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Pharmacophore Mapping via Cross-Relaxation during Adiabatic Fast Passage

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NMR spectroscopy has become an indispensable tool in chemical biology, drug discovery, and structural genomics relevant to pharmaceutical and biotech industries.[1] To date, a broad range of experiments is available to screen for or to analyze protein−ligand interactions. Broadly speaking, these experiments are either exploiting NOE effects (transferred NOE, STD, pumped NOE, waterLOGSY)[2] or, alternatively, exploiting changes of chemical shifts (most importantly ^{19}F based detection scheme, such as FAXS[3]) or molecular weights (PFG diffusion measurements).[4] Most recently, fragment-based drug design (FBDD) has demonstrated great potential in indicating valuable lead compounds for drug discovery as it allows for a better coverage of the available chemical space.[5] In early stages of the FBDD process often medium-to-weak binders are encountered, and thus reliable and sensitive detection techniques are crucial. It will be in this area that NMR spectroscopy will find most of its future applications as it not only is a very sensitive detection technique but also provides additional information about binding modes and orientations of bound ligands. Several experiments have thus been developed in the recent past, INPHARMA[6] and SALMON,[7] which exploit structural and dynamical information provided by the Nuclear Overhauser effect (NOE)[8] and rotating frame Overhauser effect (ROE).[9]

Here we introduce a novel NMR experiment, AFP-NOESY, which measures homonuclear (^{1}H−^{1}H) cross-relaxation rates (NOEs and ROEs) during adiabatic fast passage (AFP) and demonstrate its suitability for the examination of protein ligand interactions and ligand binding epitope (pharmacophore) mapping. Our experiment involves adiabatic fast passage radio frequency (RF) pulses with a parabolic phase modulation leading to a linear frequency sweep through a very large spectral window.[10] In addition to its well-established applications for broad band spin inversion and/or heteronuclear decoupling, the original AFP concept has been exploited for measuring heteronuclear spin lock relaxation rates.[11] In contrast to conventional AFP schemes the RF field intensity is not small compared to the frequency sweep range but of comparable strength and thus leads to significant contributions of transverse relaxation to the effective spin lock relaxation rate.[11] Here we present an extension of the methodology to studies of protein−ligand complexes. The pulse sequence is essentially a conventional NOESY experiment in which the original longitudinal NOESY mixing period is replaced by the AFP RF pulse (an outline of the pulse sequence is given in the Supporting Information (SI)). The adiabatic spin-lock frame is shown in Figure 1. During adiabatic fast passage cross-relaxation between spins i and j occurs and the rate (σ_{ij}) is given by $\sigma_{ij} = \sigma_{NOE} \cos^2 \theta_{eff} + \sigma_{ROE} \sin^2 \theta_{eff}$. For a macromolecule (protein) devoid of internal mobility a passage through zero occurs at a tilt angle of $\theta = 35.3°$,[9] while small molecules (ligands) show no $\sin^2 \theta$ dependence (see SI). If a ligand binds to the protein, an increase of the effective cross-relaxation rate and a pronounced dependence of the cross-relaxation rate on the effective tilt angle will be observed.

Typical results for an adiabatic spin-lock cross-relaxation experiment are shown in Figure 2, where we monitored the binding of vanillic acid to quail lipocalin Q83 (157 residues, 18 kDa).[12] The data clearly demonstrate the sensitivity of the AFP-NOESY method to probe

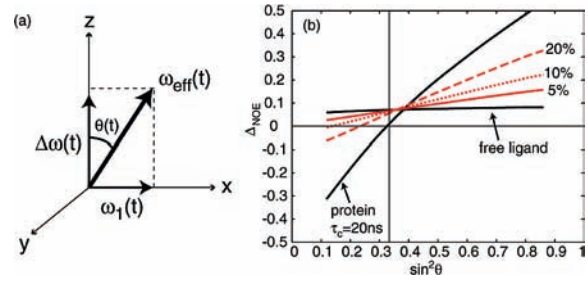

Figure 1. (a) Adiabatic spin-lock frame with offset $\Delta\omega(t)$, r.f. field ω_1(t), effective field $\omega_{eff}(t)$, and the angle $\theta(t)$ between offset and effective field. The time integral of $\sin^2 \theta(t)$ yields an effective tilt angle. The frequency of the adiabatic pulse is swept over a certain range thereby introducing a time dependent offset. Provided the adiabaticity condition ($d\theta/dt \ll \omega_{eff}$)[10] is fulfilled, the magnetization can be assumed to follow the effective field leading to a perfect inversion at the end of the pulse. (b) Changes in intensity of the NOE-enhanced signal of interest due to effective cross-relaxation from a selectively inverted signal simulated for a rigid macromolecule, e.g. a protein, and a small molecule in the extreme narrowing limit, i.e. a free ligand, as a function of the (effective) tilt angle. Upon binding to the protein (red) the effective correlation time of the ligand increases and (after dissociation) the signal of the free ligand shows protein-like behavior. Examples are given for 5% (solid), 10% (dotted), and 20% (dashed) of free ligand with macromolecular behavior.

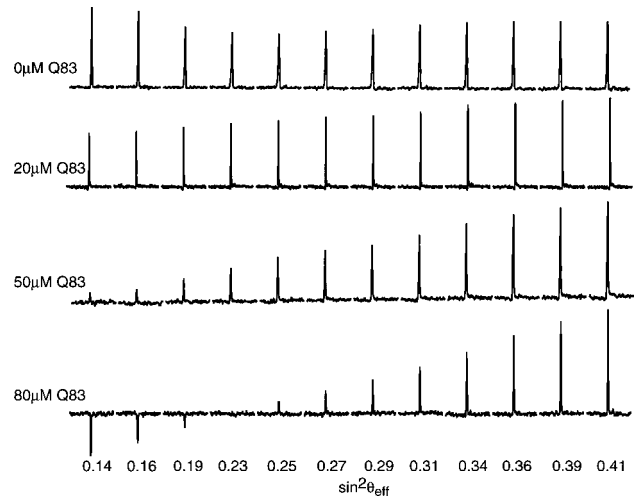

Figure 2. Selective 1d AFP-NOESY spectra for a complex formed between vanillic acid (VA, = 4-hydroxy-3-methoxybenzoic acid) and the lipocalin protein Q83. The NOE between 3-OCH_3 protons and H2 of VA is measured as a function of AFP spin lock power and protein concentration. Experimental conditions were as follows: ^{1}H Larmor frequency: 500 MHz; AFP delay: 400 ms; concentrations: VA 1 mM; Q83: (top to bottom) 0, 20, 50, and 80 μM. In the free form (top) the extreme narrowing condition holds (thus leading to a flat profile), whereas in the bound state the macromolecular cross-relaxation dominates.

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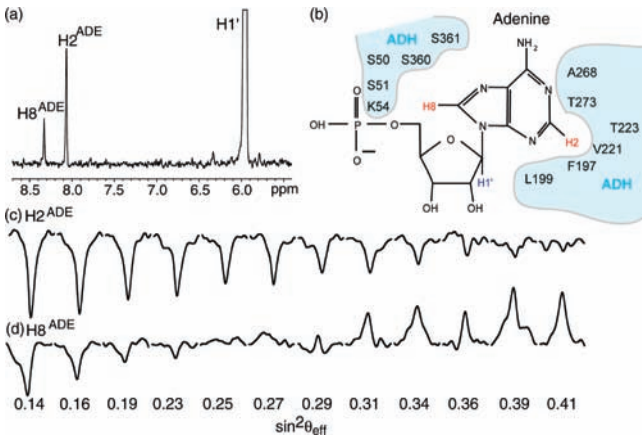

Figure 3. (a) Part of the 1D ^{1}H NMR spectrum of AMP. (b) Schematic representation of AMP contacts to ADH. (c−d) Experimental AFP cross-relaxation rates of the AMP-ADH complex, recorded with 400 ms mixing time (concentrations: AMP: 1 mM; ADH: 40 μM). The ribose proton H1′ proton was selectively inverted and acted as the source for magnetization transfer to $H2^{ADE}$ (c) and $H8^{ADE}$ (d). Experimental conditions are given in the Supporting Information.

protein ligand binding processes. At low protein concentration the extreme narrowing condition prevails and the effective cross-relaxation rate is nearly independent of the RF spin lock power, whereas at higher protein concentrations the effective cross-relaxation rate shows a profile typical for a rigid macromolecule. As expected, the experimental results correspond nicely with numerical simulations (see Figure 1b).

It is well-known that internal mobility and/or spin diffusion effects can alter the tilt angle profile of rotating-frame cross-relaxation rates.[13] While internal mobility reduces the effective correlation time relevant for the time modulation of the internuclear vector and leads to a zero passage at smaller tilt angles, spin diffusion causes the opposite effect and often leads to larger zero passage tilt angles. We have investigated the influence of spin diffusion effects by recording 2D AFP-NOESY on the quail lipocalin Q83 (SI). Unambiguous evidence for the relevance of spin diffusion was observed. The histogram of zero passage tilt angles θ_0 clearly showes a broad distribution around the theoretical θ_0 value of ~35.3°, with significant deviations toward both sides. Higher temperatures slightly alleviate spin diffusion effects (by reducing the effective correlation time). Although the observed spin diffusion effects impair a quantitative analysis of the protein AFP cross-relaxation data in terms of intramolecular dynamics, it offers a potentially rich source of information for pharmacophore mapping. In the past, protein ligand spin diffusion effects were considered problematic for the interpretation of transferred NOE measurements, and therefore experiments have been developed to quench these indirect pathways and extract reliable cross-relaxation rates devoid of spin diffusion.[14] Here these indirect relaxation pathways are actively exploited to extract structural information about ligand binding modes.

An example for experimental pharmacophore mapping is given in Figure 3. Selective AFP cross-relaxation rates were measured for the AMP/ADH and NAD^+/ADH (Alcohol Dehydrogenase from *S. cerevisiae*) protein complexes. The sugar proton H1′ was chosen as the NOE source spin. At the chosen molar ratio intraligand NOEs are dominated by the bound state and amplitude profiles typical for macromolecules are to be expected. Interestingly, however, only the cross-relaxation rate between H1′ and H8 showed an amplitude profile typical for a rigid macromolecule. Conversely, for the NOE between H1′ and H2 a clear indication of indirect spin diffusion pathways was observed. This suggests that H8 is most likely exposed to the solvent, whereas H2 is embedded in a ^{1}H dipolar coupling network and buried in a hydrophobic binding cleft. The 3D structure of AMP/ADH nicely corroborates the findings (see SI). Similar results were found for the NAD^+/ADH complex (zero crossing of $H8^{ADE}$ and no zero crossing of $H2^{ADE}$ due to spin diffusion; data not shown).

In summary, we were able to show that AFP-NOESY allows us to probe protein ligand interactions and provides detailed information about pharmacophores which will be useful for rational drug design programs. Although STD was suggested as a tool for epitope mapping, its quantitative interpretation is less straightforward. However, the pronounced differences of zero-crossing angles for H2 and H8 in Figure 3 suggest application of the AFP-NOESY method for refined analysis of protein−ligand interaction sites by quantification of proton densities for evaluation of docking models. Given the high sensitivity we anticipate widespread applications in fragment-based drug design programs, particularly as the methodology provides valuable information about potential sites for ligand extensions and/or decoration.

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Supporting Information Available: Pulse scheme, the structure of the AMP/ADH complex, simulation details, and spin diffusion effects in Q83 are given. This material is available free of charge via the Internet at http://pubs.acs.org.

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