Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

69451 Weinheim, Germany

Robust Deconvolution of Complex Mixtures by Covariance TOCSY Spectroscopy

Fengli Zhang and Rafael Brüschweiler[*]

[*] Dr. Fengli Zhang, Prof. Rafael Brüschweiler,
National High Magnetic Field Laboratory
Department of Chemistry and Biochemistry
Florida State University
Tallahassee, FL 32310
Tel. 850-644-1768
Fax: 850-644-8281
E-mail: bruschweiler@magnet.fsu.edu

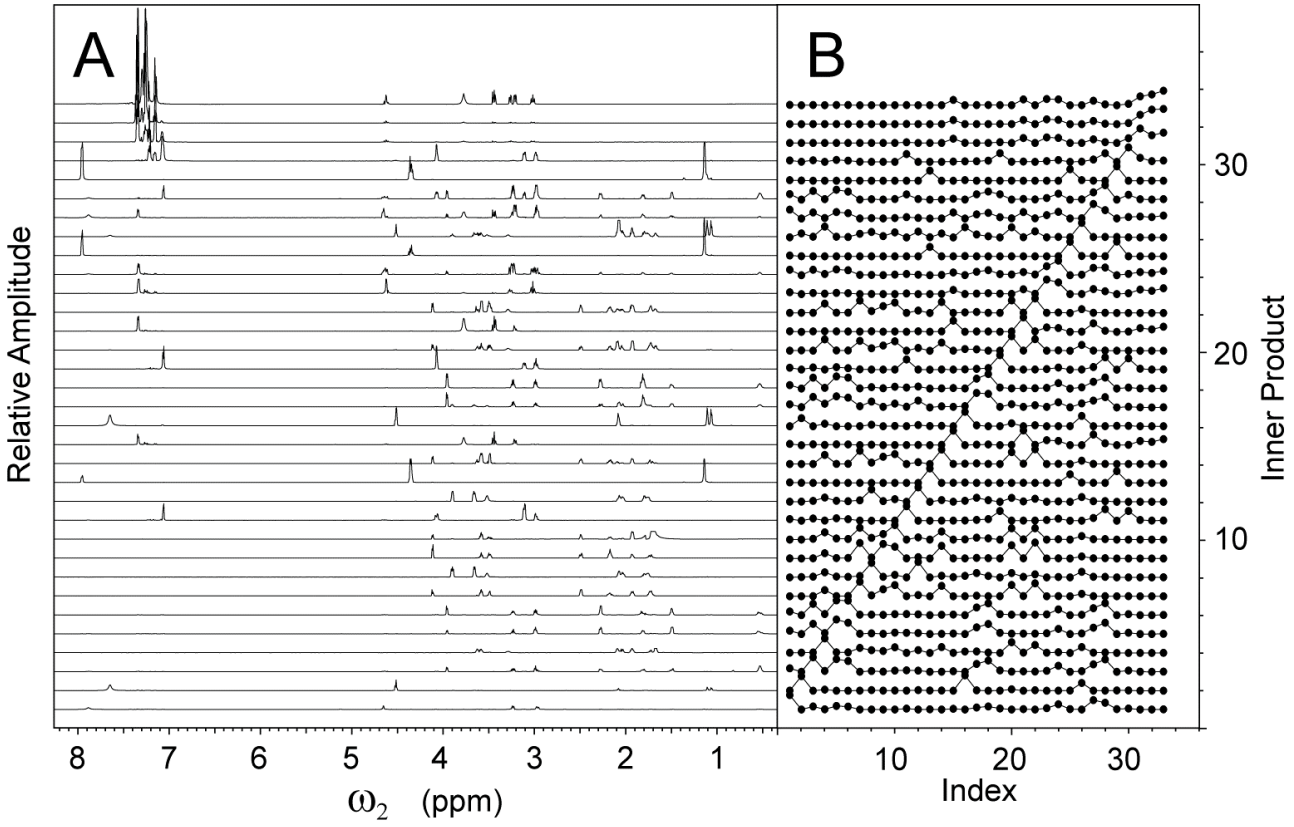

Figure S1.
Panel A: Traces of covariance TOCSY spectrum of antamanide picked according to the importance index of Figure 1F and sorted according to the intensities in importance index vector **P**. Panel B: Normalized inner products among all the traces of Panel A. The highest similarity is always achieved when a row is compared with itself.

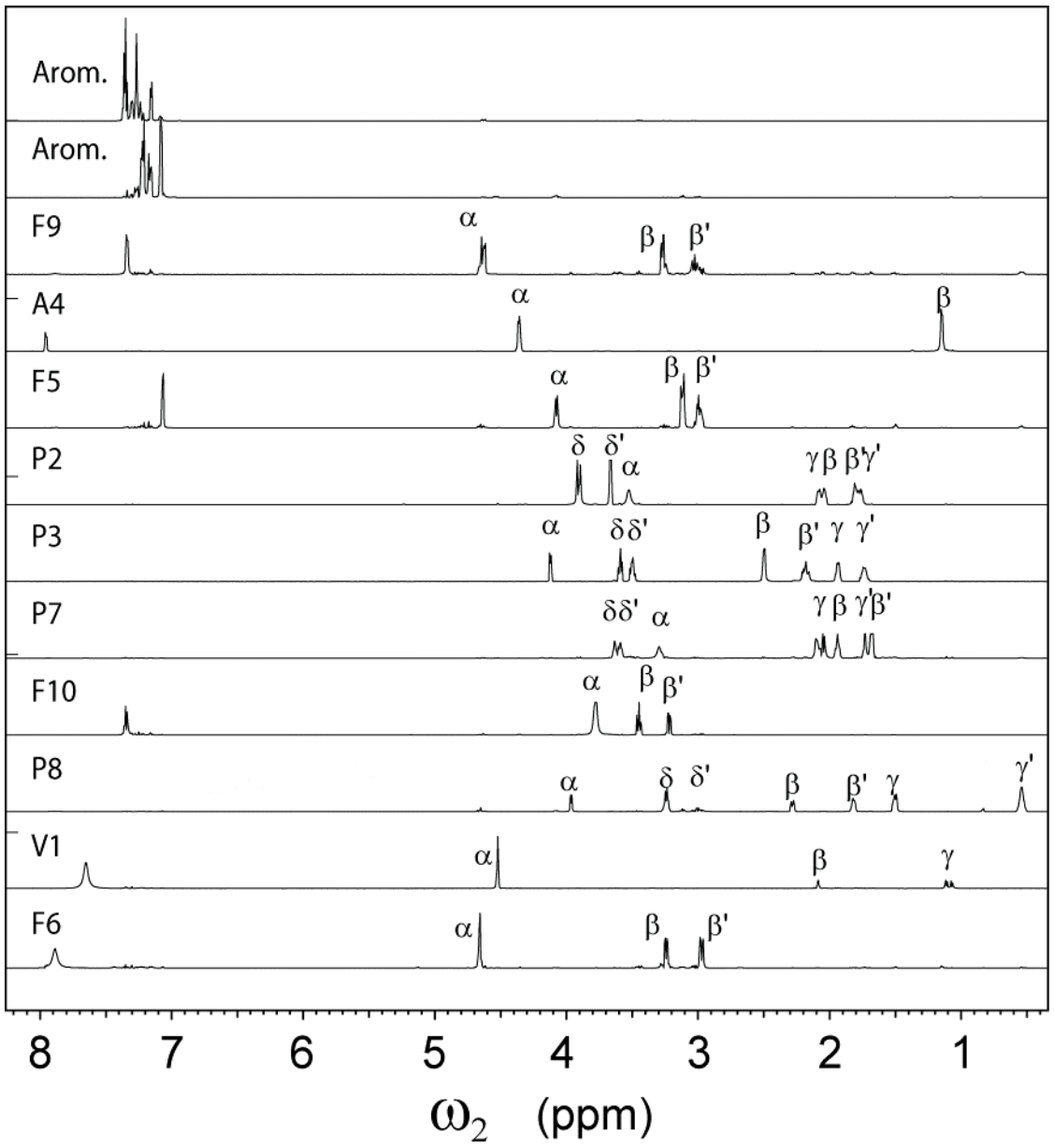

Figure S2.

The result of the DemixC method for the cyclic decapeptide antamanide using a covariance TOCSY experiment with mixing time τ_m of 76 ms. This figure is fully analogous to Figure 4, except that the mixing time was reduced from 97 ms to 76 ms. As for the 97 ms covariance TOCSY, the spin systems of the aliphatic protons of all 10 amino acids are correctly identified. This result is consistent with other tests that the exact mixing time is not critical for the method provided the mixing time is sufficiently long to allow magnetization transfer throughout the whole spin system.

Additional remarks about the DemixC method and its applications

- Due to the redundant connectivity information of TOCSY spectra it is not required that all traces belonging to a certain spin system are picked in the importance index profile. For example, in the 3-amino acid mixture (Figure 1D) the threshold was set such that traces around 1.35, 1.80, and 3.65 ppm were not picked, which does not have an adverse effect on the deconvolution result.

- Differences in resolution and multiplet patterns can be seen for the H_γ protons of Lysine (Figure 3) that are due to differences in the magnetic field strengths (400 MHz for BMRB spectra vs. 600 MHz for the mixtures).

- The mixtures used here were measured in D_2O or deuterated chloroform. For aqueous solutions NMR water suppression is a prerequisite, which has the consequence that resonances under the water or in its close vicinity cannot be observed. However, this will not affect any other TOCSY peak amplitude of resonances right or left of the water line even if magnetization is relayed via a resonance under the water. When TOCSY traces are compared with data bank spectra, the absence of the peaks under the water needs to be taken into account.

- As demonstrated in reference [10], PCA is a powerful method to uncover interdependencies between elements of a set of traces and it works well when spectral overlap between spin systems is low. In the presence of significant overlaps, however, the PCA method has the tendency to attribute too much spectral information to the largest modes, which leads to “mixed modes”, which are modes that represent a superposition of spectra of multiple spin systems, and “compensatory modes”, which are modes that correct for non-uniform spin excitation effects. These modes are hard to interpret as they do not correspond to individual 1D spectra and impede the analysis of more complex mixtures making the (semi-)automated analysis of these kinds of spectra challenging.

- Our results indicate that the method is robust for a wide range of concentrations as long as the signal-to-noise of the weakest compound is clearly distinct from the noise floor.

- Covariance spectroscopy has the advantage over Fourier transform spectroscopy that it yields high-resolution along the indirect dimension already when using only few t_1 increments. This allows one, for example, to collect in the same amount of time a TOCSY spectrum averaged over multiple mixing times to achieve more uniform magnetization transfers across the spin systems.