Supplementary Material

to

On the Use of Time-Averaging Restraints when Deriving Biomolecular Structure from ^3J-coupling Values Obtained from NMR Experiments

by

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Table S1: Average values and root-mean-square fluctuations of $^3J_{\mathrm{H_N H_\alpha}}$-couplings (in Hz) and the corresponding φ-angle values (in degree) from 2 ns MD simulations without ^3J-coupling restraining ($K^{J_\mathrm{r}} = 0$) or with instantaneous restraining (IR), for different values of the restraining force constant $K^{J_\mathrm{r}} = K^{Q,\mathrm{restr}}$ (in $\mathrm{kJ\,mol^{-1}Hz^{-2}}$) and the flat-bottom range $\Delta^3J^{\mathrm{fb}} = \Delta Q^{\mathrm{fb}}$ (in Hz) of the restraining function for 7 residues of HEWL. Initial values are from the X-ray structure with Protein Data Bank code 1AKI (Artymiuk et al, 1982) after applying the equilibration protocol described in section 2 of the main text.

		Thr 43		Ala 9		Asn 19		Thr 69		Lys 33		Met 105		Ile 124	
		$\langle^3J\rangle$	$\langle\varphi\rangle$	$\langle^3J\rangle$	$\langle\varphi\rangle$	$\langle^3J\rangle$	$\langle\varphi\rangle$	$\langle^3J\rangle$	$\langle\varphi\rangle$	$\langle^3J\rangle$	$\langle\varphi\rangle$	$\langle^3J\rangle$	$\langle\varphi\rangle$	$\langle^3J\rangle$	$\langle\varphi\rangle$
Exp. $^3J^0$		9.3	-	3.7	-	7.0	-	9.3	-	3.6	-	7.4	-	10.6	-
Initial value		9.7	-126	4.0	-58	6.7	70	8.5	-95	4.7	-64	5.2	-68	7.2	-83
K^{J_r}	Δ^3J^{fb}														
0	-	8.9 (1.0)	-129 (19)	4.1 (0.8)	-59 (7)	6.7 (0.3)	60 (11)	5.4 (1.6)	-70 (15)	5.7 (1.3)	-73 (11)	4.6 (1.3)	-59 (11)	6.9 (1.7)	-78 (18)
1	0.0	9.1 (0.6)	-127 (15)	4.0 (0.7)	-58 (7)	6.7 (0.2)	62 (9)	8.0 (1.4)	-96 (18)	5.0 (0.9)	-66 (7)	4.9 (0.8)	-66 (7)	8.4 (0.8)	-96 (10)
	0.5	9.0 (0.7)	-130 (15)	4.0 (0.8)	-58 (7)	6.7 (0.2)	62 (10)	7.4 (1.5)	-89 (18)	5.1 (0.9)	-67 (7)	4.9 (0.9)	-65 (7)	8.1 (1.5)	-95 (14)
	1.0	8.8 (0.9)	-132 (17)	4.1 (0.8)	-59 (7)	6.7 (0.3)	61 (10)	7.2 (1.5)	-86 (16)	5.2 (0.9)	-68 (7)	5.0 (0.9)	-66 (7)	8.0 (1.0)	-92 (10)
10	0.0	9.3 (0.3)	-126 (12)	3.8 (0.4)	-56 (4)	6.8 (0.1)	61 (8)	9.3 (0.4)	-112 (12)	3.9 (0.5)	-57 (4)	7.0 (0.4)	-82 (4)	9.4 (0.3)	-111 (6)
	0.5	9.2 (0.5)	-126 (14)	3.8 (0.6)	-57 (5)	6.7 (0.2)	61 (10)	9.1 (0.5)	-110 (14)	4.2 (0.6)	-59 (5)	6.4 (0.5)	-78 (4)	9.4 (0.3)	-110 (6)
	1.0	8.9 (0.6)	-133 (14)	3.9 (0.7)	-57 (6)	6.7 (0.2)	62 (10)	8.9 (0.7)	-104 (12)	4.4 (0.7)	-62 (6)	6.0 (0.5)	-75 (4)	9.2 (0.4)	-105 (7)
100	0.0	9.4 (0.2)	-126 (11)	3.7 (0.1)	-56 (1)	6.9 (0.1)	60 (5)	9.3 (0.2)	-111 (10)	3.6 (0.1)	-55 (1)	7.4 (0.1)	-85 (1)	9.7 (0.1)	-119 (3)
	0.5	9.3 (0.3)	-127 (11)	3.8 (0.4)	-56 (4)	6.8 (0.1)	62 (8)	9.3 (0.4)	-113 (12)	3.8 (0.4)	-57 (3)	7.1 (0.4)	-83 (3)	9.7 (0.1)	-118 (4)
	1.0	9.3 (0.4)	-123 (13)	3.8 (0.6)	-57 (5)	6.7 (0.2)	62 (10)	9.1 (0.5)	-108 (13)	4.2 (0.5)	-60 (4)	6.6 (0.4)	-79 (3)	9.5 (0.2)	-113 (6)

Table S2: Average values and root-mean-square fluctuations of $^3J_{\mathrm{H_N H_\alpha}}$-couplings (in Hz) and the corresponding φ-angle values (in degree) from 2 ns MD simulations without ^3J-coupling restraining ($K^{J_\mathrm{r}} = 0$) or with biquadratic time-averaging restraining (BQ-TAR), for different values of the restraining relaxation time τ_Q (in ps), the force constant $K^{J_\mathrm{r}} = K^{Q,\mathrm{restr}}$ (in kJ mol^{-1}Hz^{-4}) and the flat-bottom range $\Delta^3J^{\mathrm{fb}} = \Delta Q^{\mathrm{fb}}$ (in Hz) of the restraining function for 7 residues of HEWL. Initial values are from the X-ray structure with Protein Data Bank code 1AKI (Artymiuk et al, 1982) after applying the equilibration protocol described in section 2 of the main text.

			Thr 43		Ala 9		Asn 19		Thr 69		Lys 33		Met 105		Ile 124	
			$\langle^3J\rangle$	$\langle\varphi\rangle$	$\langle^3J\rangle$	$\langle\varphi\rangle$	$\langle^3J\rangle$	$\langle\varphi\rangle$	$\langle^3J\rangle$	$\langle\varphi\rangle$	$\langle^3J\rangle$	$\langle\varphi\rangle$	$\langle^3J\rangle$	$\langle\varphi\rangle$	$\langle^3J\rangle$	$\langle\varphi\rangle$
Exp. $^3J^0$			9.3	-	3.7	-	7.0	-	9.3	-	3.6	-	7.4	-	10.6	-
Initial value			9.7	-126	4.0	-58	6.7	70	8.5	-95	4.7	-64	5.2	-68	7.2	-83
τ_Q	K^{J_r}	Δ^3J^{fb}														
-	0	-	8.9 (1.0)	-129 (19)	4.1 (0.8)	-59 (7)	6.7 (0.3)	60 (11)	5.4 (1.6)	-70 (15)	5.7 (1.3)	-73 (11)	4.6 (1.3)	-59 (11)	6.9 (1.7)	-78 (18)
5	5	0.5	9.0 (0.9)	-130 (16)	4.1 (0.8)	-59 (7)	6.7 (0.3)	58 (11)	8.2 (1.2)	-98 (17)	4.7 (0.8)	-64 (6)	6.0 (0.7)	-74 (5)	9.2 (0.5)	-108 (9)
		1.0	9.1 (0.8)	-130 (15)	4.2 (0.8)	-59 (7)	6.7 (0.3)	60 (11)	7.7 (1.3)	-92 (16)	5.1 (0.9)	-67 (7)	5.5 (0.7)	-70 (6)	8.9 (0.6)	-105 (13)
	10	0.5	9.1 (0.7)	-128 (16)	4.0 (0.8)	-58 (7)	6.5 (0.5)	53 (13)	8.5 (1.1)	-99 (15)	4.5 (0.7)	-63 (6)	6.1 (0.6)	-75 (5)	9.3 (0.4)	-108 (7)
		1.0	8.8 (0.9)	-133 (17)	4.1 (0.8)	-59 (7)	6.7 (0.3)	60 (11)	7.9 (1.4)	-94 (18)	5.1 (0.8)	-67 (7)	5.7 (0.7)	-72 (5)	8.9 (0.5)	-101 (7)
	50	0.5	9.0 (0.8)	-130 (17)	4.0 (0.8)	-58 (7)	6.6 (0.4)	54 (12)	8.7 (0.9)	-103 (15)	4.4 (0.7)	-61 (6)	6.5 (0.5)	-78 (4)	9.5 (0.2)	-112 (6)
		1.0	8.9 (0.9)	-132 (16)	4.1 (0.9)	-59 (8)	6.9 (0.7)	30 (69)	8.1 (1.2)	-96 (17)	4.8 (0.8)	-64 (7)	6.0 (0.6)	-74 (4)	9.2 (0.4)	-107 (9)
	100	0.5	9.0 (0.8)	-130 (16)	4.1 (0.9)	-59 (8)	6.6 (0.4)	54 (11)	8.7 (0.9)	-103 (15)	4.3 (0.6)	-60 (5)	6.6 (0.6)	-79 (5)	9.6 (0.2)	-115 (6)
		1.0	8.8 (0.9)	-135 (15)	4.1 (0.8)	-59 (8)	6.6 (0.4)	56 (12)	8.2 (1.1)	-96 (15)	4.8 (0.7)	-65 (6)	6.1 (0.6)	-75 (5)	9.2 (0.3)	-107 (7)
50	5	0.5	9.0 (0.9)	-124 (19)	4.1 (0.8)	-59 (7)	6.7 (0.3)	62 (10)	8.3 (1.1)	-98 (16)	4.8 (0.8)	-65 (7)	6.0 (0.8)	-74 (6)	9.1 (0.5)	-106 (8)
		1.0	9.0 (0.9)	-129 (17)	4.1 (0.8)	-59 (7)	6.7 (0.3)	61 (10)	7.7 (1.2)	-90 (14)	5.1 (1.0)	-67 (8)	5.5 (0.7)	-71 (6)	8.8 (0.7)	-100 (9)
	10	0.5	9.0 (0.8)	-130 (16)	4.2 (0.8)	-59 (7)	6.7 (0.3)	59 (10)	8.3 (1.1)	-98 (16)	4.7 (0.7)	-64 (6)	6.1 (0.7)	-75 (5)	9.2 (0.5)	-106 (7)
		1.0	8.9 (0.9)	-129 (17)	4.1 (0.8)	-58 (7)	6.7 (0.3)	61 (10)	7.8 (1.2)	-93 (15)	5.1 (0.9)	-67 (7)	5.7 (0.7)	-72 (6)	8.9 (0.6)	-102 (9)
	50	0.5	9.0 (0.8)	-127 (17)	4.1 (0.8)	-59 (7)	6.7 (0.3)	62 (10)	8.6 (0.9)	-102 (15)	4.4 (0.7)	-61 (6)	6.4 (0.5)	-77 (4)	9.5 (0.3)	-113 (7)
		1.0	9.0 (0.8)	-131 (15)	4.1 (0.8)	-59 (7)	6.7 (0.2)	62 (10)	8.1 (1.0)	-95 (15)	4.8 (0.9)	-64 (7)	5.9 (0.6)	-74 (5)	9.1 (0.5)	-105 (7)
	100	0.5	9.0 (0.9)	-126 (18)	4.1 (0.8)	-59 (7)	6.7 (0.3)	55 (11)	8.7 (0.9)	-103 (16)	4.3 (0.7)	-61 (6)	6.6 (0.6)	-79 (5)	9.6 (0.3)	-114 (6)
		1.0	8.8 (1.0)	-135 (16)	4.1 (0.8)	-59 (7)	6.7 (0.3)	60 (11)	8.2 (1.2)	-96 (15)	4.8 (0.8)	-65 (6)	6.1 (0.6)	-75 (5)	9.3 (0.5)	-112 (11)

Table S3: Average values and root-mean-square fluctuations of $^3J_{\mathrm{H_N H_\alpha}}$-couplings (in Hz) and the corresponding φ-angle values (in degree) from 2 ns MD simulations without ^3J-coupling restraining ($K^{J_\mathrm{r}} = 0$) or with biquadratic time-averaging local-elevation restraining (BQ-TA-LER) for different values of the restraining relaxation time τ_Q (in ps), the force constant $K^{J_\mathrm{r}} = K^{Q,\mathrm{restr}}$ (in 10^{-4}kJ mol^{-1}Hz^{-4}) and the flat bottom range $\Delta^3 J^{\mathrm{fb}} = \Delta Q^{\mathrm{fb}}$ (in Hz) of the restraining function for 7 residues of HEWL. $N_{\mathrm{le}} = 36$ and $\Delta\varphi^0 = 10°$. Initial values are from the X-ray structure with Protein Data Bank code 1AKI (Artymiuk et al, 1982) after applying the equilibration protocol described in section 2 of the main text.

			Thr 43		Ala 9		Asn 19		Thr 69		Lys 33		Met 105		Ile 124	
			$\langle^3J\rangle$	$\langle\varphi\rangle$	$\langle^3J\rangle$	$\langle\varphi\rangle$	$\langle^3J\rangle$	$\langle\varphi\rangle$	$\langle^3J\rangle$	$\langle\varphi\rangle$	$\langle^3J\rangle$	$\langle\varphi\rangle$	$\langle^3J\rangle$	$\langle\varphi\rangle$	$\langle^3J\rangle$	$\langle\varphi\rangle$
Exp. $^3J^0$			9.3	-	3.7	-	7.0	-	9.3	-	3.6	-	7.4	-	10.6	-
Initial value			9.7	-126	4.0	-58	6.7	70	8.5	-95	4.7	-64	5.2	-68	7.2	-83
τ_Q	K^{J_r}	Δ^3J^{fb}														
-	0	-	8.9 (1.0)	-129 (19)	4.1 (0.8)	-59 (7)	6.7 (0.3)	60 (11)	5.4 (1.6)	-70 (15)	5.7 (1.3)	-73 (11)	4.6 (1.3)	-59 (11)	6.9 (1.7)	-78 (18)
5	5	0.5	9.2 (0.7)	-123 (16)	4.1 (0.8)	-59 (7)	6.7 (0.2)	61 (10)	9.2 (0.7)	-115 (15)	3.9 (0.8)	-57 (7)	6.8 (0.5)	35 (56)	9.4 (0.5)	-119 (14)
		1	9.2 (0.7)	-128 (15)	4.1 (0.8)	-59 (7)	6.7 (0.3)	63 (11)	9.1 (0.7)	-110 (15)	4.3 (0.9)	-60 (7)	6.8 (1.0)	-81 (10)	9.4 (0.5)	-112 (8)
	50	0.5	9.2 (0.7)	-122 (16)	4.0 (0.8)	-58 (7)	6.7 (0.3)	62 (11)	9.3 (0.5)	-117 (14)	3.6 (0.6)	-54 (8)	6.8 (0.3)	58 (19)	9.4 (0.8)	-119 (17)
		1	9.0 (0.8)	-129 (16)	4.2 (0.8)	-59 (7)	6.7 (0.2)	61 (10)	9.1 (0.7)	-109 (13)	4.1 (0.8)	-59 (7)	6.7 (0.4)	43 (50)	9.5 (0.3)	-119 (9)
50	5	0.5	8.9 (0.9)	-131 (17)	4.1 (0.8)	-59 (7)	6.7 (0.4)	60 (11)	9.0 (1.1)	-111 (20)	3.8 (0.9)	-56 (8)	7.1 (1.2)	-83 (11)	9.4 (0.7)	-119 (14)
		1	9.1 (0.8)	-125 (16)	4.2 (0.8)	-59 (7)	6.7 (0.2)	62 (10)	8.7 (1.3)	-105 (18)	4.6 (1.1)	-63 (9)	6.5 (1.2)	-78 (11)	9.3 (0.7)	-111 (10)
	50	0.5	9.2 (0.7)	-126 (14)	4.1 (0.8)	-58 (7)	6.7 (0.3)	61 (11)	9.1 (0.9)	-112 (16)	3.6 (0.8)	-54 (7)	7.4 (1.1)	-86 (12)	9.2 (1.1)	-113 (33)
		1	9.0 (0.9)	-131 (15)	4.0 (0.8)	-58 (7)	6.7 (0.3)	60 (10)	8.9 (1.1)	-106 (15)	4.1 (0.8)	-59 (7)	6.7 (0.9)	-80 (9)	9.5 (0.6)	-114 (10)

Table S4: Average values and root-mean-square fluctuations of ${}^3J_{\mathrm{H}_\alpha\mathrm{H}_\beta}$-couplings (in Hz) and the corresponding χ_1-angle values (in degree) from 2 ns MD simulations without ${}^3J$-coupling restraining ($K^{J_\mathrm{r}} = 0$) or with instantaneous restraining (IR), for different values of the restraining force constant $K^{J_\mathrm{r}} = K^{Q,\mathrm{restr}}$ (in $\mathrm{kJ\,mol^{-1}Hz^{-2}}$) and the flat-bottom range $\Delta^3J^{\mathrm{fb}} = \Delta Q^{\mathrm{fb}}$ (in Hz) of the restraining function for 5 residues with a single H_β atom of HEWL. Initial values are from the X-ray structure with Protein Data Bank code 1AKI (Artymiuk et al, 1982) after applying the equilibration protocol described in section 2 of the main text.

		Val 2		Thr 47		Thr 89		Val 99		Val 109	
		$\langle{}^3J\rangle$	$\langle\chi_1\rangle$	$\langle{}^3J\rangle$	$\langle\chi_1\rangle$	$\langle{}^3J\rangle$	$\langle\chi_1\rangle$	$\langle{}^3J\rangle$	$\langle\chi_1\rangle$	$\langle{}^3J\rangle$	$\langle\chi_1\rangle$
Exp. ${}^3J^0$		10.8	-	2.6	-	9.5	-	6.3	-	8.0	-
Initial value		12.9	-177	1.7	37	3.3	59	2.1	97	2.4	-70
K^{J_r}	Δ^3J^{fb}										
0	-	11.0 (3.6)	-171 (44)	3.1 (1.8)	49 (20)	3.8 (1.3)	59 (16)	2.3 (0.6)	75 (10)	3.2 (2.4)	-63 (40)
1	0.0	12.4 (0.6)	-175 (12)	2.6 (0.7)	50 (10)	5.6 (0.8)	76 (6)	2.9 (0.8)	67 (11)	4.5 (1.0)	-52 (8)
	0.5	12.5 (0.6)	-176 (11)	2.7 (0.8)	52 (9)	5.3 (0.8)	74 (6)	3.9 (1.5)	-35 (93)	8.0 (3.8)	-53 (60)
	1.0	12.4 (0.6)	-175 (12)	2.7 (0.8)	50 (11)	5.4 (0.9)	75 (6)	2.7 (0.9)	70 (12)	6.0 (3.6)	-37 (70)
10	0.0	11.2 (0.5)	-162 (17)	2.5 (0.4)	50 (7)	8.5 (0.6)	100 (8)	6.3 (0.6)	127 (3)	7.4 (0.5)	-31 (4)
	0.5	11.6 (0.7)	-168 (17)	2.5 (0.6)	50 (9)	8.1 (0.6)	95 (6)	6.5 (0.9)	128 (5)	7.0 (0.5)	-34 (4)
	1.0	12.1 (0.7)	-172 (14)	2.6 (0.6)	50 (9)	7.7 (0.5)	91 (4)	5.9 (1.2)	125 (7)	6.5 (0.6)	-12 (36)

Table S5: Average values and root-mean-square fluctuations of $^3J_{H_\alpha H_\beta}$-couplings (in Hz) and the corresponding χ_1-angle values (in degree) from 2 ns MD simulations without ^3J-coupling restraining ($K^{J_r} = 0$) or with biquadratic time-averaging restraining (BQ-TAR), for different values of the restraining relaxation time τ_Q (in ps), the force constant $K^{J_r} = K^{Q,restr}$ (in kJ mol^{-1}Hz^{-4}) and the flat-bottom range $\Delta^3J^{fb} = \Delta Q^{fb}$ (in Hz) of the restraining function for 5 residues with a single H_β atom of HEWL. Initial values are from the X-ray structure with Protein Data Bank code 1AKI (Artymiuk et al, 1982) after applying the equilibration protocol described in section 2 of the main text.

			Val 2		Thr 47		Thr 89		Val 99		Val 109	
			$\langle^3J\rangle$	$\langle\chi_1\rangle$	$\langle^3J\rangle$	$\langle\chi_1\rangle$	$\langle^3J\rangle$	$\langle\chi_1\rangle$	$\langle^3J\rangle$	$\langle\chi_1\rangle$	$\langle^3J\rangle$	$\langle\chi_1\rangle$
Exp. $^3J^0$			10.8	-	2.6	-	9.5	-	6.3	-	8.0	-
Initial value			12.9	-177	1.7	37	3.3	59	2.1	97	2.4	-70
τ_Q	K^{J_r}	Δ^3J^{fb}										
-	0	-	11.0 (3.6)	-171 (44)	3.1 (1.8)	49 (20)	3.8 (1.3)	59 (16)	2.3 (0.6)	75 (10)	3.2 (2.4)	-63 (40)
5	5	0.5	9.3 (0.8)	-6 (38)	2.8 (0.9)	53 (11)	7.9 (0.7)	95 (10)	4.8 (0.8)	49 (6)	6.5 (0.7)	-30 (22)
		1.0	8.8 (0.9)	-11 (34)	2.8 (0.9)	52 (11)	7.3 (0.5)	88 (4)	4.3 (0.7)	53 (6)	6.0 (0.7)	-41 (5)
	10	0.5	9.5 (0.8)	-14 (45)	2.8 (0.8)	53 (10)	8.1 (0.5)	95 (5)	5.0 (0.6)	32 (36)	6.7 (0.7)	-29 (22)
		1.0	9.0 (0.7)	-12 (27)	2.8 (0.9)	52 (11)	7.6 (0.6)	93 (12)	4.5 (0.6)	51 (5)	6.2 (0.7)	-40 (5)
	50	0.5	9.7 (0.4)	-2 (29)	2.8 (1.0)	52 (12)	8.4 (0.5)	98 (6)	5.4 (0.6)	45 (5)	7.0 (0.5)	-34 (4)
		1.0	9.3 (0.7)	-4 (33)	2.8 (0.9)	51 (12)	8.0 (0.5)	94 (4)	5.0 (0.7)	-48 (13)	6.5 (0.5)	-37 (4)
	100	0.5	9.8 (0.5)	-7 (43)	2.8 (0.9)	52 (10)	8.5 (0.4)	99 (4)	5.4 (0.6)	45 (5)	7.1 (0.5)	-26 (21)
		1.0	9.7 (1.0)	-9 (63)	2.8 (1.0)	51 (12)	8.0 (0.5)	94 (4)	5.0 (0.7)	-47 (14)	6.6 (0.6)	-8 (36)
50	5	0.5	9.8 (1.6)	-26 (84)	2.8 (0.9)	52 (10)	7.8 (0.7)	92 (6)	4.8 (0.7)	50 (6)	6.5 (0.8)	-10 (37)
		1.0	9.2 (1.6)	-19 (59)	2.8 (1.0)	52 (11)	7.4 (0.7)	89 (5)	4.3 (0.7)	54 (6)	6.0 (0.8)	-41 (5)
	10	0.5	9.4 (0.8)	-4 (27)	2.7 (0.8)	51 (11)	8.0 (0.6)	94 (6)	5.0 (0.6)	48 (5)	6.7 (0.7)	-15 (33)
		1.0	9.3 (1.4)	-18 (54)	2.9 (1.2)	51 (14)	7.5 (0.6)	90 (4)	4.4 (0.7)	52 (5)	6.2 (0.8)	-40 (6)
	50	0.5	9.7 (0.8)	-2 (38)	2.8 (0.9)	52 (11)	8.5 (0.6)	102 (13)	5.4 (0.6)	46 (5)	7.0 (0.6)	-8 (33)
		1.0	9.5 (1.1)	-9 (49)	2.9 (1.2)	51 (14)	7.9 (0.5)	93 (5)	4.8 (0.6)	49 (5)	6.5 (1.0)	-20 (47)
	100	0.5	9.8 (0.7)	-1 (45)	2.8 (0.9)	52 (11)	8.6 (0.6)	101 (9)	5.5 (0.6)	-3 (45)	7.1 (0.6)	-16 (29)
		1.0	9.5 (1.0)	-7 (43)	2.9 (1.2)	51 (14)	8.0 (0.5)	94 (4)	5.0 (0.6)	48 (5)	6.6 (0.6)	-37 (5)

Table S6: Average values and root-mean-square fluctuations of $^3J_{\mathrm{H}_\alpha\mathrm{H}_\beta}$-couplings (in Hz) and the corresponding χ_1-angle values (in degree) from 2 ns MD simulations without ^3J-coupling restraining ($K^{J_\mathrm{r}} = 0$) or with biquadratic time-averaging local-elevation restraining (BQ-TA-LER) for different values of the restraining relaxation time τ_Q (in ps), the force constant $K^{J_\mathrm{r}} = K^{Q,\mathrm{restr}}$ (in 10^{-4}kJ mol^{-1}Hz^{-4}) and the flat bottom range $\Delta^3J^{\mathrm{fb}} = \Delta Q^{\mathrm{fb}}$ (in Hz) of the restraining function for 5 residues with a single H_β atom of HEWL. $N_{\mathrm{le}} = 36$ and $\Delta\varphi^0 = 10°$. Initial values are from the X-ray structure with Protein Data Bank code 1AKI (Artymiuk et al, 1982) after applying the equilibration protocol described in section 2 of the main text.

			Val 2		Thr 47		Thr 89		Val 99		Val 109	
			$\langle^3J\rangle$	$\langle\chi_1\rangle$	$\langle^3J\rangle$	$\langle\chi_1\rangle$	$\langle^3J\rangle$	$\langle\chi_1\rangle$	$\langle^3J\rangle$	$\langle\chi_1\rangle$	$\langle^3J\rangle$	$\langle\chi_1\rangle$
Exp. $^3J^0$			10.8	-	2.6	-	9.5	-	6.3	-	8.0	-
Initial value			12.9	-177	1.7	37	3.3	59	2.1	97	2.4	-70
τ_Q	K^{J_r}	Δ^3J^{fb}										
-	0	-	11.0 (3.6)	-171 (44)	3.1 (1.8)	49 (20)	3.8 (1.3)	59 (16)	2.3 (0.6)	75 (10)	3.2 (2.4)	-63 (40)
5	5	0.5	11.2 (1.5)	-167 (26)	2.7 (1.0)	50 (12)	9.7 (1.3)	-28 (19)	6.0 (1.0)	-43 (31)	8.0 (1.7)	-82 (48)
		1.0	11.8 (1.2)	-171 (18)	2.8 (0.8)	52 (11)	8.9 (1.3)	80 (123)	5.5 (1.2)	-48 (30)	8.1 (2.0)	-44 (182)
	50	0.5	11.1 (1.1)	-7 (171)[a]	2.5 (0.6)	49 (9)	9.5 (1.1)	-27 (13)	6.3 (0.9)	-39 (15)	8.0 (1.3)	-46 (63)
		1.0	11.5 (1.1)	-171 (20)	2.8 (0.8)	53 (9)	9.8 (1.2)	-23 (30)	5.8 (0.9)	-41 (26)	8.3 (1.8)	-9 (150)
50	5	0.5	11.3 (1.5)	-170 (25)	2.8 (1.0)	51 (12)	9.9 (1.7)	-30 (24)	5.9 (1.6)	-43 (64)	8.1 (2.7)	14 (217)
		1.0	11.8 (1.5)	-172 (22)	2.9 (1.0)	53 (11)	10.1 (1.7)	-29 (18)	6.1 (2.2)	-58 (58)	8.4 (3.0)	-30 (262)
	50	0.5	11.0 (1.7)	-178 (28)	2.9 (1.0)	53 (11)	9.5 (1.4)	-24 (20)	6.0 (1.4)	-43 (49)	7.9 (2.3)	-8 (255)
		1.0	11.7 (1.6)	-174 (23)	2.8 (1.1)	52 (12)	9.1 (1.4)	-25 (45)	5.6 (1.6)	-44 (55)	8.3 (2.6)	-41 (241)

[a] average obtained from angle values mapped to interval $-180° \leq \chi_1 < 180°$

Table S7: Average values and root-mean-square fluctuations of $^3J_{\mathrm{H}_\alpha\mathrm{H}_\beta}$-couplings (in Hz) and the corresponding χ_1-angle values (in degree) from 2 ns MD simulations without ^3J-coupling restraining ($K^{J_\mathrm{r}} = 0$) or with instantaneous restraining (IR) for different values of the restraining force constant $K^{J_\mathrm{r}} = K^{Q,\mathrm{restr}}$ (in kJ mol^{-1}Hz^{-2}) and the flat-bottom range $\Delta^3J^{\mathrm{fb}} = \Delta Q^{\mathrm{fb}}$ (in Hz) of the restraining function for 5 residues with two H_β atoms of HEWL. Upper lines H_{β_2}; lower lines H_{β_3}. Initial values are from the X-ray structure with Protein Data Bank code 1AKI (Artymiuk et al, 1982) after applying the equilibration protocol described in section 2 of the main text.

		Asp 52		Asn 46		Asp 66		Glu 7		Arg 45	
		$\langle^3J\rangle$	$\langle\chi_1\rangle$	$\langle^3J\rangle$	$\langle\chi_1\rangle$	$\langle^3J\rangle$	$\langle\chi_1\rangle$	$\langle^3J\rangle$	$\langle\chi_1\rangle$	$\langle^3J\rangle$	$\langle\chi_1\rangle$
Exp.	$^3J^0$	11.6	-	11.2	-	5.1	-	6.7	-	6.9	-
		3.6	-	4.7	-	4.5	-	6.4	-	6.7	-
Initial value		11.0	-86	1.8	-152	1.8	-151	1.7	-155	12.7	-67
		1.7		10.7		10.5		11.2		2.3	
K^{J_r}	Δ^3J^{fb}										
0	-	12.2 (1.2)	-66 (15)	2.2 (0.7)	-147 (10)	2.7 (0.8)	-107 (10)	8.3 (4.6)	-49 (59)	11.3 (2.8)	-74 (29)
		3.2 (1.2)		9.8 (1.6)		12.4 (0.7)		7.4 (4.7)		3.3 (2.7)	
1	0.0	12.5 (0.6)	-64 (11)	8.8 (2.8)	-93 (24)	3.1 (0.8)	-133 (8)	8.1 (3.2)	-78 (43)	5.1 (1.7)	-93 (64)
		3.2 (1.0)		3.4 (1.0)		7.5 (1.4)		5.8 (1.7)		4.9 (1.6)	
	0.5	11.8 (1.2)	-74 (13)	11.9 (2.1)	-68 (19)	3.5 (1.2)	-42 (78)	5.9 (3.3)	-41 (242)	5.6 (2.2)	-113 (24)
		2.5 (0.8)		3.4 (1.1)		5.7 (2.9)		4.8 (1.9)		5.2 (1.8)	
	1.0	12.4 (0.6)	-65 (12)	7.5 (1.7)	-106 (10)	2.6 (0.8)	-139 (9)	5.9 (3.7)	-19 (113)	5.7 (3.2)	-72 (72)
		3.0 (1.1)		3.2 (1.1)		8.4 (1.5)		4.6 (1.8)		5.3 (2.2)	
10	0.0	12.8 (0.2)	-60 (6)	12.4 (0.6)	-48 (6)	5.1 (0.4)	-119 (3)	5.0 (0.4)	-120 (3)	8.0 (0.6)	-17 (4)
		3.4 (0.7)		5.0 (0.6)		4.9 (0.5)		5.0 (0.5)		9.0 (0.4)	
	0.5	12.6 (0.3)	-64 (81)	12.4 (0.6)	-49 (7)	4.9 (0.6)	-120 (3)	5.0 (0.4)	-120 (3)	8.3 (0.5)	-18 (4)
		3.0 (0.8)		4.9 (0.7)		5.0 (0.6)		5.0 (0.5)		8.8 (0.4)	
	1.0	12.6 (0.5)	-64 (10)	12.6 (0.7)	-55 (10)	4.5 (0.7)	-123 (4)	5.0 (0.4)	-120 (3)	8.5 (0.5)	-19 (4)
		3.1 (0.9)		4.2 (0.9)		5.5 (0.7)		5.0 (0.5)		8.7 (0.4)	

Table S8: Average values and root-mean-square fluctuations of $^3J_{\mathrm{H}_\alpha\mathrm{H}_\beta}$-couplings and the corresponding χ_1-angle values from 2 ns MD simulations without ^3J-coupling restraining ($K^{J_\mathrm{r}} = 0$) or with biquadratic time-averaging restraining (BQ-TAR), for different values of the restraining relaxation time τ_Q (in ps), the force constant $K^{J_\mathrm{r}} = K^{Q,\mathrm{restr}}$ (in kJ mol^{-1}Hz^{-4}) and the flat bottom range $\Delta^3J^{\mathrm{fb}} = \Delta Q^{\mathrm{fb}}$ (in Hz) of the restraining function for 5 residues with two H_β atoms of HEWL. Upper lines H_{β_2}; lower lines H_{β_3}. Initial values are from the X-ray structure with Protein Data Bank code 1AKI (Artymiuk et al, 1982) after applying the equilibration protocol described in section 2 of the main text.

			Asp 52		Asn 46		Asp 66		Glu 7		Arg 45	
			$\langle^3J\rangle$	$\langle\chi_1\rangle$	$\langle^3J\rangle$	$\langle\chi_1\rangle$	$\langle^3J\rangle$	$\langle\chi_1\rangle$	$\langle^3J\rangle$	$\langle\chi_1\rangle$	$\langle^3J\rangle$	$\langle\chi_1\rangle$
Exp. $^3J^0$			11.6	-	11.2	-	5.1	-	6.7	-	6.9	-
			3.6	-	4.7	-	4.5	-	6.4	-	6.7	-
Initial value			11.0	-86	1.8	-152	1.8	-151	1.7	-155	12.7	-67
			1.7		10.7		10.5		11.2		2.3	
τ_Q	K^{J_r}	Δ^3J^{fb}										
-	0	-	12.2 (1.2)	-66 (15)	2.2 (0.7)	-147 (10)	2.7 (0.8)	-107 (10)	8.3 (4.6)	-49 (59)	11.3 (2.8)	-74 (29)
			3.2 (1.2)		9.8 (1.6)		12.4 (0.7)		7.4 (4.7)		3.2 (2.7)	
5	5	0.5	12.2 (1.0)	-68 (14)	12.5 (0.8)	-59 (12)	4.4 (1.2)	-124 (8)	3.4 (0.4)	59 (12)	3.4 (0.6)	60 (13)
			3.0 (1.1)		3.8 (1.2)		5.8 (1.2)		3.5 (0.8)		3.4 (0.6)	
		1.0	12.1 (1.0)	-69 (13)	12.0 (1.6)	-68 (17)	4.0 (1.2)	-127 (8)	3.4 (0.8)	56 (26)	3.4 (0.7)	60 (8)
			2.8 (1.1)		3.1 (1.1)		6.3 (1.4)		3.5 (0.9)		3.4 (0.4)	
	10	0.5	12.2 (0.9)	-67 (13)	12.3 (0.9)	-57 (14)	4.6 (1.3)	-122 (8)	3.4 (0.6)	59 (12)	3.4 (0.9)	59 (18)
			3.0 (1.1)		4.1 (1.4)		5.5 (1.2)		3.5 (0.7)		3.5 (0.7)	
		1.0	12.2 (0.9)	-67 (13)	12.3 (1.3)	-64 (15)	4.0 (0.8)	-127 (5)	3.4 (0.4)	58 (16)	3.4 (0.6)	59 (16)
			3.0 (1.1)		3.5 (1.1)		6.2 (0.9)		3.5 (0.9)		3.5 (0.7)	
	50	0.5	12.4 (0.9)	-67 (11)	9.7 (0.5)	116 (27)	4.9 (1.3)	-121 (8)	3.6 (1.1)	56 (74)	8.6 (0.9)	137 (19)
			2.9 (0.9)		4.8 (0.6)		5.2 (1.2)		3.5 (1.0)		8.4 (1.0)	
		1.0	12.2 (1.0)	-66 (14)	12.5 (0.8)	-58 (12)	4.3 (0.9)	-125 (6)	5.0 (0.4)	-119 (3)	4.5 (2.7)	42 (640)
			3.1 (1.2)		3.8 (1.3)		5.9 (0.9)		5.0 (0.5)		4.4 (2.2)	
	100	0.5	12.4 (0.6)	-59 (13)	9.7 (0.6)	117 (21)	4.9 (1.3)	-121 (8)	5.0 (0.2)	-120 (2)	5.0 (0.8)	-110 (40)
			3.7 (1.3)		5.0 (0.7)		5.2 (1.3)		5.0 (0.3)		5.0 (0.6)	
		1.0	12.4 (0.6)	-62 (13)	9.1 (0.4)	-98 (3)	4.5 (1.4)	-124 (9)	8.5 (0.4)	-20 (7)	3.4 (0.5)	60 (14)
			3.4 (1.3)		2.2 (0.5)		5.7 (1.3)		8.8 (0.1)		3.4 (0.5)	
50	5	0.5	12.4 (0.9)	-62 (13)	10.6 (2.3)	4 (85)	4.3 (1.1)	-125 (8)	4.3 (2.5)	33 (92)	3.6 (1.5)	53 (33)
			3.4 (1.3)		4.0 (1.8)		5.8 (1.2)		4.3 (2.8)		3.5 (1.0)	
		1.0	12.4 (0.8)	-60 (12)	12.2 (1.6)	-66 (16)	3.9 (0.8)	-128 (6)	3.6 (1.4)	50 (44)	4.1 (2.4)	26 (77)
			3.6 (1.2)		3.2 (1.2)		6.3 (1.0)		3.7 (1.7)		4.1 (2.4)	
	10	0.5	12.4 (0.9)	-64 (13)	12.3 (1.1)	-58 (14)	4.3 (0.8)	-124 (5)	3.8 (1.9)	44 (57)	3.7 (1.8)	50 (41)
			3.2 (1.2)		4.0 (1.4)		5.8 (0.9)		3.7 (1.7)		3.7 (1.5)	
		1.0	12.3 (1.0)	-65 (14)	12.2 (1.5)	-64 (17)	4.0 (1.0)	-126 (7)	7.4 (2.9)	-33 (167)	3.5 (0.9)	59 (12)
			3.2 (1.2)		3.5 (1.4)		6.1 (1.1)		7.3 (3.0)		3.4 (0.3)	
	50	0.5	12.1 (1.3)	-64 (22)	12.1 (0.8)	-54 (16)	4.7 (1.1)	-122 (7)	3.4 (0.3)	59 (10)	3.5 (1.1)	55 (28)
			3.2 (1.2)		4.4 (1.7)		5.3 (1.0)		3.4 (0.6)		3.5 (1.0)	
		1.0	12.1 (1.1)	-71 (13)	12.5 (0.9)	-59 (13)	4.4 (1.7)	-124 (12)	3.9 (2.1)	36 (64)	3.5 (0.9)	59 (3)
			2.7 (1.0)		3.7 (1.3)		5.9 (1.6)		3.9 (2.1)		3.4 (0.5)	
	100	0.5	12.1 (1.3)	-67 (15)	12.0 (1.0)	-54 (17)	4.8 (1.3)	-122 (9)	3.5 (1.1)	55 (29)	3.4 (0.8)	59 (11)
			3.1 (1.2)		4.5 (1.7)		5.3 (1.3)		3.5 (1.0)		3.4 (0.4)	
		1.0	12.4 (0.6)	-64 (12)	9.0 (0.5)	-98 (4)	4.3 (1.0)	-125 (7)	3.5 (1.3)	54 (31)	3.5 (1.3)	55 (29)
			3.2 (1.3)		2.2 (0.6)		5.8 (1.1)		3.5 (1.0)		3.5 (0.8)	

Table S9: Average values and root-mean-square fluctuations of ${}^3J_{\mathrm{H}_\alpha\mathrm{H}_\beta}$-couplings (in Hz) and the corresponding χ_1-angle values (in degree) from 2 ns MD simulations without ${}^3J$-coupling restraining ($K^{J_\mathrm{r}} = 0$) or with biquadratic time-averaging local-elevation restraining (BQ-TA-LER), for different values of the restraining relaxation time τ_Q (in ps), the force constant $K^{J_\mathrm{r}} = K^{Q,\mathrm{restr}}$ (in 10^{-4}kJ mol^{-1}Hz^{-4}) and the flat bottom range $\Delta^3J^{\mathrm{fb}} = \Delta Q^{\mathrm{fb}}$ (in Hz) of the restraining function for 5 residues with two H_β atoms of HEWL. $N_\mathrm{le} = 36$ and $\Delta\varphi^0 = 10°$. Upper lines H_{β_2}; lower lines H_{β_3}. Initial values are from the X-ray structure with Protein Data Bank code 1AKI (Artymiuk et al, 1982) after applying the equilibration protocol described in section 2 of the main text.

			Asp 52		Asn 46		Asp 66		Glu 7		Arg 45	
			$\langle{}^3J\rangle$	$\langle\chi_1\rangle$	$\langle{}^3J\rangle$	$\langle\chi_1\rangle$	$\langle{}^3J\rangle$	$\langle\chi_1\rangle$	$\langle{}^3J\rangle$	$\langle\chi_1\rangle$	$\langle{}^3J\rangle$	$\langle\chi_1\rangle$
Exp. ${}^3J^0$			11.6	-	11.2	-	5.1	-	6.7	-	6.9	-
			3.6	-	4.7	-	4.5	-	6.4	-	6.7	-
Initial value			11.0	-86	1.8	-152	1.8	-151	1.7	-155	12.7	-67
			1.7		10.7		10.5		11.2		2.3	
τ_Q	K^{J_r}	Δ^3J^{fb}										
-	0	-	12.2 (1.2)	-66 (15)	2.2 (0.7)	-147 (10)	2.7 (0.8)	-107 (10)	8.3 (4.6)	-49 (59)	11.3 (2.8)	-74 (29)
			3.2 (1.2)		9.8 (1.6)		12.4 (0.7)		7.4 (4.7)		3.3 (2.7)	
5	5	0.5	12.3 (1.2)	-64 (15)	11.8 (1.4)	-38 (124)	5.2 (1.1)	-104 (45)	6.6 (2.8)	14 (381)	7.0 (2.8)	15 (531)
			3.3 (1.2)		5.0 (1.5)		4.7 (1.0)		6.5 (2.8)		6.9 (2.8)	
		1.0	12.3 (1.0)	-62 (14)	12.1 (1.3)	-42 (46)	5.5 (1.3)	-110 (32)	6.4 (2.8)	-20 (202)	7.3 (2.6)	16 (271)
			3.5 (1.3)		4.8 (1.4)		4.5 (1.1)		6.5 (2.8)		7.1 (2.6)	
	50	0.5	12.2 (1.2)	-60 (180)	11.8 (1.4)	-45 (177)	5.4 (0.9)	-118 (5)	6.5 (2.8)	5 (697)	7.1 (2.9)	18 (424)
			3.6 (1.5)		5.0 (1.6)		4.7 (0.8)		6.4 (2.9)		6.9 (2.8)	
		1.0	12.5 (0.8)	-58 (12)	12.2 (0.8)	-48 (10)	5.2 (0.8)	-119 (6)	6.4 (2.7)	-1 (658)	7.2 (2.6)	11 (432)
			3.9 (1.1)		5.1 (1.1)		4.8 (0.8)		6.5 (2.8)		7.0 (2.7)	
50	5	0.5	12.1 (1.4)	-64 (17)	12.0 (1.2)	-51 (16)	5.4 (1.4)	-116 (11)	6.6 (3.2)	16 (324)	7.1 (2.9)	20 (219)
			3.4 (1.3)		4.9 (1.6)		4.8 (1.2)		6.4 (2.9)		6.8 (2.9)	
		1.0	12.2 (1.1)	-65 (14)	12.4 (1.1)	-58 (15)	5.4 (1.5)	-110 (33)	6.3 (2.6)	-6 (339)	7.0 (2.6)	1 (105)
			3.2 (1.2)		3.9 (1.5)		4.7 (1.6)		6.4 (2.9)		7.1 (2.7)	
	50	0.5	12.0 (1.6)	-61 (179)	11.5 (1.8)	-47 (167)	5.1 (1.4)	-72 (77)	6.4 (2.9)	2 (171)	6.9 (3.0)	7 (244)
			3.5 (1.5)		4.8 (1.9)		4.8 (1.6)		6.7 (3.1)		6.7 (2.9)	
		1.0	12.2 (1.3)	-67 (15)	12.1 (1.1)	-48 (13)	5.3 (1.7)	-84 (63)	6.5 (2.8)	12 (371)	7.0 (2.9)	21 (204)
			3.1 (1.1)		5.2 (1.2)		4.7 (1.7)		6.4 (3.2)		7.0 (2.9)	

References

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