Supplementary Materials and Methods

Chemicals and Reagents

All chemicals used were reagent grade or better.

Plasmids

All plasmids used were previously constructed in our lab and are described in Maldonado [1]. pASK-Pup expresses Pup-GGQ from a tet promoter/operator, which is induced by using anhydrotetracycline. This plasmid confers ampicillin resistance, and contains an f1 origin and the *tet* gene, which encodes for Tet repressor. pRSF-*Msm* Mpa expresses *Mycobacterium smegmatis (Msm)* Mpa from a T7 promoter/lac operator, which is induced by using isopropyl 1-thio-β-d-galactopyranoside (IPTG). This plasmid confers kanamycin resistance, and contains an RSF replication origin and the *LacI* gene, which codes for Lac repressor.

Sequential over-expression of ^{15}N-Pup and Mpa

Plasmids pASK-Pup and pRSF-*Msm* Mpa were co-transformed into *E. coli* strain BL21(DE3) Codon+ (Novagen) for sequential over-expression of [*U*-^{15}N] Pup-GGQ and Mpa, respectively. [*U*-^{15}N] Pup-GGQ was overexpressed for 2 hours followed by over-expression of unlabeled Mpa. [*U*-^{15}N] labeling was achieved by growing cells in minimal medium (M9 salts containing calcium chloride (10 μM), glucose (0.4%), magnesium sulfate (2 mM), and thiamine hydrochloride (1 mM)) containing ampicillin (100 mg/L), kanamycin (35 mg/L), and [*U*-^{15}N] ammonium chloride (1 g/L) as the sole nitrogen source. Pup-GGQ over-expression was induced with anhydrotetracycline (0.2 μg/mL) in dimethylformamide (DMF) when the culture reached an OD_{595} of 0.7, and the cells grown with shaking for 2 hours at 37 ºC. Cells (100 mL) expressing labeled Pup were collected and washed twice with the NMR buffer (50 mL) (potassium phosphate buffer (10 mM, pH 6.5)) prior to NMR analysis. Cells expressing Pup were centrifuged, and washed twice with 1xM9 salts and re-suspended to a final OD_{595} of 0.7 in fresh unlabeled Luria Broth containing ampicillin (100 mg/L) and kanamycin (35 mg/L). Expression of Mpa was induced with IPTG (0.5 mM) and the cells grown with shaking at 37 ºC. Samples (100 mL) of 2 hour [*U*-^{15}N] Pup and unlabeled Mpa were collected at 3, 4, 6, 7, 8, and 18 hours post IPTG induction, for a total of six samples. Each sample was washed twice with NMR buffer

(50 mL) before being re-suspended in the NMR buffer (450 uL) and D_2O (50 uL) for a total volume of 500 uL.

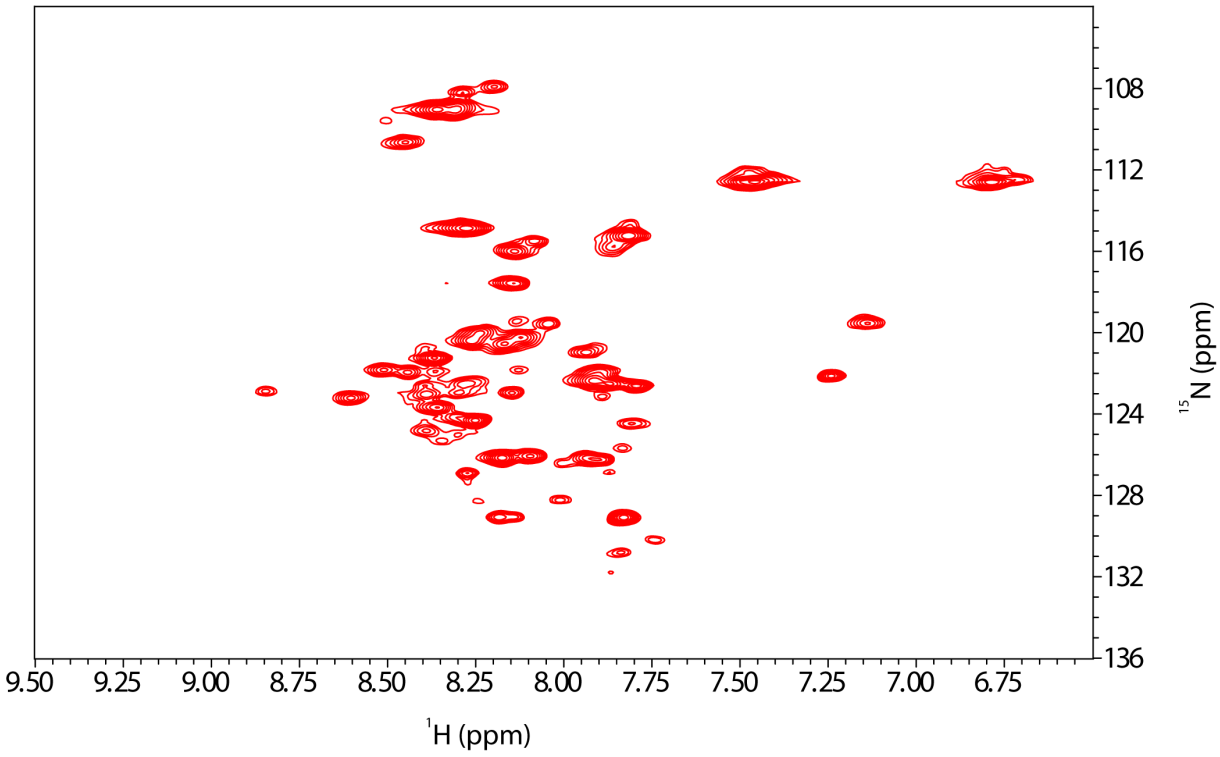

Figure S1. In-cell NMR spectra of free [*U*-^{15}N] Pup. To express [*U*-^{15}N] Pup, cells grown on [*U*-^{15}N] minimal medium were induced by anhydrotetracycline (0.2 µg/mL) for 2 hours. The cells were pelleted, washed twice with the NMR buffer (50 mL) (potassium phosphate buffer (10 mM pH 6.5), re-suspended in the NMR buffer (450 µL) and D_2O (50 µL), and transferred to a standard 5 mm NMR tube for in-cell NMR analysis.

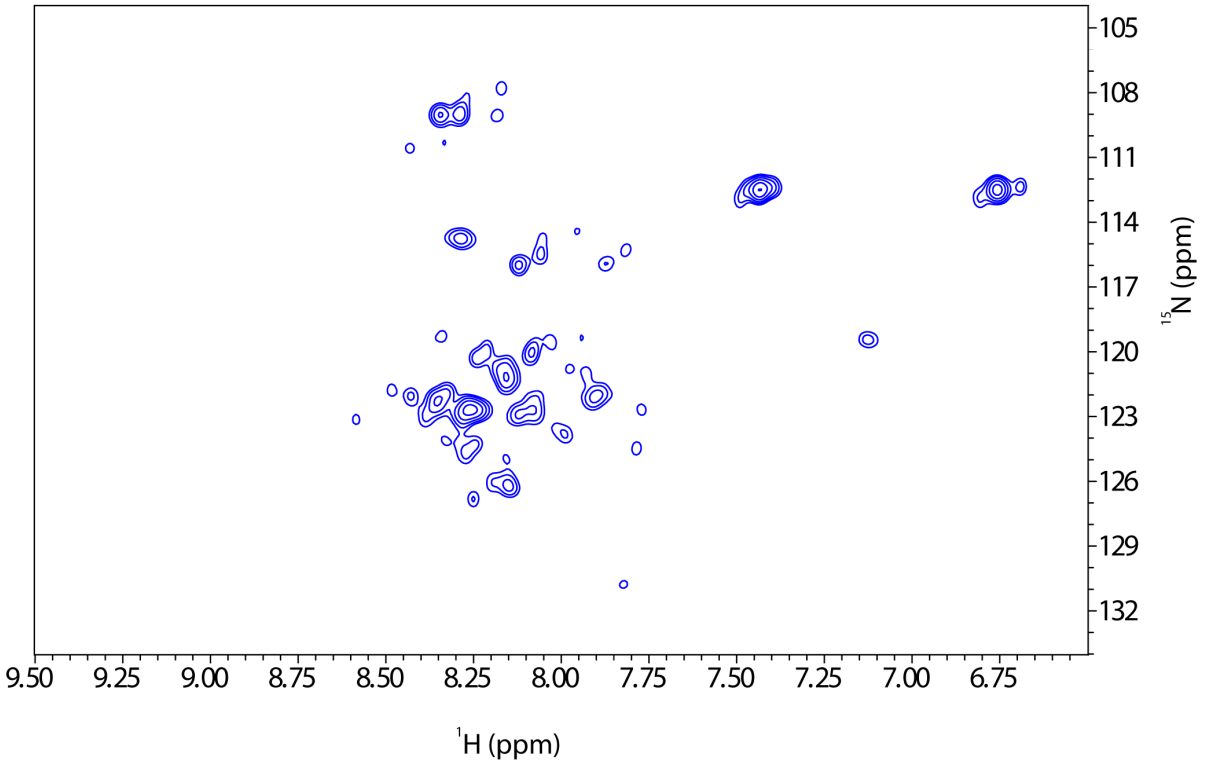

Figure S2. In-cell NMR spectrum of [*U*-^{15}N] Pup/MPA complex. To create a [*U*-^{15}N] Pup-MPA complex, after 2 hours of [*U*-^{15}N] Pup expression, the cells were re-suspended in unlabeled rich medium and expression of Mpa was induced by IPTG (0.5 mM) for 8 hours. The cells were pelleted, washed twice with the NMR buffer (50 mL) (potassium phosphate buffer (10 mM, pH 6.5), re-suspended in the NMR buffer (450 µL) and D_2O (50 µL), and transferred to a standard 5 mm NMR tube for in-cell NMR analysis.

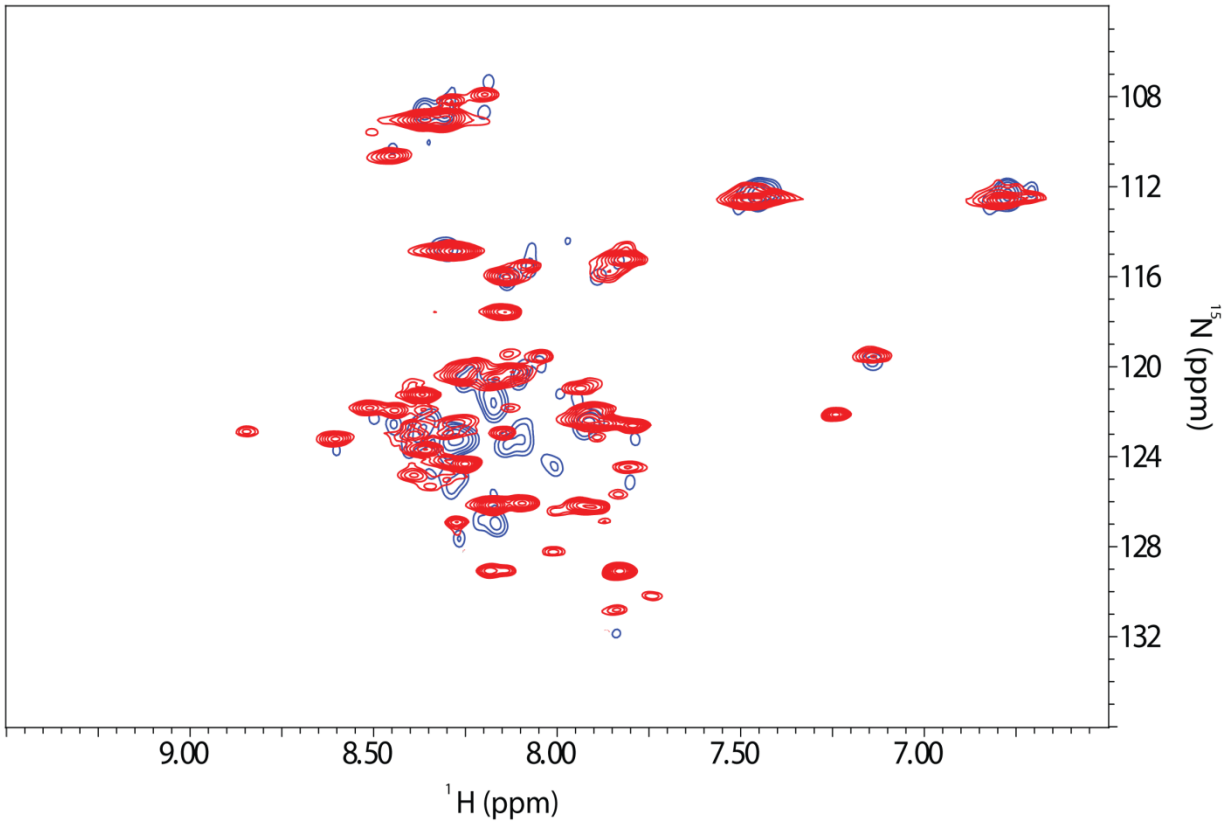

Figure S3. Overlay of in-cell NMR spectra of free [*U*-^{15}N] Pup (red) and [*U*-^{15}N] Pup/MPA complex (blue). The peaks from [*U*-^{15}N] Pup/Mpa (blue) show differential broadening compared to those of free [*U*-^{15}N] Pup (red).

Table S1. Matrix M: The scaled intensities of Pup residues at various Mpa overexpression times.

Residue #	4hrs	5hrs	6hrs	7hrs	8hrs	18hrs
1	-0.010965029	0.010313901	0.05014533	0.030400033	0.053721231	0.043644329
2	-0.101844623	-0.073658032	-0.068699048	-0.114617417	-0.129514046	-0.023239254
3	-0.088215607	-0.097832379	-0.095817042	-0.117872947	-0.120500654	-0.139916005
4	-0.061208758	-0.081909166	-0.114555562	-0.109437602	-0.125766277	-0.118433731
5	-0.05584564	-0.0602612	-0.059676737	-0.052876714	-0.058851648	-0.080175547
6	-0.023119292	-0.005230688	-0.009643726	-0.019411061	-0.039227698	0.027929839
7	-0.013348223	-0.015254886	-0.01950945	-0.03907527	-0.020968107	-0.006006758
8	-0.087926456	-0.098471411	-0.106579928	-0.111450342	-0.119753789	-0.123771822
9	0.037481674	-0.154653768	-0.006434824	-0.118009262	-0.209619901	-0.268895791
10	-0.076187975	-0.297463119	-0.284514133	-0.324464929	-0.360284099	-0.445371241
12	0.034213985	-0.112638992	0.014260747	-0.090819001	-0.196055926	-0.344998597
13	-0.069564842	-0.077139328	-0.090821184	-0.123154568	-0.150791969	-0.254764384
14	-0.086163207	-0.034394642	-0.033490074	-0.041572067	-0.27003462	-0.056771368
15	-0.064039731	-0.052575495	-0.083982479	-0.108248167	-0.099748078	-0.141057264
16	-0.020924794	-0.002145708	-0.062650448	-0.059107833	-0.073565602	-0.147671848
17	-0.003679727	0.037174421	-0.004282472	0.004491546	0.016498266	-0.035176394
18	0.01678094	0.067108806	0.025535855	0.025108097	0.036740344	0.042889379
19	-0.000612785	0.011050543	0.012895079	0.00902768	0.027991635	0.006208893
20	0.017031967	0.043460141	0.047320802	0.016587759	0.03481761	0.043898772
21	0.002625693	-0.018743765	-0.036589821	-0.034268579	-0.037146356	-0.046006109
23	-0.034249208	0.061019118	0.058370806	0.014069673	0.024263299	0.134723565

24	-0.013320864	0.033482834	0.016325117	0.012148153	-0.009187312	-0.027626495
25	0.030207864	0.062240678	0.067065746	0.055694413	0.056129566	0.033129428
26	0.04643248	0.095636564	0.07371004	0.055039754	0.064298318	0.040019059
27	0.254387347	0.330749696	0.270428554	0.248816774	0.297254513	0.384107287
28	0.183844606	0.393155328	0.28992729	0.206924862	0.378217179	0.452292746
29	0.250326814	0.353514991	0.267682578	0.335310206	0.351450488	0.355590821
30	0.315911612	0.385362	0.378349972	0.37166985	0.385785617	0.26452242
31	0.050429577	0.064069617	0.049186868	0.049425893	0.051344634	0.056915562
32	0.096068121	0.162137085	0.124323744	0.130228768	0.167316536	0.15099894
33	0.281616396	0.377072795	0.320801323	0.357364048	0.382023121	0.456292203
34	0.225761503	0.299756387	0.280741964	0.272661951	0.302996502	0.243618048
35	0.114855434	0.169452878	0.137313955	0.155092692	0.154963114	0.165130575
36	0.251413119	0.385436722	0.31267037	0.305460059	0.371461557	0.591495041
37	0.039722946	-0.003326031	-0.061561975	-0.065943578	-0.08417341	-0.182497105
38	0.12151381	0.229565521	0.165855046	0.178153123	0.196768858	0.194222212
39	0.111706432	0.1549332	0.15613129	0.150812273	0.163424016	0.349207552
40	0.240443836	0.338631355	0.254884563	0.275342316	0.172710852	0.443912627
41	0.221503574	0.353357273	0.290400331	0.219296824	0.183256631	0.450198822
42	0.055648653	0.155645828	0.117820286	0.068685495	0.137689785	0.159178061
43	0.079773928	0.122681605	0.100091489	0.124897617	0.112623788	0.232539609
44	0.258632205	0.338708577	0.314750579	0.304302368	0.305675778	0.491296543
45	0.122474439	0.122645199	0.112507292	0.136002307	0.133507253	0.16464605
48	0.251413119	0.385436722	0.30749087	0.305460059	0.371461557	0.591495041
49	0.162223235	0.239383476	0.270440538	0.289817578	0.364937707	0.316557015
50	0.107412702	0.141396898	0.116331284	0.132767302	0.151523951	0.142855654

51	0.106786478	0.153229692	0.147713751	0.154025549	0.180643628	0.182318319
52	0.114293195	0.163180652	0.153726403	0.149546689	0.18450093	0.212450971
53	0.032645503	0.074141039	0.074598121	0.058132777	-0.194103599	0.060563089
54	0.100496678	0.163085371	0.173661892	0.172758732	0.175007567	0.158934458
55	0.061367159	0.125719401	0.144694477	0.10704934	0.127837377	0.132733028
56	0.092525222	0.140857153	0.146647521	0.11605136	0.138239306	0.165190331
57	0.120085628	0.15914927	0.166323256	0.138599794	0.159274304	0.237909577
58	0.094820971	0.160982624	0.179115416	0.170479009	0.166126956	0.17598945
61	-0.01853032	0.098335151	0.003593863	0.06200133	-0.050495376	0.015057095
62	0.084016309	0.119491389	0.103486522	0.125519611	0.122909349	0.161962608
63	0.104529752	0.113936441	0.157944788	0.137532487	0.138428586	0.168061545
64	0.02906323	0.072392326	0.053649134	0.056050823	0.062852162	0.086581325

Table S2. Matrix V and Singular Values of M.

Dataset Index	4hrs	5hrs	6hrs	7hrs	8hrs	18hrs	Singular Values
1	0.2757	0.4233	0.3672	0.3723	0.4221	0.5415	3.3759
2	-0.4458	-0.1446	-0.3176	-0.2165	-0.1077	0.7881	0.5221
3	0.2436	0.2808	0.2338	0.0234	-0.8820	0.1694	0.4272
4	-0.7075	0.6304	-0.0133	0.2094	-0.0383	-0.2377	0.2199
5	0.3350	0.5683	-0.4210	-0.6058	0.1413	-0.0229	0.2144
6	-0.2304	-0.0265	0.7295	-0.6349	0.1042	-0.0014	0.1645

References

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