Supplemental data

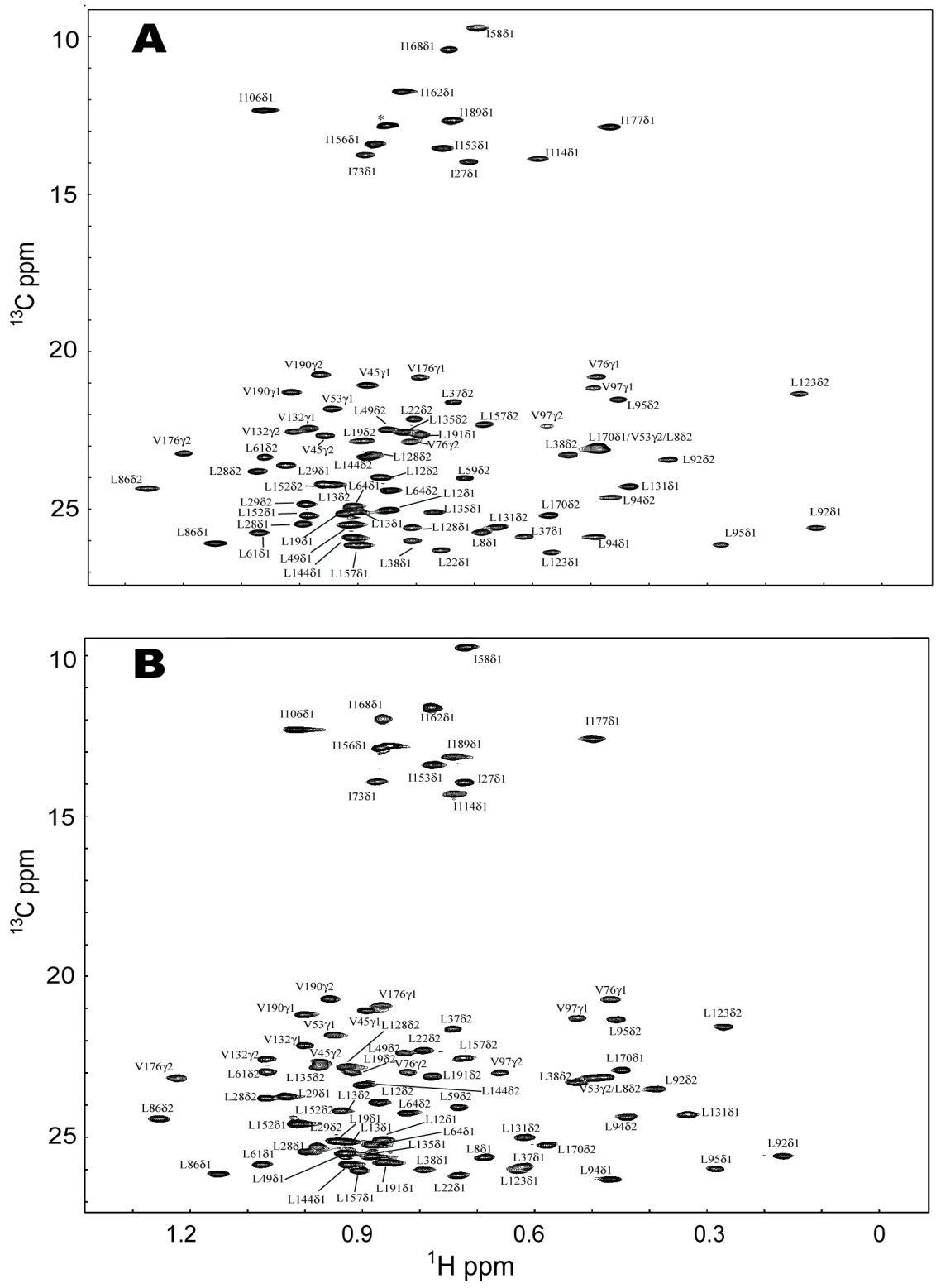
Structure calculation details

(a)Homology model preparation and metal binding coordinates A two-stage low temperature simulated annealing approach (1;2) has been employed in the structure determination for Ca^{2+}-CIB1 and Mg^{2+}-CIB1. For stage 1 of the Ca^{2+}-CIB1 structure determination, the crystal structure of Ca^{2+}-CIB1 (PDB 1XO5) was used as the starting model. After protons were added onto this crystal structure, the originally missing residues (8-11 and 137-142) were added to complete the primary sequence (res. 8-191) by using Xplor-NIH 2.18. The Ca^{2+}-ligand coordinate restraints used in structure calculation were taken from the crystal structure 1XO5 and utilized in both stage 1 and stage 2 of the structure calculation. For stage 2, the lowest energy structure of the 200 structured calculated in stage 1 was used as the new starting model, and the dihedral angle restraints were derived from this structure. The same protocol was implemented for the structure determination of Mg^{2+}-CIB1, where we used the solution structure of Ca^{2+}-CIB1 as the initial starting model. The Mg^{2+}-ligand coordination restraints for the EF-III site were essentially the same as those used for Ca^{2+}-CIB1 because the two modes of coordination are quite similar. This is consistent with simulation results for Mg^{2+}-calmodulin (CaM) (3). However, because the Glu12 residue in a canonical EF-hand loop is known to bind in a monodentate manner to Mg^{2+} (whereas Ca^{2+} binds in a bidentate manner (4)), we only used 6 coordinates for Mg^{2+}. Mg^{2+} only binds to site EF-III of CIB1 (5) which has an Asp residue in position 12 of the calcium binding loop, which is often the case for an EF-hand loop that can bind both these divalent metals. The 20 lowest energy structures obtained from 200 calculated structures in stage 2 were selected for further analysis.

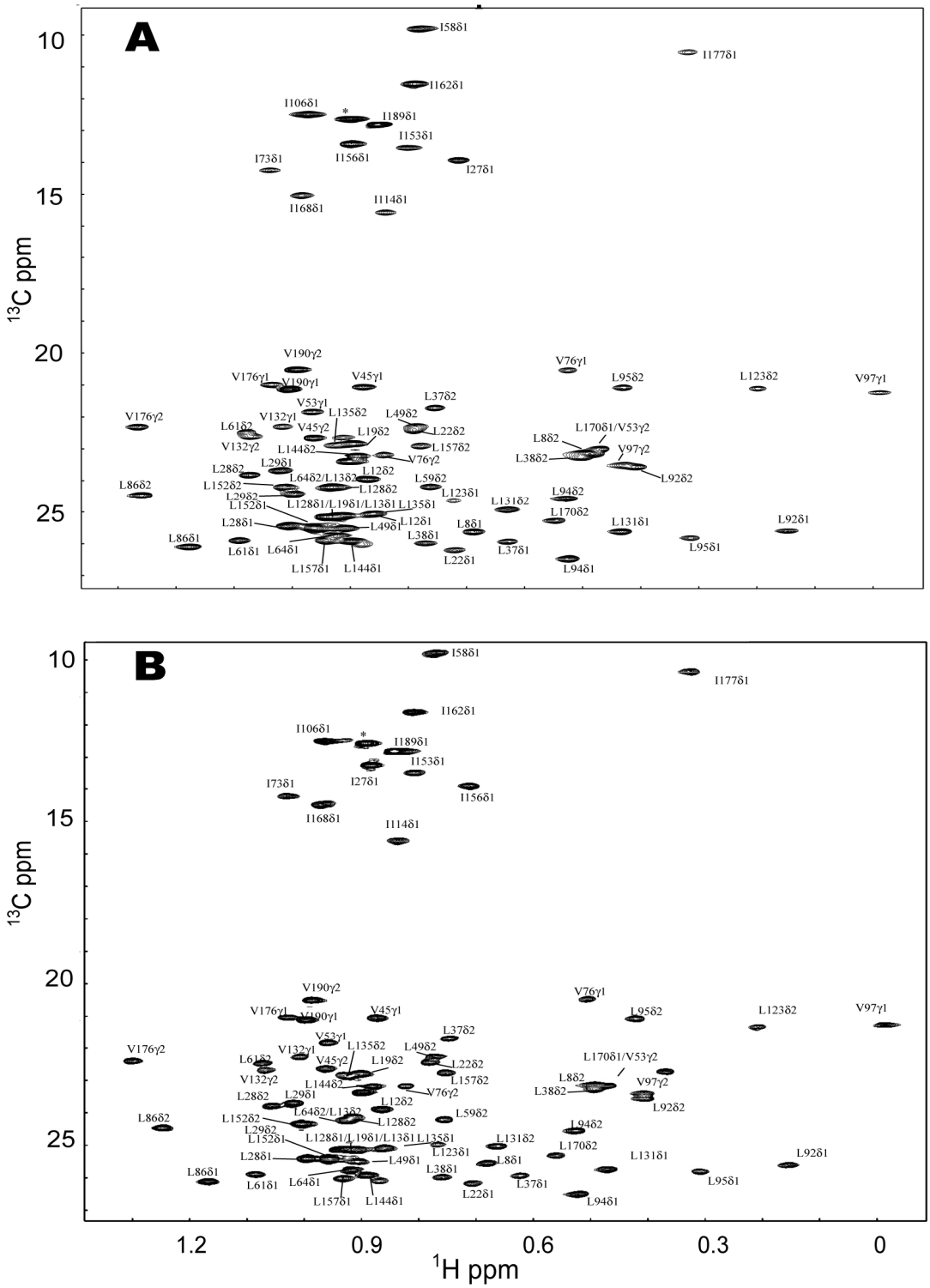
(b) *Simulated annealing protocols* This refinement protocol was initially developed to determine the structure of a protein based on the known structure of a homologous protein with same secondary structure arrangements. To avoid the RDC degeneracy problem, two stages of simulated annealing are

utilized (1). In simulated annealing stage1, cooling was achieved by lowering the temperature from 200K to 20K with each step ΔT=10K and 4 ps simulated annealing at each temperature. Experimental restraints for stage 1 include NOEs, two sets of RDCs ($^1D_{NH}$ and $^1D_{C'N}$) hydrogen bonds and metal binding coordinates. Backbone dihedral angles (φ and ψ) restraints were enforced by a strong force constant unramped at 300 kcal mol^{-1} rad^{-2}. Several other force constants (dipolar coupling, NOEs and Ramachandran potential) were ramped during the simulated annealing, in which the force constant of the dipolar coupling was ramped from 0.05 to 5 kcal mol^{-1} Hz^{-2}, the Ramanchandran force constant was ramped from 0.2 to 2.0 and the NOE force constant was ramped from 2 to 20 kcal mol^{-1} $Å^{-2}$, based on the initial protocol by Chou et al. (1). 200 structures were calculated for stage 1 and the lowest energy structure was selected as the starting model for the next stage. The structure from stage 1 still has high backbone tensions and a certain amount of dipolar energy term which needs to be lowered in stage 2. In simulated annealing stage 2, cooling was achieved by lowering the temperature from 20 K to 1 K with each step ΔT=1 K. At this stage, all the experimental restraints are the same as stage 1 except that the dihedral angle restraints were generated based on the lowest energy structure obtained in stage 1. The dihedral angle force constant was ramped down from 300 kcal mol^{-1} rad^{-2} to 50 kcal mol^{-1} rad^{-2}; other force constants were kept at the maximum values that have been reached at stage 1. Instead of using the energy term of radius of gyration, a new term called the volume of gyration (6) was implemented to improve the packing of the protein and it was kept static with a force constant of 1 kcal mol^{-1} $Å^{-3}$ throughout this two-stage simulated annealing. In the calculation, the RDC values of $^1D_{C'N}$ were normalized based on $^1D_{NH}$ RDC values.

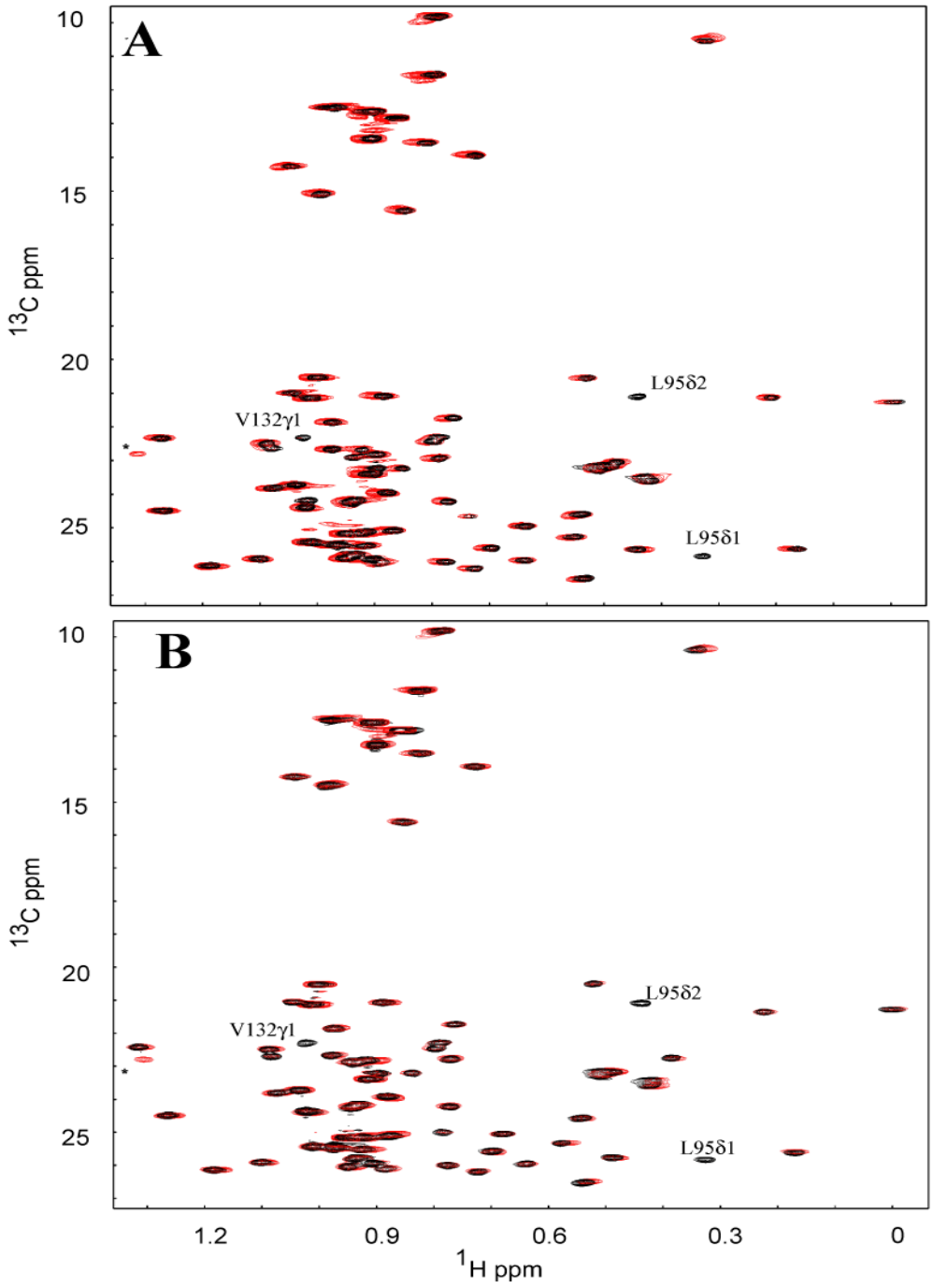
Supplemental Fig. 1: the assignment of the methyl groups (Ile/Leu/Val) of (A) Ca^{2+}-CIB1 and (B) Mg^{2+}-CIB1.



Supplemental Fig. 2: the assignment of the methyl groups (Ile/Leu/Val) of (A) Ca^{2+}-CIB1 in complex with the αIIb peptide and (B) Mg^{2+}-CIB1 in complex with the αIIb peptide.



Supplemental Fig. 3 The TEMPOL effects on the methyl groups (Ile/Leu/Val) of (A) Ca^{2+}-CIB1 in complex with the αIIb peptide and (B) Mg^{2+}-CIB1 in complex with the αIIb peptide. Black, in absence of TEMPOL; red, in presence of TEMPOL.



Reference List

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