Electronic Supplementary Information (ESI)

Lanmodulin Remains Unfold and Fails to Interact with Lanthanide Ions in *Escherichia coli* Cells

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1 Material and Methods:

Construction of LanM and LanM-His

The amino acid sequence of LanM were referred to the reported literature (Figure S1).[1] The full length gene was inserted into plasmid pUC57, was synthesized by Sangon (Shanghai, China). Gene of LanM was amplified by PCR from plasmid pUC57, and then inserted to plasmid pET24a for expression. LanM-His expression vector was constructed by fusing the C terminus of the His_6 tag to LanM without any amino acid linker between them by site-directed mutagenesis.[2]

Protein expression and purification

The plasmids pET24a-LanM-His and pET24a-LanM containing the genes for LanM-His and LanM were transformed into *Escherichia coli* BL21 (DE3) cells. Plasmid-containing cells for LanM-His and LanM were plated on LB-agar plates with 50 μg/mL of kanamycin and grown at 37°C. A single colony was inoculated and grown in 5 mL LB Media (50 μg/mL Kana in all growth media) for about 12 hours at 37°C with shaking at 220 rpm. A 100 μL volume of this culture was used to inoculate 100 mL of supplemented M9 media in a 250 mL

baffled flask. Incubate with shaking at 37°C overnight, the entire culture was used to inoculate 900 mL of the same media in a 3L flask, and the culture was grown at 37°C with shaking at 220 rpm and induced at a final concentration of 1 mM isopropyl β-D-thiogalactopyranoside (IPTG) when the optical density at 600 nm (OD_{600}) reached 0.8 to 1.0, after 4 h further incubation, the cells were pelleted by centrifugation for 10 min at 6000 rpm, 4°C. The cell pellet was stored at -80°C until purification. ^{15}N enrichment was achieved by using $^{15}NH4Cl$. For 4F-phenylalanine(4FF) labeling, 70 mg 4-Fluoro-DL-phenylalanine, 60 mg L-tyrosine, 60 mg L-tryptophan and 0.5 g glyphosate were added when OD_{600} reached ~ 0.4. 3F-tyrosine (3FY) labeling was achieved using 3-Fluoro-DL-tyrosine (3FY) plus glyphosate[3].

LanM-His was purified through a HisTrap Ni-NTA affinity column with buffer A (50 mM Tris, 500mM NaCl, 10 mM imidazole, 5% glycerol, pH7.5) and buffer B (50 mM Tris, 500 mM NaCl, 250 mM imidazole, 5% glycerol, pH7.5). The cell paste was re-suspended in 50 ml Buffer A containing 500 uL protease inhibitor cocktail (100x), 1 mg DNase. The suspension was passed several times through ATS high pressure homogenizer at 1000 bar, and debris was pelleted by centrifugation at 20000 rpm for 30 min at 4°C. The supernatant was decanted and applied to a 1.6× 10cm (20 mL) Ni-NTA agarose column, pre-equilibrated in Buffer A. The column was washed with 150 mL 5% Buffer B, and then was washed with 150 mL 10% Buffer B, followed by elution with Buffer B. The eluted protein was concentrated to ~5 mL using an Amicon Ultra 3-kDa MWCO centrifugal filter device. In order to assure sample homogeneity, 5 mM EDTA was added to the protein, then buffer exchanged into Buffer C (20 mM MOPS, 100 mM KCl, pH 7.0). The purification yielded ~20 mg LanM-His from 1 L culture.

LanM was purified by an anion exchange (DEAE Fast Flow column, GE healthcare) with buffer D (50 mM Tris-HCl, 10 mM NaCl, 1mM EDTA, 5% glycerol, and pH8.0) and buffer E (50 mM Tris, 1M NaCl, 1mM EDTA, 5% glycerol, pH8.0). The cell paste was re-suspended in 50 ml Buffer D containing 500 uL protease inhibitor cocktail (100x), 1mg DNase. The suspension was passed several times through ATS high pressure homogenizer at 1000 bar, and debris was boiled at 80℃ for 10 min, then debris was pelleted by centrifugation at 20000 rpm for 30 min at 4°C. The supernatant was decanted and applied to DEAE Fast Flow column. Fractions were concentrated to ~5 mL using an Amicon Ultra 3-kDa MWCO centrifugal filter device, then buffer exchanged into Buffer C.

Sample preparation for in vitro NMR

Protein concentration was estimated by Nanodrop 2000. The NMR samples (0.3 mM) contained six equivalents of YCl_3, and 10% D_2O (50 μL) in a total volume of 500 μL. Consistent with the reported literature, [4, 5] LanM-His is saturated with Y^{3+} in our NMR experiments.

Sample preparation for in-cell NMR

The plasmid pET24a-LanM containing the gene for LanM was transformed into *Escherichia coli* BL21 (DE3) cells. The cells were grown at 37°C in a rotary shaker at 220 rpm and induced at a final concentration of 1 mM IPTG when the OD_{600} reached 0.6. Two hours post-induction at 37°C, Cultures (usually ~200 mL) were harvested by centrifugation at 2000 g for 10 min at room temperature. The cell pellets were gently resuspended in 0.5 mL of buffer G (50 mM HEPES, pH 7.2) containing 10 % D_2O and transferred to a 5 mm tube. Another cell pellets were resuspended in 0.5 mL of buffer H (50 mM HEPES, 20 mM YCl_3/$SmCl_3$ pH7.2), leaved cell slurry at room temperature for half an hour, and then transferred to a 5 mm tube. Supernatants were collected immediately after each experiment by centrifugation (2000 g, 10 min) to assess leakage.[6] The pellets were resuspended in 1 mL of buffer G (50 mM HEPES, pH 7.2). Lysates were made from the resuspended pellets by sonication on ice for 20 min with a duty cycle of 3 s on, 6 s off. The lysate was collected after centrifugation at 16,000 *g* for 30 min prior to NMR.

Preparation of cells lysate

A suspension of BL21 Star (DE3) competent cells (10 µL) from a single colony were inoculated into 10 mL of Luria-Bertani (LB) media and incubated at 37°C for 8 to 9 h with shaking at 220 rpm. Cell pellets were obtained by centrifugation (Eppendorf, model 5804, 4°C, 2000 g, and 7 min) and resuspended in M9 media to a final volume of 100 mL. After being shaken (37°C, 220 rpm) for 4 h, the pre-cultures were transferred to M9 media to a final volume of 1.5 L. The cultures were grown at 37°C for 10 h with shaking (200 rpm). The cells were harvested by centrifugation at 6000 rpm for 10 min at 4°C. The suspension was passed several times through ATS high pressure homogenizer at 1000 bar, the supernatants, collected after centrifugation at 20000 rpm for 30 min at 4°C (Beckman CoulterTMAvanti J-26 XP), were fast frozen in N_2 (l) and lyophilized. The lyophilized lysates were stored at -20ºC.

Determination of concentrations of Y^{3+}

Two hours post-induction at 37°C **,** *Escherichia coli* BL21 (DE3) cells containing LanM from 200 mL culture were pelleted and resuspended in 50 mL buffer G (50 mM HEPES, pH 7.2), the cells were pelleted by centrifugation for 10 min at 2000 g, 4°C, repeated the procedure again, then the cell pellets were gently resuspended in 0.5 mL of buffer H (50 mM HEPES, 20 mM YCl_3, pH 7.2), leaved cell slurry at room temperature for half an hour, the cell pellets were then washed twice using buffer G (50 mM HEPES, pH 7.2), the cells were pelleted by centrifugation for 10 min at 2000 g, 4°C . The cell pellets were digested using nitric acid and analyzed by ICP-MS (Aglient 7800) .

NMR spectroscopy

Data were acquired at 298 K on a Bruker 600 MHz and 700 MHz spectrometer equipped with an H/F/ (C, N) triple resonance cryogenic probe. One-dimensional ^{19}F spectra were acquired with a sweep width 11.26 kHz, a relaxation delay 2 s and an acquisition time 0.73 s on a Bruker 600 MHz, a sweep width 10 kHz, a relaxation delay 2 s and an acquisition time 0.82 s on a Bruker 700 MHz, the number of acquisition was 512 for *E. coli* and 1280 for oocytes, chemical shifts were referenced to trifluorotoluene at -63.72 ppm. ^{1}H -^{15}NHSQC spectra were acquired with a ^{1}H spectral width of 9578.544 Hz and a ^{15}N spectral width of 2431.408 Hz on 600 MHz, a ^{1}H spectral width of 11363.64 Hz and a ^{15}N spectral width of 2838.57 Hz on 700 MHz The matrix size was 2048 by 256. The data were processed with Topspin 3.5.

Data analysis

NMR spectra were analyzed using Topspin 3.5. The integrations corresponding to unfold form of 4FF labeled LanM was calculated from -115.6 to -116.4 ppm, and the Y^{3+}- bound form was from -113.5 to -115.2 ppm and from -116.4 to -117.7 ppm. The fraction of unfold form (F_U) is calculated as:

$$F_U = \frac{Peaks\ area\ _{(from-115.6\ to\ -116.4\ ppm)}}{Peaks\ area\ _{(from-113.5\ to\ -115.4\ ppm)} + Peaks\ area\ _{(from-115.6\ to\ -116.4\ ppm)} + Peaks\ area_{(from-116.4\ to\ -117.7\ ppm)}}$$

The fraction of Y^{3+}- bound form (F_F) is calculated as:

$$F_F = 1 - F_U$$

2 Supplementary Figures

PTTTTKVD IAAFDPDKDG TIDLKEALAA GSAAFDKLDP 60

DKDGTLDAKE	LKGRVSEADL	KKLDPDNDGT	LDKKEYLAAV	100
EAQFKAANPD	NDGTIDAREL	ASPAGSALVN	LIRHHHHHH	139

Figure S1. Sequence of the LanM and LanM-His construct used in this study.

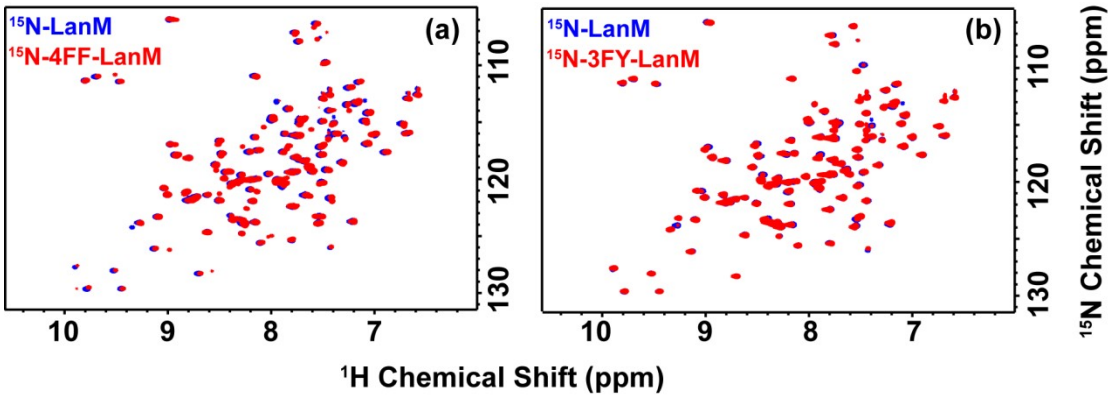

Figure S2. Overlaid ^{1}H- ^{15}N HSQC spectra of ^{15}N-enriched (blue, a) and ^{15}N/4FF double labeled Y^{3+}-LanM (red, a); Overlaid ^{1}H- ^{15}N HSQC spectra of ^{15}N-enriched (blue, b) and ^{15}N/3FY double labeled Y^{3+}-LanM (red, b).

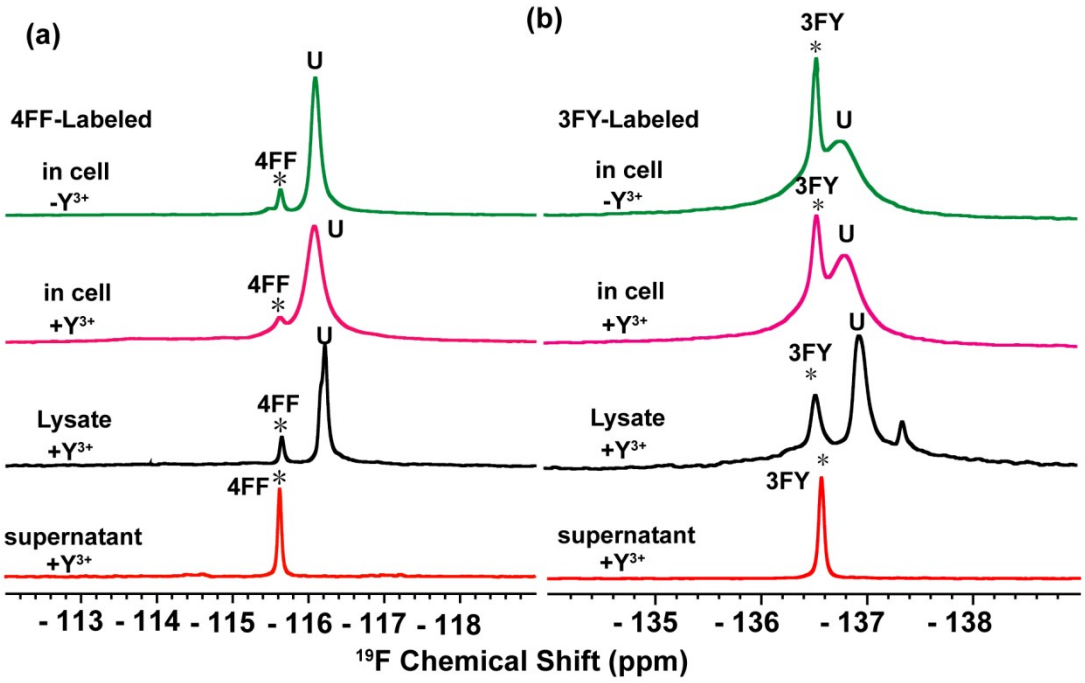

Figure S3. One-dimensional ^{19}F NMR spectra of 4FF-labeled (a) and 3FY-labeled (b) LanM in *E. coli* cells, cells

lysate and supernatant. U, unfolded form; F, folded form; asterisk, resonance of free 4FF or 3FY.

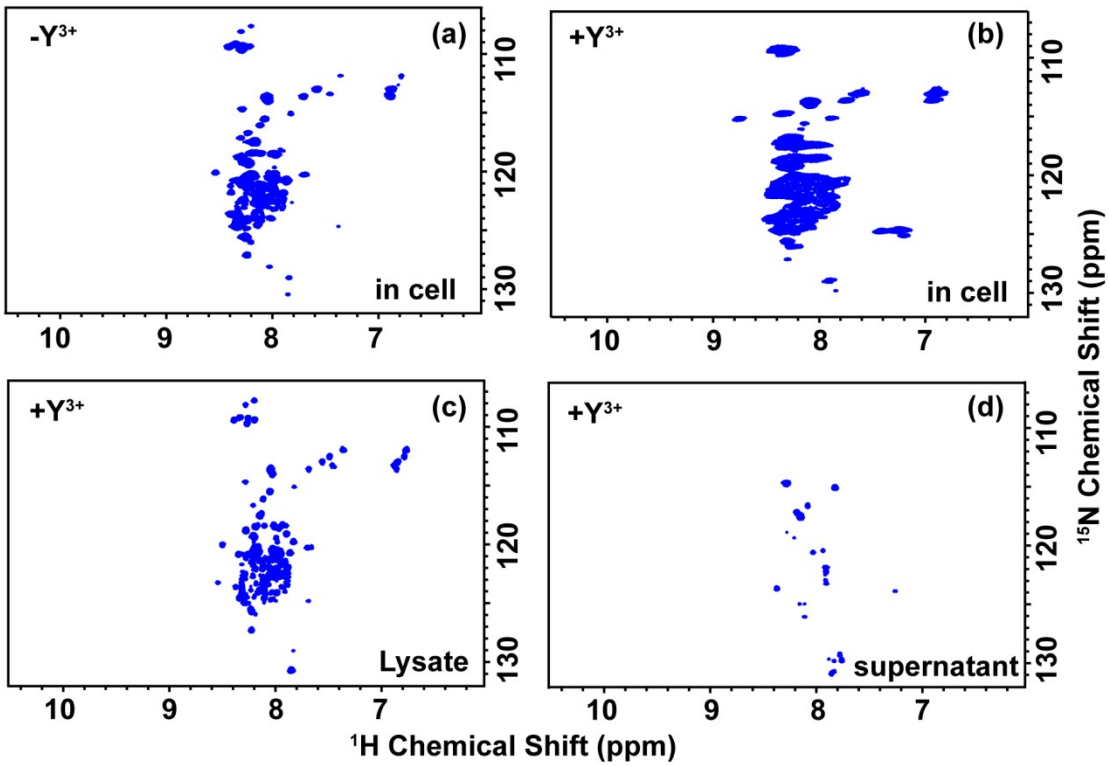

Figure S4. ^{1}H- ^{15}N HSQC spectra of ^{15}N, 4FF-labeled LanM in *E. coli* cells, cells lysate and supernatant.

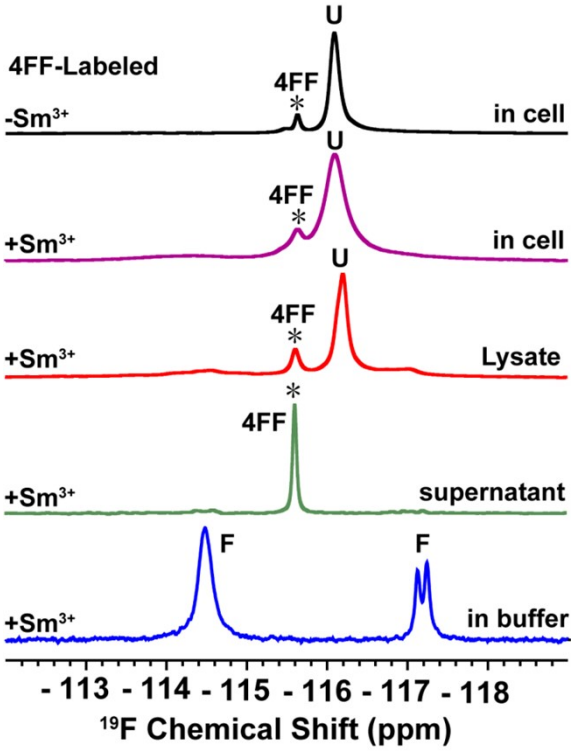

Figure S5. One-dimensional ^{19}F NMR spectra of 4FF-labeled LanM in *E. coli* cells, cells lysate, supernatant and buffer. U, unfolded form; F, folded form; asterisk, resonance of free 4FF.

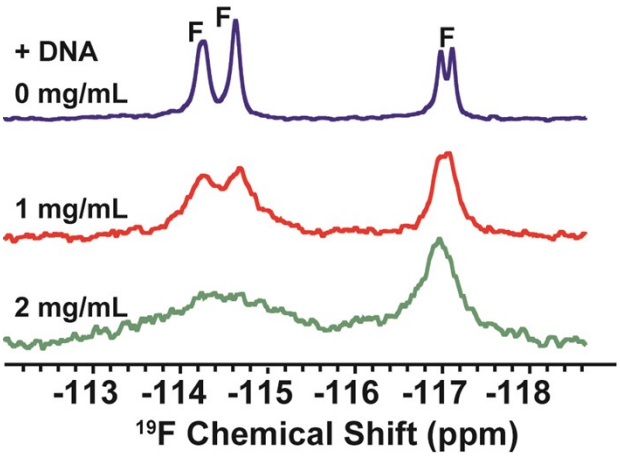

Figure S6. One-dimensional ^{19}F NMR spectra of 0.3 mM 4FF-labeled Y^{3+}-LanM in (6 equiv Y^{3+}) presence 1 and 2 mg/mL genome DNA.

References

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