

**SUPPLEMENTARY MATERIALS TO**

**Reduced Fluorescent Protein Switching Fatigue by Binding-Induced  
Emissive State Stabilization**

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## SUPPORTING METHODS

**Amino acid sequences.** Fusions of rsGreen1 and rsGreenF to Enhancer nanobody. Green letters indicate fluorescent protein residues, orange letters indicate Enhancer residues<sup>1</sup>, leucine indicated in black is an extra residue incorporated for cloning purposes.

rsGreen1-Enhancer:

MVSKGEELFTGVVPIVELDGDVNGHKFSVRGEGEGDATNGKLTMKFICTTGKLPVPWPTLVTTLTLYGVLCFARYP  
DHMKQHDFFKSAMPEGYVQERTISFEDDGYKTRAEVKFEGDTLVNRIELKIDFKEDGNILGHKLEYNFNSHDAYI  
TADKQKNGIRSNFKIRLNVEDGVSQVLADHYQQNTPIGDGPVLLPDNHYLSTQNKLSKDPNEKRDHMLLEFVTASG  
ITLGMDELYK**LQVQLVESGGALVQPGGSLRLSCAASGFPVNRYSMRWYRQAPGKEREWVAGMSSAGDRSSYEDS**  
**VKGRFTISRDDARNTVYQLQMNSLKPEDTAVYYCNVNVGFEYWGQGTQVTVS**

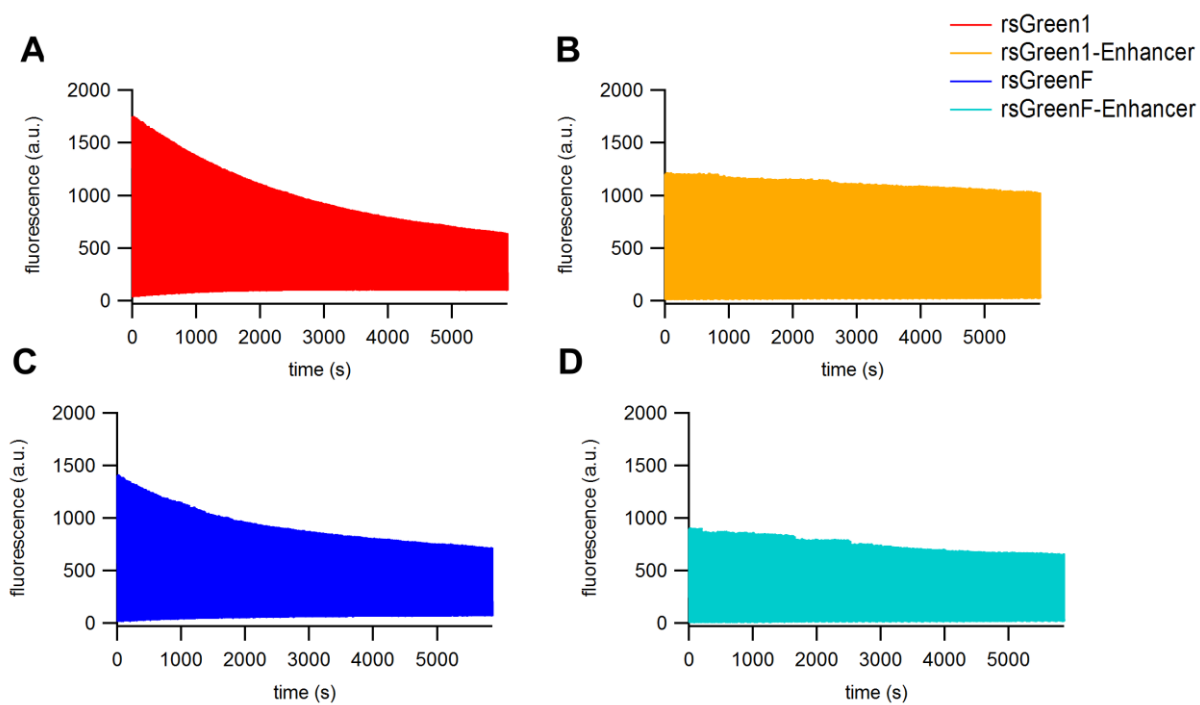
rsGreenF-Enhancer:

MVSKGEELFTGVVPIVELDGDVNGHKFSVRGEGEGDATNGKLTMKFICTTGKLPVPWPTLVTTLTLYGVLCFARYP  
DHMKQHDFFKSAMPEGYVQERTISFEDDGYKTRAEVKFEGDTLVNRIELKIDFKEDGNILGHKLEYNLNHNAYI  
TADKQKNGIRSNFKIRLNVEDGVSQVLADHYQQNTPIGDGPVLLPDNHYLSTQNKLSKDPNEKRDHMLLEFVTASG  
ITLGMDELYK**LQVQLVESGGALVQPGGSLRLSCAASGFPVNRYSMRWYRQAPGKEREWVAGMSSAGDRSSYEDS**  
**VKGRFTISRDDARNTVYQLQMNSLKPEDTAVYYCNVNVGFEYWGQGTQVTVS**

## SUPPLEMENTARY TABLES AND FIGURES

**Supplementary Table 1.** Brightness of rsGreen1, rsGreenF and Enhancer fusions when expressed in *E. coli* grown at 37°C and 20°C. From left to right: mean brightness, standard deviation, standard error on the mean, Welch's t-test: t-value, degrees of freedom, p-value.

	mean brightness	std.dev.	SEM	N	Welch's t-test		
					t	df	p
37°C							
rsGreen1	9927,19	1914,90	228,88	70	17,4712	91	<0,0001
rsGreen1-Enhancer	5615,80	949,91	92,26	106			
rsGreenF	8151,56	865,73	166,61	27	15,7288	53	<0,0001
rsGreenF-Enhancer	4955,88	1046,50	116,28	81			
20°C							
rsGreen1	10663,00	1912,09	291,59	43	1,4848	75	0,1418
rsGreen1-Enhancer	9981,51	2213,66	354,47	39			
rsGreenF	8897,09	2136,20	280,50	58	0,4383	58	0,6628
rsGreenF-Enhancer	8669,64	2507,73	436,54	33			



**Supplementary Figure 1.** Representative fluorescence photoswitching traces of rsGreen1 (A), rsGreen1-Enhancer (B), rsGreenF (C), rsGreenF-Enhancer (D) expressed in HeLa cells and submitted to 500 consecutive switching cycles.

#### SUPPORTING REFERENCES

1. Kirchofer A, Helma J, Schmidhals K, et al. Modulation of protein properties in living cells using nanobodies. *Nat Struct & Mol Biol.* 2010;17(1):133-U162. doi:10.1038/nsmb.1727.