

Supplemental Data

Table S1. Sequence analysis of the engineered YgfO mutants

Mutant	codon sequence
<i>Replacements at TM8</i>	
F259C	C/TTT/CAT/CAG/TGC/CTG/GTG/GTT/G
L260C	CAT/CAG/TTC/TGT/GTG/GTT/GGC/ACG
V261C	CAT/CAG/TTC/CTG/TGT/GTT/GGC/ACG
V262C	CAT/CAG/TTT/CTG/GTG/TGT/GGC/ACG
G263C	CTG/GTG/GTC/TGC/ACG/ATC/TAT/C
T264C	GTG/GTC/GGC/TGC/ATT/TAT/CTG/C
I265C	GTT/GGC/ACG/TGC/TAT/CTG/CTT/AGC
Y266C	GTT/GGC/ACG/ATT/TGT/CTG/CTT/AG
L267C	GGC/ACG/ATT/TAT/TGT/CTT/AGC/GTG/CTG
L268C	CG/ATT/TAT/CTG/TGC/AGC/GTG/CTG
S269C	CG/ATT/TAT/CTG/CTT/TGC/GTG/CTG/G
V270C	CTG/CTT/AGT/TGT/CTG/GAA/GCA/GTC
L271C	CTT/AGC/GTG/TGT/GAA/GCA/GTC
E272C	as in Karena & Frillingos, 2009
A273C	GTG/CTG/GAA/TGT/GTT/GGT/GAT/ATC/ACT/GCC
V274C	CTG/GAA/GCA/TGT/GGT/GAT/ATC/ACT/GCC
G275C	GAA/GCA/GTT/TGT/GAT/ATC/ACT/GCC
D276C	GAA/GCA/GTC/GGT/TGT/ATC/ACC
D276E(wt)	GAA/GCA/GTT/GGC/GAA/ATC/ACC/GC
D276N(wt)	GAA/GCA/GTC/GGC/AAT/ATC/ACC/GC
D276Q(wt)	GAA/GCA/GTT/GGC/CAA/ATC/ACC/GC
D276G(wt)	GAA/GCA/GTT/GGC/GGT/ATC/ACC
D276I(wt)	GAA/GCA/GTC/GGC/ATT/ATC/ACC
D276M(wt)	GAA/GCA/GTT/GGC/ATG/ATC/ACC/GC
D276H(wt)	GAA/GCA/GTT/GGC/CAT/ATC/ACC/GC
D276A(wt)	GAA/GCA/GTT/GGC/GCA/ATC/ACT/GC
D276T(wt)	GAA/GCA/GTT/GGC/ACA/ATC/ACC/GC
D276V(wt)	GAA/GCA/GTT/GGC/GTT/ATC/ACT/GC
<i>Replacements at the sequence 277-281</i>	
I277C	GAA/GCA/GTT/GGT/GAT/TGT/ACC/GCC/AC
I277L(wt)	GC/GAT/CTT/ACC/GCT/ACG/GCA/ATG
T278C	GTT/GGC/GAT/ATC/TGT/GCT/ACG/GCA/ATG
T278N(wt)	GAT/ATC/AAC/GCC/ACG/GCA/ATG/G
T278S(wt)	GAT/ATC/AGC/GCC/ACG/GCA/ATG/G
T278G(wt)	GAT/ATC/GGA/GCC/ACG/GCA/ATG/G
T278A(wt)	GAT/ATC/GCT/GCC/ACG/GCA/ATG/G
T278Q(wt)	GAT/ATC/CAG/GCC/ACG/GCA/ATG/G
T278V(wt)	GAT/ATC/GTC/GCC/ACG/GCA/ATG/G
A279C	GTC/GGC/GAT/ATC/ACT/TGT/ACG/GCA/ATG
A279S(wt)	GAT/ATC/ACC/TCC/ACG/GCA/ATG
A279G(wt)	GAT/ATC/ACC/GGA/ACG/GCA/ATG
A279T(wt)	GAT/ATC/ACC/ACC/ACG/GCA/ATG
A279V(wt)	GAT/ATC/ACC/GTC/ACG/GCA/ATG
A279N(wt)	GAT/ATC/ACC/GAC/ACG/GCA/ATG
T280C	GTT/GGC/GAT/ATC/ACT/GCT/TGT/GCA/ATG/G
T280S(wt)	GAT/ATC/ACT/GCC/TCG/GCA/ATG/GTT/TC
T280N(wt)	GAT/ATC/ACC/GCC/AAC/GCA/ATG/GTT/TC

T280G(wt) GAT/ATC/ACT/GCC/GGA/GCA/ATG/GTT/TC
 T280A(wt) GAT/ATC/ACT/GCC/GCA/GCA/ATG/GTT/TC
 T280Q(wt) GAT/ATC/ACT/GCC/CAG/GCA/ATG/GTT/TC
 T280V(wt) GAT/ATC/ACT/GCC/GTC/GCA/ATG/GTT/TC
 A281C GTT/GGC/GAT/ATC/ACT/GCC/ACT/TGT/ATG/GTT/TC
 A281S(wt) GAT/ATC/ACC/GCT/ACG/TCA/ATG/GTT/TC
 A281G(wt) GAT/ATC/ACC/GCT/ACG/GGA/ATG/GTT/TC
 A281T(wt) GAT/ATC/ACC/GCT/ACG/ACC/ATG/GTT/TC
 A281V(wt) GAT/ATC/ACC/GCT/ACG/GTC/ATG/GTT/TC
 A281N(wt) GAT/ATC/ACC/GCT/ACG/AAC/ATG/GTT/TC
 Quadruple(wt) GAT/ATC/GCC/ACC/GCG/ACA/ATG/GTT/TC

Replacements at the loop between TM8 and TM9a

M282C C/GAT/ATC/ACT/GCA/ACT/GCA/TGT/GTT/TCC/C
 V283C GCA/ATG/TGT/TCT/CGT/CGT/CCC/ATT/C
 S284C GCA/ATG/GTT/TGT/CGT/CGT/CCC/ATT/C
 R285C GCA/ATG/GTT/TCC/TGT/CGT/CCC/ATT/C
 R286C CA/ATG/GTT/TCC/CGT/TGT/CCC/ATT/CAG
 P287C G/GTT/TCC/CGT/CGT/TGT/ATT/CAG/G
 I288C CA/ATG/GTT/TCT/CGT/CGT/CCT/TGT/CAA/GGG/GAA/G
 Q289C CA/ATG/GTT/TCT/CGT/CGT/CCT/ATT/TGT/GGG/GAA/G
 G290C CCC/ATT/CAG/TGT/GAA/GAG/TAT/CAG/TCC
 E291C CC/ATT/CAG/GGT/TGT/GAG/TAT/CAG/TCC
 E292C CC/ATT/CAG/GGT/GAA/TGT/TAT/CAG/TCC
 Y293C G/GAA/GAG/TGT/CAG/TGT/CGT/CTG/AAA/G
 Q294C G/GAA/GAG/TAT/TGC/TCC/CGT/CTG/AAAG
 S295C GAA/GAG/TAT/CAG/TGC/CGG/CTG/AAA/G
 R296C GAG/TAT/CAG/TCT/TGC/CTG/AAA/GGC
 L297C G/TAT/CAG/TCT/CGT/TGT/AAA/GGT/GGT/GTG/CTG
 K298C G/TAT/CAG/TCT/CGT/TTA/TGT/GGT/GGC/GTG
 G299C CGG/CTG/AAA/TGT/GGT/GTG/CTG
 G299C(wt) as in G299C

Replacements at TM9a

G300C CTG/AAA/GGC/TGT/GTT/CTG/GCA/G
 V301C G/AAA/GGC/GGT/TGT/CTT/GCA/GAT/G
 L302C C/GGC/GTT/TGT/GCA/GAT/GGT/CTG
 A303C GGC/GTG/CTT/TGT/GAT/GGT/CTG
 D304C as in Karena & Frillingos, 2009
 G305C G/CTG/GCA/GAT/TGT/CTG/GTT/TCT/G
 G305A(wt) CTG/GCA/GAT/GCT/CTG/GTT/TCT/G
 G305P(wt) CTG/GCA/GAT/CCT/CTG/GTT/TCT/G
 L306C CTG/GCA/GAT/GGG/TGT/GTT/TCT/G
 V307C GAT/GGT/CTG/TGT/TCC/GTT/ATC/GC
 S308C GGT/CTG/GTT/TGT/GTT/ATC/GCC/TC
 V309C CTG/GTT/TCT/TGT/ATC/GCC/TCC/G
 I310C GTT/TCT/GTT/TGT/GCC/TCC/GCT/G
 A311C CT/GTT/ATC/TGT/TCC/GCT/GTC/GG
 S312C GTT/ATC/GCT/TGT/GCT/GTC/GGT/TC
 A313C GTT/ATC/GCC/TCT/TGT/GTC/GGT/TC
 V314C GCC/TCC/GCT/TGT/GGT/TCA/TTA/C

Replacements at TM9b

T342C GT/TAT/GTC/GGT/CGA/TGT/ATC/GCG/GTA/ATG
 I343C GGG/CGA/ACT/TGT/GCG/GTA/ATG

A344C	GG/CGA/ACC/ATC/ TGT /GTA/ATG/CTG
V345C	CGA/ACC/ATC/ <u>GCA</u> / TGT /ATG/CTG/GTT/ATC
M346C	CGA/ACC/ATC/ <u>GCT</u> / TGT /CTG/GTT/ATC
L347C	GCG/GTA/ATG/ TGT /GTT/ATC/CTC/GG
V348C	CG/GTA/ATG/CTG/ TGT /ATC/CTC/GGC
I349C	G/CTG/GTT/ TGT / <u>CTT</u> /GGC/TTA/TTT/CCG
L350C	G/CTG/GTT/ATC/ TGT /GGC/TTA/TTT/CCG
L350I(wt)	G/CTG/GTT/ATC/ ATC /GGC/TTA/TTT/CCG
L350V(wt)	G/CTG/GTT/ATC/GTT/GGC/TTA/TTT/CCG
G351C	GTT/ATC/CTC/ TGT / <u>TTG</u> / <u>TTC</u> /CCG/ATG/ATT/GG
G351P(wt)	G/GTT/ATC/CTC/ CCC /TTA/TTT/CCG
G351A(wt)	G/GTT/ATC/CTC/ GCG / <u>TTA</u> /TTT/CCG
L352C	GTT/ATC/CTC/GGC/ TGT /TTT/CCG/ATG
F353C	CTC/GGC/TTA/ TGT / <u>CCT</u> /ATG/ATT/GGC
P354C	CTC/GGC/TTA/ <u>TTG</u> / TGC /ATG/ATT/GGC
P354G(wt)	CTC/GGC/TTA/TTT/ GGC /ATG/ATT/GGC

DNA sequences are presented in the 5'→3' order with the mutated codons in *boldface* and base changes introduced outside the mutated codon in *underlined boldface*; mutants made in the wild-type background are indicated by (wt); single-Cys mutants made in the C-less background are indicated by the lack of (wt). Mutants were constructed in the biotin-acceptor domain (BAD)-tagged background. For mutants A273C, V274C, G275C, T278C, A279C, T280C and A281C, we employed one-stage PCR mutagenesis using a native EcoRV restriction site of YgfO (indicated in *italics*), followed by transfer of the PCR product to vector plasmid by BamHI-EcoRV or EcoRV-ApaI restriction fragment replacement; for all other mutants, we used two-stage PCR mutagenesis and the second-stage PCR product was transferred to vector plasmid by BamHI-ApaI restriction fragment replacement. The entire coding sequence of all constructs was verified by dsDNA sequencing. Quadruple(wt) denotes mutant T278**A**/A279**T**/T280**A**/A281**T**(wt).

References used in Table S1

Karena, E., and Frillingos, S. (2009). *J. Biol. Chem.* **284**, 24257-24268.