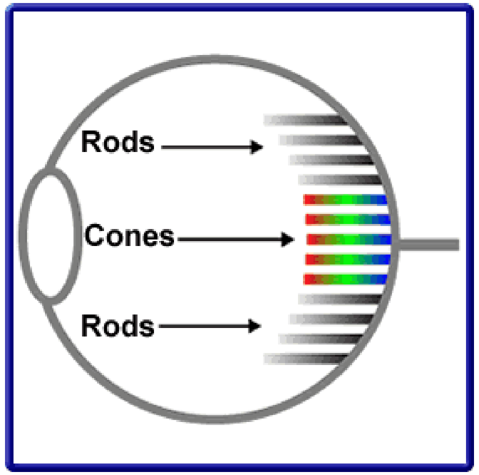
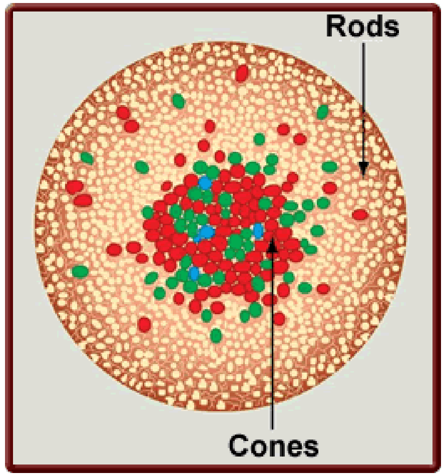
Detail mechanism of the visual process

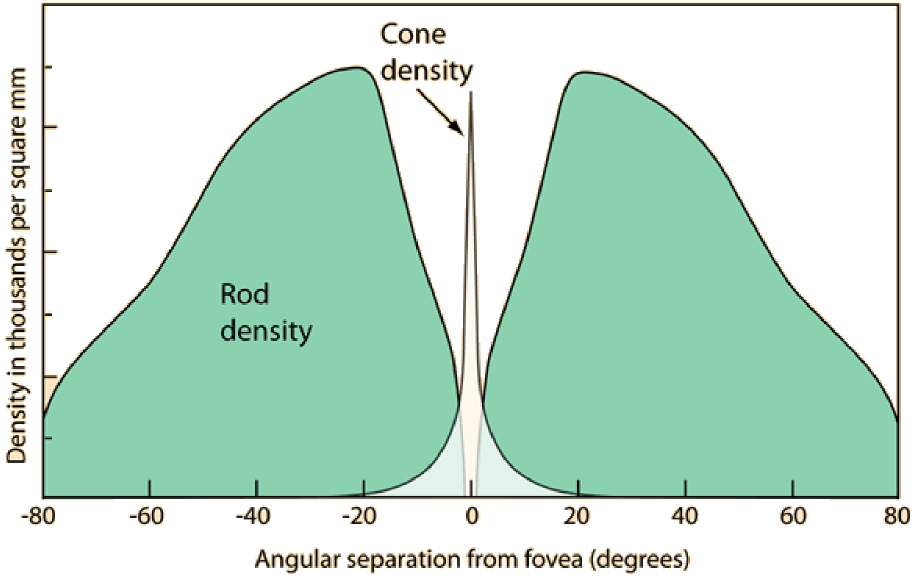
Peter M. Rentzepis

Light receptors



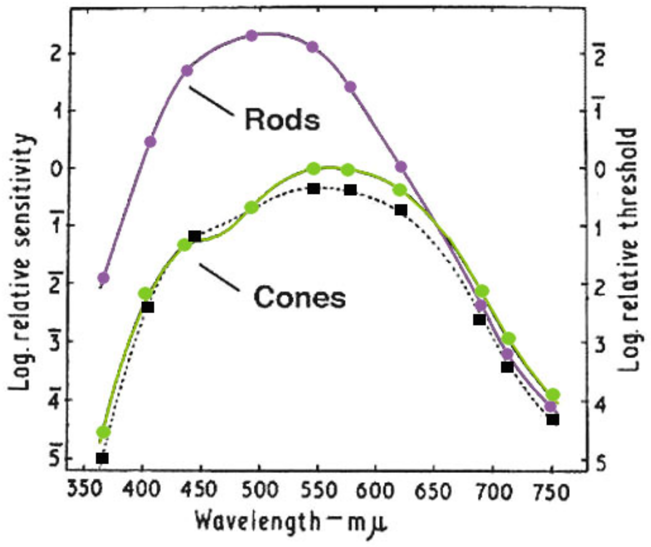


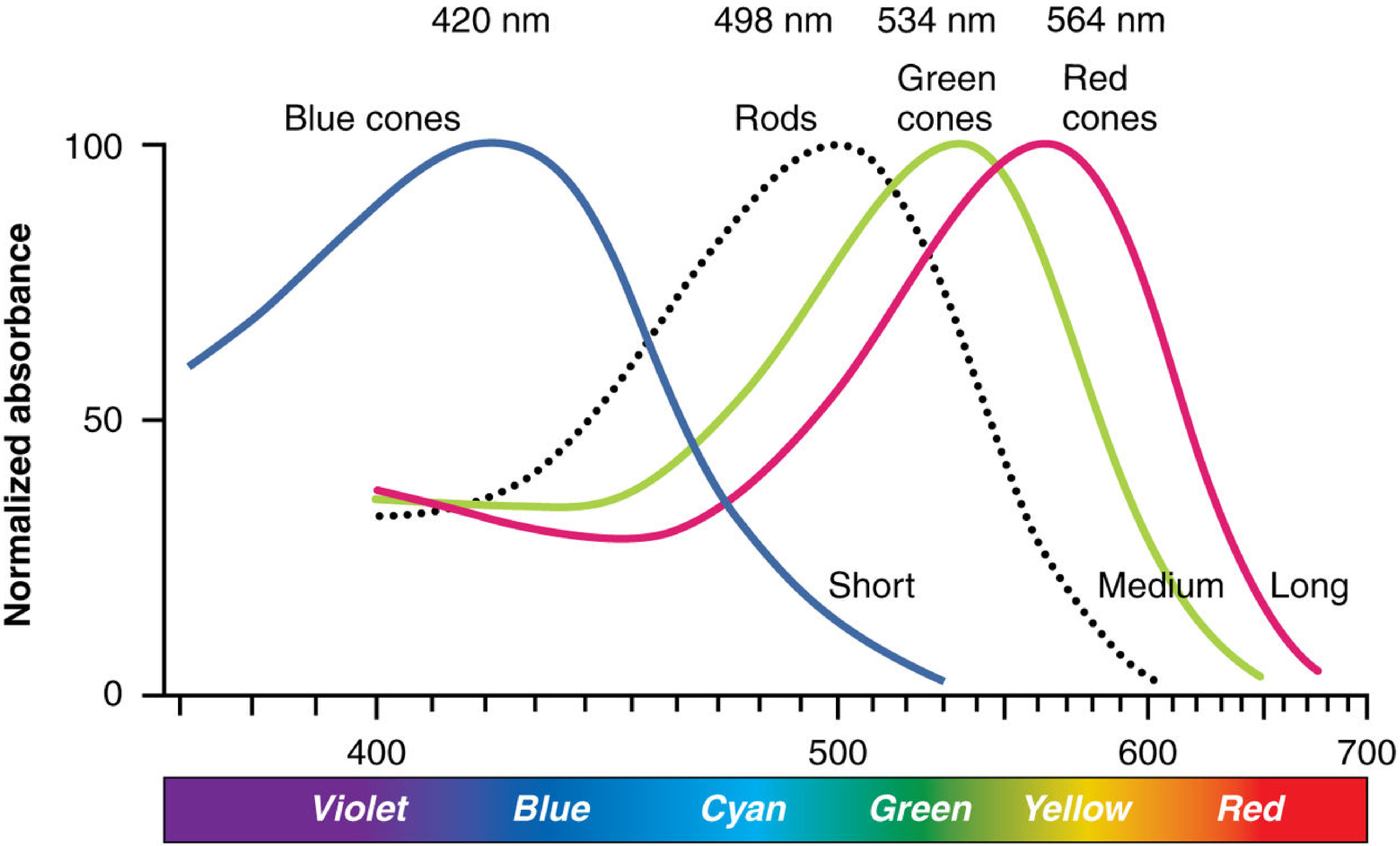
In retina 100 million rods and 7 million cones.



Scotopic, Rods)

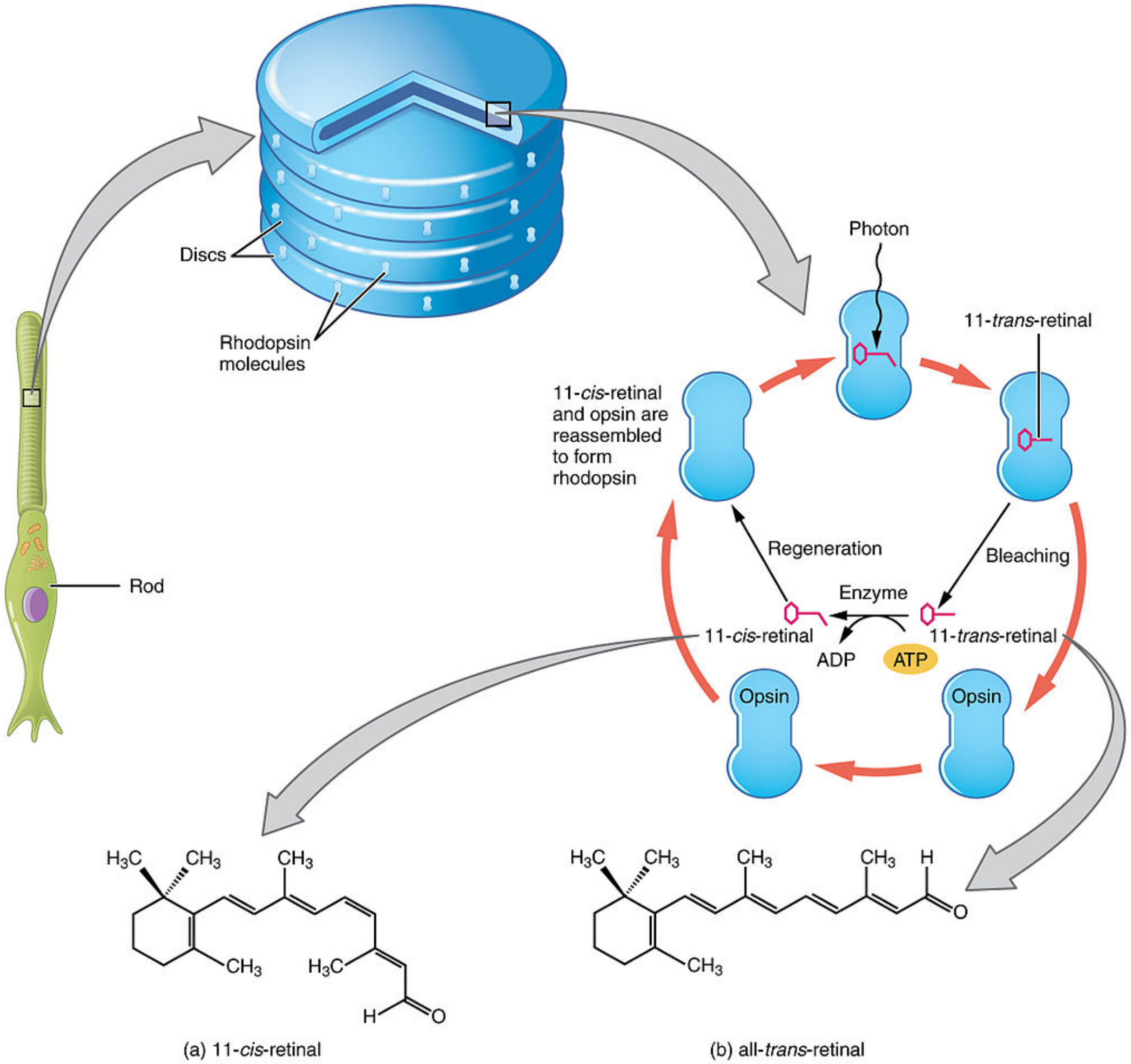
Photopic, Cones

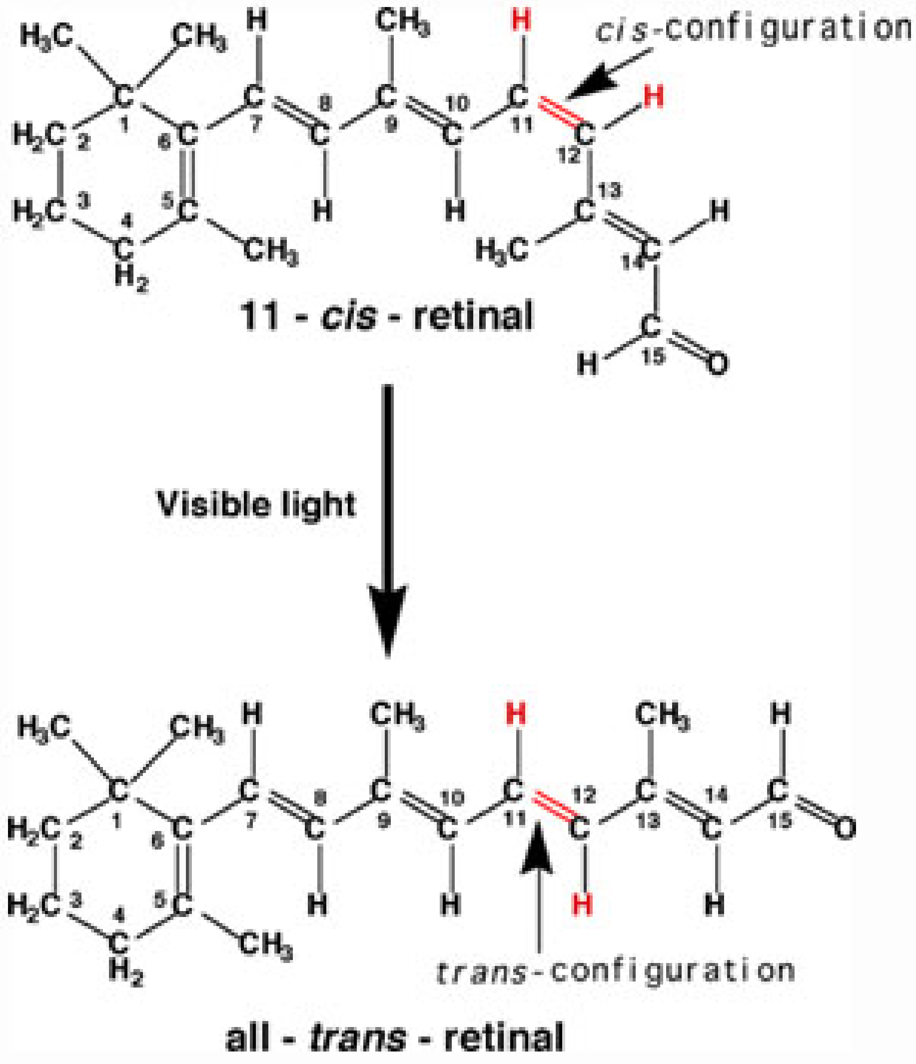


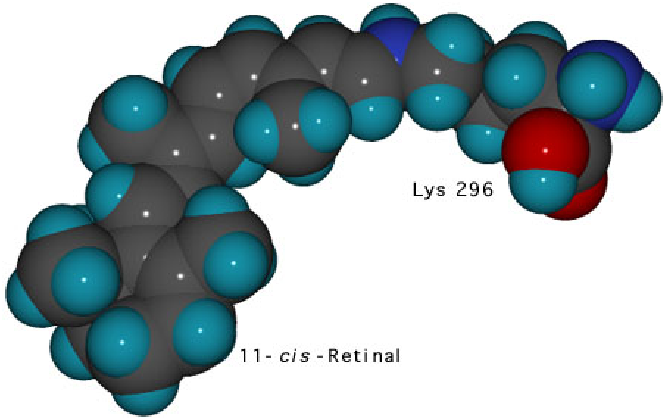

420 nm
498 nm
534 nm
564 nm
Blue cones
Rods
Green cones
Red cones
100
50
0
Normalized absorbance
Short
Medium
Long
400
500
600
700
Violet
Blue
Cyan
Green
Yellow
Red

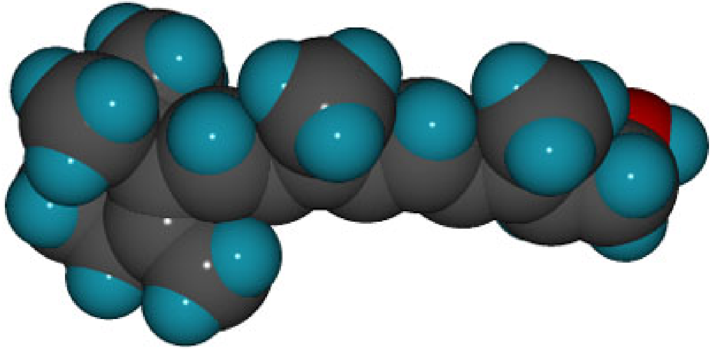
Visual pigment: Rhodopsin

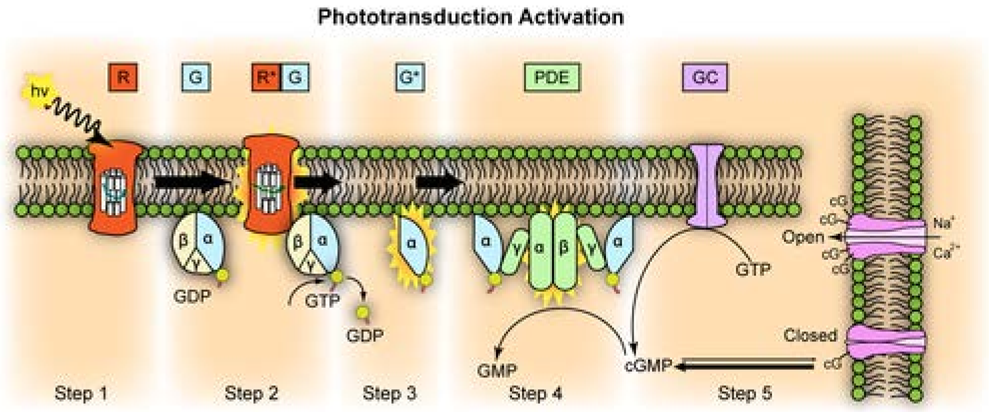
The visual cycle

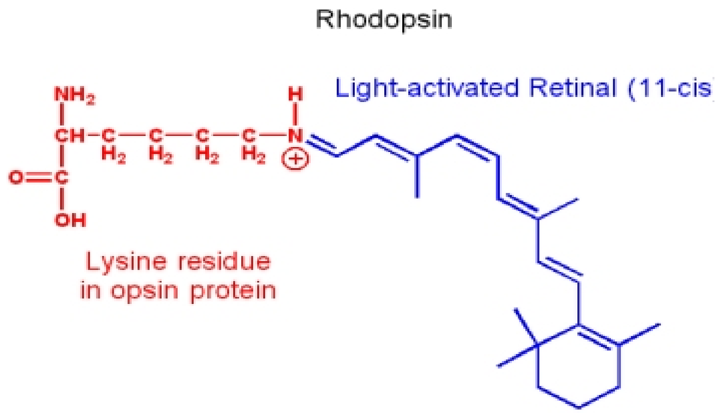



cis-configuration
11 - cis - retinal
Visible light
trans-configuration
all - trans - retinal


Lys 296
11- cis -Retinal



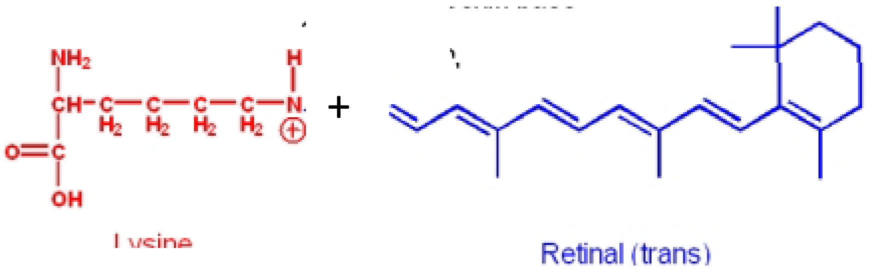




1. *11-cis to all trans* isomerisation,

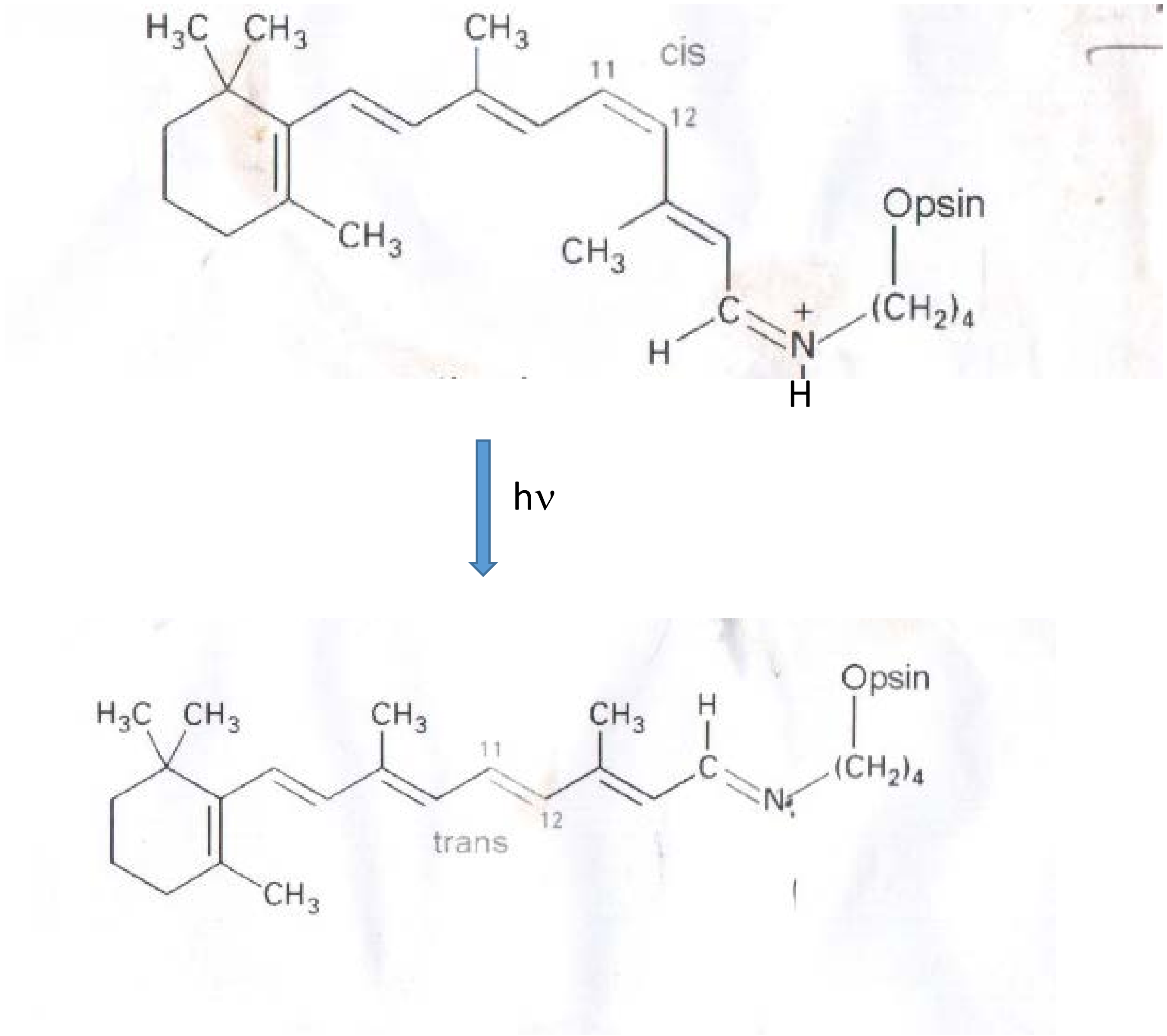
2. all-*trans* Retinal no longer fits into the opsin binding site.

3. Opsin undergoes a conformational change to metarhodopsin.
   and H^+ translocation from Schiff base

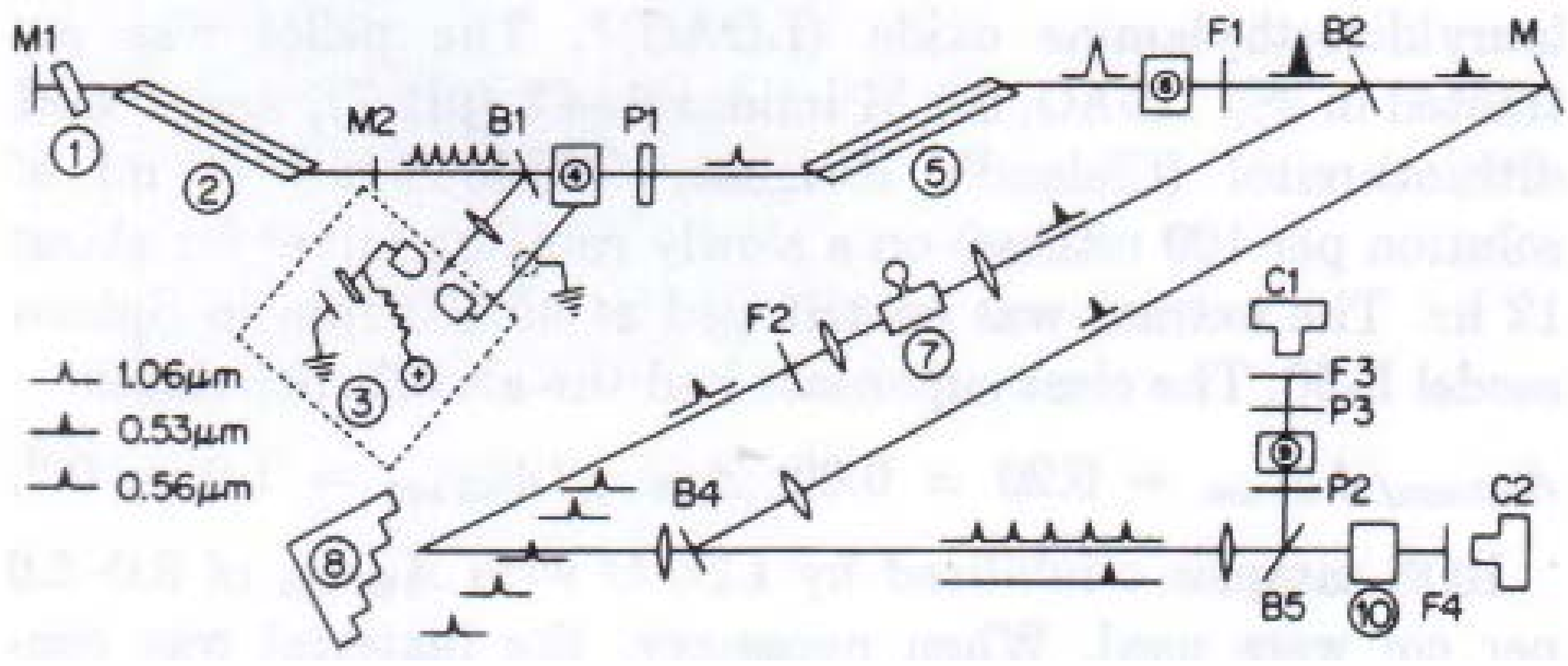


4. Metarhodopsin II is unstable and splits,to opsin and all-*trans* retinal.

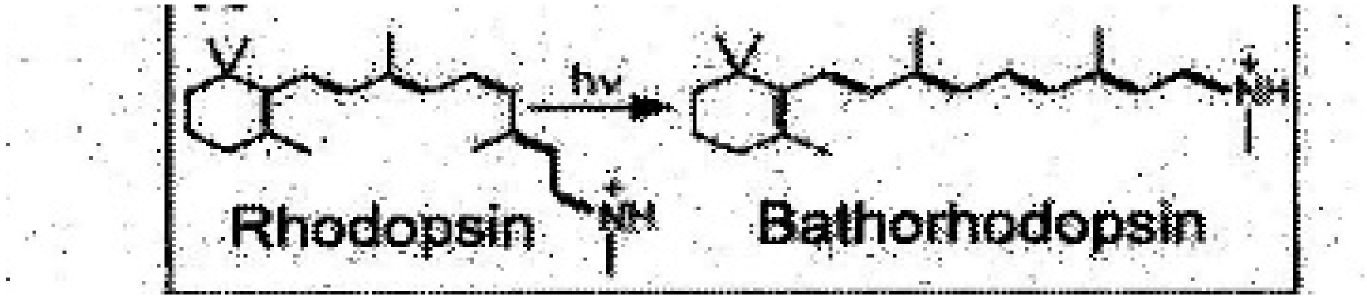
5. Rhodopsin is regenerated by enzymatic reaction.
   The Schiff base reversibly protonated

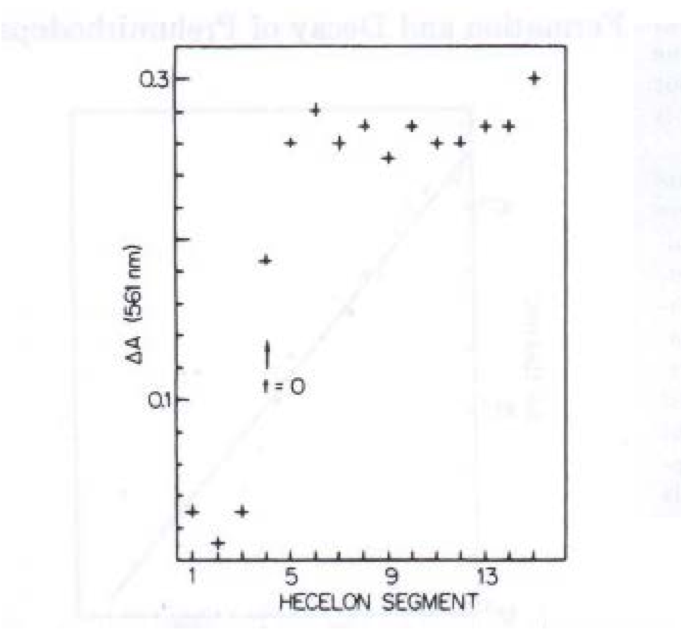


Isomerization and Proton transfer (cis N-H ↔ trans N^+

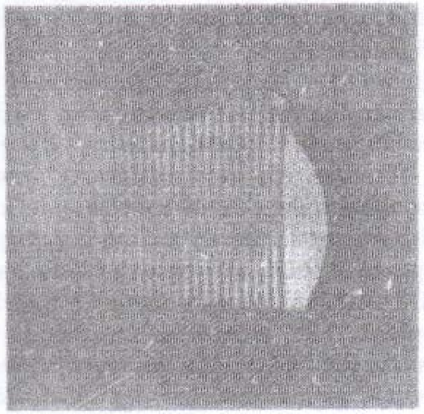


Ps time resolved laser system

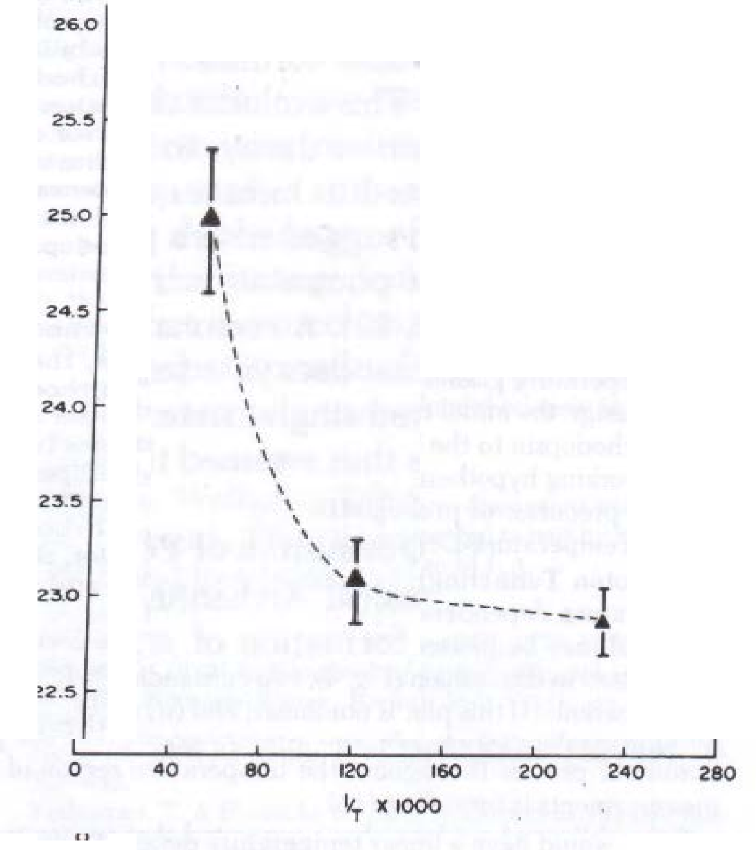




Formation of Bathorhodopsin < 4ps

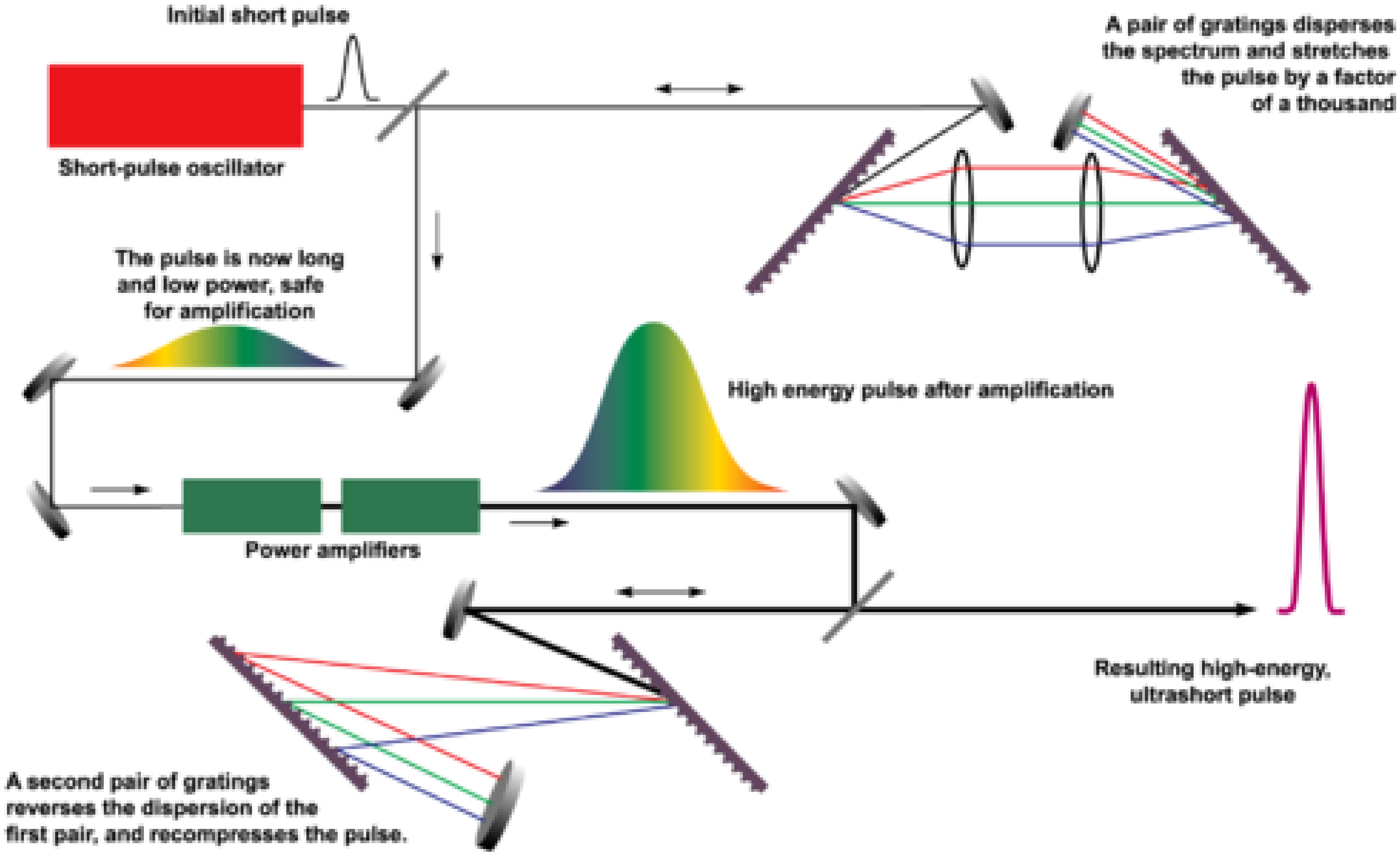


Pulse shaper

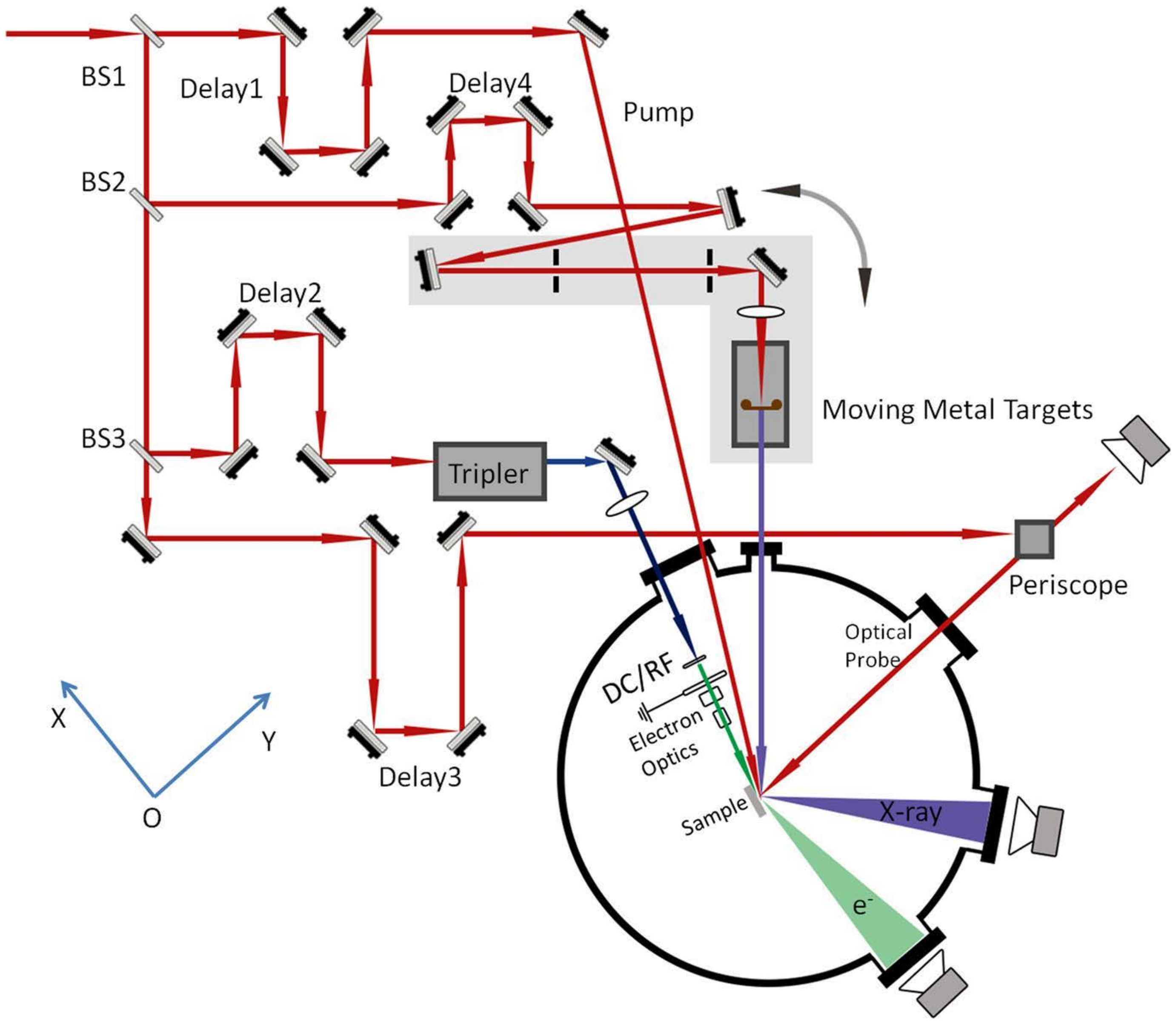


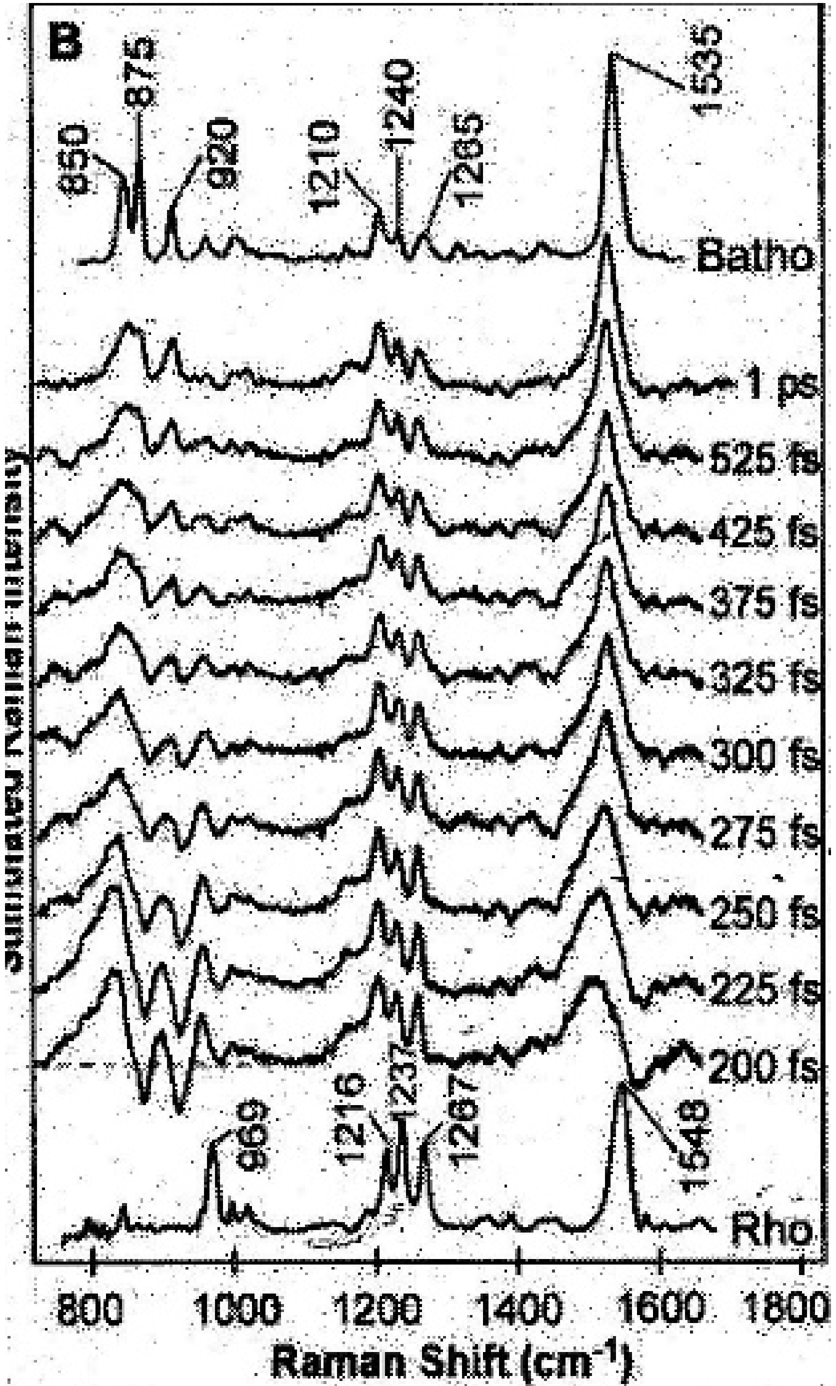
Rhodopsin decay to bathorhodopsin

Fs laser system



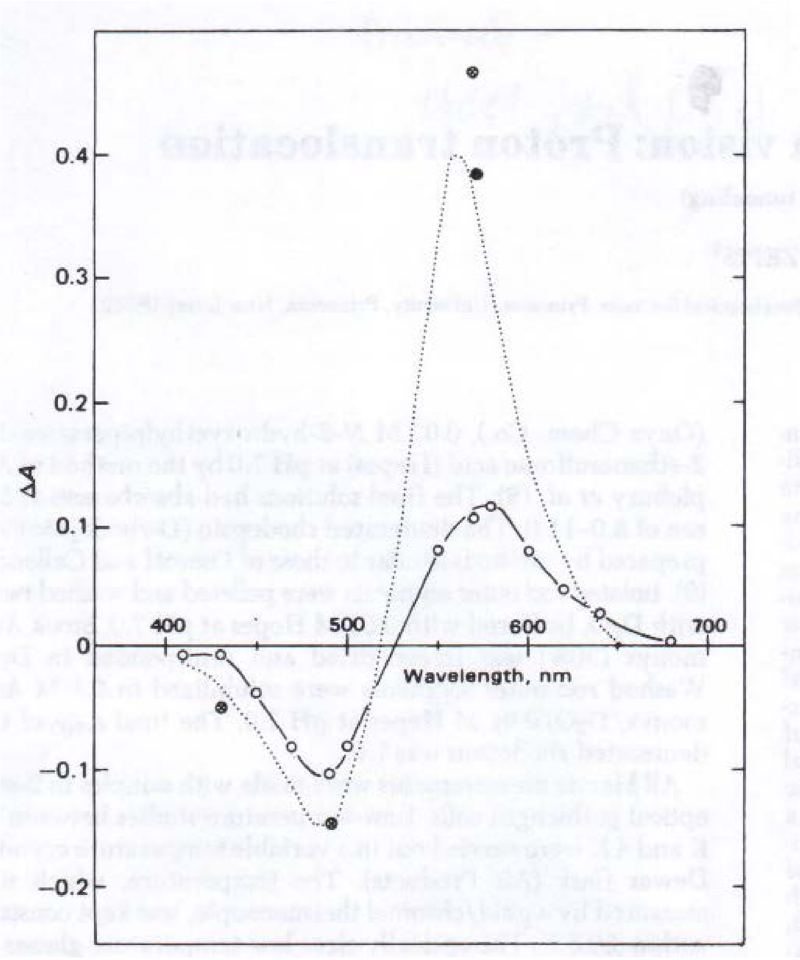
Fs Ultrafast optical, electron and x-ray system



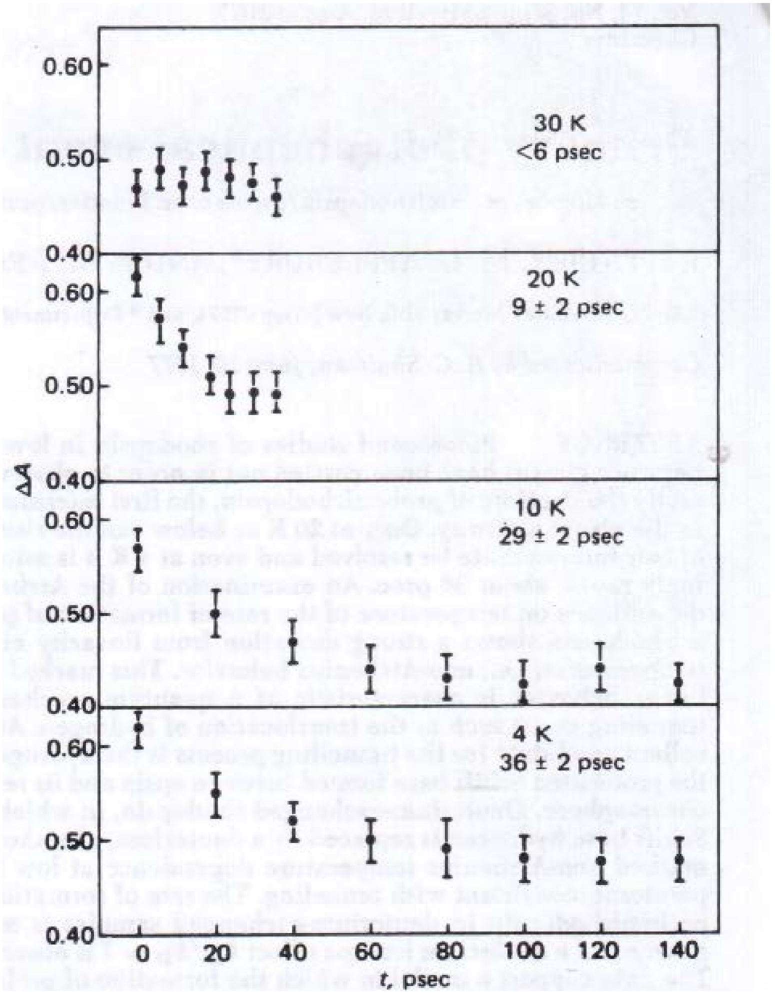


Time resolved Raman spectra

Bathorhodopsinn (H^+) translocation at low temperatures

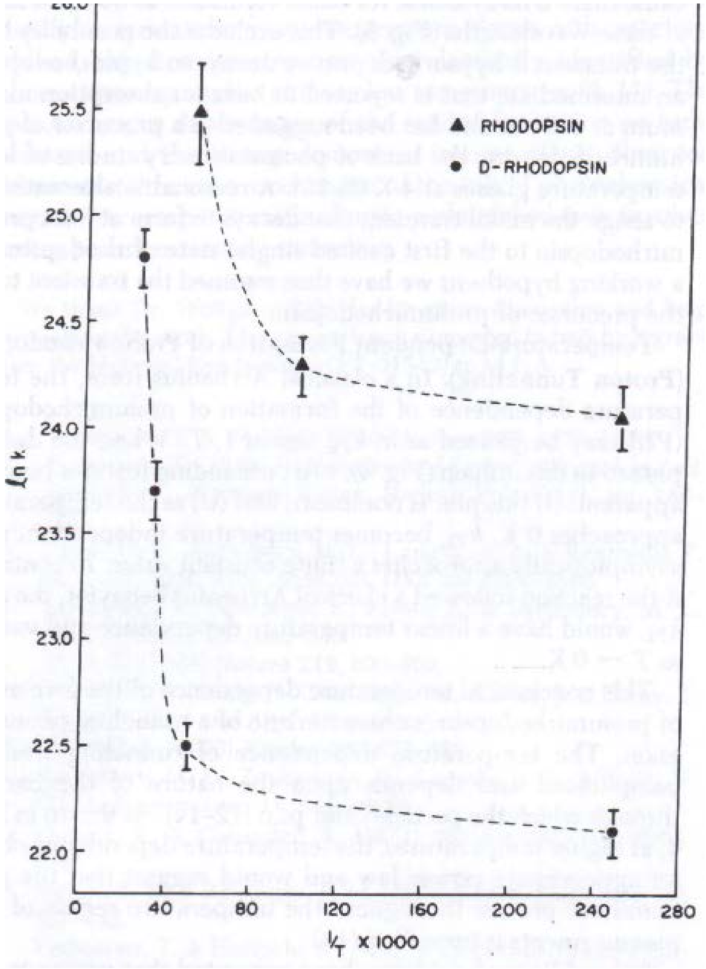


k



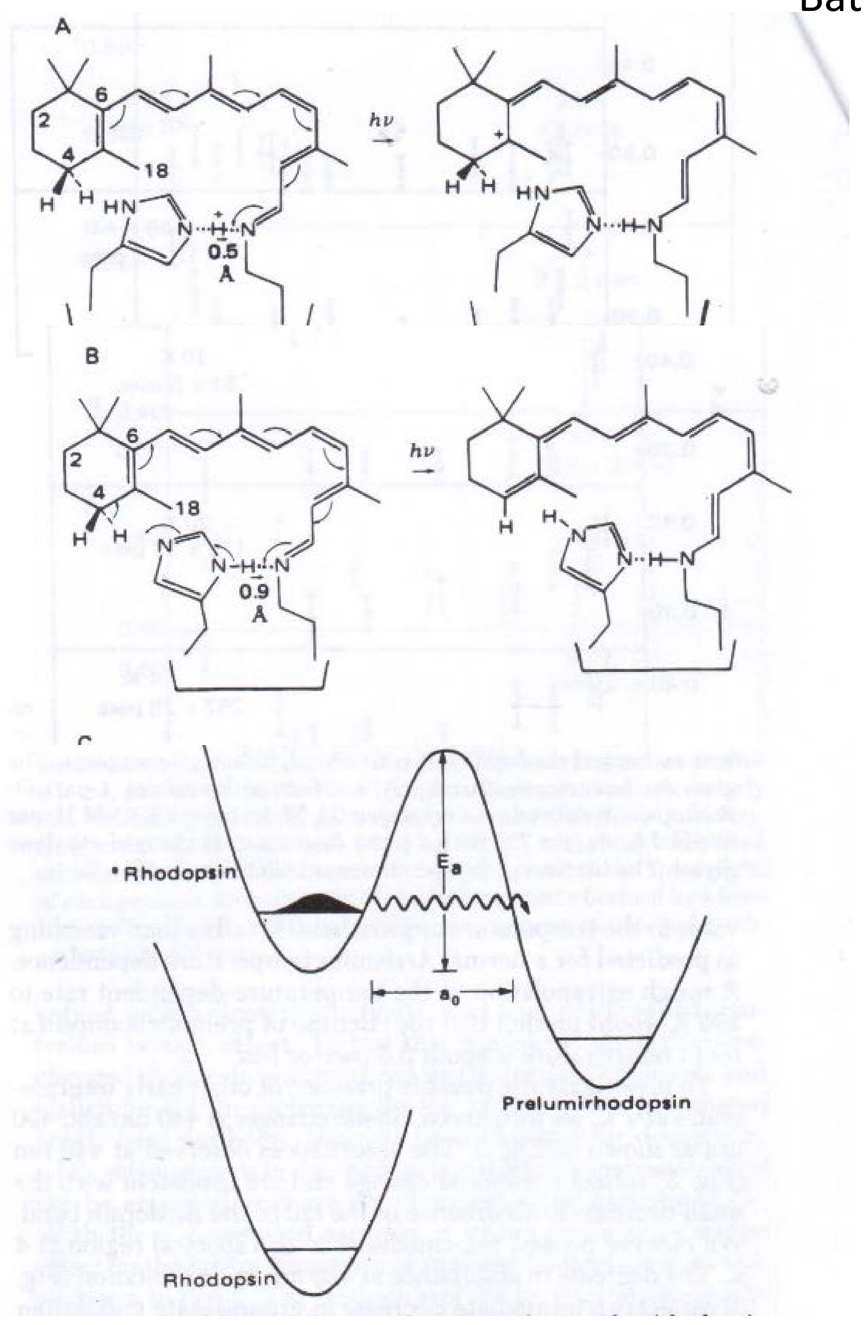
Bathorhodopsin spectra at ● 77K and o 4 K

Formation of bathorhodopsin between 4 K (36 ps) and 30 K (<6 ps)



Bathorhodopsin risetime ~ 0.3ps
Excited state: ~200 fs

Formation of H and D bathorhodopsin
Ln k (formation rate) as a function of temperature



Batho-rhodopsin proton translocation mechanisms

A. Single proton translocation

B. Concerted double proton translocation forming retro-retinal

$$k_t = \nu_0 \exp \frac{-\pi^2 \alpha_0 \kappa}{h} (2mE)^{1/2}$$

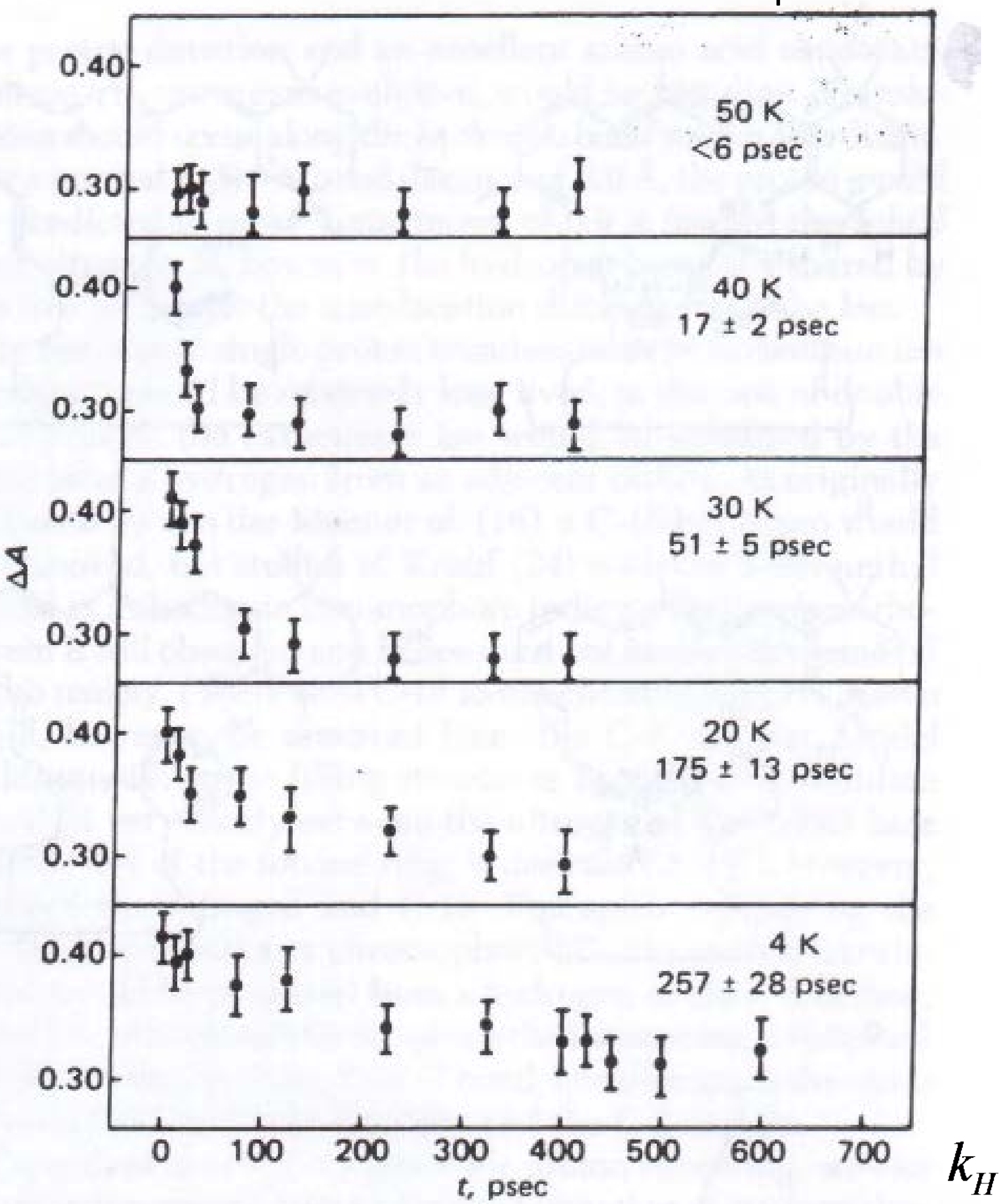
C. Tunneling potential energy barriers, E_a, for bathorhodopsin formation

Calculated energy barriers:

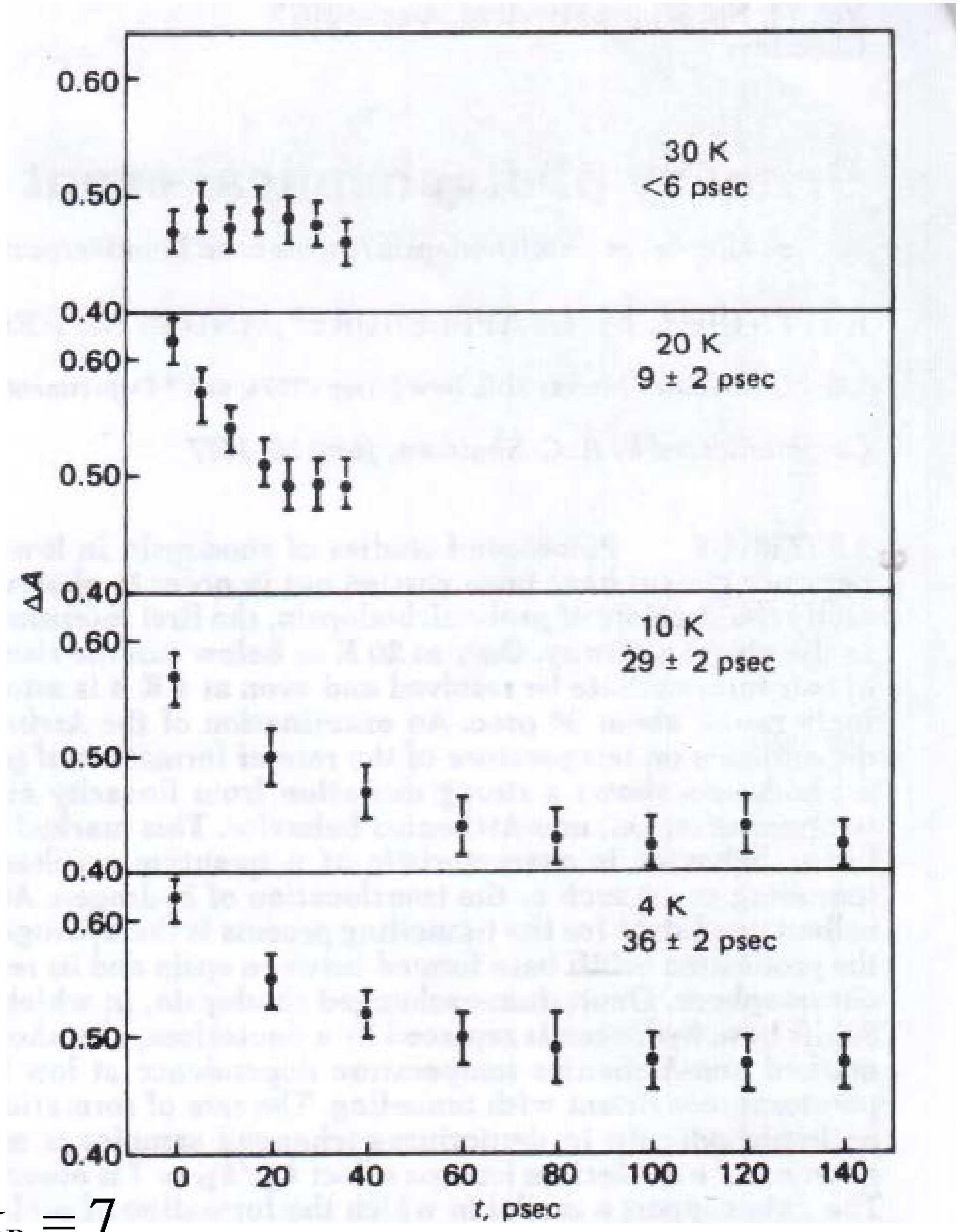
model A with a_0 = 0.5 E_a = 4.5 kcal/mol

model B with a_0 = 0.9 A E_a = 4.5 kcal/mol

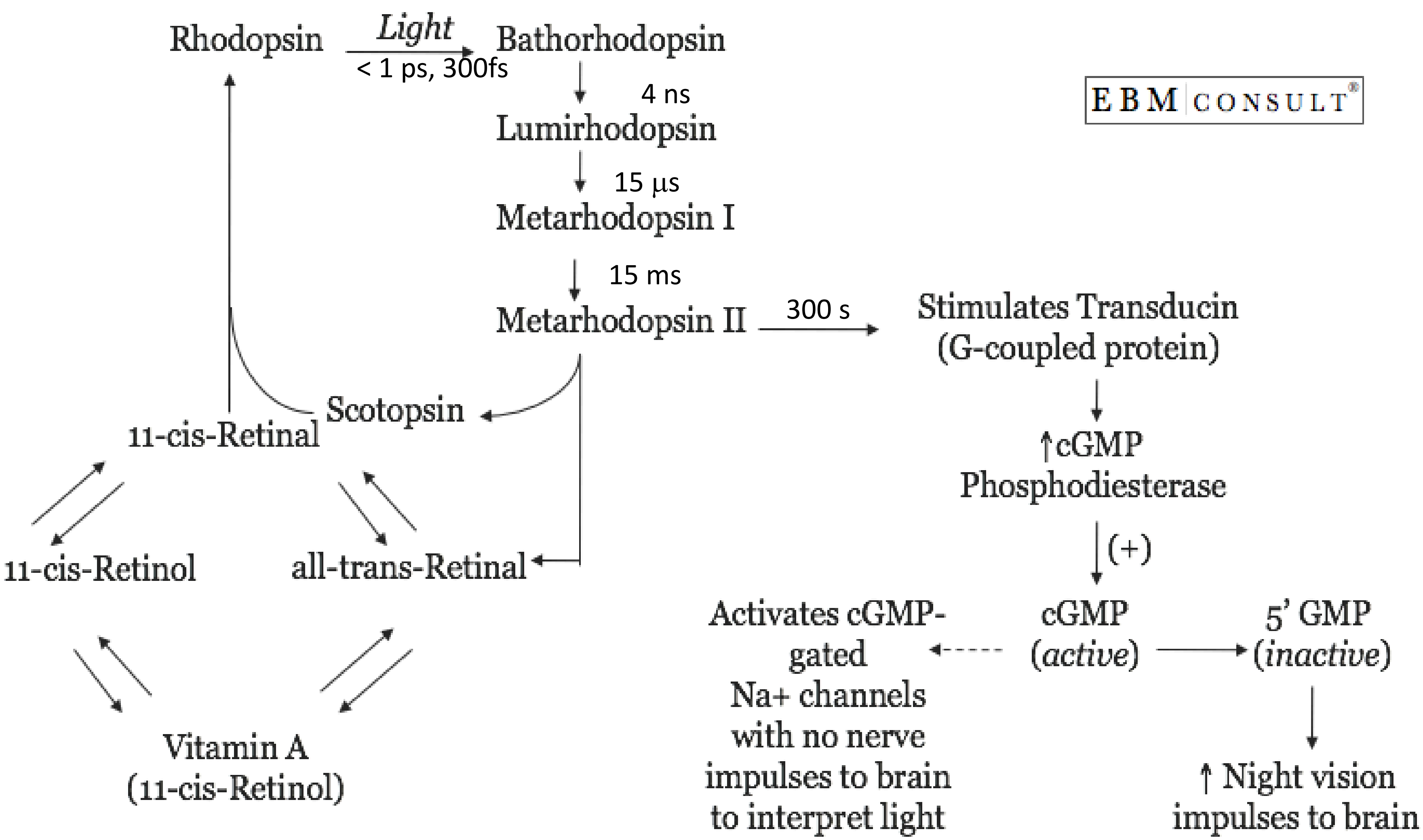
Dynamics of rhodopsin (D) at low temperatures
formation of deuterated bathorhodopsin



H rhodopsin tunneling



$$k_H / k_D = 7$$


Rhodopsin
Light
< 1 ps, 300fs
Bathorhodopsin
4 ns
Lumirhodopsin
15 μs
Metarhodopsin I
15 ms
Metarhodopsin II
300 s
Stimulates Transducin
(G-coupled protein)
↑cGMP
Phosphodiesterase
(+)
Scotopsin
11-cis-Retinal
11-cis-Retinol
all-trans-Retinal
Vitamin A
(11-cis-Retinol)
Activates cGMP-gated
Na+ channels
with no nerve
impulses to brain
to interpret light
cGMP
(active)
5' GMP
(inactive)
↑ Night vision
impulses to brain

EBM CONSULT®

Light induced visual steps:

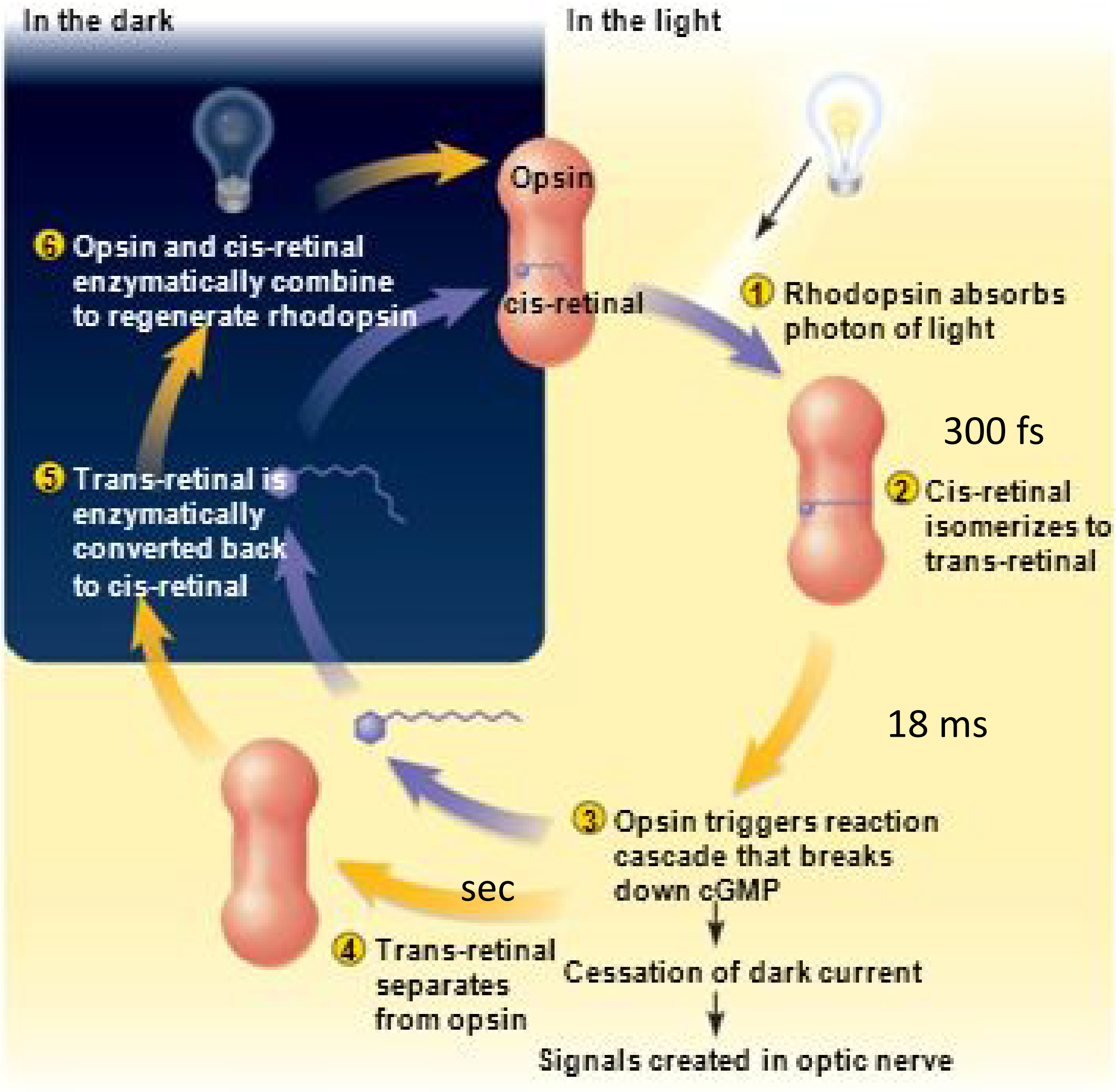
Isomerization of rhodopsin 11-cis to all trans 300fs

Proton (hydrogen bond) translocation from retinal to opsin < 2 ps (250 fs)

Dissociation of the retinal opsin bond (retinal opsin split) sec

Signal transmitted to optic nerve.

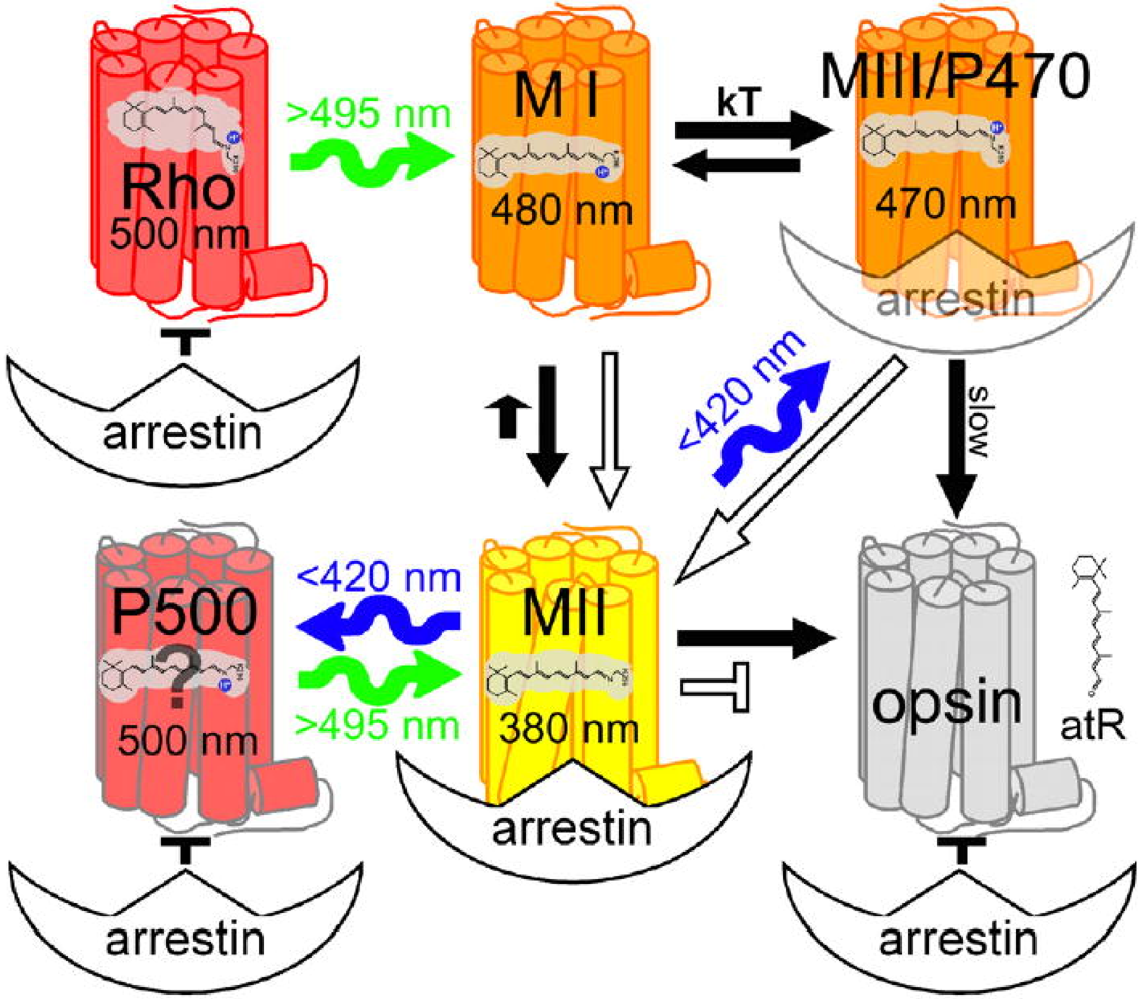
Enzimatic rhodopsin regeneration (Scotopic process) Min


In the dark
In the light
Opsin
cis-retinal
6 Opsin and cis-retinal enzymatically combine to regenerate rhodopsin
1 Rhodopsin absorbs photon of light
300 fs
2 Cis-retinal isomerizes to trans-retinal
5 Trans-retinal is enzymatically converted back to cis-retinal
18 ms
3 Opsin triggers reaction cascade that breaks down cGMP
sec
4 Trans-retinal separates from opsin
Cessation of dark current
Signals created in optic nerve

Summary

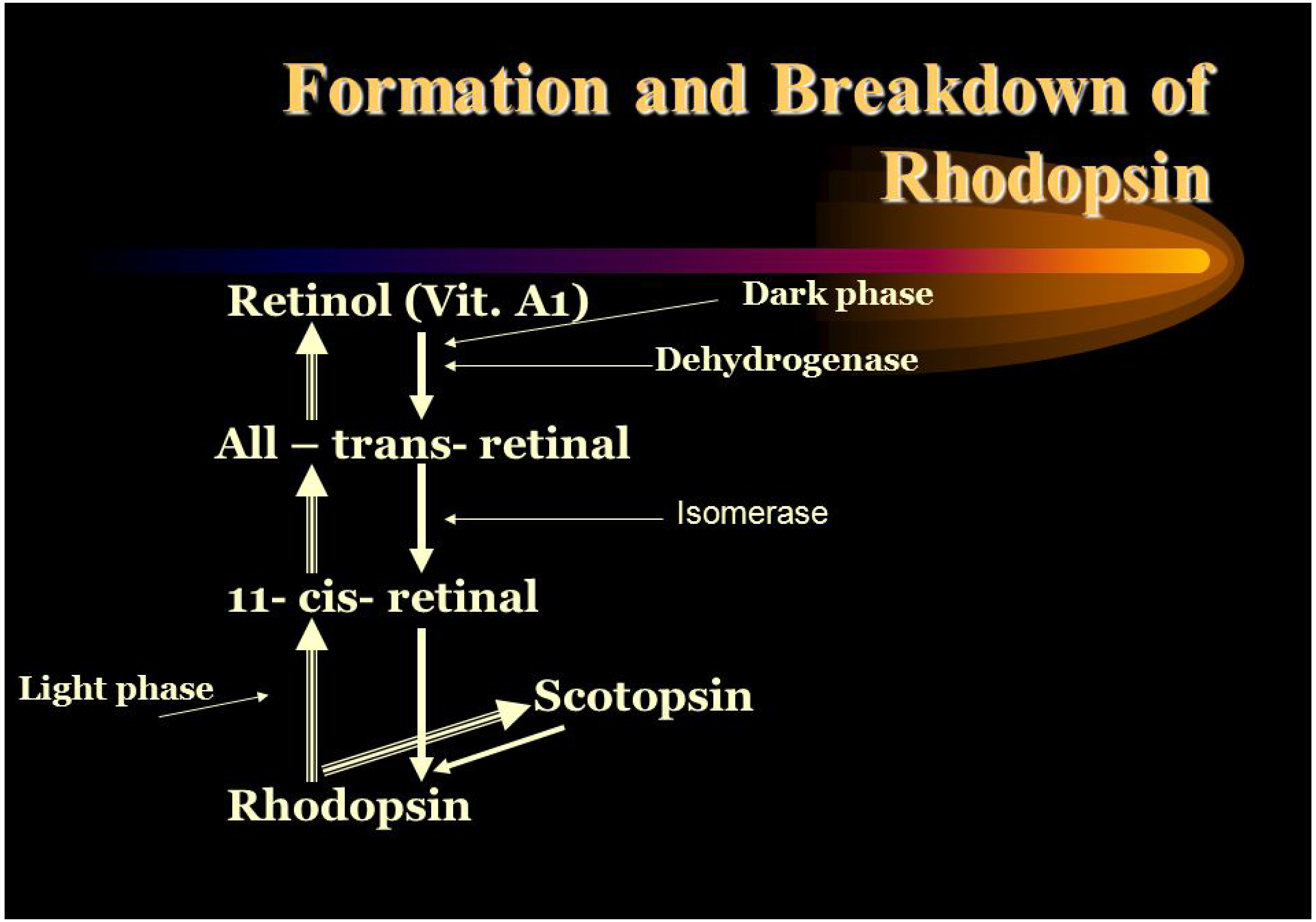
Future studies

1. Scotopic efficiency
2. Rhodopsin Regeneration
3. Extension of visual response to IR

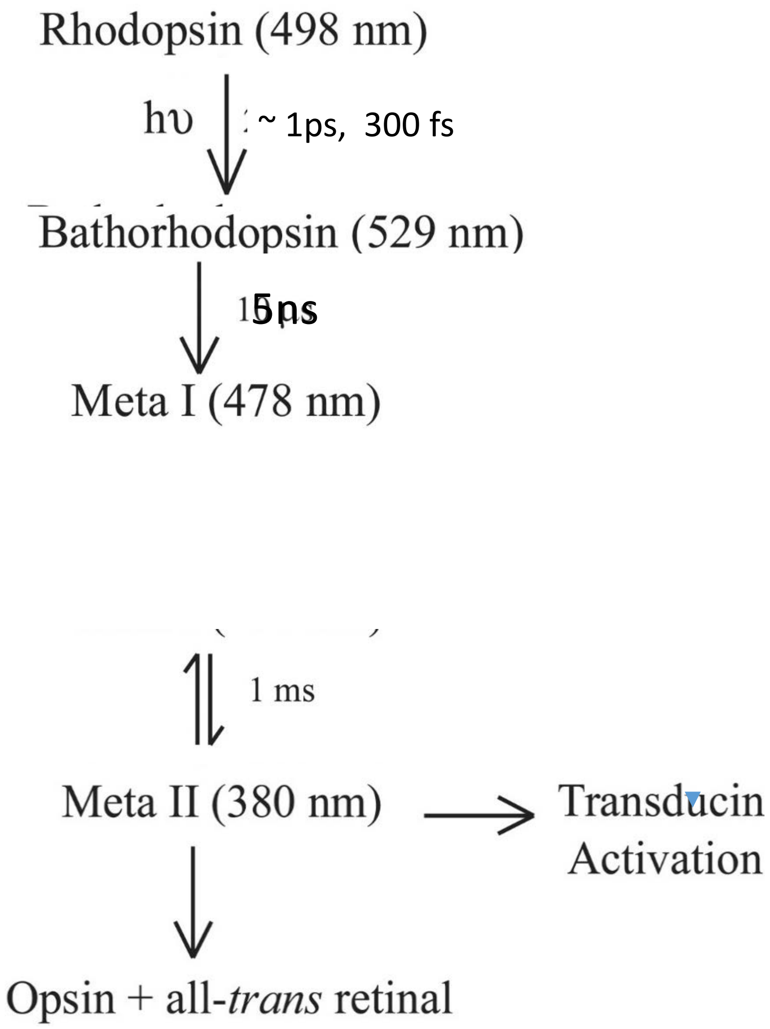


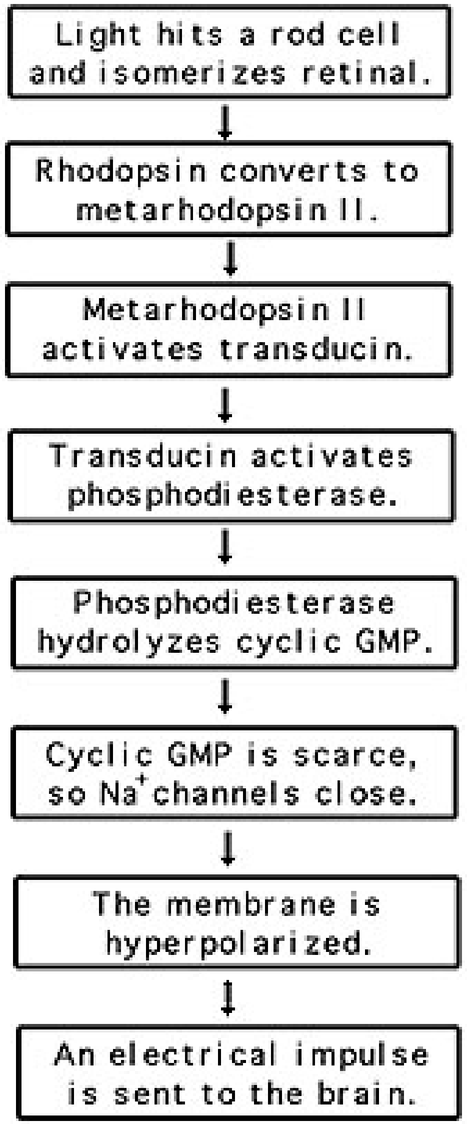
Rho
500 nm
>495 nm
M I
480 nm
kT
MIII/P470
470 nm
arrestin
arrestin
<420 nm
slow
P500
?
500 nm
<420 nm
>495 nm
MII
380 nm
arrestin
opsin
atR
arrestin
arrestin

Rhodopsin photo-transduction and regeneration

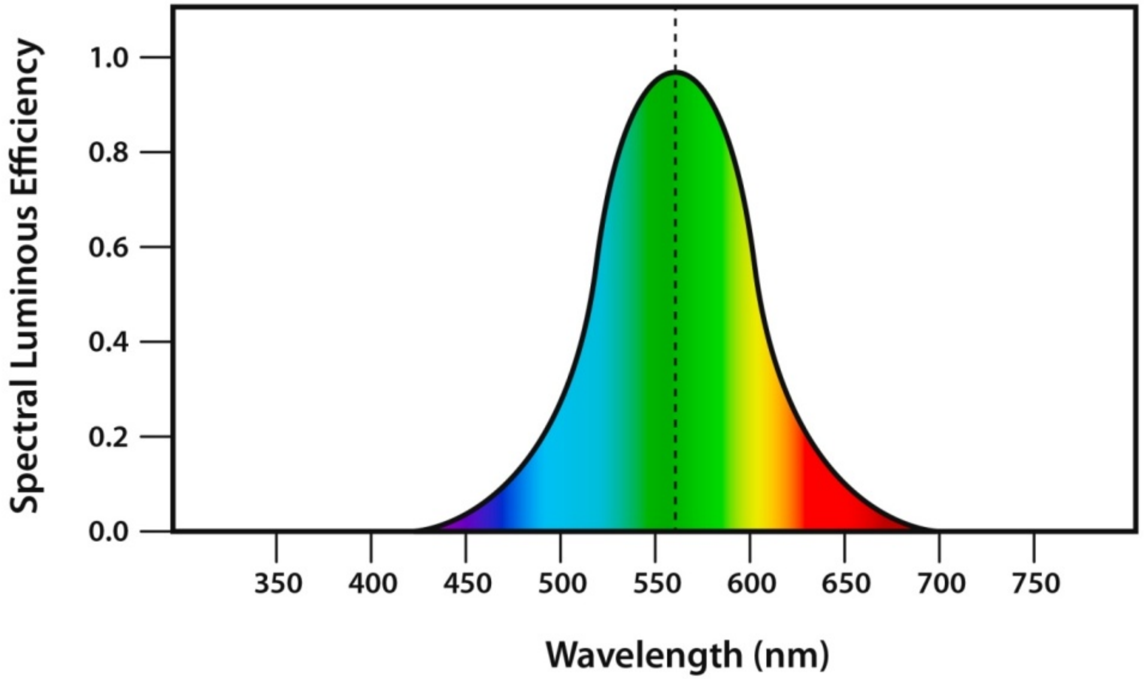


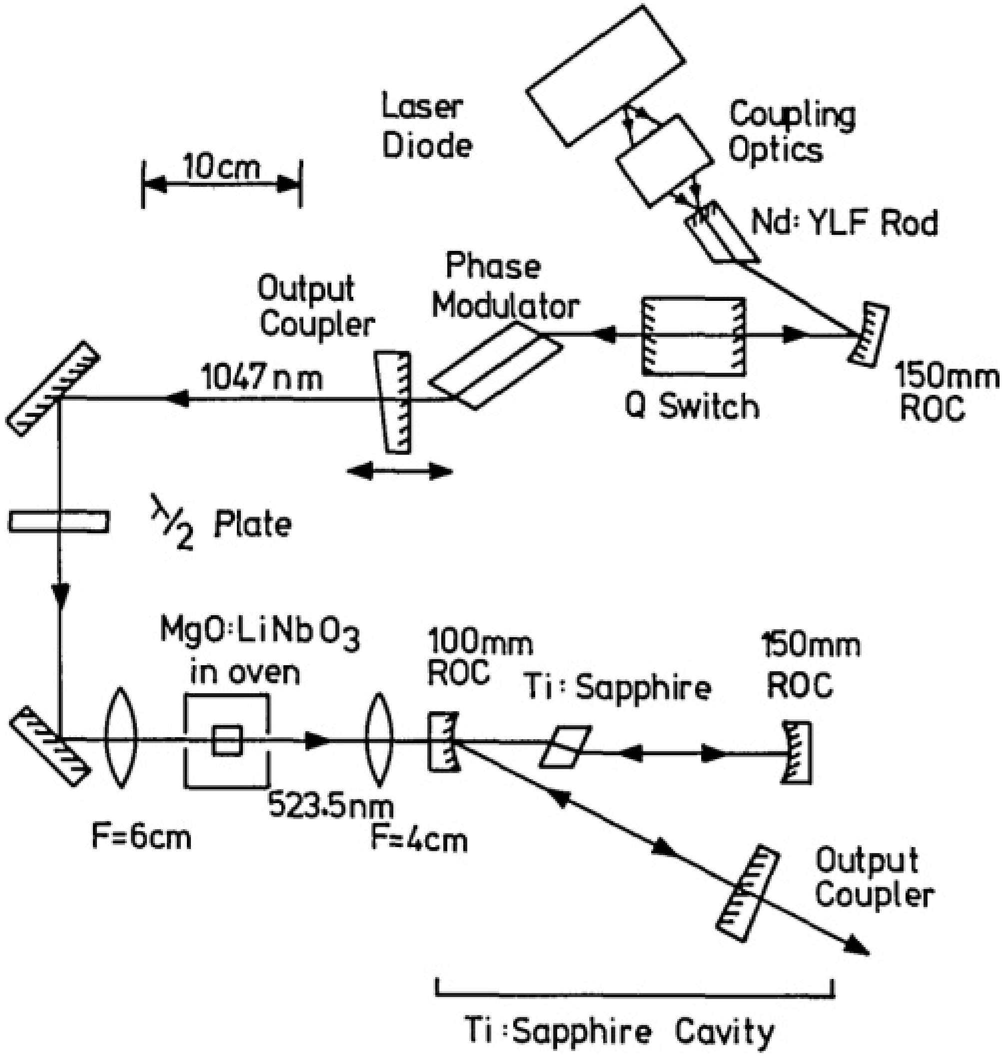
Visual Cycle

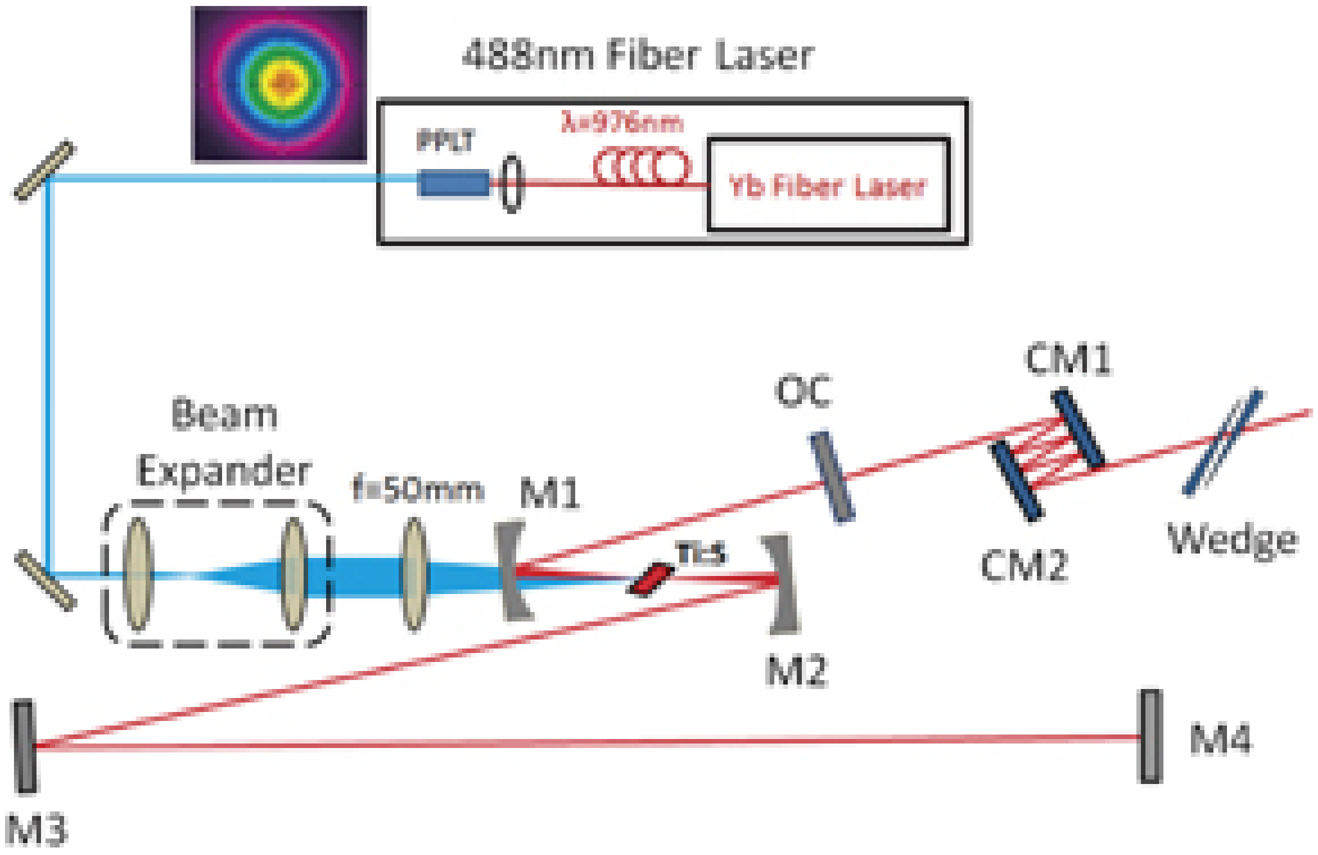




and Meta III

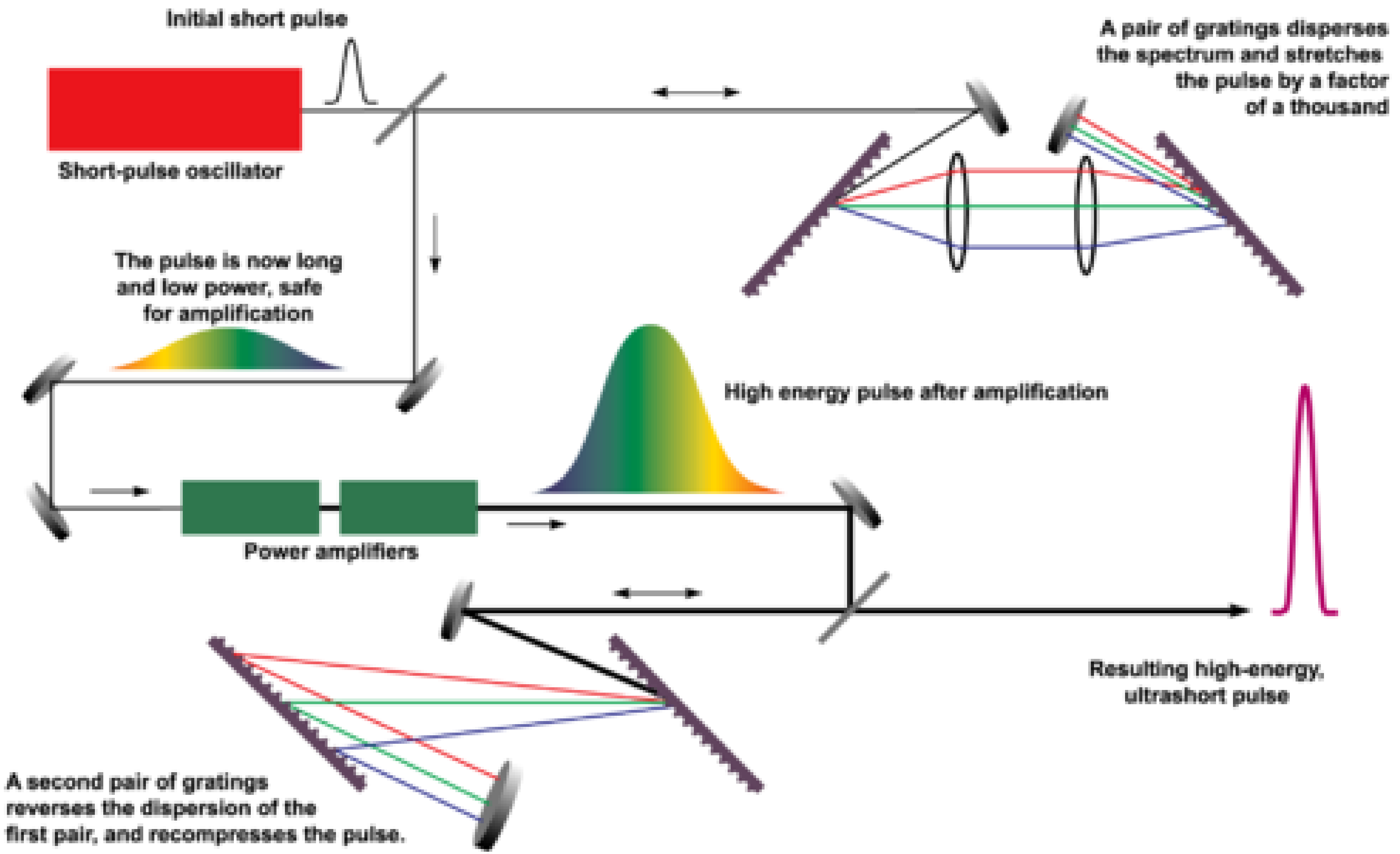

Spectral Luminous Efficiency
1.0
0.8
0.6
0.4
0.2
0.0
350
400
450
500
550
600
650
700
750
Wavelength (nm)



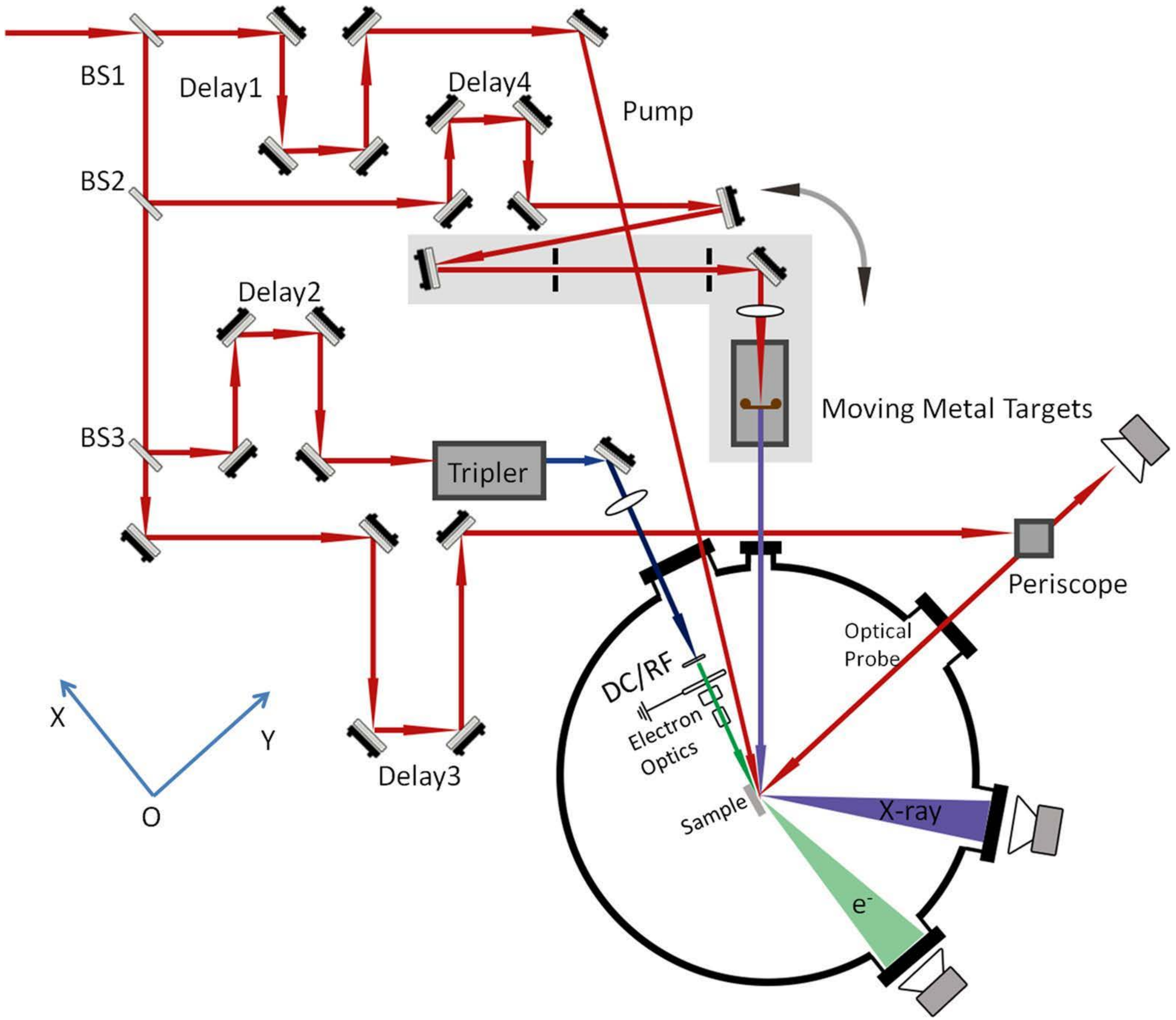


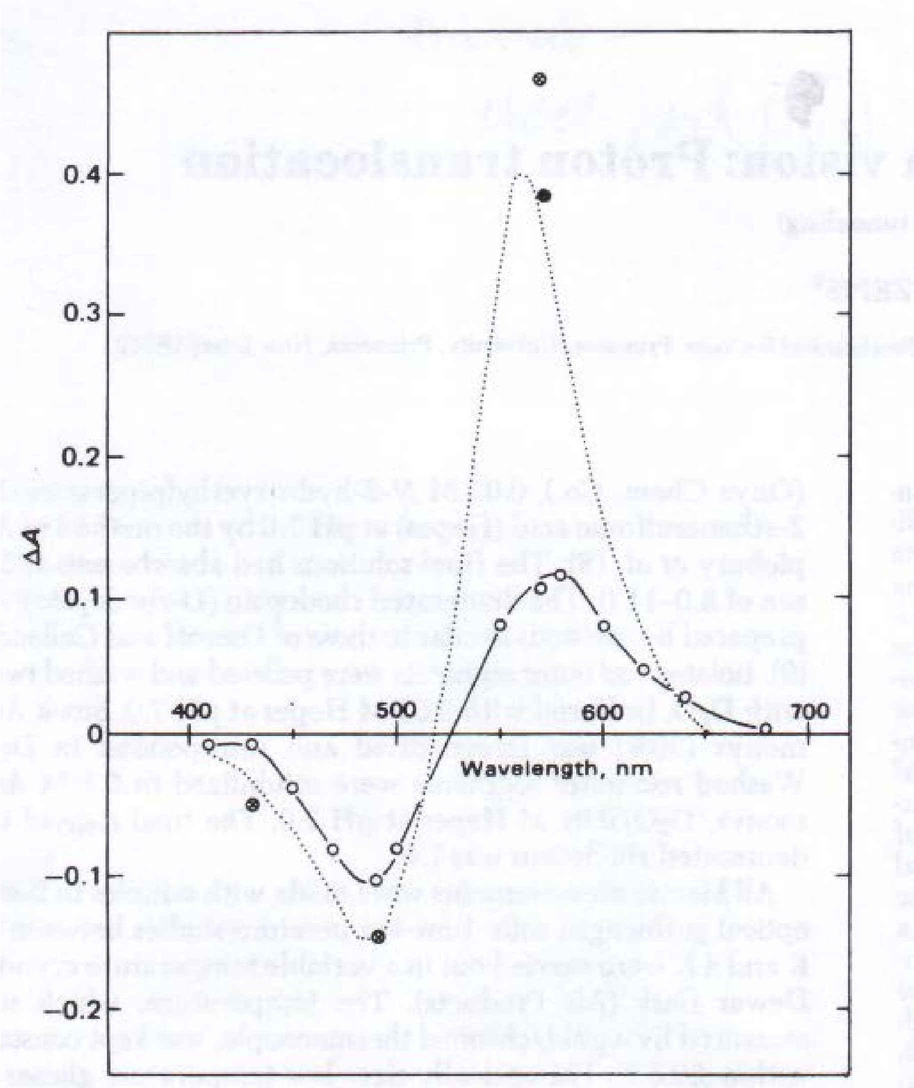
Ti: sapphire mode locked laser for ultrafast spectroscopy; $\lambda = 1064$ nm, $\tau = 3\text{–}100$ fs

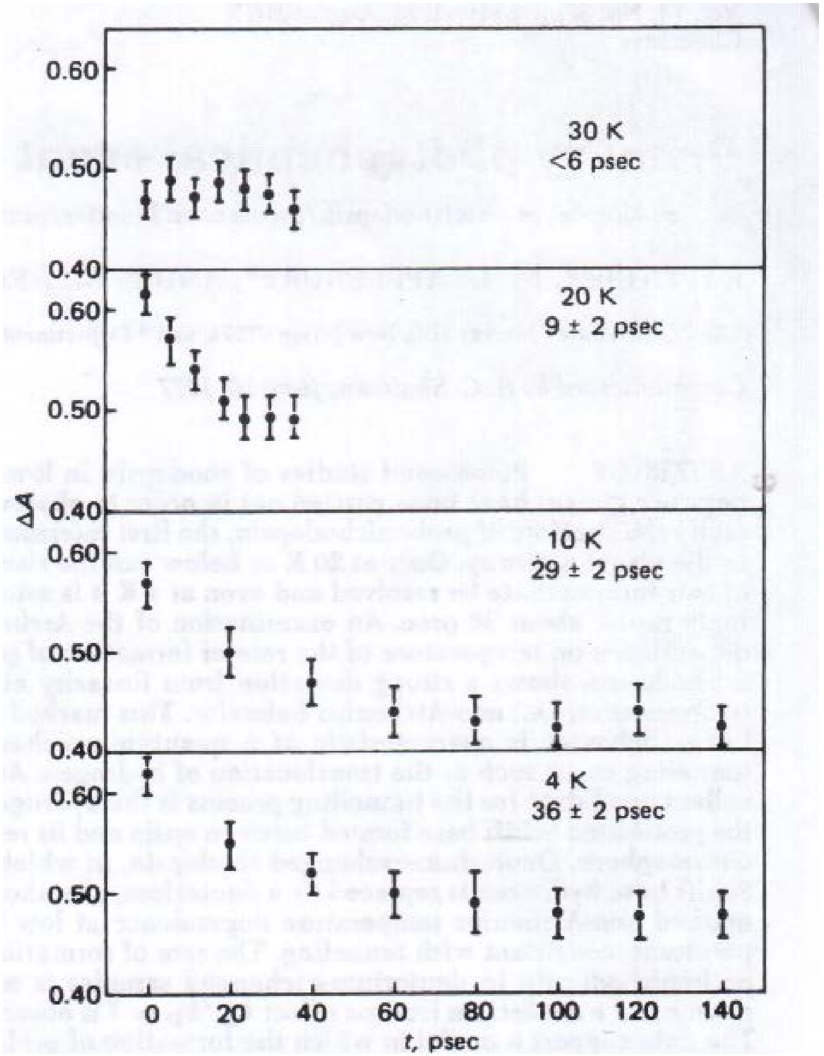
Fs laser system



Ultrafast optical, electron and xray system



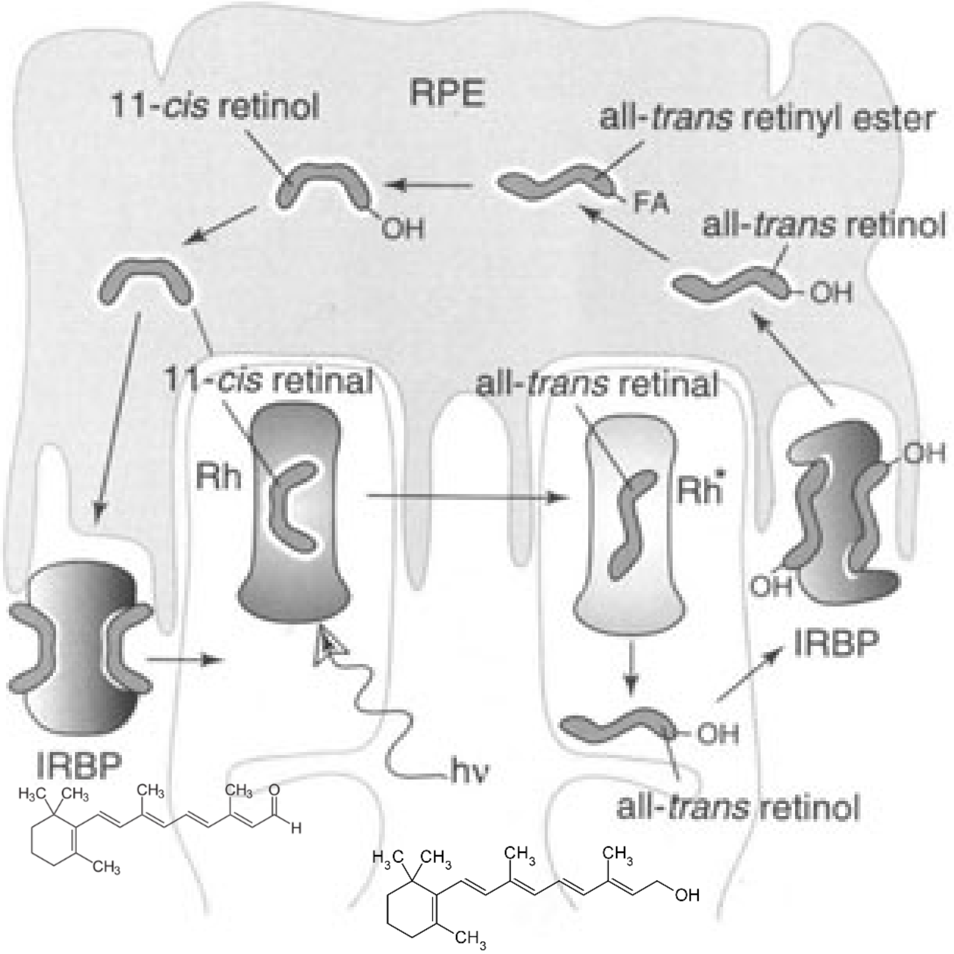




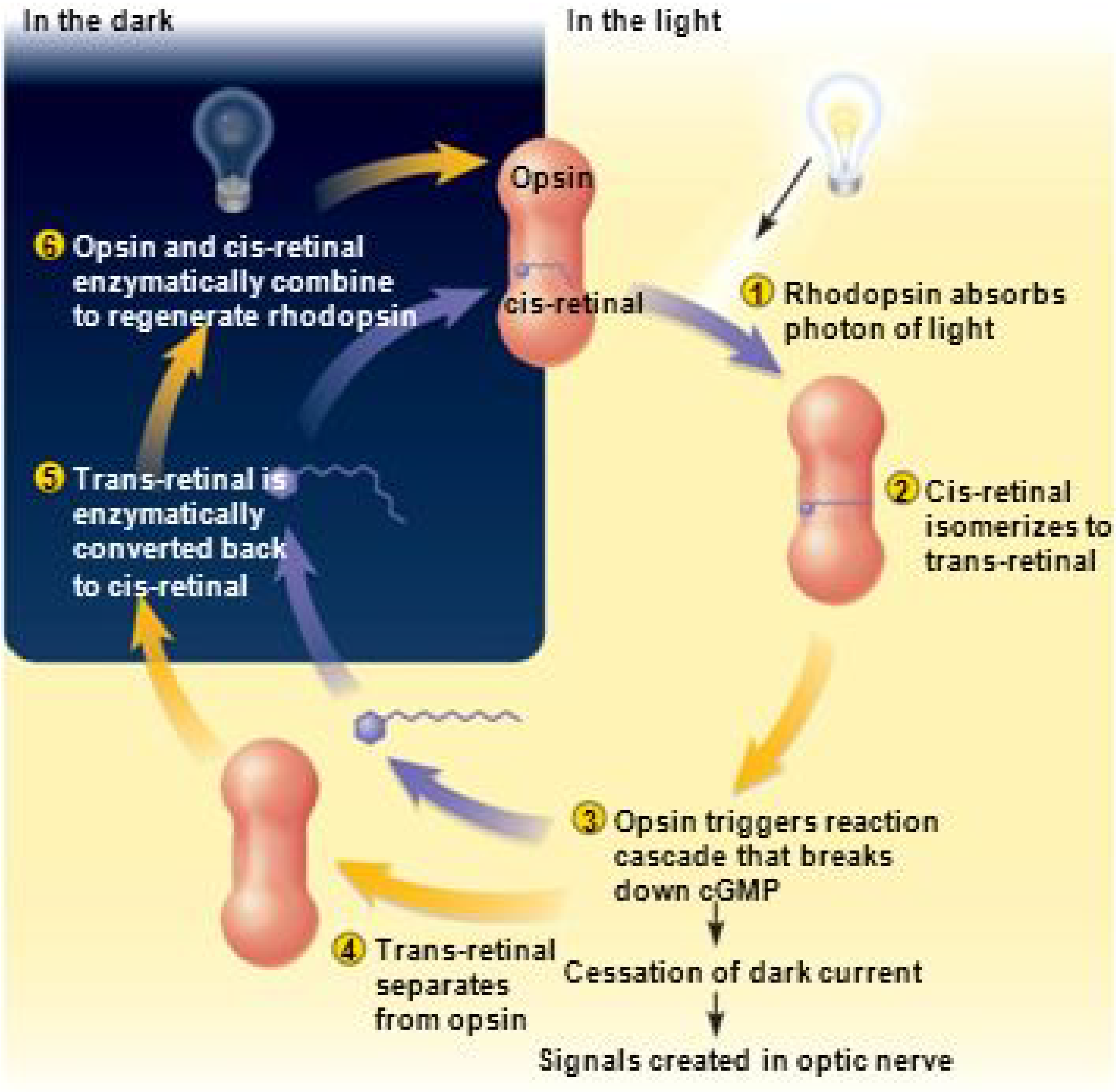
Bathorhodopsin spectra at ● 77K and o 4 K

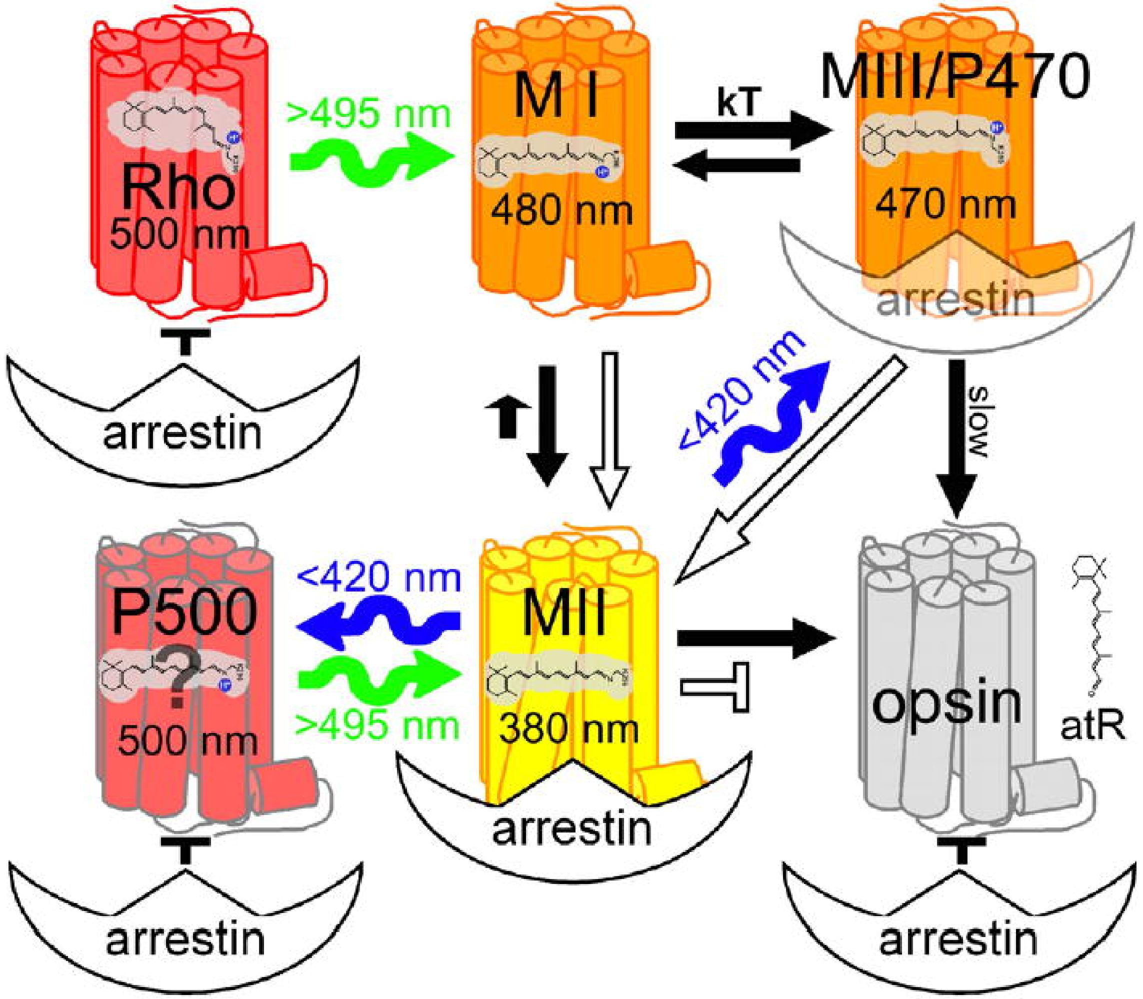
Formation of bathorhodopsin between 4 K (36 ps) and 30 K (<6 ps)

Retinal pigment epithelium

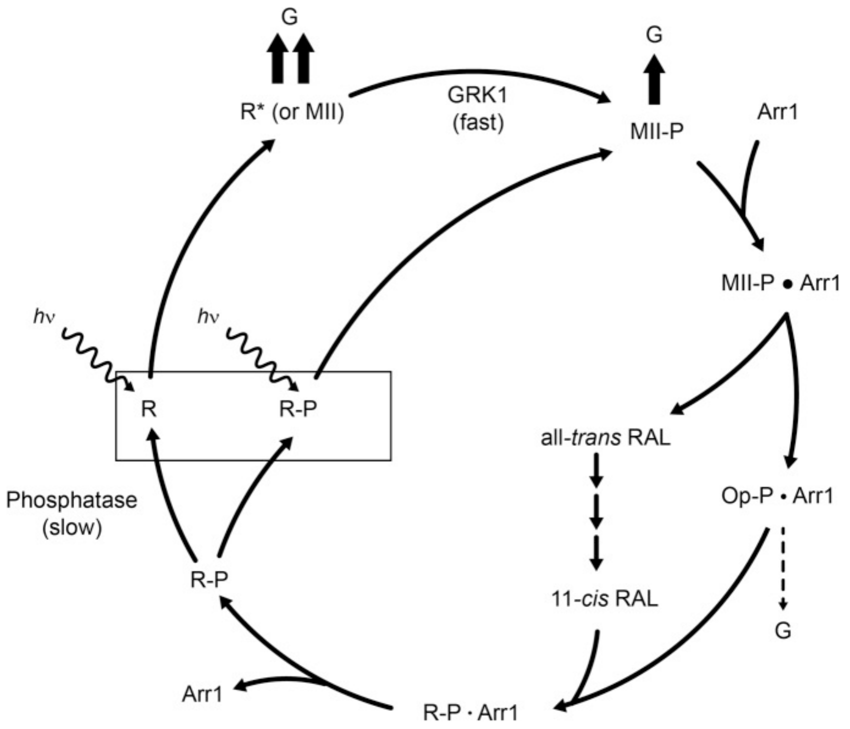


Iron Responsive binding Protein

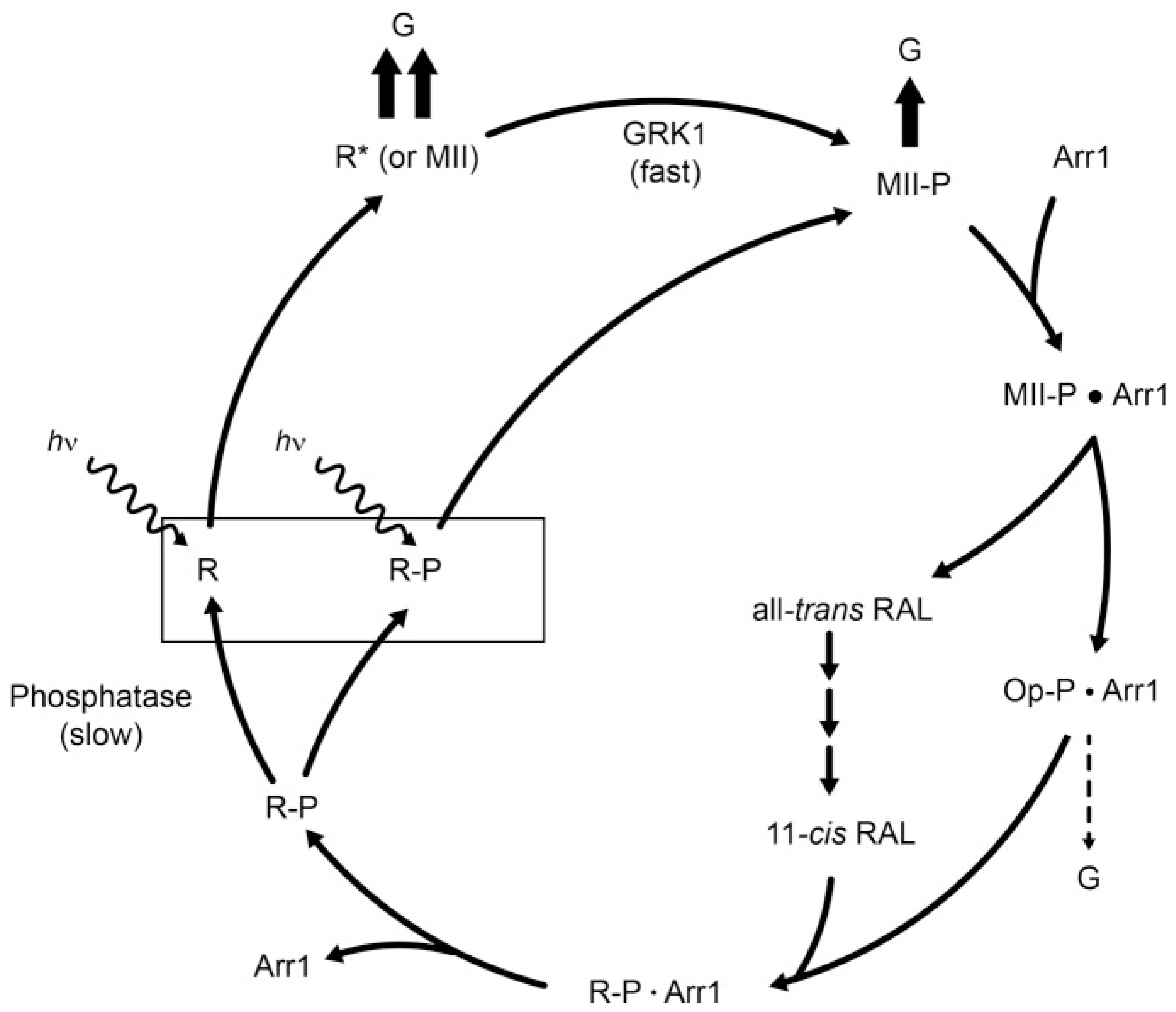

In the dark
In the light
Opsin
cis-retinal
1 Rhodopsin absorbs photon of light
2 Cis-retinal isomerizes to trans-retinal
3 Opsin triggers reaction cascade that breaks down cGMP
Cessation of dark current
Signals created in optic nerve
4 Trans-retinal separates from opsin
5 Trans-retinal is enzymatically converted back to cis-retinal
6 Opsin and cis-retinal enzymatically combine to regenerate rhodopsin



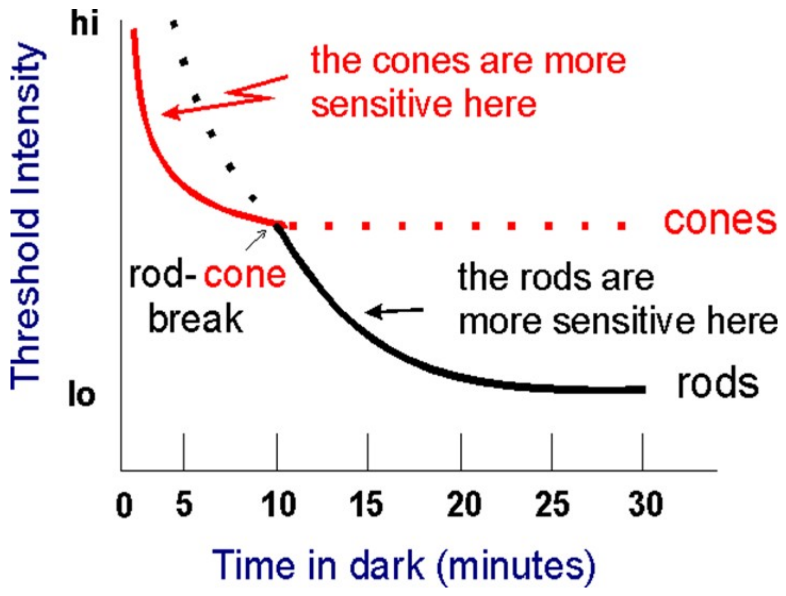
Rho
500 nm
>495 nm
M I
480 nm
kT
MIII/P470
470 nm
arrestin
arrestin
<420 nm
slow
P500
?
500 nm
<420 nm
>495 nm
MII
380 nm
arrestin
opsin
atR
arrestin
arrestin



A model illustrating the effects of R-P on the phototransduction cascade. When stimulated by light, activated rhodopsin (*R** or MII) interacts with the G-protein transducin (G) to initiate phototransduction (indicated by 2 bold arrows). Rhodopsin kinase (GRK1) quickly phosphorylates *R** to form MII-P in a process that reduces its capacity to activate transducin (as indicated with only 1 bold arrow). Shortly thereafter, the catalytic activity of the MII-P is terminated through binding with Arr1 to form MII-P · Arr1. After this step, phospho-opsin (Op-P · Arr1) is hydrolyzed from all-*trans* retinal, which is recycled in the RPE (data not shown). The dashed arrow indicates that opsin itself has very low transduction activity. Pigment regeneration occurs when phospho-opsin (Op-P) recombines with 11-*cis*-retinal (supplied from the RPE) to form phospho-rhodopsin (R–P). Phospho-rhodopsin can either be slowly dephosphorylated by rhodopsin phosphatase or reactivated by light. The latter outcome results in diminished activation of transducin (G).



G
G
R* (or MII)
GRK1
(fast)
MII-P
Arr1
MII-P • Arr1
hν
hν
R
R-P
all-trans RAL
Op-P • Arr1
Phosphatase
(slow)
R-P
11-cis RAL
G
Arr1
R-P · Arr1

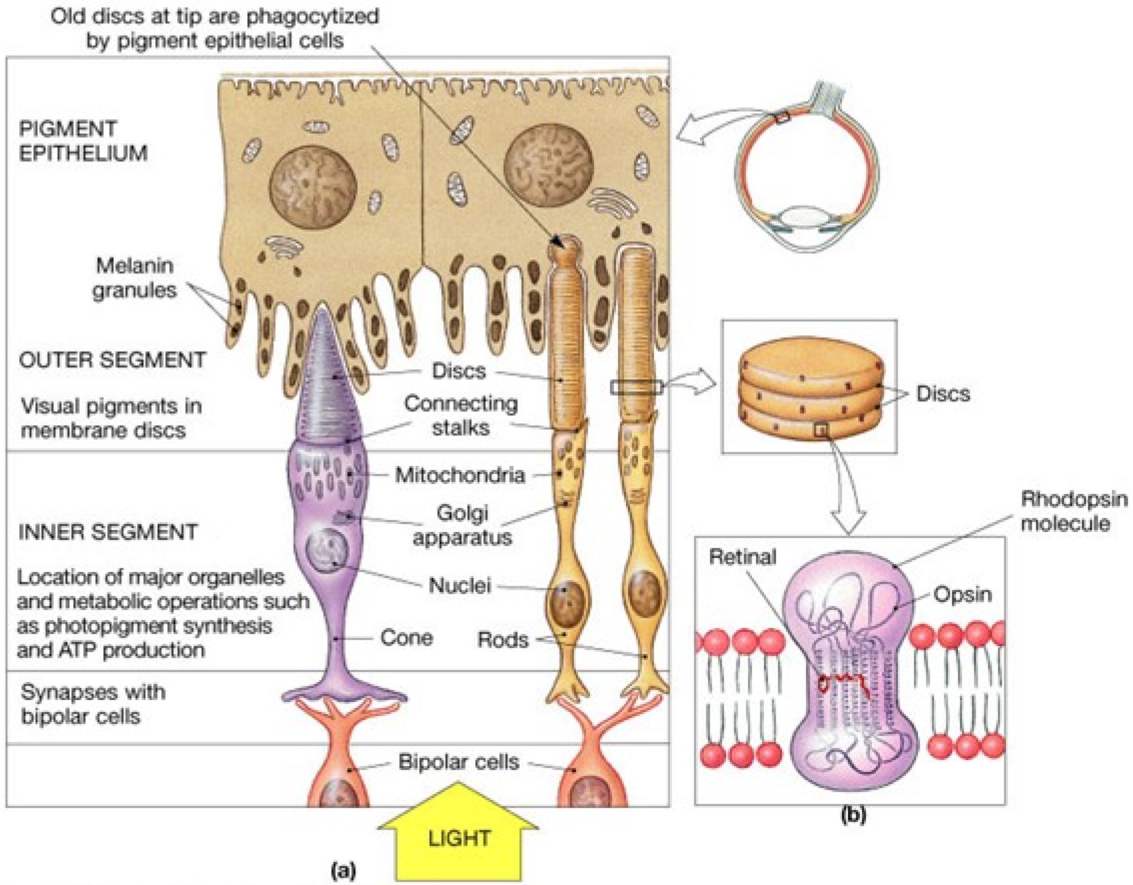


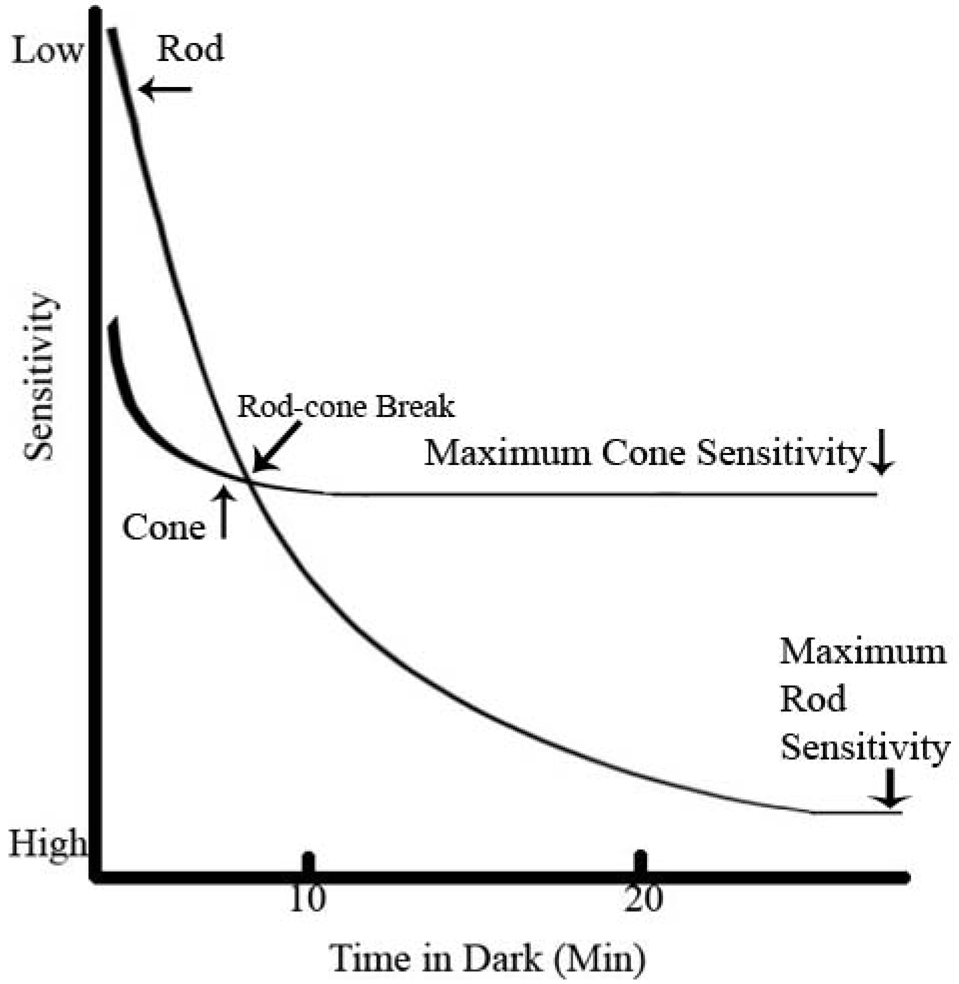
Recovery phase

1) Rhodopsin phosphorylation, retinoid recycling and regeneration - RK
2) Arrestin binding
3) cGMP restoration by guanylate cyclase activation
4) G-protein and PDE6 inactivation by RGS9-1

10^8 rods, 5×10^6 cones

Dark adaptation max in 20 min first 5 min cones threshold, 20 min rods

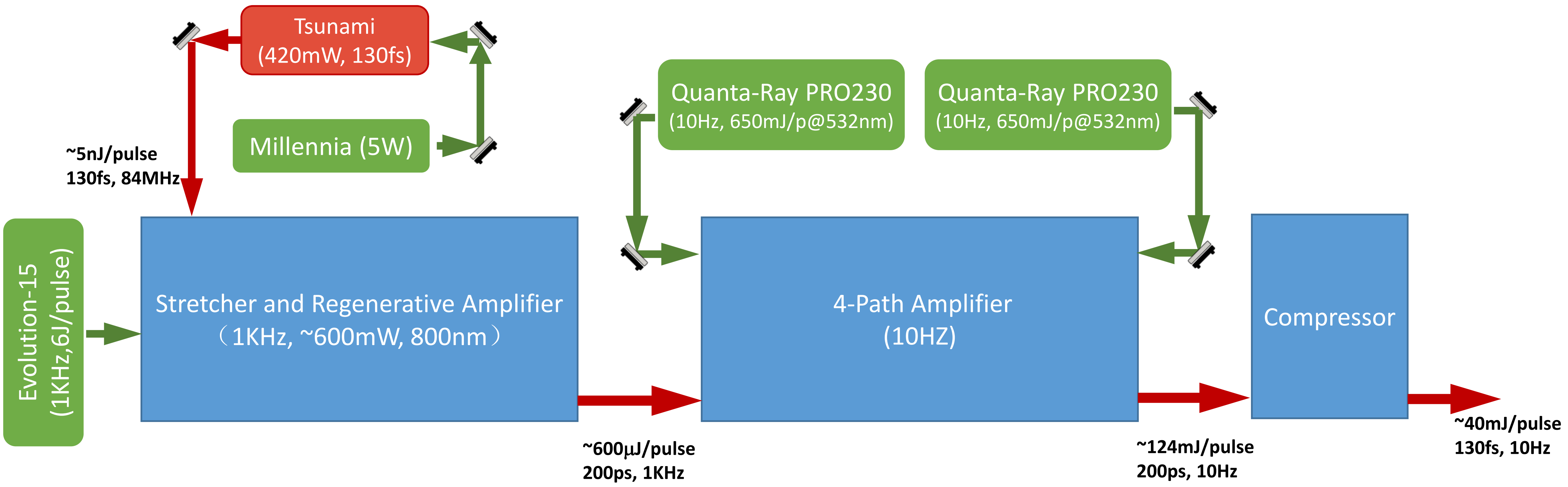



Low
Rod
Sensitivity
Rod-cone Break
Maximum Cone Sensitivity
Cone
Maximum
Rod
Sensitivity
High
10
20
Time in Dark (Min)

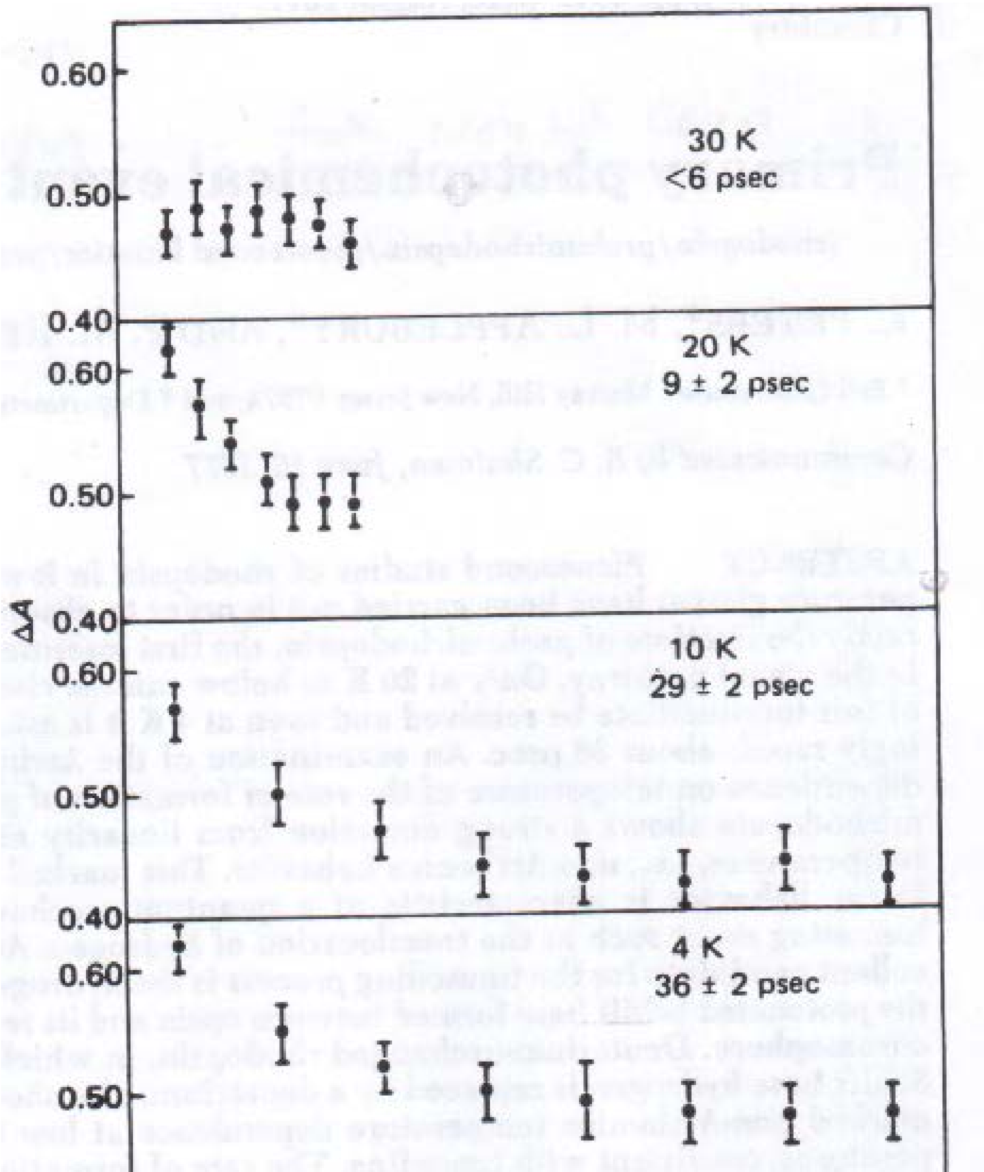
Dark Adaptation Curve

- Cones recover within 5 min while rods take up to 35 min to fully recover sensitivity
- The Rod-Cone break is the point where the rods become more sensitive than the cones
- The two plateaus represent the thresholds of the rods and cones.

Femtosecond Ti:Sapphire Laser System Layout



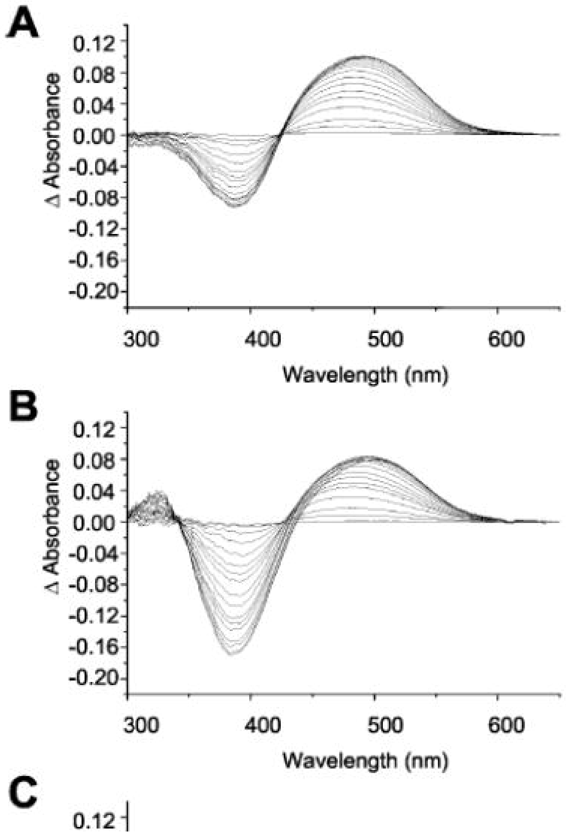
Bathorhodopsin formation at low temperatures

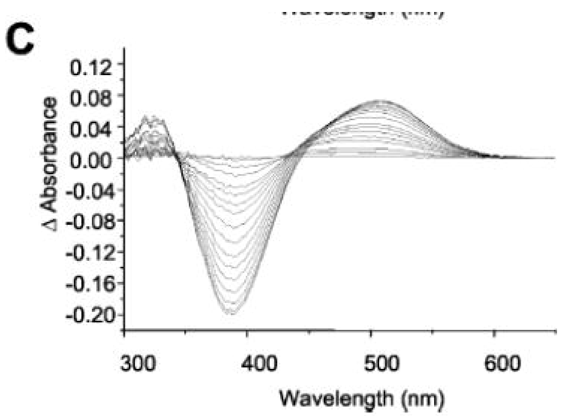


Dynamics of bathorhodopsin formation at temperatures varying from 4K to 30 K

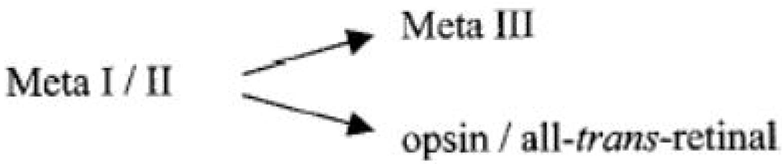
Excitation: 532 nm, 5 mJ, 6 ps pulse

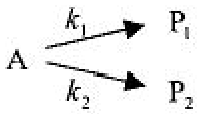
To probe the accessibility of the retinal binding pocket in the decay products, regeneration with 11-*cis*-retinal during Meta I/II decay was followed by UV-visible difference spectroscopy (Fig. 5, *A–C*). The records reflect an increase in absorbance around 500 nm and a parallel decrease around 380 nm, consistent with the formation of rhodopsin at the expense of free 11-*cis*-retinal.





UV-visible difference spectra recorded during decay of Meta II with concurrent regeneration of opsin to rhodopsin with 11-*cis*-retinal





$$[P_1] = \frac{k_1}{k_1 + k_2}[A]_0\left(1 - e^{-(k_1+k_2)t}\right) \quad [P_2] = \frac{k_2}{k_1 + k_2}[A]_0\left(1 - e^{-(k_1+k_2)t}\right)$$

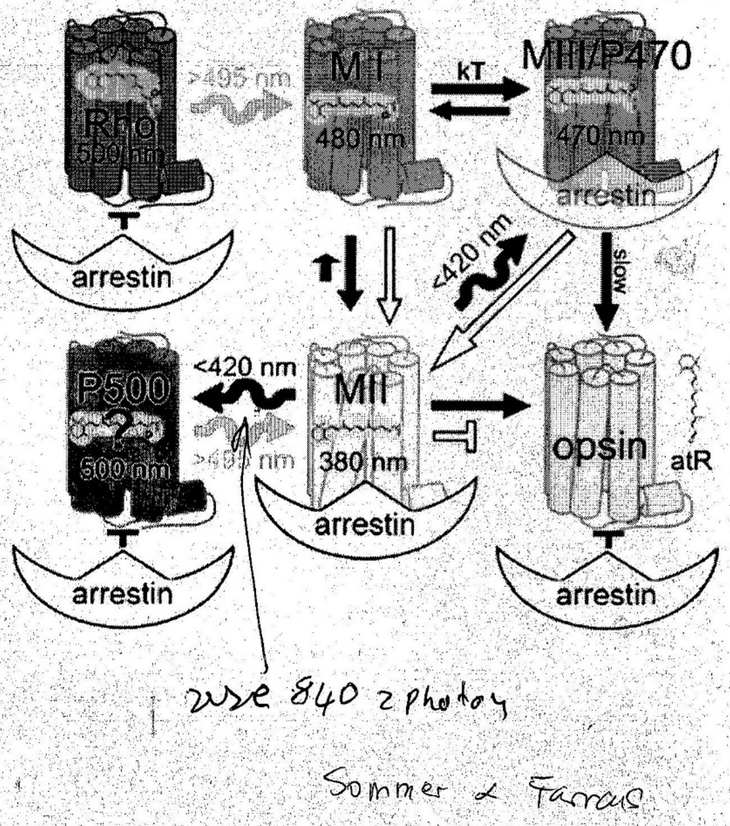
The stability of Meta III is in apparent conflict with the partial loss within minutes of Meta III-like absorbance in membrane preparations (23, 24). We can now suggest that these unstable species were not Meta III but rather NRO-like species, as discussed above. Under cellular conditions, Meta III eventually decays to opsin and all-*trans*-retinal (2–5). Here, other factors (*e.g.* G_t) come into play that may prevent (see this work and Ref. 10) or even reverse (4, 42) Meta III formation. In addition, direct hydrolysis of the Schiff base in Meta III itself was suggested (3, 5, 44) to explain the loss of 470-nm absorbance.

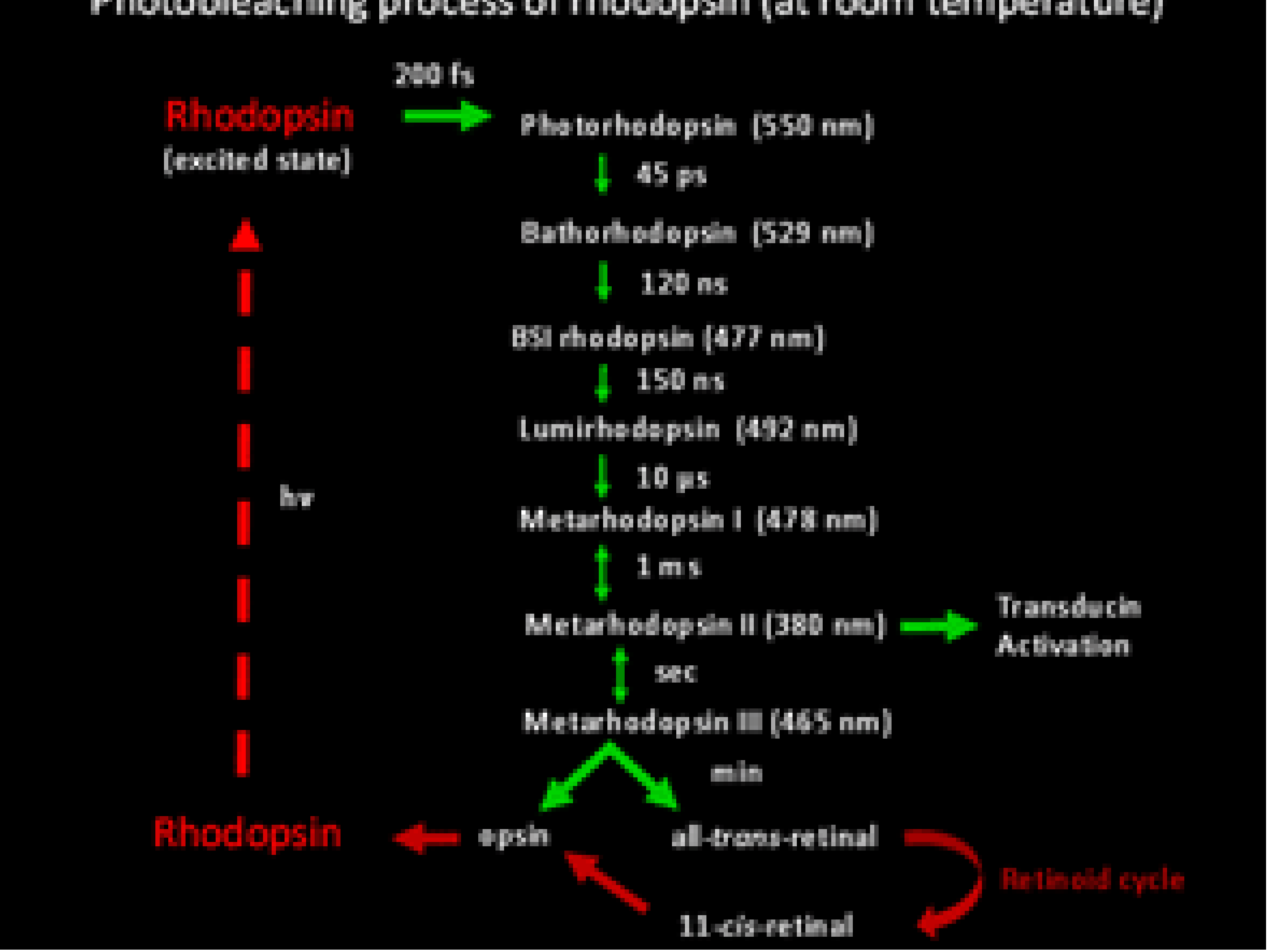
Definitions of Meta III based on visible absorbance around 460–470 nm are ambiguous because of the various products that form on a quite different chemical basis but with similar UV-visible properties. The UV-visible spectro-photometric results are consistent with the presence of a protonated Schiff base.

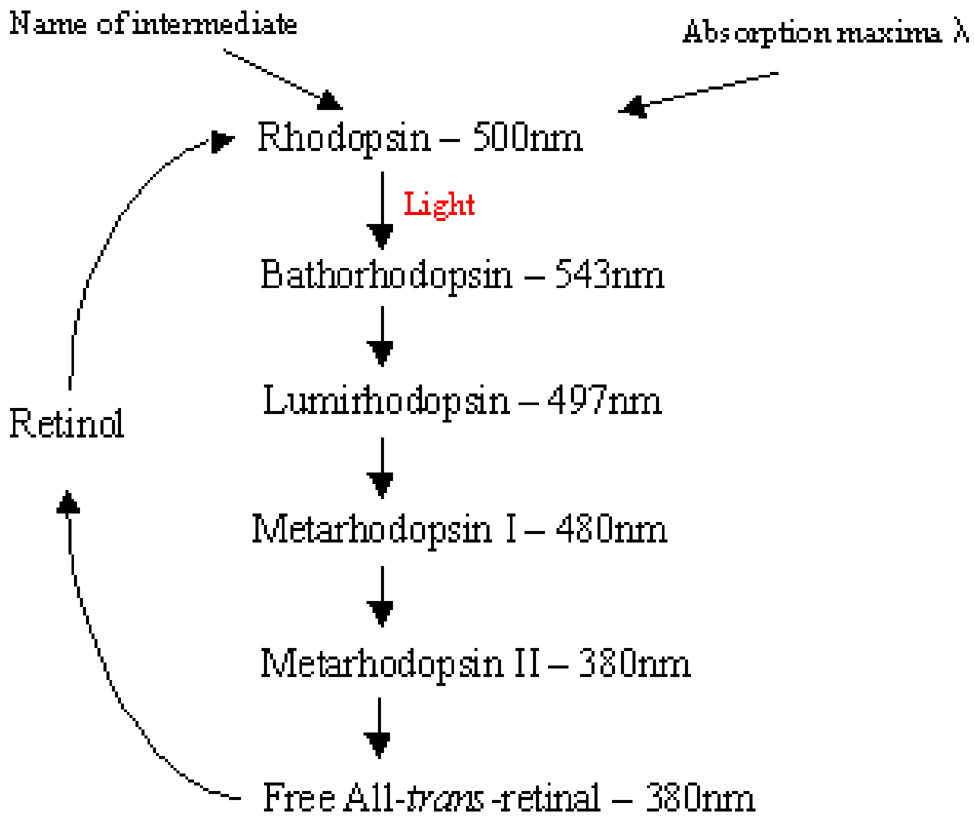
Arrestin as a regulator of Rhodopsin photochemistry

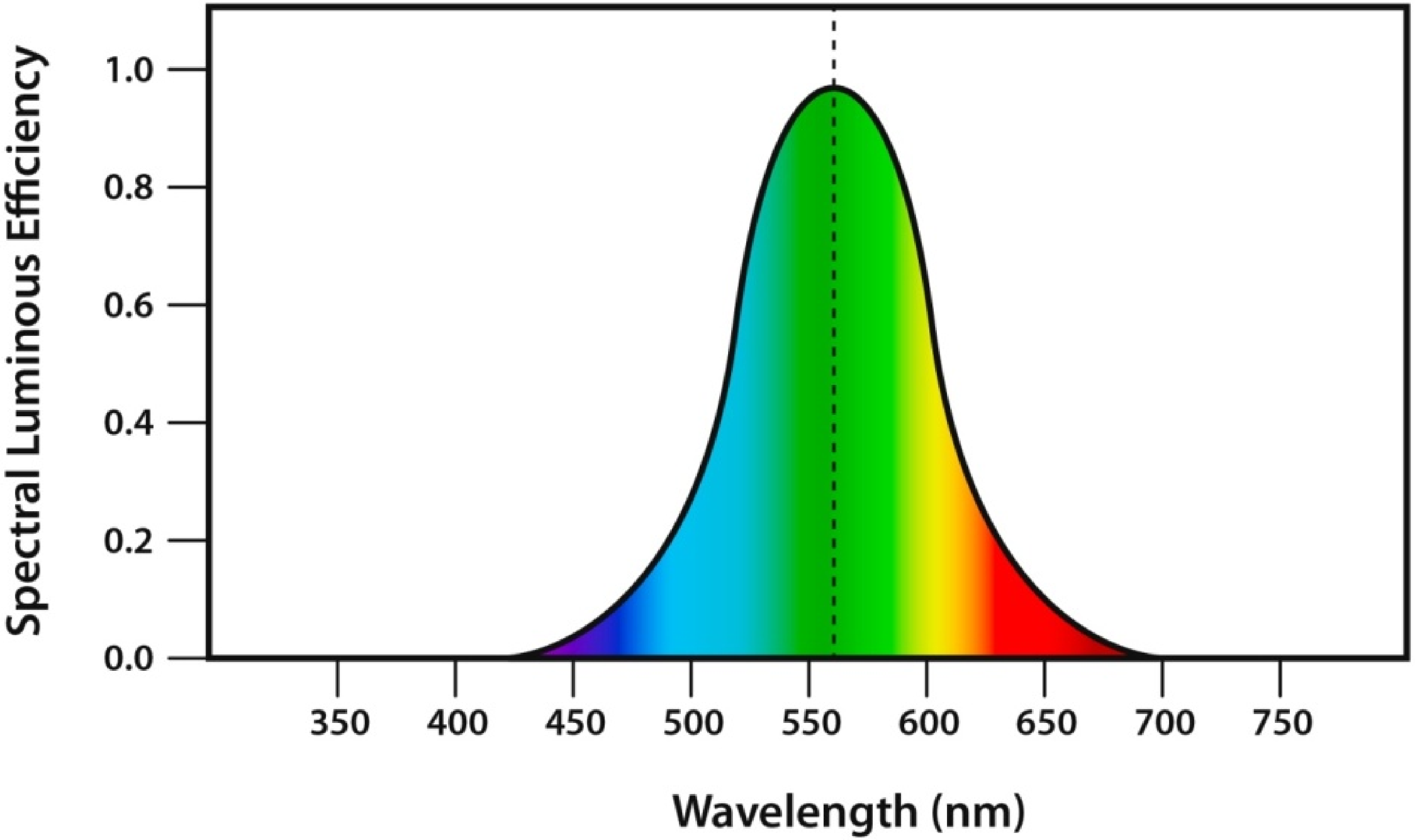
- 48 kDa protein
- Necessary for proper dark adaptation
- Binds light-activated phosphorylated rhodopsin at 500 nm
- Meta I and Meta II exist in equilibrium
- Meta II decays ultimately to opsin and all-trans retinal (atR)
- Meta III slowly decays to opsin and atR not known if this happens directly or through additional intermediates.
- Meta I decays to Meta III.
- The Meta I/Meta III transition is reversible

1. Blue-light irradiation (<420 nm) - Meta II converts to Meta III/ P470 and P500 (Inactive dark state Rhodopsin).
2. Green light can convert P500 back to

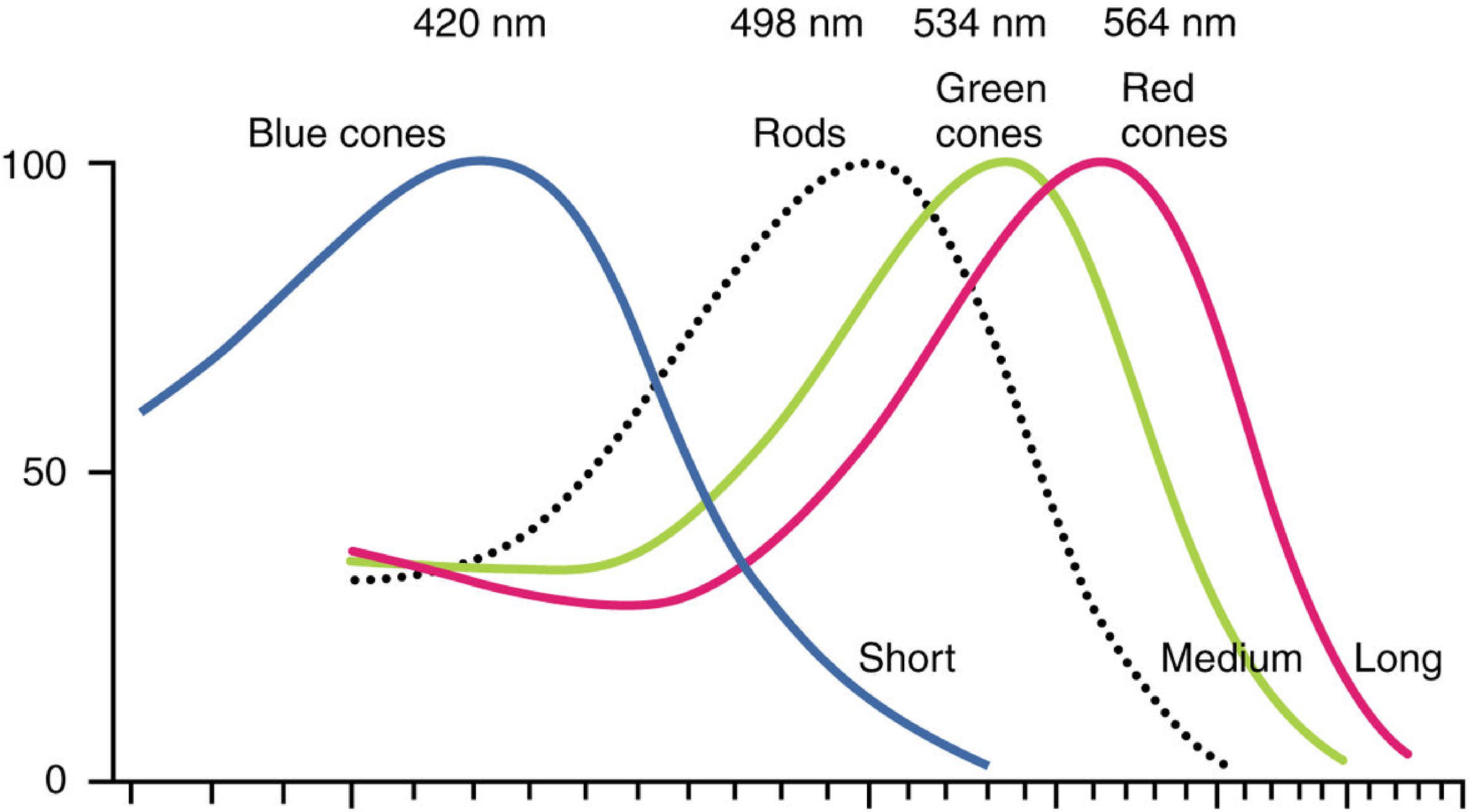



Photobleaching process of rhodopsin (at room temperature)
200 fs
Rhodopsin
(excited state)
Photorhodopsin (550 nm)
45 ps
Bathorhodopsin (529 nm)
120 ns
BSI rhodopsin (477 nm)
150 ns
Lumirhodopsin (492 nm)
10 μs
Metarhodopsin I (478 nm)
1 ms
Metarhodopsin II (380 nm)
Transducin
Activation
sec
Metarhodopsin III (465 nm)
min
hv
Rhodopsin
opsin
all-trans-retinal
Retinoid cycle
11-cis-retinal

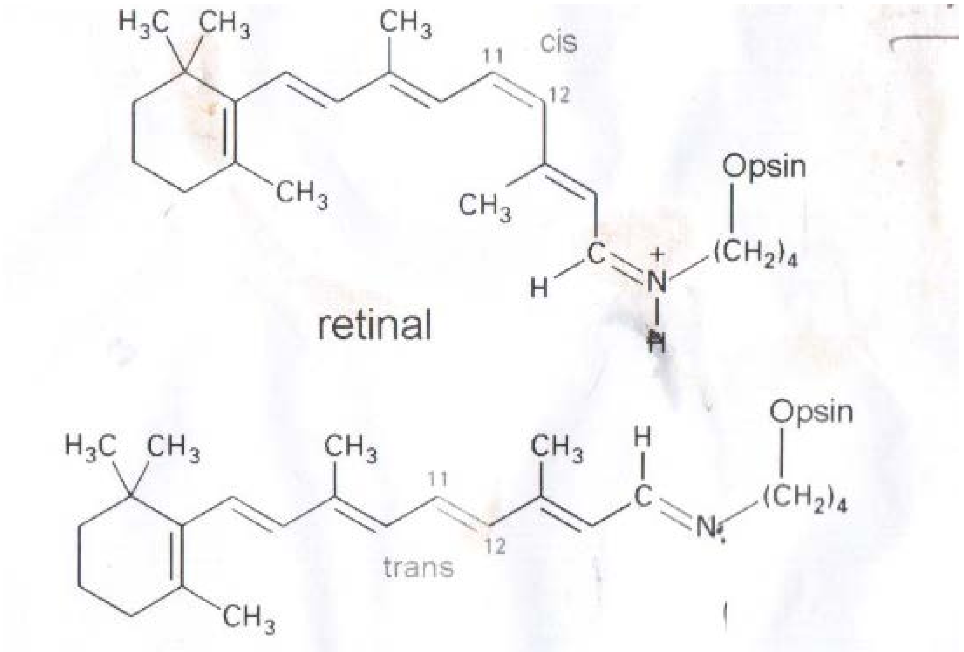

Name of intermediate
Absorption maxima λ
Rhodopsin – 500nm
Light
Bathorhodopsin – 543nm
Lumirhodopsin – 497nm
Metarhodopsin I – 480nm
Metarhodopsin II – 380nm
Free All-*trans*-retinal – 380nm
Retinol

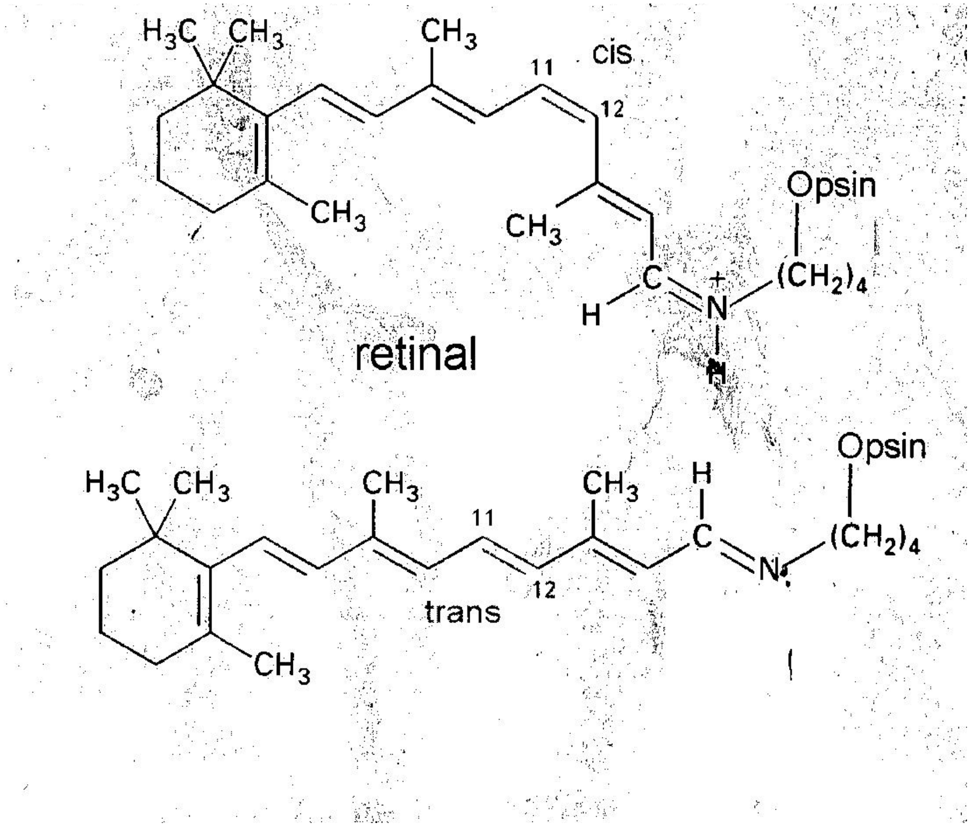


Human visual response



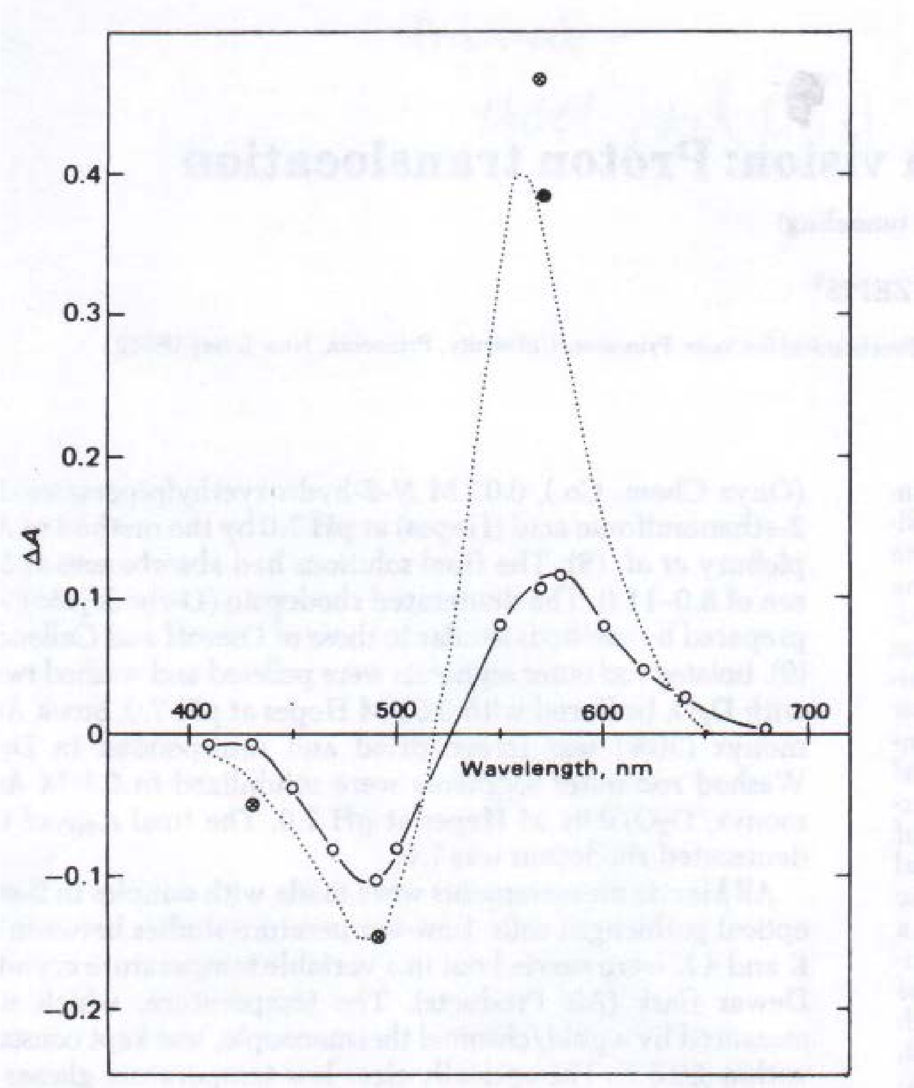
420 nm
498 nm
534 nm
564 nm
Blue cones
Rods
Green cones
Red cones
100
50
0
Short
Medium
Long



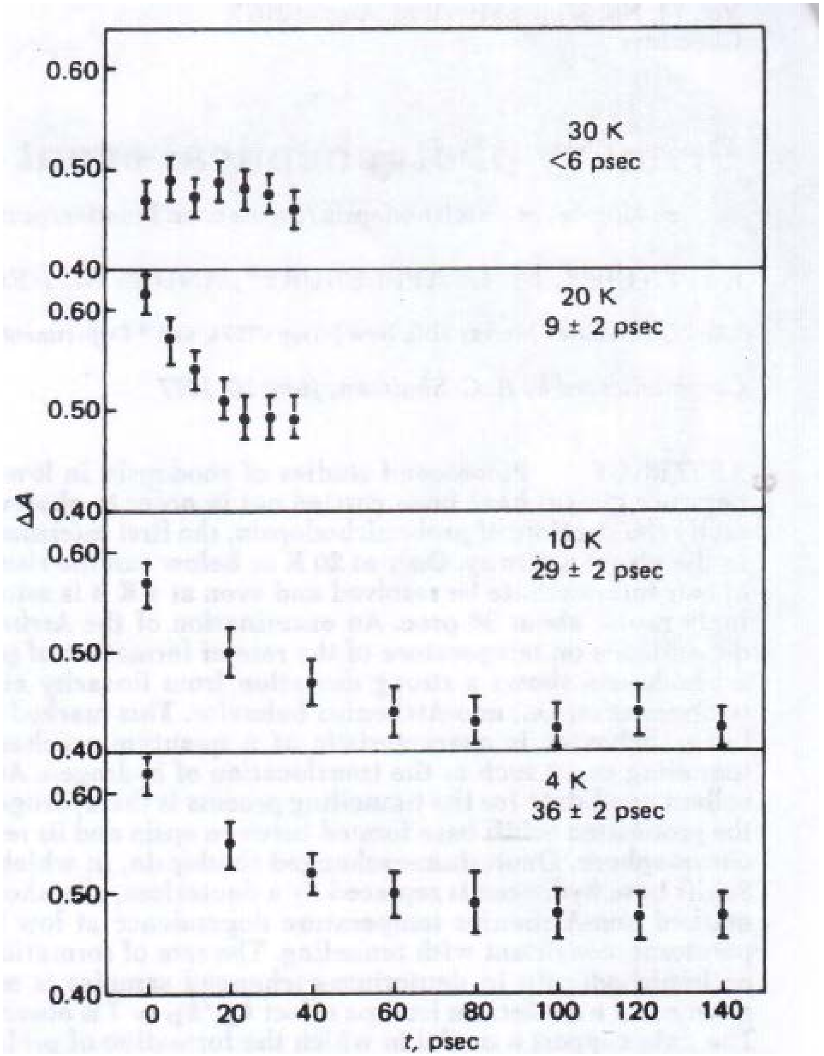


trans N^+

Isomerization and Proton transfer (cis N-H → trans N^+

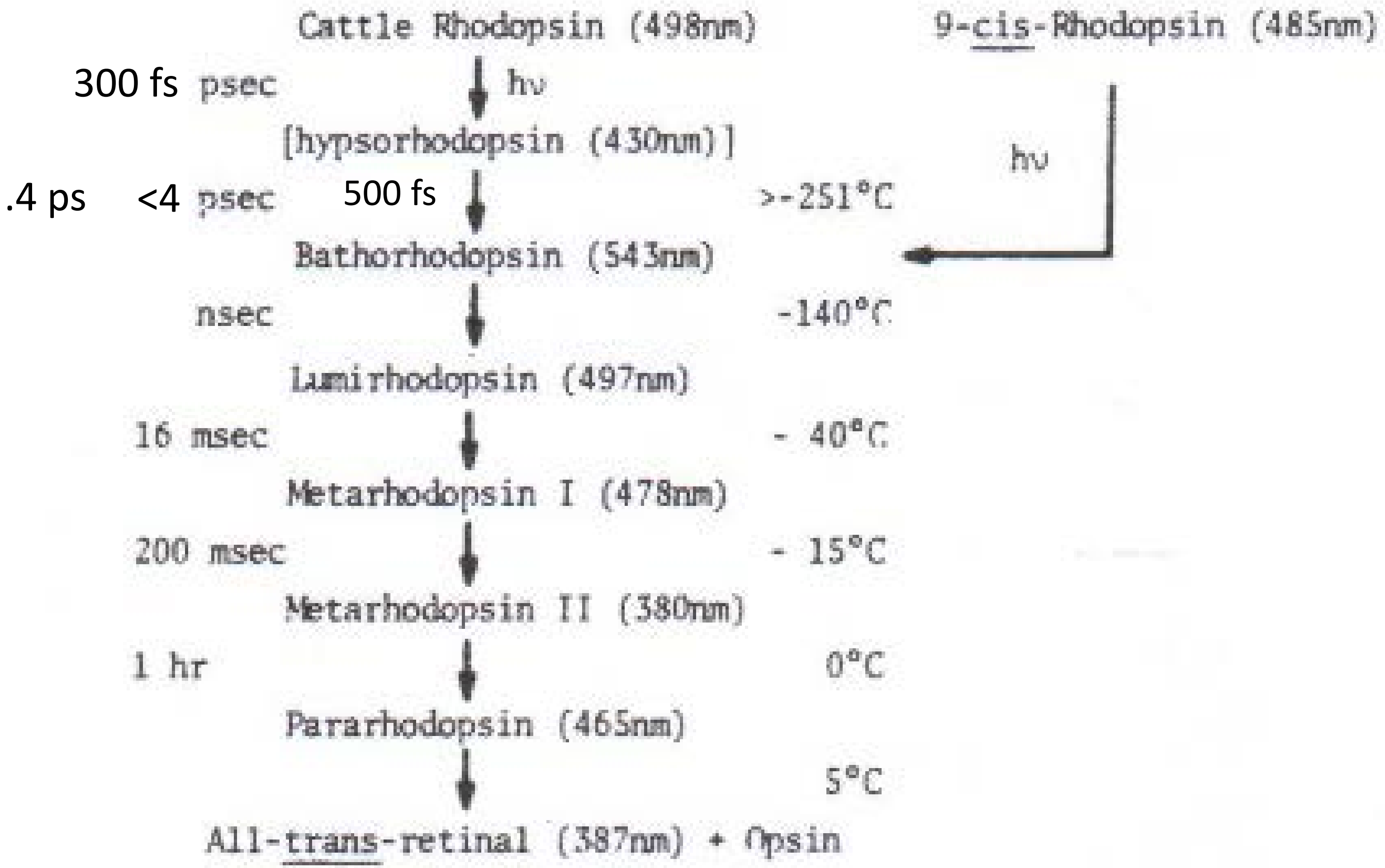


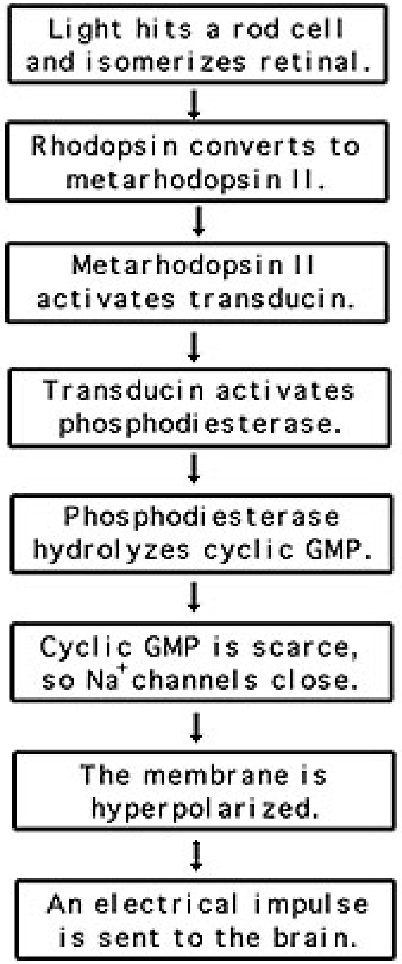
Bathorhodopsin spectra at ● 77K and o 4 K

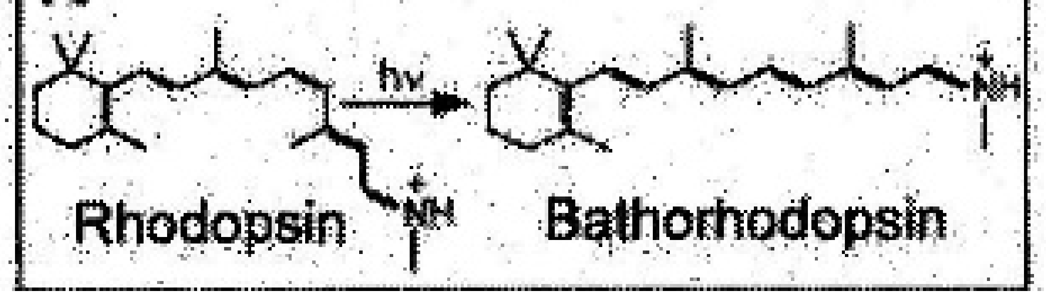


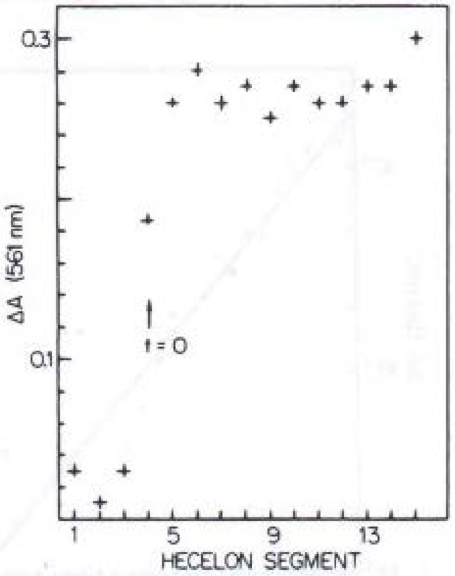
Formation of bathorhodopsin between
4 K (36 ps) and 30 K (<6 ps)

Visual process intermediates









Formation of Bathorhodopsin < 4ps

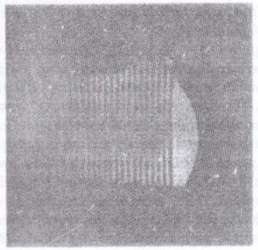
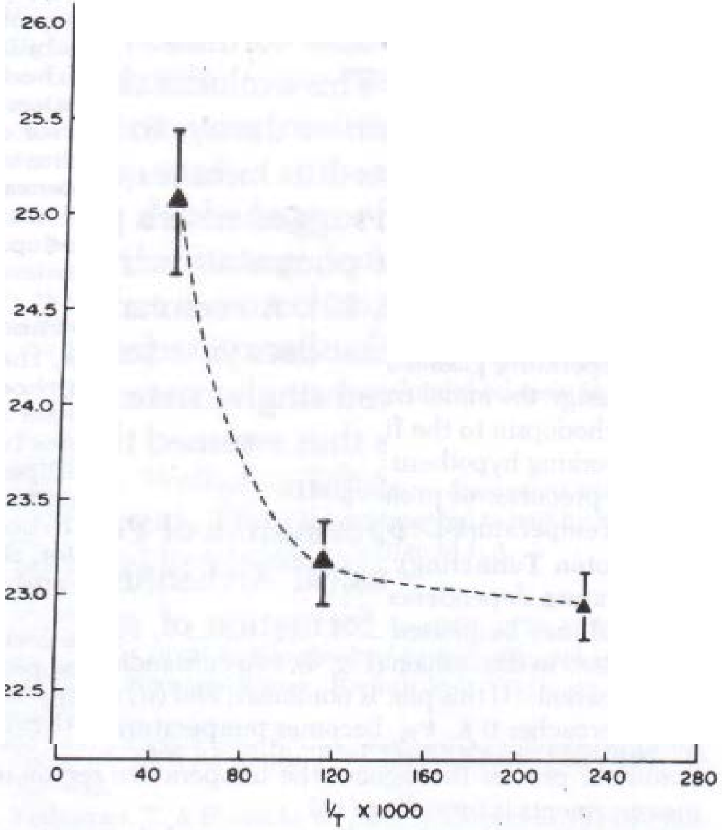
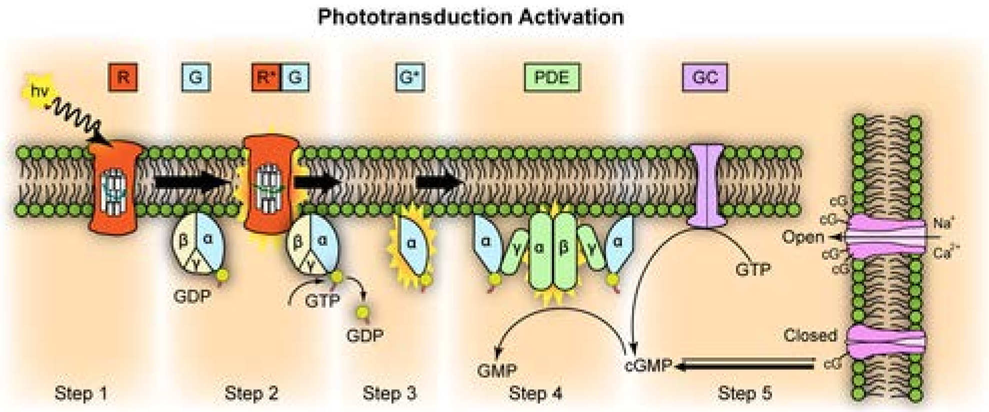
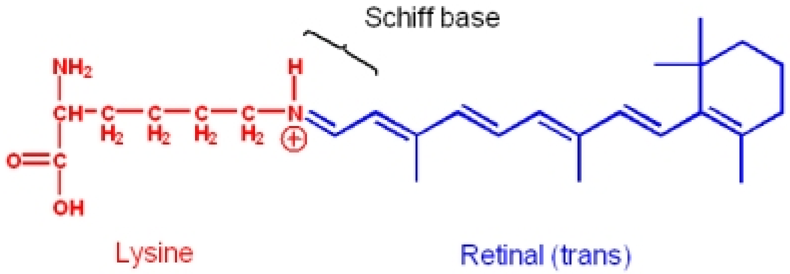


FIG. 4. A photograph similar to that in Fig. 2, but with about 2.5-psec intrasegment time separation. The absorption rises within about 2 segments, which is approximately the risetime of the exciting pulse itself.

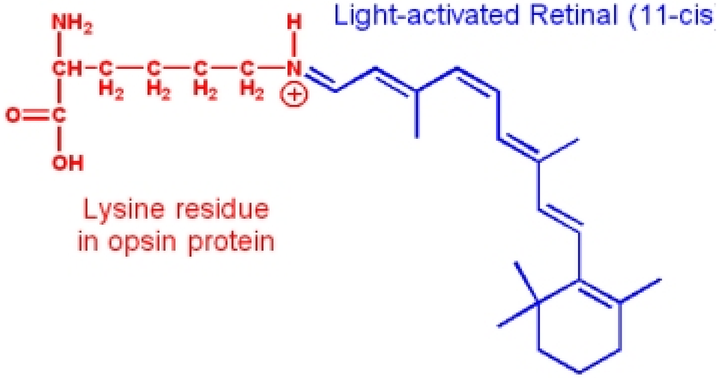


Rhodopsin decay to bathorhodopsin <4 ps
(time resolution at !1972)





Rhodopsin



1. A light photon interacts with the retinal in a photoreceptor cell. The retinal undergoes isomerisation,

2. 11-*cis* to all-*trans* Retinal no longer fits into the opsin binding site.

3. Opsin therefore undergoes a conformational change to metarhodopsin II.

4. Metarhodopsin II is unstable and splits, yielding opsin and all-*trans* retinal. Need?

5. **light-driven H^+ pump**, using the integral membrane protein **bacteriorhodopsin**

•The Schiff base can be **reversibly protonated**

•In response to light, the retinal switches between the ***trans*** and ***cis*** configurations