

*Supplementary Information*

## **Generation of Red-Shifted Cameleons for Imaging Ca<sup>2+</sup> Dynamics of the Endoplasmic Reticulum. *Sensors* 2015, 15, 13052-13068**

**Markus Waldeck-Weiermair \***, Helmut Bischof, Sandra Blass, Andras T. Deak, Christiane Klec, Thomas Graier, Clara Roller, Rene Rost, Emrah Eroglu, Benjamin Gottschalk, Nicole A. Hofmann, Wolfgang F. Graier and Roland Malli

Institute of Molecular Biology and Biochemistry, Centre of Molecular Medicine, Medical University of Graz, Harrachgasse 21, 8010 Graz, Austria;

E-Mails: bischof.helmut@gmail.com (H.B.); sandra.blass@medunigraz.at (S.B.);

andras.deak@medunigraz.at; (A.T.D.); christiane.klec@medunigraz.at (C.K.);

thomas.graier@medunigraz.at (T.G.); clara.roller@gmx.at (C.R.); rene.rost@medunigraz.at (R.R.);

emrah.eroglu@medunigraz.at (E.E.); benjamin.gottschalk@medunigraz.at (B.G.);

nicole.hofmann@medunigraz.at (N.A.H.); wolfgang.graier@medunigraz.at (W.F.G.);

roland.malli@medunigraz.at (R.M.)

\* Author to whom correspondence should be addressed: markus.weiermair@medunigraz.at;  
Tel.: +43-316-380-7562; Fax: +43-316-380-9615.

---

**Table S1.** List of primers and restriction sites for engineering red-shifted cameleons.

Constructs	Gene	Orientation	Primer Pairs	Restriction Sites
All ER targeted	ER Signal sequence of Calreticulin	N-terminal of all ER targeted sensors	Sense strand: 5'-CTAGCATGCTGCTGCCCCGTCCCCCTGCTGCTGGGCCTGCTGGGCGCCGCCGCGAC-3' Antisense strand: 5'-GTACGACGACGGGCAGGGGGACGACGACCCGGACGACCCGCGGCGGGCTGAGCT-3'	NheI/XhoI
D1ERGR, D1ERGM2	EGFP	N-terminal	Forward: 5'-AATCTCGAGTATGGTGAGCAAGGGCGAGGA-3' Reverse: 5'-GCCATCGATCTTGTACAGCTCGTCCATGC-3'	XhoI/Clal
D1ERGO-Cam2, D1ERRG-Cam2, D1ERTG, D1ERmR2G	EGFP <sub>KDEL</sub>	C-terminal	Forward: 5'-CGGGAATTCATGGTGAGCAAGGGCGAGGA-3' Reverse: 5'-GCAAGCTTACAGCTCGTCCTTGTACAGCTCGTCCATGC-3'	EcoRI/HindIII
D1ERRG-Cam1, D1ERRG-Cam2, D1ERRC	TagRFP	N-terminal	Forward: 5'-GGCTCGAGTATGGTGTCTAAGGGCGAAGAG-3' Reverse: 5'-GGATCGATATTAAGTTTGTGCCCCAGTTTGC-3'	XhoI/Clal
D1ERGR, D1ERCRCR	TagRFP <sub>KDEL</sub>	C-terminal	Forward: 5'-TCGGAATTCATGGTGTCTAAGGGCGAAGAG-3' Reverse: 5'-GCGAAGCTTACAGCTCGTCCTTATTAAGTT TGTGCCCCAG-3'	EcoRI/HindIII
D1ERTG	tdTomato	N-terminal	Forward: 5'-AATCTCGAGTATGGTGAGCAAGGGCGAGGA-3' Reverse: 5'-CGAATCGATCTTGTACAGCTCGTCCATGC-3'	XhoI/Clal
D1ERCRCR, D1ERCmR2, D1CmR2, mtD1CmR2	Clover	N-terminal	Forward: 5'-AATCTCGAGTATGGTGAGCAAGGGCGAGGA-3' Reverse: 5'-AGAATCGATAGCTCGAGATCTGAGTCCGGC-3'	XhoI/Clal
D1ERRC, D1ERmR2C	Clover <sub>KDEL</sub>	C-terminal	Forward: 5'-AGGGAATTCATGGTGAGCAAGGGCGAGGAG-3' Reverse: 5'-AGCAAGCTTACAATTCGTCTTAGCTCGAGATCTGAGTCCGGC-3'	EcoRI/HindIII
D1ERmR2G, D1ERmR2C	mRuby2	N-terminal	Forward: 5'-ACTCTCGAGTATGGTGTCTAAGGGCGAAGAGC-3' Reverse: 5'-AGCATCGATCTTGTACAGCTCGTCCATCCCAC-3'	XhoI/Clal
D1ERGM2, D1ERCmR2	mRuby2 <sub>KDEL</sub>	C-terminal	Forward: 5'-AGCGAATTCATGGTGTCTAAGGGCGAAGAGC-3' Reverse: 5'-GACAAGCTTACAGCTCGTCCTTCTTGTACA GCTCGTCCATCC-3'	EcoRI/HindIII
D1CmR2, mtD1CmR2	mRuby2	C-terminal	Forward: 5'-AGCGAATTCATGGTGTCTAAGGGCGAAGAGC-3' Reverse: 5'-GCCAAGCTTACTTGTACAGCTCGTCCATCC-3'	EcoRI/HindIII

**Table S2.** Properties of fluorescent proteins.

Colour	Protein	Length	$\lambda_{Exc}(nm)$	$\lambda_{Em}(nm)$	Brightness *	Photostability	pKA	Ref.
Cyan	ECFP	238	435	478	13	64	5.5	[1]
Yellow	Citrine	238	516	529	59	49	5.7	[2]
Green	EGFP	238	488	507	34	174	6.0	[2]
	Clover	232	505	515	84	50	6.1	[3]
Orange	mKO	217	548	559	31	122	5.0	[1]
Red	TagRFP	237	555	515	48	48	3.8	[4]
	Td-tomato	476	554	581	95	98	4.7	[2,4]
	mRuby2	238	559	600	43	123	5.3	[3]

\* Brightness: Molar Extinction Coefficient  $\times$  Fluorescence Quantum Yield/1000.

## References

1. Karasawa, S.; Araki, T.; Nagai, T.; Mizuno, H.; Miyawaki, A. Cyan-Emitting and Orange-Emitting Fluorescent Proteins as a Donor/Acceptor Pair for Fluorescence Resonance Energy Transfer. *Biochem. J.* **2004**, *381*, 307–312.
2. Shaner, N.C.; Steinbach, P.A.; Tsien, R.Y. A Guide to Choosing Fluorescent Proteins. *Nat. Methods* **2005**, *2*, 905–909.
3. Lam, A.J.; St-Pierre, F.; Gong, Y.; Marshall, J.D.; Cranfill, P.J.; Baird, M.A.; McKeown, M.R.; Wiedenmann, J.; Davidson, M.W.; Schnitzer, M.J.; *et al.* Improving FRET Dynamic Range with Bright Green and Red Fluorescent Proteins. *Nat. Methods* **2012**, *9*, 1005–1012.
4. Shaner, N.C.; Lin, M.Z.; McKeown, M.R.; Steinbach, P.A.; Hazelwood, K.L.; Davidson, M.W.; Tsien, R.Y. Improving the Photostability of Bright Monomeric Orange and Red Fluorescent Proteins. *Nat. Methods* **2008**, *5*, 545–551.

© 2015 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/4.0/>).