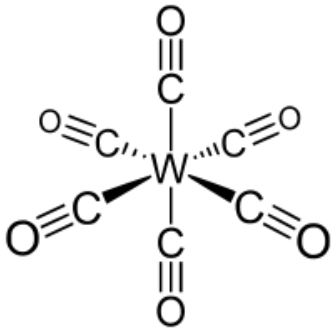
Coherent 2D IR: Introduction to a Powerful New Structural Spectroscopy and Application to Difficult Protein Systems

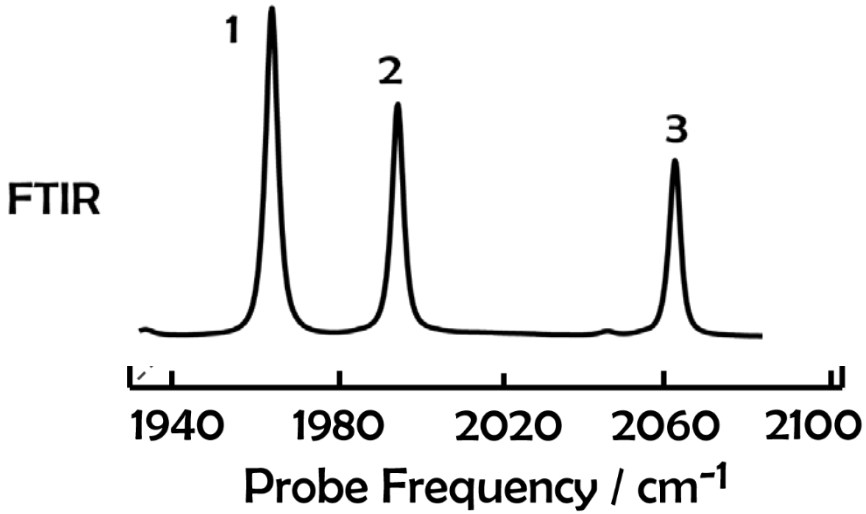
Chris Middleton, PhaseTech Spectroscopy

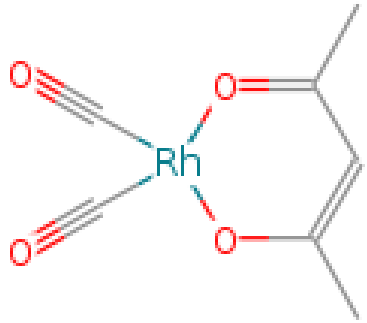
Data courtesy of Martin Zanni, University of Wisconsin-Madison

Which peaks belong to which molecule?



1 IR allowed mode

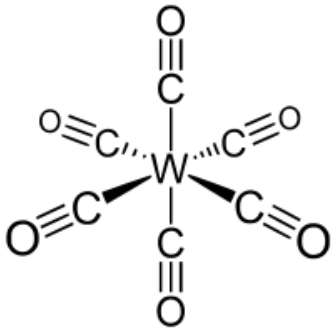




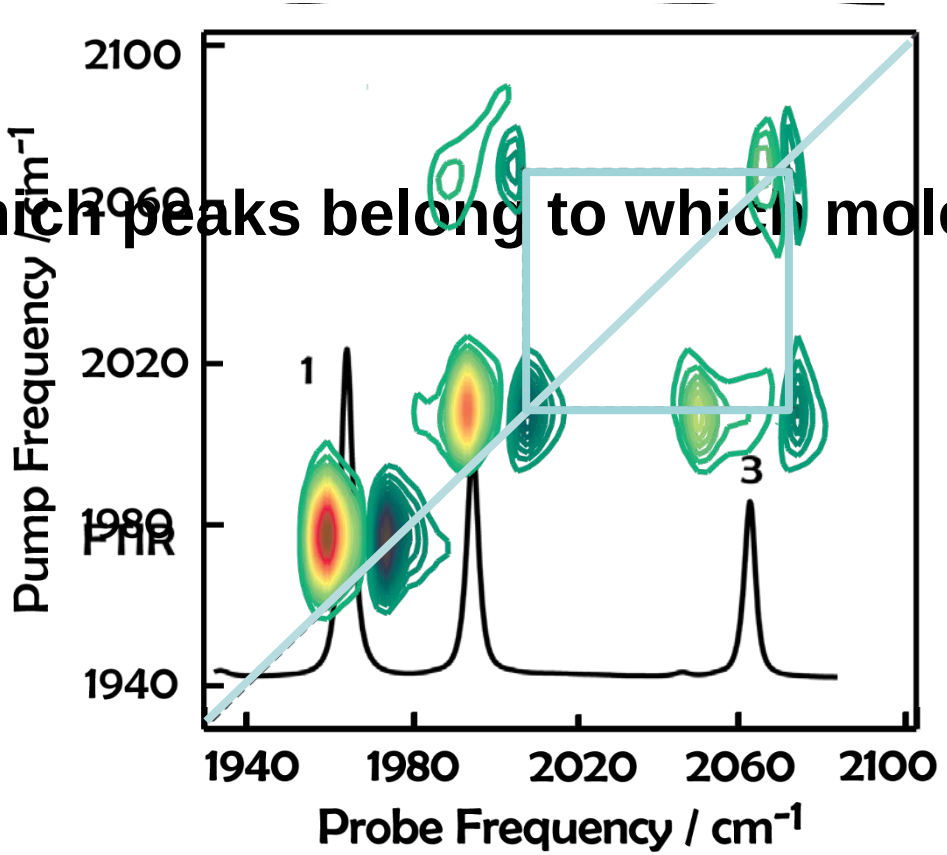
2 IR allowed modes

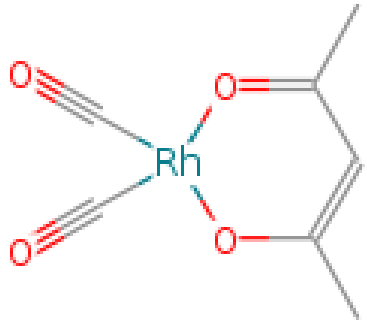
PHASETECH spectroscopy shaping science

Which peaks belong to which molecule?



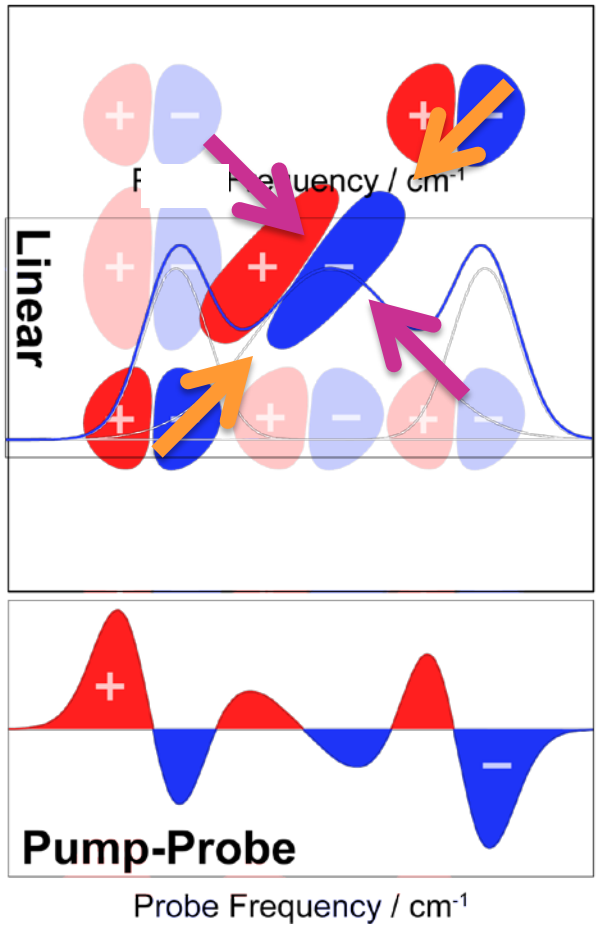
1 IR allowed mode

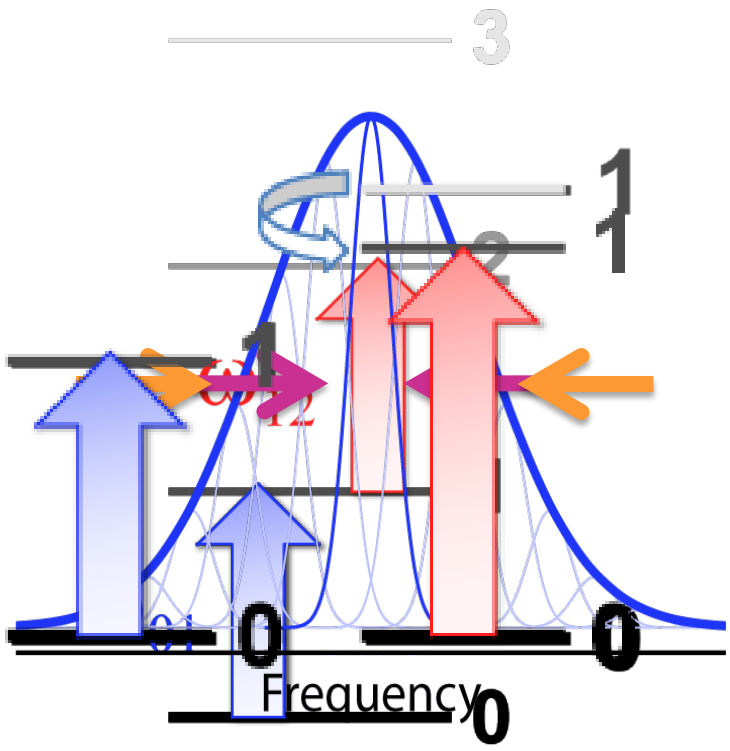




2 IR allowed modes

Coherent 2D IR spectrum





crosspeaks: interaction through-bond, through-space, or chemical exchange

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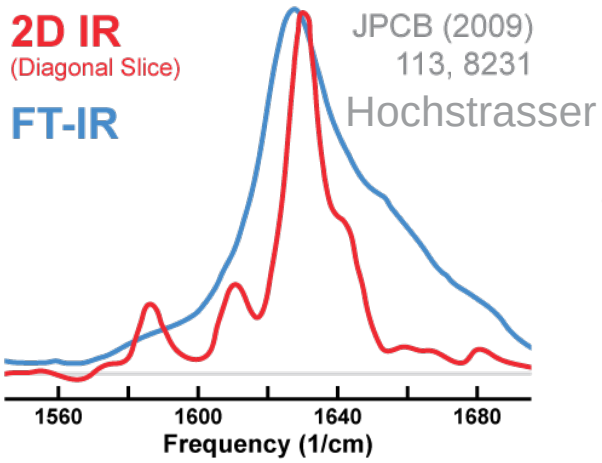
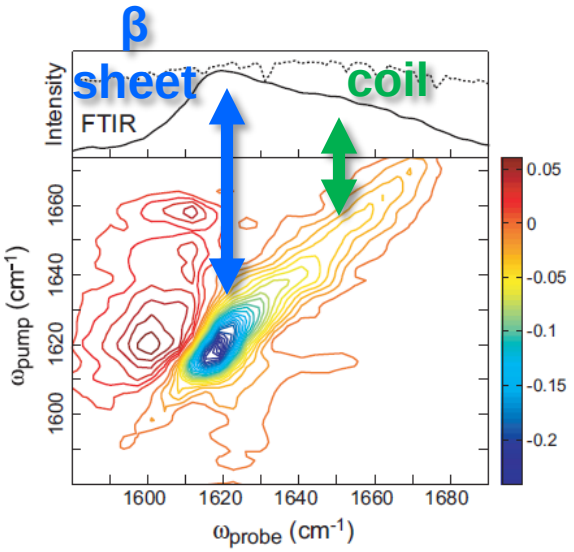
spectroscopy shaping science

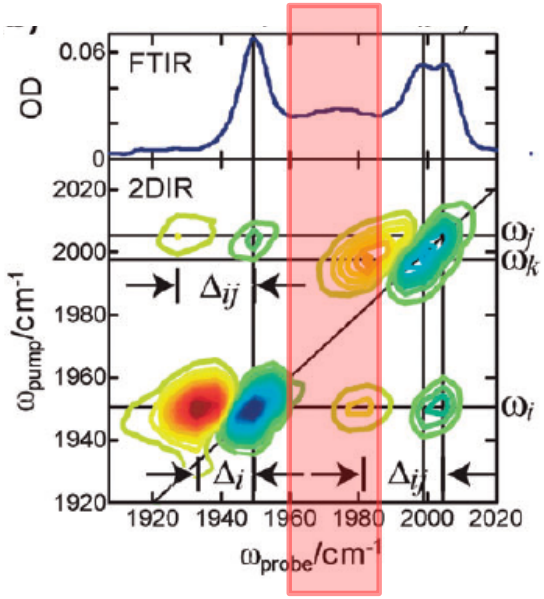
+ higher resolution than FT-IR

+ more sensitive to secondary structure than FT-IR

FT-IR ~ $|\mu|^2$
2D IR ~ $|\mu|^4$







solvent peak disappears!!

+ no reference/background samples needed

+ sample volumes < 1 uL

+ material as low as 1 ug + concentrations < 50 uM

+ flexible sampling – transmission, reflection, ATR, etc.

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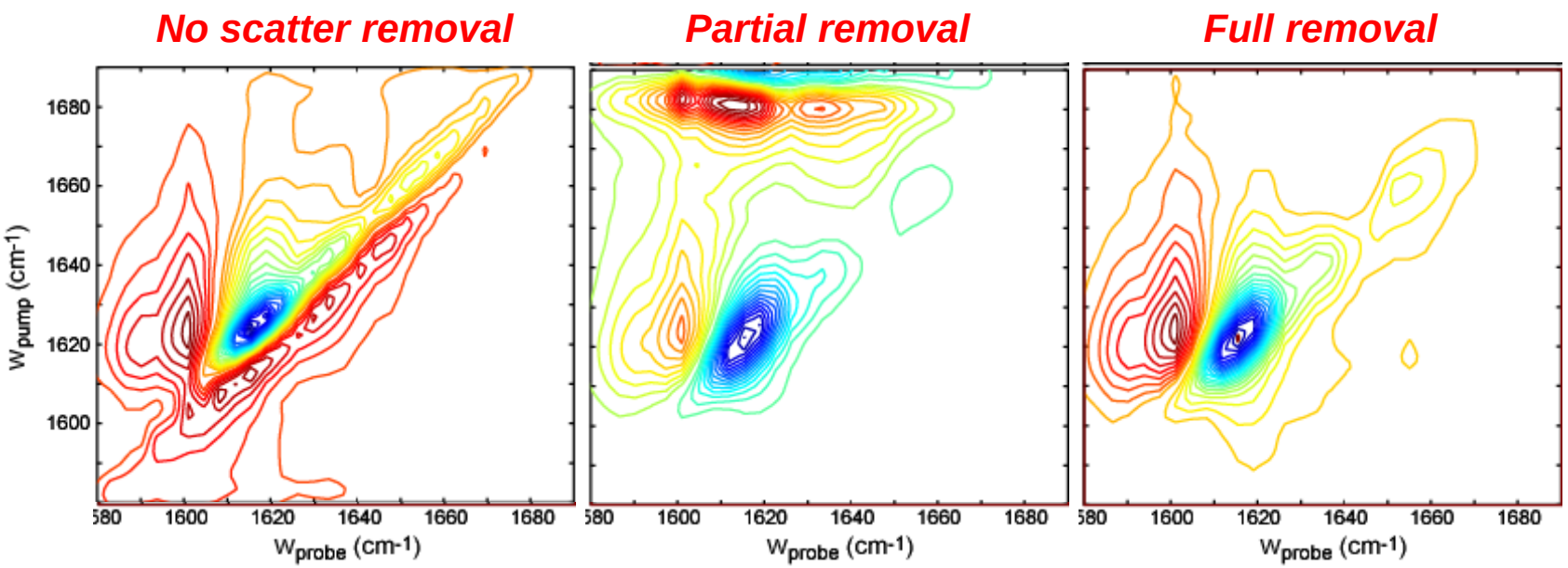
spectroscopy shaping science

+ femtosecond (10^{-15}) time resolution

+ scatter removal



+ difficult samples – aggregates, membranes, tissues, etc.

+ scan a single spectrum in < 1 sec! = study dynamic systems

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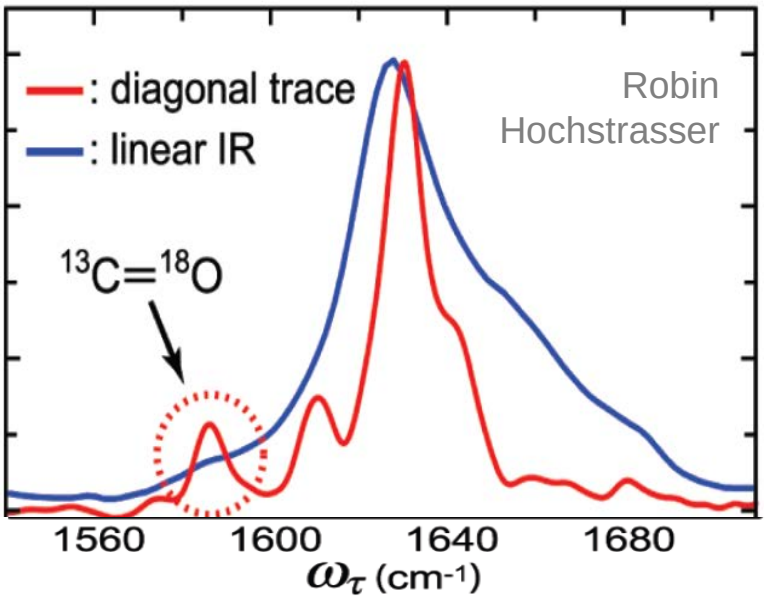
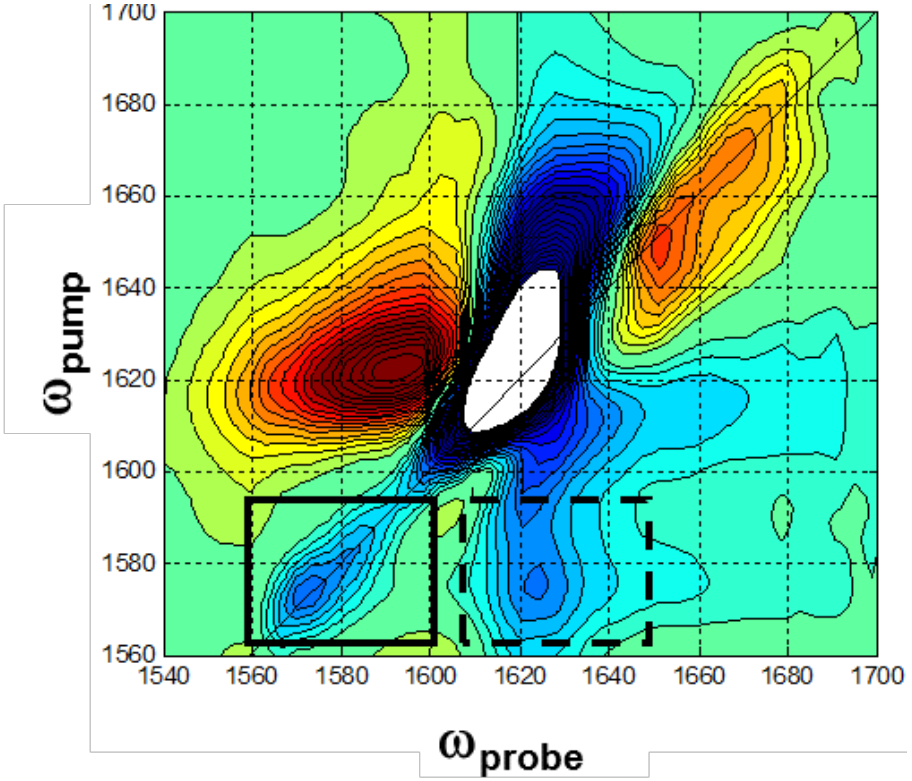
hIAPP: KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY

$^{13}C{=}^{18}O$





2D IR provides:

2D lineshapes = environment

Cross peaks = structure

STRUCTURE VIA LINEWIDTHS - HYDRATION AND DISORDER

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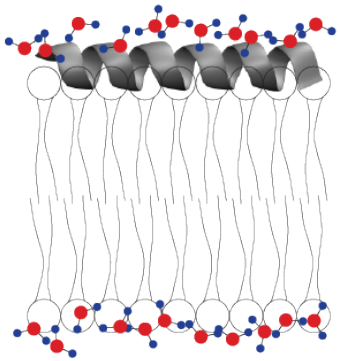
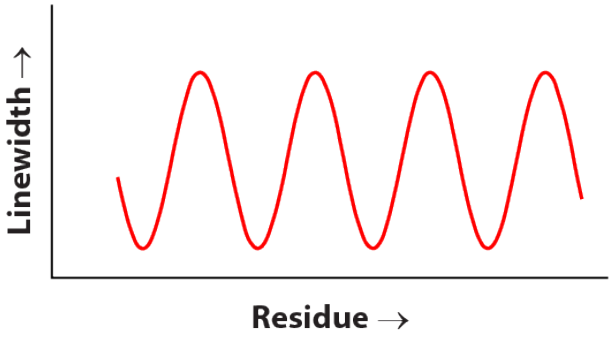
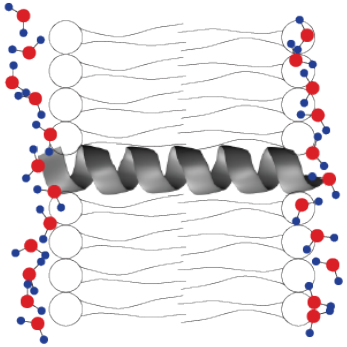
spectroscopy shaping science

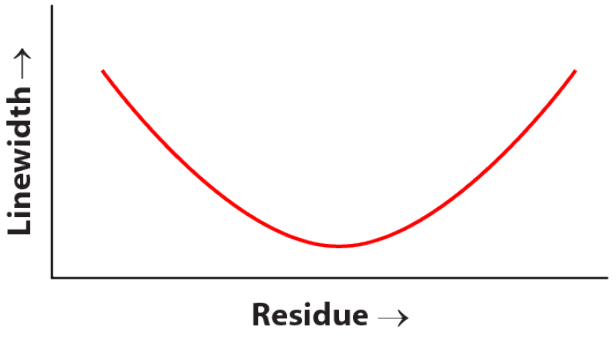
Membrane-Bound Peptide



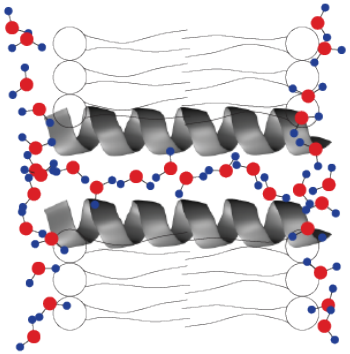


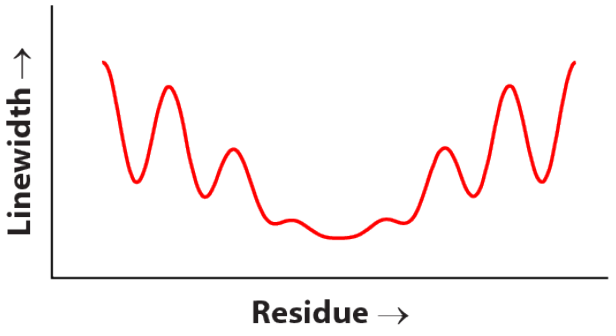
Transmembrane Protein





Transmembrane Channel





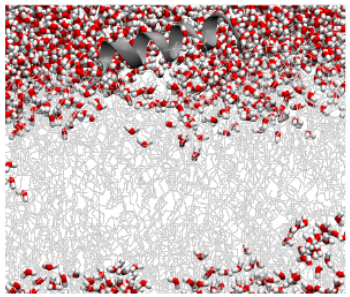
STRUCTURE VIA LINEWIDTHS - HYDRATION AND DISORDER

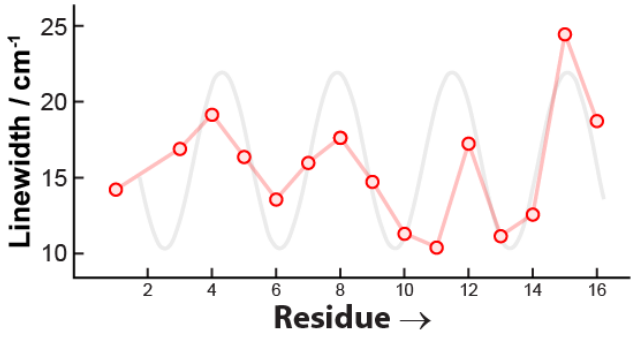
PHASETECH
spectroscopy shaping science

Membrane-Bound Peptide

Ovispirin
Antibiotic Peptide

JACS (2010) 132, 2832-2838

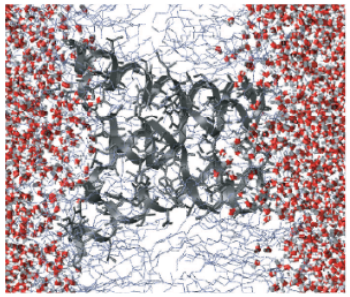


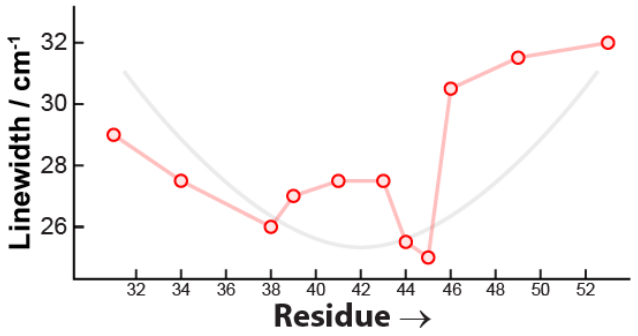


Transmembrane Protein

CD3ζ
T-Cell Receptor Expression Protein

PNAS (2006) 103, 3528-3533

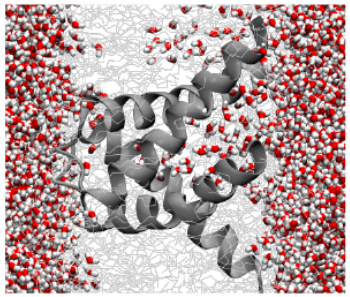


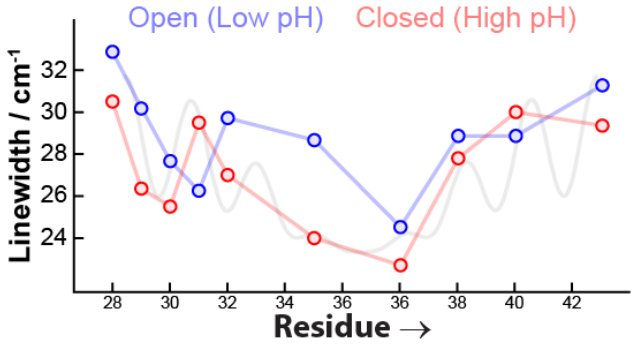


Transmembrane Channel

M2
pH-Gated Proton Channel

Structure (2009) 17, 247-254





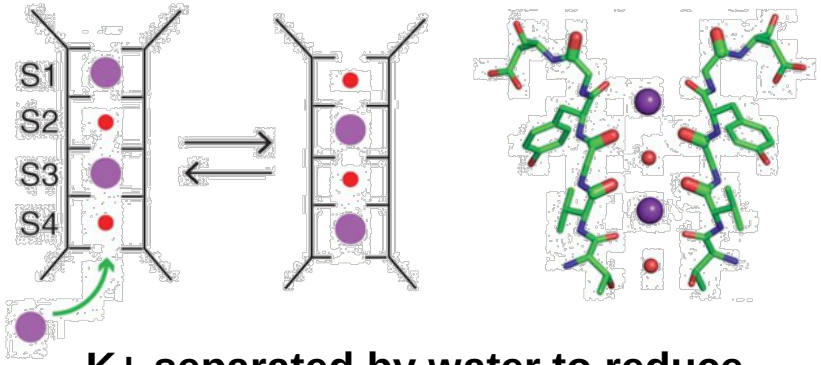
ANOTHER MEMBRANE PROTEIN - KCSA POTASSIUM CHANNEL

PHASETECH spectroscopy shaping science

Two Proposed Mechanisms for Ion Transport

"Canonical"

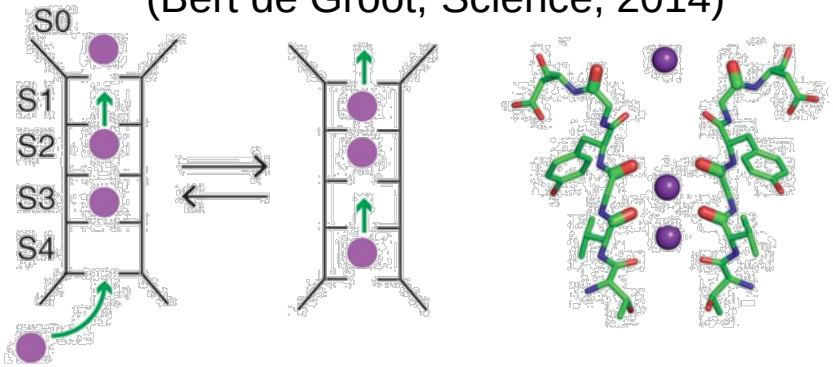
(MacKinnon, 2003 Nobel Prize)



K+ separated by water to reduce repulsion.

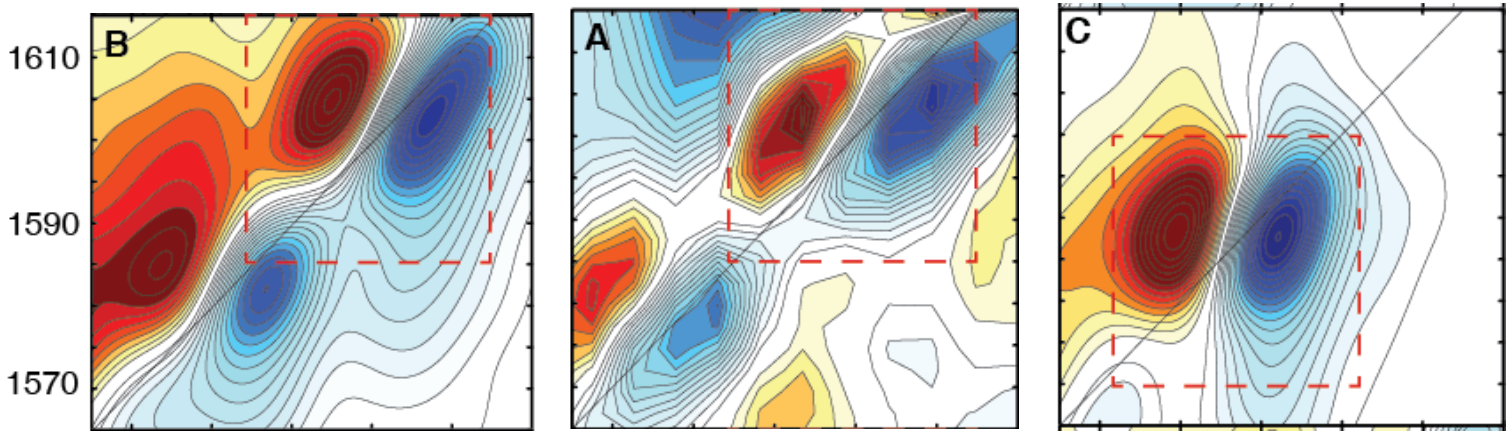
"New"

(Bert de Groot, Science, 2014)



Adjacent ions. No water. Reproduces all existing experimental data.

Simulation | **Measured** | **Simulation**



"Canonical" model is a nearly perfect match !!

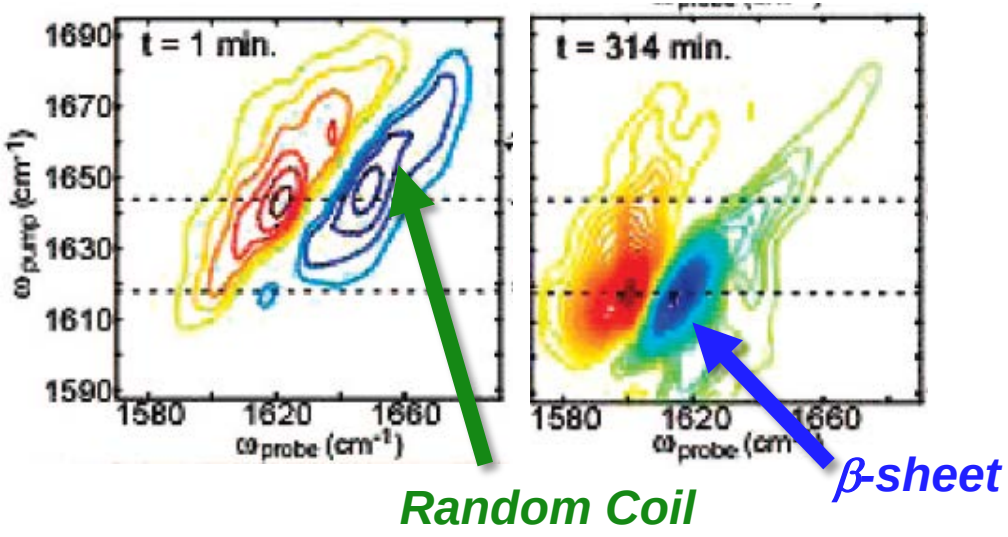
Science (2016) 353, 1040

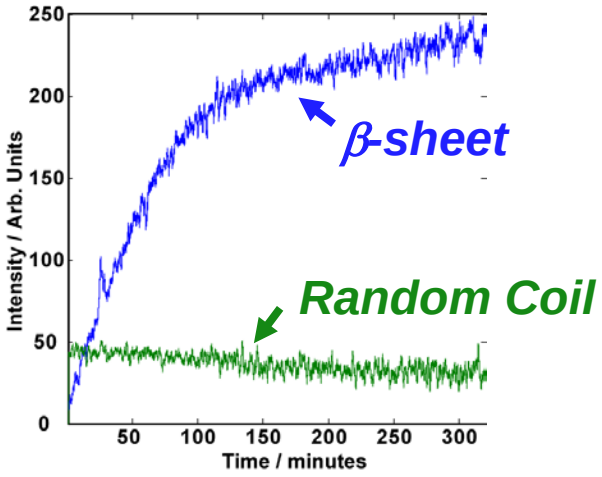
PHASETECH spectroscopy shaping science

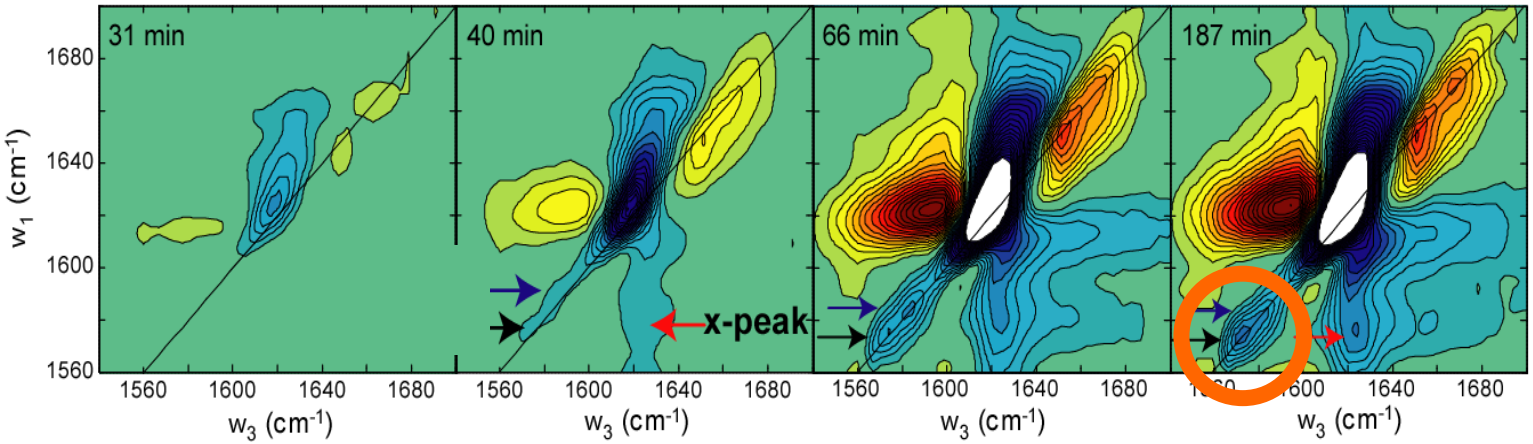
Single 2D IR spectra can be acquired with < 1 sec (average as needed)

Running averages can be used for real-time kinetics of 2D IR features

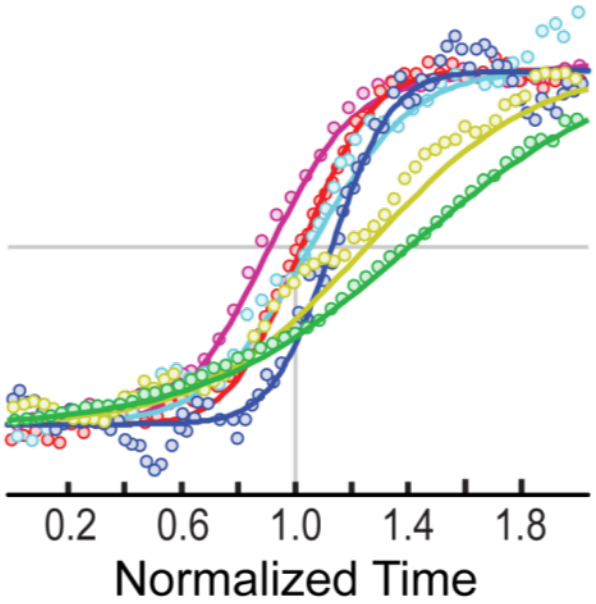
Example: Structural dynamics during amyloid aggregation into fibers

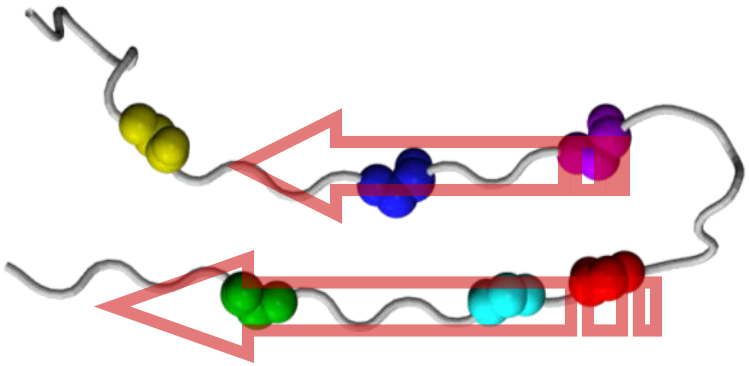






Labels exhibit different kinetic timescales !?!?

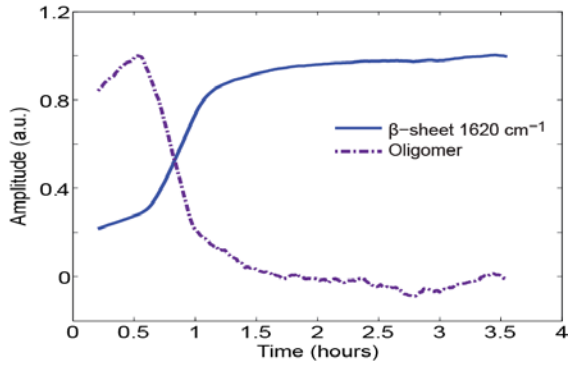




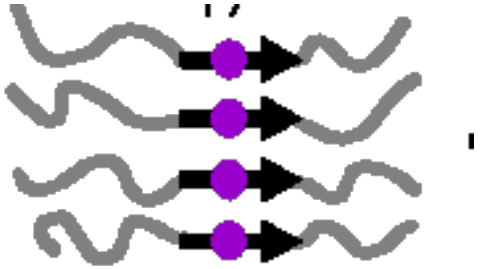
Nucleation starts near the loop and propagates down the sheets.

PNAS, 2009

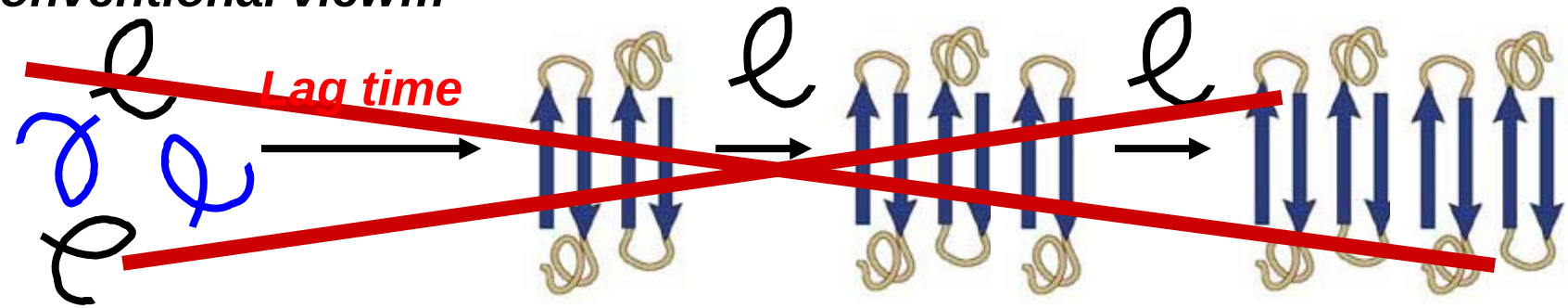
F23, G24, Ala25, L27 form a transient parallel beta-sheet !!



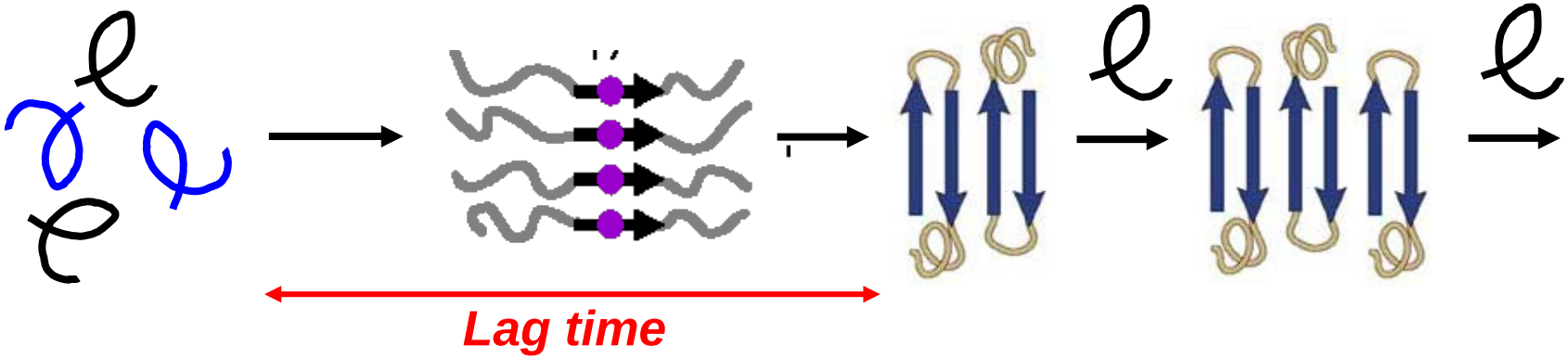
…F23-A27…

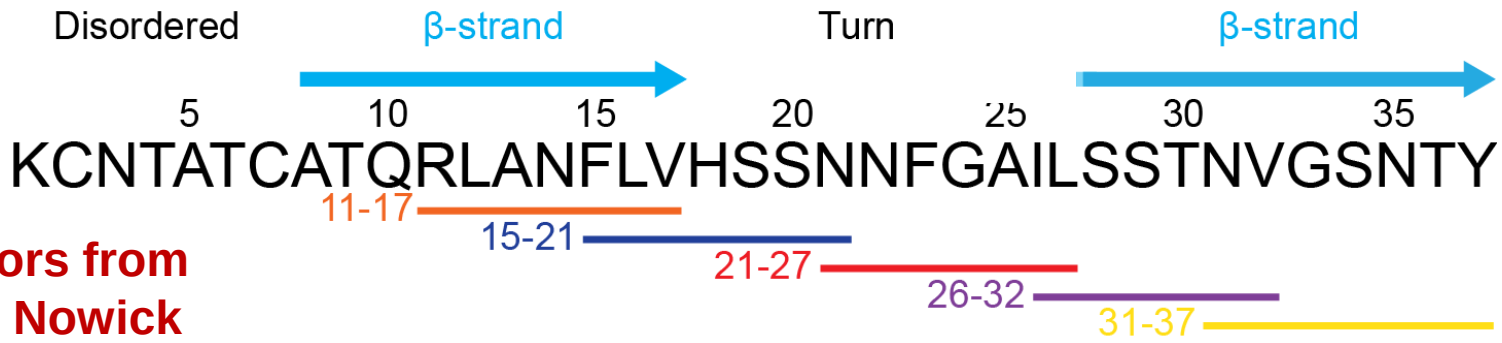


Conventional view…

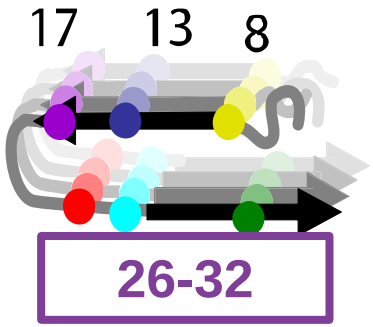


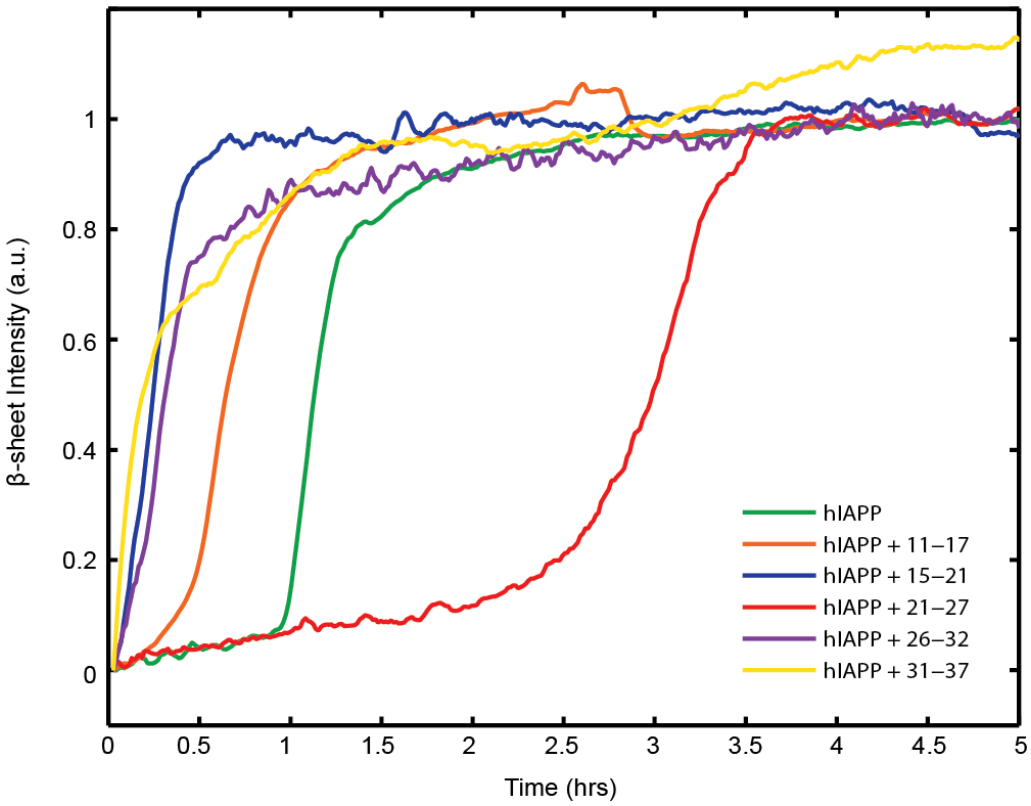
...missing a structural intermediate.

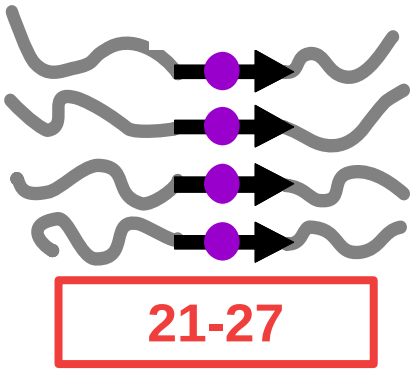




Inhibitors from James Nowick (UC-Irvine)

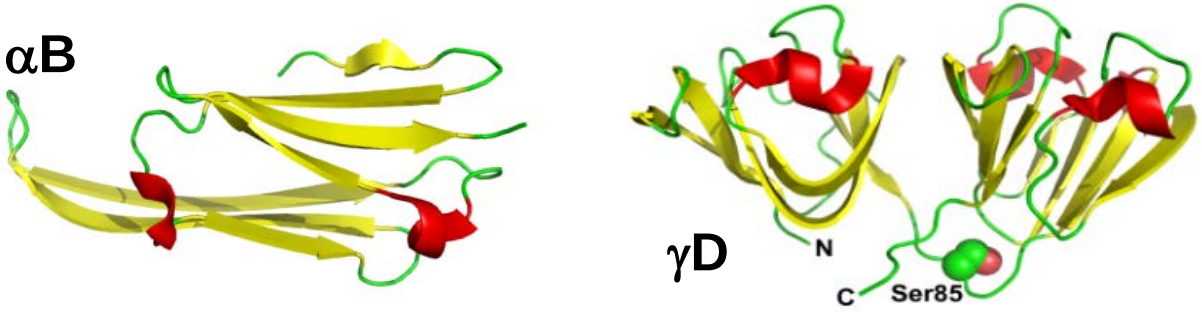






Can inhibit fiber formation by targeting intermediate !!

Cataracts are caused by aggregation of lens proteins (crystallins)



In vitro, many crystallins form amyloid fibers under denaturing conditions.

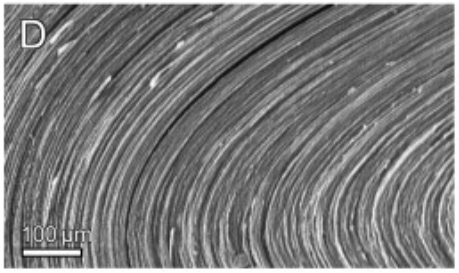
Little-to-no evidence that human cataracts contain amyloid.

SEM/TEM imaging

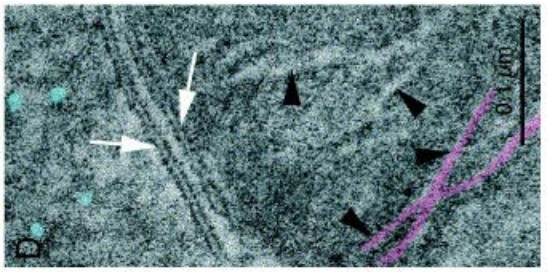
Only published examples that we can find for evidence of amyloid in lenses of animals or humans.

Human cataract lens

Michael, R. *et al. Vision Res.* 48, *4* (2008).

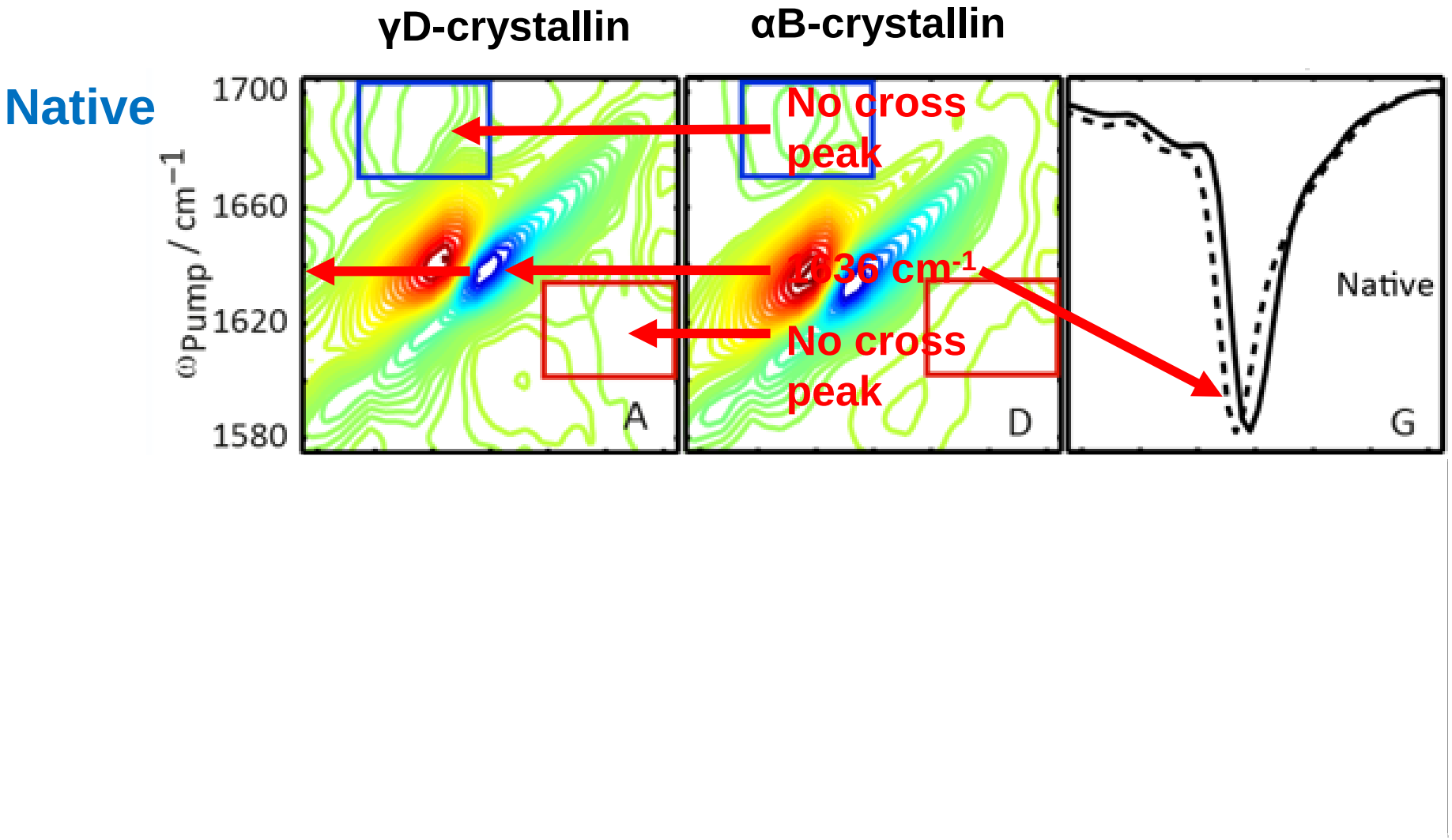


OXYS strain of rats (causes enhanced glucose transport)



S. Marsili *et al. Exp. Eye Res.* 79, *5* (2004).

In Vitro crystallins: calibration



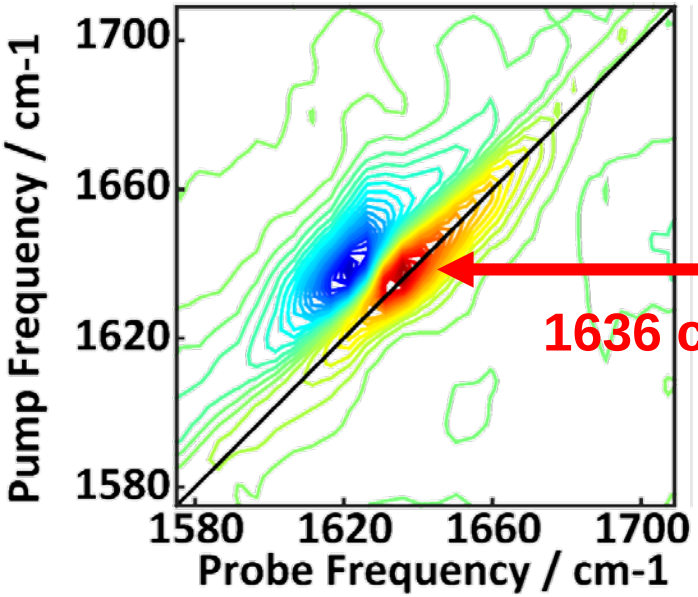
Two very different crystallin proteins both form fibers and characteristic peaks. Those are the spec signatures for amyloid secondary structure.

Strong couplings cause diagonal peaks at 1620 cm^{-1} and the cross peaks. Transition dipoles become enhanced (JCP, 2012; JPCB, 2015)

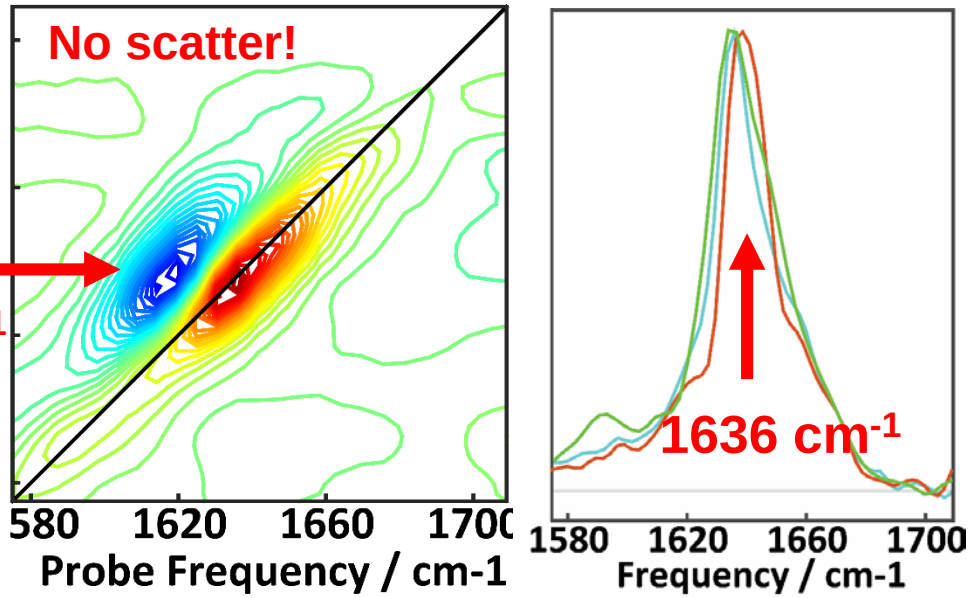
Pig Tissue: Lenses dissected from pigs.

sectioned 25 μm thick

αB-crystallin



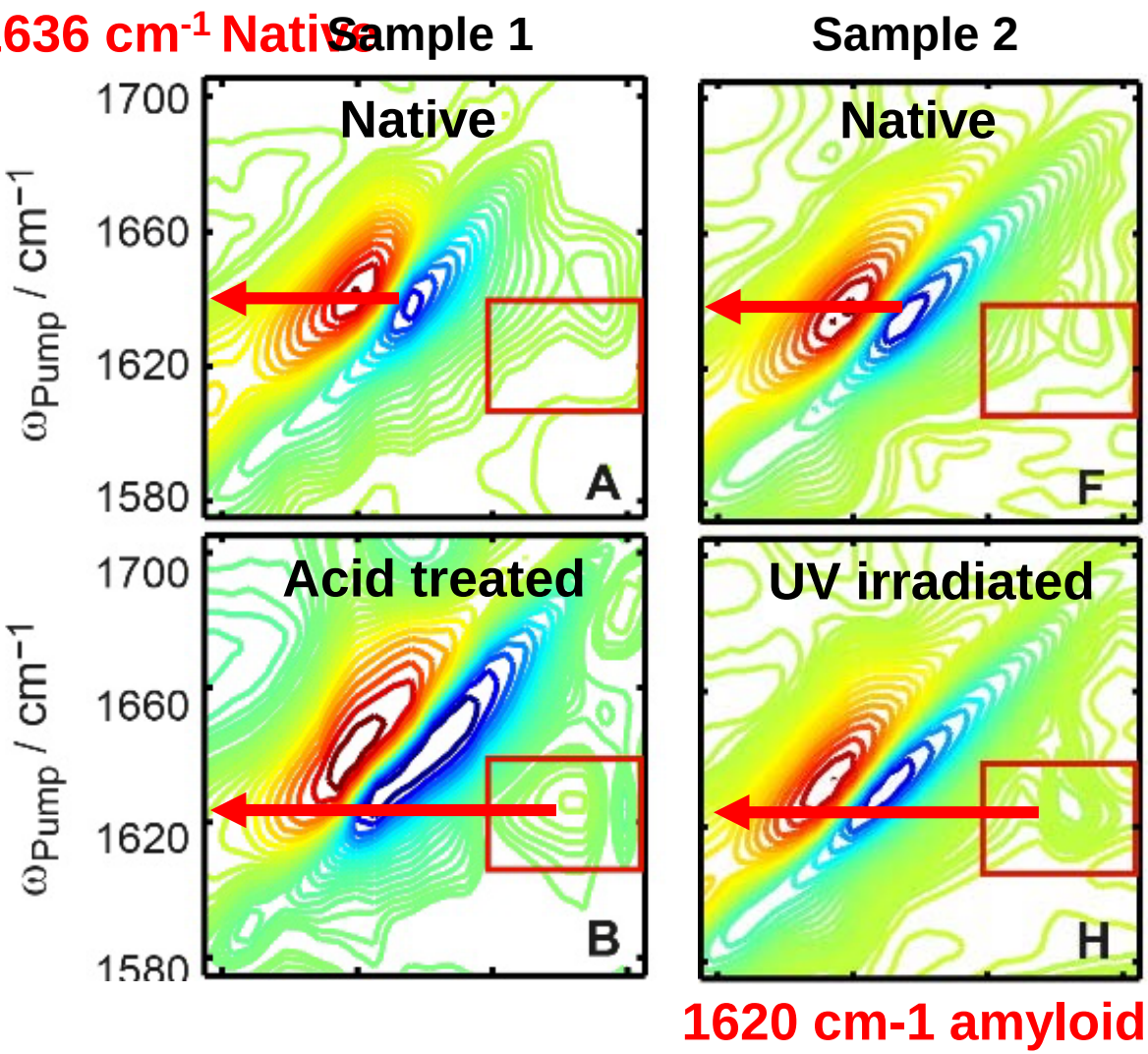
Native pig lens



1636 cm^{-1} absorption. Similar shapes. No 1620 cm^{-1} diagonal peaks nor cross peaks. Thus, no amyloid.

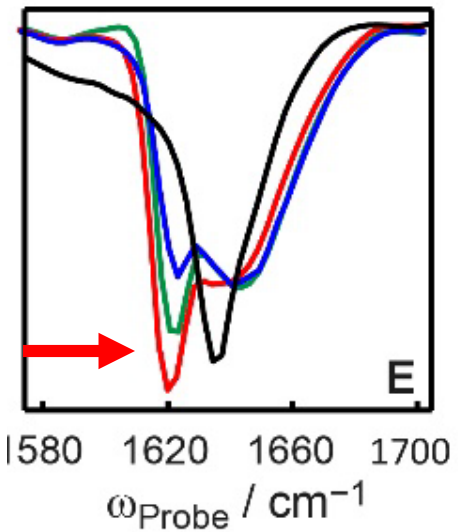
Acid and UV treated lens tissue.

1636 cm^{-1} Native

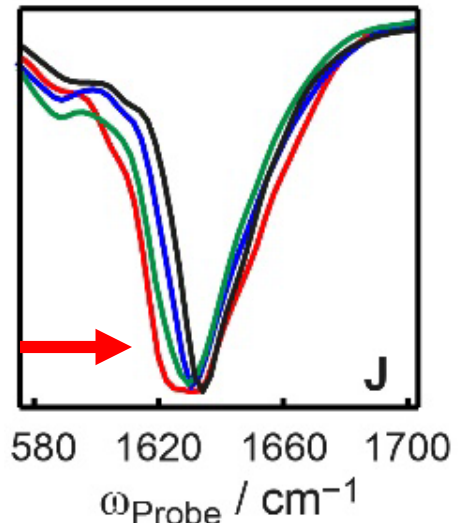


1620 cm-1 amyloid

Acid treated
0 to 150 min.



UV irradiated
0 to 24 hours.



Amyloid features increase with time.

Amyloid first observed at irradiation times equivalent to 45 years exposure.

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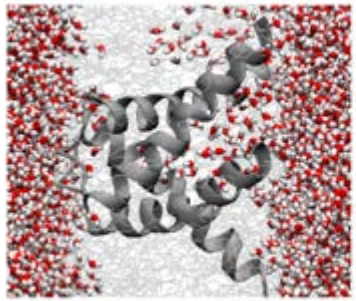
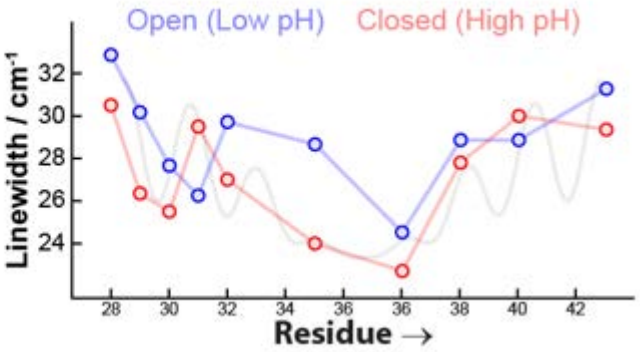
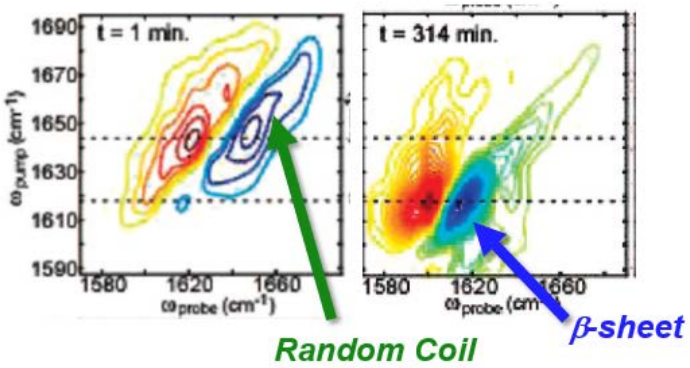
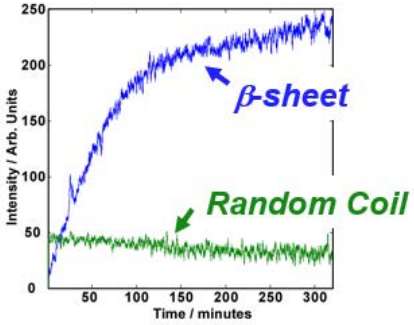


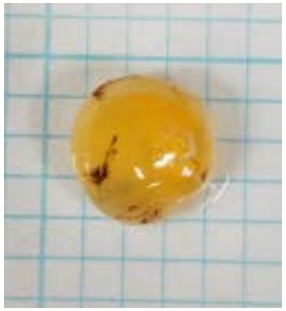


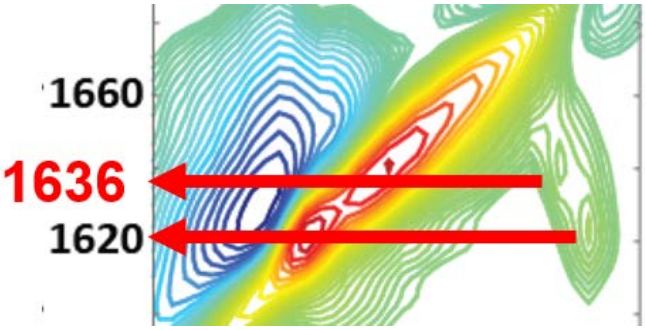
STRUCTURE

& DYNAMICS





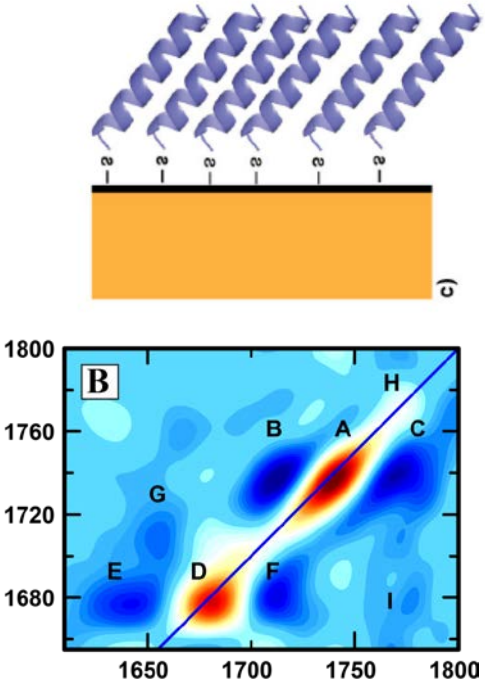




DIFFICULT SYSTEMS

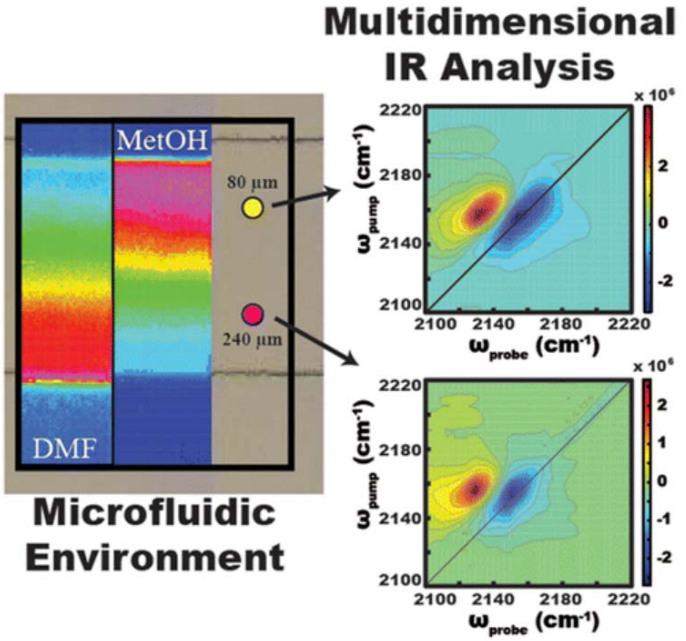
PHASETECH
spectroscopy shaping science

single monolayer sensitivity



FGAIL peptide on a model monolayer of anionic membrane

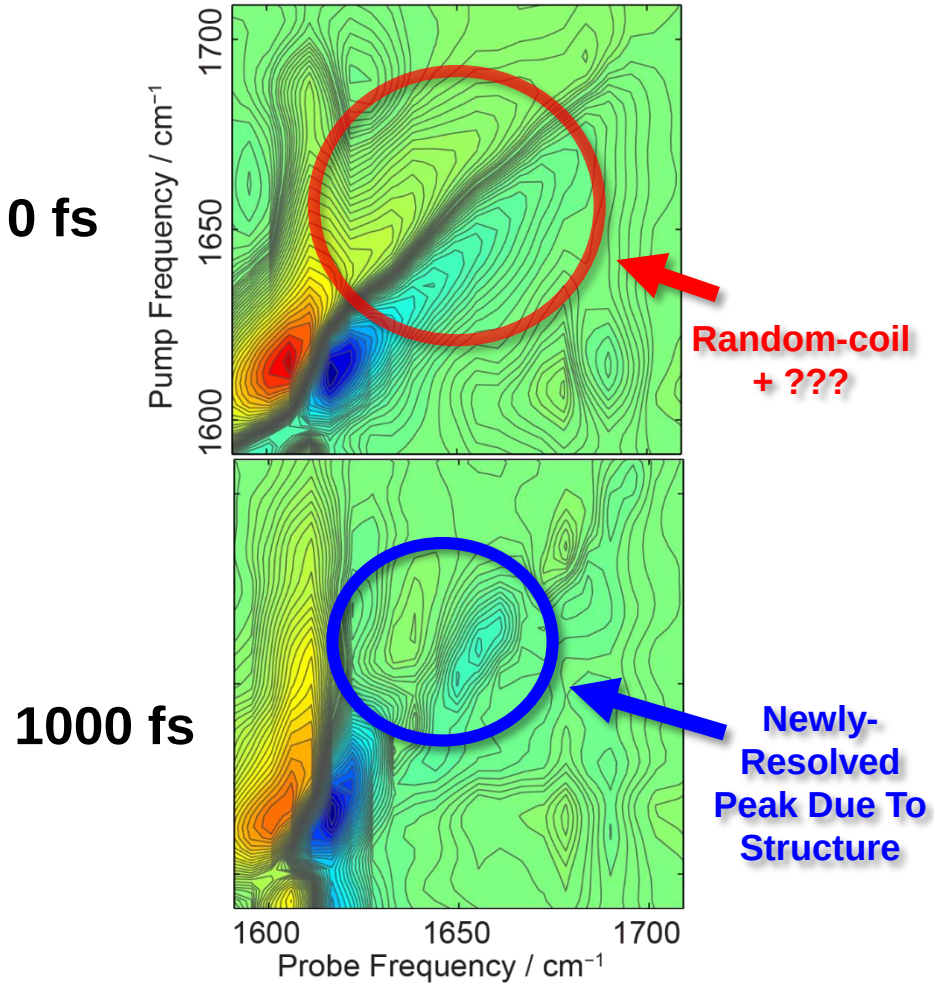
high-throughput with microfluidics



Krummel
J. Phys. Chem. Lett., **2016**, *7*, 4865

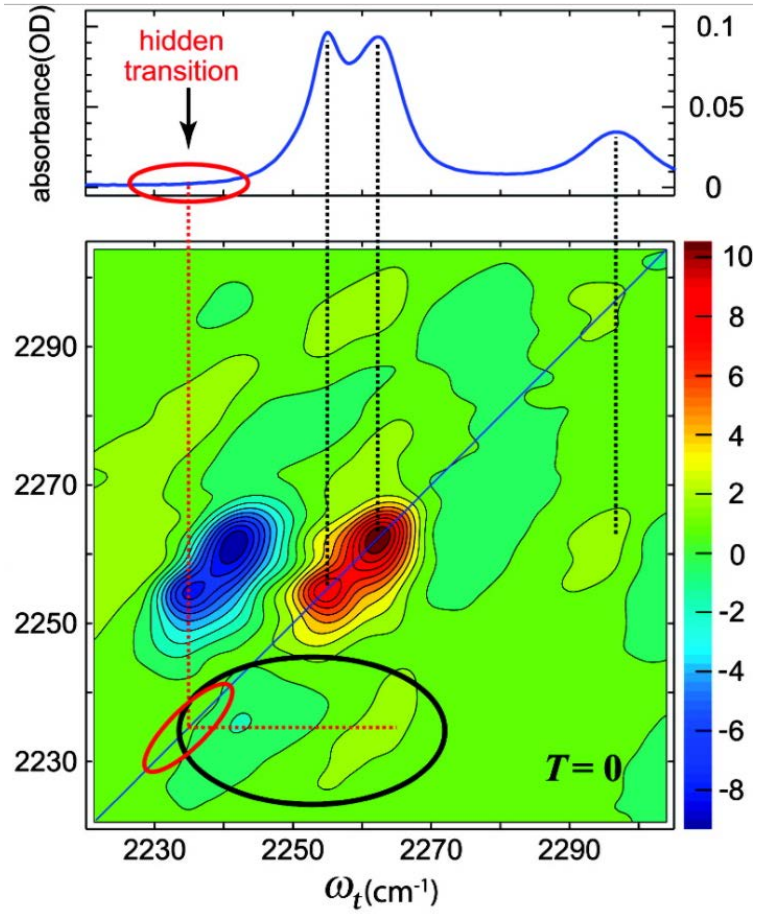
PHASETECH spectroscopy shaping science

different structures have different vibrational lifetimes



J. Phys. Chem. Lett. (2011) 2, 2357

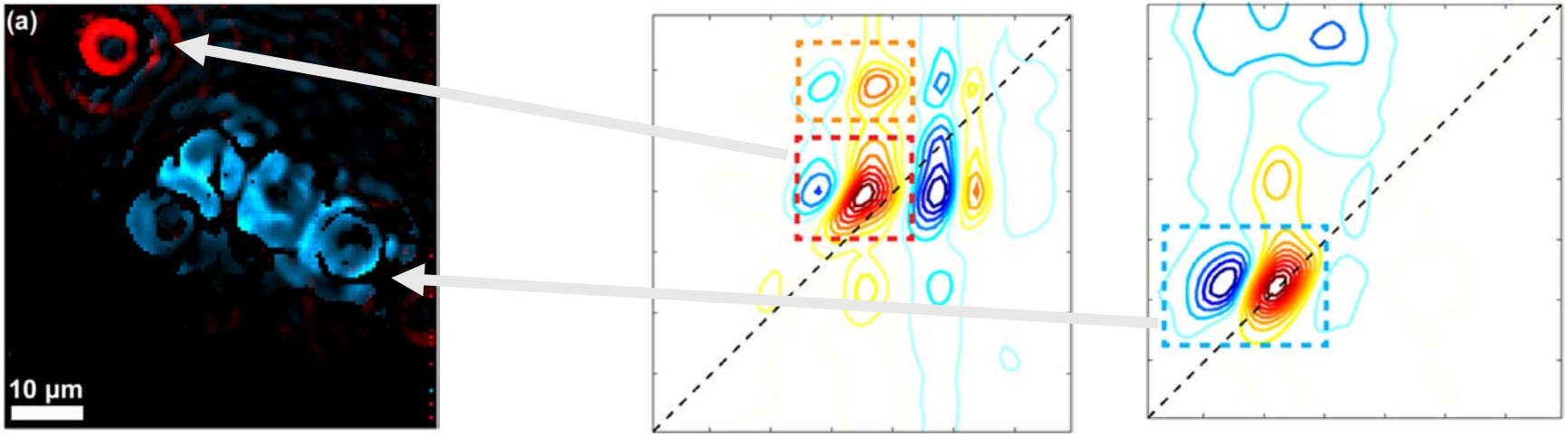
crosspeaks can reveal hidden vibrations



Hochstrasser
J. Phys. Chem. B, 2009, *113*, pp 8231

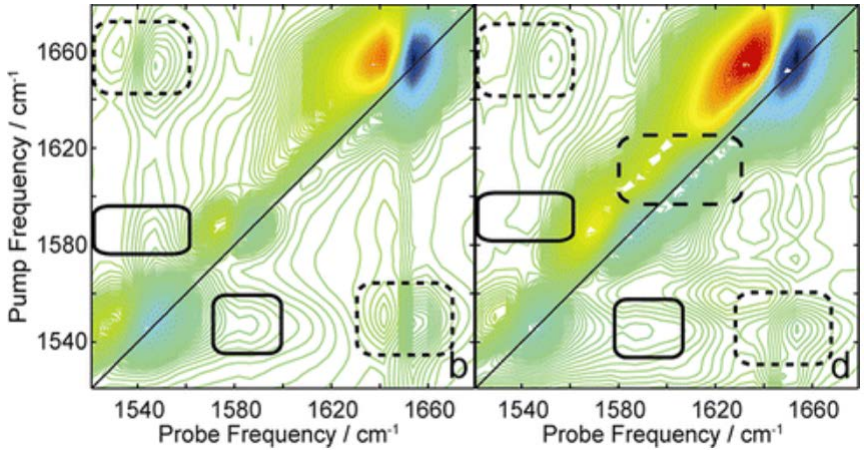
PHASETECH spectroscopy shaping science

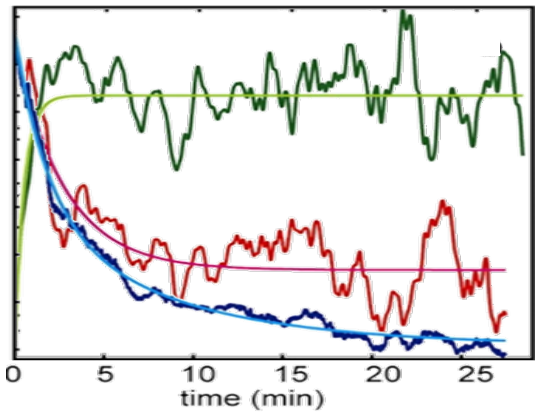
2D IR Wide-Field Microscopy



ACS Photonics (2016) 3, 1315

HDX





J. Phys. Chem. B (2013) 117, 15297

PHASETECH

spectroscopy shaping science

2DQuick IR

Currently: **Sold systems around the world to academics. Interested in learning about industrial applications, exploratory projects.**

3-5 Years: **Benchtop instrument ($100-200k)**

phasetechspectroscopy.com

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UW-Madison

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