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Standard Form 298 (Rev. 8-98)
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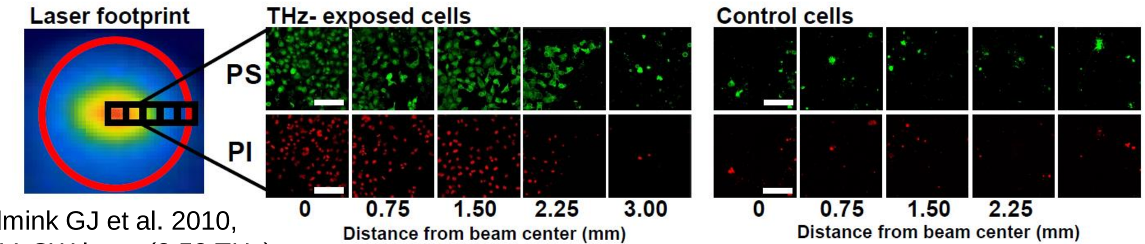
LEAD | DISCOVER | DEVELOP | DELIVER

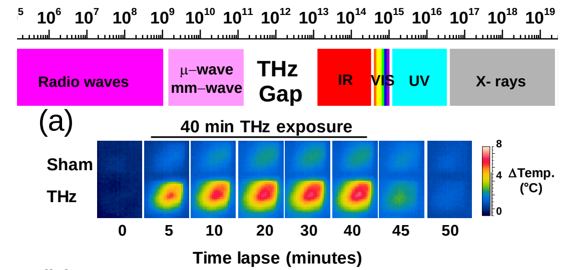
Validating the Existence of Quantum Mechanical Coherence in Cells (Fröhlich Theory)

Ibtissam Echchgadda, Ph. D.
Radio Frequency Bioeffects Branch
Bioeffects Division
711th Human Performance Wing

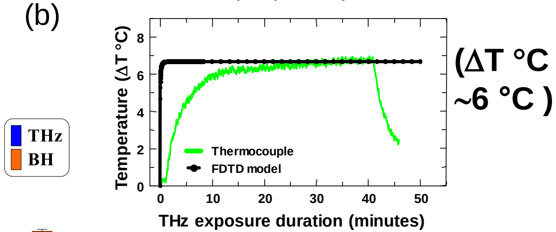
2.25 Terahertz (THz) Energy Influences Gene Expression
[THz vs. Bulk Heating (BH)]

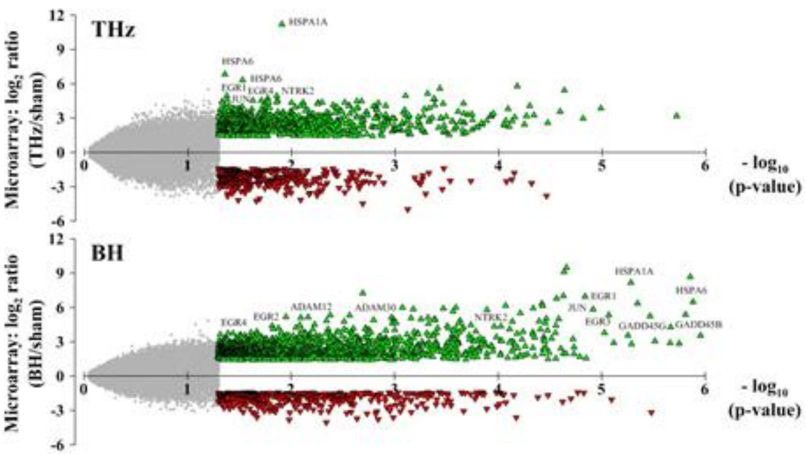
Cellular stress response, and cellular death

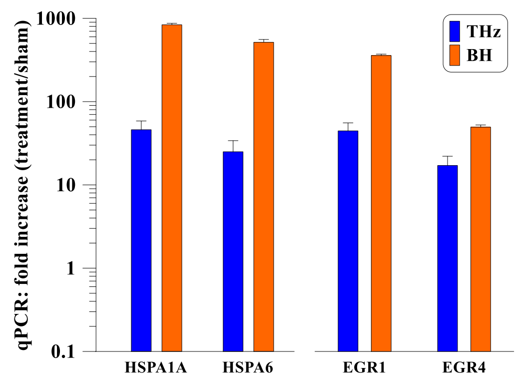


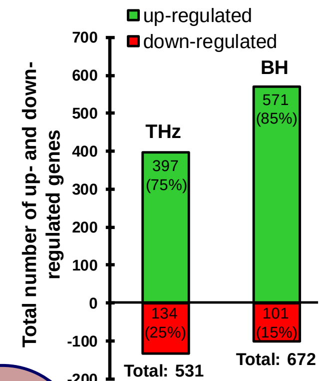


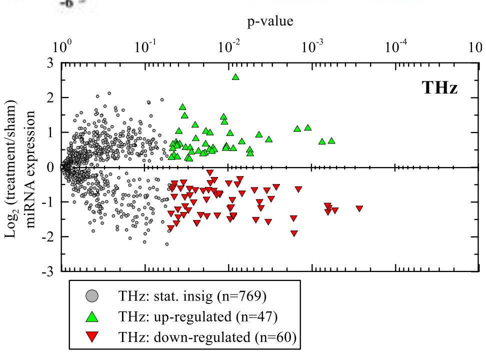
Wilmink GJ et al. 2010, 2011 CW laser (2.52 THz)

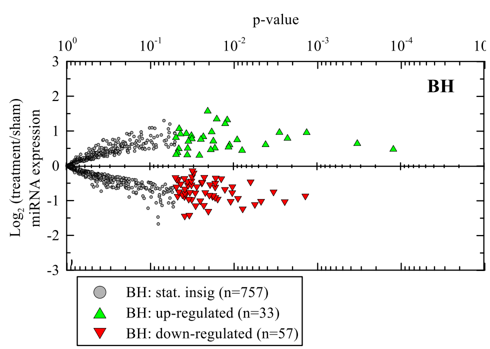


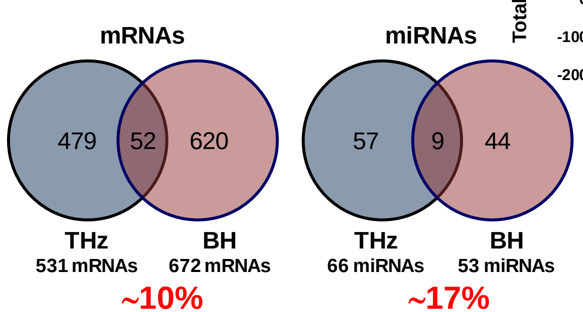








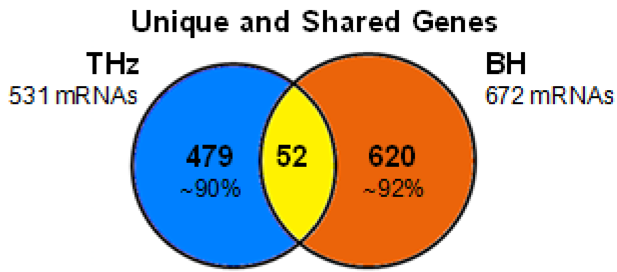




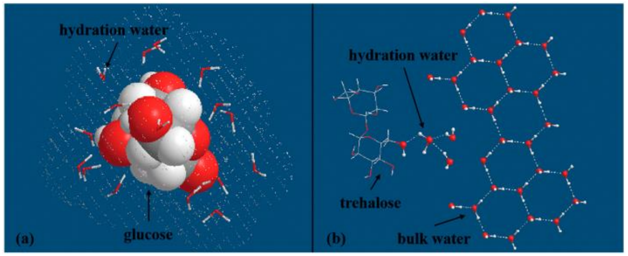
Echchgadda I. et al. IEEE Transactions on Terahertz Science and Technology, 2016

Echchgadda I. et al., IRMMW-THz). IEEE, 2014

2.52 THz Energy Stimulate Specific Signaling Pathways in Human Cells



nt. J. Mol. Sci. **2015**, *16*(4), 8454-8489



- Bulk H_2O

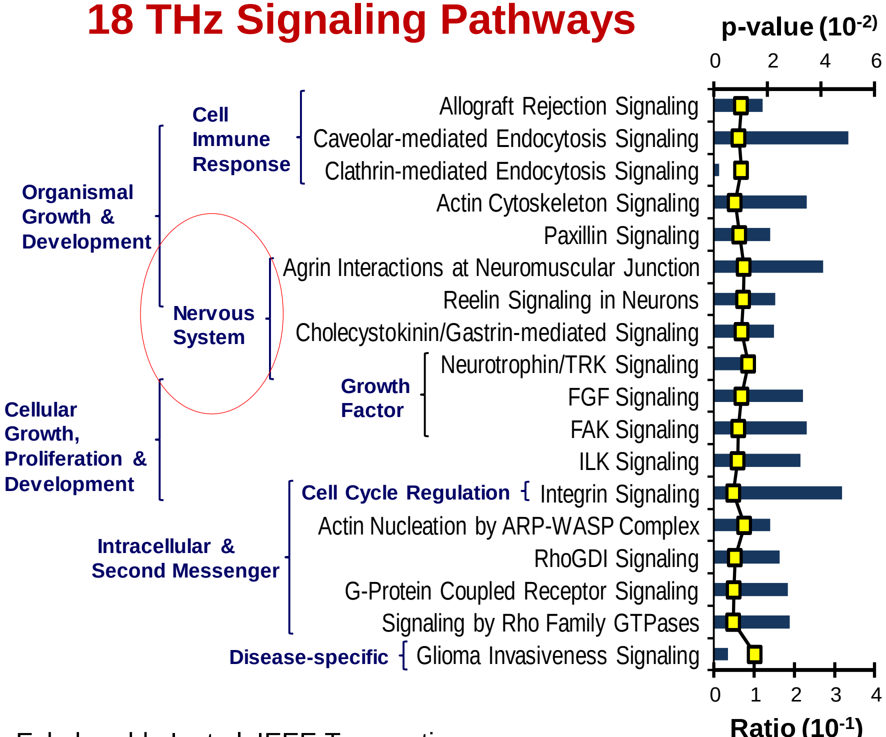
HB bend 1.5 THz

HB stretch vibration 5-6 THz

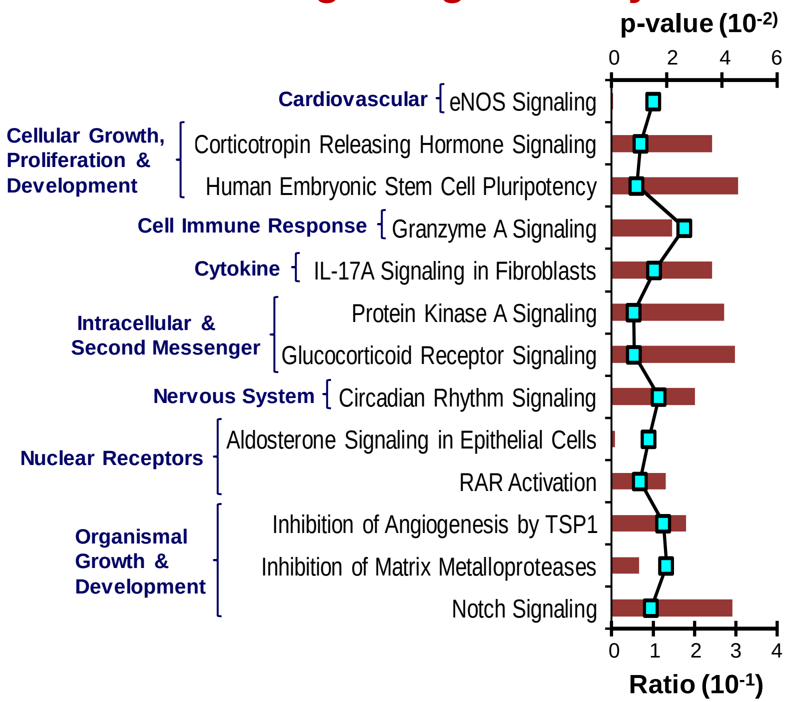
- Solvation H_2O

2.3-2.8 THz

18 THz Signaling Pathways



13 BH Signaling Pathways

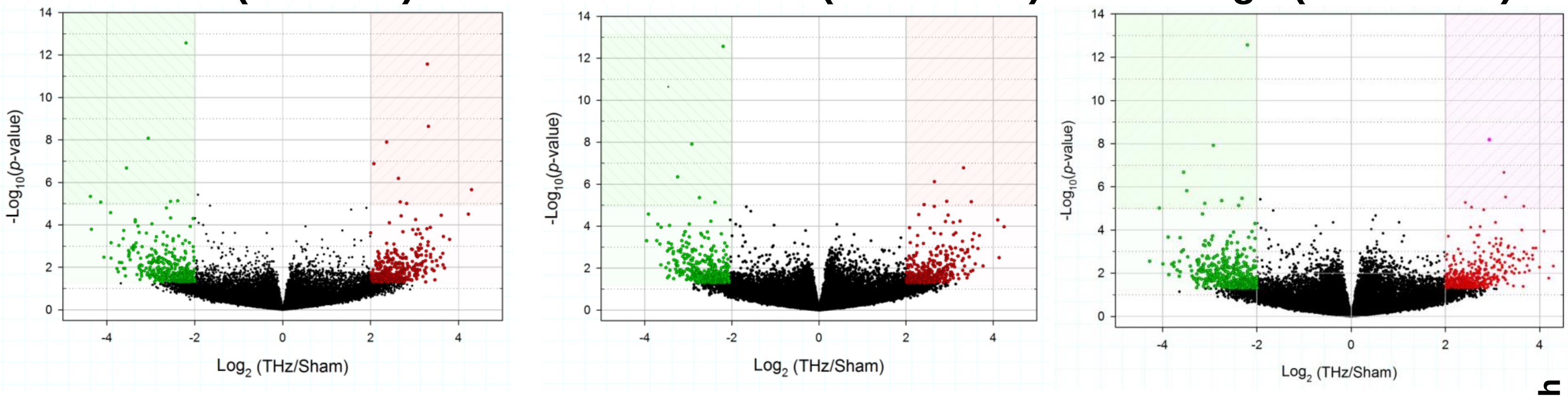


Echchgadda I. et al. IEEE Transactions on Terahertz Science and Technology, 2016

2.25 THz Energy Regulates Gene Expression
[THz at Different Intensities]

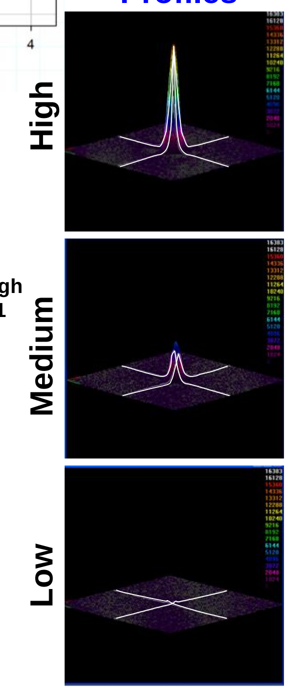
- **mRNAs Microarray**

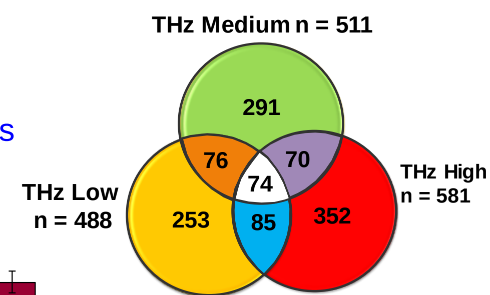
Low (no heat) | **Medium (ΔT °C~1°C)** | **High (ΔT °C~4°C)**



Output Power:
- 50.0 mW
- 22.0 mW
- 5.0 mW

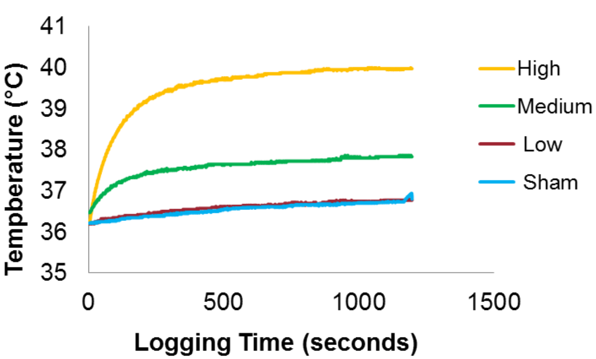
Beam Profiles



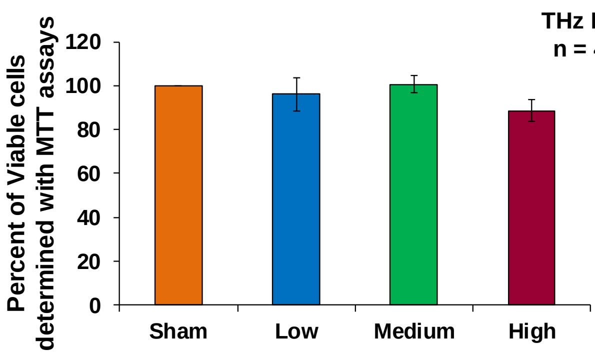


Shared and unique genes

Temperature profile during THz exposures



Cells survival post exposures

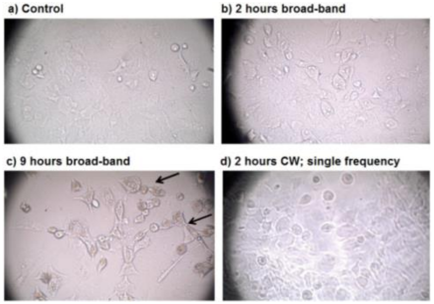


Cerna CZ. et al., SPIE 2015

2.25 THz Energy Influence Cellular Processes in Various Ways

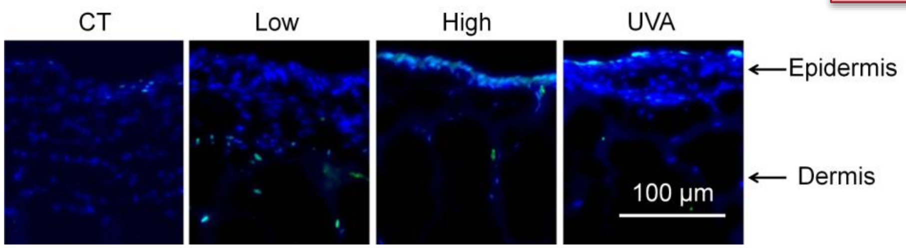
Ω=2.52THz
Pulsed broad-band or CW laser sources

Morphological changes in mouse stem cells after THz irradiations



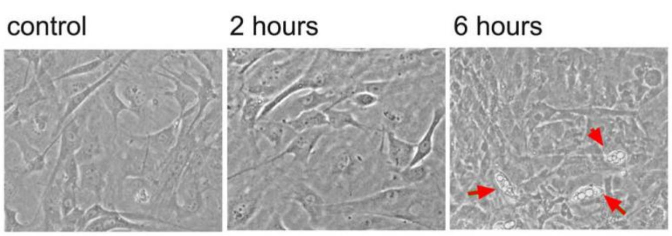
Alexandrov BS et al. 2011

THz pulses cause DNA damage and activate DNA damage repair



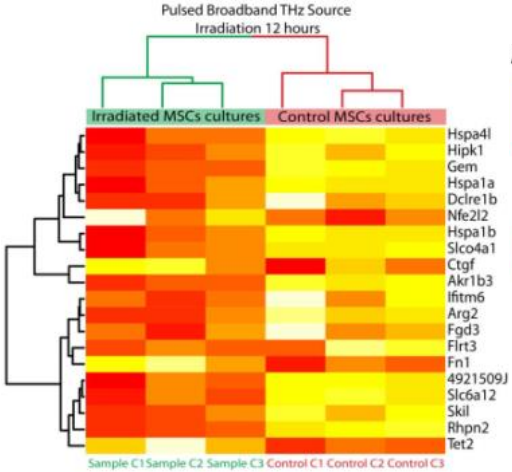
Human skin tissue models
Titova LV et al. 2013

Stem cells differentiation toward adipose phenotype

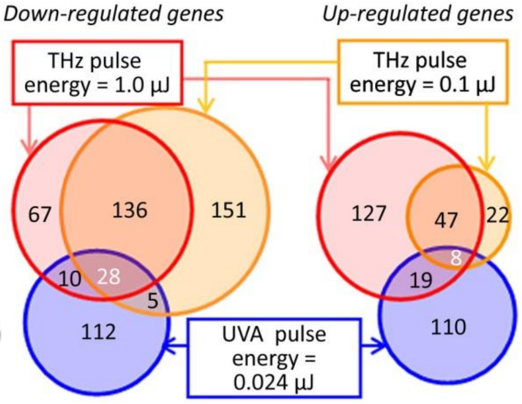


Bock J et al. 2013

Terahertz radiation effect gene expression



Alexandrov BS et al. 2013



Titova LV et al. 2015

Fröhlich Theory: Existence of Coherent Excitations in Biological Systems

Volume 26A, number 9 PHYSICS LETTERS 25 March 1968

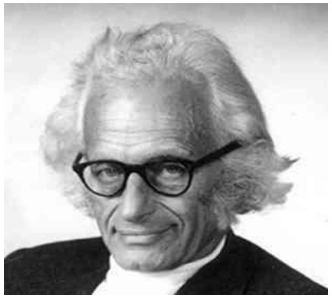
BOSE CONDENSATION OF STRONGLY EXCITED LONGITUDINAL ELECTRIC MODES

H. FRÖHLICH

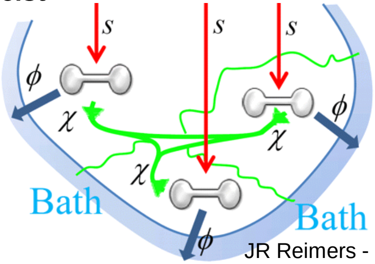
INTERNATIONAL JOURNAL OF QUANTUM CHEMISTRY, VOL. II, 641–649 (1968)

Long-Range Coherence and Energy Storage in Biological Systems

H. FRÖHLICH



Herbert Fröhlich, (1905–1991)—A Physicist



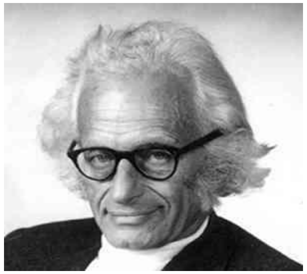
JR Reimers - 2009

- Vibrational modes within polar molecules can order and condensate in just one of the collective modes, the mode of a lowest-frequency.
- Such a condensation would have a profound influence on the organization and order in living systems
- Low-frequency collective vibrational modes of biomolecules (i.e., proteins and protein-composed structures) in the terahertz frequency range (0.1–10 THz), are expected to have a strong influence on their function
- Fröhlich's model provided a framework for cellular intra- and inter-interactions via EM fields

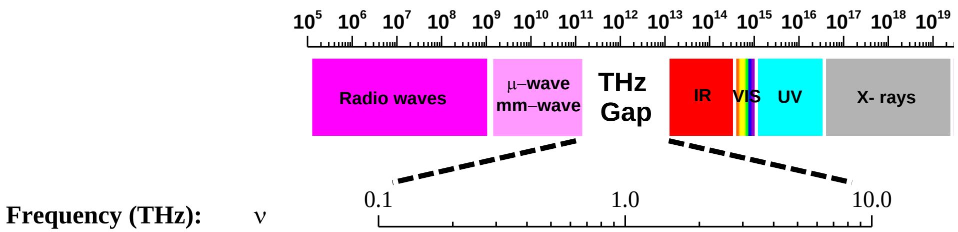
Three foundation stones

- ❑ Electrical polarity of bio-structures
- ❑ Spectral energy transfer between oscillating biomolecules
- ❑ Continuous energy supply from metabolic activity, which supports the molecular oscillations and generation of endogenous EM fields.

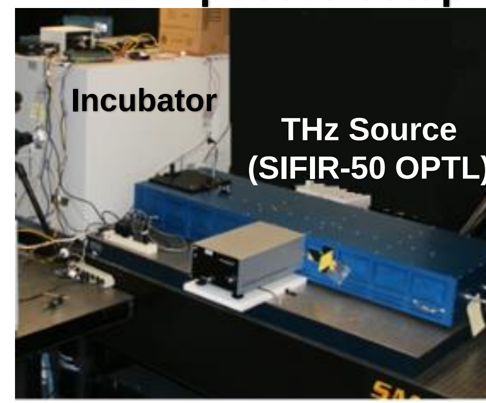
Overall Objective of The Study



Herbert Fröhlich



THz Exposure setup



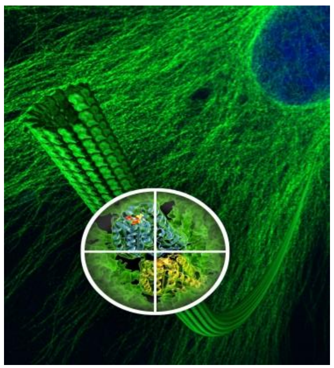
Empirically investigate the Fröhlich mechanism through interference with the intracellular oscillations, using an exogenous excitation with THz energy

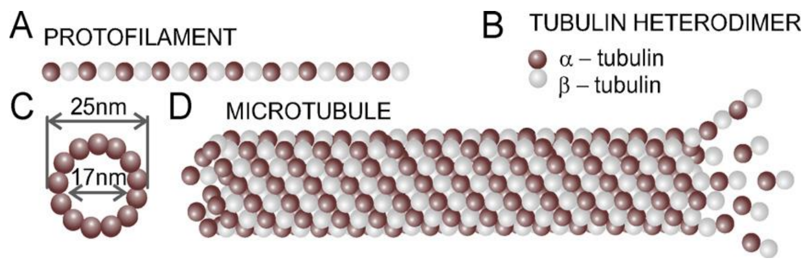
- *Would an external excitation at specific THz frequencies (or Fröhlich frequencies) interfere with the intrinsic oscillations of specific bio-structures and the endogenous EM fields they produce?*

- *Would these specific frequencies disturb the dynamic behavior and function of the target bio-structures?*

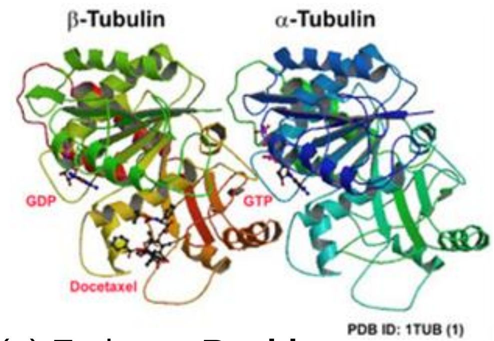
- *How would the overall intracellular organized states be affected?*

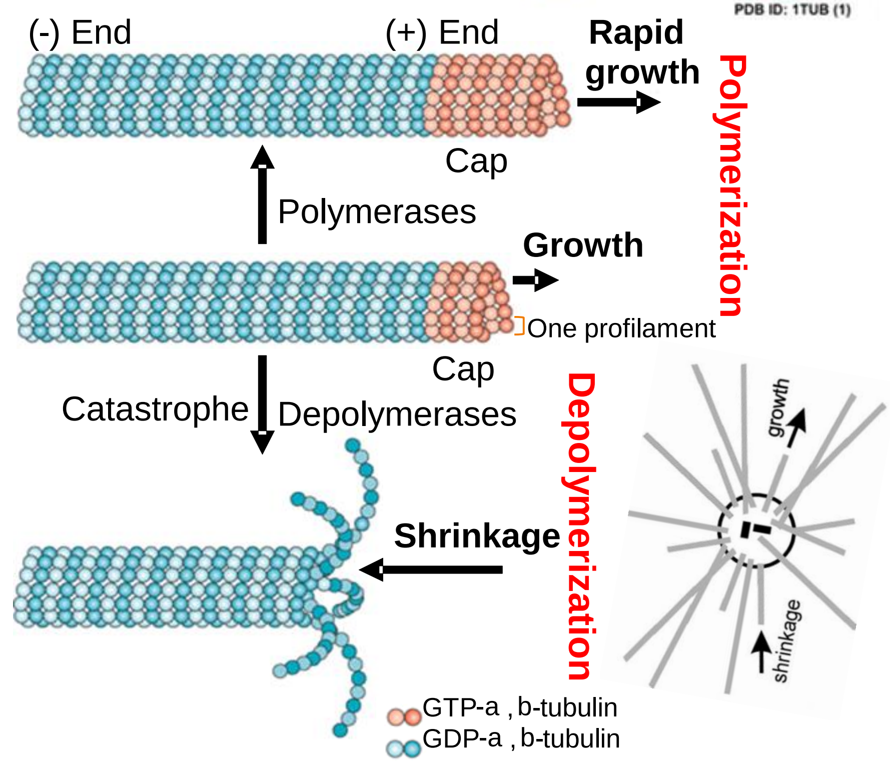
Microtubule Candidates for Fröhlich's Theory



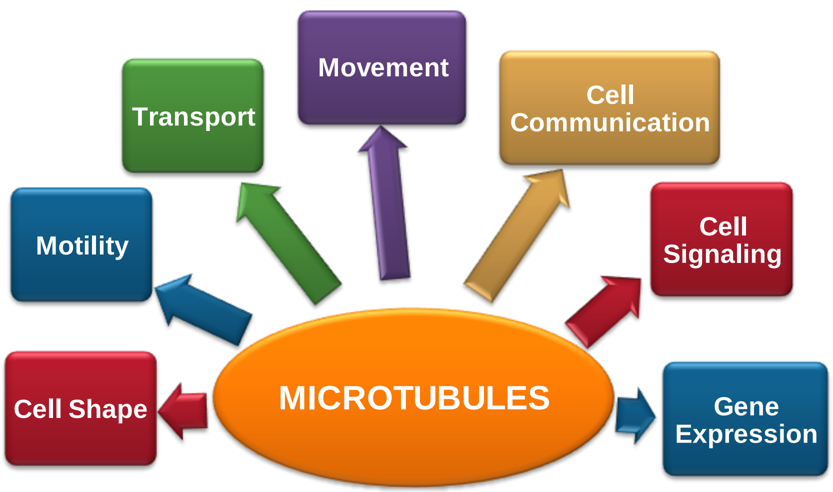


Kučera O. et al. 2012



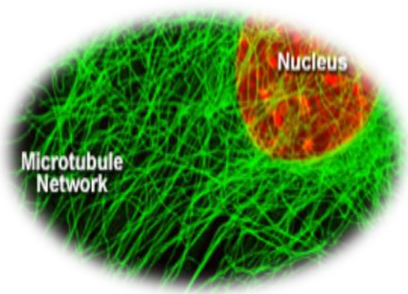


Adapted from Howard J. et al. 2009



Why Microtubules?

❑ Pokorný:



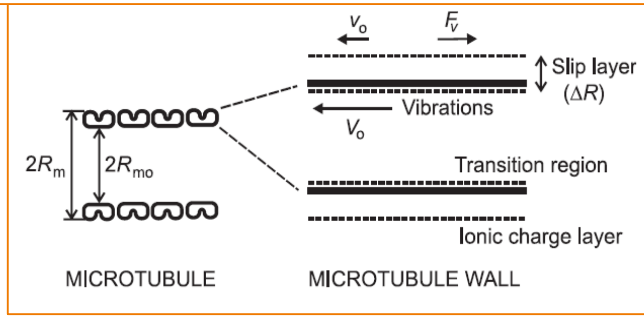
https://micro.magnet.fsu.edu/cells/microtubules/

MICROTUBULES
Protein-composed bio-structures

Astrophysics and Space Science **23:** 171–179, 1997.
© 1997 *Kluwer Academic Publishers. Printed in Belgium.*
Vibrations in Microtubules
J. POKORNÝ[1,2], F. JELÍNEK[2], V. TRKAL[2], I. LAMPRECHT[3] and R. HÖLZEL[3]

Bioelectrochemistry and Bioenergetics 45 (1998) 239–245
Electric field around microtubules
Jiří Pokorný *, František Jelínek, Viktor Trkal

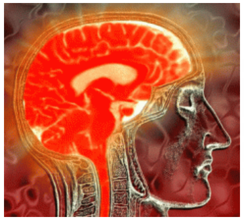
Bioelectrochemistry 63 (2004) 321–326
Excitation of vibrations in microtubules in living cells
J. Pokorný *



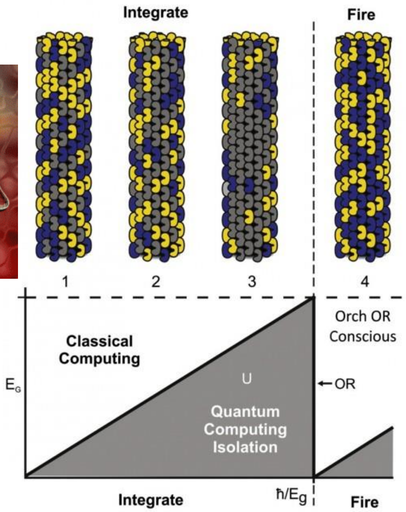
- Microtubules: Are polar and dynamic structures; Vibrations in their structure generate an oscillating electric field around them; Energy is supplied to the microtubular structures through metabolic activity

❑ The Penrose–Hameroff model ('Orch OR')

- Microtubules: function as cellular quantum computing elements, according to the Penrose-Hamerhoff "Orch OR, orchestrated objective reduction" model of consciousness.
- The physical cause of the coherent activity within the microtubules, as Penrose and Hamerhoff suggest, could be Fröhlich condensates.



http://phys.org/news/2009-03-frhlich-condensates-quantum-consciousness.html



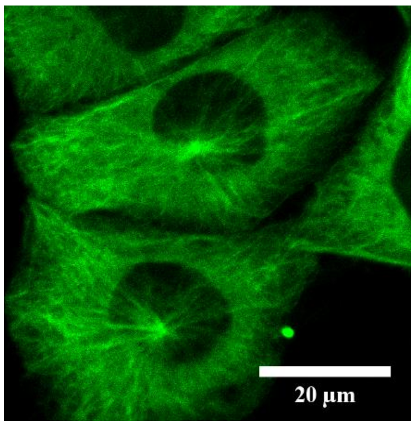
Hameroff & Penrose (2014)

Microtubule Candidates for Fröhlich's Theory

- Effects on engineered microtubules and microtubules within cells (transfected with fluorescent-tubulin)

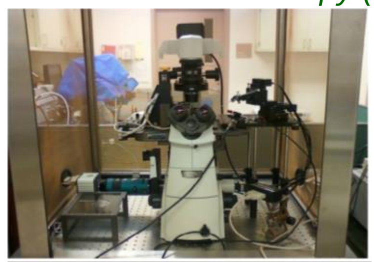
MICROTUBULES

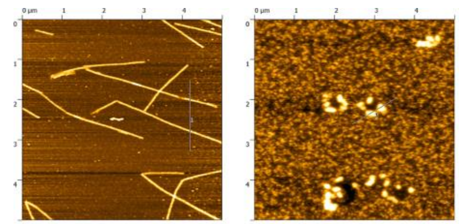
Protein-composed bio-structures



Thompson LG et al. 2014

Atomic force microscopy (AFM)

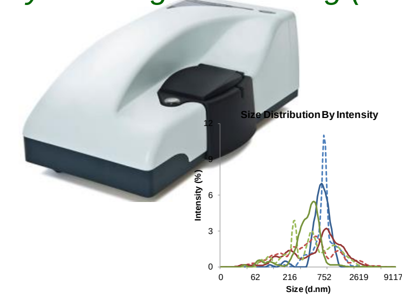




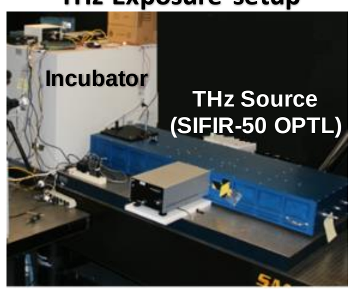
McMicken, B et al. 2014

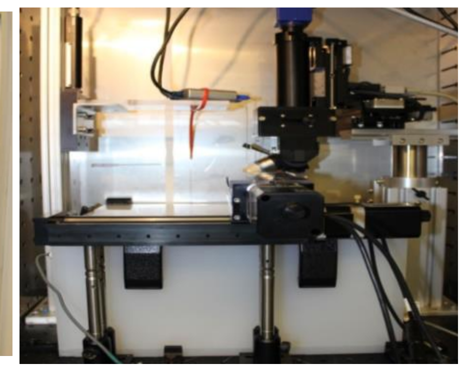
Malvern Zetasizer Nano ZS syste

Dynamic light scattering (DLS)

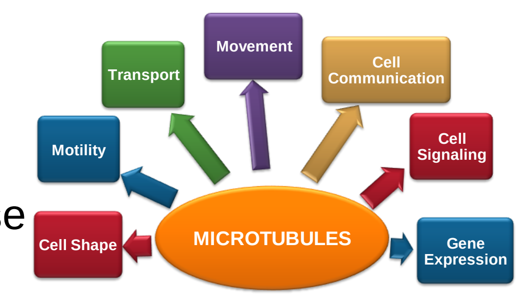


THz Exposure setup





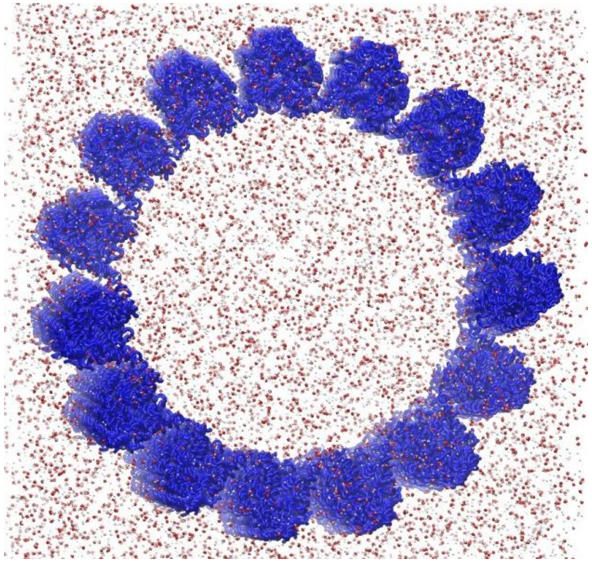
- How an exogenous THz stimulus would influence microtubules.
  - --Conformational change –Structures
  - --Formation
  - --Dynamics
- If microtubules are perturbed by THz frequencies, would that cause alterations in their fundamental processes?



Microtubule Candidates for Fröhlich's Theory

➢ *Predicting the Fröhlich frequencies that excite microtubules using molecular modeling*

*Use m*olecular dynamics simulations (**MDS**) that incorporate THz-scale, driven oscillations to determine the Fröhlich frequencies that excite the microtubules.



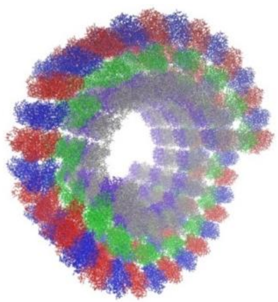
Jeremy Moix , Ph. D. (GDIT contractor)
James Parker, Ph. D. (GDIT contractor)

✓ **Large scale MDS of a solvated microtubule to**
---examine vibrational energy absorption and dissipation mechanisms
-----describe the expected behavior of the microtubule following THz excitation

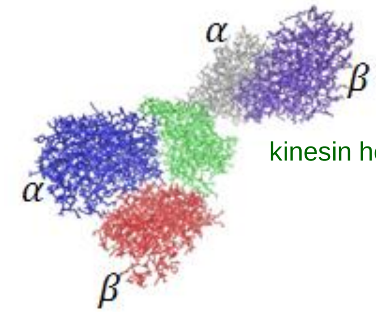
Dynamics of Low-frequency Vibrational Modes of Microtubules

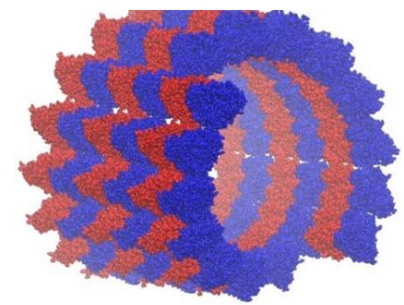
MDS Setting up the system and equilibration

- ❑ Retrieve the PDB file [3J2U] from the Protein Data Bank
- ❑ Base unit is copied, translated, and rotated

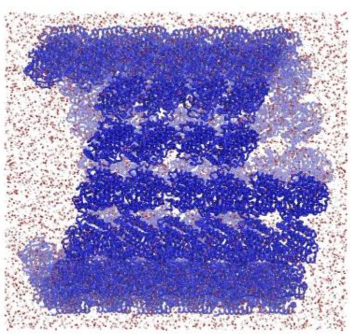


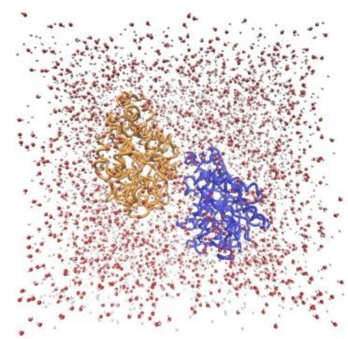
Microtubular array that resembles a doubled-walled tubular structure, with the kinesin head domains residing between the inner and outer walls.





- ❑ The inner helical microtubule is then selected for simulation
  - Comprised of slightly more than half a million atoms and forms **four full turns** of the helical assembly
  - **Outer diameter of ~25 nm**
- ❑ Simulate tublin heterodimer and the microtubule in its natural environment: solvation added (TIP3P water & Counter ion)
- ❑ Run MDS with NAMD (version 2.11) and the all-atom, CHARMM 22 protein force field



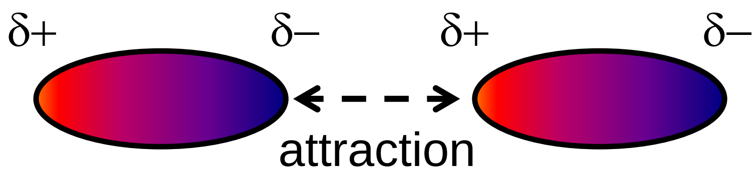


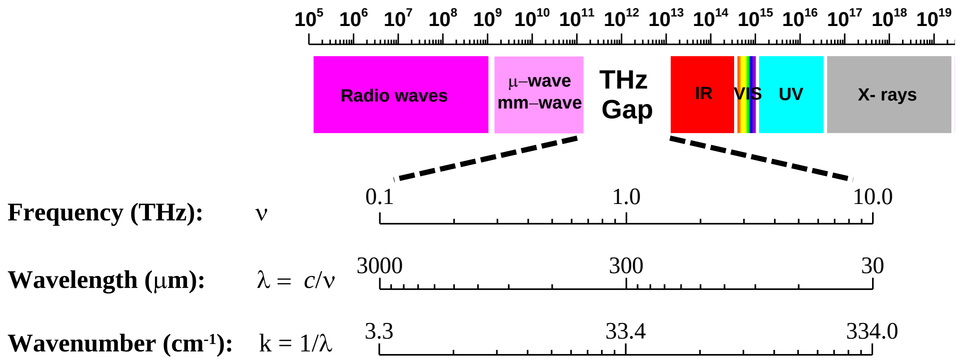
Orthorhombic simulation
325 × 325 × 352 °A
Six million total atoms

Moix J. et al., under PA approval to be submitted to J. Physical Chemistry

Dipole-dipole Correlation Function for Microtubule and Solvating Waters

MDS **Calculation**





- The total dipole moment of the subsystem is

$$\vec{M}(t) = \sum_i q_i \vec{r}_i(t) \ , \qquad (1)$$

where q_i is the fixed partial charge on each atom and r_i is the corresponding position vector

- From the time-dependence of the dipole moment, the classical absorption spectrum can be calculated from the Fourier transform of the dipole-dipole correlation function

$$A(\omega) = \frac{2\pi\omega^2}{3k_B T V c \eta(\omega)} \int_{-\infty}^{\infty} dt e^{-i\omega t} \left\langle \vec{M}(0) \cdot \vec{M}(t) \right\rangle \ , \qquad (2)$$

where k_B is Boltzmann's constant, T is the temperature, V is simulation volume, c is the speed of light, and $\eta(\omega)$ is the refractive index of the medium, taken here to be independent of frequency

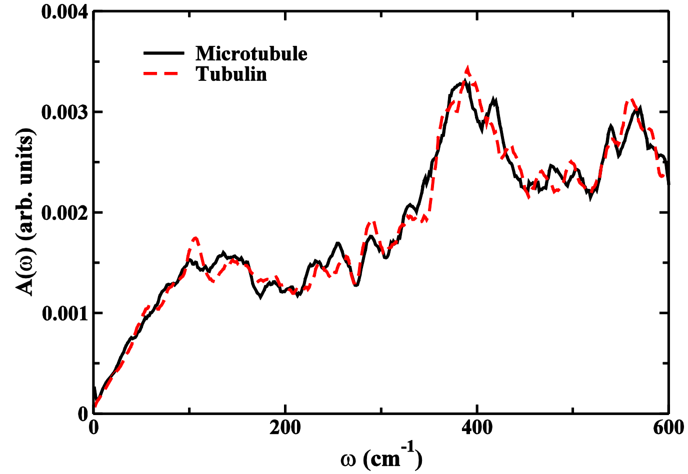
The dipole moment is recorded at least every 25 fs in order to span the frequency range from **0 to 600 cm^{-1}**

Moix J. et al., under PA approval to be submitted to J. Physical Chemistry

Absorption Spectra for the Microtubule and α- and β-Tubulins

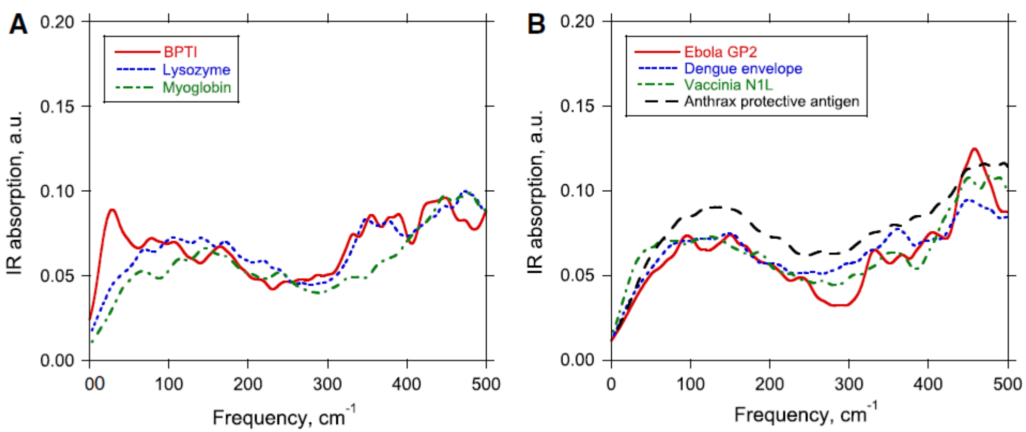
Dipole-dipole correlation function

$$A(\omega) = \frac{2\pi\omega^2}{3k_B T V c\eta(\omega)} \int_{-\infty}^{\infty} dt e^{-i\omega t} \left\langle \vec{M}(0) \cdot \vec{M}(t) \right\rangle$$



The vibrational absorption spectra of a portion of a solvated microtubule and the α- and β-tubulin monomers in solution spanning the THz Band

Minimal differences between the spectra of the tubulin monomer and the full microtubule are seen over the entire frequency range studied (0 to 600 cm^{-1})



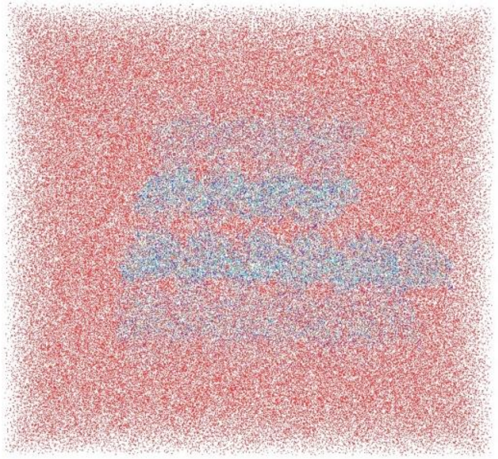
Mott A. & P Rezl, Eur. Biophys. J. 2015

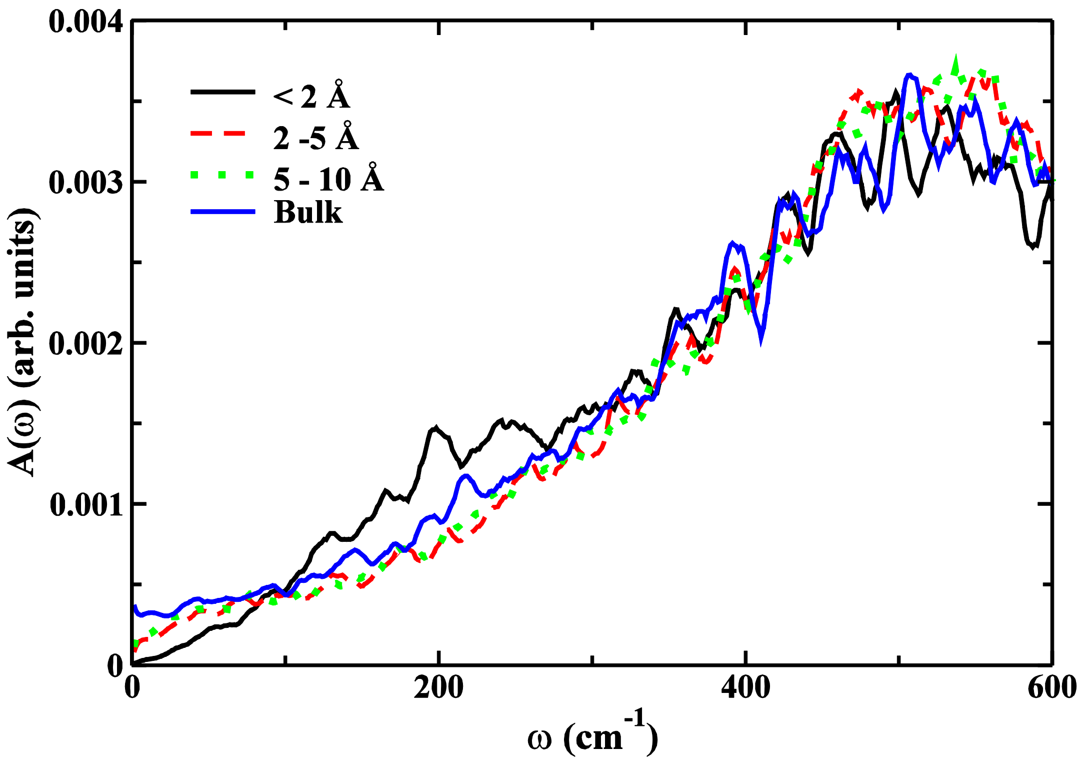
The low-frequency THz region shows very little structure up to a frequency of 300 cm^{-1}, in agreement with experimental measurements on myoglobin by Zhang et al. (2004) and lysozyme by Knab et al. (2006).

Moix J. et al., under PA approval to be submitted to J. Physical Chemistry

Vibrational Response of the Solvating Waters Surrounding a Microtubule

Absorption spectrum for various layers of water surrounding the microtubule





Moix J. et al., under PA approval to be submitted to J. Physical Chemistry

All of the spectra for the varying water layers are identical to that of the bulk (>30 Å),
with the single exception of the waters lying within 2 Å of the microtubule, displaying a slight enhancement in the librational absorption at roughly 200 cm−1 (~ 6 THz)

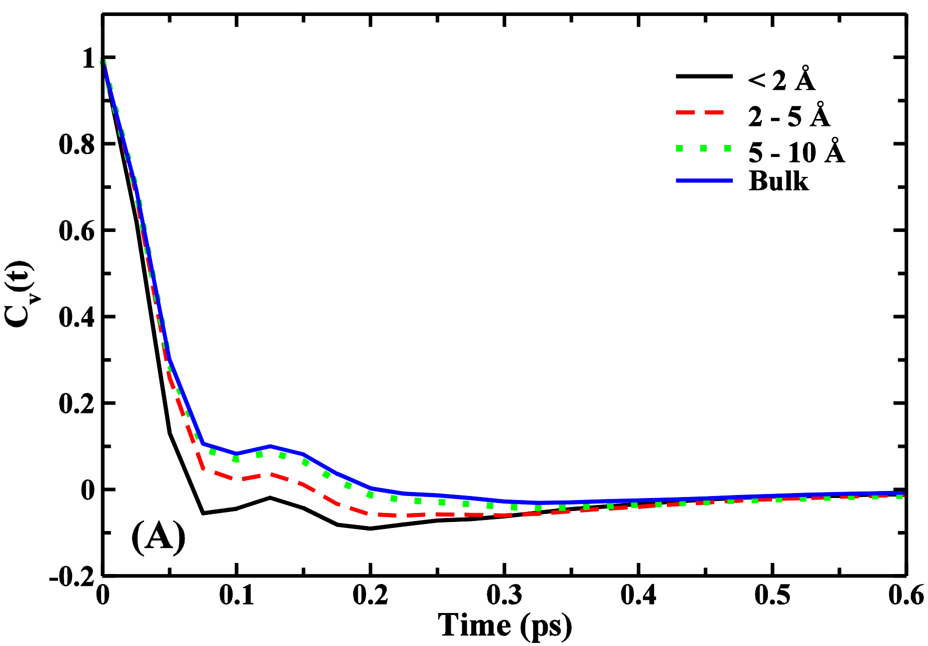
Influence of the Microtubule on the Water Dynamics

The Velocity Autocorrelation Functions (VACF) and Vibrational Density Of States (VDOS) of the various water layers

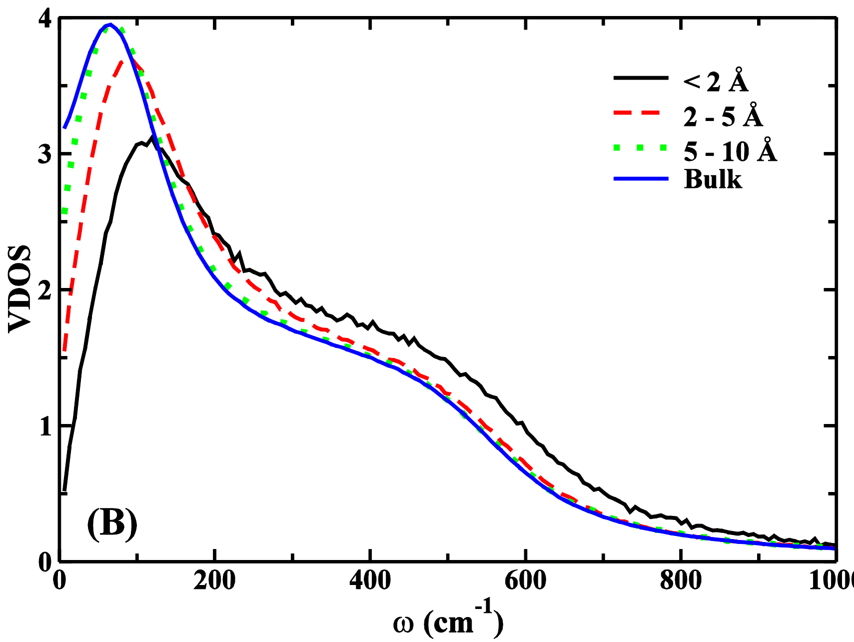
Normalized VACFs, $C_v(t) = \langle \vec{v}(t) \cdot \vec{v}(0) \rangle \, / \, \langle \vec{v}(0) \cdot \vec{v}(0) \rangle$

The normalizing factor in the autocorrelation function is a constant for all water subsets and given by the equipartition value, $\langle \vec{v}(0) \cdot \vec{v}(0) \rangle \; = \; 3k_{\rm B}T/m$

Normalized VACFs $C_v(t)$



VDOS, defined as the Fourier transform of the normalized VACF



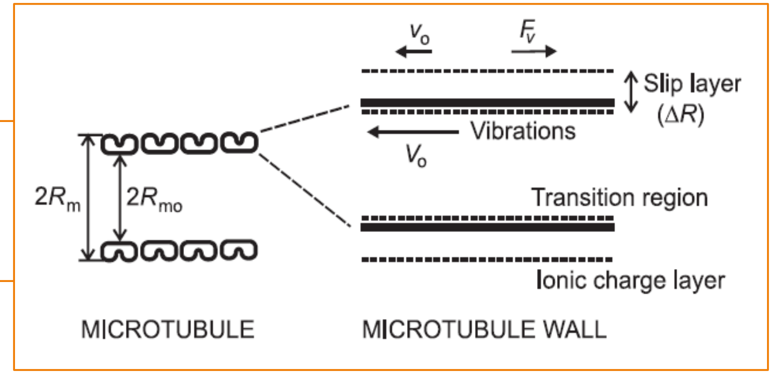
Moix J. et al., under PA approval to be submitted to J. Physical Chemistry

Qualitative Behavior of the Microtubule

Bioelectrochemistry 63 (2004) 321–326

Excitation of vibrations in microtubules in living cells

J. Pokorný*

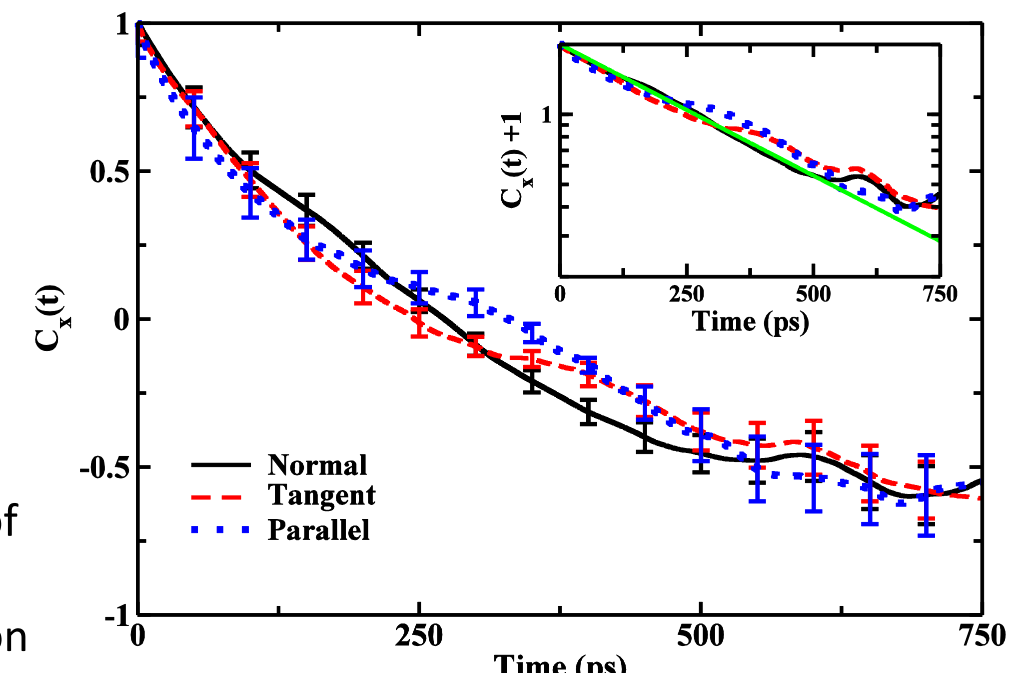


Autocorrelation function for the center of mass motion for each tubulin monomer

$$C_x(t) = \frac{\langle \delta x_c(t) \delta x_c(0) \rangle}{\langle \delta x_c(0)^2 \rangle}$$

where $\delta x_c(t) = x_c(t) - \bar{x}_c$

represents the fluctuations of the center of mass around its average position $(\bar{x}_c)$ measured over the course of the simulation



Moix J. et al., under PA approval to be submitted to J. Physical Chemistry

Summary & Conclusions
(MDS Study)

- In the low-frequency THz regime, the computed absorption spectrum of the microtubule is nearly indistinguishable from that of a simple solvated system containing only the α- and β-tubulin monomers

- Additionally below ~ 300 cm^{-1} both systems display similar spectra to other globular proteins

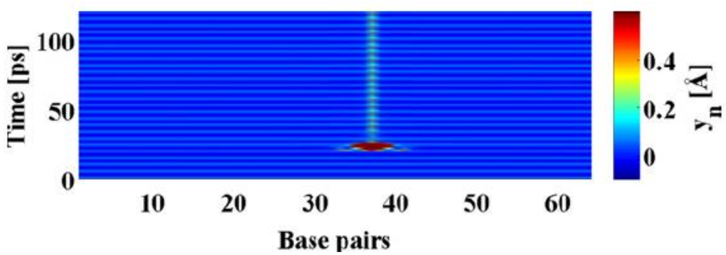
- The center of mass dynamics of the monomers in the microtubule appears to be overdamped as is generally observed for solvated systems, rather than underdamped, as has been suggested

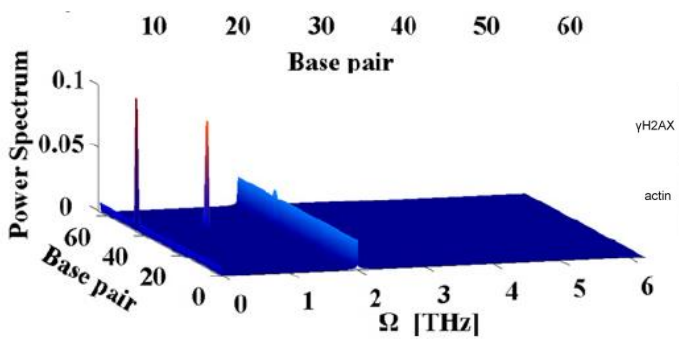
- Results on the VACFs, VDOSs, and diffusion rates indicate that waters within approximately 10 Å from the microtubule surface possess modified dynamics with respect to the bulk

  - The microtubule is quite average in comparison with the results of molecular dynamics simulations of other protein systems and is unlikely to support large scale vibrational processes such as Fröhlich condensates

What are the Mechanisms Behind THz-induced Gene Expression Changes?

Formation of a localized opening in DNA in the presence of a THz field

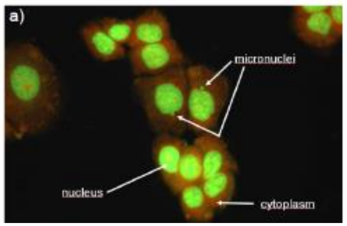




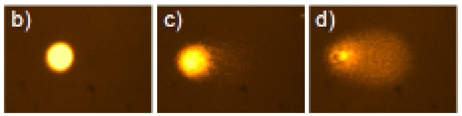
Alexandrov BS. et al., 2010

THz pulses cause DNA damage and activate DNA damage repair

Micronuclei in the HaCaT cells

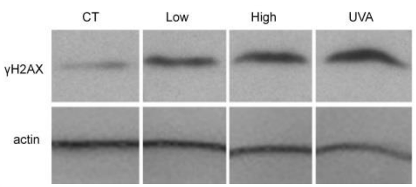


Undamaged **Damaged DNA**



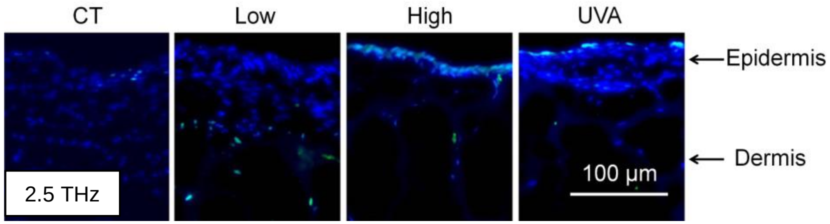
Kleine-Ostmann et al. 2009

Induction of γH2AX



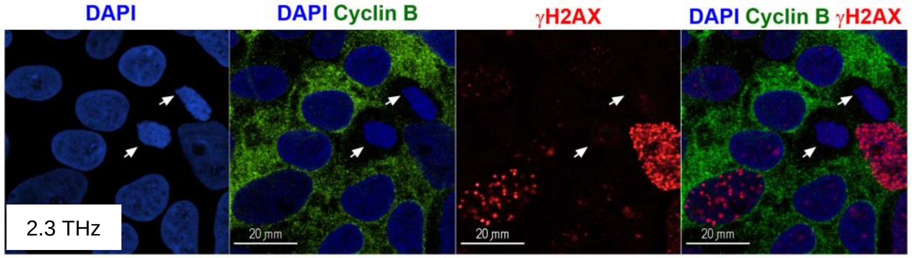
Human skin tissue models

Incidence of γH2AX foci



Titova LV et al. 2013

H2AX, H2A Histone Family Member X: a surrogate marker for double-strand breaks



Human embryonic stem cells

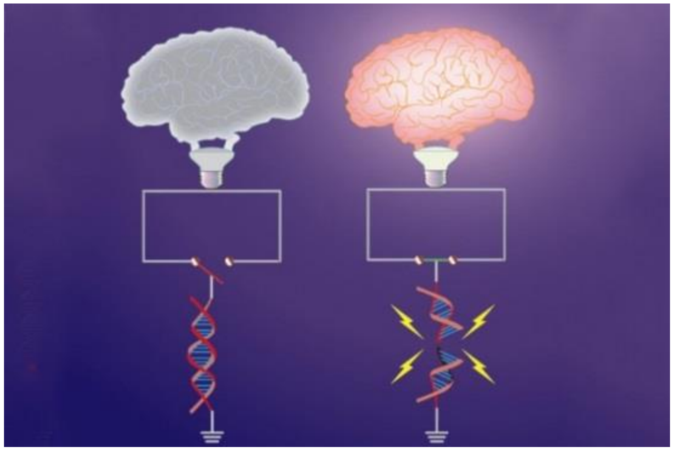
Bogomazova AN et al. 2015

Distribution A. Approved for public release; PA # TSRL 16-0321

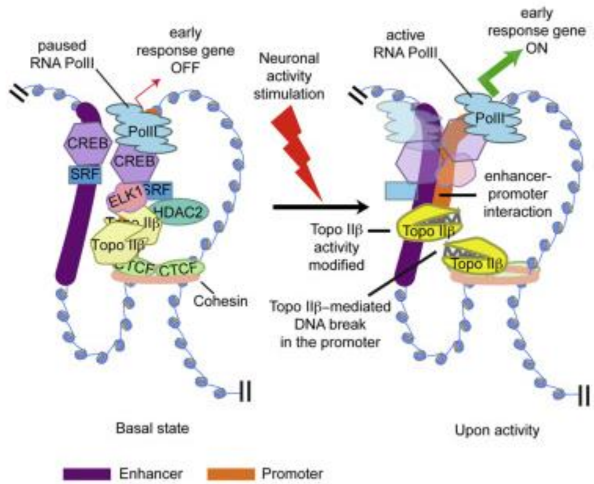
DNA Damage & Repair and Epigenetic DNA Methylation Regulate Neuronal Gene Expression

MIT News
ON CAMPUS AND AROUND THE WORLD

DNA breakage underlies both learning, age-related damage

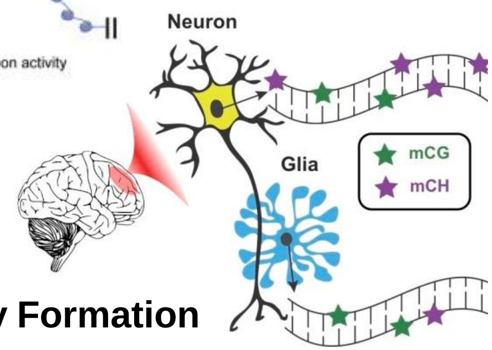


Tsai

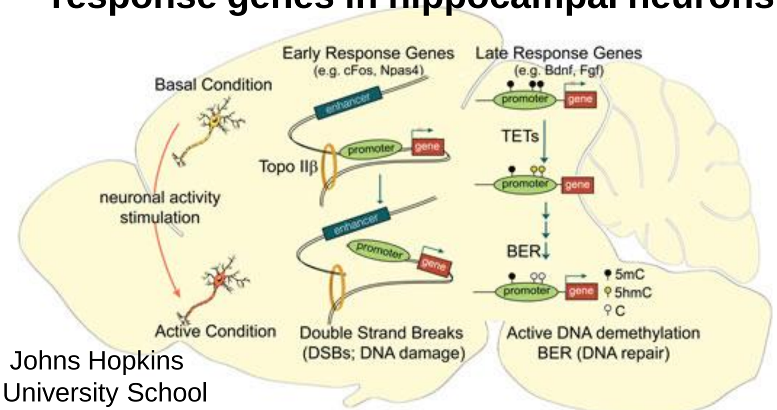


Madabhushi R et al., Cell, 2015

Activity-Induced DNA Breaks Govern the Expression of Neuronal Early-Response Genes



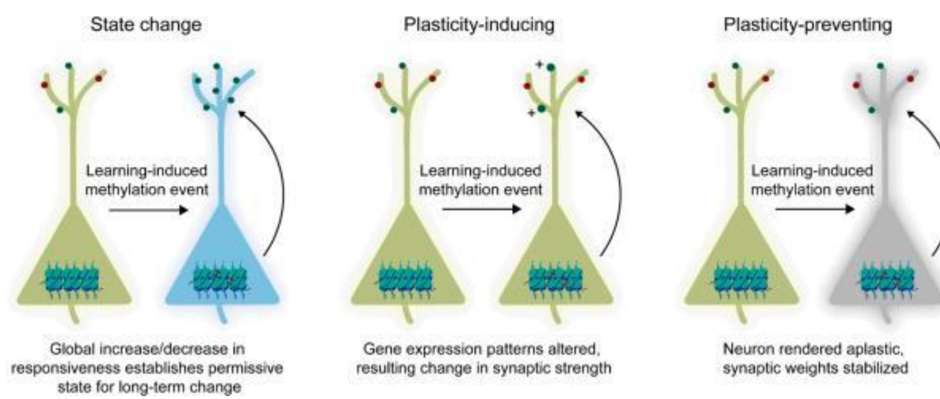
DNA damage and repair regulates expression of both early response and later response genes in hippocampal neurons



Johns Hopkins University School

Su Y el al., Cell Research, 2015

DNA methylation and Memory Formation

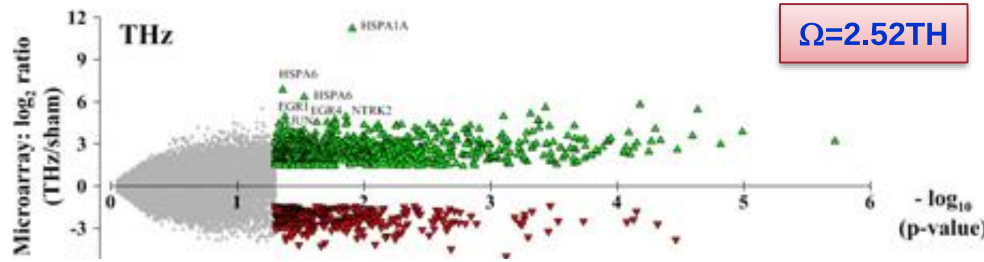


Eran Mukamel, Salk Institute

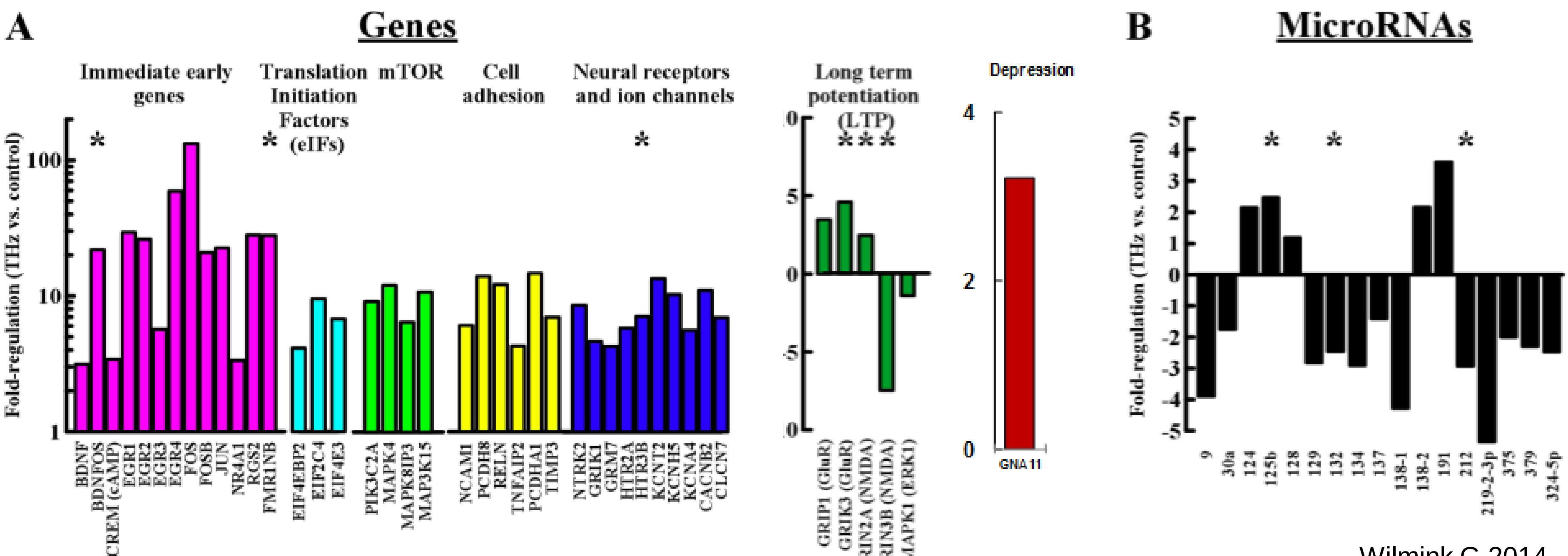
Day, JJ el al., Nature neuroscience, 2010

THz Radiation Induce mRNAs and miRNAs Involved in Synaptic Plasticity

The genes regulated by THz energy are enriched for neuronal activity-regulated (ERG, LRG) pathways



Ω=2.52TH



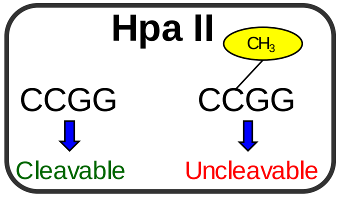
Wilmink G 2014

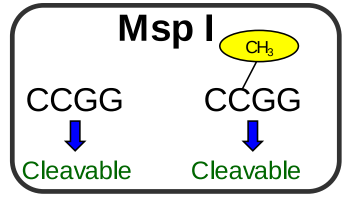
➢***<u>Genes regulation</u> plays* central role in various synaptogenetic processes:**

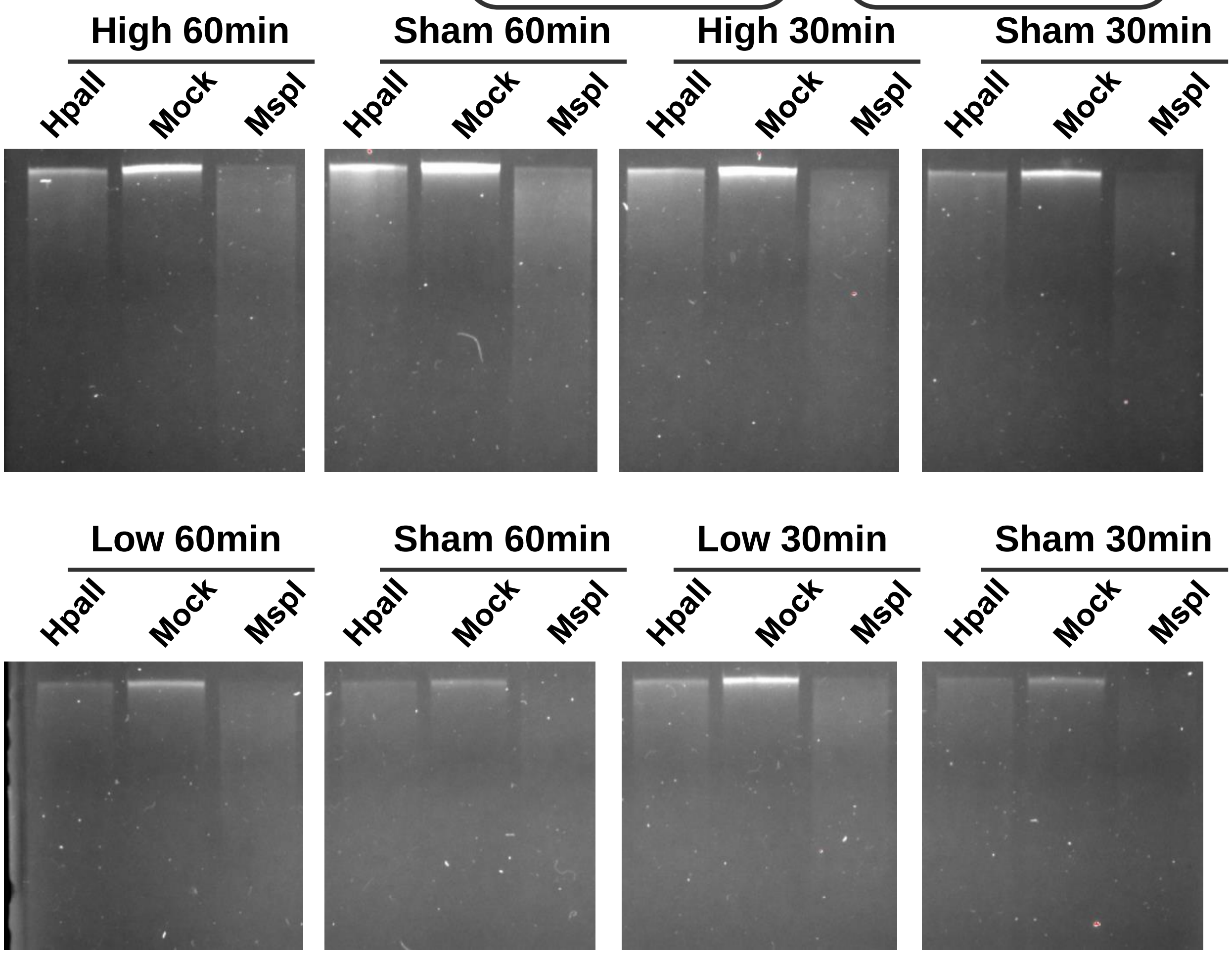
Neurite Outgrowth, Synapse Development and Maturation, Balance between Excitatory and Inhibitory Synapses, and Learning and Memory

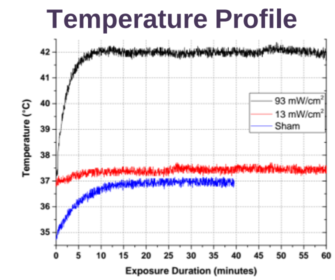
2.52 THz Affects DNA Methylation/Demethylation in NG108 Neuronal Cells

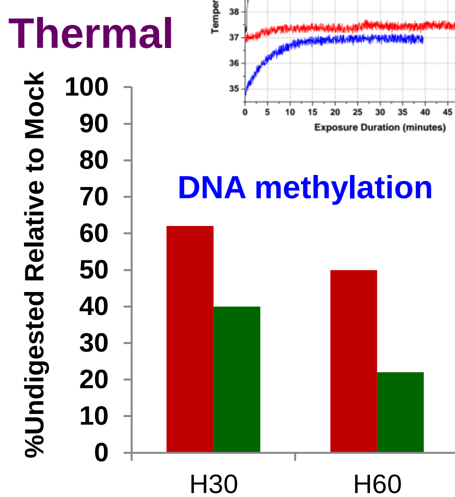
- **Catherine Millar-Haskell** (Ph.D. Student Delaware)
- **Brady McMicken**, Ph. D. (NRC Postdoc)

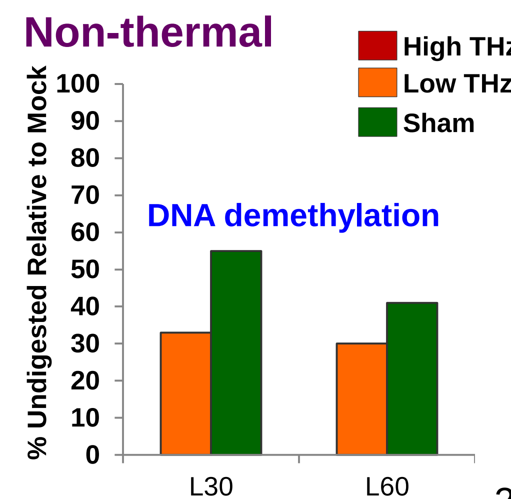




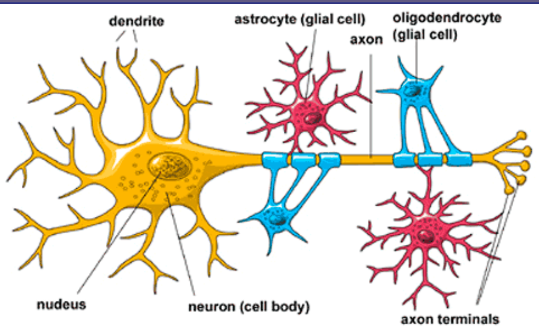








Can THz Regulate Neuronal Circuit Activity?
(DNA Damage Response and Epigenetic DNA Modifications)



Dr. Chris M. Valdez (NRC postdoc)
Dr. Ibey

- **Would THz Energy (thermal and non-thermal) elicit a neuronal activity that would orchestrate the expression of neuronal activity-regulated gene in neuron & supporting cells, and hippocampal slices?**
  - Electrophysiology (whole-cell voltage-clamp recordings, action potential, spontaneous activity, mEPSP)
  - Neurite outgrowth and spine morphology (microscopy and image analysis)
  - Synaptic plasticity RT-PCR arrays (IEGs, LTP and LTD genes)

- **What are THz mechanism of actions?**
  - Is the THz-mediated gene expression governed by an activation of a DNA damage mechanism

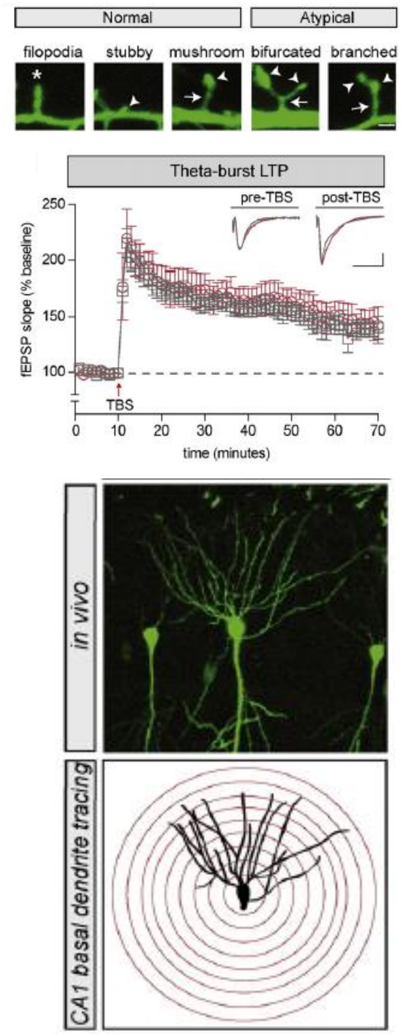
  (γH2AX levels, genome-wide γH2AX Chip-sequencing)
  - Is THz-mediated gene expression a result of an active DNA methylation/demethylation

  (Global patterns, Bisulfite-sequencing analysis)

- **Would THz energy regulate neuronal circuit activity?**

Hippocampal dendritic arborization

Synaptic transmission (Electrophysiology, fEPSP, LTP and LTD)



Valdez CM, 2016,
Molecular and Cellular Neuroscience

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Terahertz Group

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Dr. James Parker

Dr. Brady McMicken

Mr. Cesario Cerna

Ms. Bridget Endler

Ms. Catherine Millar-Haskell

THANK YOU!