

### Exercise 1.

- a) The authors would like to study the membrane bound structure. This makes FT-IR a good method since membranes can be constructed onto a so-called ATR detector cell.
- b) The Amide-I region (see figure legend); since the peptide bond carbonyl absorbs in this region it should be affected by secondary structure.
- c) B and D are second derivatives of the experimental curves in A and C. The B curve corresponds well with A (secondary structure peaks should be positioned according to corresponding features in the second derivative) but in the D curve there is little correspondence. In fact, the curve in D appears to be a mere duplicate of the B curve, so this must be a misprint/mistake.
- d) The peptide in A predominantly consists of  $\beta$ -sheet and distorted (3-10) helix. The peptide in B primarily consists of correct  $\alpha$ -helix and  $\beta$ -sheet. For evaluation see course compendium page I3.
- e) This is not so straight-forward... the peptide that is distorted could perhaps more easily sneak through the membrane and thus penetrate, whereas the peptide which has more structure could perhaps easier form correctly shaped pores and thereby punctuate the cell. More investigations are needed.

### Exercise 2.

On the indicated temperature transitions, the p53 protein appears to go from a helix structure to a mixed  $\beta$ -sheet / random coil structure, and from there to a structure dominated by  $\beta$ -turns.

FT-IR has been used since the authors needed to confirm that the structural transitions also occurred in the solid state. Using CD only solubilized material could be studied, and since they argued that aggregates could be formed, it was necessary to also study the material using a method suitable to study solid states.

### Exercise 3.

	CD	fluorescence	NMR
CH1-Zn <sup>2+</sup>	Helix (222 nm)	Molten globule (ANS binding)	Molten globule Small chemical shift dispersion, broad signals
CH1-Zn <sup>2+</sup> -HIF	Helix	Structured or unstructured (poor ANS binding)	Well structured Large chemical shift dispersion, highly resolved spectrum
CH1 alone	Random coil	Structured or unstructured (poor ANS binding)	No structure Small chemical shift dispersion, narrow signals

When  $Zn^{2+}$  binds to CH1, a molten globule structure is formed which is then further stabilized to a well-structured protein complex together with the target protein HIF.

Note that all three methods were required to solve the problem!

#### **Exercise 4.**

- a) The spectrum is recorded with NMR (nuclear magnetic resonance) and is called HSQC. The spectrum shows cross peaks between  $^{15}N$  and  $^1H$  in the NH group of the peptide bond, which thus creates one cross peak for every amino acid in the protein. In this picture the assignment of the spectrum is displayed onto the spectrum, which means that they have already found out which peak belongs to which amino acid. This process, called Assignment, requires running a number of other NMR experiments and putting together the information in a jigsaw-puzzle manner. Since the peaks are well separated and do not form a blur in the middle we can draw the conclusion that the protein is well folded – each amino acid has a unique and special chemical environment which renders its specific chemical shift.
  
- b) When adding the protein TAX it seems as if some of the peaks are shifted, that is, that their position in the spectrum changes. This indicates that the chemical environment of the amino acid is changed, which suggests that the TAX protein binds to the KIX protein. The new chemical environment can be due to the immediate presence of the TAX, thus changing the chemical environment, or to that the TAX binding results in structural changes in KIX thus changing the environment. Measurements have been performed by adding unlabelled TAX protein to  $^{15}N$ -labelled KIX (we see no peaks from Tax, only KIX-peaks that change position). By analyzing the sequence location of the amino acids that show the largest shift changes, these can be mapped onto the structure to see if the changes are located in a specific region of the protein. The amino acids that show the largest shift changes are indicated in the figure to the right. It seems as if the Tax binding results in changes in a specific surface region, which is most likely the ligand binding site.
  
- c) Since we now know which amino acids are located in the ligand binding site, and where these peaks are located in the HSQC spectrum, we can use this as a basis for SAR-by-NMR design of pharmaceutical drug candidates. We can add chemicals from a chemical library until we find the ones that bind the ligand surface. Using organic synthesis, we can combine these building blocks to form high-affinity ligands which could inhibit TAX binding. These molecules could then hinder the establishment of the viral infection.