Supporting Information

Material and Methods

All the systems were recombinantly expressed using protocols previously reported.[1–5]Although no exogenous arginine was added to the samples, previous studies indicate that both the wild-type dimer and the truncated monomer $ArgBP^{20-233}$ retain the ligand in the binding pocket.[3,6] For H/D-exchange experiments, the lyophilized powder of the different variants of the protein were dialysed in D_2O using Amicon Ultra (2 mL) centrifugation units with 10 kDa cutoff and subsequently lyophilized. 50 mM Tris buffer was chosen for the Fourier-transform infrared (FTIR) spectroscopy measurements. The pD value was adjusted to 7.5 (pH +0.4 = pD) by adding DCl or NaOD.

The measurements were performed using a MAGNA 550 (Thermo Fisher Scientific) equipped with a liquid-nitrogen cooled MCT-detector (HgCdTe). Details of the system were described elsewhere.[7] Owing to the pressure-sensitive stretching vibration of SO_4^{2-} (983.5 cm^{-1} ≙ 1 bar), $BaSO_4$ was used as an internal pressure standard.[8] A protein concentration of 50 mg mL^{-1} was used for each measurement. The spectra were averaged over 128 scans in a row by using Omnic 7.2 spectral processing software. The resolution was set at 2 cm^{-1} and the temperature (25 °C) was measured with a digital thermometer placed in the sample cell with an accuracy of ± 0.2 °C. The spectra obtained were processed and analysed with Thermo Grams 8.0 software as follows: After buffer subtraction and smoothing, the area of the amide I´ band (1700-1600 cm^{-1}) was normalized to 1. Peak numbers and peak positions for fitting of the subbands were obtained from the maxima of the Fourier self-deconvolution (FSD, Gamma factor: 13) and minima of the 2nd derivative spectra (derivative: 2nd, degree: 3, points: 11) approaches. FSD is a mathematical procedure for reducing bandwidth in order to resolve overlapping bands from each other. In order to counteract the spectral noise, the deconvolution is further multiplied by a smoothing function (smoothing: 65%).[9] Here, the amide I´ band region of the different kinds of ArgBP can be decomposed into seven (for the $ArgBP^{D1}$ domain into six) subbands. The matching peak positions provide the position of the maxima of the subbands for the fitting process relatively precisely. In addition, the range of a subband position was limited to ± 2 cm^{-1}. The start parameters number, position, height and half-width of the subbands were then fixed with reasonable limits.[10,11] To determine the relative changes in the population of secondary structure elements, mixed Gaussian-Lorentzian line shape functions were used in the fitting procedure, which were adapted to the normalized amide I´ band.[10,12] The area of each subband corresponds to the percentage of the secondary structure it is assigned to (the total amide I´ band area equals 100%).[11]

Boltzmann fits, which are based on the assumption that a two-state unfolding process of the protein takes place, to the pressure-dependent sigmoidal curve progression of the secondary structural elements were used to obtain the volume changes upon (partial) unfolding or dissociation. The pressure-induced intensity changes are given by

$$I = \frac{I_\mathrm{f} - I_\mathrm{u}}{1 + e^{-(p - p_u)\cdot\frac{\Delta V_u}{RT}}} + I_\mathrm{u} \qquad (1)$$

where I_f and I_u are the plateau values of the IR band intensities of the folded and unfolded/dissociated protein, respectively. Generally, by favoring states with a smaller partial

molar volume, pressure shifts an equilibrium towards the state with smaller overall volume, in accord with Le Châtelier's principle. The effect of pressure on any chemical equilibrium or conformational transition is given by

$$\left(\frac{\partial \ln K}{\partial p}\right)_T = -\frac{\Delta V}{RT} \tag{2}$$

at constant temperature, where K is the pressure-dependent equilibrium constant and ΔV is the associated volume change, in our case upon protein (partial) unfolding or dissociation. Hence, the K values can be expected to exponentially depend on pressure, and a transition that is accompanied by a positive ΔV will be suppressed under pressure, and vice versa.

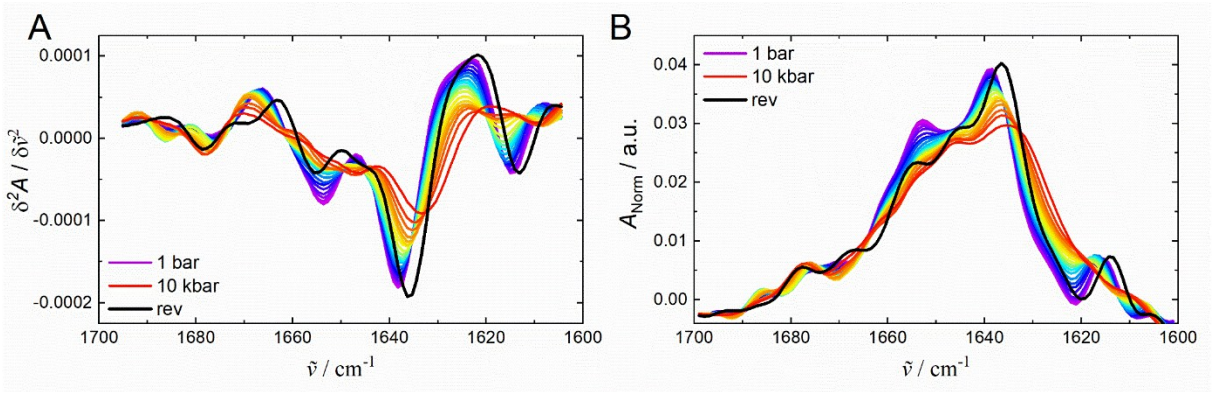
Table SI 1: Comparison of all secondary structure elements in percent of all measured ArgBP variants at 1 bar, 10 kbar and after decompression.

ArgBP variants	Secondary structure element	$\tilde{\nu}$ / cm^{-1}	Secondary structure elements in %		
			at 1 bar	at ~ 10 kbar	decompression
ArgBP^{20_246}	■ β-sheets or turns	1686	1	2	2
	● β-sheets or turns	1677	4	4	4
	▲ β-sheets or turns	1670	7	5	5
	▼ α-helices	1654	35	29	31
	◀ intramolecular β-sheets	1638	30	39	35
	◆ random coils	1643	17	14	16
	▶ side chains	1615	6	7	7
ArgBP^{20_233}	■ β-sheets or turns	1686	1	1	1
	● β-sheets or turns	1678	4	5	4
	▲ β-sheets or turns	1669	7	7	5
	▼ α-helices	1654	33	26	31
	◀ intramolecular β-sheets	1638	31	39	36
	◆ random coils	1643	17	14	16
	▶ side chains	1615	6	8	6
ArgBP^{D1}	■ β-sheets or turns	1680	6	5	7
	● β-sheets or turns	1668	9	9	8
	▼ α-helices	1654	29	25	31
	◀ intramolecular β-sheets	1638	30	34	27
	◆ random coils	1643	22	20	20
	▶ side chains	1615	4	7	5
	■ β-sheets or turns	1684	4	5	4
ArgBP^{D2}	● β-sheets or turns	1676	2	2	2
	▲ β-sheets or turns	1661	12	10	12
	▼ α-helices	1652	26	22	26
	◀ intramolecular β-sheets	1637	37	42	37
	◆ random coils	1643	15	13	15
	▶ side chains	1615	4	5	4

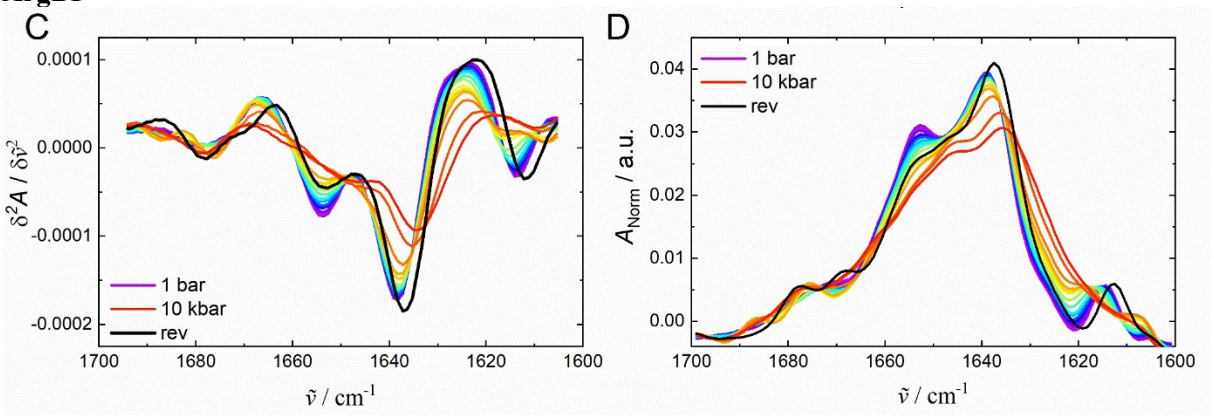
Table SI 2: Secondary structure content of the ArgBP variants as obtained from the PDB structures.

Protein	Protein Data Bank Code	α-helix (%)	β-sheet (%)
$ArgBP^{20-246}$	4PSH	38	28
$ArgBP^{20-233}$	6GGV	28	23
$ArgBP^{D1}$	6GPC	35	21
$ArgBP^{D2}$	6GPM	29	26

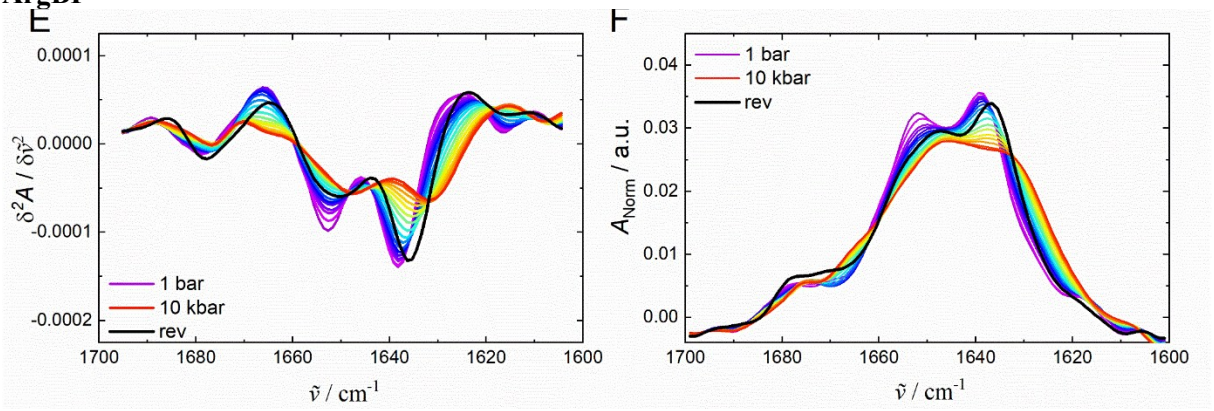
ArgBP^{20_246}



ArgBP^{20_233}



ArgBP^{D1}



ArgBP^{D2}

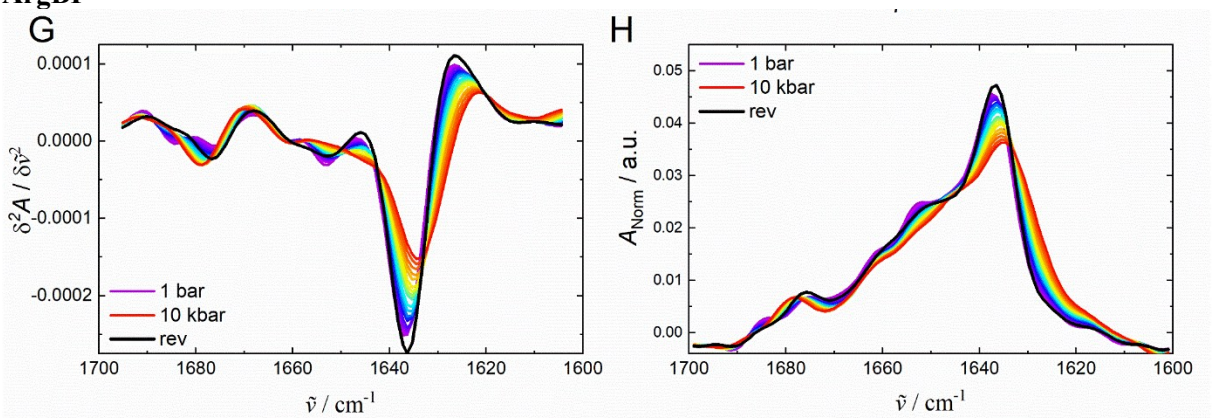


Figure SI 1: Corresponding deconvoluted amide I´ band (B, D, F, H) and 2nd derivatives (A, C, E, G) of all measured ArgBP variants at 25 °C.

Table SI 3: Unfolding temperatures (no. 75) and pressures (no. 36) of proteins with conditions as far as given in the literature by using different kinds of methods. Hyper-/thermophilic proteins are marked in red, whereas mesophilic proteins are marked in black.

Protein	T_u / °C	Ref.	Protein	$p_{u/d}$ / bar	Ref.
1. Tubulin (*porcine brain*) pH = 6.8	53 (D) 62	[13]	1. Tubulin (*porcine brain*) pH = 6.8	1300 (D) (25 °C)	[13]
2. α-Chymotrypsin (*bovine pancreas*) pH = 7.0	41	[14]	2. α-Chymotrypsin (*bovine pancreas*) pH = 7.0	4900 (21 °C)	[14]
3. Cytochrome C (*horse*) pH = 7.0	60	[15]	3. Cytochrome C (*horse*) pH = 7.0	9000 (20 °C)	[15]
4. G-Aktin (*rabbit skeletal muscle*) pH = 7.8	56	[16]	4. G-Aktin (*rabbit skeletal muscle*) pH = 7.8	2000 (20 °C)	[16]
5. Ubiquitin pD = 7.0	82	[17]	5. Ubiquitin pD = 7.0	5200 (20 °C)	[17]
6. Rs GFP (*Aequorea victoria*) pD = 5.5	78	[18]	6. Rs GFP (*Aequorea victoria*) pD = 5.5	8600 (25 °C)	[18]
7. Chymotrypsinogen pH = 2.0	42	[19]	7. Chymotrypsinogen pH = 2.0	3500 (20 °C)	[19]
8. Ribonuclease A (*bovine pancreas*) pD = 7.0	65	[20]	8. Ribonuclease A (*bovine pancreas*) pD = 7.0	7500 (30 °C)	[20]
9. Lipoxygenase (*soaked soybeans*) pH = 7.8	68	[21]	9. Lipoxygenase (*soaked soybeans*) pH = 7.8	6000	[21]
10. β-Lactoglobulin A (*bovine*) pH=7.0	60	[22]	10. β-Lactoglobulin A (*bovine*) pH = 7.0	1500 (D) (20° C)	[22]
11. Metmyoglobin (*Sperm whale*) pH = 9.0	78	[23]	11. Metmyoglobin (*Sperm whale*) pH = 9.0	6000 (20° C)	[23]
12. Staphylococcal nuclease (*Staphylococcus aureus*) pD = 5.5	48	[24]	12. Staphylococcal nuclease (*Staphylococcus aureus*) pD = 5.5	2100 (25 °C)	[24]
13. Lysozyme pD = 7.4	77	[25]	13. Lysozyme pD = 7.4	6000 (25° C)	[25]
14. Titin I27 pD = 7.0	65	[26]	14. Titin I27 pD = 7.0	11000 (30°C)	[26]
15. Fluorescein binding Lipocalin FluA	47	[27]	15. Fluorescein binding Lipocalin FluA	1700 (25 °C)	[27]
16. Equine serum albumin pD = 4.4	57	[28]	16. Equine serum albumin pD = 4.4	4000 (25 °C)	[28]
17. Arc repressor pH = 7.5	68	[29]	17. Arc repressor pH = 7.5	1000 (D)	[30]
18. Ovalbumin pH = 7.0	77	[31]	18. Lactatdehydrogenase (*Bacillus stearothermophilus*) pH = 7.6	1500 (T) (20 °C)	[32]
19. Bovine serum albumin pH = 7.0	65	[31]	19. Yeast Hexokinase pH = 7.5	800 (D) (30 °C)	[33]
20. Maltodextrin Glucosidase pH = 7.4	52	[34]	20. Yeast Glyceraldehyde-3-phosphate dehydrogenase pH = 7.5	990 (T)	[35]
21. Baker´s Yeast enolase pH = 7.2	58	[36]	21. Yeast enolase pH = 7.1	< 2400 (D)	[37]
22. Yeast 3-Phosphoglycerate kinase pH = 7.0	54	[38]	22. Phosphofructokinase (*Escherichia coli*) pH = 8.2	< 2000 (D) (25 °C)	[39]
23. GroEL (*Escherichia coli*) pH = 7.6	70	[40]	23. GroEL (*Escherichia coli*) pH = 7.6	~ 2000 (O) (20 °C)	[41]
24. GroES (*Escherichia coli*) pH = 7.6	76	[40]	24. GroES (*Escherichia coli*) pH = 7.6	2500 (O) (20 °C)	[41]
25. Papain (*latex of the papaya fruit*) pH = 5.6	83	[42]	25. Alkaline phosphatase (*Escherichia coli*)	8200	[8]
26. Immunoglobulin (monoclonal mouse anti-rat antibody of isotype 2b) pH = 8.1	71 (Fc)-domain	[43]	26. Pyruvatkinase (*Rabbit muscle*) pH = 7.5	< 3500 (T) (25 °C)	[44]

27. Carbonic anhydrase, isozyme I, human erythrocytes pH = 6.1	59	[45]	27. Lactose repressor protein (*Escherichia coli*) pH = 7.5	~ 1400 (T)	[46]
28. Bovine pancreatic trypsin inhibitor pH = 7.0	100	[47]	28. Bovine pancreatic trypsin inhibitor pD = 7.0	5500 (25°C)	[48]
29. Adenylate kinase (*Escherichia coli*) pH = 7.0	52	[49]	29. Insulin pH = 2.0	No unfolding event < 13 kbar	[50]
30. DNA Polymerase (*Thermus aquaticus*) pH = 9.5	97.6	[51]	30. N-Ras pD = 7.4	No unfolding event < 10 kbar (25 °C)	[52]
31. α-Amylase (*Bacillus sp.*) pH = 5.9	61	[53]	31. Cold shock protein A (*Escherichia coli*)	1500	[54]
32. Horse radish peroxidase pH = 7.0	74	[55]	32. Hemoglobin (*Glossoscolex paulistus*) pH = 7.5	1700 (D) (20 °C)	[56]
33. F-Actin pD = 7.8	63.1	[7]	33. F-Actin pD = 7.8	3500 (O) (20 °C)	[7]
34. Microtubuli (*calf brain*) pD = 6.8	76.3	[7]	34. HIV-1 protease pH = 8.0	2600	[57]
35. Hsp90 (*porcine brain*) pH = 7.0	62.8	[58]	35. Urate oxidase (*Aspergillus flavus*) pH = 8.0	1750 (T) (25 °C)	[59]
36. Lipase (*Pseudomonas cepacia*) pH = 7.0	75.2	[60]	36. Leucine.rich repeat domain of Anp32 tumor suppressor protein	1300 (20 °C)	[61]
37. Rubisco (*Lucerne*) pH = 7.5	67.2	[62]	**Protein**	**T_u / °C**	**Ref.**
38. Maltose binding protein (*Escherichia coli*) pH = 7.4	63	[63]	56. Small cold shock protein (*Thermotoga maritima*) pH = 6.5	88	[64]
39. Oxy-Hemoglobin (*Glossoscolex paulistus*) pH = 7.0	60.2	[65]	57. DNA ligase (*Thermus scoloductus*) pH = 7.0	95.3	[66]
40. Trisephosphate isomerase (*Saccharomyces cerevisiae*) pH = 8.5	59.1	[67]	58. Triosephosphate Isomerase (*Thermoplasma acidophilum*) pH = 7.5	74	[68]
41. Formate-Dehydrogenase (*Candida boidinii*) pH = 7.5	57	[69]	59. Triosephosphate Isomerase (*Thermotoga maritima*) pH = 7.5	102	[68]
42. Luciferase (*Firefly*) pH=7.8	42	[70]	60. Cytochrome c (*Hydrogenophilus thermoluteolus*) pH = 7.5	88.1	[71]
43. Leucoagglutinin (*Phaseolus vulgaris*) pH = 3.0	86	[72]	61. Farnesyl di-phosphate /geranylgeranyl diphosphate synthase (*Thermococcus kodakaraensis*) pH = 8.0	91	[73]
44. Dihydrofolate reductase (*Escherichia coli*) pH = 7.0	46	[74]	62. Pyrrolidone carboxyl peptidase (*Pyrococcus furiosus*) pH = 9.5	115.5	[75]
45. Leucine-isoleucine-valine binding protein pH = 7.0	67	[76]	63. Glutamate Dehydrogenase (*Pyrococcus furiosus*) pH = 8.0	113	[77]
46. Rhodopsin pH = 6.9	63.8	[78]	64. Methylguanine-DNA-methyltransferase (*Thermococcus kodakaraensis*) pH = 8.0	98.6	[79]
47. Bacteriorhodopsin pH = 7.4	67.5	[80]	65. Methylguanine-DNA-methyltransferase (*Escherichia coli*) pH = 8.0	43.8	[79]
48. α-Crystallin (*bovine lens*) pH = 7.2	61	[81]	66. Alcohol dehydrogenase (*Thermoanaerobacter brockii*) pH = 7.5	87	[82]
49. DNA ligase (*Escherichia coli*) pH = 7.0	53.9	[66]	67. Alcohol dehydrogenase (*yeast*) pH = 7.5	60	[82]

50. Tetracycline repressor pH = 8.0	59	[83]	68. Plastocyanin (*Phormidium laminosum*) pH = 5.5	Ox: 83,1 Red: 76,4	[84]
51. 3-isopropylmalate dehydrogenase (*Escherichia coli*) pH = 7.0	63	[85]	70. L-arabinose isomerases (*Bacillus halodurans*) pH = 8.0	71	[86]
52. 3-isopropylmalate dehydrogenase (*Thermus thermophilus*) pH = 7.0	83	[85]	71. L-arabinose isomerases (*Thermotoga maritima*) pH = 7.0	91	[86]
53. Aspartate aminotransferase (*Sulfolobus solfataricus)* pH = 7.5	85	[87]	72. Xylanase (*Clostridium thermocellum*) pH = 7.5	81,4	[88]
54. RNase H (*Thermus thermophilus*) pH = 5.5	82	[89]	73. Ferredoxin (*Thermus thermophiles*) pH = 7.2	114	[90]
55. Phosphoglycerate kinase (*Thermus thermophilus*) pH = 7.5	91	[91]	74. Streptavidin pH = 7.4	75.5	[92]
55. α-Amylase (thermophilic org.)	86	[93]	75. Avidin pH = 7.4	83	[92]

Table SI 4: Unfolding temperatures and pressures used to generate Figure 4B inset.

T_u / °C	$p_{u/d}$ / bar	Name
41	4900	α-Chymotrypsin (*bovine pancreas*) pH = 7.0
42	3500	Chymotrypsinogen pH = 2.0
47	1700	Fluorescein binding Lipocalin FluA
48	2100	Staphylococcal nuclease (*Staphylococcus aureus*) pD = 5.5
53	1300	Tubulin (*porcine brain*) pH = 6.8
56	2000	G-Aktin (*rabbit skeletal muscle*) pH = 7.8
57	4000	Equine serum albumin pD = 4.4
60	1500	β-Lactoglobulin A (*bovine*) pH=7.0
60	9000	Cytochrome C (*horse*) pH = 7.0
63,1	3500	F-Actin pD = 7.8
65	7500	Ribonuclease A (*bovine pancreas*) pD = 7.0
65	11000	Titin I27 pD = 7.0
68	1000	Arc repressor pH = 7.5
68	6000	Lipoxygenase (*soaked soybeans*) pH = 7.8
76	2500	GroES (*Escherichia coli*) pH = 7.6
77	6000	Lysozyme pD = 7.4
78	6000	Metmyoglobin (*Sperm whale*) pH = 9.0
78	8600	Rs GFP (*Aequorea victoria*) pD = 5.5
82	5200	Ubiquitin pD = 7.0
100	5500	Bovine pancreatic trypsin inhibitor pH = 7.0

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