

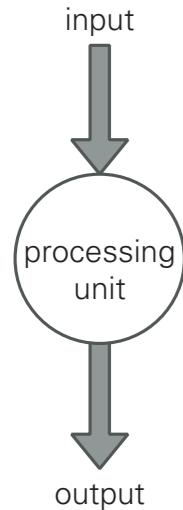
Today's class:

Emergence of allostery and structural proteins

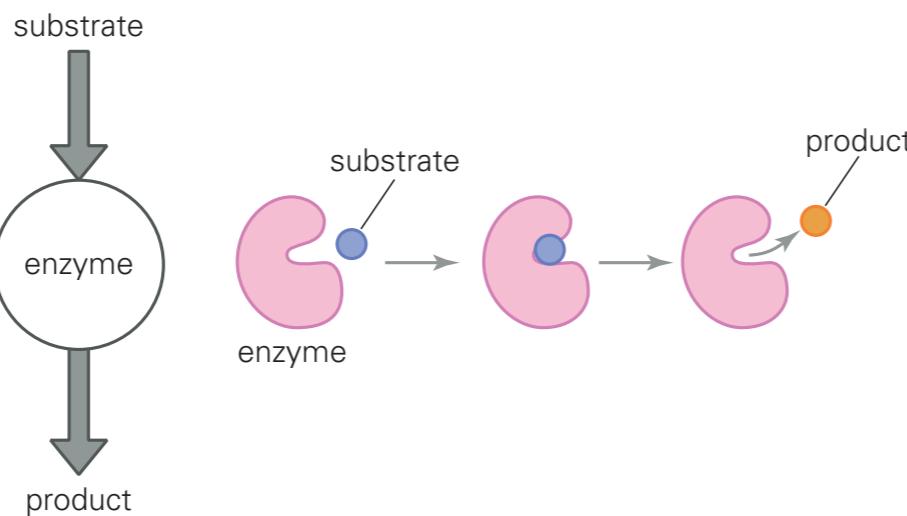
*First part of this lecture follows the chapter 14 in the book  
'The Molecules of Life' by Kuriyan, Konforti & Wemmer, 1st Ed, 2013*

# Proteins are input processing units

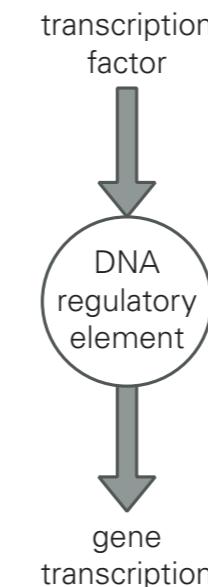
(A)



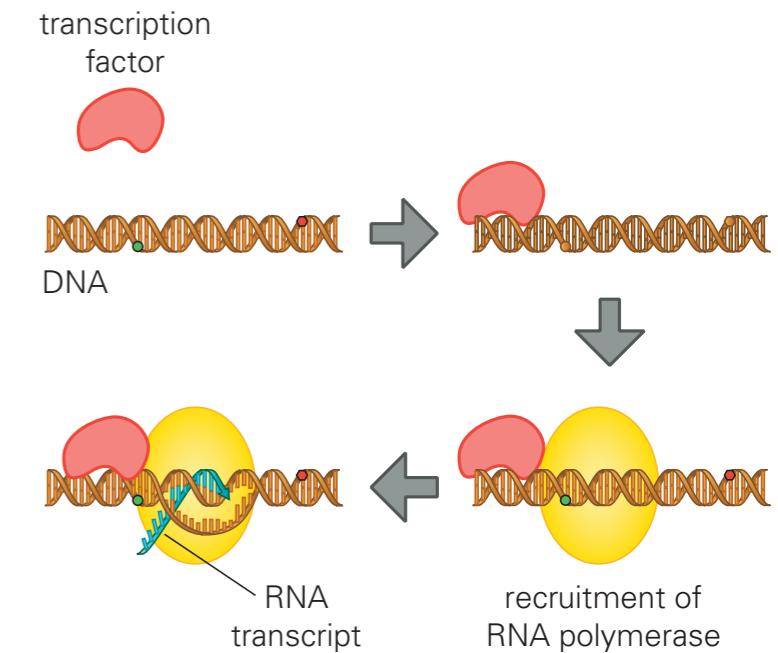
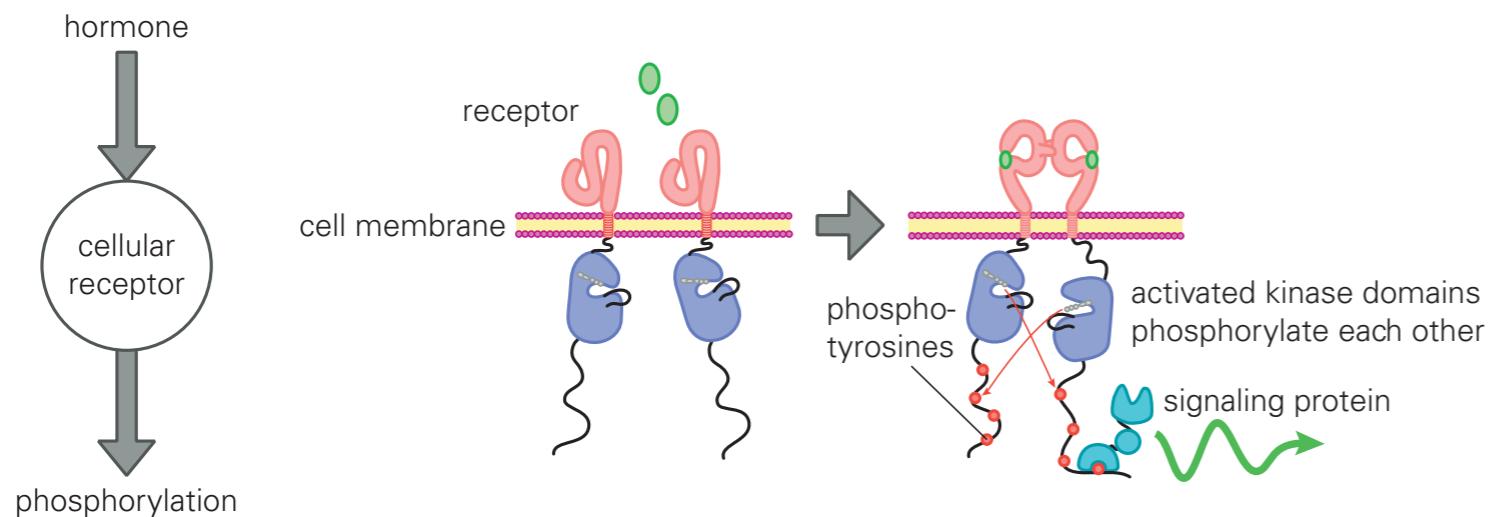
(B)



(D)



(C)

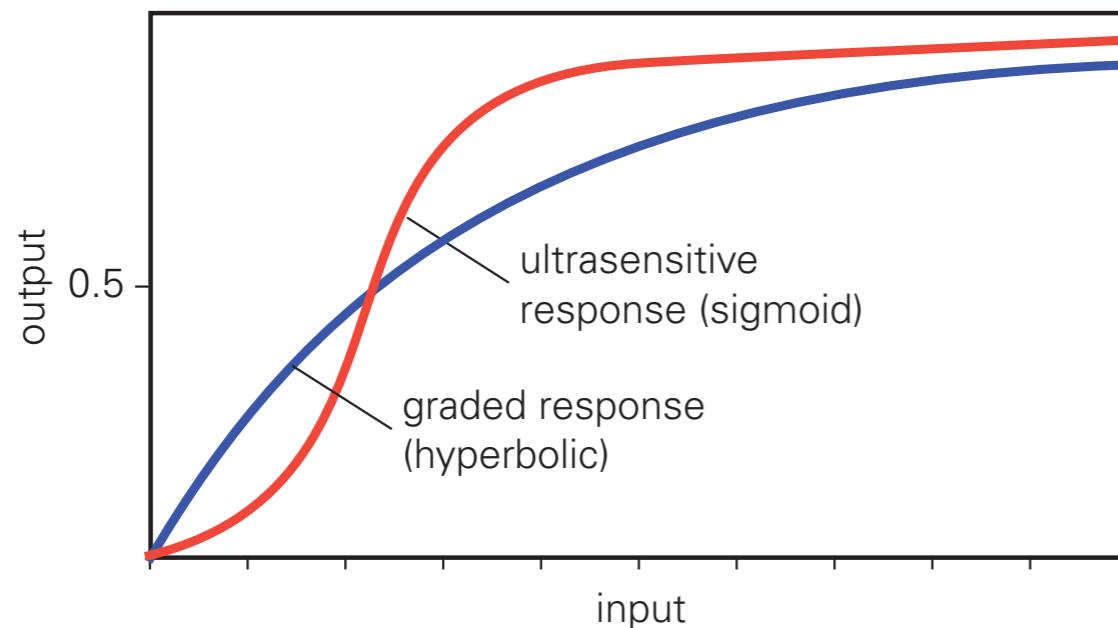


How does the output depend  
on the input?

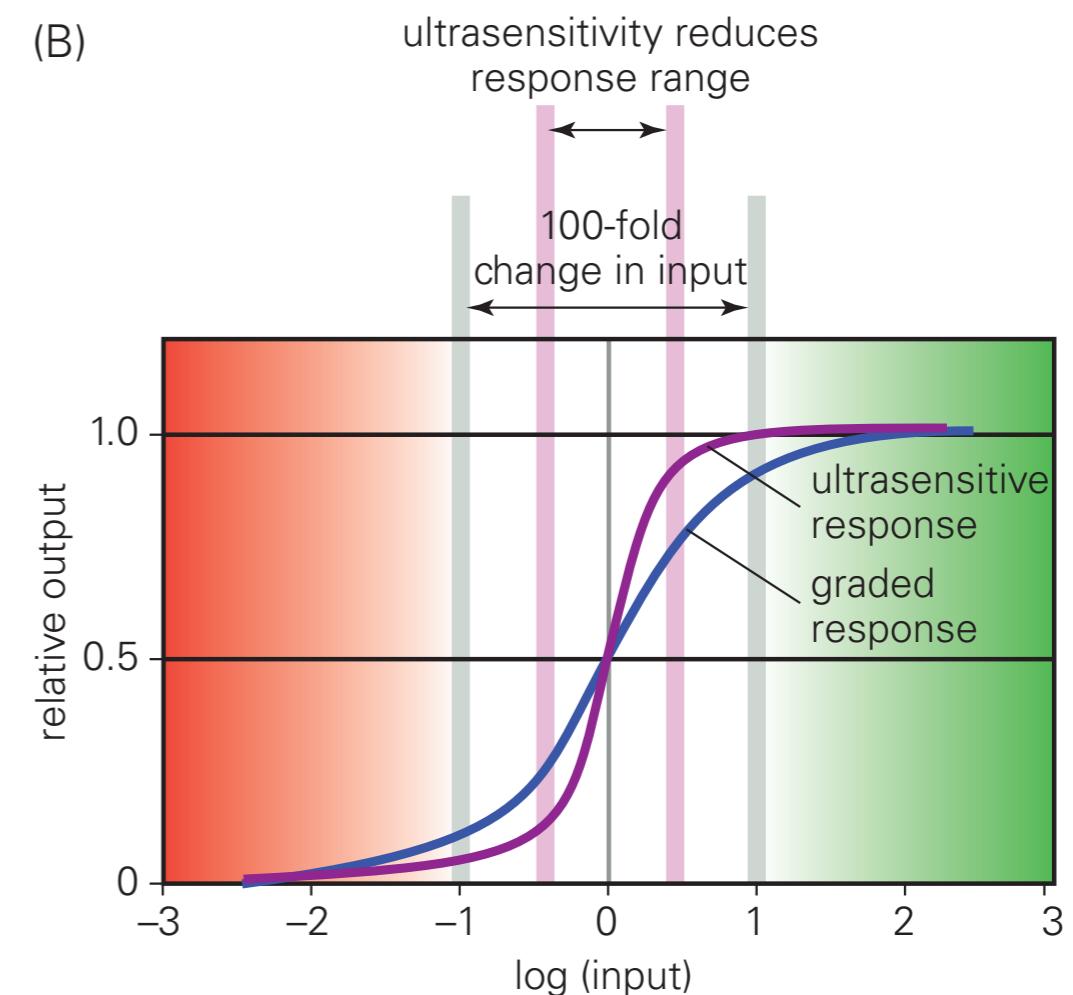
What is the nature of  
response of the system?

# Many biological processing units are ultrasensitive

(A)



(B)



## Graded response

An output function that depends on the input in a hyperbolic fashion, as in a simple binding equilibrium, is known as a graded, or linear, response. In such a response, the output switches from ~10% to ~90% of the maximum response over a 100-fold change in input strength.

$$\text{response} \propto c_{\text{ligand}}$$

## Ultrasensitivity

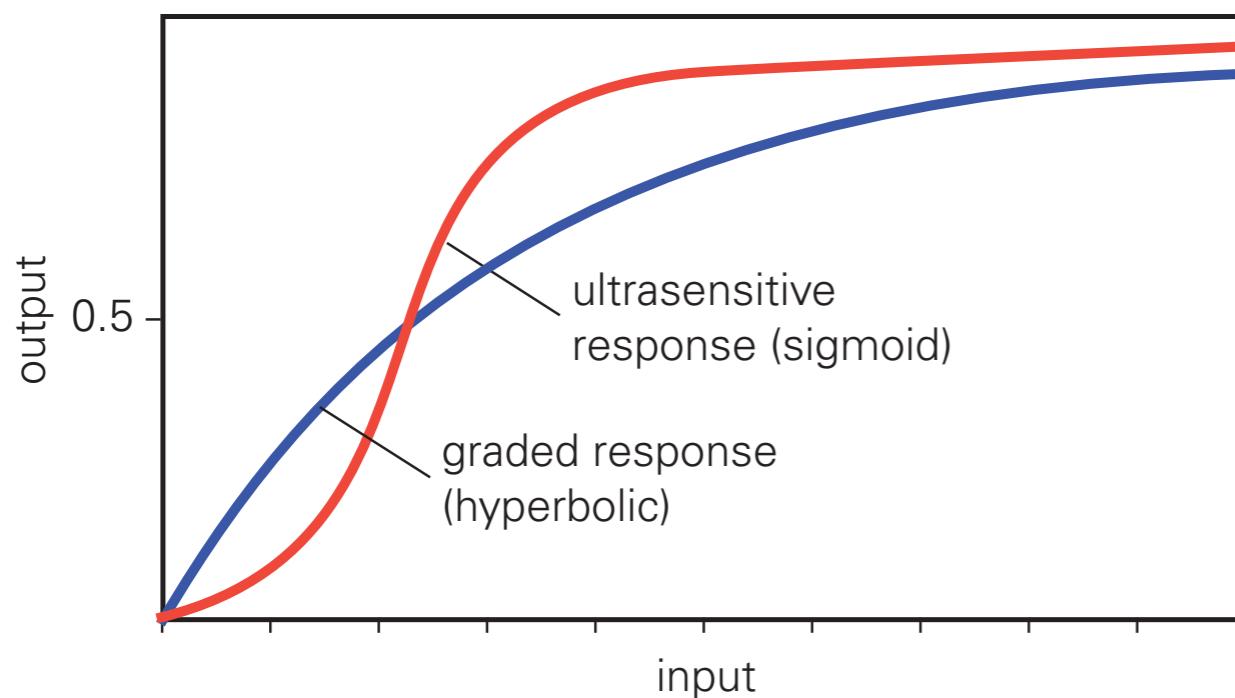
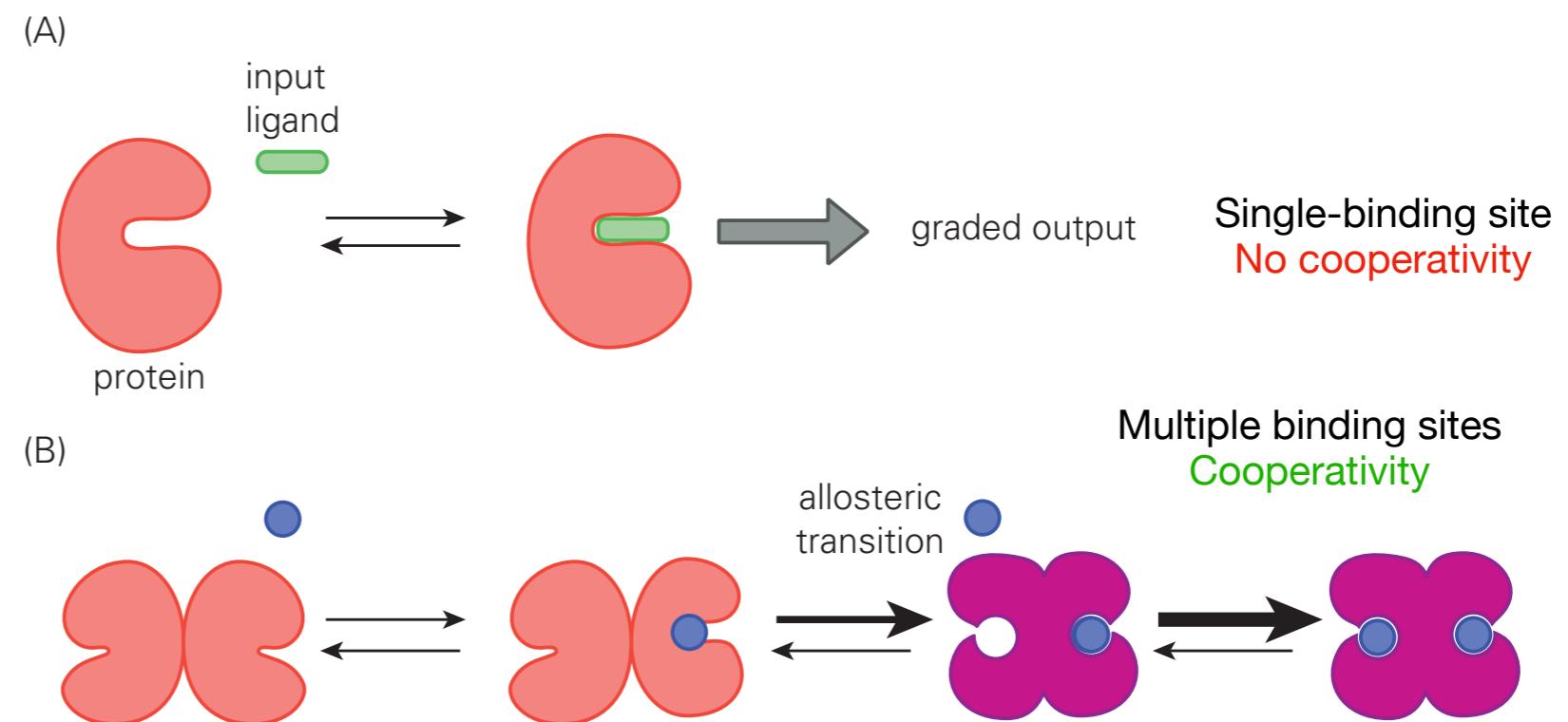
An ultrasensitive system is one in which the response to an input is sharper than expected from a simple binding equilibrium. For example, the response of a protein to a ligand can be defined as the fractional saturation,  $f$  (see Chapter 12). When  $f$  rises from 0.1 to 0.9 over a less than ~100-fold concentration range of the ligand, the system is said to be ultrasensitive.

$$\text{response} \propto c_{\text{ligand}}^\alpha, \alpha > 1$$

# Cooperativity and allostery are features of ultrasensitive systems

## Cooperativity

When the binding of a ligand to a protein is ultrasensitive, the binding is said to be cooperative. As more ligand molecules bind to the protein, the saturation of the protein increases more sharply than would be expected for a normal binding event, as if the ligand molecules "cooperate" with each other.



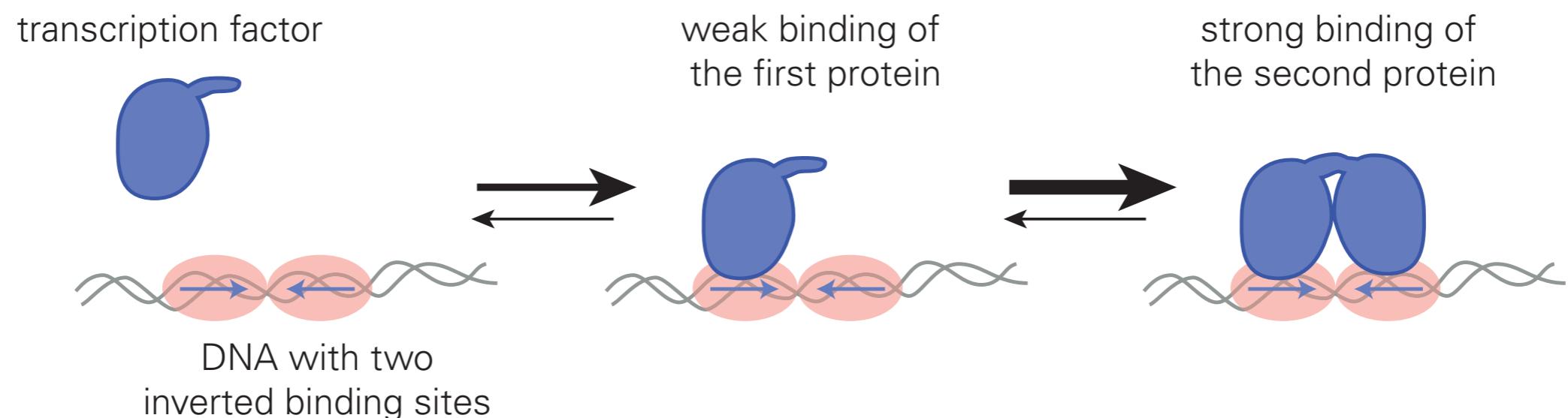
## Allotropy

An allosteric protein is one in which the activity of the protein is modulated by interactions that occur at a distance from the active site.

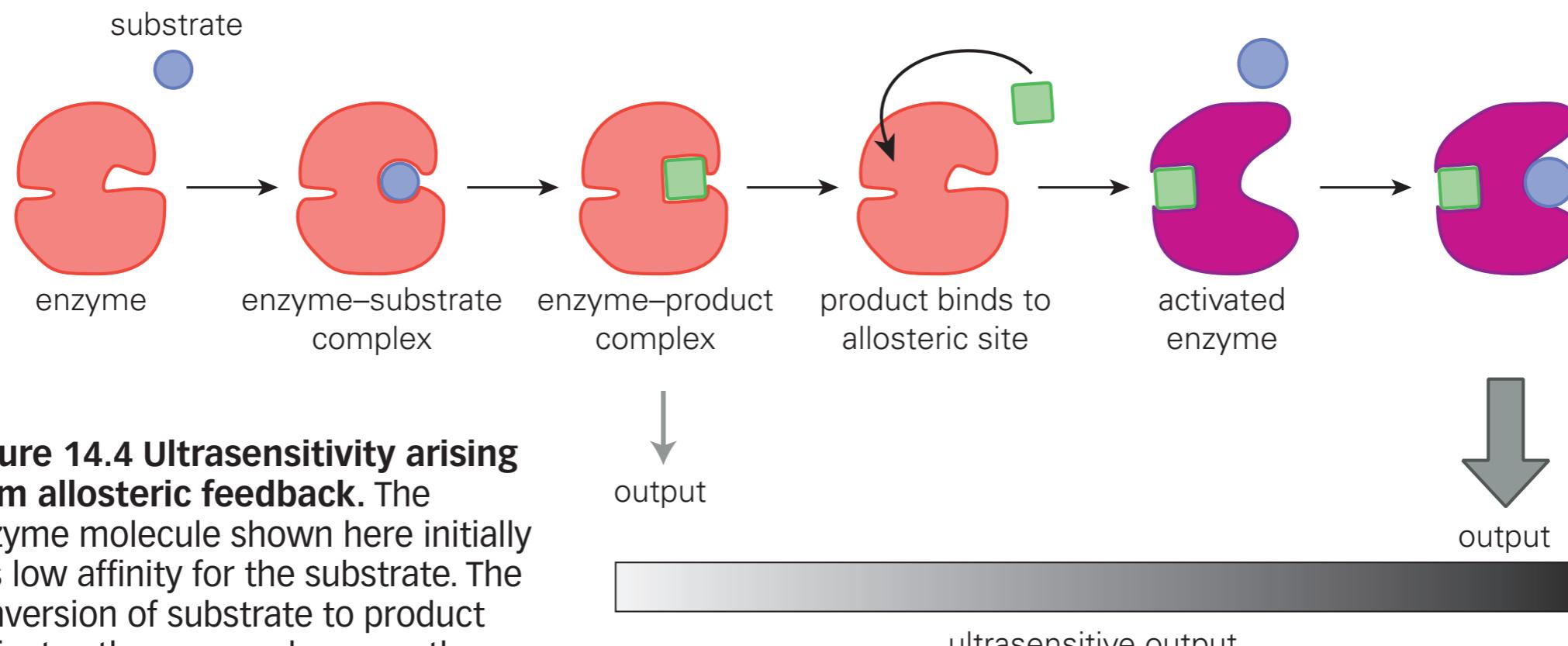
# Cooperativity usually happens due to allostery, but can without too

Main points:

- If the ultrasensitivity arises due to binding of ligands it is called cooperativity
- Cooperativity in multi-domain proteins can occur due to allosteric changes in the protein
- Cooperativity can happen without allostery



# Ultrasensitivity can also arise devoid of cooperative binding

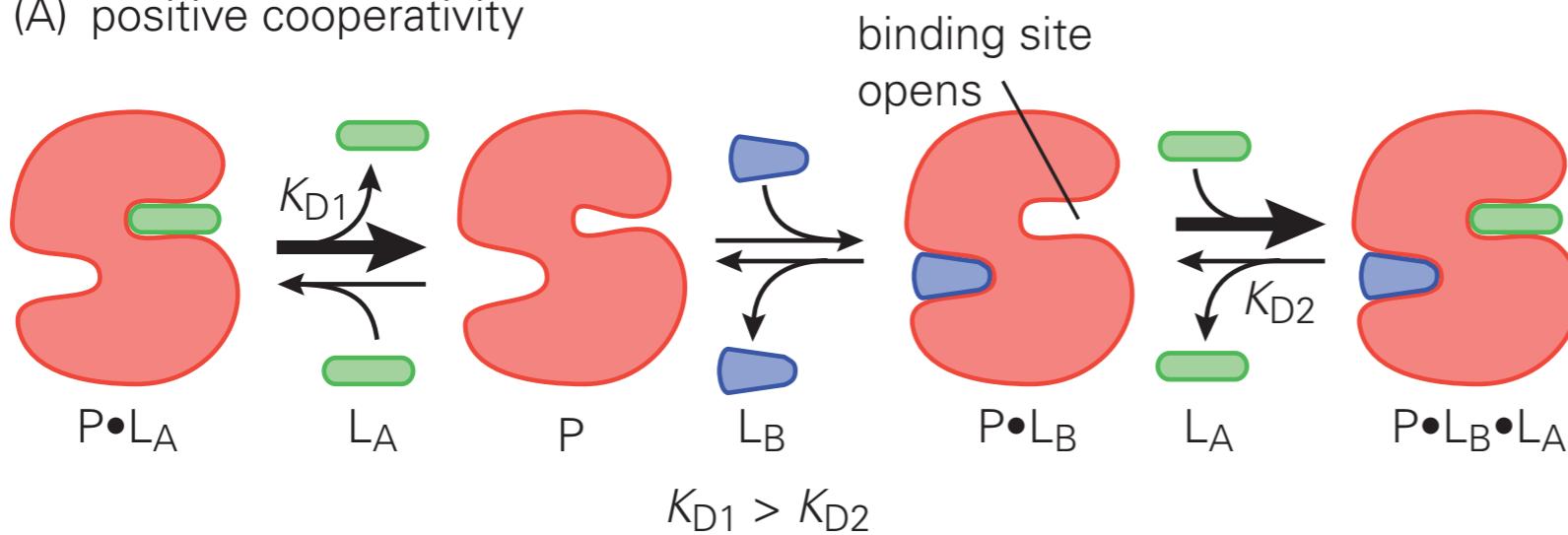


**Figure 14.4 Ultrasensitivity arising from allosteric feedback.** The enzyme molecule shown here initially has low affinity for the substrate. The conversion of substrate to product activates the enzyme because the product molecule can bind to an allosteric site on the enzyme, causing a conformational change that opens up the active site.

Allosteric feedback without cooperativity

# Allosteric proteins exhibit positive or negative cooperativity

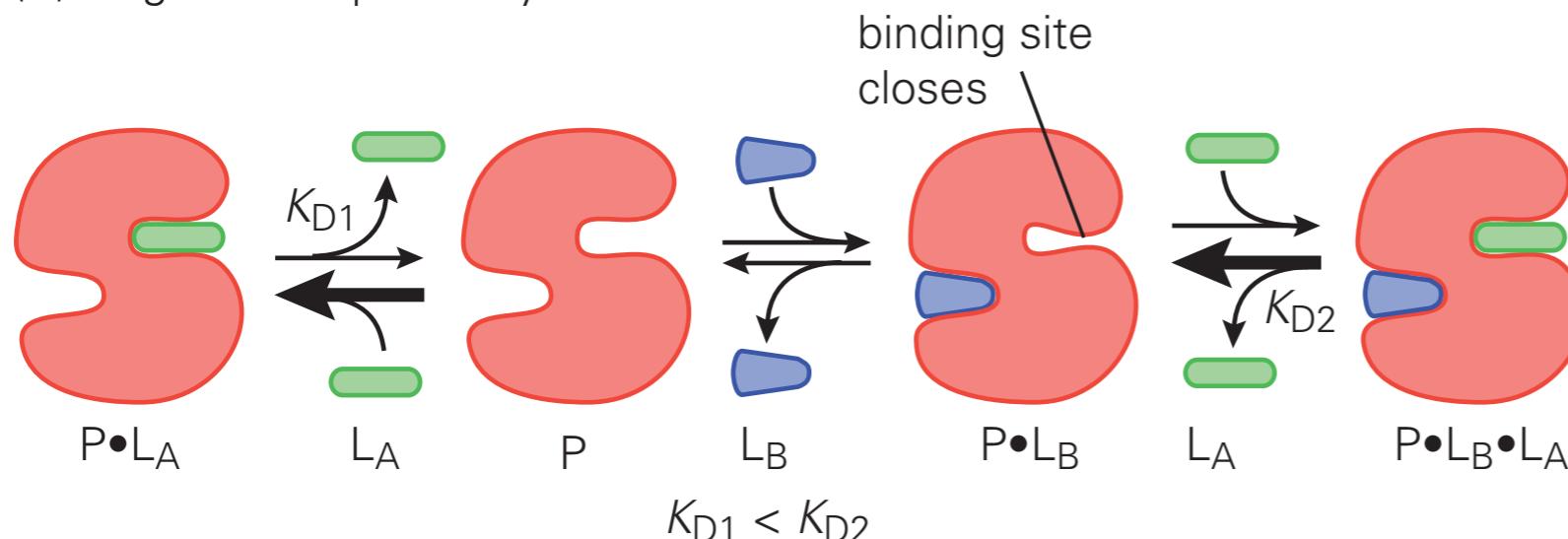
(A) positive cooperativity



## Positive and negative cooperativity

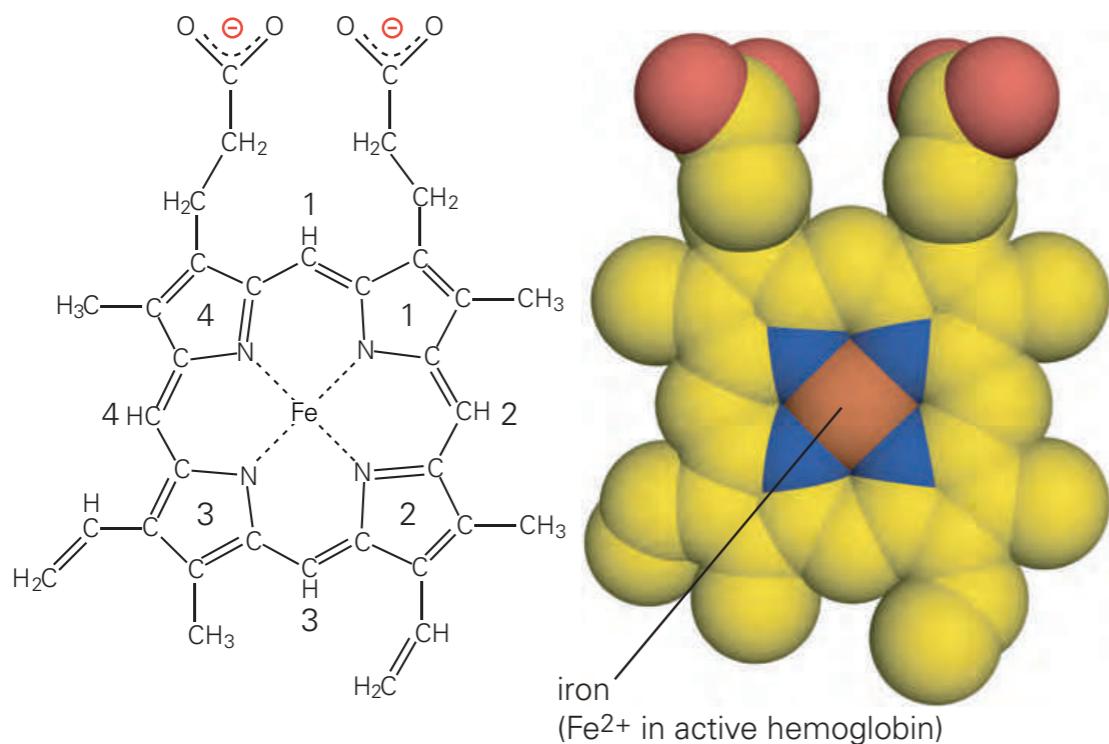
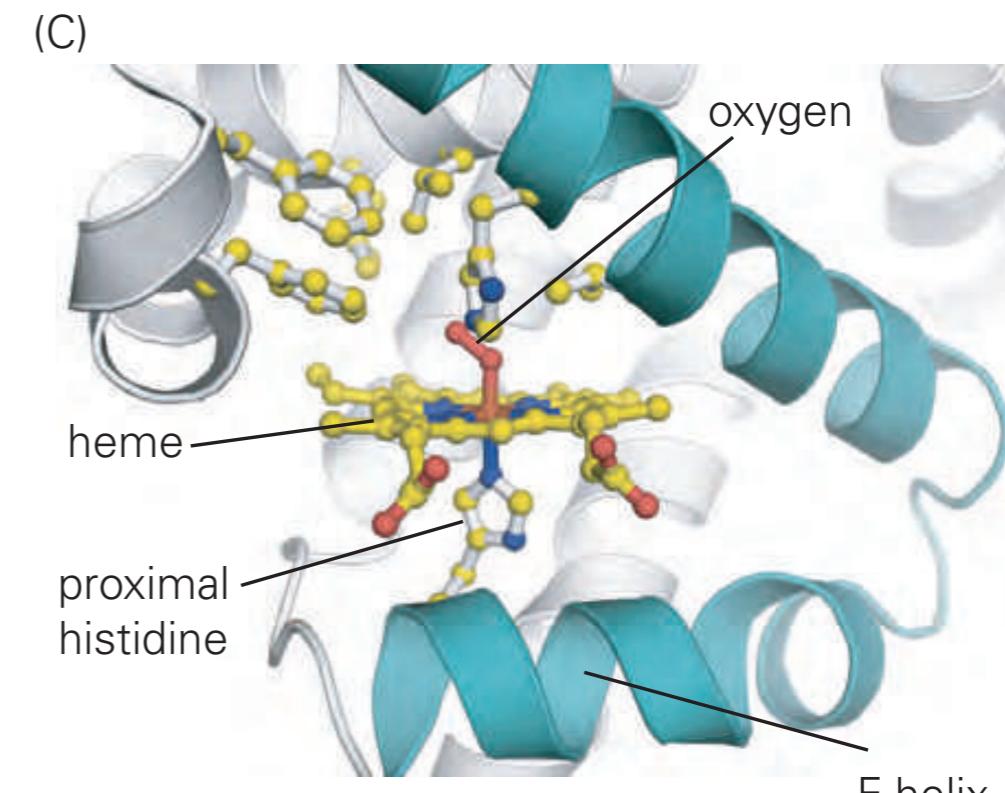
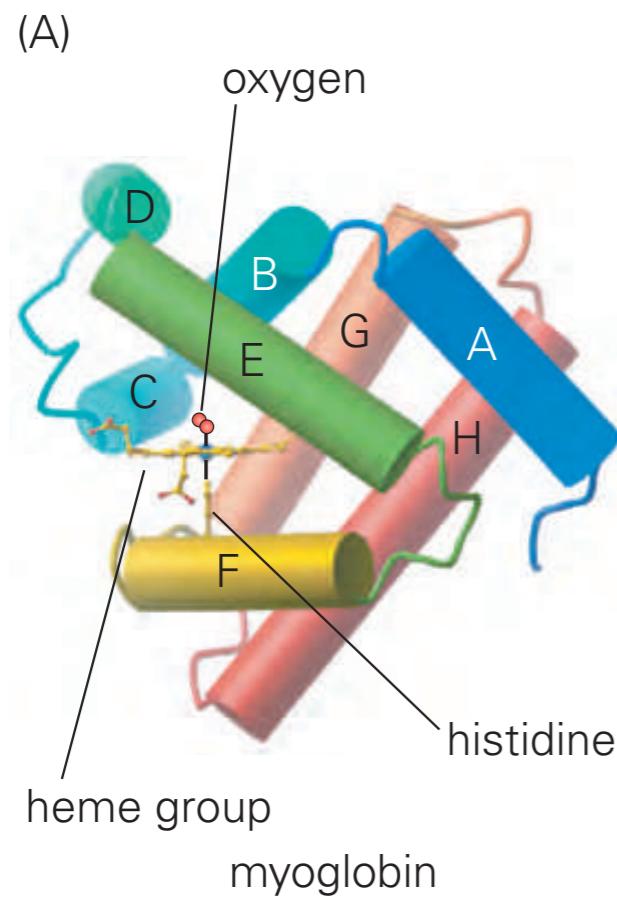
When two or more ligands bind to a protein in such a way that they mutually reinforce each of their binding affinities, the phenomenon is called positive cooperativity. If the ligands make it more difficult for each other to bind, the phenomenon is called negative cooperativity.

(B) negative cooperativity



Oxygen binding to hemoglobin is a classic example of positive cooperativity

# The heme group in hemoglobin binds oxygen reversibly



If  $Fe^{2+}$  gets oxidized to  $Fe^{3+}$  it no longer binds oxygen reversibly. The 'globin' part protects the 'heme' from getting oxidized.

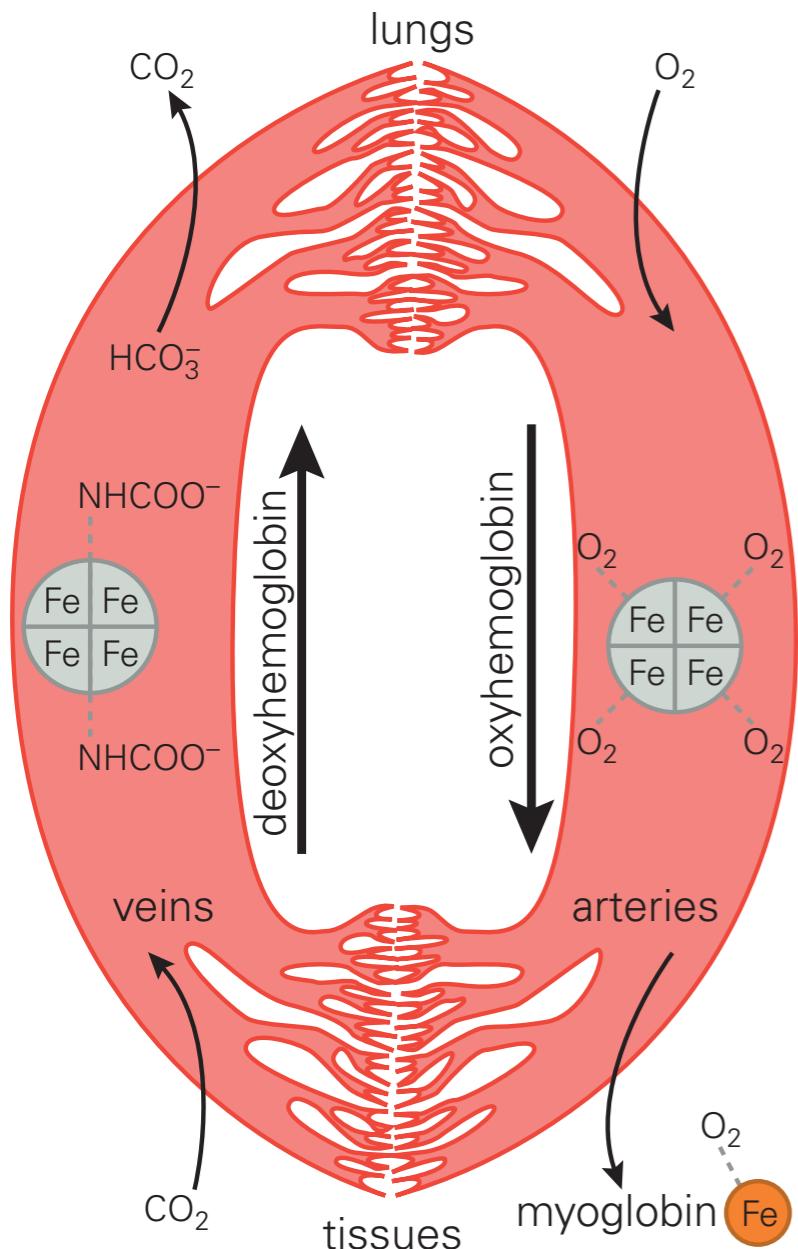
If powered by the same 'heme' group how does the functional difference of myoglobin and hemoglobin emerge?

# Myoglobin only stores oxygen, while hemoglobin transports it

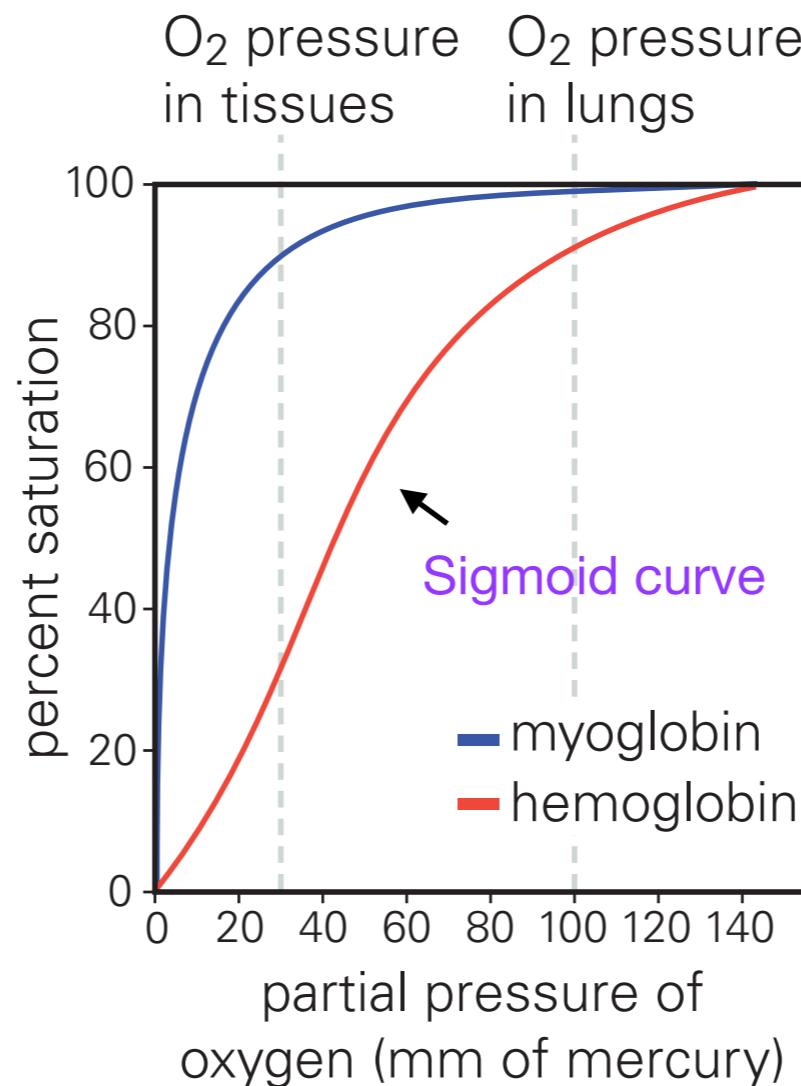
What's a good transporter?

Load the cargo efficiently

Unload the cargo efficiently



Responses of myo- and hemoglobin



Main differences here

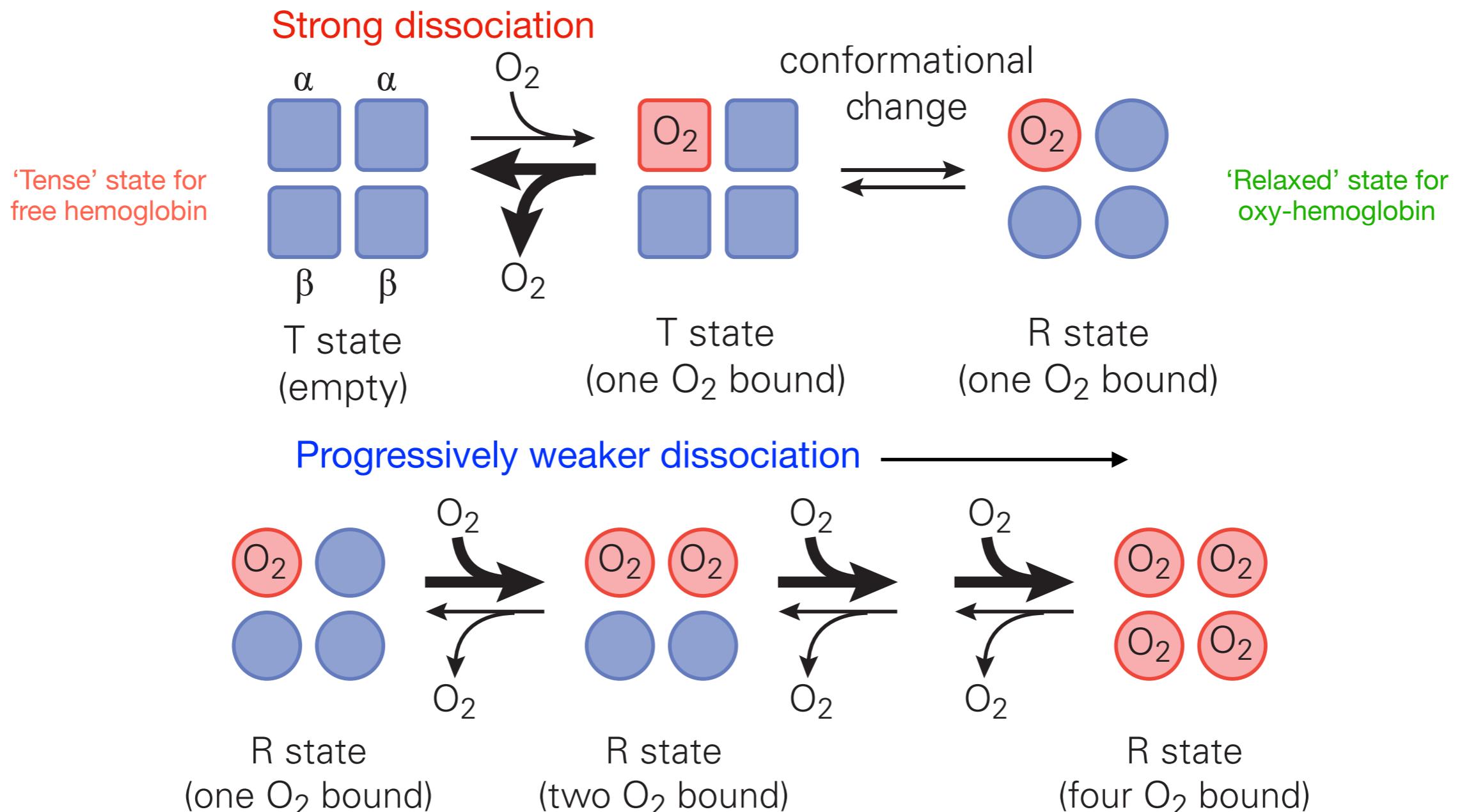
The myoglobin's response is graded - can load a lot from lungs but can't unload enough to tissues

needs a large fold change (~ 81) in  $O_2$  conc to work as a transporter that's not there (~ 3)

The hemoglobin's response is untrasensitive - can load a lot from lungs and unload a lot to tissues

Small fold change in  $O_2$  conc is enough to work as a transporter

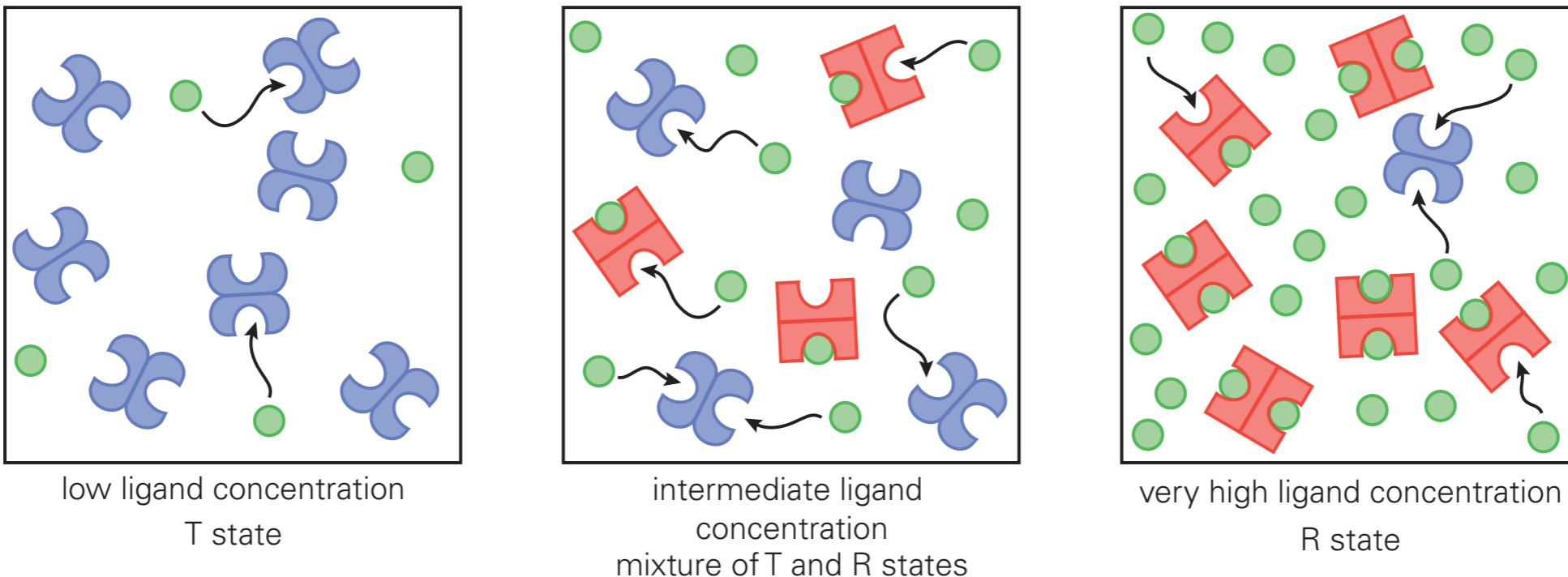
## Hemoglobin undergoes a conformational change as it binds and releases oxygen



Monod-Wyman-Changeux model

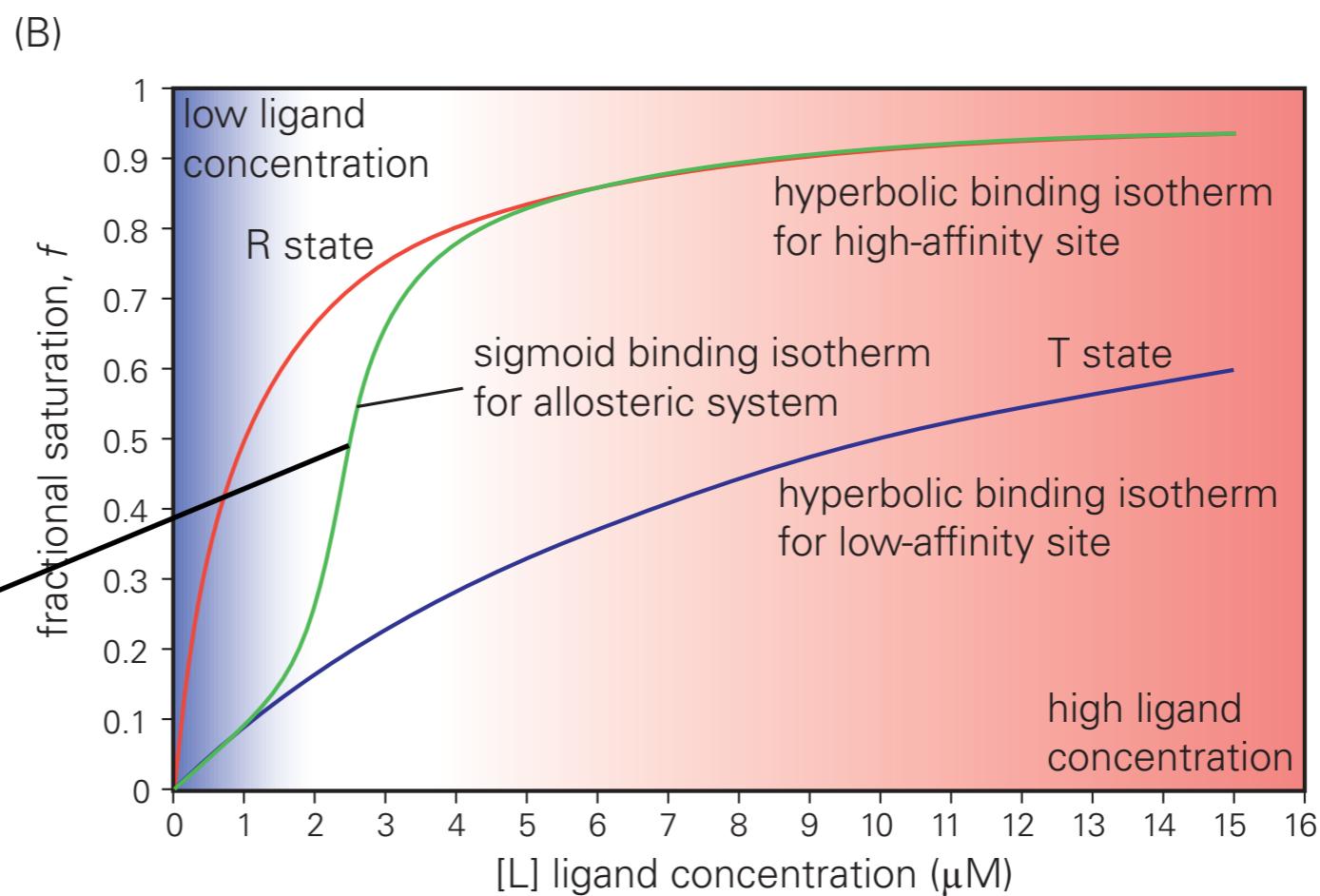
How does this picture give rise to the 'sigmoid' curve?

## Let's illustrate this for a dimeric allosteric protein

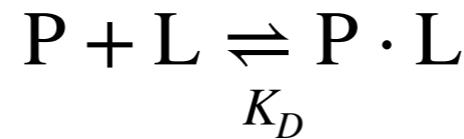


Let's also assume that the protein P has symmetrical structure that is  $P \cdot L$  undergoes conformational changes on both domains

Slope of the graph at  $f = 0.5$  provides a useful way to characterize the cooperativity



## Hill coefficient for a dimeric allosteric protein

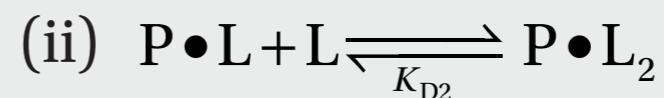


If  $P$  is a monomeric protein and  $f$  is the fractional saturation then

$$\log\left(\frac{f}{1-f}\right) = \log[L] - \log K_D$$

Let's derive this offline

For a dimeric protein we have the following reactions



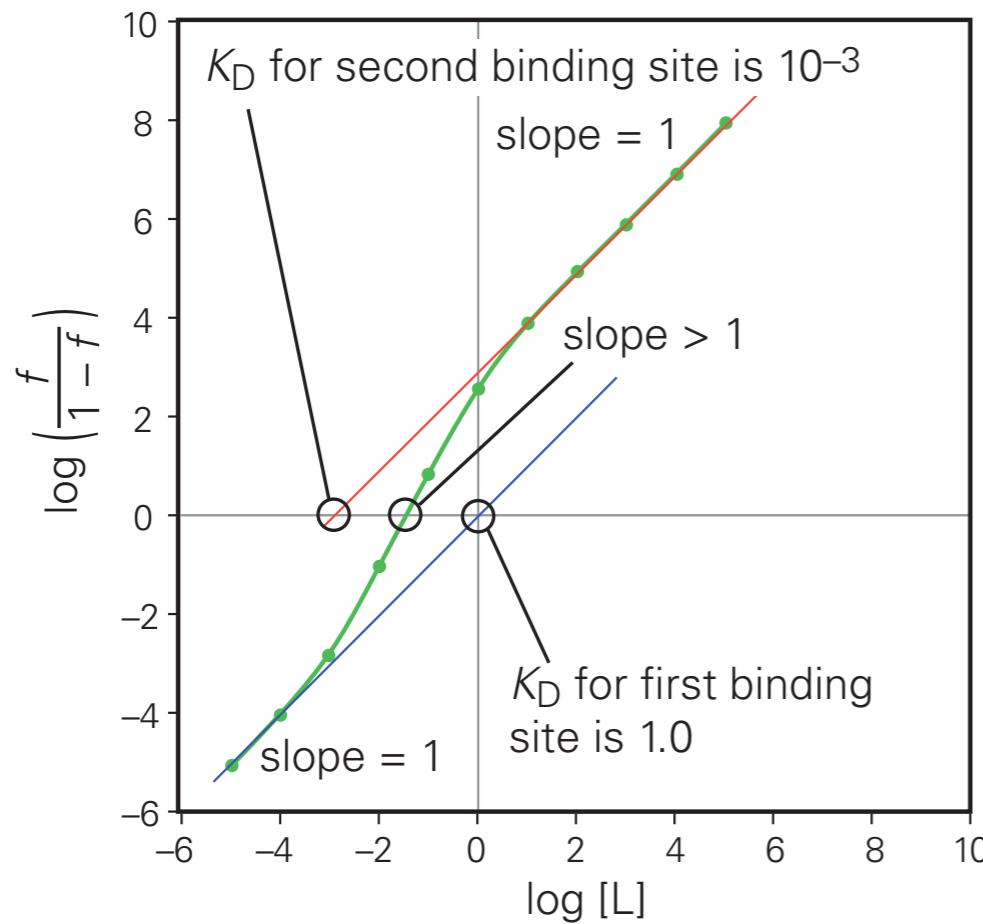
For this system one can tediously show following the derivation above

$$\frac{f}{1-f} = \frac{\frac{[L]}{K_{D1}} + \left( \frac{[L]}{K_{D1}} \right) \left( \frac{[L]}{K_{D2}} \right)}{1 + \frac{[L]}{K_{D1}}}$$

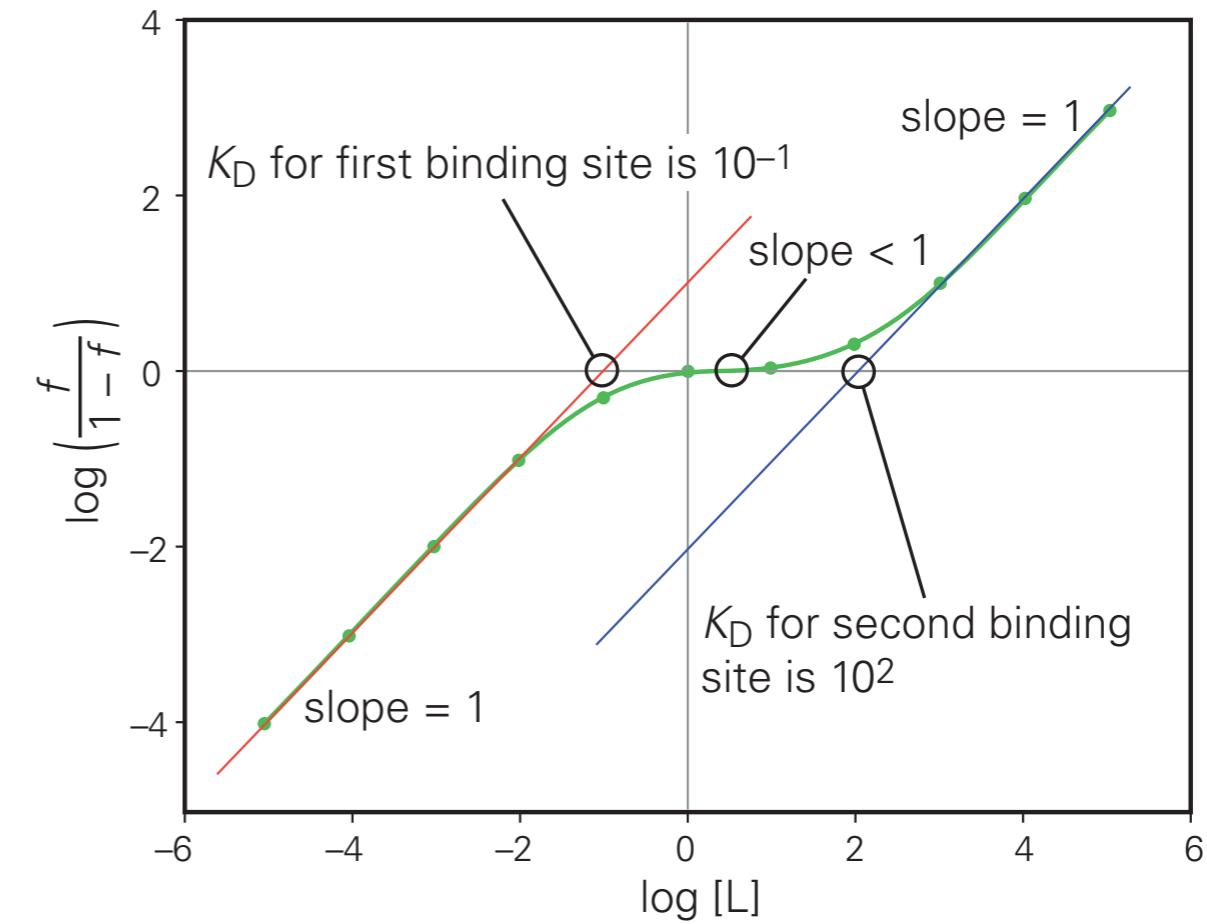
Here the slope of  $\log_{10} \left( \frac{f}{1-f} \right)$  vs  $[L]$  is the Hill coefficient  $n_H = \frac{2}{1 + \sqrt{\frac{K_{D2}}{K_{D1}}}}$

## Hill coefficient for a dimeric allosteric protein ...*contd*

Positive cooperativity



Negative cooperativity



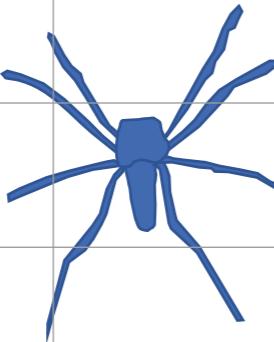
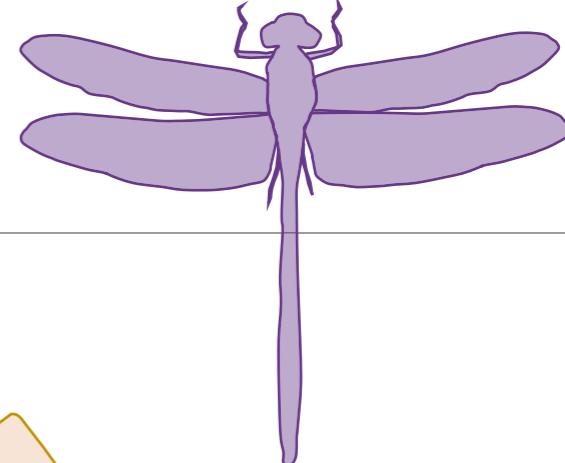
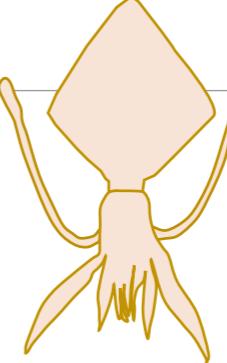
Upper limit for  $n_H = 2$  for dimeric protein

$n_H \approx 2.5 - 3.5$  for human hemoglobin

# Structural proteins

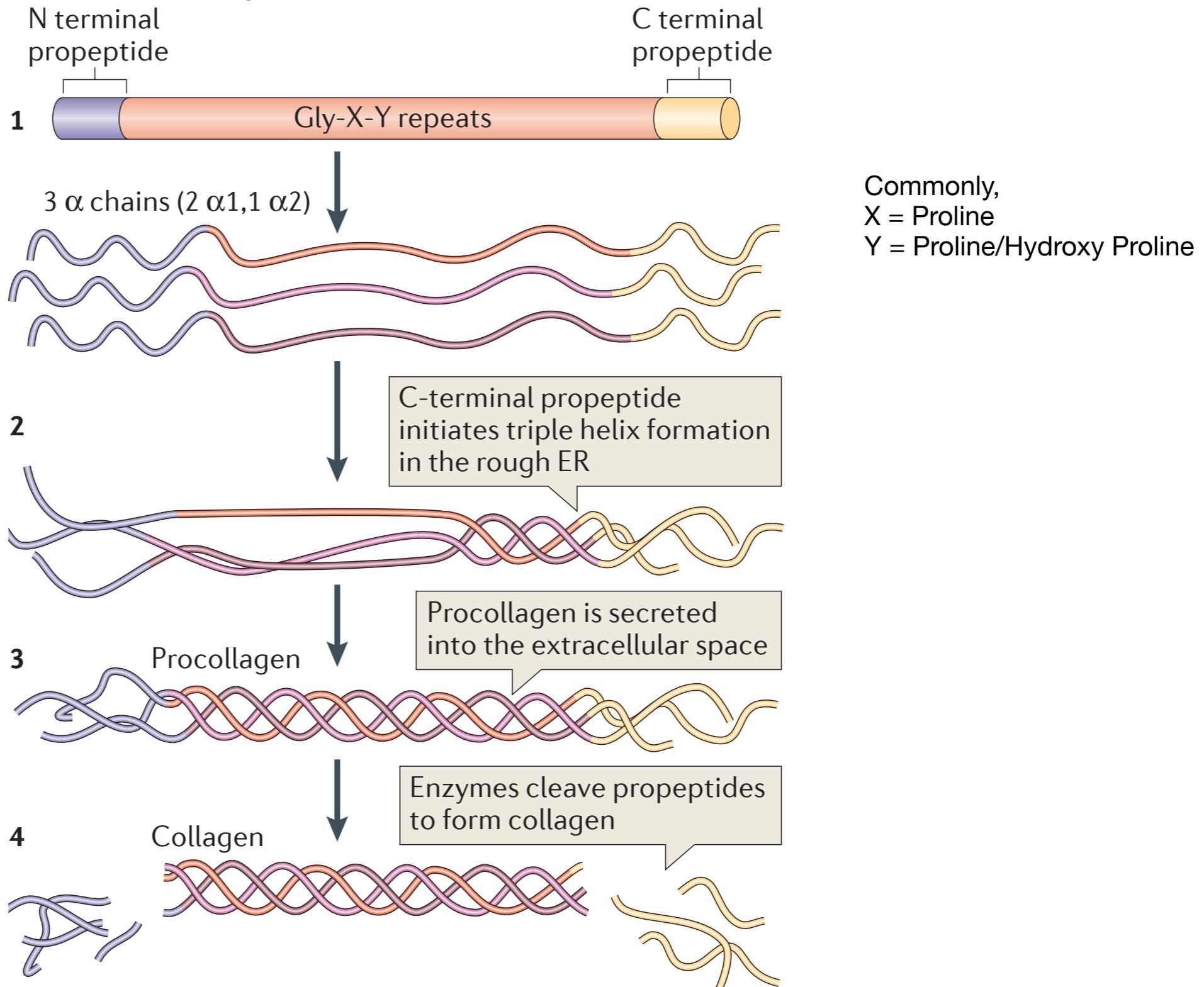
*This part of the lecture follows mostly the review papers titled  
“Extracellular matrix assembly: a multiscale deconstruction”  
by Mouw, Ou and Weaver, Nature Reviews Molecular Cell Biology, 15, 771 (2014)  
and  
“How to define and study structural proteins as biopolymer materials”  
by K. Numata, Polymer Journal (2020) 52:1043–1056*

# Structural proteins found in nature

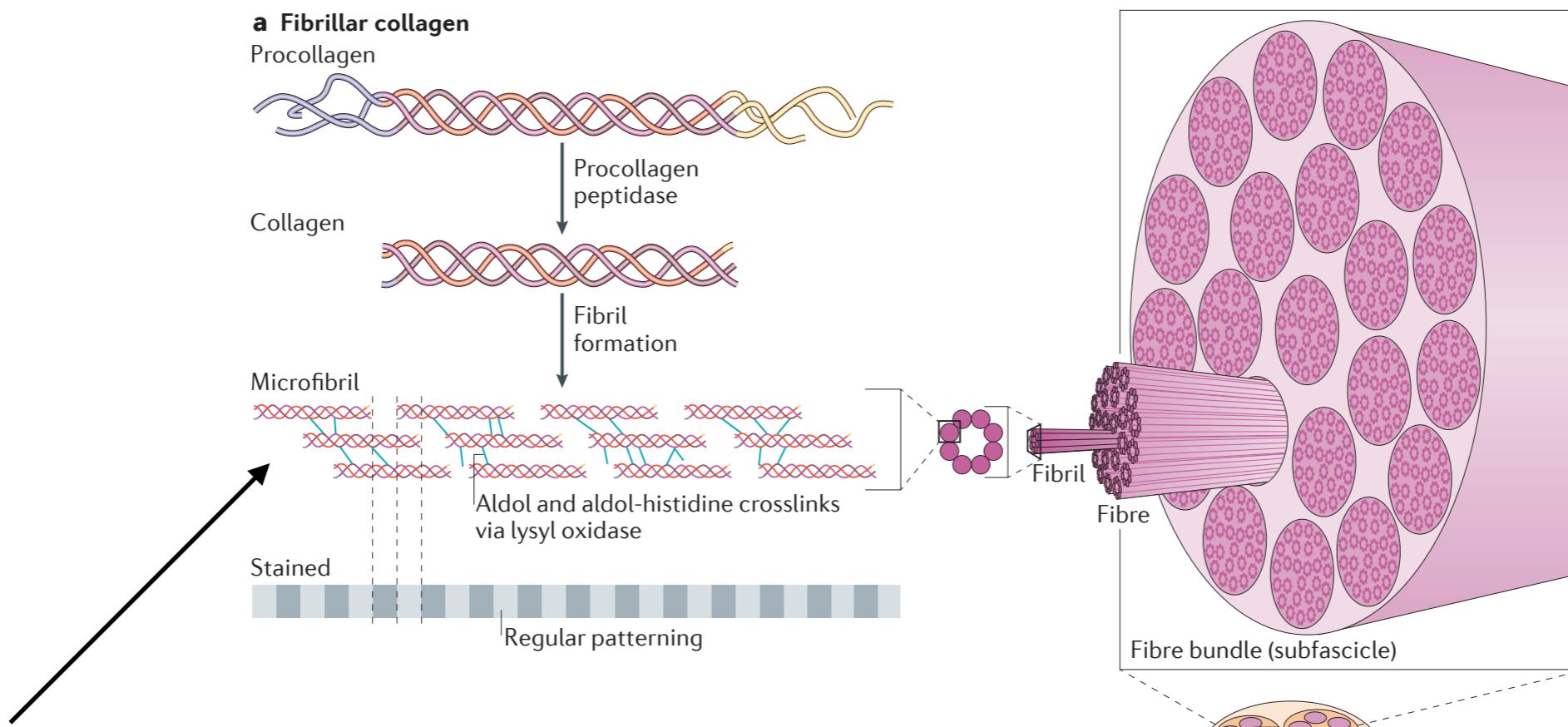
Structural Protein	In Nature	Repeat Sequence
Silkworm silk [ <i>Bombyx mori</i> ]		Cocoon  (heavy chain) [GAGAGSGAAS(GAGAGS) <sub>1-11</sub> GAGAGYGAGVGAGYGAGYGAGAGY] <sub>n</sub> GTGSSGFGPYVANGYSGYEYAWSSESDFGTGS
Silkworm silk [ <i>Antheraea spp.</i> ]		Dragline and web frame  (A) <sub>13</sub> GSGAGG(SAVR)GGGYGWGDGGYGSDS (A) <sub>13</sub> SGAGG(SA)(GGY) <sub>2-3</sub> GSDS (A) <sub>12</sub> GSGAGGRGDGGYGSGSS (A) <sub>12</sub> RRAGHDRAADS
Spider silk [ <i>Nephila clavipes</i> ]		Dragline and web frame  (Major Spidroin 1) (A) <sub>6-7</sub> GGAG[QGGYGGLG(G/S/N)QGAG] <sub>1-2</sub> RGGLGGQGAG
Collagen		Extracellular matrix in connective tissues  [Gly-Pro-Hyp] <sub>n</sub>
Elastin		[VPGVG] <sub>n</sub>
Resilin [ <i>Drosophila melanogaster</i> ]	Soft rubbery/elastic organs and tissue, such as wings and joints, of insects and other arthropods	(exon I reported as pro-resilin) [GGRPSDSY GAP GGGN] (exon III) [GYSGGRPGGQDLG]
Keratin [ <i>Homo sapiens</i> ]		Hair, wool, and horn  MKQLEDK VEELLSK NYHLENE VARLKKL
Reflectin [ <i>Doryteuthis opalescens</i> ]		Iridophores and leucophores of certain cephalopods  GMPG GMNP GMYG GMPS GMPG GMYP

# Collagen structure

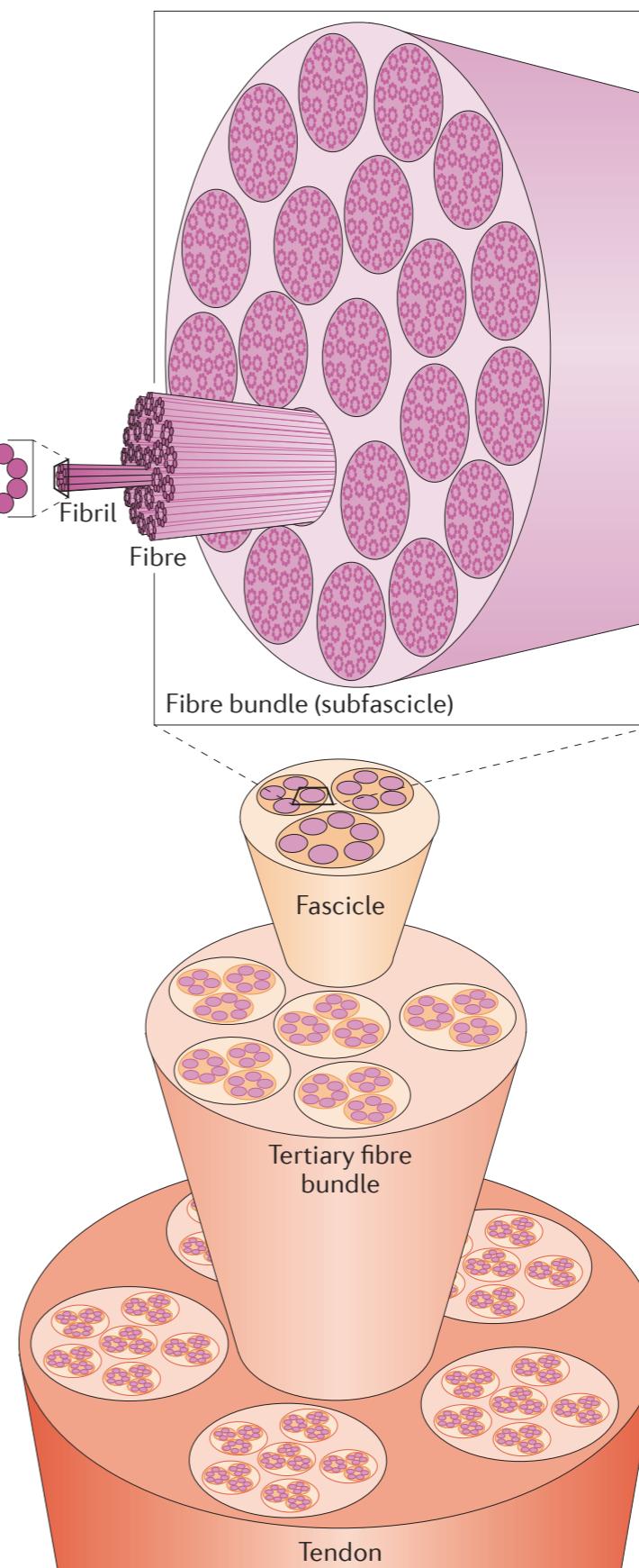
## a Standard collagen molecule



# How collagen is organized in tendons



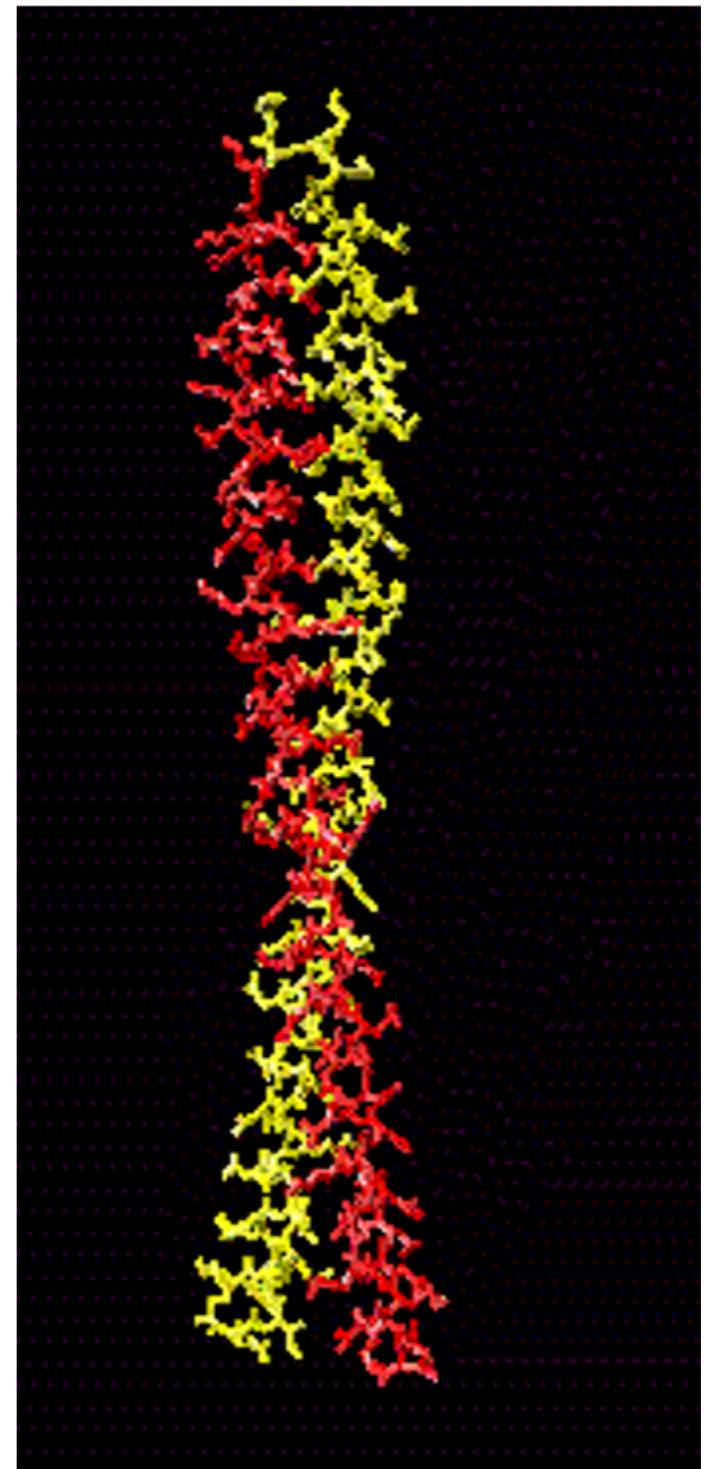
Covalent bridges hold the microfibrils together



# Keratin

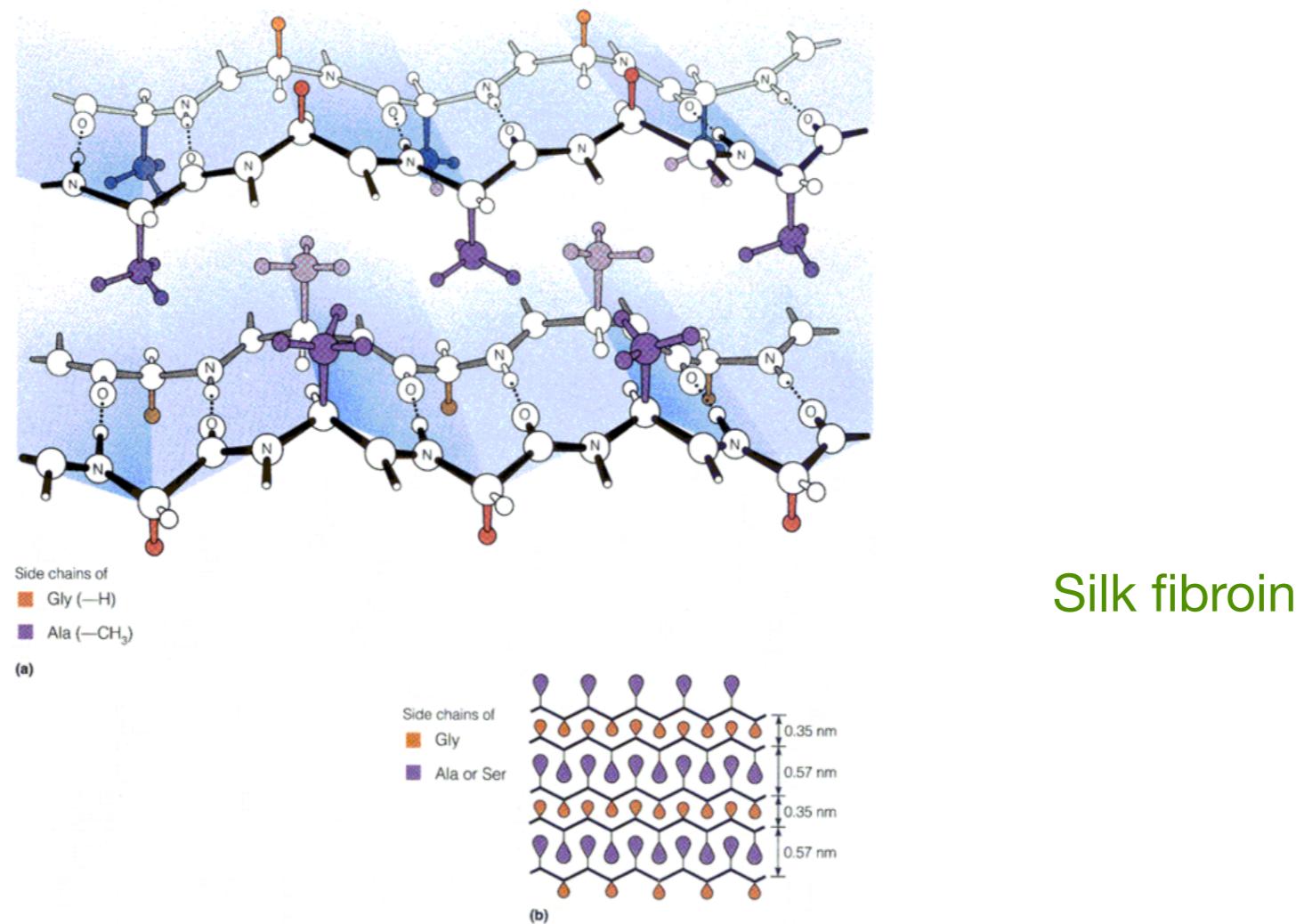
$\alpha$ -keratin is found in hair, nails, outer layer of skin. It forms almost the entire dry weight of these materials.

- It is rich in amino acids that favours helix formation (Phe, Ile, Val, Met, Ala)
- These hydrophobic side chains are on the surface-explaining its insolubility.
- $\alpha$ -keratin is also rich in Cys residues.
- Upon stretching treatment  $\beta$ -keratin forms which has different mechanical, chemical and thermal properties
- Upon reduction of disulphide bonds keratin becomes soluble



# Fibroin

- Fibroins are the silk proteins. They form the silk-worm silk and spider webs
- Made with a  $\beta$ -sheet structures with Gly on one face and Ala/Ser on the other
- Fibroins contain repeats of [Gly-Ala-Gly-Ala-Gly-Ser-Gly-Ala-Ala-Gly-(Ser-Gly-Ala-Gly-Ala-Gly)8]
- The  $\beta$ -sheet structures stack on top of each other
- Strength provided by inter-digitation of side-chains
- Bulky regions with val and tyr interrupt the  $\beta$ -sheet and allow the stretchiness



# Globular vs. Fibrous proteins

## Globular

- 1. Compact protein structure**
- 2. Soluble in water (or in lipid bilayers)**
- 3. Secondary structure is complex with a mixture of  $\alpha$ -helix,  $\beta$ -sheet and loop structures**
- 4. Quaternary structure is held together by noncovalent forces**
- 5. Functions in all aspects of metabolism (enzymes, transport, immune protection, hormones, etc).**

## Fibrous

- Extended protein structure**
- Insoluble in water (or in lipid bilayers)**
- Secondary structure is simple based on one type only**
- Quaternary structure is usually held together by covalent bridges**
- Functions in structure of the body or cell (tendons, bones, muscle, ligaments, hair, skin)**