

Supplementary Information

A superfolder variant of pH-sensitive pHLuorin for *in vivo* pH measurements in the endoplasmic reticulum

Mara Reifenrath¹ and Eckhard Boles¹

¹ Institute of Molecular Biosciences, Faculty of Biological Sciences, Goethe University Frankfurt, Max-von-Laue Straße 9, 60438 Frankfurt am Main, Germany

Figure S1. pH dependence of excitation spectra of cytosolically located, conventional pHLuorin The cells were permeabilized with digitonin and resuspended in citric acid/Na₂HPO₄ buffer of pH values ranging from 5.0 to 9.0 to an OD₆₀₀ of 0.5. The emission intensity was recorded at 508 nm.

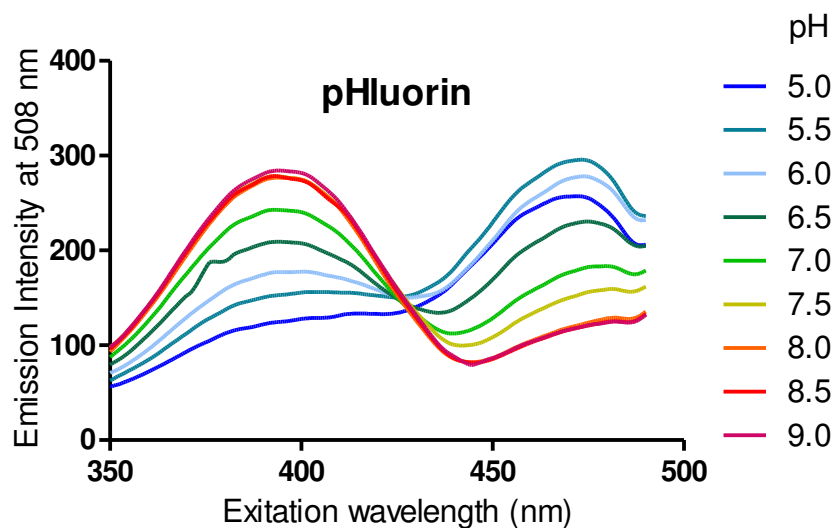
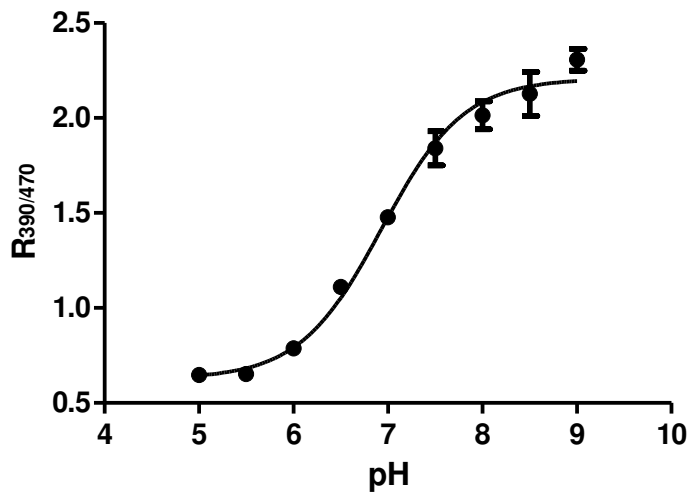


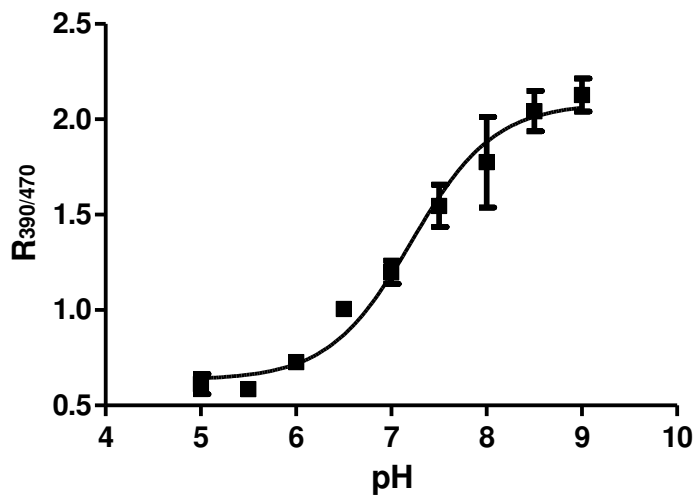
Figure S2. pH calibration and sigmoidal dose response fits for CEN.PK2-1C expressing sfpHluorin (A) and ER-sfpHluorin (B) grown in If-SCD with 0.1 mM methionine. The graphs show the mean and standard error (mean±s.d.) of biological triplicates.

S5 A)



Best-fit values	
Bottom	0,6269
Top	2,208
LogEC50	6,934
EC50	8,596e+006
Std. Error	
Bottom	0,03009
Top	0,02853
LogEC50	0,04864
95% Confidence Intervals	
Bottom	0.5648 to 0.6890
Top	2.150 to 2.267
LogEC50	6.834 to 7.035
EC50	6.822e+006 to 1.083e+007
Goodness of Fit	
Degrees of Freedom	24
R ²	0,9866
Absolute Sum of Squares	0,1415
Sy.x	0,07677

S5 B)



Best-fit values	
Bottom	0,6339
Top	2,084
LogEC50	7,212
EC50	1,628e+007
Std. Error	
Bottom	0,04070
Top	0,04838
LogEC50	0,08036
95% Confidence Intervals	
Bottom	0.5499 to 0.7179
Top	1.984 to 2.184
LogEC50	7.046 to 7.378
EC50	1.111e+007 to 2.385e+007
Goodness of Fit	
Degrees of Freedom	24
R ²	0,9643
Absolute Sum of Squares	0,3205
Sy.x	0,1156

Table S1. DNA Sequence of all GFP variants used in this study

Name	DNA sequence	Reference
Superfolder GFP	ATGAGCAAAGGAGAAGAAGCTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGG TGATGTTAATGGGCACAAATTTTCTGTCCGTGGAGAGGGTGAAGGTGATGCTACAAACG GAAAACCTACCCTTAAATTTATTTGCACTACTGGAAAACCTACCTGTTCCGTGGCCAACAC TTGCACTACTCTGACCTATGGTGTTCATGCTTTTCCCGTTATCCGGATCACATGAAAC GGCATGACTTTTTCAAGAGTGCCATGCCCCGAAGTTATGTACAGGAACGCACTATATCT TTCAAAGATGACGGGACCTACAAGACGCGTGCTGAAGTCAAGTTTGAAGGTGATACCTT TGTTAATCGTATCGAGTTAAAGGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACA CAAACCTCGAGTACAACTTAACTCACACAATGTATACATCACGGCAGACAAACAAAAGA ATGGAATCAAAGCTAACTTCAAATTCGCCACAACGTTGAAGATGGTTCGGTTCAACTA GCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAAC CATTACCTGTCGACACAATCTGTCCTTTCGAAAAGATCCCAACGAAAAGCGTGACCACAT GGTCCTTCTTGAGTTTGTAAGTCTGCTGGGATTACACATGGCATGGATGAGCTCTACA AATAA	Pédelacq et al., 2006
pHluorin	ATGAGTAAAGGAGAAGAAGCTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGT GATGTTAATGGGCACAAATTTTCTGTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACG GAAAACCTACCCTTAAATTTATTTGCACTACTGGAAAACCTACCTGTTCCATGGCCAACAC TTGCACTACTTTCTTTATGGTGTTCATGCTTTTCAAGATACCCAGATCATATGAAACG GCATGACTTTTTCAAGAGTGCCATGCCCCGAAGTTATGTACAGGAAAGAACTATATTTTT CAAAGATGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCTTGT TTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAGATGATGGAAACATTCTTGGACACA AATTGGAATACAACATAACGAGCACTTGGTGTACATCATGGCAGACAAACAAAAGAAT GGTACCAAAGCTATCTTTCAAGTTCACCACAACATTGAAGATGGAGGCGTTCAACTAGC AGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCA TTACCTGCACACACAATCTGCCCTTTCGAAAAGATCCCAACGAAAAGAGAGACCACATGG TCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACATGGCATGGATGAACTATACAAT AA	Miesenböck et al., 1998; Orij et al., 2009
ER-pHluorin (SSBiP-sfpHluorin-HDEL)	ATGTTTTTCAACAGACTAAGCGCTGGCAAGCTGCTGGTACCACTCTCCGTGGTCCTGTA CGCCCTTTTCGTGGTAATATTACCTTTACAGAATCTTTCCACTCCTCCAATGTTTTAGTT AGAGGTAGTAAAGGAGAAGAAGCTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGA TGGTGTGTTAATGGGCACAAATTTTCTGTGTCAGTGGAGAGGGTGAAGGTGATGCAACAT ACGGAAAACCTACCCTTAAATTTATTTGCACTACTGGAAAACCTACCTGTTCCATGGCCAA CACTTGTCACTACTTTCTTTATGGTGTTCATGCTTTTCAAGATACCCAGATCATATGAA ACGGCATGACTTTTTCAAGAGTGCCATGCCCCGAAGTTATGTACAGGAAAGAACTATAT TTTTCAAAGATGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACC CTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAGATGATGGAAACATTCTTGGA CACAATTGGAATACAACATAACGAGCACTTGGTGTACATCATGGCAGACAAACAAA GAATGGTACCAAAGCTATCTTTCAAGTTCACCACAACATTGAAGATGGAGGCGTTCAAC TAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACA ACCATTACCTGCACACACAATCTGCCCTTTCGAAAAGATCCCAACGAAAAGAGAGACCAC ATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACATGGCATGGATGAACTATA CAAATTCGAACACGACGAATTGTAG	This study

Superfolder pHluorin (sfpHluorin)	ATGAGCAAAGGAGAAGAAGAACTTTTCACTGGAGTTGTCCAATTCTTGTGAATTAGATGG TGATGTTAATGGGCACAAATTTTCTGTCCGTGGAGAGGGTGAAGGTGATGCTACAAACG GAAAACCTCACCTTAAATTTATTTGCACTACTGGAAAACCTGTTCCGTGGCCAACAC TTGCACTACTCTGTCTTATGGTGTTCATGCTTTTCCCGTTATCCGGATCACATGAAAC GGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAACGCACATATATCT TTCAAAGATGACGGGACCTACAAGACGCGTGCTGAAGTCAAGTTTGAAGGTGATACCCT TGTTAATCGTATCGAGTTAAAGGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACA CAAACCTCGAGTACAACCTTAAACGAGCACTTGGTATACATCACGGCAGACAAACAAAAGA ATGGAACCAAAGCTATCTTCAAGTTCACCACAACGTTGAAGATGGTTCCGTTCAACTA GCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAAC CATTACCTGCACACACAATCTGTCCTTTGAAAGATCCCAACGAAAAGCGTGACCACAT GGTCCTTCTTGAGTTTGTAACTGCTGCTGGGATTACACATGGCATGGATGAGCTCTACA AATAA	This study
ER-Superfolder pHluorin (SSBiP-sfpHluorin-HDEL)	ATGTTTTTCAACAGACTAAGCGCTGGCAAGCTGCTGGTACCCTCTCCGTGGTCCTGTA CGCCCTTTTCTGTGTAATATTACCTTTACAGAATCTTTCCACTCCTCCAATGTTTTAGTT AGAGGTAGCAAAGGAGAAGAAGAACTTTTCACTGGAGTTGTCCAATTCTTGTGAATTAGA TGGTGATGTTAATGGGCACAAATTTTCTGTCCGTGGAGAGGGTGAAGGTGATGCTACAA ACGAAAACCTCACCTTAAATTTATTTGCACTACTGGAAAACCTGTTCCGTGGCCAA CACTTGTCACTACTCTGTCTTATGGTGTTCATGCTTTTCCCGTTATCCGGATCACATGA AACGGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAACGCACATA TCTTTCAAAGATGACGGGACCTACAAGACGCGTGCTGAAGTCAAGTTTGAAGGTGATAC CCTTGTTAATCGTATCGAGTTAAAGGGTATTGATTTTAAAGAAGATGGAAACATTCTTGG ACACAACTCGAGTACAACCTTAAACGAGCACTTGGTATACATCACGGCAGACAAACAAA AGAATGGAACCAAAGCTATCTTCAAGTTCACCACAACGTTGAAGATGGTTCCGTTCAA CTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGAC AACCATTACCTGCACACACAATCTGTCCTTTGAAAGATCCCAACGAAAAGCGTGACCA CATGGTCCTTCTTGAGTTTGTAACTGCTGCTGGGATTACACATGGCATGGATGAGCTCT ACAAATTCGAACACGACGAATTGTAG	This study

Table S2. Plasmids used in this study

Plasmids	Characteristics	Reference
Plasmids used for fermentations		
p426MET25	2 μ , <i>URA3</i> , <i>Amp^r</i> , <i>MET25_{pr}</i> , <i>CYC1_{ter}</i>	(Mumberg et al., 1995)
MRV55	p426MET25, sfpHluorin	This study
MRV65	p426MET25 SSKar2-sfpHluorin-HDEL (ER-pHluorin)	This study
MRV66	p426MET25 SSKar2-sfpHluorin-HDEL (ER-sfpHluorin)	This study

Table S3. Primers used in this study

Primer name	Sequence 5'-3'	Application
MOP291	GCGTCTGTTAGAAAAGGAAGTTTTTCC	Binding in <i>MET25</i> ^{prom} , sequencing, forward
MOP290	ACCTAGACTTCAGGTTGTC	Binding in <i>CYC1</i> ^{term} , sequencing, reverse
MRP57	AATTCTATTACCCCATCCATACTACTAGAAATGAGTAAAGGAGAAACTTTTC	Binding <i>pHluorin</i> , overhang to <i>MET25</i> ^{prom} , forward
MOP252	TAAGCGTGACATAACTAATTACATGACTCGAGTTATTTGTATAGTTCATCCATGC	Binding <i>pHluorin</i> , overhang to <i>CYC1</i> ^{term} , reverse
JTP96	AATTCTATTACCCCATCCATACTACTAGAAATGAGCAAAGGAGAAACTTTTC	Binding <i>superfolderGFP</i> , overhang to <i>MET25</i> ^{prom} , forward
MRP85	TTGAACACCATAAGACAGAGTAGTGACAAGTGTGG	Introducing mutation T65S, reverse
MRP86	CTTGTCACTACTGTCTTATGGTGTTCATGCTTTTCCC	Introducing mutation T65S, reverse
MRP87	TCCATTCTTTTGTGCTGCCGTGATGTATACCAAGTCTCGTTAAAGTTGTACT CGAGTTTGTG	Introducing mutation S147E, N149L, reverse
MRP88	TACATCACGGCAGACAAAACAAAAGAATGGAACCAAAGCTATCTTCCAAAGTTC ACCACAAACGTTGAAGATGGTTC	Introducing mutation I161T, N164I, K166Q, I167V, R168H, forward
MRP89	AGGACAGATTGTGTGCAGGTAATGGTTGTCTGGTAAAAG	Introducing mutation S202H, reverse
MRP90	CAACCATTACCTGCACACACAATCTGTCTTCGAAAAG	Introducing mutation S202H, forward
JRP97	AATGTAAGCGTGACATAACTAATTACATGATTATTTGTAGAGCTCATCCATGC	Binding <i>superfolderGFP</i> , overhang to <i>CYC1</i> ^{term} , reverse
MRP07	AATTCTATTACCCCATCCATACTACTAGAAATGTTTTTCAACAGACTAAGC	Binding KAR2 signal sequence, overhang to <i>MET25</i> ^{prom} , forward
JTP89	ACCTCTAACTAAACATTGGAGGAGTGGAAAAG	Binding KAR2 signal sequence, reverse
JTP92	CTTTCCACTCCTCCAATGTTTTAGTTAGAGGTAGCAAAGGAGAAACTTTTC	Binding superfolder GFP/pHluorin, overhang to KAR2 signal sequence, forward
JRP93	GACTACAAATTCGTGCTCGAATTTGTAGAGCTCATCCATGC	Binding superfolder GFP/pHluorin, introducing ER-retention tag, reverse
MRP06	AATGTAAGCGTGACATAACTAATTACATGACTACAAATTCGTGCTGTTCCG	Binding ER-retention tag, overhang to <i>CYC1</i> ^{term} , reverse

Table S4. Sigmoidal dose-response fits**A) sfpHluorin (0 mM methionine)**

Best-fit values	
Bottom	0,6462
Top	2,316
LogEC50	6,929
EC50	8,488e+006
Std. Error	
Bottom	0,03232
Top	0,03051
LogEC50	0,04939
95% Confidence Intervals	
Bottom	0.5795 to 0.7129
Top	2.253 to 2.379
LogEC50	6.827 to 7.031
EC50	6.712e+006 to 1.073e+007
Goodness of Fit	
Degrees of Freedom	24
R ²	0,9862
Absolute Sum of Squares	0,1624
Sy.x	0,08227

B) ER-sfpHluorin (0 mM methionine)

Best-fit values	
Bottom	0,5025
Top	2,240
LogEC50	7,273
EC50	1,874e+007
Std. Error	
Bottom	0,05035
Top	0,06296
LogEC50	0,08509
95% Confidence Intervals	
Bottom	0.3986 to 0.6064
Top	2.110 to 2.370
LogEC50	7.097 to 7.448
EC50	1.251e+007 to 2.809e+007
Goodness of Fit	
Degrees of Freedom	24
R ²	0,9601
Absolute Sum of Squares	0,5114
Sy.x	0,1460