Electronic Supplementary Information for

## Role of certain amino acid residues of coelenterazine-binding cavity in bioluminescence of light-sensitive Ca<sup>2+</sup>-regulated photoprotein berovin

Ludmila P. Burakova<sup>a,§</sup>, Galina A. Stepanyuk<sup>a,§</sup>, Elena V. Eremeeva<sup>a</sup> and Eugene S. Vysotski<sup>a,\*</sup>

<sup>a</sup>Photobiology Laboratory, Institute of Biophysics, Russian Academy of Sciences, Siberian Branch, Krasnoyarsk, 660036, Russia

<sup>§</sup>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.

	10	20	30	40	50	60
			1	1	1	1
Clytin	MADTASK	YAVKLRPNFDN	JPK	WVN <mark>R</mark> HKFM <mark>F</mark> NF	L <mark>DINGDGKI</mark>	<u>LDE</u> IVS
Obelin	MSSK	YAVKLKTDFDN	JPR	WIK <mark>R</mark> HKHM <mark>F</mark> DF	L <mark>DINGNGKI</mark>	<u>CLDE</u> IVS
Mitrocomin	MSMGSR	YAVKLTTDFD	JPK	WIARHKHMFNF	LDINSNGQIN	JLNEMVH
Aequorin	MTS-EQ	YSVKLTPDFD	JPK	WIGRHKHMFNF	LDVNHNGRIS	<b>SLDE</b> MVY
Berovin	MTERLNEQNNESYF	YLRSVGNQWQE	FNVEDLH <mark>P</mark> K	MLSRLYK <mark>R</mark> FDI	FDLDSDGKME	EMDEVLY
Mnemiopsin	MPLDETNNESYF	YLRSVGNTWKE	NVEDVH <mark>P</mark> K	MLERLYKRFDI	F <mark>DLDTDG</mark> KM1	MDEIMY
Bathocyrin	MPIDQSVNESYK	YLRSVGNTWE	DVEKLH <mark>P</mark> K	MLSRLYKRFDI	FDLDCDGKMN	MEEVLY
	70	80	90	100	110	120
	1	1	Ĩ	1	ĺ.	1
Clytin	KASDDICAKLGATE	EOTKRHODAVE	EAFFKKIGM	DYGKEVEFPAF	VDGWKELANY	DLKLWS
Obelin	KASDDICAKLEATE	EOTKRHOVCVE	CAFFRGCGM	EYGKEIAFPOF	LDGWKQLATS	SELKKWA
Mitrocomin	KASNIICKKLGATE	EOTKRHOKCVE	DFFGGAGL	EYDKDTTWPEY		TELERHS
Aequorin	KASDIVINNLGATE	•EOAKRHKDAVE	EAFFGGAGM	KYGVETEWPEY	IEGWKRL <mark>A</mark> SE	EELKRYS
Berovin	WP-DRMROLVNATE	EOVEKMRDAVE	RVFFLHKGV	EPVNGLLRED	VEANRVFAE	AERERER
Mnemiopsin	WP-DRMROLVNATE	EOVEKMRAAVH	TFFFHKGV	DPVNGLKREDW	IVEANRVFAE	ERERER
Bathocvrin	WP-DRMROLVNATE	EOVEKMREAVE	RIFFLNKGV	DPEEGLKREDW	IVEANRVFAE	ERERER
1	~	~				
	130	140	150	160	170	180
					1	1
Clytin	QNKKSLIRDWGEAV	FDIF <u>DKDGSG</u>	SISLD <mark>E</mark> WKA	YGRISGICSSE	DEDAEKT <mark>F</mark> KHC	DLDNSG
Obelin	RNEPTLIREWGDAV	FDIF <u>DKDGSG</u>	CITLDEWKA	YGKISGISP <mark>S</mark> Q	EDCEATFRHC	DLDNSG
Mitrocomin	KNQVTLIRLWGDAI	FDIIDKDRNGS	SVSLDEWIQ	YTHCAGIQQSF	RGQCEATFAHO	DLDGDG
Aequorin	KNQITLIRLWGDAI	FDII <u>DKDQNG</u>	AISLD <mark>E</mark> WKA	YTKSAGIIQSS	SEDCEETFRVC	DIDESG
Berovin	RGEPSLIALISNS	YDVLDDDGDG1	<u>TVDVDE</u> LKT	MMKADVPQ	EAAYTF <mark>F</mark> EK	DTDKSG
Mnemiopsin	RGEPSLIALLSNAY	YDVLDDDGDG1	<u>IVDVE</u> LKT	MMKAFDVPQ	EAAYTF <mark>F</mark> QK	DTDKTG
Bathocyrin	RGESSLIALLSNAY	YDVLDDDGDG1	TVDVDELKT	MMKAFDVPQ	EAAYTFFEKA	DTDKSG
-						
	190	200	210			
			1			
Clytin	KLDVDEMTRQHLGE	WYT-LDPNADO	GLYGNFVP-			
Obelin	DLDVDEMTRQHLGE	WYT-LDPEADO	GLYGNGVP-			
Mitrocomin	KLDVDEMTROHLGE	WYS-VDPTCE	GL <mark>YG</mark> GAVPY			
Aequorin	QLDVDEMTRQHLGE	WYT-MDPACEF	KL <mark>YG</mark> GAVP-			
Berovin	KLERTELVHI RKF	MEPYDPQWDO	GVYAYKY			
Mnemiopsin	KLERPELVHLFRKF	WMEPYDPQWDO	GVYAYKY			
Bathocyrin	<u>RLERPE</u> LVNLFRKF	WMESYDPQWDO	GV <mark>YA</mark> FKY			

**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*<sup>29</sup>. Red and blue letters represent identical and similar residues, respectively, black letters show nonsimilar residues. The residues that form the Ca<sup>2+</sup>-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.

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

 Table S1
 The results of 2-hydroperoxycoelenterazine docking