Chapter 5

How to measure macromolecular crowding in the periplasm?

Jonas van den Berg, Arnold J. Boersma, Bert Poolman

Abstract

We wanted to address the question how condition-dependent volume regulation and protein allocation affect macromolecular crowding in the cytoplasm and periplasm of *E. coli*. We build on a previously developed FRET-based crowding sensor, which was now targeted to the periplasm via the Twin Arginine Translocation (TAT) pathway by fusing the TorA signal sequence to the N-terminus of the sensor. We find that the majority of the sensor remains in the cytoplasm, however small amounts of the sensor are detected in the periplasm, which is best visible when cell are osmotically upshifted. We thus show that the TAT system is able to transport multidomain protein, but the translocation is very inefficient. We provide some suggestions how to improve the translocation of the sensor in future experiments.

JvdB, AB and BP designed the study, AB designed the crowding sensor, JvdB carried out the experiments, analyzed the data and wrote the manuscript.
Introduction

The interior of the cell is densely packed with macromolecules at concentrations of 200-400 mg/mL [1]. The highly crowded environment alters the interaction and reaction conditions of molecules and is therefore an important physicochemical parameter that influences the physiology of the cell. The cellular content of Gram-negative bacteria is divided over four compartments, the cytoplasm, periplasm, the inner and outer membrane. In Chapter 4 we determined the volume of the cytoplasm and periplasm under different growth conditions and saw that the volume of the cytoplasm decreases and that of the periplasm increases with decreasing growth rate of the cells. Simultaneously, slow growing cells contain lower amounts of cytoplasmic and higher amounts of periplasmic proteins [2]. The condition-dependent volume and protein mass of the cytoplasm and periplasm change correspondingly, which led to the conclusion that cells keep the crowding conditions of both compartments constant over a wide range of growth conditions.

We wanted to test the hypothesis of crowding homeostasis by comparing the (macro)molecular conditions of the cytoplasm and the periplasm, using a recently developed Förster resonance energy transfer (FRET)-based crowding sensor (named GE sensor) [3]. The sensor consists of two fluorescent proteins: the donor is a cyan fluorescent protein (CFP) and the acceptor a yellow fluorescent protein (YFP). They are connected via flexible linkers and separated by two α-helices (see Figure 1), allowing the sensor to be compressed when the crowding conditions increase, which gives rise to an increase of the

![Figure 1: Schematic representation of the GE crowding sensor (see [3] for sequence and structure of the sensor). Increasing macromolecular crowding, e.g. imposed by osmotic upshift, compresses the sensor and the fluorescent proteins CFP and YFP are brought closer together, which gives an increased FRET efficiency.](image-url)
FRET signal. The sensor was successfully applied to probe the crowding of the cytoplasm of E. coli and the cytoplasm and organelles of eukaryotic cells [3]; the crowding of the E. coli cytoplasm was found equivalent to ~20% (w/v) 70 kDa Ficoll.

We aimed to target the crowding sensor to the periplasm by fusing the protein to the signal sequence TorA, which is recognized by the Twin Arginine Translocation (TAT) pathway. The TAT export machinery can transport folded proteins to the periplasm, which was successfully shown for single domain proteins like GFP and some of the native substrates of the system in E. coli [4-8]. Quantifying the crowding conditions in the periplasm is a challenging task due to the small size of the compartment. In Chapter 4 we found that the periplasm comprises 5-20% of the total cell volume and its width along the long axis is about 16 nm. Reliable FRET ratios for the periplasm can therefore only be achieved if near 100% of the sensor is located in the periplasm to avoid contamination with fluorescent signal from the cytoplasm.

Unfortunately, we did not succeed in targeting sufficient amounts of crowding sensor to the periplasm. We think this is because the crowding sensor in its compressed form is too big to be translocated by the TAT pathway. In this chapter, we describe the obstacles in exporting the GE crowding sensor to the periplasm and provide alternative suggestions for further experiments.

Material and Methods

Cloning
The cytoplasmic FRET-based crowding sensor GE carries a N-terminal CFP, followed by a sequence for flexible linkers and α-helices (GSG)₆A(EAAAK)₆A (GSG)₆A(EAAAK)₆A(GSG)₆A, where (GSG)₆ are the linkers and (EAAAK)₆ the α-helices, followed by a C-terminal YFP; versions of this protein have been made with and without N-terminal His₆-tag. To obtain the periplasmic crowding sensor the DNA coding for the signal sequence TorA was purchased from GeneArt (Germany), using their E. coli codon optimization protocol. The plasmid pMK-RQ containing TorA was digested with SalI and SacI and the TorA sequence was inserted into pACYC-GE, replacing the N-terminal His₆-tag of the cytoplasmic sensor GE. After DNA sequencing we found a ribosome binding-site (RBS) within the coding region of the gene, which was located 11 base pair upstream of an ATG. Additionally there was a GTG codon 14 base pairs downstream of the RBS, which could potentially also serve as a start codon and result in co-transcription of YFP. We used the QuikChange protocol
from Agilent to mutate M363G and V364G (pACYC-GE) and M402G and V403G (pACYC-TorA-GE), using the primers 6127 and 6128, thereby removing the start codons downstream of the unintentional ribosome binding site.

Table 1: Plasmids used in this study.

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Antibiotic resistance</th>
<th>Description</th>
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<tbody>
<tr>
<td>pACYC-GE</td>
<td>Cm</td>
<td>Cytoplasmic crowding sensor containing an N-terminal His6-tag, obtained from Boqun Liu (University of Groningen)</td>
</tr>
<tr>
<td>pACYC-TorA-GE</td>
<td>Cm</td>
<td>Periplasmic crowding sensor</td>
</tr>
<tr>
<td>pACYC-TorA-YPet</td>
<td>Cm</td>
<td>Periplasmic fluorescent protein, see Chapter 4</td>
</tr>
<tr>
<td>pRSET-GE</td>
<td>Amp</td>
<td>Cytoplasmic crowding sensor GE [3], obtained from Arnold Boersma (University of Groningen)</td>
</tr>
</tbody>
</table>

1 Cm, chloramphenicol; Amp, ampicillin

Table 2: Primers used in this study.

<table>
<thead>
<tr>
<th>Number</th>
<th>Name</th>
<th>Sequence 5’ to 3’</th>
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<tbody>
<tr>
<td>6127</td>
<td>For-mut-GE</td>
<td>GAGGTAGCGGTGGGTCCGGTGAGTTGA</td>
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<tr>
<td></td>
<td></td>
<td>GGAAC</td>
</tr>
<tr>
<td>6128</td>
<td>Rev-mut-GE</td>
<td>GTCCTCACCTTTACTCCCACCACCACCTGA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CCTC</td>
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Growth conditions

*E. coli* cells were grown under the same conditions as described in Chapter 4. In brief, all media were adjusted to 0.3 ± 0.02 Osm with NaCl and supplemented with 32 µg/mL chloramphenicol (Sigma Aldrich) or 100 mg/mL ampicillin (Sigma Aldrich). The cells were grown aerobically at 37 °C and 200 rpm shaking. For expression of the periplasmic or cytoplasmic crowding sensor in pACYC from the rha promoter we induced *E. coli* MG1655 or BL21(DE3) cells, harboring pACYC-TorA-GE or pACYC-GE, by adding 0.1% (w/v) *L*-rhamnose for 2 h, if not stated otherwise. After the induction period cells were washed twice in fresh medium without inducer and allowed to continue growing for 2 h, which is the post-induction time. TorA-YPet was induced from pACYC in *E. coli* MG1655 for 1 h with 0.01% *L*-rhamnose, followed by 1 h of post-induction time.

To express the periplasmic TorA-GE sensor in the single knockout strains from the Keio collection, *E. coli* JW0554 (ΔompT) and JW0429 (ΔLon) were made chemically competent and transformed with pACYC-TorA-GE. We induced
with 0.1% (w/v) L-rhamnose for 2 h, then washed the cells twice and used a post-induction time of 2 h.

Additionally, *E. coli* BL21(DE3) was transformed with pRSET-GE and grown in EZ rich, defined medium (EZ-RDM from Teknova) with glycerol (2 mg/mL) or M9 minimal medium with glycerol (2.2 mg/mL), galactose (2.3 mg/mL) or acetate (3.5 mg/mL) as carbon source. For exact medium composition we refer to Chapter 4. Leaky expression from the T7 promoter was used for pRSET.

**Confocal fluorescence microscopy**

All cells were grown until a final OD$_{600}$ of 0.25 – 0.4 and then prepared for microscopy. 25 µL of the cell suspension were added to a cover slide, which was cleaned in 5 M KOH for 40 min and coated with (3-Aminopropyl)triethoxysilane (Sigma Aldrich). For imaging, the coverslip was mounted on a laser-scanning confocal microscope (Zeiss LSM 710, Carl Zeiss, Jena, Germany C-apochromat 40x/1.20 w Korr M27 water immersion objective with NA of 1.2). The sample was excited with a 405-mm LED and the emission was split into a 450-505 nm (CFP) channel and a 505-797 nm (YFP) channel.

**Results**

**Optimization of expression of the periplasmic crowding sensor**

In order to export the crowding sensor to the periplasm, we first optimized the expression conditions. *E. coli* MG1655, bearing the plasmid pACYC-TorA-GE, was grown in EZ-glycerol or M9-glycerol and induced with 0.01% or 0.1% (w/v) L-rhamnose for either 1 h or 2 h. To compensate for the slow export via the TAT export system, cells were allowed to continue growing in fresh medium without inducer; the post-induction time was varied from 1, 2 to 4 h. Cells grown in EZ-glycerol showed very low levels of expression with fluorescent signals close to background intensity. We were not able to raise the expression levels to high enough amounts, even with inducer concentrations as high as 1% (w/v) L-rhamnose.

In M9-glycerol cells contained sufficient amounts of TorA-GE when induced for 2 h with 0.1% (w/v) L-rhamnose. In Figure 2A representative cells are shown after 2 h of post-induction time. The TorA-GE sensor was not detected in the periplasm. We plotted the fluorescence profile along the short axis of the cell (see Figure 2B) and compared it with cells expressing TorA-YPet that was expressed from pACYC with 0.01% (w/v) L-rhamnose and 1 h of post-induction time. TorA-YPet showed a higher fluorescence signal along the cell periphery,
indicating successful export (also see Chapter 4). In contrast, the fluorescent signal of TorA-GE seemed to emanate from the cytoplasm. With TorA-GE, we observed bright fluorescent spots with a high YPF/CFP ratio at the poles of around 10% of the cells, which may correspond to a fraction of sensor trapped in inclusion bodies. After 4 h of post-induction time the vast majority of the sensor was still in the cytoplasm. Under those conditions the fluorescent signal decreased due to distribution of the sensor over new generations of cells.

The ‘periplasmic’ crowding sensor is functional in the cytoplasm

Additionally, we expressed the GE sensor without signal sequence from the rha promoter of pACYC in E. coli MG1655 to compare it to the periplasmic sensor. The YFP/CFP ratio in the cytoplasm after a post-induction time of 2 h was found to be 1.11 and 1.10 for cells containing TorA-GE and the GE, respectively (see Figure 2C), indicating that TorA-GE, instead of being exported to the periplasm, is fully functional in the cytoplasm. It should be mentioned that the FRET ratios are dependent of the time between induction and the actual measurement, which could be caused by differences in YFP and CFP maturation times or might arise from the burst of expression of the sensor, which might alter the crowding conditions. Directly after the induction period the FRET ratio of both, GE and TorA-GE (in the cytoplasm), was ~1.4; after 1 h post-induction time it decreased to ~1.3. The FRET ratios after 2 h of post-induction time (~1.1) were still somewhat higher than those observed by Boersma et al. [3]. They observed FRET ratios of ~1.02 in the cytoplasm of E. coli BL21(DE3) with the GE sensor under control of the T7 promoter of pRSET and conditions of leaky expression.

Small amounts of crowding sensor can be found in the periplasm

To analyze the crowding conditions in the periplasmic spaces, we show two possible options. Both channels (donor: CFP and acceptor: YFP) were either combined to create a composite image of the (false-colored) red CFP and green YFP or the signals were divided to create a heatmap of FRET ratios (see Figure 2D). The first option has the advantage that one sees where most of the sensor is localized (e.g. poles, cytoplasm or periplasm), whereas the second allows the observation of heterogeneities in crowding within the cell.

In case a small fraction of the TorA-GE sensor is exported to the periplasm and crowding conditions in the periplasm and the cytoplasm are very similar then the fraction of periplasmic sensor would likely be obscured by the protein in the cytoplasm. We therefore plasmolyzed cells by addition of 500 mM NaCl, which decreases the cytoplasmic volume and should increase the periplasmic space and therefore decrease crowding conditions in the periplasm. In Figures 2D
Figure 2: Attempts to export the crowding sensor TorA-GE to the periplasm. All panels correspond to *E. coli* MG1655 growing in M9-glycerol, expressing TorA-GE or GE from pACYC with 2 h post-induction time. (A) The crowding sensor TorA-GE was only localized in the cytoplasm. In around 10% of all cells, polar spots with increased FRET ratios were observed (B) Cross-sections along the short axis of the cell show periplasmic localization of TorA-YPet, but not of TorA-GE. (C) For ~30 cells expressing GE or TorA-GE the YFP intensity was plotted versus CFP intensity. The points with intensities <5 represent blank cells, expressing no sensor, which were grown in parallel and mixed with fluorescent cells. Data points were fitted with a linear equation and the slope was used as average FRET ratio. We found FRET ratios of 1.10 for GE and 1.11 for TorA-GE, indicating fully functional TorA-GE in the cytoplasm. Cells with polar spots were not analyzed. (D) Example of a cell expressing TorA-GE and plasmolyzed with 500 mM NaCl. The cell is represented as either an overlay of CFP (donor) and YFP (acceptor) signal (composite image) or by dividing the YFP by the CFP signal to create a heat map from which the FRET ratio can be deducted. (E, F) Cells plasmolyzed with 500 mM NaCl expressing (E) TorA-GE or (F) the cytoplasmic GE. Lower FRET ratios at plasmolysis spaces are observed in (E), whereas plasmolysis spaces in (F) are free of cytoplasmic signal.
and E we see visible plasmolysis spaces in the brightfield images of cells treated with 500 mM NaCl. In the corresponding composite and heat map images, the FRET ratio is decreased in the plasmolysis spaces. The FRET value was ~0.9, which would correspond to a sensor under dilute conditions. This is what we would expect for a lower macromolecular crowding in the periplasm. We cannot exclude the possibility that the sensor is cleaved either before or during export, which would lead to CFP in the periplasm and additional YFP in the cytoplasm. In fact, the sensor could get trapped in the membrane, for example by premature closing of the TAT pore, with the different fluorophores on either side of the plasma membrane. However, in that case the CFP should be visible in the periplasm, even under non-shocked conditions.

In contrast, we did not observe any fluorescent signal in the plasmolysis spaces of cells expressing the cytoplasmic GE sensor (Figure 2F). These findings suggest that the TAT pathway is able to export a fraction of the TorA-GE sensor to the periplasm, which only becomes visible when the periplasmic space is increased by plasmolysis. In non-stressed cells, TorA-GE stays undetected, presumably because the majority of the sensor remains in the cytoplasm, which masks the periplasmic signal. Because no change in FRET ratios are visible at the cell periphery, it is tempting to say, that both compartments are similarly crowded, however solid experimental proof is missing.

**Expression of TorA-GE in E. coli BL21**

Next, we switched to *E. coli* BL21(DE3), a strain often used to express recombinant genes [9]. We transformed the cells with pACYC-TorA-GE and grew them in M9-glycerol under the same growth and induction conditions as described for *E. coli* MG1655. Surprisingly, we observed spots with extremely high YFP/CFP ratios (around 2-2.5) at the cell poles, while the cytoplasm still contained fluorescent signal (see Figure 3A). The spots were seen in around 70% of the cells and looked similar to the ones seen in about 10% of the *E. coli* MG1655 cells. Typically, they were found at only one of the cell poles. The fluorescent spots could in principle originate from the periplasmic space, which is known to be bigger at the cell poles [4]. Contrary to TorA-GE, YPet carrying a TorA signal sequence was localized throughout the periplasm and was highly mobile (see Chapter 4). From the fluorescent images it is not clear whether the spots are localized in the cytoplasm or the periplasm, because we cannot tell where the inner membrane is. However, since we did not observe export in MG1655, these protein aggregates are very likely present in the cytoplasm.
The FRET ratio of the polar spots was not affected by an osmotic upshift (500 mM NaCl; see Figure 3B), suggesting that they are aggregates of fluorescent proteins. A FRET ratio of 2-2.5 corresponds to an average distance between YFP and CFP of ~5 nm, indicating a compressed state of the sensor. By comparison, Boersma et al. showed that the fluorophores of the GE sensor in cytoplasm of cells grown in MBM glucose are on average 6.7 nm apart (at YFP/CFP = 1.02) [3]. When cells were osmotically upshifted by the addition of 500 mM NaCl the fluorophores became on average ~0.3 nm closer together (at YFP/CFP = 1.12). Due to the high compression of TorA-GE, we therefore think that in E. coli BL21(DE3) (and to some extent in E. coli MG1655) the majority of the sensor carrying the signal sequence is clustered or aggregated and in equilibrium with a non-aggregated form that is freely diffusing and fully functional in the cytoplasm.

![Figure 3](image)

**Figure 3:** (A) E. coli BL21(DE) grown in M9-glycerol expressing the periplasmic TorA-GE crowding sensor from the rha promoter in pACYC. TorA-GE shows polar spots with increased YFP/CFP ratio. (B) The same cells as in (A) when plasmolyzed with 500 mM NaCl did not show a change in FRET ratio of the polar spots.

**Lon protease is involved in degradation of aggregated sensor**

What causes the increased aggregate formation of TorA-GE sensor in E. coli BL21(DE3) compared to E. coli MG1655? We compared the genotype of both strains\(^1\) and found that two mutations in BL21(DE3) that could potentially contribute to the formation of aggregates: the gene deletions of *lon* or *ompT*. Lon is a serine protease that is required for the degradation of misfolded proteins in the cytoplasm. It digests unstructured polypeptides, often with a hydrophobic sequence. In fact, Lon is an important component of the protein quality control machinery of E. coli [10]. OmpT is an outer membrane protease

\(^1\)E. coli strain BL21(DE3): F-ompT gal dcm lon hsdSb(rk-mv-) λ(DE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5]) [malB\(^+\)]\(^{K:12}\)\(\lambda^{3}\)

E. coli strain K-12 MG1655: F- λ- ilvG- rfb-50 rph-1
with a preference for denatured periplasmic proteins [11]. We transformed chemically component cells of *E. coli* JW0554 (ΔompT) and JW0429 (ΔLon) from the Keio collection\(^2\) with pACYC-TorA-GE and induced the sensor with 0.1% (w/v) *L*-rhamnose, followed by 2 h post-induction time. In the Lon deletions strain around 80% of the cells showed polar aggregates, whereas in the OmpT deletion strain around 10% of the cells contained aggregates. We thus conclude that Lon is likely involved in the degradation of aggregated TorA-GE sensor in the cytoplasm.

**Expression of the crowding sensor in cells growing in different media**

Next, we tried to measure crowding conditions in the cytoplasm of cells grown in different media. Since expression from pACYC led to varying FRET ratios caused by the induction of the sensor (TorA-GE and GE), we decided to use the original pRSET-GE construct with leaky expression from the T7 promoter [3]. We thus used the BL21(DE3) strain and grew the cells in EZ-glycerol or M9 medium supplemented with glycerol, galactose or acetate (see Chapter 4 for details). Much to our surprise, we found that cells did not grow in M9-galactose and M9-aceate, whereas cells containing a control plasmid grew with specific growth rates of 0.3 and 0.2 h\(^{-1}\), respectively. Next, we grew the cells to exponential phase in LB medium, giving them a 'kick-start', and then washed and resuspended the cells in minimal medium. The OD\(_{600}\) did not increase in the minimal medium, even after overnight incubation. We analyzed the cells 6 h after resuspension in minimal medium and found that they were extremely bright. Cells in EZ-glycerol and M9-glycerol grew slower than expected with growth rates of 0.8 and 0.2 h\(^{-1}\), respectively, and they also displayed high fluorescence intensities. We believe that the T7 promoter of pRSET yields constitutive transcription independent of the growth conditions, which results in considerable overexpression of the sensor and slowed growth or complete growth arrest, depending on the quality of the carbon and energy source.

**Discussion**

The goal of this project was to determine and compare macromolecular crowding in the periplasm and the cytoplasm of *E. coli* under different growth conditions. In Chapter 4 we describe that protein mass and compartmental

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\(^2\)The Keio collection is a collection of systematic single-gene deletion mutants of *E. coli* K-12 BW25113. The original genotype of *E. coli* BW25113 is similar to MG1655, having the same F- λ- rfb-50 rph-1 mutations and additionally: DE(araD-araB)567, lacZ4787(del)::rrnB-3, DE(rhaD-rhaB)568, hsdR514
volume change similarly, suggesting a mechanism to maintain crowding homeostasis for both the cytoplasm and periplasm. The recently developed GE crowding sensor [3] provided a suitable tool to confirm or refute the hypothesis and yield further understanding of how volume and biomass allocation is regulated under various growth conditions. We targeted the GE crowding sensor carrying an N-terminal TorA signal sequence to the periplasm via the TAT export machinery. The vast majority of the “periplasmic” crowding sensor remained in the cytoplasm and was fully functional with FRET ratios similar to those obtained with the GE sensor. By osmotically stressing the cells we saw a decreased FRET ratio in the plasmolysis spaces, suggesting that export of TorA-GE through the TAT system is possible, but it is very inefficient when compared to single domain proteins such as TorA-YPet; the vast majority of the expressed sensor remained in the cytoplasm even after 4 h post-induction time.

The TAT system consists of three membrane components: TatB and TatC form a complex and TatA is present as dispersed monomers. When the twin-arginine recognition motif of the signal sequence binds to TatBC, TatA monomers are recruited to form a translocation complex through which the substrate is transported [6]. The TAT pathway can transport proteins with sizes ranging from 2 to 7 nm in diameter [12]. The crowding sensor is a multi-domain protein with an average distance between the fluorophores of around 6.7 nm when compressed by intracellular macromolecules. Taking the full width of both fluorescent proteins (~3 nm each) into account the average diameter of the sensor would be too big for translocation via TAT system. However, the FRET pairs are connected via flexible linkers that allow rearrangement from stretched to compressed conformations, as was shown in MD simulations (Liu et al., submitted).

We observed that the TorA-GE sensor is prone to aggregation as it accumulates in the cytoplasm of E. coli BL21(DE3), JW0429 (ΔLon) and to a lesser extent in E. coli MG1655. Protein aggregates are often formed from partially misfolded proteins, and we thus speculate that the TorA signal sequence might trigger protein aggregation. Protein aggregates are usually found at the cell poles [13, 14], excluded from the nucleoid, which is in accordance with our observations. Aggregates were not observed in cells expressing the GE sensor, the same construct without the TorA signal sequence. In E. coli strains lacking Lon protease (BL21 and JW0429) the formation of aggregates was increased roughly 7-fold compared to E. coli MG1655. In a similar manner, Rosen el al. found that in the absence of Lon the
formation of protein aggregates upon heat shock (42 °C) is 3 times more likely than in the presence of the protease [15].

We have highlighted the difficulties in exporting a relatively big, multi-domain protein to the periplasm. We propose a number of alternative approaches to improve the translocation to the periplasm. First, a different expression system should be used. The rha promoter from pACYC requires induction, which leads to a sudden increase in mRNA and protein concentrations that might affect macromolecular crowding and aggregate formation. Additionally, either of the fluorophores of the newly synthesized crowding sensor might not be fully matured at the time of measurement, which alters FRET ratios and leads to misinterpretation.

For further studies, we propose a system with constitutive expression. The T7 promoter in pRSET with leaky expression provides such a system, however the expression levels in slow growing cells were too high and the system does not allow fine-tuning. We have performed initial experiments with the GE sensor expressed from pTAC, using the tac promoter and control via multiple copies of lacIq. E. coli does not metabolize the IPTG, therefore the system can be fine-tuned by the addition of low amount of inducer to the growth medium. Second, a crowding sensor with a shorter linker would increase the likelihood of successful export, due to a smaller average diameter. Furthermore, we suggest upregulation of the tatABC genes by co-expression from a compatible plasmid. The efficiency of GFP export to the periplasm was shown to be 20-fold increased when tatABC was overexpressed [4]. Additionally, an intein could be integrated between the FRET pairs. An intein is a protein sequence that allows autocatalytic cleave and rejoining of the N and C terminal proteins (the exins) with a peptide bond, in a process called expressed protein ligation (EPL). This would require substantial protein engineering to allow cleavage of the sensor in the cytoplasm and rejoining of the parts in the periplasm [16]. Instead of analyzing the cells with a confocal microscope, fluorescence lifetime imaging (FLIM) might be an alternative to better distinguish between the cytoplasm and the periplasm.

References
