Oxygen

EVROPSKÁ UNIE
Evropské strukturální a investiční fondy
Operační program Výzkum, vývoj a vzdělávání

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Oxygen

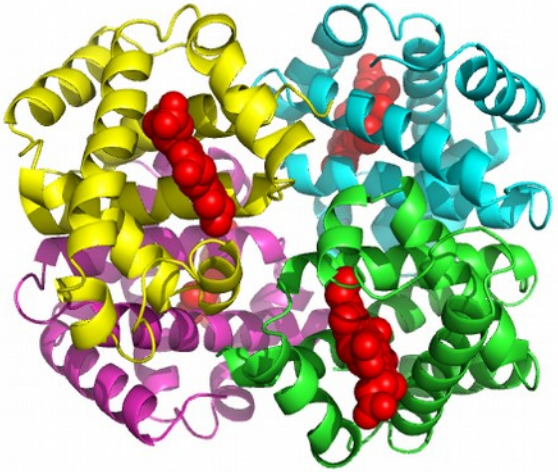
1. Transport of oxygen in the body – hemoglobin

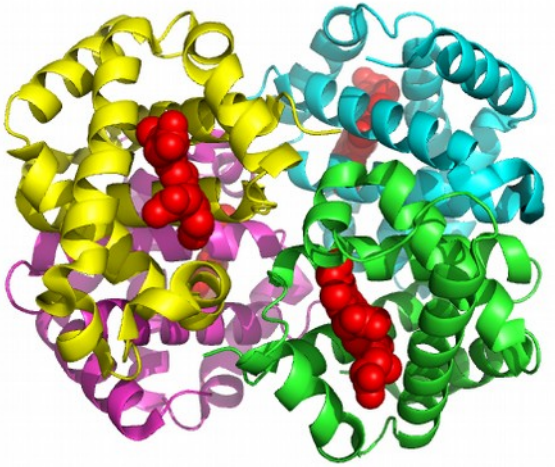
2. Electron transport chain

3. Enzymes that use oxygen as a substrate (oxidases, oxygenases),
   cyclooxygenase, glucose oxidase, cholesterol oxidase,
   collagen hydroxylation, cytochrome P450

4. Reactive oxygen species (oxidation stress)
   H_2O_2, O^{2-}●, OH●, singlet oxygen, glutathione

Hemoglobin (haemoglobin in UK)





R – relaxed (1RVW)

T – tense (2D5Z)

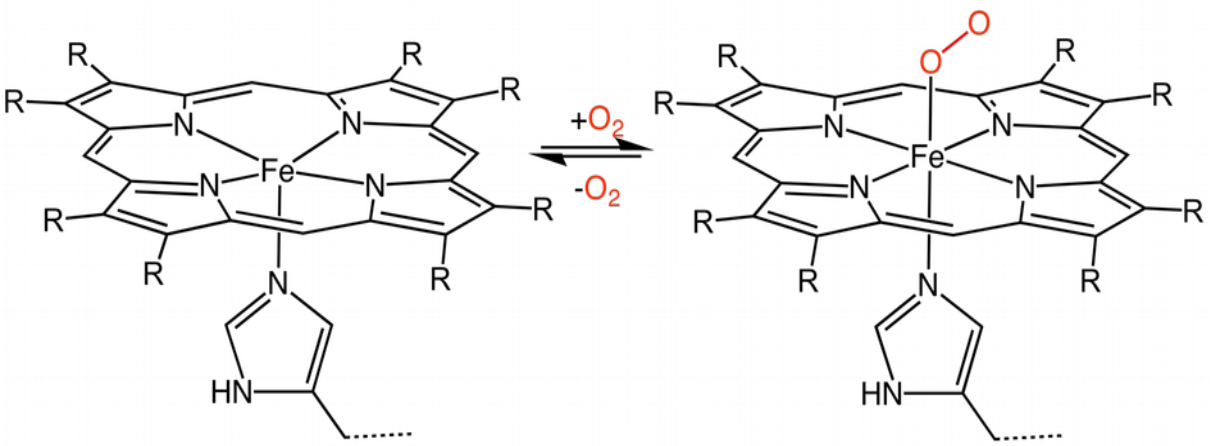
https://en.wikipedia.org/wiki/Hemoglobin#/media/File:Hemoglobin_t-r_state_ani.gif

Hemoglobin is a homotetramer. Each subunit contains heme that binds oxygen via iron atom. It can exist in two states R and T with a conformational difference. In absence of oxygen it prefers R. Binding of oxygen stabilizes T. The T form shows higher affinity to oxygen than R. As the result, binding of single oxygen increases affinity of the remaining three sites. Binding of another oxygen increases affinity of other two sites etc.

The fact that binding of oxygen in one site influences binding in another distant site gives the name of this effect – allosteric (“different” “site”).

Hemoglobin (haemoglobin in UK)

Heme – Fe(2+)

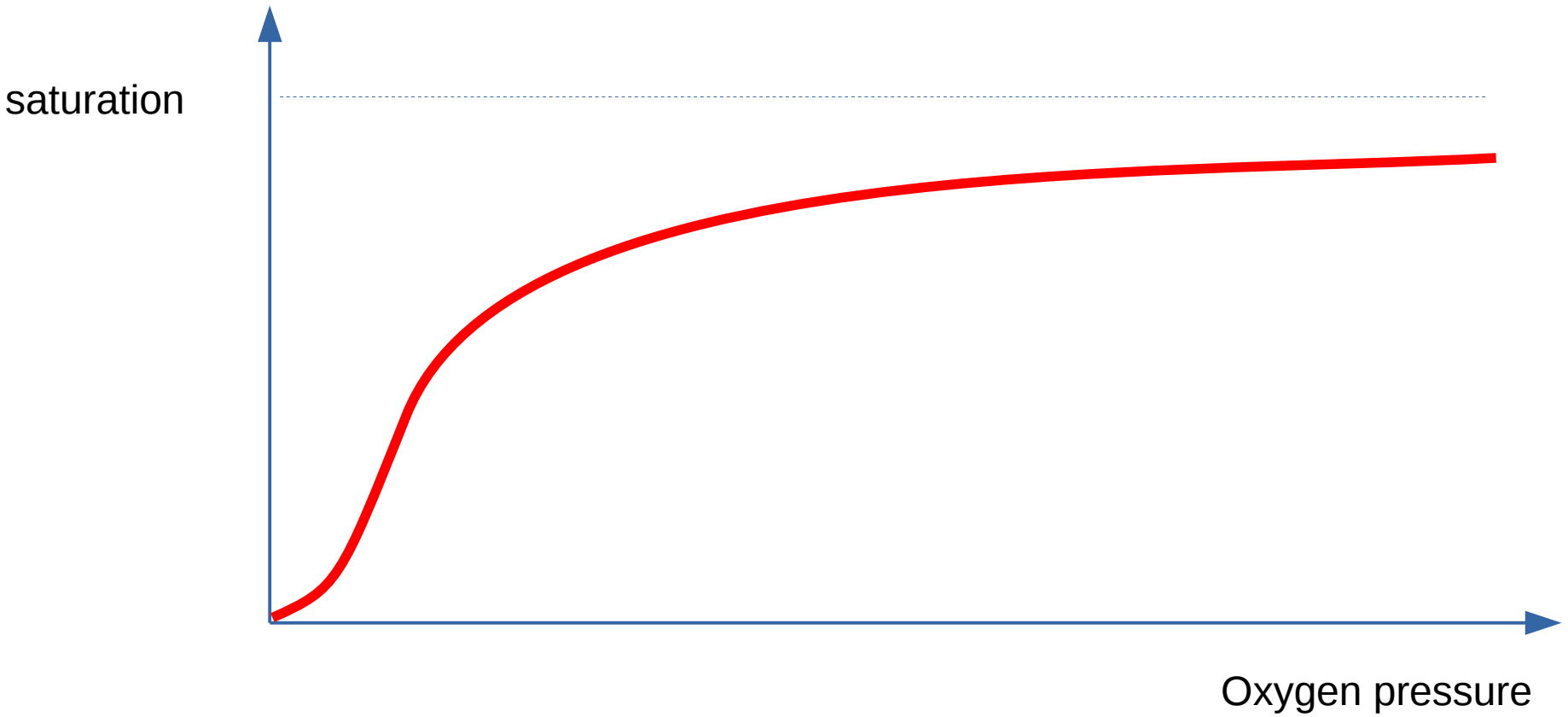


Carbaminohemoglobin – with bound CO2

may bind also CO, CN^-, NO, H_2S and other molecules

Methemoglobin – with Fe(3+) instead of Fe(2+)

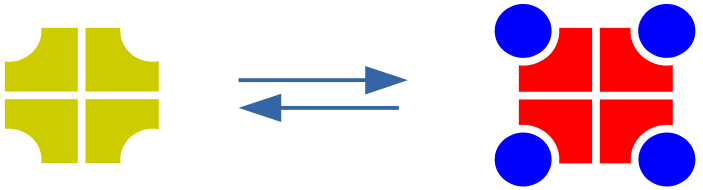
Hemoglobin



Unlike other binding of ligand by other proteins, hemoglobin does not show hyperbolic saturation curve. The curve of hemoglobin is sigmoid due to the allosteric effect. It binds oxygen weakly at low concentration and strongly at high concentrations. This is important for physiology. Hemoglobin can be almost fully loaded in lungs (high oxygen concentration) and it can release almost all oxygen in other tissues (low oxygen concentration). This cannot be achieved without allostery.

Hemoglobin

Hill's model



$$S = \frac{[A]^{\kappa}}{[A]^{\kappa} + K_S}$$

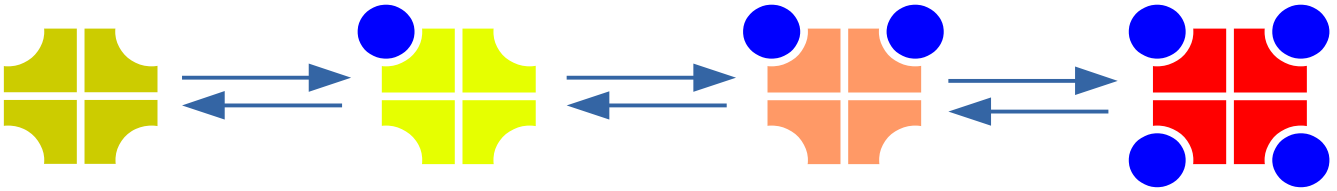
The oldest model of allostery is Hill's model. According to this model hemoglobin can bind either no oxygen or four oxygen molecules in all four sites. This model is not realistic, it is not probable that five molecules meet at the same moment. Saturation follows the Hill's equation (above).

Standard (non-allosteric) saturation curve corresponds to the Hill's equation with κ=1.

The coefficient κ is theoretically equal to four for hemoglobin. In reality it is the range 1.7-3.2. Hill's model cannot explain values of κ different from the number of binding site as well as non-integer values.

Hemoglobin

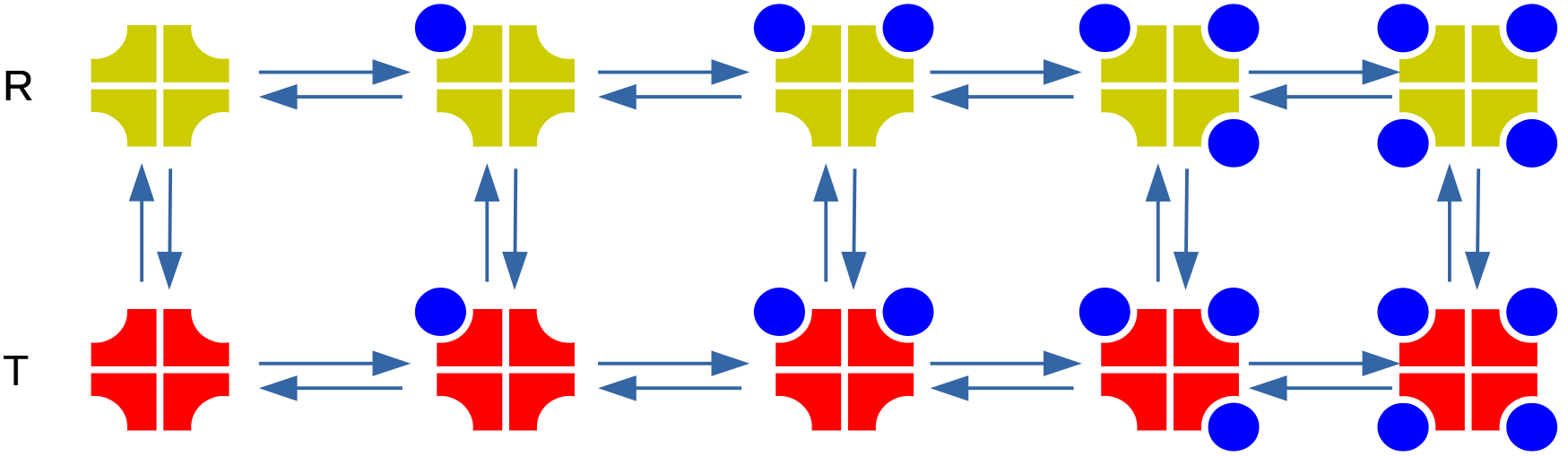
Aldair’s model



Aldair’s model of allostery is more advanced and it can explain different values of κ. Binding of each oxygen has different equilibrium constant. However, this model is bit chicken-hearted because it is easy to explain anything by quite complicated model. Aldair’s model also adds little to explanation of the structural basis of allostery.

Hemoglobin

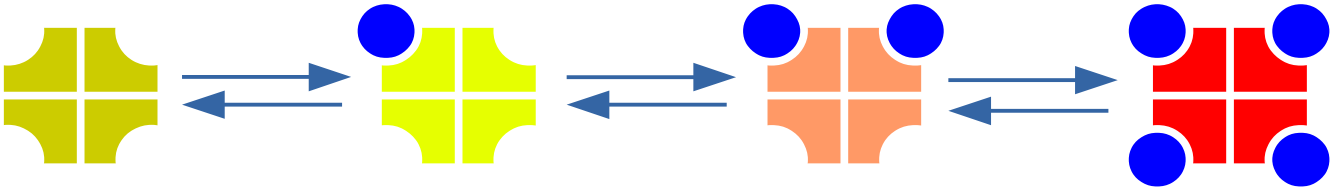
Monod-Wyman-Changeux (MWC)



One of the most respected models is the one by Monod, Wyman and Changeux (MWC). It correctly predicted existence of the two forms – relaxed and tense. According to this model hemoglobin exists in two states (conformations) R and T. R is more favored in absence of oxygen. It has also weaker affinity towards oxygen. The tense form is unpopulated in absence of oxygen. Since T binds oxygen more strongly binding of oxygen shifts R-T equilibrium in favor of T. MWC model can explain experimental values of Hill's coefficients.

Hemoglobin

Koshland-Némethy-Filmer



Koshland-Némethy-Filmer model is similar to Aldair's model. It is extended in the way that it adds a structural explanation to Aldair's model. It introduced the term induced fit, i.e. binding of oxygen introduces the changes in the hemoglobin structure.

Hemoglobin

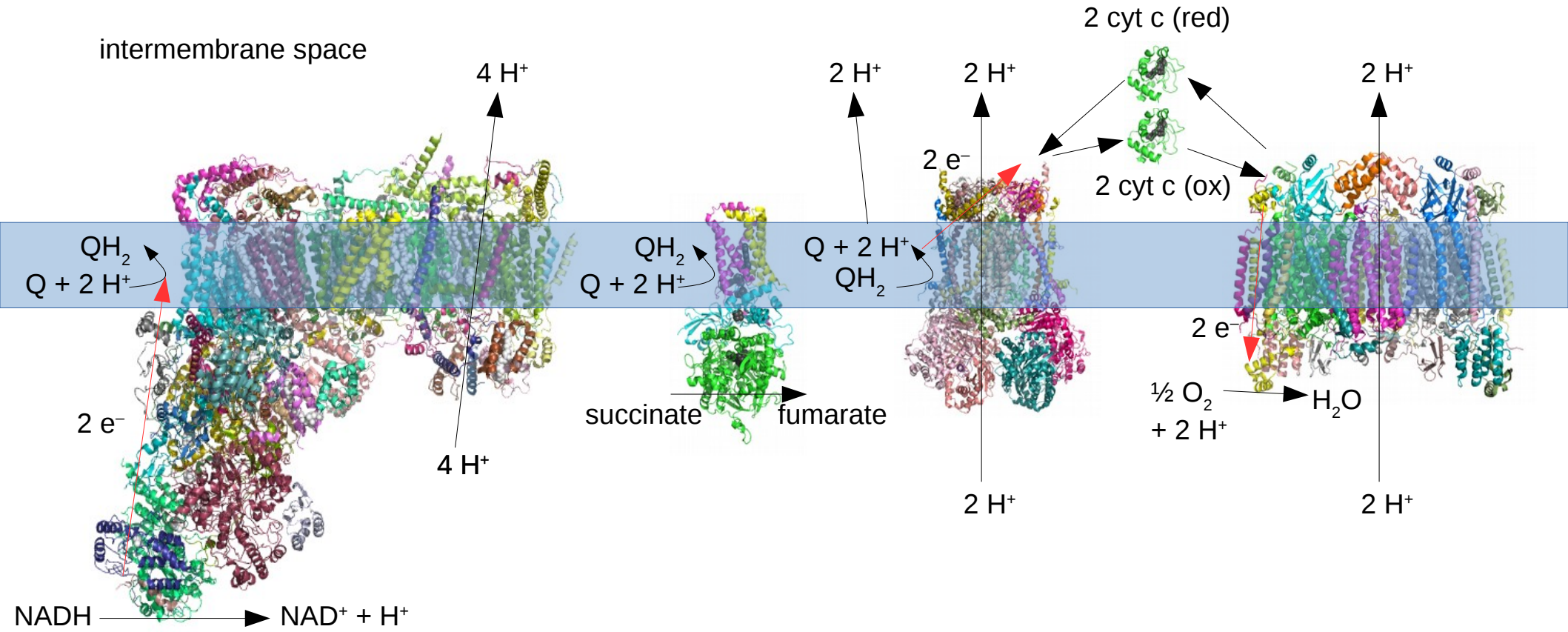
Affinity of hemoglobin for oxygen is also influenced by:

Charge state of iron – only Fe(II) binds oxygen, Fe(III) (methemoglobin) can be enzymatically reduced to Fe(II) by NADH. This reaction consumes NADH produced in erythrocytes in glycolysis.

Carbon dioxide and **acidity** (Bohr effect) shifts the dissociation curve right. It supports release of oxygen in tissues with high carbon dioxide content (and thus high acidity). Oxygenated hemoglobin is stronger acid than the deoxygenated one.

2,3-Bisphosphoglycerate also shifts the dissociation curve right. It is produced form an intermediate of glycolysis from 1,3-bisphosphoglycerate (only in erythrocytes). By this erythrocytes can respond to hypoxia or other conditions.

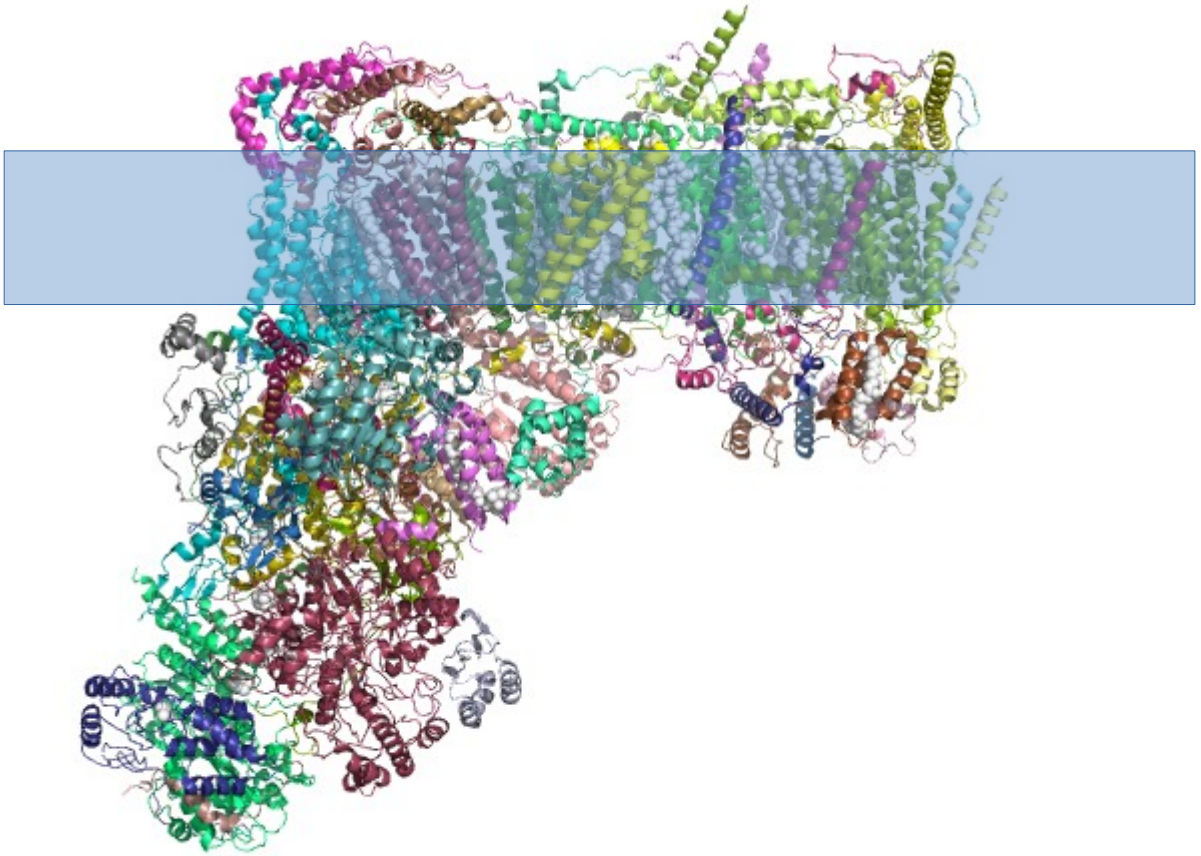
Respiratory chain



NADH from citric acid cycle and other mitochondrial processes (e.g. fatty acid degradation) is recycled in respiratory chain (also known as electron transport chain). In this case electrons flow via complex I, III and IV. At the end they convert oxygen to water. The result is pumping of protons across the membrane. Alternatively, electrons can come from other reduced substrate, such as succinate, via complex II.

Respiratory chain

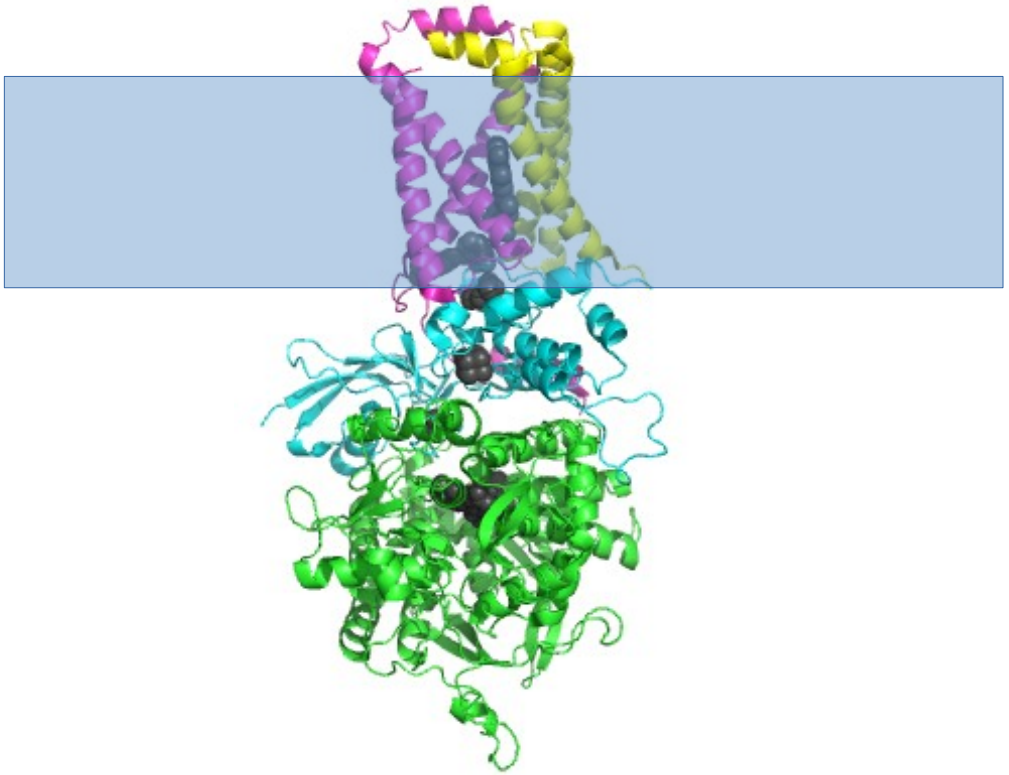
Complex I



NADH dehydrogenase (NADH:ubiquinone oxidoreductase, EC 1.6.5.3)
NADH + ubiquinone + H^+ → NAD^+ + ubiquinone-H_2 (+ 4 H^+ transported)
contains FMN and Fe-S clusters

Respiratory chain

Complex II



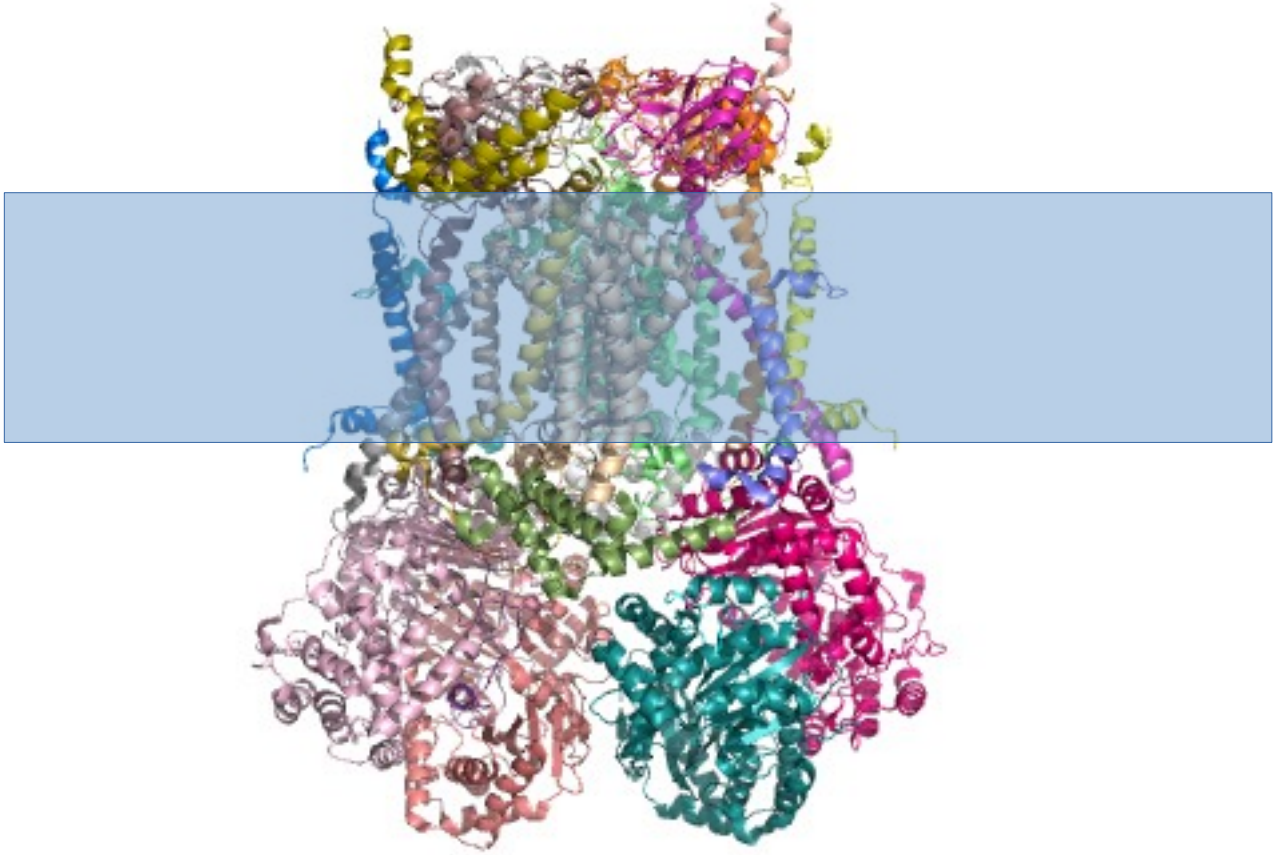
succinate dehydrogenase (succinate:quinone oxidoreductase, EC 1.3.5.1)
succinate + ubiquinone → fumarate + ubiquinone-H_2
contains FAD, Fe-S clusters and heme, does not pump protons

Alternative entry
Acyl-CoA → Acyl-CoA dehydrogenase → ETF → ETF-QO → UQ → Complex III

ETF = electron transferring flavoprotein

Respiratory chain

Complex III



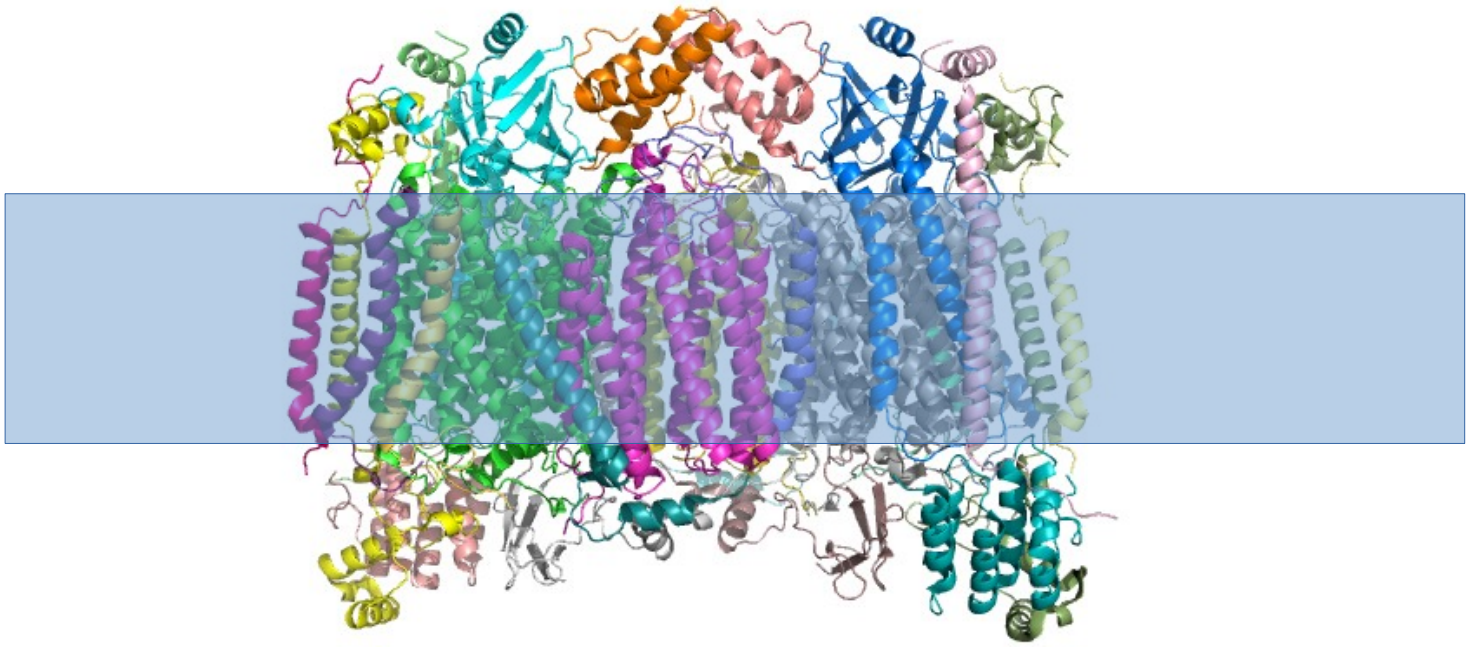
cytochrome bc_1 complex (quinol:ferricytochrome-*c* oxidoreductase, EC 1.10.2.2)

ubiquinone-H_2 + 2 ferricytochrome-*c* → ubiquinone + 2 ferrocytochrome-*c* +
+ 2 H^+ (out) (+ 2 H^+ transported)

contains Fe-S cluster and hemes

Respiratory chain

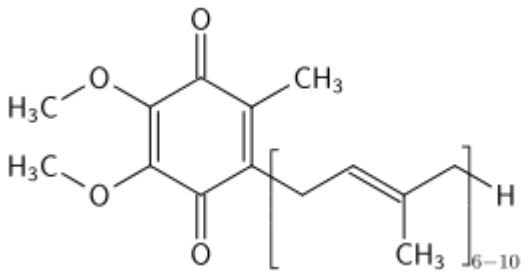
Complex IV



cytochrome-*c* oxidase (ferrocytochrome-*c*:oxygen oxidoreductase, EC 1.9.3.1)
2 ferrocytochrome-*c* + ½ O_2 + 2 H^+ → 2 ferricytochrome-*c* + H_2O (+ 2 H^+ transported)
Contains heme and copper centres

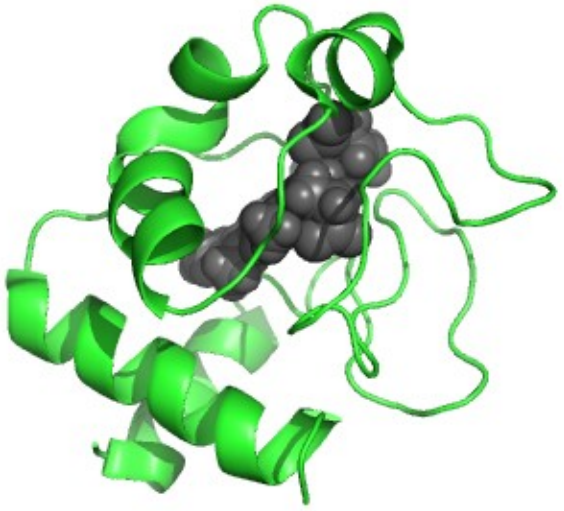
Respiratory chain

Ubiquinone (coenzyme Q_{10})



Can be reduced to hydrochinonic form

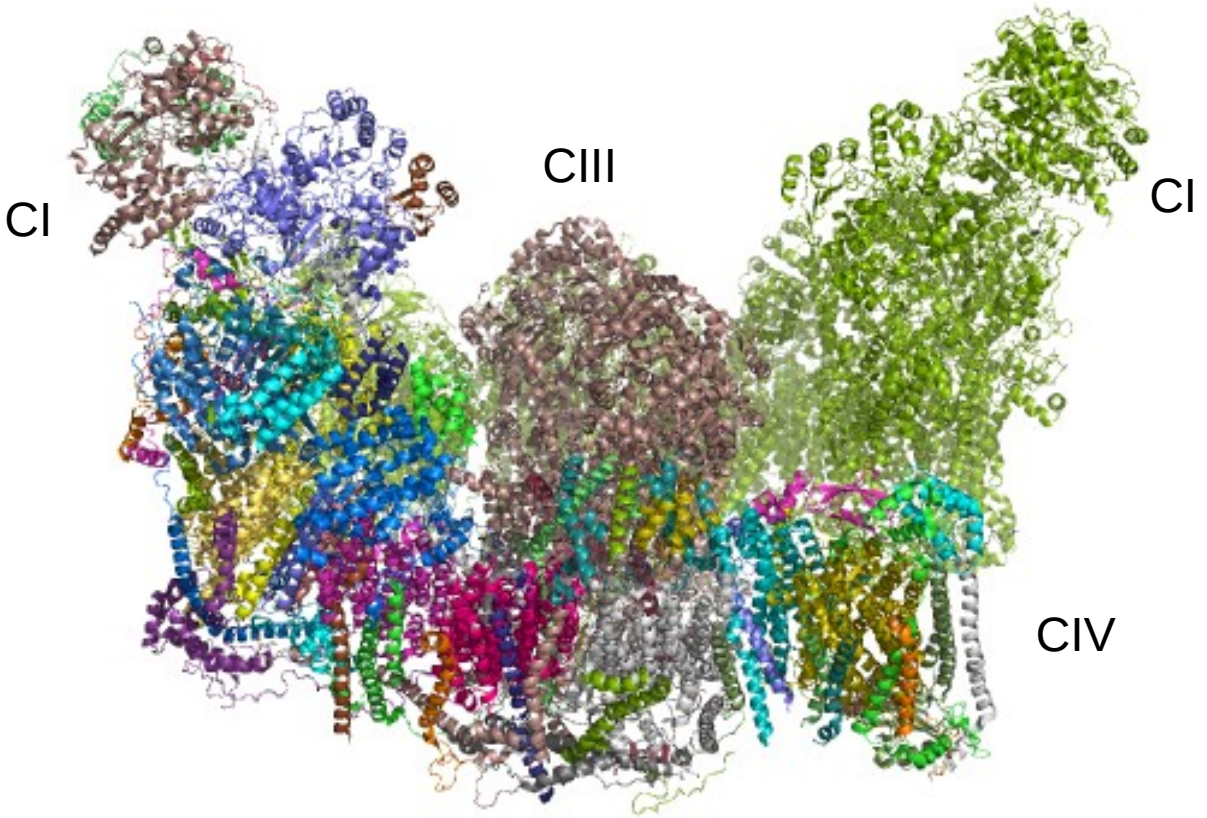
Cytochrome-*c*



oxidized: ferri-
reduced: ferro-

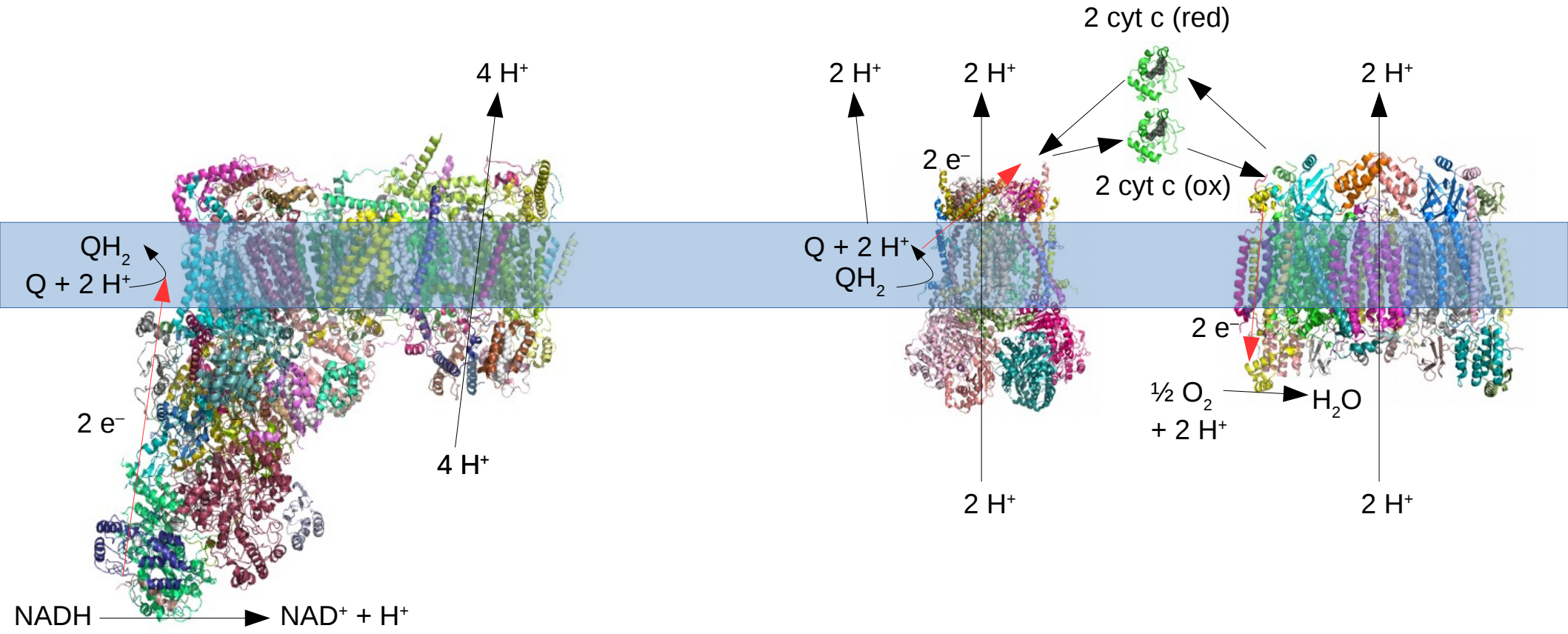
Respiratory chain

Megacomplex



Respiratory chain complexes may assemble into a large megacomplex.

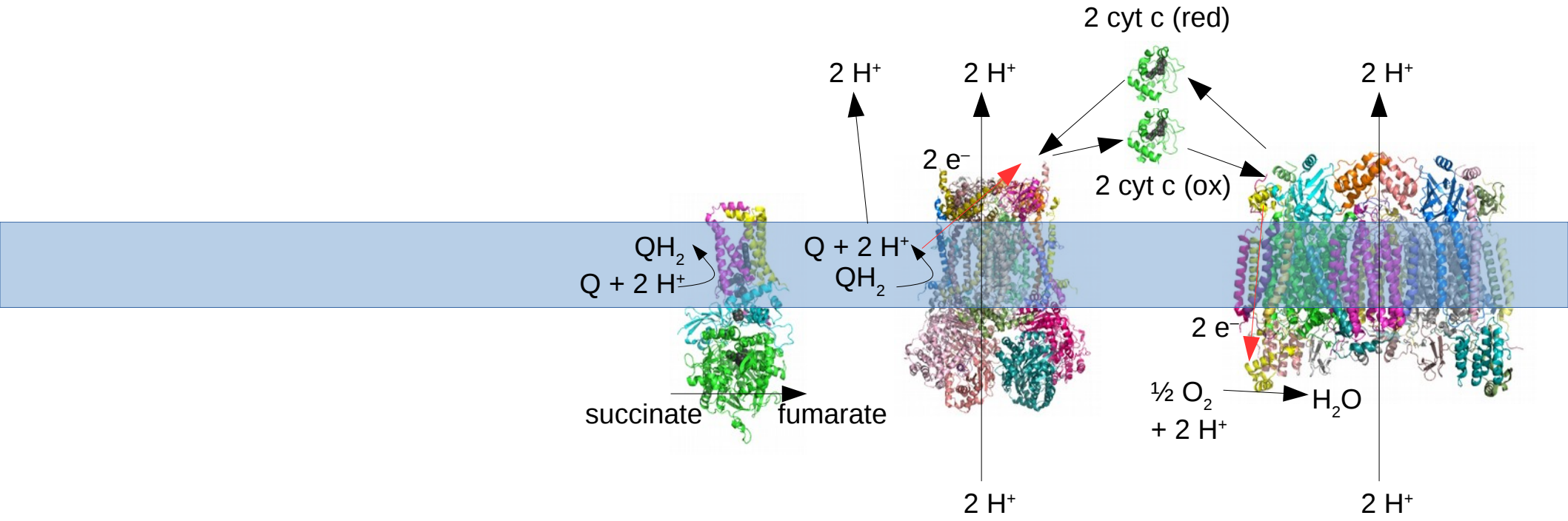
Respiratory chain



NADH as the substrate

NADH + ½ O_2 + 3 H^+ (in) → NAD^+ + H_2O + 2 H^+ (out) (+ 8 H^+ transported)

Respiratory chain

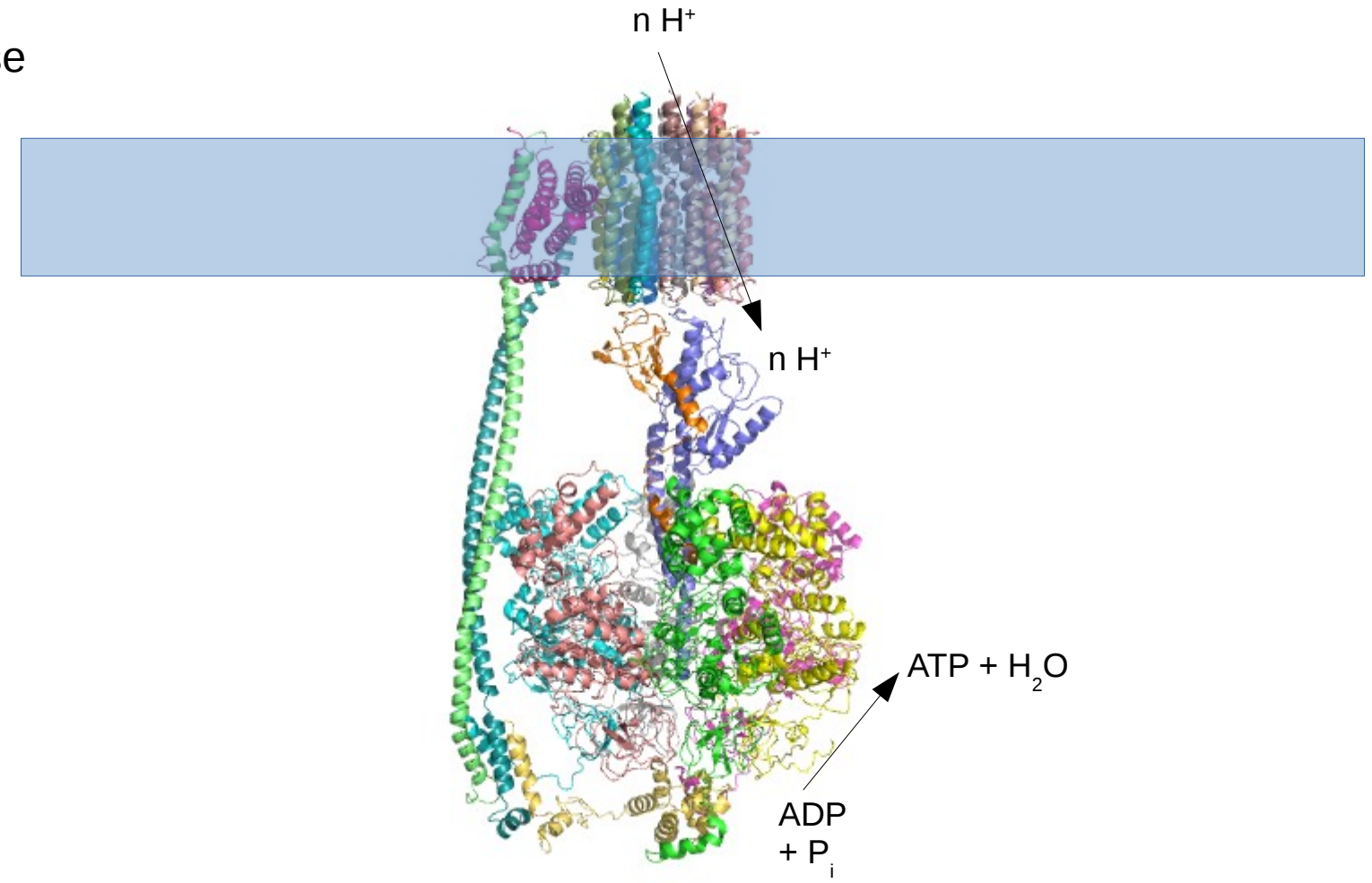


succinate as the substrate

succinate + ½ O_2 + 4 H^+ (in) → fumarate + H_2O + 2 H^+ (out) (+ 4 H^+ transported)

Respiratory chain

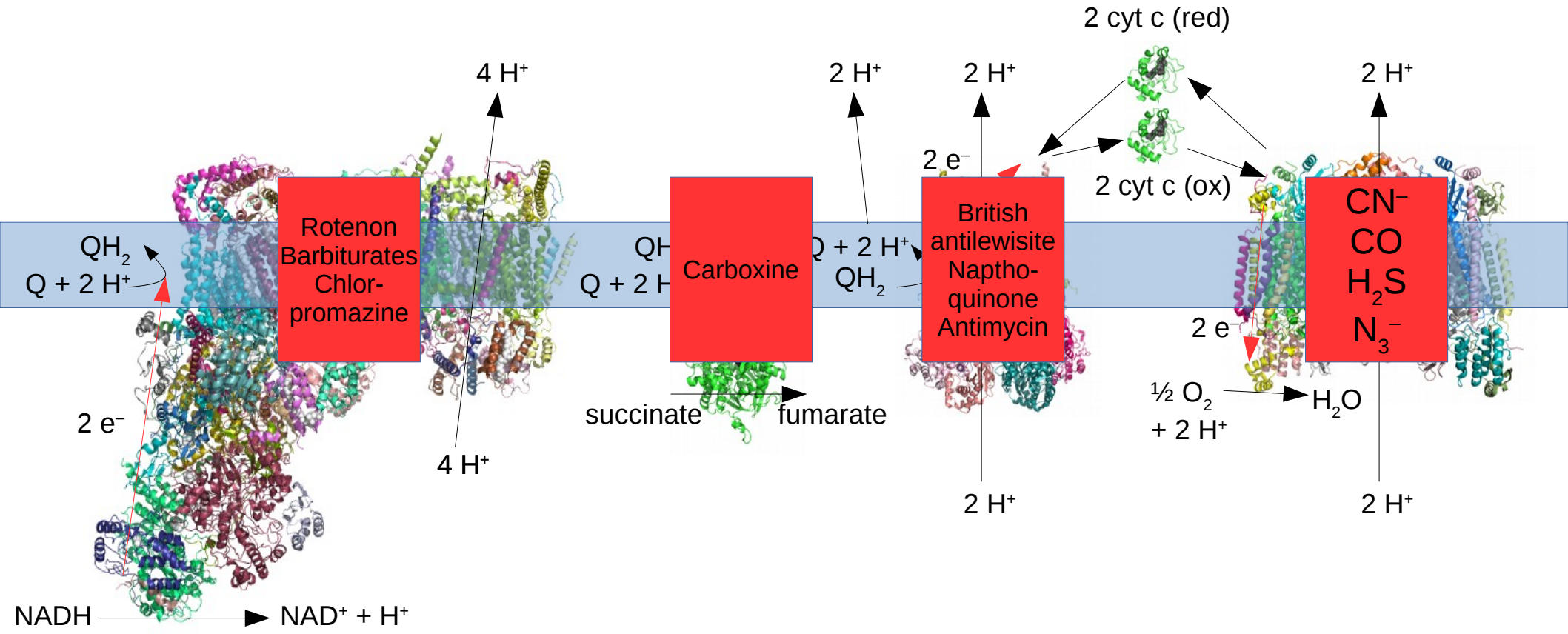
ATP synthase



https://www.youtube.com/watch?v=oFgMTdVRi6I

Respiratory chain creates the gradient of protons on the membrane by active transport. This is associated with the concentration gradient as well as membrane potential. Energy stored in this (proton motive force) is used to create ATP by ATP synthase via passive tranport. This enzyme is also known as F-ATPase or F_OF_1-ATPase (o for oligomycin inhibitor, sometimes written as F_0F_1). This enzyme converts proton motive force to rotation (watch the movie!!!) and rotation to ATP synthesis.

Respiratory chain



Research

Respiratory chain was deciphered using various approaches. Application of inhibitors turned out to be very useful. For example it is possible to prepare cytochrome-*c* in oxidized or reduced form. It is also possible to monitor the form of cytochrome-*c* by spectrophotometry. If you isolate complex IV and supply it with reduced cytochrome-*c*, it will oxidize it. If you add cyanide, it will stop. If you add cyanide to whole mitochondria, it will still reduce cytochrome-*c*. This shows that 1. cyanide inhibits complex IV and 2. complex IV is the terminal step. Similarly it is possible to test various inhibitors to determine the order of complexes.

Respiratory chain

Chemiosmotic theory (theory involving formation of proton motive force and its conversion to ATP synthesis) had been controversial for a long time. One of evidence supporting it is the function of decouplers. These are compounds reducing the proton motive force. Examples are:

2,4-dinitrophenole: Can pass the membrane in both forms and thus transport protons along the gradient. This ceases proton motive force.

Valinomycin: This peptide-based compound makes pores in the mitochondrial membrane. These pores can transport potassium cations. This does not influence concentration gradient of protons (protons cannot pass) but it ceases electrostatic potential (charge gradients). Potassium cations move into the mitochondria to compensate charge gradient caused by protons exported by respiratory chain. Therefore, valinomycin makes only partial effect compared to 2,4-dinitrophenole.

Human newborn infants and some hibernating animal can decouple proton motive force in non-shivering thermogenesis in brown adipose tissue. They can switch on the protein **thermogenin**, which leaks protons out of mitochondria, while converting proton motive force to heat. This process is controlled by GPCR (β-adrenergic receptor), which triggers formation of cAMP, cAMP activates protein kinase, this activates a lipase which produces a fatty acid. The fatty acid activates thermogenin.

Enzymes that use oxygen as a substrate (oxidases, oxygenases), cyclooxygenase, glucose oxidase, cholesterol oxidase, collagen hydroxylation, cytochrome P450

Oxidase – oxidases a substrate by molecular oxygen, but does not incorporate it into the product

examples: Glucose oxidase – fungal enzyme, oxidizes glucose by oxygen yielding hydrogen peroxide, used in diagnostics to measure glucose

Cholesterol oxidase – microbial enzyme, oxidizes cholesterol by oxygen yielding hydrogen peroxide, used in diagnostics to measure cholesterol, HDL and LDL

Oxygenase – oxidases a substrate by molecular oxygen and incorporates it into the product

examples: Cyclooxygenase – human enzyme, involved in inflammation, target of aspirin, ibuprofen, diclofenac etc.

Procollagen-proline dioxygenase – hydroxylates collagen, uses oxygen as oxidation agent and oxidizes Pro in procollagen to collagen and 2-oxoglutarate to succinate and CO_2. Requires Fe(2+) and ascorbate.

Cytochrome P450 – human detoxification factory

Reactive oxygen species (oxidation stress)
H_2O_2, $O^{2-}\bullet$, OH●, singlet oxygen, glutathione

Hydrogen peroxide – toxic compound, damages membranes, DNA and other components, can be decomposed by catalase (in animals and bacteria) or by peroxidase (by plants)

Superoxide – can be decomposed by superoxide dismutase (two superoxides yield one oxygen and one peroxide)

Hydroxyl radical

Singlet oxygen

Glutathione – peptide γ-Glu-Cys-Gly, reduced with SH group, oxidized as a dimer with a disulphide bond -S-S-, reduced by NADPH from pentose phosphate pathway, main antioxidant in the cell