Table S1. Structural statistics and root-mean-square deviations for 10 low energy structures of the KcsA / charybdotoxin complex.

Structural statistics[a]	<SA>	$<\overline{SA}>_r$
Rmsd from experimental distance restraints (Å)[b]		
All (576)	0.008±0.002	0.004
Intermolecular (13)	0.032±0.012	0.020
Charybdotoxin NOE restraints (111)	0.007±0.003	0.001
KcsA NOE restraints (452)	0.004±0.002	0.001
CNX potential energies (kcal mol^{-1}) [c]		
E_{tot}	328±18	318
E_{bond}	13±0.5	13
E_{ang}	198±4	198
E_{imp}	16±1	17
E_{repel}	45±3	44
E_{noe}	2±1	1
E_{cdih}	2±0.4	2
E_{ncs}	52±14	43
Rmsd from idealized geometry		
bonds (Å)	0.00±0.00	0.00
angles (deg)	0.33±0.00	0.30
impropers (deg)	0.17±0.01	0.12
Cartesian coordinate rmsd (Å)	N, C_α, and C'	all heavy
<SA> vs. $<\overline{SA}>$[d]	0.66±0.05	0.92±0.05
<SA> vs. $<\overline{SA}>$[e]	1.35±0.26	1.69±0.27

[a]Where <SA> is the ensemble of 10 NMR-derived solution structures of the KcsA / charybdotoxin complex.; $<\overline{SA}>$ is the mean atomic structure; $<\overline{SA}>_r$ is the energy-minimized average structure.

[b]Distance restraints were employed with a square-well potential (F_{noe} = 50 kcal mol^{-1} $Å^{-2}$). No distance restraint was violated by more than 0.3 Å in any of the final structures. In addition to 576 experimental distance restraints, 35 symmetry distance restraints for maintaining symmetry of the tetrameric subunits of KcsA were used in the calculations.

[c]The energies reported here were obtained by using F_{repel} of 0.8 times their CHARMM values with a force constant of 4.0 kcal mol^{-1} $Å^{-4}$ and by energy minimization of structures generated from the protocol as described in the experimental procedures.

[d]Rmsd for the KcsA (residues 23-119) / charybdotoxin complex.

[e]Rmsd for charybdotoxin when the KcsA (residues 23-119) / charybdotoxin complexes are superimposed.

Figure S1. The methyl regions of $^{1}H/^{13}C$-HSQC spectra of (A) $^{13}CH_3\{IVLMA\}$ /$^{1}H\{YW\}$ and (B) $^{13}CH_3\{LA\}$ labeled KcsA. All available methyl group assignments are indicated in (A).

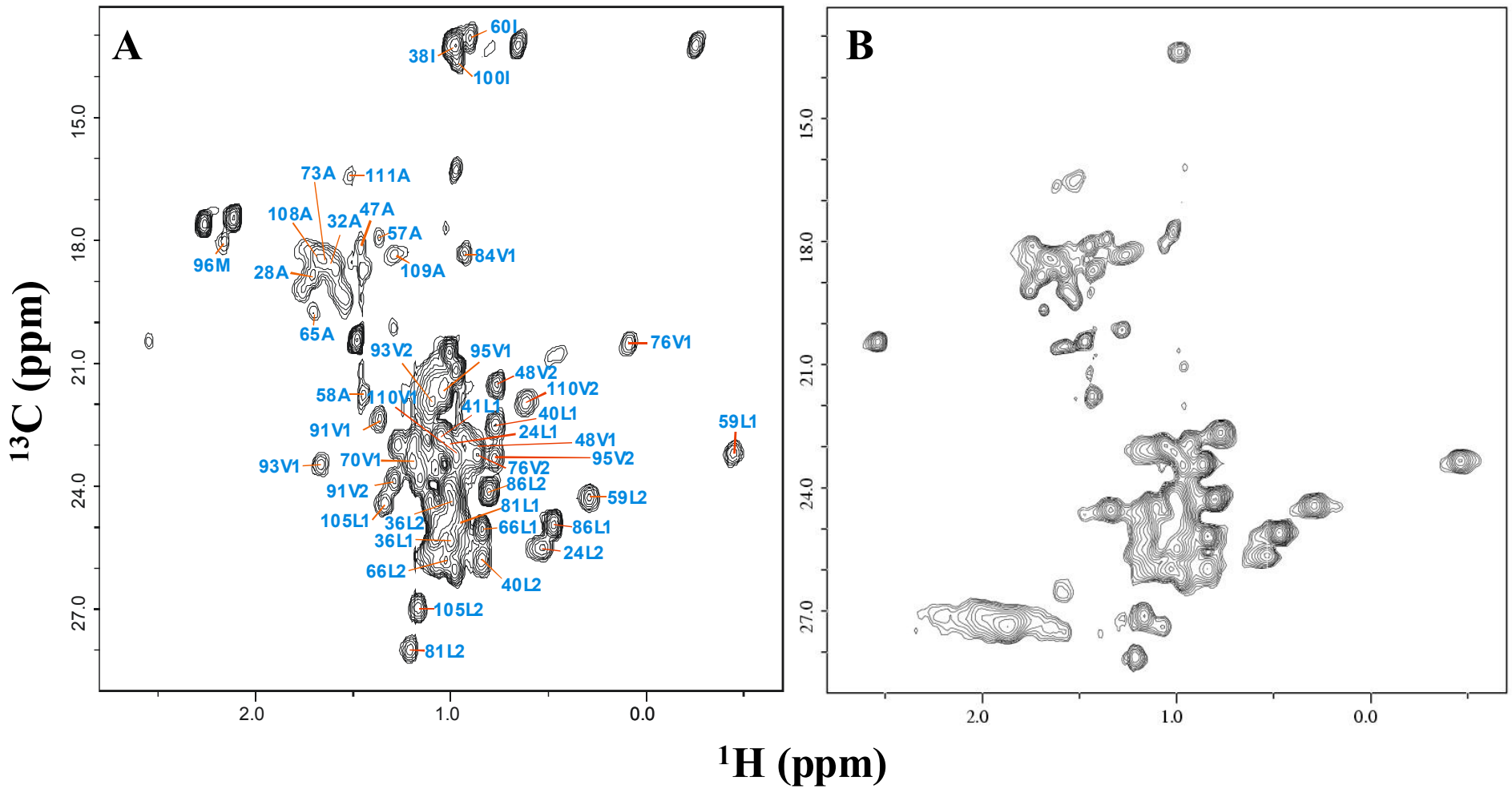

Figure S2. $^{1}H/^{1}H$ NOESY spectra of charybdotoxin in the (A) absence and (B) presence of perdeuterated KcsA. Both samples were in 20 mM sodium phosphate (pH 7.5), 5 mM KCl, 1 mM DTT, and 80 mM d38-Foscholine-12. The mixing times were 150 and 100 ms for (A) and (B), respectively.

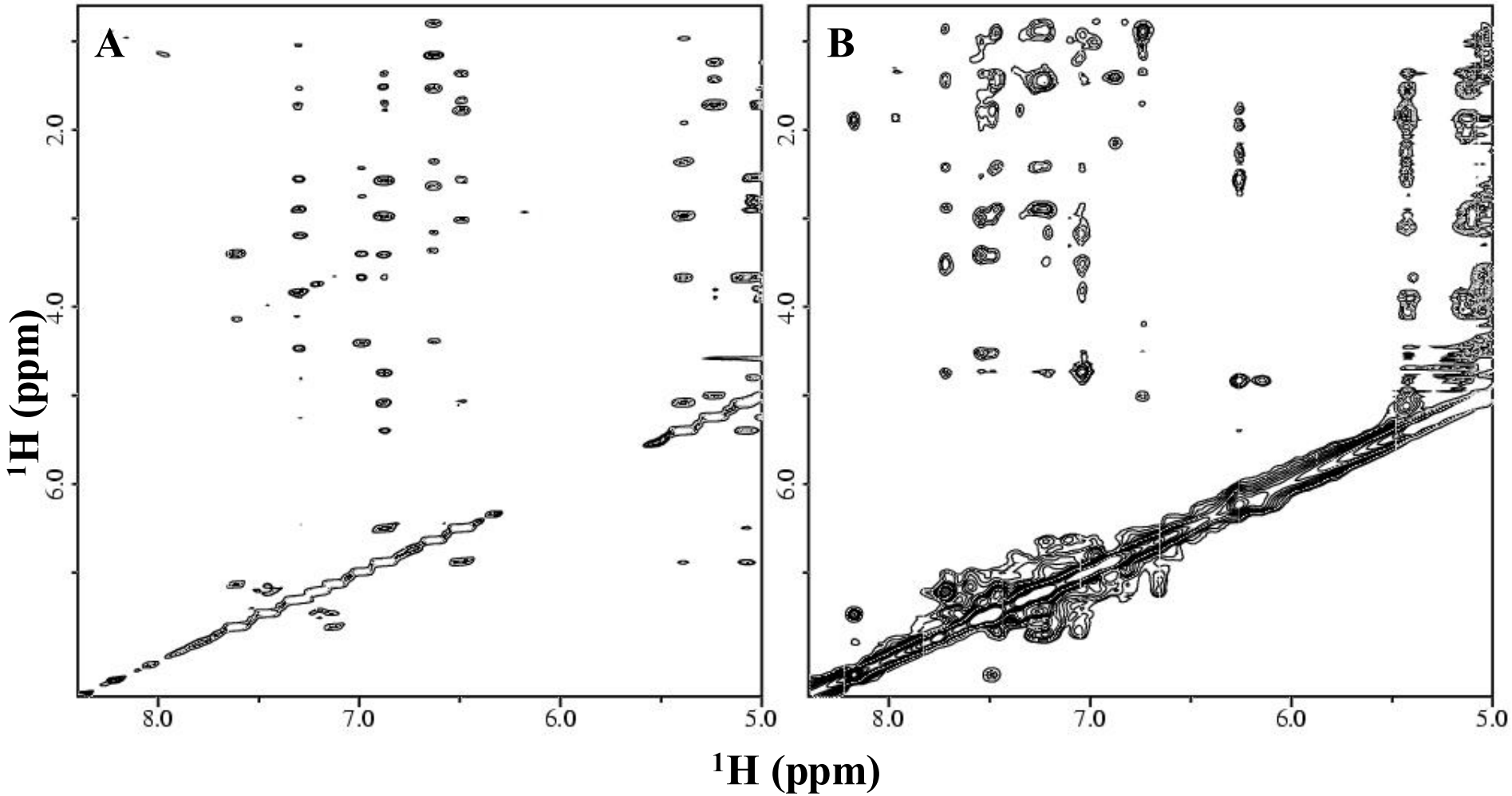

Figure S3. Stereoview of the backbone atoms (N, Cα, C') of 10 NMR-derived structures of KcsA / charybdotoxin complex when superimposed upon all the backbone atoms in the complex. KcsA and charybdotoxin are colored in black and red, respectively.