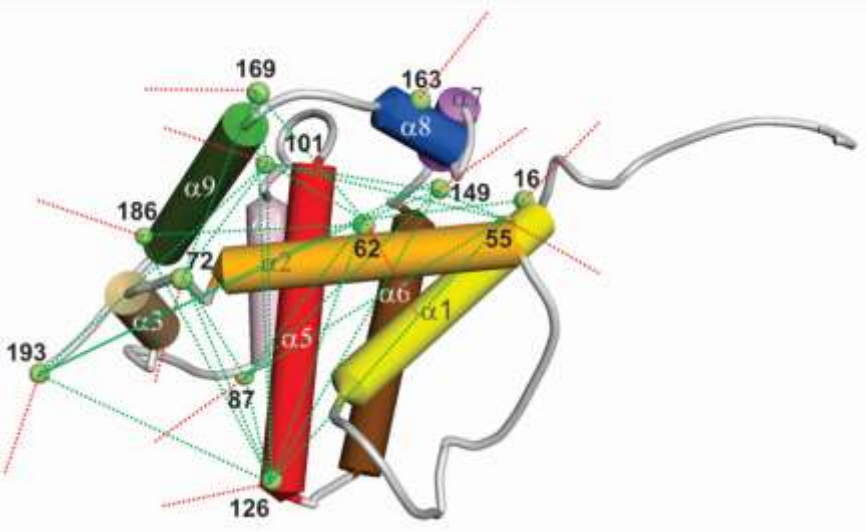
Freie Universität Berlin

Seminar: Selected Topics in Physics

Spectroscopic methods to extract intermolecular distances in biomolecules



Enrica Bordignon

Seminar

Freie Universität Berlin

Dates: Tuesdays at 16:15 in room in 1.4.03
In my office at 0.4.31 – Help for preparation of talks (arranged via e-mail)

Content: Spectroscopic methods to extract intermolecular distances in biomolecules

- After all talks: Analysis of advantages and disadvantages of different biophysical methods to monitor conformational changes in proteins, RNA and DNA.
- Talk: Introduction to one method and description of one application.

Seminar web page:

- http://www.physik.fu-berlin.de/einrichtungen/ag/ag-bordignon/Courses

- **In the web page you find:**
- Title and schedule of talks
- Abstract of talks (to be provided by the students - 1 page + figure + references)
- Slides (login/psw required)

Goals

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1. **How to give a good presentation**

- Clear structure of the talk
- Clear expression of ideas
- Slides with good visualization of the topic

2. **How to look for literature on a given topic**

- Of course Google and Wikipedia help a lot, but beyond that scientific literature is important (try Google scholar instead of Google)
- Use scientific search machines (e.g. ISI web of knowledge, pubmed)

3. **How to extract information from scientific literature**

- As you are not a specialist in the field, try to look for the keywords and from there understand the main claims of the paper
- Further look for more papers on the interesting keywords
- Look up cited papers so create your background (especially review)

4. **How to contribute to discussions**

- Ask questions!!!
- Give constructive criticism to the presentation

The seminar consists of:

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- **Preparation of a Seminar:**
  - Selection of an appropriate spectroscopic method (agreed with E Bordignon)
  - Selection of an interesting paper covering theoretical aspects or applications of the method of choice (agreed with E Bordignon)

  - Search for information on the spectroscopic technique to provide a brief but complete theoretical introduction on the spectroscopic method
  - Search for information on the specific topic of the paper
  - Identification of relevant concepts (what was new in the paper, why the technique was used?)
  - Preparation of a coherent Seminar: Introduction to the method, to the topic, to the results, conclusions, advantages/disadvantages of the method used

- **Announcement and advertisement of the seminar**
  - Email to all member of the Seminar one day in advance. The mailing list will be distributed. If you are not registered, please send an e-mail to E. Bordignon asap!
- **Presentation**
- **Questions and discussions on the content and style of presentation**
- **Questions raised to the presentations of others**

The grade depends on:

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- **Preparation of a Seminar: 40%**
  - Selection of an appropriate topic
  - Search for information,
  - Identification of relevant concepts,
  - Preparation of a coherent Seminar

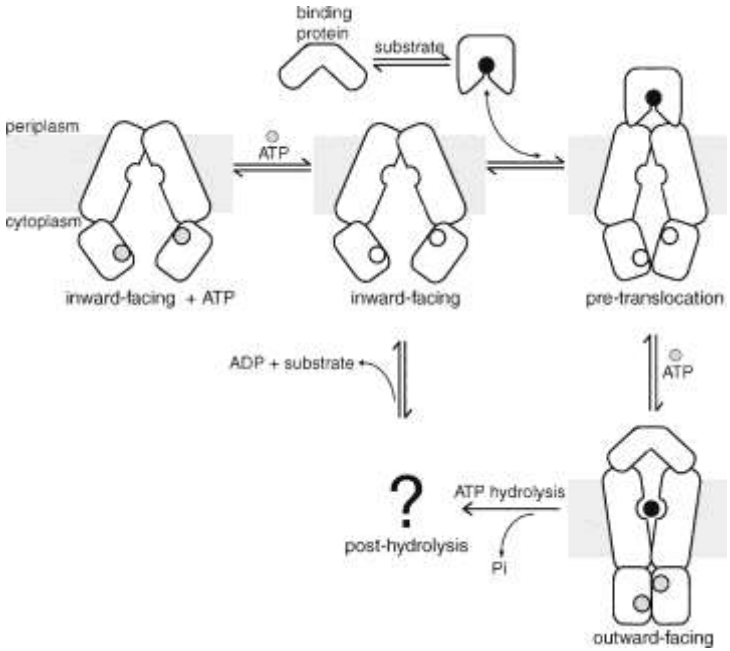
- **Announcement and advertisement of the seminar 5%**
  - Email to all member of the Seminar one day in advance

-**Presentation: 40%**
  - Quality of the presentation of the Seminar

- **Questions and discussions on the content presented**
- **Questions raised to the presentations of others**

15%

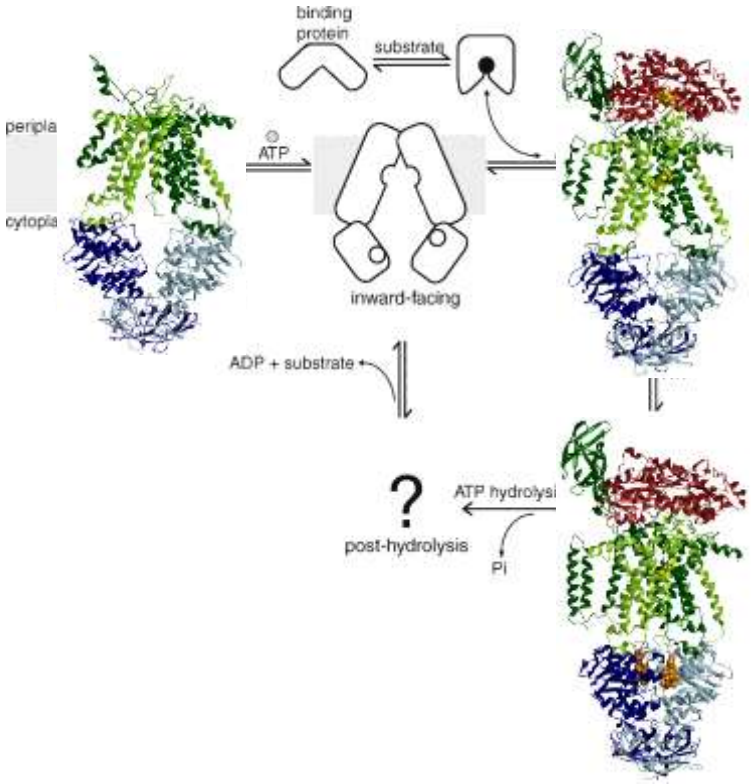
Why distances in biomolecules?



- Proteins are molecular machines: conformational changes necessary for function
- Suggested mechanism of action

Crystal Structure of the Maltose Transporter in a Pretranslocation Intermediate State
Oldham and Chen, Science, 332(6034)1202-1205

Why distances in biomolecules?



► in **few** cases it is possible to obtain crystal structures of different states

►Different snapshots in the conformational cycle

►X-ray: full atomistic detail, distances between all atoms are measured simultaneously in a crystal. NMR: more phsysiological environments but limitations.

Crystal Structure of the Maltose Transporter in a Pretranslocation Intermediate State
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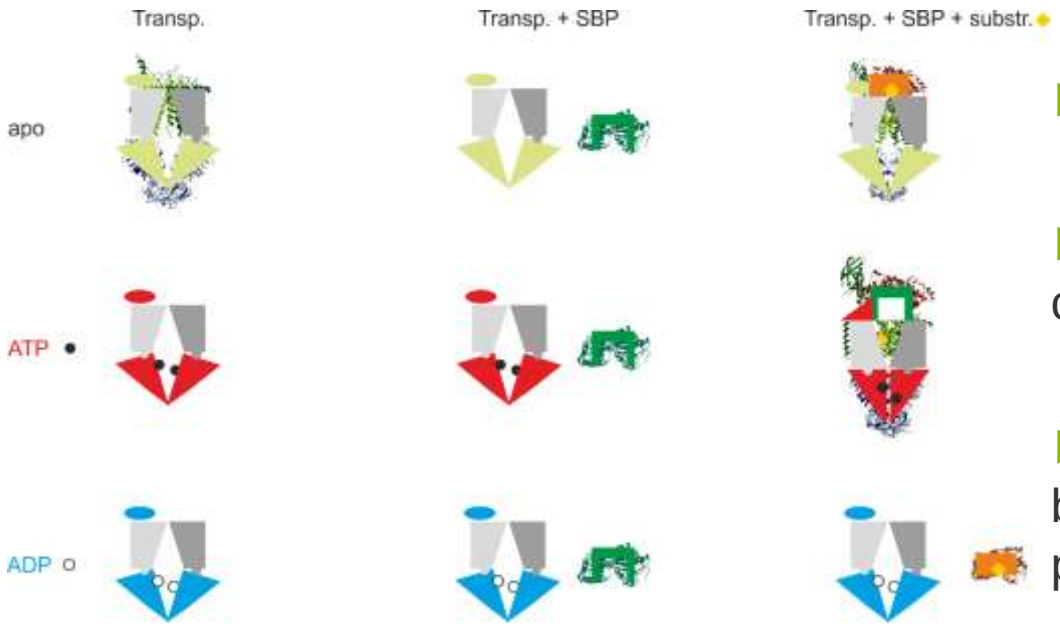
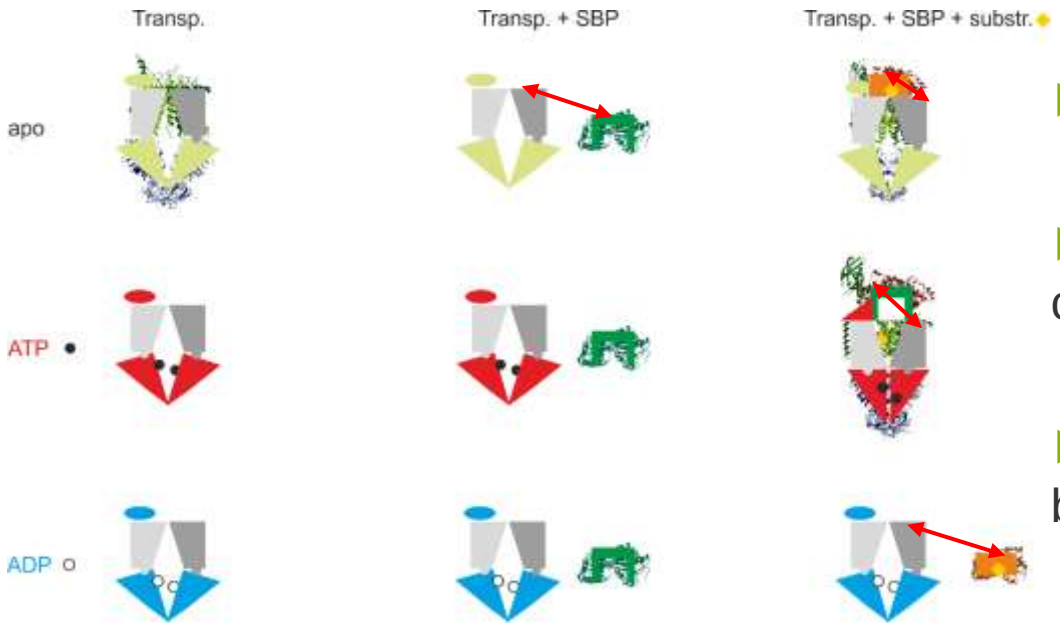
Why distances in biomolecules?



- still… missing states
- Different methods to monitor conformational changes
- Not necessarily full atomistic details, but coarse grained information in physiological environment

Why distances in biomolecules?



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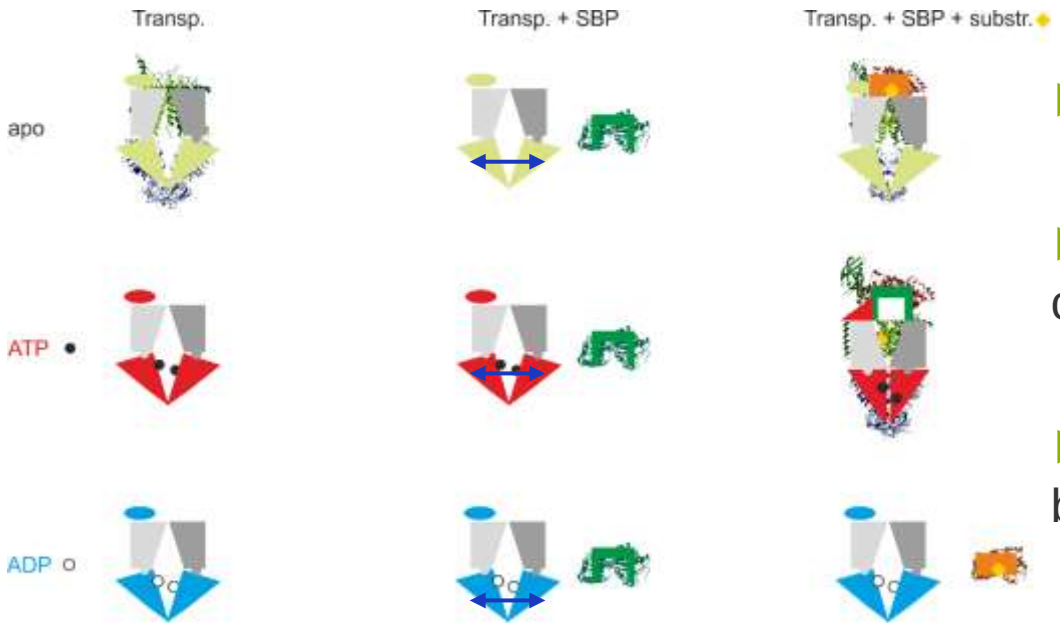
Why distances in biomolecules?



- still… missing states
- Different methods to monitor conformational changes
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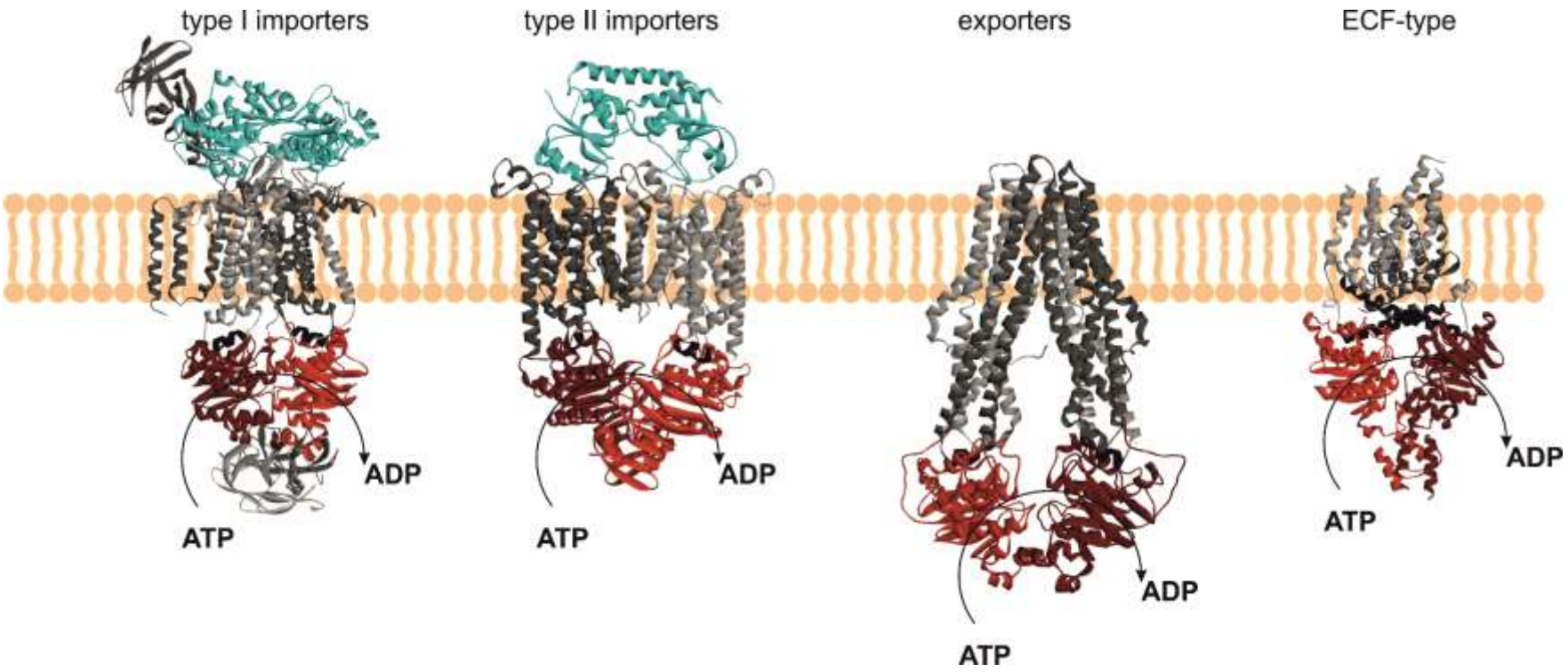
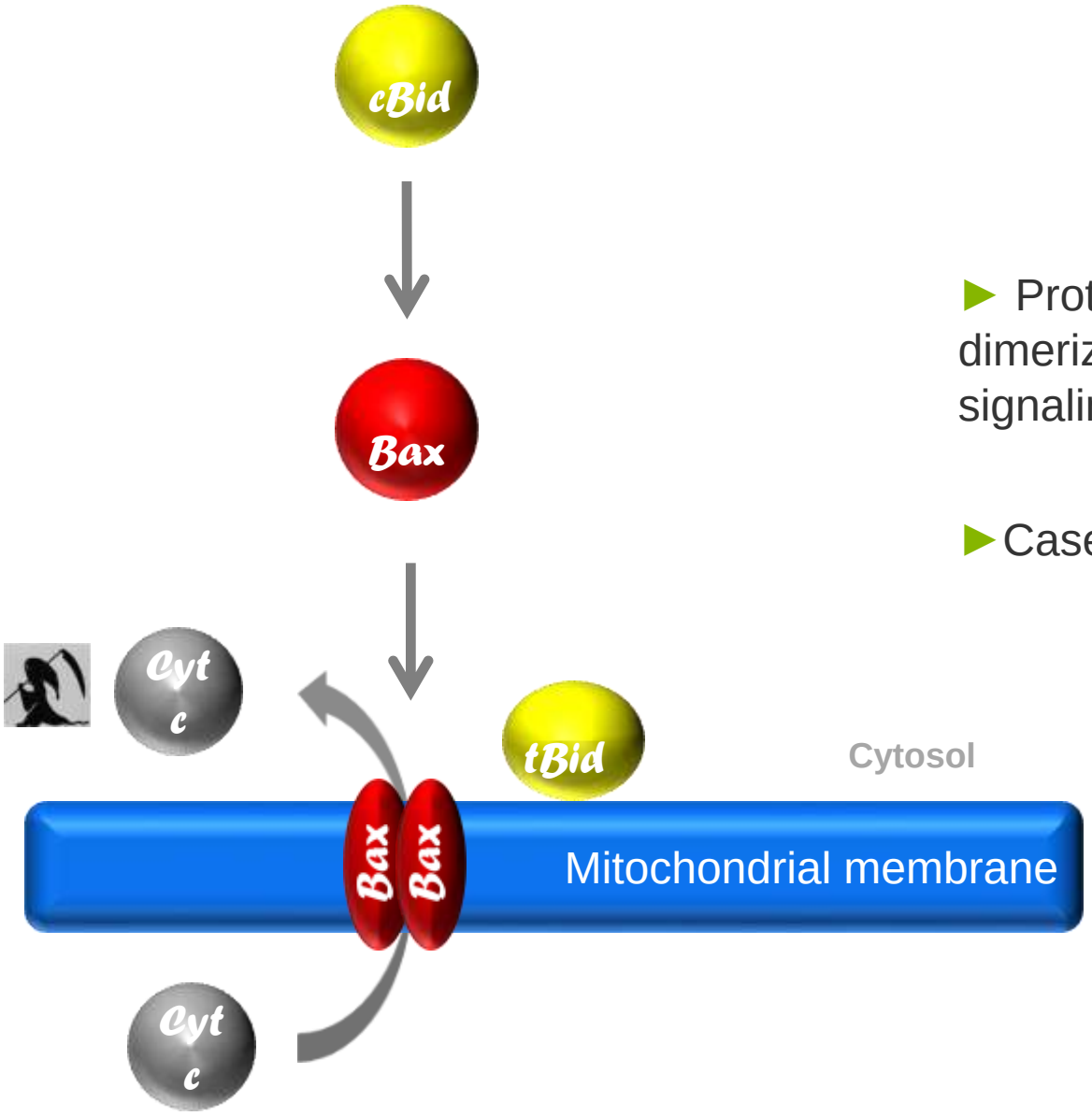
Why distances in biomolecules?



- several proteins for which some X-ray structures are available, 4 different types of transporters: different mechanisms?
- Follow conformational changes via distance measurements

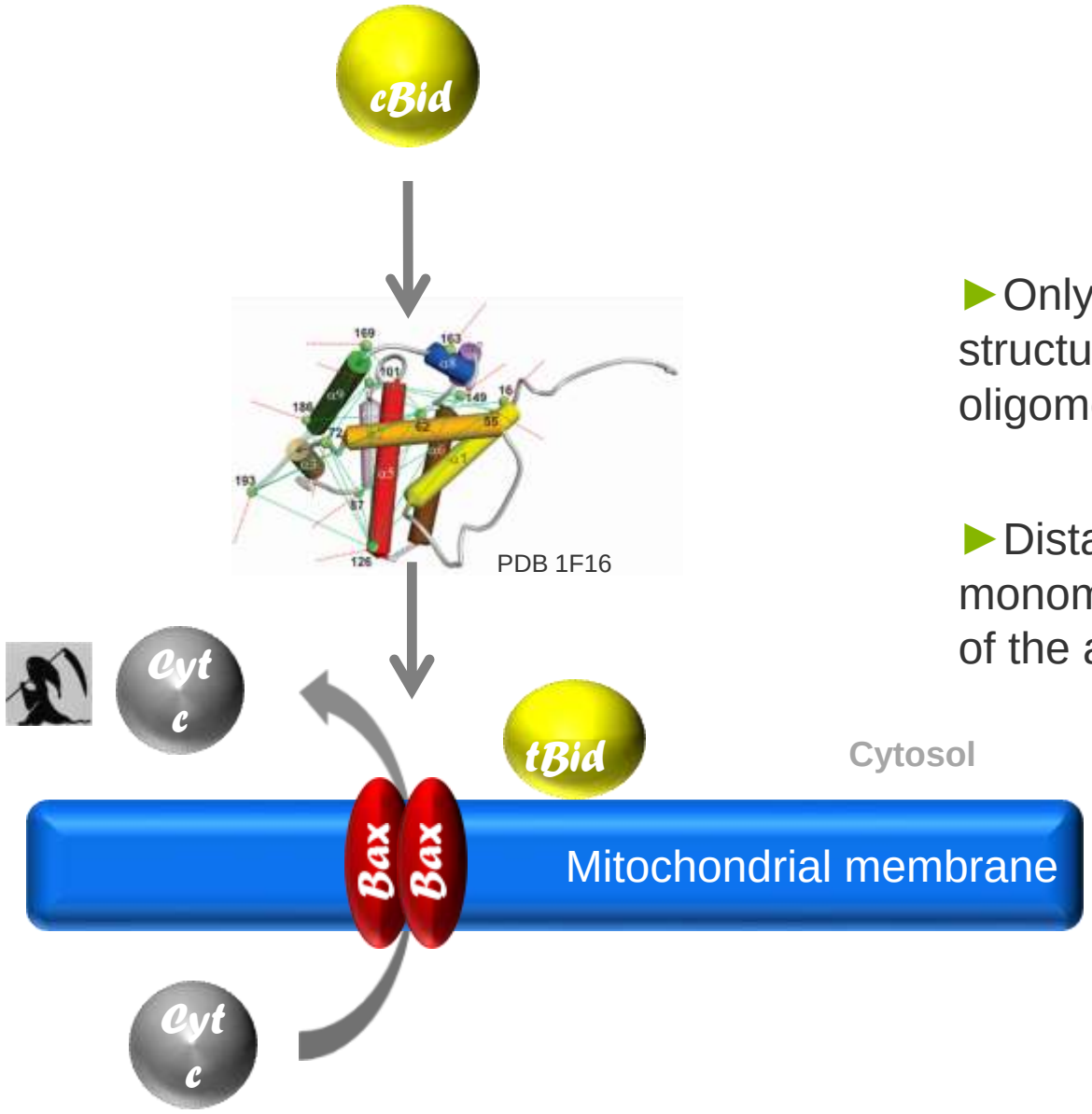
Why distances in biomolecules?



- Protein-protein interactions: dimerization-oligomerization mediates signaling

- Case study: apoptotic protein Bax

Why distances in biomolecules?

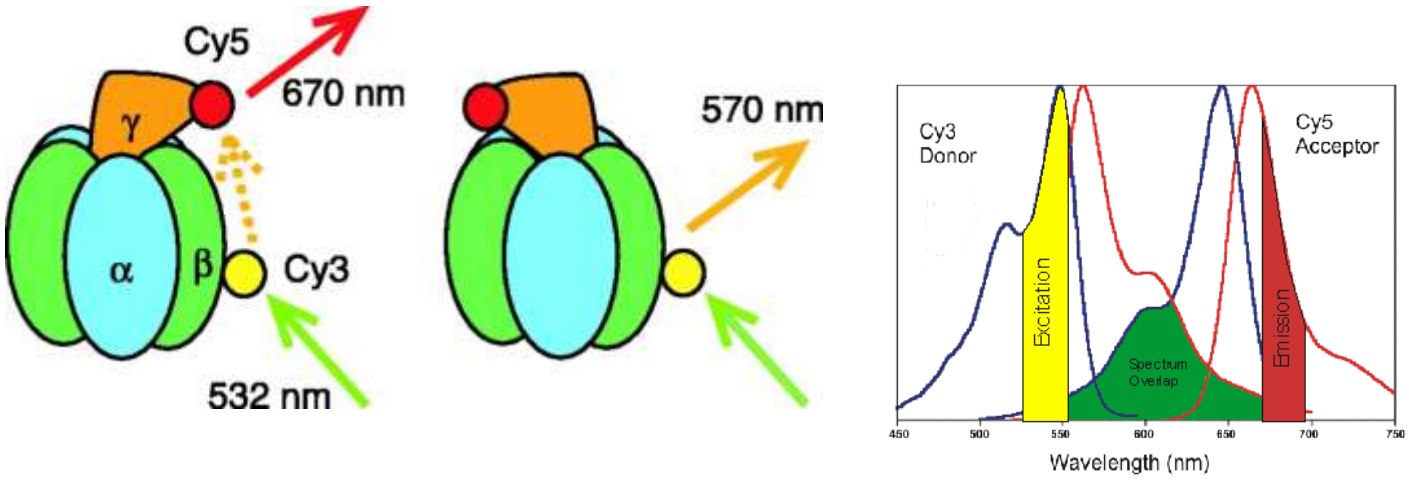


- Only the inactive monomeric structure is known (NMR): how is the oligomer built?

- Distance information between monomers can elucidate the structure of the active conformation

How to measure distances? Examples- FRET

If the two fluorophores are physically close together, the donor can transfer its energy to the acceptor, which radiates according to its emission spectrum.



- FRET (Förster Resonance Energy Transfer)
- Measuring distances between donor and acceptor molecules
- Fluorescent probes in biomolecules

FRET

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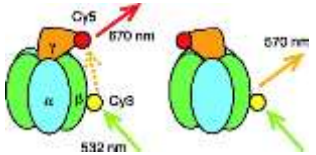
Zwischenmolekulare Energiewanderung und Fluoreszenz

Von Th. Förster

Annalen der Physik. 6. Folge. Band 2. 1948

matching of excitation / emission spectra
close physical proximity.
alignment of dipole moments



The FRET efficiency (fraction of energy transferred) is $E_{\mathrm{FRET}} = \dfrac{1}{1+(R/R_0)^6}$

where the Forster radius R_0 is $R_0(\text{Å}) \equiv \left(8.79 \times 10^{-5} Q_D n^{-4} \kappa^2 J\right)$

quantum efficiency, index of refraction, orientation between fluorophores

and the spectral overlap integral is $J(M^{-1}\mathrm{cm}^{-1}\mathrm{nm}^4) \equiv \dfrac{\int \epsilon(\lambda) f(\lambda) \lambda^4 \, d\lambda}{\int f(\lambda) \, d\lambda}$

extinction coefficient, emission spectrum, wavelength

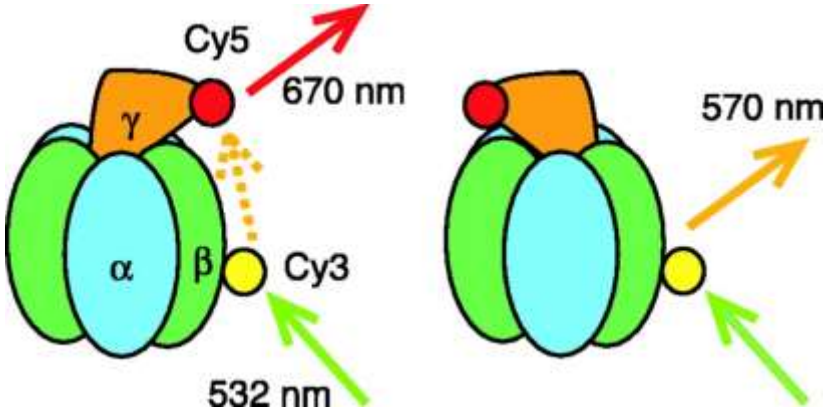
Typically, R_0 is between **20 to 100 Å**.

FRET

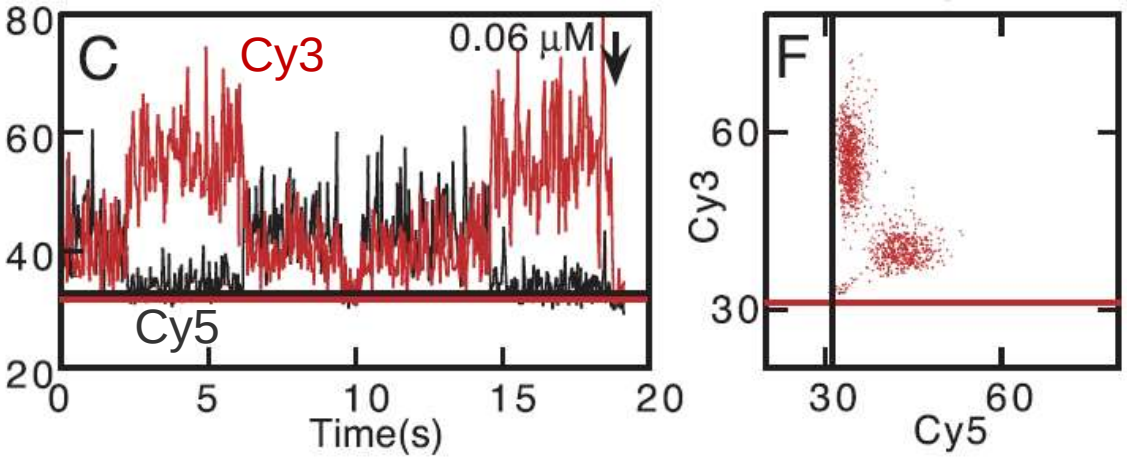
Freie Universität Berlin

The ATP-waiting conformation of rotating F_1-ATPase revealed by single-pair fluorescence resonance energy transfer

Ryohei Yasuda*†, Tomoko Masaike‡§, Kengo Adachi¶, Hiroyuki Noji‖, Hiroyasu Itoh**, and Kazuhiko Kinosita, Jr.¶



Alternate stepwise intensity changes with ATP. Rotation of the gamma subunit.



How to measure distances? Examples- LRET

If a lanthanide is a donor (Tb^{3+}, Eu^{3+}), the resonance energy transfer to an acceptor dye can be measured with higher accuracy.

IEEE JOURNAL OF SELECTED TOPICS IN QUANTUM ELECTRONICS, VOL. 2, NO. 4, DECEMBER 1996

Lanthanide-Based Resonance Energy Transfer

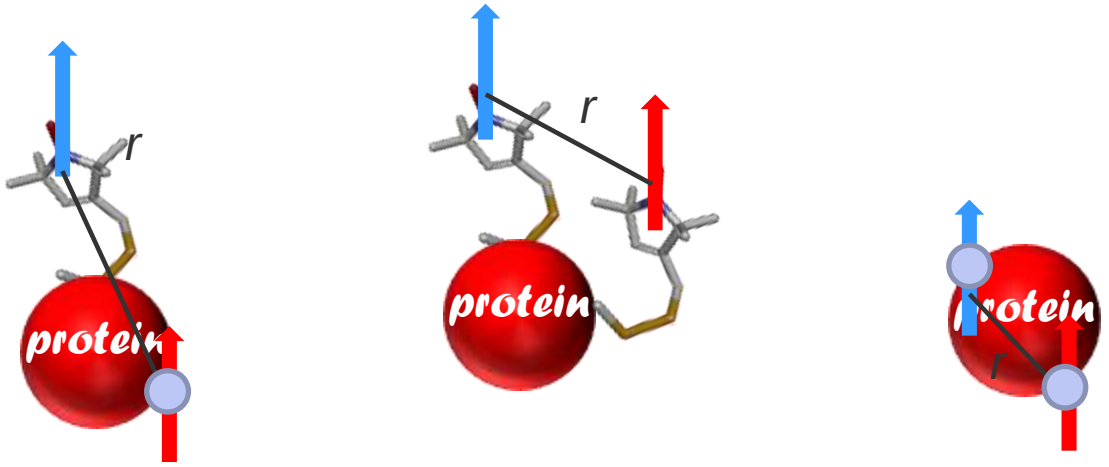
Paul R. Selvin

- LRET (lanthanide-based resonance energy transfer)

- Measuring longer distances with higher precision (larger R_0, less geometrical factor problem, close to orientational average 2/3!)

How to measure distances? Examples- EPR

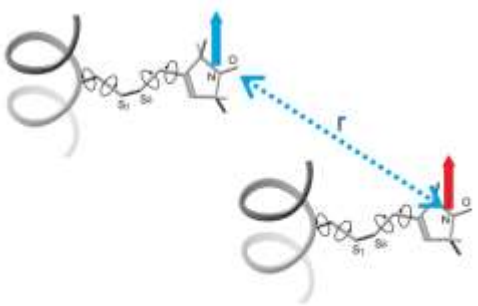
If the two paramagnetic centers are physically close together, the dipolar interaction can be measured via cw or pulsed EPR techniques.

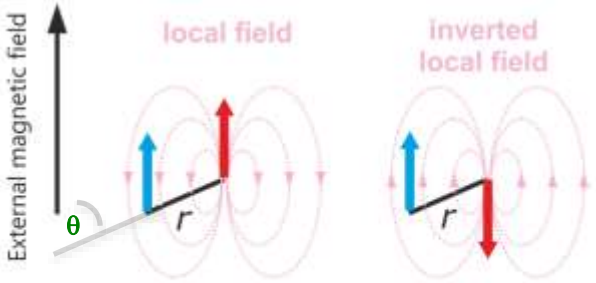


- EPR (Electron paramagnetic resonance)

- Measuring distances between unpaired electrons (metals, or spin labels attached via engineered cysteines)

EPR: Double Electron Electron resonance

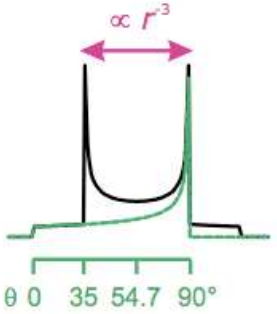




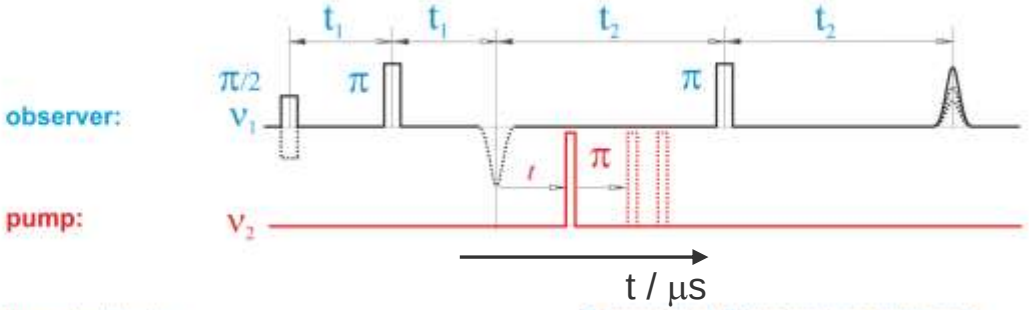
dipolar coupling

$$\nu_{dd}(\theta) = (1 - 3\cos^2\theta)d$$

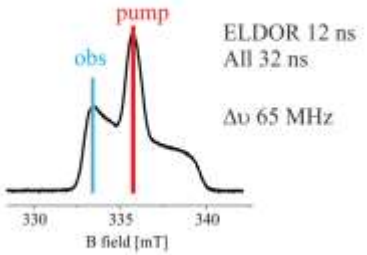
$$d = \frac{1}{r^3}\frac{\mu_0}{4\pi h} g_1 g_2 \mu_B^2$$



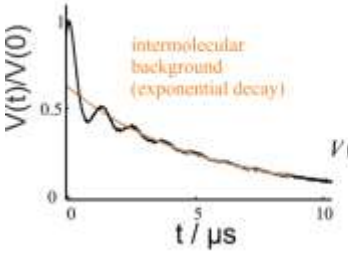
DEER sequence



X-band EPR spectrum



Primary experimental data



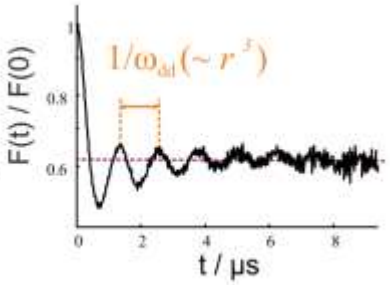
$$V(t) = F(t)B(t)$$

$$F(t) = 1 - \lambda_{AB}[1 - \cos(\omega_{dd}(\theta_{dd}, r_{AB})t)]$$

$$B(t) = \exp(-kt^{D/3})$$

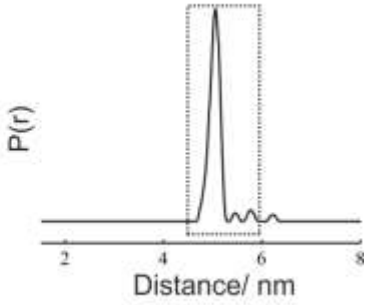
background correction

Separated intramolecular part



Tikhonov regularisation

Distance distribution



Pannier et al. (2000) JMR 142(2):331-340; Jeschke, G. Annu. Rev. Phys. Chem. (2012) 63:419–46

EPR: Double Electron Electron resonance

doi:10.1016/j.jmb.2009.08.050 J. Mol. Biol. (2009) 393, 586–597

JMB

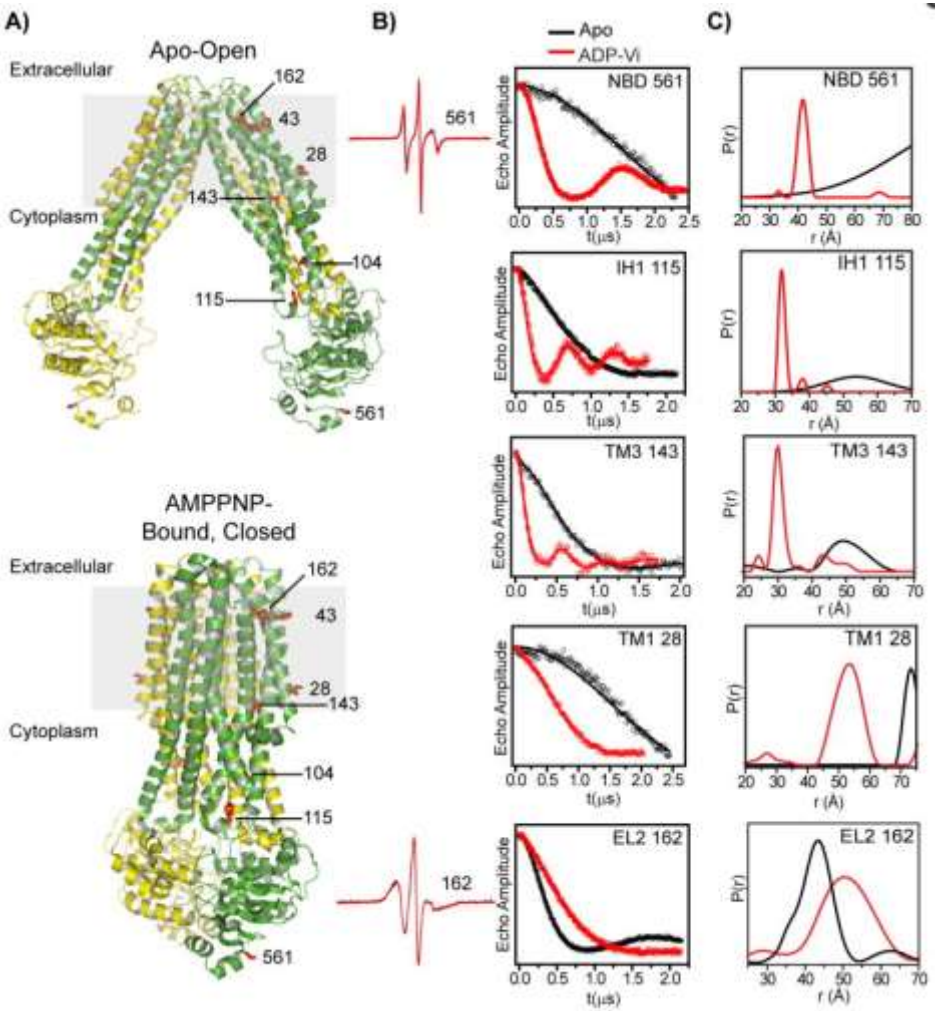
Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

Conformational Cycle of the ABC Transporter MsbA in Liposomes: Detailed Analysis Using Double Electron–Electron Resonance Spectroscopy

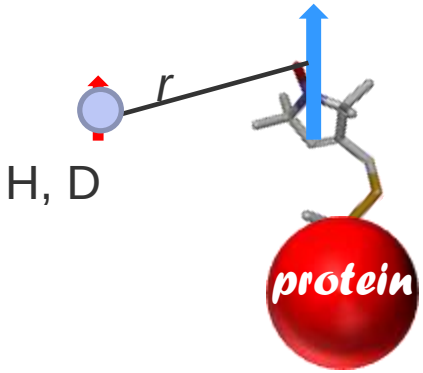
Ping Zou, Marco Bortolus and Hassane S. Mchaourab*

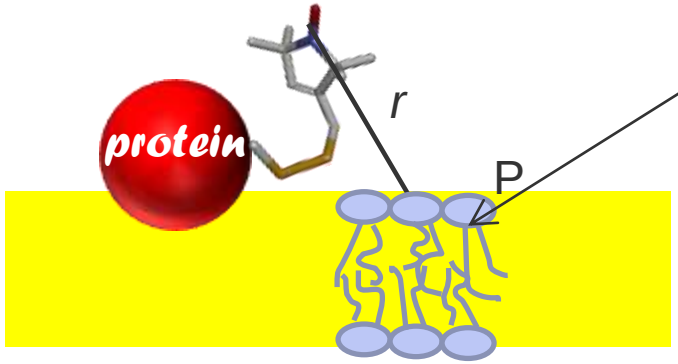


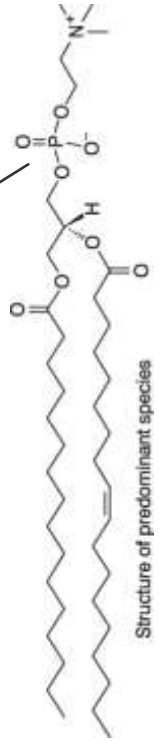
- Distance range from 1.5 to 10 nm!

How to measure distances? Examples- EPR

If one unpaired electron is physically close to an NMR active nucleus (protons, deuterons, ^{31}P), the distance can be measured by ESEEM or ENDOR.







PC, Avanti Polar Lipids

- ESEEM (Electron spin echo envelope modulation)
- ENDOR (Electron nuclear double resonance)

- Measuring distances between unpaired electrons and nuclei

ESEEM

492 Biophysical Journal Volume 90 January 2006 492–505

Utilizing ESEEM Spectroscopy to Locate the Position of Specific Regions of Membrane-Active Peptides within Model Membranes

Raanan Carmieli,* Niv Papo,† Herbert Zimmermann,‡ Alexey Potapov,* Yechiel Shai,† and Daniella Goldfarb*
*Departments of Chemical Physics and †Biological Chemistry, The Weizmann Institute of Science, Rehovot, Israel 76100; and ‡Max-Planck Institute for Medical Research, Heidelberg, Germany

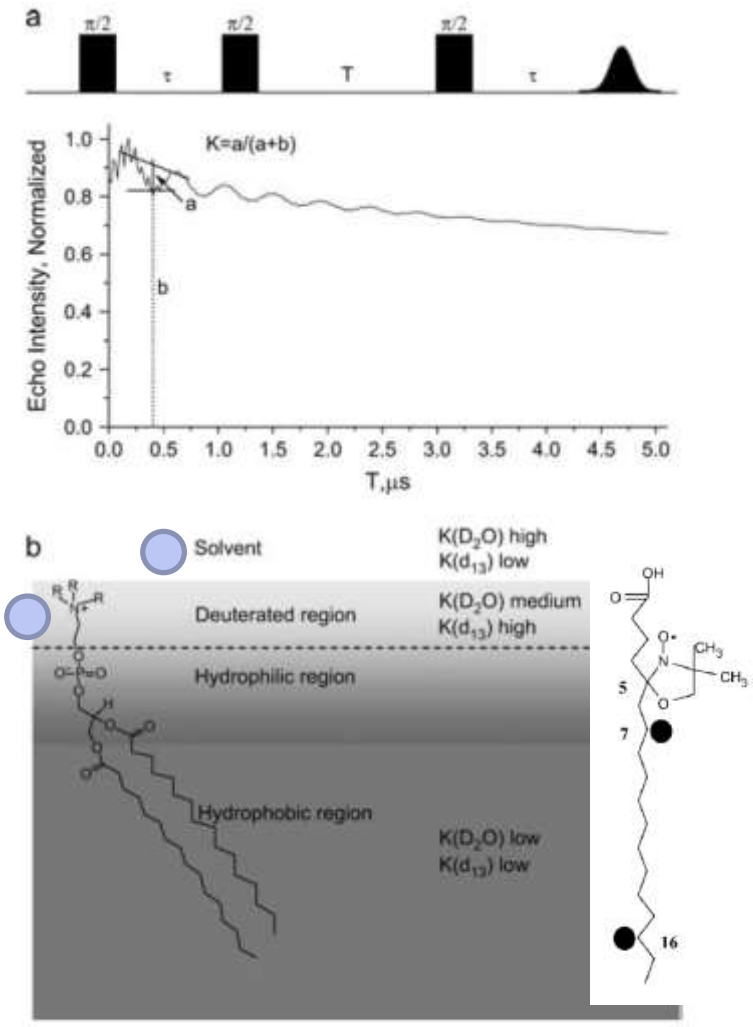


FIGURE 1 (*a*) The three-pulse ESEEM sequence and the ESEEM waveform with the definition of the modulation depth, $K(^2H)$. (*b*) A schematic representation of the various regions of the model membrane and the expected modulation depth for a spin label located within the different regions. The gradient in the gray color distinguishes between the hydrophilic and hydrophobic regions.

- water-membrane boundaries

with deuterated environments

ENDOR

492 Biophysical Journal Volume 90 January 2006 492–505

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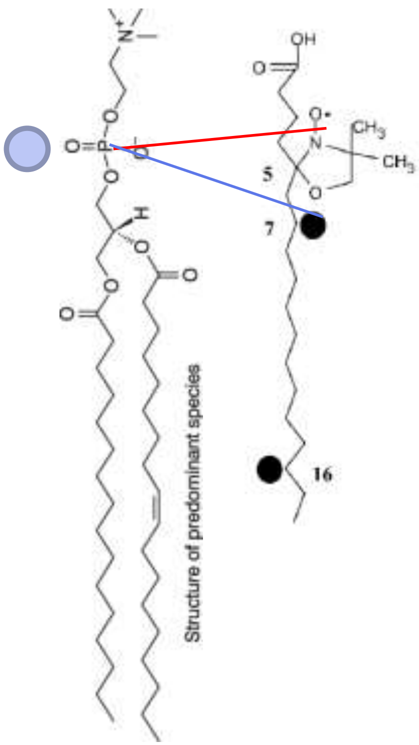
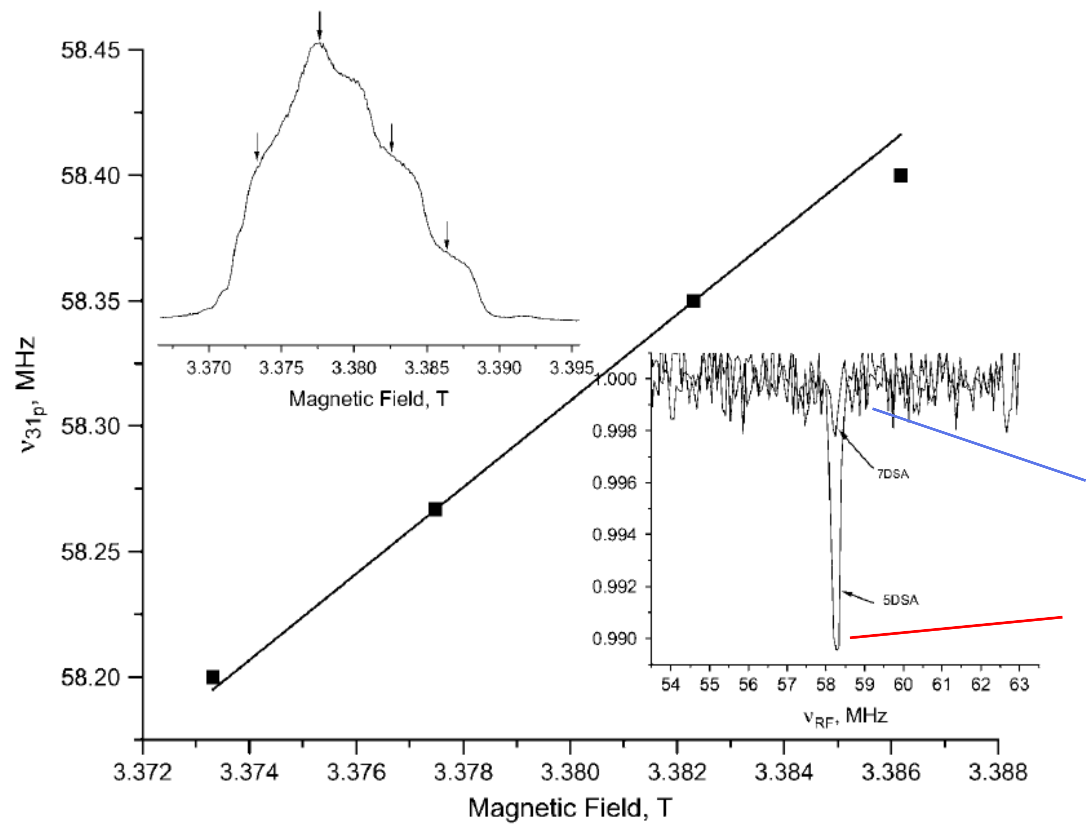
Raanan Carmieli,* Niv Papo,[†] Herbert Zimmermann,[‡] Alexey Potapov,* Yechiel Shai,[†] and Daniella Goldfarb*
*Departments of Chemical Physics and [†]Biological Chemistry, The Weizmann Institute of Science, Rehovot, Israel 76100;
and [‡]Max-Planck Institute for Medical Research, Heidelberg, Germany



PC, Avanti Polar Lipids



► positioning the spin label with respect to the membrane using the phospholipid headgroup

How to measure distances? Examples- PRE-NMR

If a paramagnetic center (metal or spin label) is close to a nucleus, it will induce an increase in the nuclear relaxation rates via magnetic dipolar interactions

4108 Chem. Rev. 2009, 109, 4108–4139

Theory, Practice, and Applications of Paramagnetic Relaxation Enhancement for the Characterization of Transient Low-Population States of Biological Macromolecules and Their Complexes

G. Marius Clore*,† and Junji Iwahara*,‡

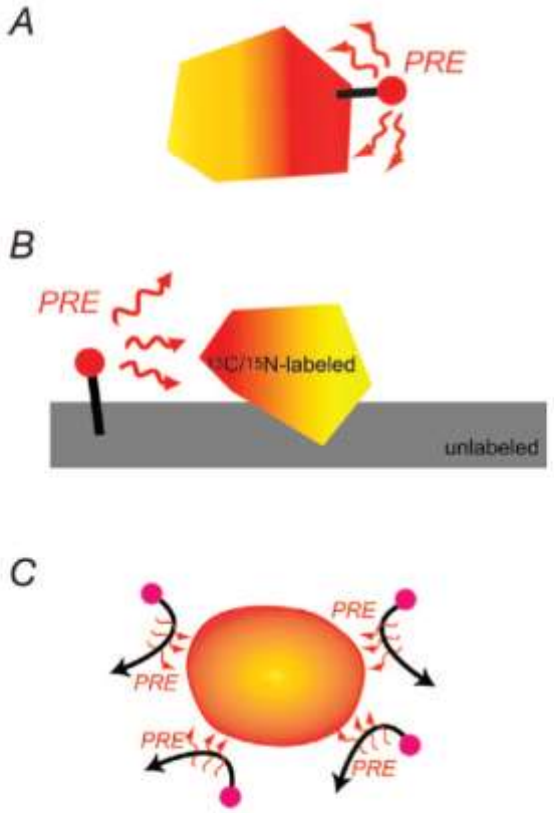


Figure 8. Three types of PRE: (A) intramolecular PREs arising from the paramagnetic group within the same molecule; (B) intermolecular PREs arising from the paramagnetic group located on the interaction partner; (C) solvent PREs arising from random collisions between a macromolecule and paramagnetic cosolute molecules.

- PRE-NMR (Paramagnetic relaxation enhancement NMR)
- $1/R^6$ dependence as in FRET, up to 3.5 nm

How to measure distances? Keywords…

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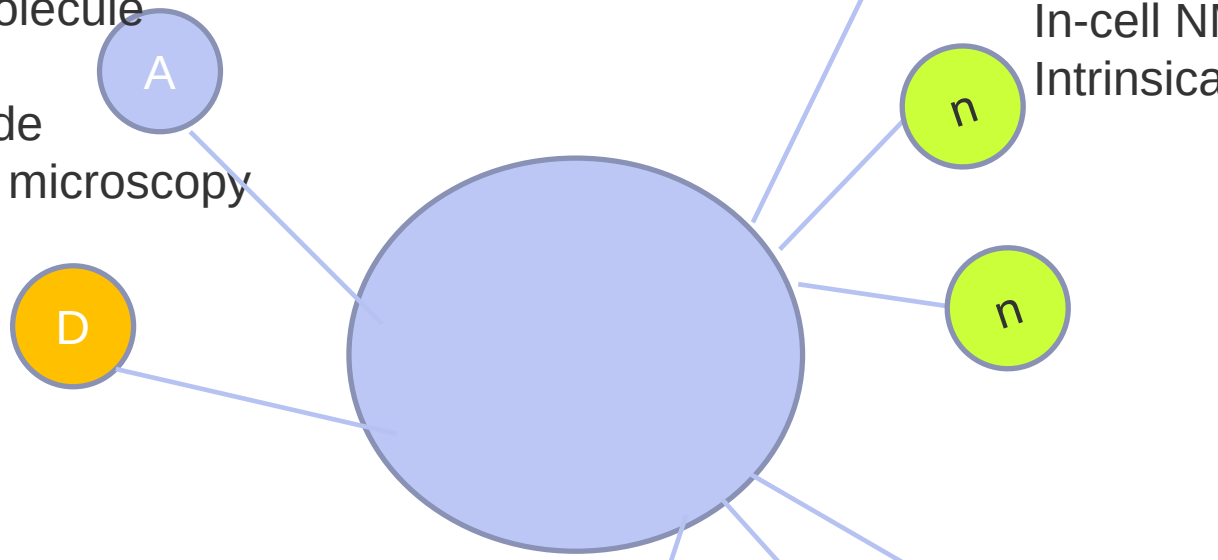


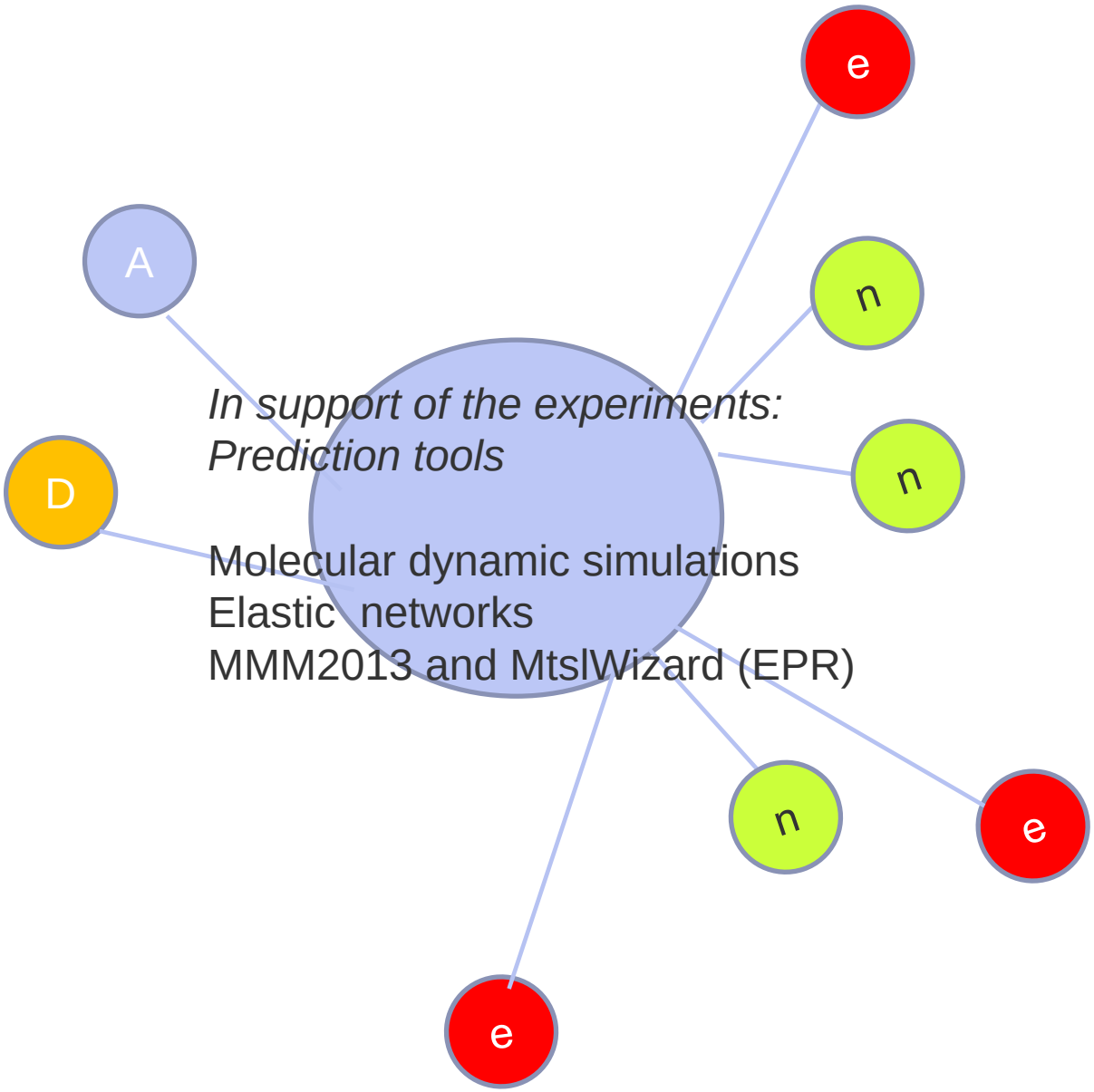
FRET
LRET
Fluorophores
Single molecule
Tags
Lanthanide
Confocal microscopy

NMR,
PRE NMR,
DNP
NMR and EPR and structure
In-cell NMR
Intrinsically disordered proteins

EPR
In-cell EPR
DEER-PELDOR
ESEEM
ENDOR
Labels
Structure protein
Membrane protein
Gadolinium
Copper
Iron
Nitroxide
Photoreceptor
Transient EPR

Saturation recovery EPR
gxx Azz proticity
H-bonds
Metal coordination
HYSCORE

How to simulate distances? Keywords…



Milestones

- **3->2 weeks** before the seminar: Topic selected and agreed
- **3->2 weeks** before seminar: Look for literature and draw an outline of the talk
- **2->1 weeks** before your talk: Prepare your talk
- **1 Week** before your talk: Discuss talk and literature used for your presentation with Enrica Bordignon
- **1->0 Weeks** before the seminar: Prepare final version of your talk
- **1 Day** before your seminar: Practice the talk loudly several times; be critical!

How to look for literature on a given topic

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Sources of information:

- Books
- Review articles
- Scientific articles
- (Wikipedia cannot be a source: not complete or even wrong)

How to find information (with keywords):

- Library
- (Google) Google scholar
- ISI web of knowledge (www.isiknowledge.com)
- Pubmed (pubmed.org)

How to extract information from scientific literature

- Extract which are the important keywords
- Try to understand their meaning
- Look for complementary literature if you are not sure that you understood everything. Usually cited papers are useful.
- Usually, review articles are a good starting point for a new topic.

Preparation:

- Who is in the audience? What do they know about the topic? What might they be most interested in?

- What is the goal of your talk? What to you want to explain to the audience? What do you want to convince them of?

- How much time do you have for your presentation?

Preparation:

- Look for the literature to support your arguments.

- Try to find a story to tell. Give an intro on the technique so that the audience will be able to understand what you say!

- Think of the structure your talk, in which the connections of the different steps are obvious.

- Look for pictures to support your ideas. A good picture says more than thousands words.

Structure of a presentation

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First slide:

- Title and author
- Maybe attractive sketch which illustrates the main focus of your presentation
- Start with a short synthesis of the main point of your talk

Motivation:

- You have to catch the interest of the audience: a nice introduction to the topic helps the audience to identify the interesting aspects of your topic and to fit your talk in a field.

Outline of your talk:

- Define the structure so that the audience can discover the red line in your talk and grasp the important steps.

Develop the structure:

- Make the different steps coherent: not just a sequence of unrelated topics.
- Develop a red line, one idea should grow from another.
- If partial conclusions are obtained, you can make a summary after each part.

Summary:

- A single sentence about the main point of the talk stays easier in memory than a mere repetition of what you have presented.

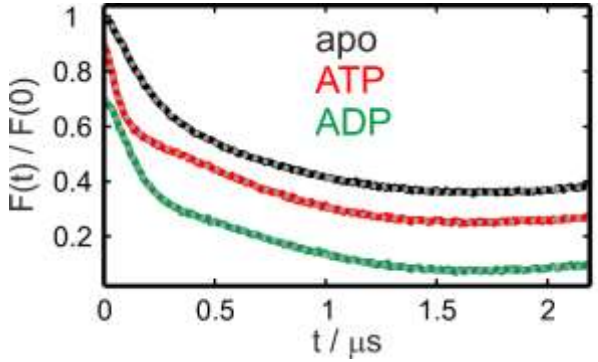
Layout

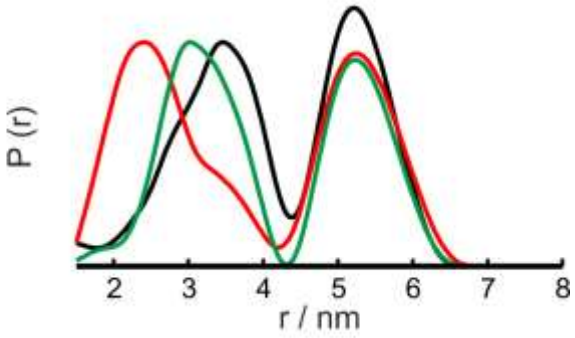
Small amount of information

if you want your audience to absorb it.

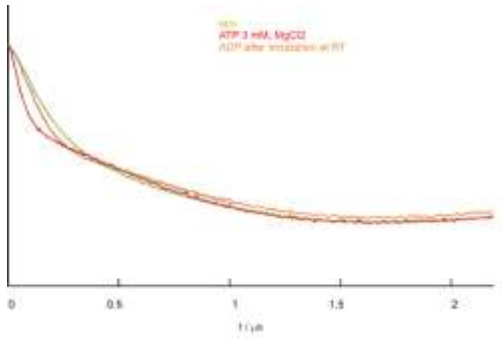
Layout

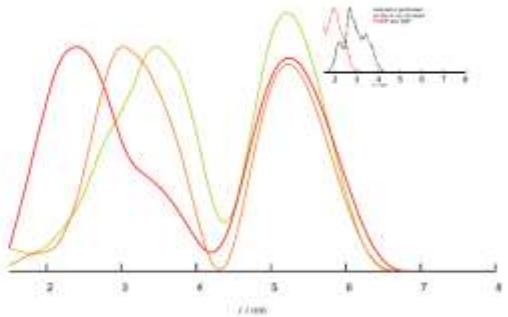
- Use plots (readable)





- Unreadable: lines too thin, labels on the axes and legends too small or absent, small symbols and fonts





Structure of the slides: some hints

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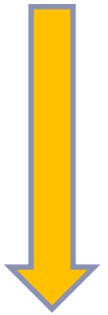
- In order to enhance the impact of your slides you may with:
  - animations
  - But use them carefully!



Dates

Freie Universität Berlin

The seminars (30 min plus discussion) will take place on **Tuesdays 16-18** in 1.4.03

date	Seminar speaker	Spectroscopic method	Title
14/10	E. Bordignon		Guidelines and selection of topics
website			