Chapter 2 Centrifugation:

Separation of Organelles and Biomolecules

Biochemistry and Molecular Biology (BMB)

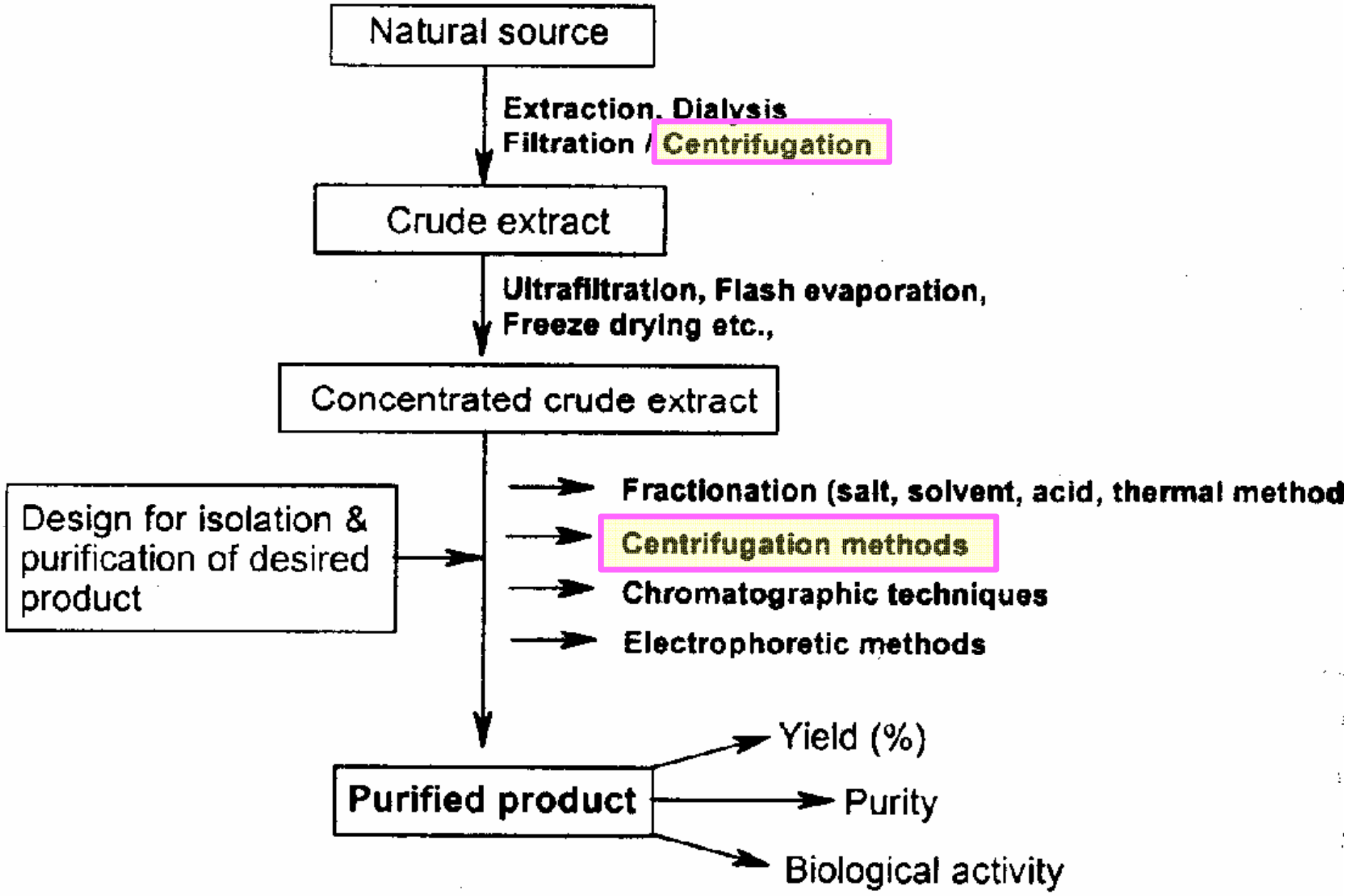
- 3.1 Introduction
- 3.2 Basic Principle of sedimentation
- 3.3 Types, care and safety of centrifuges
- 3.4 Preparative centrifugation (example on influenza virus and protein complex)
- 3.5 Analytical centrifugation

Analytical Biochemistry (AB)

- 3.4.3 Ultracentrifugation

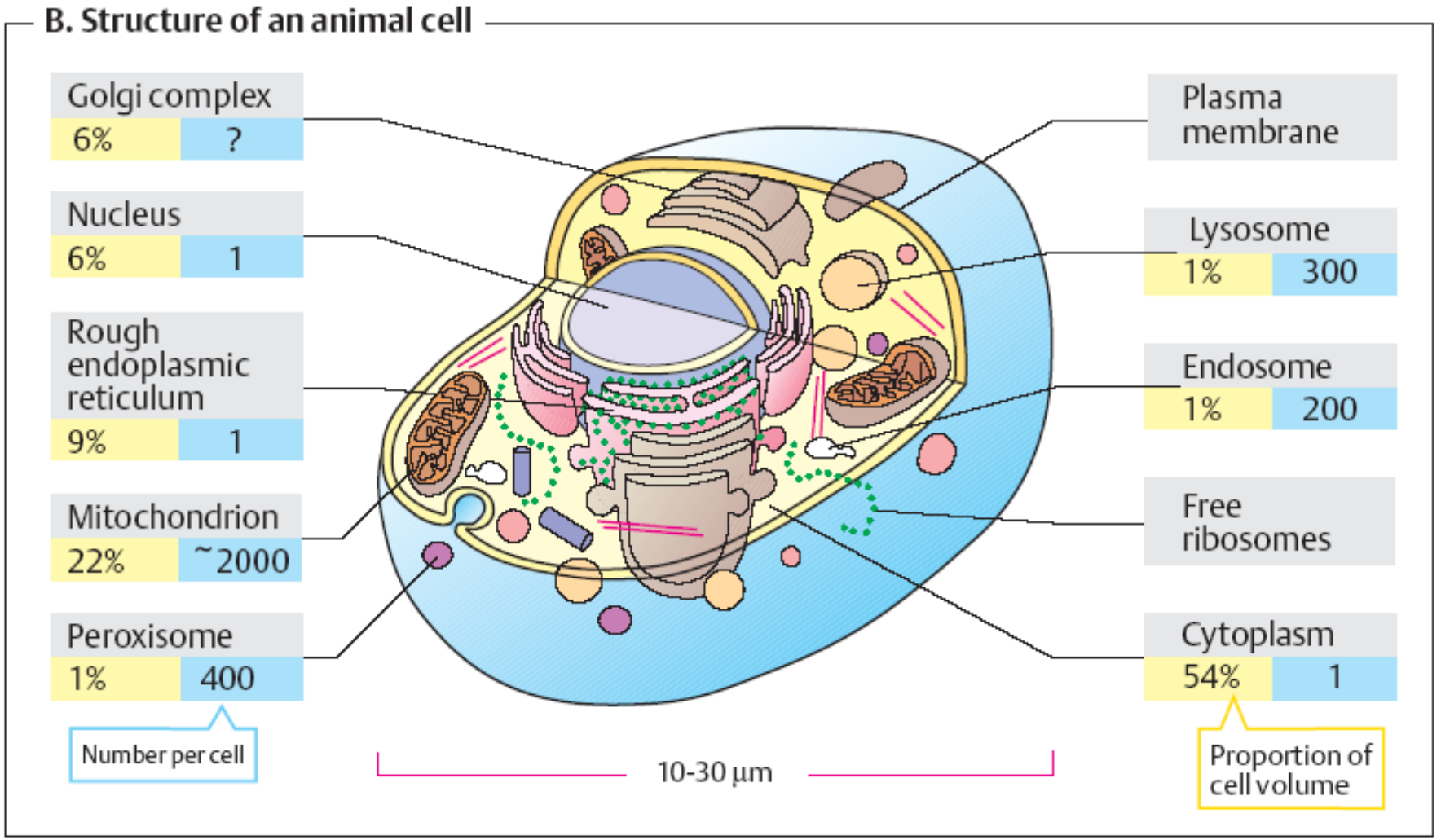
Koolman, Color Atlas of Biochemistry (CAB), 2nd edition

General Steps in Biochemical Separation



Separation of Macromolecules (CAB)

- Chromatography, precipitation
- Electrophoresis, ultracentrifugation



Introduction (MBM 3.1)

Principles of centrifugation (離心)

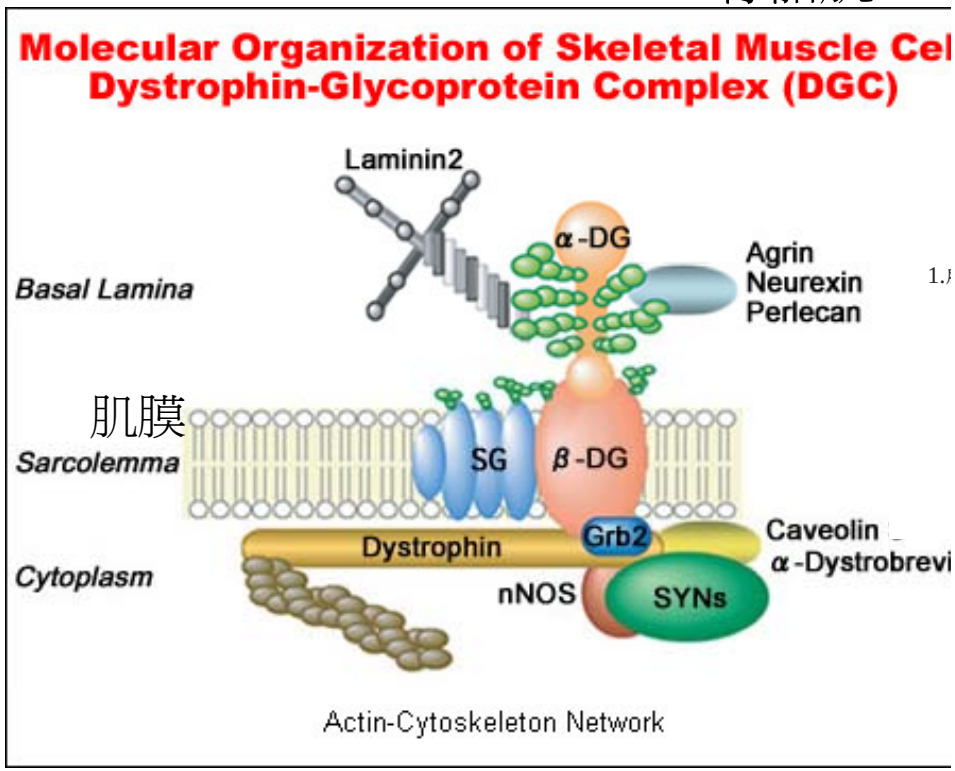
A centrifuge is a device for separating particles from a solution according to their size, shape, density, viscosity of the medium and rotor speed

In a solution, particles whose density is higher than that of the solvent **sink (sediment),** and particles that are lighter than it **float** to the top. The greater the difference in density, the faster they move. If there is no difference in density (**isopyknic conditions**), the particles **hover**. To take advantage of even tiny differences in density to separate various particles in a solution, **gravity** can be replaced with the much more powerful "**centrifugal force**" provided by a centrifuge.

Type 1: Analytical Centrifugation

Applications: *Measure the shape or mass of supermolecular molecules*

骨骼肌

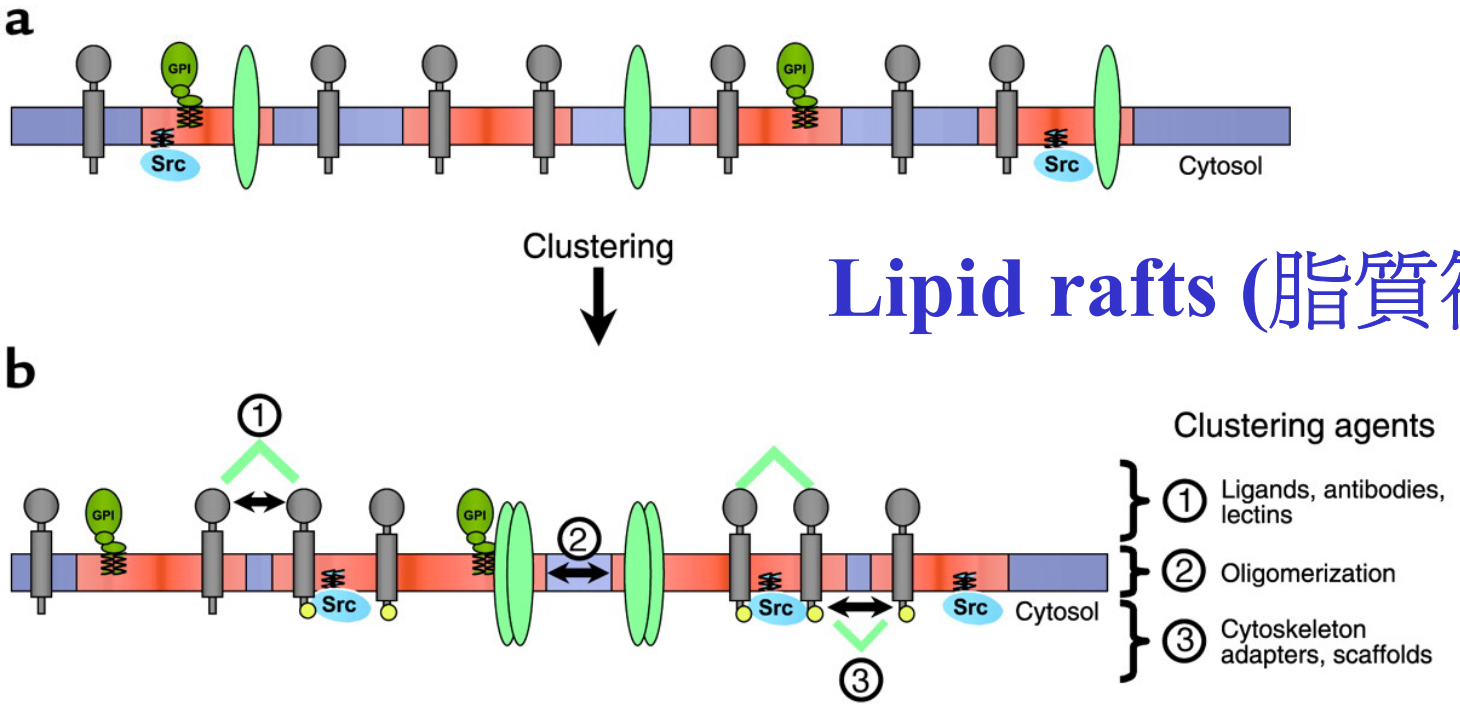


http://www.cgmh.org.tw/chldhos/intr/c4a90/new_page_50.htm

In skeletal muscle, dystroglycan is a component of the dystrophin-glycoprotein complex (DGC)(Fig. 1). -Dystroglycan is an extracellular peripheral membrane glycoprotein anchored to the cell membrane by binding to a transmembrane glycoprotein, -dystroglycan. The -dystroglycan- -dystroglycan complex is widely expressed in a broad array of tissues and is thought to stabilize the plasma membrane by acting as an axis through which the extracellular matrix is tightly linked to cytoskeleton. This is because -dystroglycan strongly binds to laminin in the extracellular matrix, and the cytoplasmic domain of -dystroglycan interacts with dystrophin, which in turn binds to the actin cytoskeleton[2].

Type 2: Preparative Centrifugation

Application: *Separation of cell, subcellular structure, membrane vesicles*



Lipid rafts (脂質筏)

Lipid rafts (脂質筏) are dynamic assemblies of proteins and lipids that float freely within the liquid-disordered bilayer of cellular membranes but can also cluster to form larger, ordered platforms. Rafts are receiving increasing attention as devices that regulate membrane function in eukaryotic cells.

Raft Proteins Are Targets fro Disease

Simons, K. et al. J. Clin. Invest. 2002;110:597-603

Alzheimer disease
Parkinson disease
Muscular dystrophy
Polyneuropathies, demyelinating diseases
Autoimmune disease, chronic inflammation, vaccine response
B cell response
T cell response
Asthma and allergic response
Neoplasia
Atherosclerosis
Hypertension, hemodynamic regulation
Diabetes
Hyperparathyroidism
Osteoarthritis
Gastrointestinal ulceration
Paroxysmal nocturnal hemoglobinuria
Lysosomal storage disease
Niemann-Pick disease
Tay-Sachs disease, morbus Fabry, metachromatic leukodystrophy
Pilzaeus-Merzbacher disease
Postsqualene cholesterol biosynthesis disorders
Pore-forming toxins (gas gangrene)
Sepsis, septic shock

Alzheimer disease
Parkinson disease
Hypertension

Sepsis, septic shock

Bacterial infections

- *Escherichia coli*
- *Mycobacteria tuberculosis* and *bovis*
- *Campylobacter jejuni*
- *Vibrio cholerae*
- *Clostridium difficile* (pseudomembranous colitis)
- *Clostridium tetani*
- *Salmonella, Shigella*

Viral infections

- Influenza virus
- HIV-1
- Measles virus
- Respiratory syncytial cell virus
- Filoviridae (Ebolavirus, Marburgvirus)
- Papillomaviridae and polyomaviridae
- Epstein-Barr virus
- Echovirus 1

Other pathogens

- *Plasmodium* (malaria)
- *Trypanosoma* (sleeping sickness)
- *Leishmania*
- Prions (Creutzfeldt-Jakob disease, Kuru, Gerstmann-Sträussler-Scheinker syndrome)
- *Toxoplasma gondii*

See supplemental reading list (www.jci.org/cgi/content/full/110/5/597/DC1) for detailed references.

3.2 Basic Principle of Sedimentation (AB 3.4.3)

Centrifugal force

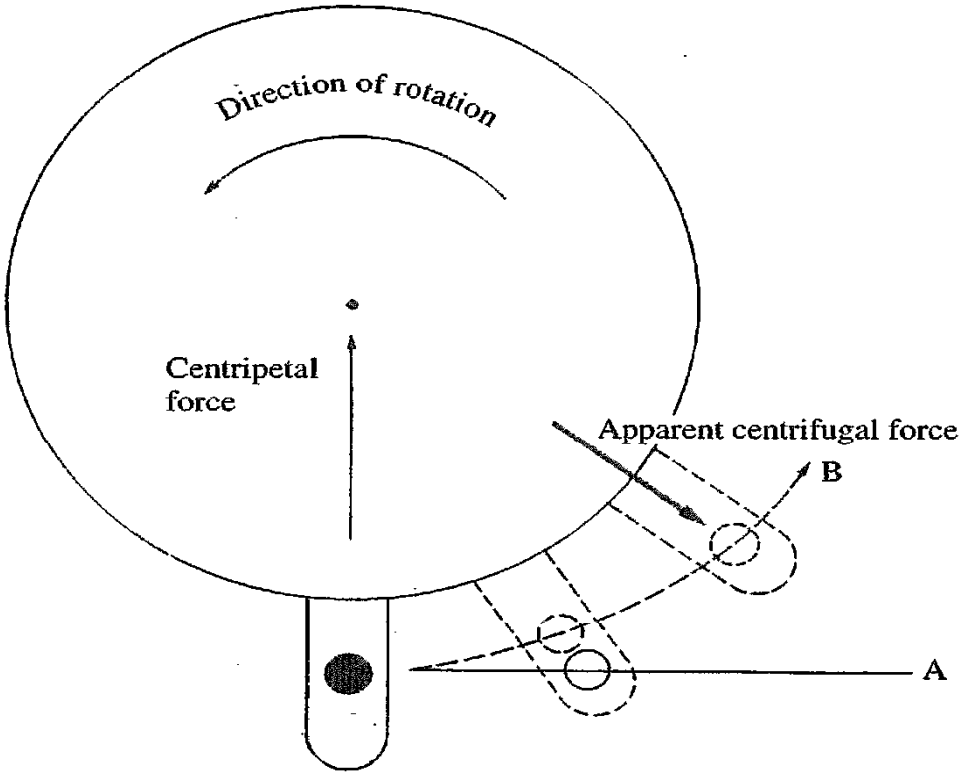


Figure 3.38 Centrifugal effects. 1

$$F = M\omega^2 r$$

M: mass of particle

r: radius of rotation (cm) (*ie* distance of particle from axis of rotation)

ω :Average angular velocity (radians/sec)

$$\omega = \frac{2\pi \text{ rev min}^{-1}}{60}$$

Rev: revolution per minute (r.p.m.)

1 revolution = 2π radians =360

Centrifugal Field

$G=r\omega^2$ depends on the radical distance of the particle from the rotation axis and the square of the angular velocity

$$G = \frac{4\pi^2 \left(\text{rev min}^{-1}\right)^2 \text{r}}{3600}$$

CALCULATION OF CENTRIFUGAL FIELD

What is the applied centrifugal field at a point equivalent to 5 cm from the centre of rotation and an angular velocity of 3000 rad s^{-1}?

The centrifugal field, G, at a point 5 cm from the centre of rotation may be calculated using the equation $G = \omega^2 r$

$G = (3000)^2 \times 5 \text{ cm s}^{-2} = 4.5 \times 10^7 \text{ cm s}^{-2}$

Angular Velocity

$$\omega = \frac{2\pi \text{ rev min}^{-1}}{60}$$

rev: revolution per minute (**r.p.m.**)

CALCULATION OF ANGULAR VELOCITY

For the pelleting of the microsomal fraction from a liver homogenate, an ultra-centrifuge is operated at a speed of 40 000 r.p.m. What is the angular velocity, ω, in radians per second?

The angular velocity, ω, may be calculated using the equation:

$$\omega = \frac{2\pi \text{ rev min}^{-1}}{60}$$

$\omega = 2 \times 3.1416 \times 40\,000/60 \text{ rad s}^{-1} = 4188.8 \text{ rad s}^{-1}$

Relative centrifugal force (different radius of rotor)

$$RCF = \frac{f_c}{f_g} = \frac{M\omega^2 r}{Mg} = \omega^2 r \times g^{-1}$$

$$RCF = \left(\frac{2\pi\, rmp}{60}\right)^2 r \times g^{-1}$$

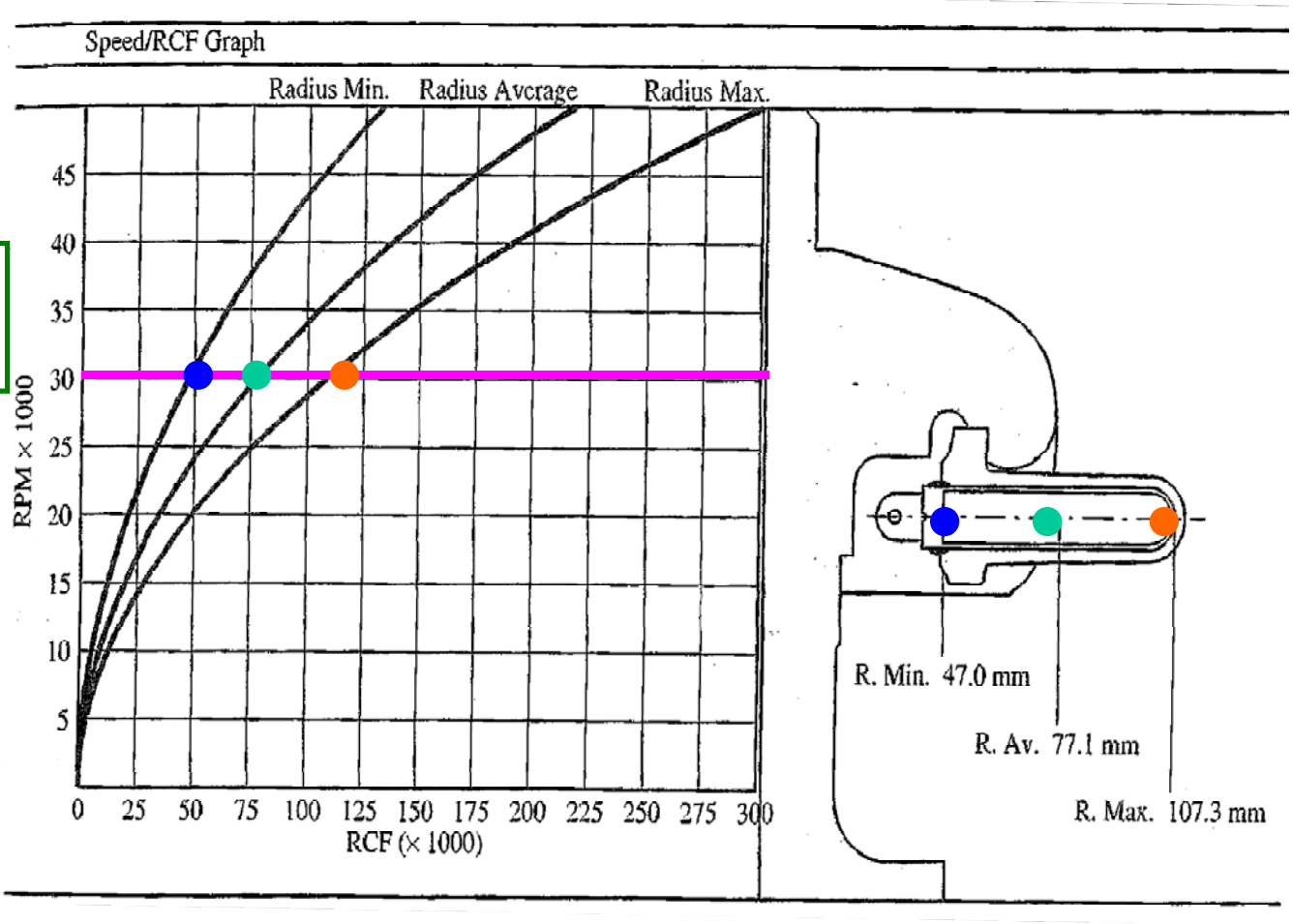
RCF value

離心力/重力，"No. x g"

(multiples of earth's gravitational force)

(離心力轉換成重力加速度的倍數).

RCF =
$1.12 \times 10^{-5} \times (rpm)^2 \times r$



Relative centrifugal force

RCF = 1.12 x 10^{-5} x $(rpm)^2$ x r

CALCULATION OF RELATIVE CENTRIFUGAL FIELD

A fixed-angle rotor exhibits a minimum radius, r_{min}, at the top of the centrifuge tube of 3.5 cm, and a maximum radius, r_{max}, at the bottom of the tube of 7.0 cm. See Fig. 3.2a for a cross-sectional diagram of a fixed-angle rotor illustrating the position of the minimum and maximum radius. If the rotor is operated at a speed of 20 000 r.p.m., what is the relative centrifugal field (RCF) at the top and bottom of the centrifuge tube?

The relative centrifugal field may be calculated using the equation:

$$RCF = 1.12 \times 10^{-5}\ \text{r.p.m.}^2\ r$$

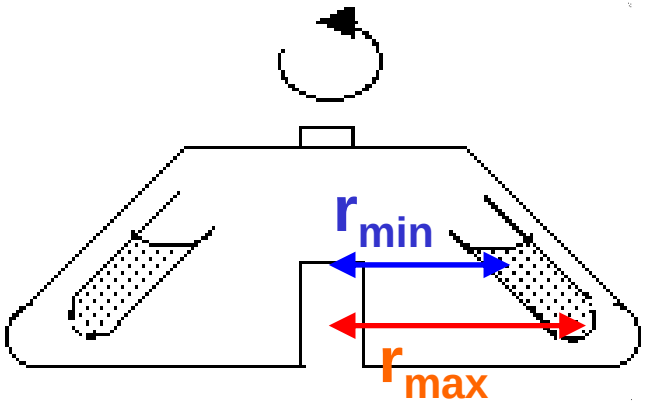
Top of centrifuge tube:

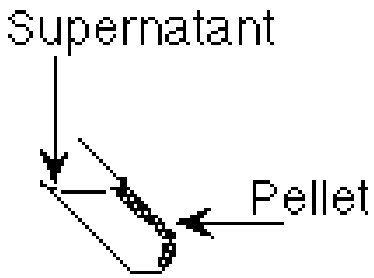
$$RCF = 1.12 \times 10^{-5} \times (20\,000)^2 \times 3.5\ g = 15\,680\ g$$

Bottom of centrifuge tube:

$$RCF = 1.12 \times 10^{-5} \times (20\,000)^2 \times 7.0\ g = 31\,360\ g$$

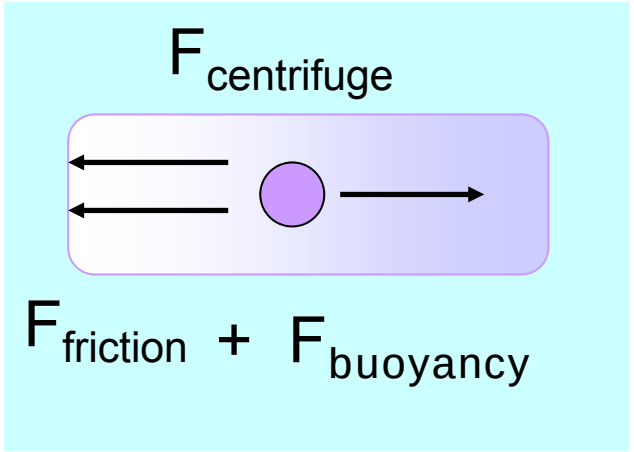
This calculation illustrates that, with fixed-angle rotors, the centrifugal field at the top and bottom of the centrifuge tube might differ considerably, in this case approximately two-fold.





Interacting Forces in Centrifugation

Sedimenting force, $M\omega^2 r$, is opposed by...



1. Frictional Resistance against particle moving through fluid.

= f.v

f = frictional coefficient of particle in the solvent

v = particle velocity

2. Flotation Force $F = Ms\,\omega^2 r$

Ms = the mass of equal volume of solvent

BALANCE between the **sedmenting force** and **counteracting force**

Net force = $\left(M_p - M_s\right)\omega^2 r - fv$

M, f: relate to the mass and shape of analyte

Sedimentation Coefficient (s), 沉降係數

When the frictional force balances the driving force, $\frac{dv}{dt} = 0$ （等速運動）

$$w^2 r(m_p - m_s) - f \cdot v = 0$$

$$S = \frac{v_t}{\omega^2 x} = \frac{m(1 - \bar{v}_2 \rho)}{f}$$

Rate of Sedimentation

where $S \equiv$ terminal velocity / unit acceleration （單位離心重力場中的沉降速度）

Sedimentation coefficients have units of sec. 10^{-13} sec is called 1 svedberg (or 1 *S*).
T. Svedberg pioneered research on sedimentation in an ultracentrifuge.

$1\ S \equiv 10^{-13}$ sec Svedberg unit

Theodor Svedberg(1884-1971)，出生於瑞典
1926 Nobel prise

1908, 發明超高速離心機並用於研究高分散膠體物質

$$F_{cent} = M\omega^2 r$$

$$F_{buoy} = M\omega^2 \not{r} \bar{v}_b \rho$$

$$F_{frict} = fv$$

$$F_{cent} = F_{buoy} + F_{frict}$$

M : *mass*

ω : angular velocity

r : distance form center of rotation

ρ : solvent density

$\bar{v}_b$: particle specific volume of particle

f : frictional coefficient

v : velocity of particle

$$v = M\omega^2 r(1 - \bar{v}\rho) / f$$

$$S = v / \omega^2 r$$ S: 離心重力場中的沉降速度

$$S = M(1 - \bar{v}_b \rho) / f$$

$$S = \frac{v_t}{\omega^2 x} = \frac{m(1-\bar{v}_2\rho)}{f}$$

M= particle mass

f = frictional coefficient of the particle in the solvent

ρ= density of solution

v = particle velocity

$\overline{\upsilon_2}$ particle specific volume (cm^3/g, 分析物密度倒數)

- S is increased for particle of larger mass

(because sedimenting force a M(1-*v*r)

- S is increased for particle of larger density (equal volume)
- S is increased for more compact structures (Shape) of

equal particle mass (frictional coefficient is less)

- S is increased with rotational speed

Mild, non-denaturing procedure is useful for protein purification, and for intact cells and organelles

$$S = \frac{v_t}{\omega^2 x} = \frac{m(1 - \bar{v}_2 \rho)}{f}$$

M= particle mass

f = frictional coefficient of the particle in the solvent

ρ= density of solution

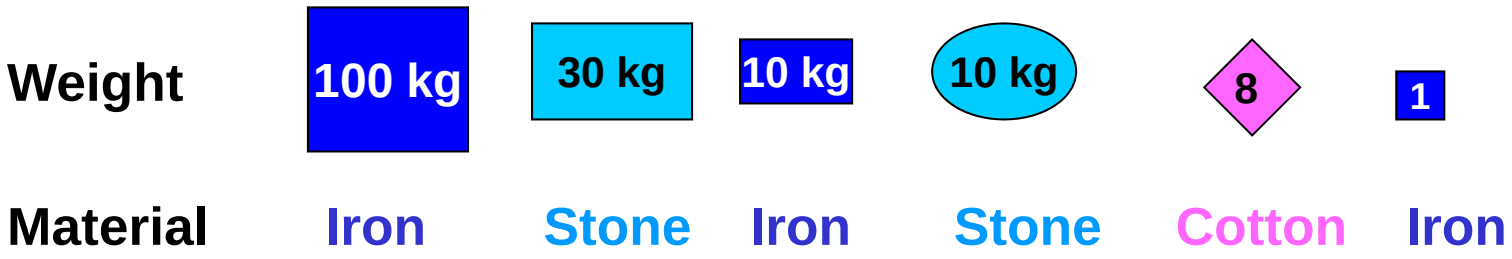
v = particle velocity

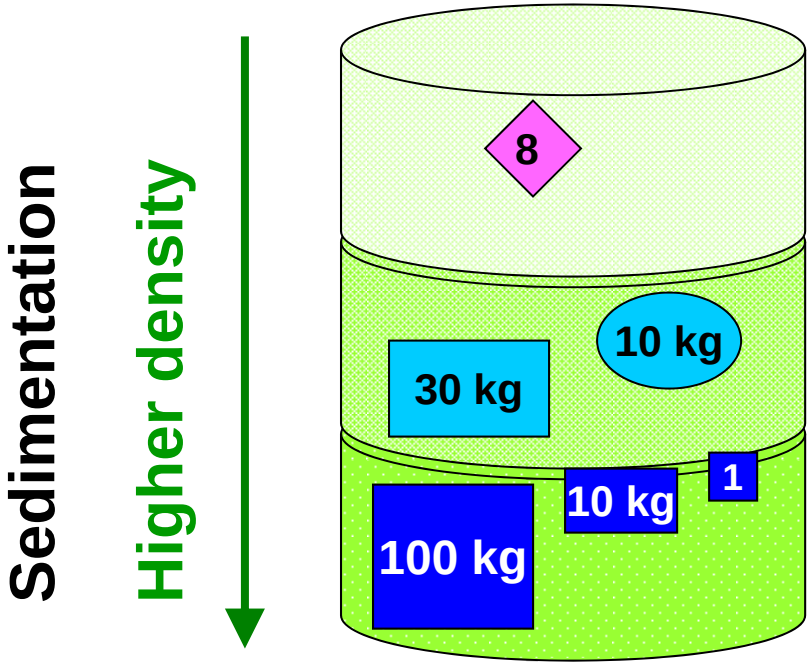
υ_2 particle specific volume (cm^3/g, 分析物密度倒數)

當 $\upsilon_2 \rho$ **<1** (分析物密度>溶液密度），分析物會沉降。

當 $\upsilon_2 \rho$ **=1** (分析物密度=溶液密度），分析物不再沉降。
無論離心力多大,

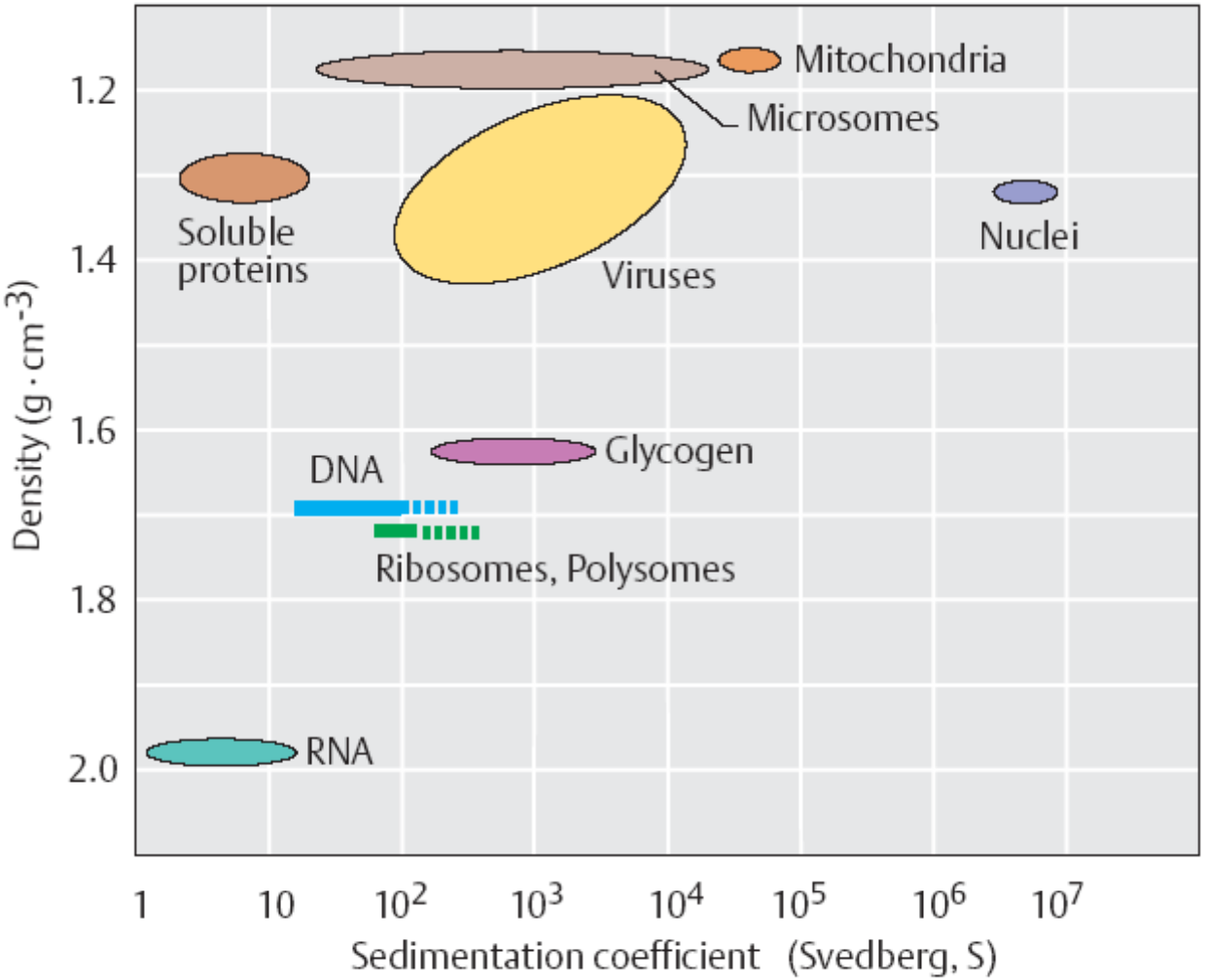
Separation by Sedimentation





- Mass
- Density
- Shape

Densities and sedimentation coefficients for biomolecules, cell organelles, and viruses.

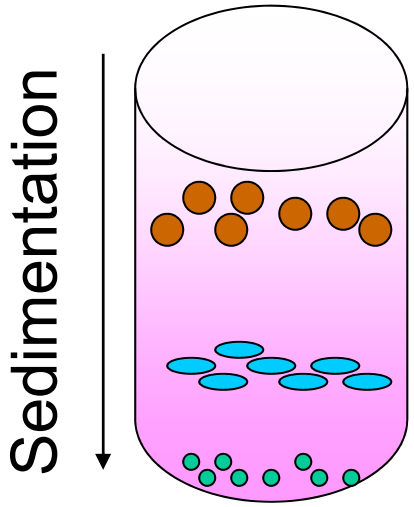


$$S = \frac{v_t}{\omega^2 x} = \frac{m\left(1 - \bar{v}_2 \rho\right)}{f}$$

Require high density media

High concentrated CsCl

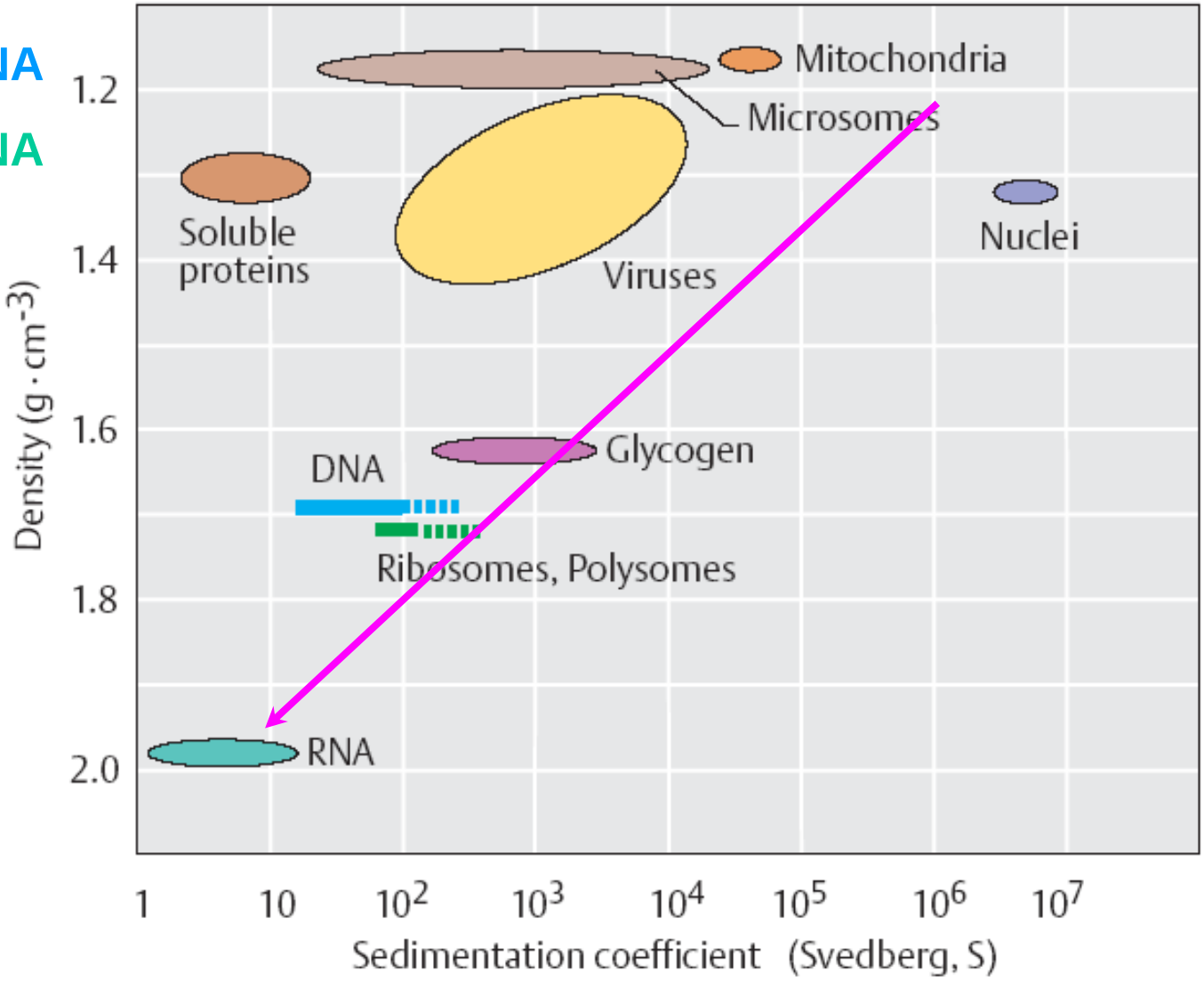
Koolman, Color Atlas of Biochemistry, 2nd edition © 2005 Thieme

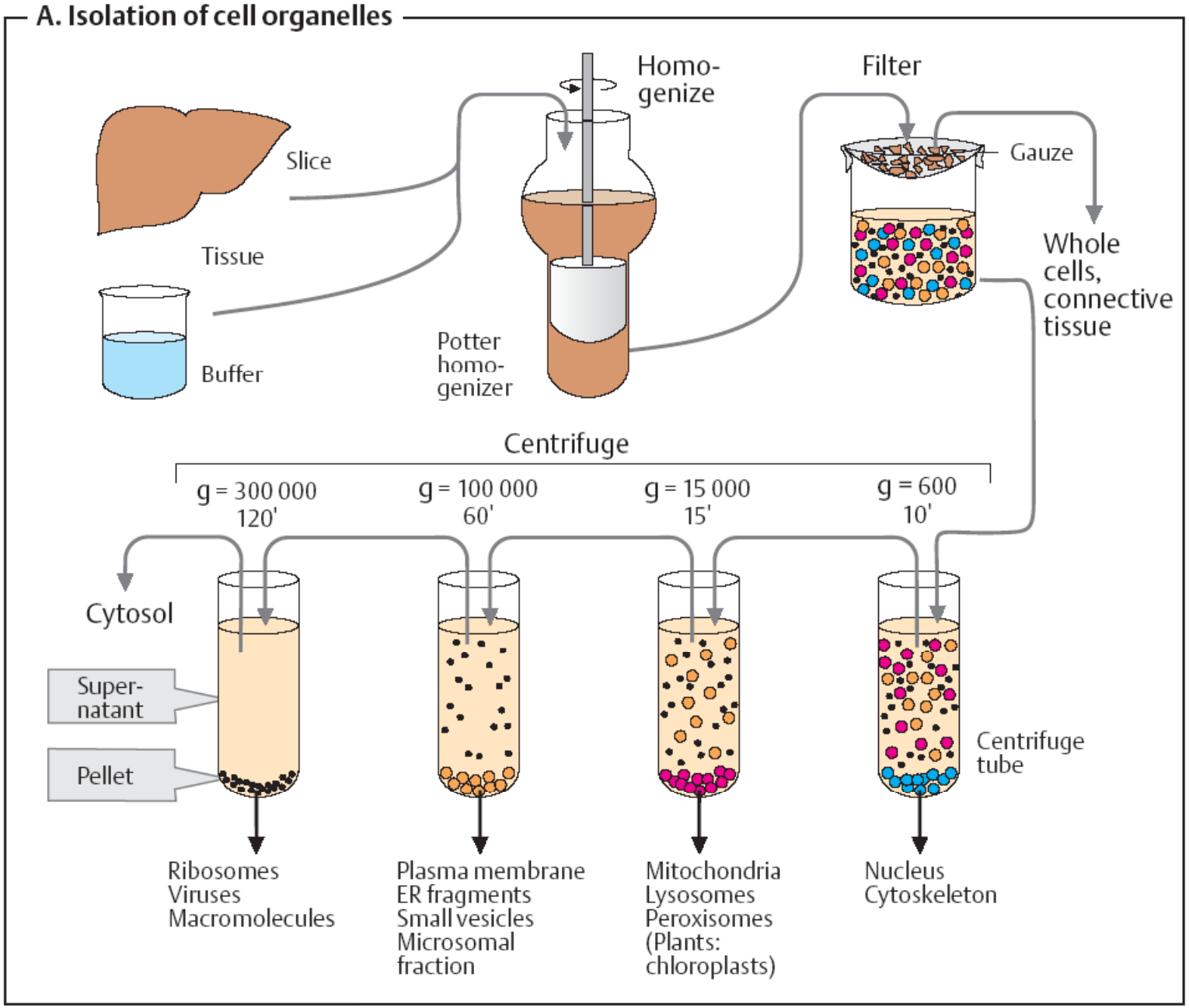


Soluble protein

DNA

RNA





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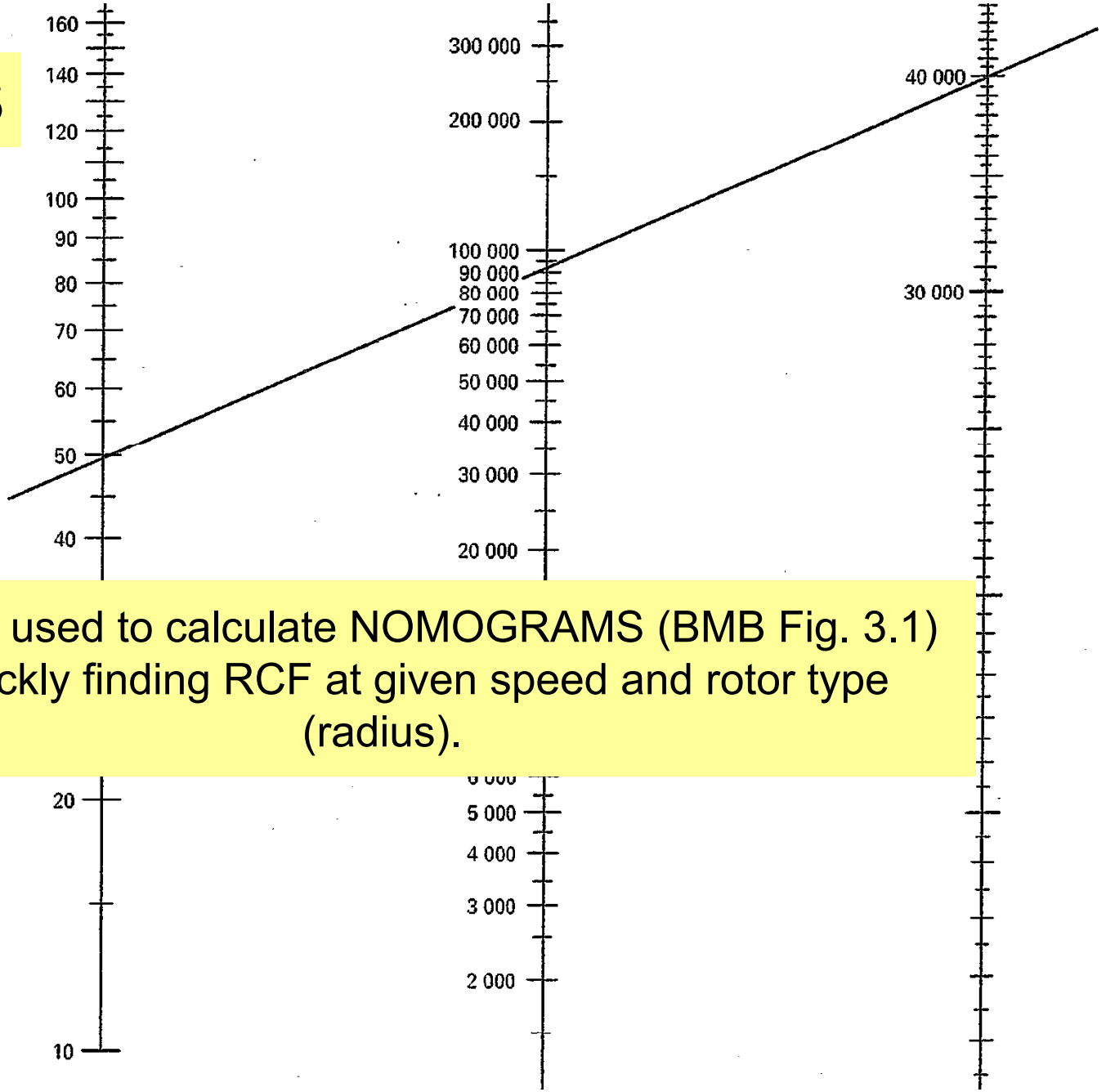
NOMOGRAMS

Conversion between relative centrifugal force



Equation used to calculate NOMOGRAMS (BMB Fig. 3.1) for quickly finding RCF at given speed and rotor type (radius).

Radical distance (mm)

Relative centrifugal field (xg)

Rotor speed (r.p.m)

Types of Centrifuge BMB 3.3.1

- Maximum speed of sedimentation
- Presence /absence of vacuum
- Temperature control refrigeration)
- Volume of sample and capacity of centrifugation tubes

- Microfuge

  0.5-1.5 cm^3, 10,000 g

  Concentration of protein samples

- Large-capacity preparative centrifuge

  5-250 cm^3, 3,000-7,000 g

H2500R-2

- High-speed refrigerated centrifuge

  5-250 cm^3, 100,000 g

Differentiation separation of nucleus, mitochondrial, protein precipitate, large intact organelle, cellular debris

- Ultracentrifugation

  5-250 cm^3, 600,000 g

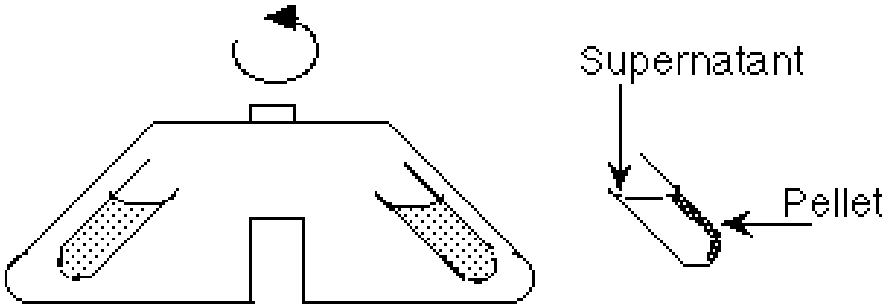
Microsomal vesicles, ribosome

Has to reduce excessive rotor temperature generated by **frictional resistance**

→ **sealed chamber, evacuated, cooling**

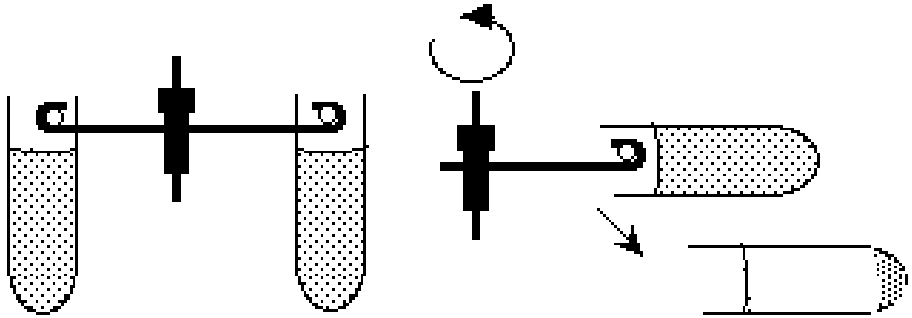
Centrifuge Rotors (MBM3.3.2)

Fixed Angle Rotor



Sedimenting particles have only short distance to travel before pelleting. **Shorter run time.**
The most widely used rotor type.

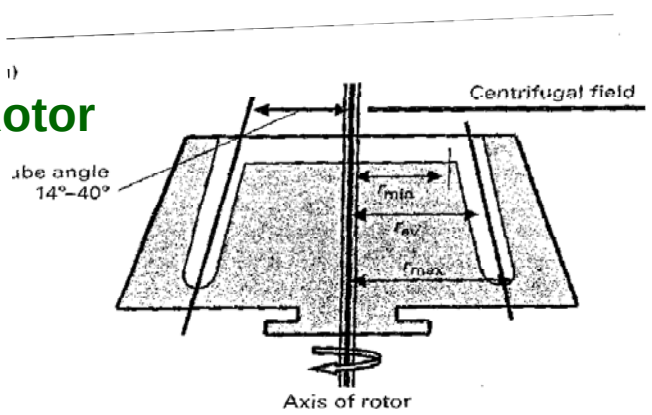
Swinging Bucket Rotor

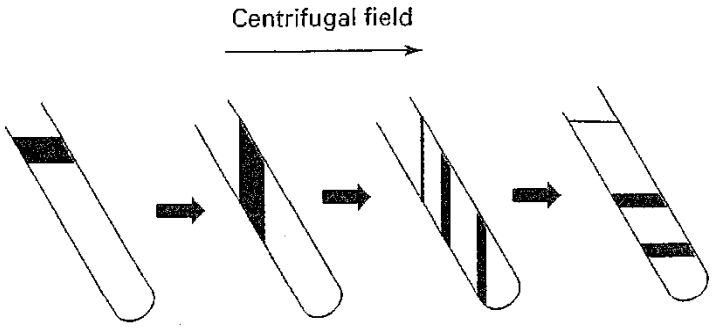


Longer distance of travel may allow better separation, such as in density gradient centrifugation. Easier to withdraw supernatant without disturbing pellet.

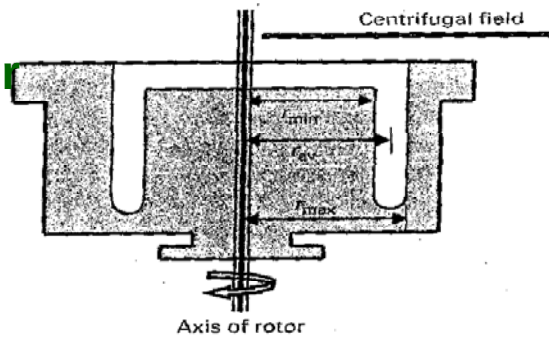
Centrifuge Rotors (MBM3.3.2)

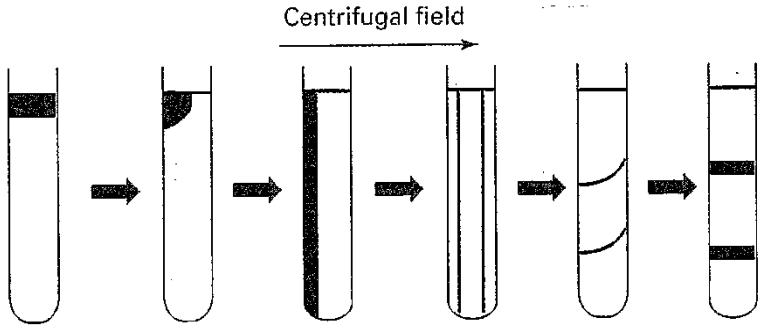
- **Fixed Angle Rotor**



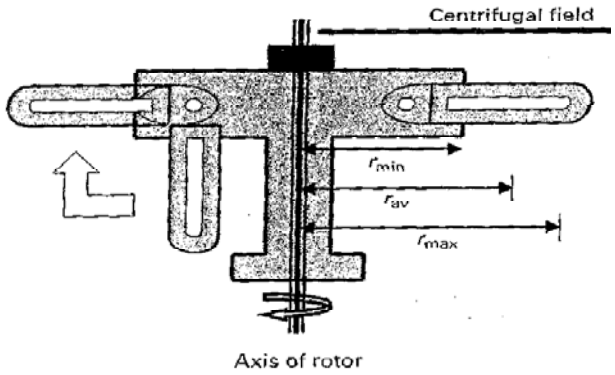


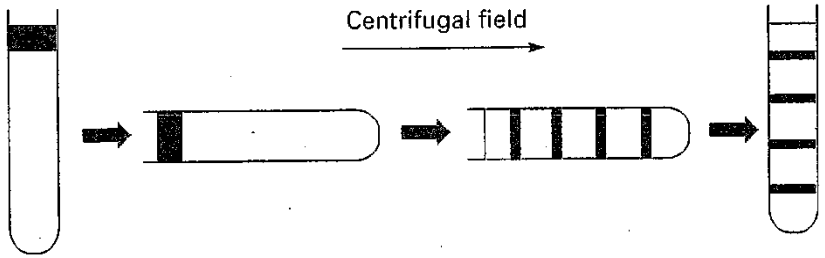
- **Vertical Tube Rotor**





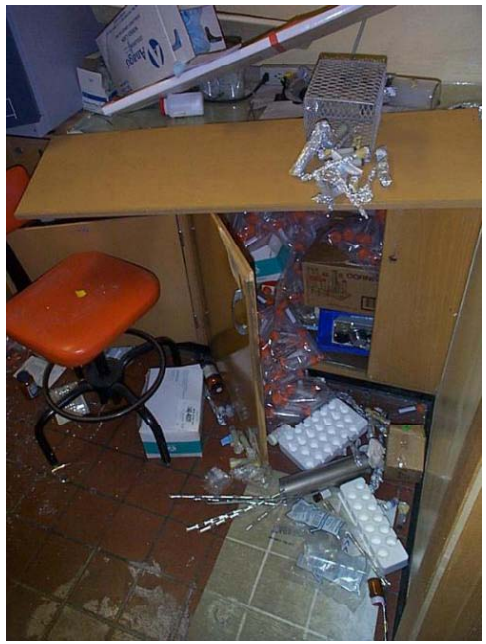
- **Swinging Bucket Rotor**





Centrifuge Its Use and Safety (BMB 3.3.4)

On December 16, 1998, milk samples were running in a Beckman L2-65B ultracentrifuge using a large aluminum rotor . The rotor failed due to excessive mechanical stress



Mechanical stress

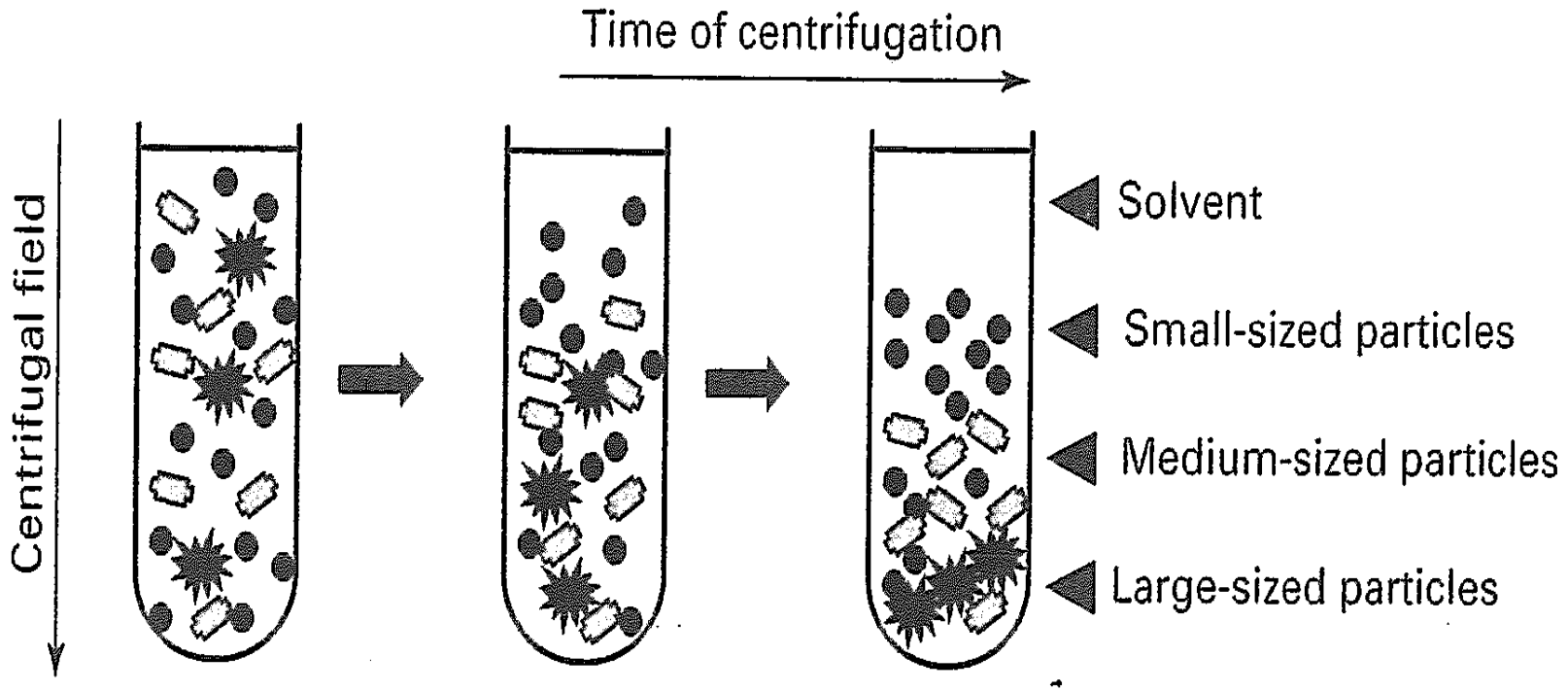
- Always ensure that loads are evenly balanced before a run.
- Always observe the manufacturers maximum speed and sample density ratings.
- Always observe speed reductions when running high density solutions, plastic adapters, or stainless steel tubes.

Corrosion

- Many rotors are made from either titanium or aluminum alloy, chosen for their advantageous mechanical properties. While titanium alloys are quite corrosion-resistant, aluminum alloys are not. When **corrosion** occurs, the metal is weakened and less able to bear the stress from the centrifugal force exerted during operation. The combination of stress and corrosion causes the rotor to fail more quickly and at lower stress levels than an uncorroded rotor

Differential Centrifugation BMB 3.4.1

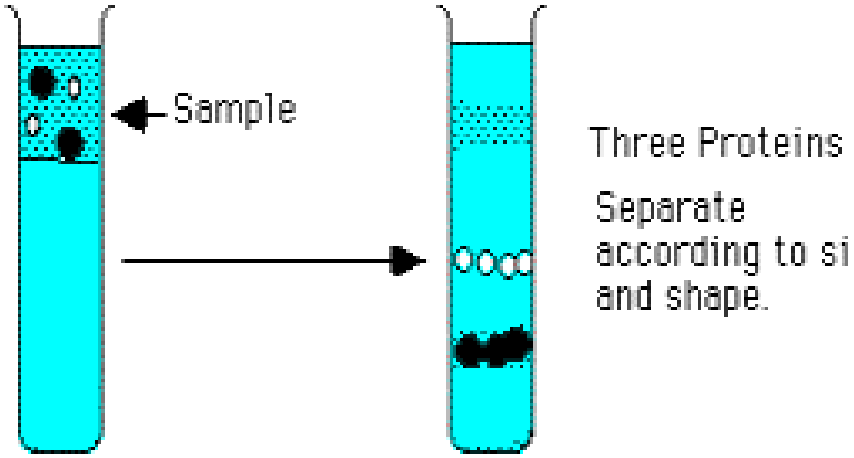
- Based on the differences in the **sedimentation rate** of the biological particles of different size, shape and density



Moving Zone (differential) Centrifugation

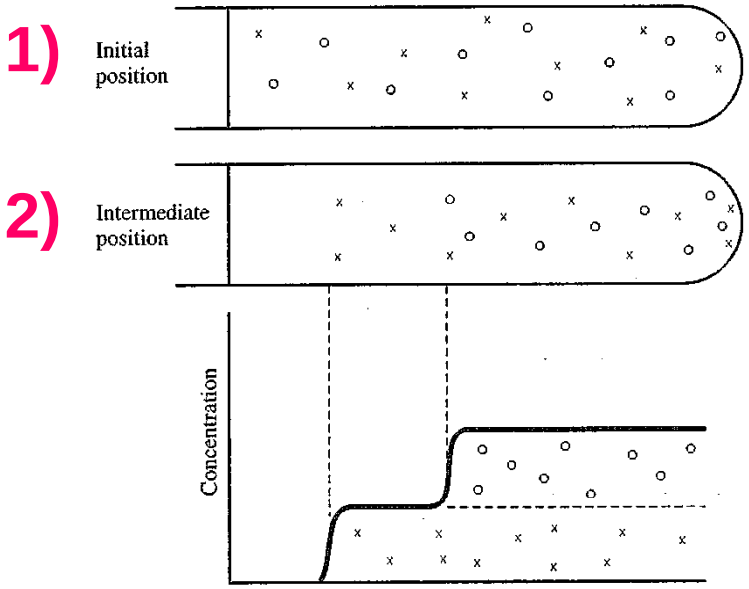
移動區帶離心法–Rate

Figure 3b: Rate zonal centrifugation.



- Incomplete sedimentation (lower operation speed).
- Control time and solution density
- Mostly used for separation with similar density and different size/shape

Moving Boundary (differential) Centrifugation



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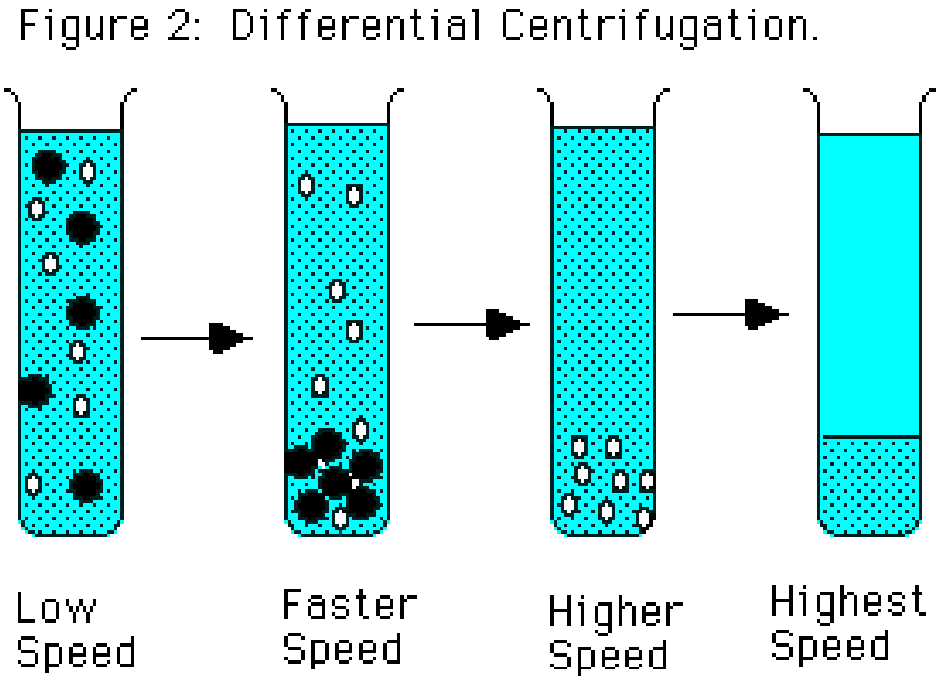
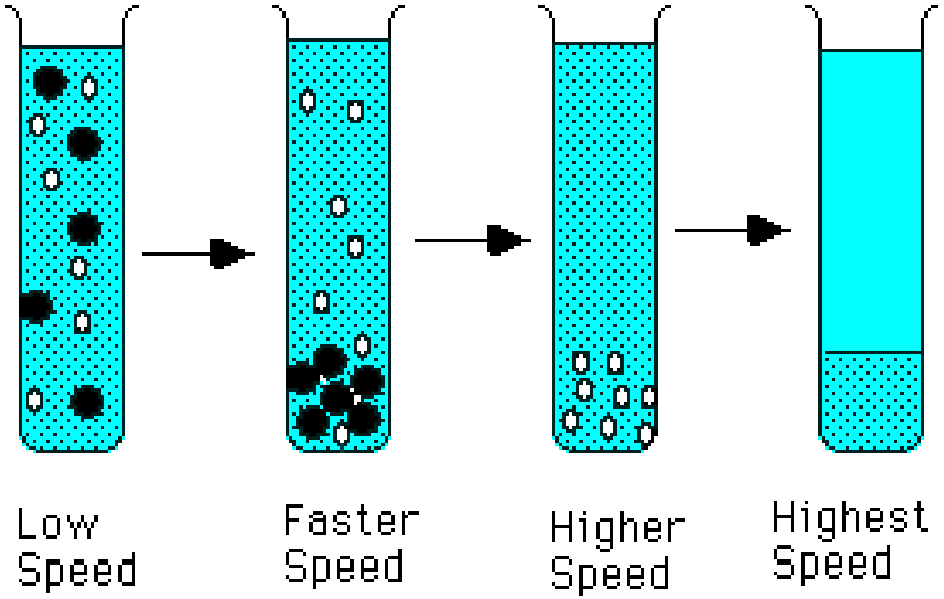


1) The entire tube is **filled with sample** and centrifuged
2) Through centrifugation, one obtains a separation of two particles but any particle in the mixture may end up in the **supernatant** or in the **pellet** or it may be distributed in both fractions, depending upon its **size, shape, density, and conditions of centrifugation**
3) Repeat sedimentation at **different speed**

Moving Boundary (differential) Centrifugation

Figure 2: Differential Centrifugation.



- Advantages: Large-scale preparation
- Disadvantages:

  Poor resolution. Poor purity

  Difficult to separate analytes with similar sedimentation coefficient

Density Gradient Centrifugation (BMB 3.4.2)

- Important technique for purifying **proteins** and particularly **nucleic acids**.

Two different types of density gradient centrifugation, for two different purposes are:

- **Zonal (or Rate Zonal) Centrifugation**
(Sucrose density gradient centrifugation)

- **Iso-density (Isopycnic) Centrifugation**
(Caesium chloride density gradient centrifugation)

Moving Zone (differential) Centrifugation

移動區帶離心法–Rate

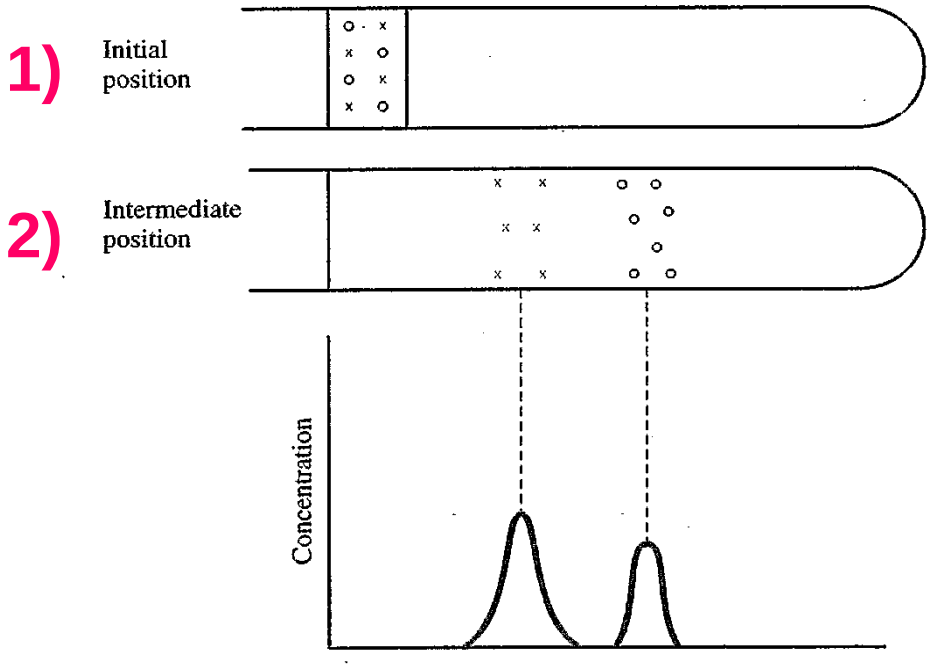
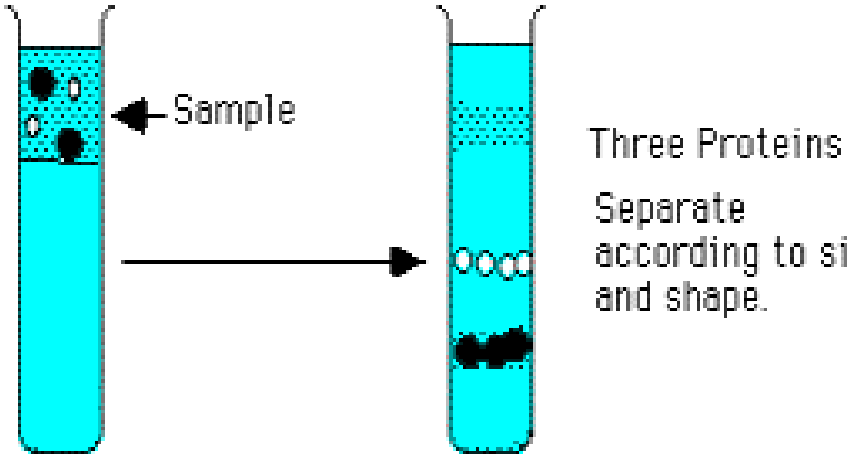


Figure 3b: Rate zonal centrifugation.



1) Sample is applied in a thin zone at the **top of the centrifuge tube** on a **density gradient solution**

2).Under centrifugal force, the particles will begin sedimenting through the gradient in separate zones **according to their size, shape and density**

Iso-density (Isopycnic) Centrifugation (AB3.4.3)

等密度平衡離心法-equilibrium

Isopycnic = same density
= density equilibrium between analyte and solution

Both analytes and solution have different densities

1) Preparation of solution with different densities

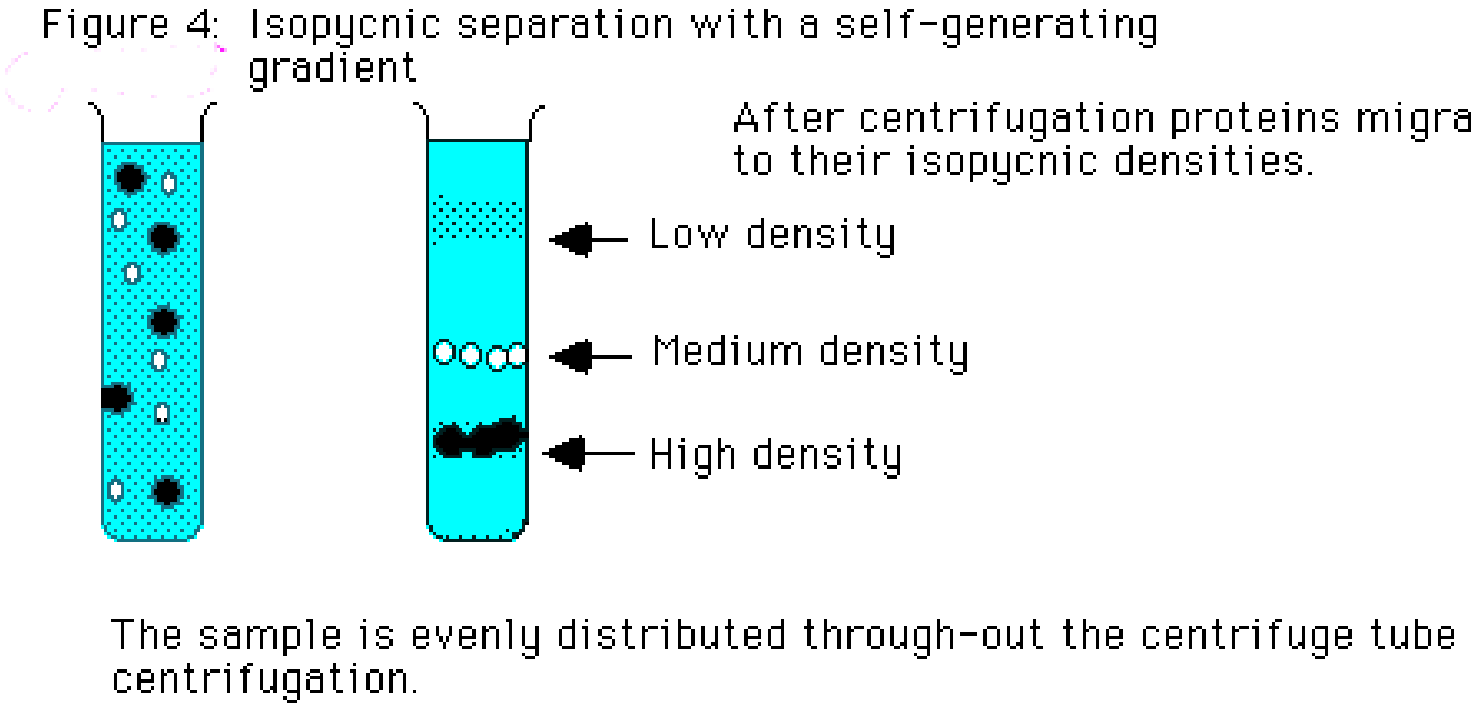
E.g. sucrose 蔗糖:

- Good water solubility for making high concentration of solution
- Cheap

Iso-density (Isopyncic) Centrifugation (AB3.4.3)

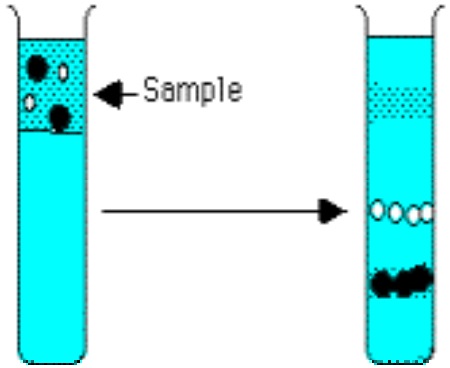
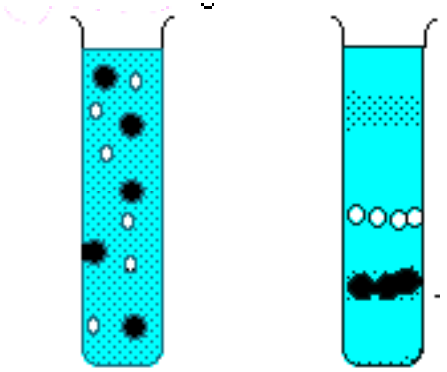
等密度平衡離心法-equilibrium

2). molecule floats or sinks to position where density equals density of CsCl solution. Then no net sedimenting force on molecules.
and separation is on basis of different densities of the particles.

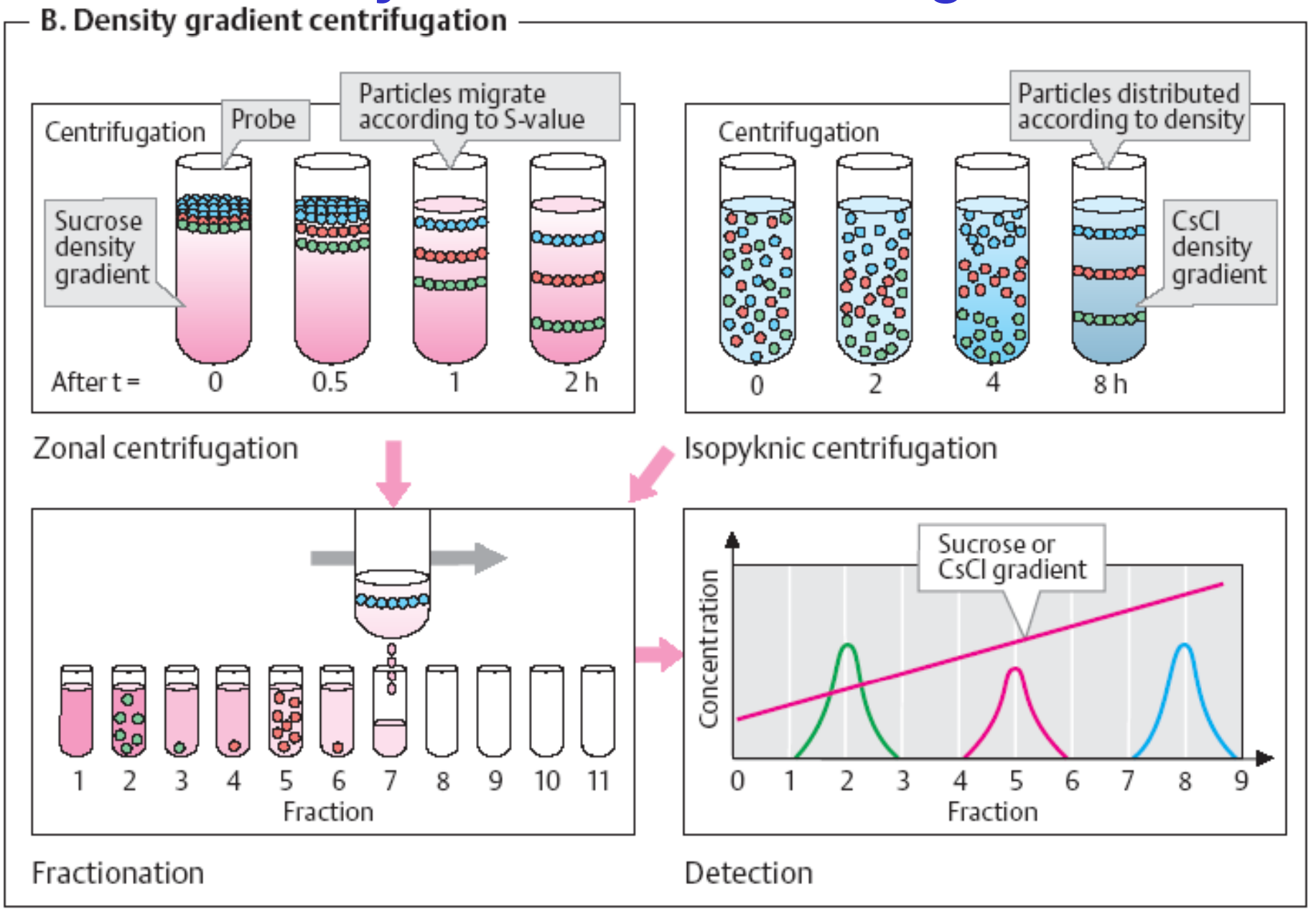


- Molecules separated on equilibrium position, NOT by rates of sedimentation.

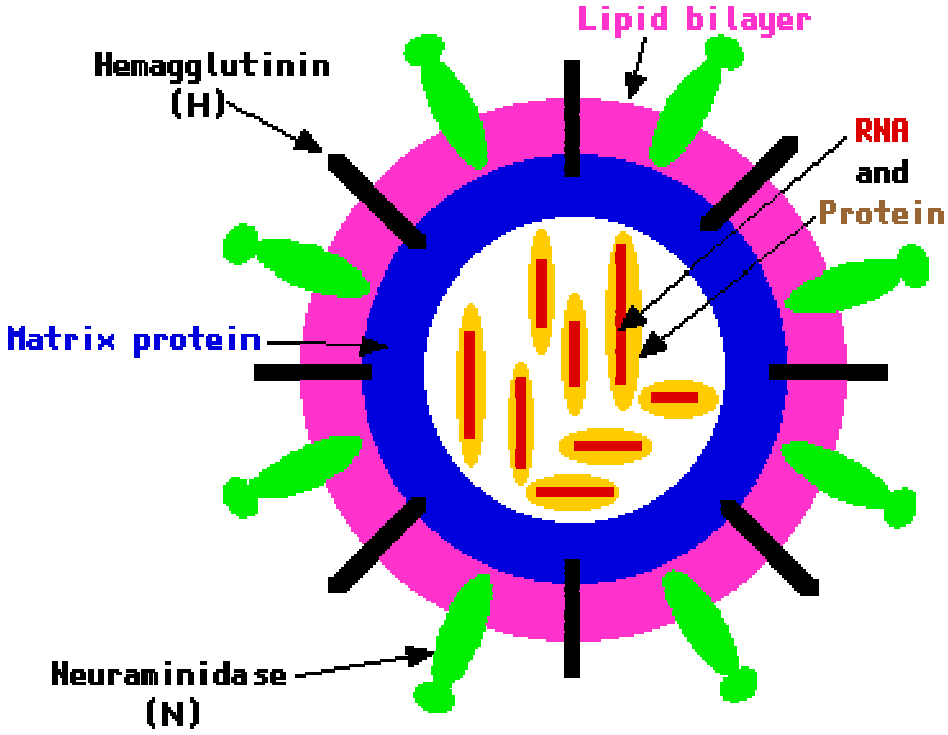
Comparison of Two Methods

	Moving Zone Centrifugation	Isopyncic centrifugation
		
Centrifugation:	**Lower speed**, not complete sedimented, stop at proper time	Completely sediment to where the density is equilibrated, **high speed**, long running time
	Sedimentation Rate	**Sedimentation equilibrium**
Sample:	**Similar density, different MW/shape**	**Similar MW, different density**
	Protein (similar density, but different in MW)	Nucleic acid / cell organelle

Density Gradient Centrifugation



The Influenza A Virus



a globular particle (about 100 nm in diameter)

sheathed in a lipid bilayer (derived from the plasma membrane of its host)

Studded in the lipid bilayer are two integral membrane proteins

some 500 molecules of **hemagglutinin** ("H") and

some 100 molecules of **neuraminidase** ("N")

Within the lipid bilayer are

some 3000 molecules of **matrix protein**

8 pieces of RNA

Each of the 8 RNA molecules is associated with

(1) many copies of a nucleoprotein

(2) several molecules of the three subunits of its RNA polymerase

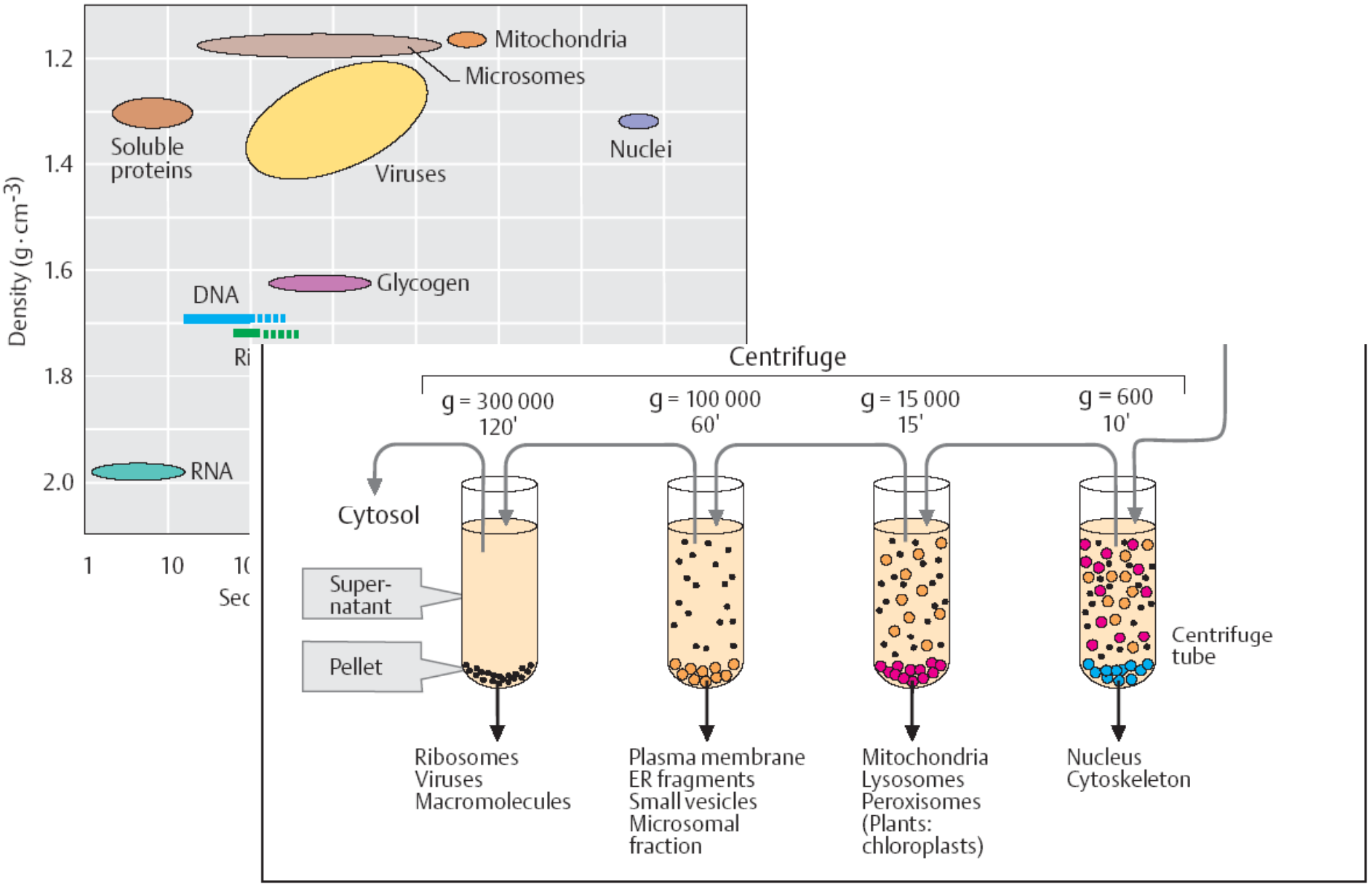
(3) some "non-structural" protein molecules of uncertain function

Virus Purification

Four commonly used methods

- **Differential centrifugation and density gradient centrifugation**
- Precipitation of viruses
- Denaturation of contaminants
- Enzymatic digestion of cell constituents

How to purify virus?



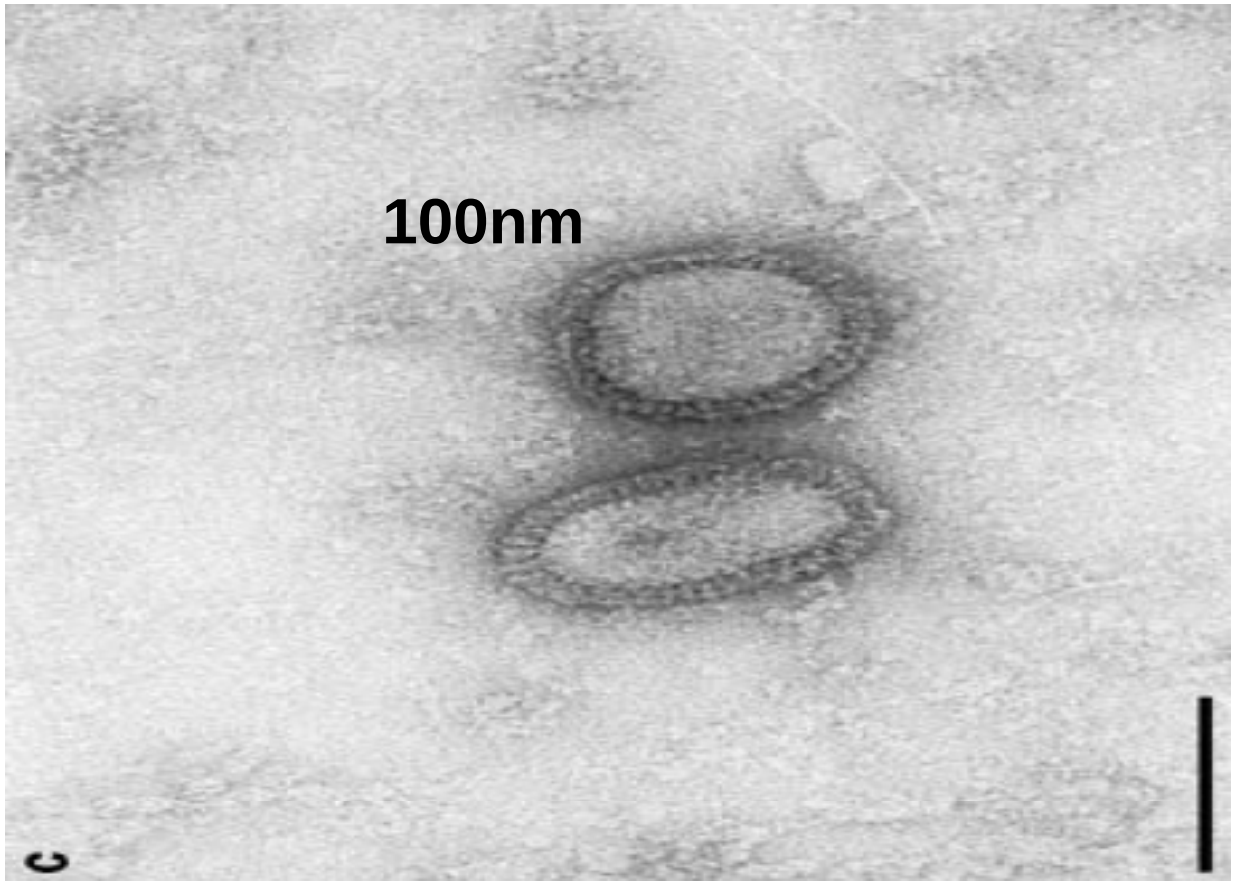
Purification of influenza A virus (H1N1) by Density Gradient Centrifugation

1. After 48 hours of incubation the allantoic fluid containing virus was purified by low speed centrifugation (4000 rpm 40 min).
2. Virus was pelleted by high speed centrifugation at 18000rpm for 1.5 h in the SW27 rotor. The virus pellet was collected, diluted in STE buffer and layered over linear 30 to 60 % sucrose gradient prepared in STE buffer.

   -----Isopyconic gradient centrifugation:(等密度離心):
3. Sample recentrifuged at 25000 rpm in the SW27 rotor for 2.5 h at 4 °C, then the virus band was collected, diluted with STE buffer and pelleted by centrifugation at 30000 rpm for 1.5 h at 4 °C. The pellet of
4. virus was collected and diluted with STE buffer.

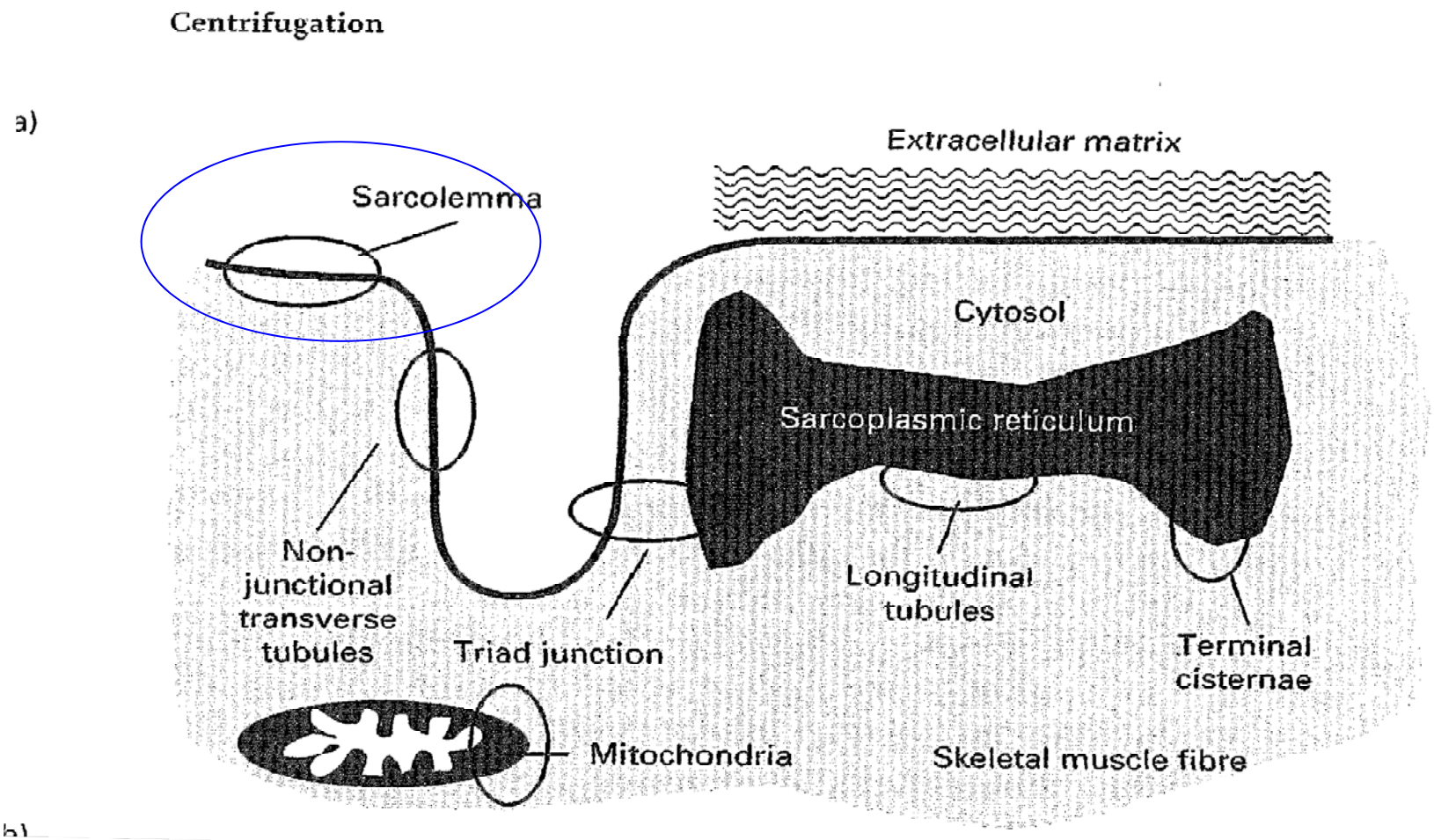
BioMarket Ltd. www.biomarket.fi

Virus Visualization by Electron Microscopy

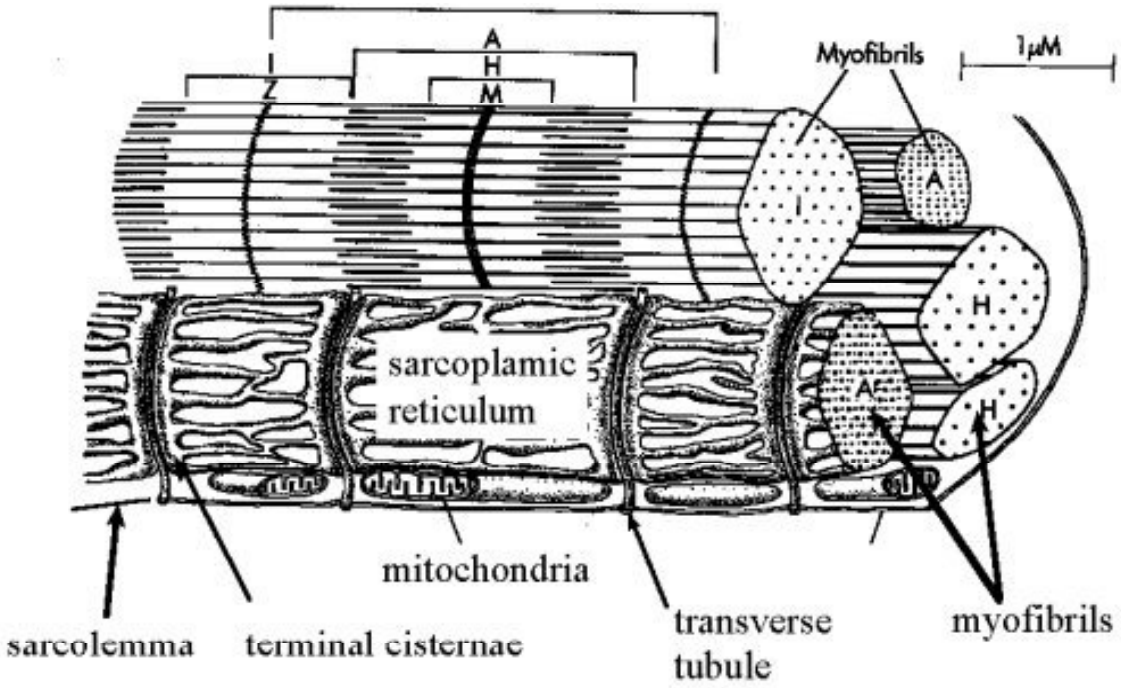


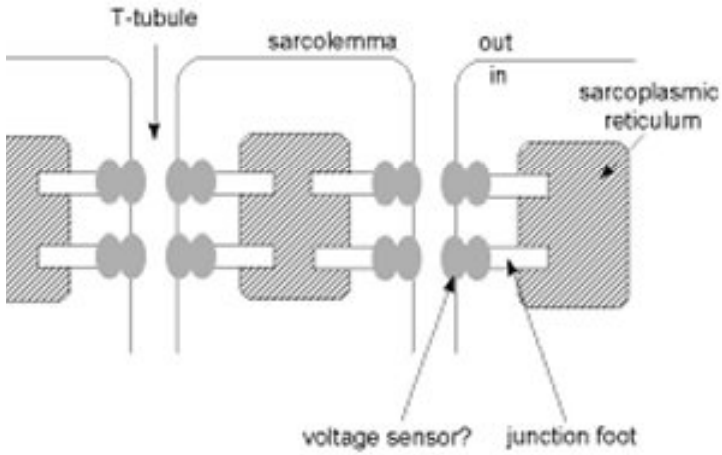
Sarcolemma (肌纖維膜）：

It is the surface membrane of the entire fiber



Skeletal Muscle





Sarcolemma :It is the surface membrane of the entire fiber
(肌纖維膜, 骨骼肌細胞膜)

T-tubular membranes

They contain extracellular fluid (high in Ca and Na ions)

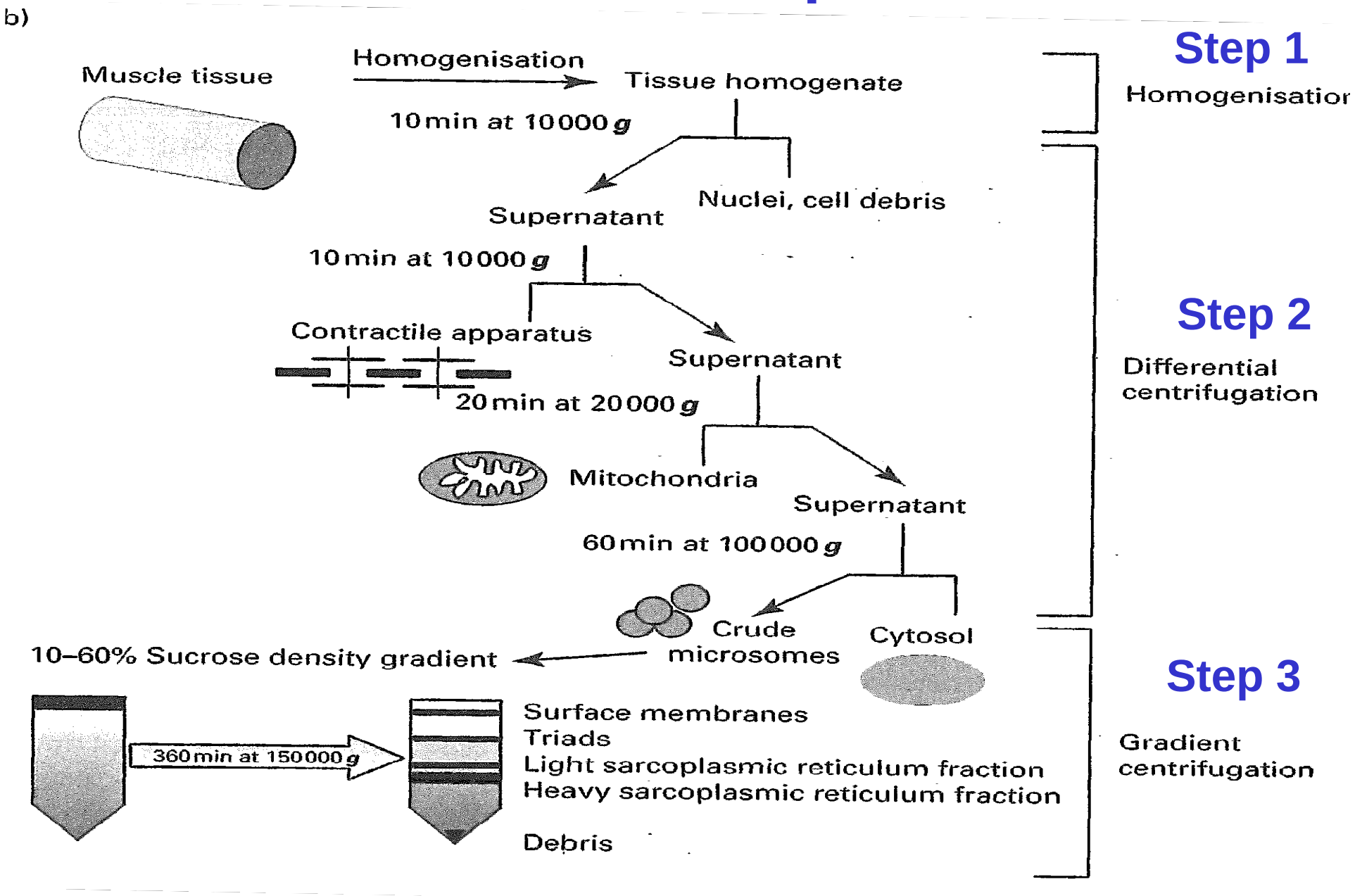
They are continuous tubes of sarcolemmal membrane that run through (transversely) the muscle fiber.

Sarcoplasmic reticulum (內質網)-\

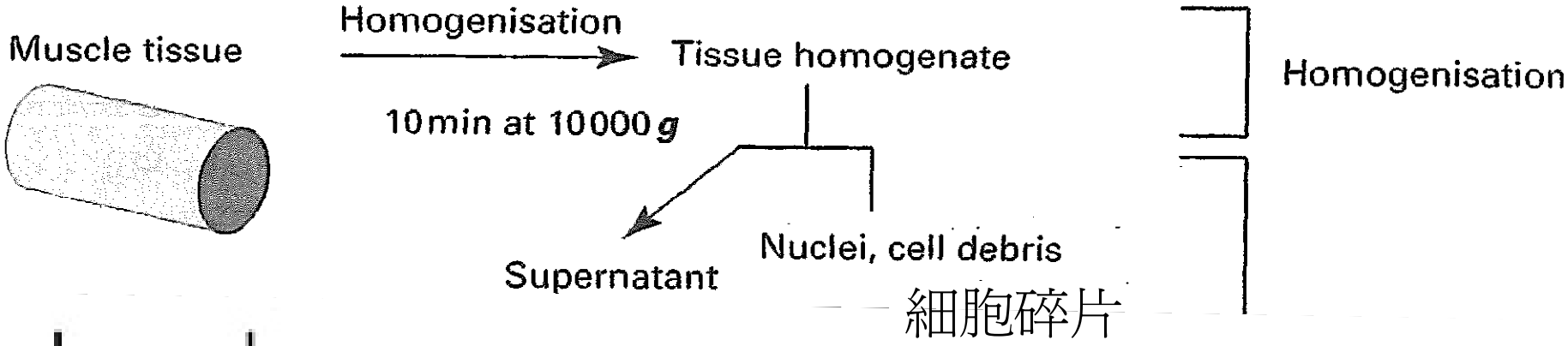
The sarcoplasmic reticlum (SR) is the Ca store.

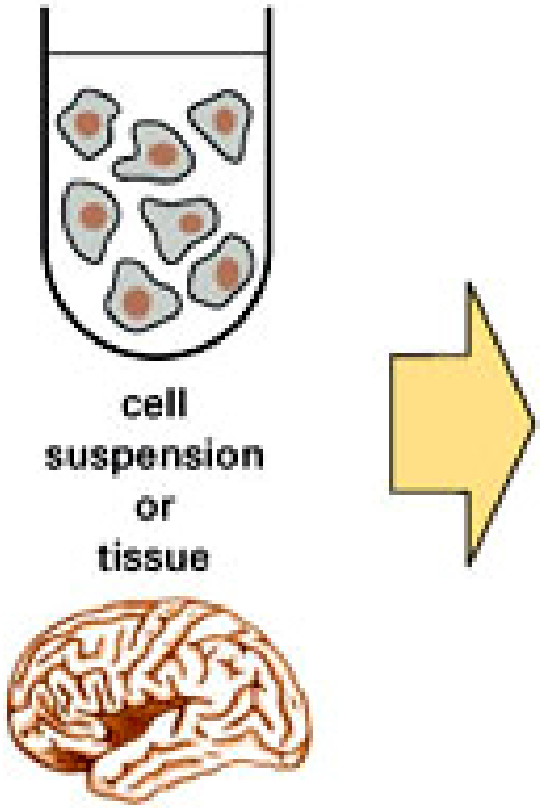
It is a diffuse membrane structure that surrounds the sarcomere

How can we do separation?



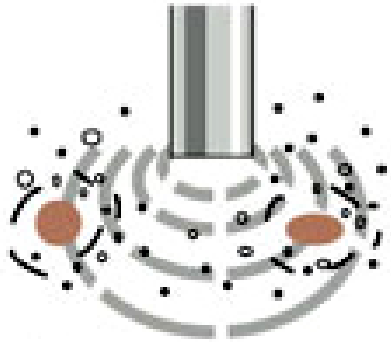
Step 1- Cell Homogenization (均質化)



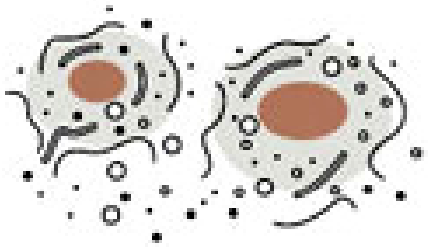


To obtain pure organelles, the cells must be ruptured, so that the cell membrane is broken, but the organelle to be studied is not. The process of rupturing a cell is known as **homogenization** of the cell and the subsequent isolation of organelles is called **fractionation.**

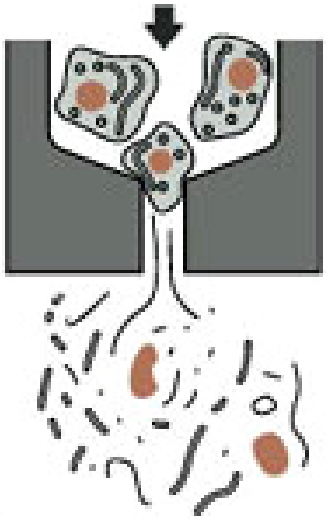
Four Common Methods



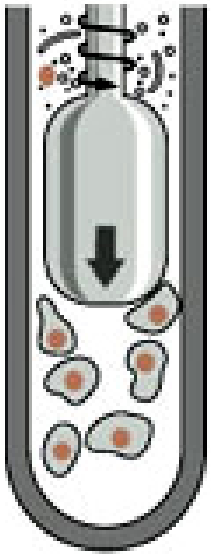
1. break cells with high frequency sound



2. use a mild detergent to make holes in the plasma membrane



3. force cells through a small hole using high pressure



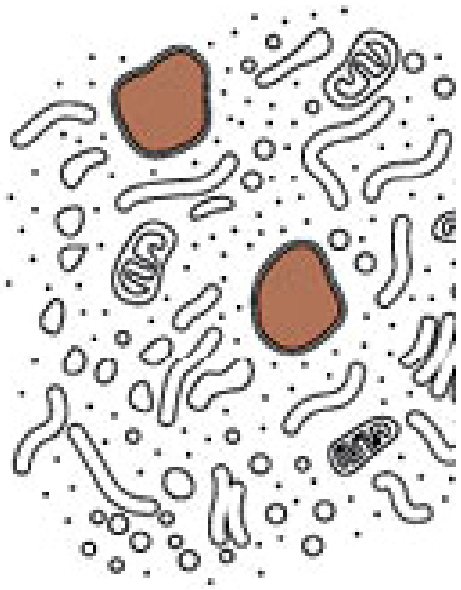
4. shear cells between a close-fitting rotating plunger and the thick walls of a glass vessel

Using gentle mechanical procedures, called **homogenization**, the plasma membranes of cells can be ruptured so that the cell contents are released.

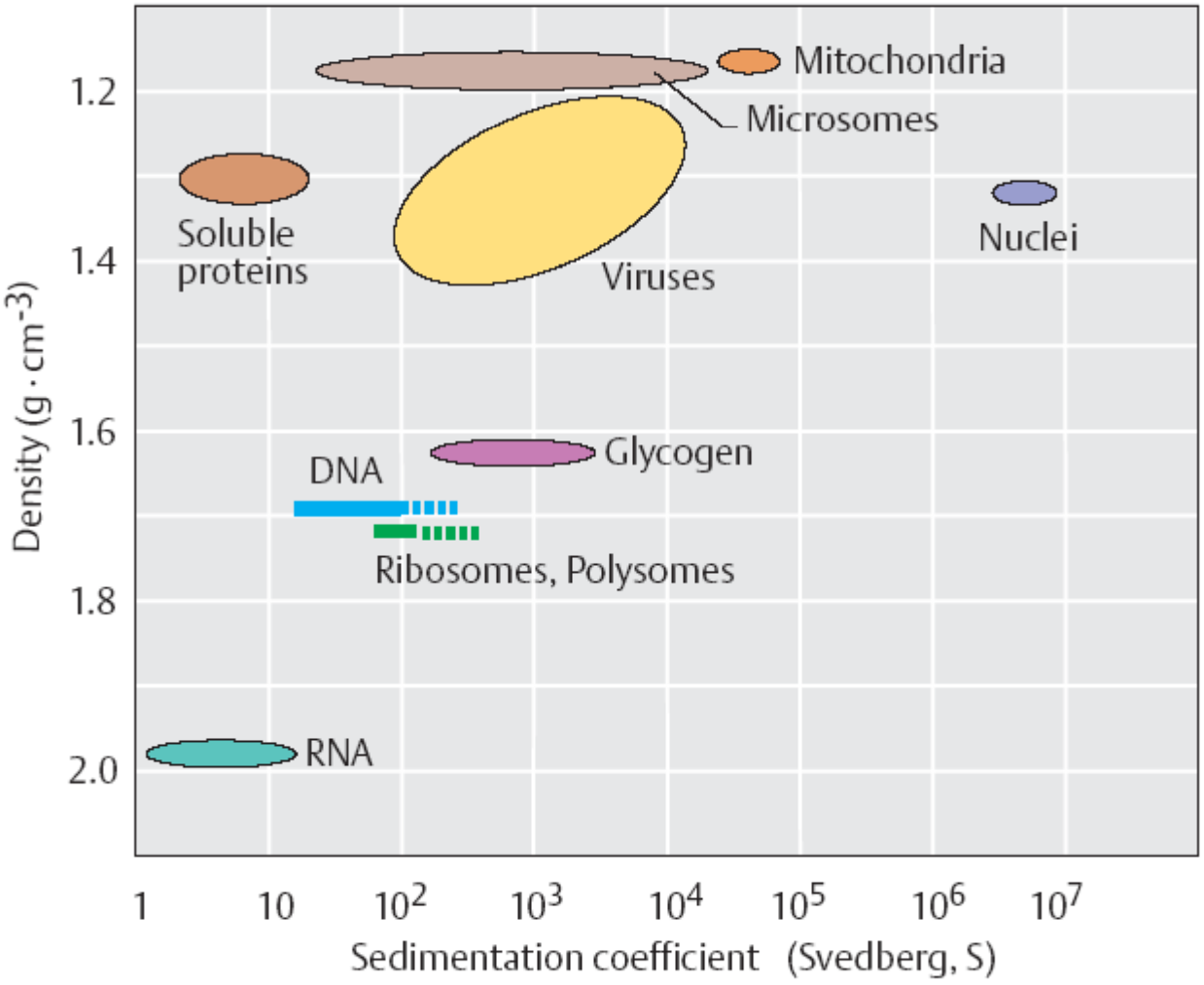
1. Sonication
2. Detergent lysis
3. French press
4. Mechanical homogenization

The resulting thick soup (called a homogenate or an extract) contains large and small molecules from the cytosol, such as enzymes, ribosomes, and metabolites, as well as all the membrane-bounded organelles.

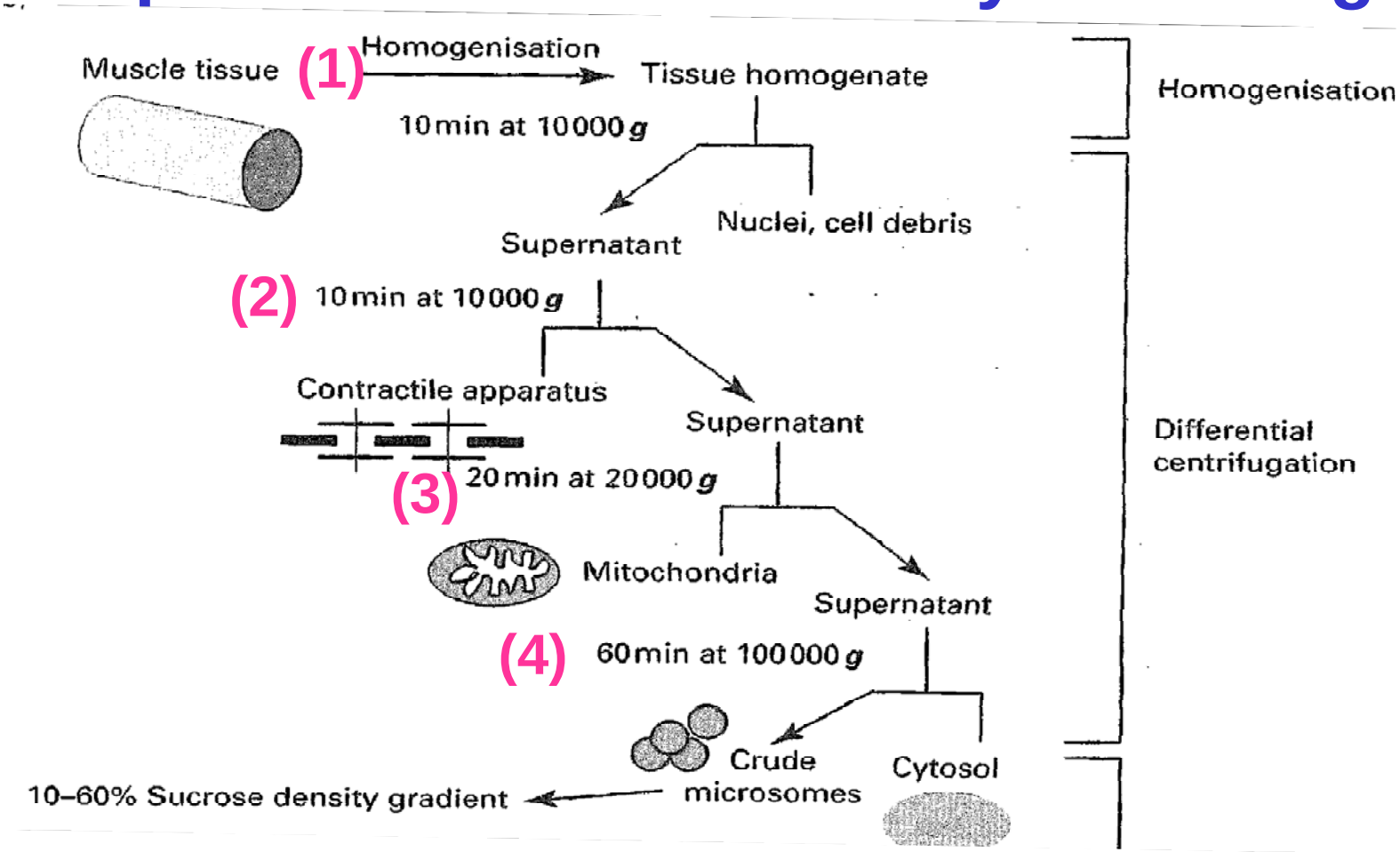
Ruptured cells producing a liquified cellular homogenate



When carefully applied, homogenization leaves mos of the membrane-bounded organelles intact.

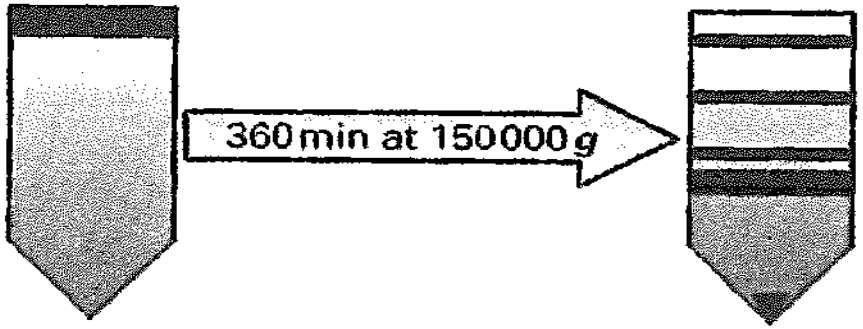


Step 2-Cell Fractionation by Centrifugation.



- Repeated centrifugation at progressively higher speeds will fractionate homogenates of cells into their components.
- In general, the smaller the subcellular component, the greater is the centrifugal force required to sediment it.

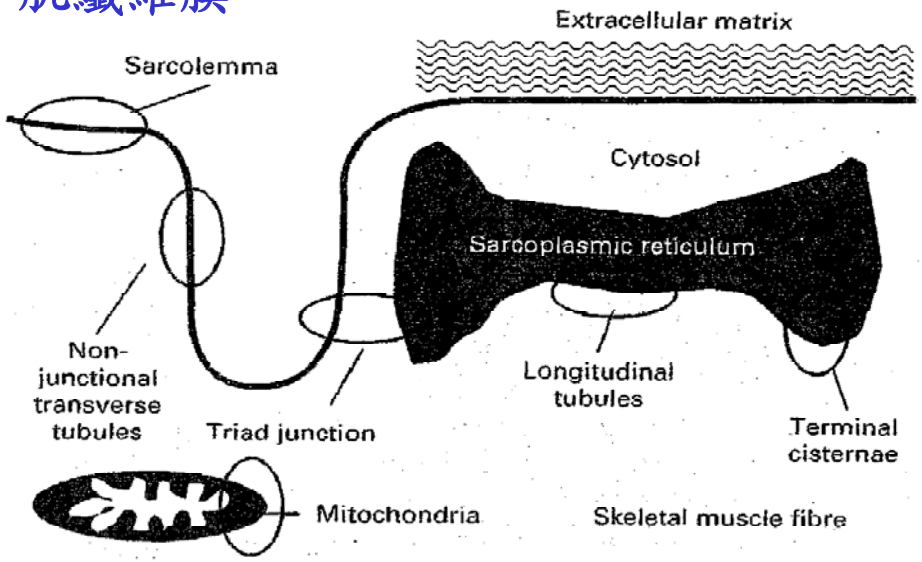
Step 3- Density Gradient Centrifugation

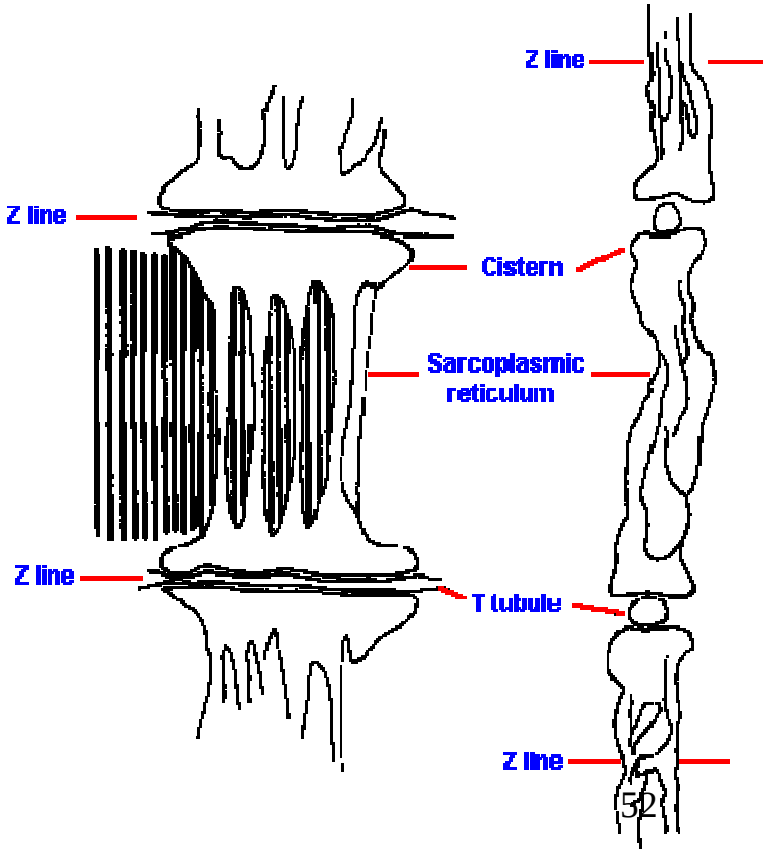


Surface membranes
Triads
Light sarcoplasmic reticulum fraction
Heavy sarcoplasmic reticulum fraction

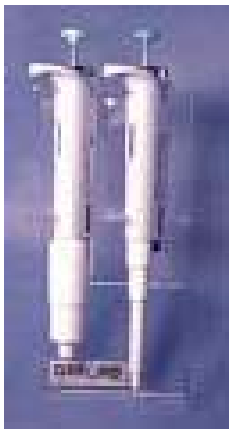
Debris

Sarcolemma
肌纖維膜

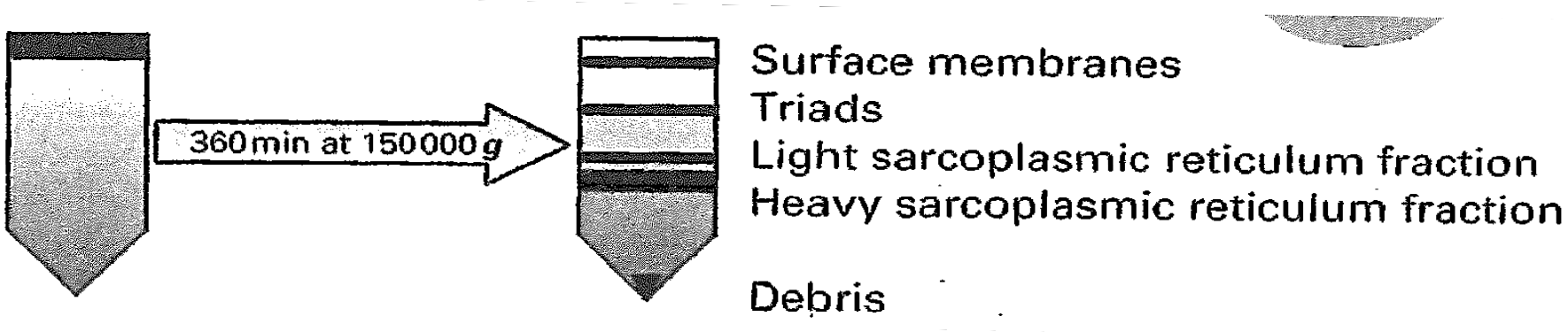




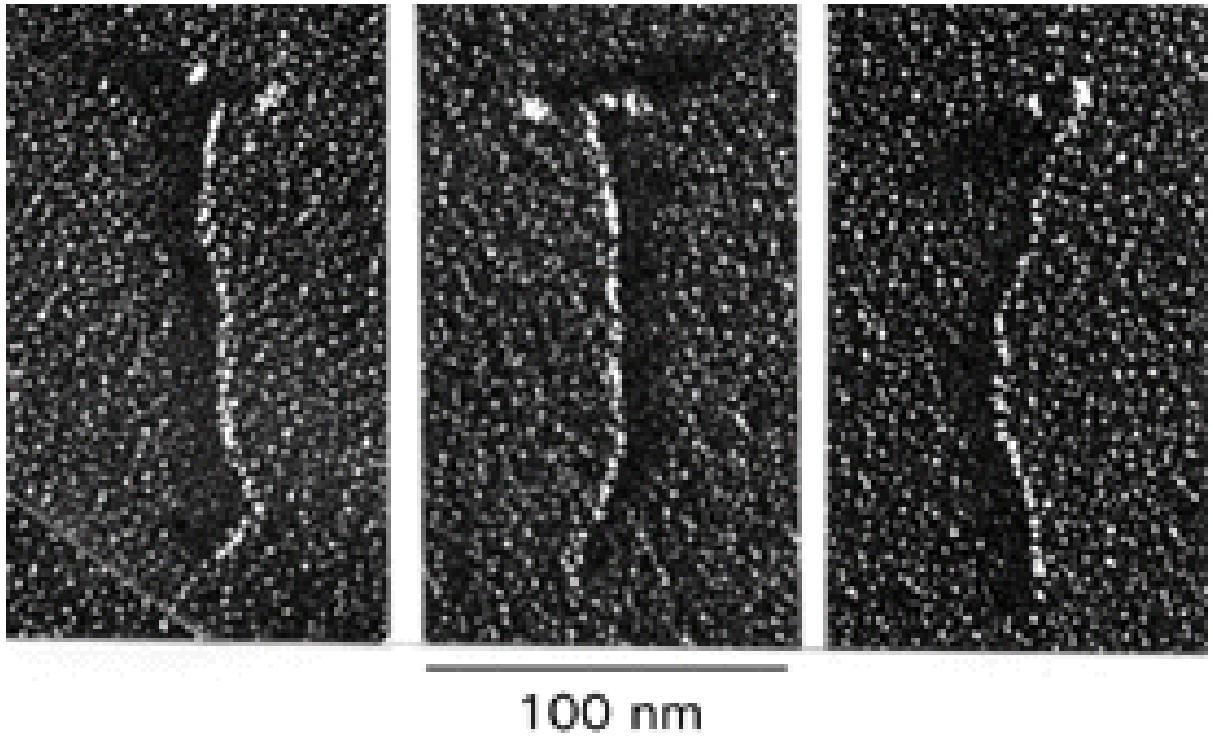
Step 4- Collection of Fractions



- Manual collection by pipette
- Automatic fraction collector for unstable gradient
- Freezing and slicing



Contractile Apparatus of Muscle



Electron micrographs of individual myosin protein molecules

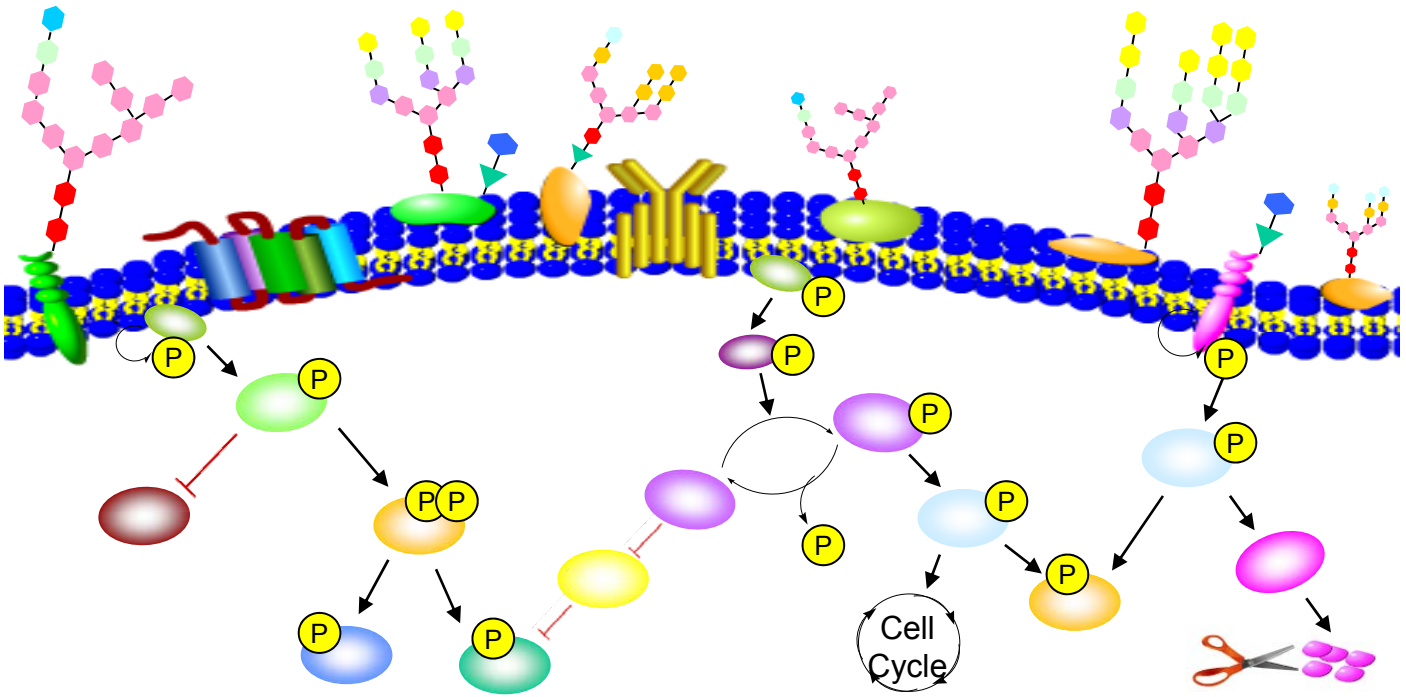
Myosin is a major component of the contractile apparatus of muscle. As shown here, it is composed of two globular head regions linked to a common rodlike tail.

Hydrophobic and Low Abundant Membrane Proteome

Immuno-modulation, Molecular recognition , Cell surface adhesion

Heterogeneous Glycosylation

Dynamic, Spacious Complexity of Phosphorylation



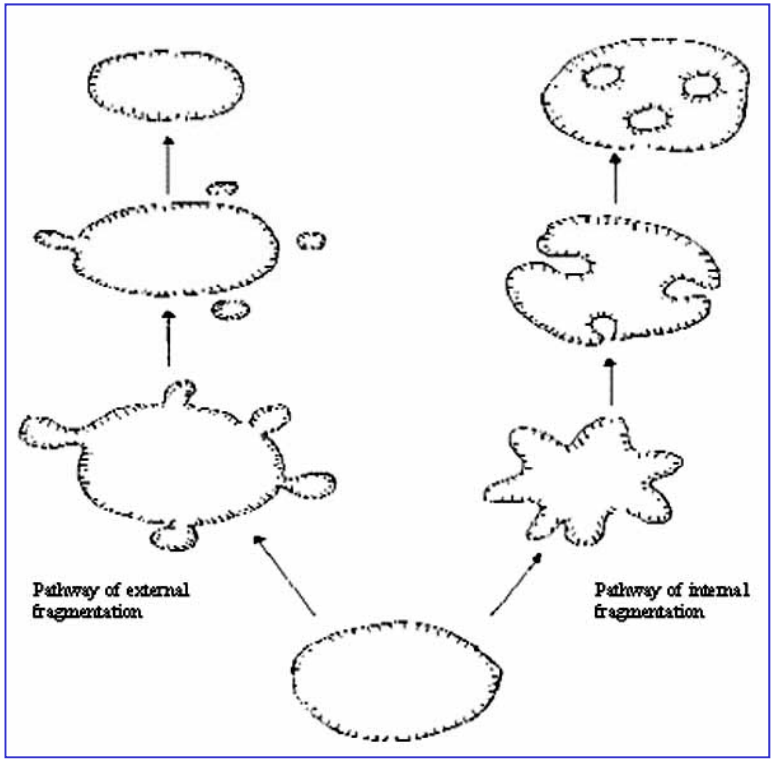
Signal transduction

Differentiation, proliferation

Protein degradation Apoptosis

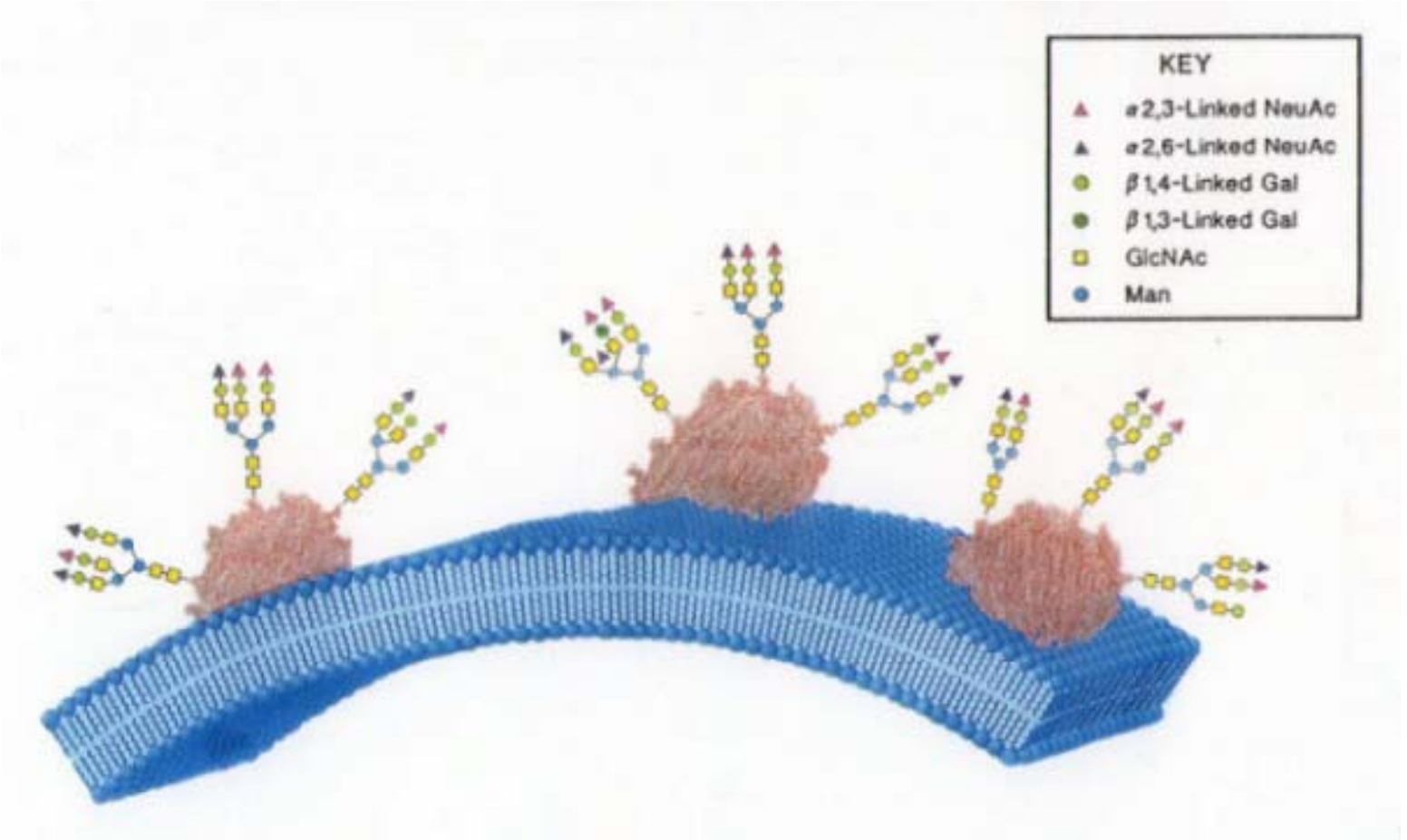
Affinity Purification of Membrane Vesicles (BMB 3.4.5)

- Cross-contamination of vesicular membrane protein
- Inside-out vesicles, right-side-out vesicle, membrane sheet, leaky vesicles
- Smaller vesicles are trapped in large vesicles



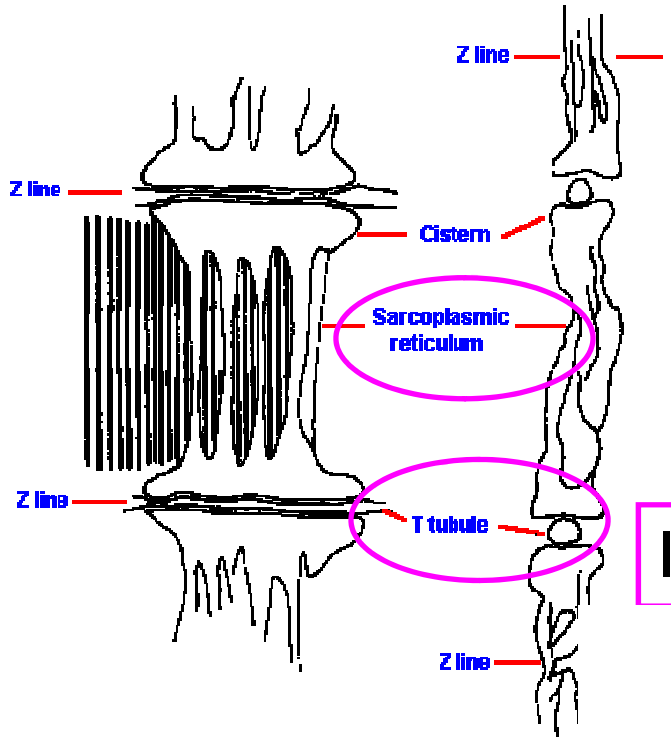
In-side-out (cytoplasmic side out)
Right-side-out (apoplastic side out) vesicles

Lectin Agglutination Method (by Lectin-carbohydrate Interaction)



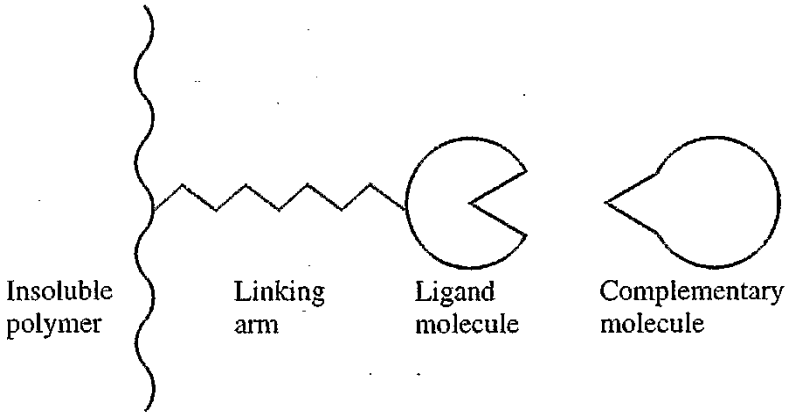
Lectin: protein that interact with carbohydrate

There are many carbohydrates on the surface of cell

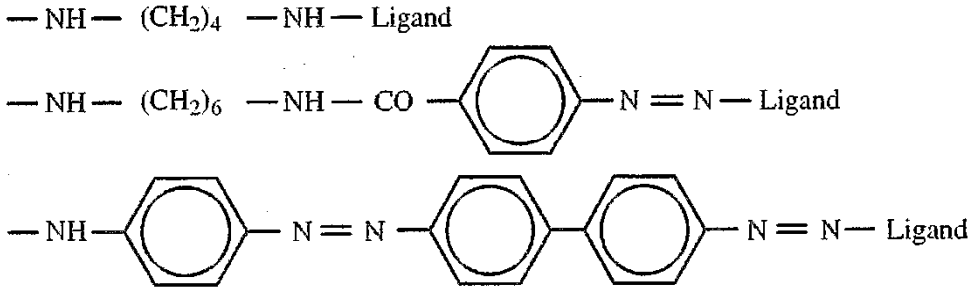


No carbohydrate

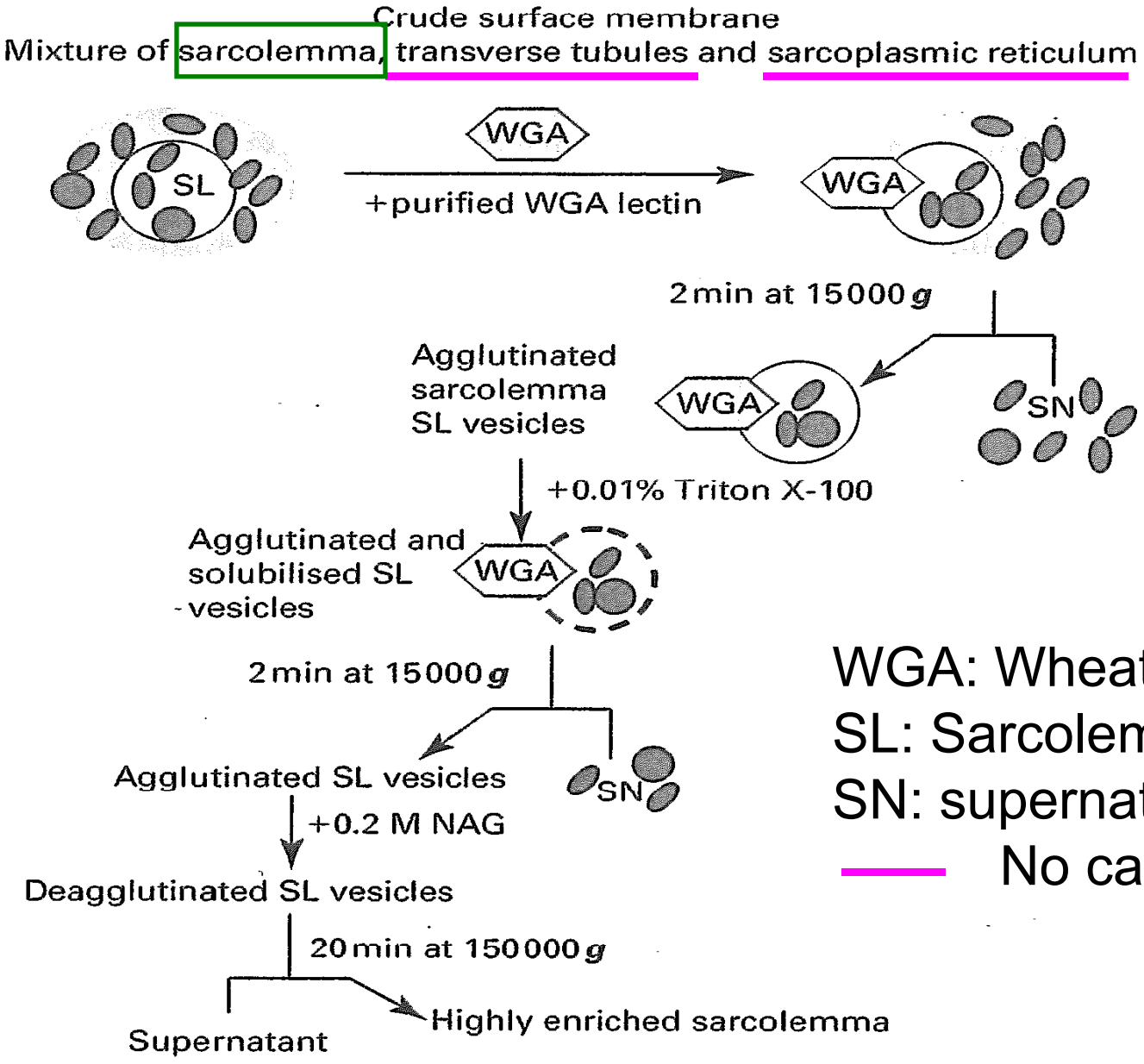
Inside-out: No carbohydrate



SOME LINKING GROUPS



Lectin Agglutination Method



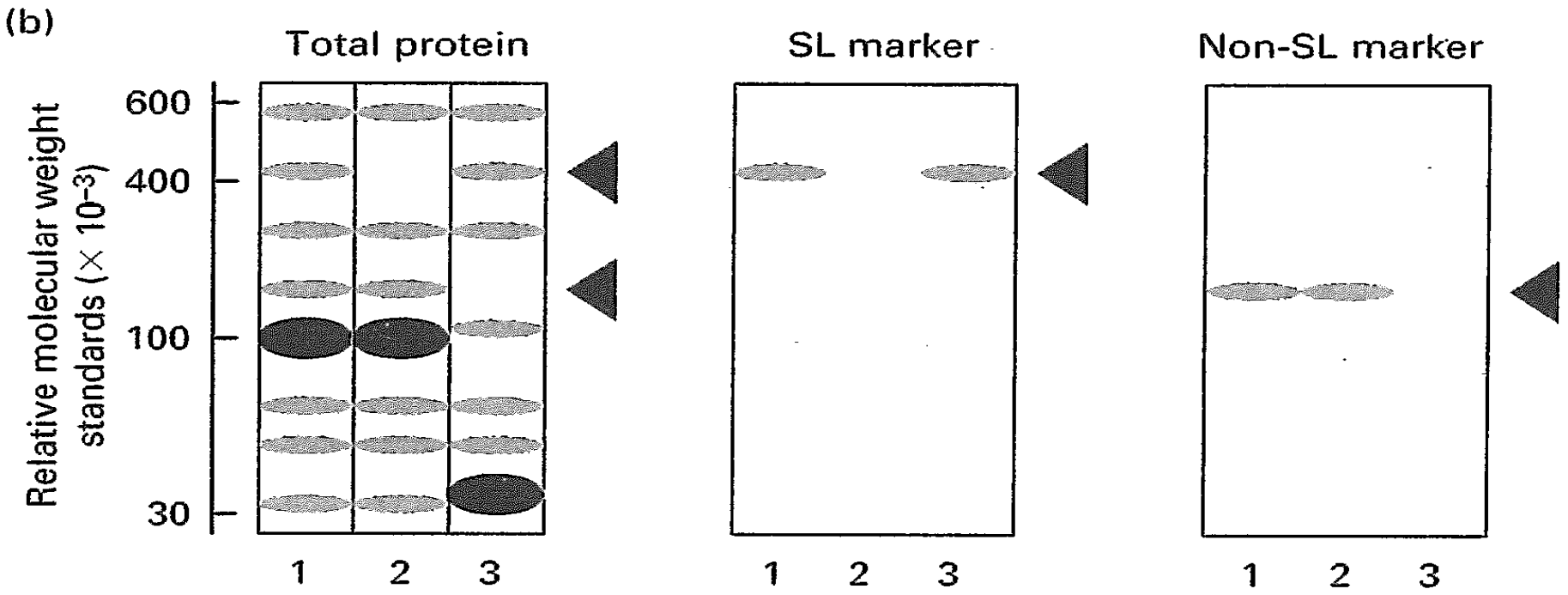
WGA: Wheat germ agglutinin

SL: Sarcolemma

SN: supernatant

—— No carbohydrate

Immunoblot Analysis for Verification of Different Subcellular Fractions



Gel/blot lane 1: Crude surface membrane
Gel/blot lane 2: Lectinvoid fraction
Gel/blot lane 3: Highly purified sarcolemma

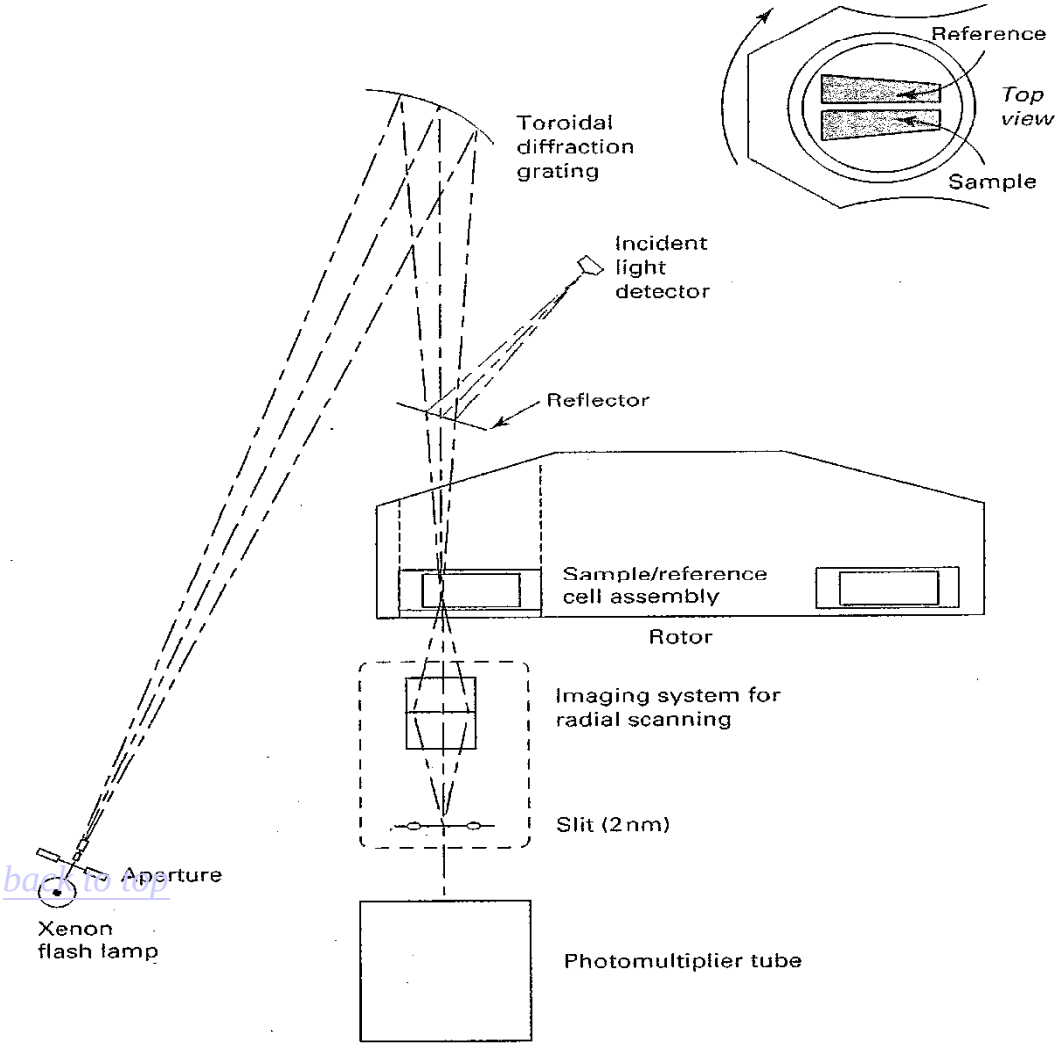
Analytical Ultracentrifugation (AUC)

MBM 3.5.1

An analytical ultracentrifuge spins a rotor at an accurately controlled speed and temperature. The concentration distribution of the sample is determined at known times using absorbance measurements. It can determine:

- Relative molecular mass of solute
- Purity of macromolecule
- Change in relative molecular mass of supermolecular complexes
- Shape, Conformational change of protein structure
- Ligand-binding study

Optical System of an Analytical Ultracentrifugation



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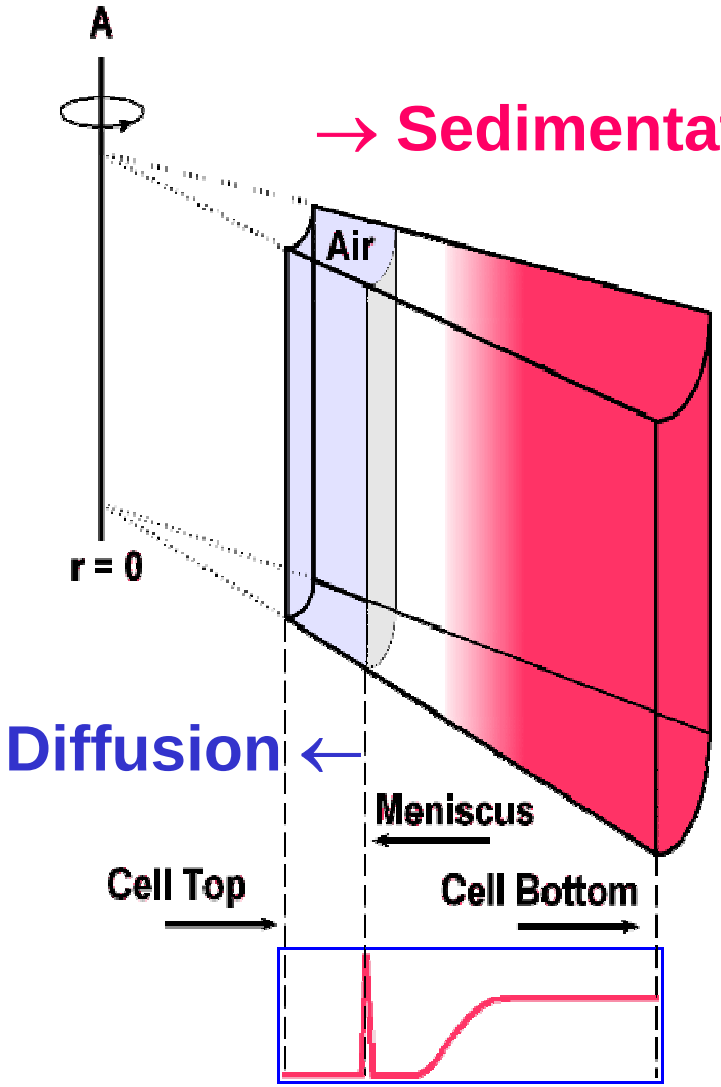
This figure displays a schematic diagram of the Beckman Optima XL-A absorbance system. A high intensity xenon flask lamp allows the use of wavelengths between 190 and 800nm. The lamp is fired briefly as a selected sector passes the detector.

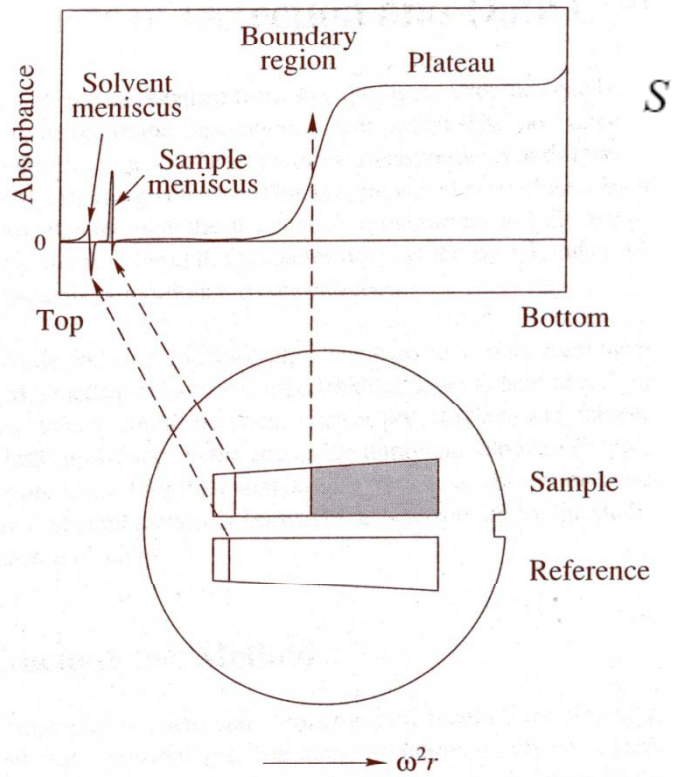
Sedimentation Velocity Method

Mass, Shape and Conformation; Higher velocity

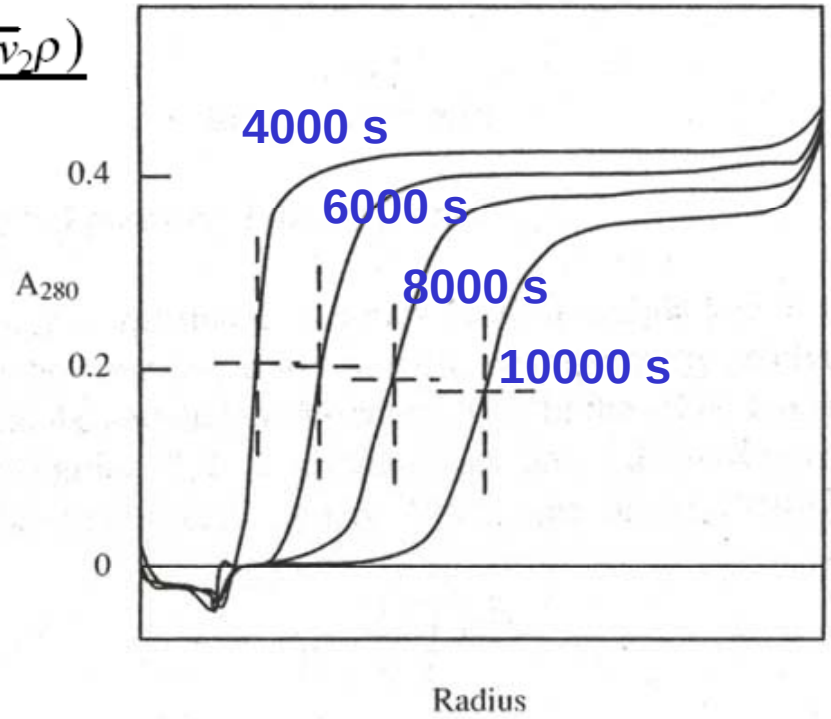


Sedimentation velocity experiments are performed at high speed to overcome the effect of diffusion. For a sedimentation velocity experiment, an initially uniform solution is placed in a cell and a sufficiently high angular velocity is applied to cause rapid sedimentation of solute towards the cell bottom. As a result, there is a depletion of solute near the meniscus, causing a characteristic spectrum as shown in the following figure. A sharp boundary occurs between the depleted region and the sedimenting solute (the plateau)

Determination of Sedimentation Coefficient (*s*)



$$S = \frac{v_t}{\omega^2 x} = \frac{m(1-\bar{v}_2\rho)}{f}$$

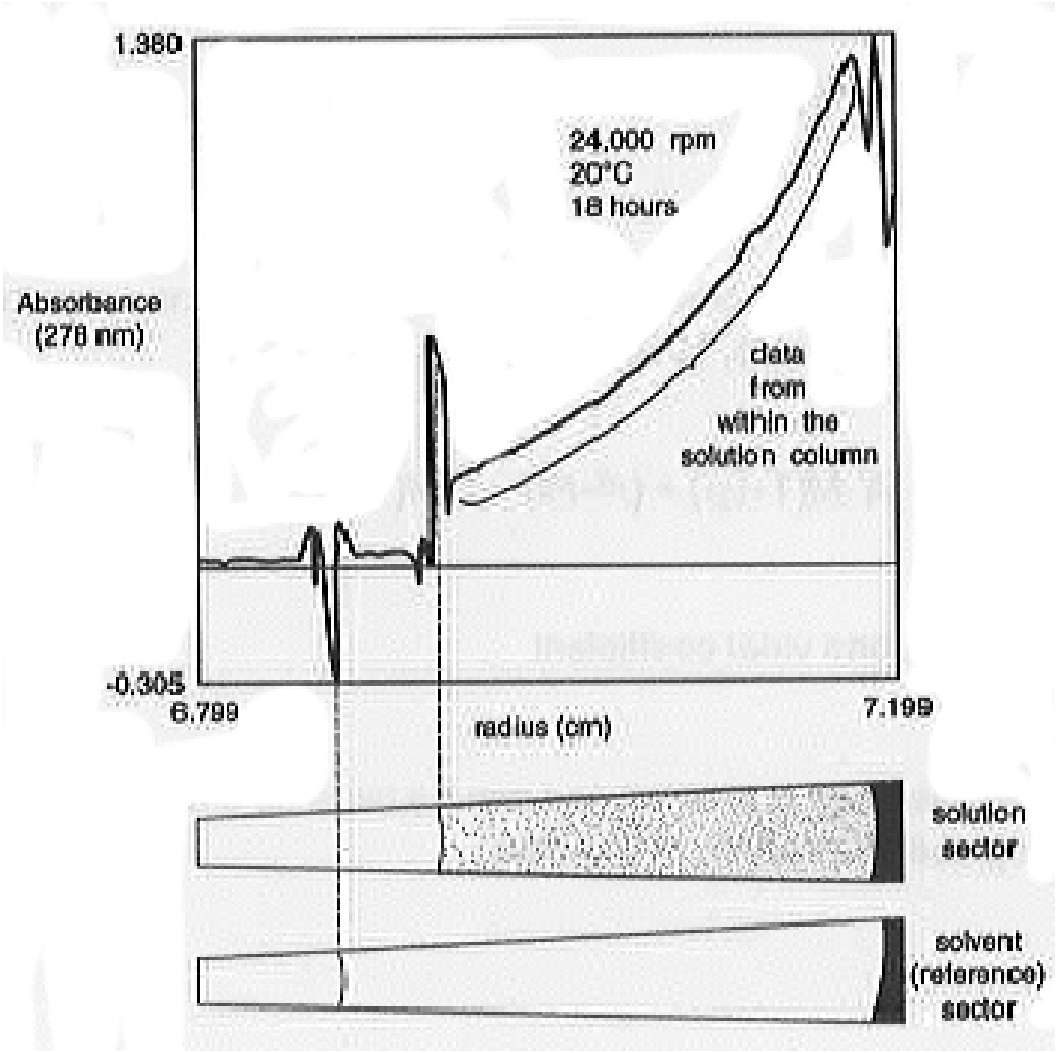


The velocity of the individual particles in SV experiments cannot be resolved, but the rate of movement of the boundary region can be measured. From this, the **sedimentation coefficient (*s*)** can be determined. Remember, *s* depends directly on the **mass** of the solute particles and inversely on the **frictional coefficient**, which is a measure of **size** of the solute particles.

http://www-bioc.rice.edu/bios576/AU/AU_Page.html#au

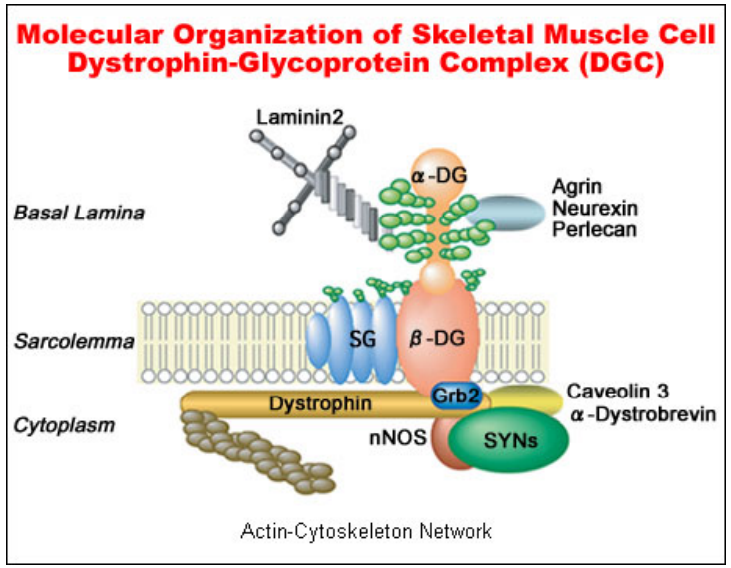
Sedimentation Equilibrium Methods

Mass, Complex formation; Lower velocity



Sedimentation equilibrium experiments have a lower rotor speed than sedimentation velocity experiments. Solute particles do not pellet at the bottom of the cell, but instead the process of diffusion opposes the process of sedimentation until after a period of time, the two opposing forces reach equilibrium and the apparent concentration profile does not change. At equilibrium, the concentration of the solute increases exponentially towards the cell bottom. Each column displays a different absorbance profile, because the concentrations of sample are varied in each.

Sedimentation Analysis of Supramolecular Protein Complex



The binding of ligands may induce conformational changes in subunits of biomolecules, which changes the supramolecular structure of complex.