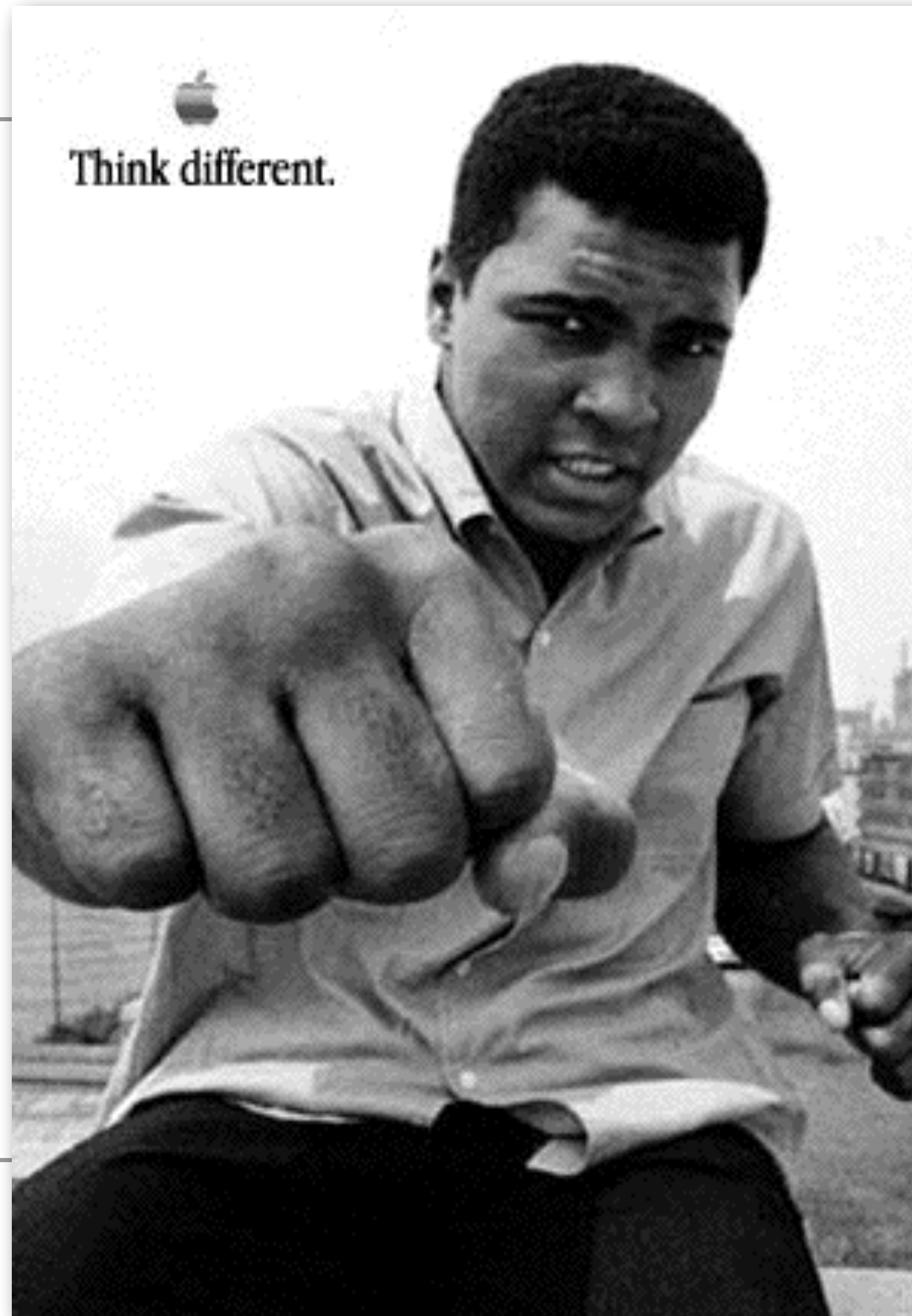


LB145-Spring 2025



1. **Pick up** Name Folder

- Pick up name folder and set it up at seat.

2. **Sit** with your group.

- laptops on outer perimeter (avoid distracting)

3. **Clicker** Attendance

- Launch your Top Hat, and get ready to click.

Announcements

- 1. *CATME GEA3 feedback is opened, due in 3 days...***
- 2. *Final Formal Talk rubric returned today via email at end of lab period, grades uploaded to D2L in 24hrs.***
- 3. *Final Exam: Monday 12:45pm in this room. Can bring 3x5 inch card with handwritten notes on both sides. Vote chose “regular multiple choice”. If haven’t yet read, look for emails from me on Friday and Monday explaining exam in more detail.***



Survey: Got bands? (2.0)

🕒 1:00

What has your group completed in your research project so far?

A Lab 1 bands

B Lab 1 bands & isolated human DNA

C Lab 1 bands & isolated human DNA & some control published primer bands

D Lab 1 bands, human DNA, control bands & some designed primer bands

E We already submitted our final manuscript (peace!)

F We completed all the required steps above and are also working on additional tests



Pop Quiz!

**Test Your Knowledge
(Reward your studying)**

Monet's 1891 canvas
"Meule" or
"Grainstack" fetched
\$81.4 million with
fees.

CHRISTIE'S IMAGES LTD

Which of the following is NOT true of a codon?

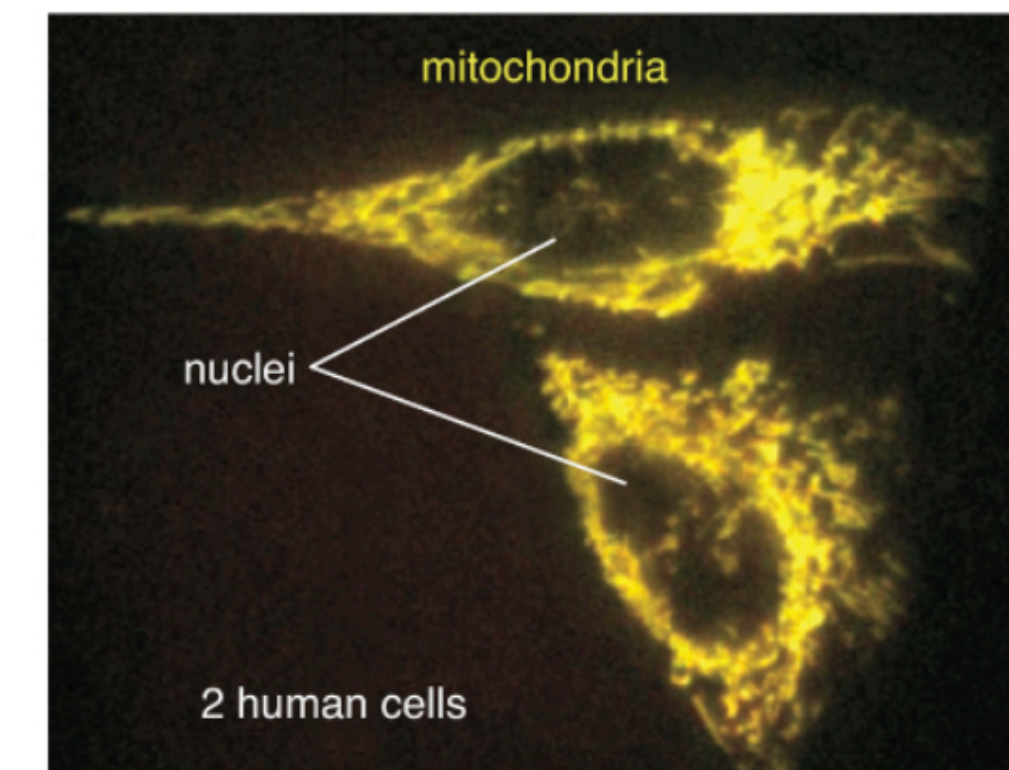
- A. It consists of three nucleotides.
- B. It may code for the same amino acid as another codon.
- C. It never codes for more than one amino acid.
- D. It extends from one end of a tRNA molecule.
- E. It is the basic unit of the genetic code.

Which of the mutations would be most likely to have a harmful effect on an organism?

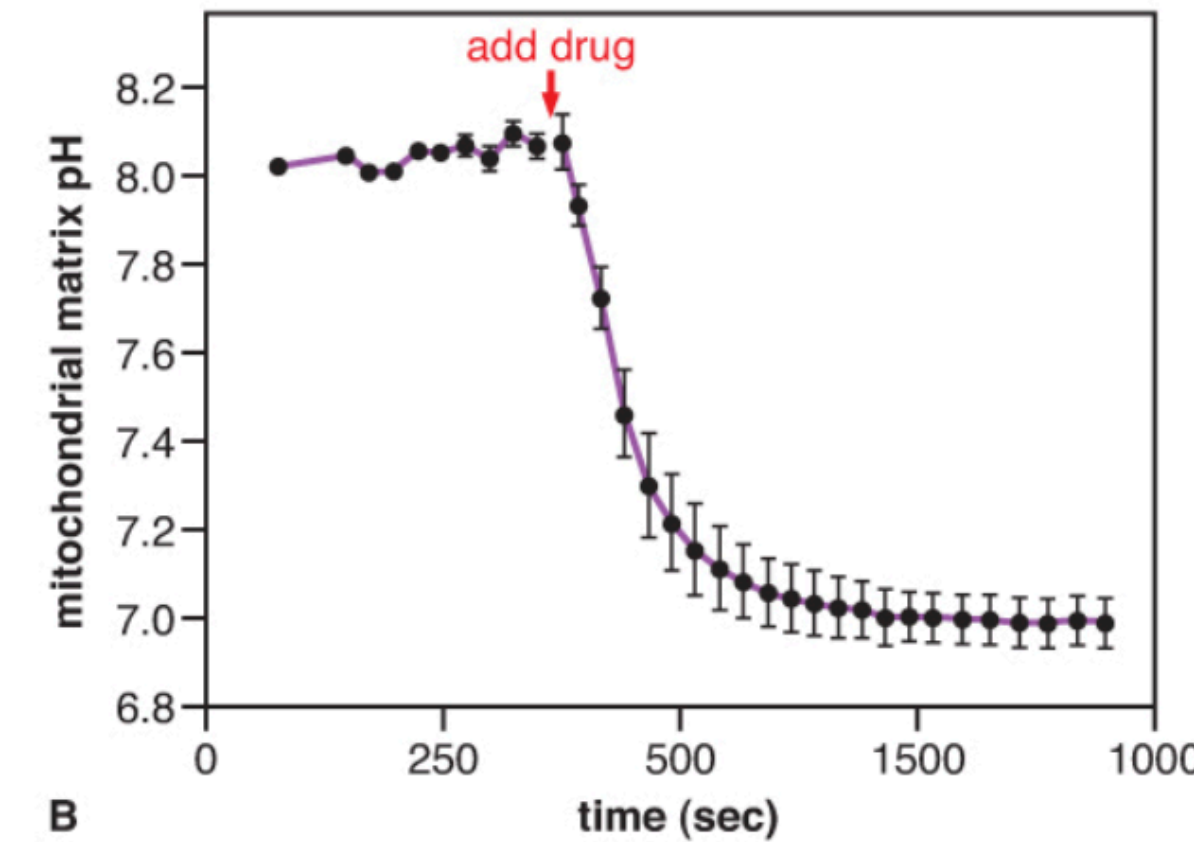
- A. a base-pair substitution
- B. a deletion of three nucleotides near the middle of a gene
- C. a single nucleotide deletion in middle of intron
- D. a single nucleotide deletion near the end of the coding sequence
- E. a single nucleotide insertion downstream of, and close to, the start of the coding sequence



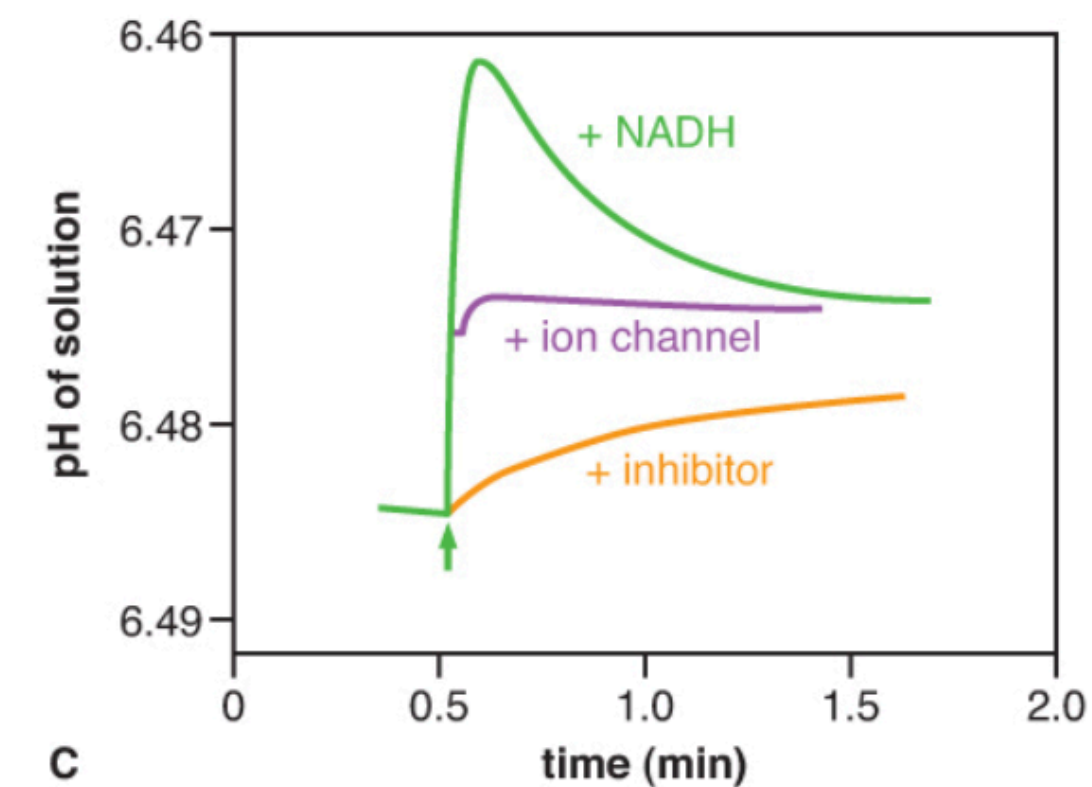
One of the keys to understanding how mitochondria convert the potential energy in NADH and FADH₂ into ATP was realizing the pH inside mitochondria differed from the pH of the cytoplasm (Figure 10.20). Using modern methods, investigators have engineered fluorescent proteins that alter their color depending on the surrounding pH. These pH indicator proteins are located in the lumen of the mitochondria, also called the **mitochondrial matrix** (Figure 10.20A). The change in colors is not readily visible to our eyes, so computer programs quantify the pH-induced color shift. To verify that the pH of the mitochondrial matrix is different from the cytoplasm, investigators monitored the pH inside mitochondria and then added a drug to insert H⁺ ion channels in the inner mitochondrial membrane (see Figure 6.15A). The added ion channels would let H⁺ ions flow down their concentration gradient until the cytoplasm and the matrix have the same pH of 7.0 (Figure 10.20B). Cell biologists had demonstrated previously that the outer mitochondrial membrane is rather porous and does not exclude



A

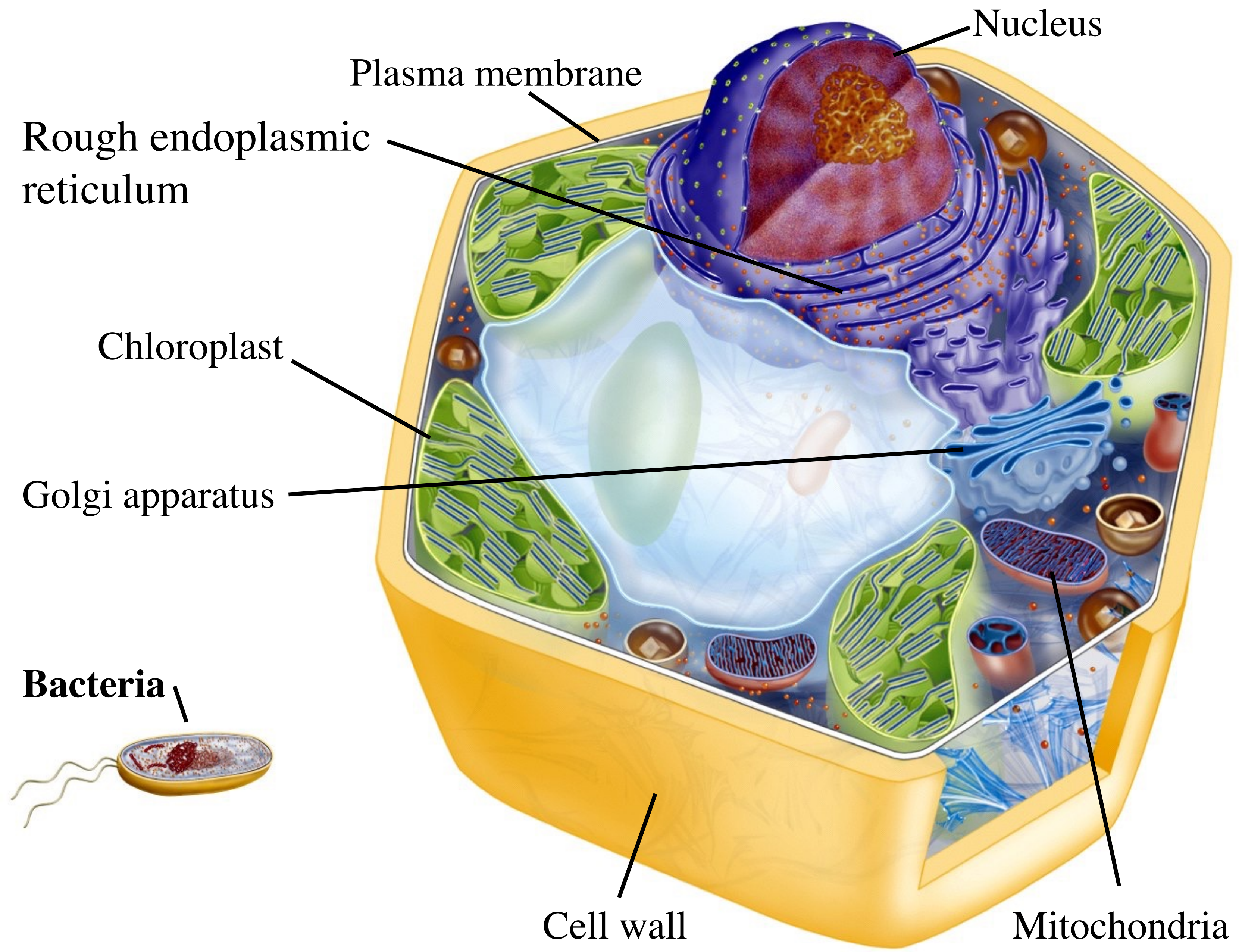


B



C

Figure 10.20 Measuring the pH inside mitochondria. **A**, Fluorescent protein inside mitochondria glows differently depending on the local pH. **B**, pH inside mitochondria before and after adding H⁺ ion channel (+/- standard error). **C**, Change in the pH of a solution containing vesicles with



Nucleus

Plasma membrane

Rough endoplasmic reticulum

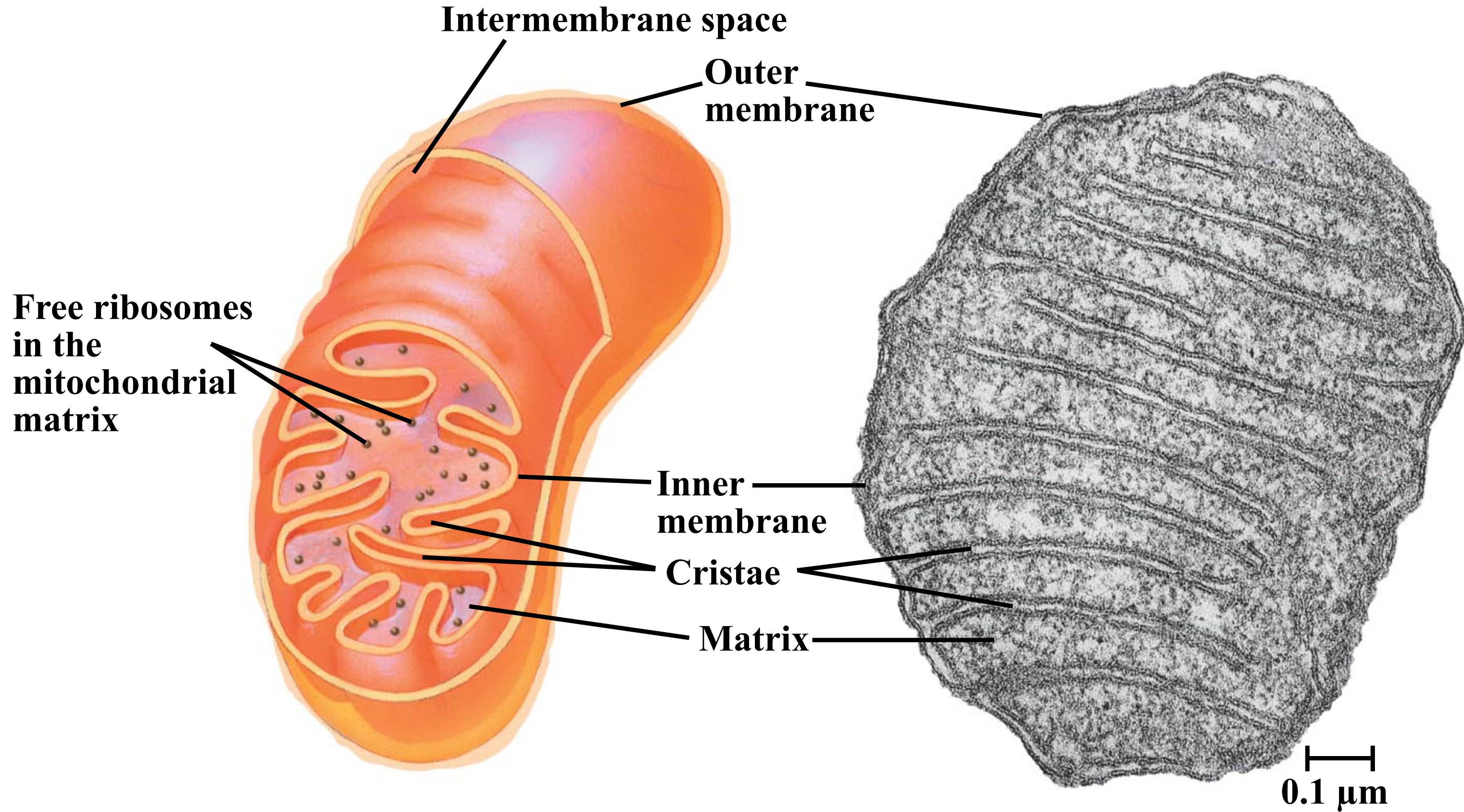
Chloroplast

Golgi apparatus

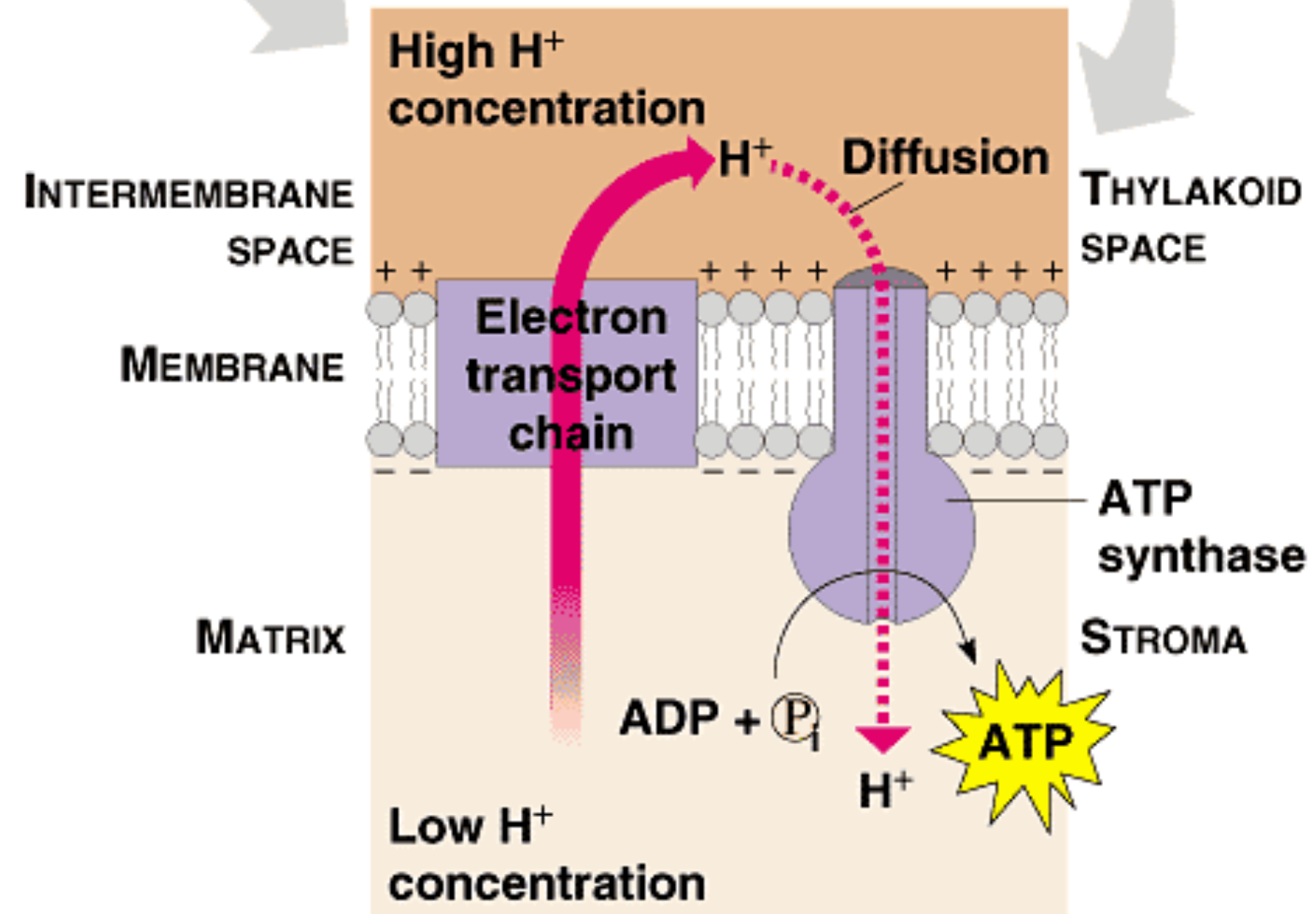
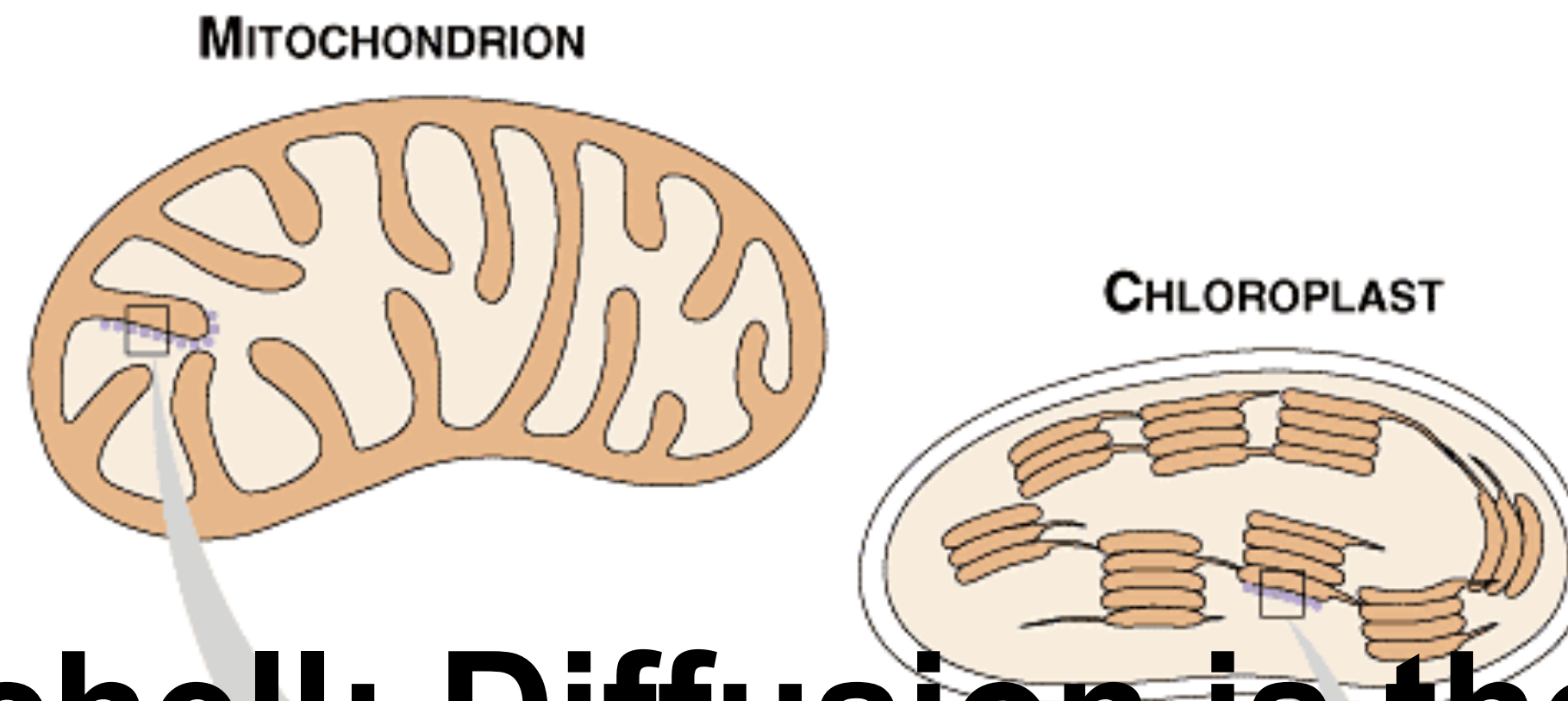
Bacteria

Cell wall

Mitochondria



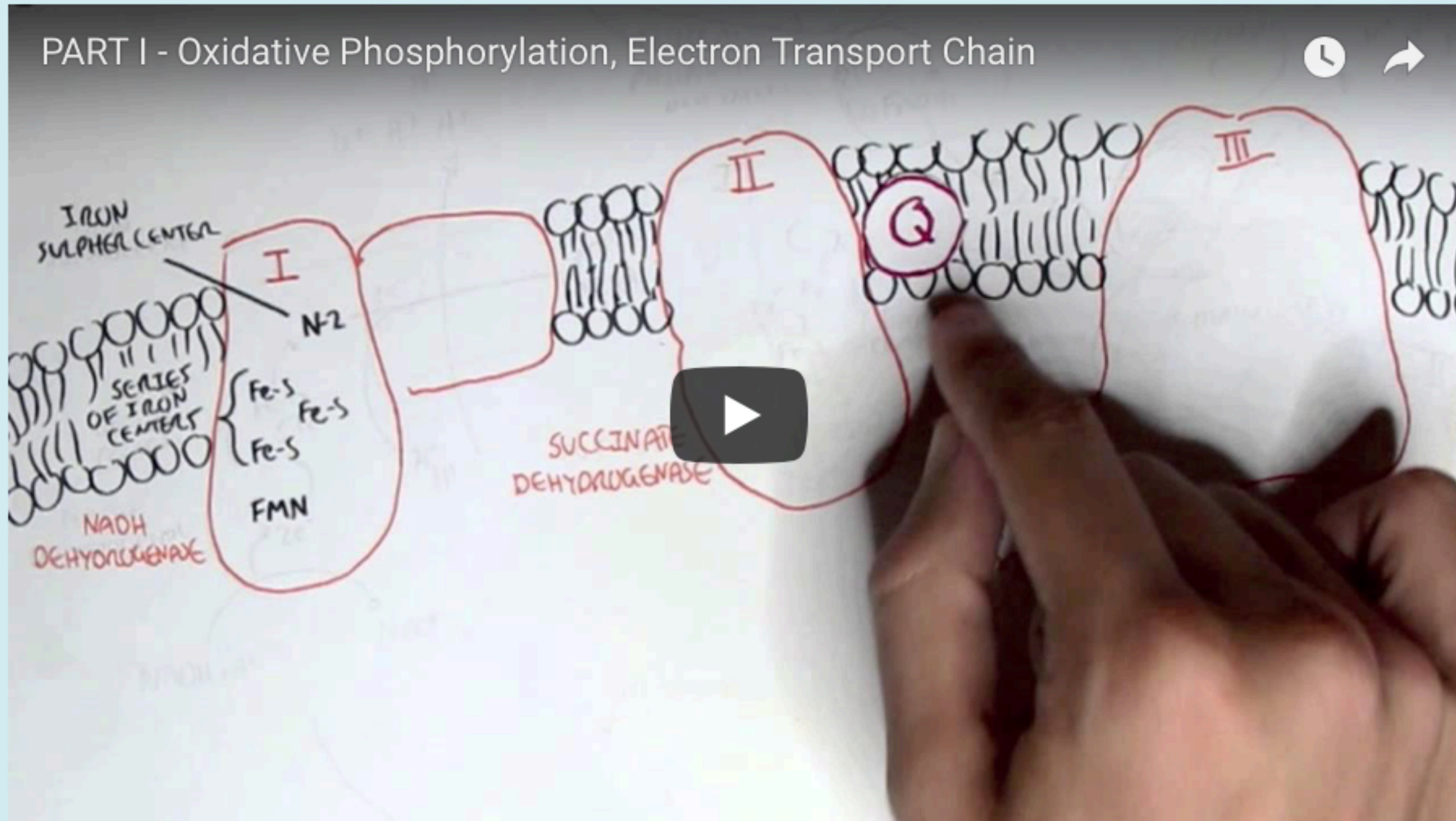
Peter Mitchell: Diffusion is the answer!

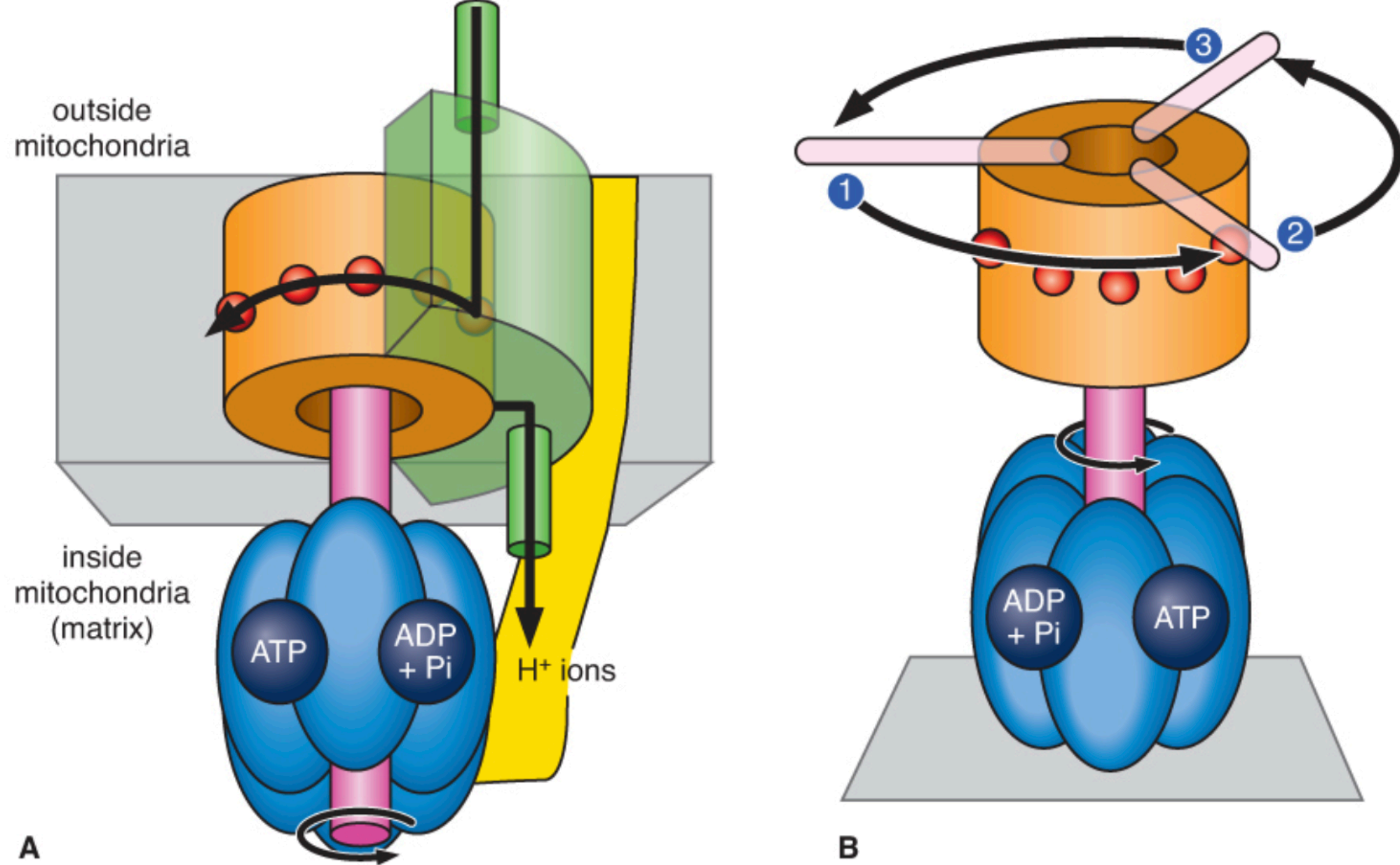


Link to Learning:

<https://youtu.be/jU2lnPwTXP0>

Video: Oxidative Phosphorylation, Electron Transport Chain.





Trifecta

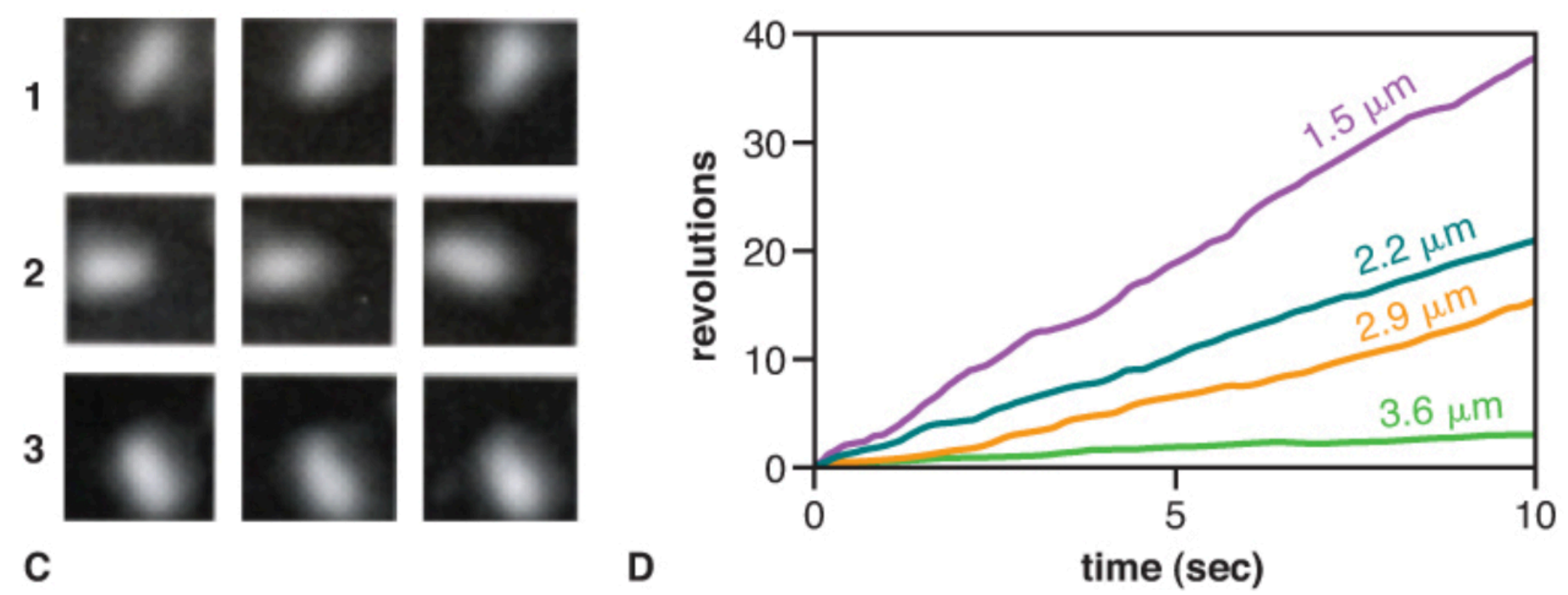


Figure 10.22 ATP synthase spins like a turbine to produce ATP. **A**, Molecular structure of ATP synthase is very similar to a human-made turbine. **B**, Immobilized ATP synthase spins its base backward when consuming ATP as visualized by attached actin rod. **C**, Photos from spinning actin from movie. **D**, Spinning rate of ATP synthase is affected by length of actin rod.

Masasuke Yoshida (1997) “check this out.”

Direct observation of the rotation of F₁-ATPase

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Hiyoshi 3-14-1, Kohoku-ku, Yokohama 223, Japan

Cells employ a variety of linear motors, such as myosin¹⁻³, kinesin⁴ and RNA polymerase⁵, which move along and exert force on a filamentous structure. But only one rotary motor has been investigated in detail, the bacterial flagellum⁶ (a complex of about 100 protein molecules⁷). We now show that a single molecule of F₁-ATPase acts as a rotary motor, the smallest known, by direct observation of its motion. A central rotor of radius ~1 nm, formed by its γ -subunit, turns in a stator barrel of radius ~5 nm formed by three α - and three β -subunits⁸. F₁-ATPase, together with the membrane-embedded proton-conducting unit F₀, forms the H⁺-ATP synthase that reversibly couples transmembrane proton flow to ATP synthesis/hydrolysis in respiring and photosynthetic cells^{9,10}. It has been suggested that the γ -subunit of F₁-ATPase rotates within the $\alpha\beta$ -hexamer¹¹, a conjecture supported by structural⁸, biochemical^{12,13} and spectroscopic¹⁴ studies. We attached a fluorescent actin filament to the γ -subunit as a marker, which enabled us to observe this motion directly. In the presence of ATP, the filament rotated for more than 100 revolutions in an anticlockwise direction when viewed from the ‘membrane’ side. The rotary torque produced reached more than 40 pN nm⁻¹ under high load.

In the crystal structure of mitochondrial F₁-ATPase⁸, rigid¹⁵ coiled-coil α -helices of the γ -subunit penetrate the central cavity of the $\alpha_3\beta_3$ and extend into the stalk region that links F₁-ATPase to the F₀ portion. The amino terminus of the β -subunits is on the side opposite the stalk region of the γ -subunit. To fix the $\alpha_3\beta_3\gamma$ subcomplex on a glass plate, the subcomplex derived from a thermophilic bacterium was expressed in *Escherichia coli*, with ten histidines (His tag) linked to the N terminus of each β -subunit. The

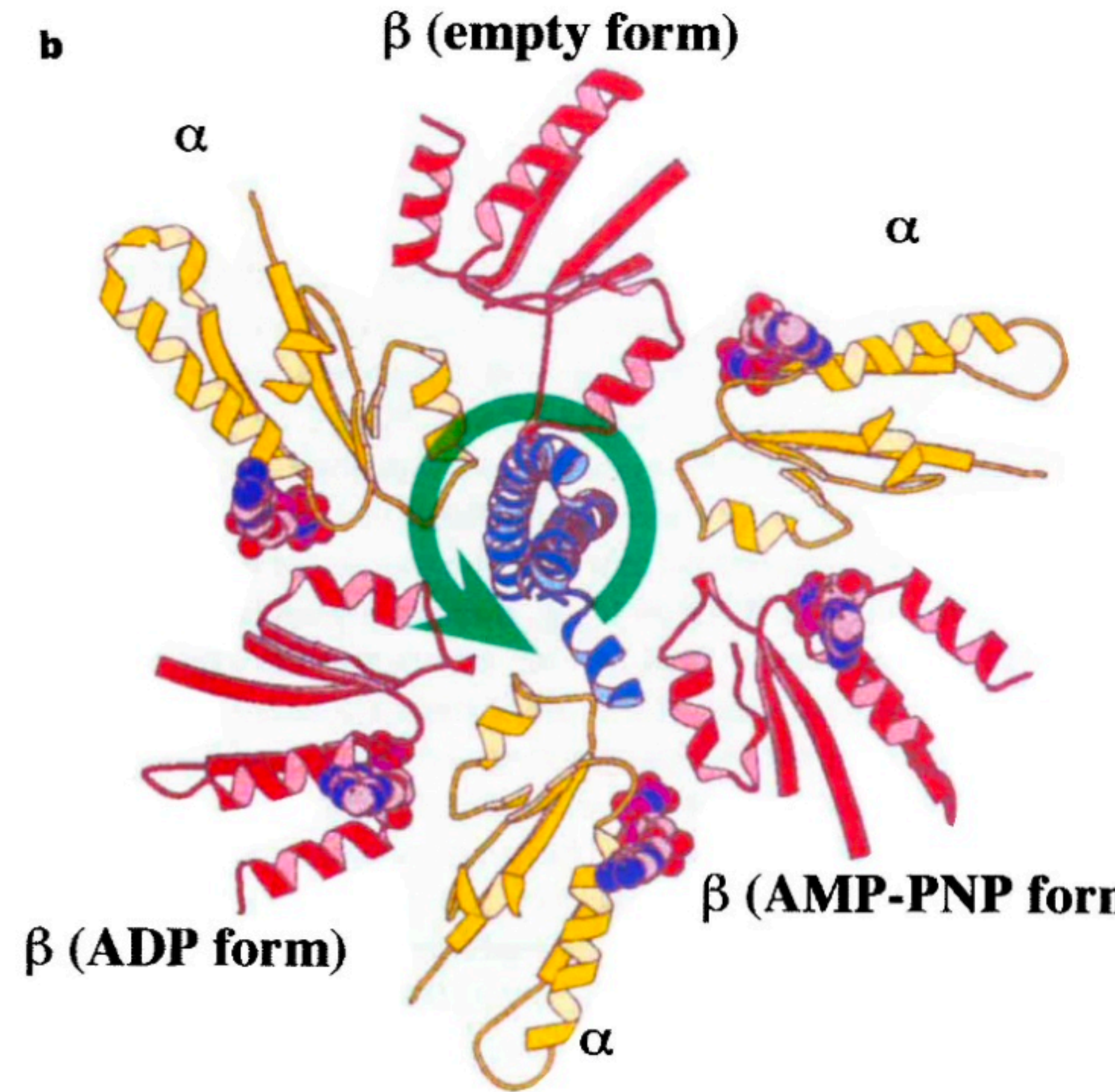
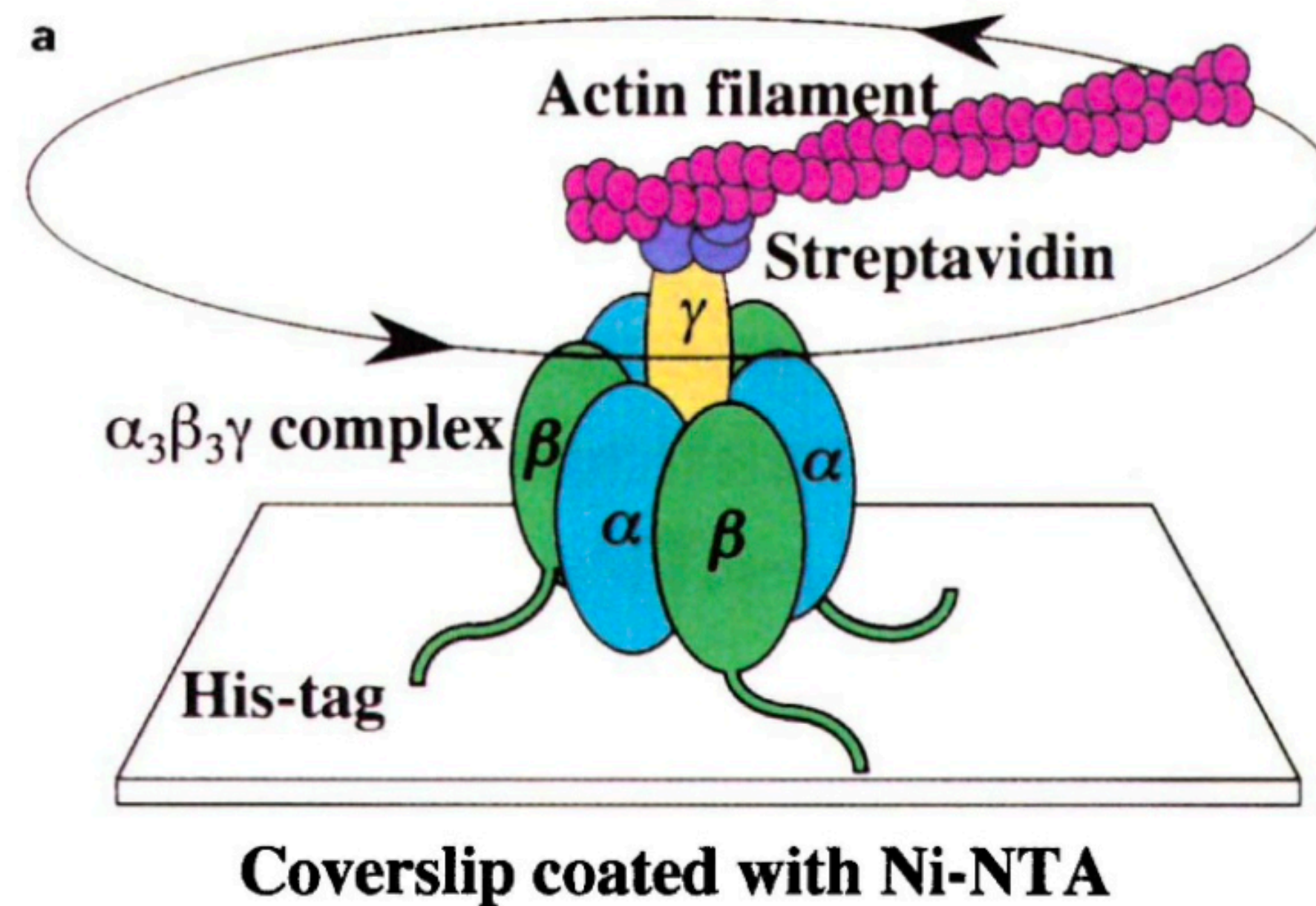
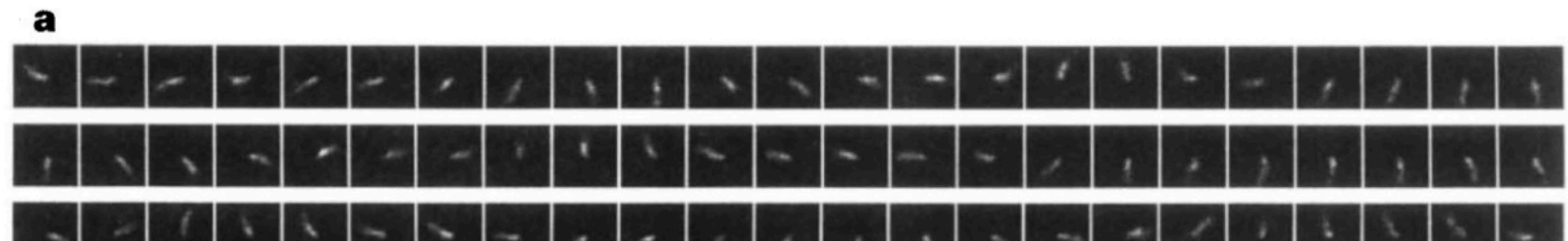
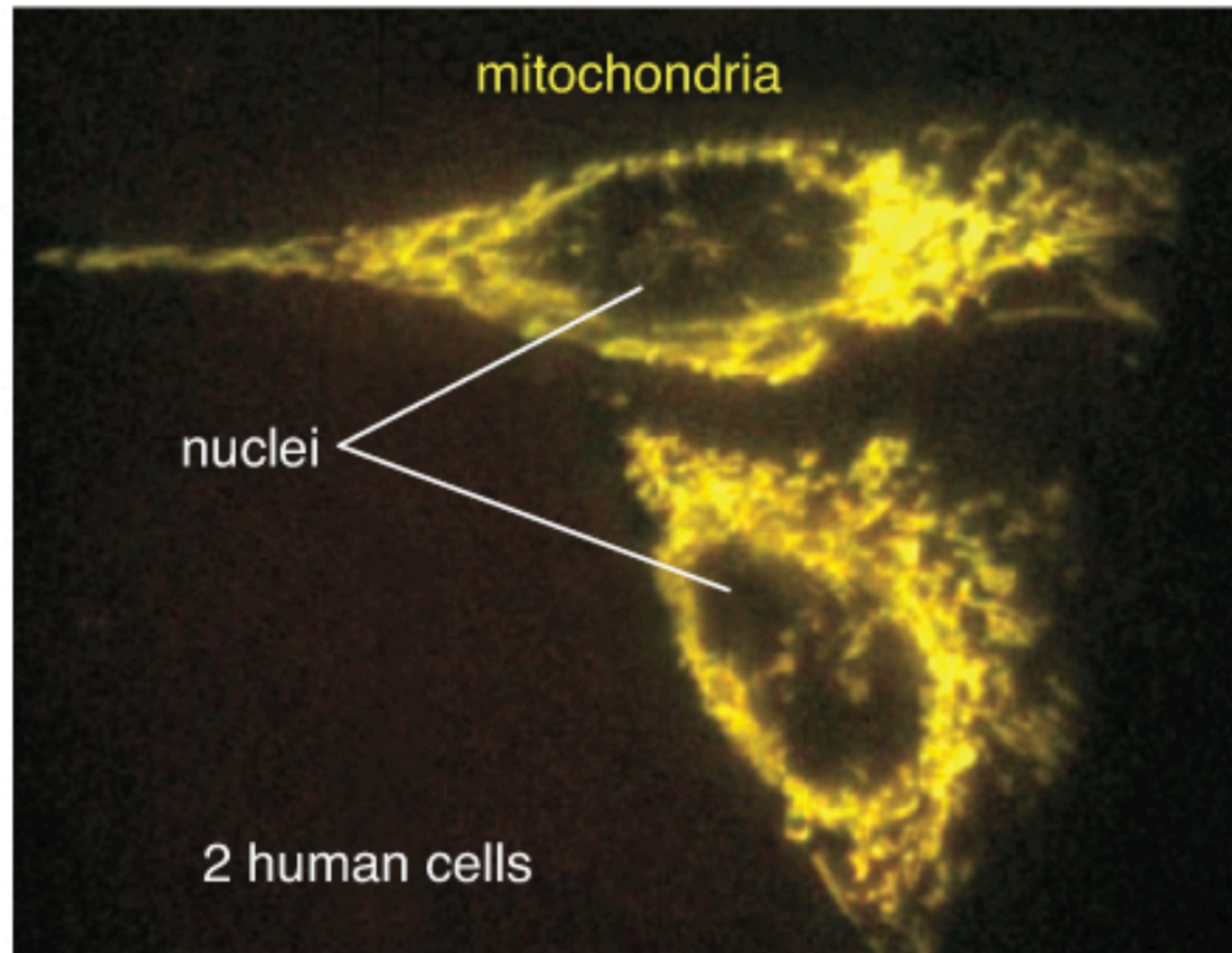
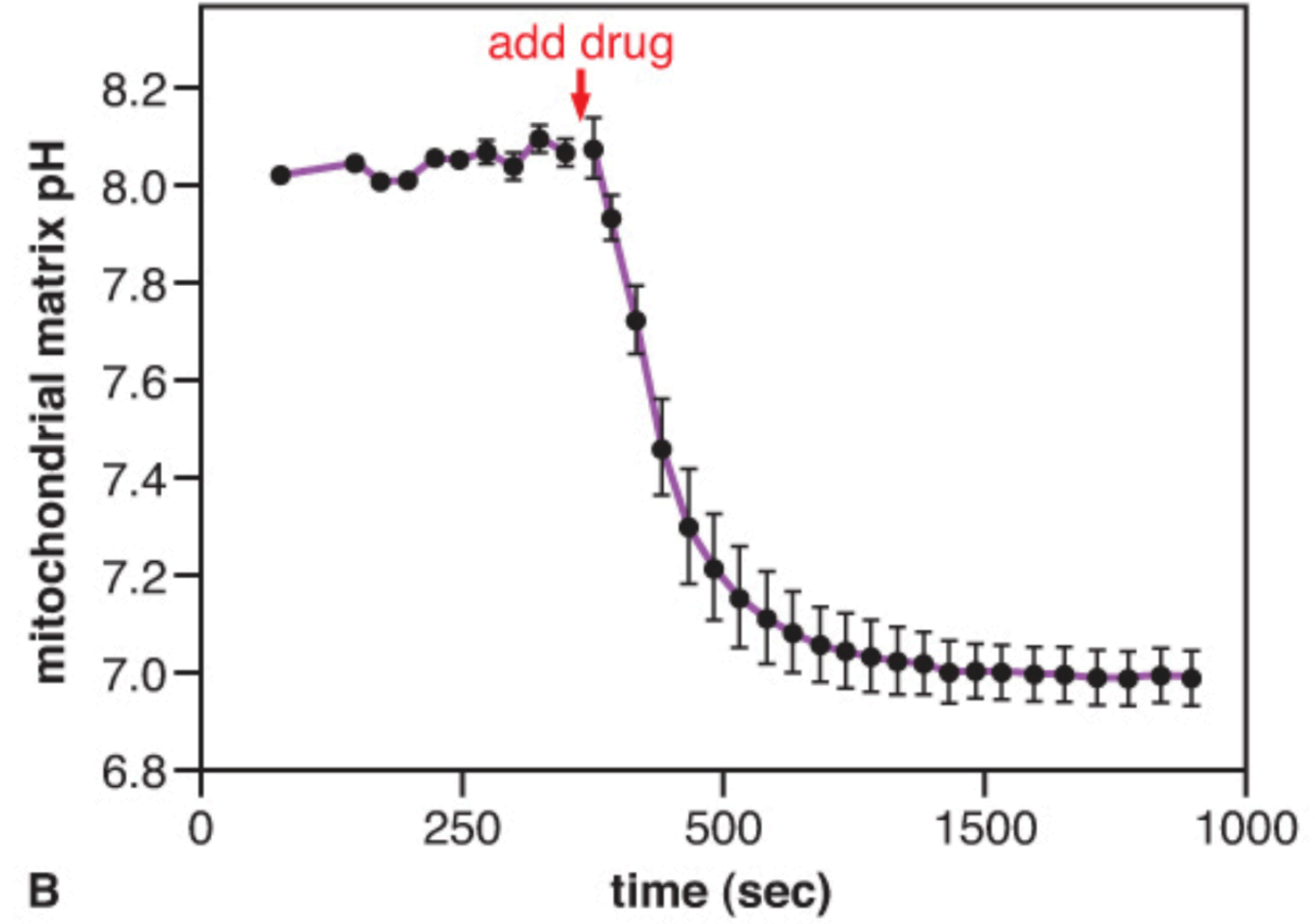


Figure 1 a, The system used for observation of the rotation of the γ -subunit in the $\alpha_3\beta_3\gamma$ subcomplex. **b**, Crystal structure of mitochondrial F₁-ATPase⁸ viewed from the membrane side, or from above the glass plate in **a**. Only a part of the structure near the nucleotide-binding site is shown. The observed direction of the rotation of the γ -subunit is indicated by an arrow.

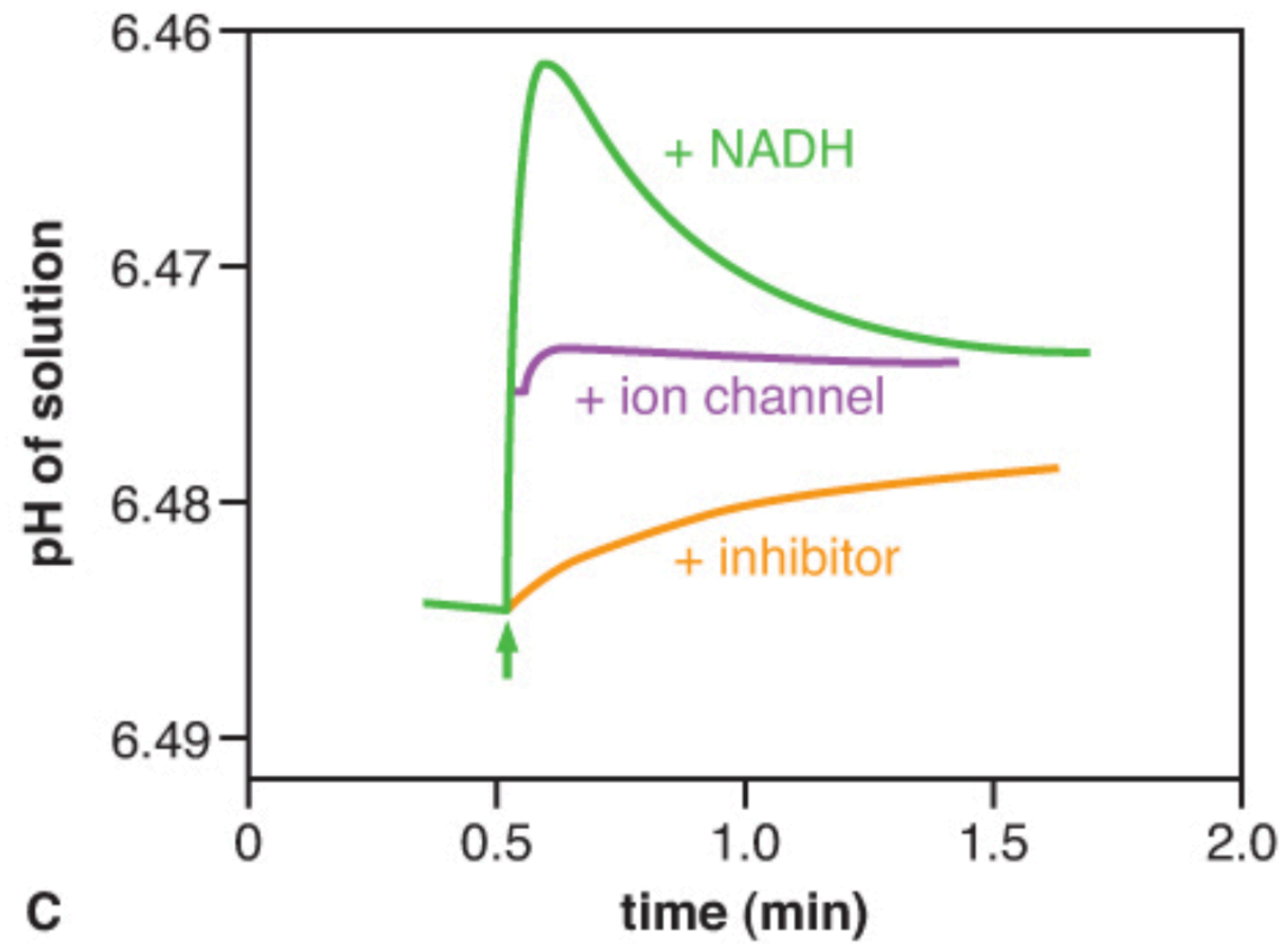




A

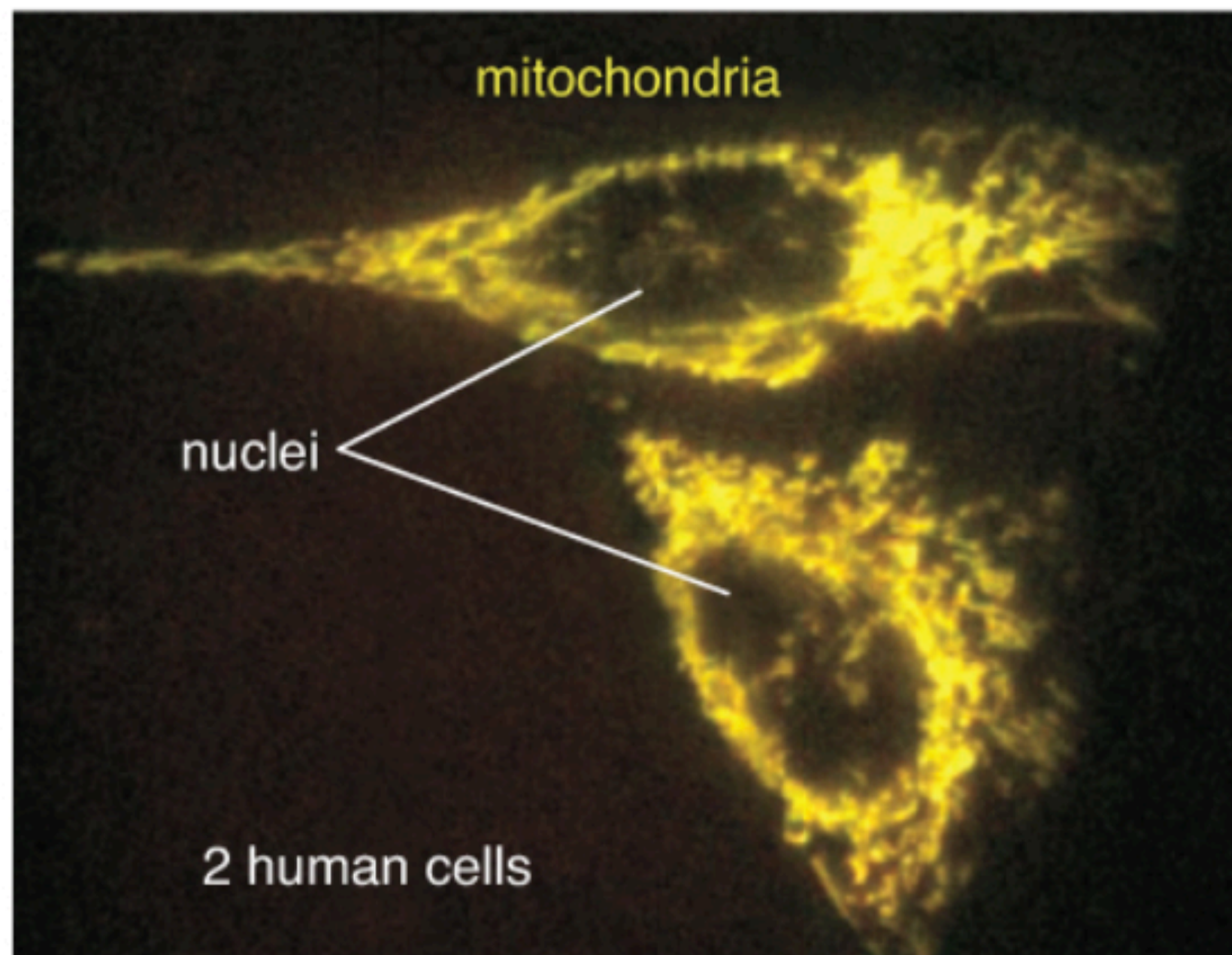


B

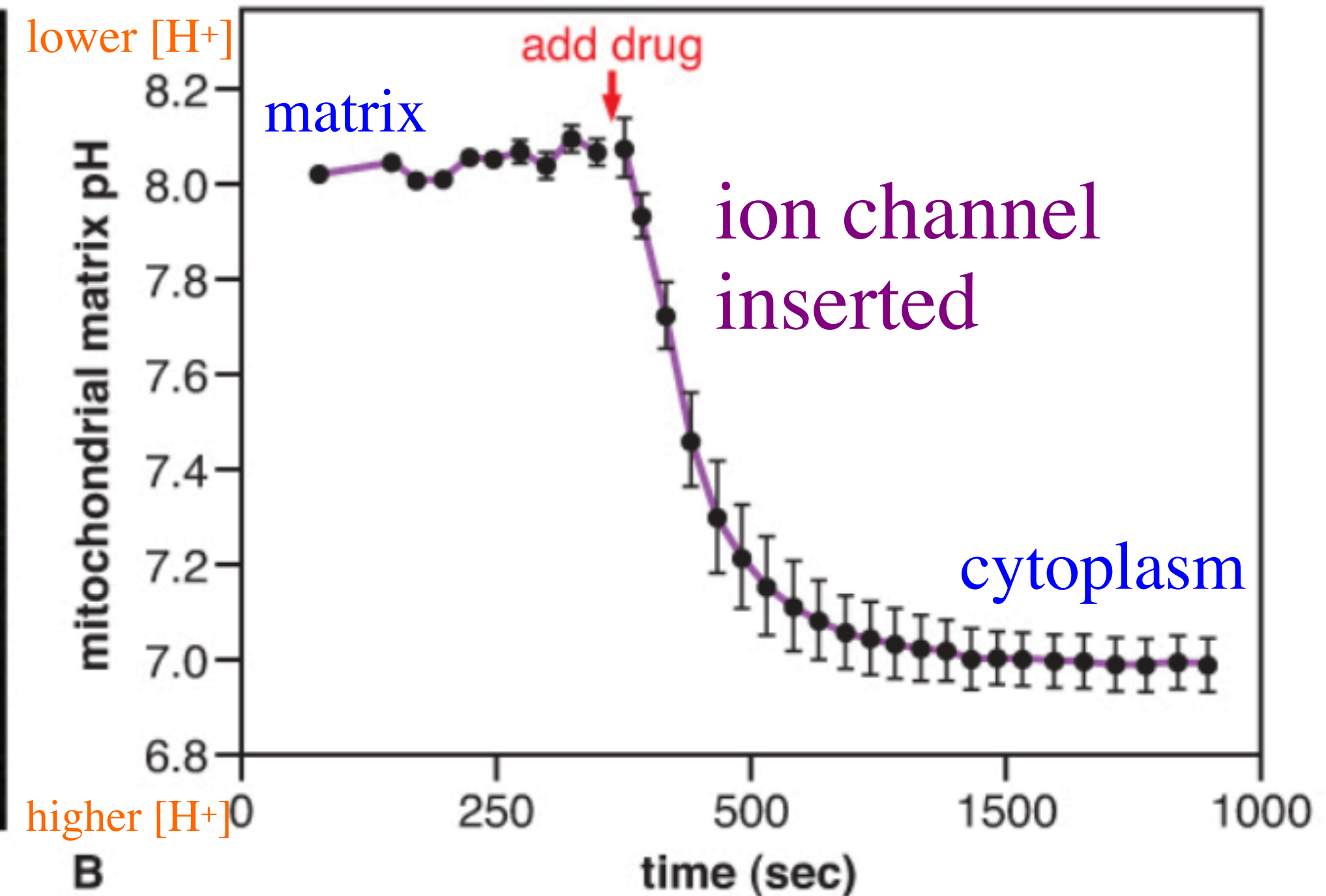


C

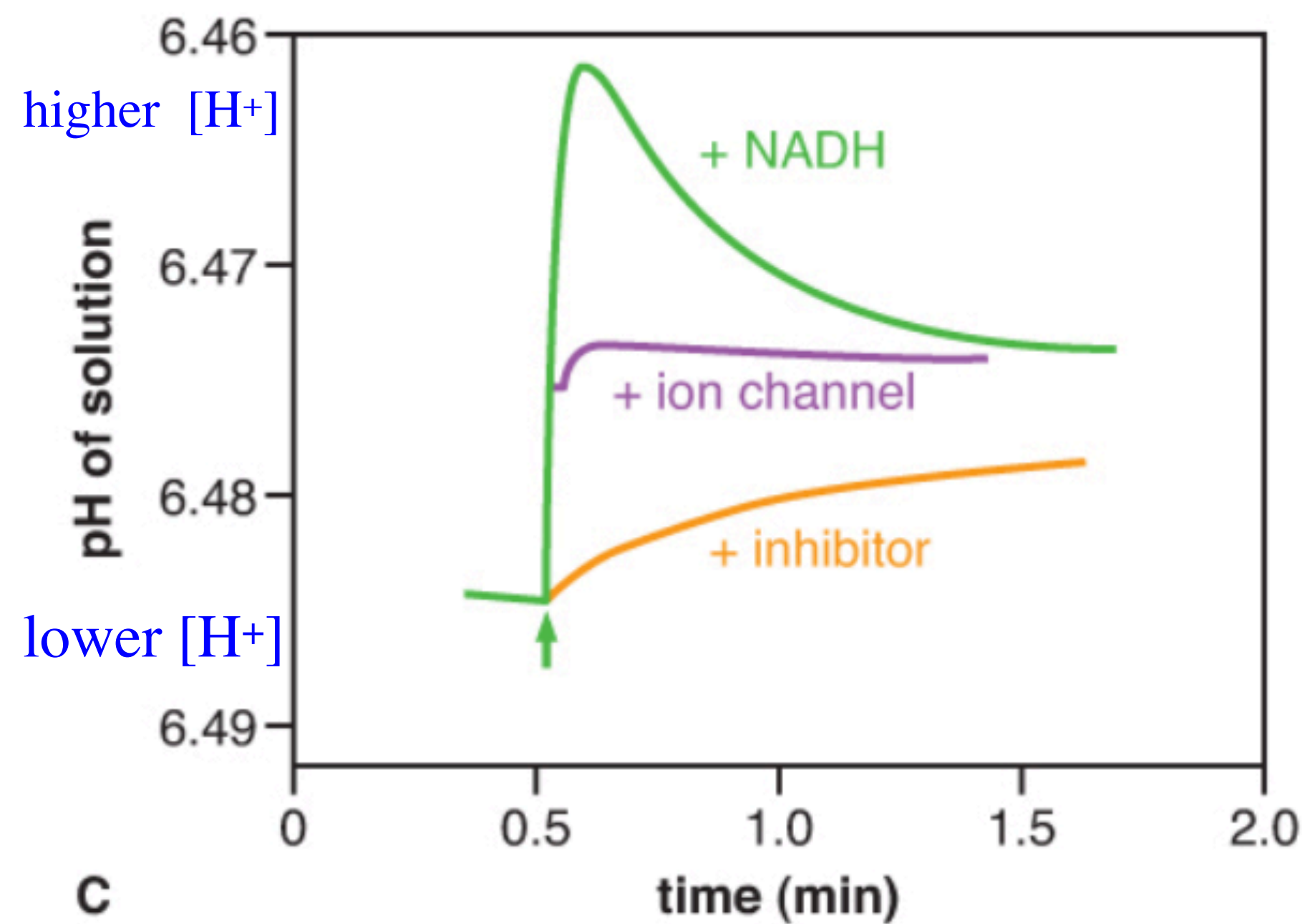
Trifecta



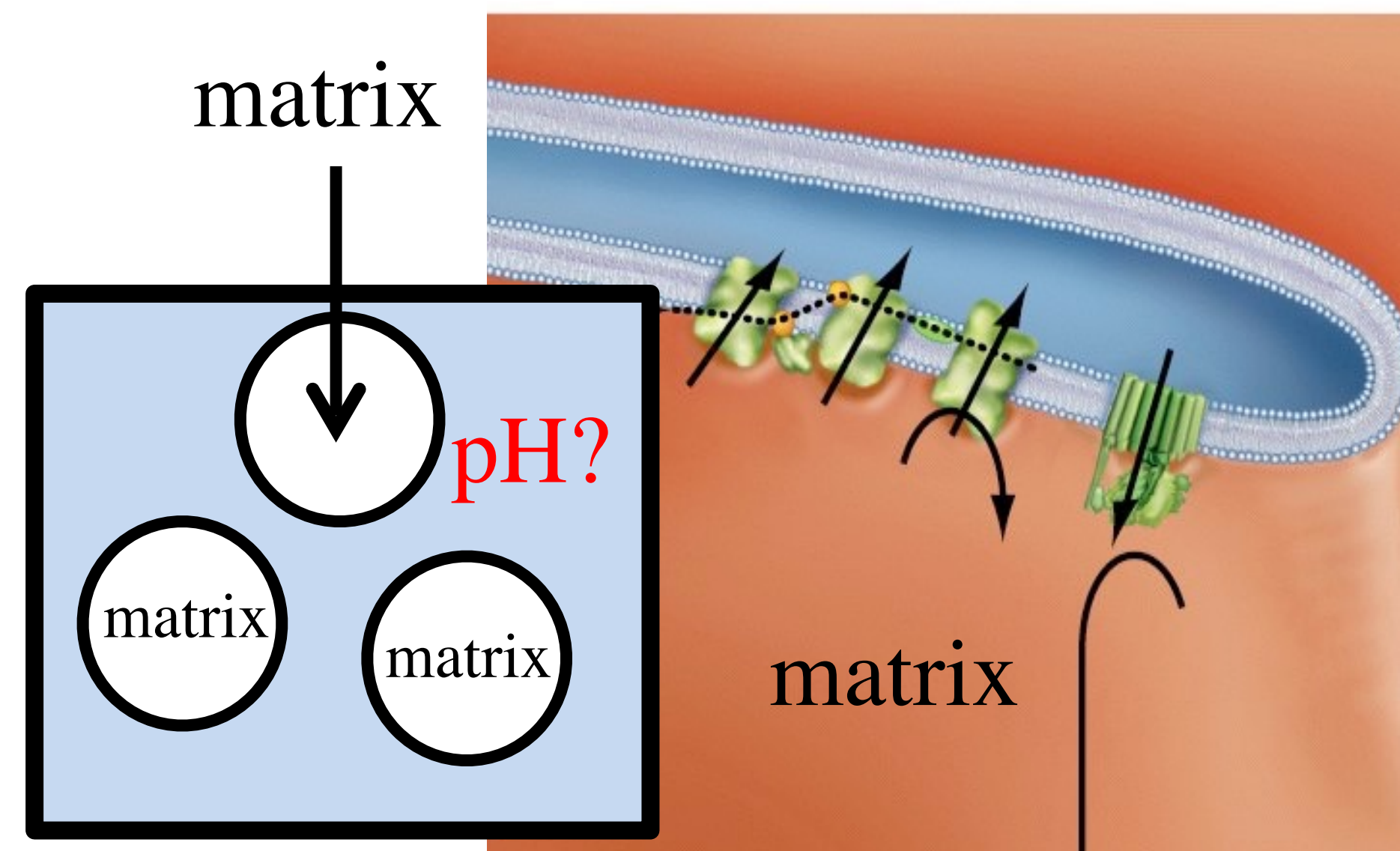
A



B



C



Δ of Mitochondrial pH

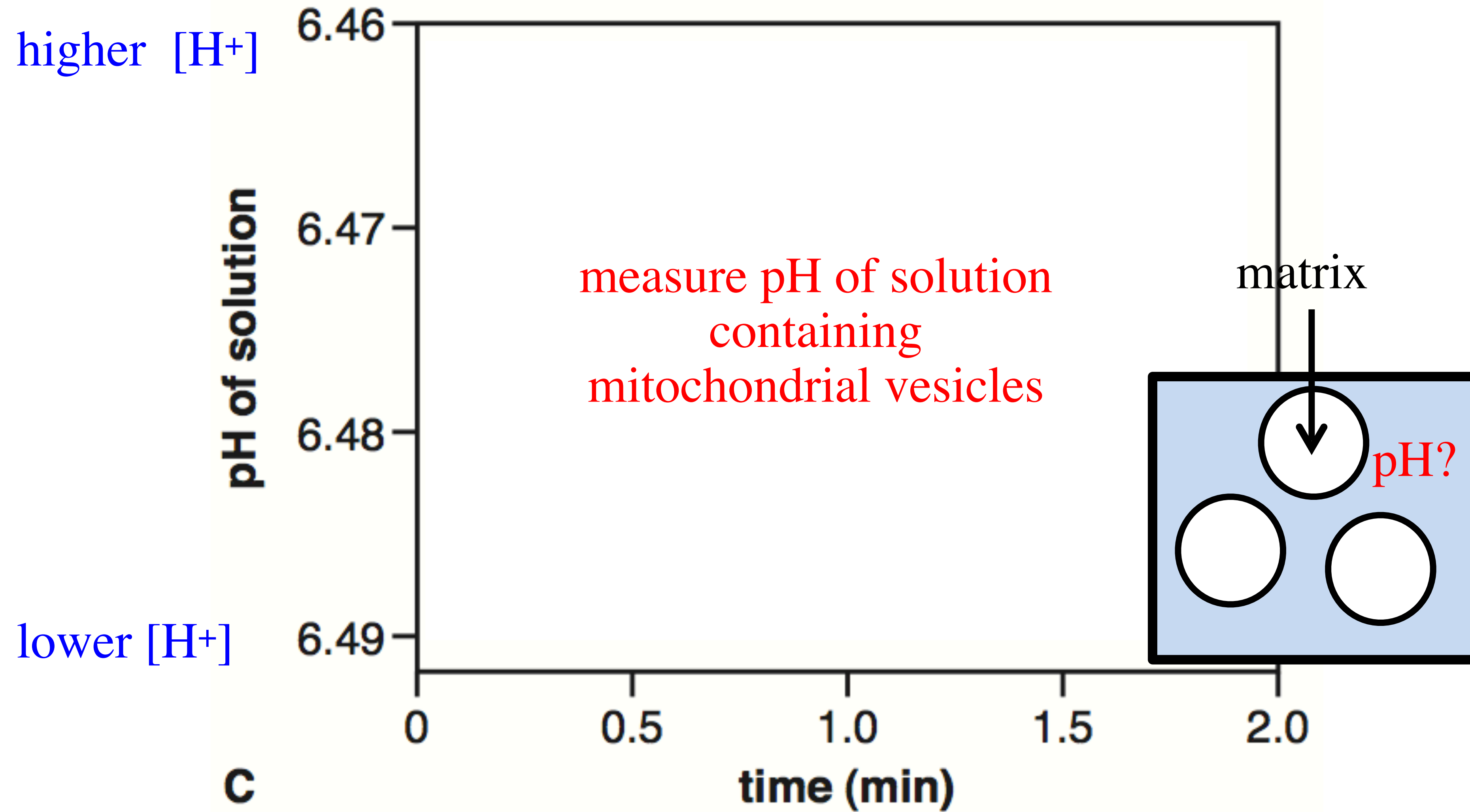


Fig. 10.20

Δ of Mitochondrial pH

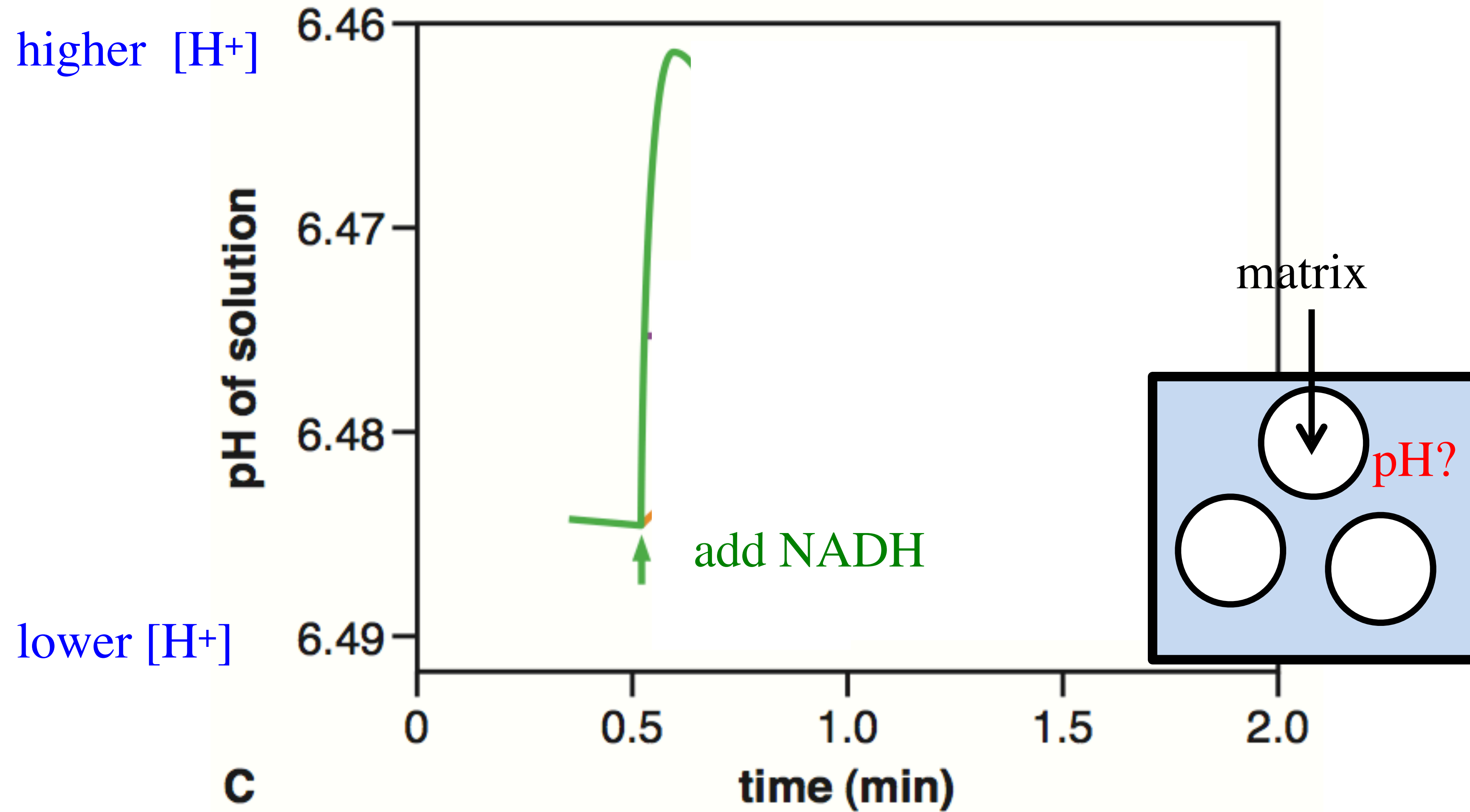


Fig. 10.20

Δ of Mitochondrial pH

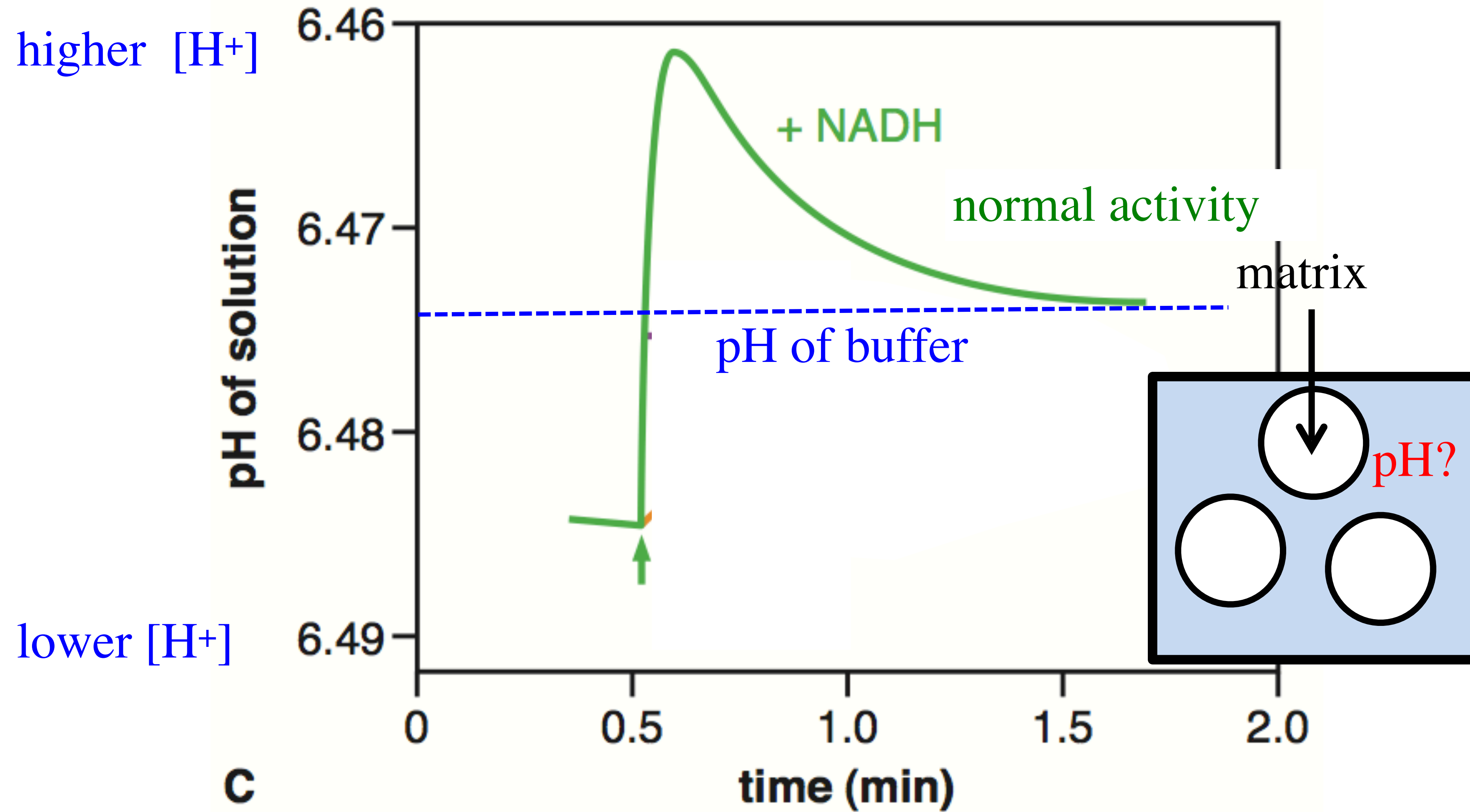


Fig. 10.20

Δ of Mitochondrial pH

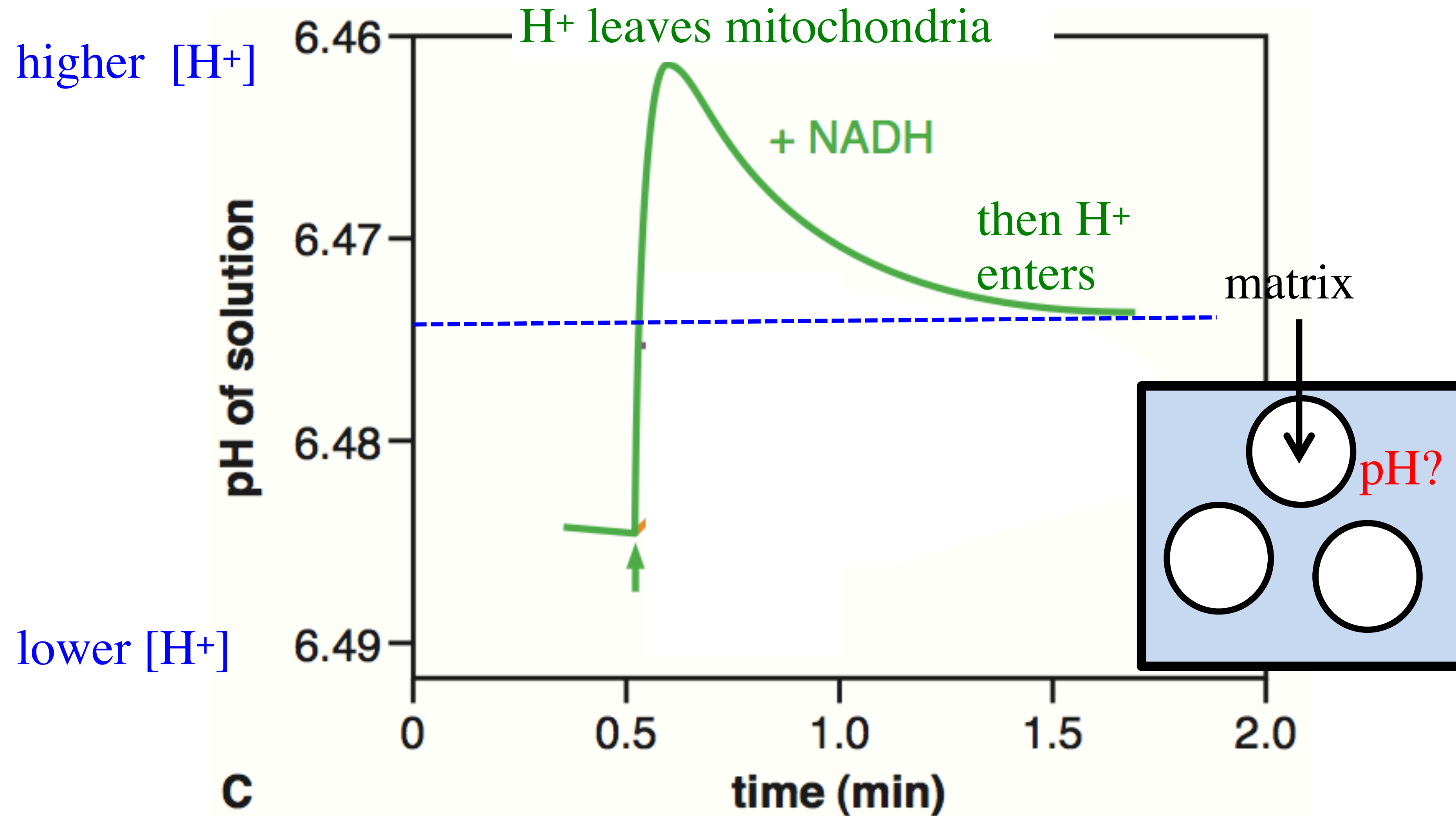


Fig. 10.20

Δ of Mitochondrial pH

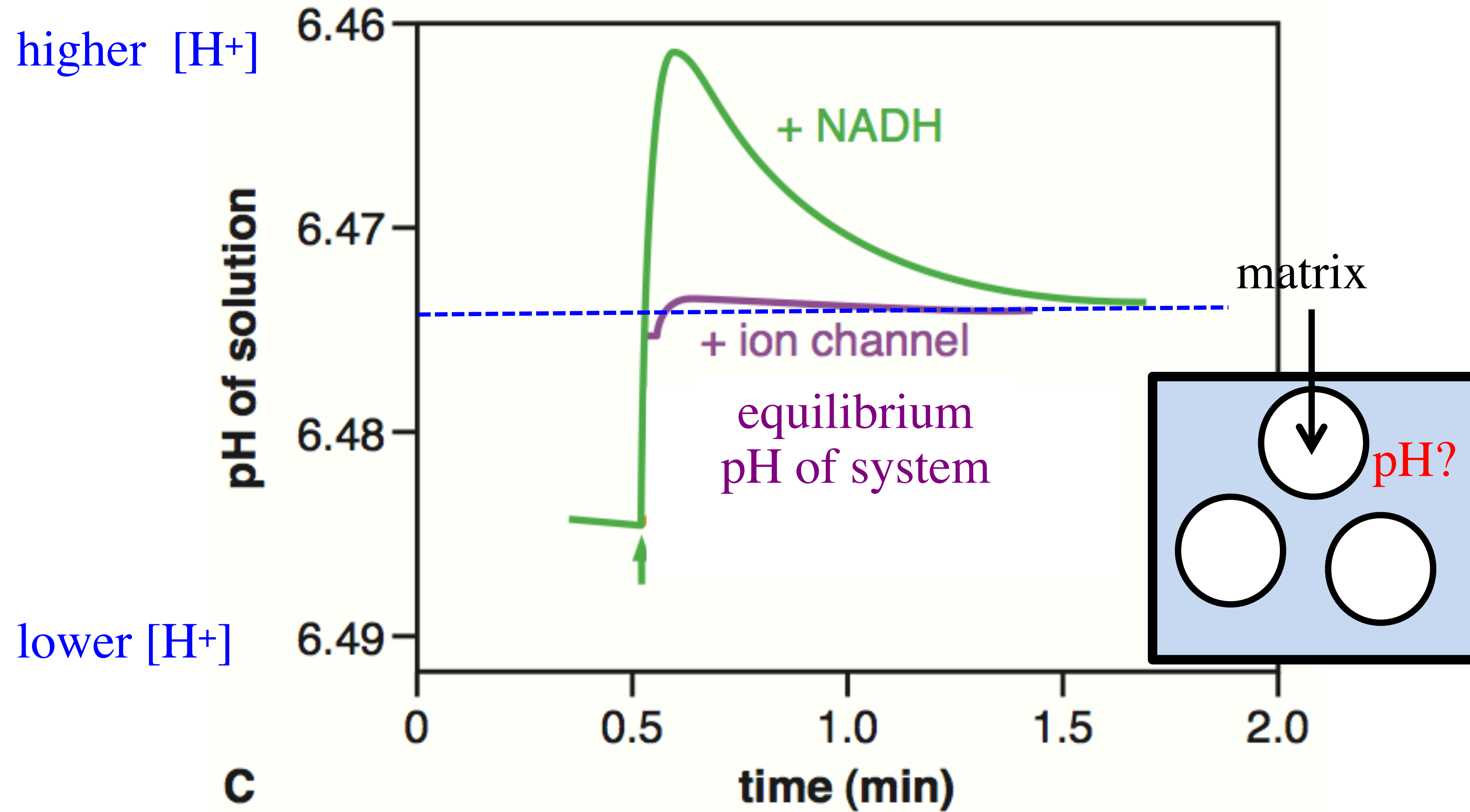


Fig. 10.20

Δ of Mitochondrial pH

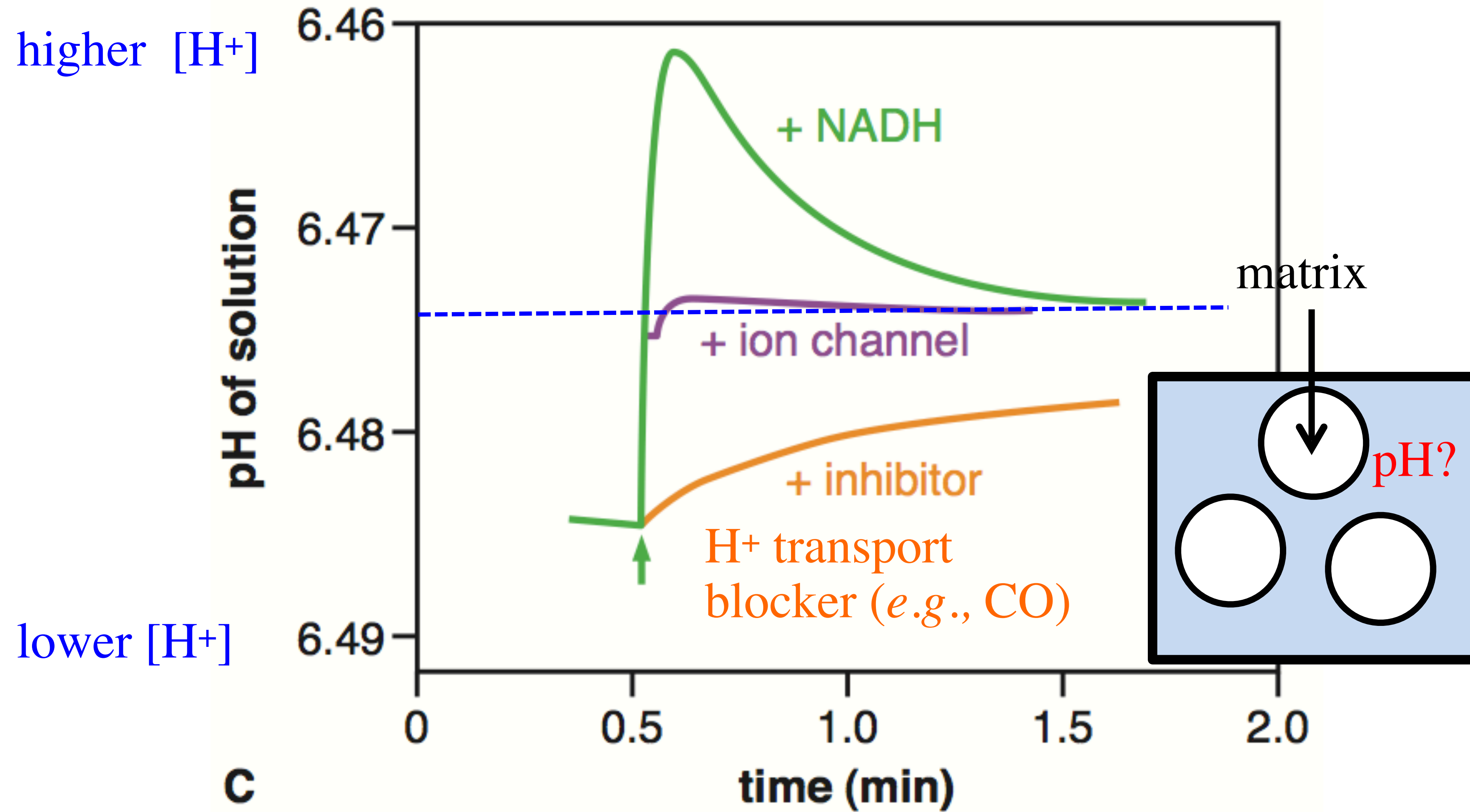
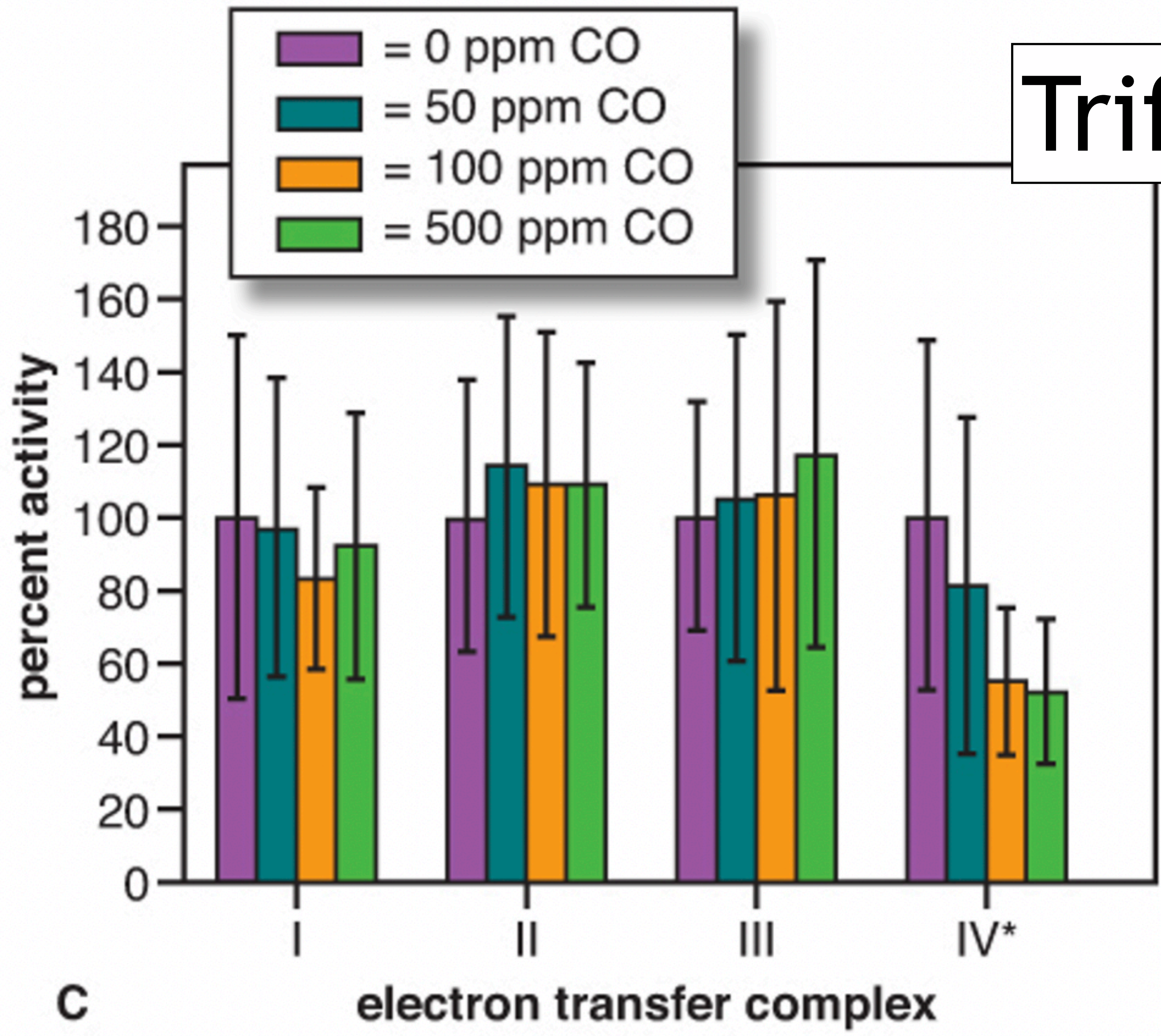


Fig. 10.20

Trifecta



C

electron transfer complex

Electron Transport and Carriers

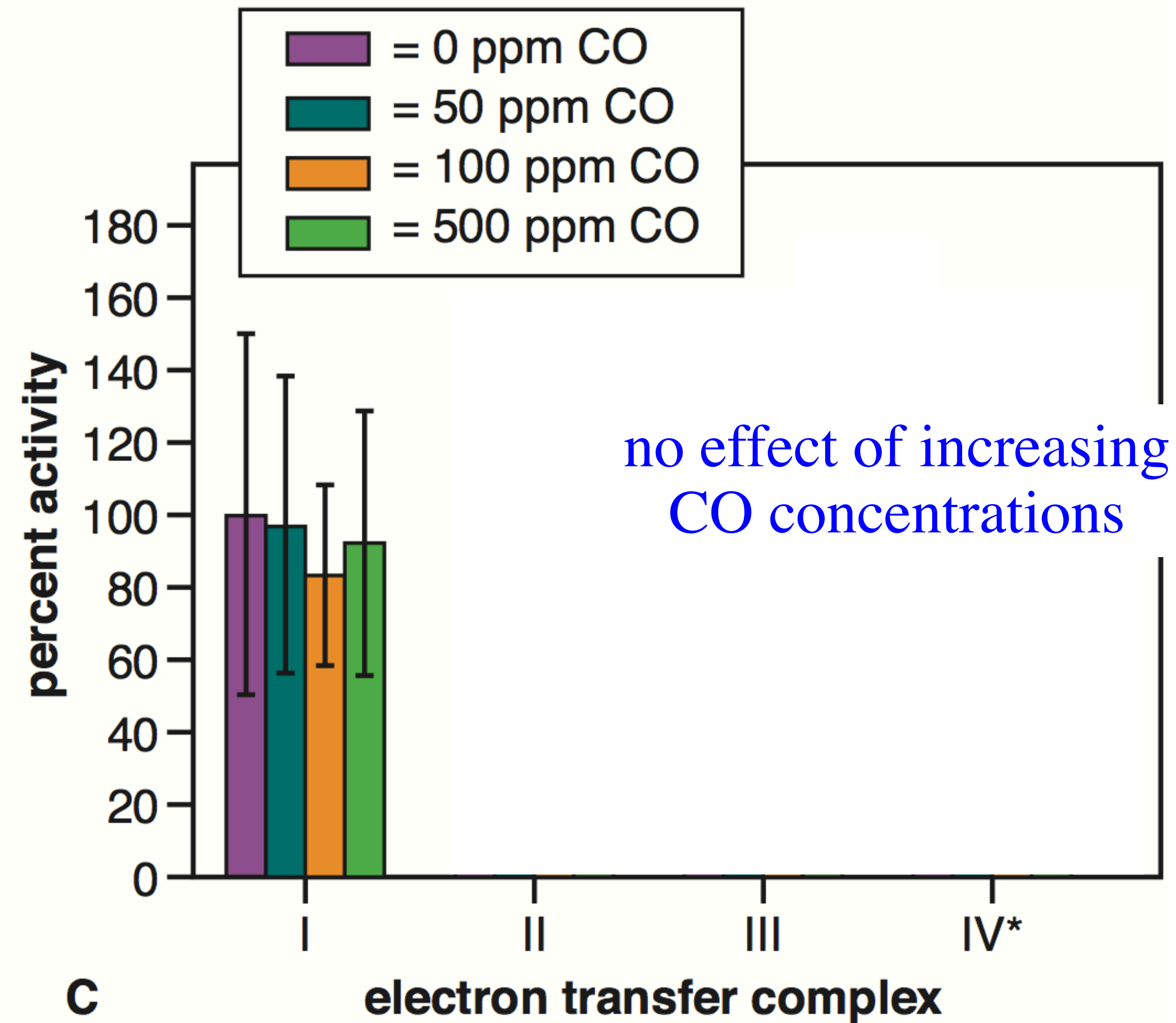


Fig. 10.21

Electron Transport and Carriers

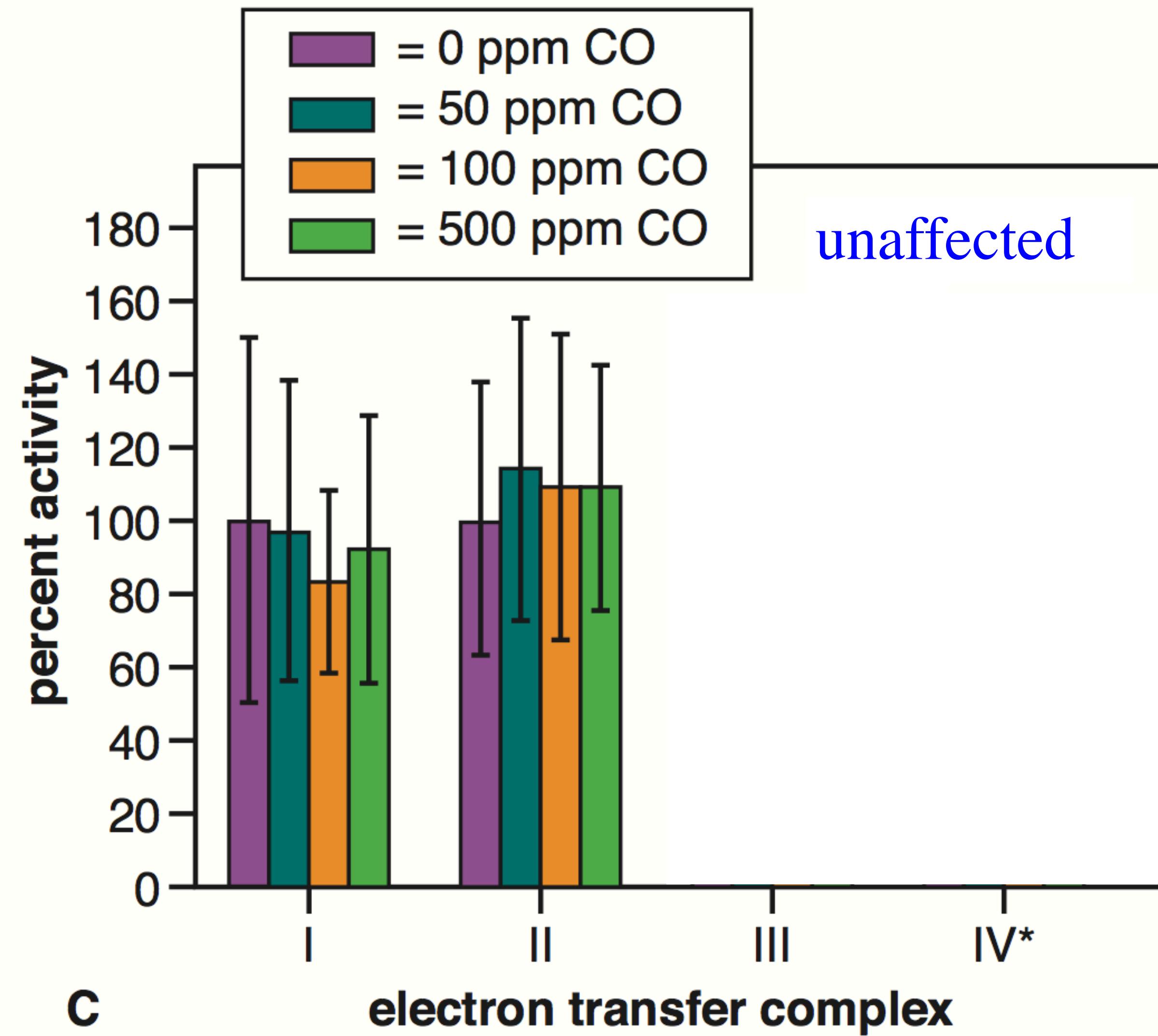


Fig. 10.21

Electron Transport and Carriers

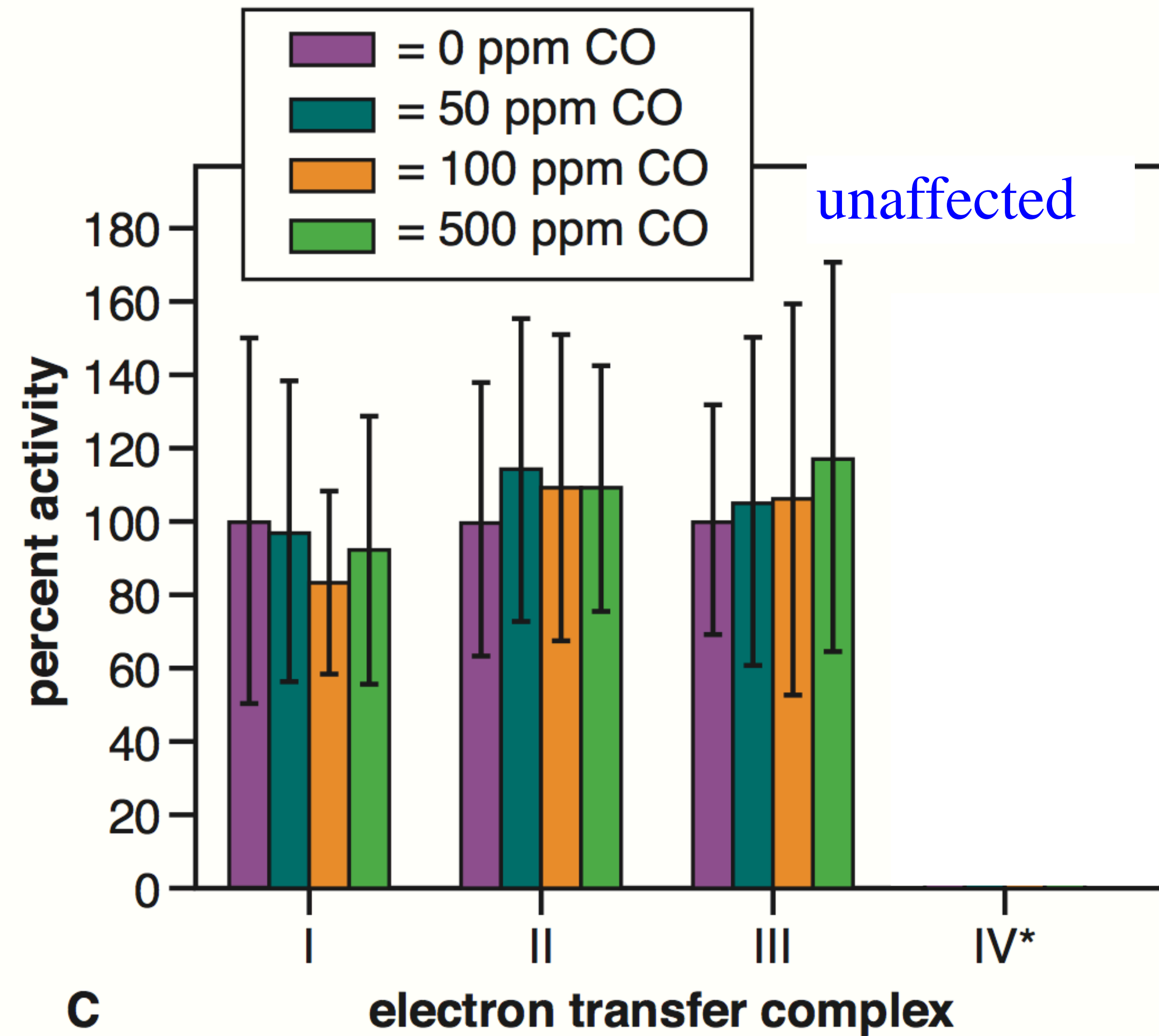


Fig. 10.21

Electron Transport and Carriers

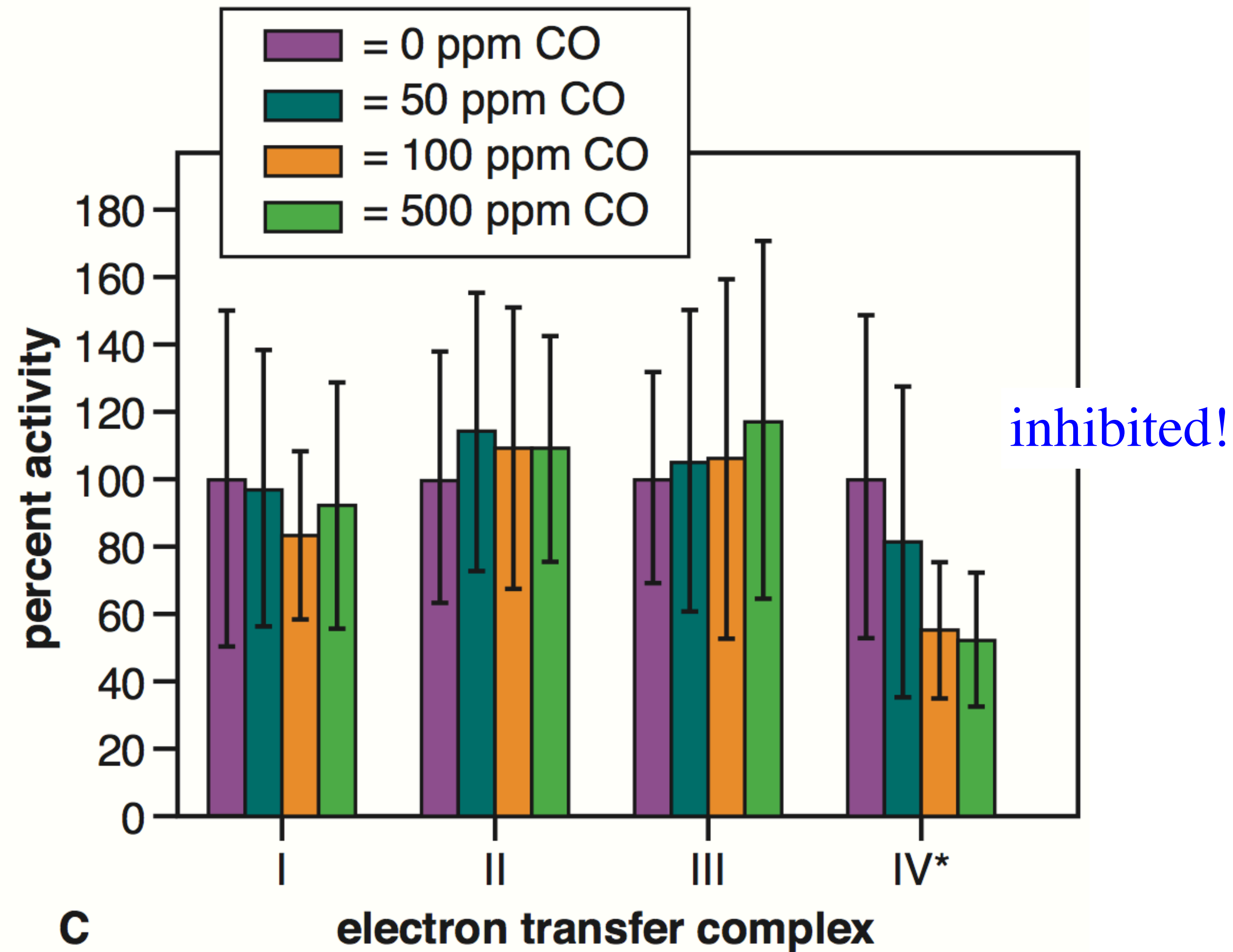
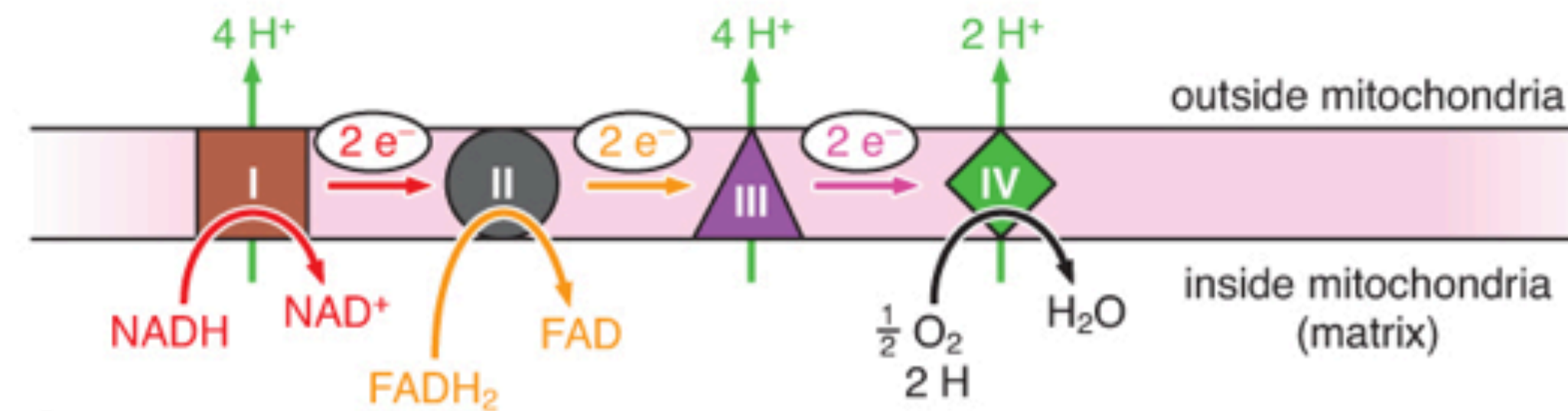
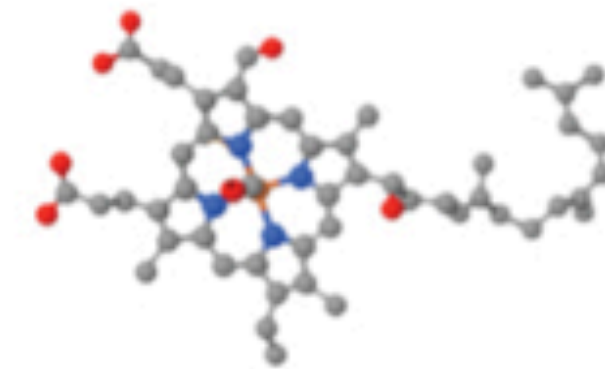


Fig. 10.21

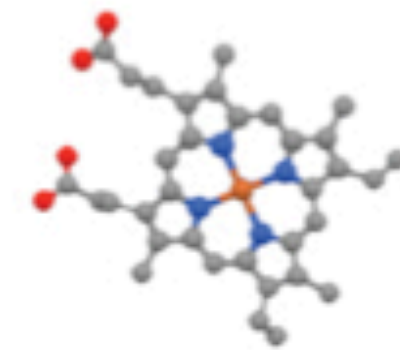
Trifecta



A



cytochrome c



B hemoglobin

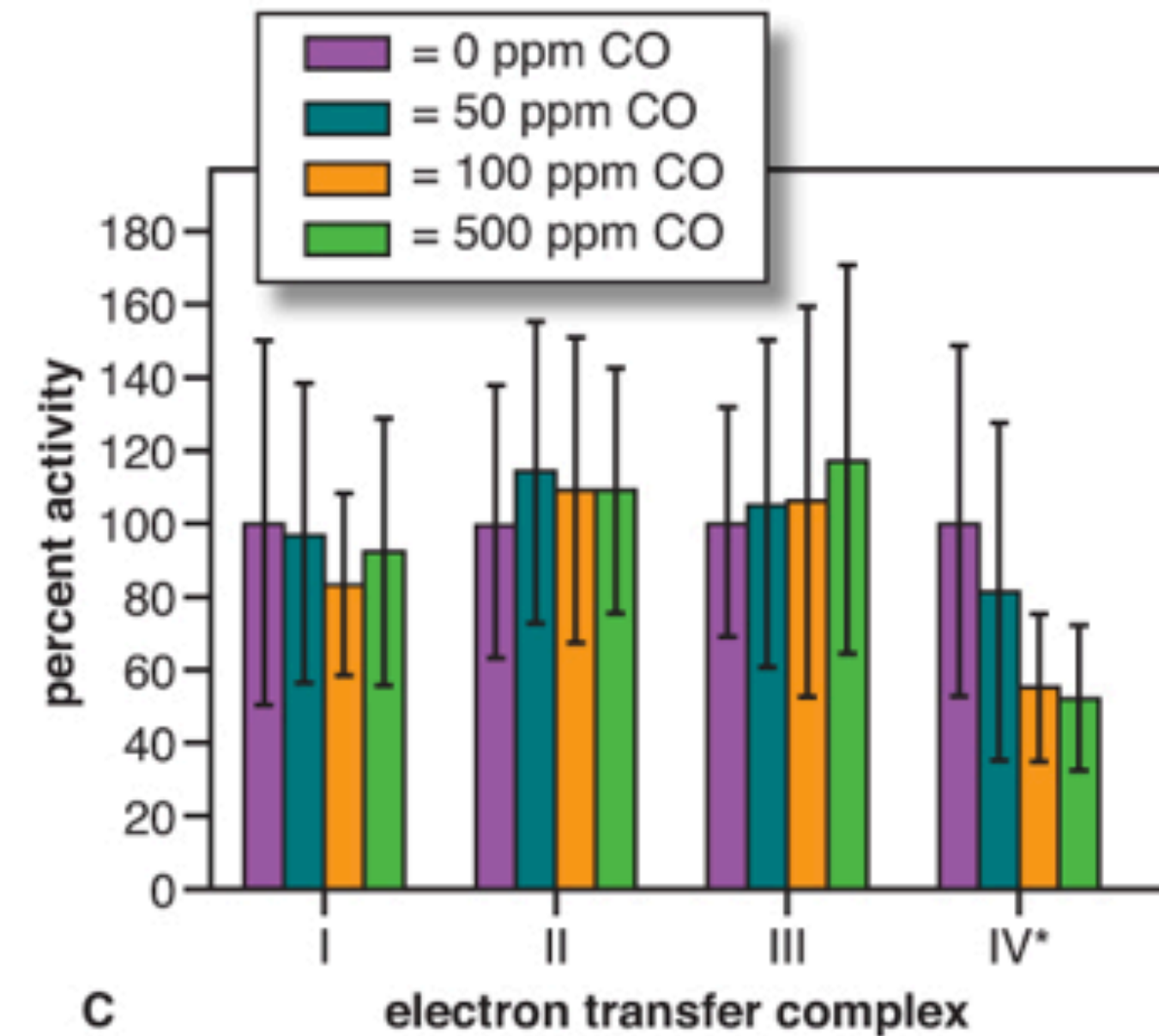
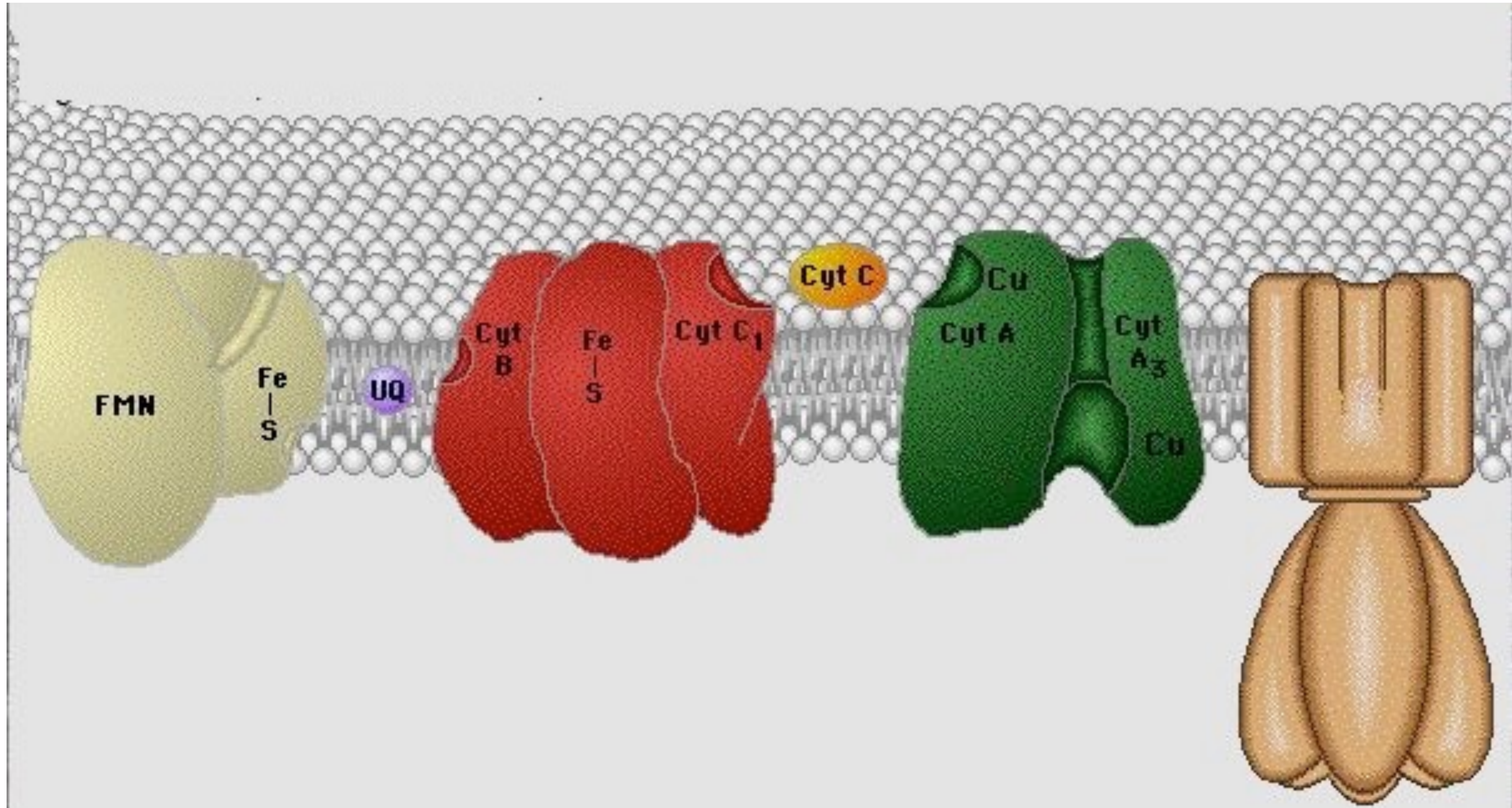
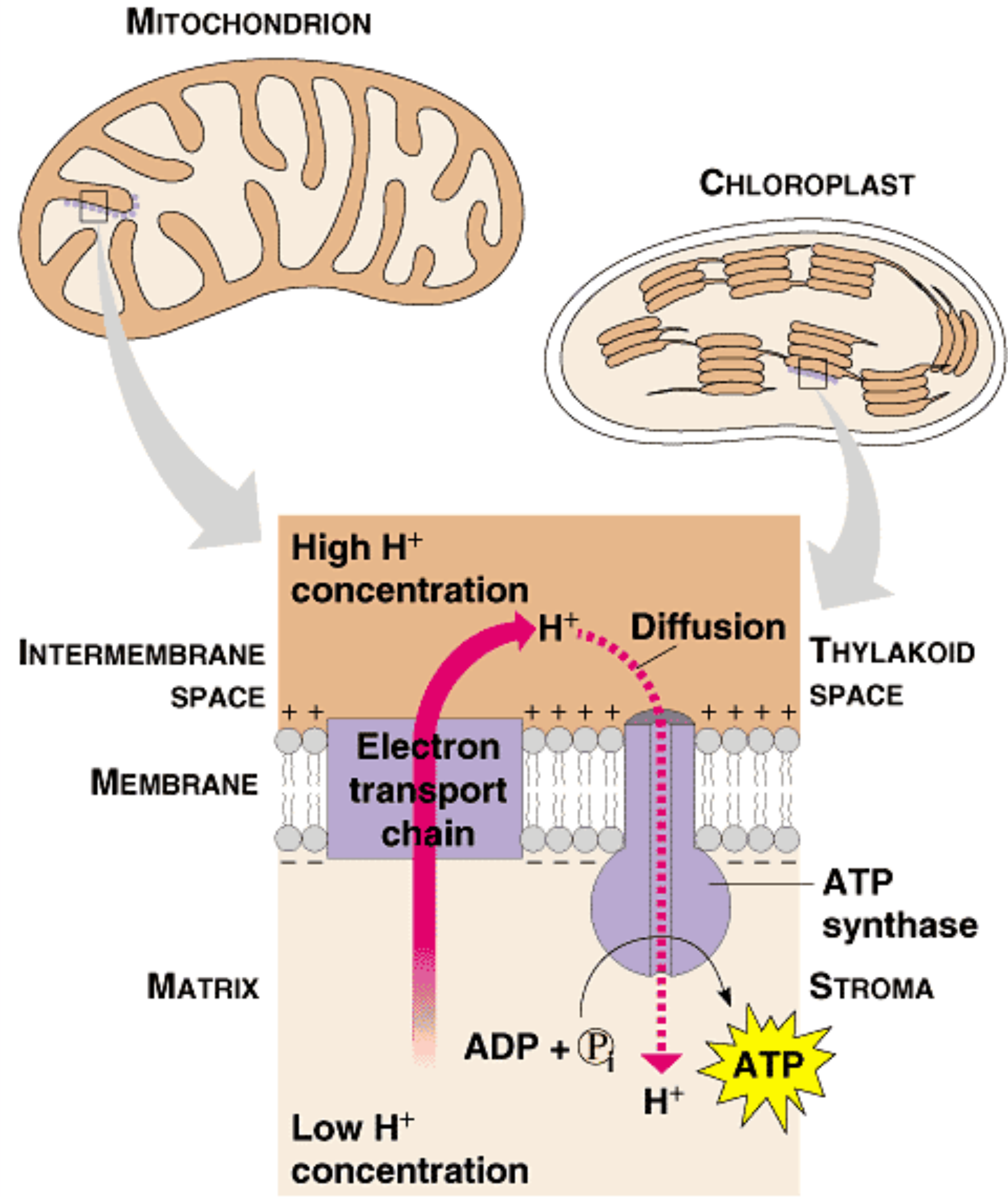


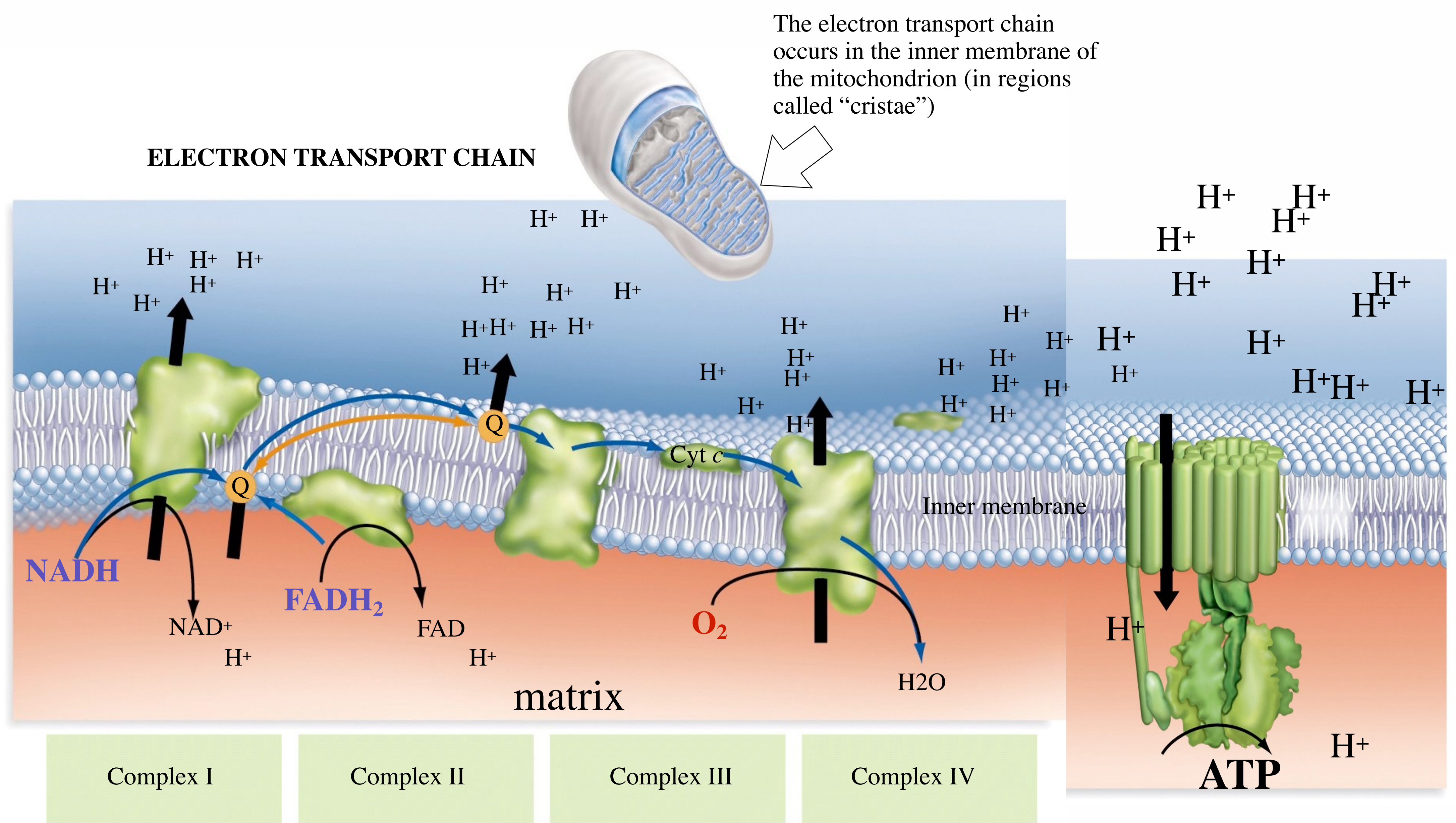
Figure 10.21 Electron transfer pathway in mitochondrial membrane. **A**, Four protein complexes contain hemes and transfer electrons from NADH and FADH₂ donors to the final acceptor molecule. **B**, Atomic structures of two similar hemes from cytochrome c of complex IV and human hemoglobin. Nitrogen atoms are blue, centrally located iron ions are orange, carbons are gray, oxygens are red. **C**, Effect of carbon monoxide (CO) on electron transfer by the four complexes shown in **A**, +/- standard deviation. * indicates $p < 0.001$. Panels A & B from common knowledge – original art. Panel C modified from Alonso *et al.*, 2003; part of their figure 2. Alonso, Jose-Ramon, Francesc Cardellach, *et al.* 2003. *Pharmacology and Toxicology*. Vol. 93: 142 – 146. Carbon Monoxide Specifically Inhibits Cytochrome C Oxidase of Human Mitochondrial Respiratory Chain.

What does this remind you of?



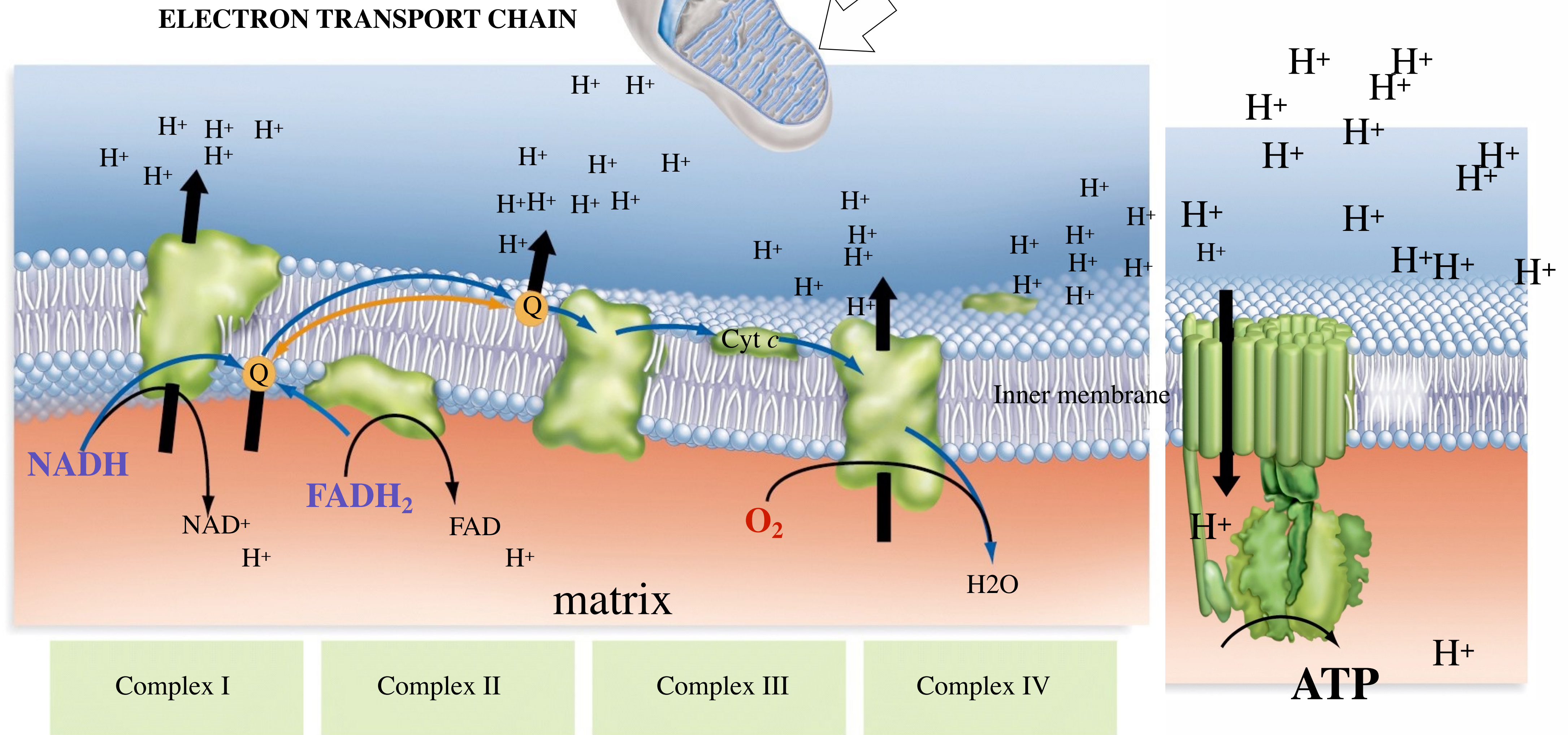


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1. What is backwards? 2. How much ATP? 3. Poke hole?

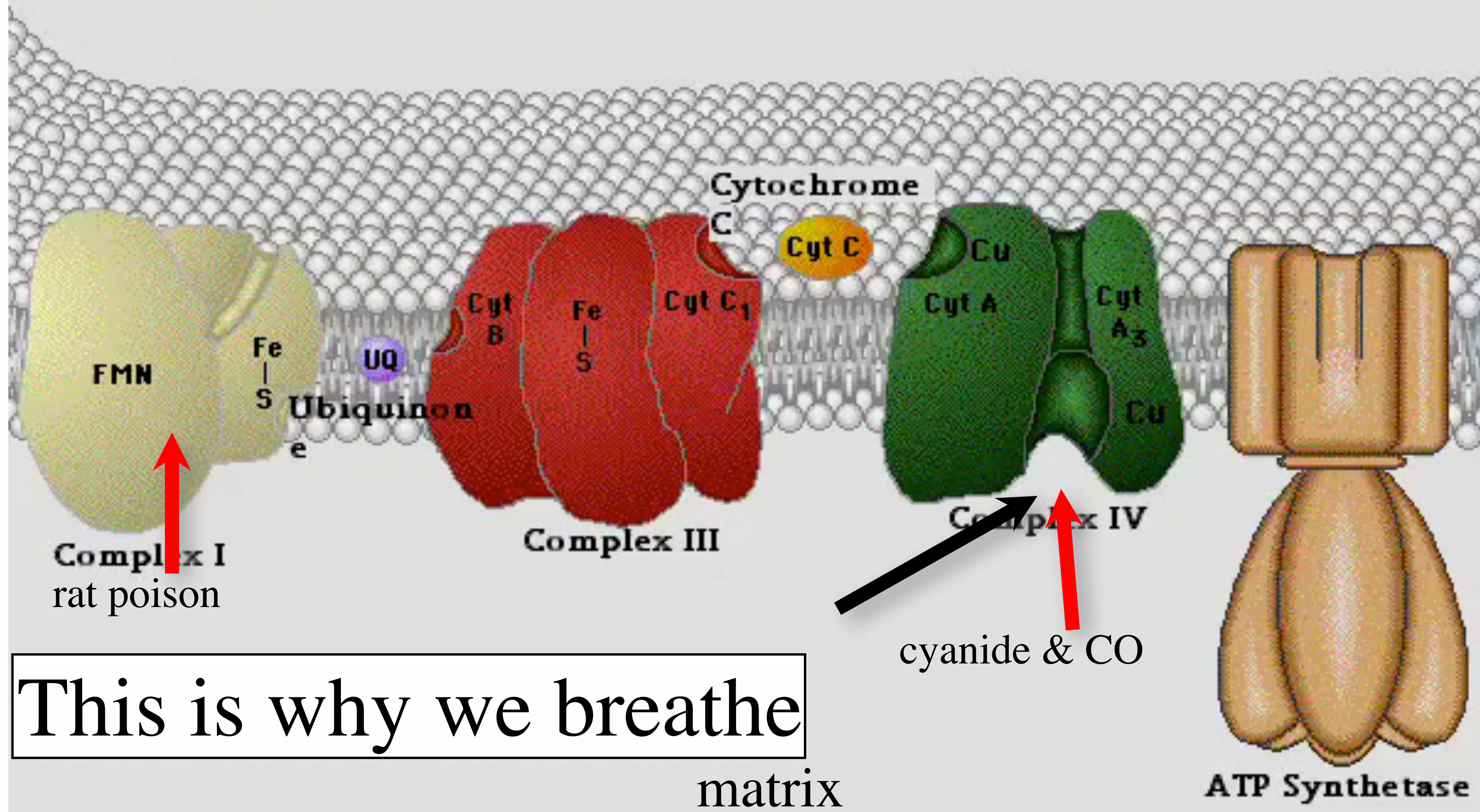
The electron transport chain occurs in the inner membrane of the mitochondrion (in regions called "cristae")



NADH-Q Reductase, FADH-Q Reductase, Cytochrome Reductase, Cytochrome Oxidase
 (NADH dehydrogenase, FADH dehydrogenase, Cytochrome complex, Cytochrome Oxidase)

The enzymes and proteins involved in oxidative phosphorylation are membrane proteins, which act as electron transporters. These are organized into just 4 or 5 large complexes embedded in the inner membrane of the mitochondrion.

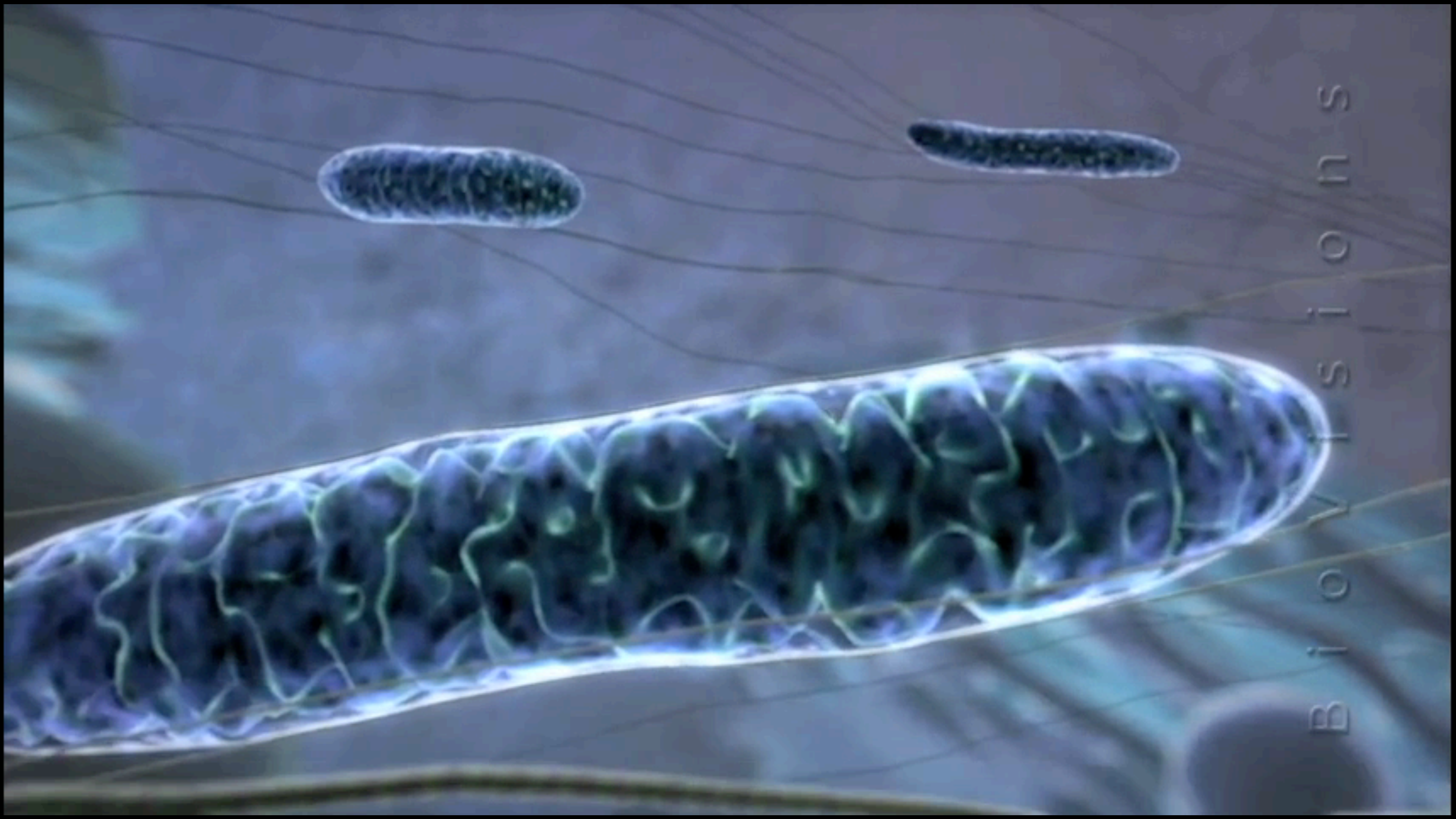
This is why we eat



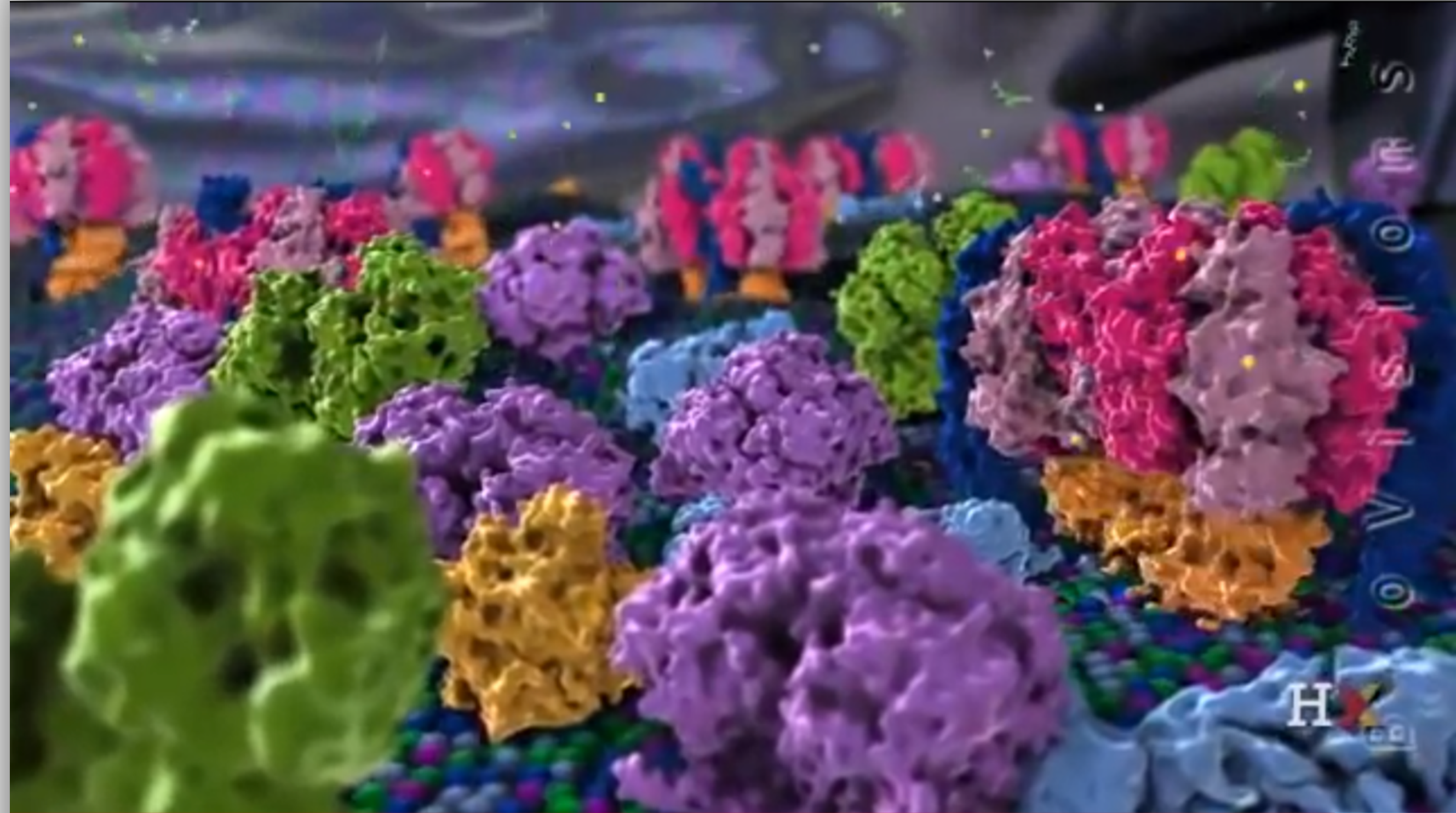
This is why we breathe

matrix

ATP Synthetase



B i o V i s i o n s



Electron Transport Chain

489,441 views



10K



91



Share



Save

<https://youtu.be/rdF3mnyS1p0>