Abstract Submitted
for the MAR06 Meeting of
The American Physical Society

Protein-Protein Interactions during Bacterial Chemotaxis using Methyl TROSY Nuclear Magnetic Resonance. DAMON HAMEL, FREDERICK DAHLQUIST, University of California Santa Barbara — During bacterial chemotaxis, the histidine autokinase CheA interacts with the chemotaxis receptors with the help of the coupling protein CheW. The CheA-CheW interaction is typical of many macromolecular complexes where protein-protein interactions play an important role. In this case a relatively small protein, CheW (18 kDalton), becomes part of a much larger complex. Here we describe a new method to map the residues at a protein-protein interface for macromolecular complexes of molecular weight greater than 100 kDalton. The method exploits the C13 methyl TROSY methodology developed in Lewis Kay's laboratory. The essence of the Kay approach is that a portion of the intensity of HMQC spectra of individual -(13)CH3 resonances in an otherwise deuterated macromolecule have much reduced dipole-dipole relaxation and remain sharp and relatively easy to detect , even in macromolecules of molecular mass 100 kD or greater. The reduction in dipolar interactions is lost if a given methyl group comes in close contact with other protons such as those supplied by the interface of a protonated interaction partner. Comparing the -(13)CH3 resonances of a protein of interest in the presence of a protonated versus deuterated interaction partner allows the methyls at the interface can be identified. The application of the approach for establishing points of contact between CheA and CheW will be discussed.

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Date submitted: 08 Dec 2005 Electronic form version 1.4