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SEMPRE: Spectral Editing Mediated by Paramagnetic Relaxation Enhancement

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NMR spectra of large biomacromolecules or complexes frequently contain a significant number of overlapping peaks hampering or even preventing structural interpretation. Selective elimination of peaks would greatly simplify this task. Paramagnetic agents enhance the relaxation rates of nuclear spins and result in line broadening of the corresponding signals in their vicinity. By increasing the paramagnetic agents (PA), concentration signals can be shifted or quantitatively suppressed, depending on the PA's nature. The paramagnetic relaxation enhancement (PRE) effect is well-known and widely used for structure elucidation and evaluation of binding interfaces of protein multimers or protein−ligand complexes.[1] Frequently, the paramagnetic agent is covalently attached to the biomacromolecule at a specific position exploiting the reactivity of free cysteine sulfhydryl groups.[2] However, freely diffusing PAs have been used to determine the surface accessibility of proteins as well as in micelle-bound peptides.[3] In this study we extend this approach by using soluble PRE agents for NMR spectral editing to facilitate interpretation of crowded NMR spectra.

We use the freely diffusing MRI contrast agent gadolinium-diethylenetriamine pentaacetic acid bismethylamide (Gd(DPTA-BMA); Omniscan), which has been used previously to detect binding interfaces *via* ^{13}C-methyl[4] and protein surfaces *via* ^{15}N-amide[5] resonances. Gd(DPTA-BMA) is preferred over other agents as it is uncharged and highly soluble in water and exhibits only small binding affinities to proteins.[4] Gd(III) causes strong relaxation enhancement but only marginally small pseudocontact shifts, if any. PRE is a distance-dependent through-space dipole−dipole interaction between the nuclear spins of the sample and the electronic spin of the unpaired electrons of the PA decreasing with $^1/_{r^6}$.[6]

For our study we used uniformly ^{15}N-labeled yeast ubiquitin (yUb) containing a 23 residue N-terminal His-tag that is disordered in solution. ^{15}N-HSQC spectra were recorded in the presence of different concentrations of Gd(DPTA-BMA). An apparent increase in the signal-to-noise ratio was detected as described before.[7] We observed concentration-dependent attenuation of NMR signals belonging to nuclei in solvent exposed regions of yUb. This is due to the increasing number of transient, short-lived contacts between the PA and the protein's surface. In cases of very fast amide exchange rates, which are typically associated with exposed or disordered parts of proteins, exchange with PRE-broadened protons from water may increase signal attenuation. In contrast, signals corresponding to nuclei that are buried in the protein and that are slowly exchanging are shielded from PRE mediated broadening. However, the penetration depth of the PRE effect can be modulated by increasing the concentration of the PA.

Therefore, by adding Gd(DPTA-BMA) we obtained spectra that contain only signals of spins distant from the protein surface and that show slow exchange. Subtracting the HSQC spectra recorded with PA from the spectra without PA yielded spectra containing only signals of spins at the protein surface. Thus, by simple addition of a soluble spin label NMR spectral editing can be achieved in a structure- and dynamics-dependent manner.

Among the signals that were broadened beyond detection even at concentrations as low as 5 mM Gd(DPTA-BMA) were the signals

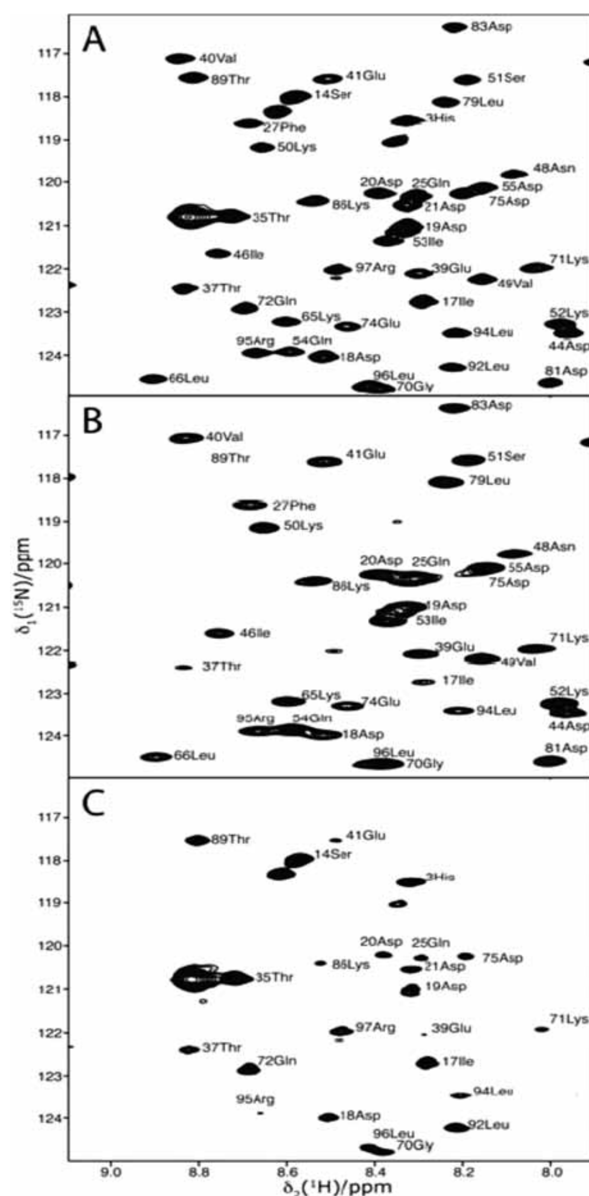

Figure 1. Section of ^{15}N-HSQC spectra of 1 mM Ubiquitin. (A) 0 mM Gd(DPTA-BMA), (B) 5 mM Gd(DPTA-BMA). (C) difference spectrum of A and B.

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corresponding to residues of the disordered N-terminus. By contrast, signals from the globular part of yUb remained essentially unchanged (Figure 2). Signals of flexible tails or linkers that often cause crowding in the region corresponding to the disordered regime of the chemical shift (~8−9 ppm for $^{1}H^{N}$) can effectively be removed without affecting globular domains. For instance, we were able to eliminate signals in the spectra of a 32 kDa scF$_v$-antibody fragment that belong to the linker region between the V_H and the V_L domains. Similarly, side-chain amide signals can also be quantitatively removed from HSQC-type spectra. In addition to spectral simplification, comparing signal loss for a particular residue while stepwise incrementing the PA's concentration thus permits a qualitative classification of spins as surface exposed, close, or distant to the surface. For yUb only few signals that correspond to spins from the interior remained unaltered at 40 mM Gd(DPTA-BMA).

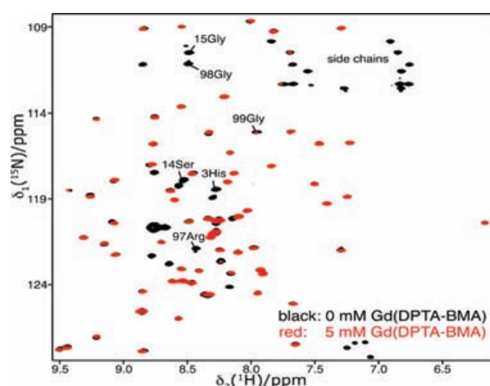

Figure 2. ^{15}N-HSQC spectra of 1 mM ubiquitin. Black: 0 mM Gd(DPTA-BMA). Red: 5 mM Gd(DPTA-BMA). Signals attenuated at 5 mM belong to the N-terminal His-tag (residues 1−23, largely unassigned), the C-terminus (residue 97 to 99), and side chain signals.

We further applied this technique to NOESY spectra, where spectral overlap often hampers unambiguous assignments. In ^{15}N-NOESY-HSQC spectra of the same samples already at 5 mM Gd(DPTA-BMA) several NOE signals were broadened resulting in beginning structure dependent simplification of the spectrum (Figure 3). Signal attenuation in ^{15}N-NOESY-ladders again depends on the surface exposure and exchange rate of the nuclear spins involved and on the PA concentration.

In summary, we have shown that freely diffusing PAs can be used not only to screen for intermolecular interaction sites[4] but also to edit NMR spectra. Particular advantages are that editing can be controlled by the concentration of PA added and that it has a structural dependence of signals affected, i.e., the distance from the solvent−analyte interface and the exchange rate. After analysis, samples can conveniently be recovered dialysing the PA out. We demonstrated that ^{15}N-HSQC-type spectra can easily be simplified and divided into spectra containing surface-exposed and buried spins. This approach can be expanded to other nuclei. We further showed that flexible parts of a protein can be masked enabling efficient structural analysis of globular domains. SEMPRE can also be applied to NOESY-type spectra to reduce overlap and facilitate assignment. Quantitative distance restraints for structure calculation, however, are recommended to be extracted from unedited NOESY spectra due to the number of different contributing parameters. Overall, PAs present a convenient way to facilitate spectral analysis of, in particular, proteins that are large, contain disordered regions, or participate in transient interactions.

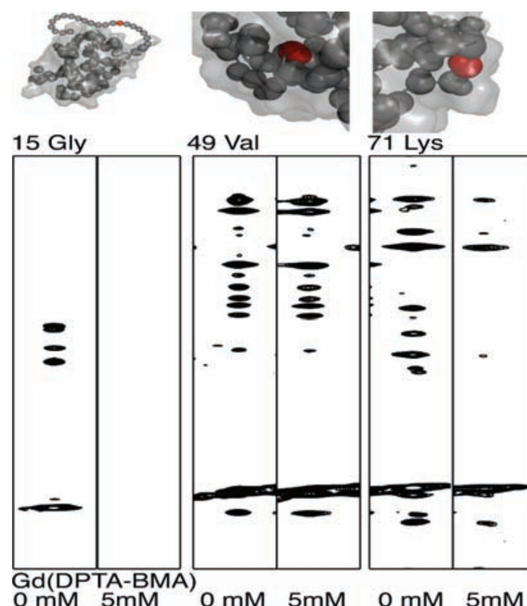

Figure 3. Strips from ^{15}N-edited NOESY spectra of 1 mM yUb for Gly 15 located in the disordered N-terminal tail, Val 49 located in the interior of yUb, and the surface exposed Lys 71 without and in presence of 5 mM Gd(DPTA-BMA). Location of the respective spins is shown in the panels above the corresponding strips.

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Supporting Information Available: Sample preparation, NMR spectra recording and assignments; additional spectra of yUb and evaluation of concentration dependence of Gd(DPTA-BMA); SEMPRE-^{15}N-HSQC of a 32 kDa scF$_v$-fragment. This material is available free of charge via the Internet at http://pubs.acs.org.

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