Solid-State NMR Spectra of Lipid-Anchored Proteins under Magic Angle Spinning

Supporting Information

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1. Synthesis of Lipid-Anchor Mimic.

General methods. All chemicals were reagent grade and purchased from Nacalai Tesque (Kyoto, Japan). All reactions were monitored using TLC on Silica Gel 60 F254 precoated glass slides (Merck) with examination under UV light (254 nm) and/or by charring with 5% 12 molybdo(VI)phosphoric acid in EtOH. Flash column chromatography was performed on silica gels (Silica Gel 60 mesh 230-400 or spherical, Nacalai Tesque). ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded at 25 °C on a Bruker DMX-500 spectrometer (Bruker Biospin) and analyzed with

solvent peaks as internal references for ^{1}H and ^{13}C NMR or 5% H_3PO_4 for ^{31}P NMR. The overall synthetic scheme is shown in Scheme 1.

***N*-(3-(maleimido)propionyl)-2-aminoethanol (1).** To an ice-cold solution of ethanolamine (189 μL, 3.14 mmol) in CH_2Cl_2 (10 mL), 3-(maleimido)propionic acid *N*-hydroxysuccimide ester (760 mg, 2.85 mmol) in CH_2Cl_2 (10 mL) was added drop-wise under an Ar atmosphere for 1 h. The reaction mixture was concentrated and purified by SiO_2-column chromatography (MeOH/$CHCl_3$1/50 → 1/9) to give the desired product **1** (490 mg, 81%) as a colorless solid. ^{1}H NMR (500 MHz, CD_3OD) δ 2.45 (t, $^{3}J_{(H,H)}$ = 7.0 Hz, 2 H; CH_2), 3.22 (dt, $^{3}J_{(H,H)}$ = 5.8, 10.9 Hz, 2 H; CH_2), 3.53 (t, $^{3}J_{(H,H)}$ = 5.8 Hz, 2 H; CH_2), 3.75 (t, $^{3}J_{(H,H)}$ = 7.0 Hz, 2 H; CH_2), 6.79 (s, 2 H; CH=CH).

3-*O*-(2-cyanoethyl (*N*-(3-(maleimido)propionyl)-2-aminoethyl)phosphono)-1,2-*O*-dimyristoyl-*sn*-glycerol(2). To a solution of 1,2-*O*-dimyristoyl-*sn*-glycerol (424 mg, 0.83 mmol)[S1] and 1*H*-tetrazole (116 mg, 1.66 mmol) in CH_2Cl_2 (5 mL), a solution of 2-cyanoethyl *N*,*N*,*N*,*N*-tetraisopropylphosphorodiamidite (500 mg, 1.66 mmol) in CH_2Cl_2 (5 mL) was added.[S2] The reaction mixture was stirred for 1 h at room temperature, concentrated *in vacuo*, and purified by SiO_2-column chromatography (hexane/AcOEt/Et_3N 6/1/0.1) to give the phosphoroamidite (524 mg, 88%) as a colorless oil. To a suspension of **1** (140 mg, 0.66 mmol) in CH_2Cl_2 (10 mL), a solution of the phosphoroamidite (426 mg, 0.60 mmol) in CH_2Cl_2 (10 mL) and 1*H*-tetrazole (210 mg, 3 mmol) was added under an Ar atmosphere. After the mixture was stirred for 1 h at room temperature, a solution of *m*CPBA (172 mg, 1 mmol) in CH_2Cl_2 (1 mL) was added. The mixture was stirred for an additional 1 h at room temperature, and extracted with AcOEt. The organic phase was washed with aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The obtained crude product was purified by SiO_2-column

chromatography (MeOH/$CHCl_3$ 1/50 → 1/20) to give the desired product **2** (398 mg, 80%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (t, $^3J_{(H,H)}$ = 6.7 Hz, 6 H; CH_3 x2), 1.16-1.32 (m, 40 H), 1.59 (m, 4 H; CH_2 x2), 2.31 (t, $^3J_{(H,H)}$ = 7.5 Hz, 2 H; CH_2), 2.34 (t, $^3J_{(H,H)}$ = 7.5 Hz, 2 H; CH_2), 2.54 (m, 2 H; CH_2), 2.78 (m, 2 H; CH_2), 3.52 (m, 2 H; CH_2), 3.84 (m, 2 H; CH_2), 4.10-4.36 (m, 8 H; CH_2 x4), 5.26 (m, 1 H; CH), 6.56 (m, 1 H; NH), 6.69 (s, 2 H; CH=CH). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.0, 19.6, 19.7, 22.6, 24.7, 28.98, 29,02, 29.2, 29.3, 29.4, 29.5-29.6, 31.8, 33.9, 34.1, 34.11, 34.4, 61.5, 62.2, 65.9, 67.4, 69.2, 116.5, 134.1, 170.1, 170.4, 172.9, 173.3. ^{31}P NMR (202 MHz, $CDCl_3$) δ -1.11.

3-*O*-(*N*-(3-(maleimido)propionyl)-2-aminoethyl phosphono)-1,2-*O*-dimyristoyl-*sn*-glycerol triethylamine salt (3). To a solution of **2** (35 mg, 0.042 mmol) in CH_2Cl_2 (2 mL), a solution of DBU (37 μL, 0.25 mmol) in CH_2Cl_2 (2 mL) was added at 0 °C under an Ar atmosphere.[S3] After being stirred for 5 min at 0 °C, the mixture was quenched with ice-cold 0.1 M HCl, and was extracted with $CHCl_3$. The organic phase was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The obtained crude product was purified by SiO_2-column chromatography (MeOH/$CHCl_3$5/95→MeOH/$CHCl_3$/Et_3N10/90/1 → 20/80/1) to give the desired product **3** (28 mg, 85%) as a pale yellow oil. ^{1}H NMR (500 MHz, 50% CD_3OD/$CDCl_3$) δ 0.85 (t, $^3J_{(H,H)}$ = 6.7 Hz, 6 H; CH_3 x2), 1.18-1.29 (m, 40 H), 1.31 (t, $^3J_{(H,H)}$ = 7.3 Hz, 9 H; CH_3 x3), 1.57 (m, 4 H; CH_2 x2), 2.26 (m, 4 H; CH_2 x2), 2.48 (t, $^3J_{(H,H)}$ = 7.5 Hz, 2 H; CH_2), 3.03 (q, $^3J_{(H,H)}$ = 7.3 Hz, 6 H; CH_2 x3), 3.41 (m, 2 H; CH_2), 3.81 (t, $^3J_{(H,H)}$= 7.4 Hz, 2 H; CH_2), 3.92-3.99 (m, 4 H; CH_2 x2), 4.14 (dd, $^3J_{(H,H)}$ = 6.6, 12.0 Hz, 1 H; CH_2), 4.36 (dd, $^3J_{(H,H)}$ = 3.2, 12.0 Hz, 1 H; CH_2), 5.21 (m, 1 H; CH), 6.65 (s, 2 H; CH=CH). ^{13}C NMR (125 MHz, 50% CD_3OD/$CDCl_3$) δ 8.5, 14.0, 22.6, 24.76, 24.80, 29.01, 29.03, 29.19, 29.20, 29.4, 29.5-29.6, 31.8, 34.0, 34.18,

34.23, 34.3, 40.91, 40.94, 45.5, 62.5, 63.50, 63.54, 63.89, 63.94, 70.23, 70.29, 134.1, 169.8, 170.4, 173.0, 173.4. ^{31}P NMR (202 MHz, 50% $CD_3OD/CDCl_3$) δ 1.11.

2. Expression and Purification of Cysteine-Tagged GB1.

The DNA sequence corresponding to the GB1-GSMNGSSGS-C (GB1-linker) construct was subcloned from a full-length GB1 construct. The DNA sequences were PCR amplified using corresponding primers and pET21a-GB1 as the template DNA. The amplified genes were ligated back into the pET21a vector (Novagen) using the *Nde*I and *Bam*HI restriction sites (New England Biolabs). Bacterial expression of the uniformly ^{13}C- and ^{15}N-labeled GB1-linker constructs in *Escherichia coli* BL21(DE3) was performed in M9 minimal media in the presence of carbenicilin at 37°C. Protein expression was induced by the addition of 1 mM isopropyl β-D-1-thiogalacto-pyranoside (IPTG) overnight at 25°C. The cell pellet was lysed by sonication in buffer A (50 mM Tris-HCl, 10 mM DTT, pH 8) at 4°C. To precipitate nucleic acids and unwanted proteins, the lysate was acidified by 0.1% trifluoroacetic acid (TFA). Following centrifugation, the supernatant was purified using a C4 HPLC packed column (200×150 mm, Waters) with a water/acetonitrile gradient in the presence of 0.1% (v/v) TFA at 40 °C.

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Scheme

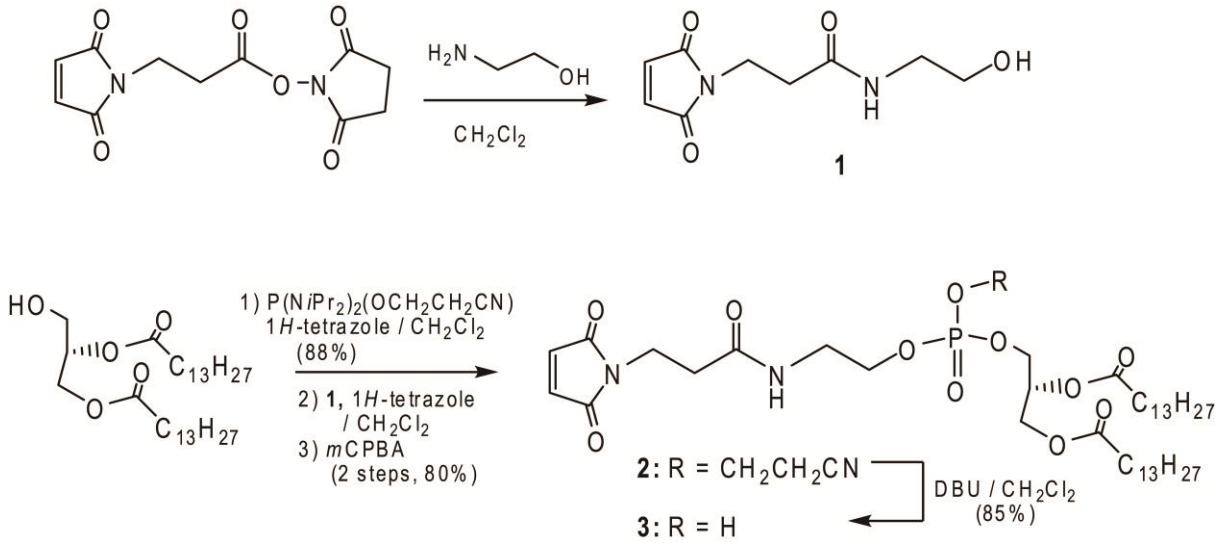
Scheme 1. Synthetic scheme of the GPI anchor mimic.

Figures

Figure S1. Gel filtration profiles of the reaction mixture and each eluted fraction from gravity-flow column filtration of the reaction mixture between GB1 and the bicelle-containing anchor. Fraction numbers are shown at the top left in each profile. Beginning at fraction 7, the uncoupled GB1 signal gradually appeared.

Figure S2. Schematics of NMR pulse sequences used for measurements of backbone amide ^{15}N longitudinal relaxation rate constant R_1^N. Narrow and wide black rectangles correspond to 90˚ and 180˚ pulses, respectively. Phases of RF pulses in the sequences are as follows: ϕ_1 = x, x, -x, -x; ϕ_2 = y, -y; ϕ_3 = x, x, -x, -x; ϕ_4 = x, x, x, x, y, y, y, y, -x, -x, -x, -x, -y, -y, -y, -y; ϕ_5 = x, x, -x, -x, y, y, -y, -y; ϕ_6 = -y, y, -y, y, x, -x, x, -x; ϕ_7 = y, -y, y, -y, -x, x, -x, x; receiver = x, -x, x, -x, y, -y, y, -y, -x, x, -x, x, -y, y, -y, y.

Figure S3. Comparison of the backbone amide ^{15}N longitudinal relaxation rate constant R_1^N of GB1. The R_1^N values of the anchored GB1 in the bicelles prepared in this study are shown in red, and those reported by the solution NMR experiment at 30°C, pH 7.2 performed by Idiyatullin *et al*. in blue.[53] The R_1^N values of the residues whose quantitative measurement could not be made were set to zero.


H2N
OH
CH2Cl2
1
HO
1) P(NiPr2)2(OCH2CH2CN)
1H-tetrazole / CH2Cl2
(88%)
2) 1, 1H-tetrazole
/ CH2Cl2
3) mCPBA
(2 steps, 80%)
C13H27
2: R = CH2CH2CN
3: R = H
DBU / CH2Cl2
(85%)

Scheme S1

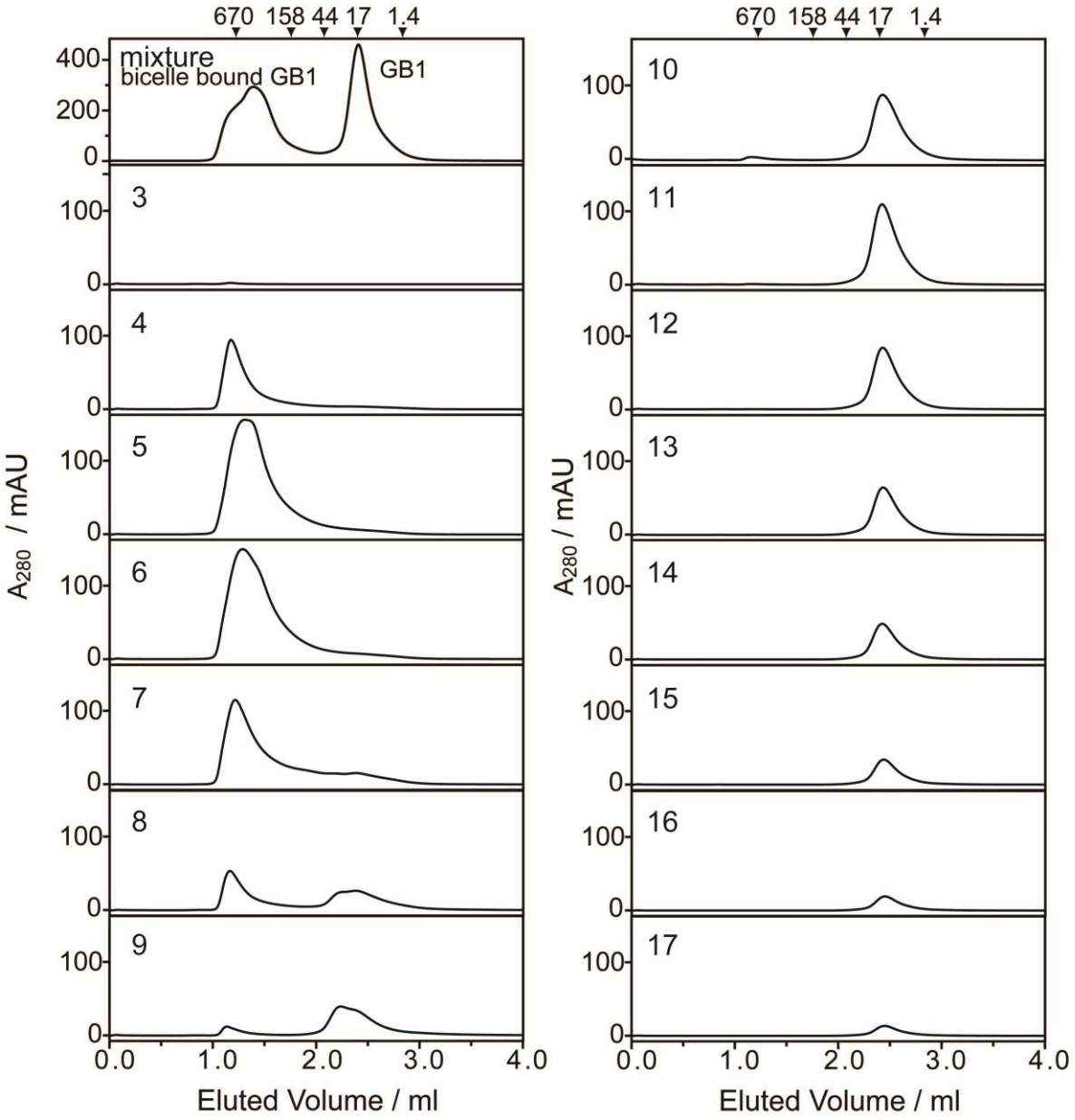
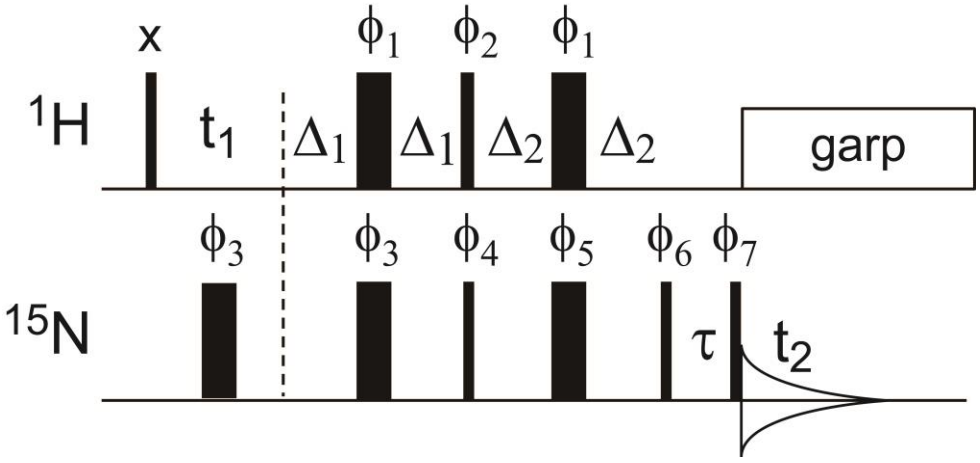

670 158 44 17 1.4
mixture
bicelle bound GB1
GB1
400
200
0
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
100
0
A280 / mAU
0.0
1.0
2.0
3.0
4.0
Eluted Volume / ml

Figure S1


x
ϕ_1
ϕ_2
ϕ_1
^{1}H
t_1
Δ_1
Δ_1
Δ_2
Δ_2
garp
ϕ_3
ϕ_3
ϕ_4
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ϕ_6
ϕ_7
^{15}N
τ
t_2

Figure S2

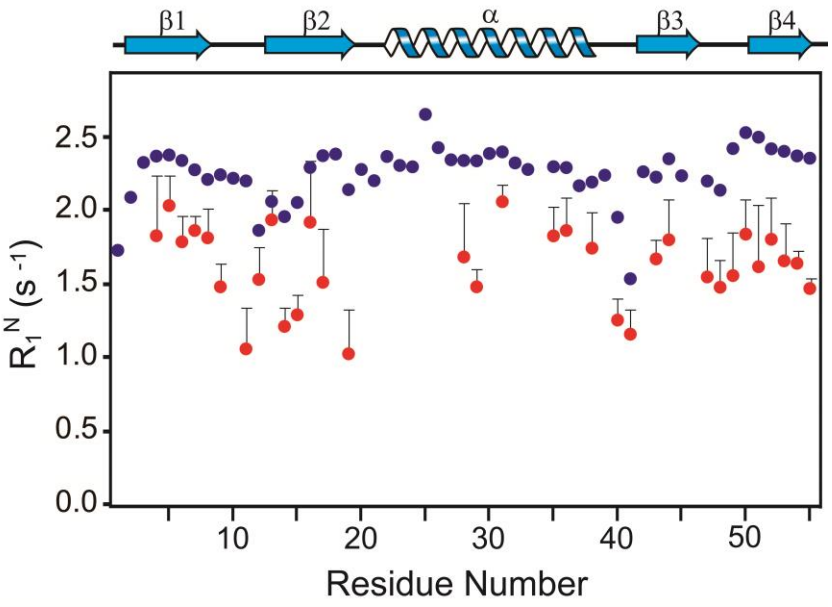

Figure S3.