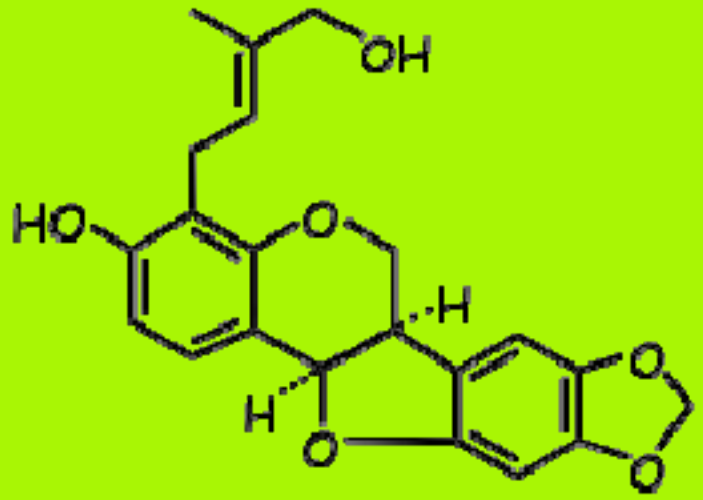
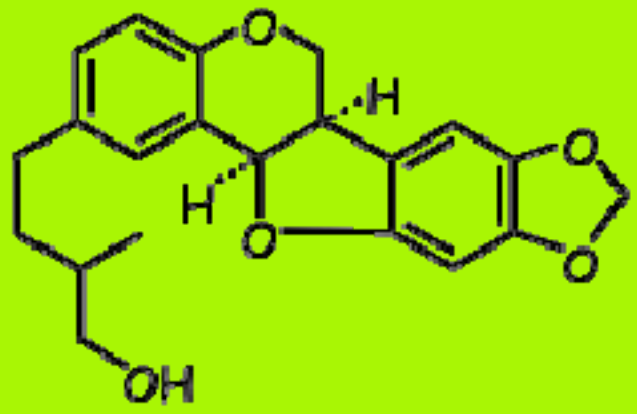
anti-snake venom from Cabeca de negra

aq. alcoholic extract "especifico pessoa" of root, 135 ml

cabenegrin A-I, 44 mg



cabenegrin A-II, 1 mg

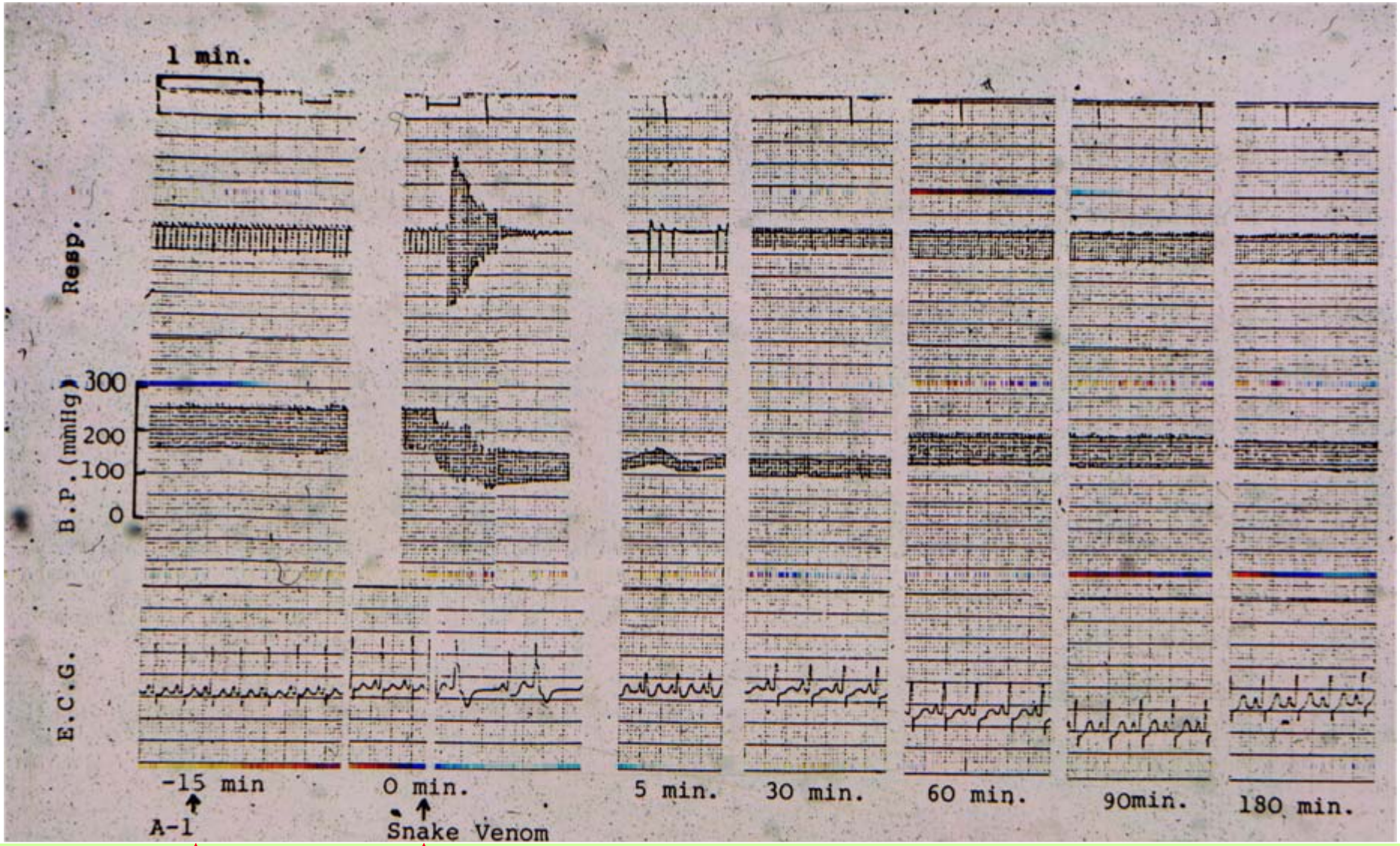


antidote against Fer de lance (*Bothrops atrox*) snake venom

injection of 2.5 mg / kg venom (lethal dose) into 9 kg beagle dog leads to hypotension, respiratory and cardiac arrest.

injection of 1.0 mg / kg A-I, 15 min before or aftger venom inection restores or reverses all effects in 90 min

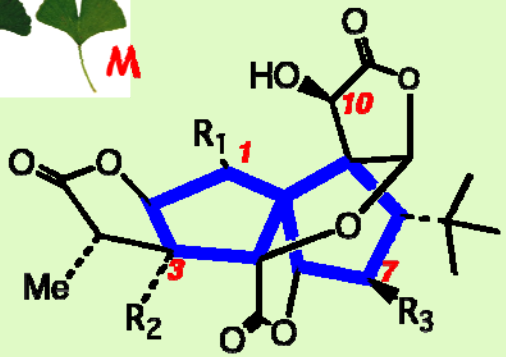
nakagawa, Nakanishi, Darko, Vick, *Tetrahedron Lett.* 38, 3855 (1982)



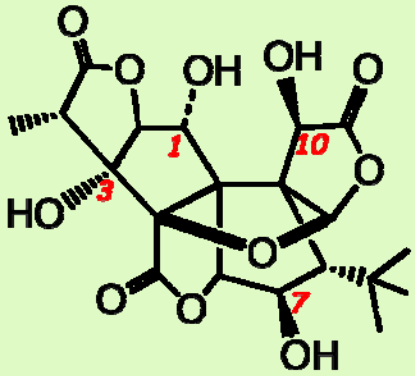
Effect of A-I on respiratory and cardiovascular responses to snake Venom (Bathrops atorax) in anaesthetized dogs

Ginkgo biloba

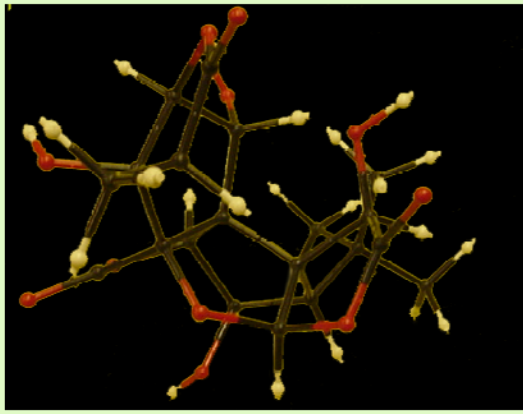
Division Ginkgophyta
Order Ginkgoales
Family Ginkgoaceae



ginkgolides



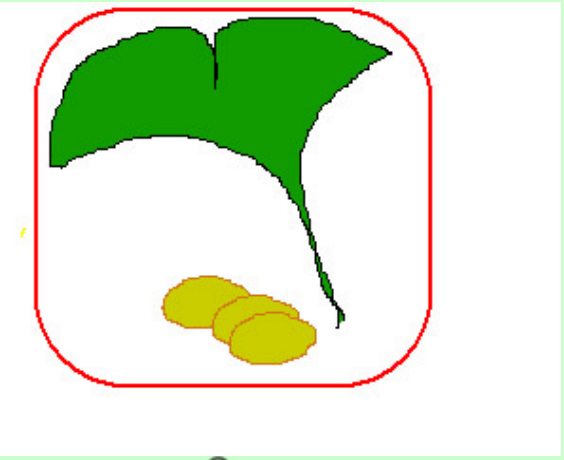
ginkgolide C

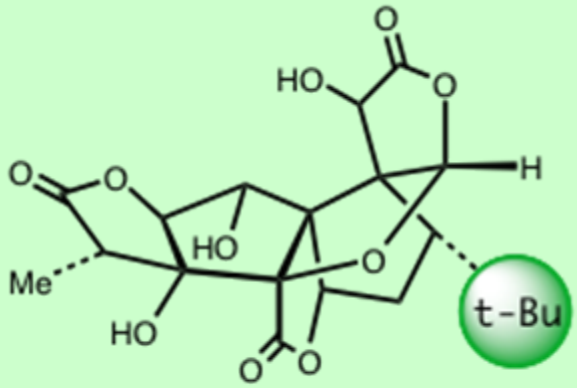


Ginkgo biloba, the ginkgo tree

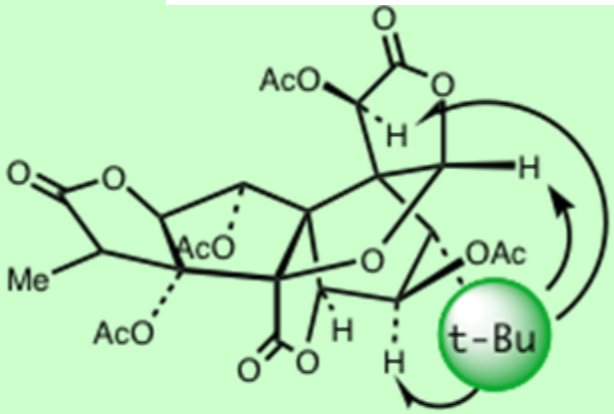
One of the first tree, *Ginkgoales*, appeared 250 million years ago.

All species vanished, except one, *Ginkgo billoba*, which remains unchanged 150 illion years.





Maruyama, Terahara, Itagaki ('67)



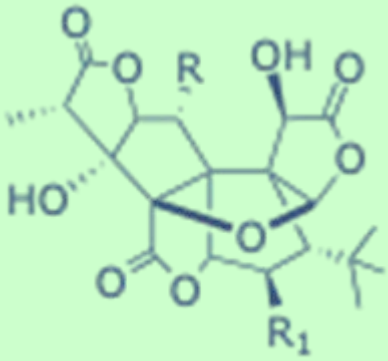
nuclear Overhauser effect
Woods, Miura ('67)

Mentioned in Chinese materia medica, 2800 B.C.
Inhibitor of platelet activating factor.
$ 3 billion of *Ginkgo* tree extract sold over-the-counter annually.

Summary of ginkgolide isolation from *Bioginkgo* extract

1. Solid-liquid Extraction
2. Bilobalide separation by chromatography
3. Ginkgolide functionalization (benzylation, etc.)
4. Separation by chromatography
5. De-functionalization (debenzylation, etc.)

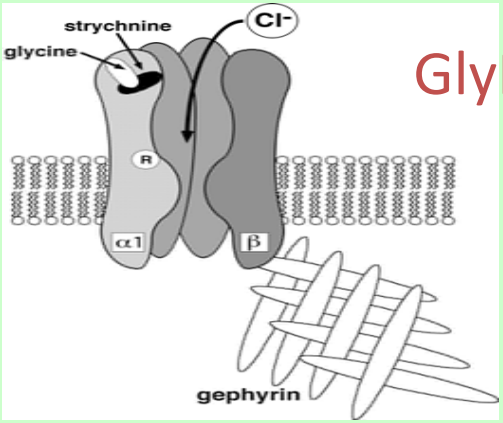
1-2 days



R, R_1 = H, OH

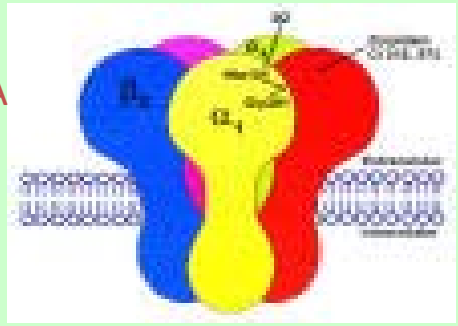
K. Nakanishi, S. Jaracz, S. Malik, H. Ishii, S. V. Dzyuba, *PCT Int. Appl*. WO 2005046829, 2005, pp 61.

GlyR



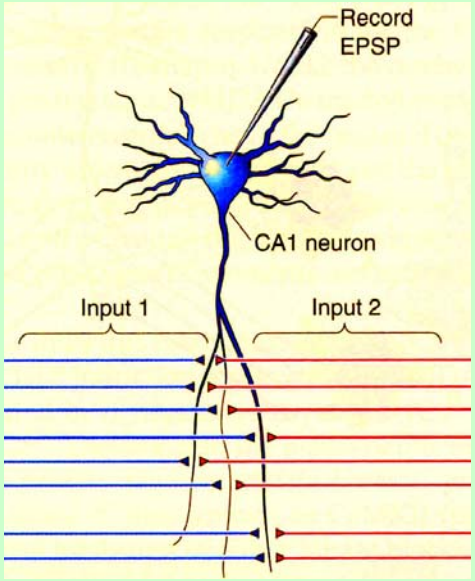
S. Jaracz, K. Nakanishi, A. A. Jensen, K. Stromgaard, *Chem. Eur. J.*, 2004, *10*, 1507

$GABA_A$

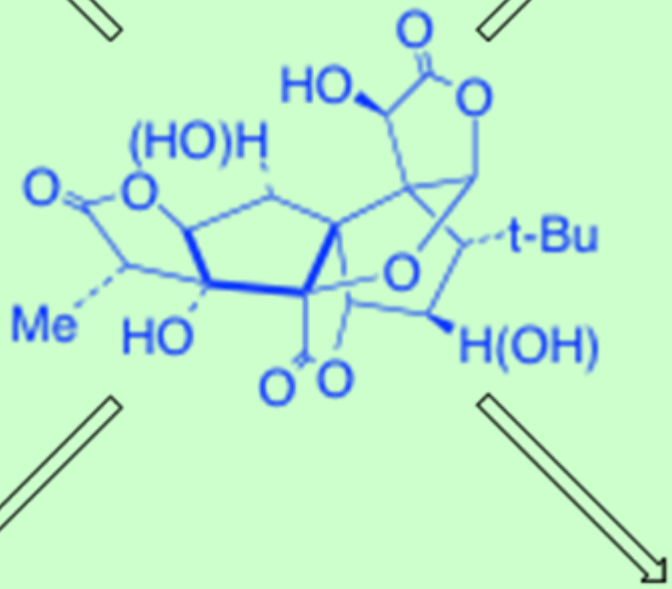


L.Ivic, T. T. J. Sands, N. Fishkin, K. Nakanishi, A. R. Kriegstein, K. Stromgaard, *J. Biol. Chem.*, 2003, *278*, 49279

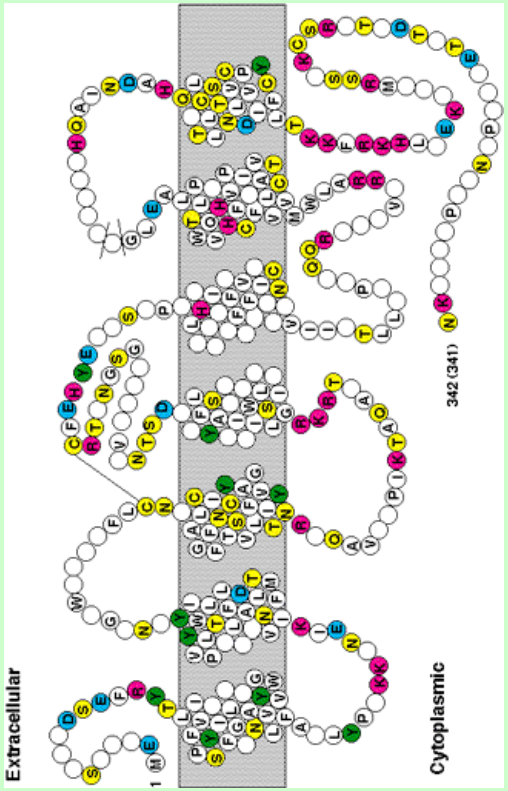
Neuronal cell



O. Vitolo, Z. Cao, H. Ishii, S. Jaracz, K. Nakanishi, O. Arancio, S. V. Dzyuba, M. Shelanski, *Neurobiol. Aging, in press*



PAFR



K. Stromgaard, D. R. Saito, H. Shindou, S. Ishii, T. Shimizu, K. Nakanishi, *J. Med. Chem.*, 2002, *45*, 4038.

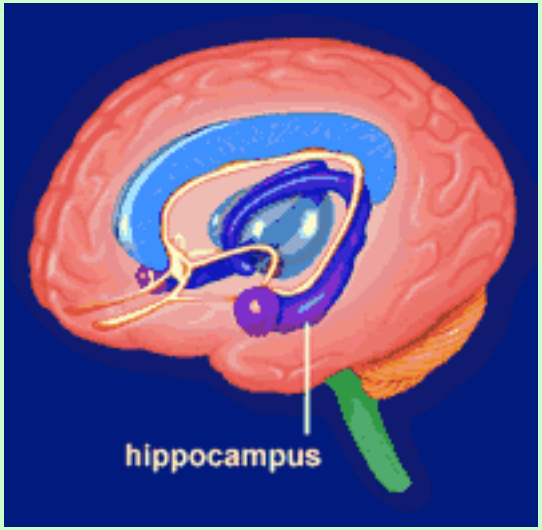
Syntheses of 7-[^{3}H]-GB & 7-[^{18}F]fluoro-GB

^{18}F: positron emitter, $T_{1/2}$ = 110 min. produced from $H_2{}^{18}O$ in in-house cyclotron.

Goal: to visualize ginkgolide action sites in living brain by PET (Positron Emission Tomography).

Suehiro, Simpson, Underwood, Castrillon, Nakanishi, van Heertum, *Planta Medica*, 2005, *71*, 622

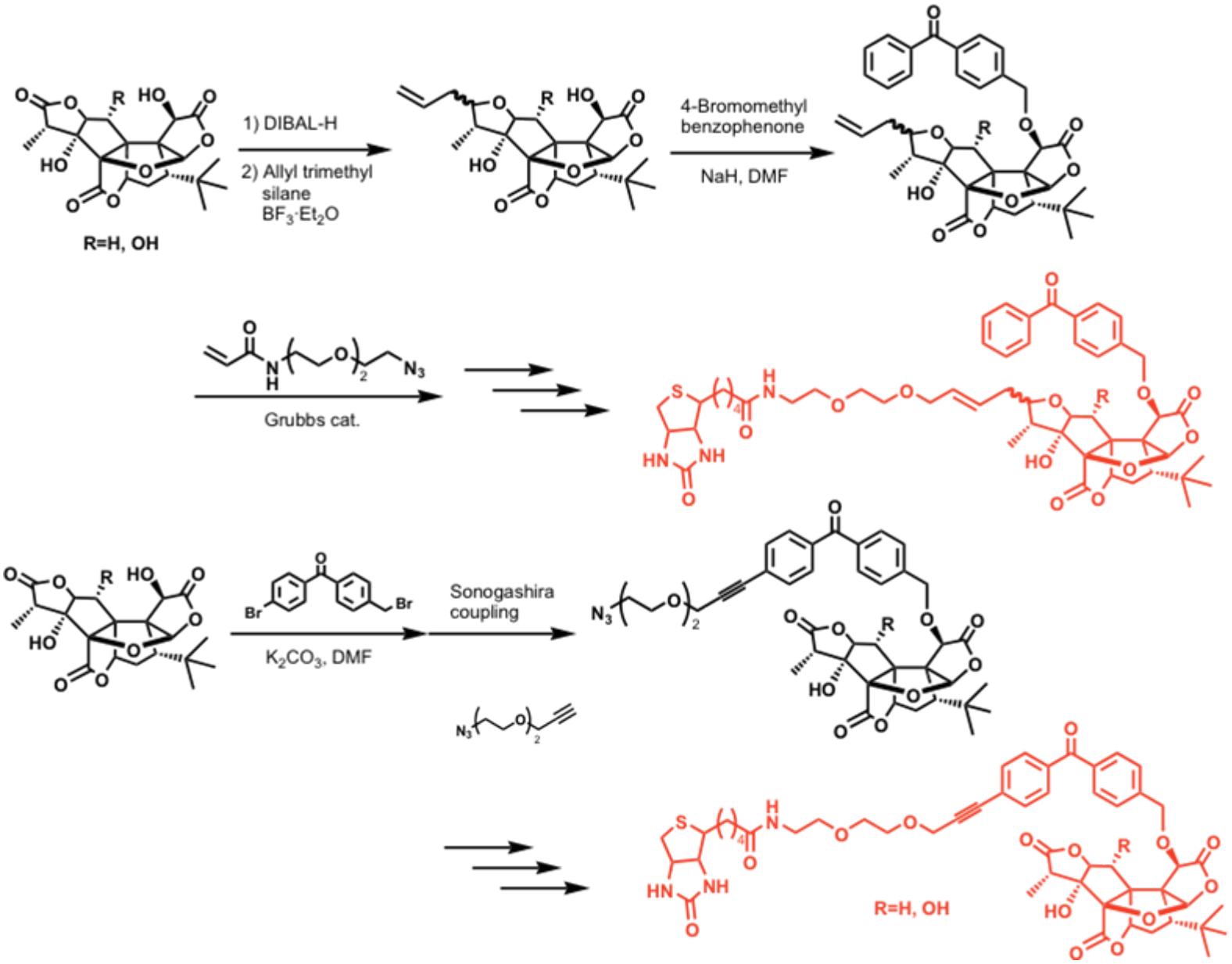
Goal: to study GB and its action site interactions by in vivo autoradiography and in vitro competitive binding studies.



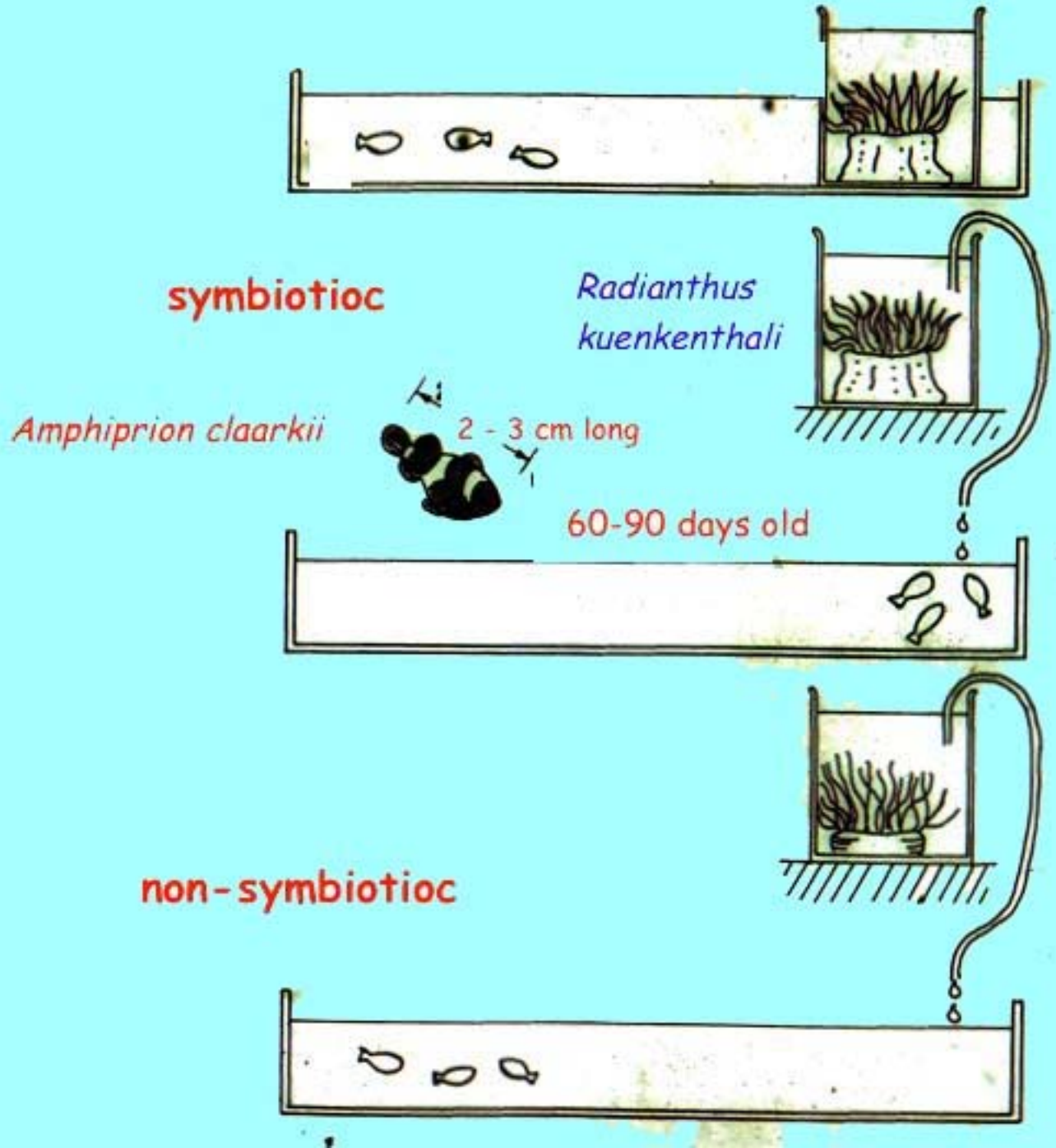
Strømgaard, Suehiro, Nakanishi, *Bioorg. Med. Chem*. Let., 14, 5673-75 (2004)

hippocampus: site of long term potentiation (LTP)

Biotinylated derivs. for photo-crosslinking



E. Kato, R. Howitt, S. V. Dzyuba, K. Nakanishi, *Org. Biomol. Chem.*, 5, 3758-3761 (2007)


symbiotioc
Radianthus
kuenkenthali
Amphiprion claarkii
2 - 3 cm long
60-90 days old
non-symbiotioc

Amphikuemine, aymbiosis inducing substance

Secreted by the sea anamone Radianthus kukenthali
Specifically attracts the anemone fish Amphiprion perideraion.

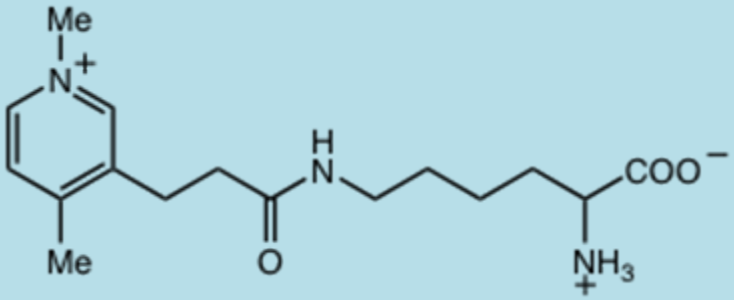
15 kg of sea anemone homogenate

1 % acetic acid /20 % *aq*. MeOH

extract

residue

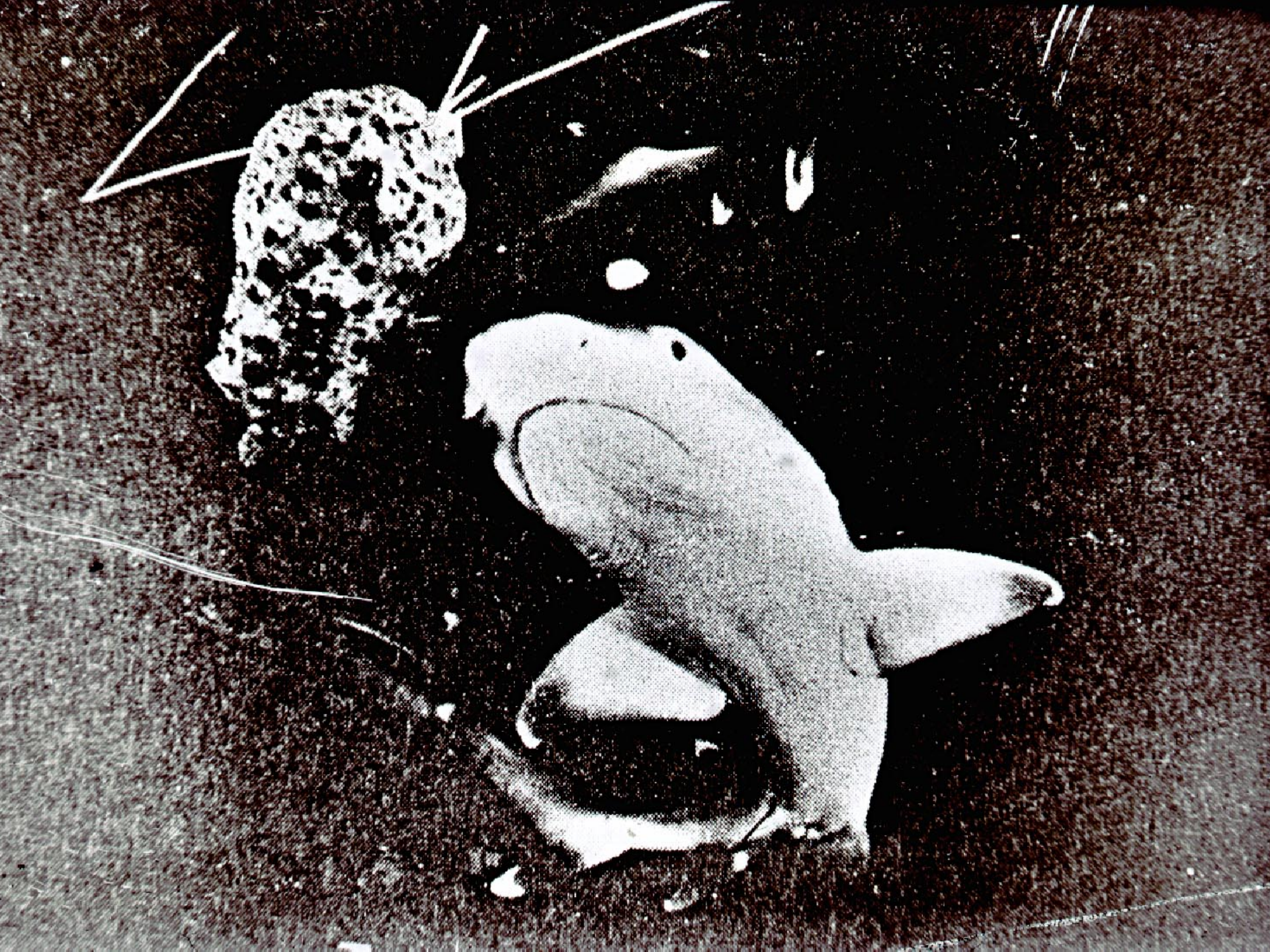
0.048 mg of symbiont (corresponding to 70% yield)



Structure; Murata, Naya…
Science, 234, 585 (1986)
Synthesis; konno, Naya (1990)

Murata, Miyagawa-Kohshima, Nakanishi, Y. Naya, *Science*, 234, 585 (1986)
Konno, Qin, Nakanishi, Murata, Naya, *Heterocycles*, 30,247 (1990)


Pardachiruspavoninus

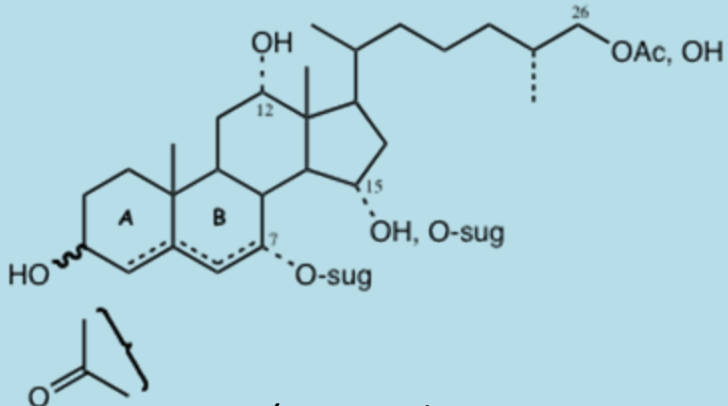


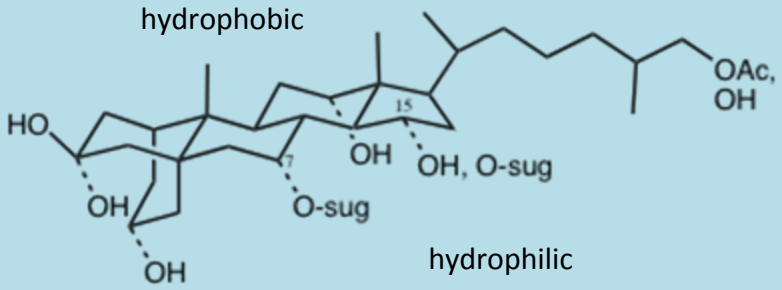
Shark Repellents from Flatfish

Pardachirus marmoratus, "Moses sole"(Red Sea) : Clark (1974)
mixture of detergent-like compounds very difficult to separate
also present in P. pavoninus (Indian Ocean, Ryukyus)

I) Steroidal saponins

Tachibana, Sakaitanai Nakanishi, *Science*, *226*, 703 (1984).





rings A/B cis and trans

pavoninins : glcNAc at C-7 or C-15
mosesins : gal or gal-6-Ac at C-7

Tachibana, Gruber (1985~88)
Gargiulo (1989) synthesis

II) Amphiphilic 33-peptides

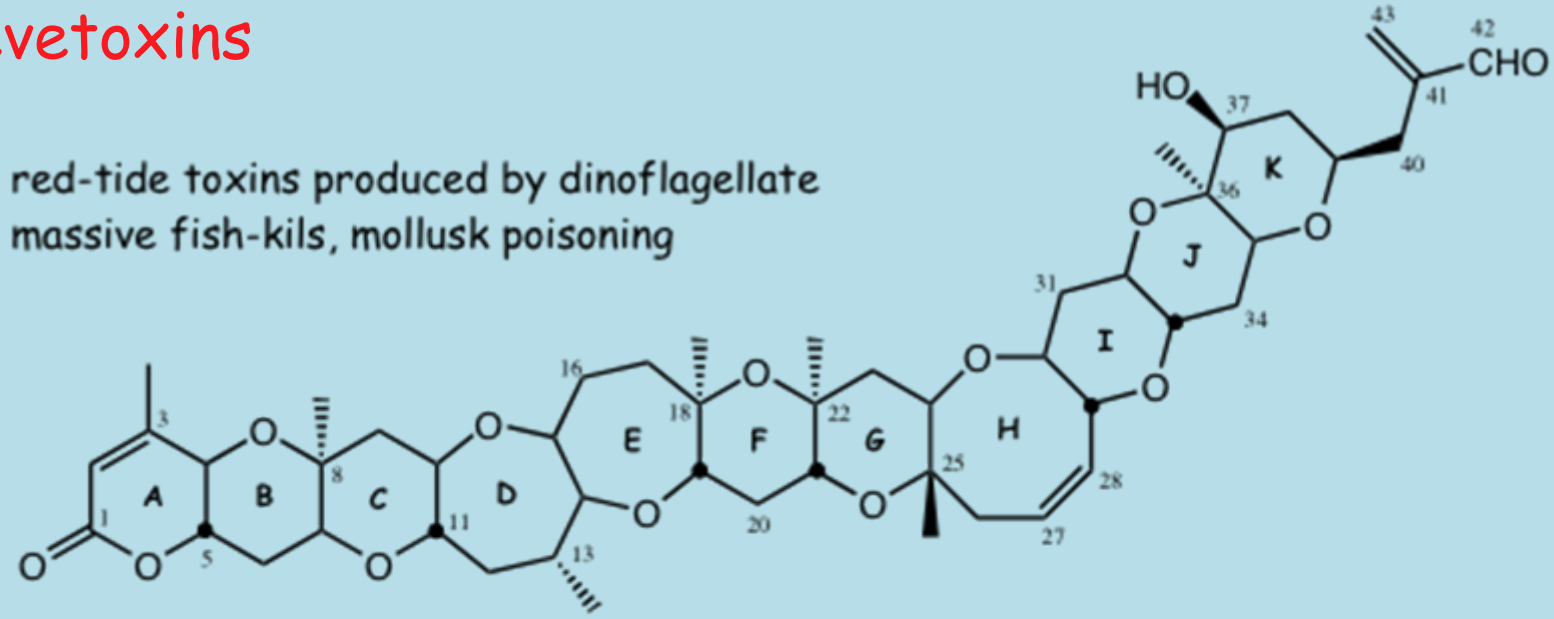
similar to bee venom "mellitin"

Tachibana, Thompson, Kubota (1986~89)

Thompson, Tachibana, Nakanishi, Kubota, *Science*, 233, 341-343 (1986).

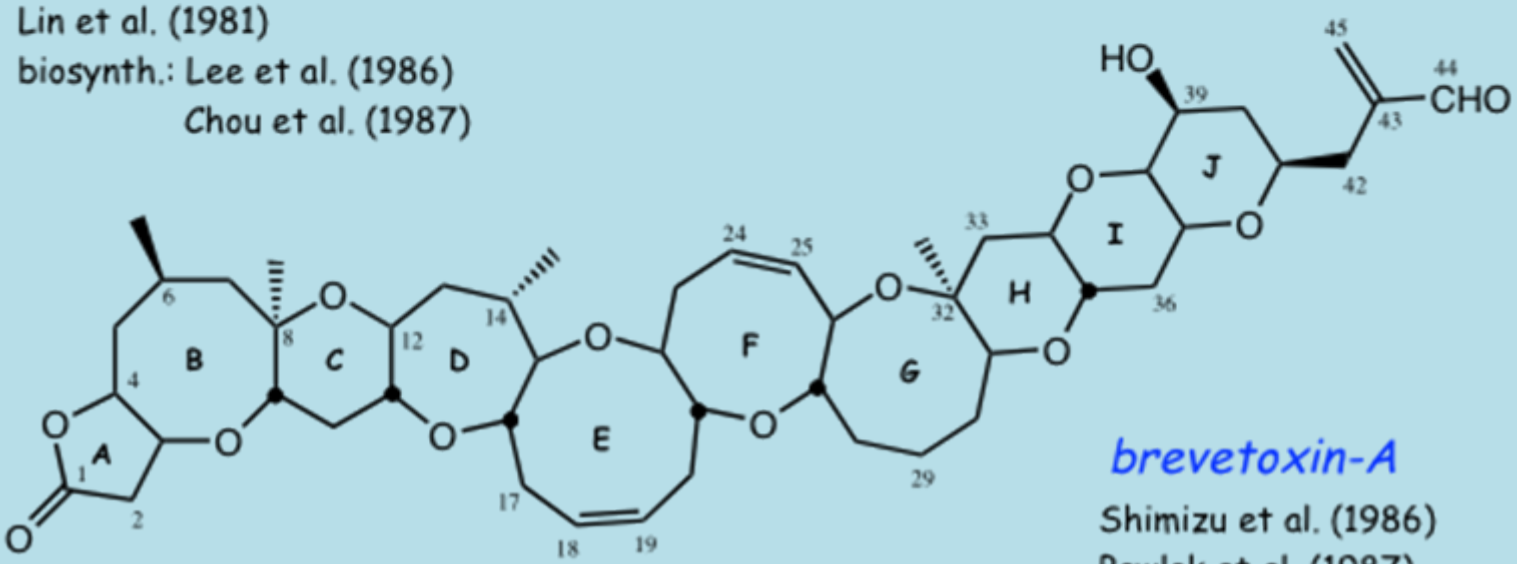
Brevetoxins

red-tide toxins produced by dinoflagellate
massive fish-kils, mollusk poisoning



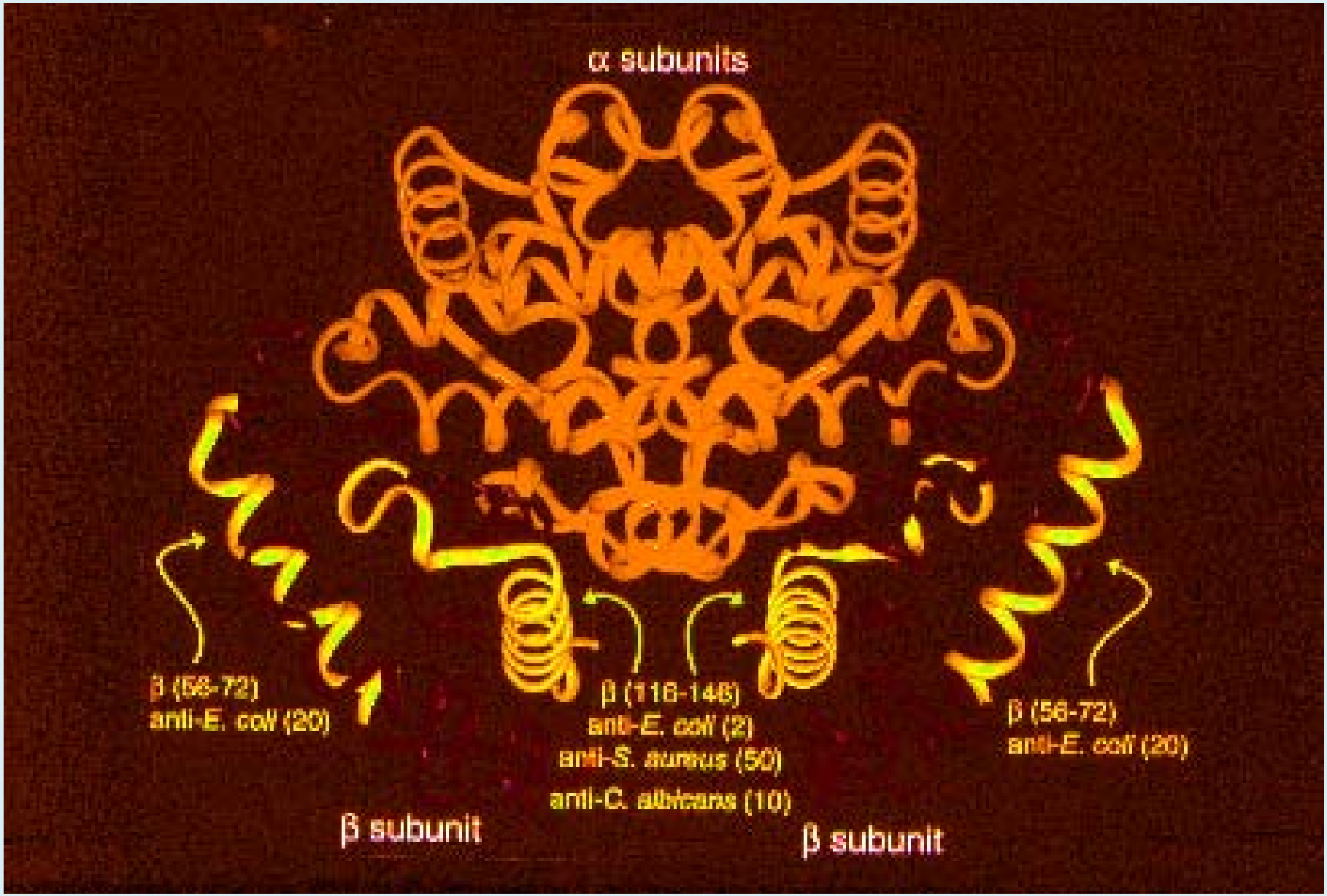
brevetoxin-B

Lin et al. (1981)
biosynth.: Lee et al. (1986)
Chou et al. (1987)

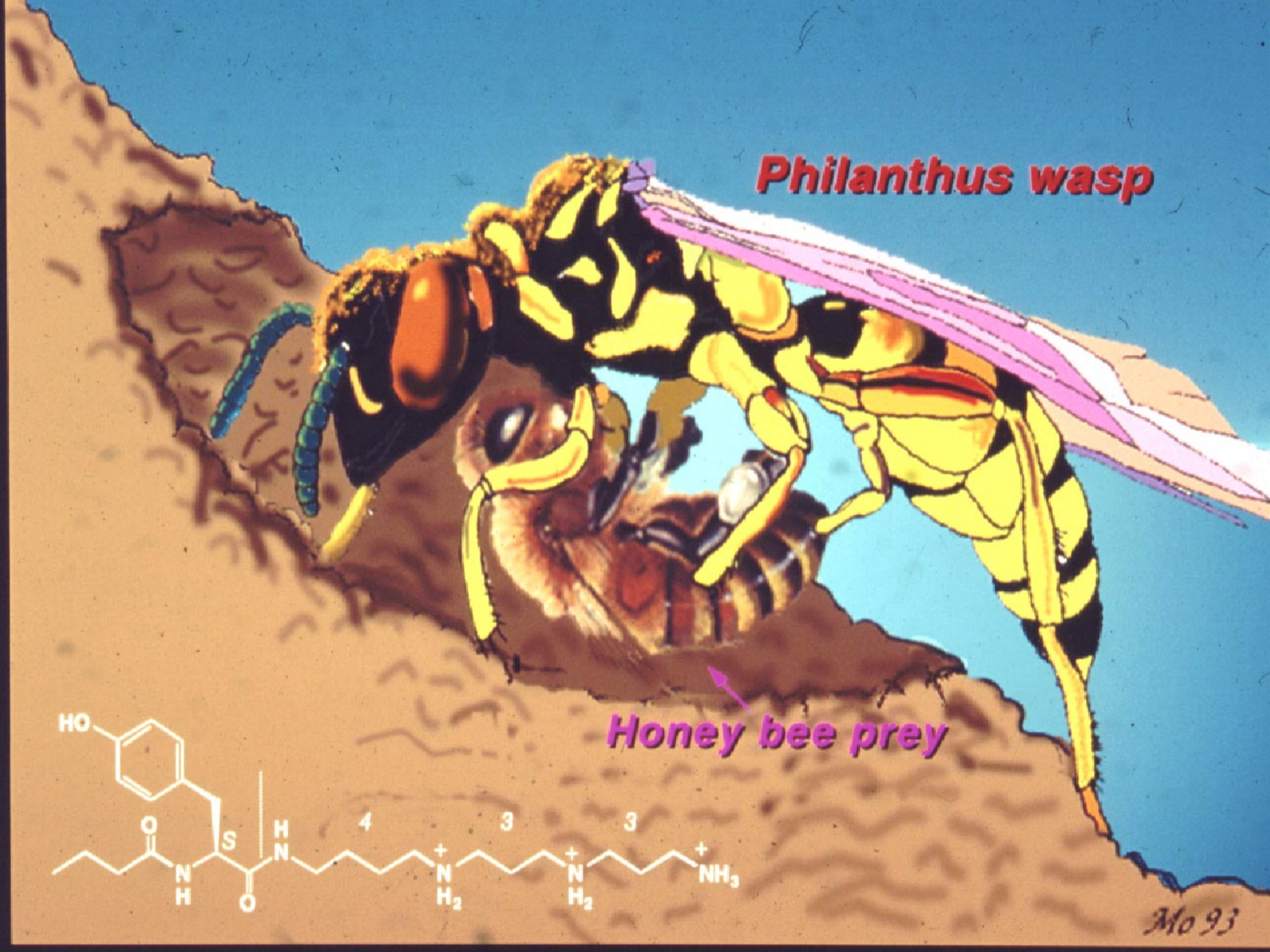


brevetoxin-A

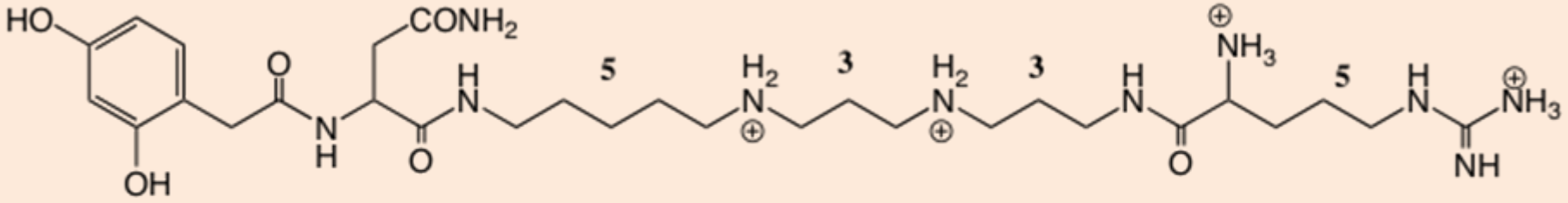
Shimizu et al. (1986)
Pawlak et al. (1987)



Parish, Jiang, Tokiwa, Berova, Nakanishi, McCabe, Zuckerman, Xia, Gabay, *Bioorg. & Med. Chem*. **9** 377 (2001)

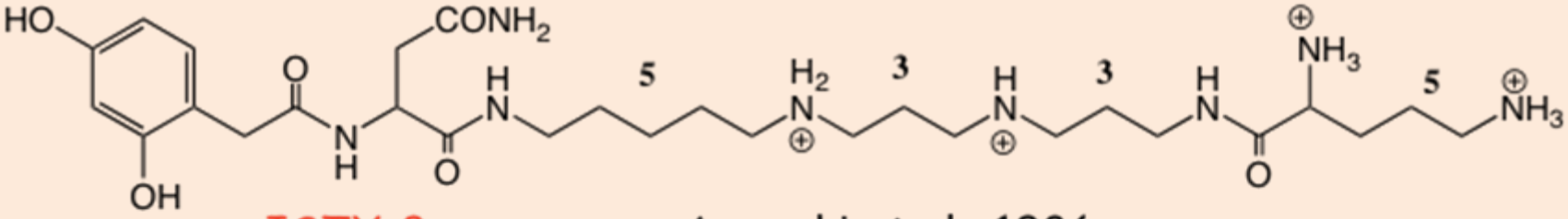

Philanthus wasp
Honey bee prey
HO
O
N
H
S
H
N
O
4
3
3
NH3
Mo 93

Spider toxins



argiopine
argiotoxin 636

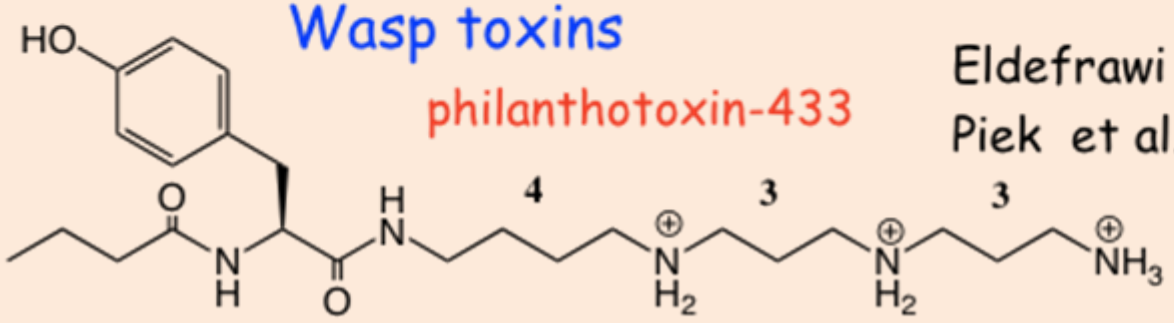
Grishin et al. 1986
Adams et al. 1987



JSTX-3

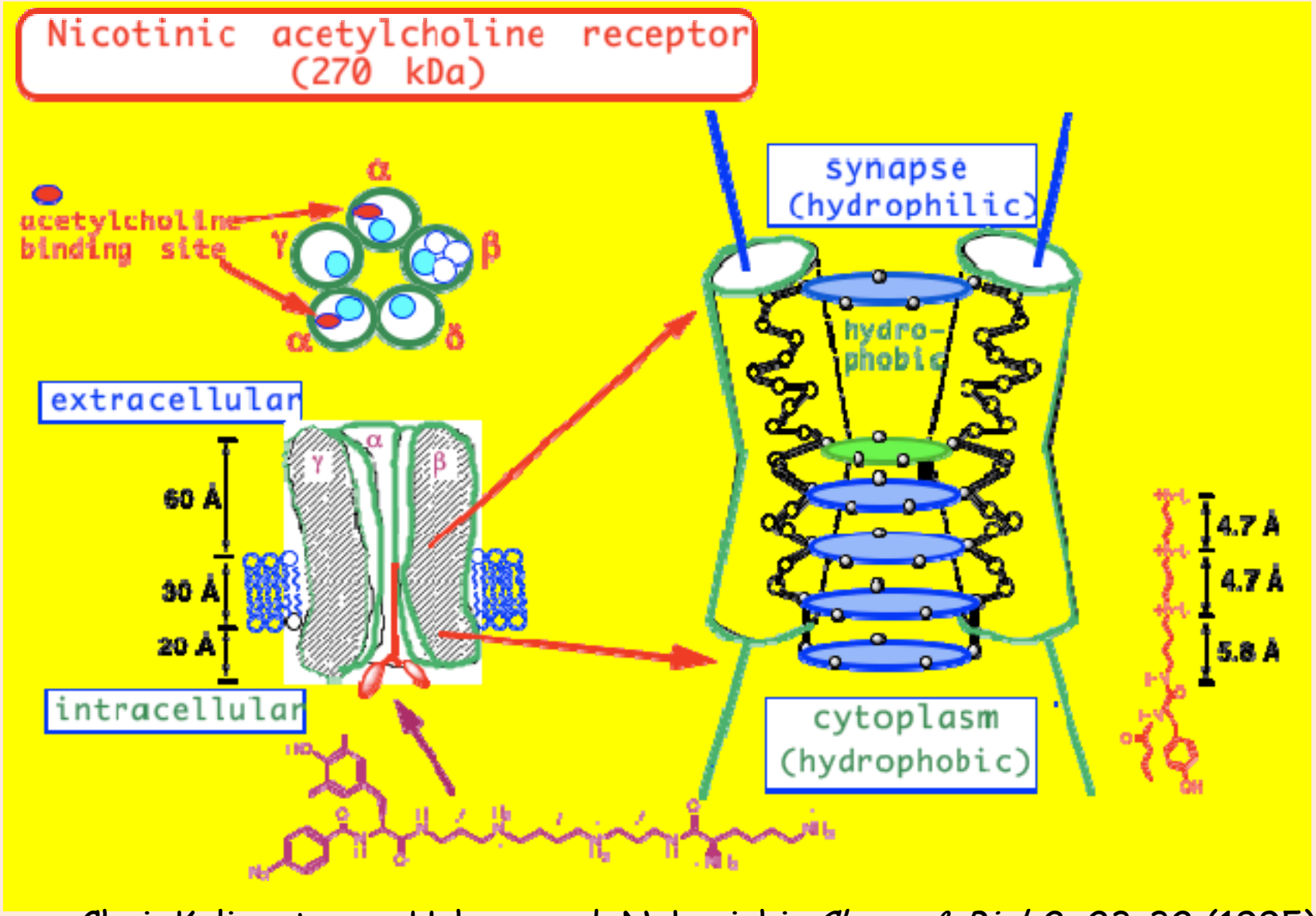
Aramaki et al. 1986

Wasp toxins



philanthotoxin-433

Eldefrawi et al. 1988
Piek et al. 1988

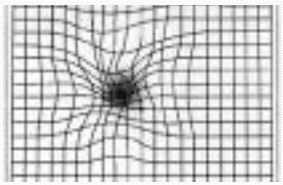


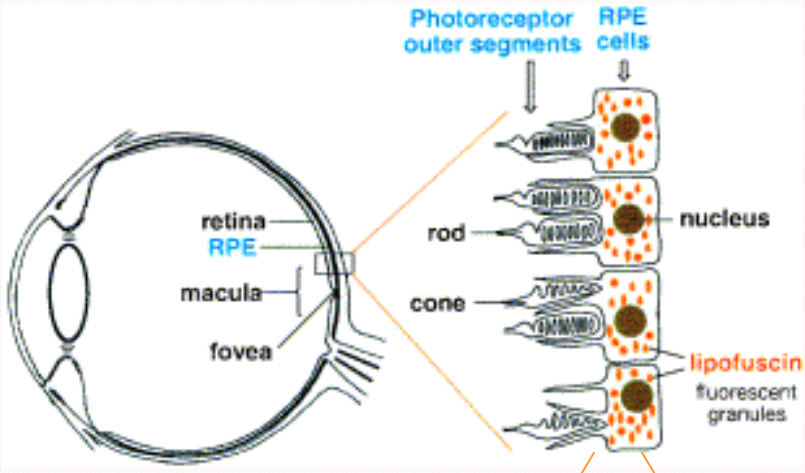
Choi, Kalivretenos, Usherwood, Nakanishi, *Chem. & Biol.*,2, 23-32 (1995).

Age-Related Macular Degeneration (AMD)

- Leading cause of blindness in people over 60
- No cure or treatment
- Progression of atrophic (dry) AMD
- RPE cell atrophy leads to photoreceptor cell death and loss of vision

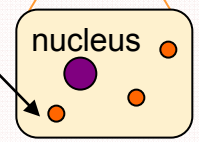
Vision loss with AMD





- RPE: retinal pigment epithelium
  - Critical for vision since it stores and supplies nutrients, e.g., retinol
  - Phagocytoses used outer segment disks

Lipofuscin (fluorescent granules)



RPE cell

Review: Ben-Shabat, Parish, Hashimoto, Liu, Nakanishi, Sparrow, *Bioorg. Med. Chem. Lett*. **2001**, *11*, 1533-1540.

Age-Related Macular Degeneration (AMD)

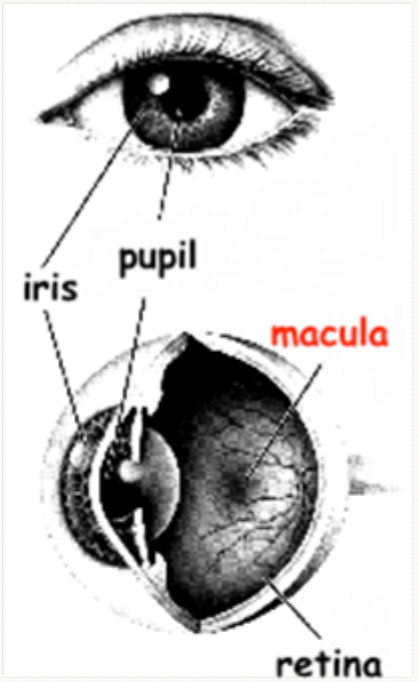
*Macular degeneration is damage or breakdown of macula

*Close work becomes difficult or impossible

*Many elderly people develop AMD as part of natural aging process, eventually leading to untreatable blindness.

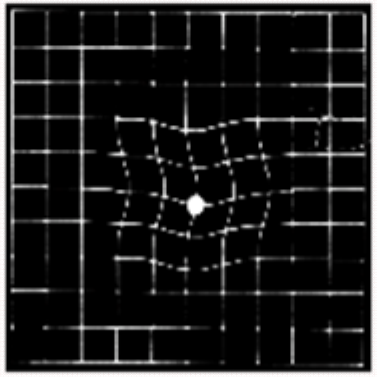
*AMD affects >10 M in U.S.A., and ca. one in every three people over the age of 65.

*There is no remedy for this disease.

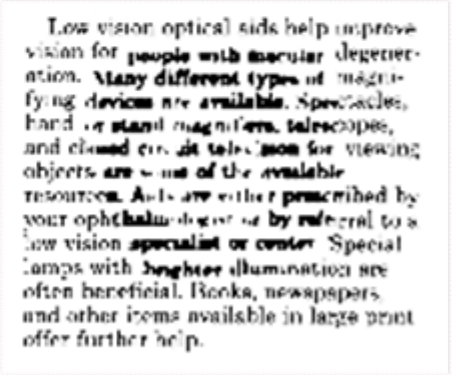


Symptoms associated with AMD

A dark or empty area in the center of sight



Straight lines distorted



Low vision optical aids help improve vision for people with macular degeneration. Many different types of magnifying devices are available. Spectacles, hand or stand magnifiers, telescopes, and closed circuit television for viewing objects are some of the available resources. Aids are either prescribed by your ophthalmologist or by referral to a low vision specialist or center. Special lamps with brighter illumination are often beneficial. Books, newspapers and other items available in large print offer further help.

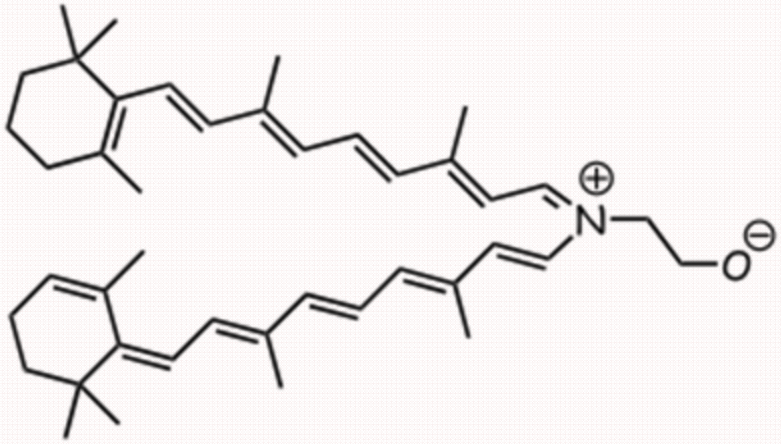
Words look blurred

(from Am. Acad. of Ophthalmology "Keeping an eye on your sight.")

A2E Fluorophore

Human RPE cells
(from > 250 eyes, > 40 yrs. old)

Orange fluorophore < 100 μg



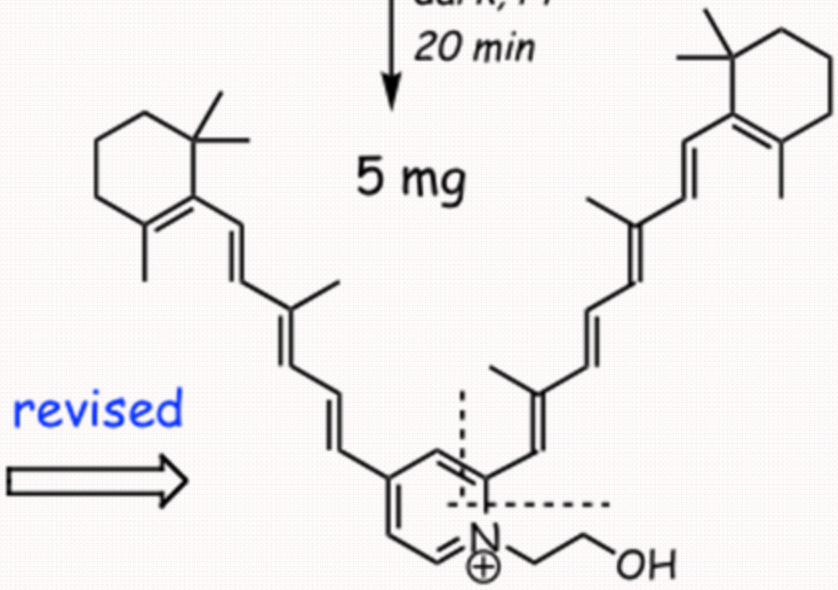
Eldred & Lasky
Nature 361, 724 (1993)

all-trans retinal (1 g),
ethanolamine, pH 5.2 with AcOH

dark, rt
20 min

5 mg

revised



geometrically rigid wedge shape

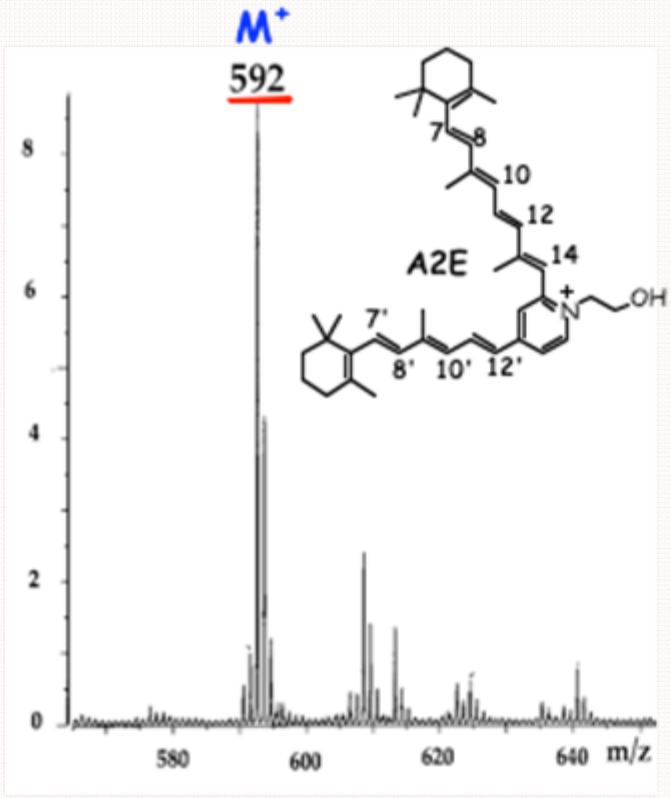
structure: N. Sakai et al. *JACS* 118, 1559 (1996)

synth.: R. Ren et al. *JACS* 119, 3619(1997)

A2E old/new structure

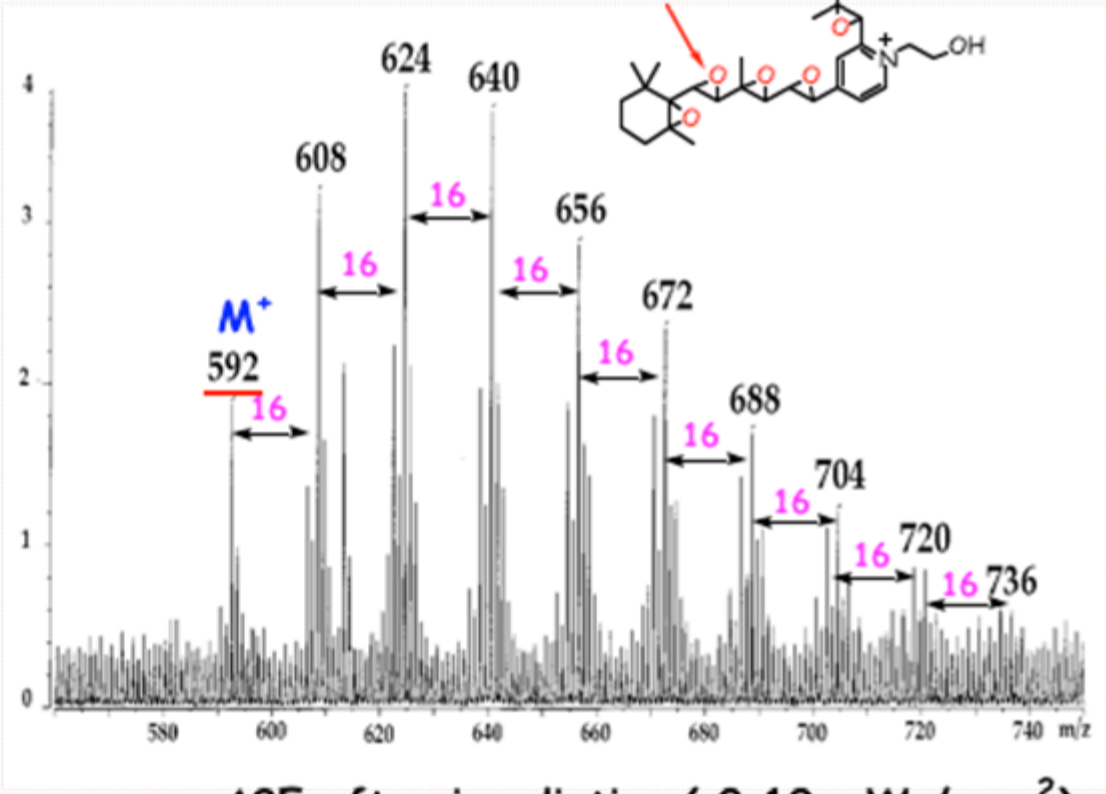
A2E FAB-MS w/wo irr. blue light, 450 nm, 10 min, PBS

JMS HX110A/110A MS/MS
accel. volt. 10 kV
collision cell floating : 8 kV
collision gas : He



A2E control (in dark)

first oxidation

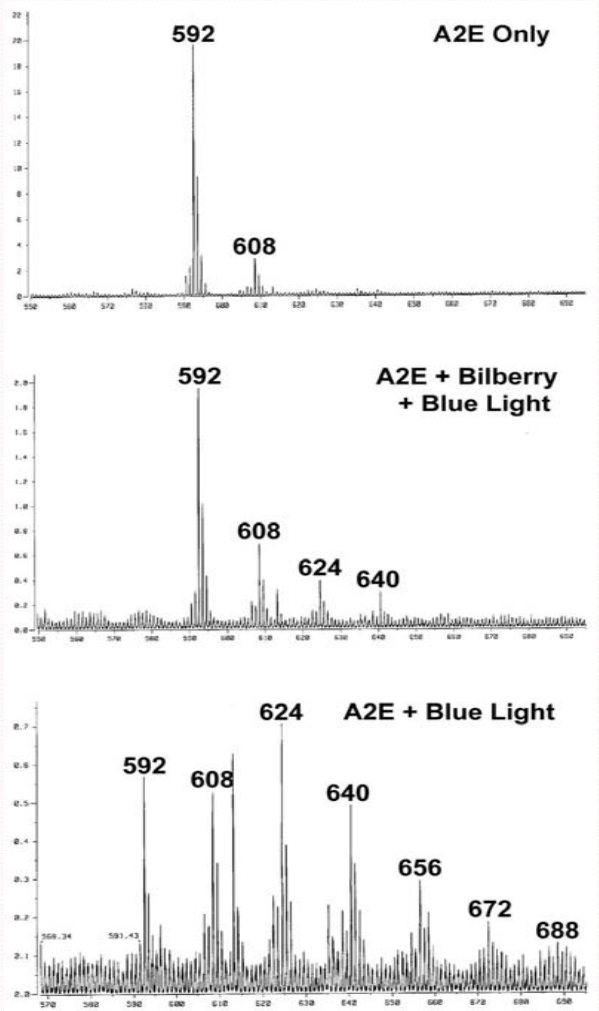


A2E after irradiation (0.19 mW / mm^2)

Ben-Shabat, Itagaki, Jockusch, Sparrow, Turro, Nakanishi, Angew. Chem.,41, 814 (2002)

Bilberry extract vs. blue-light induced A2E epoxidation

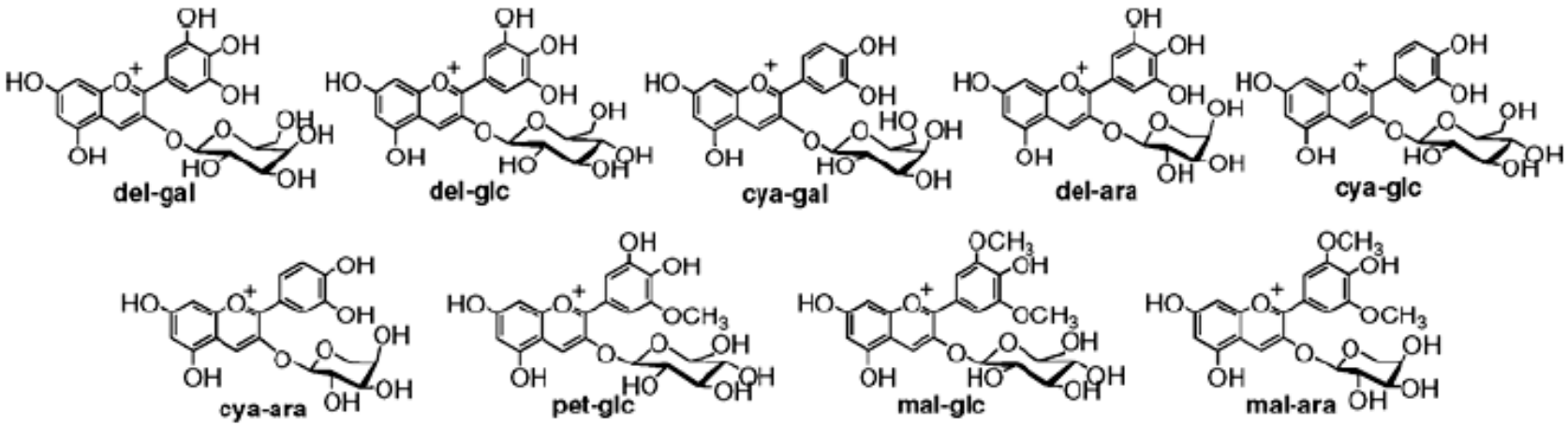
- Common name : bilberry, blueberry
- Northern Europe, western Asia, and western North America
- Edible fruits with history of medicinal usage
- Clinical application
  - Ophthalmologic disorders
  - Vascular disorders
  - Diabetes mellitus

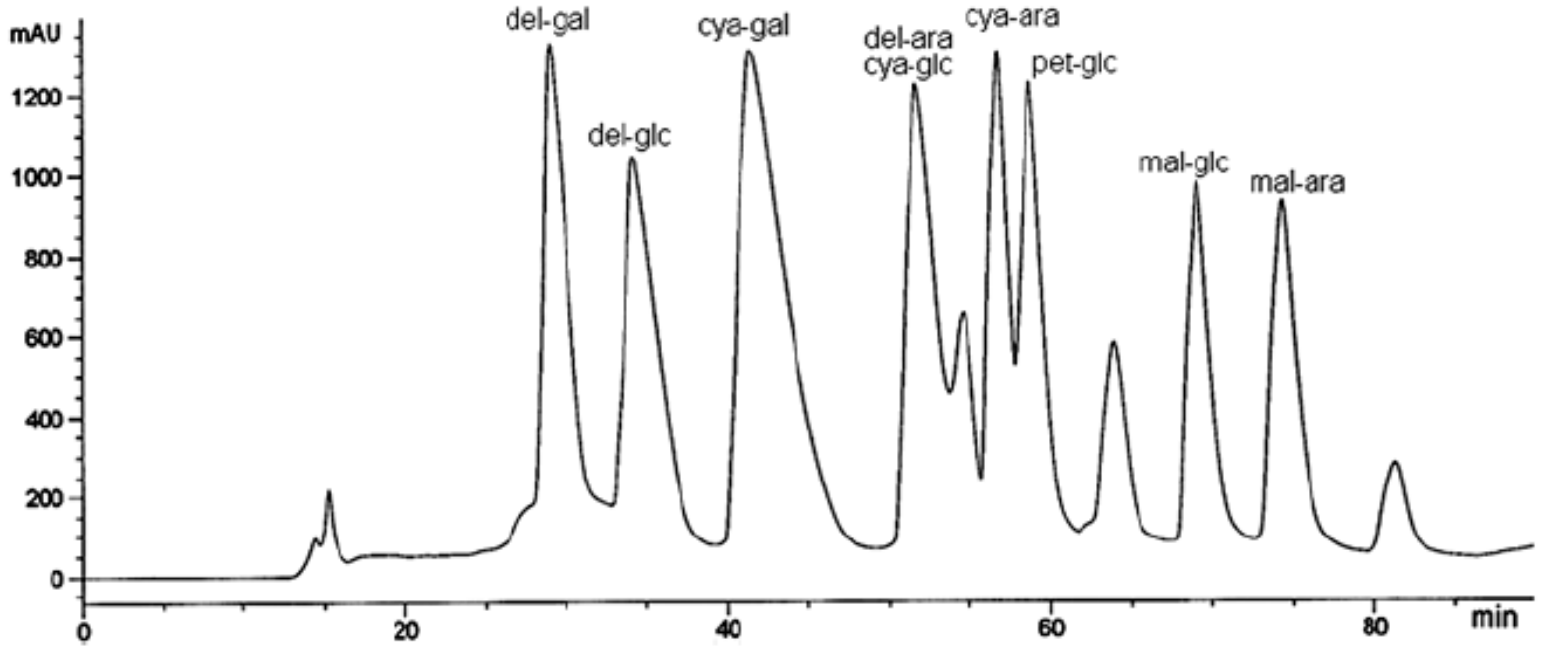


- A2E in PBS; Tungsten halogen source (470±20nm; 0.4mW/mm^2); 8 min exposure
- FAB-MS (3-Nitrobenzyl alcohol as matrix)

Y. Jang *et al., Photochem. Photobiol.* 81, 529 (2005)

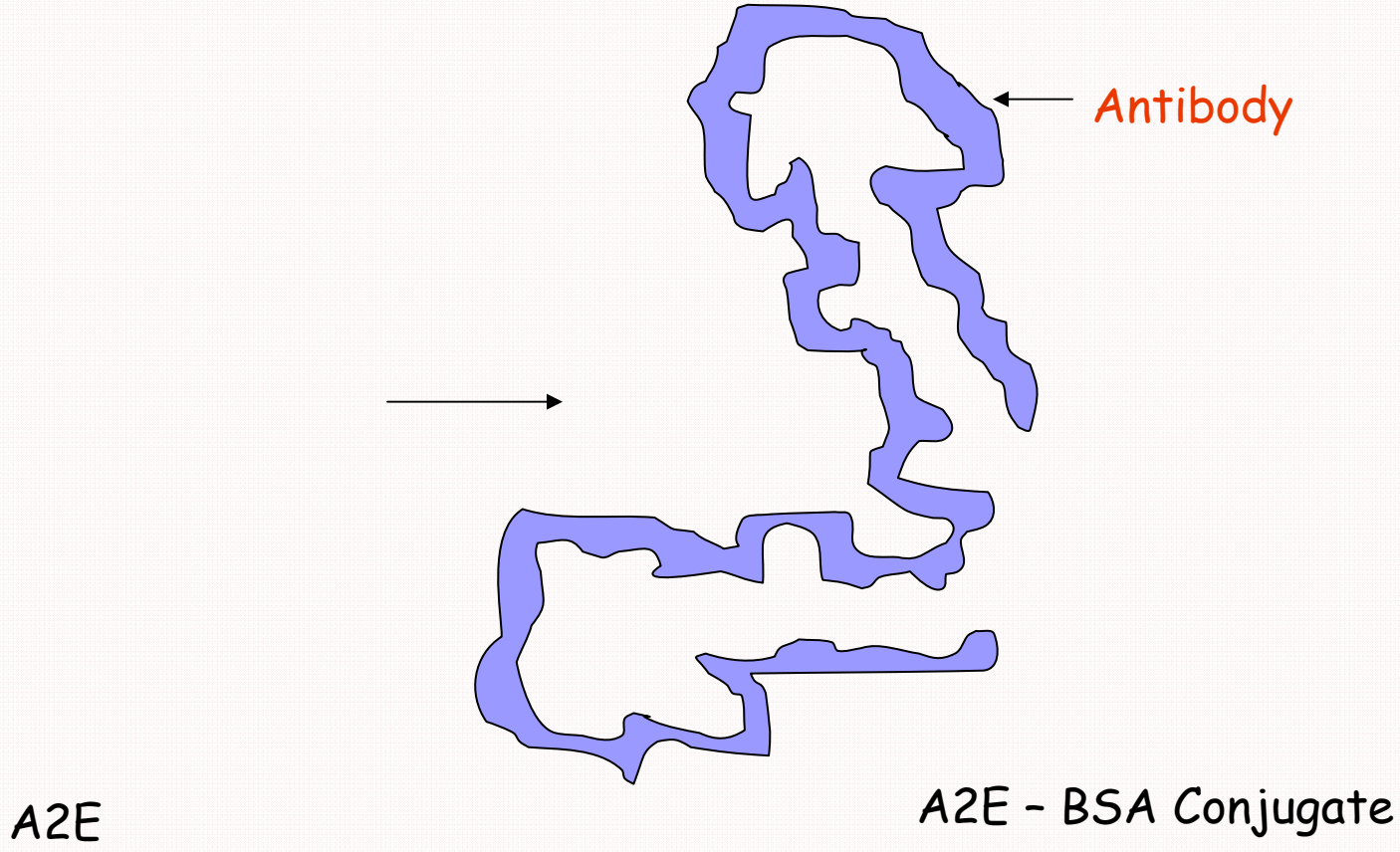
Anthocyanins from bilberry extract





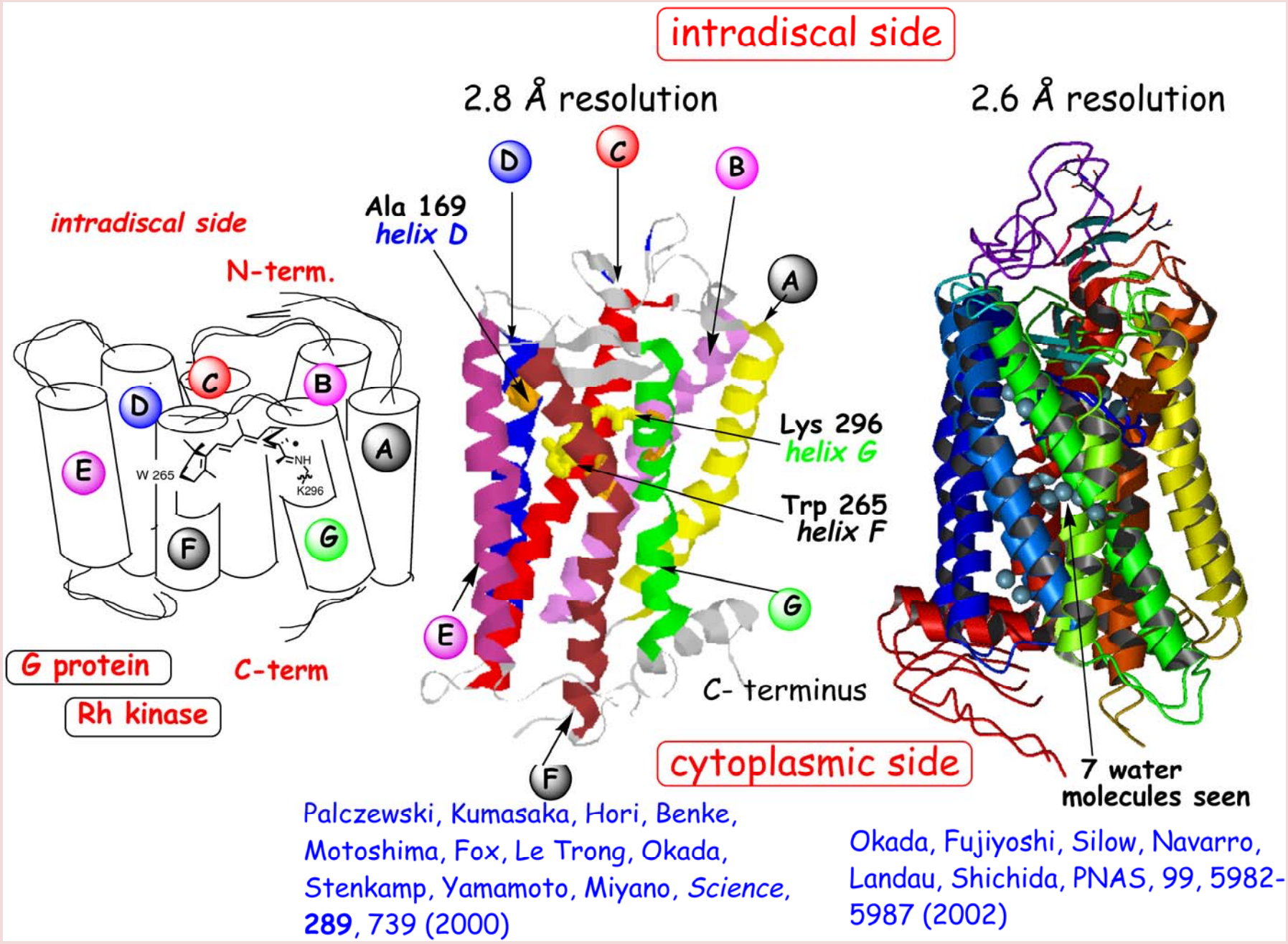
C18 5µm, 22 X 250 mm, 5% Formic acid:CH_3CN gradient mode

Young Jang *et al.*, *Photochem. Photobiol.* 81, 531 (2005)



Antibody generation against two polyene moieties of A2E

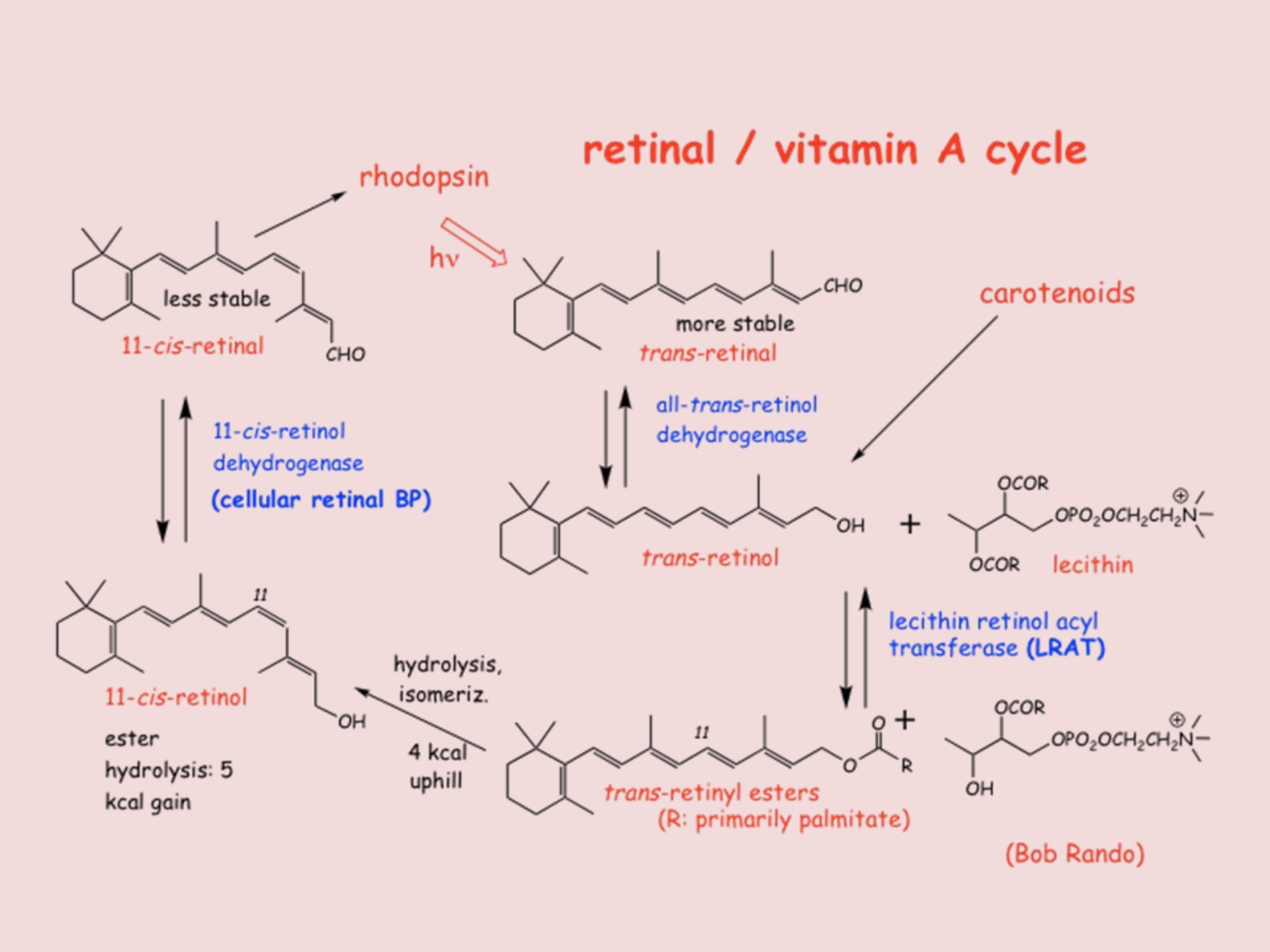
Abeywickrama *et al.*, *PNAS,* 2007, *104*, 14610-14615



Palczewski, Kumasaka, Hori, Benke, Motoshima, Fox, Le Trong, Okada, Stenkamp, Yamamoto, Miyano, *Science*, **289**, 739 (2000)

Okada, Fujiyoshi, Silow, Navarro, Landau, Shichida, PNAS, 99, 5982-5987 (2002)

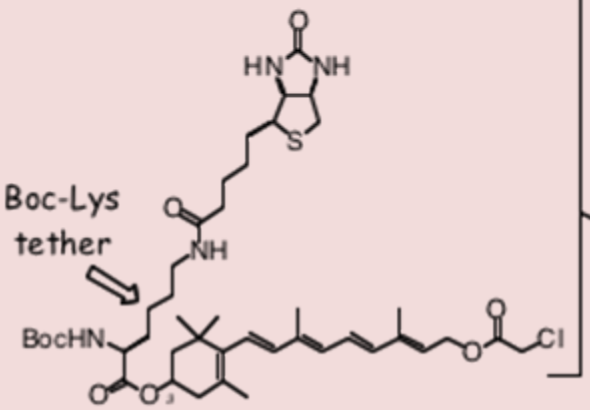
retinal / vitamin A cycle



(Bob Rando)

Selective capture of retinoid binding proteins

retinal pigment
epithelium membrane



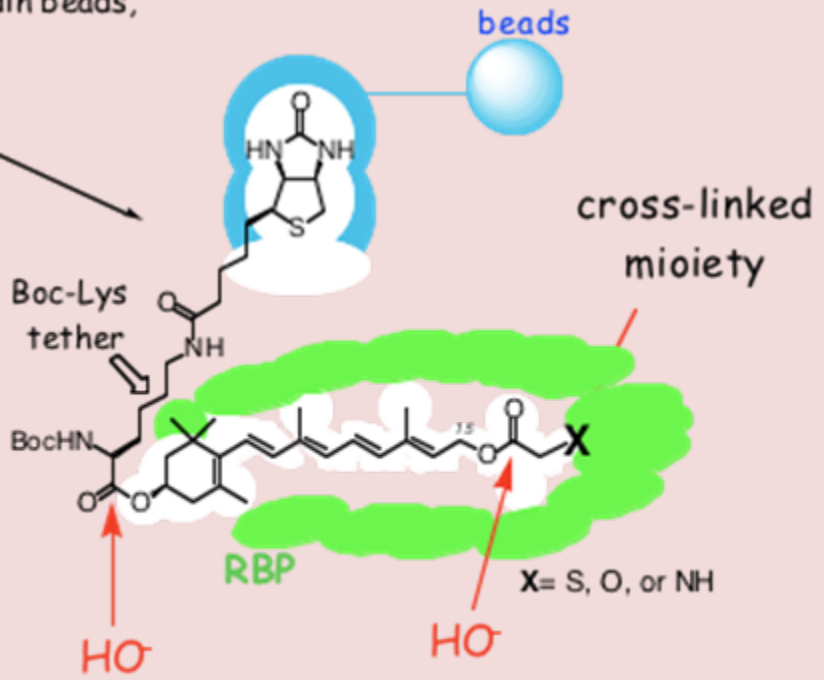
biotinylated trans-retinyl chloroacetate

[Nesnas, Rando, Nakanishi, *Tetrahedron*, **2002**, *58*, 6577]

(i) incubation, 12 h, rt

(ii) dialysis

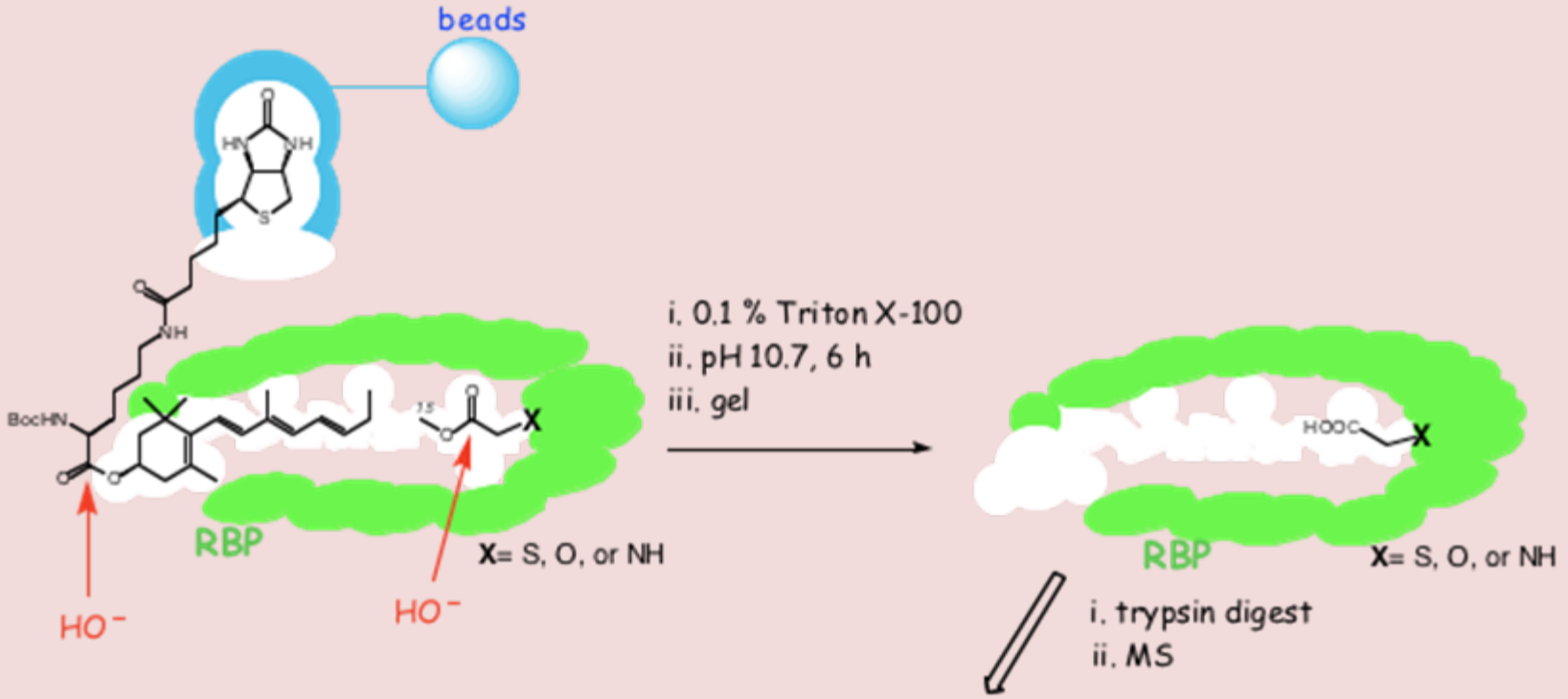
(iii) Neutravidin beads,
3 h, 4°C



labeling blocked by *trans*-retinyl
bromoacetate but not by oleyl acetate

Jahng ,David, Nesnas, Nakanishi, Rando, *Biochem.*, **42**, 6159 (2003)

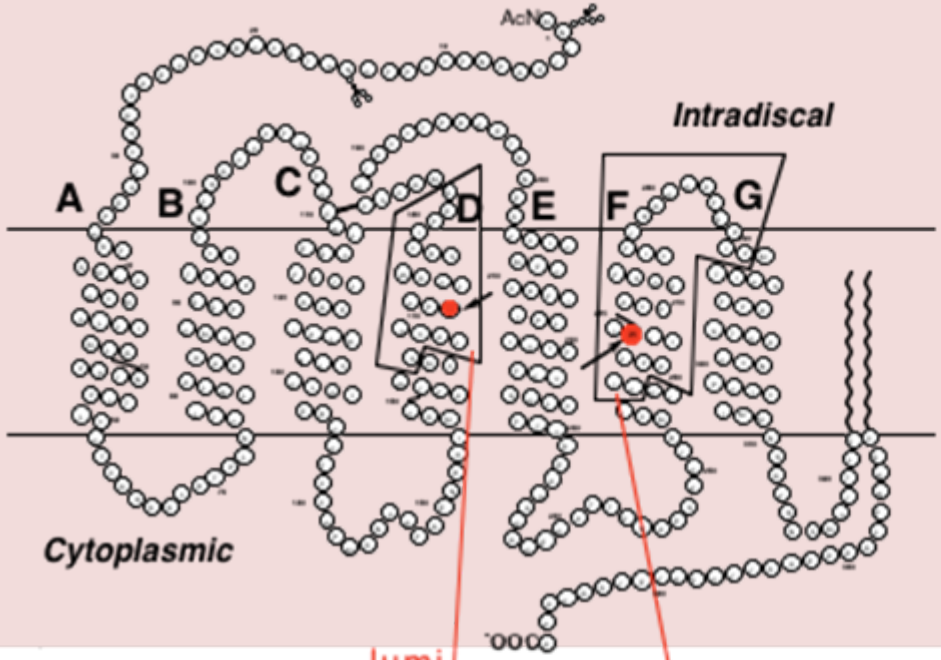
Selective capture of proteins from bovine RPE

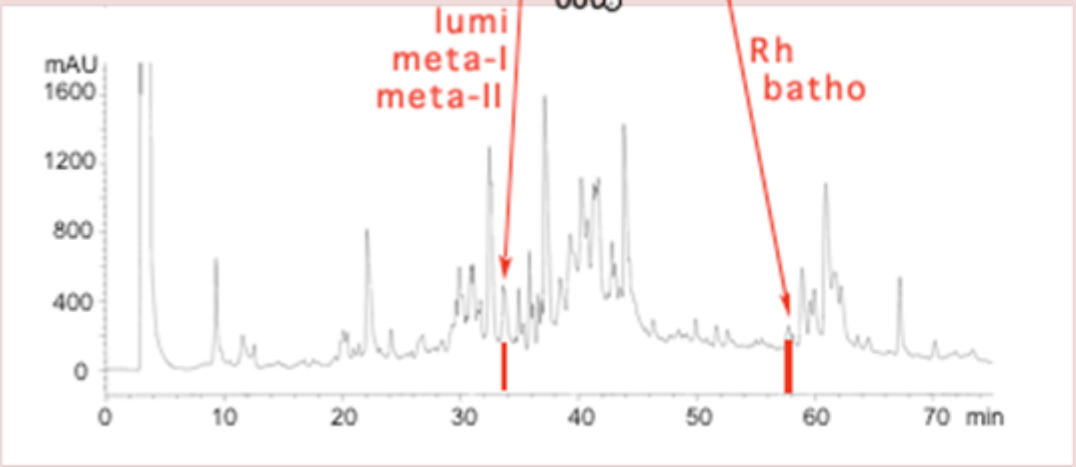


selective capture of only few proteins from a mixture of perhaps thousands

- 25 kDa LRAT: lecithin retinal acyltransferase
- 31 kDa RGR: retinal G protein coupled R (11-*cis*-retinol dehydrogenase)
- 63 kDa RBP

Jahng, David, Nesnas, Nakanishi, Rando, *Biochem.*, **42**, 6159 (2003)



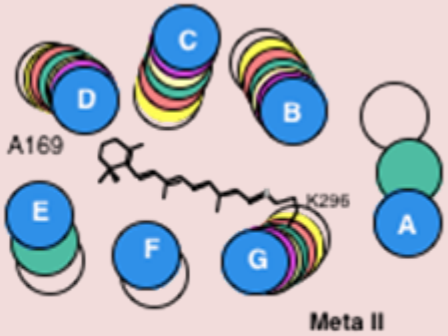


Borhan, Souto, Imai, Shichida, Nakanishi, *Sience* **288**, 2209 (2000)

Rh (dark) & batho (-196°)

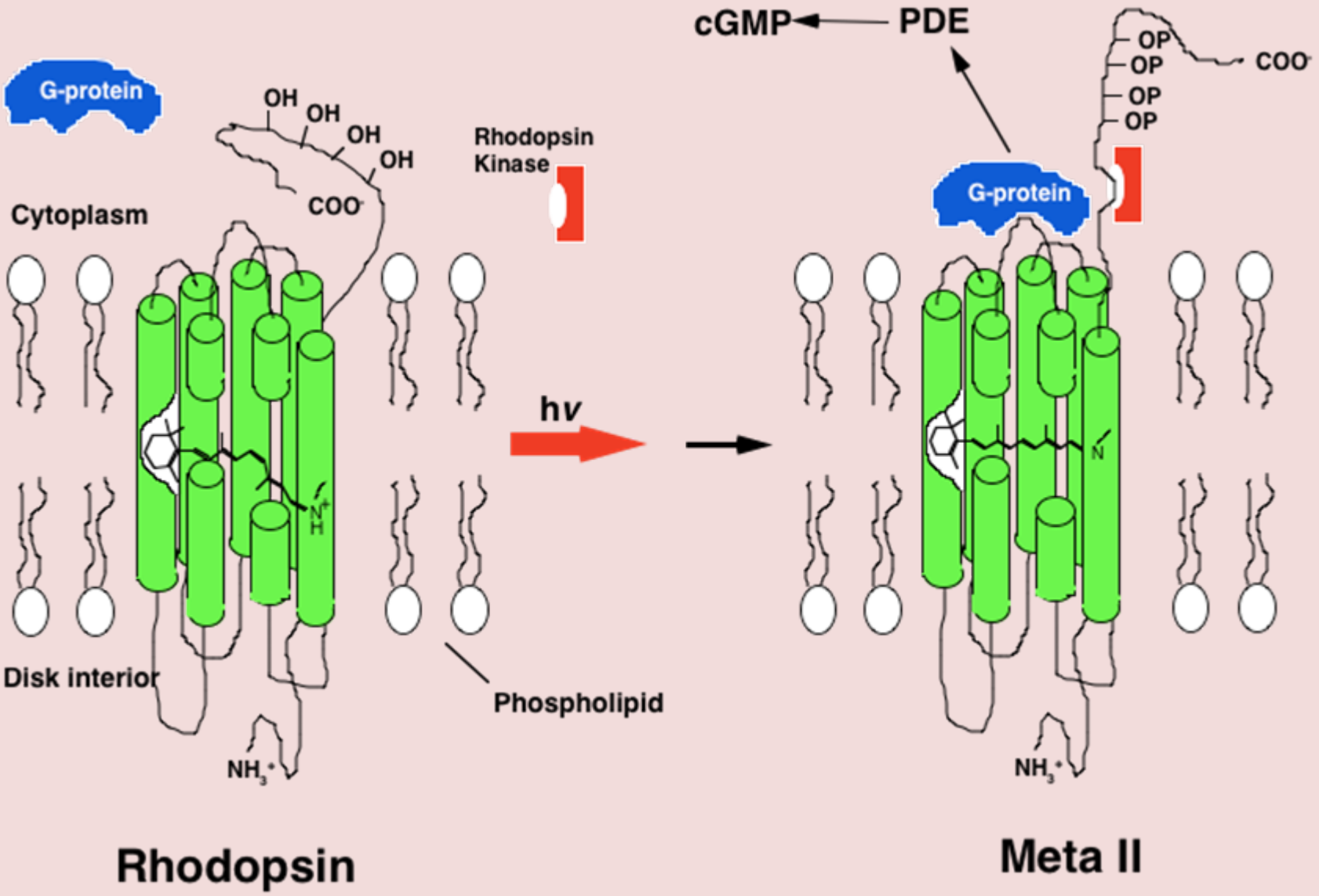
CN-13 (Val258 - Phe287)

hυ

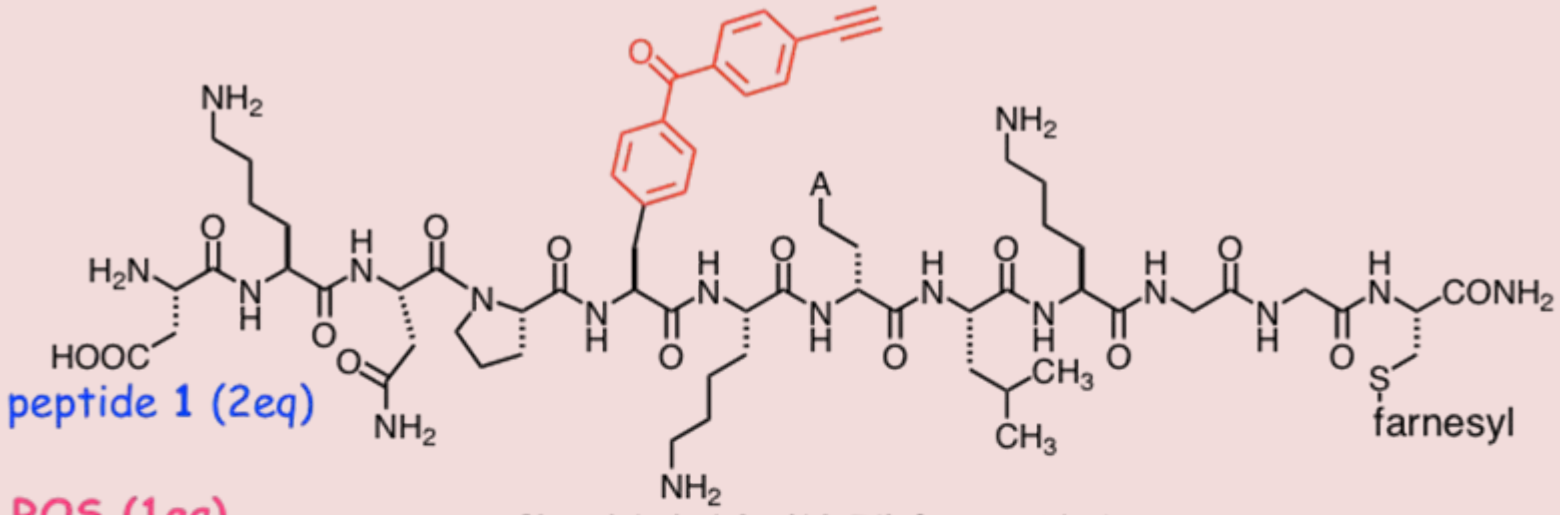


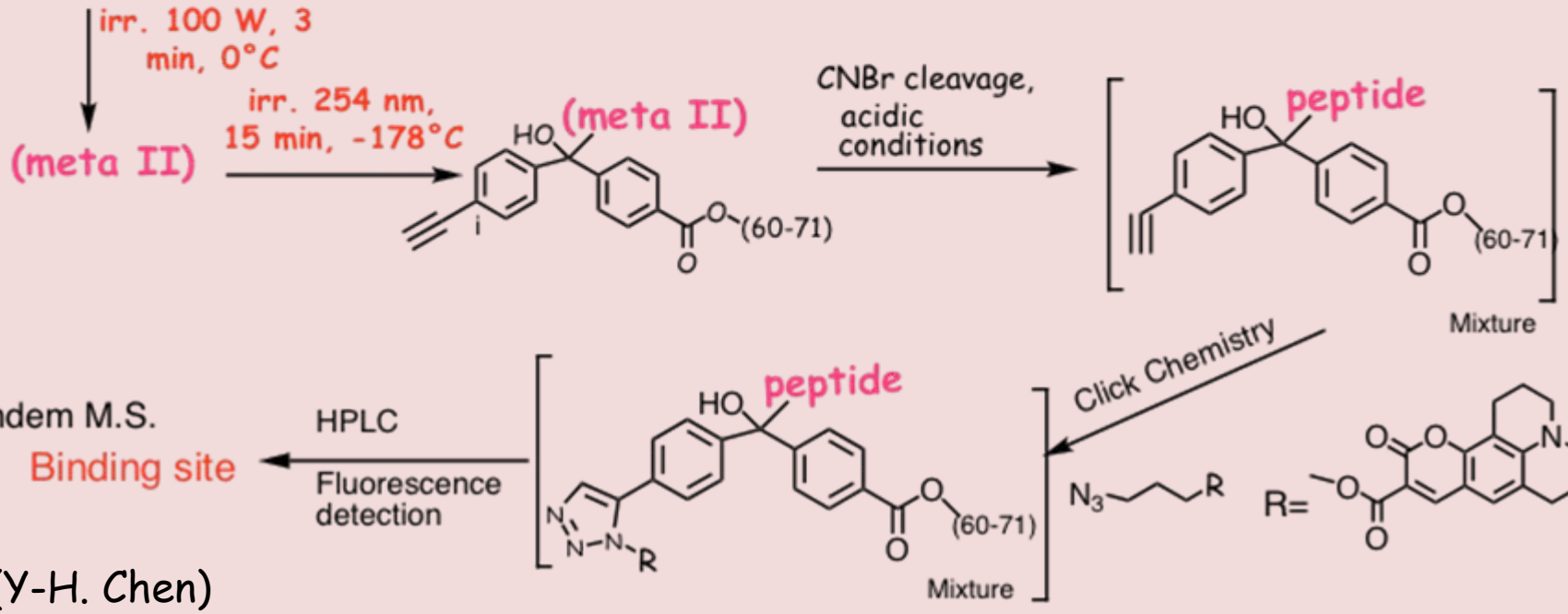
lumi (-80°), meta-I (-40°) & meta-II (0°)

CN-9 (Ala164 - Gly182)


cGMP
PDE
G-protein
OH
OH
OH
OH
COO
Rhodopsin
Kinase
Cytoplasm
OP
OP
OP
OP
COO
G-protein
hν
N
H
Disk interior
Phospholipid
NH3
NH3
Rhodopsin
Meta II

Rh / G protein cross-linking

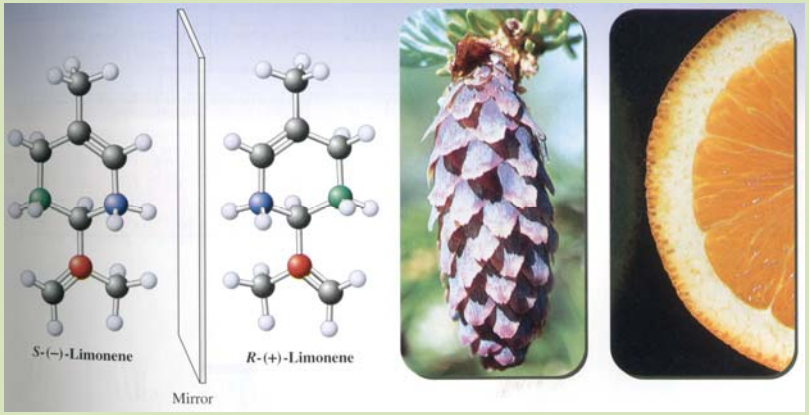




(Y-H. Chen)

Does bee vision differentiate two enantiomers of flower pigment?

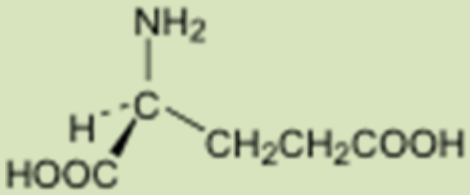
olfactory



(*S*)-Limonene (*R*)-Limonene

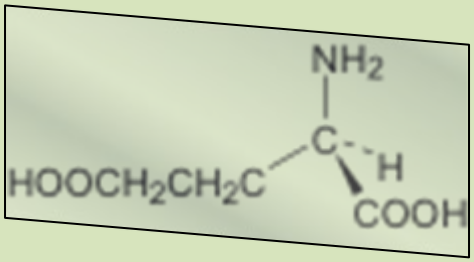
turpentine orange

taste



L-glutamate

umami
(the fifth taste sensation)



D-glutamate

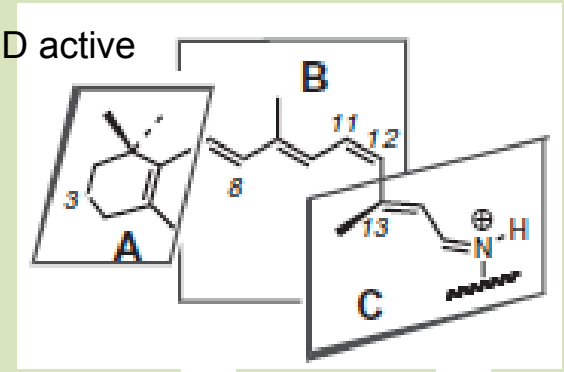
tasteless

Can vision recognize chirality?

Light vs. Rodopsin

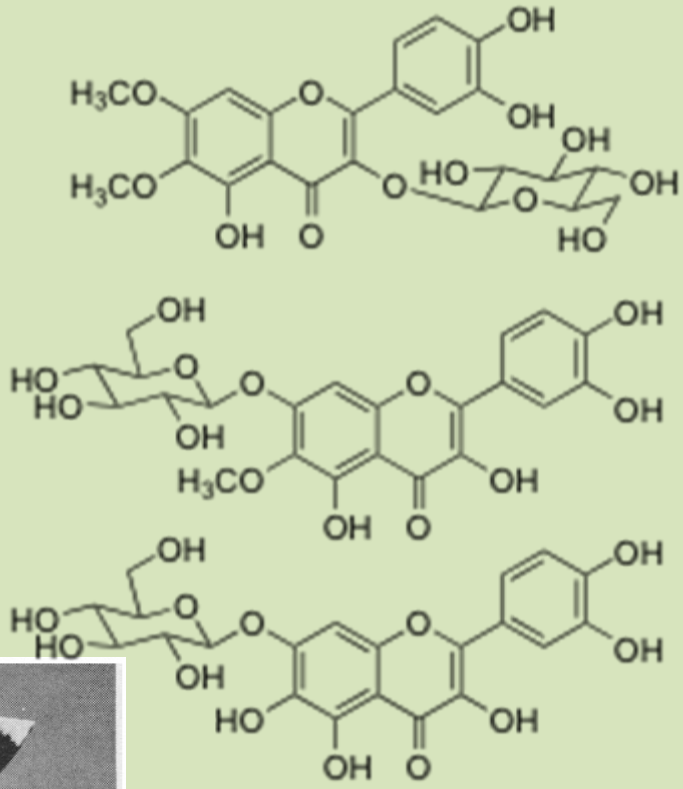
Fishkin, Berov a, Nakanishi
The Chemical Record, 4, 120 (2004)

Retinal is in chiral environment, thus CD active



Flavonol Glycoside Pigments are responsible for Ultraviolet Absorption in Nectar Guide of Flower

Rudbeckia hirta (black-eyed susan)



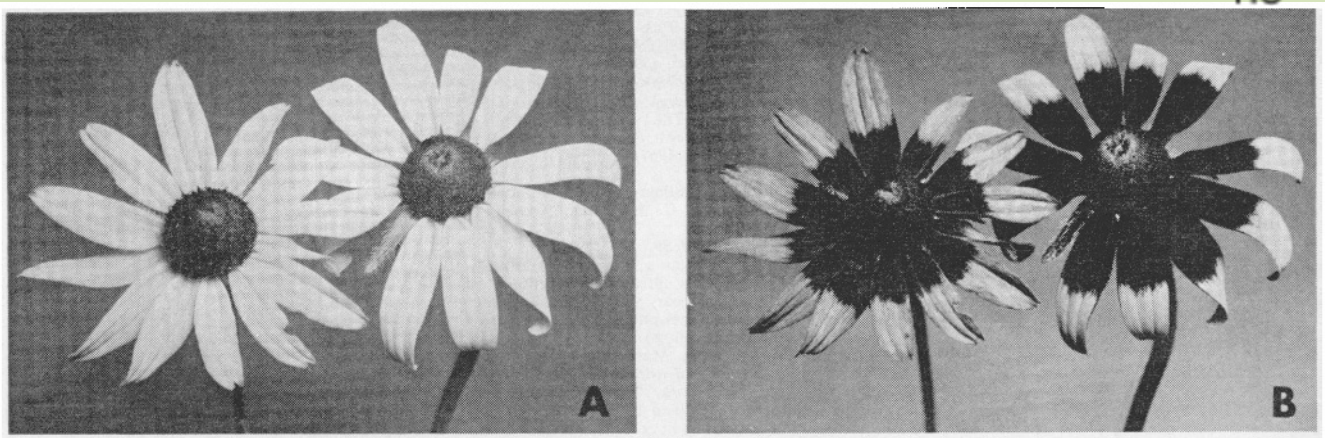


Fig. 1. (A) *Rudbeckia hirta*, photographed in visible light. (B) Same, photographed with ultraviolet transmitting lens and filter, in ultraviolet light. The absorbent basal portions of the petals are the "nectar guides."

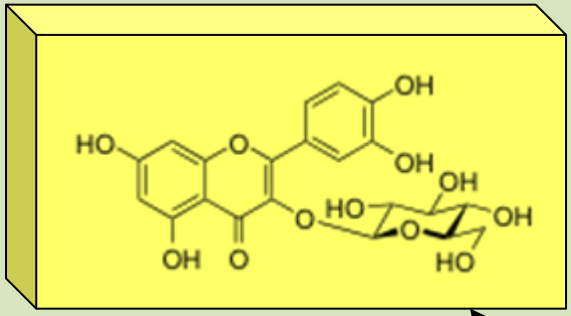
*Science***1972**, *177*, 528-530.

*PNAS***2001**, *24*, 13745-13750.

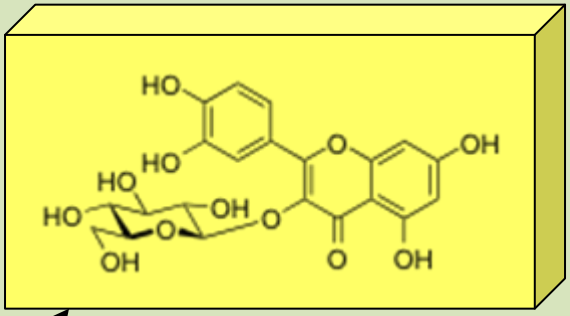
Can vision recognize chirality?

-Biological Assays using bees-

Quercetin D-glucoside

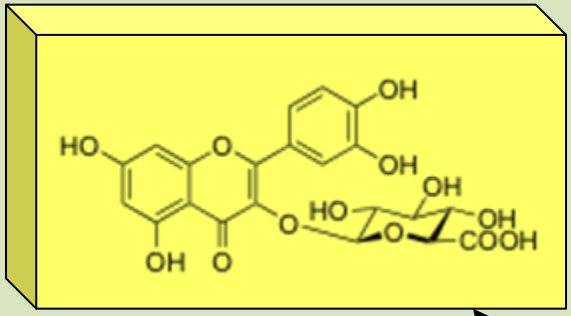


Quercetin L-glucoside

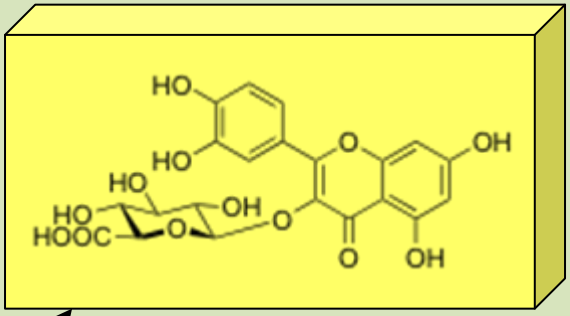


? ?

Quercetin D-glucuronide



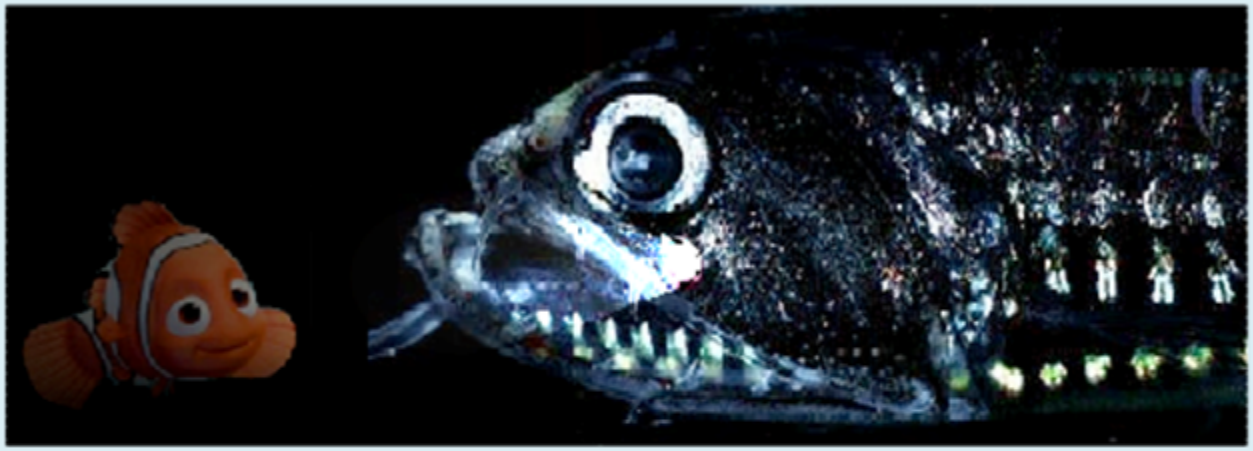
Quercetin L-glucuronide

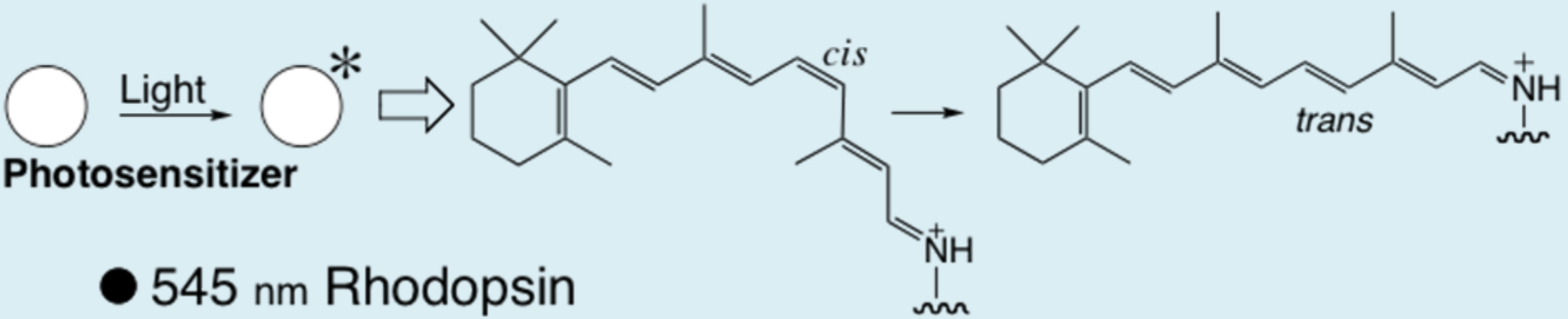


? ?

Tohru Taniguchi

Loose Jaw Fish and Photosensitizers

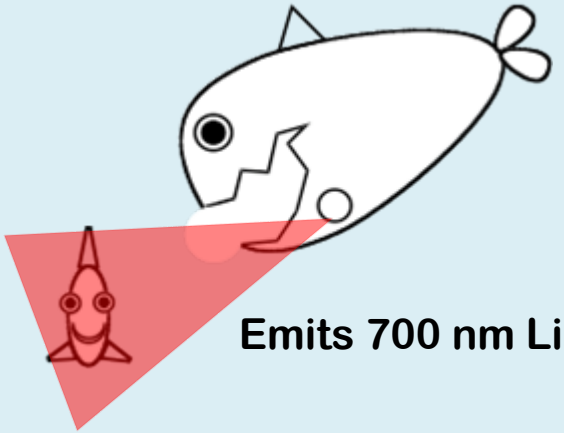




- 545 nm Rhodopsin
- Chlorophyll derivatives as sensitizers
- Bleaching at 671 nm faster than at 554 nm

Photosensitizers for Vision

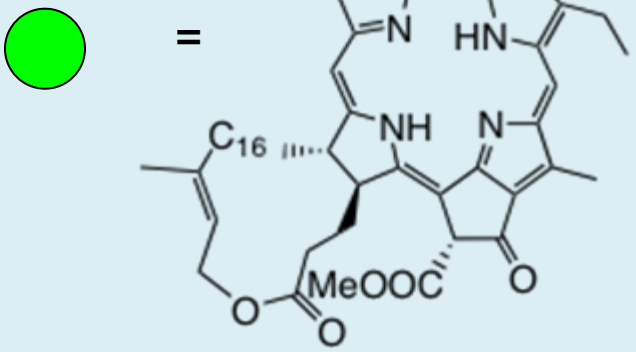
Loose Jaw Fish



Emits 700 nm Light

But Only Contains a 545 nm Rhodopsin

Phaeophytin b

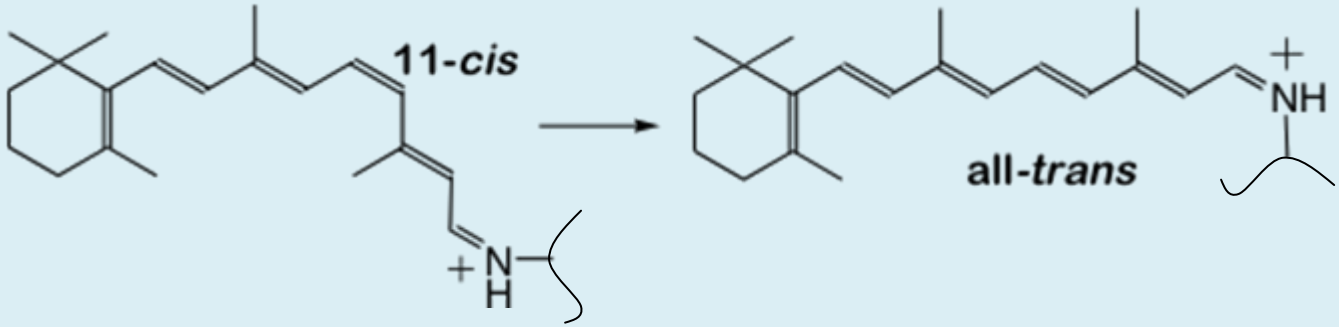


Absorbs 680 nm

671 nm

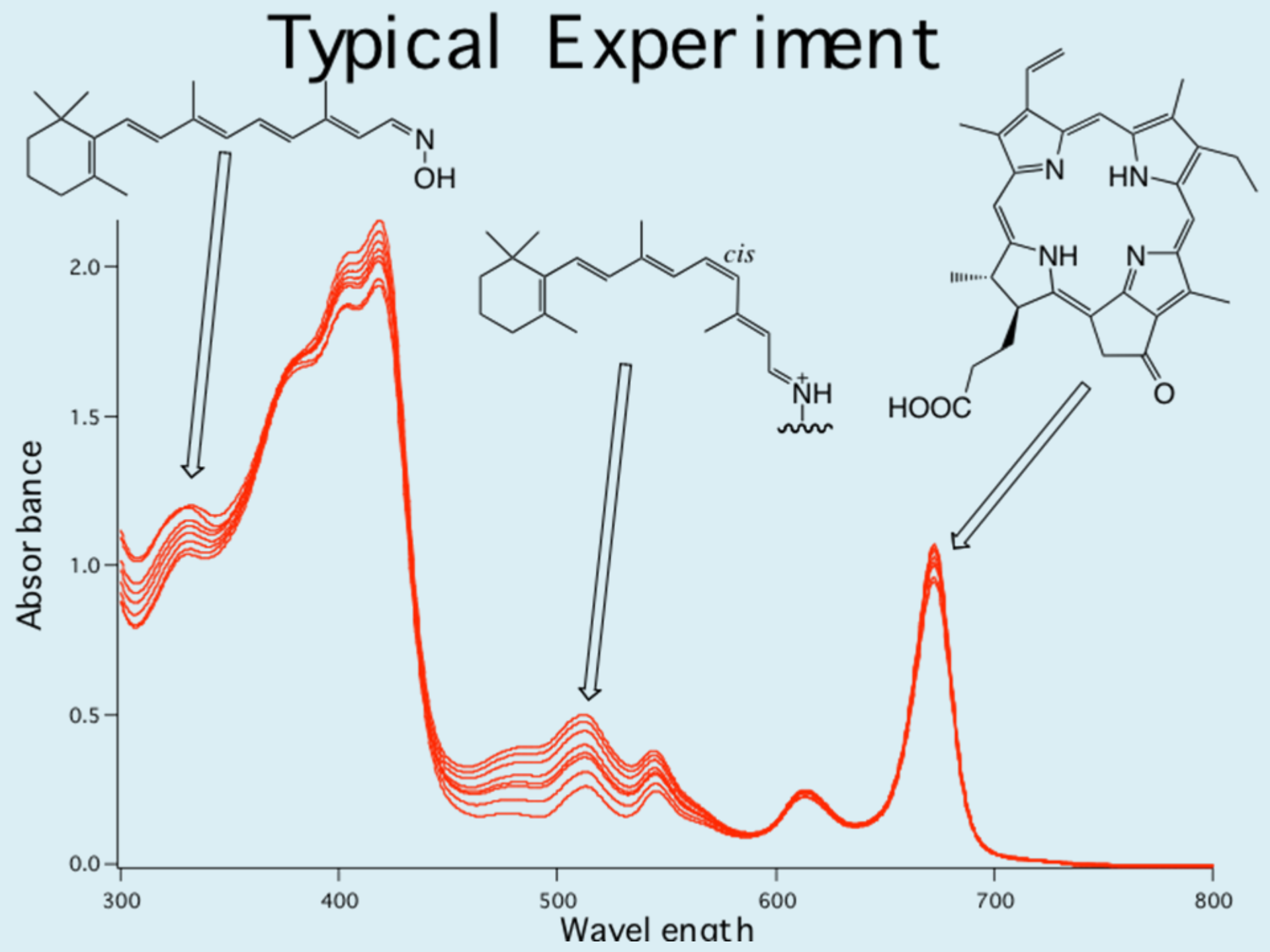
Faster !

554 nm



Douglas, Partridge, Dulai, Hunt, Mullineaux, Tauber, Hynninen, Nature 1998, 423.

Typical Experiment



Studying Nature and learning from Nature

* Nature is efficient and sophisticated
* Intriguing problems lie in borderline areas
* Interdisciplinary approach is essential
* Such approaches have become feasible only recently
* As questions are answered, further enigmas emerge
* Broad and imaginative thinking is essential