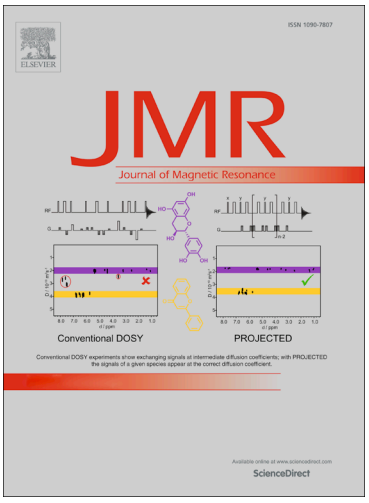
Accepted Manuscript



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PII: S1090-7807(17)30220-3
DOI: http://dx.doi.org/10.1016/j.jmr.2017.08.012
Reference: YJMRE 6151

To appear in: *Journal of Magnetic Resonance*

Received Date: 9 June 2017
Revised Date: 15 August 2017
Accepted Date: 27 August 2017

Please cite this article as: V. Chevelkov, K. Giller, S. Becker, A. Lange, Measurement of backbone hydrogen-deuterium exchange in the type III secretion system needle protein PrgI by solid-state NMR, *Journal of Magnetic Resonance* (2017), doi: http://dx.doi.org/10.1016/j.jmr.2017.08.012

Measurement of backbone hydrogen-deuterium exchange in the type III secretion system needle protein PrgI by solid-state NMR

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ABSTRACT

In this report we present site-specific measurements of amide hydrogen-deuterium exchange rates in a protein in the solid state phase by MAS NMR. Employing perdeuteration, proton detection and a high external magnetic field we could adopt the highly efficient Relax-EXSY protocol previously developed for liquid state NMR. According to this method, we measured the contribution of hydrogen exchange on apparent ^{15}N longitudinal relaxation rates in samples with differing D_2O buffer content. Differences in the apparent T_1 times allowed us to derive exchange rates for multiple residues in the type III secretion system needle protein.

INTRODUCTION

Hydrogen exchange provides important information on the structure and dynamics of proteins. In structured protein regions, amide protons are locked into hydrogen bonds and may be solvent-obstructed, which makes hydrogen exchange significantly slower in comparison to amide sites located in unstructured, solvent-exposed regions. Local and/or cooperative protein motion can open hydrogen bonds, and protons can be exchanged. Linderstrøm-Lang introduced a two-step kinetic model to describe this process [1].

Numerous NMR methods have been developed to map exchange rates, which span over several orders of magnitude [2].Exchange rates in the timeframe of minutes to months can be obtained by monitoring the amide proton signal decay of lyophilized protein dissolved in D_2O buffer [3]. Modifications to this approach allow the monitoring of faster exchange processes [4, 5].

To access even faster exchange rates, which are also very important for protein characterization, a variety of pulse sequences have been introduced. Cross peaks between water and amides can be monitored in 3D homonuclear EXchange SpectroscopY experiments (EXSY)[6, 7], which require longer measurement time compared to 2D experiments. Saturation transfer techniques allow the use of 2D HSQC experiments to monitor variation in amide magnetization during the hydrogen exchange period defined by a proper experimental scheme [8-12]. However, a number of experimental drawbacks decrease the accuracy of these approaches (see detailed discussion in [13]), in particular amide magnetization evolution due to dipolar couplings with HA, water and hydroxyl protons, and water radiation damping. Recent developments [13-15] aimed to minimize these unwanted effects.