Chapter 1

Introduction

One of the most remarkable phenomenon in nature is the ability of proteins to fold from a polypeptide chain into structures which impart their functions as molecular machines of life. Proteins play a key role in all aspects of life, from cell cycle regulation to signal transduction.

Much of the critical regulation of biological activity within a cell is mediated by receptor-ligand interactions, often in the form of binding interactions between a receptor and an inhibitory ligand. REFs Furthermore, apoptosis (3) and autophagy (4), cellular mechanisms that are in place to prevent unregulated cell division, are also dependent on well-orchestrated receptor-ligand interactions. REFs

When these systems are dysregulated, the consequences can be devastating to the cell and/or organism. For example, disruptions to the regulation of cell cycle progression and proliferation lead to diseases such as cancer (1, 2). (Adapted from tummino and copeland 2008) It is not surprising that many diseases arise from the improper functioning of proteins, for example, when mutations occur or when they denature or misfold (fail to adopt their native functional state). Hence, about 40% of modern medicine targets the G protein-coupled receptors, a large protein family of receptors found in eukaryotes that sense molecules outside the cell and activate inside signal transduction pathways,

and ultimately cellular responses.

Since the historical experiment performed by Anfinsen and colleagues which demonstrated that the structure of a folded protein is encoded in its amino acid sequence and solvent environment, protein folding and structure determination have gained much attention in the fields of biochemistry and biophysics. The static models produced by NMR, X-ray crystallography, and homology modeling provide valuable insights into macromolecular structure, but molecular recognition and drug binding are very dynamic processes. When a small molecule like a drug (for example, a ligand) approaches its target (for example, a receptor) in solution, it encounters not a single, frozen structure, but rather a macromolecule in constant motion. Understanding both protein structure and dynamics enable us to elucidate the molecular basis of disease pathways and ultimately will aid in the discovery of novel therapeutics.

As ligand binding and the important macromolecular motions associated with it are microscopic events that take place in mere millionths of a second, a complete understanding of the atomistic energetics and mechanics of binding is unattainable using current experimental techniques. In recent years, molecular dynamics (MD) simulation of biomolecules, a physics-based computer simulation technique, became a tool of choice to investigate protein dynamics and function, and ligand-binding

Currently, MD simulations is the most accurate computational method for probing small-molecule binding, and are useful for filling in the details where experimental methods cannot.[11] In the past few years, MD simulations are able to routinely reach microseconds in sampling time. With the ever increase in the availability of computing power and data storage, simulations are a promising technique for aiding in the structure-based drug discovery process.

In recent years, a class of proteins without a uniquely folded state known as intrinsically disordered proteins (IDPs) have gained attention because of their involvement in a multitude of physiological pathways and diseases. For example, proteins associated

with cell signalling and cancer in humans are predicted to be enriched in protein disorder (Iakoucheva et al. 2002). In particular, 79% of cancer-associated proteins (Dunker et al. 2008a) and 60% of proteins associated with cardiovascular disease are predicted to contain contiguous regions of disorder longer than 30 residues (Uversky et al. 2009). A detail review of disordered proteins and their roles in biology is beyond the scope of this thesis and is provided elsewhere.[38,47]

A subset of IDPs under certain in vivo conditions are able to aggregate to form amyloid. Amyloid is known to be associated with incurable diseases such as prion disorders, neurodegenerative diseases, Type II diabetes, and systemic amyloidosis.

1.1 The amyloid state of proteins

150 years ago, amyloids were first discovered when tissue deposits of extracellular filaments were observed[15,43] These fibrillar deposits were microscopically visible deposits found on various organs in many seemingly unrelated diseases. It is now well-known that there are numerous diseases, many of which are neurodegenerative, involving the amyloid formation of a distinct aggregation-prone protein or peptides (Table **??**).

However, the ability of polypeptide chains to form amyloid is not restricted to the proteins associated with recognized clinical disorders. In particular, under certain in vitro solution conditions, even polypeptide chains capable of adopting well-defined tertiary structures were capable of forming amyloid fibrils (eg. myoglobin and lysozyme), suggesting that the amyloid state may be the globally-stable state for all polypeptides. Although the ability of proteins to form amyloid fibrils appear to be generic, the propensity for a given peptide is highly dependent on the formation condition, and vary for different sequences. For example, for globular protein to form amyloid, the protein must be first partly unfolded before conversion into amyloid fibrils is possible.

Kinetically, the mechanism of amyloid formation is akin to that of nucleation-polymerization

processes, such as crystallization. and micelle formation. During nucleation, a lag phase occurs where energetic barriers of aggregation must be overcome to form the initial aggregation nucleus. Following the lag phase, free monomers may bind to the nucleated aggregates and polymerize into mature fibrils.[29] Seeding, a process where preformed aggregates is introduced into solutions, may eliminate the lag phase.[?][?]

Amyloid fibrils are formed via a complex aggregation pathway in vivo where many intermediate species may be formed. Much of what we understand about amyloids comes from the use of in vitro models of amyloid-forming peptides. Monomers self-assemble into oligomers of different morphologies and sizes, which exist in equilibrium with amyloid fibrils, a visible endpoint of aggregation. Oligomers may either be on-pathway to fibril formation, that is, they serve only as intermediates, while others themselves may be the endpoints of aggregation.

In the sections below we review the structure of fibrillar and non-fibrillar amyloid oligomers that are common to all amyloid-forming polypeptides. Because much of the detailed biochemical and biophysical characterization of amyloid formation is centered upon the amyloid-beta peptide (implicated in Alzheimer's disease) and hence our discussion will be focused on this peptide.

1.1.1 Fibrils

Fibrillar amyloid deposits have several physical properties in common. Amyloid fibrils exhibit specific optical behavior upon binding certain dye molecules. After staining with Congo Red, amyloids exhibit bright green birefringence under polarized light. Fibrils are both protease resistant and are insoluble in the presence of sodium dodecyl sulfate (SDS).

Despite having dramatically different sequences, amyloid fibrils formed from different polypeptides all adopt a similar morphology known as the cross-β structure. To
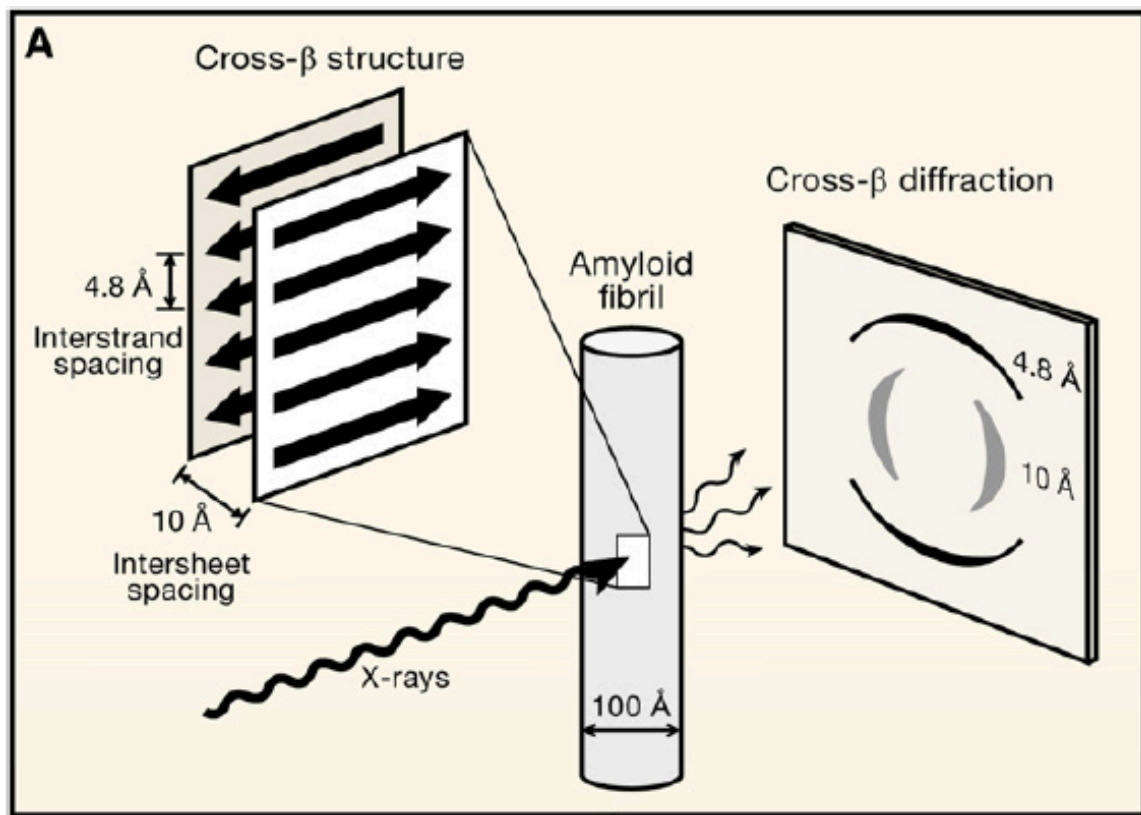
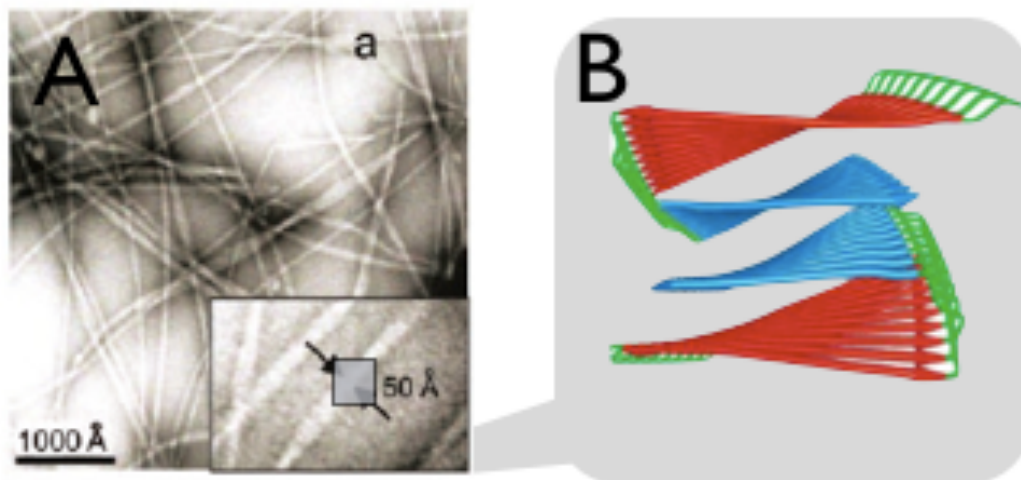Figure 1.1: This is adapted from Eisenberg, 2012

Figure 1.2: A Example EM images of oligomers. Adapted from Bitan G. et al. 2003 and Walsh D. 1999 C TEM image of fibrils D SSNMR model proposed by Tycko et al.

date, independent measurements of fibrillar structure from different instruments have all confirmed the presence of cross-β structure as the core structure of amyloid fibrils. **Initial structural studies, done using X-ray fiber diffraction, showed that fibril diffraction patterns are characterized by two major orthogonal reflections: meridional direction (along the fibril long axis) corresponding to a 4.8 Å interpeptide separation, and perpendicular to the fiber axis (equatorial direction) has a 10 Å intersheet spacing** (Figure 1.1). These defining measurements in fiber diffraction data have been adopted by biophysicists to be indicative of the presence of cross-β structure, and hence, of amyloid fibrils. Furthermore, under the transmission electron microscope (TEM), fibrillar structures are visible as long, unbranched, and ribbon-like structures with diameters between 50 - 100 nms (Figure 1.2).

Advances in solid-state NMR (SSNMR) and X-ray crystallography in the last decade have made major contributions to our knowledge of the molecular structure of amyloid fibrils. One of the early SSNMR model of an amyloid fibril was of the Aβ40 peptide, a protein implicated in Alzheimer's Disease. The study by Petkova et. al.[35] indicated
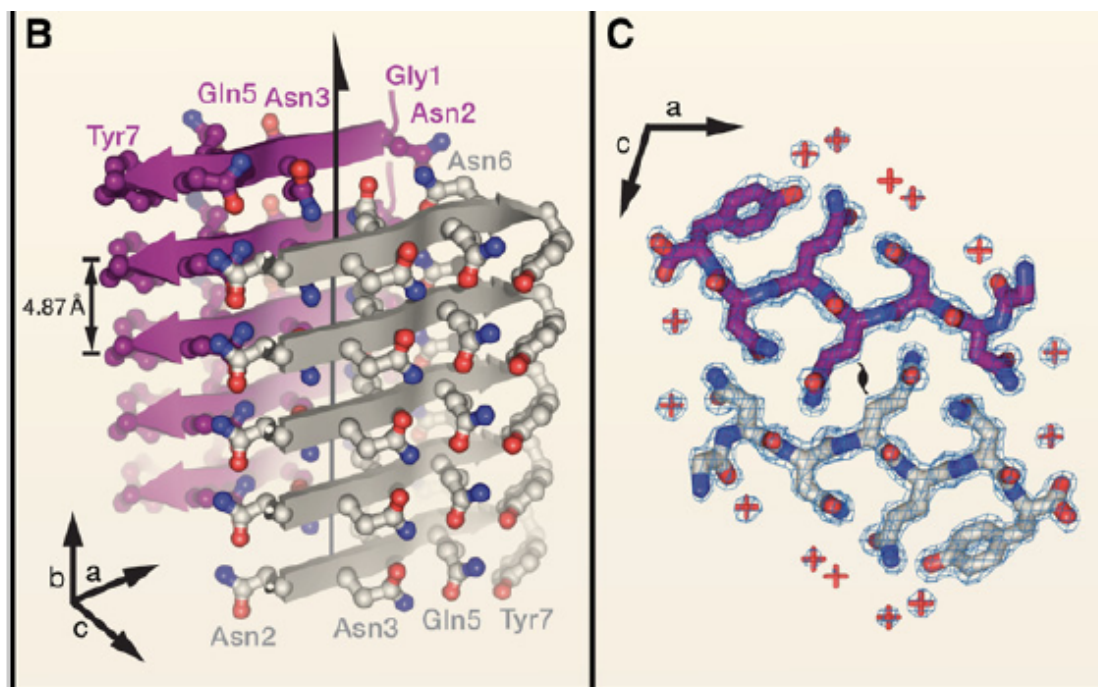
Figure 1.3: This is adapted from Eisenberg, 2012

that the β-sheet core of Aβ40 involves residues 10-22 and 30-40, and is linked by a loop formed by residues 23-29. The core fibril unit was found to consist of a parallel in-register β-sheet, where each strand is a β-hairpin with peptide-peptide backbone hydrogen-bond running along the long axis of the fibril (Figure 1.2).

Aggregates formed from many different small peptide fragments of larger amyloido-genic peptides, which were amenable to single crystal X-ray diffraction analysis, produced crystal structures similar in structure to those resolved using SSNMR. These structures are composed of multiple layers of β-sheet with a dehydrated ("dry") stacking interface (Figure 1.4). Taken together, these recently proposed structures demonstrate that the core region is composed of two to four sheets that interact closely with each other.

Although all fibrils share the cross-β structure, fibrils formed a peptide may exhibit polymorphism at the molecular level. Depending on the experimental conditions under which they are formed, fibrils may vary in the length of the β-strand involved, side chain orientation and inter-protofilament packing.[19] Fibril polymorphism may have important

implications in amyloid diseases because, depending on which residues are exposed at the surface, different morphologies may have differing fibril toxicities. For example, in vitro, quiescently formed fibrils of Aβ(1-40) have been shown to be more toxic than agitated fibrils.[34] A polymorphic structure introduced by a seed have been shown to be able to propagate in vitro.[32] A recent study propagated a brain-derived fibril fragment in order to obtain structural information on fibrils that most closely resembles those formed in the AD brain.[33]

1.1.2   Non-fibrillar oligomers

Due to their structural disorder and transient nature, obtaining the molecular details of amyloid oligomers using traditional structural determination techniques have been impeded. However, advances in instrumentation and experimental techniques in recent years have begun to shed light on the structure of non-fibrillar oligomers. Like fibrils, oligomers formed from disparate sequences appear to share similar overall morphologies.[18] EM and AFM experiments have shown that transient, unstable particles may appear prior to the formation of fibrils.[2,6,8] In particular, soluble Aβ protofibril assemblies that are annular, spherical, or curvilinear in shape have been reported in literature.[15] Protofibrils may also bind to dyes Thioflavin T (ThT) and Congo Red (CR), suggesting the presence of substantial β-sheet content.[15,19,49] Although these particles may be β-sheet-rich, they are often morphologically distinct and are typically much smaller than fibrillar structures (Figure ??).[51] However, it has been recently suggested that large non-fibrillar oligomers may contain fragments cross-β like in structure.[7,45,50]

1.2   Amyloid involvement in diseases

Because many diseases were found to have amyloid pathologies in common, it was initially hypothesized that fibrils found in amyloid plaques may be the toxic species in amyloid
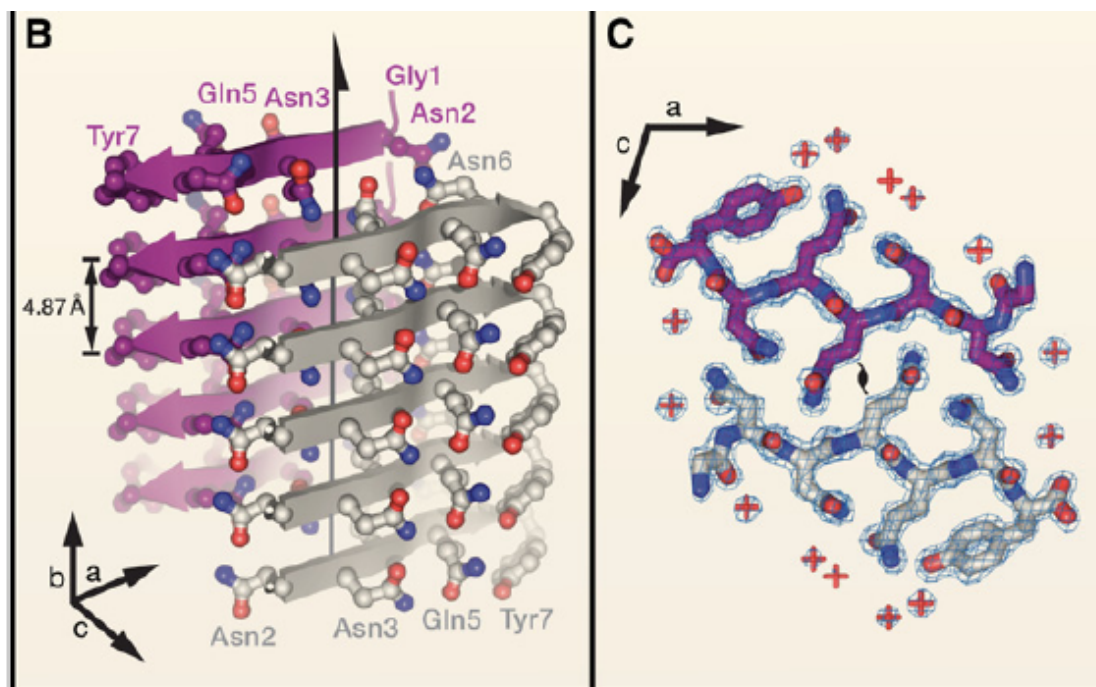
Figure 1.4: This is adapted from Eisenberg, 2012

disorders. However, recent research has implicated soluble oligomers to be the most likely causative agent for toxicity in several neurodegenerative diseases: Alzheimer's, Parkinson's, Huntington's, and Prion-related diseases.[15,53] Currently, the molecular mechanism of toxicity caused by amyloid oligomers is not understood, and is an area that is under intensive research.

Upon the addition of oligomers formed from $A\beta$ peptides, cells showed characteristic symptoms of neurotoxicity, leading to eventual apoptosis.[5,21,40,48] In addition, oligomeric species formed from short fragments of full length amyloidogenic peptides may also exhibit toxicity: for example, KLVFFAE or $A\beta$(16-22), a fragment of $A\beta$(1-42).[?] Furthermore, oligomers formed from a variety of peptides, including those that are not implicated in amyloid disorders (e.g. lysozyme, β2-microglobulin, transthyretin), suggesting that amyloid oligomers may have a generic mechanism of toxicity that is independent of the peptide sequence. Currently, it is thought that oligomers may cause toxicity by interacting with the cellular membrane, which may disrupt the ionic equilibrium leading
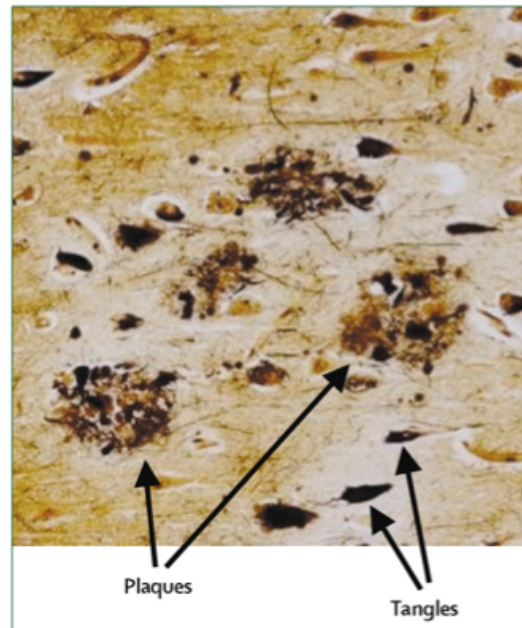
Figure 1: Plaques and tangles in the cerebral cortex in Alzheimer's disease
Plaques are extracellular deposits of Aβ surrounded by dystrophic neurites, reactive astrocytes, and microglia, whereas tangles are intracellular aggregates composed of a hyperphosphorylated form of the microtubule-associated protein tau.

Figure 1.5: This is adapted from Blennow, 2006

to cell death.[24,49] **Add more references of oligomer toxicity mechanisms**

1.3 Alzheimer's Disease

Alzheimer's Disease (AD) is a devastating neurodegenerative disease that is most common cause of dementia in persons of age 65 or older. Upon examination, the post-mortem brains of AD patients show significant neuronal dystrophy. Pathologically, AD is characterized by the presence of extracellular deposits of senile plaques and neurofibrillary tangles, which appear as lesions on stained neuronal tissue under light microscopy (Figure 1.5).

Although it has been more than one hundred years since Dr. Alois Alzheimer first presented the association between the presence of neuronal plaques and the clinical symptoms of presenile dementia characteristic of Alzheimer's disease (AD), the exact relation-
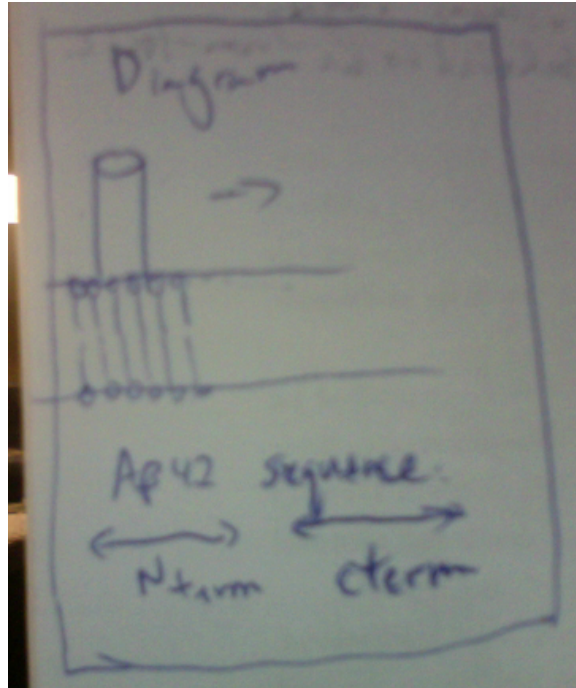
Figure 1.6: Production of Abeta from APP

ship between the two is still under much contention. It was not until in the 1980s, the amyloid-β protein or Aβ was identified as the largest component of these plaques.

1.3.1   The Aβ peptide

Monomeric Aβ is an approximately 4 kDa peptide produced by the intramembrane proteolytic cleavage of the larger amyloid-β precursor protein (APP). Aβ is produced constitutively as part of the normal cellular metabolism.[16] APP is sequentially processed by the aspartyl proteases β-secretase and γ-secretase, where depending on the position of the cleavage by γ-secretase, a pool of Aβ peptides of lengths varying from 38 to 43 residues are produced. The peptides spanning residues 1-40 (Aβ40) or 1-42 (Aβ42) are predominantly found in AD-associated plaques. Neuritic plaques is composed of mainly Aβ42, whereas Aβ40 is more commonly found in cerebral vascular plaques. REFS

The ubiquitous presence of amyloid plaque deposits found in the brains of deceased dementia patients led to the formulation of the long-standing amyloid hypothesis, which

posits that the amyloidogenesis of Aβ plays a key role in the initiation of AD.[16] Support for the amyloid hypothesis comes from genetic evidence, particularly in those with trisomy 21 (occurring in Down's syndrome), the chromosome responsible for encoding APP. Furthermore, in familial AD, genetic mutations on the APP, which is responsible for developing early-onset AD were also found to have increased aggregation propensities in vitro.

Multiple lines of evidence indicate that Aβ42 is likely to be the more deleterious form of Aβ. Genetic studies showed that mutations which cause early-onset AD also in turn increases the ratio of Aβ42 to Aβ40.[?] Moreover, in vitro, Aβ42 displays significantly higher propensity for aggregation than Aβ40, despite differing by only two amino acids. In addition, Aβ40 and Aβ42 also have distinct aggregation pathways in vitro: Aβ42 is found to form a morphologically more diverse population of intermediate oligomers than Aβ40.[3]

In addition to the presence of amyloid plaques, another hallmark lesion found in the brains of patients with AD is neurofibrillary tangles, which are composed of hyperphosphorylated forms of the microtubule-associated protein tau.[?] Although the roles of tau and its interactions with amyloid?? in the pathogenesis of AD are still being established, evidence from mouse models currently suggest that the role of NFTs in AD may be secondary to that of Aβ. In particular, Aβ production have been shown to drive the formation of tau pathologies: Abeta plaque pathology is not developed in a tau transgenic mouse model, whereas amyloid?β formation in APP transgenic mice causes hyperphosphorylation of tau.

A key question is what is causing AD? Aβ aggregates have been found to be present in a variety of morphologies in the brain. Although plaques are often visible in the dementia patients, the plaque load does not correlate with disease progression and severity, a puzzling aspect of AD. Instead, synaptic loss correlated well with the concentration of soluble Aβ oligomers in the brain. **MORE DETAILS ON THE CURRENT**

ACCEPTED MECHANISM OF TOXICITY

1.4 Amyloid Inhibition by small molecules: A promising method of treatment for AD

With the longevity of our population, AD is approaching epidemic proportions with no cure or preventative therapy available.[4] In 2010, there are an estimated 36 million people in the world currently suffering from AD, and this number is projected to grow to 115 million people by the year 2050.[1] Furthermore, there are no drugs which may target the underlying disease: approved treatments today such as donepezil (a cholinesterase inhibitor), and memantine (a N-methyl-D-aspartate antagonist) only mitigates cognitive symptoms.

Hence, it is imperative that efforts be made towards the development of therapeutics for AD. The vast number of structural and biochemical studies on amyloid structure have been crucial for the development of potential therapeutics for treating the underlying disease. A promising method of treatment of AD is by preventing amyloid aggregation, and decreasing amyloid production. Here is a paper summarizing current treatments for underlying disease[39]

A key pharmacological challenge of developing a drug for AD and other neurodegenerative diseases involves developing a small-molecule candidates, which also penetrate the blood-brain barrier (BBB) at sufficient concentrations for their therapeutic effects (ie. to inhibit amyloid formation).

In recent years, in vitro screening has led to the discovery of a large number of small-molecules which were found to affect the amyloid aggregation pathway. Some inhibited amyloid fibrils, whereas others arrested or reduced non-fibrillar oligomer formation. Many of these small molecules are thought to act by directly binding to amyloidogenic peptides and aggregates. The main classes of molecules that have demonstrated promise as
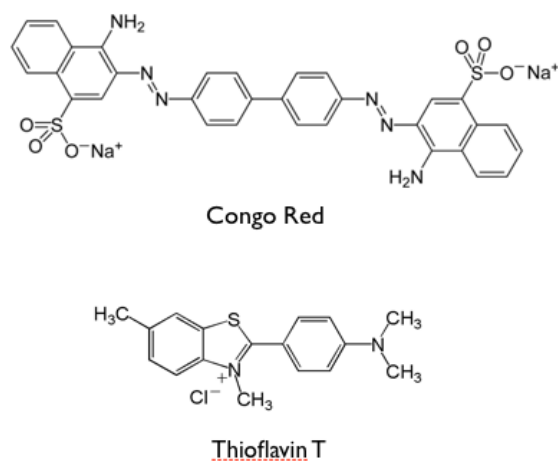
Figure 1.7: Amyloid binding dyes Congo Red and Thioflavin T

amyloid fibrillation inhibitors are reviewed in the sections below.

1.4.1 Dye molecules

Among the first molecules known to bind amyloid fibrils were dyes molecules used to identify their presence (Figure 1.7). Early histological detection of amyloid binding was done using congo red (CR), where upon binding with CR, fibrils exhibit red-green bire-fringence when viewed with polarized light.[?] The binding affinity of CR to various fibrils are estimated to be in the range of 0.1 - 1.5 micromolar. Putative binding modes of CR molecules with the amyloid fibril are most likely to be bound along the long axis of the fibril. Binding studies where chemical modifications to CR suggest that both ionic and hydrophobic interactions may be important for its binding as disruption of either can affect its binding affinity. Although CR is typically used to detect the presence of fibrillar aggregates, CR may also interact with non-fibrillar conformations such as monomers of alpha-synuclein, and alpha-helical form of poly-L-lysine.[?]

Early studies of amyloid using CR led to the finding that CR not only binds to fibrils, but also affect the amyloid aggregation pathway by interacting with one or more amyloidogenic species.[?] CR may inhibit and promote amyloid formation in a dosage-

dependent manner: Fibril formation of amyloidogenic proteins Abeta,[?] prion,[?] and immunoglobulin light chain variable domain (SMA)[?] were found to be promoted by the presence of CR at low molar ratios, and inhibited at high molar ratios. Furthermore, it can also self-aggregate in solution to form complexes, which may play a role in its binding mechanism to amyloid peptides and aggregates.[?] [?]

Although CR exhibits anti-amyloidogenic and anti-prion properties, it has low penetrability through the BBB and is carcinogenic, making it a poor therapeutic candidate. Therefore, efforts were applied to find CR-based analogues, which maintain their anti-fibrillar aggregation activities, but with improved toxicity profile and bioavailability. Chrysamine G is one such analogue of CR. **ADD: How is it different? Better binder? less toxic? Maybe just a sentence or two.**

The use of congo red to detect the presence of amyloid formation is often a laborious process, and only gives a qualitative measurement of the amount of amyloid present. Thioflavin-T (ThT) is a benzathiole fluorescent dye also used to detect the presence of amyloid fibrils in post-mortem brain tissue samples, and monitor fibril formation in vitro. ThT exhibits a dramatic shift in the excitation spectrum maximum and an emission enhancement upon binding to fibrils, making it a sensitive and efficient report for the presence of amyloid fibrils. ThT is soluble in water and have K_d in the low μM range: 0.033 to 23 micro molar.[?]

Due to the ubiquitous use of ThT as a amyloid fibril probe, fibril binding by ThT is well-characterized. Studies have suggested that ThT binds at the surface of the fibril, parallel to its long-axis, and may depend on the physiochemical properties of the amyloid fibril.[?] [?] [?] [?] More than one binding site with similar affinities for ThT may be present on fibrils. Moreover, ThT binding is not limited to fibrils, and was found to bind in hydrophobic pockets of human serum albumin, a globular protein, with comparable affinity to many drug-like molecules.[14][?]
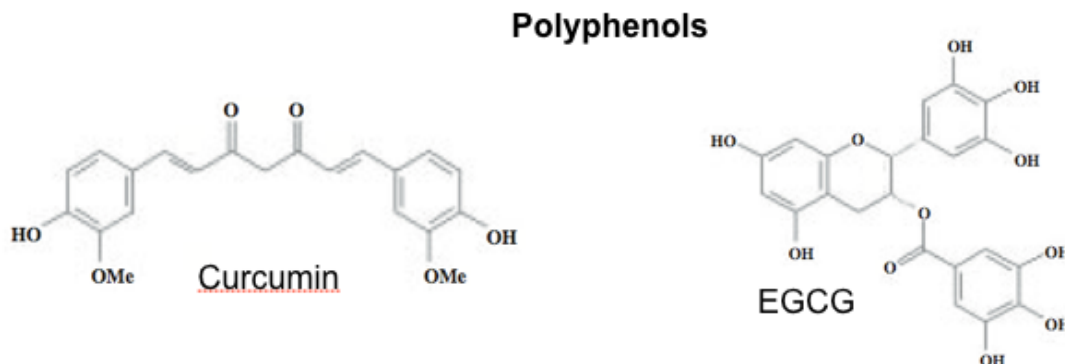
Figure 1.8: Polyphenols

1.4.2 Polyphenols

Polyphenols is a class of molecules found both naturally in plants. They can also be made synthetically. Recently experimental evidence suggests that three such molecules X, Y, Z modulate Aβ aggregation, and hence may be developed into therapeutics for Alzheimer's Disease and related neurodegenerative diseases. In this section, the discussion will be focused on these compounds and their role in fibril inhibition.

Most common subgroup of phenolic compounds are flavonoids, known for their anti-oxidant properties, were shown to modulate amyloid formation (Fig. 1.8). They have a high EC50 for the inhibition of fibrils. -epigallocatechin-3-gallate EGCG is the major polyphenolic component of green tea and A variety of experimental in vitro cell culture paradigms have shown micromolar concentrations of EGCG to be protective against Ab-induced cell death [Bastianetto et al., 2006; Kim et al., 2007; Levites et al., 2003].

Only begun to explore their molecular mechanism of binding [Do they have similar mechanism of action? What are their advantage and disadvantages as a therapeutic for AD?]

1.4.3 Non-steroidal Anti-inflammatory compounds (NSAIDs)
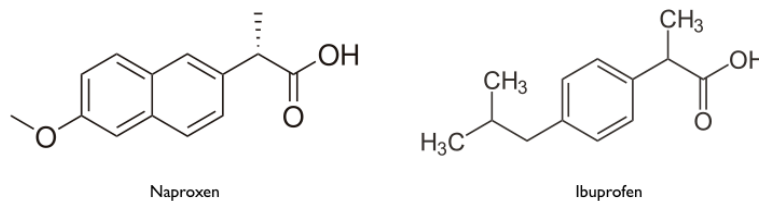
CUT THIS SECTION DOWN

Figure 1.9: NSAIDs

One of the potential candidates is a nonsteroidal anti-inflammatory drug (NSAID) naproxen and ibuprofen (1.9).? Epidemi- ological studies have shown that chronic pro-phylactic intake of naproxen moderately reduces the risk of AD.12,13 Furthermore, reex-amination of the results of large-scale clinical trials suggests that under certain conditions naproxen can reduce the AD risk by 67%.11

Biomedical studies suggest that treatment with ibuprofen reduces the amount of Ab deposits and alleviates memory deficits in mice models (17,18). Ibuprofen intake also correlates with a decrease in the amount of Ab oligomers in mice brain tissues (18). A prophylactic long-term use of ibuprofen appears to reduce the risk of AD (19), but the effectiveness of this drug against preexisting AD cases is unclear (20).

Several recent experimental studies have investigated the molecular aspects of inter-actions between Ab and ibuprofen. Binding of ibuprofen to Ab fibrils has been demon-strated when the ligand/peptide stoichiometric ratio approximates or exceeds 1 (21,22).

Experimental in vitro studies have shown that ibuprofen reduces accumulation of Ab fibrils by apparently interfering with fibril elongation (23). Furthermore, ibuprofen demon- strates an ability to at least partially dissociate preformed Ab fibrils (21,23).

1.4.4 Inositol

Inositol with the molecular formula of $C_6H_12O_6$, is a simple polyol with nine naturally occurring stereoisomers. Out of these nine isomers, seven are optically inactive, and the remaining two (L- and D-chiro-inositol) are chiral enantiomers.(Figure 1.10) Myo-
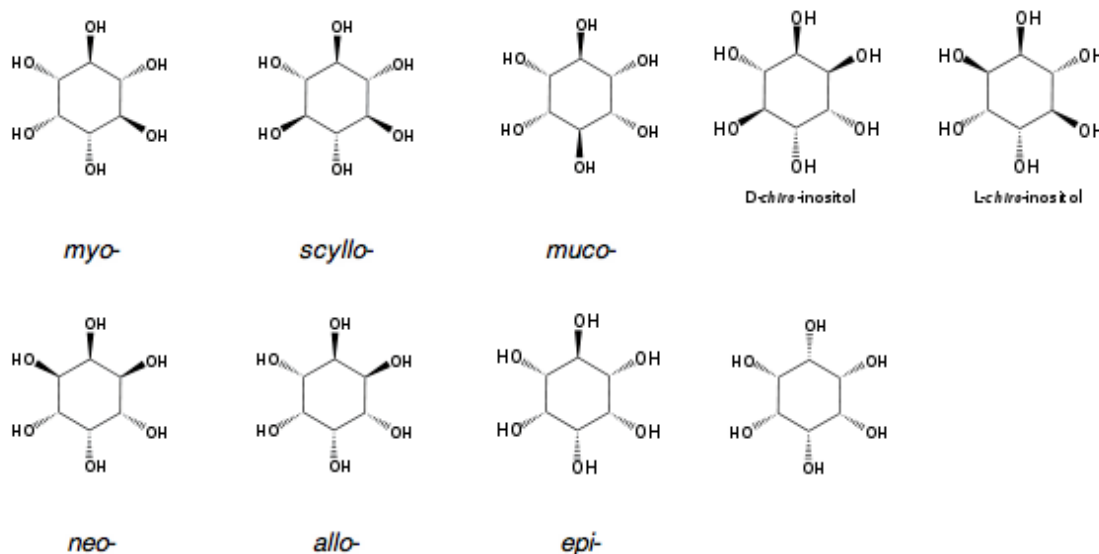
Figure 1.10: Inositol stereoisomers

inositol, the most abundant isomer, is ubiquitous in all eukaryotes and is a physiologically important osmolyte. Furthermore, myo- is a precursor for inositol lipid synthesis: It is a constituent of phosphatidylinositol, an important phospholipid in membranes and second messenger systems. Once phosphorylated, myo-inositol phosphatides act as second messengers in intracellular signal transduction pathways.[12] Some specific pathways which involves inositol are XXX ... YYY.[28]

Inositol is found in high concentrations in tissues of the human central nervous system (CNS): myo- And scyllo-inositol have approximate concentrations of 5 and 0.1-0.5 mM in the CNS, respectively.[12] Accordingly, inositols also function as osmolytes in the CNS, where alterations in their concentrations are known to be associated with neuropathological conditions.[12,27]

In recent years, scyllo-inositol have been identified as a promising therapeutic candidate for the treatment of Alzheimer's Disease. **ADD MORE DETAILS ON HOW IT WAS DISCOVERED. IT MUST SOMEWHERE IN JOANNES PAPERS** Moreover, inositol exhibits stereochemistry-specific effects on $A\beta$ fibril inhibition and cytotoxicity:[25] Scyllo-, myo-, and epi-, but not chiro-inositol, have been shown to in-

hibit Aβ42 fibril assembly, stabilize an oligomeric complex of Aβ42, and attenuate Aβ-oligomer-induced neurotoxicity in vitro.

In vivo studies with a transgenic mouse model of AD demonstrated that alleviation of symptoms after inositol treatment was correlated with a decrease in the levels of soluble Aβ oligomers, suggesting that the beneficial effects of scyllo-inositol may be attributed to the inhibition and/or disaggregation of high-order Aβ oligomers.[26] Presently, scyllo-inositol has completed both phase I and II of human clinical trials. Inositol was found to be non-toxic to healthy individuals at concentrations effective for amyloid inhibition. Taken together, these results suggest that scyllo-inositol, and its derivatives, are a potential therapy for AD with the ability to change the course of the disease.[30,46] A central focus of this thesis is on elucidating the molecular mechanism of inositol binding to amyloidogenic proteins and peptides (in particular Aβ). **ADD MORE DATA ON CLINICAL TRIALS**

1.4.5   Mechanism of action of small-molecule aggregation modulators

Some small molecules inhibit fibril formation, where as others may prevent oligomerization, but not fibrillation. A high concentration is often required to observe activity (micromolar to millimolar), which suggests that they may be non-specific, and have much weaker binding affinities compared to classical inhibitors of enzymes, which typically binds with nanomolar Kds. EGCG, one such polyphenol, is known to have the lowest IC50. These small molecule inhibitors which act on species often without a well-defined morphology such as those of globular proteins, do not fit entirely within the classical enzyme inhibition model. They are able to be active at much lower affinities, and do not bind in one specific binding pocket. Although the concentration of their activity is high compared with classical inhibitors, they are still below co-solvent and osmlarity levels, which suggests these small molecule inhibitors may affect aggregation by directly binding
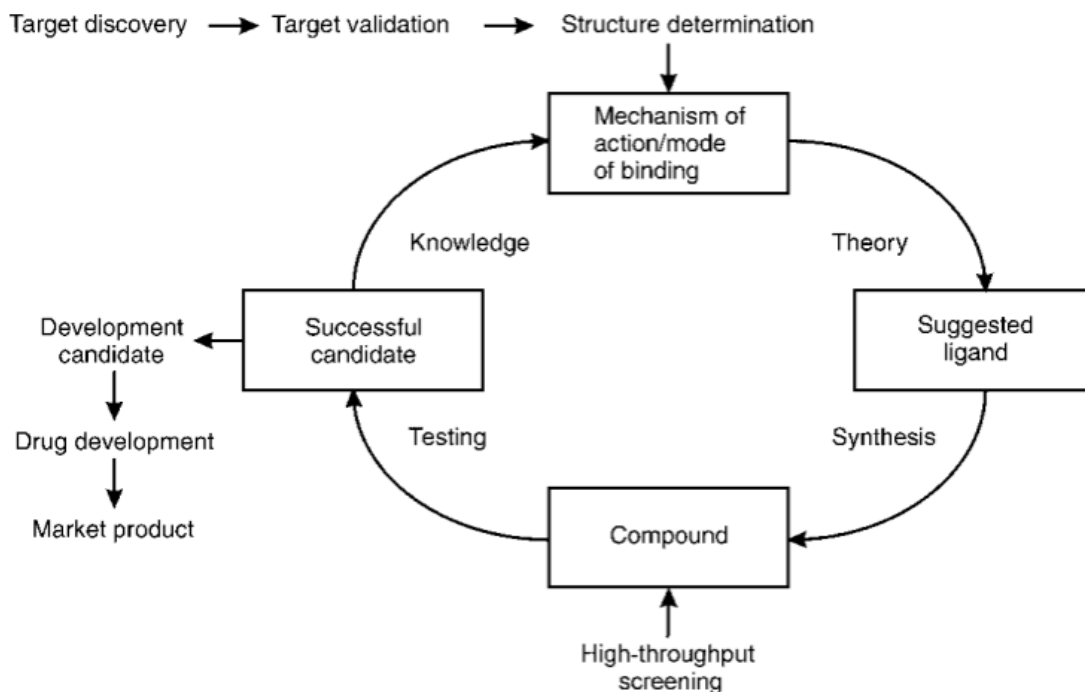
Figure 1. General approach for the rational design of inhibitors. Starting with discovered or previously synthesized compounds and biological testing, information about the mechanism of action or binding mode is used as a starting point for a subsequent design cycle assisted by computational methods.

Figure 1.11: Diagram explaining rational drug design adapted from[13]

to amyloidogenic peptides and / or aggregates.

What is the evidence for direct binding? NMR studies indicate direct binding activity for several small molecules.[?] . EGCG was found to directly bind to alpha-s and Abeta.

A single small molecule may inhibit the fibril formation of different peptides.

Small molecule inhibitors share common chemical features and groups. They are typically planar in geometry, have many aromatic rings, and polar functional groups (hydroxyl groups) around the edge of these aromatic rings.[22,36,41,44]

1.5 Rational drug design

Below is a summary of an excerpt from Tom's thesis on structure-based drug discovery.

Design of antibiotics example:

1) Target determination (biochemical)

2) Structural determination (Xray, NMR, or homology); active site identified; Here would be useful to get the holo structure of the protein

3) Screen for inhibitors against a chemical library or in silico docking.

(see Figure 1.11)

1.6 Protein-ligand binding

These references will help me in part flesh this section out. [9,10,20,23,31,37,42]

Kinetics and equilibria metrics used to characterize a lead compound. The in vitro interaction between a receptor and ligand is quantitatively assessed by equilibrium measures of binding affinity, such as IC50, the equilibrium dissociation constant or the Gibbs free energy of binding. These quantities are related to each other. Go on to discuss each of these in a little more detail: introduce terms such as EC50 or IC50

Binding kinetics is concerned with the rate constant of ligand association (k_{on}) and ligand dissociation (k_{off}). The ratio of the dissociation to the association rate constants establishes the equilibrium dissociation metric of the ligand (Kd = koff/kon), which determines the fraction of receptor occupancy at specific ligand concentrations;

In mechanism 1, the receptor (R) and ligand (L) combine to form a binary complex RL with association/dissociation rate con- stants k1 (kon) and k2 (koff), respectively (Eqn (1))

In mechanism 2, the ligand encounters the receptor (R) in a conformational state that is suboptimally complementary to the ligand for binding. Subsequent to the initial encounter (RL), the receptor undergoes a conformational change to a more committed

state (R*) where the new binary complex (R * L) competent binding affinity than RL (Eqn (2)). Two equilibrium dissociation constants are required to describe this so-called 'induced fit' mechanism – do I really care about this?

1.6.1 Binding equilibria

The dissociation constant, K_d, is a measure of the affinity of a ligand for its binding site on the host protein. Pharmacologically, it can be interpreted as the concentration at which 50% of the drug is bound to the protein. In experimental studies, K_d is used as a measurement of drug potency and is used to quantitatively screen for potential drug candidates. A low K_d indicates that the ligand is tightly bound (high affinity binding) to its binding site on the protein. Enzyme and its putative ligand typically bind specifically high affinity binding. Rational drug design is often applied to optimize ligand binding specificity in an effort to increase the efficacy of the putative drug, and decrease adverse side effects (toxicity) in the human body.

The chemical equilibrium for the binding reaction is given by,

$$[Protein \cdot Inositol] \rightleftharpoons [Protein] + [Inositol] \tag{1.1}$$
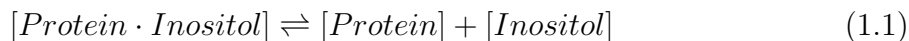
The binding free energy of a ligand to a protein is directly related to its dissociation constant, K_d, the equilibrium constant of the above reaction

$$K_d = f_{ub} \frac{[Protein] \, [Inositol]}{[Protein \cdot Inositol]}, \tag{1.2}$$

$$K_d = e^{\frac{-\Delta G}{RT}} \tag{1.3}$$

$$\Delta G = -RT \ln K_d \tag{1.4}$$

Inhibitors of enzymes bind with affinities typically in the nanomolar to micromolar range. For example, XXX (where can I find some examples?) However, a class of protein-ligand binding interaction, where a high K_d (weak affinity binding) is found are protein that bind carbohydrates is one such class of proteins, where weak interactions (low affinities in the millimolar range) between carbohydrates enable their function.

1.6.2   Multivalency in binding

Above describes the quantitative measure for a ligand binding to a single specific binding site on its target. However, there are cases where a ligand can binding in several binding sites at once, where the a single interaction of the ligand with the receptor site is fairly weak, but the ligand achieves its specificity of binding via forming multiple interactions. In this case, we talk about a binding avidity.

1.6.3   Intermolecular forces involved in binding

In protein-ligand binding and recognition non-covalent interactions are important. These interactions may be roughly divided into two types: hydrophobic or electrostatic.

Nonpolar molecules experience London dispersion forces, which are a form of weakly attractive intermolecular force. **Define these forces**

Hydrogen bonding is a type of interaction important in biology, and in particular, for molecular recognition contributing to both protein-ligand affinity and specificity. Furthermore, hydrogen bonding is ubiquitous in water, where a single water molecule is able to tetrahedrally coordinate four other water molecules by forming a hydrogen bonding network, and gives rise to its unique properties such as high heat capacity.

Proteins can form hydrogen bonds with other proteins, the surrounding solvent, carbohydrates, and lipids. In proteins, intra- and intermolecular hydrogen bonds formed by the peptidic backbone of polypeptide chains define their secondary structures such as helices and β-sheets and impart stability to a protein's overall tertiary structure.[?]

Large role in amyloid formation, where the polypeptide backbone are hydrogen bonded to form in-registered β-sheets (as reviewed in Section 1.1).

Hydrogen bonds are electrostatic interactions formed between two dipoles, where the acceptor group is composed of an electronegative heavy atom such as F, Cl, N, O, and the donor group is composed of electropositive atom with an attached proton (CHECK THIS XXX). In proteins, hydrogen bonds between peptide groups involves N-H and O=C as the donor and acceptor groups, respectively. Furthermore, amino acids with polar or charged side chains are also able to hydrogen bond.

Energetically, hydrogen bonds can range from XXX to YYY kcal/mol, and are typically much stronger than van Der Waals forces. The energy of N-H ... C=O hydrogen bond has been estimated to be XXX kcal/mol in gas phase, but is only about 0.5 to 1.5 kcal/mol in solution.[?] The energy of a water-water hydrogen bond is about -3.2 kcal/mol.[?] Furthermore, hydrogen bonds have directionality.

[INSERT FIGURE OF GEOMETRY OF HYDROGEN BONDS]

In a molecular mechanics forcefield, the energetics of hydrogen bonds are accounted for implicitly via the electrostatic and Lennard-Jones potentials. (geometry of hydrogen bond here measured experimentally as well?) In simulation, hydrogen bonds are typically counted using a geometric criteria.

[Describe the geometry of a hydrogen bond used in simulations]

Methods used for studying protein-protein and protein-ligand interactions.[52] This paper[11] has a nice picture of the thermodynamic cycle which I think I shall discuss in parts of this theses in methods.

1.7   Protein-carbohydrate

1.7.1   Binding modes

Here I will describe general features of protein-carbohydrate binding modes. Binding is typically specific to the stereochemistry of the sugar molecule, whether if its a sugar oligosaccharride or a monosaccharride. Atomic features of protein-carbohydrate interactions are aromatic stacking and hydrogen bonding.[?] At a sugar binding site many aromatic residues, and charged residues such as glu and asp may be found. A monosaccharide often packs "face-to-face" against the planar nonpolar aromatic residues at binding sites. An example is depicted in 1.12. The hydroxyl groups provide specificity to the binding. Depending on the residue at the binding site, they act as both hydrogen bonds donors and acceptors. Taken together both hydrogen bonding and van der waals interactions stabilize the sugar-protein complex.

Examples of sugar protein binding. L-arabinose.

Lectins are proteins which are known to bind monosaccharrides carbohydrates. Lectin structure[?]

Molecular simulations of carbohydrates and protein-carbohydrate interactions: motivation, issues and prospects[?]

This paper has a pretty good summary of the drug discovery process in the context of CNS. It also has summaries of the experimental techniques used to measure binding affinity[17]

1.8   Thesis objectives and rationale

[Challenges of developing small molecule for amyloid inhibition] Structure-based drug discovery approach developed to target folded proteins such as enzymes cannot be directly applied to discovery of small molecules which targets amyloid formation. Because the
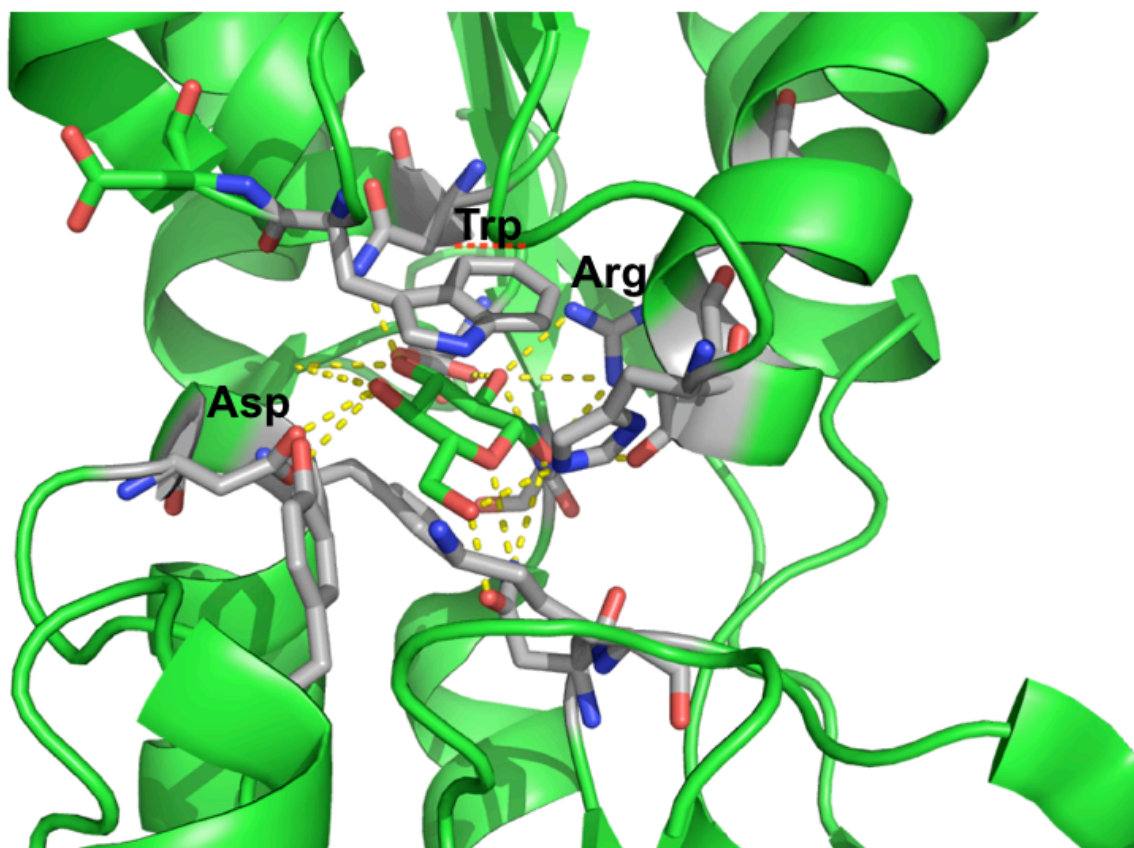
Figure 1.12: Example of sugar-lectin mode: glucose binding to galactose chemoreceptor protein (Ref: Vyas, N. et al, 1991; Rendered from PDB ID: 2GP)

Aβ amyloid aggregate pathway encompasses a variety of species, some of which are disordered, a single conformation cannot be assumed for binding. Furthermore, structural information of amyloidogenic species lags behind those of enzymes, which tends to be globular proteins amenable for X-ray crystallography. This means that the putative binding sites are not known. Furthermore, amyloid inhibitors are found to be very weak binders. In AD, there is the added challenge of the drug being able to cross the brain barrier, while remaining non-neurotoxic. What kind of drugs cross the BBB? Typically hydrophobic drugs.

We don't know if a small-molecule like inositol acts by binding. if ligands bind, and if they bind, we do not know where they bind, and it is experimentally challenging to identify the binding sites using available techniques such as SSNMR, NMR, and X-ray. Although there are some data other there with experimental data, we cannot yet obtain fully atomistic detail on the interactions between the ligands and proteins.

Because amyloid is a group of different species, where the structures of which are largely unknown, we don't know (and we cannot assume) which aggregate species along the amyloid aggregation pathway that inositol may interact with. We want to examine representative species within the aggregation pathway, that is, monomers, disordered oligomers, and fibril like aggregates. Furthermore, we cannot assume putative binding sites - that is these binding sites are not known a priori. Unlike the case with enzymatic inhibition, a single bound molecule is unlikely to inhibit fibrillation. Instead, amyloid aggregates such as fibrils present surfaces for binding.

Although amyloid fibrils of different peptide sequences share the overall cross-beta, there are specific differences in their surface properties due to their amino acid composition. Hence, we look at different species of different peptides.

We took a reductionist approach to solve this problem. Beginning with the simplest model systems for an amyloidogenic peptide, the alanine dipeptide, we systematically examine binding of inositol with systems of both increasing sequence and structural
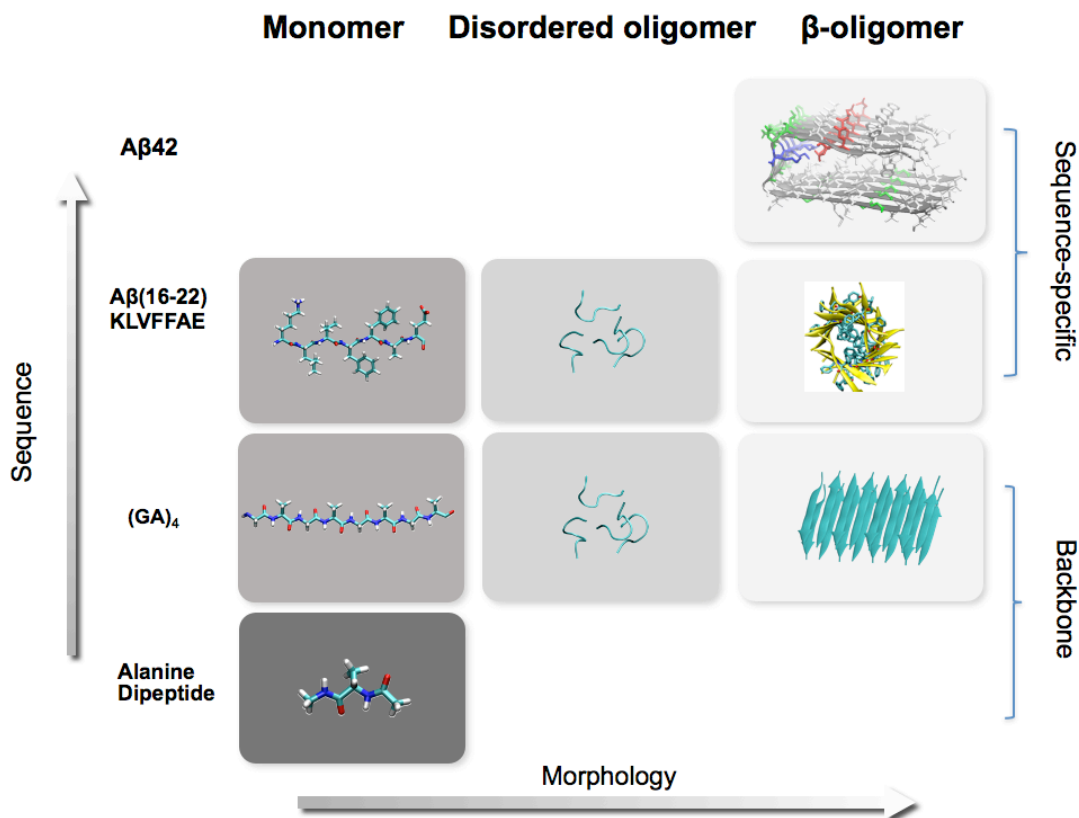
Figure 1.13: Shows the progression from small, model systems to larger and structurally more complex systems involving the full-length Aβ42 peptide.

complexity.

We wanted to isolate the problem to something simple to examine the backbone binding mechanism of inositol. Because inositol had many hydrogen bonding groups, and it is shaped like it is geometrically suited to bind the peptidic backbone. Why did we first choose to look at the backbone? Because it is the common element in all polypeptides, and the common cross-beta where the backbone of polypeptide chains are organized in the same manner in the structure of amyloid fibrils suggests that backbone plays an important role in amyloid formation. We examine aggregates of varying sequence and structural complexity. By examining a simple beta-sheet forming peptide, (GA)4, an amyloid inhibitor may bind to a simple amyloidogenic peptide. An illustration of this

approach is shown in Figure 1.13.

1.8.1   Study Design

Simulation challenge - The structural disorder of the peptides involved poses a challenge for obtaining converged properties from MD simulations. To date, few studies have attempted to provide statistically meaningful results pertaining to general mechanisms of protein self-aggregation and amyloid formation. Furthermore, despite the abundance of MD studies of Aβ, few studies have systematically examined the mechanism of action of small molecule inhibitors of amyloids

For sampling, we exploit conventional MD simulation techniques because simulation approaches used for understanding enzyme-ligand binding is not applicable. Conventional MD simulations were combined with many repeated independent replicas to obtain statistics on the binding equilibria. We use many repeat simulations - why ? We can observe binding and unbinding events within several nanoseconds. This is an approach that is typically used to trivially parallelizing simulations to speed up sampling. Because inositol acts at higher than normal (what do you mean by this) concentrations (in vitro), we were able to approach this problem using a "bulk" approach, by examining binding between multiple molecules of inositol with different amyloid aggregates. Inositol's interactions are weak (but I shouldn't be stating this as an assumption.. because 90

1.8.2   Hypotheses

The central hypotheses of the research proposal are as follows: (1) inositol exerts its effects through direct binding to one or more of the monomeric, aggregated, or fibrillar forms of amyloidogenic peptides and proteins; and (2) binding involves hydrogen bonding interactions with the polypeptidic backbone.

Two important lines of evidence support the above hypotheses. First, preliminary experimental data from the McLaurin lab are showing that in addition to Aβ42, inositol

can also inhibit superoxide dismutase (an amyloidogenic protein involved in amyotrophic lateral sclerosis), α-synuclein (Parkinson's disease), and poly-Q (Huntington's disease) amyloid assemblies in a similar manner to Aβ42 (J. McLaurin unpublished data). Since all amyloid structures have the cross β-sheet structure in common, their findings suggest that inositol is capable of binding the polypeptide backbone. Secondly, our own systematic MD study of inositol with alanine dipeptide, a model of the peptidic backbone, has demonstrated that inositol binds to the peptidic backbone through hydrogen bonding interactions (See Section 5.1 below).

1.8.3 Rationale and Specific Aims

1.8.4 Rationale

Multiple sources of experimental evidence have demonstrated the existence of species that are morphologically distinct from fibrils in the amyloid aggregation pathway.[6, 13, 29] At least two intermediates on pathway to fibril formation have been observed: oligomers and protofibrils. These species together with the monomer and fibrils, constitute a possible pathway by which Aβ aggregates to form fibrils. In order to characterize the molecular mechanism of action of inositol, it is imperative that the interaction of inositol with various aggregate species be examined. To this end, I will carry out systematic comparative studies of amyloidogenic peptides and their aggregates of increasing sequence and composition complexity and characterize the respective role of specific interactions of inositol with the backbone and sidechains. Inositols have been shown to have stereochemistry-dependent activity in vitro and in vivo.[18, 20] Chiro-inositol is a stereoisomer that was shown to be inactive in inhibiting amyloid formation. Thus, I will comparatively study chiro- and scyllo-inositol with each of the aggregates in the pathway to determine the stereochemical basis of the activity of inositol.

Specific Aim 1: To determine the effect of inositol on the structure and thermody-

namics of the self-aggregation of different amyloidogenic peptides

Specific Aim 2: To determine the effect of inositol on the kinetics of aggregation

1.8.5   Choice of Model Systems

Following the approach of studying systems of increasing complexity, we have chosen the following peptides and sequences: (1) Alanine Dipeptide; (2) (GA)4; (3) KLVFFAE; (4) SNNFGAIL; (5) Aβ40/42. Amyloid-forming proteins involved in disease, such as Aβ, Tau (Alzheimer's) or hIAPP (diabetes), are often many residues in length and high in sequence complexity, which renders the collection of meaningful statistics from large-scale computer simulations of these protein aggregates difficult. However, many of these longer sequences have been found to have shorter segments that preserve the amyloidogenicity and cytotoxicity of the longer parent protein.[30, 31] These shorter peptides not only have been shown to retain the physiochemical properties for studying general mechanisms of amyloid formation experimentally, but also are much simpler systems suitable for detailed computational studies.

4.4 Alanine Dipeptide Alanine dipeptide (N-acetyl-L-alanine-N'-methylamide or AcAlaNHMe) is a minimalist model of the peptidic backbone with its two backbone dihedral angles (φ,ψ) as its significant degrees of freedom. The dominant conformations of its conformational equilibrium span all possible dihedral angles representative of α-helices and β-sheets in (Figure 1A). Due to the simplicity of alanine dipeptide, all relevant local peptidic backbone binding modes of inositol can be explored efficiently computationally.

4.5 GAGAGAGA An octamer with repetitive sequence involving only nonpolar side-chains, (GA)4 is the simplest amyloidogenic peptide[32] of relevance to study the interaction of inositol with the peptidic backbone. GA is a commonly occurring sequence motif in the β-sheet forming (crystalline) domains of a variety of spider silks.[33] The tetramer GAGA is a part of the B. Mori silk fibroin, which is found to contain β-sheet structure.[34] The repetitive nature of (GA)4 and its low sequence complexity allow simulations to be

computational tractable and thus, permits timescales needed to determine the effects of inositol on amyloid aggregation in a statistically meaningful way. Finally, the simplicity of (GA)4 allows the detailed examination and dissection of the different possible types of inositol-peptide interactions.

4.6 KLVFFAE The peptide KLVFFAE, a segment spanning residues 16 to 22 of Aβ1-40 and Aβ1-42, has been well studied both experimentally and computationally.35-38 KLVFFAE is the central hydrophobic core thought to be important in full-length Aβ assembly and is affected by four AD causing mutations: A31G (Flemish), E22Q (Dutch), E22K (Italian), and E22G (Arctic). This peptide not only contains side chains with more bulk, but also those capable of forming charged and aromatic interactions. The protofibrillar structure has been shown by SSNMR to have anti-parallel β-sheet organization with possible parallel stacking of the β-sheets.36

4.8 Full length Aβ40 or Aβ42 Inositol does not necessarily act on the shorter peptide models and their aggregates. To address this possibility, I will study inositol with the Aβ peptide and aggregates. The monomeric, oligomeric, and fibrillar structures of Aβ40 and Aβ42 have been studied both experimentally and computationally.4, 16, 40-45 Thus, I will utilize the structural data available to construct oligomeric and fibrillar species and comparatively analyze the interaction of chiro- and scyllo-inositol with these species.

1.9   Thesis Organization

The first chapter introduces the thesis in the context of the field. The second chapter introduces the main methods used in the work in the thesis. Chapters 3, 4, and 5 are the results of simulations of inositol with amyloid like peptides and aggregates. Chapter 6 shows work of the general applicability of our methods developed throughout this thesis to MD simulations to understand protein - carbohydrate binding. Chapter 7 provides discussion, suggestions for future work, and perspectives.

Bibliography

[1] 2012 alzheimer's disease facts and figures. *Alzheimer's & dementia : the journal of the Alzheimer's Association*, 8(2):131–168, 03 2012.

[2] Mahiuddin Ahmed, Judianne Davis, Darryl Aucoin, Takeshi Sato, Shivani Ahuja, Saburo Aimoto, James I Elliott, William E van Nostrand, and Steven O Smith. Structural conversion of neurotoxic amyloid-β1–42 oligomers to fibrils. *Nat Struct Mol Biol*, pages 1–8, November 2010.

[3] G Bitan, MD Kirkitadze, A Lomakin, SS Vollers, GB Benedek, and DB Teplow. Amyloid β-protein (Aβ) assembly: Aβ40 and Aβ42 oligomerize through distinct pathways. *Proceedings of the National Academy of Science*, 100:330–335, 2003.

[4] Kaj Blennow, Mony J De Leon, and Henrik Zetterberg. Alzheimer's disease. *Seminar*, 368:387–403, 2006.

[5] Roberto Cappai and Kevin J Barnham. Delineating the mechanism of Alzheimer's disease A beta peptide neurotoxicity. *Neurochem Res*, 33(3):526–532, August 2007.

[6] Byron Caughey and Peter T Lansbury. Protofibrils, pores, fibrils, and neurodegeneration: separating the responsible protein aggregates from the innocent bystanders. *Annual review of neuroscience*, 26:267–298, 2003.

[7] Sandra Chimon, Medhat A Shaibat, Christopher R Jones, Diana C Calero, Buzulagu Aizezi, and Yoshitaka Ishii. Evidence of fibril-like b-sheet structures in a neurotoxic

amyloid intermediate of Alzheimer ' s b-amyloid. *Nat Struct Mol Biol*, 14(12):1157–1164, 2007.

[8] Brett A Chromy, Richard J Nowak, Mary P Lambert, Kirsten L Viola, Lei Chang, Pauline T Velasco, Bryan W Jones, Sara J Fernandez, Pascale N Lacor, Peleg Horowitz, Caleb E Finch, Grant A Krafft, and William L Klein. Self-assembly of Abeta(1-42) into globular neurotoxins. *Biochemistry*, 42(44):12749–12760, November 2003.

[9] Robert A Copeland. Conformational adaptation in drug–target interactions and residence time. *Future Medicinal Chemistry*, 3(12):1491–1501, September 2011.

[10] Robert A Copeland, David L Pompliano, and Thomas D Meek. Drug-target residence time and its implications for lead optimization. *Nat Rev Drug Discov*, 5(9):730–739, September 2006.

[11] Jacob D Durrant and J Andrew McCammon. Molecular dynamics simulations and drug discovery. *BMC Biol*, 9(1):71, 2011.

[12] SK Fisher, JE Novak, and BW Agranoff. Inositol and higher inositol phosphates in neural tissues: homeostasis, metabolism and functional significance. *J Neurochem*, 82(4):736–754, 2002.

[13] Holger Gohlke and Gerhard Klebe. Approaches to the description and prediction of the binding affinity of small-molecule ligands to macromolecular receptors. *Angew Chem Int Ed Engl*, 41(15):2644–2676, August 2002.

[14] Minna Groenning, Lars Olsen, Marco van de Weert, James M Flink, Sven Frokjaer, and Flemming S Jørgensen. Study on the binding of Thioflavin T to beta-sheet-rich and non-beta-sheet cavities. *J Struct Biol*, 158(3):358–369, June 2007.

[15] Christian Haass and Dennis J Selkoe. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. *Nat Rev Mol Cell Biol*, 8(2):101–112, February 2007.

[16] John Hardy and Dennis J Selkoe. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science*, 297(5580):353–356, July 2002.

[17] Roderick E Hubbard. Structure-based drug discovery and protein targets in the CNS. *Neuropharmacology*, 60(1):7–23, January 2011.

[18] Rakez Kayed, Elizabeth Head, Jennifer L Thompson, Theresa M McIntire, Saskia C Milton, Carl W Cotman, and Charles G Glabe. Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science (New York, N.Y.)*, 300(5618):486–489, 2003.

[19] Ravindra Kodali and Ronald Wetzel. Polymorphism in the intermediates and products of amyloid assembly. *Curr Opin Struct Biol*, 17(1):48–57, 2007.

[20] Sandhya Kortagere and Sean Ekins. Troubleshooting computational methods in drug discovery. *Journal of Pharmacological and Toxicological Methods*, 61(2):67–75, March 2010.

[21] M Lambert, A Barlow, B Chromy, C Edwards, R Freed, M Liosatos, T Morgan, I Rozovsky, B Trommer, K Viola, P Wals, C Zhang, C Finch, G Krafft, and W Klein. Diffusible, nonfibrillar ligands derived from $A\beta 1$-42 are potent central nervous system neurotoxins. *Proc Natl Acad Sci USA*, 95(11):6448–6453, June 1998.

[22] Justin A Lemkul and David R Bevan. The Role of Molecular Simulations in the Development of Inhibitors of Amyloid β-Peptide Aggregation for the Treatment of Alzheimer's Disease. *ACS Chem. Neurosci.*, 3(11):845–856, November 2012.

[23] Hao Lu and Peter J Tonge. Drug-target residence time: critical information for lead optimization. *Curr Opin Chem Biol*, 14(4):467–474, August 2010.

[24] Ivo Cristiano Martins, Inna Kuperstein, Hannah Wilkinson, Elke Maes, Mieke Vanbrabant, Wim Jonckheere, Patrick Van Gelder, Dieter Hartmann, Rudi D Hooge, Bart De Strooper, Joost Schymkowitz, and Frederic Rousseau. Lipids revert inert Ab amyloid fibrils to neurotoxic protofibrils that affect learning in mice. *EMBO JOURNAL*, 27(1):224–233, 2008.

[25] J McLaurin, R Golomb, A Jurewicz, J Antel, and P Fraser. Inositol stereoisomers stabilize an oligomeric aggregate of Alzheimer amyloid β peptide and inhibit Aβ-induced toxicity. *J Biol Chem*, 275(24):18495–18502, July 2000.

[26] JoAnne McLaurin, Meredith E Kierstead, Mary E Brown, Cheryl A Hawkes, Mark H L Lambermon, Amie L Phinney, Audrey A Darabie, Julian E Cousins, Janet E French, Melissa F Lan, Fusheng Chen, Sydney S N Wong, Howard T J Mount, Paul E Fraser, David Westaway, and Peter St George-Hyslop. Cyclohexanehexol inhibitors of Aβ aggregation prevent and reverse Alzheimer phenotype in a mouse model. *Nat Med*, 12(7):801–808, July 2006.

[27] Thomas Michaelis, Gunther Helms, Klaus-Dietmar Merboldt, Wolfgang Hänicke, Harald Bruhn, and Jens Frahm. Identification of scyllo-inositol in proton NMR spectra of human brain in vivo. *NMR Biomed.*, 6(1):105–109, January 1993.

[28] Robert H Michell. Inositol derivatives: evolution and functions. *Nat Rev Mol Cell Biol*, 9(2):151–161, February 2008.

[29] Regina M Murphy. Peptide aggregation in neurodegenerative disease. *Annu Rev Biomed Eng*, 4:155–174, 2002.

[30] Mark Nitz, Daniela Fenili, Audrey A Darabie, Ling Wu, Julian E Cousins, and

JoAnne McLaurin. Modulation of amyloid-β aggregation and toxicity by inosose stereoisomers. *FEBS J*, 275(8):1663–1674, April 2008.

[31] Sara Núñez, Jennifer Venhorst, and Chris G Kruse. Target–drug interactions: first principles and their application to drug discovery. *Drug Discov Today*, 17(1-2):10–22, January 2012.

[32] Anant K Paravastu, Aneta T Petkova, and Robert Tycko. Polymorphic Fibril Formation by Residues 10–40 of the Alzheimer's β-Amyloid Peptide. *Biophys J*, 90(12):4618–4629, June 2006.

[33] Anant K Paravastu, Isam Qahwash, Richard D Leapman, Stephen C Meredith, and Robert Tycko. Seeded growth of beta-amyloid fibrils from Alzheimer's brain-derived fibrils produces a distinct fibril structure. *Proc Natl Acad Sci USA*, 106(18):7443–7448, May 2009.

[34] Aneta T Petkova, Richard D Leapman, Zhihong Guo, Wai-Ming Yau, Mark P Mattson, and Robert Tycko. Self-propagating, molecular-level polymorphism in Alzheimer's beta-amyloid fibrils. *Science*, 307(5707):262–265, January 2005.

[35] Aneta T Petkova, Wai-Ming Yau, and Robert Tycko. Experimental constraints on quaternary structure in Alzheimer's β-amyloid fibrils. *Biochemistry*, 45(2):498–512, January 2006.

[36] Yair Porat, Adel Abramowitz, and Ehud Gazit. Inhibition of amyloid fibril formation by polyphenols: structural similarity and aromatic interactions as a common inhibition mechanism. *Chem Biol Drug Des*, 67(1):27–37, January 2006.

[37] Heino Prinz and André Schönichen. Transient binding patches: a plausible concept for drug binding. *J Chem Biol*, 1(1-4):95–104, September 2008.

[38] Sarah Rauscher and Régis Pomès. Molecular simulations of protein disorder. *Biochem Cell Biol*, 88(2):269–290, April 2010.

[39] Salvatore Salomone, Filippo Caraci, Gian Marco Leggio, Julia Fedotova, and Filippo Drago. New pharmacological strategies for treatment of Alzheimer's disease: focus on disease modifying drugs. *British Journal of Clinical Pharmacology*, 73(4):504–517, March 2012.

[40] Ganesh M Shankar, Shaomin Li, Tapan H Mehta, Amaya Garcia-Munoz, Nina E Shepardson, Imelda Smith, Francesca M Brett, Michael A Farrell, Michael J Rowan, Cynthia A Lemere, Ciaran M Regan, Dominic M Walsh, Bernardo L Sabatini, and Dennis J Selkoe. Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med*, 14(8):837–842, August 2008.

[41] Hila Shoval, Dov Lichtenberg, and Ehud Gazit. The molecular mechanisms of the anti-amyloid effects of phenols. *Amyloid*, 14(1):73–87, March 2007.

[42] DMN Sikazwe. The Multi-Target Drug Design Era is Here, Consider it. *Drug Design*, 1:e101, 2012.

[43] J D Sipe and A S Cohen. Review: history of the amyloid fibril. *J Struct Biol*, 130(2-3):88–98, June 2000.

[44] Shiri Stempler, Michal Levy-Sakin, Anat Frydman-Marom, Yaniv Amir, Roni Scherzer-Attali, Ludmila Buzhansky, Ehud Gazit, and Hanoch Senderowitz. Quantitative structure-activity relationship analysis of β-amyloid aggregation inhibitors. *Journal of Computer-Aided Molecular Design*, 25(2):135–144, February 2011.

[45] James C Stroud, Cong Liu, Poh K Teng, and David Eisenberg. Toxic fibrillar oligomers of amyloid-β have cross-β structure. *Proc Natl Acad Sci USA*, 109(20):7717–7722, May 2012.

[46] Yedi Sun, Guohua Zhang, Cheryl A Hawkes, James E Shaw, JoAnne McLaurin, and Mark Nitz. Synthesis of scyllo-inositol derivatives and their effects on amyloid β peptide aggregation. *Bioorg Med Chem*, 16(15):7177–7184, August 2008.

[47] Vladimir N Uversky, Christopher J Oldfield, and A Keith Dunker. Intrinsically disordered proteins in human diseases: introducing the D2 concept. *Annual review of biophysics*, 37:215–246, 2008.

[48] Dominic M Walsh, Igor Klyubin, Julia V Fadeeva, William K Cullen, Roger Anwyl, Michael S Wolfe, Michael J Rowan, and Dennis J Selkoe. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature*, 416(6880):535–539, April 2002.

[49] Dominic M Walsh and Dennis J Selkoe. A beta oligomers - a decade of discovery. *J Neurochem*, 101(5):1172–1184, 2007.

[50] Patrick Walsh, Philipp Neudecker, and Simon Sharpe. Structural Properties and Dynamic Behavior of Nonfibrillar Oligomers Formed by PrP(106−126). *J Am Chem Soc*, 132(22):7684–7695, June 2010.

[51] Patrick Walsh, Jason Yau, Karen Simonetti, and Simon Sharpe. Morphology and Secondary Structure of Stable β-Oligomers Formed by Amyloid Peptide PrP(106−126). *Biochemistry*, 48(25):5779–5781, June 2009.

[52] W Wang, O Donini, C M Reyes, and P A Kollman. Biomolecular simulations: recent developments in force fields, simulations of enzyme catalysis, protein-ligand, protein-protein, and protein-nucleic acid noncovalent interactions. *Annual review of biophysics and biomolecular structure*, 30:211–243, 2001.

[53] Wei-Feng Xue, Andrew L Hellewell, Walraj S Gosal, Steve W Homans, Eric W Hewitt, and Sheena E Radford. Fibril fragmentation enhances amyloid cytotoxicity. *Journal of Biological Chemistry*, 284(49):34272–34282, December 2009.