Expression of MycG for NMR

Plasmid and transformation

The MycG was originally expressed from a PET28b vector in *E. coli* BL21, but due to difficulties with expression in perdeuterated media using the PET28b construct, the MycG gene was removed by double digestion (HindIII, NdeI) and ligated into a pAW5775 plasmid construct. The pAW5775 plasmid was a gift from Dr. Luet-Lok Wong , and originally contained the P450cam C334A gene. The gene transfer removed the N-terminal 6x His tag from the MycG and placed the MycG gene into a system that has proven reliable for expression of deuterated P450 enzymes.

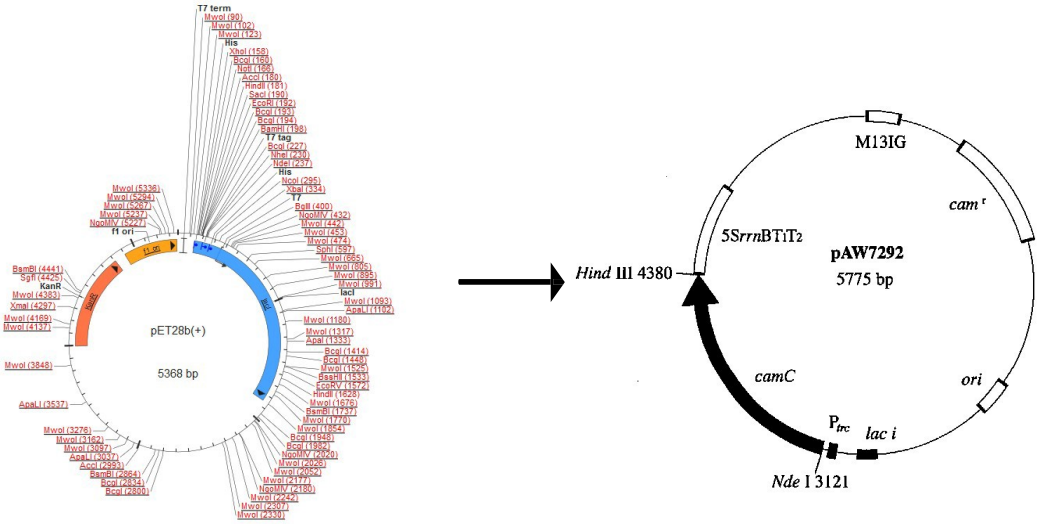


Figure S1: The MycG gene was transferred from the pET28B(+) vector to a pAW7292 plasmid by utilizing the HIND III and NDE I cut sites.

Approximately 100 ng of the resultant plasmid was transformed into 50 uL of *Escherichia coli* NMC533 by electroporation. Transformants were spread onto LB plates containing 25 mg/mL of chloramphenicol and 30 mg/mL kanamycin and incubated at 37^{o} C for 18 hours.

Expression of uniformly ^{15}N-labeled MycG. A single isolated colony was picked from plates of freshly transformed NCM533 *E. coli* and used to inoculate 5 mL of LB containing 25 mg/mL chloramphenicol and 30 mg/mL kanamycin. The inoculant was grown for 4 hours at 37^{o} C with vigorous shaking until significant turbidity was observed. The 5 mL culture was then scaled up to 1 L of LB containing the same antibiotic concentrations. When an A_{600} of 0.5 was reached the culture was centrifuged at 5,000 RPM for 20 minutes, and the pellet resuspended in M9+ minimal media containing $^{15}NH_4Cl$ and the appropriate antibiotics. When the A_{600} reached 0.8, heme precursor γ-aminolevulinic acid (ALA) was added to a concentration of 0.5 mM and the temperature was adjusted to 28^{o}C. When A_{600} =1 was reached, expression was induced by the addition of 1 mM isopropyl β-thiogalactoside (IPTG) and cultured with vigorous agitation for 18 hours. Cells were harvested by centrifugation at 5,000 RPM for 30 minutes and the pellets immediately stored at -80^{o}C.

Expression of uniformly -$^{13}C,^{15}N,^{2}H$ labeled MycG. A single isolated colony was picked from fresh transformants as described above and used to inoculate 5 mL of LB with 25 mg/mL chloramphenicol and 30 mg/mL kanamycnin. The inoculant was grown for 4 hours at 37^{o}C with vigorous shaking until significant turbidity was observed. A 1 mL aliquot of the LB culture was transferred directly to 50 mL of M9+ media, then incubated at 37^{o}C for 4 hours, at which time A_{600} was 0.61. A 10 mL aliquot of the 50 mL culture was used to inoculate a 250 mL volume of 70% D_2O, 30% H_2O, M9+ media. The 70% D_2O culture was incubated at 37^{o} C for 6 hours until the A_{600} reached 0.25. The cells were then spun down at 5,000 RPM for 20 minutes and resuspended in 250 mL of M9+ media prepared with 98 % D_2O. The culture was then incubated at 37^{o}C for 8 hours at which time the A_{600} was 0.82. The cells

were again collected by centrifugation and resuspended in the final expression media, 1.5 L of M9+ prepared with 99% D_2O (DLM-4-99-1000, CIL), 1.5 g of $^{15}NH_4Cl$, and 5 g of $^{13}C_6$, d_7 glucose (CIL). All components of the M9+ media were added solid and subsequently filter sterilized with the exception of $CaCl_2$ and $FeCl_3$ which were autoclaved separately and added later. Note that autoclave sterilization of deuterated media should be avoided to prevent dilution of deuterons, and all salts and additives should be prepared using D_2O. After cells were added, the final expression culture was incubated at 37^{o}C for 8 hours until an A600 of 0.70 was reached. At this time, incubation temperature was lowered to 28^{o}C, followed by the addition of 2 g of ^{13}C,^{15}N,^{2}H Celtone base powder (CGM-1030P-CDN, CIL) and 105 mg of ALA. After one hour of incubation, expression was induced with 357 mg of IPTG. The culture was harvested 36 hours later by centrifugation at 5,000 RPM for 30 minutes. The harvested cell pellet (4.7 g) was immediately stored at -80^{o}C.

M9+ minimal media (1 L volume):
800 mL ddH_2O
200 mL 5x M9 salts
2 mL P1 metal mix
2 mL 1 M $MgSO_4$
0.1 mL 0.1 M $FeCl_3$
0.1 mL 1 M $CaCl_2$ 16 uL
2% thiamine 4 g glucose
25 mg chloramphenicol
30 mg kanamycin

5x M9 salts (1 L) (all masses are for anhydrous material)
34 g Na_2HPO_4
15 g KH_2PO_4
2.5 g NaCl
4.0 g NH_4Cl

P1 metal mix (1 L)
34.36 g H_3BO_3
4.32 g $MnCl_2 \cdot 4H_2O$
0.315 g $ZnCl_2$
0.03 g MoO_3
0.003 g $CuSO_4 \cdot 5H_2O$
0.012 g $CoCl_2 \cdot 6H_2O$

Expression of MycG with selectively ^{15}N labeled amino acids. The preparation of a selectively labeled amino acid sample is similar to that of a uniformly ^{15}N labeled sample, except an amino acid mixture prepared as described below is substituted for $^{15}NH_4Cl$. Autoclavable amino acids can be autoclaved together and filter-sterilized amino acids are added to the media after it cools. To produce a selectively labeled sample, a ^{15}N version of the amino acid of interest is substituted for the natural abundance version. For preparation of the ^{13}C-Pro U-^{15}N-sample used for the selective Pro-X HNCO experiment, the recipe for ^{15}N-labeled M9+ media is used, with the addition of filter-sterilized U-^{13}C-Pro (0.1 g) upon induction.

Ala	0.50 g	Autoclave
Arg	0.40 g	Autoclave
Asp	0.40 g	Filter, dissolve by titrating with NaOH
Asn	0.40 g	Filter
Gln	0.40 g	Filter, dissolve by titrating with HCl
Glu	0.65 g	Filter
Gly	0.55 g	Autoclave
His	0.15 g	Autoclave
Ile	0.23 g	Autoclave
Leu	0.23 g	Autoclave
Lys	0.42 g	Autoclave
Met	0.25 g	Autoclave
Phe	0.13 g	Autoclave
Pro	0.10 g	Autoclave
Ser	2.00 g	Autoclave
Thr	0.23 g	Autoclave
Tyr	0.17 g	Filter, dissolve by titrating with NaOH
Val	0.23 g	Autoclave
Trp	0.05 g	Filter
Cys	0.05 g	Filter

Preparation of the amino acid mixture is adapted from methods described in: Cheng, H., Westler, W. M., Xia, B., Oh, B. H. & Markley, J. L. Protein Expression, Selective Isotopic Labeling, and Analysis of Hyperfine-Shifted NMR Signals of Anabaena 7120 Vegetative [2Fe- 2S] Ferredoxin. *Archives of Biochemistry and Biophysics* 316, 619–634 (1995).

Purification of MycG. The described procedure is scaled for 5 g of cell paste, the typical yield for 1 L of growth media. The methods described can be scaled up to 15 grams of cell paste. Above this amount of cell mass multiple purifications are required to maintain efficiency. A frozen cell pellet (5g) was thawed and resuspended in 50 mL of lysis buffer. The cells were disrupted using a Misonix sonicator 3000 (Farmingdale, NY). The total sonication time was 6 minutes using a 20 seconds on, 40 seconds off program at a power level of 6.0 while the metal sonication beaker was kept cool with an ice bath. The lysate was centrifuged at 15,000 RPM in a Beckman JA20 rotor for 45 minutes to remove cell debris. The supernatant was decanted, placed in an ice-bath with stirring and precipitated by addition of 70 % w/v ammonium sulfate (approximately 25 g). The ammonium sulfate was ground to a fine powder, added in small (~0.5 g) increments over several minutes and allowed to stir for 30 minutes. The precipitated protein was collected by centrifugation at 15,000 RPM for 25 minutes. The protein pellets obtained were gently rinsed with water before being dissolved in 8 mL of low salt buffer. The re-dissolved protein was applied to a 20 mL HiTrap desalting column equilibrated with low salt buffer (see below). A properly desalted sample was obtained by loading 1 mL protein solution per 5 mL of column bed volume flowing at 3 mL/min. Within these guidelines, it was necessary to run and re-equilibrate the column a total of 3-4 times per prep. Loss of protein due to the multiple batch requirements was deemed negligible. The pooled desalted fractions were diluted to 100 mL with low salt buffer and loaded on a 25 mL bed volume DEAE ion exchange column equilibrated with low salt buffer. The protein was loaded by gravity and washed with 200 mL of low

salt buffer. The protein was eluted with a linear gradient from low salt to high salt buffer over a total volume of 200 mL. Fractions of 5 mL each were collected throughout the elution gradient. Purity of each fraction was assessed spectroscopically by A_{417}/A_{280}, with fractions having $R > 0.5$ pooled and concentrated to 1 mL total volume. The pooled, and concentrated fractions were then loaded onto a HiPrep 26/60 Sephacryl S200 gel filtration column previously equilibrated with gel filtration buffer using an ATKA FPLC system flowing at 0.5 mL/min and collecting 3 mL fractions (see Figure S2). Fractions were again assessed for purity by measuring A_{417}/A_{280}, fractions with $R > 1.5$ were pooled, concentrated to approximately 1 mL, and immediately stored in liquid nitrogen. All buffers are created fresh, filtered and degassed the day of use.

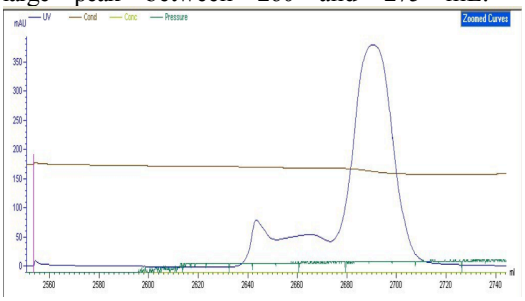
Lysis Buffer
50 mM TRIS pH 7.4
50 mM KCl
0.5 mM PMSF protease inhibitor
10 ug/mL DNaseI
10 ug/mL RNaseA
100 ug/mL lysozyme

Low salt buffer
50 mM Tris HCl pH 7.4
50 mM KCl

High salt buffer
50 mM Tris HCl pH 7.4
250 mM KCl

Gel Filtration buffer
50 mM KPi pH 7.4
200 mM KCl

Fig. S2. To the left, an S200 size exclusion column loaded with MycG. Below, chromatogram of the S200 run. MycG is the large peak between 260 and 275 mL.



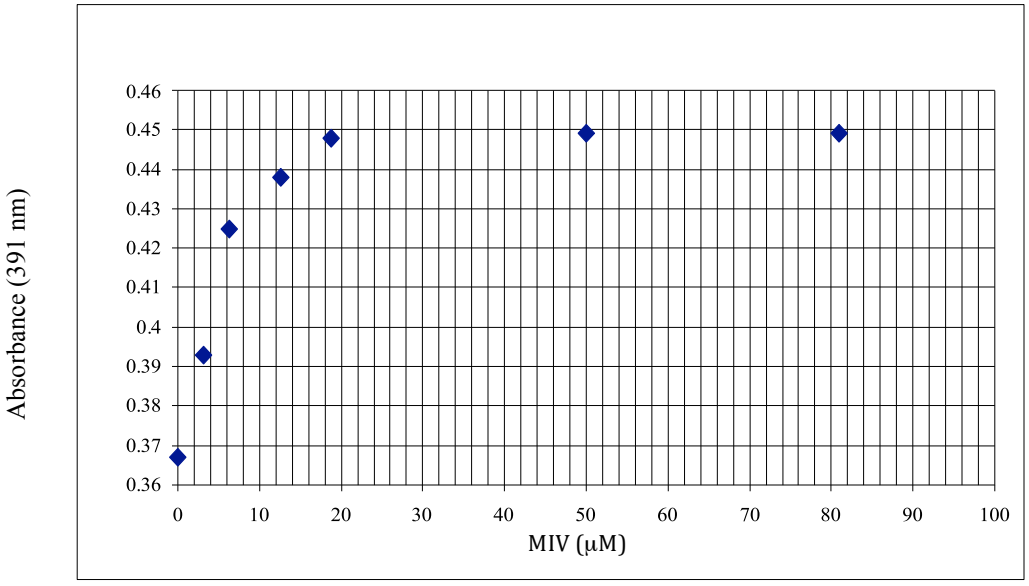


Figure S4: The absorbance increase at 391 nm as a function of MIV addition to 10 μM MycG.

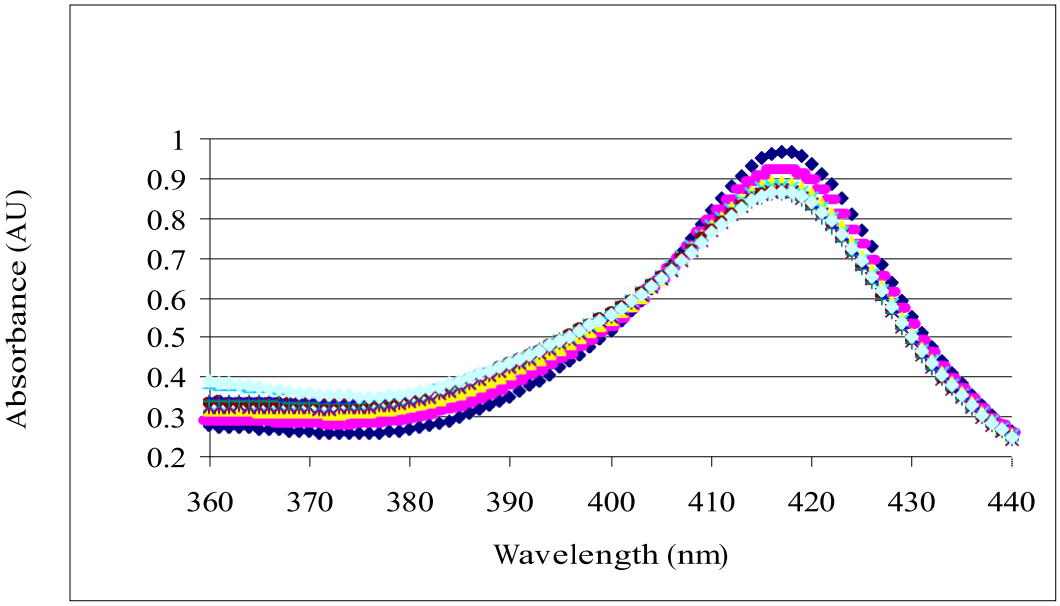


Figure S3: Overlay of UV/VIS spectra with MycG at 10 μM and substrate ranging from 0 – 550 μM (0, 3.2, 6.4, 9.5 , 12.6, 18.8, 50, 81, 263, 550 μM) As MIV in added a decrease absorbance is seen at 417 nm and an increase at 391 nm. Saturation of the transition is seen by 12.5 μM MIV.

AMBER 14 Scripts

Heating:

Heating from 0-40 with constant volume

```
 &cntrl
  imin=0,
  irest=0,
  nstlim=20000,
  dt=0.002,
  ntc=2,
  ntf=2,
  nmropt=1,
  ntpr=100,
  ntwx=100,
  cut=16,
  ntb=1,
  ntt=3,
  gamma_ln=2.0,
  tempi=0.0,
  temp0=40.0,
/
 &wt type='END' /
LISTOUT=POUT
DISANG=paraFeRestraint.dist
&end
```

Equilibration (constant volume):

equilibrate at 300 constant volume

```
 &cntrl
  imin=0,
  irest=1,
  nstlim=100000,
  dt=0.002,
  ntc=2,
  ntx=5,
  ntf=2,
  nmropt=1,
  ntpr=100,
  ntwx=1000,
  cut=16,
  ntb=1,
  igb=0,
  ntt=3,
  gamma_ln=2.0,
  tempi=300.0,
  temp0=300.0,

  /
 &wt type='END' /
LISTOUT=POUT
DISANG=paraFeRestraint.dist
&end
```

Equilibration (constant pressure):

```
1ns equilibium with constant pressure
 &cntrl
  imin=0,
  irest=1,
  nstlim=100000,
  dt=0.002,
  ntc=2,
  ntx=5,
  ntf=2,
  nmropt=0,
  ntpr=250,
  ntwx=10,
  cut=16,
  ntb=2,
  ntp=1,
  tautp=2.0,
  igb=0,
  ntt=3,
  gamma_ln=2.0,
  tempi=300.0,
  temp0=300.0,

    /
 &wt type='END' /
LISTOUT=POUT
DISANG= paraFeRestraint.dist
&end
```

Production runs:

```
RDC restrained 0.25 ns
 &cntrl
  imin=0,
  irest=1,
  nstlim=125000,
  dt=0.002,
  ntc=2,
  ntx=5,
  ntf=2,
  nmropt=1,
  ntpr=1000,
  ntwx=1000,
  cut=16,
  ntb=2,
  ntp=1,
  tautp=2.0,
  igb=0,
  ntt=3,
  gamma_ln=2.0,
  tempi=300.0,
  temp0=300.0,
 &end
```

/
&wt type='END' /
LISTOUT=POUT
DISANG=paraFeRestraint.dist
DIPOLE=edited_RST.dip
&end

Table S1. Chemical shift perturbation (Δδ) upon saturation of MycG with MIV, and time point in minutes at which a signal is no longer observed in HD exchange (HDX) experiments.

Res #	MIV titration exchange behavior	Δδ ^{1}H	Δδ ^{15}N	Free HDX	MIV-bound HDX
3	Intermediate	0	20		
4	Slow	22.4	0	0	0
7	Fast	13.6	10	1200	1200
8	None	0	0	0	0
9	Intermediate	12	3	0	0
14	Slow	14	31	0	0
15	Fast	14	5	0	0
16	Slow	40	16	0	0
17	Slow	22	6	1200	1200
20	Slow	28	8	0	0
21	Intermediate	6	12	0	0
23	Fast	16	6	0	0
24	Intermediate	0	17	1200	1200
25	Sharpens	0	0	0	0
27	Sharpens	0	0	0	0
28	Slow	35	11	0	0
29	Fast	0	8	800	800
30	Intermediate	19	11	1000	1000
32	Sharpens	0	0	0	0
33	Sharpens	0	0	1200	1200
34	Slow	28	24	0	0
35	Fast	13	16	120	120
36	No Change	0	0	1200	1200
39	Intermediate	116	6	800	600
40	Fast	18	8	0	0
41	Sharpens	0	0	0	0
42	Fast	12	0	0	0
43	Fast	0	11	600	600
44	Sharpens	0	0	0	0
45	Intermediate	35	3	180	180
46	Intermediate	0	12	0	0
47	Sharpens	0	0	1200	1200
48	Sharpens	0	0	1200	1200
49	Intermediate	0	7	120	120
50	Intermediate	2	12	120	120
51	Intermediate	60	10	120	60
52	Broadens	0	0	1200	60
53	Intermediate	39	22	0	0

54	Intermediate	0	23	600	1200
55	Grows in	0	0	0	0
56	No change	0	0	1200	1200
57	Slow	64	0	180	0
58	Sharpens	0	0	0	0
60	Slow	53	23	0	0
61	Slow	48	16	600	30
62	Slow	14	45	1200	0
63	Sharpens	0	0	0	0
64	Slow	141	55	0	0
66	Sharpens	99	5	1200	0
67	Slow	19	26	150	0
68	Slow	35	2	150	150
70	Intermediate	7	12	0	0
73	Fast	6	1	480	60
74	Slow	68	32	0	0
77	Intermediate	28	8	0	0
81	None	0	0	0	0
84	Slow	56	2	1200	1200
87	Fast	0	46	0	0
92	Slow	26	2	1200	1200
93	Fast	14	11	60	0
94	None	0	0	0	0
95	slow	28	0	0	0
97	Sharpens	0	0	0	0
108	Sharpens	0	0	0	0
109	Slow	26	7	1200	1200
110	None	0	0	0	0
111	Intermediate	7	24	300	300
112	Sharpens	0	0	0	0
113	Sharpens	0	0	1200	1200
114	Intermediate	24	0	0	0
115	Slow	56	12	480	120
116	Sharpens	0	0	0	0
118	Sharpens	0	0	1200	1200
123	Sharpens	13	24	0	0
126	Fast	6	10	300	120
127	Sharpens	0	0	0	0
128	Slow	35	47	1200	0
129	Slow	70	4	60	60
130	Intermediate	2	19	1200	1200
132	Fast	12	24	60	180
133	Broadens	0	0	0	0
134	Sharpens	0	0	0	0
136	Sharpens	0	0	0	0
138	Slow	29	0	300	120
146	Intermediate	4	34	0	0
156	None	0	0	0	0
157	None	0	0	0	0
174	Slow	36	7	30	30

176	Intermediate	68	11	60	60
177	Slow	9	12	1200	1200
190	Slow	30	23	60	60
191	Intermediate	13	62	0	0
193	Slow	67	24	60	0
198	Slow	18.4	18	0	0
199	Fast	20	0	480	30
202	Intermediate	20	2	0	0
207	Slow	25	16	120	0
208	Intermediate	66	2	600	600
215	Fast	14	10	600	600
216	Fast	5	0	0	0
217	None	0	0	0	0
225	Intermediate	40	15	0	0
235	Intermediate	88	15	180	180
251	None	0	0	1200	1200
252	Fast	8	4	480	120
256	Fast	7	0	0	0
258	No Exchange	0	0	1200	1200
259	Intermediate	29	12	1200	800
260	Fast	8	0	800	800
265	None	0	0	600	600
266	None	0	0	0	0
270	Intermediate	42	0	0	0
289	Sharpens	0	0	0	0
291	Slow	24	26	30	180
294	Sharpens	0	0	0	0
295	None	0	0	180	180
296	Fast	0	6	180	180
298	Sharpens	0	0	600	600
300	None	0	0	120	120
301	Fast	4	0	600	600
302	Intermediate	12	3	480	1200
303	Fast	5	0	0	0
304	Intermediate	35	4	30	30
305	Intermediate	48	24	0	0
307	Intermediate	29	32	180	180
308	Sharpens	0	0	0	0
311	Slow	40	5	600	600
318	Intermediate	22	20	0	0
320	Intermediate	46	12	240	180
323	None	0	0	60	60
324	Intermediate	13	12	0	0
325	Intermediate	24	11	120	60
326	Intermediate	8	15	0	0
327	Fast	4	2	30	0
328	Intermediate	27	4	180	120
329	Fast	0	50	0	0
337	Sharpens	0	0	30	30
353	None	0	0	1200	1200
366	Intermediate	54	15	0	0
369	Fast	4	8	0	0
370	Sharpens	0	0	0	0
372	Fast	6	0	0	0
373	Intermediate	26	2	0	0
374	None	0	0	480	480
377	Slow	56	2	30	180

380	Sharpens	0	0	0	0
381	Sharpens	0	0	0	0
382	Intermediate	17	18	0	0
384	Slow	4	32	0	0
385	Sharpens	0	0	0	0
389	Slow	21	23	0	0
391	Broadens	0	0	0	0
392	Fast	14	9	600	600
395	Intermediate	28	14	1200	0
396	Intermediate	8	13	1200	1200
397	Fast	9	9	0	0

Table S2. ^{15}N R_1 relaxation times for MIV-bound MycG

Res #	T_1 (s)	T_2(ms)
3	2.03	36.2
4	1.04	172.3
7	2.10	22.1
8	2.12	28.7
9	2.17	27.0
14	2.07	120.5
16	8.88	56.7
17	1.89	40.6
20	3.90	18.3
21	3.08	20.9
23	2.24	25.5
28	2.45	21.1
29	2.87	19.8
30	2.49	26.5
33	2.53	24.1
34	3.27	20.6
35	2.84	18.3
36	1.78	42.9
39	1.09	55.6
40	2.11	20.7
41	3.36	21.5
42	2.50	14.5
44	2.13	28.7
46	3.26	36.9
48	3.63	26.8
50	2.57	21.8
51	3.18	19.7
52	3.04	27.9
53	2.39	20.0
54	3.04	21.3
57	2.23	18.3
58	2.02	27.4
59	5.10	25.7
60	2.78	23.2
61	2.43	22.2

62	5.29	23.5
63	3.43	20.8
66	2.44	26.8
67	2.20	17.5
68	1.94	18.2
70	2.47	21.1
73	2.80	24.7
74	1.27	18.7
77	1.25	430.2
83	1.38	52.6
84	3.29	20.8
87	2.37	20.3
92	1.32	140.8
94	2.04	21.4
95	3.14	23.5
97	1.97	21.9
109	1.96	28.6
111	2.26	25.5
113	2.70	21.9
114	3.13	20.9
115	3.02	21.3
123	3.18	19.9
126	2.68	19.0
129	1.61	34.0
132	2.58	23.5
133	3.09	22.0
137	2.88	21.9
138	2.39	23.3
174	1.07	23.9
176	2.12	26.8
177	2.76	21.5
178	2.05	21.8
178	2.14	22.3
189	1.76	114.7
190	2.62	24.8
192	2.16	23.8
193	2.05	24.3
198	3.01	24.6
199	2.13	21.8
200	2.27	33.7
203	2.96	23.4
207	2.40	28.8
208	1.97	23.2
215	2.89	23.2
216	2.30	21.2
217	1.02	236.4
225	2.89	20.8
230	2.35	18.4
235	2.69	24.4
251	2.84	22.0
252	2.84	20.1
256	3.04	24.3
260	2.53	22.2
261	2.34	23.3
262	2.70	24.0

265	2.32	20.6
266	1.56	68.9
270	2.33	21.6
272	1.90	20.2
273	2.00	19.3
293	2.87	24.2
295	2.48	31.1
296	2.91	23.1
298	2.11	21.2
300	2.04	40.7
301	2.46	19.4
302	1.54	19.8
302	1.54	20.8
303	3.57	23.5
304	2.93	19.9
305	3.21	21.0
307	6.17	20.7
311	2.45	24.7
313	1.82	38.3
314	1.88	53.7
318	2.49	23.5
319	3.01	21.3
320	1.53	19.9
321	2.82	25.5
323	2.48	23.4
324	2.09	28.2
325	2.26	23.8
326	1.98	32.9
327	2.82	41.4
328	2.43	13.5
329	3.09	20.4
339	2.05	21.0
342	2.95	40.1
366	2.19	25.7
367	2.71	27.5
369	3.00	19.0
372	3.12	20.5
373	3.53	13.3
374	2.44	21.0
376	2.97	26.0
377	2.80	19.8
377	2.80	19.8
382	2.24	23.7
384	1.30	55.4
391	2.56	21.8
392	2.65	61.0
395	4.44	21.4
396	3.78	22.6
397	2.79	22.2