Supplemental Data

Conversion of Red Fluorescent Protein

into a Bright Blue Probe

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	10	ט	20	30	40	50	60
EGFP	MVSKGEELFT	GVVPILVE	LDGDVNGHR	FSVSGEGEGD	ATY-GKLTL	KFICTT-GKLP	VPWPTL
mCherry	MVSKGEEDNMAIIK	EFMRFKVH	MEGSVNGHE	FEIEGEGEGR	P-YEGTQTA	KLKVTKGGPLP	FAWDII
mCherry-Blue	MVSKGEEDNMAIIK	EFMRFKVH	MEGSVNGHE	FEIEGEGEGR	P-YEGTQTA	KLKVTKGGPLP	FAWDII
HcRed1	MVS-GLLK	ESMRIKMYN	MEGTVNGHY	FKCEGEGDGN	P-FAGTQSM	RIHVTEGAPLP	FAFDII
HcRed1-Blue	MVS-GLLKI	ESMRIKMYN	MEGTVNGHY	FKCEGEGDGN	P-FAGTQSM	RIHVTEGAPLP	FAFDII
M355NA	MAS LLTI	ETMPFRTT:	LEGTVNGHY	FKCTGKGEGN	P-LEGTQEM	KIEVIEGGPLP	FAFHII
M355NA-Blue	MAS LLTI	STMPFRTT:	LEGTVNGHY	FKCTGKGEGN	P-LEGTQEM	KIEVIEGGPLP	FAFHII
mKeima	MVSVIA	KQMTYKVYI	MSGTVNGHY	FEVEGDGKGK	P-YEGEQTV	KLTVTKGGPLP	FAWDII
mKeima-Blue	MVSVIAN	KQMTYKVYI	ISGTVNGHY	FEVEGDGKGK	P-YEGEQTV	KLTVTKGGPLP	FAWDII
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	*** '	70	80	90	100	110	120
EGFP	VTTFTYGV	OCFSRYPDI	HMKOHDFFF	SAMPEGYVOE	RTIFFKDDG	NYKTRAEVKFE	GDTLV
mCherry	SPQFMYGSI	KAYVKHPAI	DIPDYLF	LSFPEGFKWE	RVMNFEDGG	VVTVTQDSSLQ	DGEFI
mCherry-Blue	SPQF <mark>L</mark> YGSI	KAYVKHPAI	DIPDYLF	LSFPEGFKWE	RVMNFEDGG	VVTVTQDSSLQ	DGEFI
HcRed1	APCCEYGSI	RTFVHHTA I	SIPDFFF	QSFPEGFTWE	RTTTYEDGG	ILTAHQDTSLE	GNCLI
HcRed1-Blue	APCC <mark>H</mark> YGSI	TFVHHTAI	SIPDF <mark>W</mark> F	QSFPEGFTWE	RTTTYEDGG	ILTAHQDTSLE	GNCLI
M355NA	STSCMYGSI	KAFIKYVS	GIPDYFF	QSLPEGFTWE	RTTTYEDGG	FLTAHQDTSLD	GDCLV
M355NA-Blue	STSC <mark>H</mark> YGSI	KAFIKYVSO	GIPDYFF	QSLPEGFTWE	RTTTYEDGG	FLTAHQDTSLD	GDCLV
mKeima	SPQLQYGS	IPFTKYPEI	DIPDYFF	QSFPEGYTWE	RSMNFEDGA	VCTVSNDSSIQ	GNCFI
mKeima-Blue	SPQL <mark>H</mark> YGSI	<mark>PFTKYPE</mark> I	DIPDY <mark>W</mark> F	QSFPEGYTWE	RSMNFEDGA	VCTVSNDSSIQ	GNCFI
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	13	30	140	150	160	170	180
EGFP	NRIELKGII	FKEDGNI	LGHKLEYNY	NSHNVYIMAD	KQKNGIKVN	FKIRHNIEDGS	VQLAD
mCherry	YKVKLRGTI	NFPSDGPVI	NQ-KKTMGV	VEASSERMYP-	-EDGALKGE	IKQRLKLKDGG	HYDAE
mCherry-Blue	YKVKLRGTI	NFPSDGPVI	1Q-KKTMGV	VEA <mark>F</mark> SERMYP-	-EDGALKGE	IKQRLKLKDGG	HYDAE
HcRed1	YKVKVLGTI	NFPADGPVI	K-NKSGGV	VEPSTEVVYP-	-ENGVLCGR	NVMALKVGDR-	HLICH
HcRed1-Blue	YKVKVLGTI	NFPADGPVI	K-NKSGGV	VEP <mark>I</mark> TEVVYP-	-ENGVLCGR	AVMALKVGDR-	HLICH
M355NA	YKVKILGNI	NFPADGPVI	NC-NKAGRV	EPSTEIVYE-	-VDGVLRGQ	SLMALECPGGR	HLTCH
M355NA-Blue	YKVKILGNI	NFPADGPVI	NQ-NKAGRV	NEP <mark>R</mark> IEIVIE-		ALMALECPGGR	HLTCH
mKeima mKeima Dlue	YNVKISGEI	NFPPNGPVI	NQ-KKTQGV	EPSTERLFA-	- RDGMLIGN	DYMALKLEGGG	HYLCE
mkeima-Biue	INVKISGEI	NF PPNGPVI	NQ-KKTQGV ∽	VEP <mark>I</mark> TERLFA-	-RDGMLIGN		HILCE
		> _	$\langle \rangle =$			\equiv	
	190	ט	200	210	220	230	
EGFP	HYQQNTPIGI	D-GPVLLPI	DNHYLSTQS	SALSKDPNEKR	DHMVLLEFV	TAAGITHGMDE	LYK
mCherry	VKTTYKAKK	PVQLPC	JAYNVNIKI	DITSHNEDYT	-IVEQYERA	EGRHSTGGMDE	LYK
mcnerry-Blue	AKTTYKAKK ·		JAYNVN <mark>Y</mark> KI	DITSHNEDY1	- IVEQYERA	EGRHSTGGMDE	LYK
HCREQI Hapadi Diwa	HYTSYRSKKA AVECVDCKKA		FHFTDIRI	QMLRKEKDE -	- IFELIEAS	VARISDLPEKA VARISDLPEKA	.N N
MOREGI-BIUE		AVRALIMP	JEHERDUDI	QMLRKERDE-	- IFELIEAS	VARISDLPERA	
M355NA-Blue	TUTTVDQVVI		JEHEEDUKI JEHEEDUKI	ETLEEVERGE ETLEEVERGE	-CINQIDAA	VGRICDAAPSK	T.CHN
mKeima	THE TROKE		SEHELDERI 1919 - Selevi	DVTSHNRD	VTSVEOC	ETATAPHGI.I.C	
mKeima-Blue	AKSTYKAKK	PVRMP	GRHEIDKKI	DVTSHNRD	YTSVEOC	ETATARHSLIG	ļ
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Figure S1. Alignment of the Amino Acid Sequences of the Best Blue Mutants of mCherry, HcRed1, M355NA, and mKeima with Their Red Analogs and EGFP

Structurally important regions are highlighted in grey, β -strands are shown with arrows, α -helixes are shown with ribbons. The chromophore forming residues are marked with asterisks. Site-specific mutations resulted in conversion of RFP into the blue mutants are shown in red. The alignment numbering follows that for EGFP.



Figure S2. mTagBFP- β -Actin and mTagBFP- α -Tubulin Fusion Proteins Expressed in Mammalian Cells

(a and b) Live HeLa cells expressing (a) mTagBFP- β -actin and (b) mTagBFP- α -tubulin fusion constructs were imaged 48 hours after transfection using Leica AFLX 6000 inverted fluorescent microscope equipped with 63x glycerol objective lens. The fusions exhibit the monomeric behavior and are well incorporated into the endogenous cytoskeleton structures. Statistical quality of mTagBFP in fusions with β -actin and α -tubulin was compared to that of the respective EGFP fusions for approximately 200 transfected cells. Both EGFP and mTagBFP fusions gave about the same 70% of positive cells determined as clear visibility of prolonged filaments in the case β -actin or microtubules in the case of α -tubulin EGFP and mTagBFP fusions.



Figure S3. Determination of Quantum Yields for mTagBFP and EBFP2

The linear plots of integral fluorescence versus absorbance for mTagBFP and EBFP2. The gradient for each fluorescent protein is proportional to the quantum yield. The experiments were repeated three times.



Figure S4. Alignment of the Amino Acid Sequences of mTagGFP with EGFP and TagGFP Structurally important regions are highlighted in grey, beta-strands are shown with arrows, and alphahelixes are shown with ribbons. The chromophore forming residues are marked with asterisks.

Monomerizing A203K mutation in mTagGFP is shown yellow. Mutations induced by site-directed saturated mutagenesis that enhance mTagGFP maturation rate and extinction coefficient are shown green. The sequence differences of TagGFP and mTagGFP from EGFP are highlighted by cyan. The alignment numbering follows that for EGFP.





Normalized absorbance (black curve), fluorescence excitation (blue curve) and fluorescence emission (dark green curve) spectra for freshly purified mTagGFP are shown. Maxima of the absorbance, excitation and emission spectra are indicated.





Dashed lines indicate maturation half-times of 11 min and 18 min for mTagGFP (dark green curve) and TagGFP (light green curve), respectively. Recording of protein maturation was started when about 7% from their maximal fluorescence has been detected. Time point "0" was defined using an approximation of the beginning of the maturation curves with straight lines.



Figure S7. Fluorescence Spectra of the Purified EBFP2-mTagGFP Fusion Construct Emission spectra of the purified EBFP2-mTagGFP construct before and after its cleavage with trypsin were measured when excited at 383 nm (maximum of the absorbance for EBFP2 donor).

Table S1. Properties of the Purified mTagGFP in Comparison with Parental TagGFP and EGFP

Protein	Excitation maximum, nm	Emission maximum, nm	Extinction coefficient, M ⁻¹ cm ⁻¹	Quantum yield	Brightness relative to EGFP	Effective pK _a	Reference
EGFP	489	508	55,000	0.60	1.00	5.9	(Patterson et al., 2001)
TagGFP	481	505	55,000 ± 500	0.60 ± 0.02	1.00	5.0 ± 0.1	this paper
mTagGFP	483	506	56,500 ± 300	0.61 ± 0.02	1.05	5.0 ± 0.1	this paper

Supplemental Reference

Patterson, G.H., Day, R.N., and Piston, D.W. (2001). Fluorescent protein spectra. J. Cell Sci. 114, 837-838.