The "Future" of protein dynamics experiments on FELs

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If you want to sleep, I think the key word here is:

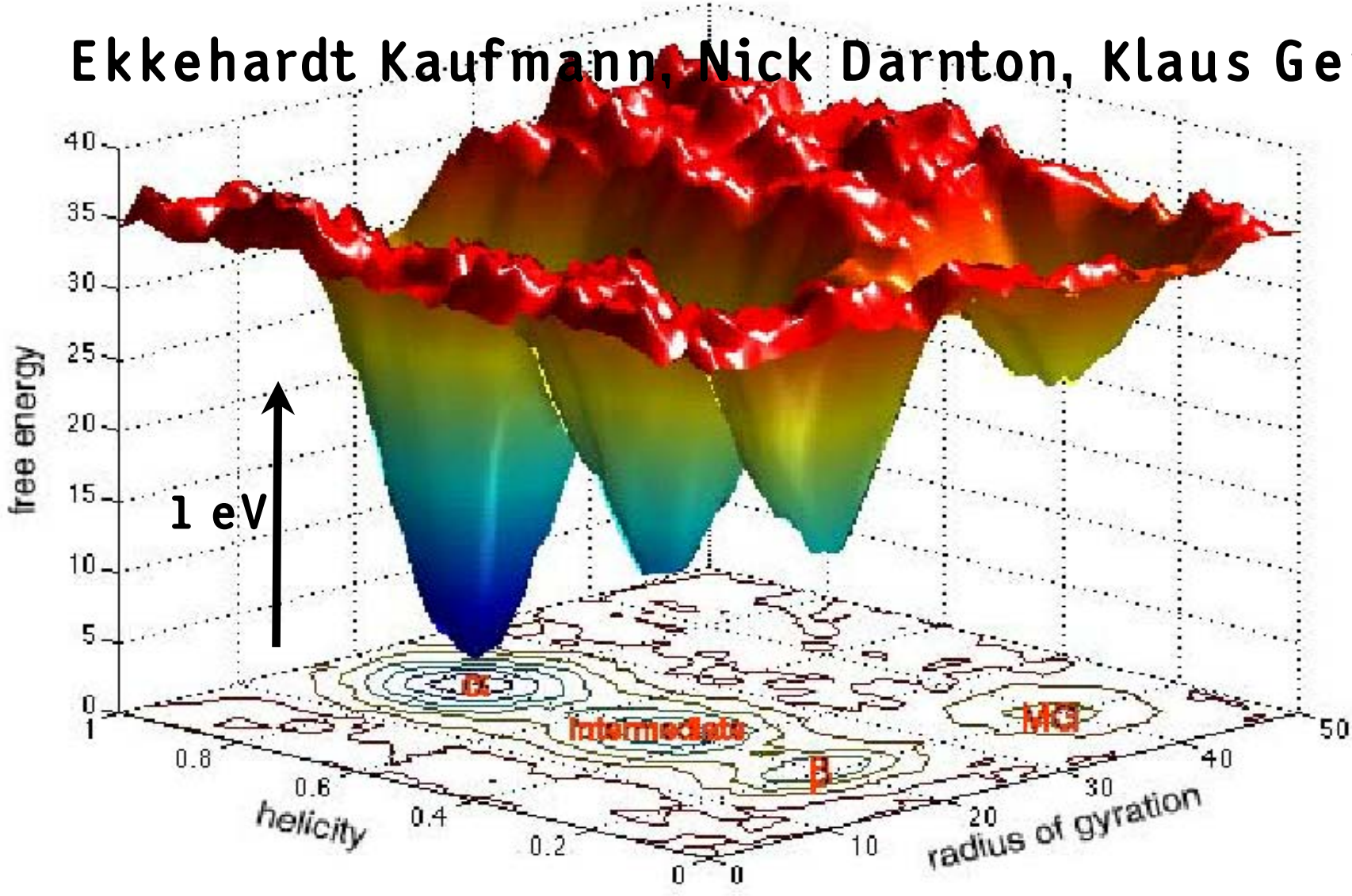
MULTIWAVELENGTH

Probing the full range of protein
dynamics will need "light" from the X-ray to the FIR (THz). The JLAB can put

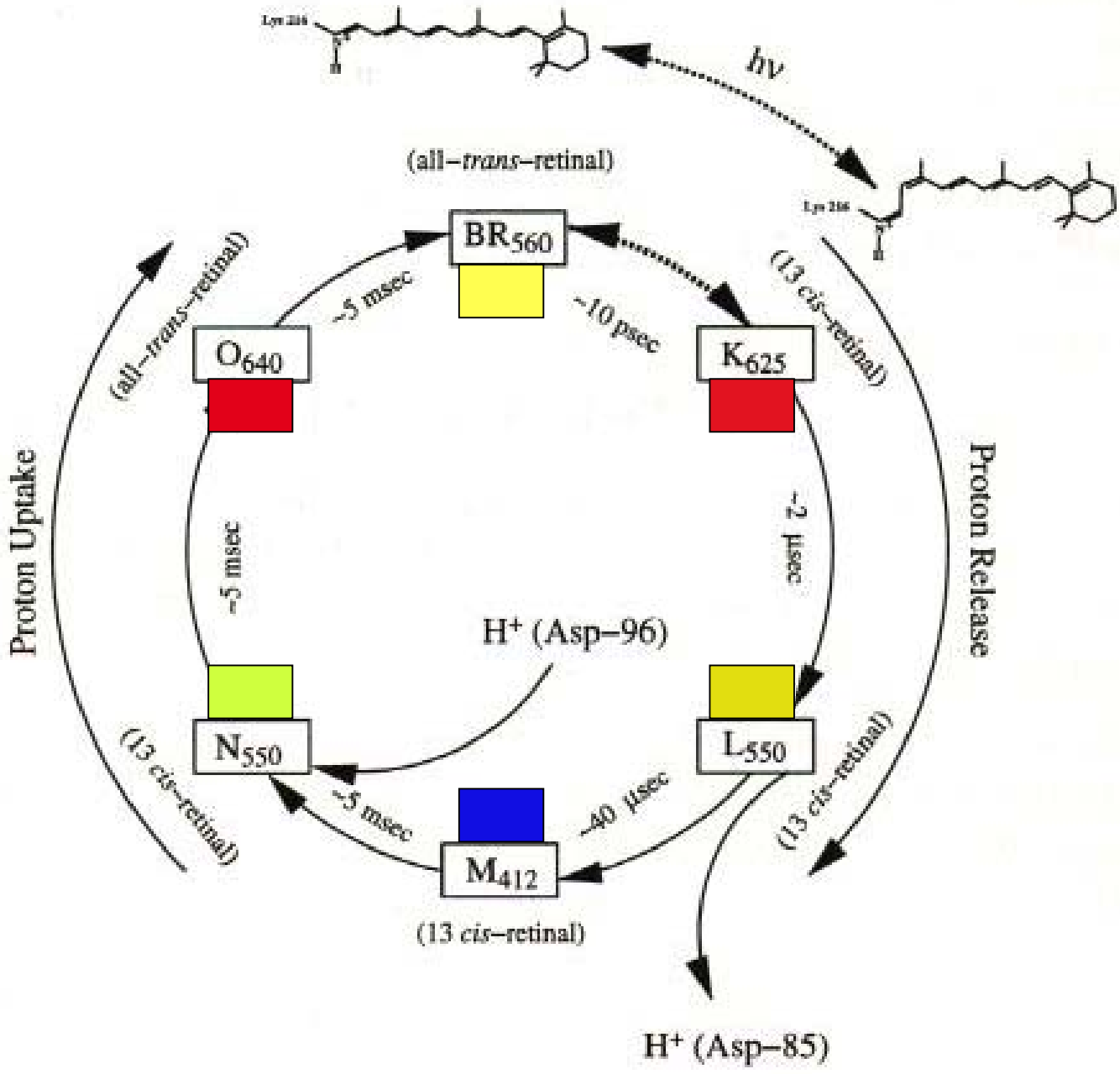
Protein Functional Important Motions are the large scale switching motions.

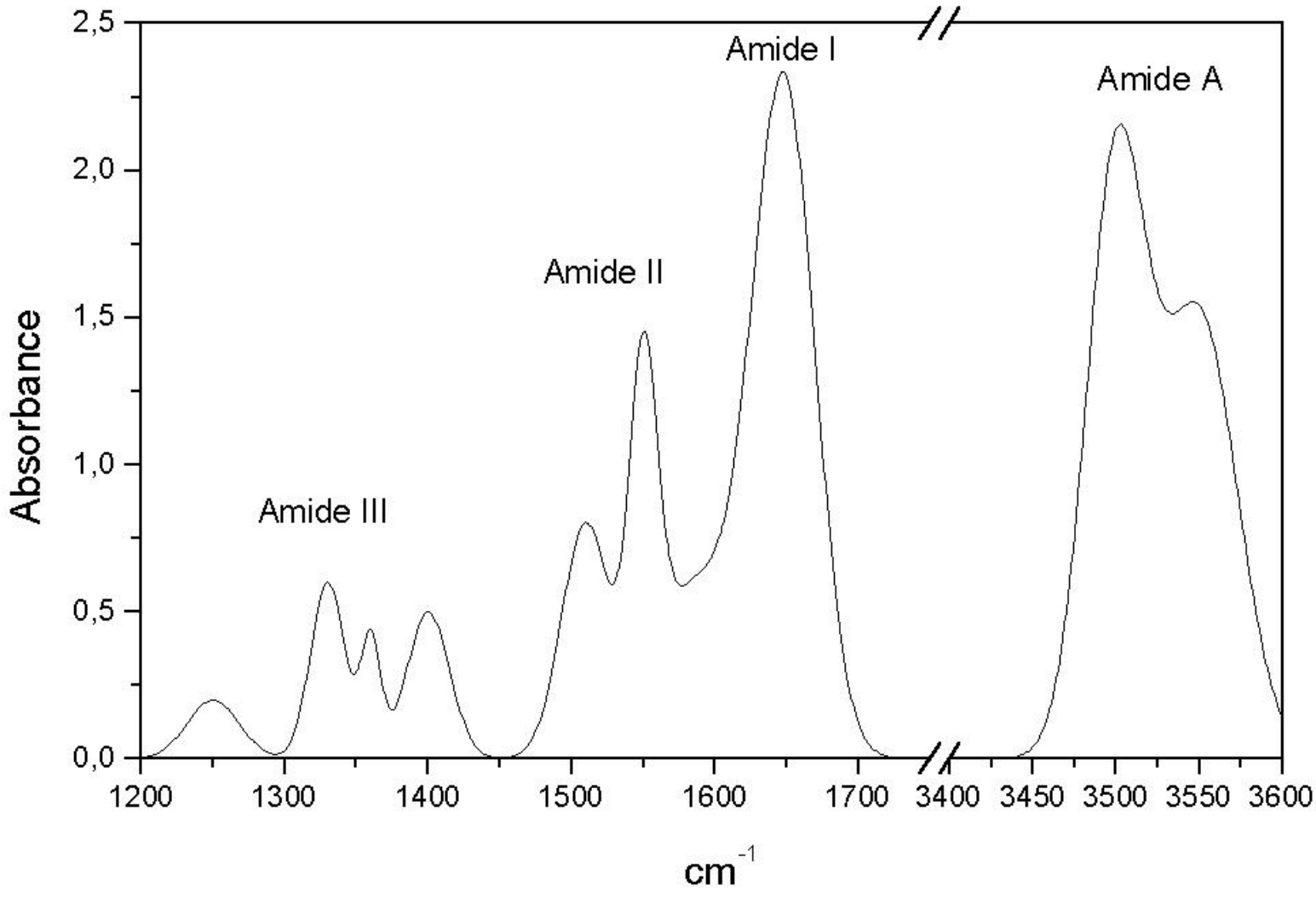
It is the conformational switching that makes a protein a true nanomachine, and the system fundamentally anharmonic. Few people view functionally important motions of proteins as collective and anharmonic motions. These motions involve both a few atom motions and motions involving most of the atoms in a protein.

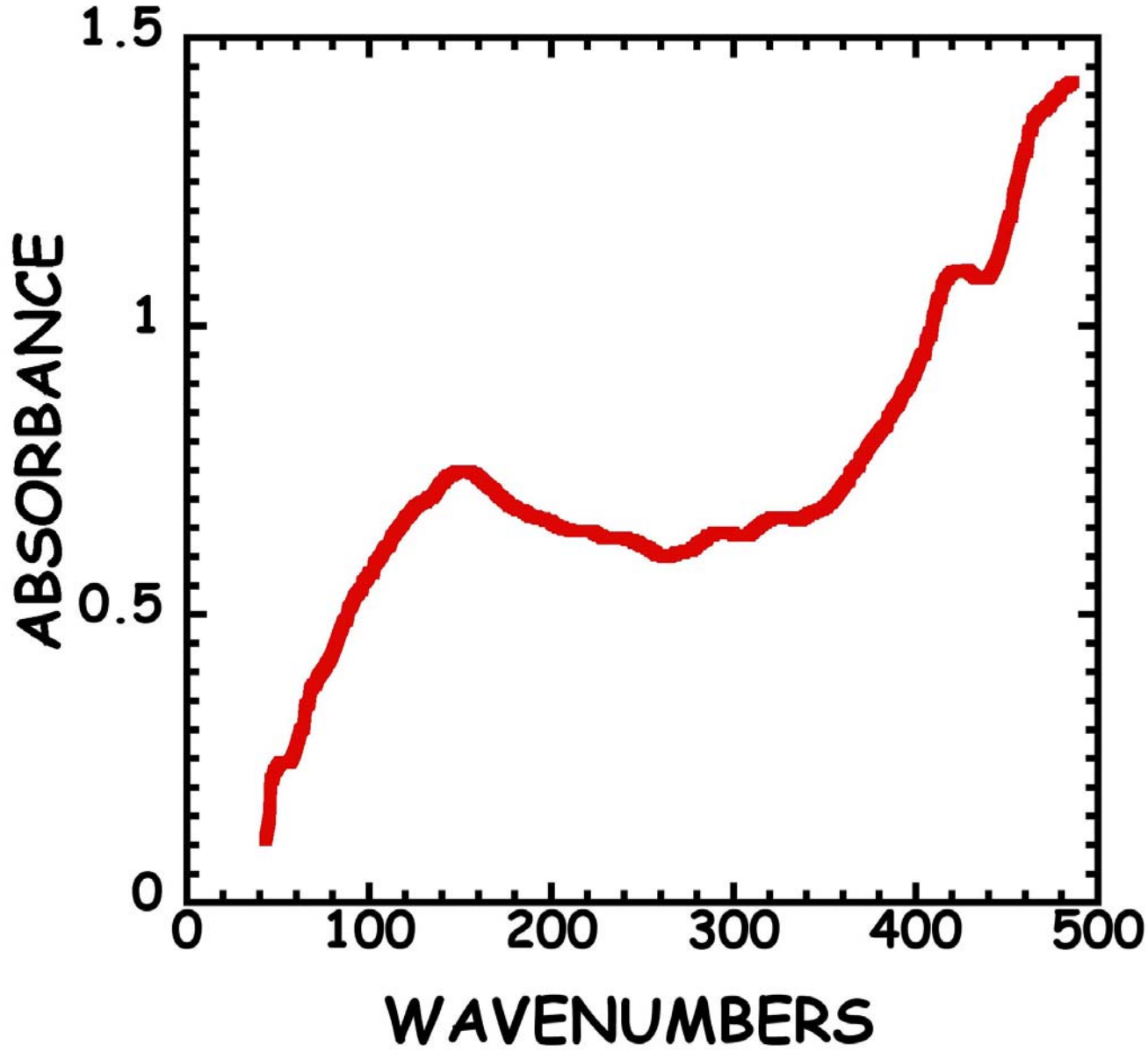
Ekkehardt Kaufmann, Nick Darnton, Klaus Gewert

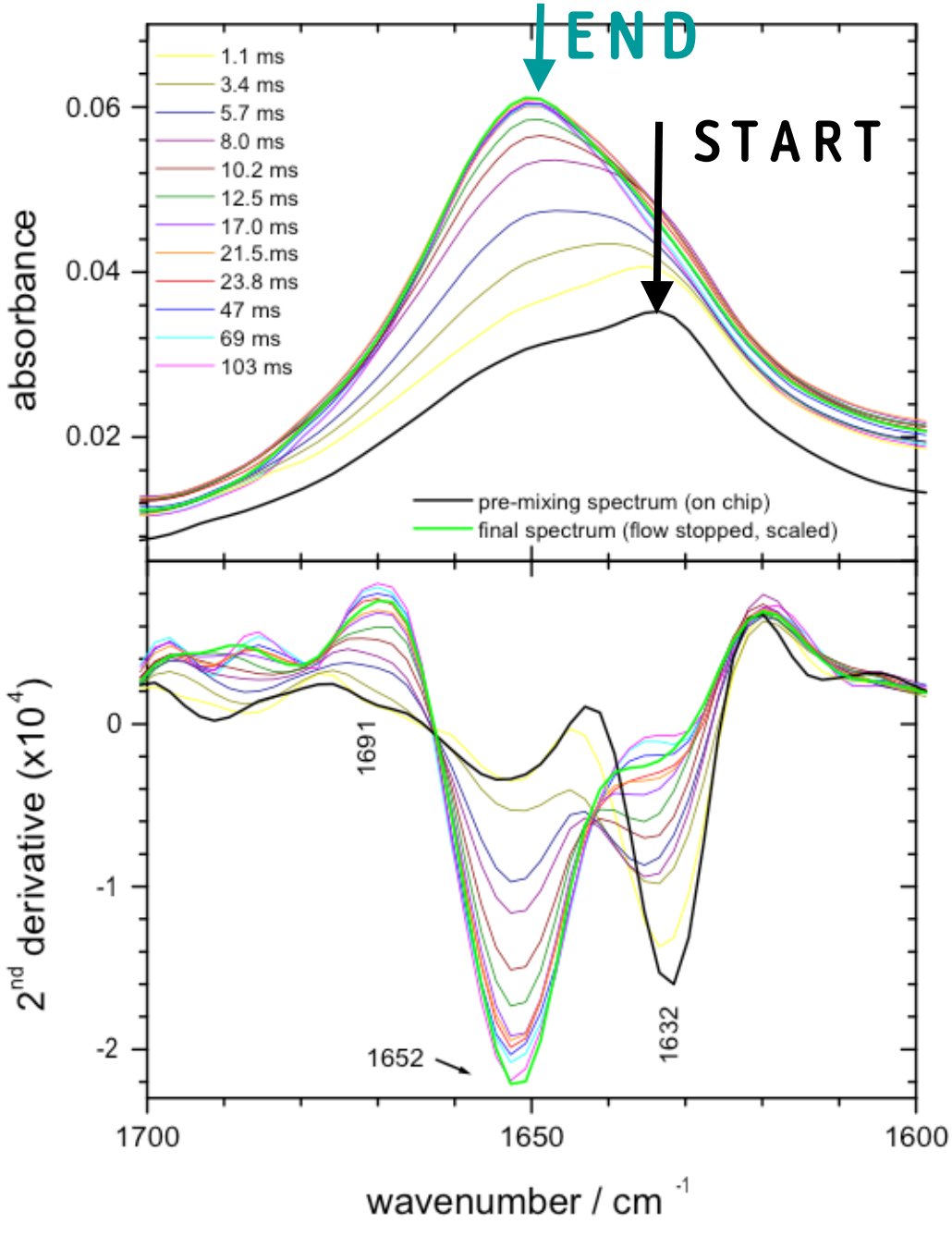


Although electronic chromophores have been extremely important in elucidating the rough energy landscape of (a few) proteins, the infrared region is sensitive for all proteins to the tertiary and secondary structural changes that signal large scale conformational changes.


hν
(all–trans–retinal)
BR560
K625
L550
M412
N550
O640
~10 psec
~2 μsec
~40 μsec
~5 msec
~5 msec
~5 msec
(13 cis–retinal)
(13 cis–retinal)
(13 cis–retinal)
(all–trans–retinal)
Proton Release
Proton Uptake
H+ (Asp–96)
(13 cis–retinal)
H+ (Asp–85)


Absorbance
2,5
2,0
1,5
1,0
0,5
0,0
Amide I
Amide A
Amide II
Amide III
1200
1300
1400
1500
1600
1700
3400
3450
3500
3550
3600
cm-1


1.5
1
0.5
0
ABSORBANCE
0
100
200
300
400
500
WAVENUMBERS



These are time-resolved spectral changes seen in beta-lactoglobulin as it changes from a 41% beta sheet to 81% alpha helix structure.

These kinds of structural changes can be triggered by chemical bonding (including photolysis), solvent changes, temperature changes. The biologically relevant changes are believed to be mostly triggered by chemical bonding. Note that the energy surfaces must ultimately be highly metastable, since only a "few" bonds need to be broken out of hundreds to trigger large conformation changes.

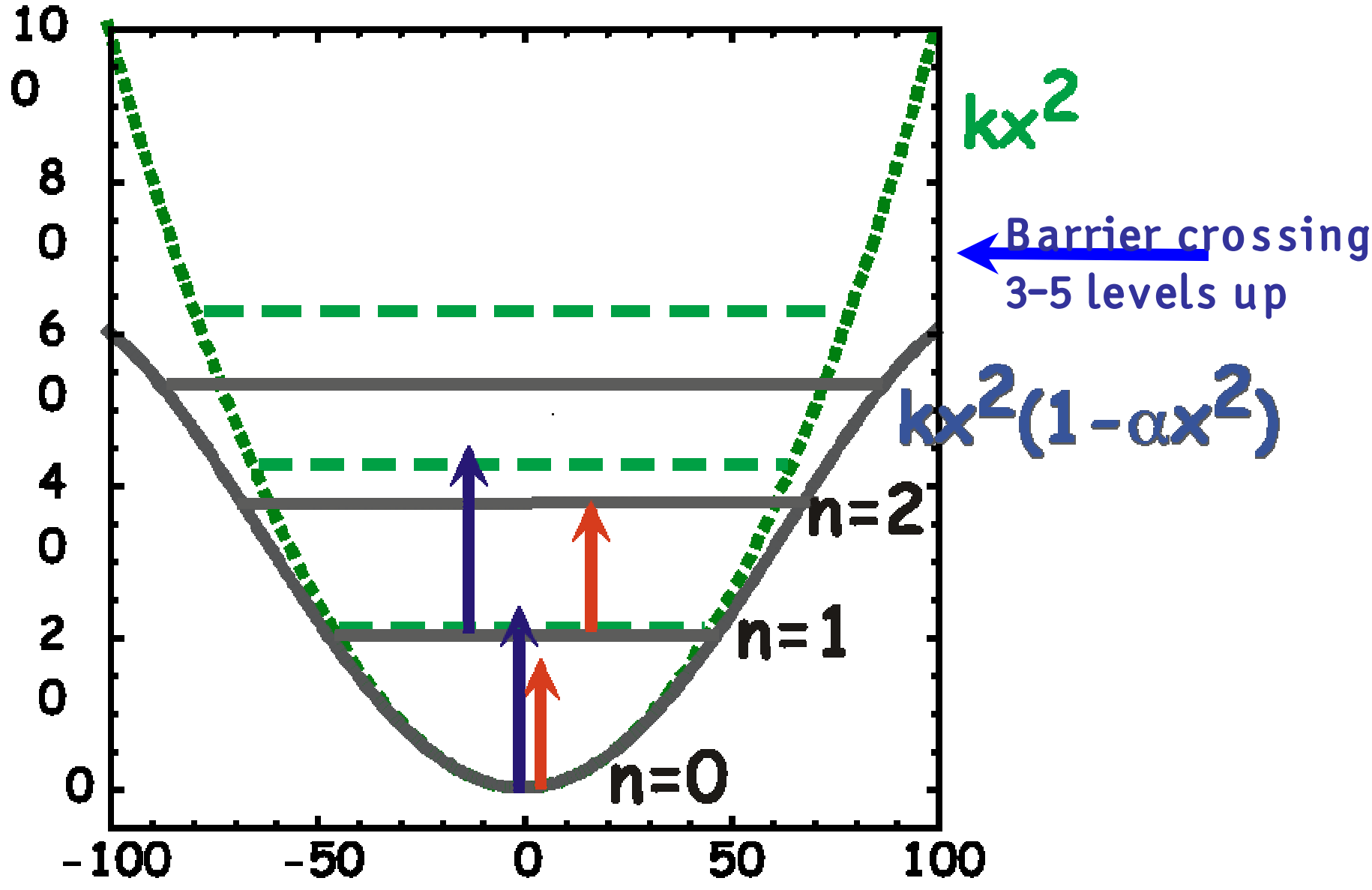
Aihua Xie (Oklahoma State) and I have for 6 years (and I for 16 years total as a FEL gypsy) have been doing pump-probe experiments on the Dutch Free Electron Laser FELIX (Lex van der Meer has been our colleague there). The main idea has been to put enough mode-specific energy into delocalized vibrational bands to drive a conformational change.

WHY FELIX?

(1) I like getting up in the pitch dark at 8 AM in the winter and riding my folding bike past laughing Dutch women over icy roads. And, there is the fine Dutch "pizza"..

(2) FELIX, like KAL, is an extremely well designed and reliable laser that runs. The user has total control, and USA style OSHA crap is non-existent.

(3) Rapid tuning from 3 microns to 200 microns! Wow!


10
0
8
0
6
0
4
0
2
0
0
-100
-50
0
50
100
kx2
Barrier crossing
3-5 levels up
kx2(1-αx2)
n=2
n=1
n=0

As you climb the vibrational ladder up to the metastable crossing levels the system become extremely anharmonic and shows a generalized loss of absorbance across the spectrum.

From Per-Anker Lingard, these thoughts on 3 possible ways to view system response:

1) Soliton. A soliton in biomolecules offers one possible way to
understanding how energy released by ATP hydrolysis can be propagated over a fairly long distance without significant dissipation. The idea is that there's a motion (coherent in some sense) enabled by an anharmonic interaction between conventional vibrations and the C=O mode
(Davidov 1977).

2). Excitons. In the conventional condensed matter case, there are two types of large and small (Stoneham 1989). Both concern neutral "hole-carrier" pairs and the associated strain and polarisation they cause in the host solid. Almost all the theory is harmonic: there's no need for anharmonicity. But see Hochstrasser and his students, off-diagnonal ps IR spectroscopy shows coupling.

3). Self-trapping. There's another regime at low temperatures when the scattering vibrations are not excited. Self-trapping localises the carrier, so gives exciton localization (Itoh and Stoneham 2000). When the excitons self-trap, they give energy localisation and the localised energy can cause local atomic changes and defect production. CM speak for protein conformations

You can roughly guess how much energy in the IR it will take to create a self-trapped state, if they exist at all.

The amide I vibron has a energy of about 0.2 eV. You might guess it will take about n=5 IR photons in that mode to move it so far up the potential energy surface as to make the $1/2kx^2$ approximation very bad. This amounts to 1 eV/amide I bond.

It turns out that a 10 mM protein concentration in a 10 micron thick layer has an OD of 1.0 at 6 microns. If you assume that each aa residu`e in a protein absorbs 1 eV of IR photons, you need about 1 uJ in a 50 um diameter spot to "saturate" the system. FELIX can deliver that, modern OPO systems are also there now (perhaps), KAL can hammer this.

In order to do mode-specific excitation, the pulse width should be no LESS than 1 ps (10 cm^{-1}). I have become an amateur ps pump/probe IR laser jock.

Critical: true single ps pulse experiments: 1 uJ at 5 Hz to avoid local heating issues that have plagued so many experiments.

You will see statements in the literature like:

" there is no significant temperature dependence to the absorption spectrum of proteins"

" proteins can make no structural changes below 200 K"

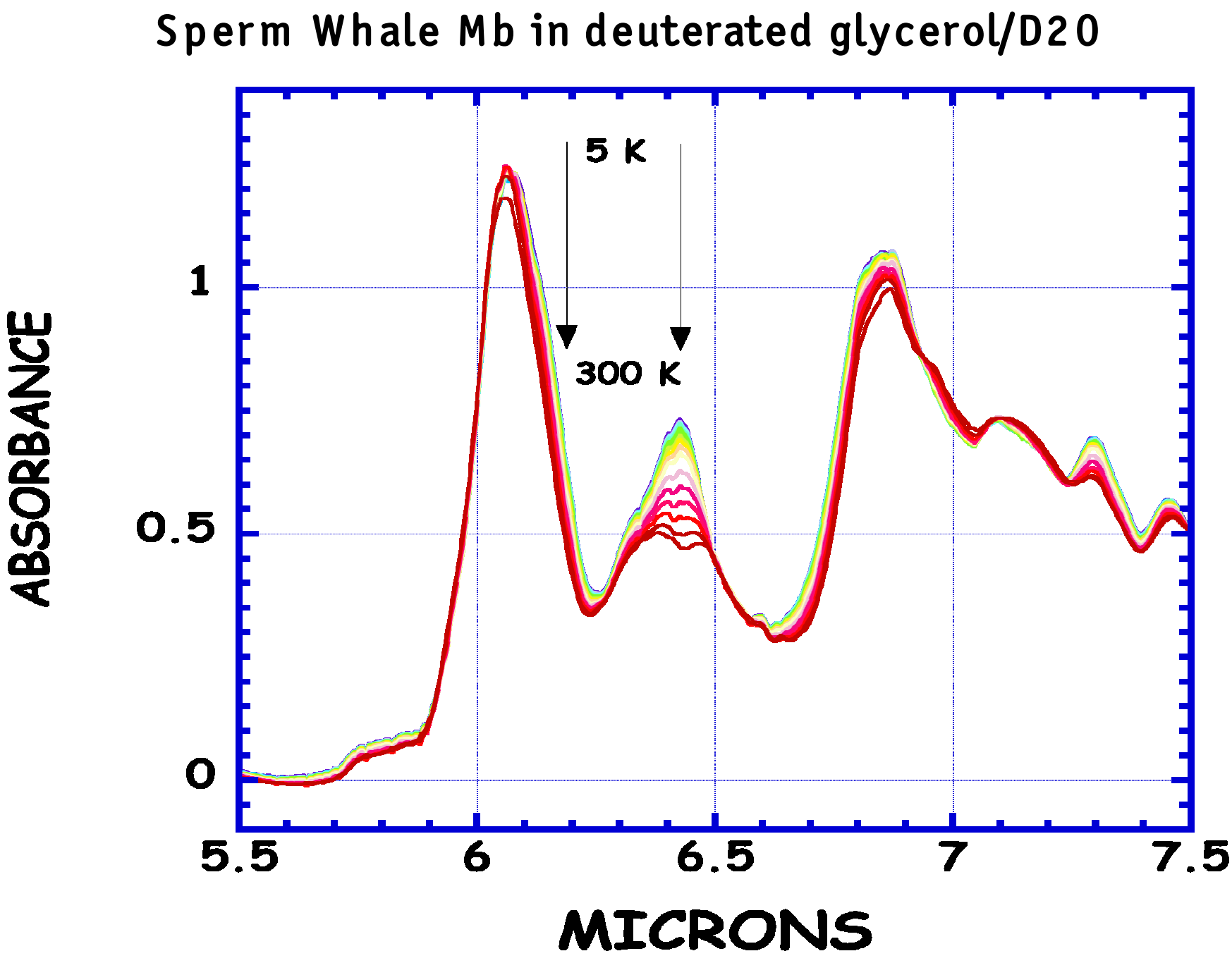
"there is no significant temperature dependence to amide I relaxation rates"

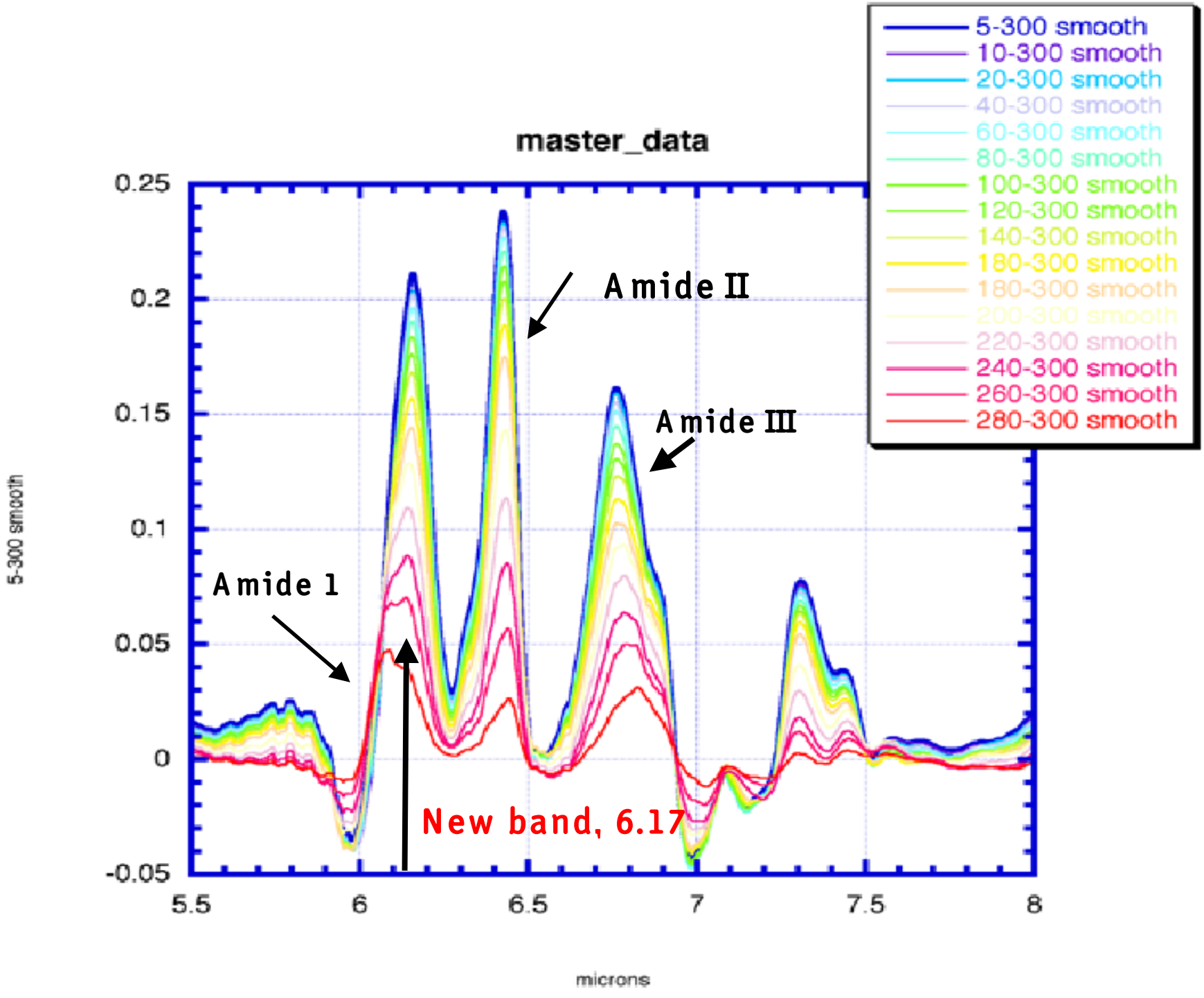
Ithink this is wrong. There are VERY significant changes to the IR spectra of proteins as you cool them in a d-glycerol/D_2O solvent, easily as significant as the beta sheet-alpha helix transitions I showed you earlier. This is much more than the well-known and beautiful changes that Frauenfelder et al. have seen in the CO band at 5 microns, these are changes throughout the amide I and amide II regions, and no doubt further in the IR where I haven't measured yet.

Careri et al. made two mistakes that hurt them:

1) They neglected to mention that the IR spectrum of ACN shows temperature dependent changes in MANY other regions of the spectrum. This is dumb, full spectra are just a mouseclick away. If there are spectra changes across the board, don't obsess on just one band.

2) They never moved beyond simple model system crystals. Proteins are far more complex than simple molecular crystals, but far more interesting.


Sperm Whale Mb in deuterated glycerol/D20
5 K
300 K
ABSORBANCE
1
0.5
0
5.5
6
6.5
7
7.5
MICRONS


master_data
5-300 smooth
10-300 smooth
20-300 smooth
40-300 smooth
60-300 smooth
80-300 smooth
100-300 smooth
120-300 smooth
140-300 smooth
160-300 smooth
180-300 smooth
200-300 smooth
220-300 smooth
240-300 smooth
260-300 smooth
280-300 smooth
Amide II
Amide III
Amide 1
New band, 6.17
0.25
0.2
0.15
0.1
0.05
0
-0.05
5.5
6
6.5
7
7.5
8
5-300 smooth
microns

What's going on?

One possibility is the annealing of an alternate helical structure within the protein.

There is a form of the alpha helix called the 3_{10} helix which has 2 hydrogen bond chains rather than 3 chains, lies about 10 cm-1 to the red of the alpha helix. This helix could act as a defect state within the basic alpha helix.

What's going on?

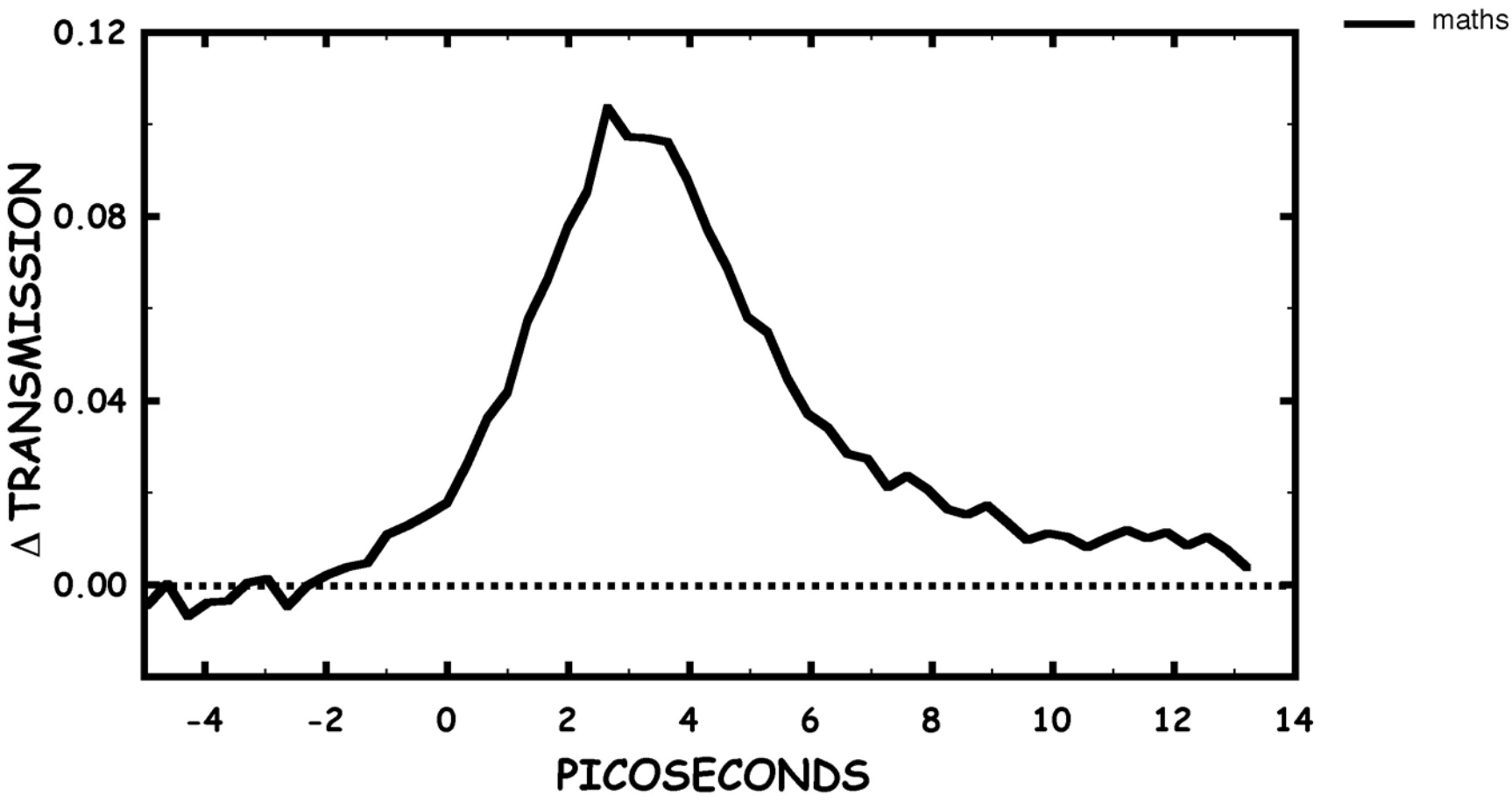
(1) Confromational substate equilibrium. Hydrogen bonds? Biophysics speak.

(2) Self-localized excitations (a dynamic effect due to interaction with lattice phonons). Low-lying states are not stable. Condensed matter speak.

(3) Davydov splitting. Davydov splitting is (I think) splitting due to inequivalent molecules in a lattice. Chemist speak.

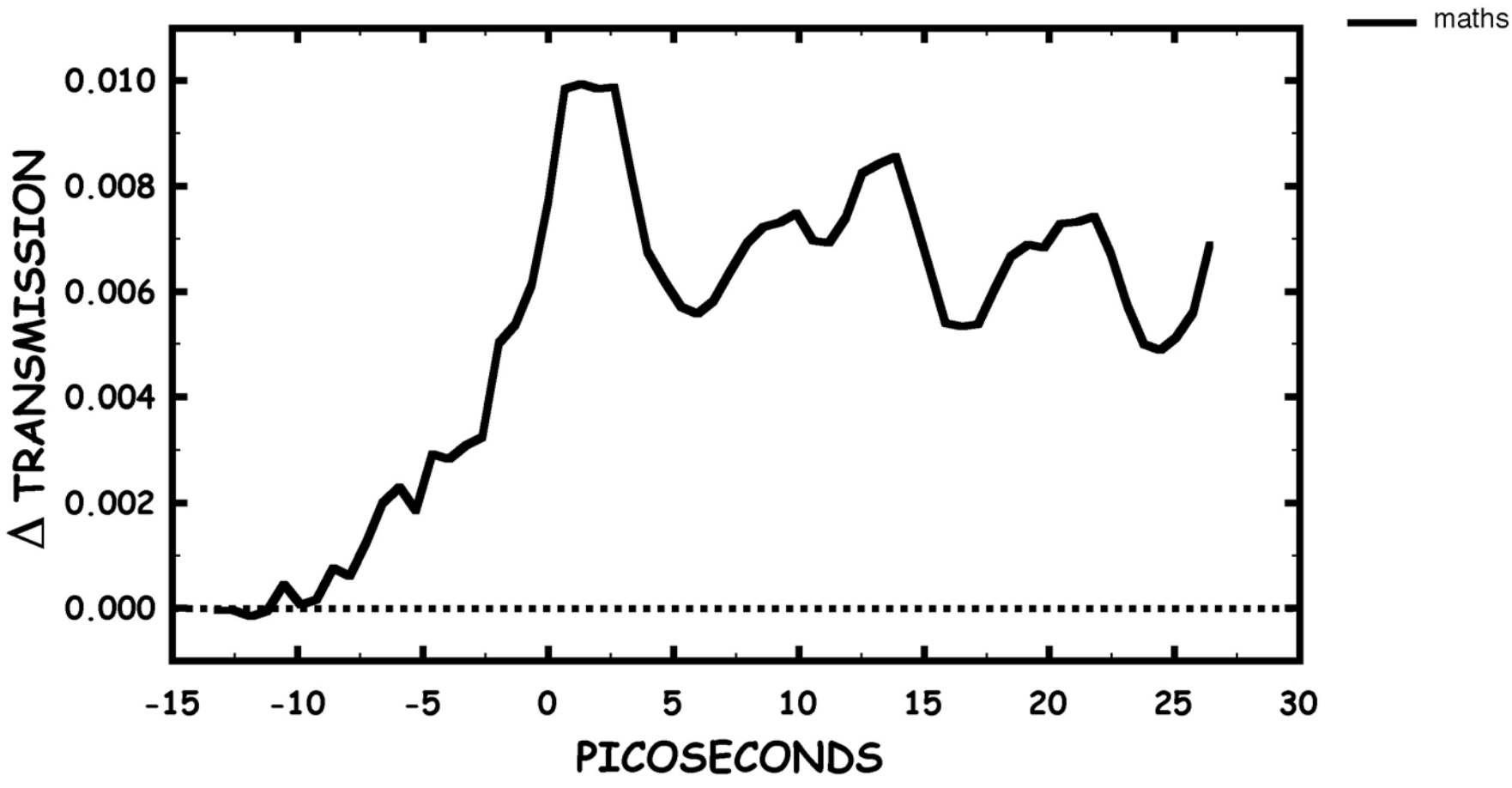
The next obvious question is (obvious after it took me 6 years of screwing around at FELIX while I learned physical optics and how to do single ps pump/probe IR measurements in a cryostat at KAL and finally did the full temperature IR spectrum and realized it was non-trivial):

Do the low temperature bands (say, the 6.17 um amide I band) have different relaxation dynamics then the "normal" one at 6.0 um?

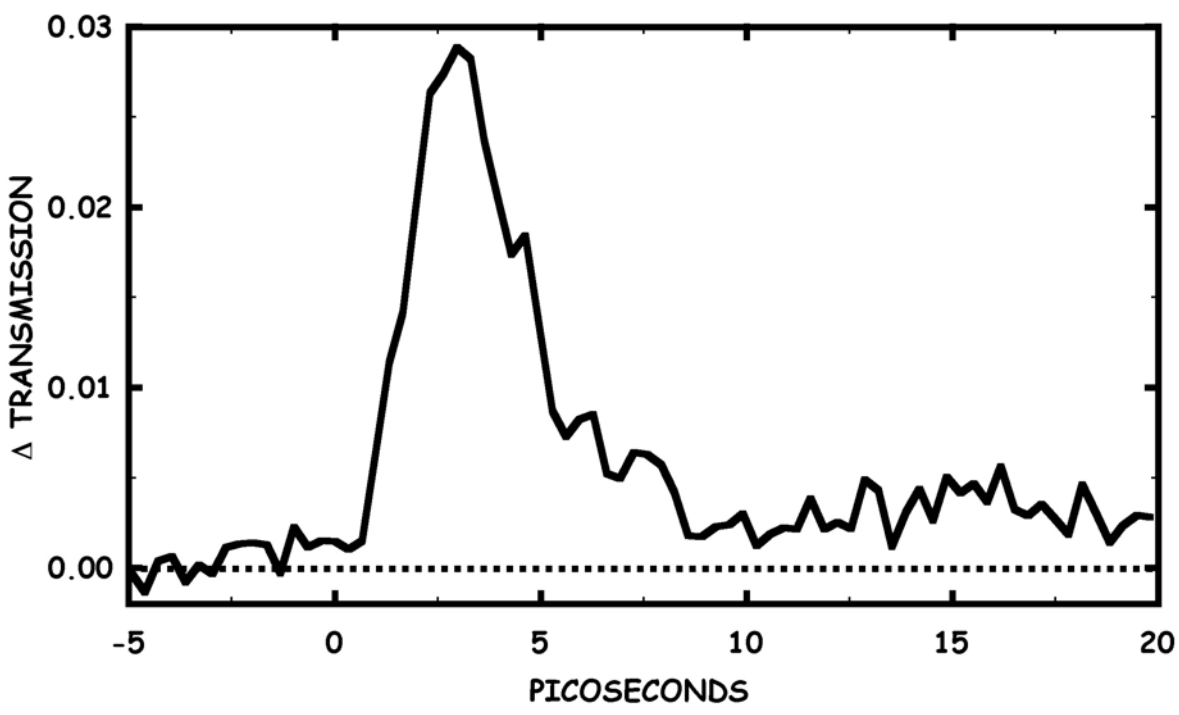


Mb at 290 K, 6.00 um . (blue side amide I, bleach)
10% bleach! This is serious saturation. A real whack.
"We" needed 50 micron spot sizes at full FELIX energy
to obtain this kind of pump/probe signal, only

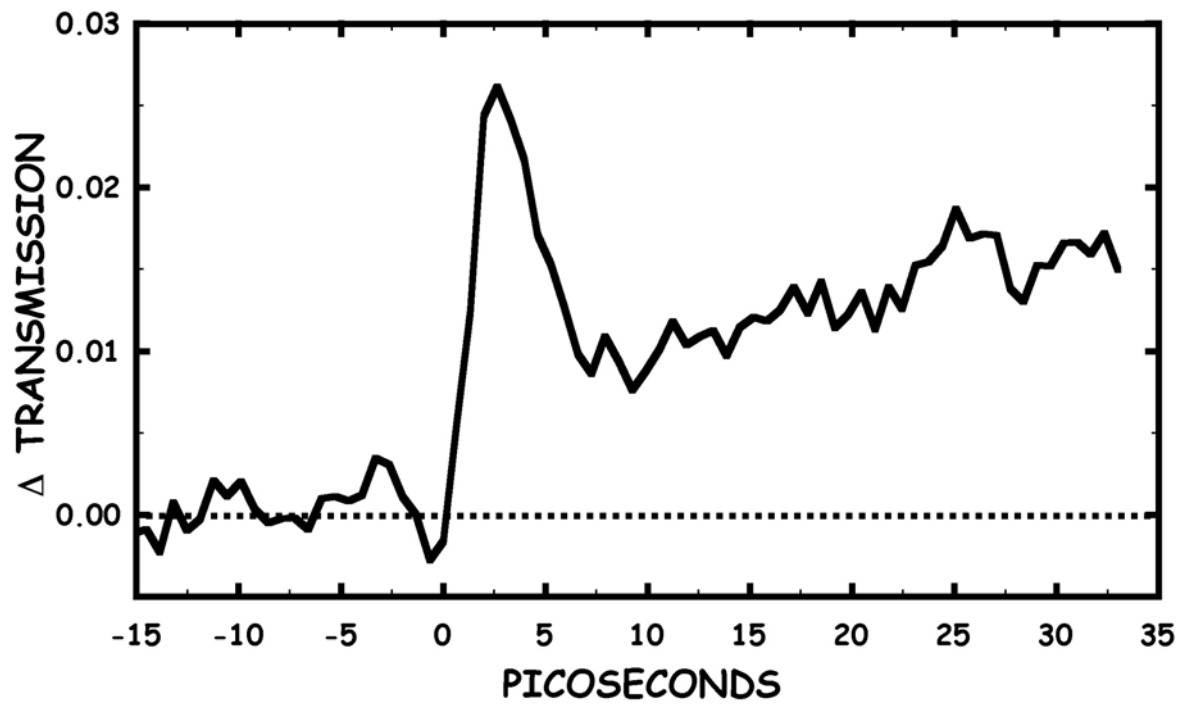
Mb, 290 K, 6.17 um band



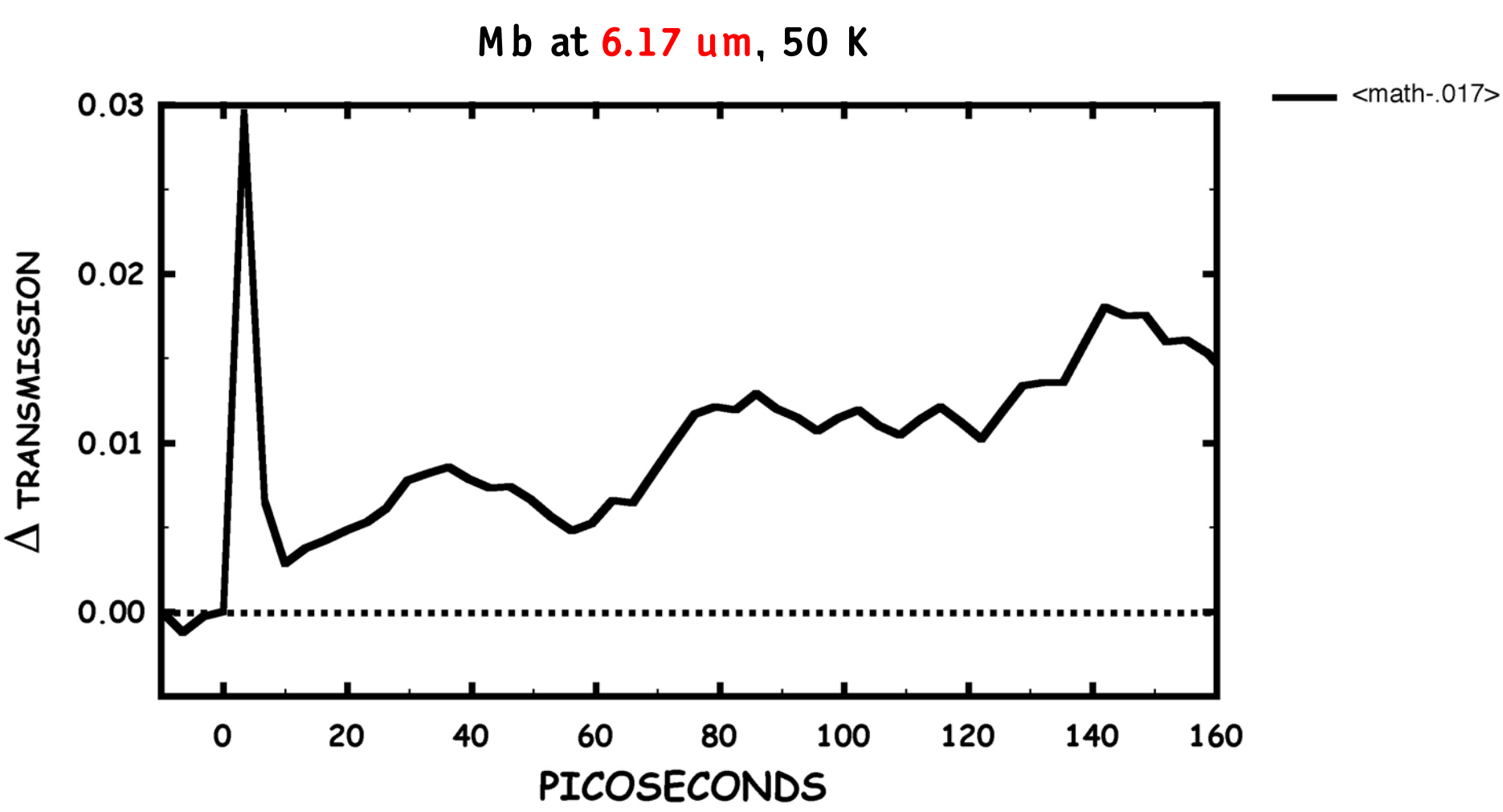
At the 6.17 um band at room temperature we see a long lived state, and one can even imagine seeing beating happening there. Signal size 1/10 of 6.0 um signal.



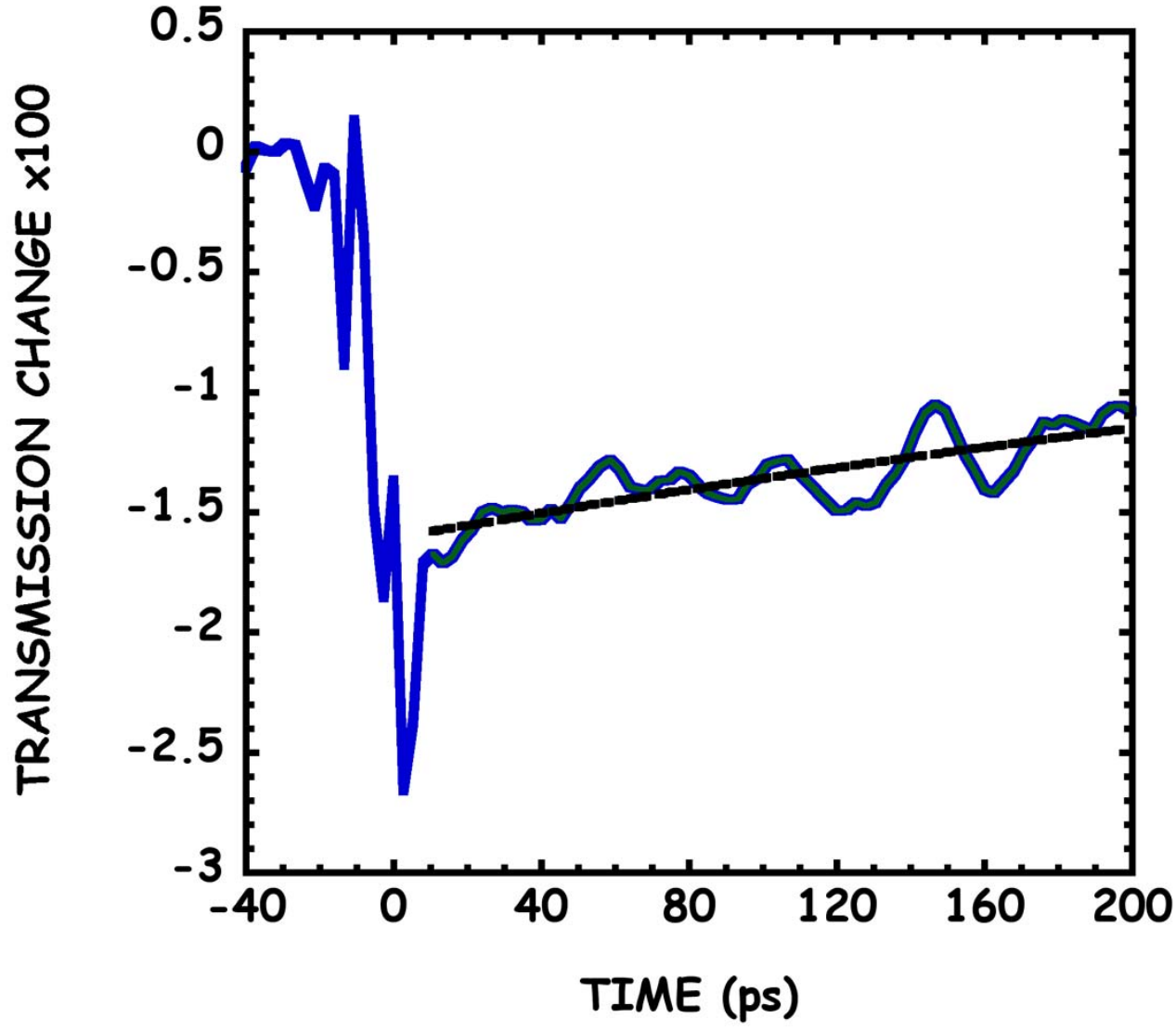
50 K, 6.00 um. Blue side of "normal" band.



50 K, 6.17 um, low temperature band.



The protein seems to be undergoing a slow (100 ps) time scale change to a new conformation. There may be beating. The structural transition rate is slowed relative to 290 K, but it still happens.



bR FIR pump/probe response at 120 microns

Conclusions:

- For some mysterious reason nobody has ever looked even casually at the temperature dependence of a protein's IR spectrum out to 10 microns. There are a number of new bands that appear in Mb (and other proteins) as you cool. Transition temp about 300 K, coupled to solvent IR spectra changes

- Single ps pump/probe IR spectroscopy can be done far up the vibrational ladder (5 photons/residue).

- Mb can be driven into long-lived states. Something special about these new bands. Little is known at this point about the anharmonic dynamics of these states, they are possibly functional.

What is it going to take to really open this
field up:

(1) 10 uJ "single" ps pulses, mid to far IR.
Driving up to the inverted potential.

(2) "2" colors: mid to far IR, broad spectral
range of at least 100 cm-1 locked to pump