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# Residue Specific Studies of NH Exchange Rates Performed on Ubiquitin

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## 1. Introduction

The aim of the present study was to establish a new method to investigate NH exchange rates in proteins. Experiments, which are based on residue specific diffusion measurements, were performed on human ubiquitin since it is a relatively small protein consisting of 76 residues, resulting in a molecular weight of 9 kDa. Furthermore, the availability of resonance assignment [1] as well as an NMR structure [2] is advantageous.

## 2. Experimental

For NMR studies a 1.8 mM solution of uniformly $^{13}C$,$^{15}N$-labelled ubiquitin in 90% $H_2O$, 10% $D_2O$, buffered to pH 5.8 was used. Experiments were carried out at 300 K on a Bruker Avance 700 spectrometer equipped with a cryo probe. **D**iffusion **O**rdered **S**pectroscop**y** [3] was used to obtain an apparent diffusion coefficient *D* for each separated signal. In order to observe resolved signals for most residues of the protein $^{1}H$,$^{15}N$-DOSY-HSQC spectra were recorded.

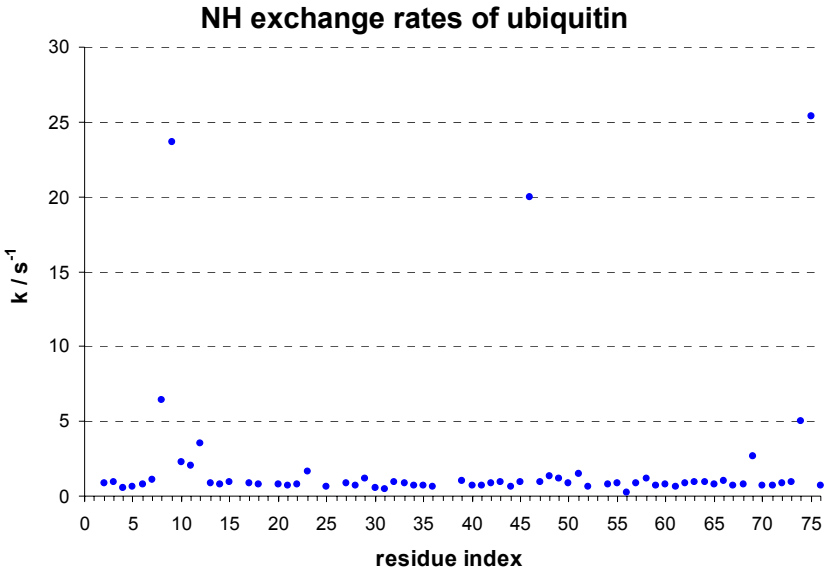
$^{1}H$,$^{13}C$-DOSY-HSQC spectra were used as control experiments since CH protons cannot be affected by exchange. All separated CH signals of ubiquitin showed apparent diffusion coefficients which are in agreement with the actual size of the protein.

Further control experiments using suppression of convection artefacts by double stimulated echo [4] were performed to prove that convection has no significant influence on apparent diffusion coefficients under the conditions used in this study. Due to sensitivity reasons, measurements for the determination of exchange rates were performed without suppression of convection artefacts.

## 3. Results

Analysis of the apparent diffusion coefficients showed several “fast moving” NH protons. Subsequently, the exchange rates were derived directly from the decay data of the diffusion experiment by applying a model deduced from the assumption of two-site exchange with water and the “pure” diffusion coefficients of water and protein.

The “pure” diffusion coefficient of the protein was determined in an experiment with selective excitation of the amide protons in order to prevent the influence of magnetization transfer from water to amide protons on the decay data. This value was in agreement with those diffusion coefficients obtained in $^{1}H,^{13}C$-DOSY-HSQC experiments. Figure 1 shows the exchange rates obtained for ubiquitin.




We plan to compare our results to exchange rates determined by other NMR methods such as the MEXICO sequence [5]. These experiments are topic of current research.

## 4. Conclusion

Diffusion measurements are a convenient tool for the detection of residues undergoing fast NH exchange and can complement relaxation studies. Exchange rates can also be quantitatively evaluated.

## References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