Amyloid β-Protein Oligomers and Alzheimer Disease

Speaker: CHENG Guijuan

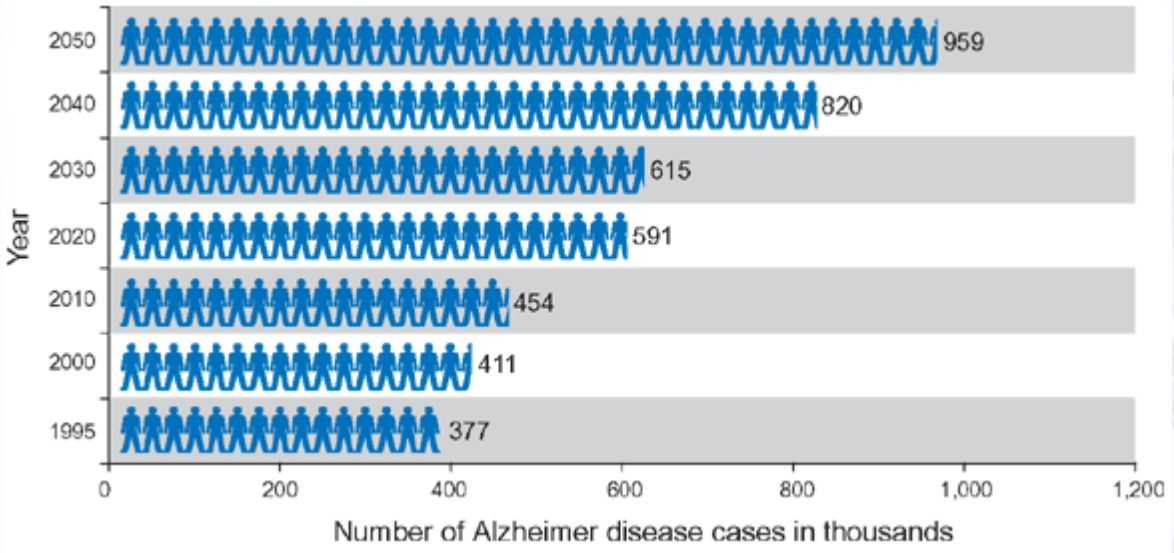
Dec. 27, 2013

Preface

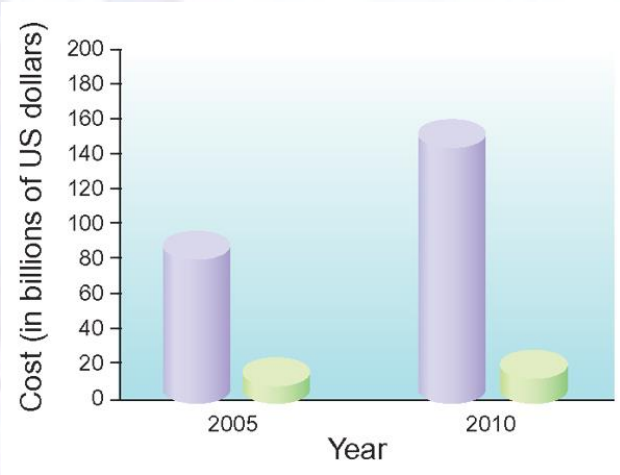
- Alzheimer's Disease (AD):

The most common form of progressive senile dementia primarily affecting individuals over the age of 65. The disease leads to the inevitable destruction of neurons, and ultimately death within 7 to 10 years.

Actual and estimated number of new Alzheimer disease cases in the US through the year 2050.



Actual and estimated financial costs of Alzheimer disease in the US



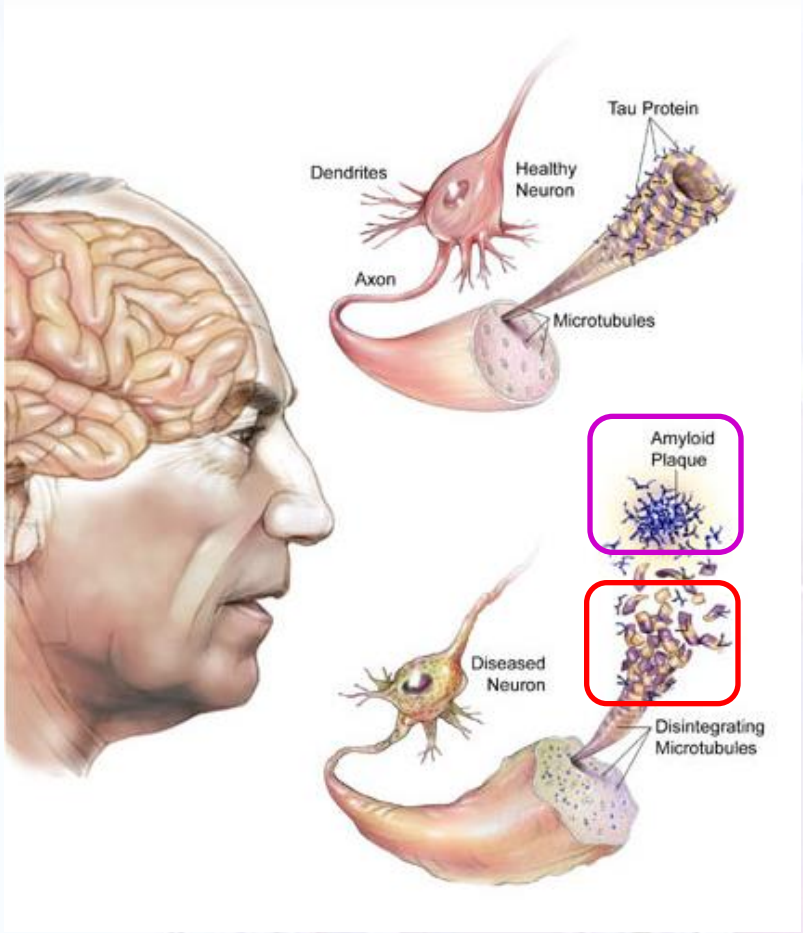
Mount, C.; Downton, C. *Nat. Med*. **2006**, *12*, 780

Outline

- **Background**
  1. The hallmarks of Alzheimer Disease
  2. Amyloid Hypothesis
- **Oligomer Toxicity**
- **Approaches to Study the Oligomers**
- **Summary**

The Hallmarks of AD

- The Hallmarks of AD : Plaques and Tangles

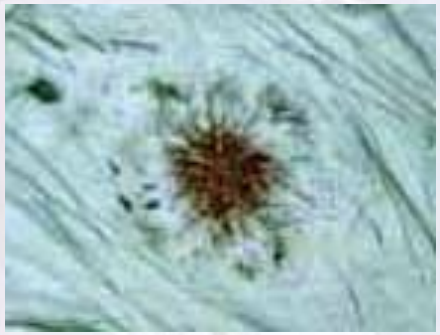


- Amyloid β plaques:

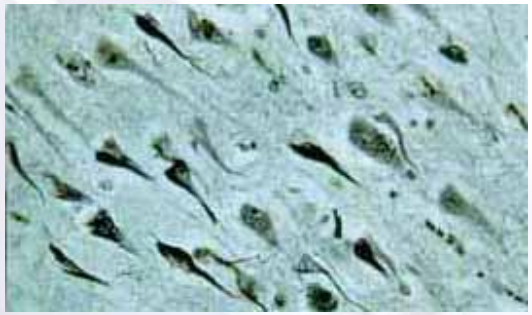
dense deposits of protein that accumulate outside and around nerve cells

- neurofibrillary tangles:

twisted fibers that build up inside the nerve cell



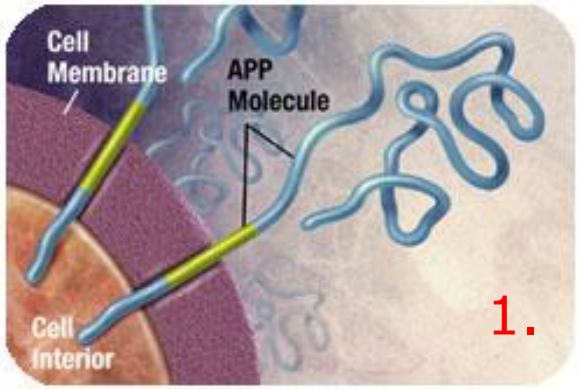
Amyloid β plaques

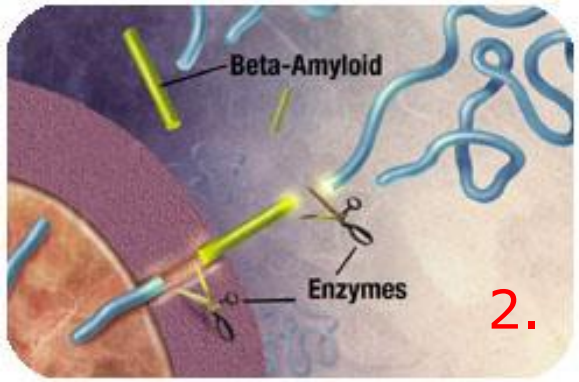


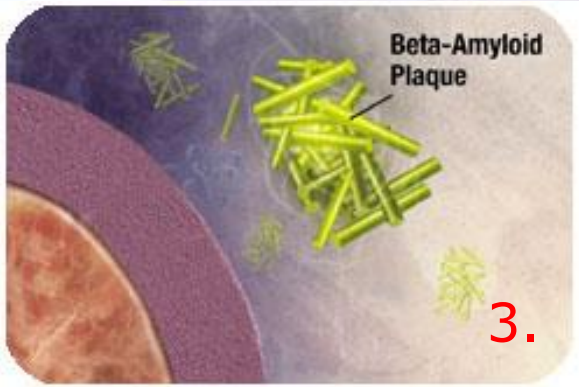
neurofibrillary tangles

http://abdellab.sunderland.ac.uk/lectures/Neurodegeneration/References/Brain_Neurons_AD_Normal.html

Amyloid-β plaques







Amyloid precursor protein (APP) is membrane protein that sits in the membrane and extends outward. It is thought to be important for neuronal growth, survival, and repair.

1. APP sticks through the neuron membrane.

2. Enzymes cut the APP into fragments of protein, including beta-amyloid.

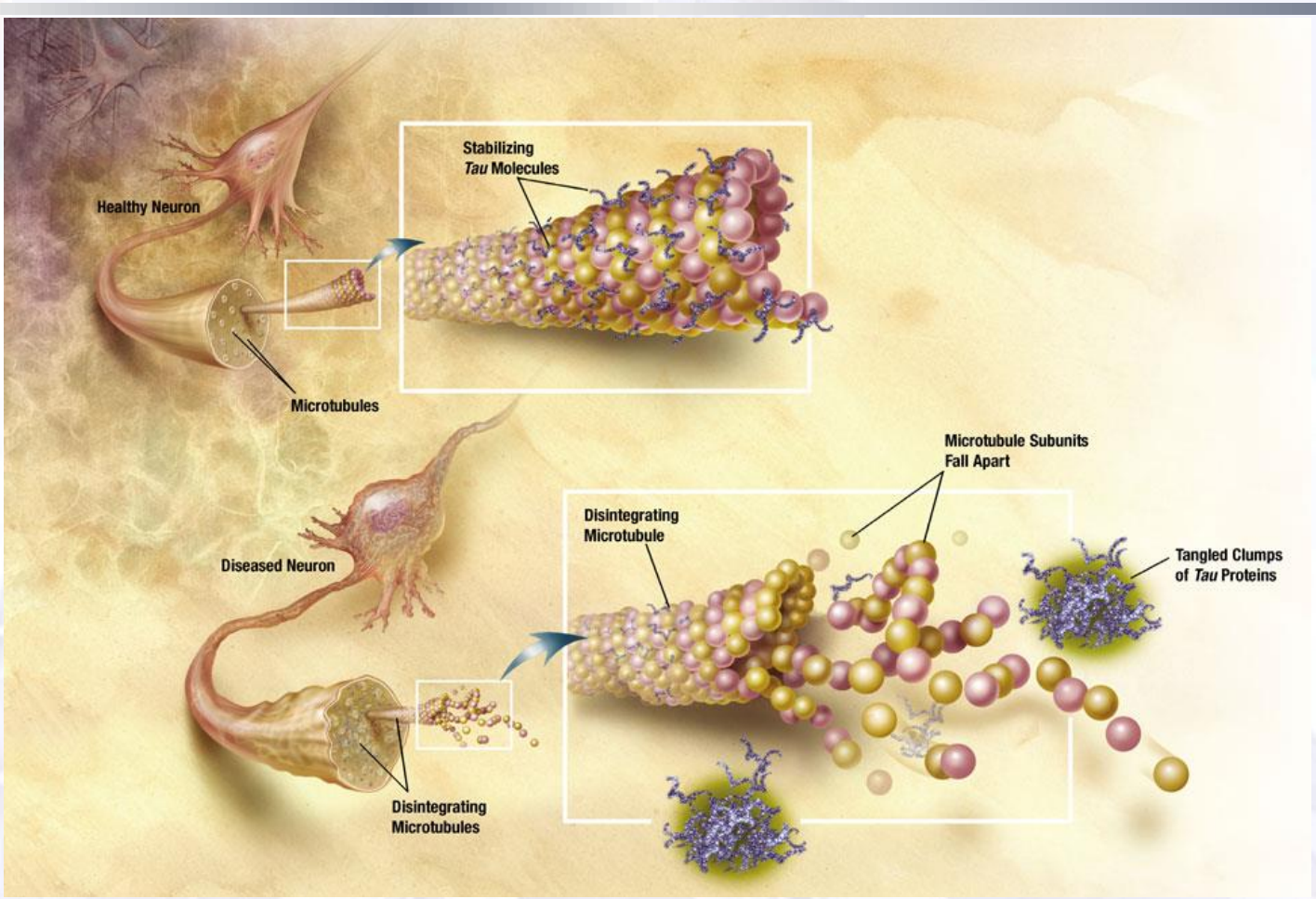
3. Beta-amyloid fragments come together in clumps to form plaques.

APP Protein:

Aβ40: Aβ42=10:1 in vivo

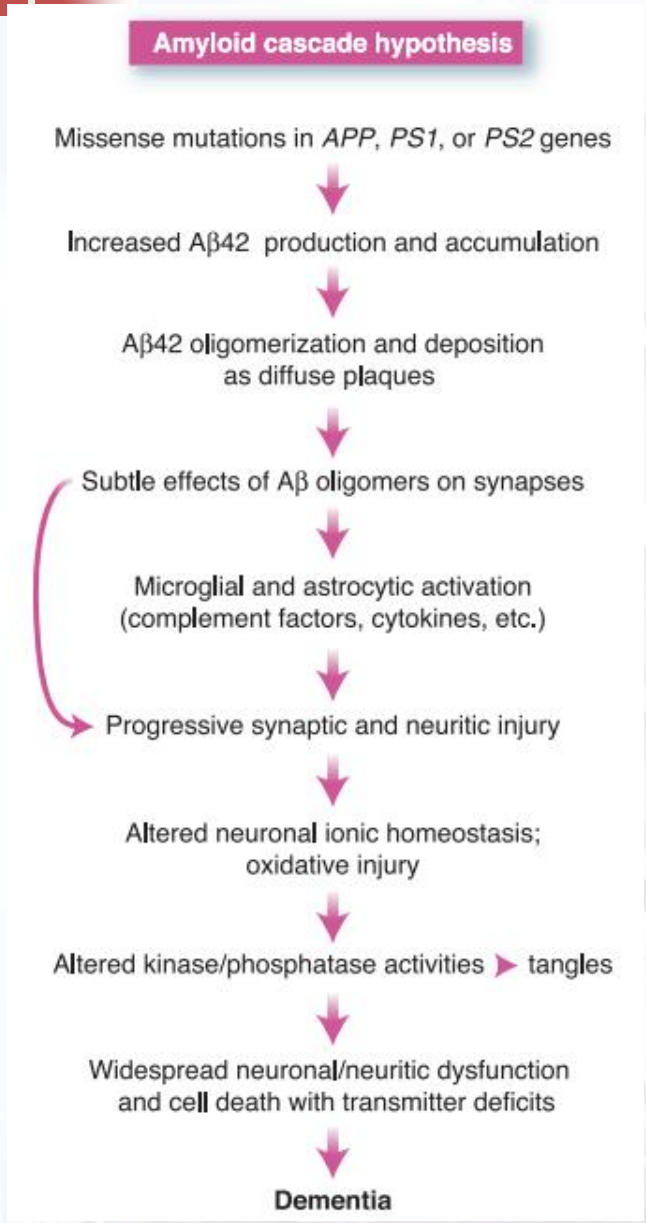
Aβ42 forms fibrils more rapidly and more neurotoxic.

Neurofibrillary Tangles



Microtubules are like railroad tracks that transport nutrition and other molecules. Tau-proteins act as "ties" that stabilize the structure of the microtubules. In AD, tau proteins become tangled, unstabilizing the structure of the microtubule. Loss of axonal transport results in cell death.

The Amyloid Hypothesis



Accumulation of Aβ in the brain is the primary influence driving AD pathogenesis.

1. Some humans without symptoms of AD have many critical A-beta deposits.
2. Transgenic mice expressing Aβ has revealed neurological deficits prior to amyloid deposition.

Soluble oligomers of A-beta are the proximate neurotoxins in AD.

Hardy, J.; Selkoe, D. J. *Science* **2002**, *297*, 353.

Oligomer Toxicity

- Interact with membrane

amphipathic peptide

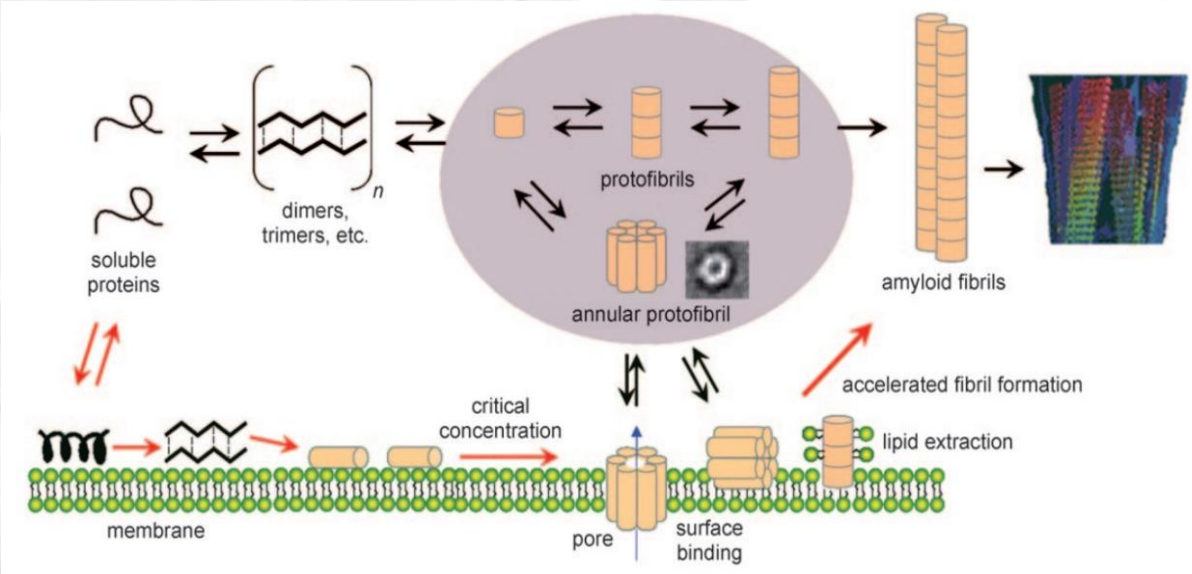
```
    5        10        15        20        25        30        35        40
DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA
```

I. Disrupt membrane integrity.

II. Increase membrane permeability.

Ion channel formation.
Membrane thinning.

facilitate charge translocation across the bilayer, release of membrane components (including cholesterol, phospholipids)



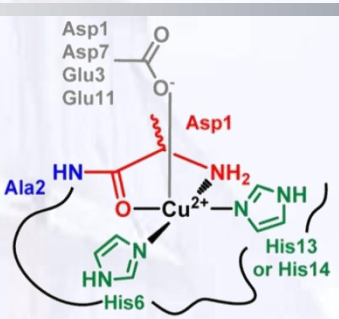
Butterfield, S. M.; Lashuel, H. A. *Angew. Chem. Int. Ed.* **2010**, *49* , 5628.

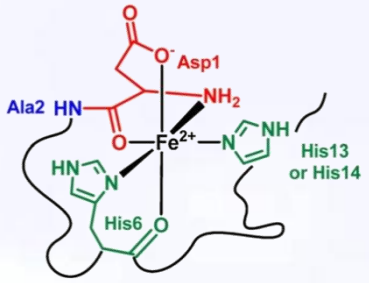
Oligomer Toxicity

- Bind with metals

1. Disrupt the metal homeostasis

2. Produce radical, reactive oxygen species, oxidative stress



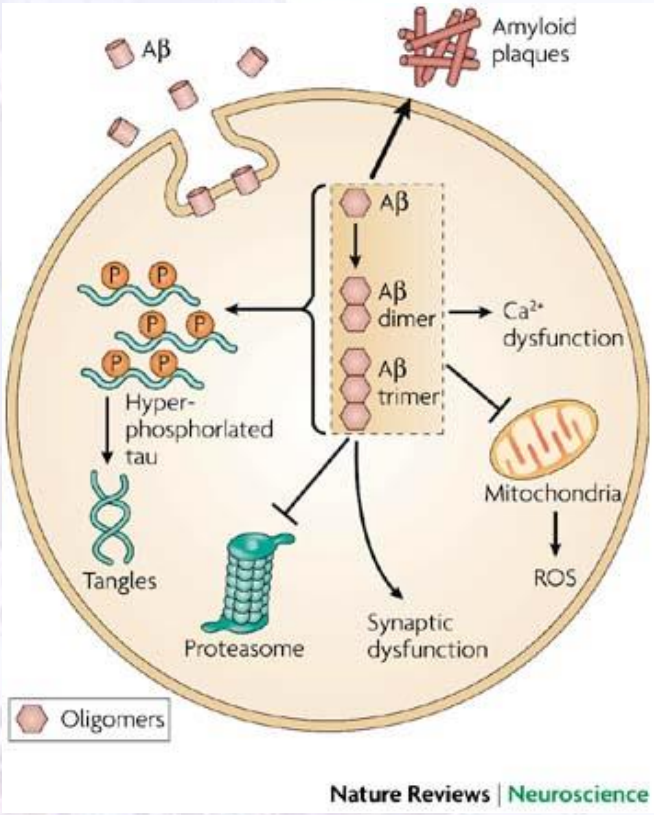


Catalyze Fenton reaction:

$$M^{n+} + H_2O_2 \rightarrow M^{(n-1)+} + HOO\cdot + H^+$$

- Mitochondrial dysfunctions

Tau hyperphosphorylation, disrupting proteasome(蛋白酶体) and mitochondrial(线粒体) function, and triggering calcium and synaptic dysfunction.



LaFerla, F. M.; Green, K. N.; Oddo, S. *Nat. Rev. Neurosci.* **2007**, *8*, 499.

Oligomer

- Fundamental Questions:

(1) What's the structures and relative abundance of small oligomers?

(2) Which types of oligomers are involved in the disease?

(3) How these oligomers are assembled?

- Difficulties in studying oligomers:

(1) Aβ is "natively unfolded" and preferentially form amyloid fibrils rather than protein crystals. X-ray diffraction×

(2) Aβ oligomers often are metastable and comprise structurally heterogeneous populations in equilibrium with monomers and fibrils.

pure populations of conformers: CD×, FT-IR×, SDS-page×

Teplow, D. B.; Lazo, N. D.; Bitan, G.; Bernstein, S.; Wyttenbach, T.; Bowers, M. T.; Baumketner, A.; Shea, J.-E.; Urbanc, B.; Cruz, L.; Borreguero, J.; Stanley, H. E. *Acc. Chem. Res.* **2006**, *39*, 635.

Oligomer

(3) Some conformations of Aβ oligomers are rarely-populated and variations of conformations can occur under different experimental conditions.

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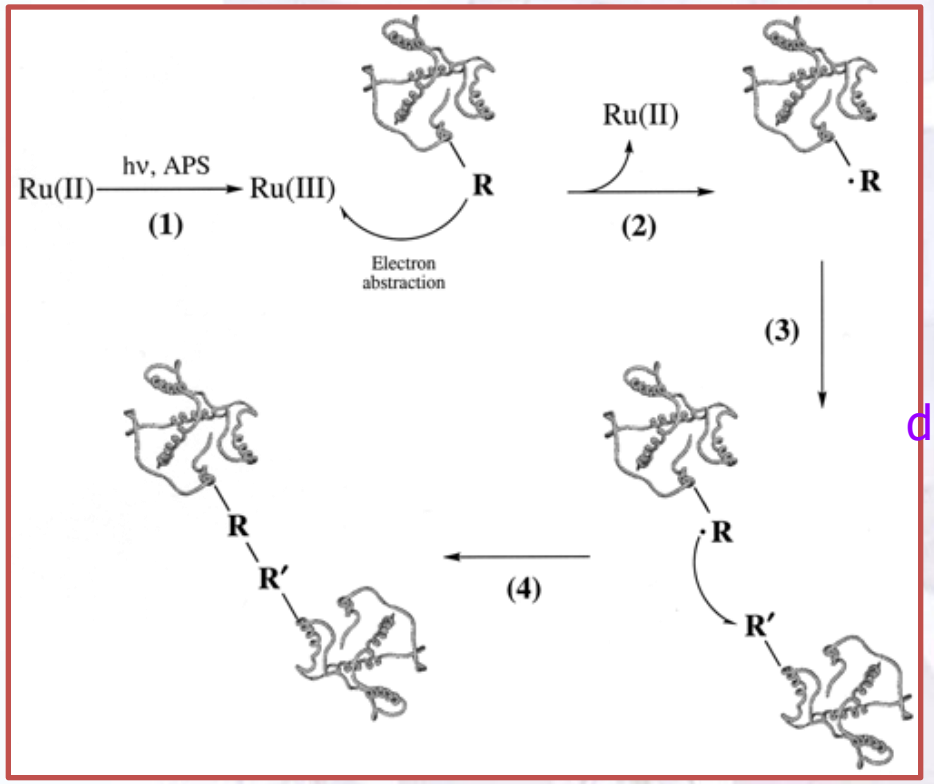
UCLA Alzheimer's Disease Research Center
Brain Research Institute
UCLA Health System Research
UCSF California Alzheimer's Disease Centers

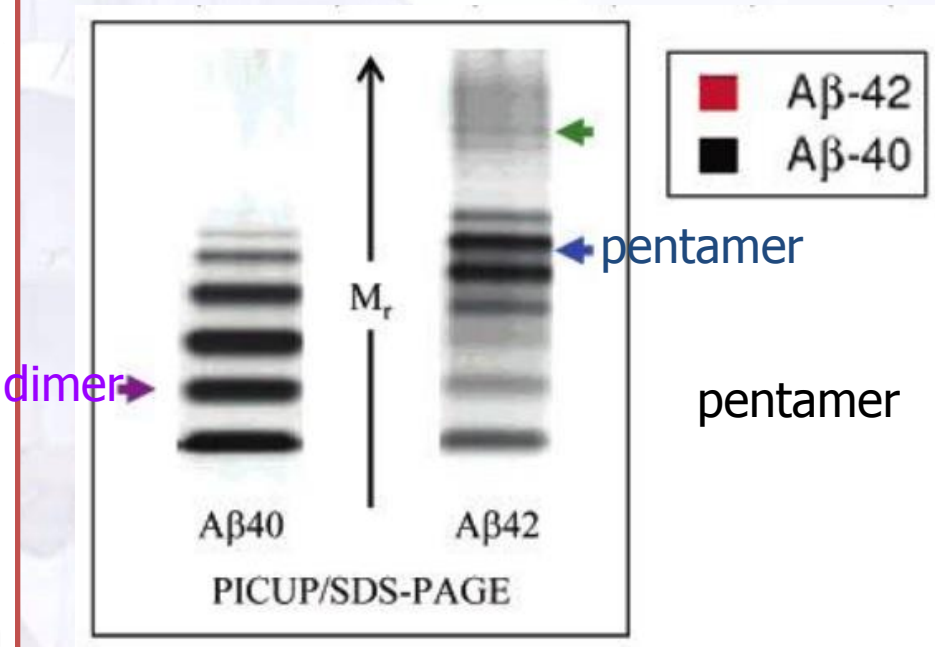
Teplow, D. B.; Lazo, N. D.; Bitan, G.; Bernstein, S.; Wyttenbach, T.; Bowers, M. T.; Baumketner, A.; Shea, J.-E.; Urbanc, B.; Cruz, L.; Borreguero, J.; Stanley, H. E. *Acc. Chem. Res.* **2006**, *39*, 635.

Study of Oligomer

- *In hydro* Studies---provide information about oligomers in solution

1. photoinduced cross-linking of unmodified proteins (PICUP)



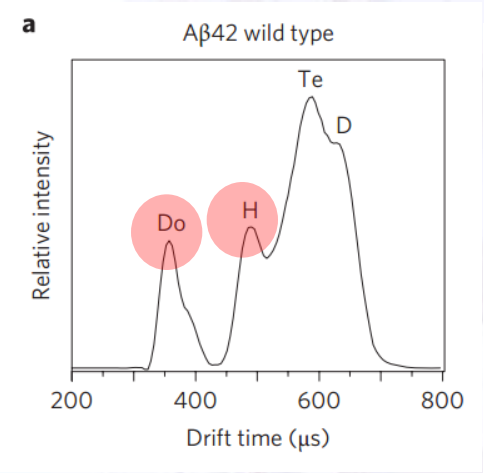


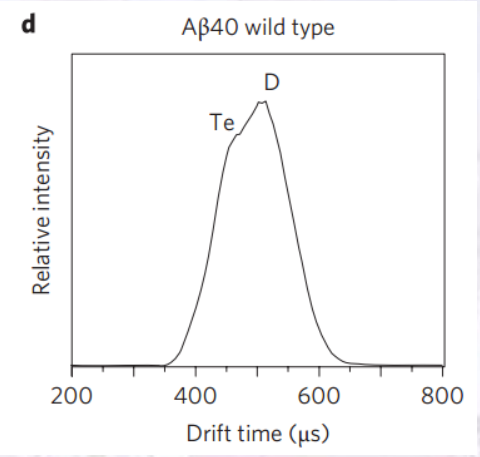
Bitan, G.; Teplow, D. B. Acc. Chem. Res. 2004, 37, 357.
Bitan, G.; Kirkitadze, M. D.; Lomakin, A.; Benedek, G. B.; Teplow, D. B. Proc. Natl. Acad. Sci. 2003, 100 , 330

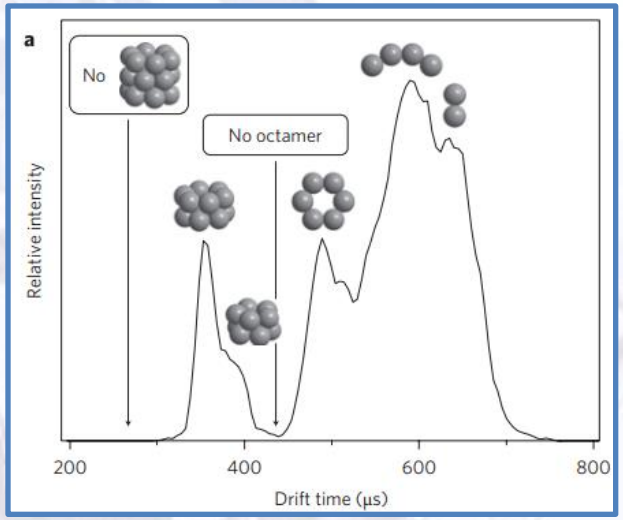
Study of Oligomer

- *In Vacuo* Studies:

1. ESI–MS has been shown to be capable of preserving non-covalently bound species
2. Detect rarely-populated conformers of highly dynamic proteins
3. IMS–MS is now accepted as a powerful method to determine conformational properties of unfolded and partially folded species, as well as natively folded proteins
4. IMS–MS determinates oligomer mass and shape and studies of self-association kinetics







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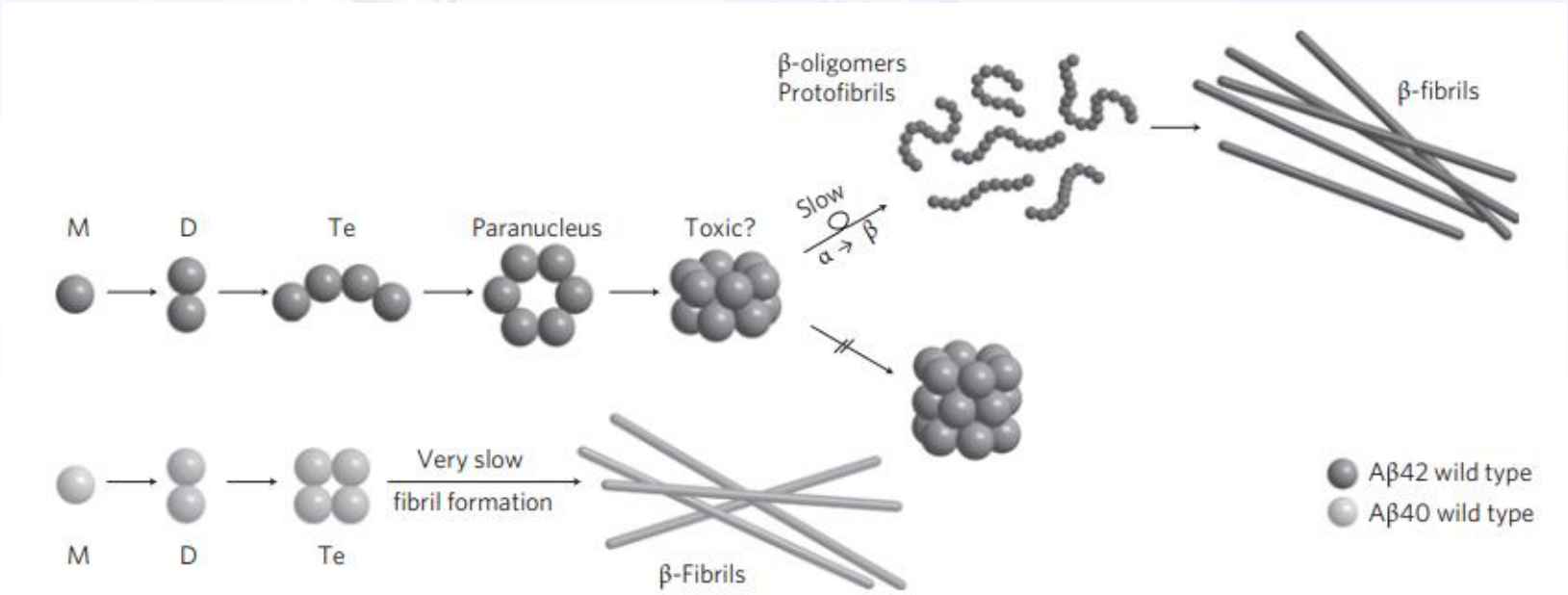
Mechanism of oligomerization



In Aβ42 an 'open' tetramer promotes the formation of the planar hexamer (paranucleus) and the stacked dodecamer.

For Aβ40 and other studied alloforms the key structure is the tetramer that resists further monomer or dimer addition.

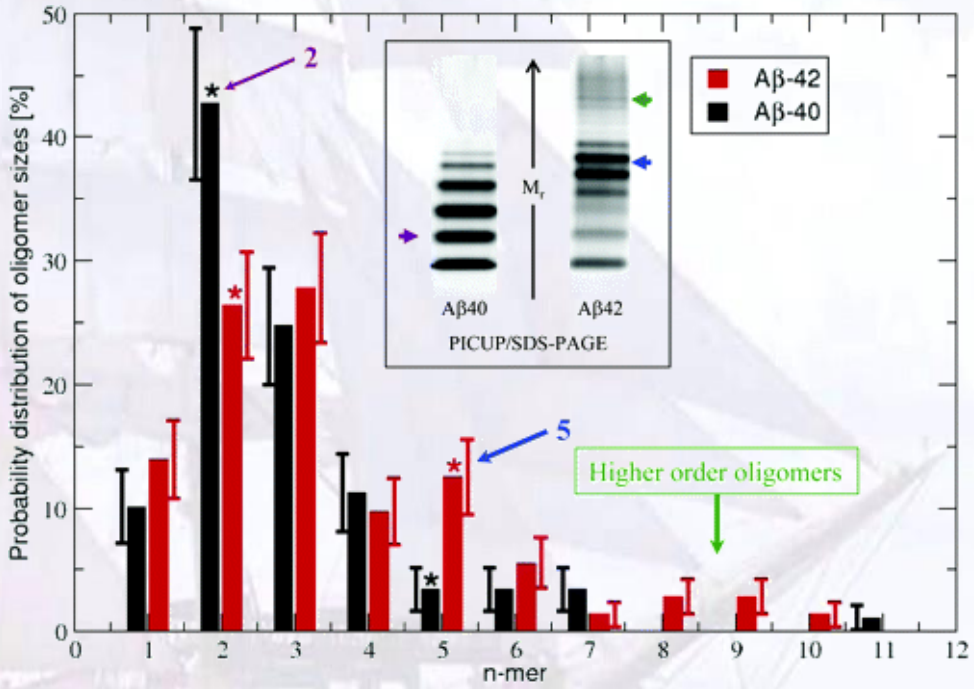
Bernstein, S. L.; Dupuis, N. F.; Lazo, N. D.; Wyttenbach, T.; Condron, M. M.; Bitan, G.; Teplow, D. B.; Shea, J.-E.; Ruotolo, B. T.; Robinson, C. V.; Bowers, M. T. *Nat. Chem.* **2009**, *1*, 326.

Study of Oligomer

- *In Silico* Studies:
  1. Provide structural information at atomic resolution.
  2. Provide information of assembly dynamics for oligomers.
- *In silico study of amyloid β-protein folding and oligomerization*

Method: discrete MD (DMD) algorithm with a coarse-grained protein model (four-bead protein model)

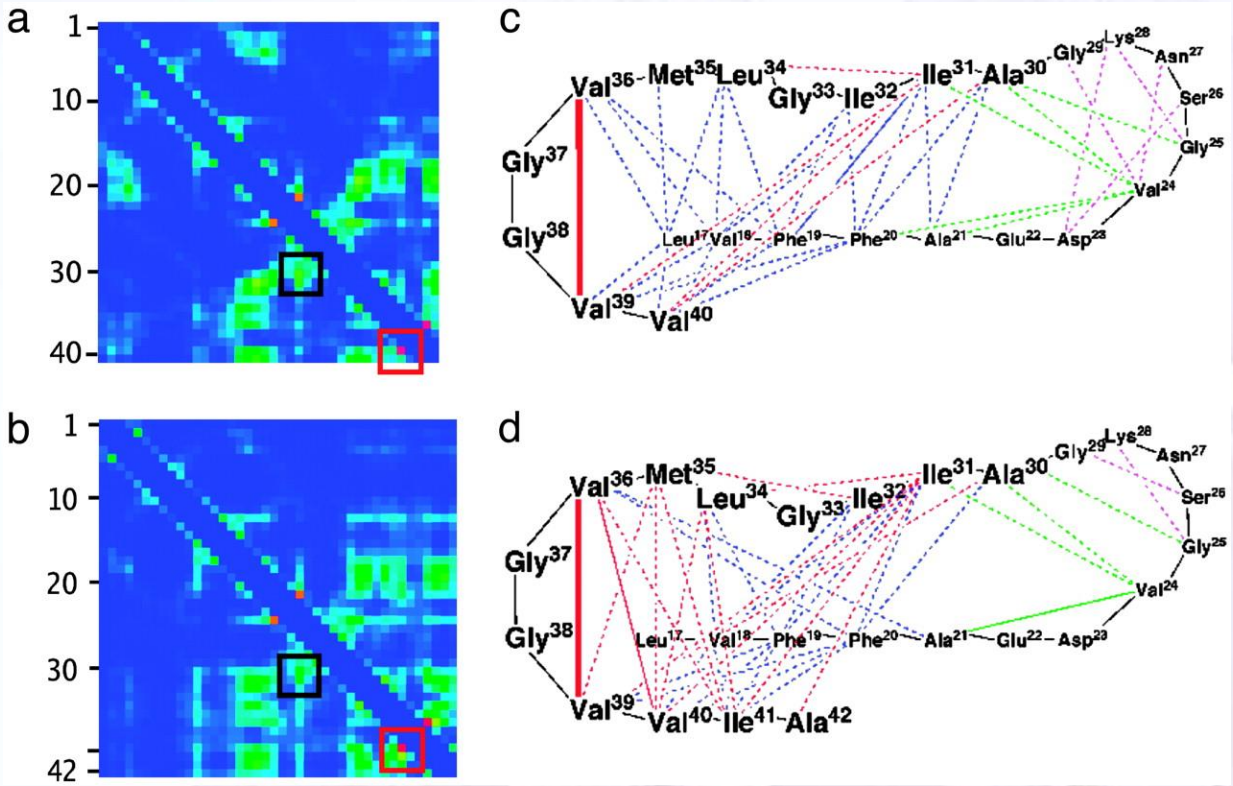
1: Oligomer Size Distributions of Aβ 40 and Aβ 42.



Urbanc, B.; Cruz, L.; Yun, S.; Buldyrev, S. V.; Bitan, G.; Teplow, D. B.; Stanley, H. E. *Proc. Natl. Acad. Sci.* **2004**, *101*, 17345.

Study of Oligomer

2: Intramolecular contacts in pentamers.



Average contact maps of intramolecular contacts within a pentamer conformation. The averages are calculated by using individual contact maps of 11 Aβ40 and 34 Aβ42 pentamers using three fixed simulation steps (9 million, 9.5 million, and 10 million simulation steps).

the Aβ42 contact map contains a significantly greater number of contacts.

These additional contacts do not involve only the Ile−Ala residues but result from the involvement of residues in the 1−40 region establishing contacts where none existed before.

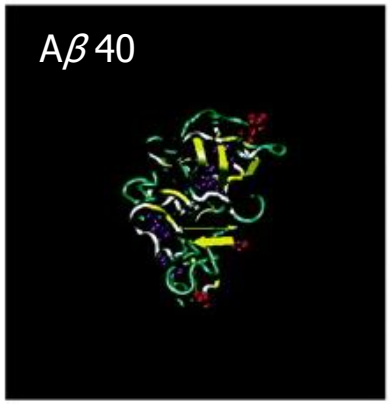
Met35 contacts the Aβ C-terminus in Aβ42 but not in Aβ40.

Oxidation of Met35 in Aβ42 blocked paranucleus formation and produced oligomers indistinguishable in size and morphology from those produced by Aβ40.

Urbanc, B.; Cruz, L.; Yun, S.; Buldyrev, S. V.; Bitan, G.; Teplow, D. B.; Stanley, H. E. *Proc. Natl. Acad. Sci.* **2004**, *101*, 17345.

Study of Oligomer

3: Geometrical Characteristics of Aβ Pentamers.



silver tube (random coil-like structure), light-blue tube (turn), and yellow ribbon (β-strand).
Red spheres:the N-terminal Asp-1.
Purple: the C-terminal amino acids Val-39 and Val-40
Green: the C-terminal amino acid Ile-41
Blue: Ala-42

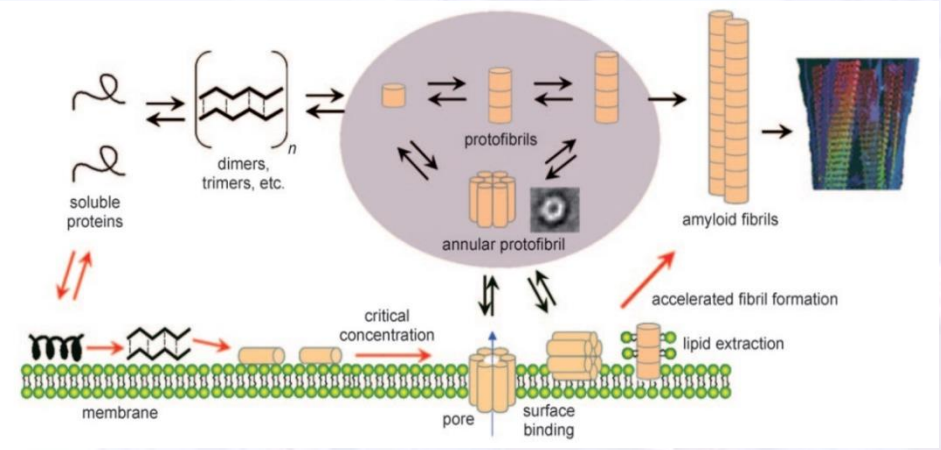
Commonalities: globular, C termini is in the assembly core and N termini on the surface.
Difference: the N termini of the Aβ42 pentamers are more extended and less structured.

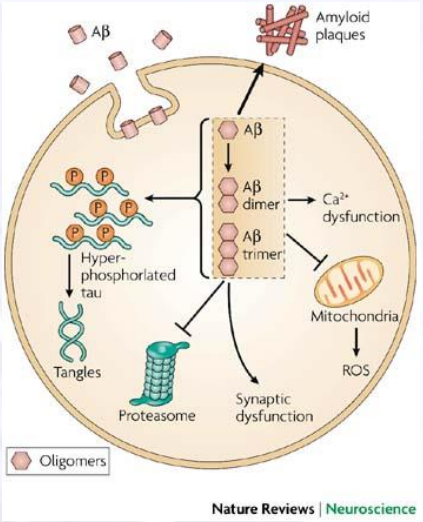
the N-terminal β-strand in Aβ40 may shield the hydrophobic core of the oligomer, hinder the intermolecular interactions among hydrophobic cores of multiple oligomers, thus hinder higher-order association reactions.

Urbanc, B.; Cruz, L.; Yun, S.; Buldyrev, S. V.; Bitan, G.; Teplow, D. B.; Stanley, H. E. *Proc. Natl. Acad. Sci.* **2004**, *101*, 17345.

Summary

- Soluble oligomers of Aβ are the proximate neurotoxins in AD.





- Study of oligomers is difficult task.

- Approaches to study oligomres:

(1) in hydro studies---PICUP method:

provide information in solution and a standard to MS and MD methods.

(2) in vacuo studies---IMS-MS method:

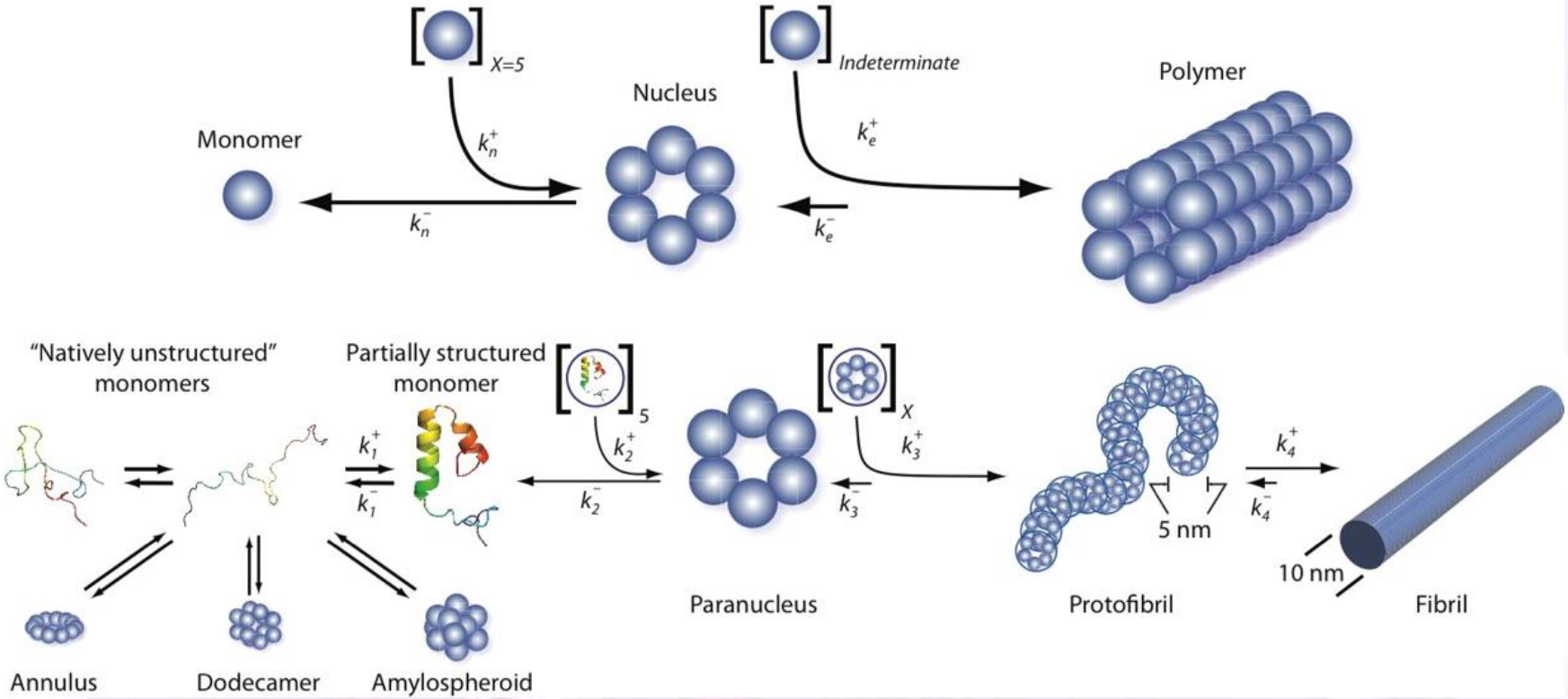
determinates oligomer mass and shape, detect rarely-populated conformers

(3) in silico studies---MD simulations:

provide high resolution structural information and information of assembly dynamics

Thanks for your attention!

Amyloid β-Protein Assembly



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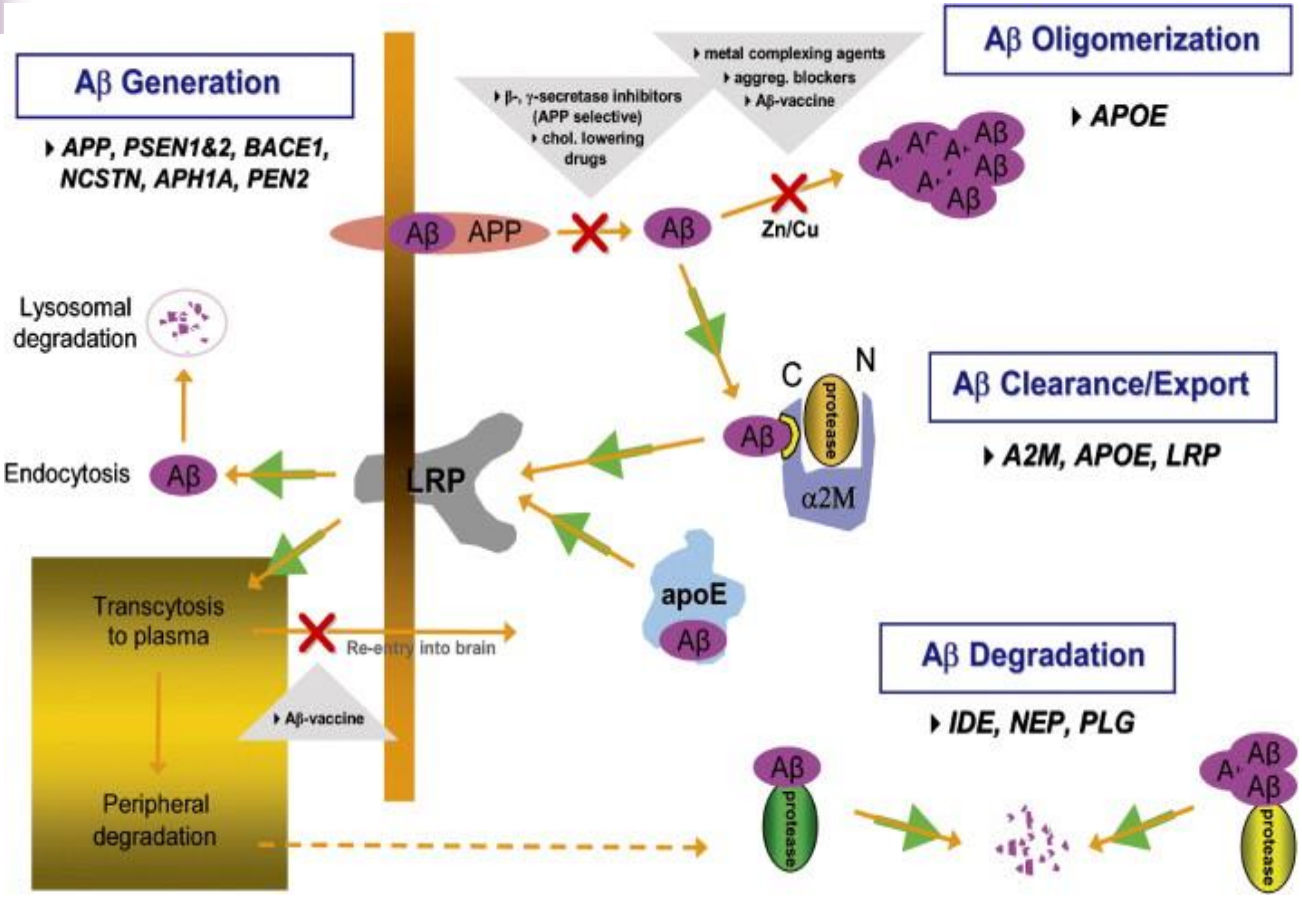
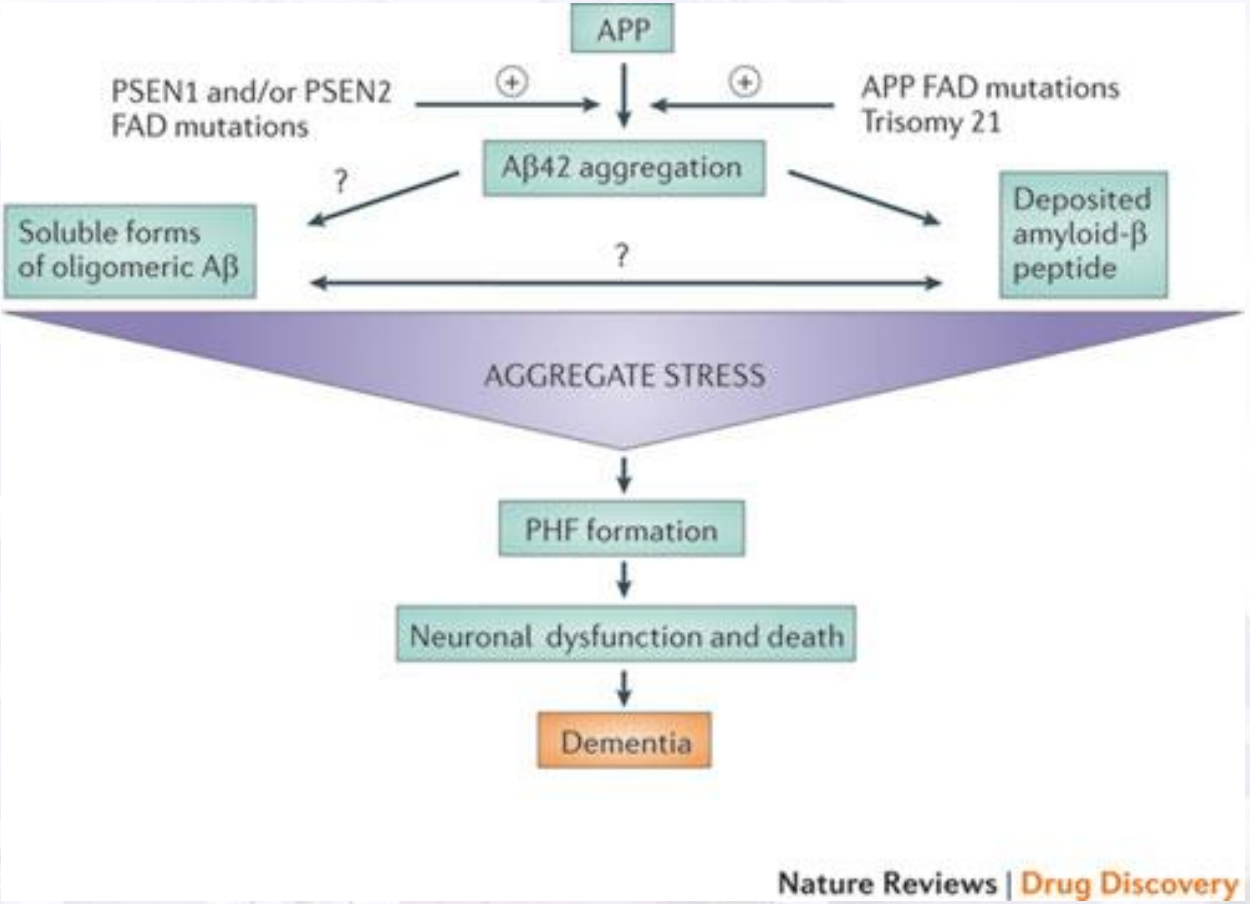

Aβ Generation
APP, PSEN1&2, BACE1, NCSTN, APH1A, PEN2
β-, γ-secretase inhibitors (APP selective)
chol. lowering drugs
metal complexing agents
aggreg. blockers
Aβ-vaccine
Aβ Oligomerization
APOE
Aβ
APP
Zn/Cu
Lysosomal degradation
Endocytosis
LRP
C
N
protease
α2M
Aβ Clearance/Export
A2M, APOE, LRP
apoE
Transcytosis to plasma
Re-entry into brain
Aβ-vaccine
Peripheral degradation
Aβ Degradation
IDE, NEP, PLG


APP
PSEN1 and/or PSEN2
FAD mutations
APP FAD mutations
Trisomy 21
Aβ42 aggregation
?
Soluble forms
of oligomeric Aβ
?
Deposited
amyloid-β
peptide
AGGREGATE STRESS
PHF formation
Neuronal dysfunction and death
Dementia
Nature Reviews | Drug Discovery

Therapy of AD

Drug	Manufacturer	Mechanism of action	Launched	US sales (2005)	Side effects
Donepezil (Aricept)	Eisai Inc.	Cholinesterase inhibitor; prevents the breakdown of acetylcholine in the brain	1997	$1.1 billion	Nausea, diarrhea, insomnia, vomiting, muscle cramps, fatigue, anorexia
Memantine (Namenda)	Forest Pharmaceuticals	NMDA receptor antagonist; blocks toxic effects associated with excess glutamate and regulates glutamate activation	2003	$498 million	Dizziness, headache, confusion, constipation
Rivastigmine (Exelon)	Novartis Pharmaceuticals Corporation	Cholinesterase inhibitor; prevents the breakdown of acetylcholine and butyrylcholine in the brain	2000	$226 million	Nausea, vomiting, loss of appetite, indigestion, weakness/ lack of energy, dizziness, diarrhea, headache, stomach pain
Galantamine (Razadyne)	Ortho-McNeil Neurologics Inc.	Cholinesterase inhibitor; prevents the breakdown of acetylcholine and stimulates nicotinic receptors to release more acetylcholine	2001	$223 million	Nausea, vomiting, diarrhea, anorexia, weight loss
Galantamine (Razadyne ER)	Ortho-McNeil Neurologics Inc.	Cholinesterase inhibitor; prevents the breakdown of acetylcholine and stimulates nicotinic receptors to release more acetylcholine	2005	$24 million	Nausea, vomiting, diarrhea, anorexia, weight loss

Source: IMS Health

Weak weapons: The drugs available for Alzheimer disease have shown only modest benefits

	Drug	Manufacturer	Mechanism of action	Stage of development	Side effects
New drugs	3APS (Alzhemed)	Neurochem, Inc.	inhibits amyloid-beta aggregates, binds and reduces soluble amyloid-beta	Phase 3	Nausea, vomiting
	MPC-7869 (Flurizan)	Myriad Pharmaceuticals	NSAID derivative; inhibits amyloid-beta aggregates and reduces their levels of amyloid-beta with little or no anti-inflammatory effect	Phase 3	None disclosed
	AAB-001	Elan Pharmaceuticals	monoclonal antibody binds to and clears amyloid-beta, is designed to directly deliver antibodies to amyloid-beta	Phase 2	None disclosed
	Neramexane	Forest Laboratories	NMDA receptor antagonist: blocks the effects of excessive glutamate at the receptor	Phase 3	None disclosed
Drugs for other conditions	Simvastatin (Zocor)	Merck	Statin; reduces cholesterol-carrying protein that promotes amyloid-beta aggregation	Phase 3	None disclosed for the trial, but Zocor has been known to cause nausea, diarrhea, abdominal pain and muscle cramps
	VP4896	Voyager Pharmaceutical	Hormone drug leuprolide acetate; decreases amount of luteinizing hormone in body, might prevent brain cell death	Phase 3	None disclosed
	Valproate	Manufacturer not disclosed	Anticonvulsant drug; neuroprotective properties may delay clinical progression of Alzheimer disease	Phase 3	None disclosed
Dietary supplements	Gingko biloba		Antioxidants neutralize free radicals and may reduce or prevent the damage they cause in brain cells	Phase 3	Headache, upset stomach, allergic reactions
	Vitamin E Selenium		Antioxidants neutralize free radicals and may reduce or prevent the damage they cause in brain cells	Phase 3	None disclosed

Target practice: Most candidates being tested for Alzheimer disease are based on the amyloid hypothesis

New strategy: Immunotherapy

Model	Antibody or antigen	Route of immunization	Effect	Refs
Pheochromocytoma cells	Amyloid-β_{1-28}-specific antibody	*In vitro*	Inhibition and solubilization of fibrils of amyloid-β peptide through antibody recognition of the amino-terminal Glu-Phe-Arg-His epitope	61–64
APP (V717F)-transgenic (PDAPP) mice	Amyloid-β_{1-42}	Subcutaneously with adjuvant	Reduced amyloid-β plaques, neuritic dystrophy and astrogliosis	65
APP (V717F)-transgenic (PDAPP) mice	Amyloid-β_{1-42}	Nasally	Reduced cerebral amyloid burden	66
APP (K670N, M671L, V717F)-transgenic (CRND8) mice and *APP* (K670N, M671L), *PSEN1* (M146L) double-transgenic mice	Amyloid-β_{1-42}	Subcutaneously with adjuvant	Reduced behavioural impairment and amyloid-plaque deposition	14,15
APP (K670N, M671L)-transgenic (Tg2576) mice	A non-fibrillar amyloid-β_{1-30} homologous peptide	Subcutaneously with adjuvant	Reduced Alzheimer's-disease-associated pathology	70
APP (V717I)-transgenic mice	Filamentous phage displaying amyloid-β_{1-6} (Glu-Phe-Arg-His epitope)	Intraperitoneally	Reduced amyloid-β plaques and behavioural impairment	79,80
APP (K670N, M671L)-transgenic (Tg2576) mice	Recombinant adeno-associated virus vector expressing amyloid-β_{1-21}	Orally	Reduced amyloid-β plaques	77
APP (K670N, M671L)-transgenic (Tg2576) mice	Amyloid-β-encoding DNA vaccine	Intramuscularly	Decreased amyloid burden due to antibodies induced by DNA vaccination	76
APP (V717F)-transgenic (PDAPP) mice	Amyloid-β_{1-6}- and amyloid-β_{3-6}-specific antibodies	Passive	Reduced amyloid-β plaques	67
APP (V717F)-transgenic (PDAPP) mice	Amyloid-β_{13-28}-specific antibody	Passive	Reversion of memory deficits without reduction of brain amyloid-β burden	68,69
APP (K670N, M671L, V717F)-transgenic (CRND8) mice	Amyloid-β_{1-40}- and amyloid-β_{1-42}-specific antibodies	Passive	Attenuation of amyloid deposition	82
APP (V717F)-transgenic (PDAPP) mice	Amyloid-β_{4-10}-specific antibody	Passive	Reduced amyloid-β plaques	83
APP (K670N, M671L)-transgenic (Tg2576) mice	Oligomeric amyloid-β_{1-40}-specific antibody	Passive	Reduced amyloid-β plaques, improvement in learning and memory	81

bapineuzumab
solanezumab

Amyloid-β-specific antibody as a mediator of amyloid clearance following active or passive immunization

Howard L. Weiner and Dan Frenkel . Nature 2006; 6: 404-417