The Potential of Ultrafast 2D NMR in Kinetic Studies of Protein Folding

Or Szekely[1] , Gad Armony[2], Maria Baias[1], Talia Harris[1], Deborah Fass[2], and Lucio Frydman[1]

[1]Department of Chemical Physics, [2]Department of Structural Biology, Weizmann Institute of Science, Rehovot, Israel

Abstract:

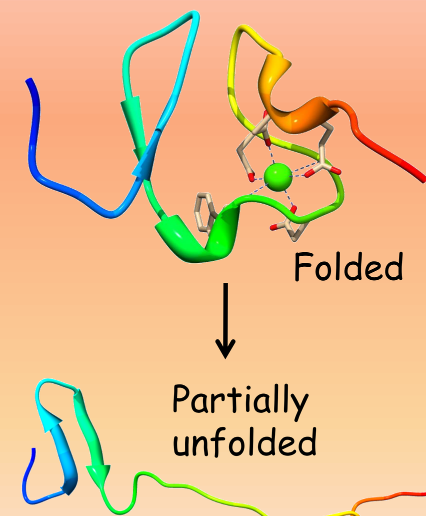
Two-dimensional Nuclear Magnetic Resonance (2D NMR) plays a uniquely important role in structural biology. Conventional acquisitions of 2D NMR spectra are generally on the time scale of minutes to hours, which is too long to observe dynamic processes of interest. Recent developments in the Frydman group [1] have made it possible to acquire 2D spectra in a single scan. The protocols developed in the Frydman group bypass the need for collecting a step-by-step incremented indirect dimension evolution time, encoding instead the same information along a spatial dimension of the sample.

This *spatio-temporal encoding* is done using a combination of frequency-swept pulses and gradients, and of an acquisition sequence where the data is decoded during free precession under gradients [2-4]. This form of *ultrafast* (UF) NMR thus accelerates the acquisition of a two-dimensional spectrum by reducing the number of required transients to just a single scan.

Excitation | Evolution (t_1) | Mixing | Acquisition t_2

Repeat N times, for $t_1 = \Delta t_1, 2\Delta t_1, \ldots, N\Delta t_1$

Spatial Encoding | EPSI

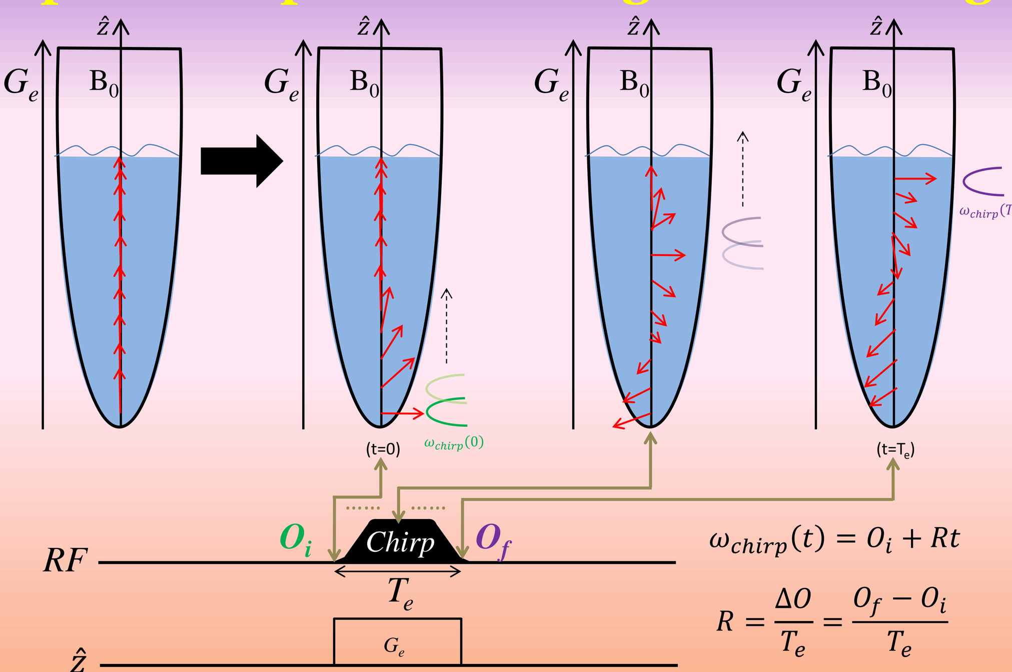


Biophysical processes, such as protein folding, may take place on a timescale of milliseconds-to-seconds, and are therefore suitable candidates for studies by means of ultrafast 2D NMR. Thus, with the reduced acquisition time inherent to UF 2D NMR, and with additional sensitivity gained from a careful optimization of the experimental parameters, the method can be adapted to monitor folding processes of proteins at mM concentrations. This work aims to find the appropriate conditions and parameters to increase signal-to-noise ratio (SNR) and to produce a high-quality time-resolution series for the application of UF NMR in the kinetic studies of protein folding and protein dynamics.

1. Frydman, L., Scherf, T., and Lupulescu, A., The acquisition of multidimensional NMR spectra within a single scan. Proceedings of the National Academy of Sciences, 2002. 99(25): 15858-15862.
2. Gal, M., and Frydman, L., Ultrafast Multidimensional NMR: Principles and Practice of Single-Scan Methods, in Encyclopedia of Magnetic Resonance. 2008, John Wiley & Sons, Ltd.
3. Mishkovsky, M., and Frydman, L., Principles and Progress in Ultrafast Multidimensional Nuclear Magnetic Resonance. Annual Review of Physical Chemistry, 2009. 60(1): 429-448.
4. Tal, A., and Frydman, L., Single-scan multidimensional magnetic resonance. Progress in Nuclear Magnetic Resonance Spectroscopy, 2010. 57(3): 241-292.

Ultrafast 2D NMR:

Spatio-temporal encoding and decoding



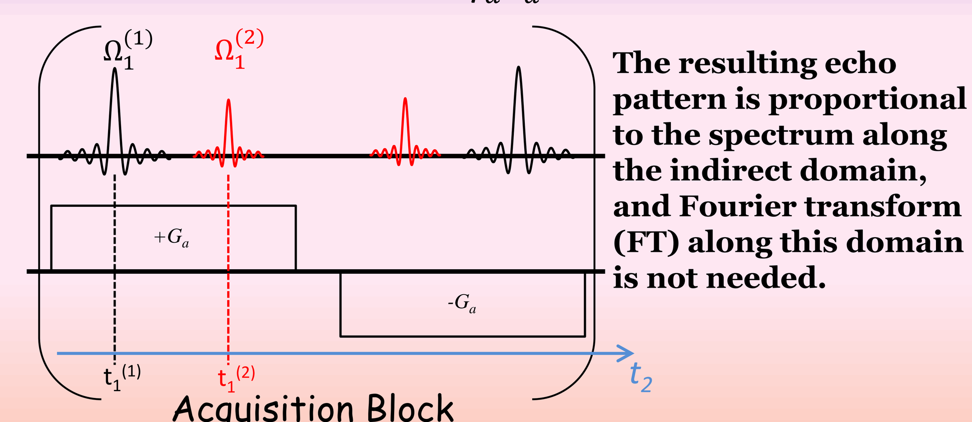
The combination of the chirped π pulse and the gradient causes the spins to acquire a phase with a quadratic term.

The application of a second chirp with the same sweep in the presence of a gradient with opposite polarity, leads to the desired linear dependence on position:

$$\phi(z) = t_1(z)\Omega_1 = Cz\Omega_1$$

Constant - Time (CT) Encoding Scheme

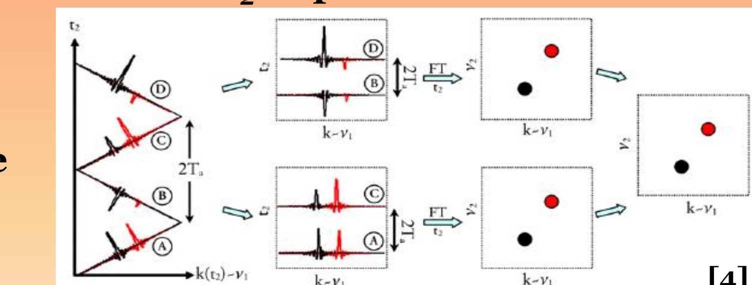
Decoding is achieved during acquisition using a gradient G_a, causing each spin to produce an echo at a time: $t_{echo}^{(j)} = \frac{C}{\gamma_a G_a}\Omega_1^{(j)}$



The resulting echo pattern is proportional to the spectrum along the indirect domain, and Fourier transform (FT) along this domain is not needed.

This acquisition block is repeated multiple times during the acquisition time t_2.

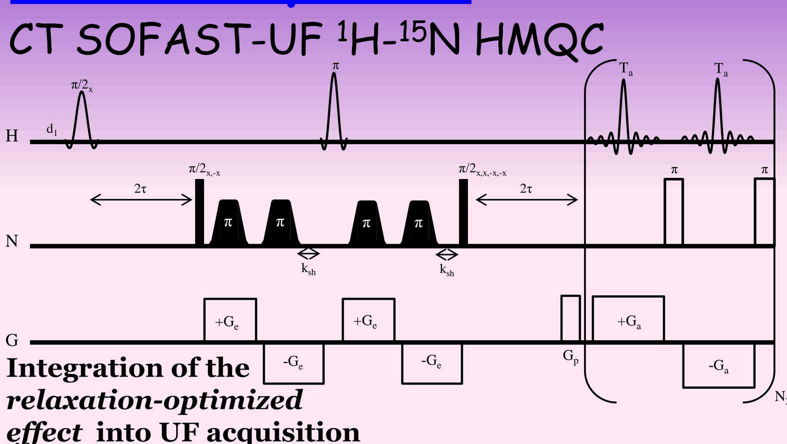
The amplitude of the echoes is modulated by inherent t_2-dependent chemical shifts, Ω_2.



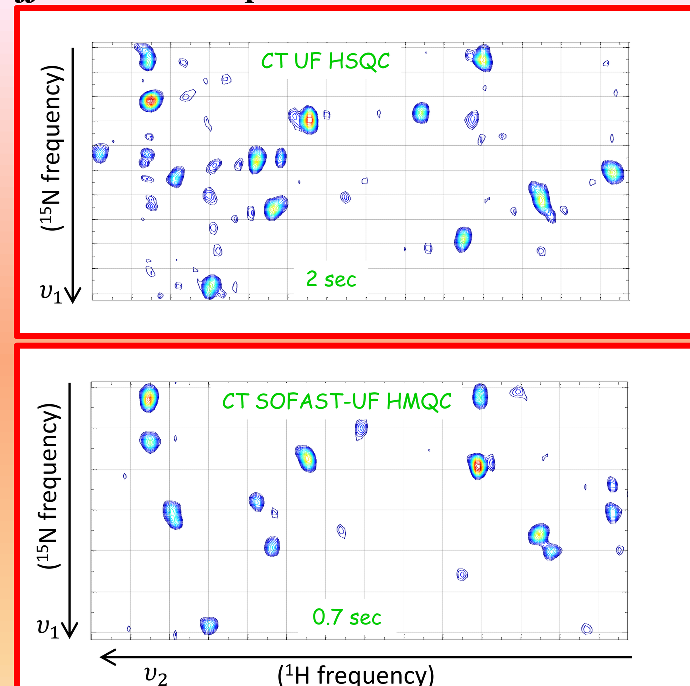
Re-arranging the data [4] into two sets (arising from the positive and negative G_a) and applying Fourier transform along the t_2 dimension, results in the full 2D spectrum.

Selective pulses:

CT SOFAST-UF ^1H-^{15}N HMQC



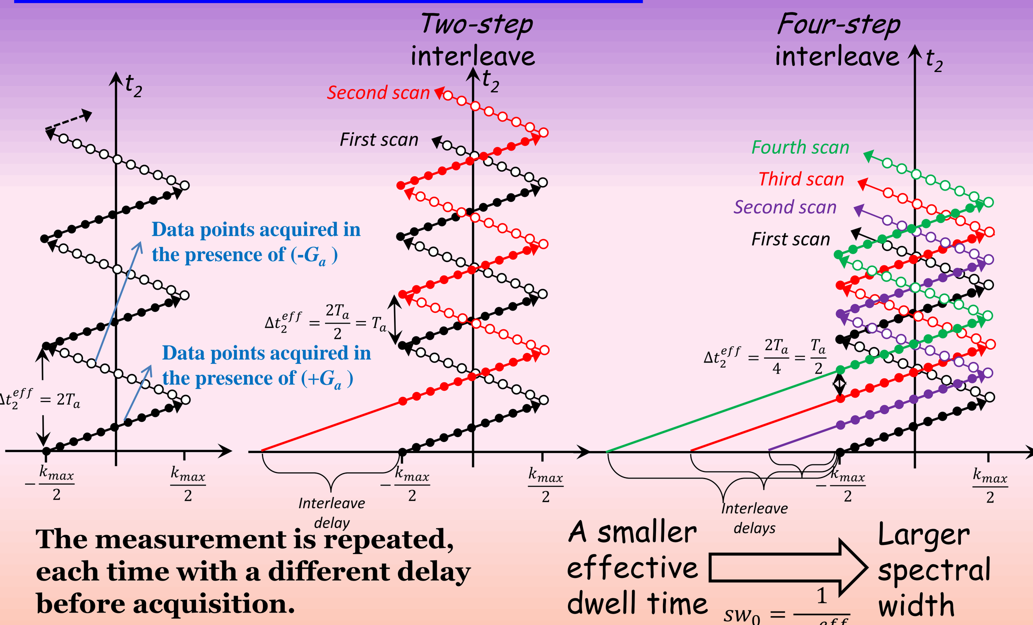
Integration of the *relaxation-optimized effect* into UF acquisition



Measured on a Varian iNova 600 MHz console equipped with a triple-axis HCN probe.

Cutting the repetition rate determines the time resolution of the experiment, and thus the biological processes we can observe using it.

Interleaved data sets:



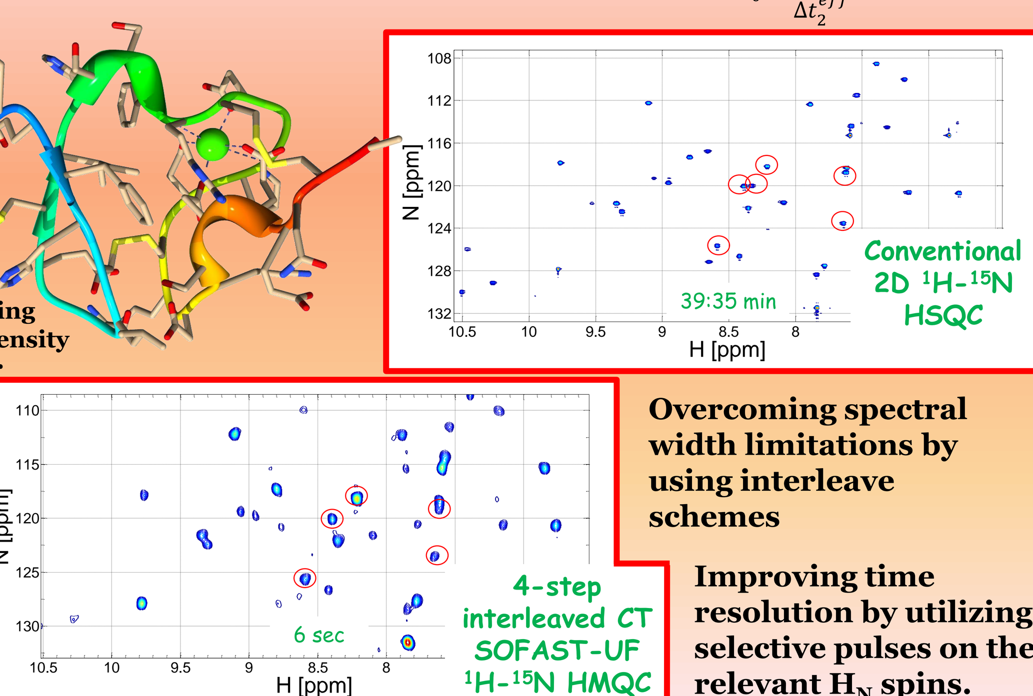
The measurement is repeated, each time with a different delay before acquisition.

A smaller effective dwell time ⟹ Larger spectral width

$sw_0 = \frac{1}{\Delta t_2^{eff}}$

40 amino acids, 4.5 kDa

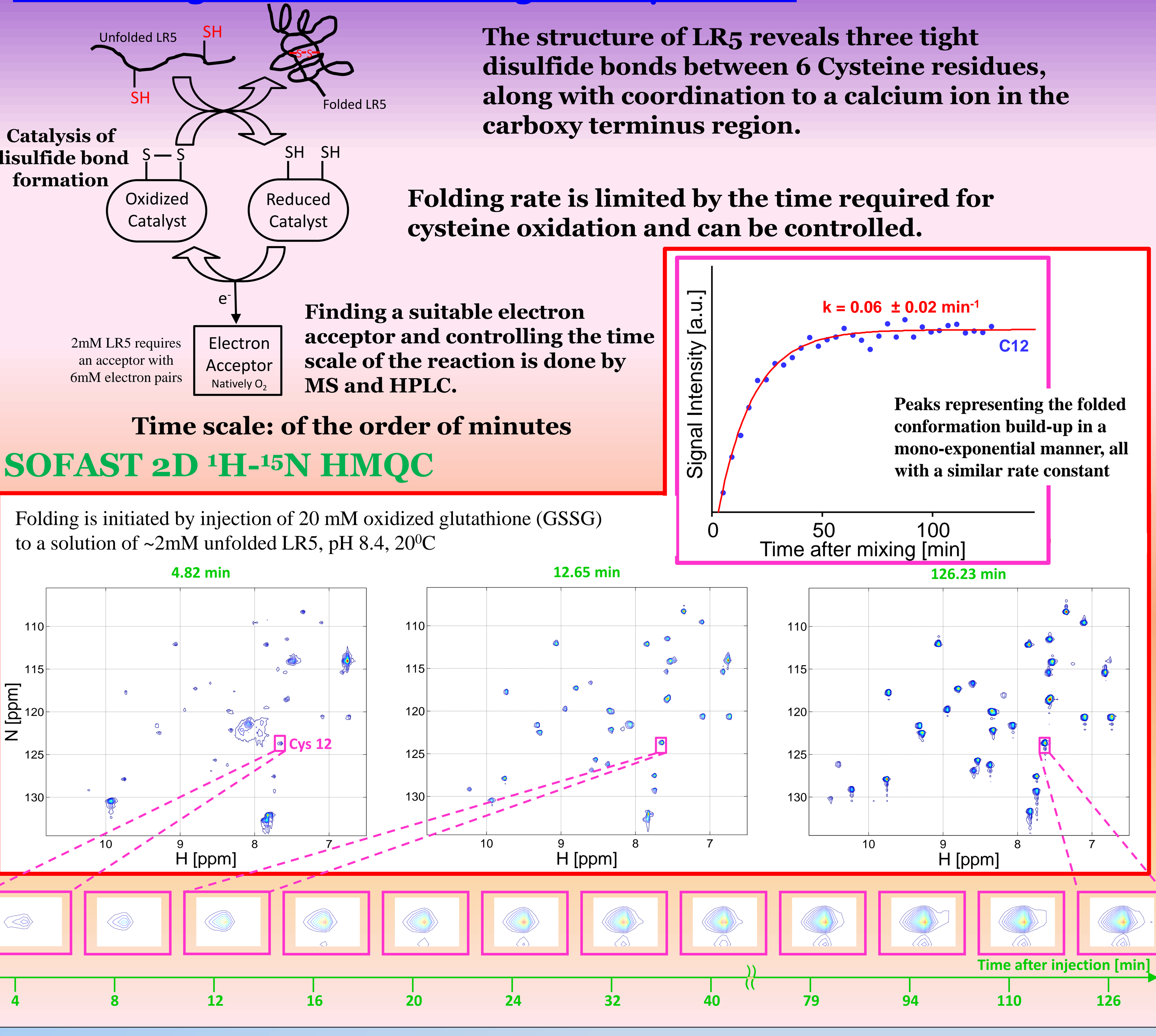
LR5 is the fifth binding domain of the low density lipoprotein receptor (LDLR)



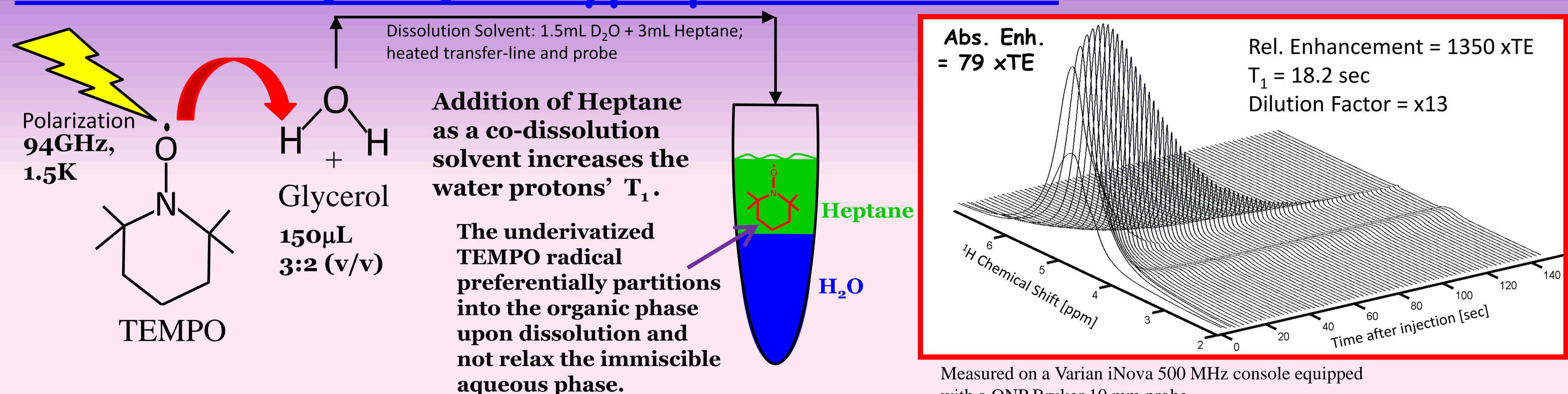
Overcoming spectral width limitations by using interleave schemes

Improving time resolution by utilizing selective pulses on the relevant H_N spins.

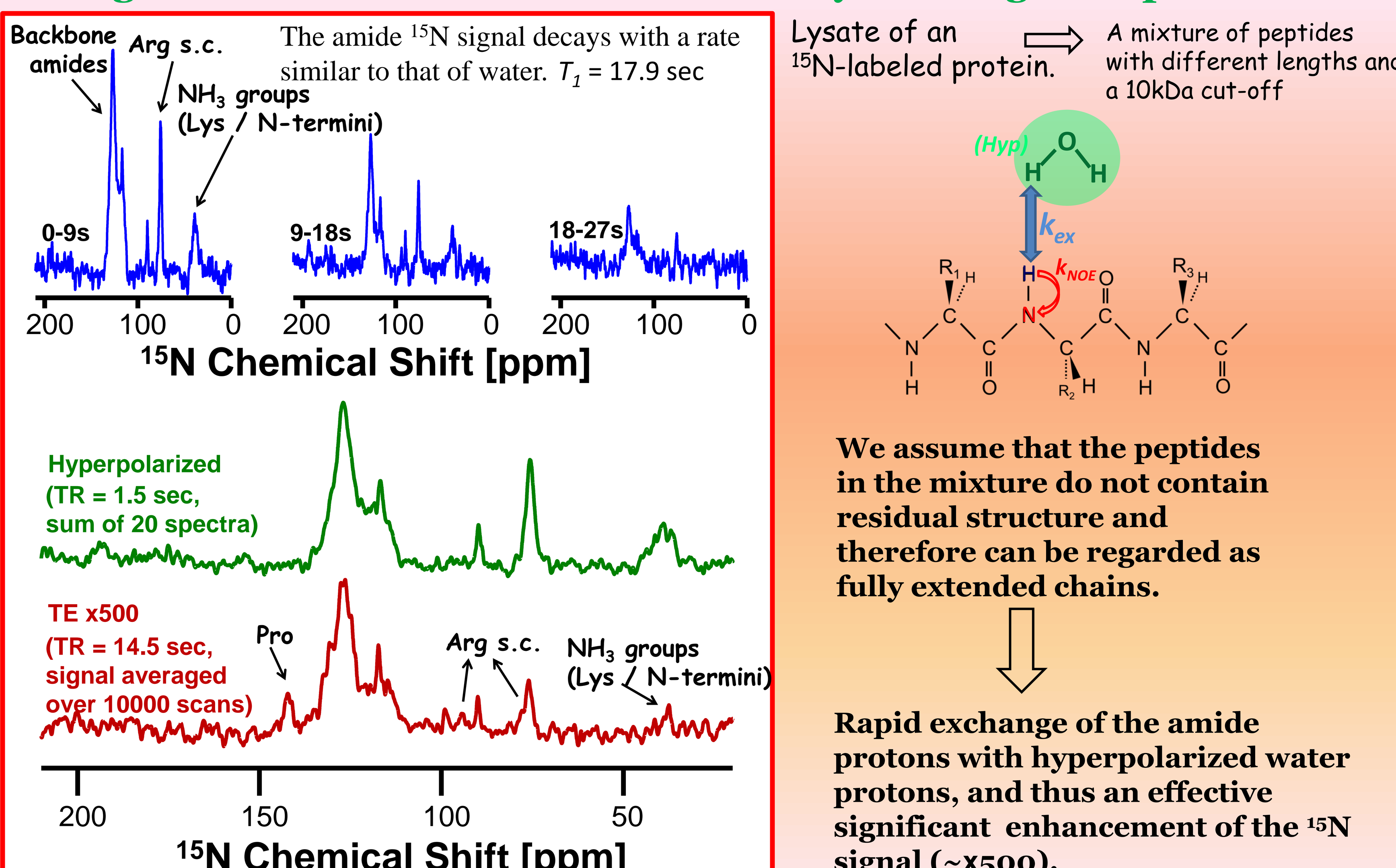
Monitoring oxidative folding of a protein:



Heteronuclei signal enhancement by using Dynamic Nuclear Polarization (DNP) to hyperpolarize water:



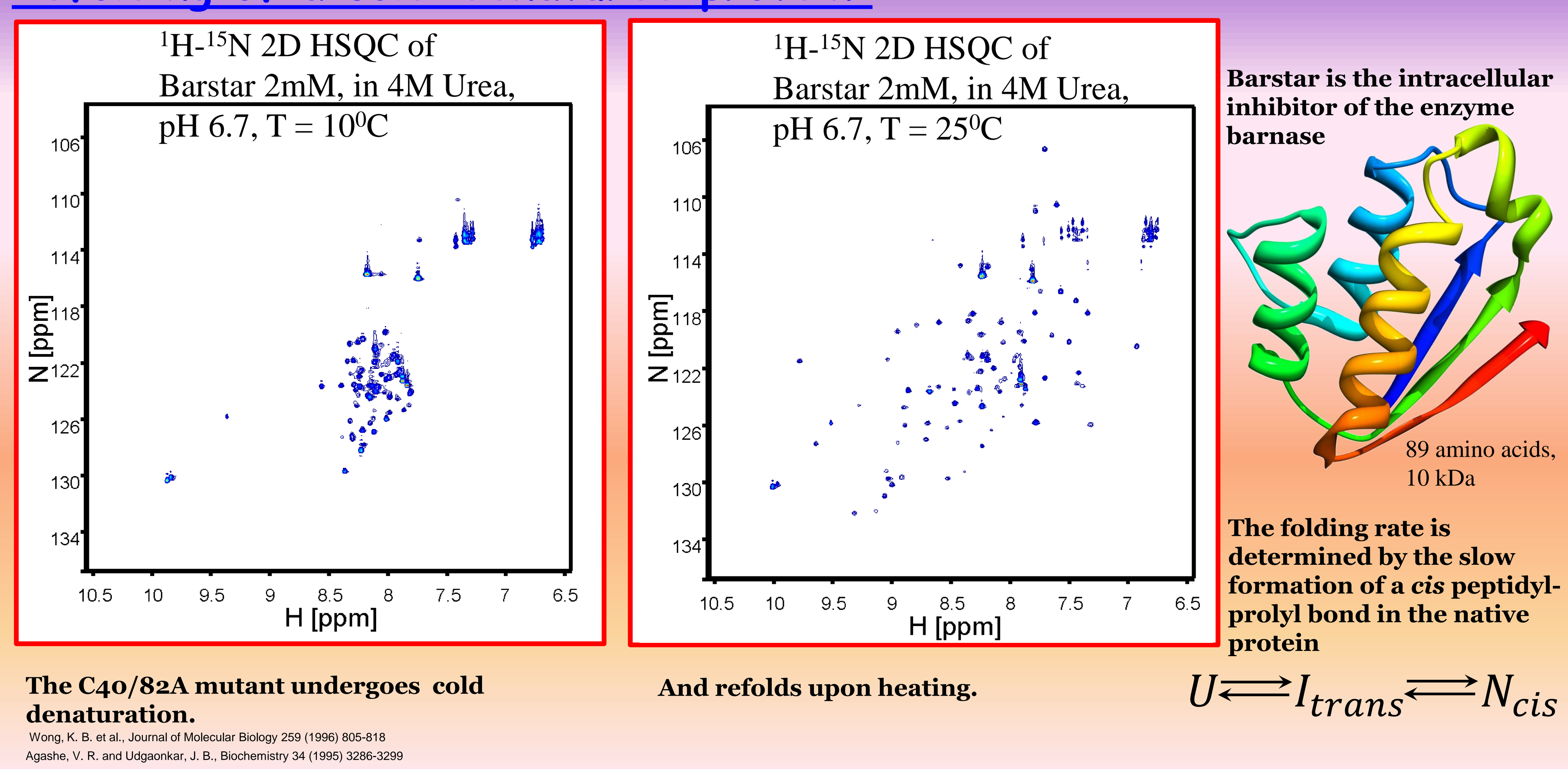
^{15}N signal enhancement of biomolecules by exchangeable proton NOE:



We assume that the peptides in the mixture do not contain residual structure and therefore can be regarded as fully extended chains.

Rapid exchange of the amide protons with hyperpolarized water protons, and thus an effective significant enhancement of the ^{15}N signal (~x500).

Refolding of a cold-denatured protein:



The C40/82A mutant undergoes cold denaturation. **And refolds upon heating.**

$$U \rightleftharpoons I_{trans} \rightleftharpoons N_{cis}$$

Wong, K. B. et al., Journal of Molecular Biology 259 (1996) 805-818
Agashe, V. R. and Udgaonkar, J. B., Biochemistry 34 (1995) 3286-3299

Future Plans:

- Monitor the folding process of unfolded LR5 using interleaved CT SOFAST-UF HMQC upon injection of GSSG in order to observe folding intermediates.
- Substitute Ca^{2+} ion by a paramagnetic shift agent to elucidate the coupling between S-S bond formation and metal binding in the folding process of LR5.
- Monitor proline rearrangements as a rate-determining step in protein refolding upon sudden heating cold-denatured Barstar.
- Utilizing the enhanced long-lived ^{15}N magnetization of biomolecules upon injection of hyperpolarized water in measurement of ultrafast 2D spectra. Thus, we shall gain sensitivity, and eventually can follow the folding of an unfolded protein upon injection of a hyperpolarized refolding buffer.