Supplementary Materials

Table S1. ^{1}H and ^{15}N chemical shifts assignment* of ψRGDechi in HBSS buffer (pH 7.4) at 298K on 600 MHz spectrometer.

Residue	H_N	N	$H\alpha$	$H\beta$	Others
Lys^1	8.59	126.2	4.13	1.85,1.76	γCH_2 1.51, 1.37
					δCH_2 1.71
					εCH_2 3.01
Arg^2	8.51	117.6	4.41	1.90, 1.85	γCH_2 1.61
					δCH_2 3.20
					εCH_2 7.53
Gly^3	8.52	109.5	4.15, 3.78		
Asp^4	8.46	119.6	4.62	2.84, 2.73	
DGlu^5	8.14	118.3	4.36	2.17, 2.13	γCH_2 2.49
Met^6	8.09	121.0	4.51	2.06, 1.86	γCH_2 2.60, 2.50
					εCH_3 2.10
Asp^7	8.37	119.6	4.65	2.80, 2.69	
Asp^8	8.25	120.7	4.92	2.81, 2.62	
Pro^9			4.40	2.23, 1.80	γCH_2 1.99, 1.81
					δCH_2 3.73
Gly^{10}	8.47	108.3	3.95, 3.88		
Arg^{11}	7.93	120.0	4.32	1.86, 1.73	γCH_2 1.63, 1.58
					δCH_2 3.19
					εNH 7.24
Asn^{12}	8.42	121.0	4.96	2.86, 2.74	δNH_2 7.70, 7.02
Pro^{13}			4.41	2.26, 1.99	γCH_2 2.03, 1.81
					δCH_2 3.79

His[14]	8.51	117.7	4.70	3.31, 3.20	δ2H 6.97 ε1H 7.26
hCyt[15]	8.09	122.5	4.37	1.86, 1.76	γCH$_2$ 1.36 δCH$_2$ 1.50 εCH$_3$ 3.02
Gly[16]	8.33	110.4	4.08,4.04		
Pro[17]			4.42	2.14, 1.80	γCH$_2$ 2.07, 1.81 δCH$_2$ 3.79
Ala[18]	8.38	119.7	4.11	1.56	
Thr[19]	8.34	120.9	4.20	4.25	γCH$_2$ 1.20

*^{1}H ,^{15}N chemical shifts error values are ± 0.01 ppm and ± 0.4 ppm, respectively.

Table S2. [13]C chemical shifts assignment* of ψRGDechi in HBSS buffer (pH 7.4) at 298K on 600 MHz spectrometer.

Residue	Cα	Cβ	Others
Lys[1]	58.4	33.7	Cγ 25.1
			Cδ 29.1
			Cε 42.1
Arg[2]	56.1	30.4	Cγ 27.2
			Cδ 43.3
Gly[3]	46.5		
Asp[4]	53.8	39.0	
DGlu[5]	56.9	28.3	Cγ 33.5
Met[6]	55.6	32.7	Cγ 32.2
			εCH₃ 17.1
Asp[7]	53.8	40.0	
Asp[8]	52.1	40.5	
Pro[9]	63.6	32.2	Cγ 27.1
			Cδ 50.7
Gly[10]	45.4		
Arg[11]	56.0	30.9	Cγ 27.2
			Cδ 43.4
Asn[12]	51.4	39.2	
Pro[13]	63.6	32.1	Cγ 27.3
			Cδ 49.7
His[14]	55.3	28.6	
hCyt[15]	56.6	33.7	Cγ 22.6

				Cδ 31.3
				εCH₂ 42.2
Gly¹⁶	44.7			
Pro¹⁷	63.9	31.2		Cγ 27.1
				Cδ 50.8
Ala¹⁸	51.3	18.1		
Thr¹⁹	63.6	70.4		Cγ 22.2

$*^{13}$C chemical shifts error values is $\pm$ 0.3 ppm

Table S3. ^{1}H and ^{15}N chemical shifts assignment* of RGDechi1-14 in HBSS buffer (pH 7.4) at 298K on 600 MHz spectrometer.

Residue	H$_N$	N	Hα	Hβ	Others
Lys¹	8.99	125.6	4.16	1.80	γCH₂ 1.51, 1.44
					δCH₂ 1.69
					εCH₂ 3.00
Arg²	8.40	119.4	4.37	1.87, 1.68	γCH₂ 1.55
					δCH₂ 3.21
					εCH₂ 7.22
Gly³	8.31	112.0	4.15, 3.56		
Asp⁴	8.21	124.2	4.78	2.90, 2.65	
DGlu⁵	8.06	121.9	4.46	2.07, 1.90	γCH₂ 2.38
Met⁶	8.22	125.3	4.32	2.06, 1.99	γCH₂ 2.61, 2.56
					εCH₃ 2.10
Asp⁷	8.57	119.7	4.63	2.83	

Residue					
Asp[8]	8.20	120.3	4.95	2.86, 2.62	
Pro[9]			4.43	2.30, 2.00	γCH_2 2.03 δCH_2 3.78
Gly[10]	8.44	108.2	3.92, 3.85		
Arg[11]	7.92	119.9	4.31	1.86, 1.76	γCH_2 1.59 δCH_2 3.20 ϵNH 7.24
Asn[12]	8.42	120.7	4.96	2.83, 2.67	δNH_2 7.62, 6.94
Pro[13]			4.40	2.23, 1.91	γCH_2 1.96, 1.84 δCH_2 3.75, 3.70
His[14]	8.11	122.4	4.50	3.26, 3.13	$\delta 2H$ 7.30 $\epsilon 1H$ 8.59

*[1]H , ^{15}N chemical shifts error values are ± 0.01 ppm and ± 0.4 ppm, respectively.

Table S4. [13]C chemical shifts assignment* of RGDechi1-14 in HBSS buffer (pH 7.4) at 298K on 600 MHz spectrometer.

Residue	Cα	Cβ	Others
Lys[1]	58.4	32.9	Cγ 25.2 Cδ 29.0 Cε 42.2
Arg[2]	55.4	30.0	Cγ 27.3 Cδ 43.4
Gly[3]	46.3		
Asp[4]	52.4	38.2	
DGlu[5]	54.6	29.7	Cγ 33.6

Met[6]	56.3	32.7	Cδ 32.0 εCH₃ 16.9
Asp[7]	53.5	39.2	
Asp[8]	51.6	39.9	
Pro[9]	64.0	32.1	Cγ 27.2 Cδ 50.7
Gly[10]	45.4		
Arg[11]	56.0	30.9	Cγ 27.1 Cδ 43.3
Asn[12]	51.5	38.9	
Pro[13]	63.6	32.1	Cγ 27.2 Cδ 50.7
His[14]	56.6	29.7	

*^{13}C chemical shifts error values is ± 0.3 ppm

Table S5. $^{13}C\beta$ and $^{13}C\gamma$ chemical shifts, their difference $\Delta\beta\gamma$ and predictions of *cis* Xaa-Pro likelihood for prolines of ψRGDechi and RGDechi1-14.

ψRGDechi				
Residue	*Cβ*	*Cγ*	*Δβγ[a]*	*Normalized likelihood for Pro to be in cis[b]*
Pro[9]	32.2	27.1	5.1	0.004
Pro[13]	32.1	27.3	4.8	0.016
Pro[17]	31.2	27.1	4.1	0.005
RGDechi1-14				
Pro[9]	32.1	27.2	4.9	0.002
Pro[13]	32.1	27.2	4.9	0.019

[a] Values of $\Delta\beta\gamma$ ($\Delta\beta\gamma = \delta[C\beta] - \delta[C\gamma]$) for prolines are function of the Xaa-Pro peptide bond conformation: $\Delta\beta\gamma$ Pro trans = 4.51 ± 1.17 and $\Delta\beta\gamma$ Pro cis = 9.64 ± 1.27.
[b]The predictions were performed by using the Promega server from the amino acid sequence and chemical shift data.

Table S6. ^{3}JHNHα coupling constants of ψRGDechi in HBSS buffer (pH 7.4) at 298K on 600 MHz spectrometer.

Residue	$^3J_NH\alpha(Hz)$
Lys[1]	7.61
Arg[2]	7.39
Gly[3]	
Asp[4]	5.64
DGlu[5]	7.32
Met[6]	
Asp[7]	7.22
Asp[8]	6.21
Pro[9]	
Gly[10]	
Arg[11]	7.08
Asn[12]	8.14
Pro[13]	
His[14]	7.58
hCyt[15]	7.07
Gly[16]	
Pro[17]	
Ala[18]	6.84
Thr[19]	5.92

Table S7. ^{3}JHNHa coupling constants of RGDechi1-14 in HBSS buffer (pH 7.4) at 298K on 600 MHz spectrometer.

Residue	$^3J_NH\alpha$(Hz)
Lys^1	6.57
Arg^2	7.79
Gly^3	
Asp^4	5.57
DGlu^5	7.75
Met^6	5.48
Asp^7	7.70
Asp^8	6.87
Pro^9	
Gly^{10}	
Arg^{11}	6.74
Asn^{12}	7.18
Pro^{13}	
His^{14}	5.11

Figure S1. 2D [^{1}H-^{1}H] TOCSY and 2D [^{1}H-^{1}H ROESY] spectra of RGDechi1-14 (A, B) and ψRGDechi (C, D) peptides acquired at 298K using a 600 MHz spectrometer.
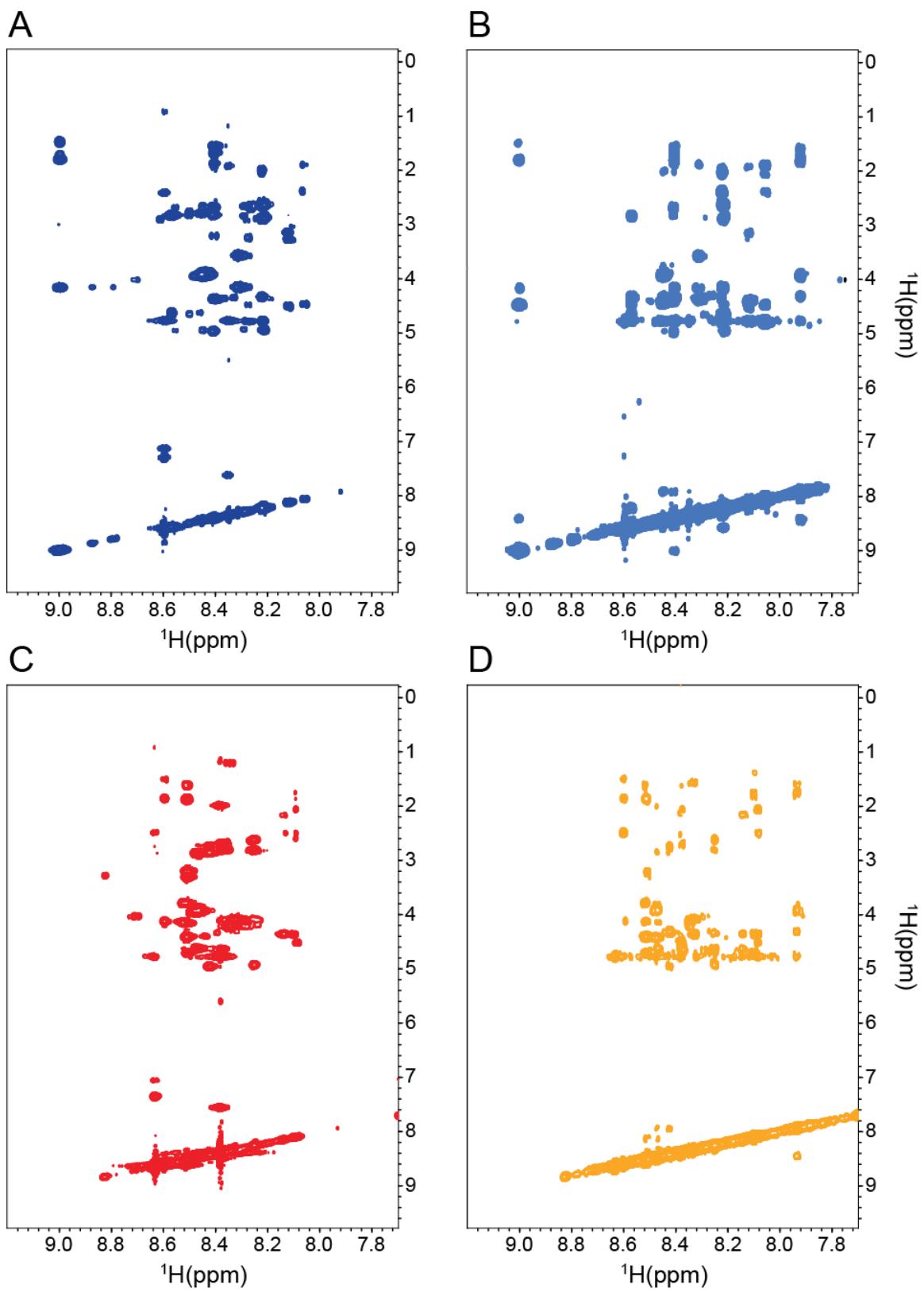
Figure S2. 2D [^{1}H-^{15}N] HSQC spectra of RGDechi1-14 (A) and ψRGDechi (B) peptides acquired at 298K using a 600 MHz spectrometer.
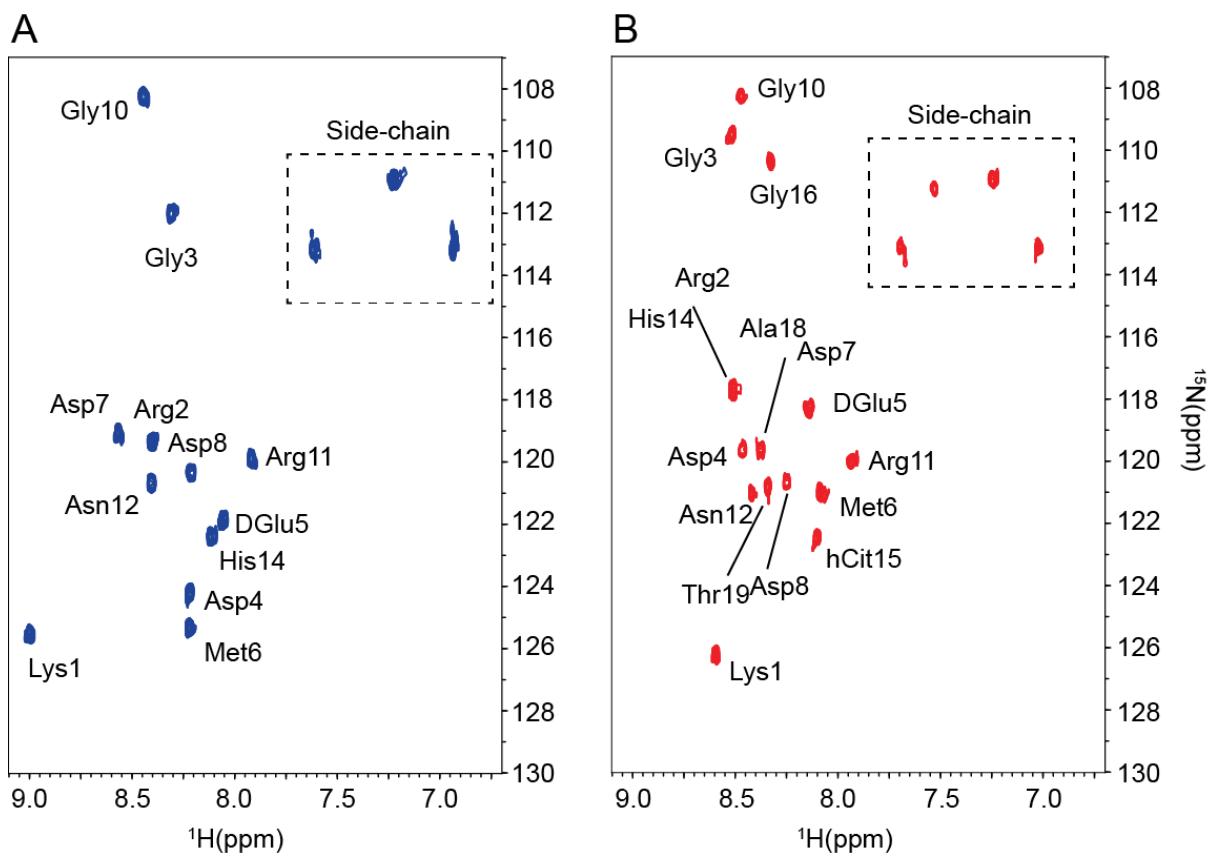
Figure S3. (A) Illustration of the expected NOE/ROE peaks depending on the *cis* and *trans* configuration of the peptidyl- prolyl peptide bond. Arrows indicate protons that are close through the space for each conformation. (Left) In the *cis* configuration of the Xaa-Pro peptide bond an NOE/ROE cross-peak(s) is expected between the Hα of the proline and the Hα of the previous residue. (Right) In the *trans* Xaa-Pro peptide bond configuration NOE/ROE cross-peak(s) are expected between the Hα of the preceding residue and one or both

of the prolyl Hδ2 and Hδ3. (B) A portion of the 2D [^{1}H-^{1}H] ROESY spectra acquired for RGDechi1-14 (Left) and ψRGDechi (Right) displaying Hα-Hδ ROEs from residue pairs D8-P9 and N12-P13 for both peptides and G16-P17 for only ψRGDechi. (C) A portion of the 2D [^{1}H-^{13}C] HSQC spectra acquired on the RGDechi1-14 (Left) and ψRGDechi (Right) at 298 K using a 600 MHz spectrometer. The black boxes indicate the Cβ and Cγ chemical shifts regions for in *cis* and *trans* Xaa-Pro peptide bond conformations.

A

Cis

ROEs/NOEs

Hα3   Hα

Hα2

Gly   **Pro**

Trans

Pro

Gly

Hα3

Hα2   Hδ3   Hδ2

ROEs/NOEs

B

Hβ3   Hβ3

Hγ   Hγ

Hβ2   Hβ2

Hδ Pro9

Hβ2 D8   Hβ2 N12

Hα M6

Hα   Hδ2 Pro 13

Hα D8   Hα N12

Hγ2   Hβ3   Hγ3

Hβ2   Hγ2   Hβ3

Hβ2   Hβ2

Hδ Pro 17   Hδ Pro 13   Hδ Pro 9

Hα2 G16   Hα R11

Hα   Hα

Hα N12   Hα D8

C

Cγ *Cis* Pro

Cγ *Trans* Pro

Cβ *Trans* Pro

Cβ *Cis* Pro

Cγ *Cis* Pro

Cγ *Trans* Pro

Cβ *Trans* Pro

Cβ *Cis* Pro
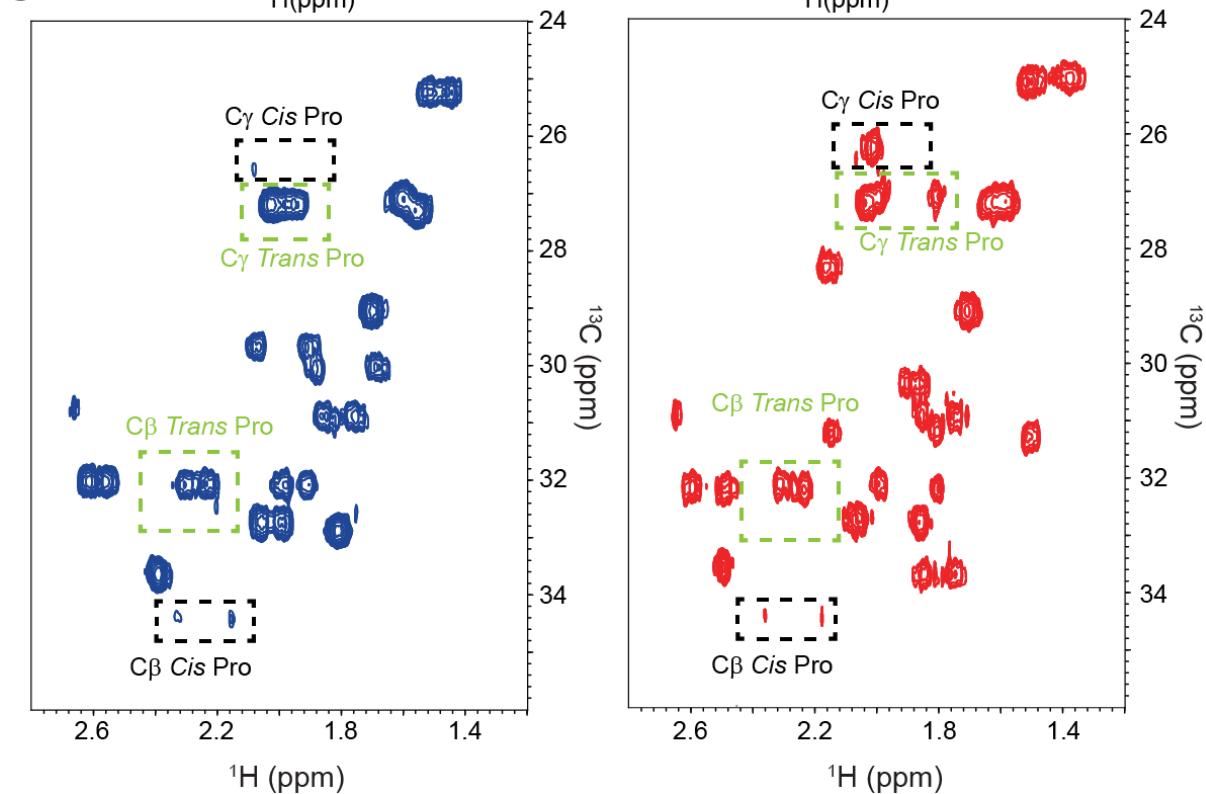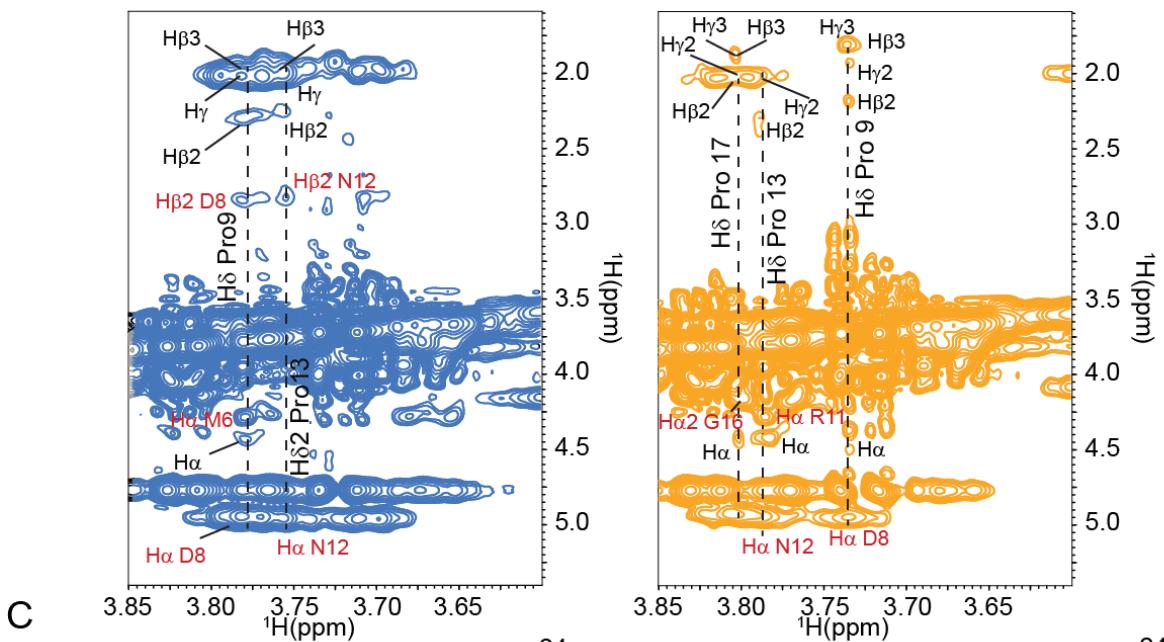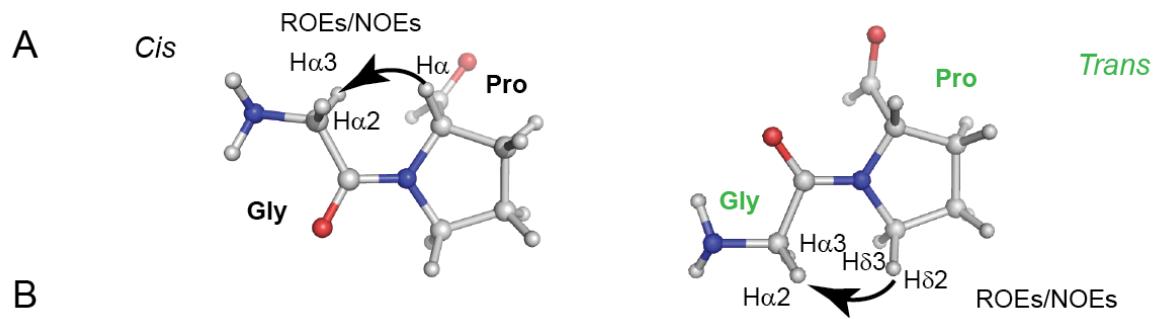
Figure S4. Flow-chart of the integrated approach applied to generate the conformational ensemble using a combination of NMR and MD for RGDechi1-14 (A) and ψRGDechi (B) peptides. (C, D) Evaluation by using the experimental ^{1}H, ^{15}N and ^{13}C chemical shifts of each representative structure obtained for RGDechi1-14 and ψRGDechi peptides by analysis of the MD conformational ensemble. The plots report the global CS-RMSD calculated, as reported in the materials and methods, for each model by evaluating the predicted versus observed chemical shifts.
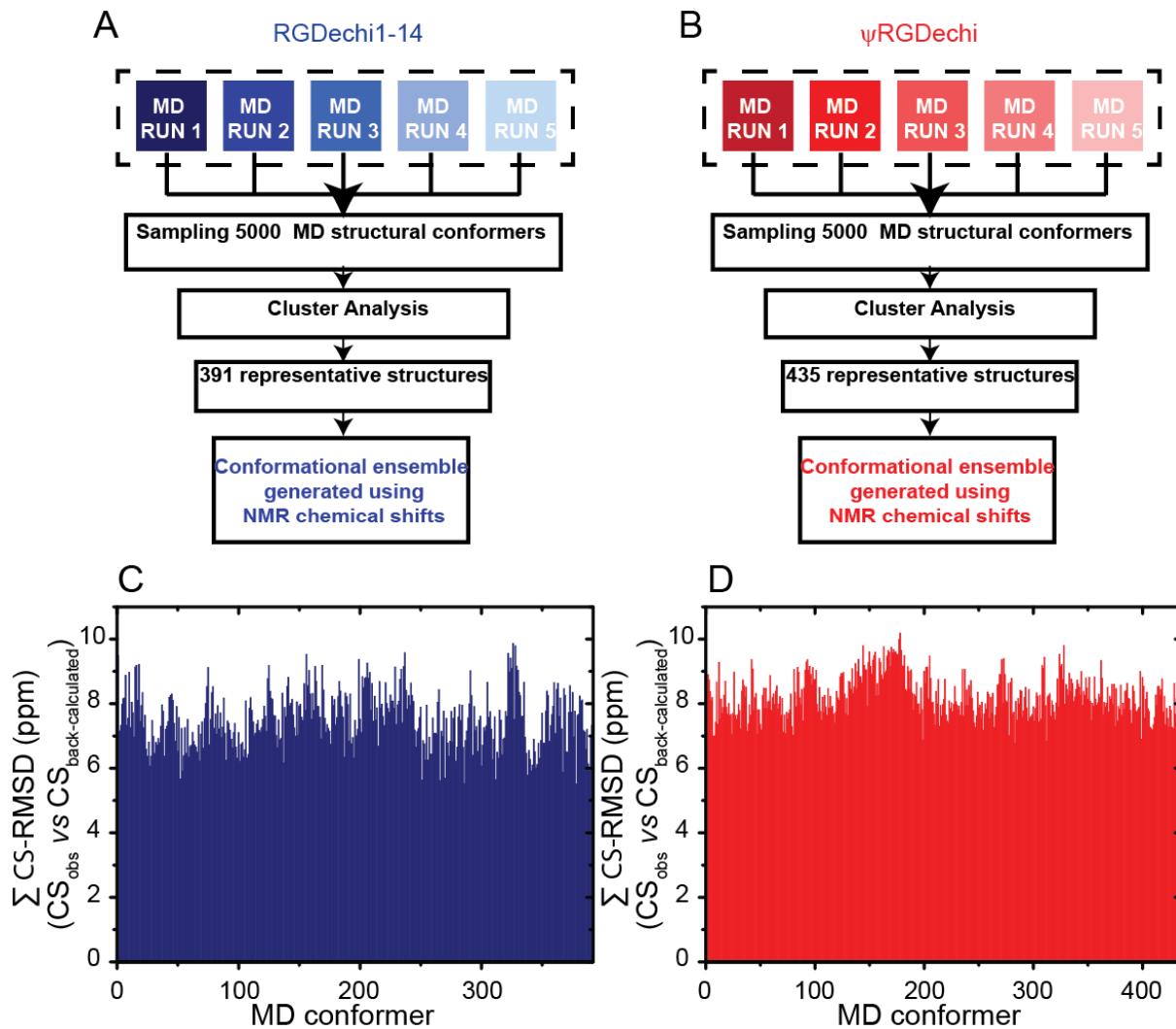
Figure S5. Evaluation of three MD ensembles generated for RGDechi1-14 and ψRGDechi using the experimental ^{1}H, ^{15}N and ^{13}C chemical shifts. The MD ensemble 50x was generated selecting the 50 conformers with the lowest global CS-RMSD; the MD ensemble2 50x was obtained including the 50 conformers with the highest global CS-RMSD; the MD ensemble3 50x was created considering 50 randomly selected conformers. The yellow and blue dash lines indicate the lowest global CS-RMSD values obtained by fitting each MD model for RGDechi1-14 and ψRGDechi, respectively.
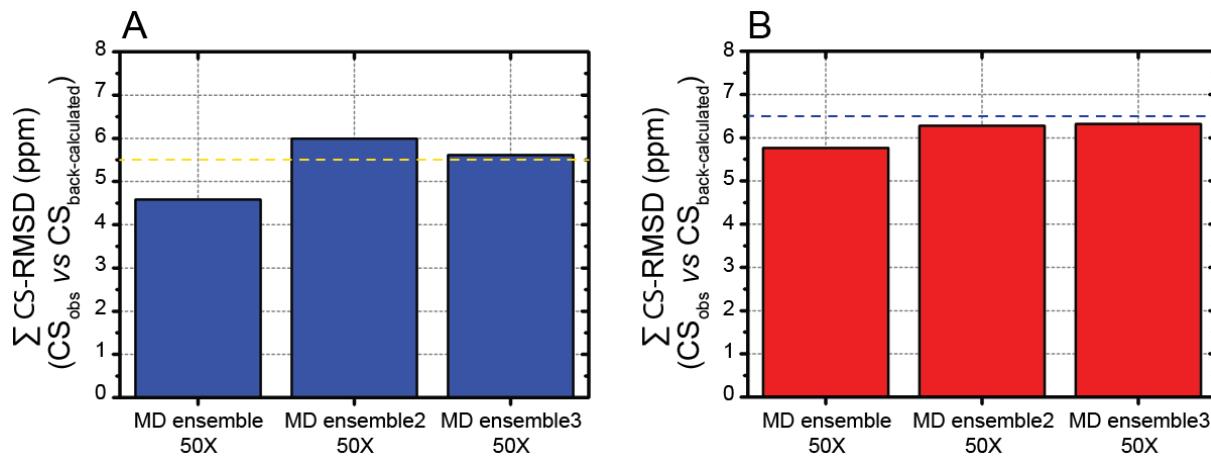
Figure S6. Correspondence between experimental and MD ensemble 50X derived $^3J_{HN,H\alpha}$ coupling constants for RGDechi1-14 (A) and ψRGDechi (B).
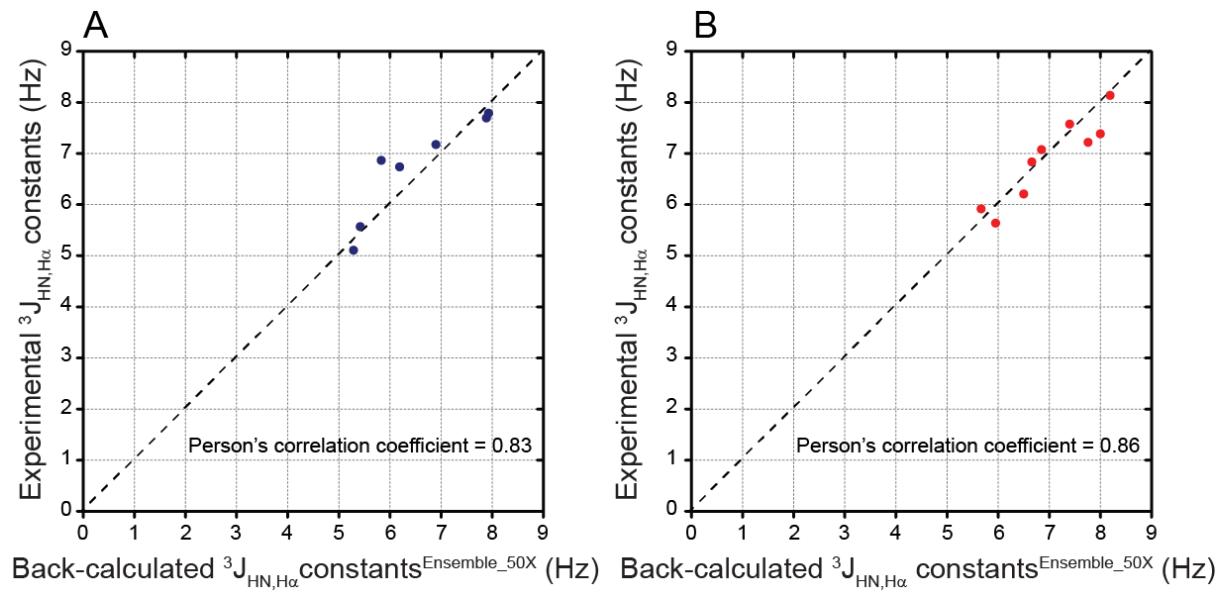
Figure S7. Back-calculated versus experimental proton (A), Cα/Cβ (B) and nitrogen (C) chemical shifts. The predicted shifts were obtained, as reported in the material and methods, using the ψRGDechi-bound conformation reported by Comegna *et al..* (D) Cumulative ^{1}H,^{13}C and ^{15}N chemical shift differences between predicted and observed shifts plotted as a function of residue number.