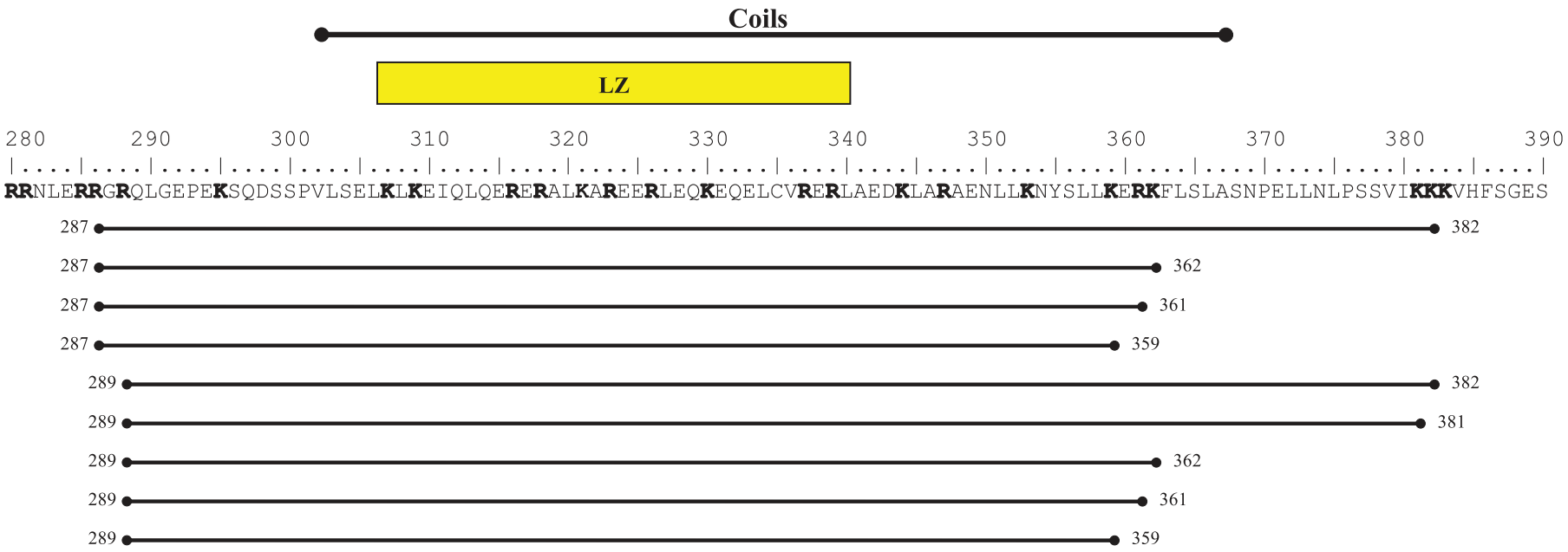

Figure S1: Diagram of the limited proteolytic fragments relating to the Nek2 leucine zipper/coiled coil region. Nek2 residues 280-390 are illustrated with tryptic sites in bold. The leucine zipper motif (LZ) and the total coiled-coil region (CC) predicted by 'COILS' are shown for reference.

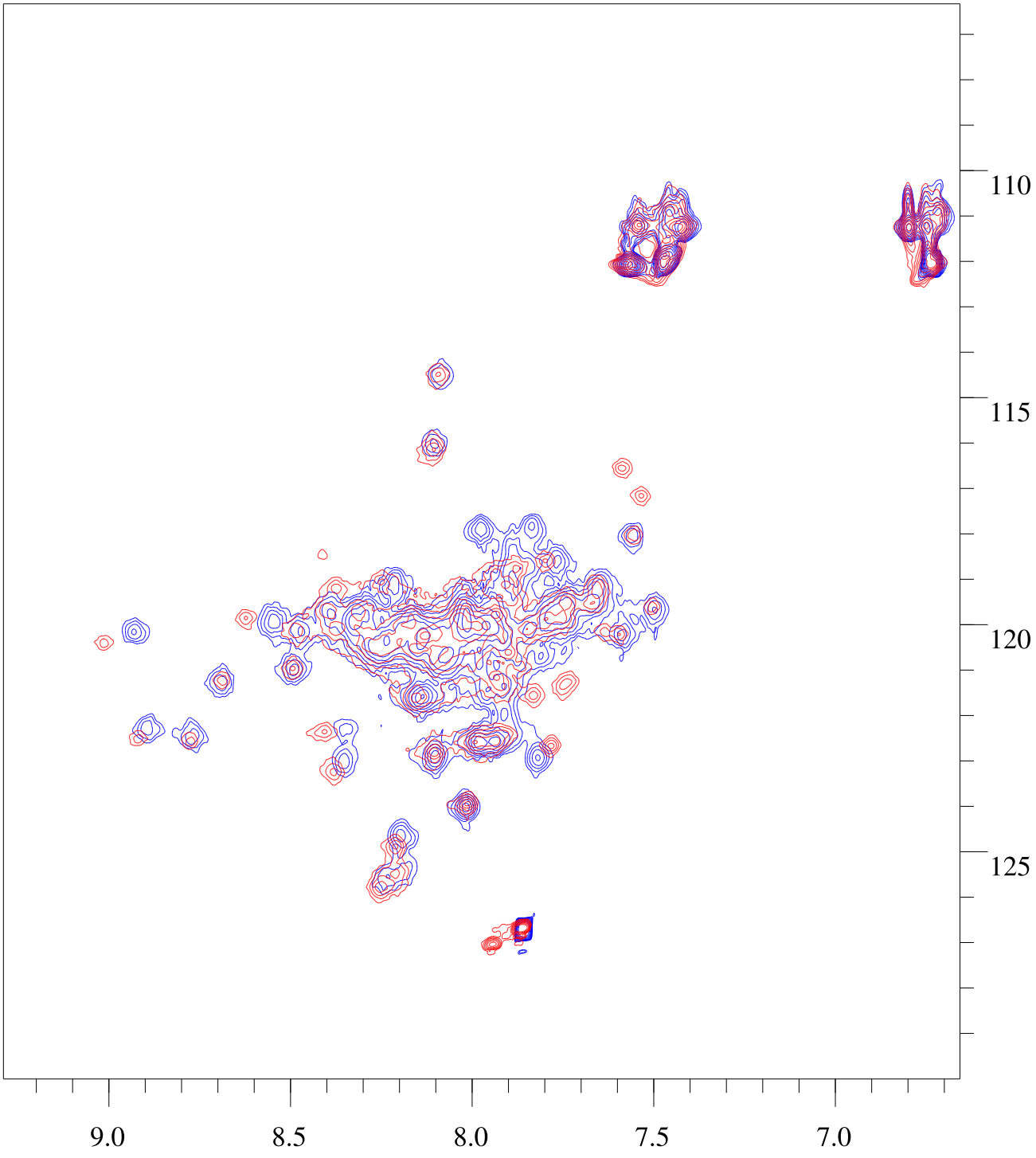

Figure S2: effects of mutations on the spectrum of LZ5
Shown is the superposition of 15N HSQC spectra of LZ5 wildtype in blue and of LZ5 C335A in red.

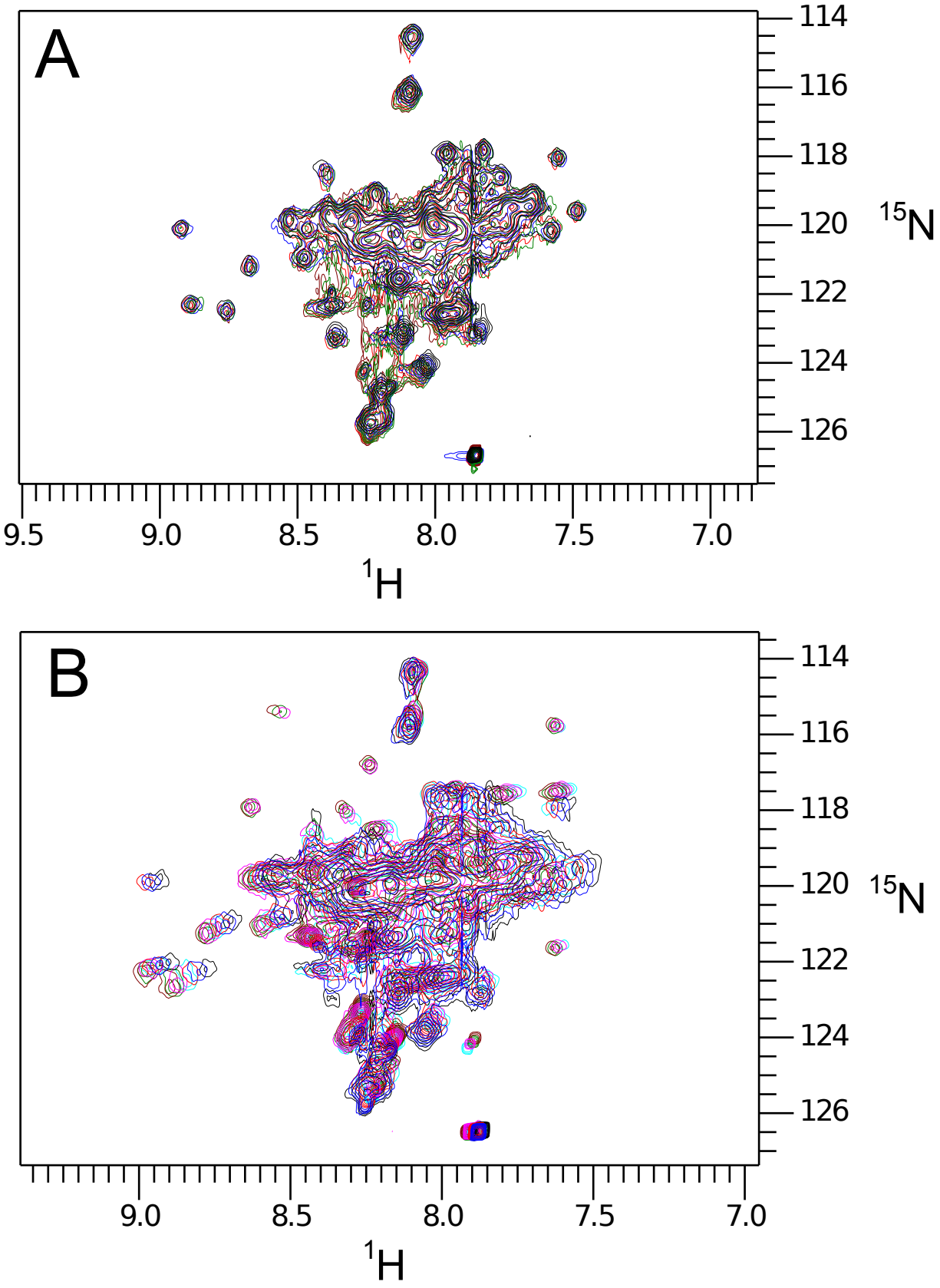

Figure S3: effects of buffer and concentration on spectra of LZ5 A: 15N HSQC spectra of LZ5 over a wide range of concentrations from 2 (black), 1 (blue), 0.5 (red), 0.25 mM (green) to 0.125 mM (brown). B: Effects of acetonitrile on the 15N-HSQC spectrum of LZ5. Black - 0%, Blue - 5%, Red - 10%, Cyan - 15%, Magenta - 20%, Green - 25%, Brown - 30%. All experiments were carried out in standard measurement buffer at pH 7.1 and 298K.

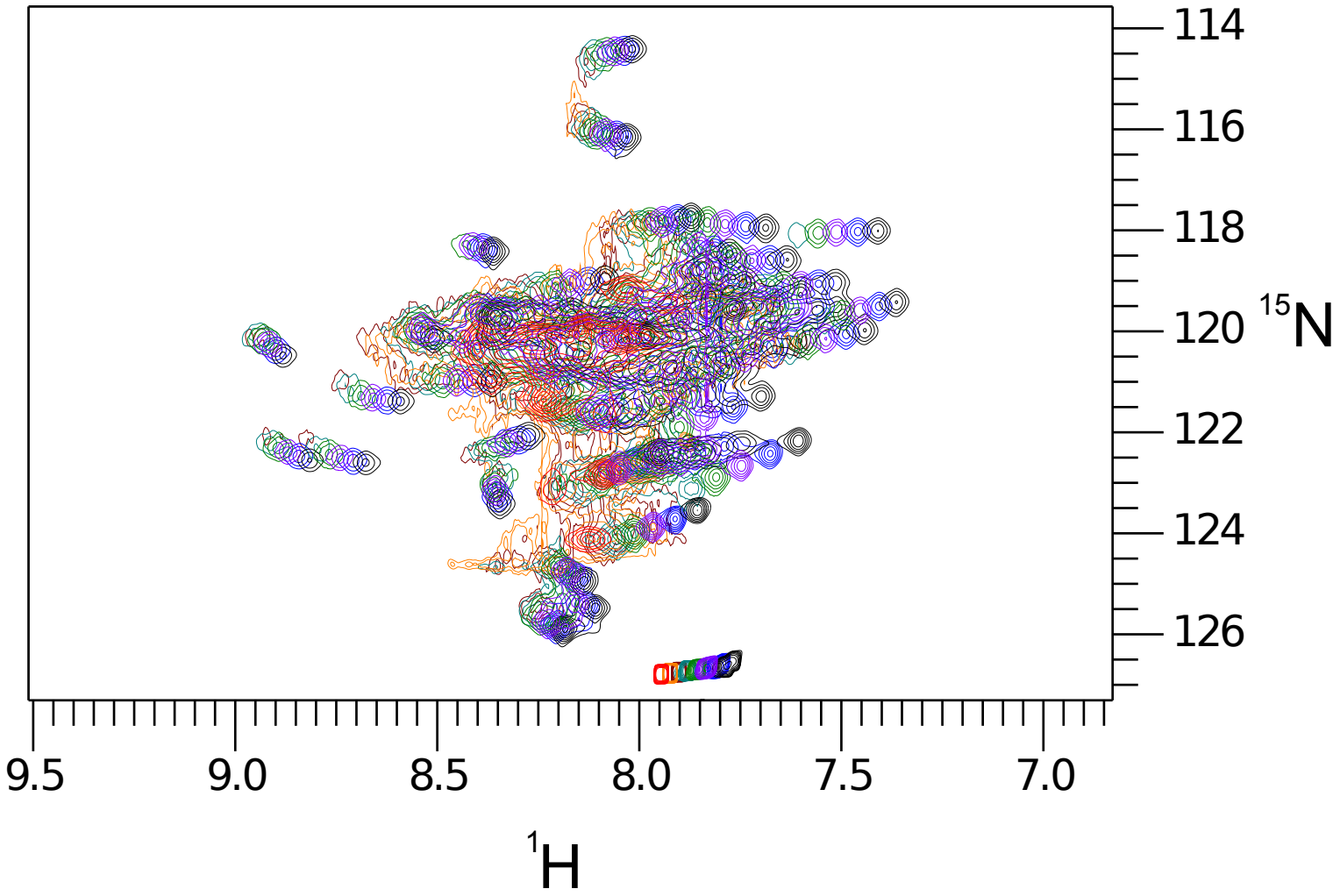

Figure S4: Temperature effects on spectra and exchange of LZ5: HSQC spectra of 0.5mM LZ5 in measurement buffer pH 7.1 were recorded at temperatures of 283K (black), 288K (blue), 293K (mauve), 298K (green), 303K (teal), 308K (brown), 313K (orange) and 318K (red).

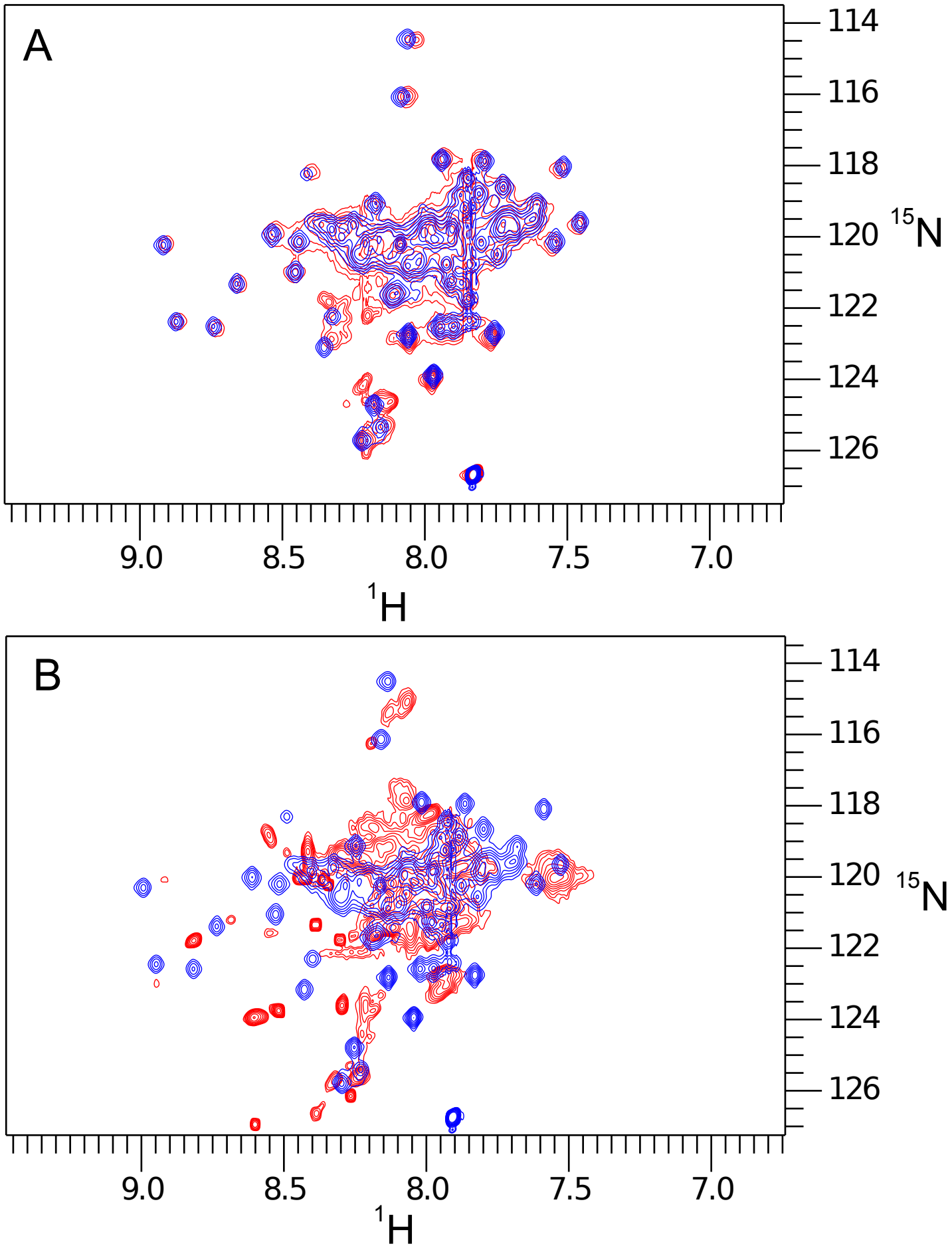

Figure S5: Buffer effects on spectra and exchange of construct LZ5 A : Effect of sucrose on the HSQC spectrum of LZ5. Blue - measurement buffer pH7.1, Red - plus 0.5M sucrose. B: pH effects on the HSQC spectrum of LZ5. Blue - measurement buffer, pH 7.1; Red - pH 3. All spectra were recorded at 298K with a sample of 1.0 mM.

Figure S6: Summary of secondary structure specific NMR data for LZ5 K309C/C335A
Secondary structure summary for the construct LZ5 K309C,C335A in non-reducing buffer. The sequence is shown at the top. The following 7 lines indicate short and medium range helix specific distances between backbone protons extracted from a 15N resolved 3D NOESY-HSQC experiment. The following 3 lines are secondary chemical shifts for alpha and beta carbons as well as alpha protons. Positive values for alpha carbons, negative values for beta carbons and alpha protons are indicative of a helical structure. These three are summarised automatically by CCPN analysis in the chemical shift index which has a value of -1 for a helix, +1 for a beta-strand and 0 for an undefined secondary structure. At the bottom of the diagram the extent of helical structure in the construct is indicated, ranging from V302 to R339.

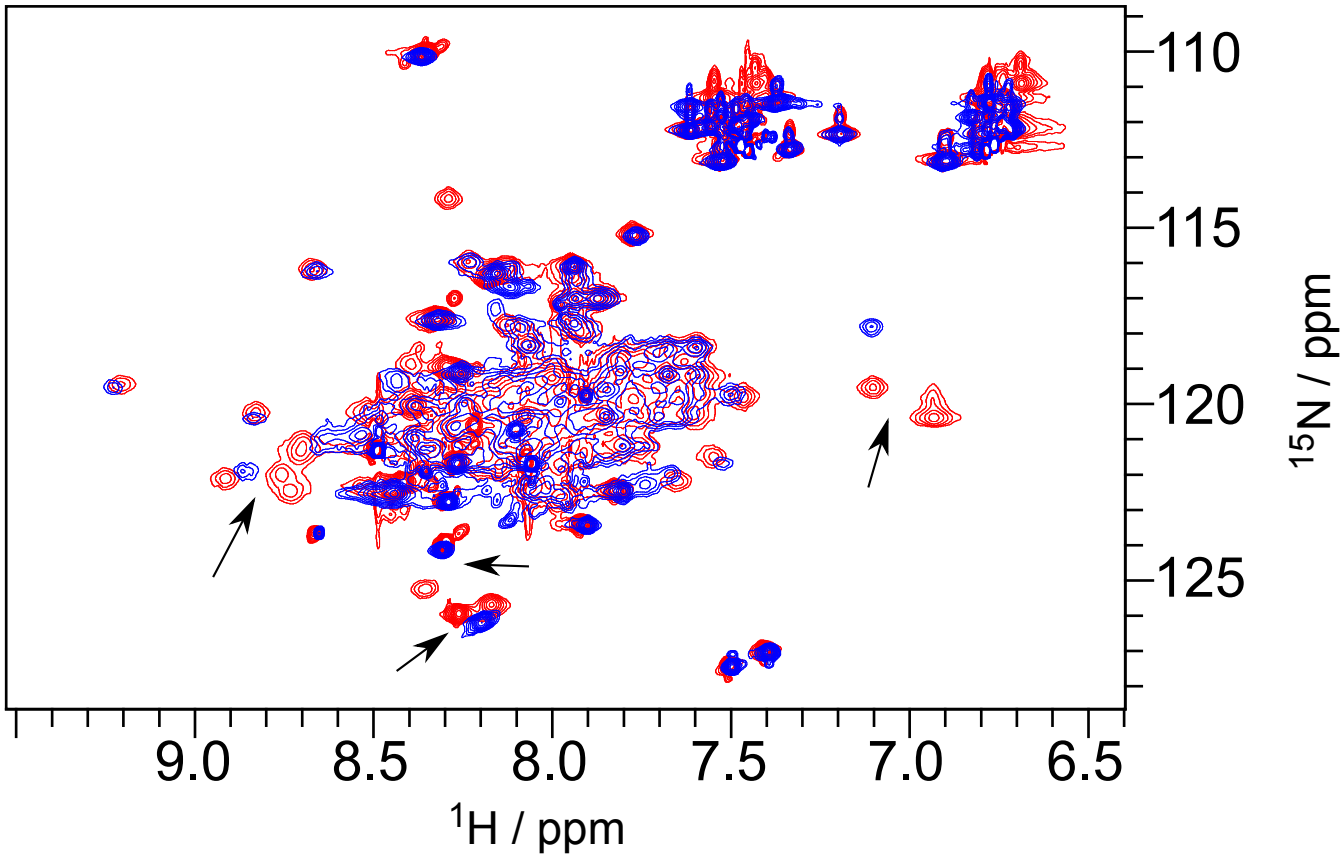

Figure S7: NMR analysis of disulphide formation in LZ0 K309C/C335A

Effect of the formation of a disulphide bond in the double mutant K309C/C335A in construct LZ0. All experimental conditions were identical to those used for LZ5 K309C/C335A. The quality of the spectra of LZ0 were generally significantly inferior to those obtained for LZ5. As a result, the reduction in number of peaks and the reduction in linewidth is not as strongly apparent as in LZ5. Nevertheless, the characteristic effects can be seen for the few well resolved resonances, some of which are indicated by arrows. Especially the pair of exchanging peaks at 8.8/122 ppm which is the easiest to monitor due to its prominent position is reduced to a single, sharp peak. The spectrum in blue is that of LZ0 K309C/C335A in non-reducing buffer, the spectrum in red is that of LZ0 wildtype.

Table S1: Potential artefacts as a cause of slow chemical exchange in the Nek2 leucine zipper

Potential cause	**Experimental evidence to the contrary:**
Degradation: intact <-> fragments	Exchange spectra are symmetric; no degradation noticed on SDS gels
Oxidation of C335 : oxidised <-> reduced	Exchange observed independent of age of sample; presence of 2 mM DTT; exchange also present in C335A mutant
End effects as result from cutting	Wide range of constructs from the shortest LZ5 (299-343) to the longest LZ0 (290-360) show evidence of the two exchanging conformations
Changes in oligomeric state	Always a dimer by AUC independent of construct or condition; exchange seen from concentrations of 50 μM to 2 mM
Exchange of folded <-> unfolded	Well resolved HN resonances of both conformers show virtually identical, extensive, helix specific crosspeak patterns
Exchange affects only part of the LZ	Number of exchanging pairs of HN resonances in 3D exchange spectra (~35) suggeststst that essentially the entire structured part of the LZ participates.
Side effect of pH, salt, temperature, solvent conditions...	Exchange identified under a wide range of conditions