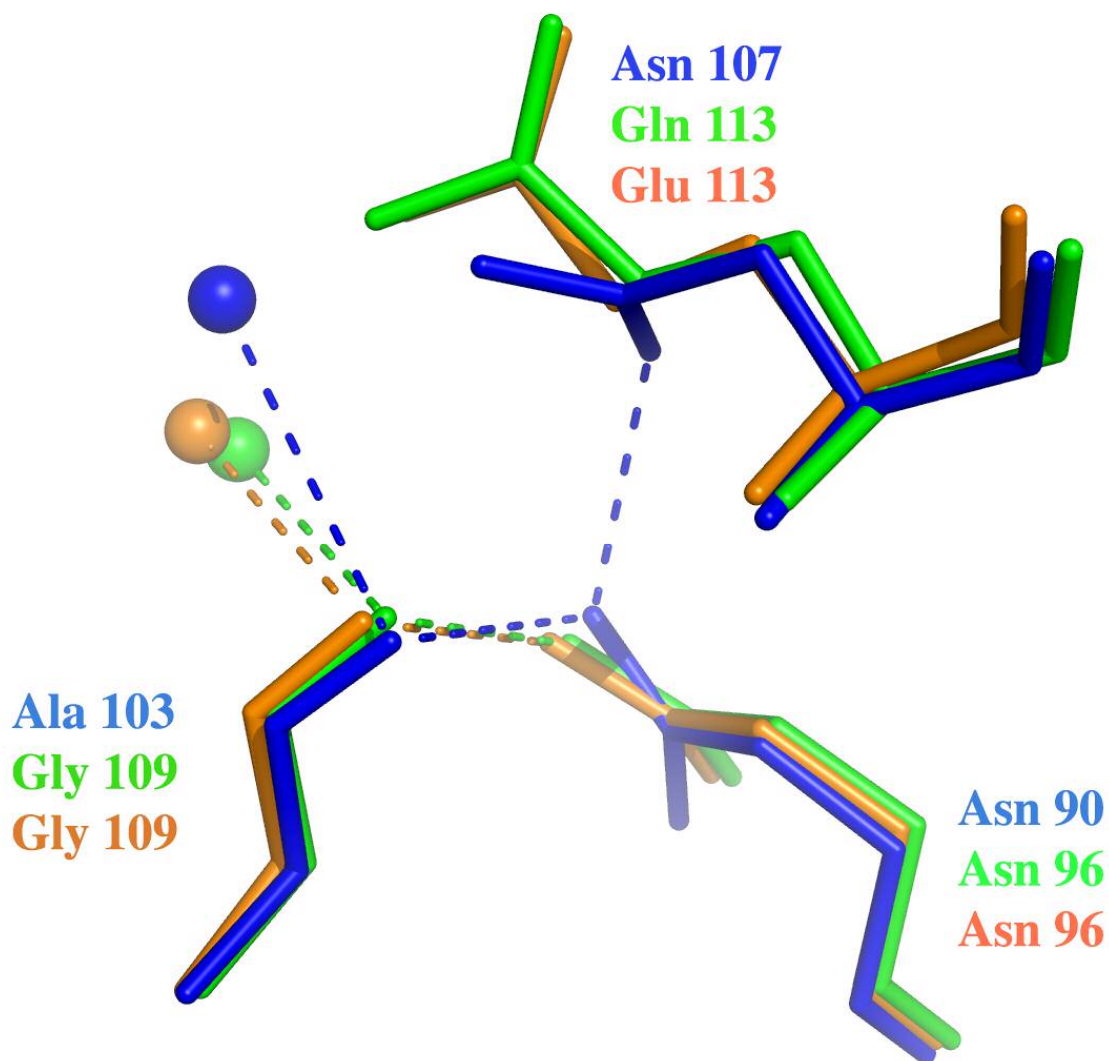


### Figure S1

Residues Asn 90, Ala 103 and Asn 107 of *AxNiR* (blue) superimposed with their equivalents from *AcNiR* (green) and *AfNiR* (orange). Water molecules are shown as spheres. In *AxNiR*, a hydrogen bond is present between Asn 90 and Asn 107. In the green *NiRs*, Asn107 is replaced by Gln 113 (*AcNiR*) or Glu (*AfNiR*) and this bond is abolished. However, in the green *NiR* structures a reorientation of the Asn 90 (96) side chain together with the substitution of Ala 103 (*AxNiR*) with Gly 109 (*AcNiR* and *AfNiR*) allow a hydrogen bond between Asn 90 (96) and the carbonyl of Gly 109 which is in turn hydrogen-bonded to a water molecule leading to bulk solvent. Note that in *AxNiR* the Asn 90-Ala 103 and Ala 103–water distances are extended and this route is unlikely to be efficient for electron transfer.



**Figure S1**