Chapter 10

Structure and Function of Stefin B Oligomers – Important Role in Amyloidogenesis

Ajda Taler-Verčič, Mira Polajnar and Eva Žerovnik

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/57570

1. Introduction

Many functional proteins act as oligomers. Oligomerization is a well-controlled and regulated process. Those proteins cannot adopt their physiological functions without oligomerization, while the protein misfolding and subsequent unphysiological oligomerization influence the primary protein functions and produce a "gain in toxic function" as the prefibrillar oligomers are toxic for the cells. Misfolding, oligomerization and aggregation are the reasons for the so called conformational diseases. Several of them are neurodegenerative, but some also affect other vital organs. The accumulation of intracellular protein aggregates (various inclusions) and of extracellular protein deposits cause severe cellular degeneration, such as neurodegeneration of affected neurons. Different proteins form rather similar but not identical fibrillar structures, all showing cross-β-structure, where continuous β-sheets run perpendicular to the long axis of the fibrils.

Neurodegenerative diseases like Alzheimer's disease, Parkinson's disease, Huntington's disease, prion diseases and many others have in common protein aggregation to amyloid fibrils. Type II diabetes is also an amyloid disease although it is not neurodegenerative. Nowadays it is believed that ordered prefibrillar oligomers or protofibrils may be responsible for cell death and that mature fibrils may even be neuroprotective. Most amyloid prone proteins form different oligomers in the lag phase of the amyloid fibril formation. Conformational diseases (Table 1) are difficult to diagnose in the early stage, because they are usually asymptomatic during their development. Even when they can be diagnosed in the early stage there is an ethical reason for avoiding diagnosis (i.e. to hesitate letting know the patient or the relatives), because there are still no therapies which would slow down or even stop the progression of this type of diseases. Many different proteins are being studied in order to understand the common molecular mechanism of the conformational disease and to develop

appropriate treatments. Studies use *in vitro* (different spectroscopic methods), *ex vivo* (different cell cultures) and *in vivo* (mouse models) systems to clarify the changes of the protein conformation and the down-stream effects during the whole process.

Conformational diseases (organ/systemic))	Protein
Alzheimer's disease, Down's syndrome (Trisomy 21), Hereditary cerebral angiopathy (Dutch)	Amyloid precursor protein (Aβ 1-42)
Kuru, Gerstmann-Straussler-Scheinker Syndrome (GSS), Creutzfeld-Jacob disease, Scrapie (sheep), Bovine spongiform encephalopathy ("mad cow")	Prion protein
Type II diabetes mellitus (adult onset)	Islet amyloid polypeptide (amylin)
Dialysis-associated amyloidosis	β_2-microglobulin
Senile cardiac amyloidosis	Atrial natriuretic factor
Familial amyloid polyneuropathy	Transthyretin
Reactive amyloidosis familial Mediterranean fever	Serum amyloid A
Familial amyloid polyneuropathy (Finnish)	Gelsolin
Macroglobulinemia	Gamma-1 heavy chain
Primary systemic amyloidosis	Ig-lambda, Ig-kappa
Familial polyneuropathy – Iowa (Irish)	Apolipoprotein A1
Hereditary cerebral myopathy – Iceland	Cystatin C
Nonneuropathic hereditary amyloid with renal disease	Fibrinogen alpha, Lysozyme
Familial British dementia	FBDP
Familial Danish dementia	FDDP
Diffuse lewy body disease, Parkinson's disease	α-synuclein
Fronto-temporal dementia	Tau
Amyotropic lateral sclerosis	Superoxide dismutase-1
Triplet repeat diseases: (Huntinghton's, Spinocerebellar ataxias	Polyglutamine tracts (Huntingtin)
Spinal and bulbar muscural atropy	Androgen receptor
Spinocerebellar ataxias	Ataxins
Spinocerebellar ataxia 17	TATA box-binding protein

Table 1. Diseases of protein misfolding: amyloidoses and non-amyloidoses (reviewed in [1])

Both the amyloid forming proteins involved in certain conformational disease and model proteins are used (Table 2). Stefin B is a model protein for studying amyloid fibril formation.

SH3 domain p 85 phosphatidylinositol 3-kinase	Fibronectin type III phosphoglycenite linase acylphosphatase
HypF N-terminal domain (*E. coli*)	Amphoterin (human)
Apomyoglobin (equine)	Apocytochrome c
Endostatin (human)	Met aminopeptidase
Stefin B (human)	ADA2H
Fibroblast growth factor (*N. viridescens*)	Apolipoprotein CII
VI domain (murine)	B1 domain of IgG binding protein
Curlin CgsA subunit	Monellin
Serpins	

Table 2. Nondisease related amyloid forming proteins/peptides – model proteins (reviewed in [1]) serpins leads to diseases when there are mutations inducing aggregation or change of conformation (e.g. see serpinopathies and the Conformational dementias by *David A. Lomas and Robin W. Carrell* [2].

2. Human stefin B

Stefins are endogenous cysteine protease inhibitors [3], which are ubiquitously expressed in human tissues [4]. They are specific for the papain-family of cysteine proteases and classified as the IH clan in the MEROPS scheme [5]. They are mainly intracellular inhibitors, although have also been found outside the cell in body fluids [6]. They do not have a signal peptide, and they bear a cystatin motif QXVXG, which is the main site involved in binding to target enzymes.

Human stefin B (also termed cystatin B) possesses 98 amino acid residues (Mr= 11 kDa) and no carbohydrate groups or disulphide bridges [7], although it contains one free cysteine [8]. It is an intracellular protein, which tightly and reversibly binds to papain-like cysteine proteases. We will simply call it stefin B from now on.

Stefin B main function is protection against inappropriate proteolysis of lysosomal cysteine proteases [9]. It is an inhibitor of cathepsins B, L, H and S [7, 10, 11]. However, it exerts some additional functions. It was found to interact with five known non-protease proteins (neurofilament light chain (NFL), brain β-spectrin, RACK-1, human myotubularin related protein 8 (Mtrp) and human T-cell activation protein (Tcrp)) [12]. NFL and β-spectrin are specific to the nervous system. Stefin B (cystatin B) knock-out mice show neurological disorder (loss of the cerebellar granule cells, because of apoptotic bodies, chromatin condensation and some other changes). This suggests that stefin B has an essential anti apoptotic role in the cerebellum [12].

Stefin B is overexpressed in patients with hepatocellular carcinoma and is therefore in combination with some other proteins used as a marker for this disease (it is elevated in the

serum already at the early stage of the disease development and therefore easy to detect) [13]. In different types of cancer (human colorectal cancer [14], gastric cancer [15], esophageal carcinoma [16], prostatic adenocarcinoma [17], bladder cancer [18]…) both lower expression level and higher activity due to higher expression level have been detected.

Stefin B is localized both in the cytosol and in the nucleus [19, 20]. It is expressed in neurons and glial cells in the brain, but with slightly different localizations; in neurons it localizes to the nucleus while in astrocytes it is localized in the nucleus and in the cytoplasm [21]. It inhibits cathepsin L in the nucleus, whose substrates are transcription factors. Interaction with nucleosome – histones H2A.Z, H2B and H3, and cathepsin L in the nucleus has also been reported [19]. Therefore, it likely regulates transcription. Furthermore, stefin B was reported to regulate cell cycle progression into the S phase – entry into the S phase is delayed [19]. Increased expression of stefin B in the nucleus of T98G astrocytoma cells delays caspase-3 and caspase-7 activation and this delay is independent of cathepsin inhibition [22].

Stefin B plays an important role in the immune system. It upregulates the release of nitric oxide from interferon-γ-activated macrophages [23]. The protein is involved in innate immune response to bacterial challenge of the leech *Theromyzon tessulatum* [24]. It has an essential role in protection of the central nervous system from apoptosis [25] and from oxidative stress [26]. Increased level of stefin B was found in the senile plaques of Alzheimer's and Parkinson's diseases and in samples of patients suffering from senile dementia [27].

Stefin B functions are summarized in Table 3.

Loss of functions because of alterations in the cystatin B gene (dodecameric repeat expansions in the promoter region or point mutations in the coding gene) is the cause of progressive myoclonus epilepsy of type 1 also known as Unverricht-Lundborg disease (EPM1) [25, 28]. EPM1 mutans are polymeric and aggregate prone *in vivo* [29]. *In vitro* and *ex vivo* G4R, R68X, [30, 31], G50E and Q71P [32, 33] have been studied. Only G4R mutant folds like the wild type protein [31], all others lack tertiary structure and are partially unfolded [31, 33]. G4R and R68X also form amyloid fibrils [31]. Both G50E and Q71P mutants are more susceptible to cleavages by proteases, because of the partially unfolded structure [33], which likely contributes to loss of function in cells. Stefin B deficiency triggers neurodegeneration by impaired redox homeostasis [26]. G4R and R68X do not have any inhibitory activity, while G50E and Q71P are much less active than the wild type protein [32]. All three missense mutants, except for G4R form rather large but diffuse aggregates in cells when over-expressed, while G4R forms small aggregates similar to those of the wild type protein [32].

Cystatin C, a secreted and extracellular protein, is another cysteine protease inhibitor and mutations in its gene are the cause of hereditary cerebral amyloid angiopathy [34]. Human cystatin C is a risk factor for late onset Alzheimer's disease. The protein co-deposits with amyloid β peptide (Aβ peptide) amyloid plaques in patients with Alzheimer's disease [35]. Moreover, cystatin C binds to both the whole amyloid precursor protein (APP) and to Aβ peptide and it inhibits Aβ peptide amyloid fibril formation *in vitro* [36].

Stefin B functions
inhibition of cathepsins B, L, H and S [7, 10, 11];
interaction with five non protease proteins: neurofilament light chain (NFL), brain β-spectrin, RACK-1, human myotubularin related protein 8 (Mtrp) and human T-cell activation protein (Tcrp) (NFL and β-spectrin are specific to the nervous system) – essential anti apoptotic role in the cerebellum [12];
involved in immune-response to bacterial challenge of the leech *Theromyzon tessulatum* [24];
increased level in the senile plaques of Alzheimer's and Parkinson's and of patients suffering from senile dementia [27];
essential role in some of the neurons in the central nervous system, protecting the cells against apoptosis [25];
protects thymocytes against cell death [37];
cell specific expression [21];
inhibits Aβ peptide amyloid fibril formation in oligomer specific manner [38];
positive/negative progression of cancer [13-18];
upregulation of nitric oxide release from interferon-γ-activated macrophages [23];
found mainly in the nucleus of proliferating cells and both in the nucleus and cytoplasm of differentiated cells;
regulates cell cycle [19];
increased expression in the nucleus delays caspase activation [22];
protecting cells from oxidative stress [26].

Table 3. Stefin B has many different functions.

3. Stefin B oligomers *in vitro* and in cells

Stefin B can adopt different oligomeric states *in vitro* and in cells. On the size-exclusion chromatography (SEC) the wild type protein elutes as a set of well-defined oligomers apart from monomers, dimers, tetramers and even higher oligomers (Figure 1) [39]. Y31 isoform is predominantly dimeric [31], while Y31 P79S mutant is tetrameric [40]. All oligomers can be isolated as separate peaks by SEC and stay stable for weeks at pH 7.0 and 4 °C [38].

SEC results have been confirmed up to decamers by electrospray-ionization mass spectrometry (ESI-MS) [41]. In cells stefin B is present both in monomeric and oligomeric forms. Oligomers size ranges between 10 and 250 kDa. The higher oligomeric species are resistant to 1% SDS and 8 M urea and partially resistant to reducing agents (DTT treatment). The low molecular species comprise monomers, dimers, trimers and pentamers. Stefin B polymers *in vivo* seem to grow by monomer addition and not by domain-swapped dimer addition. The protein binds to many different proteins of various sizes [29]. Already the endogenous stefin B forms small punctate aggregates in cells and after overexpression the aggregates amount increases [30].

Oligomers have been observed for many other proteins, especially the amyloidogenic ones. Oligomeric species appear in the lag phase of the reaction and are usually more stable under

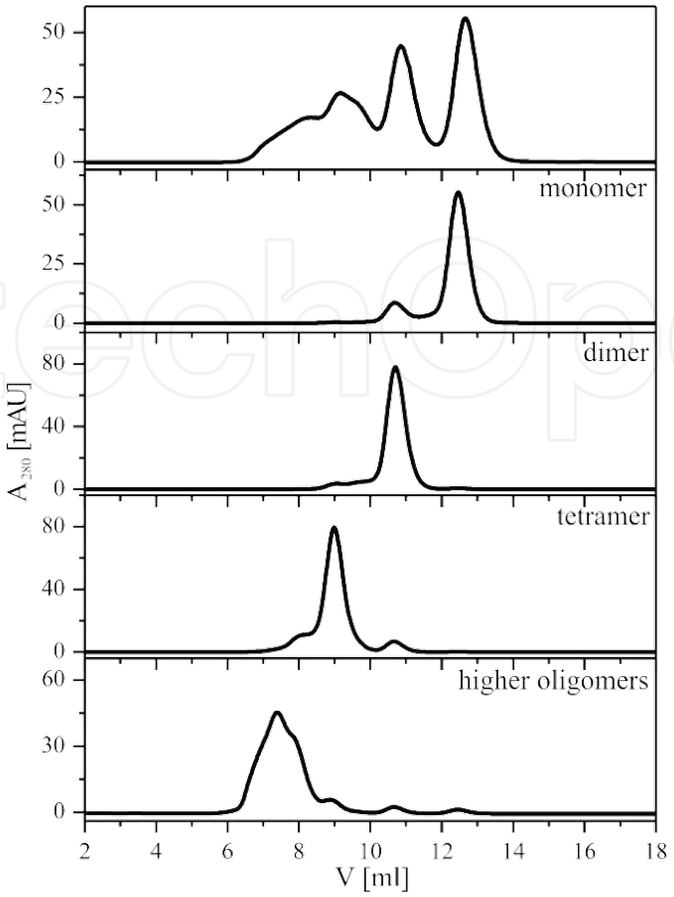

Figure 1. Elution profile of stefin B wild type protein (S3E31 form) at pH 7.0 from SEC. The whole sample (top trace) and separated oligomers as indicated, are shown.

non-amyloidogenic conditions. Size and morphology of those oligomers can be determined by SEC, mass spectrometry, dynamic light scattering and analytical ultracentrifugation. Some examples are given below.

Serpins are serine protease inhibitors also used as a model for studies of amyloid fibril formation. They have key regulatory functions in the inflammatory, complement, coagulation and fibrinolytic cascades [2]. The mechanism of their fibril formation is known in crystallographic details [42, 43]. They form amyloid fibrils through domain-swapping [44]. Serpins preferably oligomerize than interact with other proteins [45]. They form both smaller oligomers and condensed longer polymers [46]. Monomers of some serpins are meta-stable with the rate limiting step representing the transition to the dimer. Once dimers are formed, they can connect to each other to form tetramers or recruit monomers to form trimers and also much longer oligomers [47].

Aβ peptide forms a set of oligomers from monomer to hexadecamer; all even and odd numbered oligomers have been detected by ESI MS [48]. Also for many other amylod forming proteins the whole set of oligomeric species has been observed (insulin [49], β_2-microglobulin [50, 51], SH3 domain [52]…).

http://dx.doi.org/10.5772/57570

4. Inhibitory activity of stefin B oligomers

For a long time it has been thought that monomer is the only active form of stefin B. All other oligomeric forms would serve as a reservoir of monomers or possess additional functions. Now we have shown that all types of oligomers are active against cysteine protease papain (Figure 2). Dimers which are presumably domain-swapped (discussed in the structural characterization part) show the lowest activity in the enzyme to inhibitor 1:2 ratio. They, as well as other oligomers – tetramers and higher, are fully active in the enzyme to inhibitor 1:4 ratio (Fig. 2). SEC-isolated oligomers are all >95% pure and the amount of monomer present in other oligomers samples has been tested to show that the inhibitory effect is not due to monomer contamination. Higher oligomers are a mixture of various oligomers, starting from hexamers. The highest peak represents octamers, which are the prevalent species and therefore we propose that the average size of the higher oligomer is an octamer (this was used for determining the molar ratio). The sample of the higher oligomers contains a small amount of all other oligomers and we suspect that some inhibitory effect could be due to this contamination, but possibly not the whole effect. The results are in agreement with the detection of different oligomers in cells [29] and explain that all detected species could retain inhibitory function.

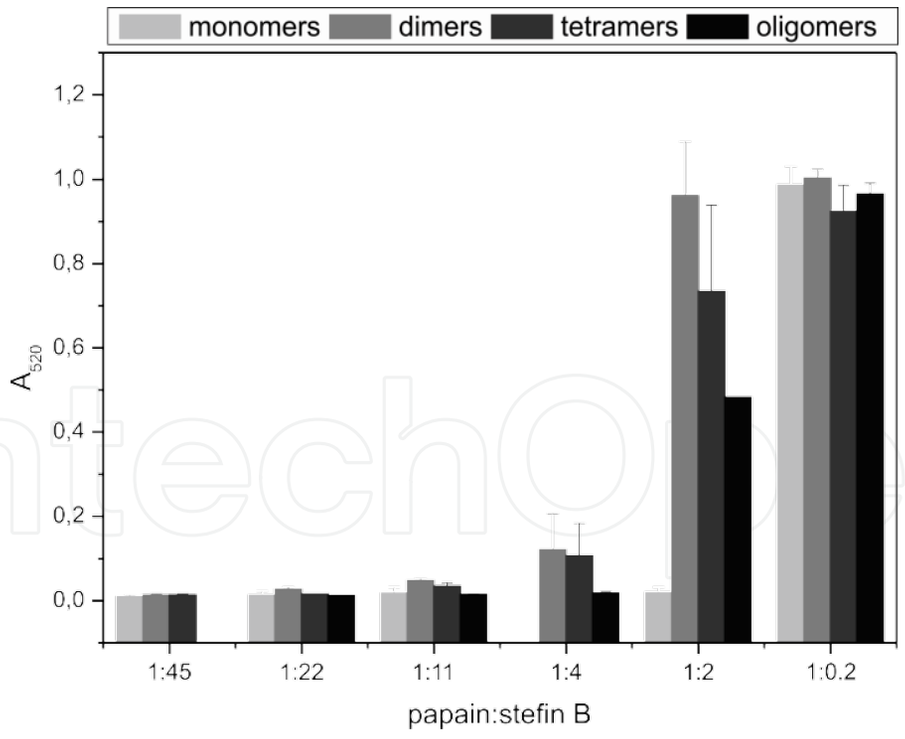

Figure 2. Different stefin B oligomers all exert inhibitory activity against the cysteine protease papain. To evaluate the inhibitory activity of stefin B, BANA test was performed [53]. Stefin B monomers, dimers, tetramers and oligomers were prepared in eight different molar ratios [E]:[I]– 1:45, 1:22, 1:11, 1:4, 1:2, 1:0.2. Higher absorbance means lower inhibitory activity. The average oligomeric state (the highest peak from SEC) in the sample of higher oligomers are the octamers.

5. Structural characterization of different oligomeric states of stefin B

Crystal or solution structure of stefin B monomer and oligomers have been so far characterized from monomer in complex with the target enzyme [54], dimer under amyloid forming conditions [55], tetramer [40] to model of amyloid fibrils [56]. Domain-swapped dimer of stefin A has also been determined by heteronuclear NMR [57].

Stefin B was first crystallized in complex with the cysteine protease papain (Figure 3A) [54]. The structure of the monomeric stefin B consists of a five stranded β-sheet wrapped around a five turn α-helix and with an additional carboxy terminal strand running along the convex side of the β-sheet. This type of the tertiary structure is conserved throughout the cystatin family (Fig. 3A)

High resolution structure of stefin B dimer was determined by heteronuclear NMR [55]. Furthermore, changes within the dimer under amyloid forming conditions were observed and flexible residues were defined. Even under amyloid forming conditions the structure remains largely folded, the main differences are in the flexible loops regions. Prolines on positions 74 and 79 play an important role in the orientations of the loop between strands 4 and 5 (the same loop is involved in "hand-shaking" in the tetramer formation). Four different dimer states have been observed in solution.

When the proline at position 79 was mutated into serine, the tetrameric form of the protein became favourable [40]. This tetramer has been crystallized and its structure determined (Figure 3B) [40]. The tetramer consists of two domain-swapped dimers connected with the so-called "hand-shaking" mechanism to each other. Hand-shaking occurs along with *trans* to *cis* isomerization of the proline at position 74. This proline is conserved throughout the cystatin family. Both domains preserve the fold of the monomer (the same as observed in the complex with papain); each domain is composed of the N-terminal part from one chain (strand 1, the α-helix and strand 2) and C-terminal part from the other chain (strands 3, 4 and 5), between both domains there is a linker region (two peptides, belonging each to one chain).

The structured core of human stefin B amyloid fibrils has been determined (Figure 3C) [56]. Based on the H/D exchange rates the structure could be divided onto protected region (inside the fibril core) and unprotected region (striking out of the fibril). Strands 2, 3, 4 and 5 are protected, while strand 1, α-helix and loops between strands 3 and 4 and 4 and 5 are unprotected. The loop between strands 2 and 3 is the same as the one involved in the domain-swapping mechanism. The fibril core would therefore be made of domain-swapped dimers but with the loop between strands 4 and 5 in position of a tetramer.

6. Initial oligomers on the way to fibril formation

That stefin B forms amyloid fibrils *in vitro* under relatively mild conditions was first shown in 2002 [58] and various solution conditions were probed, fibrils and protofibrils were imaged by AFM and TEM [59]. The fibril formation of stefin B was compared to that of stefin A, a much

http://dx.doi.org/10.5772/57570

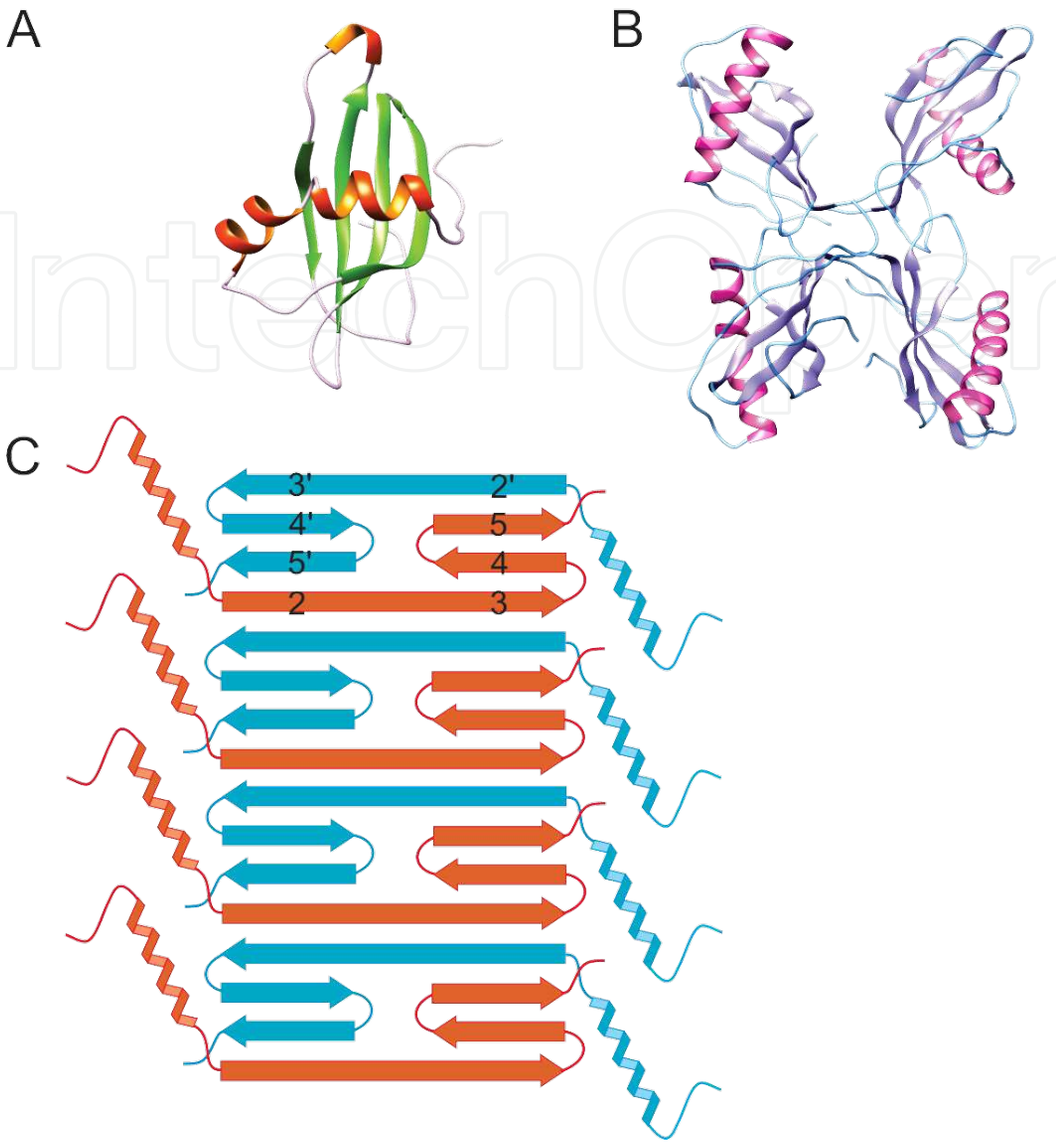

Figure 3. Stefin B structure. (A) stefin B monomer (PDB 1stf) [54], (B) stefin B tetramer (PDB 2oct) [40], (C) secondary structure representation of stefin B fibril [56]

more stable homologue [60], to proline mutants [61], EPM1 mutants [62] and to chimeras between stefins A and B [63].

A model for the mechanism of stefin B amyloid fibril formation has been proposed based on temperature and concentration dependence of the kinetics [64]. Following changes during the lag phase of the reaction by SEC and ESI MS we were able to improve the model of amyloid fibril formation (Figure 4), further explaining the role of various off-pathway oligomers [41]. Dimers seem to be the building block from which fibril formation starts and the fibrils grow [56]. When the process was started from any kind of oligomers, these transformed into dimers [41]. NMR studies have shown that there are actually four types of dimers observed in the lag phase under amyloid forming conditions [55].

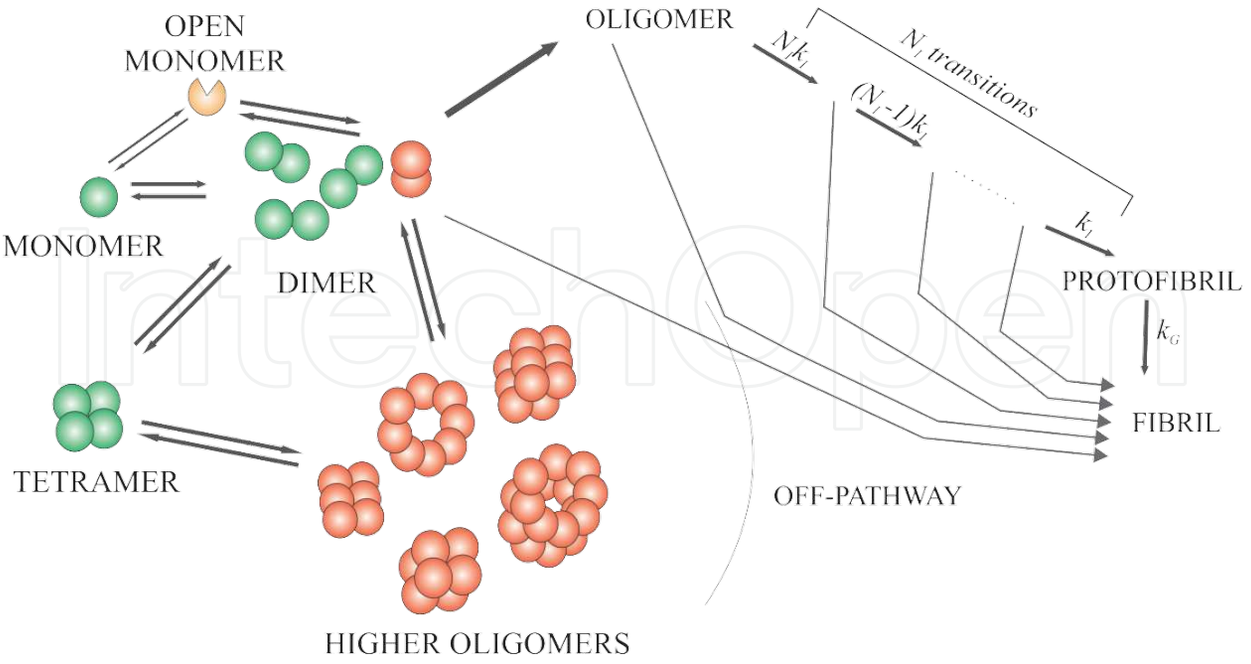

Figure 4. The proposed mechanism for amyloid fibril formation by stefin B [41]. Non-toxic species are coloured green, toxic are coloured red and potentially toxic are coloured orange.

7. Oligomeric state related toxicity

Stefin B higher oligomers are equally toxic to cells as the molten globule state (mutant P74S Y31) [39]. Higher oligomers still have native like CD spectra and increased ANS binding, which would mean that they are properly folded. The toxicity seems to correlate with the ANS binding (ANS binding is indicator of exposed hydrophobic patches in the protein structure) [39]. MTS test has shown that isolated dimers and tetramers are not toxic (Figure 5A), while higher oligomers show some toxicity to neuroblastoma cells (SH-SY5Y). In accordance, low ordered oligomers, monomers, dimers and tetramers do not increase caspase-3-like activity (Figure 5B) [39]. All those results correlate with insertion into lipid membranes, where toxic species insert more effectively than not toxic. Monomers do not get internalized into the cytoplasm when added to the cell medium, while higher oligomers do [39].

Confirming the above results, in another study [65] it was demonstrated that a mutated P74S Y31 form of stefin B, which is molten globule at neutral pH and prefibrillar aggregates at pH 3, which are also in the molten globule conformation, are most toxic.

A growing body of evidence shows that the soluble oligomers formed during amyloid fibril formation exert toxicity and likely cause neurodegeneration [66]. Small soluble oligomers are the cause of synaptic disfunction, whereas large and insoluble aggregates are likely the reservoir of those toxic species [66]. In the case of Aβ peptide a significant correlation has been found between the levels of soluble oligomers and the degree of synaptic alteration, neurodegeneration and cognitive decline, while the correlation between insoluble deposits and those

http://dx.doi.org/10.5772/57570

symptoms has not been demonstrated [67]. Non fibrilar dimers or oligomers of α-synuclein could play a major role in Parkinson's disease progression [68]. In accordance with a generic process of amyloid type of protein aggregation, not only oligomers of amyloidogenic proteins related to amyloid diseases exert toxicity, also HypF-N from *E. coli*, SH3 domain of PI3 kinase and some other proteins form oligomers that are toxic to fibroblasts and neurons, whereas amyloid fibrils of the same proteins show only low toxicity [69, 70].

Conformation dependent antibodies distinguish between soluble oligomers [71-73] and amyloid fibrils [74, 75]. Many of those antibodies recognize the generic epitopes regardless of the amino acid sequence and this suggests that different proteins form structurally similar oligomers and fibrils. It was shown that the antibody recognizing Aβ peptide fibrils, recognize also fibrils of transthyretin, islet amyloid polypeptide, β_2-microglobulin and polyglutamine and that the same antibody does not recognize soluble oligomers of those proteins nor the native forms [75]. Antibodies recognizing soluble oligomers also inhibit their toxicity which suggests that soluble oligomers do not share only structural properties but also the mechanism of toxicity [72].

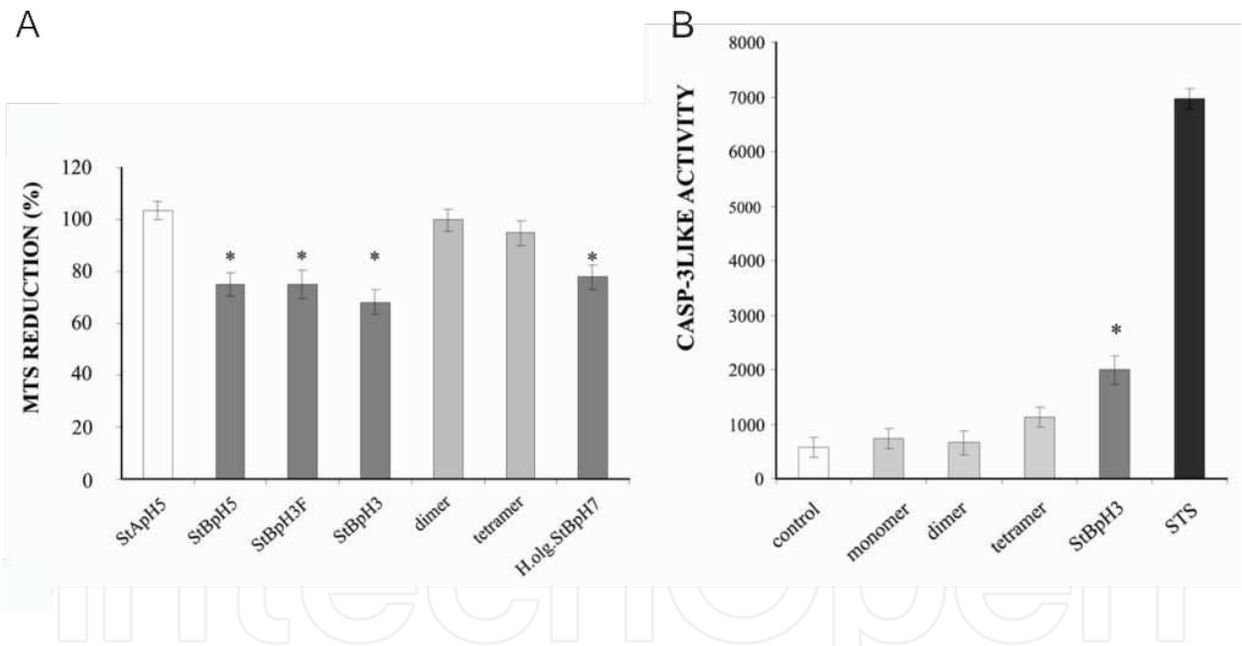

Figure 5. Viability of cells exposed to prefibrillar aggregates and oligomers of stefin B. (A) MTS test-viability of the SHSY5Y cells was measured after exposure for 16 hours to serum free medium, which contained prefibrillar oligomers of stefin B – StBpH3, StBpH5, StBpH3F (filtered), and separated dimers, tetramers and higher-order oligomers of stefin B at pH 7 – StBpH7; each at a final concentration of 44 μM. As a control for the effect of a non-toxic protein, cells were exposed for the corresponding length of time to 44 μM of soluble stefin A prepared at pH 5 (StApH5). All the results are relative to the cells alone, with no added substance or protein in the medium. (B) DEVD-ase activity after exposing SHSY5Y cells to prefibrillar aggregates. 200 μl of concentrated StBpH3 aggregates were added to 800 μL of cell medium to a final concentration of 44 μM (final pH was checked to be neutral). The cells were treated for four hours with 0.5 μM staurosporine (STS). Statistics-as described [39].

Both oligomers and amyloid fibrils are harmful for the cells; oligomers present mostly the toxic effect, while amyloid fibrils present steric obstacles.

• the disruption of the tissue architecture and functions promoted by the invasion of the extracellular space of organ by amyloid [76, 77]
• the destabilization of intracellular and extracellular membranes by oligomers [78, 79]
• the apoptotic cell death and receptor-mediated toxicity triggered by the oligomer interaction with various neuronal receptors [80]
• the oligomer-mediated impairment of the P/Q-type calcium currents [81]
• the impaired maturation of autophagosomes to lysosome mediated by the oligomer accumulation [82]
• the dysfunction of autophagy, a lysosomal pathway for degrading organelles and proteins [83]
• the oxidative damage-induced disruption of the cell viability promoted by the incorporation of redox metals into amyloid fibrils and subsequent generation of reactive oxygen species [84-88]
• the general disorganization of cellular protein homeostasis associated with the exhaustion of the cell defence mechanisms, such as chaperone system [89, 90]
• proteasome inhibition [91]
• the loss of crucial protein functions and/or gain of toxic functions

Table 4. Cellular degeneration after protein oligomerization/deposition (summarized in [92])

8. Oligomeric state related membrane interactions

Amyloid forming proteins interact with biological membranes and even form pores, which are similar to those formed by the pore-forming proteins [1, 93]. Interactions between stefin B and membranes have been extensively studied [65, 94, 95]. Three different proteins have been studied: wt protein, Y31 isoform and G4R mutant (mutant involved in EPM1 disease). Also some comparisons have been made between native and prefibrillar states of the protein (prepared with incubation at pH 3.3 or 4.8) (Figure 6) [94]. Prefibrillar oligomers may be organized in such a way that they are more amphipatic than the native protein and therefore acquire a higher surface-seeking potential. All forms of the protein insert into acidic lipid membranes, cause permeabilization of unilamelar vesicles and destabilize the membranes [94, 95]. Nevertheless there are some significant differences between them concerning pore formation. The mutant G4R does not form pores, but breaks the membrane very fast (in a few minutes), while both the wt protein and Y31 isoform form pores; wt pores are cation selective (and may not be deleterious for the cell) and Y31 pores are anion selective [95]. Wt sample was separated into different oligomers apart from monomers, into dimers, tetramers and higher oligomers. Monomers, dimers and tetramers insert into lipid membranes with approximately the same critical pressure, while the higher oligomers (average size is around octamers) insert more effectively [65].

http://dx.doi.org/10.5772/57570

The exact mechanism of amyloid induced toxicity is yet controversial; nevertheless the pore formation into plasma or mitochondrial membrane is now the leading theory of pathogenesis. Aβ peptide needs anionic phospholipids for binding and insertion into membranes [96-101]. Monomeric form does not interact with membranes. Amylin (islet amyloid polypeptide) readily forms pores into planar lipid bilayer, preferring negatively charged lipids and furthermore the channel activity is inversely proportional to the amount of negative surface charge in the membrane [102]. Native form of prion protein does not form pores, while the mutant prion protein forms irreversible pores in the lipid bilayer. They claim that the pore formation is the main cause of the prion disease [103]. α-synuclein, a natively unfolded protein, also forms pores into lipid membranes but in contrast to others described it is not known how does the oligomeric form increase membrane permeability without forming discrete channels [104]. In the literature we can find contradictory results what effects amyloid proteins exert on membranes. For the same proteins we can find reports that they affect membranes by a non-channel mechanism and other reports that they affect membranes by the formation of ion channels. Two mechanisms of pore formation into membranes by the amyloid forming proteins are summarized in Table 5.

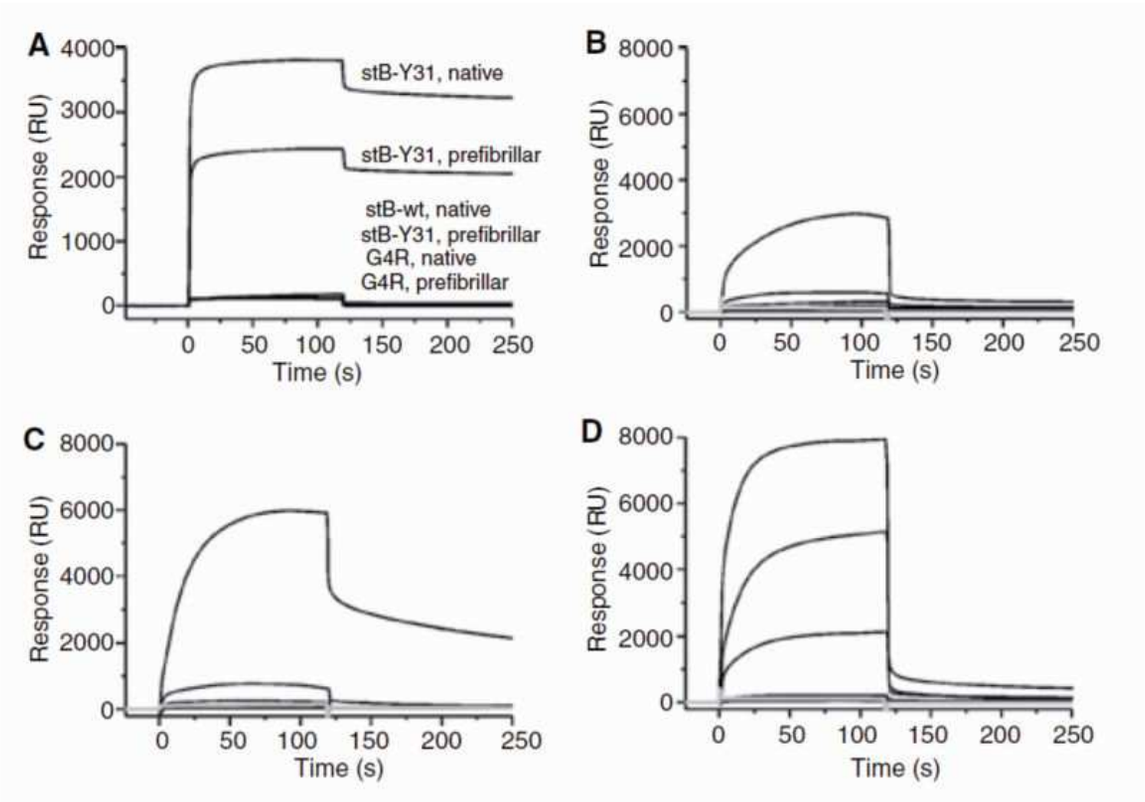

Figure 6. Binding of stefin B variants to liposomes as measured by SPR. (A) Binding to PC LUV. A comparison of binding of 70 μM stefin variants to PC LUV immobilized on the surface of a L1 sensor chip. (B–D) A comparison of the binding of prefibrillar form of stefin variants to PG LUV. The concentration of the protein was 10, 20, 40, 50, 60 and 70 μM (curves from the bottom to the top) in each case. The thick gray line represents binding of native stefin variants at 70μM. (B) StB-wt; (C) StBY31; (D) G4R [95].

Membrane poration followed by nonspecific membrane leakage – increased conductivity of the membranes by a non-channel mechanism	Specific ion transport through ion channel followed by destabilized ionic homeostasis
Aβ40 peptide [105]	Aβ40 peptide [106, 107]
Aβ42 peptide [105]	Aβ42 peptide [108]
α-synuclein [105]	Aβ22-35 peptide [109]
IAPP or amylin [105, 110]	α-synuclein [111]
Polyglutamine [105]	IAPP or amylin [112]
Prion (106-126) H1 [105]	stefin B [95]
SOD1 [113]	

Table 5. The mechanisms underlying globular peptides induced cell dysfunction

9. Copper binding to stefin B and its inhibition of amyloid fibril formation

Divalent metal ions (Cu (II), Zn (II), Fe (II)) are often observed to colocalize with amyloid plaques *in vivo* in much higher concentrations than usually present in the normal environment. This has led to the hypothesis that this metal ions bind to mature fibrils and influence the fibril formation reaction [114].

Indeed it was proven that human stefin B is a copper binding protein [114]. It shows a picomolar affinity to copper at pH 7 and a nanomolar affinity at pH 5. Both the wild type protein and the Y31 isoform bind copper, while the Y31 P79S, which is tetrameric, does not. Monomers and dimers are able to bind copper, while other oligomers are not. Copper binding does not change the conformation of the protein; however it inhibits amyloid fibril formation (Figure 8). Of note it does not prevent aggregation to prefibrillar oligomers, which can be even more toxic to cells. The protein binds two Cu^{2+} ions. Copper binding promotes protein dimerization at neutral pH, while at acidic pH protein undergoes dimerization, already without added copper [114].

Later-on, NMR was used to detect the three possible copper binding sites of stefin B. The first is in the α-helix facing away from the β-sheets together with the loop between strands 4 and 5. The second potential binding site is the C-terminal together with the loop between strands 3 and 4 (this binding site is more likely in the dimeric form of the protein). The third binding site is in the dimer only and is the loop between strands 2 and 3, which is the stretched loop in the domain-swapped dimer together with the few residues at the N-terminal [55].

It was confirmed that copper affects the stefin B fibril formation. In more details, it slows down the elongation phase. It was also shown that the presence of copper destabilizes the protein structure and therefore it was concluded that it preferably binds to the slightly unfolded state of the protein. The final fibril morphology does not differ if copper ions are present or not [55].

Metal ions have different effect on amyloid forming proteins. They can speed up the fibril formation, reduce the lag phase, retard the fibrillization or even stop the process completely [115-117].

http://dx.doi.org/10.5772/57570

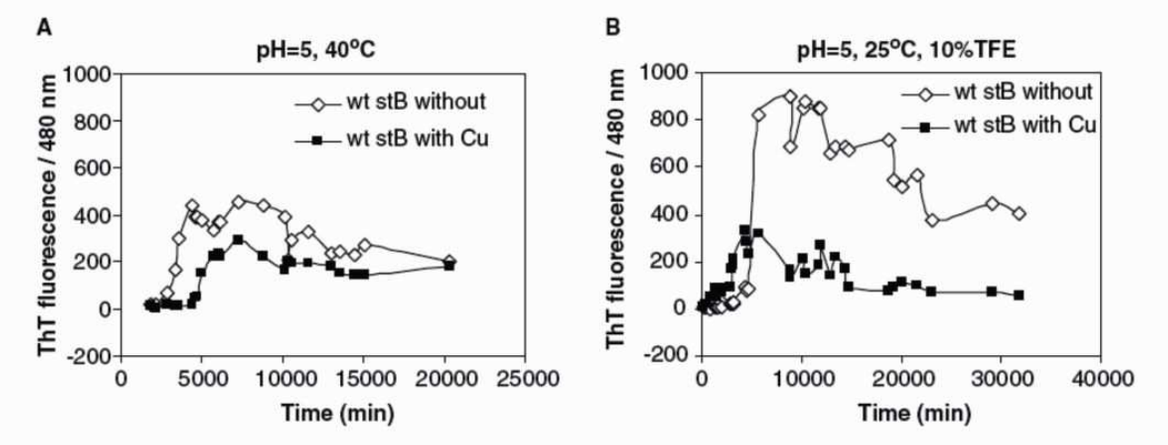

Figure 7. Inhibition of fibrillation of stefin B by Cu^{2+} as probed by ThT fluorescence. Final protein concentration was in all cases 45 μM and final concentration of Cu^{2+} 46 μM, leading to 1 : 1 of protein to Cu^{2+} ratio. (A) Stefin B wildtype (E31 isoform) at pH 5, 40 °C, 0 and 50 μM Cu^{2+} in the buffer. (B) Stefin B wildtype (E31 isoform) at pH 5, 10% TFE, 25 °C, 0 and 50 μM Cu^{2+} in the buffer [114].

Binding of metal ions to prion protein increases proteolysis resistance and structural changes which might play an important role in the conversion process [118].

SOD1 aggregation is enhanced and modulated by Ca^{2+} ions; at physiological pH Ca^{2+}induces conformational changes that increase β-sheet content, they can also divert the aggregation from amyloid fibrils to amorphous aggregates [119]. Cu^{2+} and Zn^{2+} accelerate deposition of Aβ40 peptide and Aβ42 peptide, which results in the amorphous aggregates [120, 121]. Fe^{3+} induces the deposition of fibrillar amyloid plaques of Aβ40 peptide and Aβ42 peptide [120]. Prion protein can directly influence neuronal zinc concentrations [122]. In prion plaques there was found a significant disregulation of copper and manganese, copper was depleted and manganese was enriched [123]. Fe^{3+} and Al^{3+} enhance both formation of mixed oligomers and recruitment of α-synuclein in pre-formed tau oligomers [124]. Copper and selenium inhibit amylin fibril formation, while aluminium and manganese promote it [125]. Copper amylin complex has an anti-aggregating and anti-apoptotic properties, quenching the metal catalysed ROS [126]. Zinc also inhibits amylin fibril formation, furthermore, it favours the formation of amylin hexamers and inhibits the formation of dimers [127].

10. Interaction between oligomers of stefin B and Aβ peptide *in vitro* and in cells-"amateur chaperones"

"Professional chaperones" (heat shock protein family) prevent protein misfolding and subsequent protein aggregation. "Amateur chaperones" bind amyloidogenic proteins and may affect their aggregation process. Both types of chaperones colocalize with pathological lesions of Alzheimer's disease, may be involved in Aβ peptide conformational changes, clearance of Aβ peptide from the brain. Both types of chaperones may be involved in the

aggregation, accumulation, persistence and clearance of Aβ peptide and may therefore serve as a potential targets for medical treatment of those patients [128].

Aβ peptides are produced by proteolytic cleavage of the amyloid precursor protein (APP) by α-secretase, β-secretase and γ-secretase. Cathepsin B, which can be inhibited by stefin B, is likely contributor to β-secretase activity [129]. Cysteine protease inhibitors reduce both Aβ peptide level in the brain and β-secretase activity *in vivo* [129]. Stefin B has an effect on production of Aβ peptides and furthermore it also inhibits Aβ peptide fibril formation [38].

Two isoforms of stefin B (Y31 and wt E31) have been studied. Y31 isoform is predominantly a dimer [31] while the wild type protein exist as mixture of monomers, dimer, tetramers and even higher oligomers [39]. ThT fluorescence and transmission electron microscopy (TEM) have shown that Y31 isoform completely inhibits Aβ peptide fibril formation (Figure 9I). The direct interaction between those two proteins has also been shown by SPR measurements, where concentration dependent interaction has been reported and by ESI MS where the complex between dimer stB and monomer Aβ peptide has been detected (Figure 9II) [38]. Furthermore, isolated oligomers of the wild type protein have been studied and it was shown that only the tetramer inhibits Aβ peptide fibril formation and that the higher oligomers show only a weak inhibition. Stefin B also colocalizes with the Aβ peptide aggregates in cells (shown by confocal microscopy) and with the C-terminal fragment of APP (comprising Aβ peptide sequence) (shown by immunoprecipitation).

Molecule/protein	Type of interaction
stefin B	Inhibits Aβ peptide fibril formation, interaction *in vitro* and in cells [38].
laminin	Inhibits Aβ peptide fibril formation, induce depolymerisation of preformed fibrils [130].
polyphenols	Inhibits Aβ peptide fibril formation [131].
heme	Prevents Aβ peptide aggregation [132].
GroEL	Prevents Aβ peptide aggregation [133].
apolipoprotein E	Slows down the oligomerization of Aβ peptide [134].
myelin basic protein	Inhibitor of Aβ peptide fibrilar assembly [135].
cystatin C	Concentration dependent inhibition of Aβ peptide fibril formation [36].
ferulic acid	Inhibits Aβ peptide oligomer formation from monomers and at the same time accelerates fibril formation from already formed oligomers [136].
S14G humanin	Inhibits aggregation into fibrils and disaggregates preformed fibrils, reduces cytotoxicity effect of Aβ peptide [137].
albumin	Increases the lag phase and decreases the total amount of fibrils [138]
crocetin	Inhibits Aβ peptide fibril formation, destabilizes preformed fibrils, stabilize Aβ peptide oligomers and prevents their conversion into fibril [139].

Table 6. Molecules interacting with Aβ peptide

http://dx.doi.org/10.5772/57570

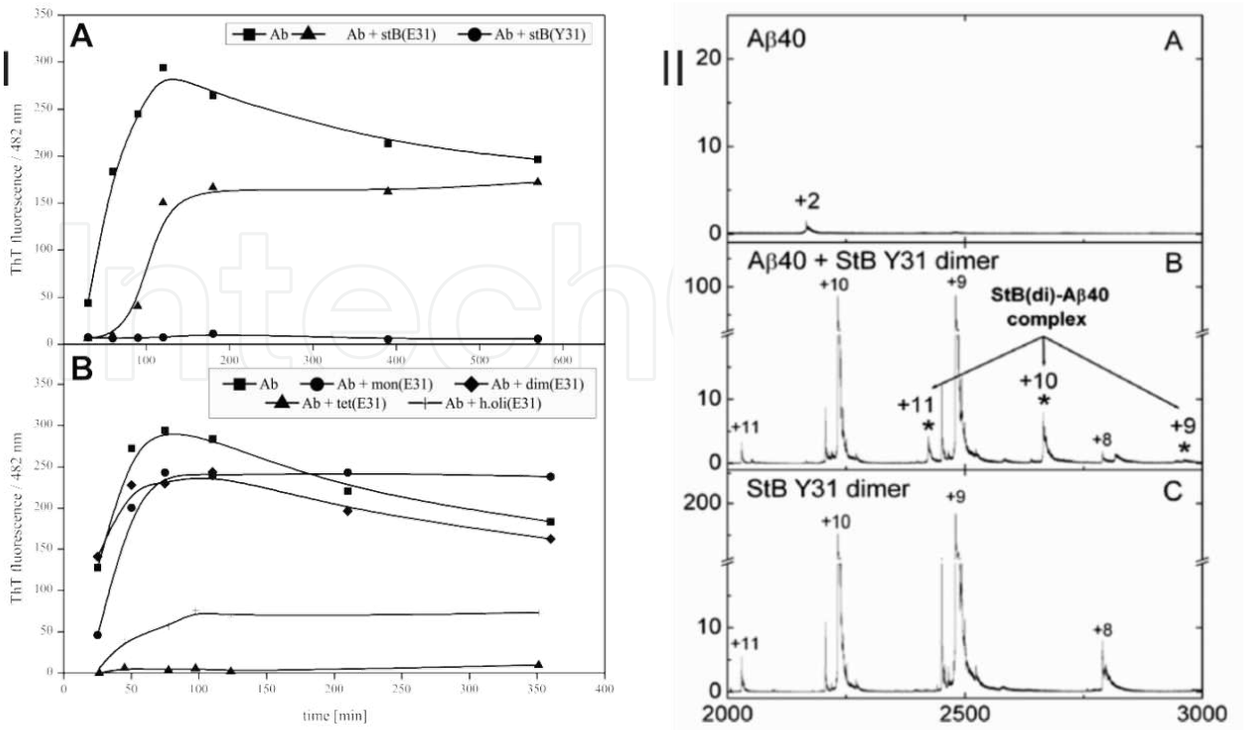

Figure 8. (I) Inhibition of Aβ peptide fibril formation by stefin B measured by ThT fluorescence and (II) the detection of the complex by ESI MS. (I) The Aβ peptide concentration was 17 μM throughout, pH 7.3, 40 °C. (I, A), Aβ peptide alone, 1:1 molar ratio of Aβ to Y31 stefin B (complete inhibition) and 1:1 molar ratio of Aβ to E31 stefin B. (I, B), Aβ peptide alone, and 1:1 molar ratios to E31 stefin B monomers, dimers, tetramers, and higher oligomers. The protein concentrations of stefin B were 17 μM. (II) Complex detected by ESI-MS. (II, A) ESI-MS spectra of Aβ(1– 40) peptide, (II, C) stefin B dimer (Y31 variant) and (II, B) their mixtures were recorded: (II, A), 2 μM Aβ(1– 40) peptide; (II, B), a mixture of 2 μM Aβ peptide and 2 μM stefin B; and (II, C), 2 μM stefin B. Peaks corresponding to the Aβ peptide-stefin B complex are denoted with an asterisk and numbers above the peaks denote charge state of the ions [38].

In order to show if binding between domain-swapped dimers and Aβ peptide is a generic property, other cystatins have been used. It was shown that only cystatin C dimers inhibit Aβ peptide fibril formation (30% inhibition) while stefin A dimers exhibited no such effect [140].

Several molecules interacting with Aβ peptide have been reported by now (Table 6).

11. Conclusions and perspectives

Stefin B has been so far found as a good model system for studying amyloid fibril formation. It exhibits nearly all features shared with other amyloid forming proteins: it forms mature fibrils under mildly acidic conditions or even at neutral pH at somewhat higher temperature, forms membrane pores and therefore promotes membrane leaking, binds copper ions and its' oligomers are toxic. It is not a model protein only, but also could be termed an "amateur chaperone" affecting Aβ peptide fibril formation both *in vitro* and *in vivo* [38].

We are trying to extend our *in vitro* knowledge to cell cultures to contribute even more to the understanding of conformational disease. It is hoped that new knowledge of protein oligo-

merization and aggregation on the molecular and cellular levels will contribute to the development of new therapeutic strategies for patients with various conformational diseases, including the neurodegenerative ones.

Acknowledgements

All this work has been done by several contributors over past years. We are thankful to Manca Kenig, Sabina Rabzelj, Slavko Čeru, Katja Škerget, Aida Smajlović and Saša Jenko Kokalj, similarly to some collegues: Vito Turk, Nataša Kopitar Jerala (both JSI, Slovenia), Selma Berbić (University of Tuzla, BiH). Many collaborators from different fields helped us to achieve different perspectives on the problem; Peep Palumaa (TTU, Estonia) – mass spectrometry, Dušan Turk (JSI, Slovenia) – structural biology, Magda Tušek Žnidarič (NIB, Slovenia) – transmission electron microscopy, Andrej Vilfan (JSI, Slovenia) – calculations and mathematical models, Rosemary A. Staniforth (University of Sheffield, UK) – NMR, Miha Škarabot (JSI, Slovenia) – atomic force microscopy and many others.

This work was supported by the program P1-0140 and the projects J7-4050 (led by E. Z.) via the Slovenian Research Agency (ARRS) and ARRS young investigators grants (A. T.-V.).

Author details

Ajda Taler-Verčič[1,2], Mira Polajnar[1,2] and Eva Žerovnik[1,2*]

*Address all correspondence to: eva.zerovnik@ijs.si

1 Department of Biochemistry, Molecular and Structural Biology, Jožef Stefan Institute, Ljubljana, Slovenia

2 Jožef Stefan International Postgraduate School, Ljubljana, Slovenia

References

[1] Kagan B.L.,J. Thundimadathil, Amyloid peptide pores and the beta sheet conformation. Adv Exp Med Biol 2010; 677: p. 150-167.

[2] Lomas D.A.,R.W. Carrell, Serpinopathies and the conformational dementias. Nat Rev Genet 2002; 3(10): p. 759-768.

[3] Turk V.,W. Bode, The cystatins: protein inhibitors of cysteine proteinases. FEBS Lett 1991; 285(2): p. 213-219.

[4] Jerala R., M. Trstenjak, B. Lenarcic, V. Turk, Cloning a synthetic gene for human stefin B and its expression in E. coli. FEBS Lett 1988; 239(1): p. 41-44.

[5] Rawlings N.D., D.P. Tolle, A.J. Barrett, MEROPS: the peptidase database. Nucleic Acids Res 2004; 32(Database issue): p. D160-164.

[6] Abrahamson M., A.J. Barrett, G. Salvesen, A. Grubb, Isolation of six cysteine proteinase inhibitors from human urine. Their physicochemical and enzyme kinetic properties and concentrations in biological fluids. J Biol Chem 1986; 261(24): p. 11282-11289.

[7] Brzin J., M. Kopitar, V. Turk, W. Machleidt, Protein inhibitors of cysteine proteinases. I. Isolation and characterization of stefin, a cytosolic protein inhibitor of cysteine proteinases from human polymorphonuclear granulocytes. Hoppe Seylers Z Physiol Chem 1983; 364(11): p. 1475-1480.

[8] Pol E.,I. Bjork, Role of the single cysteine residue, Cys 3, of human and bovine cystatin B (stefin B) in the inhibition of cysteine proteinases. Protein Sci 2001; 10(9): p. 1729-1738.

[9] Turk B., V. Turk, D. Turk, Structural and functional aspects of papain-like cysteine proteinases and their protein inhibitors. Biol Chem 1997; 378(3-4): p. 141-150.

[10] Bromme D., R. Rinne, H. Kirschke, Tight-binding inhibition of cathepsin S by cystatins. Biomedica biochimica acta 1991; 50(4-6): p. 631-635.

[11] Turk V., J. Brzin, M. Kotnik, B. Lenarcic, T. Popovic, A. Ritonja, M. Trstenjak, L. Begic-Odobasic, W. Machleidt, Human cysteine proteinases and their protein inhibitors stefins, cystatins and kininogens. Biomedica biochimica acta 1986; 45(11-12): p. 1375-1384.

[12] Di Giaimo R., M. Riccio, S. Santi, C. Galeotti, D.C. Ambrosetti, M. Melli, New insights into the molecular basis of progressive myoclonus epilepsy: a multiprotein complex with cystatin B. Human molecular genetics 2002; 11(23): p. 2941-2950.

[13] Lee M.J., G.R. Yu, S.H. Park, B.H. Cho, J.S. Ahn, H.J. Park, E.Y. Song, D.G. Kim, Identification of cystatin B as a potential serum marker in hepatocellular carcinoma. Clin Cancer Res 2008; 14(4): p. 1080-1089.

[14] Sheahan K., S. Shuja, M.J. Murnane, Cysteine protease activities and tumor development in human colorectal carcinoma. Cancer research 1989; 49(14): p. 3809-3814.

[15] Plebani M., L. Herszenyi, R. Cardin, G. Roveroni, P. Carraro, M.D. Paoli, M. Rugge, W.F. Grigioni, D. Nitti, R. Naccarato, et al., Cysteine and serine proteases in gastric cancer. Cancer 1995; 76(3): p. 367-375.

[16] Shiraishi T., M. Mori, S. Tanaka, K. Sugimachi, T. Akiyoshi, Identification of cystatin B in human esophageal carcinoma, using differential displays in which the gene ex-

pression is related to lymph-node metastasis. International journal of cancer. Journal international du cancer 1998; 79(2): p. 175-178.

[17] Mirtti T., K. Alanen, M. Kallajoki, A. Rinne, K.O. Soderstrom, Expression of cystatins, high molecular weight cytokeratin, and proliferation markers in prostatic adenocarcinoma and hyperplasia. The Prostate 2003; 54(4): p. 290-298.

[18] Feldman A.S., J. Banyard, C.L. Wu, W.S. McDougal, B.R. Zetter, Cystatin B as a tissue and urinary biomarker of bladder cancer recurrence and disease progression. Clinical cancer research : an official journal of the American Association for Cancer Research 2009; 15(3): p. 1024-1031.

[19] Ceru S., S. Konjar, K. Maher, U. Repnik, I. Krizaj, M. Bencina, M. Renko, A. Nepveu, E. Zerovnik, B. Turk, N. Kopitar-Jerala, Stefin B interacts with histones and cathepsin L in the nucleus. J Biol Chem 2010; 285(13): p. 10078-10086.

[20] Riccio M., R. Di Giaimo, S. Pianetti, P.P. Palmieri, M. Melli, S. Santi, Nuclear localization of cystatin B, the cathepsin inhibitor implicated in myoclonus epilepsy (EPM1). Experimental cell research 2001; 262(2): p. 84-94.

[21] Brannvall K., H. Hjelm, L. Korhonen, U. Lahtinen, A.E. Lehesjoki, D. Lindholm, Cystatin-B is expressed by neural stem cells and by differentiated neurons and astrocytes. Biochem Biophys Res Commun 2003; 308(2): p. 369-374.

[22] Sun T., V. Turk, B. Turk, N. Kopitar-Jerala, Increased expression of stefin B in the nucleus of T98G astrocytoma cells delays caspase activation. Front Mol Neurosci 2012; 5: p. 93.

[23] Verdot L., G. Lalmanach, V. Vercruysse, S. Hartmann, R. Lucius, J. Hoebeke, F. Gauthier, B. Vray, Cystatins up-regulate nitric oxide release from interferon-gamma-activated mouse peritoneal macrophages. J Biol Chem 1996; 271(45): p. 28077-28081.

[24] Lefebvre C., C. Cocquerelle, F. Vandenbulcke, D. Hot, L. Huot, Y. Lemoine, M. Salzet, Transcriptomic analysis in the leech Theromyzon tessulatum: involvement of cystatin B in innate immunity. Biochem J 2004; 380(Pt 3): p. 617-625.

[25] Pennacchio L.A., D.M. Bouley, K.M. Higgins, M.P. Scott, J.L. Noebels, R.M. Myers, Progressive ataxia, myoclonic epilepsy and cerebellar apoptosis in cystatin B-deficient mice. Nat Genet 1998; 20(3): p. 251-258.

[26] Lehtinen M.K., S. Tegelberg, H. Schipper, H. Su, H. Zukor, O. Manninen, O. Kopra, T. Joensuu, P. Hakala, A. Bonni, A.E. Lehesjoki, Cystatin B deficiency sensitizes neurons to oxidative stress in progressive myoclonus epilepsy, EPM1. J Neurosci 2009; 29(18): p. 5910-5915.

[27] Ii K., H. Ito, E. Kominami, A. Hirano, Abnormal distribution of cathepsin proteinases and endogenous inhibitors (cystatins) in the hippocampus of patients with Alzheimer's disease, parkinsonism-dementia complex on Guam, and senile dementia and in

the aged. Virchows Archiv. A, Pathological anatomy and histopathology 1993; 423(3): p. 185-194.

[28] Pennacchio L.A., A.E. Lehesjoki, N.E. Stone, V.L. Willour, K. Virtaneva, J. Miao, E. D'Amato, L. Ramirez, M. Faham, M. Koskiniemi, J.A. Warrington, R. Norio, A. de la Chapelle, D.R. Cox, R.M. Myers, Mutations in the gene encoding cystatin B in progressive myoclonus epilepsy (EPM1). Science 1996; 271(5256): p. 1731-1734.

[29] Cipollini E., M. Riccio, R. Di Giaimo, F. Dal Piaz, G. Pulice, S. Catania, I. Caldarelli, M. Dembic, S. Santi, M. Melli, Cystatin B and its EPM1 mutants are polymeric and aggregate prone in vivo. Biochim Biophys Acta 2008; 1783(2): p. 312-322.

[30] Ceru S., R. Layfield, T. Zavasnik-Bergant, U. Repnik, N. Kopitar-Jerala, V. Turk, E. Zerovnik, Intracellular aggregation of human stefin B: confocal and electron microscopy study. Biol Cell 2010; 102(6): p. 319-334.

[31] Rabzelj S., V. Turk, E. Zerovnik, In vitro study of stability and amyloid-fibril formation of two mutants of human stefin B (cystatin B) occurring in patients with EPM1. Protein science : a publication of the Protein Society 2005; 14(10): p. 2713-2722.

[32] Polajnar M., S. Ceru, N. Kopitar-Jerala, E. Zerovnik, Human stefin B normal and patho-physiological role: molecular and cellular aspects of amyloid-type aggregation of certain EPM1 mutants. Front Mol Neurosci 2012; 5: p. 88.

[33] Polajnar M., R. Vidmar, M. Vizovisek, M. Fonovic, N. Kopitar-Jerala, E. Zerovnik, Influence of partial unfolding and aggregation of human stefin B (cystatin B) EPM1 mutants G50E and Q71P on selective cleavages by cathepsins B and S. Biol Chem 2013; 394(6): p. 783-790.

[34] Olafsson I., L. Thorsteinsson, O. Jensson, The molecular pathology of hereditary cystatin C amyloid angiopathy causing brain hemorrhage. Brain pathology 1996; 6(2): p. 121-126.

[35] Levy E., M. Sastre, A. Kumar, G. Gallo, P. Piccardo, B. Ghetti, F. Tagliavini, Codeposition of cystatin C with amyloid-beta protein in the brain of Alzheimer disease patients. Journal of neuropathology and experimental neurology 2001; 60(1): p. 94-104.

[36] Sastre M., M. Calero, M. Pawlik, P.M. Mathews, A. Kumar, V. Danilov, S.D. Schmidt, R.A. Nixon, B. Frangione, E. Levy, Binding of cystatin C to Alzheimer's amyloid beta inhibits in vitro amyloid fibril formation. Neurobiol Aging 2004; 25(8): p. 1033-1043.

[37] Kopitar-Jerala N., A. Schweiger, R.M. Myers, V. Turk, B. Turk, Sensitization of stefin B-deficient thymocytes towards staurosporin-induced apoptosis is independent of cysteine cathepsins. FEBS Lett 2005; 579(10): p. 2149-2155.

[38] Skerget K., A. Taler-Vercic, A. Bavdek, V. Hodnik, S. Ceru, M. Tusek-Znidaric, T. Kumm, D. Pitsi, M. Pompe-Novak, P. Palumaa, S. Soriano, N. Kopitar-Jerala, V. Turk, G. Anderluh, E. Zerovnik, Interaction between oligomers of stefin B and amyloid-beta in vitro and in cells. J Biol Chem 2010; 285(5): p. 3201-3210.

[39] Ceru S., S.J. Kokalj, S. Rabzelj, M. Skarabot, I. Gutierrez-Aguirre, N. Kopitar-Jerala, G. Anderluh, D. Turk, V. Turk, E. Zerovnik, Size and morphology of toxic oligomers of amyloidogenic proteins: a case study of human stefin B. Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis 2008; 15(3): p. 147-159.

[40] Jenko Kokalj S., G. Guncar, I. Stern, G. Morgan, S. Rabzelj, M. Kenig, R.A. Staniforth, J.P. Waltho, E. Zerovnik, D. Turk, Essential role of proline isomerization in stefin B tetramer formation. Journal of molecular biology 2007; 366(5): p. 1569-1579.

[41] Taler-Vercic A., T. Kirsipuu, M. Friedemann, A. Noormagi, M. Polajnar, J. Smirnova, M.T. Znidaric, M. Zganec, M. Skarabot, A. Vilfan, R.A. Staniforth, P. Palumaa, E. Zerovnik, The role of initial oligomers in amyloid fibril formation by human stefin B. Int J Mol Sci 2013; 14(9): p. 18362-18384.

[42] Dunstone M.A., W. Dai, J.C. Whisstock, J. Rossjohn, R.N. Pike, S.C. Feil, B.F. Le Bonniec, M.W. Parker, S.P. Bottomley, Cleaved antitrypsin polymers at atomic resolution. Protein Sci 2000; 9(2): p. 417-420.

[43] Huntington J.A., N.S. Pannu, B. Hazes, R.J. Read, D.A. Lomas, R.W. Carrell, A 2.6 A structure of a serpin polymer and implications for conformational disease. Journal of molecular biology 1999; 293(3): p. 449-455.

[44] Guo Z.,D. Eisenberg, Runaway domain swapping in amyloid-like fibrils of T7 endonuclease I. Proc Natl Acad Sci U S A 2006; 103(21): p. 8042-8047.

[45] Carrell R.W., A. Mushunje, A. Zhou, Serpins show structural basis for oligomer toxicity and amyloid ubiquity. FEBS Lett 2008; 582(17): p. 2537-2541.

[46] Mushero N.,A. Gershenson, Determining serpin conformational distributions with single molecule fluorescence. Methods Enzymol 2011; 501: p. 351-377.

[47] Zhou A.,R.W. Carrell, Dimers initiate and propagate serine protease inhibitor polymerisation. Journal of molecular biology 2008; 375(1): p. 36-42.

[48] Kloniecki M., A. Jablonowska, J. Poznanski, J. Langridge, C. Hughes, I. Campuzano, K. Giles, M. Dadlez, Ion mobility separation coupled with MS detects two structural states of Alzheimer's disease Abeta1-40 peptide oligomers. Journal of molecular biology 2011; 407(1): p. 110-124.

[49] Nettleton E.J., P. Tito, M. Sunde, M. Bouchard, C.M. Dobson, C.V. Robinson, Characterization of the oligomeric states of insulin in self-assembly and amyloid fibril formation by mass spectrometry. Biophys J 2000; 79(2): p. 1053-1065.

[50] Smith A.M., T.R. Jahn, A.E. Ashcroft, S.E. Radford, Direct observation of oligomeric species formed in the early stages of amyloid fibril formation using electrospray ionisation mass spectrometry. Journal of molecular biology 2006; 364(1): p. 9-19.

[51] Smith D.P., L.A. Woods, S.E. Radford, A.E. Ashcroft, Structure and dynamics of oligomeric intermediates in beta2-microglobulin self-assembly. Biophys J 2011; 101(5): p. 1238-1247.

[52] Ruzafa D., B. Morel, L. Varela, A.I. Azuaga, F. Conejero-Lara, Characterization of oligomers of heterogeneous size as precursors of amyloid fibril nucleation of an SH3 domain: an experimental kinetics study. PLoS One 2012; 7(11): p. e49690.

[53] Lenarcic B., I. Krizaj, P. Zunec, V. Turk, Differences in specificity for the interactions of stefins A, B and D with cysteine proteinases. FEBS Lett 1996; 395(2-3): p. 113-118.

[54] Stubbs M.T., B. Laber, W. Bode, R. Huber, R. Jerala, B. Lenarcic, V. Turk, The refined 2.4 A X-ray crystal structure of recombinant human stefin B in complex with the cysteine proteinase papain: a novel type of proteinase inhibitor interaction. The EMBO journal 1990; 9(6): p. 1939-1947.

[55] Paramore R., G.J. Morgan, P.J. Davis, C.A. Sharma, A. Hounslow, A. Taler-Vercic, E. Zerovnik, J.P. Waltho, M.J. Cliff, R.A. Staniforth, Mapping local structural perturbations in the native state of stefin B (cystatin B) under amyloid forming conditions. Frontiers in molecular neuroscience 2012; 5: p. 94.

[56] Morgan G.J., S. Giannini, A.M. Hounslow, C.J. Craven, E. Zerovnik, V. Turk, J.P. Waltho, R.A. Staniforth, Exclusion of the native alpha-helix from the amyloid fibrils of a mixed alpha/beta protein. J Mol Biol 2008; 375(2): p. 487-498.

[57] Staniforth R.A., S. Giannini, L.D. Higgins, M.J. Conroy, A.M. Hounslow, R. Jerala, C.J. Craven, J.P. Waltho, Three-dimensional domain swapping in the folded and molten-globule states of cystatins, an amyloid-forming structural superfamily. The EMBO journal 2001; 20(17): p. 4774-4781.

[58] Zerovnik E., M. Pompe-Novak, M. Skarabot, M. Ravnikar, I. Musevic, V. Turk, Human stefin B readily forms amyloid fibrils in vitro. Biochim Biophys Acta 2002; 1594(1): p. 1-5.

[59] Zerovnik E., M. Skarabot, K. Skerget, S. Giannini, V. Stoka, S. Jenko-Kokalj, R.A. Staniforth, Amyloid fibril formation by human stefin B: influence of pH and TFE on fibril growth and morphology. Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis 2007; 14(3): p. 237-247.

[60] Jenko S., M. Skarabot, M. Kenig, G. Guncar, I. Musevic, D. Turk, E. Zerovnik, Different propensity to form amyloid fibrils by two homologous proteins-Human stefins A and B: searching for an explanation. Proteins 2004; 55(2): p. 417-425.

[61] Smajlovic A., S. Berbic, C. Schiene-Fischer, M. Tusek-Znidaric, A. Taler, S. Jenko-Kokalj, D. Turk, E. Zerovnik, Essential role of Pro 74 in stefin B amyloid-fibril formation: dual action of cyclophilin A on the process. FEBS Lett 2009; 583(7): p. 1114-1120.

[62] Kenig M., S. Berbic, A. Krijestorac, L. Kroon-Zitko, M. Tusek, M. Pompe-Novak, E. Zerovnik, Differences in aggregation properties of three site-specific mutants of recombinant human stefin B. Protein Sci 2004; 13(1): p. 63-70.

[63] Kenig M., S. Jenko-Kokalj, M. Tusek-Znidaric, M. Pompe-Novak, G. Guncar, D. Turk, J.P. Waltho, R.A. Staniforth, F. Avbelj, E. Zerovnik, Folding and amyloid-fibril formation for a series of human stefins' chimeras: any correlation? Proteins 2006; 62(4): p. 918-927.

[64] Skerget K., A. Vilfan, M. Pompe-Novak, V. Turk, J.P. Waltho, D. Turk, E. Zerovnik, The mechanism of amyloid-fibril formation by stefin B: temperature and protein concentration dependence of the rates. Proteins 2009; 74(2): p. 425-436.

[65] Ceru S.,E. Zerovnik, Similar toxicity of the oligomeric molten globule state and the prefibrillar oligomers. FEBS Lett 2008; 582(2): p. 203-209.

[66] Haass C.,D.J. Selkoe, Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. Nature reviews. Molecular cell biology 2007; 8(2): p. 101-112.

[67] McLean C.A., R.A. Cherny, F.W. Fraser, S.J. Fuller, M.J. Smith, K. Beyreuther, A.I. Bush, C.L. Masters, Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. Annals of neurology 1999; 46(6): p. 860-866.

[68] El-Agnaf O.M., S.A. Salem, K.E. Paleologou, L.J. Cooper, N.J. Fullwood, M.J. Gibson, M.D. Curran, J.A. Court, D.M. Mann, S. Ikeda, M.R. Cookson, J. Hardy, D. Allsop, Alpha-synuclein implicated in Parkinson's disease is present in extracellular biological fluids, including human plasma. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2003; 17(13): p. 1945-1947.

[69] Bucciantini M., G. Calloni, F. Chiti, L. Formigli, D. Nosi, C.M. Dobson, M. Stefani, Prefibrillar amyloid protein aggregates share common features of cytotoxicity. The Journal of biological chemistry 2004; 279(30): p. 31374-31382.

[70] Malisauskas M., J. Ostman, A. Darinskas, V. Zamotin, E. Liutkevicius, E. Lundgren, L.A. Morozova-Roche, Does the cytotoxic effect of transient amyloid oligomers from common equine lysozyme in vitro imply innate amyloid toxicity? The Journal of biological chemistry 2005; 280(8): p. 6269-6275.

[71] Hrncic R., J. Wall, D.A. Wolfenbarger, C.L. Murphy, M. Schell, D.T. Weiss, A. Solomon, Antibody-mediated resolution of light chain-associated amyloid deposits. Am J Pathol 2000; 157(4): p. 1239-1246.

[72] Kayed R., E. Head, J.L. Thompson, T.M. McIntire, S.C. Milton, C.W. Cotman, C.G. Glabe, Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. Science 2003; 300(5618): p. 486-489.

[73] Moretto N., A. Bolchi, C. Rivetti, B.P. Imbimbo, G. Villetti, V. Pietrini, L. Polonelli, S. Del Signore, K.M. Smith, R.J. Ferrante, S. Ottonello, Conformation-sensitive antibod-

ies against alzheimer amyloid-beta by immunization with a thioredoxin-constrained B-cell epitope peptide. J Biol Chem 2007; 282(15): p. 11436-11445.

[74] Kayed R., E. Head, F. Sarsoza, T. Saing, C.W. Cotman, M. Necula, L. Margol, J. Wu, L. Breydo, J.L. Thompson, S. Rasool, T. Gurlo, P. Butler, C.G. Glabe, Fibril specific, conformation dependent antibodies recognize a generic epitope common to amyloid fibrils and fibrillar oligomers that is absent in prefibrillar oligomers. Mol Neurodegener 2007; 2: p. 18.

[75] O'Nuallain B.,R. Wetzel, Conformational Abs recognizing a generic amyloid fibril epitope. Proc Natl Acad Sci U S A 2002; 99(3): p. 1485-1490.

[76] Merlini G.,V. Bellotti, Molecular mechanisms of amyloidosis. N Engl J Med 2003; 349(6): p. 583-596.

[77] Tan S.Y.,M.B. Pepys, Amyloidosis. Histopathology 1994; 25(5): p. 403-414.

[78] Caughey B.,P.T. Lansbury, Protofibrils, pores, fibrils, and neurodegeneration: separating the responsible protein aggregates from the innocent bystanders. Annu Rev Neurosci 2003; 26: p. 267-298.

[79] Lashuel H.A., D. Hartley, B.M. Petre, T. Walz, P.T. Lansbury, Jr., Neurodegenerative disease: amyloid pores from pathogenic mutations. Nature 2002; 418(6895): p. 291.

[80] Ferreira S.T., M.N. Vieira, F.G. De Felice, Soluble protein oligomers as emerging toxins in Alzheimer's and other amyloid diseases. IUBMB Life 2007; 59(4-5): p. 332-345.

[81] Nimmrich V., C. Grimm, A. Draguhn, S. Barghorn, A. Lehmann, H. Schoemaker, H. Hillen, G. Gross, U. Ebert, C. Bruehl, Amyloid beta oligomers (A beta(1-42) globulomer) suppress spontaneous synaptic activity by inhibition of P/Q-type calcium currents. J Neurosci 2008; 28(4): p. 788-797.

[82] Nixon R.A., Autophagy in neurodegenerative disease: friend, foe or turncoat? Trends Neurosci 2006; 29(9): p. 528-535.

[83] Powers E.T., R.I. Morimoto, A. Dillin, J.W. Kelly, W.E. Balch, Biological and chemical approaches to diseases of proteostasis deficiency. Annu Rev Biochem 2009; 78: p. 959-991.

[84] Barnham K.J., G.D. Ciccotosto, A.K. Tickler, F.E. Ali, D.G. Smith, N.A. Williamson, Y.H. Lam, D. Carrington, D. Tew, G. Kocak, I. Volitakis, F. Separovic, C.J. Barrow, J.D. Wade, C.L. Masters, R.A. Cherny, C.C. Curtain, A.I. Bush, R. Cappai, Neurotoxic, redox-competent Alzheimer's beta-amyloid is released from lipid membrane by methionine oxidation. J Biol Chem 2003; 278(44): p. 42959-42965.

[85] Cuajungco M.P., L.E. Goldstein, A. Nunomura, M.A. Smith, J.T. Lim, C.S. Atwood, X. Huang, Y.W. Farrag, G. Perry, A.I. Bush, Evidence that the beta-amyloid plaques of Alzheimer's disease represent the redox-silencing and entombment of abeta by zinc. J Biol Chem 2000; 275(26): p. 19439-19442.

[86] Huang X., C.S. Atwood, M.A. Hartshorn, G. Multhaup, L.E. Goldstein, R.C. Scarpa, M.P. Cuajungco, D.N. Gray, J. Lim, R.D. Moir, R.E. Tanzi, A.I. Bush, The A beta peptide of Alzheimer's disease directly produces hydrogen peroxide through metal ion reduction. Biochemistry 1999; 38(24): p. 7609-7616.

[87] Huang X., M.P. Cuajungco, C.S. Atwood, M.A. Hartshorn, J.D. Tyndall, G.R. Hanson, K.C. Stokes, M. Leopold, G. Multhaup, L.E. Goldstein, R.C. Scarpa, A.J. Saunders, J. Lim, R.D. Moir, C. Glabe, E.F. Bowden, C.L. Masters, D.P. Fairlie, R.E. Tanzi, A.I. Bush, Cu(II) potentiation of alzheimer abeta neurotoxicity. Correlation with cell-free hydrogen peroxide production and metal reduction. J Biol Chem 1999; 274(52): p. 37111-37116.

[88] Opazo C., X. Huang, R.A. Cherny, R.D. Moir, A.E. Roher, A.R. White, R. Cappai, C.L. Masters, R.E. Tanzi, N.C. Inestrosa, A.I. Bush, Metalloenzyme-like activity of Alzheimer's disease beta-amyloid. Cu-dependent catalytic conversion of dopamine, cholesterol, and biological reducing agents to neurotoxic H(2)O(2). J Biol Chem 2002; 277(43): p. 40302-40308.

[89] Macario A.J.,E. Conway de Macario, Sick chaperones, cellular stress, and disease. N Engl J Med 2005; 353(14): p. 1489-1501.

[90] Muchowski P.J.,J.L. Wacker, Modulation of neurodegeneration by molecular chaperones. Nat Rev Neurosci 2005; 6(1): p. 11-22.

[91] Almeida C.G., R.H. Takahashi, G.K. Gouras, Beta-amyloid accumulation impairs multivesicular body sorting by inhibiting the ubiquitin-proteasome system. J Neurosci 2006; 26(16): p. 4277-4288.

[92] Uversky V.N., Mysterious oligomerization of the amyloidogenic proteins. Febs J 2010; 277(14): p. 2940-2953.

[93] Anderluh G.,E. Zerovnik, Pore formation by human stefin B in its native and oligomeric states and the consequent amyloid induced toxicity. Front Mol Neurosci 2012; 5: p. 85.

[94] Anderluh G., I. Gutierrez-Aguirre, S. Rabzelj, S. Ceru, N. Kopitar-Jerala, P. Macek, V. Turk, E. Zerovnik, Interaction of human stefin B in the prefibrillar oligomeric form with membranes. Correlation with cellular toxicity. Febs J 2005; 272(12): p. 3042-3051.

[95] Rabzelj S., G. Viero, I. Gutierrez-Aguirre, V. Turk, M. Dalla Serra, G. Anderluh, E. Zerovnik, Interaction with model membranes and pore formation by human stefin B: studying the native and prefibrillar states. Febs J 2008; 275(10): p. 2455-2466.

[96] Alarcon J.M., J.A. Brito, T. Hermosilla, I. Atwater, D. Mears, E. Rojas, Ion channel formation by Alzheimer's disease amyloid beta-peptide (Abeta40) in unilamellar liposomes is determined by anionic phospholipids. Peptides 2006; 27(1): p. 95-104.

[97] Bokvist M., F. Lindstrom, A. Watts, G. Grobner, Two types of Alzheimer's beta-amyloid (1-40) peptide membrane interactions: aggregation preventing transmembrane

anchoring versus accelerated surface fibril formation. Journal of molecular biology 2004; 335(4): p. 1039-1049.

[98] Hertel C., E. Terzi, N. Hauser, R. Jakob-Rotne, J. Seelig, J.A. Kemp, Inhibition of the electrostatic interaction between beta-amyloid peptide and membranes prevents beta-amyloid-induced toxicity. Proc Natl Acad Sci U S A 1997; 94(17): p. 9412-9416.

[99] Hirakura Y., M.C. Lin, B.L. Kagan, Alzheimer amyloid abeta1-42 channels: effects of solvent, pH, and Congo Red. J Neurosci Res 1999; 57(4): p. 458-466.

[100] McLaurin J.,A. Chakrabartty, Characterization of the interactions of Alzheimer beta-amyloid peptides with phospholipid membranes. Eur J Biochem 1997; 245(2): p. 355-363.

[101] Terzi E., G. Holzemann, J. Seelig, Interaction of Alzheimer beta-amyloid peptide(1-40) with lipid membranes. Biochemistry 1997; 36(48): p. 14845-14852.

[102] Janson J., R.H. Ashley, D. Harrison, S. McIntyre, P.C. Butler, The mechanism of islet amyloid polypeptide toxicity is membrane disruption by intermediate-sized toxic amyloid particles. Diabetes 1999; 48(3): p. 491-498.

[103] Lin M.C., T. Mirzabekov, B.L. Kagan, Channel formation by a neurotoxic prion protein fragment. J Biol Chem 1997; 272(1): p. 44-47.

[104] Zakharov S.D., J.D. Hulleman, E.A. Dutseva, Y.N. Antonenko, J.C. Rochet, W.A. Cramer, Helical alpha-synuclein forms highly conductive ion channels. Biochemistry 2007; 46(50): p. 14369-14379.

[105] Kayed R., Y. Sokolov, B. Edmonds, T.M. McIntire, S.C. Milton, J.E. Hall, C.G. Glabe, Permeabilization of lipid bilayers is a common conformation-dependent activity of soluble amyloid oligomers in protein misfolding diseases. J Biol Chem 2004; 279(45): p. 46363-46366.

[106] Kawahara M., N. Arispe, Y. Kuroda, E. Rojas, Alzheimer's disease amyloid beta-protein forms Zn(2+)-sensitive, cation-selective channels across excised membrane patches from hypothalamic neurons. Biophys J 1997; 73(1): p. 67-75.

[107] Lin H., Y.J. Zhu, R. Lal, Amyloid beta protein (1-40) forms calcium-permeable, Zn2+-sensitive channel in reconstituted lipid vesicles. Biochemistry 1999; 38(34): p. 11189-11196.

[108] Rhee S.K., A.P. Quist, R. Lal, Amyloid beta protein-(1-42) forms calcium-permeable, Zn2+-sensitive channel. J Biol Chem 1998; 273(22): p. 13379-13382.

[109] Di Scala C., J.D. Troadec, C. Lelievre, N. Garmy, J. Fantini, H. Chahinian, Mechanism of cholesterol-assisted oligomeric channel formation by a short Alzheimer beta-amyloid peptide. J Neurochem 2013.

[110] Green J.D., L. Kreplak, C. Goldsbury, X. Li Blatter, M. Stolz, G.S. Cooper, A. Seelig, J. Kistler, U. Aebi, Atomic force microscopy reveals defects within mica supported lip-

id bilayers induced by the amyloidogenic human amylin peptide. Journal of molecular biology 2004; 342(3): p. 877-887.

[111] Fantini J.,N. Yahi, The driving force of alpha-synuclein insertion and amyloid channel formation in the plasma membrane of neural cells: key role of ganglioside-and cholesterol-binding domains. Adv Exp Med Biol 2013; 991: p. 15-26.

[112] Zhao J., Y. Luo, H. Jang, X. Yu, G. Wei, R. Nussinov, J. Zheng, Probing ion channel activity of human islet amyloid polypeptide (amylin). Biochim Biophys Acta 2012; 1818(12): p. 3121-3130.

[113] Oladzad Abbasabadi A., A. Javanian, M. Nikkhah, A.A. Meratan, P. Ghiasi, M. Nemat-Gorgani, Disruption of mitochondrial membrane integrity induced by amyloid aggregates arising from variants of SOD1. International journal of biological macromolecules 2013; 61C: p. 212-217.

[114] Zerovnik E., K. Skerget, M. Tusek-Znidaric, C. Loeschner, M.W. Brazier, D.R. Brown, High affinity copper binding by stefin B (cystatin B) and its role in the inhibition of amyloid fibrillation. Febs J 2006; 273(18): p. 4250-4263.

[115] Atwood C.S., R.D. Moir, X. Huang, R.C. Scarpa, N.M. Bacarra, D.M. Romano, M.A. Hartshorn, R.E. Tanzi, A.I. Bush, Dramatic aggregation of Alzheimer abeta by Cu(II) is induced by conditions representing physiological acidosis. The Journal of biological chemistry 1998; 273(21): p. 12817-12826.

[116] Raman B., T. Ban, K. Yamaguchi, M. Sakai, T. Kawai, H. Naiki, Y. Goto, Metal ion-dependent effects of clioquinol on the fibril growth of an amyloid {beta} peptide. The Journal of biological chemistry 2005; 280(16): p. 16157-16162.

[117] Zou J., K. Kajita, N. Sugimoto, Cu(2+) Inhibits the Aggregation of Amyloid beta-Peptide(1-42) in vitro We thank JEOL for the AFM measurement. This work was supported in part by Grants-in-Aid from the Japanese Ministry of Education, Science, Sports, and Culture, and a Grant from "Research for the Future" Program of the Japan Society for the Promotion of Science to N.S. Angewandte Chemie 2001; 40(12): p. 2274-2277.

[118] Kuczius T.,R. Kelsch, The effect of copper and zinc binding on the solubility and resistance to proteolysis of physiological prion protein PrP depends on the tissue source and the PrP glycotypes. Journal of cellular biochemistry 2013.

[119] Leal S.S., I. Cardoso, J.S. Valentine, C.M. Gomes, Calcium ions promote superoxide dismutase 1 (SOD1) aggregation into non fibrillar amyloid: a link to toxic effects of calcium overload in amyotrophic lateral sclerosis (ALS)? The Journal of biological chemistry 2013.

[120] Ha C., J. Ryu, C.B. Park, Metal ions differentially influence the aggregation and deposition of Alzheimer's beta-amyloid on a solid template. Biochemistry 2007; 46(20): p. 6118-6125.

[121] Tougu V., A. Karafin, K. Zovo, R.S. Chung, C. Howells, A.K. West, P. Palumaa, Zn(II)-and Cu(II)-induced non-fibrillar aggregates of amyloid-beta (1-42) peptide are transformed to amyloid fibrils, both spontaneously and under the influence of metal chelators. J Neurochem 2009; 110(6): p. 1784-1795.

[122] Watt N.T., H.H. Griffiths, N.M. Hooper, Neuronal zinc regulation and the prion protein. Prion 2013; 7(3): p. 203-208.

[123] Johnson C.J., P.U. Gilbert, M. Abrecht, K.L. Baldwin, R.E. Russell, J.A. Pedersen, J.M. Aiken, D. McKenzie, Low copper and high manganese levels in prion protein plaques. Viruses 2013; 5(2): p. 654-662.

[124] Nubling G., B. Bader, J. Levin, J. Hildebrandt, H. Kretzschmar, A. Giese, Synergistic influence of phosphorylation and metal ions on tau oligomer formation and coaggregation with alpha-synuclein at the single molecule level. Mol Neurodegener 2012; 7: p. 35.

[125] Mirhashemi S.M.,M.E. Shahabaddin, Evaluation of aluminium, manganese, copper and selenium effects on human islets amyloid polypeptide hormone aggregation. Pakistan journal of biological sciences: PJBS 2011; 14(4): p. 288-292.

[126] Lee E.C., E. Ha, S. Singh, L. Legesse, S. Ahmad, E. Karnaukhova, R.P. Donaldson, A.M. Jeremic, Copper(ii)-human amylin complex protects pancreatic cells from amylin toxicity. Physical chemistry chemical physics : PCCP 2013; 15(30): p. 12558-12571.

[127] Salamekh S., J.R. Brender, S.J. Hyung, R.P. Nanga, S. Vivekanandan, B.T. Ruotolo, A. Ramamoorthy, A two-site mechanism for the inhibition of IAPP amyloidogenesis by zinc. Journal of molecular biology 2011; 410(2): p. 294-306.

[128] Wilhelmus M.M., R.M. de Waal, M.M. Verbeek, Heat shock proteins and amateur chaperones in amyloid-Beta accumulation and clearance in Alzheimer's disease. Mol Neurobiol 2007; 35(3): p. 203-216.

[129] Hook G., V.Y. Hook, M. Kindy, Cysteine protease inhibitors reduce brain beta-amyloid and beta-secretase activity in vivo and are potential Alzheimer's disease therapeutics. Biol Chem 2007; 388(9): p. 979-983.

[130] Morgan C.,N.C. Inestrosa, Interactions of laminin with the amyloid beta peptide. Implications for Alzheimer's disease. Braz J Med Biol Res 2001; 34(5): p. 597-601.

[131] Porat Y., A. Abramowitz, E. Gazit, Inhibition of amyloid fibril formation by polyphenols: structural similarity and aromatic interactions as a common inhibition mechanism. Chem Biol Drug Des 2006; 67(1): p. 27-37.

[132] Zhao L.N., Y. Mu, L.Y. Chew, Heme prevents amyloid beta peptide aggregation through hydrophobic interaction based on molecular dynamics simulation. Phys Chem Chem Phys 2013; 15(33): p. 14098-14106.

[133] Yagi-Utsumi M., T. Kunihara, T. Nakamura, Y. Uekusa, K. Makabe, K. Kuwajima, K. Kato, NMR characterization of the interaction of GroEL with amyloid beta as a model ligand. FEBS Lett 2013; 587(11): p. 1605-1609.

[134] Ly S., R. Altman, J. Petrlova, Y. Lin, S. Hilt, T. Huser, T.A. Laurence, J.C. Voss, Binding of apolipoprotein E inhibits the oligomer growth of amyloid-beta peptide in solution as determined by fluorescence cross-correlation spectroscopy. J Biol Chem 2013; 288(17): p. 11628-11635.

[135] Kotarba A.E., D. Aucoin, M.D. Hoos, S.O. Smith, W.E. Van Nostrand, Fine mapping of the amyloid beta-protein binding site on myelin basic protein. Biochemistry 2013; 52(15): p. 2565-2573.

[136] Cui L., Y. Zhang, H. Cao, Y. Wang, T. Teng, G. Ma, Y. Li, K. Li, Ferulic Acid Inhibits the Transition of Amyloid-beta42 Monomers to Oligomers but Accelerates the Transition from Oligomers to Fibrils. Journal of Alzheimer's disease : JAD 2013.

[137] Zhang W., Y. Du, M. Bai, Y. Xi, Z. Li, J. Miao, S14G-humanin inhibits Abeta1-42 fibril formation, disaggregates preformed fibrils, and protects against Abeta-induced cytotoxicity in vitro. Journal of peptide science : an official publication of the European Peptide Society 2013; 19(3): p. 159-165.

[138] Stanyon H.F.,J.H. Viles, Human serum albumin can regulate amyloid-beta peptide fiber growth in the brain interstitium: implications for Alzheimer disease. The Journal of biological chemistry 2012; 287(33): p. 28163-28168.

[139] Ahn J.H., Y. Hu, M. Hernandez, J.R. Kim, Crocetin inhibits beta-amyloid fibrillization and stabilizes beta-amyloid oligomers. Biochem Biophys Res Commun 2011; 414(1): p. 79-83.

[140] Taler-Vercic A.,E. Zerovnik, Binding of amyloid peptides to domain-swapped dimers of other amyloid-forming proteins may prevent their neurotoxicity. BioEssays : news and reviews in molecular, cellular and developmental biology 2010; 32(12): p. 1020-1024.