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Mechanisms of interaction (RF and ELF): established vs hypothesized ones

Guglielmo d'Inzeo
DIET, "La Sapienza" University of Rome
ICNIRP Member
guglielmo.dinzeo@uniroma1.it

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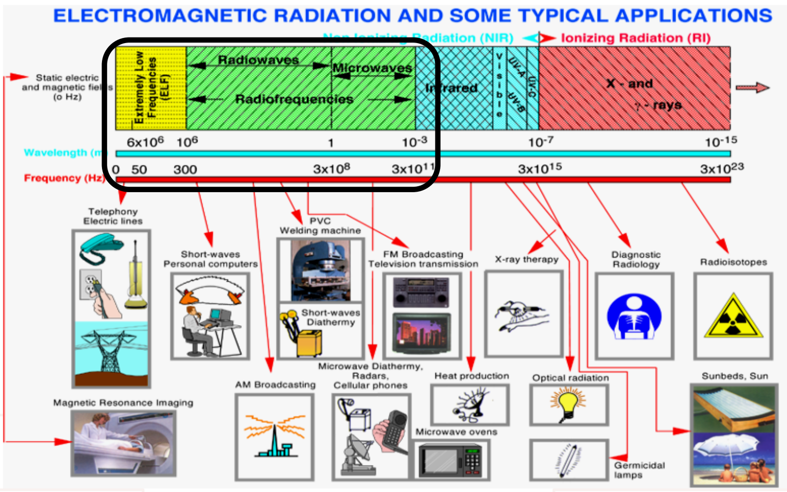
ELF and RF (MW and mmW)



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Long-term Effects

Research on a health effect finishes when:

The effect has been established

or

It is concluded that further studies cannot change the overall pattern and substantially reduce the uncertainties

EBEA 2011 - Rome, 21-24 February 2011

By courtesy of Paolo Vecchia

Where are we now with respect to long-term effects of EMF?

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EM

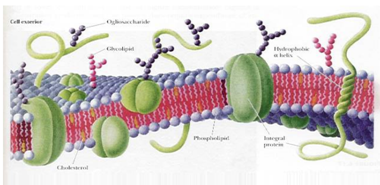
Temperature increasing

⇩

THERMAL EFFECTS

Assessed mechanisms

EM



Cellular parameters variation

⇩

SPECIFIC EFFECTS

Controversial

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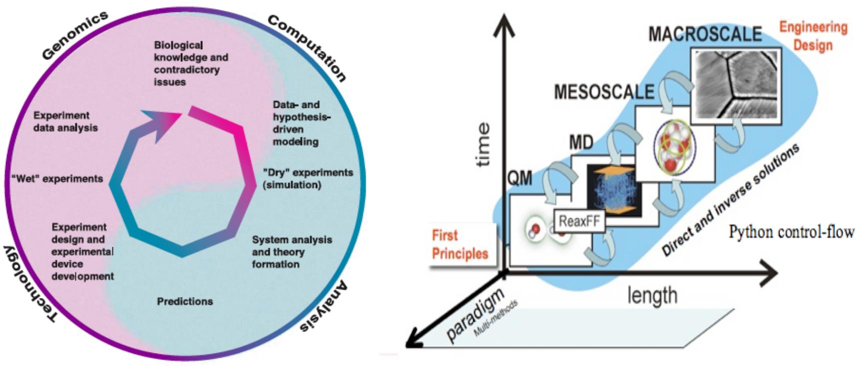
BioEM Modelling

To solve physical problems with an important impact at different levels of the biological complexity scale



The growing of computational power allows us to use new instruments to analyse problems at different levels of complexity

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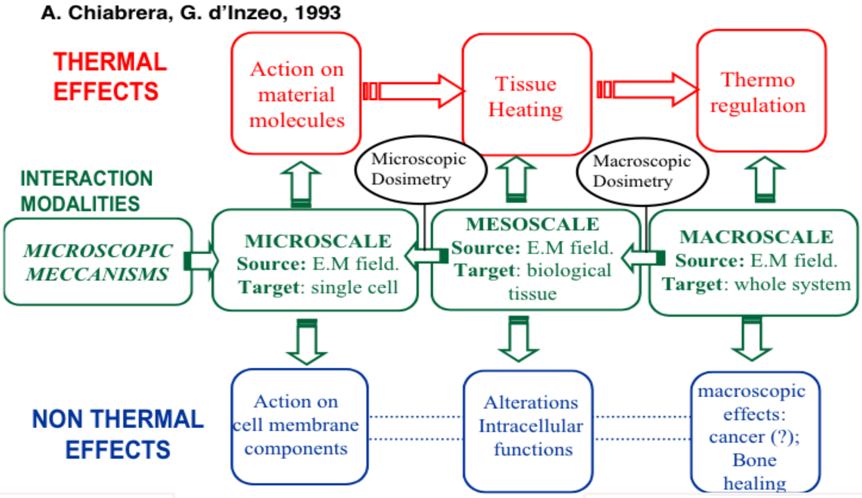
Modelling Interaction Mechanisms

A. Chiabrera, G. d'Inzeo, 1993



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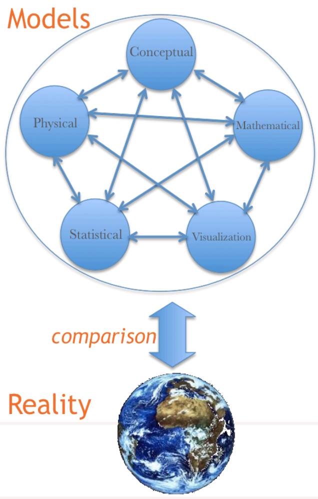
Scientific Modelling

Modelling => *Only mathematical formulas ?*

Modelling is an essential and inseparable part of all scientific activity

Scientific modelling is the process of generating abstract, conceptual, graphical and/or mathematical models (and finding the links among them)

The aim is to built up a formal system for which reality is the only implementation.



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Scientific Modelling II

Some examples in Bioelectromagnetism

Biological Level:
in vitro (Glassware: e.g. cells, tissue culture)
in vivo (Living models: e.g. mice, rats)

Physical Level:
EM fields: Maxwell Equations
Matter: Schrödinger Equation

For the scientists, a model is also a way for extending their thoughts

Models, that can be rendered in software, allow scientists to leverage computational power to simulate, visualize, manipulate and gain intuition about the phenomenon or process being represented.

Such computer models are called ***in silico***.

Churchman, 1968, Wikipedia, 2010

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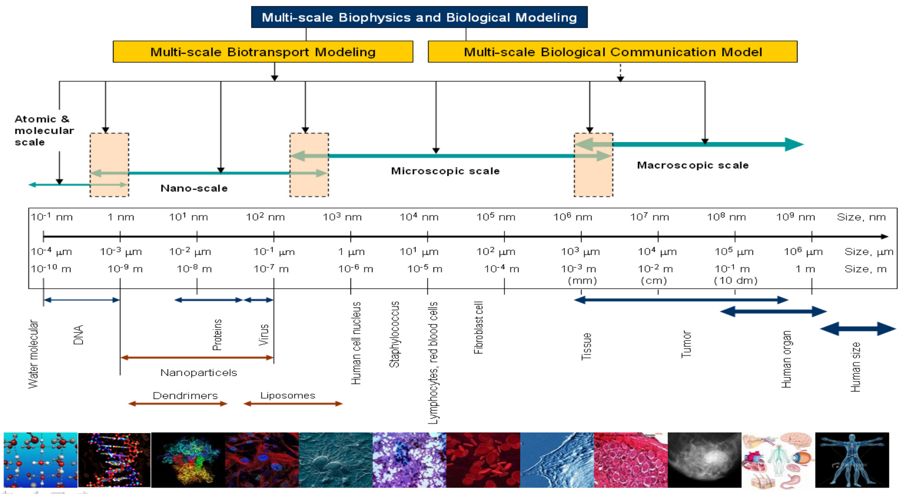
Multiscale Approach to Biology



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Electromagnetic Field Basic Laws

J.C. Maxwell
The "electromagnetic field" is that particular state produced into the environment by the interaction between charged bodies, polarized or crossed by currents

$E = E(r,t)$	electric field	$[V/m]$
$H = H(r,t)$	magnetic field	$[A/m]$
$D = D(r,t)$	electric displacement	$[C/m^2]$
$B = B(r,t)$	magnetic induction	$[Wb/m^2]$

Maxwell Equations

$$\nabla \times \boldsymbol{E} = -\frac{\partial \boldsymbol{B}}{\partial t}$$

$$\nabla \times \boldsymbol{H} = \frac{\partial \boldsymbol{D}}{\partial t} + \boldsymbol{J}$$

$$\nabla \cdot \boldsymbol{D} = \rho$$

$$\nabla \cdot \boldsymbol{B} = 0$$

$\boldsymbol{J} = \boldsymbol{J}(\boldsymbol{r}, t)$ $[A/m^2]$

$\rho = \rho(\boldsymbol{r}, t)$ $[C/m^3]$

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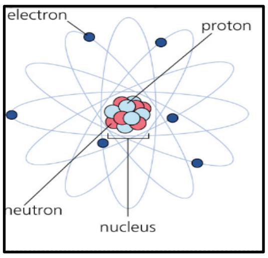
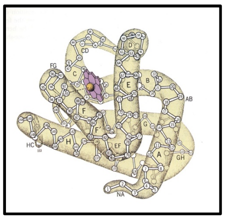
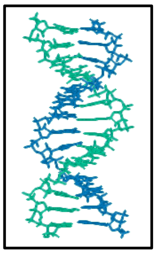
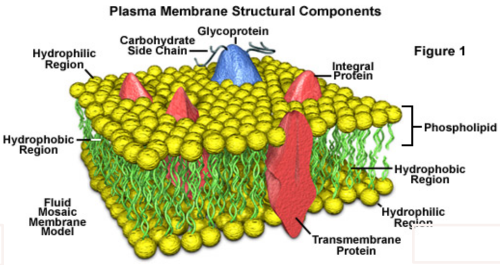
Electric-, magnetic- and EM-fields interact with charged component(s)









By courtesy of Q. Balzano, 2010

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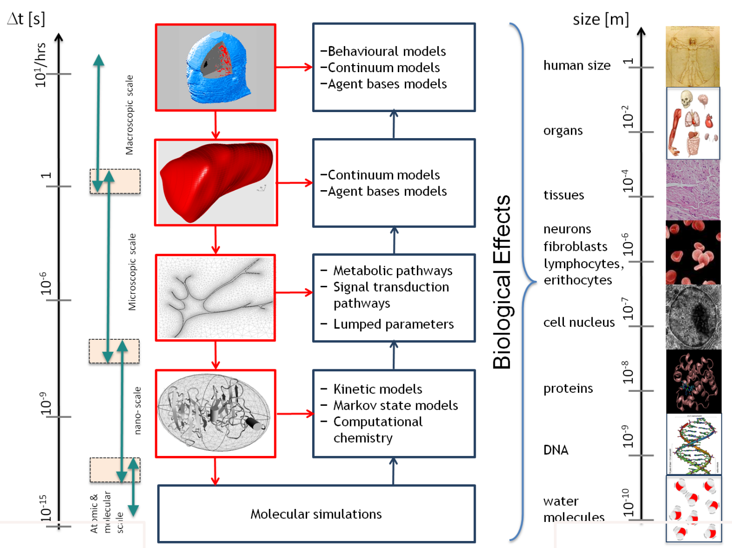
Bioeletromagnetism: A multiscale problem



Apollonio et al. 2010

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A common approach down to a molecular level

Applied External Fields

At **low frequencies**: Static and ELF magnetic and electric fields can be applied separately

At **higher frequencies: E** and **B** must be considered coupled and acting in the same instants. Time changes of **B** generate **E** and viceversa

Fields induced at tissue level

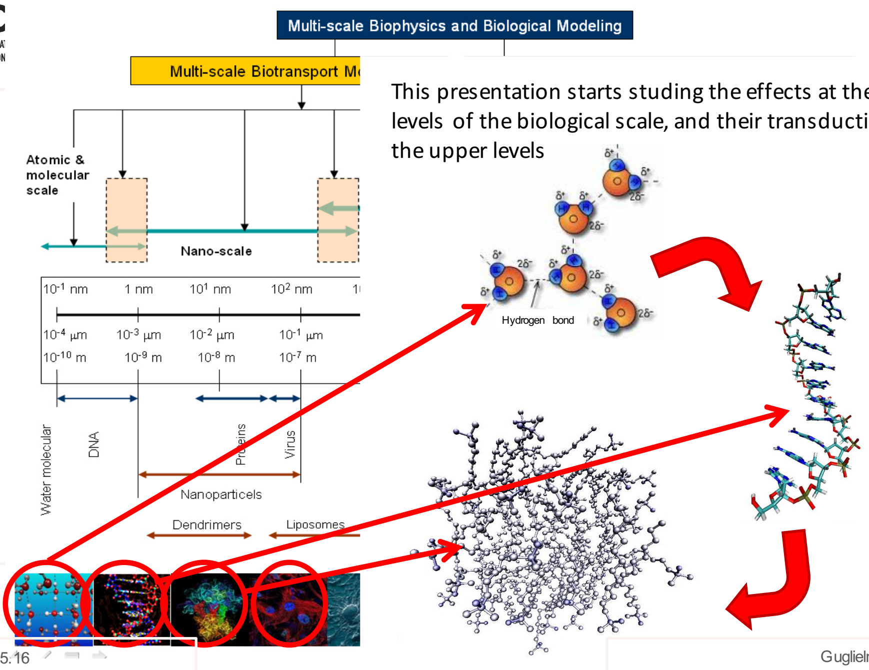
At **ELF:** both **E** and **B** fields can induce into tissue (macroscopic) an electric field

At **higher frequencies: E** and **B** must be considered coupled and depending on the tissues properties

Fields induced at molecular level

The induced **E** and **B** have to be considered acting separately and directly on each charge (microscopic)

$$m_i\,\mathbf{a}(t) = q_i\mathbf{E}(t) + q_i\mathbf{v}_i(t) \times \mathbf{B}(t)$$



This presentation starts studing the effects at the lower levels of the biological scale, and their transduction to the upper levels

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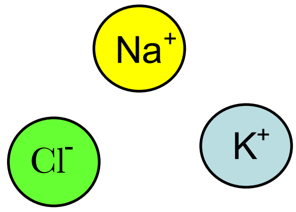
Forces at molecular level

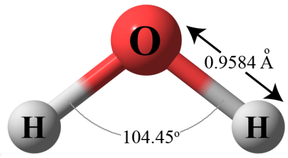
Interaction with monopoles (ions):

- $\mathbf{F} = q\mathbf{E}$ Electric force
- $\mathbf{F} = q(\mathbf{v} \times \mathbf{B})$ Magnetic force



Interaction with dipoles (molecules):

- $\mathbf{T} = \mathbf{m}_e \times \mathbf{E}$ Electric couple
- $\mathbf{T} = \mathbf{m}_m \times \mathbf{B}$ Magnetic couple
- $\mathbf{F} = \nabla(\mathbf{m}_e \cdot \mathbf{E})_{\theta=cost}$ Dielectrophoretic force



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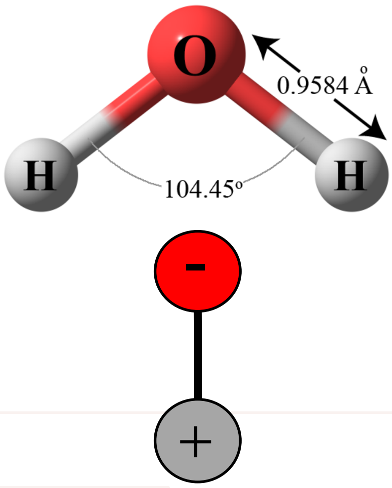
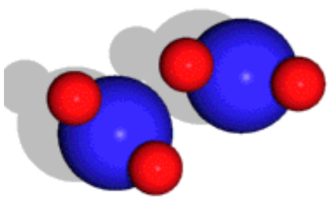
EM fields acting on Molecules





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Well known biological effects at ELF

There are a number of well established acute effects of exposure to low-frequency EMFs on the nervous system:

- Phosphenes Stimulation **50 to 100 mV/m**

The most robustly established effect of electric fields below the threshold for direct nerve or muscle excitation is the induction of magnetic phosphenes, a perception of faint flickering light in the periphery of the visual field. They are thought to result from the interaction of the induced electric field with electrically excitable cells in the retina. This is formed as an outgrowth of the forebrain and can be considered a good but conservative model of processes that occur in CNS tissue in general. The threshold for induction of phosphenes in the retina has been estimated to lie between about 50 and 100 mV m^{-1} at 20 Hz. The evidence for neurobehavioral effects on brain electrical activity, cognition, sleep and mood in volunteers exposed to low frequency electric and magnetic fields is much less clear.

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Proposed Interaction Mechanisms

	Ions-Molecules	Proteins-Macromolecules	Membrane	Cell	Network
Resonance		• Pickard and Rosenbaum (1978) • Swicord (1984) • Kohli (1981)			
Stochastic Resonance		• Kruglikov and Dertinger (1994) • Bezrugof and Voydanov (1995-1998) • Astumian et al (1997-99)	• Bezrugof and Voydanov (1998) • Fulinsky (1998)	• Bezrugof and Voydanov (1997)	• Weaver et al. (1998-2002)
Non-Linear	• Chiabrera et al. (2000) • Pokorny (1998-2001) • PicKard et al (1995-2001)	• Gailey (1996-1999) • Bystrov et al. (1994) • Albanese and Bell (1984) • Cain (1980) • Tsong and Astumian (1984) • Walleczek (1990-2000) • D'Inzeo et al (1993) • Barnes (1996)	• Blank et al.(1982-1987) • Gailey (1996-1999) • Barnes (1977-1996) • Bernardi and D'Inzeo (1984) • Casaleggio et al. (1984) • Franceschetti and Pinto (1984) • Bruner (1998) • Markin et al. (1990-92)	• Weaver and Astumian (1992-2000) • Robertson and Astumian (1991) • Kaiser et al. (1982-1995) • Astumian et al. (1995) • Litovitz et al. (1991-1997) • Bernardi et al (1994)	• Pilla et al. (1999) • Apollonio et al. (2000)
E-B Fields	• Chiabrera et al.(1984) • D'Inzeo et al. (1990-91) • Durney (1989) • Liboff & McLeod (1984-1997) • Lednev (1991, 96) • Blanchard and Blackman (1992-1994) • Bianco et al. (1990-1997) • Zadhin (1990-2001) • Edmonds (1993) • Bihni (2000-2002) • Ramundo et al. (2000) • Grissom (1994-1995)	• Liboff & McLeod (1992) • Balcavage et al. (1996) • Blank & Goodman (2002) • Grissom (1994-1995)			
Cooperative		• Frohlich (1968-1986)	• Frohlich (1968-1986) • Grodsky (1975) • Thompson et al. (2000)	• Frohlich (1968-1986)	•
Cascade /integration	•	•		• Adey (1988) • Engstrom (1997)	Barnes (1998)
Others	• Zago et al. (1999)	• Scott (1982) • Lawrence and Adey (1982)			

Liberti et al.
URSI GA, 2005

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WHERE?

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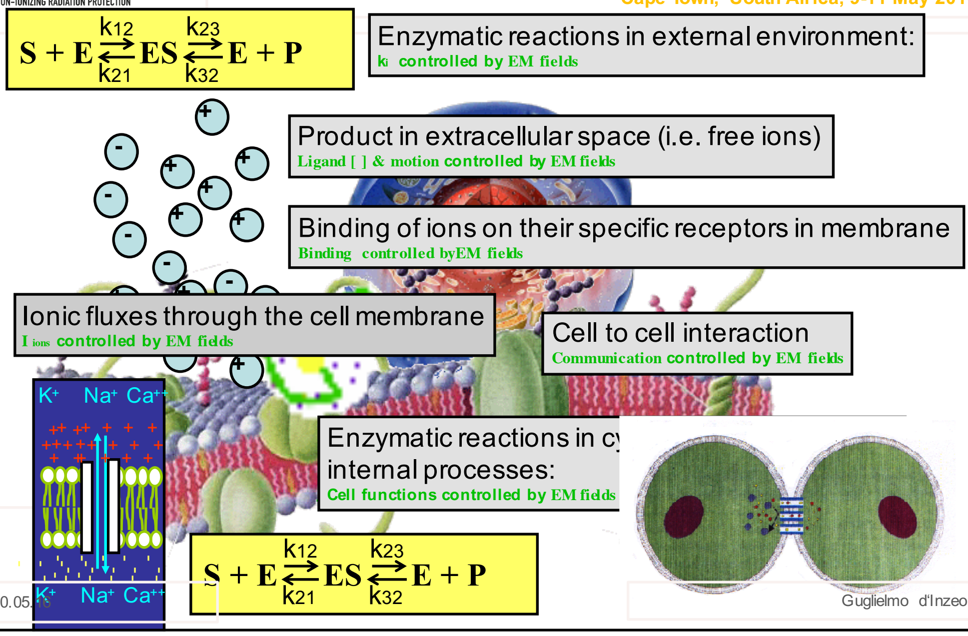

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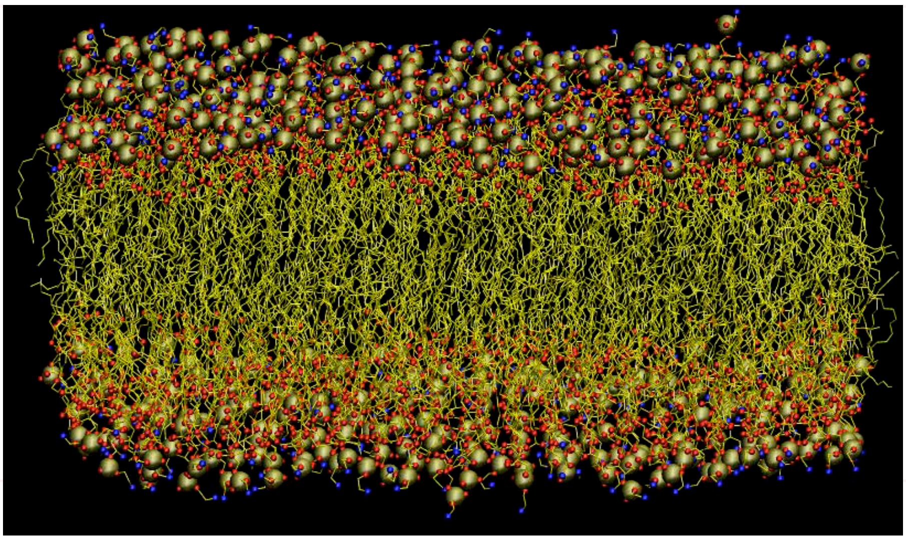

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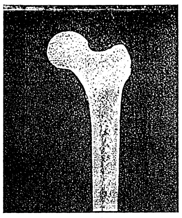
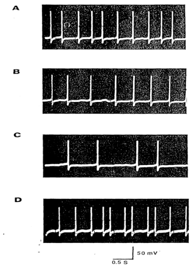
Our Scope

- To identify models valid to ELF (E,B), RF and MW, modulated RF





- Use the same approach for fields of different intesity (and frequencies)
- Enhance connections from theory and experiments

Criteria of reviewing

Databases: Web of Science & Medline

d'Inzeo et al., EBEA 2011

- Included low- and high- intensity fields;
- Included static and pulsed MF in the ELF range;
- Disregarded works with inadequate exposure conditions:
  a. no values of SAR or field intensity
  b. no exposure system description (e.g use of cell phone)
  c. not clear end points

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END-POINT\SYSTEM	NERVOUS	CARDIOV.	REPROD.	IMMUNE	BONE/CARTILAGE/MUSCLE	CONNECTIVE/EPITHELIAL
GENE EXPRESSION	Pirozzoli (2003) Reyes-Guerrero (2010)				De Mattei (2005)	
PROTEIN SYNTHESIS	Piacentini (2008)	Martino (2010)			Fioravanti (2002) De Mattei (2003), (2004), (2007) Shen (2010)	Patruno (2009)
CELL SIGNALLING Kinase Ca c-AMP	Hogan (2004) Manikonda (2007) (a) Manikonda (2007) (b) Jeong (2006)		Bernabò (2007)	Tenuzzo (2006) Dini (2009)	Brighton (2001) Varani (2002), (2003), (2008)	
OXYDATIVE STRESS (ROS)	Falone(2007) Di Loreto (2010)			Varani (2002), (2003) Simko (2004)	Morabito (2010)	
MORPHOLOGY			Bernabò (2007)	Dini (2009)		Cricenti (2008)
MEMBRANE PROTEINS Receptors enzymes	Massot (2000) Piacentini (2008) Manikonda (2007) Morelli (2005) Ravera (2006) Ravera (2010) Sieron (2001) Sieron (2004) Shin (2007) Wang (2008) Cahill (2007)	Morelli (2005)	Sun (2008) Ravera (2006)	Varani (2002), (2003)	Varani (2008) De Mattei (2009)	
EEG (ALFA RYTHM)	Cook (2004) Cook (2006)					
NEUROTRANSMITER	Wierastzko (2005)					
PAIN PERCEPTION	Choleris (2002) Shupak (2004) Ghione (2005)					
PROLIFERATION/DIFFERENTIATION		Martino (2010)			De Mattei (2001), (2005) Dimitriou (2007) Varani (2008) De Mattei (2009) Perez (2009)	Vianale (2008) Patruno (2009)

ELF

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Classification

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END-POINT\SYSTEM	NERVOUS	CARDIOV.	REPROD.	IMMUNE	BONE/CARTILAGE/MUSCLE	CONNECTIVE/EPITHELIAL
DNA (genotoxicity)		Belayev (2009)		Kim (2007)		Schwarz et al. (2008)
GENE EXPRESSION	Lee (2008)			Gerner (2010)		
PROTEIN SYNTHESIS						Karinen (2008) Gerner (2010)
CELL SIGNALLING Kinase Ca c-AMP	Lee (2008) Ammari (2008)			Grigoriev (2010)		
OXYDATIVE STRESS (ROS)	Lee (2008) Xu (2010); Del Vecchio (2009) (a)		De Iuliis (2009)			Yao (2008)
MORPHOLOGY	Bas (2009) (a) Bas (2009) (b) Del Vecchio (2009) (b) Sonmetz (2010) Xu (2006) Maskey (2010)	Curcio (2009)				
FUNCTION			Salama (2010) Falzone (2008)			
MEMBRANE PROTEINS Receptors enzymes	Ammari (2008)					
EEG (ALFA RYTHM) & MEMORY	Carruba (2009) Lowden (2011) Wiholm (2009)					
PAIN PERCEPTION	Gapeyev (2009)					

RF

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Classification

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SYSTEM ENDPOINT	CARDIOV.	REPROD.	BONE CARTILAG. MUSCLE	CONNECTIVE EPITHELIAL	INTESTINE, LUNG LIVER	FOOD
MEMBRANE STRUCTURE	Zhang (2008) Jarm (2010) Garon (2007) Chen (2004) Beebe (2004)	Heller (2010) Ibey (2009)	Sersa (2000) Kranjc (2003) Golzio (2007)	Gehl (2000) Daud (2008) Rageh (2007) Nuccitelli (2006)	Hall (2007) Labanauskiene (2006) Larkin (2005) Edd (2006)	Lebovka (2010)

Micro & Nanopulses

d'Inzeo et al., EBEA 2011

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IEEE TRANSACTIONS ON MICROWAVE THEORY AND TECHNIQUES, VOL. 61, NO. 5, MAY 2013 2031

Feasibility for Microwaves Energy to Affect Biological Systems Via Nonthermal Mechanisms: A Systematic Approach

Francesca Apollonio, *Member, IEEE*, Micaela Liberti, *Member, IEEE*, Alessandra Paffi, *Member, IEEE*, Caterina Merla, *Member, IEEE*, Paolo Marracino, Agnese Denzi, Carmela Marino, and Guglielmo d'Inzeo, *Member, IEEE*

+

Francesca Camera and Maura Casciola (PhD students)

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TABLE I
SCHEMATIC VIEW OF THE MAIN LITERATURE MODELS PROPOSED TO DESCRIBE THE INTERACTION BETWEEN BIOSYSTEMS AND RF EM FIELDS. MODELS ARE ORGANIZED ACCORDING TO THE MULTISCALE APPROACH

Complexity level	Modeled mechanism	Comments	References
Atoms-molecules	Direct action of E-B fields on ion binding	Coupling with thermal bath has to be accurately modeled	Chiabrera et al, 1985 [21]; Chiabrera and Bianco 1987 [22]; Moggia et al., 1997 [23]; Bianco et al., 1997 [24]; Chiabrera et al., 2000 [25]; Barnes and Kwon, 2005 [26];
	Ferromagnetic resonance	Absorbed energy must be > kT	Kirschvink, 1996 [27];
	Radical pair	f < 100 MHz in geomagnetic static fields	Woodward et al., 2001 [33]; Henbest et al.,2004 [153]; Hansen and Pedersen, 2006 [30]
Macromolecules	Direct action of E field on protein conformation	E fields comparable to the endogenous ones (10^9 V/m)	English and Mooney, 2007 [36]; Astumian, 2003 [35]; Apollonio et al., 2008 [43];
	Resonant absorption by macromolecules	f > 100 GHz	Edwards et al., 1984 [48]; Bohr and Bohr, 2000 [37]; Adair, 2002 [28]; Prohofsky, 2004 [38];
	Conformational changes due to transient local heating	Too small temperature rise for exposure below the guidelines	Laurence et al., 2000 [39]; Laurence et al., 2003 [40];
	Demodulation by channel non-linearity	No results available for modulating frequency below 50 MHz	Stoykov et al.,2004 [44]
Cell compartments	Demodulation/rectification by membrane non-linearity	f < 10 MHz low demodulation efficiency	Barnes and Hu, 1977 [49]; Pickard and Rosenbaum 1978 [57]; Bernardi and d'Inzeo, 1984 [50]; Franceschetti and Pinto, 1984 [51]; Astumian et al., 1995 [54]; Barnes, 1996 [55]; Balzano 2002 [59]; Balzano 2008 [58];
	Soliton	Requires strong coupling	Lawrence and Adey, 1982 [65];
	Resonant absorption by microtubules	Absence of absorption bands	Frohlich, 1968 [66]; Foster and Baish, 2000 [67]; Adair 2002 [28]
Cells-Aggregates of cells	Cooperativity	Based on assumption on the first interaction step	Thompson et al., 2000 [68];
	Pearl-chain	f < 100 MHz	Schwan, 1985 [70]; Krasil'nikov 1999 [71]; Adair 1994 [73]; Sernelius 2004 [72]; Adair 2004 [74]

Apollonio et al., 2013

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TABLE II
SCHEMATIC VIEW OF THE MAIN BIOLOGICAL EFFECTS INDUCED BY RF AND MW EM FIELDS IN THE LAST DECADE. THE REVIEWED EFFECTS ARE ORGANIZED ON THE BASIS OF THE INVESTIGATED BIOLOGICAL SYSTEMS AND OF THE ANALYZED BIOLOGICAL ENDPOINT

End-point/System	Nervous	Reproductive	Immune	Connective and Epithelial	Blood	Yeast cultures /Drosophila model
Genotoxicity: gene expression & DNA alteration	Diem et al., 2005 [83]; Paulray and Behari 2006 [84]; Buttiglione et al., 2007 [85]; Zhao et al., 2007 [86];	-	Sykes et al., 2001 [95]; D'Ambrosio et al., 2002 [93]; Remondini et al. 2006 [82]; Schwarz et al., 2008 [92]; Kim et al., 2008 [94]; Belyaev et al., 2009 [141];	Diem et al., 2005 [83]; Nylund and Leszczynski 2006 [90]; Schwarz et al., 2008 [92]; Franzellitti et al., 2008 [87];	-	Lee et al., 2008 [88]; Chen et al., 2012 [89]
Oxidative Stress (ROS)	Meral et al., 2007 [103]; Hoyto et al., 2008 [106]; Luukkonen et al., 2009 [107]; Luukkonen et al., 2010 [108]; Del Vecchio et al. 2009 [109]; Xu et al., 2010 [105];	De Iuliis et al., 2009 [99];	Zmyslony et al., 2004 [100]; Grigoriev et al., 2010 [104];	Yao et al., 2008 [98];	Zmyslony et al., 2004 [100];	Lee et al., 2008 [88]; Crouzier et al., 2009 [110];
Morphology	Bas et al., 2009 [113], [114]; Del Vecchio et al., 2009 [111]; Sonmetz et al. 2010 [112]; Maskey et al., 2010 [115], [116];	-	-	-	-	-
Protein synthesis	-	-	Gerner et al., 2010 [118];	Karinen et al., 2008 [117]; Gerner et al., 2010 [118];	-	-
Cell signaling (kinase, Ca, c-AMP)	-	-	-	Xu et al., 2006*	-	Lee et al., 2008 [88];
Function	-	Falzone et al., 2008**	-	-	-	-
Pain / Perception	Gapeyev et al., 2009***	-	-	-	-	-

10.05.16 Apollonio et al., 2013

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ENDPOINTS

Major reported endpoints refer to
a) genotoxicity,
b) oxidative stress,
c) cell morphology (a role for microdosimetry),
d) protein synthesis.

However, the different SAR levels, frequencies, and modulations of the EM fields used in the reviewed experiments led to a great variability of outcomes making their comparison extremely difficult, as well as their systematic organization and comprehension.

Apollonio et al., 2013

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Plausible mechanisms

These plausible hypotheses have been restricted to five:

1) selective/microthermal heating;
2) nonthermal effects on reactions;
3) action on hydrogen bonds in the hydration layer;
4) radical pair mechanism;
5) iron-ion mediated mechanisms.

Comments:

a) Among these, the first is, in principle, capable of justifying all the effects reported, but it lacks of an experimental assessment of microthermal properties of the biological cells.

b) Conversely, each of the others is only able to partially explain some of the effects reviewed.

Apollonio et al., 2013

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Multiscale approach and molecular scale activation

The authors conclude that only through a multiscale methodology it is possible to perform a comprehensive study on nonthermal effects if a basic condition is fulfilled: the entire modeling has to be founded on a plausible hypothesis of processes that can be affected by the field at the molecular scale, thus acting as a first transduction step.

Once such a multiscale strategy has been accomplished, it will open the challenge of affordable and realistic in silico models, which could help researchers involved in bioelectromagnetics to design their activities on hypothesis driven experiments.

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Apollonio et al., 2013

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How to go further?

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How to go further?

The most effective approach is to develop a macromolecule dynamics model (MDM) which uses the information provided by the Protein Data Bank, e.g. the spatial co-ordinates of all the constituent atoms, as initial condition of the computer simulation. The MDM must include not only the interplay of all the endogenous forces acting on the atoms of the messenger molecule interacting with the atoms of a given macromolecule, but also the contribution of the solvent molecules (water) and of the exogenous forces due to the electromagnetic exposure.

Chiabera et al., COST 244 (Bordeaux, April 99)

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That means ”Virtual Experiment” !

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A rigorous approach : Molecular Simulations

- ✓ They provide a **realistic representation**, at atomic scale, of microscopic systems in their own environment (e.g. proteins in cell membrane, liposomes, biochemical reaction….)
- ✓ They provide a **dynamic description**, at atomic scale, of the *behavior* and the *properties* of microscopic systems

In Bioelectromagnetic research:

The big challenge of molecular simulations is to provide more insights into the interaction between EM fields and biological systems by means of **virtual experiments at molecular level**

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Methodology for virtual molecular experiments

a) Solve electromagnetic fields distribution:

Maxwell Equations

b) Describe biological matter in a rigourous way:

Schrödinger Equation

Our target is:

to link **Maxwell equations** to **Schrödinger *equation***

or better

to apply electric, magnetic, EM fields as exogenous stimuli to molecular behaviour using a **"First Principles"** quantitatitative approach

In conclusion

to solve Schrödinger Equation in the presence of EM Fields

Computational Chemistry

Methods that allow the study at molecular level of biological targets having different complexity

MM
(Molecular Mechanics)

$$\mathbf{F}_i = m_i \mathbf{a}_i \quad i = 1, 2, ..., N$$

Solving Newton equations

- MM methods allows the study of conformational and energetical dynamics of complex molecular systems

- **SD (Stochastic Dynamic)**
- **BD (Brownian Dynamic)**
- **MD (Molecular Dynamic)**

QM
(Quantum Mechanics)

$$H\Psi(r,t) = i\hbar \frac{\partial}{\partial t} \Psi(r,t)$$

Solving Schrödinger equation

- QM methods allows the study, usig a rigourous approach of biochemical reactions based on electro or proton transfers

- **DFT (Density Functional Theory)**
- **HF (Hartree Fock theory)**
- **post HF methods**
- **semiempirical methods**

System Complexity

10^5 atoms

10^3 atoms

10^2 atoms

Accuracy

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This approach can be applied to

These plausible hypotheses have been restricted to five:

1) selective/microthermal heating;
2) nonthermal effects on reactions;
3) action on hydrogen bonds in the hydration layer;
4) radical pair mechanism;
5) iron-ion mediated mechanisms

Two quick examples!

Species Used to Study True Navigation all have magnetic compasses

Sea Turtles

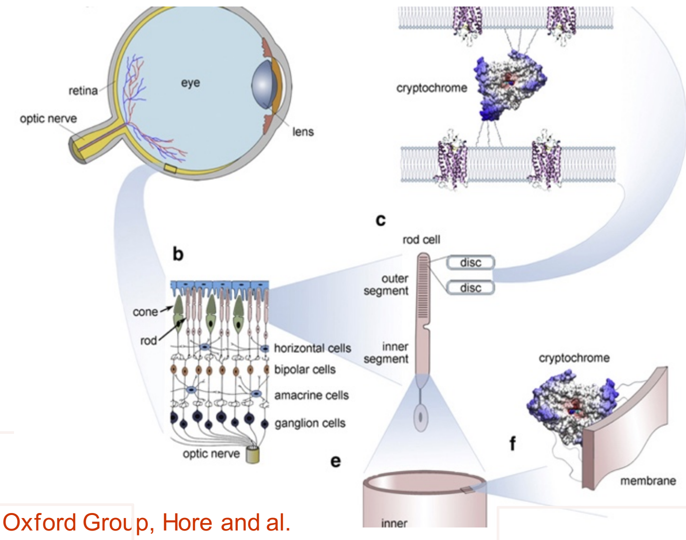
Homing Pigeon

Migratory Birds

Newts

Salmon

Chryptochrome



Oxford Group, Hore and al.

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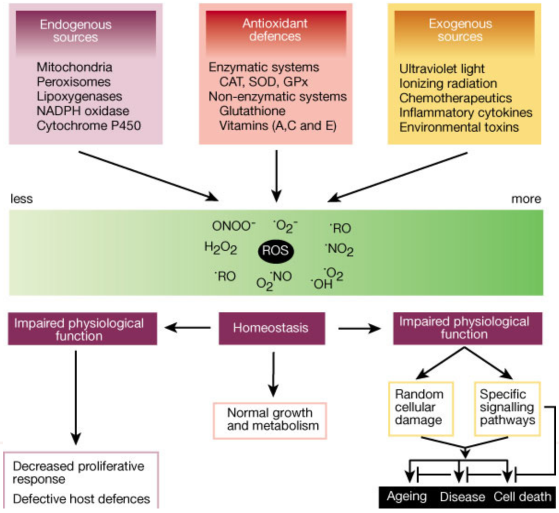
Reactive Oxygen Species (ROS)



Nature et al., 2000

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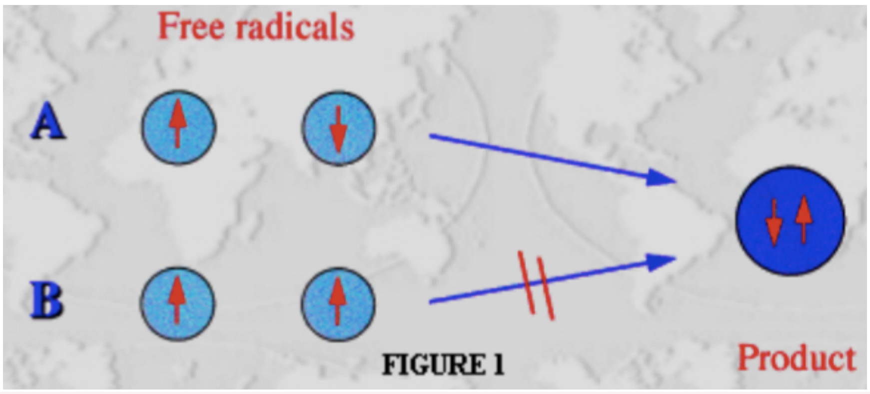

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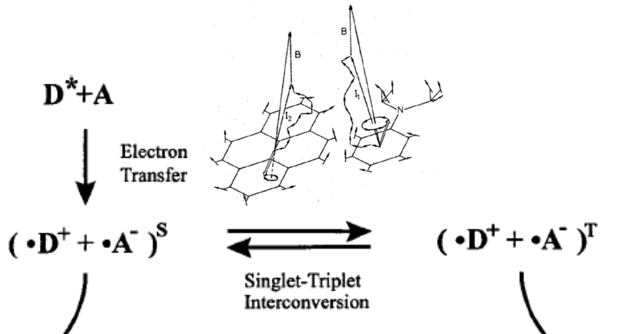
Radical-Pair Mechanism



$$H(\vec{B}) = H_1(\vec{B}) + H_2(\vec{B})$$

$$H_j(\vec{B}) = g\mu_B\vec{S}_j \cdot (\vec{B} + \mathbf{A}_j\vec{I}_j)$$

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Radical pair magnetic field effects

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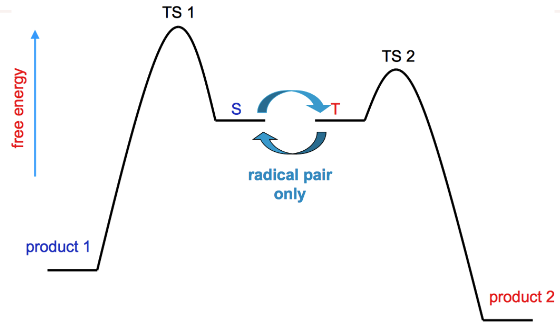

A radical pair, most generally, is a pair of molecules,each of which have an unpaired electron. If the radical pair is formed so that the spins on the two unpaired electrons in the system are entangled (i.e. they begin in a singlet or triplet state), and the reaction products are spin-dependent (i.e., there are distinct products for the cases where the radical pair system is in an overall singlet vs. triplet state), then there is an opportunity for an external magnetic field to affect the reaction by modulating the relative orientation of the electron spins.

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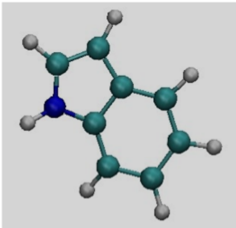
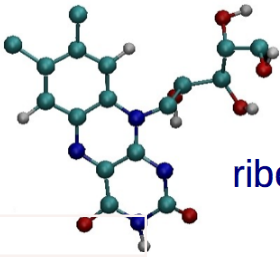
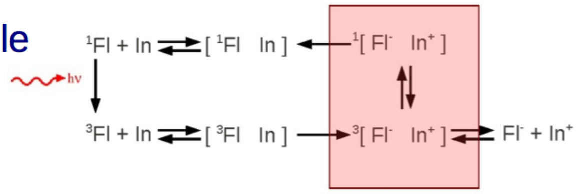
Indole and Riboflavin



indole



riboflavin



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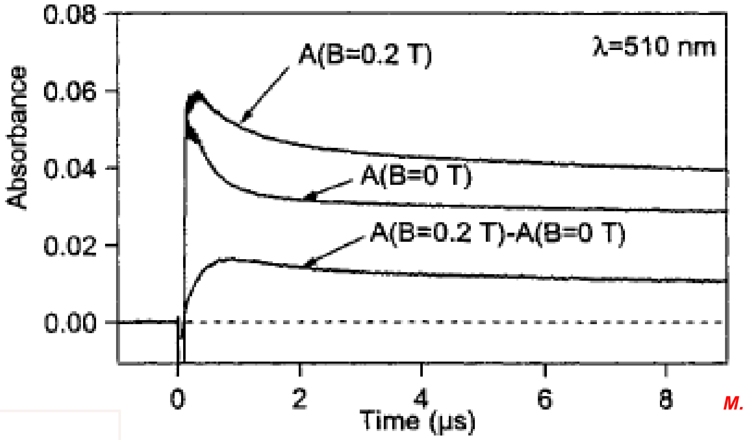
Why?

a) Small Molecules

b) Experimental results



M. Horiuchi, 2003

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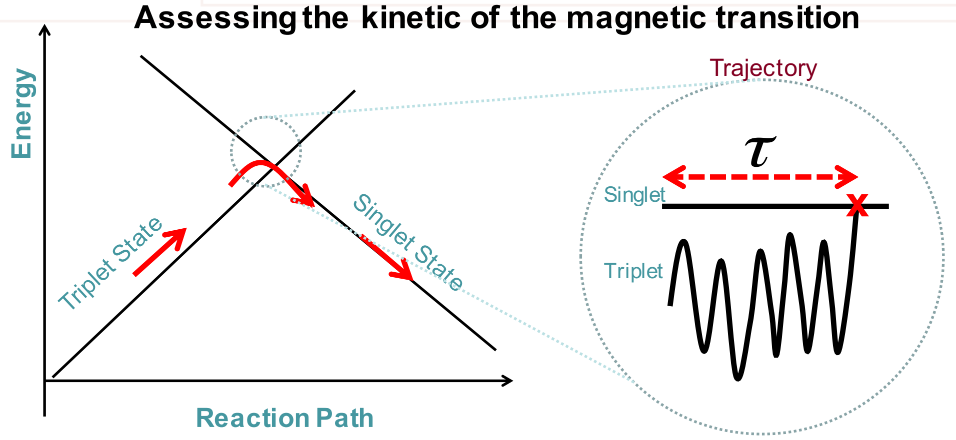
Assessing the kinetic of the magnetic transition



Crossing: Magnetic transition between the triplet and the singlet states

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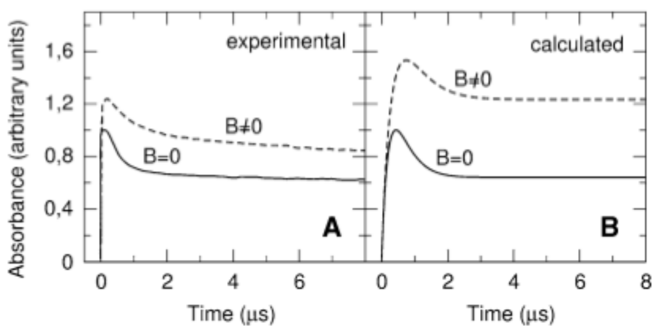

Fig. 5 Comparison between experimental and calculated data. *Panel A* absorption time profiles (normalized to 1) experimentally obtained by Horiuchi et al. [44] in the absence of an exogenous magnetic field (*continuous line*) and in the presence of a 200 mT magnetic field (*dashed line*). *Panel B* normalized concentration of the ionic reaction species as a function of time obtained by the calculated kinetic model and using the kinetic constants discussed in the Sect. 4. The kinetic constants were set both hypothesizing the absence (*continuous line*) and the presence (*dashed line*) of an exogenous magnetic field

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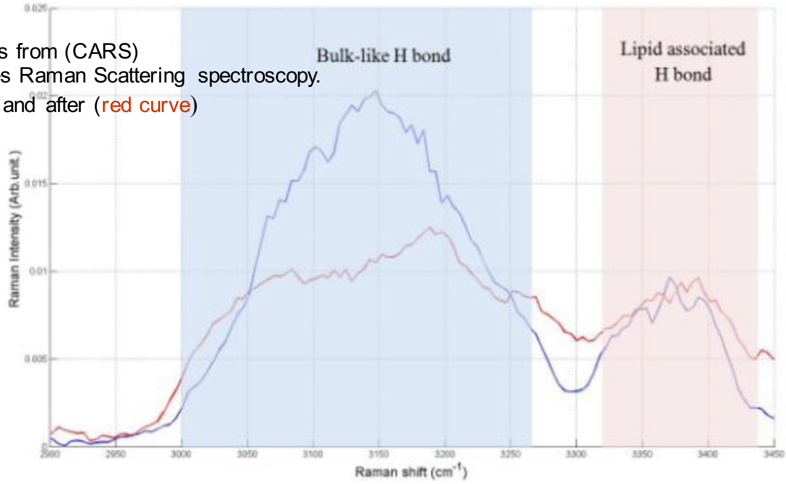
Lluis Mir and collaborators, AT-RASC 2015

Experimental results from (CARS)
Coherent Anti-stokes Raman Scattering spectroscopy.
Before (blue curve) and after (red curve)



Nanopulses on DC-3F cells (Chinese hamster lung fibroblasts)

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Lluis Mir and collaborators, AT-RASC 2015

After the delivery of the electric pulses, the relative intensities of these spectral components changed: the "lipid associated" band became dominant, whereas the "bulk-like" bands decreased (Figure 1).

The changes in the bands pattern are attributed to the alteration of the "bulk-like" hydrogen bonds and thus of the organization of the interfacial water molecules which are actually the first to be affected by the electric pulses delivery, as expected from the molecular dynamics simulations.

These results reinforce the mechanistic details in the numerical models and provide experimental information about the behavior of the interfacial water after electroporation.

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Thank You

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