Analytical Ultracentrifugation

Mücke N et al.:
Molecular and Biophysical Characterization of Assembly-Starter Units of Human Vimentin.
J Mol Biol. 2004 Jun 25;340(1):97-114.

Outline

- Analytical Ultracentrifugation
  - Applications
  - Design and principles of an analytical ultracentrifuge
  - Sedimentation velocity vs. sedimentation equilibrium experiments
  - Fundamental mathematics
  - Data analyses
- Vimentin
- Characterization of Assembly-Starter Units of Human Vimentin
- References

Analytical Ultracentrifugation – Applications

- determine sample purity
- characterize assembly and disassembly mechanisms of biomolecular complexes
- determine subunit stoichiometries
- detect and characterize macromolecular conformational changes
- measure equilibrium constants and thermodynamic parameters for self- and hetero-associating systems

→ characterize the solution-state behavior of macromolecules under various conditions

Analytical Ultracentrifugation – Applications

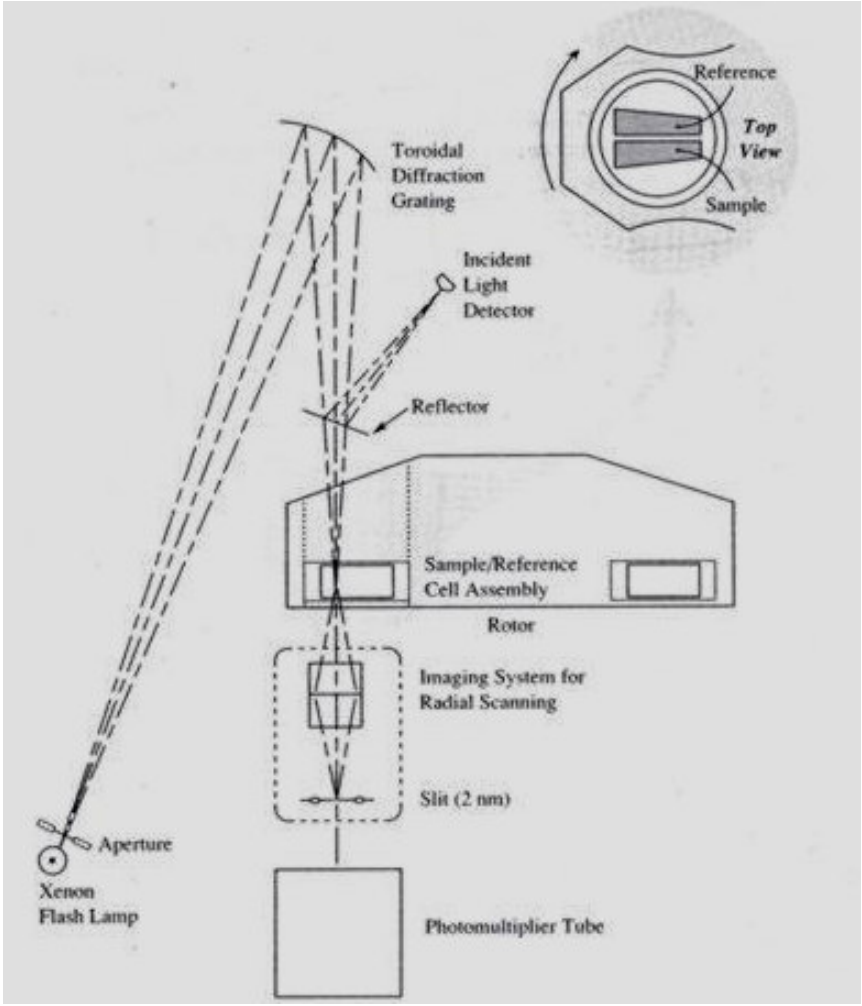
- determine sample purity
- characterize assembly and disassembly mechanisms of biomolecular complexes
- determine subunit stoichiometries
- detect and characterize macromolecular conformational changes
- measure equilibrium constants and thermodynamic parameters for self- and hetero-associating systems

→ thermodynamic and hydrodynamic information

Analytical Ultracentrifugation – Design

- analytical ultracentrifuge = preparative ultracentrifuge + optical detection system
  → measure sample concentration inside the centrifuge cell during or after sedimentation
- centrifugation parameters and data acquisition under computer control
  → experiments lasting many days performed with minimal operator intervention

Analytical Ultracentrifugation – Design



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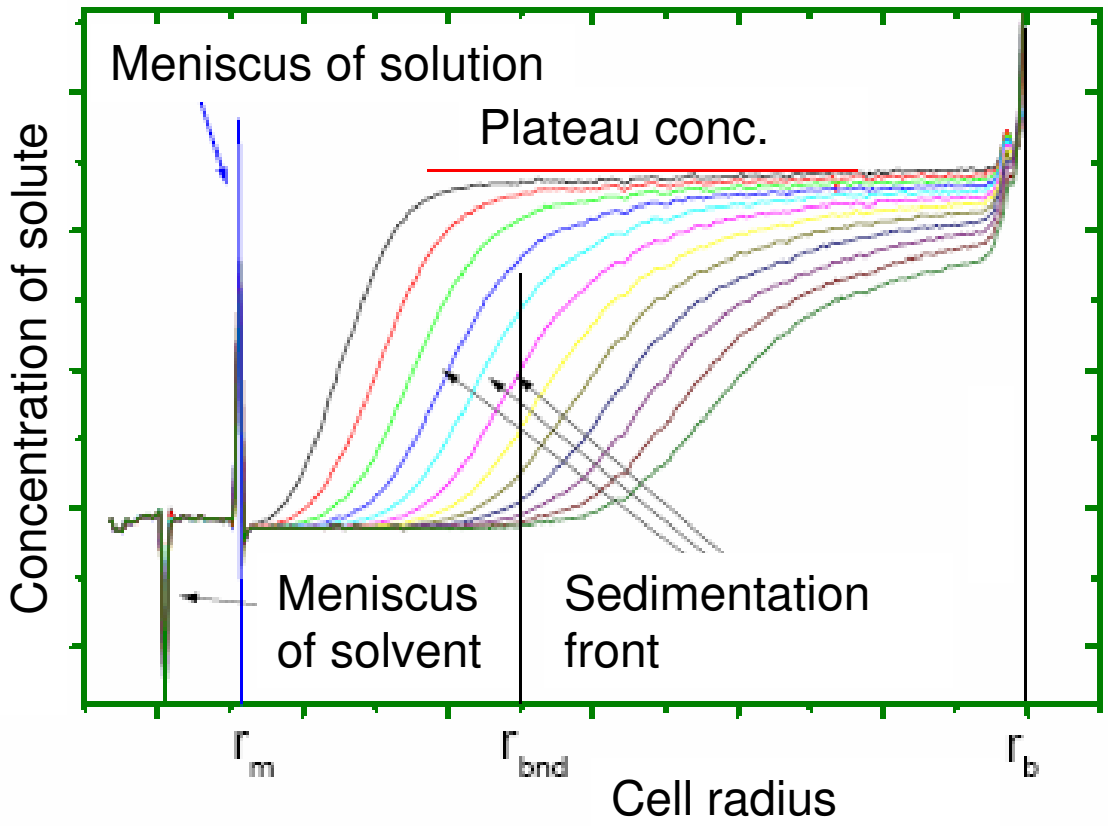
Analytical Ultracentrifugation – Design: Optical systems

- Absorbance optical system:
  - → measurement of sample concentration at wavelengths from 200 to 800 nm
    - → detection of macromolecules containing strong chromophores
- Rayleigh interference optical system:
  - → measurement of sample concentration based on refractive index changes
    - → analyze macromolecules lacking intense chromophores (eg, polysaccharides) and samples that contain strongly absorbing buffer components (eg, ATP/GTP, DTToxidized)

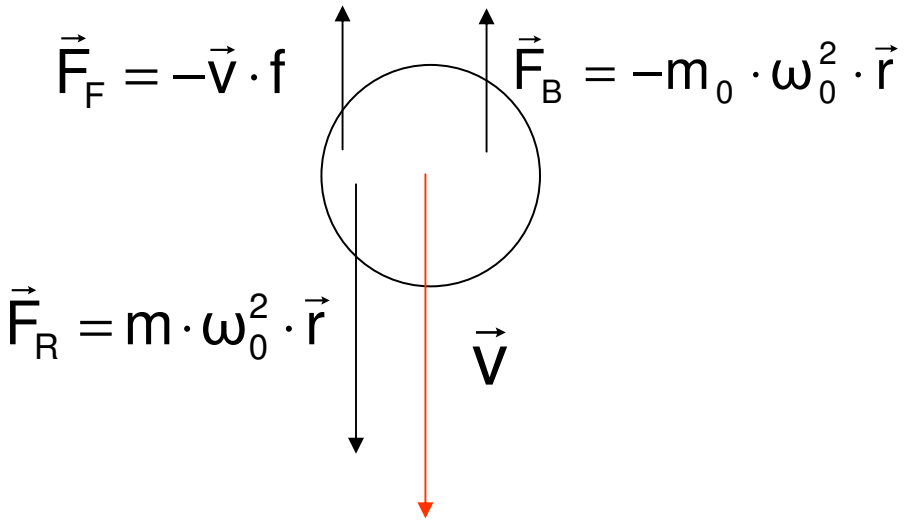
Analytical Ultracentrifugation – Sedimentation velocity experiments



Modified from http://www.kolloidanalytik.de/uz/sed/uzsedhr.gif

Analytical Ultracentrifugation – Sedimentation velocity experiments

- Spherical particle with radius R moves with constant velocity v in a centrifuge at the radial distance r:



- $\vec{F}_R + \vec{F}_B + \vec{F}_F = m \cdot \varpi_0^2 \cdot \vec{r} - m_0 \cdot \varpi_0^2 \cdot \vec{r} - \vec{v} \cdot f = 0$

Analytical Ultracentrifugation – Sedimentation velocity experiments

- Spherical particle moves with constant velocity v in a centrifuge at the radial distance r:

$$\rightarrow \quad \vec{F}_R + \vec{F}_B + \vec{F}_F = m \cdot \omega_0^2 \cdot \vec{r} - m_0 \cdot \omega_0^2 \cdot \vec{r} - \vec{v} \cdot f = 0$$

- Possibility to determine molecular mass of a spherical molecule
- Possibility to determine shape of a molecule using the friction factor of an idealized spherical particle compared to the measured friction factor
  → axial ratio of oblate or prolate elipsoide

Analytical Ultracentrifugation – Sedimentation velocity experiments

- Svedberg equation:

$$\vec{F}_R + \vec{F}_B + \vec{F}_F = m \cdot \omega_0^2 \cdot \vec{r} - m_0 \cdot \omega_0^2 \cdot \vec{r} - \vec{v} \cdot f = 0$$

$$\Rightarrow v = r \cdot \omega_0^2 \cdot \frac{m \cdot (1 - \bar{v}\rho_0)}{f}$$

$$\Rightarrow s \equiv \frac{m \cdot (1 - \bar{v}\rho_0)}{f} = \frac{v}{r \cdot \omega_0^2}$$

→ influenced by density and viscosity of solvent

→ standard solvent (water, 20 °C): $s_{20,w}$

- Boundary spreading: Flux *J*

$$J = s \cdot \omega^2 \cdot r \cdot c - D \cdot \left(\frac{dc}{dr} \right) \text{ with } D = \frac{R \cdot T}{N \cdot f}$$

Analytical Ultracentrifugation – Sedimentation velocity experiments

- Hydrodynamic information
- Experimentally determined parameters:
  - Sedimentation coefficient *s*
  - Diffusion constant D or friction factor *f*
  - Molecular mass M
  - Estimation of the molecule's shape in solution
- High rotor speeds
  → sedimentation dominates diffusion

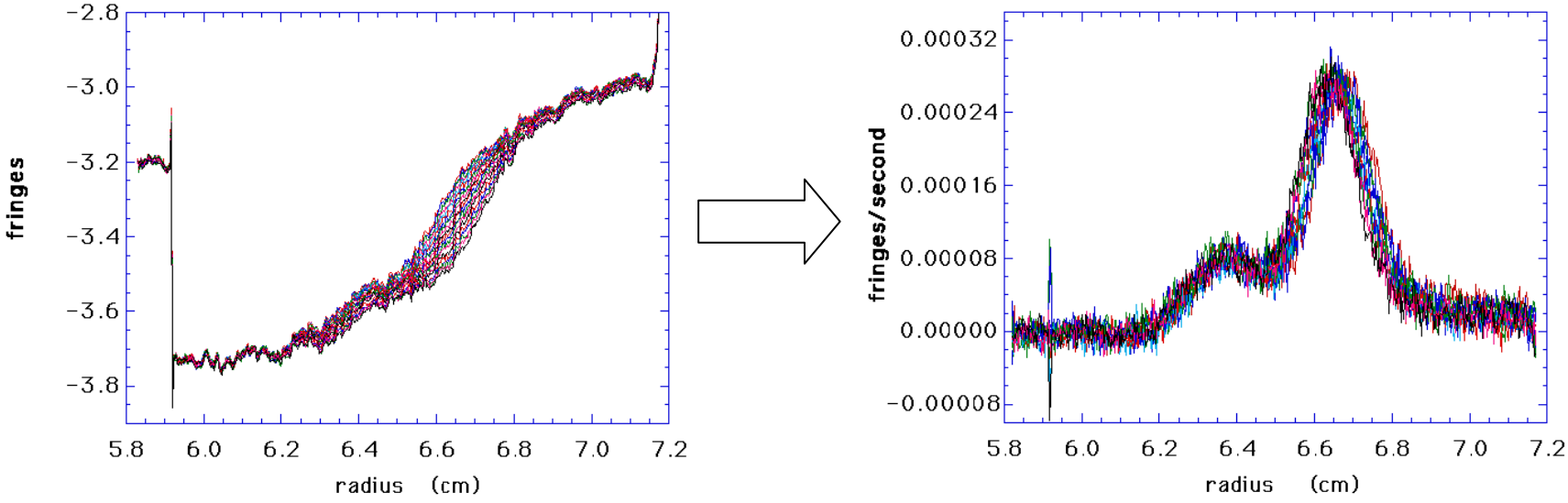
Analytical Ultracentrifugation – Data analyses: sedimentation velocity

- plot natural logarithm of boundary midpoint versus time
  - → single-point boundary analyses
  - → slope of straight line yields sedimentation coefficient *s*
- time derivative (DCDT) method (Stafford)
  - → subtract different scans
  - → convert the boundaries into apparent differential distribution of *s*, g(*s**) and plot g(*s**) versus *s**

Analytical Ultracentrifugation – Data analyses: sedimentation velocity

- time derivative (DCDT) method (Stafford)

  → subtract different scans



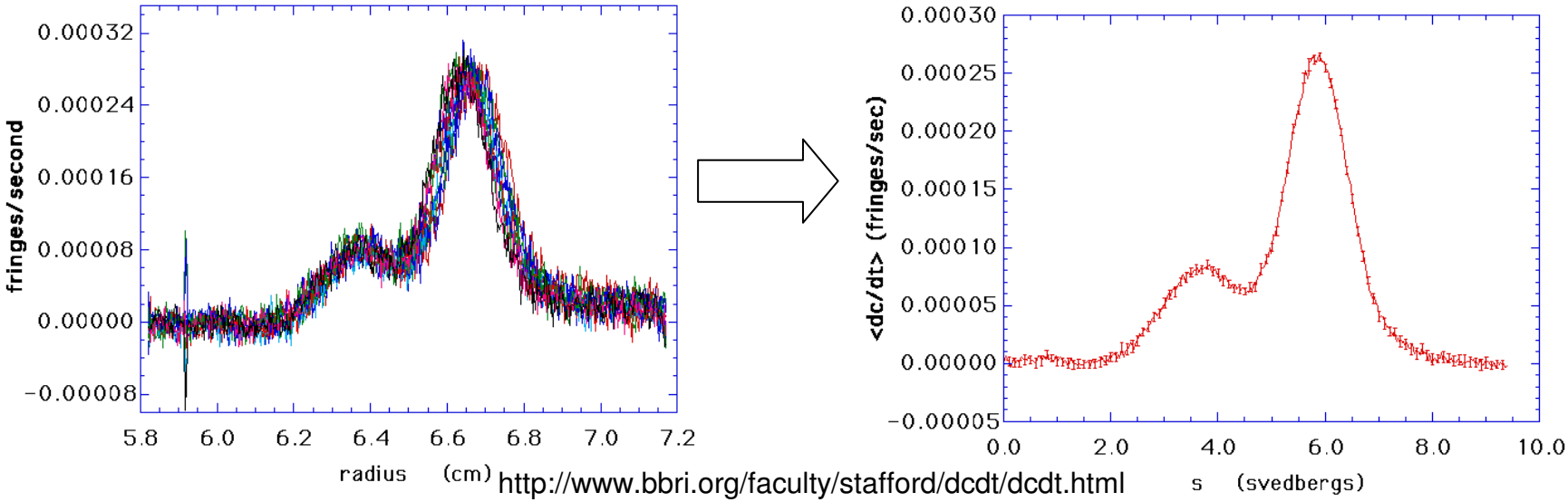
http://www.bbri.org/faculty/stafford/dcdt/dcdt.html

Analytical Ultracentrifugation – Data analyses: sedimentation velocity

- time derivative (DCDT) method

  → convert boundaries into distribution of *s*

$$s^* = \frac{1}{\omega^2 t} \ln\left(\frac{r}{r_m}\right)$$



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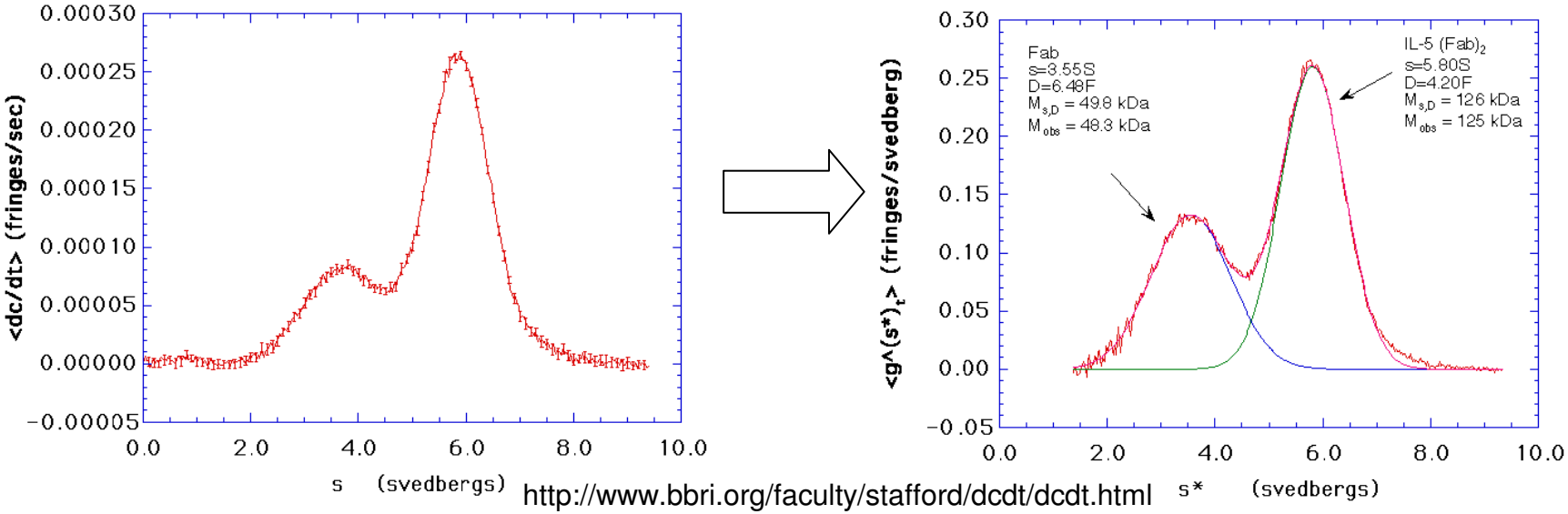
Analytical Ultracentrifugation – Data analyses: sedimentation velocity

- time derivative (DCDT) method
  - → recalculate to obtain g(*s**)
    - → area under the peak equals plateau concentration

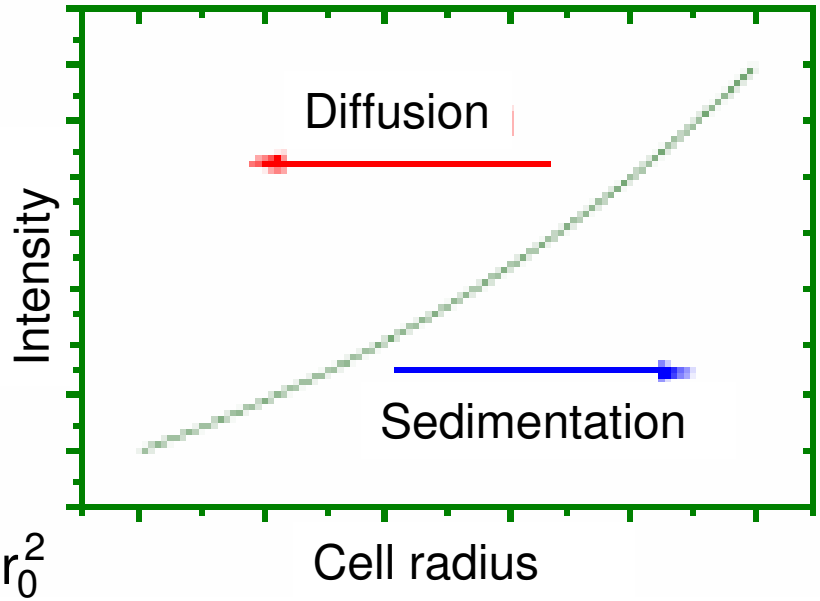


http://www.bbri.org/faculty/stafford/dcdt/dcdt.html

Analytical Ultracentrifugation – Sedimentation equilibrium experiments

- Slower rotor speeds
  - → balance between sedimentation and diffusion forces
  - → no net transport
  - → no influence of shape factors
- Determination of M:

$$c(r) = c_0 \cdot e^{\frac{M(1-\bar{v}\cdot\rho_0)\cdot\omega_0^2\cdot r_0^2}{2\cdot R\cdot T}}$$



http://www.kolloidanalytik.de/uz/equil/hequil.pdf

Analytical Ultracentrifugation – Sedimentation equilibrium experiments

- Thermodynamic information
- Experimentally determined parameters:
  - Molecular mass M
  - Solution assembly state
  - Thermodynamic parameters like the equilibrium constant K
    → calculation of the free energy of the association reaction
  - Other thermodynamic parameters

Analytical Ultracentrifugation – Data analyses: sedimentation equilibrium

- Graphical data analysis methods
  - Plot ln(c) versus r^2

    → straight line with slope proportional to M:

$$c(r) = c_0 \cdot e^{\frac{M\cdot(1-\bar{v}\cdot\rho_0)\cdot\omega_0^2\cdot r_0^2}{2\cdot R\cdot T}} \Rightarrow \frac{d\ln(c)}{dr^2} = \frac{M\cdot(1-\bar{v}\cdot\rho_0)\cdot\omega^2}{2\cdot R\cdot T}$$

- Alternative for more complex systems:

  → direct fitting of sedimentation equilibrium concentration gradients to mathematical functions

Analytical Ultracentrifugation – Examples of Applications

- Sedimentation velocity
  - Biomolecular Shape
  - Biomolecular Conformational Changes
  - Assembly and Disassembly of Biomolecular Complexes
  - Molecular Mass and Subunit Stoichiometry
  - Equilibrium Constants for Self-Associating Systems

- Sedimentation equilibrium
  - Molecular Mass and Subunit Stoichiometry
  - Equilibrium Constants for Hetero-associating Systems
  - Equilibrium Constants for Self-Associating System

Vimentin

- Intermediate filament of eukaryotic cells
- Structure:

  - monomer with central α-helical domain, capped with non-helical head/tail

    → two monemers: coiled-coil dimer

    → further oligomerisation

  - α-helical sequences with "hydrophobic seal" on the surface of the helix

    → allows coiling

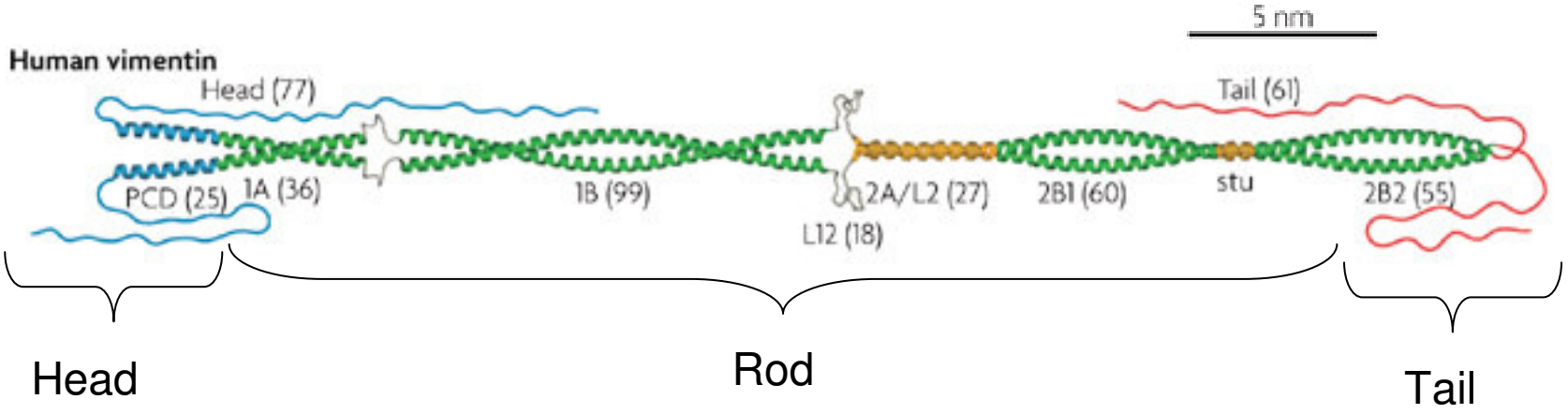
  - homopolymeric filaments

Vimentin

- Intermediate filament of eukaryotic cells
- Function:
  - anchoring the position of organelles in the cytosole
  - important for the flexibility of cells and cell integrity
  - stabilization of cytoskeletal interaction
  - transport of LDL inside the cell
  - no enzymatic activity (unlike actin and tubulin)

Vimentin

- Structure of a dimer of human vimentin:

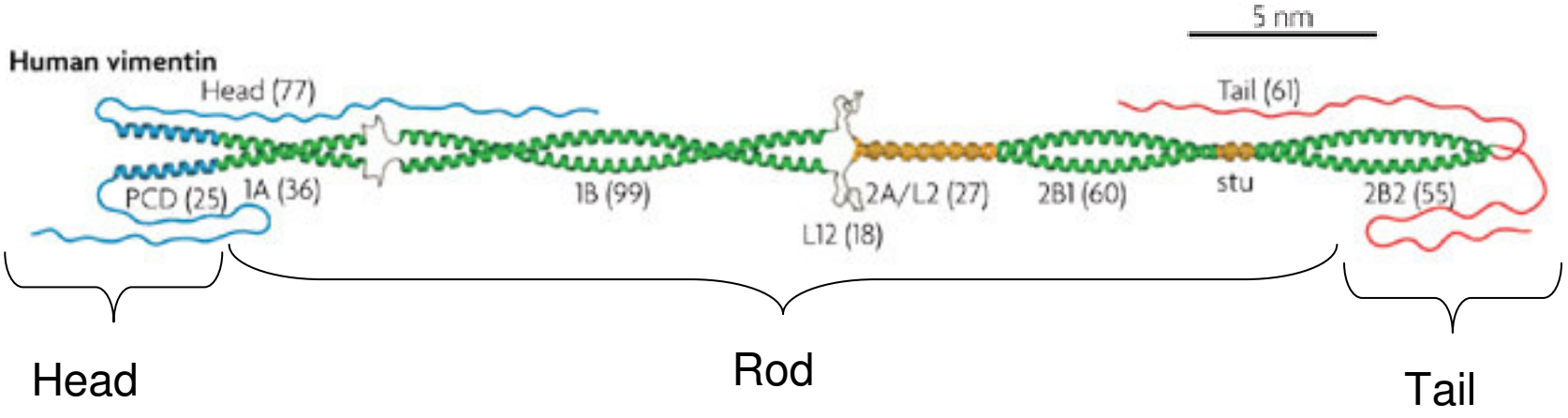


Herrmann H, *Nat Rev Mol Cell Biol,* 2007

- Formation of tetramers in vitro

Characterization of Assembly-Starter Units of Human Vimentin

- Structure of a dimer of human wt vimentin:



Herrmann H, *Nat Rev Mol Cell Biol,* 2007

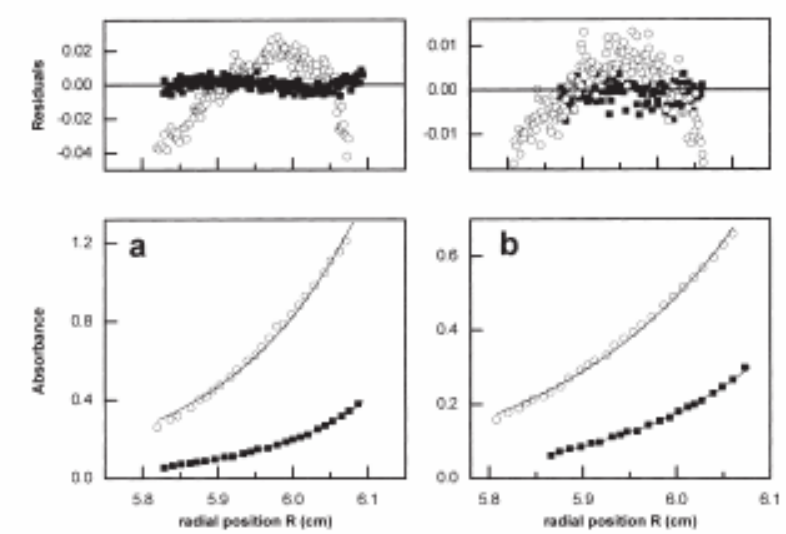
- Study of the assembly of wt, headless, tailless vimentin and vimentin rod

Characterization of Assembly-Starter Units of Human Vimentin: Aims and Questions

- Investigation of complex assembly of wt vimentin in low salt and physiological buffer
  - → Investigation of the homogeneity of the vimentin complexes
- Quantify influence of truncation of the non-α-helical head and tail domains
  - → Determination of the association constants of wt and headless vimentin
  - → Determination of s-values
  - → Modeling of the shape of different vimentins

Characterization of Assembly-Starter Units of Human Vimentin: Results

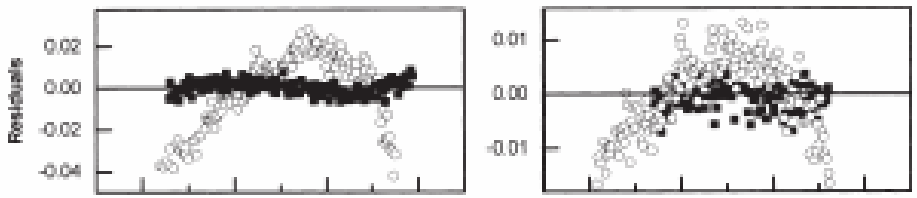
- Investigation of complex assembly of wt vimentin in low salt and physiological buffer
  → analytical ultracentrifugation



Mücke N, *J Mol Biol*. 2004

Characterization of Assembly-Starter Units of Human Vimentin: Results

- Investigation of complex assembly of wt vimentin in low salt and physiological buffer
  - → by sedimentation equilibrium runs
  - → concentration dependent deviation
    - → non-ideal sedimentation behavior caused by rod domain rather than by the head
      - → extrapolation of values for molecular mass to zero concentration



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Characterization of Assembly-Starter Units of Human Vimentin: Results

- Investigation of complex assembly of wt vimentin in low salt and physiological buffer

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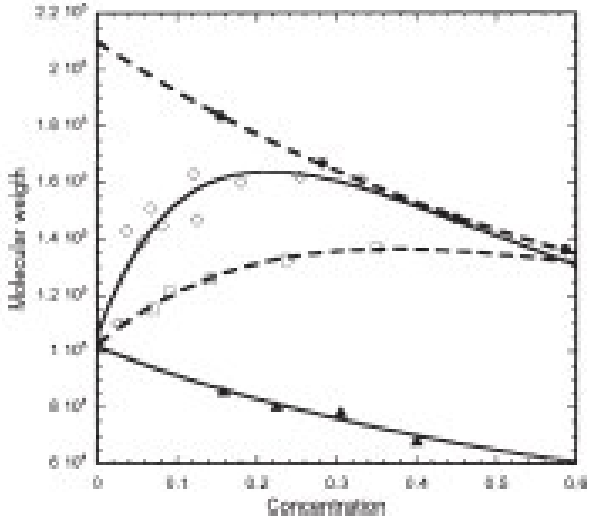


Mücke N, *J Mol Biol*. 2004

Characterization of Assembly-Starter Units of Human Vimentin: Results

- Investigation of complex assembly of vimentin in low salt and physiological buffer
  - → extrapolation of values for molecular mass to zero concentration
  - → wt vimentin: 2.1×10^5
    - → tetrameric complex
  - → headless vimentin: 1.0×10^5
    - → dimeric complex
    - → at higher ionic strength: tetramers
- Results confirmed using a non-linear global fit program

Characterization of Assembly-Starter Units of Human Vimentin: Results

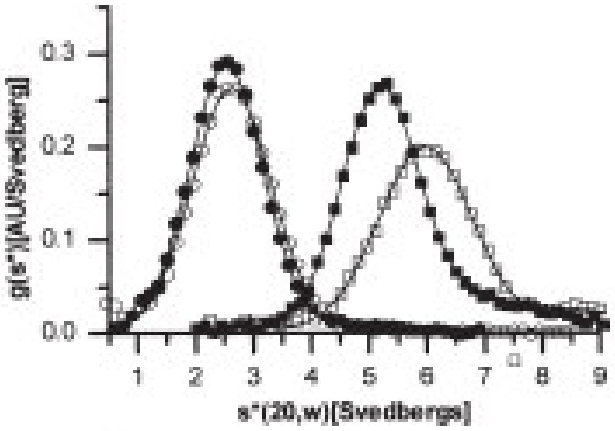
- Determination of the association constants of wt and headless vimentin
  - → increase in the ionic strength results in a shift of the equilibrium towards higher oligomers of wt vimentin
    → association of tetramers to octamers
  - → small effect of salt addition of headless vimentin
    → association of dimers to tetramers

Characterization of Assembly-Starter Units of Human Vimentin: Results

- Determination of s-values
  - → by sedimentation velocity runs using low protein concentrations (avoid non-ideality)
  - → pH dependent sedimentation coefficients of wt and tailless vimentin
    - → pH dependent changes in molecule size, shape or stiffness
    - → wt: good agreement with data obtained from sedimentation equilibrium runs
    - → tailless: second species with higher s value (<10%)
  - → headless vimentin and vimentin rod: sedimentation as homogenous species

Characterization of Assembly-Starter Units of Human Vimentin: Results

- Determination of s-values
  - → pH dependent sedimentation coefficients of wt and tailless vimentin
    - → wt: homogenous species
    - → tailless: second species with higher s value (<10%)
  - → headless vimentin and vimentin rod: sedimentation as homogenous species



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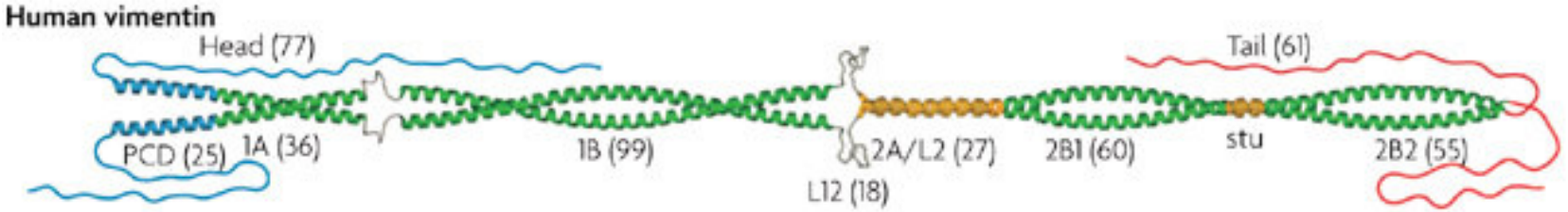
Characterization of Assembly-Starter Units of Human Vimentin: Results

- Modeling of the shape of different vimentins
  - → using SEDNTERP
  - → electron microscopy: elongated, rod-like shape
    - → modeling as prolate ellipsoids
      - → wt: 73 nm length, 3.3 nm width
      - → tailless: 53 nm length
      - → rod (dimeric): 49 nm length
      - → headless (dimeric): 59 nm length



Herrmann H, *Nat Rev Mol Cell Biol,* 2007

Characterization of Assembly-Starter Units of Human Vimentin: Results

- Modeling of the shape of different vimentins
  - → at higher pH values: increasing lengths
    - → correlation with lower s values
  - → description of vimentin oligomers as prolate ellipsoids
- Results obtained from analytical ultracentrifugation and other methods
  - → similar complex sizes determined

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