Chapter 3

The APP Proteolytic System and Its Interactions with Dynamic Networks in Alzheimer's Disease

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Abstract

Diseases of aging are often complex and multifactorial, involving many genetic and life course modifiers. Systems biology is becoming an essential tool to investigate disease initiation and disease progression. Alzheimer's disease (AD) can be used as a case study to investigate the application of systems biology to complex disease. Here we describe approaches to capturing biological data, representing data in terms of networks and interpreting their meaning in relation to the human population. We highlight issues that remain to be addressed both in terms of modeling disease progression and in relating findings to the current understanding of human disease.

Key words Alzheimer's disease, Amyloid precursor protein, Amyloid-beta-protein, Presenilin, Network modeling, Systems biology

1 Introduction

Diseases of aging, such as cancer and neurodegeneration, are complex and multifactorial, involving many genetic and life course modifiers. As more evidence becomes available, many links between different diseases of aging are becoming apparent [1], such as the roles of cell cycle proteins in cancer and neurodegeneration [2] or the contributions of Alzheimer's disease (AD) related and cardiovascular related genes in both normal aging and neurodegeneration [3]. Systems biology, a field that aims to integrate data from diverse biological areas, is becoming an essential tool to investigate processes relating to initiation and progression in complex disease. AD is the most common form of dementia associated with aging and is increasingly being accepted as a complex multifactorial neurodegenerative syndrome. AD can be used as a case study to investigate the application of systems biology to complex molecular disease pathways and relate these to brain behavior and ultimately treatment strategies.

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2 Overview of Alzheimer's Disease (AD)

AD is characterized clinically by memory loss, cognitive impairments and dementia [4, 5]. These symptoms lead to impairments in activities of daily living with the result that individuals with AD require an increasing degree of support and care as the disease progresses. Neuropathologically, the hallmarks of AD include intracellular neurofibrillary tangles (NFT) composed of paired helical filaments of the microtubule associated protein tau, extracellular senile plaques containing aggregated amyloid-beta-protein (Aβ) and neuritic plaques and dystrophic neurites that are tau reactive and are also often associated with aggregated Aβ [6, 7].

The importance of the amyloid precursor protein (APP) proteolytic system to dementia initiation and progression in AD is highlighted by both neuropathological and genetic evidence. Various mutations within APP and the γ-secretase associated Presenilin (PS) genes, PS1 and PS2, are associated with early onset familial Alzheimer's disease (FAD) [8]. The genetic data is further linked to disease progression by the deposition of the Aβ, a proteolytic fragment of APP, in neuritic and senile plaques. Additionally, the deposition of Aβ in the brain vasculature as congophilic amyloid angiopathy (CAA) is common in AD and may have independent effects on cognitive function [9, 10]. For late onset AD, accounting for >95 % of cases, the genetic contributions to disease are estimated to be between 48 and 79 % [11, 12] and include contributions from genes such as ApoE [13], CLU and PICALM [14] and CR1 [15] amongst others (reviewed in [16, 17]). Lifestyle modifiers that may contribute to dementia risk include education [18], exercise [19] and diet [20].

The relationship between neuropathology and cognitive status is not straight forward [21]. While considered as neuropathological hallmarks of AD, clinicopathological population studies show that the relationships between various neuropathologies, age and dementia status are complex [22] and that very few "pure" AD cases exist [23]. Population studies of the aging brain commonly find the neuropathological hallmarks of AD in cognitively normal individuals, albeit generally at lower severities, and demented individuals may show little neuropathology [21, 22, 24]. This raises questions around how these neuropathologies, and the neurochemistry associated with them, contribute to disease initiation and progression and how AD is defined both clinically and neuropathologically. If the aim is to devise treatment strategies, where some medication may alleviate or prevent the clinical manifestation of dementia, then the relationships between the human genome, (the complete set of genetic material in a cell), the transcriptome, (the entire collection of gene transcripts both destined to be expressed as proteins and as regulatory elements), the proteome,

(the complete set of expressed proteins in a specific cell type), the interactome, (the complete set of molecular interactions in a cell), the functional brain connectome, (the complete set of neural and synaptic connections in the human), and the whole body within its ever-changing environment must be elucidated. Computational models can be a tool to investigate these relationships and how they change due to disease.

3 Basic Background for Biomolecular Networks

Molecular pathways are dynamic functional systems involving multiple players often with complex regulatory systems involving both direct and indirect feedback loops. Flow of biological information through these pathways can be represented as computational networks based on molecular communication theories [25]. Within a cell as a whole, the probability that an interaction or biological reaction will occur between specific molecules and not others depends on many factors including, compartmentation, relative affinity, concentration, half-life, protein modifications, the presence of co-factors, and the formation of biologically active protein complexes.

3.1 Compartmentation

A cell is divided into compartments and forms organized structures that allow cellular processes to occur in a controlled way. Organelles, such as the nucleus, endoplasmic reticulum and mitochondria, isolate specific cellular processes within semi-permeable membranes that concentrate components of a particular cellular process and increase the chance that they will combine. Compartmentation also isolates reactions that would otherwise be deleterious for the whole cell, such as lysosomal reactions involved in the breakdown of proteins tagged for destruction. Within organelles, specific compartments can be defined by further interactions between factors, such as relatively rigid cholesterol-rich lipid raft areas within a more fluid phospholipid membrane. In order to maintain cellular compartments, the cell must express all the various components in the correct place and at the appropriate time and this involves the complex process of cellular trafficking.

3.2 Relative Affinity

The relative affinity of one protein for another contributes to the probability that they will react and this affinity depends on shape and charge distribution which ultimately depend on the amino acid sequence and protein folding. Protein shape and charge distribution are altered by the protein modifications described below and by many other factors including pH, metal ion binding and interactions with other cellular molecules.

3.3 Concentration

The concentration of the active form of a protein depends on many factors including gene expression, protein synthesis, protein modification, trafficking and storage mechanisms and protein degradation amongst others. Concentration is usually tightly regulated and over- or under- expression of active proteins can be disruptive to normal cellular processes.

3.4 Half Life

The rate at which a protein is synthesized and degraded is its turnover and this is characterized by its half-life, i.e. the time it takes for half the amount of a particular protein to be degraded. The length of time a protein is active and available can contribute to the likelihood that it will be involved in a cellular reaction. The concentration of a protein with a short half-life is more easily manipulated by the cell.

3.5 Protein Modifications

After translation, proteins are often processed and/or modified before achieving an active form and more than 200 different types of modification are known [26]. Modifications can be permanent or transient. Permanent modifications include proteolytic processing, where an immature protein, such as immature PS, requires cleavage to attain its active form [27, 28]. Transient and reversible enzymatic modifications are fundamental to the regulation cellular processes and include (1) glycosylation, the addition of sugar groups, (2) phosphorylation and dephosphorylation, the addition and removal of phosphate groups and (3) acetylation and deacetylation, the addition or removal of acetyl groups. Phosphorylation and dephosphorylation in particular form a major mechanism by which cells can switch processes on or off or change the flow through a biochemical pathway. Additionally, proteins may be modified non-enzymatically by metabolites, e.g. the modification of various lysine residues by the glycolytic metabolite 1,3-bisphosphoglycerate [29].

3.6 Co-factors

Co factors are molecules or ions that are required for biological functions or reactions to occur. For many proteins, metal ions are central to their mechanism of action. For example, the *N*-methyl D-Aspartate (NMDA) glutamate receptor allows calcium ions into a neuron when both electrical and neurotransmitter signals are received. The Ca^{2+} channel is normally blocked by Mg^{2+}. This block is removed briefly when a previous electrical signal changes the electrical potential of the membrane surrounding the NMDA glutamate receptor. If glutamate binds at this time, the calcium channel opens to allow Ca^{2+} ions into the cell. With no change in electrical potential, glutamate binding cannot open the channel. In effect, Mg^{2+} contributes mechanistically to the way the NMDA receptor senses coincidence in electric and neurotransmitter signals and this process contributes to one mechanism of synaptic plasticity. Other examples of co-factors include small molecules such as

vitamins which are often involved in enzyme reactions as part of the chemical process.

3.7 Protein Complexes

The formation of tightly associated proteins within large complexes is often required for biological activity. An example of this is the endopeptidase γ secretase complex, discussed later, where at least four different proteins are required to form an active enzyme [30]. These include one of the presenilins, either PS1 (UniProt P49768) or PS2 (UniProt P49810), which forms the catalytic core and the proteins Pen-2 (UniProt Q9NZ42), nicastrin (UniProt Q92542) and APH-1 (UniProt Q96BI3) that may contribute to the activation of the protein complex and regulate how the complex interacts with its various substrates [31].

3.8 Environmental Factors

In addition to processes regulated by the cell via gene and protein expression, features such as temperature, pH or redox state associated with the cellular environment may also affect the likelihood of a reaction, for example pH modulates Aβ aggregation [32, 33] and oxidative stress may increase Aβ production and also be increased by Aβ [34].

3.9 Describing Protein Interactions

The properties of affinity and concentration for active forms of a protein in relation to its biological outcomes can be illustrated by dose response curves (Fig. 1). Further, interactions such as enzyme reactions can be described by various kinetic constants such as the affinity constant $K_{(a)}$, the catalytic efficiency $K_{(cat)}$, maximal reaction velocity $V_{(max)}$ and K_m, an inverse measure of affinity defined as the amount of substrate at half V_{max}. These values are calculated from experimental data using equations such as the Michaelis-Menten equation [35] and associated variations. The basic biochemical properties should be captured in any mechanistic model of a molecular pathway. Some pathways will be more complex than others but most will feature these properties in regulatory mechanisms. It must be remembered that molecules and signaling pathways in different cell types may be associated with different functions and these may also vary between species making a generally applicable model of any one molecular pathway impossible.

4 Networks and Their Analysis as Tools to Investigate Complexity in Molecular Pathways

One approach to teasing apart the complexity of molecular pathways is to model molecular interactions as networks to describe and characterize the complex relationships and components within and between pathways. A molecular system can be represented as a graph in the form of a collection of nodes (objects) and edges (relationships). The functional relevance of nodes and edges can be

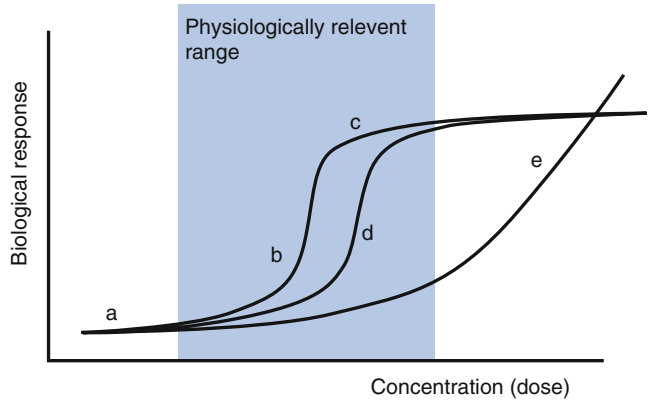

Fig. 1 A generalized dose response curve. Where the concentration of an active protein is very low, the probability that it will interact with its target is also very low and any associated biological outcome will be minimal (**a**). As concentration increases towards a physiologically relevant range, the high affinity biological outcome will also increase (**b**). At a certain point the system is maximally active and any further increase in protein concentration will not increase the high affinity biological outcome as other features of the system may be rate limiting and the biological outcome reaches a steady state (**c**). At increasing concentrations of the active protein, other pathways may become more relevant as the chances of lower affinity reactions increase (**d**); other features of the lower affinity systems may be rate limiting for the relevant biological outcomes which will reach a steady state. At very high concentrations, there are increased chances of aberrant or inappropriate reactions/interactions between the active protein and other pathways with which it would not normally associate (**e**), and these may not be rate limited

described by assigning various attributes derived from the molecular system in question.

Nodes can be used to represent molecules and annotations can represent the various factors such as concentration, affinity and compartmentation. Edges can be either directed, specifying a source (starting point) and a target (endpoint), or non-directed. Directed edges are suitable for representing flow while non-directed edges are used to represent mutual interactions. Mixed graphs contain both directed and undirected edges and have various sets of relations.

A network of molecular relationships can be built in several ways. One way is to iteratively search literature databases using keywords relevant to the system being investigated [36]. An iterative procedure can be used to develop the search strategy, with input from clinician advisors, neuropathologists, information specialists etc. A search of PubMed (28 August 2013) for the keywords systems biology AND Alzheimer disease retrieved 183 results and the increasing number of references over time indicates that the application of systems biology to AD research is of increasing importance.

Not all of these references will be relevant and manual curation will be required. A search of PubMed (28 August 2013) using the MeSH terms ("Systems Biology"[Mesh]) AND "Alzheimer Disease"[Mesh] retrieved 24 results, with some relevant references missing. A comprehensive search of several bibliographic databases as well as hand searches of key journals would also need to be undertaken to ensure all literature would be identified. All titles and abstracts should be screened by two independent reviewers and a third reviewer would resolve any disagreements about inclusion. This underlines the importance of a reliable and repeatable search strategy.

Once a collection of papers has been generated, there are various ways to filter these results to obtain only those papers of interest, involving either automated text search, human search of abstracts or both. Using this approach, networks can be built based on the information available, analyzed and then used to generate questions for further experimentation.

It must be remembered that any defined literature search, whilst being reproducible, may not retrieve all the papers of interest and a manual search of paper references may be required until no more useful references are found. Specific molecules in older literature may not be named in a standard way and in one network construction study [37], two APP interacting proteins were excluded as they could not be identified with certainty due to inconsistent naming. Additionally, only information that is published is available, leading to an unquantifiable bias in network construction due to missing information and this has important consequences for the analysis and interpretation of any resultant molecular network.

Molecular interactions can also be extracted from databases such as those listed in Table 1. While each database may be slightly different, there are now systematic ways to query such databases and extract relevant information in standard formats [38]. However, these databases are built from the existing literature and will therefore share the unquantifiable bias due to missing information. Automated methods of text searching are often used in database construction as they can be fast and repeatable. However, automation can lead to errors of misclassification and manual curation is used in most databases to minimize this. Manual curation can also lead to errors which must be repaired when found.

Most molecular databases are built using data from a variety of sources and are annotated with the experimental system from which the data were derived; this generally includes the species, whether in-vivo or in vitro and the exact method used, such as co-immunoprecipitation, various gene [39, 40] and protein [41, 42] expression systems or co-migration in sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), all methods have strengths and weaknesses.

Table 1
Examples of molecular pathway and interaction databases

Database	Database description	Reference/link
MINT	Experimentally verified protein interactions; uses automated literature mining and expert curation	http://mint.bio.uniroma2.it/mint/Welcome.do
IntAct	Molecular interactions derived from literature mining or from direct user submissions; expert curation	http://www.ebi.ac.uk/intact/?conversationContext=1
DIP	Experimentally verified protein interactions; data from a variety of sources including automated literature mining and expert curation	http://dip.doe-mbi.ucla.edu/dip/Main.cgi
KEGG	A collection of databases covering various areas including ontology, genomics and molecular networks	http://www.genome.jp/kegg/
HPRD	Database of human specific protein interactions, expert curation, no automation	http://www.hprd.org
BioGRID	Protein interactions from a number of species models, automatic literature mining and expert curation	http://thebiogrid.org/
STRING	Known and predicted direct and indirect protein interactions; uses automated literature mining and expert curation	http://string-db.org/

The studies listed in Table 2 have approached map construction in different ways, using different combinations of protein-protein interaction (PPI) databases, with different literature searching protocols and different inclusion or exclusion criteria. The networks generated in these studies do not always correspond and different studies highlight different pathways or biological processes, e.g. Fe^{2+} [43], apoptosis [44], or cardiovascular disease/diabetes [3]. Each study has different starting points, inclusion/exclusion criteria and network construction methods, so this lack of agreement is to be expected. It is difficult to assess the degree to which the various starting points, criteria and network construction methods bias results towards an outcome. For example, the study by Soler-Lopez et al. [45] may not represent the interactions of full length APP in the membrane adequately, as many of the extracellular matrix (ECM) proteins that might be expected to interact with APP are excluded due to difficulties involved in expressing them in the experimental microarray used. This may shift the focus of their network more towards intracellular interactions. Given the importance of the various interactions of APP with components of the ECM (*see* Fig. 2), any network excluding such proteins and proteoglycans could be seriously confounded and any findings would have to be interpreted carefully. Additionally, the correspondence between gene expression as mRNA and viable functional proteins within a cell is not absolute, varying from 9 to 87 % depending on which genes are investigated [46].

Table 2
Protein–Protein interaction (PPI) network studies (adapted from [36])

Reference	Selection criteria	Exclusion criteria	Main focus
[37]	Evidence of direct PPI from literature searches	Non-protein molecules and metals, poorly characterized proteins, specific peptides are included as parent genes	Direct PPI involving APP and associated fragments by domain with reference to APP770 isoform; molecular networks with reference to biological processes
[45]	Genes in close proximity with 12 "seed" genes previously associated with AD	Proteins without open reading frames, highly glycosylated proteins, transcription factors, extracellular proteins, proteins with several transmembrane regions	Identification of genes in AD with reference to direct PPI and biological processes
[3]	Co-expressed genes that differ between controls and AD	Probe-sets not mapping to any gene or mapping to hypothetic proteins are removed	Variations in transcriptomes of AD similar to cardiovascular disease and diabetes. Cis regulatory elements identified in several diseases known to co-occur with AD
[51]	Genes with variable expression between human and mouse datasets	Outlier removal, removed datasets with low interspecies expression and connectivity; top 5,000H and 3,000 M connected genes included, rest removed to reduce noise	Mouse and human networks are similar—expression levels more preserved than connectivity, species differences in gene co-expression in astroglia and microglia but not neurons, human specific role of PSEN1 in myelination and evidence of species differences in glial cells linked to neuroinflammation

Molecular networks built from PPI databases or literature searches do not explicitly take into account differences between cell types arising through the processes of differentiation during development which can lead to different susceptibilities of different cell types to neuropathology, such as the well-recognized difference in susceptibility to tau reactive NFT pathology of Ca4, Ca3, Ca2 and Ca1 neurons in the hippocampus as reflected in Braak Staging [47]; a widely accepted semi-quantitative measure of NFT pathology. Different cellular systems may have very different functions depending on cell type: an example of this is the way many cell cycle proteins, involved in regulating cell proliferation, are involved in synaptic plasticity in non-proliferative neurons [2]. The differences between cell types potentially undermine many of the current network approaches, especially where different experimental systems have been used to generate interaction data. Ideally there should be a database for each cell type, and for the brain this would need to include different neuron types as not all neurons necessarily share similar signaling and interaction pathways.

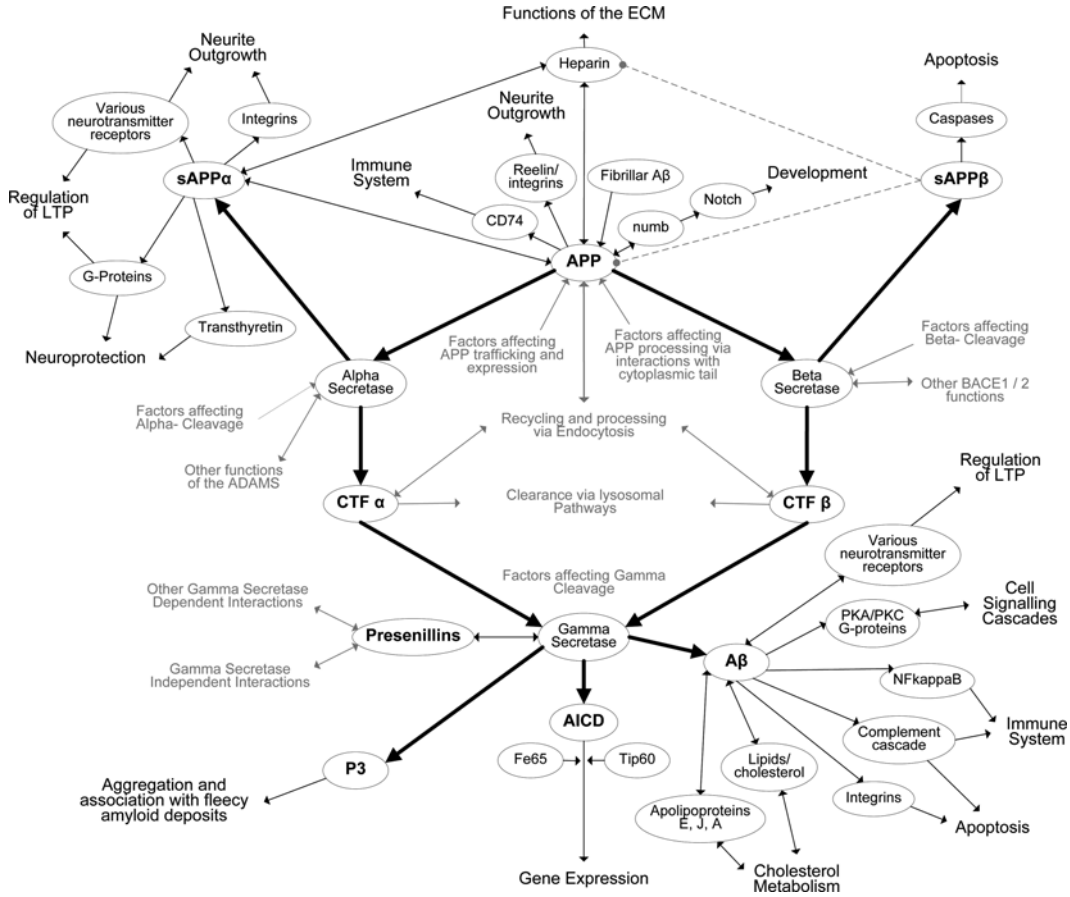

Fig. 2 A simplified view of selected interactions of the APP proteolytic system (adapted from [36]) Nodes represent molecules or molecular assemblies and interactions between them as arrows. Some complex interactions have been collapsed into general processes shown in grey. Multiple sequence variants and conformations of APP and Aβ have been collapsed into a single node for each. Aβ, amyloid beta protein; ADAM, a disintegrin and metalloproteinase domain-containing protein; AICD, APP intracellular domain; APP, amyloid precursor protein; BACE, beta-site amyloid precursor protein cleaving enzyme; CD74, HLA class II histocompatibility antigen gamma chain; CTF, carboxy- terminal fragment; ECM, extracellular matrix; Fe65, Amyloid beta A4 precursor protein-binding family B member 1; LTP, long-term potentiation; PKA, protein kinase A; PKC, protein kinase C; sAPP, secreted amyloid precursor protein; Tip60, Histone acetyltransferase KAT5. With permission from BioMed Central (part of Springer Science + Business Media) under the Open Access License Agreement (http://www.biomedcentral.com/about/license)

A major problem with all molecular map type networks is their inability to include dynamic information relating to the way molecular networks are regulated in living systems. Transient protein modifications, such as phosphorylation, regulate molecular interactions and are central to cellular function are not easily captured, for example, differential phosphorylation of the tyrosine (tyr) residues Tyr_{682} and/or Threonine Thr_{668} of the APP_{695} cytoplasmic domain regulates many interactions with small binding proteins

and kinases [48]. Other dynamic processes that may not be fully represented include transient changes in gene expression via epigenetic mechanisms, changes in protein expression via RNA interference, responses to environmental perturbations such as infection and activity lead changes, such as the up-regulation of synaptic proteins in response to synaptic activity.

Inter-species differences in the way cellular signaling systems are organized, especially in the brain, are well recognized [49, 50] and this should be taken into account when designing animal disease models and building networks. Miller et al. [51] confirm this in the comparison between human and mouse networks, revealing an additional function of PS in oligodendrocytes and myelination in humans that is not seen in the mouse. Given the association of PS mutations in FAD, this difference is likely to impact on the suitability of the mouse as a model for AD.

The development of animal models that represent AD disease processes in humans is crucial in the search for effective therapeutic interventions. Early transgenic mouse models did not completely replicate the neuropathology associated with human disease [52] nor the more fundamental aspects of Aβ biochemistry in humans [50]. Attempts to fully represent AD in humans are on-going with the development of new animal models that can be used to investigate the links between various features of AD. Using multiply transgenic animal models allows the investigation of molecular interactions and signaling pathways involved in different aspects of the disease in a way not possible in humans. For example, the TgF344-AD rat [53] displays oligomeric Aβ species and plaque pathology, tau pathology, behavioral change and neuronal loss, combinations not always present together in other animal models, and this model can be used to study the connections between Aβ and tau pathologies. Different animal models may be used to highlight different aspects of human disease, such as the association between Aβ and cholesterol metabolism in the triple transgenic mouse model over-expressing the sterol regulatory element-binding protein-2 [54] or the relationship between age and cognitive decline in the senescence-accelerated mouse [55].

The success of all animal models depends on being comparable to disease presentation in humans, and this is where the main problems lie. The characteristics of AD in humans are constantly being updated as new disease processes and pathologies are found. Disease processes, such as hippocampal sclerosis [7], or other pathologies such as the Tar-DNA binding protein of 43 kDa (TDP-43) [56] may independently contribute to cognitive status and are yet to be fully characterized in the human population. Population studies highlight the existence of multiple pathologies including contributions from the vascular system in the development of Alzheimer- like dementia in the aging population, with relatively few cases of "pure" AD [23, 57]. Additionally, the

relationship between age, neuropathology and disease is not straight-forward, with many pathologies showing an age related distribution [22].

Fresh human brain tissue that may be of use in functional studies is extremely rare and to a great extent, interaction databases rely on various animal, cell culture and in vitro based models, all of which have yet to be fully characterized with respect to the normal human system. If only animal or cell based systems are used as experimental models, functions that are human specific could be misrepresented or missed completely. The full range of pathologies associated with age and AD in humans still remains to be replicated in any animal model.

5 APP: A Dynamic and Complex Proteolytic System

A review of the complexity of the APP proteolytic system has been described [36]. In summary, APP is a member of a wider family of similar proteins that also includes the APP like proteins (APLP)1 and APLP2 that have significant functional redundancy [58] complicating investigations. It is expressed in various isoforms due to mRNA splicing, with APP_{695} being expressed predominantly in the brain and linked to amyloid deposition. It is a type I, single pass transmembrane protein with diverse functions including associations with cell differentiation [59], neurite outgrowth [60, 61], cell adhesion [62], synapse formation, maintenance and plasticity [62, 63] and many cell signaling pathways [36, 64, 65] including apoptosis [66]. APP is post-translationally glycosylated [67] and phosphorylated [48] at various residues and these modifications may contribute to the regulation of the various APP functions and proteolytic pathways.

Full length APP has a large N-terminal domain that interacts with various components of the ECM including heparin and other proteoglycans [68, 69], other proteins such as reelin [70], DAB1 [71] and also forms homodimers regulated by heparin and Zn^{2+} [72]. The transmembrane region has been implicated in the process of homodimerization and also interacts with various proteins including Notch [73]. The C-terminal domain of full length APP also interacts functionally with a variety of proteins including FE65 [74], the low density lipoprotein receptor protein (LRP) [75, 76], a variety of small binding proteins [48, 77] and several kinases [48, 78, 79] that phosphorylate the residues Y_{682} of the binding and signaling sequence $GY_{682}ENPTY$ and T_{668} of APP_{695} [48, 79, 80]. Phosphorylation regulates the interaction of the C-terminal domain with other proteins [48, 77], may modulate proteolytic processing [80] and allows cross talk between diverse cellular systems [48].

Full length APP can remain at the cell surface, be recycled via endocytosis or proteolytically processed and has a high turnover, with a half-life ranging from 30 min [76, 81–83] to 4 h [84–86]. Unprocessed APP is degraded or recycled via the endosomal or lysosomal pathways and may be recycled back to the membrane and processed within ~30 min [82], with perhaps one third to one half being processed via the cleavage pathways as measured by secreted sAPPα/β [82]. APP is proteolytically processed to more than 40 fragments [87]. There are two main cleavage pathways, α- and β- pathways that then converge on a shared γ-cleavage, summarized in Fig. 2. These cleavages have been well reviewed [88, 89]. Additional cleavage pathways (not shown in Fig. 2) include caspase cleavages producing an alternative C-terminal cytoplasmic fragment C31 that is associated with apoptosis [90, 91] and the alternative cleavages by β-site APP cleaving enzyme (BACE)1, 11 residues within the Aβ sequence [88, 92] leaving a membrane bound fragment C88 and BACE2 at the θ-cleavage site between the phenylalanine residues, F_{615} and F_{616} of APP_{695} downstream of both the Aβ and P3 cleavage sites, producing a membrane bound fragment C80 [93].

5.1 α Cleavage

α-cleavage occurs between residues Lys_{612} and Leu_{613} within the Aβ sequence of APP_{695}, releasing the N-terminal sAPPα and leaving a membrane bound C83 C-terminal fragment [88]. α-secretase activity has been observed by several membrane-anchored zinc-dependent metalloproteinase enzymes including A Disintegrin and Metalloproteinase (ADAM)9, ADAM10, ADAM17 [94–96] and possibly the matrix metalloproteinase (MMP)9 [97]. α-cleavage is both constitutive and regulated, with the various ADAMs responding in different ways depending on many factors [95, 98]. In addition to APP, α-secretases also cleave alternative substrates such as Notch [99], pro-TNF-α and the epidermal growth factor receptor [100] which may lead to competition between different pathways with consequences for many cellular processes including development, synaptic plasticity and the cell cycle and cancer [96, 100, 101]. How the balance between these alternative pathways is regulated is not known.

The soluble N-terminal fragment released by α-cleavage, sAPPα, retains two heparin binding sites and has been shown to bind heparin as a dimer [102]. The ability of sAPPα to disrupt APP dimerization at the cell surface may contribute to its neuroprotective actions [103–105] and may partly explain why sAPPα is ~100× more neuroprotective against excytotoxicity, glucose deprivation and the addition of Aβ in hippocampal cultures than sAPPβ, which lacks the second C-terminal heparin binding site [104]. Additionally, neuroprotective actions of sAPPα may be mediated by its antagonism of stress signaling by the JNK stress signaling pathway [106]. Dementia status has been associated with both reduced sAPPα

levels in human CSF [107] and an increased half-life of sAPPα [86] in transgenic mice, however, as yet, there has been no systematic study of the α-pathway proteolytic fragments in the human population.

5.2 β Cleavage

β cleavage occurs between residues Met_{596} and Asp_{597} of APP_{695} within the second heparin binding site, releasing the N-terminal sAPPβ from the membrane bound C99 C-terminal fragment [88, 92]. Two membrane bound aspartyl proteases are associated with β-cleavage, BACE 1 and to a lesser extent, BACE2 [88, 92]. Additionally, Cathepsins D and B have shown β-cleavage activity to release Aβ [108]. BACE1 and BACE2 are differentially regulated and have different functions [109]. In addition to APP, BACE1 may also cleave alternative substrates including APLP1 and APLP2 [110] and P-selectin glycoprotein ligand-1 [111]. Heparin and heparin sulfates may be involved in regulating APP cleavage by BACE1 [112]. In addition to interactions with sAPPα and APP, the large soluble sAPPβ fragment may be associated with apoptotic signaling and axonal degeneration via the death receptor DR6 and caspase6 [113], though the interactions of sAPPβ are not fully characterized and require further detailed investigation.

5.3 γ Cleavage

Cleavage of APP by the γ-secretase complex occurs within the membrane to release the variable length 38–46 residue Aβ peptide following β-cleavage, the variable length 21–29 residue P3 (Aβ17-X) fragment following α-cleavage, with both pathways releasing the APP intracellular domain, (AICD) [8, 88, 114, 115]. There is some uncertainty as to how γ-cleavage occurs; γ-secretase cleavage may occur via successive ζ and ε cleavages producing progressively shorter Aβ fragments [116–118], though there may also be distinct cleavage mechanisms that may be separately modulated [119].

There are a number of alternative γ-secretase substrates, e.g. APLP1, APLP2, Notch, cadherins, LRP [120, 121], and syndecan-1 [114, 122]. In addition to γ-secretase dependent functions, some PS functions are independent of γ-secretase, so that in effect, γ-secretase may compete for presenilins with other γ-secretase independent PS functions including cell adhesion, trafficking of various proteins [123], and Ca^{2+} homeostasis [114]. How the γ-secretase is regulated between the different substrates is not fully understood but may involve other binding proteins such as numb [65] and Rac1 [124], regulation of PS trafficking, including a possible reciprocal interaction with APP [125] and localization of PS within specific organelles and cellular membrane compartments [126].

Aβ is produced in a range of sequence lengths [87] and can form monomers, dimers, oligomers and fibrils [8] which have been difficult to study due to their dynamic instability [127]. At physiological concentrations Aβ is associated with numerous normal

cellular functions [128] and in AD progression has multiple interactions that have been described as either neuroprotective or neurotoxic [36]. It is deposited in the brain in various pathological forms including CAA, diffuse and cored senile plaques and is often associated with neuritic plaques. Different sequence lengths have different propensities to aggregate [32, 129] and aggregation is also affected by amino acid substitution in mutant forms [130, 131] and various factors such as proximity to membranes [132], and pH or metal ion availability [133]. Different sequence lengths and different aggregation states can have different functional roles [36], making investigations into the exact roles of Aβ in the brain difficult. These associations may be better approached experimentally as a matrix, where the various sequence lengths, aggregation states and mutant forms should be assessed for each interaction.

While it is likely that P3 is produced in alternative sequence lengths following γ cleavage, very little evidence can be found in the literature for the contributions of P3 to disease progression. There is currently little interest in characterizing the contributions of P3 to normal brain function or AD, even though P3 is known to aggregate [134–136], has been associated with in cotton wool type amyloid plaques [137] enhances the aggregation of Aβ1-40 [138] and may have a signaling role in apoptosis via caspase activation [139].

Regulation of expression and proteolysis of APP involves multiple factors, some of which are summarized in Fig. 2 (adapted from [36]). How signals from these multiple factors in various cellular locations are integrated to produce a specific outcome in any one cell is not known. Regulation of APP proteolysis, from both outside and within the APP proteolytic system, can be in response to a wide variety of cellular signals and various modulators including glycosylation, phosphorylation, dimerization, associations with heparin glycoproteins and other binding proteins. Feedback routes can be simple and short range such as the promotion of APP expression associated with fibrillar Aβ and prion protein [140]. Indirect and complex feedback routes also exist, such as the effects of heparin on regulating β-cleavage with low concentration promoting and high concentration inhibiting the activation of BACE1 [141] and the effects of Aβ on heparin. Aβ interacts with heparins in the ECM and at high levels may prevent the catabolism of proteoglycans and promote amyloid formation [142]. Reciprocally heparins modulate many of the interactions involving Aβ such as enhancing both nucleation and elongation processes in the aggregation of Aβ [143], limiting the neurotoxic and pro-inflammatory activity of Aβ in a dose dependent manner [144] and contributing to the uptake of Aβ by a pathway shared with ApoE [145].

6 Modeling the APP Proteolytic System. Practical Considerations

As a summary of interactions, maps, such as Fig. 2, can highlight particular areas that may be of interest such as hubs or regulatory interactions that may be open to modification by medications, or may highlight areas where data are missing, leading to further research. While molecular networks involving APP can be constructed, how these relate to the actual network of molecular interactions in any one human cell type at any one stage of development cannot yet be fully assessed. As reviewed above, different criteria and network construction methods can generate different networks, each with strengths, weaknesses and different behaviors in analysis. The impact of missing data, such as interactions that have not yet been identified, is difficult to assess. For the APP network, the contributions of alternative proteolytic fragments, such as sAPPα, sAPPβ, P3 and the various longer Aβ fragments, e.g. Aβ43, Aβ45, Aβ46 and Aβ48, in various states of aggregation have yet to be fully described. It is still unclear which Aβ sequence or aggregation state is linked to disease progression [146]. These alternative fragments may yet provide further interactions that have the potential to affect network behavior as a whole, as suggested by the predisposition to form Aβ42 from γ cleavage due to the accumulation of γ secretase substrates, C99 and longer Aβ fragments [147].

There are great difficulties in representing an iterative and dynamic proteolytic system, such as APP, as a static network map of connections. One of the first questions raised is what exactly does a static network represent? If a network represents interactions, and these interactions change with protein modifications such as phosphorylation, is it best to represent each functional protein version as a separate node? Should the alternative isoforms of APP be included and if so, should they have separate nodes? How do we best represent Aβ with around 40 possible sequence lengths [87] and various states of aggregation [32, 146]? In Fig. 2, Aβ has been collapsed into a single node for clarity. How would over 40 nodes in this space with potentially different connections affect computational and analytical methods? Given the different conformations [148] and functional actions [149–151] of Aβ(1–40) and Aβ(1–42), a single node for these peptides cannot fully represent the APP functional network.

If the aim is to understand the role of PS in AD, perhaps with a view to developing treatment strategies that modulate its probability to react between its various substrates, then a network of its interactions could be constructed and this could be the basis for a dynamic computational model. This dynamic model would need to include calculations of a protein's probability of reaction, where the basic molecular features described previously, (concentration, half-life etc.), could be represented as values in a computational matrix.

Table 3
Kinetic values for human synthetic wild-type PS1. N/A, not available

Reaction	K_m	V_{max}	Cell/model system	Ref
APP C99 → Aβ and AICD Notch (S3) → NICD	0.40 ± 0.05 μM (C99) 1.08 ± 0.17 μM	175.6 ± 8.4 pM/min (AICD) 95.7 ± 7.5 pM/min (NICD)	Mouse embryonic fibroblasts (MEF) derived membrane cell free assay system	[152]
APP C99 → AICD	874 ± 252 nM (AICD)	15 ± 1.82 nM/h (AICD)	MEF derived membrane cell free assay system	[147]
APP C99 → Aβ	N/A	Maximal activity: 217 ± 110 pM/106 cells (Aβ)	HeLa cells transfected with APP WTC99 cDNA construct	[153]
Aβ42 → Aβ38	370 ± 40 nM	N/A	Hek293 cells transfected with wild type PS1	[118]

This approach could be developed iteratively and different versions of a network could be compared in terms of flow through the network. Experimental data relating to basic biomolecular properties that are relevant to modeling the probability that a reaction will occur can be extracted from the literature, including V_{max}, K_m, and $K_{(cat)}$. However, characterizing enzyme reactions in order to model the probability of reaction is not an easy task as demonstrated in the following example.

Recent studies [118, 147, 152, 153] have looked at γ secretase enzyme kinetics for a variety of PS mutations, substrates and products. Different experimental models have been used and different features of the system have been reported in different formats. Table 3 gives values for K_m, and V_{max} for human synthetic wild-type PS1 and its interaction with various substrates extracted from the associated references.

Values for K_m have been given in μM or nM and values for maximum reaction rate have been given as V_{max} (pM/min or nM/h) or maximal activity (pM/10^6 cells). While manual extraction from the literature could easily convert μM to nM or nM/h to pM/min, automated text based searches could introduce error due to units reported. It is not possible to convert pM/10^6 cells into nM/h or pM/min, making comparisons between these studies difficult. The degree to which the experimental system used affects the values gained is difficult to assess, mouse embryonic fibroblast (MEF) derived membrane cell free assays, Hek293 or HeLa cell based systems are likely to have very different environments and each system will have experimental advantages and disadvantages. None of these systems accurately represent aging in the human brain. Indeed, which values of K_m and V_{max} in Table 3 would be most representative

of the situation in any human neuron? Standard reporting formats for proteomic data exist [154, 155] and are annotated by experimental system used to derive the information such as species used, etc. so that data from different studies can be integrated but it is difficult to choose those values that may best represent the human system as it has not yet been fully characterized.

Attempts to dynamically model the human cognitive system are on-going with a diversity of approaches. For example, Kasabov et al. [156, 157] have combined gene and protein expression networks with a probabilistic spiking neural network and compared this to real human electroencephalograms [158] and used this to investigate pathways involved in AD [157]. In these models, dynamic behavior is captured in the network output, represented as spiking neurons, which can be controlled by networks representing gene and protein expression data. These gene and protein networks are in turn re-modeled iteratively by the spiking neural network. While a computational model of the AD process would be very useful to investigate how the system might be perturbed by changes to gene and protein expression, their current usefulness is open to question. Connectionist network models contain unquantifiable modules, as the weights of connections between the nodes in a network are stochastically modified during the training process. The relationships between the nodes and weighted connections with any feature of the human system are not certain: the nodes do not necessarily represent real human neurons and the connections do not necessarily represent connections between neurons. Populations of trained networks will consist of individual network models, each of which will have different connection weights. The difficulty here is in relating the distributions of the weights in any network to the living human system: the extraction of potentially useful information from the structure of the network is problematic.

7 Applying Systems Biology Approaches in Other Areas

7.1 Pattern Recognition and the Early Diagnosis of AD

Various computational methods such as principle component analysis [159, 160], linear regression methods [161, 162], machine learning methods [163–165] and random forests [166] are being used to investigate automated pattern recognition in magnetic resonance imaging (MRI) image analysis [161] or various imaging methods coupled with multiple biomarker analysis [160, 163, 165, 166] with some success in separating normal aging from mild cognitive impairment (MCI) and AD. Although the use of new computational methods for multiple markers for AD increases the specificity and sensitivity in categorizing normal aging, MCI or AD, there is still no combination of markers that can identify those with MCI that may convert to dementia and AD with certainty and this is an urgent requirement.

7.2 The Human Connectome Project

Beyond mapping gene and protein expression or interaction networks, the effects of the human connectome on dementia risk is another complex area that presents huge challenges. The human connectome project [167] aims to map the human connectome at the macroscopic scale, (~1 mm^3) using a variety of neuroimaging methods. This project aims to create a map of healthy human connectivity. There is great inter-individual heterogeneity, both in the vascular system, that may affect certain imaging methods and in cortical folding, so any resultant map can only be an idealized reference map. Further, how this connectivity changes with progression in dementia may also be highly heterogeneous between individuals and this has yet to be fully investigated.

8 Relating the Systems Biology of APP to Normal Cognition and Disease Progression in AD

For any neuron, signals received via synapses must be integrated into dynamic responses of the cell as a whole and this requires signaling between any specific synapse on a dendrite and its nucleus, possibly located some distance from the synapse. Changes to gene and protein expression in response to synaptic signaling must be transmitted back to the synapse via protein trafficking so that receptors and signaling molecules are in the correct cellular positions. There may be different signals arriving via different pathways, both electrical and metabolic, and these must be integrated into a coherent neuronal response. There is a temporal coherence, where everything must be in the right place at the right time, as the synaptic response builds on the previous state of the synapse. These synapses are further organized within a neural network connectome of different cell types and different functional brain areas from which cognition and human behavior arise that may include inputs from the whole body as it interacts with its environment. Figure 3 illustrates the interdependence of the areas involved in normal brain function, where gene expression may be modified by behavior which in turn may change protein expression and interaction leading to further changes in behavior as the whole system iteratively and stochastically changes over time. Attempts to isolate any specific area, such as protein expression, can be undermined by this interdependence and contributions to cognitive processes may be misrepresented, simply due to the assumptions of independence in experimental design.

In order to understand this coherent system, research has necessarily had to break it into smaller parts giving rise to discrete research fields investigating all the areas involved from genomes and proteomes to interactomes and connectomes. Traditionally, the reductionist approach aims to characterize individual pathways by introducing changes that are meant to impact on specific

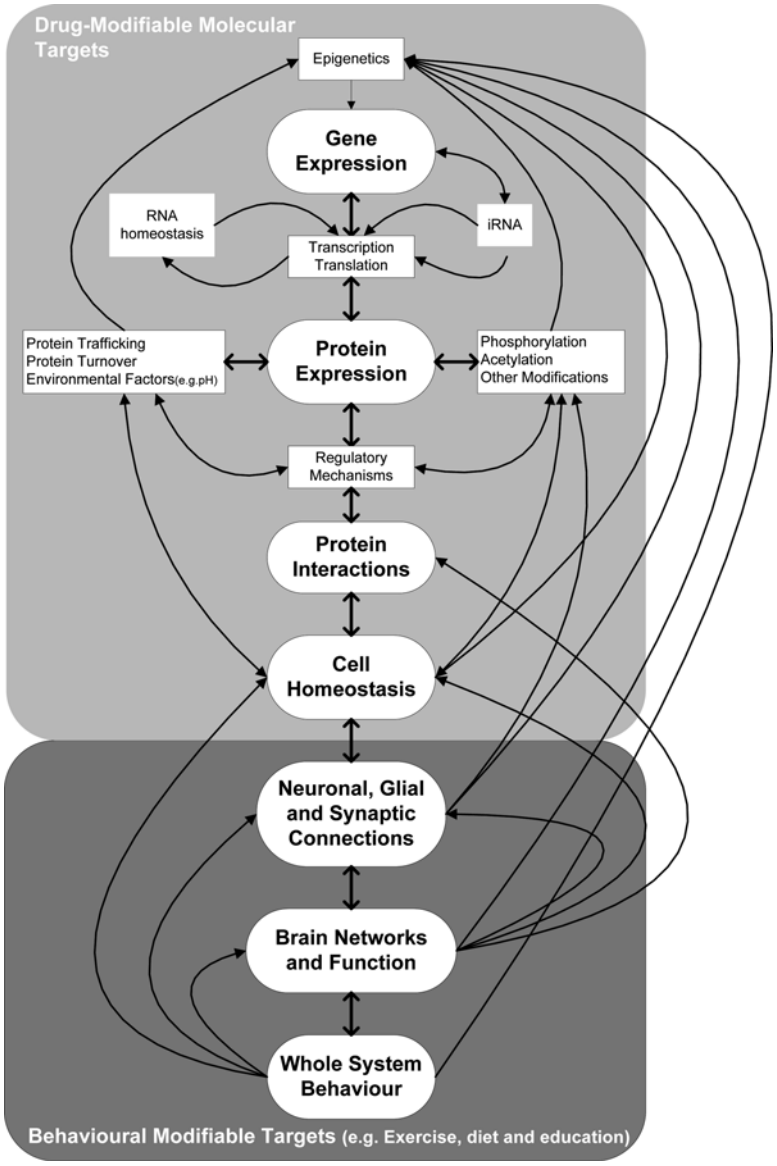

Fig. 3 The interdependence between 'omics research areas. General discrete research areas discussed in the text appear as nodes, selected feedback and feed forward relationships are shown as *arrows*

components in potentially well understood ways. This can lead to a limited view of complex processes, for example, the amyloid cascade hypothesis suggests that Aβ, in some form, is the sole cause of AD and that therefore removal of Aβ should modify the disease course. This can be understood in terms of a more linear infection type model. However, treatments based on this model have been unsuccessful in clinical trials so far and have failed to change the

course of the disease [168], questioning its validity. Population studies highlight complexity in the presentation of AD, bringing wider research areas such as aging, diet, exercise, education, the vascular system and other biochemical pathways into consideration. Few complex biological mechanisms can be reduced to simple in vitro, cell based or animal based experimental models [50] and poorly characterized or unsuitable experimental systems may lead to erroneous interpretations.

In contrast to reductionist approaches, in which molecular systems may be treated as isolated and independent mechanisms, systems biology aims to integrate evidence from diverse areas into a representation of living processes as a whole. Even simple molecular systems present enormous challenges in terms of modeling biological outcomes as theories of molecular communication, i.e. how biological information is transmitted through a molecular network, are still being developed [25] and any computational representations of biological processes are necessarily limited to the data we currently have. In complex maps of protein interactions, many pathways are possible and whether any specific interactions are central, peripheral or involved in only subtypes of disease progression cannot yet be fully assessed.

Integrating networks constructed at the level of gene expression, with networks constructed at the levels of protein expression, protein interaction and cellular behavior is currently difficult as there isn't correspondence between them. As reviewed above, not all genes expressed as mRNA transcripts become functional proteins and not all functional proteins necessarily interact due to dynamic regulation. Additionally, while the human connectome is being mapped at ever increasing resolution [167], how information is represented and stored across the human brain as a dynamic neural system of synaptic connections and how this changes with disease progression is not known.

Given that there is no qualitative marker for AD, diagnosis has relied on various clinical [4, 5] and neuropathological [6, 7] criteria that are quantitative and involve the application of thresholds: no single measure yet defines AD. Further, biomarkers used in the diagnosis of clinical disease remain to be standardized and harmonized [169]. Aβ fragments, commonly employed as biomarkers of disease, may have both protective and aberrant behaviors associated with disease and multiple disease pathways may exist. Additionally, no Aβ fragment has been identified as the "neurotoxic" disease related species [146]. How can poorly defined neurodegenerative diseases be diagnosed at an early stage when treatment strategies could have the best chance of preserving cognitive functions? This has consequences for how we understand AD, whether it is a single process that will respond to a single intervention strategy or whether AD is a syndrome, requiring multiple different interventions depending on disease types, yet to be characterized.

This is of great importance to the design of experimental investigations and clinical trials. Selection of participants and controls relies on how we understand the disease process and how any disease process is reflected in clinical markers. How do we know that in any given clinical trial, the subjects selected represent homogenous disease or non-disease groups? There may be other disease processes, such as hippocampal sclerosis [7] and other pathologies such as TDP-43 [56] that may contribute to disease pathways and are yet to be fully characterized. Additionally, individuals may vary in the degree to which cognitive reserve and compensation to neuronal injury may limit the impact of pathological changes that occur during aging to better preserve cognitive functions [170].

In terms of health care planning, given the lack of progress towards a reliable dementia treatment strategy, in the immediate future perhaps dementia prevention and dementia care are areas where progress can best be made. The association of education [18], exercise [19] and diet [20] throughout life with a lower dementia risk in old age suggests that Public Health strategies devised to promote these activities would be worthwhile. Without a cure or ameliorating treatment, we need to be able to care for dementia sufferers in the most appropriate and efficient manner to maintain an individual's independence and quality of life for as long as possible.

While applying the systems biology approach to represent complex dynamic proteolytic systems such as APP may not yet be entirely feasible, useful perspectives can still be generated. For APP, the complexity of its interactions and regulatory features suggest that multiple initiation and progression pathways are possible: the analysis of networks to highlight those disease pathways that may be most likely to occur in humans presents major challenges. Capturing this complexity in any network model and being able to relate network behavior to real human brains is the ultimate goal. Whether it will ever be possible to build a dynamic model of the AD disease processes at all levels of consideration (genome, proteome, interactome, connectome and whole body) is not clear. There is no best way to build a network and all networks constructed so far are incomplete. Additionally, both AD and normal aging in humans have yet to be fully characterized. How this missing data impacts on the reliable prediction of events from an incomplete network cannot yet be known. However, this chapter suggests some initial steps and proposals on how we could build more sophisticated networks. The challenge to the AD biomedical research community is to iteratively integrate data generated via a variety of approaches, both reductionist and systems biology, and then to use any insights gained to integrate the information and design further experiments to generate new data. It is clear that no single approach, reductionist or systems biology can tackle this problem alone.

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References

1. Tacutu R, Budovsky A, Yanai H, Fraifeld VE (2011) Molecular links between cellular senescence, longevity and age-related diseases—a systems biology perspective. Aging 3:1178–1191
2. Arendt T (2003) Synaptic plasticity and cell cycle activation in neurons are alternative effector pathways: the 'Dr. Jekyll and Mr. Hyde concept' of Alzheimer's disease or the yin and yang of neuroplasticity. Prog Neurobiol 71:83–248
3. Ray M, Ruan J, Zhang W (2008) Variations in the transcriptome of Alzheimer's disease reveal molecular networks involved in cardiovascular diseases. Genome Biol 9:R148
4. Morris JC, Heyman A, Mohs RC et al (1989) The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. Neurology 39:1159–1165
5. McKhann GM, Knopman DS, Chertkow H et al (2011) The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 7:263–269
6. Mirra SS, Heyman A, McKeel D et al (1991) The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology 41: 479–486
7. Hyman BT, Phelps CH, Beach TG et al (2012) National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. Alzheimers Dement 8:1–13
8. Selkoe DJ (2001) Alzheimer's disease: genes, proteins, and therapy. Physiol Rev 81:741–766
9. Zekry D, Duyckaerts C, Belmin J et al (2003) Cerebral amyloid angiopathy in the elderly: vessel walls changes and relationship with dementia. Acta Neuropathol 10:367–373
10. Attems J, Jellinger K, Thal DR, Van Nostrand W (2011) Review: sporadic cerebral amyloid angiopathy. Neuropathol Appl Neurobiol 37: 75–93
11. Gatz M, Reynolds CA, Fratiglioni L et al (2006) Role of genes and environments for explaining Alzheimer disease. Arch Gen Psychiatry 63:168–174
12. Pedersen NL, Gatz M, Berg S, Johansson B (2004) How heritable is Alzheimer's disease late in life? Findings from Swedish twins. Ann Neurol 55:180–185
13. Cedazo-Minguez A, Cowburn RF (2001) Apolipoprotein E: a major piece in the Alzheimer's disease puzzle. J Cell Mol Med 5:254–266
14. Harold D, Abraham R, Hollingworth P et al (2009) Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat Genet 41: 1088–1093
15. Crehan H, Holton P, Wray S et al (2012) Complement receptor 1 (CR1) and Alzheimer's disease. Immunobiology 217:244–250
16. Bertram L, Tanzi RE (2012) The genetics of Alzheimer's disease. Prog Mol Biol Transl Sci 107:79–100
17. Schellenberg GD, Montine TJ (2012) The genetics and neuropathology of Alzheimer's disease. Acta Neuropathol 124:305–323
18. Brayne C, Ince PG, Keage HA et al (2010) Education, the brain and dementia: neuroprotection or compensation? Brain 133: 2210–2216
19. Ma Q (2008) Beneficial effects of moderate voluntary physical exercise and its biological mechanisms on brain health. Neurosci Bull 24:265–270
20. Mattson MP, Chan SL, Duan W (2002) Modification of brain aging and neurodegenerative disorders by genes, diet, and behavior. Physiol Rev 82:637–672
21. Xuereb JH, Brayne C, Dufouil C et al (2000) Neuropathological findings in the very old. Results from the first 101 brains of a population-based longitudinal study of dementing disorders. Ann N Y Acad Sci 903:490–496
22. Savva GM, Wharton SB, Ince PG et al (2009) Age, neuropathology, and dementia. N Engl J Med 360:2302–2309

23. MRC-CFAS (2001) Pathological correlates of late-onset dementia in a multicentre, community-based population in England and Wales. Neuropathology Group of the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS). Lancet 357: 169–175
24. Brayne C, Richardson K, Matthews FE et al (2009) Neuropathological correlates of dementia in over-80-year-old brain donors from the population-based Cambridge city over-75 s cohort (CC75C) study. J Alzheimers Dis 18:645–658
25. Nakano T, Moore MJ, Wei F et al (2012) Molecular communication and networking: opportunities and challenges. IEEE Trans Nanobioscience 11:135–148
26. Jensen ON (2006) Interpreting the protein language using proteomics. Nat Rev Mol Cell Biol 7:391–403
27. Ratovitski T, Slunt HH, Thinakaran G et al (1997) Endoproteolytic processing and stabilization of wild-type and mutant presenilin. J Biol Chem 272:24536–24541
28. da Costa CA, Ancolio K, Checler F (1999) C-terminal maturation fragments of presenilin 1 and 2 control secretion of APP alpha and A beta by human cells and are degraded by proteasome. Mol Med 5:160–168
29. Ogishima S, Mizuno S, Kikuchi M et al (2013) A map of Alzheimer's disease-signaling pathways: a hope for drug target discovery. Clin Pharmacol Ther 93:399–401
30. Kimberly WT, LaVoie MJ, Ostaszewski BL et al (2003) Gamma-secretase is a membrane protein complex comprised of presenilin, nicastrin, Aph-1, and Pen-2. Proc Natl Acad Sci U S A 100:6382–6387
31. Kimberly WT, Wolfe MS (2003) Identity and function of gamma-secretase. J Neurosci Res 74:353–360
32. Kirkitadze MD, Condron MM, Teplow DB (2001) Identification and characterization of key kinetic intermediates in amyloid beta-protein fibrillogenesis. J Mol Biol 312: 1103–1119
33. Rubinstein A, Lyubchenko YL, Sherman S (2009) Dynamic properties of pH-dependent structural organization of the amyloidogenic beta-protein (1–40). Prion 3:31–43
34. Smith MA, Hirai K, Hsiao K et al (1998) Amyloid-beta deposition in Alzheimer transgenic mice is associated with oxidative stress. J Neurochem 70:2212–2215
35. Berg JM, Tymoczko JL, Stryer L (2006) Biochemistry, 6th edn. W. H. Freeman and Company, New York, NY
36. Hunter S, Brayne C (2012) Relationships between the amyloid precursor protein and its various proteolytic fragments and neuronal systems. Alzheimers Res Ther 4:10
37. Perreau VM, Orchard S, Adlard PA et al (2010) A domain level interaction network of amyloid precursor protein and Abeta of Alzheimer's disease. Proteomics 10:2377–2395
38. Aranda B, Blankenburg H, Kerrien S et al (2011) PSICQUIC and PSISCORE: accessing and scoring molecular interactions. Nat Methods 8:528–529
39. Murphy D (2002) Gene expression studies using microarrays: principles, problems, and prospects. Adv Physiol Educ 26:256–270
40. Baranzini SE (2004) Gene expression profiling in neurological disorders: toward a systems-level understanding of the brain. Neuromolecular Med 6:31–51
41. Hu S, Xie Z, Qian J et al (2011) Functional protein microarray technology. Wiley Interdiscip Rev Syst Biol Med 3:255–268
42. Sutandy FX, Qian J, Chen CS, Zhu H (2013) Overview of protein microarrays. Curr Protoc Protein Sci. Editorial board, John E Coligan et al. Chapter 27: Unit 27.1. doi:10.1002/0471140864.ps2701s72
43. Kell DB (2010) Towards a unifying, systems biology understanding of large-scale cellular death and destruction caused by poorly liganded iron: Parkinson's, Huntington's, Alzheimer's, prions, bactericides, chemical toxicology and others as examples. Arch Toxicol 84:825–889
44. Alberghina L, Colangelo AM (2006) The modular systems biology approach to investigate the control of apoptosis in Alzheimer's disease neurodegeneration. BMC Neurosci 7(Suppl 1):S2
45. Soler-Lopez M, Zanzoni A, Lluis R et al (2011) Interactome mapping suggests new mechanistic details underlying Alzheimer's disease. Genome Res 21:364–376
46. Suarez RK, Moyes CD (2012) Metabolism in the age of 'omes'. J Exp Biol 215:2351–2357
47. Braak H, Braak E (1991) Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 82:239–259
48. Tamayev R, Zhou D, D'Adamio L (2009) The interactome of the amyloid beta precursor protein family members is shaped by phosphorylation of their intracellular domains. Mol Neurodegener 4:28
49. Raiteri M (2006) Functional pharmacology in human brain. Pharmacol Rev 58:162–193
50. Kokjohn TA, Roher AE (2009) Amyloid precursor protein transgenic mouse models and

Alzheimer's disease: understanding the paradigms, limitations, and contributions. Alzheimers Dement 5:340–347

51. Miller JA, Horvath S, Geschwind DH (2010) Divergence of human and mouse brain transcriptome highlights Alzheimer disease pathways. Proc Natl Acad Sci U S A 107: 12698–12703
52. Codita A, Winblad B, Mohammed AH (2006) Of mice and men: more neurobiology in dementia. Curr Opin Psychiatry 19:555–563
53. Cohen RM, Rezai-Zadeh K, Weitz TM et al (2013) A transgenic Alzheimer rat with plaques, tau pathology, behavioral impairment, oligomeric abeta, and frank neuronal loss. J Neurosci 33:6245–6256
54. Barbero-Camps E, Fernandez A, Martinez L et al (2013) APP/PS1 mice overexpressing SREBP-2 exhibit combined Abeta accumulation and tau pathology underlying Alzheimer's disease. Hum Mol Genet 22:3460–3476
55. Butterfield DA, Poon HF (2005) The senescence-accelerated prone mouse (SAMP8): a model of age-related cognitive decline with relevance to alterations of the gene expression and protein abnormalities in Alzheimer's disease. Exp Gerontol 40:774–783
56. Davidson YS, Raby S, Foulds PG et al (2011) TDP-43 pathological changes in early onset familial and sporadic Alzheimer's disease, late onset alzheimer's disease and Down's syndrome: association with age, hippocampal sclerosis and clinical phenotype. Acta Neuropathol 122:703–713
57. Keage HA, Ince PG, Matthews FE et al (2012) Impact of less common and "disregarded" neurodegenerative pathologies on dementia burden in a population-based cohort. J Alzheimers Dis 28:485–493
58. Heber S, Herms J, Gajic V et al (2000) Mice with combined gene knock-outs reveal essential and partially redundant functions of amyloid precursor protein family members. J Neurosci 20:7951–7963
59. De Strooper B, Annaert W (2000) Proteolytic processing and cell biological functions of the amyloid precursor protein. J Cell Sci 113: 1857–1870
60. Small DH, Clarris HL, Williamson TG et al (1999) Neurite-outgrowth regulating functions of the amyloid protein precursor of Alzheimer's disease. J Alzheimers Dis 1:275–285
61. Small DH, Nurcombe V, Reed G et al (1994) A heparin-binding domain in the amyloid protein precursor of Alzheimer's disease is involved in the regulation of neurite outgrowth. J Neurosci 14:2117–2127
62. Priller C, Bauer T, Mitteregger G et al (2006) Synapse formation and function is modulated by the amyloid precursor protein. J Neurosci 26:7212–7221
63. Hoe HS, Rebeck GW (2008) Functional interactions of APP with the apoE receptor family. J Neurochem 106:2263–2271
64. Okamoto T, Takeda S, Murayama Y, Ogata E, Nishimoto I (1995) Ligand-dependent G protein coupling function of amyloid transmembrane precursor. J Biol Chem 270: 4205–4208
65. Roncarati R, Sestan N, Scheinfeld MH et al (2002) The gamma-secretase-generated intracellular domain of beta-amyloid precursor protein binds Numb and inhibits notch signaling. Proc Natl Acad Sci U S A 99: 7102–7107
66. Kogel D, Schomburg R, Schurmann T et al (2003) The amyloid precursor protein protects PC12 cells against endoplasmic reticulum stress-induced apoptosis. J Neurochem 87:248–256
67. Georgopoulou N, McLaughlin M, McFarlane I, Breen KC (2001) The role of post-translational modification in beta-amyloid precursor protein processing. Biochem Soc Symp 67:23–36
68. Bush AI, Pettingell WH Jr, de Paradis M et al (1994) The amyloid beta-protein precursor and its mammalian homologues. Evidence for a zinc-modulated heparin-binding superfamily. J Biol Chem 269:26618–26621
69. Williamson TG, Nurcombe V, Beyreuther K et al (1995) Affinity purification of proteoglycans that bind to the amyloid protein precursor of Alzheimer's disease. J Neurochem 65:2201–2208
70. Hoe HS, Lee KJ, Carney RS et al (2009) Interaction of reelin with amyloid precursor protein promotes neurite outgrowth. J Neurosci 29:7459–7473
71. Hoe HS, Tran TS, Matsuoka Y et al (2006) DAB1 and Reelin effects on amyloid precursor protein and ApoE receptor 2 trafficking and processing. J Biol Chem 281:35176–35185
72. Dahms SO, Hoefgen S, Roeser D et al (2010) Structure and biochemical analysis of the heparin-induced E1 dimer of the amyloid precursor protein. Proc Natl Acad Sci U S A 107:5381–5386
73. Fassa A, Mehta P, Efthimiopoulos S (2005) Notch 1 interacts with the amyloid precursor protein in a Numb-independent manner. J Neurosci Res 82:214–224
74. McLoughlin DM, Miller CC (2008) The FE65 proteins and Alzheimer's disease. J Neurosci Res 86:744–754
75. Rebeck GW, Moir RD, Mui S et al (2001) Association of membrane-bound amyloid precursor protein APP with the apolipoprotein E

receptor LRP. Brain Res Mol Brain Res 87:238–245
76. Pietrzik CU, Busse T, Merriam DE et al (2002) The cytoplasmic domain of the LDL receptor-related protein regulates multiple steps in APP processing. EMBO J 21:5691–5700
77. Taru H, Suzuki T (2009) Regulation of the physiological function and metabolism of AbetaPP by AbetaPP binding proteins. J Alzheimers Dis 18:253–265
78. Muresan Z, Muresan V (2005) c-Jun NH2-terminal kinase-interacting protein-3 facilitates phosphorylation and controls localization of amyloid-beta precursor protein. J Neurosci 25:3741–3751
79. Suzuki T, Nakaya T (2008) Regulation of amyloid beta-protein precursor by phosphorylation and protein interactions. J Biol Chem 283:29633–29637
80. Lee MS, Kao SC, Lemere CA et al (2003) APP processing is regulated by cytoplasmic phosphorylation. J Cell Biol 163:83–95
81. Perez RG, Soriano S, Hayes JD et al (1999) Mutagenesis identifies new signals for beta-amyloid precursor protein endocytosis, turnover, and the generation of secreted fragments, including Abeta42. J Biol Chem 274:18851–18856
82. Loerch PM, Lu T, Dakin KA et al (2008) Evolution of the aging brain transcriptome and synaptic regulation. PLoS One 3:e3329
83. Weidemann A, Konig G, Bunke D et al (1989) Identification, biogenesis, and localization of precursors of Alzheimer's disease A4 amyloid protein. Cell 57:115–126
84. Savage MJ, Trusko SP, Howland DS et al (1998) Turnover of amyloid beta-protein in mouse brain and acute reduction of its level by phorbol ester. J Neurosci 18:1743–1752
85. Lyckman AW, Confaloni AM, Thinakaran G et al (1998) Post-translational processing and turnover kinetics of presynaptically targeted amyloid precursor superfamily proteins in the central nervous system. J Biol Chem 273: 11100–11106
86. Morales-Corraliza J, Mazzella MJ, Berger JD et al (2009) In vivo turnover of tau and APP metabolites in the brains of wild-type and Tg2576 mice: greater stability of sAPP in the beta-amyloid depositing mice. PLoS One 4:e7134
87. Wang R, Sweeney D, Gandy SE, Sisodia SS (1996) The profile of soluble amyloid beta protein in cultured cell media. Detection and quantification of amyloid beta protein and variants by immunoprecipitation-mass spectrometry. J Biol Chem 271:31894–31902
88. Turner PR, O'Connor K, Tate WP, Abraham WC (2003) Roles of amyloid precursor protein and its fragments in regulating neural activity, plasticity and memory. Prog Neurobiol 70:1–32
89. Selkoe DJ (1994) Normal and abnormal biology of the beta-amyloid precursor protein. Annu Rev Neurosci 17:489–517
90. Pellegrini L, Passer BJ, Tabaton M et al (1999) Alternative, non-secretase processing of Alzheimer's beta-amyloid precursor protein during apoptosis by caspase-6 and -8. J Biol Chem 274:21011–21016
91. McPhie DL, Golde T, Eckman CB et al (2001) beta-Secretase cleavage of the amyloid precursor protein mediates neuronal apoptosis caused by familial Alzheimer's disease mutations. Brain Res Mol Brain Res 97: 103–113
92. Cole SL, Vassar R (2007) The Alzheimer's disease beta-secretase enzyme, BACE1. Mol Neurodegener 2:22
93. Sun X, He G, Song W (2006) BACE2, as a novel APP theta-secretase, is not responsible for the pathogenesis of Alzheimer's disease in Down syndrome. FASEB J 20:1369–1376
94. Slack BE, Ma LK, Seah CC (2001) Constitutive shedding of the amyloid precursor protein ectodomain is up-regulated by tumour necrosis factor-alpha converting enzyme. Biochem J 357:787–794
95. Allinson TM, Parkin ET, Turner AJ, Hooper NM (2003) ADAMs family members as amyloid precursor protein alpha-secretases. J Neurosci Res 74:342–352
96. Yang P, Baker KA, Hagg T (2006) The ADAMs family: coordinators of nervous system development, plasticity and repair. Prog Neurobiol 79:73–94
97. Talamagas AA, Efthimiopoulos S, Tsilibary EC et al (2007) Abeta(1–40)-induced secretion of matrix metalloproteinase-9 results in sAPPalpha release by association with cell surface APP. Neurobiol Dis 28:304–315
98. Deuss M, Reiss K, Hartmann D (2008) Part-time alpha-secretases: the functional biology of ADAM 9, 10 and 17. Curr Alzheimer Res 5:187–201
99. Hartmann D, Tournoy J, Saftig P et al (2001) Implication of APP secretases in notch signaling. J Mol Neurosci 17:171–181
100. Edwards DR, Handsley MM, Pennington CJ (2008) The ADAM metalloproteinases. Mol Aspects Med 29:258–289
101. Arribas J, Bech-Serra JJ, Santiago-Josefat B (2006) ADAMs, cell migration and cancer. Cancer Metastasis Rev 25:57–68

102. Gralle M, Oliveira CL, Guerreiro LH et al (2006) Solution conformation and heparin-induced dimerization of the full-length extracellular domain of the human amyloid precursor protein. J Mol Biol 357:493–508
103. Gralle M, Botelho MG, Wouters FS (2009) Neuroprotective secreted amyloid precursor protein acts by disrupting amyloid precursor protein dimers. J Biol Chem 284: 15016–15025
104. Furukawa K, Sopher BL, Rydel RE et al (1996) Increased activity-regulating and neuroprotective efficacy of alpha-secretase-derived secreted amyloid precursor protein conferred by a C-terminal heparin-binding domain. J Neurochem 67:1882–1896
105. Ghosal K, Vogt DL, Liang M et al (2009) Alzheimer's disease-like pathological features in transgenic mice expressing the APP intracellular domain. Proc Natl Acad Sci U S A 106:18367–18372
106. Copanaki E, Chang S, Vlachos A et al (2010) sAPPalpha antagonizes dendritic degeneration and neuron death triggered by proteasomal stress. Mol Cell Neurosci 44:386–393
107. Sennvik K, Fastbom J, Blomberg M et al (2000) Levels of alpha- and beta-secretase cleaved amyloid precursor protein in the cerebrospinal fluid of Alzheimer's disease patients. Neurosci Lett 278:169–172
108. Hook V, Schechter I, Demuth HU, Hook G (2008) Alternative pathways for production of beta-amyloid peptides of Alzheimer's disease. Biol Chem 389:993–1006
109. Sun X, Wang Y, Qing H et al (2005) Distinct transcriptional regulation and function of the human BACE2 and BACE1 genes. FASEB J 19:739–749
110. Li Q, Sudhof TC (2004) Cleavage of amyloid-beta precursor protein and amyloid-beta precursor-like protein by BACE 1. J Biol Chem 279:10542–10550
111. Lichtenthaler SF, Dominguez DI, Westmeyer GG et al (2003) The cell adhesion protein P-selectin glycoprotein ligand-1 is a substrate for the aspartyl protease BACE1. J Biol Chem 278:48713–48719
112. Scholefield Z, Yates EA, Wayne G et al (2003) Heparan sulfate regulates amyloid precursor protein processing by BACE1, the Alzheimer's beta-secretase. J Cell Biol 163:97–107
113. Nikolaev A, McLaughlin T, O'Leary DD, Tessier-Lavigne M (2009) APP binds DR6 to trigger axon pruning and neuron death via distinct caspases. Nature 457:981–989
114. Vetrivel KS, Zhang YW, Xu H, Thinakaran G (2006) Pathological and physiological functions of presenilins. Mol Neurodegener 1:4
115. Selkoe DJ (1994) Cell biology of the amyloid beta-protein precursor and the mechanism of Alzheimer's disease. Annu Rev Cell Biol 10:373–403
116. Zhao G, Cui MZ, Mao G et al (2005) gamma-Cleavage is dependent on zeta-cleavage during the proteolytic processing of amyloid precursor protein within its transmembrane domain. J Biol Chem 280:37689–37697
117. Qi-Takahara Y, Morishima-Kawashima M, Tanimura Y et al (2005) Longer forms of amyloid beta protein: implications for the mechanism of intramembrane cleavage by gamma-secretase. J Neurosci 25:436–445
118. Okochi M, Tagami S, Yanagida K et al (2013) Gamma-secretase modulators and presenilin 1 mutants act differently on presenilin/gamma-secretase function to cleave Abeta42 and Abeta43. Cell Rep 3:42–51
119. Chen F, Hasegawa H, Schmitt-Ulms G et al (2006) TMP21 is a presenilin complex component that modulates gamma-secretase but not epsilon-secretase activity. Nature 440:1208–1212
120. Lleo A, Waldron E, von Arnim CA et al (2005) Low density lipoprotein receptor-related protein (LRP) interacts with presenilin 1 and is a competitive substrate of the amyloid precursor protein (APP) for gamma-secretase. J Biol Chem 280:27303–27309
121. von Arnim CA, Kinoshita A, Peltan ID et al (2005) The low density lipoprotein receptor-related protein (LRP) is a novel beta-secretase (BACE1) substrate. J Biol Chem 280: 17777–17785
122. Shen J, Kelleher RJ 3rd (2007) The presenilin hypothesis of Alzheimer's disease: evidence for a loss-of-function pathogenic mechanism. Proc Natl Acad Sci U S A 104:403–409
123. Uemura K, Kuzuya A, Shimohama S (2004) Protein trafficking and Alzheimer's disease. Curr Alzheimer Res 1:1–10
124. Boo JH, Sohn JH, Kim JE et al (2008) Rac1 changes the substrate specificity of gamma-secretase between amyloid precursor protein and Notch1. Biochem Biophys Res Commun 372:913–917
125. Liu Y, Zhang YW, Wang X et al (2009) Intracellular trafficking of presenilin 1 is regulated by beta-amyloid precursor protein and phospholipase D1. J Biol Chem 284: 12145–12152
126. Vetrivel KS, Cheng H, Kim SH et al (2005) Spatial segregation of gamma-secretase and substrates in distinct membrane domains. J Biol Chem 280:25892–25900
127. Bitan G, Teplow DB (2004) Rapid photochemical cross-linking–a new tool for studies

of metastable, amyloidogenic protein assemblies. Acc Chem Res 37:357–364

128. Pearson HA, Peers C (2006) Physiological roles for amyloid beta peptides. J Physiol 575:5–10
129. Saito T, Suemoto T, Brouwers N et al (2011) Potent amyloidogenicity and pathogenicity of Abeta43. Nat Neurosci 14:1023–1032
130. Miravalle L, Tokuda T, Chiarle R et al (2000) Substitutions at codon 22 of Alzheimer's abeta peptide induce diverse conformational changes and apoptotic effects in human cerebral endothelial cells. J Biol Chem 275: 27110–27116
131. Murakami K, Irie K, Morimoto A et al (2002) Synthesis, aggregation, neurotoxicity, and secondary structure of various A beta 1–42 mutants of familial Alzheimer's disease at positions 21–23. Biochem Biophys Res Commun 294:5–10
132. Kakio A, Yano Y, Takai D et al (2004) Interaction between amyloid beta-protein aggregates and membranes. J Pept Sci 10: 612–621
133. Klug GM, Losic D, Subasinghe SS et al (2003) Beta-amyloid protein oligomers induced by metal ions and acid pH are distinct from those generated by slow spontaneous ageing at neutral pH. Eur J Biochem 270:4282–4293
134. Zhao JH, Liu HL, Liu YF et al (2009) Molecular dynamics simulations to investigate the aggregation behaviors of the Abeta(17–42) oligomers. J Biomol Struct Dyn 26:481–490
135. Zheng J, Jang H, Ma B et al (2007) Modeling the Alzheimer Abeta17-42 fibril architecture: tight intermolecular sheet-sheet association and intramolecular hydrated cavities. Biophys J 93:3046–3057
136. Miller Y, Ma B, Nussinov R (2009) Polymorphism of Alzheimer's Abeta17-42 (p3) oligomers: the importance of the turn location and its conformation. Biophys J 97:1168–1177
137. Thal DR, Sassin I, Schultz C et al (1999) Fleecy amyloid deposits in the internal layers of the human entorhinal cortex are comprised of N-terminal truncated fragments of Abeta. J Neuropathol Exp Neurol 58:210–216
138. Liu R, McAllister C, Lyubchenko Y, Sierks MR (2004) Residues 17–20 and 30–35 of beta-amyloid play critical roles in aggregation. J Neurosci Res 75:162–171
139. Wei W, Norton DD, Wang X, Kusiak JW (2002) Abeta 17–42 in Alzheimer's disease activates JNK and caspase-8 leading to neuronal apoptosis. Brain 125:2036–2043
140. White AR, Maher F, Brazier MW et al (2003) Diverse fibrillar peptides directly bind the Alzheimer's amyloid precursor protein and amyloid precursor-like protein 2 resulting in cellular accumulation. Brain Res 966: 231–244
141. Beckman M, Holsinger RM, Small DH (2006) Heparin activates beta-secretase (BACE1) of Alzheimer's disease and increases autocatalysis of the enzyme. Biochemistry 45:6703–6714
142. Bame KJ, Danda J, Hassall A, Tumova S (1997) Abeta(1–40) prevents heparanase-catalyzed degradation of heparan sulfate glycosaminoglycans and proteoglycans in vitro. A role for heparan sulfate proteoglycan turnover in Alzheimer's disease. J Biol Chem 272:17005–17011
143. Klajnert B, Cortijo-Arellano M, Bryszewska M, Cladera J (2006) Influence of heparin and dendrimers on the aggregation of two amyloid peptides related to Alzheimer's and prion diseases. Biochem Biophys Res Commun 339:577–582
144. Bergamaschini L, Donarini C, Rossi E et al (2002) Heparin attenuates cytotoxic and inflammatory activity of Alzheimer amyloid-beta in vitro. Neurobiol Aging 23:531–536
145. Winkler K, Scharnagl H, Tisljar U et al (1999) Competition of Abeta amyloid peptide and apolipoprotein E for receptor-mediated endocytosis. J Lipid Res 40:447–455
146. Benilova I, Karran E, De Strooper B (2012) The toxic Abeta oligomer and Alzheimer's disease: an emperor in need of clothes. Nat Neurosci 15:349–357
147. Svedruzic ZM, Popovic K, Smoljan I, Sendula-Jengic V (2012) Modulation of gamma-secretase activity by multiple enzyme-substrate interactions: implications in pathogenesis of Alzheimer's disease. PLoS One 7:e32293
148. Yan Y, Wang C (2006) Abeta42 is more rigid than Abeta40 at the C terminus: implications for Abeta aggregation and toxicity. J Mol Biol 364:853–862
149. Lee DH, Wang HY (2003) Differential physiologic responses of alpha7 nicotinic acetylcholine receptors to beta-amyloid1-40 and beta-amyloid1-42. J Neurobiol 55: 25–30
150. Zou K, Kim D, Kakio A et al (2003) Amyloid beta-protein (Abeta)1-40 protects neurons from damage induced by Abeta1-42 in culture and in rat brain. J Neurochem 87:609–619
151. Parameshwaran K, Sims C, Kanju P et al (2007) Amyloid beta-peptide Abeta(1–42) but

not Abeta(1–40) attenuates synaptic AMPA receptor function. Synapse 61:367–374

152. Chavez-Gutierrez L, Bammens L, Benilova I et al (2012) The mechanism of gamma-secretase dysfunction in familial Alzheimer disease. EMBO J 31:2261–2274
153. Svedruzic ZM, Popovic K, Sendula-Jengic V (2013) Modulators of gamma-secretase activity can facilitate the toxic side-effects and pathogenesis of Alzheimer's disease. PLoS One 8:e50759
154. Orchard S, Salwinski L, Kerrien S et al (2007) The minimum information required for reporting a molecular interaction experiment (MIMIx). Nat Biotechnnol 25: 894–898
155. Taylor CF, Paton NW, Lilley KS et al (2007) The minimum information about a proteomics experiment (MIAPE). Nat Biotechnol 25:887–893
156. Kasabov N (2010) To spike or not to spike: a probabilistic spiking neuron model. Neural Netw 23:16–19
157. Kasabov N, Schliebs R, Kojima H (2011) Probabilistic computational neurogenetic modeling: from cognitive systems to Alzheimer's disease. IEEE Trans Auton Ment Dev 3:300–311. doi:10.1109/TAMD.2011.2159839
158. Kasabov N, Benuskova L, Wysoski SG (2005) Biologically plausible computational neurogenetic models: modeling the interaction between genes, neurons and neural networks. J Comput Theor Nanosci 2:569–573
159. Habeck C, Foster NL, Perneczky R et al (2008) Multivariate and univariate neuroimaging biomarkers of Alzheimer's disease. Neuroimage 40:1503–1515
160. Haense C, Buerger K, Kalbe E et al (2008) CSF total and phosphorylated tau protein, regional glucose metabolism and dementia severity in Alzheimer's disease. Eur J Neurol 15:1155–1162
161. Desikan RS, Cabral HJ, Hess CP et al (2009) Automated MRI measures identify individuals with mild cognitive impairment and Alzheimer's disease. Brain 132:2048–2057
162. Wang H, Nie F, Huang H et al (2011) Identifying AD-sensitive and cognition-relevant imaging biomarkers via joint classification and regression. Med Image Comput Comput Assist Interv 14:115–123
163. Cui Y, Liu B, Luo S et al (2011) Identification of conversion from mild cognitive impairment to Alzheimer's disease using multivariate predictors. PLoS One 6:e21896
164. Hinrichs C, Singh V, Xu G, Johnson SC (2011) Predictive markers for AD in a multimodality framework: an analysis of MCI progression in the ADNI population. Neuroimage 55:574–589
165. Zhang D, Wang Y, Zhou L et al (2011) Multimodal classification of Alzheimer's disease and mild cognitive impairment. Neuroimage 55:856–867
166. Gray KR, Aljabar P, Heckemann RA et al (2013) Random forest-based similarity measures for multi-modal classification of Alzheimer's disease. Neuroimage 65:167–175
167. Van Essen DC, Ugurbil K (2012) The future of the human connectome. Neuroimage 62:1299–1310
168. Castellani RJ, Perry G (2012) Pathogenesis and disease-modifying therapy in Alzheimer's disease: the flat line of progress. Arch Med Res 43:694–698
169. Carrillo MC, Rowe CC, Szoeke C et al (2013) Research and standardization in Alzheimer's trials: reaching international consensus. Alzheimers Dement 9:160–168
170. Stern Y (2012) Cognitive reserve in ageing and Alzheimer's disease. Lancet Neurol 11:1006–1012