Electronic Supplementary Information for

Role of certain amino acid residues of coelenterazine-binding cavity in bioluminescence of light-sensitive Ca^{2+}-regulated photoprotein berovin

Ludmila P. Burakova[a,§], Galina A. Stepanyuk[a,§], Elena V. Eremeeva[a] and Eugene S. Vysotski[a,*]

[a]*Photobiology Laboratory, Institute of Biophysics, Russian Academy of Sciences, Siberian Branch, Krasnoyarsk, 660036, Russia*

[§]These authors contributed equally to this work.

*Corresponding author:
Fax: +7 (391) 243-3400; Tel: +7 (391) 249-4430;
E-mail: eugene.vysotski@gmail.com or eugene_vysotski@ibp.ru

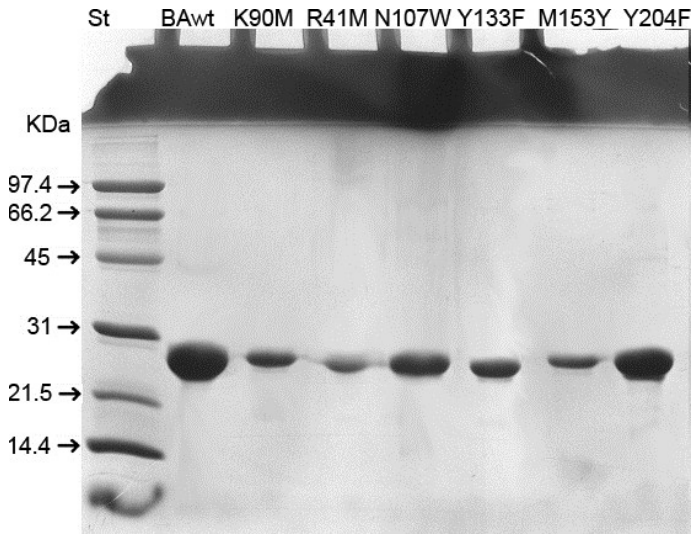

Fig. S1 SDS-PAGE purity analysis of the wild type berovin and its certain mutants after purification on Mono Q column. Lanes: 1, standard proteins; 2, wild type berovin; 3-8, berovin mutants. 12.5% polyacrylamide gel was stained with Coomassie Brilliant Blue.

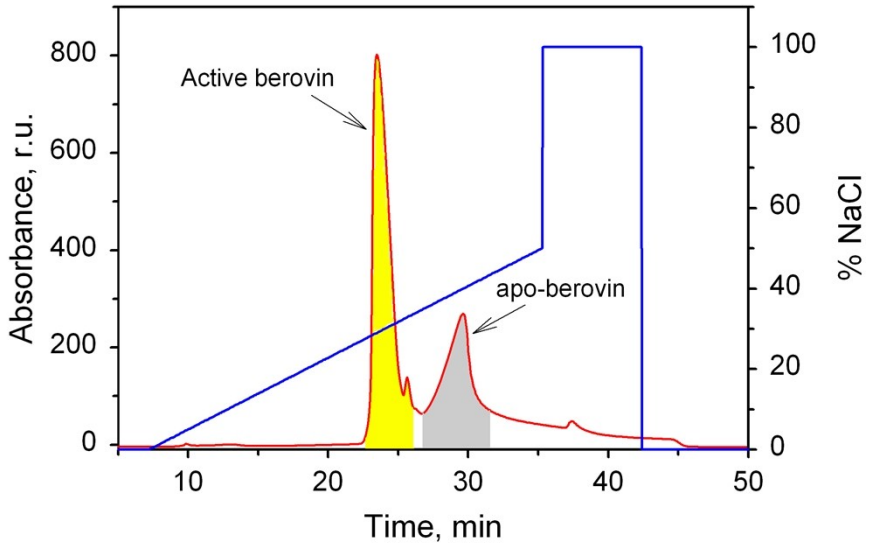

Fig. S2 Example of chromatographic separation of an active photoprotein from apoprotein on Mono Q column. The berovin was eluted with a linear salt gradient of NaCl from 0 to 0.5 M (blue line). The fractions corresponding to the active berovin and apo-berovin are highlighted by yellow and grey, respectively.

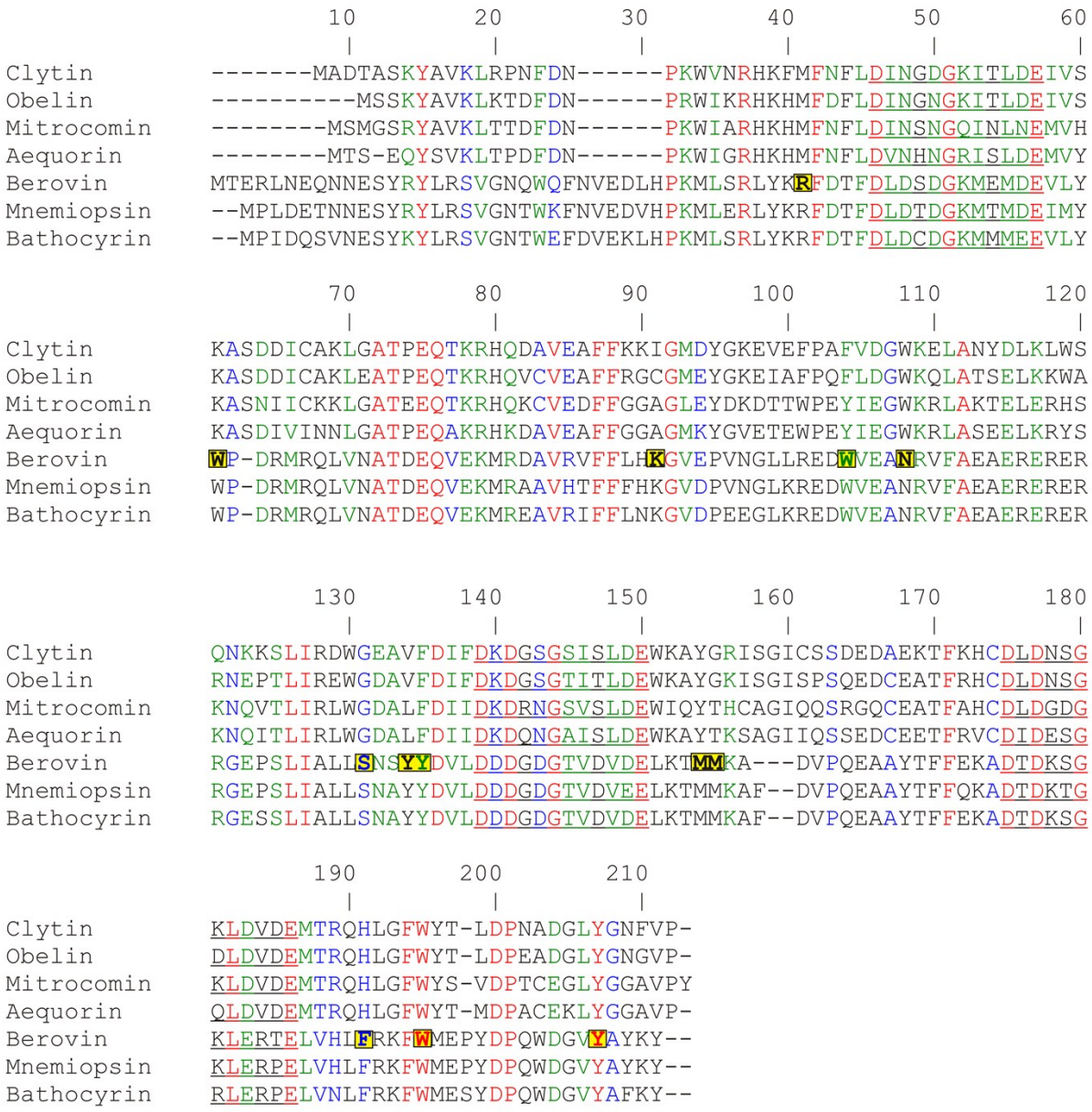

Fig. S3 Comparison of the amino acid sequence of berovin (AFE88612) with those of hydroid photoproteins: obelin from *Obelia longissima* (Q27709), aequorin from *Aequorea victoria* (P07164), clytin from *Clytia gregaria* (CAA49754), mitrocomin from *Mitrocoma cellularia* (AIU48027), and ctenophore photoproteins: mnemiopsin from *Mnemiopsis leidyi* (ADD70248) and bathocyrin from *Bathocyroe fosteri*[29]. Red and blue letters represent identical and similar residues, respectively, black letters show nonsimilar residues. The residues that form the Ca^{2+}-binding site are underlined. Gaps are shown by dashes. The amino acid residues forming the inner coelenterazine-binding cavity of the berovin are highlighted by yellow boxes.

Table S1 The results of 2-hydroperoxycoelenterazine docking

Model	Estimated free energy of bonding (kcal/mol)	Estimated inhibition constant	vdW + H-bond + dissolve energy (kcal/mol)	Electrostatic energy (kcal/mol)	Total intermolecular energy (kcal/mol)	Frequency	Interaction surface
M.UHK	-6.07	35.42 μM	-5.83	-0.24	-6.07	30%	1035.59
M.1JF0	-3.54	2.55 mM	-3.39	-0.14	-3.54	20%	992.883
M.2F8P	-9.05	230.97 nM	-9.05	-0.01	-9.05	100%	1277.823