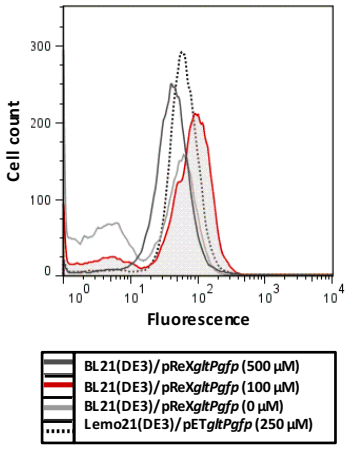
Supplementary Table 1: The primers used in this study.

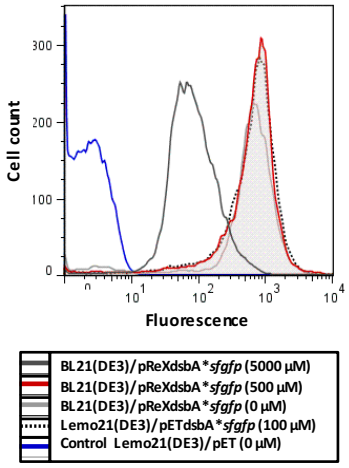
Primer name	Primer sequence	Primer description
P57_KpnIMod_fw	5'- ATATATGGTACCTCGCCGAAAATGACCCAG-3'	Forward primer to introduce a *Kpn*I restriction site in the pMOD fragment of pReX
P58_EagIMod_rv	5'- ATATATCGGCCGTCAAGAAGATCCTTTGATCTTTTC-3'	Reverse primer to introduce an *Eag*I restriction site in the pMOD fragment of pReX
P59_EagILemo_fw	5'- ATATATCGGCCGTCACAGGTATTTATTCGGC-3'	Forward primer to introduce an *Eag*I restriction site in the pLemo fragment of pReX
P60_KpnILemo_rv	5'- ATATATGGTACCAGGGCAGGGTCGTTAAATAG-3'	Reverse primer to introduce a *Kpn*I restriction site in the pLemo fragment of pReX
pLemoMOD_pMB1_ins_fw	5'- ACACCCCTTGTATTACTGTTTATG-3'	Forward primer to amplify the pMB1 origin of replication
pLemoMOD_pMB1_ins_rv	5'- CTGACTTCAGGTGCCTGTGGAACACCTACATC-3'	Reverse primer to amplify the pMB1 origin of replication
pLemoMOD_Vec_p15A_fw	5'- CACCTGAAGTCAGCCCC-3'	Forward primer to amplify the vector backbone of pReX
pLemoMOD_Vec_p15A_rv	5'- CATAAACAGTAATACAAGGGGTGT-3'	Reverse primer to amplify the vector backbone of pReX
pET-T7-Ins_fw	5'- ATTAATACGACTCACTATAGGG-3'	Forward primer to subclone target genes into pReX
pET-Ter-Ins_rv	5'- ATCCGGATATAGTTCCTCC-3'	Reverse primer to subclone target genes into pReX
pET-Ter-Vec_fw	5'- GGAGGAACTATATCCGGAT-3'	Forward primer to amplify the vector backbone of pReX
pET-T7-Vec_rv	5'- CCCTATAGTGAGTCGTATTAAT-3'	Reverse primer to amplify the vector backbone of pReX

Supplementary Figure 1: Flow cytometry-based analysis of GltP-GFP and secreted SfGFP production. BL21(DE3) cells harboring a pReX expression vector with either *gltPgfp* or *dsbA*sfgfp* and Lemo21(DE3) cells harboring a pET expression vector with either *gltPgfp* or *dsbA*sfgfp* were cultured in LB medium at 30°C at varying L-rhamnose concentrations. Cells where induced with IPTG as described in the Methods section. **A.** Using flow cytometry the amount of GltP-GFP fusion produced in the cytoplasmic membrane per cell was assessed. Traces of BL21(DE3)/pReX*gltpgfp* and Lemo21(DE3)/pET*gltpgfp* cells cultured at the optimal L-rhamnose concentration (maximal amount of fluorescent protein per milliliter of culture) are in red and black (dotted), respectively. The trace of the BL21(DE3)/pReX*gltpgfp* cells cultured in the absence of L-rhamnose (0 μM L-rhamnose; control) is in light gray. The trace of the BL21(DE3)/pReX*gltpgfp* cells cultured in the presence of a suboptimal concentration of L-rhamnose (500 μM L-rhamnose; control) is in dark gray. Cells were isolated 8 h after the addition of IPTG. **B.** Using flow cytometry the amount of SfGFP produced in the periplasm per cell was assessed. Traces of BL21(DE3)/pReX*dsbA*sfgfp* and Lemo21(DE3)/pET*dsbA*sfgfp* cells cultured at the optimal L-rhamnose concentration (maximal amount of fluorescent protein per milliliter of culture) are in red and black (dotted), respectively. The trace of the BL21(DE3)/pReX*dsbA*sfgfp* cells cultured in the absence of L-rhamnose (0 μM L-rhamnose; control) is in light gray. The trace of the BL21(DE3)/pReX*gltpgfp* cells cultured in the presence of a suboptimal concentration of L-rhamnose (5000 μM L-rhamnose; control) is in dark gray. The trace of the Lemo21(DE3)/pET*dsbA*sfgfp* cells cultured in the absence of L-rhamnose (0 μM L-rhamnose; control) is in blue. Cells were isolated 4 h after the addition of IPTG.

A


Cell count
300
200
100
0
10^0 10^1 10^2 10^3 10^4
Fluorescence
BL21(DE3)/pReX*gltPgfp* (500 µM)
BL21(DE3)/pReX*gltPgfp* (100 µM)
BL21(DE3)/pReX*gltPgfp* (0 µM)
Lemo21(DE3)/pETg*ltPgfp* (250 µM)

B


Cell count
300
200
100
0
10^1 10^2 10^3 10^4
Fluorescence
BL21(DE3)/pReXdsbA**sfgfp* (5000 µM)
BL21(DE3)/pReXdsbA**sfgfp* (500 µM)
BL21(DE3)/pReXdsbA**sfgfp* (0 µM)
Lemo21(DE3)/pETdsbA**sfgfp* (100 µM)
Control Lemo21(DE3)/pET (0 µM)

Supplementary Figure 2: Color version of Figure 4C.