CHARACTERIZATION OF A BIOLOGICAL SYSTEM AS RADIATION BIOSENSOR

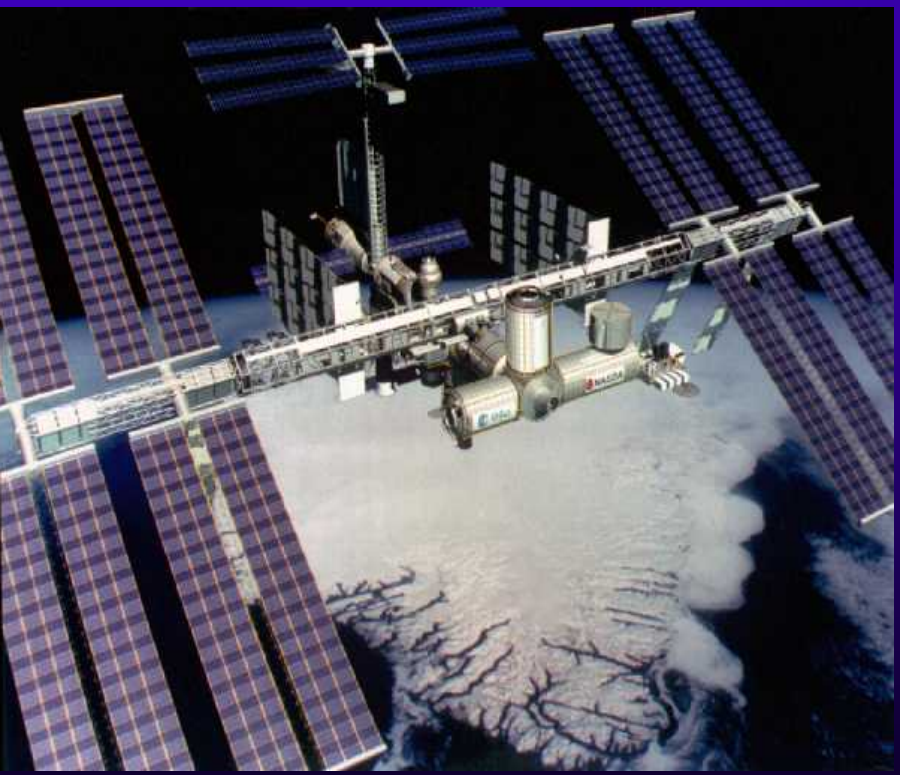
OUTLINE

- Space dosimetry
- Radiation biosensor
- Photosynthesis
- Radiation Target Theory
- Test and calibrations
- Experimental set-up
- Measurements
- Future developments
- Application fields

SPACE DOSIMETRY

Long periods of permanence in space and hence exposure to cosmic radiation are foreseen for the next space missions

- **INTERNATIONAL SPACE STATION (ISS)**
- **HUMAN MISSIONS TO MARS**



One of the most important hazard for the crews in long term space mission is:

RADIATION EXPOSURE

SPACE DOSIMETRY

Primary cosmic radiation: 95% protons, 3,5% α particles, HZE (High Z Elements)

- **GCR (Galactic Cosmic Rays): galactic origin, generated outside of the solar system**
- **SCR (Solar Cosmic Rays): are in general large clouds of charged particles (mainly protons and helium nuclei in a wide range of energy) released from the sun by gigantic eruptions during solar storms.**

In the spacecraft habitat a secondary radiation is produced by the interaction of primary cosmic rays with the spacecraft shielding

Secondary particles: neutrons, protons, electrons, photons, muons, pions, nuclear fragments

Aircrew are exposed to

↓

- ✓ **MIXED RADIATION FIELDS**
- ✓ **WIDE ENERGY SPECTRA**

SPACE DOSIMETRY

BIOLOGICAL EFFECTS OF RADIATION DURING SPACE MISSIONS

Few literature data are available; however, there might be a synergistic effect between:

- **different types of radiations;**
- **radiation and environmental factors such as microgravity**

RADIATION BIOSENSOR

Evaluation of the absorbed dose through

A DIRECT ASSESSMENT OF DAMAGES

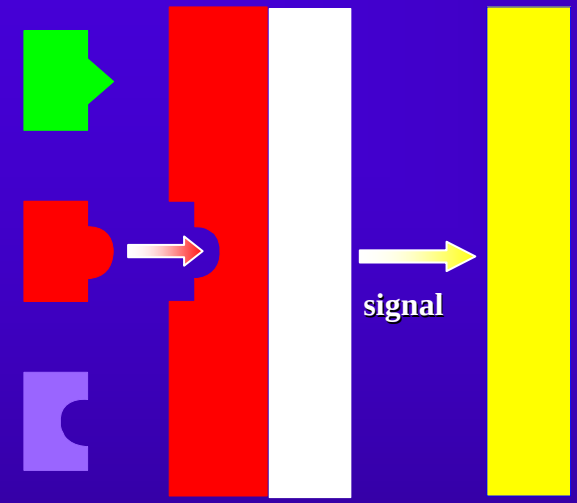
at biochemical level on biological elements

Final goal:

SPACE DOSIMETRY

- ✓ **To develop a device which is sensitive to EVERY KIND OF RADIATION, as long as it is harmful at biological level.**
- ✓ **To answer questions concerning the possible synergy between radiation and other factors that characterize the space environment.**

BIOSENSORS



"Detector molecule" (BIOMEDIATORS) are fixed on a surface.

The information is obtained when they react selectively with the biological molecules that must be monitored in each sample.

INHIBITION BIOSENSORS

Information is given by the measurement of the TOXIC EFFECT of the substance to be detected, on the BIOCHEMICAL ACTIVITY of enzymes or enzymatic complexes, which are used as biomediators.

RADIATION BIOSENSOR

It is a **INHIBITION BIOSENSOR: the incident radiation has a HARMFUL EFFECT** and causes a variation of the biological activity of the biomediator.

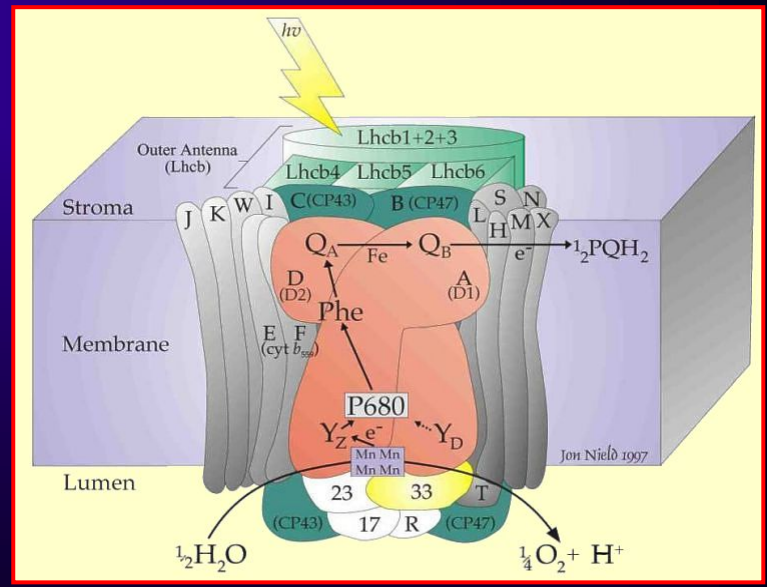
The chosen biomediator, for its natural biochemical activity, if stimulated with visible light, emits fluorescence.

If the sample is exposed to radiation, then the fluorescence intensity depends on the absorbed dose.

The biomediator is:

PHOTOSYSTEM II

enzymatic complex that is included in photosynthetic organisms



Photosynthetic organisms

- Photosynthesis : transformation of light energy in chemical energy

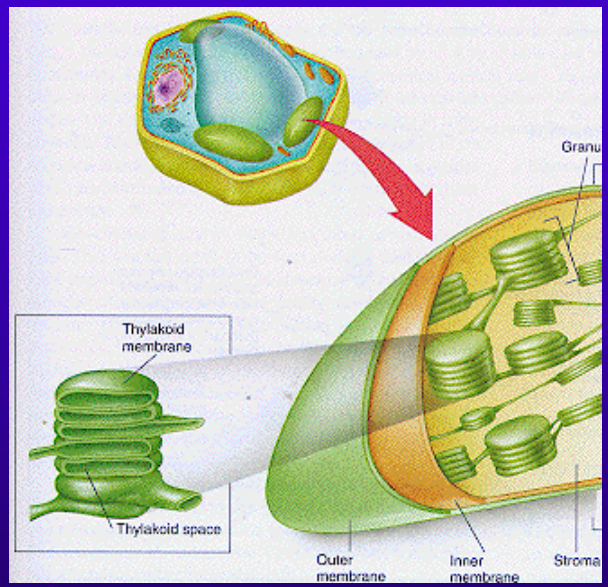
- Photosystem II: multi enzymatic complex in plants and microorganisms (algae, cyanobacteria) able to catalyze the light to produce redox chain with electron transport and separation of water in molecular oxigen

- On the basis of the life

PHOTOSYNTHESIS and PS II

LIGHT ENERGY ⟶ **CHEMICAL ENERGY**

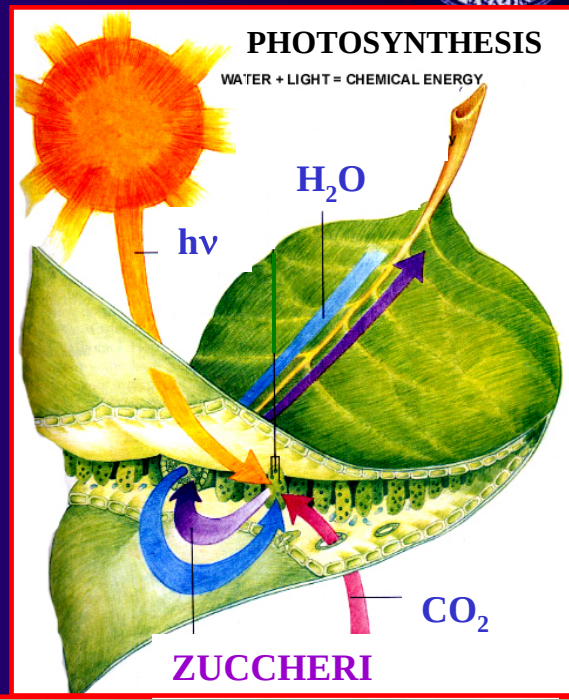
$$6CO_2 + 6H_2O + nh\nu \longrightarrow 6O_2 + C_6H_{12}O_6$$



Where:

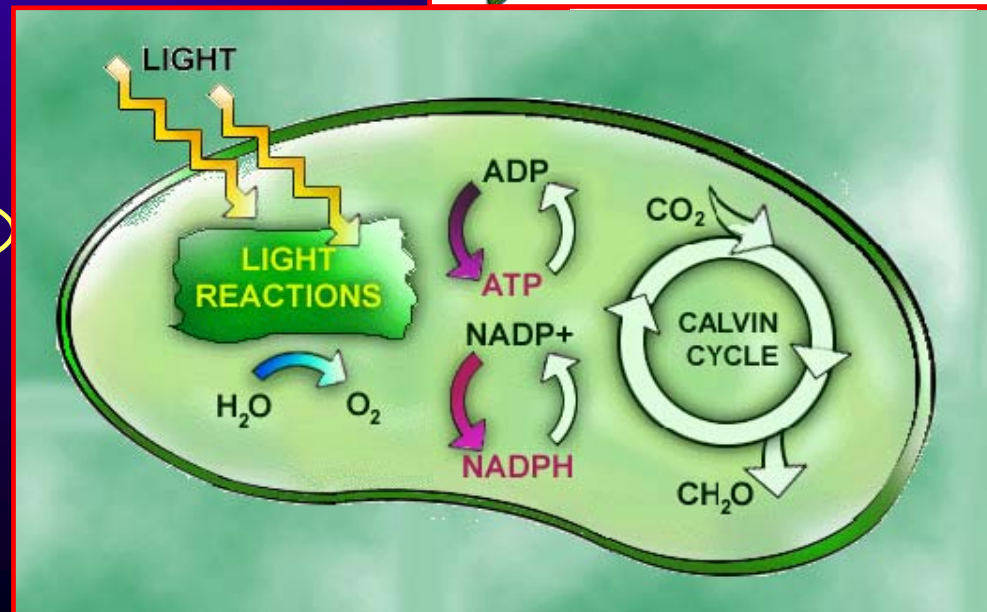
in thylacoids,

within chloroplasts

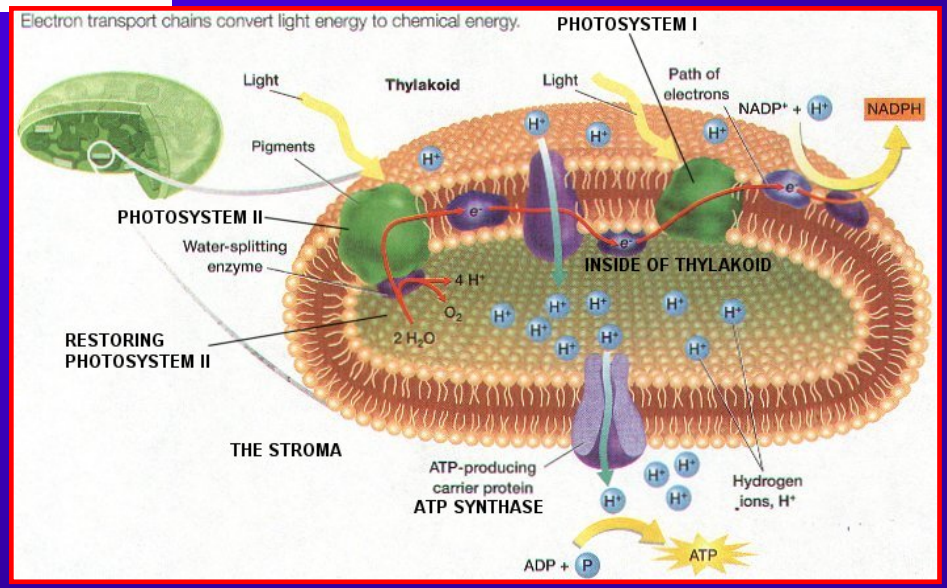


2 phases:

- LIGHT reactions
- DARK reactions

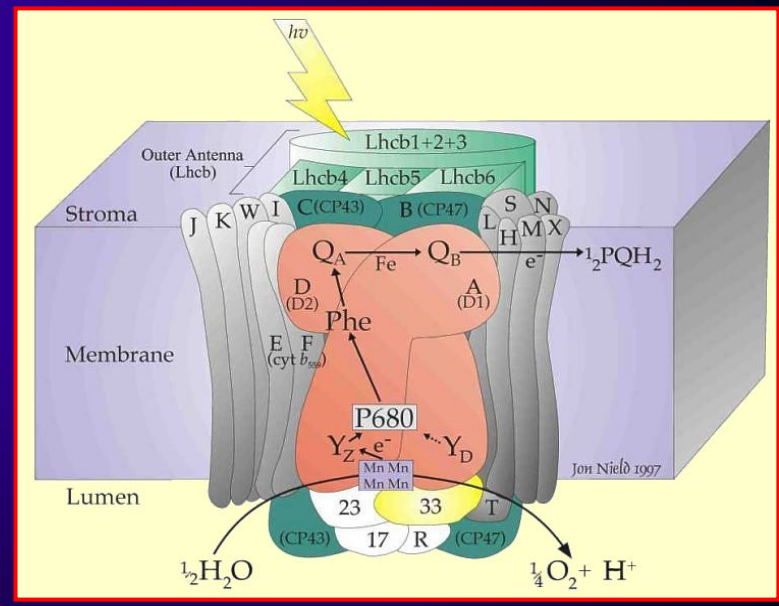


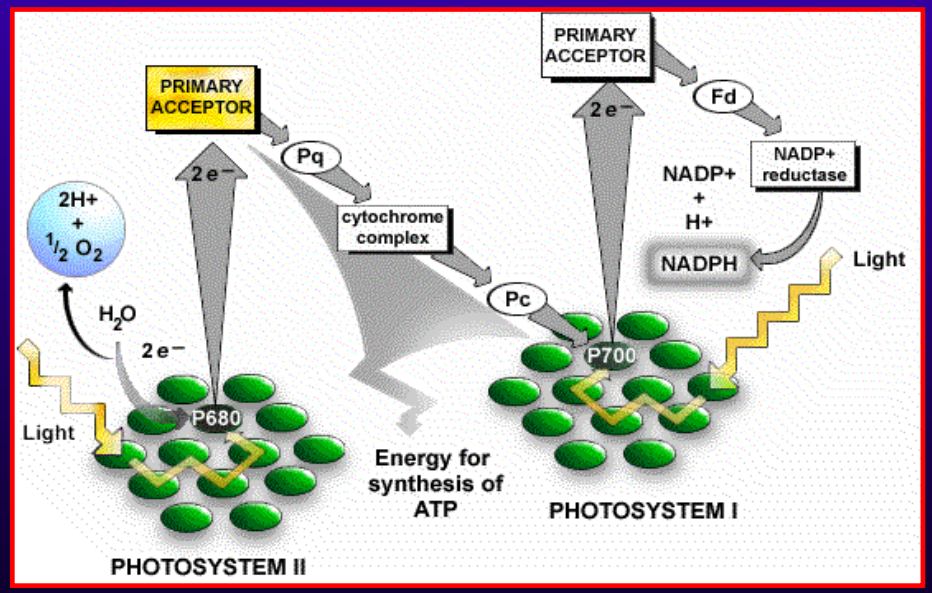
LIGHT REACTIONS



Absorption of photons in the range of visible light

ATP and NADPH synthesis



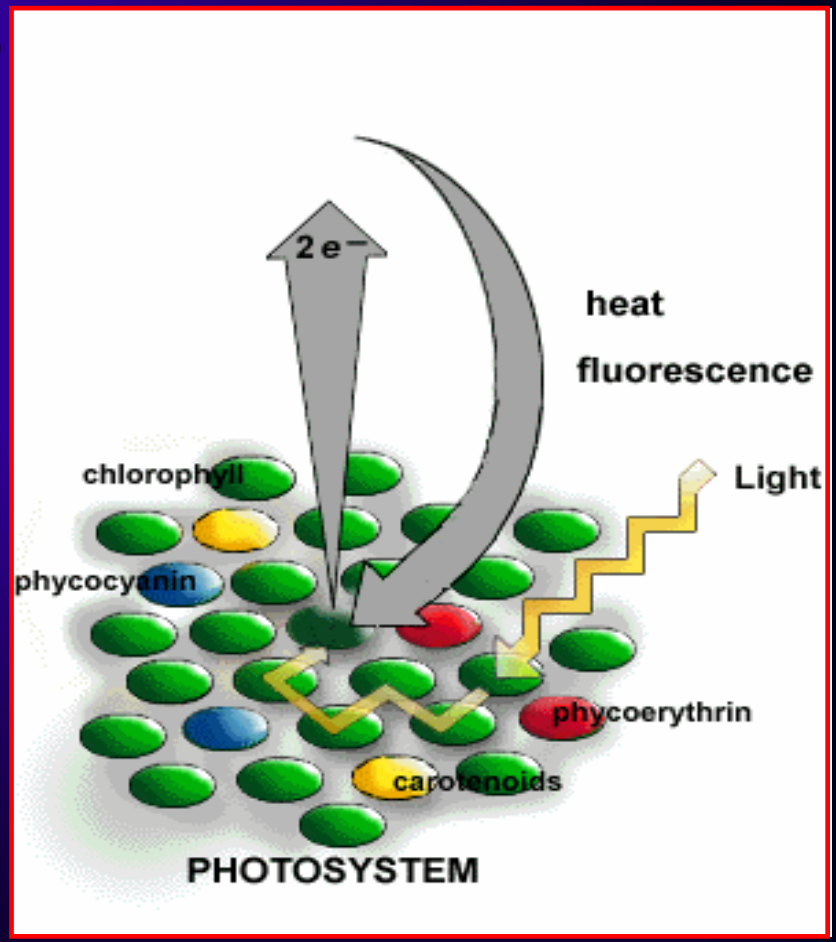


CHAIN of REDOX REACTIONS

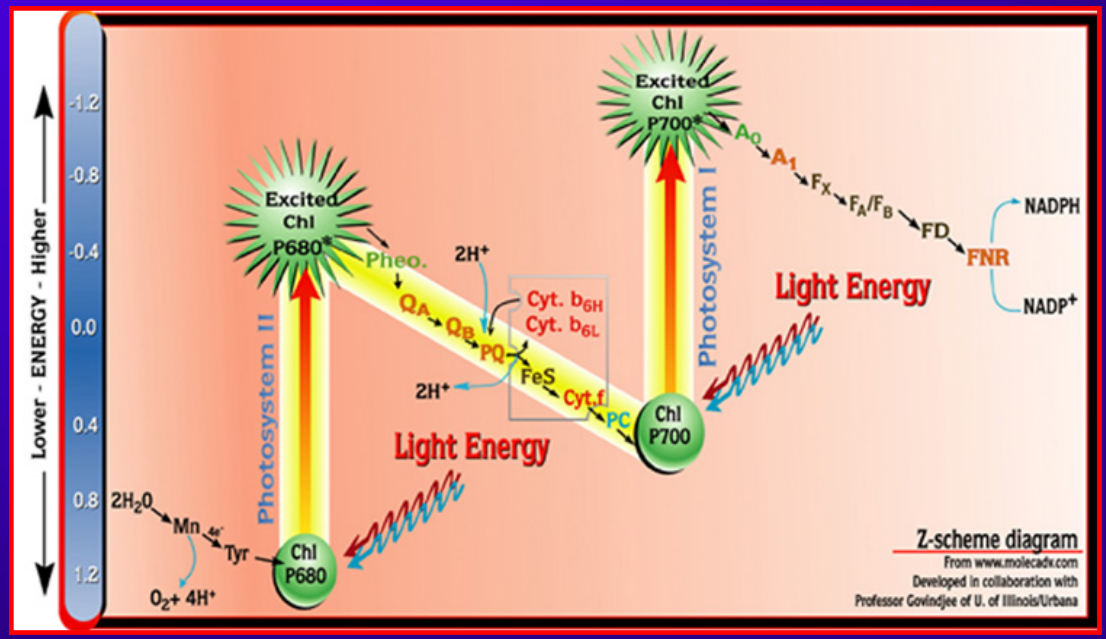
FLUORESCENCE AND PHOTOSYNTHESIS

Processes that are competitive to photosynthesis:

- **Radiative decay (FLUORESCENCE)**
- **Non-radiative decay (heat).**



FLUORESCENCE IN PHOTOSYNTHESIS



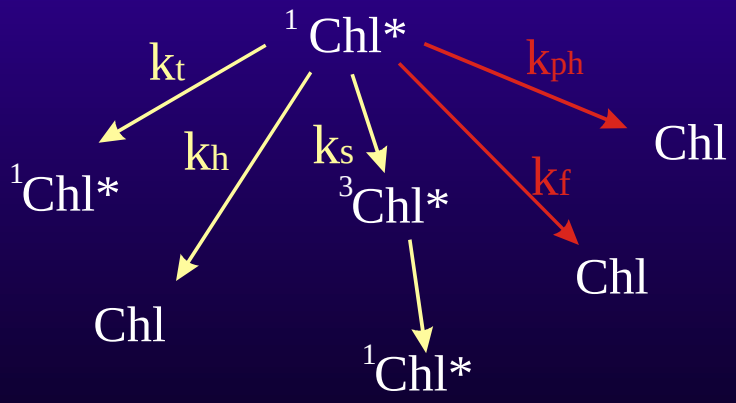
If $Q_A \longrightarrow Q_A^-$

$\downarrow$

$[Q_A]$ decreases

$\downarrow$

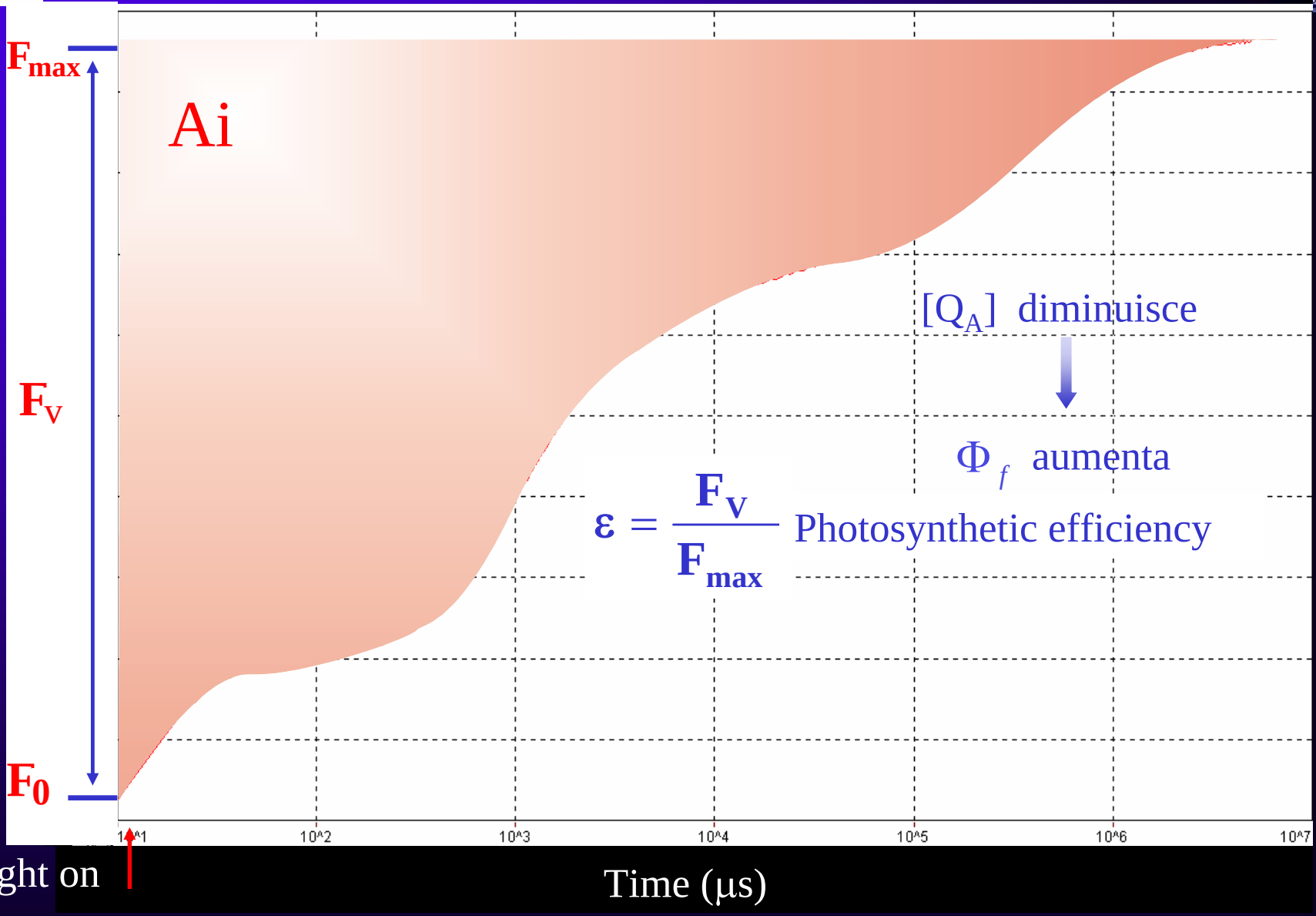
Φ_f raises



$$\Phi_f = \frac{k_f}{k_f + k_e + k_h + k_t + k_p[Q_A]}$$

FLUORESCENCE as a index of PHOTOSYNTETIC ACTIVITY

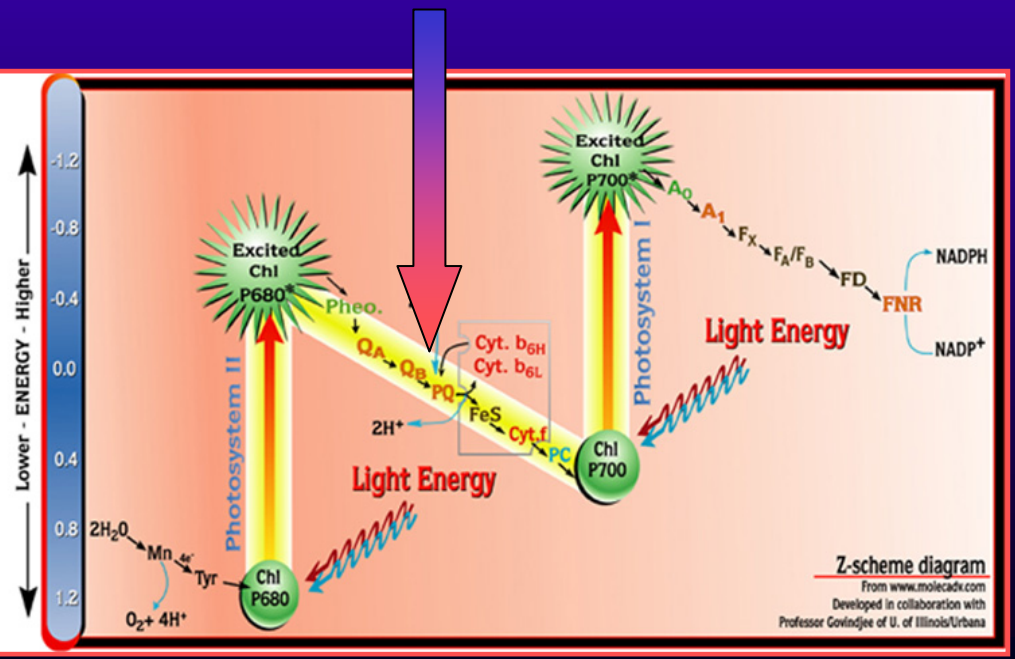
FLUORESCENCE INDUCTION CURVE

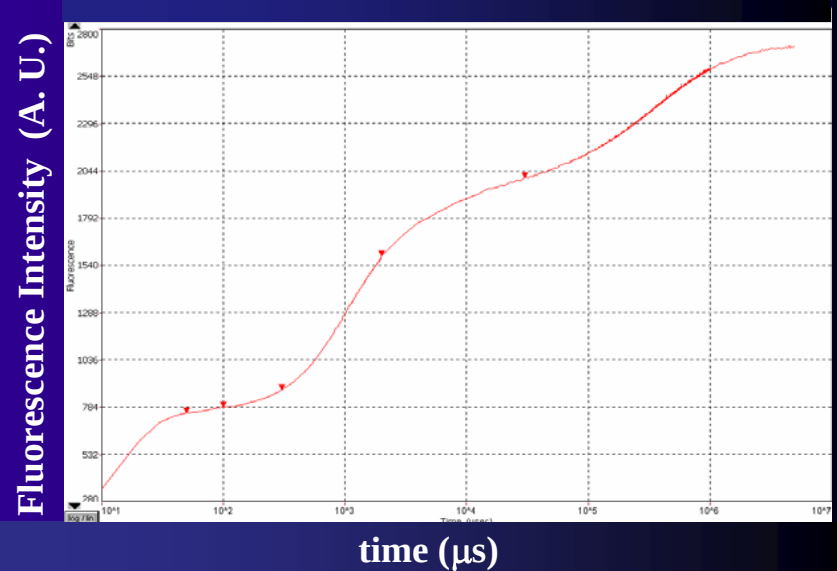


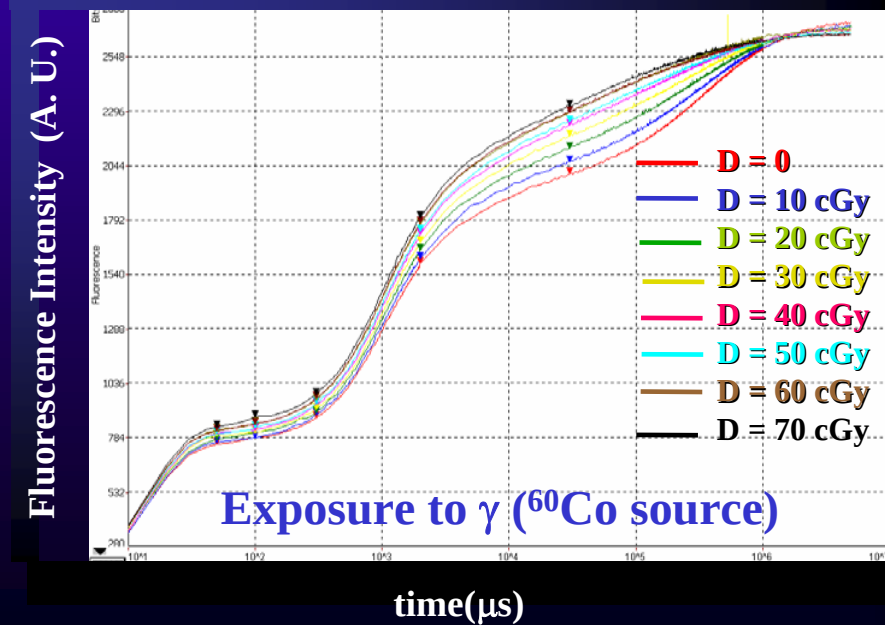
PHOTOSYNTHESIS AND STRESS

Various factors may affect the complex chain of photosynthetic reactions:

- ✓ Weedkiller
- ✓ Thermic Stress
- ✓ Toxic substances
- ✓ Radiation
- ✓ ….



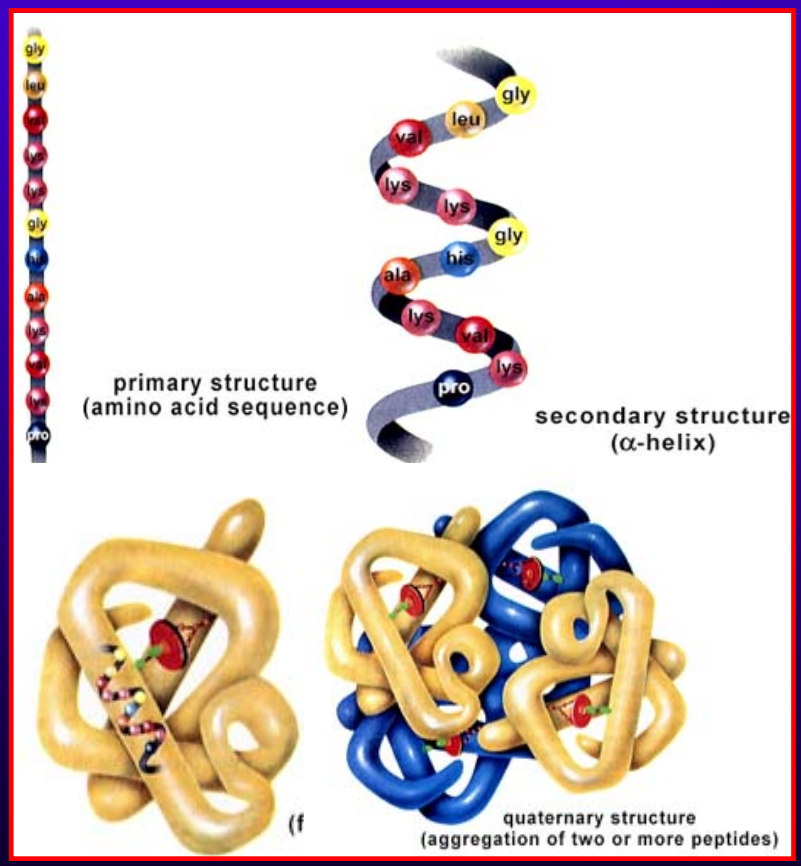




ENZYME RADIOINDUCED INACTIVATION

Direct effect

Radiation-enzyme interaction:



Indirect effect:
due to free radical generated from water radiolysis

ionization → biochemical damage

- fragmentation of polypeptide chains
- solubility variation
- break of enzyme structures

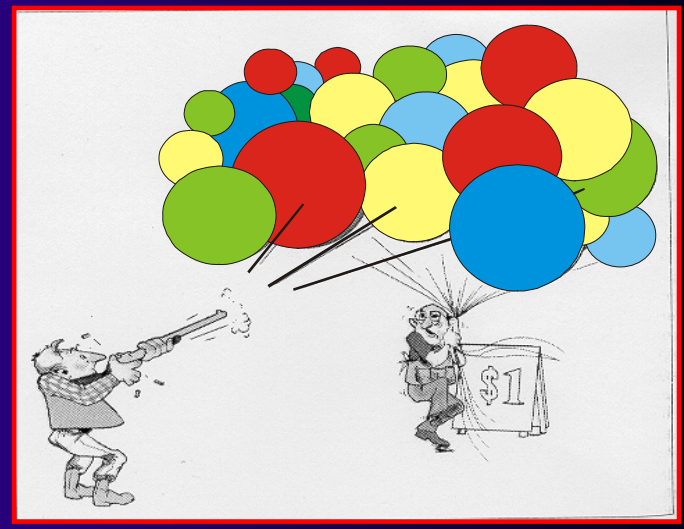
RADIATION TARGET THEORY

It allows to predict an exponential decrease of biochemical activity (K) of an enzymatic complex with the absorbed dose (D)

Assumptions:

1. **Existence of functional, molecular units (TARGET)**
2. **Only primary random ionizations can occur**
3. **∀ primary ionization leads to a loss of functionality**

$$K = K_0 e^{-\frac{D}{D_0}}$$



K= biochemical activity
(e.g.photosynthetic efficiency)

K_0= initial biochemical activity

D = dose

D_0= dose to reduce activity to 37%

Simple relationship dose – effects
why?

- ✓ **No repair mechanisms**
- ✓ **Only direct effects**

1 /D_0 and Mr

$$K = K_0 e^{-\frac{D}{D_0}} \longrightarrow \ln K = \ln K_0 - \frac{1}{D_0} D$$

The trend in the exponential loss of biological activity depends on the **FUNCTIONAL MASS OF ESSENTIAL ENZYMES Mr,** (mass of sensitive target)

$$\mathbf{n = \frac{D}{D_0} = qM_rD} \longrightarrow \mathbf{\frac{1}{D_0} \propto M_r}$$

For γ e^-: $Mr = 6.4 * 10^5 \left(\frac{1}{D_0}\right)$ (Kempner G.R. Macey R.I. (1968) Biochim.biophys.Acta, **163**)

CHOICE OF PARAMETER

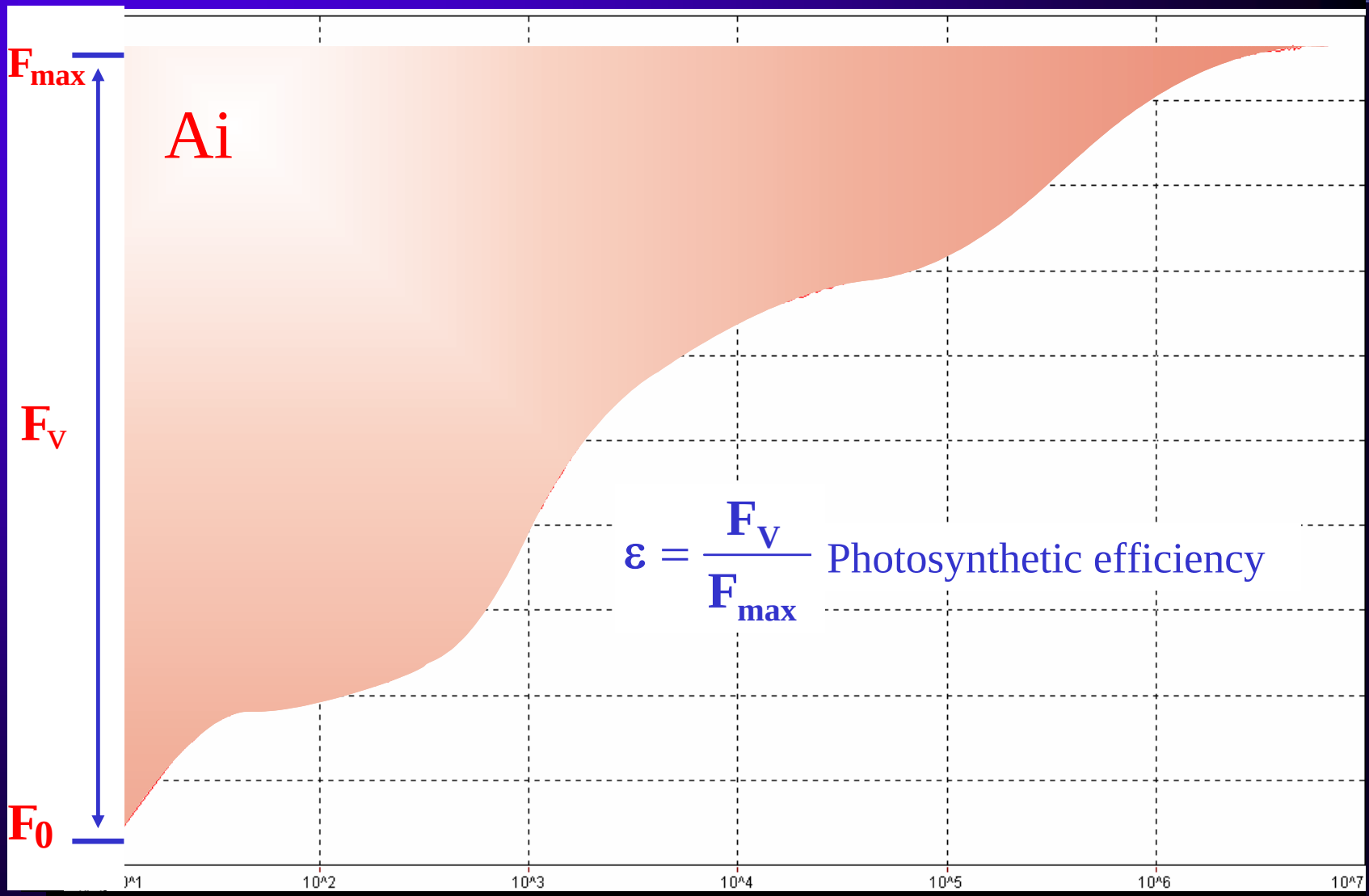
$$\ln \varepsilon = \ln \varepsilon_0 - \frac{1}{D_o} D \qquad \log \varepsilon = 0{,}43 \log \varepsilon_0 - \frac{0{,}43}{D_o} D$$

Considering the typical literature value of M_r (M_r =334 kDa)

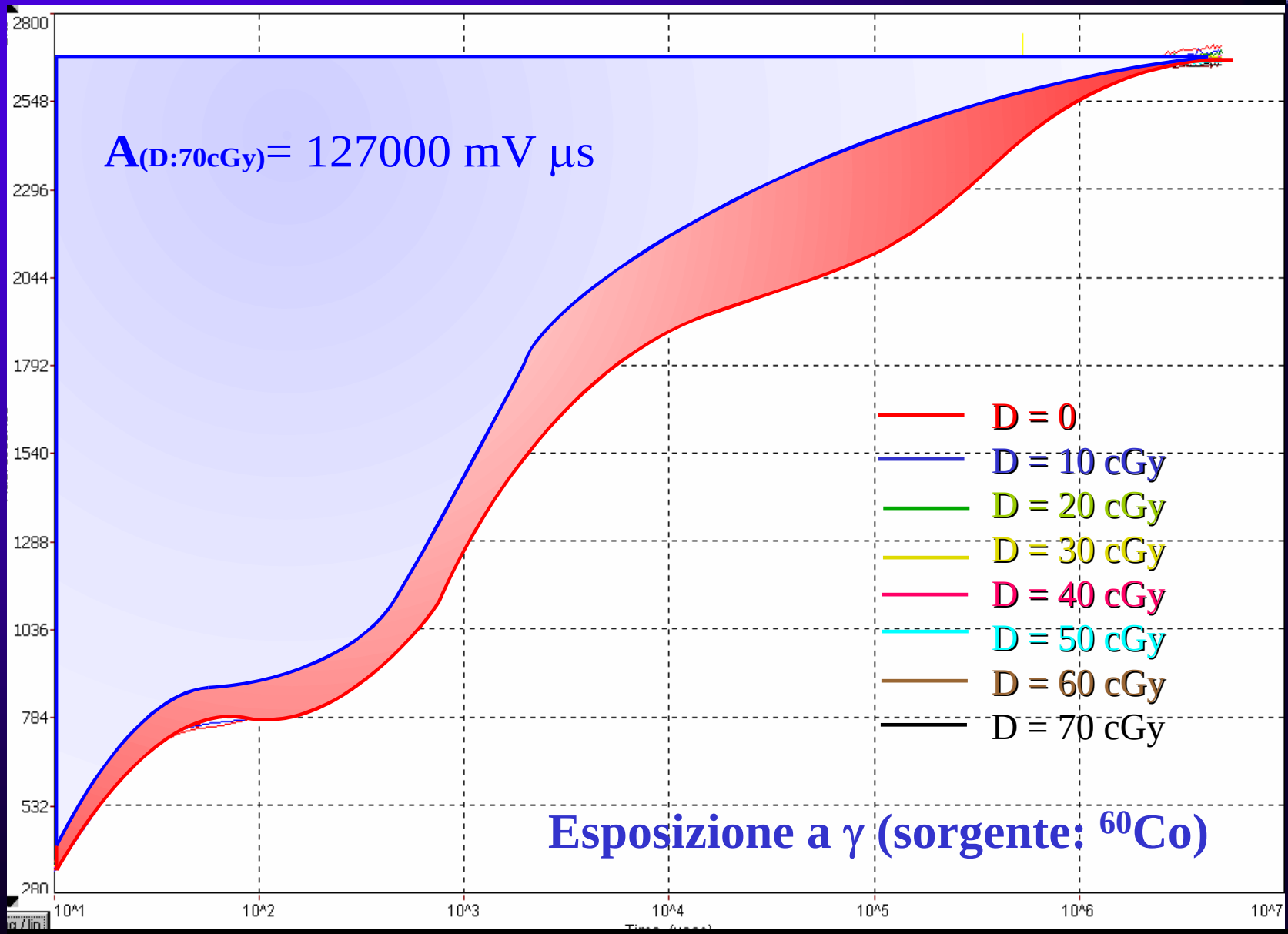
$$\frac{1}{D_0} \sim 10^{-8}\ \text{mGy}^{-1}$$

To see biological effects due to radiation absorption, especially at LOW doses, we need a much more sensitive parameter to radiation, different from photosynthetic efficiency

CHOICE OF THE PARAMETER



ESTIMATED PARAMETER



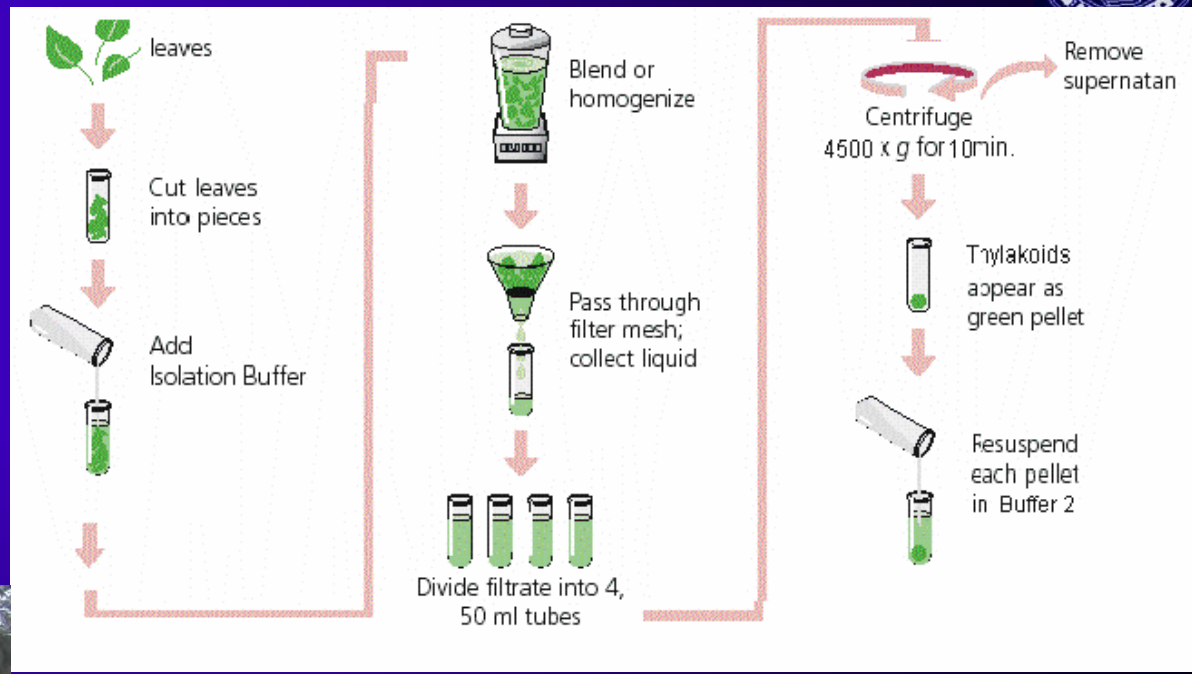
Fluorescence intensity (mV)

Time (µs)

PREPARATION PROCEDURE

To extract tylakoids from spinach leaves, a defined preparation protocol is accurately followed.

(Berthold D.A, et al., 1981)



MEASUREMENT PROCEDURE

To guarantee Radiation Target Theory hypothesis during radiation exposure

NO FREE RADICAL must determine biological damages

so the sample can be irradiated:

FROZEN

Free radical can't spread to react with functional molecules

The measure can be automated, at different time during the exposure time

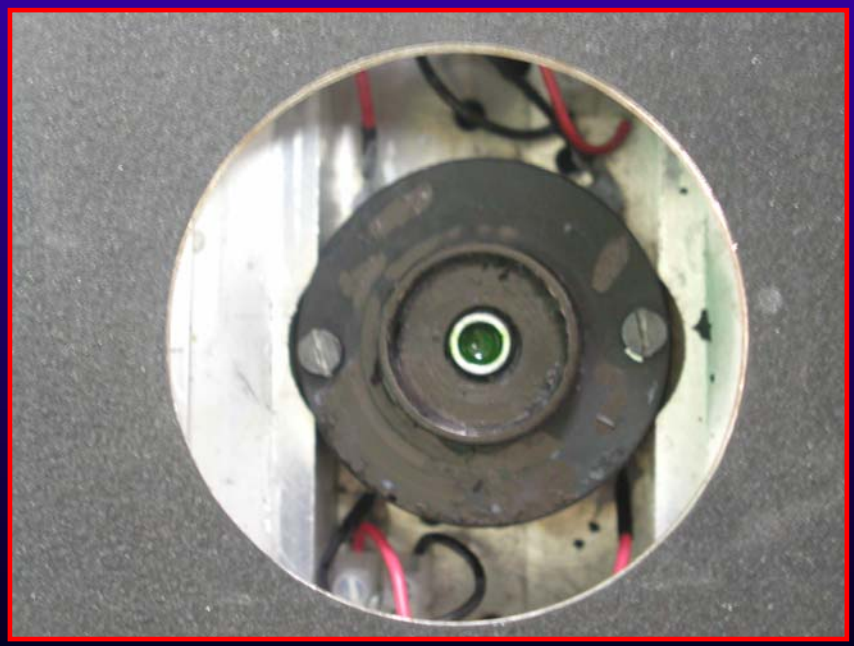
FREEZED-DRIED

Free radical are not generated because no water molecules are present during the exposure.

The measure can't be automated because the sample must be hydrated before measure

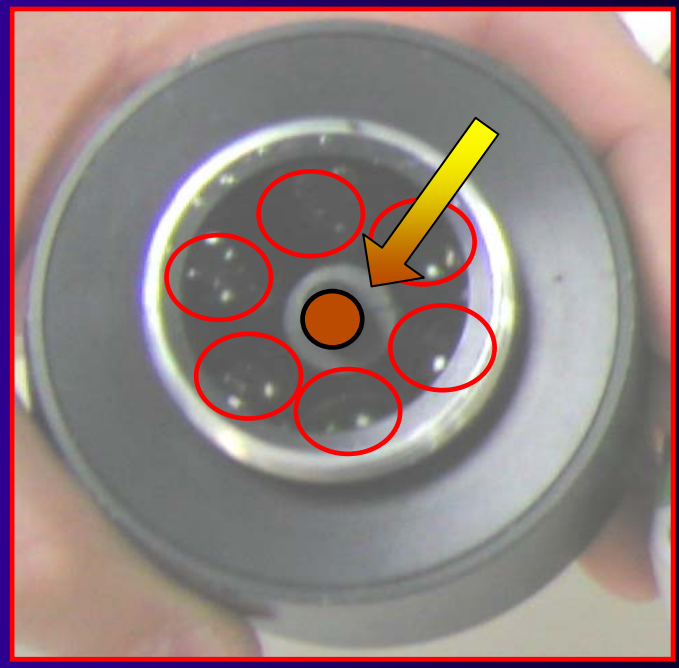
MEASUREMENT PROCEDURE

The frozen sample is placed in the measurement seat where it is maintained at T= -13°C



Sample Volume: 150 μl

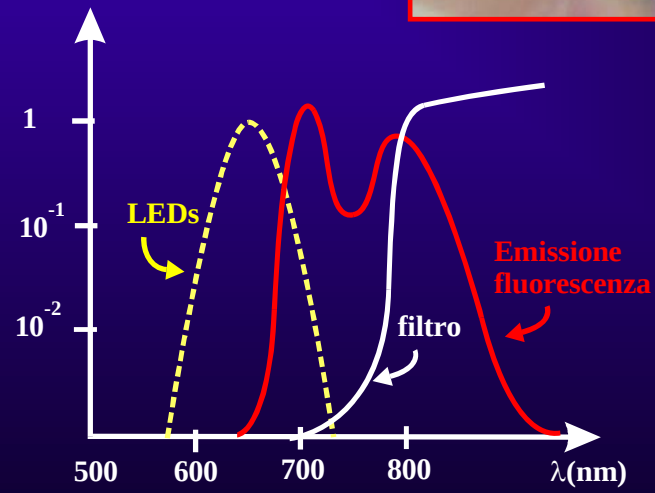
MEASUREMENT PROCEDURE



6 light emitting diodes (LEDs)

Are focused on the sample surface and they provide the light flash (5s) necessary to stimulate fluorescence

$\lambda_{recieved}$= 650 nm



1 PIN photodiode

Allows the detection of the fluorescence signal at:

$\lambda_{fluorescence}$ = 750 nm

MEASUREMENT APPARATUS

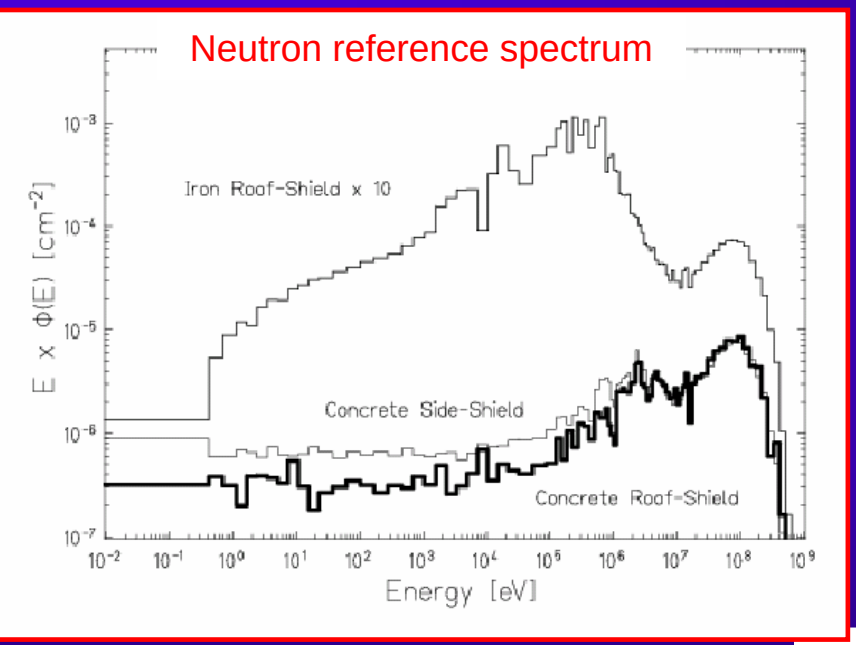
CALIBRATION IN DIFFERENT RADIATION FIELDS

To characterize biosensor behaviour, different exposures have to be performed:

- ✓ **To different radiation fields**
- ✓ **To different energy beams**
- ✓ **To different dose and dose rate**

neutrons	Am-Be	0,1-11 MeV	JRC (I)
	Am-Be	0,1-11 MeV	CRE Casaccia(I)
	CERF	2E-8 - 800 MeV	CERN(CH)
	Accelerator	40 -180 MeV	TSL Uppsala(SW)
protons	Accelerator	180 MeV	TSL Uppsala(SW)
gamma	Accelerator	250MeV	MAX L (SW)
	^{137}Cs	660 keV	CRE Casaccia(I)
	^{60}Co	1,17 - 1,33 MeV	Torino (I)
HZE	C12	500 MeV/n	GSI (D)
	F56		

EXPOSURE TO NEUTRONS: CERF



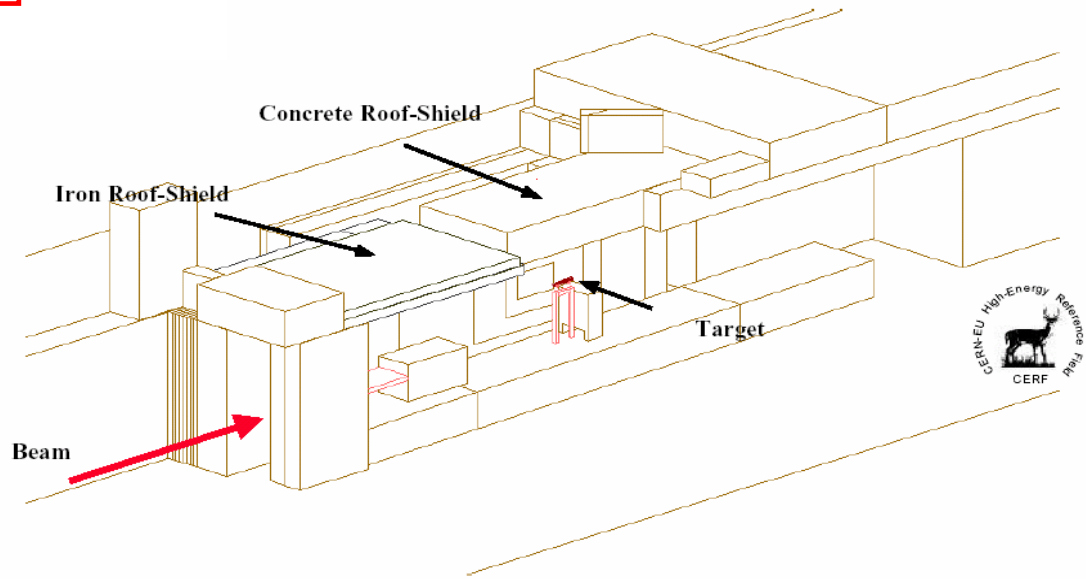
CERN Geneve

Exposure to neutrons: (2E-8 - 800MeV)

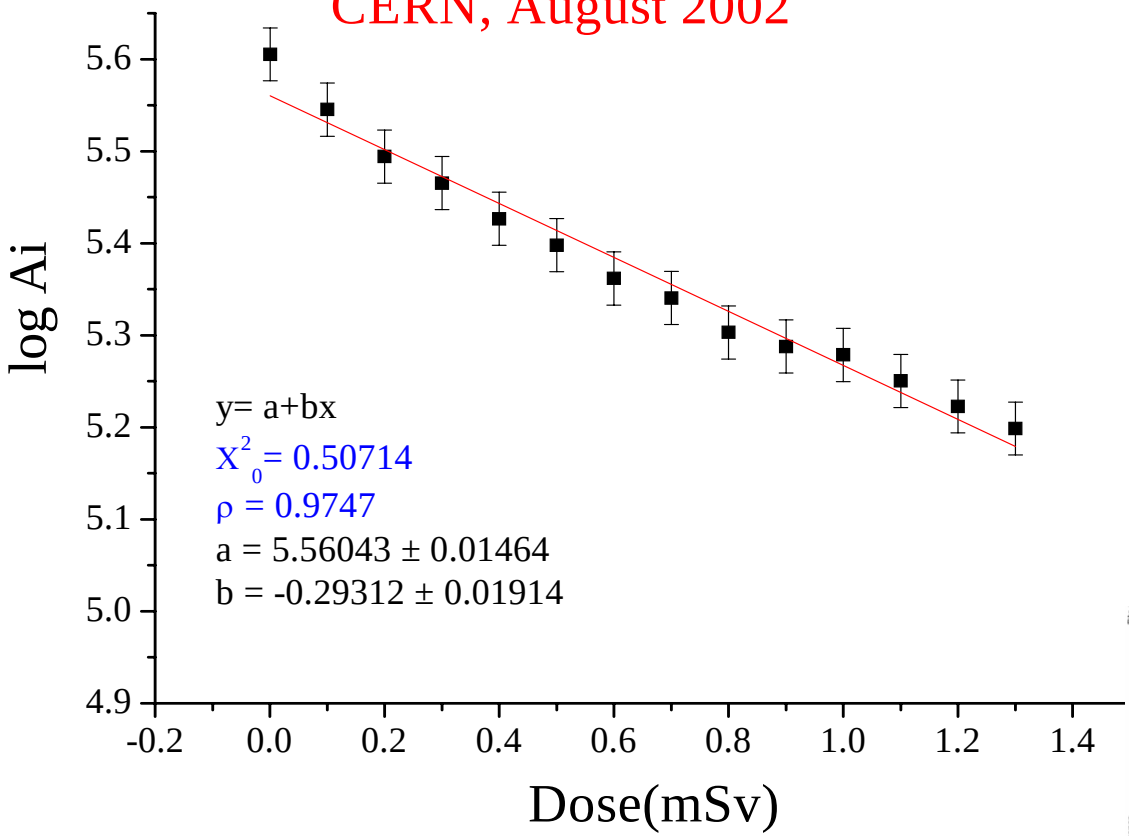
CERF (*Cern Eu Reference Field*), Line H6 (SPS beam)

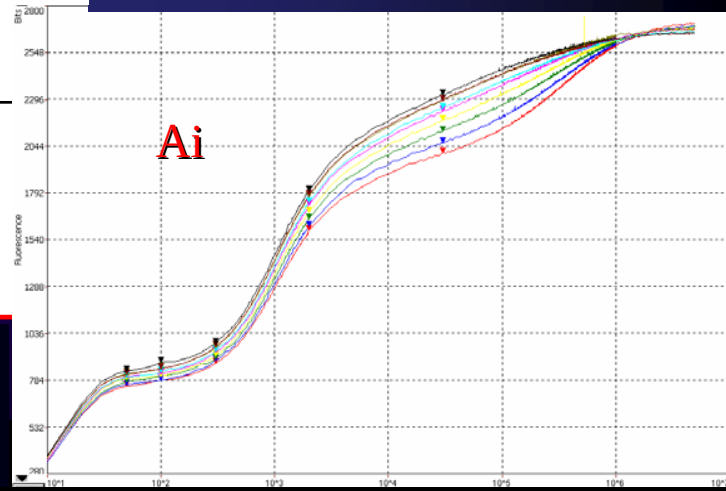
It provides particle composition and spectral fluences similar to those in the cosmic radiation field,

a reference base for testing, intercomparing and calibrating passive and active instruments before their use on-board aircraft.



EXPOSURE TO NEUTRONS: CERF



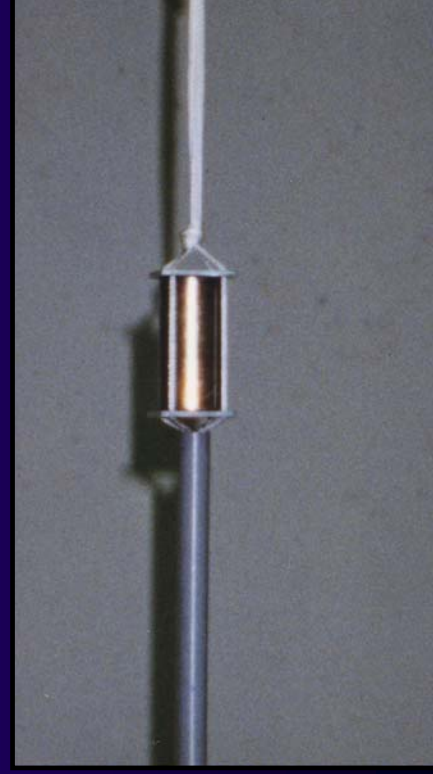


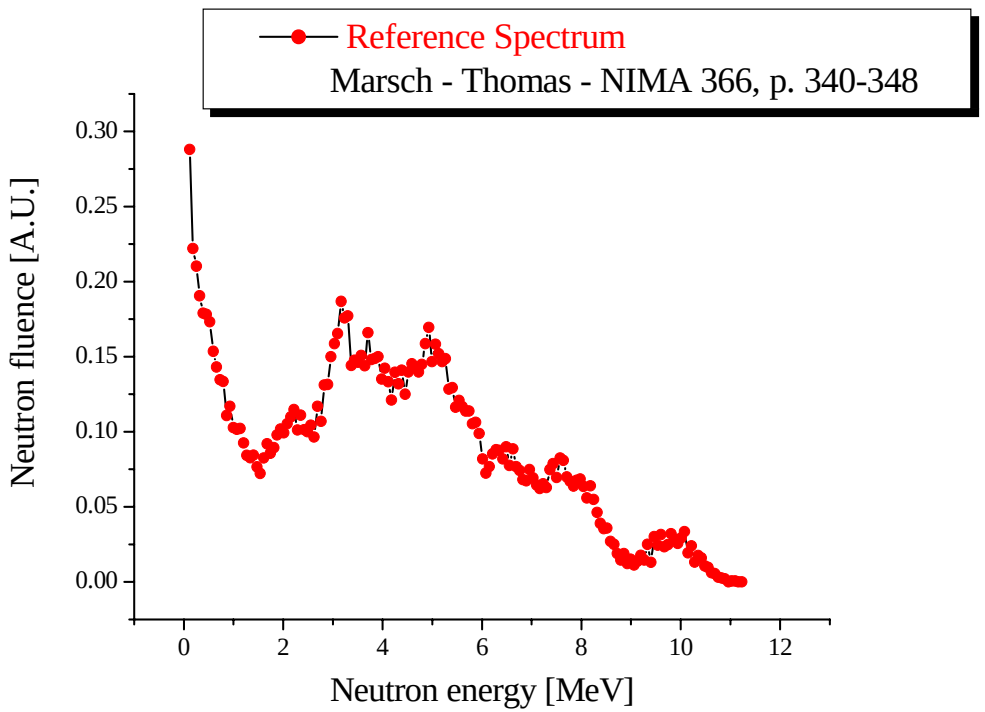
EXPOSURE TO NEUTRONS: Am-Be

JOINT RESEARCH CENTRE, Ispra

Exposure to neutrons: (0,1-11MeV)

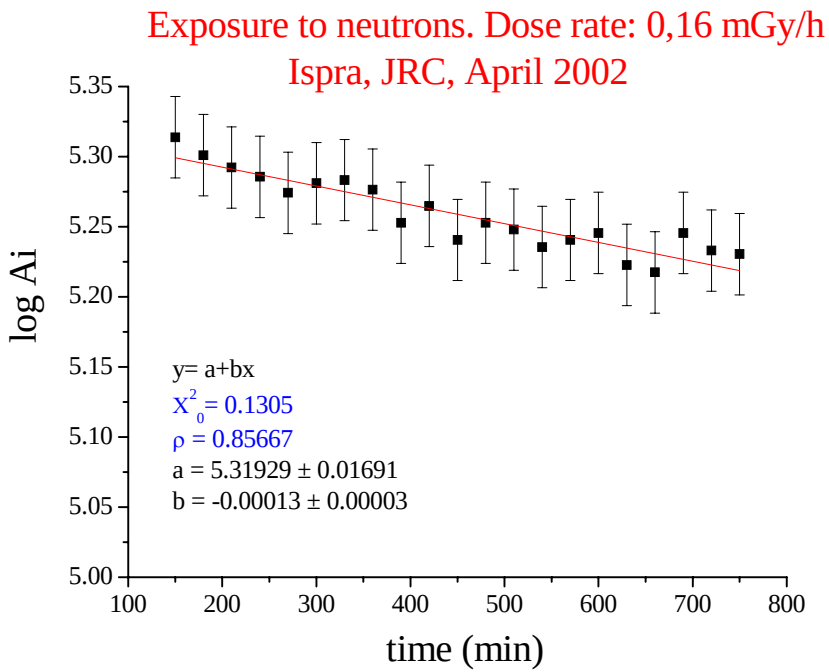
Source: Am-Be

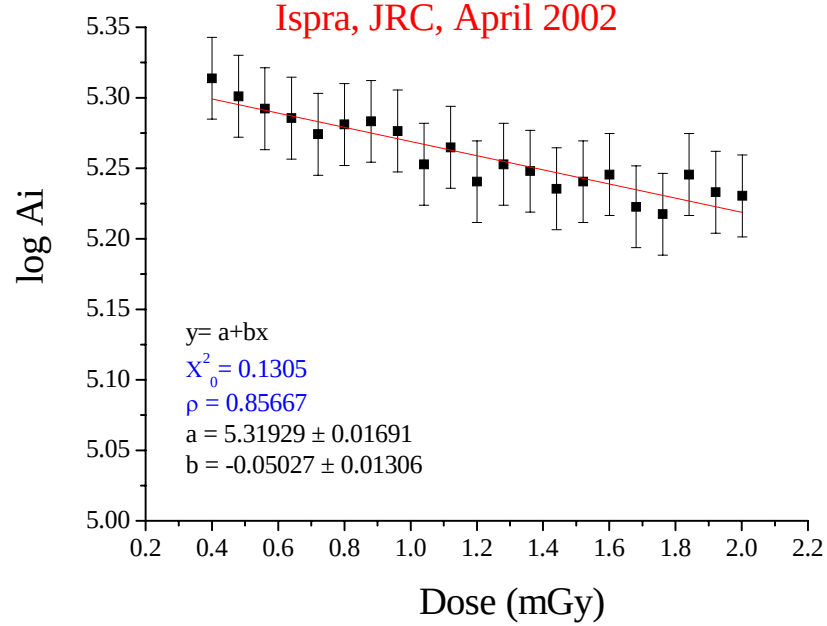




characteristic	description
material	$^{241}_{95}Am$ $^{9}_{4}Be$
Am activity	15,5 Ci
neutron emission rate	$2,2\ 10^{6}$ per Ci = $3,4\ 10^{7}$ n/s

EXPOSURE TO NEUTRONS: Am-Be





CONCLUSIONS ABOUT NEUTRON EXPOSURES

Conclusions about CERN e JRC measurements:

GOOD LINEAR RELATIONSHIP

log Ai vs D

Main goal:

REPRODUCIBILITY IN THE RELATIONSHIP

log Ai vs D

Open questions:

- Possible dependence on the time break Δt between measurements
- Possible dependence on the "age" of the samples
- Possible differences between samples extract from different stock

EXPOSURE TO γ: San Giovanni Hospital

Exposure to γ

^{60}Co: $E_{\gamma 1}$ = 1173.2 keV

$E_{\gamma 2}$ = 1332.5 keV

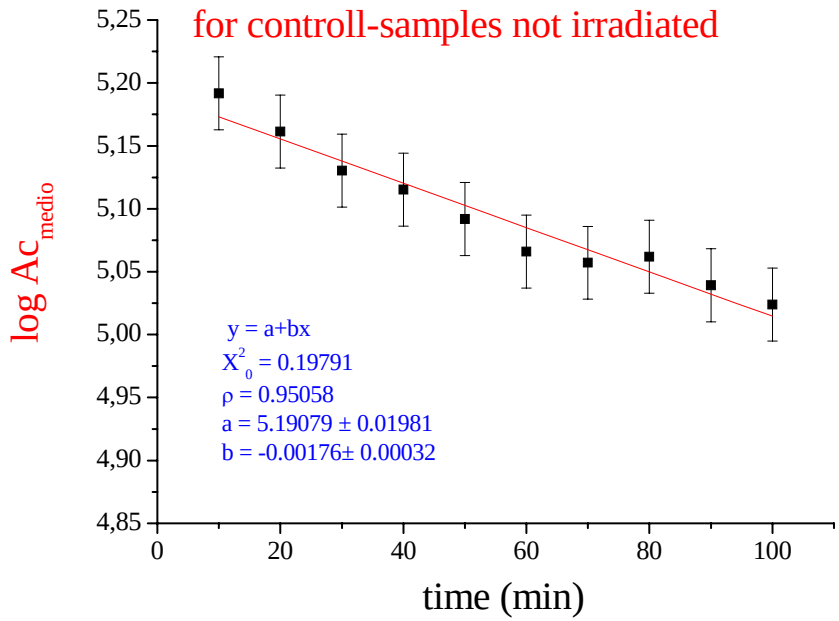
Irradiation tests :

- with different Δt;
- on sample with different "age".

To guarantee that the slope depends only on the absorbed Dose and not on other factor (e.g. health status of leaves used for the thylacoid extraction)

same measurements were performed on controll-samples not irradiated

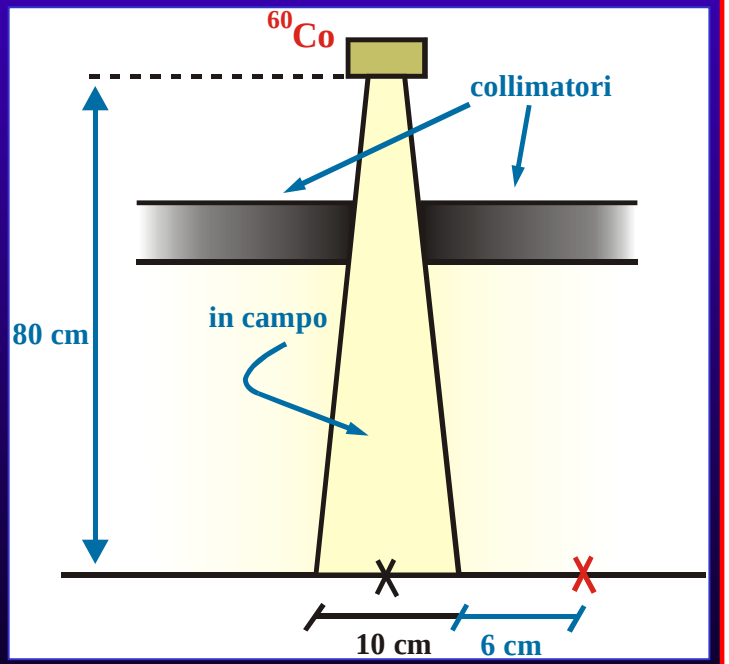
Trend of the mean value Ac measured for controll-samples not irradiated

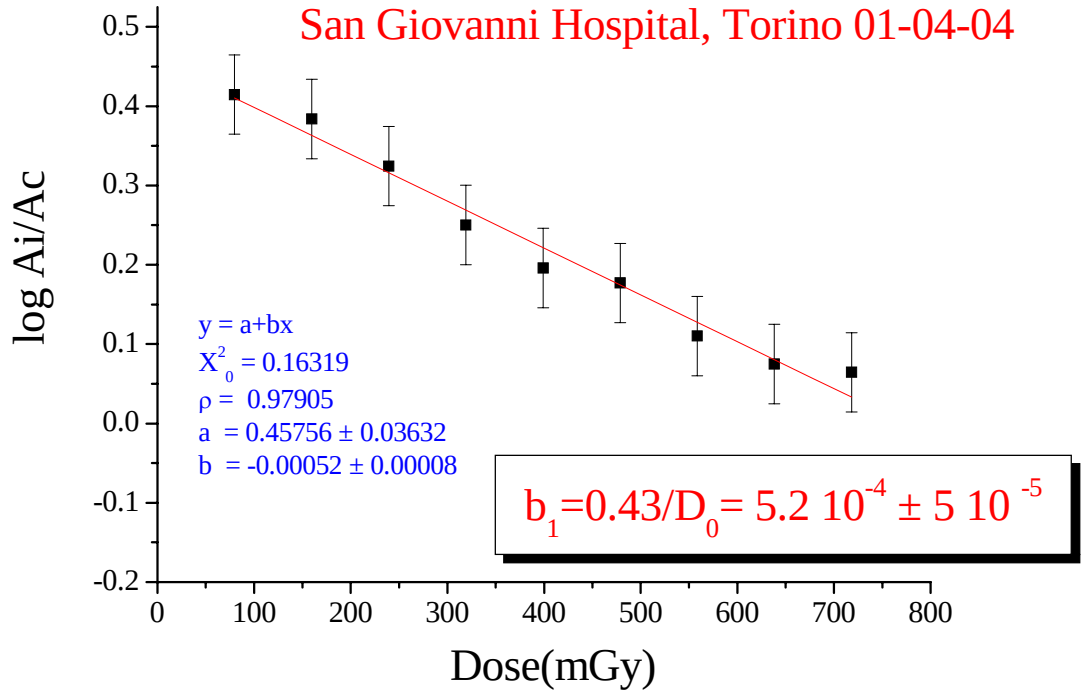


EXPOSURE TO γ

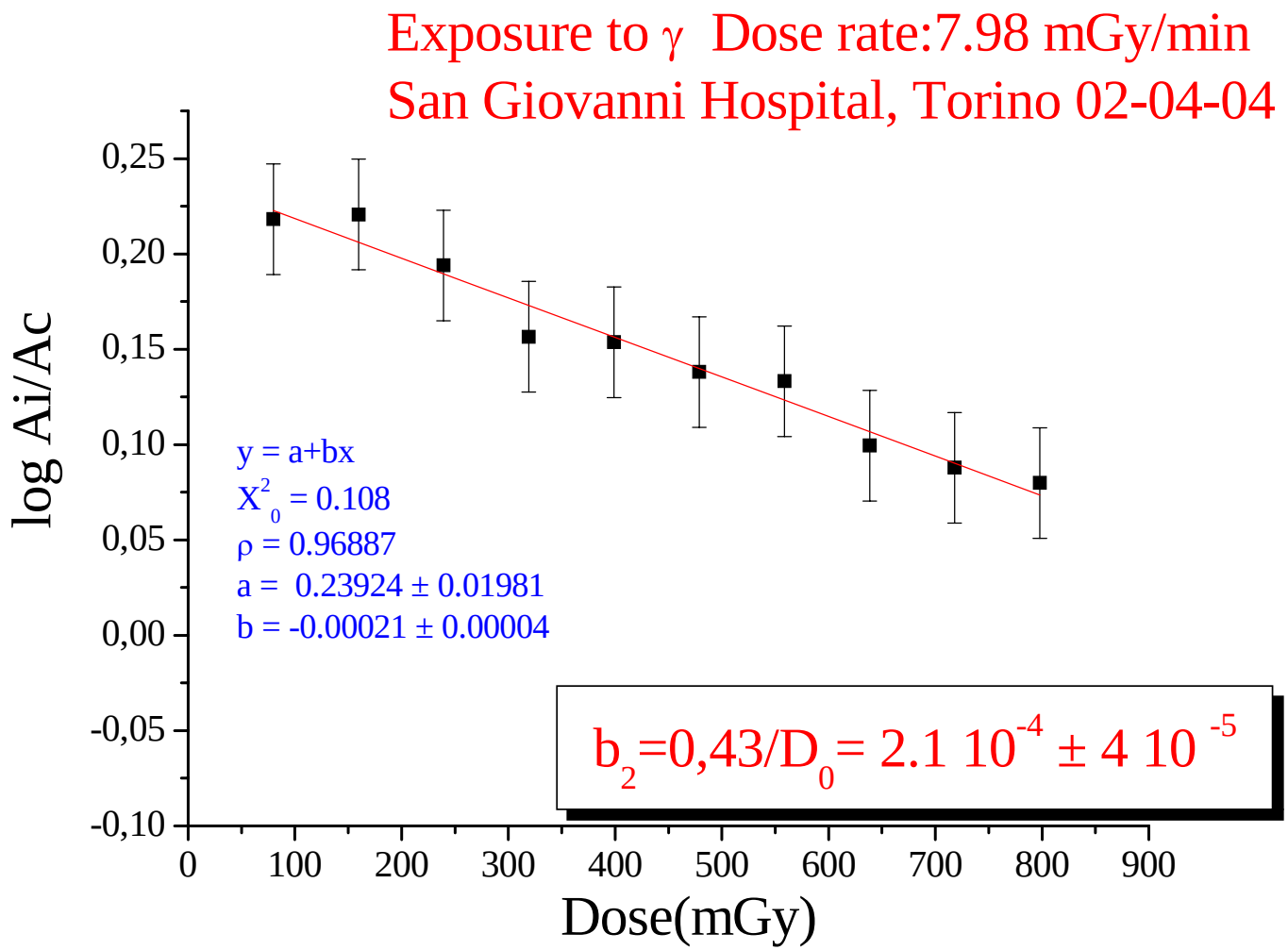
Since values Ai are normalized to Ac, the new parameter $\left(\frac{Ai}{Ac}\right)$

is independent of peculiar characteristics of the leaves and its decrement depends only on radiation induced damages.

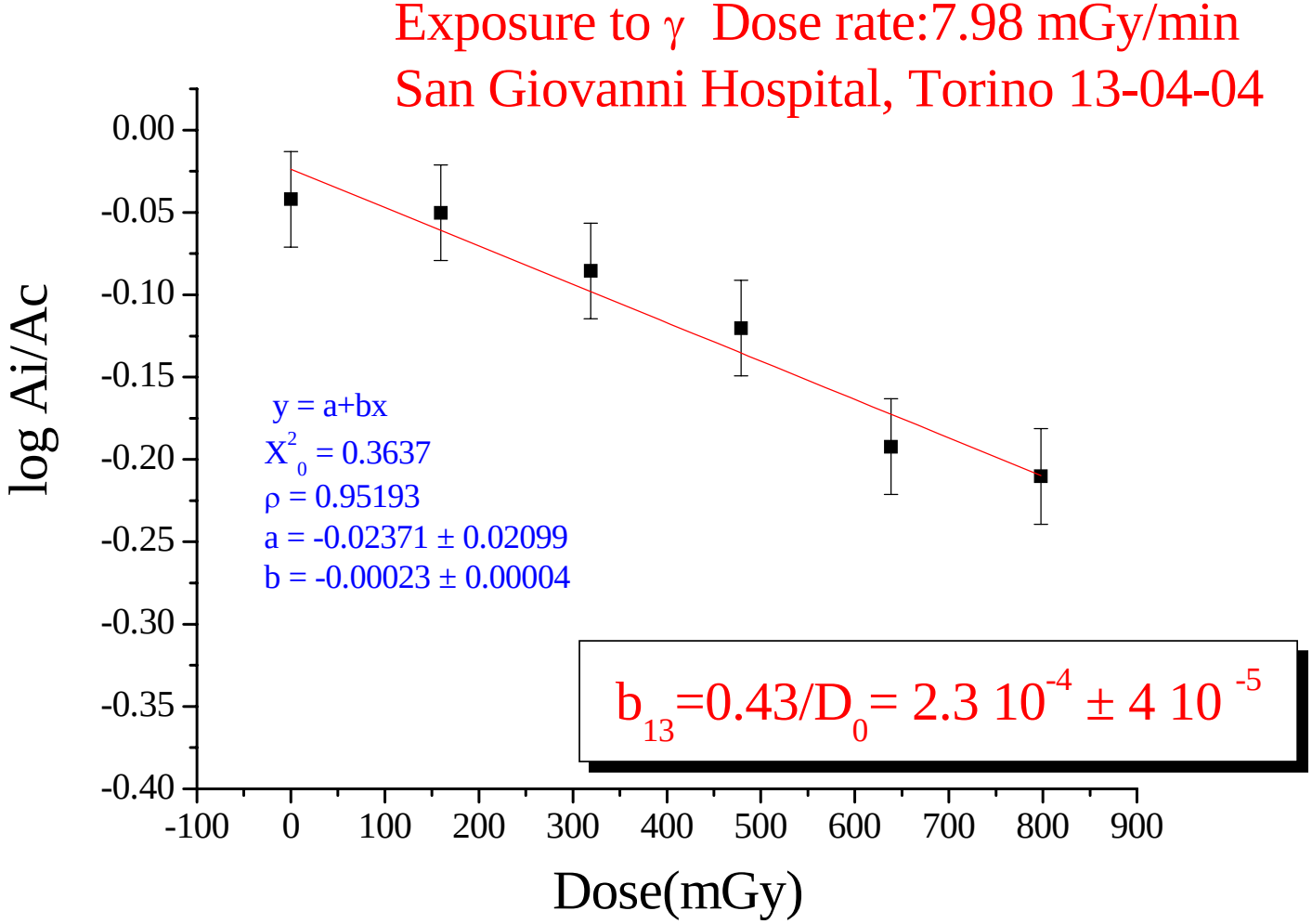




EXPOSURE TO γ



EXPOSURE TO γ



CONCLUSIONS ABOUT EXPOSURE TO γ

$$b_2 = 0{,}43/D_0 = (\mathbf{2{,}1 \cdot 10^{-4}} \pm 4 \cdot 10^{-5})\ \text{mGy}^{-1}$$

$$b_{13} = 0{,}43/D_0 = (\mathbf{2{,}3 \cdot 10^{-4}} \pm 4 \cdot 10^{-5})\ \text{mGy}^{-1}$$

Resolved problems:

- **Dependence on the time break Δt between measurements** ✓ **OK!**
- **Dependence on the "age" of the samples** ✓ **OK!**
- **Differences between samples extract from different stock** ✓ **OK!**

Actual final goal:

CALIBRATION

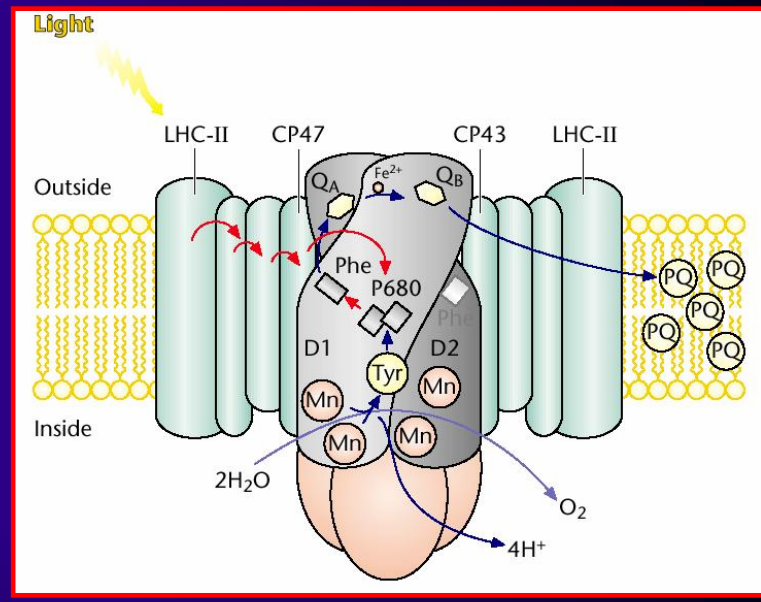
in different radiation fields, with different energy beams, with different dose and dose rate

THRESHOLD BIOSENSOR

$\frac{1}{D_o}$ is linked to biosensor sensitivity to radiation (sensitive molecular target)

Mutants:
organisms in which at least one nucleotide is replaced

If mutants organisms have a different functional enzymatic organization



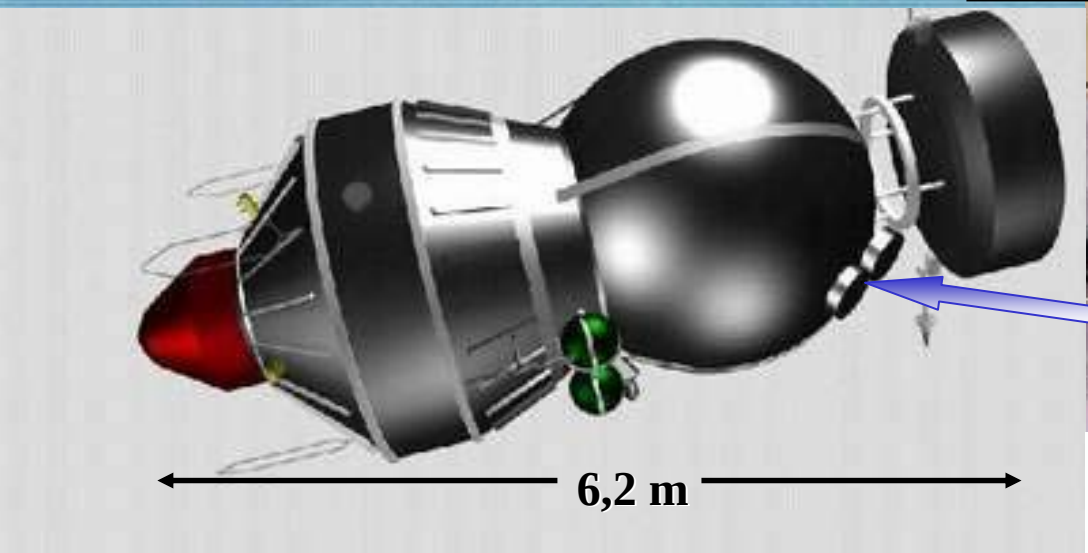
DIFFERENT MOLECULAR TARGET UNITS

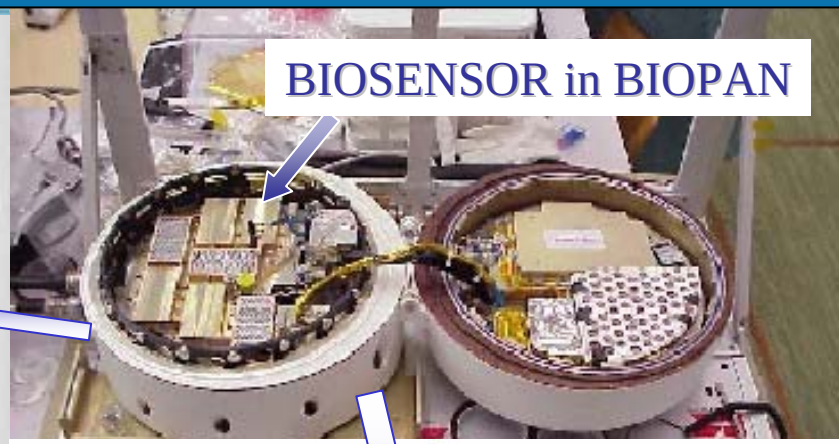
DIFFERENT SENSIBILITY TO RADIATION

ESA FLIGHT

Scientific & technological meeting

Trieste, 28-29 Aprile 2004





Foton-M2

Reflight: Spring 2005

Biopan:

Facility installed on the external surface in Foton, designed for exposure experiments in space biology and dosimetry

APPLICATION FIELDS:

RADIOBIOLOGY:

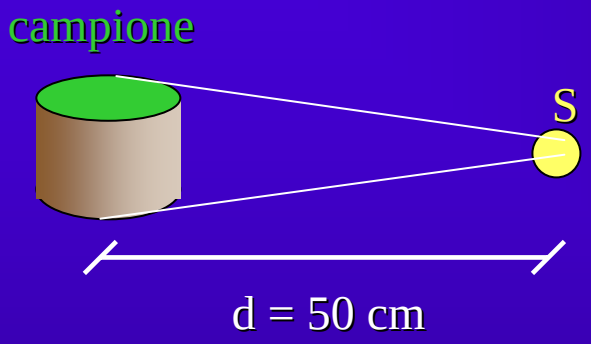
- ✓ Improvement in knowledge about radiation interaction mechanisms at biomolecolar level.
- ✓ Improvement in knowledge about space environment effects on photosynthetic organisms

ANTI-TERRORISM

Detection of “dirty bombs”: devices that combine traditional explosive material (dinamite, nitroglicerina…) with radioactive material (Caesium, Cobalt, Plutonium) in form of dust or thin layers.

Photosystem II is sensitive both to some chemical components in traditional explosive material and and emitted radiation.

VALUTAZIONE DELLA DOSE ASSORBITA, PRESSO LA SORGENTE DI Am-Be



Rate di emissione n su 4π: $3{,}4 \cdot 10^{7}\ s^{-1}$

Rate di neutroni sul campione: $4{,}2 \cdot 10^{2}\ s^{-1}$

Fattori di kerma utilizzati per la conversione flusso-dose, da ICRU, Report 44 espressi in Gy m^2

Dose rate totale:

D_{rate} = 0,16 mGy/h