Improving coiled-coil stability by optimizing ionic interactions

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Sequences of parallel left-handed coiled-coil proteins are characterized by a heptad repeat pattern of seven amino acids denoted **a** to **g** harboring mostly apolar residues in their **a** and **d** positions [1]. Stability of coiled coils is achieved by the distinctive packing of the side chains of the amino acids in the **a** and **d** positions into a hydrophobic seam, called "knobs-into-holes" packing, which was first postulated by Crick. However, sometimes even relatively long heptad-repeat-containing polypeptide segments derived from stable coiled-coil domains fail to associate into coiled coils. In this context, it has been shown that distinct coiled-coil trigger sites within heptad-repeat-containing amino acid sequences may be necessary to mediate coiled-coil formation and the coiled-coil trigger site of the actin bundling protein cortexillin I was shown to harbor a distinct inter- and intrahelical salt bridge pattern in addition to the hydrophobic interactions occurring along the dimer interface [2]. Intrahelical salt bridges can stabilize monomeric α-helices while interhelical salt bridges specify the proper alignment and orientation of the coiled coil and provide additional stability to the coiled-coil oligomer which is primarily hold together by the hydrophobic seam. Accordingly, the design principles for a shortest possible coiled-coil peptide can be divided into factors contributing to either monomeric or to oligomeric α-helical stability. We have recently designed a two-heptad repeat long coiled-coil which is stabilized by a complex network of salt bridges in addition to an optimal hydrophobic seam [3]. Using this parent peptide as a template we have now extended our design into a more detailed analysis of ionic interactions in coiled coils in order to test and improve coiled-coil stability of these *de novo* designed peptides.

To compare the high-resolution features of the novel optimized *de novo* designed coiled coil with the parent peptide, we determined the X-ray structure of a novel peptide at 1.45 Å resolution. Also the structure of the parent peptide was solved as native peptide from a crystal which was not soaked in trimethyl lead acetate (cf. ref [3]). The structure of the novel peptide was solved by molecular replacement using the polyalanine model of the parent peptide as a search model. The crystal form of the novel peptide contains only one monomer in the asymmetric unit. The coiled-coil trimer is built up from the three-fold crystallographic symmetry in this space group. This implies that the coiled coil of the novel peptide displays perfect three-fold symmetry while the parent peptide was found to be slightly asymmetric with respect to its coiled-coil trimer axis. But apart from that the packing of the hydrophobic core residues is virtually identical between the two peptides as is the **g-e' Arg to Glu** interhelical ionic interaction between Arg 6 and Glu 11.

Using these structural data in combination with biophysical analyses of these peptides we have demonstrated that ionic interactions can improve coiled-coil stability considerably. In particular, the interhelical salt bridge of the **g-e' Arg to Glu** type strongly stabilizes the oligomeric structure, while the intrahelical salt bridges stabilize the monomeric α-helix. The **i to i+3 Glu - Arg** and **i to i+4 Arg - Glu** types seem to be more stabilizing than the opposite arrangements **i to i+3 Arg to Glu** and **i to i+4 Glu to Arg** due to a better ionic interaction because of the specific geometry of the α-helix. Combining as many optimal inter- and intrahelical ionic interaction together with an optimal hydrophobic seam (Leu in **d** position and Ile in **a** position) and ideal helix capping boxes we were able to design an improved two-heptad repeat long peptide which forms a very stable α-helical coiled coil.

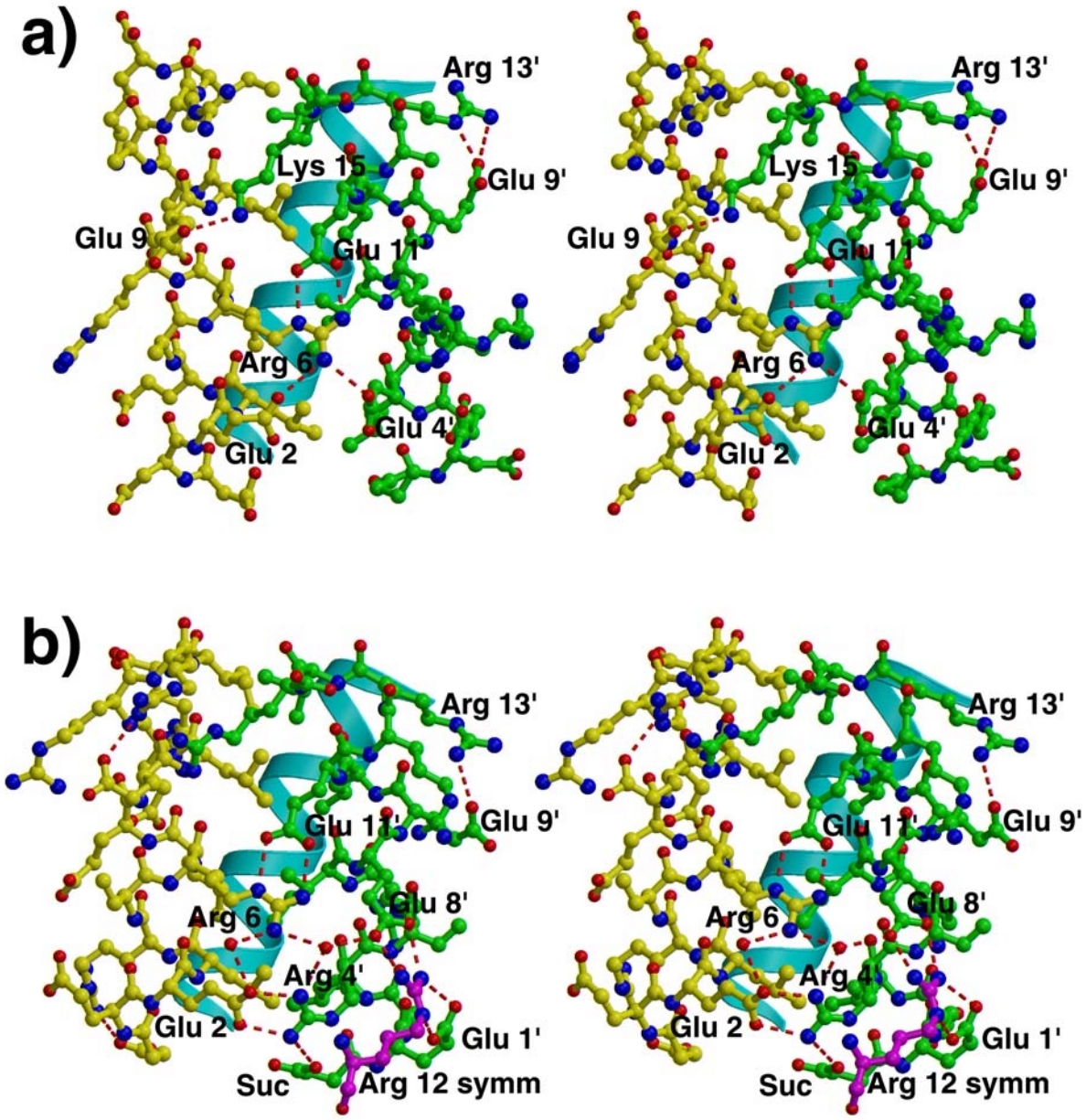

Figure 1: Structural comparison of the parent peptide (**a**) with the novel peptide (**b**). For two helices all atoms are drawn as ball-and-stick model colored according to atom type with the carbon atoms in yellow and green, respectively. The third helix of the trimer is depicted as a sketch in cyan. The ionic interactions are drawn as dashed red lines.

References

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