

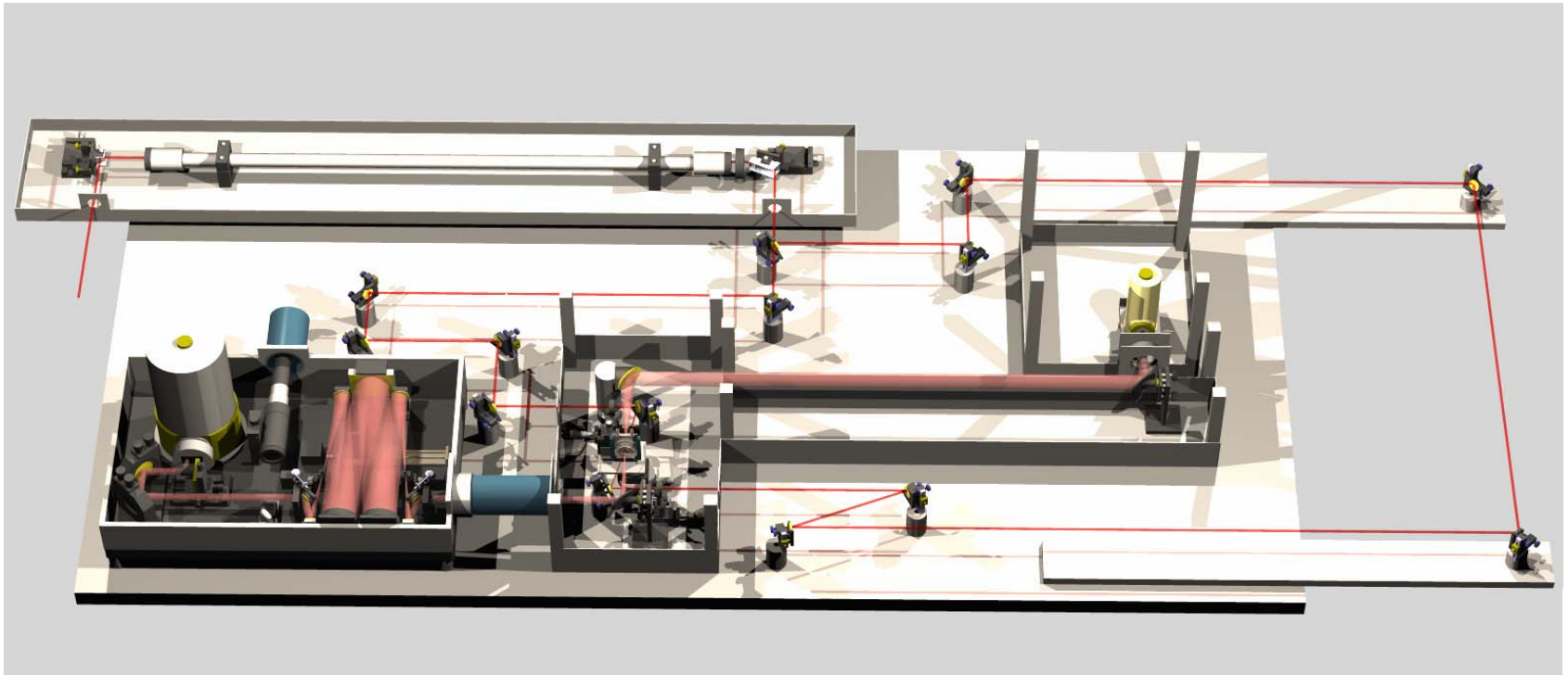
Trpzip-based beta hairpin temperature jump IR studies enhanced by site-specific isotope labeling

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Laser-Induced Temperature Jump & Single Wavelength Detection

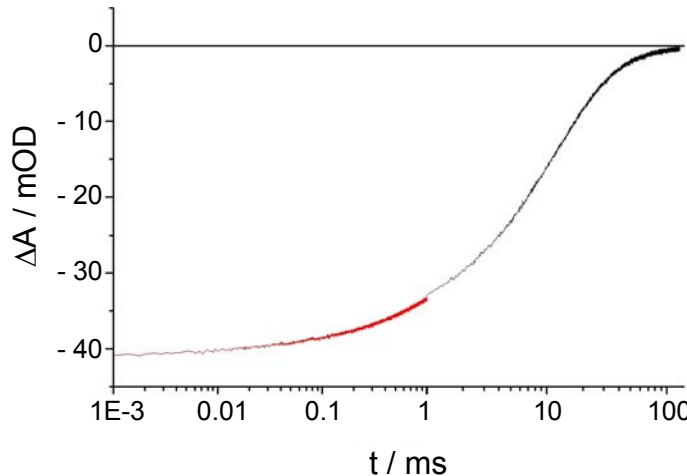
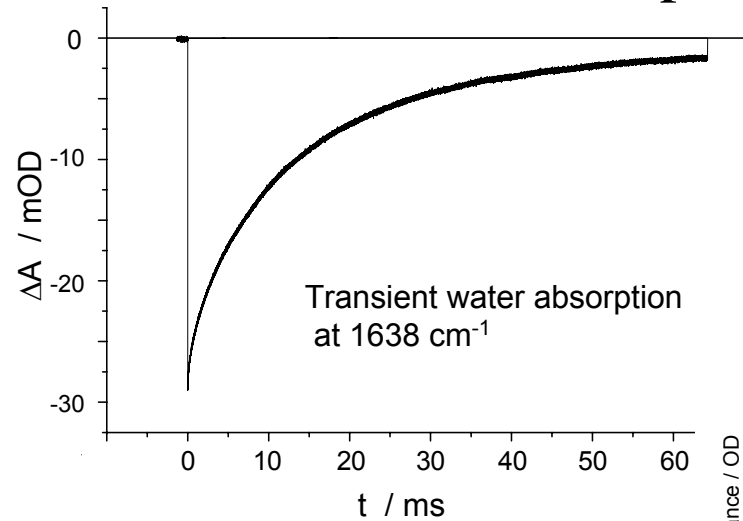


- Conformational relaxation dynamics in the ns- μ s time range
 - Excitation of water overtone/combination vibration at 1906 nm
-

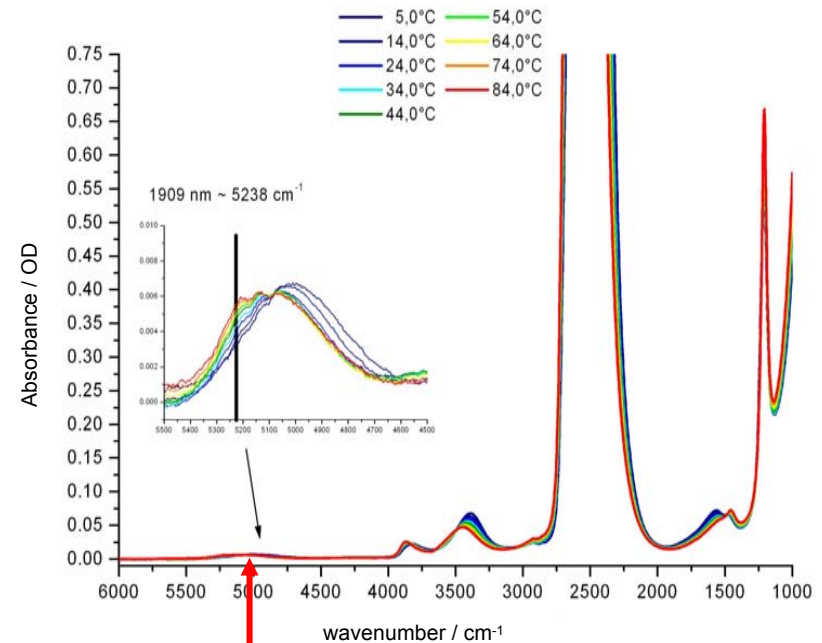
Absorption Changes of the Solvent Induced by T-Jump

Linear scale – re-cool solvent after pulse, 10s ms

$\Delta T \cong 10^\circ\text{C}$
(in 10 ns)



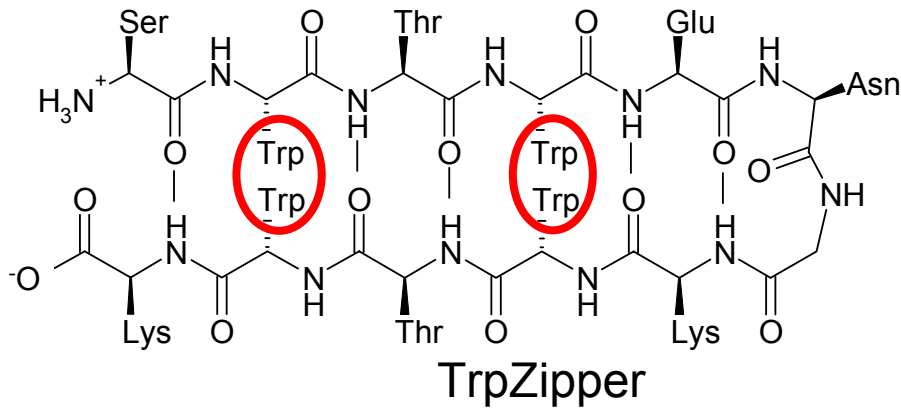
D_2O absorb pump,
thermometer also



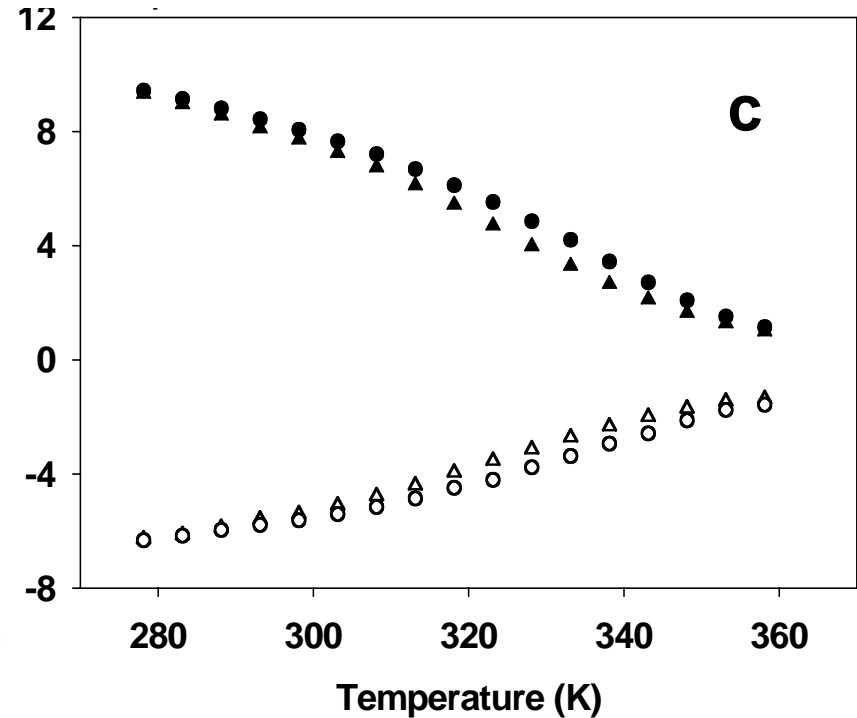
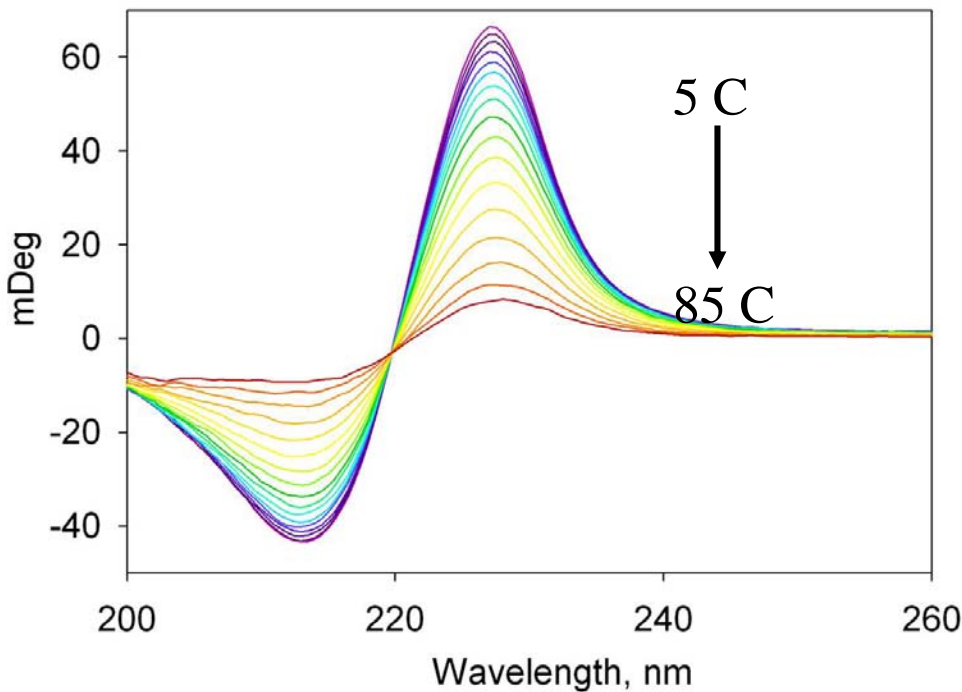
Pump Raman shifted YAG

Log scale = temperature relatively constant to 1 ms

Stable hairpin – cross-strand hydrophobic interaction



Trp interfere in CD – provides no secondary structure information
Effectively tertiary

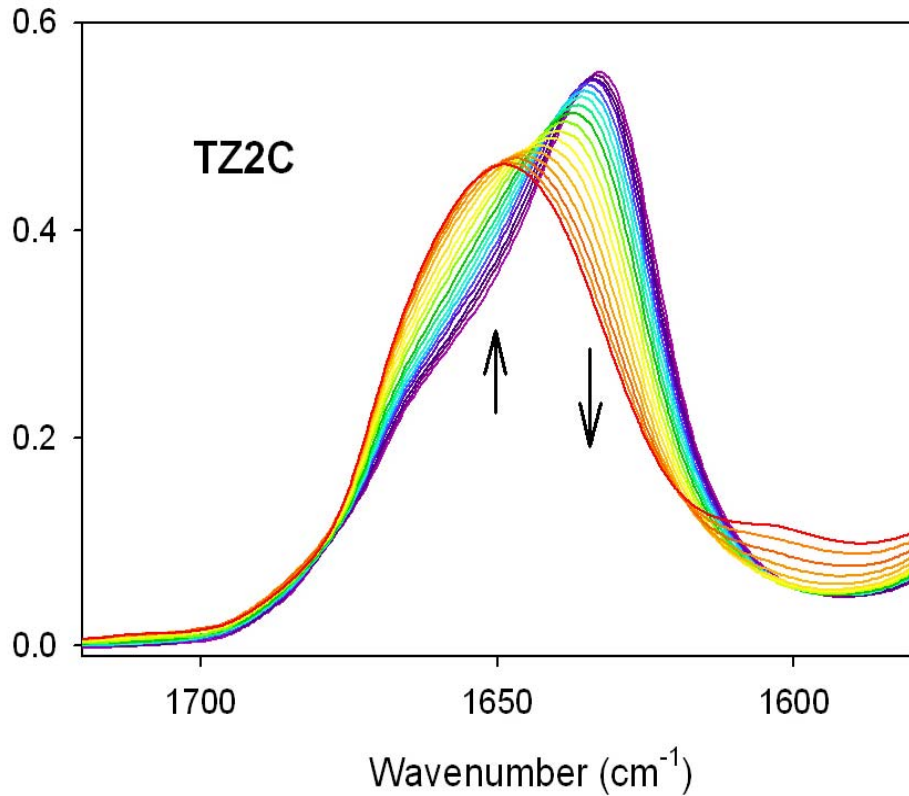


heating from 5 to 85°C, step 5°C

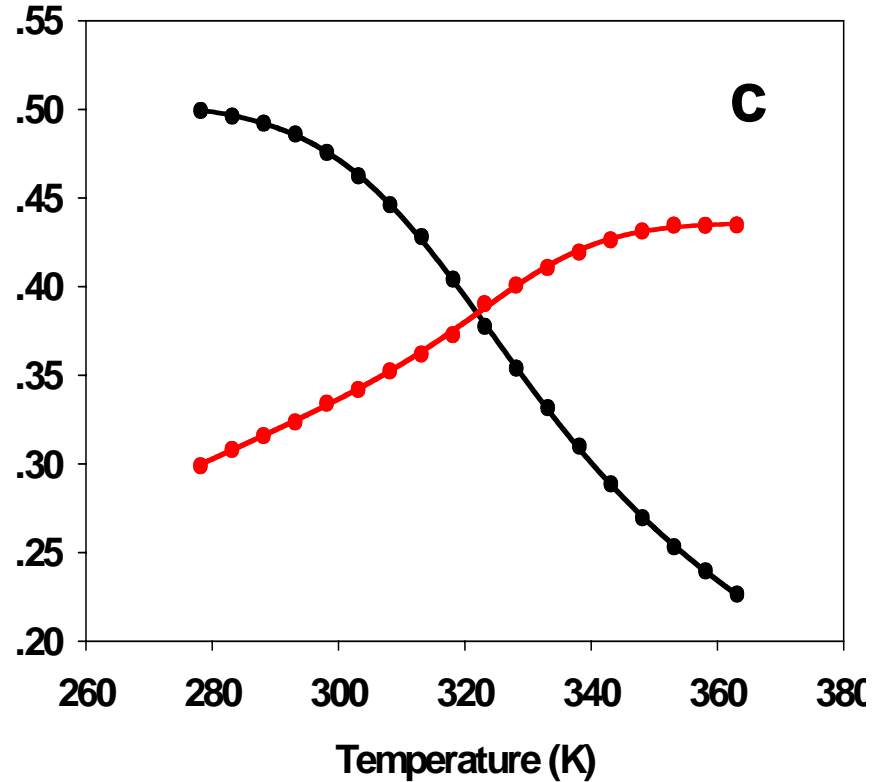
Reversible, 215, 230 nm same transition

Trp-Trp interactions stabilize hairpin - Cochran et al. *PNAS* 2000

Secondary Structure Change with Temperature - Monitor with IR

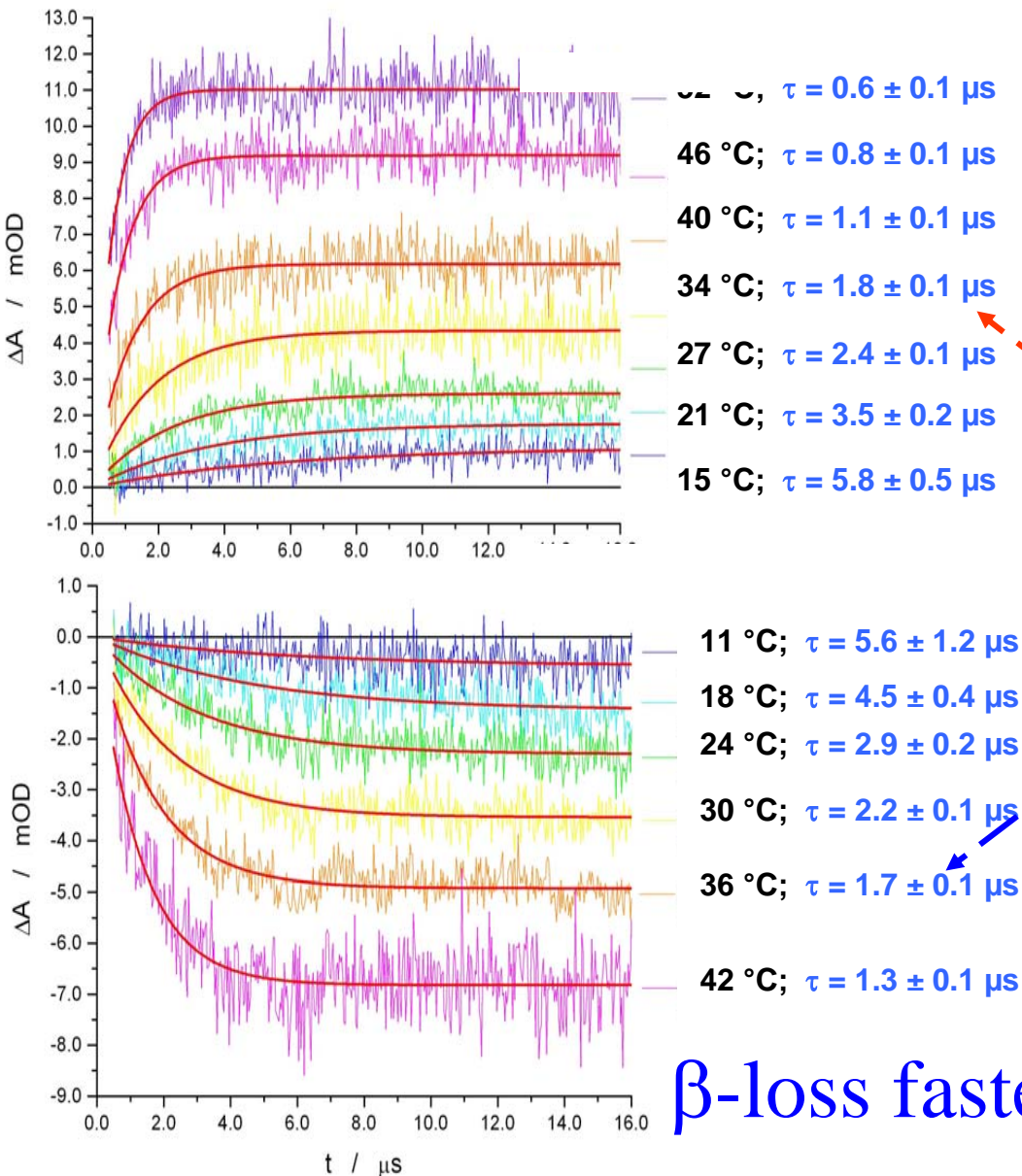


Thermal transition from β -hairpin (low temp, 1625-30 cm^{-1} max.) to disordered (high temp, 1645-50 cm^{-1} max.)

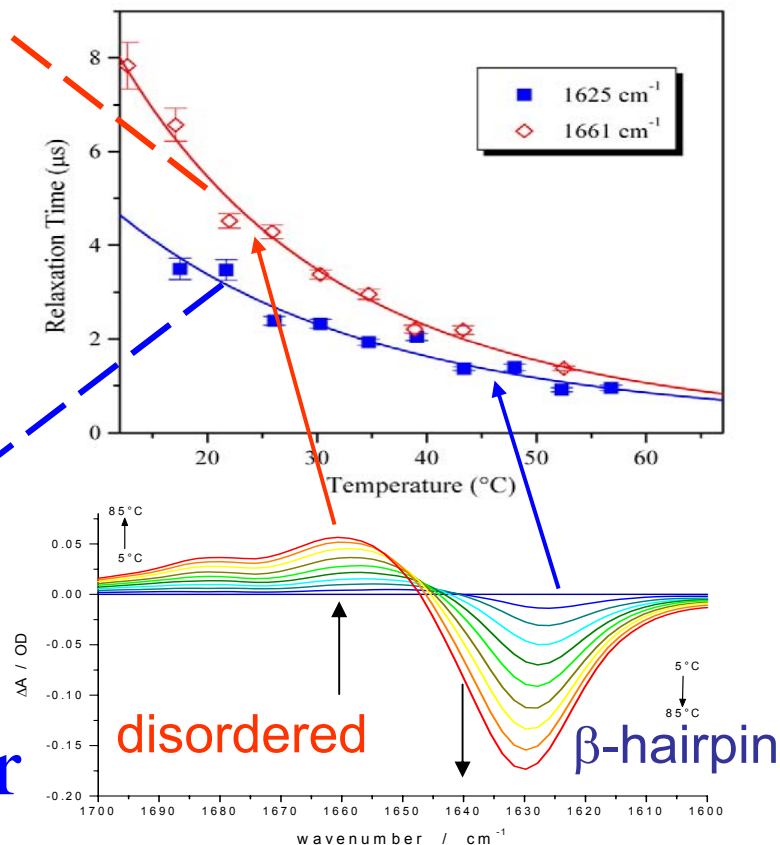


IR intensity change at 1632 cm^{-1} (β -sheet, black), 1652 cm^{-1} , (disordered, red), fit to a two state model - transition temperatures of 321 K and 333 K
→ not really two-state!!

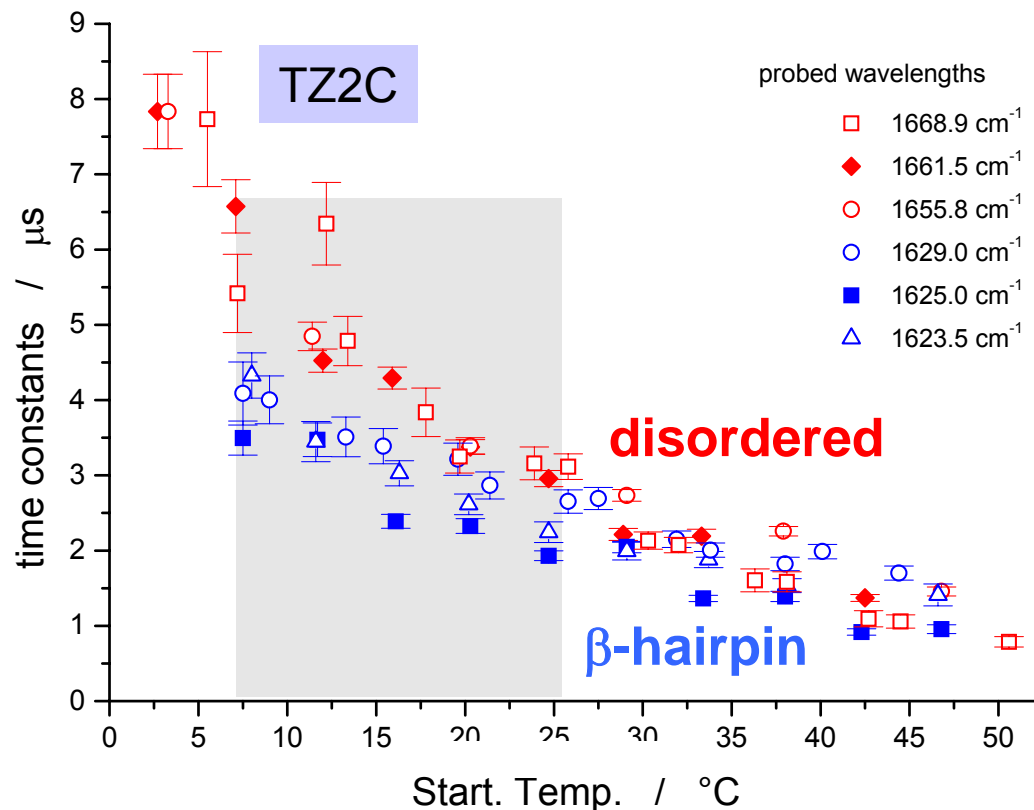
Unfolding Dynamics of TZ2C



- relaxation kinetics at initial temperatures
- single exponential fit:
 $\Delta A(t) = B \exp(-t/\tau)$



Unfolding Dynamics of TZ2C—two regimes



• at 30°C (pD=1.5):

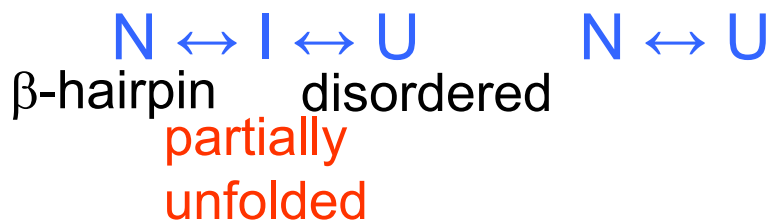
□ $\tau = 2.2 \pm 0.1 \mu$ s

• the time constants increase with decreasing starting temperature

• at low starting temperatures the β -hairpin band decays faster than the disordered band appears

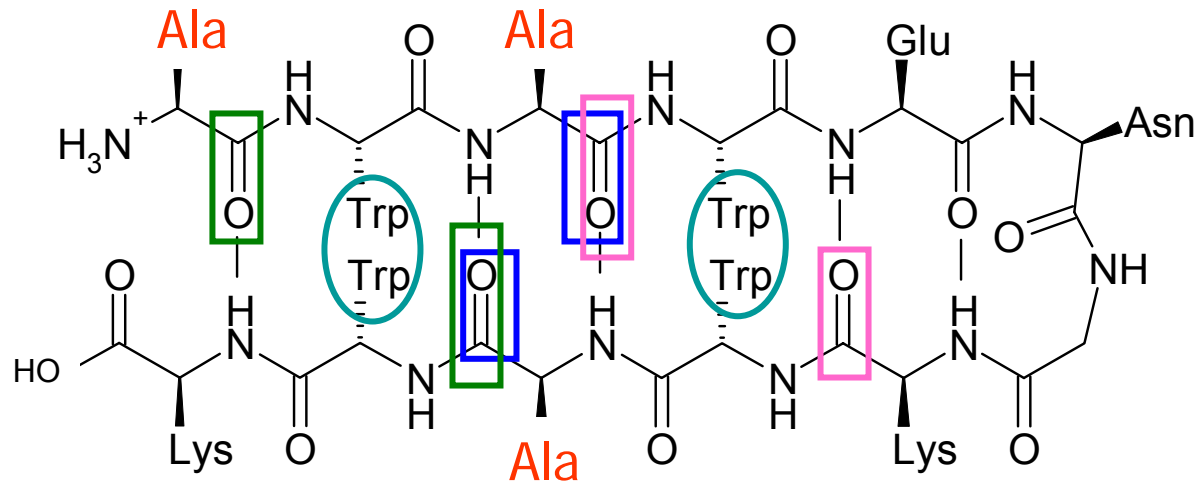
High temp, processes same

Must have intermediate Free energy path dependent



TrpZip2C Labeling Scheme

Mutations for Isotopic Labeling: Thr → Ala



Single Labeled Peptides: A1, A3, A10

Provide measure of diagonal FF effect, little site coupling

Double Labeled Peptides: A1A10, A3A10, A3K8

Designed to emphasize cross-strand coupling

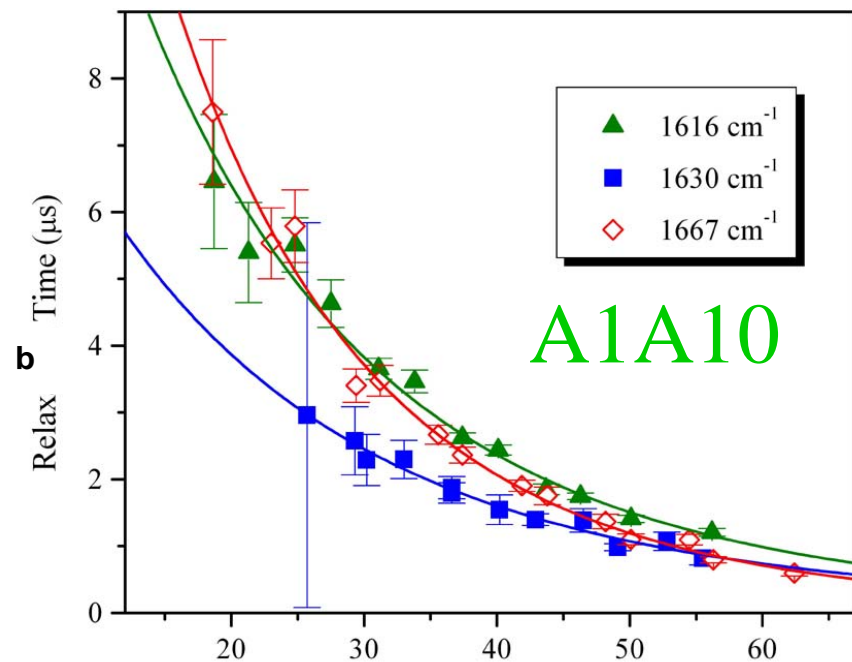
Dynamics Labeled TZ2C

$^{13}\text{C}=\text{O}$ dynamics picks out character of region

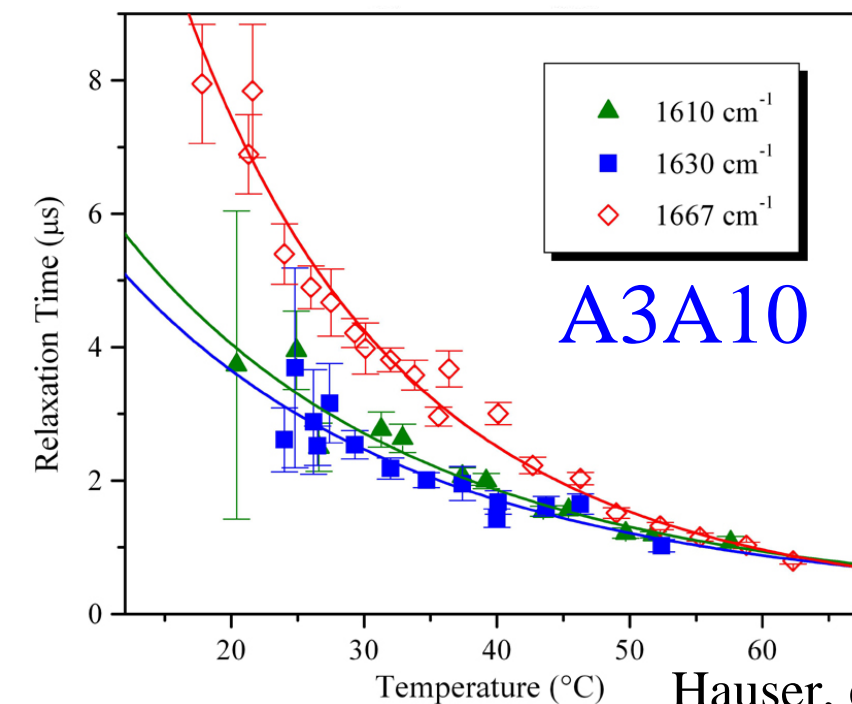
Blue — β -strand

Red — disordered

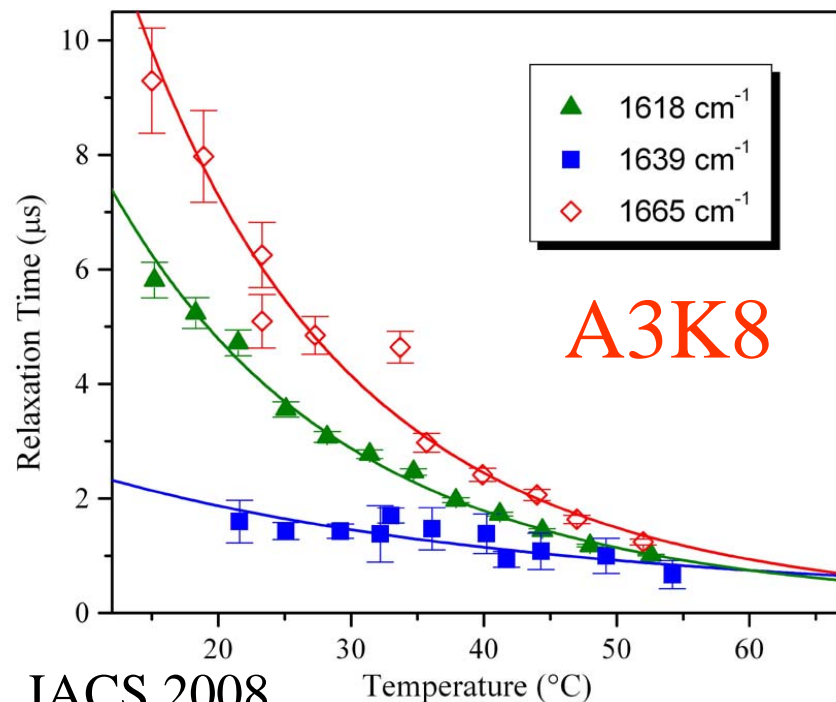
Green — $^{13}\text{C}=\text{O}$ relaxation



A1A10



A3A10



A3K8

Arrhenius activation energies, E_a , for T-jump relaxation for labeled Trpzip2C

	^{12}C -strand		^{13}C		disordered	
	ν / cm^{-1}	$E_a / \text{kJ mol}^{-1}$	ν / cm^{-1}	$E_a / \text{kJ mol}^{-1}$	ν / cm^{-1}	$E_a / \text{kJ mol}^{-1}$
unlabeled	1625	27.8 ± 2.3			1661	33.2 ± 1.4
A1A10	1630	33.6 ± 1.9	1616	37.9 ± 1.4	1667	46.2 ± 1.3
A3A10	1630	28.9 ± 3.1	1610	29.6 ± 2.2	1667	41.4 ± 1.3
A3K8	1639	18.7 ± 4.1	1618	37.6 ± 0.9	1665	41.6 ± 2.5

^a determined with $\ln(k) = -E_a/(RT) + \ln(A)$

Note: A3K8 (large ring) and A3A10 (small ring) differ for ^{13}C and strand
 Disruption of exciton make ^{12}C differ for different isotope patterns

Summary

- **Cross-strand vibrational coupling**
 - Cross-strand coupling is closely related to local structure of the peptide
 - ^{13}C amide I band can serve as a local probe for thermal unfolding studies and ^{12}C amide I band can only monitor the thermal transition of the whole peptide
- **Site-specific folding dynamics**
 - TrpZip2C and labeled variants unfold in a few μs
 - Relaxation kinetics of the ^{13}C amide I band provides site-specific structural dynamic information
 - Kinetic data indicates fraying of the hairpin end residues upon unfolding