Supplementary material *B*

The protonation state of aspartic and glutamic acids in native (Mn, Ca) concanavalin A at 0.94, 1.20 and 0.92 Å resolutions.

All the graphs that have error bars are derived from the full matrix inversion in *SHELXL*-97 and the bond lengths are derived from the CGLS refinement in *SHELXL*-97. In figure 1 the carboxyl group bond lengths (restrained) in aspartic acids for native (Mn,Ca) concanavalin A at 0.94Å resolution is shown (as reported by Deacon *et al.,* 1997; PDB code 1nls). The result shows that Asp28 is clearly protonated with weaker indication of Asp82 and Asp136. After resolution truncation to 1.20 Å (figure 2) there remains protonation indication of Asp28, Asp82 and Asp136 although degraded compared with 0.94 Å. At 1.50 Å resolution (figure 3) Asp28 surprisingly still shows an indication of protonation in spite of the truncation to 1.50 Å. When the restraints were removed at 0.94 Å resolution (figure 4) the protonation indication for Asp28 and Asp82 is clear but that for Asp136 is marginal. The determined distances for Asp28 agree closely with the expected values from the Cambridge Structural Database (CSD) values but those of Asp82 deviate somewhat from the CSD values. At 1.20 resolution (figure 5) Asp28 protonation indication remains confident but Asp82, Asp136 and Asp151 behaviour show differences from the CSD. When the resolution is truncated to 1.50 Å (figure 6), Asp28 protonation indication remains clear but Asp16, Asp58, Asp80, Asp82, Asp136, Asp151 and Asp203 could equally well be assigned 'protonated', which is incorrect. Hence, by 1.50 Å there is no confidence in the evidence that Asp28 is protonated. Figure 7 shows the carboxyl group bond lengths in aspartic acids for native (Mn,Ca) concanavalin A at 1.20 Å resolution, reported by Parkin *et al.* (1996); PDB code 1jbc. The impact of restraints here is to largely force the distances to 1.25 Å but curiously Asp82 here is still correctly showing indication of the protonation. In figure 8 the carboxyl group bond lengths in aspartic acids for native (Mn,Ca) concanavalin A at 0.92 Å resolution is shown (as reported by Price, 1999). The impact of restraints here does not alter the fact that Asp28, Asp82 and Asp136 are protonated as expected. The estimated single and double bond distances are of course slightly in error being pulled by the 1.25 Å restraint.

Figure 15 shows the carboxyl group bond lengths (restrained) in glutamic acids for Mn,Ca concanavalin A at various resolutions (Deacon *et al.,* 1997). Glu102 shows indication of protonation up to 1.20 Å resolution. When the restraints are removed (figure 16) Glu102 shows indication of protonation up to 1.20 Å resolution. Glu8 indication is not protonated up to 1.20 Å (figure 16).

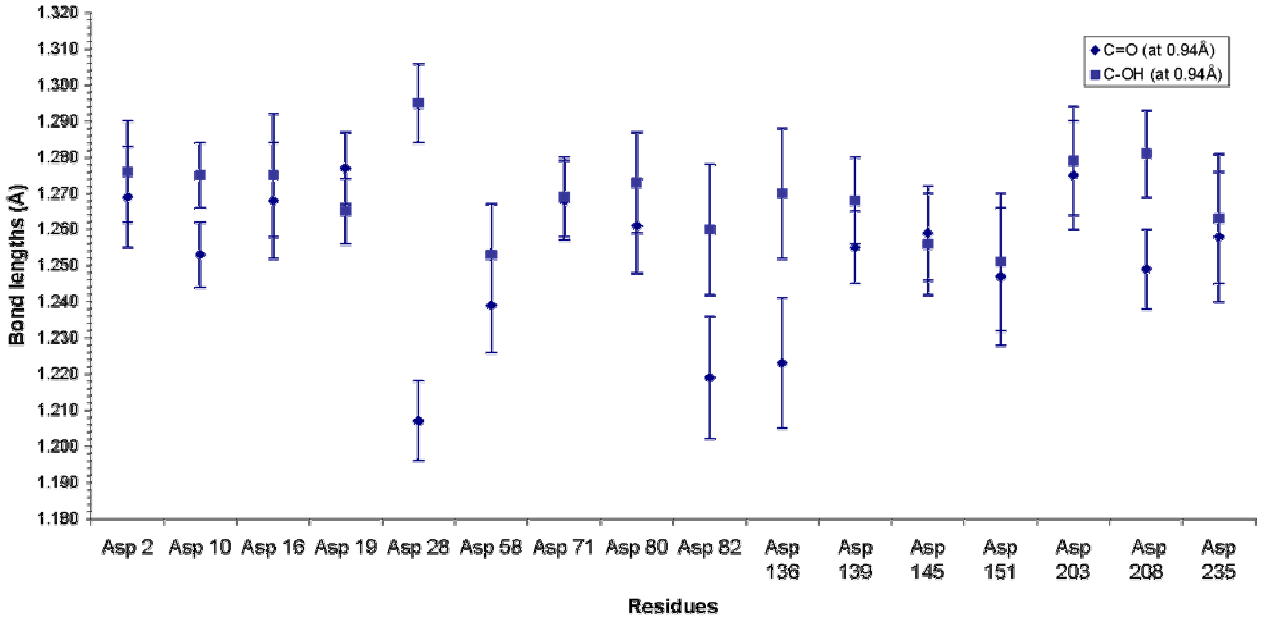

Figure 1. Carboxyl-group bond lengths (restrained) in aspartic acids for native (Mn,Ca) ConA at 0.94 Å resolution as reported by Deacon et al. (1997); PDB code 1nls. Asp82 and 136 show weaker indication of protonation.

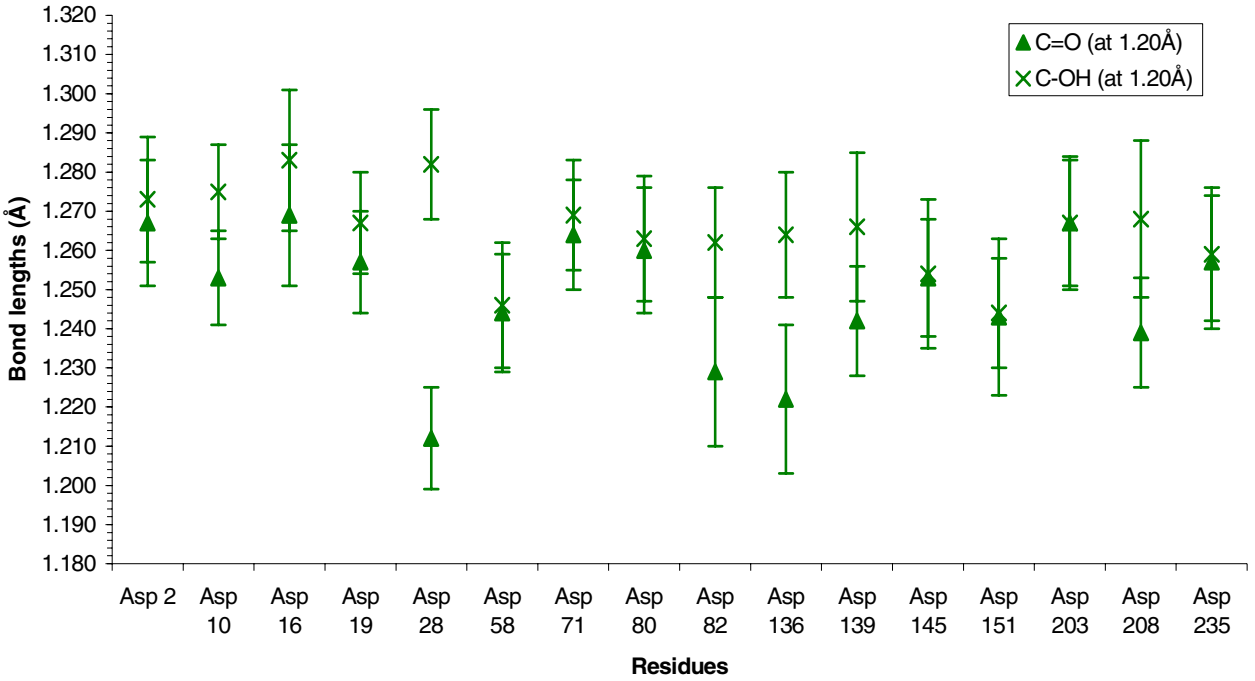

Figure 2. Carboxyl-group bond lengths (restrained) in aspartic acids for native (Mn,Ca) ConA truncated at 1.20 Å resolution. After truncation to 1.20 Å there remains marginal protonation indication although degraded compared with 0.9 4Å.

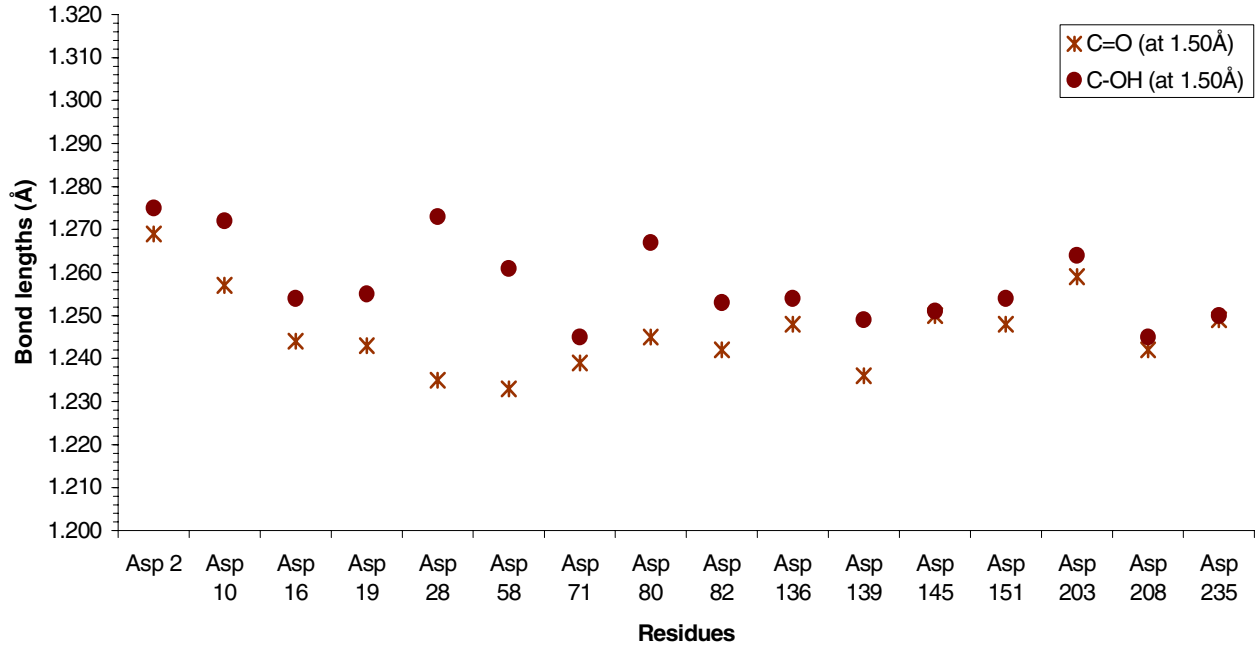

Figure 3. Carboxyl-group bond lengths (restrained) in aspartic acids for native (Mn,Ca) ConA truncated at 1.50 Å resolution. Surprisingly Asp28 still shows an indication of protonation in spite of the truncation to 1.50 Å.

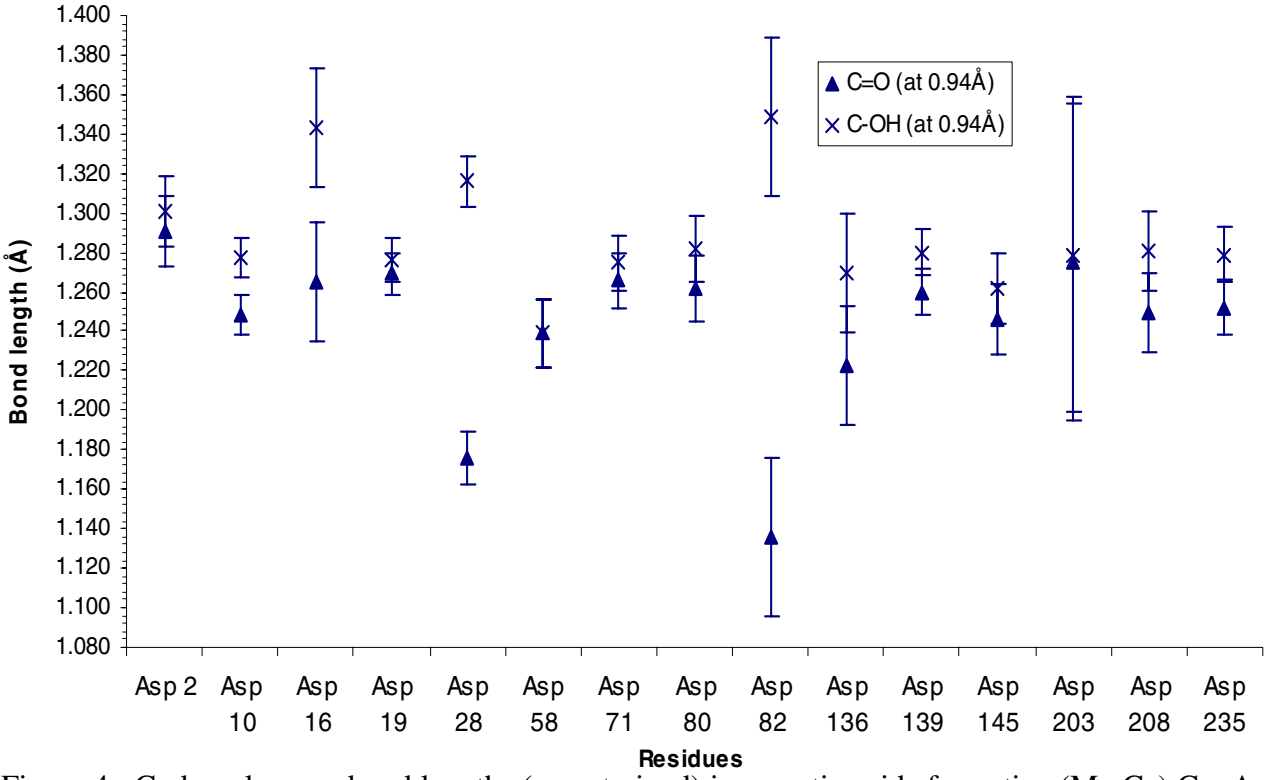

Figure 4. Carboxyl-group bond lengths (unrestrained) in aspartic acids for native (Mn,Ca) ConA at 0.94 Å resolution. With restraints removed the protonation indication for Asp28 and sp82 is clear but that for Asp136 is not reproduced. The determined distances for Asp28 agree closely with expectation values from the Cambridge Structural Database (CSD) values but those of Asp82 deviate somewhat from the CSD values. Asp151 has been omitted due to large bond length values which distorts the *y* scale.

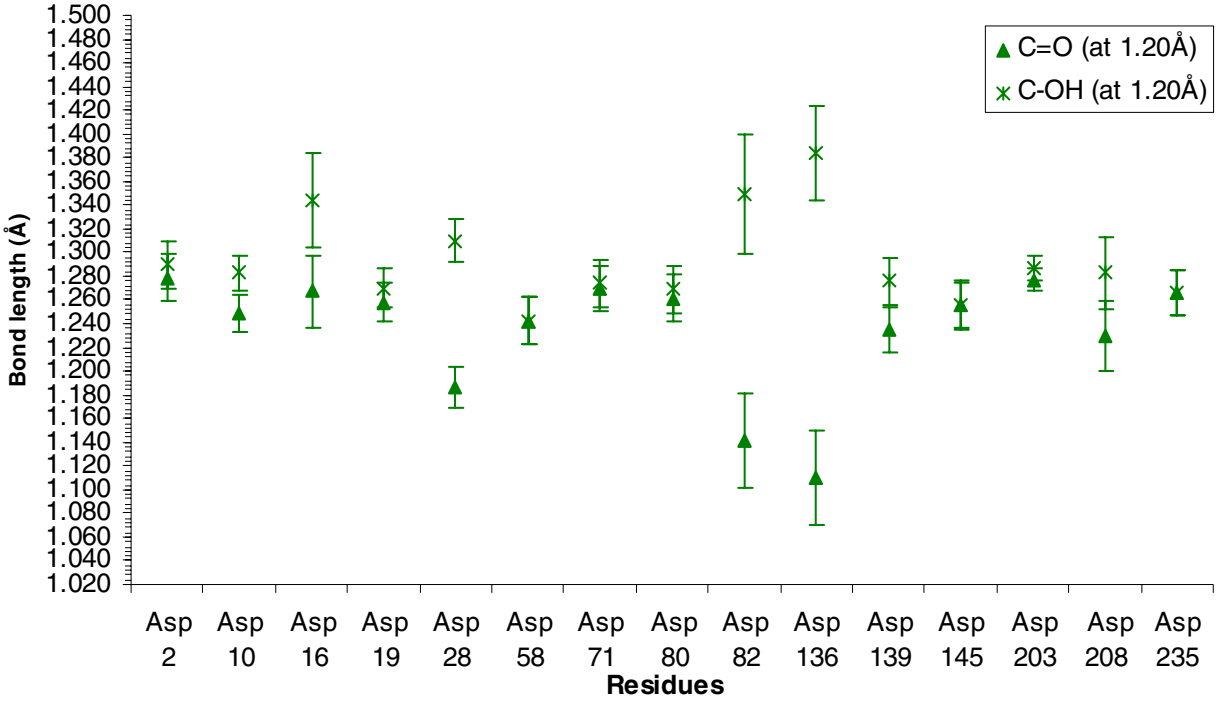

Figure 5. Carboxyl-group bond lengths (unrestrained) in aspartic acids for native (Mn,Ca) ConA truncated at 1.20 Å resolution. Asp28 protonation indication remains confident but the behaviour of Asp82 and Asp136 show differences from the CSD. Asp151 has been omitted due to large bond length values which distorts the *y* scale.

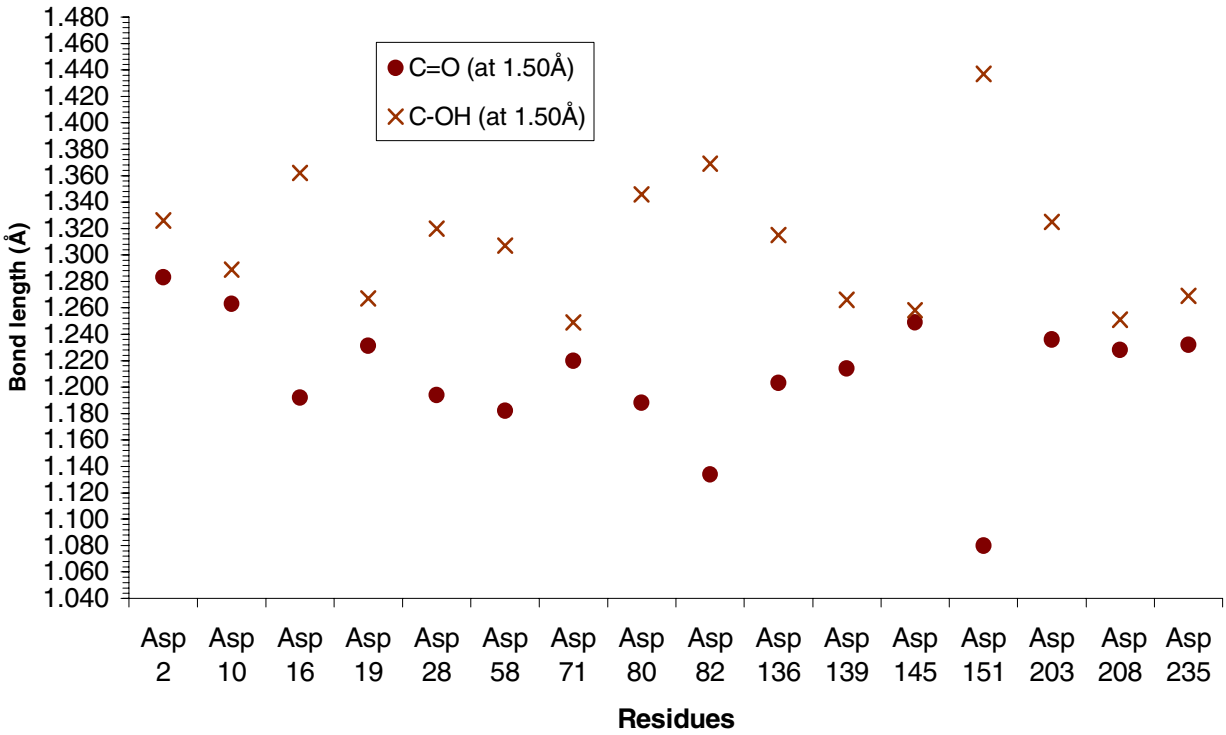

Figure 6. Carboxyl-group bond lengths (unrestrained) in aspartic acid for native (Mn,Ca) ConA truncated at 1.50 Å resolution. Asp28 protonation indication remains clear but Asp16, 58, 80, 82, 136, 151, and 203 could equally well be assigned 'protonated', which is incorrect and unlikely; their behaviour means that the evidence for Asp28 being protonated is now compromised.

Previous work performed by Parkin et al. (1996) and Price (1999) on Mn,Ca concanavalin A

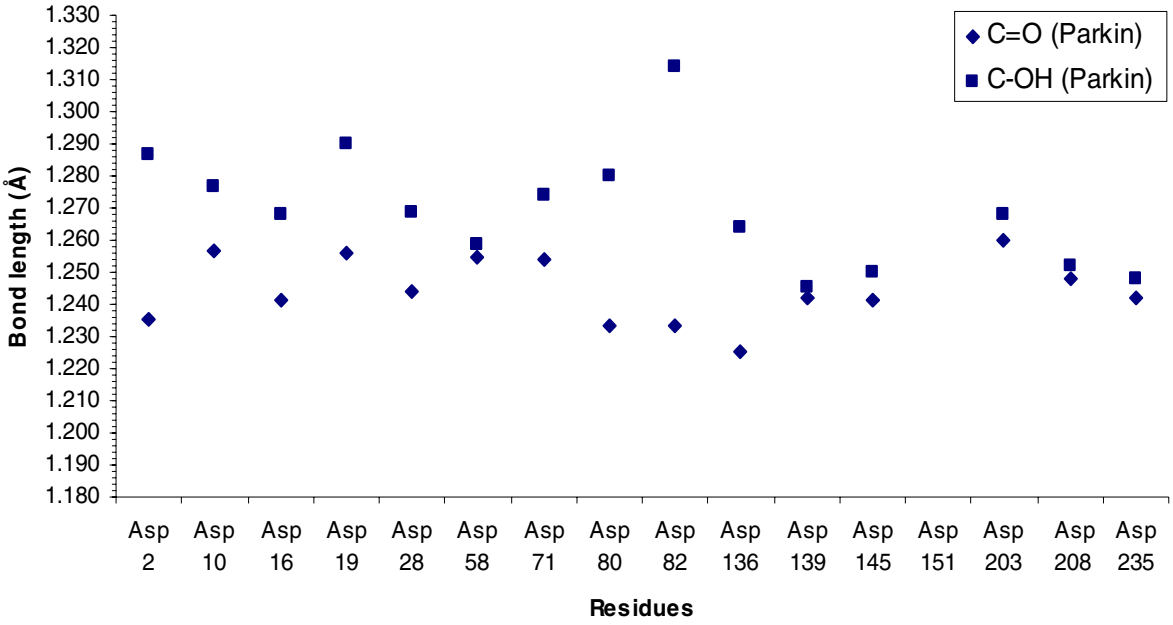

Figure 7. Carboxyl-group bond lengths in aspartic acids for native (Mn,Ca) ConA at 1.20 Å resolution; as reported by Parkin et al. (1996); PDB code 1jbc. The impact of restraints here is to largely force the distances to 1.25 Å but curiously Asp82 here is still correctly showing indication of the protonation. Asp151 is in double conformation and therefore not plotted here.

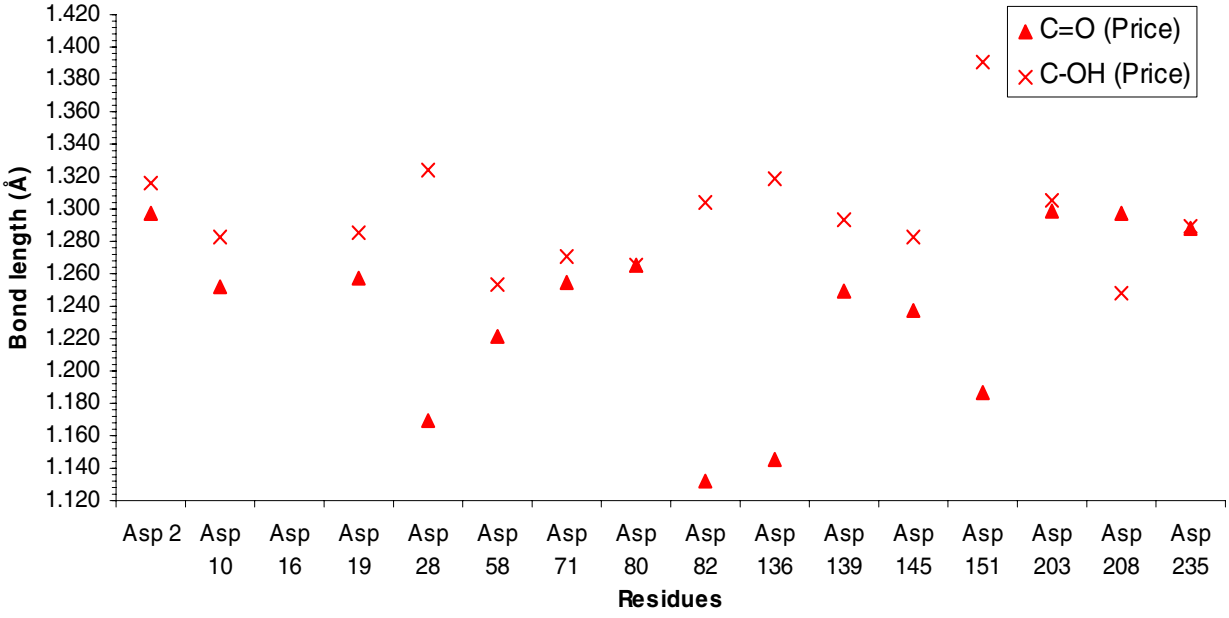

Figure 8. Carboxyl-group bond lengths (restrained) in aspartic acids for native (Mn,Ca) ConA at 0.92Å resolution as reported by Price (1999). The impact of restraints here does not alter the fact that Asp28, 82 and 136 are protonated as expected. The estimated single- and double-bond distances are of course slightly in error being pulled by the 1.249 Å restraint in *SHELXL*-97.

Ni,Ca concanavalin A

The protonation state of aspartic and glutamic acids in Ni,Ca concanavalin A at 0.94 Å resolution

Figure 9 shows the carboxyl-group bond lengths in aspartic acids for Ni,Ca concanavalin A at 0.94 Å resolution. Asp28 and Asp82 show protonation, whereas Asp136 shows weaker signs of protonation. At 1.20 Å resolution (figure10) Asp28 and Asp82 show weaker indications of protonation, whereas at 1.50 Å resolution (figure 11) the protonation indications for Asp28 and Asp82 are now lost. When the restraints are removed at 0.94 Å resolution (figure 12), Asp28 and Asp151 are close to ideal bond-length values, whereas surprisingly Asp82 and Asp136 are not. At 1.20Å resolution (figure 13) Asp28 bond lengths are still close to the ideal values. At 1.50 Å resolution (figure 14) Asp28 bond lengths become closer in their estimated bond distance, i.e. the protonation state has not been indicated.

Asp82 was initially assigned with double conformation. However, the refinement program (*SHELXL*-97) could not refine the occupancies as the output atomic coordinates for the alternative conformation had high *B*-factors and no $2F_o$ - F_c electron density (contoured at 1σ). The bond lengths exhibited were those of a carboxylate group. After removing the double conformation, further cycles of refinement were performed and the F_o - F_c electron density, which was separate from the 2 F_o - F_c, became smaller. The bond lengths of the carboxylate group were now significantly different indicating protonation (a carboxylic group), and the appearance of F_o - F_c electron density (at 3σ, after unrestrained refinement) adjacent to Oδ1 further supported the fact that it was protonated (see main paper, section 1.1, figure 1b). Overall, this residue was a marginal case for being considered a double conformation side chain; treating it as a predominantly single occupancy was possible and with that the protonation state determination became possible.

In figure 17 the carboxyl group bond lengths (restrained) in glutamic acids for Ni,Ca concanavalin A at various resolutions are shown. Only Glu102 shows indication of protonation, and as Glu8 is involved in a hydrogen bond with Asp28, it is not protonated. This is also seen in the other plots. When the resolution is truncated to 1.20 Å, Glu102 still shows indication of protonation even at 1.50 Å resolution (marginally). When the restraints are removed (figure 18) Glu122 had to be excluded from the graph because the bond lengths were beyond the ideal lengths, consequently distorting the *y* scale. We see that Glu102 retains its protonation evidence up to 1.20 Å resolution, whereas at 1.50 Å the bond lengths exceed the ideal lengths.

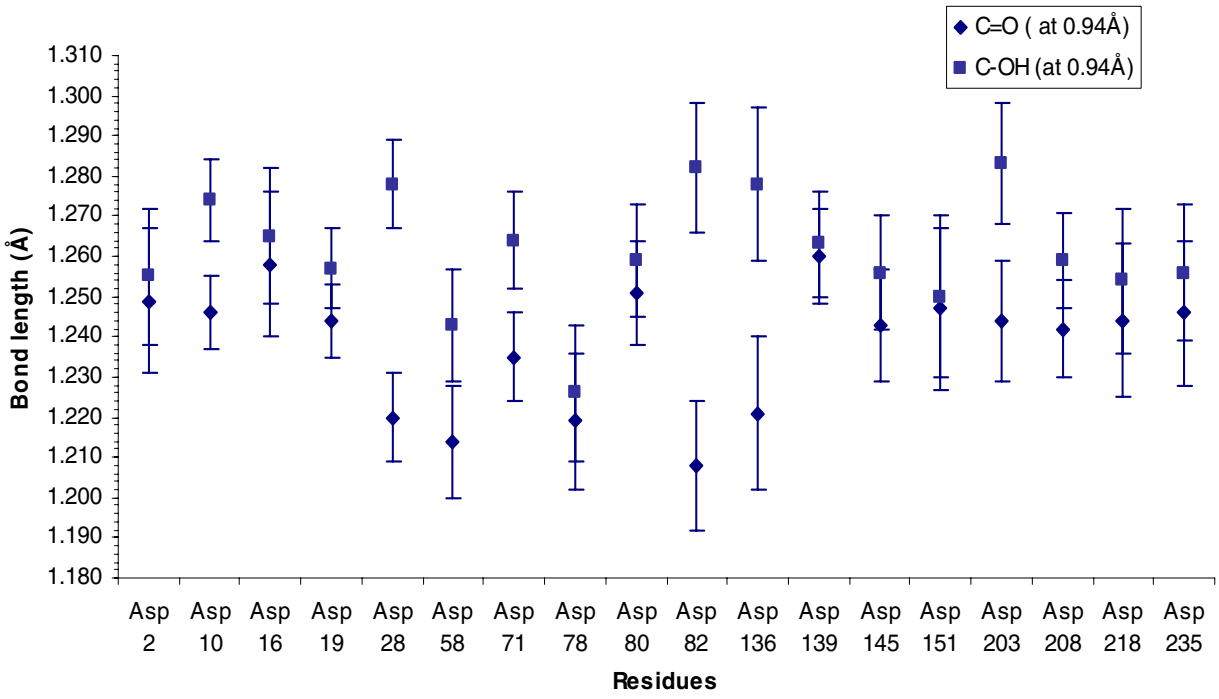

Figure 9. Carboxyl-group bond lengths (restrained) in aspartic acids for Ni,Ca ConA at 0.94 Å resolution. Asp 28 and Asp 82 show protonation. Asp136 shows weaker indication of protonation state. This figure is the same as Figure 5(*a*) in the main paper text but is shown again here for ease of comparison.

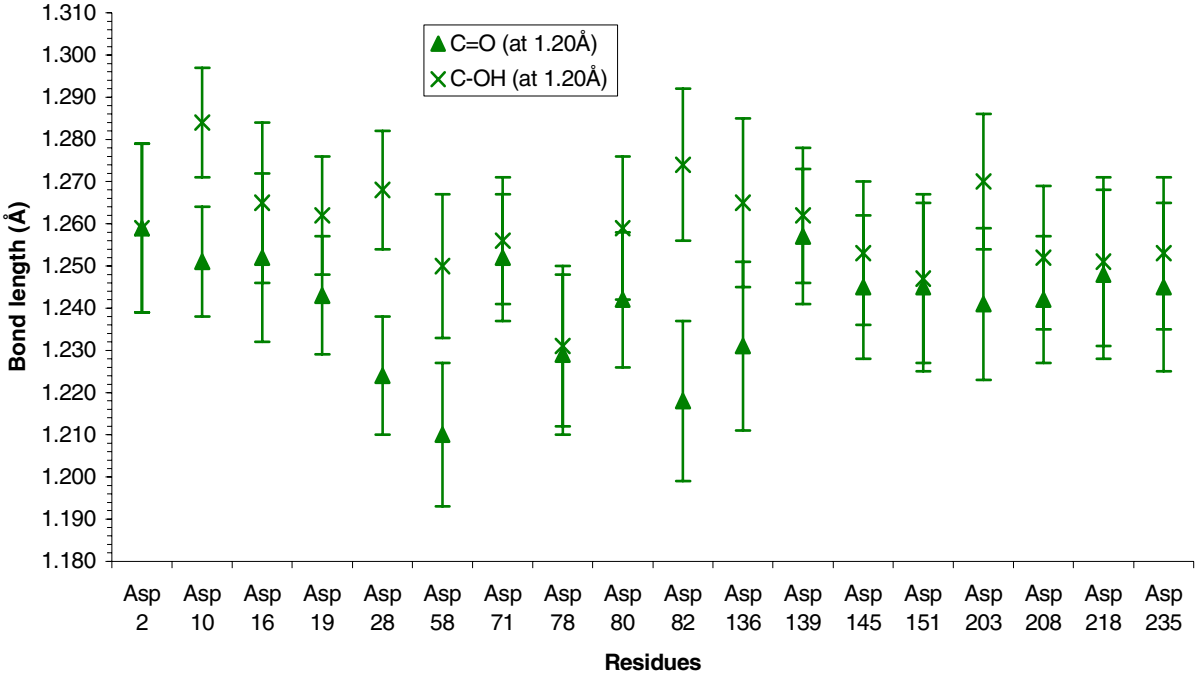

Figure 10. Carboxyl-group bond lengths (restrained) in aspartic acids for Ni,Ca ConA truncated at 1.20 Å resolution. Asp28 and Asp82 show weaker indication of protonation.

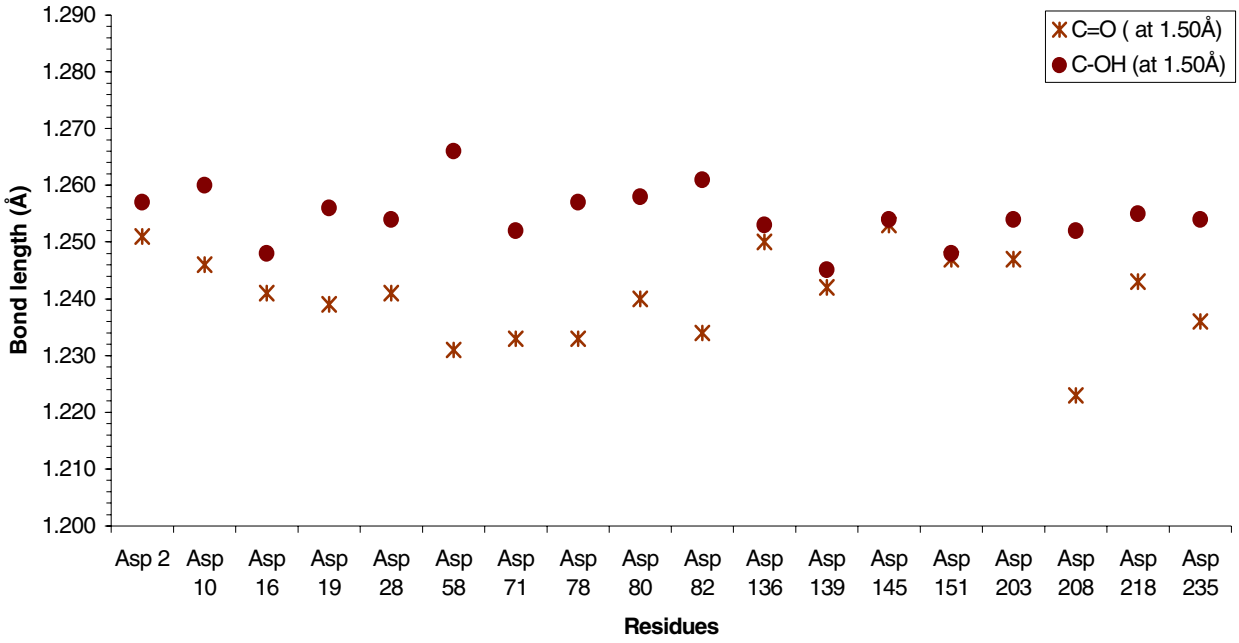

Figure 11. Carboxyl-group bond lengths (restrained) in aspartic acids for Ni,Ca ConA truncated at 1.50 Å resolution. The protonation indications for Asp28 and Asp82 are now lost

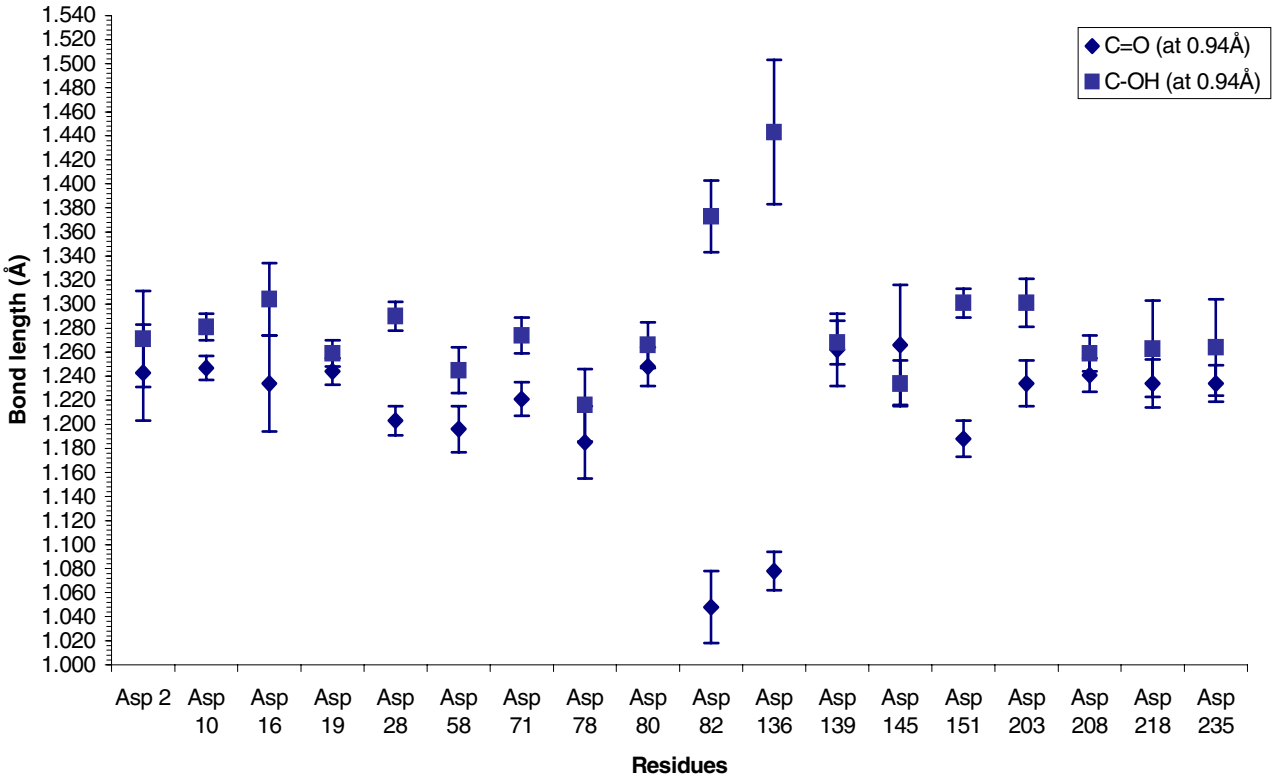

Figure 12. Carboxyl group bond lengths (unrestrained) in aspartic acids for Ni,Ca ConA at 0.94Å resolution. Asp28 and 151 are close to ideal bond length values whereas surprisingly Asp82 and

136 are not.

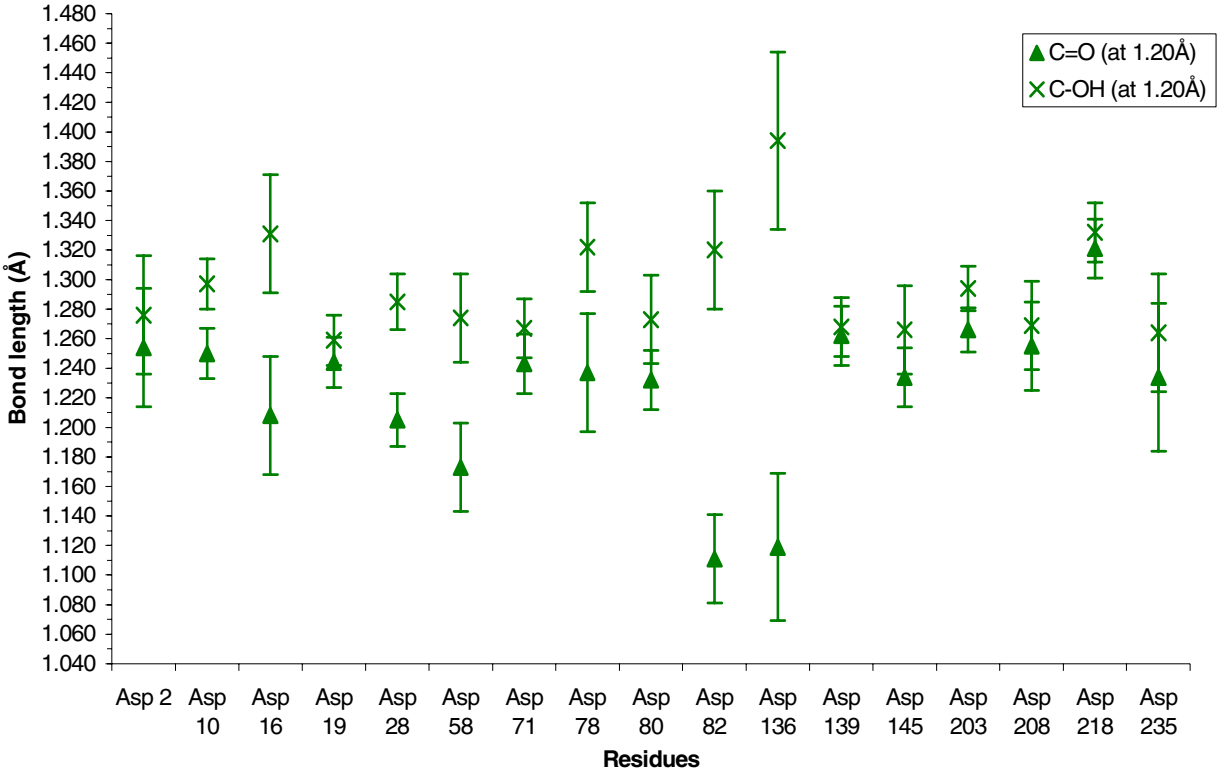

Figure 13. Carboxyl-group bond lengths (unrestrained) in aspartic acids for Ni,Ca ConA truncated at 1.20 resolution. Asp28 distances are still close to ideal values. Asp151 has been omitted due to large bond length differences which distorts the *y* axis.

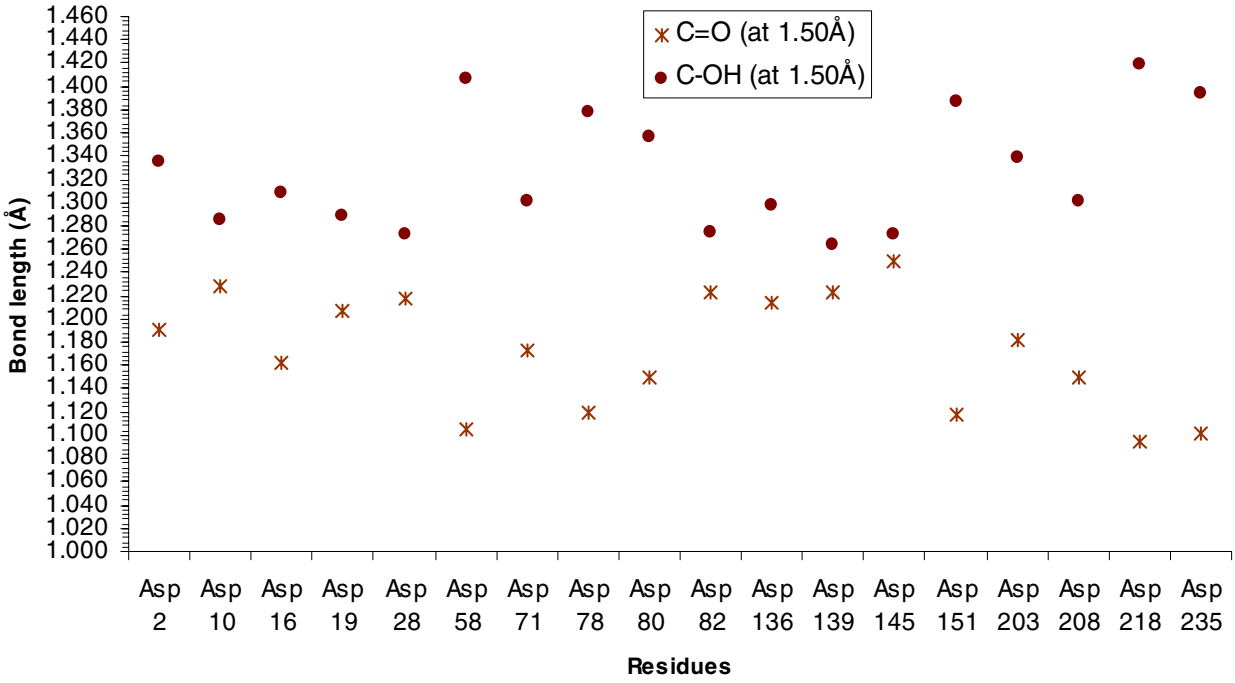

Figure 14. Carboxyl-group bond lengths (unrestrained) in aspartic acids for Ni,Ca ConA truncated at 1.50 Å resolution. Asp28 distances are now not indicating clearly enough the protonation and the other residues also confuse the picture.

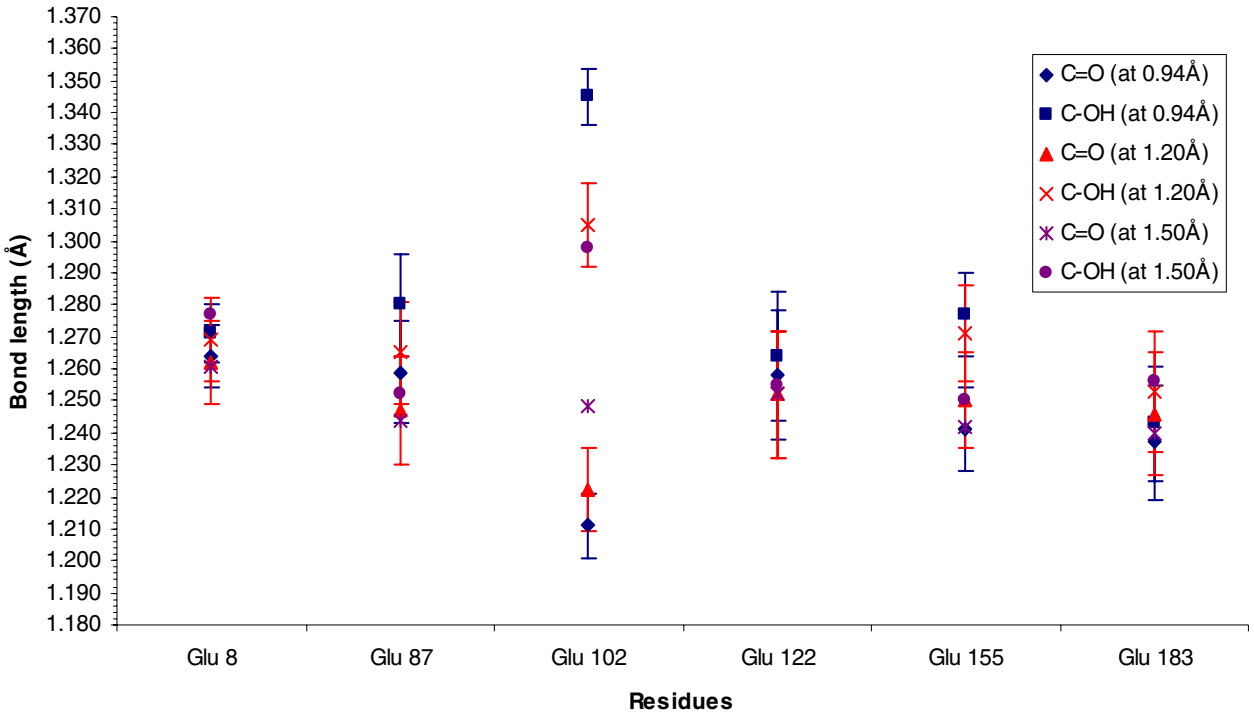

Figure 15. Carboxyl-group bond lengths (restrained) in glutamic acids for Mn,Ca ConA at various resolutions (Deacon et al., 1997). Glu102 shows indication of protonation up to 1.20 Å resolution, even with the restraints in place.

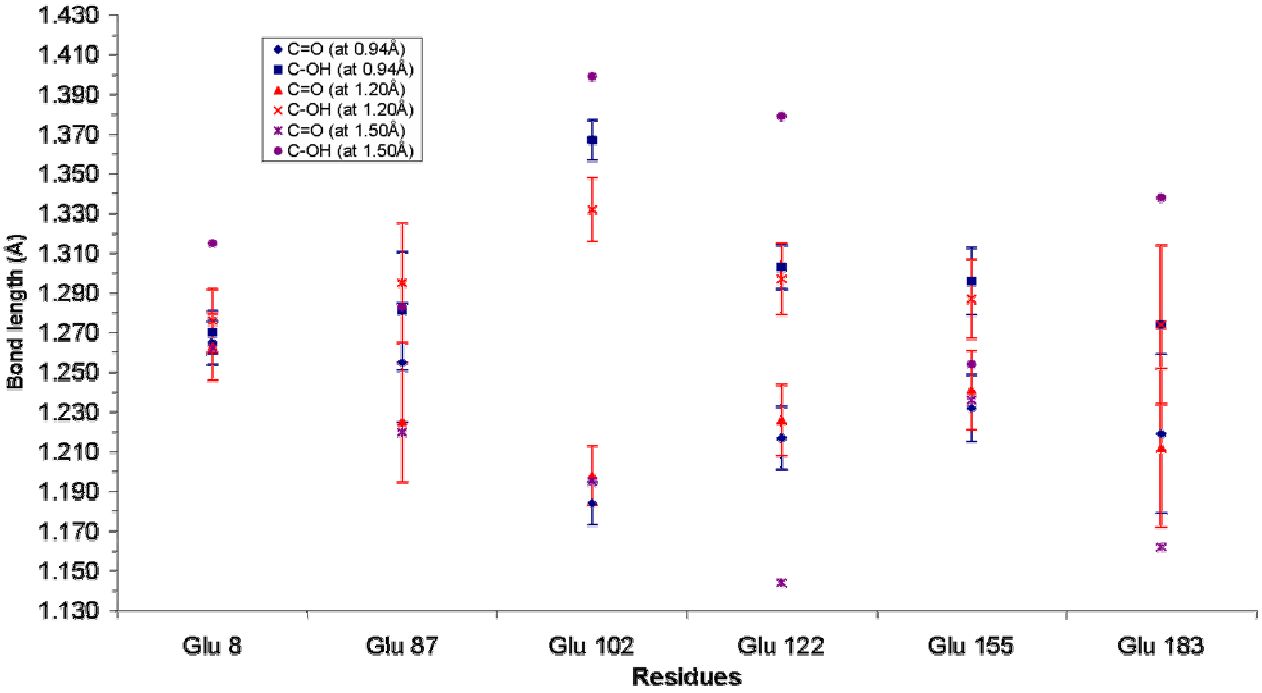

Figure 16. Carboxyl-group bond lengths (unrestrained) in glutamic acids for Mn,Ca ConA at various resolutions (Deacon et al., 1997). Glu102 shows indication of protonation up to 1.20 Å resolution; its behaviour at 1.50 Å is clearly anomalous. Glu8 is again clearly indicated as not protonated up to 1.20Å.

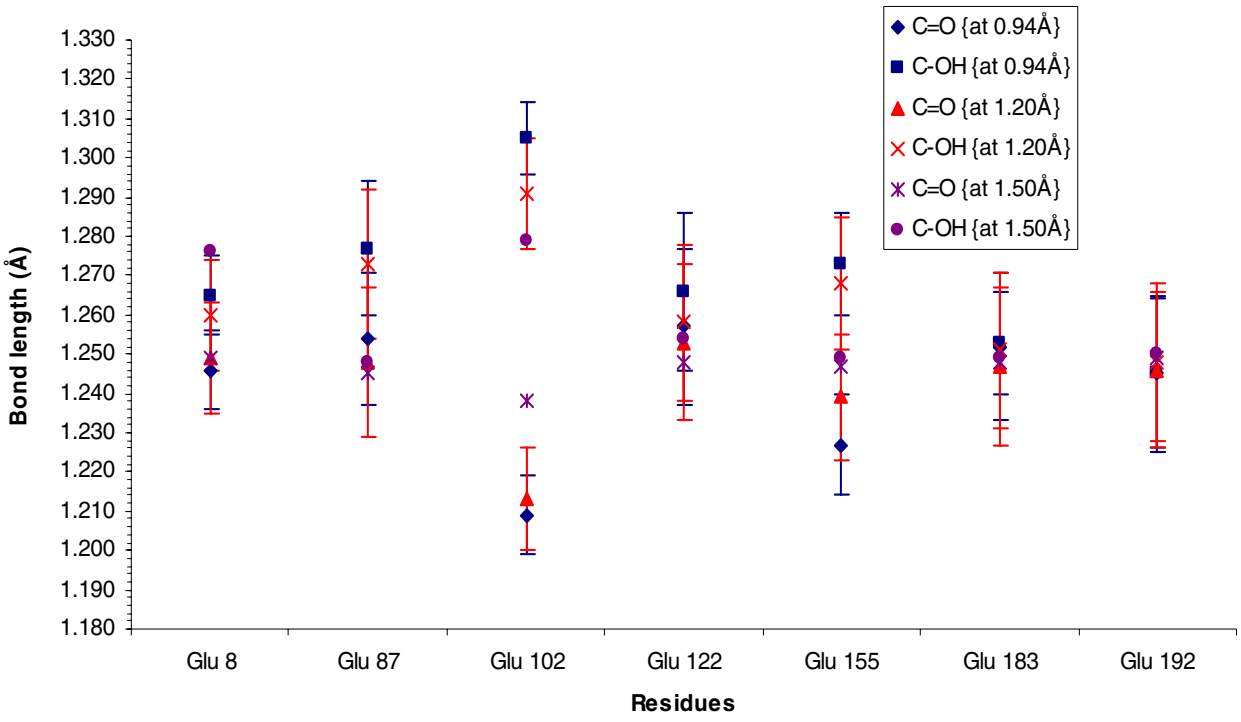

Figure 17. Carboxyl-group bond lengths (restrained) in glutamic acids for Ni,Ca ConA at various resolutions. Glu102 shows good indication of protonation. Glu8 is involved in a hydrogen bond with Asp28 and therefore it is not protonated as is also clearly indicated; at 1.50 Å the evidence is marginal.

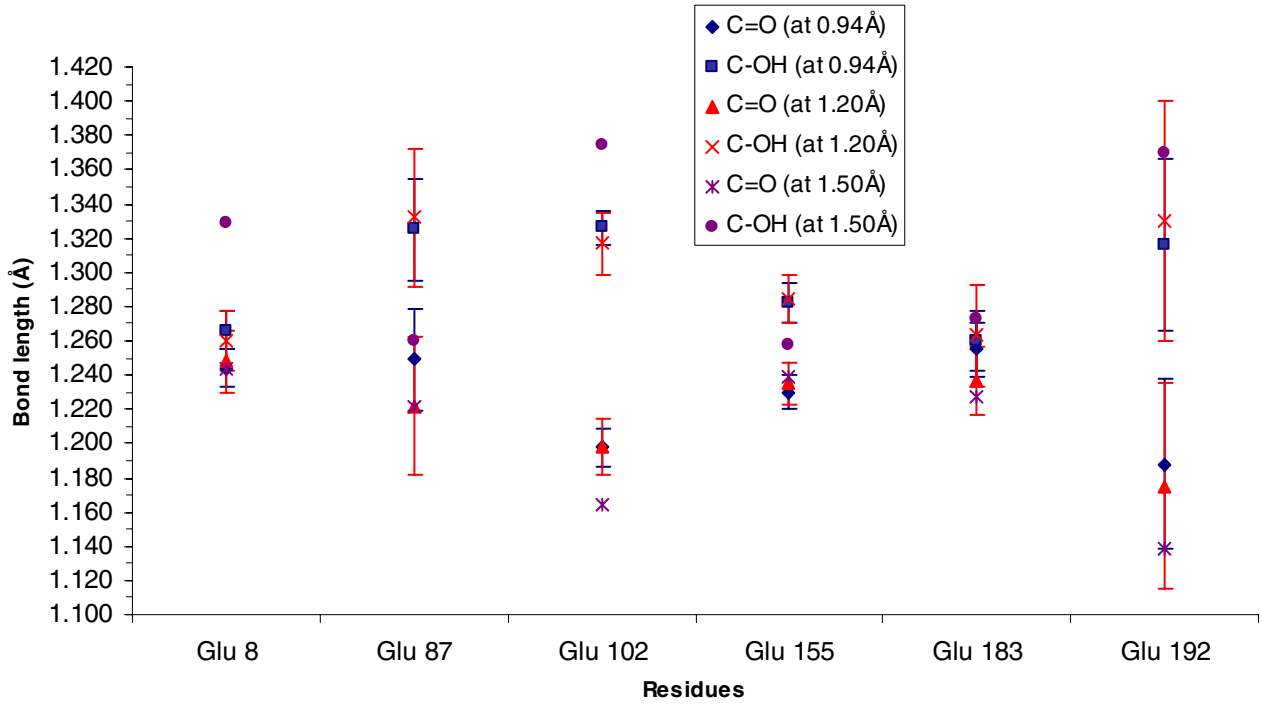

Figure 18. Carboxyl-group bond lengths (unrestrained) in glutamic acids for Ni,Ca ConA at various resolutions. Glu102 again shows good indication of protonation up to 1.20 Å resolution. Glu122 has been excluded from the graph because the bond lengths were beyond the ideal lengths,

consequently distorting the y scale.