Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Material

Monitoring fast reactions by spatially-selective and frequency-shifted continuous NMR spectroscopy. Application to rapid-injection protein unfolding.

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Materials

100% D_2O was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) in the highest purity available and used without further purification except for horse heart myoglobin, which was dissolved in NMR buffer and purified by centrifugation as described previously.[1] NMR buffer (20 mM potassium phosphate, 0.02% NaN_3, pH 6.5) was prepared in H_2O, lyophilized and dissolved in 100% D_2O. This procedure was repeated twice in order to remove residual H_2O.

NMR Spectroscopy

NMR spectra of the imidazole alanine mixture (1.2 M imidazole, 0.32 M alanine in 100% D_2O) were measured on a Bruker Avance III 500 MHz spectrometer using a TXI triple-resonance probe. The NMR spectra of the myoglobin unfolding were acquired on a Bruker Avance III 700 MHz NMR spectrometer using a 5 mm TCI (HCN) cryo probe. 300µL of a 38.5 mg/ml myoglobin sample in NMR buffer were provided in a Shigemi tube and 25 µL diluted (1:5) acetic acid was injected. The rapid injection device and the experimental setup were used basically as described by Mok et al.[2]. However, the coaxial insert was modified, as no optical fiber was needed for our experiments. Therefore we simply replaced it by a PTFE tube.

Supplementary Figures:

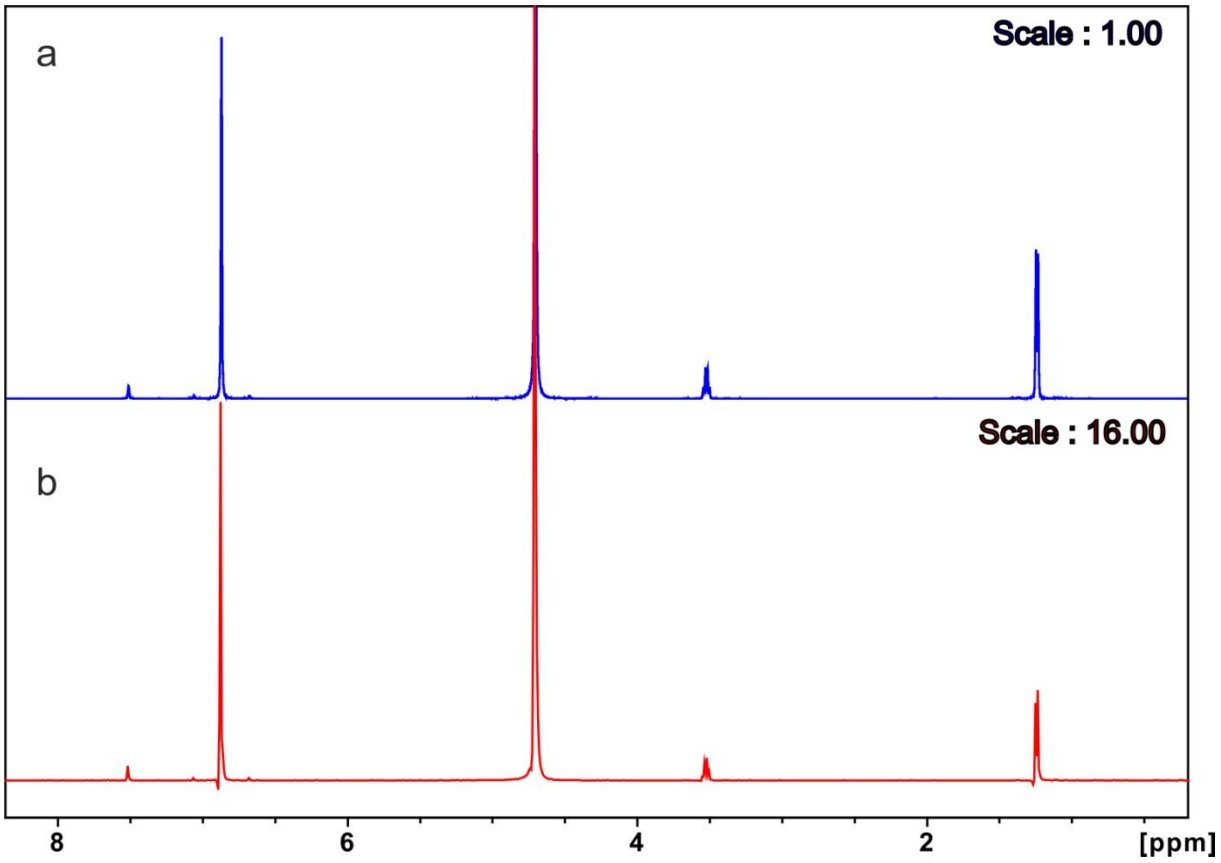

Figure S1:

Sensitivity comparison of a regular (non-slice-selective) ^{1}H NMR spectrum of a mixture of alanine and imidazole in D_2O in a) and one acquired with the spatially selective excitation scheme using a 1.5 G/cm gradient in b). The spectrum in b) is increased by a factor of 16.

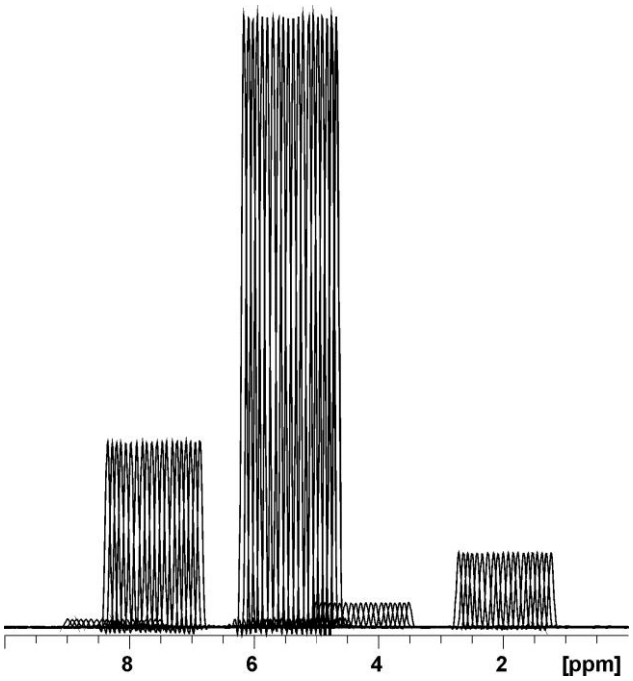

Figure S2:

First 20 spectra of a mixture of alanine and imidazole in D_2O. The scale corresponds to the first spectrum (rightmost) and the subsequent increments are shifted horizontally to the left. The time between two consecutive increments is 48.5 ms corresponding to a total duration of 970 ms for the acquisition of all 20 spectra. Uniform signal intensities indicate the absence of saturation. For the abovementioned example the gradient was chosen to permit 40 successive increments before the originally excited frequency is excited again and thus allows almost 2 seconds of actual relaxation, which is enough for most systems.

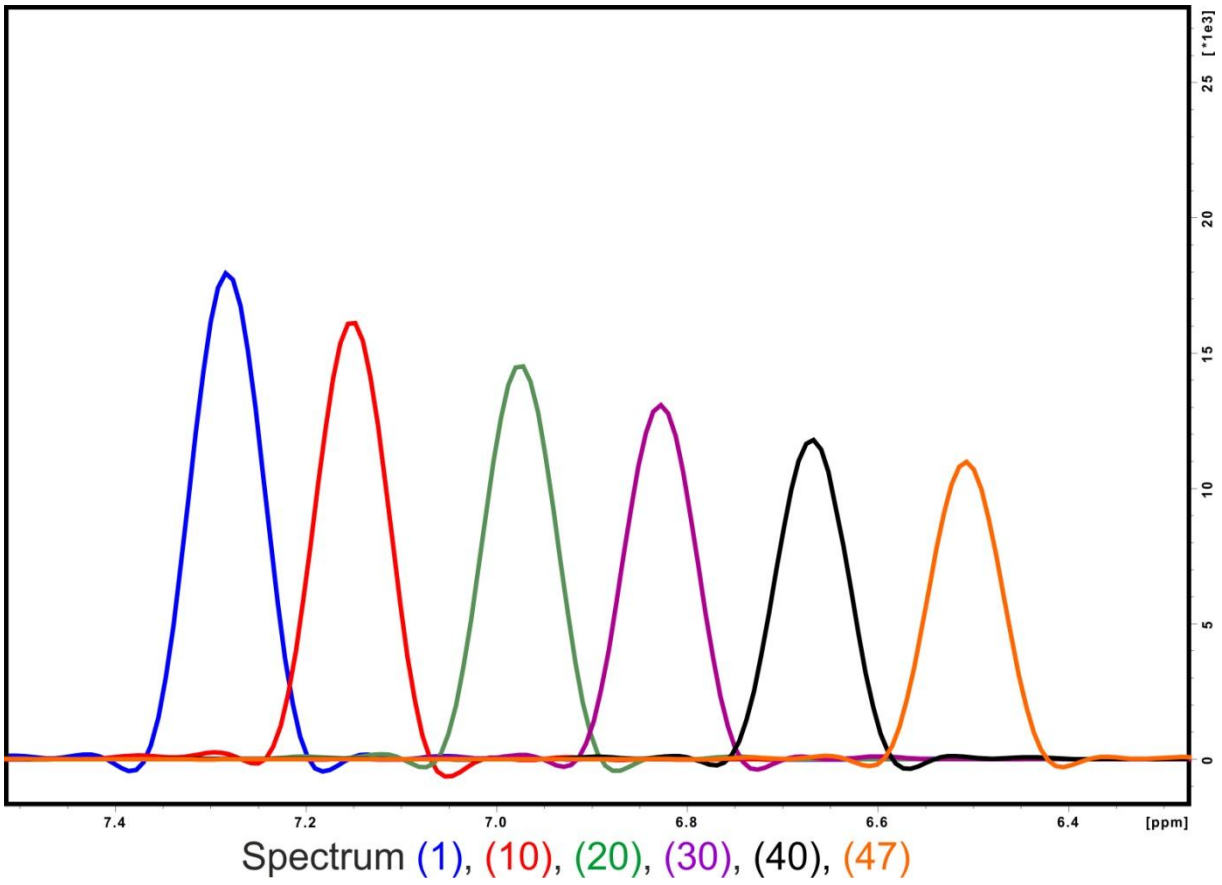

Figure S3:

Spectra of chloroform acquired sequentially using short flip angle pulses of 1μs and 512 data points. The total time for each spectrum (pulse-sequence plus data acquisition) was 48.5 ms. Progressive saturation can be seen by the steady decrease of signal intensity.

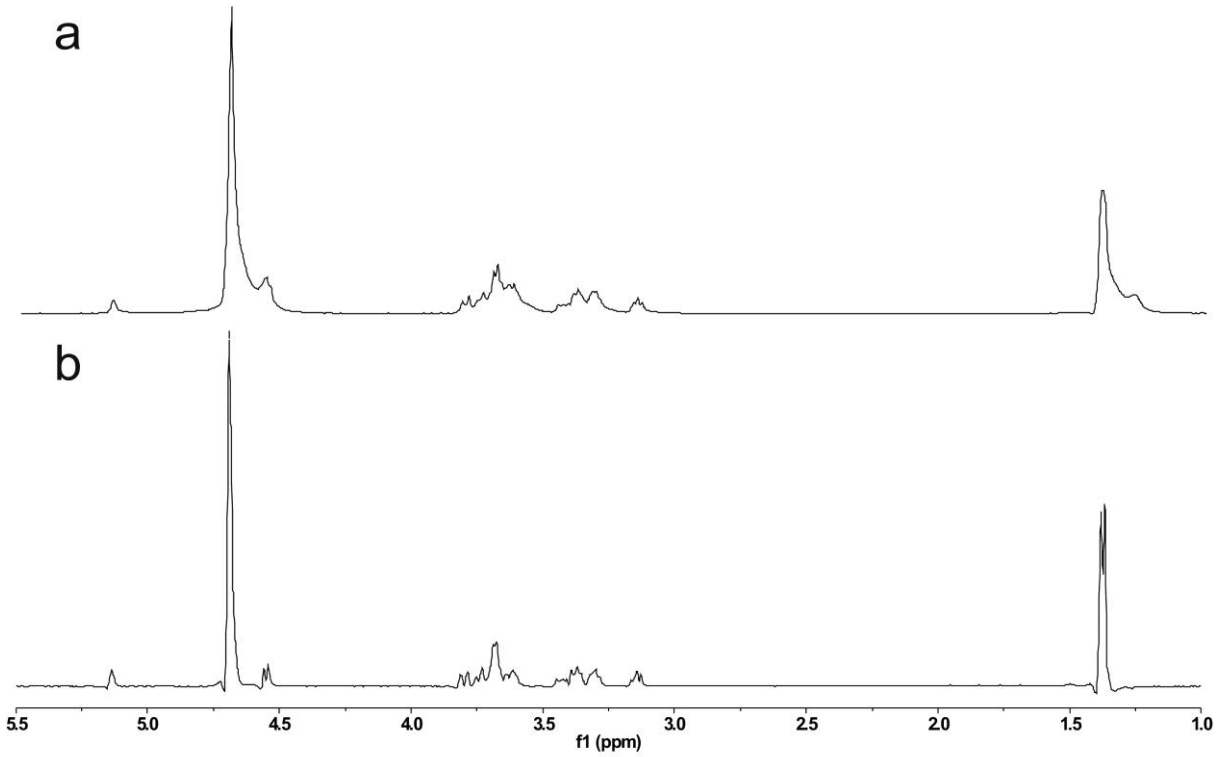

Figure S4:

Spectra of a solution of 300 µl of 13.3 mg alanine to which 30 µl of a glucose solution (12.1 mg) was injected. Due to the increased sample volume the magnetic field homogeneity is deteriorated, which is clearly visible in spectrum a), which was acquired using a small flip angle pulse. In contrast, spectrum b), which was acquired using the presented rapid spatially-selective pulsing approach yields a much better resolution since each signal is excited in a single slice and therefore magnetic field inhomogeneities are drastically reduced.

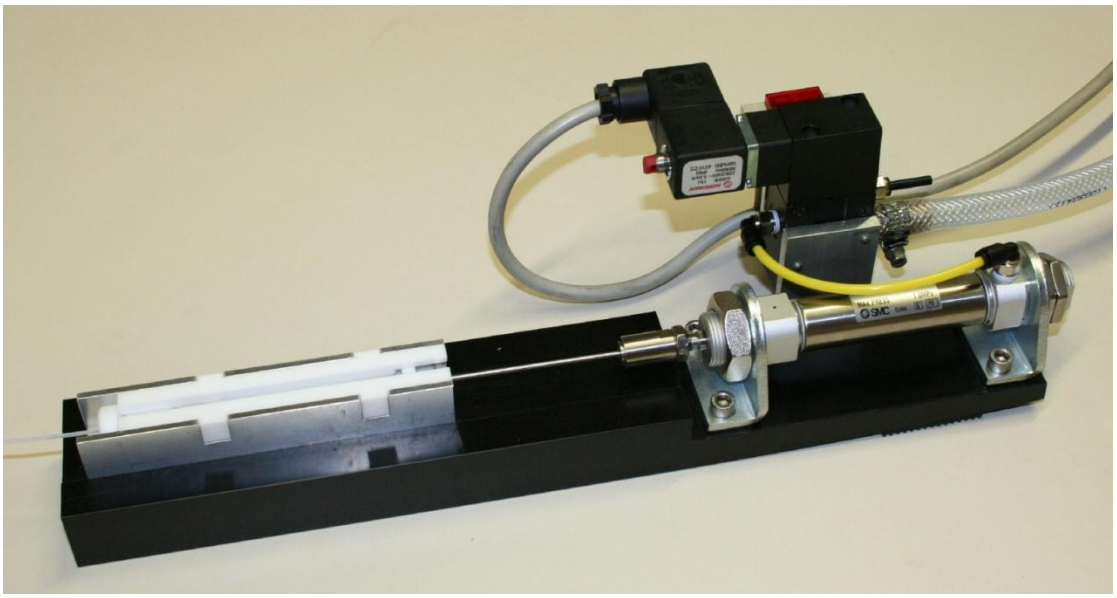

Figure S5:

The rapid injection device was built similar to the one described by Hore et al.[2].

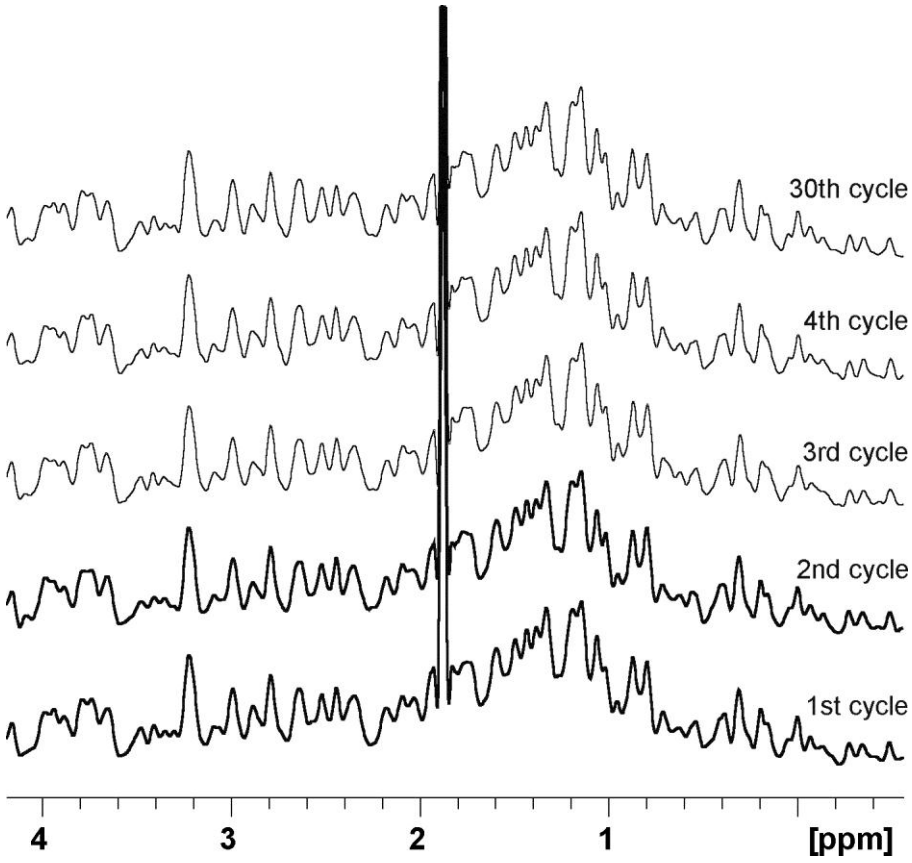

Figure S6:

Spectra of the first four and the 30th cycle (each consisting of 16 scans) of myoglobin acquired using the spatially selective and frequency-shifted continuous acquisition scheme. The time per scan was 78 ms amounting to a total of 1.25 seconds per cycle. This is enough for hMg to prevent any significant intensity changes between the individual cycles, as one also benefits from the selective excitation and the associated relaxation enhancement[3].

References:

(1) Sogbein, O. O.; Simmons, D. A.; Konermann, L. *Journal of the American Society for Mass Spectrometry* **2000**, *11*, 312.

(2) Mok, K. H.; Nagashima, T.; Day, I. J.; Jones, J. A.; Jones, C. J. V.; Dobson, C. M.; Hore, P. J. *Journal of the American Chemical Society* **2003**, *125*, 12484.

(3) Pervushin, K.; Vögeli, B.; Eletsky, A. *Journal of the American Chemical Society* **2002**, *124*, 12898.