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chapter

# 5

## Protein Function

1. **Relationship between Affinity and Dissociation Constant** Protein A has a binding site for ligand X with a  $K_d$  of  $10^{-8}$  M. Protein B has a binding site for ligand X with a  $K_d$  of  $10^{-9}$  M. Which protein has a higher affinity for ligand X? Explain your reasoning. Convert the  $K_d$  to  $K_a$  for both proteins.

**Answer** Protein B has a higher affinity for ligand X. The lower  $K_d$  indicates that protein B will be half-saturated with bound ligand X at a much lower concentration of X than will protein A. Because  $K_a = 1/K_d$ , protein A has  $K_a = 10^8 \text{ M}^{-1}$ ; protein B has  $K_a = 10^9 \text{ M}^{-1}$ .

2. **Negative Cooperativity** Which of the following situations would produce a Hill plot with  $n_H = 1.0$ ? Explain your reasoning in each case.

- (a) The protein has multiple subunits, each with a single ligand-binding site. Binding of ligand to one site decreases the binding affinity of other sites for the ligand.
- (b) The protein is a single polypeptide with two ligand-binding sites, each having a different affinity for the ligand.
- (c) The protein is a single polypeptide with a single ligand-binding site. As purified, the protein preparation is heterogeneous, containing some protein molecules that are partially denatured and thus have a lower binding affinity for the ligand.

**Answer** All three situations would produce  $n_H = 1.0$ . An  $n_H$  (Hill coefficient) of 1.0 generally suggests situation (a)—the classic case of negative cooperativity. However, closer examination of the properties of a protein exhibiting apparent negative cooperativity in ligand binding often reveals situation (b) or (c). When two or more types of ligand-binding sites with different affinities for the ligand are present on the same or different proteins in the same solution, apparent negative cooperativity is observed. In (b), the higher-affinity ligand-binding sites bind the ligand first. As the ligand concentration is increased, binding to the lower-affinity sites produces an  $n_H = 1.0$ , even though binding to the two ligand-binding sites is completely independent. Even more common is situation (c), in which the protein preparation is heterogeneous. Unsuspected proteolytic digestion by contaminating proteases and partial

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**D Kirk**



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## **Table of Contents Lehninger Biochemistry Solutions**

1. Understanding the eBook Lehninger Biochemistry Solutions
  - The Rise of Digital Reading Lehninger Biochemistry Solutions
  - Advantages of eBooks Over Traditional Books
2. Identifying Lehninger Biochemistry Solutions
  - Exploring Different Genres
  - Considering Fiction vs. Non-Fiction
  - Determining Your Reading Goals
3. Choosing the Right eBook Platform
  - Popular eBook Platforms
  - Features to Look for in an Lehninger Biochemistry Solutions
  - User-Friendly Interface
4. Exploring eBook Recommendations from Lehninger Biochemistry Solutions
  - Personalized Recommendations

- 
- Lehninger Biochemistry Solutions User Reviews and Ratings
  - Lehninger Biochemistry Solutions and Bestseller Lists
5. Accessing Lehninger Biochemistry Solutions Free and Paid eBooks
    - Lehninger Biochemistry Solutions Public Domain eBooks
    - Lehninger Biochemistry Solutions eBook Subscription Services
    - Lehninger Biochemistry Solutions Budget-Friendly Options
  6. Navigating Lehninger Biochemistry Solutions eBook Formats
    - ePub, PDF, MOBI, and More
    - Lehninger Biochemistry Solutions Compatibility with Devices
    - Lehninger Biochemistry Solutions Enhanced eBook Features
  7. Enhancing Your Reading Experience
    - Adjustable Fonts and Text Sizes of Lehninger Biochemistry Solutions
    - Highlighting and Note-Taking Lehninger Biochemistry Solutions
    - Interactive Elements Lehninger Biochemistry Solutions
  8. Staying Engaged with Lehninger Biochemistry Solutions
    - Joining Online Reading Communities
    - Participating in Virtual Book Clubs
    - Following Authors and Publishers Lehninger Biochemistry Solutions
  9. Balancing eBooks and Physical Books Lehninger Biochemistry Solutions
    - Benefits of a Digital Library
    - Creating a Diverse Reading Collection Lehninger Biochemistry Solutions
  10. Overcoming Reading Challenges
    - Dealing with Digital Eye Strain
    - Minimizing Distractions
    - Managing Screen Time
  11. Cultivating a Reading Routine Lehninger Biochemistry Solutions
    - Setting Reading Goals Lehninger Biochemistry Solutions
    - Carving Out Dedicated Reading Time
  12. Sourcing Reliable Information of Lehninger Biochemistry Solutions
    - Fact-Checking eBook Content of Lehninger Biochemistry Solutions

- 
- Distinguishing Credible Sources
13. Promoting Lifelong Learning
- Utilizing eBooks for Skill Development
  - Exploring Educational eBooks
14. Embracing eBook Trends
- Integration of Multimedia Elements
  - Interactive and Gamified eBooks

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