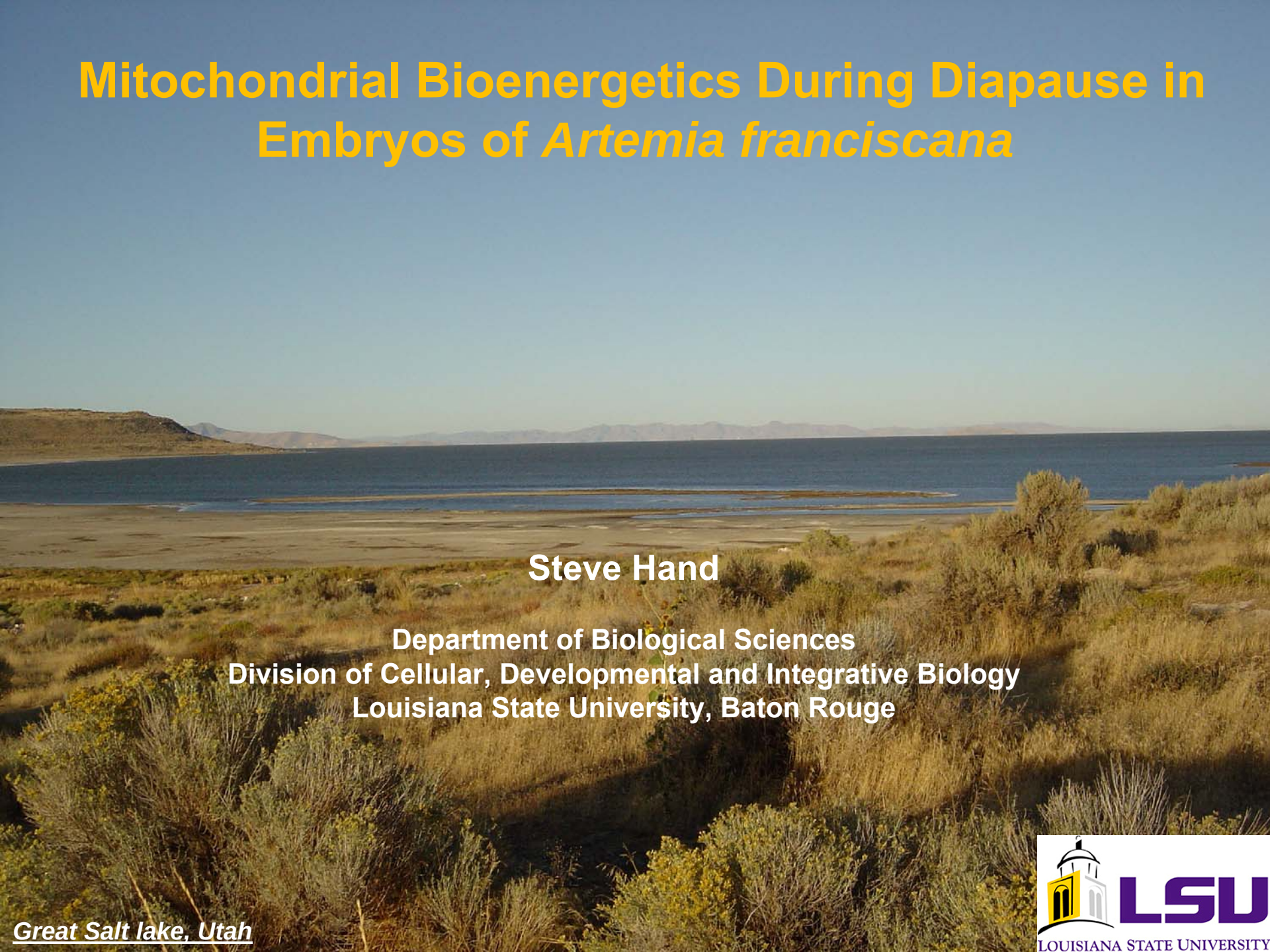
Mitochondrial Bioenergetics During Diapause in Embryos of *Artemia franciscana*



Steve Hand

Department of Biological Sciences
Division of Cellular, Developmental and Integrative Biology
Louisiana State University, Baton Rouge

Great Salt lake, Utah

LSU
LOUISIANA STATE UNIVERSITY

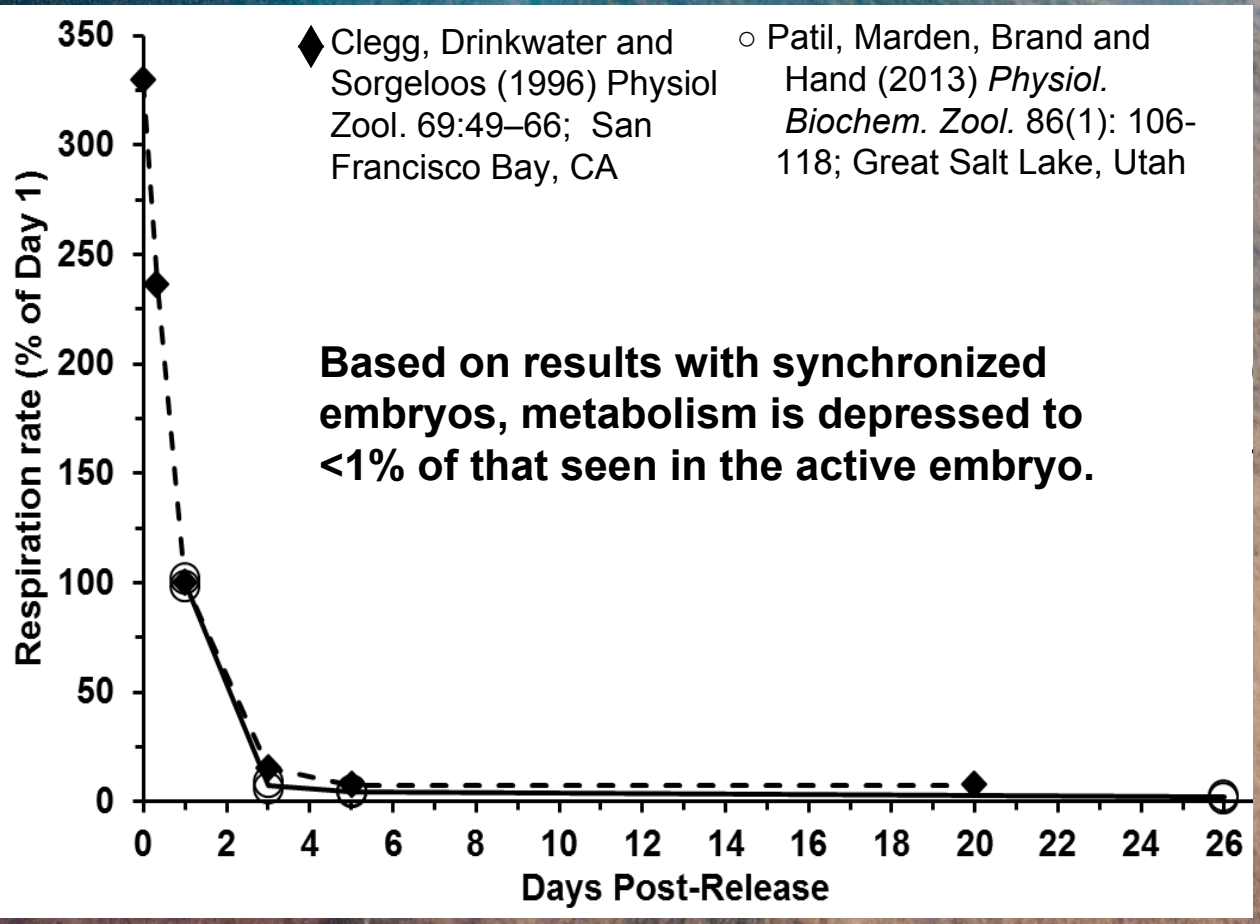
Background and Aims of the Presentation

Diapause seen many invertebrates is a developmentally-programed reduction of development and often metabolism, the depth of which can be profound – particularly in *Artemia franciscana*. Metabolic depression is positively correlated with extended survival during environmental stress.

We have recently provided data suggesting the membrane potential ($\Delta\Psi_m$) across the inner mitochondrial membrane is compromised during diapause, which has major implications for **[1]** *cellular ATP status* and **[2]** *cell death pathways*.

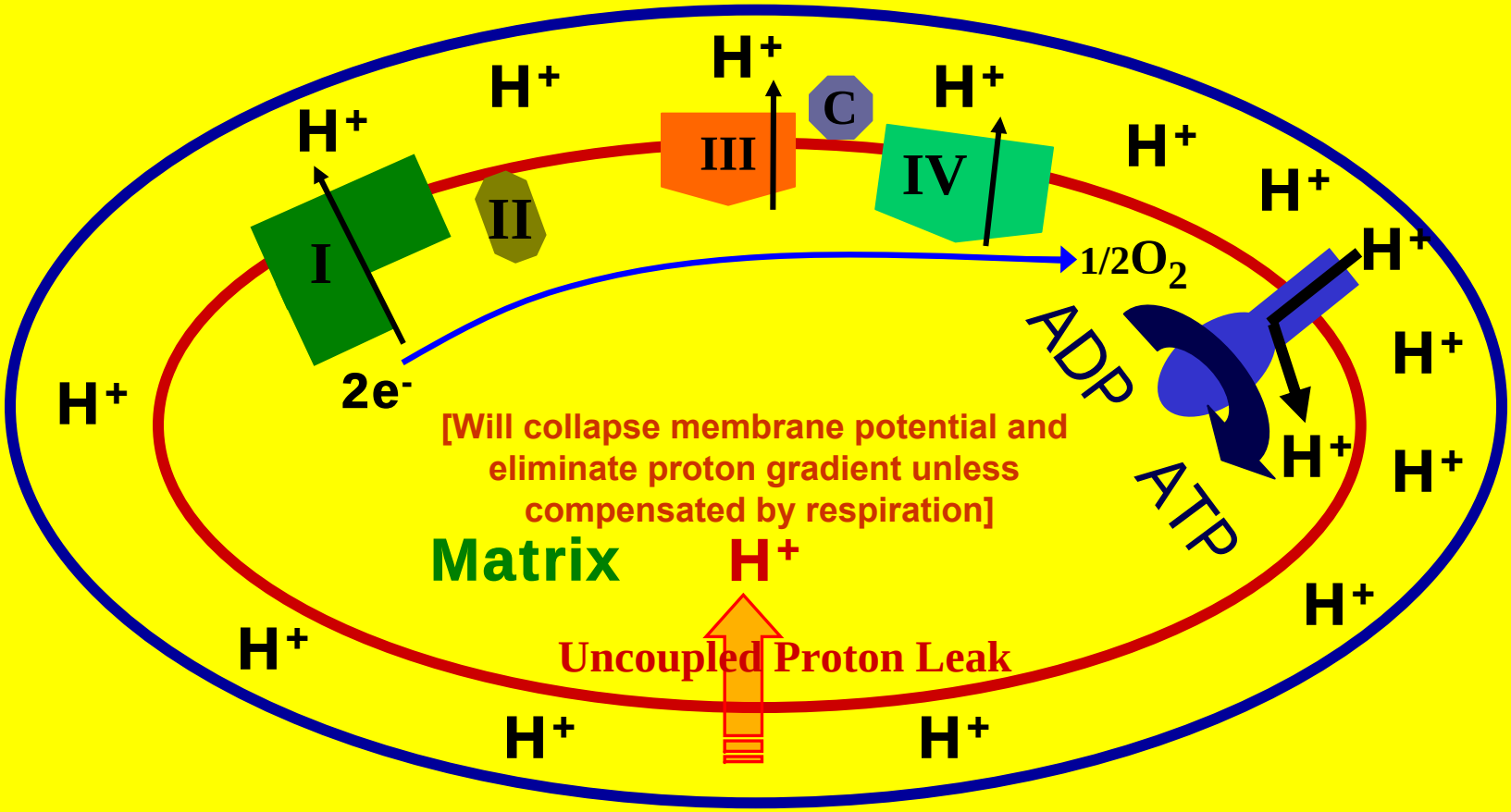
Knowledge of the complete sequence identities for the 22 or so subunits of the *Artemia* ATP synthase and the Inhibitor of F_1 Protein (IF_1) would advance our understanding of the above physiological aspects of diapause. Genomic data are essential.

Time Course for Respiratory Depression During Entry into Diapause



Great Salt Lake, Utah

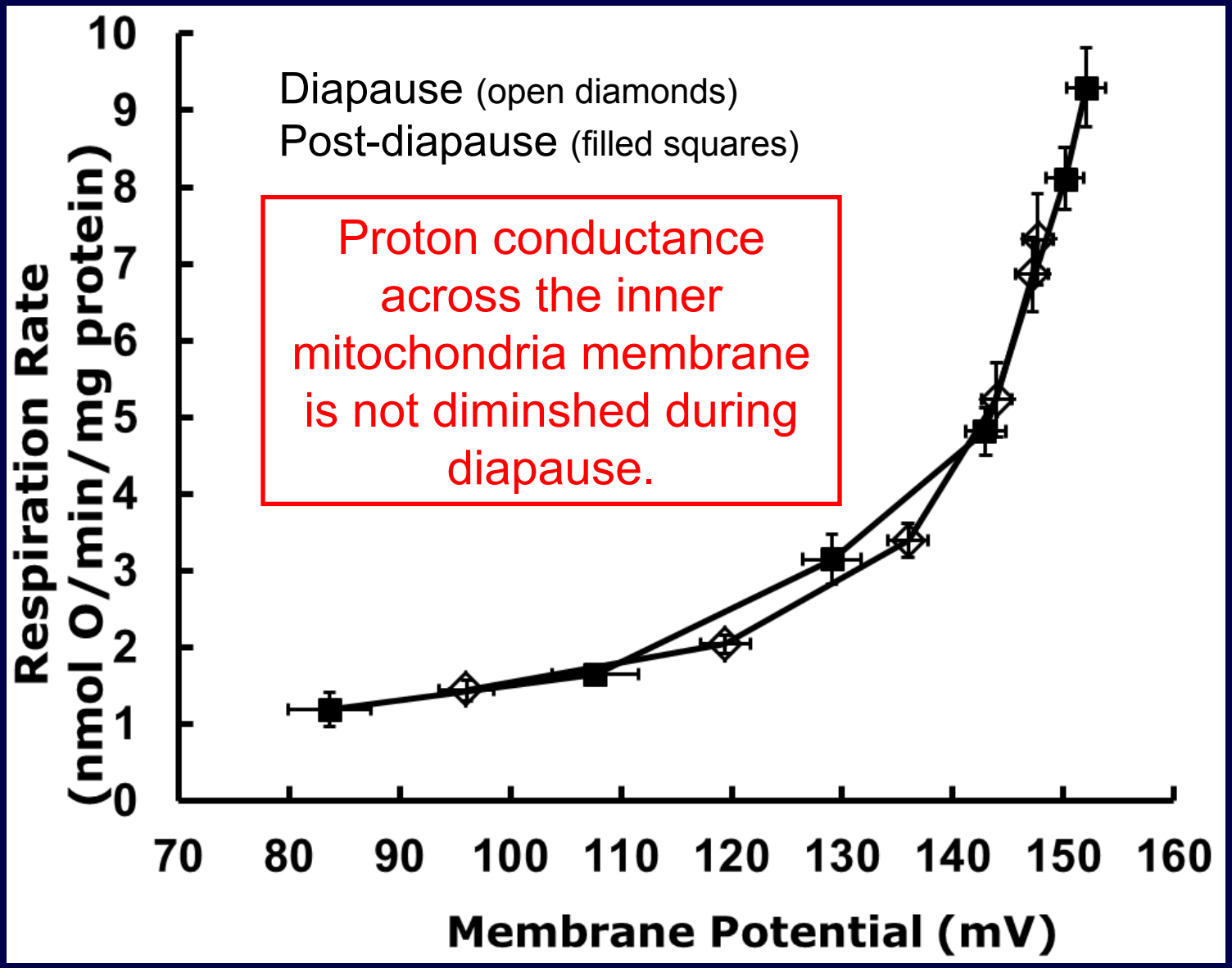
Proton re-entry (leak) into the matrix and compensatory 'leak respiration'



Proton leak respiration generally represents 15 % or more of basal metabolism (respiration).

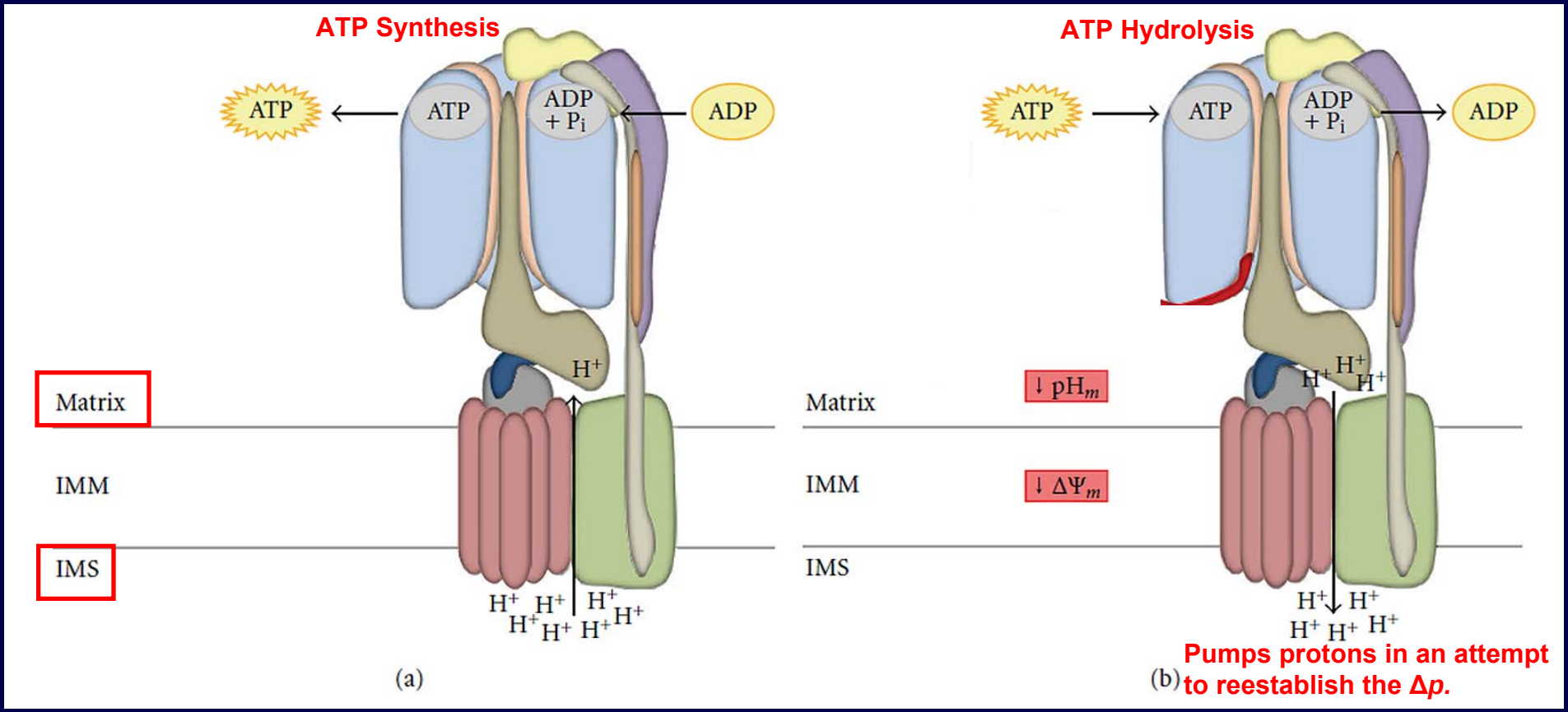
Proton Leak Respiration Versus Membrane Potential

Isolated Mitochondria from *Artemia franciscana* embryos



Patil, Marden, Brand, and Hand (2013) *Physiol. Biochem. Zool.* 86(1): 106- 118.

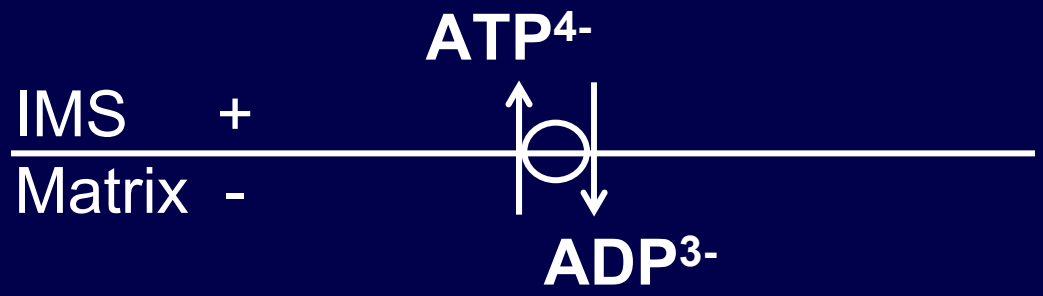
Consequences? Under such conditions of declining ATP, compromised $\Delta\Psi$ and acidic matrix pH, the ATP synthase and the adenine nucleotide translocator (ANT) can reverse causing hydrolysis of all cellular ATP



[modified after: Faccenda D, Campanella M (2012) *Internat. J. Cell Biol.*, Article ID 367934, doi:10.1155/2012/367934]

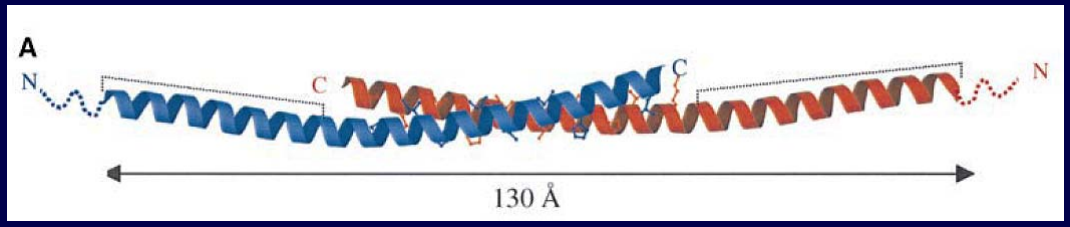
The Adenine Nucelotide Translocator also Reverses

When there is a normal $\Delta\Psi_m$, ATP_{out}/ADP_{in} is the favored direction for the ANT, because:



In the $\Delta\Psi_m$ is compromised, there is no favoritism for the ANT – direction just depends on concentration gradient for ATP. So ATP enters from the cytoplasm and can be readily cleaved by the ATP synthase, now functioning as a F_1F_0 ATPase.

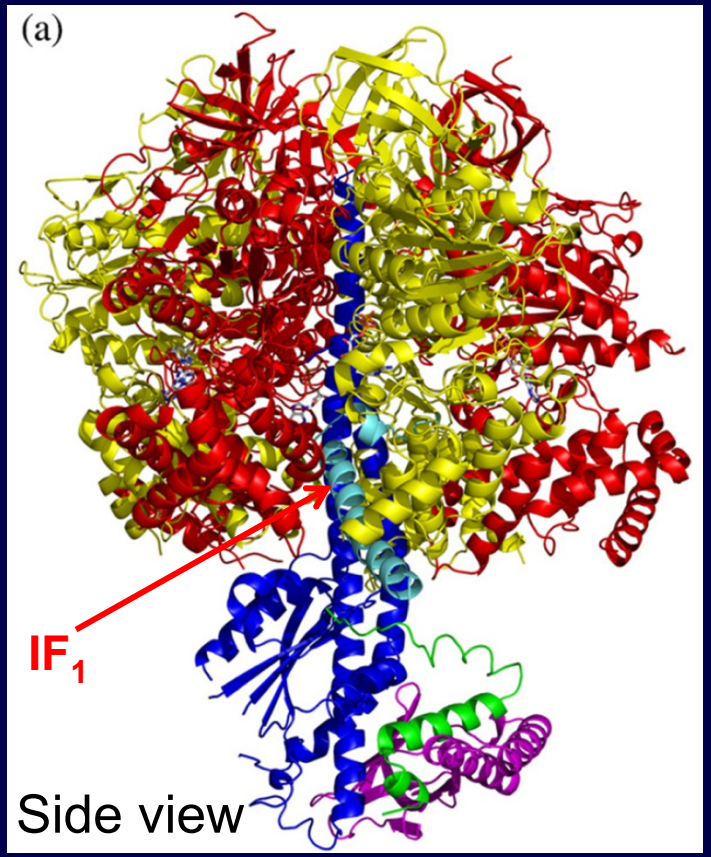
Binding of IF_1 to Bovine ATP Synthase

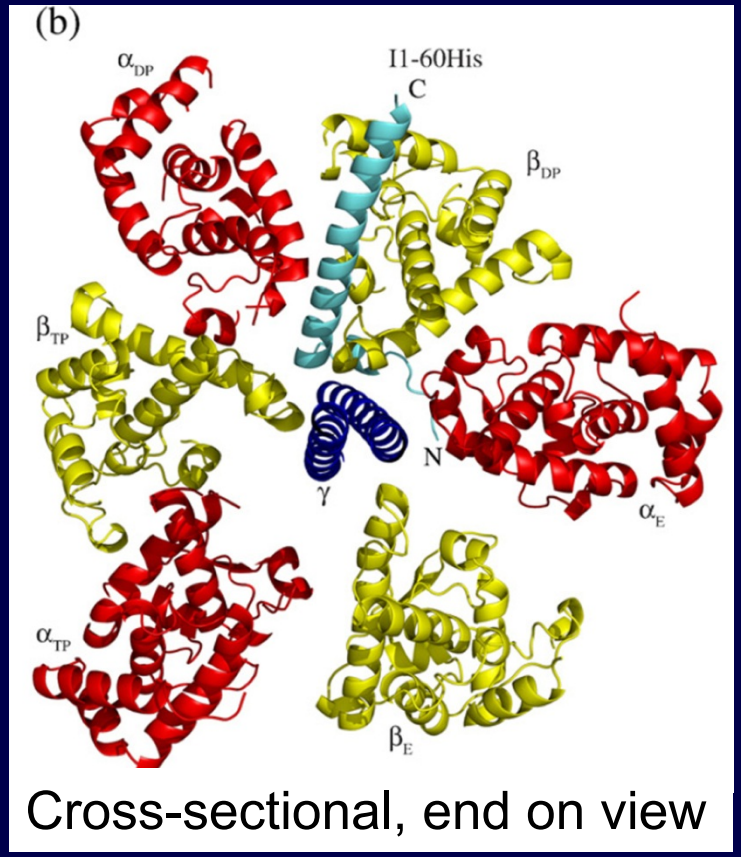


Ribbon diagram of the *active dimer* of bovine IF_1 protein. Dashed lines represent the minimal inhibitory sequence. [from: Cabezon, Runswick, Leslie, Walker (2001) *EMBO J.* 20(24): 6990-6996]

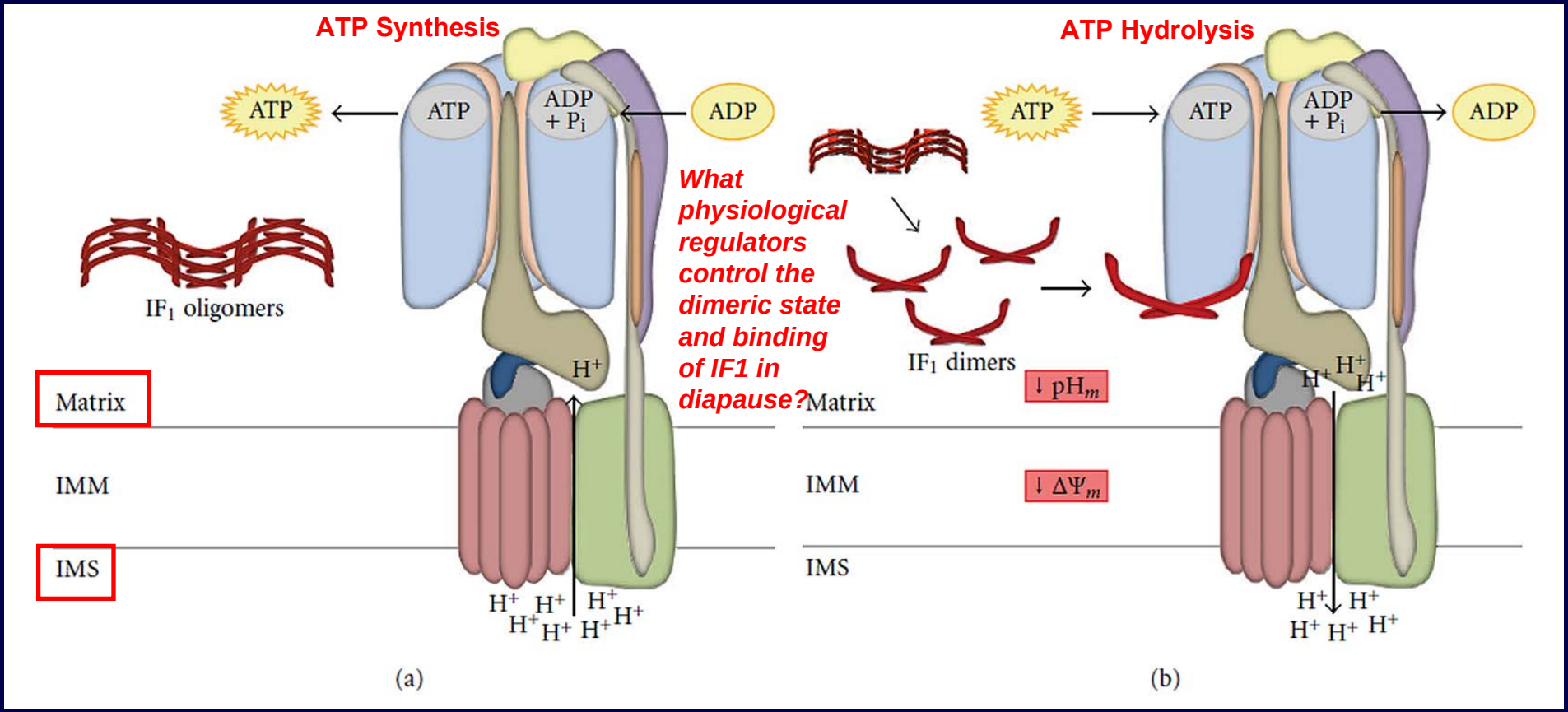
The structure of bovine F_1-ATPase inhibited with residues 1–60 of the bovine inhibitor protein IF_1 (light blue). The inhibitor is bound at a catalytic interface between *the β_{DP}- and α_{DP}-subunits*.

[modified from: Gledhill, Montgomery, Leslie, Walker (2007) *Proc. Natl. Acad. Sci. USA* 104 (40): 15671–15676]





Normally IF_1 exists as large oligomers, but these can depolymerize to free dimers that bind to the ATP synthase and inhibit hydrolysis



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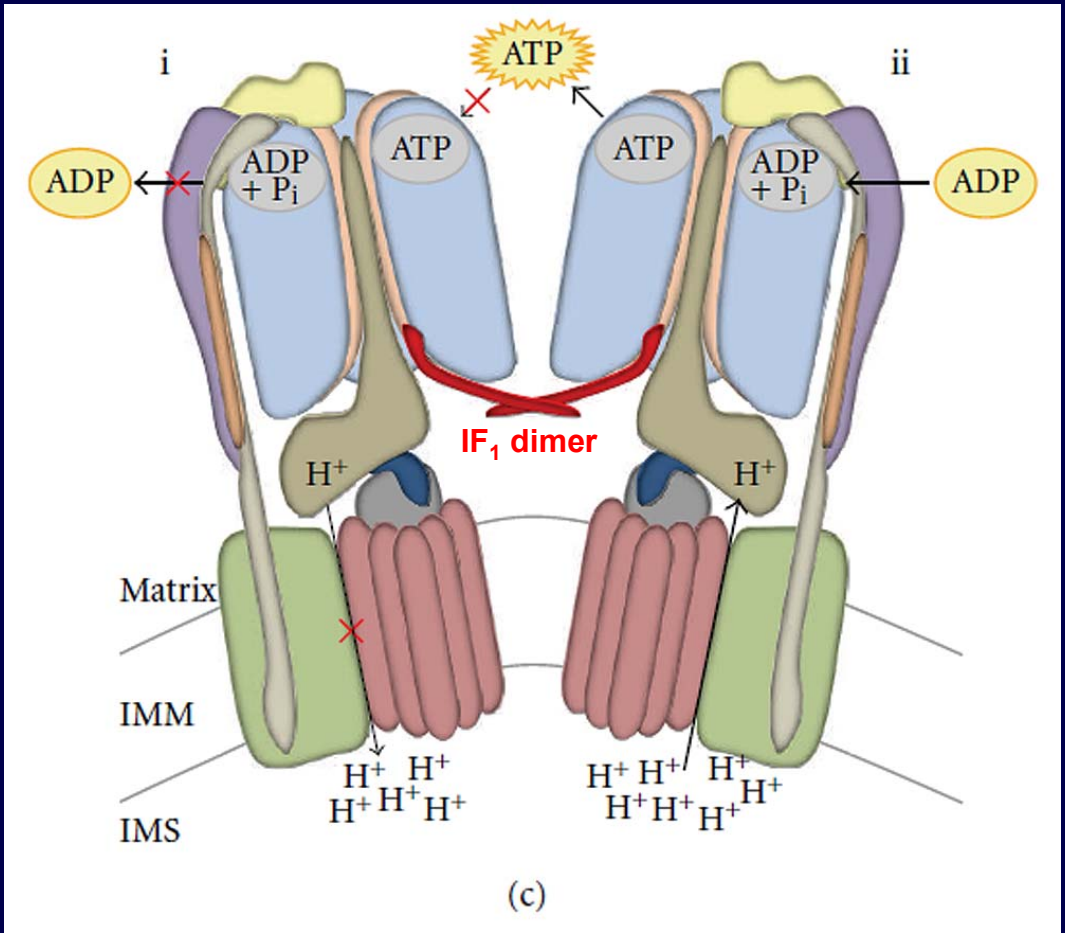
ATP Synthase Inhibited by IF1



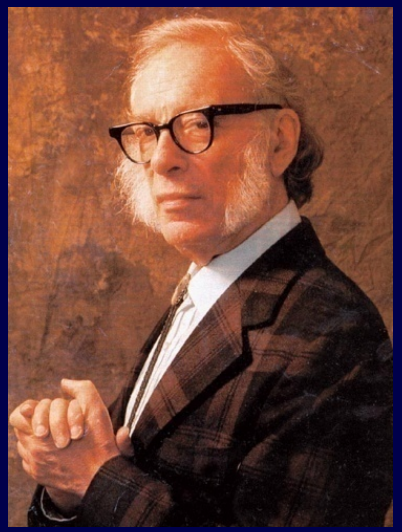
[Faccenda D, Campanella M (2012) *Internat. J. Cell Biol.* Article ID 367934, doi:10.1155/2012/367934]

Kinetically characterization of the interaction of IF_1 and the synthase in *Artemia* embryos requires the sequence of *Artemia* IF_1 protein so that the recombinant protein can be prepared.

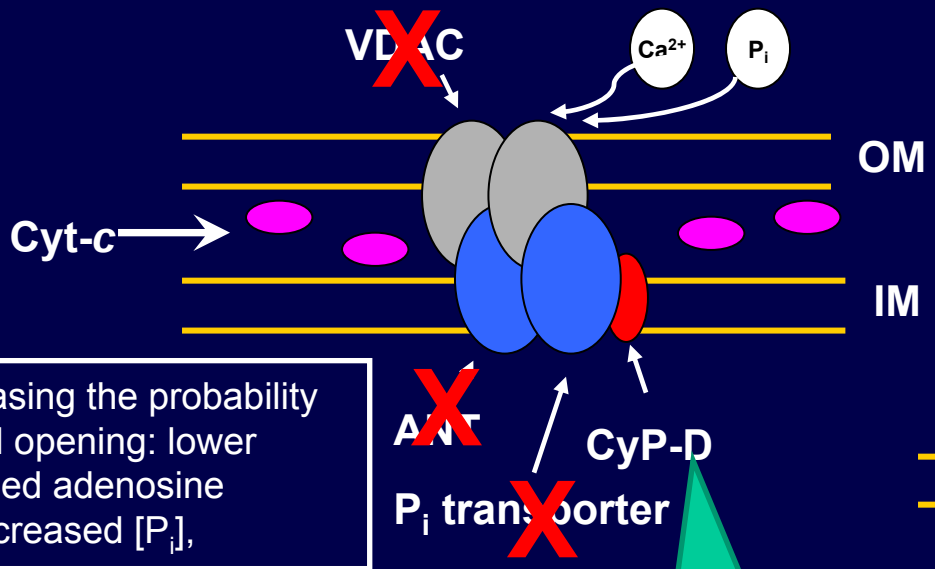
A second consequence of a disrupted mitochondrial $\Delta\Psi_m$?

Life is pleasant. Death is peaceful. It's the transition that is troublesome.

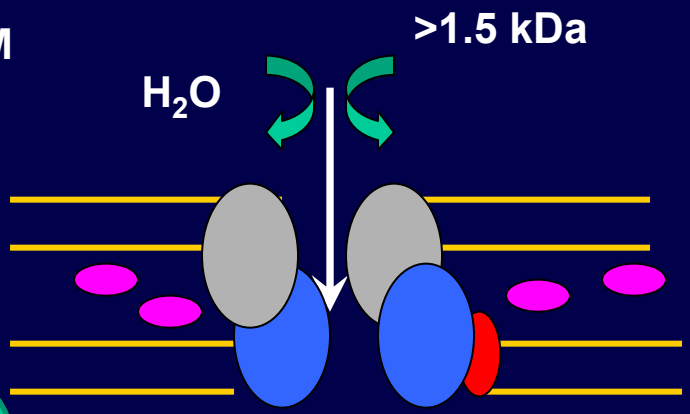
Isaac Asimov (January 2, 1920 – April 6, 1992)



MPTP: A voltage dependent, cyclosporin A sensitive, and calcium-induced inner membrane channel with a ~1500 Da molecular weight cut off (Bernardi, 1992)



Factors increasing the probability of Ca-induced opening: lower $\Delta\Psi_m$, decreased adenosine nucleotide, increased $[P_i]$, oxidative stress.



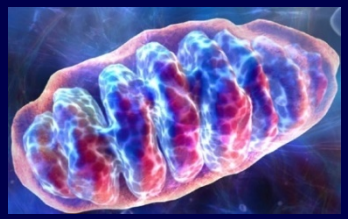
CyPD is the mitochondrial receptor for CsA and is a key modulator of the MPTP, but not a structural component.

Necrosis

PCD

Cyt-*c*

Rupture of OM

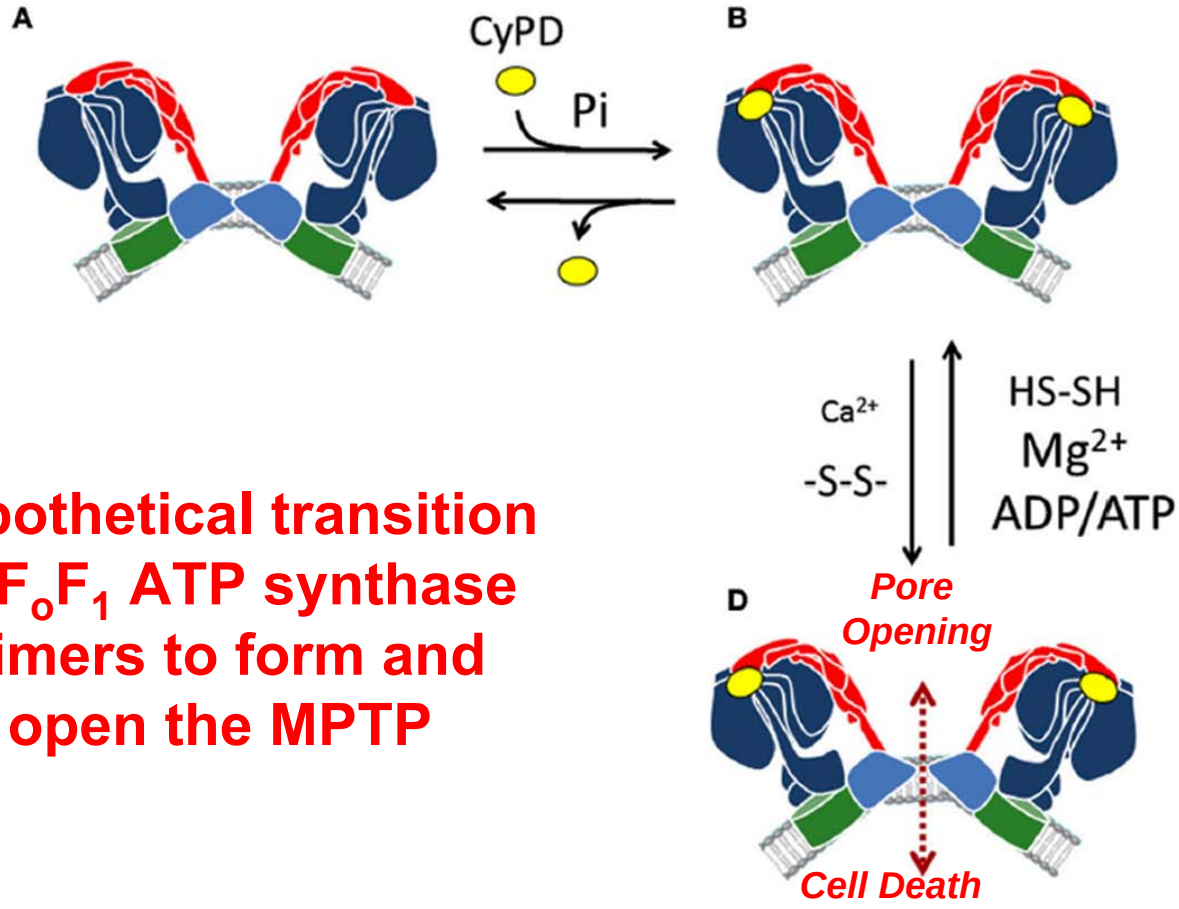


We have previously documented that the MPTP does not open in *A. franciscana* embryos in response to known physiological inducers or strong artifical inducers of pore opening.

[Menze, Hutchinson, Laborde, and Hand (2005) *Amer. J. Physiol*. 289: R68-R76]

What is the basis for the absence of this phenomenon in A. franciscana*?*

Recent Evidence Suggests the MPTP is Formed by an Altered Conformation of the Dimeric ATP Synthase



Hypothetical transition of F_oF_1 ATP synthase dimers to form and open the MPTP

Modified after: Giorgio et al (2013) *Proc. Natl. Acad. Sci. USA* 110: 5887–5892; Bernardi (2013) *Frontiers in Physiology* 4 doi: 10.3389/fphysiol.2013.00095

What structural/functional differences exist in the ATP Synthase from *A. franciscana* embryos that preclude opening of the MPTP?

Identification of subunits with genomic sequence data and comparison to the mammalian enzymes are important for resolving the MPTP issue in *A. franciscana* and would improve our understanding cell death and its prevention in general.

Additionally, the mechanisms for mitochondrial *outer* membrane permeabilization (involving Bcl-2 family proteins, Bax, Bak, etc.) need resolution in *Artemia* embryos.

Genomic data for Artemia are key for advancing this work.

Lab Group, Collaborators, Support

Ph.D. Students:

John Anderson
Apu Borcar
Leaf Boswell
Daniel Moore
Yuvraj Patil

Senior Research Associate:

Shumin Li

Undergraduates:

Dwayne Brown
Jane Craig
Emily Hodges
Suman Nag

Collaborators:

Martin D. Brand (Buck Inst. for Research on Aging, Novato, CA)
Michael Menze (Eastern Illinois University)
Erich Gnaiger (Medical University of Innsbruck, Austria)
Mehmet Toner and colleagues (Harvard Med. School/MIT)

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