

## Supplementary Information

### Fluorescent Proteins and Genetically Encoded Biosensors

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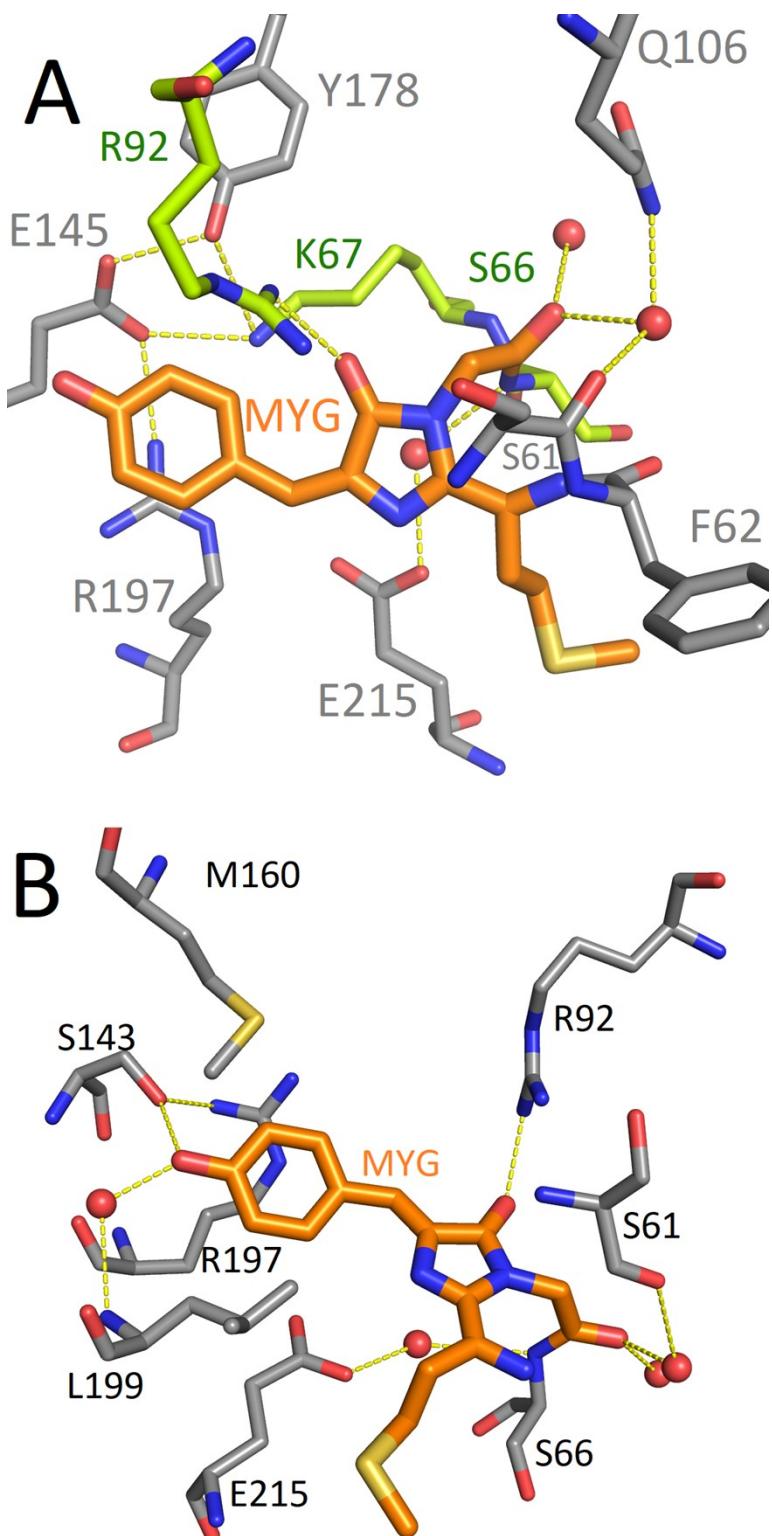
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**Table S1.** List of some genetically encoded fluorescent sensors of which analytes are metal ions, neuron transmitters, amino acids and nucleotides.

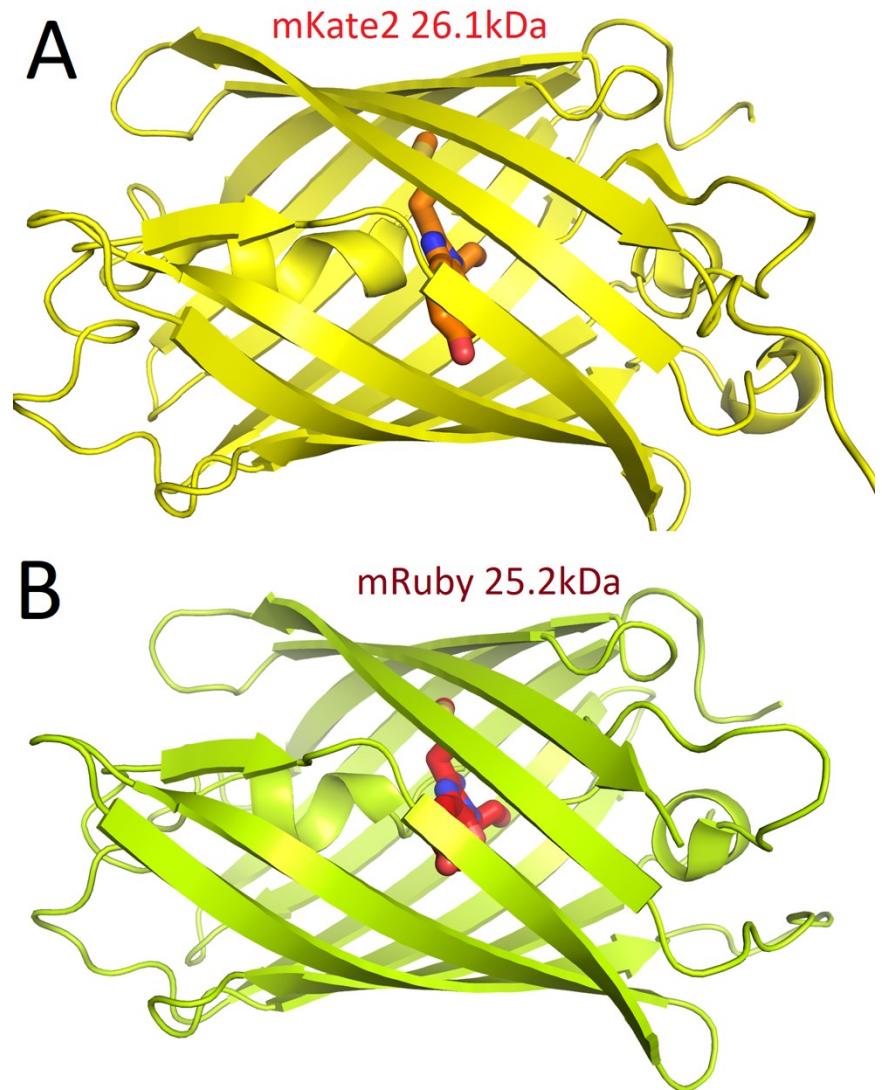
Name	Type	Ex/Em	FP1	FP2	ΔF/F or ΔFRET	Sensor	analyte	Kd	Ref
ΔFRET									
GCaMP2	single	487/508	EGFP	-	1.5	CaM	Ca <sup>2+</sup>	146nM	Ref <sup>1</sup>
					M13				
GCaMP6f	single	496/513	EGFP	-	19.0	CaM	Ca <sup>2+</sup>	375nM	Ref <sup>2</sup>
					M13				
RCaMP1h	single	575/602	mRuby	-	10.0	CaM	Ca <sup>2+</sup>	1.9μM	Ref <sup>3</sup>
					M13				
Camga-roo-1	single	487/512	EYFP	-	7.0	CaM	Ca <sup>2+</sup>	7μM	Ref <sup>4</sup>
cameleon-2 yellow	FRET	433/535	ECFP	EYFP	1.5	CaM	Ca <sup>2+</sup>	5.4μM	Ref <sup>5</sup>
					M13				
GAF-CAM3-sfGFP	single	648/676	GAF-FP	sfGFP	2.0	CaM	Ca <sup>2+</sup>	433nM	Ref <sup>6</sup>
				1-172		M13			
GINKO1	single	502/514	GFP	-	2.5	Kbp	K <sup>+</sup>	420μM	Ref <sup>7</sup>
GEPII 1.0	FRET	456/525	CFP	Venus	-3.2	BON	K <sup>+</sup>	2.6mM	Ref <sup>8</sup>
					LysM				
ZifCY	FRET	434/535	ECFP	EYFP	2.2	Zif268	Zn <sup>2+</sup>	1.7μM	Ref <sup>9</sup>
ZinCh-9	FRET	(475/527)	ECFP	EYFP	~4	ECFP-	Zn <sup>2+</sup>	500nM	Ref <sup>10</sup>
					Y39H			88μM	
					S208C				
BLZinCh-3	BRET	Auto/(530)	Nanoluc	Cerulean	0.5	H206/Citrine	Zn <sup>2+</sup>	15.6pM	Eef <sup>11</sup>
					C208				
ZapCY1	FRET	434/535	ECFP	EYFP	4.2	Zap1	Zn <sup>2+</sup>	2.5pM	Ref <sup>12</sup>
ZapCY2	FRET	434/535	ECFP	EYFP	1.4	Zap1	Zn <sup>2+</sup>	811pM	Ref <sup>12</sup>
CALWY	FRET		CFP	YFP	0.15	AtoX1	Zn <sup>2+</sup>	0.2pM	Ref <sup>13</sup>
					WD4				
eCALWY-C2M	FRET	420/527	Ceru-lean	Citrine		AtoX1	Cu <sup>+</sup>	96pM	Ref <sup>14</sup>
					WD4		Zn <sup>2+</sup>		

Name	Type	Ex/Em	FP1	FP2	$\Delta F/F$ or $\Delta FRET$	Sensor	analyte	Kd	Ref
ΔFRET									
Amt1-	FRET	433/527	CFP	YFP	0.16	Amt1	Cu <sup>+</sup>	2.5aM	Ref <sup>15</sup>
(Tran)									
Ace1-	FRET	433/527	CFP	YFP	<0.2	Ace1	Cu <sup>+</sup>	4.7aM	Ref <sup>16</sup>
(Tran)									
GZnP1	single	488/513	GFP	-	2.6	Zap1	Zn <sup>2+</sup>	58pM	Ref <sup>17</sup>
ZnGreen1	single	(460/ 500)	mTFP1	-	-26.3	Zap1	Zn <sup>2+</sup>	633nM	Ref <sup>18</sup>
ZnGreen2	single	(460/ 500)	mTFP1	-	-8.7	Zn hook	Zn <sup>2+</sup>	2.7μM	Ref <sup>18</sup>
ZnRed1	single	(575/ 600)	mApple	-	3.8	M13	Zn <sup>2+</sup>	166nM/ CaM	Ref <sup>18</sup>
(no name)	BiFC	(488)/510	EGFP	-	(-0.5)	Amt1	Cu <sup>+</sup>	0.46aM	Ref <sup>19</sup>
AR-18	single	(550/590)	RFP	-	-0.3	Amt1	Cu <sup>+</sup>		Ref <sup>20</sup>
AR-41	single	(550/590)	RFP	-	-0.4	Amt1	Cu <sup>+</sup>		Ref <sup>20</sup>
AR-59	single	(550/590)	RFP	-	-0.4	Amt1	Cu <sup>+</sup>		Ref <sup>20</sup>
SenSil	FRET	(420/530)	ECFP	Venus	(0.5)	CusF	Ag <sup>+</sup>	5.1μM	Ref <sup>21</sup>
(HcRed)	single	592/645	HcRed	-	~-0.8	(HcRed)	Cu <sup>+</sup>	3.6μM	Ref <sup>22</sup>
PbGFP	single		GFP	-	-1.0	Cys145/ Cys205	Pb <sup>2+</sup>	700nM	Ref <sup>23</sup>
Met-Cad	FRET	458/535 1.57	ECFP	Venus	0.8	CadR	Cd <sup>2+</sup>	271nM	Ref <sup>24</sup>
H <sub>39</sub> GFP	single	(488)/513	EGFP	-	-1.0	39 Histidines	Cu <sup>2+</sup>	16.2nM	Ref <sup>25</sup>
LaMP1	FRET	(433/529)	ECFP	Citrine	~3.0	LanM	La <sup>3+</sup>	9.4pM	Ref <sup>26</sup>
							Ce <sup>3+</sup>	16pM	
							Pr <sup>3+</sup>	13pM	
							Nd <sup>3+</sup>	12pM	
							Sm <sup>3+</sup>	12pM	
							Eu <sup>3+</sup>	16pM	
							Gd <sup>3+</sup>	10pM	
							Tb <sup>3+</sup>	18pM	
							Dy <sup>3+</sup>	26pM	
							Ho <sup>3+</sup>	29pM	
							Er <sup>3+</sup>	36pM	
							Yb <sup>3+</sup>	44pM	
SenALiB	FRET	(420/530)	ECFP	Venus	(0.15)	ArsR	As <sup>III</sup>	676nM	Ref <sup>27</sup>
dLight1.1	single	(488)/513	EGFP	-	2.3	DRD1	Dopamine	330nM	Ref <sup>28</sup>
GRAB <sub>NEIm</sub>	single		EGFP	-	2.3	α2AR <sub>NE</sub>	Norepinephrine	930nM	Ref <sup>29</sup>
							(EC50)		
GRAB <sub>DAIm</sub>	single		EGFP	-	0.9	D2R <sub>DA</sub>	Dopamine	97nM	Ref <sup>30</sup>
							(EC50)		
GRAB <sub>SHT</sub>	single		EGFP	-	2.5	5-HT2C	5-HT	22nM	Ref <sup>31</sup>

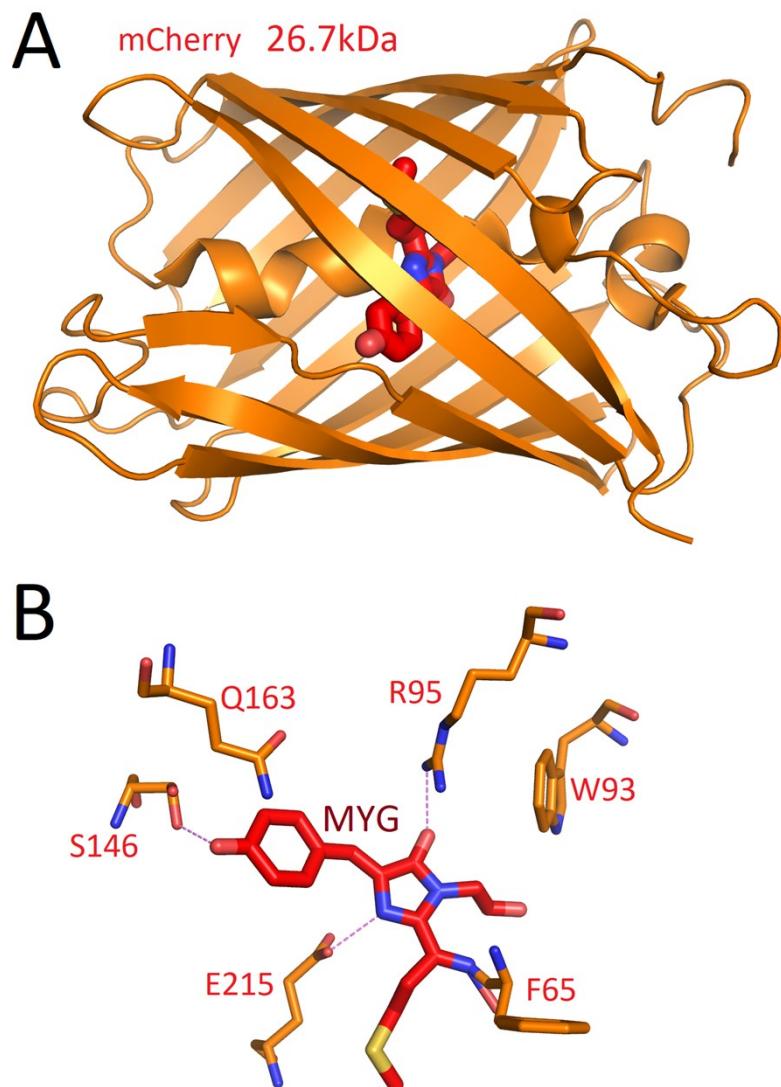
Name	Type	Ex/Em	FP1	FP2	$\Delta F/F$ or $\Delta FRET$	Sensor	analyte	Kd	Ref
(EC50)									
GACh2.0	single		EGFP	-	0.94	M3R <sub>ACh</sub>	Acetyl-choline	1.9μM	Ref <sup>32</sup>
OxLight1	single		EGFP	-	1.3	OX2R	Orexin-A	47nM	Ref <sup>33</sup>
MBP165-cpGFP	single	(485/515)	EGFP	-	2.5	MBP	maltose	3μM	Ref <sup>34</sup>
FLIPglu-600μΔ13	FRET	433/(525)	ECFP	EYFP	-0.28	MgIB	glubose	(0.6μM)	Ref <sup>35</sup>
Super GluSnFR	FRET	(420/526)	CFP	Citrine	-0.44	GltI	Glutamate	2.5μM	Ref <sup>36</sup>
SF-iGluSnFR	single		sfGFP	-	0.3	GltI	Glutamate		Ref <sup>37</sup>
iGAGA-SnFR	single		sfGFP	-	2.5	Pf622	γ-amino-butyric acid	9μM	Ref <sup>38</sup>
FLIP-Leu50	FRET	430/535	CFP	YFP	0.3	LivK	leucine	50μM	Ref <sup>39</sup>
FLIP-M	FRET	430/535	CFP	YFP	0.2	MetN	methionine	203μM	Ref <sup>40</sup>
FLIPK	FRET	430/535	CFP	YFP	0.2	LAO	lysine	97μM	Ref <sup>41</sup>
GBOS	FRET	(435/535)	CFP	YFP	0.3	ProX	Glycine-betaaine	400μM	Ref <sup>42</sup>
GRAB <sub>ATP</sub> 1.0	single		EGFP	-	5.0 – 10.0	hP2Y1	ATP	6.7μM	Ref <sup>43</sup>
iATPSnFR	single	(435/530)	sfGFP	-	2.4	PS3 F <sub>0</sub> F <sub>1</sub>	ATP ATP Synthase	120μM	Ref <sup>44</sup>
NS2 C3L194E	single	421, 500/518	YFP	-	9.0	T-Rex	NADH	3.7μM	Ref <sup>45</sup>
NS2 C8N120E	single	421, 500/519	YFP	-	-0.66	T-Rex	NADH	40nM	Ref <sup>45</sup>
Peredox	single	400/510	T-Sapphire	-	1.5	T-Rex	NADH	<5nM	Ref <sup>46</sup>
(no name)	single	(500/520)	Venus	-	~0.5	(Bacterial DNA ligase)	NAD+	65μM	Ref <sup>47</sup>



**Fig. S1.** Sticks representation showing the micro-environment of the fluorophore of (A) mKate under pH 2.0 (the trans- form, PDB: 3BX9) and (B) mKate under pH 7.0 (the cis- form, PDB: 3BXB). The fluorophore and the surrounding residues are shown as sticks whilst the water molecules are shown as red spheres. Important hydrogen bonds are shown as dash lines.



**Fig. S2.** The overall structure of mKate2 (A, an eqFP578 derivative, PDB: 3BX9) and mRuby (B, an eqFP611 derivative, PDB: 3U0L) in cartoon representation. The  $\alpha$ -helices are shown as helices whilst  $\beta$ -sheets are shown as arrows.



**Fig. S3.** The overall structure in cartoon representation (A) and the micro-environment of the fluorophore (B) in the DsRed-derivative fluorescent protein, mCherry. (PDB: 2H5Q) In (A),  $\alpha$ -helices are shown as helices whilst  $\beta$ -sheets are shown as arrows. In (B), the fluorophore and surrounding residues are shown as sticks. Important hydrogen bonds are shown as dash lines.

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