

PATHOPHYSIOLOGY AND MECHANISMS OF RADIOPHARMACEUTICAL LOCALIZATION

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20141020 morning meeting

The Pathophysiological basis of Nuclear Medicine 2nd ed. Springer.

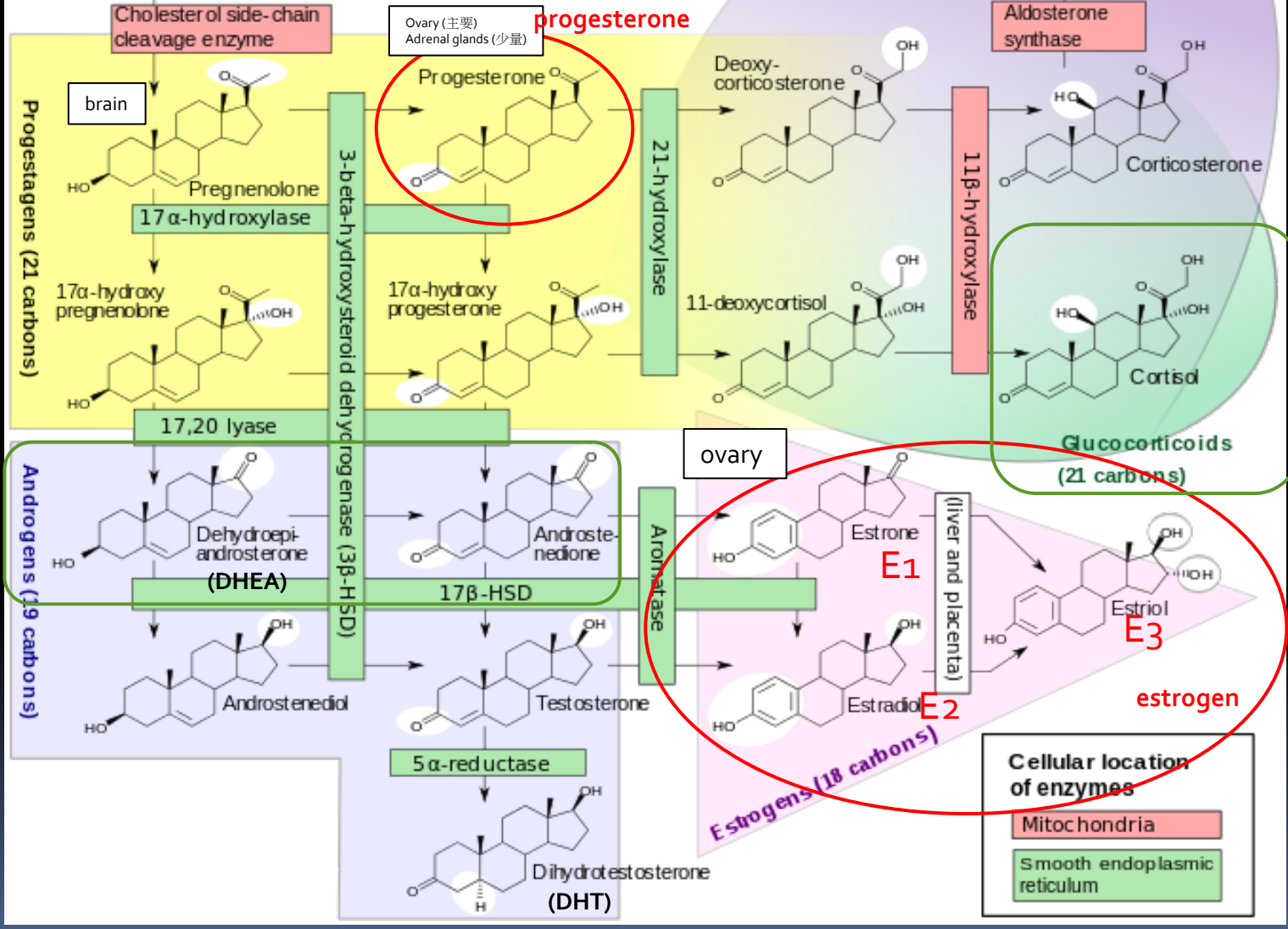
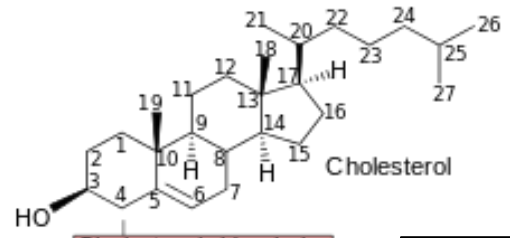
The mechanisms of radioisotope localization

1. Isotope dilution In vivo, MUGA, RBC scan
2. Capillary blockade MAA lung perfusion
3. Physicochemical adsorption MDP bone scan
4. Cellular migration and sequestration WBC scan, denatured RBC spleen scan
5. Membrane transport Xe-133 ventilation
 - Simple diffusion
 - Diffusion and intracellular metabolism/binding HMPAO/ECD brain perfusion scan
 - Diffusion and mitochondrial binding Tc-99m MIBI
 - Diffusion and increased capillary and plasma membrane permeability Gallium
 - Facilitated diffusion FDG, IDA derivatives
 - Active transport Radioiodine, pertechnetate, TlCl, Rb +
 - Phagocytosis SC
 - Receptor-mediated endocytosis Gallium

The mechanisms of radioisotope localization

6. Metabolic Substrates and Precursors **FDG**
 - Precursors: Radiolabeled Amino Acids **Amino acids**
7. Tissue Hypoxia **F-MISO**
8. Cell Proliferation **Ribonucleic acids, ex: F-18 FLT**
9. Specific Receptor Binding
 - Radiolabeled Peptides **SST analogues, VIP**
 - Steroid Hormone Receptors **F-18 FES, F-18 FENP, Z-[123 I]MIVE**
 - Adrenergic Presynaptic Receptors and Storage **I-131 MIBG**
 - LDL Receptors
 - Radiolabeled Antibodies
10. Imaging Gene Expression

Zona reticularis



Mineralocorticoids (21 carbons)

CC(=O)CC12CC[C@H]3[C@H]([C@@H]1CC[C@@H]2O)CCC4=CC(=O)CC[C@]34C
 Aldosterone

Zona glomerulosa

Zona fasciculata

Estrogens (18 carbons)

CC12CC[C@H]3[C@H]([C@@H]1CC[C@@H]2O)CCC4=CC(=O)CC[C@]34C
 Estrone (E1)

CC12CC[C@H]3[C@H]([C@@H]1CC[C@@H]2O)CCC4=CC(=O)CC[C@]34C
 Estradiol (E2)

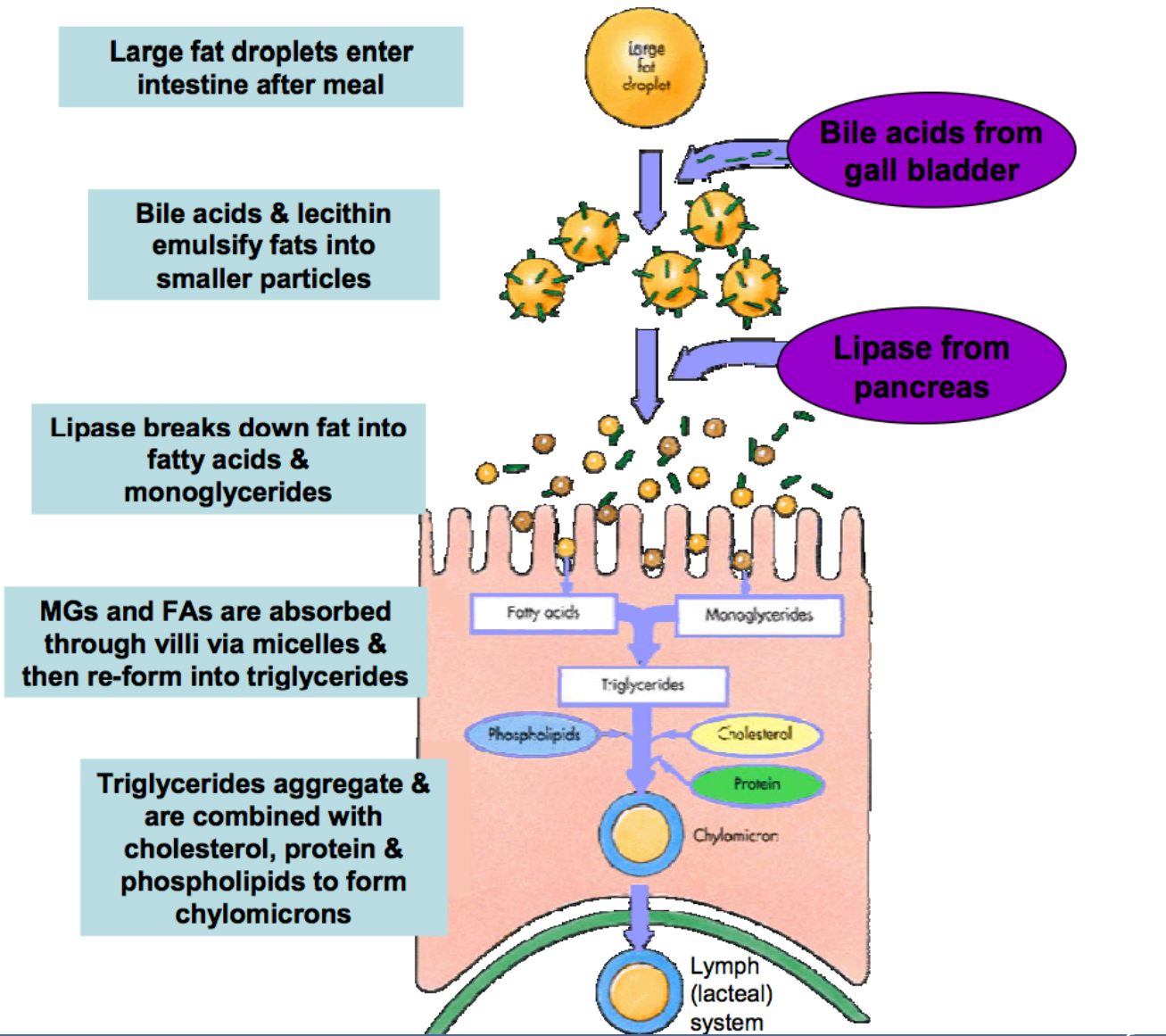
CC12CC[C@H]3[C@H]([C@@H]1CC[C@@H]2O)CCC4=CC(=O)CC[C@]34C
 Estriol (E3)

(liver and placenta)

Cellular location of enzymes

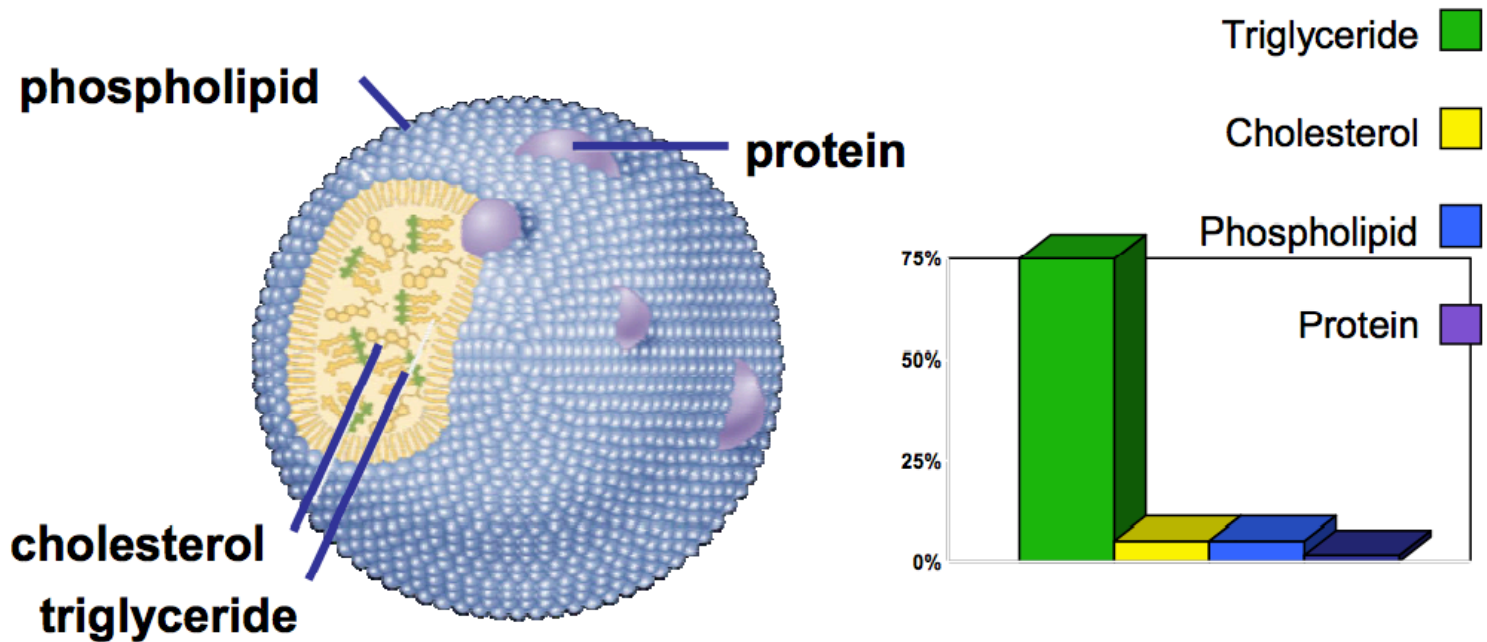
Mitochondria
Smooth endoplasmic reticulum

Fat digestion



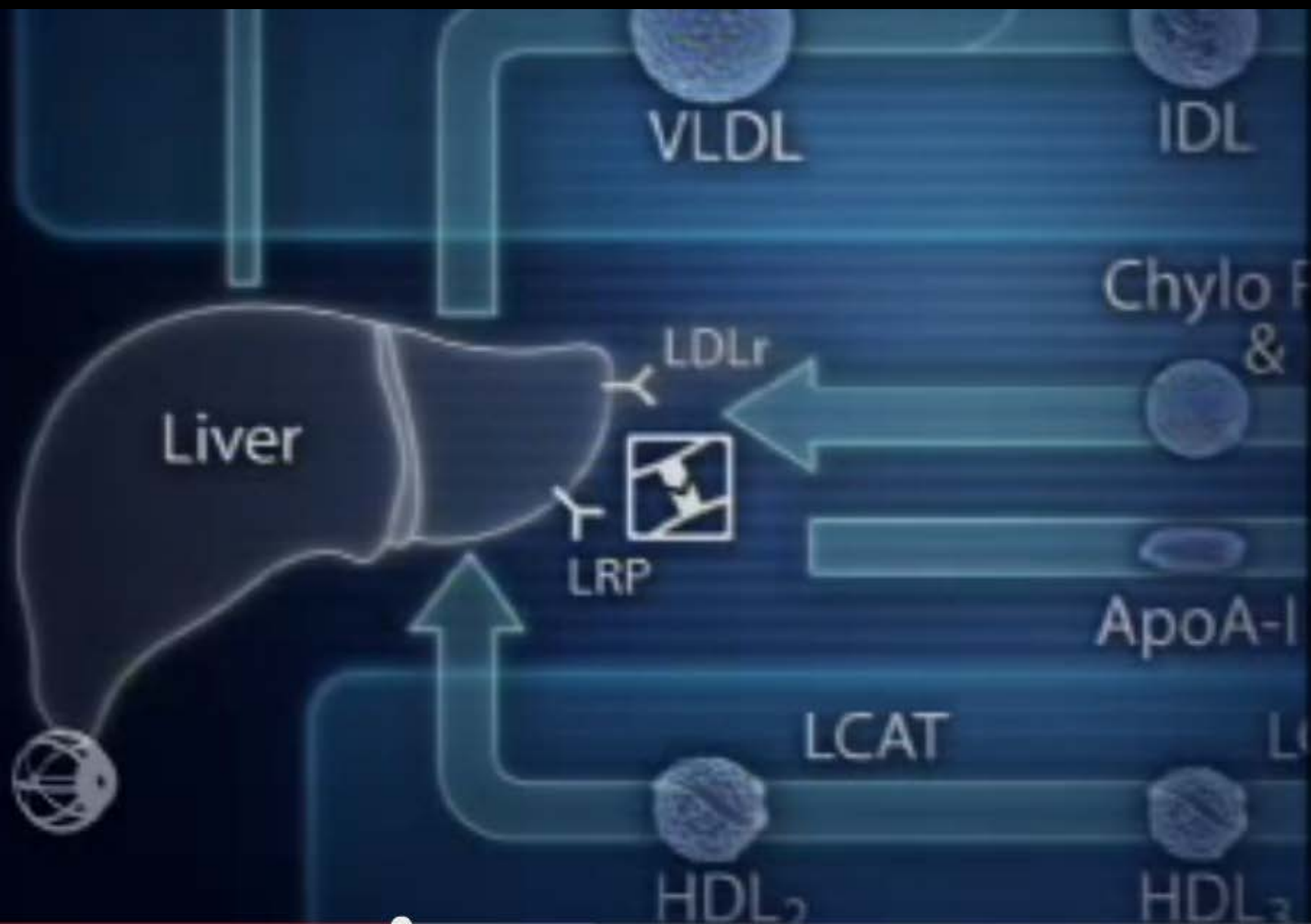
Composition of chylomicron

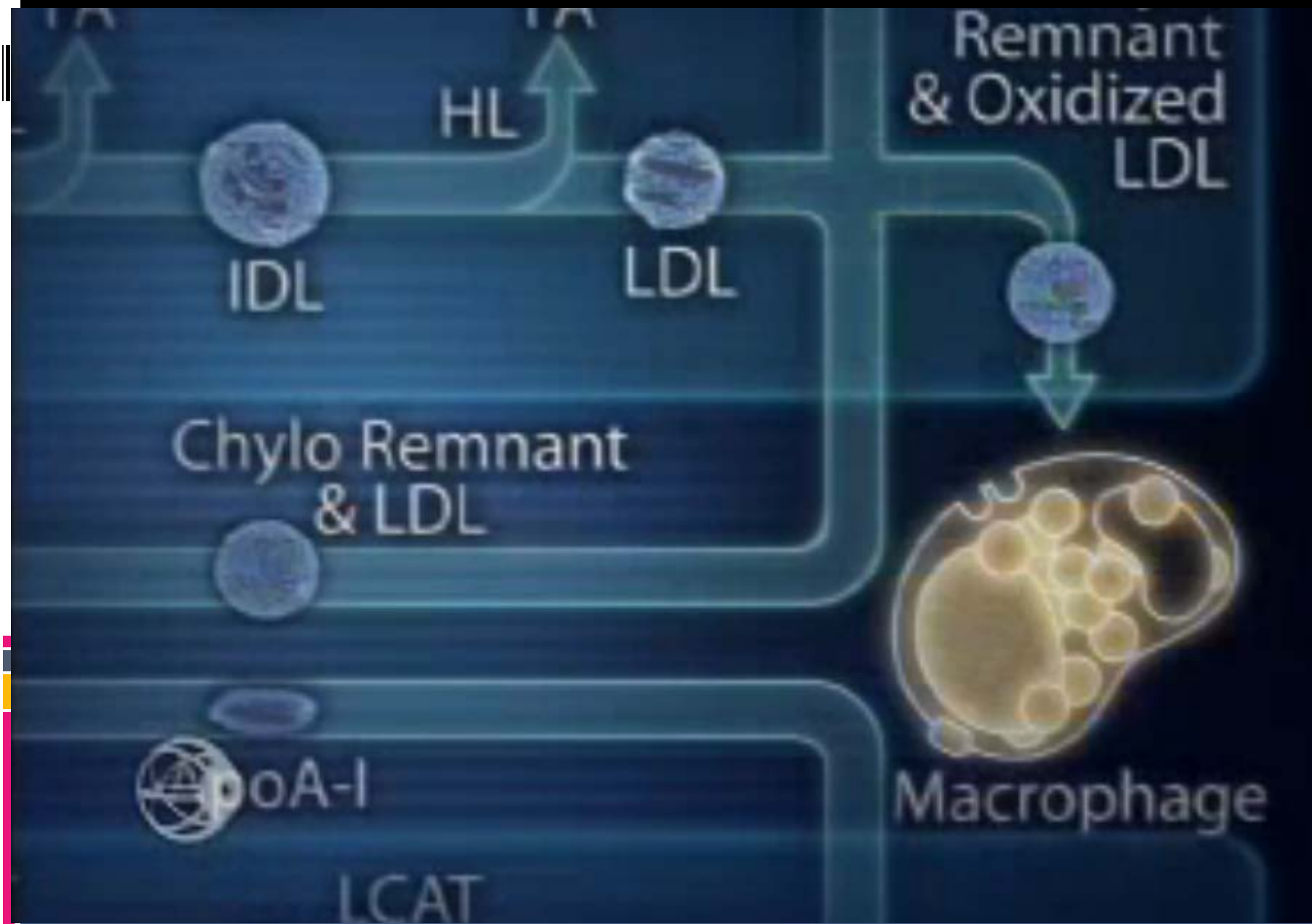
Chylomicrons



B Lipoproteins (NOT HDL)









- Chylomicrons carry triglycerides (fat) from the intestines to the liver, to skeletal muscle, and to adipose tissue.
- Very-low-density lipoproteins (VLDL) carry (newly synthesised) triglycerides from the liver to adipose tissue.
- Intermediate-density lipoproteins (IDL) are intermediate between VLDL and LDL. They are not usually detectable in the blood.
- Low-density lipoproteins (LDL) carry cholesterol from the liver to cells of the body. LDLs are sometimes referred to as the "bad cholesterol" lipoprotein.
 - large buoyant LDL (lb LDL) particles
 - small dense LDL (sd LDL) particles
 - Lipoprotein(a) is a lipoprotein particle of a certain phenotype
- High-density lipoproteins (HDL) collect cholesterol from the body's tissues, and take it back to the liver. HDLs are sometimes referred to as the "good cholesterol" lipoprotein.

Density (g/mL)	Class	Diameter (nm)	% protein	% cholesterol	% phospholipid	% triacylglycerol & cholesterol ester
>1.063	HDL	5–15	33	30	29	4
1.019–1.063	LDL	18–28	25	50	21	8
1.006–1.019	IDL	25–50	18	29	22	31
0.95–1.006	VLDL	30–80	10	22	18	50
<0.95	Chylomicrons	100-1000	<2	8	7	84

Animation of lipoprotein metabolism:
<https://www.youtube.com/watch?v=g7uiV4RiSAY>

from: Wikipedia, lipoproteins

Specific Receptor Binding— LDL receptors

- ^{131}I -6 β -iodomethyl-19-norcholesterol (NP-59)
- ^{131}I -6-iodocholesterol (Ioderin)
- ^{75}Se - β -iodomethyl-19-norcholesterol (Scintadren)
- Mechanism of uptake:
 - transported by plasma LDL and are accumulated in the adrenal cortex via **LDL receptors**
 - **esterified** like cholesterol and **stored** intracellularly **without** further metabolism or incorporation into adrenocortical steroid hormones

NP-59 Adrenal Cortical Scintigraphy

- 利用NP-59檢查時有不同的步驟：
 - 當懷疑是庫辛氏症時直接注射NP-59檢查即可，藉著影像表現的不同來判斷是ACTH依賴型或是非依賴型
 - 若懷疑是原發性醛固酮過多症則必須先以dexamethasone(DS)進行抑制ACTH分泌再注射NP-59
- 適應症：
 - 1、診斷原發性醛固酮過多症是腺瘤或是腎上腺皮質增生所引起
 - 2、當CT、MRI、超音波發現腎上腺有腫塊時可以鑑定腫塊的功能及特性
 - 3、診斷庫辛氏症(Cushing's syndrome)是ACTH依賴型或是非依賴型
 - 4、診斷女性多毛症或無月經是否因腎上腺雄性素分泌過高引起

NP-59 Adrenal Cortical Scintigraphy

掃瞄前準備

- 1、受檢者最好先做血液生化檢查，再來作腎上腺皮質掃描，若是cortisol高則進行不服用DS的NP-59 scan，若是aldosterone高則進行DS suppression的NP-59 scan。
- 2、為預防I-131聚積而傷害甲狀腺，打針前三天要服用Lugol's solution，每天4滴，滴入開水中稀釋服用，一直持續到檢查結束為止。
- 3、DS抑制：採小劑量長期抑制的方式進行，於注射前一星期開始服用，每天4次每次2顆(0.5 mg/顆)共8顆4 mg，持續到檢查結束。
- 4、NP-59時必須於2~5分鐘內**緩慢注射**完畢，以防止產生過敏反應，因為NP-59含有Tween 80安定劑是一種脂肪酸酯類之物會刺激體內分泌histamine。
- 5、去除腹部之金屬物品。
- 6、向受檢者或家屬解釋檢查之詳細流程，希望能全力配合以完成繁瑣的檢查。

VGHTPE protocol

With dexamethasone suppression

For 5 days (prior to exam)

dexamethasone suppression 0.5mg/1# tid?

Lugol's solution amount

溶液劑：含碘5%、碘化鉀10%

exam day (Day 0)

Day 0 1.2 mCi I-131 NP-59 IV, 30mins later, 3 mCi Tc-99m
DMSA IV

take renal image (of DMSA), mark renal countour

Day 1 take NP-59 image

Day 2 take NP-59 image

Day 3 take NP-59 image

Day 4 take NP-59 image

NP-59 Adrenal Cortical Scintigraphy

3、原發性醛固酮過多症影像類型

影像類型	診斷
只見到單側影像(<5天)	Adenoma腺瘤
可見到兩側影像(<5天)	Hyperplasia增生
可見到兩側影像(>5天)	Hyperplasia增生或正常

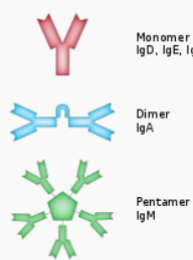
NP-59 Adrenal Cortical Scintigraphy

4、庫辛氏症(Cushing's syndrome)影像類型。

影像類型	診斷
兩側對稱	ACTH依賴型
兩側不對稱	非ACTH依賴型
只見到單側影像	Adrenal adenoma
兩側影像皆看不到	Adrenal carcinoma

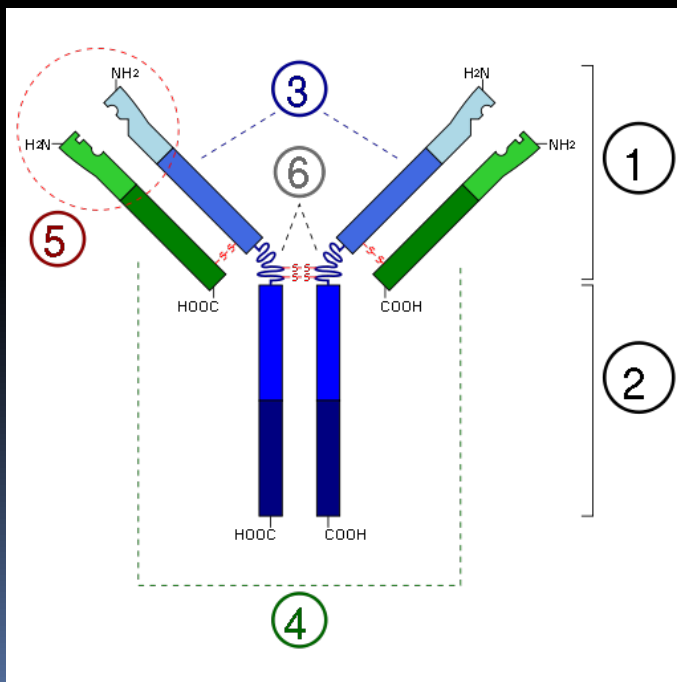
Specific Receptor Binding— Radiolabeled antibodies

- Antibodies (Ab)= immunoglobulins (Ig)
 - Glycoproteins, produced by B-lymphocytes, 5 isotypes: M, E, D, A, G
 - IgG is the major immunoglobulin in human serum
 - IgG: around 146 KD, T_{1/2}: 21 days

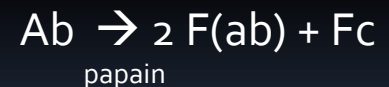
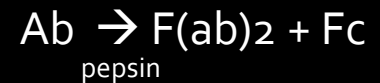
Antibody isotypes of mammals			
Name	Types	Description	Antibody Complexes
IgA	2	Found in mucosal areas, such as the gut, respiratory tract and urogenital tract , and prevents colonization by pathogens . ^[13] Also found in saliva, tears, and breast milk.	 <p>Monomer IgD, IgE, IgG</p> <p>Dimer IgA</p> <p>Pentamer IgM</p>
IgD	1	Functions mainly as an antigen receptor on B cells that have not been exposed to antigens. ^[14] It has been shown to activate basophils and mast cells to produce antimicrobial factors. ^[15]	
IgE	1	Binds to allergens and triggers histamine release from mast cells and basophils , and is involved in allergy . Also protects against parasitic worms . ^[3]	
IgG	4	In its four forms, provides the majority of antibody-based immunity against invading pathogens. ^[3] The only antibody capable of crossing the placenta to give passive immunity to the fetus .	
IgM	1	Expressed on the surface of B cells (monomer) and in a secreted form (pentamer) with very high avidity . Eliminates pathogens in the early stages of B cell-mediated (humoral) immunity before there is sufficient IgG. ^{[3][14]}	

Specific Receptor Binding— Radiolabeled antibodies

- Antibodies (Ab)= immunoglobulins (Ig)
 - A monoclonal antibody (MAb) is specific for a particular epitope \leftrightarrow a particular antigen can have several different epitopes



1. Fab region
2. Fc region
3. Heavy chain (blue) with one variable (V_H) domain followed by a constant domain (C_H1), a hinge region, and two more constant (C_H2 and C_H3) domains.
4. Light chain (green) with one variable (V_L) and one constant (C_L) domain
5. Antigen binding site (paratope)
6. Hinge regions.



Specific Receptor Binding— Radiolabeled antibodies

- murine Mabs: Mab derived from mice
 - The Fc portion of the murine Ab is antigenic in human and induces the formation of HAMA (huma anti-mouse-antibody)
- Chimeric Abs: murine variable regions of Fab + constant region of human IgG

Specific Receptor Binding— Radiolabeled antibodies

- Important requirements:
 - Identify an antigen/epitope specific to a particular type of cancer or tissue
 - TAA (tumor-associated antigens), ex: CEA, TAG-72, PSA, PSMA
 - The used radionuclide should match the pharmacokinetics of the antibody
 - Tc-99m, I-123: suited for Ab fragments, with faster blood clearance
 - In-111, I-131: better for labeling intact Abs
 - Imaging: Tc-99m, I-123, In-111
 - Tx (with β): I-131, Y-90, Re-188
 - **High-specific-activity** labeling but preserving the **immunoreactivity** of the Abs

Specific Receptor Binding— Radiolabeled antibodies

- Radioiodinated antibodies
 - Iodogen method → high-specific-activity Ab (10-15 mCi/mg): labeling the tyrosine residues, it is possible that tyrosine residues of the **variable** region of the IgG molecule are labeled, resulting in significant **loss of immunoreactivity**
 - Ex:
 - I-131 antiB1 antibody (BEXAR)... NHL

Specific Receptor Binding— Radiolabeled antibodies

- Tc-99m labeled antibodies

不需
ligand

- **Direct**: reduction of disulfide groups of the Ab molecule and subsequent labeling of reduced Tc-99m to the **sulfide moieties** of the Ab
 - Most commonly used to label Ab fragments, such as CEA-scan & Verluma
 - CEA scan: for colorectal ca., binds to CEA ~200 kD
 - Verluma scan: small cell lung ca., binds to a 40 kD cell surface glycoprotein
- Indirect

Schwarz method: a direct labeling method

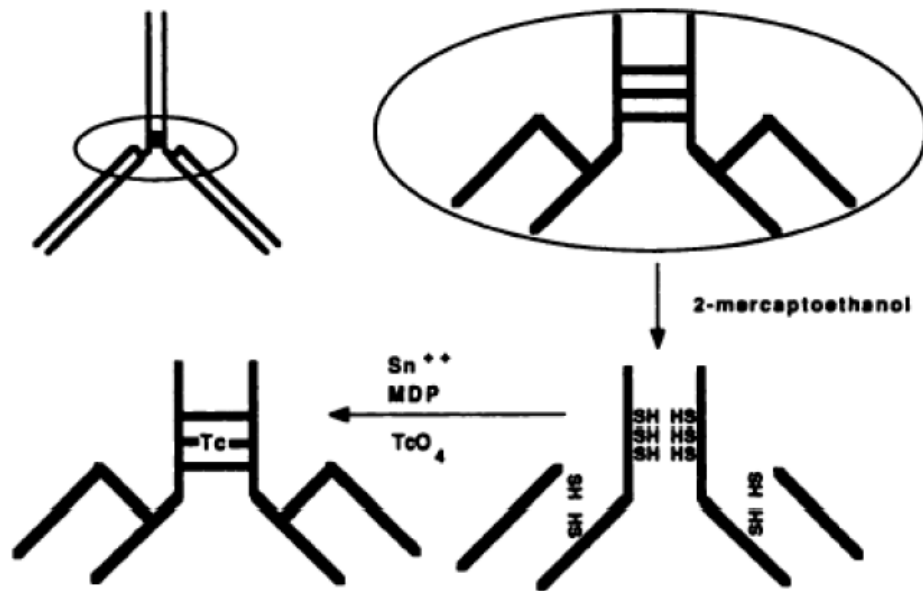


FIGURE 1
General schema showing outline of labeling procedure.

- Incubate Ab with β -ME (1000:1)
30 min, RT
- Purify Ab by Gelfiltration
- Add transchelator
(gluconate, MDP, tricine)
- Add TcO_4^-
- Incubate 10 min, RT
- QC with ITLC

Mather & Ellison. J Nucl Med 1990; 31: 692-697

Specific Receptor Binding— Radiolabeled antibodies

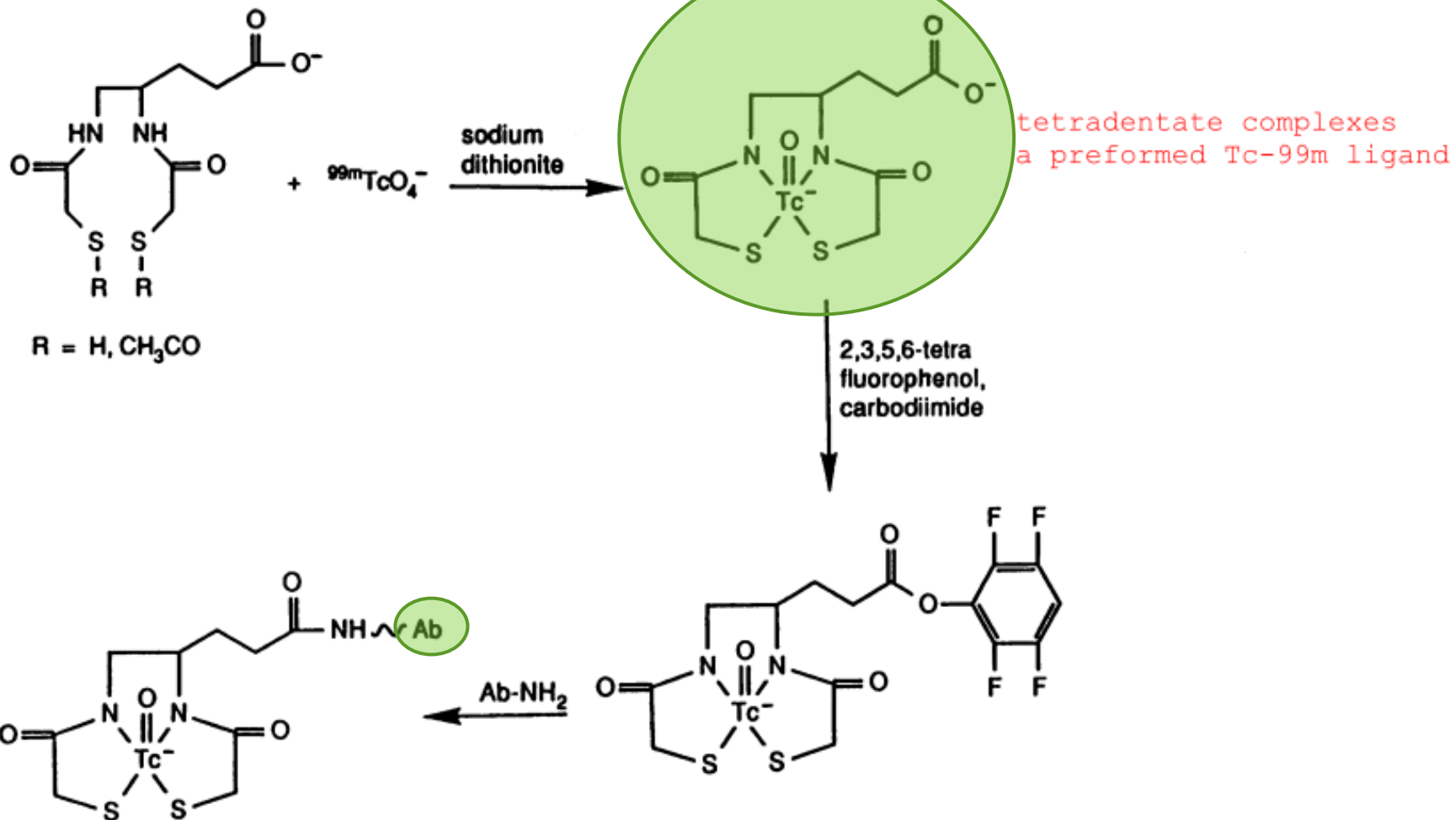
- Tc-99m labeled antibodies

- Direct

需ligand □ **Indirect**: linking Tc-99m to the Ab through a ligand which binds to the amino groups of the lysine and arginine residues of the Ab

- Depending on the ligand used, the Tc-99m is bound either to the Ab or to the ligand, ligands: iminothiolane, N₂S₂ or N₃S₂ tetradentate
 - Method of preformed Tc99m-ligand
 - Tc-99m diamide dithiolate (二胺二硫系統; DADS= N₂S₂): Tc is bound to the ligand
 - Derivatized (衍生) method (ligand-Ab first, Tc-99m labeling then)
 - Iminothiolane—Tc is bound to Ab through –SH group

Example of preformed Tc-99m ligand method (indirect method)



Specific Receptor Binding— Radiolabeled antibodies

- **In-111** & Y-90 labeled antibodies

- Bifunctional chelating agent (BFC)

Choice of a chelating agent depends on the radiometal to be complexed

- DTPA

- isothiocyanatobenzyl-DTPA

→ provide high in vivo stability to In-111 Ab

Disadvantage

- High concentration in liver, spleen, bone marrow and intestine

Advantage

- Possibility of kit formation and reasonable long physical half-life

Specific Receptor Binding– Radiolabeled antibodies

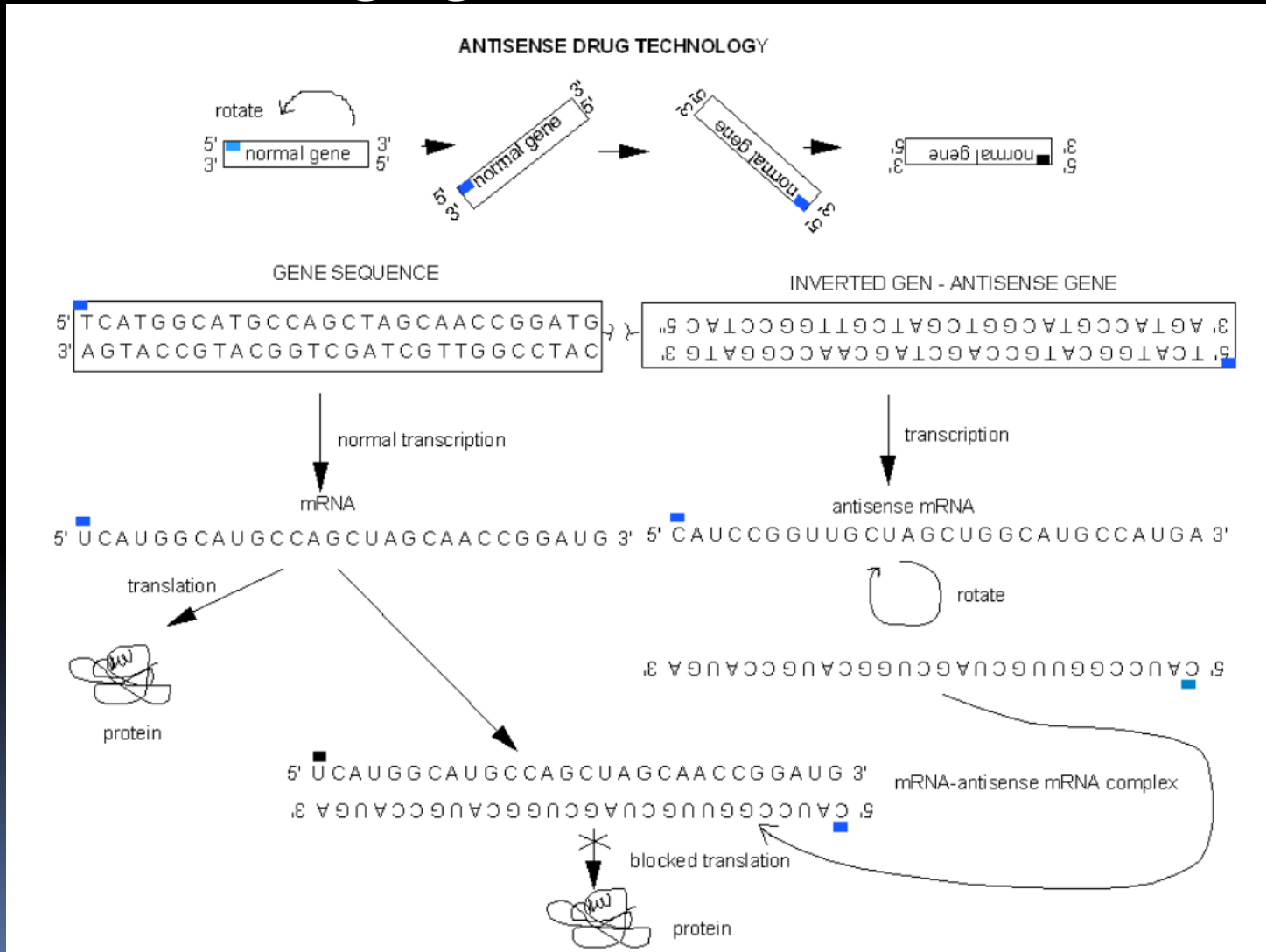
- In-111 & Y-90 labeled antibodies
 - Macrocyclic bifunctional chelating agent (BFC)
Provide greater in vivo stability for Y-90 or Cu-67 radiometals
 - DOTA
 - TETA
 - Conjugated at Fc segment:
 - OncoScint® → TAG-72 Ag, colorectal & ovarian ca.
 - ProstaScint® → PSMA, prostate ca.
 - Conjugated randomly
 - Y-90 IDEC-Y2B8

10. Imaging Gene Expression

- Human genome sequencing...2003
 - Discovery of molecular mechanisms of carcinogenesis
 - Advances in gene therapy
- it may be possible to assess **gene function** and **regulation** by **radionuclide imaging** of gene expression:
1. Antisense imaging
 2. Reporter gene imaging

Imaging Gene Expression

Antisense imaging



Imaging Gene Expression

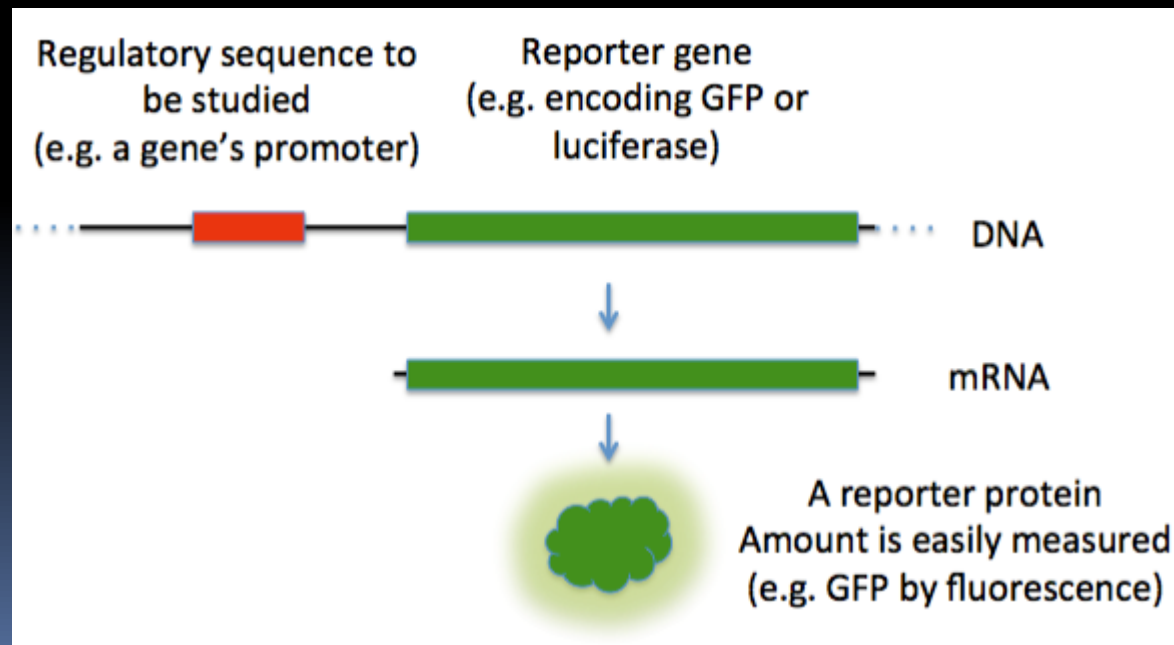
Antisense technique/ imaging:

- Expression of specific genes in vivo
- gene radiotherapy
- therapeutic monitoring of the effort to block the expression of the specific gene

- Unfortunately, oligonucleotides are poor pharmaceuticals because of their large size, low stability and poor membrane permeability. Hence, **chemically modified** oligonucleotides
- ex: c-myc oncogene

Imaging Gene Expression

- Reporter gene :
a reporter gene (often simply reporter) is a gene that researchers attach to a regulatory sequence of another gene of interest



Imaging Gene Expression

Reporter gene imaging

- For imaging, reporter genes can be classified into two categories:
 - those that lead to the production of an **enzyme** that is capable of metabolizing or trapping a reporter probe
 - those that lead to the production of a protein that acts as a **receptor** for binding intracellularly or extracellularly with the reporter probe

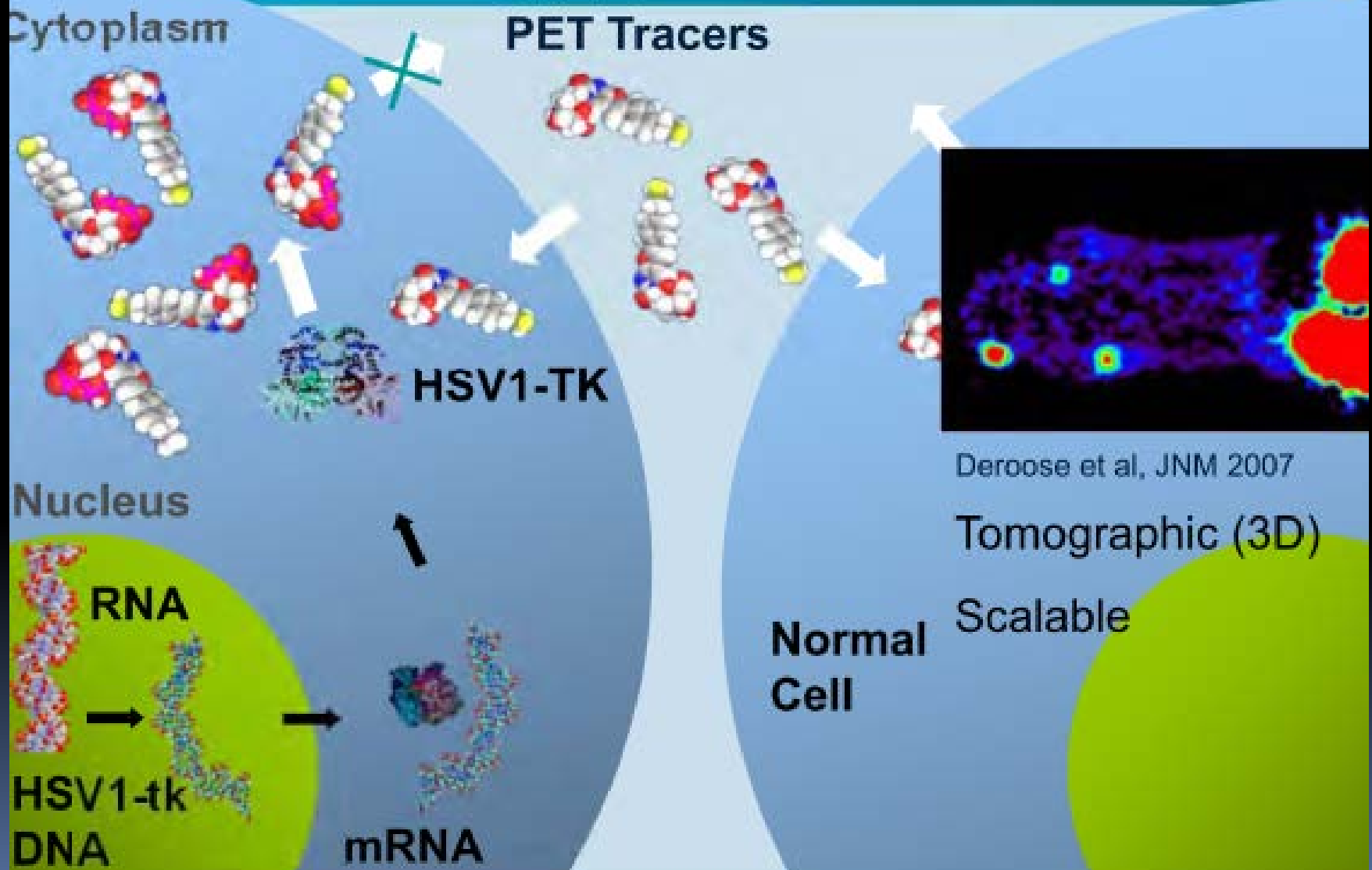
Imaging Gene Expression

Example:

Various reporter probes for *HSV type 1 thymidine kinase (HSV1-tk) reporter gene* have been developed:

- 9-(4-¹⁸F-fluoro-3-[hydroxymethyl]butyl)guanine (FHBG)
- 2'-fluoro-2'-deoxy-1 β -arabinofuranosyl-5-iodo-uracil (FIAU)

HSV1-tk Reporter Gene Imaging



Deroose et al, JNM 2007

Tomographic (3D)

Scalable

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- Thanks for your attention.