Art and Destiny

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Art and Destiny - Protein Chemistry of Disease

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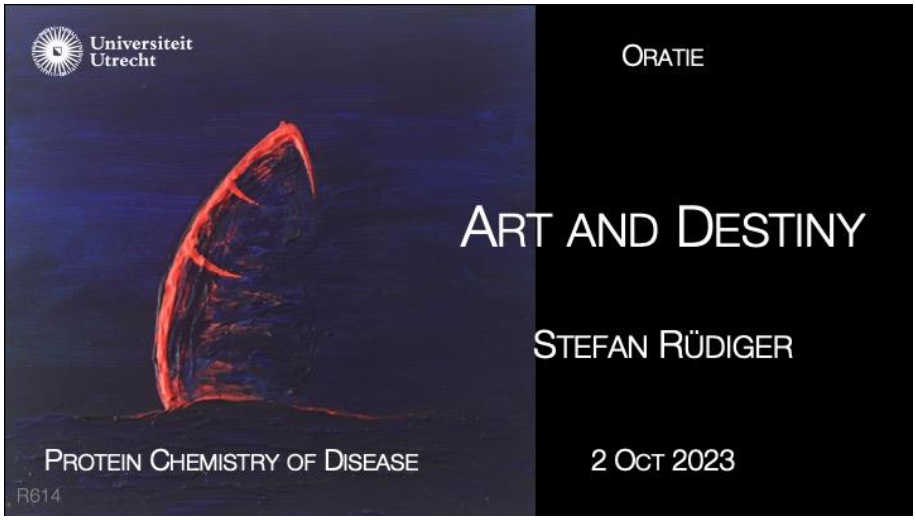
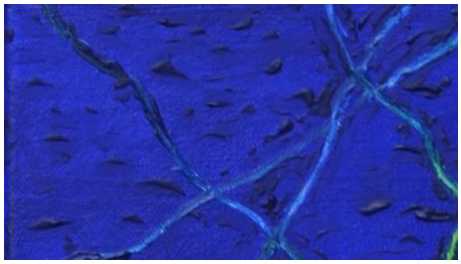
Abstract
All vital biological processes involve proteins. To get a grip on our destiny at molecular level, we need to master the art to understand, control and making proteins. This art is the application of science on disease, and the science we need to apply is protein chemistry. Here I outline the potential and ambition of Protein Chemistry of Disease.



Sailing into the unknown.

Dear Rector Magnificus
Dear Dean
Dear students
Dear colleagues
Dear and friends and family

1. Protein Chemistry of Disease



Threads of life, fragment I.

I define Chemistry as the science of understanding, controlling and making matter. I understand Science as knowledge-creating research, aiming to understand the world following systematic methodology based on evidence. Disease is any harmful deviation from the normal structural or functional state of an organism, according to the World Health Organisation. Protein Chemistry of Disease thus aims to control proteins, the most important and most versatile class of macromolecules, to revert harmful deviations within an organism. Protein Chemistry of Disease is the science of a rational approach to diseases at nanometer scale - this is the dimension of molecules.

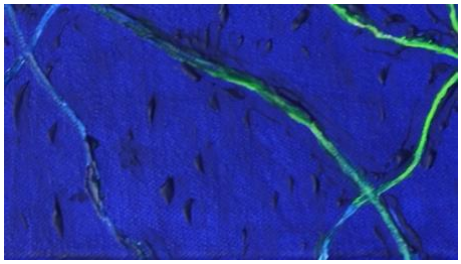
Fig. 1. Norns. In the Nordic Edda, three Norns are weaving the thread of life, determining the life of any human being. The thread of the Norns is controlling the shape of the proteins. Loss of control of the shape of the proteins is fatal. In Greek mythos three Moiras have the same function as the Norns.

The figure depicts the essence of Protein Chemistry of Disease **(Fig. 1)**. On the right is the human. On the left there are three mystic, scary figures. This trio controls the thread of the life of the human. In fact, the thread of life depicted here are the chains of the proteins in our cells. The proteins are complex

molecular machines that run all vital processes throughout our body[1]. Health requires the proper function of 20,000 different proteins in all parts of our body. Failure of control leads either to loss of vital function or to proteins gaining new, possibly dangerous functions[2-4].

The three figures controlling the thread of life are known in the Nordic Edda saga as the three Norns. The Norns also appear in Wagner's Götterdämmerung, where breaking of the thread triggers the dramatic end of the gods. In remarkable parallel evolution of mythos, according to Greek tradition three Moiras control the threads of life. Thus, it is an eternal wish of mankind to regain control on the threads of life. This means breaking the frame that boxes us in and controlling the fate of proteins ourselves. As all vital biological processes involve proteins, we need to master the art to understand, control and making proteins to get a grip on our own destiny at molecular level. This art is the application of science on disease, and the science we need to apply is protein chemistry. Here I will outline the potential and ambition of protein chemistry of disease.

2. Chemical thinking



Threads of life, fragment II.

Science is intrinsically connected to thinking, and thinking takes time. Deep thoughts together with intensity, often hard work and a bit of luck are breeding ground for breakthroughs. Breakthroughs, therefore, take time, often years, sometimes decades. Typically, ground-breaking science is slow science.

As an example, take a middle-aged scientist who finally became a professor, having been a prolific writer before. After finally being appointed, he did not publish any notable papers for 11 years. Then, in 1781, Immanuel Kant published "Kritik der Reinen Vernunft" - Critics of Pure Reason[5]. It ended the pursuit of metaphysics and paved the way to rational science as we know it today. Kant's work was a qualitative leap, it is a prime example of what we call today slow science.

Critics of Pure Reason provided the framework that natural science can produce conclusions with apodictic certainty based on evidence. Kant differentiated between what is given to us before any empirical evidence, which he terms "*a priori*", and what after empirical evidence, "*a posteriori*"[6].

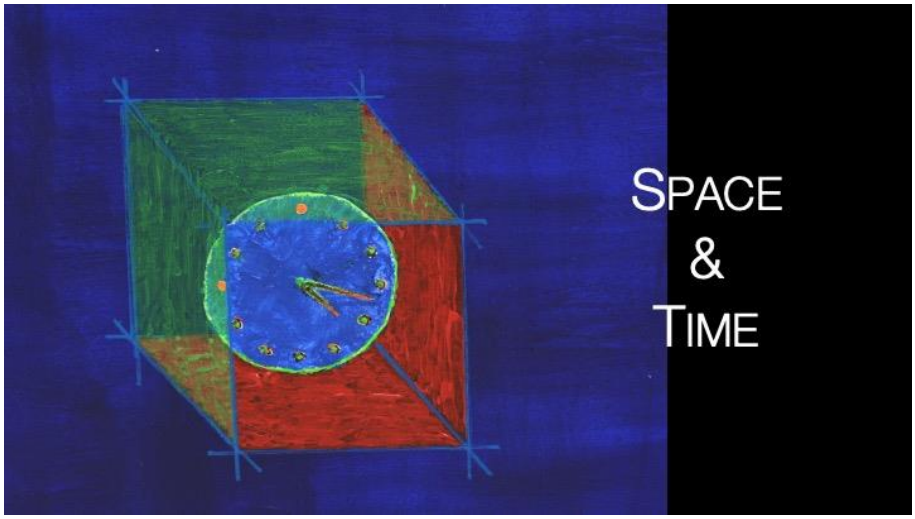


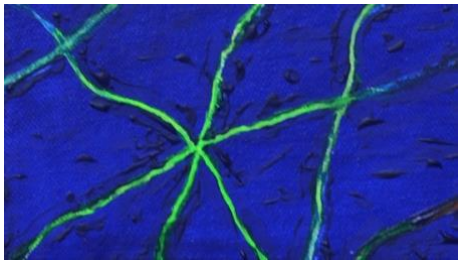
Fig. 2. Space and time - necessary a priori to recognise objects.

He concluded that the perception of space and time is necessary condition *a priori* to recognise any object (**Fig. 2**)[6]. The objects themselves are never given to us *a priori*. Recognising the properties of objects requires empirical facts, by observations or experiments. Based on these empirical facts, we can draw quantitative conclusions and make stringent predictions, with apodictic certainty. This is the basis for the laws of natural sciences[7].

It is important to differentiate between the law and the objects. A law that provides apodictic certainty has an *a priori* element in it[6]. Mathematical concepts are *a priori*. We know that 5 + 7 = 12. The number 12 is not given in the numbers 5 or 7. It is the application of a theoretical framework that reveals the correct answer. It is possible to verify this by counting 5 and 7 apples. The theoretical framework, however, is valid independent of the existing world and thus independent of having any apples. Mathematics will also reveal the correct answer for summing up 5 and 7 unicorns, without the need of ever catching one. This is a conclusion *a priori*. In contrast, it is impossible to draw conclusions *a priori* about the smell of unicorns. This requires evidence that can only be given by observations.

According to Kant, science is a framework of rules, which requires empirical observations in combination with concepts *a priori* [7]. Apodictic certainty is in essence mathematical. Kant concluded that in each natural science is as much science as it is mathematics in it. What does this mean for Chemistry?

Kant states that understanding that is purely based on empirical certainties is only knowledge[7]. Science does go further than that. Science refers to the systematic connection of the entirety of our understanding, of causes and consequences. We should not, according to Kant, use the term "science" for a collection of mere empirical rules without apodictic and stringent predictability[7]. He provides an example: Chemistry should be called a "systematic art**"**, but not a "science"[7].



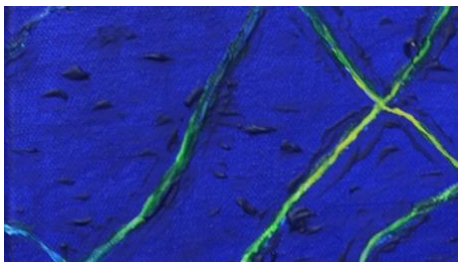
Threads of life, fragment III.

3. Chemistry as science

Kant reached this conclusion, almost a century before the discovery of the periodic table of elements by Medelejew in 1871. We can hardly argue with Kant that back then Chemistry lacked apodictic certainty and quantitative reasoning. Today's Chemistry, however, does exactly that. It connects empirical facts with apodictic laws and allows quantitative conclusions about matter.

Is chemistry still a systematic art? I define art as a creative act generating a product that can inspire aesthetic imagination. Thus, it is about a man-made process. This means that things we just find in nature are not art. Chemistry creates, and chemical creation can have a sense of beauty in them. Thus, Chemistry is an art indeed. Chemistry also acts within an apodictic and quantitative framework, which is what makes today's Chemistry science. Chemistry is unique by being a synthetic science, thus, it can create its own study objects. This makes Chemistry both, art and science.

The term "Chemistry of Disease" declares ambition develop therapeutic strategies based on rational understanding of the molecular processes of life, to quantitatively and systematically influence processes in our body.



Threads of life, fragment IV.

4. Incurable diseases

Progress in chemical research did drive development of drugs, increasing life expectancy and in quality of life. Still, there are diseases for which no cure exists[8]. A large unmatched need is tackling dementia. Currently there are 55 million people with dementia, increasing to 78 million in 2030, according to the World Health Organisation. The total costs worldwide are currently 1.3 trillion dollars. There is no cure is a lack of understanding the basis of the disease. The chemical approach to this is to understand the chemical laws of the processes

leading to the disease. Strikingly, at molecular level there is common ground between various apparently unrelated forms of dementia.

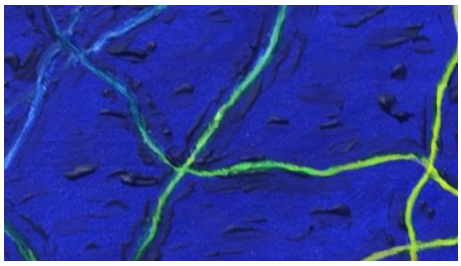
5. Inclusions in neurodegeneration

Alzheimer's Disease is the most common cause of dementia, causing more than half of all cases[8]. In 1906, Alois Alzheimer described the case of a 56-year-old woman whom he had followed for five years, Auguste Deter[9]. Her symptoms included memory disturbances and progressive confusion, what we today know as typical symptoms of Alzheimer's Disease. Alzheimer was clinical psychiatrist and neuroanatomist in Frankfurt. When analysing her brain, he observed what he described as plaques and neurofibrillary tangles. Alzheimer guided later teams in Munich and Breslau, today's Wroclaw, that characterised features of other neurodegenerative diseases. Alzheimer himself identified characteristics bodies in Frontotemporal Dementia, another neuropathy. In 1912, his co-worker Friedrich Lewy described the inclusions in the brainstem of Parkinson's Disease, which are now known as Lewy Bodies[10, 11]. Parkinson is a disease which the British surgeon James Parkinson described in 1817 as "involuntary tremulous motion" while, in contrast to Alzheimer's Disease, "senses and intellects [are] uninjured". Alzheimer's former group members Creutzfeld and Jakob characterised inclusions in what is now known as Creutzfeld-Jakob Disease or Mad Cow Disease.

The discovery of inclusions in neurodegenerative diseases is still an ongoing process. George Huntington of Columbia University, New York, had described already in 1872 "Chorea", a genetic disease, characterised by disturbances of mobility and cognitive functions and which is now named after him[12]. It was only in 1998 that Isabelle Gourfinkel-An and co-workers at INSERM in Paris identified inclusions in Huntington's Disease[13].

Remarkably, all these various diseases are characterised by inclusions. Each inclusion represents aggregation of a protein, and it is poorly understood why such protein aggregates lead to death of neurons.

6. Origin protein science



Threads of life, fragment V.

The term "protein" was coined by the Swedish chemist Jöns Jakob Berzelius[14, 15]. He was based at Karolinska, Stockholm, and he was actively exchanging letters with colleagues all over the world. On 10 July 1838 he wrote a letter to the Dutch Chemist G.J. Mulder, Rotterdam, proposing the term protein. Berzelius derived the word "protein" from the Greek word "prΩteios", meaning

"first", as it is the prime nutrition for animals[14, 15]. Mulder followed this suggestion and used it in a publication submitted on 30 July 1838. Remarkably, the protein species Berzelius and Mulder were referring to was in fact denatured, aggregated protein, as it is produced by heat denaturation of the active proteins. Thus, the history of Protein Chemistry started in fact with what we now know as aggregated and inactive protein.

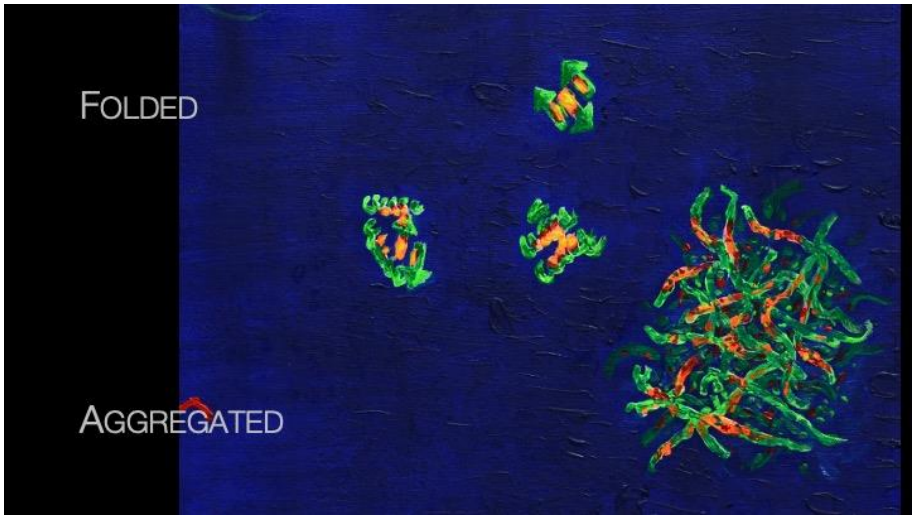


Fig. 3. Folds and aggregates. (LEFT) Proteins fold around a hydrophobic core (yellow-orange), leaving hydrophilic, water-interacting parts on the outside (green). This results in characteristic protein structures. (RIGHT) When proteins lose structure, e.g. due to heat, they turn inside out and the elements fthat formed the hydrophobic core are sticking together. This results in an irregularly structured aggregate.

Proteins consist of chains of linearly connected amino acids. The genome encodes 20 different amino acids, each of which has different properties. The linear chain in many proteins adopt a defined three-dimensional structure[16]. The building principle of these structures is that hydrophobic amino acids are inside the protein while hydrophilic proteins are outside, interacting with water (**Fig. 3 LEFT**; hydrophobic amino acids are depicted yellow). Upon heating the protein or by adding acid the protein loses its defined structure, exposing the hydrophobic groups that had been inside now to the outside (**Fig. 3 RIGHT**)[16]. As results the protein chains clump together in a chaotic way, forming unstructured, amorphous aggregates - what Berzelius called the prime nutrition of animals[14, 15].

7. Protein aggregates in neurodegeneration

It appears that the common ground between various neurogenerative diseases are protein aggregates that are found in inclusion.

Fig. 4. Chaos and order. (LEFT) If a folded, globular protein unfolds it form a chaotic, unstructured aggregate. (RIGHT) Fibril aggregates are highly ordered structures, each layer is identical to the layer on top and bottom.

However, there is also a striking difference between the aggregates in brain and the aggregates Berzelius and Mulder referred to as prime nutrition for animals. The aggregates in the neurodegeneration have a very specific shape[17, 18]. They form needle-like fibrils, build up from identical layers (**Fig. 4 RIGHT**). The proteins aggregating in e.g Alzheimer, Parkinson's Disease or Huntington's Disease are entirely unrelated[19-21]. What they share is that they can form such highly ordered aggregates, protein fibrils. In contrast, heating up proteins leads to entirely chaotic aggregates (**Fig. 4 LEFT**)[16].

8. The fibril-building principle is universal

If the proteins that form fibrils in these diseases are different, what do they have in common that they form these aggregates? Counterintuitively, they have much less hydrophobic amino acids compared to most proteins, which would form the Berzelius aggregates upon boiling.

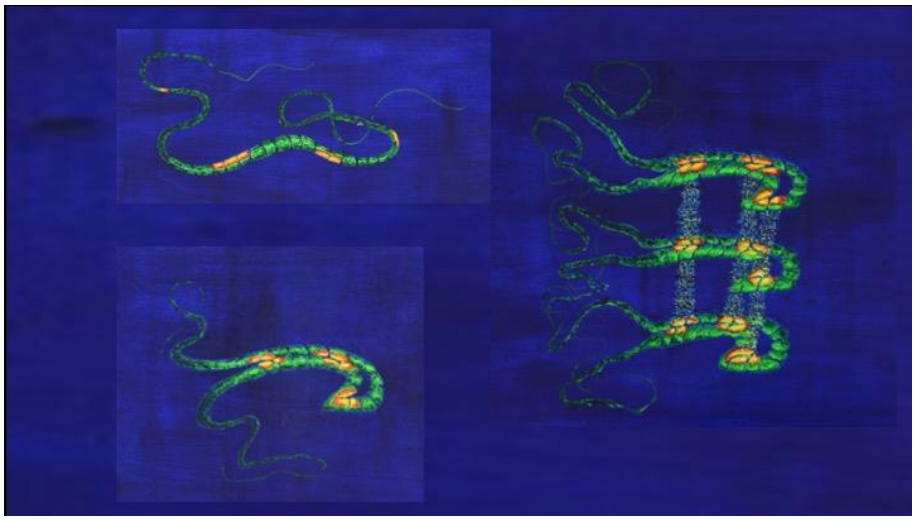


Fig. 5. Fibril formation. (TOP LEFT) A disordered protein has only few hydrophobic stretches (orange-yellow), not enough to fold. (BOTTOM LEFT) The few hydrophobic residues can form

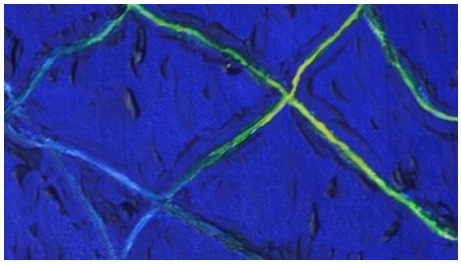
a two-dimensional layer, which are unstable as the hydrophobic patches are exposed to the water at top and bottom. (RIGHT) The layers can staple on top of each other into a fibril. This is stable as the each hydrophobic patch is now saturated at top and bottom.

The protein Tau that forms the tangles Alois Alzheimer spotted inside neurons does not aggregate at all when boiled[22]. It does not have sufficient hydrophobic residues to clump together. The protein Tau does not even have enough hydrophobic residues to form a hydrophobic core around which the protein could possibly fold, it remains always unfolded (**Fig 5 TOP LEFT**)[23, 24]. Unless - it forms a fibril.

While Tau does not have enough hydrophobic residues to form a three-dimensional core, it has sufficient hydrophobic residues to form a two-dimensional layer (**Fig 5 BOTTOM LEFT**). This layer exposes a hydrophobic surface at top and bottom (yellow in **Fig. 5**), which is instable in water, unless these patches could find other hydrophobic partners.

When several of these layers staple on top of each other, the hydrophobic patches are satisfied at both the ends (**Fig. 5 RIGHT**)[18, 19]. This is the nucleus of a fibril; it can now grow in both directions. When it breaks, both fragments are again a passepartout for two fibrils[25-27]. This is a self-replicating system. Up to now we cannot effectively stop this process in Alzheimer's Disease.

This fibril forming principle is similar in other neurodegenerative diseases. In Parkinson's Disease it is the protein α-synuclein, in Huntington's Disease the huntingtin protein that form inclusions[20, 21, 28].



Threads of life, fragment VI.

Remarkably, the proteins Tau, α-synuclein, huntingtin and other fibril forming proteins do not share any similarity in sequence or composition. Yet, they all have in common that they form fibril structures in disease[18-21, 29]. How fibril formation is related to the origin of the disease, why would fibrils be toxic, and are they toxic at all?

9. Traditional drugs target pockets

At present there are no answers to these questions, and this is in the heart of the question why there are no curative drugs for any of these diseases.

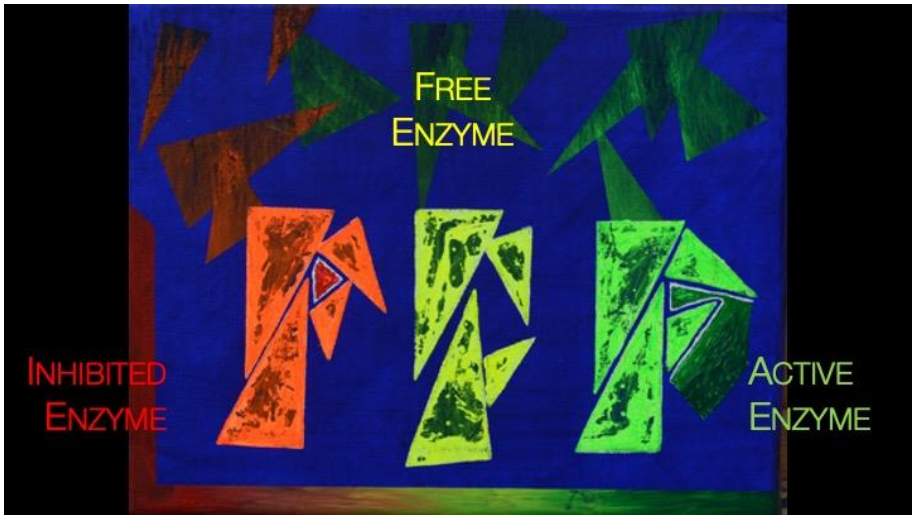
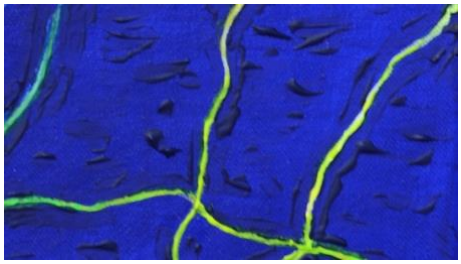


Fig. 6. Action of typical drugs. Enzymes have typically a pocket that is open in the absence of any substrate (yellow). The active pocket of the enzyme takes up the substrate (green). The pocket closes around the substrate, leading to chemical modification of the substrate. Small molecule drugs block the active pocket of an enzyme (red). `The drug binds better than the substrate, the enzyme cannot function anymore.

The traditional approach in drugging is targeting active sites in enzymes[30]. These are protein machines that transform small molecules. The active site in which the small molecules are transformed are typically in pockets inside the protein, which protect the active site from the surrounding water and other molecules. Active sites can be targeted by small molecules (**Fig. 6**). Such small molecules take the space of the normal substrate (green in the Figure) and can block the pathway in which the enzyme is active.

An example is acetyl salicylic acid, the most sold drug ever. Chemists at the company Bayer synthesized acetyl salicylic acid in 1897 and applied it under the name "Aspirin" as a pain killer in 1899[31]. At the time it was unknown why aspirin reduces pain. It was only in 1995 when the structure of the COX protein bound to aspirin was solved[32]. The COX proteins are the cyclooxygenases that are thought to inhibit the prostaglandin synthesis[33]. Aspirin irreversibly inhibits the active centre of the COX protein, by acetylating a key amino acid in the active centre[32, 33].

Bayer developed Aspirin empirically, neither knowing the target nor the mechanism. Today we know the structure of the complex, we understand the mechanism but also its side effects. For traditional developing of drugs, it is not essential to have knowledge of protein structures and mechanisms. Aggregation diseases require an entirely different approach than traditional drug screening. Protein Chemistry of Disease aims to develop drugging strategies based on mechanistic knowledge. Protein fibrils do not have enzymatic pockets that could be inhibited by small molecules such as Aspirin, and no desired activities that could be blocked and inhibited. Instead, targeting protein fibrils requires to understand the machinery that controls protein quality, their shape and aggregation.



Threads of life, fragment VII.

10. Failure of protein quality control

Most of these diseases are not genetic. In fact, in every human brain are large quantities of the proteins Tau or α-synuclein[34]. For decades, they do not aggregate, suggesting that there is an effective control system preventing this. This protein quality control system has two major tools: molecular chaperones that support folding or refolding damaged protein structures and degradation machineries that remove useless or dangerous proteins in the cell[2, 4, 35-39]. To understand how the protein quality control machinery acts - and fails - in protein folding diseases it is important to understand the molecular basis of the sensing system and what it does when calling the system into action. The quality control system controls the quality, but it does not determine the shape of the folded protein[38]. The human genome has 20,000 genes, which equal 20,000 proteins. For each protein, the building plan is encoded in the amino acid sequence[16].

11. Protein quality control

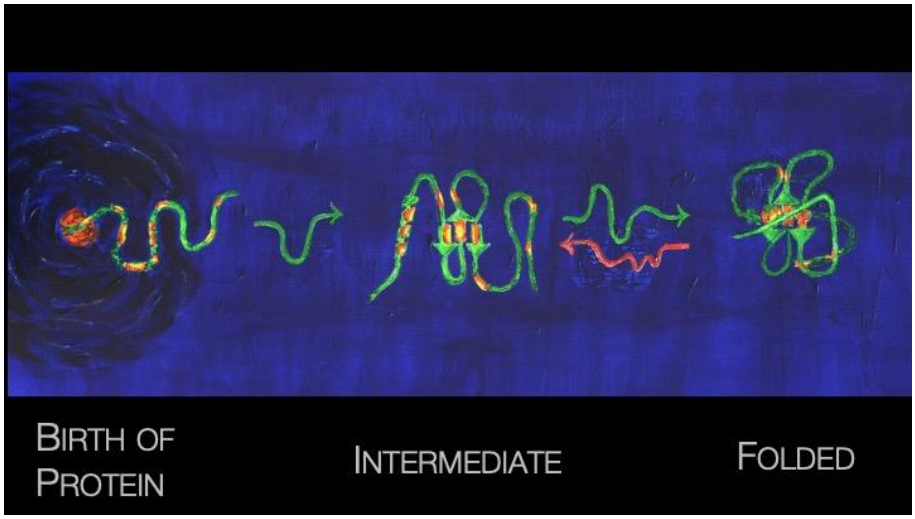


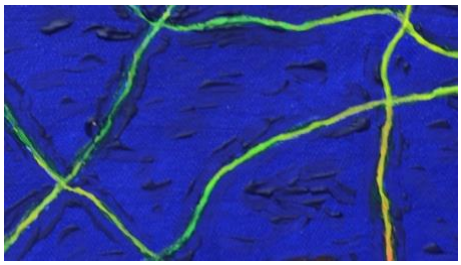
Fig. 7. Folding path of a protein. Proteins are born at the ribosome, leaving the ribosomal tunnel (red). After leaving the ribosome, the protein forms intermediates in which not all hydrophobic stretches (yellow) found the right partners, they are still accessible from the outside. When folding is finished, hydrophobic stretches are buried. Protein structures are dynamic, they can unfold again (red arrow).

Proteins are born at the ribosome, leaving as linear chains (**Fig. 7**)[40]. At this moment, the proteins appear inside out, exposing the hydrophobic groups (yellow in the Figure) that can clog together, with themselves or other proteins.

Proteins remain vulnerable until they folded into the native state, when their hydrophobics are buried, and the outside is hydrophilic[41-43]. This calls for help, and this call is answered by a conserved set of molecular chaperones.

Help remains a life-long need for proteins. Folded proteins can also unfold again (**Fig. 7**, red arrow). Proteins are dynamic, their structures are breezing[44-46]. Many of them act as machines, and machines have moving parts. Proteins have evolved to perform a specific task, such as catalysing a particular reaction, binding a particular partner, open or close a gate or building up a structure[47]. The structure is just means to perform the task. It is the need for the work to be done, not the structure itself that had been selected for in evolution.

As evolution selected for function, proteins are just sufficiently stable to be able to do their job. Thus, dynamics is more important that stability[47]. The price every organism pays for this is the need to deal with damaged proteins that lose shape and function. Proteins that lose function disturb other processes by accidentally exposing hydrophobic groups by turning inside out.



Threads of life, fragment VIII.

The protein quality control network ensures protein health[37, 48]. Supporting protein health is one of the most basic processes of life, and despite its importance still poorly understood. The protein quality control system enables the proper function of all 20,000 proteins in human cells. It is a huge network, which consists of almost 3,000 proteins[49]. The protein quality control system protects the organism well for most of the life. The onset of a protein folding disease is the fatal consequences when this system does not work properly anymore[4, 37, 38, 50, 51]. Strikingly, the activity of this network changes throughout all phases of life, from the embryo over adulthood to old age[37, 52, 53]. Thus, it is important is understand how we can play this system to restore health when the system fails. This could lead to novel strategies to develop drugs that either cooperate with the system or mimic the actions of its components.

The central chaperone cascade of life consists of two major players, historically named heat shock proteins 70 and 90, in short Hsp70 and Hsp90[54-56]. The names originate from increased production upon heat stress and their molecular weight. They are protein families that are conserved from bacteria to man[38, 54, 57, 58]. They are reptiles of protein evolution. These two chaperone families are energy consuming machines[54, 56, 57, 59-63]. This energy consumption is needed to influence protein folding processes. Protein folding steps from unfolded to folded states and back are reversible[42]. A system that does influence this equilibrium needs to burn energy to introduce a direction.

12. Hsp70 as the central chaperone system

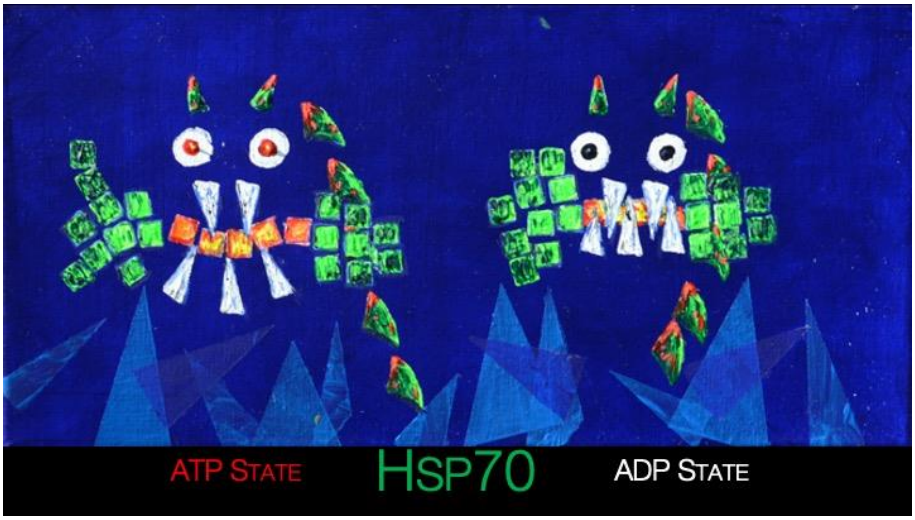


Fig. 8. The Hsp70 chaperone machine. (LEFT) In the ATP-bound state, the substrate binding cleft is open, hydrophobic stretches can bind and leave the substate binding site fast. (RIGHT) In the ADP-bound state the substrate binding site closes, the substrate is trapped.

The most central chaperone is the Hsp70 system (**Fig. 8**). It acts like a monster that puts its teeth into the hydrophobic segments (yellow in **Fig. 8**) before they can aggregate. Hsp70 the most versatile chaperone machine developed during evolution. It is the central node in a large web of molecular chaperones and their partner proteins[64-68]. Life evolved in the presence of this chaperone family. Thus, bacterial Hsp70 functions in the same manner as the human Hsp70[69, 70].

The Hsp70 machine consists of two domains[69]. The substrate binding domain recognises unfolded proteins, and the ATPase domain triggers the action of the substrate binding domain. The secret how the Hsp70 machine can recognise all 20,000 proteins in human cells resides in its substrate binding domain [71, 72]. It is the mouth of the reptile that sets its teeth into the unfolded protein. Hsp70 needs to bind unfolded proteins but not folded proteins. The substrate binding site is adapted to short, very hydrophobic stretches that are around five amino acids long and build the core of a folded protein (**Fig 8 LEFT**)[71-73]. Typically, these sides reside inside a folded protein and are hidden from the outside. Thus, under normal circumstances there are no proteins Hsp70 can bind to. On average, there is one binding site every 36 amino acids [72]. A small domain of 100 amino acids will have two to three Hsp70 binding sites. When the protein is unfolded, these sites become available for Hsp70, but they disappear when the protein folds[54]. This makes Hsp70 a tailor-made sensor for unfolded proteins.

The binding of Hsp70 to its substrates is controlled by the ATPase domain[69]. This domain binds and hydrolyses Adenosine Triphosphate, ATP. ATP is the energy currency of the cell.

ATP cleavage in Hsp70 triggers opening and closing of the mouth of the substrate binding cavity[60, 69]. In the ATP-bound state, the substrate binding cleft is open. It binds substrate very fast, but substrate is also leaving fast (**Fig. 8 LEFT**)[60, 69, 71]. When ATP is hydrolysed into Adenosine Diphosphate, ADP, the

mouth closes, and the short hydrophobic substrate stretch is enclosed (**Fig. 8 RIGHT**). Now substrate binds only slowly, but it is released only slowly. When ADP leaves, ATP can rebind and the mouth opens, and we are back in the fast binding-fast release mode. This allows the bound protein to leave - and to fold. Interestingly, the energy of the ATP hydrolysis is not used to fold the protein, it gives the system a direction, to break reversibility of folding/unfolding equilibrium. Only energy consuming chaperones can do that.

How does this fold proteins? The answer is - not at all. Hsp70 binds a stretch that is needed to build the nucleus of the folded protein structure[54, 55]. As long as Hsp70 is bound, the core of the protein cannot fold. The specificity of Hsp70 is tailored to Unfold proteins, not to fold them. If Hsp70 would bind to the outside of the protein instead of the inside, in this case it could stabilise the protein. What is the point of having an Hsp70 chaperone machinery that is designed to unfold proteins?

The function of Hsp70 is to disentangle hydrophobic stretches[54, 74]. After release from Hsp70, these stretches have a new chance to find the correct partner stretch, and if they do the protein quickly forms a folding nucleus that ultimately leads to the native state.

Repetitive cycles of binding and release from Hsp70 can lead to the native state. This process stalls, however, if several Hsp70s bind to various binding sites, or if Hsp70 re-binding after release is faster than folding[54]. Channelling folding into the right direction is the task of the other chaperone reptile, Hsp90.

13. The Hsp90 machinery

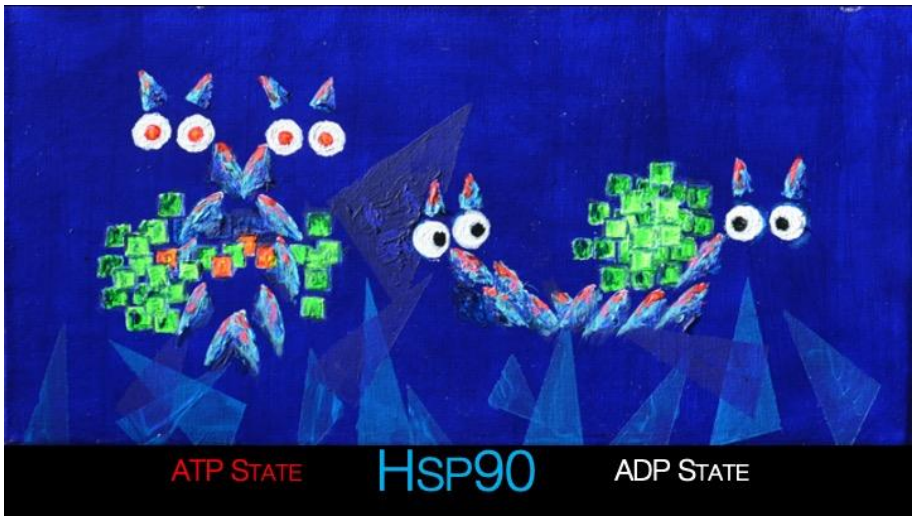


Fig. 9. The Hsp90 chaperone machine. (LEFT) Hsp90 forms a dimer. In ATP-bound state, a substrate stretch of various hydrophobicity can cross the central ring of the Hsp90 dimer, while the parts of the substrate on both sides of this stretch bind in folded form on the outside Hsp90. (RIGHT) In ADP-bound state the ring opens on one side. Hsp90 can bind substrate proteins until they are folded.

In contrast to Hsp70, the Hsp90 chaperone does not have a highly hydrophobic substrate binding mouth, it does not have teeth[75, 76]. It acts like a double-headed snake that cuddles around its prey (**Fig. 9**). Hsp90 is a dimeric prote[77]in

that binds its substrate on its outside, on a surface scattered with hydrophobic and hydrophilic amino acids[59, 77, 78]. Like Hsp70, Hsp90 is an ATPase[79]. The ATP state determines the shape of the dimer, and thereby the interaction with substrate[80]. In ATP-bound conformation, both halves of the dimer form a ring-shaped structure (**Fig. 9 LEFT**)[80, 81].

A stretch of the substrate can pass through the opening of the ring[82-85]. The inner surface of the ring allows for some mildly hydrophobic contacts (**Fig. 9 LEFT,** depicted orange) to the stretch crossing its centre[78]. On both sides of this stretch, the substrate protein folds and makes contacts to the outside of the Hsp90 snake[78, 85].

Upon ATP hydrolysis the Hsp90 ring opens on one side, the double-headed snake stretches into an elongated shape (**Fig. 9 RIGHT**)[80, 86]. The substrate remains bound on the outer skin of Hsp90[77]. Thanks to the scattered nature of the Hsp90 surface, the protein can continue folding into the native state, even while being bound to Hsp90[77, 87].

14. The Hsp70-Hsp90 cascade

Chaperone function is determined by specificity. The less hydrophobic nature of the large Hsp90 surface positions is adapted to take over the substrate from the claws of Hsp70[54, 55]. While the aggressively hydrophobic mouth of Hsp70 blocks folding, the Hsp90 skin does not[54]. Together, Hsp70 and Hsp90 form the central folding cascade in all kingdoms of life, increasing the yield of folded protein[54].

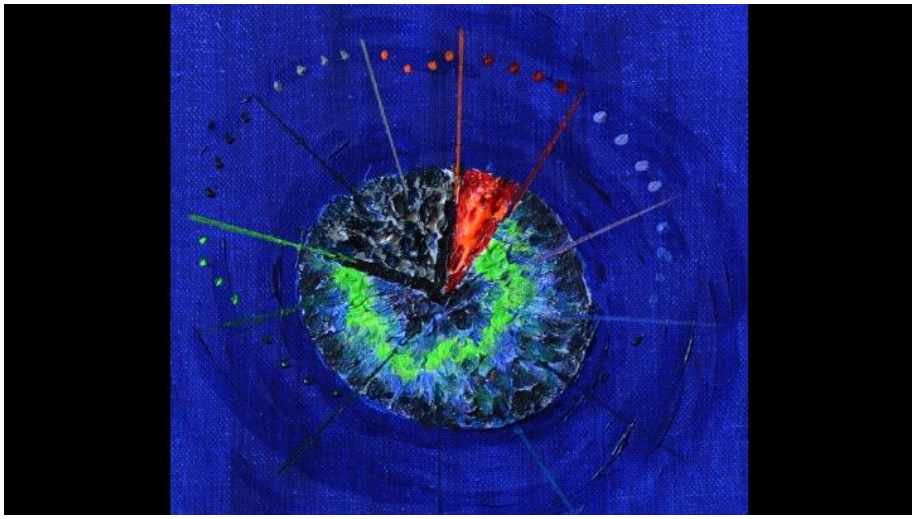


Fig. 10. Timing of chaperone action. The Hsp70-Hsp90 chaperone cascade is active in the first few minutes of the folding reaction, the red-hot phase. The folding protein reaches a conformation from which it can fold on its own, taking the long, green path over the energy hypersurface towards the native state, without further influence by the chaperone cascade.

Fig. 10 depicts the timing of the chaperone cascade, in form of a folding clock. The first few minutes are the red-hot phase for chaperone action, bringing the protein into a state from which it can fold on its own[55]. The function of the chaperone cascade is to influence the earliest stages of the folding process such that the protein forms a folding nucleus, from which embarks on a productive

folding path on its own, following a long journey via the green path into its active structure[55].

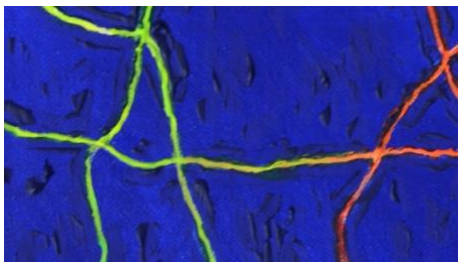
The ATP cycle enables a plethora of partner proteins to tune the activity by accelerating or delaying the ATPase cycle, up to 3 orders of magnitude[59, 60, 69, 88]. The human cytoplasm has nine different Hsp70 machines and two Hsp90s[49]. Both systems together have around 100 co-factors that tune their activity[49]. Thus, there are ten times more factors to tune and regulate the machines than machines itself.

For its function in the conserved protein folding cascade, Hsp90 does not need any further partner proteins[54, 55, 89]. In fact, the bacterial Hsp90 does not have any known co-factors[54]. Thus, while dispensable for protein folding, co-factors link human Hsp90 to complex processes that are absent in bacteria, such as the hormones that communicate between different organs in our body[54, 90, 91].

15. Chaperoning fibril diseases

Partner proteins can adapt the chaperone machinery for specific needs of substrate proteins beyond effective folding[92, 93]. A particular challenge of the human cell is to deal with disordered proteins such as Tau or α-synuclein[94]. Such proteins are rare in bacteria. They are lacking sufficient hydrophobic residues to form a stable folding nucleus[94]. Still, they interact with Hsp70 and Hsp90[95-97]. Tau is a particular interesting case. The role of Tau is to stabilise the cytoskeleton, by lining on the outside of rod-like structures, the microtubules[98]. These rods are dynamic, and when they disassemble the cell needs to remove the Tau protein that is now set free[98].

The protein that scavenges the free Tau is Hsp90[99]. Hsp90, however, does not fold Tau [75, 76]. It would be a Sysiphos task to do so, given that Tau lacks the hydrophobic residues to ever fold. Instead, the Tau-bound Hsp90 binds to a co-factor of the degradation machinery, the CHIP protein[99]. CHIP transfers a degradation label on the Hsp90-bound Tau. This label is a small protein, ubiquitin. The cell disposes of ubiquitin labelled proteins, and Tau any free Tau can be removed via this path[100, 101]. Thus, Hsp90 does not fold Tau, instead it applies a kiss of death to any Tau protein bound. This highlights a key function of molecular chaperones: They are not only effective folding factors, but they are also nodes for deciding the fate of the protein - between folding, holding and removal[102, 103].



Threads of life, fragment IX.

16. Strategy to tackle protein diseases

As long as the Hsp90-controlled removal of Tau works a human is protected from Alzheimer's Disease[4, 100, 104, 105]. It is unclear why this process stops in disease. This offers two strategic options for early intervention: 1. Restoring normal business in which the quality control machinery removes Tau. 2. Actively target fibrils at the earliest moment and flag them for removal.

In most cases dementia is not caused by mutations, thus in principle all humans possibly get it when old enough[34]. Most 90-year-olds have Tau tangles in the brain, some above the pathological threshold, others below. Thus, the effectivity of the control machinery decreases with age[37]. The extent of the decline determines whether the balance tips towards the pathological state[4]. Thus, engaging the protein quality control system is an attractive strategy in dementia[4, 106-108].

The potential for traditional small molecule drugs in fibril diseases is limited. Fibrils do not have pockets, and they do not need to be inhibited. Also, the protein quality control system is more likely to need activation than inhibition[4]. The increase of knowledge on the protein quality control system offers to use a different type of drugs, biologics. Biologics are derived from biomolecules such as proteins, protein fragments or RNA strands[109, 110]. A widely known example are the RNA vaccines against covid19, for which the 2023 Nobel Prize for Medicine was awarded[111]. A biologic has more potential to engage the natural system than small molecule drugs.

17 Biologics in Alzheimer's Disease

A type of biologics that recently showed some success in delaying the progression of Alzheimer's Disease had been antibodies[112, 113]. The antibodies Leqembi and Donanemab target precursors of the amyloids that form outside neurons in the process that leads to the disease[113-115]. These antibodies reduce amyloid levels, and they prove that directly targeting aggregates can be a promising aim. There is an urgent need for better drugs, though. These antibodies have strong side effects, which can be deadly, they delay the progression of the disease by only 5 months, and they are very expensive, requiring fortnightly infusions of an antibody solution over a period of 18 months[112-114, 116, 117].

The application of antibodies is limited to processes that happen outside cells. Most aggregation events take place inside cells, though[17, 20, 28, 29, 118]. This includes the aggregation of Tau in Alzheimer, a-synuclein in Parkinson or huntingtin in chorea Huntington. General challenges for developing biologics for processes inside the cell is to get the drug into the cell, ensure sufficient stability and limit potential side effects. Most important is, however, is to identify and target a relevant process, though.

18. Fibril paint

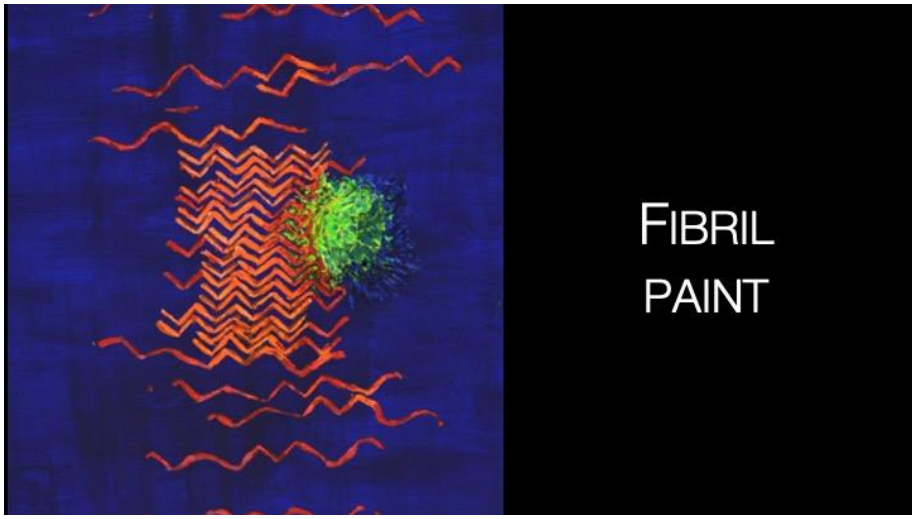


Fig. 11. Fibril paints (green) are peptides that bind to protein fibrils (orange) but not to their monomeric precursors.

A strategy for developing a biologic that would target protein fibrils inside cells could be a drug that mimics the action of Hsp90: recognising the target protein and link it to the degradation machinery[119]. The first step to develop such a drug is to specifically recognise protein fibrils, preferentially at the earliest possible moment.

The chemist's approach is to identify unique properties that make protein fibrils different from monomers. Chemically protein fibrils are characterised by a repetitive structure[17, 18]. The repetitive nature of fibril structure would allow compounds with multiply weak binding sites to bind to fibril polymers, but they would not recognise individual molecules. Interactors that are interesting for this purpose are so-called π-stackers[119-121]. These are aromatic or polar groups that can engage in such weak interactions. This work resulted in a family of peptides that bind to Tau fibrils[119]. Peptides are short protein fragments, in this case around 20 amino acids long. The peptides carry a fluorescent group, which allows easy monitoring of fibril binding. They are named "Fibril paint", (**Fig. 11**, green dot)[119]. They bind to the fibril rod but not to the free protein at the top and bottom, which have not yet joined the fibril. FibrilPaint1 binds sufficiently strong to fibrils that they detect Tau fibrils derived from patient's brain of Alzheimers's Disease, Frontotemporal Dementia and Corticobasal Degeneration[119].

19. Fibril paints as Diagnostics

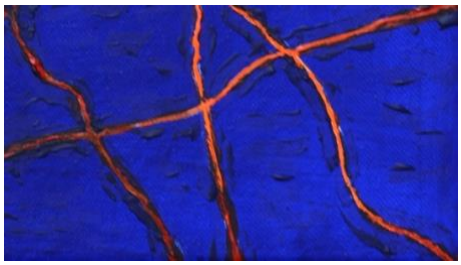
FibrilPaint1 is a promising drug lead to develop diagnostic assays for fibril disease. Importantly, Fibril paint peptides do not bind to denatured proteins, they require the specific fibril structure[119]. Remarkably, FibrilPaint1 does not only bind to Tau, but also the chemically very different fibrils of huntingtin[119]. The common feature of both types of fibrils is indeed the repetitive structure[19, 21].

The broad but specific recognition of fibrils makes Fibril paint peptides interesting compounds to develop diagnostic assays. The holy grail for diagnostic assays in this field would be the detection of protein fibrils in body fluids such as blood[122-125]. This would enable early detection of fibrils in the

body. Any future cure of aggregation diseases requires improved diagnostic methodology to for monitoring the disease state and the efficacy of the treatment.

In case of Parkinson's Disease, it is known that fibrils spread from the brain to other organs[126-128]. Sufficiently sensitive detections may be interesting also other aggregation diseases, for example the IAPP protein that form fibrils in diabetes[129]. The current fibril paint peptides are an attractive start point to develop a sensitive fibril detection platform.

19. Fibril removal



Threads of life, fragment X.

A peptide family that binds strongly and specific to protein fibrils can be an interesting start point for drugs that marks fibrils for degradation. Fibril paints can be combined with various recognition motifs for the protein removal machinery. Indeed, it is now in at least one case possible to use FibrilPaint1 to connect a degradation marker to Tau fibrils[119]. This allows the fibrils paint compound to act like the Hsp90. It cuts out the middleman and links the fibrils directly to the degradation, machinery, without the need of the chaperone.

Fibril paints peptides are an exciting start point to develop drugging strategies that cooperate with the cellular protein quality control machinery. Can we make molecules that increase the efficiency of molecular chaperones? Can we switch between various pathways to remove unwanted fibrils? This could be important as in case one pathway does not work very well in one specific cell type, maybe another one could take over? The recent antibody-based results showed that protein aggregates can be targeted[112, 130]. The fibrils paints may lead to compounds facilitating removal of fibrils at the earliest possible stage.

20. Predictability makes chemistry a science

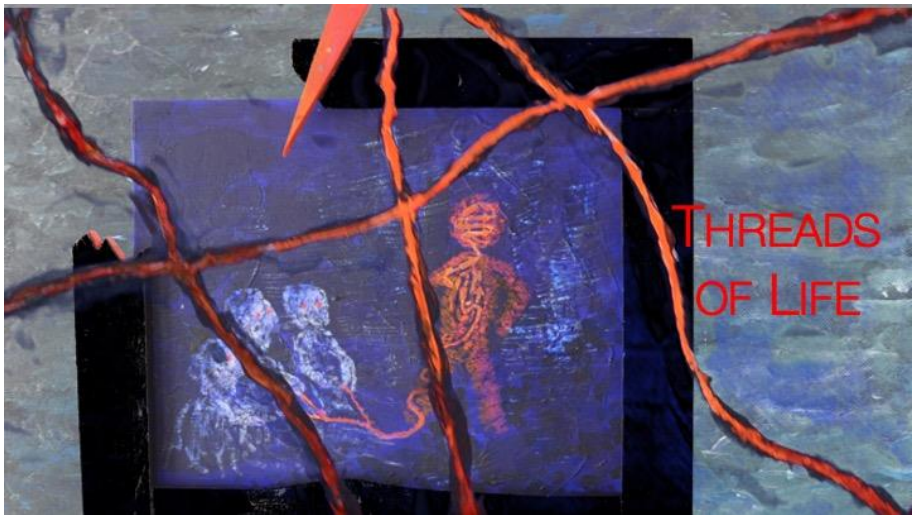
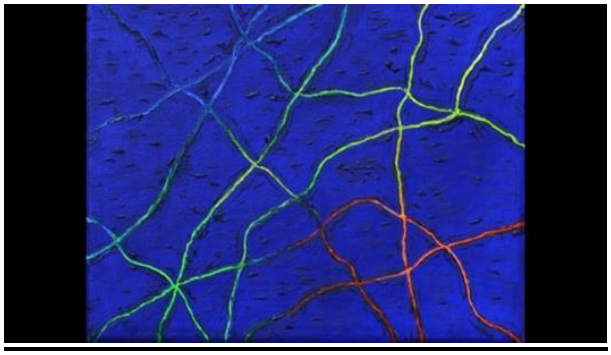


Fig. 12 The ultimate goal of Protein Chemistry of Disease - regaining control of the threads of life from the Norns (Fig. 1).

Fibril paint peptides are an example how fundamental research in Protein Chemistry can contribute a rational approach for an urgent but unsolved medical riddle. The start point is mechanistic understanding of the protein quality control system. The recent progress in structural understanding of biomolecules by leaps cryo-electron microscopy and by artificial intelligence enables a rational approach that was very recently impossible. From here we can embark on a reductionist strategy, targeting diseases that had been the incarnation of incurable. This fuels the dream to take over the threads of life from the norns and moiras (**Fig. 12**).

Referring to Kant, it is a prerequisite for doing science that space and time are given *a priori* [6, 7]. Predictability and apodictic certainty are the basis of the scientific approach. They make chemistry a science, but a very special one. By creating molecules that play the networks in cell it is also an art, the art that may allow us to control our destiny.

21. Threads of life of a project



Threads of life - the full picture.

Space and time have also concrete relevance for doing science.

Space:

Modern research is about sharing - sharing thoughts, sharing data, sharing equipment and sharing resources. Effective sharing requires that researchers using the same space. Key for the success of sharing and collaboration is that research institutes always find themselves in one location, to be frugal with resources and to maximise quality.

And then for Time:

Research quality is about providing conceptual advance. Conceptual advances take time. In 2018, Tania Morán Luengo in my team published the discovery on how the conserved chaperone cascade operates, in collaboration with the group of Matthias Mayer in Heidelberg[55]. It provided the answer to the central question I was aiming for in 2004 when I started by laboratory in Utrecht. The finding is based on understanding the substrate binding modes of both Hsp70 and Hsp90. Elif Karagöz published in 2014 a structural model of Hsp90 and Tau, in collaboration with the group of Rolf Boelens[76]. She started her PhD with me in 2006 and her key publication ultimately came out when she was already postdoc in California. The binding motif of the Hsp70 machine was my own discovery when I was PhD student with Bernd Bukau in Heidelberg and Freiburg, in the previous millenium[72]. Some of my current co-workers were born in the year when the study was published.

Back in 2006, Madelon Maurice at the UMCU and myself started a joint project on the relation of protein stability and cancer, funded by High Potential fund of Utrecht University. 10 years later, in 2016, we finally published that concept[131]. Based on this study, we started working with peptides interfering with aggregation, in collaboration with Assaf Friedler at Hebrew University, Jerusalem. Luca Ferrari found that peptides can inhibit Tau aggregation[120]. Martina Radli and Lena Wawrzyniuk started on to work on Hsp90 partner proteins, key for understanding peptide interactions with the chaperone machinery. Margreet Koopman transferred our work to protein purified from patients' brain. In 2022, Júlia Aragonés Pedrola and Françoise Dekker establishing the fibril paint concept[119].

Protein chemistry is slow science. It is fundamental science and as such it requires faith into long term strategy aiming for years into the future.

It does help to meet people with spark when pursuing long term goals - co-workers, colleagues and students. It is a privilege working at Utrecht University to teach in enthusiastic students. This morning our master students joined the symposium "Chemistry of Disease". This is what university should be about, the unity of teaching and research.

Acknowledgement

I am grateful to the Executive Board of Utrecht University for creating the Chair Protein Chemistry of Disease and for appointing me. I am obliged to the Dean of the Faculty of Sciences, Isabel Arends, for supporting this and also for trusting me with the Department of Chemistry. I thank Ineke Braakman for getting me to Utrecht. Thank you also to all friends and colleagues over the years, including those who infected me with schaatskoorts, one of the diseases for which there is no cure yet. Last and most importantly I thank Silke and Gesa, Pia and Esther for all the things that are even more important than science.

Dixit.

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Relevant quotations in reference [7], Immanuel Kant, "**Metaphysische Anfrangsgründe der Naturwissenschaft**" on Chemistry as a science, reproduced from "Kants gesammelte Schriften" edited by the Preussische Akademie der Wissenschaften.

p.468 l.17

*"Eigentliche Wissenschaft kann nur diejenige genannt werden, deren Gewissheit apodiktisch ist; Erkenntnis, die bloss empirische Gewissheit enthalten kann, ist nur uneigentlich so genanntes Wissen. Dasjenige Ganze der Erkenntnis, was systematisch ist, kann schon darum Wissenschaft heissen und, wenn die Vernüpfung der Erkenntnis in diesem System ein Zusammenhang von Gründen und Folgen ist, sogar rational Wissenschaft. Wenn aber diese Gründe op der Prinzipien in ihr, wie z. B. in der **Chemie**, doch zuletzt bloss empirisch sind, und die Gesetze, aus denen die gegebenen Facta durch die Vernunft erklärt werden, bloss Erfahrungsgesetze sind, so führen sie kein Bewusstsein ihrer Notwendigkeit bei sich (sind nicht apodiktisch-gewiss), und darum alsdann verdient das Ganze in strengem Sinne nicht den Namen einer Wissenschaft, und **Chemie** sollte daher eher systematische Kunst als Wissenschaft heissen."*

p.470 l.13

"Ich behaupte daher, dass in jeder Naturlehre nur so viel eigentliche Wissenschaft angetroffen werden könne, als darin Mathematik anzutreffen ist."

p.470 l.27

"Also mag zwar einen reine Philosophie der Natur überhaupt, d. i. diejenige, die nur das, was den Begriff einer Natur im Allgemeinen ausmacht, untersucht, auch ohne Mathematik möglich sein, aber eine reine Naturlehre über bestimmte Naturdinge (Körperlehre und Seelenlehre) ist nur vermittelst der Mathematik möglich, und da in jeder Naturlehre nur so viel eigentliche Wissenschaft angetroffen wird , als sich darin Erkenntnis a priori befindet, so wird Naturlehre nur so viel eigentliche Wissenschaft enthalten, als Mathematik in ihr angewandt werden kann."

p.470 l.36

*"So lange also noch für die **chemische Wirkung der Materien** auf einander kein Begriff ausgefunden wird, der sich construieren lässt, d. i. kein Gesetz der Annäherung der Teile angeben lässt, nach welchem etwas in Proportion ihrer Dichtigkeiten u.d.g. ihre Bewegungen samt ihren Folgen sich im Raume a priori anschaulich machen und darstellen lassen (eine Forderung, die schwerlich jemals erfüllt werden wird), so kann Chemie nichts mehr als eine systematische Kunst oder Experimentallehre, niemals aber eigentliche Wissenschaft werden, weil die Principien derselben bloss empirisch sind und keine Darstellung a priori in der Anschauung erlauben, folglich die **Grundlage chemischer Erscheinungen** ihrer Möglichkeit und nicht im mindesten begreiflich machen, weil sie der Anwendung der Mathematik unfähig sind."*

p.471 l.21

*"Aber nicht einmal als systematische Zergliederungskunst oder Experimentallehre kann sie [die Mathematik] der **Chemie** jemals nahe kommen, weil sich in ihr das Mannigfaltige der inneren Beobachtung nur durch blosse Gedankenteilung von einander absondern , nicht aber abgesondert aufbehalten und abgesondert aufbehalten und beliebig wiederum verknüpfen, noch weniger aber ein anderes denkendes Subjekt sich unseren Versuchen der Absicht angemessen von uns unterwerfen lässt, und selbst die Beobachtung an sich schon den Zustand des beobachteten Gegenstandes alteriert und verstellt. Sie kann daher niemals etwas mehr als eine historische und, als solche, so viel möglich systematische Naturlehre des inneren Sinnes, d. i. eine Naturbeschreibung der Seele, aber nicht der Seelenwissenschaft, ja nicht einmal psychologische Experimentallehre werden; welches dann auch die Ursache ist, weswegen wir uns zum Titel dieses Werkes, welches eigentlich die Grundlage der Körperlehre enthält, dem gewöhnlichen Gebrauche gemäss des allgemeinen Namens der Naturwissenschaft bedient haben, weil ihr diese Benennung im eigentlichen Sinne allein zukommt und also hierdurch kein Zweideutigkeit veranlasst wird."*