



*Men occasionally stumble over the truth, but most of them pick themselves up and hurry off as if nothing happened.*

--- Winston Churchill

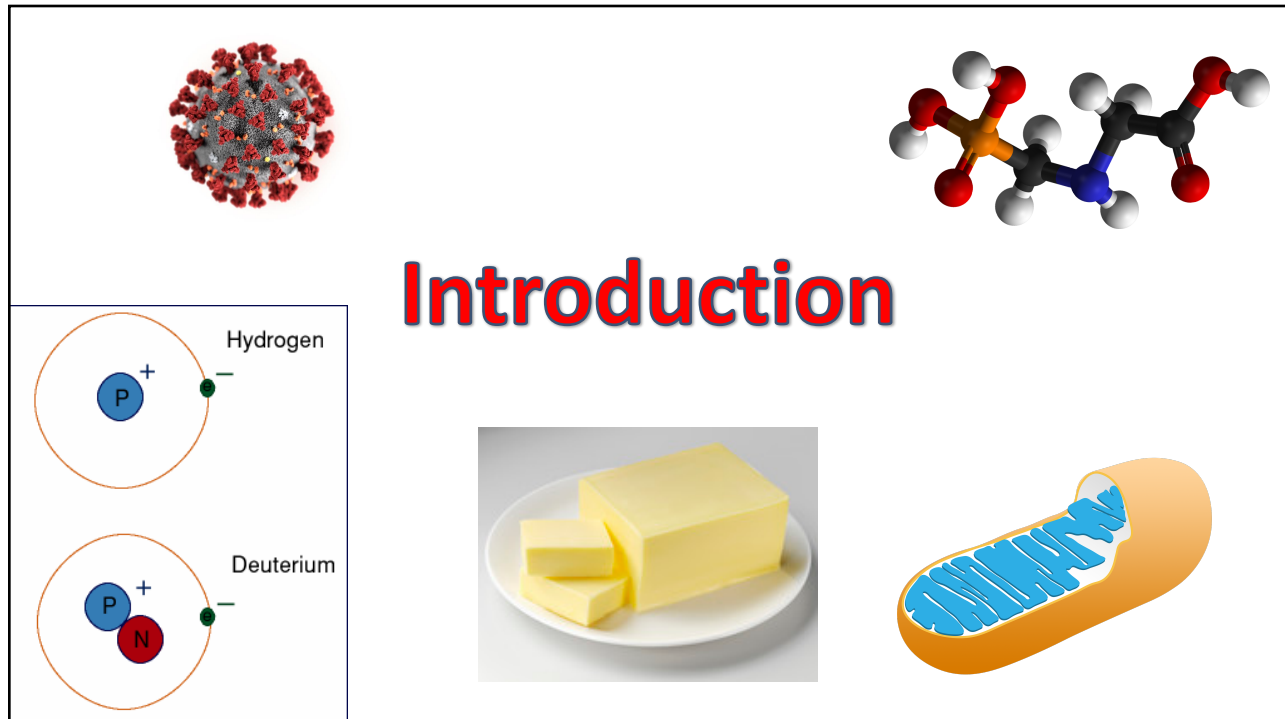
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## Outline

- Introduction
- NAD(P)(H) is Essential to Support Mitochondria
- Glyphosate, Glycine and Flavoproteins
- Lipid Pathways and Deuterium
- Prions and Neurodegenerative Disease
- SARS-CoV-2, Deuterium and Glyphosate
- Healthy Lifestyle
- Summary

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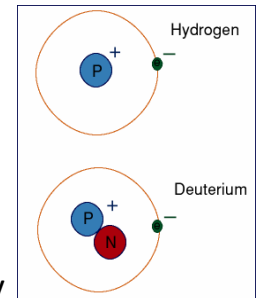


# Introduction

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## The Big Picture

- Deuterium is a natural rare isotope of hydrogen with distinct physical properties
  - Exposure to high concentrations of  $D_2O$  is life threatening
  - Living organisms have developed sophisticated strategies involving specialized enzymes to deal with it
- Mitochondria require low-deuterium water to function properly
- Choices in water and food consumption can influence deuterium exposure
- Glyphosate disrupts deuterium homeostasis, causing deuterium intoxication
- Hypothesis: prion proteins promote recovery from deuterium overload
- In severe reactions to COVID-19, a complex response ensues aimed at restoring mitochondrial health in immune cells

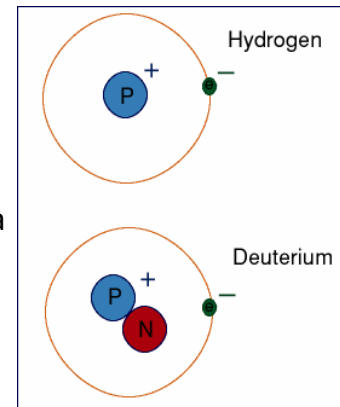


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## Deuterium = “Heavy” Hydrogen

- Hydrogen has one proton and one electron
- Deuterium has one proton, one electron and one neutron
  - ~ Twice as heavy as hydrogen
  - Present in ocean water at 155.8 ppm
  - Present in the blood at 6x the concentration of calcium
  - Has distinct physical and chemical properties compared to hydrogen
  - Deuterium disrupts the ATPase pumps in the mitochondria

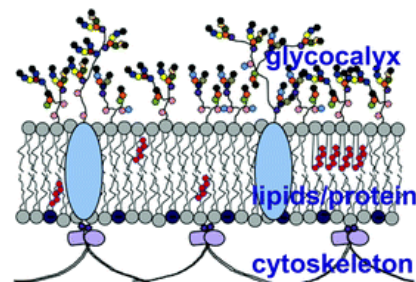
*Deuterium management in the body involves trapping deuterium in hydrogel and invoking specialized enzymes that choose hydrogen over deuterium for their reaction in order to fuel the mitochondria with hydrogen rather than deuterium*



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## Sulfate’s Critical Role for Maintaining Exclusion Zone Water\*

- The glycocalyx which lines blood vessels generates electricity to supply the cells
- (Hypothesis) The glycocalyx traps deuterium and extrudes D-depleted protons
  - Protons enter the cells along cytoskeletal “wires”
  - They fuel the mitochondrial intermembrane space
- Sulfate is crucial for maintaining gelled water in the glycocalyx
- Sulfated glycosaminoglycans (GAGs) become depleted in sulfate when chronically exposed to glyphosate



\*S Seneff and G Nigh. Water 2019; 11: 22-42.

<https://waterjournal.org/current-volume/seneff-summary/>

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## Ketogenic Diet and DDW: Health Benefits\*

- *Deuterium depleted water* (DDW) is essential for mitochondria to function properly
- People whose water supply is naturally depleted in deuterium are healthier
- Deuterium depletion maintains strong hydrogen bond networks in DNA (keeps it stable)
- DDW inhibits tumor progression
- A ketogenic diet is a deuterium-depleted diet



\*Laszlo G Boros et al. Medical Hypotheses 2016; 87: 69-74.

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## "The Pathologic Anatomy of Deuterium Intoxication"\*

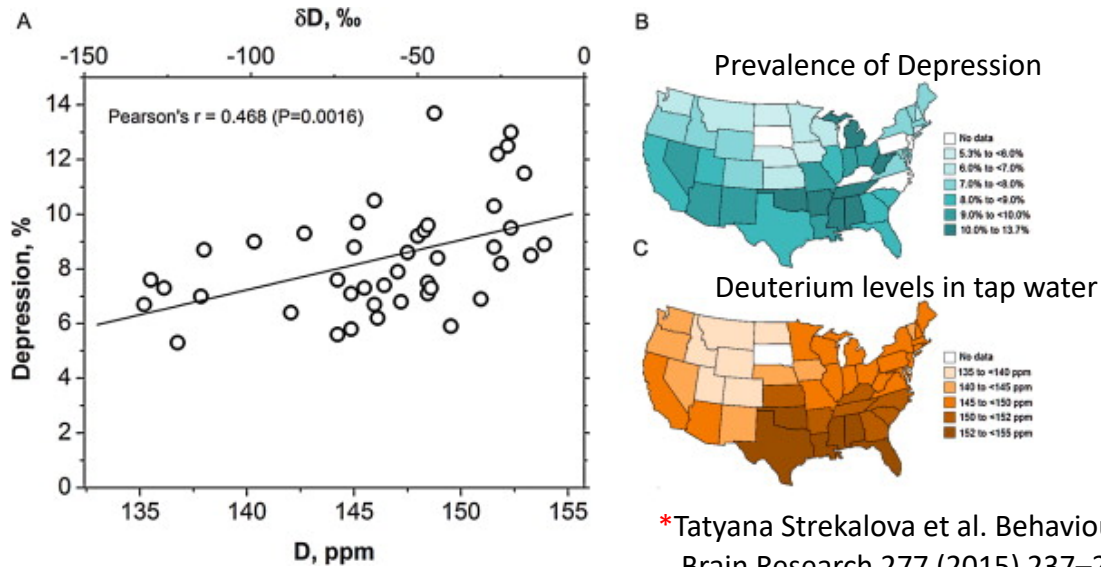
- Mice received 5% dextrose solution in D<sub>2</sub>O water
- "Their appetites were voracious, and constant savage attacks upon each other were continually in progress."
- "Eventually, at varying intervals, the animals became hypoactive, lethargic, and dramatically weak."
- "Death occurred in all cases within 6-10 days."



\*Paul Bachner et al. PNAS 1964; 51: 464-471.

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## Deuterium in Water and Depression\*



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## Deuterium Levels are Variable in Fluids\*

- Deuterium in Antarctic glacier water is only 89 ppm (compared to 155 in sea water).
  - People who get their water from glaciers live longer
- Deuterium is higher in rainwater at the equator than at the poles
  - Due to higher evaporation off of descending water droplets
- Deuterium levels are different in different body fluids:
  - Saliva > Blood > Breast milk
  - Salivary glands selectively secrete deuterium
  - Breast milk is a low-deuterium nutrient

\*Alexander Basov et al. Nutrients 2019; 11: 1903.

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## Glyphosate: The Big Picture

- Glyphosate is the active ingredient in the herbicide Roundup
  - Pervasive in the food supply
  - Considered to be safe for humans but there is increasing doubt that this is true
- Glyphosate kills weeds by suppressing an enzyme in the shikimate pathway called EPSP synthase
  - *Our gut bacteria use this enzyme to make essential aromatic amino acids for the host*
- There are strong correlations between the rise in glyphosate usage in the US and the rise in multiple neurological, oncological, autoimmune and metabolic diseases\*

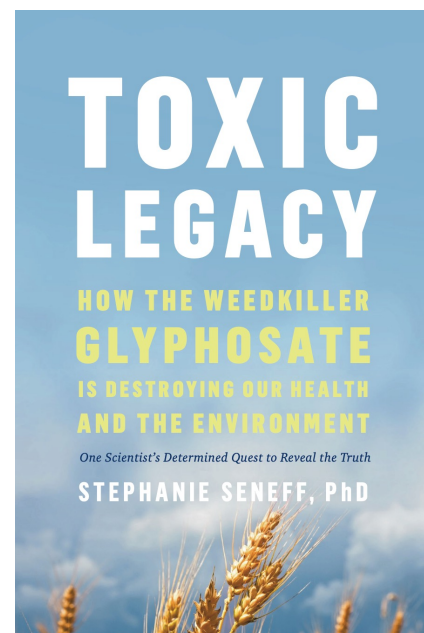


\*NL Swanson et al. J Org Systems 2014; 9: 6-37.

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## My New Book!

- Released by Chelsea Green in July 2021
- Presents extensive data on glyphosate toxicity to animals and humans
- Shows how glyphosate interferes with sulfate homeostasis
- Argues that glyphosate is insidiously, cumulatively toxic through its diabolical insertion into proteins by mistake in place of the coding amino acid glycine
  - This unique feature explains why it is causal in so many diseases



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# NAD(P)(H) is Essential to Support Mitochondria

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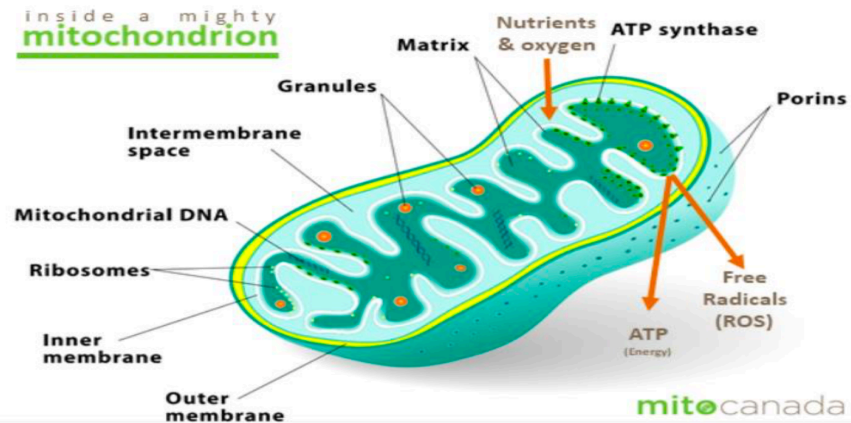
## The Big Picture

- NAD (Nicotinamide Adenine Dinucleotide) is an essential cofactor in oxidation/reduction reactions
  - It exists in four forms: with and without hydrogens and with and without phosphate

NAD <sup>+</sup>	(no H, no P)	The enzymes that supply NAD <sup>+</sup> and NADP <sup>+</sup> with H are specially designed to avoid deuterium
NADH	(with H, no P)	
NADP <sup>+</sup>	(no H, with P)	
NADPH	(with H, with P)	
- Mitochondria are organelles inside cells that supply energy in the form of ATP
- Mitochondria depend critically on a proton-ATPase pump to make ATP
  - Protons derived from NADH and NADPH are pumped into the intermembrane space
- Deuterium “gums up” the pump (like sugar in the gas tank)
- Glyphosate interferes with the supply of NADH and NADPH to the organism

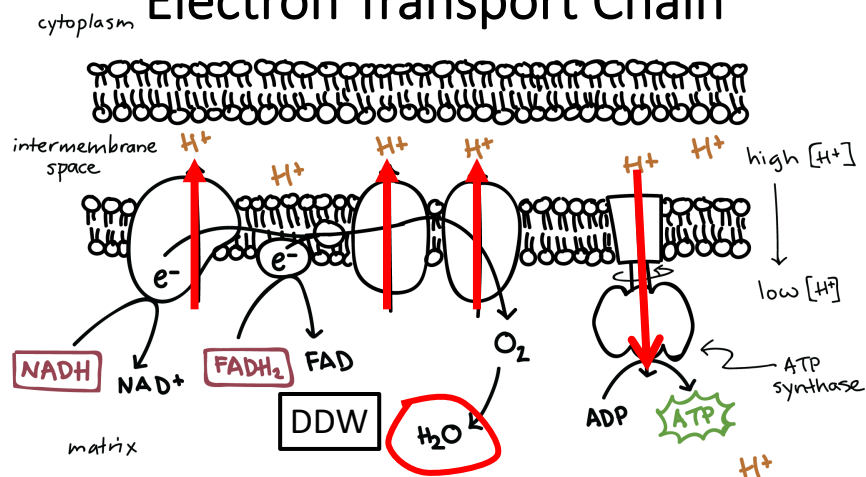
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## A Mitochondrion



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## Electron Transport Chain

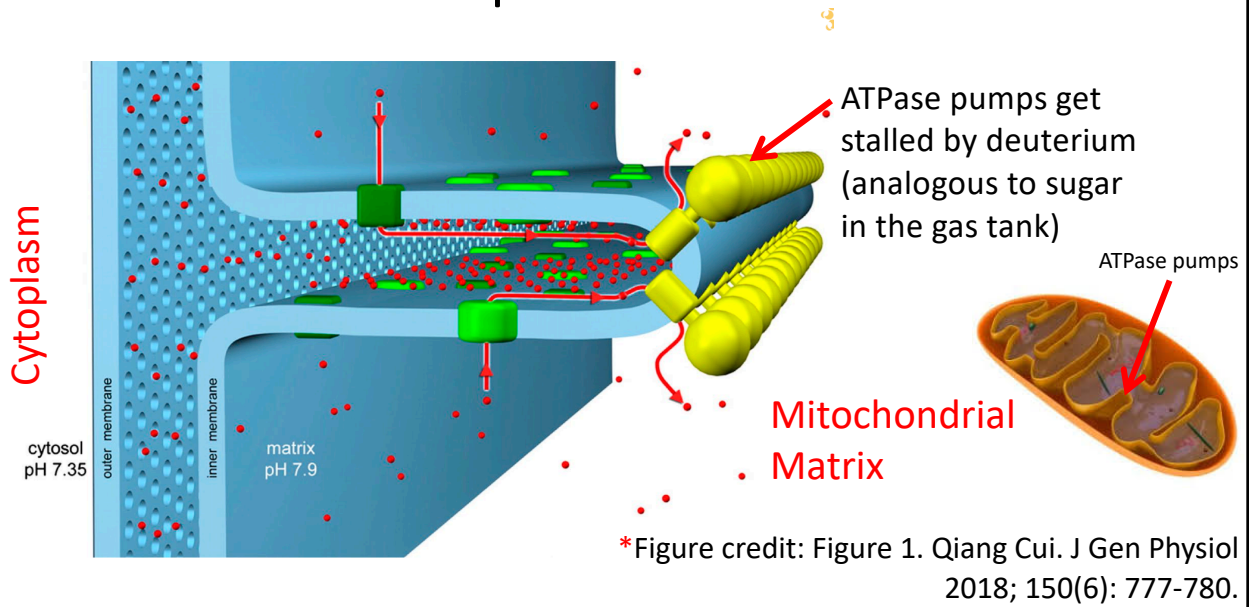


Protons from NADH and  $FADH_2$  are pumped across the membrane and then flow back through the ATPase pump, reacting with oxygen to form deuterium-depleted "metabolic water"

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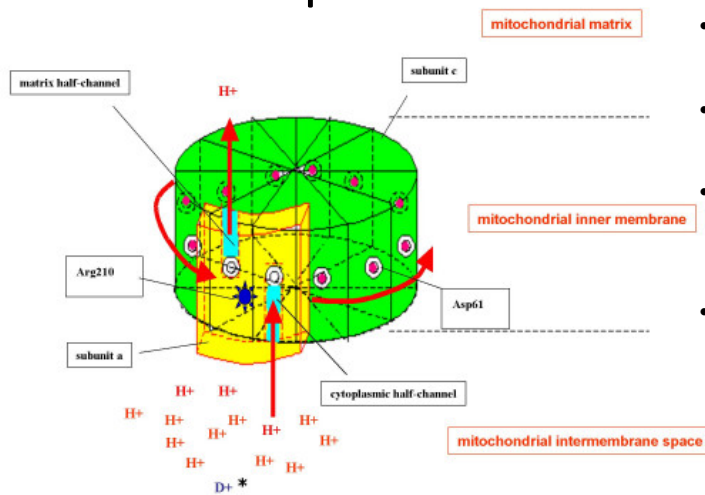


## ATPase Proton Pump\*



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## Deuterons Disrupt the ATPase Pump\*



- There are around 15,000 ATPase pumps in a single mitochondrion
- Proton force rotates the ATPase pumps at a rate of 1,000 cycles per second
- Deuterons resist letting go and stall the pump, producing a stutter
- Deuterons also disrupt proton-coupled electron transport (PCET) which is based on proton tunneling
- This causes decreased production of ATP and increased production of reactive oxygen species, damaging the pumps.

\*Abdullah Olgun. Theoretical Biology and Medical Modelling 2007, 4: 9.

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## G6PD & Fatty Acids\*

- Glucose 6 phosphate dehydrogenase (G6PD) converts  $\text{NADP}^+$  to  $\text{NADPH}$  (has strong preference for **H** over **D**)\*\*
- $\text{NADPH}$  is the largest source of hydrogen in fatty acid biosynthesis
- E coli and Bacillus subtilis produce *depleted fatty acids (FAs)*
  - Due to high dependency on G6PD to restore H in  $\text{NADPH}$



*Photosynthetic organisms (e.g., algae (chlorella)) produce highly deuterium-depleted FAs*

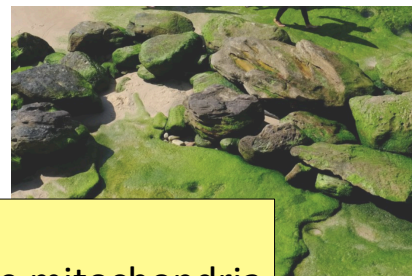
- \*RS Wijker et al. PNAS 2019; 116(25): 12173-12182.
- \*\*Xinning Zhang et al. PNAS 2009; 106: 12580-12586.



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  - E coli and Bacillus subtilis produce *depleted fatty acids (FAs)*
    - Due to high dependency on G6PD to restore H in  $\text{NADPH}$
- Fatty acids are a preferred fuel for the mitochondria because they are deuterium depleted



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- \*RS Wijker et al. PNAS 2019; 116(25): 12173-12182.
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# Glyphosate, Glycine and Flavoproteins

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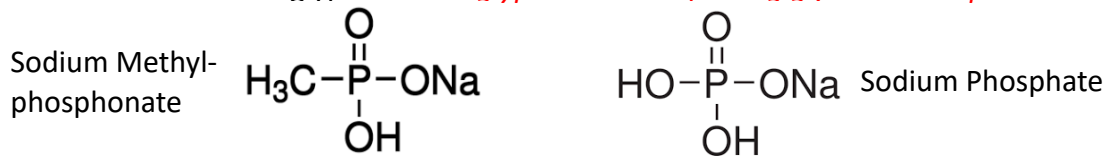
## The Big Picture

- Flavoproteins are an important class of proteins that transfer hydrogens to and from NAD(P)H using flavins (FAD, FMN) as intermediaries
- Glyphosate's disruption of the shikimate pathway in gut microbes leads to deficiencies in tryptophan – a precursor to NAD
  - Tryptophan is also a precursor to serotonin, the “feel good” hormone
  - Serotonin deficiency is linked to depression, obesity and violent behavior
  - Both glyphosate and excess deuterium deplete serotonin and cause depression
- My research strongly suggests that glyphosate suppresses enzymes that bind phosphate
  - NAD(P)(H), FAD and FMN all contain multiple phosphate anions
- Flavoproteins contain essential glycine residues at all the sites where they bind phosphate
  - Tremendous susceptibility to glyphosate's mischief through glycine substitution

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## Hypothesis: Glyphosate Disrupts Proteins that Bind Phosphate\*

- Glyphosate kills plants by suppressing EPSP synthase
- Glyphosate blocks EPSP synthase binding to the phosphate in PEP
- Glyphosate is a glycine molecule with a methylphosphonate unit attached to the nitrogen atom
- The binding site for PEP has a highly conserved glycine residue
  - If this glycine is swapped out for alanine, the enzyme becomes completely insensitive to glyphosate → *glyphosate is displacing glycine in the protein??*

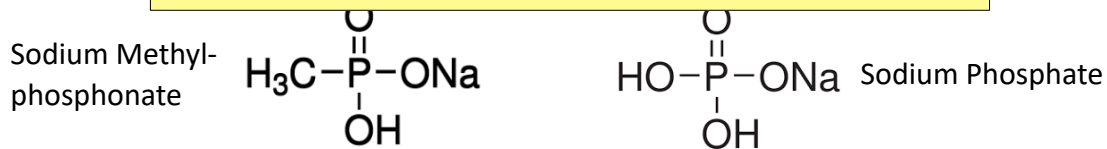


\*S Gunatilake et al., Int J Environ Res Public Health 2019; 16(15): 2734.

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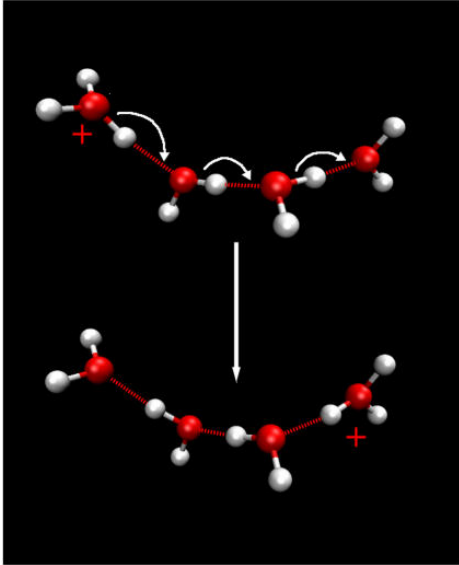
- Glyphosate kills plants by suppressing EPSP synthase
  - Glyph
  - Glyph
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    - If t
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- Glyphosate's methylphosphonate unit fits nicely in the pocket that is reserved for phosphate, blocking phosphate binding.



\*S Gunatilake et al., Int J Environ Res Public Health 2019; 16(15): 2734.

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## Grotthuss Effect: Water Wires

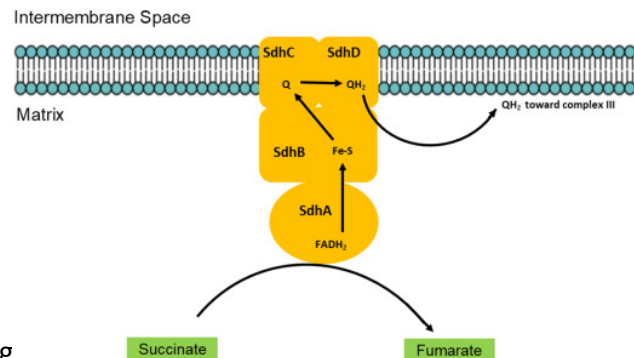


- A few water molecules settle into the reaction center and facilitate proton transfer
  - Deuterium is a reluctant participant
- This assures that a proton rather than a deuteron ends up in the reaction product
- Flavoproteins and many other enzymes take advantage of this effect to select hydrogen over deuterium
- *Flavin-binding is essential for this to work*

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## Succinate Dehydrogenase

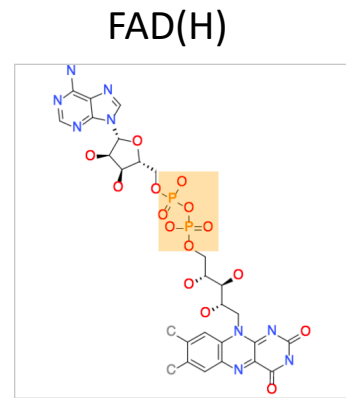
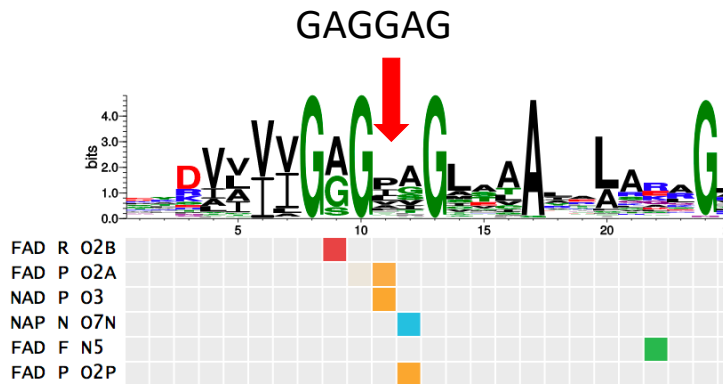
- Succinate dehydrogenase (Complex II) is the only enzyme in the mitochondria that is intimately involved in both the citric acid cycle and oxidative phosphorylation
- It is a flavoprotein (binds FAD)
- It extracts two protons from succinate and embeds them in the membrane (by converting ubiquinone to ubiquinol (coenzyme Q10))
- Genetic mutations in succinate dehydrogenase are associated with multiple cancers:
  - E.g., neuroblastoma, breast cancer, colon cancer, renal cancer, melanoma and uterine cancer, prostate cancer, endometrial cancer, bladder cancer, and gastrointestinal stromal tumor



\*Marianne Dubard Gault et al., Cold Spring Harb Mol Case Stud. 2018 Aug; 4(4): a002584.

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## Succinate Dehydrogenase: GxGGxG Motif\*



Binds FAD as a cofactor to catalyze electron transport in the mitochondria

\*<http://nbd.bii.a-star.edu.sg/search>

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## Glyphosate Suppresses Succinate Dehydrogenase

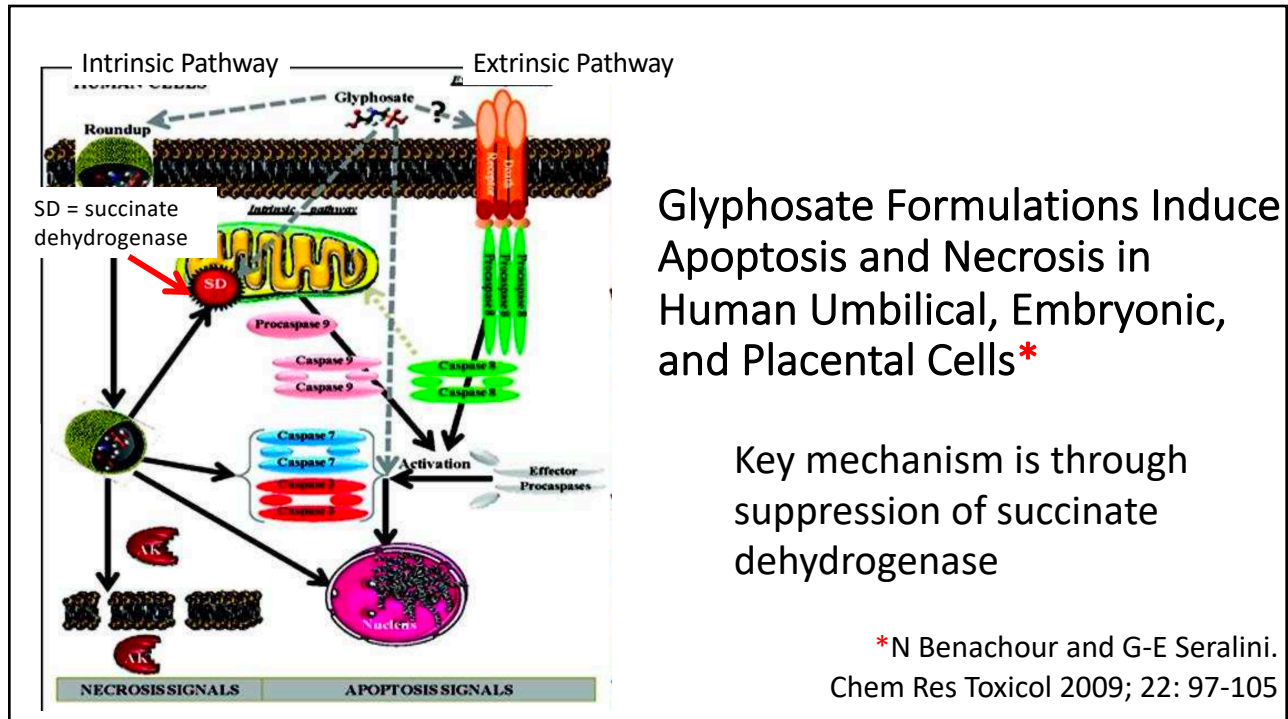
- Study on glyphosate's effects on E. coli proteins found that glyphosate significantly suppressed (by 3-4-fold) three different components of succinate dehydrogenase complex\*
- In vitro study on rat liver mitochondria in isolation exposed to Roundup and glyphosate alone\*\*
  - Roundup significantly suppressed succinate dehydrogenase although glyphosate did not – authors proposed that surfactants in Roundup enabled glyphosate transport across mitochondrial membranes
- Analysis of mechanism of glyphosate suppression suggested it disrupted *binding of succinate dehydrogenase to FAD\*\*\**

\*W Lu et al., Mol. Biosys. 9 (2013) 522–530.

\*\*Francisco Peixoto Chemosphere 61 (2005) 1115-1122.

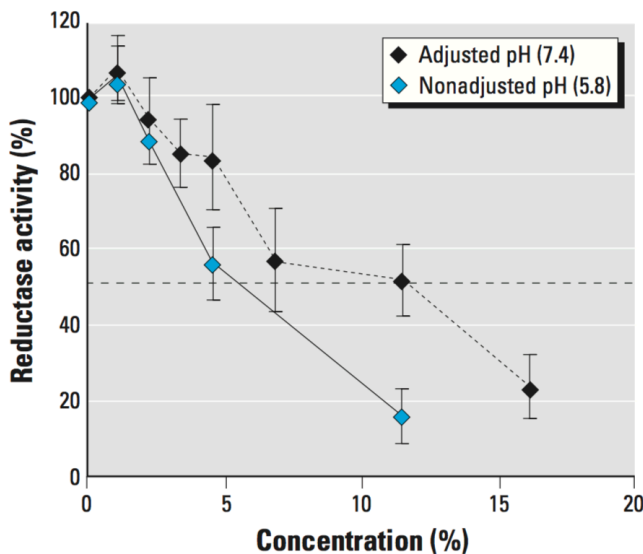
\*\*\*R Ugarte. Computational Theor. Chem. 1043 (2014) 54–63.

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## Glyphosate Suppresses NADPH Reductase\*



This enzyme supplies NADPH to the enzyme aromatase that converts testosterone to estrogen and to many other enzymes involved in steroid metabolism

\*Sophie Richard et al. Environmental Health Perspectives 2005; 113(6): 716-720.

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Besides succinate dehydrogenase and NADPH reductase, other phosphate-binding enzymes that glyphosate has been shown to suppress include cytochrome P450 enzymes, NAD transhydrogenase (transfers hydrogen from NAD to NADP), G6PD and RuBisCo (in plants, the most common enzyme in the world)

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## **Lipid Pathways and Deuterium**

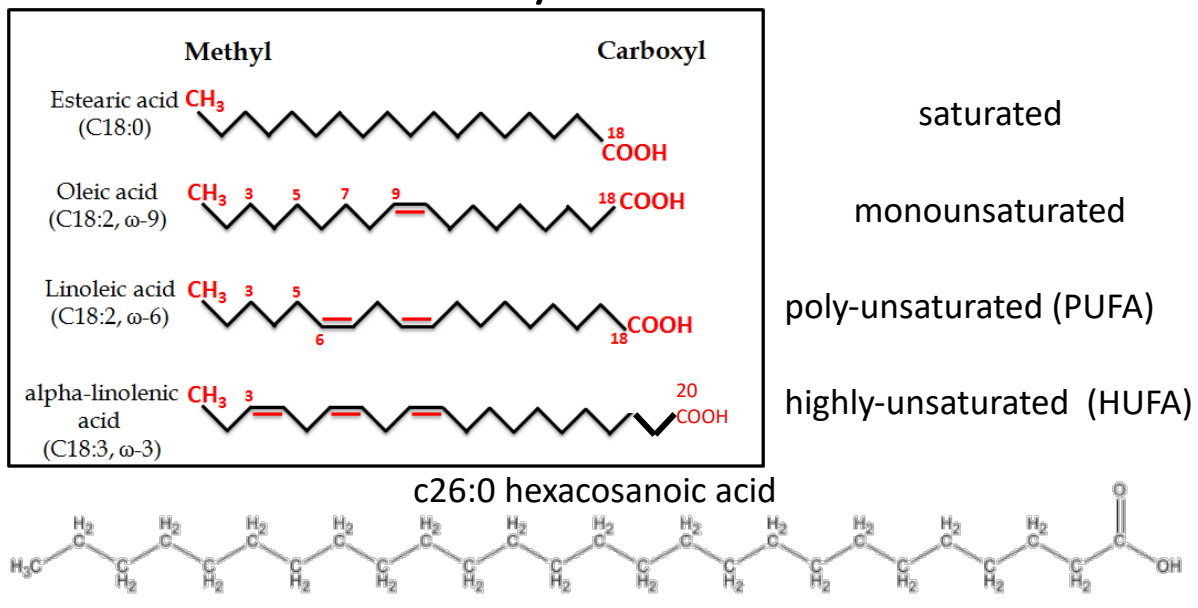
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## The Big Picture

- The synthesis of long chain polyunsaturated fatty acids involves a series of reactions catalyzed by elongases and desaturases
- Longer chains in the membrane protect from hydrogen leakage
- Membrane lipids are released under stress conditions
- Released membrane lipids can be processed through three primary pathways
  - CYP-enzyme-based production of *endogenous cannabinoids*
  - Lipoxygenase-based production of *leukotrienes*
  - Cyclooxygenase-based production of *prostaglandins*
- The diverse products of lipid modifications are powerful signaling molecules
- *Most, if not all, of these pathways produce deuterium-depleted water, which might be the primary product*
- *Glyphosate likely disrupts many of these pathways*

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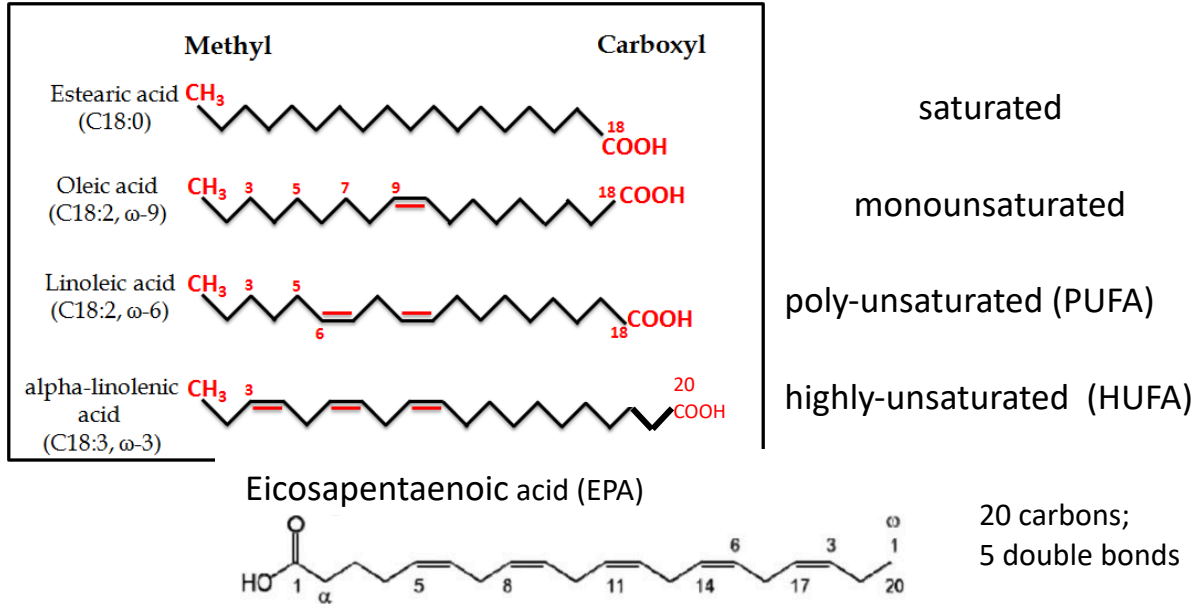
## A Quick Tutorial on Fatty Acids



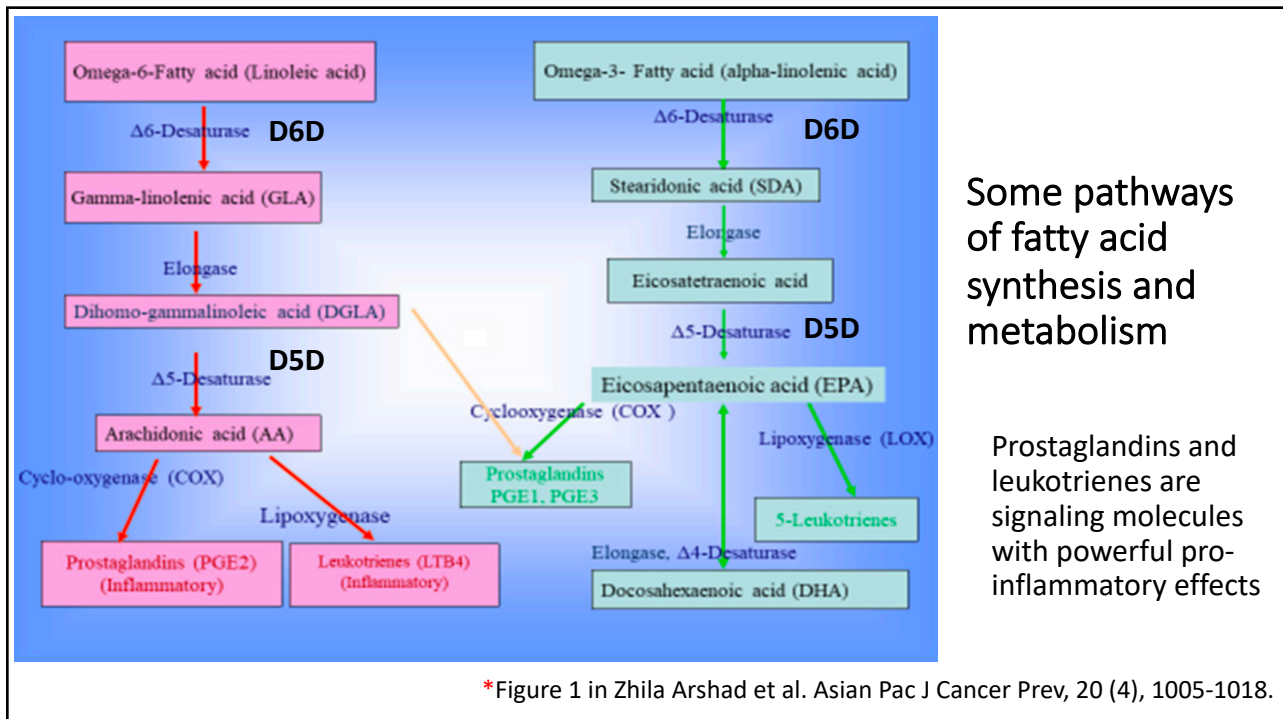
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# A Quick Tutorial on Fatty Acids



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## “Inflammation, Cancer and Oxidative Lipoyxygenase Activity are Intimately Linked”\*

- Lipoyxygenases oxidize arachidonic acid (AA) and its derivatives producing leukotrienes (pro-inflammatory) and ultimately lipoxins (anti-inflammatory)
- Leukotrienes activate NF-κB pathway and TNF-alpha expression (inflammatory signals)
- 15-LOX is highly expressed in white blood cells
- Over-expression of lipoyxygenases has been implicated in many human acute and chronic inflammatory diseases such as asthma, atherosclerosis, rheumatoid arthritis, inflammatory bowel diseases, dermatitis, and cancer
- Leukotriene LTB4 is a potential stimulator for cancer cell growth, and it also plays a role in the formation of reactive oxygen species in response to hypoxia

\*Rosalina Wisastra and Frankj J Dekker. Cancers 2014, 6, 1500-1521.

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Lipoyxygenase has a fantastic ability to select hydrogen over deuterium despite not being a flavoprotein\*

\*Pengfei Li et al. J Phys Chem Lett 2018; 9(22): 6444-6449.

\*Rosalina Wisastra and Frankj J Dekker. Cancers 2014, 6, 1500-1521.

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## “The Endocannabinoid System: A Target for Cancer Treatment”\*

- Cannabinoid receptor (CB-R) agonists inhibit cancer cell proliferation through various receptor-mediated mechanisms
  - Slowed growth of Lewis lung adenocarcinoma in culture and in a mouse model after oral administration
- Many types of cancer cells upregulate cannabinoid receptor expression
- Cannabinoids suppress metastasis and angiogenesis
- In HER2-overexpressing breast cancer cells, CBD arrested cancer cell proliferation in vitro and in vivo by inhibiting Akt and ERK signaling
- Receptor activation locks cell cycle at the G1/S phase and at the G2/M phase
- Anti-tumor properties have also been seen in pancreatic cancers



\*Chiara Laezza et al. Int. J. Mol. Sci. 2020, 21, 747.

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## Prions and Neurodegenerative Disease

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## Some Mysteries

- Several “prion-like” proteins expressed in neurons are linked to neurodegenerative disease (amyloid beta, alpha-synuclein, TDP-43, FUS, the prion protein (PrP), tau, ...)
- Researchers have been unable to figure out what is the toxic form of these prion-like proteins (soluble oligomers? Insoluble fibrils in plaque and Lewy bodies?) and, more importantly, how they induce toxicity
  - Impairments may even arise from a *loss-of-function* effect
- Researchers have also been unable to figure out what these proteins actually *do*, even though it is established that mutations in most of them are embryologically lethal

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## The Big Picture (Hypothesis)

- Do prion-like proteins play a role in maintaining low deuterium in the cytoplasm??
  - **Hypothesis:** These proteins can trap deuterium inside water-inaccessible precipitates

### **Bold claims:**

- Misfolded proteins in Alzheimer’s plaque or Lewy bodies are a sign of impaired deuterium homeostasis
- Prion diseases begin with stressed immune cells in the gut and spleen
- Cells with impaired ability to clear misfolded proteins release them inside exosomes and deliver them to other cells, propagating the disease process
- Impaired sulfate homeostasis plays a significant role in the pathology

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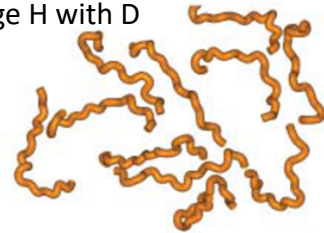
## “Structural Organization of Brain-derived Mammalian Prions as Probed by Hydrogen Exchange”\*

“The H/D exchange method exploits the rapid exchange of backbone amide hydrogens within the unstructured regions of proteins compared to relatively slow exchange of those involved in systematically H-bonded structures such as  $\beta$ -sheets or  $\alpha$ -helices.”

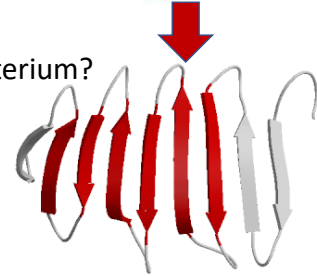
Prion protein gathers deuterium from the medium and traps it within  $\beta$ -sheets when there is too much?

\*V Smirnovas et al. Nat Struct Mol Biol 2011; 18(4): 504-506.

Unstructured:  
Exchange H with D

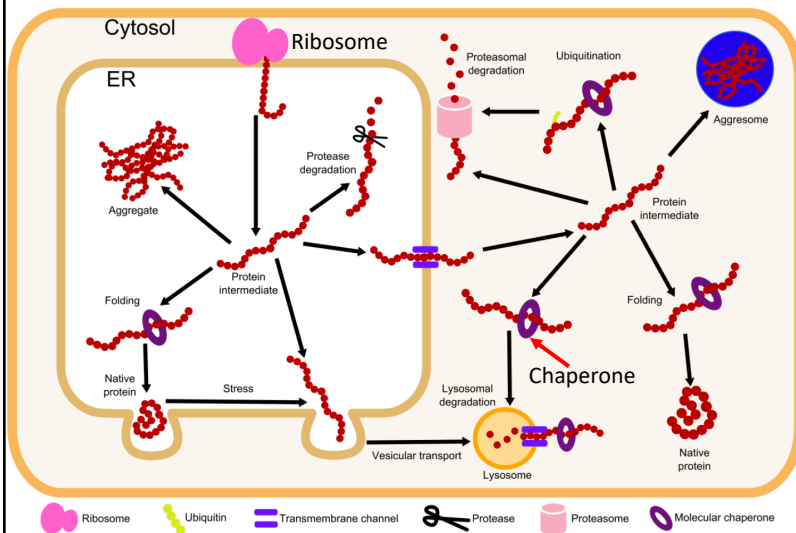


$\beta$ -sheets:  
Trap deuterium?



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## Protein Misfolding\*

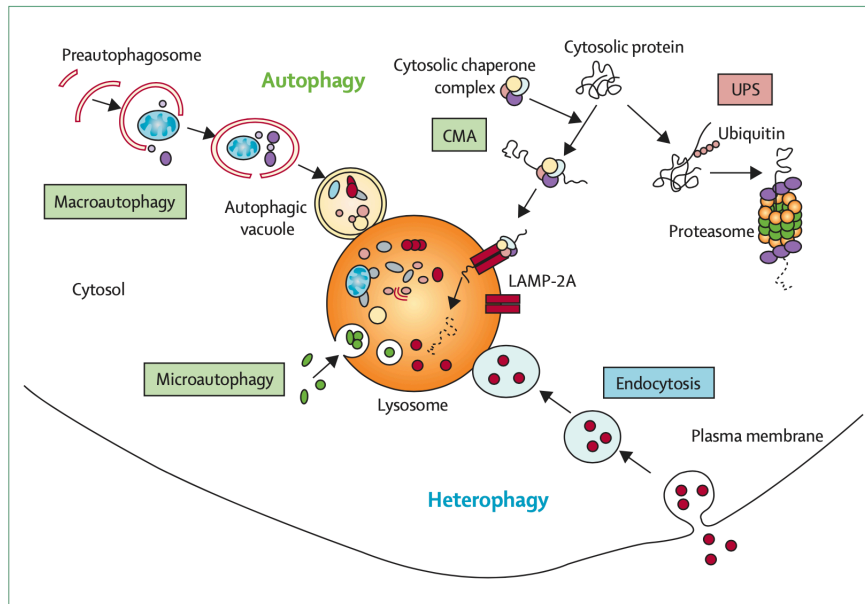


- Proteins synthesized in the endoplasmic reticulum (ER) can fold incorrectly
- Molecular chaperones carry misfolded proteins to the lysosome or proteasome for clearance
- Insoluble aggregates accumulate when these processes are impaired

\*Figure 1. Dezerae Cox et al. Biochim Biophys Acta 2014;1842(9):1830-43.

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## How “damaged” proteins are cleared\*



- Lysosome:
  - Macroautophagy
  - Microautophagy
  - Endocytosis
- Proteasome:
  - Ubiquitin-mediated protein degradation

\*Figure 1. Marta Martinez-Vicente and Ana Maria Cuervo. Lancet Neurol 2007; 6: 352-61.

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## “Amyloid proteotoxicity initiates an inflammatory response blocked by cannabinoids”\*

- Amyloid- $\beta$  ( $A\beta$ ) is the protein that misfolds and accumulates as plaques in the brain in association with Alzheimer’s disease
- Intraneuronal  $A\beta$  increases production of leukotrienes by 5-lipoxygenase (5-LOX) ( $\rightarrow$  *deuterium-depleted water!*)
- Leukotrienes increase  $A\beta$  accumulation (positive feedback loop) and nerve cell death through production of proinflammatory cytokines
- Cannabinoids reduce intraneuronal  $A\beta$  accumulation and improve memory (*possibly by catalyzing flavoproteins*)

\*Antonio Currai et al. *npj Aging Mech Dis* 2016; 2: 16012.

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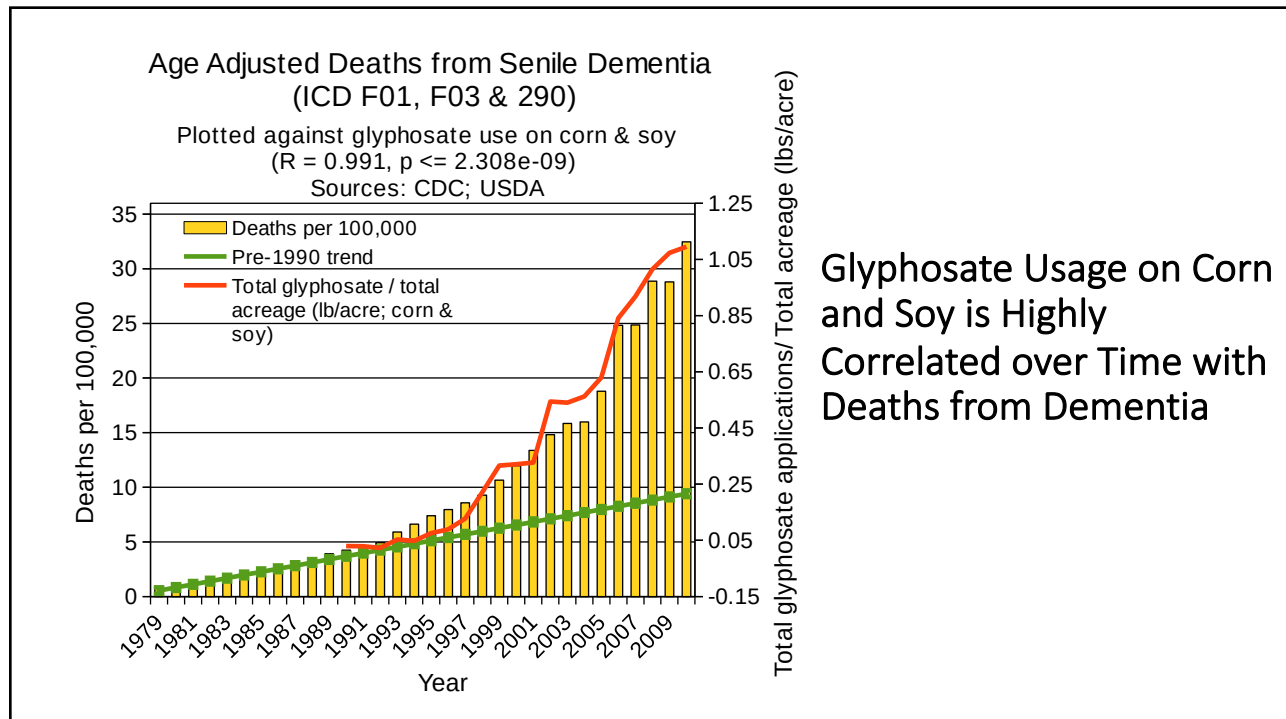
“Amyloid proteotoxicity initiates an inflammatory response blocked by cannabinoids”\*

- Amyloid-β (Aβ) initiates an inflammatory response in the brain
- Intraneuronal Aβ accumulation leads to neuroinflammation and neurodegeneration
- Leukocytes and nerve cells release pro-inflammatory cytokines
- Cannabinoids reduce intraneuronal Aβ accumulation and improve memory (possibly by catalyzing flavoproteins)

The endogenous cannabinoids derived from arachidonic acid would do the same thing, but the CYP enzymes that are needed to synthesize them are blocked by glyphosate and other toxic environmental chemicals

\*Antonio Currai et al. snpj Aging Mech Dis 2016; 2: 16012.

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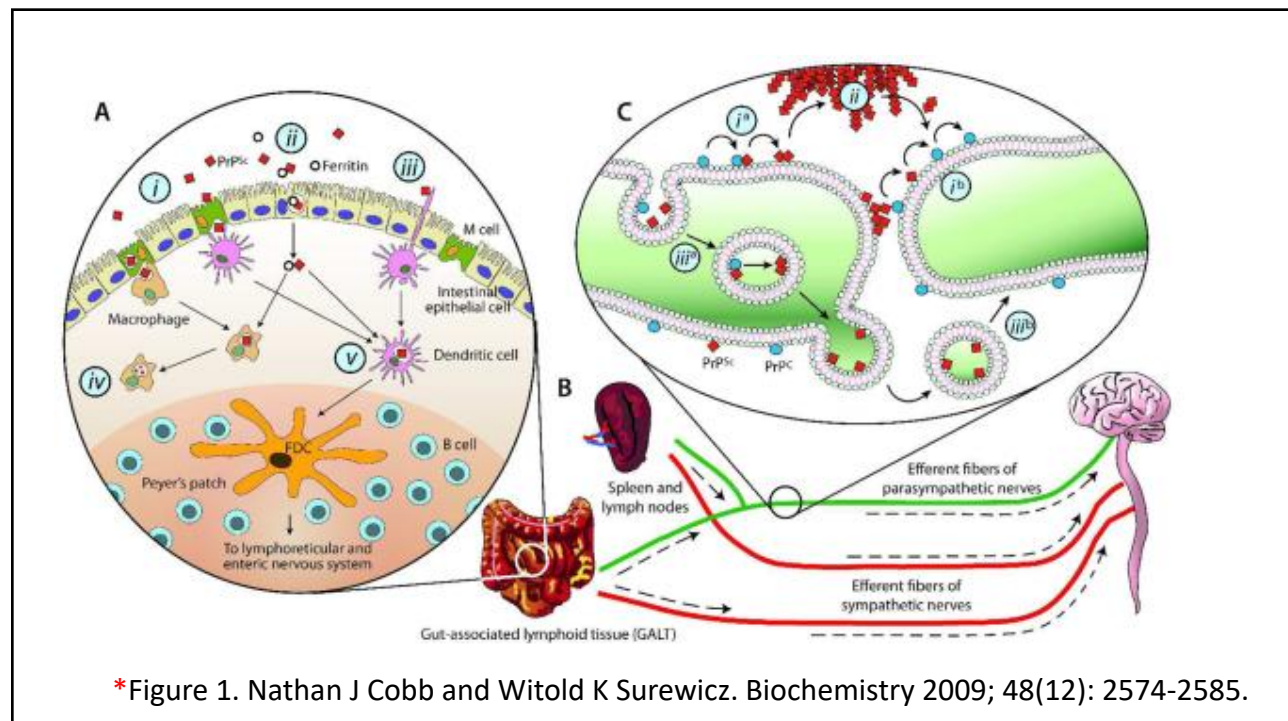
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## "Prion Diseases and their Biochemical Mechanisms"\*

- Creutzfeldt Jakob Disease is the human form of Madcow
  - Neurodegenerative disorder caused by prion protein misfolding
- Ingested prions can seed the disease process
  - Taken up by dendritic cells and carried into the gut-associated lymphoid tissue (GALT) and the spleen
  - Act like a crystal to induce further misfolding of human prion proteins
- Exosomes are released by the dendritic cells in the GALT and spleen, and they travel along retrograde nerve fibers (splanchnic nerve and vagus nerve) to the brain stem
- Neurons in the brain take up the misfolded proteins which bind to prion proteins in the brain and induce further misfolding

\*Nathan J Cobb and Witold K Surewicz. Biochemistry 2009; 48(12): 2574-2585.

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\*Figure 1. Nathan J Cobb and Witold K Surewicz. Biochemistry 2009; 48(12): 2574-2585.

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## “Deuterium Isotope Effects on Lymphoid Tissues and Humoral Antibody Responses in Mice”\*

- Mice were exposed to water enriched with 30% D<sub>2</sub>O
- Deuteration resulted in depression of antibody response to a toxin

“A particularly striking weight loss of the thymus and spleen, and a marked systemic lymphocyte depletion were apparent, particularly in the thymus, white pulp of the spleen, lymph nodes, and gut-associated lymphoid tissue (GALT)”

The spleen was especially sensitive to the deuterium compared to all other organs

\*JA Laissue and RD Stone. Virchows Arch. A Path Anat and Histol 1979; 383: 149-166.

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- “A particularly striking weight loss of the thymus and spleen, and a marked systemic lymphocyte depletion were apparent, particularly in the thymus, white pulp of the spleen, lymph nodes, and gut-associated lymphoid tissue (GALT)”

Perhaps the spleen plays an essential role in maintaining low deuterium levels in the blood?

The spleen was especially sensitive to the deuterium compared to all other organs

\*JA Laissue and RD Stone. Virchows Arch. A Path Anat and Histol 1979; 383: 149-166.

58

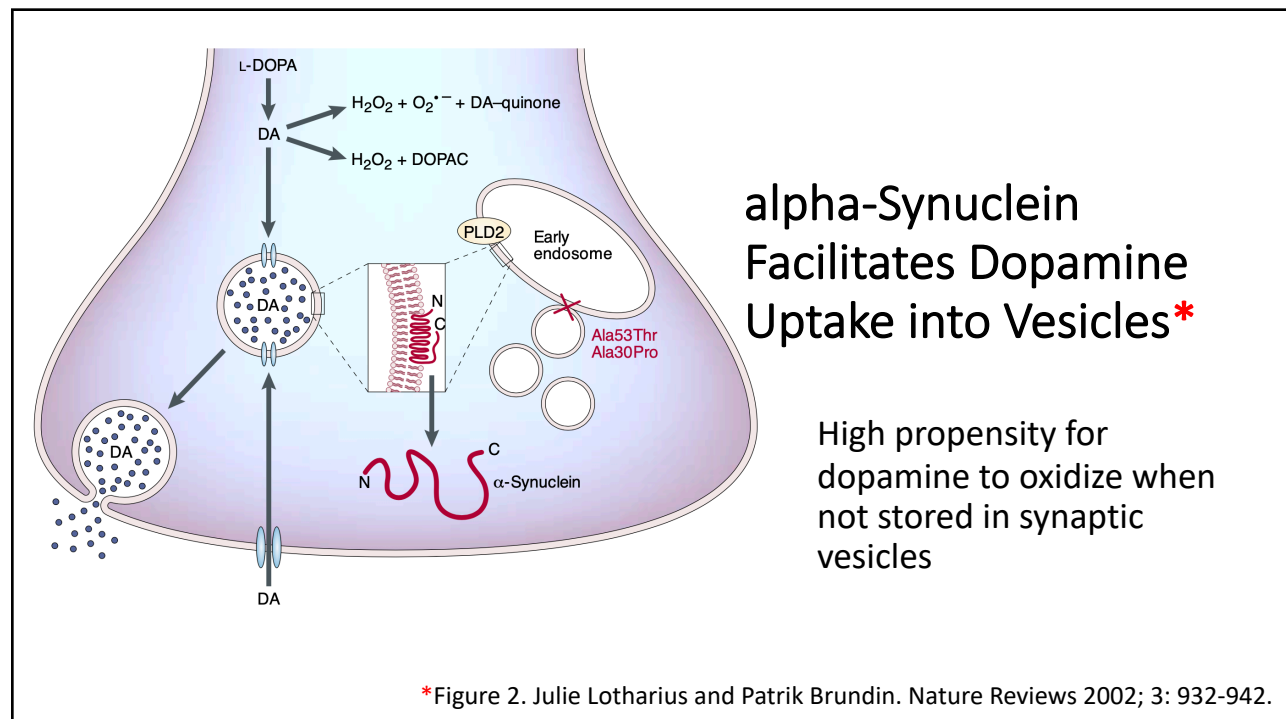
## Alpha-Synuclein & Parkinson's Disease

"When autophagic mechanisms fail to digest the toxic  $\alpha$ -SYN oligomers, exosome-mediated release could be a way to clear the cells of the toxic species, unfortunately allowing uptake of these exosomes by neighboring cells"

- Alpha-synuclein binds to membranes in its N-terminal domain
- Alpha-synuclein has a C-terminal domain that is intrinsically disordered
- Excessive phosphorylation and/or crowding induce the formation of soluble oligomers that block access of the hydrophobic domain *to water*

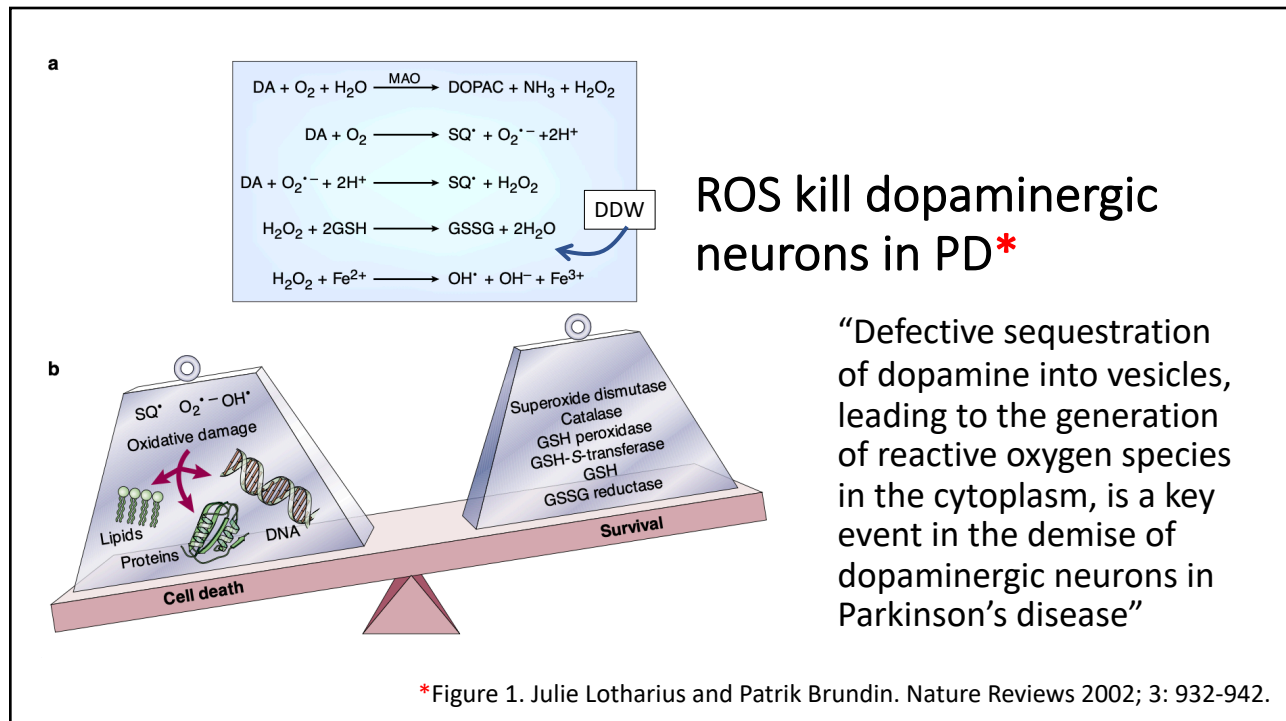
\*Angélique Deleersnijder et al. Trends in Molecular Medicine 2013 19(6): 368-377.

59



\*Figure 2. Julie Lotharius and Patrik Brundin. Nature Reviews 2002; 3: 932-942.

60



61

## Prion-like Proteins: A Hypothesis

- Prion-like proteins are normally “intrinsically disordered”:
  - They have broad access to the water-based medium and freely exchange hydrogen with deuterium
- Prion-like proteins bind to membranes in order to gain access to structured water where deuterium is enriched and pH is elevated
- Prion-like proteins spontaneously organize into oligomers under conditions of high pH, deuterium rich exposure (in structured water)
  - Excess bound deuterium/hydrogen ratio facilitates this refolding
- *Prion-like proteins ultimately trap deuterium in fibrils that precipitate out into Alzheimer’s plaque and Lewy bodies*
  - This serves to lower the deuterium levels in the cell
- When there is impaired ability to clear “misfolded” proteins, they are shipped out as exosomes
  - Exosomes can then get taken up by other cells better equipped to degrade the prions

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## A Bold Claim

- Researchers have been unable to figure out exactly how misfolded prion-like proteins cause disease
- I propose that they are looking under the wrong light!
  - Lewy bodies and plaque are an indicator of impaired ability to maintain low deuterium levels in the mitochondria and lysosomes
  - The proteins misfold when deuterium levels rise too high
  - Their misfolding works to deplete the deuterium – helping to solve the problem
- *Impaired ability to clear misfolded proteins can be caused by insufficient heparan sulfate in the membrane glycoproteins*
- *The plaque regions are the solution to, not the cause of, the toxicity*

63

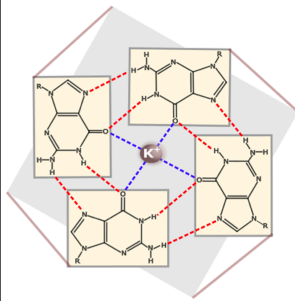
## G-Quadruplexes (G4s) and Prion Proteins

- Guanosine and its corresponding nucleotides are well known gelators
  - This unique gelating ability is due to propensity of guanine to self-associate into stable higher-order assemblies, such as ... G-quadruplexes\*
- PrP(C) binding to G4 RNAs destabilizes its structure and is thought to trigger its conversion to PrP(Sc)\*\*
- PrP messenger RNA (mRNA) itself contains several G4 motifs, located in the octarepeat region
  - PrP binds to G4s *in its own mRNA !!* Both the protein and the mRNA are affected
- “Our results allowed to surmise a quadruplex unwinding-activity of PrP, that may have a feedback in vivo”\*\*\*
  - \*Gretchen Peters and Jeffery Davis. Chemical Society Reviews 2016; 45: 3188-3206.
  - \*\*René CL Olsthoorn. Nucleic Acids Research, 2014; 42(14): 9327-9333
  - \*\*\*Paola Cavaliere et al. Nucleic Acids Research 2013; 41(1): 327–339

64

# The Intricate Coding of G4s

- GxxxG motif is a signature motif of prion proteins and prion-like proteins – two glycines spaced by three wild cards
- Glycine (G) DNA code is GGx – two guanine nucleotides followed by any nucleotide
- Tryptophan (W) DNA code is UGG
- GGGWG is a motif in the human prion protein that repeats many times in the protein
- Glycine-glycine-glycine-tryptophan-glycine = GGGWG



GGx	GGx	GGx	UGG	GGx
G	G	G	W	G
G	x	x	x	G

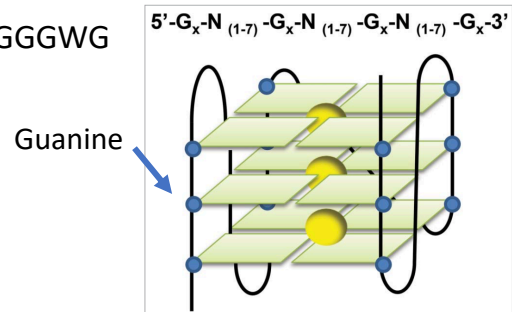
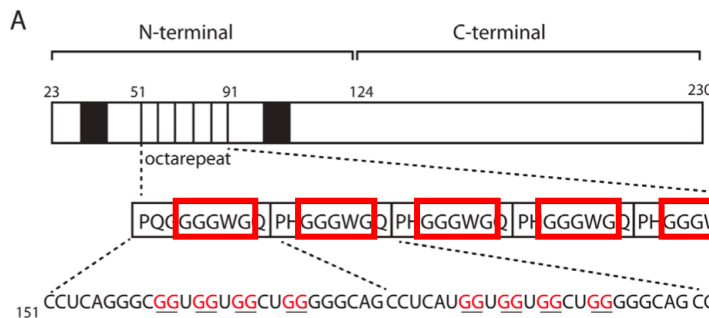


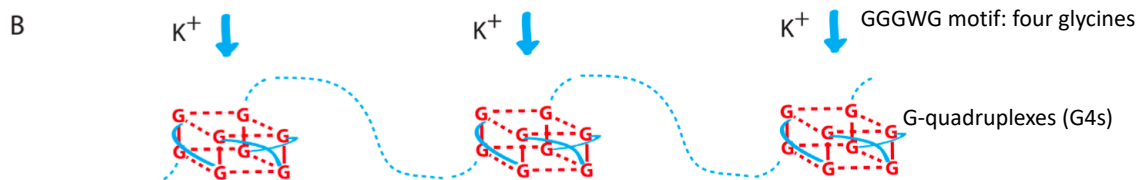
Figure 1. Jingwen Song et al. Translation 2016; 4:2, e1244031.

65

## “G-quadruplexes within prion mRNA: the missing link in prion disease?”\*

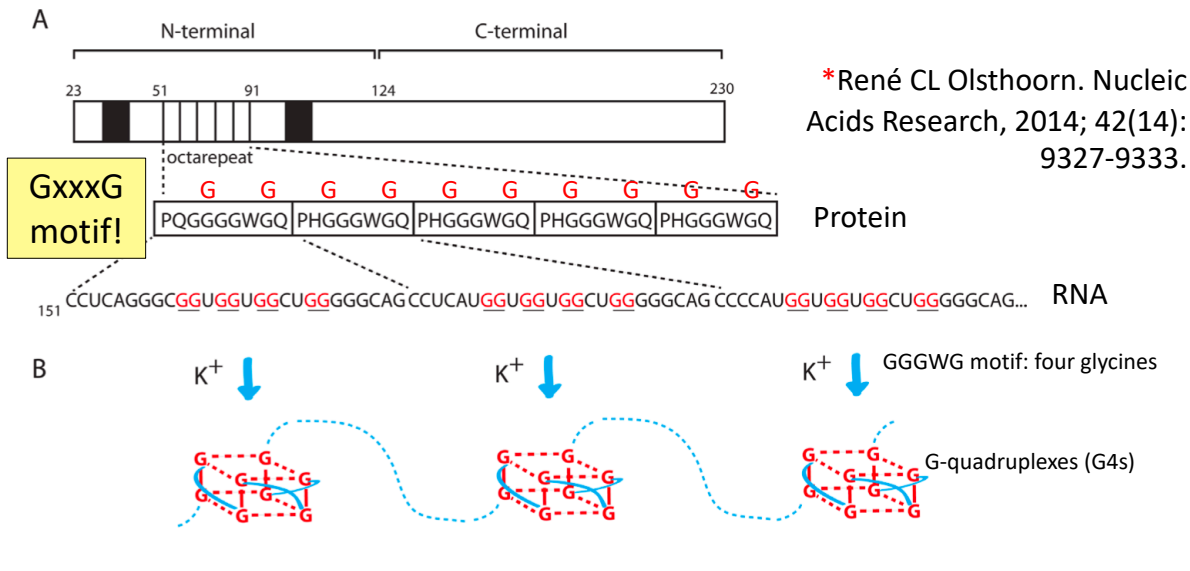


\*René CL Olsthoorn. Nucleic Acids Research, 2014; 42(14): 9327-9333.



66

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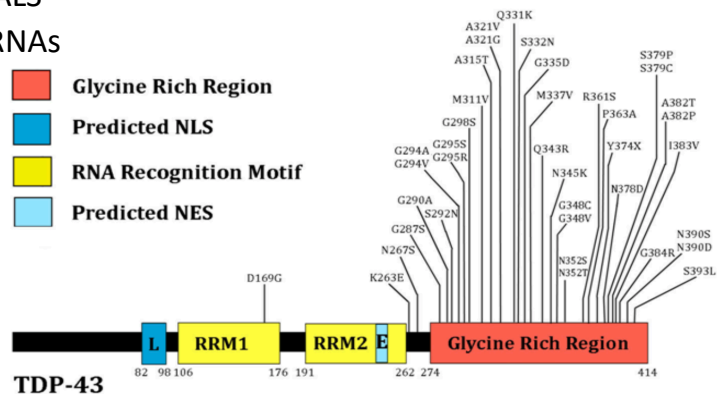


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# “Does Glyphosate Acting as a Glycine Analogue Contribute To ALS?”\*

- TAR (transactive response) DNA-binding protein 43 (TDP-43) is a protein that misfolds in association with Amyotrophic Lateral Sclerosis (ALS)
- TDP-43 has a long glycine-rich region where there are many mutations in glycines that are linked to familial ALS
- TDP-43 binds to G4s in multiple mRNAs
- Fused in Sarcoma (FUS) is another protein linked to ALS
- FUS has an RGG/RG domain that binds RNA

\*S Seneff et al. Journal of Bioinformatics and Proteomics Review 2016; 2(3): 1-21.



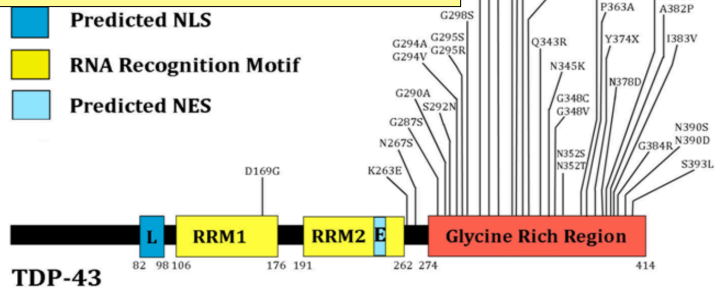
68



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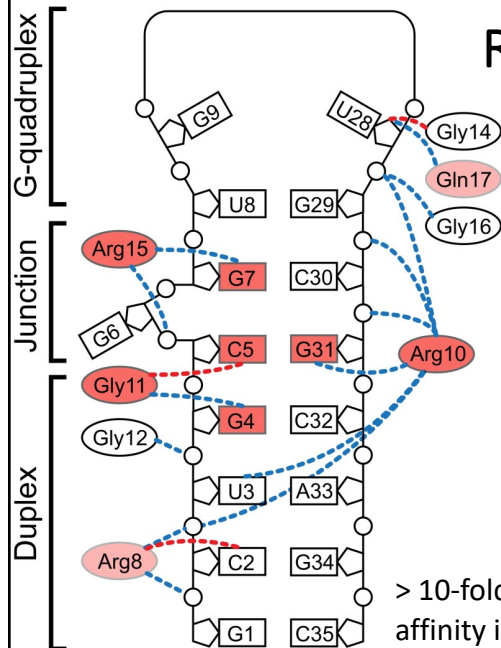
Whenever a protein binds RNA, it is binding to phosphate. Phosphate-binding sites are typically enriched in glycine. This is a glyphosate-susceptibility motif.



\*S Seneff et al. Journal of Bioinformatics and Proteomics Review 2016; 2(3): 1-21.

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# RGG motif of FMRP\*



Fragile Mental Retardation Protein

Species	Sequence
hum	RRGDGRRRGGGGRG-QGGRGRGGGFVK
rat	RRGDGRRRGGGGRG-QGGRGRGGGFVK
dog	RRGDGRRRGGGGRG-QGGRGRGGGFVK
ops	RRGDGRRRGGGPRG-QGGRGRGG-FVK
ckn	RRGDGRRRGGGARG-QGGRGRGG-FVK
frg	RRGDGRRRGG-TRG-QGMRGRGG-FVK

\*\*\*\*\* ↑ \*\*\*\*\*

> 10-fold reduction in binding affinity if Gly-11 is not glycine

\*Nikita Vasilyeva et al. PNAS 112(39): 2015; E5391-E5400.

70

## Glycine-to-glutamate mutation in FMRP causes fragile-X syndrome\*

- G266E (glycine at residue 266 substituted by glutamate)
- Mutated protein was unable to bind to RNA
- “Structurally, position 266 likely requires a small, flexible, and nonpolar amino acid, all the characteristics of glycine. Glutamic acid, on the other hand, is large and negatively charged and is predicted to clash sterically and ionically with surrounding amino acids”
- Glyphosate, like glutamate, is a bulky negatively charged amino acid

\*Joshua A Suhl and Stephen T Warren. J Exper Neurosci 2015: 9(s2): 35-41.

71

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About a third of individuals with Fragile X Syndrome have features of autism such as problems with social interactions and delayed speech

\*Joshua A Suhl and Stephen T Warren. J Exper Neurosci 2015: 9(s2): 35-41.

72

## The Big Picture (Hypothesis)

- Do prion-like proteins play a role in maintaining low deuterium in the cytoplasm??
  - **Hypothesis:** These proteins can trap deuterium inside water-inaccessible precipitates

### ***Bold claims:***

- Misfolded proteins in Alzheimer's plaque or Lewy bodies are a sign of impaired deuterium homeostasis
- Prion diseases begin with stressed immune cells in the gut and spleen
- Cells with impaired ability to clear misfolded proteins release them inside exosomes and deliver them to other cells, propagating the disease process
- Impaired sulfate homeostasis plays a significant role in the pathology

73

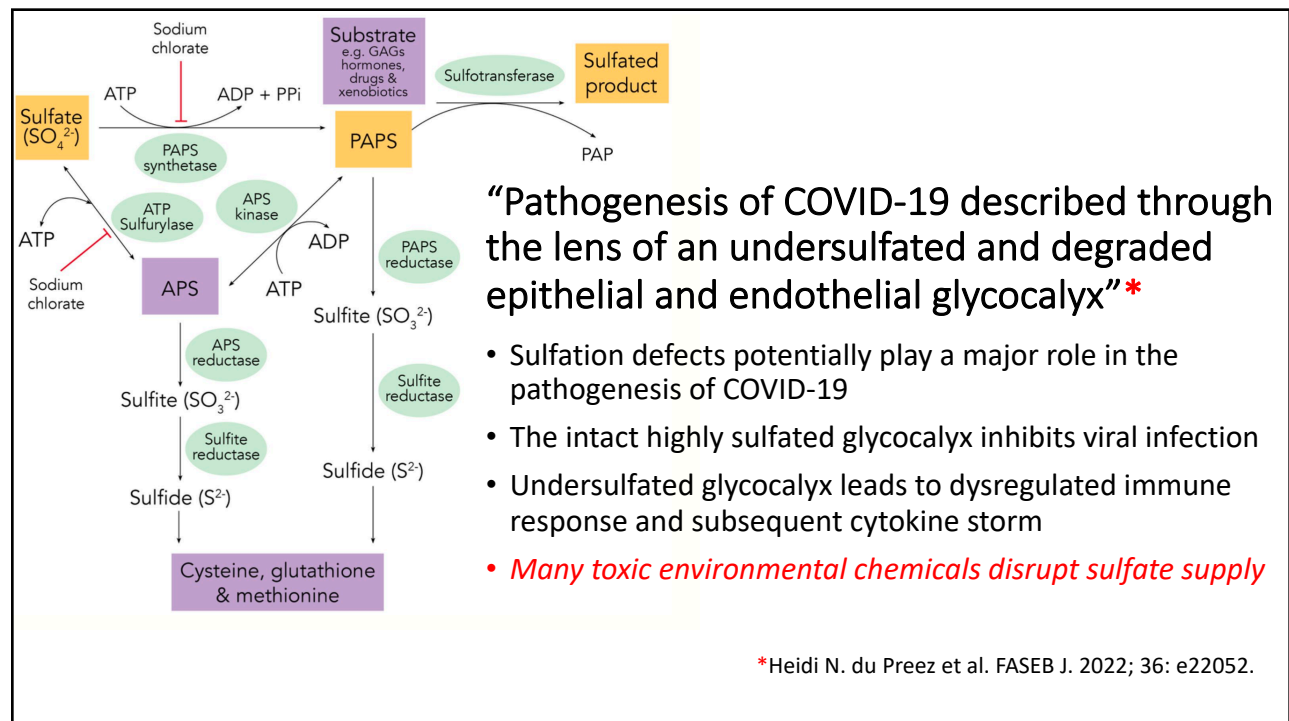
# **SARS CoV-2, Deuterium and Glyphosate**

74

## The Big Picture

- People who suffer from acute symptoms with COVID-19 are immune compromised due to system-wide defective mitochondria
- The virus invades the lungs and proliferates there, inducing an inflammatory response
  - This allows lipids in the viral membranes to be metabolized by lipoxygenase, producing deuterium-depleted water
- Sharp upregulation of bradykinin results in a dramatic drop in blood pressure, and infiltration of water and macrophages into the lung alveoli
  - Massive overproduction of hyaluronan
  - A hydrogel encasing the hyaluronan fills the alveoli and traps deuterium
- The signaling cascade causes myeloid cells from the bone marrow and platelets to infiltrate the lungs and release their mitochondria into the medium, which is rich in deuterium-depleted water
  - These mitochondria resupply the immune cells, thus revitalizing them

75



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**“Pathogenesis of COVID-19 described through sulfated and degraded glycoalkaloids”\***

Both PAPS synthetase and sulfotransferase bind phosphate at sites that have highly conserved glycine residues: GxxGxGK motif

- Undersulfated glycoalkaloid leads to dysregulated immune response and subsequent cytokine storm
- *Many toxic environmental chemicals disrupt sulfate supply*

\*Heidi N. du Preez et al. FASEB J. 2022; 36: e22052.

77

**“Pathogenesis of COVID-19 described through sulfated and degraded glycoalkaloids”\***

Sulfate maintains gelled water lining the walls of the blood vessels and the gel traps deuterium, leaving the fluid blood plasma depleted in deuterium

- Undersulfated glycoalkaloid leads to dysregulated immune response and subsequent cytokine storm
- *Many toxic environmental chemicals disrupt sulfate supply*

\*Heidi N. du Preez et al. FASEB J. 2022; 36: e22052.

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## Immune System 101

- The innate system is actually very powerful if it is healthy
- If the innate system can't clear the virus, the adaptive system gets involved
- The adaptive system releases antibodies to help tag and trap the viruses, and inflammatory cytokines which induce release of reactive oxygen species

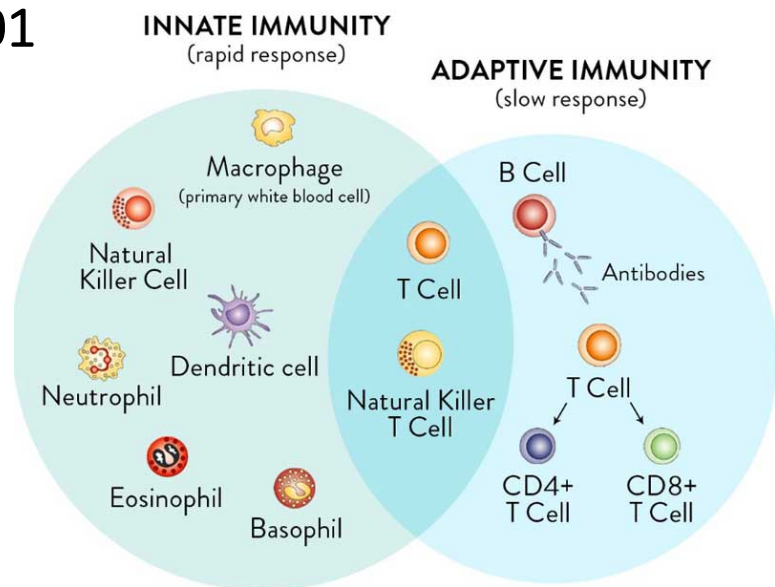


Figure from <https://articles.mercola.com/sites/articles/archive/2020/10/14/herd-immunity-coronavirus.aspx>

79

## Mouse Study Shows Link Between Mitochondrial Dysfunction and Virus Susceptibility\*

- Aged mice had dysfunctional T-cells with reduced mitochondrial oxidative phosphorylation
  - Skew towards Th1 type response, with higher secretion of TNF- $\alpha$  and IFN- $\gamma$  (inflammatory response)
- Aged mice had weakened immune system
  - Infection with a mouse pox virus caused all the elderly mice to die, but none of the young mice
- In humans, "inflammaging" predicts susceptibility to cardiovascular diseases, neurodegeneration, frailty, and multi-morbidity

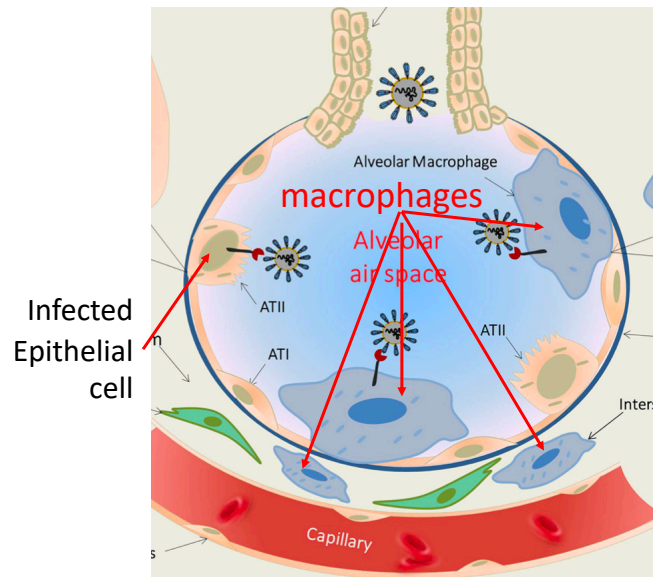


\*Desdín-Micó et al., Science 2020; 368: 1371-1376.

80

## Initial COVID-19 Events

- SARS-CoV-2 viruses enter through the bronchioles and infect epithelial cells lining the alveoli
- Residential macrophages fail to clear the virus because they are defective
- Viruses proliferate wildly
- Macrophages send out alarm signals which draw in CD4+ and CD8+ T-cells
- Capillary wall becomes leaky to support invasion
- Blood pressure drops; fluid begins to fill the alveolar space
- Person feels as if they are drowning



\*Frank L van de Veerdonk et al. eLife 2020; 9: e5755.

81

## Is a Bradykinin Storm Brewing in COVID-19?\*

- Hypertension is a risk factor for COVID-19, but *hypotension* develops instead during the disease process
  - ACE2 receptor is upregulated by 199-fold in the lungs in severe COVID-19 patients, and ACE is downregulated (8-fold)
    - ACE normally degrades (clears) bradykinin
  - Bradykinin receptors are upregulated by nearly 3000-fold!
  - Bradykinin induces vasodilation and hypotension
- Inflammatory cytokines induce capillary leakage and inhibit alveolar fluid reabsorption leading to alveolar flooding\*\*

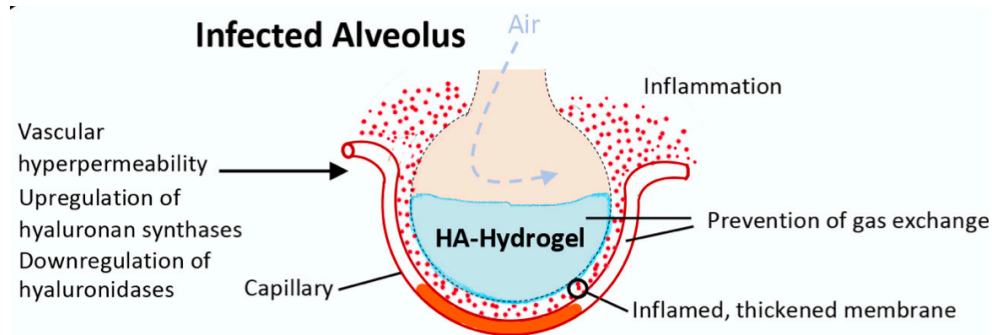
\*<https://www.the-scientist.com/news-opinion/is-a-bradykinin-storm-brewing-in-covid-19-67876>  
Michael R Garvin et al. eLife 2020; 9: e59177.

\*\* Andrew M Luks and Erik R Swenson. Ann Am Thorac Soc 2020 Apr 24 [Epub ahead of print]

82



## Bradykinin-induced hyperpermeability of the lung capillaries causes formation of hyaluronic-acid hydrogel that inhibits gas exchange\*



\*Michael R Garvin et al. eLife 2020; 9: e59177.

83

## SARS CoV-2 causes massive overproduction of hyaluronic acid in the lungs\*

- "Hyaluronic acid can trap roughly 1000 times its weight in water and when bound to water the resulting hydrogel obtains a stiff viscous quality similar to 'Jello' "
- Multiple enzymes that synthesize hyaluronic acid are massively upregulated in COVID-19 lungs: HAS1 (9,113-fold), HAS2 (493-fold), and HAS3 (32-fold)
- Excess hyaluronic acid is associated with pulmonary thrombosis, ground glass opacities, and acute respiratory distress syndrome
- "Hyaluronic acid in the bronchoalveolar space of the lungs could form a *viscous hydrogel* that would negatively impact gas exchange"

\*Michael R Garvin et al. eLife 2020; 9: e59177.

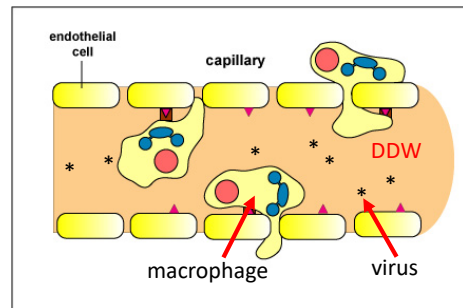
84



## How the Viral Response Attempts to Repair the Deuterium Problem - Hypothesis

- Virus contains lipids such as linoleic acid in its membrane (stolen from host cell)
- Inflammatory response due to weak innate immunity causes release of lipoxygenase
  - Lipoxygenase extracts protons from lipids in viral membrane and converts oxygen into deuterium depleted water (DDW)
- Produced leukotrienes are powerful signaling molecules which induce further reaction
  - Arterioles constrict access to capillary
  - Venules open up leaks
- Macrophages and T-cells “drink the sweet nectar” – and supply their mitochondria with much-needed deuterium depleted water ??
  - This empowers them to clear the virus

Lipoxygenase has a fantastic ability to select hydrogen over deuterium despite not being a flavoprotein\*

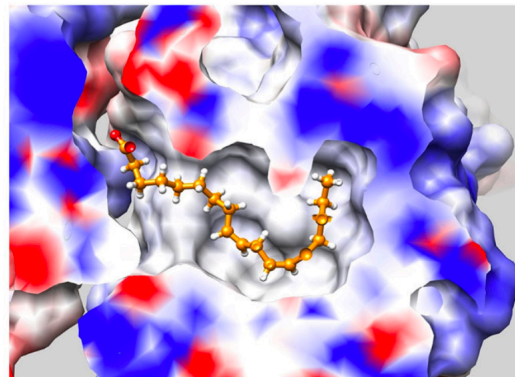


\*Pengfei Li et al. J Phys Chem Lett 2018; 9(22): 6444-6449.

85

## Virus traps linoleic acid in its membrane which then gets oxidized by lipoxygenase to make DDW

- Linoleic acid fits perfectly in three binding pockets of SARS CoV-2 membrane S glycoprotein\*
- Patients with severe COVID-19 show reduced levels of fatty acids\*\*



\*Christine Toelzer et al. Science Sept 21 2020 [Epub ahead of print]

\*\*Bo Shen et al. Cell 2020; 182: 59-72.

86

## Viruses are stabilized by excess deuterium!

- The nucleocapsid protein in SARS-CoV-2 starts to unfold at 35 degrees Centigrade (95 degrees Fahrenheit) and is completely denatured at 55 degrees Centigrade (131 degrees Fahrenheit)\*
  - A fever is a natural defense against the virus
- Viruses take up deuterium and trap it in their protein coat and in their internal single strand of RNA\*\*
  - Deuterium stabilizes the viral protein and protects from temperature denaturation
  - The virus removes deuterium from the body fluids
- When the deuterium level in the body fluids is high, the virus becomes more stable

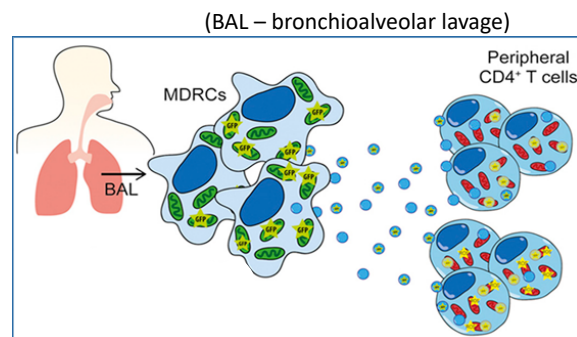
\*Milan Surjit and Sunil K Lal. Infection, Genetics and Evolution 8 (2008) 397-405.

\*\*Jiangsen MAO et al., Chinese Science Bulletin 2004 Vol. 49 No. 3 253-257.

87

## “Exosomal transfer of mitochondria from airway myeloid-derived regulatory cells to T cells”\*

- Myeloid-derived regulatory cells (MDRCs) are cells that emerge from the bone marrow and infiltrate inflammatory sites, e.g., in asthma
- MDRCs in the airways transfer mitochondria to T cells via *exosomes*
  - These mitochondria have been shown to be functional in the recipient cell
- Hypothesis: Immune cells (e.g., T cells, macrophages, dendritic cells, ...) can sweep up DDW and mitochondria via macropinocytosis
- *Intercellular communication is essential for resolving inflammation*



\*Kenneth P. Hough et al. Redox Biol 2018; 18: 54-64.

88

## “Coronavirus (Covid-19) sepsis: revisiting mitochondrial dysfunction in pathogenesis, aging, inflammation, and mortality”\*

- Dysregulated host response to an infection leads to sepsis
  - Destructive downstream effects manifested as profound persistent hypoxia, profuse blood clots and lactic acidosis
  - Multiple organ failure – high mortality risk
- Elderly disproportionately suffer from impaired mitochondrial function
- Depleted glutathione weakens antioxidant defenses
- Damaged mitochondria release their DNA which upregulates HIF-1 $\alpha$
- Depleted NAD<sup>+</sup> supplies impede recovery phase

\*Santosh Shenoy. Inflamm Res 2020 Aug 7: 1-9.

89

## Heme Oxygenase 1 (HO-1) and COVID-19\*

- Some cases of COVID-19 cascade into a hyper-inflammatory state leading to thrombosis and multiple organ failure
  - HIF-1 $\alpha$  upregulates HO-1 which normally tames the inflammation
- For every molecule of heme that HO-1 converts to biliverdin, it produces three molecules of deuterium depleted water (DDW) from oxygen and NADPH
- Glyphosate substitution for a critical glycine residue in HO-1 would convert it to a pro-inflammatory enzyme\*\*
  - Fails to metabolize heme to bilirubin and biliverdin (doesn't produce DDW)
  - Releases ferryl iron (Fe<sup>+4</sup>) that reacts with ROS to damage the vascular wall
  - Positive feedback loop is highly destructive

\*J Greil et al. Haematologica 2016; 101:e437; Y Liu et al. JBC 2000; 275(44): 34501-34507

\*\*S Seneff. Glyphosate, Deuterium and COVID-19. Masters of Health. <https://joom.ag/xCOC>

90

## Recapitulation

- Innate immune system in the lungs is impaired due to chronic exposure to glyphosate (and other toxic chemicals)
  - Macrophages can't trap and clear viruses due to defective mitochondria
- Viruses proliferate and cause a strong reaction from the adaptive immune system
  - Inflammatory response leads to complex cascade causing a cytokine storm, eventually damaging the systemic circulatory system
  - Heme oxygenase is upregulated and would normally tame inflammation, but glyphosate turns it into a rogue version of itself
  - This planned response has a goal of temporarily shutting down the mitochondria so that they can be supplied with deuterium depleted water obtained through direct assistance of the viruses
  - It backfires when many of the proteins are disrupted by glyphosate

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# Healthy Lifestyle

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## A High-Fat Diet is a Low-Deuterium Diet

- Butter, lard and coconut oil were among the foods with the lowest detected levels of deuterium among foods tested
- The synthesis of fats involves transfer of hydrogen from NADPH into the growing fatty acid chain



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## Amounts of Deuterium in Various Foods

Flour: 150 ppm



Sugar: 146 ppm



Cottage Cheese: 136 ppm



Coconut Oil: 110 ppm



Ghee: 112 ppm



Butter: 124 ppm

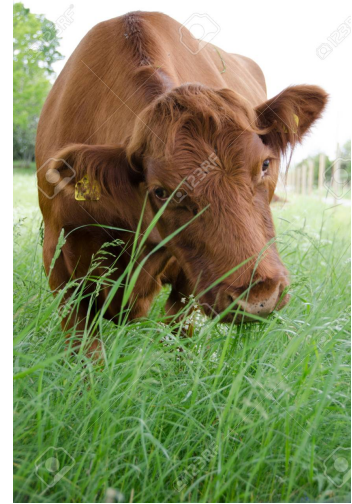


Olive Oil: 130 ppm

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## Deuterium in Foods and Mast-Cell Activation Syndrome\*

- Mast cells release histamines during an allergic reaction
- Deuterium increases histamine release from mast cells
- Plants get rid of deuterium by storing it in sugar and starch
  - Fruits, root vegetables (potato) and grains are high in deuterium
  - Meats from grain-fed animals are high in deuterium
- Leafy green vegetables, animal fats (lard, tallow, butter) and plant fats (avocado, coconut, olive oil) are low in deuterium
- Grass-fed beef and the dairy products derived from pastured cows are excellent sources of low-deuterium fat

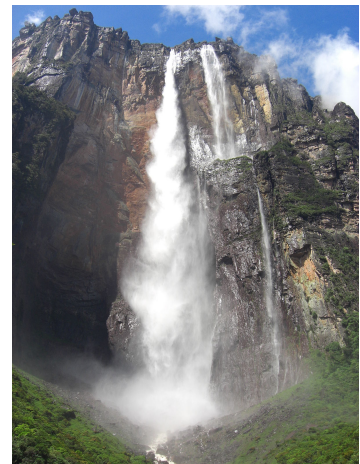


\*<https://healinghistamine.com/deuterium-histamine-intolerance/>

95

## Waterfalls are Therapeutic!\*

- Waterfalls produce inhalable, negatively charged nano-water particles known as “Lenard ions” or ballo-electric ions
  - These particles are likely to be deuterium depleted
- “Conclusions: Our study provides new data, which strongly support an “added value” of exposure to waterfall microclimate when combined with a therapeutic sojourn at high altitude including regular physical activity.”
- Ocean waves probably have a similar effect



\*C Grafetstätter et al. *Physiol Anthropol.* 2017; 36: 10.

96



## "Life expectancy in Iceland among the highest in Europe, infant mortality rate is lowest"\*

"In 2015 the infant mortality rate in Iceland was 1.9 per 1,000 live births"

"The average in the European Union is 4.0, while the US has an infant mortality rate of 5.87"



Sólheimajökull Glacier



Smoked puffin



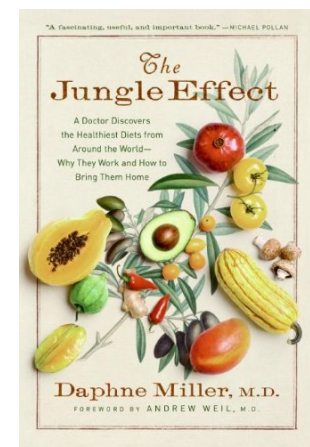
black sand beach of town Vík í Mýrdal in Iceland.

\*<https://icelandmag.is/article/life-expectancy-iceland-among-highest-europe-infant-mortality-rate-lowest>

97

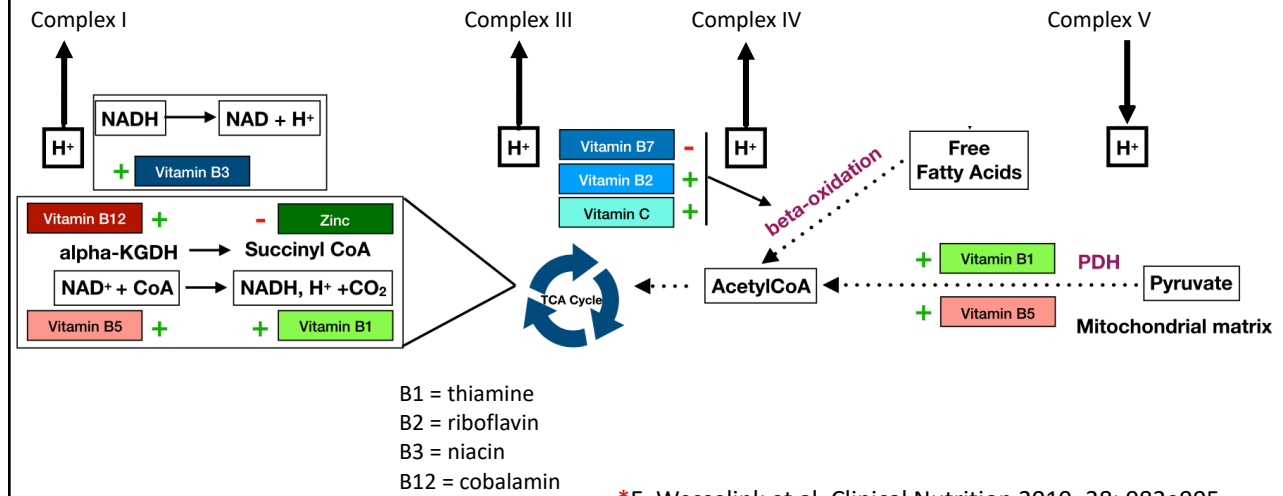
## "The Jungle Effect: Healthiest Diets from Around the World" by Daphne Miller, MD

- Iceland was one of the places she studied
- Diet consists of fish, sheep, seabirds, potatoes, and other simple vegetables
- Iceland also has glacier water which is low in deuterium, and basalt which is high in sulfur (maintain sulfate levels)
  - Many sulfur hot springs with therapeutic benefit
- After a major eruption in the 1800's, many islanders moved to northern Canada
  - They lost their health benefits



98

# Many Nutrients Contribute to Mitochondrial Function\*



99

# Eat Foods Containing Sulfur



100



## Supplemental Sources of Sulfur\*

- glucosamine sulfate
- chondroitin sulfate
- glutathione
- N-acetylcysteine
- alpha lipoic acid
- taurine
- DMSO, MSM
- S-adenosylmethionine (SAMe)
- Epsom salts (Mg-sulfate)

These can have many beneficial effects and are nearly nontoxic

My personal favorite is Epsom salt baths: Magnesium sulfate uptake through the skin

\*S Parcell, Alternative Medicine Review 7(1), 2002, 22-44

101

## Foods High in Niacin → NAD(P)(H)

- Many foods are rich in niacin, especially animal products like meat, fish and poultry.
- Vegetarian sources include avocado, peanuts, whole grains, mushrooms, green peas and potatoes.



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## Riboflavin: Source for FAD in Flavoproteins

Foods high in riboflavin include beef, tofu, milk, fish, mushrooms, pork, spinach, almonds, avocados, and eggs



103

## DDW, Glacier Water and Hydrogen Water



104

# Flavonoids and Polyphenols!



105



106

## Summary

- Deuterium is pervasive in the environment, and it is very damaging to the ATPase motors in the mitochondria
  - Biological organisms have devised sophisticated mechanisms to deal with it
- A class of enzymes called flavoproteins have amazing skills to select hydrogen over deuterium in their product
  - These enzymes are disrupted by glyphosate
- Heparan sulfate proteoglycans play an essential role in supplying protons to mitochondria by trapping deuterium in the extracellular matrix
- Prion misfolding diseases are likely caused by an inability to maintain low deuterium in the organelles
- SARS-CoV-2 induces a reaction in the lungs that supports renewal of mitochondrial health to the immune cells
- Glacier water, foods rich in niacin and riboflavin, healthy fats and an organic diet all help protect from deuterium toxicity