

## Titration analysis of UbcH5B upon complexation with Not4 Ring finger.

NMR is a very powerful technique to map the interacting site of a protein upon complexation to its partner. The technique is based on the analysis of  $^{15}\text{N}$ -HSQC spectra. In an HSQC spectrum, one can observe peaks arising from every amide proton and the connected amide nitrogen. The nitrogens have to be of the  $^{15}\text{N}$  isotope for this NMR experiment; so one needs an  $^{15}\text{N}$ -labelled protein. An HSQC spectrum is like a nice "fingerprint" of the protein because each peak corresponds to an NH of each residue. You will get as many peaks as the number of residues in the protein (except for the first amino acid and the prolines). Side chain NH groups (from Asn, Gln, His and Trp) can also give a peak in the spectrum. The chemical shift in both the nitrogen and the proton dimension is sensitive to the chemical environment of the two nuclei. Therefore, this experiment is a nice technique to map the interacting site of a protein with its partner. One first records an HSQC of the  $^{15}\text{N}$  labelled protein alone and then HSQC spectra of the protein complexed with increasing amounts of its unlabelled partner. The peaks showing a change in the chemical shift in the HSQC of the complex compared to the HSQC of the protein alone have a different chemical environment. This shift can be due to two phenomena:

- When adding the partner, chemical shifts of the residues at the interface will be perturbed by the proximity of the partner.
- The addition of the partner may induce structural changes, or conformational changes in the protein itself.

The analysis of the shifts and the mapping of these shifts on the protein structure will give information on the binding interface and the conformational changes of the protein. In this NMR practical, you will have to analyse the HSQC spectra of a protein, UbcH5B, upon complexation to its physiological partner, the Not4 Ring finger. By mapping shifted residues on the protein structure, you will gain insight into the binding interface of UbcH5B. UbcH5B and Not4 fulfil an important function in the ubiquitination pathway when they are in complex with one another. Therefore, the study of the complex is important to gain insight into the molecular basis of this interaction. The assignment of the protein has already been done. Due to various reasons, it can be difficult to solve the molecular structure of the complex by NMR. But, as you know by now, NMR is a powerful tool to study the interaction with titration experiments, for instance. You will assign the HSQC spectrum of the protein in its bound form and plot the shifted residues on the structure of UbcH5B to visualise the binding area of UbcH5B. You may also be able to draw a binding curve and calculate a binding constant.

Spectra to analyse:

- ubch5 is the  $^{15}\text{N}$ -labelled UbcH5B protein alone.
- ubch5\_not\_1-8 is UbcH5B, added with 1/8 of the unlabelled Not4 Ring finger protein
- ubch5\_not\_1-4 is UbcH5B, added with 1/4 of Not4
- ubch5\_not\_1-2 is UbcH5B, added with 1/2 of Not4
- ubch5\_not\_1-1 is the 1:1 complex of UbcH5B and Not4



Titration analysis of UbcH5B – Report card

Name of student(s):

Date:

The amino acids that move the most are the following:

Threshold used: