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Supporting Material

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SUPPORTING INFORMATION

Materials

Electrophoresis grade acrylamide, ultrapure TRIS base, and ultrapure Na_2EDTA were purchased from ICN Biomedicals (Costa Mesa, CA). Most other chemicals were reagent grade and acquired from either Fisher Scientific (Unionville, ON) or Sigma-Aldrich (Oakville, ON). The Ni^{2+}-NTA (nitrilotriacetic acid) agarose beads were purchased from Qiagen (Mississauga, ON). The lipids 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1,2-dimyristoyl-*sn*-glycero-3-[phosphor-rac-(1-glycerol)] (sodium salt) (DMPG), and cholesterol (Chol) were purchased from Avanti Polar Lipids (Alabaster, AL). The phosphorus assay standard was purchased from Sigma-Aldrich (Oakville, ON). The BCA assay kit was purchased from Pierce (Rockford, IL). For uniform labeling and selective-labeling of protein for NMR spectroscopy, the stable isotopic compounds D_2O, $^{15}NH_4Cl$, $^{13}C_6$-glucose, and uniformly $^{13}C,^{15}N$- labeled Asn and Val were obtained from Cambridge Isotope Laboratories (CIL, Andover, MA). For evaluation of reverse-labeling of protein, natural abundance Asn and Val were purchased from Sigma-Aldrich (Oakville, ON).

Evaluation of selective Val and Asn labeling in rmC1 and rmC8 variants.

The unmodified classic 18.5 kDa recombinant murine myelin basic protein (rmC1), and its quasi-deiminated variant (rmC8), were expressed in *E. coli* BL21-CodonPlus(DE3)-RP cells (Stratagene, La Jolla, CA) and purified as previously described [(1, 2)]. Uniformly $^{13}C,^{15}N$-labeled protein was derived from cells grown in M9 minimal medium supplemented with $^{15}NH_4Cl$ and $^{13}C_6$-glucose [(3, 4)]. Selective-labeling of the Val and Asn residues in rmC1 and rmC8 was optimized as next.

In all preparations, both rmC1 and rmC8 concentrations were determined by measuring the absorbance at 280 nm, using $\varepsilon=0.627\ Lg^{-1}cm^{-1}$ (as calculated by SwissProt for protein in 6.0 M guanidine hydrochloride, 0.02 M phosphate buffer, pH 6.5). Protein purity was routinely assayed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Following purification, all proteins were freeze-dried and stored at -20ºC prior to their use.

It was first necessary to test the selectivity of isotopic-labeling of valine and asparagine, and to optimize the level of added amino acids to M9 media during selective-labeling of protein, reverse-labeled protein was derived from cells grown in M9 medium supplemented with natural abundance Asn and Val at different molar concentrations ranging from 0.2 to 1 mM.

As the yield of rmC8 was always less than that of rmC1, more accurate measurement of the level of the degree of [Asn,Val]-isotopic labeling in each protein variant was performed using solution NMR. Three samples were investigated: the first sample was uniformly $^{13}C,^{15}N$-labeled, whereas the other two were grown on M9 media supplemented with natural abundance NH_4Cl and glucose, and $^{13}C,^{15}N$-labeled Val and Asn added at molar concentrations of 0.35 mM and 0.5 mM.

All samples examined by solution NMR were prepared by dissolving the freeze-dried protein in 500 µL of 30% (vol%) perdeuterated 2,2,2-trifluoroethanol (CF_3-C^2H_2-O^2H, TFE-d_2). Six samples were prepared from rmC1 and three from rmC8. **Table SI-1** shows the details of labeling of each sample.

Table SI-1. Composition of samples used for solution NMR spectroscopy.

Sample Name	Protein	Labeling	Concentration of added Asn and Val (mM)
S1	rmC1	U-^{13}C, ^{15}N	0
S2	rmC1	U-^{13}C, ^{15}N + NA-N, V[a]	0.2
S3	rmC1	U-^{13}C, ^{15}N + NA-N, V	0.35
S4	rmC1	U-^{13}C, ^{15}N + NA-N, V	0.5
S5	rmC1	U-^{13}C, ^{15}N + NA-N, V	0.75
S6	rmC1	U-^{13}C, ^{15}N + NA-N, V	1
S7	rmC8	U-^{13}C, ^{15}N	0
S8	rmC8	NA, U-^{13}C, ^{15}N-N, V	0.35
S9	rmC8	NA, U-^{13}C, ^{15}N-N, V	0.5

[a]N and V stands for asparagine and valine added to M9 media, respectively. Both N and V were natural abundance for rmC1 samples and U-^{13}C,^{15}N for rmC8 samples.

To characterize the degree of isotopic labeling and scrambling, we collected a series of standard ^{1}H-^{13}C and ^{1}H-^{15}N heteronuclear single quantum correlation (HSQC) spectra for rmC1 and rmC8 samples (s1-s9) dissolved in 30% TFE-d_2, and at room temperature. All experiments were performed on a Bruker Avance III spectrometer operating at a proton Larmor frequency of 600.13 MHz, using standard parameter sets [(5)]. All spectra were processed using NMRPipe. The peak assignments for rmC1 were published previously [(3, 4)]. Integration of peaks was done using SPARKY 3 (T. D. Goddard and D. G. Kneller, University of California, San Francisco), as well as using in-house written LUA script (www.lua.org) executed using the CARA (computer-aided resonance assignment) [(6)] built-in LUA interpreter.

We have collected ^{1}H-^{15}N and ^{1}H-^{13}C HSQC spectra on rmC1 (samples S1-S6), for which the spectroscopic assignments are known [(3, 4)]. The analysis of individual peak intensities corresponding to Val and Asn showed minimal transamination effects to Asn backbone and side chain ^{15}N, but some non-negligible transamination for Val.

The analysis of individual peaks or groups of peaks corresponding to different amino acid types in the ^{1}H-^{13}C HSQC spectra of samples S1-S6 revealed that for all amino acids but Asn, Val, and Leu, the HSQC peak intensities were comparable to their counterparts in the uniformly-labeled sample (S1), and that the degree of ^{13}C scrambling was generally low (albeit non-zero), and independent of the molarity of Asn and Val added to the M9 media (**Figure SI-1**). In other words, all the examined amino acid types but Asn, Val, and Leu, are mainly synthesized by the internal metabolic mechanisms of the *E. coli* cell strain used for over-expression of rmC1.

On the other hand, the Asn and Val cross peaks showed a dramatic reduction in their intensities. The degree of reverse-labeling was found to be relatively independent of the amount of added Asn and Val in the range 1 to 0.5 mM (**Figure SI-1**). In addition, leucine side chain signals were of relative intensities comparable to those of Asn and Val. This observation indicates that the Leu C^{β}, C^{γ} and $C^{\delta1/\delta2}$ are strongly scrambled because of the addition of Val to the growth media [(7, 8)].

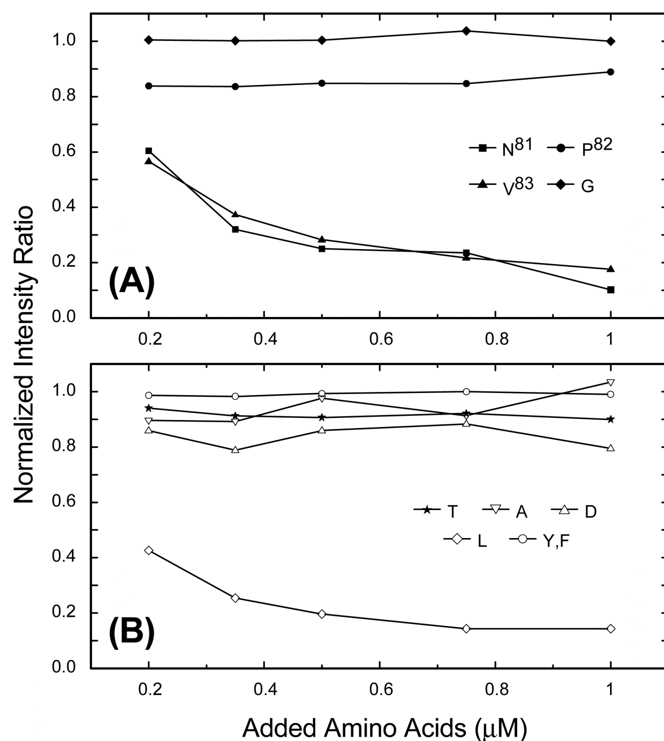

Figure SI-1. Normalized intensity ratios for selected peaks in the ^{1}H-^{13}C HSQC (heteronuclear single quantum coherence) spectrum of rmC1: (A) H^{α}-C^{α}, and (B) H^{β}-C^{β}. The intensity/sum-over-box ratios of each spectrum at a given added amount of amino acids was first normalized by dividing the ratios by the median of intensity ratios of the central 75% of the population in each spectrum. The selected peaks are plotted here as a function of the molarity of the added amino acids in M9 medium during recombinant murine MBP (rmC1 or rmC8) over-expression.

Similar experiments were performed on the quasi-deiminated variant rmC8, and showed reduced levels of over-expression compared to rmC1, which is why a more precise test of the level of scrambling was performed. Because the spectroscopic assignments of rmC8 are as yet unknown, a [U-^{13}C, ^{15}N]-labeled rmC8 (S7) and two [U-^{13}C,^{15}N-Val, Asn]-labeled samples (S8 and S9, **Table SI-1**) were prepared, and the peak intensities were compared. The level of valine and asparagine labeling was estimated to be ~33-40% when 0.35 mM of the amino acids were added, and increased to 66-75% for 0.5 mM of added amino acids. The Leu C^{β}, C^{γ} and $C^{\delta1/\delta2}$ correlations were found to be of comparable intensities to Val, whereas most other residues were labeled in the range of 2-13%.

In summary, supplementing the media with the appropriate concentration of isotopically-labeled valines and asparagines resulted in the incorporation of labeled amino acids into the over-expressed protein at relatively high levels. Most other amino acids were synthesized from the internal metabolic mechanisms of the *E. coli* strain that we used. The only noticeable exception was leucine, in which the C^{β}, C^{γ}, and C^{δ} carbons were synthesized from Val C^{α}, C^{β}, and C^{γ} [(8)].

Solid-state NMR spectroscopy

Table SI-2. Acquisition parameters for two-dimensional spectra[a].

Spectrum-Sample	Temperature (°C)	t_1- points	Number of scans
(CO)N(CO)CA	-25	60	1792
^{13}C-^{13}C-rmC1[b]	-25	512	152
^{13}C-^{13}C-rmC1[c]	10	320	200
^{13}C-^{13}C-rmC1[c]	20	320	160
^{13}C-^{13}C-rmC8[b]	-25	512	128
^{13}C-^{13}C-rmC8[d]	10	320	256
^{13}C-^{13}C-rmC8[d]	20	320	256
^{1}H-^{13}C INEPT-HETCOR[e]	35	128	192
^{1}H-^{13}C CP-HETCOR[e]	35	128	384

[a]All spectra were acquired on a 600 MHz spectrometer with a 12 kHz MAS rate, except for the ^{1}H-^{13}C correlations of rmC1 which were acquired on a 800 MHz spectrometer with a 14.3 kHz MAS rate.
[b]DARR (dipolar assisted rotational resonance) mixing of 10 and 20 ms for rmC1 and rmC8, respectively.
[c]Spin-diffusion mixing of 150 ms.
[d]DARR mixing of 150 ms.
[e]The same acquisition parameters were used for rmC1 and rmC8.

Table SI-3. Chemical shift assignments at -25°C for membrane-associated rmC1 and rmC8.

Nucleus	MBP isoform	
	rmC1	rmC8
Asn81- C^{α}	52.1	52.4
Asn81- C^{β}	39.9	40.1
Val83- C^{α}	66.3	66.3
Val83- C^{β}	31.3	31.4
Val84- C^{α}	67.0	66.8
Val84- C^{β}	31.9	31.8
Asn89- C^{α}	52.9	53.5
Asn89- C^{β}	40.3	40.4
Val91- C^{α}	62.3	62.3
Val91- C^{β}	32.1	32.3

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