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The Business of Innovation

Live-cell Vibrational Spectroscopy for Nanotoxicity and Nanomedicine

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Outline

- Introduction
  - Nanotoxicity
  - Need rapid screening
  - Biomarker discovery
- ATR - FTIR spectroscopy
  - Transmission vs. ATR
  - Challenges and solutions
- Particokinetics - Silica settling
- Silica settling on C-10 cell and interacting
- Connection to high level cellular processes
- Summary
- Acknowledgements

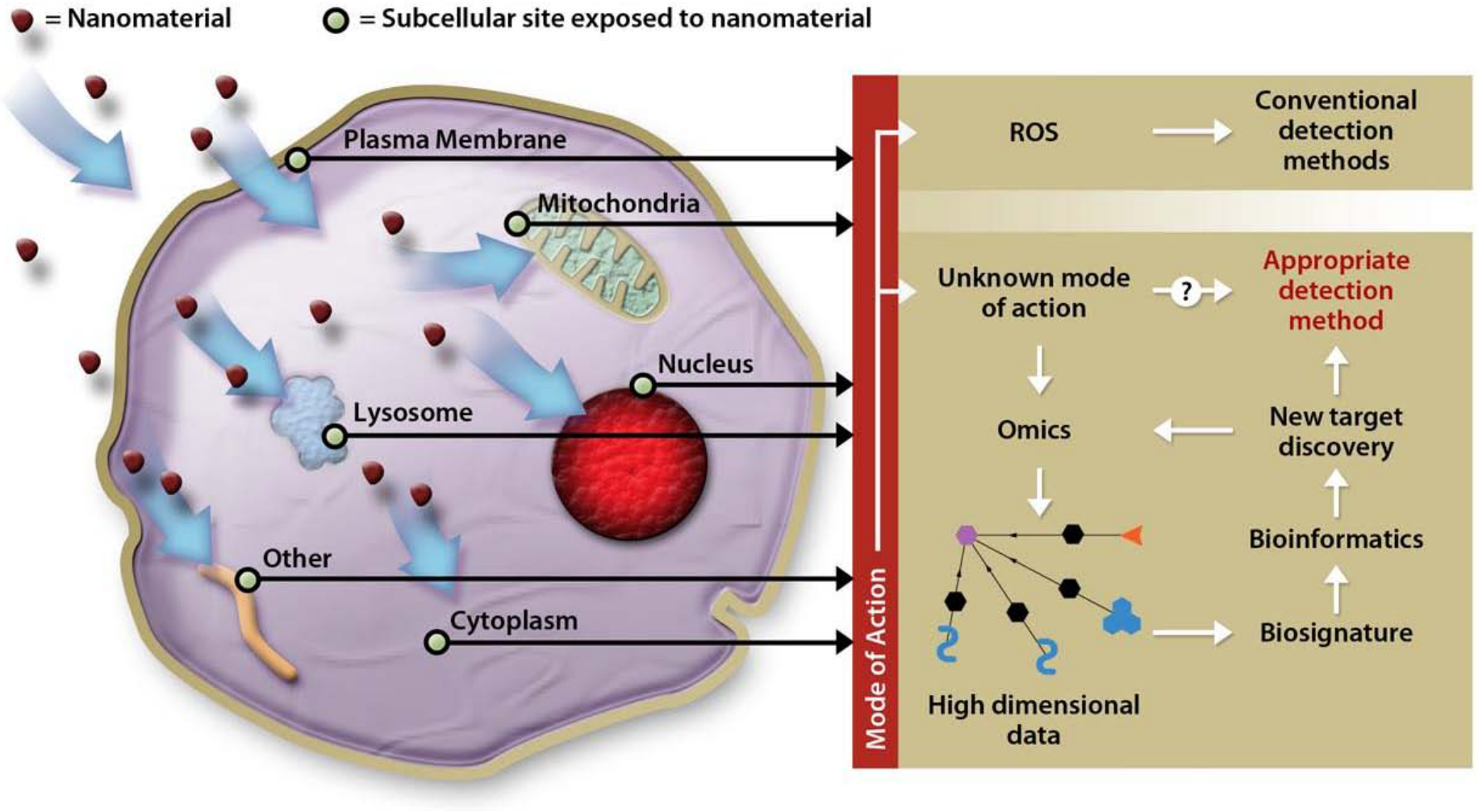
Health Effects

- The adverse health effects of particulate matter (PM) are well documented
  - Pulmonary
  - Cardiovascular
- Nanoparticle toxicity studies encompass complex processes
  - Partico/chemico/biokinetics
  - Composition-dependent
    - Intentional functionalization
    - Unintentional functionalization and contaminants
- Impossible to test every variable - needs a rapid screening tool

Biomarkers of particulate matter toxicity may not adequately predict nanoparticle toxicity
(Warheit et al., Toxicol. Sci. 77:117-125, 2004).

What method can be used to rapidly detect biologically active nanoparticles?

Need for Rapid and Cost Effective Global Profiling Technologies: Mode of Action is Unclear



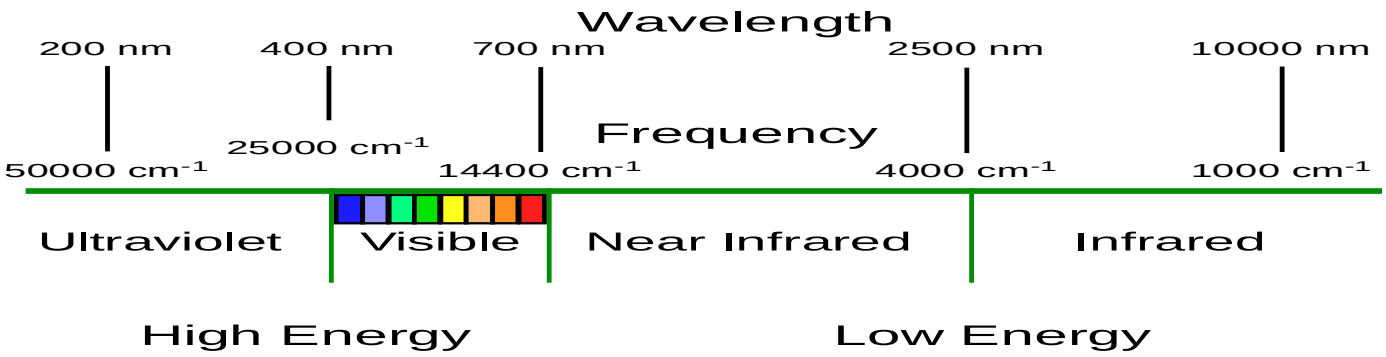
Comparison of Modalities

- **IR Spectroscopy Modality**
  - Can track IR-observable changes in viable cells in real-time without the need for a specific probe
  - Expected to provide a semi-quantitative if not quantitative way to know when to look for emerging biomarkers based on real-time monitoring of IR spectra
  - Expected to provide new supporting bonding data by correlating IR spectral data to fluorescence imaging data of known cell responses to model toxicants
- **Fluorescence Imaging Modality**
  - Real-time sensitivity
  - Dependent on availability of probes for pre-determined pathways/responses

Why Combine IR Spectroscopy and Fluorescence Microscopy Capabilities?

- Difficult to interpret IR spectra when the origin of the IR-observable signal is unknown
- Fluorescence capability can exploit a battery of established fluorescence-based assays to improve our understanding of IR observable signals
  - e.g., changes in pH, membrane fluidity, organelle status, etc…
- Fluorescence imaging modality will train the IR spectroscopy modality

Electromagnetic Radiation

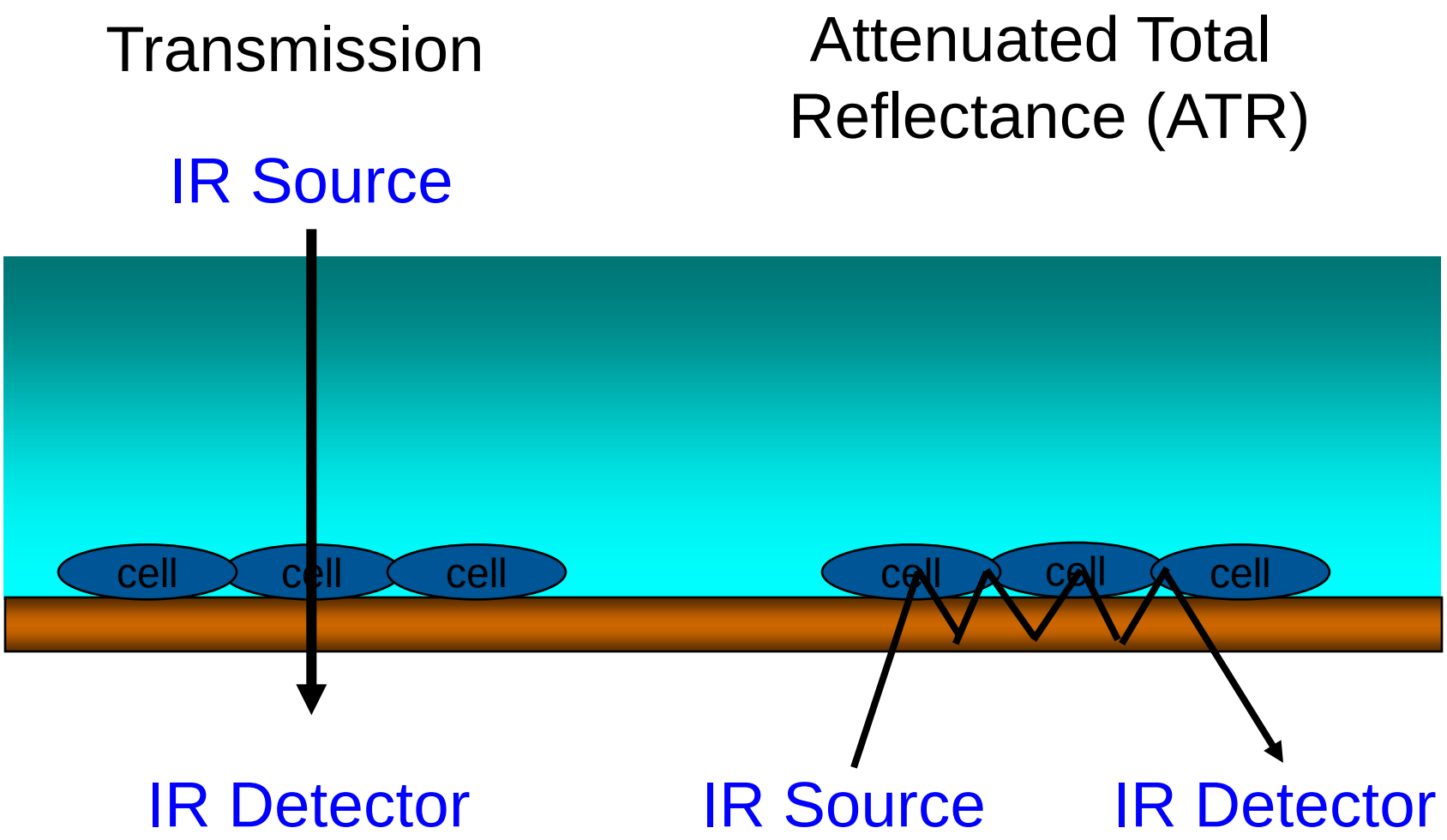


- Infrared region:
  - Low energy radiation
  - Causes net change in the dipole moment
  - Changes amplitude of rotation, stretching, and bending of molecules
  - Non-symmetric bonds (H_2O, CH, CH_2, NH, NH_3)

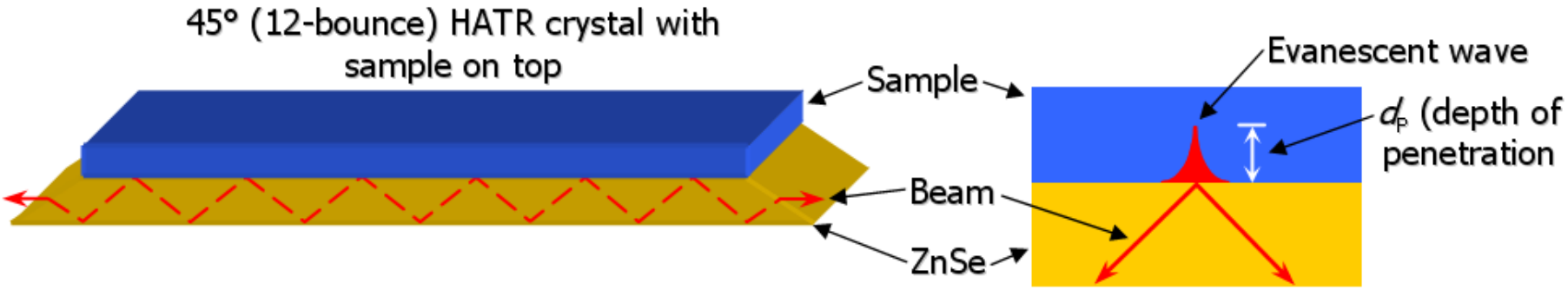
Monitoring Cellular Response By FTIR Spectroscopy

- Cells make a good model system.
  - Respond to a wide range of materials
  - Provide complex response
  - Rapid screening
  - Cost effective
  - Animal welfare
- Emphasis placed on detecting biological activity of nanomaterial
  - No *bias* in the interpretation of biological activity
  - Detect biologically active nanoparticles without knowledge of cellular targets *a priori*
  - Do not need a contrast agent or specific reagent to detect biological activity
  - Near real-time (minutes)
- Prioritize materials for further investigation

Transmission vs. ATR Modes of FTIR Spectroscopy

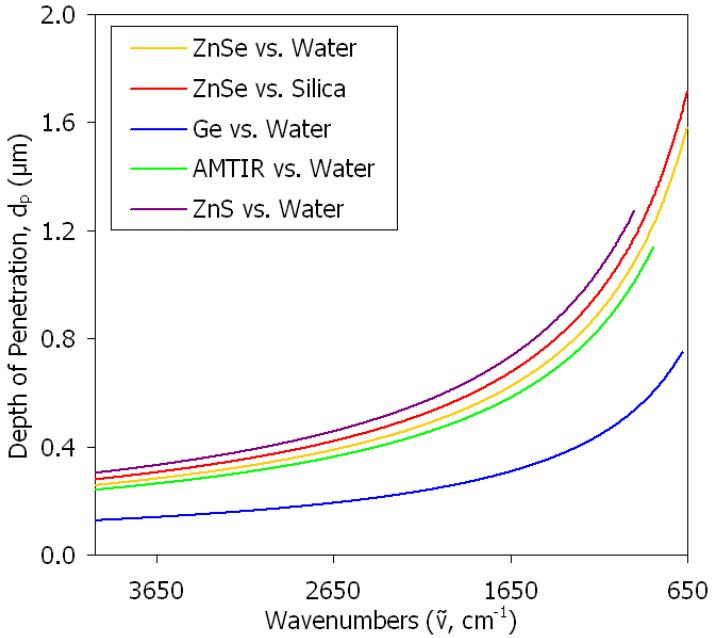


ATR-FTIR Spectroscopy



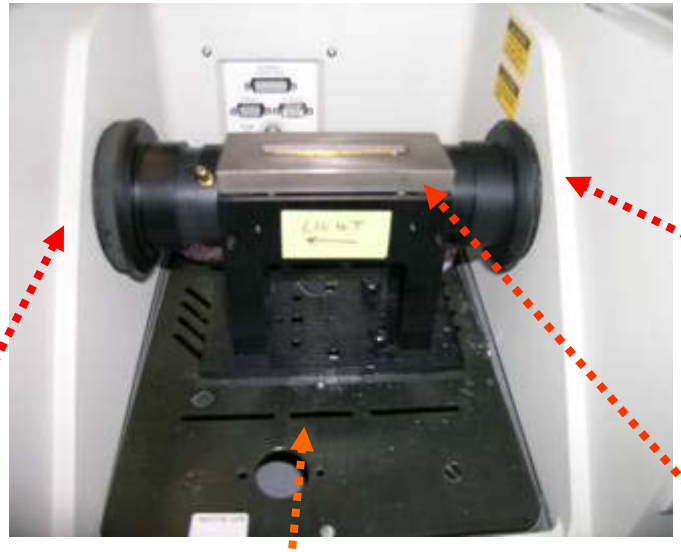
Material	*n* (RT)
ZnSe	2.40
ZnS	2.20
AMTIR	2.50
Ge	4.0
Water	1.330
Silica	1.460

$$d_P = \frac{\lambda_0}{2\pi\left(n_1^2 \sin^2\theta_1 - n_2^2\right)^{1/2}}$$



ATR-FTIR Spectroscopy (continued)

ZnSe crystal



Light Source

ZnSe crystal

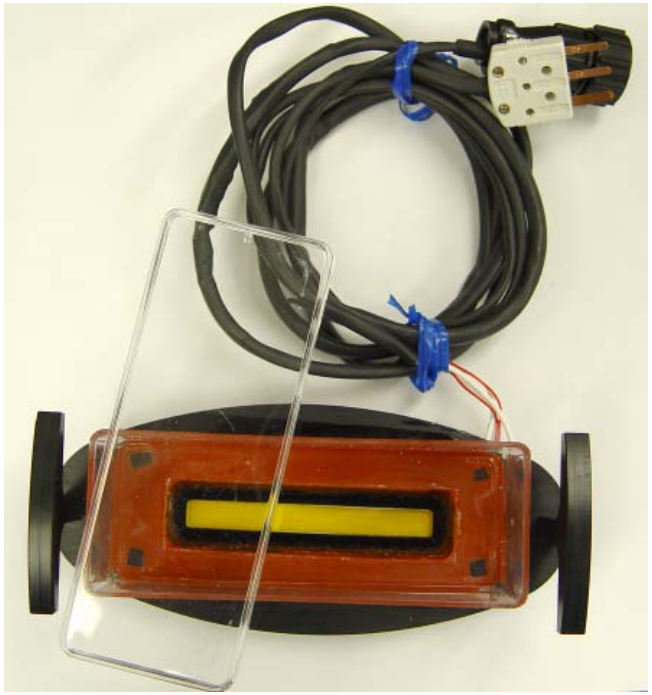
Light Detector

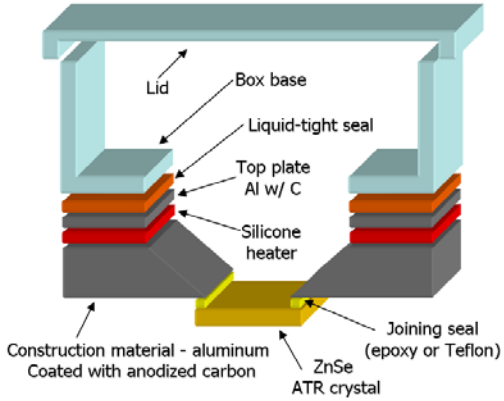
Sample holder

ATR-FTIR Spectroscopy (continued)

Unheated plate

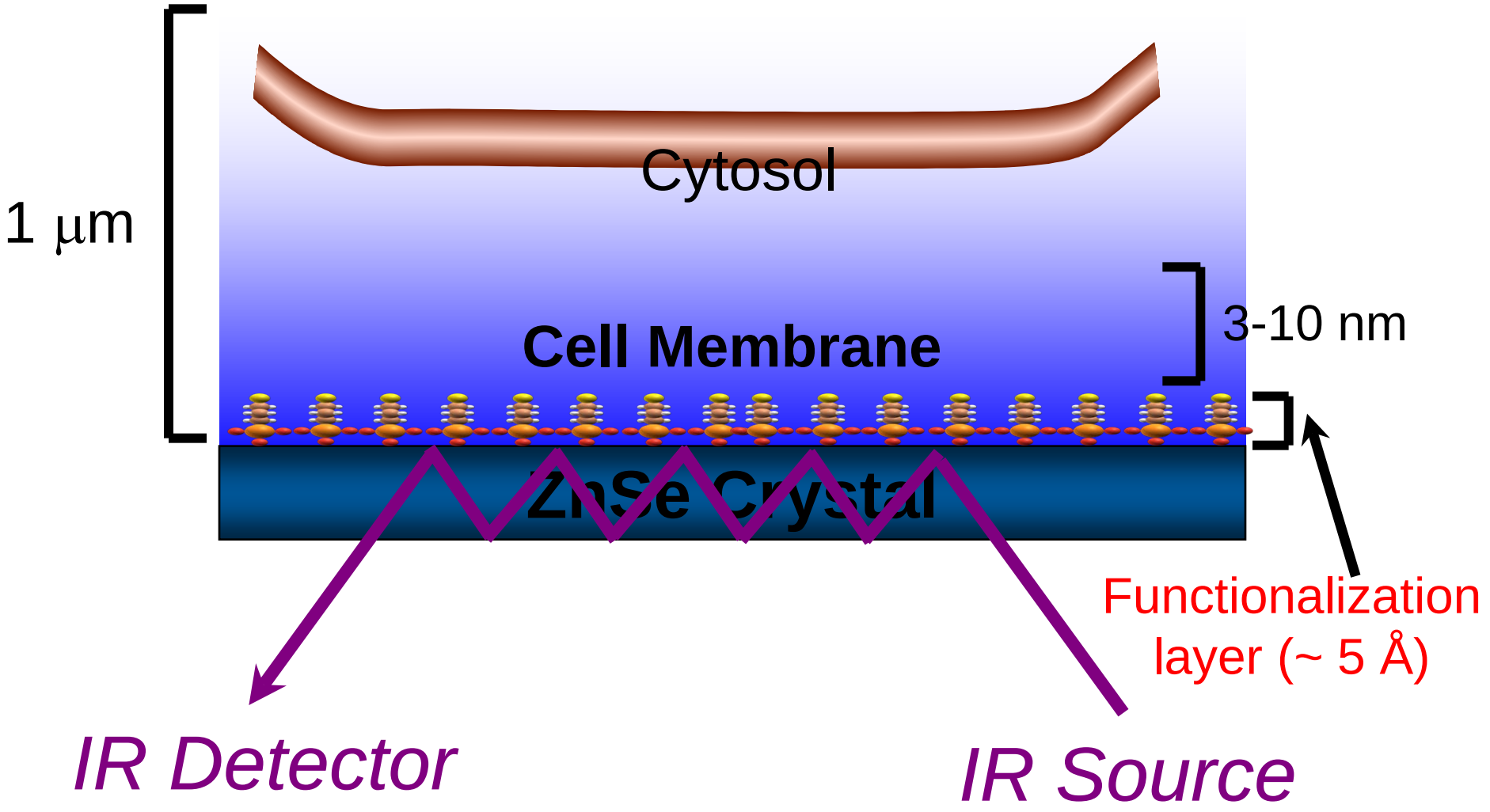
Heated plate





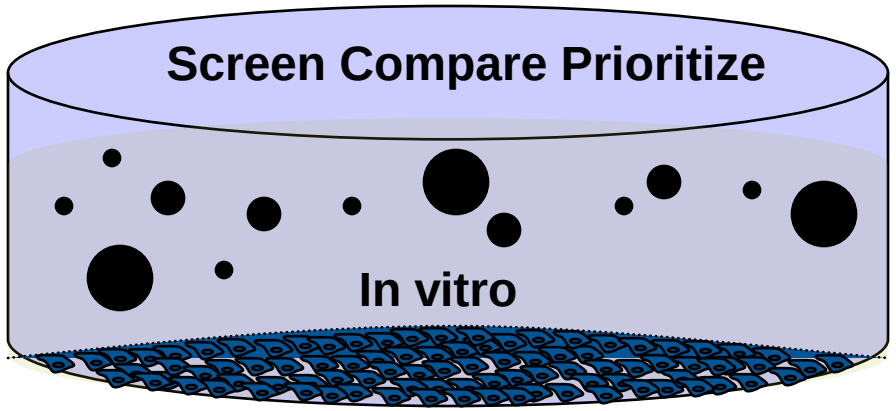
A Dedicated FTIR Facility

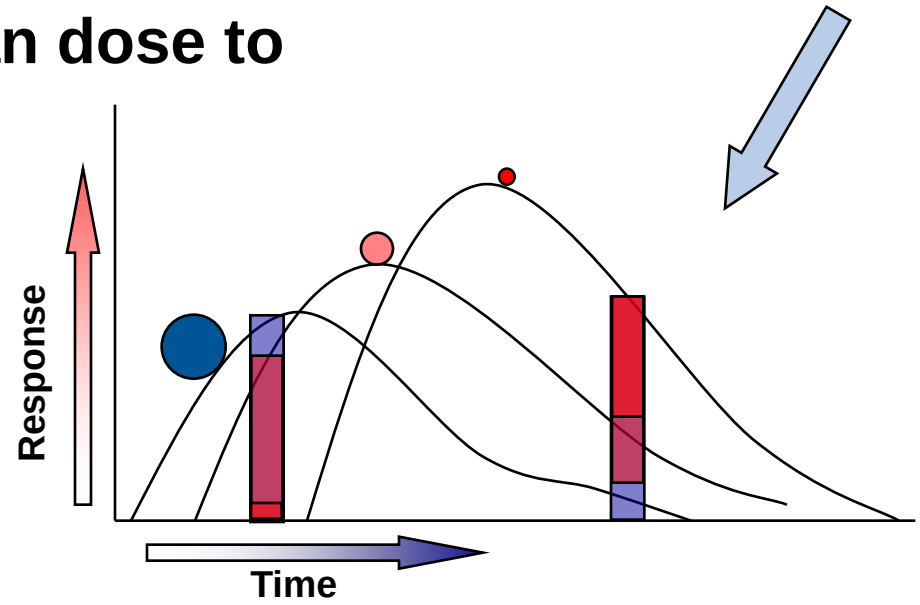
Problems in ATR Measurements: How to Improve?



Particokinetics - In Vitro

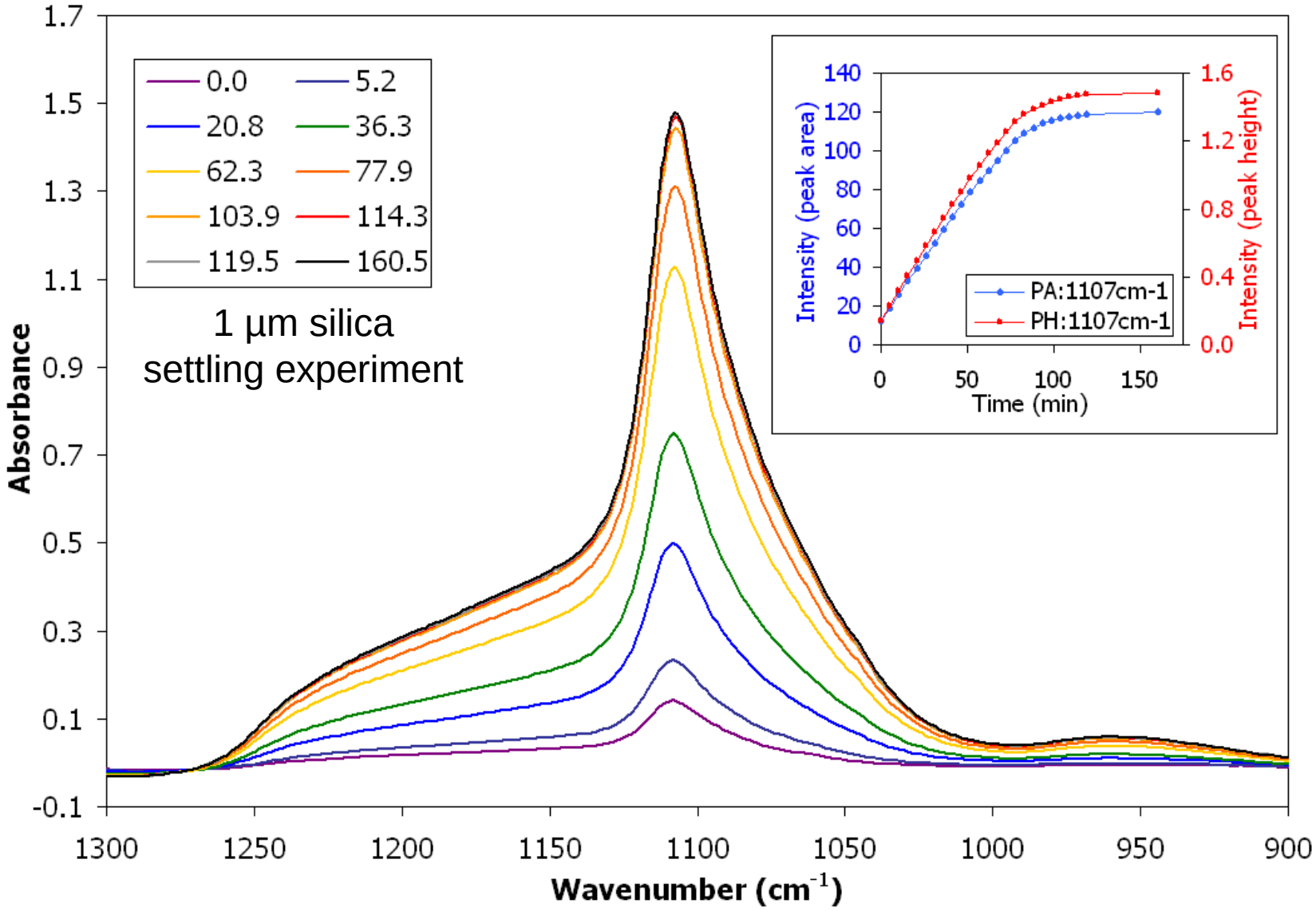
- **Shape, size, and density affect settling rate.**
- **Settling time impacts timing/magnitude of response**
- **Media "dose" is different than dose to the cell**



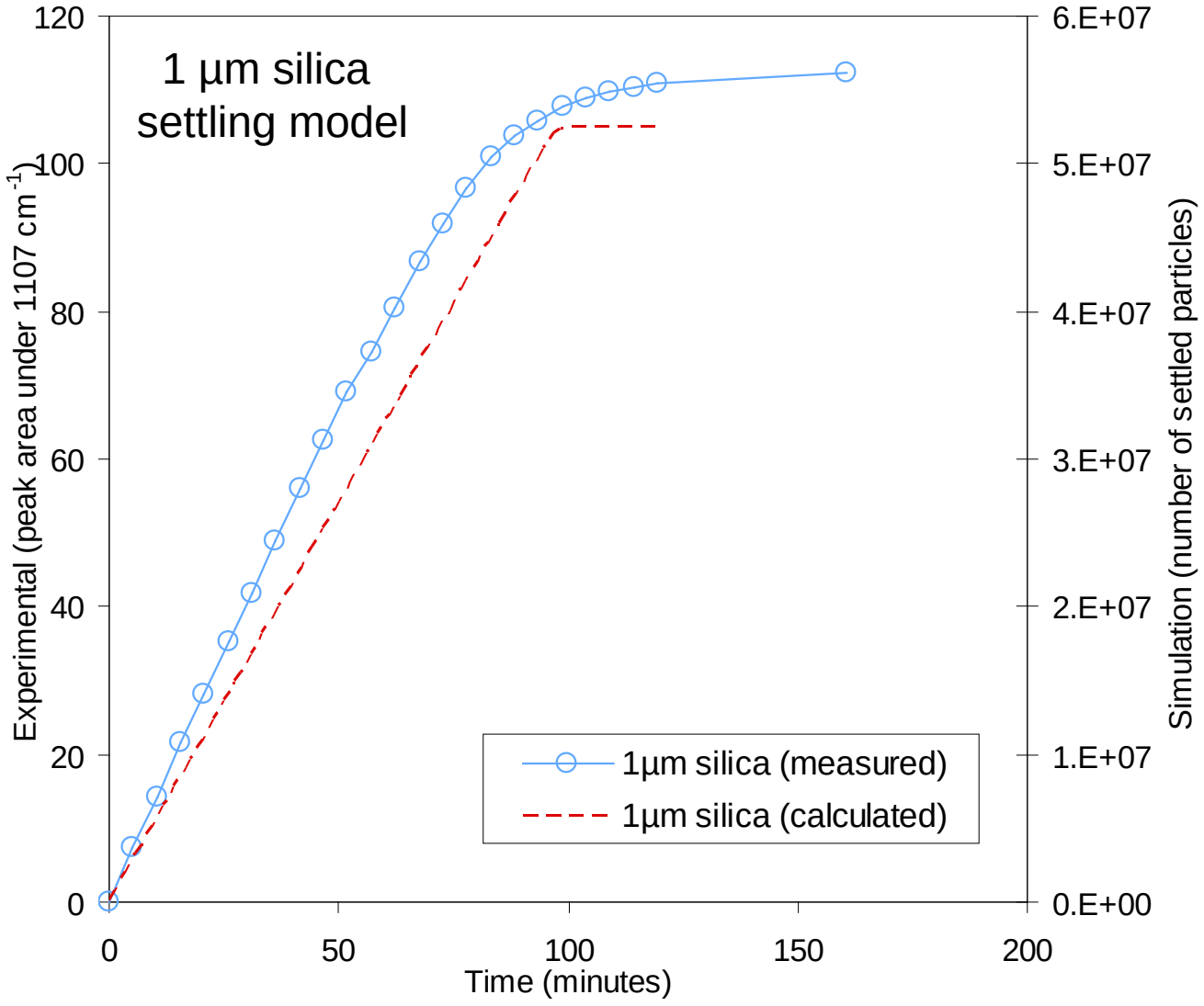


J. G. Teeguardian, P. M. Hinderliter, G. Orr, B. D. Thrall, and J. G. Pounds, "Particokinetics In Vitro: Dosimetry Considerations for In Vitro Nanoparticle Toxicity Assessments," *Toxicol. Sci.*, **95**, pp 300-312 (2007).

Particokinetics - Silica Particles



Particokinetics - Silica Particles (continued)



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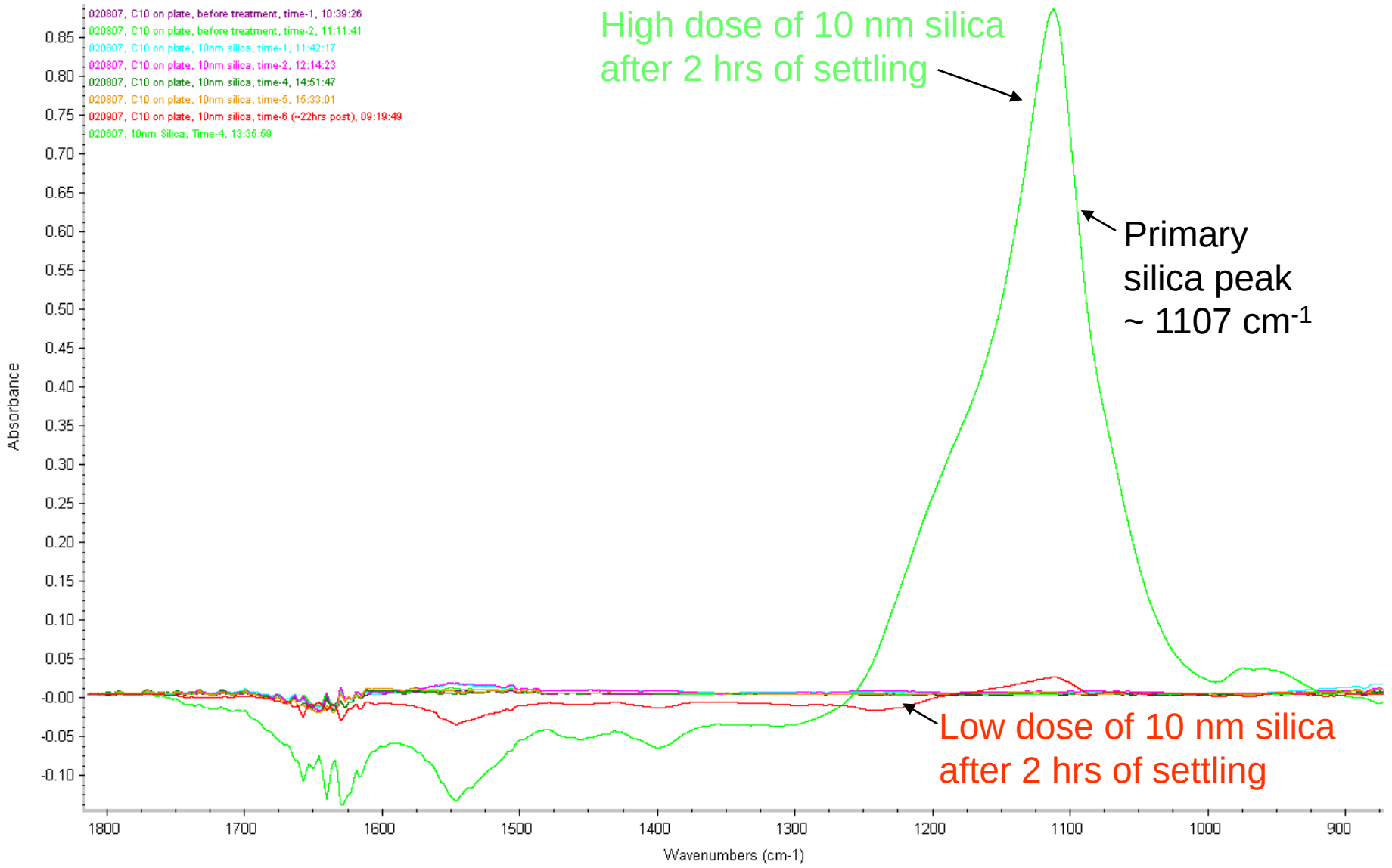
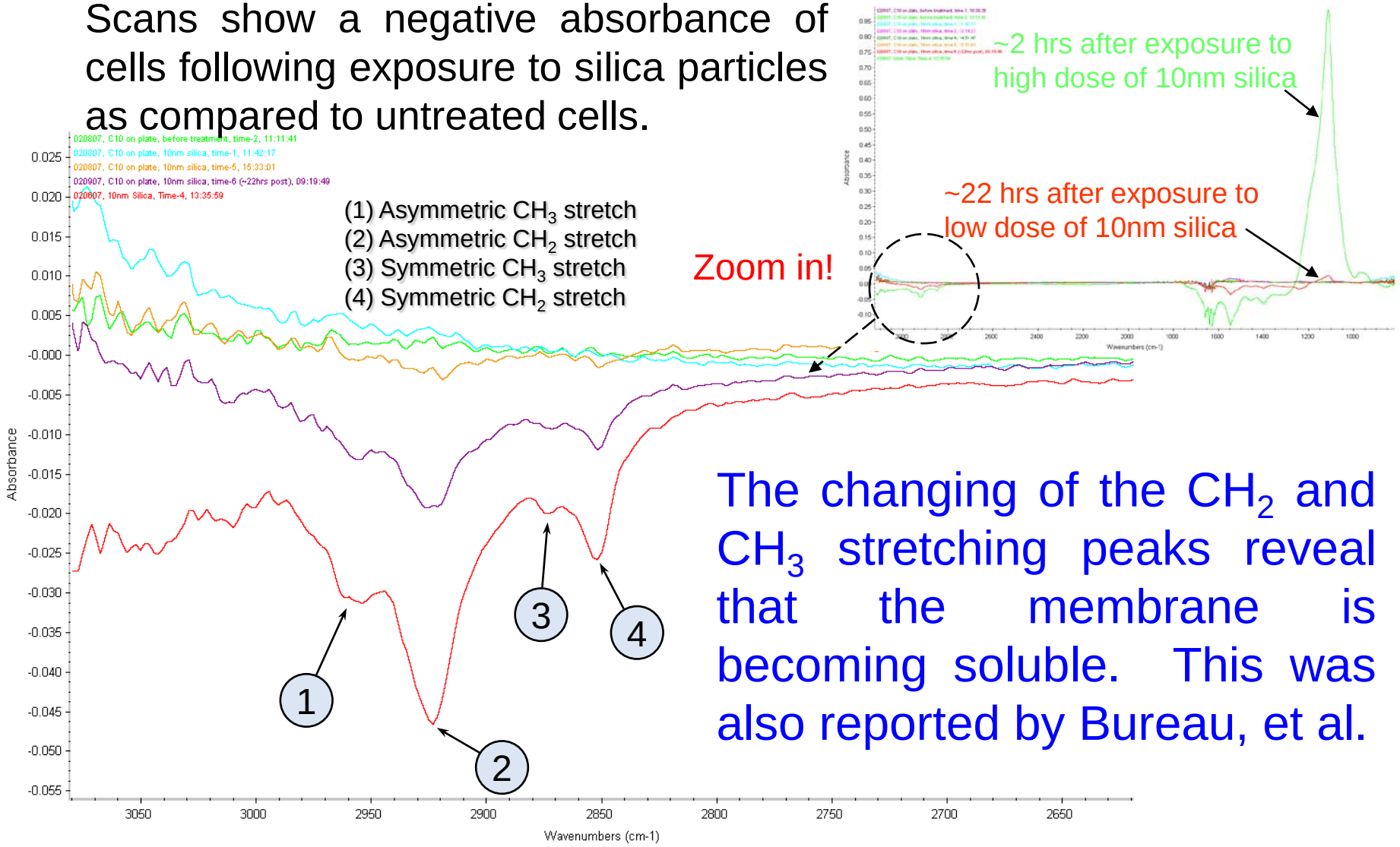
Nano Silica Particles Settling



Nano Silica Particles Settling

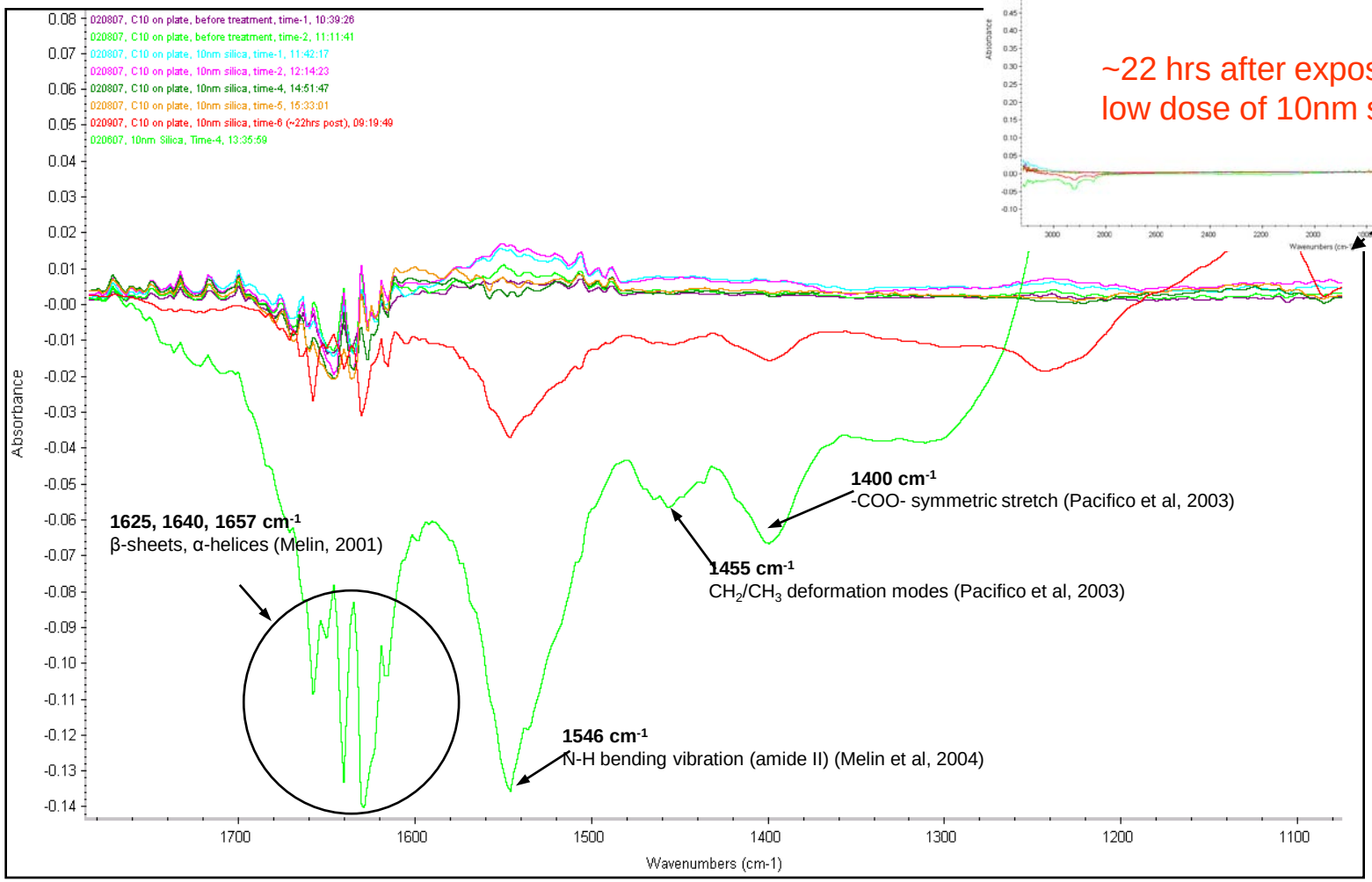
Scans show a negative absorbance of cells following exposure to silica particles as compared to untreated cells.

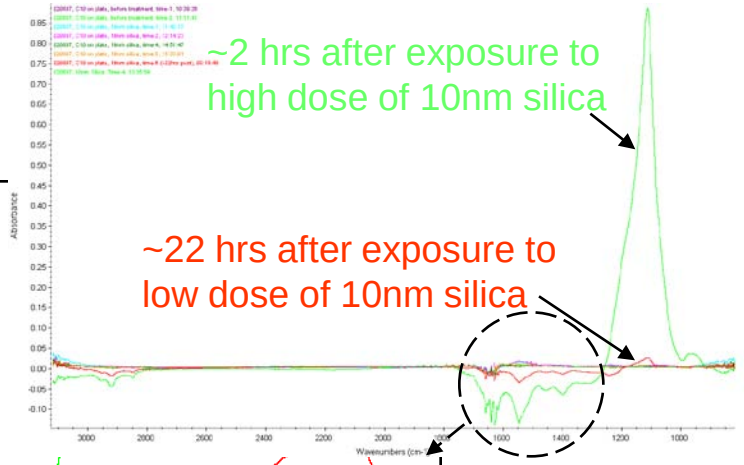


The changing of the CH_2 and CH_3 stretching peaks reveal that the membrane is becoming soluble. This was also reported by Bureau, et al.

B. Bureau, X. H. Zhang, F. Smektala, J.-L. Adam, J. Troles, H.-I. Ma, C. Boussard-Ple`del, J. Lucas, P. Lucas, D. Le Coq, M. R. Riley, J. H. Simmons, "Recent advances in chalcogenide glasses," *Journal of Non-Crystalline Solids*, **345&346**, pp 276-283 (2004).

Nano Silica Particles Settling



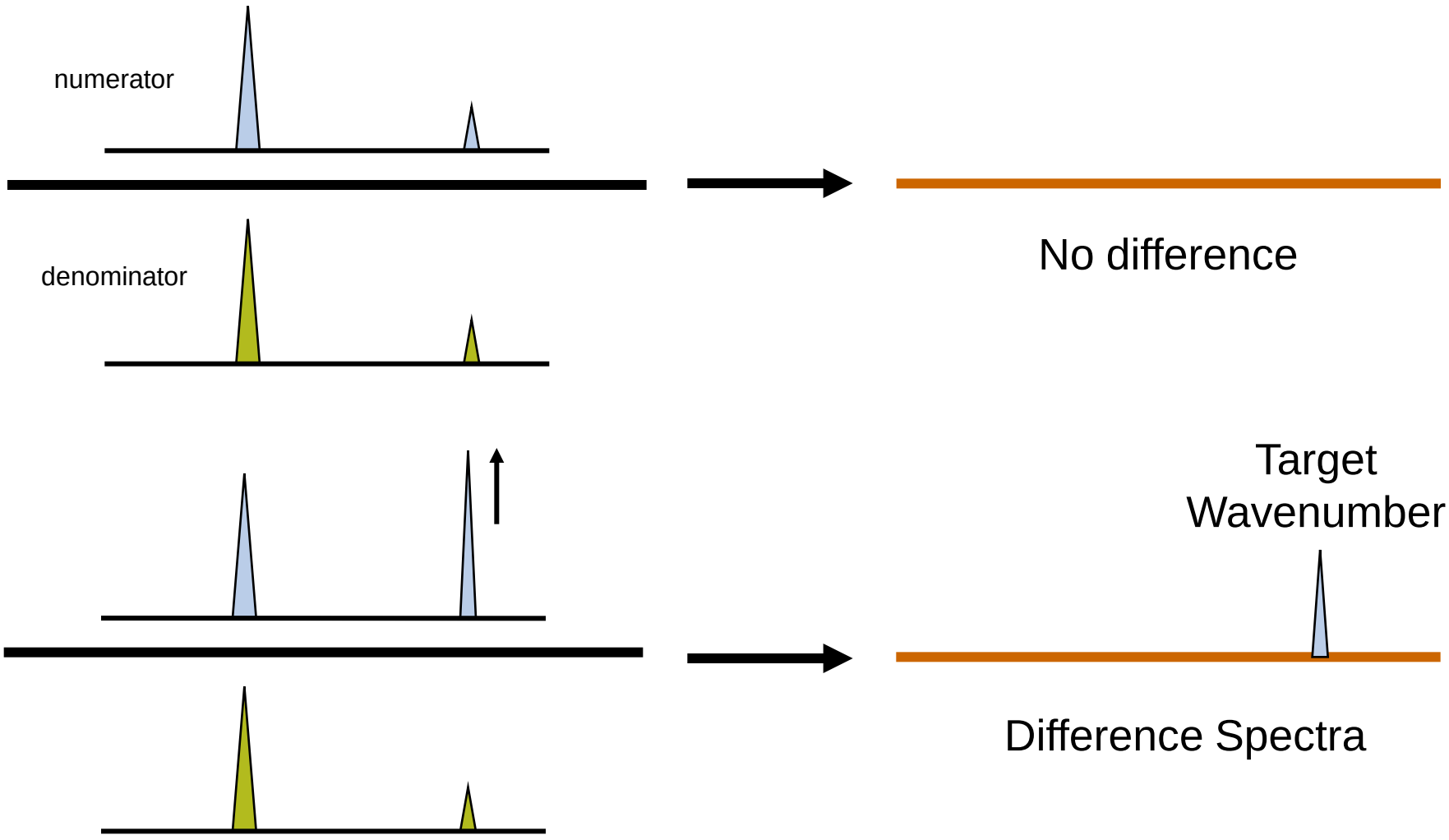


Zoom in!

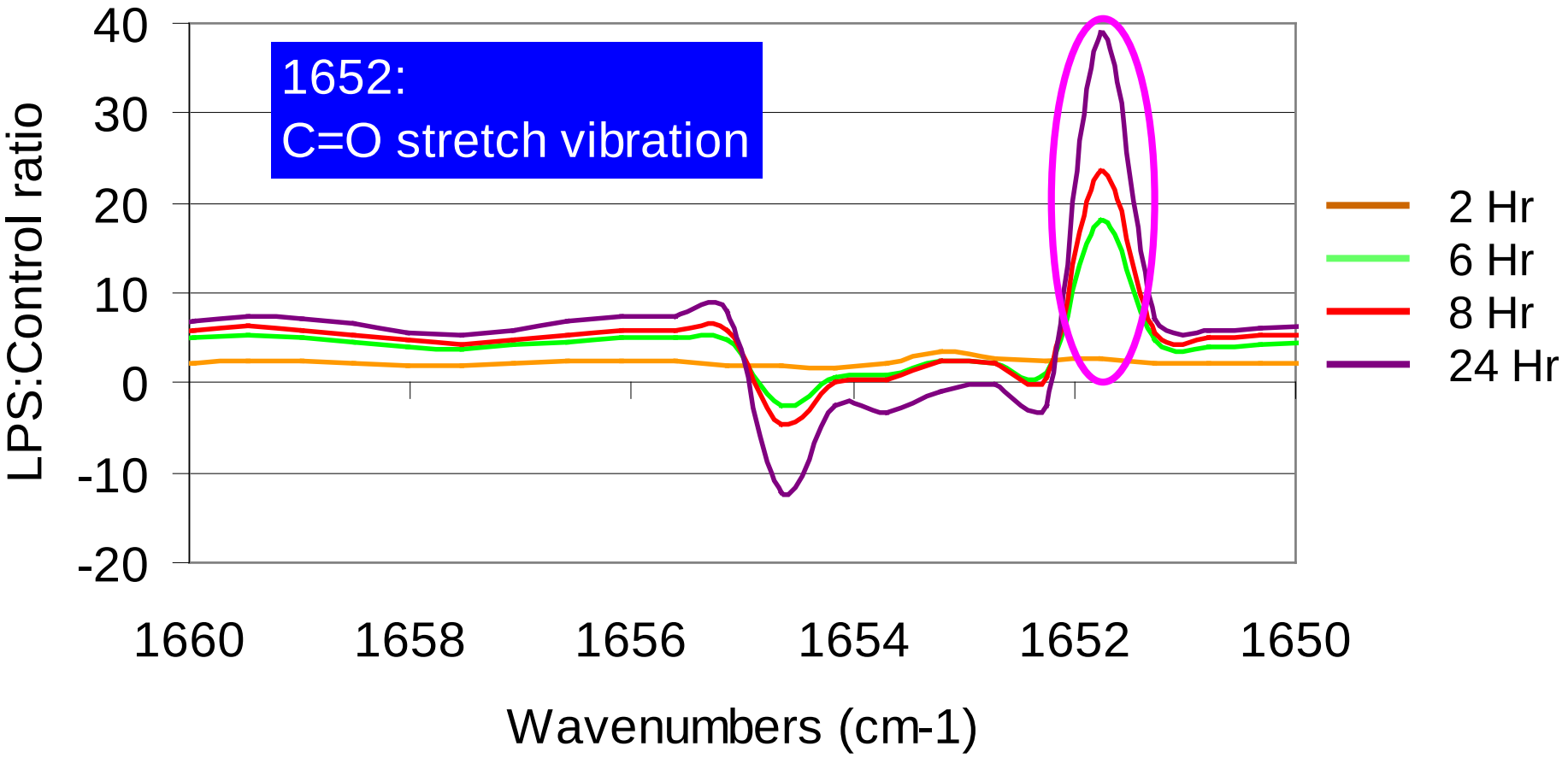
A. M. Melin, G. Perromat, G. Déléris. 2001. Effect of radical attach on bacteria: an application of FT-IR spectroscopy. *Applied Spectroscopy* **55**:23-28.
A. Pacifico, L. A. Chiriboga, P. Lasch, M. Diem. Infrared spectroscopy of cultured cells: II. Spectra of exponentially growing, serum-deprived and confluent cells. *Vibrational Spectroscopy*. 2003, **32**, 107-122.
A. M. Melin, A. Allery, A. Perromat, C. Bébéar, G. Déléris, B. Barbeyrac. Fourier transform infrared spectroscopy as a new tool for characterization of mollicutes. *Journal of Microbiological Methods*. 2004, **56**, 73-82.

Can We Detect Induced Biological Activity In Live Cells By FTIR?

Ratio Spectra To Identify Differences



Temporal Changes in RAW 264.7 Cells Treated With LPS



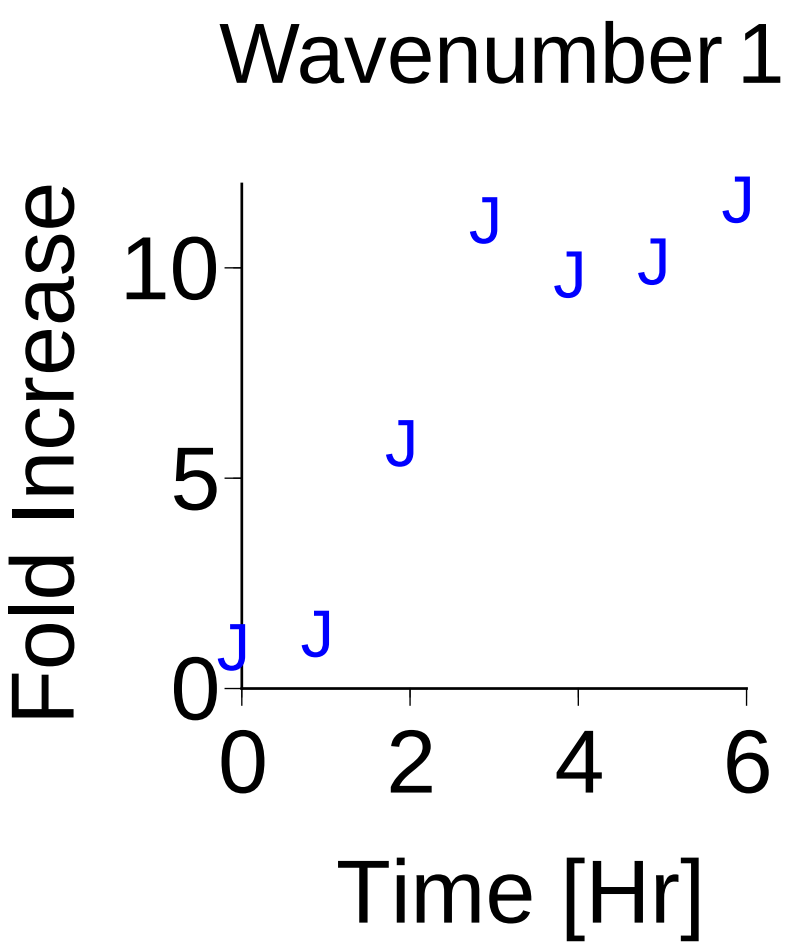
Macrophage Activation

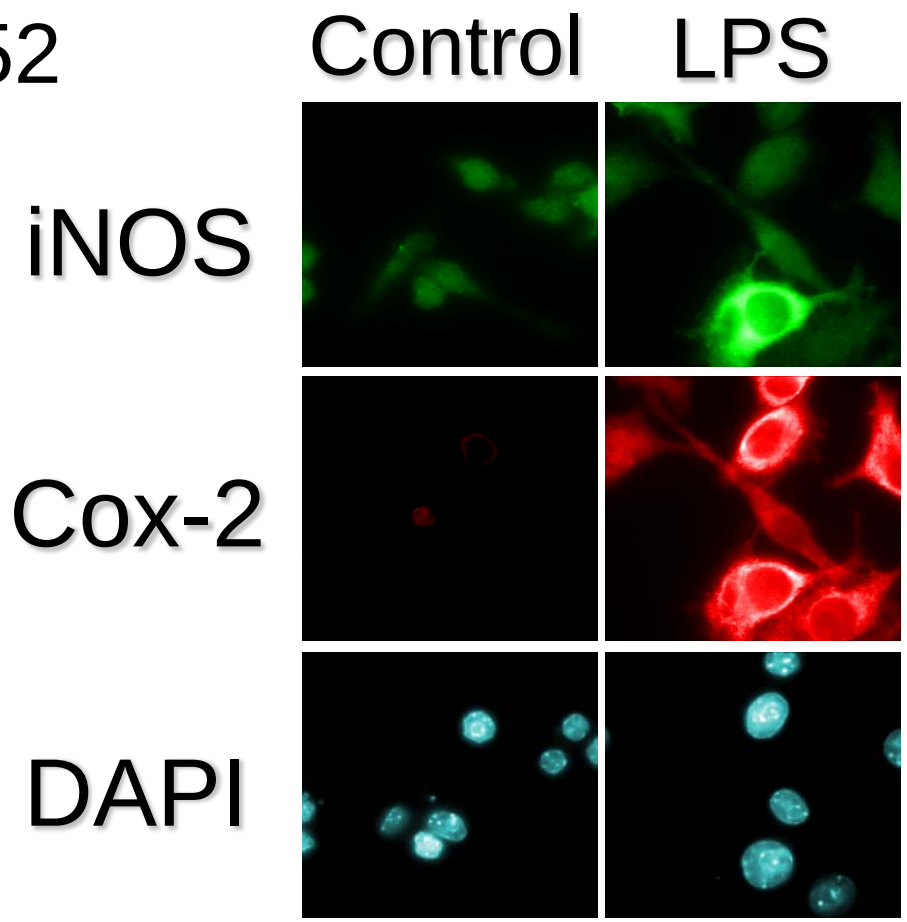
- Wavenumber 1652 frequently modulated by endotoxin treatment
  - C=O
- Endotoxin induces an inflammatory response in macrophages
  - reactive oxygen and nitrogen species (oxidative/ nitrative stresses)
    - Cox-2
    - iNOS
- Oxygen free radical damage to proteins results in protein carbonyl formation
  - C=O

Hypothesis

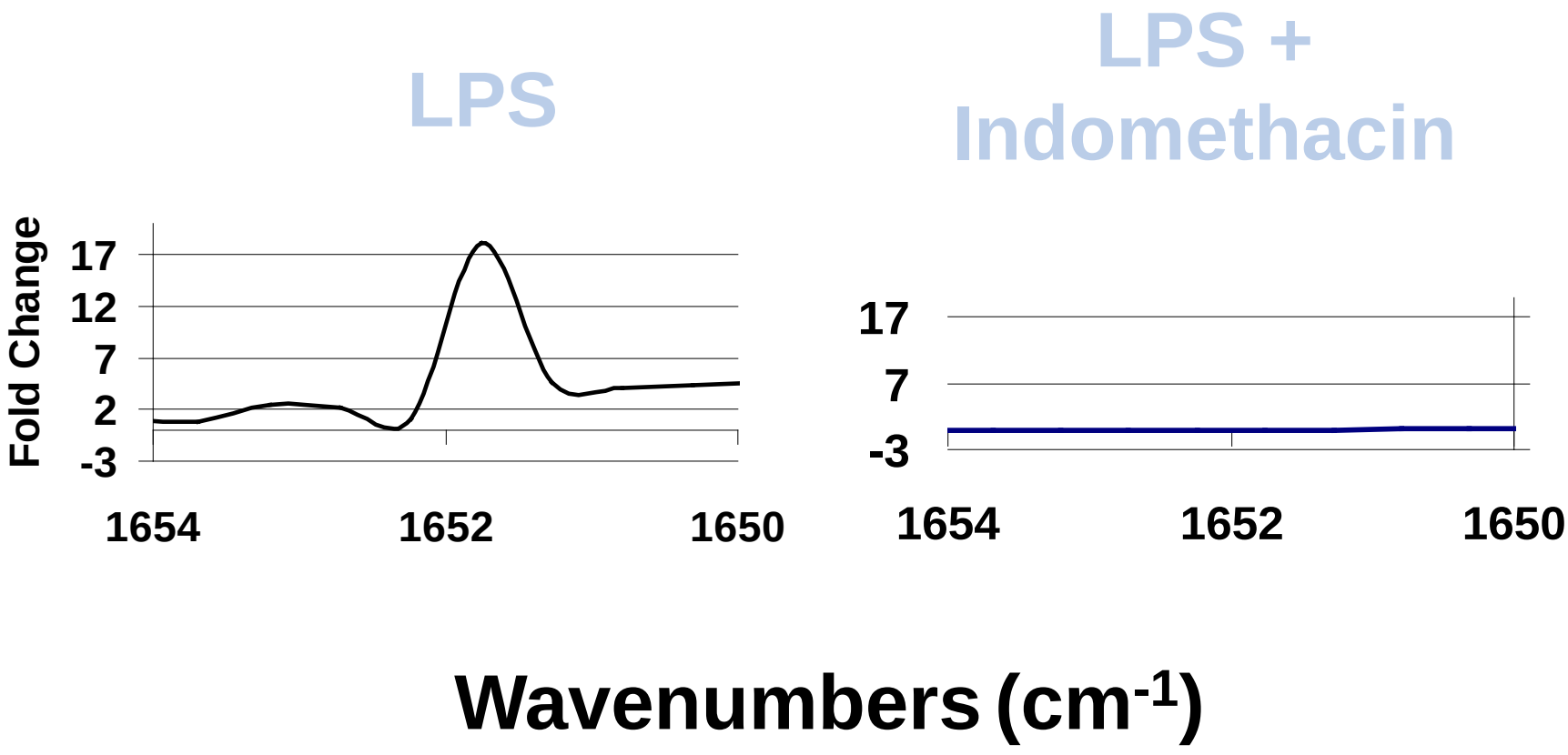
Inflammatory processes can be detected in live cells by FTIR.

Wavenumber 1652 cm^{-1} Temporal Profile Following LPS-Treatment

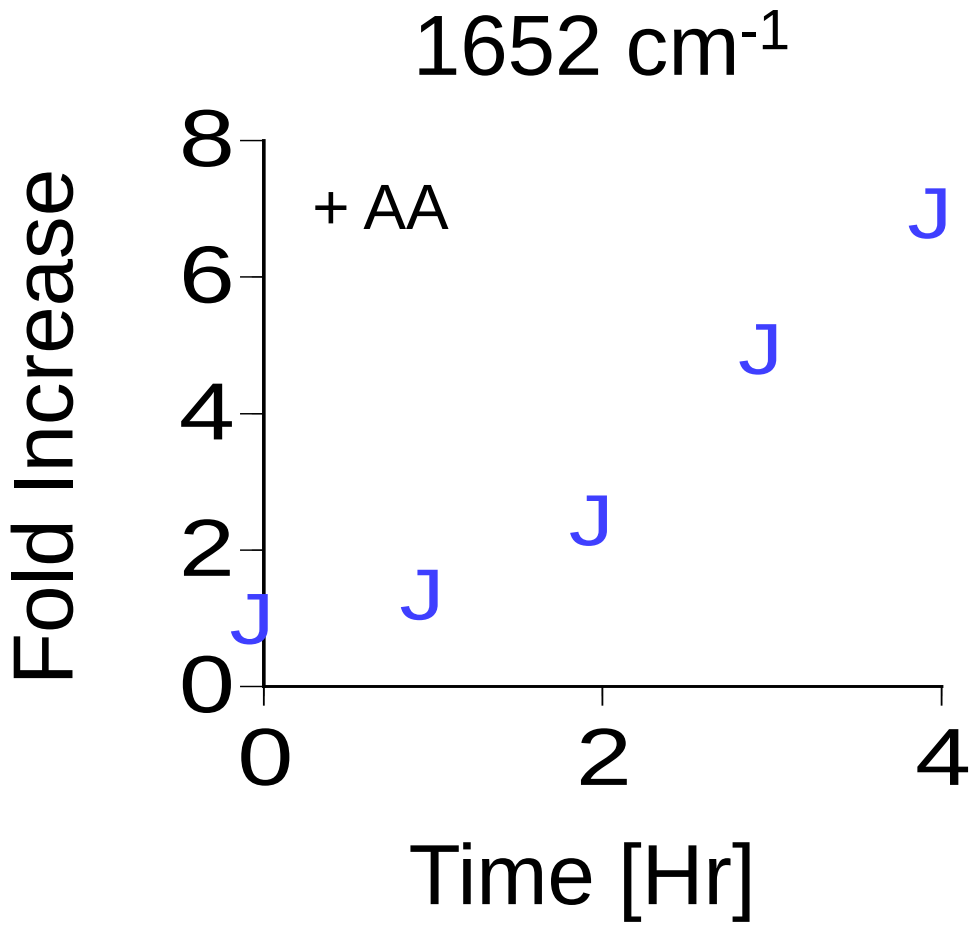




Indomethacin Effects on Peak 1652 cm^{-1}



Cox-2 Transfected Cells Treated With Arachidonic Acid

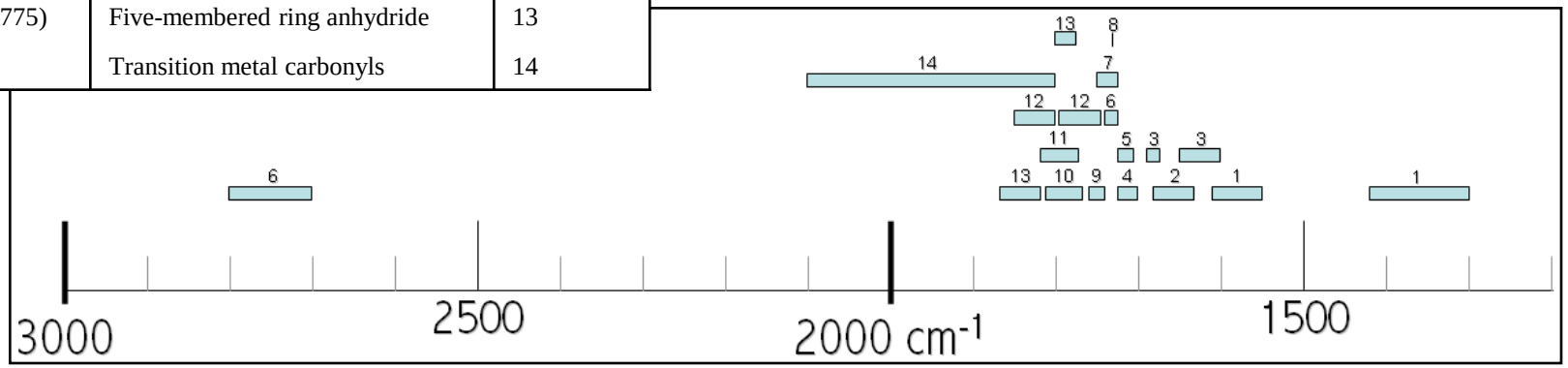


Carbonyl bond frequencies found in different functional groups

Group Frequency (cm^{-1})	Functional Group	Figure Reference
1610-1550 / 1420-1300	Carboxylate (carboxylic acid salt)	1
1680-1630	**Amide**	**2**
1690-1675/(1650-1600)[a]	Quinone or conjugated ketone	3
1725-1700	Carboxylic acid	4
1725-1705	Ketone	5
1740-1725/(2800-2700)[b]	Aldehyde	6
1750-1725	Ester	7
1735	Six-membered ring lactone	8
1760-1740	Alkyl carbonate	9
1815-1770	Acid (acyl) halide	10
1820-1775	Aryl carbonate	11
1850-1800/(1790-1740)	Open-chain acid anhydride	12
1870-1820/(1800-1775)	Five-membered ring anhydride	13
2100-1800	Transition metal carbonyls	14

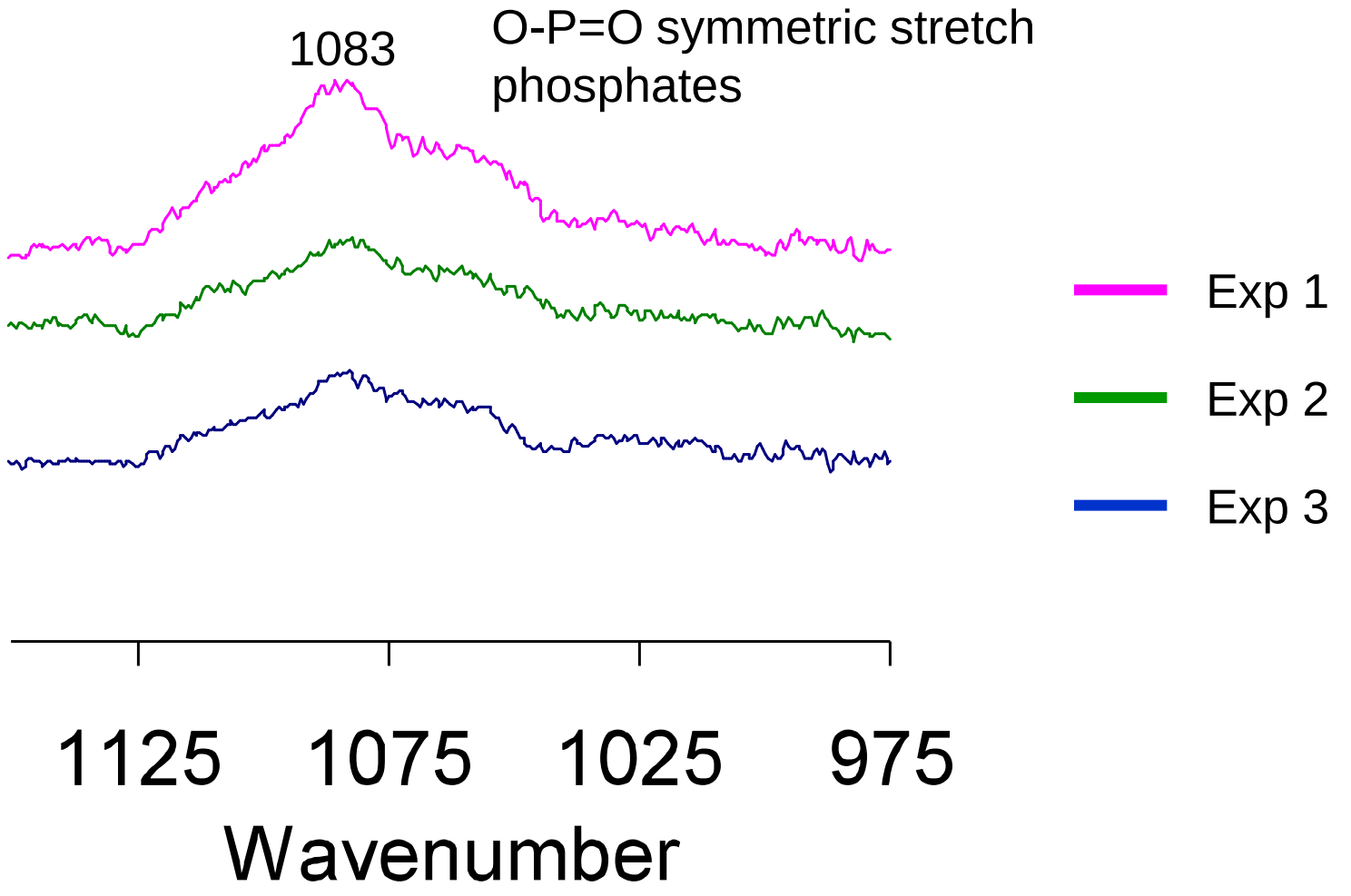
[a] Lower frequency band is from the conjugated double bond

[b] Higher frequency band characteristic of aldehydes, associated with the terminal aldehydic C-H stretch

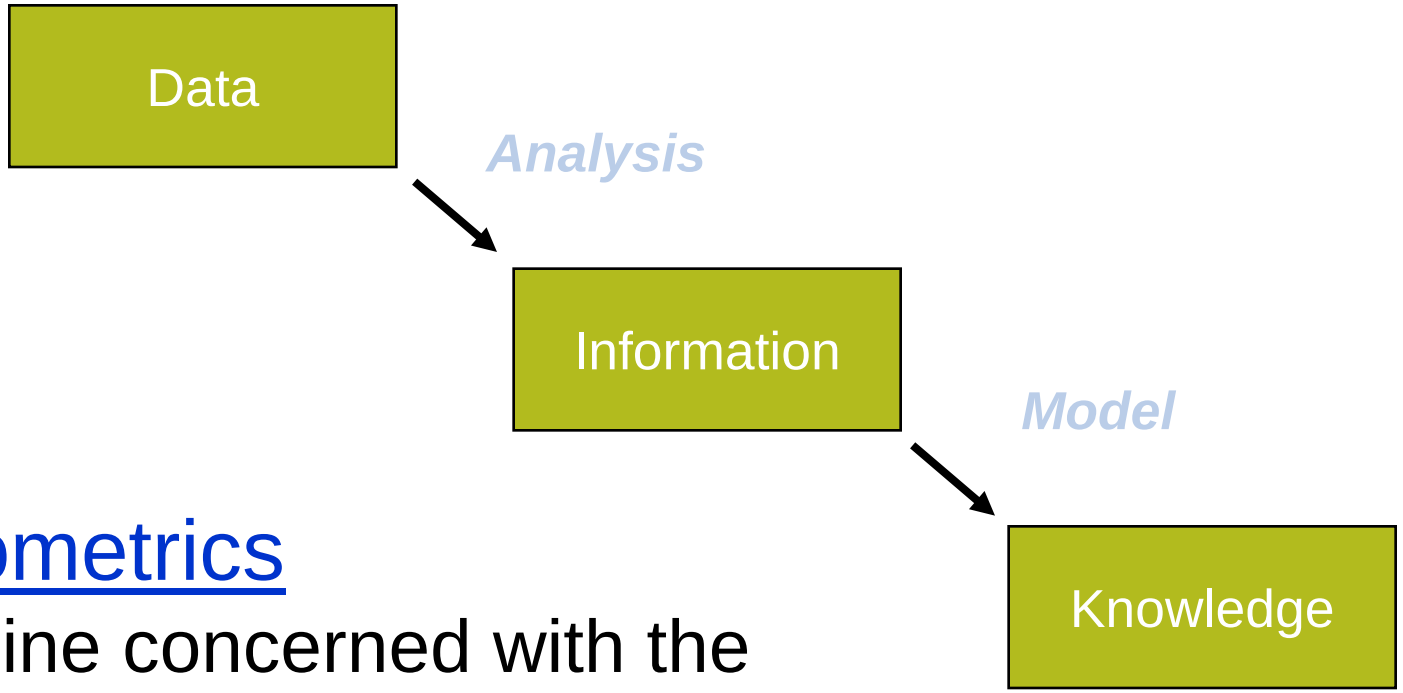


REFERENCE: J. Coates. Interpretation of infrared spectra, a practical approach in *Encyclopedia of Analytical Chemistry*. R. A. Meyers (Ed.), pp. 10815-10837. Copyright © John Wiley & Sons Ltd, Chichester. 2000.

MWCNT-Treated RAW 264.7 Cells



Transformation and Analysis



Chemometrics

- Discipline concerned with the application of statistics and mathmatical methods of chemistry
- Complex, interrelated data sets

Qualitative vs. Quantitative

- Mostly qualitative or semi-quantitative
- Quantitative analysis
  - Water absorption
  - Base-line shift
  - Temperature control
  - Atmosphere
- Chemometrics
- Mechanisms
- Integration for rapid screening

Summary

- Live cell FTIR spectroscopy is a promising tool to identify biologically active nanomaterials
  - Intuitive temporal patterns of regulation
  - Changes are complex
    - Complementary to genomic and proteomic approaches
- Functionalization monolayer chemistries support cell attachment and proliferation
  - Goal is to identify chemistry that is biologically inert or non-perturbing to improve ATR spectroscopy in live cell experiments
- Particochembiokinetics
  - Can be studied in near real-time
- Development of pathway- or process-specific spectral profiles is needed to enable spectra interrogation
  - Cell cycle, Cell death, Target pathways, Toxicants with well defined modes of action, etc.

Acknowledgements

- Cheryl Cejka for support under Technology Maturation program
- Joel G. Pounds, Ellyn Murphy, Environmental Biomarker Initiative (EBI)
  - Support of LDRD project
- Pacific Northwest National Laboratory (PNNL) is a multiprogram national laboratory operated by Battelle Memorial Institute for the United States Department of Energy under DE-AC06-76RLO 1830.