Kinetics with IR for Protein folding

or other dynamic processes

Time dependent data with FTIR

Stop-flow methods - msec limits so far

Continuous, micro-flow methods - < 100 μsec

Rapid scan FT-IR - msec

T-jump and Flash photolysis -nsec time scales
using step scan methods

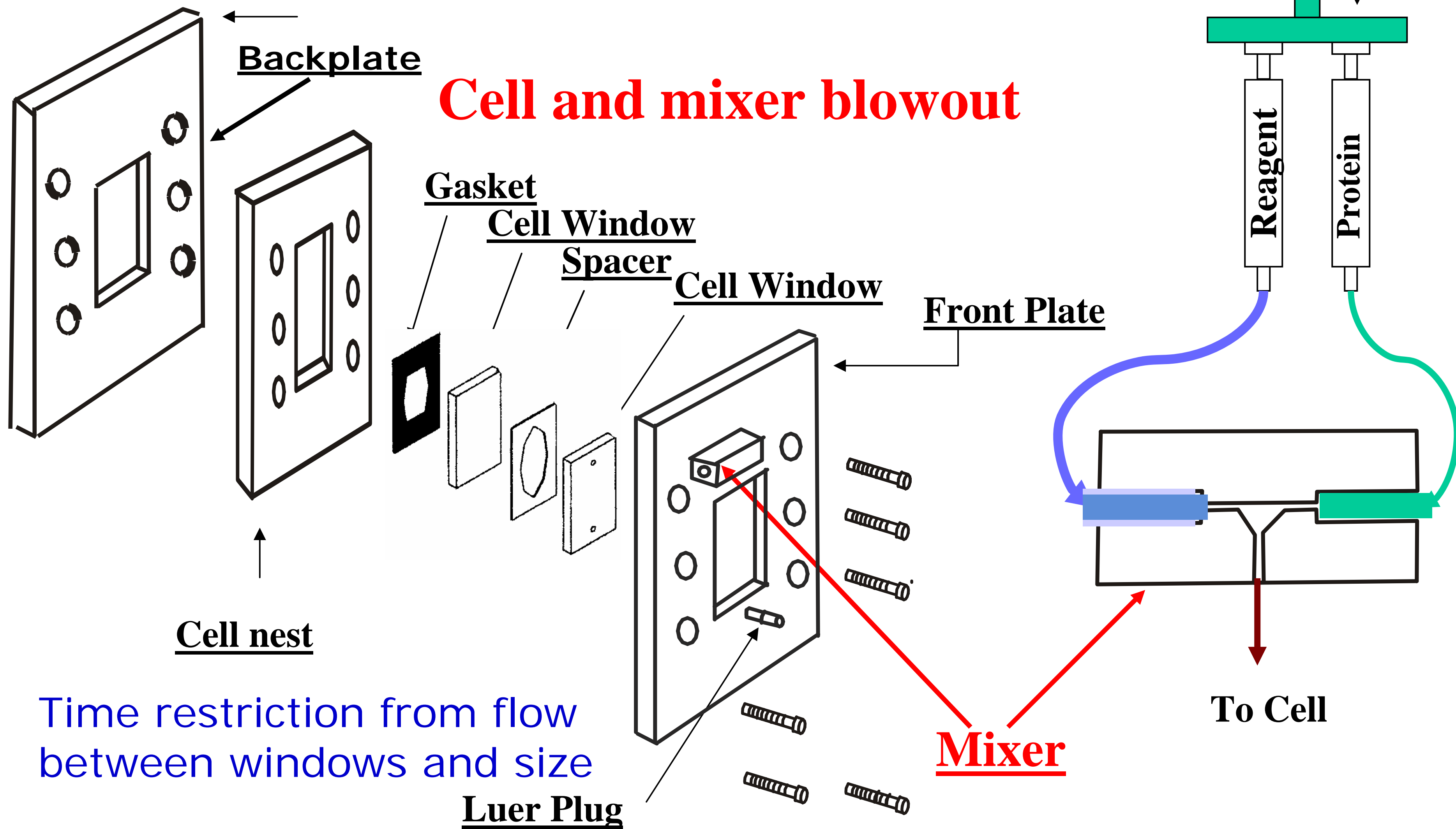
2-D IR, pump-probe, and THz laser based IR
spectra can sample spectrum on fsec scale

Most T-jump single ν with tunable IR laser for S/N, filtering and . .

a. Stop-flow concepts

b. Micromixer Continuous-Flow IR Characteristic

Scheme of Stop Flow— initialize by rapid mixing

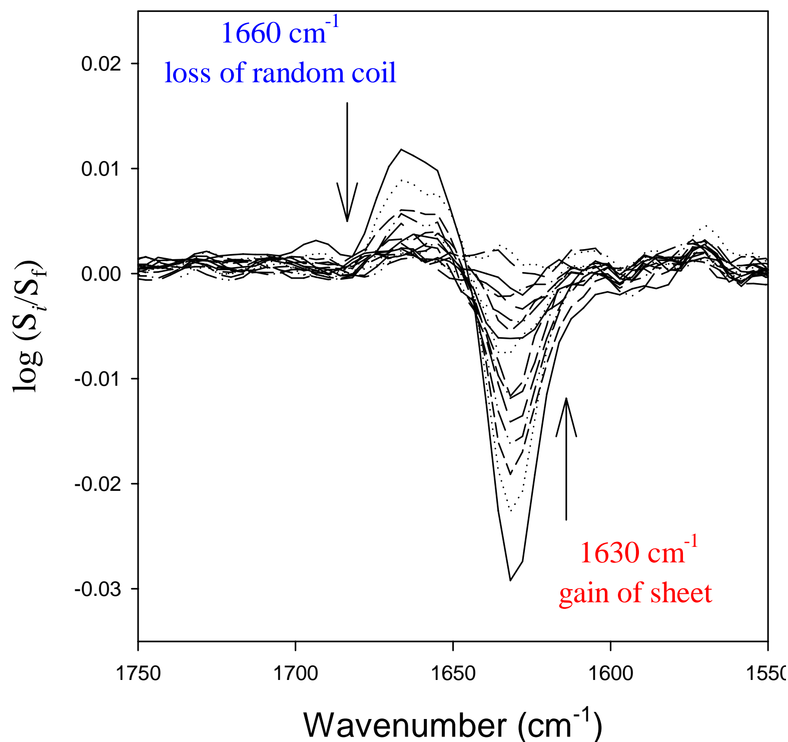


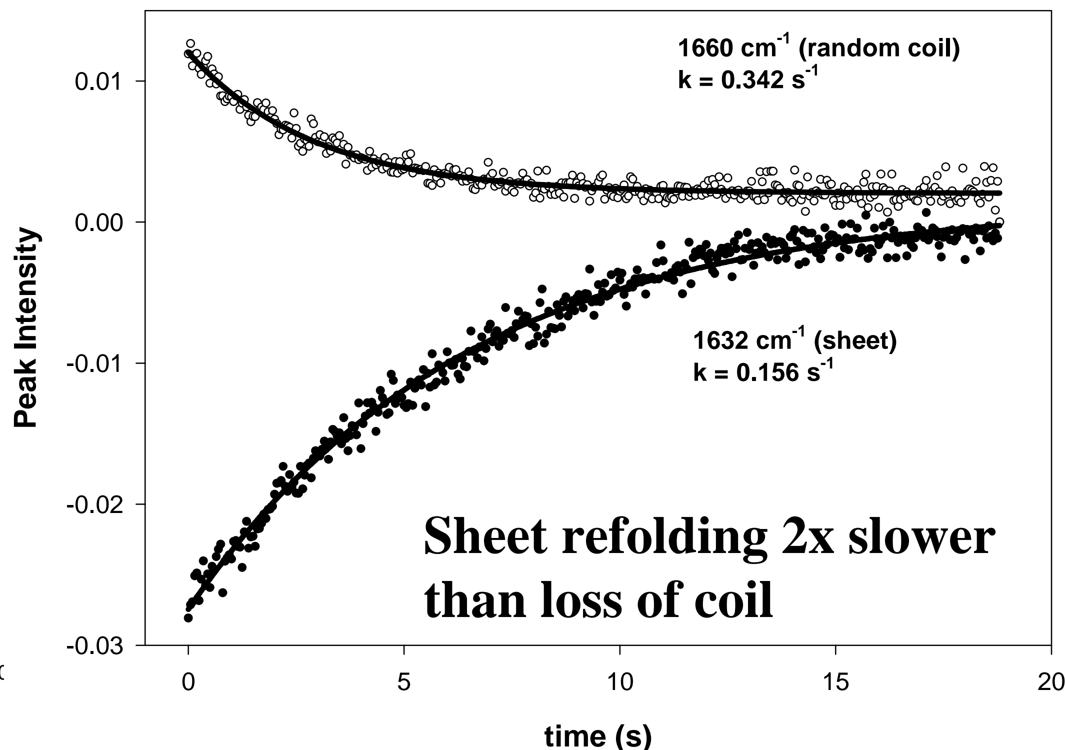
Time restriction from flow between windows and size

Mix protein and perturbant rapidly to get new state, follow spectra

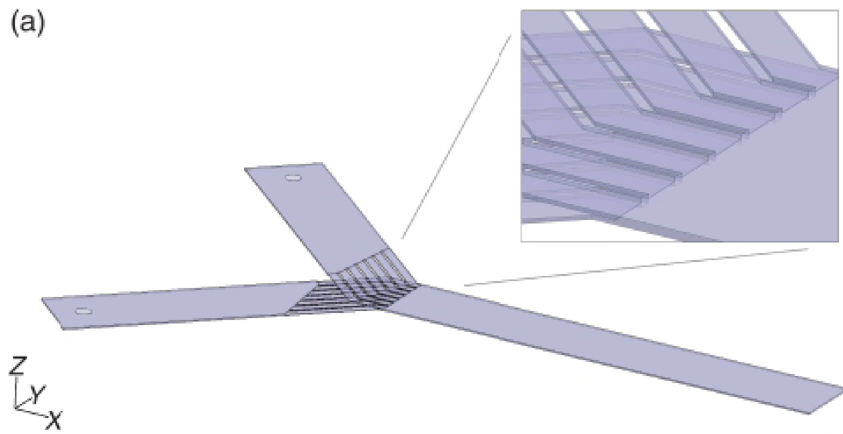
Refolding of Ribonuclease A by FTIR

Inverse T-jump: Refolding initiated by injecting Ribo A stored in syringe at 80 °C into IR cell at 25 °C





<u>One</u> single beam spectrum (IF scan) is collected for each time point. Time resolution = 50 ms, but could be faster, if modify. IR resolution 8 cm^{-1} sufficient to separate increase in sheet, decrease in coil as folds.



Flow mixer to bring reagents together on micro-scale

IR spectra measure through chip, distance is time

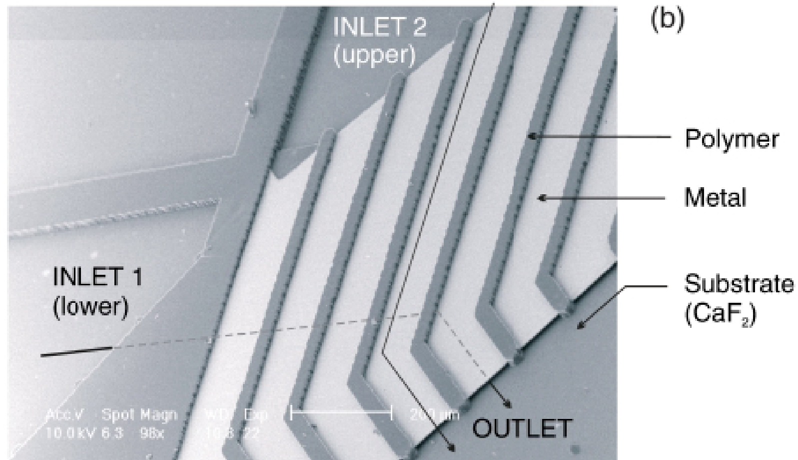
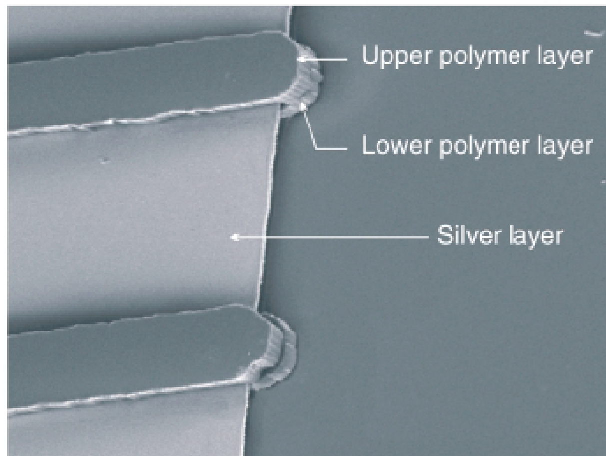


Fig. 2 (a) Schematic view of the whole micromixing pattern with a close up view of the microchannels. (b) Scanning electron micrographs to illustrate the sandwich construction of polymer and silver layer.



Design, simulation and application of a new micromixing device for time resolved infrared spectroscopy of chemical reactions in solution

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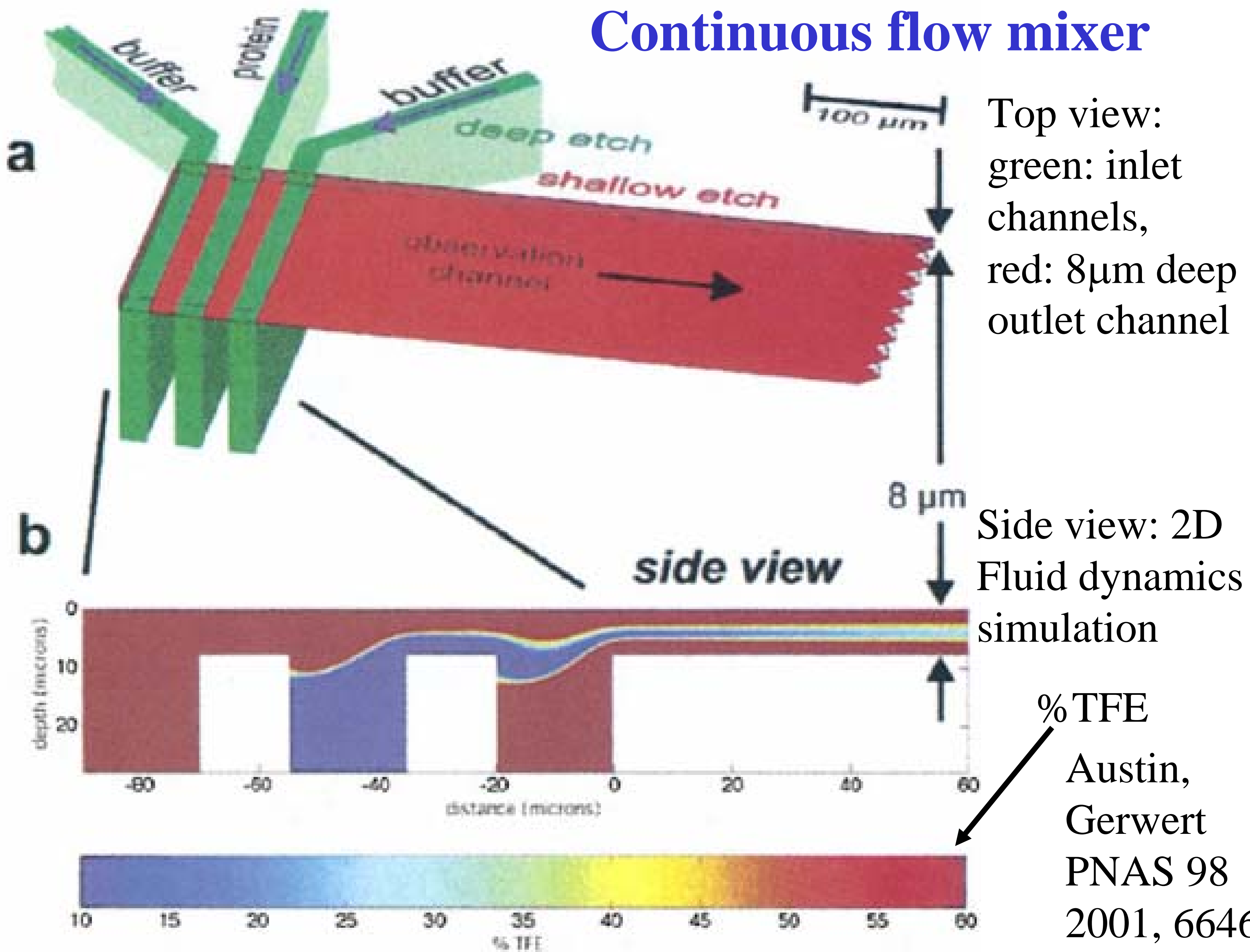
Peter Hinsmann,[a] Johannes Frank,[b] Peter Svasek,[c] Michael Harasek[d] and Bernhard Lendl*[a]

Lab on a Chip, 2001, **1**, 16–21


Continuous flow mixer
a
buffer
protein
buffer
deep etch
shallow etch
100 μm
Top view:
green: inlet channels,
red: 8μm deep outlet channel
8 μm
b
side view
Side view: 2D Fluid dynamics simulation
0
10
20
-80
-60
-40
-20
0
20
40
60
%TFE
10
15
20
25
30
35
40
45
50
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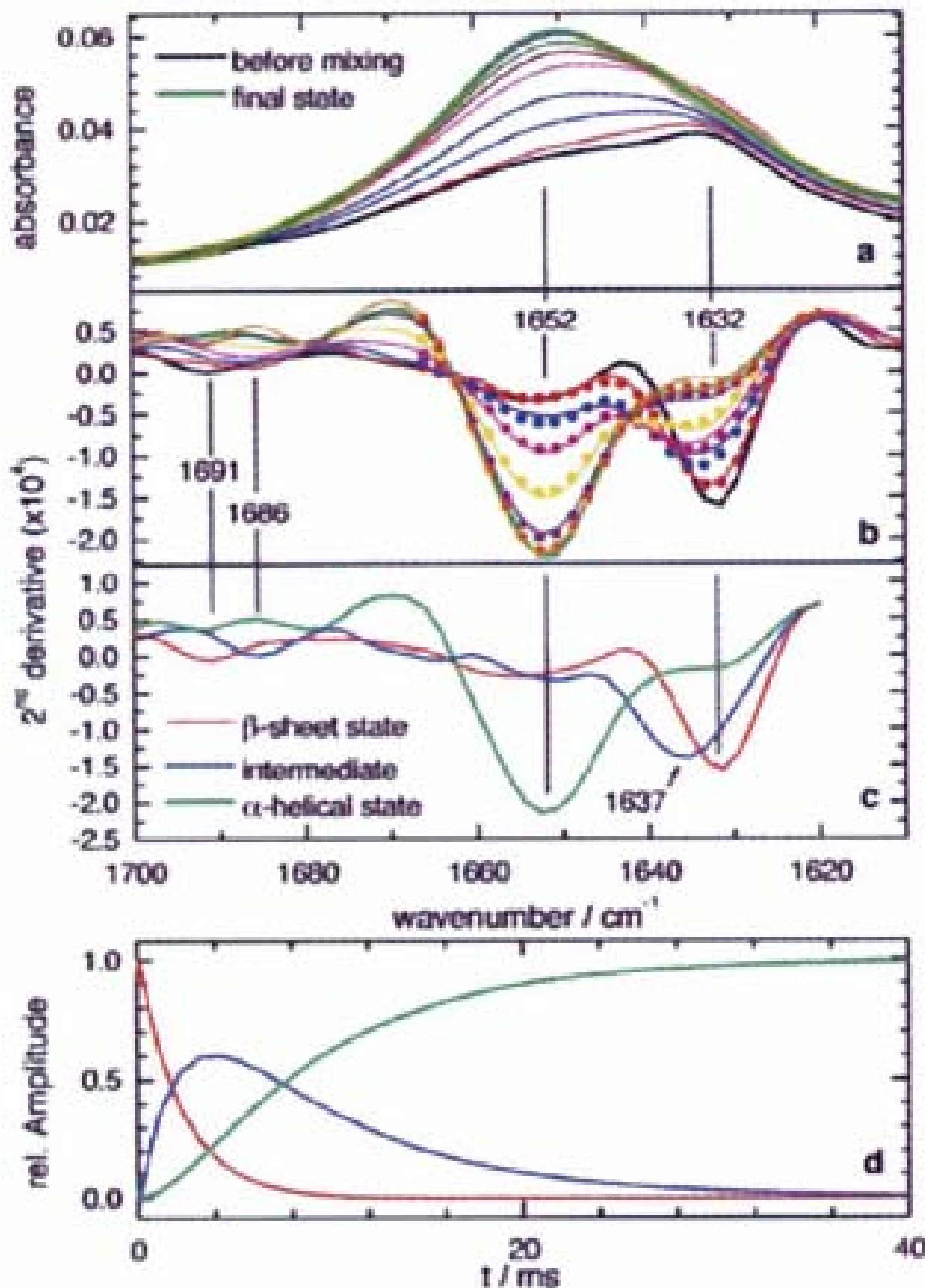
60
Austin, Gerwert PNAS 98 2001, 6646



Lifetimes of intermediates in the β-sheet to α–helix transition of β-lactoglobulin using diffusional IR mixer

E. Kauffmann, N. C. Darntont, R. H. Austin, C. Batts, and K. Gerwert

PNAS 2001 98 6646-6649

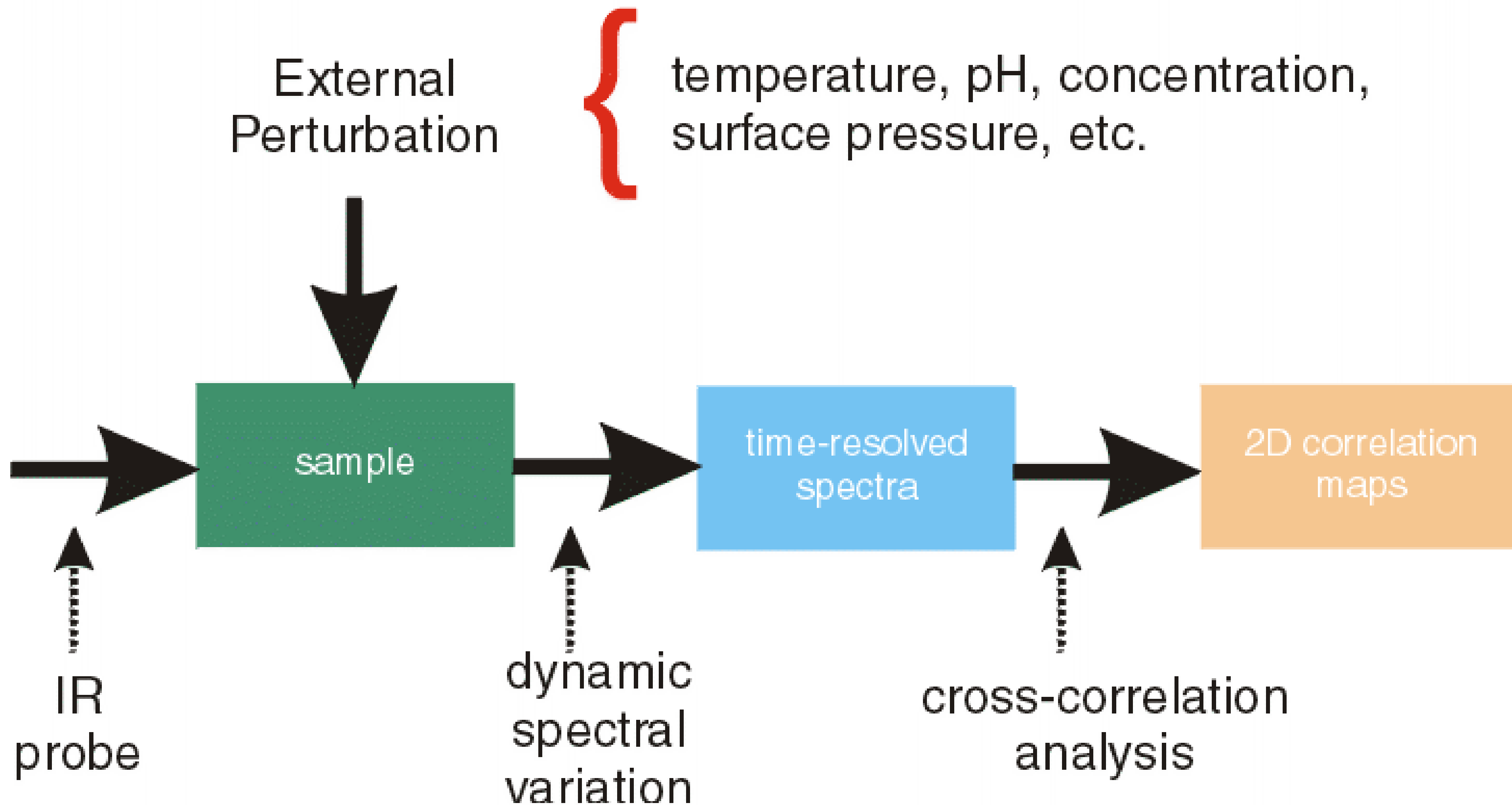
a) Spectra along channel: 1.1,3.4,5.7,10.2,21.6,103 ms
b) 2nd deriv. & 3-state fit
c) 3 basic spectra derived
d) Time course of 3 states

2-Dimensional analyses of spectra-(Noda)

- Correlation of peaks due to perturbation
- Identification of structural dependencies (novel development)
  - homospectral -- vary with secondary structure
  - heterospectral -- correlate to well known dependence
- Allow assignment of unknown features

Generalized 2D IR Procedure

I. Noda *Applied Spectroscopy* (1993) **47**, 1329.



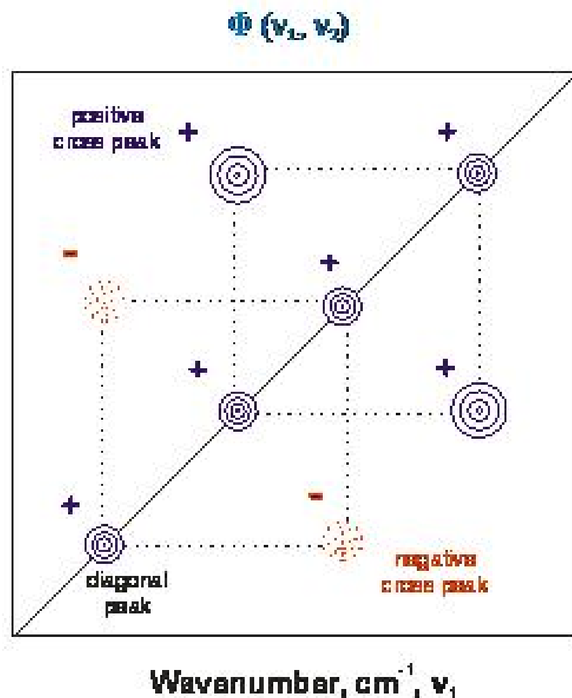
From Rich Dluhy WebSite, Univ. Georgia

2D IR Correlation Maps

Synchronous Plot

- similar changes at (ν_1, ν_2)
- In phase signal variations

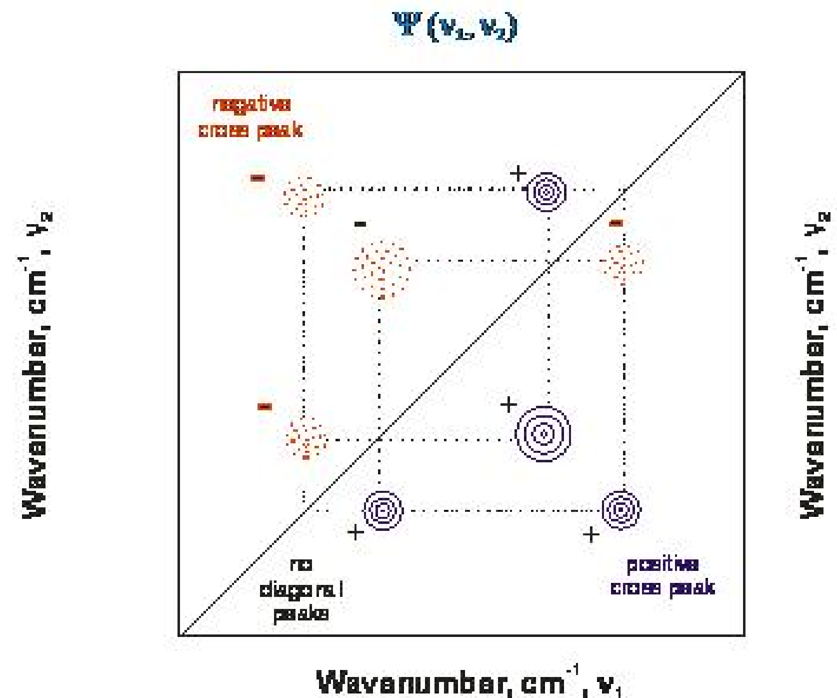
$\Phi(\nu_1, \nu_2)$



Asynchronous Plot

- dissimilar changes at (ν_1, ν_2)
- Out of phase signal variations

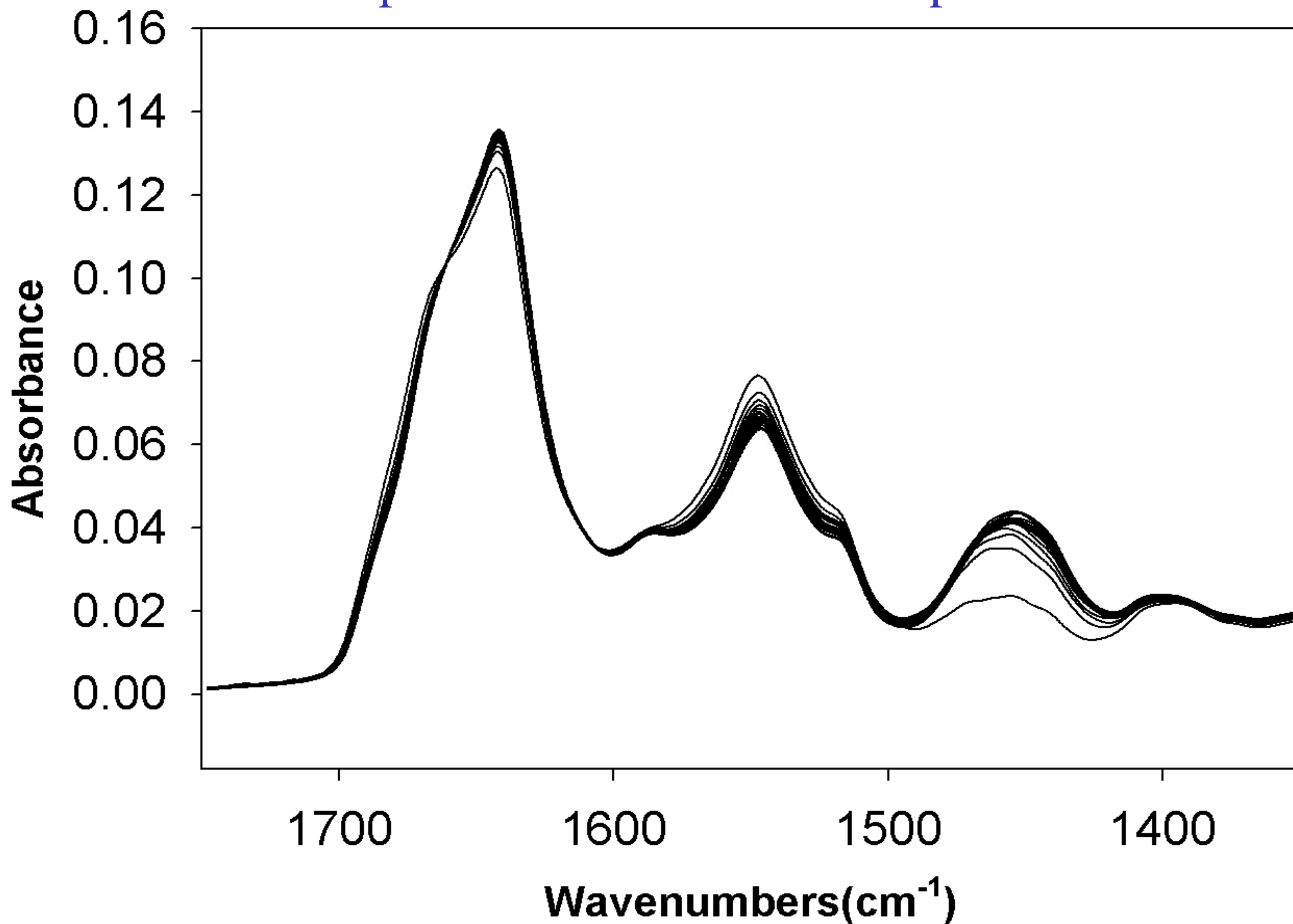
$\Psi(\nu_1, \nu_2)$



From Rich Dluhy WebSite, Univ. Georgia

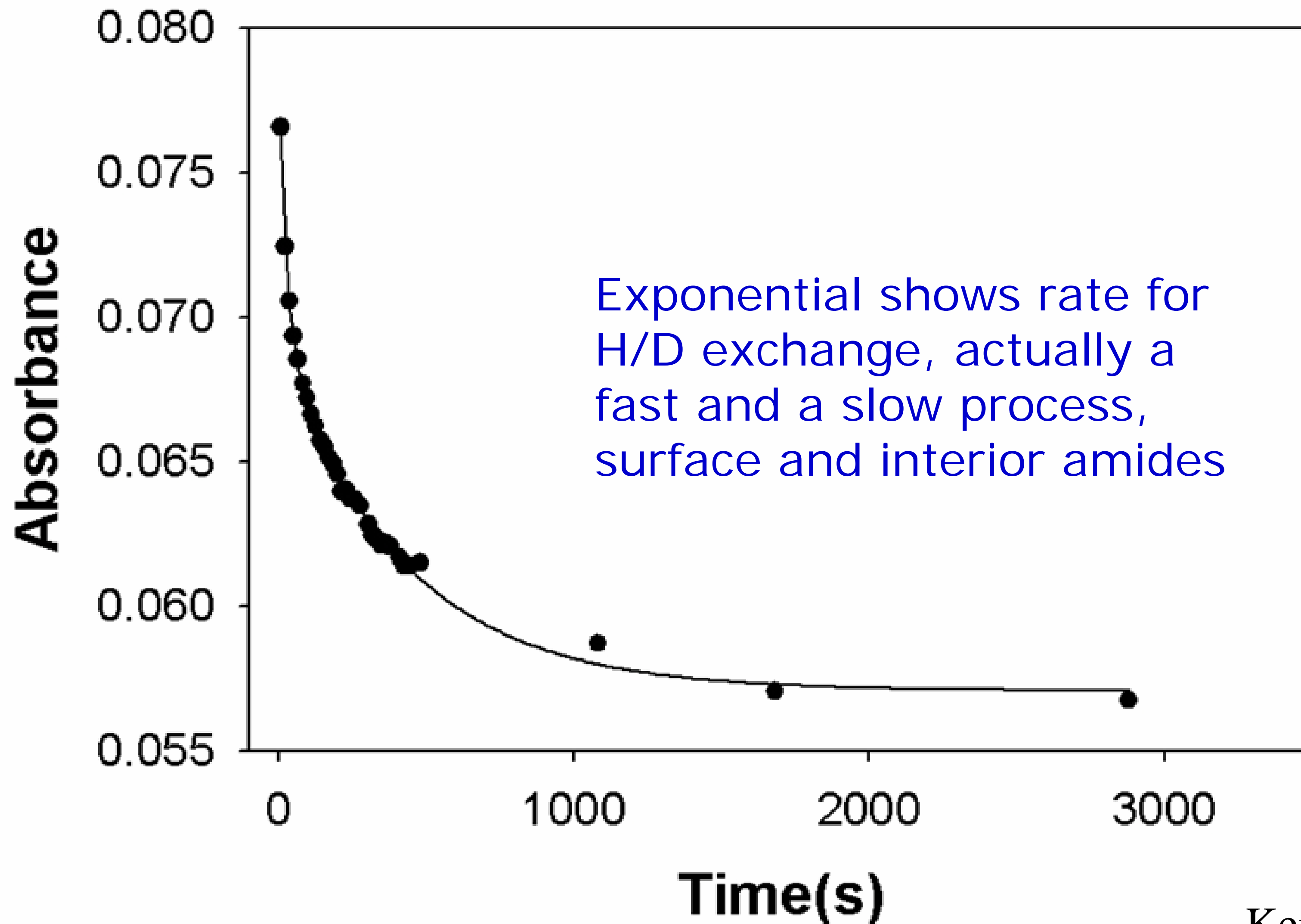
IR Spectra of H/D exchange of Rnase A

Time Dependent variation after Stop-Flow insertion



Keiderling/Qi Xu - unpublished

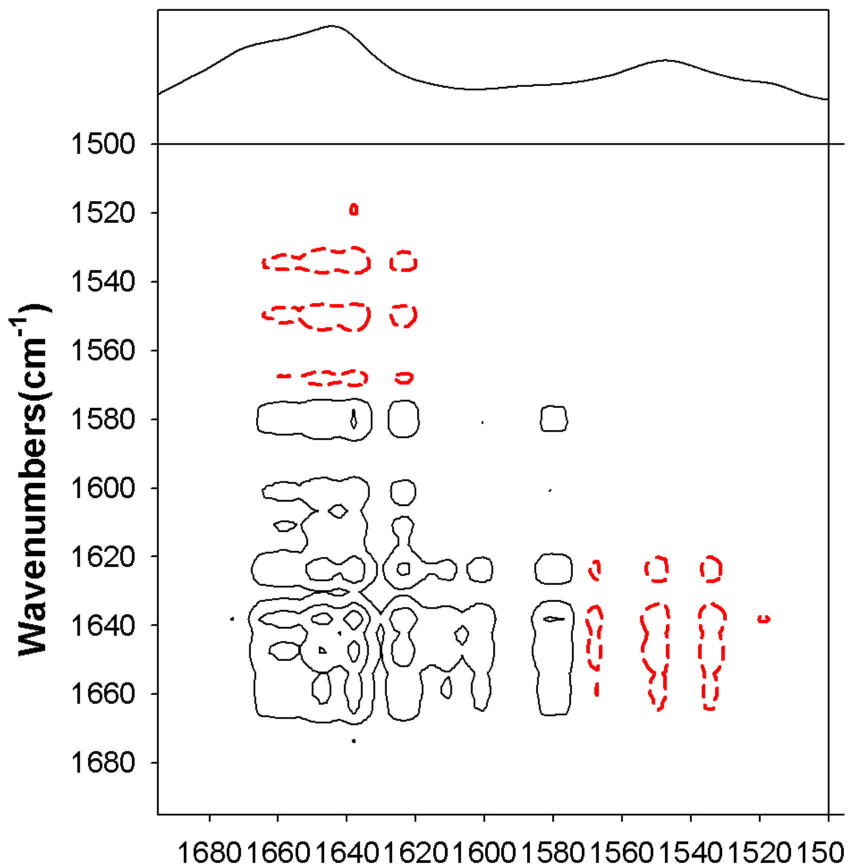
100mg/ml Rnase A in H2o dilute with 4 times d2o pH=4.6



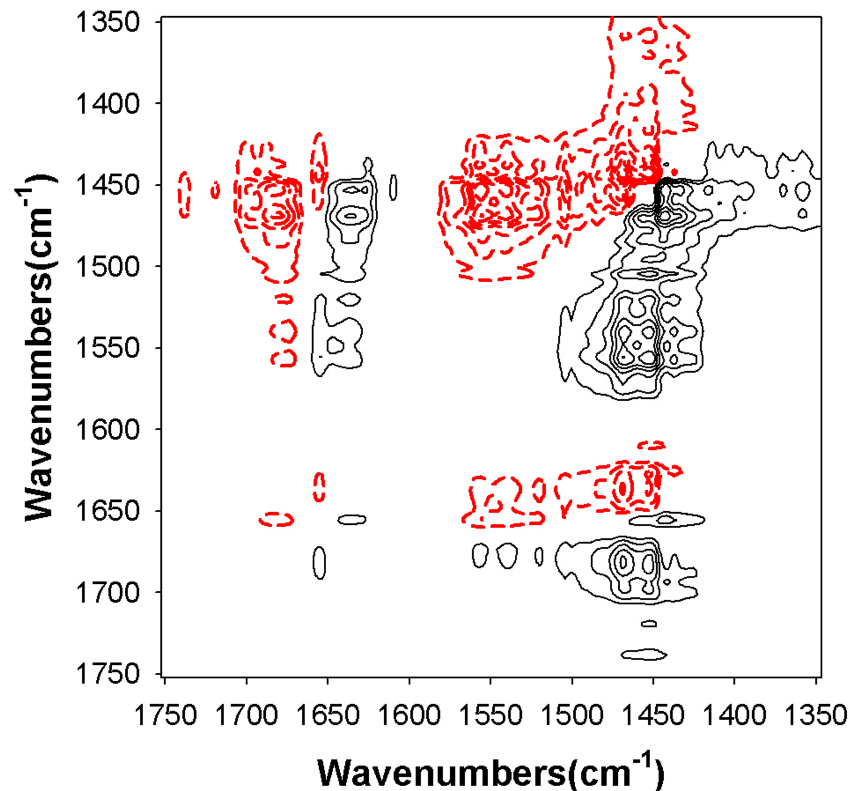
Keiderling/Qi Xu
- unpublished

This remains an interesting way to categorize folds!

2-D Maps of time correlation of H/D exchange



Solid line: Positive
Dotted Line : Negative

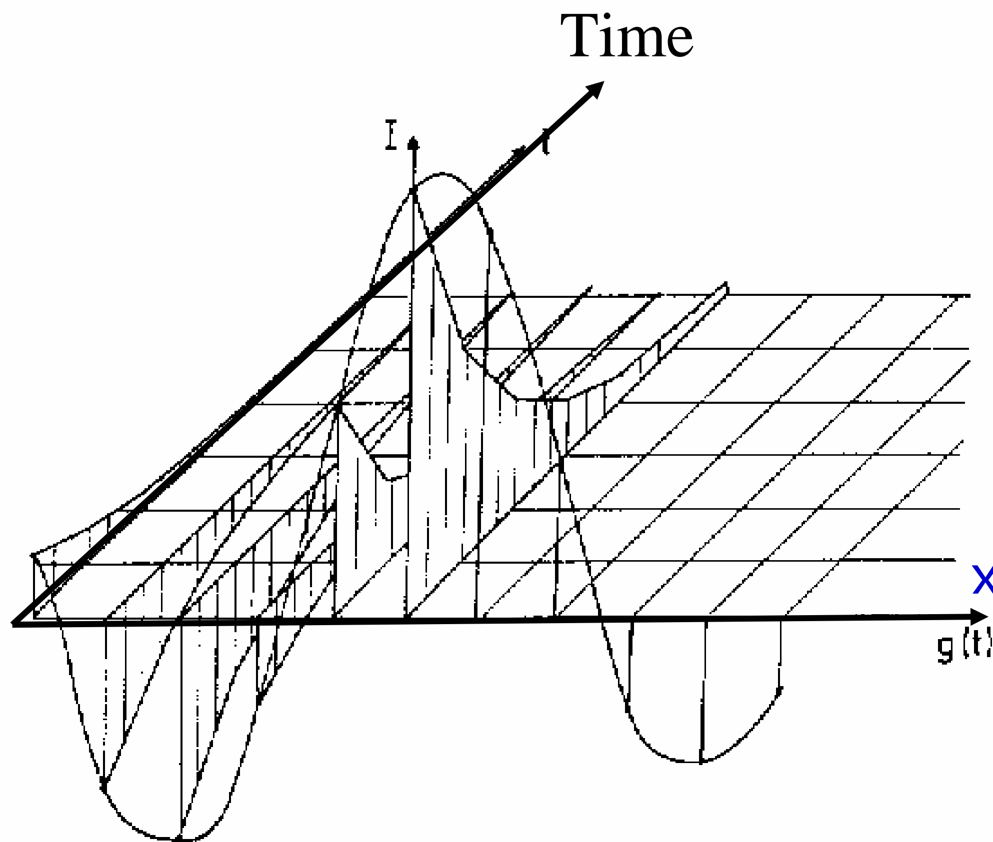


Solid line : Positive
Dotted line : Negative

Keiderling/Qi Xu - unpublished

- **Processes that can be repeated many times (thousands) can be studied at very fast (ns) rates using step scan techniques**

Step-Scan FTIR based Time-resolved Experiments



At each mirror position, pulse the sample, then collect signal vs. time (nsec resolution). Move to the next step, repeat. When complete, data from same time delay following the pulse at each step can be combined to form an interferogram for that time. FT gives the spectrum. Requires sample to be cyclic, must reversibly relax.

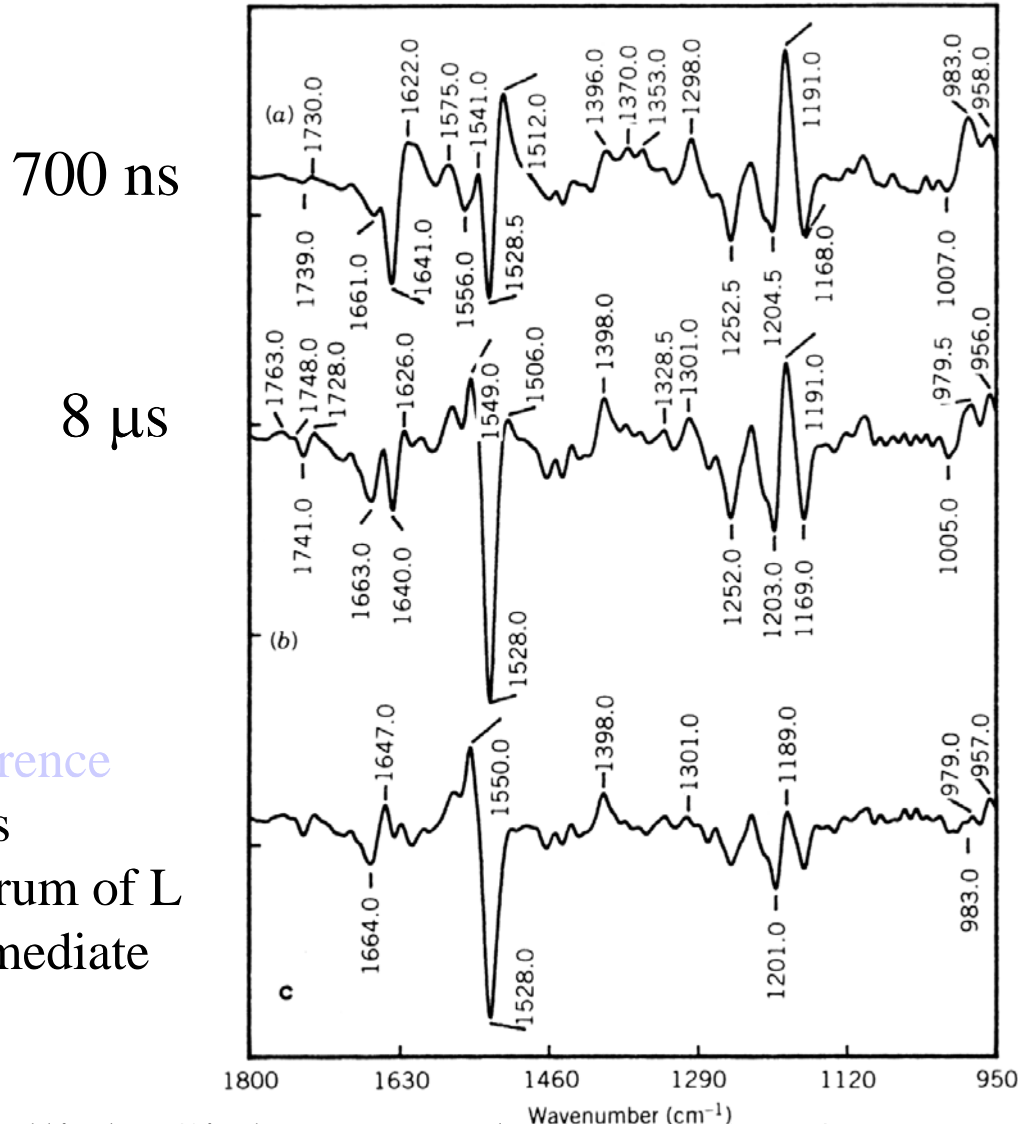
Step-scan is slow, but at each step can measure very fast decay

Bacteriorhodopsin - flash photolysis

time resolved step-scan

700 ns

8 μs



Difference yields spectrum of L intermediate

Terrific sensitivity from measuring the baseline for each pulse by recording the signal just before the strobe—no drift

Systems that can be photo initiated to new state (like BR) and relax back reversibly offer possibility of fast kinetics, specific sites

Weidlich, Siebert, Appl. Spect. 1993

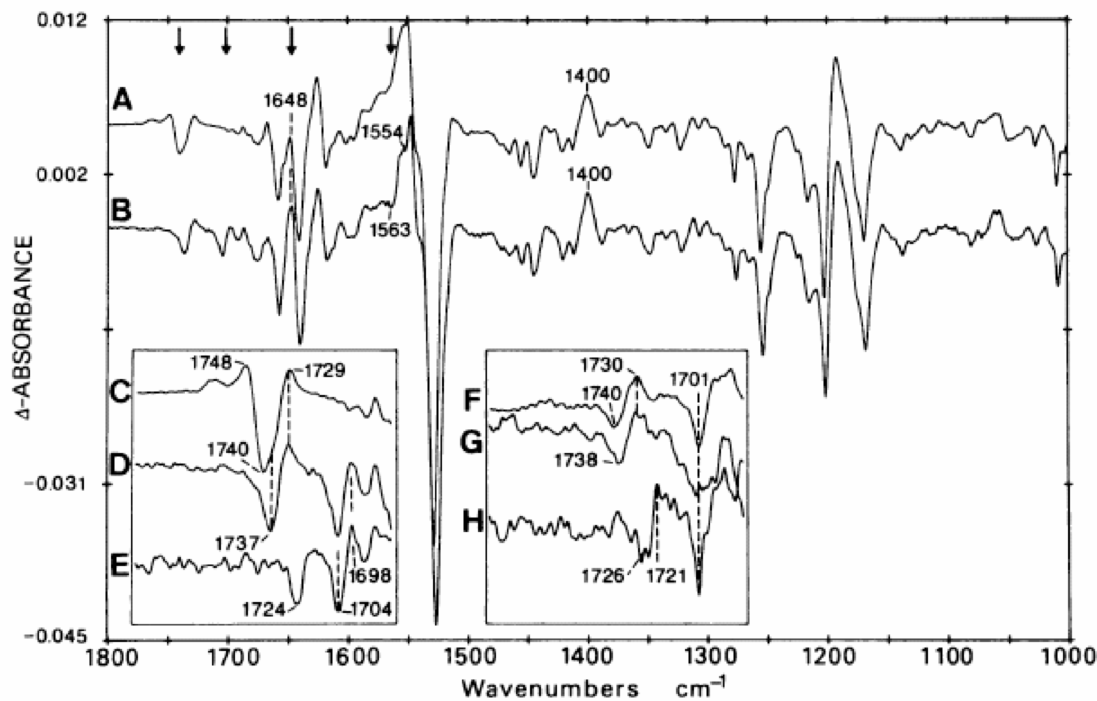


FIG. 1. FTIR difference spectra of bacteriorhodopsin. A, BR − L difference spectrum taken in H_2O at 170 K with spectral resolution of 2 cm^{-1}. B, Corresponding difference spectrum of the mutant in which Asp-96 is changed to Asn-96, recorded under the same conditions as A. C, Expansion of the spectral region 1800–1680 cm^{-1} of A. D, Expansion of the spectral region 1800–1680 cm^{-1} of B. E, BR − L difference spectrum of the mutant in deuterium oxide (D_2O) at 1800–1680 cm^{-1}. F, BR − K difference spectrum of the wild type at 1800–1680 cm^{-1} taken at 70 K in H_2O. G, BR − K difference spectrum of the mutant taken in H_2O at 1800–1680 cm^{-1}. H, BR − K difference spectrum of the mutant taken in D_2O at 1800–1680 cm^{-1}.

Gerwert co-workers PNAS mutant bR

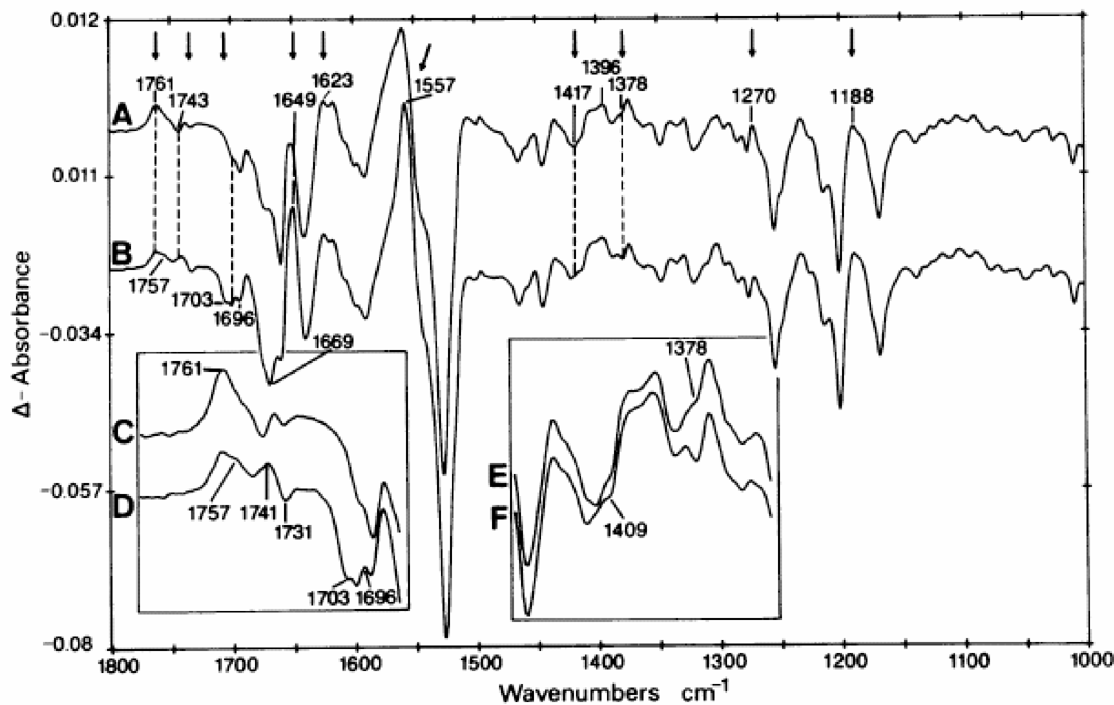


FIG. 2. BR − M difference spectra of bacteriorhodopsin taken at 272 K in H_2O with 2 cm^{-1} spectral resolution. The M intermediate is stabilized under photostationary conditions. Thereby small contributions of the N intermediate are present, but in the same ratio in both samples, as indicated in the fingerprint region. A, wild type; B, mutant; C, expansion of A from 1800 to 1680 cm^{-1}; D, expansion of B from 1800 to 1680 cm^{-1}; E, expansion of A from 1450 to 1350 cm^{-1}; F, expansion of B from 1450 to 1350 cm^{-1}.

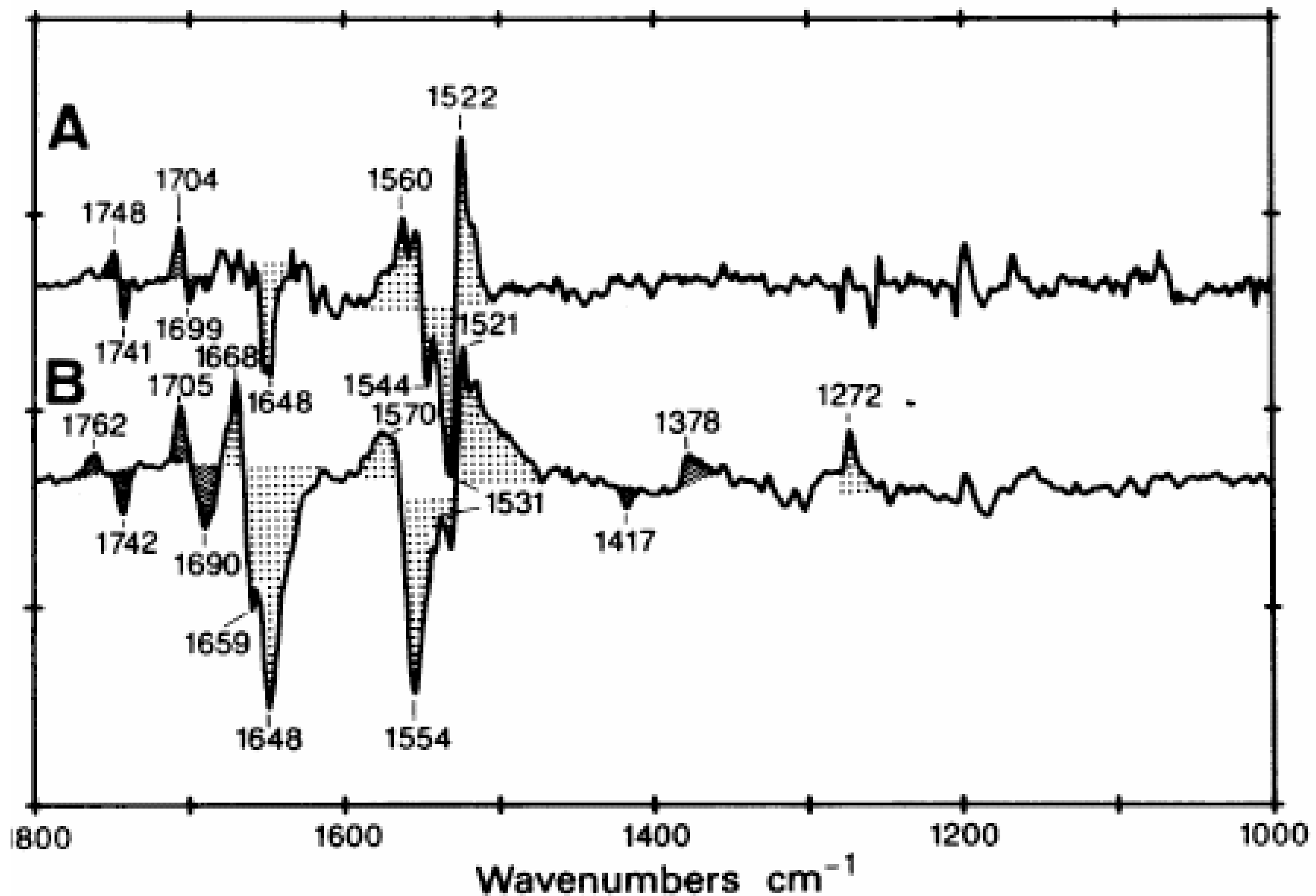
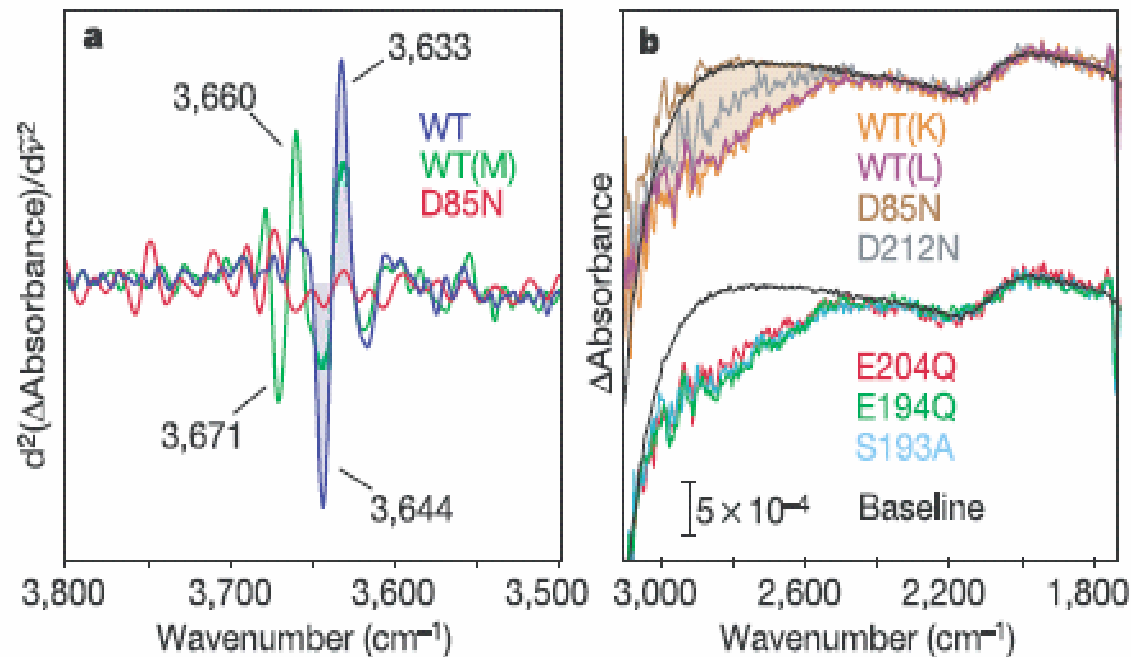


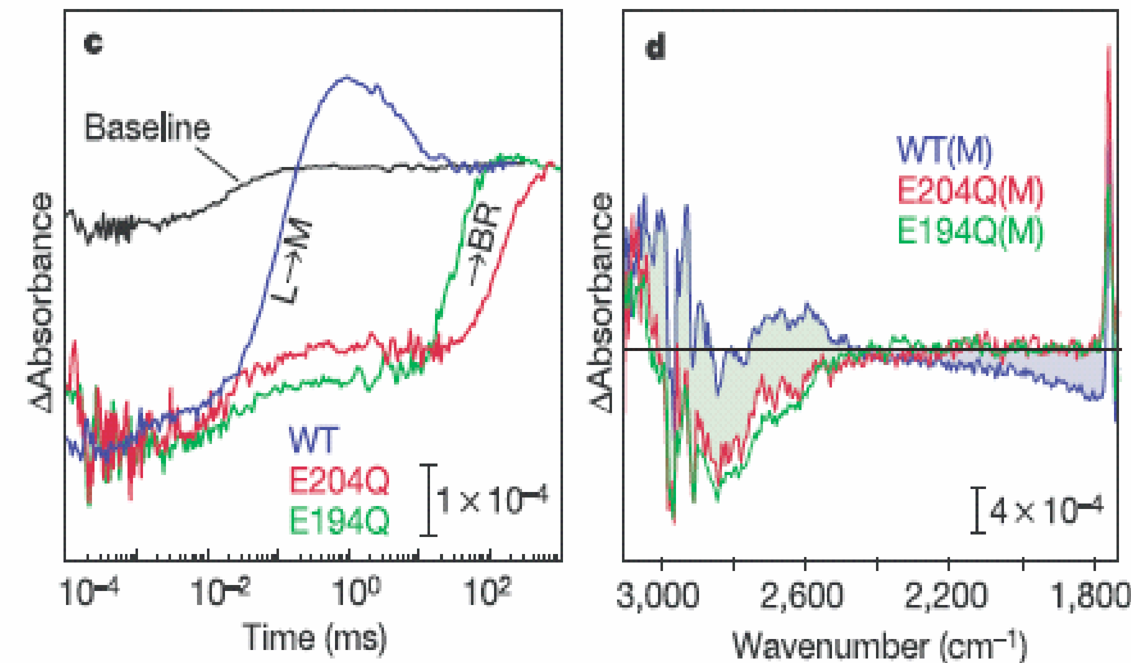
FIG. 3. A, Subtraction of the BR − L difference spectra of the wild type and the mutant; B, corresponding subtraction of the BR − M difference spectra. For details see text.

Role of Asp-96 in Proton Translocation by Bacteriorhodopsin
Klaus Gerwert, Benno Hess, Jorg Soppa, and Dieter Oesterhelt



Functional waters in intraprotein proton transfer monitored by FTIR difference spectroscopy

F.Garczarek & K. Gerwert



a) $^{16}O/^{18}O$ exch. WT(blue), D85N (red), M intermed. (green). OH shift 3644-3633

b) time resolv. Diff. IR K&L

c) time course 2900-2600 (break H-bond <0, make>0)

d) asb: M-intermed