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

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Data courtesy of Martin Zanni, University of Wisconsin-Madison

Which peaks belong to which molecule?

![Graph showing FTIR spectrum with peaks labeled 1, 2, and 3.]

1 IR allowed mode

2 IR allowed modes
Which peaks belong to which molecule?

Coherent 2D IR spectrum

1 IR allowed mode

2 IR allowed modes
crosspeaks: interaction through-bond, through-space, or chemical exchange
2D IR SPECTROSCOPY

+ higher resolution than FT-IR
+ more sensitive to secondary structure than FT-IR
+ no reference/background samples needed
+ sample volumes < 1 uL
+ material as low as 1 ug  + concentrations < 50 uM
+ flexible sampling – transmission, reflection, ATR, etc.

FT-IR $\sim |\mu|^2$

2D IR $\sim |\mu|^4$

solvent peak disappears!!
2D IR SPECTROSCOPY

- femtosecond (10\(^{-15}\)) time resolution
- scatter removal

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

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

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

2D IR provides: 2D lineshapes = environment
Cross peaks = structure
STRUCTURE VIA LINEWIDTHS - HYDRATION AND DISORDER

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
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
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 !!

Conventional view...

...missing a structural intermediate.

Lag time
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

Human cataract lens


OXYS strain of rats (causes enhanced glucose transport)


Only published examples that we can find for evidence of amyloid in lenses of animals or humans.
In Vitro crystallins: calibration

$\gamma$D-crystallin  $\alpha$B-crystallin

Native

Strong couplings cause diagonal peaks at 1620 cm$^{-1}$ and the cross peaks. Transition dipoles become enhanced (JCP, 2012; JPCB, 2015) secondary structure.
Pig Tissue: Lenses dissected from pigs.

sectioned 25 \( \mu \text{m} \) thick

1636 cm\(^{-1}\) absorption. Similar shapes. No 1620 cm\(^{-1}\) diagonal peaks nor cross peaks. Thus, no amyloid.
Acid and UV treated lens tissue.

Amyloid features increase with time. Amyloid first observed at irradiation times equivalent to 45 years exposure.
**single monolayer sensitivity**

FGAIL peptide on a model monolayer of anionic membrane

**high-throughput with microfluidics**

Krummel

**JUST FYI...**

**different structures have different vibrational lifetimes**

**crosspeaks can reveal hidden vibrations**

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**0 fs**

**Random-coil + ???**

**1000 fs**

**Newly-Resolved Peak Due To Structure**

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Hochstrasser
2D IR Wide-Field Microscopy

HDX

ACS Photonics (2016) 3, 1315

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

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