Speaker – Prof. Khodonov A.A.

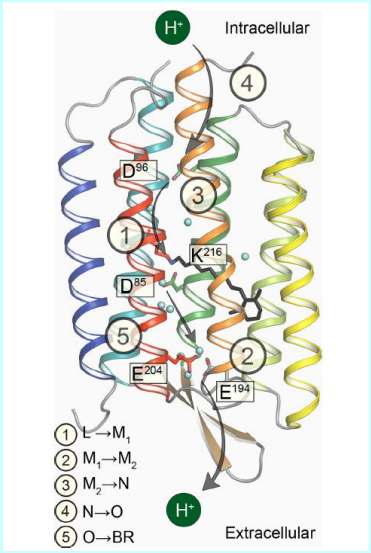
The effect of chromophoric group modifications on the spectral properties of proteorhodopsin from *E. sibiricum* (ESRh)

Belikov N.E.[1], Melnikova I.A.[1,2], Demina O.V.[1], Lukin A.Yu.[2], Petrovskaya L.E.[3], Chupin V.V.[4], Chizhov I.[5], Varfolomeev S.D.[1], Khodonov A.A.[1]*

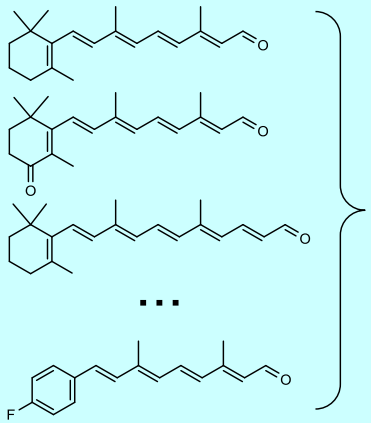
1 – N.M. Emanuel Institute of Biochemical Physics RAS, Moscow, Russia
2 – MIREA – Russian Technological University, Moscow, Russia
3 - Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia
4 – Moscow Institute of Physics and Technology, Dolgoprudny, Russia
5 – Institute for Biophysical Chemistry, Hannover Medical School, Hannover, Germany

**** khodonov@gmail.com***

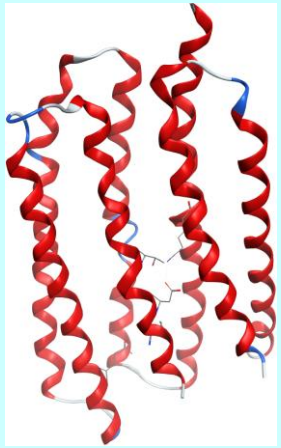
Introduction

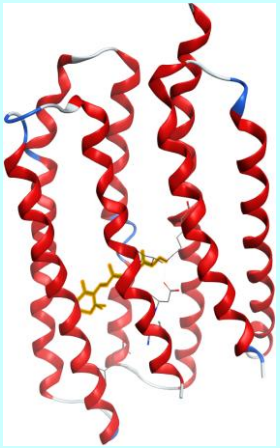


bacteriorhodopsin (BRh)


Retinal analogs
AR1-AR12

+


E. sibiricum opsin

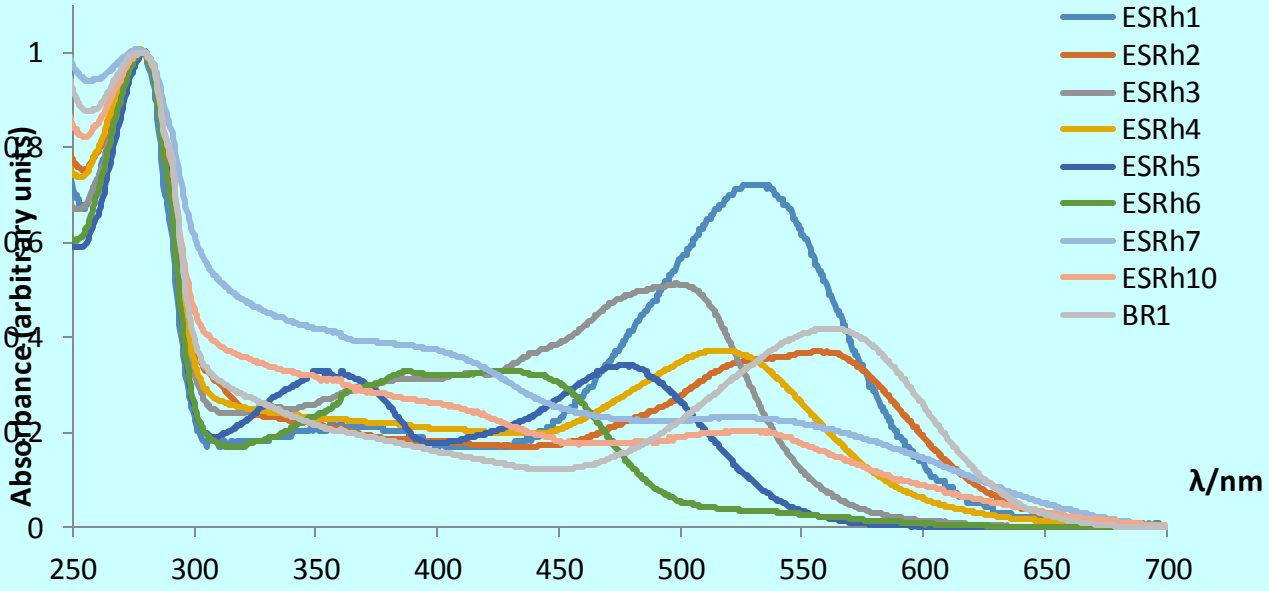

Artificial pigments ESRh

In this work, the following retinal based proteins were studied: **(1) bacteriorhodopsin (BRh),** the well-known light-driven proton pump from the extremely halophilic microorganism *H. salinarum*, for which a whole arsenal of modern methods for determining structure–function relationships has been developed in past 30 years and (2) a new member from the retinal based protein family, the unique **proteorhodopsin, tundra-rhodopsin from microorganism *E. sibiricum* (ESRh)**, which was isolated from permafrost aged at about three million years.

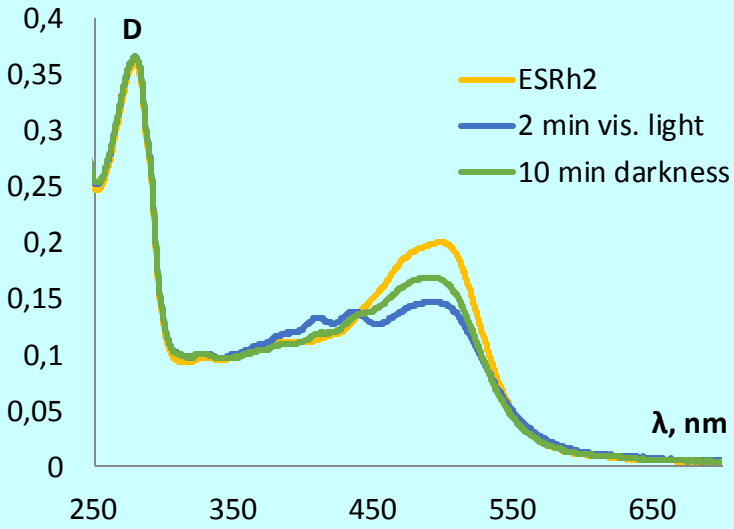
The light driven proton pump ESRh from the psychrotrophic bacterium *E. sibiricum* utilizes Lys96 as a proton donor to a Schiff base that distinguishes it from related retinal proteins – bacteriorhodopsin (BRh) and xanthorhodopsin (XRh), wherein the donor function is performed by a carboxyl side chain. Similarly to other members of the proteorhodopsin family, but unlike BRh, the proton acceptor in ESRh, Asp85, is tightly coupled with His57, which exerts profound influence upon the properties of the acceptor. Light-induced changes in retinal proteins are associated with charge redistribution in the excited retinal chromophore and are driven by the isomerization of chromophore moiety around a 'critical' double bond. **Chromophoric group modifications allow one to tune the spectral properties and other characteristics of microbial rhodopsins, providing their optimization for biomedical and nanotechnological applications**.

In the present study, for the estimation of the influence of the chromophoric group structure on the functional properties of ESRh, expressed in *E. coli*, we used the following modification types of natural *all-E*-retinal **(AR1):** analogs, modified at the ring – 4-oxoretinal **(AR2)**, 3,4-didehydroretinal **(AR3)**, 5,6-dihydro-5,6-epoxyretinal **(AR5)** and 4-fluorophenylretinal **(AR10)**; analog, modified at the polyenic chain – 13-desmethylretinal **(AR4)**, analogs with altered length of polyenic chain – C22 **(AR7),** C25 **(AR8)**, C15 **(AR9)**; the acyclic retinal derivative (without the ionone ring) **(AR6)** .

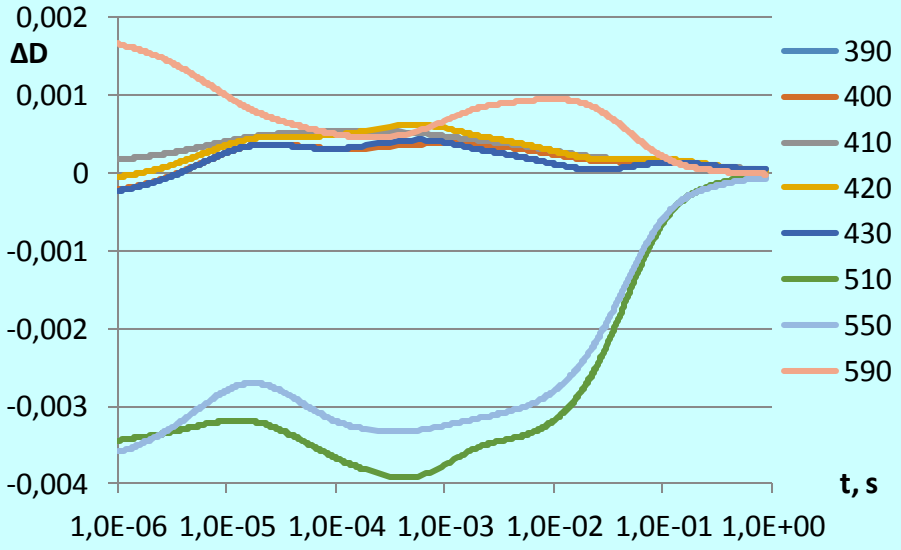
The effects of chromophoric group structures on the functional properties of proteorhodopsin from *E. sibiricum* (ESRh) were studied. ESRh retinal binding site was found as preserving the similar stereo- and spatial restrictions on the chromophore structure during the retinal protein reconstitution process (except for C25-analog **(AR8)** and C15 **(AR9)**). It was revealed that the structure peculiarities of the chromophore analog molecules affect the optical parameters of ESRh and BRh pigment families in similar ways.



UV-vis spectra of artificial pigments ESRh1-10 and BR

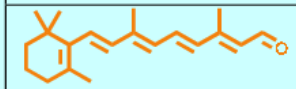
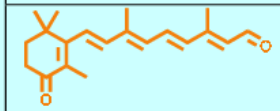
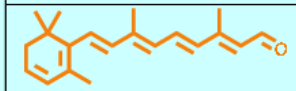
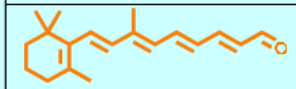
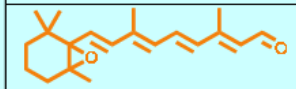
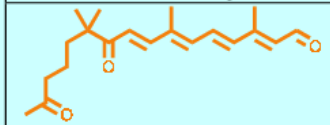
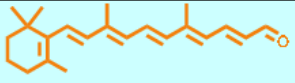
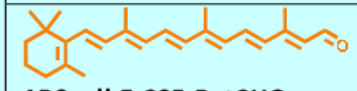
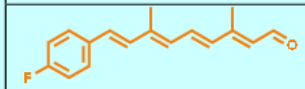


Absorption spectra of ESRh2 before and after irradiation with a halogen lamp light source

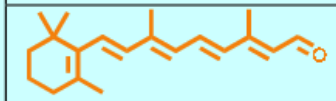
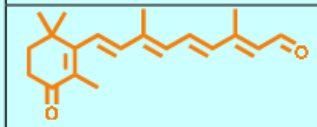
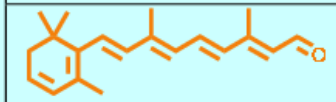
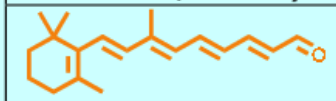
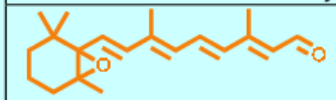
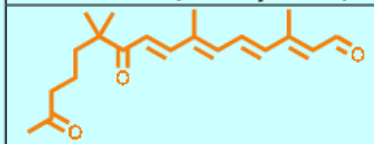
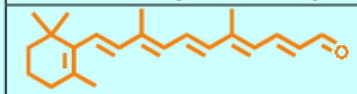
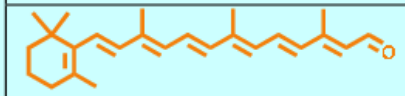
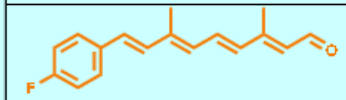


The preliminary multi-exponential global analysis of laser flash photolysis data for ESRh3

Retinal based protein – Bacteriorhodopsin and its analogs

Chromophore analogs RetCHO	λ_{max} RetCHO [ε] EtOH	λ_{max} BR	Photocycle / H^+-transport efficiency	NH_2OH reaction	all-E-RetCHO replacement
 AR1: natural all-E-RetCHO	380[43800]	560 / 570	light-dark adaption + M_{412} τ_{Mform} 50 µs τ_{Mdecay} 5 ms H^+-transport efficiency +++++	Stable in dark	-
 AR2: all-E-4-oxoRetCHO	294[11400], 380[50500]	504 / 527	$M_{410-412}$ τ_{Mdecay} extremely decelerated H^+-transport efficiency ++	Moderately stable in 20 mmol $\tau_{1/2}^{repl}$ 1h	Stable
 AR3: all-E-3,4-didehydroRetCHO	398 [45700]	585 / 600	light-dark adaption + M_{434} H^+-transport efficiency +++++ 66%-70%	Stable in dark	Stable
 AR4: all-E-13-desmethylRetCHO	377	565	M_{420} τ_{Mdecay} decelerated H^+-transport efficiency ++ 16%		
 AR5: all-E-5,6-dihydro-5,6-epoxyRetCHO	362[53500]	452	light-dark adaption no H^+-transport efficiency ++ 3-16%		Stable
 AR6: all-E-5,6-dioxo-5,6-secoRetCHO	269, 370[58400]	450	No photocycle Photodestruction 1 min H^+-transport efficiency 0		Moderately stable $\tau_{1/2}^{repl}$ 2h
 AR7: all-E-C22-RetCHO	392	555	H^+-transport efficiency ++	Unstable	Unstable
 AR8: all-E-C25-RetCHO	400 (in methanol)	460sh 523		-	-
 AR10: all-E-4-Fluorophenyl-RetCHO	388[47700]	524 / 510		-	-

tundra-rhodopsin (ESRh) analogs

Chromophore analogs RetCHO	λ_{max} RetCHO [ε] EtOH	λ_{max} ESRh / Photocycle	NH_2OH reaction	all-E-RetCHO replacement
 AR1: natural all-E-RetCHO	380[43800]	280, ~350, 531	Unstable $\tau_{1/2}^{repl}$ 30 min	-
 AR2: all-E-4-oxoRetCHO	294[11400], 380[50500]	280, 475sh, 499	Unstable $\tau_{1/2}^{repl}$ 1 h	Stable $\tau_{1/2}^{repl}$ 1 day
 AR3: all-E-3,4-didehydroRetCHO	398 [45700]	280, 485sh, 530sh, 558	Moderately stable $\tau_{1/2}^{repl}$ 2 h	Stable
 AR4: all-E-13-desmethylRetCHO	377	280,~410sh, ~360sh, 518	Stable	Unstable $\tau_{1/2}^{repl}$ 15 min
 AR5: all-E-5,6-dihydro-5,6-epoxyRetCHO	362[53500]	280, 353, 370sh, ~425sh, 476	-	Stable $\tau_{1/2}^{repl}$ 5 h
 AR6: all-E-5,6-dioxo-5,6-secoRetCHO	269, 370[58400]	280, 367sh, 388, 428	Unstable $\tau_{1/2}^{repl}$ 30 min	Moderately stable $\tau_{1/2}^{repl}$ 2 h
 AR7: all-E-C22-RetCHO	392	280, ~395sh, 522 Photocycle ?	Unstable $\tau_{1/2}^{repl}$ 15 min	Unstable $\tau_{1/2}^{repl}$ 15 min
 AR8: all-E-C25-RetCHO	400 (in methanol)	Does not form a pigment	-	-
 AR10: all-E-4-Fluorophenyl-RetCHO	388[47700]	280, ~400sh, 527	Moderately stable $\tau_{1/2}^{repl}$ 2 h	Unstable $\tau_{1/2}^{repl}$ 15 min

Research results

1. A family of new tundra-rhodopsin (ESRh) and Bacteriorhodopsin (ABRh) analogs has been prepared. The effects of chromophoric group structures on the functional properties of proteorhodopsin from *E. sibiricum* (ESRh) were studied. It has been shown that recombinant ESRh easily forms artificial pigments with all retinal derivatives in dodecylmaltoside micelles, except for the long-chain C25 and short-chain C15 derivative (see table). The kinetics of formation of ESRh analogs was much faster than that of ABRh.

2. To establish the differences in the photocycle and the mechanism of proton transport of tundra-rhodopsin and bacteriorhodopsin, we carried out a comparative study of the effect of modification of their chromophore groups on the properties of these proteins. Their optical parameters were studied. For the ESRh series: ESRh1-5,7,10 displayed expressed cycles of photoconversions. Both of BRh6 and ESRh6 analogs undergone the irreversible destruction under light illumination.

3. ESRh retinal binding site was found as preserving the similar stereo- and spatial restrictions on the chromophore structure during the retinal protein reconstitution process (except for C25-analog (AR8) and C15 (AR9)). It was revealed that the structure peculiarities of the chromophore analog molecules affect the optical parameters of ESRh and BRh pigment families in similar ways.

4. The accessibility and stability of the protonated aldimine bond in ESRh and its analogs were studied. It has been shown that the resistance to hydrolysis by hydroxylamine in the dark, as well as to the action of all-E-retinal, varies significantly depending on the structure of the analog. The protonated Schiff base bond between retinal analogs formyl group and the Lys residue in the binding site of ESRh is moderately stable against hydrolysis by hydroxylamine in the dark, but slowly undergoes replacement by excess *all-E*-retinal (except (ESRh 4, ESRh 7, ESRh 10).

References

[1] Belikov N.E., Melnikova I.A., Demina O.V., Petrovskaya L.E., Kryukova E.A., Dolgikh D.A., Kuzmichev P.K., Chupin V.V., Lukin A.Yu., Shumsky A.N., Chizhov I., Levin P.P., Kirpichnikov M.P., Varfolomeev S.D., Khodonov A.A. The effect of the chromophoric group modification on the optical properties of retinal proteins. // *Mendeleev Commun.* **28**, 406–408 (2018).

Funding: The reported study was funded by RFBR, project number 20-03-00139