science and Technology for 2006. In 2010, I received the University of Utah's Distinguished Scholarly and Creative Research Award, and in 2014 I was awarded the University of Utah's Distinguished Innovation and Impact Award. In 2010, I received the "Rooster Prize" of the International Society for Hyaluronan Science ("ISHAS") for outstanding contributions to HA-derived products; I am currently the Vice President of the Applied Division of ISHAS. In 2013, I was inducted as a Fellow of the National Academy of Inventors, and in 2018, I was elected as a Fellow of the American Association for the Advancement of Science.

19. I have been a member of the American Chemical Society since 1970, a member of the American Association for the Advancement of Science since 1975, a member of Sigma Xi since 1977, an American Associate of the Colleges of Pharmacy since 1998, the Alternate Councilor for the Division of Medicinal Chemistry for the American Chemical Society between 2002 to 2004, a member of the American Association for Cancer Research from 2003 to 2017, a member of the Society for Biomaterials since 2003, and a member of the International Society for Hyaluronan Sciences since 2004.

20. I have been a reviewer of manuscripts and grants for more than 50 journals, including *Nature*, *Science*, *Journal of Biological Chemistry*, *Journal of Organic Chemistry*, *Proceedings of the National Academy of Sciences of the United*

States of America, and *Journal of the American Chemical Society*. I have also served on the editorial and advisory boards for many journals, including: *Bioconjugate Chemistry*, *Macromolecular Biosciences*, *Current Opinion in Chemical Biology*, *Perspectives in Medicinal Chemistry*, and *Advanced Therapeutics*. Currently, I am on the editorial advisory board for *Science Translational Medicine*.

21. Accordingly, I am an expert in the fields of organic and medicinal chemistry, biological chemistry, chemical biology, biochemistry, and bioengineering. Without limitation, this includes the synthesis and analysis of deuterium- and tritium-labeled lipids, and the uses of such isotopically-labeled lipids to better understand of mechanistic details of biochemical reactions and transport processes.

22. A copy of my curriculum vitae, which further details my qualifications and experience, is attached as **Exhibit 2053**.

II. Legal Principles

A. Effective Filing Date

23. I understand that claims of a patent have an “effective filing date” and that obviousness is considered based on the prior art as of that date.

24. I have been asked to use September 12, 2007, as the effective filing date for the '045 patent.

B. Person of Ordinary Skill in the Art

25. I understand that obviousness is assessed from the viewpoint of a person of ordinary skill in the art (or “POSA”). I understand that a person of ordinary skill in the art is a hypothetical person presumed to have a certain level of knowledge and expertise, including knowledge of all relevant prior art references at the time of invention.

26. I understand that Gene Company Pty, Ltd and Drs. Guengerich and Calman have offered the following definition of a person of ordinary skill in the art:

A [POSA] of the ’045 patent in September 2007 would have had an advanced degree (e.g., Ph.D., M.D., M.S., or equivalent), in chemistry, biochemistry, pharmaceutical science, medicine, or a related field. This individual may have worked in consultation with a team including, e.g., a practicing physician who diagnoses and treats macular degeneration. Moreover, skilled artisans would have had practical knowledge about biological studies, pharmaceutical formulation, and the deuterium isotope effect, including the patents and publications discussed herein.

Pet. 7; EX1002 ¶¶52–55; EX1004 ¶¶47–49.

27. In my opinion and based on my experience having worked in the field, Petitioner’s definition does not accurately reflect the level of skill presumed by the ’045 patent. The claims of the ’045 patent are directed to methods of “slow[ing]

lipofuscin formation/accumulation in order to treat or ameliorate an ophthalmologic disorder, such as, e.g., macular degeneration,” and more specifically to “slowing or limiting the accumulation of age pigments or lipofuscin in the retinal pigment epithelium (‘RPE’) cells of the retina.” EX1001 at 1:25–39. This is not an area where a POSA is a generalist, such as an ophthalmologist generally or advanced science degree. Rather, it is an area where a POSA has specialized skill with respect to the biosynthesis and structure of related natural products and mechanisms of retinal degeneration.

28. I understand that Patent Owner contends that the skilled artisan:

would have an advanced degree (e.g., M.D., Ph.D., M.S., or equivalent), in a field related to medicine (with training in ophthalmology), and/or in chemistry, biochemistry, pharmaceutical science, or a related field with at least three years of experience researching biosynthesis and structure of naturally-occurring terpenoids (e.g., retinoids and bis-retinoids such as lipofuscin pigments, carotenoids, steroids) and/or methods to slow lipofuscin formation or accumulation to treat an ophthalmologic disorder, such as AMD or Stargardt’s disease. The POSA may consult with others, such as a biochemist or formulation scientist, to understand or explain the contents of the prior art.

29. In my opinion and based on my experience having worked in the field, Patent Owner’s definition accurately reflects the level of skill in the art at the time.

30. The structure elucidation of A2E and other bisretinoids from 1996 to 2005 originated in collaboration with Columbia University laboratory of Professor Koji Nakanishi. *E.g.*, EX2079 (Sakai 1996) at 2; EX1018 (Parish 1998) at 1 (citing ref. 14 EX2079 (Sakai 1996)). I was trained in diterpene structure determination and biosynthesis by Professor Nakanishi and worked with members of his team from 1974 to 1977 while I was an NIH Postdoctoral Associate. I isolated and purified novel diterpenes from termite soldiers and used NMR and x-ray crystallography to determine the structures of these novel diterpenes. This research was published in Papers Nos. 9, 12, 15, and 67 on my CV, attached here as Exhibit 2053.

31. Further, Dr. Marcus Boehm¹ worked in my laboratories as a graduate student at Stony Brook University. Dr. Boehm and I published seven papers (CV Paper Nos. 90, 91, 94, 101, 109, and 120) and two book chapters (CV Book Chapter Nos. 12, 16). All but one of these papers dealt with Dr. Boehm's synthesis, characterization, biochemical studies of selectively tritium-labeled terpenoid and related molecules important in insect developmental biology. After completing his

¹ I understand that Dr. Boehm's patent application describing methods for synthesis of 9-*cis*-monotritium-labeled retinoids, which Dr. Guengerich refers to as *Boehm* (EX1052), was addressed during the prosecution history of the '045 patent. EX1002 ¶¶40, 44–47.

Ph. D degree, Dr. Boehm then pursued postdoctoral studies in Prof. Nakanishi's laboratory where he worked on synthesis and uses of isotopically-labeled retinoids.

32. Based on my personal experience in the field at the time and knowledge of those who were also working in the field at the time, the person of ordinary skill was not a generalist as in Petitioner's POSA definition but one with specialized experience as in Patent Owner's definition.

33. I have been at least a POSA according to both Patent Owner's definition and Petitioner's definition since 1974.

34. While I have applied Patent Owner's definition, unless stated otherwise my opinions set forth in this declaration remain the same under either Petitioner's or Patent Owner's definition.

C. Obviousness

35. I am not an attorney but understand that the analysis of obviousness of a claimed invention relies on four factual inquiries: (1) the scope and content of the prior art, (2) the differences between the prior art and the claimed invention, (3) the level of ordinary skill in the art at the time of the invention, and (4) "objective evidence" or "secondary considerations" of nonobviousness.

36. I have also been informed that a showing of obviousness requires that, as of the patent's effective filing date, (1) a POSA would have been motivated to combine prior art references to arrive at the claimed subject matter and (2) there

would have been a reasonable expectation of success in achieving the claimed invention from such combination.

37. I have also been informed that “objective indicia” or “secondary considerations” must also be considered in an obviousness analysis. I understand that these can include a long-felt but unmet need, failure of others, praise from industry and academia, and unexpected results.

III. The '045 Patent

A. The Claims

38. The '045 patent includes 54 claims, all of which are directed to methods of retarding the accumulation of a lipofuscin pigment in a retina or treating the effects of a disease (e.g., macular degeneration) characterized by the accumulation of a lipofuscin pigment in a retina. I understand that the Petitioner has challenged all claims of the '045 patent.

1. Independent Claims

39. I have been advised that 3 of the 54 claims (claim 1, claim 28 and claim 29) are “independent” claims because they do not refer back to or depend on any other claim.

40. Claim 1 is directed to a method of retarding the accumulation of a lipofuscin pigment in a retina. Claim 1 reads as follows:

1. A method of retarding the accumulation of a lipofuscin pigment in a retina, comprising administering an effective amount of a

pharmaceutical composition to a patient in need thereof, wherein the pharmaceutical composition comprises a substituted C₂₀-D₁₋₃-retinoid, wherein the C₂₀-D₁₋₃-retinoid is selected from the group consisting of C₂₀-D₁₋₃-retinol, C₂₀-D₁₋₃-retinol ester, C₂₀-D₁₋₃-retinal, and C₂₀-D₁₋₃-pro-vitamin A carotenoids, and a pharmaceutically acceptable carrier.

41. Claims 28 and 29 are each directed to a method of treating a macular degeneration. Claim 28 reads as follows:

28. A method of treating a macular degeneration, comprising administering an effective amount of a substituted C₂₀-D₁₋₃-retinoid to a patient in need thereof, wherein the substituted C₂₀-D₁₋₃-retinoid is selected from the group consisting of C₂₀-D₁₋₃-retinol, C₂₀-D₁₋₃-retinol ester, C₂₀-D₁₋₃-retinal and C₂₀-D₁₋₃-pro-vitamin A carotenoids.

42. Claim 29 differs from claim 28 only in that the substituted C₂₀-D₁₋₃-retinoid is in a pharmaceutical composition comprising a pharmaceutically acceptable carrier. Specifically, claim 29 reads as follows:

29. A method of treating a macular degeneration, comprising administering an effective amount of a *pharmaceutical composition* to a patient in need thereof, wherein the *pharmaceutical composition* comprises a substituted C₂₀-D₁₋₃-retinoid, wherein the C₂₀-D₁₋₃-retinoid is selected from the group consisting of C₂₀-D₁₋₃-retinol, C₂₀-D₁₋₃-retinol ester, C₂₀-D₁₋₃-retinal and C₂₀-D₁₋₃-pro-vitamin A carotenoids, and a *pharmaceutically acceptable carrier*.

2. Dependent Claims

43. Claims 2–27 depend directly or indirectly from claim 1. No claims depend from claim 28. Claims 30–54 depend directly or indirectly from claim 29. These dependent claims recite additional limitations summarized below:

- Dependent claims 2, 6–8, 12–14, 17, 30, 33–35, 39–41, and 44 each specify the C₂₀-D₁₋₃-retinoid to be administered in the method.
- Dependent claims 19 and 46 specify that the amount of C₂₀-D₁₋₃-retinoid present in the pharmaceutical composition is sufficient to deliver on average about 0.1 to about 90 mg/day to a patient.
- Dependent claims 9, 15, 26, 36, 42, and 53 specify that the deuterated retinoid forms A2E about 7 times slower than the non-deuterated retinoid.
- Dependent claims 3 and 31 specify that the pharmaceutical composition is suitable for use as a nutraceutical composition.
- Dependent claims 20 and 47 recite that the composition comprises an additional active, and dependent claims 21–25 and 48–52 specify the additional active.
- Dependent claims 4, 5, 10, 11, 16, 18, 37 specify the disease for which the patient is in need of treatment; for example, macular degeneration (claim 4), age-related macular degeneration (claim 5), dry (non-

neovascular) age-related macular degeneration (claims 10 and 37), and Stargardt disease (claim 11).

- Dependent claims 32, 38, 43, and 45 specify the macular degeneration to be treated by the claimed method; for example, age-related macular degeneration (claim 32) and Stargardt disease (claim 38).
- Dependent claims 27 and 54 specify that the pharmaceutical composition is administered directly to the eye.

IV. Claim Construction

44. I have been informed that for purposes of my analysis in this proceeding, the terms in the claims of the '045 patent should be interpreted based on their ordinary and customary meaning as understood by a POSA.

45. Independent claims 1, 28 and 29 each recite an introductory phrase that I understand is referred to as a preamble: “[a] method of retarding the accumulation of a lipofuscin pigment in a retina” (claim 1), and “[a] method of treating a macular degeneration” (claims 28 and 29). I understand that a preamble may or may not act as a distinct limitation in a patent claim. I understand that for method of treatment claims, the preamble is deemed limiting when it is essential to define the patients receiving the treatment and/or when it introduces terms that are referenced in the rest of the claim.

46. I understand that the Board in this proceeding has preliminarily construed the preamble of claim 28 as limiting and given the phrase “method of treating a macular degeneration” patentable weight. Institution Decision at 30–31. Specifically, the Board explained that the phrase is a positive limitation of the claim because it prescribes “a specific purpose (i.e., treating a macular degeneration)” (*id.* at 30) and “is necessary to understand the patients to whom the substituted C20-D1-3-retinoid should be administered.” Institution Decision at 31.

47. I agree with the Board’s construction of claim 28. In my opinion, a POSA would understand the identical claim 29 preamble (“A method of treating a macular degeneration”) to be limiting for the same reasons. Further, “macular degeneration” in the claim 29 preamble provides antecedent for its dependent claims, including:

32. The method according to claim 29, wherein the macular degeneration is age-related macular degeneration.

38. The method according to claim 29, wherein the macular degeneration is Stargardt disease.

48. In my opinion, the preamble of claim 1, “method of retarding the accumulation of a lipofuscin pigment in a retina,” is similarly limiting. As with claims 28 and 29, this preamble is necessary to identify the “patient in need thereof” to whom the treatment is administered. Moreover, the preamble is limiting because it provides essential context for understanding dependent claims 9, 15, and 26, which

refer back to claim 1. These dependent claims specify the rate at which the accumulation of a specific lipofuscin pigment, A2E, is reduced, stating that the compound “forms A2E in vitro about 7 times slower than non-deuterium-enriched retinal retinol acetate.”

49. In presenting my opinions below, I have applied these constructions of the claim 1, 28, and 29 preambles as limiting. Were the Board to construe the claims according to the construction proposed by Drs. Guengerich and Calman, my opinions that the claims of the '045 patent would not have been obvious would not change.

V. Technology Background

A. Biochemistry and the Rate-Limiting Step

50. Fundamental principles of chemical kinetics dictate that in multistep chemical reactions the overall rate of product formation is generally determined by the “rate-limiting” step. This rate-limiting step, also known as the slowest step, the bottleneck, or the rate-determining step, acts as a control point in the reaction sequence.

51. Faster steps before or after the rate-limiting in a multistep chemical reaction do not significantly influence the overall reaction rate, as the system can only proceed as quickly as its slowest component allows. This is illustrated in a standard analogy of a series of funnels (reactions), where the overall rate (filling the

flask) is controlled by the funnel (reaction step) with the smallest opening (slowest rate).²



Figure 1.

52. As evident from these illustrations, narrowing the opening of a non-rate-limiting funnel will only impact the steady-state rate of the reaction if is narrowed to be smaller than that of the current rate-determining funnel.

53. The rate-limiting step in a biochemical reaction *in vivo* is determined by more than the kinetics of individual chemical reaction steps. This is because

² Illustration from *Reaction Rates – A Microscopic View*, LIBRETEXTS CHEMISTRY, https://chem.libretexts.org/Courses/Howard_University/General_Chemistry:_An_Atoms_First_Approach/Unit_6:_Kinetics_and_Equilibria/Chapter_14:_Chemical_Kinetics/Chapter_14.6:_Reaction_Rates_-_A_Microscopic_View

biochemical reactions are influenced by a myriad of different biological and physicochemical processes that provide the *in vivo* context for the chemical transformations. These processes include, without limitation, (i) substrate or product transport across lipid bilayer membranes, (ii) substrate or product transport between or among two or more intracellular compartments, (iii) ligand binding to, and release from, a protein, (iv) catalysis via a transient intermediate, and (v) the nature of the physiological (or pathophysiological) environment in which the chemical transformations are occurring.

54. Moreover, any one of these context-dependent biological or physicochemical processes may affect the rate of an individual chemical reaction, and may also be rate-limiting for the overall process. In other words, in such biochemical systems, the chemical reaction steps may be relatively rapid, while one or more biologically-mediated or physicochemical processes could become a rate-limiting factor.

B. Complexity of the Visual Cycle and Lipofuscin Formation

55. Lipofuscin is a generic name for the heterogenous mixture of lipids and proteins that accumulate with age in a number of different cells throughout the body. In the eye, lipofuscin accumulates in retinal pigment epithelial (“RPE”) cells and comprises fluorescent pigments, such as A2E (*N*-retinylidene-*N*-retinylethanolamine) and ATR-dimer (all-*trans*-retinal dimer). *E.g.*, EX1011

(Fishkin) at 4–6. The buildup of these pigments was associated with macular degenerative diseases, including Stargardt’s disease and age-related macular degeneration (“AMD”). *E.g.*, EX1011 (Fishkin 2005) at 1.

56. As shown below in Figure 2, these products were believed to be produced as a byproduct of the visual cycle from excess “all-*trans*-retinal.”³ EX1034 (Wolf 2003) at 1; EX1036 (Fishkin 2003) at 1–2.⁴

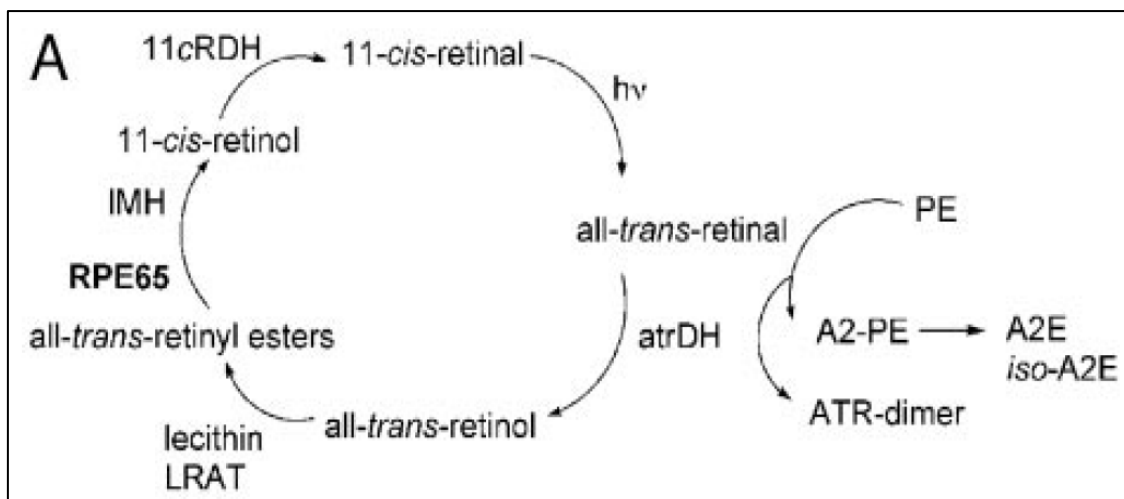


Figure 2. EX2058 (Kim 2004) at 1.

³ Various prior art references herein abbreviate all-*trans*-retinal as “atRAL,” others use “ATR.” These abbreviations are synonymous and are used interchangeably herein.

⁴ EX1036 (Fishkin 2003) at 1 (“As a consequence of the continual light-related vitamin A cycling in the retina, the orange-emitting fluorophore A2E (Fig. 1) is deposited in retinal pigment epithelial (RPE) cells”).

1. The Visual Cycle

57. The visual cycle in the retina of the vertebrate eye is an intricate, multi-step process that embodies the complex relationship between chemical reactions and biological processes. *See supra*, Section V.A.

58. The visual cycle can be conceptualized as the combination of two components: (1) the regeneration of visual chromophore and (2) the uptake of retinoids from the choroidal circulation. EX1050 (Kuska) at 1.

59. The regenerative component of the visual cycle refers to the continuous recycling of the diterpenoid vitamin A. EX2059 (Rando 2001) at 1–3. This cycle regenerates 11-*cis*-retinal (“11CR”), a required component of 11CR Schiff base chromophore that initiates the phototransduction cascade that allows for vision in vertebrates. EX2059 (Rando 2001) at 1; EX2060 (Saari 1998) at 1–2.

60. The visual cycle begins with the absorption of light by rhodopsin in photoreceptor cells, which causes the Schiff base chromophores of rhodopsin 11CR to photoisomerize to all-*trans*-retinal (“atRAL” or “ATR”). EX2059 (Rando 2001) at 1; Fig 3, below. ATR is then released from rhodopsin in the photoreceptor cells, followed by enzymatic reduction by all-*trans*-retinol dehydrogenases (“atRDH”), converting ATR to all-*trans*-retinol (“atROH” or vitamin A). EX2059 (Rando 2001) at 1.

depend on a number of other proteins, probably indirectly involved in retinoid trafficking”).

64. Upon absorption of light, the 11CR Schiff base chromophore is again isomerized to the atRAL Schiff base, initiating another cycle. EX2059 (Rando 2001) at 1.

65. As of September 2007, many aspects of the visual cycle remained incompletely understood. *E.g.*, EX2059 (Rando 2001) at 1; EX1050 (Kuska) at 3. As *Saari 1998* explained, this included the specific molecular mechanisms for enzymatic conversions, the full portfolio of enzymes involved, and the precise nature of various intraocular biological or physicochemical transport steps:

[T]he reactions of visual pigment regeneration are poorly characterized. With one exception, *none of the enzymes has been molecularly characterized*, the regulation of the pathway *is not understood*, the molecular bases of differences in rod and cone visual pigment regeneration *are not known*, and the relationship of visual cycle defects to retinal pathology *has not been examined*.

EX2060 (Saari 1998) at 7; *see also* EX1050 (Kuska) at 3 (“There are many unanswered questions in the retinoid cycle . . .”).⁵

⁵ All emphasis added unless otherwise noted.

66. Thus, a POSA would have understood that the visual cycle incorporates a wide variety of processes beyond simple chemical reactions, many of which were incompletely understood. *E.g.*, EX2059 (Rando 2001) at 1–6; EX2062 (Rózanowska 2005) at 7.

2. Lipofuscin Biosynthesis

67. As of September 2007, it was known that the lipofuscin pigments, such as A2E, were a byproduct of the visual cycle. The chemical, biological, and physicochemical processes that were involved in this process, however, were the subject of ongoing research and debate. *E.g.*, EX2062 (Rózanowska 2005) at 15–17.

68. The prevailing belief was that lipofuscin pigments were generated from excess ATR.

69. Following absorption of light, 11CR is converted to ATR. It was thought that ATR was then released from rhodopsin, and reduced by atRDH, and then proceeded through the visual cycle where it is eventually converted back into 11CR. *See supra*, Section V.B.1.

70. Sometimes, however, instead of proceeding through the visual cycle, ATR would accumulate prior to its reduction to atRDH, creating excess ATR in the

retina. EX2066 (Maiti 2006) at 2.⁶ This excess ATR would then react with a lipid component of the of the OS membrane (phosphatidylethanolamine, or “PE”) to form a condensation product, *N*-retinylidene-phosphatidylethanolamine (“NRPE”⁷). *E.g.*, EX1036 (Fishkin 2003) at 2.

71. As of September 2007, researchers proposed several different hypothetical pathways for how this condensation product (NRPE) would be converted to A2E.

⁶ EX2066 (Maiti 2006) at 1 (“Most of the all-*trans*-retinal generated is reduced by the RDH mentioned above. However, some of the chemically reactive all-*trans*-retinal partitions into a chemically mediated pathway that generates a set of photoreactive flourogenic molecules. These fluorophores, which include A2E . . .”).

⁷ Most the references reviewed herein refer to this condensation product as “NRPE.” EX1036 (Fishkin 2003) at 2; EX1011 (Fishkin 2005) at 5, Fig 10; EX2019 (Kim 2007) at 2; EX1034 (Wolf 2003) at 2. At least *Mata 2000*, however, refers to the same condensation product as “APE.” EX1010 (Mata 2000) at 1. As used herein, I use “NRPE” to refer to both NRPE and APE.

a) A2E Intermediates and Chemical Reactions

72. **Parish 1998.** A2E was the first fluorescent lipofuscin pigment identified by Sakai et al. in 1996. EX1018 (Parish 1998) at 1. Building on their earlier identification of A2E, *Parish 1998* aimed to optimize an *in vitro* biomimetic synthesis⁸ of A2E to aid in further characterizing RPE lipofuscin pigments. EX1018 (Parish 1998) at 1.⁹

73. *Parish 1998* proposed a hypothetical A2E biomimetic/biosynthetic pathway that began with the formation of a Schiff base formed from “ethanolamine or phosphatidylethanolamine” and ATR. EX1018 (Parish 1998) at 1. The authors emphasize the speculative nature of this proposed mechanism, and indicate the need for further research. EX1018 (Parish 1998) at 1.

74. **Mata 2000.** *Mata 2000* sought to characterize intermediates in the biosynthesis of A2E by studying compounds extracted from mouse and human eye

⁸ Biomimetic synthesis is the creation of chemical compounds or materials that mimic natural processes or structures found in living organisms.

⁹ EX1018 (Parish 1998) at 1 (“[b]ecause the biological *properties of A2E and its relationship to AMD have yet to be clarified*, the availability of an efficient and simple method for the preparation of large quantities of A2E becomes critical for further chemical and biological studies.”).

tissues. EX1010 (Mata 2000) at 1. *Mata 2000* identified what they believed were three intermediates: *N*-retinylidene-phosphatidylethanolamine (“APE”, which later became known as “NRPE”), *N*-retinylidene-*N*-retinylphosphatidylethanolamine (“A2PE”), and dihydro-*N*-retinylidene-*N*-retinylphosphatidylethanolamine (“A2PE-H₂”). EX1010 (Mata 2000) at 1. *Mata 2000* proposed a pathway that rationalized how A2E could be formed from these intermediates. EX1010 (Mata 2000) at 5. This hypothetical pathway included a “1,5 sigmatropic rearrangement” of NRPE.¹⁰ EX1010 (Mata 2000) at 5.

75. *Mata 2000* concluded that the availability of ATR was the primary factor controlling the rate of A2E formation, stating, “[o]ne prediction of the

¹⁰ Dr. Guengerich and various references discussed herein variously refer to this reactions as e.g., a “1,5-sigmatropic shift” (EX1002 ¶58); “[1,5] hydrogen-shift” (EX1002 ¶86); “[1,5]-sigmatropic rearrangement” (EX1002 ¶93, EX1010 (Mata 2000), 5); “[1,6]-proton shift” (EX1002 ¶91); “[1,6]-proton tautomerization” (EX1018 (Parish 1998) at 1); “[1,6] H-shift” EX1011 (Fishkin 2005) at 5. As explained below (§VII.B), these reactions are equivalent in that a hydrogen atom moves from the same place on the molecule to form the same product, but the mechanisms by which that process occurs are distinct. For the sake of clarity, I refer to all of these reactions as a “[1,5] shift” throughout this declaration.

pathway proposed in Fig. 5 is that A2E accumulation should depend on the availability of all-*trans*-RAL [ATR] and hence on light exposure.” EX1010 (Mata 2000) at 6. Based on this, *Mata 2000* further concluded that “[p]harmacologic inhibition of one or more steps in the A2E synthetic pathway may thus be an effective treatment strategy for other macular degenerations associated with lipofuscin accumulation.” EX1010 (Mata 2000) at 6.

76. ***Fishkin 2003.*** *Fishkin 2003* proposed a different hypothetical pathway for A2E formation than that proposed by *Mata 2000*. EX1036 (Fishkin 2003) at 1. As with *Mata 2000*, *Fishkin 2003* identified the availability of ATR as critical to the formation of A2E and noted that the “reduction [of ATR] to retinol is the rate-limiting step in the vitamin A cycle.” EX1036 (Fishkin 2003) at 2.

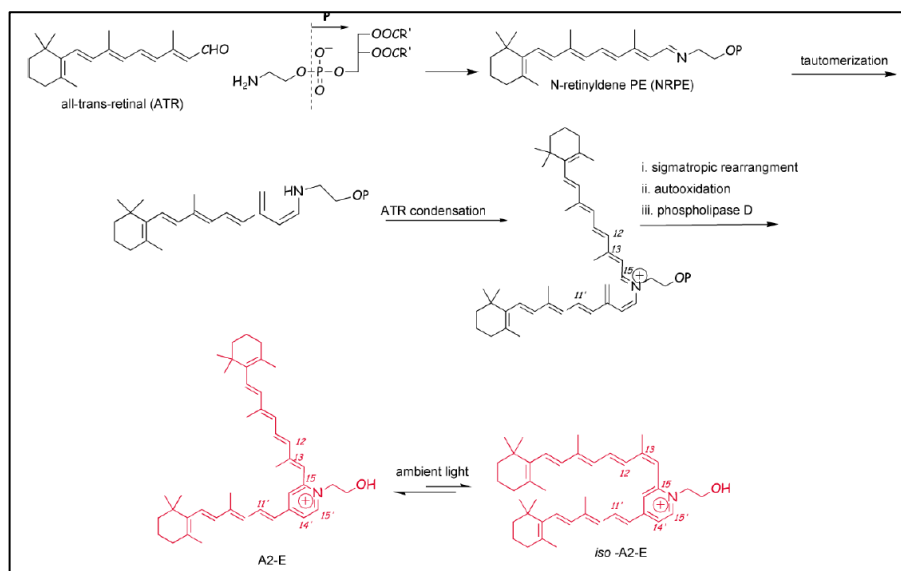


Figure 5. Proposed A2E Pathway of Fishkin 2003. EX1036 (Fishkin 2003) at 1.

77. ***Fishkin 2005.*** *Fishkin 2005* sought to further characterize the lipofuscin pigments by studying compounds extracted from bovine rod outer segments and mouse eye tissues. EX1011 (*Fishkin 2005*) at 1. *Fishkin 2005* presented findings that were inconsistent with some of the conclusions drawn by *Mata 2000*, explaining that “several lines of reasoning suggested that the 500-nm pigment isolated by *Mata et al.* was not A2-PE-H₂.” EX1011 (*Fishkin 2005*) at 5. The authors proposed that rather than being an intermediate in the A2E pathway, A2PE-H₂ was actually a new product unaccounted for in *Mata 2000*, ATR-dimer. EX1010 (*Fishkin 2005*) at 4–5.

78. These findings prompted *Fishkin 2005* to propose an alternative biosynthetic pathway, distinct from the A2E pathways in *Mata 2000* and *Fishkin 2003*. EX1011 (*Fishkin 2005*) at 5. Referencing Figure 6, below, *Fishkin 2005* explained:

Both ATR dimer and A1-PE may form from the same tautomer x arising from a (1,6) H-shift of NRPE. Tautomer x can form an iminium ion with a second ATR according to path a, and after 6 π -aza electrocyclization and autooxidation, A2-PE is formed. Conversely, in path b, C20 of NRPE reacts with C13 of free ATR in a Michael-type addition, followed by a Mannich reaction to close the ring, and elimination of the amino group of PE, yielding the ATR dimer.

EX1011 (Fishkin 2005) at 5, Fig. 10.

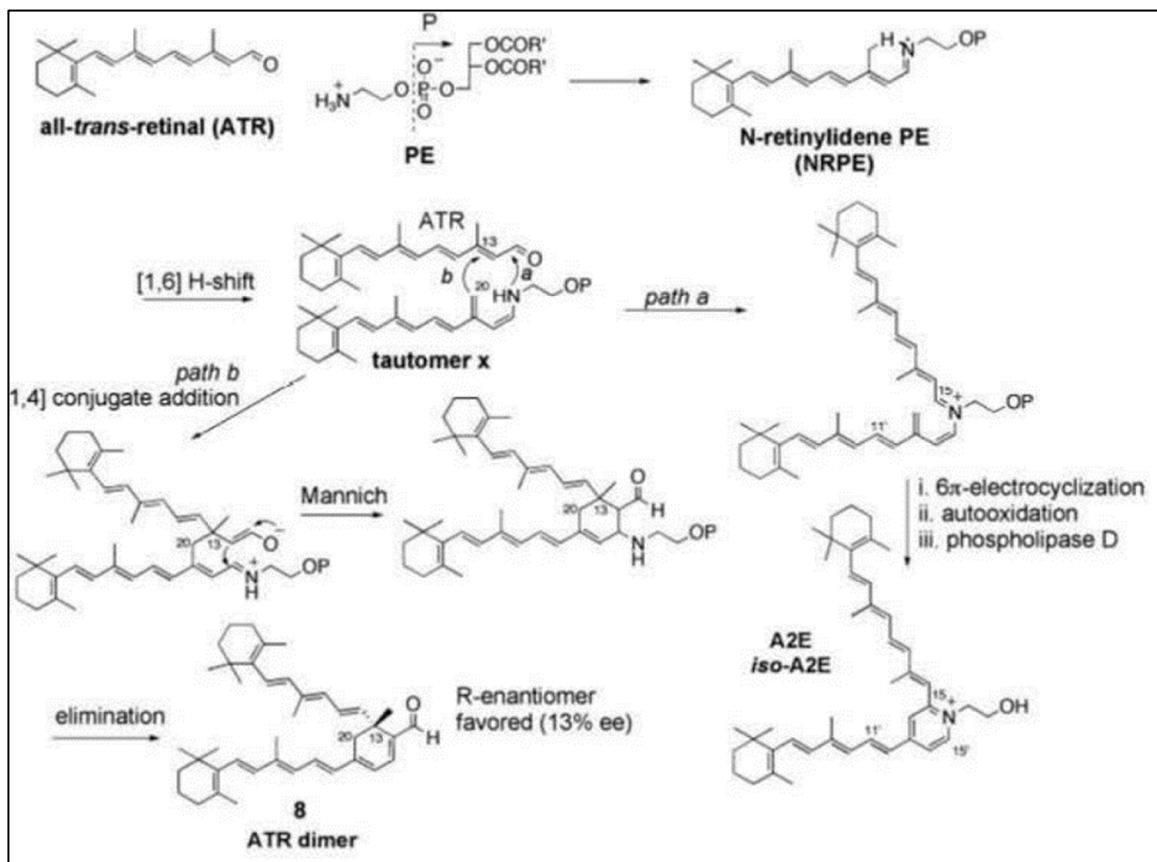


Figure 6. *Fishkin 2005's Proposed A2E Pathway.* EX1011 (Fishkin 2005) at 5, Fig. 10.

79. In 2007, *Kim 2007* proposed another hypothetical pathway for the formation of A2E. *Kim 2007* also explained that “[a] number of observations over the years have shown that the deposition of lipofuscin fluorophores in RPE is dependent on the availability of vitamin A and/or vitamin A derivatives.” EX2019 (Kim 2007) at 1.

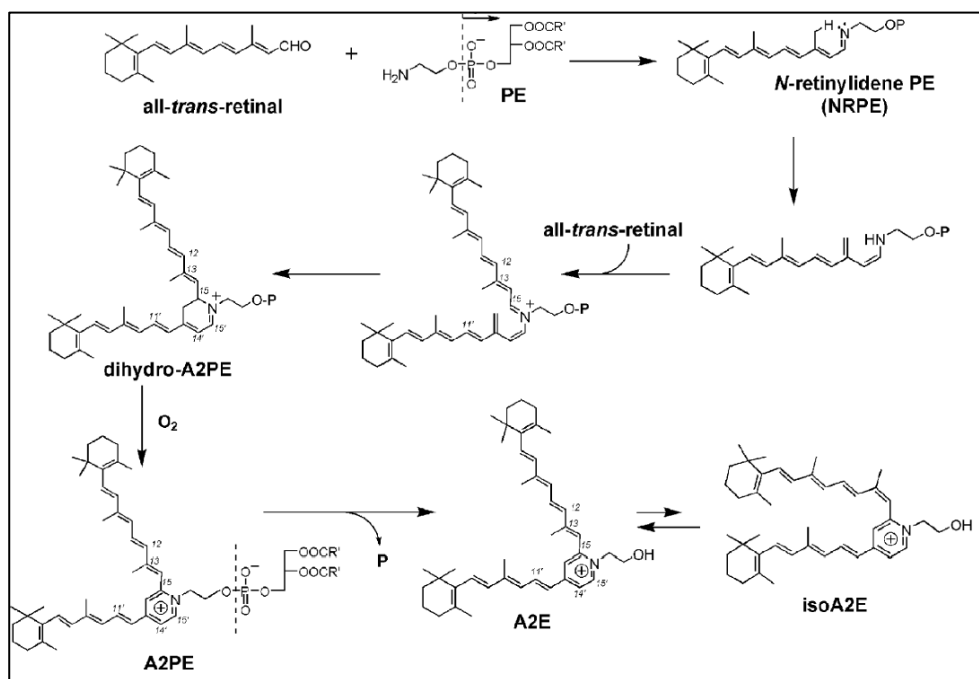


Figure 7. Kim 2007's Proposed A2E Pathway.
EX2019 (Kim 2007) at 10.

80. Thus, as of September 2007, a POSA would have understood that (1) the formation of lipofuscin pigments was widely regarded as dependent on the availability of excess ATR¹¹ and (2) the chemical reactions and the specific intermediates involved in the formation of lipofuscin pigments remained subjects of ongoing research and active debate.

¹¹ As explained below (§ V.B.3), this understanding was one of the reasons many researchers later sought to reduce the availability of excess ATR via the use of visual cycle inhibitors. *E.g.*, EX1035 (Radu 2003) at 1; EX1030 (Radu 2005) at 1; EX2064 (Maeda 2006) at 1; EX2066 (Maiti 2006) at 1; EX1009 (Widder) at 2–3.

b) Biological Processes Involved in Lipofuscin Accumulation

81. Importantly, the hypothetical reaction¹² schemes discussed above, represent only one piece of the puzzle. While these proposed reaction schemes allow for bookkeeping of atoms and electrons during chemical reactions, they do not account for the full array of biological and physicochemical processes involved in the overall inflow of reactants and outflow of products.

82. As of September 2007, a POSA would have recognized that the proposed reaction schemes for lipofuscin formation occur across different compartments in the eye and involve a complex interplay of biological and physicochemical processes. These dynamic processes include, without limitation, (i) enzymatic reactions, (ii) substrate transport across membranes and between (and among) different cellular compartments, (iii) interaction with complex chiral protein-lipid environments, (iv) protein binding and release events, (v) enzyme interactions, (vi) phagocytosis, and (vii) product transport across membranes and between (and among) different cellular compartments. *E.g.*, EX2082 (Weng 1999)

¹² Dr. Guengerich does not identify any mechanistic studies for these hypothetical reaction schemes, and I am not aware that any such studies had been completed as of September 2007.

at 13 (“Analysis of retinas and RPE from these *abcr*^{-/-} mutants revealed a complex phenotype involving physiological, biochemical, and ultrastructural changes.”).

(1) Enzyme Interactions

83. Numerous enzymes play critical roles in the visual cycle’s production of lipofuscin pigments. EX2059 (Rando 2001) at 11–12; EX1050 (Kuska) at 2–3.

84. The generation of ATR, a necessary prerequisite to the biosynthesis of the lipofuscin pigments¹³, is directly influenced by a diverse collection of different

¹³ As *Mata 2000* recognized, the availability of excess ATR modulates the rate of both the initial Schiff base reaction between ATR and PE as well as the second condensation reaction between NRPE and a second molecule of ATR. EX1010 (Mata 2000) at 5–6; EX2066 (Maiti 2006) at 1–2; EX1030 (Radu 2005) at 8 (“It is well established that the genesis of ocular lipofuscin fluorophores is dependent on the presence of retinol and/or retinaldehyde (e.g., atRAL).”); EX2019 (Kim 2007) at 1 (“A number of observations over the years have shown that the deposition of lipofuscin fluorophores in RPE is dependent on the availability of vitamin A and/or vitamin A derivatives.”); EX2062 (Rózanowska 2005) at 4 (“For accumulation of any additional ATR, rhodopsin chromophore, 11-*cis*-retinal, must be efficiently synthesized in the RPE and delivered to OS to regenerate rhodopsin, which subsequently needs to be photobleached.”).

enzymatic processes. For example, the amount of ATR available to supply these reactions was known to be regulated in part by atRDH:

The results suggest that the rate-limiting step of the visual cycle is the reduction of all-trans-retinal to all-trans-retinol by all-trans-retinol dehydrogenase. The accumulation of all-trans-retinal during illumination may be responsible, in part, for the reduction in sensitivity of the visual system that accompanies photobleaching and may contribute to the development of retinal pathology associated with light damage and aging.

EX2060 (Saari 1998) at 1; *see also* EX1036 (Fishkin 2003) at 2 (“Since the reduction of retinal to retinol is the rate-limiting step in the vitamin A cycle, the sequestration of all-*trans*-retinal as NRPE and access of the latter to all-*trans*-retinol dehydrogenase [atRHD], appears to be important in limiting the accumulation of all-*trans*-retinal in the outer segment.”)

85. Elsewhere, *Maiti 2006* looked to the involvement of the enzyme RPE65. EX2066 (Maiti 2006) at 2 (“RPE65 is also on the rate-limiting pathway to A2E formation.”).

86. As explained above, many of these enzymatic processes were poorly understood, both as individual reactions and as components of various reaction sequences or cascades of successive transformations. § V.B.1; EX2060 (Saari 1998) at 7.

87. Thus, returning to *Mata 2000*'s proposed reaction scheme (above)¹⁴, a POSA would have recognized that the rate of least (i) the initial formation of NRPE from ATR and PE, (ii) and the bimolecular condensation reaction between APE and a second molecular of ATR were directly influenced by numerous enzymatic processes, many of which were incompletely understood. EX1010 (*Mata 2000*) at 5, Fig. 5. A POSA would have therefore been aware that the involvement of a number of enzymes in, for example, the conversion of A2PE to A2E remained unresolved.

(2) Transport Proteins and Processes

88. It was known that transport process also played a critical role in the determining the amount of ATR and hence the rate of A2E formation. Lipofuscin formation occurs across different cellular compartments. Movement between these different cellular compartments was known to be facilitated by both active transport (e.g., transport and chaperone proteins) and passive transport (diffusion)

¹⁴ The scheme in *Mata 2000* is used here to exemplify the effects of the different biological processes not shown in these chemical reaction schemes, but the same principles apply to the other schemes discussed above (e.g., Fishkin 2005). Of course, given the different hypothetical reactions, each of the biological processes discussed herein apply in separate ways and to different degrees depending on which hypothetical scheme one relies on. EX1010 (*Mata 2000*) at 5, Fig. 5.

mechanisms. *E.g.*, EX2062 (Rózanowska 2005) at 4–6; EX2059 (Rando 2001) at 1–2.

89. For example, the Rim protein (“RmP”) was believed to be act as a “flippase”, i.e., an energy requiring enzyme that uses ATP to facilitate the reduction of ATR by physically flipping the NRPE from the inner face of ROS disc membrane to the outer leaflet of the disc membrane. EX2062 (Rózanowska 2005) at 6.¹⁵ In the absence of RmP, NRPE and ATR accumulate in the disc interior rather than being translocated to the outer leaflet, thus providing the “excess” ATR believed to be the substrate for A2E formation in Mata’s proposed pathway. EX2062 (Rózanowska 2005) at 6. *Rózanowska 2005* further explained that the absence of this transport system, i.e., the ABCR-encoded RmP flippase, played a critical role in the rate at which ATR could be cleared from the ROS, and hence on the rate at which lipofuscin

¹⁵ EX2062 (Rózanowska 2005) at 4 (“the rate of accumulation of ATR in the inner leaflet of OS disc membrane must exceed the rate of removal of ATR, where removal refers to its direct enzymatic reduction to all-*trans*-retinol, or formation of a reversible Schiff base linkage with phosphatidylethanolamine (PE) and *N*-retinylidene phosphatidylethanolamine (NRPE) and its transport across to the outer leaflet of OS disc membrane, where subsequent reduction to all-*trans*-retinol can occur.”).

pigments could accumulate. EX2062 (Rózanowska 2005) at 6 (“It may be speculated that in the absence of ABCR [the gene encoding the flippase RmP] the transverse diffusion of ATR/NRPE to the outer leaflet becomes the limiting factor in the reduction of ATR to all-*trans*-retinol.”)

90. As explained above, dozens of other transport and chaperone proteins were thought to be involved in the visual cycle. § V.B.1; *see also* EX2059 (Rando 2001) at 3–12.

(3) Location of Reactions

91. The formation of lipofuscin in the visual cycle was known to occur in both the ROS and the RPE. A POSA would have understood that the rate of the chemical reactions in the above reaction schemes would vary significantly depending on the physiological compartment or compartments in which each of these reactions occurred.

92. As of September 2007, there was no consensus over where many of the individual chemical reactions leading to the formation of bisretinoid lipofuscin pigments took place.

93. *Mata 2000* disclosed that the conversion of A2P2-H2 to A2PE and the conversion of A2PE to A2E most likely occur within the RPE phagolysosomes.

EX1010 (Mata 2000) at 1, 5.¹⁶ In 2007, *Travis 2007* echoed *Mata 2000*'s conclusion, disclosing that the conversion of A2PE-H2 occurs in the “acidic and oxidizing conditions” of the RPE. EX2003 (Travis 2007) at 7.¹⁷

94. In contrast, *Ben-Shabat 2002* reached the opposite conclusion, explaining: “the observation that A2-PE formation from all-*trans*-retinal and PE is optimal at neutral pH *are incompatible with the formation of A2E in the acidic environment of RPE cell lysosomes.*” EX2051 (Ben-Shabat 2002) at 6. Similarly, *Fishkin 2005* disclosed that A2PE formed in the ROS, not in the RPE as suggested by *Mata 2000* and *Travis 2007*. EX1011 (Fishkin 2005) at 1.¹⁸ *Kim 2007* also

¹⁶ EX1010 (Mata 2000) at 1 (“The latter two reactions probably occur within RPE phagolysosomes.”), 5 (“These observations suggest that the final two reactions in Fig. 5 occur only within the oxidizing and acidic environment of RPE phagolysosomes.”).

¹⁷ EX2003 (Travis 2007) at 7 (“A2PE-H2 is converted to A2E and A2E-epoxides upon exposure to short-wavelength light in the acidic and oxidizing conditions of RPE phagolysosomes”).

¹⁸ EX1011 (Fishkin 2005) at 1 (“A2-PE, the phosphatidyl-pyridinium bisretinoid precursor that forms in rod outer segments (ROS).”).

provided “[s]everal lines of investigation” suggesting that A2PE formed in the photoreceptor outer segments. EX2019 (Kim 2007) at 6.¹⁹

95. *Fishkin 2005* further suggested that the formation of the lipofuscin pigment ATR-dimer conjugate, the compound that *Mata 2000* identified as the A2PE-H₂ intermediate in its A2E pathway, likewise formed in the ROS and was only subsequently deposited in the RPE via phagocytosis. EX1011 (Fishkin 2005) at 4–5.²⁰

96. One of skill in the art would have recognized that the ROS and RPE are decidedly different cellular environments. The ROS environment is characterized by a neutral pH and a reducing atmosphere maintained to protect against oxidative stress. In contrast, the RPE, particularly within its lysosomes, comprises an acidic

¹⁹ EX2019 (Kim 2007) at 6 (“Several lines of investigation also demonstrated that A2PE forms within photoreceptor outer segments.”).

²⁰ EX1011 (Fishkin 2005) at 4 (“ATR dimer . . . formation in photoreceptors is likely the result of *general acid-base catalysis* by membrane-bound proteins in the outer segment . . .”), 5 (“it seems reasonable that ATR dimer forms a conjugate with PE *in* the outer segment and that the resulting pigment then becomes deposited in RPE cells through photoreceptor outer segment disk phagocytosis”).

environment (pH around 4.5-5) with oxidizing conditions and a variety of hydrolytic enzymes. *E.g.*, EX2069 (Ben-Shabat 2001) at 6–7.

97. Returning to the hypothetical biosynthetic pathway proposed by *Mata 2000*, it is apparent that the rate of the conversion of A2PE-H2 to A2PE and the final hydrolysis of A2PE to form A2E, were likely to proceed much more rapidly in the acidic, oxidizing environment of the RPE lysosomes, but would proceed slowly in the ROS. EX1010 (*Mata 2000*) at 5, Fig. 5. Consequently, a POSA, attempting to determine the reaction rates in the A2E pathway in September 2007, would have recognized the need to account for the influence of these differing cellular environments and that there was insufficient knowledge at the time with which to do so.

(4) Cellular Environment and Lipid Membrane

98. A POSA would have understood the viscosity of specific cellular environments would also influence both the rates of diffusion of the reacting species as well as the rate of reaction.

99. Metabolic reactions in living cells are limited by diffusion of reagents in the cytoplasm. The cytoplasm is a complex, crowded fluid and it was known that the viscosity of the cytoplasm is not constant, but spatially heterogeneous. Any attempt to quantify the kinetics of biochemical reactions in the cytosol requires

careful consideration and knowledge of the physical properties of the cellular interior.

100. Moreover, a POSA would have understood that many of steps in the A2E pathway, including the initial reaction between ATR and PE, as well as the bimolecular condensation reaction between NRPE and a second molecular of ATR, were known to occur at, and be influenced by, the complex chiral transmembrane protein-containing lipid bilayer of the ROS. *E.g.*, EX1034 (Wolf 2003) at 1.²¹

101. The lipid membrane is comprised of a phospholipid bilayer structurally augmented with cholesterol, proteins, and other constituents. EX2082 (Weng 1999) at 6–7; EX2062 (Rózanowska 2005) at 2, 13. The lipid membrane acts as a flexible interface separating the cell interior from the extracellular environment. EX2082 (Weng 1999) at 6–7; EX2062 (Rózanowska 2005) at 2, 13. As with other lipid bilayer membranes, the rod outer segments (ROS) are composed of phospholipids arranged with their hydrophilic head groups facing either inward to the cytoplasm (for the inner leaflet of the bilayer) or outward (for the outer leaflet) to the extracellular space. EX2082 (Weng 1999) at 6–7, Fig. 7. The hydrophobic tails of

²¹ EX1034 (Wolf 2003) at 1 (“The formation of lipofuscin begins in the photoreceptor outer segments, where [ATR] reacts spontaneously (i.e. non-enzymatically) with the membrane lipid [PE] to form [NRPE]”)

both inner and outer leaflets face inward to the hydrophobic core of the bilayer. *Weng 1999* graphically illustrates how these attributes of the bilayer membrane pertain to the ROS inner and outer DM compartments, showing several visual cycle-relevant proteins, and where the NRPE and potential ATR adducts might occur. EX2082 (*Weng 1999*) at 6–7, Fig. 7.

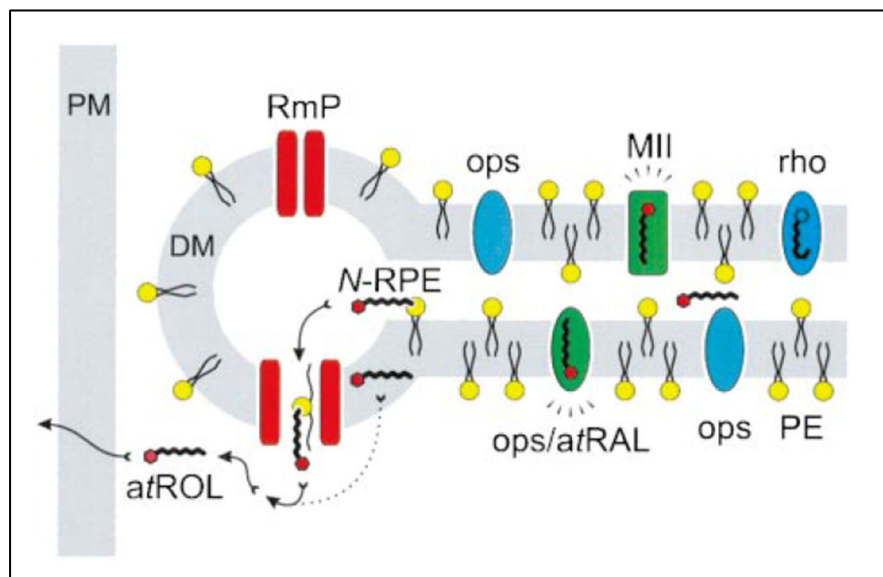


Figure 8. Proposed Model of the ROS Lipid Membrane.
EX2082 (*Weng 1999*) at 7, Fig. 7.

102. *Fishkin 2005* further proposed that this lipid bilayer environment was likely a *chiral* environment based on key experimental observations from *Fishkin 2005*. EX1011 (*Fishkin 2005*) at 4. In particular, *Fishkin 2005* explained how ATR-dimer likely forms by “general acid-base catalysis by membrane-bound proteins in the outer segment which gives rise to a 13% enantiomeric excess of the 13’ (*R*) configuration.” EX1011 (*Fishkin 2005*) at 4. This observation led the authors to

conclude that “this enantioenrichment is *consistent with the involvement of a chiral protein environment* in the catalysis.” EX1011 (Fishkin 2005) at 4.

103. Returning to *Mata 2000*’s hypothetical pathway, a POSA would have understood that the rates of each of the reactions that took place within the immediate proximity of this protein-chiral lipid environment of the ROS²² would be influenced by the crowded, protein and lipid-rich nature of this dynamic environment.

104. The rate of the bimolecular condensation reaction between (NRPE) and a second molecule of ATR would likely have been particularly susceptible to this influence. This is because bimolecular reactions are dependent not only on the concentration of the reactants—which determines in part the frequency of intermolecular collisions—but also on the orientation of reactants when they collide.

105. The frequency of productive reactions occurring from bimolecular collisions is often referred to as the reaction cross-section. EX2068 (Greene 1968)

²² By September 2007, it was known that the initial reaction between ATR and PE and the bimolecular condensation reaction took place as described in the preceding section (§ V.B.2.b.3). However, as also discussed, there was no consensus as to where the subsequent steps in the reaction took place.

at 5.²³ Absent the correct orientation, the reaction will not proceed to products. The crowded, chiral protein lipid environment in which this reaction was assumed to take place could significantly limit the frequency with which these collisions occurred in the correct orientation. EX1011 (Fishkin 2005) at 4.

(5) Phagocytosis

106. Phagocytosis of the ROS by the RPE would also have been recognized as a necessary step in the formation of lipofuscin.

107. Phagocytosis is the process by which cells ingest and eliminate foreign materials, such as microorganisms, dead cells, or other cellular debris. *E.g.*, EX1020 (Shaban Richter) at 1.²⁴ Each day in the visual cycle, RPE cells phagocytose portions of the ROS. EX1020 (Shaban Richter) at 1; *see also* EX2003 (Travis 2007) at 13.

²³ The reaction cross-section is defined by *Greene 1968* as “a proportionality constant with the dimensions of area per molecule which measures the effective size of the molecules for reaction to occur, that is, the likelihood that a collision between a pair of molecules will lead to reaction.” EX2068 (Greene 1968) at 5.

²⁴ EX1020 (Shaban Richter) at 1 (“Phagocytosis, degradation and recycling by RPE cells of outer segment material [] are pivotal for the physiology and pathology of the retina.”)

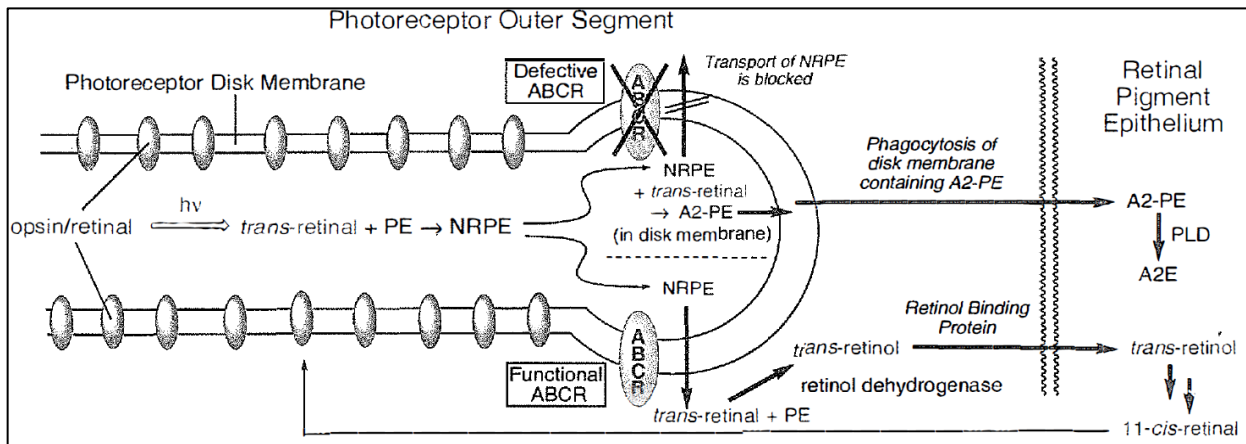


Figure 9. Phagocytosis of the ROS by the RPE.
EX2069 (Ben-Shabat 2001) at 8.

108. As of September 2007, phagocytosis of the ROS was understood to take place “*via membrane receptor-mediated processes that are not yet well defined.*” EX2080 (Bok 1993) at 1. Bok 1993 theorized that during the ingestion of the ROS by the RPE cell, there was a two-step lysosomal response: “First, small lysosomes fuse with the outer segment to form phagolysosome. Then larger lysosomes, formed by the fusion of smaller precursors, interact with the phagolysosomes through what appear to be bridge-like structures.” EX2080 (Bok 1993) at 2.

109. As with the other biological processes discussed in this section (§V.B.1.b), phagocytosis is not depicted in the simplified chemical accounted for in *Mata 2000*. Nonetheless, phagocytosis is a necessary prerequisite to the proposed reaction scheme of *Mata 2000* uses to rationalize the formation of A2E. Although

not accounted for in the proposed reaction scheme,²⁵ *Mata 2000* implicitly recognizes that phagocytosis occurs when explaining that NRPE forms “[i]n [rod] outer segments after a photobleach” but that the final two reactions occur “only within the oxidizing and acidic environment of RPE phagolysosomes.” EX1010 (*Mata 2000*) at 5; *see also* EX1034 (*Wolf 2003*) at 3.²⁶

110. Finally, a POSA would have recognized that none of these processes occur in isolation, but rather that the processes are intimately interconnected. Changes to any one process could, and likely would, have multiple effects elsewhere in the tightly interconnected system. As *Różanowska 2005* explains, numerous processes contribute the accumulation of ATR—the necessary step in the formation of lipofuscin pigments.

Presently, we do not know what the worst case scenario for accumulation of ATR within OS is. *We can only speculate* that in the case of exposure to *intensive light* rapidly bleaching all the rhodopsin resulting in very low *steady-state levels of rhodopsin*, the rate of

²⁵ EX1010 (*Mata 2000*) at 5, Fig. 5.

²⁶ EX1034 (*Wolf 2003*) at 3 (“NRPE accumulates inside the lumen of the discs, where increasing levels of all-*trans*-retinal, produced by light-activation of rhodopsin, increase A2PE formation. Phagocytosis by the RPE and hydrolysis of the A2PE then lead to accumulation of the stable A2E.”)

removal of ATR, the content of retinyl esters stored in retinosomes and the regulatory pathways responsible for their mobilization will determine the concentrations of ATR transiently accumulating in the OS.

EX2062 (Rózanowska 2005) at 9.

111. In sum, as of September 2007, a POSA would have understood that lipofuscin formation involved a complex interplay of chemical reactions, physicochemical and biological processes, occurring in a variety of physiological compartments, many of which were known to be not fully understood at the time. A POSA would have recognized that one or more of these processes could influence the rate of any of the reactions in the postulated reaction scheme of *Mata 2000*. Given this complexity, the simplified chemical rationalization of a potential biosynthetic pathway, and the lack of a complete understanding of the full biological context of this proposed pathway, a POSA would have considered it virtually impossible to accurately assess the rate (or relative rates) of any individual step in the formation of A2E.

3. Prior Art Attempts to Reduce Lipofuscin Accumulation

112. Prior art approaches to reducing lipofuscin accumulation were focused on reducing the amount of excess ATR available to form lipofuscin pigments. EX2003 (Travis 2007) at 14 (“Several approaches have been tested or proposed to

slow accumulation of A2E. *All . . . ultimately limit the formation of all-trans-RAL.*”); *see also* EX2003 (Travis 2007) at 9.

113. As explained above, the art taught that it was the availability of excess ATR that controlled, i.e., was rate limiting, on the formation of lipofuscin pigments, such as A2E. EX1010 (Mata 2000) at 6 (“One prediction of the pathway proposed in Fig. 5 is that A2E accumulation should depend on the availability of all-*trans*-RAL”); *see also* EX1036 (Fishkin 2003) at 2; EX1030 (Radu 2005) at 1, 8.²⁷

114. Consistent with this, researchers at the time consistently sought to limit the availability of excess ATR. The belief was that limiting the amount of excess ATR available to participate in the initial reaction between ATR and PE and the condensation reaction between NRPE and second molecule of ATR would lower the frequency with which they occur, and, in turn, reduce the formation of A2E. *Travis 2007* explains:

All-trans-RAL released by photoactivated rhodopsin condenses with phosphatidylethanolamine (PE) in disc membranes to form *N*-

²⁷ EX1030 (Radu 2003) at 1 (“Because A2E biosynthesis relies ultimately on circulating retinol, therapies that lower retinol should lower A2E levels These findings *demonstrate the vitamin A-dependent nature of A2E biosynthesis*”), 8 (“It is well established that the genesis of ocular lipofuscin fluorophores is dependent on the presence of retinol and/or retinaldehyde”).

retinylidene-PE (*N*-ret-PE). Secondary condensation of *N*-ret-PE with another all-*trans*-RAL, followed by electrocyclization, hydrolysis of the phosphate ester, and oxidation yields A2E. Pharmacologic interventions that reduce light-dependent formation of all-*trans*-RAL greatly reduce the probability of this secondary condensation and inhibit formation of A2E.

EX2003 (Travis 2007) at 35, Fig. 5; *see also* EX2066 (Maiti 2006) at 2.²⁸

115. By September 2007, several different methods of pharmacologically reducing ATR had been considered and attempted. A number of researchers sought

²⁸ EX2066 (Maiti 2006) at 1 (“The evidence . . . indicates that the excessive accumulation of A2E and related fluorophores plays a direct and essential role in the causation of the ABCR-associated disease. It follows that inhibition of A2E formation in diseases caused by ABCR defects, *by limiting the visual cycle and, therefore, the formation of all-trans-retinal, represents a therapeutic approach to the treatment of AMD*. A specific antagonist directed against a rate-limiting element in the visual cycle could emerge as a preventative, lipitor-like drug for the treatment of retinal degenerative disorders.”)

to reduce the amount of ATR available in the visual cycle by inhibiting the conversion of retinoids in the visual cycle. EX2064 (Maeda 2006) at 2.²⁹

116. For example, *Radu 2003* studied Isotretinoin (Accutane®). Isotretinoin inhibits 11-*cis*-retinol dehydrogenase (“11-*cis*-RDH”), an enzyme that converts 11-*cis*-ROL to 11-*cis*-RAL in visual cycle. EX1035 (Radu 2003) at 1. Reducing the production of 11-*cis*-RAL reduces the subsequent production of ATR (which is produced from 11-*cis*-RAL). EX1035 (Radu 2003) at 5. *Radu 2003* reported that treatment of *abcr*^{-/-} mice with isotretinoin “completely suppressed” accumulation of A2E and concluded “isotretinoin or other inhibitors of 11cRDH [11-*cis*-RDH] may be effective treatments for retinal or macular degenerations of different etiologies

²⁹ EX2064 (Maeda 2006) at 2 (“another strategy proposed by Travis and colleagues was to reduce the flow of retinoids through the inhibition of the visual cycle and thus slow the production of the component of A2E, *all-trans*-retinal (Mata et al., 2000; Radu et al., 2003).” Notably, the “Mata et al., 2000” publication that Maeda cites for this proposition is the same paper as *Mata 2000* (EX1010) discussed herein. EX2064 (Maeda 2006) at 10.

associated with A2E accumulation.” EX1035 (Radu 2003) at 6; *see also* EX2003 (Travis 2007) at 14;³⁰ EX1034 (Wolf 2003) at 1.³¹

117. *Gollapalli 2004* studied the ability of atRA, isotretinoin (Accutane®), and 4-HPR to bind to and inhibit RPE65. EX2063 (Gollapalli 2004) at 1. RPE65 functions as a chaperone for all-*trans*-retinyl esters, making them available for isomerization to 11-*cis*-ROL. EX2063 (Gollapalli 2004) at 1; EX2066 (Maiti 2006) at 3.³² The authors suggested that inhibiting this function would slow the production of ATR (which is produced from 11-*cis*-ROL), reducing A2E accumulation. EX2063 (Gollapalli 2004) at 1, 5; *see also* EX2003 (Travis 2007) at 15.

118. *Maiti 2006* also targeted RPE65 but instead used two (*E,E*)-farnesyl-containing analogues, TDT and TDH. EX2066 (Maiti 2006) at 2. The authors explained that “limiting the visual cycle and, therefore, the formation of all-*trans*-retinal” represents a “therapeutic approach to the treatment of AMD.” EX2066

³⁰ EX2003 (Travis 2007) at 14.

³¹ EX1034 (Wolf 2003) at 1 (reviewing *Radu 2003* and noting “*isotretinoin inhibits* a key step in the visual cycle”)

³² EX2066 (Maiti 2006) at 3 (“Its functional role is to stereoselectively bind hydrophobic all-*trans*-retinyl esters, making them available for isomerization and hydrolysis in the visual cycle”) (citations omitted).

(Maiti 2006) at 2. The authors reported that *abcr*^{-/-} mice treated with these compounds “abolished the formation of A2E.” EX2066 (Maiti 2006) at 2. They concluded that: “Our studies suggest that these, or similar, molecules may be efficient and nontoxic candidates as drugs aimed at preventing the onset of lipofuscin-sensitive form of macular degeneration, including STGD and a prevalent form of AMD (geographic atrophy).” EX2066 (Maiti 2006) at 10; *see also* EX2003 (Travis 2007) at 15.

119. *Maiti 2006* also highlights the fact that since ATR is required for the formation of A2E, a rate limiting step in the visual cycle can thus also be rate-limiting in the A2E pathway. That is, this rate limiting process in the visual cycle was also the considered rate-limiting in the formation of A2E. *Maiti 2006* explains:

RPE lipofuscin, including the fluorophore A2E, forms in large part as a byproduct of the visual cycle. Inhibiting visual cycle function with small molecules is required to prevent the formation of the retinotoxic lipofuscins. This in turn requires identification of rate-limiting steps in the operation of the visual cycle.

EX2066 (Maiti 2006) at 2.

120. The authors of *Maiti 2006* further explain that the studies it described “provide strong evidence that mRPE65 is rate-limiting in visual cycle function *and* in A2E formation and that mRPE65 is a valid target for therapies designed to prevent the accumulation of lipofuscin.” EX2066 (Maiti 2006) at 3.

121. *Maeda 2006* used retinylamine (Ret-NH₂), which had been shown to inhibit the isomerization of 11-*cis*-retinol. EX2064 (*Maeda 2006*) at 2; EX2003 (*Travis 2007*) at 15. *Maeda 2006* explained: “[t]he target for this inhibitor seems to be either a subfraction of RPE65 or another protein essential for the isomerization reaction.” EX2064 (*Maeda 2006*) at 2. The authors concluded that: “[i]nhibiting A2E formation should therefore slow the progression of visual loss in diseases associated with A2E over accumulation.” EX2064 (*Maeda 2006*) at 11. Moreover, they noted that “the current study is an important prelude for understanding the metabolism, the proper doses, timing, and mechanism, of the drug [Ret-NH₂] action.” EX2064 (*Maeda 2006*) at 11; *see also* EX2003 (*Travis 2007*) at 15.

122. On the other hand, other researchers proposed limiting the amount of retinoids introduced into the visual cycle (as opposed to limiting the conversion of retinoids already in the visual cycle to ATR). EX1030 (*Radu 2005*) at 1; EX1009 (*Widder*) at 16, 24.

123. For example, *Radu 2005* studied *N*-(4-hydroxyphenyl)retinamide (“HPR,” or “4-HPR”) as a “therapeutic approach based on reduction of serum retinol.” EX1030 (*Radu 2005*) at 1. The authors explained that “[b]ecause A2E biosynthesis relies ultimately on circulating retinol, therapies that lower retinol should lower A2E levels.” EX1030 (*Radu 2005*) at 1. *Radu 2005* notes that while lipofuscin was known to be dramatically reduced by a retinol deficient diet, this

approach was impractical due to deleterious effects associated with long-term retinol deficiency. Thus, the authors suggested that serum retinol could be regulated by “pharmacological means.” EX1030 (Radu 2005) at 1–2.

124. Retinol-binding protein (“RBP”) is part of a complex that plays a role in transporting vitamin A from the liver to the eye. E.g., EX1030 (Radu 2005) at 2. 4-HPR (Fenretinide®) binds to RBP, inhibiting this usual function and thus reducing the amount of dietary vitamin A that is taken up by the eye. EX1030 (Radu 2005) at 2. The authors reported that “HPR-mediated reductions in serum retinol led to proportionate reductions in toxic A2E-fluorophores.” EX1030 (Radu 2005) at 7; *see also* EX2003 (Travis 2007) at 14.

125. The strategy for using HPR described in Radu 2005 was also disclosed in the *Widder* patent application filed the same year. EX1009 (Widder) at 3 (explaining the patent application describes pharmacological agents that lower “the levels of serum retinal in the body of a patient”), at 14 (describing testing showing administration of HPR lowered serum levels of all-*trans*-retinol and the formation of A2E).

126. In 2007, the senior author of *Mata 2000*, Dr. Gabriel Travis, reviewed many of the strategies discussed above in *Travis 2007*. *Travis 2007* concluded that all-*trans*-RAL A2E production can be prevented using “*pharmacological therapies* aimed at reducing the rate of the retinoid cycle and/or limiting uptake of serum

retinal into the RPE.” EX2003 (Travis 2007) at 18. Dr. Travis also suggested that “[p]ossible future therapeutic interventions could involve the regulation of the rate of the retinoid cycle.” EX2003 (Travis 2007) at 18.

127. Notably, having considered the art including *Mata 2000*, neither *Travis 2007* nor any of the papers discussed in this section (§ V.B.3) identify the [1,5] shift described in *Mata 2000* as an actual or potential target for preventing A2E production. EX2003 (Travis 2007) at 17–18; EX1035 (Radu 2003) at 6; EX2063 (Gollapalli 2004) at 5; EX2066 (Maiti 2006) at 7–10; EX2064 (Maeda 2006) at 11; EX1030 (Radu 2005) at 7–8; EX1009 (Widder 2009) at 46.

128. Thus, as of September 2007, a POSA would have understood the availability of excess ATR was rate-limiting on the formation of A2E and that reducing the excess ATR available for the initial ATR-PE condensation and the ATR-NRPE could reduce lipofuscin formation. A POSA would have recognized that researchers focused on treating lipofuscin-associated disorders were targeting a reduction in excess ATR by slowing the conversion of retinoids in the visual cycle and limiting the uptake of new retinoids from the bloodstream.

C. Deuterium Isotope Effect

1. The Kinetic Isotope Effect

129. As of September 2007, the deuterium kinetic isotope effect was recognized as a versatile tool for studying chemical and biological processes. Its

applications in these fields included analytical techniques like mass spectrometry, pharmacokinetic studies, metabolic investigations, mechanistic studies, structural elucidation, and isotope tracing, to name but a few. *See generally* EX1013 (Baillie); EX1047 (Blake) at 1;³³ EX1046 (Kushner 1999) at 1, 5–7.³⁴

130. The kinetic isotope effect (KIE) is generally defined as the difference in the rate of reaction caused by substituting an atom in a molecule with one of its heavier isotopes, e.g., replacing hydrogen with deuterium. EX2038 (Anslyn) at 24; EX1047 (Blake) at 3.

131. Within this general category however, it is important to distinguish between “intrinsic” isotope effects and “observed” isotope effects. An intrinsic isotope effect (k_H/k_D) represents the “true” difference in the rate of C-H bond

³³ EX1047 (Blake) at 1 (“Deuterated drugs have proved useful in studying isotope effects, in permitting a better understanding of the mechanism of drug action, and in elucidating metabolic and biosynthetic pathways.”).

³⁴ EX1046 (Kushner 1999), 1, 5–7 (Explaining deuterated compounds had “been widely used” to study metabolic reactions, “in studies of metabolism and movement of drugs and toxic substances in humans and other animals,” and “as biochemical tracers.”).

breaking and the rate of a C-D bond-breaking in a reaction, independent of other steps in the reaction mechanism. EX2085 (Nelson 2003) at 1.

132. Intrinsic effects are measured using techniques that isolate the bond-breaking step from any other steps in overall reaction mechanism, using e.g., intramolecular competition experiments. EX2085 (Nelson 2003) at 1–2. Intrinsic isotope effects provide insight into transition state structure and reaction mechanisms. A large intrinsic effect indicates a symmetrical transition state, while smaller values suggest an asymmetrical transition state. EX1015 (Roth), 3–4; EX2038 (Anslyn) at 28. But while intrinsic isotope effects can provide valuable information about the nature of the bond-breaking step, such as the nature of the transition state, they do not provide information on the rate of this step *relative* to other steps in the overall reaction mechanism. EX2085 (Nelson 2003) at 1.

133. *Roth* (EX1015) is an example of a study leveraging the *intrinsic* isotope effect. *Roth* examined the intrinsic isotope effect for the 1,5-hydrogen shift in *cis*-pentadiene using specially synthesized deuterated compounds.³⁵ EX1015 (Roth) at 5–6. By comparing rate constants for hydrogen (k_H) and deuterium (k_D) migration,

³⁵ 1,1-dideutero-pentadiene-(1,3) (compound 3) and *cis*-5,5,5-trideutero-pentadiene-(1,3) (compound 4).

the authors obtained an intrinsic KIE of 12.2. EX1015 (Roth) at 6, n. 11, 8, n. 13.³⁶ This large KIE led them to conclude that the reaction proceeds through a symmetrical transition state. EX1015 (Roth) at 1.

134. In contrast to *intrinsic* isotope effects, an *observed* isotope effect $(k_H/k_D)_{\text{obs}}$ is the ratio of reaction rates between protium and deuterium substrates for an entire reaction process. EX2085 (Nelson 2003) at 1. Unlike intrinsic isotope effects, this measure depends on and reflects the combined influence of all the biological and physical processes that together determine the overall rate of reaction. EX2085 (Nelson 2003) at 1; EX2083 (Krauser 2005) at 1. In a multi-step reaction, this includes steps in the reaction mechanism before and after the bond-breaking step.

135. *Observed* effects can be measured through experiments comparing overall reaction rates or product ratios between hydrogen and deuterium substrates. EX2085 (Nelson 2003) at 1; EX2083 (Krauser 2005) at 1. The observation of an effect can provide insights into which step in the reaction mechanism might be rate-limiting within the overall reaction. EX2085 (Nelson 2003) at 3–4; EX1051 (Miwa)

³⁶ EX1015 (Roth) at 6, n. 11 (“ k_H is defined as the rate constant for a single hydrogen displacement, taking into account the statistical conditions”), 7, n. 13 (“ k_D was defined analogous to quotation¹¹”).

at 1.³⁷ A large observed KIE suggests that the C-H bond breaking step is rate-limiting, whereas the absence of a KIE suggests that other steps or processes in the reaction could be rate-limiting. EX1014 (Van Langehove) at 1–2; EX2083 (Krauser 2005) at 9–10.

136. The existence of an intrinsic isotope effect on a reaction will only result in an observed isotope effect for the reaction when the C-H bond breaking step is rate-limiting for the overall reaction. EX1047 (Blake) at 3;³⁸ EX2084 (Westheimer) at 3. This is because the contributions to the reaction rate from breaking the C-H bond (measured with the intrinsic isotope effect) can be “masked” by the contributions of other steps in the reaction (measured by the observed isotope effect).

³⁷ EX1051 (Miwa) at 1 (“[The] kinetic isotope effect, provides useful information about the importance of this step in the overall reaction and the nature of the transition step.”).

³⁸ EX1047 (Blake) at 3 (“An observable isotope effect will only be apparent, of course, where the breaking of a C-H or C-D bond is involved in the rate-determining step.”).

E.g., EX2085 (Nelson 2003) at 2-3, 7; EX2083 (Krauser 2005) at 1;³⁹ EX1013 (Baillie) at 34.⁴⁰

137. The difference between intrinsic and observed isotope effects is often discussed in the context of enzymatic reactions, where intrinsic isotope effects are masked by the multistep enzymatic reaction. *E.g.*, EX2083 (Krauser 2005) at 1; EX1013 (Baillie) at 34. This concept applies equally to the multistep biological process in the hypothetical pathway described by *e.g.*, *Mata 2000*, *Fishkin 2005*. EX2084 (Westheimer) at 3.⁴¹ In both cases, the overall reaction proceeds through

³⁹ EX2083 (Krauser 2005) at 1 (“The use of kinetic isotope effect methods with enzymes is subject to the complexity of these multi-step systems and the isotope effect on the chemical step can be masked by the contributions of other steps.”).

⁴⁰ EX1013 (Baillie) at 34 (“In multistep enzymatic processes . . . many factors may influence, or ‘mask,’ this intrinsic isotope effect and thus observed (or ‘apparent’) kinetic deuterium isotope effects are normally much less than the above figures.”).

⁴¹ *Westheimer* explains: “the kinetic isotope effect obtains only when a bond to a hydrogen atom is broken (or formed) in the rate controlling step of a reaction.” EX2084 (Westheimer) at 3. The authors further describe examples of non-enzymatic reactions where the C-H bond breaking step was not rate limiting. EX2084 (Westheimer) at 3.

multiple intermediates, each step potentially masking the intrinsic isotope effect.⁴² On the other hand, for single step chemical reactions (such as the [1,5] sigmatropic rearrangement studied by *Roth* and *Itou*), the intrinsic isotope effect is generally representative of the observed isotope effect, as there are no other steps in the reaction that can mask the contribution of the C-H bond breaking step.

138. This masking effect is intuitive—any reaction can proceed only as fast as its slowest step allows, and that slowest step will control the overall reaction rate. § V.A. If the C-H bond breaking step is fast relative to the other steps and processes in the reaction, there is no basis to expect that slowing the C-H bond breaking step (even when there is an intrinsic KIE) will result in an observed KIE for a multistep reaction in a complex reaction environment.

2. Therapeutic Uses of the Kinetic Isotope Effect

139. At the time of invention, researchers had also attempted to use deuterium substitution to improve drugs by slowing or redirecting their enzymatic metabolism. *E.g.*, EX1012 (Haskins) at 1–2; EX1051 (Miwa) at 1. Drs. Guengerich and Calman conflate this relatively narrow use with an understanding in the art that

⁴² In addition to the contributions of the other chemical steps, biological processes (e.g., transport mechanisms) can also mask the observed isotope effect. EX1013 (Baillie) at 35; EX1047 (Blake) at 10, 18.

deuterium substitution was broadly useful therapeutically to slow *any* undesirable reaction. EX1002 ¶¶99–123; EX1004 ¶¶96–102.

140. I fundamentally disagree with the overly broad, as well as overtly hindsight driven, interpretation of the prior art from Drs. Guengerich and Calman. As detailed below, none of the references they cite suggest using deuteration to slow undesirable reactions *generally* for therapeutic purposes. Rather, these references show that deuterium substitution had been used in specific circumstances to attempt to improve the known function of a drug by altering enzyme mediated metabolism.

141. Dr. Guengerich asserts “[u]sing *deuteration to slow undesirable reactions* was one of *several known uses* of deuteration by 2007.” EX1002 ¶100. Dr. Guengerich relies on *Haskins 1982* (EX1012), *Baillie 1981* (EX1013), and *Miwa 1987* (EX1051). EX1002 ¶¶99–115. I disagree. None of these references taught or suggested the use of deuterium to slow undesirable reactions *generally*.

142. ***Haskins* (EX1012)** is a review article titled “The Application of Stable Isotopes in Biomedical Research.” EX1012 (*Haskins*) at 1. *Haskins* reviews the use of deuterium for stable isotope dilution assays, metabolite identification, bioavailability studies, and pharmacokinetics in studies of chronic administration. EX1012 (*Haskins*) at 1–5.

143. *Haskins* includes a single section (“biochemical stable isotope effects”) that discusses potential therapeutic uses of the deuterium isotope effect. EX1012

(Haskins) at 5–6. This section discusses the use of deuterium to slow enzymatic metabolism to improve the duration of effect of the parent compound and using metabolic switching to mitigate the effect of toxic metabolites. EX1012 (Haskins) at 5–6.

144. *Haskins* does not disclose or otherwise suggest that deuterium has been, or could be, used therapeutically to “slow undesirable reactions” in general. Nor did *Haskins* suggest that deuterium could be used to create a new compound to target a step in a biological pathway that had not been targeted before. Instead, *Haskins* concludes that “*Careful substitution of protium by deuterium in a drug can lead to enhanced activity and reduced or altered metabolism.*” EX1012 (Haskins) at 6.

145. **Baillie (EX1013)** is a review article entitled “The Use of Stable Isotopes in Pharmacological Research.” EX1013 (Baillie) at 1. *Baillie* reviews a range of different uses of deuterium as a tool to *study* drug metabolism. EX1013 (Baillie) at 2.⁴³

⁴³ EX1013 (Baillie) at 2 (“The object of this review is to summarize those areas of pharmacological research in which stable-isotope-labelling techniques have made an important contribution, and also to emphasize those developing applications where stable isotopes are likely to play an important role in future years”).

146. As to therapeutic uses, the author explains that “the future *use* of stable-isotope-labelled drugs “must be considered to be a more speculative area of application in pharmacological research.” EX1013 (Baillie) at 41. *Baillie* explains that such use was by virtue of the “*kinetic isotope effects on metabolism*.” EX1013 (Baillie) at 41. The author further notes that the possibility of metabolic switching “has led to some concern over the possible toxicological consequences” of deuterium modification. EX1013 (Baillie) at 41.

147. *Baillie* does not disclose or otherwise suggest that deuterium has or could be used therapeutically to “slow undesirable reactions” in general, much less be used to create a new compound to create a new intervention in a biological pathway.

148. *Miwa* (EX1051) is a review article addressing the concept of “metabolic switching.” EX1051 (Miwa) at 1. *Miwa* explains that substitution of deuterium at the primary site of metabolism on a compound can cause an enzyme to “switch” to metabolize that compound at a minor site of metabolism. EX1051 (Miwa) at 2. *Miwa* explains this occurs because the C-D bond at the major site of metabolism becomes harder to break, causing the enzyme to “switch” to another location where it can metabolize a C-H bond:

The substitution of the hydrogen isotopes ^2H and ^3H for the hydrogen on a carbon atom that undergoes *oxidation by the hepatic cytochrome P-450* system often results in the reduction of this pathway for

metabolism and ‘metabolic switching’ or a *compensatory increase in the metabolism to another site on the substrate molecule*.

EX1051 (Miwa) at 2.

149. *Miwa* reviews several examples where deuterium caused a decrease in the rate of a metabolism to a major metabolite and a compensatory increase in production of the minor metabolite. EX1051(Miwa) at 3–4.

150. *Miwa* does not disclose or otherwise suggest that deuterium has or could be used therapeutically to “slow undesirable reactions” in general, much less do so as an independent means to target a biological pathway. *Miwa*’s conclusion is carefully constrained to specific instances where the use of metabolic switching may be useful: “achieving less toxic therapeutic agents through the *conservative strategy* of deuterium substitution *on carbon atoms susceptible to the cytochrome P-450 oxidation critical to the genesis of toxic metabolites*.” EX1051 (Miwa) at 5.

151. Dr. Calman opines that “[a] person of ordinary skill in the art before 2007 would have understood that there was *significant interest* in making deuterated versions of known therapeutics in order to *slow rates of undesirable reactions*.” EX1004 ¶96. Dr. Calman relies on Kushner (EX1046), Blake (EX1047), and McCarty (EX1048). EX1004 ¶¶96–102. I disagree. None of these references prior to September 2007 support Dr. Calman’s hindsight-driven proposition.

152. *Kushner* (EX1046) is a review paper entitled “Pharmacological uses and perspectives of heavy water and deuterated compounds.” EX1046 (Kushner) at 1. In Kushner’s section on the “properties and uses of deuterated drugs,” the authors discuss the use of deuterium substitution to alter the properties of known pharmaceuticals. *Kushner* explains:

Sometimes changed properties are due to different transport or penetration of the drug itself. Wenzel (1989) showed that deuterated amphetamines (also labelled with ^{131}I) were taken up more readily into mouse and rat brains than the hydrogenated forms of the same compounds. More often, changes in pharmacological action seem due to *changes in drug metabolism. Of special interest are reactions catalyzed by the hepatic cytochrome P450 system.*

EX1046 (Kushner) at 5.

153. With respect to this effect on “drug metabolism” *Kushner* further explains that “[o]ne of the key steps in all such reactions is the breaking of a C–H bond, and compounds that have C–D structures *at the site of enzymatic attack are more resistant to P450 change.*” EX1046 (Kushner) at 5. *Kushner* also discusses how deuterium “can also cause ‘metabolic switching,’ whereby the *paths of metabolism of a drug* are quantitatively changed” and notes that “[t]he *metabolic products of many drugs* are toxic, and deuteration can *lower such toxicity by reducing metabolism.*” EX1046 (Kushner) at 5–6.

154. *Kushner* does not disclose or otherwise suggest that deuterium has been used or could be used therapeutically to “slow undesirable reactions” in general, much less do so as an independent means to target a biological pathway. Rather, the authors conclude that deuterated drugs have had “few practical therapeutic consequences” and note that as of 1999 there were “no drugs on the market that contain deuterium in the molecule.” EX1046 (Kushner) at 7.

155. *Blake* (EX1047) is a review article entitled “Studies with Deuterated Drugs.” EX1047 (Blake) at 1. *Blake* reviews attempts to study the metabolism of or improve the metabolic properties of a number of different drugs via deuterium substitution. Without exception, each and every one of these studies involved the use of deuterium to alter enzymatic metabolism as a means to either *study* how the drug was metabolized or to alter *enzymatic* metabolism as means to improve the known activity of the parent drug. EX1047 (Blake) at 9–25.

156. *Blake* does not disclose or otherwise suggest that deuterium has or could be used therapeutically to “slow undesirable reactions” in general, much less do so as an independent means to target a biological pathway. Rather, *Blake* concludes that despite the substantial number of studies of deuterated pharmaceuticals, none have found a therapeutic application in drug therapy:

An impressive number of drugs have been synthesized or biosynthesized that are variously substituted with deuterium; while they have not found application in drug therapy, they are being used to study

the mechanism of drug action, to elucidate metabolic and biosynthetic pathways, and to study kinetic isotopic effects.

EX1047 (Blake) at 22.

157. Thus, a POSA would have understood that researchers had attempted to use deuterium substitution in the narrow context of attempting to improve the efficacy of known drugs for their intended indication. A POSA would have further understood that such improvements were limited to (1) increasing the resistance of the drug to enzymatic metabolism and (2) retaining the efficacy of the drug for its known indication while reducing the level of toxic metabolites the drug produced. A POSA would not have understood deuterium substitution was a generally applicable strategy for slowing *any* undesirable reaction.

VI. A POSA Would Not Have Been Motivated to Use Deuterated Vitamin A to Slow A2E Formation With a Reasonable Expectation of Success

A. *Mata 2000* Did Not Suggest Targeting the [1,5] Shift Specifically

158. Dr. Guengerich asserts four reasons why a POSA would have been motivated to target the [1,5] shift in *Mata 2000*'s proposed A2E pathway:

1. *Mata 2000*'s disclosures to "target" the intermediates the authors identified and the use of "pharmacological inhibition" against one or more steps in the *Mata 2000*'s proposed A2E pathway (EX1002 ¶¶97–98, 181, 221, 269, 302, 306, 321);

2. The fact that the [1,5] shift was upstream of A2E but downstream of the formation of ATR (EX1002 ¶¶98, 184);

3. *Mata 2000*'s disclosure that the [1,5] shift was "pre-requisite" to A2E formation (EX1002 ¶¶260, 269, 302, 321); and

4. A POSA would have recognized that the [1,5] shift was "likely" the rate-limiting step in *Mata 2000*'s proposed A2E pathway and thus a POSA would have expected that slowing the [1,5] shift would slow the formation of A2E (EX1002 ¶¶98, 178, 181, 184, 260–262, 353).

159. I disagree. None of these lines of reasoning would have motivated a POSA to target the [1,5] shift in the hypothetical A2E pathway described by *Mata 2000*. The first three of Dr. Guengerich's alleged motivations are merely generic suggestions to treat ocular disorders by targeting the pathway associated with lipofuscin formation, entirely non-specific to the [1,5] shift. They provide no reason why a POSA would have been motivated to target the [1,5] shift in *Mata 2000*'s proposed A2E pathway to slow A2E formation with a reasonable expectation of success. § VI.A.1–VI.A.3

160. As to the fourth, the myriad factors beyond the hypothetical chemical mechanisms in the proposed A2E pathway described by *Mata 2000* that were known to influence the overall process of production of A2E would prevent a POSA from reasonably expecting what step was "likely" rate-limiting. Even if a POSA were to attempt such analysis (which they would not have been motivated to do), there was insufficient information available to do such an analysis and the prior art suggested

several other steps in the pathway were more likely to be rate-limiting than the [1,5] shift. § VI.A.4

1. *The Disclosure of Mata 2000* of “Pharmacological Inhibition” Would Not Have Motivated a POSA to Try to Slow the [1,5] Shift Specifically

161. Dr. Guengerich asserts that *Mata 2000*’s instruction that “[p]harmacologic inhibition of one or more steps in the A2E synthetic pathway” may be “an effective treatment strategy for other macular degenerations associated with lipofuscin accumulation” provided “good reason” to inhibit the [1,5] shift in the proposed pathway of *Mata 2000*. EX1002 ¶¶97–98; *see also* EX1002 ¶¶181, 221, 269, 302, 306, 321. I disagree. These statements would not have directed a POSA to the [1,5] shift specifically and “pharmacological inhibition” would not have been understood as suggesting introducing additional vitamin A to the visual cycle.

162. Dr. Guengerich relies on two statements from *Mata 2000*:

[1] The precursors of A2E identified in this study may represent pharmacological targets for the treatment of ABCR-mediated macular degeneration.

and

[2] Pharmacologic inhibition of one or more steps in the A2E synthetic pathway may thus be an effective treatment strategy for other macular degenerations associated with lipofuscin accumulation.

EX1002 ¶¶97–98, 181, 221, 269, 302, 306, 321 (citing EX1010 (*Mata 2000*), 1, 5–6).

163. **First**, the comment in *Mata 2000* that “may represent pharmacological targets” is no more than speculation that any of the “precursors” *Mata 2000* purports to identify may be a target. EX1010 (*Mata 2000*) at 1. It does not suggest targeting any particular precursors and also does not suggest targeting the [1,5] shift that occurs (according to the *Mata 2000* scheme) after formation of NRPE (APE) and before the second bimolecular reaction with ATR.

164. The second statement from *Mata 2000* is even less specific, providing nothing more than a hope that inhibiting “one or more” of *any step* in the “A2E synthetic pathway” would be therapeutically relevant. EX1010 (*Mata 2000*) at 6. The speculation in *Mata 2000* as to what “may thus be an effective treatment strategy” does not suggest the [1,5] shift as a target over other “one or more steps” and does not provide a motivation to target the [1,5] shift to slow A2E formation with a reasonable expectation of success.

165. **Second**, these disclosures of “targeting” and “pharmacological inhibition” would not have suggested administering a precursor of A2E (C₂₀-D₃-vitamin A in place of or in addition to unlabeled vitamin A) as a means to slow the [1,5] shift. Rather, a POSA would have understood these terms as they are used in the context of pharmaceutical research, i.e., suggesting the use of a pharmacological means (e.g., small molecule) to bind to receptors or proteins to prevent or circumvent their usual function.

166. Specifically, in pharmaceutical research, “pharmacologic inhibition” refers to the use of drugs or compounds that interact with specific biological targets to prevent or circumvent a particular process. This generally involves molecules binding to enzymes, receptors, or other proteins to interfere with their normal function. Such an enzyme, receptor, or other protein would be referred to as “druggable” or as a “drug target.”⁴⁴

167. By contrast, deuterium substitution was, at best, understood as a means to improve the way in which a drug was enzymatically metabolized. § V.C. A POSA would not have understood the speculative suggestion of *Mata 2000* of “pharmacological inhibition” to teach or suggest administering C₂₀-D₃-vitamin A at all, much less with a reasonable expectation of success to retard A2E formation.

168. This natural reading of “target” and “pharmacological” inhibition also aligns with how those of skill in the art actually interpreted *Mata 2000*.

169. In this regard, numerous prior art references (1) cite *Mata 2000* and (2) attempted to identify a means to limit the formation of A2E. For example, *Radu 2003*

⁴⁴ EX2072 (Oxford) at 214 (“**drug target** a biological entity (usually a protein or gene) that interacts with, and whose activity is modulated by, a particular compound. If its biochemical profile makes it a suitable candidate for drug testing it is deemed a druggable target.”).

used isotretinoin to inhibit the natural function the enzyme 11cRDH. EX1035 (Radu 2003) at 1, 4–6. *Radu 2005* used HPR to prevent the natural function of RBP from binding to vitamin A, thereby inhibiting the natural function of RBP (facilitating vitamin A uptake into the visual cycle). EX1030 (Radu 2005) at 2, 7–8. *Gollapalli 2004* studied atRA, isotretinoin (Accutane[®]), and 4-HPR to ability to bind to and inhibit the enzyme RPE65. EX2063 (Gollapalli 2004) at 1, 5. *Maiti 2006* used TDT and TDH for the same purpose; both groups of researchers sought to prevent the natural function of RPE65 (facilitating the conversion of all-*trans*-retinyl esters to 11-*cis*-ROL). EX2066 (Maiti 2006) at 1–2, 9.

170. Each and every one of these examples describe the use of a pharmacological means to affect a *druggable target*. EX2072 (Oxford) at 214 (“drug target”). Thus, these references reflect that those of skill in the art reading *Mata 2000* understood its disclosures as teaching conventional means of treatment, not the novel form of intervention claimed in the ’045 patent.

171. Thus, in my opinion neither of the disclosures in *Mata 2000* relied on by Dr. Guengerich would have motivated a POSA to target the [1,5] shift in the proposed A2E pathway of *Mata 2000* by administering C₂₀-D₃-vitamin A. Nor do these disclosures support a reasonable expectation that doing so would slow A2E, retard lipofuscin accumulation, or treat any macular degeneration.

2. Dr. Guengerich's Upstream/Downstream Argument Would Not Have Motivated a POSA to Slow the [1,5] Shift Specifically

172. Dr. Guengerich further asserts that a POSA would have been motivated to target the [1,5] shift because “the [1,5]-sigmatropic shift step in the pathway is just upstream of the intermediate that results in both A2E and ATR-dimer but sufficiently downstream of ATR formation, thus reducing the formation of undesirable lipofuscin without interfering with the visual cycle.” EX1002 ¶¶98; *see also* EX1002 ¶¶85, 184

173. I disagree. As with the disclosures from *Mata 2000* discussed above (§ VI.A.1), this line of reasoning is entirely non-specific to the [1,5] shift. That is, *all* of the intermediates in *Mata 2000* and *all* of the steps required to form them are also “upstream” of A2E formation and “downstream” of ATR formation.

174. Thus, this purported teaching would not have motivated a POSA to target the [1,5] shift in the proposed A2E pathway of *Mata 2000* by administering C₂₀-D₃-vitamin A. Nor would it support a reasonable expectation that doing so would slow A2E, retard lipofuscin accumulation, or treat any macular degeneration.

3. The Disclosure of Deprotonation of C20 by *Mata 2000* as a “Pre-Requisite” for A2E Formation

175. Dr. Guengerich further urges that the disclosure of *Mata 2000* that C-20 bond breaking was a “pre-requisite” for A2E formation would have motivated a POSA to use C₂₀-D₃-vitamin A. Specifically, he contends:

There was also good reason to replace non-deuterated forms of vitamin A being used to treat or prevent macular degeneration or to reduce the accumulation of a lipofuscin pigment in a retina with the C20-deuterated vitamin A disclosed in Olson 1988. For example, Mata taught that deprotonation at C-20 of retinal was a pre-requisite for undesirable A2E formation

EX1002 ¶260; *see also* EX1002 ¶¶269, 302, 321.

176. I disagree. As with the prior two disclosures on which Dr. Guengerich relies (§§ VI.A.1–§ VI.A.2), this line of reasoning is non-specific to the [1,5] shift disclosed by *Mata 2000*. *Mata 2000* does not call out the [1,5] shift as a specific requirement for the formation of A2E anymore than it calls out any of the other myriad steps and processes described in the paper.

177. That is, while, according to *Mata 2000*, the breaking of a C-H bond is required, so too are *all* of the steps and processes described above (§ V.B.2). Insomuch as *Mata 2000* disclosed that the deprotonation of C-20 was a “pre-requisite” for the formation of A2E, *Mata 2000* equally disclosed that all of the steps in its proposed pathway in Figure 5 were “pre-requisites” to the formation of A2E. Unlike the effect of reducing the formation of ATR—also pre-requisite for the formation of A2E—*Mata 2000* does not support a reasonable expectation that merely slowing C-H bond breaking will have any efficacy.

178. Thus, in my opinion, this purported teaching would not have motivated a POSA to target the [1,5] shift in the proposed A2E pathway of *Mata 2000* by administering C₂₀-D₃-vitamin A. Nor do either of these references support a reasonable expectation that doing so would slow A2E, retard lipofuscin accumulation, or treat any macular degeneration.

4. A POSA Would Not Have Reasonably Expected the [1,5] Shift Was Rate-Limiting

179. Dr. Guengerich further asserts that a POSA would have been motivated to inhibit the [1,5] shift specifically based on the alleged recognition that “[1,5]-sigmatropic shift was likely a rate-limiting step”:

A person of ordinary skill in the art would thus have had good reason to inhibit the [1,5]-sigmatropic shift disclosed in the '045 patent to reduce lipofuscin formation associated with ophthalmic disorders including macular degeneration. . . . Second, a person of ordinary skill in the art with the perspective of a chemist would have recognized that the [1,5]-sigmatropic shift was likely a rate-limiting step due to the requirement that the bond between the hydrogen and Carbon 20 will break so that the hydrogen can shift to the nitrogen.

EX1002 ¶98; *see also* EX1002 ¶181 (“This provides a person of ordinary skill in the art with very good reason *to inhibit the rate-limiting step in Mata’s pathways, which, as I explained above, is the [1,5]-sigmatropic rearrangement.*”), ¶261 (“[A] person of ordinary skill in the art would have recognized that *the 1,5-sigmatropic*

shift disclosed in Mata . . . was likely a rate-limiting step in A2E-formation”);

¶¶178, 181, 184, 260, 262, 353.

180. I disagree with his hindsight-driven speculation, which is contradicted by the state of art as of September 2007.

181. First, Dr. Guengerich’s contention that the [1,5] shift in the *Mata 2000* pathway is “likely” rate-limiting is premised on oversimplifying a host of complex biochemical and biological processes, and is not supported by the state of the art at the time. Given the complexity of the other biological and physicochemical processes involved in A2E formation and the significant unknowns in these processes, a POSA would have had insufficient information from which they could have concluded that the [1,5] shift was “likely” rate-limiting in A2E formation. § VI.A.4.a.

182. Second, even if the skilled artisan had attempted to identify the rate-limiting step in *Mata 2000* by merely reviewing the hypothesized pathway on paper, they would have concluded that the literature identified other steps as likely to be slow. Moreover, Dr. Guengerich provides no basis from which a POSA would have reasonably concluded the [1,5] shift was relatively slow under physiological conditions or likely rate limiting in the formation of lipofuscin pigments. § VI.A.4.b.

**a) A POSA Would Have Understood that *Mata 2000*'s
Hypothesized Chemical Reactions Could Not be
Viewed in Isolation**

183. Dr. Guengerich asserts that a POSA would have recognized the [1,5] shift as “likely” rate-limiting in the hypothetical pathway proposed by *Mata 2000*. EX1002 ¶181. I disagree. A POSA could not have reasonably expected any particular step in the hypothetical pathway of *Mata 2000* to be “likely” rate-limiting given the multitude of non-chemical processes that are also implicated in the biological context of the hypothetical chemical reaction pathway of *Mata 2000* and the unsettled literature regarding the formation of lipofuscin pigments.

184. To reasonably determine which, if any, of the *chemical* steps in the hypothetical pathway of *Mata 2000* for A2E formation were rate-limiting, the relative magnitudes of the rate constants of the individual chemical steps would need to be known. In the multistep reaction described by *Mata 2000*, this necessarily requires at least an understanding of the rate constants and concentration of the reactants involved in each individual step.⁴⁵ This level of information was not available as of September 2007, and determining these variables would entail

⁴⁵In practice, this would require experiments be designed and performed to determine these rate constants and concentrations.

consideration of myriad different biological and physicochemical processes that could influence the rate of each individual step. § V.B.

185. For example, a POSA would have recognized that the rate constant of the bimolecular condensation reaction between NRPE and ATR was influenced by at least numerous enzymatic reactions in the visual cycle (e.g., RDH, RPE65); the viscosity of the cellular environment and the interaction of NRPE and ATR with the chiral lipid membrane; and active and passive transport of NRPE and ATR within the ROS. § V.B.2.b. *Each* of these considerations would necessitate accounting for numerous potential effects on the reaction rate. For instance, evaluating the effect of the lipid membrane environment alone would require accounting for effects on local concentration, mobility of the reactants, orientation of the reactants, viscosity effects, and lipid composition, to name but a few.

186. As described above, this analysis would need to be carried out for every step in the A2E pathway, each of which was influenced by a variety of physical and biological processes. § V.B.2.b.

187. Dr. Guengerich does not establish that the approximate rate of any chemical step was known in the art, let alone attempt to compare the relative rates of *each* step in the hypothetical A2E pathway of *Mata 2000*. Moreover, Dr. Guengerich's blinkered approach, focusing solely on *Mata 2000*, misrepresents how

a POSA would have considered the A2E pathway.⁴⁶ The cursory analysis that Dr. Guengerich does purport to perform—i.e., that a POSA would have recognized the kinetics of the [1,5] shift were “likely” slow—does not account for *any* of the biological or physicochemical processes that would have influenced the rates of the individual steps, much less show that such an analysis was or could have been done as of September 2007.⁴⁷

188. Lacking this analysis, Dr. Guengerich provides no basis with which to support his proposition that a POSA would have recognized the [1,5] shift was “likely” the rate-limiting step in the proposed A2E pathway of *Mata 2000*.

189. Further, the complexity of accounting for these myriad processes was compounded by a compendium of unknowns and would have prevented a POSA from having a reasonable expectation as to whether any step was rate-limiting. Contrary to Dr. Guengerich’s opinion, the mechanism of the conversion of ATR to

⁴⁶ Dr. Guengerich’s admits his analysis is from the “perspective of a chemist,” further underscores his oversimplified approach. EX1002 ¶98.

⁴⁷ In practice one could identify the rate-limiting step in the proposed reaction scheme in *Mata 2000* by conducting kinetic isotope studies, as the inventors did in the ’045 patent. Dr. Guengerich does not identify any such studies in the prior art.

A2E was not “well known.” EX1002 ¶182.⁴⁸ Rather, as of September 2007, there was significant uncertainty regarding which physical processes and biological systems acted on which steps in the reaction, as well as to what extent such processes and systems would affect the rate of each reaction. §V.B.2.b. There was also a lack of consensus as to which series of chemical reactions in which tissues led to lipofuscin formation and accumulation. §V.B.2.a.

190. For instance, *Mata 2000* suggested a pathway involving the intermediate AE2-PE-H₂. EX1010 (*Mata 2000*) at 5. In contrast, *Fishkin 2005* argued identified same compound was not an intermediate, but an entirely new

⁴⁸ Dr. Guengerich asserts that *Mata 2000* and *Fishkin 2005* describe the same hypothetical pathway. EX1002 ¶¶181–82. The only common features of these pathways that Dr. Guengerich identifies, however, are the [1,5] shift and the end product, A2E. EX1002 ¶182 (“As I explained above, the prior art biosynthetic pathway that Mata corroborated is the same pathway that the ’045 patent later identified as the reason that C-20 deuteration slows lipofuscin formation. Specifically, step 1 of the biosynthetic pathway in Mata (which is provided below), features *N*-retinylidene-*N*-retinylethanolamine (A2E) and a [1,5]-sigmatropic rearrangement.”). As described above, there were several differences between the pathway disclosed by *Mata 2000* and *Fishkin 2005*. § V.B.2.a.

lipofuscin pigment, ATR-dimer, unaccounted for in the mechanism of *Mata 2000*. EX1011 (Fishkin 2005) at 1, 5; § V.B.2.a.

191. The lack of consensus as to the specific intermediates in the A2E pathway makes a difference, because to identify the rate-limiting step, one must understand the *relative rates* of the individual steps. § V.A. Such comparison is not possible where the individual steps are not even agreed upon by experts in the field.

192. Consider the two hypothetical mechanisms in the below illustration, for the same product, each with three steps, but where steps “B” and “C” differ between the two hypothesized pathways:

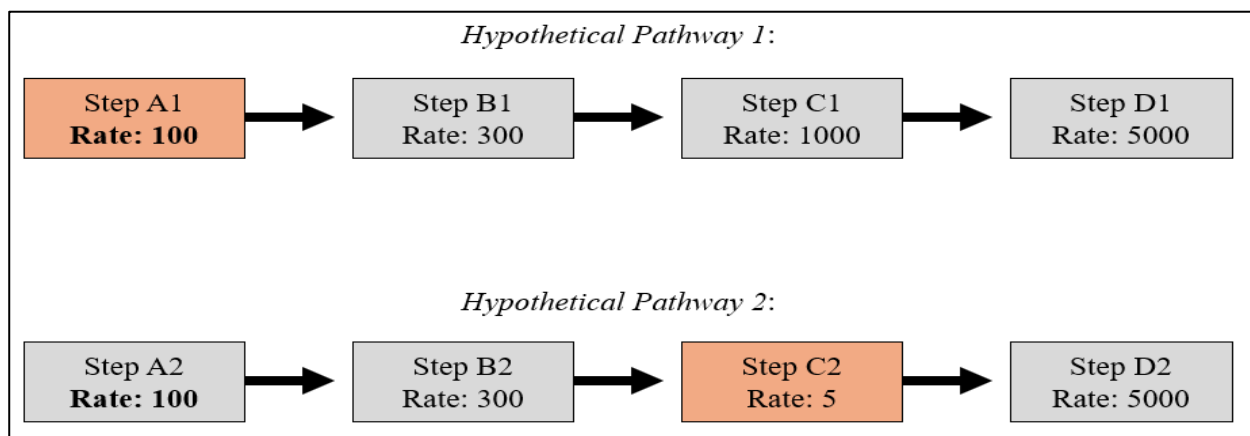


Figure 10. Hypothetical Reaction Mechanisms.

193. In this hypothetical, the same step A exists in both pathways and proceeds at the same rate (100). Despite this, Step A is rate-limiting only in Pathway 1. In Pathway 2, by contrast, the significantly slower Step C2 controls the overall

rate. Thus, even if one knew the rate of Step A with certainty,⁴⁹ it is not possible to form any reasonable expectation as to whether Step A is rate-limiting without knowing which pathway was correct together with the knowing the relative rates of each other step.

194. Without knowing which of these pathways was correct, or in what biological compartment each step in each pathway might actually occur, there would have been no evidence-driven basis to identify any one particular step as “likely” rate-limiting, because one could not have compared the relative rates of all necessary reaction steps where those steps are unknown or in dispute.

195. Thus, in my opinion, the complexity and uncertainty surrounding A2E formation precluded any reasonable expectation that the [1,5] shift was rate-limiting. Without such an expectation, a POSA would not have been motivated to select the [1,5] shift from the dozens of possible intermediates, steps, pathways, locations, or fluorophores on which to base a research and development program to develop a rational, targeted drug discovery effort. Without such an expectation, a POSA could not have had a reasonable expectation that administering C₂₀-D₃-vitamin A would slow A2E, retard lipofuscin accumulation, or treat any macular degeneration.

⁴⁹ Dr. Guengerich does not provide data on the rate of *any* individual step in *any* pathway.

b) The Prior Art Suggested Other Steps in the *Mata 2000* Pathway were More Likely to be Slower than the [1,5] Shift

196. To the extent that one of skill in the art would have even attempted to identify the rate-limiting step in the proposed A2E pathway of *Mata 2000*, they would have concluded (1) that the literature pointed to at least two other steps as likely to be slow, and (2) that the [1,5] shift was likely to be a relatively fast step.

(1) Bimolecular Condensation Reaction to Form A2PE-H₂

197. The first step a POSA would have looked to in the hypothesized chemical pathway of *Mata 2000* as more likely to be rate-limiting is the bimolecular condensation reaction between APE and a second molecule of ATR that forms A2PE-H₂. EX1010 (*Mata 2000*) at 1 (“biosynthesis of A2E . . . (ii) *condensation of the amine product with a second all-trans-RAL to form a bis-retinoid [A2PEH₂]*”).

198. As an initial matter, in the context of the A2E pathway, the multi-component nature of bimolecular reactions suggested the NRPE-ATR condensation was likely to proceed at a slower rate than the [1,5] shift. In contrast to unimolecular processes, bimolecular reactions involve two molecules coming together to form a product, and as such, the rate of such reactions depends on the concentrations of each reactant. Moreover, for such a reaction to occur, the reactant molecules must not only collide, but they must do so in a “productive” fashion, that is, with the correct orientation and with sufficient energy to allow specific functional groups of the

molecules to interact productively. This reaction cross-section is concentration dependent, and a productive fashion is defined as one that leads to the product. EX2068 (Greene 1968) at 1. As discussed above, a POSA would have expected that the complex chiral protein-lipid membrane environment in which the NRPE-ATR reaction was assumed to occur would limit the frequency with which this occurred. § V.B.2.b.

199. Based on these textbook principles, the bimolecular condensation reaction between NRPE and a second ATR molecule would have been a strong candidate for the rate-limiting step. This is due to (1) the need for sufficient concentrations of each molecule, (2) the crowded amphiphilic interfacial environment where the reaction is proposed to occur, and (3) the need for the reactants to collide with a productive reaction cross-section.

200. Further there is no need to speculate: numerous prior art references, including *Mata 2000*, expressly taught that the availability of ATR *was rate-limiting* on the formation of A2E. *E.g.*, EX1010 (Mata) at 6; EX1036 (Fishkin 2003) at 2; EX2003 (Travis 2007) at 14. Indeed, in contrast to the [1,5] shift—which is not called out in any of the prior references as being particularly slow (§ V.B)—the literature is replete with references specifically identifying the ATR-NRPE bimolecular reaction as a slow, “rare,” and “infrequent” step in the overall pathway. EX1035 (Radu 2003) at 2; EX1034 (Wolf 2003) at 2; EX1033 (Sparrow 2003) at 2.

201. For instance, *Radu 2003* explains that “after light exposure, newly released atRAL condenses reversibly with phosphatidylethanolamine to form *N*-ret-PE (step 1). “*Rarely*, a second molecule of atRAL will condense with *N*-ret-PE to form A2PE-H2 (step 2).” A second review paper from the same group echoes this characterization. EX1034 (Wolf 2003) at 2.

202. Elsewhere, *Sparrow 2003* explained that “[t]he reaction of NRPE with a second all-*trans*-retinal to form a molecule of A2-PE, ***is likely to occur very infrequently*** and probably only when circumstances, including high levels of illumination, increase the availability of all-*trans*-retinal.” EX1033 (Sparrow 2003) at 2 (citations omitted). That is, *Sparrow 2003* suggests that this reaction only occurs when levels of excess ATR are increased. Thus, a POSA would have understood that this suggests that it is the availability and/or likelihood of condensation reaction is rate-limiting, not the [1,5] shift of NRPE.

203. Consistent with this understanding that the APE-ATR condensation reaction was the rate-limiting step, those of skill in the art were actually targeting this step in the proposed pathway of *Mata 2000*. For example, *Travis 2007* reviewed several prior art therapies intended to reduce A2E formation, including HPR, Ret-NH₂, TDH and TDT, and isotretinoin. EX2003 (Travis 2007) at 13–15, 35, Fig. 5. The authors summarize their proposed A2E pathway, which includes the bimolecular condensation reaction:

All-trans-RAL released by photoactivated rhodopsin condenses with phosphatidylethanolamine (PE) in disc membranes to form *N*-retinylidene-PE (*N*-ret-PE). *Secondary condensation of N-ret-PE with another all-trans-RAL*, followed by electrocyclization, hydrolysis of the phosphate ester, and oxidation yields A2E.

EX2003 (Travis 2007) at 35.

204. *Travis 2007* explains that these prior art visual cycle inhibitors (HPR, Ret-NH₂, TDH and TDT, and isotretinoin) function by reducing “light-dependent formation of all-trans RAL” which “greatly reduce[s] the probability of this secondary condensation reaction and inhibit A2E formation.” EX2003 (Travis 2007) at 35. Thus, consistent with *Radu 2003*, *Wolf 2003*, and *Sparrow 2003*, *Travis 2007* suggests that the rate-limiting mechanism in step 2 in the *Mata 2000* pathway is the “probability of the secondary condensation reaction” occurring—and that the way to target this reaction was not to inhibit the [1,5] shift, but rather to reduce the availability of the reactants. EX2003 (Travis 2007) at 35. This suggestion is consistent with, and is a logical conclusion that a POSA would draw, the discussion of the parameters controlling the rate of bimolecular reactions as elaborated above.

205. Notably, the first author of *Travis 2007* was also the senior author on *Mata 2000*. EX2003 (Travis 2007) at 1; EX1010 (Mata 2000) at 1. Thus, seven years after *Mata 2000* was published, Dr. Travis, who was deeply involved in the original research, highlighted efforts to target the bimolecular condensation reaction

described in *Mata 2000*. In describing these efforts, Dr. Travis did not suggest that the [1,5] shift preceding the condensation reaction was rate-limiting or otherwise a target for inhibition (the summary of the A2E pathway does not even mention the [1,5] shift). This is inconsistent with Dr. Guengerich's proposition that *Mata 2000* identified the [1,5] shift as likely to be rate-limiting or otherwise specifically called it out as a step in the *Mata 2000* pathway to focus on as a potential drug target.

206. Thus, based on the nature of bimolecular reactions, express statements in the art concerning the bimolecular condensation reaction, and the *Mata 2000* authors' description of efforts to target *Mata 2000* step 2, a POSA would have recognized that the bimolecular condensation reaction between NRPE and ATR was more likely to be rate-limiting than the proposed [1,5] shift.

(2) Acid Hydrolysis of A2PE to A2E

207. A POSA would have recognized that the conversion of A2PE to A2E was also more likely to be rate-limiting on A2E formation in *Mata 2000*'s proposed biosynthetic pathway than the [1,5] shift. EX1010 (*Mata 2000*) at 5 ("Finally, A2E is formed within RPE phagolysosomes on acid hydrolysis of the phosphate ester and release of phosphatidic acid (reaction 4).").

208. *Mata 2000* reported that A2PE-H₂ was found in both the retina and the RPE, whereas A2PE and A2E were exclusively present in the RPE. EX1010 (*Mata 2000*) at 5. The authors further disclosed that the conversion of A2PE to A2E

requires oxidizing and acidic conditions found only in the RPE, not in the ROS. EX1010 (Mata 2000) at 3–4. Based on these findings, *Mata 2000* concluded that the conversion of A2PE to A2E must occur exclusively within the acidic environment of the RPE phagolysosomes. EX1010 (Mata 2000) at 5; *see also* EX2079 (Sakai 1996) at 2 (explaining formation of A2E “*in vivo* within the acidic environment of the RPE lysosome.”); EX2003 (Travis 2007) at 7 (“A2PE-H2 is converted to A2E and A2E-epoxides upon exposure to short-wavelength light in the acidic and oxidizing conditions of RPE phagolysosomes.”).

209. *Mata 2000* further reported *in vitro* experiments demonstrating the conversion of A2PE to A2E under acidic conditions was particularly slow, requiring overnight incubation in acid. EX1010 (Mata 2000) at 3–4.

210. Similar results (a slow rate of this acid hydrolysis reaction) were reported by *Ben-Shabat 2002*, which explained “A2E Is Generated by Acid Hydrolysis of A2-PE *at a Slow Rate*” and that “the generation of A2E from A2-PE by acid hydrolysis *was found to occur very slowly*. EX2051 (Ben-Shabat 2002) at 4, 7; *see also* EX2073 (Lamb 2004) at 3;⁵⁰ EX1018 (Parish 1998) at 1–2 (explaining

⁵⁰ *Ben-Shabat 2002* and *Lamb 2004* theorized that because acid hydrolysis was slow, the reaction may be enzymatically catalyzed by PLD in the ROS. EX2051 (Ben-Shabat 2002) at 1, 4, 7; EX2073 (Lamb 2004) at 3. Still, as late as 2006, *Mata 2000*

preparation of A2E in a biomimetic fashion is “highly sensitive” to acid additives and pH, that “[w]hen this reaction was carried out in the absence of acetic acid, only a trace amount of A2E was observed after 1 week”).

211. Thus, even if a POSA were seeking to identify the rate-limiting step in the hypothetical biosynthetic pathway for A2E of *Mata 2000*, they would have concluded the acid hydrolysis of A2PE to A2E was more likely than the [1,5] shift identified by Dr. Guengerich.

(3) Phagocytosis of the ROS

212. Another step more likely to rate-limiting than the [1,5] shift in the hypothetical A2E pathway of *Mata 2000* was the transport of A2PE-H2 from ROS to RPE *via* phagocytosis—which *Mata 2000* identifies as prerequisite to the acid

was cited for the proposition that “*A2E forms by non-enzymatic reactions.*” EX2074 (Rattner 2006) at 4 (citing Ref. 22, EX1010 (*Mata 2000*)). These differing interpretations of the same data set underscore the POSA’s inability to reasonably identify what was rate-limiting in the proposed A2E pathways in September 2007. While Dr. Guengerich does not address the A2PE-to-A2E conversion, he asserts, without citation to any prior art, “that *enzymatic catalysis was very unlikely* because lipofuscin formation was undesirable,” which would mean, as *Mata 2000* concludes, that the reaction occurs slowly in the RPE. EX1002 ¶¶98, 178.

hydrolysis of A2PE to A2E. Notably, while *Mata 2000* identifies this biological step as prerequisite to the acid hydrolysis of A2PE to A2E, it is not addressed in the simplified chemical reaction scheme Dr. Guengerich evaluates from his narrow “perspective of a chemist.” EX1002 ¶98.

213. Figure 5 of *Mata 2000* is limited to hypothesized chemical reactions and does not show phagocytosis. EX1010 (*Mata 2000*) at 5. Nevertheless, the hypothetical A2E pathway of *Mata 2000* necessarily requires this transport step as its proposed process for A2E formation begins in the ROS and is completed in the RPE. EX1010 (*Mata 2000*) at 5.⁵¹ *Mata 2000* discloses that it is fusion of phagosomes with lysosomes (i.e., phagocytosis) that creates the acidic environment necessary for A2PE to A2E conversion. EX1010 (*Mata 2000*) at 3–5; *see also* EX1034 (Wolf 2003) at 3 (“Phagocytosis by the RPE and hydrolysis of the A2PE then lead to accumulation of the stable A2E”).⁵²

⁵¹ EX1010 (*Mata 2000*) at 5 (explaining APE (also known as NRPE) forms from PE and ATR “In [rod] outer segments after a photobleach” and that the final two reactions in Fig. 5 occur only within the oxidizing and acidic environment of RPE phagolysosomes”).

⁵² EX1034 (Wolf 2003) at 3 (“NRPE accumulates inside the lumen of the discs, where increasing levels of all-*trans*-retinal, produced by light-activation of

214. As explained above, the phagocytosis of ROS by RPE cells is a complex, multi-step, and incompletely understood process. § V.B.2.b; EX2080 (Bok 1993) at 1. According to *Shaban Richter*, the phagocytosis only occurs periodically, specifically, “[e]ach RPE cell serves about 50 photoreceptors and engulfs and degrades 10% of 50 outer segments per day.”⁵³ EX1020 (Shaban Richter) at 1; *see also* EX2003 (Travis 2007) at 13.

215. Based on this, a POSA would have understood that this transport step is a discontinuous biological process, the rate of which is independent of the rate of the prior chemical reaction steps. By way of analogy, the periodic and discontinuous nature of phagocytosis is like a ferry transporting cars from one road on a mainland to a road on an island. In general, even a slowdown in traffic on the way to a ferry will not impact the overall trip time if the wait for the ferry takes a much longer time

rhodopsin, increase A2PE formation. Phagocytosis by the RPE and hydrolysis of the A2PE then lead to accumulation of the stable A2E”).

⁵³ A POSA would have recognized the rate of phagocytosis may have been further slowed by A2E itself. EX2073 (Lamb 2004) at 6 (“the rate at which RPE cells degraded the POS was slowed by the presence of A2E”).

than other parts of the trip.⁵⁴ This discontinuous step is more likely to be rate-limiting.

216. Thus, even if a POSA were seeking to identify the rate-limiting step in forming A2E, they would have concluded the phagocytosis of the ROS by the RPE was more likely than the [1,5] shift identified by Dr. Guengerich.

(4) A POSA Would Not Have Recognized the [1,5] Shift as a Slow Reaction

217. Dr. Guengerich asserts “[a] person of ordinary skill in the art would have recognized that cleavage of this carbon-hydrogen bond would already have been relatively slow under physiological conditions absent enzymatic catalysis (especially on the C-20 methyl moiety, even as compared to a methylene moiety), and that enzymatic catalysis was very unlikely because lipofuscin formation was undesirable.” EX1002 ¶98; *see also* EX1002 ¶184.

⁵⁴Even aside from this discontinuity, the ROS-RPE interface was relatively poorly characterized on a molecular basis from a biochemical or physicochemical perspective. A POSA would know that, given that the critical formation of the A2E lipofuscin pigment forms in this incompletely understood and complex set of biological compartments, identification of the slow step in both the biological context as well as the chemical mechanistic context are required to ultimately define the rate-limiting step.

218. I disagree. To the extent that one of skill in the art would have considered the rate of the various steps in *Mata 2000*, nothing suggested a POSA would have recognized that the [1,5] shift was likely to be slow under physiological conditions or rate limiting in the pathway proposed by *Mata 2000*.⁵⁵

219. **First**, *Mata 2000* does not provide any indication that the proposed [1,5] shift is likely the rate-limiting step. Rather, the authors expressly suggest that it is the availability of ATR that is rate-limiting on A2E formation. *Mata 2000* explains: “[o]ne prediction of the pathway proposed in Fig. 5 is that A2E accumulation *should depend on the availability of all-trans-RAL* and hence on light exposure.” EX1010 (*Mata 2000*) at 6. Since one way in which ATR is generated is from light exposure, the authors further suggest that limiting light exposure—and hence limiting the generation of ATR—“may reduce the rate of disease progression in humans with recessive *ABCR*-mediated diseases.” EX1010 (*Mata 2000*) at 6.

220. **Second**, as explained further below Dr. Guengerich’s reliance on the gas phase reaction of *cis*-pentadiene from *Roth* and *Ito* is misplaced. *E.g.*, EX1002

⁵⁵ Dr. Guengerich’s opinion is also contradicted by his recognition in a 2005 publication he authored that “many of the expressed kinetic isotope effects cited in the literature are low and *there has been a tendency to regard C–H bond breaking as generally being relatively rapid*.” EX2083 (*Krauser 2005*) at 1.

¶¶116–122. A POSA would have recognized that the isotope effect reported by *Roth* and *Itou* pertains only to a single-step 1,5 sigmatropic rearrangement and thus would not predict the observed isotope effect for NRPE in the proposed multi-step A2E pathway of e.g., *Mata 2000*, *Fishkin 2005*. § VII.A. As also explained below, based on the significant differences in reaction mechanisms, experimental conditions (gas-phase studies versus NRPE in aqueous solution *in vivo*), and structural distinctions between (Z)-1,3-pentadiene and NRPE, a POSA would have understood the sigmatropic rearrangement described by *Roth* was not an appropriate model for the [1,5] shift reaction attributed to NRPE in the proposed A2E pathways. § VII.B.

221. **Third**, Publications citing *Mata 2000* demonstrate that researchers in the field did not interpret *Mata 2000* as identifying or otherwise suggesting the [1,5] shift was rate-limiting in A2E formation.

222. Although Dr. Guengerich relies on numerous prior art references that cite *Mata 2000*, he fails to identify any reference reading *Mata 2000* as teaching or suggesting the C-20 C-H bond breaking as rate-limiting in lipofuscin formation. EX1002(citing EX1009 (Widder), EX1011 (Fishkin 2005), EX1033 (Sparrow 2003), EX1034 (Wolf 2003), EX1035 (Radu 2003), EX1036 (Fishkin 2003)). Likewise, none of the references provided with my declaration draw such a conclusion from *Mata 2000*. In other words, persons of skill in the art in 2007 interpreted *Mata 2000* very differently from how Dr. Guengerich reads it in 2024.

223. Consistent with the understanding that the formation of A2E “depend[s]” on the availability of ATR, prior art published after and relying on *Mata 2000* consistently sought to identify means to inhibit the generation of ATR. EX1035 (Radu 2003) at 5; EX1009 (Widder) at 3; EX2003 (Travis 2007) at 14; EX1030 (Radu 2005) at 8.

224. In this regard, the art recognized that *Mata 2000* disclosed that the rate-limiting step in A2E formation and the rate-limiting step in the visual cycle were one in the same—not that there was some distinct rate-limiting step in the A2E pathway. Consistent with this, *Maeda 2006* explained the authors’ understanding of *Mata 2000*:

Because it is unknown how to speed up the ABCR4 activity, another strategy proposed by Travis and colleagues was to *reduce the flow of retinoids through the inhibition of the visual cycle and thus slow the production of the component of A2E, all-trans-retinal*.

EX2064 (Maeda 2006) at 2 (citing EX1010 (Mata 2000) and EX1035 (Radu 2003)).

225. Also inconsistent with Dr. Guengerich’s assertion that a POSA would have recognized *Mata 2000* as disclosing the [1,5] shift as rate-limiting, subsequent literature demonstrates that researchers were actively searching for a rate-limiting step in A2E formation. For instance, *Maiti 2006* cited *Mata 2000* and was actively researching steps in the visual cycle that could be targeted to reduce A2E formation:

RPE lipofuscin, including the fluorophore A2E, forms in large part as a byproduct of the visual cycle. Inhibiting visual cycle function with small molecules *is required* to prevent the formation of the retinotoxic lipofuscins. This in turn *requires identification of rate-limiting steps* in the operation of the visual cycle.

EX2066 (Maiti 2006) at 1.

226. *Maiti 2006* reviews several papers examining the A2E pathway, including *Mata 2000*. EX2066 (Maiti 2006) at 3. The authors did not conclude that the [1,5] shift was rate-limiting in A2E formation; instead, they found that “*these studies provide strong evidence that mRPE65 is rate-limiting in A2E formation and that mRPE65 is a valid target for therapies designed to prevent the accumulation of lipofuscin and possibly the onset and progression of macular degeneration.*” EX2066 (Maiti 2006) at 3.

227. The absence of discussion in these papers about the proposed [1,5] shift is inconsistent with Dr. Guengerich’s hindsight interpretation of what *Mata 2000* suggested to those of skill in the art. As Dr. Guengerich points out, many of these papers expressly disclosed the [1,5] shift in *Mata 2000*’s. EX1002 ¶¶84–94. Nonetheless, none of these subsequent papers mention the [1,5] shift as rate-limiting or a potential therapeutic target. The lack of discussion, by leading researchers in the field working on bisretinoids, lipofuscin, and ophthalmic diseases, is inconsistent

with Dr. Guengerich's assertion that those of skill in the art would have readily recognized the [1,5] shift as rate-limiting for A2E formation.

228. Further, in contrast to the way the art understood and relied on *Mata 2000*, numerous papers cite the work of the inventor of the '045 patent (Ilyas Washington) as identifying the rate-limiting step in A2E formation.

229. Specifically, the work described in the '045 patent was published as "Deuterium Enrichment of Vitamin A at the C20 Position Slows the Formation of Detrimental Vitamin A Dimers in Wild-type Rodents" (*Kaufman 2011*). EX2006 (Kaufman 2011) at 1. Since publication, this paper has been widely cited as discovering the rate-limiting step in A2E formation:

- EX2014 (Huang 2022) at 13: cites *Kaufman 2011* (Ref. 173) as teaching that "cleavage of the C20-H bond is the rate-limiting step in synthesis of vitamin A dimers;"
- EX2015 (Hussain 2018) at 8: cites *Kaufman 2011* (Ref. 33) as teaching that "[t]he rate determining step in vitamin A dimerization is the cleavage of a C20 carbon-hydrogen;"
- EX2016 (Zernant 2019) at 23: cites *Kaufman 2011* (Ref. 51) for teaching "cleavage of a C20 carbon-hydrogen bond in the retinaldehyde-PE conjugate is the rate determining step in vitamin A dimerization;" and

- EX2017 (Ciulla 2017) at 2: cites *Kaufman 2011* (Ref. 10) for teaching that “[t]he rate-determining step in vitamin A dimerization is the cleavage of a C20 carbon-hydrogen bond of the retinaldehyde-PE Schiff base.”
- EX2075 (Pirali 2019) at 12: cites *Kaufman 2011* (Ref. 16) as teaching “the rate-determining step in vitamin A dimerization is the cleavage of a C20 C–H bond in retinaldehyde-PE Schiff base”

230. Thus, contrary to Dr. Guengerich’s opinion, those skilled in the art did not recognize *Mata 2000* as identifying the rate-limiting step in A2E formation and did not read *Mata 2000* as calling out the [1,5] shift specifically. Rather, it was not until the discoveries in the ’045 patent that the [1,5] shift was identified as a potential target to prevent A2E accumulation.

B. A POSA Would Not Have Been Motivated to Use Deuterium to Modify Vitamin A as a Means to Slow Lipofuscin Formation

231. Dr. Guengerich asserts that “[u]sing deuteration to *slow undesirable reactions* was one of several known uses of deuteration by 2007.” EX1002 ¶100. He further contends the claims of the ’045 patent would have been obvious in view of the “well-known teachings about the primary kinetic isotope effect (KIE) and the use of selective deuterium substitution of pharmaceutical compounds *to slow reactions* involving removal of a hydrogen at a particular position.” EX1002 ¶50.

232. I disagree. One of skill in the art would not have been motivated to use deuterium to intervene in the A2E path based on limited prior attempts to use deuterium to alter enzymatic metabolism of a drug to improve its efficacy for known indications. The therapeutic uses of deuterium described by Dr. Guengerich's references (slowing or redirecting enzymatic metabolism) would not have been viewed as useful in reducing lipofuscin formation.

1. The Use of Deuterium to Attempt to Improve the Known Function of A Drug is Fundamentally Different than Attempting to Provide A Treatment Effect By Intervening in a Biological Process

233. As explained above, the art that Drs. Guengerich and Calman rely on do not teach that deuterium was broadly used to slow *all* undesirable biological reactions. § V.C. Rather, at best, it shows that deuteration was used to alter enzymatic metabolism to improve the efficacy of known drugs for their intended purposes. § V.C. This approach represents an attempt at incremental improvement within the paradigm of drug optimization.

234. In contrast, the inventor's use of deuterium to slow a non-enzymatic reaction in the A2E pathway represents a fundamentally different concept—intervening in a natural biochemical process to create a new method of treatment, rather than merely optimizing an existing drug.

235. Put another way, deuterium substitution of known drugs in the prior art was premised on strategic substitution of a hydrogen atom in a drug molecule with

a deuterium atom to give what could become an “improved” deuterated drug. Under Dr. Guengerich’s theory, C₂₀-D₃-vitamin A *is the treatment*. It does not replace a drug that otherwise would have been administered, C₂₀-D₃-vitamin A would be administered *in addition* to a baseline level of vitamin A that patients would obtain naturally from their usual diet.

236. These are two different ways of thinking about both the problem and the potential solutions.⁵⁶ In this regard, Dr. Guengerich conflates the technical ability to use deuterium with the conceptual framework that would lead someone to consider using it in this new context. The question is not merely whether the deuterium substitution *could* work, but rather whether a POSA would have conceived of and been motivated to apply deuterium substitution to limit lipofuscin accumulation and treat macular degeneration.

⁵⁶ The A2E context requires inhibiting an irreversible pathway that leads to accumulation of an undesired pathological pigment, which the body is unable to clear by physical or enzymatic means. This presents a fundamentally different challenge compared to the deuterium substitution within an existing drug described in prior art (which approach aims to slow the removal of a drug from the body by potentially increasing its resistance to normal enzymatic processes).

237. At best, Dr. Guengerich's references demonstrate the use of deuterium for investigating or optimizing drug metabolism, they did not suggest its application as an independent therapeutic modality to modulate an endogenous pathway. The inventor's approach required integrating knowledge from biochemistry, organic chemistry, and the proposed molecular pathophysiology of diseases leading to lipofuscin accumulation, not a straightforward extension of the known uses of deuterium substitution in drug metabolism. This novel approach claimed in the '045 patent is well beyond what a POSA would have gleaned from the art on which Dr. Guengerich relies. *E.g.*, EX1002 ¶¶99–123.

2. Neither Slowing or Altering Enzymatic Metabolism were Applicable to Reducing Lipofuscin Accumulation

238. As explained above, the prior art Dr. Guengerich and Calman rely on suggest two means of using deuterium to improve a known drug: (1) slowing the rate at which the drug was enzymatically metabolized and (2) altering the metabolism of the drug via “metabolic switching” to reduce its production of toxic metabolites. § V.C. Neither of these would have been seen as useful to retarding the accumulation of lipofuscin pigments.

a) Slowing Metabolism Would Not Be Expected to Retard Lipofuscin Accumulation

239. In the first scenario, replacing a drug with its deuterated analog, could potentially *slow* the rate at which a drug is metabolized. The potential benefit is the

drug remains in circulation for a longer and thus could provide its therapeutic effect for an extended period of time.

240. For instance, *Haskins* explains how deuterium substitution slowed the metabolism of 5-(3,3-dideuterio-*n*-butyl)-5-ethylbarbituric acid. EX1012 (Haskins) at 6. This extended the half-life of the drug and created a prolonged effect of the drug (i.e., longer sleep time in mice):

For example, an extension of sleep time in mice administered 5-(3,3-dideuterio-*n*-butyl)-5-ethylbarbituric acid was observed. The major metabolite of butethal is 5-(3-hydroxy-*n*-butyl)-5-ethyl barbituric acid, so by slowing the rate of hydroxylation of the butyl side chain with deuteration at the 3 position, the active parent compound had an extended biological half-life.

EX1012 (Haskins) at 6.

241. Similarly, *Haskins* explains that deuterium was used to slow the metabolism of Merck's antibacterial 3-fluoro-*D*-alanine, which enhanced the antibacterial activity of the deuterated analog relative to the hydrogen parent:

Van den Heuvel announced that Merck were trying to market a deuterated drug based on the antibacterial 3-fluoro-*D*-alanine. By administering 2-deutero-3-fluoro-*D*-alanine the rate of *in vivo* metabolism is reduced several-fold. However, the heavy analogue has the same antibacterial activity as the parent, and thus the deuterated drug has an enhanced duration of activity.

EX1012 (Haskins) at 6.

242. In such scenarios in which deuterium is used to slow metabolism, the goal is generally to prolong the desired clinical effect of the drug that is administered. *E.g.*, EX1046 (Kushner) at 5–6.⁵⁷ If the active drug stays in the body longer, this may provide a longer duration of the desirable action (*e.g.*, sleep time). EX1012 (Haskins) at 6. This can be beneficial to improve a pharmaceutical agent, because it could increase the efficacy of the drug and/or allow the patient to take the drug less frequently. *E.g.*, Nevertheless, despite this longer duration of action, the drug is still ultimately metabolized and removed from the body, at which point the patient would need to take another dose.

243. In the A2E scenario, however, the goal is to retard accumulation of the end product, A2E, which builds up over a lifetime. In this context, marginally slowing A2E biosynthesis would not be expected to produce any benefit. Regardless of how long it takes for deuterated-vitamin A to be turned into A2E, by applying what was known using deuterium to slow drug metabolism the POSA would expect that C₂₀-D₃-vitamin A would still generate A2E. That is, a POSA would not expect that administration of C₂₀-D₃-vitamin A would cause less total A2E to be formed.

⁵⁷ EX1046 (Kushner) at 5–6 (“Resistance to P450-induced changes may lead to an increase in duration of pharmacological action.”).

244. Consider the following hypothetical. Assume that two molecules of vitamin A produces one molecule of A2E. Even if deuterium worked as Dr. Guengerich proposes, two molecules of C₂₀-D₃-vitamin A would still be expected to produce one molecule of A2E. That is, even if the conversion of one molecule of C₂₀-D₃-vitamin A occurred at a slower rate than its hydrogen-vitamin A counterpart, a POSA would not expect that to retard the total accumulation of A2E.⁵⁸

245. Thus, prior art teaching that deuterium could slow enzymatic metabolism to improve the efficacy of known drugs for their intended indication would not have motivated a POSA to use deuterium substitution of vitamin A to prevent the gradual and irreversible accumulation of lipofuscin. Nor would it support a reasonable expectation that doing so would slow A2E, retard lipofuscin accumulation, or treat any macular degeneration

⁵⁸ Moreover, as noted above, Dr. Guengerich's theory proposes the addition of C₂₀-D₃-vitamin A on top of the patient's usual dietary intake of vitamin A. E.g., EX1002 ¶¶142–159, 259–260 (citing EX1008 (Ortho)). Thus, continuing with this hypothetical, Dr. Guengerich's theory proposes its two molecules of vitamin A (from the patient's diet) plus two molecules of C₂₀-D₃-vitamin A. The expected result would not be less A2E than the patient would have otherwise accumulated had they not taken C₂₀-D₃-vitamin A.

b) Metabolic Switching Would Not Be Expected to Reduce Lipofuscin Accumulation

246. Likewise, disclosures of “metabolic switching” in the prior art would not have motivated a POSA to use deuterium substitution to retard the accumulation of lipofuscin pigments. As Dr. Guengerich and Calman’s references demonstrate, metabolic switching is a narrow concept limited to creating a more optimal deuterated drug to replace its hydrogen analog *via* modulation of enzymatic metabolism of the hydrogen drug. This would not have suggested the use of deuterium substitution to target an endogenous biological pathway.

247. *Miwa* (EX1051) explains that metabolic switching refers to the phenomenon where a deuterium “switches” metabolism of a drug from the primary route of enzymatic metabolism to a minor route. EX1051 (*Miwa*) at 2. Because the C–D bond at position A is harder to break, the enzyme instead metabolizes the drug at position B. *Miwa* explains:

The substitution of the hydrogen isotopes ^2H and ^3H for the hydrogen on a carbon atom that undergoes *oxidation by the hepatic cytochrome P-450* system often results in the reduction of this pathway for metabolism and ‘metabolic switching’ or a *compensatory increase in the metabolism to another site on the substrate molecule*.

EX1051 (*Miwa*) at 2.

248. Therapeutic applications of this strategy were limited to specific situations involving enzymatic metabolism. As *Miwa* further explains:

These observations suggest the possibility of achieving less toxic therapeutic agents through the conservative strategy of deuterium substitution on carbon atoms susceptible to the cytochrome P-450 oxidation critical to the genesis of toxic metabolites

EX1051 (Miwa) at 4; *see also* EX2076 (Fisher 2006) at 7 (explaining metabolic switching “is not expected to be an effective strategy for CYPs except in highly specific situations”).

249. Thus, a POSA would have understood that the goal of “metabolic switching” was to reduce the amount of the toxic metabolite that is formed from metabolism of the parent drug. For example, a drug has a beneficial effect (e.g., sleep time), but produces a toxic metabolite causing negative side effects. Strategic substitution with deuterium could redirect the metabolism of the drug to a less toxic metabolite, mitigating the adverse effects. Thus, the benefit of metabolic switching is the drug can be administered for the positive benefits (sleep time, antibacterial effect) without the negative effects caused by the toxic metabolite.

250. The concept of metabolic switching does to apply in the context of using C₂₀-D₃-vitamin A to reduce lipofuscin accumulation. Metabolic switching is premised on the enzyme metabolizing the parent drug at different position, thus producing less of the toxic metabolite and more of the non-toxic metabolite. *E.g.*,

EX1051 (Miwa) at 1. Here, according to Dr. Guengerich,⁵⁹ there is no enzyme that would be redirected by metabolic switching. Even if there were, Dr. Guengerich does not identify some alternative, non-toxic metabolite that vitamin A would “switch” to and produce instead of ATR.

251. Moreover, by analogy, the “toxic metabolite” of vitamin A is ATR. As explained, it is excess ATR that produces A2E. § V.B. Researchers at the time were expressly trying to limit the availability of this excess ATR to prevent lipofuscin accumulation. § V.B.3. Dr. Guengerich, however, asserts the motivation for using deuterium was to prevent lipofuscin accumulation *without* limiting the availability of ATR. *E.g.*, EX1002 ¶¶68–69, 98.

252. Thus, prior art teaching the use deuterium substitution as a means to switch the enzymatic metabolism known drugs for their intended indication would not have motivated a POSA to use deuterium substitution of vitamin A to prevent the accumulation of lipofuscin. Nor would it support a reasonable expectation that doing

⁵⁹ EX1002 ¶98 (“A person of ordinary skill in the art would have recognized that cleavage of this carbon-hydrogen bond would already have been relatively slow under physiological conditions *absent enzymatic catalysis* . . . and that *enzymatic catalysis was very unlikely because lipofuscin formation was undesirable*.”).

so would slow A2E, retard lipofuscin accumulation, or treat any macular degeneration.

c) Neither *Roth* Nor *Itou* Suggest Using Deuterium Substitution to Intervene in the A2E Pathway

253. *Roth* and *Itou* would not have motivated a POSA to administer C₂₀-D₃-vitamin A to reduce lipofuscin formation or to treat macular degeneration. *Roth* and *Itou* are even farther removed from the use of deuterium to slow lipofuscin accumulation than the prior art discussed above using deuterium to improve resistance to enzymatic metabolism. § V.C; VI.2.a–b.

254. *Roth* is a study to understand the mechanism of a [1,5] sigmatropic rearrangement in a model compound, (Z)-1,3-pentadiene. EX1015 (*Roth*) at 3. The authors measured intrinsic isotope effects to confirm whether the reaction proceeds through a symmetrical transition state, as predicted by theoretical considerations. EX1015 (*Roth*) at 3–4. *Roth* reported a high intrinsic kinetic isotope effect, which the authors interpreted as evidence that the [1,5]-sigmatropic rearrangement of (Z)-1,3-pentadiene proceeds through a highly symmetrical transition state. EX1015 (*Roth*) at 5–6.

255. *Itou* sought to improve the theoretical calculation of kinetic isotope effects for the [1,5]-sigmatropic rearrangement of (Z)-1,3-pentadiene by accounting for “the direct quantum effect of migrating H and D nuclei in the multi-component molecular orbital-Hartree-Fock (MC_MO-HF) method.” EX1016 (*Itou*) at 1–2. The

authors compared their results using the MC_MO-HF method to conventional computational methods and experimental data. EX1016 (*Itou*) at 2–3. The authors conclude that their method produces KIE values closer to experimental results than traditional computational approaches. EX1016 (*Itou*) at 5. *Itou* does not report separate experimentally-determined data.⁶⁰ EX1016 (*Itou*) at 1–2.

256. Both of these papers are narrowly focused on understanding reaction mechanisms in organic chemistry. EX1015 (*Roth*) at 3–4; EX1016 (*Itou*) at 1. Neither mentions or otherwise suggests the use of deuterium substitution in the therapeutic or pharmacological context, much less suggests the novel use of deuterium substitution to intervene in a natural biological process.

VII. A POSA Would Not Have Reasonably Expected the KIE from Expect Roth to Slow the Formation of A2E in the Hypothetical Pathway of *Mata 2000*

257. Dr. Guengerich asserts: “Roth further discloses that there is a large kinetic isotope effect associated with the 1,5-sigmatropic shift. . . . It is therefore my opinion that *the mechanism of the [1,5] H-shift at C-20*, which is disclosed in the

⁶⁰ Dr. Guengerich does not cite data disclosed in *Itou* beyond that originally reported by *Roth*. E.g., EX1002 ¶¶117–122 (*Itou*, referring to an article by Roth *Itou* further discloses that the authors in the Roth article experimentally determined a large kinetic isotope effect”), 262 (“*Itou*, citing Roth”), 352 (“*Itou*, citing Roth *Itou* discloses that Roth”).

'045 patent and which I have reproduced below, *would similarly have a large kinetic isotope effect* as experimentally determined in Roth.” EX1002 ¶262; *see also* EX1002 ¶¶121–122, 352.

258. I disagree. *Roth* and *Itou* describe the isotope effect for a single chemical step and would not have provided a reasonable expectation of a large observed isotope effect for NRPE in the proposed multistep A2E pathway of e.g., *Mata 2000*, *Fishkin 2005*. Further, due to the differences in their reaction mechanisms, the test conditions between *Roth* (gas phase) and NRPE (aqueous *in vivo*), and the significant structural differences between (Z)-1,3-pentadiene and NRPE, a POSA would not have considered *Roth* relevant to the proposed A2E pathways of, e.g., *Mata 2000*, *Fishkin 2005*, much less use it as a basis to expect a large KIE for NRPE.

A. The Isotope Effect Reported by *Roth* Would Not Have Provided a Reasonable Expectation of a Large Observed Isotope Effect for NRPE

259. The results of *Roth* and *Itou* describe the isotope effect for a single chemical reaction, the [1,5] sigmatropic rearrangement for (Z)-1,3-pentadiene. A POSA would have understood that such results would not predict the observed isotope effect for NRPE in the proposed multistep A2E pathway of e.g., *Mata 2000*, *Fishkin 2005*.

260. As explained above (§ V.C.1), an *intrinsic* isotope effect measures the difference between the energy required to break the C-H bond and the energy required to break the C-D bond. In contrast, an *observed* isotope effect measures the effect of deuterium on the rate of the overall reaction.

261. *Roth* measured only the *intrinsic* isotope effect for the single step [1,5] shift.⁶¹ Specifically, the KIE of 12.2 that Dr. Guengerich relies on from *Roth* was determined by dividing the measured rate constant for hydrogen migration (k_H) by the rate constant for the migration of deuterium (k_D). EX1015 (*Roth*) at 6, n. 11 (“ k_H is defined as the rate constant for a single hydrogen displacement, taking into account the statistical conditions”)⁶², 8, n. 13 (“ k_D was defined analogous to

⁶¹ In *Roth*, the intrinsic isotope effect for the [1,5] sigmatropic rearrangement of (Z)-1,3-pentadiene is predicted to be similar to the observed isotope effect because, unlike the proposed A2E pathway *in vivo*, there are no other steps and no environmental or biological processes that mask the contribution of the C-H bond breaking step. § V.C.1.

⁶² In the English translation of *Roth* (EX1015) “ k_H ” appears as “ k_{kn} .” EX1015 (*Roth*) at 6 (“ k_{kn} is defined as the rate constant for a single hydrogen displacement, taking into account the statistical conditions”). This is clearly a typographical error in the translation, as the original document (provided with the declaration of Irina

quotation¹¹”). *Itou* describes an effort to more effectively model the same intrinsic kinetic isotope effect. EX1016 (*Itou*) at 5.⁶³

262. A POSA would recognize that these intrinsic isotope values from *Roth* and *Itou* do not inform the *relative rate* of [1,5] shift in the pathway shown in *Mata 2000*. § V.C.1. Thus, even if a POSA expected a direct 1:1 translation of the KIE for [1,5] sigmatropic rearrangement in *Roth* to the proposed [1,5] shift for NRPE (which they would not have, *see* § VII.B), this still would not suggest that the [1,5] shift was rate-limiting in the formation of A2E.

263. Further, neither *Roth* nor *Itou* would have directed a POSA to the [1,5] shift in *Mata 2000* in the first place.

264. Starting from the teachings of *Roth* and *Itou*—and assuming the exact same rate of reaction for the [1,5] shift disclosed in *Roth* would be seen in *Mata*

Hinrichs) uses “ k_H ”. EX1024 (Declaration of Irina Hinrichs) at 9 (“ k_H ist definiert als Geschwindigkeitskonstante für eine einzelne Wasserstoffverschiebung unter Berücksichtigung der statistischen Gegebenheiten.”).

⁶³ EX1016 (*Itou*) at 5 (“By including the quantum effect of the migrating H or D nucleus in the MC_MO-HF method, the KIE can be directly evaluated from the difference in structure, and the k_H/k_D of 8.28 calculated using the MC_MO-HF method is substantially closer to the measured k_H/k_D of 12.27 [from *Roth*]”).

2000—this information reveals nothing about whether the [1,5] shift is rate limiting in *Mata 2000*. Nor could it because *Mata 2000* does not contain dedicated kinetic studies for its hypothetical biosynthetic pathway. EX1010 (*Mata 2000*) at 5, Fig. 5. This would include, for example the experiments described in the '045 patent in which the inventors compared the rate of formation of A2E following administration of vitamin A with the rate of formation of A2E following administration of deuterated vitamin A. EX1001 at 18:50–20:59.

265. Dr. Guengerich did not identify any such kinetic studies in the prior art. Instead, he bridges this gap by asserting that a POSA would have reasonably expected the [1,5] shift to be rate-limiting “*based at least on the teachings of Roth and Itou.*” EX1002 ¶178. This logic is entirely circular.

266. Dr. Guengerich’s further assertion that this reasonable expectation that deuterium-labeled vitamin A would slow A2E formation would have been “*been reasonably confirmed* even using *in vitro* experiments and then subsequently using *in vivo* experiments” underscores the circularity of his argument:

As I explain in detail below, it was well-known before 2007 that replacing hydrogens with deuteriums in carbon-hydrogen bonds slowed reactions such as the [1,5]-sigmatropic shift [from *Roth* and *Itou*] that required cleavage of the carbon-hydrogen bond. *This reasonable expectation* [that C20 deuterated vitamin A would slow A2E formation in *Mata 2000*’s proposed pathway] *would have been reasonably*

confirmed even using in vitro experiments and then subsequently using in vivo experiments.

EX1002 ¶¶99.

267. This logic presupposes that a POSA would have looked to the [1,5] shift in the proposed pathway of *Mata* 2000 in the first place. But as explained, without the doing the experiments disclosed in the '045 patent (or independently calculating the rate of each step in the proposed pathway of *Mata* 2000), there was no reason to look at the [1,5] shift. In essence, Dr. Guengerich's argument can be summarized as: to recognize the significance of the [1,5] shift, one must conduct testing; to conduct testing, one must recognize the significance of the [1,5] shift.

B. A POSA Would Not Have Reasonably Expected the KIE Observed in Roth to Translate to the A2E Pathway *In Vivo*

268. Dr. Guengerich asserts that the KIE seen in *Roth* would also be seen for NRPE because both undergo a 1,5 sigmatropic rearrangement. In particular, Dr. Guengerich asserts:

persons of ordinary skill in the art would have understood that deuterating C₂₀ would slow A2E formation because 1,5-sigmatropic shifts were known to be subject to strong primary deuterium kinetic isotope effects.

EX1002 ¶262 (citing EX1015 (*Roth*)); *see also* EX1002 ¶¶98, 116–123, 178.

269. I disagree. A POSA would have understood that the sigmatropic rearrangement of *Roth* is not an appropriate model for the [1,5] shift reaction

attributed to NRPE in the proposed A2E pathways of, *e.g.*, *Mata 2000*, *Fishkin 2005*. Due to the differences in reaction mechanisms, test conditions (Roth in gas phase versus NRPE in aqueous solution *in vivo*), and structural differences between (Z)-1,3-pentadiene and NRPE, a POSA would not have considered *Roth* relevant, let alone use it as a basis to expect a large KIE for NRPE.

270. Dr. Guengerich reasons that the reactions in *Roth* and *Mata 2000* are the same “1,5 sigmatropic rearrangement” disclosed by *Mata 2000*. *E.g.*, EX1002¶¶93–94, 98, 116–122, 178. This characterization, however, is inconsistent with the majority of the literature, which predominantly describes this reaction as a “[1,6]-proton tautomerization” or simply a “[1,6] H-Shift,” not a sigmatropic rearrangement. *E.g.*, EX1036 (*Fishkin 2003*) at 2; EX1018 (*Parish 1998*) at 3. While the net result of these reactions are similar, the mechanisms by which they reach that end are fundamentally different.

271. A POSA would have recognized that the 1,5 sigmatropic hydrogen shift for (Z)-1,3-pentadiene studied by *Roth* is a pericyclic rearrangement. EX1015 (*Roth*)

at 8;⁶⁴ EX1016 (Itou) at 2–3.⁶⁵ This reaction proceeds via a concerted mechanism where bonds are broken and formed simultaneously through a cyclic transition state, without the formation of intermediates. EX2045 (March) at 315. As shown in Figure 11, below, the pericyclic reaction mechanism shifts the same hydrogen atom from one carbon atom in the (Z)-1,3-pentadiene molecule to another carbon atom within the molecule in a single, concerted (continuous) process.

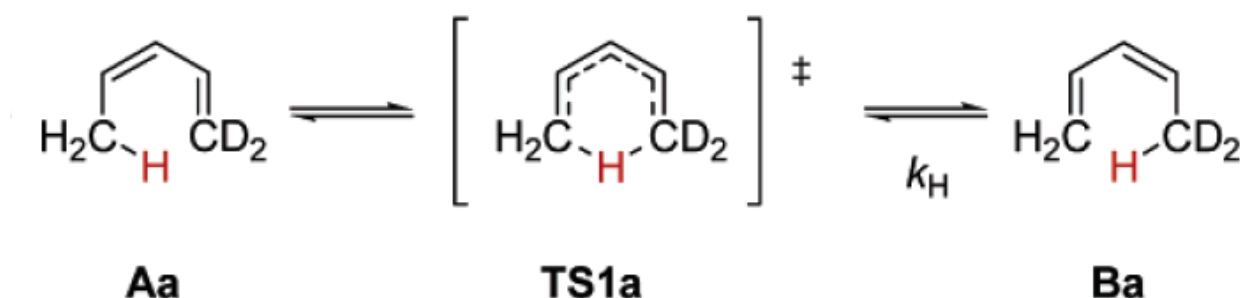


Figure 11. Pericyclic reaction studied by *Itou* from EX1016 (Itou) at 2, Fig. 2.

⁶⁴ EX1015 (Roth) at 3, 6, 8 (“Mass spectroscopic analyses . . . and of the equilibrium mixture . . . also ensure a strictly intramolecular course for this reaction.”),

⁶⁵ EX1016 (Itou) at 2–3 (“In this study, we therefore report on the continued research of pericyclic reactions and use the MC_MO method to analyze pericyclic reactions by demonstrating the importance of the quantum mechanical treatment of the KIE for the proton or deuteron transfer in a thermal [1,5]-sigmatropic hydrogen (or deuterium) shift of (Z)-1,3-pentadiene.”).

272. In contrast, a POSA would have expected the [1,5] shift in the proposed pathways involving NRPE to be a general acid-base-mediated tautomerization. These reactions proceed via a stepwise mechanism involving the formation of an anionic intermediate.

273. In general, tautomerization is defined as a reaction that interconverts two structural isomers. Examples include enol-keto tautomerization, amine-imine tautomerization, lactam-lactim tautomerization, and the interconversion of the neutral and zwitterionic forms of amino acids. EX2045 (March) at 116–120. Tautomerization typically associated with polar molecules and ions that possess weakly acidic functional groups. In proton tautomerization, bases can cause deprotonation; protonation then occurs at a different position in the molecule.

274. Figure 12, below, illustrates a general acid-base mediated tautomerization of NRPE. A POSA would be aware that the transition state involves a highly asymmetric transient intermediate species that includes a hydrophobic diterpene chain on one side (at left), the polar aqueous head group of the phosphatidylethanolamine Schiff base (center), and the hydrophobic diacylglycerol lipid chains (at right). (The Figure depicts the dihexadecanoyl (or dipalmitoyl) phosphatidylethanolamine as the PE phospholipid.). The tautomerization reaction is depicted with a base removing a proton from C20, resulting in an anionic species that is stabilized by delocalization into the conjugated ene-imine functionality, which

includes a lone (unbonded) electron pair on the nitrogen. Subsequently, the nitrogen atom is protonated by a general acid, e.g., a protonated protein base, water, or a protonated phospholipid head group. This sequence of deprotonation and re-protonation involves discrete steps with transient intermediates. This reaction mechanism is fundamentally different from the concerted pericyclic for (Z)-1,3-pentadiene described by *Roth*.

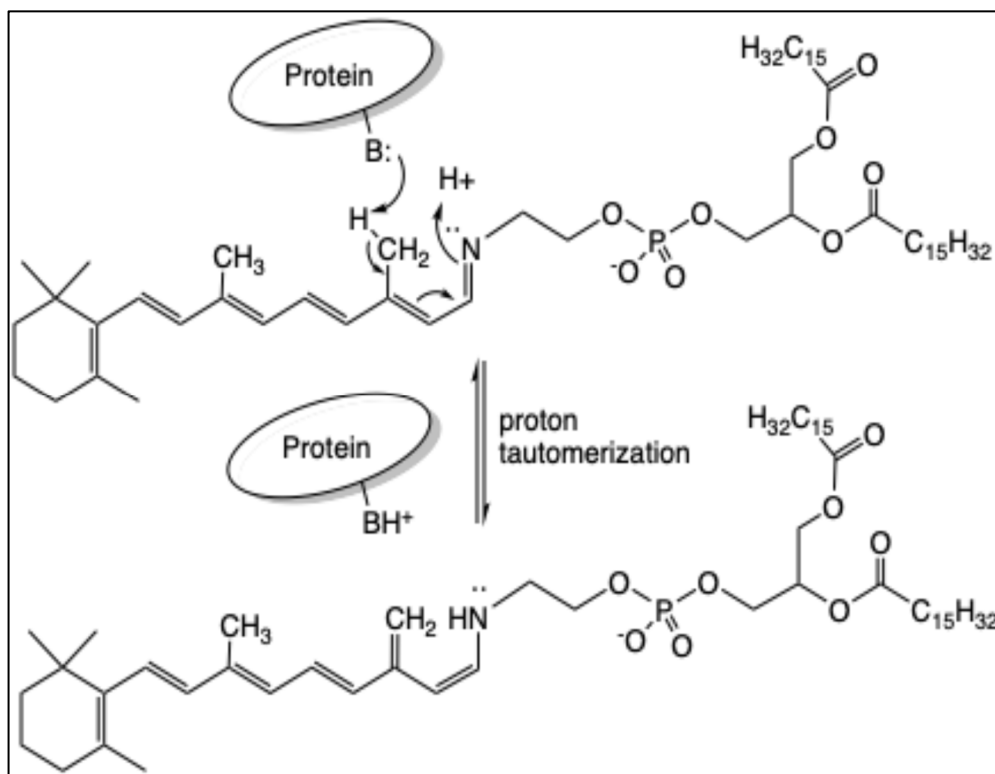


Figure 12. NRPE General Acid-Base Tautomerization.

275. Unlike the reaction described by *Roth*, the same hydrogen atom does not shift from one part of the molecule to the other. Instead, the existing hydrogen atom at C20 on NRPE is deprotonated in a general acid-basis mediated reaction.

Then, a new proton is added to the basic nitrogen of NRPE, resulting in a net “shift” of hydrogen. Unlike the 1,5 sigmatropic rearrangement, however, the hydrogen atom that ends up on the nitrogen is not necessarily the same one that was originally on the carbon.

276. There are multiple reasons why a POSA would have understood that the proposed [1,5] shift in NRPE proceeds through a general acid-base mediated tautomerization, not through the pericyclic sigmatropic rearrangement studied by *Roth* and *Itou*.

277. **First**, the description of this reaction in *Mata 2000* as a “sigmatropic rearrangement” is a clear outlier from the rest of the prior art, which expressly describes the reaction as a “tautomerization” or a “proton tautomerization.” In my opinion, a POSA considering the totality of the literature, would have understood the mechanism in NRPE to be proton tautomerization based on the predominant description of the reaction as such:

- EX1036 (Fishkin 2003) at 2 (“this adduct undergoes a [1,6]-*proton tautomerization*”);
- EX1034 (Wolf 2003) at 1 (“After *tautomerization*, this compound reacts with a second molecule of all-*trans*-retinal”);

- EX1018 (Parish 1998) at 1 (“a [1,6]-*proton tautomerization* to enamine”);⁶⁶
- EX2019 (Kim 2007) at 10 (“a [1,6]-*proton tautomerization* generating the phosphatidyl analogue of enamine which reacts with a second molecule of all-*trans*-retinal.”);
- EX2051 (Ben-Shabat 2002) at 1 (“This adduct undergoes a [1,6]-*proton tautomerization* generating a phosphatidylethanolamine-retinyl enamine”);
- EX1011 (Fishkin 2005) at 5, Fig. 10 (describing “*tautomer x*”).

278. **Second**, analogous reactions had been studied in the prior art under aqueous conditions, and had been shown to take place via general acid-base chemistry involving anionic intermediates, not a [1,5] sigmatropic rearrangement as in *Roth*. EX2077 (Mowery) at 1; EX2078 (Verdegem) at 1. In the gas phase experiment of *Roth* a base catalysis from the environment is not possible. In contrast to *Roth*, these studies were done on retinoids under aqueous conditions where both general base (deprotonation) and general acid (protonation) can contribute to the reaction process, as with *in vivo* systems.

⁶⁶ *Parish 1998* expressly distinguishes between “[1,6 H-Shift]” and the subsequent “[3,3] sigmatropic rearrangement.” EX1018 (Parish 1998) at 3, Fig. 2.

279. For example, *Mowery* studied 13-*cis*-retinylidene-*n*-butylamine.⁶⁷ The reaction described in *Mowery* is very similar to the proposed [1,5] shift with NRPE in the hypothetical A2E pathway: like NRPE, which is a Schiff base formed from a retinoid (all-*trans*-retinal) and PE, 13-*cis*-retinylidene-*n*-butylamine is a Schiff base formed from a retinoid (retinal) and *n*-butylamine. EX2077 (*Mowery*) at 1; EX1010 (*Mata* 2000) at 1. *Mowery* reported that under aqueous conditions “13-*cis*-retinylidene-*n*-butylamine *easily isomerizes* to the *trans* imine by a mechanism involving *general base catalysis*.” EX2077 (*Mowery*) at 3. A POSA would have understood that the reaction described in *Mowery* proceeds via general acid-base mediated deprotonation forming an anionic intermediate, characteristic of a stepwise mechanism.

⁶⁷ As explained above, NRPE (*N*-retinylidene-*N*-retinylphosphatidylethanolamine), is a Schiff base formed between the retinal and phosphatidylethanolamine (PE). EX1010 (*Mata* 2000) at 1, 5; EX1011 (*Fishkin* 2005) at 1, 5.

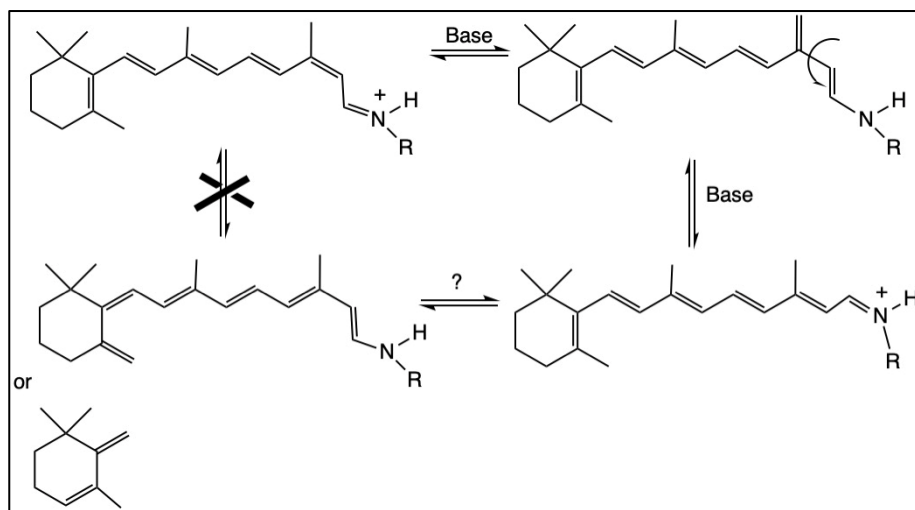


Figure 13. General base catalysis for 13-*cis*-retinylidene-*n*-butylamine. The C13 methyl group, which would be numbered C20) in *Mowery* is the same as Carbon 20 (C20) in NRPE in, e.g., *Mata 2000*, *Fishkin 2005*. EX2077 (*Mowery*) at 3, Fig. 5 (redrawn).

280. Similarly, *Verdegem* described a base-induced condensation reaction of two molecules of ATR to form ATR-dimer, analogous to the formation of ATR-dimer described by *Fishkin 2005*. EX2078 (*Verdegem*) at 1; EX1011 (*Fishkin 2005*) at 5, Fig. 10. *Verdegem* reported that its reaction proceeded via a base-induced reaction mechanism, explaining:

It is well known, that α,β -unsaturated aldehydes possessing a β -methyl substituent are prone to base induced condensations. Under the basic conditions provided by the sodium hydride, one of the relative acidic

C20-methyl protons^[68] is abstracted by the strong base. The resulting anion of retinal reacts in a Michael addition with a neutral retinal molecule at C13, resulting in the formation of a noncyclic C₄₀ adduct (I) as shown in Scheme 1.

EX2078 (Verdegem) at 1.⁶⁹

281. *Verdegem* concludes that the “condensation of all-*E*-retinal can be easily performed by exposing retinal to sodium hydride, a non-nucleophilic strong base. The reaction is general for three compounds possessing the β -methyl ethene aldehyde moiety, providing a promising synthetic tool for the preparation of chemically modified retinoids.” EX2078 (Verdegem) at 3.

282. **Third**, *Fishkin 2005* further suggests a general acid-base mediated tautomerization mechanism for the 1,5 shift in NRPE. *Fishkin 2005* disclosed that the [1,6] H shift (the same reaction Dr. Guengerich characterizes as a [1,5]

⁶⁸ The mention of the “relative acidity of C20-methyl protons” by *Verdegem* acknowledges the unique acidity of the vinylogous allylic hydrogens in conjugation with the protonated iminium species shown at the upper left structure of Figure 13.

⁶⁹ EX2078 (Verdegem) at 1 (“The basic conditions during the work up of e.g. a dibal-H reduction of C20-nitrile yielding retinal, promotes the formation of a dark-red byproduct.”).

sigmatropic rearrangement⁷⁰) forms “tautomer X.” EX1011 (Fishkin 2005) at 5. *Fishkin 2005* explains that its proposed pathway *requires* C20 to be nucleophilic and that this nucleophilicity at C20 is accounted for by “tautomer x:” EX1011 (Fishkin 2005) at 5.

Indeed, that ATR dimer forms in ROS lends support to the proposed existence of this tautomer [tautomer x], as this intermediate would account for the nucleophilicity of C20 and its participation in the (1,4) conjugate addition. According to the scheme in Fig. 10, C20 of NRPE would react with C13 of free ATR in a Michael-type addition, and after ring closure and elimination of the amino group of PE, ATR dimer would be formed.

EX1011 (Fishkin 2005) at 5.

283. A POSA would have readily understood that the nucleophilicity at C20 necessarily requires that C20 becomes nucleophilic due to deprotonation in the prior reaction (the [1,6] H shift), thus indicating the [1,6] H-shift is a general base-mediated tautomerization. EX1011 (Fishkin 2005) at 5, Fig. 10.

⁷⁰ EX1002 ¶84 (“Fishkin thus discloses that *the same reaction step*, which involves a [1,6] H-shift and which Fishkin describes as converting *N*-retinylidene PE (NRPE) into ‘tautomer x,’ results in the formation of both ATR dimer and A2E.”).

284. **Fourth**, a POSA would have recognized that, in the aqueous environment of photoreceptor cells, the [1,5] shift reaction described in, e.g., *Mata 2000* and *Fishkin 2005* made a general acid-base mediated tautomerization was more likely. This is because, in the cellular environment, such reactions are more favorable than pericyclic 1,5 sigmatropic rearrangements as the polarity of the aqueous environment stabilizes the intermediates. Moreover, basic species such as amino acid side chains (e.g., lysine, arginine) and other nucleophilic moieties present in the complex chiral protein-lipid environment of the ROS facilitate the deprotonation of acidic hydrogen atoms, promoting general acid-base mediated reactions.

285. The difference between the gas phase pericyclic sigmatropic shift for the (Z)-1,3,-pentadiene in *Roth* and the general acid-base mediated *in vivo* tautomerization of NPRE undermines the basis for Dr. Guengerich's opinions, including for instance, Dr. Guengerich's opinions that:

“deuteration of the C₂₀ methyl would have a similar kinetic profile as the well-studied 1,3-pentadiene system outlined in both Roth and Itou because C₂₀ forms a similar diene system as in 1,3-pentadiene” (EX1002 ¶122); and

“based at least on the teachings of Itou and Roth, it is my opinion that the kinetics of the (1,5)-sigmatropic rearrangement would be significantly slower than the (3,3)-sigmatropic rearrangement in Mata

and thus likely the rate limiting step in the mechanism.” (EX1002 ¶178).

286. Dr. Guengerich’s reliance on *Roth* and *Itou* assumes that these papers describe the same reaction mechanism theorized to be involved in the formation of A2E. EX1002 ¶¶116–123; 261–264.⁷¹ Without this alleged common thread, a POSA would have no reason to even consider *Roth*, much less expect that the KIE observed in *Roth* would also be observed for NRPE. The molecules and mechanisms are fundamentally different: the reaction described in *Roth* is a concerted pericyclic process, while a POSA would recognize that the NRPE reaction proceeds via a stepwise, general acid-base deprotonation-reprotonation mechanism involving intermediates.

⁷¹ EX1002 ¶261 (“Further, persons of ordinary skill in the art would have understood that deuterating C₂₀ would slow A2E formation because 1,5-sigmatropic shifts were known to be subject to strong primary deuterium kinetic isotope effects. For example . . . Itou, citing Roth, provides an analysis of the kinetic isotope effect of a 1,5-sigmatropic shift in a 1,3-pentadiene system. Roth further discloses that there is a large kinetic isotope effect associated with the 1,5-sigmatropic shift.”) (citing EX1015 (Roth) and EX1016 (Itou)).

287. *Roth* and *Itou* are both studying the [1,5]-sigmatropic hydrogen shift—a pericyclic reaction mechanism. As *Itou* explains, these papers focus on the “thermal [1,5]-sigmatropic hydrogen-shift reaction,” which proceeds through a cyclic, concerted transition state:

The thermal *[1,5]-sigmatropic hydrogen-shift* reaction is not only one of the most important sigmatropic rearrangement reactions, but it is also the most fundamental hydrogen-transfer reaction (Z)-1,3-Pentadiene . . . is the simplest model *for representing thermal [1,5]-sigmatropic hydrogen-shift reactions*

EX1016 (*Itou*) at 1; *see also* EX1016 (*Itou*) at 5 (“We used the MC_MO-HF method to study the KIE and GIE in the thermal *[1,5]-sigmatropic H (or D) shift* of (Z)-1,3-pentadiene.”).

288. Neither *Roth* nor *Itou* purport to be relevant to *all* types of hydrogen-transfer reactions. Dr. Guengerich also does not contend that *Roth* and *Itou* are broadly applicable to all hydrogen transfer reactions. A POSA would have understood that the factors influencing the KIE, such as the degree of C-H bond cleavage in the transition state, differ significantly between these mechanisms. EX1047 (*Blake*) at 3.⁷² Thus, a POSA would understand that the KIE reported by

⁷² EX1047 (*Blake*) at 3 (“In certain acid-base-catalyzed reactions, *depending on the mechanism*, an inverse isotope effect may take place.”).

Roth and *Itou* would not suggest a similar KIE for the different reaction mechanism seen with NRPE.

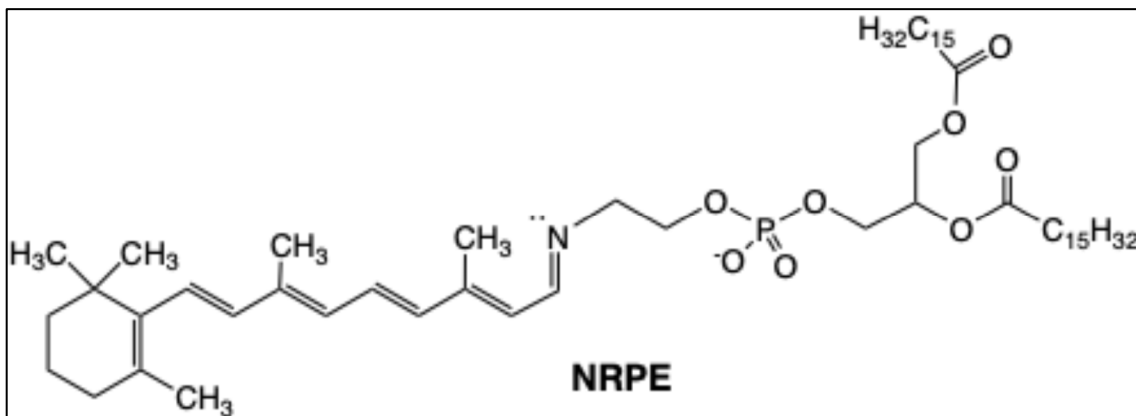


Figure 14. NRPE.

289. Further, a POSA would expect from closer chemical analogies that the interconversions characterizing the tautomerization of NRPE were likely to be relatively fast reactions with relatively low activation energies, particular due to the involvement of imines and enamines in the reaction and the biphasic aqueous-lipid environment in which it takes place. EX2077 (Mowery) at 3 (“It is *clear* that 13-*cis*-retinylidene-*n*-butylamine *easily isomerizes*.”); EX2078 (Verdegem) at 1.

290. For instance, *Mowery* reported that under aqueous conditions “13-*cis*-retinylidene-*n*-butylamine *easily isomerizes* to the *trans* imine by a mechanism involving *general base catalysis*.” EX2077 (Mowery) at 3. *Mowery* further explains that this is due to the relative acidity of the C20 hydrogen due to the presence of the nitrogen in the retinoid:

The 13-methyl hydrogens of the imine are relatively acidic, because they are homoallylic to the nitrogen, whereas the C4, C18, or C19 hydrogens are less so, because the electron-withdrawing nitrogen substituent effect is attenuated as it is broadcast down the chain.

EX2077 (Mowery) at 3.

291. In other words, the hydrogen atoms attached to C13-methyl group (C20 in NRPE) in the imine are relatively acidic because they are homoallylic to the imine Schiff base. In contrast, the hydrogens at carbons 4, 18, and 19 are less acidic because the electron-withdrawing effect of the nitrogen weakens as it extends down the carbon chain. This reduced acidity at more distant positions explains the “X” across the equilibrium on the left side *Mowery* Figure 5,⁷³ indicating that deprotonation of these remote methyl hydrogens is unfavorable. EX2077 (Mowery) at 3, Fig. 5.

292. Based on the analogous reaction in *Mowery*, a POSA would have expected the hydrogen at C20 of NRPE similarly was more acidic due to its proximity to the nitrogen. This increased acidity makes it easier for a general base to remove the proton from C20. Moreover, a POSA would understand that the lone pair of electrons on the nitrogen can delocalize the negative charge that forms when the hydrogen at C20 is removed. EX2047 (Anslyn) at 45. This delocalization through

⁷³ Shown in Figure 13, above.

extended conjugation to the Schiff base stabilizes the negative charge on C20, lowering the activation energy needed for the reaction and increasing its rate. EX2047 (Anslyn) at 45.

293. The combined inductive and resonance effects of the nitrogen in NRPE result in a more acidic C-H bond at C20. The increased acidity means that the proton is more readily removed under basic conditions, accelerating the reaction compared to a system that does not have a nitrogen, such as *Roth's* (Z)-1,3-pentadiene.

294. Thus, based on the significant differences in both reaction mechanisms and molecular structures, a POSA would not have reasonably expected that the KIE observed in *Roth's* pericyclic, concerted reaction would also be observed in the base-catalyzed, stepwise tautomerization of NRPE. Instead, a POSA would have expected the NRPE rearrangement to be a lower-energy and faster tautomerization, as it is described in most of the primary publications and reviews prior to September 2007.

295. Further, even if a POSA believed NRPE could undergo the same sigmatropic rearrangement as described by *Roth*, they still would not have reasonably expected the KIE for the 1,3-(Z)-pentadiene of Roth to predict the KIE for NRPE. This is because a POSA would understand the significant structural differences between the two compounds would meaningfully alter the symmetry of NPPE's transition state during the [1,5] shift, a key determinant of the KIE.

296. A POSA would have understood that the presence and magnitude of a KIE depend on the symmetry of the transition state. A more symmetrical transition state produces a larger KIE, whereas a more asymmetrical transition state results in a smaller or undetectable KIE. For instance, *Roth* explains that the “extent [of the KIE] is largely determined by the symmetry of the transition state and reaches a maximum value with a linear, symmetrical arrangement of the activated complex.” EX1015 (Roth) at 3–4; *see also* EX1015 (Roth) at 4 (“The detection of only a small effect would be a reliable criterion for a non-symmetrical transition state”); EX2047(Anslyn) at 22 (“hydrogen transfers that proceed through non-linear transition states have lower KIEs.”).

297. *Roth* explicitly selected (*Z*)-1,3-pentadiene as a model compound specifically because its symmetrical structure was expected to produce a significant KIE due to a highly symmetrical transition state. EX1015 (Roth), 3–4 (“*cis*-pentadiene-(1.3) therefore seemed particularly suitable for investigating the isotope effect of the 1.5-hydrogen displacement.”); *see also* EX1016 (Itou) at 1 (“(*Z*)-1,3-Pentadiene A (Figure 1) is the *simplest model* for representing thermal [1,5]-sigmatropic hydrogen-shift reactions.”).

298. In contrast to the pentadiene of *Roth*, NRPE is a significantly larger and more complex compound that includes a bulky and amphiphilic phosphatidylethanolamine group connected to the retinylidene moiety. *E.g.*, EX1010

(Mata 2000) at 5, Fig. 5. Additionally, the nitrogen in NRPE would have been expected to introduce additional electronic interactions that are absent in *Roth's* simple pentadiene system. These interactions can stabilize the transition state differently and may involve partial negative charge development on nitrogen, altering the reaction pathway and affecting the KIE. A POSA would have recognized that the steric hindrance and electronic effects introduced by the enamine-imine tautomerization in addition to the lipid bilayer-bound phosphatidylethanolamine group would dramatically disrupt the symmetry of the transition state.

299. Thus, even assuming a POSA believed the [1,5] shift in the hypothetical A2E pathways was a [1,5] sigmatropic rearrangement (which they would not have), the significant differences between NPPE and the simple pentadiene model of *Roth* would have precluded a POSA from reasonably expecting the KIE observed in *Roth* would also be observed with NPPE.

VIII. Researchers in the Field were Actively Using all the Tools Dr. Guengerich Asserts Were Obvious to Combine

300. Further underscoring further underscoring Dr. Guengerich's hindsight analysis, a review of the literature post-*Mata 2000* reflects the art proceeding in directions contrary to that which he now alleges would have been obvious.

301. As of September 2007, there was no cure for Stargardt's Disease or macular degeneration and no treatments for Stargardt's Disease whatsoever. As the litany of prior art references studying lipofuscin accumulation demonstrates, there

was intense interest throughout the field on this area of research. EX2003 (Travis 2007) at 17 (“the focus of many laboratories on diseases of the retina and RPE”); *see also* EX2058 (Kim 2004) at 4 (“there is considerable interest in retarding A2E formation as a means to prevent vision loss in Stargardt disease and, perhaps, age-related macular degeneration”); EX1009 (Widder) at 3 (“Currently, treatment options for the macular degenerations and macular dystrophies are limited “successes have been limited and there continues to be a strong desire for new methods and treatments to manage and limit vision loss associated with the macular degenerations and dystrophies.”).

302. These researchers had all the elements on which Dr. Guengerich bases his obviousness argument⁷⁴ and researchers were using hydrogen isotopes to study the visual cycle, including lipofuscin formation specifically. EX1035 (Radu 2003) at 4–6; EX2064 (Maeda 2006) at 10; EX2059 (Rando 2001) at 4.

303. For example, *Radu 2003* reported the use of isotretinoin (Accutane®) to inhibit lipofuscin accumulation. EX1035 (Radu 2003) at 1. In doing so, the authors

⁷⁴ EX1002 ¶¶124–184.

used tritium labeled *N*-ret-PE (also known as NRPE), the same intermediate that undergoes the [1,5] shift in *Mata 2000*. EX1035 (Radu 2003) at 3, 5.⁷⁵

304. *Maeda 2006* used isotopically labeled chromophores to follow their path through the visual cycle. EX2064 (Maeda 2006) at 10 (“Finally, replacement of the chromophore will allow us to follow the flow (e.g., the *isotope-labeled synthetic chromophore*”) and to investigate how the chromophore modifications affect rhodopsin regeneration.” Like *Radu 2003*, *Maeda 2006* was studying the ability of a small molecule inhibitor to prevent the formation of lipofuscin accumulation. EX2064 (Maeda 2006) at 1–2, 10; § V.B.3.

305. Thus, after *Mata 2000* was published, researchers (including the authors of *Mata 2000*) were using hydrogen isotopes, studying the A2E pathway,⁷⁶ and were attempting to solve the very problem (preventing lipofuscin accumulation) addressed by the ’045 patent. Despite having all the tools these researchers did not even hint at potentially using the [1,5] shift as—depicted on page 2 of *Radu 2003*—

⁷⁵ EX1035 (Radu 2003) at 5 (“We injected radio-labeled vitamin A . . . and measured levels of labeled *N*-ret-PE. This design permitted us to consider only *N*-ret-PE formed during the treatment period. We observed a . . . reduction in labeled *N*-ret-PE.”).

⁷⁶ EX1035 (Radu 2003) at 2.

as a means to prevent lipofuscin accumulation. Rather, *Radu 2003* attempted to reduce the amount of excess ATR available to form lipofuscin pigments, just like all the other treatment approaches in the prior art described above. § V.B.3; EX1035 (*Radu 2003*) at 1.

306. Macular degeneration is immensely complex and challenging to treat. *E.g.*, EX2074 (*Rattner 2006*) at 11 (“Macular degeneration falls into a class of diseases that are generally considered difficult therapeutic targets: those that are characterized by the slow destruction of a complex non-renewing tissue, as seen, for example, in Parkinson’s disease and Alzheimer’s disease.”). Leading researchers were using the very tools that Dr. Guengerich claims were obvious to combine, yet nothing in the prior art suggests targeting the [1,5] shift or using deuterium. Dr. Guengerich’s analysis is nothing more than a hindsight recreation of the novel methods described in the ’045 patent.

IX. Conclusion

307. For the reasons set forth herein, it is my opinion that all of the Challenged Claims are patentable and are not rendered obvious by the alleged prior art.