Supporting Information

Unravelling the conformational plasticity of the extracellular domain of a prokaryotic nAChR homologue in solution by NMR

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Materials and Methods

Sample preparation:

The $GLIC_{ECD}$ construct (1) was expressed in *Escherichia coli* and purified using affinity chromatography via a C-terminal His6 tag. The U-$^{2}H/^{15}N/^{13}C$ labeled sample for NMR studies was prepared using minimal (M9) medium supplemented with 0.1 g/l ($^{15}N,^{13}C,^{2}H$) ISOGRO® (Sigma), 1 g/l $^{15}NH_4(SO_4)_2$ and 4 g/l of $^{2}H_7/^{13}C_6$-glucose as nitrogen and carbon sources, respectively, in 99.9% $^{2}H_2O$. Protein samples for NMR experiments were prepared in a mixed solvent of 90% H_20 (50 mM KPi, pH 6.9) and 10% $^{2}H_2O$.

A brief overview of the selective labeling efforts is given in Table S1.

Selective unlabeling strategy in *E. coli* BL21(DE3): The amino acid selective unlabeling of ^{15}N-$GLIC_{ECD}$ with ^{14}N-Lys, ^{14}N-Arg, ^{14}N-His, and ^{14}N-Asn, respectively, was achieved by addition of 150 mg/l of each unlabeled amino acid (except 1 g of ^{14}N-Asn) to ^{15}N labeled minimal (M9) medium one hour prior to induction by IPTG.

Selective ^{15}N labeling strategy in *E. coli* BL21(DE3): The amino acid selectively ^{15}N labeled samples of $GLIC_{ECD}$ were expressed in otherwise unlabeled minimal (M9) medium. To incorporate ^{15}N labeled Lys, Leu, Val, or Ile into ^{14}N-$GLIC_{ECD}$, 150 mg/l ^{15}N-Lys, 85 mg/l ^{15}N-Leu (with 510 mg/l each of unlabeled Val and Ile), 170 mg/l ^{15}N-Val (with 1g/l each of unlabeled Leu and Val) and 90 mg/l of ^{15}N-Ile (with 540 mg/l each of unlabeled Leu and Val), respectively, were added to the culture one hour prior to induction.

Selective ^{15}N labeling strategy using the auxotrophic *E. coli* strain DL39: All the amino acid selective ^{15}N labeling of $GLIC_{ECD}$ using *E. coli* DL39 was done on unlabeled minimal (M9) medium, supplemented with the following mixture of unlabeled amino acids: 150 mg/l Phe, 90 mg/l Tyr, 400 mg/l Asp, 200 mg/l Leu, 200 mg/l Ile, 200 mg/l Val, 400 mg/l Ala, 200 mg/l Asn, 500 mg/l Gly, 100 mg/l Met and 210 mg/l Lys. To incorporate ^{15}N labeled Leu, Phe, Ala, or Tyr into ^{14}N-$GLIC_{ECD}$, the respective ^{14}N amino acid was omitted from the above mixture. Instead, 200 mg/l, 150 mg/l, 300 mg/l or 70 mg/l, respectively, of the desired ^{15}N amino acid were added to the medium at the beginning of the cell culture.

NMR experiments:

The complete set of heteronuclear NMR experiments used for the sequence specific assignments of H^N, N, Ca, Cb and C' is listed in Table S2. Internal 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was used as a chemical shift reference for ^{1}H. The backbone resonance assignments are available from the BioMagResBank (http://www.bmrb.wisc.edu/) under accession no. 17695.

The ^{15}N *R*1 and *R*2 relaxation rates (2) and $\{^{1}H^{N}\}$–^{15}N NOE values (3) were measured at 600 MHz (298 K) on a Bruker Avance spectrometer equipped with a TXI CryoProbe ™ [Figure S2].

Molecular Dynamics Simulations and Normal Mode Analysis.

Both calculations were performed on the ECD of one subunit from the crystal structure of the pentameric full-length GLIC (PDB: 3EAM). Chain A with the minimal number of undefined residues was selected.

Table S1: Overview of the selective labelling efforts for the preparation of $GLIC_{ECD}$ samples for NMR studies.

^{15}N Selective labeling using prototrophic E. coli BL21(DE3):			
Amino acid	^{14}N (reverse labeling)	^{15}N labeling	Scrambling
Leu	✓		+
Arg	✓		-
Val	✓		+
Phe	✓		+
Lys	✓		-
Ala	✓		+
Lys		✓	-
Leu		✓	+
^{15}N Leu+ ^{14}N (Val+Ile)		✓	+
^{15}N Val + ^{14}N (Leu+Val)		✓	-
^{15}N Ile + ^{14}N (Leu+Val)		✓	-
His	✓		-
Asn	✓		-
^{15}N selective labeling using auxotrophic E. coli DL39:			
Amino acid	^{15}N labeling		Scrambling
Leu	✓		-
Phe	✓		-
Ala	✓		-
Tyr	✓		-
Asp	✓		+
Glu	✓		+

Table S2: Acquisition parameters of the NMR experiments performed on $GLIC_{ECD}$ at 298 K.

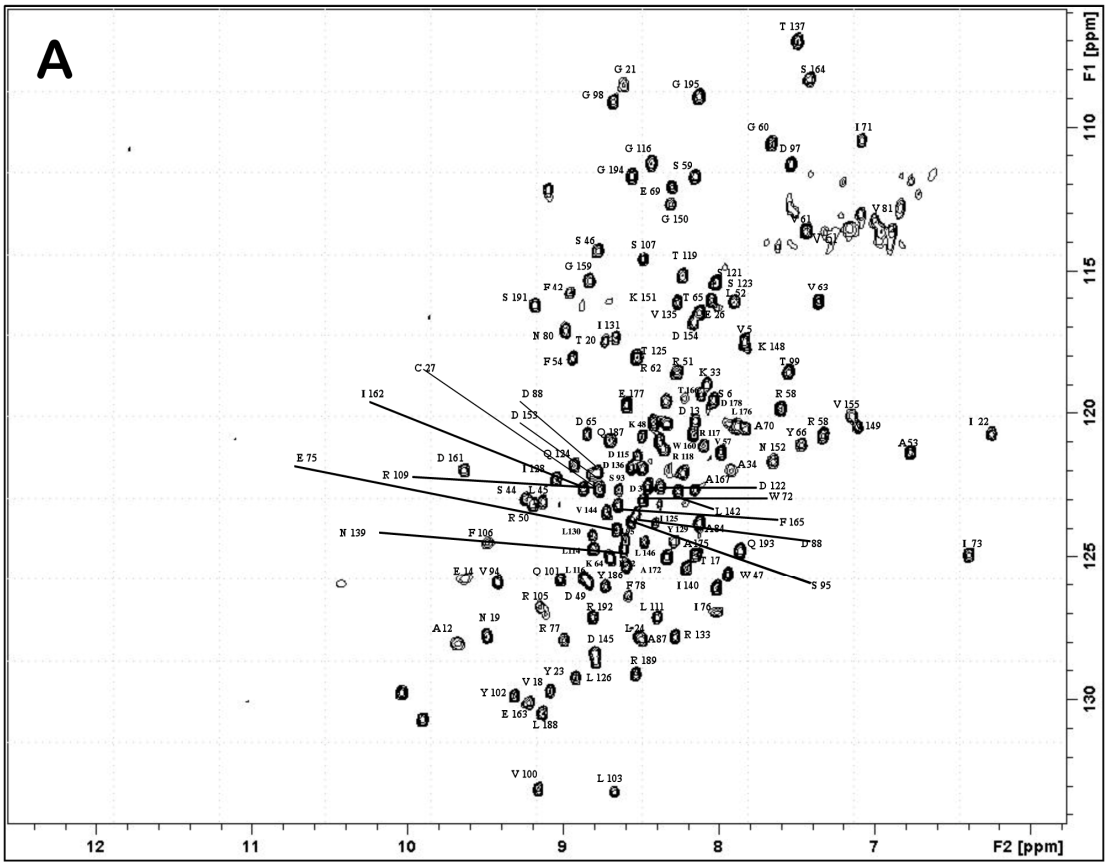
Experiments[a]	Dimension of acquired data (nucleus)			Spectral width (ppm)			n[b]
	t_1	t_2	t_3	F_1	F_2	F_3	
[^{1}H-^{15}N]-TROSY-HSQC	192(^{15}N)	1024(^{1}H)		28.4	15.5		64
TROSY-HNCA	72(^{13}C)	38(^{15}N)	1024(^{1}H)	31.55	28.4	12.5	64
TROSY-CBCA(CO)NH	64(^{13}C)	38(^{15}N)	1024(^{1}H)	60.2	28.4	12.5	64
TROSY-CBCANH	64(^{13}C)	38(^{15}N)	1024(^{1}H)	60.2	28.4	12.5	64
TROSY-HN(CO)CA	56(^{13}C)	38(^{15}N)	1024(^{1}H)	28.4	12.5	12.4	80
HNCO	48(^{13}C)	38(^{15}N)	1024(^{1}H)	14.0	24.3	12.0	16
^{15}N-edited [^{1}H-^{1}H]-NOESY	92(^{1}H)	38(^{15}N)	1024(^{1}H)	12	24.3	12.9	48
^{15}N R_1	256(^{15}N)	1024(^{1}H)		26	15		24
^{15}N R_2	256(^{15}N)	1024(^{1}H)		26	15		24
steady-state heteronuclear NOEs	256(^{15}N)	1024(^{1}H)		26	15		56

[a] Data acquired on a Bruker Avance 600 MHz spectrometer equipped with a 5 mm triple resonance cryoprobe (TXI) with pulsed field gradients along the z-axis. The ^{15}N-edited NOESY was acquired with a mixing time of 100 ms.

[b] number of acquired scans.

All 2D and 3D spectra were collected at 298 K, processed with the Bruker software TOPSPIN and analyzed with the programs CARA (*4*) and XEASY (*5*).

Figure S1. **(A)** 2D ^{1}H-^{15}N TROSY NMR spectrum and **(B)** Circular dichroism spectrum of $GLIC_{ECD}$ at pH 7.0, 298 K (20 mM TrisHCl.). Data analysis was performed using the server DICHROWEB (http://dichroweb.cryst.bbk.ac.uk/html/home.shtml) *(6)*



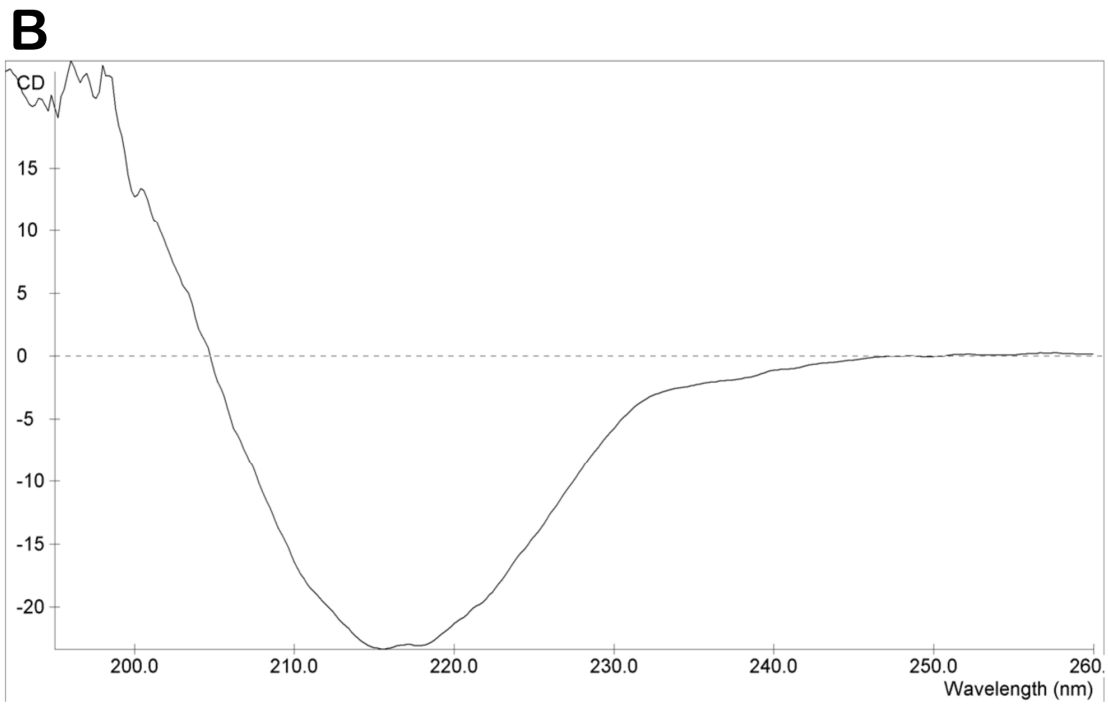


Figure S2. (Upper panel) Assignment status of the $GLIC_{ECD}$-{Gly2His6} polypeptide at 298 K (yellow: assigned, light blue: unassigned, dark blue: Pro). The secondary structure in the crystal structure of $GLIC_{ECD}$ (PDB: 3IGQ) as attributed by PROCHECK *(7)* is shown above the sequence. (Lower panel) ^{15}N R_1, R_2, and heteronuclear $\{^1H^N\}$–^{15}N NOE values.

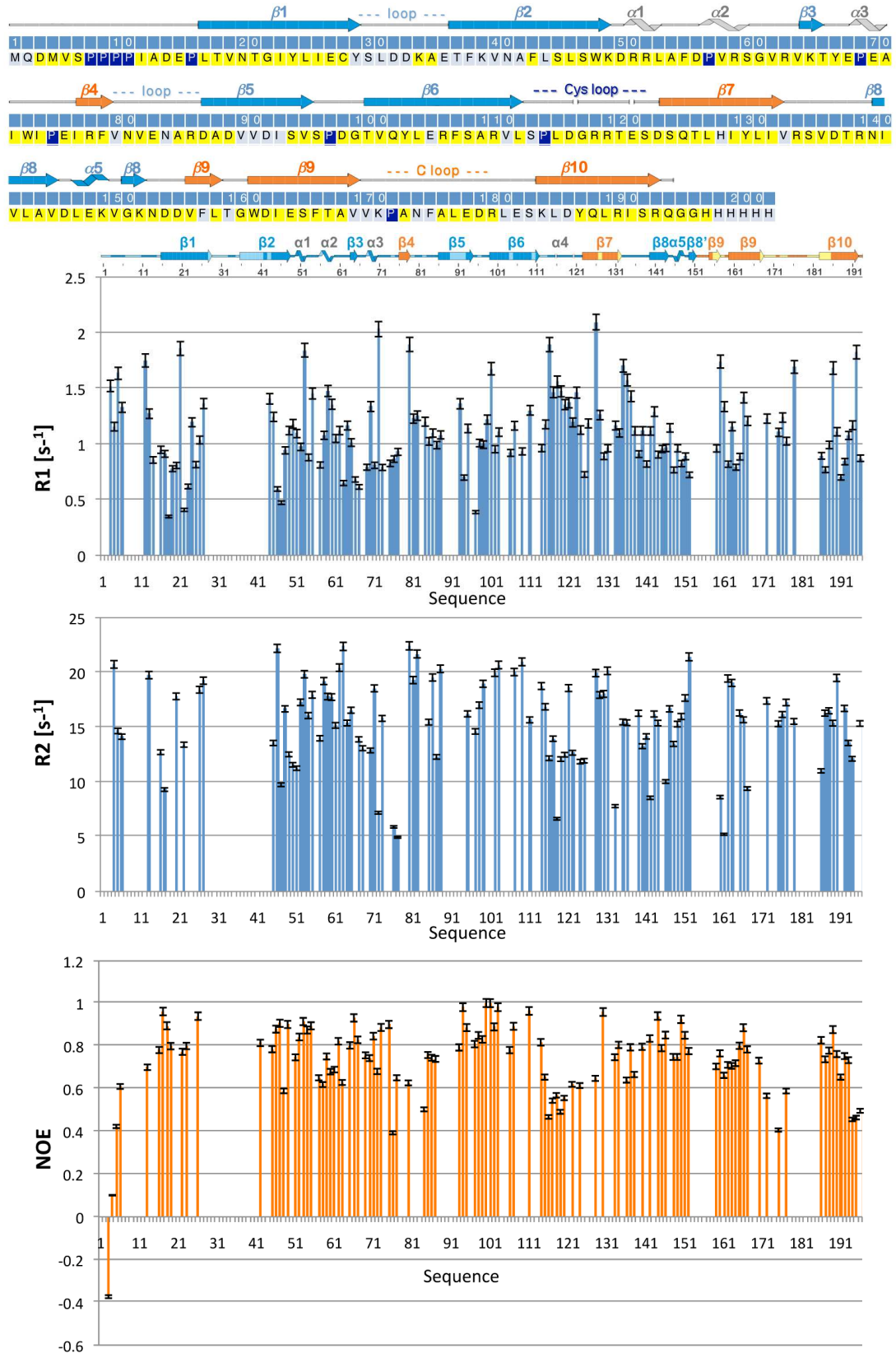


Figure S3. Atomic RMS fluctuations extracted from a 10 ns MD calculation of $GLIC_{ECD}$ (PDB: 3EAM, chain A) in water as a function of sequence. Unassigned regions of $GLIC_{ECD}$ are also shown for comparison. Assigned residues are indicated with "0", unassigned ones are indicated with "1". An ECD of the pentameric structure of full-length GLIC (3EAM) was chosen instead of a chain of the hexameric $GLIC_{ECD}$ structure (3IGQ), since the subunits of the latter contain undefined residues.

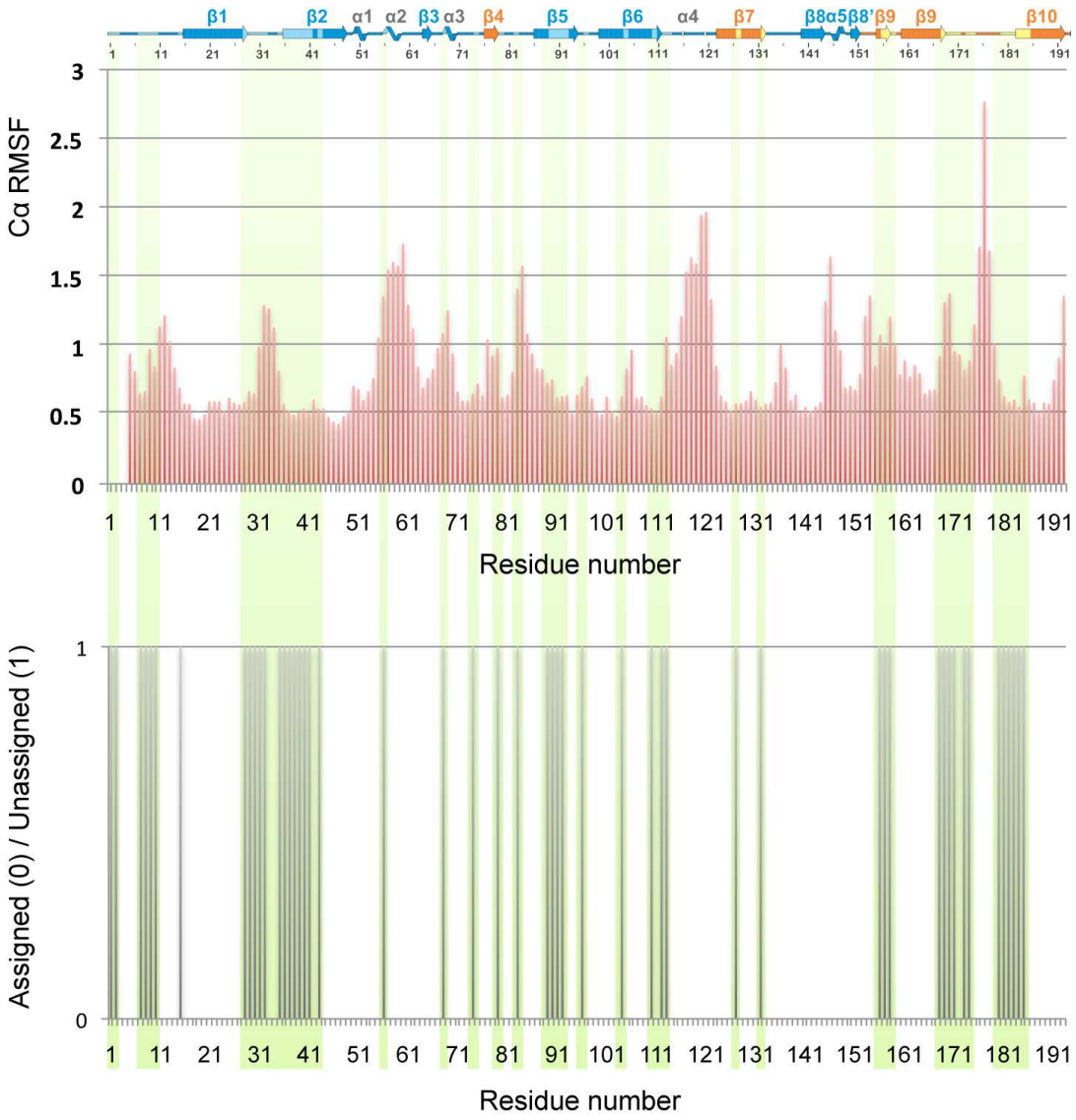


Figure S4. H/D exchange of $GLIC_{ECD}$ as a function of time monitored through ^{1}H-^{15}N HSQC NMR spectra: **(A)** ^{1}H-^{15}N HSQC spectrum of deuterated $GLIC_{ECD}$ (600 MHz; 50 mM KP_i, pH 7.0, 298 K), **(B)** ^{1}H-^{15}N HSQC spectrum of $GLIC_{ECD}$ after 90 min of H/D exchange, **(C)** ^{1}H-^{15}N HSQC spectrum of $GLIC_{ECD}$ after 23 h of H/D exchange, and **(D)** ^{1}H-^{15}N HSQC spectrum of $GLIC_{ECD}$ after 6d 20h of H/D exchange

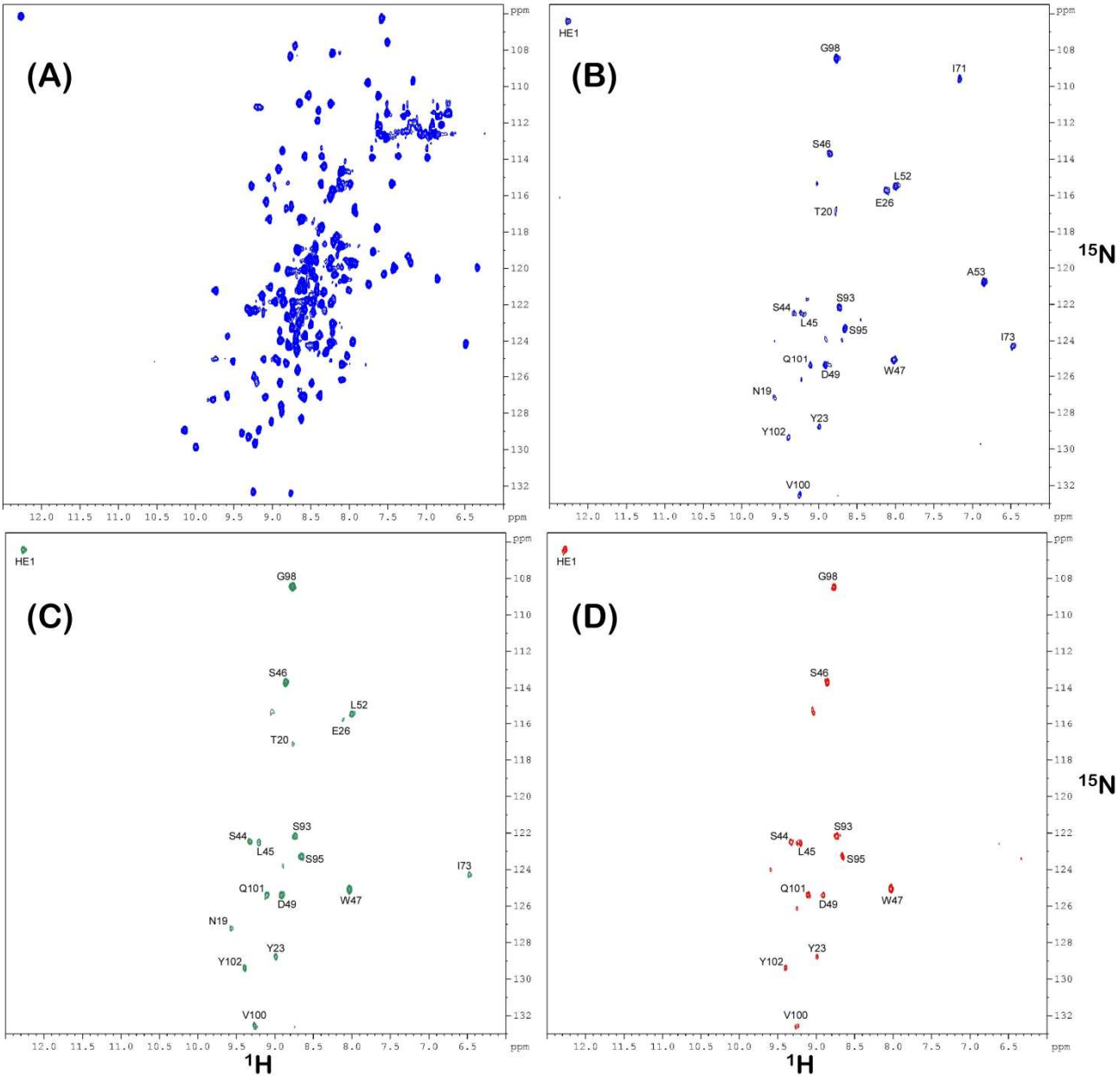
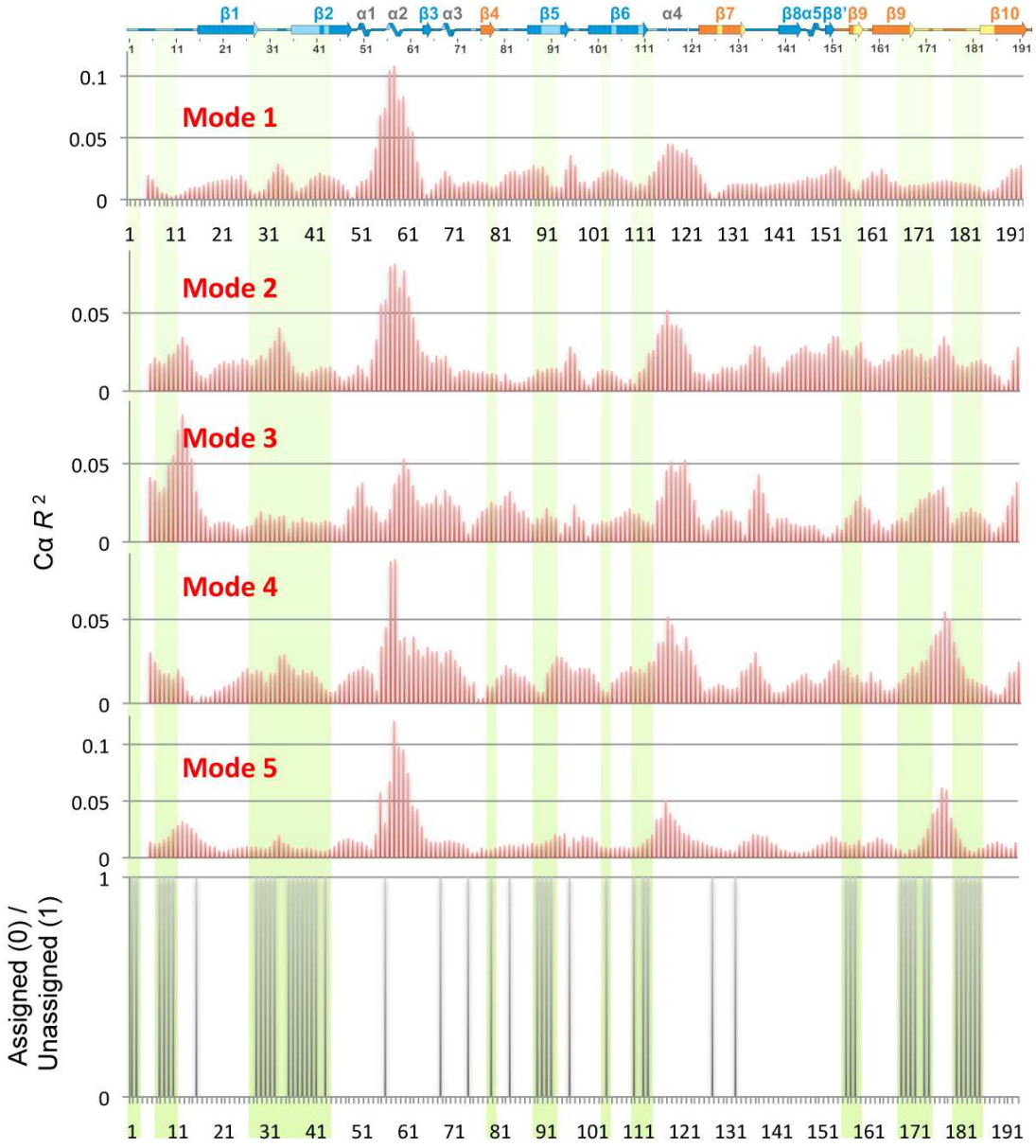


Figure S5. *Ab initio* normal mode analysis of GLIC ECD_{5-193} (PDB: 3EAM, chain A) as a function of sequence through the web interface of *The Elastic Network Model* (http://www.igs.cnrs-mrs.fr/elnemo/)*(8)*. The *ElNémo* server considers the first 6 modes to represent the translational and rotational motions of the system and calculates the next 5 modes. These modes are presented here, and numbered from 1 to 5. Unassigned regions of $GLIC_{ECD}$ are also shown for comparison. Assigned residues are indicated with "0", unassigned ones are indicated with "1". An ECD of the pentameric structure of full-length GLIC (3EAM) was chosen instead of a chain of the hexameric $GLIC_{ECD}$ structure (3IGQ), since the subunits of the latter contain undefined residues.



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