Oxidative stress, DNA damage, and human disease: from patients to single molecules.

Ben Van Houten, PhD
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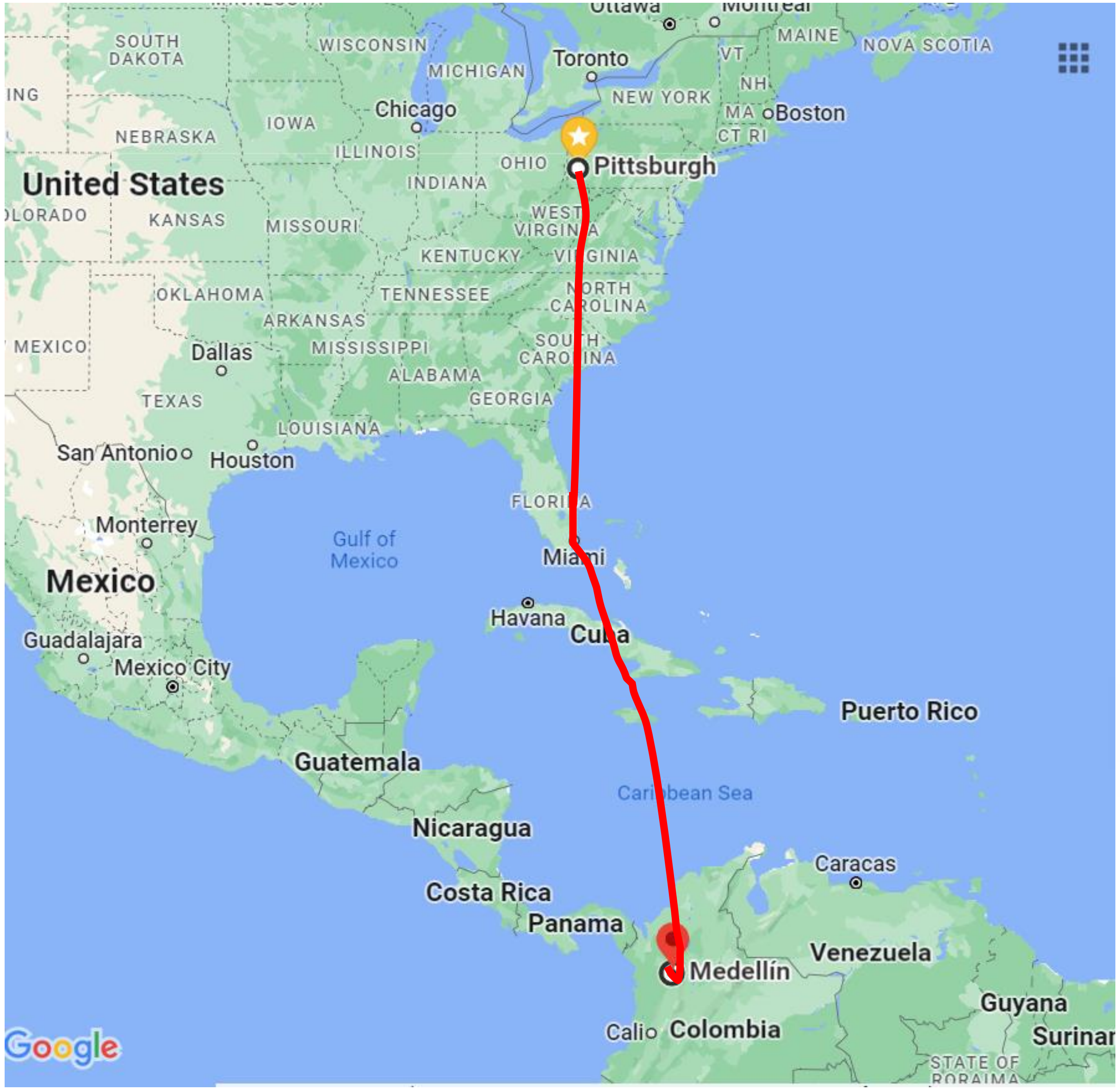
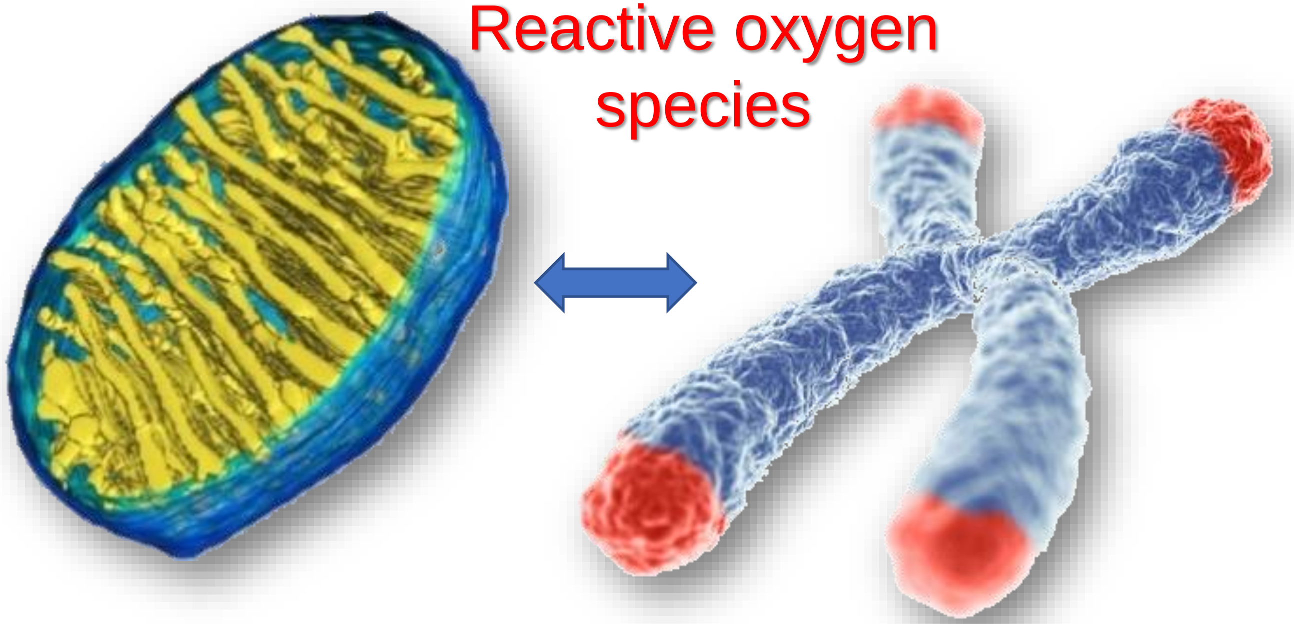
Today's journey



Interaction between two libraries of life: mitochondrial DNA and nuclear DNA.

Mitochondrial dysfunction

Reactive oxygen species



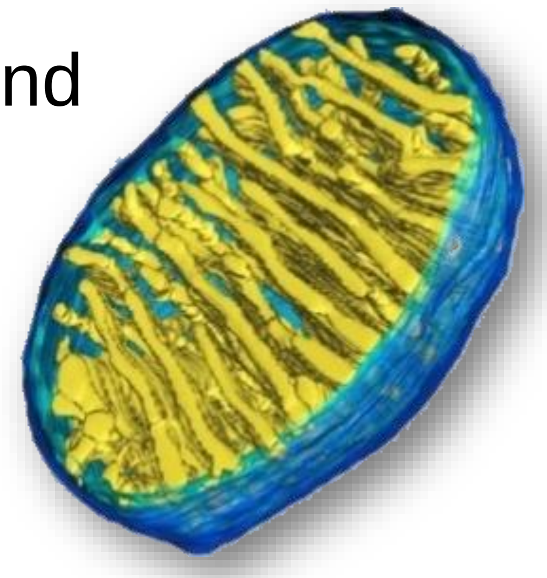
Nuclear damage

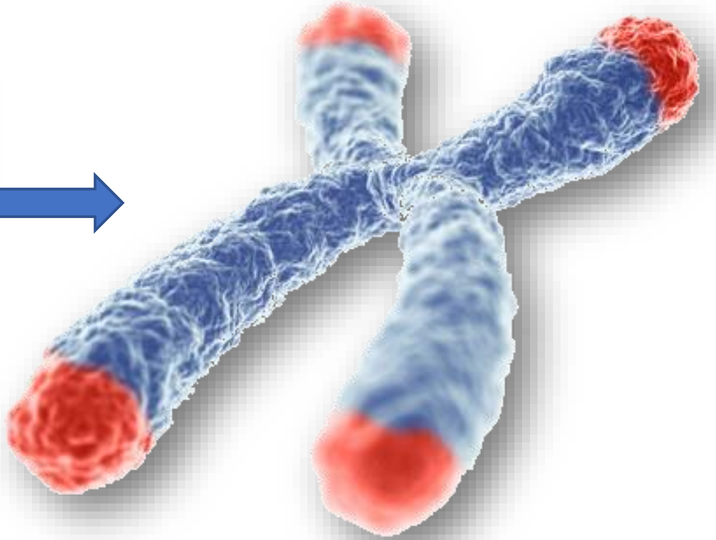
Today's presentation:

1. Mitochondria and ROS and human disease: Friedreich's Ataxia

2. Using a chemoptogenetic "nanosurgery" approach to understand how mitochondria generate ROS and consequences of telomere DNA damage

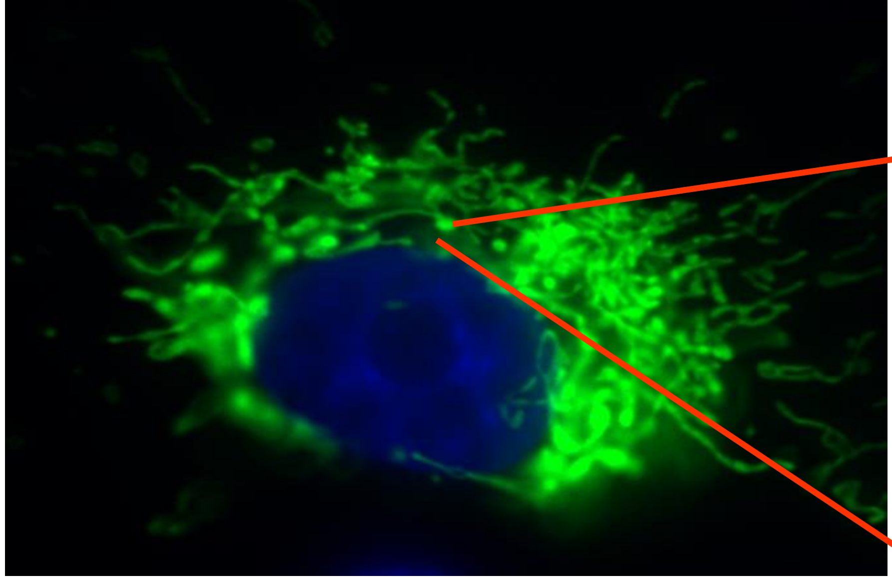
3. Mitochondria-telomere crosstalk and associated diseases: PD & cancer

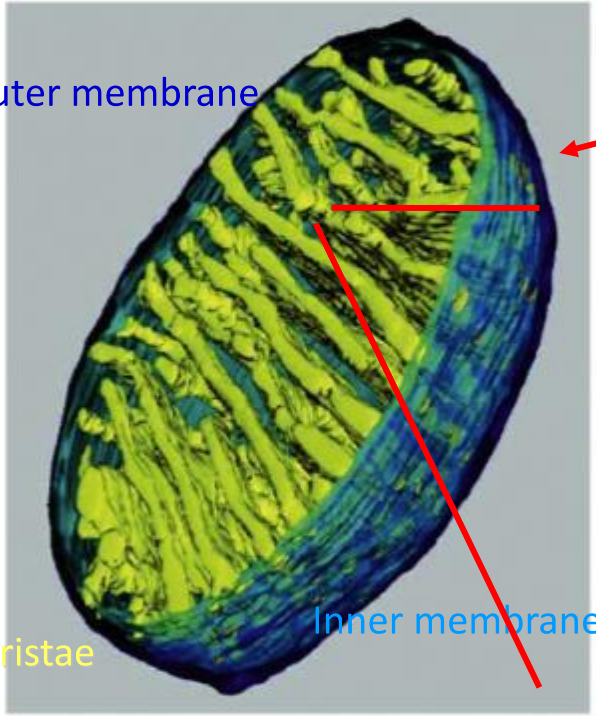




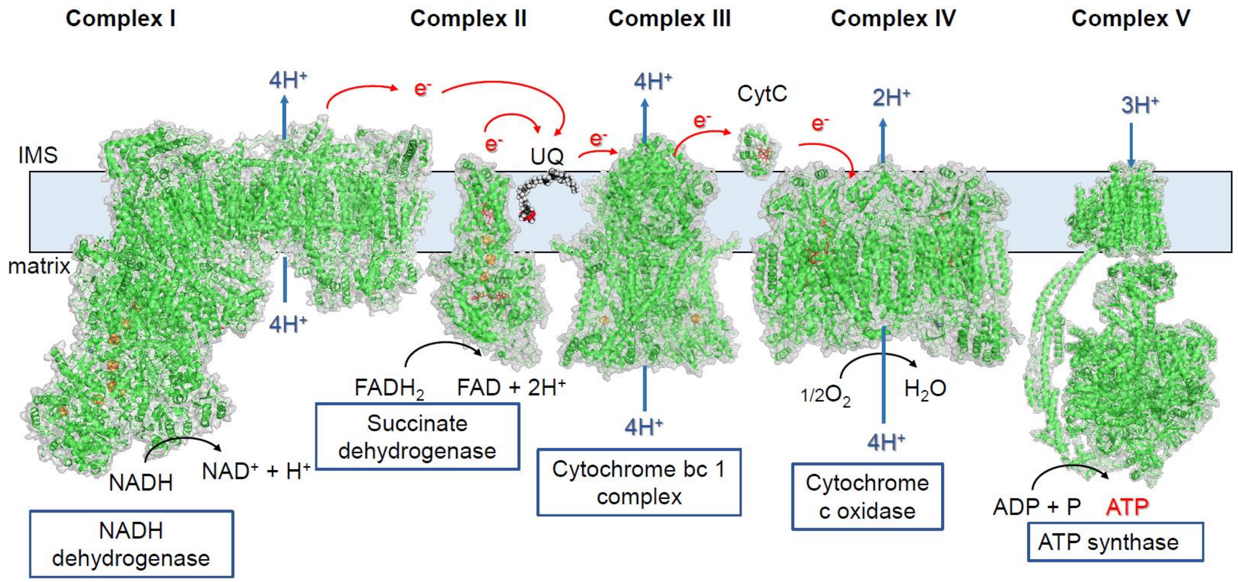
4. UVA causes ROS from mitochondria and nuclear mutation signatures of melanoma.

5. Watching DNA repair in real-time at the single molecule level





Electron transport chain = site of **oxidative phosphorylation**



Importance of mtDNA and oxidative phosphorylation

mtDNA codes for:

7 subunits of Complex I (46)

0 subunits of Complex II (4)

1 subunit of Complex III (11)

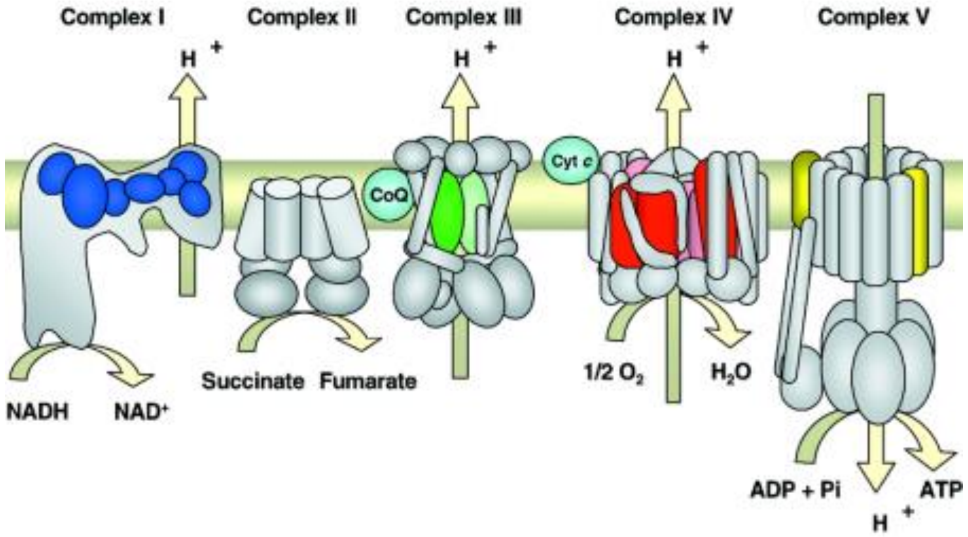
3 subunits of Complex IV (13)

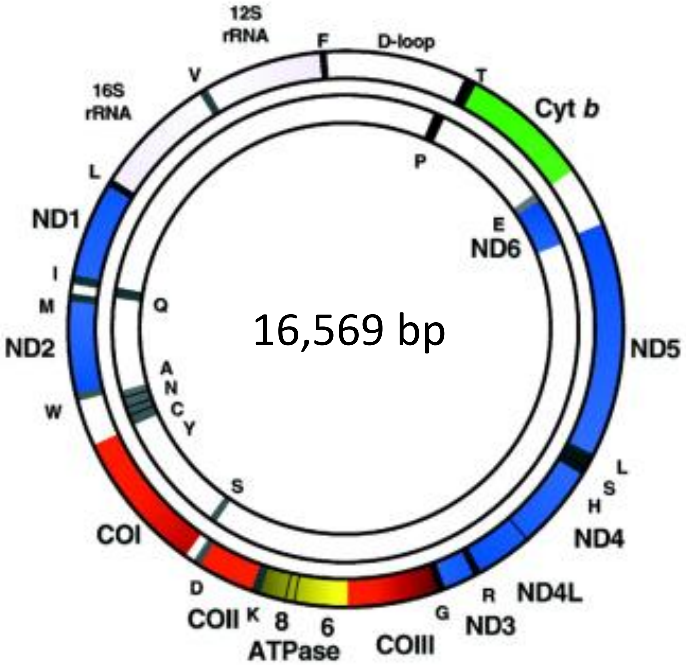
2 subunits of Complex V (14)

PLUS

2 rRNAs

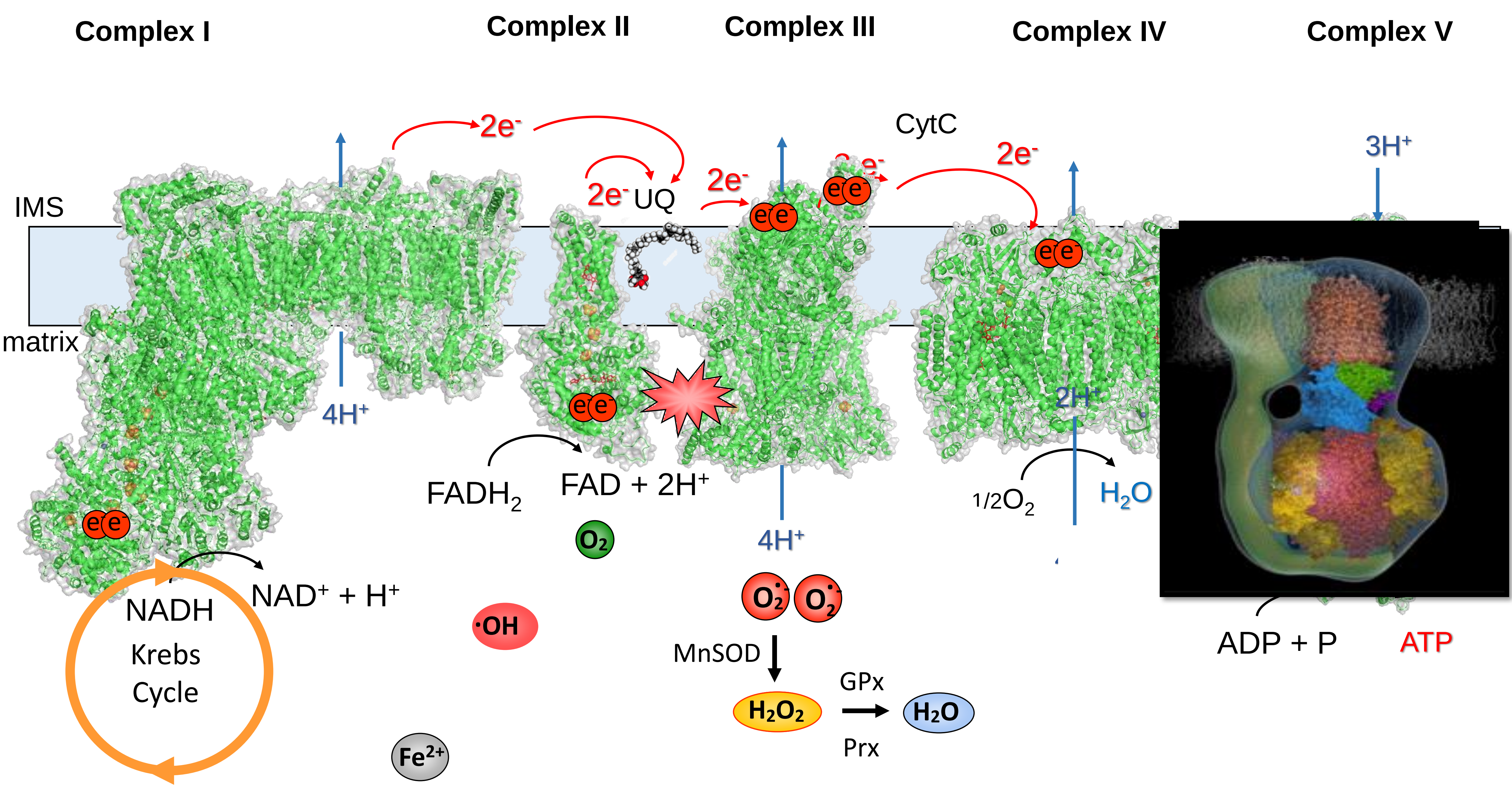
22 tRNAs





Toño Enriquez

Brain (2004), 127, 2153–2172



Complex I
Complex II
Complex III
Complex IV
Complex V
IMS
matrix
2e-
2e- UQ
2e-
CytC
2e-
3H+
4H+
4H+
2H+
FADH2
FAD + 2H+
O2
1/2O2
H2O
NADH
NAD+ + H+
Krebs
Cycle
·OH
Fe2+
O2
O2
MnSOD
H2O2
GPx
Prx
H2O
ADP + P
ATP

ROS and mtDNA damage

- Hydrogen peroxide leads to rapid mtDNA damage; protracted treatments lead to persistent mtDNA

ROS → *MtDNA damage* ↓ *mRNA* →

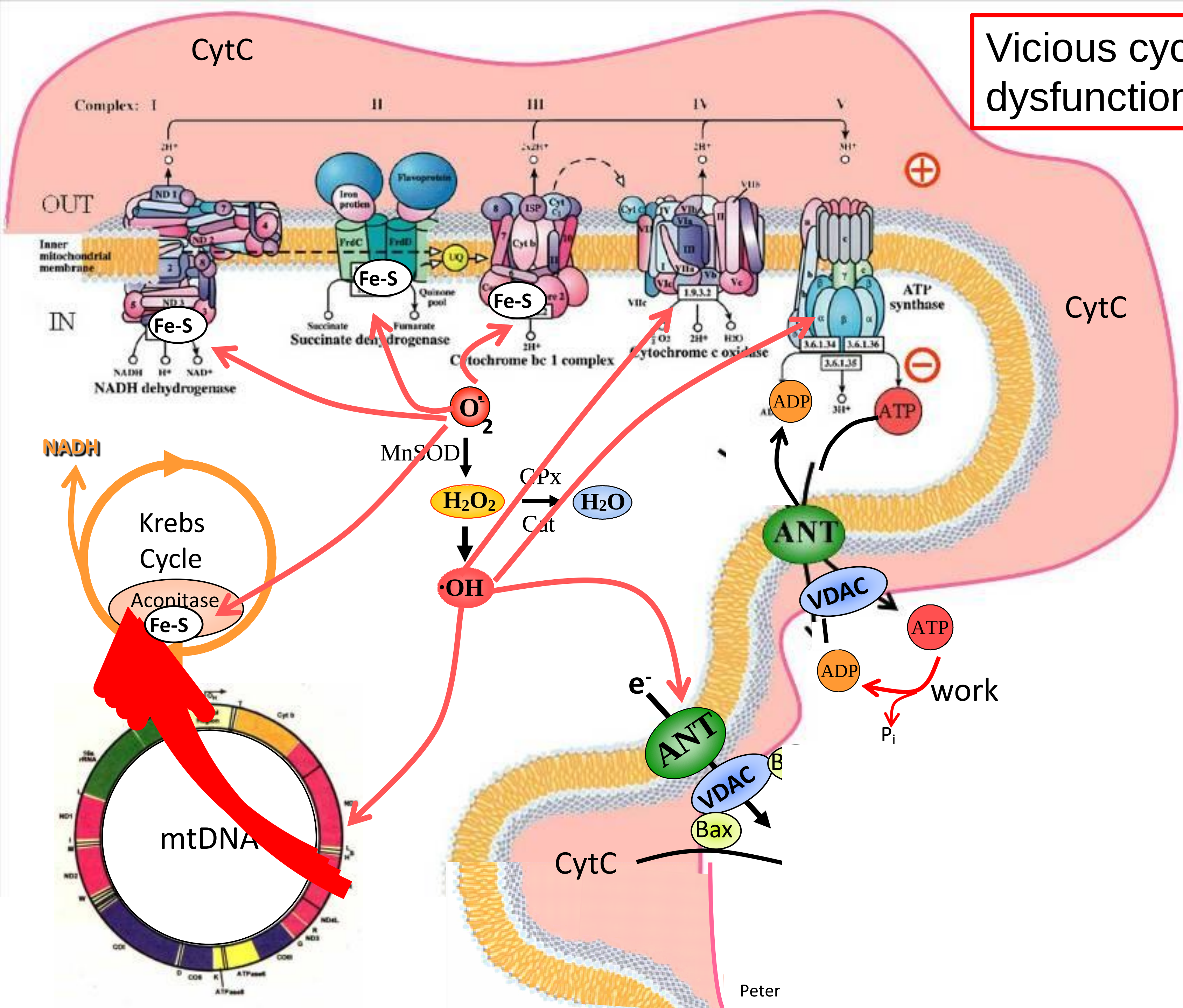
ROS ↓ *electron transport* ← ↓ *13 proteins*

loss of PMF, decrease of OXPHOS & ATP, cytC release, apoptosis/necrosis

- Persistent mtDNA damage could be caused by repair enzyme oxidation and/or lack of transport into the mitochondria.

Yakes and Van Houten, 1997*PNAS, 94(2):514-9. 94:514-519.*

Vicious cycle of mitochondrial dysfunction due to mtDNA damage

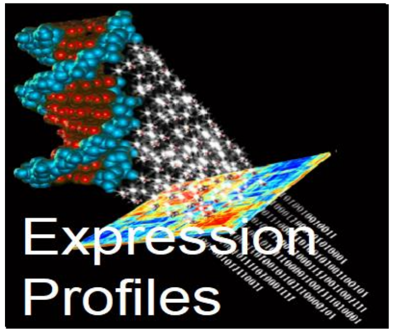


Peter

PLoS Genetics | www.plosgenetics.org 1 January 2010 | Volume 6 | Issue 1 | e1000812

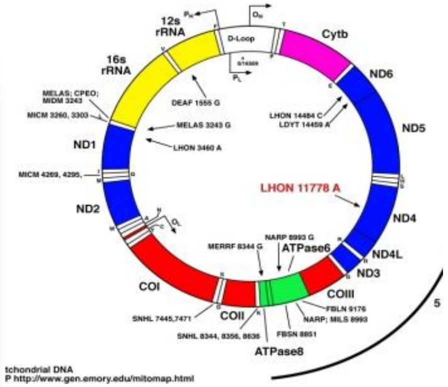
Altered Gene Expression and DNA Damage in Peripheral Blood Cells from Friedreich's Ataxia Patients: Cellular Model of Pathology

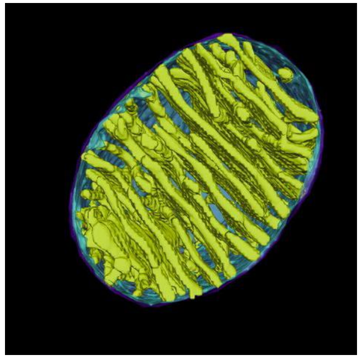
Astrid C. Haugen[1], Nicholas A. Di Prospero[2], Joel S. Parker[3], Rick D. Fannin[4], Jeff Chou[4], Joel N. Meyer[5], Christopher Halweg[1], Jennifer B. Collins[4], Alexandra Durr[6,7], Kenneth Fischbeck[2], Bennett Van Houten[8,9]*



- Total Blood RNA for microarray profiling on the 22K Agilent chip – 28 patients; 10 controls

- Total Blood DNA for analysis of mitochondrial and nuclear DNA damage – 47 patients; 15 controls

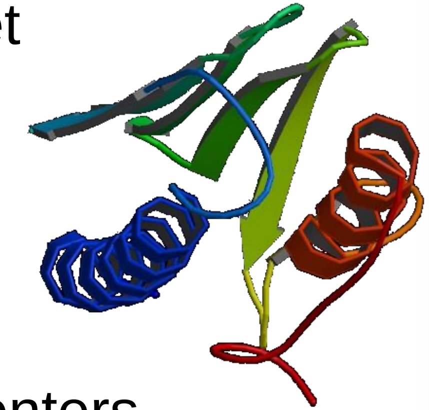


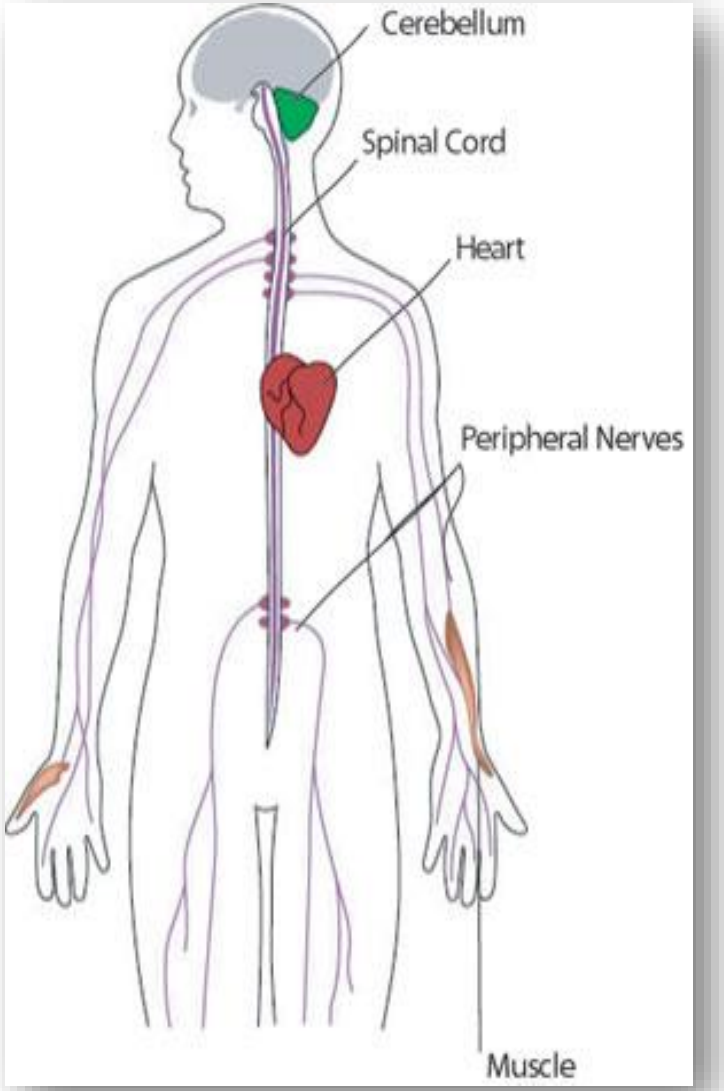


Astrid Haugen

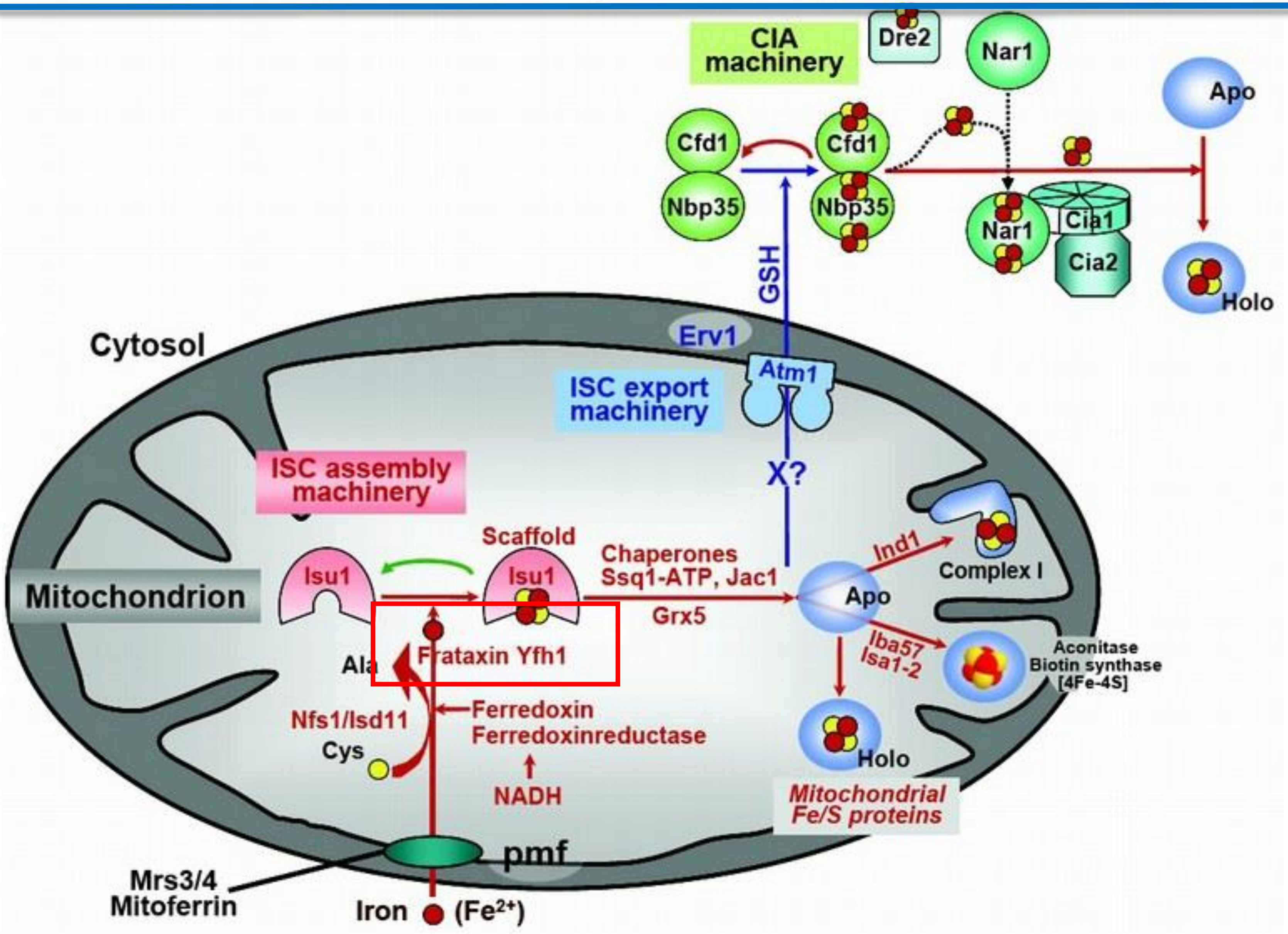
Friedreich Ataxia: an iron homeostasis disease

- autosomal-recessively inherited: caused by triplet repeat expansion
- reduced levels of frataxin
- KO is embryonic lethal
- more mitochondrial iron and loss of iron-sulfur centers
- symptoms usually begin between the ages of 5 and 15
- progressive damage to the nervous system, muscle weakness, speech problems, heart disease.
- hypertrophic cardiomyopathy leads to cardiac arrest





A current model of Fe/S protein biogenesis in eukaryotes



https://www.uni-marburg.de/fb20/cyto/lill/research

Adapted from J.O. Fuss et al. / Biochimica et Biophysica Acta 1853 (2015) 1253–1271 1255

FeS containing proteins involved in genome maintenance!

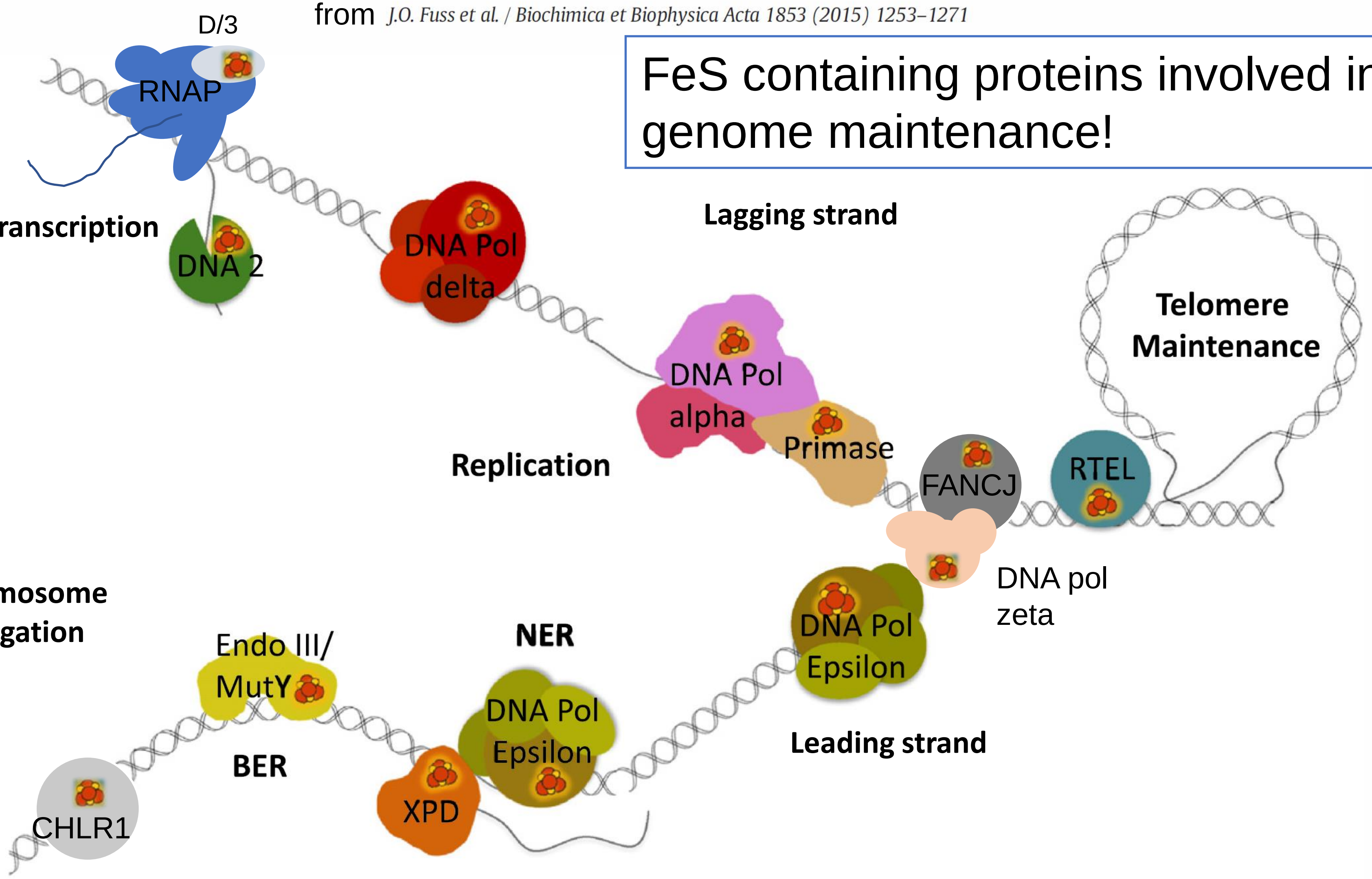
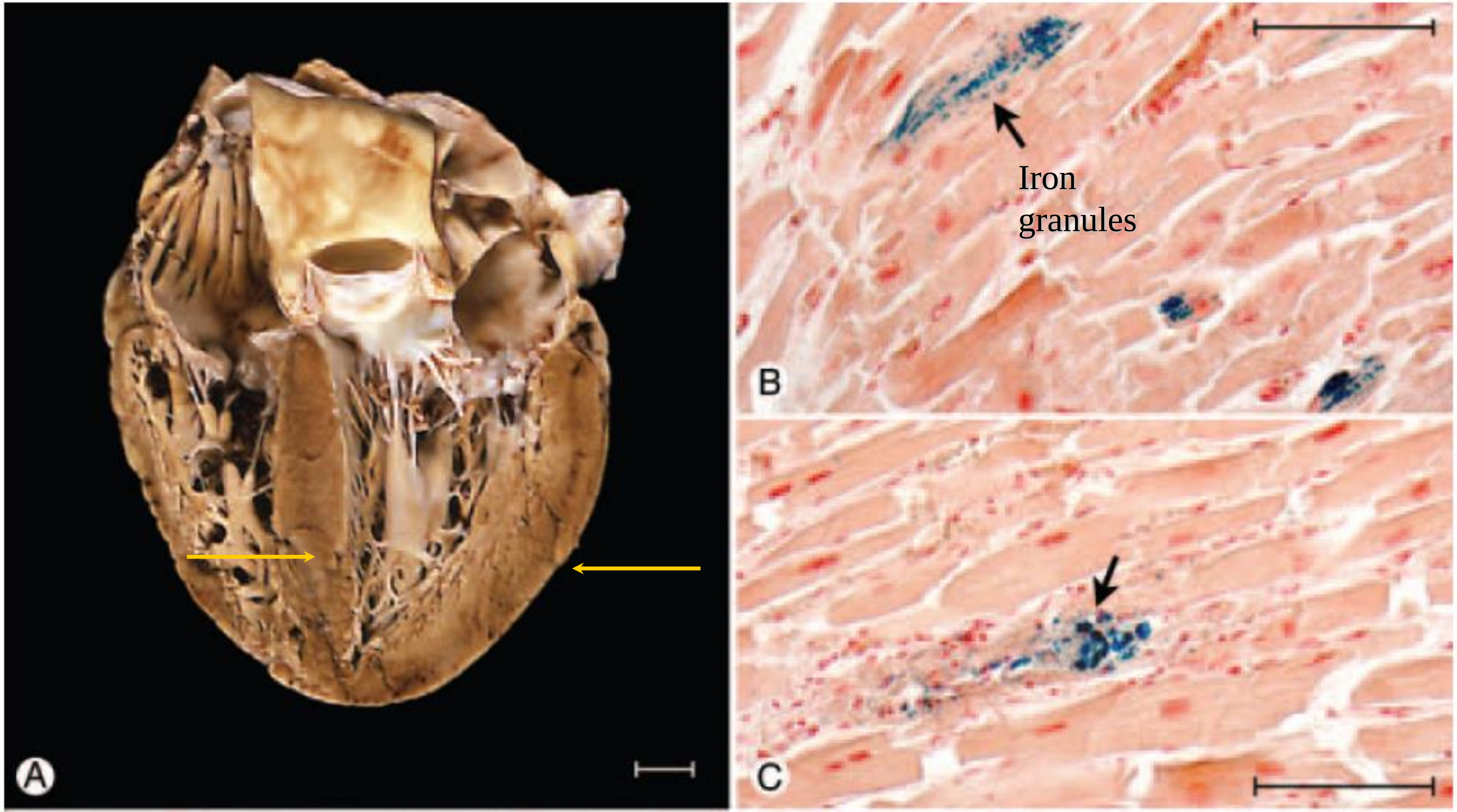


Fig. 1. The emerging roles of Fe–S cluster enzymes in DNA replication and repair. Replication: Fe–S clusters are critical elements of DNA primase, all replicative DNA polymerases (DNA pols α and δ shown), and the nuclease/helicase Dna2 (shown on lagging strand 5′ flaps). Nucleotide Excision Repair (NER): the 5′->3′ Fe–S cluster helicase XPD opens a single stranded bubble around duplex distorting DNA damage allowing excision of the damaged strand by endonucleases and the gap filling by DNA polymerase (DNA pol ε shown). Base Excision Repair (BER): glycosylases Endo III / MutY and their role in the discovery and removal of damaged and mispaired bases. Telomere Maintenance: the helicase RTEL is involved in the unwinding of telomeric D-loops that affects telomere length maintenance and HR in the region.

Hypertrophic cardiomyopathy



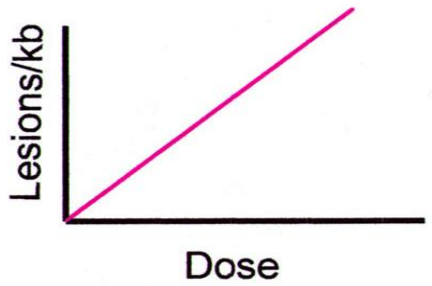
SUSAN MICHAEL[1], SIMONE V. PETROCINE[2], JIANG QIAN[2], JACQUES B. LAMARCHE[4], MITCHELL D. KNUTSON[5], MICHAEL D. GARRICK[6] & ARNULF H. KOEPPEN[1,2,3,7]

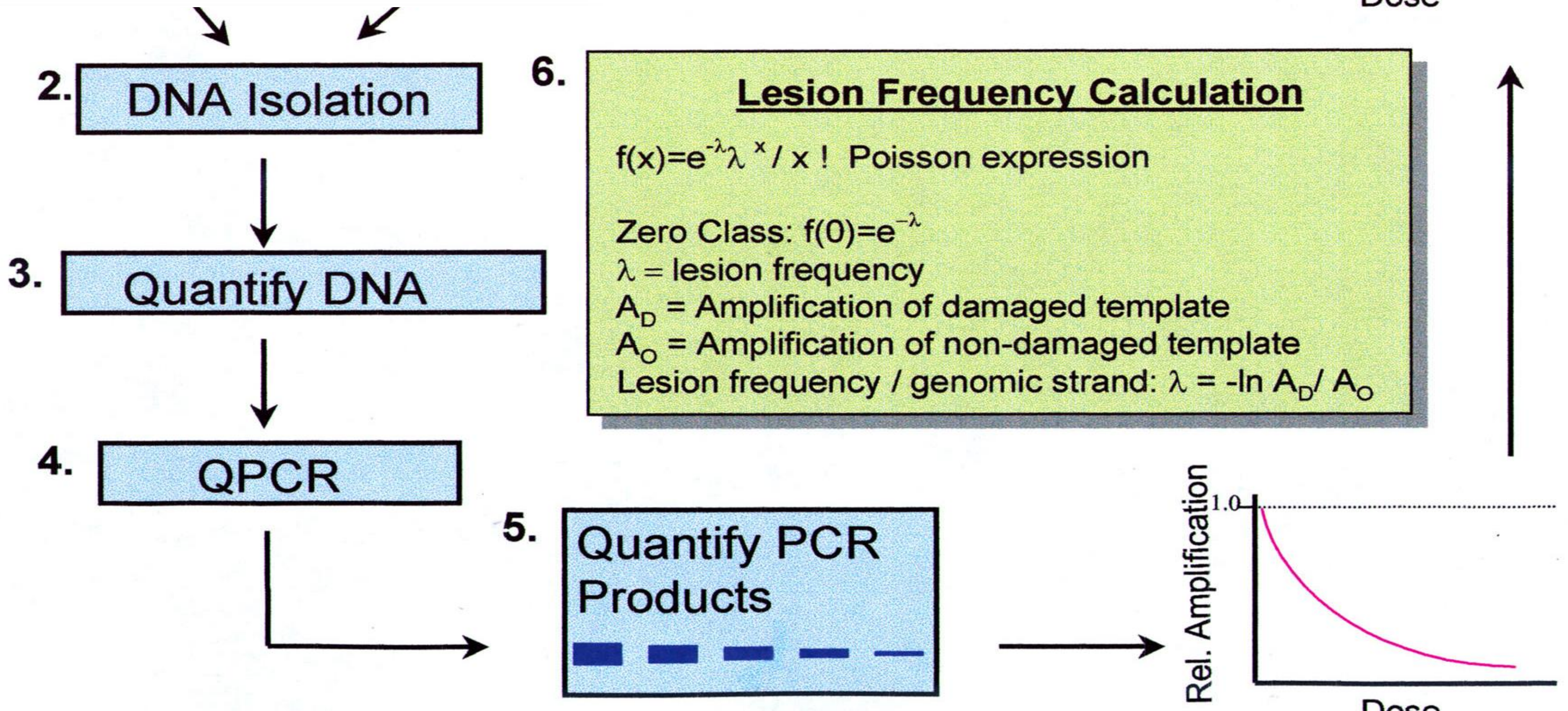
The Cerebellum. 2006; 5: 257–267

How to measure DNA damage in mitochondrial and nuclear DNA

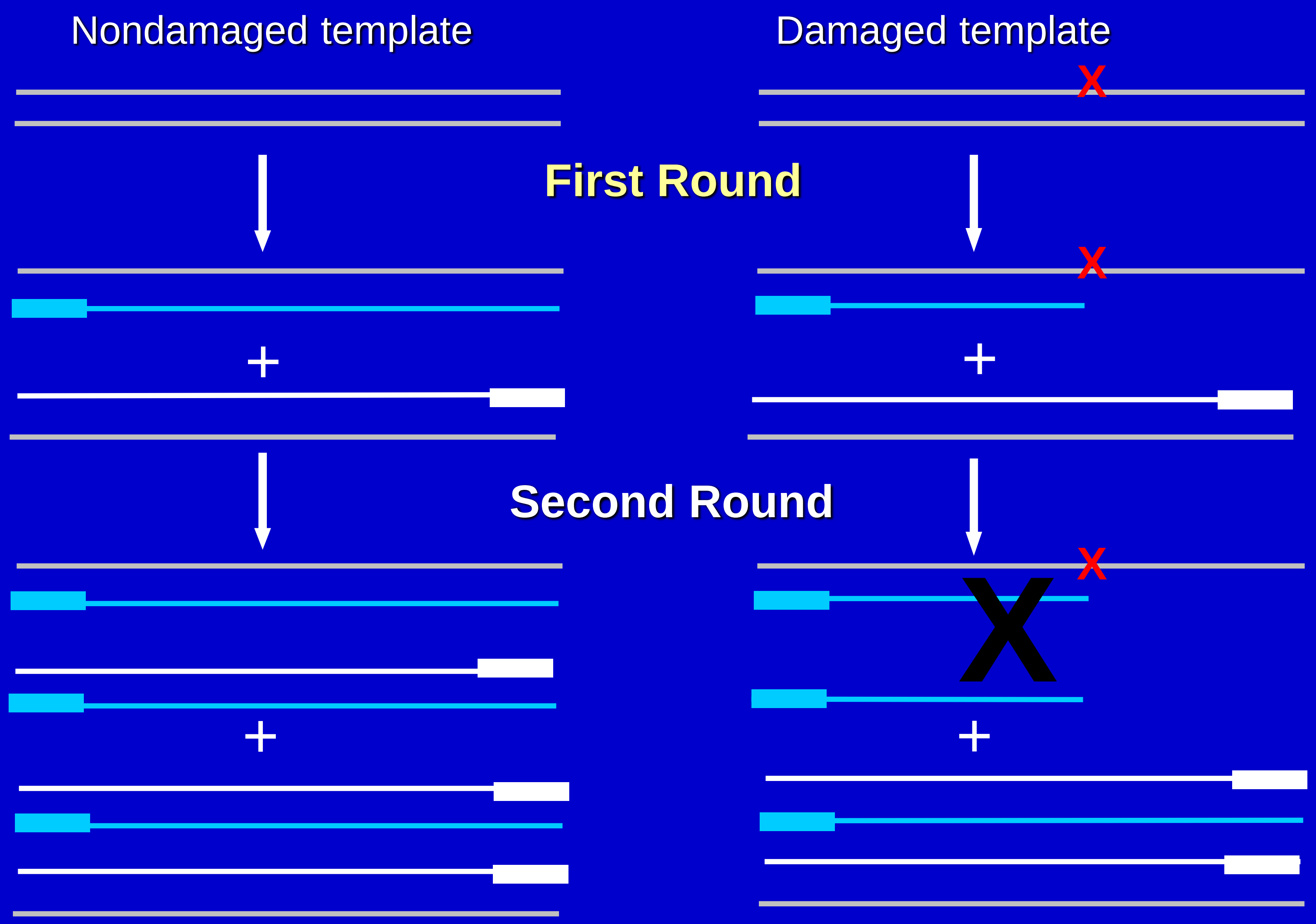
1.

PAX Gene blood DNA tubes 8.5 ml sample – stored at -80 ^{o}C

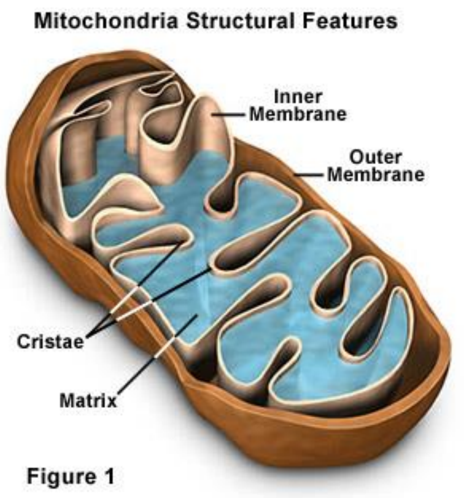


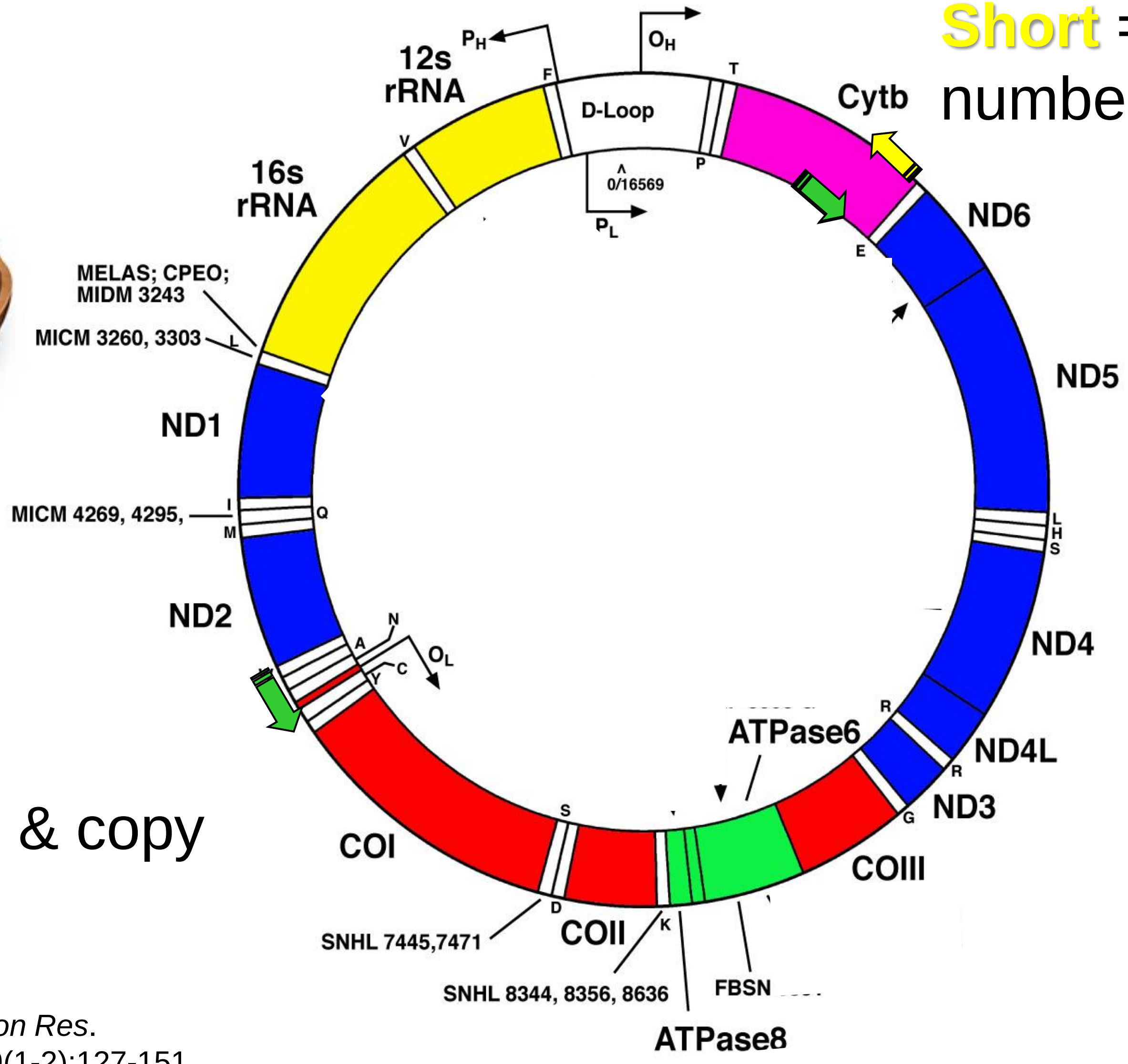


Detection of gene-specific damage by QPCR



How to study mtDNA damage and repair?





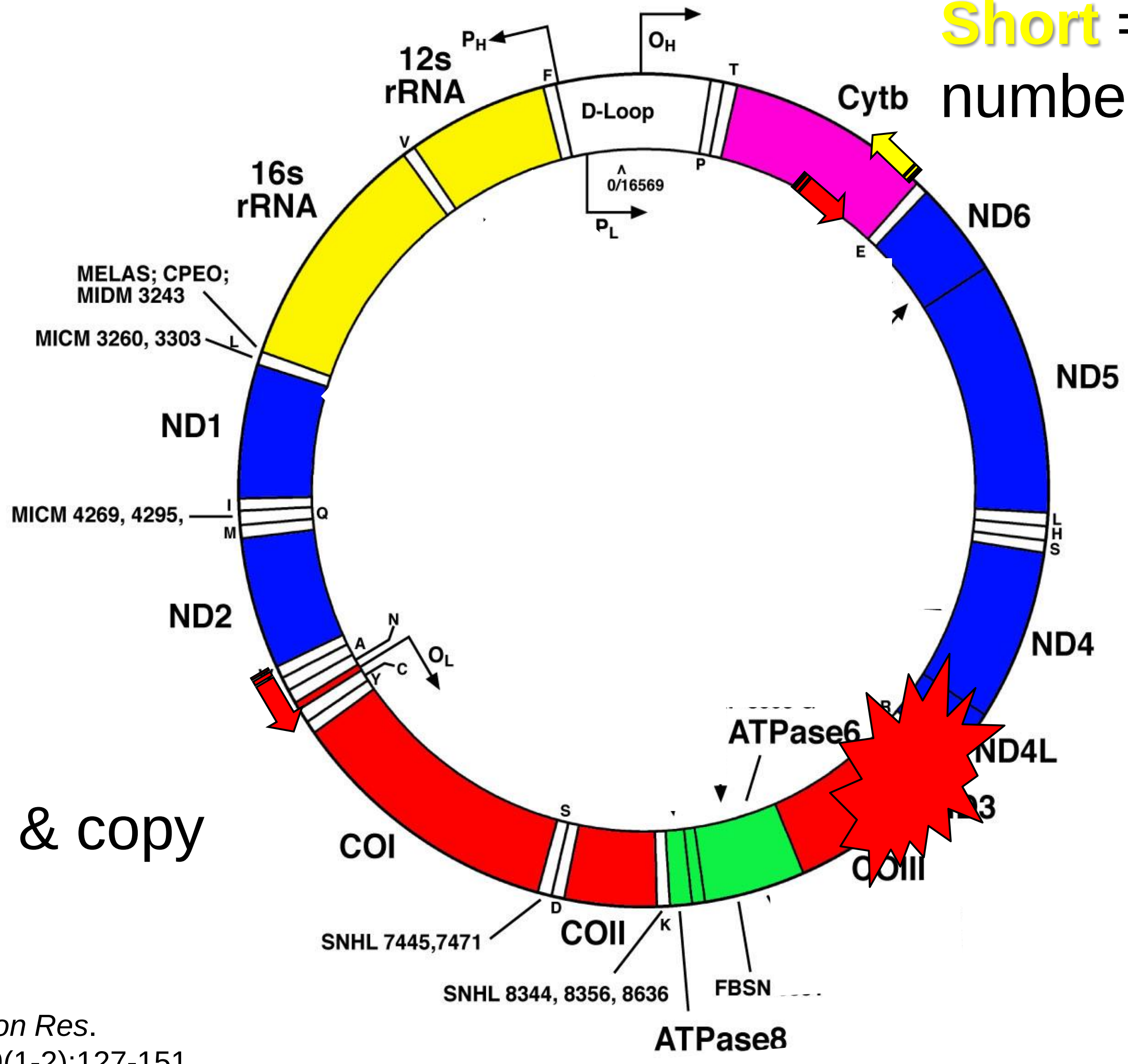
Short = copy number

Long = damage & copy number

B. S Mandavilli,. 2002. *Mutation Res. Fundam. Mol. Mech. Mut*. 509(1-2):127-151.

How to study mtDNA damage and repair?

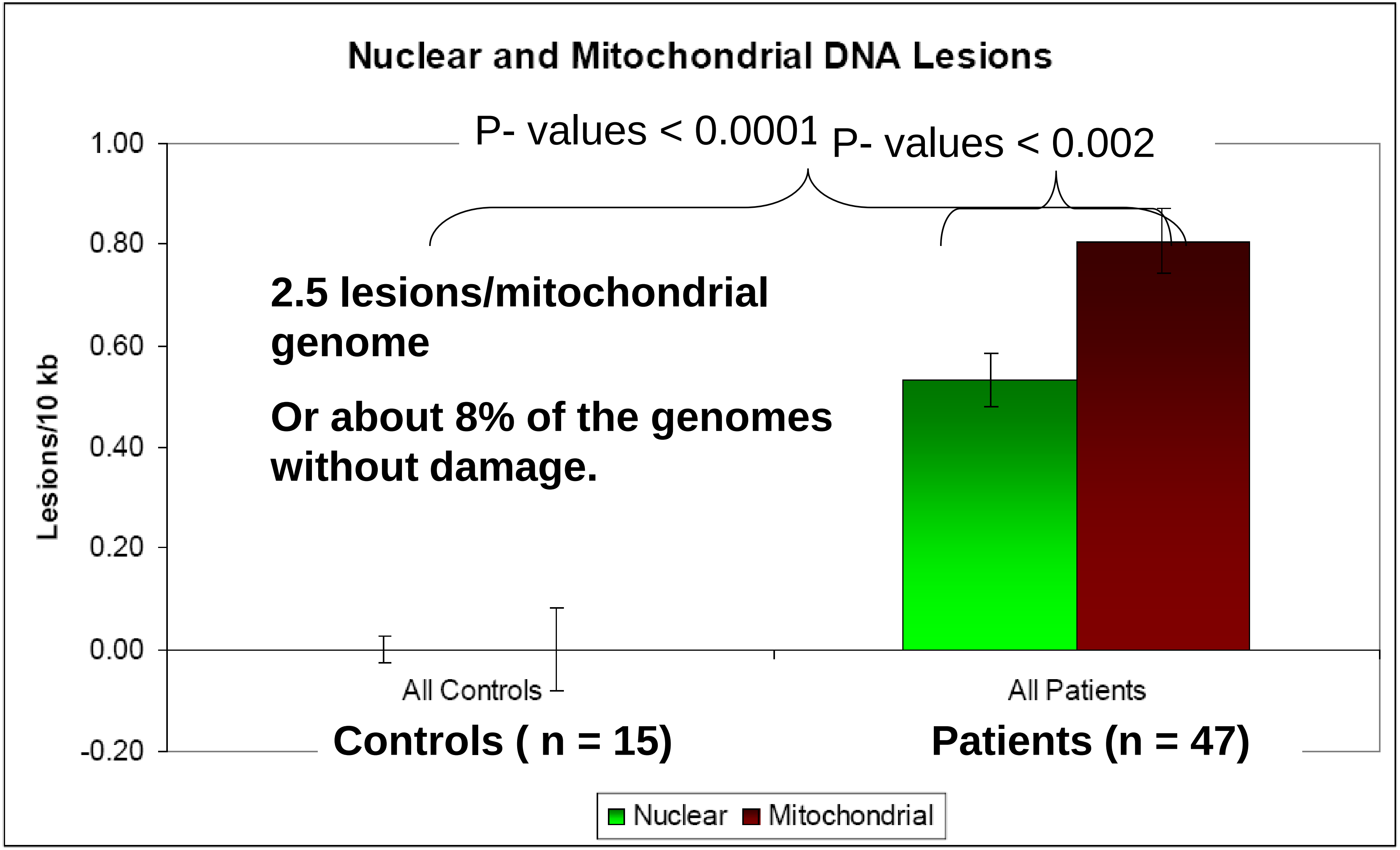
Short = copy number



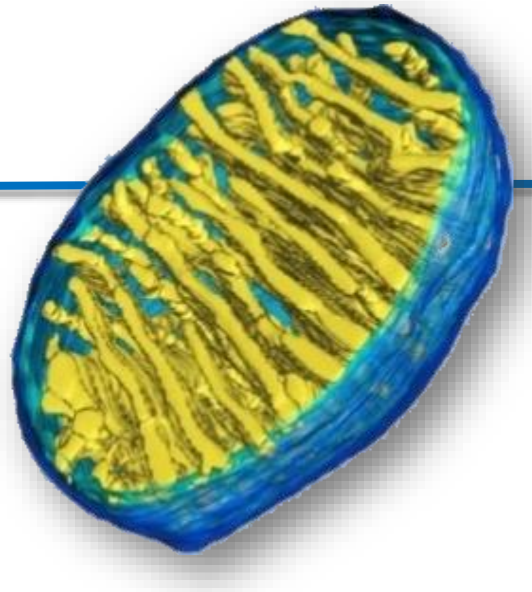
Long = damage & copy number

B. S Mandavilli,. 2002. *Mutation Res. Fundam. Mol. Mech. Mut*. 509(1-2):127-151.

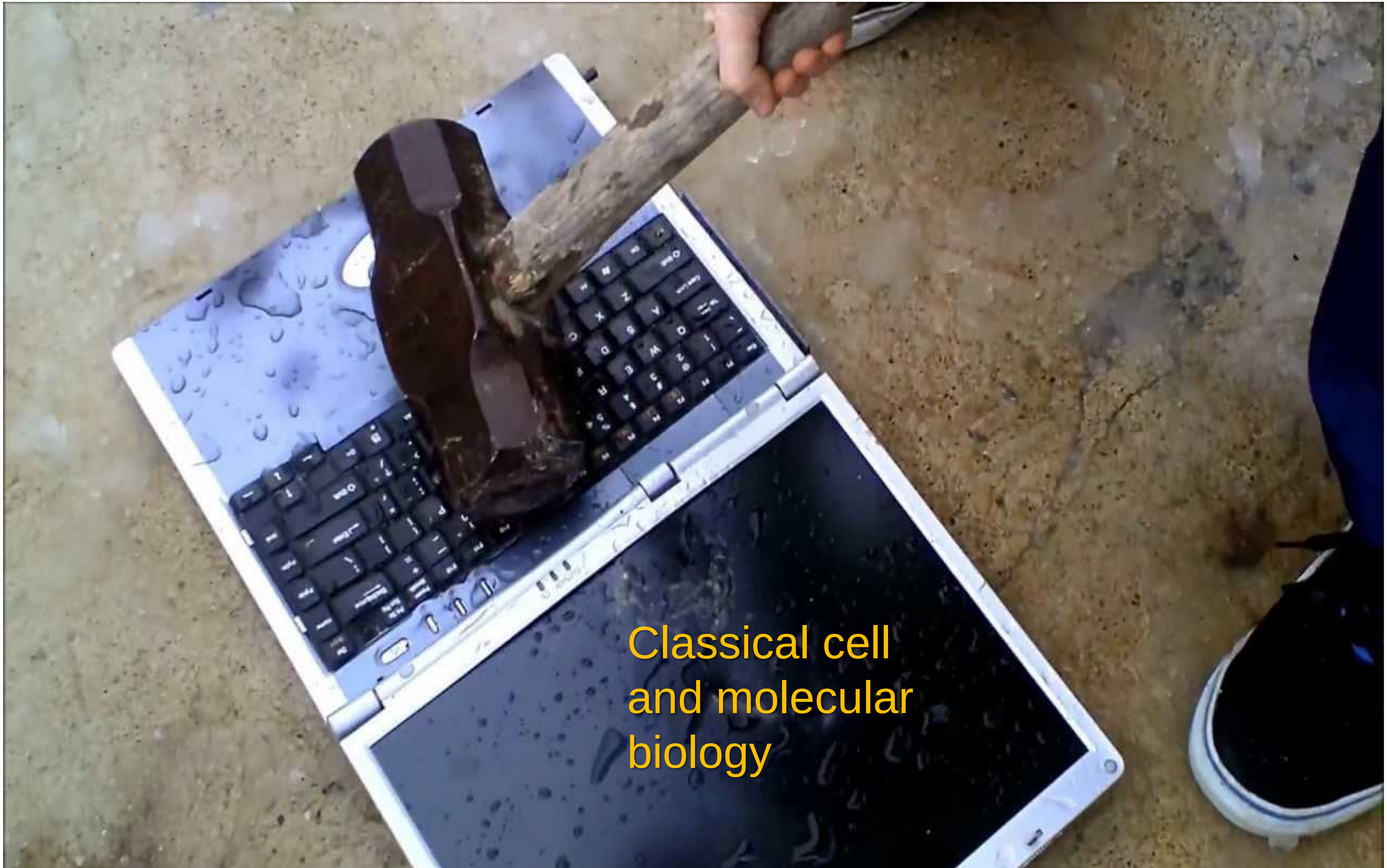
QPCR assay indicated significant DNA damage in children with FRDA



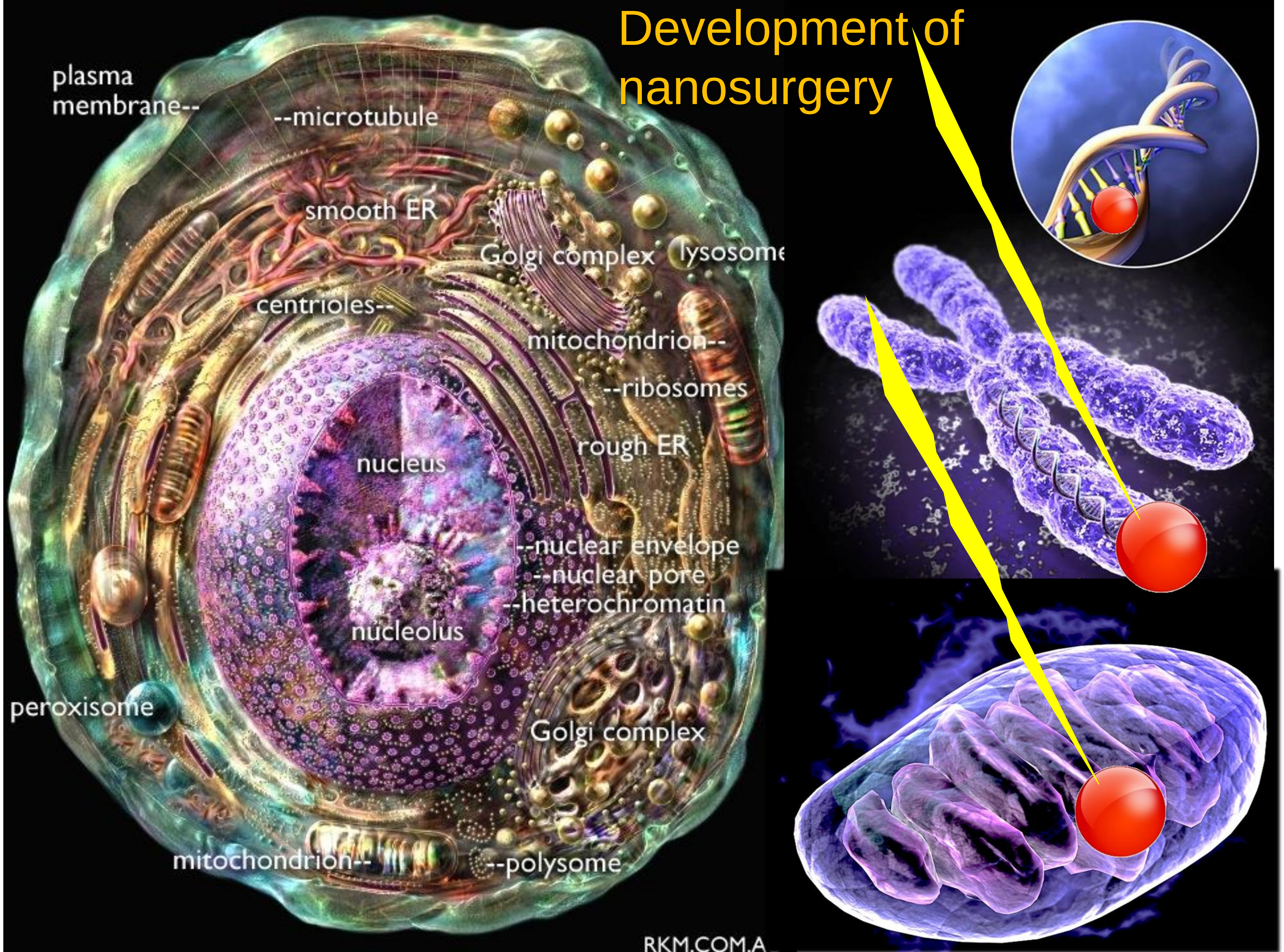
Summary



- QPCR assay could be used effectively on human samples
- Young patients with Friedreich's Ataxia displayed:
  - early heart damage
  - Increased gene expression suggesting nuclear damage
  - Increased mtDNA and nuclear damage


Classical cell
and molecular
biology

Development of nanosurgery

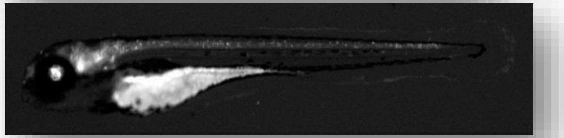


Co-Principal Investigators

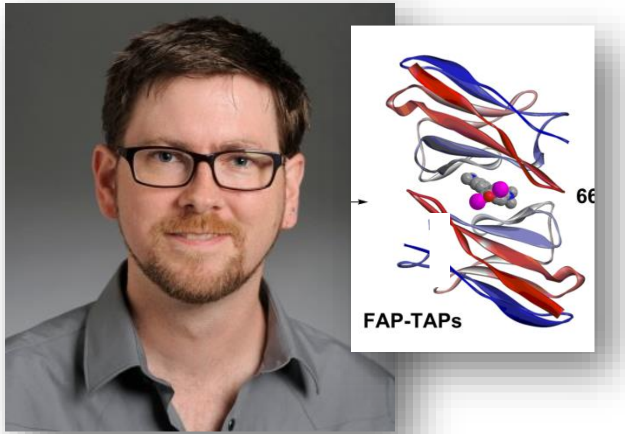
Ben Van Houten, PhD, Genome stability group Dept. Pharmacology & Chemical biology

Patty Opresko, PhD, Genome stability group Dept. of Environmental & Occupational Health

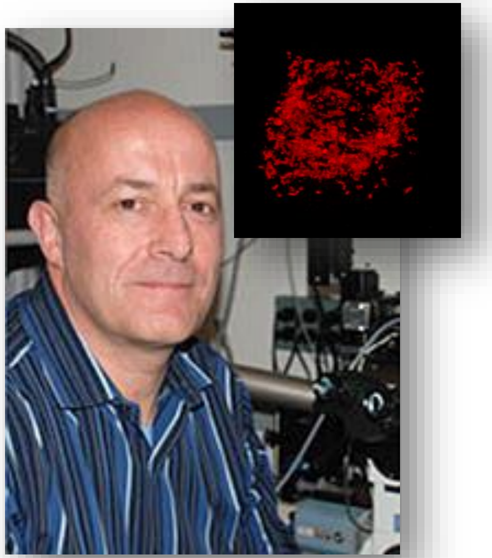
DREAM TEAM of Co-investigators



Ed Burton, MD, PhD Pittsburgh Institute of Neurodegenerative Diseases

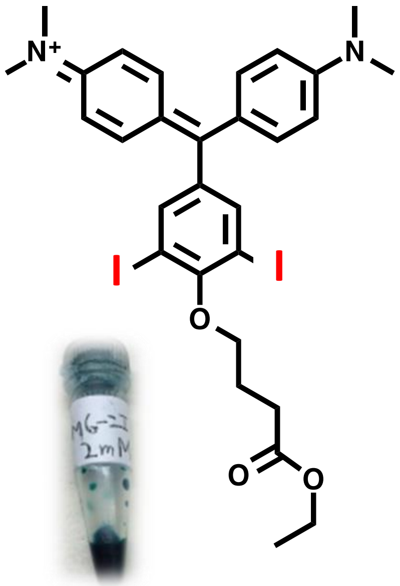


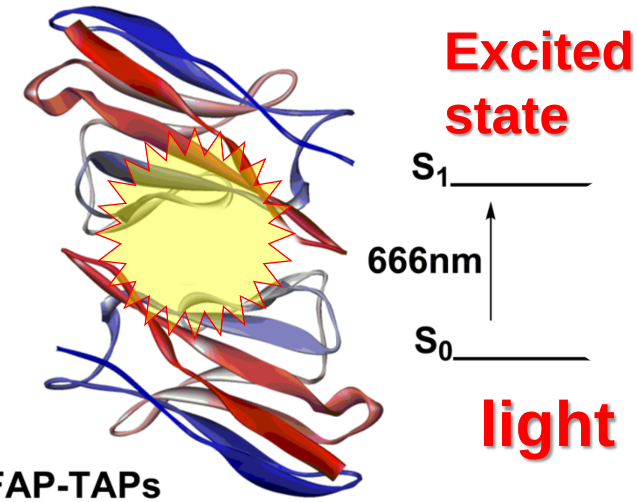
Marcel Bruchez , Depts. of Chemistry & Biology, Carnegie Mellon University



Simon Watkins, PhD, Center for Biologic Imaging, Univ. of Pittsburgh

Chemoptogenetic Innovative technology: targeting singlet oxygen damage using Fluorogen Activating Peptide (FAP)



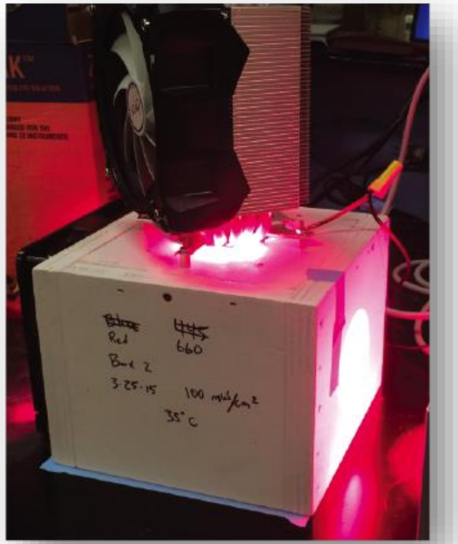


targeted and activated photosensitizer

Malachite green dye (inactive)

MG2I

Marcel Bruchez ,
Depts. of Chemistry & Biology, Carnegie Mellon University



Nature Methods, Mar;13(3):263-8, 2016

Versatility of FAP technology: multiple cellular targets & senor dyes

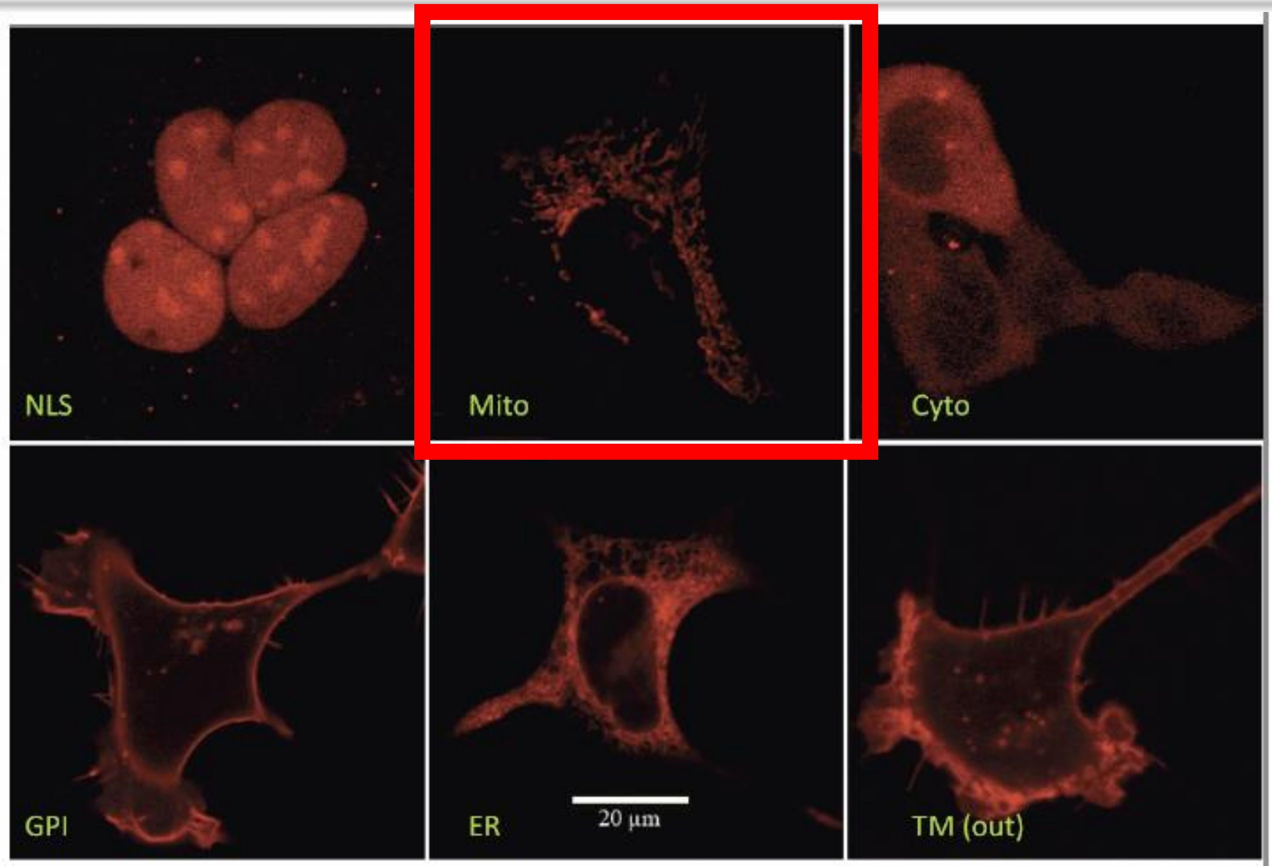


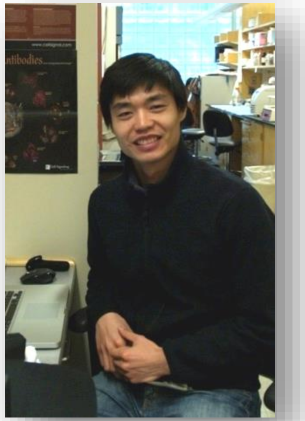
Figure A1.2 Subcellular targeting of MG-binding FAPs. Using targeting sequences, HEK293 cells labled with MG-lys-$(SO_3^-)_2$ or MG-Ester were imaged on a Zeiss LSM510 MetaNLO with 633 nm excitation. Scalebar is 20 µm.

Targeted locations

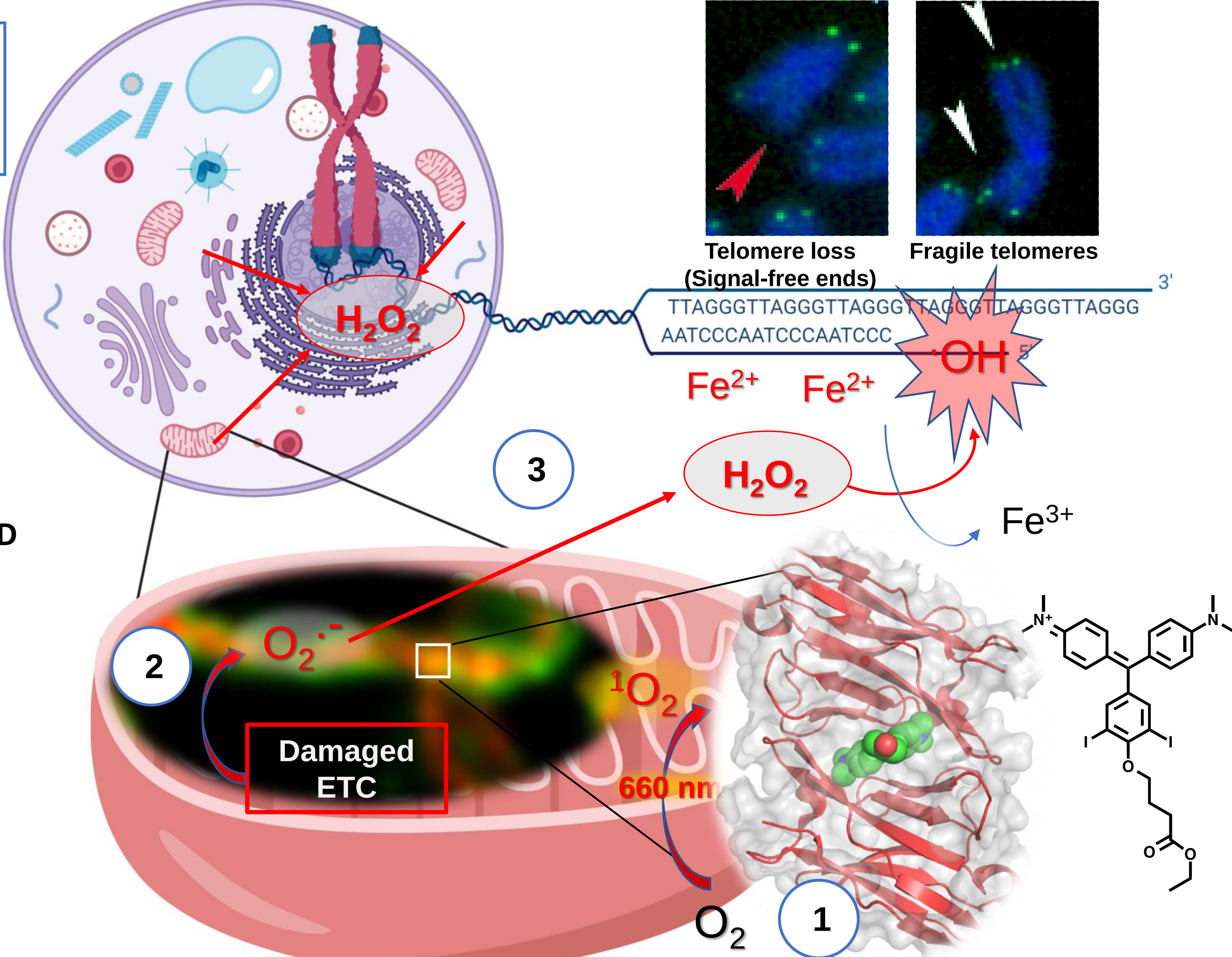
Nature Methods, Mar;13(3):263-8, 2016

Proving that mitochondrially generated ROS can damage telomeres


Namrata Kumar and Elise Fouquerel, PhD

Vera Roginskaya **Wei Qian, PhD**



Mito-FAP & MG-2I

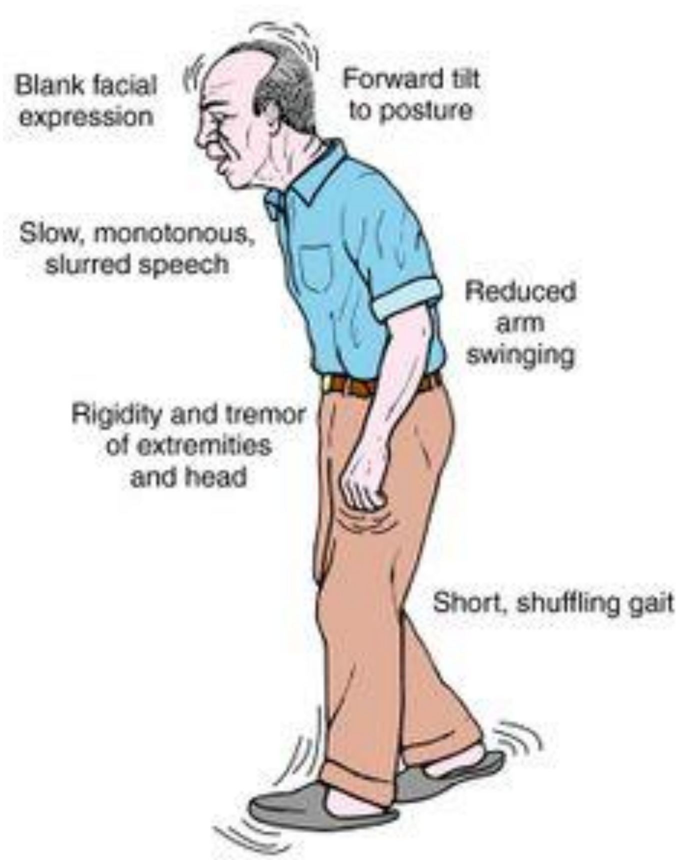
MG-2I dye

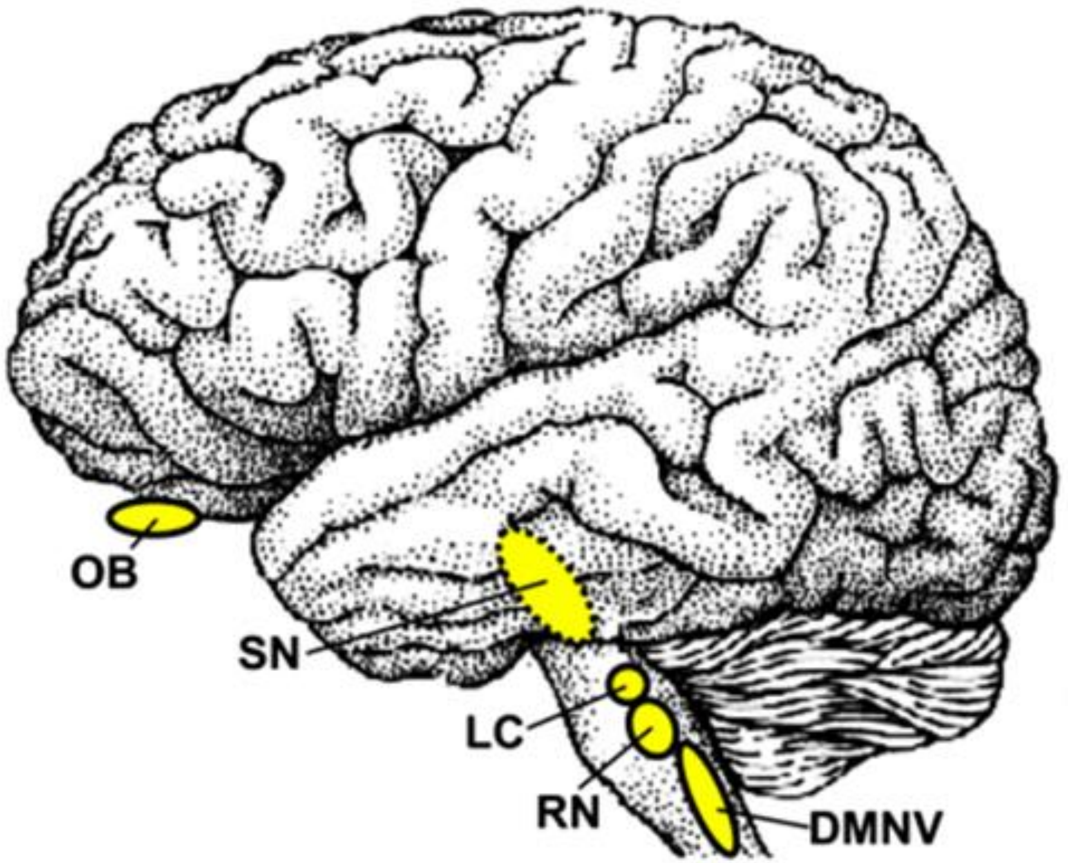
Qian W, et al,. (2019). *Proc Natl Acad Sci U S A.* Sep 10;116(37):18435-18444.

As many as one million Americans live with Parkinson's disease

Approximately 60,000 Americans are diagnosed with Parkinson's disease each year

Regions of brain affected in Parkinson's Disease



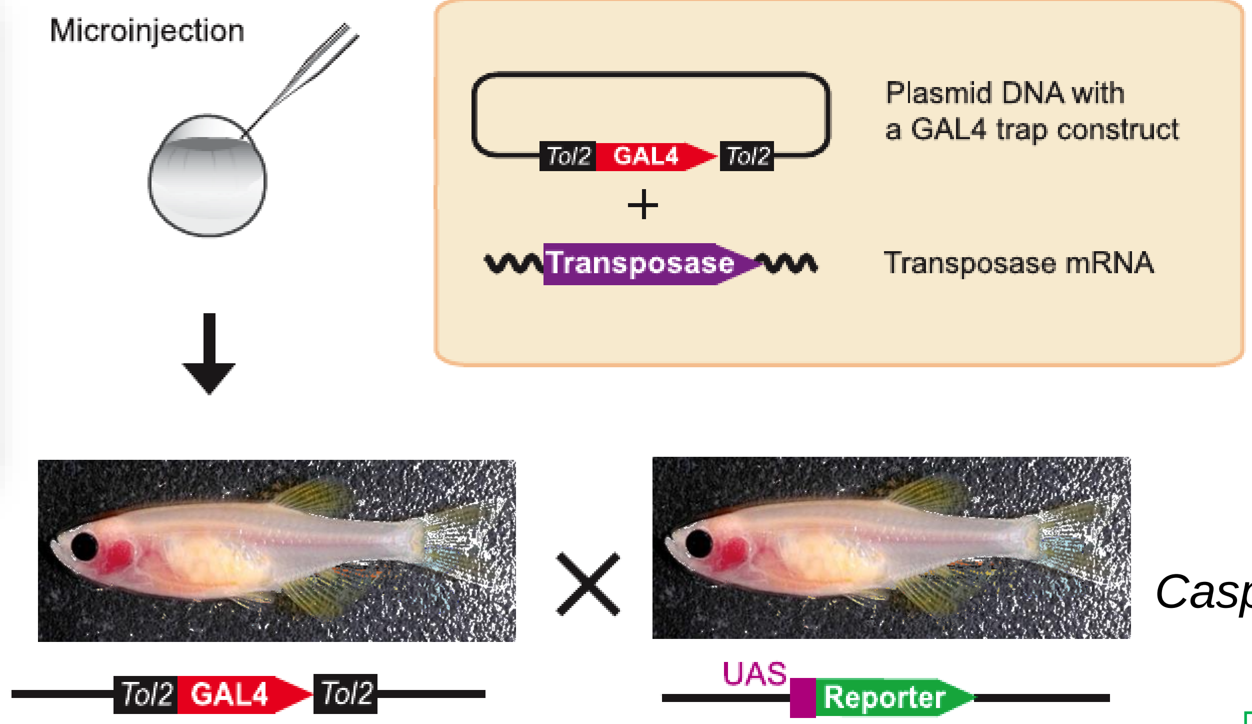


Several lines of evidence suggest that mitochondrial dysfunction is part of the pathophysiology of PD.

PD patients show shorter telomeres in their peripheral blood.

Bipartitie gal4/UAS expression system with cell-type expression of FAPs using Gal4 driver lines.

Ed Burton, MD, PhD



Qing Bai, PhD

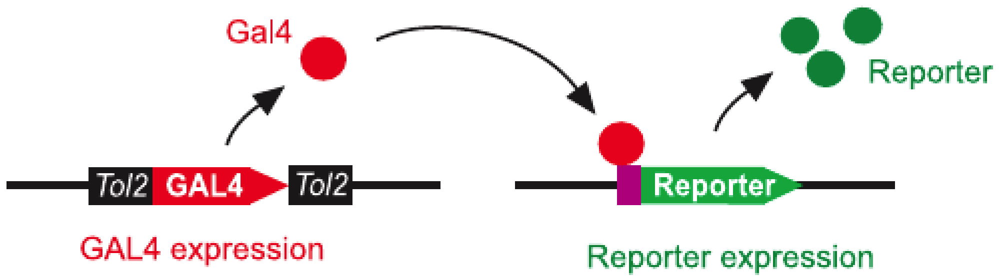
Casper background

Driver lines

Eno2

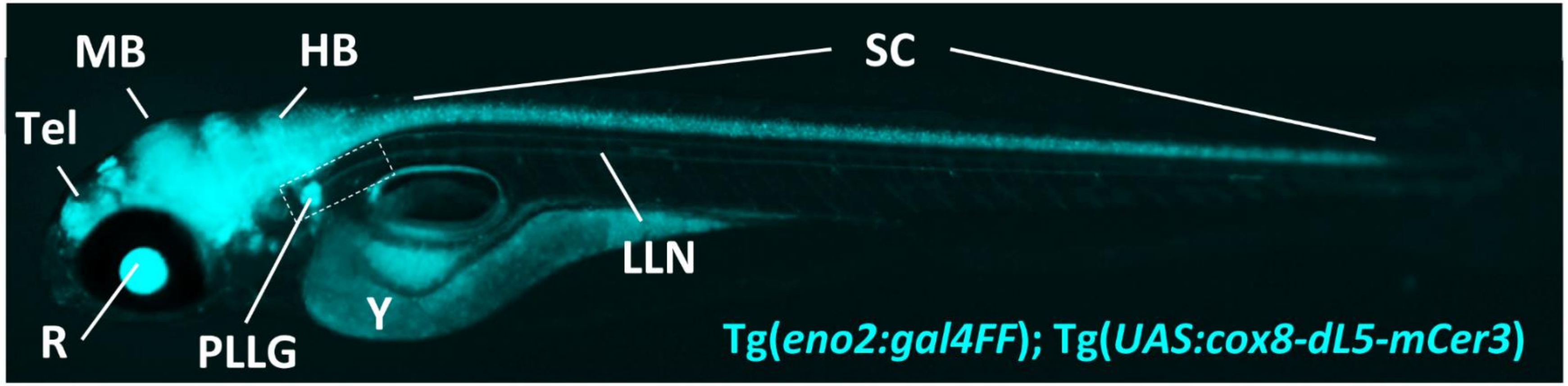
UAS:Mito-FAP

Responder line



ENO2, neuron specific promoter.

Mito-FAP-mCer expression in CNS of zebrafish

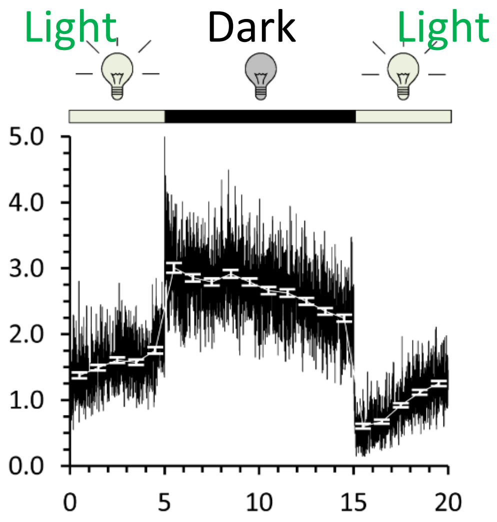


Tel: telencephalon
MB: midbrain
HB: hindbrain
R: retina
PLLG: posterior lateral line ganglion
Y: yolk sac (autofluorescence)
LLN: lateral line neurons
SC: spinal cord

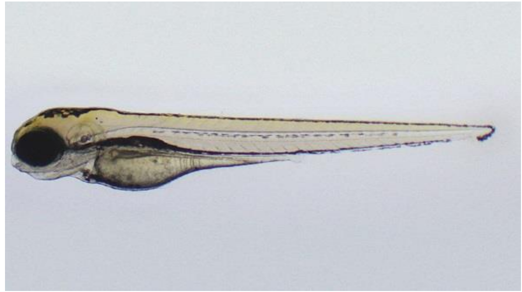
Expression only in neurons and not other brain cells such as glial cells or astrocytes.

Xie W, et al, Chemoptogenetic ablation of neuronal mitochondria in vivo with spatiotemporal precision and controllable severity. *Elife.* 2020 Mar 17;9:e51845.

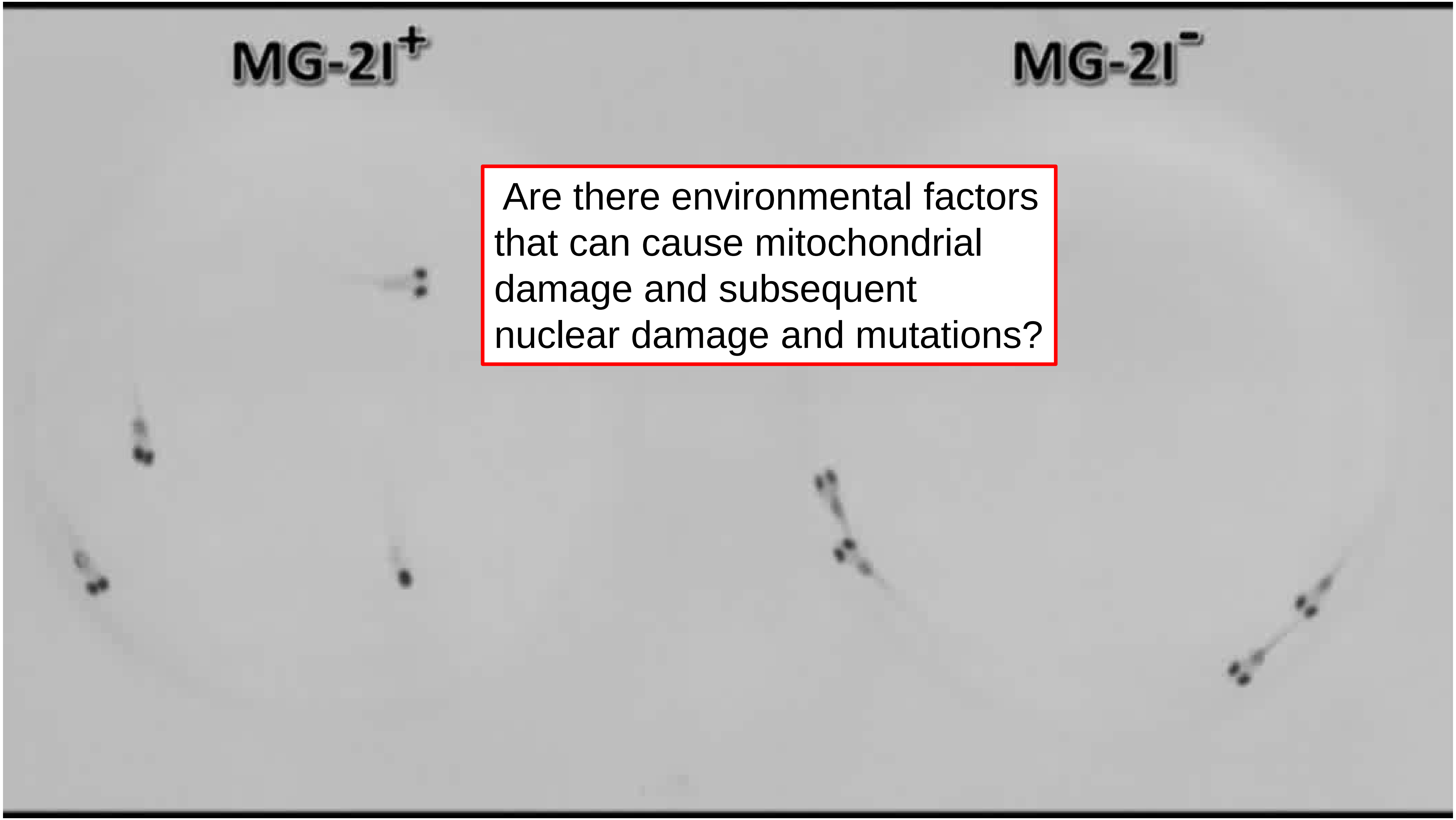
Light & dye causes movement deficits in the in CNS of zebrafish



Visual motor response in zebrafish embryos

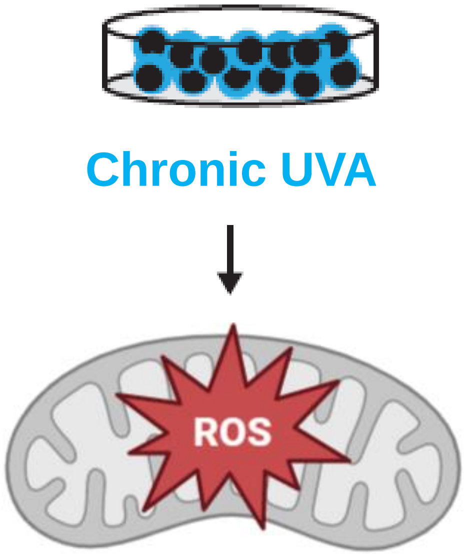


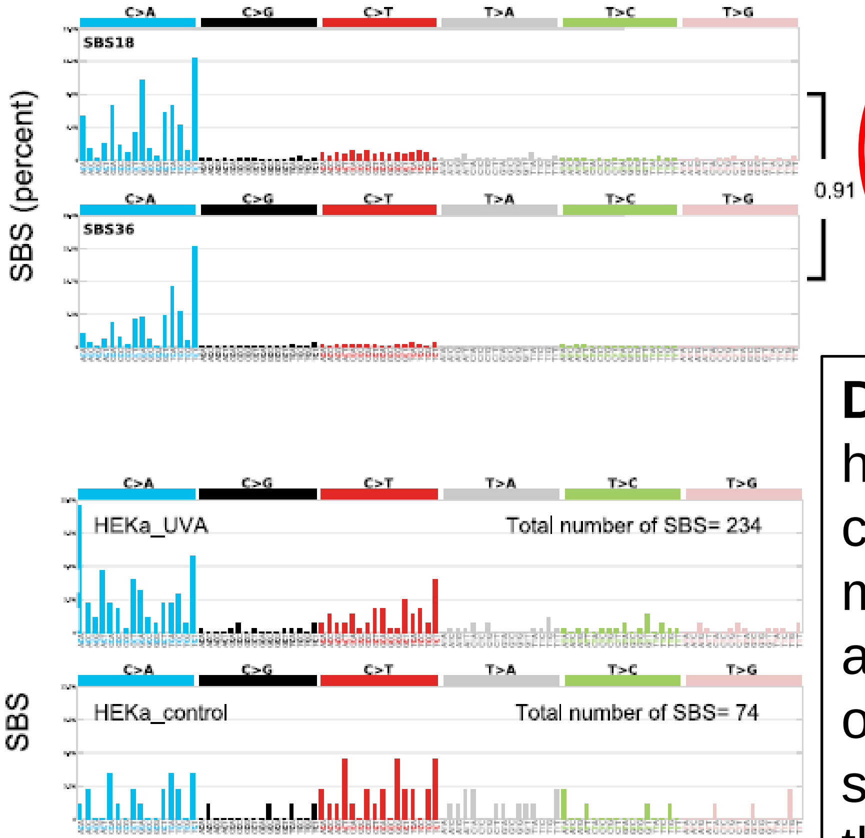
Wenting Xie
Tsinghau Visiting Scholar

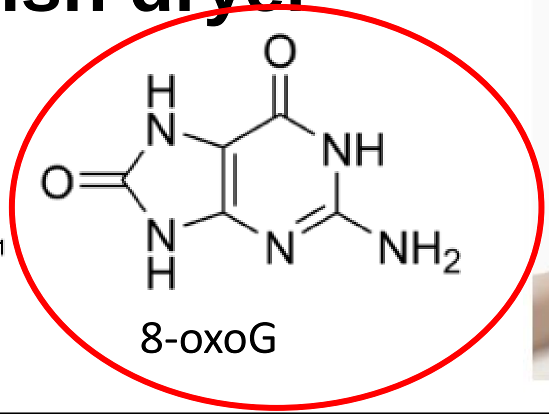


Are there environmental factors that can cause mitochondrial damage and subsequent nuclear damage and mutations?

DNA damage and somatic mutations in mammalian cells after UVA irradiation with a nail polish dryer







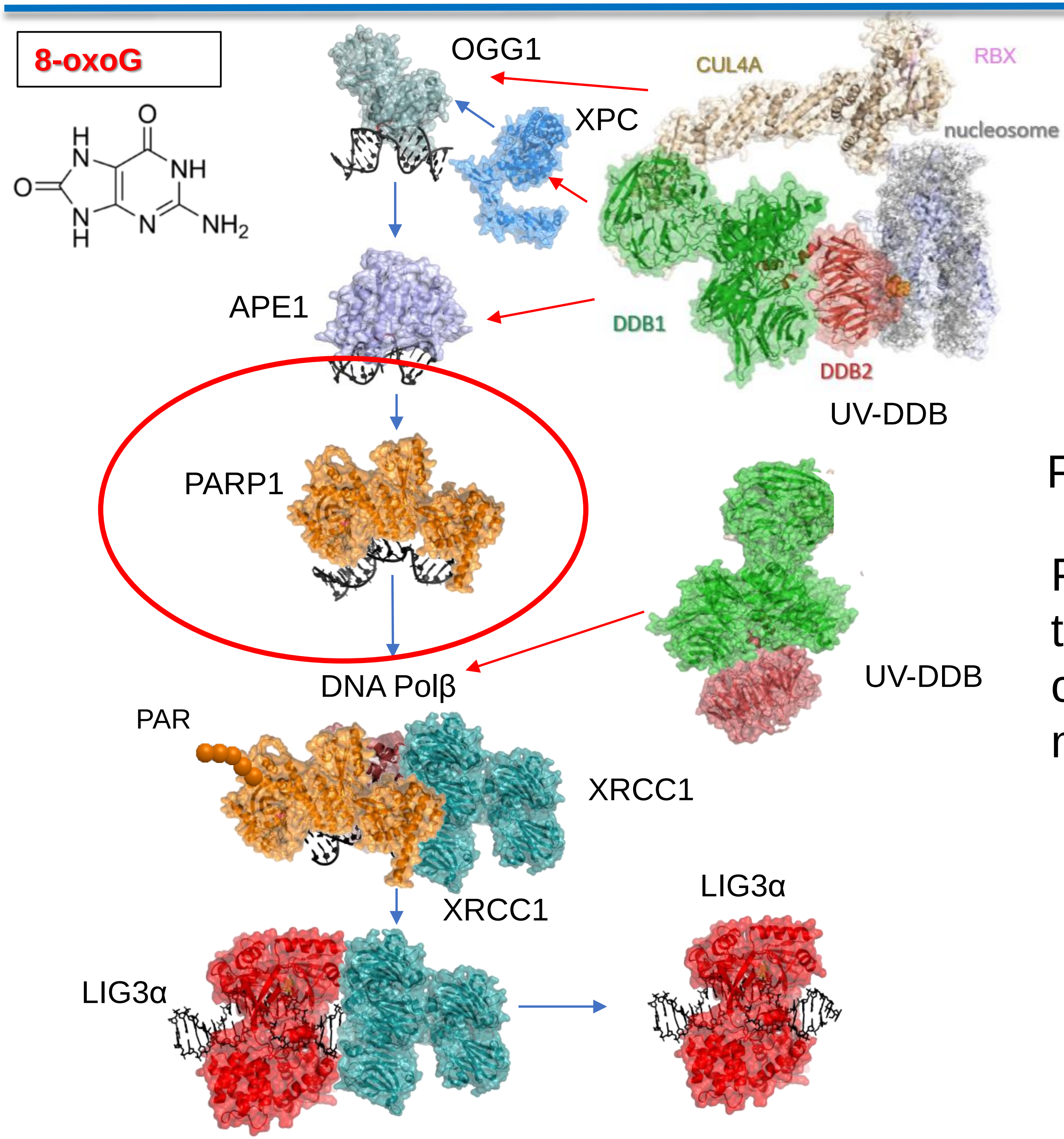
Discovery: UVA-nail polish dryer causes high levels of reactive oxygen species, consistent with 8-oxoguanine damage and mitochondrial dysfunction. Somatic mutation analysis reveals a dose dependent increase of C:G>A:T substitutions in irradiated samples with mutagenic patterns similar to those attributed to reactive oxygen species.

Ludmil Alexandrov

Impact: This study demonstrates that UVA radiation emitted by nail polish dryers can both damage DNA and permanently engrave mutations on the genomes of mammalian cells and is consistent with driver mutations in melanoma. Caution is recommended for a large population using this apparatus.

Zhivagui et al, Nature Comm. 2023

Base excision repair with the help of UV-DDB and XPC



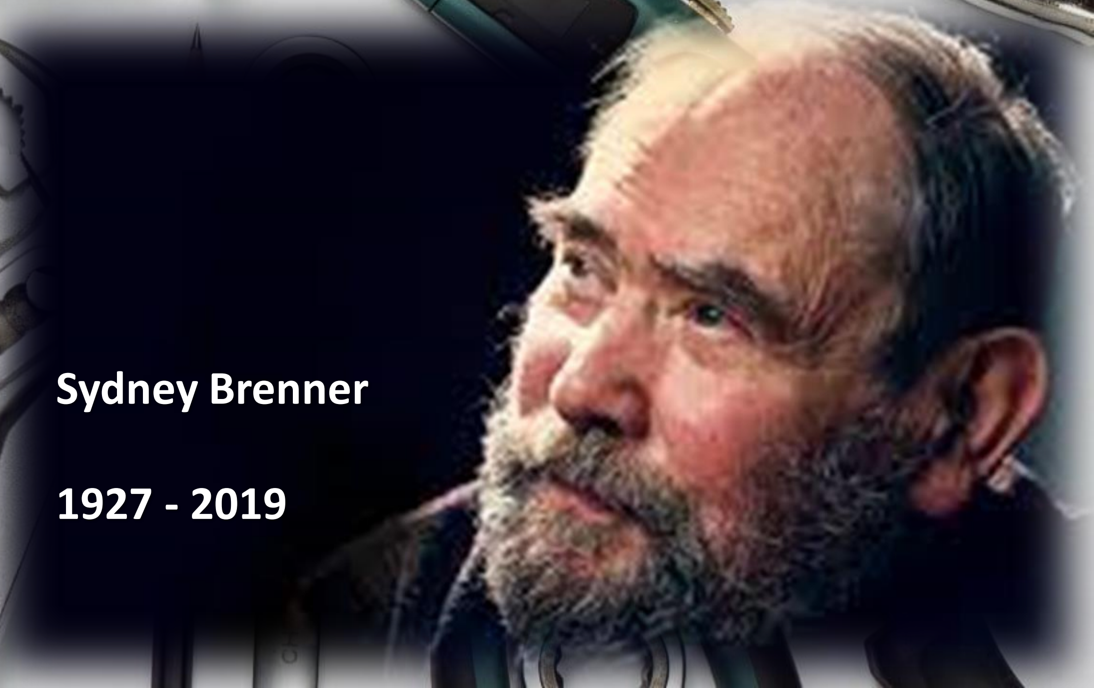
Goal: To observe the kinetics of all the intermediate steps in BER for these complexes in chromatin

PARP1 = poly(ADP)-ribose polymerase

PARP1 inhibitors are used widely in the treatment of breast and ovarian cancers that are BRCA1 or BRCA2 mutated

Jang *et al*., *NSMB*, 2019, 26(8):695-703;
Jang *et al*., *NAR*, 2021,49(14):8177-8188
Jang et al., *NAR*, 2022, 2022, 50(22):12856-12871.
Jang & Raja et al, NAR, 2023 (in press).
Kumar *et al., Nature Comm.,* 2022, 13(1):974
Nagpal, A. *et al., Biochem. Society Tran.* (2022),
Schaich et al., *NAR*, 2023.

“Progress in science depends on new techniques, new discoveries and new ideas, probably in that order”
- Sydney Brenner

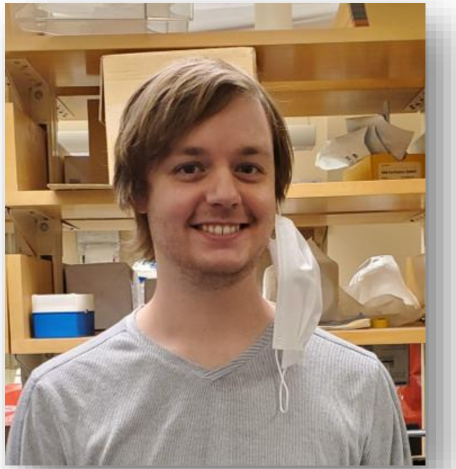


Sydney Brenner

1927 - 2019

SMADNE (Single Molecule Analysis of DNA binding proteins from Nuclear Extracts)

- Can follow your favorite fluorescently-tagged protein
- Protein binding kinetics to DNA targets
- Multiple colors allows assessment of order of assembly and dissociation
- Allows rapid survey of protein variants
- Can assess the affects of post-translation modifications
- Contains protein chaperones which may help improve activity



Matt Schaich, PhD

Brittani Schnable

Namrata Kumar, PhD

Schaich *et al, NAR* (2023)

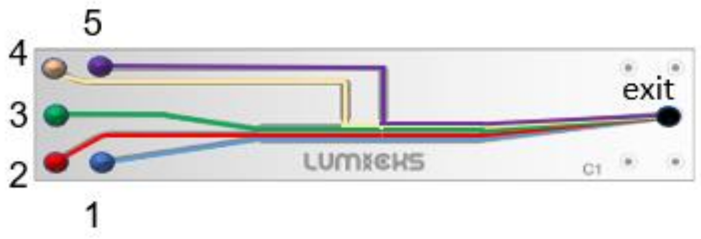
SMADNE (Single Molecule Analysis of DNA binding proteins from Nuclear Extracts): workflow

From construct to extract to C-trap in one week!

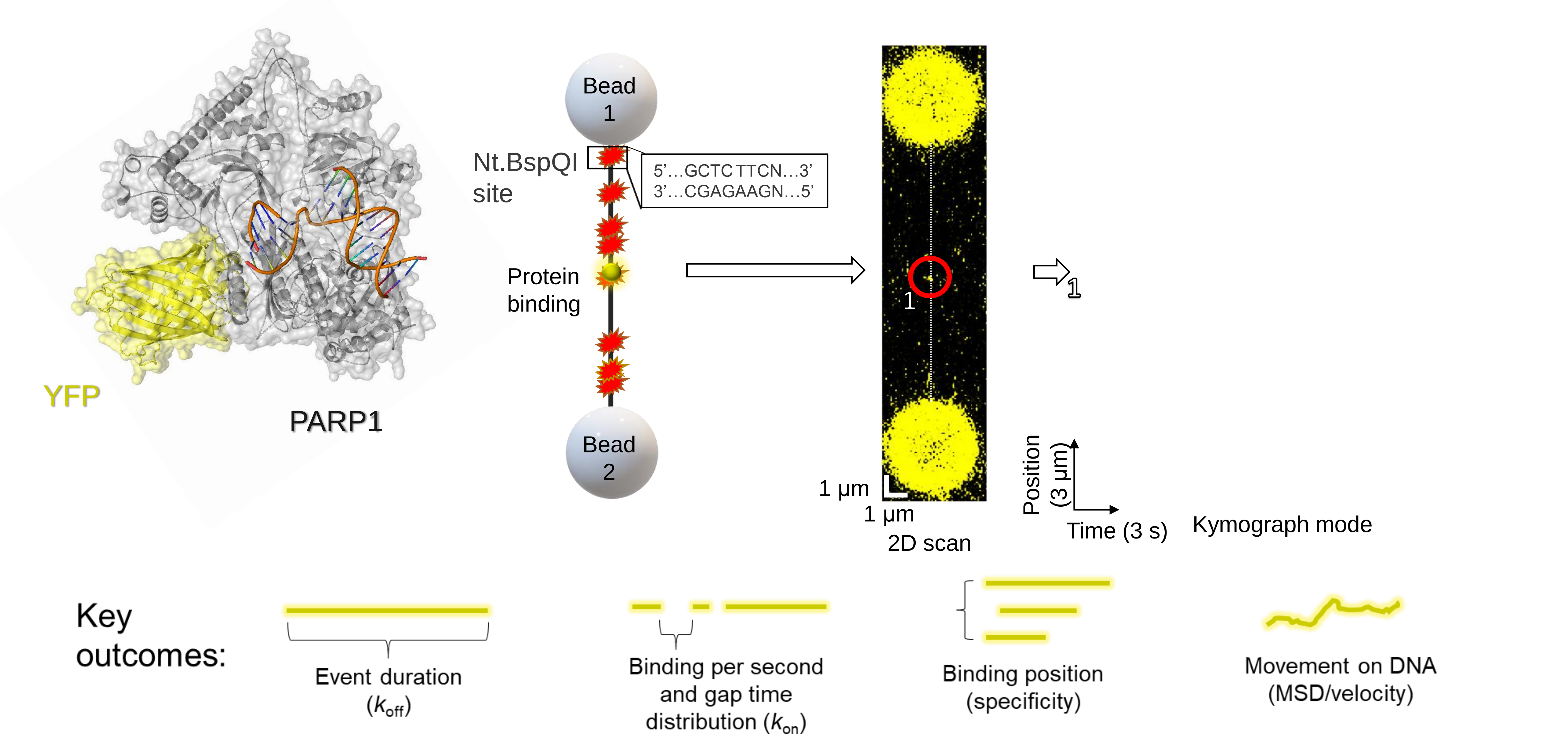
LUMICKS C-trap

- Laser tweezer
- 5 chamber microfluidics
- 3 color confocal

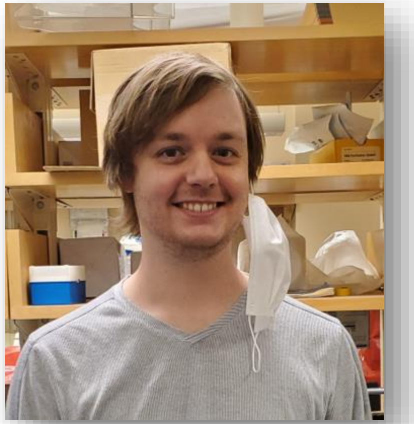
1. Five chambered flow cell



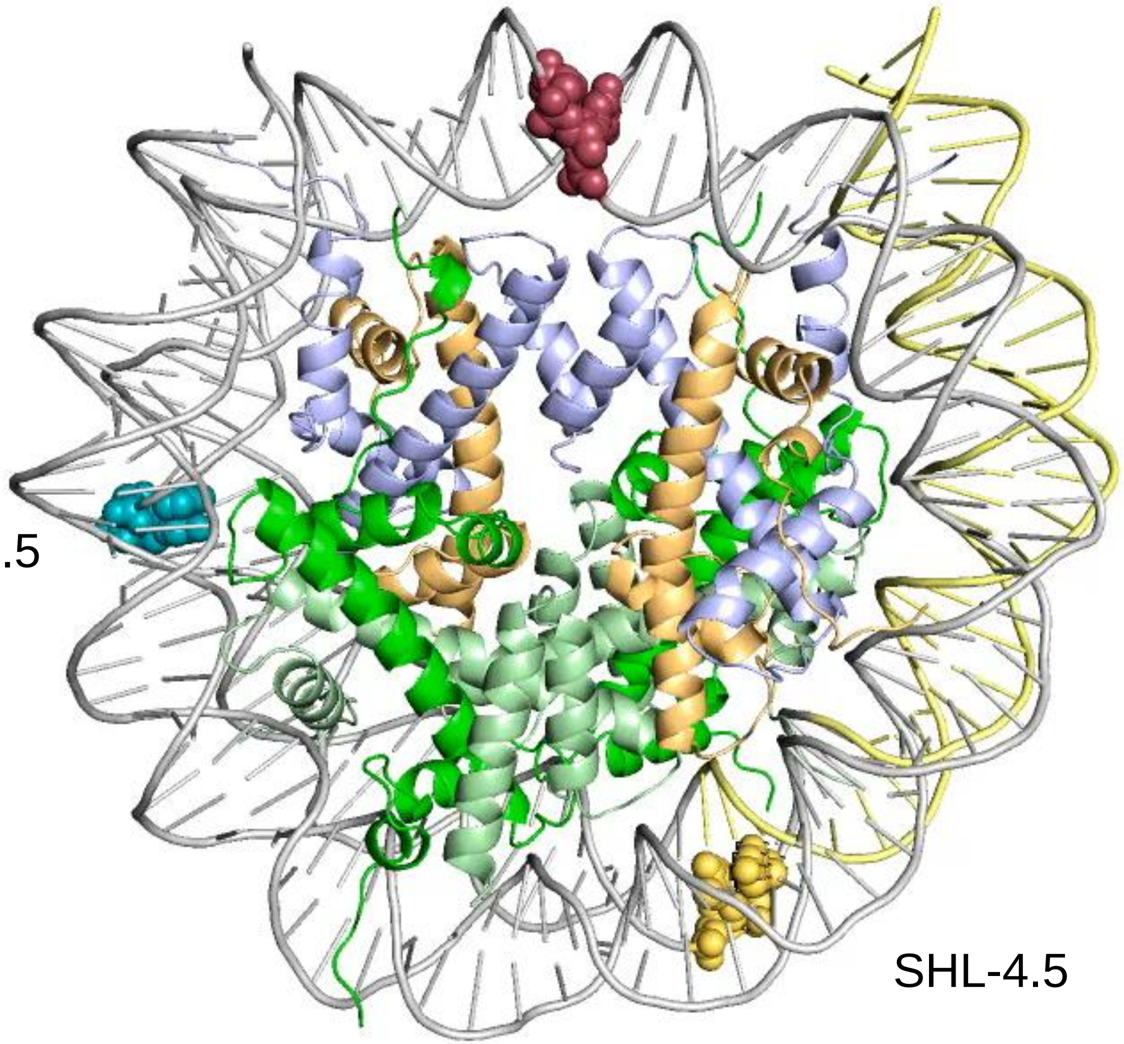
YFP-PARP1 binds nicked DNA



Can we study repair protein interactions on a nucleosome containing a non-ligatable nick?



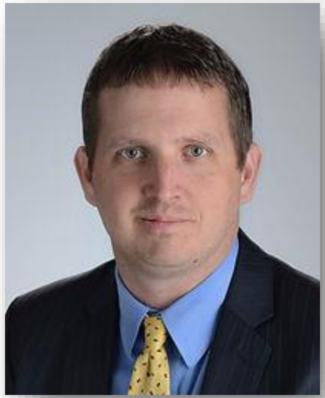
Matt Schaich, PhD



H2A
H2B
H3
H4

Positions of nicks on Cy3-labeled H2A nucleosomes

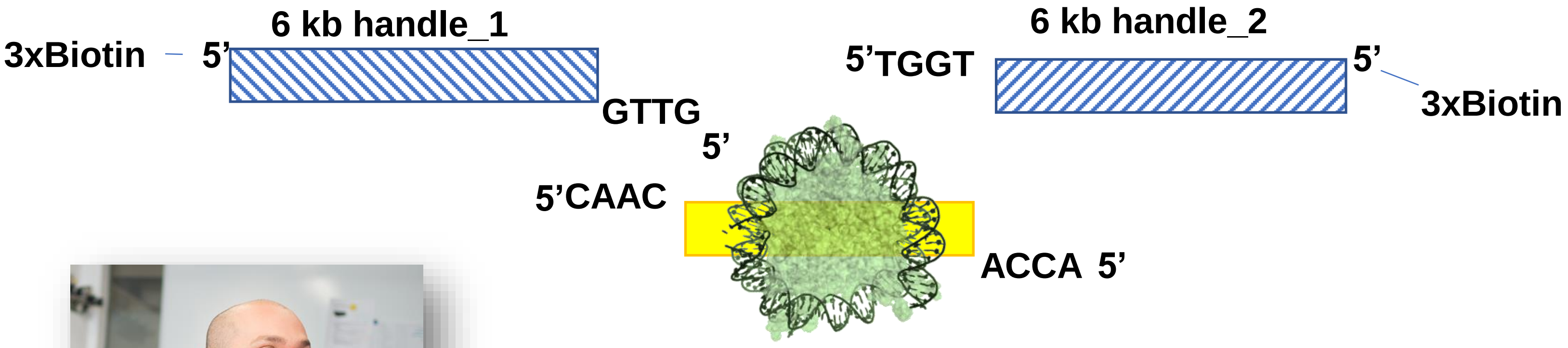
SHL – superhelical location
SHL0 = dyad



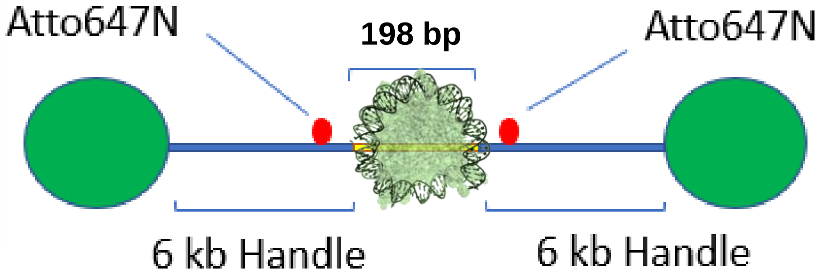
Bret D. Freudenthal, PhD
UKMC

Tyler Weaver, PhD

Strategy for making nucleosomes containing nicks

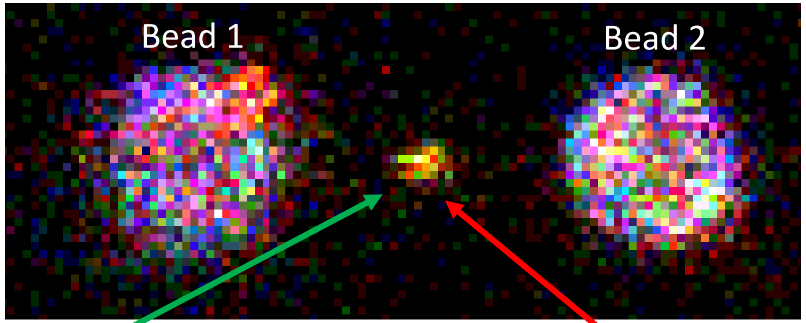


Marco Simonetta, PhD



Ligated product suspended in the C-trap

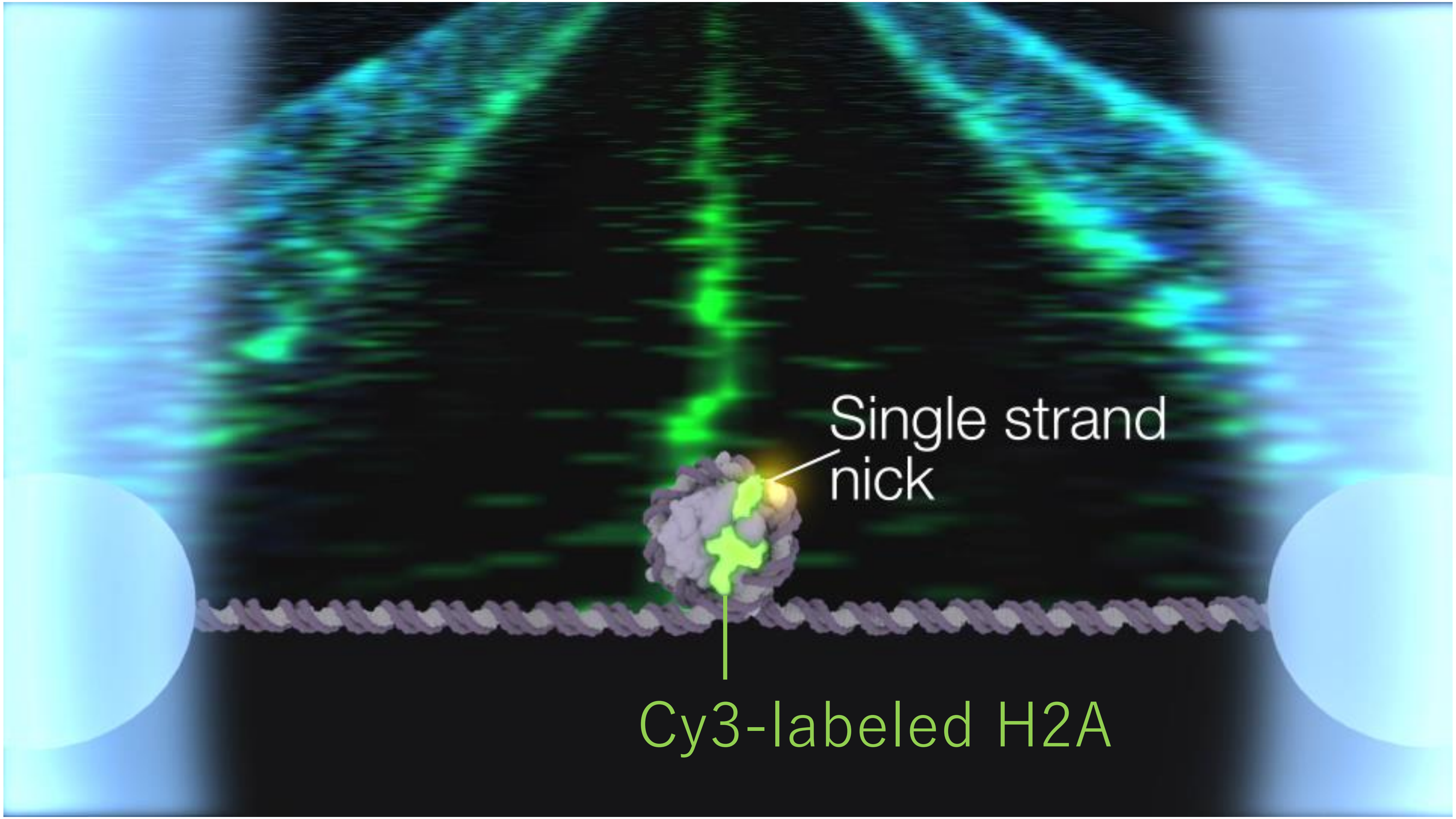
Bas Groen



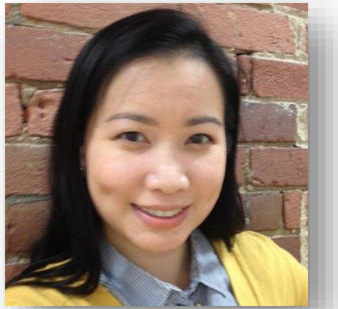
Cy3-labeled H2A nucleosome and Atto647 labeled 601 DNA attached to 6 kb handles

LUMICKS

Can YFP-PARP1 interact with a nick within a nucleosome?



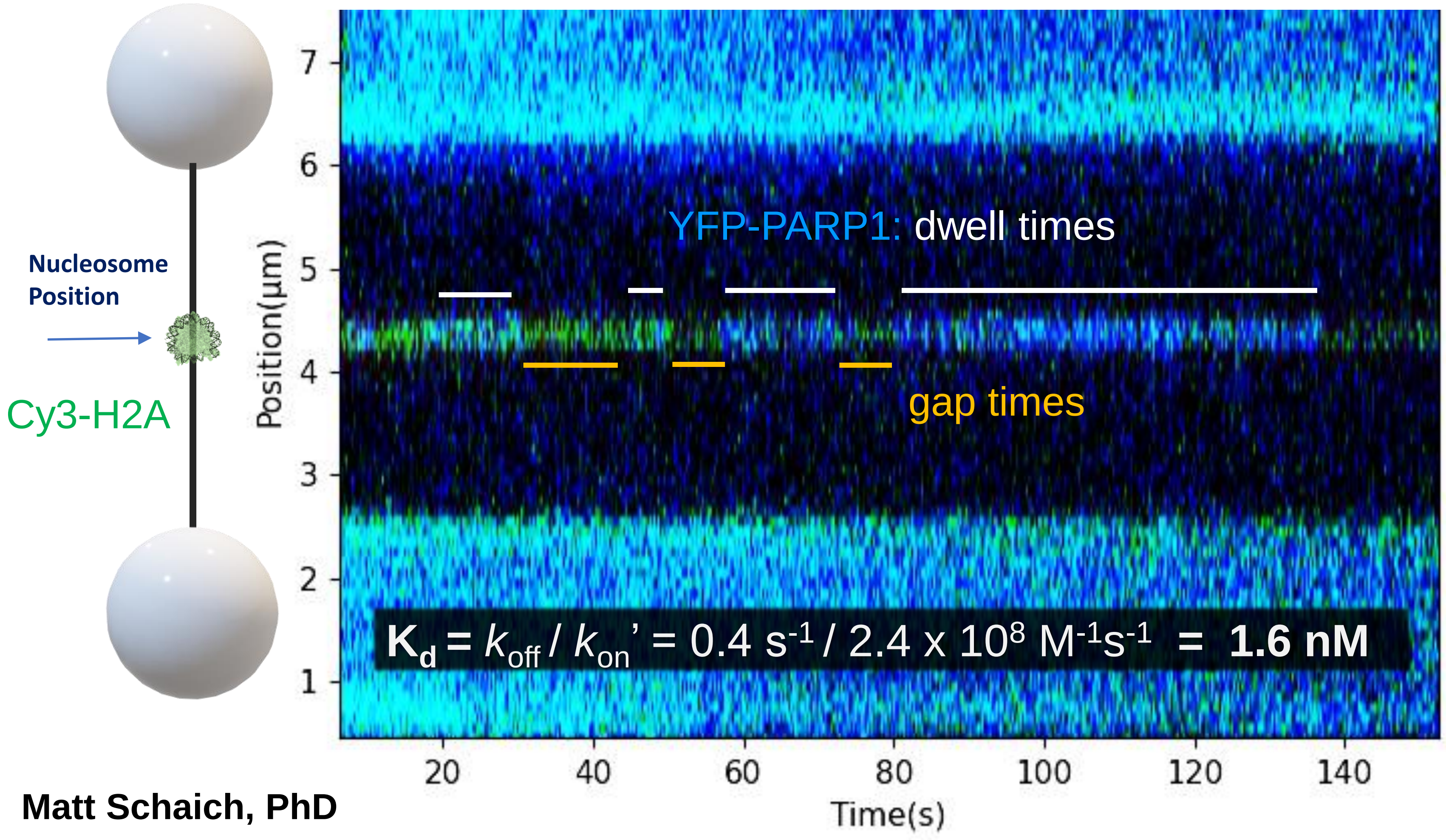
Janet Isawa PhD, Univ. of Utah



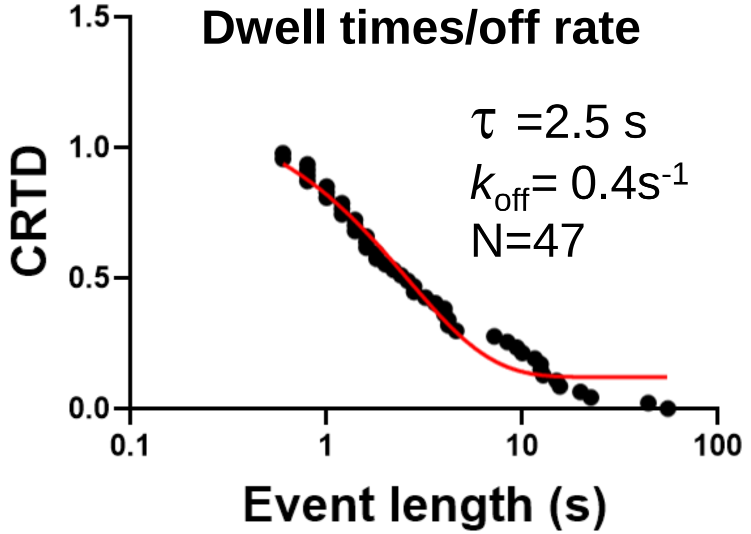
Grace Hsu

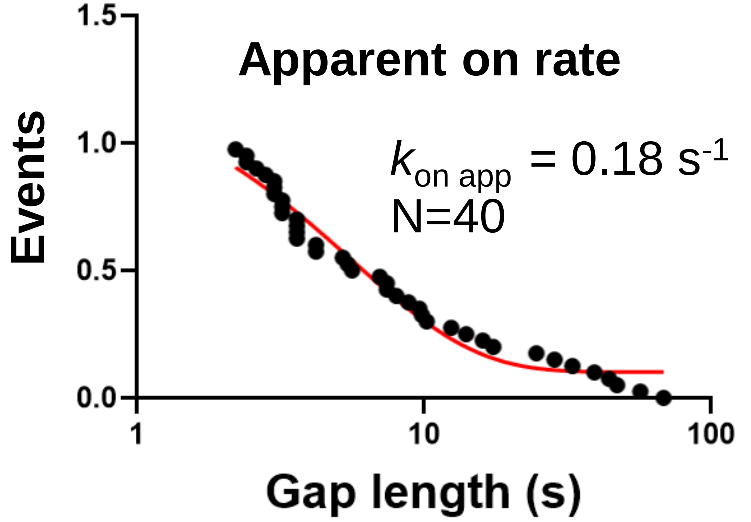
YFP-PARP1 binding events at SHL 0 location at 4 pN

Obtaining a K_d from on and off rates



Matt Schaich, PhD





YFP-PARP1 = 0.7 nM

k_{on}' = 2.4 x 10^8 $M^{-1}s^{-1}$

Similar to the on rate for DSB by Rudolph J, *et al* Luger K. *Elife.* 2018

PARP1:

Matt Schaich, PhD

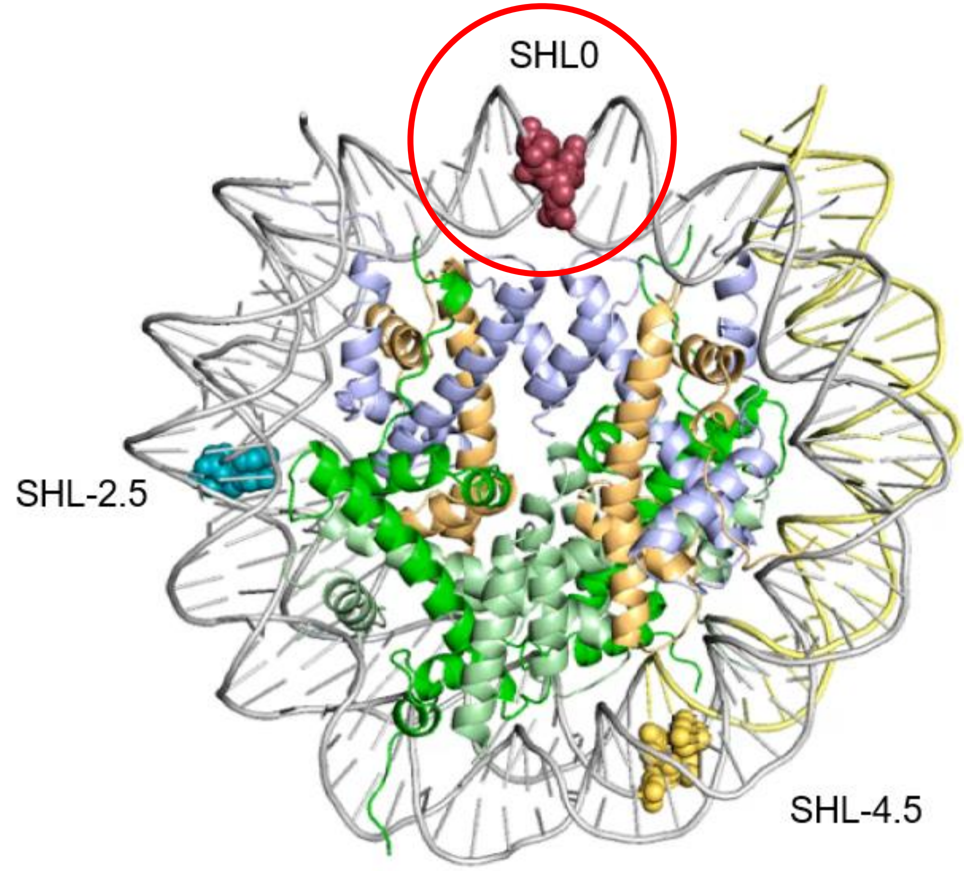
- Binding to undamaged nucleosome increases ~ 4-fold from 12.8 to 3.0 nM, at 4 pN and 10 pN respectively.
- Binding to naked nicked DNA has the highest affinity (0.9 nM).
- Binding to nicked nucleosomes (SHL0) at the dyad at 4 pN (1.6 nM) is 8-fold tighter than the undamaged nucleosome.

$$K_D \text{ (nM)} = k_{off}/k_{on}$$

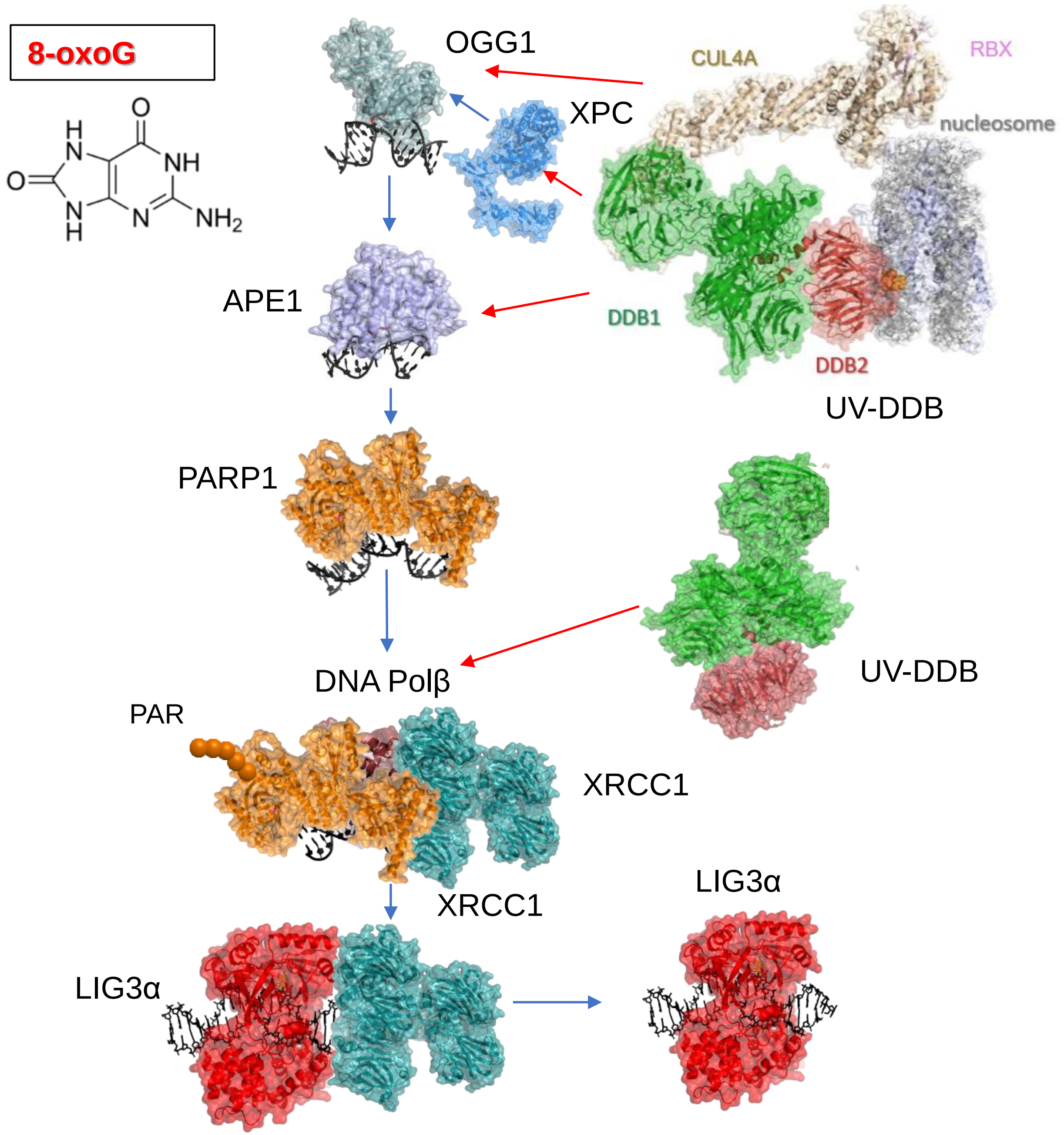
sec^{-1}
1/dwell time

M^{-1} sec^{-1}

SHL0/dyad



Base excision repair with the help of UV-DDB and XPC



Protein	Function	fluorescent tag N- or C-	Substrate	weighted corrected lifetime (S)
AAG	glycosylase BER	C-GFP	Hx	2.8
APE1	nicking BER	N-GFP	DNA nicks	0.3
APOBEC3A	deamination	N-GFP	ssDNA	6.0
DDB1	recog. NER	N-eGFP	UV	43.7
DDB2	recog. NER	N-Halo	UV	39.0
K244E		N-mNeonGreen	UV	27.2 (motile)
HPF1	modifying PARP activity	C-GFP	Nicked nucleosome + PARP1	27.5
LIG3	nick sealing BER	N-Halo	DNA nicks	5.4
ATP + Mg		N-Halo	DNA nicks	1.7
K421A		N-Halo	DNA nicks	1.7
ZNF1-BRCT		N-Halo	DNA nicks	11
ΔZNF1		N-Halo	DNA nicks	11.5
OGG1	glycosylase BER	C-GFP	8-oxoG	2.0
K249Q		C-GFP	8-oxoG	47.2
SPRTN-E112A	DPC removal	N-Halo	DPC	184.5
PARP1	nick recog. BER	N-Halo	DNA nicks	4.3
F44A		N-Halo	DNA nicks	8.3
ZNF1&2		N-Halo	DNA nicks	2.3
H862A/Y896A/E988A		N-Halo	DNA nicks	97.4
PARP2	BER	N-YFP	DNA nicks	11.7
Pol-β	gap filling BER	N-GFP	DNA nicks	2.0
TDG		C-Halo	nondamaged DNA	7.5 (motile)
WT		C-Halo	formyl-C	72.1
N140A		C-Halo	formyl-C	1.9
R275A		C-Halo	nondamaged DNA	2.8
R275L		C-Halo	nondamaged DNA	1.8
XPC	recog. NER	N-eGFP	UV	75.5 (motile)
ATP + Mg		N-Halo	[illegible]	[illegible]
XRCC1	nick sealing BER	C-YFP	DNA nicks	6.9

Goal: To observe the kinetics of all the intermediate steps in BER for these complexes

Nagpal, A. *et al.*, *Biochem. Society Tran.* (2022), Schaich et al., *NAR*, 2023.

Acknowledgements (1-4)

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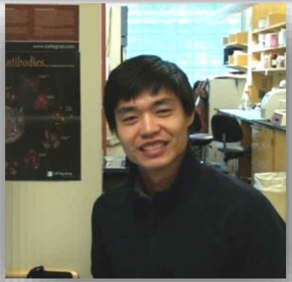
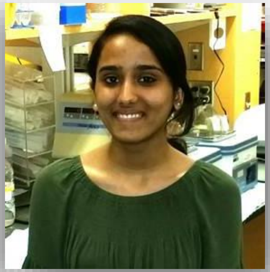
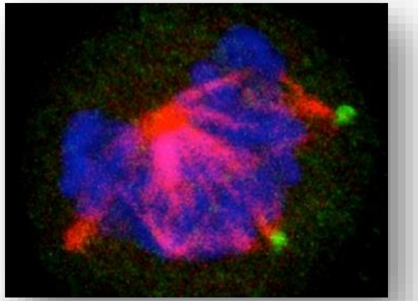





Sruti Shiva **Simon Watkins** **Claudette St Croix**

Patty Opresko
Elise Fouquerel
Ryan Barnes





Ed Burton
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Vlad Llin




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Jianjun He
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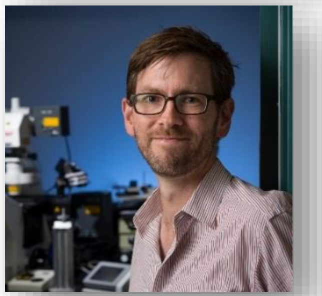
Dmytro Kolodieznyi

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Suo Tan
Zhou Zhong

University of Pittsburgh

UPMC | HILLMAN CANCER CENTER

Genome Stability Program

- DNA Pitt Crew -

Marcel Bruchez (CMU)
Elise Fouquerel
Sarah O'Melia
Patty Opresko/ Ryan Barnes
Vesna Rapic-Otrin

Positions available !

Pharmacology and Chemical Biology

mb+sb

NIH National Institute of Environmental Health Sciences

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LUMICKS

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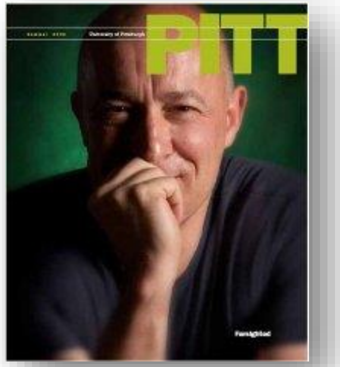

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Co-leader of the Genome Stability