

## NMR Studies of *E. coli trp* Repressor and Protease Inhibitor from *Arabidopsis thaliana*

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This talk is composed of two parts.

1. The oligomeric state of *E. coli trp* Repressor (TR) was studied by NMR spectroscopy. The purpose of this study was to investigate the structural changes occurring upon oligomerization of TR molecules. By utilizing the gel-filtration chromatography, the optimum condition for maximizing the tetrameric state was determined. Because of the complexity of oligomeric states, selective labeling strategy was employed, which resulted in the significantly simplified spectra. From the NMR data, the oligomeric state was found to have a locally unfolded DNA-binding region, similar to the acid-denatured state. Thus, the oligomers prefer the dissociation from the operator DNA as well as effectively reducing the active TR concentration. This pathway provides additional level of gene expression.
2. The three-dimensional structure of a novel protease inhibitor (ATT) from *Arabidopsis thaliana* was determined by NMR spectroscopy. ATT shares little sequence homology with the previously known proteases. Many NMR experiments were performed to determine sequence-specific resonance assignments and to identify NOEs. Despite the size and heat-resistance, ATT looks quite flexible, especially the active site region. According to the calculation, ATT has 2  $\alpha$  helices and 3  $\beta$  strands. Interestingly, ATT was found to have structural similarity with brazzein, the sweet-tasting protein. Efforts are being made to interconvert the functions of these two proteins.