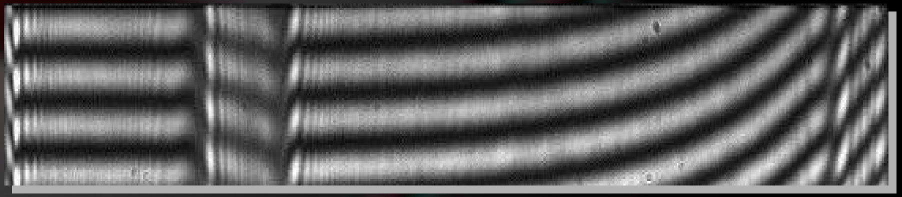
Sedimentation equilibrium



- Thermodynamic information
  - Mass
  - Stoichiometry
  - Equilibrium constants
  - Nonideality

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Preliminaries

- *What do you want to know?*
- *Sample handling*
- *Sample type*
- *Optical system*

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What do you want to know?

- Monomer molecular weight
- Stoichiometry of a complex
- Association constants
- Nonideality

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Preliminaries

- What do you want to know?
- Sample handling
- Sample type
- Optical system

Sample Handling

Sample Arrives

If sample has not been gel filtered during purification, do so before analysis

General Sample Handling

- Estimate concentration and volume
- Bring sample to dialysis equilibrium with buffer
- Choose centerpiece material

Sedimentation Equilibrium

Sedimentation Velocity

Short column (Sedimentation Equilibrium)

- Quick survey
- Heteroassociations
- Titrations

"Long" column (Sedimentation Equilibrium)

- Detailed analysis
- Low molecular weight
- Heterogeneity

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Preliminaries

- What do you want to know?
- Sample handling
- Sample type
- Optical system

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Sample type

Protein

Choice of optics

1 $A_{230 \text{ or } 280}$

1 mg/ml

Polysaccharide

Interference optics

C > 1 mg/ml

Nonideality

Nucleic Acid

Absorbance optics

1 A_{260}

Nonideality

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Preliminaries

- *What do you want to know?*
- *Sample handling*
- *Sample type*
- *Optical system*

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Choosing optical system

- ***Use absorbance if:***
  - *Need selectivity*
  - *Added sensitivity*
  - *Cannot dialyze sample*
- ***Use both:***
  - *Determine extinction coefficient*
  - *Test for sample purity*
  - *Extend concentration range*
- ***Use interference if:***
  - *Buffer absorbs*
  - *Sample does not absorb*
  - *Precision required*
  - *g(s)*
  - *Extinction coefficient varies*
  - *Short columns*

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What do you want to know?

- Monomer molecular weight
- Stoichiometry of a complex
- Association constant
- Nonideality

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Monomer molecular weight

- Use denaturing conditions
  - 6 M guanidinium chloride
  - 8 M urea
- Determine partial specific volume
  - Measurement
  - Calculation
- Density
- Viscosity (for sedimentation velocity)

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What do you want to know?

- Monomer molecular weight
- Stoichiometry of a complex
- Association constant
- Nonideality

- Sedimentation equilibrium
  - Shape of a complex
    - K_s the effect of C on s
  - Molecular weight (discrete mixtures)
  - Association constant (favorable conditions)
- Sedimentation velocity

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Stoichiometry of a complex

- Examine each component individually
  - Isolate components for heterooligomer
- M_d in denaturing buffer
- M_n in nondenaturing buffer
  - Isolated components (if stable alone)
  - Holocomplex
- Stoichiometry
  - Homooligomer $N = M_n/M_d$
  - Heterooligomer N can be harder to determine
  - Uncertainty rises as N increases

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What do you want to know?

  - Monomer molecular weight
  - Stoichiometry of a complex
  - Association constant
  - Nonideality
- Sedimentation equilibrium
  - Shape of a complex
    - K_s the effect of C on s
  - Molecular weight (discrete mixtures)
  - Association constant (favorable conditions)
- Sedimentation velocity

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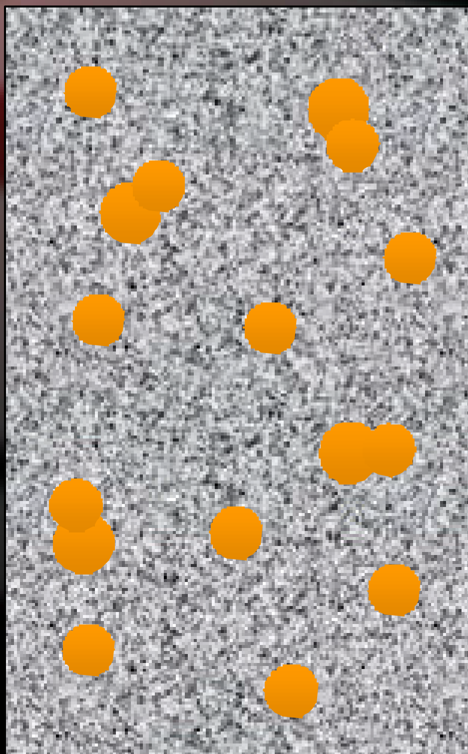
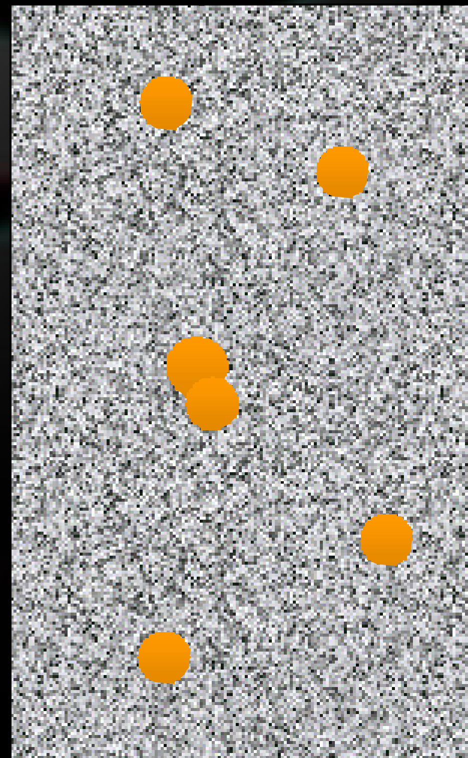
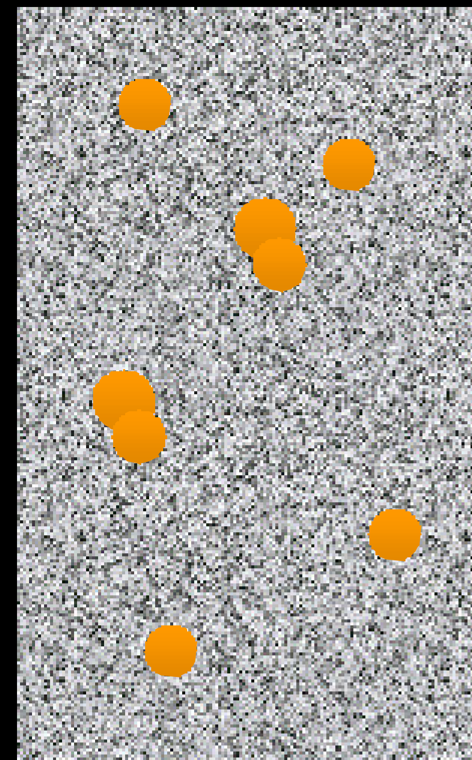
Association Constant

- Depends on type of association
- Self association
  - Must have correct assembly model
  - Must cover correct concentration
- Hetero association
  - Test using different ratios of subunits
  - Model as self association for 1:1 assemblies
    - $Ds < 3.5$

Is an interaction reversible?

$$K_{app} = [D]/[M]^2$$

Stock	1:2 Equilibrating	1:2 Aggregate
		
$K_{app} = 4/8^2 = 1/16$	$K_{app} = 1/4^2 = 1/16$	$K_{app} = 2/4^2 = 1/8$

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Nonideality

- Always present
- Two major sources
  - Excluded volume
    - Constant and small for spheres
    - Worse for rods and coils
  - Charge-charge repulsion
    - Dominates for proteins of any shape at low G/ 2
    - Minimize by adding salt G/ 2> 0.1 or more
- Minimize by extrapolation to C=0

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Two types of equilibrium expts

Sample Arrives

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Sedimentation Equilibrium

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Short Column Equilibrium

Solution Column < 1 mm

Select Conditions

Rotor speed

Temperature

Use interference optics

General requirements

>30 uL sample at >1 mg/ml

- Dialyzed against solvent
- Use 15 uL in channel A
- Make 3 serial 1:2 dilutions for channels B->D

Run duplicate sample treated with 6 M GuHCl

- Cannot use centrifugal gel filtration w/ 6 M GuHCl

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Two types of equilibrium expts

Sample Arrives
If sample has not been gel filtered during purification, do so before analysis

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Estimate concentration and volume
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Choose centerpiece material

Sedimentation Equilibrium

Sedimentation Velocity

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Quick survey
Heteroassociations
Titrations

"Long" column
Detailed analysis
Low molecular weight
Heterogeneity

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“Long” Column Equilibrium

Solution Column > 2 mm

Select Conditions:

Rotor speed
Temperature
Optical system

General requirements

250 uL @ 1 unit (e.g. 1 OD or 1 mg/ml)
Use 110 uL in channel A
Make 2 1:3 serial dilutions for B and C

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Notes on rotor speeds

- Use 4 rotor speeds
  - Go from lowest to highest
  - Lowest should have monomer $s \sim 2$
    - starting rpm ~ $5 \times 10^6 (1/M)^{1/2}$
    - start at 3000 rpm if working with complete unknown
- Space rotor speeds to cover 2 to 3 fold range
  - $s \sim 2 - 15$

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General considerations

- Correcting for buoyancy
  - Determining density
  - Partial specific volume
- Correcting for viscosity

Useful references

Books:

Analytical Ultracentrifugation in Biochemistry and Polymer Science. (1992) S.E. Harding, A.J. Rowe, and J.C. Horton, eds. Royal Society of Chemistry, Cambridge.

Modern Analytical Ultracentrifugation. (1995) T.M. Schuster and T.M. Laue, eds. Birkhauser, Boston.
Two fairly recent books devoted entirely to this field

K.E. van Holde, Physical Biochemistry. (1985) Prentice Hall, Englewood Cliffs, New Jersey.
Good introductory text for general theory of sedimentation, frictional coefficients, diffusion, and other hydrodynamic analysis

Freifelder, D. (1982). Physical Biochemistry: Applications to biochemistry and molecular biology. W.H. Freeman, New York.
Regarded as a good introductory text that is strong on centrifugation methods

van Holde, K.E., W.C. Johnson, Jr., and P.S. Ho. .(1998). Principles of physical biochemistry. Prentice-Hall, Upper Saddle River.

Cantor, C.R. and Schimmel, P.R. (1980). Biophysical chemistry. Part II: Techniques for the study of biological structure and function. W.H. Freeman, San Francisco.
These two are more advanced texts with good coverage of centrifugation methods

Special Journal Issue:

Chemtracts Biochemistry and Molecular Biology, vol. 11 no. 13 (pp. 933-1004), December 1998 (Jeffrey C. Hansen, Guest Editor)
Several review articles and condensation commentaries on current research

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Useful references

Review Articles:

Stafford, W.F. III. (1997). Sedimentation velocity spins a new weave for an old fabric. *Curr. Opin. Biotechnol*. 8, 14-24.

Laue, T.M. (1995). Sedimentation equilibrium as thermodynamic tool. Methods Enzymol. 259, 427-452.

Laue, T.M. Stafford, W.F., III (1999). Modern Applications of Analytical Ultracentrifugation. *Annu. Rev. Biophys. Biomol. Struct.* 28, *75-100.*

Articles:

Laue, T.M., Shah, B.D., Ridgeway, T.M., and Pelletier, S.L. (1992). Computer-aided interpretation of analytical sedimentation data for proteins. In: Analytical ultracentrifugation in biochemistry and polymer science. S.E. Harding, A.J. Rowe, and J.C. Horton, eds. Royal Society of Chemistry, Cambridge, pp. 90-125.
Procedures for calculating partial specific volume, density, sedimentation coefficient (corrected for water @ 20°C and extrapolated to zero concentration) hydration, frictional rations, ellipsoidal shapes, etc; basis for SEDNTERP software (but note that the formulae and tables contain a number of typographical errors that were corrected in SEDNTERP – see the SEDNTERP Help file for corrected formulas)

Stafford, W.F., III. (1992). Boundary analysis in sedimentaiton transport experiments: A procedure for obtaining sedimentation coefficient distributions using the time derivative of the concentration profile.
Anal. Biochem. 203, 295-301.
Initial publication describing the dc/dt method

Johnson, M.L. and Frasier, S.G. (1985). Nonlinear least-squares analysis. *Methods Enzymol*. 117:301-342.
Good overview of the fitting of experimental data