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PROJECT

Energy storage in organisms

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Glossary

amino acid	building block of proteins
anabolism	processes whose result is the construction of larger molecules from smaller units
ATP	Adenosine Triphosphate or ATP is the prevalent high-energy molecule in organisms and is often referred to as its energy currency. It effectively transports energy to where it is needed.
ATP Synthase	protein in the Electron Transport Chain responsible for converting an electrical gradient into chemical energy in form of ATP
Calvin Cycle	process in plants that produces glucose using ATP and NADPH as energy-containers
catabolism	processes whose result is the decomposition of large molecules into smaller ones
catalysis	increment of reaction rate due to the presence of an additional substance (catalyst)
cellular respiration	metabolic reactions and disposal of waste products in the cell
chloroplast	unit responsible for glucose production using the energy of photons (photosynthesis)
coenzyme	necessary compound for functioning of enzymes
contour length	full length of stretched polymer
Electron Transport Chain (ETC)	system of protein complexes in the mitochondrial inner membrane responsible for the synthesis of a high-energy molecule called Adenosine Triphosphate (ATP)
endosymbiotic theory	evolutionary theory consisting in the advent of eukaryotes from prokaryotes
enzyme	biological catalyst

eukaryotic cell	cell consisting of a cell nucleus and other membrane-enclosed organelles
FADH2	Flavin adenine dinucleotide or $FADH_2$ is a high-energy molecule responsible for donating electrons to the Electron Transport Chain for energy production
fatty acid	high-energy macromolecule that is exceptionally energy-dense
glucose	high-energy macromolecule that delivers energy quickly
glycogen	high-energy macromolecule stored in muscles
glycolysis	decomposition of glucose molecules
hepatocyte	liver cell
hydrolysis	Separation of chemical bonds by addition of H_2O
hydrophilic	attracted by water
hydrophobic	repelled by water
Krebs Cycle / TCA Cycle / Citric Acid Cycle	process in the mitochondrion in which the Acetyl Coenzyme A is decomposed producing high-energy molecules
macronutrient	molecule responsible especially for providing energy to the organism
metabolism	the sum of processes of construction (anabolism) and decomposition (catabolism) of molecules in the cell
micronutrient	molecule from which no energy can be extracted, but that is still very important for the correct functioning of the metabolism
mitochondrion	unit in the cell responsible for energy conversion through ATP synthesis
NADH	Nicotinamide adenine dinucleotide or NADH is a high-energy molecule responsible for donating electrons to the Electron Transport Chain for energy conversion

NADPH	Nicotinamide adenine dinucleotide phosphate or NADPH is a high-energy molecule responsible for donating electrons in the Calvin Cycle to produce glucose in plants
oxidation	donation of electrons
persistence length	length over which direction of small length-segments changes statistically (tangent vectors of such segments lose correlation)
pH	potential of hydrogen, measured as pH=-$log_{10}[H^+]$
phosphorylation	addition of phosphate group to a compound, especially to ADP, i.e. $ADP + P_i \rightarrow ATP$
polymer	compound consisting of repeating structural units
PPAK/PEVK	important sequence in titin (a muscle protein) responsible for its elasticity
prokaryotic cell	cell containing no membrane-bound organelles
protein	macromolecule composed of amino acids with specific functionality in the cell that can also be decomposed for energy conversion
pyruvate	building block of glucose; can be further divided to produce ATP
reactant/product	in a chemical reaction the reacting molecules are called reactants and the resulting ones products
reduction	reception of electrons
stroma	space surrounding thylakoid
thermogenesis	production of heat in the cell
thylakoid	enclosed region in the chloroplast responsible for producing the high-energy molecules ATP and NADPH
triacylglycerol	macromolecule consisting of three fatty acid molecules held together by glycerol
UCP	Uncoupling proteins or UCPs uncouple the process of oxidation and phosphorylation in the Electron Transport Chain through proton leakage, causing random motion, i.e. heat

Chapter 1

Introduction

Energy storage is one of the most important topics in the modern world and is essential for a sustainable development on our planet. According to statistics from the OECD/IEA the world energy demand has been increasing continually, reaching 1919.502 kg of oil equivalent per capita (unit used to compare energetic content with oil) in 2014 [2]. This is a shocking figure, especially in light of the fact that the percentage of fossil fuel energy consumption with respect to other energy sources has not significantly changed since the 1990s.

An "energy storage revolution" has been highlighted for example as the potential catalyst for clean energy production. The reason is that renewable energies produce electricity in an intermittent manner, given the intermittent fashion of solar irradiation and fluctuating wind speeds, among others. These fluctuations have to be smoothed out, in order to deliver the electricity to the end consumer. That can be achieved by temporary energy storage. The problem is that even though batteries are becoming less expensive, their cost is still relatively high. Hence the enormous importance and potential of innovation in the efficiency of energy storage.

Having said that, it is undoubtably the case, that nature has been able to find its ways to optimize energy storage through natural selection. Triacylglycerols for example are the reason why the American Golden Plover (*Pluvialis dominica*) is able to travel non-stop over large distances over open ocean (3800km on trip from Alaska to the southern tip of South America).[8]

Organisms are able to store different kinds of energy in very different ways. In this project different mechanisms of storage of electrical, chemical, thermal and mechanical energy as well as the conversion from one type into another are going to be explored. Interestingly, the complexity of the task of storing a given kind of energy can be reduced to one single protein. For example, the conversion between electrical and chemical energy is done by the ATP Synthase. Understanding the physics behind each one of these proteins is crucial to create new technologies that hopefully get closer to achieving the incredibly high efficiencies inside living organisms.

That approach is called biomimetics and could greatly contribute to solve many technological challenges and work towards achieving the goals of the Millenium Project as described in the article *"A gaze into the crystal call: biomimetics in the year 2059"*[19]. Many important innovations, such as the airplane, were inspired by nature, but the strategic innovative approach is quite recent.

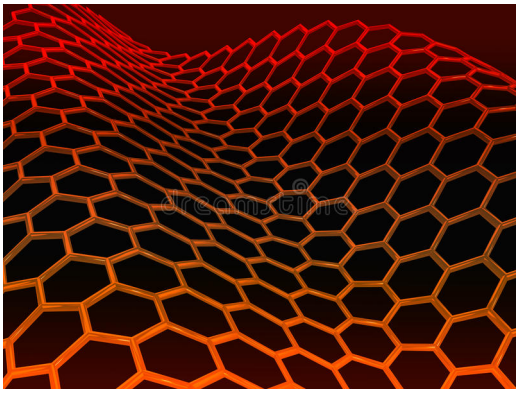
Chapter 2

How nature inspires technology

Technology faces many challenges; from products to make our everyday life more efficient, to medical applications, all the way through its most fundamental aspect on which we depend now more than ever: energy conversion and storage.
As previously discussed, those two topics go hand in hand. To achieve disruptive innovation one has to think outside of the box and explore new territories. Nature is already doing lots of processes much more efficiently than current human technology could achieve. That is because organisms had the chance to evolve over billions of years through natural selection to continuously optimize processes that were important for survival; processes such as energy storage in their bodies. Fat is an incredibly energy-dense substance. To illustrate that statement, let us look at some numbers:

Table 2.1.

energy storage device	**energy density [Wh/kg]**
fat	10611
good lead acid battery used in cars	42
best Li-ion battery	190
All-graphene battery [20]	225



That means a human being with about **20 kg of fat**, stores the same amount of energy as about **1000 kg of the best batteries available on the market**. As we can see, fat is incredibly energy-dense. If its energy could be effectively extracted in industry, this would mean a breakthrough in energy storage.
This method of extracting important innovations in clue technological issues from nature is called Biomimetics. Once a technological problem is posed, one asks the question: how would nature do it? Biophysical/biochemical processes are explored to try to discover better solutions to current problems. Fundamental principles are then abstracted and efforts of technological implementation are made.

A **Best Practice example** of biomimetics is the Shinkansen Bullet Train[4] which belongs to the fastest trains on the planet, reaching speeds of up to $320\frac{km}{h}$. Engineers were troubled about the noise it generated. Soon Eiji Nakatsu, chief engineer of the train, realised that learning from birds flying from one medium to another would help to design a more efficient vehicle. It just so happens that kingfishers whose beak dives into water to catch fish without much splash inspired the design of a train that was quieter, consumed 15% less electricity and travelled 10% faster.

Chapter 3

Basics of metabolism

Energy storage is part of a bigger set of biophysical/biochemical processes that maintain the energetic balance inside of the cell. This project aims to discuss the physics of particular proteins involved in energy storage. To do that, nevertheless, it is necessary to understand some background about the metabolism of the cell. The specific proteins will be the protagonists of key cellular metabolic processes.
So what is metabolism? It basically maintains the balance between synthesizing molecules and storing them (anabolism) and breaking big molecules down particularly for energy extraction (catabolism). Different molecules have different amounts of energy that can be extracted and used inside an organism. High-energy molecules will be discussed in detail once the foundations of the metabolic pathways are established.

Map of metabolic processes

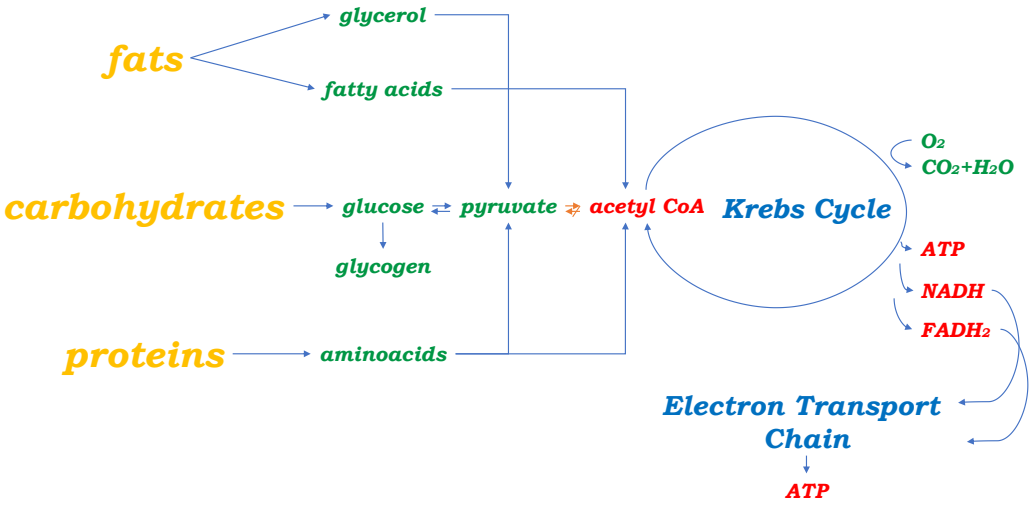


Figure 3.1: Stages of metabolism

3.1 Metabolic processes

As can be seen in the graph above, different macronutrients (proteins, carbohydrates and fats) can be metabolized into each other through several stages of reactions that are not further specified here to avoid too much complexity. According to its needs, the organism can then store the energy in one form or another. For energetic utilization the macronutrients have to be split into smaller constituents, ultimately leading to the production of the Acetyl Coenzyme A (Acetly-CoA) that can then be metabolized in the Krebs Cycle.

3.1.1 Krebs Cycle

Once a compound has been converted into Acetyl-CoA, the process is irreversible and the so-called Krebs cycle or TCA cycle (Tricarboxylic Acid Cycle) is entered. This and the subsequent Electron Transport Chain happens in the mitochondria, which are considered to be the power generators of the cell. During the Krebs Cycle for each original pyruvate one ATP molecule is formed thanks to enzymes that catalyze the reaction $ADP + P_i \rightarrow ATP$. Additionally three $NADH$ molecules and one $FADH_2$ molecule are synthesized which have no direct use but serve as energy carriers for further energy release in the Electron Transport Chain.

3.1.2 Electron Transport Chain

During the Electron Transport Chain the electrons in the $NADH$ and $FADH_2$ molecules are used for transporting protons from inside the mitochondria (matrix) to the intermembrane space. This creates an electrostatic gradient that forces the protons to re-enter the matrix. In this process a protein called ATP Synthase is involved, which together with the incoming protons synthesizes ATP.
As we can see, ATP production is the ultimate goal of cellular respiration which suggests that this compound plays an important role. It contains large amounts of energy and is well-suited for energy transport. For that reason ATP and other high-energy molecules will be looked at in more detail.

Note: Thylakoids in chloroplasts, where photosynthesis happens, do also have an Electron Transport Chain. ATP is produced by the exact same mechanism, the only difference being the energy source which is photons.

3.2 High-energy molecules (HEM)

As we have seen in Chapter 2, there are some molecules that serve as energy-carriers. The so-called "high-energy" molecules are of great importance for energy storage. They contain different amounts of energy and fulfil different tasks. In this chapter the most important such energy-carriers are going to be explored and special attention will be given to their energy density and the efficiency by which energy can be extracted from them.
Fundamentally the energy budget of the high-energy molecules below will be measured based on the change in the thermodynamic Gibbs Free Energy G before and after a reaction.
The change in Gibbs Free Energy is given by [15]

$$\Delta G = \Delta G^0 + RTln\left(\frac{c_{products}}{c_{reactants}}\right) \tag{3.1}$$

ΔG^0 is the free energy change under the assumption of standard conditions (T=298K, pH=7) when reactants and products coexist in equal concentrations. The former is usually the case, but concentrations of reactants and products are usually distinct. They reach an equilibrium at $\Delta G = 0$ which delivers the following relationship:

$$\Delta G^0 = -RTln\left(\frac{c_{products}}{c_{reactants}}\right) \tag{3.2}$$

Measurement[27]: ΔG^0 is not directly measured. To determine it, accurate information about intracellular concentrations is needed. These measurements can be done using different methods depending on the organism in question. In the case of ATP hydrolysis in humans for example, a nuclear magnetic resonance measurement is needed. In *E.coli* on the contrary an ATP bioluminescence assay is sufficient.

3.2.1 HEM 1: ATP

The ultimate energy-carrier in the cell is called Adenosine Triphosphate (ATP). Many reactions in the cell are endergonic ($\Delta G^0 > 0$) which means they require additional energy input to become favourable in the forward direction. ATP is often called the energy currency of the cell. This is so because ATP hydrolysis, i.e. the reaction

$$ATP \rightleftharpoons ADP + P_i \tag{3.3}$$

is highly exergonic ($\Delta G^0 < 0$) meaning it releases large amounts of energy. A great number of reactions inside the cell are endergonic and can therefore not happen spontaneously. When coupled with ATP hydrolysis however the total sum of the Gibbs Free Energies is still negative, meaning the reaction happens spontaneously because it releases some overall energy. Why exactly ATP and not another compound has been chosen by nature is not yet fully understood, but it is widely known that nucleoside phosphates are excellent energy carriers because the oxygen atoms attached to the phosphorus atoms are negatively charged leading to electrostatic repulsion.
The energy released by ATP would generally go into heat, i.e. random motion. Nevertheless, enzymes have the task of catalysing unfavourable reactions. They can couple reactions for example using ATP hydrolysis to fulfil specific tasks. In figure 3.2 a depiction of the chemical structure of

ATP is presented.

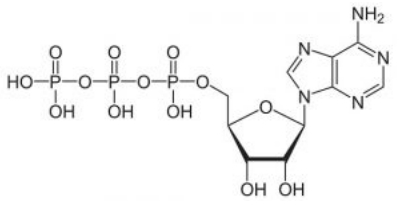


Figure 3.2: Chemical structure of Adenosine Triphospate (from [16])

Thanks to the oxygen atoms that are bound to the phosphates, the electrostatic repulsion causes a large ΔG^0 which can be measured to be about $-30.5\frac{kJ}{mol}$[15].
In the table below a comparison of the hydrolysis enthalpy in human muscle cells during stress and recovery, as well as in *E.coli* and in standard conditions is compared.

Table 3.1. [27]

physiological condition of organism	**ATP conc.**	**ADP conc.**	P_i **conc.**	**Inferred Δ G** $[kJ/mol]$
standard conditions	1 M	1M	1M	-36 to -38
E. coli aerobic exponential growth on glucose	10 mM	0.6 mM	20 mM	-54
E. coli anaerobic exponential growth on glucose	3 mM	0.4 mM	10 mM	-54
E. coli aerobic exponential growth on glycerol	7 mM	0.7 mM	10 mM	-55
Homo sapiens - resting muscle	8 mM	9 μM	4 mM	-68
Homo sapiens - muscle recovery from severe exercise	8 mM	7 μM	1 mM	-72

As we can see, the amount of energy that can be extracted is largely dependent on the intracellular conditions. The resulting ΔG in physiological conditions is generally much higher than in standard conditions.

3.2.2 HEM 2: NADH

The importance of ATP as the energy currency in the cell has already been highlighted. In order to produce all of the ATP however, a coenzyme called NADH is incredibly important. NADH can be described as the "biological hydrogen".[9] It is a crucial electron donator that is the main driver of the Electron Transport Chain. By far most of the energy in the cell (in form of ATP) is produced by the Electron Transport Chain. To illustrate this, the amount of energy produced by a glucose molecule in form of ATP in each metabolic stage can be seen below:

Glycolysis	2 ATP
Krebs Cycle	2 ATP
Electron Transport Chain (in an efficient cell)	34 ATP

The ETC is clearly the protagonist of mitochondrial metabolism being NADH the primary reduction agent. So it is important that NADH is present in sufficient concentrations for the metabolism to work efficiently.

The Gibbs Free Energy change under standard conditions for NADH oxidation, i.e.

$$NADH \rightarrow NAD^+ + H^+ + 2e^- \tag{3.4}$$

is

$$\Delta G_0 = -158.2kJ/mol \quad (from[23]) \tag{3.5}$$

Nevertheless this reaction is accompanied by the reduction of oxygen:

$$\frac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_2O \tag{3.6}$$

which adds an additional energy contribution of

$$\Delta G_0 = -61.9kJ/mol \quad (from[23]) \tag{3.7}$$

3.2.3 HEM 3: NADPH

The energy-carrying compound NADPH, which is synthesized during photosynthesis and used to synthesize glucose, has basically the same reduction/oxidation properties and therefore the same oxidation energy as NADH. The only difference lies in the reactions it triggers (enzymes are sensitive to the additional phosphate).

3.2.4 HEM 4: $FADH_2$

$FADH_2$ does, just like NADH, act as an important reduction agent, donating electrons to the ETC to build up a proton gradient along the inner mitochondrial membrane. Nevertheless, smaller quantities of it are produced during the Krebs Cycle.
The Gibbs Free Energy change under standard conditions for $FADH_2$ oxidation, already taking into account the reduction of oxygen, i.e.

$$FADH_2 + \frac{1}{2}O_2 \rightarrow FAD + H_20 \tag{3.8}$$

provides us the following value

$$\Delta G_0 = -181.6kJ/mol \quad (from[23]) \tag{3.9}$$

3.2.5 Glucose

Glucose provides rapid energy to an organism. It contains a Gibbs Free Energy of

$$\Delta G^0 = -2845kJ/mol \quad (from[15]) \tag{3.10}$$

weighing $180\frac{g}{mol}$ and therefore yielding an energy density of about 15.8 kJ/g.

Energy balance after glycolysis and Krebs Cycle:

Produced high-energy molecules	Energy $[kJ/mol]$
$4ATP + 10NADH + 2FADH_2$	2686.2

Therefore up until this point only about 5.6% of the energy got lost into heat. In the ETC however, $NADH$ and $FADH_2$ are converted into "only" 34 ATP meaning that 59.6% of the energy was not converted into ATP. Around 40% efficiency is a significant amount when compared to efficiencies of human-built machines, nevertheless it is the most inefficient part of the metabolic chain. It has to be further added that this is the maximum efficiency, assuming no additional losses. In reality however, as will be explained in the chapter about thermal energy release, uncoupling proteins in the inner mitochondrial membrane could disturb this process leading to lower efficiencies.

Maximum overall efficiency:

$$\eta_{max} = \frac{38 \cdot \Delta G^0_{ATP}}{\Delta G^0_{glucose}} \approx 40.7\% \tag{3.11}$$

3.2.6 Fatty acids

Fat molecules are composed of three fatty acids and glycerol. It is the nutrient with the highest energy density. A 16-carbon fatty acid (palmitic acid) provides a Gibbs Free Energy of

$$\Delta G^0 = -9782.2kJ/mol \quad (from[15]) \tag{3.12}$$

weighing $256\frac{g}{mol}$, yielding an energetic density of about $38.2\frac{kJ}{g}$, approximately 2.4 times the amount of glucose. That is the reason organisms predominantly store fat for later energy use.

Energy balance after lipolysis and Krebs Cycle:

Produced high-energy molecules	Energy $[kJ/mol]$
$7ATP + 31NADH + 15FADH_2$	9760.6

This implies an incredible efficiency of about 99.8%, meaning that fat is not only much more energy dense but also more efficient in its initial stages of metabolism. After conversion to ATP in the ETC, providing a total of 130 ATP molecules, the maximum efficiency drops to

$$\eta_{max} = \frac{130 \cdot \Delta G^0_{ATP}}{\Delta G^0_{palmiticacid}} \approx 40.5\% \tag{3.13}$$

signifying no significant efficiency deviation from glucose.

Chapter 4

Energy storage in the living cell

4.1 Thylakoids

Thylakoids are the enclosed regions within chloroplasts where photosynthesis takes place. They consist of a space enclosed by a phospholipid membrane as depicted in figure 4.1.

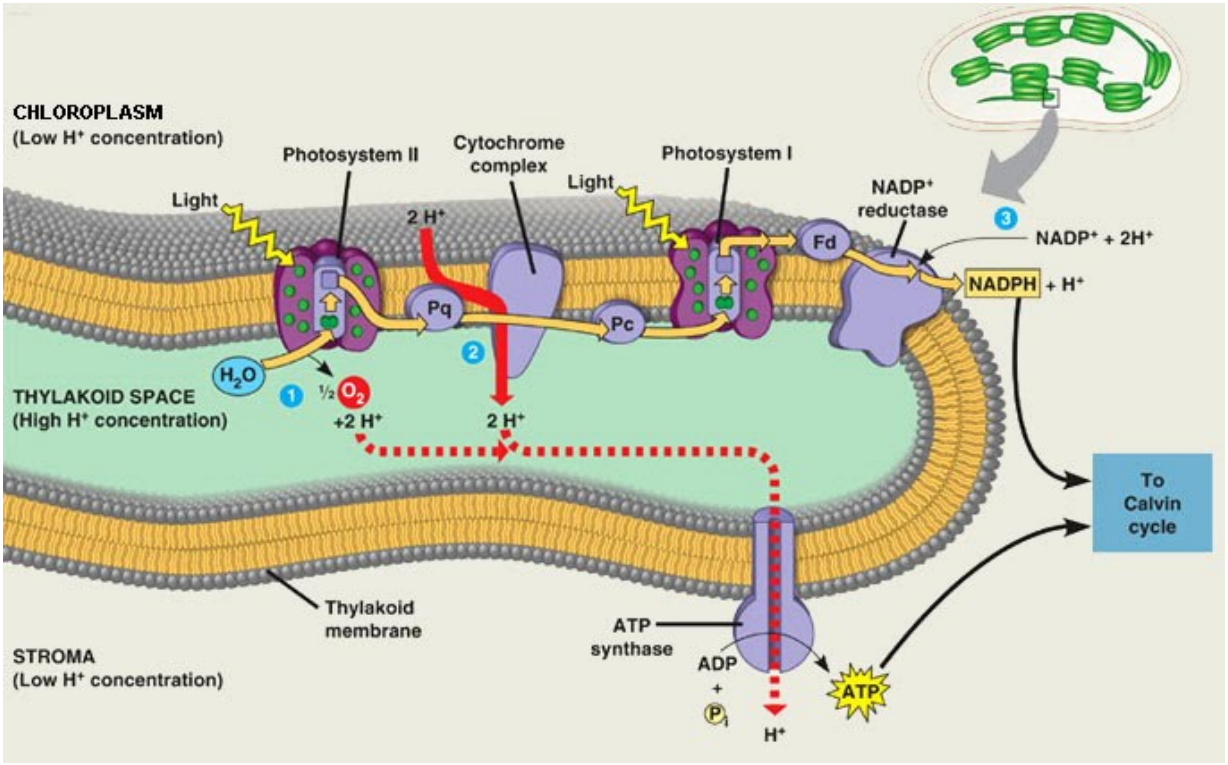


Figure 4.1: Thylakoid (from [3])

This membrane includes a very similar Electron Transport Chain as the one in the inner membrane of the mitochondrion that will be discussed in detail later. Basically energy is "produced"

in the following way:

1. Photophosphorylation: Photons excite electrons in the so-called Photosystem II that retains the energy. As the electron travels through the chain, several proteins cause protons to flow into the thylakoid space (space enclosed by membrane) creating a proton gradient between thylakoid space and stroma (outside of the membrane). The proton motive force (PMF) drives the ATP production through ATP Synthase that will also be discussed in the section about mitochondria in more detail.

2. Synthesis of NADPH: In Photosystem I the energy of photons is used to excite electrons that cause the synthesis of NADPH from NADP and H.

3. Calvin Cycle: NADPH and ATP go into the Calvin Cycle to produce glucose which is then in later stages brought to mitochondria that produce more ATP with it.

During photophosphorylation and NADPH synthesis 8 photons are needed to produce 3 ATP and 2 NADPH molecules. The photosynthetically active radiation range lies within 400-740nm with absorption peaks on the extremes.[36] As a simplifying assumption for the following calculations it will be estimated that the average absorbed photon, independent of the photosystem, has a wavelength of 570nm which lies exactly at half the range. This would imply the following:

Energy input [aJ]	Energy output [aJ]	Efficiency [%]
2.788	0.883	31.67

So photophosphorylation is quite inefficient, contrary to the Calvin Cycle. 18 ATP and 12 NADPH are necessary to synthesize one glucose molecule.[15] That means:

Energy input [kJ/mol]	Energy output [kJ/mol]	Efficiency [%]
3190.2	2845	89.18

All of the above calculations assumed all photons to have a 570nm wavelength and therefore be in the photosynthetically active range. Several losses lead to a decrease in efficiency. All in all the efficiency is approximated to be around:

$$\eta_{photosynthesis} \approx 4.6\% - 6\% \,[36] \tag{4.1}$$

Photosynthesis is therefore far less efficient than ATP production in the mitochondrion. Because of that and because both work on the principle of Electron Transport Chains with the main difference of the energy source (light versus macromolecules) we will now focus on the mitochondrion. However the advantage of thylakoids, being the independence from already preexisting macromolecules, has to be noted.

4.2 Mitochondria - the batteries of the cell

Mitochondria are remarkable organelles inside the eucaryotic cell. It is believed they originate from procaryotic cells like bacteria, since their metabolism is very similar (endosymbiotic theory). What is so interesting about mitochondria is that they quite literally serve as the power generators of the eucaryotic cell. They produce electrical, chemical and thermal energy and are able to convert from one type into another. What exactly is meant by "power generators" or "batteries" of the cell is explored in more detail now.

4.2.1 Electrical energy storage

When the different stages of metabolism were explored in Chapter 1, it was mentioned that the last two stages, namely the Krebs Cycle and the Electron Transport Chain (ETC) occurred inside the Mitochondria. Let us now focus on the ETC since it is the stage that produces the most energy and is directly tied to the energy conversion processes.

In figure 4.2 the Electron Transport Chain is depicted. This chain is located in the inner membrane of the mitochondrion.

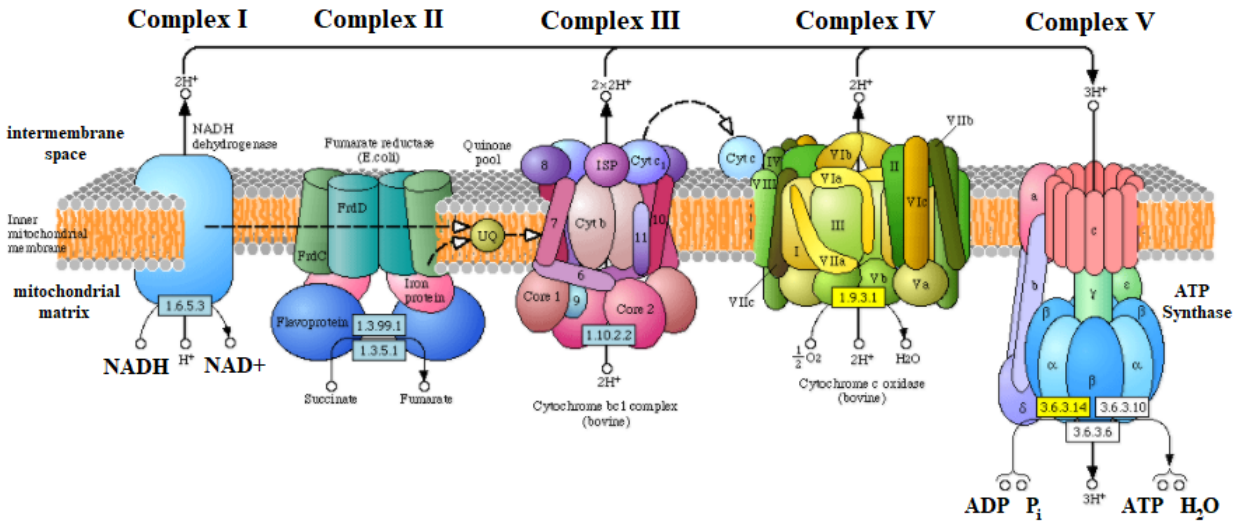


Figure 4.2: Electron Transport Chain (from [18])

As can be seen, several protein complexes participate in the process. To summarize, Complexes I, III and IV are responsible for the active transport of protons from the matrix to the intermembrane space, using two electrons from NADH. Complex II does the same, but extracts its electrons from the high-energy molecule $FADH_2$ instead of NADH. Now, the amount of protons transported by each Complex is listed below:

Complex I	4 H^+
Complex III	2 H^+
Complex IV	4 H^+
Total per NADH	10 H^+

So once the electrons from a single NADH molecule have passed the Electron Transport Chain, a total of 10 protons have been transported from the matrix to the intermembrane space. This creates an electrostatic gradient between matrix and intermembrane space as mentioned before and the voltage can be measured to be about **155.46 mV** under normal conditions of $\Delta G_{ATP} = -50kJmol^{-1}$ and a temperature of 310K.[12] This is important because through this mechanism electrical energy has been effectively stored. Parting from this point, the electrical energy can be converted into chemical or thermal energy.

4.2.2 Chemical energy storage

In the cases reported above, electrical energy was stored by the creation of a proton gradient. Nevertheless storing the energy chemically as ATP is much more useful for the cell in many cases, one reason being that energy needs to be transported and used to catalyse reactions using enzymes. That is where a very special protein called ATP Synthase comes into play. The electrostatic gradient forces the protons to re-enter the matrix passing through this enzyme that is also referred to as Complex V. It causes the synthesis of ATP from ADP and inorganic phosphate. So this enzyme effectively converts electrical into chemical energy in form of ATP. But stunningly it can also work in reverse and functions at near 100% efficiency (when in equilibrium), far surpassing human technology.[12]
Let us take a closer look into the structure, functioning, thermodynamics and kinetics of this remarkable molecular machine. It is at the very core of the cell's energy production.

ATP Synthase (Complex V)

This astonishing molecular machine, capable of converting back and forth between electrical and chemical energy through a quite simple rotary motor mechanism, is key to the energy storage in the cell. This is what basically makes mitochondria the batteries or power generators of the cell. That is why understanding the physical mechanisms behind it is of such utmost importance and has been subject to lots of research.

Structure: The enzyme basically consists of two sectors. One that is contained in the inner mitochondrial membrane and is mostly hydrophobic (F_0) and one inside the matrix (F_1) as is depicted in figure 4.3.

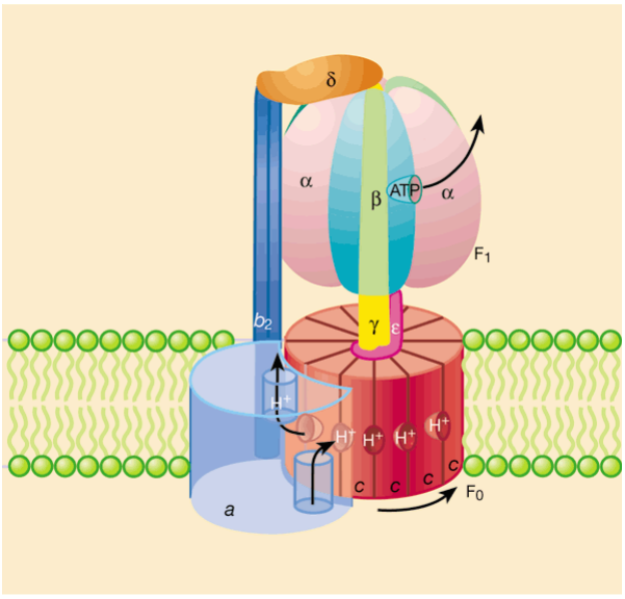


Figure 4.3: ATP Synthase (from [11])

The structure of each region is shortly mentioned:

- F_0 **region:** This region consists of one a-subunit with two half-channels where protons flow through and 10 to 14 c-subunits organized in a cylindric shape that will rotate due to the PMF (proton motive force).
- F_1 **region:** This region contains an $\alpha_3\beta_3$ hexamer ring structure connected to the a-subunit in the F_0 region via a δ-subunit. The β-subunits are where ATP synthesis takes place. The central structure formed by the γ- and ϵ-subunits is called central stalk. It is connected to the c-structure in the F_0 region and reaches the central cavity of the hexamer ring.

Functioning of the complex: While the ATP Synthase is functioning, there is a stationary part (a-subunit in F_0 connnected to hexamer ring in F_1) and a rotating part (c-ring and central stalk). The part where the proton motive force originates is in the c-ring. The protons move through one half-channel, generate a rotation and exit the ring in the second half-channel. This rotation changes the state of the β-subunits in the hexamer ring bringing the reactants ADP and P_i closer together to form ATP.

A natural question to ask would be how many protons are required for the synthesis of one ATP, which is denoted by n. A full rotation of the central stalk produces 3 ATP, which requires 10 to 14 protons depending on the amount of c-subunits in the c-ring. Hence

$$n = \frac{360°rotation}{3ATP} \cdot \frac{(10-14)H^+}{360°rotation} \approx (3.33 - 4.67)\frac{H^+}{ATP}$$

As can be clearly seen, the efficiency of ATP synthesis depends on the amount of c-subunits in the F_0 region. The efficiency will be discussed in detail later.

Thermodynamics, Kinetics and Efficiency of ATP Synthase

The relationship between the logarithmic ratio of forward to reverse 120° rotations and the applied torque, which provides a thermodynamic description of the ATP synthesis in ATP Synthase, is reported to be linear by Toyabe *et al.*[33] Efficiencies of ATP production in the Electron Transport Chain were previously discussed in the "High-energy molecules" section. Now the role of ATP Synthase in influencing these efficiencies will be explored.
As suggested by Toyabe et al. the following equation applies:

$$k_B T ln \frac{p_S}{p_H} = (N - N_{stall})d \tag{4.2}$$

where p_S and p_H are the fractions of rotational steps in synthetic (ATP producing) and hydrolytic (ATP separating) direction and their sum must equal unity. N is the applied torque and d is the rotational stepsize in degrees. N_{stall} and d can be experimentally fitted and result in values of $N_{stall} = 31.2 pN nm rad^{-1}$ and $d = 55°$.[12] This value for d is inconsistent with previous measurements from Noji *et al.* where steps of 120° were suggested but not completely confirmed since Brownian fluctuations hampered a more precise analysis. This, according to Chapman and Loiselle[26], is caused by non-physiological molecular friction because the biomembrane was experimentally removed.
In any case the value for N_{stall} suggests a near 100% efficiency of the protein since

$$N_{stall} \cdot \frac{2\pi}{3} \approx 65.3 pN nm \approx \Delta\mu \tag{4.3}$$

where $\Delta\mu$ is the ATP synthesis/hydrolysis free energy. This rough equality suggests that there is almost no loss of energy, implying almost 100% efficiency. While this is true, it is misleading because it suggests that this efficiency could be achieved even in a non-equilibrium situation, which would contradict the Second Law of Thermodynamics.
The efficiency can be calculated as follows:

$$\eta = \frac{\Delta G_{ATP}}{\Delta G_{dissipation} + \Delta G_{ATP}} \tag{4.4}$$

The dissipation free energy $\Delta G_{dissipation}$ is only zero if ATP Synthase is in equilibrium, meaning the working in forward direction is just as likely as in backward direction. Otherwise it depends on the forward rate r_f and the backward rate r_b as follows:

$$\Delta G_{dissipation} = RT ln \left(\frac{r_f}{r_b} \right) \tag{4.5}$$

Different values for r_f and r_b and the resulting efficiencies were extracted from table 1 of the work of Chapman and Loiselle[26]:

Table 4.1.

r_f/r_b	$r_f[mM/s]$	$r_b[mM/s]$	$\Delta G_{dissipation}[kJ/mol]$	$efficiency[\%]$
1.01	1010	1000	0.026	99.95
1.1	110	100	0.246	99.51
11	11	1	6.177	89.00
101	10.1	0.1	11.889	80.79
10^3	10.01001	0.01001	17.795	73.75
10^4	10.001	0.001	23.727	67.82

4.2.3 Thermal energy storage

It is fascinating how heat is generated in living organisms. And astoundingly the mechanism is very similar for animals and plants. As previously discussed, the Electron Transport Chain consists of basically two parts: the oxidation that takes place in Complexes I through IV and creates a proton gradient, and the phosphorylation taking place in the ATP Synthase. This phosphorylation requires a Proton Motive Force (PMF) which in essence means oxidation and phosphorylation are coupled.
Thermogenesis is caused by the uncoupling of these processes and the responsible proteins are called uncoupling proteins as explained in [30]. There are different such proteins denoted by the abbreviation UCP. The most important UCP in mammals and the first to be discovered is UCP1. pUCP1 is its version for plants. It is believed that these proteins evolved before the animal and plant kingdom were separated conducing to a common functioning of thermogenesis.
The general functionality of UCPs is quite straightforward although the exact mechanism behind them is not yet fully understood. They uncouple oxidation and phosphorylation by allowing proton leakage through the inner mitochondrial membrane as depicted in figure 4.4. The leaked protons cannot be used for ATP synthesis anymore, increasing the concentration of ADP in the matrix. This ADP can then be further used in the Krebs Cycle to produce more $NADH$ and $FADH_2$ which again pump out protons.

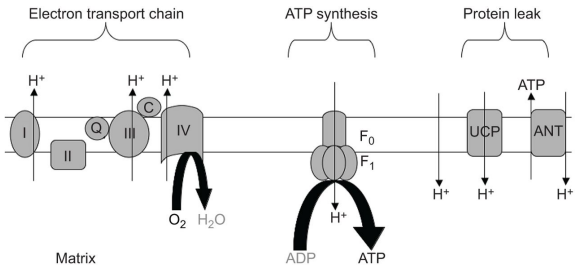


Figure 4.4: Uncoupling of oxidative phosphorylation (from [14])

Energy has to be invested to maintain the proton gradient intact and keep producing enough ATP. This energy goes into random motion (heat). Experiments in rats have yielded that about 25% of the energy in hepatocytes go into counteracting proton leakage, even though oxygen consumption in the liver cells is known to be low. In rat muscle cells the energy expenditure reaches the incredible amount of 50% which clearly shows the important role for metabolism.[30]

The phenomenon of thermogenesis through uncoupling is not only present in warm-blooded mammals, but also in thermogenic plants like *Amorphophallus paeoniifolius*, *Sauromatum guttatum* and *Victoria cruziana*. Excess temperatures of up to 10K were observed in conjunction with intensive odours.[21]

4.3 Mechanical energy storage

Not only electrochemical or thermal energy can be accumulated in living organisms. It is often very important to store mechanical energy for rapid release or to guarantee the elasticity of tissues. There are various key elastic proteins that take on that function. They are characterized by different possible combinations and variations of interesting properties, such as high flexibility, low stiffness and large strain compared to other proteins and molecules. These properties give rise to many biotechnological applications. For a protein to be elastic the stretching process has to be reversible without energy loss. So elasticity does not imply a linear force-extension relationship as is often thought; it just refers to reversibility. As will be seen there are many polymers that have highly non-linear force-extension relationships and are still elastic. This can be explained by cross-linking. Having a large elastic region, as is the case for resilin, elastin, gluten, titin, spider silks and other proteins, is synonymous to the capacity of storing large amounts of energy without doing any damage to the stretched protein.

4.3.1 Polymer elasticity models

Polymers are in essence entropic springs. They consist of many domains that are cross-linked. At large extensions the entropy is low (there are very few possible configurations) while at small extensions it is high (many configurations). This causes a natural force to refold the polymer when it is unfolded, since large entropy is preferred (Second Law of Thermodynamics).
To show that polymers are in fact entropic springs, some polymer thermodynamics originating from the "Mechanical Behavior of Materials" lectures from Prof. Niezgoda[25] will be presented. But first it should be clarified that the protein does not significantly change in volume during stretching or compression.[25] This will be important later on.

First Law of Thermodynamics, assuming a reversible process:

$$dU = dQ + dW = TdS + dW = fdl + TdS \tag{4.6}$$

f...applied force
l...extension

As discussed before the stretching and compression process is nearly isochoric, making the Helmholtz Free Energy $A = U - TS$ the most suitable thermodynamic variable to use. Given constant volume and temperature the following relationships are derived:

$$dA = dU - TdS - SdT \tag{4.7}$$

$$\rightarrow dA = fdl - SdT \tag{4.8}$$

$$\rightarrow \left(\frac{\partial A}{\partial l}\right)_T = f, \qquad \left(\frac{\partial A}{\partial T}\right)_l = -S \tag{4.9}$$

The symmetry of second derivatives implies that

$$\left(\frac{\partial S}{\partial l}\right)_T = -\left(\frac{\partial f}{\partial T}\right)_l \tag{4.10}$$

and therefore

$$f = \left(\frac{\partial U}{\partial l}\right)_T + T\left(\frac{\partial f}{\partial T}\right)_l \tag{4.11}$$

In 1935 Meyer and Ferri found out that the relationship between force and temperature in rubber is linear if the length is held constant.

$$f = \alpha T \tag{4.12}$$

$$\left(\frac{\partial f}{\partial T}\right)_l = \alpha \tag{4.13}$$

That means

$$\left(\frac{\partial U}{\partial l}\right)_T = 0 \tag{4.14}$$

which implies that the internal energy in the stretching or compression process is never changed as long as the process is isothermal. So it can be said to a good approximation that the elasticity in polymers is only a consequence of changes in entropy. Therefore polymers can be well described as entropic springs.

Given some parameters like the size of the protein, the number of domains and the amount of cross-linking, its elasticity - given by its force-extension relationship - can vary greatly. Not always is it possible to assume a linear relationship between those two quantities, and therefore it sometimes does not even make sense to define a Young's modulus. In some cases more complex models have to be applied. Their basis lies in statistical physics as will be explained hereafter.

4.3.2 Statistical Physics

To understand the elasticity of a polymer one has to examine carefully the interactions taking place between the atoms in the compound. A polymer can be seen as a chain consisting of beads and links. The beads can interact; the main interactions are depicted in figure 4.5. There exists a Lennard-Jones and/or stretch interaction between two successive beads (a), a bending interaction between three beads with an optimal angle θ_0 (b) and a torsional interaction (c).

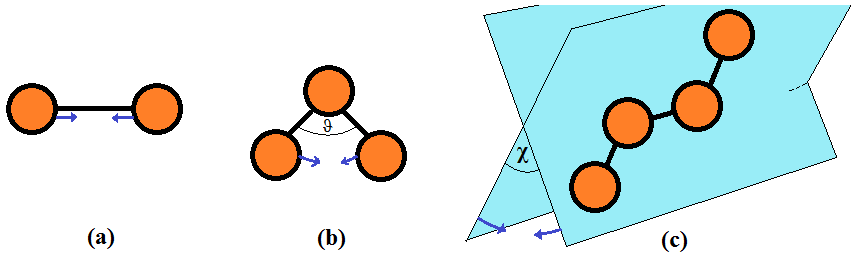


Figure 4.5: Interactions between beads in a polymer chain

Freely Jointed Chain Model

The simplest imaginable model as explained by Dr. Jos Thijssen [32] is the Freely Jointed Chain Model which is characterized by the fact that the beads, connected to each other with a fixed length s_0 can be randomly oriented. That can only be the case if there are no interactions between them. Assuming N+1 beads with position $\vec{R}_i$ connected with N links with directionality $\vec{r}_i$ the probability distribution for a given configuration of the chain where the i-th bead is in the position $\vec{R}_i$ is given by:

$$P_N(\vec{R}_0, \vec{R}_1, ..., \vec{R}_N) = \left(\frac{1}{4\pi s_0^2}\right)^N \Pi_{i=0}^{N-1} \delta(|\vec{r}_i| - s_0) \tag{4.15}$$

Much more interesting is the probability distribution of finding a chain in a configuration where the end-to-end distance is given by $\vec{R}$ as shown below:

$$P_N^E(\vec{R}) = \int P_N(\vec{R}_0, \vec{R}_1, ..., \vec{R}_N)\delta(\vec{R}_N - \vec{R}_0 - \vec{R}) d^3\vec{R}_0 ... d^3\vec{R}_N \tag{4.16}$$

After a Fourier Transform and several steps one arrives at

$$P_N^E(\vec{k}) = \left(\frac{sin(ks_0)}{ks_0}\right)^N \tag{4.17}$$

which can be approximated as

$$P_N^E(\vec{k}) \approx e^{-N\frac{k^2 s_0^2}{6}} \tag{4.18}$$

$$\rightarrow P_N^E(\vec{R}) \approx \left(\frac{3}{2\pi N s_0^2}\right)^{3/2} e^{-\frac{3R^2}{2Ns_0^2}} \tag{4.19}$$

Since most chains do have stretching interactions it is useful to expand the Freely Jointed Chain model to a Gaussian Chain which can be interpreted as containing stretching interaction. A Gaussian Chain is composed of N segments of freely jointed chains, resulting in the following distribution:

$$\rightarrow P_N(\vec{R}_0, \vec{R}_1, ..., \vec{R}_N) \approx \frac{1}{V}\left(\frac{3}{2\pi s_0^2}\right)^{\frac{3}{2}N} e^{-\frac{3}{2s_0^2}\Sigma_{i=0}^{N-1}(\vec{R}_{i+1} - \vec{R}_i)^2} \tag{4.20}$$

From this distribution we can extract the Gaussian Hamiltonian:

$$H_{Gauss} = \frac{3}{2}k_B T \frac{1}{a^2}\Sigma_{i=0}^{n-1}(\vec{R}_{i+1} - \vec{R}_i)^2 = \frac{3}{2}k_B T \frac{1}{a^2}\Sigma_{i=0}^{N-1}(\vec{r}_i)^2 \tag{4.21}$$

$$\rightarrow H = \frac{3}{2} k_B T N \tag{4.22}$$

This model in itself is quite unspectacular because there is no resulting force to restore the initial configuration. There is no spring-like behaviour. To account for interactions leading to a restoring force other Hamiltonians have to be added.

Interaction Hamiltonians [32]

As previously discussed in the Gaussian Chain model it is possible to consider a polymer as a compound of freely jointed segments and add interactions between them. The length of these segments is called Kuhn length and is denoted by the letter b. In the context of bending interactions there exists a quantity called persistence length P which is about half of the Kuhn length and is defined as the length over which directionality does not correlate with the tangent.[17] In the context of elongation interactions the parameter K signifies the elastic modulus. Furthermore the contour length of the polymer is given by s.
The Hamiltonians for the previously discussed interactions (except the torsional one that is not as significant) are specified below:

- bending: $H_b = \frac{1}{2} k_B T \int_0^L P(L,s) \left(\frac{\partial^2 \vec{r}}{\partial s^2} \right) ds$
- elongation: $H_e = \frac{1}{2} \int_0^L K(L,s) \left(\frac{s}{s_0(s)} - 1 \right)^2 ds$
- tension: $H_t = -\vec{f} \cdot (\vec{r}(l) - \vec{r}(0))$

Using combinations of these Hamiltonians based on the properties of the relevant polymer it is possible to deduce force-extension relationships. Those in turn can be used to determine the amount of energy that can be stored in a polymer when stretching it.

Worm-like chain model (WLC)

The simplest such model is the worm-like chain model. It assumes that polymers are made up of different domains that are crosslinked and once certain extensions are reached, certain domains completely unfold giving rise to jumps in the force-extension diagram. The extension until a given jump is called the persistence length A, while the maximum length that can be reached is referred to as the contour length L.
The mentioned force-extension relationship can be measured with different devices such as the Atomic Force Microscope or with help of optical tweezers, resulting in diagrams like the following:

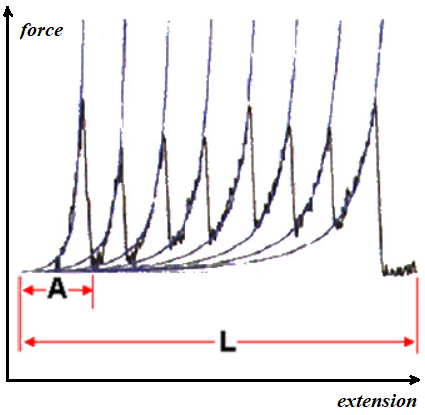


Figure 4.6: Force-extension diagram of a typical polymer (from [1])

As derived by Marko and Siggia[24] the force-extension relationship in the case of DNA and many other polymers is given by the following equation:

$$F = \frac{k_B T}{P}\left[\frac{1}{4}\left(1 - \frac{x}{L_c}\right)^{-2} - \frac{1}{4} + \frac{x}{L_c}\right] \tag{4.23}$$

x...extension
L_c...contour length
P...persistence length

For molecules following this behaviour, it is therefore very easy to determine the energy that can be stored in it. It just corresponds to the integral under the curve shown in figure 4.6. Once the contour length and the respective persistence lengths are known, an integration over the different domains provides us the stored energy. So the determination of the energy storage reduces to the determination of the contour length of the overall polymer and the individual persistence lengths of the domains.

Extended WLC

The WLC model fits many polymers sufficiently well, but it does not take into account enthalpic contributions to the stretching process. To accurately incorporate these contributions, this model can be extended. The extended WLC model and the following ones are both entropic and enthalpic ones.
The extended WLC force-extension relationship is stated below:

$$F = \frac{k_B T}{P}\left[\frac{1}{4}\left(1 - \frac{x}{L_c} + \frac{f}{K_0}\right)^{-2} - \frac{1}{4} + \frac{x}{L_c} - \frac{f}{K_0}\right] \tag{4.24}$$

f...external force
K_0...stretch modulus

4.3.3 Actual research techniques for protein elasticity

In recent times different experimental and computational techniques have been applied to study the mechanical/elastic behaviour of proteins. Such techniques include but are not limited to the application of ultrasonic waves, Atomic Force Microscopy, Molecular Dynamics simulations and optical tweezers.[5]

Based on the work of Cheng et al. [13] the elastic properties as determined from MD simulations are presented and contrasted with experimental results. They determined that there is a significant correlation between amino acid sequence and elasticity. In particular glycine, proline and PPII contents were highlighted. Glycine is flexible, while proline having a cyclic side-chain is rather stiff and hampers compaction of the polymer. Different abundances and configurations in those residues lead to respective elastic properties.

Titin

Titin is the human body's largest protein and is responsible for the muscle's passive elasticity and static tension. It serves as a shock absorber in sarcomeres-the basic contractile unit of muscle tissue.[7] It reaches a size of 3,700kDa and an *in vivo* physical length of about 2 μm.[6] Dalton is a unit equivalent to the atomic mass unit and often used in biochemistry. Being such an enormous molecule, lots of research is still to be done to fully understand it. Many NMR and X-ray crystallography measurements have been made on several of its domains, but we are far from understanding the molecule in detail. Still, many correlations have been observed. One of the most important sequences for titin elasticity is the PEVK region that will be analysed as follows. Specifically the PPAK motif will be explored.
PPAK has high content of:

$$\textit{prolines} \quad 35\%$$

As previously discussed this leads to a stiffening of the protein.

$$\textit{Persistence lengths: 0.3-2.3nm}$$

The higher the persistence length the less flexibility is exhibited.
All analysed polypeptides were fitted by the WLC model, but PEVK showed significant deviations, especially at low extensions which indicates that hydrophobic effects should be considered. In experiments[22] the protein could be reasonably well fitted using the WLC model, although for greater extensions enthalpic contributions had to be considered (Extended WLC).

Resilin

Resilin is a remarkable protein. Its elastic properties include:

- high resilience (recovery after deformation)
- low stiffness
- high strain

Some mechanical properties are summarized in the table below:

Table 4.2. [31]

Resilience (%)	92
Tensile modulus (kPa)	640-2,000
Unconfined compressive modulus (kPa)	600-700
Strain-to-break (%)	300

Resilin is also very rich in:

$$\textit{glycine} \quad \textit{40\%}$$

That is what gives the compound its elasticity. Resilin allows some insects to fly and plant- and froghoppers to jump reaching the necessary acceleration for take-off within just a few milliseconds.[29] Full-size natural resilin has a size of about 60kDa (more than 60 times smaller than Titin), although the proteins of interest that are analyzed in most studies are just segments of it and therefore smaller.[28] Being a hydrogel, its properties are highly dependent on the presence or absence of water. Hydrophobic effects have to be taken into account. That is the reason why according to the MD simulations the WLC model was not a perfect fit.

Silk

Silk is a truly unique material. Its remarkable mechanical properties include:

- high tensile strength
- high toughness
- high elongation
- small diameter
- low density

Here are some data to support this:

Table 4.3.

Property	Value	References
Young's modulus $[GPa]$	4.0-10	[35]
Toughness $[MJ/m^3]$	131-160	[35]
strain-to-break [%]	4-19	[35]
Diameter $[\mu m]$	1-6	[10]

Note: All of the data in Table 4.3., except the strain-to-break percentage, refer to the silk of the spider *Araneus diadematus* which was examined in the work of Cheng et al. [13]

In contrast to titin and resilin the silk of the spider *Araneus diadematus* is rich in both

glycine 54% and proline 25%

The special feature about silk is its amorphous structure of glycine residues in between crystalline portions.

Energy storage

The mechanical energy that can be stored in elastomers like the ones above can be determined in a very simple way. It corresponds to the integral over the stress-strain curve of the compound. In figure 4.7 the stress-strain relationships from the work of Cheng et al. are depicted for the three mentioned proteins.

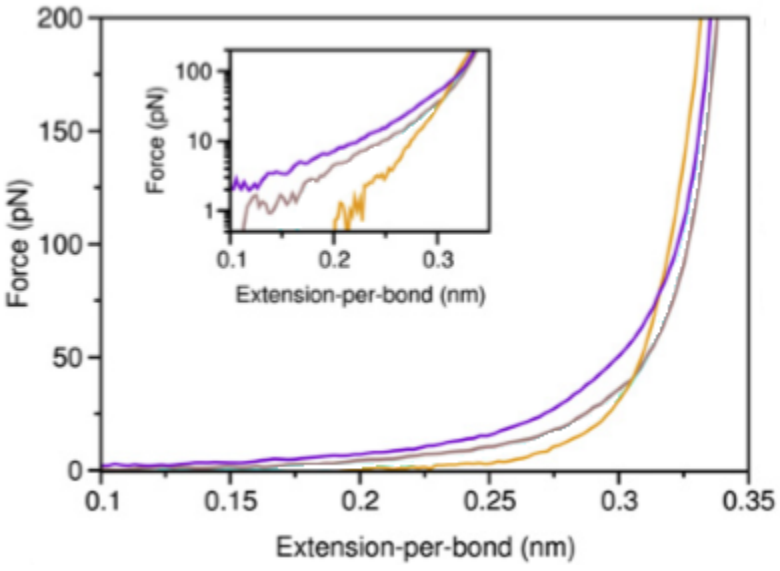


Figure 4.7: Stress-strain curve for resilin(brown), PPAK(orange) and silk(violet) (from [13])

It can be clearly seen that the energy densities have the following order:

$$E_{silk} > E_{resilin} > E_{PPAK}$$

It was nevertheless not possible to find raw data from which the integral could be computed. Assuming a rupture force of 200pN and using the WLC parameters reported in the work of Cheng et al. it is possible to make a rough estimate of the storable energy density per bond by integrating over the force-extension relationships up to the mentioned maximal force. However, to get a reliable result the force-extension curve should be acquired either experimentally or through MD simulation. It is important that the same materials are used, as even between the silk of different spiders there could be important differences.[34]
Rough estimate of the energy density per bond:

Table 4.4.

	Energy density per bond [meV]
Titin	16.3431
Resilin	28.2362
Silk	36.5254

From the studied materials only titin could be found as a commercially available material. At Abcam (http://www.abcam.com/recombinant-human-titin-protein-ab131751.html) $10\mu g$ of Recombinant Human Titin protein is available at a price of $US\$435$. It must be stored at $-80°C$ without freeze/thaw cycles at a pH of 8.

Chapter 5

Conclusion

Several energy storage mechanisms in organisms, along with their storable energy densities and efficiencies were presented. It is thinkable to develop interesting applications from some of them depending on the desired properties.
The efficiency of electrical and chemical energy storage is about 40%, much higher than engines built by humans, although this process could be disturbed by uncoupling proteins. Because of them, in rats 25%-50% of the energy input goes into heat. Depending on the desired application it is thinkable to manipulate the amount of specific proteins to engineer organelles and optimize them for storage of a specific form of energy.
Mechanical energy in elastomers was also widely discussed. The energy in them can be very efficiently stored, although it is difficult to quantify because it depends on the assumptions that are made about interaction mechanisms in the underlying models. Pure entropic models lead to storage without heat loss, a case that from the analysed materials could only be satisfactorily applied to spider silk. Elastomers could also be potentially engineered to optimize their properties. It is known that their elasticity depends on the content of specific residues like glycine and proline. The possibility for applications from biophysical processes like the ones presented is broad given their significant efficiencies and storing capabilities.

Acknowledgement

I am deeply grateful to my professor Ille C. Gebeshuber. This project would not have been possible without her careful, expert and kind supervision. Her research was an extraordinary source of inspiration for the project and her comments always stimulated me to learn more about this fascinating topic.

There is no way I could forget to mention Julian Gamboa González, a dedicated and brilliant researcher and great friend of mine, with whom I always enjoy talking about science and technology. With great interest I constantly shared my discoveries with him, leading to amazing discussions which inspired me further.

I also want to hugely thank my family for their incredible support and interest that constantly motivated me.

Annotated Bibliography

[19] Ille C Gebeshuber, Petra Gruber, and Manfred Drack. A gaze into the crystal ball: biomimetics in the year 2059. *Proceedings of the Institution of Mechanical Engineers, Part C: Journal of Mechanical Engineering Science*, 223(12):2899–2918, 2009

Biomimetics is a fascinating field that is capable of reshaping the way we solve technological problems. "A gaze into the crystal ball: biomimetics in the year 2059" is a work that describes precisely that: its important role in solving technological challenges and in working towards completing the Millenium Project containing the 15 most important global problems to be solved for a prosperous future. Very detailed and hands-on examples of how major global challenges could be tackled using this powerful tool are provided.
Also the importance of causal information and integration of knowledge across related fields is highlighted. Increasing causal knowledge could drive innovations, although the understanding of basic principles does not necessarily imply easier applicability. Even without causal insights innovations can be inspired by nature.
The publication is definitely a must-read for everybody trying to understand the potential of biomimetics in transferring knowledge from nature to applications.

[15] GM Cooper. The Cell: A Molecular Approach, 2nd edn. The Cell: A Molecular Approach. Sunderland, MA, 2000

This book offers a good picture of the workings inside the cell. From its evolution to its chemistry, structure, molecular biology, physiology and pathology, the work offers a complete description of the current knowledge on the topic. It shows the intrinsic relationship between cell biology and medicine and tries to illustrate the influence the former has on the latter.

[23] Harvey Lodish, Arnold Berk, S Lawrence Zipursky, Paul Matsudaira, David Baltimore, and James Darnell. Molecular cell biology 4th edition. *National Center for Biotechnology Information, Bookshelf*, 2000

What is interesting about this book is its unifying character of metabolic processes in organisms. It describes the structure and functionality of molecules in cells, their relationship, and makes emphasis on the similarities in functioning across different organisms.
It highlights genomics and proteomics as two key areas of our millenium since they are responsible for the functions carried out by the cell.

[36] Xin-Guang Zhu, Stephen P Long, and Donald R Ort. What is the maximum efficiency with which photosynthesis can convert solar energy into biomass? *Current Opinion in Biotechnology*, 19 (2):153–159, 2008

Understanding the efficiency with which plants convert solar energy into biomass is especially important for agriculture, although it could have interesting implications for other applications where photosynthesis is used. This article carefully examines the efficiencies for different plant types and suggests solutions for maximizing them.

[12] Brian Chapman and Denis Loiselle. Thermodynamics and kinetics of the FoF1-ATPase: application of the probability isotherm. *Royal Society Open Science*, 3(2):150379, 2016

This article portrays the thermodynamics and kinetics of one of the most important proteins and molecular machines in all organisms: ATP Synthase. It produces ATP parting from a proton gradient. Current publications on the topic are discussed and alternative solutions to existing modeling problems proposed. It also very clearly shows how the efficiency of the protein depends on flow rates.

[26] Hiroyuki Noji, Ryohei Yasuda, Masasuke Yoshida, and Kazuhiko Kinosita. Direct observation of the rotation of F1-ATPase. *Nature*, 386(6622):299–302, 1997

This is one of the most fundamental articles about energy conversion in the ATP Synthase. For the first time the rotation of the protein during ATP synthesis was observed under the microscope and the functioning of this molecular rotary machine was looked at in detail confirming and completing existing theories.

[30] Jeff A Stuart, Kevin M Brindle, James A Harper, and Martin D Brand. Mitochondrial proton leak and the uncoupling proteins. *Journal of Bioenergetics and Biomembranes*, 31(5):517, 1999

This article gives important insight into the biophysical mechanisms of thermal energy production in organisms. It explains how uncoupling proteins (UCPs) cause random motion by uncoupling oxidation and phosphorylation in the Electron Transport Chain.

[13] Shanmei Cheng, Murat Cetinkaya, and Frauke Gräter. How sequence determines elasticity of disordered proteins. *Biophysical Journal*, 99(12):3863–3869, 2010

The elasticity of polymers can be a very complex issue. It depends on a variety of factors, but certain correlations among highly elastic polymers are observed. This article talks about significant correlations regarding the amino acid sequence. Contents of specific amino acids influence elastic properties. The results are based on a Molecular Dynamics simulation.

[7] Itamar Benichou and Sefi Givli. The hidden ingenuity in titin structure. *Applied Physics Letters*, 98(9):091904, 2011

This article explains how titin, the human body's largest molecule, is able to store and release energy in a very efficient manner, with very little energy dissipation or hysteresis.
A simple mechanical chain model is used to show the dependence of dissipation on the system size and the energetic barrier between equilibrium configurations.

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