Supporting Information for:

# Protein $^{19}$F NMR in *Escherichia coli*

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## Materials and Methods

**$^{15}$N Enrichment and 3FY Labeling.** Ten mL of Luria-Bertani (LB) media (10 g Bacto-Tryptone, 5 g Bacto-yeast extract and 10 g NaCl in 1 L of $H_2O$) containing the appropriate antibiotic were inoculated with a single colony and incubated overnight at 37 $^oC$ with shaking at 250 rpm. These overnight cultures were added to 100 mL of Tryptone-Yeast media (16 g/L Bacto-tryptone, 10 g/L Bacto-yeast extract, 5 g/L NaCl, 1 mM NaOH) containing antibiotic. These pre-cultures were grown with shaking at 37 $^oC$ until the absorbance at 600 nm ($A_{600}$) reached between 0.8 and 1.0. The pre-cultures were pelleted at 25 $^oC$ for 10 min at 1,600*g*. One L of $^{15}$N-enriched M9 media[1] plus 1 mL of 1 mg/L thiamine HCl was used to resuspend the cell pellet. This culture was grown with shaking at 37 $^oC$ to an $A_{600}$ of 0.4. Seventy mg of 3-fluoro-*D*, *L*-tyrosine (96%, Lancaster), 60 mg of *L*-phenylalanine (Sigma), 60 mg of *L*-tryptophan (Sigma) and 0.5 g of N-(phosphonomethyl)glycine (96%, Sigma) were dissolved in 1 L of media. This mixture was added 30 min before induction. The induced culture was grown overnight with shaking at 37 $^oC$.

**tfmF Labeling.** A single DH10B colony containing both the appropriate pBAD and pDule-tfm-Phe vectors was picked from an ampicillin/tetracycline plate and used to inoculate 50 mL of LB media containing 100 mg/L ampicillin and 25 mg/L tetracycline. The culture was grown overnight at 37 °C with shaking at 250 rpm. A 2.5 mL sample of the saturated overnight cultures was added to 500 mL of warm arabinose autoinduction medium[2] and the culture was shaken (250 rpm) at 37 °C for 1 h. tfmF was added to a final concentration of 1 mM after 30 min from a 100 mM stock solution prepared by

dissolution in 20 mM NaOH. The cultures were shaken at 37°C for additional 40 h ($A_{600}$ ~5). Cells were harvested by centrifuging at 1,200*g* for 20 min. For in-cell NMR studies, 100 mL cultures were centrifuged at 1,200*g* for 20 min, washed twice with 100mL of LB and resuspended in 1 mL of LB.

**αSYN.** The protein was purified as described,[3] except that the freeze-thaw step was eliminated. Purity was assessed by using SDS-PAGE and its expected molecular weight was confirmed with mass spectrometry (NanoESI-MS).

**PDZ3.** The $^{15}$N-enrichment procedure was similar to that described by Serber *et al.*[4] Luria Bertani (100 mL) media containing 60 μg/mL kanamycin was inoculated with a single colony and incubated overnight at 37 $^{o}$C with shaking at 250 rpm. The overnight culture was pelleted for 10 min at 1,600*g* (Sorvall RC-5B, GSA rotor). One L of $^{15}$N-enriched Spectra 9 media (Cambridge Isotope Laboratories, Inc.) containing the antibiotic was used to resuspend the cell pellets. This culture was grown with shaking at 37 $^{o}$C to an $A_{600}$ nm of 0.8. Expression was induced with isopropyl-D-thiogalactoside at a final concentration of 1 mM, and allowed to proceed for four h.

**Protein Concentration.** Purified proteins were used as standards. The concentration of each pure protein was measured spectrophotometrically [ubiquitin, $\varepsilon_{280nm}$ = 1280 $cm^{-1}M^{-1}$;[5] PDZ3, $\varepsilon_{280\ nm}$ = 2560 $cm^{-1}M^{-1}$ [6] calmodulin, $\varepsilon_{276nm}$ = 3300 $cm^{-1}M^{-1}$;[7] GFP, $\varepsilon_{475\ nm}$= 32500 $cm^{-1}M^{-1}$ (as reported by the manufacturer)].

For each culture, 1 mL aliquots were centrifuged at 16,000*g* for 10 min after induction. The pellets were resuspended in 20 mM potassium phosphate buffer (pH 7.5). The proteins in lysates and standards were resolved by electrophoresis on 10-20% gradient SDS polyacrylamide gels (Criterion, Bio-Rad) for 65 min at 200 V. Gels were analyzed by Coomassie staining with a VersaDoc MP imager (Bio-Rad). Quantity-One software (Bio-Rad) was used to quantify the band intensities.

The concentration of the protein under study in the NMR tube, $C_{tube}$, was determined from the SDS PAGE experiment described above. Cell densities in the NMR tube, $C$, were determined by serial dilution and plating. The protein concentration in cells, $C_{cell}$, was calculated from the equation:

$$C_{cell} = \frac{C_{tube}}{C * V_{cell}}$$

$V_{cell}$ is the volume of an *E. coli* cell [$1\times10^{-15}$ L[8]]. Measurements were performed in triplicate.

**Protein Localization.** Two methods, osmotic shock[9,10] and osmotic shock plus lysozyme,[11] were used to determine the location of expressed protein.

**References**

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**Table S1**. Cell and protein concentrations

| Protein | MW, kDa | Cell concentration, cells/mL x $10^{-11}$ | Protein concentration, mM | |
|---|---|---|---|---|
| | | | NMR tube | cells |
| ubiquitin | 8.5 | 6.3 | 1.8 | 2.9 |
| PDZ3 | 10.8 | 3.1 | 1.3 | 4.2 |
| calmodulin | 16.8 | 4.9 | 2.3 | 4.7 |
| GFP | 26.9 | 5.9 | 1.2 | 2.0 |

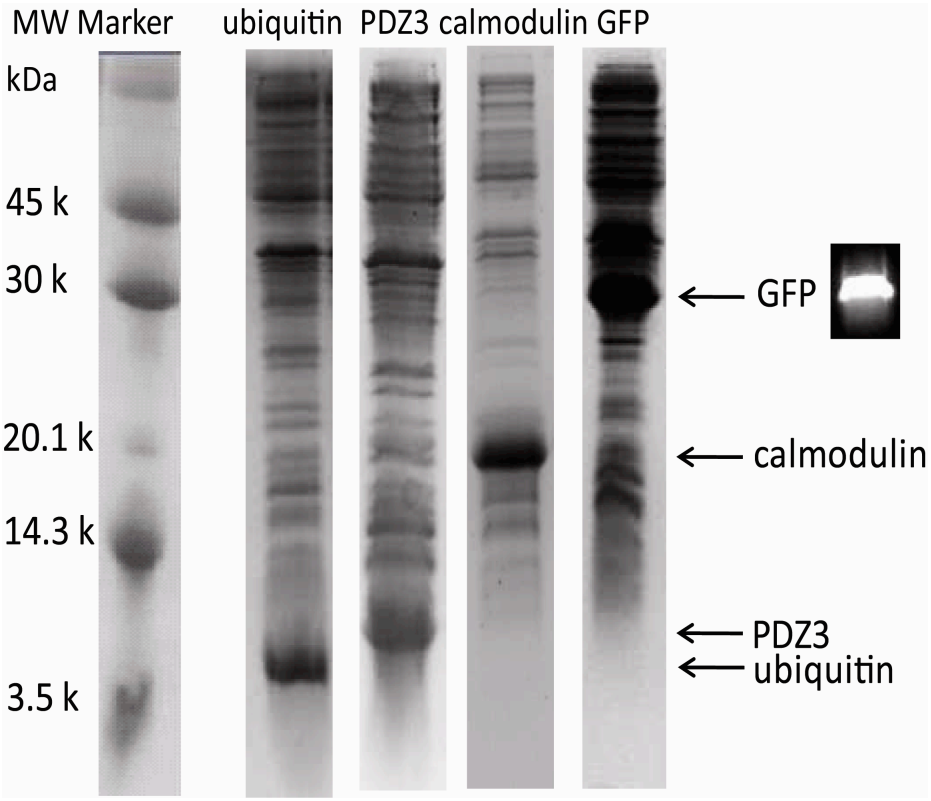



**Figure S1.** SDS-PAGE of protein expression level in cells. Cell lysates were separated on an 18% gel and visualized with Coomassie staining. GFP was also visualized by using fluorescence.

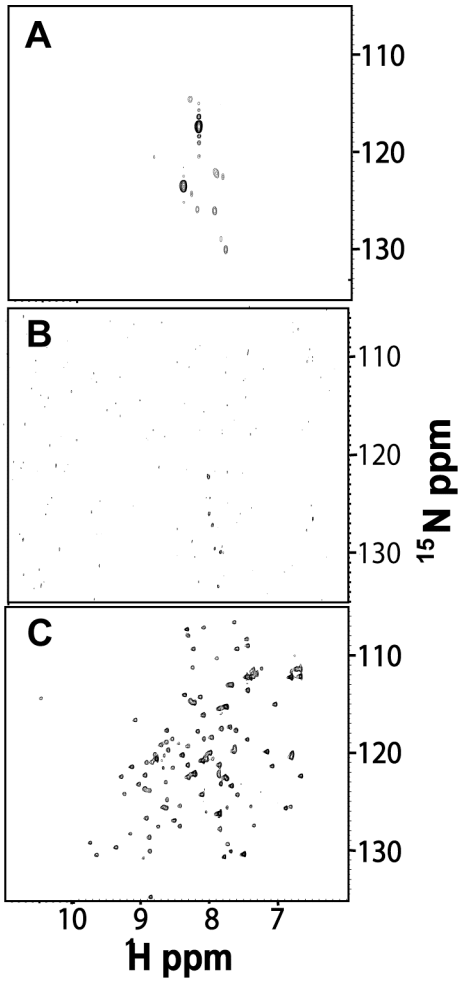



**Figure S2.** $^{1}$H-$^{15}$N HSQC- spectra of $^{15}$N-enriched PDZ3. Cell slurry (A). Supernatant collected immediately after completing the in-cell spectrum (B). Supernatant from the cell lysate (C).

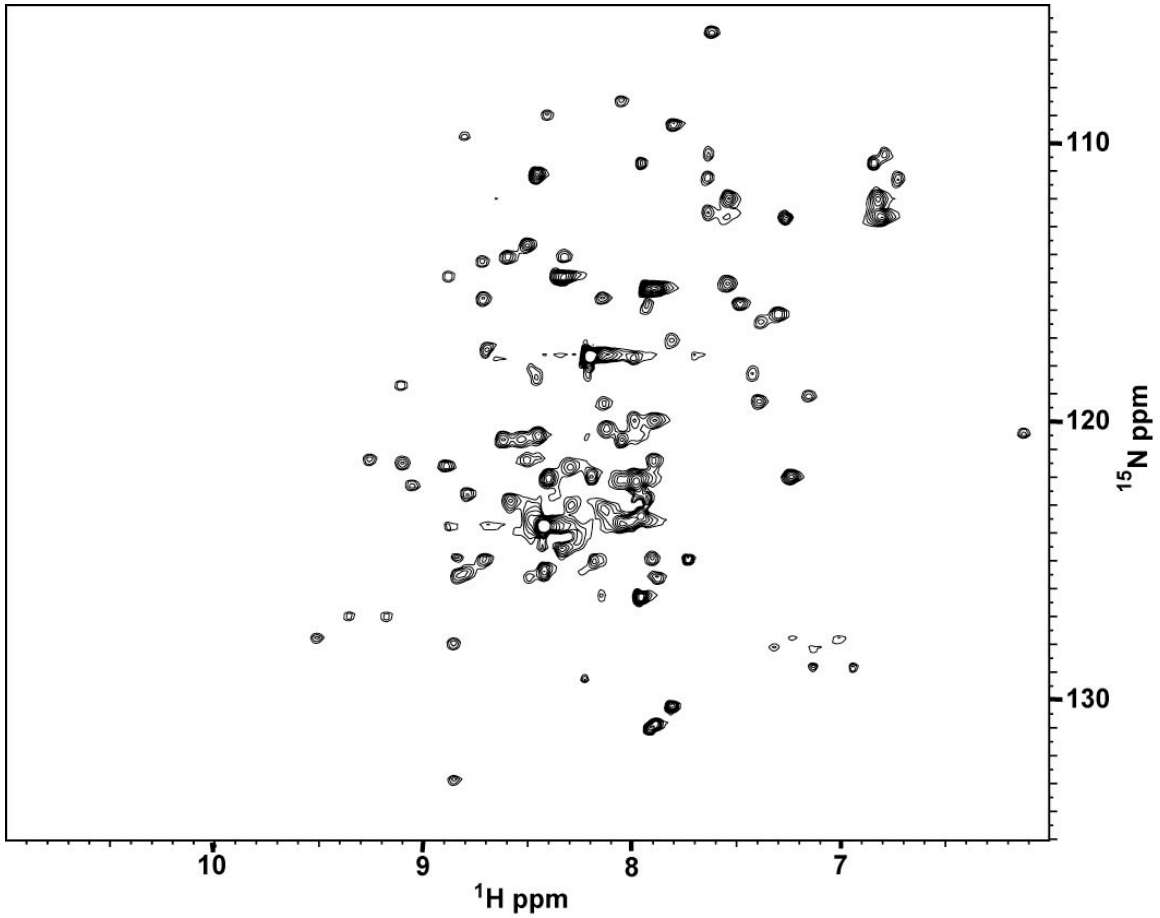
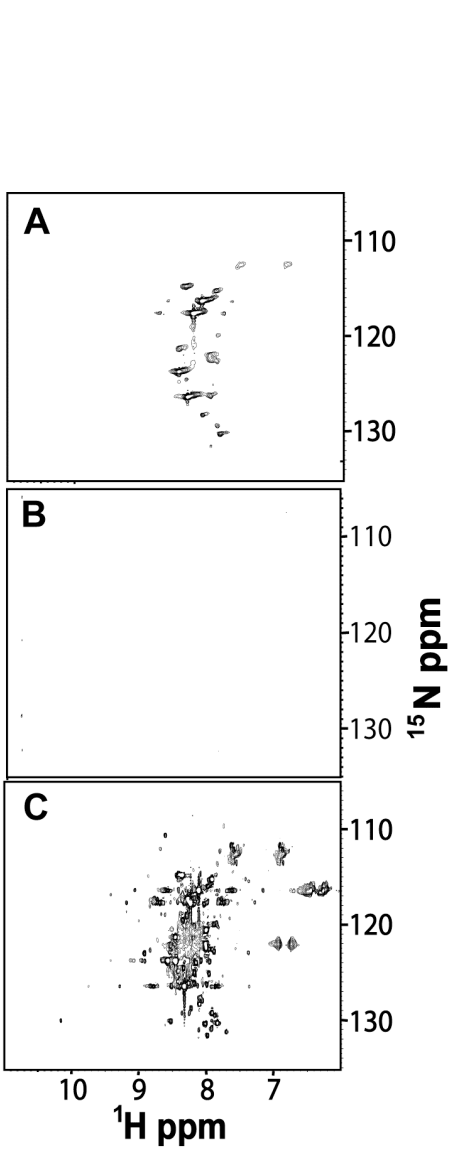



**Figure S3.** $^{1}H$-$^{15}N$ HSQC- spectra of an in-cell UBQ sample after storage at -20 $^{o}C$ overnight.



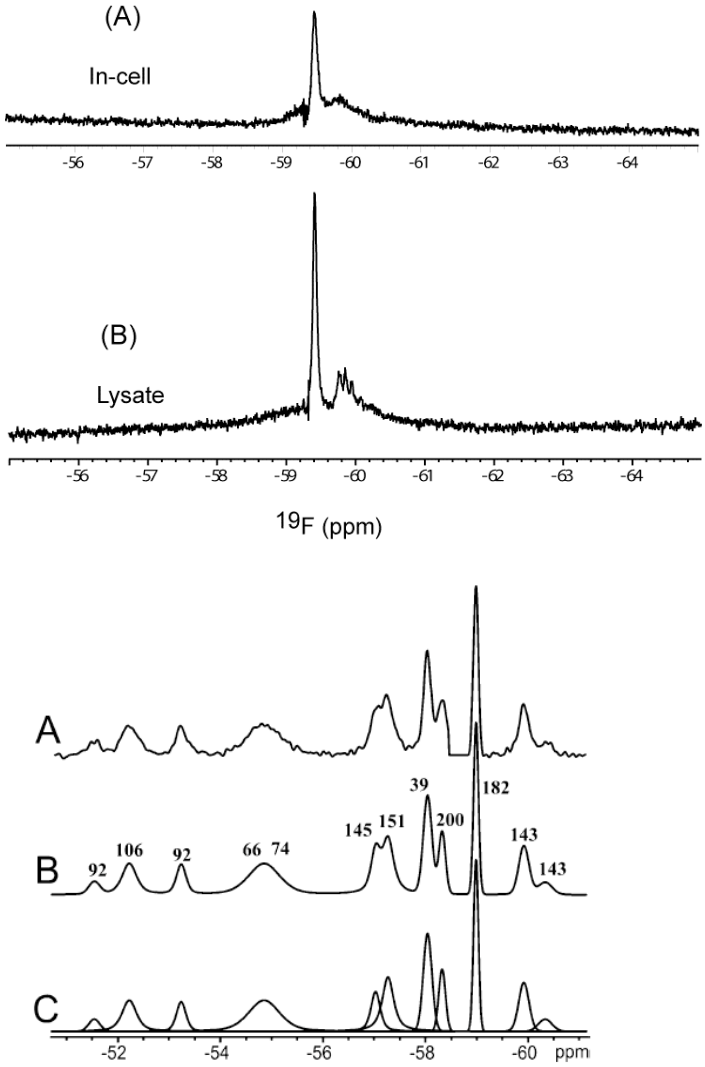




***Figure 5.*** (A) $^{19}$F spectrum (564 MHz) of GFP with Lorentzian-to-Gaussian resolution enhancement. (B) Line-fitting. (C) Individual lines in the fitting. The narrow resonance of free 3-fluorotyrosine at −58.7 ppm has been zeroed to facilitate least squares line-fitting. The assignments of Y39 and Y200 are tentative, as described in the text.

**Figure S4.** GFP data.

**Left column:** $^{1}$H-$^{15}$N HSQC- spectra of $^{15}$N-enriched GFP. Cell slurry (A). Supernatant collected immediately after completing the in-cell spectrum (B). Supernatant from the cell lysate (C).

**Right column:** $^{19}$F spectra of 3FY labeled GFP in cells (A) and the cell lysate (B). The in vitro spectrum of 3FY GFP [from [12]] is shown below panel B.

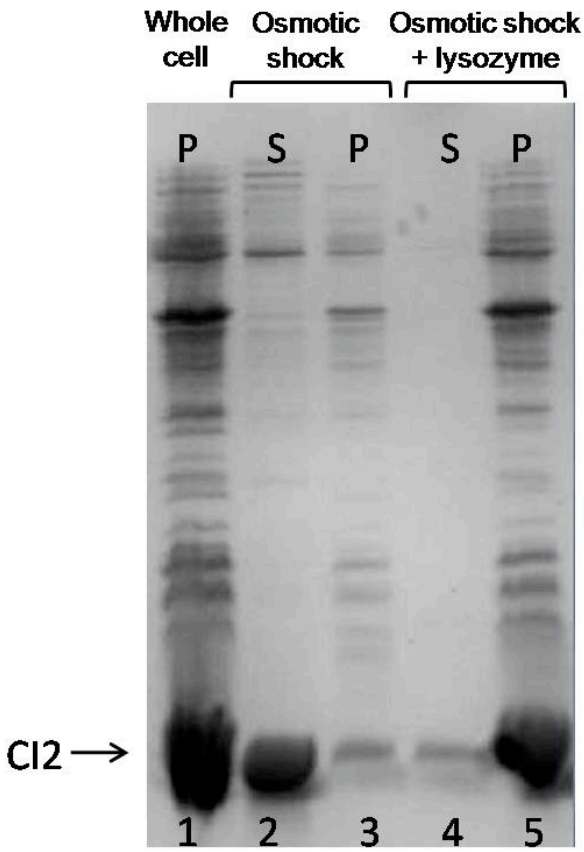



**Figure S5.** Protein location of CI2. Aliquots of *E. coli* [BL-21 (DE3)] expressing CI2 were centrifuged and the pellets exposed to osmotic shock (lanes 2, 3) and osmotic shock plus lysozyme (lanes 4, 5). The pellets (P) and supernatants (S) were resolved by SDS-PAGE (18% gel) with Coomassie staining. Lane 1 is the untreated cell lysate. Proteins in the supernatants are periplasmic. Proteins in the pellets are cytoplasmic.

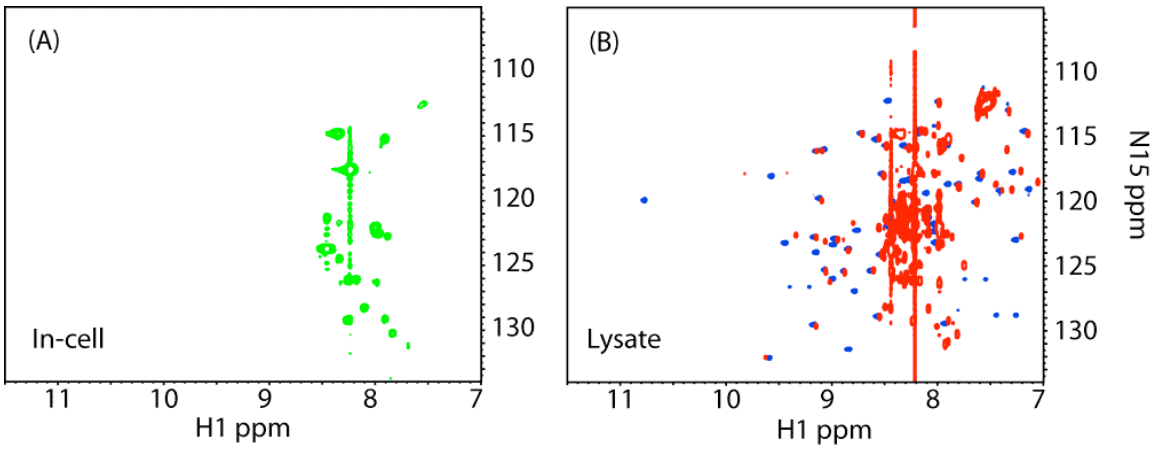



**Figure S6.** $^{1}$H-$^{15}$N HSQC- spectra of $^{15}$N-enriched CI2 expressed from the pBAD promoter in BL21(DE3-Gold) cells [13]. Cell slurry (A). Cell lysate (Red) and purified CI2 (blue) (B).