CH40001 Biochemical Engineering
Module 3, Chapter 8

# Receptor-Ligand Binding

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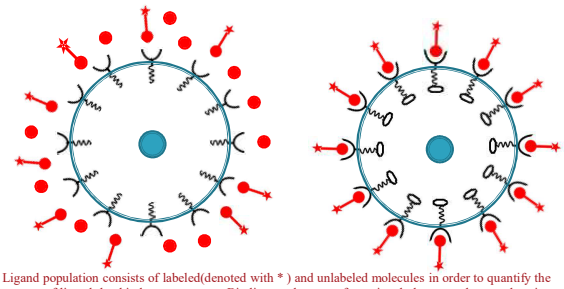
## RECEPTOR–LIGAND BINDING

- Receptors : Proteins present on cell membrane and on that of organelles , including the nucleus.
  - Role : Transfer signal between cell surface to cell interior or between different parts of the cell.
  - Types : G-type protein coupled receptor , ion channel receptor, Enzyme linked receptor, receptor with intrinsic enzyme activity.
- Ligands : A ligand could be a peptide, protein or hormone that binds **specifically** to the receptors and helps in **transfer of information**.
- R-L Binding : Produces a conformational change in the cytoplasmic portion of the receptor, which in turn initiates a cascade of signaling events that result in functional changes in the cell.
  Subsequently – small molecule second messengers may be activated or enzymes that modify proteins may be activated.



Ligand population consists of labeled(denoted with * ) and unlabeled molecules in order to quantify the amount of ligand that binds to receptors – Binding produces conformational change to the cytoplasmic portion of the receptors

$$L + R \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} C$$

$k_1$: association rate constant ($M^{-1}\ min^{-1}$ or $M^{-1}\ s^{-1}$)
$k_{-1}$: dissociation rate constant ($min^{-1}$ or $s^{-1}$)

## Examples of Cell Surface Receptors

| Receptor | Classification | Effect |
|---|---|---|
| Epinephrine | G-protein-coupled receptor | Neurotransmitter and hormone that affects metabolic activity. |
| Serotonin | Same | Neurotransmitter that causes constriction of blood vessels in the brain. |
| Acetylcholine | Ion channel receptor | Neurotransmitter that stimulates or inhibits muscle activity |
| Cytokines | Tyrosine Kinase Activator | Stimulates immune cells |
| Interferon | Same | Cytokine that interferes with replication of viruses |
| Insulin | Monomer | Increases glucose uptake by cells and functions as a growth factor |
| Growth Factors | Receptors have intrinsic tyrosine kinase activity | Stimulates cell division |

## Kinetics of R-L Binding

$$L + R \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} C$$

L: ligand, R: receptor, C: complex

$L + R \rightleftharpoons C$
(Ligand+Receptor ⇌ Complex)

$C_R, C_L, C_C$ : Concentrations of receptor,ligand,complex respectively

$$\frac{dC_C}{dt} = k_1 C_R C_L - k_{-1} C_C \quad \text{.....(1)}$$

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$N_R$ = no. of receptors/cell (free)
$N_C$ = no. of complexes/cell
$N_{RT}$ = Total no of receptors/cell (free+bound)
$N_{RT} = N_C + N_R$ .....(2)[valid under limited conditions]

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In experiments, ligand is added in solution in a ligand form at an initial conc. of $C_{L0}$.If ligand is not metabolized by cells,then either it is in solution or bound to receptors.
If $n$=no of cells/volume && $N_A$ = Avogadro no.

$$C_{L0} = C_L + \left(\frac{n}{N_A}\right) N_C \quad \text{.....(3)}$$

Using eqns. (1) & (3) ,& $C_C = \frac{n}{N_A} N_C$ && $C_R = \frac{n}{N_A} N_R$

$$\frac{dN_C}{dt} = k_1 (N_{RT} - N_C)(C_{L0} - \frac{n}{N_A} N_C) - k_{-1} N_C \quad \text{.....(4)}$$

In experiments, ligands are typically in excess.

$$\left(\frac{n}{N_A}\right) N_C << C_{L0} \quad \text{.....(5)}$$

$$\therefore \frac{dN_C}{dt} = k_1 N_{RT} C_{L0} - (k_{-1} + k_1 C_{L0}) N_C \quad \text{.....(6)}$$

with $N_C = N_{C0}$ at $t = 0$

$$N_C = N_{C0} \exp[-(k_{-1} + k_1 C_{L0})t] + \frac{k_1 N_{RT} C_{L0}}{k_{-1} + k_1 C_{L0}} [1 - \exp\{-(k_{-1} + k_1 C_{L0})t\}] \quad \text{.....(7)}$$

Using $K_D = k_{-1}/k_1$, eqn (7) becomes

$$N_C = N_{C0} \exp[-k_{-1}(1 + \frac{C_{L0}}{K_D})t] + \frac{N_{RT} C_{L0}}{K_D + C_{L0}} \left[1 - \exp\left\{-k_{-1}\left(1 + \frac{C_{L0}}{K_D}\right)t\right\}\right] \quad \text{.....(8)}$$

## Half-Time

For any given ratio of $N_C/N_{RT}$, time required to reach half its maximum value(i.e.$N_{C,max}$)

$$N_{C,max}=\left(\frac{K_D+C_{L0}}{N_{RT}C_{L0}}\right)^{-1}=\frac{N_{RT}C_{L0}}{K_D+C_{L0}}\quad(\text{for } N_{C0}=0 \;\&\&\; t\to\infty)$$

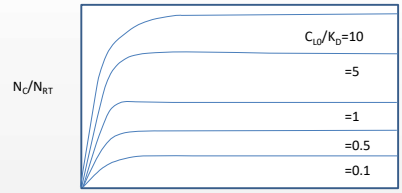
$$\frac{N_C}{N_{C,max}}=0.5=\left[1-\exp\left\{-k_{-1}\left(1+\frac{C_{L0}}{K_D}\right)t_{1/2}\right\}\right] \quad \ldots\ldots(9)$$

Solving (9) for $t_{1/2}$

$$t_{1/2}=\frac{\ln 2}{k_{-1}\left(1+\frac{C_{L0}}{K_D}\right)} \quad \ldots\ldots(10)$$

For small ligand conc.(i.e. $C_{L0}$small),$t_{1/2}=\frac{\ln 2}{k_{-1}}$(rxn. is dissociation limited)

For large ligand conc.,$C_{L0}$is large & $K_D$is small since binding is rapid, & $t_{1/2}\to 0$.




$K_{-1}t$(scaled time ), dimensionless

- PROBLEM : Determine the half-time for binding of endothelin to endothelin1 receptor under the following conditions:

$$k_{-1}=0.005\,\text{min}^{-1}, K_D=16\,pM, C_{L0}=1,10,100\,pM.1\,pM=1*10^{-12}\,M$$

## Determination of Rate Constants for R-L Binding

$$N_C=\frac{N_{RT}C_{L0}}{K_D+C_{L0}}\left[1-\exp\left\{-k_{-1}(1+\frac{C_{L0}}{K_D})t\right\}\right]$$

- Kinetic parameters:$k_1,k_{-1},N_{RT}$ –determined by performing experiments at various values of initial ligand concentration $C_{L0}$,where some of the ligands are labeled or tagged with radioactive or fluorescent labels($N_{L0}^*$).
- Approach 1

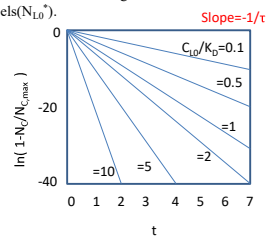
(1) Measure $N_C^*$ as a function of time for different values of $C_{L0}$.

$$\ln\left(1-\frac{N_C^*}{N_{C,max}^*}\right)=-k_{-1}\left(1+\frac{C_{L0}}{K_D}\right)t$$

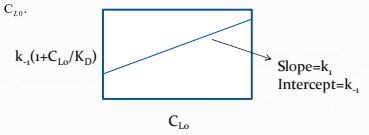
where $N_{C,max}^*=N_C$ @ steady state.

Obtain slopes for different $C_{L0}$.

Measure slope$=-k_{-1}\left(1+\frac{C_{L0}}{K_D}\right)$ for different $C_{L0}$.




(2) Plot(-slope)=$k_{-1}\left(1+\frac{C_{L0}}{K_D}\right)$ vs $C_{L0}$.



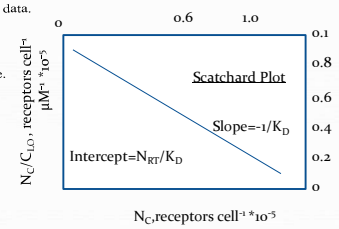

(3)To obtain $N_{RT}$,use steady state data.

$$N_{C,max}=\frac{N_{RT}C_{L0}}{K_D+C_{L0}}$$

$$N_{RT}=\left(1+\frac{K_D}{C_{L0}}\right)N_C \quad \text{, steady state.}$$

$$\Rightarrow \frac{N_{C,max}}{C_{L0}}=\frac{N_{RT}}{K_D}-\frac{N_{C,max}}{K_D}$$

Plot $N_C/C_{L0}$ vs $N_C$.




- Problem: Use the following data to determine the specific binding of a ligand to a receptor , the values of $K_D$ and the number of receptors per cell.

| $C_{L0}$,M | Amount bound without unlabeled ligand | Amount bound with 100 excess unlabeled ligand |
|---|---|---|
| 1*10$^{-10}$ | 15,000 | 5,000 |
| 5*10$^{-10}$ | 58,000 | 25,000 |
| 1*10$^{-9}$ | 100,000 | 50,000 |
| 5*10$^{-9}$ | 330,000 | 250,000 |
| 1*10$^{-8}$ | 590,000 | 500,000 |

## DEVIATIONS FROM SIMPLE BIMOLECULAR KINETICS

- CAUSES OF DEVIATIONS FROM BM KINETICS

A. Ligand depletion ($C_L \neq C_{L0}$)

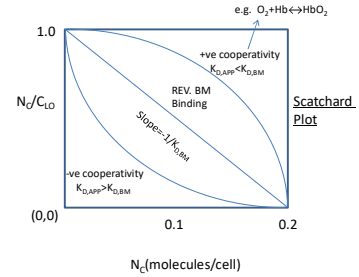
B. Multiple receptor populations of different affinity.

C. Multivalent ligand binding .

$$R+2L \rightleftharpoons RL_2$$

D. Receptor aggregation (receptors may form complex with each other before binding to ligand )

$$R+R \rightleftharpoons R_2 \quad R_2+L \rightleftharpoons LR_2 \quad LR_2+L \rightleftharpoons LR_2L$$



## A. LIGAND DEPLETION

Earlier assumption of $C_L \square C_{L0}$ when $\left\{\frac{n}{N_A}\right\}\frac{N_{RT}}{C_{L0}} << 1$ is not valid in this case.

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Dimensionless groups::

$u = \frac{N_C}{N_{RT}}$ , $\tau = k_{-1}t$ , $\eta = \frac{nN_{RT}}{N_A C_{L0}}$ , $\alpha = \frac{C_{L0}}{K_D}$

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*Model* :: $\frac{du}{d\tau} = (1-u)(1-\eta u)\alpha - u$

with $u = u_0 = \frac{N_{C0}}{N_{RT}}$ at $\tau = 0$.

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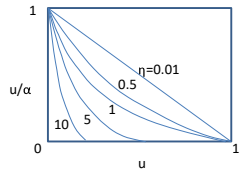
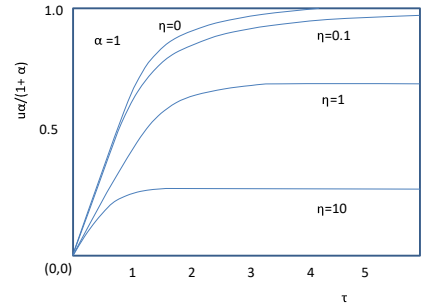
Steady State Model: $(1-u)(1-\eta u)\alpha - u = 0$




Dimensionless Scatchard Plot:

$\frac{u}{\alpha} = (1-u)(1-\eta u)$

## Solution of Unsteady State Model




*Errors* :: $\eta = 0.1, \quad \alpha = 1, \quad \varepsilon = 2.3\%$

$\eta = 0.1, \quad \alpha = 0.1 \text{ or } 10, \quad \varepsilon = 0.9\%$

## B. TWO OR MORE RECEPTOR POPULATION

Receptor 1$(K_{D1}, N_{RT1})$ && Receptor 2$(K_{D1}, N_{RT1})$

At Steady State: $N_C = \frac{C_{L0}N_{RT1}}{C_{L0}+K_{D1}} + \frac{C_{L0}N_{RT2}}{C_{L0}+K_{D2}}$ (Each receptor acts independently)

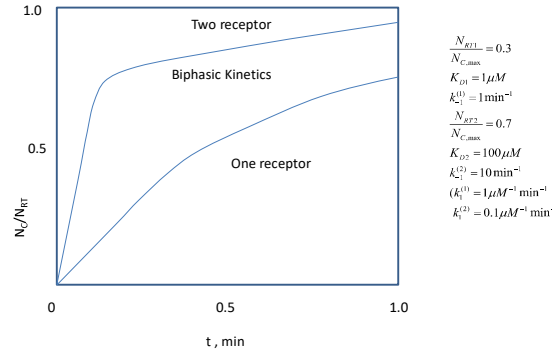
N.B: It may not be possible to distinguish between the two values of $K_D$ unless they differ by more than 1 order of magnitude.

Model: $\frac{dN_{C1}}{dt} = k_1^{(1)}N_{RT1}C_{L0} - (k_{-1}^{(1)} + k_1^{(1)}C_{L0})N_{C1}$

$\frac{dN_{C2}}{dt} = k_1^{(2)}N_{RT2}C_{L0} - (k_{-1}^{(2)} + k_1^{(2)}C_{L0})N_{C2}$

with $N_{C1} = N_{C2} = 0$ at $t = 0$.

$N_C = N_{C1} + N_{C2} = \frac{C_{L0}N_{RT1}}{C_{L0}+K_{D1}}\left[1-\exp\left\{-k_{-1}^{(1)}\left(1+\frac{C_{L0}}{K_{D1}}\right)t\right\}\right] + \frac{C_{L0}N_{RT2}}{C_{L0}+K_{D2}}\left[1-\exp\left\{-k_{-1}^{(2)}\left(1+\frac{C_{L0}}{K_{D2}}\right)t\right\}\right]$




$\frac{N_{RT1}}{N_{C,max}} = 0.3$

$K_{D1} = 1\mu M$

$k_{-1}^{(1)} = 1\text{min}^{-1}$

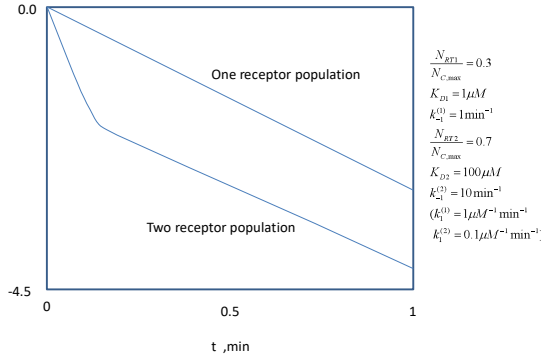
$\frac{N_{RT2}}{N_{C,max}} = 0.7$

$K_{D2} = 100\mu M$

$k_{-1}^{(2)} = 10\text{min}^{-1}$

$(k_1^{(1)} = 1\mu M^{-1}\text{min}^{-1}$

$k_1^{(2)} = 0.1\mu M^{-1}\text{min}^{-1})$

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$K_{D2} = 100\mu M$

$k_{-1}^{(2)} = 10\text{min}^{-1}$

$(k_1^{(1)} = 1\mu M^{-1}\text{min}^{-1}$

$k_1^{(2)} = 0.1\mu M^{-1}\text{min}^{-1})$

- PROBLEM 2:

Show that by rearranging equation:-

$$N_C = \frac{C_{L0}N_{RT1}}{C_{L0}+K_{D1}} + \frac{C_{L0}N_{RT2}}{C_{L0}+K_{D2}} \quad ,$$

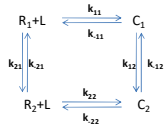
You can determine the 4 parameters ($N_{RT1}$,$N_{RT2}$,$K_{D1}$,$K_{D2}$)for 2 receptor populations from a Scatchard plot . Assume that receptor population 1 is high affinity receptor(i.e. $K_{D1}<K_{D2}$)

## INTERCONVERTING RECEPTOR SUBPOPULATION

- Often, receptor undergoes a conformational change after binding to the ligand. The change however does not affect subsequent ligand receptor binding . In the most general case, the receptor is present as inter-converting subpopulations, with differences in rates of dissociation and association between receptor and ligand.

Model:

A.




Special case:

B. $R_1 + L \underset{k_{-11}}{\overset{k_{11}}{\rightleftarrows}} C_1 \underset{k_{-12}}{\overset{k_{12}}{\rightleftarrows}} C_2$

A. It could be shown that the general model (model a) follows Scatchard relationship , in which :

$$N_C = N_{C1} + N_{C2} = \frac{C_{L0} N_{RT}}{C_{L0} + K_{Dapp}}$$

where, $N_{RT} = N_{RT1} + N_{RT2} + N_{C1} + N_{C2}$

and, $K_{Dapp} = K_{D11}\left(\frac{1+\frac{1}{K_{D21}}}{1+\frac{1}{K_{D12}}}\right) = K_{D22}\left(\frac{1+K_{D21}}{1+K_{D12}}\right)$

in which, $K_{D11} = \frac{k_{-11}}{k_{11}}$ , $K_{D21} = \frac{k_{-21}}{k_{21}}$ , $K_{D12} = \frac{k_{-12}}{k_{12}}$ , $K_{D22} = \frac{k_{-22}}{k_{22}}$ .

Special Case:Model (b) is a special case of model (a) when $\frac{1}{K_{D21}} = 0$.

$R_1 + L \underset{k_{-11}}{\overset{k_{11}}{\rightleftarrows}} C_1 \underset{k_{-12}}{\overset{k_{12}}{\rightleftarrows}} C_2$

At steady state ,$C_2$ & $C_1$ are related by $N_{C2} = \frac{k_{12}}{k_{-12}} N_{C1} = \frac{N_{C1}}{K_{D12}}$

Total no of receptors: $N_{RT} = N_R + N_{C1} + N_{C2}$ ($N_R$=free receptors)

$$= N_R + N_{C1}\left(1 + \frac{1}{K_{D12}}\right)$$

Also at steady state, $k_{11} N_R C_{L0} = k_{-11} N_{C1} \Rightarrow N_R = K_{D11} \frac{N_{C1}}{C_{L0}}$ .

$$or, K_{D11} = \frac{N_R}{N_{C1}} C_{L0} = \frac{C_{L0}}{N_{C1}}\left\{N_{RT} - N_{C1}\left(1 + \frac{1}{K_{D12}}\right)\right\}$$

$$or, N_{C1} = \frac{N_{RT} C_{L0}}{K_{D11} + C_{L0}\left(1 + \frac{1}{K_{D12}}\right)}$$

$$\therefore \boxed{N_C = N_{C1} + N_{C2} = \frac{N_{RT} C_{L0}}{C_{L0} + \left(\frac{K_{D11} K_{D12}}{1 + K_{D12}}\right)}}$$

### Summary of Various Kinetics of Ligand-Receptor Binding

| Type of Binding | Scatchard Plot | Dissociation Kinetics |
|---|---|---|
| Single R | Linear | Single Exponential |
| Single R-L depletion | Non-linear | Same |
| 2 R Population | Non-linear | Double Exponential |
| R-L Inter-conversion | Linear | 2/more Exponential |
| Inter-conversion of ligand to a non-dissociable form. | Linear | Double Exponential |
| True Co-operativity | Non-linear | Double Exponential |

### Problem:

- G proteins are activated as a result of ligand binding to a G-protein coupled receptor . The process can be schematized as

$$L + R \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} LR$$

$$G + LR \xrightarrow{k_a} G^* \xrightarrow{k_i} G \quad ,$$

where $G^*$ is the activated G complex .Note that in the second reaction , the ligand-receptor complex is not consumed , but merely activates the G protein . Thus a balance on the receptor is : $C_{RT}$=$C_R$+$C_{LR}$ , where $C_{LR}$ is either free or bound to G protein . The activated G protein is deactivated with a rate constant $K_i$.

(a)A certain method was developed to measure the rate constants $K_a$ and $K_i$ . In the ensuing experiments different amounts of ligands were bound to cells containing $5*10^4$ receptors per cell . The number of activated G proteins was measured after steady state is reached . The total G protein concentration was $1*10^5$ molecules/cell . Results from a typical experiment are shown in the Table 1. Use these results to determine the ratio $K_a/K_i$.

(b)To determine $K_i$ , levels of $G^*$ were allowed to reach steady state . At that point the ligand rapidly dissociated from the receptor by incubating with a high affinity anti-body for the ligand . $G^*$ vs. time was measured and reported in Table 2 . Use this result to determine $K_i$

**Table-1**

| $C_{LR}$(molecules/cell) | $C_G^*$ (molecules/cell) |
|---|---|
| 5000 | 250 |
| 10,000 | 500 |
| 20,000 | 1000 |
| 30,000 | 1500 |
| 40,000 | 2000 |
| 50,000 | 2500 |

**Table-2**

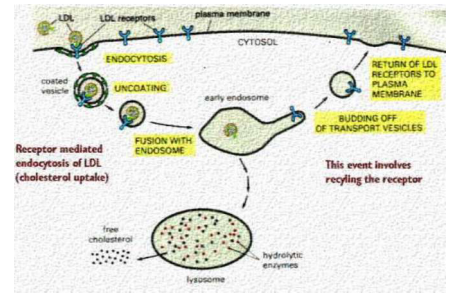
| t (sec) | $C_G^*$ (molecules/cell) |
|---|---|
| 0 | 2500 |
| 1 | 2047 |
| 2 | 1676 |
| 5 | 920 |
| 10 | 338 |

## RECEPTOR – MEDIATED ENDOCYTOSIS

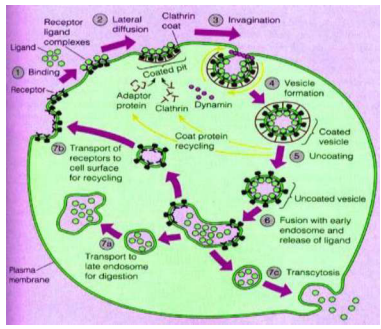
- DEFINITION & FUNCTION: -

  Receptor – mediated endocytosis is the major pathway by which macromolecules enter the cell. Receptor – mediated endocytosis acts to internalize proteins for metabolism, for the generation of signals, and for the regulation of receptor numbers. Molecules transported by this mechanism include nutrients (low-density lipoprotein (LDL) or Transferrin), immunoglobulins, hormones (insulin), growth factors, viruses.

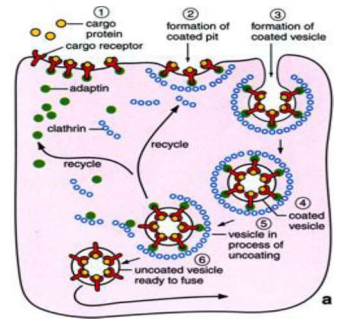
## RECEPTOR – MEDIATED ENDOCYTOSIS




## RECEPTOR – MEDIATED ENDOCYTOSIS




## RECEPTOR – MEDIATED ENDOCYTOSIS




Mechanism of Endocytosis: -

- Through the interaction of cytoplasmic proteins, cell membrane components form vesicles and transport membrane, fluid and solutes from one region of the cell to another. Endocytosis is the process of vesicle formation from plasma membrane. Exocytosis is a process by which membrane vesicles form from Golgi apparatus, fuse with plasma membrane and release newly synthesized proteins to the extracellular fluid.

- Endocytosis can result in turnover of plasma-membrane lipids every 1 to 10hrs at a rate much faster than membrane synthesis. Vesicles do not diffuse in the cytoplasm, but are transported along a network of microtubules.

Steps in Endocytosis (Ref. to Figure)

1. Binding of protein (ligand) to specific receptor. Receptors migrate into specialized regions of the cell membrane known as coated pits, identifiable by dark appearance in TEM. Coated pits typically cover a few percent of the cell membrane.
2. Coated pits continuously form coated vesicles every 2 or 3 minutes. If receptor – bound ligand is in the coated pit, when a vesicle is formed, then it is internalized.
3. Within 1 to 2 minutes after internalization of vesicles, Clathrin (a cytoplasmic protein present in the coated pits that coats the vesicles) dissociates from vesicle. The vesicle is now referred to as early endosome.
4. At this point, ion pumps in the vesicle membrane pump in $H^+$ to lower the pH within the endosome. Often the receptor-ligand affinity decreases with decreasing pH.
5. As a result, ligands and receptors dissociate and segregate.
6. Most ligand molecules remain in vesicles that are directed to lysosomes, where proteins are degraded to amino acids. From the time a ligand molecules finds to a receptor, if takes it around 15 minutes to reach a lysosome.
7. Most types of receptors are sorted and sent to tubular structures that return the receptors to the cell membrane. e.g. LDL receptor makes a round trip in 12 mins & each receptor makes 150 round trips before degradation.

Classification of receptors involved in receptor –mediated endocytosis

1. Class 1 receptors – receptors for hormones and nutrients. These receptors bind to ligands before binding to coated pits.

2. Class 2 receptors – e.g. LDL, transferrin receptors. These are bound to coated pits without ligand. L-R binding follows endosome formation.

- Kinetic model of LDL receptor-mediated endocytosis:

  shall include LDL protein binding, internalization and degradation, as well as identify the effects of mutation on the process.

KINETIC MODEL FOR LDL RECEPTOR – MEDIATED ENDOCYTOSIS

- LDL (low density lipoprotein ) plays an important role in delivery of cholesterol to cells. The LDL molecule itself consists of a core of cholesterol ester surrounded by cholesterol, phospholipids, and proteins. LDL is internalized by a class 2 receptor.
- Understanding the dynamics of the LDL receptor helps in understanding the basis for the genetic disease familial hypercholesterolemia. This disease is characterized by extremely high levels of plasma cholesterol and the early onset of cardio- vascular disease.
- Several mutations of the LDL receptor produce the disease: -
  a. In some cases, the affinity between LDL and its receptor is reduced, so that $K_D$ increases, leading to impaired binding.
  b. In other cases, there is a defect in the internalization of the receptor.
  c. Several rare mutations involve normal binding to LDL receptors, but little localization in coated pits.
- Whichever be the mutation, the net effect is that the number of functional LDL receptors that can undergo binding and internalization of LDL is reduced. This reduces the LDL uptake of the cell.

## EXPERIMENT TO MEASURE KINETICS

- At 4°C, internalization of LDL does not occur. As a result, at 4°C it is possible to separate the internalization process from the binding process.

- Pulse – chase experiment: -

(a) Cells are incubated with radio-labeled LDL at 4°C. this period of incubation is called *pulse period*.

(b) Solution is removed and cells are rinsed at 4°C to remove LDL that is not bound to them.

(c) Solution is then replaced with a solution of unlabeled LDL and warmed to 37°C.

(d) During this period, known as chase period, the disappearance of labeled LDL from the cell surface is measured.

(e) The appearance of labeled LDL in the medium is also measured, to determine the amount of LDL dissociation from its receptors.

(f) Also, amount of LDL that remains bound to the receptors, internalized, and degraded is also measured.

## RESULTS OF THE EXPERIMENT:

- Kinetics of labeled LDL disappearance are first order reactions.

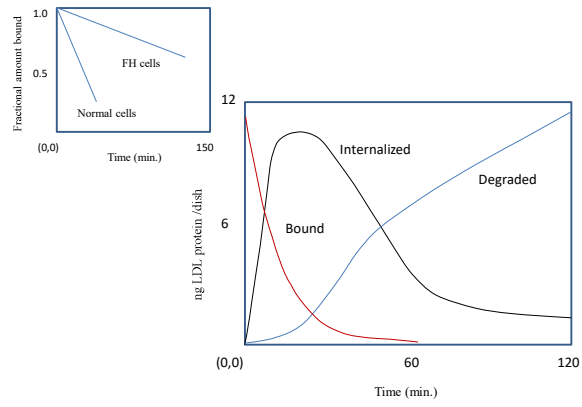
Rate constants $\overline{K} = 0.257\ \text{min}^{-1}$ for normal cells.

$\overline{K} = k_{-1} + k_2$, where $k_{-1}$ & $k_2$ are:

$$LDL + R \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} LDLR \xrightarrow{k_2} LDL_i \xrightarrow{k_3} LDL_d$$

Binding of LDL receptor on cell surface | Internalization via coated pits | Degradation in lysosomes

- From measurement of appearances of labeled LDL in the medium at 37°C & 4°C,
- $K_{-1}$=0.01 min$^{-1}$ && $K_2$=0.247 min$^{-1}$
  => $K_{-1} << K_2$ for normal cells
- From diseased cells, is similar to that in normal cells, but K2,FH<K2,normal , suggesting that for this mutation (FH) LDL receptor is not internalized.



## Kinetic model for Experiment

$LDL + R \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} LDLR \xrightarrow{k_2} LDL_i \xrightarrow{k_3} LDL_d$

CHASE PHASE: No association; $k_{-1} << k_2$.

$$\frac{dN_{LDLR}}{dt} = -k_2 N_{LDLR}$$

$$\frac{dN_{LDL_i}}{dt} = k_2 N_{LDLR} - k_3 N_{LDL_i}$$

$$\frac{dN_{LDL_d}}{dt} = k_3 N_{LDL_i}$$

At $t = 0, N_{LDLR} = N_{LDLR_0}, N_{LDL_i} = N_{LDL_{i0}}, N_{LDL_d} = 0.$

Solving the above equations:

$$N_{LDLR} = N_{LDLR_0} \exp(-k_2 t)$$

$$N_{LDL_i} = \frac{N_{LDLR_0} k_2}{k_3 - k_2}\left[\exp(-k_2 t) - \exp(-k_3 t)\right] + N_{LDL_{i0}} \exp(-k_3 t)$$

$$N_{LDL_d} = \frac{N_{LDLR_0} k_2 k_3}{k_3 - k_2}\left[\left(\frac{1-\exp(-k_2 t)}{k_2}\right) - \left(\frac{1-\exp(-k_3 t)}{k_3}\right)\right] + N_{LDL_{i0}}\left[1-\exp(-k_3 t)\right]$$

## Full Kinetic Model

$$\frac{dN_{LDLR}}{dt} = k_1 C_{LDL} N_R - (k_2 + k_{-1}) N_{LDLR}$$

$$\frac{dN_{LDL_i}}{dt} = k_2 N_{LDLR} - k_3 N_{LDL_i}$$

$$\frac{dN_{LDL_d}}{dt} = k_3 N_{LDL_i}$$

Assume, $C_{LDL} \approx C_{LDL,0}$ (initial conc.)

Constraint: $N_{RT} = N_R + N_{LDLR}$

Solution:

$$N_{LDLR} = \frac{C_{LDL0} N_{RT}}{K + C_{LDL0}}\left[1-\exp(-Kt)\right]$$

$$N_{LDL_i} = \frac{N_{RT} k_2 C_{LDL0}}{K + C_{LDL0}}\left[\frac{1-\exp(-k_3 t)}{k_3} - \frac{\exp(-Kt) - \exp(-k_3 t)}{k_3 - K}\right]$$

$$N_{LDL_d} = \frac{N_{RT} k_2 C_{LDL0}}{K + C_{LDL0}}\left[t - \left(\frac{1-\exp(-k_3 t)}{k_3}\right) - \left(\frac{k_3}{k_3 - K}\right)\left(\frac{1-\exp(-Kt)}{K} - \frac{1-\exp(-k_3 t)}{k_3}\right)\right]$$

where, $K = \frac{k_{-1} + k_2}{k_1}, k = k_1(K + C_{LDL0})$

For, $k_2 = 0.2\,\text{min}^{-1}$, $k_3 = 0.02$. Show good model-experiment agreement;
$K = 25$ μg/ml, $k_{-1} = 0.0084$ ml.μg$^{-1}$min$^{-1}$, $C_{LDL,0} = 10$ μg/ml.