Supporting information for

Protection by desiccation-tolerance proteins probed at the residue level

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Supplemental figures

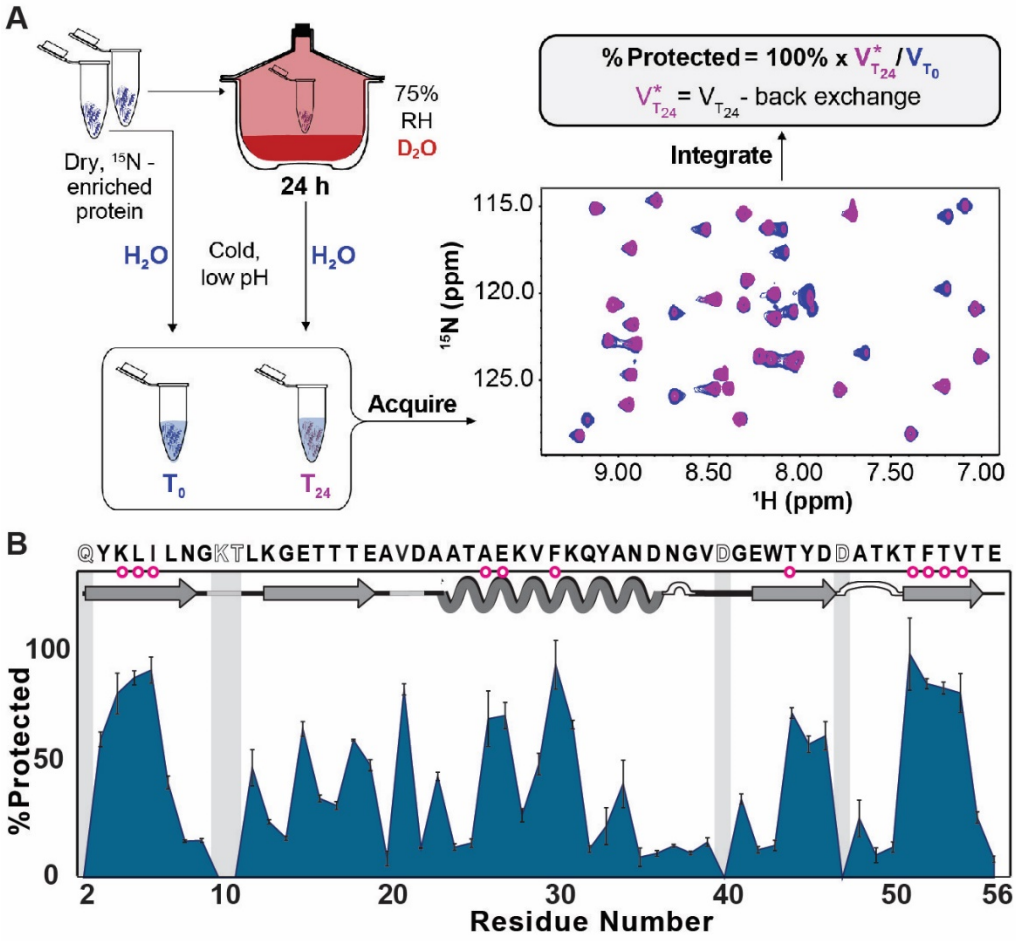
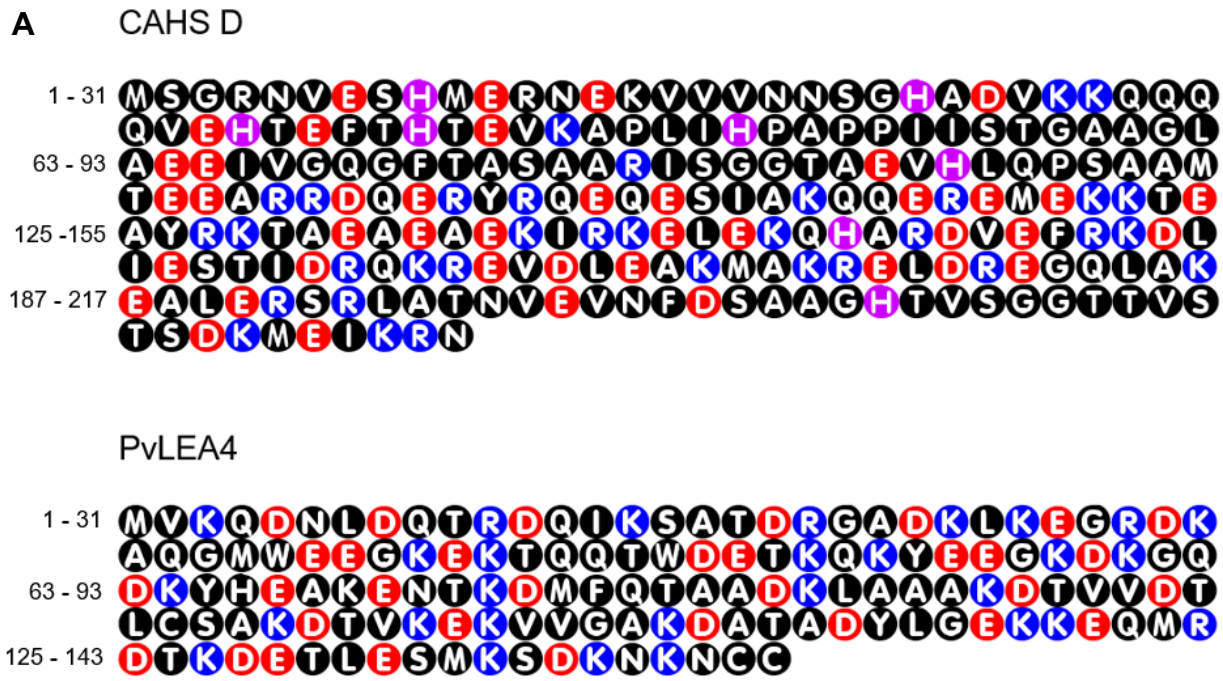


Figure S1. LOVE NMR workflow and output. A) LOVE NMR workflow. Identical samples of ^{15}N-enriched protein dried alone or in the presence of a cosolute are resuspended in cold, acidic buffer before (T_0) or after (T_{24}) 24 h exposure to D_2O vapor at 75% relative humidity (RH). Amide protons unprotected in the dry state exchange with deuterons from the vapor, resulting in smaller cross peak volumes in the T_{24} ^{15}N-^{1}H HSQC spectrum relative to the T_0 spectrum (pink and blue cross peaks, respectively). To correct for solution back-exchange that occurs before and during spectrum acquisition, serial HSQC spectra are obtained for the T_{24} sample, integrated, and fit to the equation $V_{T24}(t) = A(1-e^{-bt})+V_{T24}^*$, where V_{T24} is peak volume, t is time since resuspension, A is a scaling factor, b is the observed rate of exchange, and V_{T24}^* the peak volume before any back exchange (see Materials and Methods). The fitted V_{T24}^* value is then divided by the maximum possible peak volume, V_{T0}, and multiplied by 100 to obtain %Protected. B) LOVE profile of model protein GB1 freeze-dried in 1.5 mM HEPES, pH 6.5. Open letters indicate residues with undefined dry-state protection because they are 100% quench-labelled. Secondary structure (β-strands; undulations, helix; white bumps, turns; gray lines, bends) is shown at top, with magenta circles indicating solution global unfolding residues. Gray areas indicate the absence of data. Error bars represent uncertainty propagated from standard deviations from the mean from triplicate analysis.

A



B

Job Title	**CAHS D**
RID	JK899JMW11N *Search expires on 08-29 10:36 am* Download All
Program	Blast 2 sequences Citation
Query ID	lcl|Query_84359 (amino acid)
Query Descr	CAHS D
Query Length	227
Subject ID	lcl|Query_84361 (amino acid)
Subject Descr	PvLEA4
Subject Length	143

⚠ No significant similarity found. For reasons why, click here

Figure S2. Primary structure comparisons of CAHS D and PvLEA4. A) Amino acid sequences of CAHS D and PvLEA4. Red, blue, gray, and purple circles indicate negatively charged residues, positively charged residues, neutral residues, and histidines, respectively. B) Results of sequence alignment of CAHS D and PvLEA4 using protein BLAST with default algorithm parameters.[1]

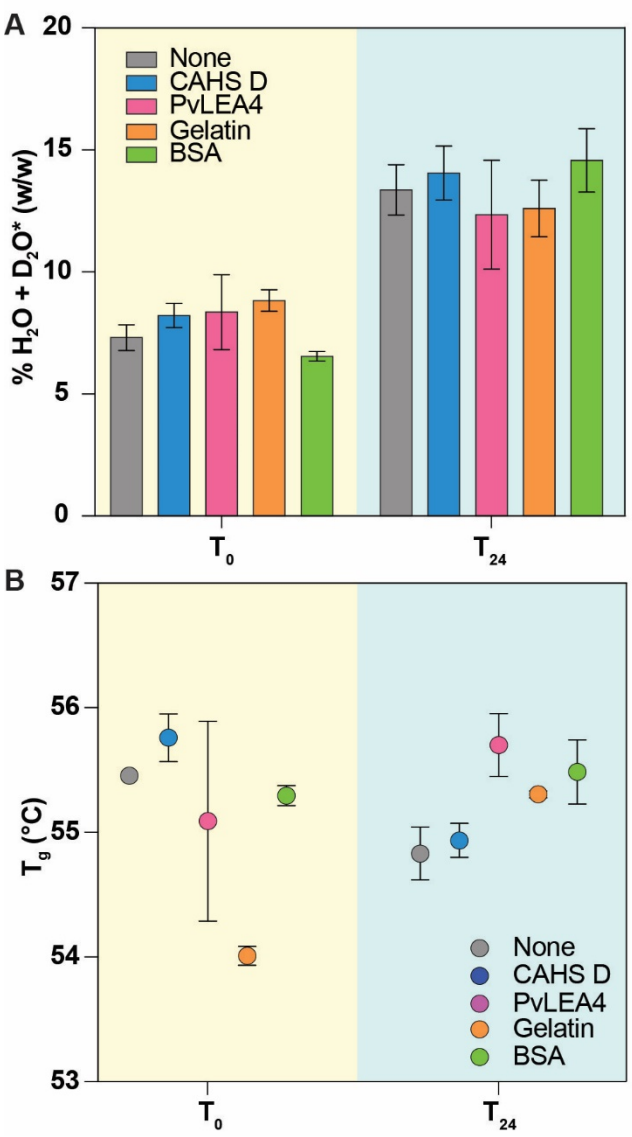


Figure S3. Water content and glass transition temperature of dehydrated protein mixtures before and after exposure to 75% relative humidity (D_2O). A) Percent *H_2O (T_0) or H_2O + D_2O (T_{24}) by weight of samples lyophilized from 650 μL of 500 μM GB1 and 5 g/L of indicated protectants before and after (T_0 and T_{24}, respectively) incubation in 75% RH D_2O. Error bars represent the standard deviation from 3 independent measurements for GB1 with CAHS D, 4 independent measurements for GB1 with PvLEA4, and the range of 2 independent measurements for other data. B) Glass transition temperature, T_g, of formulations of lyophilized 650 μL aliquots of 500 μM GB1 and 5 g/L of indicated protectants before and after (T_0 and T_{24}, respectively) incubation in 75% RH D_2O. Error bars represent the range of 2 independent measurements.

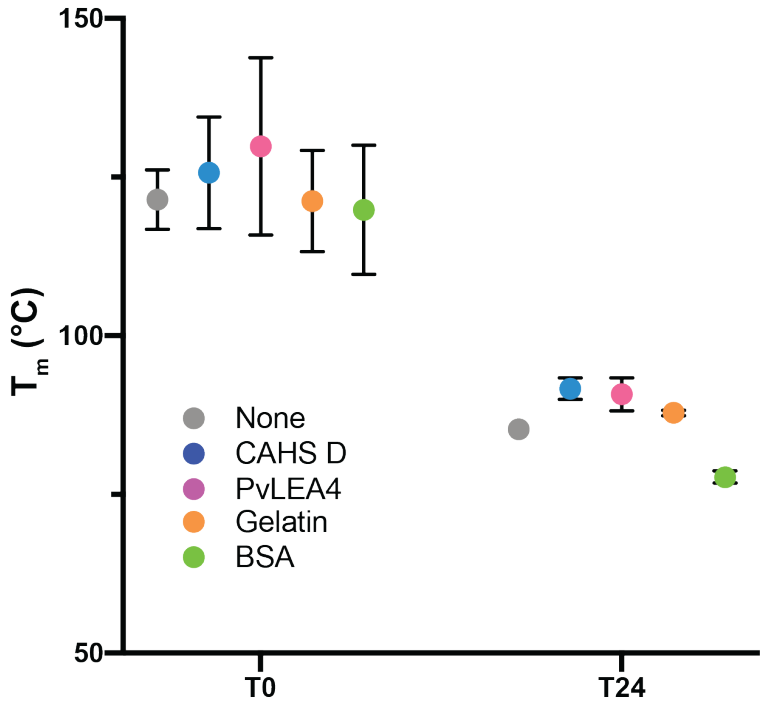


Figure S4. Denaturation temperature, T_m, of dehydrated protein mixtures before and after exposure to 75% relative humidity (D_2O). T_m of GB1 lyophilized from 650 μL aliquots of 500 μM GB1 alone or with 5 g/L of the indicated protectant before and after incubation at 75% RH D_2O (T_0 and T_{24}, respectively.) As expected, as moisture content increases, T_m of GB1 decreases.[2-3] T_m is similar for all protectants, except for BSA, T_m is lower at T_{24}. Error bars are the standard deviation from 3 independent measurements for GB1 with CAHS D and PvLEA4 and represent the range of 2 independent measurements other data.

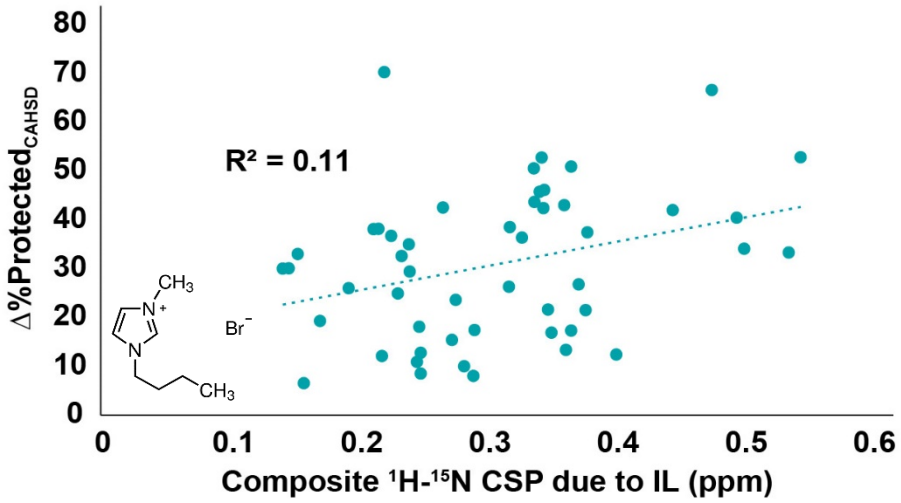


Figure S5. Correlation of composite chemical shift perturbations induced by an ionic liquid and Δ%Protected induced by drying with 5 g/L CAHS D. Non-quench-labelled GB1 residues are included (n=50). The ^{1}H-^{15}N composite chemical shift perturbations (CSPs) induced by 50% v/v 1-butyl-3-methylimidazolium bromide ([C_4-mim]Br), an ionic liquid (IL) are from the data of Warner *et al*.[4] Chemical structure of [C_4-mim]Br is shown in the inset. The probability that the R arises from uncorrelated data is less than 5%.[5]

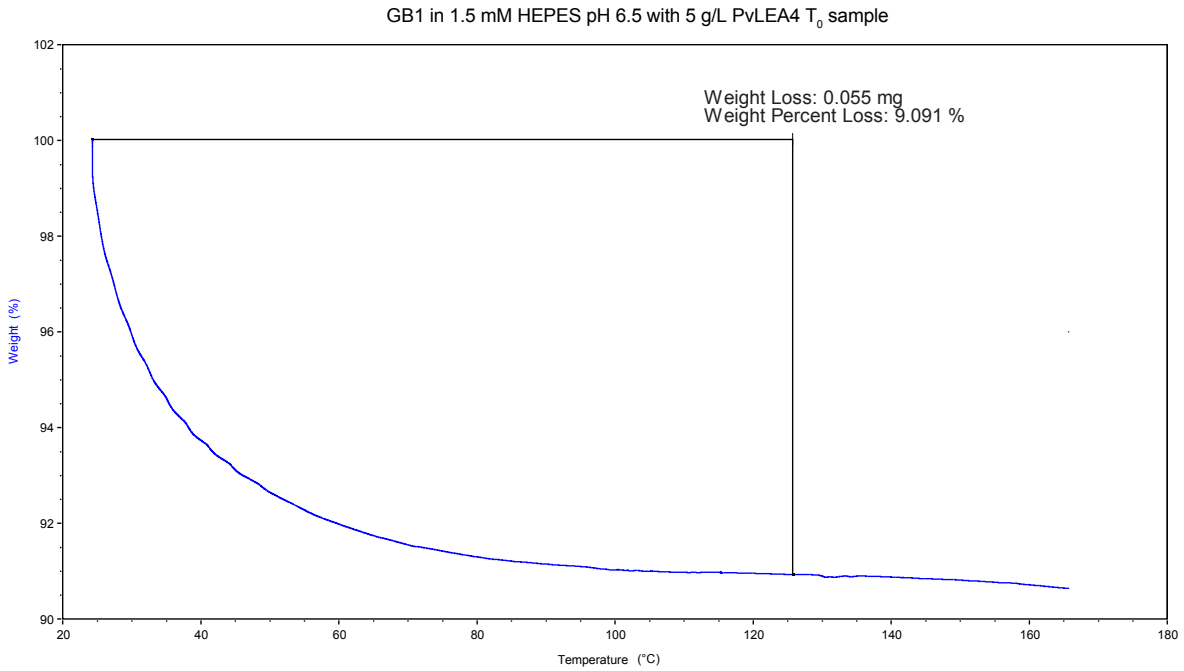


Figure S6. Representative data from thermogravimetric analysis. The initial change in weight due to water loss is shown.

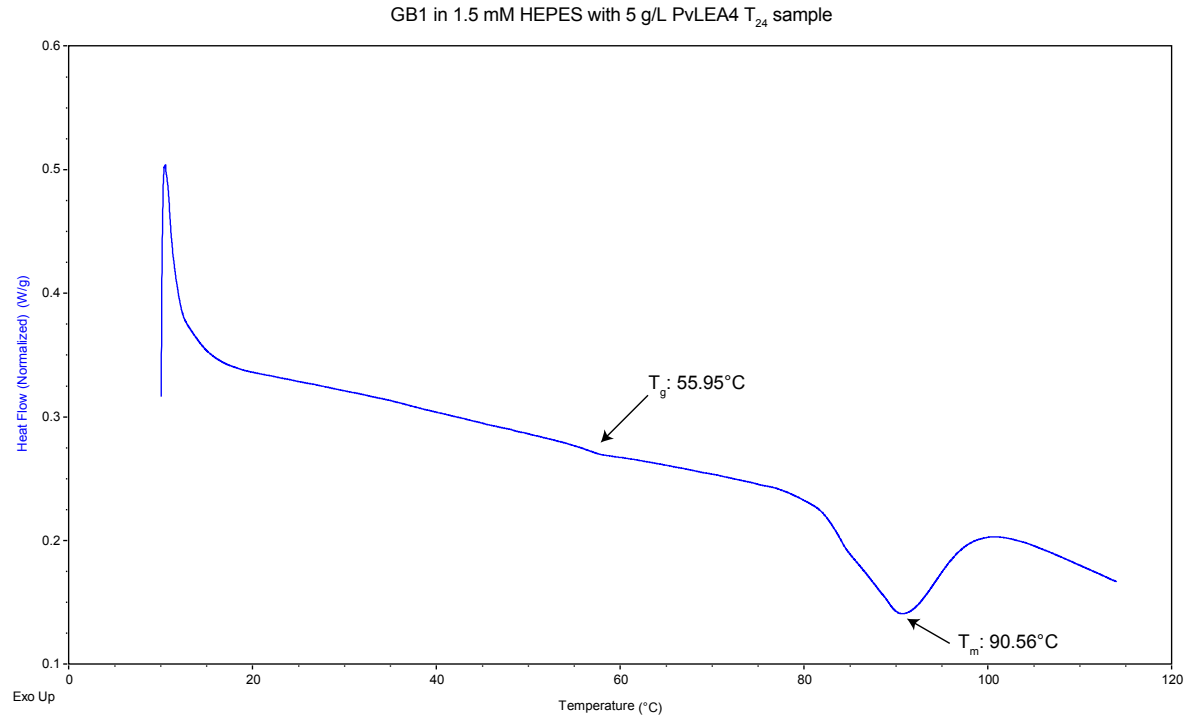


Figure S7. Representative differential scanning calorimetry (second scan). The glass transition- (T_g) and denaturation- (T_m) temperatures are indicated.

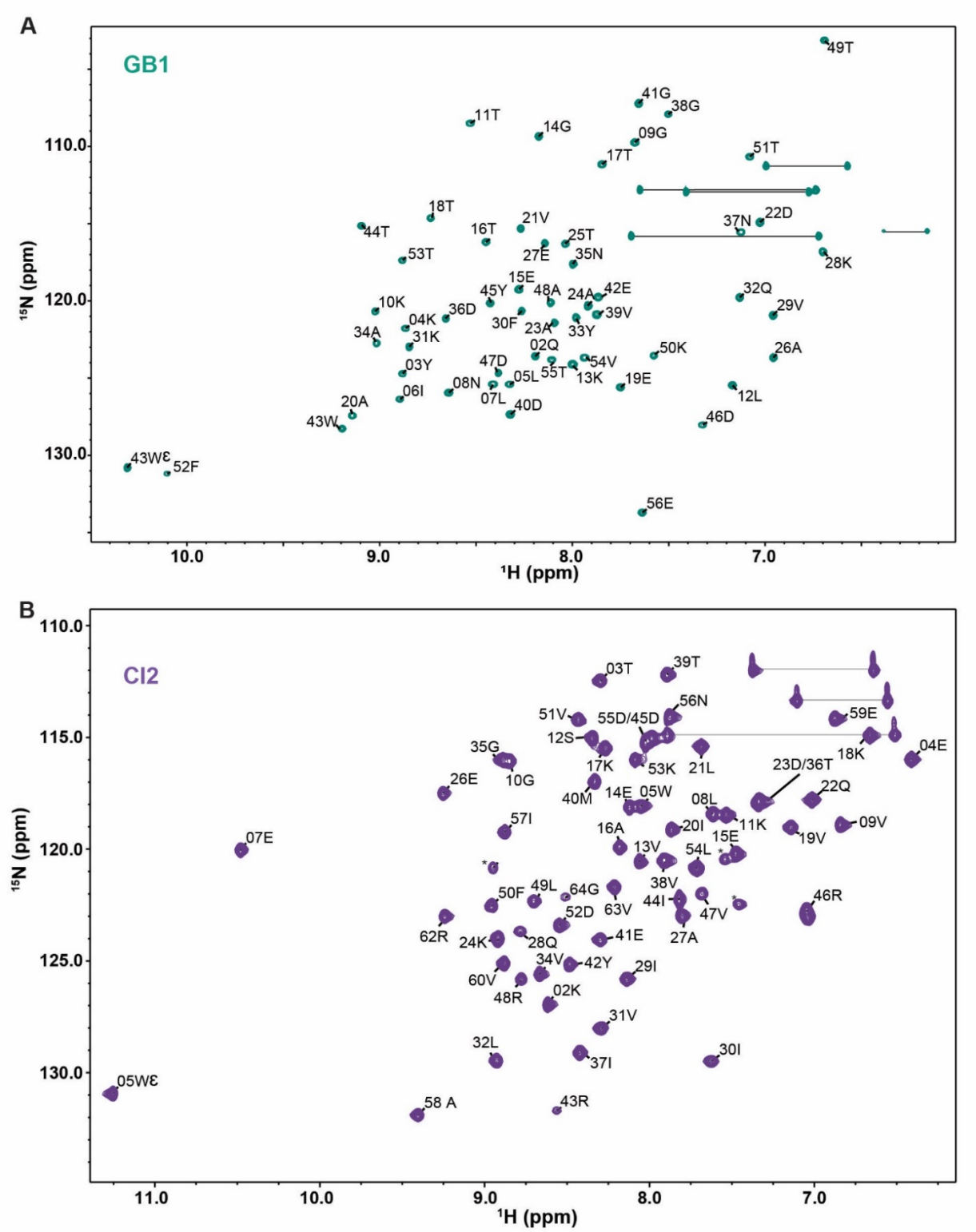


Figure S8. Assigned ^{1}H-^{15}N HSQC spectra of client proteins under acquisition conditions for LOVE NMR. A) GB1 and B) CI2 in 100 mM citrate in 90%H_2O, 10% D_2O, pH 4.5, 4°C. *Starred resonances for CI2 probably arise from an alternative conformation.

Supplemental Tables

Table S1. Hydrated fraction of GB1 solvent-accessible surface area (SASA)

Hydrated fraction of GB1 SASA ± uncertainty		
Protectant	**T_0**	**T_{24}**
None	0.40 ±0.03	0.70 ±0.06
CAHS D	0.44 ±0.03	0.74 ±0.07
PvLEA4	0.46 ±0.09	0.6 ±0.1
Gelatin	0.49 ±0.03	0.65 ±0.07
BSA	0.35 ±0.01	0.77 ±0.08

We assumed that all water is bound to GB1, that all water in the T_0 sample is H_2O, and that all water in the T_{24} sample is D_2O. The values are calculated by multiplying the molar ratio of water to GB1 (as measured by TGA) by the average amount of protein surface covered by a water molecule (20 $Å^2$),[6] and then dividing that value by the surface area of the native solution structure of GB1 (3727 $Å^2$) as determined by the PyMOL get_area function for PDB 2QMT. Origins of uncertainties are described in the caption of Fig. 2.

Table S2. Average %Protected values of GB1 dried alone or in the presence of 5 g/L protectant.

Residue	No protectant	+CAHS D	+PvLEA4	+Gelatin	+BSA
Y3	61 ± 3	90 ± 10	90 ± 1	88 ± 6	69
K4	81 ± 9	108 ± 8	95 ± 1	94 ± 1	76
L5	88 ± 3	102 ± 9	99.2 ± 0.3	96 ± 6	91
I6	92 ± 6	130 ± 10	100.2 ± 0.2	89 ± 3	87
L7	42 ± 3	110 ± 10	109 ± 20	90 ± 10	52
8N	16 ± 1	34 ± 4	40 ± 1	31 ± 3	19
9G	16 ± 1	36 ± 9	25 ± 4	19 ± 2	17
12L	49 ± 8	90 ± 20	82 ± 1	60 ± 20	58
13K	25 ± 1	60 ± 10	51 ± 4	32 ± 2	26
14G	17 ± 1	34 ± 7	37 ± 2	26 ± 2	19
15E	66 ± 3	112 ± 6	90 ± 10	100 ± 10	81
16T	35 ± 2	78 ± 5	81 ± 9	73 ± 7	39
17T	32 ± 2	70 ± 20	48 ± 7	33 ± 4	31
18T	60.7 ± 0.4	110 ± 20	96 ± 1	90 ± 10	64
19E	50 ± 2	80 ± 10	60 ± 10	60 ± 10	51
20A	8 ± 3	16 ± 4	25 ± 1	17 ± 1	6
21V	83 ± 3	117 ± 6	90 ± 20	80 ± 10	64
22D	13.1 ± 0.3	22 ± 3	21 ± 1	16 ± 1	15
23A	45 ± 2	80 ± 10	58 ± 3	54 ± 7	40
24A	13 ± 1	26 ± 4	17 ± 3	16 ± 1	16
25T	15 ± 2	22 ± 1	28.0 ± 0.1	18 ± 4	18
26A	70 ± 10	116 ± 7	107 ± 1	99 ± 7	78
27E	72 ± 6	110 ± 30	96 ± 8	90 ± 10	64
28K	28 ± 3	70 ± 20	78 ± 1	54 ± 2	39
29V	50 ± 5	100 ± 10	92.0 ± 0.4	78 ± 5	53
30F	90 ± 10	116 ± 5	101 ± 1	110 ± 10	87
31K	68 ± 2	98 ± 3	91 ± 8	91 ± 7	60
32Q	13 ± 1	46 ± 8	52 ± 1	30 ± 2	21
33Y	23 ± 8	74 ± 2	85 ± 1	62 ± 5	34
34A	40 ± 10	110 ± 10	99 ± 1	87 ± 5	44
35N	9 ± 4	50 ± 4	52 ± 4	37 ± 3	17
36D	11 ± 1	37 ± 2	32 ± 1	23.3 ± 0.4	14
37N	14 ± 1	52 ± 3	57 ± 9	51 ± 2	22
38G	11 ± 1	28 ± 2	15.2 ± 0.5	14 ± 1	14
39V	16 ± 2	54 ± 7	55 ± 7	56 ± 5	18
41G	35 ± 2	60 ± 20	60 ± 10	45 ± 8	45
42E	12 ± 1	23 ± 3	23 ± 1	20 ± 1	14
43W	14 ± 2	38 ± 9	25 ± 5	16 ± 2	14
44T	73 ± 2	110 ± 7	98.1 ± 0.2	90 ± 4	72
45Y	59 ± 4	112 ± 8	80 ± 10	70 ± 20	63
46D	63 ± 6	90 ± 10	86 ± 2	79 ± 3	62
48A	26 ± 8	60 ± 10	46 ± 9	38 ± 6	35
49T	10 ± 3	20 ± 1	17 ± 1	14 ± 1	15
50K	13 ± 2	26 ± 2	42 ± 1	25 ± 3	19
51T	100 ± 20	120 ± 10	102 ± 2	97 ± 5	86
52F	86 ± 2	120 ± 10	110 ± 20	110 ± 20	87
53T	84 ± 3	117 ± 3	98 ± 1	95 ± 5	83
54V	81 ± 9	100 ± 20	98.2 ± 0.4	81 ± 4	63
55T	27 ± 2	56 ± 6	72 ± 1	61 ± 3	32
56E	8 ± 1	20 ± 5	21 ± 2	19 ± 1	16

Standard deviation from the mean for three independent measurements is reported for all conditions except for GB1 dried in the presence of BSA, which was measured once.

Table S3. Average %Protected values of CI2 dried alone or in the presence of 5 g/L protectant.

Residue	No protectant	+CAHS D	+PvLEA4	+Gelatin	+BSA
4	11.3 ± 0.7	12 ± 1	11.7 ± 0.3	12.1 ± 0.5	12
5	60 ± 4	71 ± 2	65.9 ± 0.4	76 ± 3	68
7	37 ± 3	32 ± 2	40 ± 9	43 ± 2	40
8	68 ± 9	90 ± 8	73.8 ± 0.7	78 ± 3	77
9	38 ± 9	39 ± 1	35 ± 1	37.6 ± 0.9	41
11	86 ± 1	94.6 ± 0.6	92 ± 1	93 ± 5	90
12	11.0 ± 0.1	16 ± 6	11.6 ± 0.5	12.0 ± 0.5	13
13	18 ± 5	16 ± 4	17.0 ± 0.1	18.9 ± 0.8	17
14	13.9 ± 0.5	20 ± 10	16 ± 1	16.7 ± 0.4	16
15	15.2 ± 0.6	18 ± 3	15.2 ± 0.3	15.9 ± 0.7	16
16	90 ± 20	95 ± 3	85.7 ± 0.9	91 ± 4	87
17	16 ± 3	16 ± 1	17 ± 1	17.6 ± 0.9	18
18	24 ± 6	24 ± 2	25.1 ± 0.8	25.0 ± 0.7	25
19	85 ± 5	99 ± 8	90 ± 2	92 ± 5	89
20	94 ± 2	110 ± 10	94 ± 1	103 ± 5	99
21	91 ± 1	105 ± 10	92.5 ± 0.8	100 ± 5	96
22	71 ± 8	86 ± 5	74.4 ± 0.9	82 ± 4	75
24	79 ± 5	94 ± 3	85.0 ± 0.6	91 ± 5	81
26	24 ± 2	30 ± 20	24 ± 2	27.4 ± 0.5	26
27	18 ± 1	22 ± 1	20.9 ± 0.1	24.6 ± 0.9	20
28	19 ± 4	24 ± 3	24 ± 4	27 ± 2	17
29	12.2 ± 0.3	15 ± 5	12.5 ± 0.1	13.1 ± 0.7	13
30	93.5 ± 0.6	110 ± 10	98 ± 2	100 ± 4	106
31	79 ± 3	78 ± 2	82 ± 2	82 ± 5	70
32	96.4 ± 0.5	110 ± 10	101 ± 2	103 ± 5	100
34	13 ± 2	15.1 ± 0.4	17.1 ± 0.7	15.6 ± 0.8	15
35	58 ± 7	75 ± 1	63.4 ± 0.1	70 ± 3	62
37	24 ± 1	22.1 ± 0.4	26.8 ± 3.2	27 ± 1	26
38	14 ± 4	20 ± 9	15.8 ± 0.8	16.2 ± 0.5	15
39	60 ± 20	46 ± 4	60 ± 10	68 ± 4	62
42	40 ± 10	41 ± 4	50 ± 10	54 ± 2	50
43	13 ± 3	16 ± 4	15 ± 9	21 ± 2	15
44	13.3 ± 0.8	19 ± 6	16.5 ± 0.4	13.6 ± 0.6	14
46	89 ± 3	98 ± 2	85 ± 2	92 ± 22	93
47	83.9 ± 0.7	98 ± 9	82.4 ± 0.2	92 ± 5	87
48	50 ± 10	58 ± 1	62.4 ± 0.8	60 ± 5	49
49	98 ± 2	110 ± 10	102 ± 2	104 ± 5	102
50	86 ± 4	98 ± 8	92 ± 1	90 ± 5	88
51	87 ± 2	99 ± 7	92 ± 2	96 ± 6	87
52	85 ± 2	91 ± 4	92 ± 2	92 ± 5	82
54	65 ± 3	70.3 ± 0.2	70.8 ± 0.9	71 ± 3	61
56	94.4 ± 0.4	101 ± 2	98 ± 2	100 ± 5	91
57	86 ± 3	98 ± 8	88 ± 1	95 ± 5	89
58	90 ± 2	98 ± 8	96 ± 3	99 ± 5	91
59	92 ± 3	102 ± 6	97 ± 1	98 ± 5	92
60	80 ± 10	110 ± 10	93 ± 1	100 ± 5	88
62	56 ± 7	64 ± 3	63.4 ± 0.5	61 ± 4	55
63	32 ± 6	40 ± 2	37.3 ± 0.5	41 ± 2	37
64	62 ± 3	69 ± 2	70 ± 2	76 ± 7	55

Standard deviation from the mean for three independent measurements is reported for all conditions except for CI2 dried in the presence of BSA, which was measured once.

Table S4. Probability that observed protection is random

	CAHS D			PvLEA4			Gelatin			BSA		
	+	-	*p*	+	-	*p*	+	-	*p*	+	-	*p*
GB1	48	2	1.1 E-12	47	3	1.7 E-11	41	9	2.2 E-06	27	23	9.6 E-02
CI2	25	24	1.1 E-01	20	29	5.0 E-02	30	19	3.4 E-02	16	33	6.0 E-03

Key: +, number of successes (Δ%Protected is positive and not within error of zero)
-, number of failures (Δ%Protected within error of zero or negative)
p, probability that observed protection arises from a binomial distribution with 50% chance of protection, i.e. protection (or lack thereof) is random
green p-values, positive protection is non-random ($p<.05$) → protects
red p-values, lack of protection is non-random ($p<.05$) → does not protect
gray p-values, observed protection trend is random ($p>.05$) → not conclusive

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