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Electronic supplementary information for:

Abnormal incorporation of amino acids into the gas hydrate crystal lattice

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1. Experimental

Materials

Deionized water was distilled in the laboratory. Ultra-high purity CO_2 (99.999%) was supplied by Deokyang Energen Corporation, Korea. Glycine, L-alanine and L-valine were obtained from Sigma-Aldrich, USA. All amino acids were of reagent grade (≥99%) and were used without further purification.

Experimental apparatus

Gas hydrate samples were prepared using the same experimental system as has been in our previous work.[1] Two high pressure cells with a volume of 250 cm^3 were immersed in an ethanol bath to control the temperature of the system. The cell contents were mixed by an impeller driven by magnetic motors. The maximum errors of thermocouple and pressure transmitter were ±0.1 K and ±0.5%, respectively.

Sample preparation

Amino acids were mixed with 40 g water, which was then placed in a high pressure cell. Water without amino acids was used for comparison. The cell was assembled and then immersed in an ethanol bath. After pressurizing the cell with CO_2 to 36 bar at 284.05 K, the system was left with agitation at 450 rpm for at least 3 h to allow an equilibrium to be reached. An isothermal method described in our previous work was used[32]. The system was cooled to 273.45 K without agitation, and then agitation was applied to induce hydrate crystallization. Gas hydrate samples were formed over a period greater than 15 h to provide a high conversion ratio. After hydrate crystallization was completed, the cell was transferred to liquid N_2 for 30 min, and CO_2 gas was removed from the cell. After grinding the gas hydrates

into powders, the samples were stored in a liquid N_2 bath to prevent dissociation.

Synchrotron PXRD

PXRD experiments were performed using the 9B high-resolution powder diffraction beamline (9B) at the Pohang Accelerator Laboratory. Details of the PXRD experimental system were described in our previous work.[1] The wavelength of the incident X-rays was 1.54720 Å. The scan was performed from 80 to 200 K in step mode, with a fixed time of 1 s and a step size of 0.0005° or 0.02°.

^{13}C CP NMR spectroscopy

^{13}C CP NMR experiments were performed using a Bruker 400 MHz solid state NMR spectrometer at the Korea Basic Science Institute. After placing powdered hydrate samples in 4 mm o.d. zirconia rotor loaded into a variable temperature probe, ^{13}C CP NMR spectra were recorded at 240 K. A pulse length of 2 μs and a pulse repetition of 10 s were employed.

Raman spectroscopy

The experimental system used for the Raman measurements is well described in a previous report.[2] A Sentinel Raman spectrometer coupled with a Unilab II probe, and a charge coupled device detector was used. The laser employed was a 532 nm Nd:YAG laser at 100 mW power. The spectra were collected in the range of 1230 to 1430 cm^{-1}, and the spectral resolution was 1 cm^{-1}.

Growth kinetics measurement

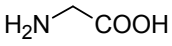
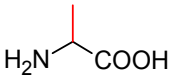
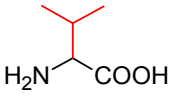
When a sudden increase in temperature was observed due to the hydrate nucleation, the rate of hydrate growth was measured immediately. The observed temperature and

pressure values were converted to the number of moles of gas consumed (gas uptake) following calculations described in our previous work.[1]

2. Model amino acid system

Glycine, L-alanine, and L-valine were selected as a model amino acid system as they vary only in the size of their alkyl side chain. The physicochemical properties of these amino acids are summarized in Table S1.

Table S1. The physicochemical properties of amino acids.

Amino acid	glycine (gly)	alanine (ala)	valine (val)
Molecular structure			
Side chain	-H	$-CH_3$	$-CH(CH_3)_2$
Hydrophobicity[a]	-0.4	1.8	4.2
Solubility in water at 298.15 K (mol%)	5.728[b]	3.245[b]	0.884[c]
Solubility in water at 273.15 K (mol%)	3.320[b]	2.390[b]	0.763[c]

[a] Hydrophobicity values were taken from the literature.[3]

[b] Solubility values were taken from the literature.[4]

[c] Solubility values were taken from the literature.[5]

3. Lattice parameter calculation for pure CO_2 hydrate

The lattice parameters were calculated from the patterns by 2 phases (structure I hydrate + hexagonal ice) full pattern (10° to 110.5°) matching using the FullProf program.[6] The results of these calculations demonstrate that the lattice parameter values obtained in the present work were consistent with those taken from previous reports.[7-9]

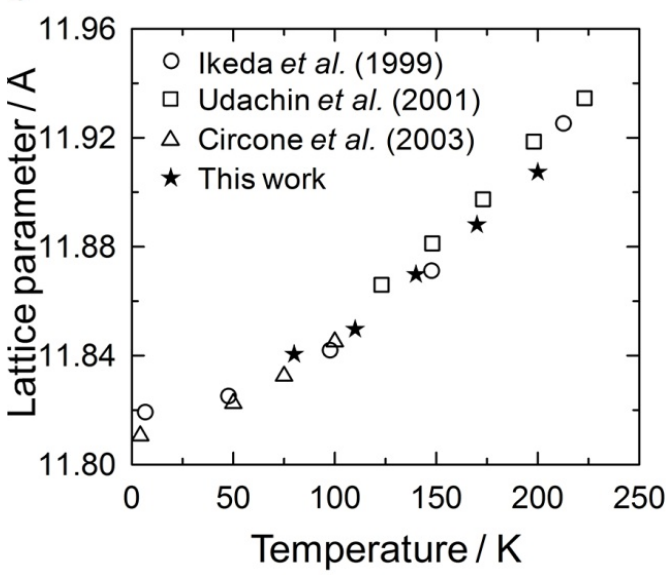


Figure S1. Lattice parameters of CO_2 hydrate calculated from PXRD patterns.

4. PXRD patterns of CO_2 hydrate in the presence of 0.1 mol% amino acids

PXRD patterns of CO_2 hydrate in the presence of amino acids were obtained in the temperature range of 80 to 200 K.

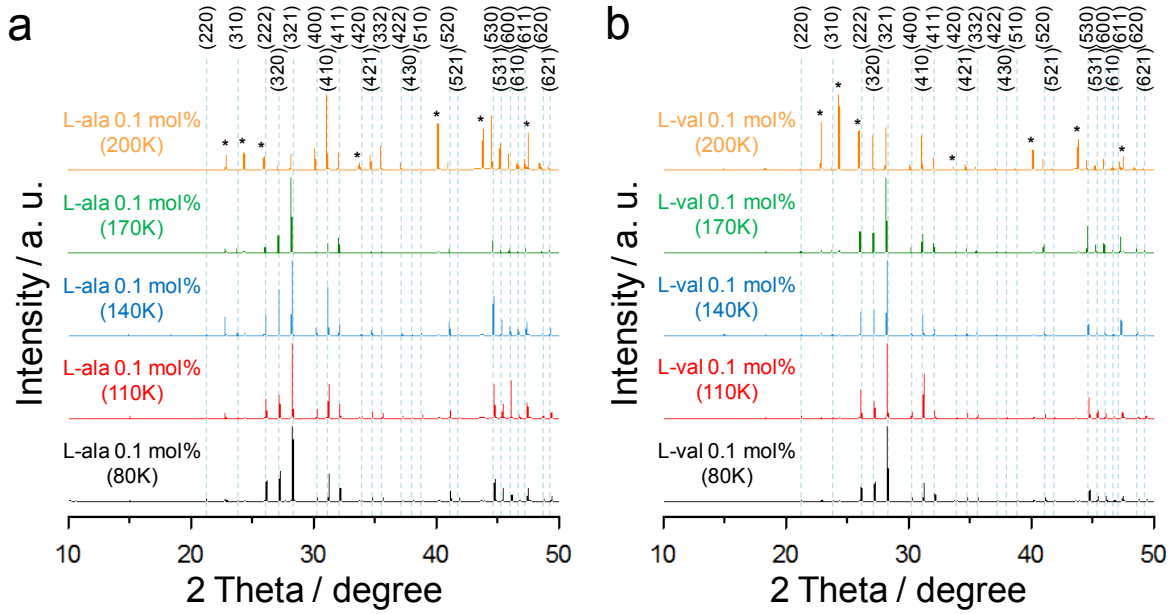


Figure S2. PXRD patterns of CO_2 hydrate in the presence of 0.1 mol% (a) L-alanine and (b) L-valine with increasing temperature.

5. Lattice parameter calculation of CO_2 hydrate in the presence of 0.1 mol% amino acids

The lattice parameters were calculated from the patterns by 2 phases (structure I hydrate + hexagonal ice) full pattern (10° to 110.5°) matching using the FullProf program.[6] Before starting the calculations, the initial lattice parameter values of a structure I hydrate[7-9] and hexagonal ice[10] in the temperature range of 80 to 200 K were taken from the literature.

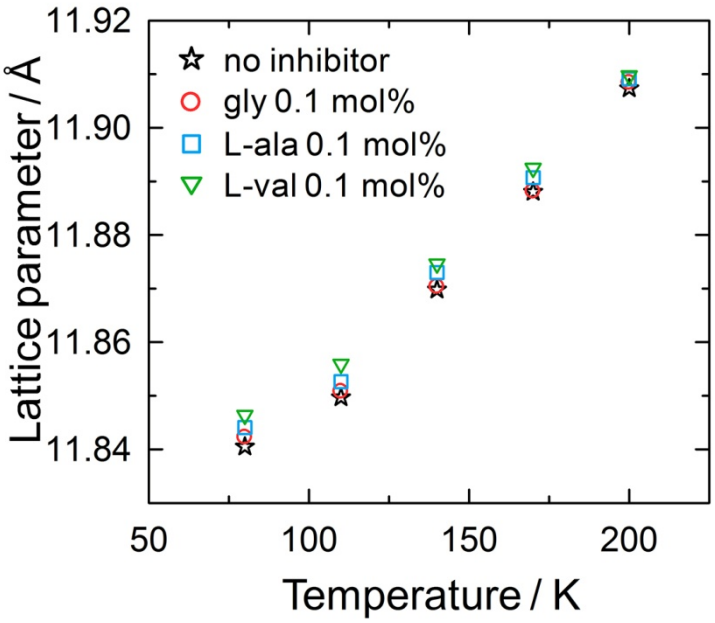


Figure S3. Lattice parameters calculated from PXRD patterns of CO_2 hydrate in the presence of 0.1 mol% amino acids.

Table S2. Lattice parameters of CO_2 hydrate in the presence of 0.1 mol% amino acids.

Temperature (K)	Lattice parameter (Å)			
	CO_2 hydrate	CO_2 hydrate + glycine 0.1 mol%	CO_2 hydrate + L-alanine 0.1 mol%	CO_2 hydrate + L-valine 0.1 mol%
80	11.840503 ± 0.000042	11.842253 ± 0.000039	11.844065 ± 0.000046	11.846368 ± 0.000034
110	11.849692 ± 0.000039	11.850787 ± 0.000043	11.852629 ± 0.000056	11.855887 ± 0.000033
140	11.869809 ± 0.000053	11.870359 ± 0.000031	11.872991 ± 0.000106	11.874619 ± 0.000040
170	11.888054 ± 0.000061	11.888142 ± 0.000026	11.890693 ± 0.000040	11.892442 ± 0.000028
200	11.907361 ± 0.000035	11.908436 ± 0.000080	11.909125 ± 0.000101	11.909729 ± 0.000023

6. Assignment of ^{13}C CP NMR spectra

The peak at the chemical shift of 123.8 ppm (Figure 1d) corresponds to CO_2 molecules occupied in 5^{12} cages of a structure I hydrate. The anisotropic motion of CO_2 molecules in asymmetric $5^{12}6^2$ cages led to a broad pattern. The low resolution of the data was attributed to the fact that $^{12}CO_2$ was used for sample preparation rather than $^{13}CO_2$, and the cage symmetry significantly influences the axially symmetric chemical shift tensor of CO_2 molecules.[11,12]

The ^{13}C CP NMR spectrum of pure glycine powder was also recorded. Two characteristic peaks at 46.9 ppm and 179.5 ppm, which correspond to the methylene carbon and carboxyl carbon, respectively.[13]

When 3.0 mol% glycine was added to the system, the ^{13}C CP NMR spectrum showed negligible differences in the chemical shifts of all the peaks. This result indicates that glycine molecules did not occupy into the hydrate cages even though glycine was added at a high concentration.

7. Crystallographic information

Table S3. Crystallographic information for amino acid crystals.

Amino acid	Crystal system	Space group	Temperature (K)	Cell dimension (Å, °)	Reference
α-glycine	Monoclinic	$P2_1/n$ (No. 14)	298	a = 5.1020(8), b = 11.971(2), c = 5.458(2) α = γ = 90, β = 111.42(3)	*Acta Cryst.* **11**, 654-663 (1958).
			120	a = 5.084(1), b = 11.820(2), c = 5.4579(9) α = γ = 90, β = 111.95(2)	*Acta Cryst.* **B36**, 3052-3059 (1980).
β-glycine	Monoclinic	$P2_1$ (No. 4)	298	a = 5.077(4), b = 6.267(6), c = 5.379(9) α = γ = 90, β = 113.12	*Acta Cryst.* **13**, 35-45 (1960).
γ-glycine	Trigonal	$P3_1$ (No. 144)	298	a = b= 7.037, c = 5.483 α = β = 90, γ = 120	*Acta Cryst.* **11**, 225-226 (1958).
			83	a = b= 6.975(2) , c = 5.473(2) α = β = 90, γ = 120	*Acta Cryst.* **B36**, 115-120 (1980).
L-alanine	Orthorhombic	$P2_12_12_1$ (No. 19)	298	a = 6.032(1), b = 12.343(1), c = 5.784(1) α = β = γ = 90	*Acta Cryst.* **20**, 550-555 (1966).
			23	a = 5.928(1), b = 12.260(2), c = 5.794(1) α = β = γ = 90	*J. Phys. Chem.* **92**, 966-973 (1988).
L-valine	Monoclinic	$P2_1$ (No. 4)	298	a = 9.71(1), b = 5.27(2), c = 12.06(2) α = γ = 90, β = 90.8(2)	*Acta Cryst.* **B26**, 1317-1326 (1970).
			120	a = 9.682(2), b = 5.247(1), c = 11.930(2) α = γ = 90, β = 90.57(1)	*Acta Chem. Scand.* **50**, 544-548 (1996).

8. Phase identification

PXRD patterns were analyzed using the FullProf program[6] for phase identification. The crystallographic information summarized in Table S3 was used to give the initial parameters for the calculations. The red circles indicate observed data points, and the black lines indicate a model peak shape calculated from the FullProf. Green ticks indicate the calculated diffraction peak positions for each crystalline phase. The blue lines indicate the differences between the observed and calculated peak intensities. The calculation procedures for full pattern profile matching using the FullProf are summarized below.

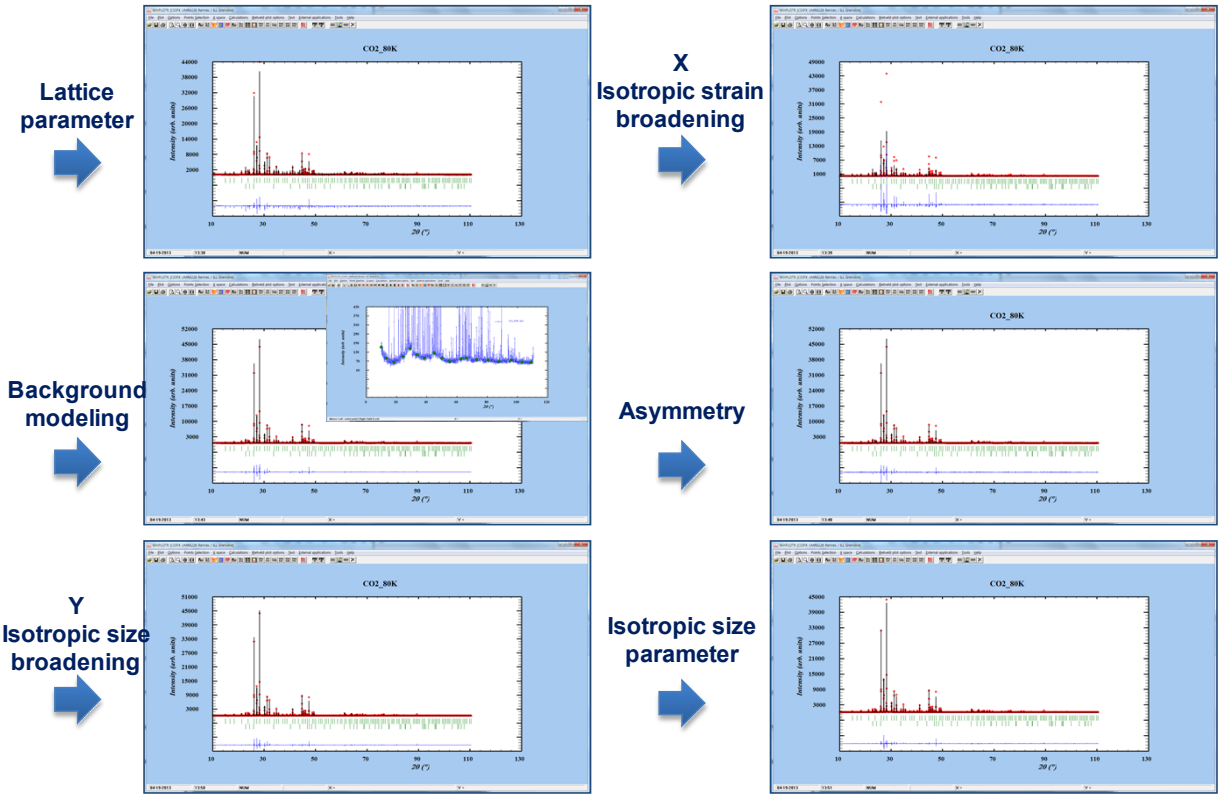


Figure S4. Procedures for full pattern profile matching calculations.

a. CO_2 hydrate + glycine 3.0 mol%

- 3 phases (structure I hydrate + hexagonal ice + α-glycine) profile matching

From the full pattern (10° to 110.5°) matching using the FullProf program,[6] diffraction peaks for α-glycine phase were clearly observed.

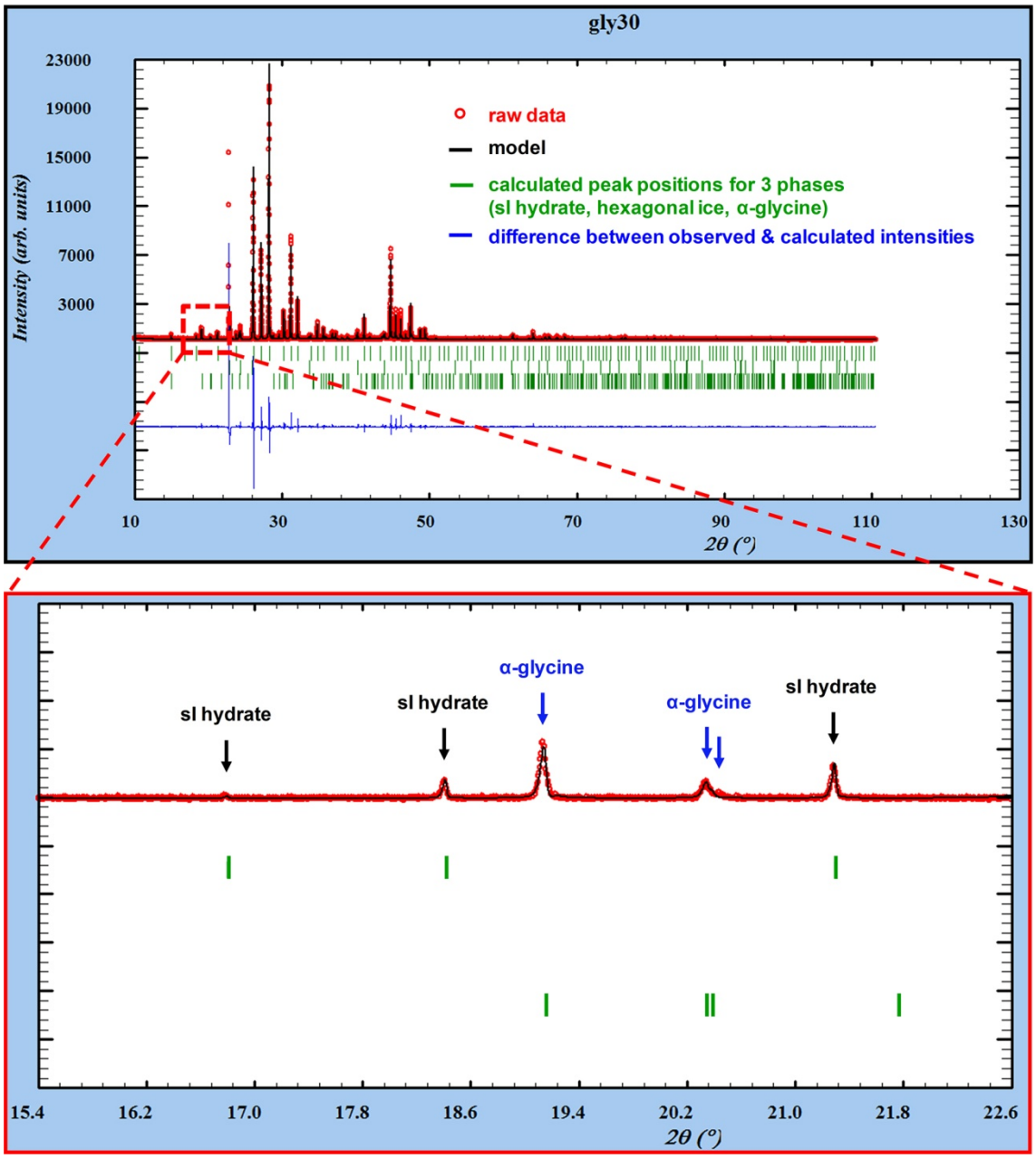


Figure S5. 3 phases (structure I hydrate + hexagonal ice + α-glycine) profile matching result.

- 3 phases (structure I hydrate + hexagonal ice + β-glycine) profile matching

From the full pattern (10° to 110.5°) matching using the FullProf program,[6] diffraction peaks for β-glycine phase were not observed, and several diffraction peaks were not identified.

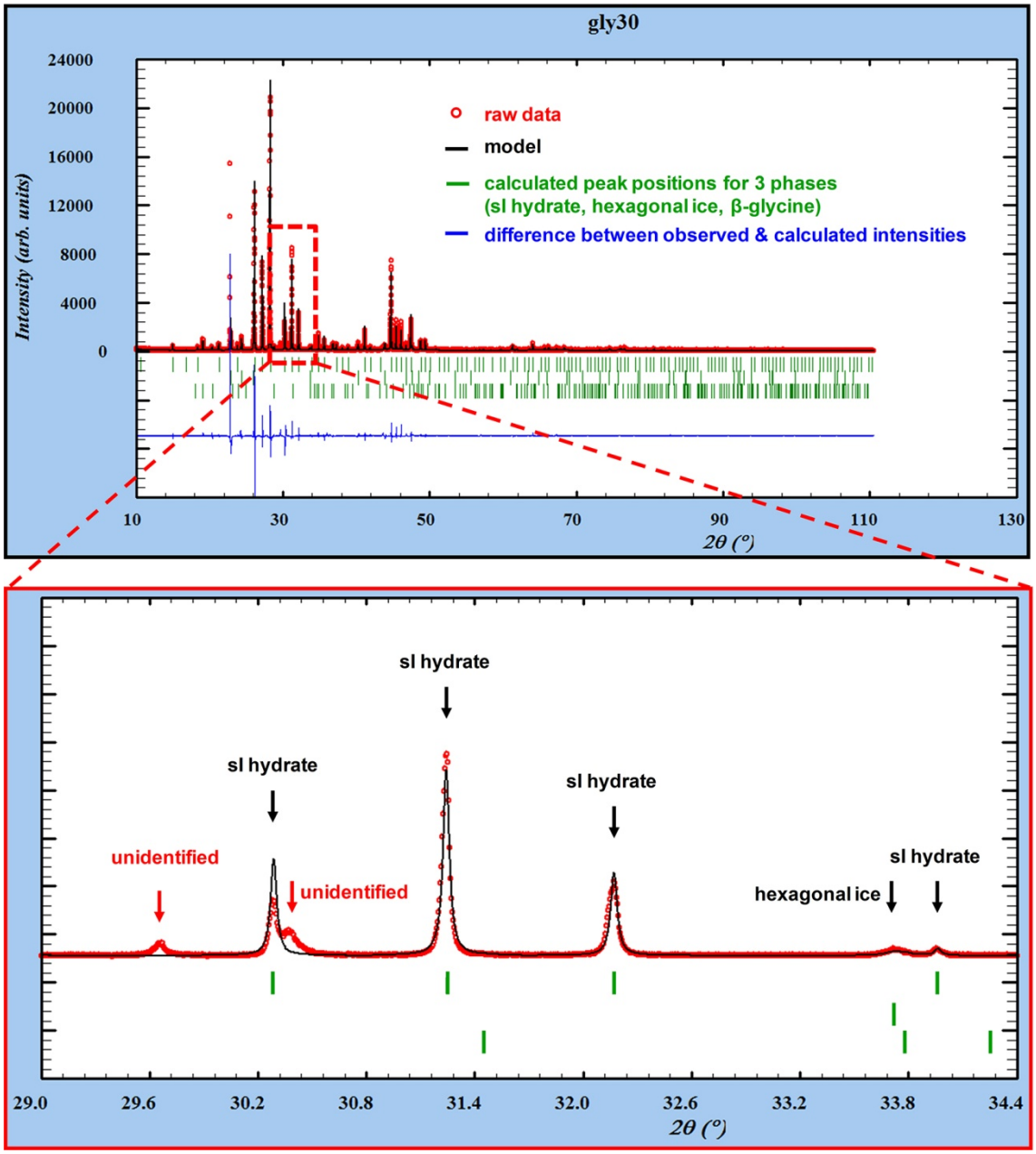


Figure S6. 3 phases (structure I hydrate + hexagonal ice + β-glycine) profile matching result.

- 3 phases (structure I hydrate + hexagonal ice + γ-glycine) profile matching

From the full pattern (10° to 110.5°) matching using the FullProf program,[6] diffraction peaks for γ-glycine phase were not observed, and several diffraction peaks were not identified.

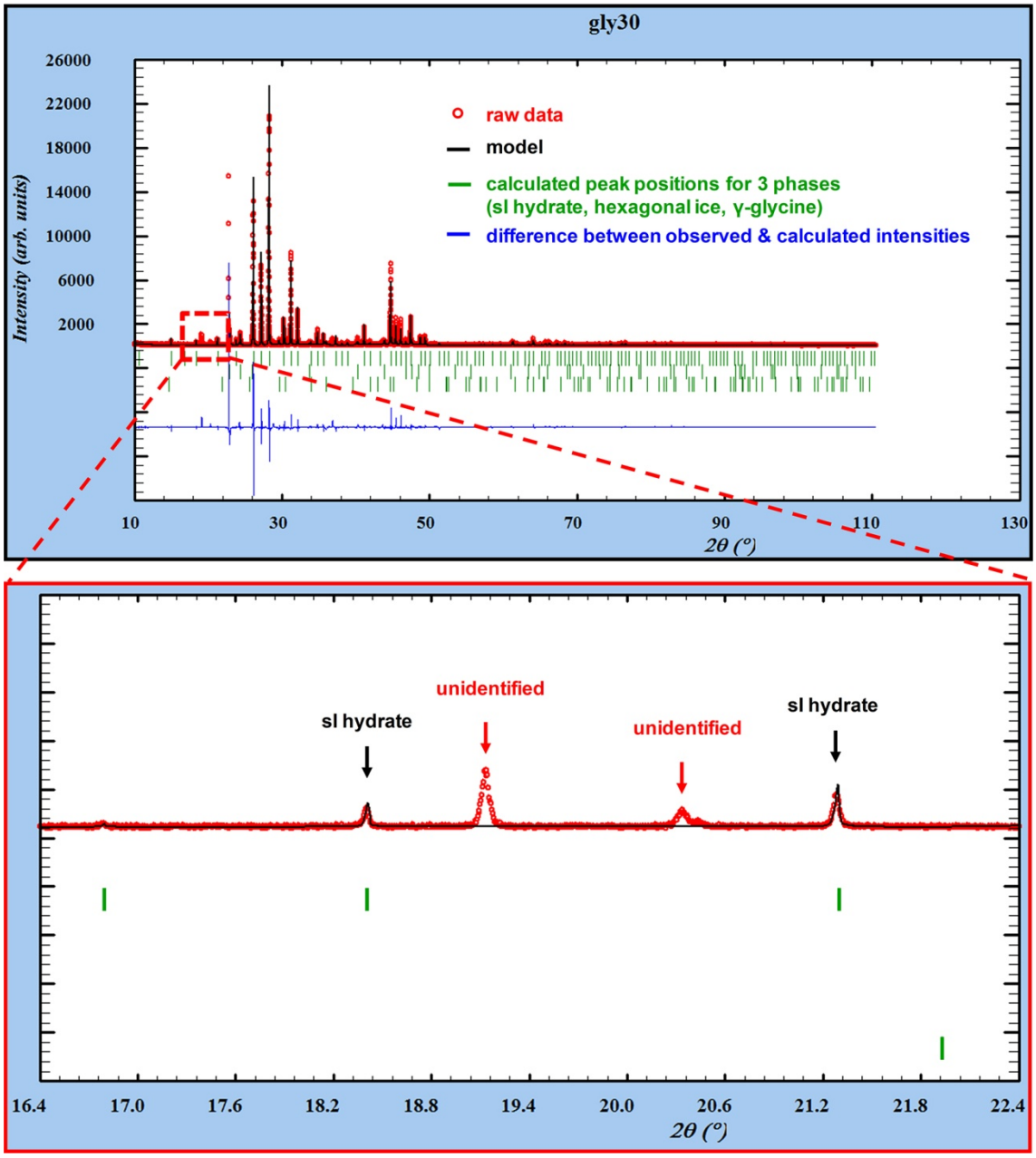


Figure S7. 3 phases (structure I hydrate + hexagonal ice + γ-glycine) profile matching result.

b. CO_2 hydrate + L-alanine 2.2 mol%

- 3 phases (structure I hydrate + hexagonal ice + L-alanine) profile matching

From the full pattern (10° to 110.5°) matching using the FullProf program,[6] diffraction peaks for L-alanine phase were clearly observed.

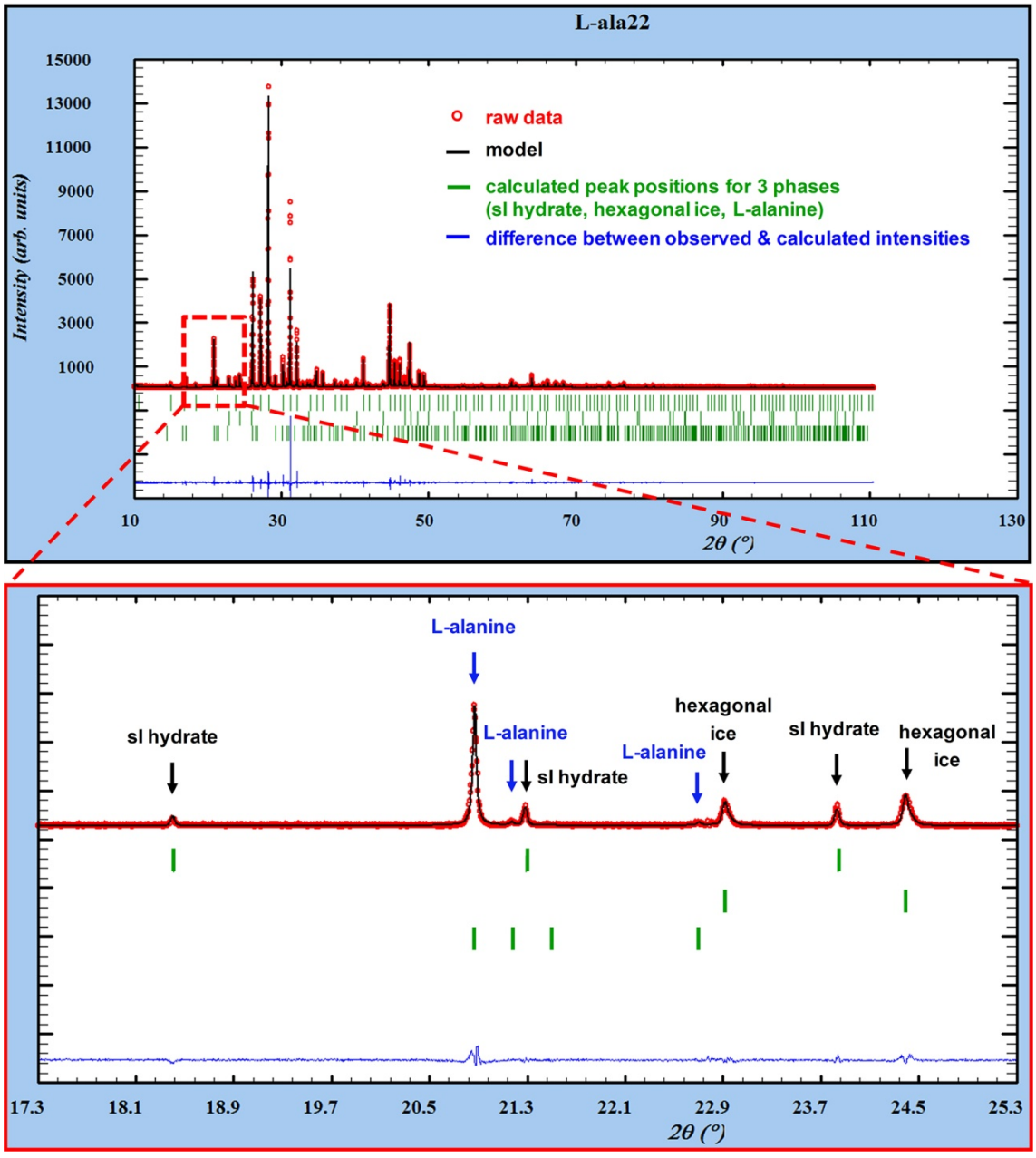


Figure S8. 3 phases (structure I hydrate + hexagonal ice + L-alanine) profile matching result.

c. CO_2 hydrate + L-valine 0.5 mol%

- 3 phases (structure I hydrate + hexagonal ice + L-valine) profile matching

From the full pattern (10° to 110.5°) matching using the FullProf program,[6] diffraction peaks for L-valine phase were clearly observed.

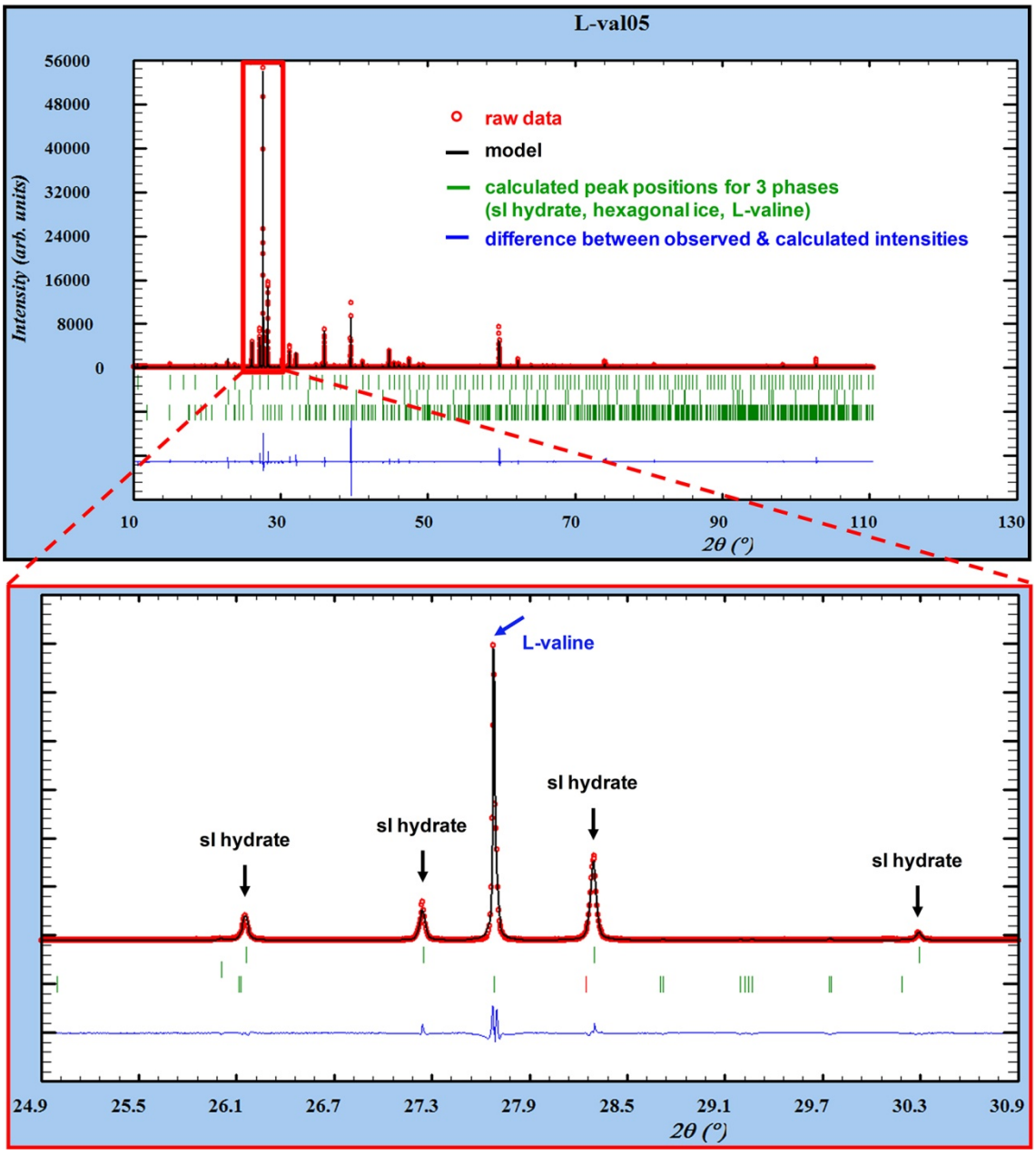


Figure S9. 3 phases (structure I hydrate + hexagonal ice + L-valine) profile matching result.

9. Lattice parameter calculations for CO_2 hydrate in the presence of high concentrations of amino acids

The lattice parameters were calculated from the PXRD patterns of CO_2 hydrate in the presence of a high concentration of amino acids by 3 phases (structure I hydrate + hexagonal ice + amino acid) full pattern (10° to 110.5°) matching using the FullProf program.[6] Before starting the calculations, initial lattice parameter values of crystals of amino acids were taken from the literature (Table S3).

Table S4. Lattice parameters of CO_2 hydrate in the presence of amino acids at 80 K.

Concentration (mol%)	Lattice parameter (Å)		
	CO_2 hydrate + glycine	CO_2 hydrate + L-alanine	CO_2 hydrate + L-valine
0.1	11.842253 ± 0.000039	11.844065 ± 0.000046	11.846368 ± 0.000034
0.5	11.843864 ± 0.000091	11.845690 ± 0.000036	-
1.3	11.844856 ± 0.000069	11.845851 ± 0.000072	-
2.2	11.846091 ± 0.000150	11.845954 ± 0.000037	-
3.0	11.846135 ± 0.000110	-	-

10. Kinetics of CO_2 hydrate crystallization

To investigate the influence of amino acid incorporation on the kinetics of hydrate crystallization, the rate of CO_2 hydrate growth was measured. In the systems containing glycine or L-alanine (Figure S10a,b), the initial growth rate and the amount of gas uptake after the hydrate growth finished were significantly reduced, and the extent of growth inhibition was enhanced with increasing concentration. Both glycine and L-alanine clearly had the ability to inhibit the crystal growth of CO_2 hydrate, while L-valine had no significant effect (Figure S10c). The extent of reduction in gas uptake by the amino acids also correlated well with their hydrophobicity (Figure S10d), consistent with previous results.[1]

An interesting point is that while the extent of lattice expansion by the incorporation became saturated at around 2.2 mol% glycine or 0.5 mol% L-alanine (Figure 3f), both the initial growth rate (Figure S10e) and the amount of gas uptake (Figure S10f) were further decreased above these concentration limits. Although L-valine was found to reduce the initial growth rate at a very early stage, it had negligible effect on the overall hydrate growth kinetics. These results demonstrate that amino acids can inhibit hydrate crystallization by interacting with surrounding free water molecules without a direct incorporation into the hydrate crystal lattice. The mechanism of hydrate inhibition by amino acids has been interpreted in terms of the hydrophobic effect[14,15] and the perturbation phenomenon whereby molecules with high hydrophobicity strengthen the local water structure[16,17] and induce easier hydrate crystallization.[1] However, in the systems with 0.1 mol% amino acids, while most of the amino acids were expected to be incorporated into the hydrate crystal lattice based on the extent of lattice expansion, they were found to be still efficient in delaying hydrate nucleation and retarding hydrate growth.[1] When gas hydrates crystallize in the presence of amino acids, competition for lattice incorporation between them, and the resulting lattice distortion, would

alter the kinetics of hydrate crystallization. Consequently, the incorporation of amino acids into the hydrate crystal lattice as well as the perturbation of the hydrogen bond network of surrounding water molecules is crucial for understanding their crystallization behavior. In addition, any molecules capable of incorporating into the hydrate crystal lattice could affect the kinetics of crystallization.

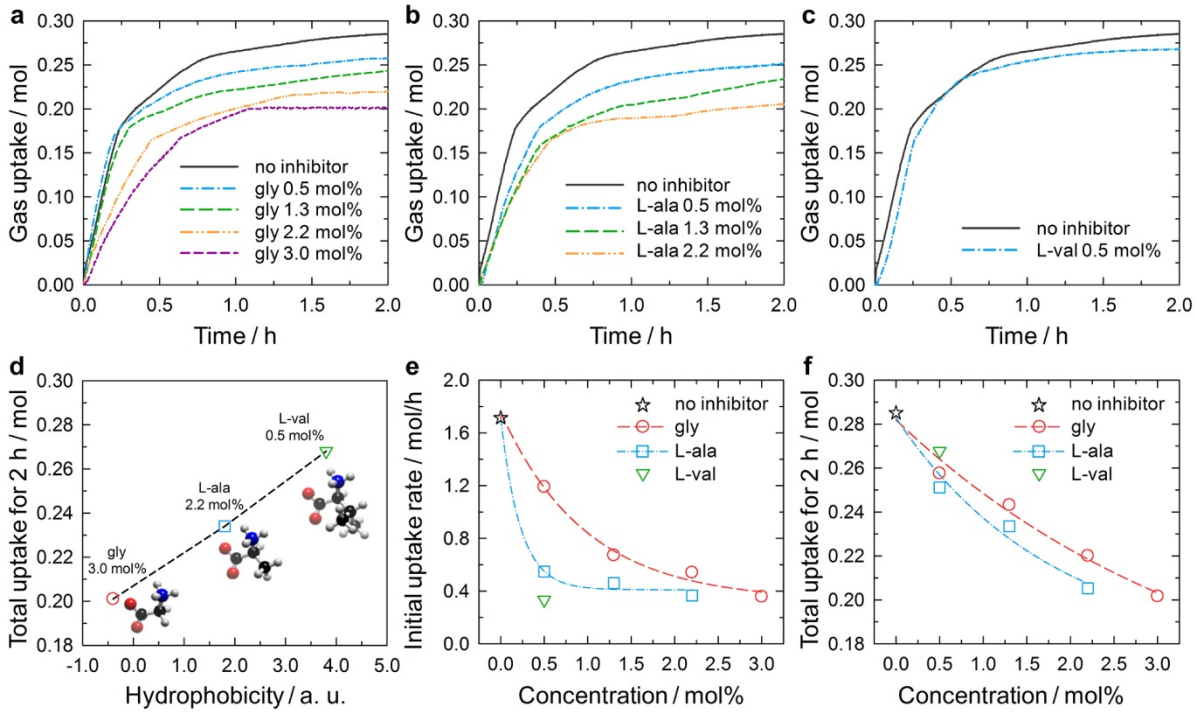


Figure S10. Gas uptake rate of CO_2 hydrate in the presence of increasing concentrations of (a) glycine, (b) L-alanine, and (c) L-valine at 273.45 K. Measurements were initiated immediately after the onset of hydrate nucleation. The initial rate of gas uptake generally decreased with increasing amino acid concentration. The gas uptake curves were almost saturated after 2 h of hydrate growth. Both glycine and L-alanine considerably reduced the initial growth rate and the amount of gas uptake. These amino acids clearly have the ability to inhibit hydrate growth. However, L-valine showed no significant influence on hydrate growth kinetics due to its high hydrophobicity. (d) The amount of gas uptake correlated with the hydrophobicity of the amino acids. The kinetics of hydrate crystallization strongly depended on the hydrophobicity of amino acids in the system. (e) The initial growth rate and (f) the amount of gas uptake in the presence of increasing concentrations of amino acids. The lines are visual fits to clarify the trend. The extent of growth inhibition by amino acids was further increased above the concentration limits of the hydrate crystal lattice expansion.

11. Enlargement of the PXRD patterns

We added the enlargement of the PXRD patterns shown in the manuscript.

Figure 1a.

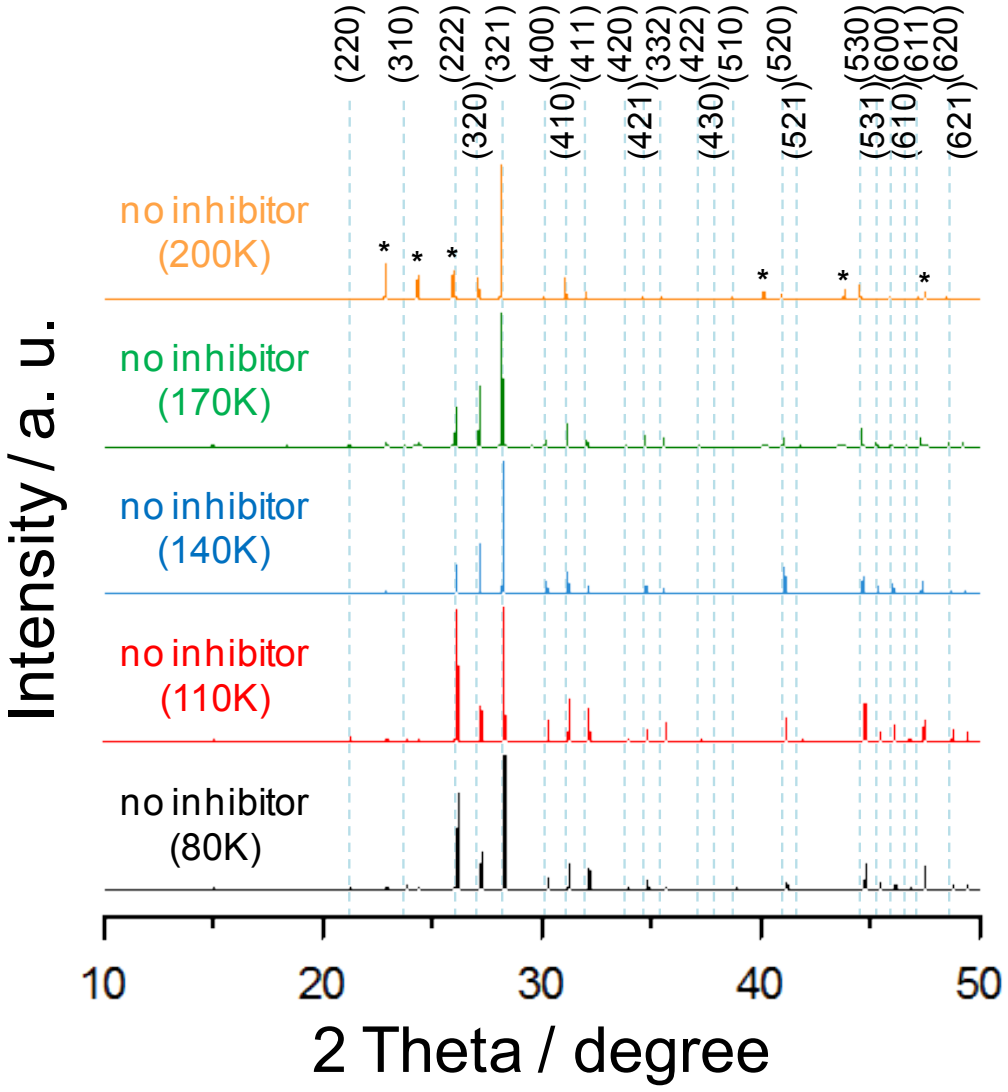


Figure 1b.

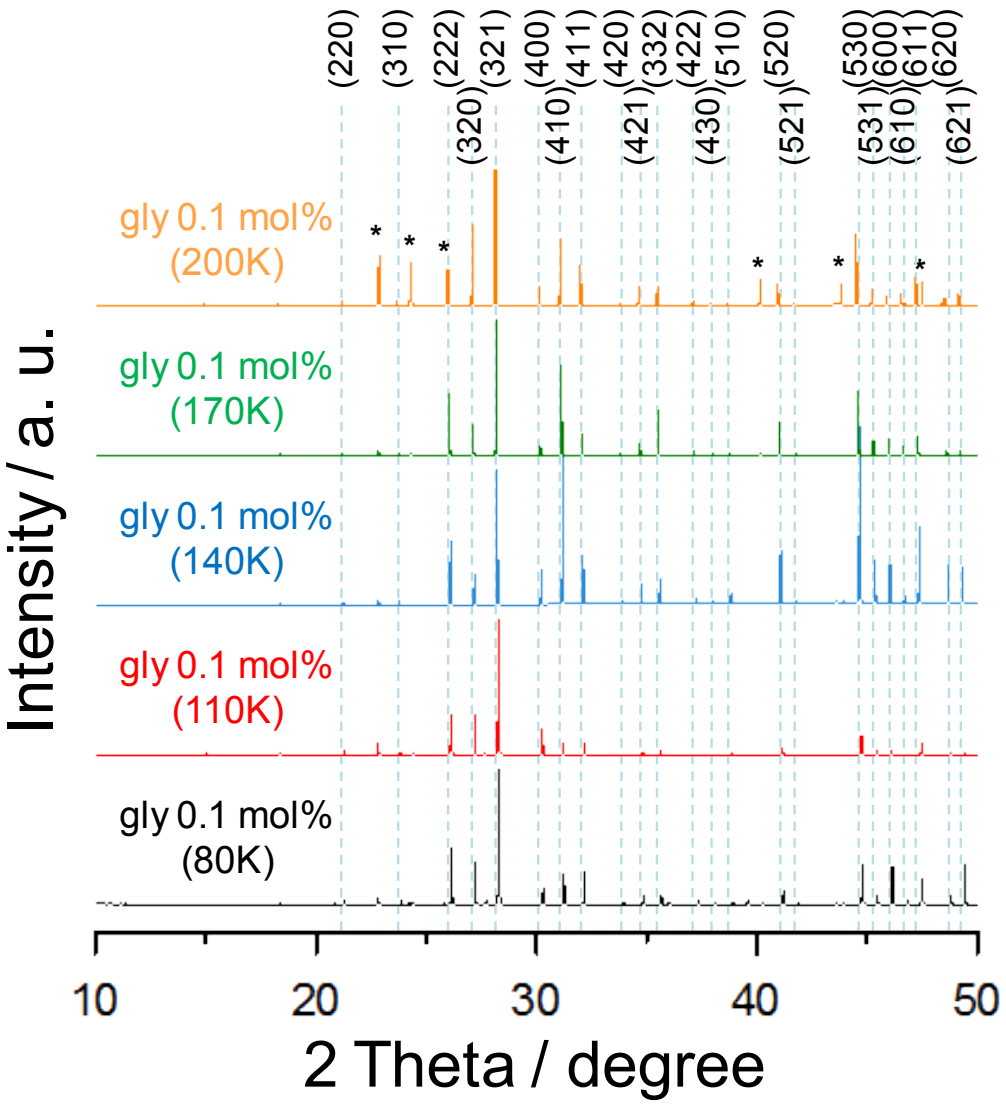
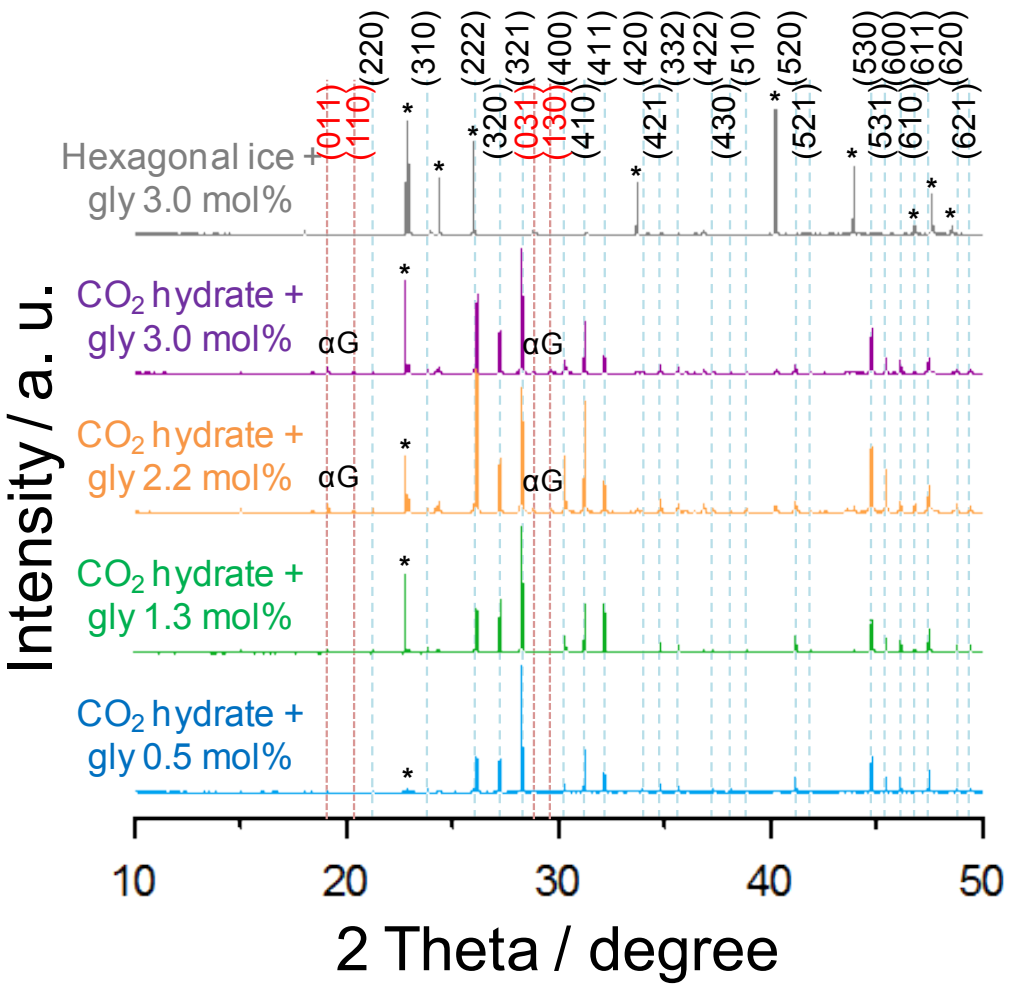

(220)
(310)
(222)
(321)
(320)
(400)
(410)
(411)
(420)
(421)
(332)
(422)
(430)
(510)
(520)
(521)
(530)
(531)
(600)
(610)
(611)
(620)
(621)
gly 0.1 mol% (200K)
gly 0.1 mol% (170K)
gly 0.1 mol% (140K)
gly 0.1 mol% (110K)
gly 0.1 mol% (80K)
Intensity / a. u.
10
20
30
40
50
2 Theta / degree

Figure 3a.


Hexagonal ice + gly 3.0 mol%
CO2 hydrate + gly 3.0 mol%
CO2 hydrate + gly 2.2 mol%
CO2 hydrate + gly 1.3 mol%
CO2 hydrate + gly 0.5 mol%
αG
αG
αG
αG
(011)
(110)
(220)
(310)
(222)
(320)
(031)
(130)
(321)
(400)
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(411)
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(430)
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(620)
(621)
Intensity / a. u.
10
20
30
40
50
2 Theta / degree

Figure 3c.

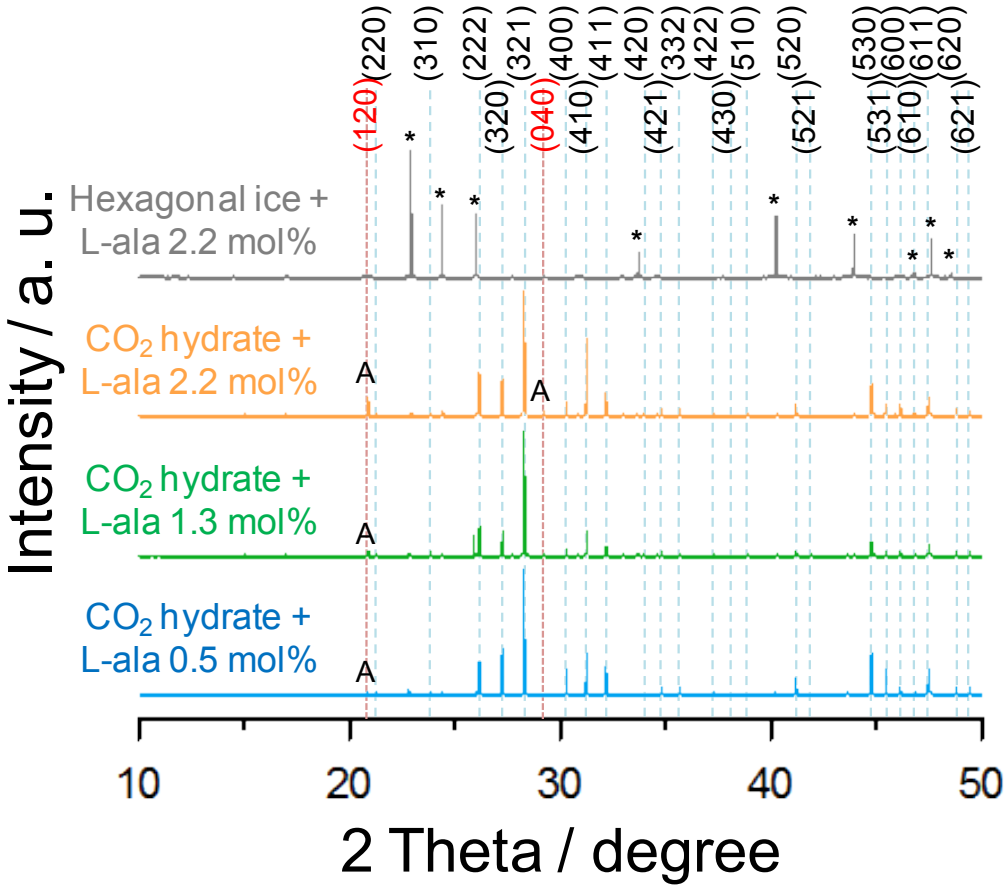
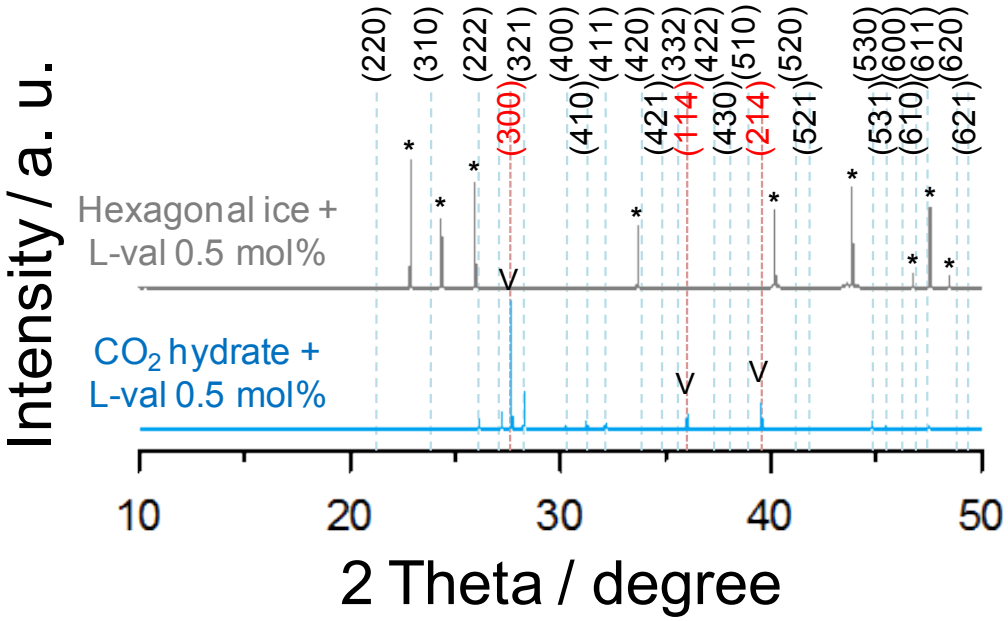


Figure 3e.



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