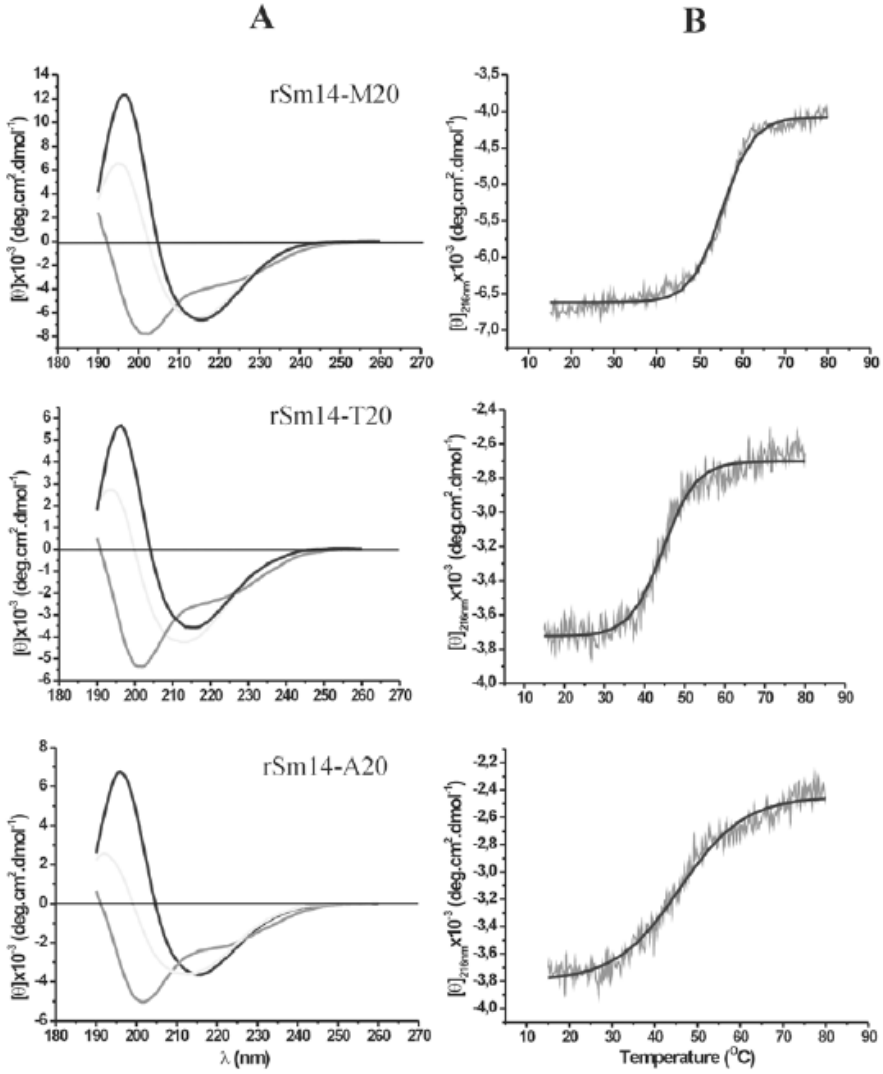
Biomeasurement technologies: CD spectroscopy

Exercise 1.

The protein Sm14 is a lipid-binding protein and a tentative vaccine candidate against parasites Fasciola hepatica and Schistosoma mansoni. In this experiment, the researchers investigate if the protein could be stabilized by mutations to make it more suitable for vaccine trials. The effects of three separate point mutations in position 20 of the protein Sm14 were studied by CD spectroscopy, both by scanning the spectrum in the wavelength region 190-270 nm at 15 and 80 °C, and by studying the ellipticity as a function of temperature at 216 nm. In figure A, one of the spectra shows the folded protein and the other overlaid spectrum shows the denatured protein.

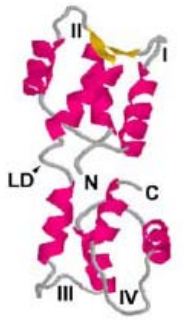
a) Which of the spectra in Figure A, the grey or the black one, shows the folded protein? Motivate your answer.
b) Which of the mutants was most highly structured before denaturation? How would you describe the structure of the native state?
c) Describe the denatured state!
d) Which of the mutants was most stable? Motivate using the data shown below!



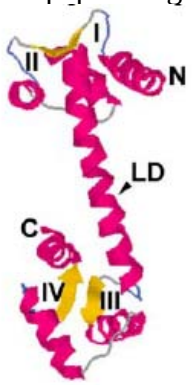
(Ramos et al., J. Biol. Chem 2003, 278, 12745-12751)

Exercise 2.

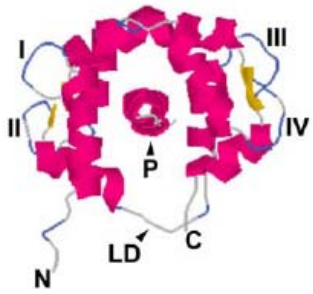
Calmodulin (CaM), a calcium binding protein, regulates calcium ion mediated events such as muscle contraction and signal transfer. The activity of CaM is regulated by binding of calcium and/or hydrophobic target peptides. The structures of these complexes (CaM+Ligand) can look different depending on which peptide/ligand that has been bound.



CaM utan Ca^{2+}
(apo)



med fyra Ca^{2+}
(holo)



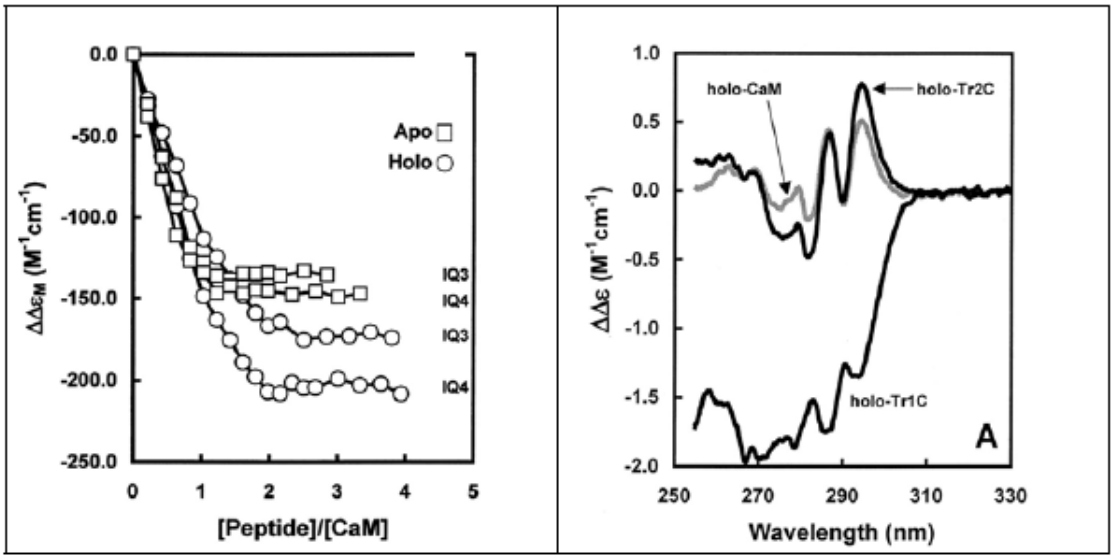
Exempel på struktur
med peptidligand

The left figure below shows how apo- and holo CaM spectra have been affected at 222 nm as a function of added peptide. The peptides Q3 and Q4 have been studied.

a) What is usually measured at 222 nm with this technique?
b) What does the change indicate?
c) What is the stochiometry of peptide binding to CaM for the apo and holo forms respectively? Motivate your answer.

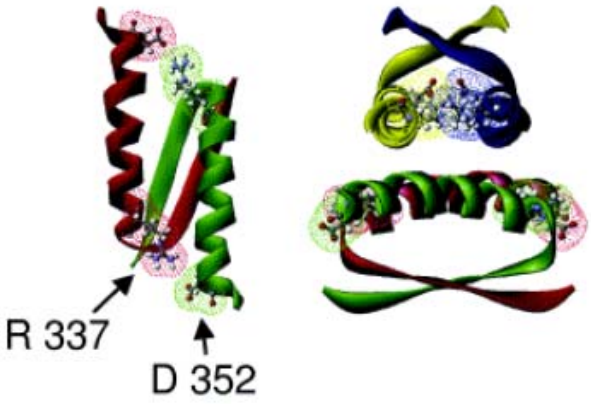
In the right figure below, difference spectra for the holo forms of CaM as well as for the CaM homologues TR1C and TR2C are shown (spectra of protein complexed with peptide, where spectra of protein and peptide alone have been subtracted as background).

d) What is measured in this wavelength region?
e) TR1C gives the largest signal in the difference spectrum when peptide binds. How would you interprete this?



Exercise 3.

Mutated tumor suppressor p53 is one of the most characteristic properties of cancer cells. Scientists have recently found that mutant forms of p53 accumulate in cancer cells. Central to the function of p53 is a tetramerization domain. The structure of this domain is known and is shown below, where you see the structure of a symmetric dimer to the left, and the structure of the entire tetramer to the right (two dimers positioned with their helices facing each other). The indicated positions correspond to known sites for cancer mutations.



Lee et al (JMB 327, 2003) have studied how the tetramerization domain of wt (wild type, native) p53 performs at denaturation/renaturation, and have recorded the CD spectra shown below.

a) How could you describe the structure of the domain at different temperatures?
b) What do you think happens with the protein? Could it affect cellular behaviour?
c) What would you like to study as the next step of the investigation given the information given in the figure above?

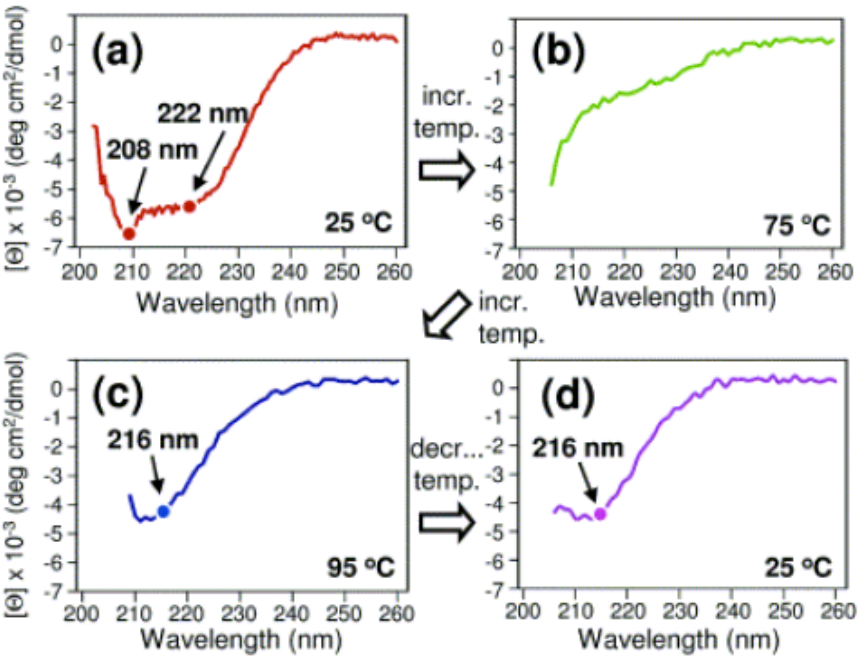


Figure 2. CD spectra during the thermal denaturation of 80 μM p53tet-wt* at pH 4.0, 250 mM NaCl and 25 °C (a), 75 °C (b), 95 °C (c), or after cooling from 95 °C to 25 °C (d).