Metals, proteins and neurodegeneration

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INTRODUCTION
Schematic model for brain iron homeostasis.

E. L. Que, D.W. Domaille, C. J. Chang
Schematic model of neuronal copper homeostasis.

E. L. Que, D.W. Domaille, C. J. Chang

Fluorescent Zinc Sensors

Imaging of Zn\(^{2+}\) secretion from pancreatic β-cells

- Adaptation of commercial Ca\(^{2+}\) sensor based on bis(o-aminophenoxy)-ethane-\(N,N,N',N'\)-tetraacetic acid (BAPTA) chelator
- The sensor binds Zn\(^{2+}\) with high affinity \((K_d = 15 \text{ nM})\) and a large fluorescence increase (over 200-fold)
- It can detect local bursts of endogenous Zn\(^{2+}\) excreted by pancreatic β-cells as far as 15 μm away from the cell
Redox-active metal ions (Fe, Cu) can generate oxidative stress by production of ROS

- James Fenton (1894) showed that Fe$^{2+}$ in the presence of H$_2$O$_2$ could oxidise tartaric acid:
  \[ \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \cdot\text{OH} + \text{OH}^- \] [1]
- It has been suggested that O$_2^-$ can reduce Fe$^{3+}$:
  \[ \text{Fe}^{3+} + \text{O}_2^- \rightarrow \text{Fe}^{2+} + \text{O}_2 \] [2]
- The sum of reactions [1] and [2] gives [3], the Haber Weiss (or Haber-Willstätter) reaction (1931-34)
  \[ \text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + \cdot\text{OH} + \text{OH}^- \] [3]
- Potentially, free Cu$^+$/$\text{Cu}^{2+}$ can do the same.
OXIDATIVE STRESS

ROS: $\bullet O_2^- \quad H_2O_2 \quad HO^\cdot \quad LOO^\cdot \quad LOOH$

BIOLOGICAL TARGETS

MITOCHONDRIAL MEMBRANE

CELLULAR MEMBRANE
Polygalloyl tannins due to stacking interactions and strong tetradsentate coordination are able to accumulate iron(III) cations.
Diseases that result from aberrant protein linkage

<table>
<thead>
<tr>
<th>β-linked protein</th>
<th>Inclusions</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuroserpin</td>
<td>Collins body</td>
<td>Familial encephalopathy with neuroserpin inclusion bodies</td>
</tr>
<tr>
<td>α-Synuclein</td>
<td>Lewy body</td>
<td>Familial Parkinson disease</td>
</tr>
<tr>
<td>Prion protein</td>
<td>vCJD amyloid</td>
<td>Creutzfeldt–Jakob disease</td>
</tr>
<tr>
<td>β-amyloid peptide</td>
<td>β-amyloid plaque</td>
<td>Alzheimer disease</td>
</tr>
<tr>
<td>Tau protein</td>
<td>Pick body</td>
<td>Frontotemporal dementia (Pick disease)</td>
</tr>
<tr>
<td>Huntingtin</td>
<td>Huntingtin inclusion</td>
<td>Huntington disease</td>
</tr>
</tbody>
</table>
Drugs for neurodegenerative diseases

- Alzheimer
- Parkinson
- ALS
<table>
<thead>
<tr>
<th>DISEASE</th>
<th>LESION/CORES AND COMPONENTS</th>
<th>LOCATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alzheimer's Disease (AD)*</td>
<td>Senile Plaques/β-amyloid, NAC Neurofibrillary tangles/PHF&lt;sub&gt;τ&lt;/sub&gt;</td>
<td>Extracellular</td>
</tr>
<tr>
<td>Amyotrophic Lateral Sclerosis (ALS)</td>
<td>Spheroids/NF subunits</td>
<td>Intracytoplasmic</td>
</tr>
<tr>
<td>Dementia with Lewy Bodies (DLB)#</td>
<td>Lewy Bodies/NF subunits, α-synuclein</td>
<td>Intracytoplasmic</td>
</tr>
<tr>
<td>Lewy Body Variant Alzheimer's Disease (AD + DLB)#</td>
<td>Senile Plaques/β-amyloid, NAC Neurofibrillary tangles/PHF&lt;sub&gt;τ&lt;/sub&gt; Lewy Bodies/NF subunits, α-synuclein</td>
<td>Intracytoplasmic</td>
</tr>
<tr>
<td>Multiple System Atrophy (MSA)#</td>
<td>Glial Cell Inclusions/α-synuclein</td>
<td>Intracytoplasmic</td>
</tr>
<tr>
<td>Neuronal intranuclear inclusion disease</td>
<td>Inclusions/Expanded polyglutamine tracts</td>
<td>Intranuclear</td>
</tr>
<tr>
<td>Parkinson's Disease (PD)#</td>
<td>Lewy Bodies/NF subunits, α-synuclein</td>
<td>Intracytoplasmic</td>
</tr>
<tr>
<td>Prion diseases</td>
<td>Amyloid plaques/Prion</td>
<td>Extracellular</td>
</tr>
<tr>
<td>Tauopathies*</td>
<td>Neurofibrillary tangles/AD-like PHF&lt;sub&gt;τ&lt;/sub&gt;</td>
<td>Intracytoplasmic</td>
</tr>
<tr>
<td>Trinucleotide repeat diseases</td>
<td>Inclusions/Expanded polyglutamine tracts</td>
<td>Intranuclear</td>
</tr>
<tr>
<td>Trinucleotide repeat diseases</td>
<td>Inclusions/Expanded polyglutamine tracts</td>
<td>Intranuclear</td>
</tr>
</tbody>
</table>

This table summarizes neurodegenerative disorders characterized by filamentous brain lesions in the extracellular space or within neurons or glia.

* AD is a heterogeneous dementing disorder, and one of several tauopathies. Other tauopathies are: progressive supranuclear palsy, Pick's Disease, corticobasal degeneration, FTDP-17 and Guam amyotrophic lateral sclerosis/parkinsonism dementia complex.

No treatments are known that will stop or reverse the progress of Alzheimer’s disease.

- Four FDA-approved drugs available that may be able to relieve symptoms for patients for a limited time such as memory loss, and other problems with thinking or reasoning.

- For patients with mild to moderate symptoms: Aricept®, Exelon®, Razadyne® or Galantamine®

- Moderate to severe symptoms of Alzheimer’s disease can be treated with Aricept®, Exelon® and Namenda®.
The only way to prevent neurodegeneration...
What is Parkinson’s disease?

- **Definition**
  - Degeneration of dopaminergic neurons which cause a decrease of dopamine in the brain.

- **Symptoms**
  - Tremor
  - Rigidity
  - Akinesia
  - Postural changes
  - Monotonous speech

Signals that control body movements travel along neurons that project from the substantia nigra to the caudate nucleus and putamen (collectively called the striatum). These "nigro-striatal" neurons release dopamine at their targets in the striatum. In Parkinson's patients, dopamine neurons in the nigro-striatal pathway degenerate for unknown reasons.
EXCLUSIVE
Michael J. Fox
THE FIGHT OF HIS LIFE
For the first time, the star tells all about his secret battle with Parkinson's disease: "I'm not crying 'What a tragedy,' because it's not. It's a reality"
Drugs in clinical development for Parkinson’s disease

They include several indirect and direct modulators of oxidative stress

Meissner et al. 2011
<table>
<thead>
<tr>
<th>Drug/Therapy</th>
<th>Function</th>
<th>Phase</th>
<th>Effect</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coenzyme Q10</td>
<td>Modulator of mitochondrial function</td>
<td>III</td>
<td>Change in UPDRS total score</td>
<td></td>
</tr>
<tr>
<td>Creatine</td>
<td>Modulator of mitochondrial function</td>
<td>III</td>
<td>Disease progression over 5 years</td>
<td></td>
</tr>
<tr>
<td>Deferiprone</td>
<td>Iron chelator</td>
<td>II/III</td>
<td>Decrease in substantia nigra iron overload (UPDRS I–IV)</td>
<td></td>
</tr>
<tr>
<td>Inosine</td>
<td>Urate precursor</td>
<td>II</td>
<td>Tolerability and safety</td>
<td></td>
</tr>
<tr>
<td>Isradipine CR</td>
<td>Calcium antagonist</td>
<td>II</td>
<td>Tolerability (UPDRS II and III)</td>
<td></td>
</tr>
<tr>
<td>G-CSF</td>
<td>Haematopoietic growth factor</td>
<td>II</td>
<td>UPDRS III</td>
<td></td>
</tr>
<tr>
<td>Green tea polyphenols</td>
<td>Antioxidant</td>
<td>II</td>
<td>Delay of progression of motor dysfunction</td>
<td></td>
</tr>
<tr>
<td>AAV2-Neurturin (CERE-120)</td>
<td>Neurotrophic growth factor; intraputaminal and intranigral injection</td>
<td>I/II</td>
<td>Change from baseline in UPDRS III in OFF condition</td>
<td></td>
</tr>
<tr>
<td>PDGF (sNN0031)</td>
<td>Intracerebroventricular injection of PDGF</td>
<td>I/II</td>
<td>Safety and tolerability (UPDRS)</td>
<td></td>
</tr>
<tr>
<td>Cogane (PYM-50028)</td>
<td>Oral neurotrophic factor modulator</td>
<td>II</td>
<td>Change from baseline in UPRDS II and III</td>
<td></td>
</tr>
</tbody>
</table>

Modified from: Meissner et al., Nature Reviews 2011
Levodopa (Bendopa®, Dopar®, Larodopa®)

- a dopamine precursor, increases dopaminergic activity of surviving DA neurons in substantia nigra.
- converted to DA by aromatic amino acid decarboxylase (AAAD) also called DOPA decarboxylase
Beneficial Effects of L-dopa
- Least Improved: Tremor
- Most Improved: Bradykinesia, Rigidity, Depression

Side-Effects of L-dopa
- Tachycardia, Arrhythmia
- Nausea, vomiting, anorexia
- Anxiety, Agitation, Insomnia
- Dyskinesia
- Orthostatic hypotension
- Hallucination, Delirium, Psychosis
Carbidopa

(Lodosyn® alone or Sinemet®, Atamet®, Parcopa® - when combined with L-dopa)

✓ blocks conversion of L-dopa to DA by L-aromatic amino acid decarboxylase (also AAAD or DOPA decarboxylase) outside the brain.

✓ neither carbidopa nor DA cross the blood brain barrier → more unmetabolized L-dopa gets into the brain and less is converted to DA in the periphery lessening nausea-related side-effects.
Anticholinergics (antimuscarinic)

Trihexyphenidyl (Artane®)

Benztropine (Cogentin®)

Drugs for Parkinson's Disease
Muscarinic Cholinergic Receptor (mAChR) Blockers

Older H₁ 'Antihistamines'

Orphenadrine (Dispiper®)

Diphenhydramine (Benadryl®)

Have antimuscarinic side-effects that are useful here

Amantadine HCl - has several possible mechanisms
Alzheimer’s disease (AD)

Mattson, M. 2003  Nature
Brain neuropathology in Alzheimer’s Disease:

✓ substantial neuronal loss in basal forebrain, hippocampus and associative cerebral cortex

✓ decreases in acetylcholine levels in these brain areas with reduction in choline acetyltransferase activity of 30-70%

Drugs Improving Cognition in Alzheimer's Disease

Acetylcholine Esterase Inhibitors

Increase ACh levels in brain areas important for memory / cognition in early Alzheimer's disease.

**Tacrine** (Cognex®) - side effects include nausea, abdominal cramping, anorexia and diarrhea - not CNS selective - limited use currently.

**Donepezil** (Aricept®), **rivastigmine** (Excelon®) and **galantamine** (Razadyne®) - more selective inhibition of acetylcholinesterase in brain, fewer peripheral side effects than the tacrine.
Drugs Improving Cognition in Alzheimer's Disease (Cont.)

N-Methyl-d-Aspartate Receptor (NMDAR) Inhibitors

Inhibit glutamate activation of NMDARs which may reduce Ca^{2+}-related neuronal excitotoxicity?

Ironically, NMDAR inhibitors are well known for blocking learning and memory in experimental models (e.g., prevent LTP - long-term potentiation).

**Memantine** (Namenda®) - for treatment of moderate to severe Alzheimer's disease - side effects include dizziness and headache.

Improves cognitive function – no clear evidence that disease progression is actually slowed.
Therapeutic strategies in Alzheimer’s disease:

...difficult to identify a target (oxidative stress? protein aggregation? redox active metal ions?)...

✓ antioxidants
✓ excitotoxicity modulators
✓ alternative approaches:
  ✓ inhibition of the expression of amyloidogenic protein
  ✓ inhibition of the release of amyloidogenic peptide
  ✓ inhibition of Ab-amyloid aggregation

Chelation therapy seems to be a reasonable strategy.
Design of clinically useful metal chelators:

✓ Metal selectivity:

iron selective chelators for the treatment of PD;
iron/copper/zinc chelators for the investigation and eventual treatment of AD.

✓ Clear guidelines for the design of iron-selective chelating agents;
not so clear in the case of copper and zinc

✓ Any agent that binds copper(II) tightly will also bind iron(II), zinc(II), nickel(II), cobalt(II) and manganese(II), thereby causing a potential toxic insult to most cell types

✓ Permeation of the blood–brain barrier (BBB): size of chelator should be less than 300Da → this excludes hexadentate ligands and seriously limits the potential for the design of selective copper(II) and zinc(II) chelators (In order to achieve greater than 70% oral absorption, the chelator molecular weight should be 500).
Most common iron(III) chelators:

1. [Structural formula](#)
2. [Structural formula](#)
3. [Structural formula](#)
4. [Structural formula](#)
5. [Structural formula](#)
6. [Structural formula](#)
7. [Structural formula](#)
8. [Structural formula](#)
9. [Structural formula](#)
10. [Structural formula](#)
11. [Structural formula](#)
Chelators with potential in the treatment of neurodegenerative diseases

Chelators with a broad selectivity for transition metals generally use nitrogen atoms as ligands.

However, the metal complexes of these ligands are positively charged, tend to bind to membranes and, by virtue of their net charge, do not penetrate membranes readily.
Amotrophic Lateral Sclerosis (ALS)

The loss of motor neurons leads to muscle weakness and atrophy as well as eventual denervation leading to loss of control of all voluntary movement, including swallowing and inspiration.

Riluzole (Rilutek®) – extends survival by several months in those with difficulty swallowing and ventilation. But: degenerative loss of motor neurons is not stopped! Mechanism of action – not clear; appears to reduce release of glutamate and increase inactivation of voltage-gated sodium channels, possibly slowing excitotoxicity.

Riluzole (Rilutek®)

World renowned Physicist
Stephen Hawking has ALS
The diagram illustrates a protein domain with various functional regions. Key features include:

- **Signal peptide** (beginning of the protein)
- **Cysteine-rich** domain
- **Negatively charged** region
- **Protease inhibitor** domain
- **Transmembrane** section
- **Cytoplasmic** domain (end of the protein)

The protein sequence shown includes:

```
-KM-673DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIAT-713VIVITLVML-KKK-
```

Specific regions of interest are highlighted:

- **β-amyloid peptide**
- **β-secretase**
- **γ-secretase**
Effect of pH on metal ion-induced Aβ1-40 aggregation: proportion of aggregated Aβ1-40 following incubation (30 min. 37°C) with various metals ions (30 μM) at pH 6.6 or 7.4 after centrifugation (10,000 × g, 20 min.) expressed as a percentage of starting peptide (2.5 μM).
The best ten structures of the free Aβ28 from rat (A) and from human (B) obtained through restrained simulated annealing based on the experimental data. (C) Superimposition of the first structure from rat (blue) and human (red) Aβ28. The figure was created with MOLMOL 2K.1.0.

Snapshots from the MD simulation of Zn$^{2+}$- rAβ28 complex, superimposed with the structure obtained from experimental data. Two different regions are obtained: (A) the C-terminal one showing helical structural elements among 16-24 residues; (B) the N-terminal one illustrating the metal binding domain with Zn$^{2+}$ tetrahedrally coordinated to Asp-1 NH$_2$, His-6 N$\delta$, Glu-11 COO$^-$ and His-14 N$\varepsilon$. The figure was created with MOLMOL 2K.1.0.

Snapshots from the MD simulation of Zn$^{2+}$- hAb28 complex, superimposed with the model based on experimental data. (A) global view of the peptide conformation; (B) metal binding domain with Zn$^{2+}$ coordinated to Asp-1 NH$_2$, His-6 Nδ, Glu-11 COO$^-$, His-13 Nε and His-14 Nε. The figure was created with MOLMOL 2K.1.0.

Snapshots from the MD simulation of the Cu(II)-rAβ complex: (A) the backbone is shown as ribbon, the copper ion and the two coordinating histidines in green. (B) the Cu(II) binding region: the Asp-1, His-6 and His-14 nitrogen donors (blue spheres) bound to Cu(II) (green sphere) and the distances Cu(II)-COO$^-$ from Asp-1 are shown.

### Synucleinopathies

<table>
<thead>
<tr>
<th>Parkinson’s disease (PD)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sporadic</td>
<td></td>
</tr>
<tr>
<td>Familial with αS mutations</td>
<td></td>
</tr>
<tr>
<td>Familial with mutations other than αS</td>
<td></td>
</tr>
</tbody>
</table>

**Dementia with Lewy bodies**

- “Pure” Lewy body dementia
- Lewy body variant of Alzheimer disease
- Familial Alzheimer disease with APP<sup>a</sup> mutations
- Familial Alzheimer disease with PS-1<sup>b</sup> mutations
- Familial Alzheimer disease with PS-2<sup>b</sup> mutations
- Down syndrome

**Multiple system atrophy**

- Shy-Drager syndrome
- Striatonigral degeneration
- Olivopontocerebellar atrophy

**Neurodegeneration with brain iron accumulation, type 1**

- Hallervorden-Spatz syndrome
- Neuroaxonal dystrophy

**Other diseases that may have synuclein-immunoreactive lesions**

- Traumatic brain injury
- Pick disease
- Amyotrophic lateral sclerosis

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α-Synuclein (αS) and Neurodegeneration

**Hallmark of synucleinopathies**

Loss of dopaminergic neurons in SN pars compacta and inclusion bodies accumulation.

The major component of Lewy bodies is fibrillar αS protein.

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<sup>a</sup> APP=amyloid precursor protein; <sup>b</sup> PS-1 and PS-2 = presenilin-1 and -2.
Primary structure of α-synuclein

R1 R2 R3 R4 R5 R6

NAC region Acidic tail

Region essential for assembly

A30P A53T

<table>
<thead>
<tr>
<th></th>
<th>1-140</th>
<th>4-60</th>
<th>61-95(NAC)</th>
<th>104-140</th>
</tr>
</thead>
<tbody>
<tr>
<td>αS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>w. t.</td>
<td>4.7</td>
<td></td>
<td>9.7</td>
<td>4.5</td>
</tr>
<tr>
<td>E46K</td>
<td>4.8</td>
<td>9.9</td>
<td></td>
<td>3.1</td>
</tr>
</tbody>
</table>
Schematic representation of plausible Cu$^{2+}$ binding modes to $\alpha$-synuclein
High-affinity ($K_d \sim 0.1 \mu M$) binding sites are located at the N-terminal domain:

1MDVFMKGLS$^9$

...VGSKTKEG$^48$VVHGVATV$^{52}$AEKTKE...

$\alpha S_{45-55}$  Ac-$^45$KEG$^50$VVHG$^55$VATV$^{55}$-NH$_2$

A53T  Ac-KEG$^53$VVHGVT$^{55}$TV-NH$_2$

E46K  Ac-K$^46$GVVHG$^{53}$VATV-NH$_2$
Structure of Cu(II) complexes of $\alpha S_{45-55}$ fragments

### K_d values (M) calculated from stability constants

<table>
<thead>
<tr>
<th>Ref.</th>
<th>N-terminus</th>
<th>His-50 site (WT)</th>
<th>His-50 site (E46K)</th>
<th>His-50 site (A53T)</th>
<th>pH</th>
<th>Exp. conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>$0.7 \pm 0.2 \times 10^{-9b}$</td>
<td>$1.5 \pm 0.4 \times 10^{-6}$</td>
<td>$1.0 \pm 0.4 \times 10^{-6}$</td>
<td>$2.0 \pm 0.5 \times 10^{-6}$</td>
<td>7.5</td>
<td>25 °C, no buffer, KCl 0.1 M</td>
</tr>
<tr>
<td>a</td>
<td>$3.1 \pm 0.7 \times 10^{-8b}$</td>
<td>$1.2 \pm 0.2 \times 10^{-4}$</td>
<td>$1.2 \pm 0.2 \times 10^{-4}$</td>
<td>$1.0 \pm 0.2 \times 10^{-4}$</td>
<td>6.5</td>
<td>25 °C, no buffer, KCl 0.1 M</td>
</tr>
<tr>
<td>27</td>
<td>$1.2 \pm 1.6 \times 10^{-7}$</td>
<td>$0.4 \pm 0.4 \times 10^{-4}$</td>
<td>—</td>
<td>—</td>
<td>6.5</td>
<td>15 °C, 20 mM MES, NaCl 0.1 M</td>
</tr>
<tr>
<td>21</td>
<td>$2.0 \pm 0.2 \times 10^{-7}$</td>
<td>$0.6 \pm 0.2 \times 10^{-4}$</td>
<td>—</td>
<td>—</td>
<td>6.5</td>
<td>15 °C, 20 mM MES, NaCl 0.1 M</td>
</tr>
<tr>
<td>24</td>
<td>$1.0 \times 10^{-7}$</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>7.0</td>
<td>25 °C, 20 mM MOPS, NaCl 0.1 M</td>
</tr>
<tr>
<td>19</td>
<td>$2.1 \pm 0.2 \times 10^{-10}$</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>7.4</td>
<td>37 °C, No buffer</td>
</tr>
</tbody>
</table>

*This work. b Complex-formation constant data taken from ref. 29.*

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Conclusions

- α-synuclein His$_{50}$ spanning fragment ($\alpha$S$_{45-55}$) binds Cu(II) effectively within physiological pH in the manner independent from familiar mutations. However, at subphysiological pH (~6.5) Glu$_{46}$ dependent binding was observed.

- α-synuclein His$_{50}$ spanning segment $\alpha$S$_{45-55}$ may constitute Cu(II) anchoring site independent from the N-terminus. However, due to controversy, there is still extensive debate on that within scientific community.

- Competition between the N-terminus and His$_{50}$ Cu(II) binding domains at physiological pH, might indicate the importance of this anchoring site in affecting protein aggregation rates and oligomerization especially at high concentration of copper.
Prion Diseases

- Creutzfeldt-Jakob Disease (CJD)- human 85% of all human prion disease. NO link to any other disease - sporadic
- Gerstmann-Sträussler-Scheinker Syndrome - human prion disease linked to point mutations - inherited
- Variant CJD - over 150 cases since 1995 with most cases in the UK and a small number in other countries - cause unknown (possibly BSE)
- Scrapie - disease of sheep - sporadic
- Chronic Wasting Disease - disease of deer and elk - sporadic
- Bovine Spongiform Encephalopathy - Europe wide,
- Experimental scrapie (rodents) - “infection”
Kuru was among the first of the diseases to be identified. Beginning in the 1950s, D. Carleton Gajdusek studied a neurological disorder common among the Fore people of New Guinea.
Mad cow questions?

Any other ideas for turning people vegan?

Mad Cow Disease
Avian flu
Swine flu
Mad Cow Disease and Creutzfeldt – Jakob (CJD)

• Similar to Alzheimer’s disease in that long neurofibrillary tangles are formed by aggregated prion protein

• The protein is the infectious particle aggregating in the cell
Prion Disease in Human

Cow brain with BSE  Human brain with CJD
PrP and Prion Hypothesis

Models for the three manifestations of prion diseases
Scrapie Pathology

Plaque - PrP\textsuperscript{Sc}

Spongiform Changes
Prion Protein Fibrils

View of Purified Protein under an Electron Microscope
The α-helical structure of Syrian hamster recombinant PrP 90-231, which presumably resembles that of the cellular isoform (PrP<sub>C</sub>)

**A plausible model of the tertiary structure of human PrP<sub>Sc</sub>**

*S. B. PRUSINER*  
The Essence of Prion Disease

Conversion of Normal cellular prion protein to an abnormal form

PrP<sub>c</sub> → ???? → PrP<sub>Sc</sub>

- GPI anchored
- Monomeric
- Protease sensitive
- Binds copper
- Synaptic association

- Extracellular
- Aggregated
- Protease resistant
- No specific copper bound
- High beta sheet content
- Associated with infectivity

D. R. Brown
Three-dimensional structure of human prion protein hPrP (23-230)

The mammalian prion protein (PrP\textsuperscript{C}) is a cell surface protein consisting of a flexibly disordered N-terminal segment (residues 23-120) and a structured C-terminal domain (residues 121-231).

- **Sugar moieties**
- **Unstructured N-terminal**
- **Octarepeats (residues 60-91): (PHGGGWGQ\textsubscript{4})**
- **Structured C-terminal**
- **Glycosylphosphatidylinositol (GPI) anchor**
- **Plasma membrane**
“The cellular prion protein binds copper in vivo”

Copper Stimulated Internalisation of GFP tagged PrP and Mutants

<table>
<thead>
<tr>
<th></th>
<th>GFP-PrP</th>
<th>Δ23-38</th>
<th>Δ51-89</th>
<th>Δ67-89</th>
<th>Δ112-119</th>
</tr>
</thead>
<tbody>
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<td>0</td>
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<td>10 min</td>
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<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
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<td><img src="image14.png" alt="Image" /></td>
<td><img src="image15.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Confocal images of individual cells exposed to 100 mM Cu

D. R. Brown
Mutants in Culture

Signal Peptide  Octameric Repeats  Hydrophobic Region  GPI Anchor Signal Peptide

WT

△23-38

△51-89

△67-89

△112-119

GFP - GPI Contro

D. R. Brown
Copper Stimulated Internalisation of GFP tagged PrP and Mutants

<table>
<thead>
<tr>
<th></th>
<th>GFP-PrP</th>
<th>Δ23-38</th>
<th>Δ51-89</th>
<th>Δ67-89</th>
<th>Δ112-119</th>
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<td>0</td>
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<td><img src="image2.png" alt="Image" /></td>
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</tr>
</tbody>
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Confocal images of individual cells exposed to 100 mM Cu

D. R. Brown
Schematic showing the mechanism of internalisation of PrP$^C$. Upon Cu$^{2+}$ binding to the octapeptide repeats (blue), the protein undergoes a conformational change that dissociates it from the raft-resident partner and PrP$^C$ then moves laterally out of rafts into detergent-soluble regions of the plasma membrane. The polybasic N-terminal region then interacts with the ectodomain of a transmembrane protein that engages, via its cytoplasmic domain, with the adaptor protein AP-2 and the endocytic machinery of clathrin-coated pits.

Recombinant Prion Protein Refolded with Cu is a Potent Antioxidant

Inhibition of Xanthine Oxidase Toxicity to Cerebellar Neurones in Culture

% Inhibition of Toxicity

Protein added in µg/ml

Refolded

+ Cu

- Cu
Prion Protein Structure

**PrP^Sc**
- Region constituting proteinase resistant core
- N-terminal Cleavage
- Sugar chain attachment

**PrP^c**
- Signal Peptide
- Hydrophobic core
- Disulfide bridge
- GPI Anchor Signal Peptide

**WGQ (PHGGGWGQ)_4**

**Copper Binding region**
Primary Structure of Prion Octapeptide Repeat

Pro-\textbf{His}-\textbf{Gly}-\textbf{Gly}-\textbf{Gly}-\textbf{Gly}-\textbf{Trp}-\textbf{Gly}-\textbf{Gln}
Plausible structure of Cu(II) complex with PHGGGWG in neutral pH

M. Luczkowski et al
(2002) 2269–2274
Primary Structure of Prion Octapeptide Repeat

Pro-His-Gly-Gly-Gly-Gly-Trp-Gly-Gln
Schematic representation of structure of Cu$^{2+}$ complex of octarepeat tetramer
The comparison of Cu,Zn-SOD active site with the fragment of ChPrP
Other regions of PrPC can bind copper ions…
Possible Cu$^{2+}$ binding sites in PrP$^C$
Neurotoxic Prion Peptide PrP106-126

PrP106-126 is a good model for PrPSc, because:
- it is neurotoxic
- it requires the expression of PrPC
- it form fibrills
Structure hypothesis for the complex \([\text{CuLH}]^{3+}\)

pH = 5.7

E. Gaggelli, F. Bernardi, R. Pogni, D. Valensin, G. Valensin, M. Remelli, M. Luczkowski H. Kozlowski,
_J. Am. Chem. Soc._ 127, 996-1006, 2005
Mad cow answers

EAT
MOR
CHIKIN
SUPRISE!

Dude! Not yet.
REALITY-TV
Chicken Lifestyle

In Put

Out Put

Kaput
\((\text{HNPGYP})_5 - \text{QNPGYYPHNP} \text{GYP}\)

\[
\begin{align*}
\text{Ac-}[\text{HNPGYP}]_2 - \text{NH}_2 & \div 1\text{Cu}^{2+}, 2\text{Cu}^{2+} \\
\text{Ac-}[\text{HNPGYP}]_4 - \text{NH}_2 & \div 1\text{Cu}^{2+}, 2\text{Cu}^{2+}, 3\text{Cu}^{2+}, 4\text{Cu}^{2+}
\end{align*}
\]
Model of CuH₄L complex \{4N_{im}\}
Primary Structure of Chicken Prion Hexapeptide Repeat

Ac-His-Asn-Pro-Gly-Tyr-Pro-NH$_2$
Best five structures of Cu(II)Ch-PrP hexapeptide trans-trans (a) and cis-trans (b) conformers

P. Stanczak, D. Valensin, P. Juszczyk, Z. Grzonka, G. Valensin, F. Bernardi, E. Molteni, E. Gaggelli and H. Kozlowski,
ChemComm. 26, 3298-3300, 2005
Avian Prion Protein

FSKKGKGKPSGGGWGAGSHRQPSYPRQ PGYP
HNPGYP HNPGYP HNPGYP HNPGYP HNPGYP QNPGYP HNPGYP GWGQGYN PS

SGGSYH\textsuperscript{110}NQ KPWKPPKTNFKH\textsuperscript{124}VAGAAAAGA

VVGGLGGYAMGRVIQ

Amyloidogenic region

A. Pietropaolo et al.
ChemPhysChem, 10, 2009,
Human peptide with neurotoxic part

Fmoc-QGGGTHTSQWNNKPSKPKTNMKHMGAAAGAAGA

Chicken peptide with neurotoxic part

Ac-SGGSYHNNQKWPWPKPPKTNFKHVAGAAAGAG-NH₂

Comparison of the metal ion binding abilities of human and chicken prion protein fragments with Cu²⁺ ions.


*Mol. BioSyst.*, 5, 2009, 497-510,
The neurotoxic part:
Structure hypothesis of the complex \([\text{CuH-1L}]\) for chicken prion protein fragment with Ni2+ ions

D. Valensin, K. Gajda, E. Gralka, G. Valensin, W. Kamysz, H. Kozlowski,
Japanese pufferfish fugu (*Fugu rubripes*)
The cloning *Fugu rubripes* genes that encode a structurally conserved prion proteins which were termed “similar to PrPs” (st1PrPs and st2PrPs) display characteristic structural features among PrPs family:

- similar pl (9.14)
- the conservative signal sequence at N-terminus
- a Gly-Pro-rich region (parallelly to repeat domain in mammals and birds)
- two typically positioned cysteine residues,

\[
\text{Ac-GHG}YG\text{VYGH-NH}_2 \\
\text{Ac-GHG}YG\text{VYGHPGYG}G\text{HGYG}V\text{YG-NH}_2 \\
\text{Ac-GHG}YG\text{VYGHPGYG}G\text{HGYG}V\text{YGHPGYG}G\text{HG}F\text{HGR-NH}_2
\]
Ac-PVHTGHMGHIGHTGHTGH-NH₂/ Cu(II) 1/1

The model of CuL

Zebrafish
PrP-rel2Δ63-80
Zebra fisch prion protein (zrel-PrP)

mhskflfsf lncllllav1 lpvaqsrrgg gfgrrggrgg
gwggssssgra gwgaaggghhr appvhtghmg hightghtgh
tgsgghvgk vagaaaagal ggmlvghgls smgrpgygyg
yggygghgyg yghgyghghg hghghghsgd hnetdadyyl
dgaasghays cvtvfglmms flighfls
zPrP$_{63-87}$

Ac-PVHTGHMGHGHTGHTGHTGGSSGHHG-NH$_2$

Ac-PVHTGHMG-NH$_2$

Ac-PVHTGHMGHGHIGH-NH$_2$

Ac-PVHTGHMGHGHTGHTGH-NH$_2$

Ac-PVHTGHMGHGHTGHTGHTGGSSGHHG-NH$_2$
Copper binding to: Ac-PVHTGHMGHIGH-NH$_2$

Species distribution profile for Cu$^{2+}$ complexe

Structures of the Cu$^{2+}$-zPrP63-74 complex obtained through restrained simulated annealing and molecular dynamics

Zinc binding to:

\[
\text{Ac-PVHTGHMGHIGHTGHTGH-NH}_2
\]

\[
\text{Ac-PVHTGHMGHIGHTGHTGHTGGSSGHG-NH}_2
\]
Copper and zinc ions bind to:

Ac-PVHTGHMGHIGHTGHTGHTGH-NH$_2$
Competition plots showing the affinity of Cu\(^{2+}\) towards:

- zebra: Ac-PVHTGHMGHIGHTHTGHTGHTGGSSGHG-NH\(_2\)
- human: Ac-PHGGGWGQPHPHGGGWGQPPHGGGWGQPPHGGGWGQ-NH\(_2\)
- bird: Ac-HNPGYPHNPGYPHNPGYPHNPGYP-NH\(_2\)
- fugu: Ac-HGYGVYGHPGYGGHGYZGVYGHPGYGGHGFGHGR-NH\(_2\)

\[\text{pH} \quad 3 \quad 4 \quad 5 \quad 6 \quad 7 \quad 8 \quad 9 \quad 10\]

\(\text{free Cu(II)}\)

\(\text{Cu[zebra]}\)

\(\text{Cu[fugu]}\)

\(\text{Cu[bird]}\)

\(\text{Cu[human]}\)
Biology and chemistry show that prion is potentially a multicopper binding protein (Copper Transporter).

„Unstructured” or flexible N-terminal domain serves the major binding sites for 4-6 Cu (II).

Multi-imidazole binding for low copper very efficient.

Prion-Copper complexes induce antioxidant (super oxide dismutase) activity: 

\[ \text{Cu(II)} \cdot (N_{\text{imid}})_4 \] is much better redox site than tetragonal one!

From mammals to fishes multi-HIS binding occurs: it could be relevant to biological activity of prions and prion-like proteins.
Drugs for neurodegenerative diseases

- Alzheimer
- Parkinson
- ALS
no treatments that will stop or reverse the progress of Alzheimer’s disease

four FDA-approved drugs available that may be able to relieve symptoms for patients for a limited time such as memory loss, and other problems with thinking or reasoning.

For people with mild to moderate symptoms, doctors may prescribe Aricept®, Exelon®, Razadyne® or Galantamine®.

Moderate to severe symptoms of Alzheimer’s disease can be treated with Aricept®, Exelon® and Namenda®.
Most common iron(III) chelators:
Chelators with potential in the treatment of neurodegenerative diseases
no treatments that will stop or reverse the progress of Alzheimer’s disease

four FDA-approved drugs available that may be able to relieve symptoms for patients for a limited time such as memory loss, and other problems with thinking or reasoning.

For people with mild to moderate symptoms, doctors may prescribe Aricept®, Exelon®, Razadyne® or Galantamine®.

Moderate to severe symptoms of Alzheimer’s disease can be treated with Aricept®, Exelon® and Namenda®.
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