Amphoid-related diseases are a group of illnesses in which an abnormal accumulation of proteins into fibril structures is evident. Results from a wide range of studies, ranging from identification of amyloid-β dimers in the brain to biophysical characterization of the interactions between amyloidogenic peptides and lipid membranes during fibril growth shed light on the initial events which take place during amyloid aggregation. Accounts of fibril disaggregation and formation of globular aggregates due to interactions with lipids or fatty acids further demonstrate the complexity of the aggregation process and the difficulty to treat amyloid-related diseases. There is an inherent difficulty in generalizing from studies of aggregation in vitro, but the involvement of too many cellular components limits the ability to follow amyloid aggregation in a cellular (or extracellular) context. Fortunately, the development of experimental methods to generate stable globular aggregates suggests new means of studying the molecular events associated with amyloid aggregation. Furthermore, simulation studies enable deeper understanding of the experimental results and provide useful predictions that can be tested in the laboratory. Computer simulations can nowadays provide molecular or even atomistic details that are experimentally not available or very difficult to obtain. In the present review, recent developments on modelling and experiments of amyloid aggregation are reviewed, and an integrative account on how isolated interactions (as observed in vitro and in silico) combine during the course of amyloid-related diseases is presented. Finally, it is argued that an integrative approach is necessary to get a better understanding of the protein aggregation process.

Key words: Alzheimer’s disease, computer simulation, integrative biology, molecular dynamics, Parkinson’s disease.

INTRODUCTION

Amyloid-related diseases comprise a group of several dozen pathologies. The most common among the human amyloid-related diseases are AD (Alzheimer’s disease), PD (Parkinson’s disease) and T2DM (Type 2 diabetes mellitus). Other notable amyloid-related diseases include Huntington’s disease, transmissible spongiform encephalopathies, ALS (amyotrophic lateral sclerosis) and systemic amyloidosis. What amyloid-related diseases have in common is an underlying molecular mechanism which involves abnormal aggregation of many copies of the same protein into well-ordered filamentous β-sheet-rich structures [1,2], termed amyloid fibrils (Figure 1). The fibrils accumulate in the damaged tissue, e.g. as senile plaques (in the brain of AD patients), Lewy bodies (in the brain of PD patients) or pancreatic amyloid deposits (in T2DM).

Amyloid fibrils are formed by aggregation of proteins or peptides into β-sheet-rich structures. Aggregates of a segment of 39–43 residues of the Aβ (amyloid-β) peptide are located in senile plaques, whereas fibrils made up of multiple copies of α-synuclein, IAPP (islet amyloid polypeptide) and SOD (superoxide dismutase) are associated with the tissue damage in PD, T2DM and ALS respectively. The formation of the insoluble fibrils is a complex process which is not completely understood, and may be tissue- or peptide-sequence-specific.

Since amyloid fibrils are common to all of these diseases, prevalent exclusively in the damaged tissue and thermodynamically stable, it seems plausible that fibrils are the primary cause of amyloid-related diseases. This is, however, not a common held view today. Apparently, the amount of senile plaques in the brain of AD patients does not correlate with the degree of dementia [3]. Likewise, Lewy bodies are not evident in all cases of PD [4]. Amyloid aggregation is in fact a multi-step process, where peptides initially cluster together into soluble bodies comprised of dimers, trimers or other oligomers, whose shape and composition are not completely understood (for a schematic representation, see Figure 2). Yet, a link has been established between soluble prefibrillar oligomers comprised of the aggregating proteins and cellular toxicity [5–7].

Various cellular and molecular components are involved in the toxicity of amyloid oligomers. In particular, lipid membranes and metal ions (Zn2+, Cu2+ and Fe3+) are believed to play a role in several amyloid-related diseases. It is argued that disruption of membrane integrity is the most likely explanation for membrane-related amyloidogenic toxicity [8,9], whereas various multivalent ions accelerate protein aggregation and catalyse the formation of toxic radicals [10], providing an alternative or additional mechanism for toxicity. Another proposed mechanism is binding of the oligomers to cellular receptors [11].

PROTEINS AND PEPTIDES INVOLVED IN AMYLOID-RELATED DISEASES

Several examples of proteins or peptides that aggregate into fibrils in several amyloid-related diseases are given in Table 1. Strikingly, whereas some of the peptides are natively unfolded, others have clearly defined structures with no similarities between them.
Amyloidogenic peptides and proteins come in a variety of sizes, from dipeptides [12] to large proteins of several thousand residues. Bioinformatics studies have been successful in predicting the amyloidogenic tendency of protein sequences [13–15]. Web servers, such as PASTA (http://protein.cribi.unipd.it/pasta/) [16] and FoldAmyloid (http://antares.protres.ru/fold-amyloid/oga.cgi) [17], provide such predictions based on various algorithms.

STRUCTURAL STUDIES OF AMYLOID AGGREGATES

Common approaches for structural studies of amyloid aggregates are discussed in this section, with several examples. More details about interactions of biological relevance will be given in the following sections.

Structures of fibrils and oligomers by microscopy

EM (electron microscopy) is very useful to view and characterize amyloid fibrils, and even protofibrils and oligomers, through negative staining [18]. The main advantages of EM are that samples can be studied directly and that this can be done without the need for structural transformations (e.g. formation of crystals). Fine cryo-EM pictures clearly show the rope-like structure of fibrils (Figure 4) and their sizes (typically several nanometres wide and up to several metres long). STEM (scanning transmission EM) is also widely used to study amyloids fibrils [19]. STEM allows a direct measurement of the mass-per-length of an amyloid fibril. Together with cryo-EM, this enables modelling of the fibrils by reconstructing monomers of known mass into filaments [20].

Furthermore, STEM has been used to determine the mass of prefibrillar aggregates with different shapes and sizes [21], which aids in the understanding of the aggregation process. Interestingly, EM techniques can also be applied to biological preparations, such as ex vivo fibrils [22]. AFM (atomic force microscopy) has been used for determination of the dimensions of fibrils and prefibrillar structures. AFM can also be used to study ex vivo samples. For example, the anatomy of fibril growth during disease progression has been revealed by AFM (Figure 5).

Small-angle scattering

Small-angle scattering methods, in particular using X-ray [SAXS (small-angle X-ray scattering)], have become increasingly useful to study fibril formation in solution. Unlike EM, SAXS is not a direct method, but it allows measurements of sizes and can be used to study the shapes of molecules in solution. An advantage of SAXS is that models can be built to fit the scattering intensity [23]. Such models have been used together with SAXS measurements to suggest fibrillation pathways [24,25] (see Figure 6). SAXS excess intensities can also be calculated from MD (molecular dynamics) simulations, as recently demonstrated [26]. Given that MD simulations are widely used for modelling of amyloid aggregation (see below), a combination of the two approaches can provide high-resolution models of aggregation in solution.

Atomic resolution structures: X-ray crystallography and NMR

X-ray structures of proteins provide atomistic details on the structure of single proteins and protein assemblies. Monomers
Despite the success of high-resolution methods, and particularly solid-state NMR, to shed light on the atomic structure of fibrils, relatively few systems yield to such experiments. Oligomers, and to a lesser extent also fibrils, are very heterogeneous and evolve rapidly in solution. Methodological advancements of solution NMR have already been applied to large macromolecular assemblies (e.g. the 20S proteasome core particle, with a mass of 670 kDa [39]). XFELs (X-ray-free electron lasers), such as that being built near Hamburg, Germany (http://www.xfel.eu), are also expected to provide atomic structures of large biomolecular complexes [40] that are difficult or even impossible to crystalize. The first XFEL instrument is already operating in the U.S.A., and has recently been used to provide the sub-nanometre resolution structure of a large macromolecular complex [41].

**COMPUTER SIMULATIONS OF AGGREGATING PROTEINS**

Computer simulations are an essential tool in the study of dynamic processes in biology and chemistry, providing data on sizes and time scales that are not accessible to the experiment. Although it is possible to conduct single-molecule experiments, it is very difficult to conduct experiments with single oligomers or prefibrillar arrangements which change their structure and composition. Accordingly, computer simulations are widely used to study protein aggregation. MD simulations are particularly applicable to study the time-dependent evolution of (bio)molecular systems [42–46]. In MD simulations, the potential energy of the system is calculated for an initial state, and the Newton equations of motion are solved, providing a new set of co-ordinates. The procedure is then repeated numerous times. The output is a trajectory, revealing how the system evolves in time, as in a motion picture. Various observables can be calculated from the trajectory, from interaction energies to distribution functions and spectra.

MD is one of the most popular techniques to study amyloids. MD simulations of amyloid aggregation have therefore been the subject of several review papers [47–50]. Many studies have concentrated on identifying the structure of amyloidogenic proteins as monomer or dimers. In the present review, however, I limit the discussion only to studies of formation of fibrils and oligomers.

Several approaches can be used to simulate amyloid fibrils or oligomers. Perhaps the most straightforward way is to use experimental structures as starting points (for example see [51,52]), which can shed light on the molecular structure in solution as long as it does not deviate too much from the initial conformation. Direct simulation of the aggregation process, or even the formation of oligomers by amyloidogenic peptides, is currently not achievable by atomistic simulations. Although the simulations are limited to the sub-microsecond time scale (extended to milliseconds with specialized hardware that is not commonly available [46]), the formation of oligomers may take minutes. In other cases (for example, with Aβ oligomers are formed right away, but the conformational space of the monomer cannot be studied effectively by atomistic MD. Oligomers can be studied in silico by modelling them based on low-resolution structures and simulating the system by conventional MD [53]. Such simulations can show whether the proposed models are stable for several tens of nanoseconds.

The difficulty in obtaining any structures of oligomers limits the ability to model such structures, and necessitates the use of different approaches. One of the most popular methods is CGMD (coarse-grained MD). In CGMD, a molecule is represented by

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**Figure 2  Schematic representation of the amyloid aggregation process**

An animated version of this Figure is available at http://www.BiochemJ.org/bj/438/0415/bj4380415add.htm.
### Table 1  Amyloidogenic peptides involved in several amyloidogenic diseases

<table>
<thead>
<tr>
<th>Associated disease (main)</th>
<th>Peptide</th>
<th>Number of amino acids</th>
<th>Molecular mass (kDa)</th>
<th>Biological role</th>
<th>Defined structure (yes/no)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>Aβ</td>
<td>39–43</td>
<td>4.6</td>
<td>Unknown</td>
<td>No</td>
</tr>
<tr>
<td>PD</td>
<td>α-Synuclein</td>
<td>101</td>
<td>11.4</td>
<td>Unknown</td>
<td>No</td>
</tr>
<tr>
<td>T2DM</td>
<td>IAPP</td>
<td>37</td>
<td>4.2</td>
<td>Unknown</td>
<td>No</td>
</tr>
<tr>
<td>Huntington's disease</td>
<td>Huntingtin*</td>
<td>&gt; 3000</td>
<td>&gt;330</td>
<td>Hormone</td>
<td>No</td>
</tr>
<tr>
<td>ALS</td>
<td>SOD1</td>
<td>154</td>
<td>32</td>
<td>Enzyme</td>
<td>Yes</td>
</tr>
<tr>
<td>Dialysis-related amyloidosis</td>
<td>β2-Microglobulin</td>
<td>99</td>
<td>11.6</td>
<td>Various</td>
<td>Yes</td>
</tr>
<tr>
<td>Non-neuropathic systemic amyloidosis</td>
<td>Lysozyme</td>
<td>130</td>
<td>15</td>
<td>Enzyme</td>
<td>Yes</td>
</tr>
<tr>
<td>Meretoja's disease†</td>
<td>Gelsoline</td>
<td>782</td>
<td>82</td>
<td>Regulatory</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*Huntingtin includes a variable number of polyglutamine repeats. Proteins with >40 repeats aggregate to form amyloid fibrils.
†Also known as Finnish amyloidosis.

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**Figure 3  Structures of several proteins that participate in amyloid-related disease**

Note the difference in sizes and secondary structure, which stands in contrast with the relatively uniform organization of the fibrils. (A) Na, Zn SOD [135]. (B) β2-Microglobulin [136]. (C) Lysozyme [137]. (D) Gelsoline [138]. The Figure was produced with the computer program VMD [139].

a set of beads rather than atoms, where each bead corresponds with several atoms or residues. This reduces the accuracy of the simulations, but enables the study of larger systems and longer time scales. Applications of CGMD have become very successful for simulations of biomolecules [54,55], but their applicability for studies involving drastic secondary structure changes, such as the refolding of amyloidogenic peptides, are limited. Physics-based CG representation of peptides, without the need to pre-impose secondary structures, may become useful for aggregation studies, as demonstrated in a study of Aβ1–40 [56].

Specific models have been developed directly for the study of amyloid aggregation [57–60]. These models aim at representing specific peptides based on their sequence. Alternatively, models can be developed based on a certain process...
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Figure 4 EM of oligomers (left-hand panel), protofibrils (middle panel) and fibrils (right-hand panel), visible through negative staining

Reproduced from [18] (permission not required).

Figure 5 Surface plots of an AFM image (height data) of \( \beta \)-2-microglobulin ex vivo amyloid material obtained from a patient affected by dialysis-related amyloidosis

The original Figure is shown on the left-hand side. A processed Figure is shown on the right-hand side to highlight the difference between the amyloid and collagen fibrils. The amyloid fibrils (thin threads; yellow/green) extend from a collagen fibril (larger cylindrical body, blue). The original Figure is reproduced from [140], with permission from the American Society for Biochemistry and Molecular Biology.

(e.g. aggregation) rather than dealing with peptide sequences. Such \textit{ad hoc} representations are called phenomenological CG models. Phenomenological CGMD simulation studies have been conducted by representing each peptide by two states only (aggregation-prone and aggregation-protected). In particular, these simulations led to the conclusion that the aggregation pathway (e.g. formation of oligomers and protofilaments) crucially depends on the amyloidogenic tendency of the aggregating peptides [61–63]. In other words, the free energy difference between the aggregation-prone and aggregation-protected forms determines the aggregation pathway, even if the end-state of the simulations is always a fibril.

CG models can be supplemented by computational methods that enable more efficient sampling. DMD (discrete MD) simulations are particularly appealing for amyloid aggregation studies [64]. In such simulations, the relatively sophisticated interactions between the simulated particles are replaced by simplified functions that are only dealt with when two particles physically interact. This offers a gain of speed, but reduces the accuracy of the simulations. MC (Monte Carlo) simulations, in which the system is simulated by subsequent ‘trial moves’ that are accepted based on energetic criteria, rather than evolved in time (as in MD), are also used for the study of amyloid aggregation. For example, modifying a backbone dihedral of a certain residue can be considered such a ‘trial move’, without the need to consider the motions of each atom separately. MC simulations have been used to study amyloid aggregation and formation of oligomers [65–69], with peptide representations that range from atomistic to phenomenological.

**MAPPING DISEASE PROGRESSION IN CELLULAR MODELS AND IN VIVO**

The most common amyloid-related diseases do not naturally occur in model animals such as mice and rats, and take many years to develop in humans. PD, for example, may progress over 40 years, in different areas of the brain [70]. Techniques to visualize or characterize amyloid aggregation in patients or in tissues can therefore be of high benefit for research and clinical development, and much progress has been made in this respect over the last few years.

Several chemical agents that bind A\( \beta \) and can be used for PET (positron emission tomography) imaging have been reported. The most widely used agent is known as PiB (Pittsburg compound B). PiB contains \(^{11}\text{C}\) as a tracer. Using PiB-PET, regions in the brain that contain a high amount of A\( \beta \) can be imaged, revealing the difference between healthy and sick individuals [71] (Figure 8). Unfortunately, the use of PiB is limited because \(^{11}\text{C}\) has a half-life of 20 min, and its preparation must therefore be carried out.
Figure 6  The full oligomerization/fibrillation model for glucagon


Figure 7  Solid-state NMR structure of a fibril of the HET-s fungal amyloid [36]

The fibril is made up of five monomers of the amyloidogenic core of HET-s (residues 218–289). The Figure was produced with the computer program VMD [139].

in the medical centre itself. Another imaging agent, florbetapir, specifically binds to A\(\beta\) in the brain cortex and has recently been successfully tested in a clinical trial [72]. Florbetapir is labelled with \(^{18}\)F, which has a half-life of 120 min. It has the potential to be widely used, but further studies should carried out to identify its predictive ability when the cognitive impairment is still mild [72]. There is currently no imaging agent in use that directly binds to \(\alpha\)-synuclein to monitor aggregation in PD and related diseases [73].

Monomers and small aggregates of proteins have been isolated from the cerebral cortex of AD patients, revealing that A\(\beta\) dimers have toxic effects and are more common than other oligomers [74,75]. An assay to identify A\(\beta\) dimers in blood has recently been developed, and it has shown that elevated levels are found in the blood of AD patients [76]. Yet it is not widely accepted that dimers are the most harmful oligomeric species, and less is known about the size of toxic amyloid aggregates in other diseases. Whereas amyloid aggregation is extracellular in AD, \(\alpha\)-synuclein fibrils develop in neurons. It is therefore important to understand how the disease progresses from cell to cell. A recent
study has demonstrated that α-synuclein propagates between cells [77]. Interestingly, cells were able to take up not only monomers, but also oligomers and fibrils [78]. Advanced imaging [79] or modelling techniques may be useful to gain a better understanding of the process, particularly in the case of fibril transfer from cell to cell. Cell-to-cell transmission may be common to many amyloid-related diseases, as mammalian cells can take up fibrils made up of polyglutamine aggregates [80], suggesting a prion-like mechanism in which the disease progresses via an infectious protein agent [81]. This demonstrates the need to consider cellular systems in studies aimed at a better understanding of PD and other amyloid-related diseases, and particularly for drug design. In this context, it is worth mentioning that a mutant form of α-synuclein abrogates wild-type aggregation in vitro, but not in cells [82], further stressing the importance of cellular interactions for disease progression.

FIBRIL GROWTH ON THE MEMBRANE

Studies of amyloidogenic peptides in pure solutions have proven very useful to learn about the kinetics of amyloid aggregation, whereas studies involving cells or in vivo investigations approach the biological disease-relevant interactions. In between these there are in vitro experiments that take into account not only the peptides and solvent, but also other molecules that participate in the aggregation process. Biological membranes, for example, seem to play an important role in fibrillation and toxicity, as demonstrated for several amyloid-related diseases.

A convincing demonstration of the role for lipid membranes in the aggregation process was given in a study of leakage from lipid vesicles induced by IAPP [83]. Membrane leakage was maximal during fibril growth. Once the fibrils matured, they could bind the vesicles, but leakage was much slower. In a study of α-synuclein, addition of amyloid oligomers led to leakage from giant unilamellar vesicles, but the vesicles appeared intact following the process [79] (Figure 9). The leakage from lipid vesicles owing to fibril growth or binding of oligomers can be explained following computer simulations with a phenomenological model [84]. Leakage during fibril growth occurs because the fibrils grow on the lipid surface and penetrate the vesicle structure during the process. Once the fibrils are fully grown and stable, they are released from the vesicle surface. Oligomers may disrupt the vesicle structure by adsorption to its surface, where they interfere with the packing of lipids, or by growing into fibrils.

Membrane composition can influence the outcome of aggregation in many ways. For example, negatively charged membranes have a higher tendency to interact with IAPP [85,86], Aβ [87–90] and α-synuclein [91,92] compared with neutral or zwitterionic membranes. Other effects, such as the membrane phase [93,94] and the presence of polyunsaturated fatty acids [95] also seem to play a role. Computer simulations of fibril disaggregation have shown that such interactions can be taken into account by treating the degree of attraction between membranes of certain compositions and the peptides as a variable [96].

Recently, much attention has been given to the role of lipid rafts in amyloid aggregation [97]. Lipid rafts are membrane domains that are rich in lipids with a rigid structure, such as cholesterol and sphingolipids. Their presence in the brain seems to be affected by age [98] and diet [99], which is also true for many amyloid-related diseases. Lipids rafts are apparently involved in the aggregation process, but data on their interactions is not yet conclusive. There is some discrepancy in the literature as to whether raft domains accelerate disease progression and toxicity [100].

AGGREGATES OF PEPTIDES AND FATTY ACIDS OR DETERGENTS

When it became clear that globular oligomers of Aβ and α-synuclein are neurotoxic [101,102], efforts have been made to isolate stable forms of such oligomers. Stable α-synuclein oligomers from brain tissue appeared to be associated with lipids [103]. Similarly, incubation of synthetic Aβ1–42 with fatty acids or SDS led to the formation of stable oligomers (known as globulomers); antibodies that bind to such oligomers recognized epitopes in the brains of AD patients [104]. NMR measurements reveal that the globulomers may have some β-sheet content [105]. Likewise, titration of Aβ1–40 [106] and opioid neuropeptides [107]
with SDS, monitored by CD, revealed an increase in $\beta$-sheet content. SDS-induced oligomers of proinsulin C-peptide led to the formation of structures containing a high ratio of $\beta$-sheets, and the same is true for toxic oligomers prepared from equine lysozyme and oleic acid [108]. Taken together, these results suggest that oligomers in the presence of detergent molecules are rich in $\beta$-sheets. Yet the structure of globular amyloid oligomers is not known in detail and may depend on the aggregating peptide. Low-resolution SAXS structures [109] and computer simulations are expected to yield more data on the conformation of such oligomers. It remains to be seen whether fatty acid or detergent-associated oligomers are the common form.

**MULTIVALENT IONS AND ADDITIONAL CELLULAR AND EXTRACELLULAR FACTORS**

Multivalent ions such as Zn$^{2+}$ were shown to promote amyloid aggregation more than 15 years ago [110]. Senile plaques in brains of AD patients contain elevated concentrations of Zn$^{2+}$.
In parallel, peptide synthesis continues, more oligomers are formed. Such oligomers may differ from those formed on-pathway to interactions with lipid membranes [127], forming oligomers that end with fibril formation, since the fibrils can disaggregate due to regions which inhibit fibril formation [126]. The process does not always progress in the same manner and can be influenced by other factors.

Alternatively, the oligomers may bind to certain membrane receptors, which can induce ruptures and eventually lead to cell death, depending on the cellular location. If fibrils are formed on the membranes, they can aggregate further into fibrils, which can aggregate into larger aggregates in the brain to form amyloid fibrils ([118]).

Interestingly, interactions with Zn$^{2+}$ may lead to the formation of non-fibrillar aggregates [118]. Moreover, an NMR study of Aβ$^{1-42}$ together with lipid membranes shows that the structure of the peptide in the membrane is influenced by the metal ions [119]. Thus combined effects should be taken into account to approach the biological environment. The cellular (and extracellular) environment contains a wide range of macromolecules, collectively known as macromolecular crowders, which makes it different from pure water or saline solutions. Macromolecular crowders can alter the protein structure in various ways. Simplicistically, low concentrations of crowders stabilize the native structure, and thus disfavour aggregation, whereas high concentrations force the peptides together and result in increased aggregation rates [120]. Yet crowding effects depend on the nature of the crowders (e.g. size, chemical head group and solubility [121,122]) and the involvement of other molecules such as lipids [123] and ions. Results from recent simulation studies reveal that the effect of crowders also depends on the intrinsic amyloidogenic propensity of the peptides [124]. Moreover, the energy landscape of peptides may be affected by the crowders [125].

### INTEGRATIVE BIOLOGY APPROACH TO AMYLOID-RELATED DISEASES

Let us consider a peptide that is naturally unstructured and which aggregates in the brain to form amyloid fibrils (α-synuclein for example). The peptide is synthesized in the brain, but is also degraded. With age, the degradation machinery wears out, and the concentration of the peptides is increased. This raises the probability for the formation of oligomers. Other age-related modifications, such as the increase in Zn$^{2+}$ concentration, may speed up the oligomerization. Oligomers can undergo many changes. They can aggregate further into fibrils, bind to receptors and interact with membranes. The biological consequences depend not only on whether or not fibrils are formed, but also on the cellular location. If fibrils are formed on the membranes, they can induce ruptures and eventually lead to cell death, whereas if they are rapidly formed in solution the damage can be milder. Alternatively, the oligomers may bind to certain membrane regions which inhibit fibril formation [126]. The process does not end with fibril formation, since the fibrils can disaggregate due to interactions with lipid membranes [127], forming oligomers again. Such oligomers may differ from those formed on-pathway [96]. In parallel, peptide synthesis continues, more oligomers are formed (perhaps also due to interactions with fatty acids and other detergent-like molecules), and cellular and tissue damage accumulate. Monomers, oligomers and even fibrils may progress to nearby cells. Age-related modifications in membrane composition may contribute to the aggregation process.

The interactions described above can only be realized by a wide range of experiments, from aggregation of peptides in vitro to monitoring changes in brain lipid composition with age. Indeed, many experimental and theoretical techniques have been used in the study of amyloid-related diseases; only a few have been discussed above. Systems ranging from small-scale atomistic simulations to studies of peptides in aqueous solutions, and further to brain samples, have been used. Amyloid-related diseases probably start with small and fast interactions, such as binding of multivalent ions by peptides (t < 10^{-7} s, involving few molecules) to development of age-related diseases (t > 10 years, multiple tissues). This calls for an integrative approach that bridges molecular and cellular sizes and time scales, as well as different experimental and computational studies.

One of the most difficult aspects of developing an integrative biology approach to amyloid-related diseases is the shear amount of literature available. No review paper, or even book, can deal with such significant amounts of studies as devoted to AD and PD. Efforts have been made to systematically gather the data by experts, e.g. in the Alzheimer’s Research Forum (http://www.alzforum.org), but scientists may still miss crucial data. Computational methods are being developed to deal with this difficulty [128]. Such an approach has been used to build a Web-server that deals with interactions of proteins in AD and related drugs [129]. It should be stressed that such studies should involve both computational scientists and specialists that act as biological curators.

Once key interactions are mapped, the next challenge is to realize how these are quantitatively related, which is often not possible without computer simulations. This has recently been demonstrated in an analysis of kinase activity following polyglutamine aggregation (which takes place in Huntington’s disease [130]). Experiments were conducted to deal with kinase inhibition upon aggregation, cellular toxicity and formation of inclusion bodies. The results have been used to derive a model of the process that could be simulated (Figure 10). Dealing with interactions that involve more players (e.g. several processes taking place simultaneously in the brain of a patient) leads to difficulty in presenting the data, which calls for the development of computer visualization techniques (for example see [131]).

### CONCLUSIONS

Mounting evidence attributes aberrant protein aggregation as the cause for amyloid-related diseases. In recent years, scientists and clinicians have concentrated on the toxicity of oligomeric structures made by proteins and peptides, and on the interactions between such structures and cellular components (chiefly lipid membranes). These diseases, however, develop over many years and are affected by a multitude of factors. A wide range of experimental evidence is available, which spans the molecular to cellular levels and beyond. Understanding how the molecular interactions operate at the cellular context and lead to amyloid aggregation and toxicity is desired. Biophysical and structural experiments have shed light on the shape of aggregation intermediates and the kinetics of their formation under various conditions, whereas studies at the cell and organ level have been used to identify the molecular entities that are associated with the disease. Modelling and simulation studies aid in the understanding of molecular processes and may provide atomistic
details. Stabilization of oligomers by mutation [132] or chemical modifications can be used to gather structural data. In addition, novel instruments, such as XFELs, are expected to contribute significantly to our understanding of amyloid aggregation at the molecular level.

Even a simple two-state toy model of aggregating peptides can yield a very complex energy landscape [62] and provide interesting insights, which only exemplifies the complexity of the intra- and extra-cellular interactions that lead to amyloid aggregation in vivo. A wide range of experiments and simulations is needed to provide a better understanding of amyloid-related diseases. These studies should be supplemented by an integrative approach. Software tools and new techniques for visualization and analysis of the data should be developed for this purpose. Prototypes [130] show that this should be achievable.

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