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# ABSTRACT

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This thesis focuses on the structural characteristics of macromolecules such as DNA, peptides, and proteins. The structure of three DNA sequences and a peptide is discussed and also the problem of understanding how proteins fold into a native structure. In total, five projects are discussed.

The structure of the palindromic sequence 5' CTC(tC)ACGTGGAG with the tricyclic cytosine base analogue tC in position 4 was determined by NMR. Correct and specific Watson-Crick base pairing of the base and retained B-DNA conformation for the duplex are verified.

The hairpin sequences from two strains, human (HSV-1) and horse (EHV4) of the Herpes Simplex virus, were studied by NMR and were found to adopt normal B-DNA structure except for an A-G mismatch in the middle of the stem for HSV-1.

The *de novo* designed helix-loop-helix peptide GTD-43ce was uniformly labelled with  $^{15}\text{N}$  and  $^{13}\text{C}$  by expression in *Escherichia coli*. The homodimer formed in solution was structurally characterised by NMR. Isotope-filtered NOESY experiments were performed but an almost total lack of observed intermolecular contacts in combination with the indirect detection of conformational exchange from relaxation experiments suggest that the peptide has stable secondary structure elements but exhibit conformational exchange.

The folding behaviour of the apo azurin mutant C112S, with and without core mutations introduced, is probed by equilibrium tryptophan fluorescence and folding kinetics. A two state model describes the folding, and the folding rate is shown to correlate with the folding free energy. Fractional  $\Phi$  values were observed for three of the core mutants. This in combination with a possible ground state effect for the unfolded state suggests that the removed hydrophobic interactions are important in the unfolded state as well as in the transition state.

Cyt *c'* was studied under native as well as non-native conditions using fluorescence resonance energy transfer between the heme group and a tryptophan placed at two different positions. The distance measured for the native proteins correspond well to the distance measured in the crystal structures, while in the unfolded state, equilibrium between more or less compact structures is observed. Also, folding kinetics studied by tryptophan fluorescence as well as heme absorbance reveals a sequence of events; the upper (non-heme coordinating) part of the four helix bundle is formed rapidly, followed by the lower part and last is the coordination of the heme group to reach the native structure.

**KEYWORDS:** Azurin; Cytochrome *c'*; *de novo* design; DNA; fluorescent base analogue; NMR; protein folding; tryptophan fluorescence.

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