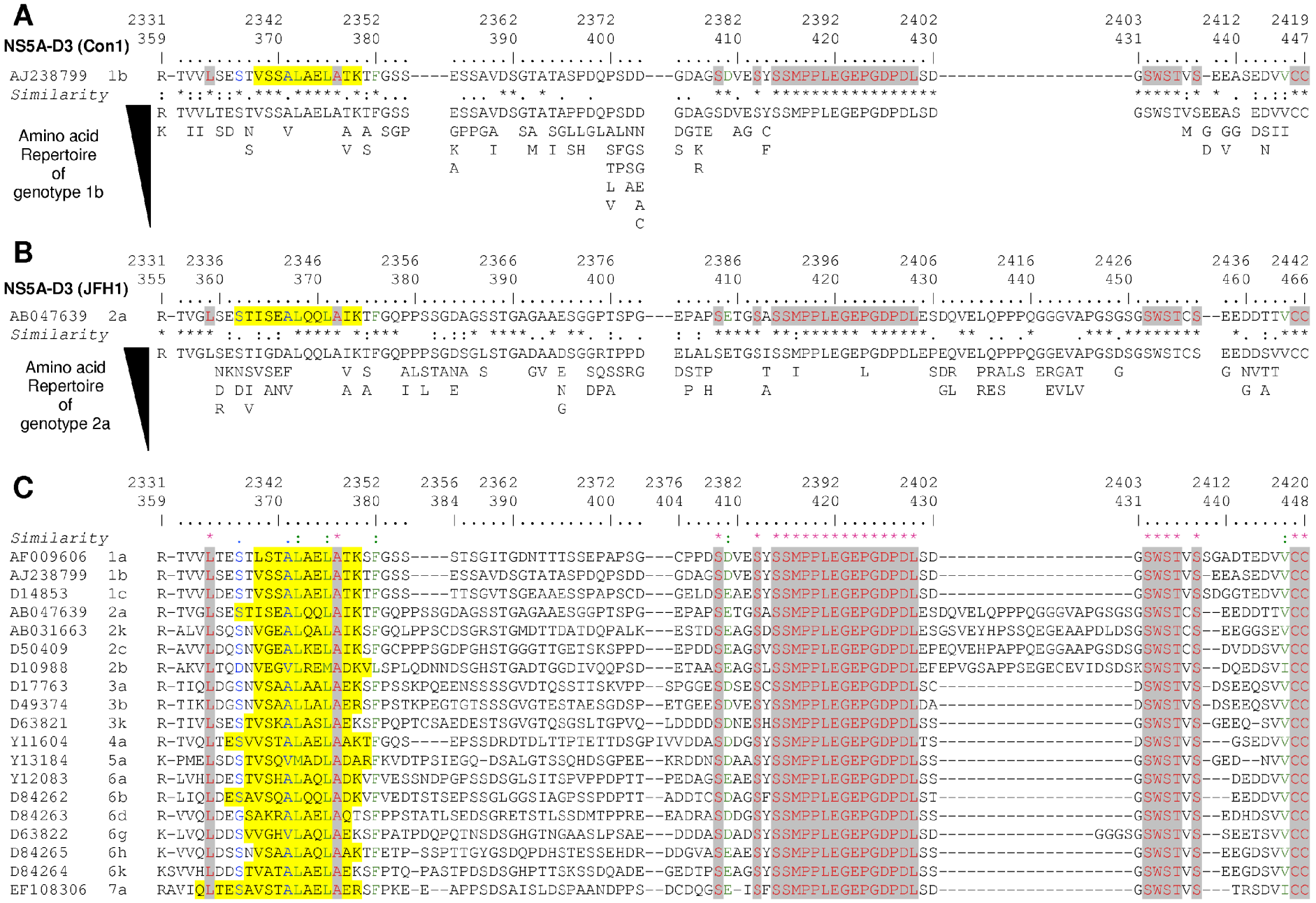
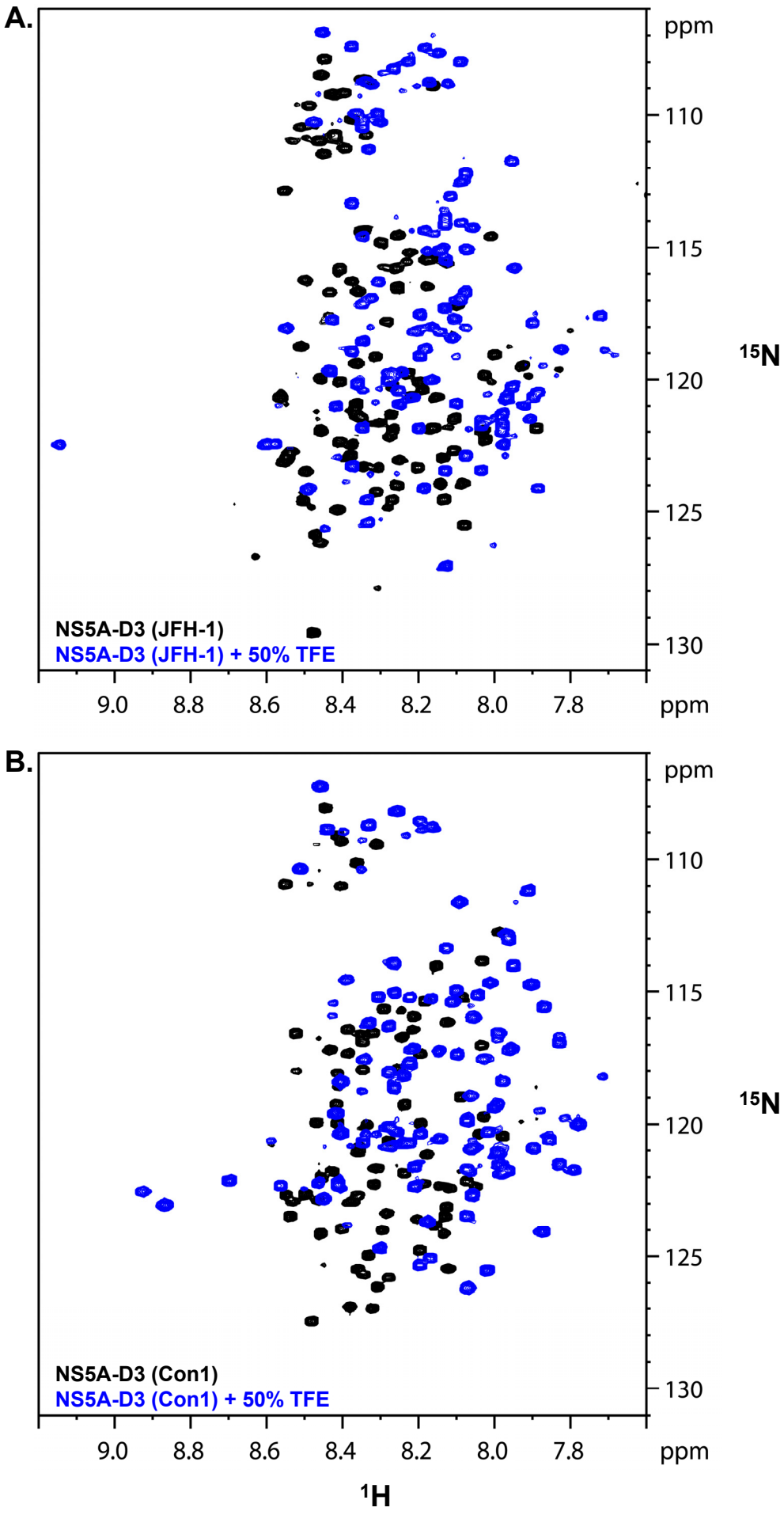

A
NS5A-D3 (Con1)
Amino acid Repertoire of genotype 1b
B
NS5A-D3 (JFH1)
Amino acid Repertoire of genotype 2a
C
Supplemental Figure 1

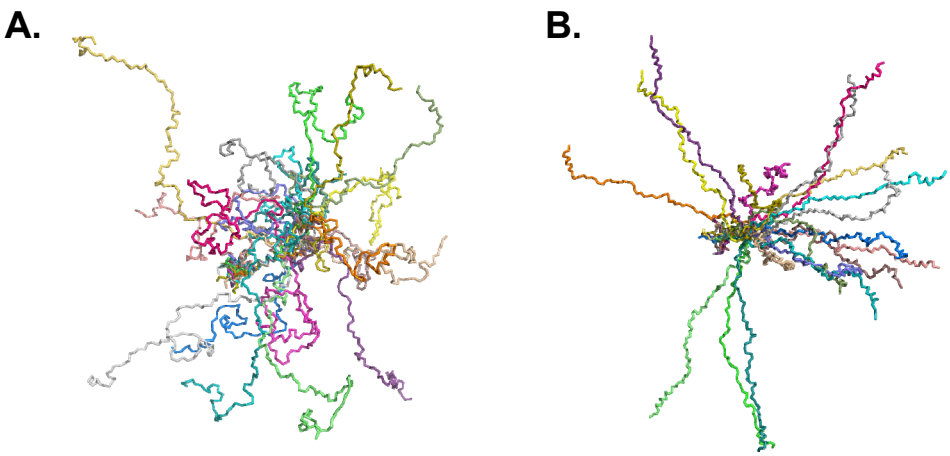
Conservation and variability of NS5A domain D3 amino acids sequence from various HCV genotypes. ***A*** and ***B***, amino acid repertoires of NS5A domain D3 from genotype 1b and 2a, respectively. The NS5A-D3 aa 359–447 sequence from HCV Con1 strain (AJ238799 ; genotype 1b) and aa 355–466 sequence from HCV JFH1 strain (AB047639 ; genotype 2a) are indicated with respect to the respective NS5A proteins numbering and polyproteins numbering. The *hyphens* indicate the amino acid gap compared with the multiple sequence alignment of D3 from representative HCV genotypes (panel ***C***, see below). The aa repertoires were deduced from the ClustalW multiple alignments of 363 and 21 NS5A sequences of genotypes 1b (panel ***A***) and 2a (panel ***B***), respectively. Amino acids observed at a given position in fewer than three distinct sequences (1%) for the former and two sequences for the latter were not included. The degree of aa conservation at each position can be inferred from the extent of variability (with the observed aa listed in decreasing order of frequency from top to bottom) together with the similarity index according to ClustalW convention (*asterisk*, invariant; *colon*, highly similar; *dot*, similar ; (53)). ***C***, multiple alignments of NS5A domain D3 from representative confirmed HCV genotypes and subtypes (listed with accession numbers in Table 1 in (106); for details, see the European HCV Database (http://www.euhcvdb.fr,(52)). Numbering of residues refers to NS5A protein of genotype 1a, H77 strain (AF009606), as recommended (107). Amino acids that are invariant, highly similar, or similar in any genotypes are colored in red, green and blue, respectively. Amino acid positions shaded in grey indicate the amino acids that are strictly conserved in any HCV reference genotypes. Amino acid positions consensually predicted to adopt an alpha helical fold are shaded in yellow. These consensus helical folds were deduced from a large set of prediction methods available at the NPSA website, including DSC, HNNC, MLRC, PHD, Predator, SOPM, and SIMPA96 (http://npsa-pbil.ibcp.fr/; (51) and references therein).

Supplemental Figure 2



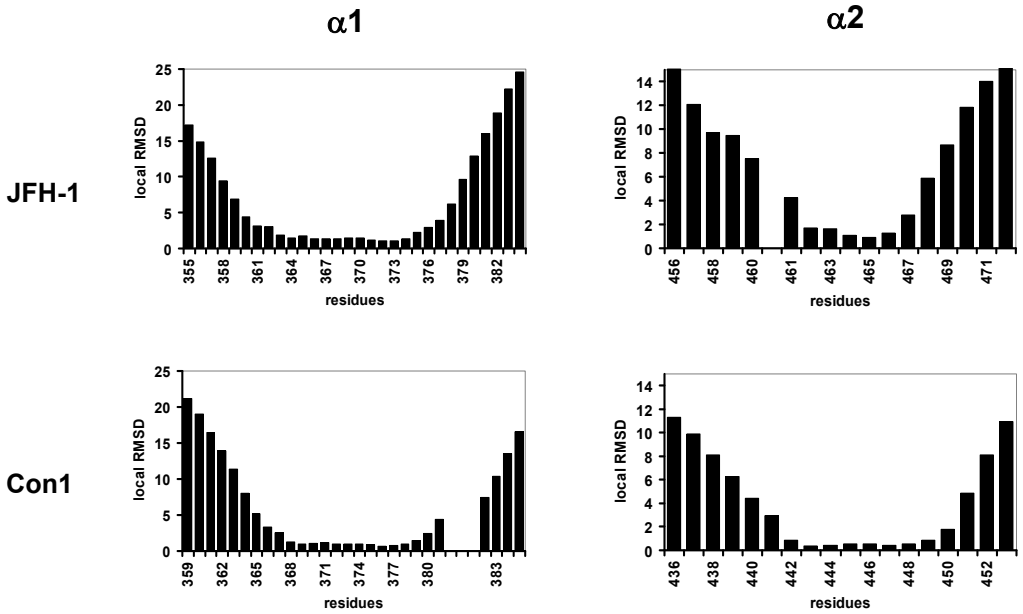
^{1}H,^{15}N HSQC spectra of NS5A-D3 with or without 50% TFE. Each panel corresponds to the superimposition of ^{1}H,^{15}N-HSQC spectra that have been acquired on NS5A-D3, from the JFH-1 (***A***) or the Con1 (***B***) HCV strains, without (black spectrum) or in the presence of 50% TFE (blue spectrum).

Supplemental Figure 3



NMR structure model of NS5A-D3 from JFH-1 (*A*) and Con1 (*B*) HCV strains in 50%TFE. The 20 final structures for each NS5A-D3 have been superimposed on the backbone atoms (N, C_α and C_O) of the α1 helix located in the N-terminus. This illustrates that NS5A-D3 remains mainly unfolded even in presence of 50% TFE.

Supplemental Figure 4



Local RMSD (Å) values for the backbone atoms (N,C_α and C_O) of α1 (left panels) and α2 (right panels) helices in the final set of calculated structures of NS5A-D3 (JFH-1) (upper panels) and NS5A-D3 (Con1) (lower panels). The sequences of NS5A-D3 (JFH-1) and NS5A-D3 (Con1) were kept aligned as in Fig. 1.

Supplemental Table 1

Assignments of minor NMR resonances in the $^{1}H,^{15}N$-HSQC spectrum of NS5A-D3 (JFH-1) that have been recorded in aqueous medium.

residue	^{1}H	^{15}N	$^{13}C\alpha$	$^{13}C\beta$	$^{13}C'$
T375	8.068	114.965	61.676	70.049	174.135
F376	8.328	122.296	58.156	39.774	176.302
G377	8.360	110.895	45.235		172.744
Q378	7.809	118.148	52.417		173.893
Q378	8.127	120.466	53.385	29.095	173.491
T400c	8.558	115.898	62.288	69.809	*nd*
S401	8.118	118.227	55.674	64.204	173.867
S401	8.529	119.778	56.413	63.484	174.339
E404	7.837	119.620	53.505	31.385	174.615
A406c	8.640	126.699	50.755	17.893	175.835
A406	8.190	124.368	49.984	19.732	175.591
E409	8.694	123.273	56.763	30.335	176.889
S415	8.282	117.993	58.255	63.840	*nd*
M416	8.127	121.843	52.158	34.170	174.077
L419c	8.695	124.739	55.214	42.037	177.357
E420	8.473	121.535	56.636	30.468	176.903
G421	8.483	110.438	45.313		173.162
G421	8.436	110.342	44.991		173.682
E422	7.919	119.893	53.817	30.832	174.821
G424	8.530	109.621	45.077		172.633
D425	7.919	119.890	51.857	42.420	174.825
D427c	8.556	120.951	54.144	41.094	176.770
L428	8.350	123.914	55.256	42.455	178.072
E429	8.477	121.233	56.752	30.130	176.903
S430	8.231	116.322	58.903	63.844	*nd*
E434	8.491	124.852	56.278	30.398	176.046
E434	8.519	124.280	56.522	30.228	176.195
L435	8.290	124.869	55.231	42.580	175.790
Q436	8.015	120.106	52.424	30.718	174.134
G442	8.283	108.075	45.385		174.796
V444	7.972	118.767	61.893	33.072	175.272
V444	8.042	118.882	61.845	32.966	*nd*
A445	8.314	127.898	50.602	18.919	175.966
G447c	8.563	111.483	45.600		174.073

c, indicates that the minor form is directly following a Proline residue in *cis* conformation

Supplemental Table 2

Statistics of the NMR structures models calculations of NS5A-D3 JFH-1 and Con1 in 50%TFE.

Conditions	NS5A-D3_JFH1 (50% TFE)	NS5A-D3_Con1 (50% TFE)
Restraints used		
Distance restraints		
Intra-residue	0	0
Sequential	48	44
Medium range	25	30
Longe range	0	0
Total distance restraints	73	74
Angle restraints		
$^{3}J_{HNH\alpha}$ couplings	16	19
Chemical shifts		
$^{13}C\alpha$	113	93
$^{13}C\beta$	95	86
Statistics for the CNS accepted structures		
Number of accepted structures	111	65
CNS energy (kcal.mol^{-1})	450±216	111±24
NOE violations		
Number > 0.5 Å	0	0
NOE r.m.s.d. (Å)	0.007±0.006	0.006±0.005
$^{3}J_{HNH\alpha}$ coupling violations (for ^{3}J < 5Hz)		
Number > 1 Hz	0	0
$^{3}J_{HNH\alpha}$ r.m.s.d. (Hz)	0.257±0.073	0.211±0.063
Deviations from idealized covalent geometry		
r.m.s.d. bonds (Å)	0.002±0.0006	0.002±0.0005
r.m.s.d. angles (deg.)	0.356±0.0285	0.316±0.0166
r.m.s.d. impropers (deg.)	0.232±0.0777	0.151±0.0220
Statistics for the 20 lowest energy final CNS structures		
Number of structures in the final set	20	20
CNS energy (kcal.mol^{-1})	92.204±11.037	61.098±7.217
NOE r.m.s.d. (Å)	0.007±0.007	0.005±0.004
$^{3}J_{HNH\alpha}$ r.m.s.d. (Hz)	0.273±0.078	0.208±0.061
Deviations from idealized covalent geometry		
r.m.s.d. bonds (Å)	0.002±0.0004	0.001±0.0004
r.m.s.d. angles (deg.)	0.326±0.019	0.300±0.009
r.m.s.d. impropers (deg.)	0.175±0.044	0.133±0.010
Ramachandran data - α1 helix	**res. S360-G377**	**res. E365-G381**
Residues in most favoured regions (%)	100.0	81.2
Residues in allowed regions (%)	0.0	12.5
Residues in generously allowed regions (%	0.0	6.2
Residues in disallowed regions (%)	0.0	0.0
Ramachandran data - α2 helix	**res. D461-V464 (+3 His)$_{tag}$**	**res. A440-V445 (+6 His)$_{tag}$**
Residues in most favoured regions (%)	80.0	70.0
Residues in allowed regions (%)	20.0	20.0
Residues in generously allowed regions (%	0.0	10.0
Residues in disallowed regions (%)	0.0	0.0
Ramachandran data - full chain	**R355-V464 (+His-tag)**	**R359-V445 (+His-tag)**
Residues in most favoured regions (%)	48.8	51.2
Residues in allowed regions (%)	38.1	37.5
Residues in generously allowed regions (%	7.1	10.0
Residues in disallowed regions (%)	6.0	1.2