BBSG501
Section 1
Fall 2003 Semester

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DATE: 08/25/03 **Lecturer: Tomasz Heyduk**

Lecture 1: Review of thermodynamics and kinetics I.

1. Criteria for spontaneity**:**
- $ both energy and entropy are important for determining the direction of the process
- $ free energy

2. Free energy and the equilibrium constant:

3. Examples of entropy changes in some biologically important processes:
- $ entropy changes of mixing
- $ entropy changes of solvation
- $ entropy changes of ionization
- $ entropy changes of protein-ligand complex formation

4. Thermodynamics of hydrophobic effect**.**

5. Thermodynamics of polyanion effect.

6. Applications of free energy calculations in biochemistry:
- $ calculation of free energy change of the reaction from known standard free energies of formation
- $ driving the unfavorable reactions by coupling to favorable processes

Suggested reading: ABiochemistry@, Goeffrey L. Zubay (4th edition), chapter 2.
ABiochemistry@, Donald and Judith Voet (2nd edition), chapter 3.

Date: 08/25/03 **Lecturer: Tomasz Heyduk**

Lecture 2: Review of thermodynamics and kinetics II.

1. Transition-state theory of chemical reactions in solution.

2. How fast a reaction can be ?

3. Basic laws of chemical kinetics:
   - $ dependence of the rates of reactions on concentrations of reagents
   - $ first-order reactions
   - $ second-order reactions

4. Equilibrium vs kinetics:
   - $ lifetimes of biological complexes

5. Biological process can be under thermodynamic or kinetic control.

Suggested reading: ABiochemistry@, Goeffrey L. Zubay (4th edition), chapter 8.
ABiochemistry@, Donald and Judith Voet (2nd edition), chapter 3.

Date: 08/25/03 **Lecturer: Tomasz Heyduk**

Lecture 3: Amino-acids and polypeptides

1. Structure of the amino-acid:
   - $ nomenclature
   - $ optical isomers

2. Common amino-acids found in proteins**:**
   - $ apolar
   - $ uncharged polar
   - $ basic
   - $ acidic

3. Acid-base properties of amino-acids:
   - $ titration curves of amino acids
   - $ isoelectric point

4. Chemical reactivity of amino-acids side chains:
   - $ reactivity of cysteine
   - $ reactivity of lysine
   - $ preparation of bioconjugates

5. Aromatic amino-acids: absorption of light in near-UV.

Date: 08/25/03 **Lecturer: Tomasz Heyduk**

Lecture 3: Amino-acids and polypeptides (continued)

6. Peptide bond:

- $ geometry of the peptide bond
- $ properties of the polypetide chain
- $ stability of the peptide bond

Suggested reading: ABiochemistry@, Goeffrey L. Zubay (4th edition), chapter 4.
ABiochemistry@, Donald and Judith Voet (2nd edition), chapter 6.

Date: 08/27/03 **Lecturer: Yie-Hwa Chang**

Lecture 4: Protein Folding

1. The information for folding is contained in the primary structure.

2. The Ramachandran plot predicts sterically permissible structures.

3. Protein folding reveals a hierarchy of structual organization.

4. Two secondary structures are found in most proteins:

- $ The helix
- $ The sheet

5. Pauling and Corey provided the foundation for our understanding of fibrous protein structures:

- $ Collagen forms a unique triple-stranded structure

6. In globular proteins, secondary structure elements are connected in simple motifs

Suggested reading: ABiochemistry@, Goeffrey L. Zubay (4th edition), chapter 5.

Date: 08/27/03 **Lecturer: Yie-Hwa Chang**

Lecture 5: 3-D structure of proteins

1. The domain is the basic unit of tertiary structure

2. The helix-loop-helix motif is the basic component found in a-domain structures

3. / domains exploit the -- motif

Date: 08/27/03 **Lecturer: Yie-Hwa Chang**

Lecture 5: 3-D structure of proteins (continued)

4. Antiparallel domains show a great variety of topologies

5. Some proteins or domains require additional features to account for their stability

6. Many proteins contain more than one domain

7. Quaternary structure depends onn the interaction of two or more protein subunits

8. Predicting protein tertiary structure from protein primary structure

9. Methods for determining protein conformation

- $ X-ray diffraction analysis of fibrous proteins
- $ X-ray diffraction analysis of protein crystals
- $ Nuclear magnetic resonance (NMR) complements X-ray crytallography

Suggested reading: ABiochemistry@, Goeffrey L. Zubay (4th edition), chapter 5.

Date: 08/28/03 **Lecturer: Tomasz Heyduk**

Lecture 6: Nucleic acid structure.

1. Chemical structure of nucleic acids:

- $ purine and pyrimidine bases

2. Base-paring and higher order structure:

- $ Watson-Crick base pairs
- $ comparison of B-DNA and A-DNA helices
- $ Z-DNA: left-handed helix
- $ accessibility of functional groups in DNA duplex
- $ alternative base pairing: formation of DNA triple helices

3. Stability of DNA duplex

4. Sequence dependent variation of DNA helix structure

- $ local sequence-dependent changes of DNA helix structure
- $ sequence-directed DNA bending

Suggested reading: ABiochemistry@, Goeffrey L. Zubay (4th edition), chapter 30.
ABiochemistry@, Donald and Judith Voet (2nd edition), chapter 28.
ADNA: Structure and Function@, R.R. Sinden, chapter 1 and 2.

Date: 08/29/03 **Lecturer: Tomasz Heyduk**

Lecture 7: Hydrodynamic properties of macromolecules

1. Diffusion of macromolecules in solution:

- $ concentration gradient dependence
- $ viscosity dependence
- $ size and shape dependence
- $ rotational diffusion

2. Determination of hydrodynamic properties of macromolecules

- $ movement of macromolecules in solution under external force
- $ analytical centrifuge
- $ concentration dependence of sedimentation coefficient
- $ light scattering and correlation spectroscopies

3. Effect of cellular environment on hydrodynamic properties of macromolecules.

Assigned reading: APhysical Biochemistry@, D. Freifelder, chapter 9 and 11.

Date: 08/29/03 **Lecturer: Tomasz Heyduk**

Lecture 8: Modern physical methods for studying macromolecules I

1. Absorbance spectroscopy:

- $ rules of absorption of light by molecules, absorption spectra
- $ biological chromophores
- $ reporter groups
- $ practical applications of absorption spectroscopy
- $ infrared and CD spectroscopies

2. Fluorescence spectroscopy:

- $ origin of light emission by the molecules
- $ fluorescence as a sensitive means of detection of tagged biomolecules
- $ fluorescence studies of single molecules
- $ sensitivity of fluorescence to microenvironment
- $ resonance energy transfer and fluorescence polarization
- $ practical applications of fluorescence spectroscopy

Suggested reading: APhysical Biochemistry@, D. Freifelder, chapter 14, 15 and 16.
Proc. Natl. Acad. Sci. USA, **96,** 9077-9082 (1999).
Trends in Cell Biology, **9**, 77-80 (1999).
Current Opinion in Biotech. **10,** 22 - 28 (1999)

Date: 09/02/03 **Lecturer: Tomasz Heyduk**

Lecture 9: Modern physical methods for studying macromolecules II

1. Mass spectrometry of biological molecules:
- $ MALDI-TOF
- $ ESI
- $ mass spectrometry in proteomics

2. Single-molecule experiments
- $ techniques for observation single molecules
- $ techniques for manipulating single molecules
- $ examples:
  - single-molecule FRET
  - single-molecule dynamics

Date: 09/03/03 **Lecturer: Ali Shilatifard**

Lecture 10: Protein purification

1. Protein purification via centrifugation
- $ Low speed centrifugation
- $ High speed centrifugation

2. Protein purification via Dialysis

3. Protein purification via salt precipitation
- $ N_2H_8SO4 Cut
- $ $MgCl_2$ Cut
- $ $CaCl_2$ Cut

4. Protein purification via high and low speed column chromatography
- $ Cation-exchange chromatography
- $ Anion-exchange chromatography
- $ Size exclusion chromatography
- $ Hydrophobic interactions chromatography

5. Protein purification via affinity chromatography
- $ Lectin affinity chromatography
- $ Antibody chromatography
- $ Nickel chromatography

6. Protein purification via crystallization

Date: 09/03/03 **Lecturer: Ali Shilatifard**

Lecture 10: Protein purification (continued)

7. Recombinant proteins expression and purification

- $ Expression in bacterial systems
- $ Baculovirus expression system
- $ Expression in Mammalian cells

8. Protein purification via gel electrophoresis

- $ Isoelectric focusing
- $ SDS/PAGE

Suggested reading: ABiochemistry@ L. Stryer (4th Edition) Chapter 3**.**

Date: 09/04/03 **Lecturer: Ali Shilatifard**

Lecture 11: Protein characterization

1. Protein identification via staining methodologies

- $ Coomassie Blue staining
- $ Colloidal Blue staining
- $ Silver staining

2. Protein identification via Amino Acid analysis

- $ HPLC-method for amino acid analysis
- $ Ion-exchange method for amino acid analysis
- $ Mass spectrometry methods for amino acid analysis

3. Protein identification via Protein sequencing

- $ Protein sequencing via Edman degradation
- $ Protein sequencing via Mass spectrometry method

4. Protein identification via Western analysis

- $ SDS/PAGE
- $ Protein transfer
- $ Primary and secondary antibodies

5. Identification of recombinant proteins

- $ N-terminal and C-terminal tags

Suggested reading: ABiochemistry@ L. Stryer (4th Edition) Chapter 3**.**

Date: 09/05/03 **Lecturer: Tomasz Heyduk**

Lecture 12: Binding phenomena: basic concepts

1. Basic properties of the equilibrium constant.

2. Binding of a ligand to a single site:
- $ graphical representations of binding data

3. Binding of the ligand to two independent binding sites:
- $ statistical effects: macroscopic and microscopic equilibrium constants
- $ interpretation of Scatchard plot
- $ "specific" vs "nonspecific" binding

4. Methods for studying binding:
- $ equilibrium techniques
- $ nonequilibrium techniques
- $ indirect techniques

5. Macromolecular interactions: test tube vs cell
- $ macromolecular crowding

Suggested reading: APhysical Biochemistry@, D. Freifelder, chapter 18.

Date: 09/08/03 **Lecturer: Tomasz Heyduk**

Lecture 13: Binding phenomena: cooperativity and allostery.

1. Positive and negative cooperativity:
- $ biological significance of cooperativity

2. Detection of cooperativity:
- $ shape of the binding curve
- $ Scatchard plot
- $ Hill plot

3. Molecular interpretations of cooperativity:
- $ direct interaction between bound ligands
- $ indirect effects - allostery

Date: 09/08/03 **Lecturer: Tomasz Heyduk**

Lecture 13: Binding phenomena: cooperativity and allostery. (continued)

4. Models of allosteric protein:

- $ Monod-Wyman-Changeux (MWC) model
- $ Koshalnd-Nemethy-Filmer (KNF) model
- $ allosteric inhibitors and allosteric activators

Suggested reading: APhysical Biochemistry@, D. Freifelder, chapter 18.

Date: 09/08/03 **Lecturer: Ray Rezaie**

Lecture 14: Enzyme kinetics

1. Basic aspects of chemical kinetics

- $ Rate laws for elementary chemical reactions
- $ Rate enhancement through transition state stabilization

2. Kinetics of enzyme-catalyzed reactions

- $ The Michaelis-Menten treatment of enzyme kinetics
- $ The steady-state model of enzyme kinetics
- $ Experimental measurements of Km and kcat
- $ Significance of Km and kcat constants

Suggested reading: ABiochemistry@, Goeffrey L. Zubay (4^{th} edition), chapter 8

Date: 09/09/03 **Lecturer: Ray Rezaie**

Lecture 15: Mechanisms of enzyme catalysis

1. General mechanisms
- $ Proximity effect
- $ Electrostatic effects
- $ General-acid and general-base catalysis
- $ Nucleophilic or electrophilic catalysis
- $ Induced fit

2. Detailed mechanisms of enzyme catalysis
- $ Mechanism of catalysis by serine proteases as a model system
- $ Role of cofactors in catalytic reactions

Suggested reading: ABiochemistry@, Goeffrey L. Zubay (4th edition), chapter 9

Date: 09/10/02 **Lecturer: Ray Rezaie**

Lecture 16: Regulation of enzyme activities

1. Regulation by covalent modifications
- $ Irreversible partial proteolysis
- $ Reversible phosphorylation, adenylylation and disulfide reduction

2. Enzyme Inhibition
- $ Reversible competitive, noncompetitive and uncompetitive inhibitors
- $ Irreversible inhibitors

3. Allosteric regulation
- $ Positive cooperativity
- $ Negative cooperativity
- $ Advantages of the allosteric regulation

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Suggested reading: ABiochemistry@, Goeffrey L. Zubay (4th edition), chapter 8 and 10.

Open day: 09/11/03

Exam: 09/12