

**Protein-nucleotide interactions studied by magnetic resonance**T. Wiegand<sup>1</sup>, R. Cadalbert<sup>1</sup>, C. Copéret<sup>2</sup>, A. Böckmann<sup>3</sup>, B. H. Meier<sup>1\*</sup><sup>1</sup>Laboratorium für Physikalische Chemie, ETH Zürich, Switzerland, <sup>2</sup>Laboratorium für Anorganische Chemie, ETH Zürich, Switzerland, <sup>3</sup>IBCP BMSSI, Lyon, France

In this contribution we present strategies to characterize protein-nucleotide interactions in large biological assemblies using the example of the (double-) hexameric DnaB helicase from *Helicobacter pylori* with a molecular weight of 672 kDa. The binding of nucleotides (ATP-analogues and single-stranded DNA, ssDNA) to the helicase is monitored by <sup>31</sup>P,<sup>1</sup>H cross-polarization experiments. The observed <sup>31</sup>P chemical-shift values allow a clear discrimination between the different nucleotides used in this work and reveal that two nucleotides of bound ssDNA can be structurally distinguished. <sup>15</sup>N,<sup>13</sup>C correlation experiments highlight the role of arginine sidechains in binding to single-stranded DNA.

We further present dipolar-coupling based polarization transfer experiments from <sup>31</sup>P spins of bound nucleotides to <sup>13</sup>C or <sup>15</sup>N spins of the protein (or vice versa) to describe nucleotide binding on a molecular level (see Figure 1). Residues involved in protein-nucleotide interactions are identified in CHHP and NHHP correlation experiments.

While conventional NMR experiments on such large protein assemblies typically suffer from low signal-to-noise ratios and thus long experimental measurements times, we used Dynamic Nuclear Polarization (DNP) enhanced MAS experiments to increase the sensitivity. The samples for DNP were prepared in the absence of glycerol which results in the highest sensitivity for DnaB. The obtained spectra allow to identify spin systems of the helicase in the neighborhood of <sup>31</sup>P spins of the nucleotides. The extensive line-broadening at cryogenic temperatures is not a severe issue in this case due to the sparsity of the obtained spectra.

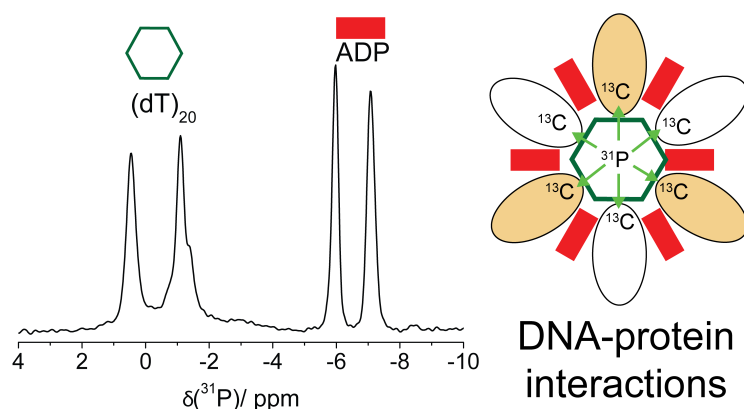


Figure 1: <sup>31</sup>P spins of bound nucleotides can be used in solid-state NMR experiments to identify helicase-nucleotide interactions.