Hybrid quantum simulations of biomolecules: the role of copper in neurodegenerative diseases

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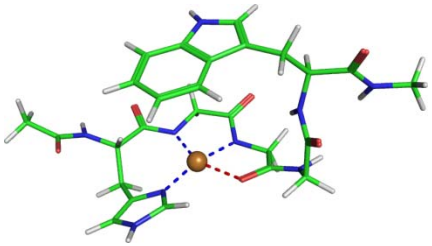
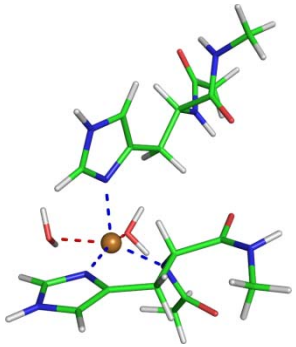
I. Introduction to molecular simulations in biology

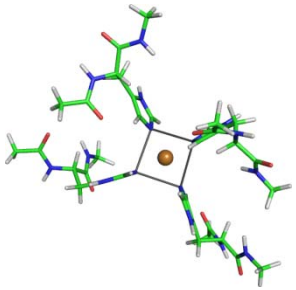
II. DFT/approximate-DFT hybrid method for biosimulations
- Orbital-free linear-scaling DFT for the solvent
- Full DFT for the biomolecule and its first solvation shells

III. Applications
- Prion protein and the role of copper
- Cu-induced misfolding in Parkinson disease

IV. Summary

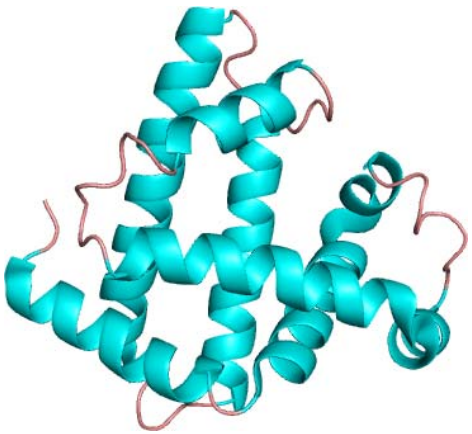


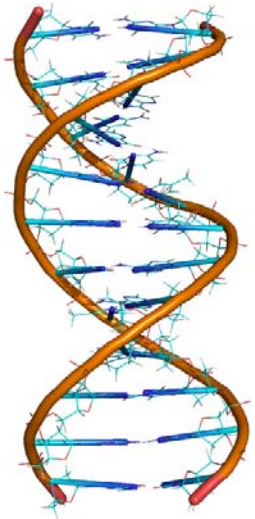




Biosimulations

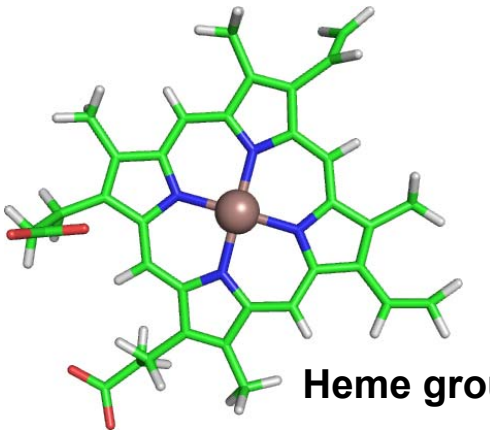
- Computer simulations involving organic molecules
  - Proteins, lipids, DNA, etc.
  - Biological macromolecules
    - amino acids, nucleotides, etc.
- Computational challenges
  - Size
    - Thousands, even millions of atoms
  - Time scale
    - Processes involving structural changes (folding etc.) take place over μs or more
  - Solvation
    - Increases size of the system
      - Solvent usually outnumbers solute



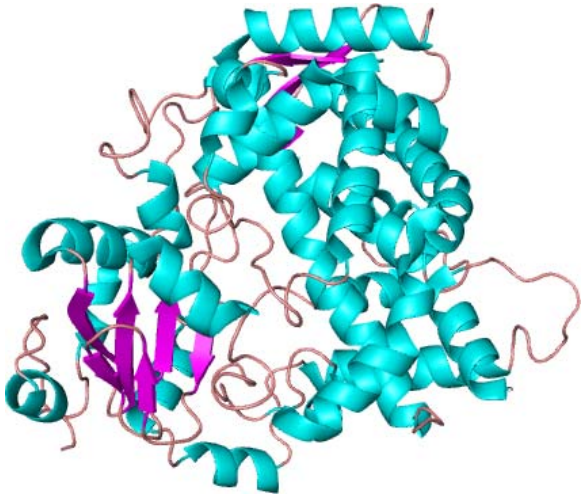


Biosimulations: methods

- Quantum molecular dynamics
  - can describe chemical processes
    - bond formation, bond breaking, charge transfer
  - ~10^2-10^3 atoms
  - timescale: ps
  - fragments and localized processes
- Classical molecular dynamics
  - force fields
  - cannot describe chemical processes
  - ~10^6 atoms
  - timescale: ns
- Coarse grained and lattice models
  - more simplified description
  - can describe protein folding



Heme group

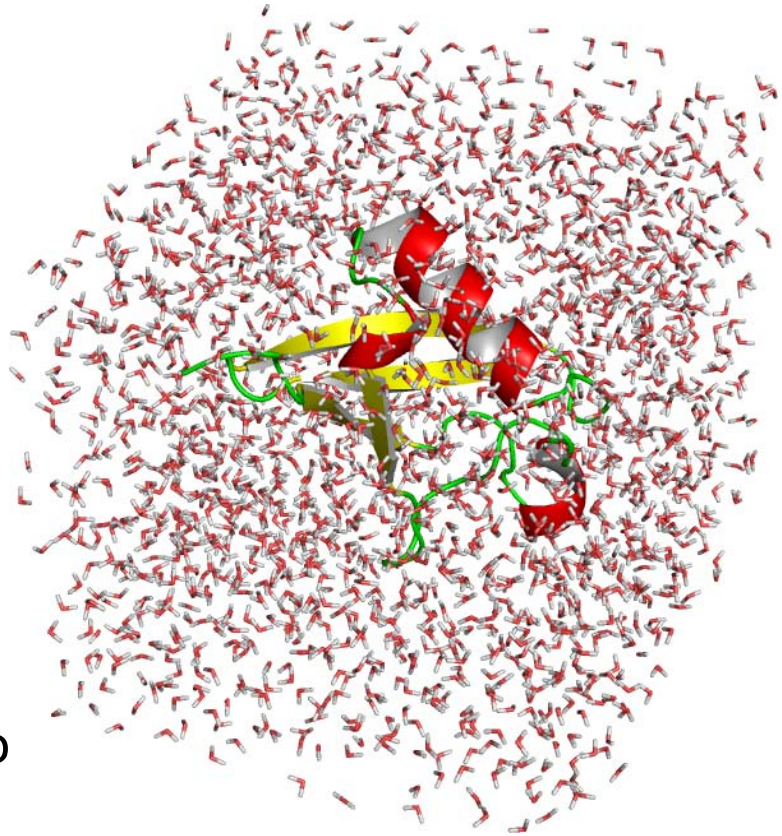


P4503A4 Oxidoreductase

Solvation in biosimulations

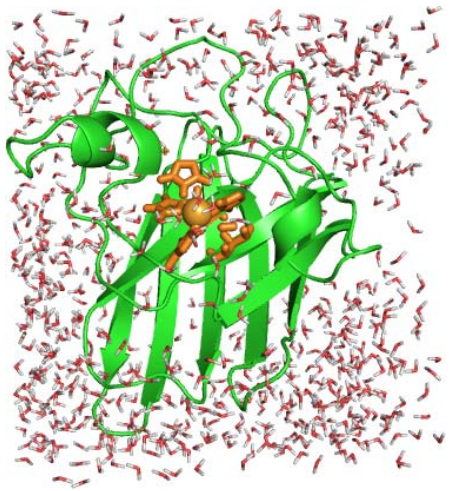
- Implicit
  - Surrounding solvent treated as continuum
  - Efficient
  - Incorrect results in certain cases
    - protein folding pathways etc.
- Explicit
  - More accurate than implicit
  - Solvent outnumbers solute
- DFT simulations
  - Implicit solvation is often used
    - Polarizable continuum model (PCM)
  - Simplified treatment of solvent has to be used for explicit solvation
    - Hybrid QM/MM approach

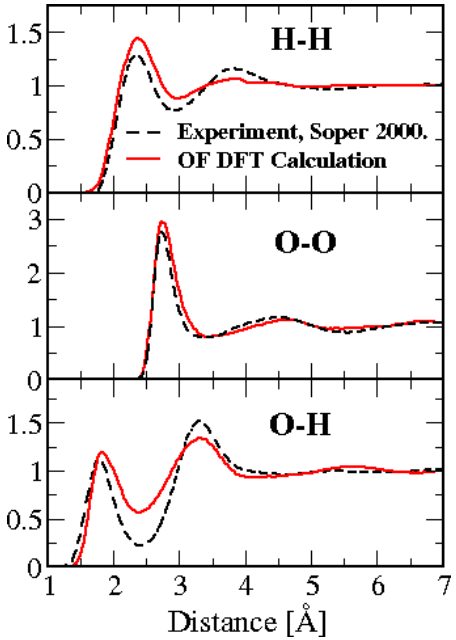
Fully solvated *ubiquitin*
Water: 83% of atoms



Hybrid DFT/DFT calculations

- QM/MM
  see e.g. **Warshel, Levitt, J. Mol. Biol. 1976.**
  - QM: chemically active part
    - small part of the system
  - MM: rest of the system
  - QM-MM interface
    - special interaction terms have to be defined
      - electrostatic, repulsion, etc.
      - MM parameters are used
      - problem with exchange of atoms
- Our approach: hybrid DFT/DFT method
  Hodak, Lu, Bernholc, JCP, 2008.
  - Kohn-Sham DFT for the chemically active part of biomolecule
  - Orbital-free DFT for the solvent in the system





Real-space Multi-Grid method (RMG)

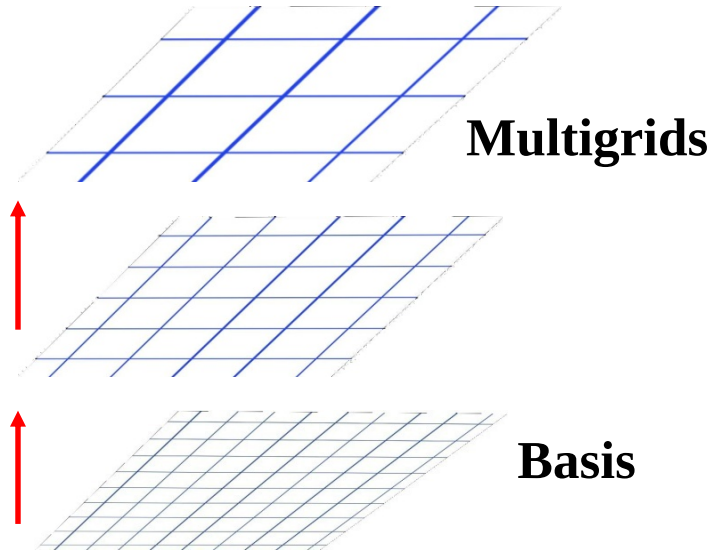
- Density functional equations solved directly on the grid
- Multigrid techniques remove instabilities by working on one length scale at a time
- Convergence acceleration and automatic preconditioning on all length scales
- Non-periodic boundary conditions are as easy as periodic
- Compact "Mehrstellen" discretization

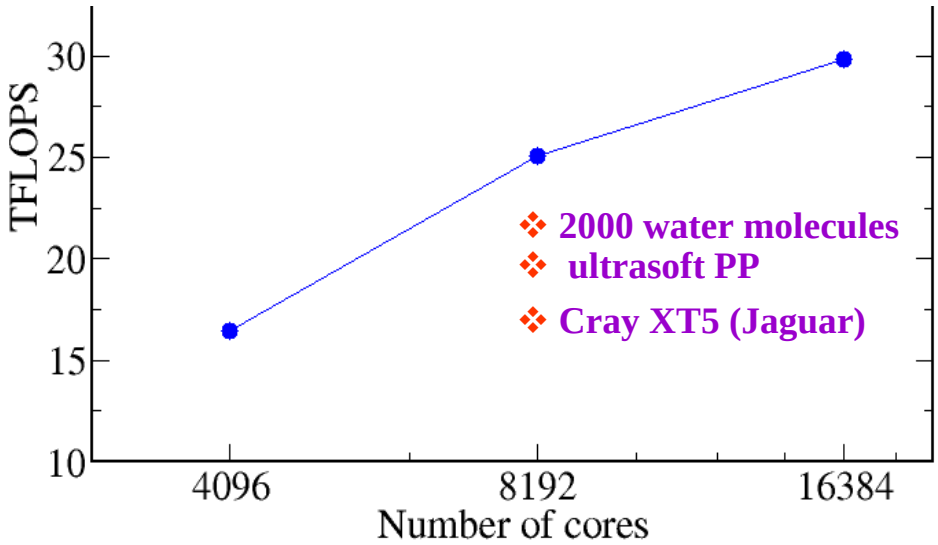
$$A[\phi_i] + B[(V_{eff} + V_{NL})\phi_i] = \varepsilon_i B[S\phi_i]$$

- Allows for efficient massively parallel implementation

❖ *See E. L. Briggs, D. J. Sullivan and J. Bernholc Phys. Rev. B 54, 14362 (96).*

❖ Ultrasoft pseudopotentials: *M. Hodak, S. Wang, W. Lu and J. Bernholc, PRB 76, 085108 (07)*

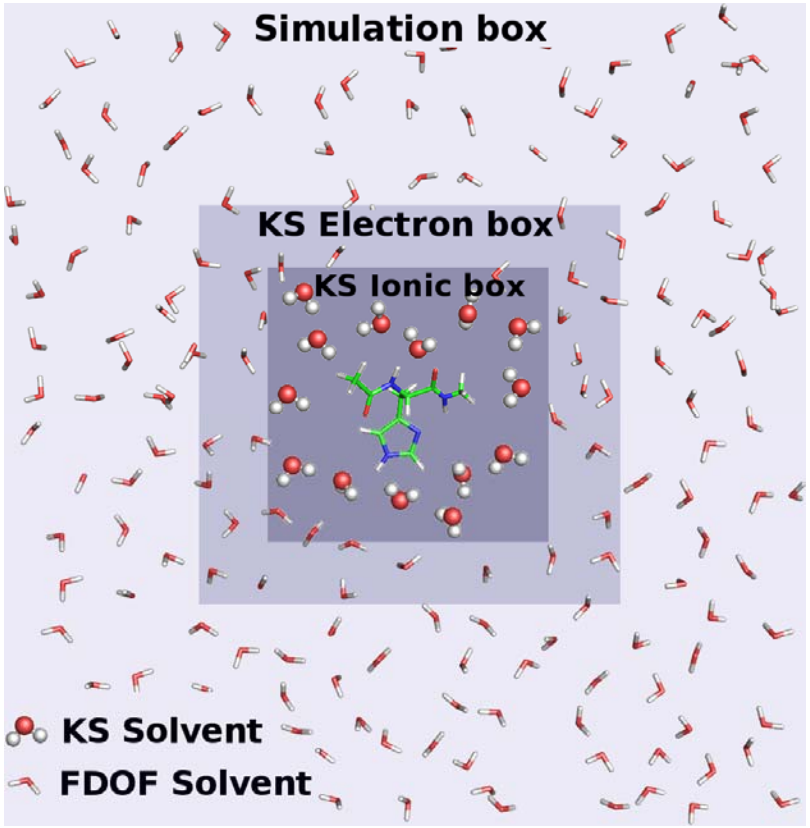




Kohn-Sham DFT/Orbital-free DFT calculation

- Kohn-Sham DFT for chemically active part
  - KS Ions must be located inside KS Ionic Box
    - biomolecules must be trimmed at the boundary
  - KS equations solved in KS electron box with zero boundary conditions (non-periodic)
    - extra space needed for wavefunctions and density to decay to zero
- Orbital-free DFT for the rest of the system (Lee, Lee, Parr functional, 1991)
  - takes 5% or less even when treating 100× more atoms
  - Generalization of Gordon-Kim approach for closed-shell molecules

    Gordon and Kim, JCP, 1972, see also Barker and Sprik, Mol. Phys. 2003



Orbital-free DFT

- **Minimize energy functional without using orbitals**
- **Efficient, linear scaling**
- **Mixed success in reproducing K-S electron densities**
- **Kinetic energy functional has to be explicit in *ρ* (Thomas-Fermi)**
- **Frozen-density orbital-free model for liquid water**

  LLP KE functional (TF + GGA) **Lee, Lee, Parr, PRA, 1991.**

$$T_{LLP} = C_{TF} \int_{\Omega} \varrho_{TF}^{5/3} \left(1 + \frac{0.0045x^2}{1 + 0.0253x \sinh^{-1} x} \right) d\mathbf{r}, \; x = 2^{1/3} \frac{|\nabla \varrho_{TF}(\mathbf{r})|}{\varrho_{TF}^{4/3}(\mathbf{r})}$$

  - **Individual molecules have fixed geometries and frozen densities**
  - **Ultrasoft and norm-conserving pseudopotentials (only local parts needed)**
  - **Charge density given by gaussians**
    - **Constants determined from dipole moment and RDF**

      see also Barker and Sprik, Mol. Phys. 2003

- **Electronic energy functional**

$$E_{FDOF}[\varrho] = T_{LLP}[\varrho] + E_H[\varrho] + E_{XC}[\varrho] + E_{PPlocal}[\varrho] + E_{ii}$$

Hybrid KS DFT/OF DFT energy

- System consisting of KS and OF ions
  - Total density: $\varrho_{tot}(\mathbf{r}) = \varrho_{OF}(\mathbf{r}) + \varrho_{KS}(\mathbf{r})$
  - Total kinetic energy

$$T_{tot}[\varrho_{OF}, \varrho_{KS}] = T_{OF}[\varrho_{OF}] + T_{KS}[\varrho_{KS}] + T_{nadd}[\varrho_{OF}, \varrho_{KS}]$$

  - Non-additive kinetic energy can be estimated

Wesolowski and Warshel, JPC 1993

$$T_{nadd}[\varrho_{OF}, \varrho_{KS}] = T_{OF}[\varrho_{tot}] - T_{OF}[\varrho_{OF}] - T_{OF}[\varrho_{KS}]$$

  - Hybrid electronic energy functional

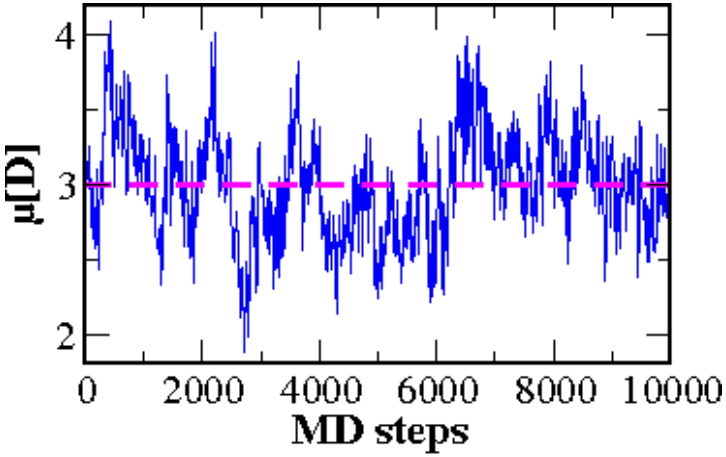
$$E_{Hybrid}[\varrho_{OF}, \varrho_{KS}, \{\mathbf{R}_i\}_{OF}, \{\mathbf{R}_i\}_{KS}] = T_{tot}[\varrho_{OF}, \varrho_{KS}] + E_H[\varrho_{tot}]$$

$$+E_{XC}[\varrho_{tot}] + E_{PP_{nonloc}}[\varrho_{KS}, \{\mathbf{R}\}_{KS}] + E_{PPloc}[\varrho_{tot}, \{\mathbf{R}\}] + E_{ii}[\{\mathbf{R}\}]$$

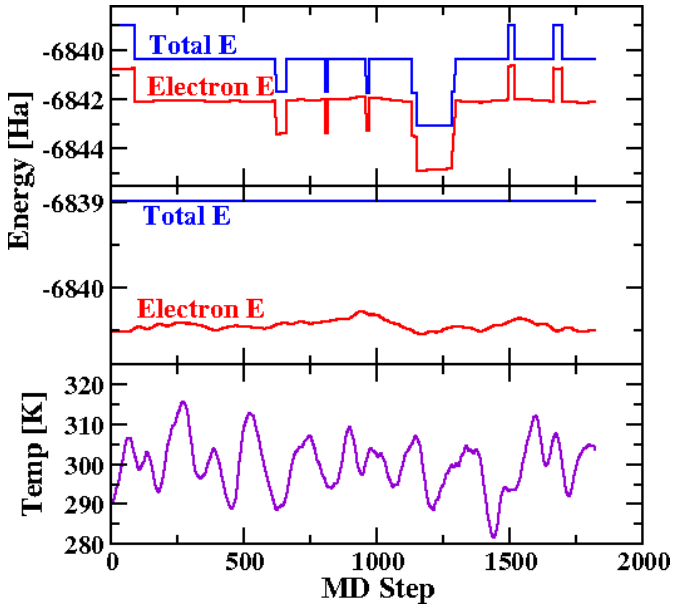
  - Leads to modified KS equations for ρ_{KS}
  - Interactions between subsystems on quantum (DFT) level

Solvent exchange and solvation effects

- Water molecule polarization:
  - Gas phase: 1.85 D
  - Liquid phase:
    - Experiments: 2.9-3.15 D
    - ab initio calculations: ~3.0 D
      - Silvestrelli, Parrinello, JCP 3572, 1999; PRL 3311, 1999.
    - **Hybrid: 3.0 D**



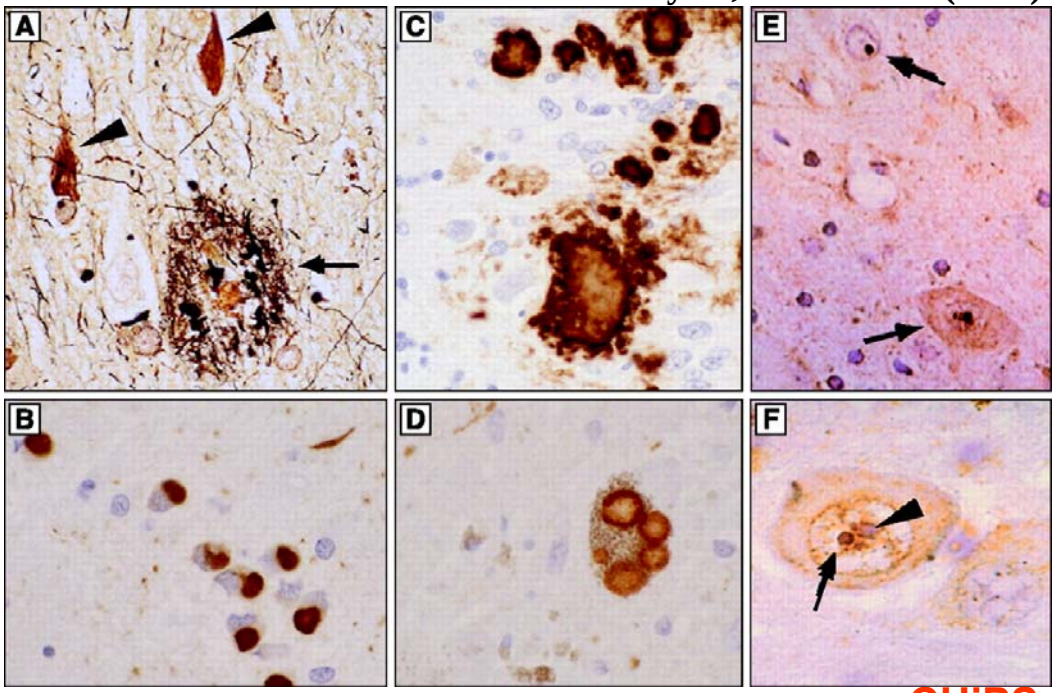
- Exchange of solvent between OF and KS regions
  - smooth energy curves can be obtained
  - energy conserving MD



Applications: Neurodegenerative diseases

- **Diseases related to neuronal damage**
  - **characterized by insoluble deposits (plaques, amyloids) composed of proteins**
  - **Protein-only hypothesis: Diseases are caused by improperly folded proteins unable to perform their functions**
  - **New class of infectious diseases, no DNA or RNA involved**
  - **Metals ions may play a role**
  - **Alzheimer's disease (A)**
  - **Pick's disease (B)**
  - **Prion disease(C)**
  - **Parkinson's disease (D)**
  - **Machado-Joseph's disease (E,F)**
  - **Huntington disease**

❖Taylor, Science 296 (2002).



Pathogenesis in Neurodegenerative diseases

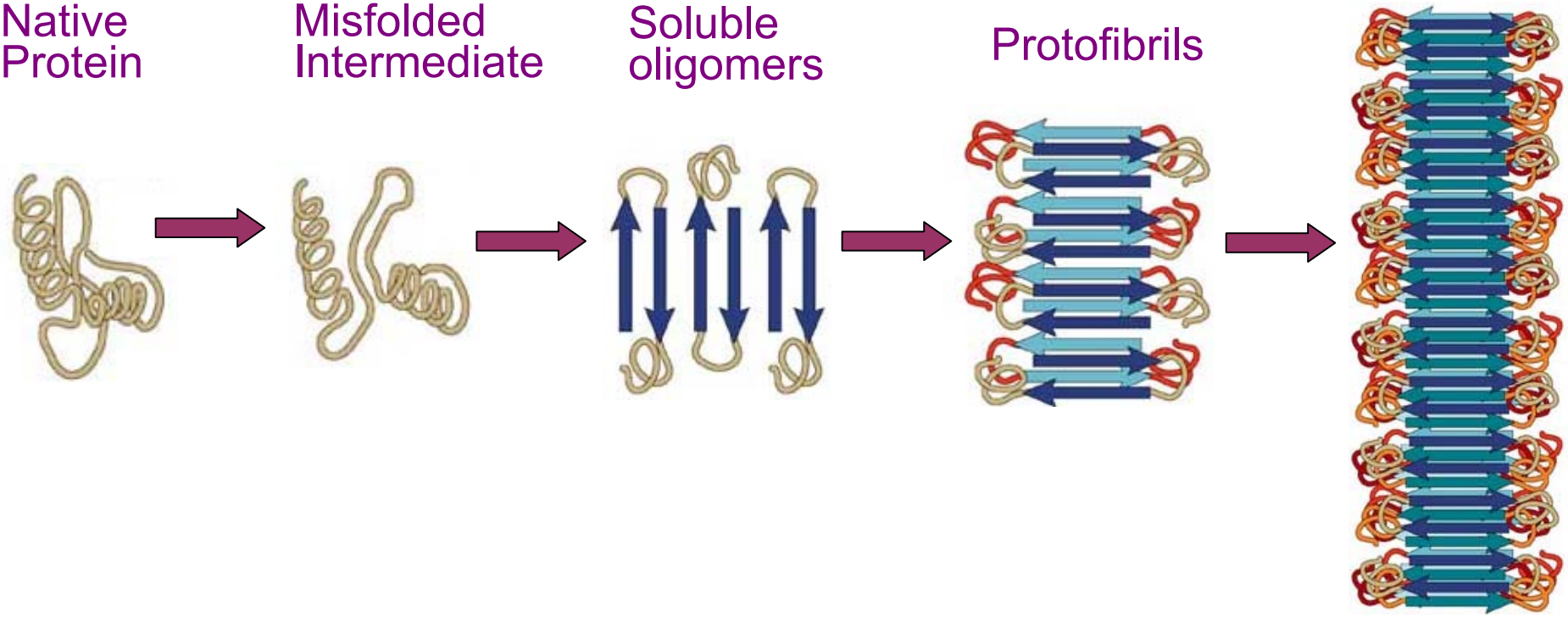
Amyloid Fibrils
- Cross-β sheet
- β-strands perpendicular to fiber axis

Native Protein

Misfolded Intermediate

Soluble oligomers

Protofibrils

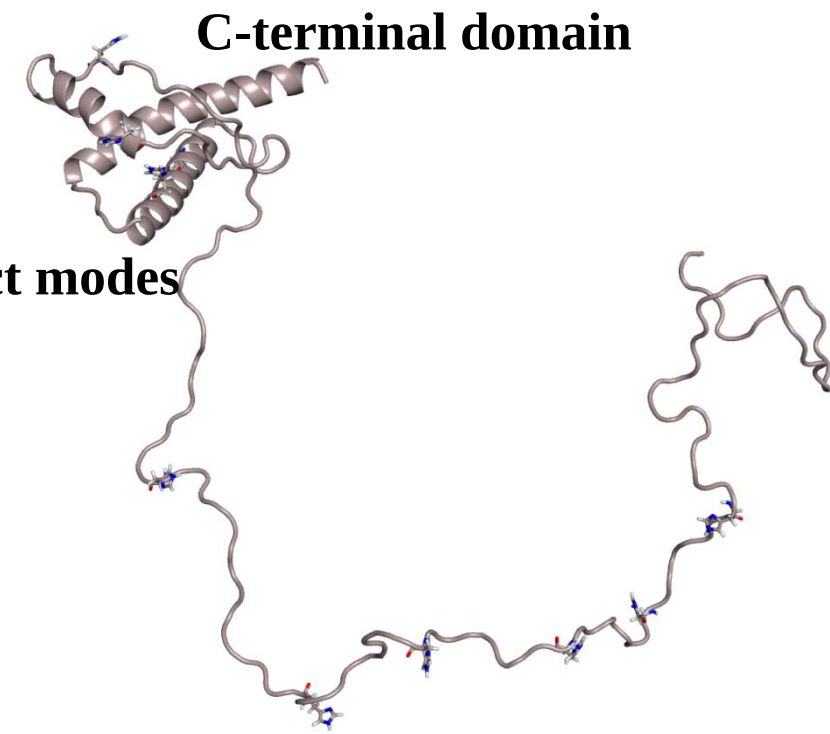


Exact nature of neurotoxic species is unknown

C. Soto,
Nat. Rev. Neurosci, 2003.

Prion protein

- **Involved in prion diseases**
  - **mad cow disease (cattle), scrapie (sheep, goat)**
  - **Creutzfeldt-Jakob disease, kuru (human)**
- **Prion protein (PrP)**
  - **Folded and unfolded regions**
  - **Unknown normal function**
  - **Efficiently binds Cu(II)**
    - **Cu may be bound in three distinct modes**
    - **May be related to PrP's function**
      - **Copper buffer?**
  - **Role of copper in prion diseases?**



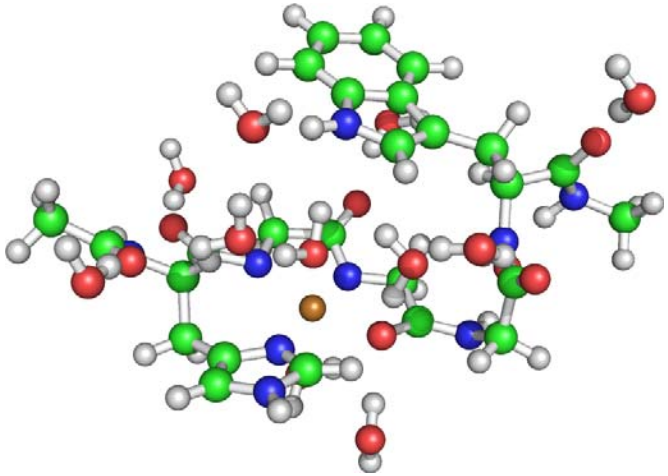
Hybrid calculation setup

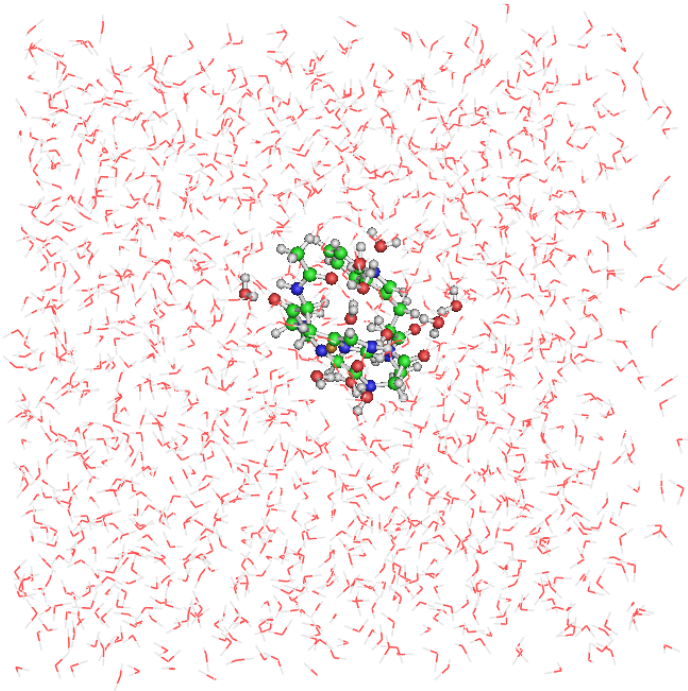
Quantum system:

- PrP-Cu binding fragment
- 1st solvation shell around Cu

Hybrid system:

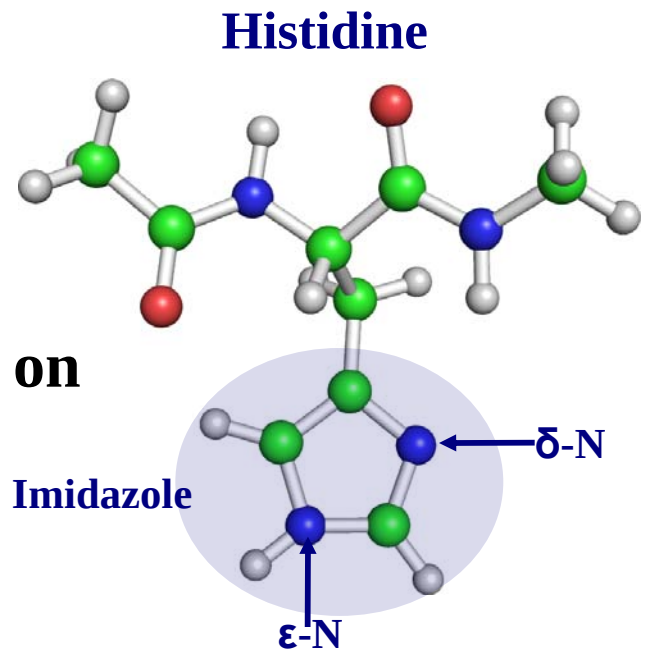
- Full solvation
- 2000-3000 water molecules treated by OF DFT



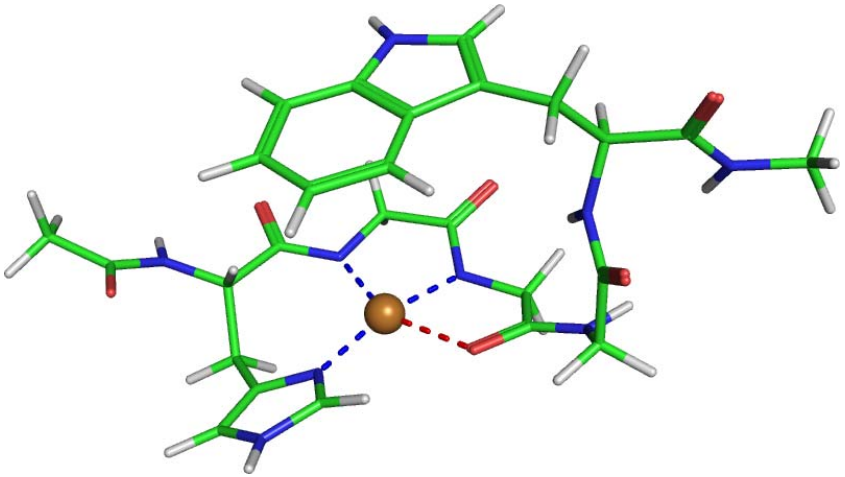


Copper binding to PrP

- **High affinity binding sites located in octarepeat region**
  - **copper anchored by histidine residues**
  - **octarepeat contains four histidines**
- **Three distinct binding modes depending on copper concentration**

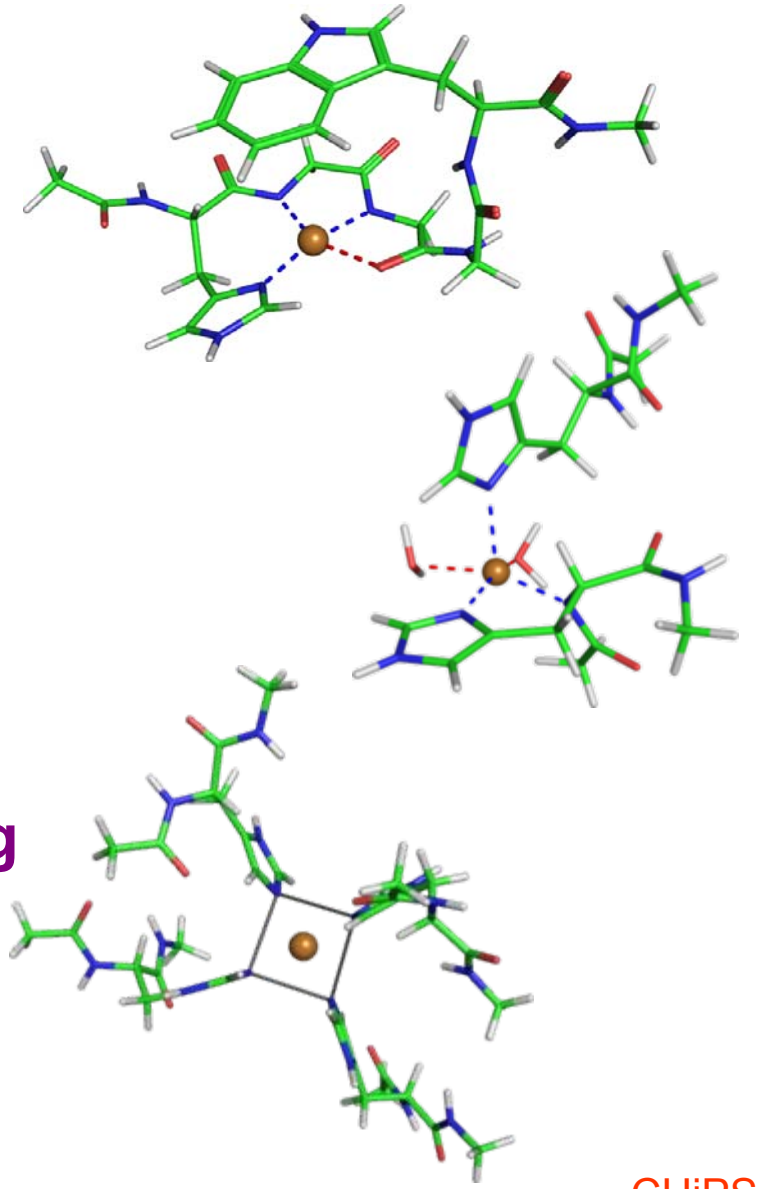


- **High concentration binding**
  - **Sequential residues HGGGW**
  - **One histidine involved**
  - **Four Cu ions can be bound**
  - **Three N and 1 O**
  - **Planar binding**



Concentration dependent Cu^{2+} binding to PrP

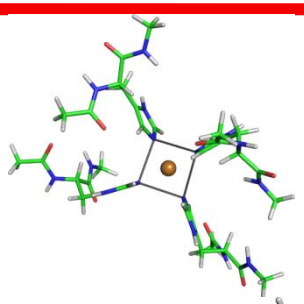
- **High Cu^{2+}concentration**
  - Sequential residues HGGGW
  - One histidine involved
  - Four Cu ions can be bound
  - ~ 150 KS atoms, 3000 OF water molecules
- **Medium Cu^{2+}concentration**
  - Two histidines are involved
    - One in axial position
  - Two in-plane waters
  - Two Cu ions can be bound
- **Low Cu^{2+}concentration binding**
  - Four histidine are involved
    - All histidines in octarepeat domain
  - Coordinating atoms arranged on square
  - One Cu ion can be bound



Energetics of PrP's function as a copper buffer

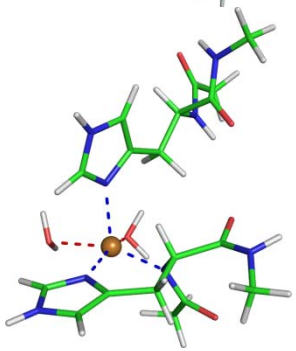
Cu concentration

Low: 1 Cu bound @ 5.22 eV



Intermediate: 2 Cu bound, each @ 2.75 eV

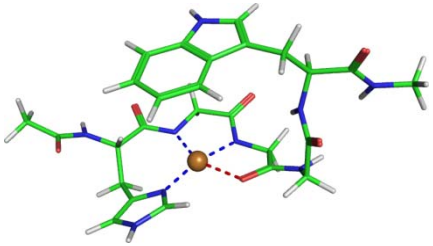
Energy balance: 2*2.75 eV – 5.22 eV = 0.28 eV
Entropy*T increase (at 300 K): 0.16 eV
calculated using Schlitter's formula, CPL 1993.
Addition of 2nd Cu is **exothermic**



High: 4 Cu bound, each at 1.76 eV

Energy balance: 4*1.76 eV – 2*2.75 eV = 1.54 eV
Entropy*T increase relative to previous case: 0.23 eV

Addition of more Cu is **exothermic**

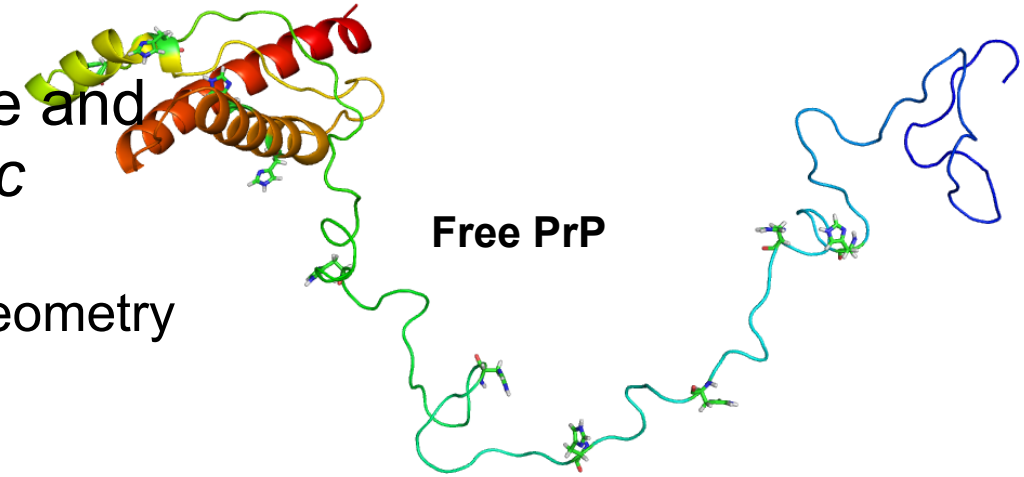


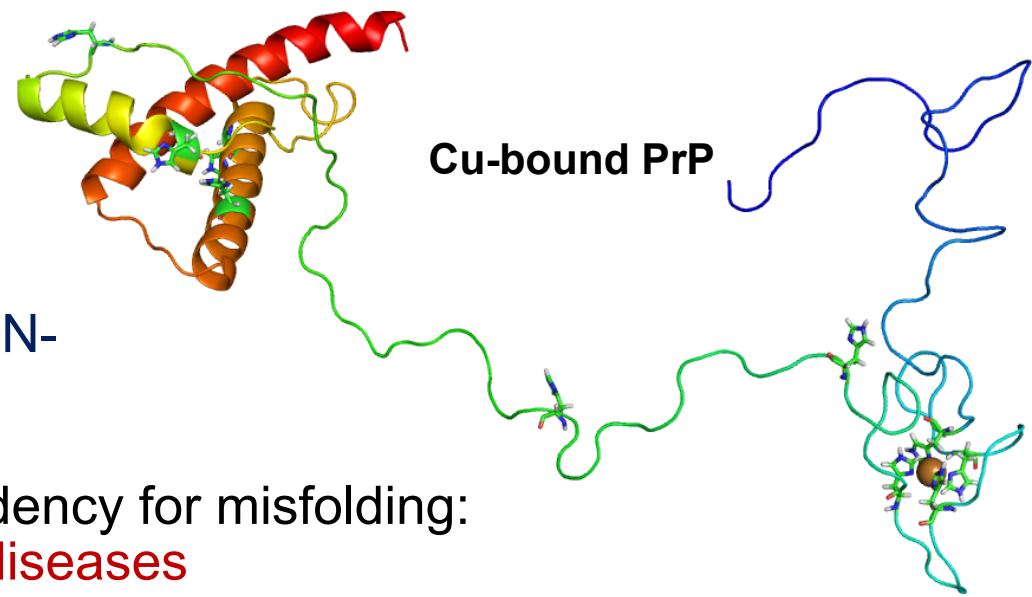
Conclusion: It is favorable to bind more copper ions when they are available

PrP can function as a copper buffer !

Effect of Cu on full-length PrP

- Classical MD (NAMD) on free and Cu-bound PrP at low Cu *conc*
  - Cu modeled by either fixing the binding site to the calculated geometry or using Cu-bond "springs"
- Flexible N-terminal part
  - Free PrP
    - only temporary turns
  - Cu-bound PrP
    - Stable turns around Cu
    - Metastable short β-hairpins
  - Cu enhances stability of whole N-terminal domain

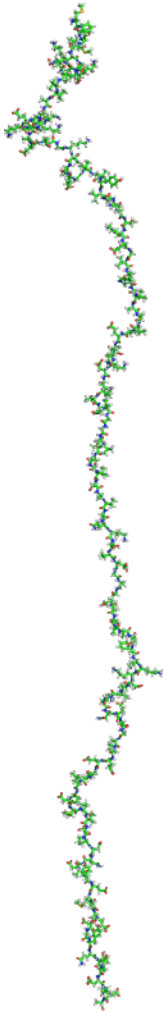




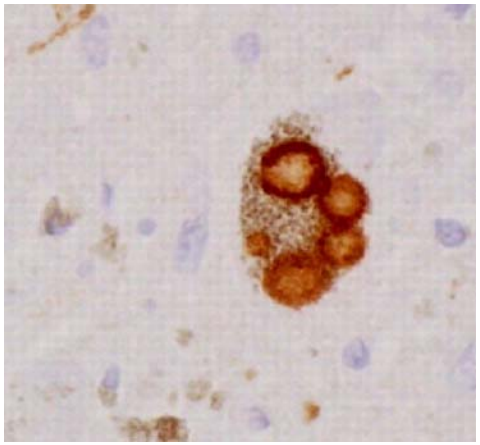
Higher stability → lesser tendency for misfolding:
Protective role of copper in prion diseases

Hodak, Chisnell, Lu, Bernholc, Proc. Nat. Acad. Sci. 2009

Parkinson's disease



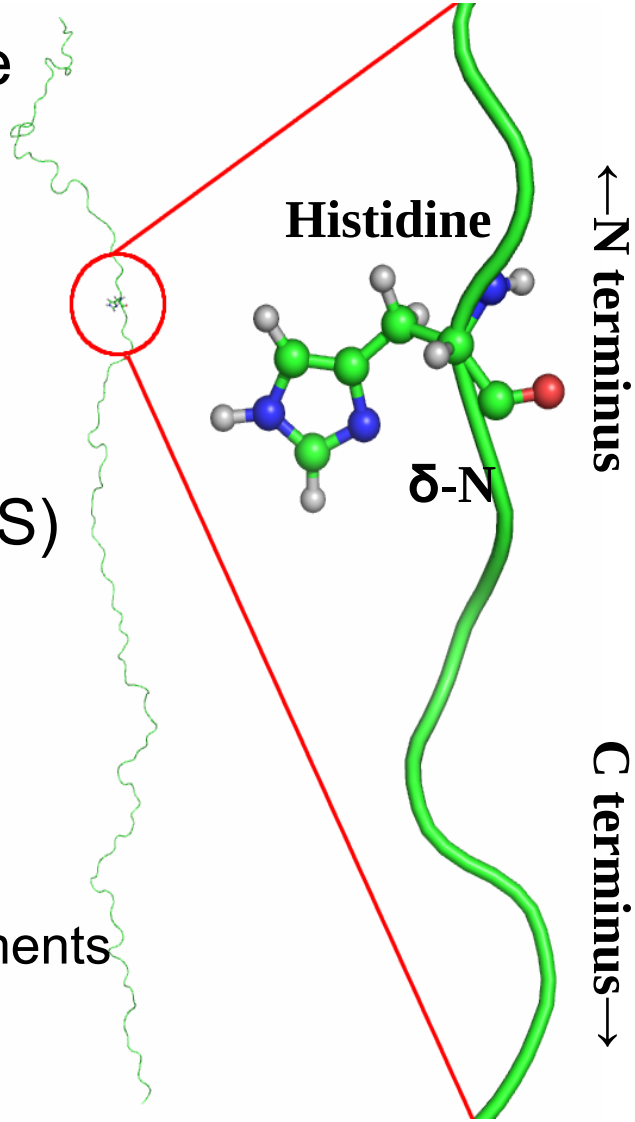
- Involves α-synuclein
  - 140 aminoacids
  - misfolded proteins form Lewy bodies
  - present in neurons of the central nervous system
  - natively unstructured
  - Unknown function
    - May affect neuron plasticity and neurotransmitter release
  - binding of metal ions causes misfolding and subsequent oligomerization
    - Cu(II), Al(III), Fe(III), Co(III), Mn(II)
    - Cu(II) most effective



Taylor, Science 296 (2002).

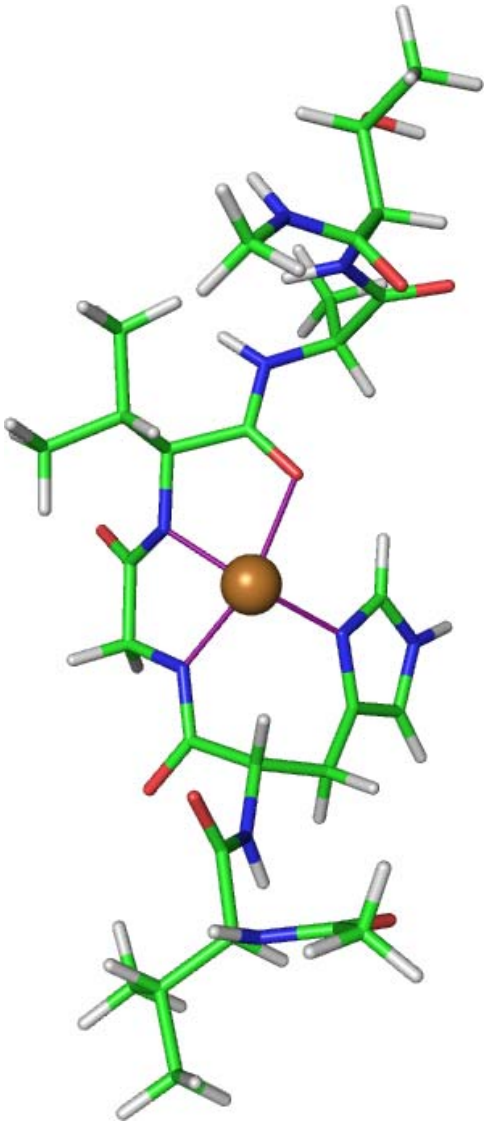
Cu^{2+} and α-synuclein

- Cu^{2+} is the most effective metal ion in the formation of α-synuclein plaques
- Cu^{2+} is found in α-synuclein plaques of Parkinson's disease patients and is speculated to be causal
- NMR Structural characterization of Cu binding to α-synuclein (Rasia et al., PNAS)
  - Preferentially bound to δ-N of Histidine 50
  - Secondary binding occurs at N terminus
- Our Objective:
  - Validate Cu binding as a viable progenitor of stable β-sheet formation
  - Explore the extent of available β-sheet alignments

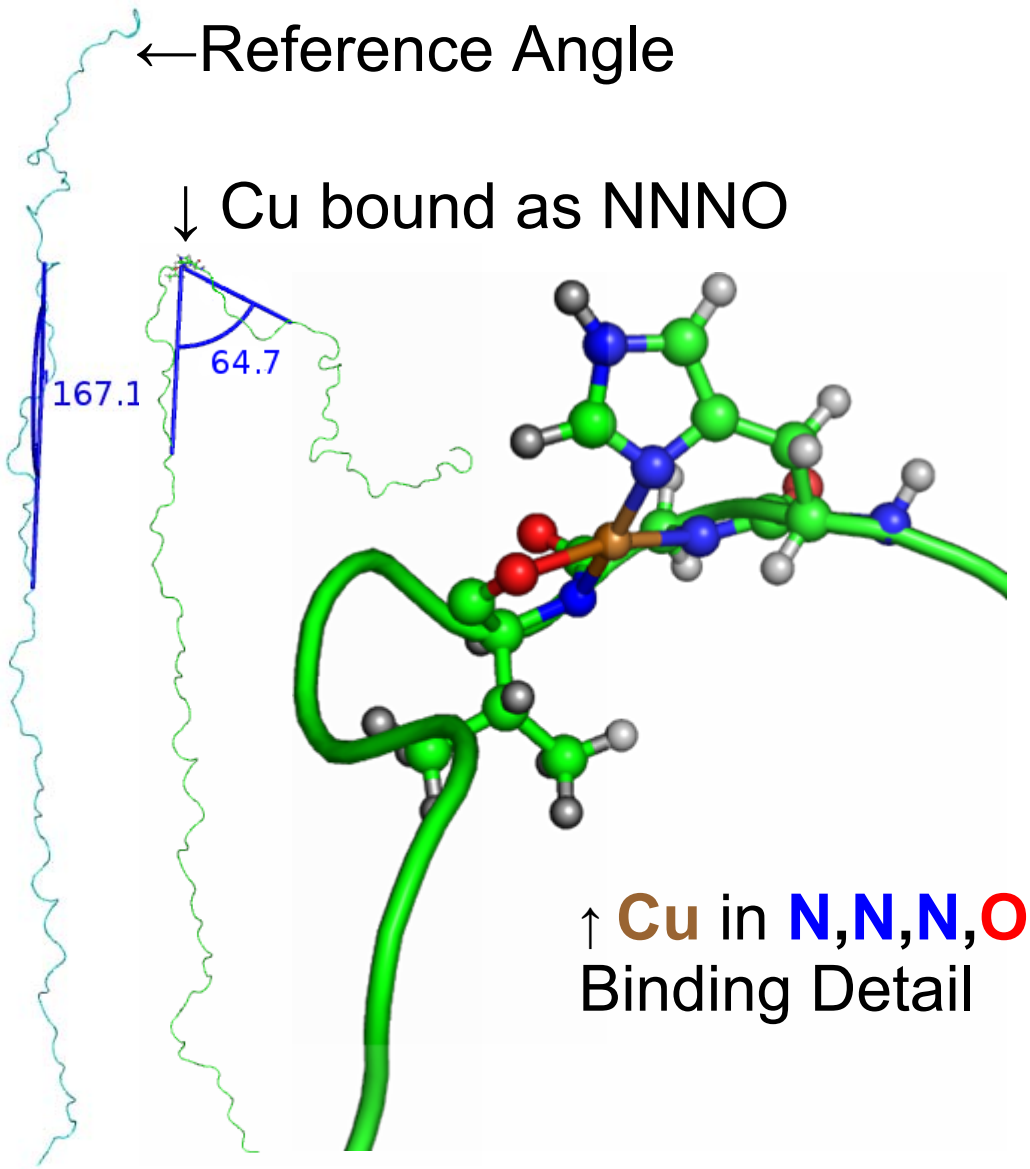


Cu(II) binding to α-synuclein fragment

- **Experiment: NMR**
  - **primary binding site: His50**
  - **residues 49-54 most affected**
- **Our model:**
  - **residues 49-54: VHGVAT**
  - **Cu(II) anchored by δ-N on His50 imidazole**
  - **deprotonated N and O in protein backbone**



NNNO Binding Geometry



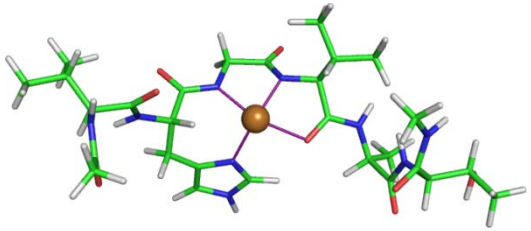
- *ab-initio* fragment consists of 91 atoms
- Backbone deforms from reference 167° angle to 65° angle
- Neutrally charged system
- NNNO is energetically preferred

NNNO	**0.0 eV (ref)**
NNOO	**+ 0.82 eV**
NONO	**+ 1.56 eV**

Cu(II)-bound α-synuclein

Free

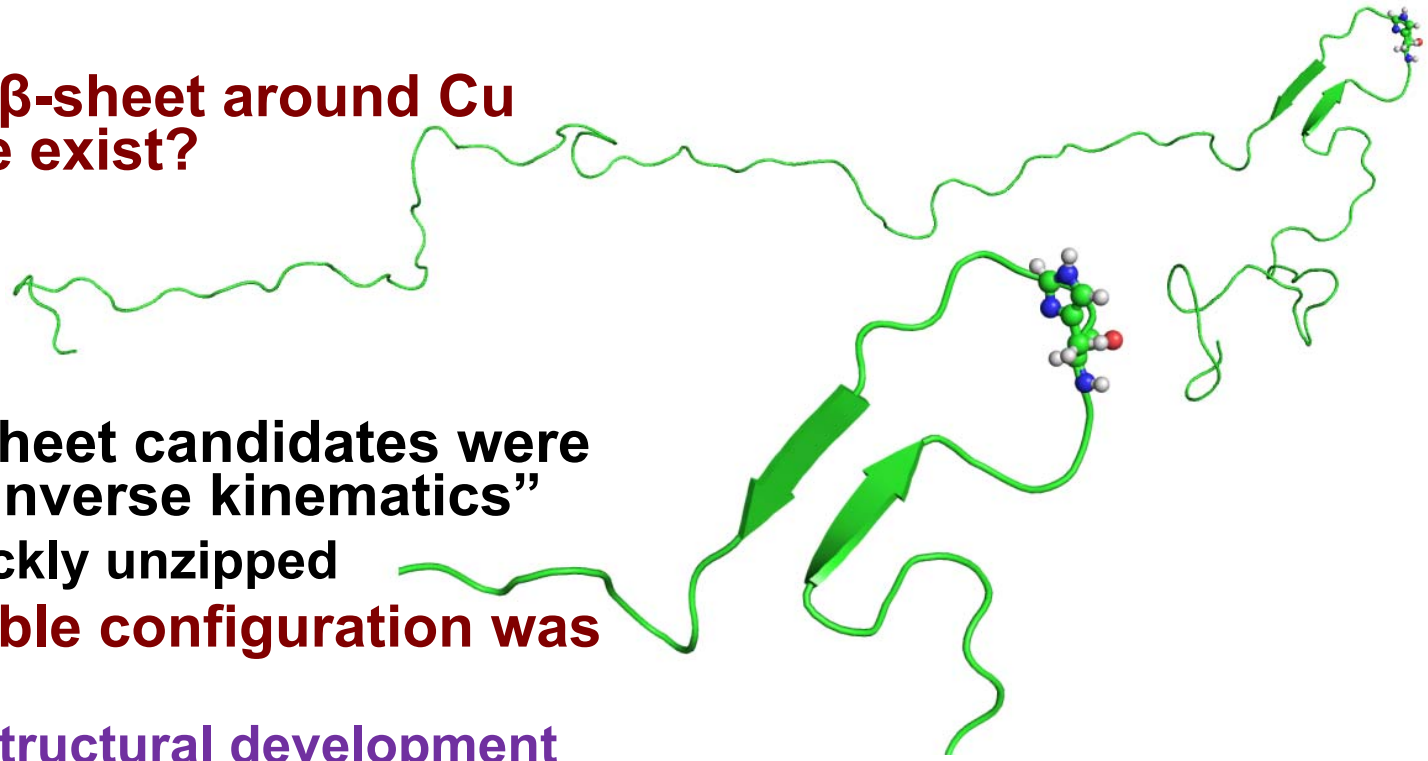
- Free α-synuclein
  - mostly linear
  - angle around His50: 167°
- Cu-bound α-synuclein
  - imposing binding site geometry introduces turn to the protein structure
  - angle around His50: 65°
  - continued development using classical MD (NAMD) shows hydrogen contacts around binding site and alignment of the backbone
    - Possible β-sheet development
    - Misfolded α-synuclein contains β-sheets !



Cu-bound

β-sheet in Cu-bound α-synuclein

- Can stable β-sheet around Cu binding site exist?



- Several β-sheet candidates were tested by "inverse kinematics"
  - Most quickly unzipped
- But... a stable configuration was found
  - Further structural development observed in MD
    - Backbone starts aligning next to existing β-sheet

Suggests mechanism for Cu-initiated misfolding of α-synuclein!

Mechanism of Cu-initiated misfolding of α-synuclein (α-syn)

Free α-syn

- Mostly linear
- Little tendency for structural development

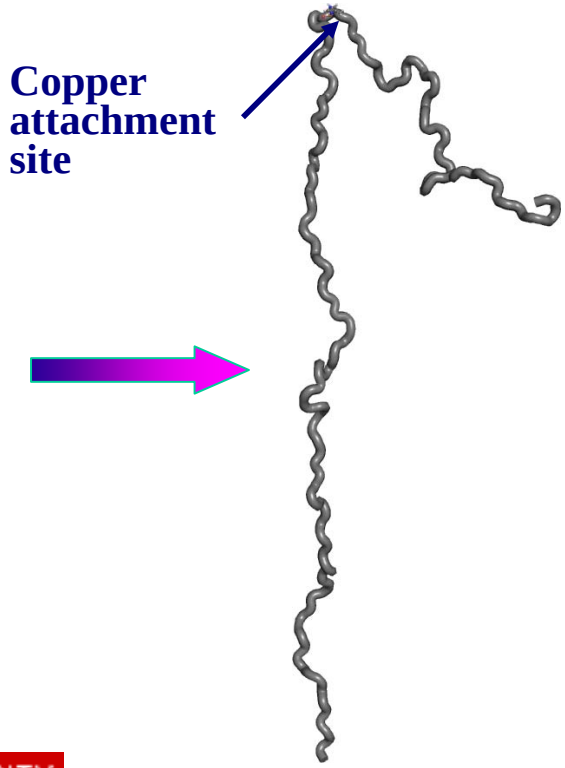
Cu-bound α-syn

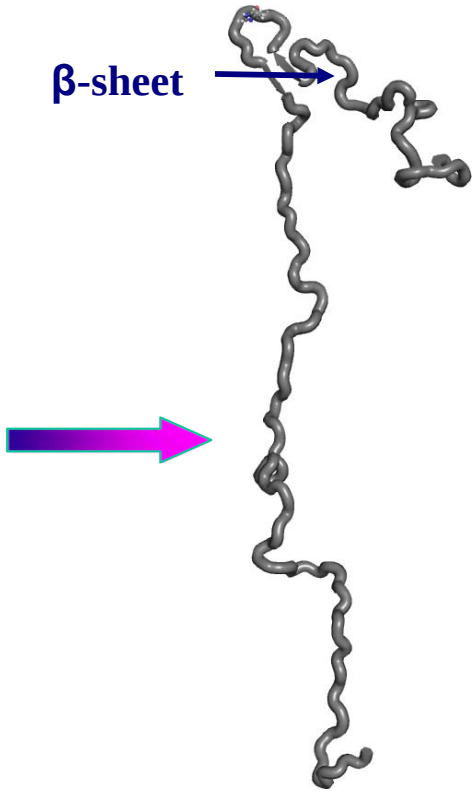
- Cu attachment causes a turn-like feature at the binding site
- Turn creates favourable conditions for β-sheet development

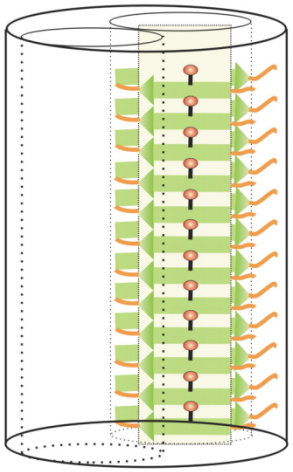
β-sheet development

- β-sheet is formed around the binding site
- Structural development continues

Structural development continues until the protein is completely misfolded







Oligomerization

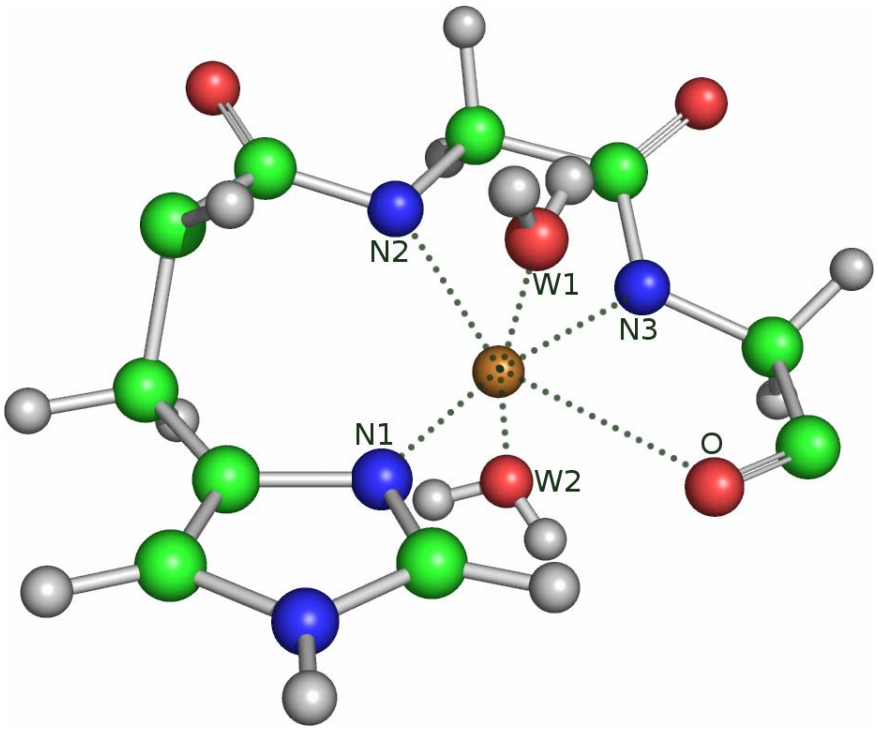
Summary

- New orbital-free DFT method for including solvent effects at low cost
  - Large-scale hybrid DFT/approximate-DFT quantum molecular dynamics
  - Biomolecule and its first solvation shells treated by full DFT
  - Solvent treated by orbital-free DFT: cheap & linear-scaling
  - Exchange of solvent molecules between full and approximate DFT regions is allowed; total energy is conserved
- Suitable for Exascale
  - Linear-scaling full DFT & "beyond DFT": multi-level hierarchical *ab initio*
  - Replica dynamics and multiple time-steps acceleration
- Applications to transition metal centers in proteins
  - Prion protein serves as a copper storage medium: multistage binding
  - Copper plays a protective role in the onset of prion diseases
  - Copper in Parkinson's disease protein
  - Accelerates misfolding and the onset of Parkinson's disease

Cu-PrP binding site geometry

- Copper bond distances (Å):

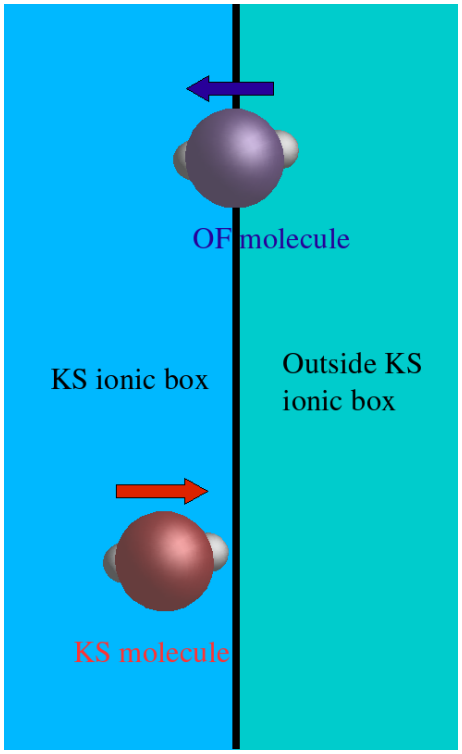
	Dry protein	partial solvation	full solvation
Cu-N1	1.99	1.98	1.98
Cu-N2	2.01	2.00	2.00
Cu-N3	1.91	1.91	1.92
Cu-O	2.19	2.38	2.4
Cu-W1	-	2.83	2.88
Cu-W2	-	2.71	2.64



- Solvation is important for proper description of binding
- Gap between occupied and unoccupied states
  - No solvation: **2.11** eV
  - Partial solvation: **2.25** eV
  - Full solvation: **2.34** eV
- Solvation is important for electronic properties and cannot be neglected

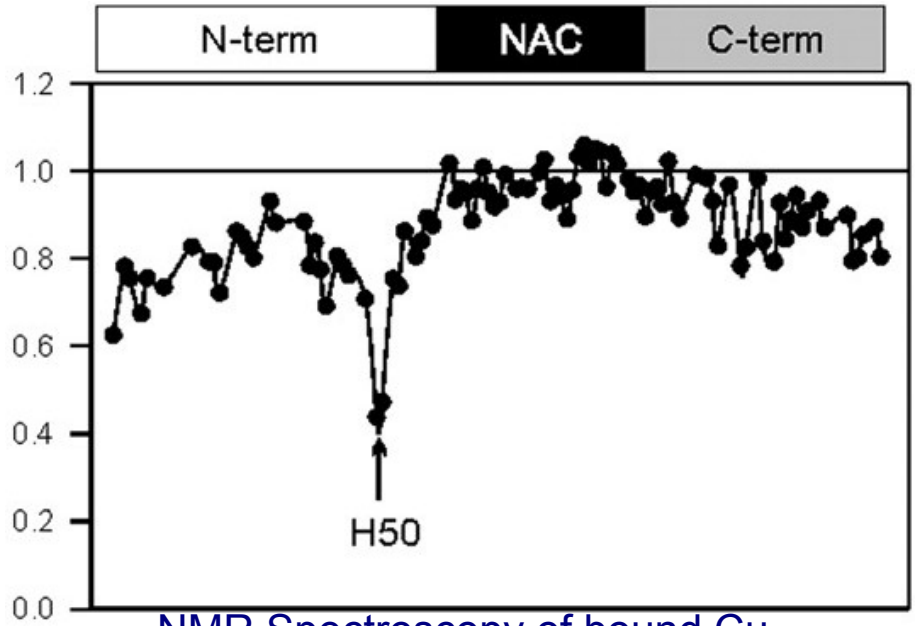
Exchange of solvent II.

- Comparing hybrid simulations with enabled and disabled switching
  - 1 exchange event: OF -> KS
  - Difference at the end of simulation: 200 MD steps after the exchange
    - Displacement: 5.6 % (0.05 Bohr)
      - timestep = 20 a.u (0.5 fs), T= 300K



Protein based Onset Mechanisms

Copper (II) binding at residue 50 of α-synuclein has been experimentally verified to accelerate oligomerization



NMR Spectroscopy of bound Cu
(Rasia et al., PNAS 2005)

Inherited mutation of α-synuclein at residue 53 (Alanine to Threonine - A53T) invariably leads to early onset PD

α-synuclein sub-sequence

40 50 60
... GSKTKEGVVH GVATVAEKTK ...

40 50 60
... GSKTKEGVVH GVTTVAEKTK ...