

BIOC530 AU 2014
Protein NMR Unit
Homework Assignment

The recent paper published in Science “Structural Basis for Protein Antiaggregation Activity of the Trigger Factor Chaperone” used a wide variety of NMR experiments to obtain remarkable insights into how a molecular chaperone works. The senior author of the paper, Prof Charalampos Kalodimos, gave a Biochemistry Dept. seminar in October 2014 that you should have attended. This provided an excellent introduction to the paper and should help to make it more accessible.

Your assignment: Read the paper (attached) and answer the questions below.

1. What information was used to generate Figure 2A?

ANS: Chemical shifts for the following nuclei are used to predict secondary structure from NMR data: ^1HN , ^{15}NH , $^{13}\text{C}\alpha$, $^{13}\text{C}\beta$, $^{13}\text{C}'$

2. What samples (what proteins were in the NMR tube? Which one(s) has isotopic labels? What were the labels?) were used to generate the following:

- spectra shown in Fig. 3B: gray and pink

ANS: ^{15}N -labeled TF (TFSBD-PPD) on its own (gray) and in the presence of natural abundance (unlabeled) PhoA sites, as indicated

- spectra shown in Fig. 3B: blue

ANS: ^{13}C -labeled TF plus natural abundance (unlabeled) PhoA sites

3. What experiments provided the exchange rates depicted in Fig. 3K?

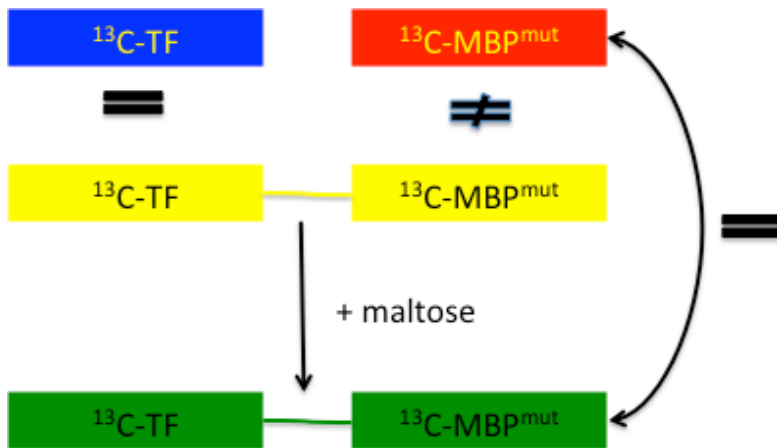
ANS: ^{15}N relaxation dispersion experiments (also called CPMG relaxation)

4. Draw a cartoon or scheme to explain Fig. 7C, showing:

- what are the molecules that generated the spectra
- what molecule(s) are isotopically labeled and with what isotope(s)/

Also use your cartoon to depict the results/interpretation of the experiments.

ANS: see next page



1. There are yellow peaks that overlay with blue peaks. So, the TF is unperturbed by the fusion w/ MBP^{mut} .
2. Yellow peaks do not overlay with red peaks, so the conformation of MPB^{mut} is different when it's fused to TF.
3. Addition of maltose to the fusion results in green peaks that overlap with red spectrum. Maltose will stabilize the MBP, so can infer that the MBP fused to TF is destabilized/unfolded.