

薬物動態基盤解析法の構築と その応用に関する研究

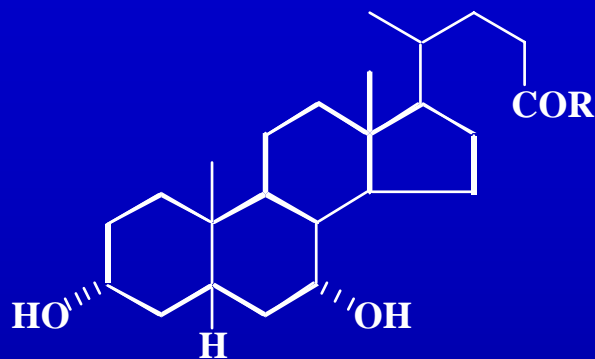
東北大学病院、東北大学大学院薬学研究科

後藤 順 一





**Cholic acid
(CA)**



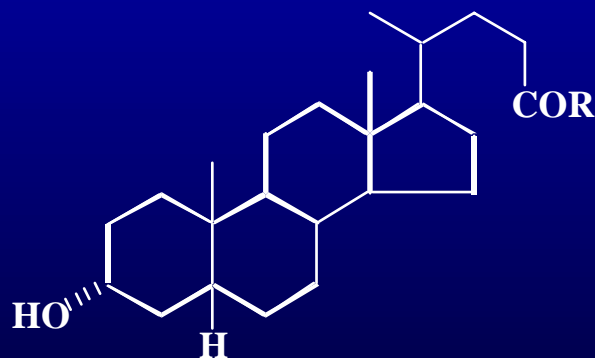
**Chenodeoxycholic acid
(CDCA)**



**Deoxycholic acid
(DCA)**



**Ursodeoxycholic acid
(UDCA)**



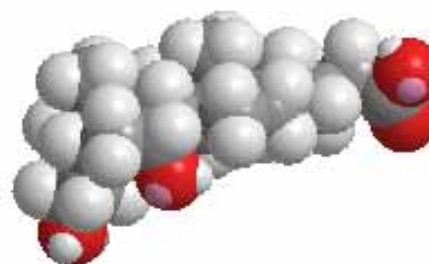
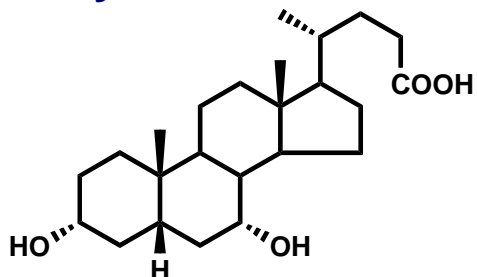
**Lithocholic acid
(LCA)**

R = OH
= NHCH₂COOH
= NHCH₂CH₂SO₃H

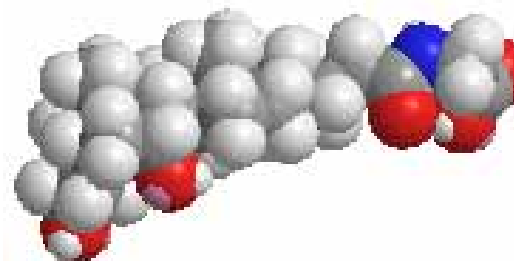
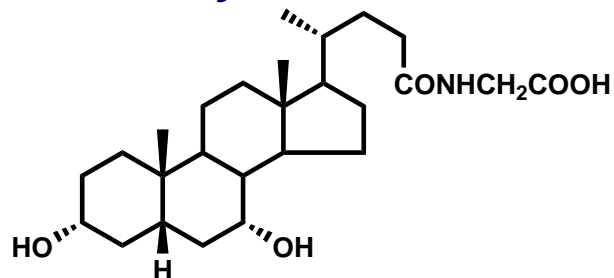
Structures of Bile Acids



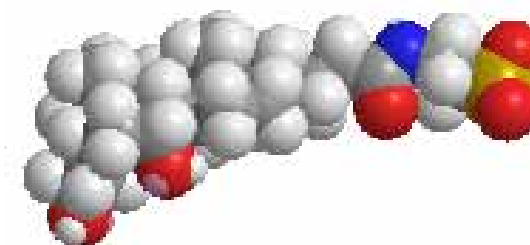
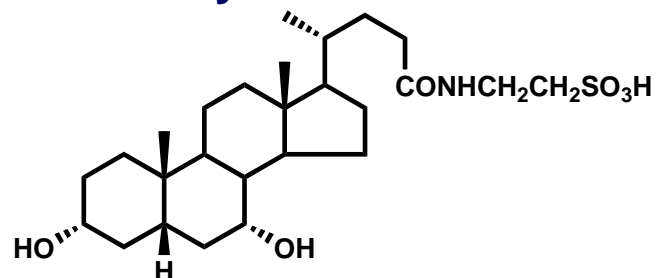
Chenodeoxycholic acid



Glycochenodeoxycholate

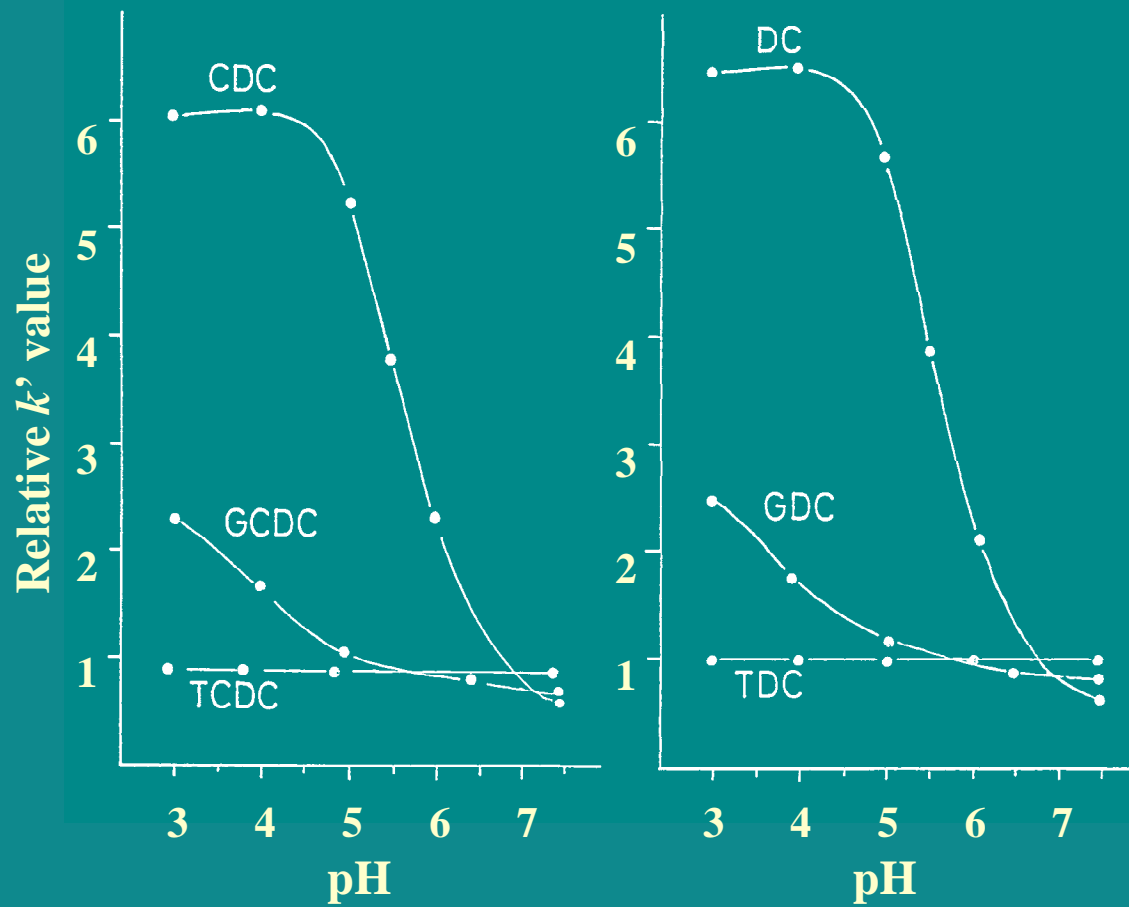


Taurochenodeoxycholate

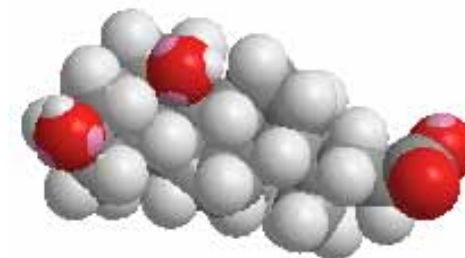
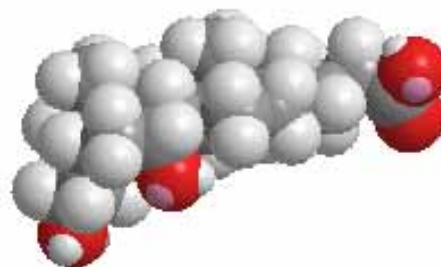
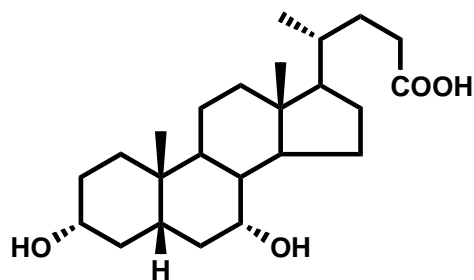


Structures of Nonamidated, Glycine- and Taurine-Conjugated Chenodeoxycholic Acid

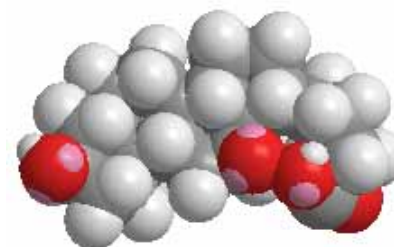
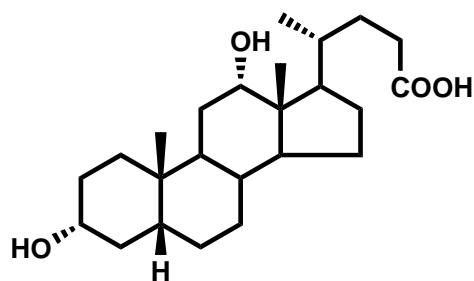




Chenodeoxycholic acid



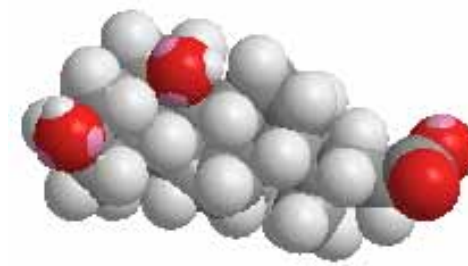
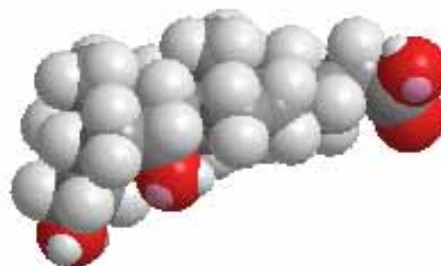
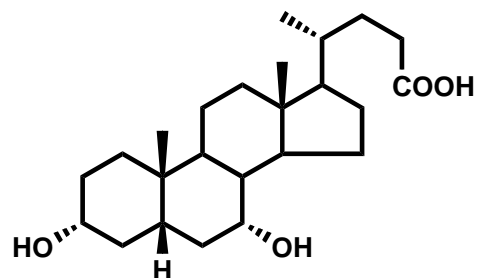
Deoxycholic acid



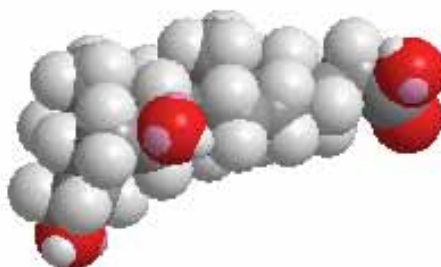
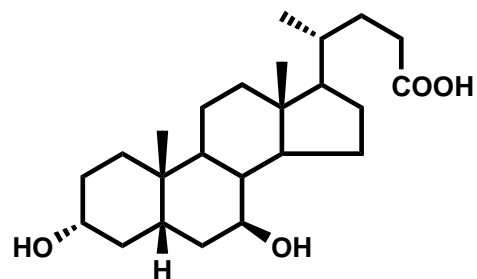
Structures of Chenodeoxycholic Acid and Deoxycholic Acid



Chenodeoxycholic acid

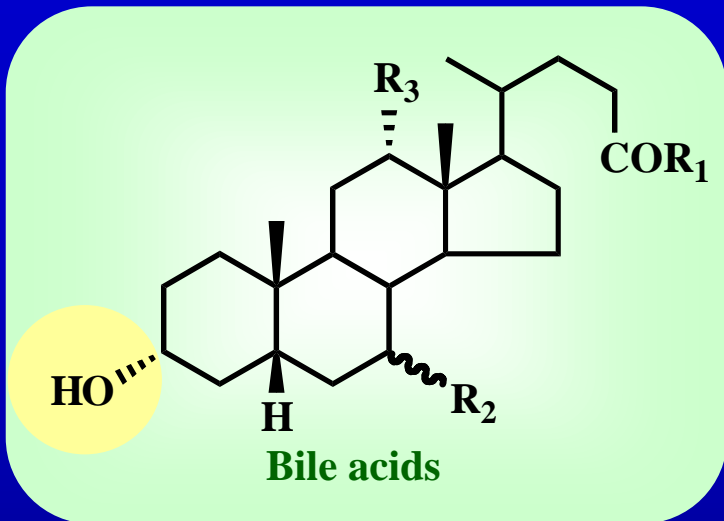


Ursodeoxycholic acid



Structures of Chenodeoxycholic Acid and Ursodeoxycholic Acid



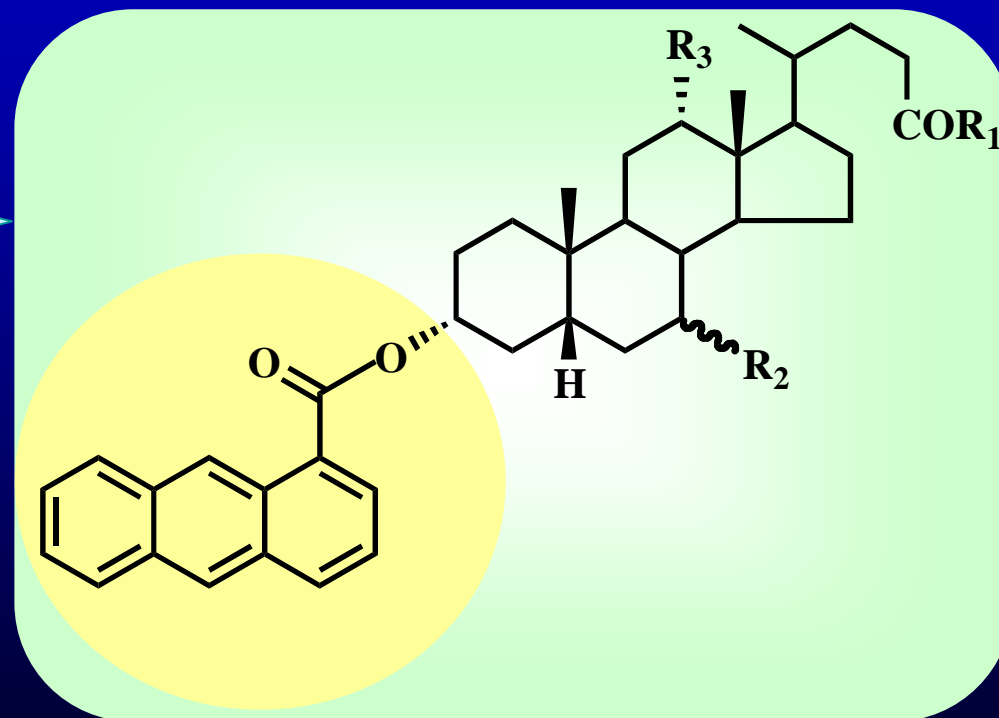
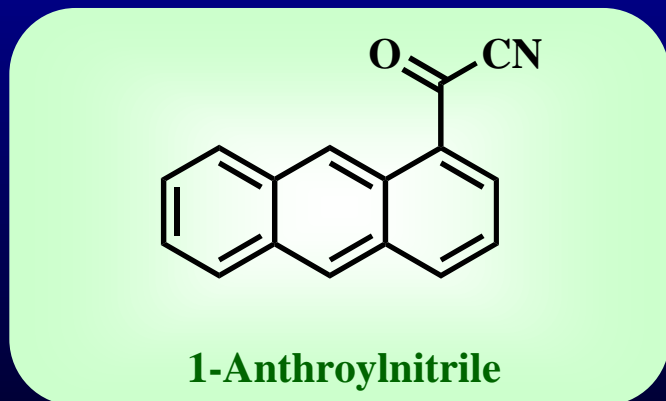


	Unconjugates	Glycine conjugates		Taurine conjugates	
R ₁ :	OH	NHCH ₂ COOH		NHCH ₂ CH ₂ SO ₃ H	
	CA	CDCA	DCA	LCA	UDCA
R ₂ :	-OH	-OH	H	H	-OH
R ₃ :	OH	H	OH	H	H

+

0.08% quinuclidine
in acetonitrile

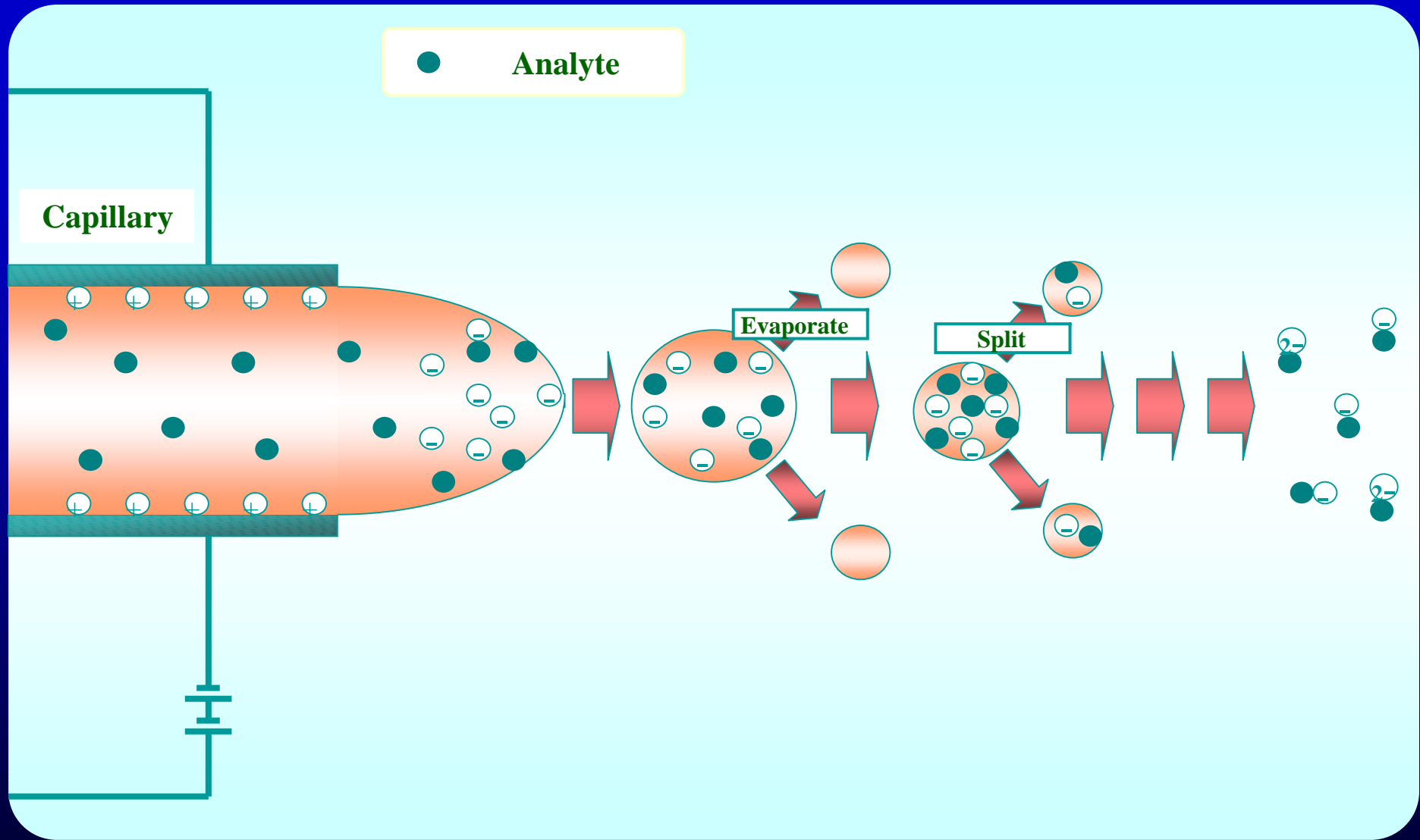
60



Derivatization of Bile Acids by 1-Anthroynitrile

J. Goto, M. Saito, T. Chikai, N. Goto and T. Nambara, *J. Chromatogr.*, **276** (1983) 289-300.






Mechanism of Negative Electrospray Ionization



Analyte -OH

PAPS

Enzyme : Sulfotransferase(SULT2A1)

Derivative -OSO₃H

LC/ESI-MS/MS

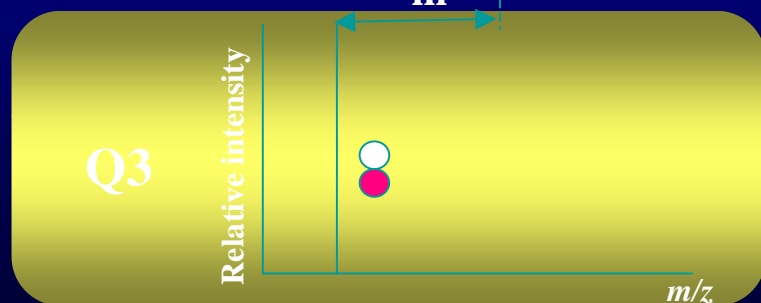
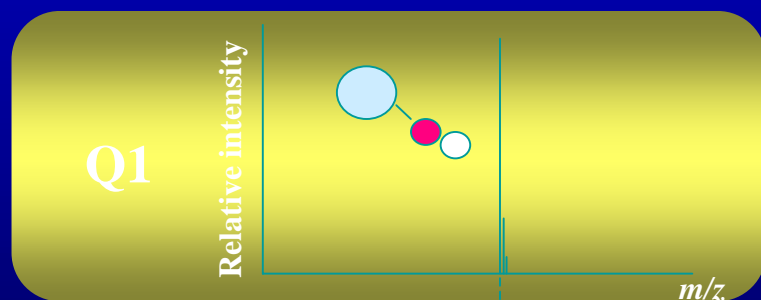
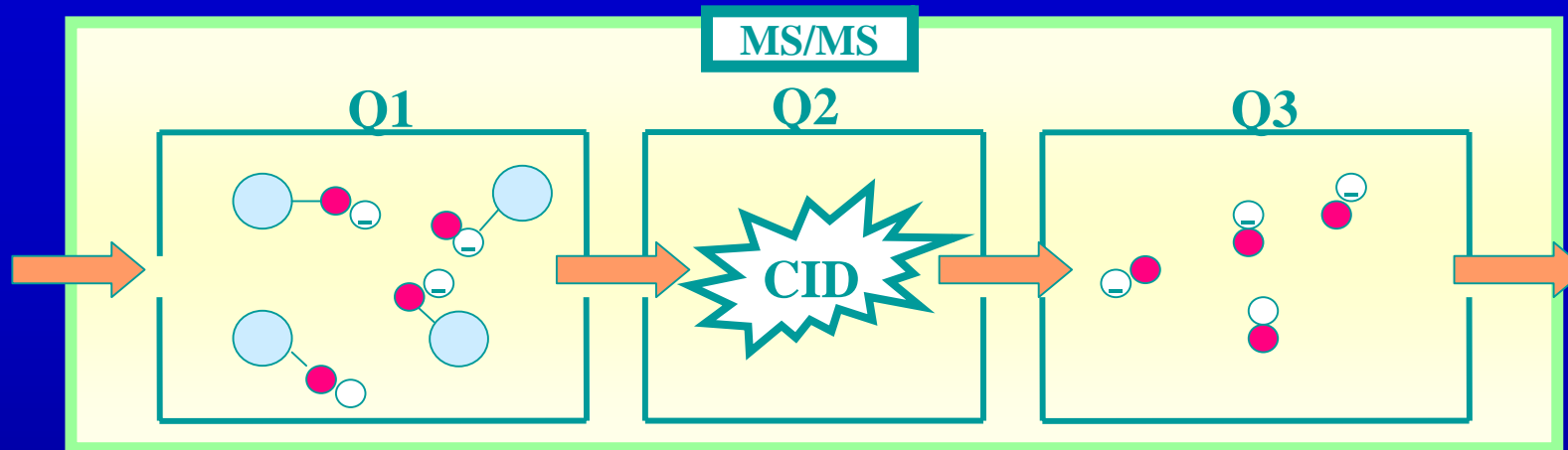
CID fragmentation

-OSO₃⁻


+
HSO₄⁻

Derivatization by Sulfotransferase





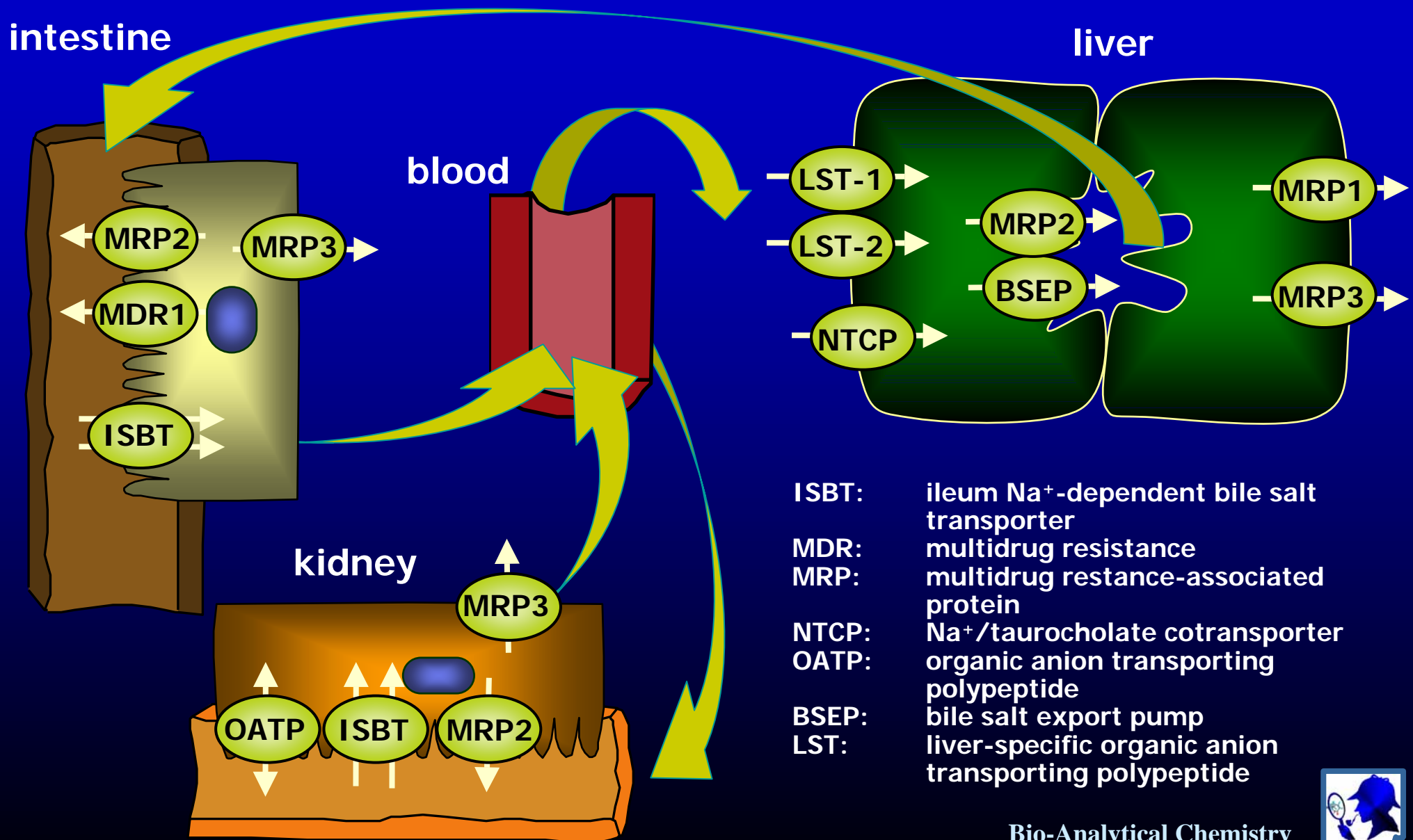
- Steroid**
- Sulfur containing function group**

$m+18 =$ molecular mass of underivatized steroid

Tandem Mass Spectrometry of the Sulfated Steroids



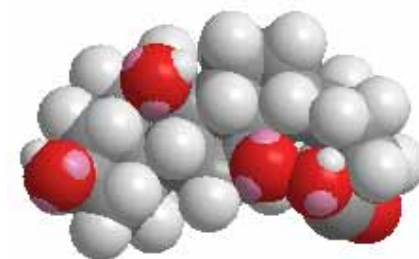
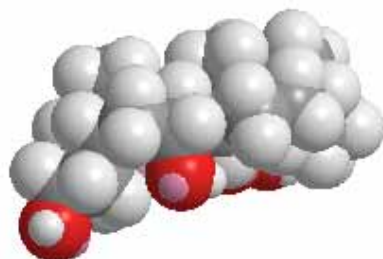
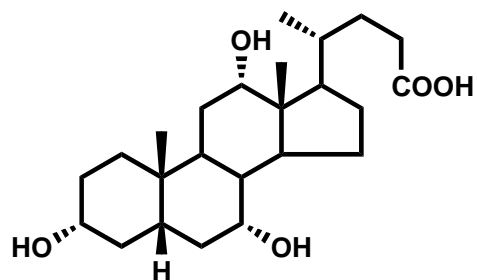
Bile Acids Transport Systems



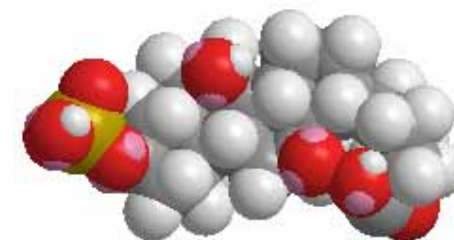
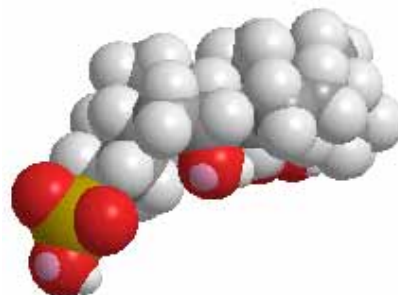
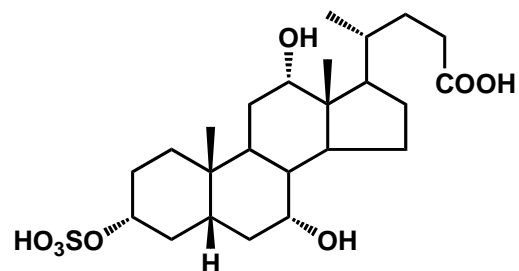
- ISBT: ileum Na⁺-dependent bile salt transporter
- MDR: multidrug resistance
- MRP: multidrug resistance-associated protein
- NTCP: Na⁺/taurocholate cotransporter
- OATP: organic anion transporting polypeptide
- BSEP: bile salt export pump
- LST: liver-specific organic anion transporting polypeptide



Cholic acid



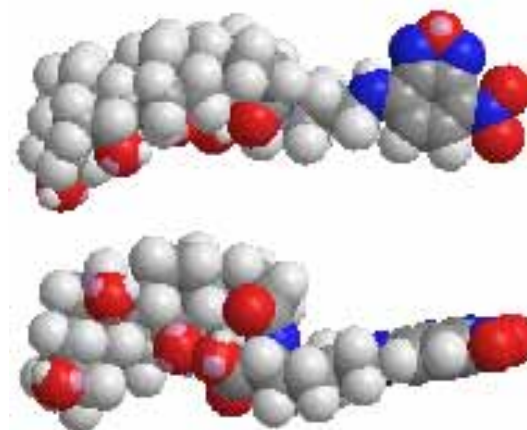
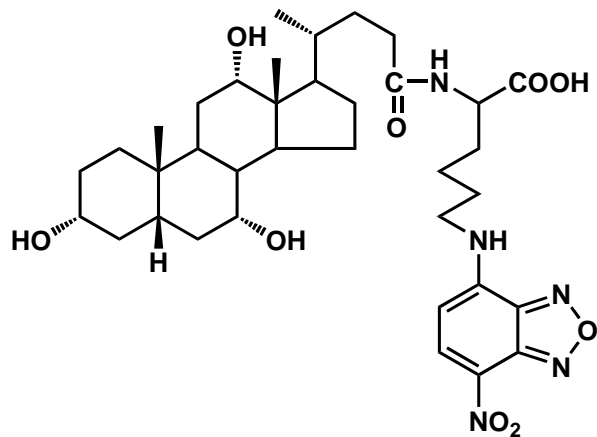
Cholic acid 3-sulfate



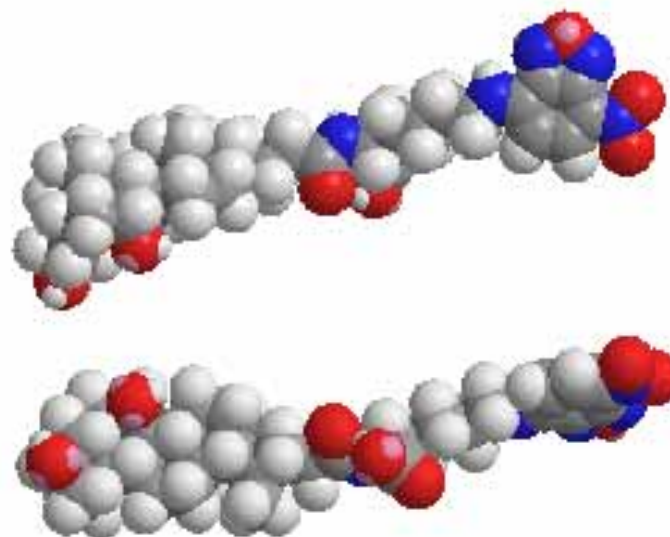
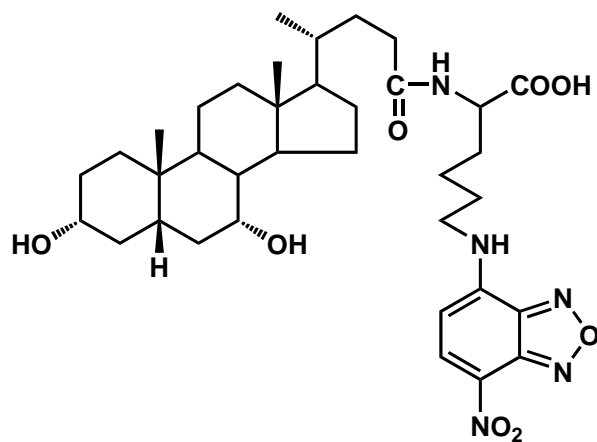
Structures of Cholic Acid and Cholic Acid 3-Sulfate



CA-(N ϵ -NBD)-lysine



CDCA-(N ϵ -NBD)-lysine



Structures of CA-(N ϵ -NBD)-lysine and CDCA-(N ϵ -NBD)-lysine



Analysis Mode

IN Cell Analyzer 1000 Workstation

File Edit View Image Mode Settings Sample Application Help

Summary

Hyperbola

Operations

Data: D:\IWATA\TOHOKUDAI\1449 A\DR.YAMAGUCHI 001.L02

Plate: 1449 A Well: A - 4... 0 ms Cell: Summary Clear

Well	Cell	Nuclei Count	Mean Nuclei Intensity
A - 6Hd 1	Summary	21.000	306.968
A - 6Hd 1		23.000	320.718
A - 6Hd 1		23.000	343.232
A - 6Hd 2		139.000	331.361
A - 6Hd 2		130.000	327.564
A - 6Hd 2		140.000	325.972
A - 1Hd 1		32.000	269.811
A - 1Hd 1		94.000	263.971
A - 1Hd 1		93.000	258.926
A - 1Hd 2		102.000	272.604
A - 1Hd 2		102.000	298.032
A - 1Hd 2		103.000	308.124
A - 2Hd 1		196.000	270.291
A - 2Hd 1		199.000	272.252
A - 2Hd 1		197.000	266.477
A - 2Hd 2		136.000	265.124
A - 2Hd 2		134.000	261.285
A - 2Hd 2		134.000	261.554
A - 2Hd 1		167.000	273.088
A - 2Hd 1		166.000	272.007
A - 3Hd 1		171.000	269.402
A - 3Hd 2		121.000	269.562
A - 3Hd 2		120.000	265.925
A - 3Hd 2		119.000	263.229
A - 4Hd 1		197.000	291.769
A - 4Hd 1		199.000	306.639
A - 4Hd 1		202.000	316.889
A - 4Hd 1		149.000	340.942
A - 4Hd 2		149.000	368.365
A - 4Hd 2		151.000	365.027
A - 5Hd 1		192.000	289.505
A - 5Hd 1		191.000	301.765
A - 5Hd 1		194.000	316.926
A - 5Hd 2		127.000	324.016
A - 5Hd 2		127.000	328.828
A - 5Hd 2		127.000	305.525

Image View - Ch 1 - D:\IWATA\TOHOKUDAI\1449 A\A - 4(Hd 2 wv 5475_20K - D535_30M - time 1 - 0 ms).tif

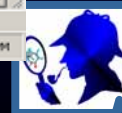
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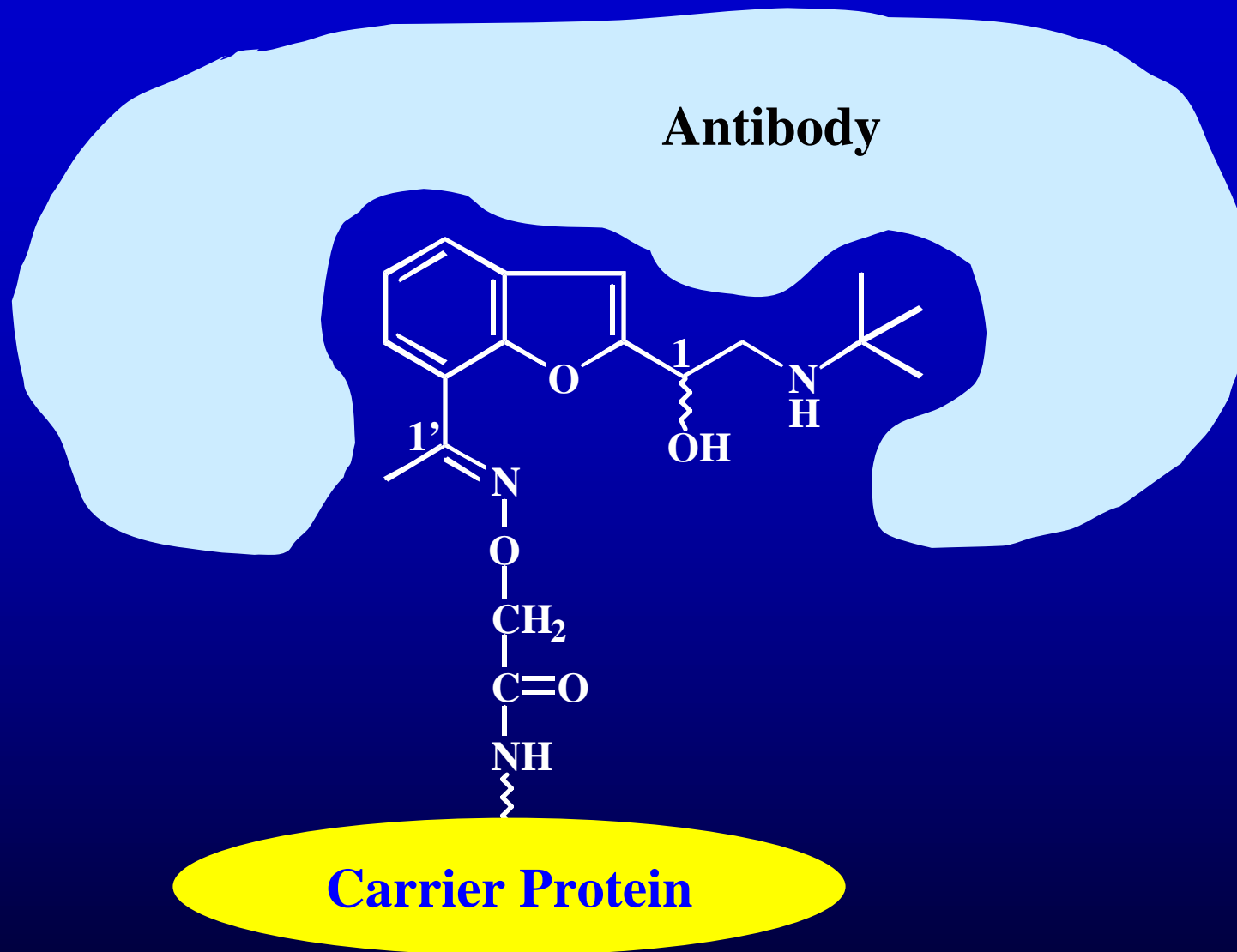
Image View - Ch 3 - D:\IWATA\TOHOKUDAI\1449 A\A - 4(Hd 2 wv TL - Bright field - open - time 1 - 0 ms).tif

Image Map - Ch 1

Start IN Cell Analyzer 1000 1315 Zwell IN Cell Analyzer 1000 W... Dr.Yamaguchi 001.graph Dr.Yamaguchi 001-ABCD IN Cell Analyzer 1000 ... Microsoft PowerPoint - [...]

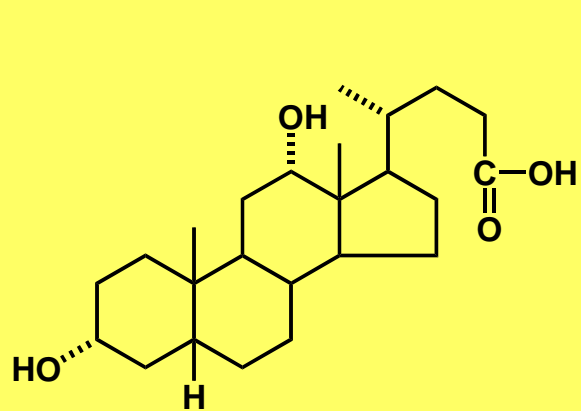
4:27 PM



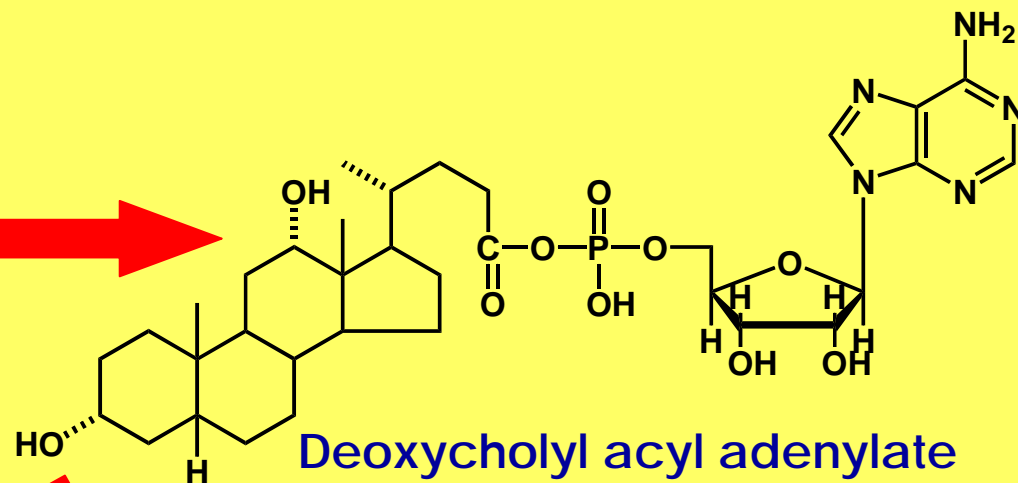


Design of Immunogen for Producing Antibody with Broad Affinity Spectrum

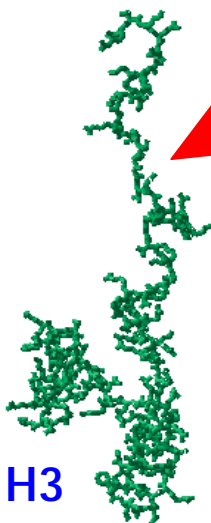




Deoxycholic acid

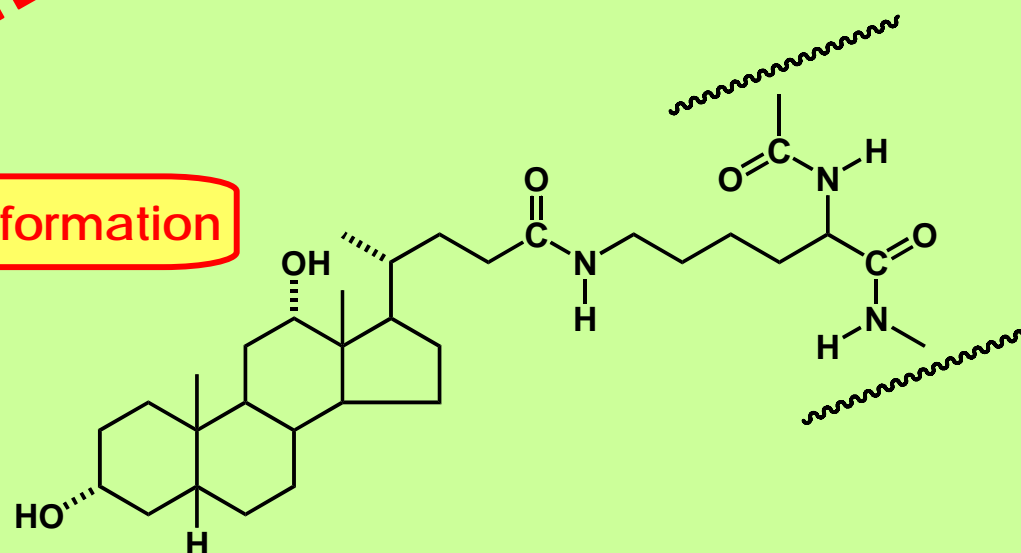


Deoxycholyl acyl adenylate



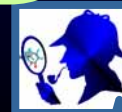
Histone H3

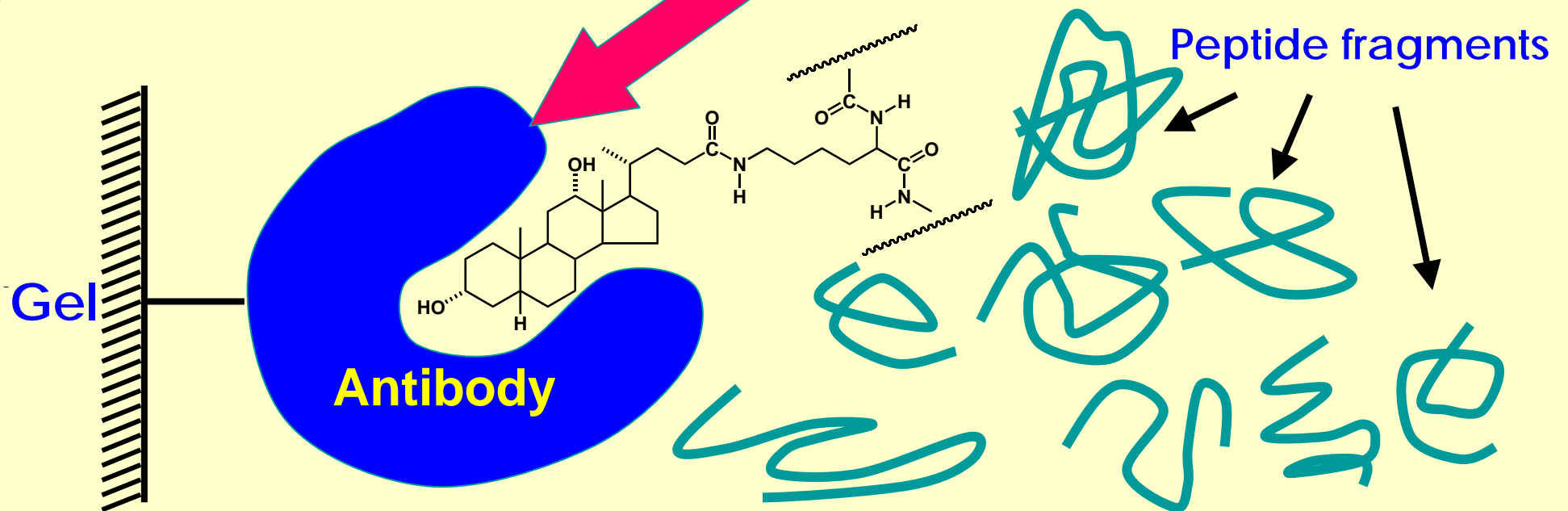
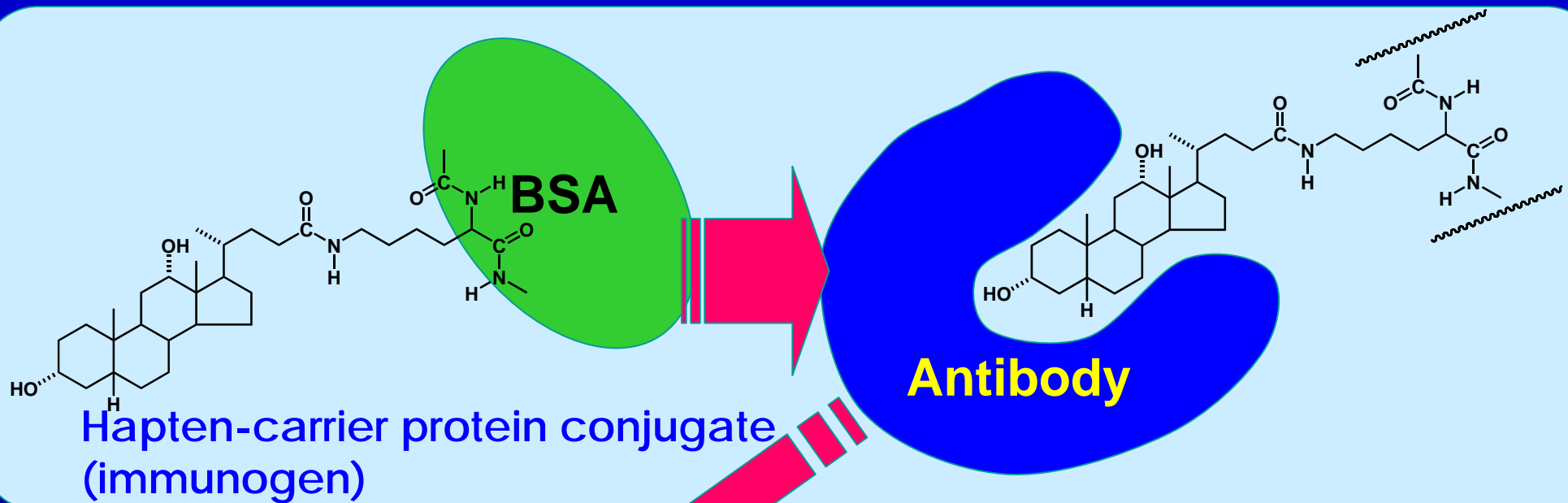
Adduct formation



Deoxycholic acid-histone H3 adduct
via lysine residue

Deoxycholic Acid-Histone H3 Adduct Formation via Acyl Adenylate



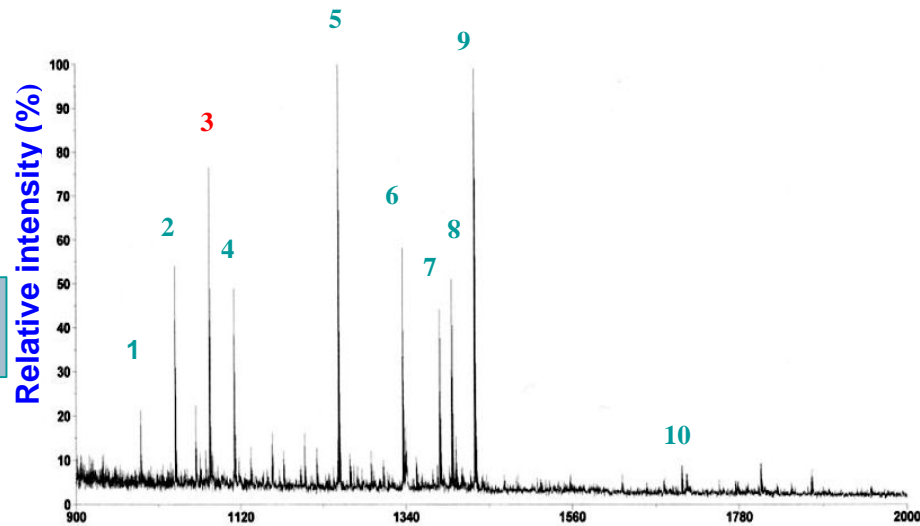


Preparation of Anti-DCA Monoclonal Antibody-Immobilized Gel

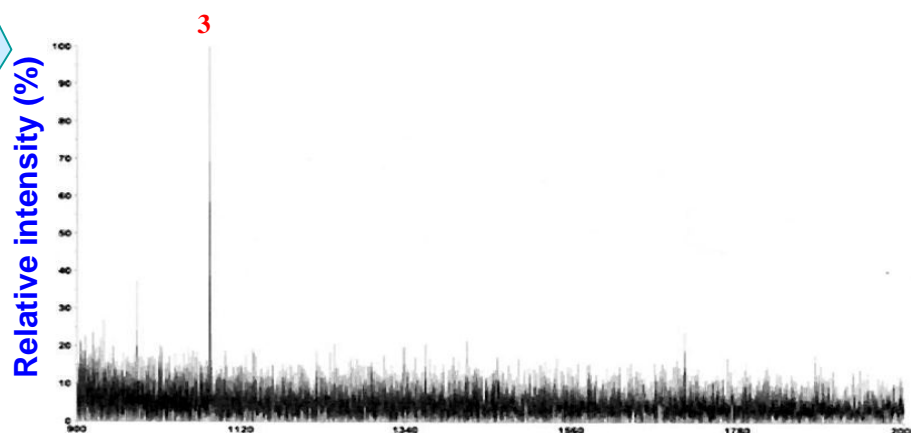


DCA-AMP:Histone H3=1:1

Before immunoaffinity extraction

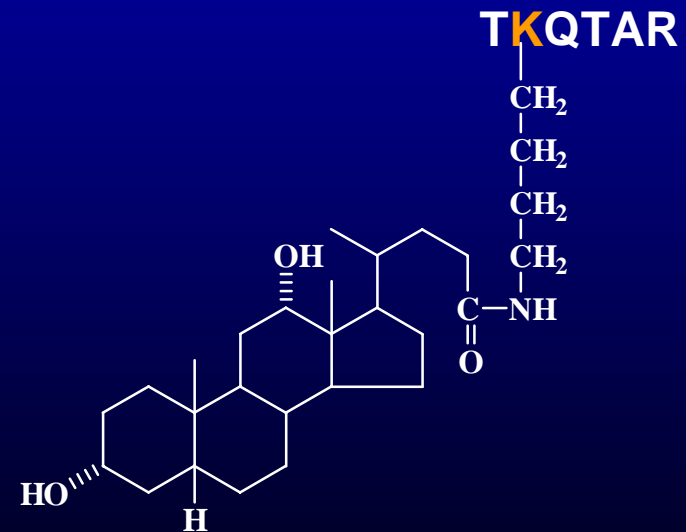


After immunoaffinity extraction



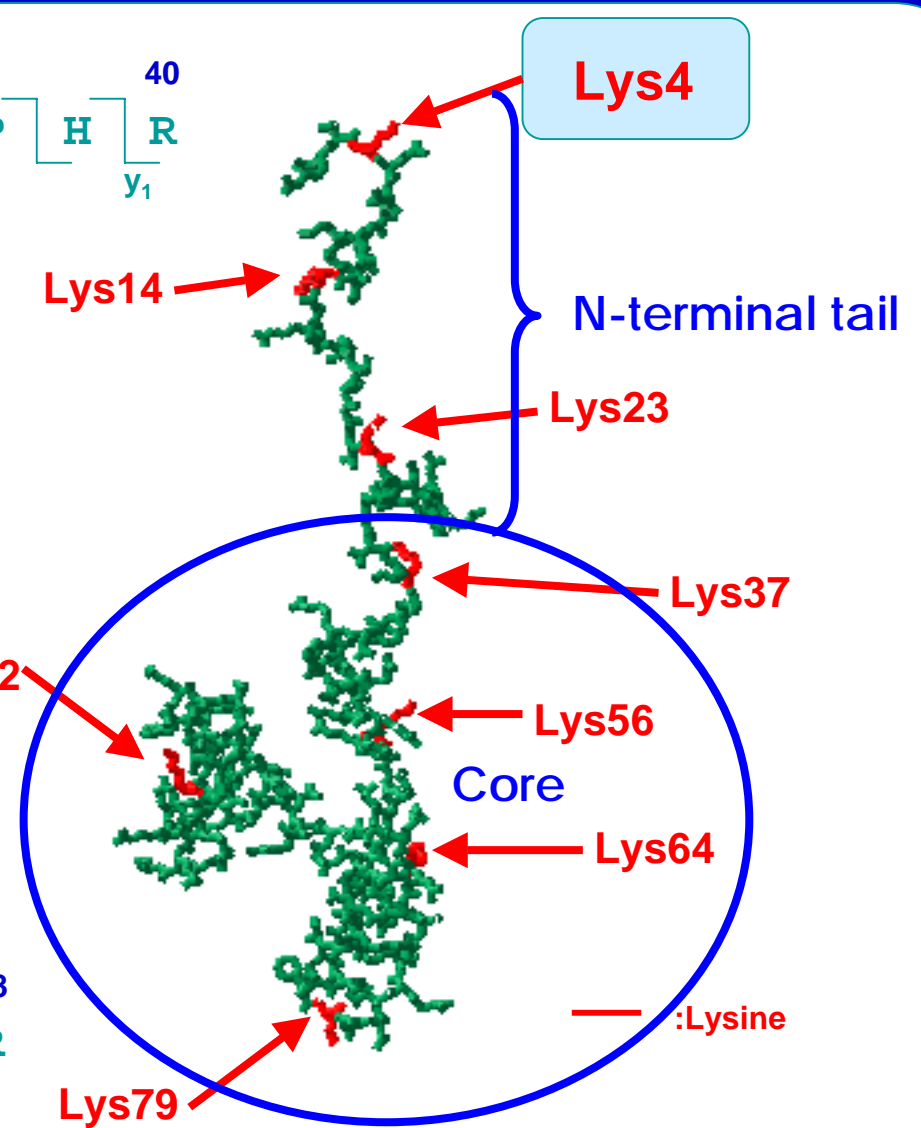
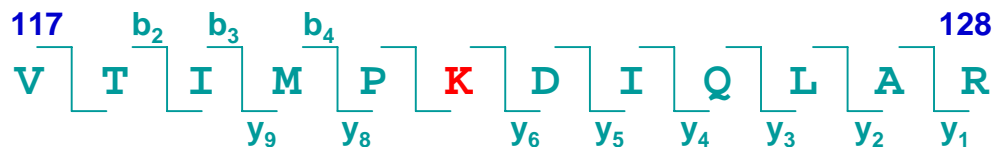
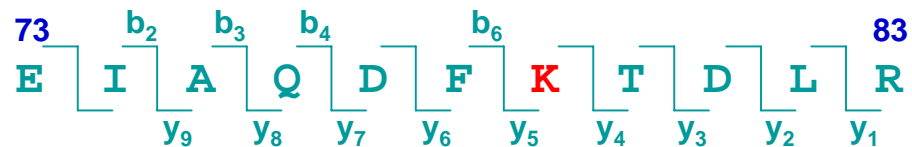
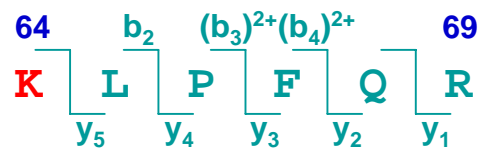
- 1: KQLATKAAR (18-26)
- 2: YRPGTVALR (41 - 49)
- 3: **TKQ**TAR (3 - 8) + DCA
- 4: PGTVALREIR (43-52)
- 5: YQKSTELLIR (54 - 63)
- 6: EIAQDFKTDLR (73 - 83)
- 7: VTIMPKDIQLAR (117 -128)
- 8: VTIM_{ox}PKDIQLAR (117 -128)
- 9: YRPGTVALREIR (41 - 52)
- 10: LVREIAQDFKTDLR (70-83)

K: predicted DCA binding site



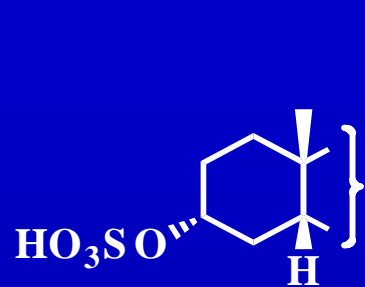
Predominantly Adduct Formation at Lys4 in Histone H3





DCA Binding Sites at Histone H3





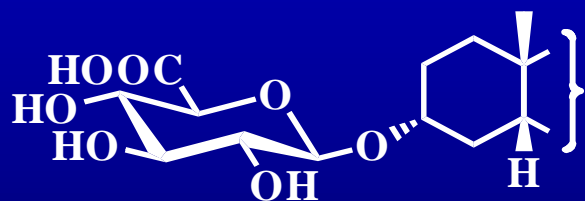
3-Sulfates



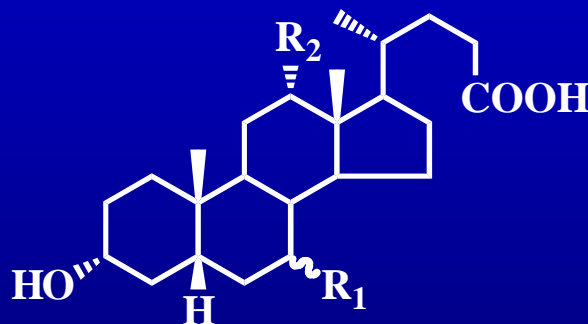
Taurine conjugates



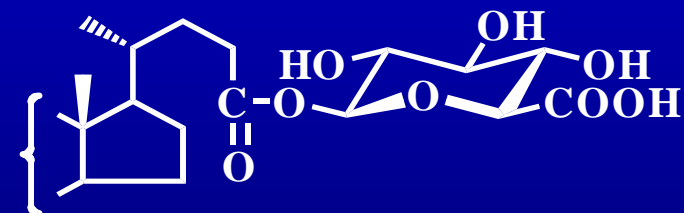
Glycine conjugates



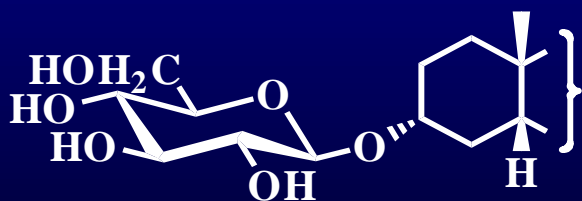
3-Glucuronides



$R_1 = \text{H}, \alpha\text{-OH}, \beta\text{-OH}$
 $R_2 = \text{H}, \text{OH}$



24-Glucuronides



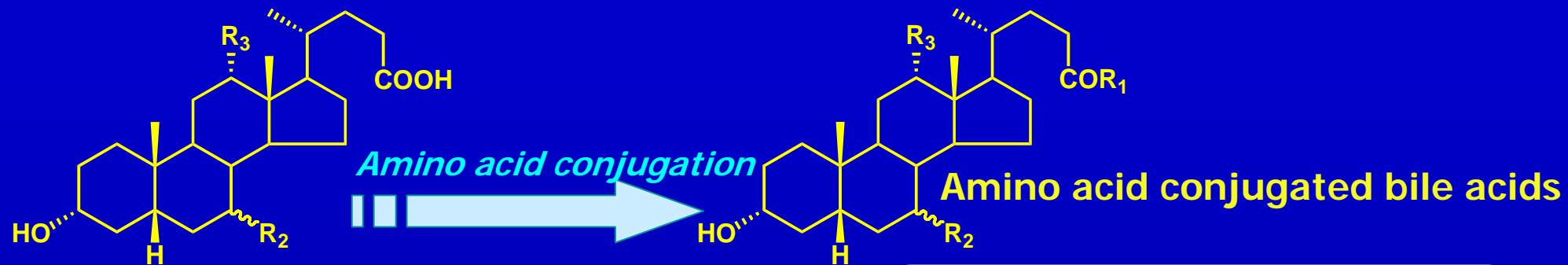
3-Glucosides



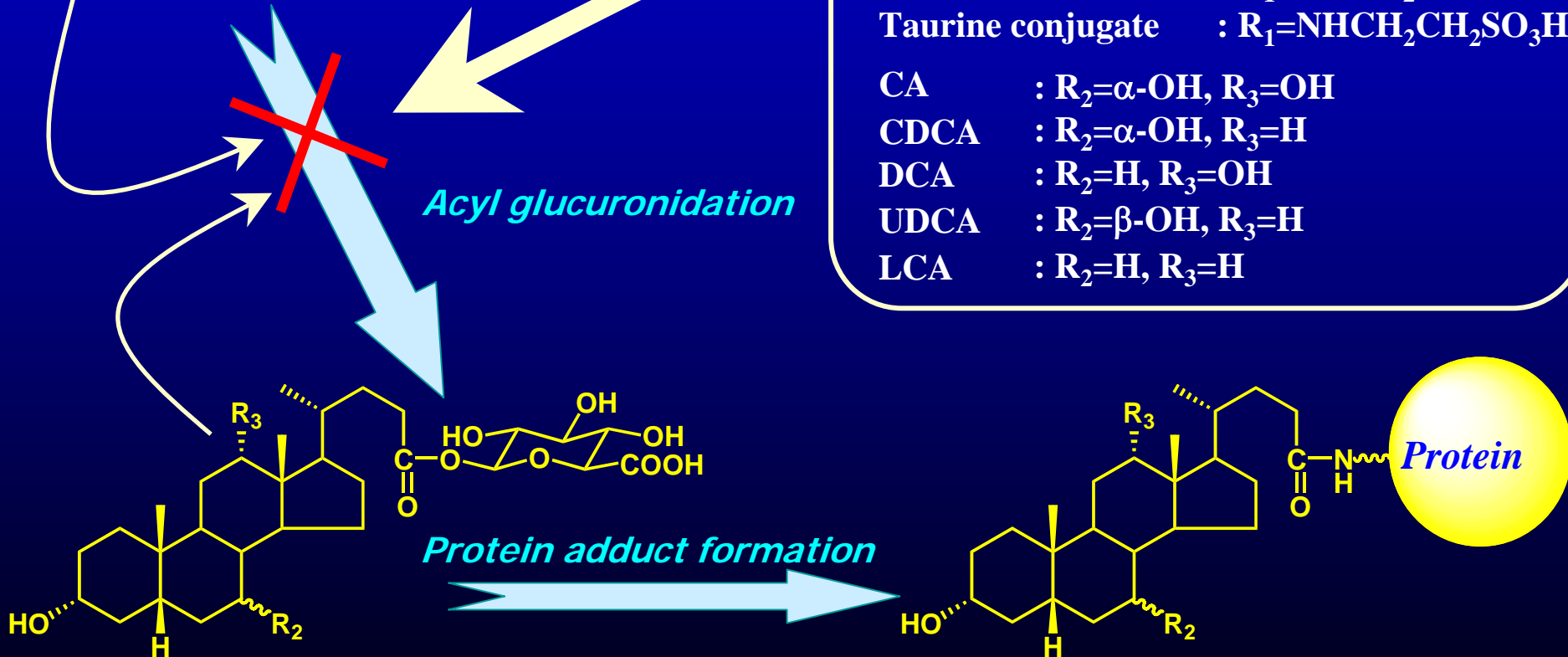
7-N-Acetylglucosaminides

Various Conjugation of Bile Acids



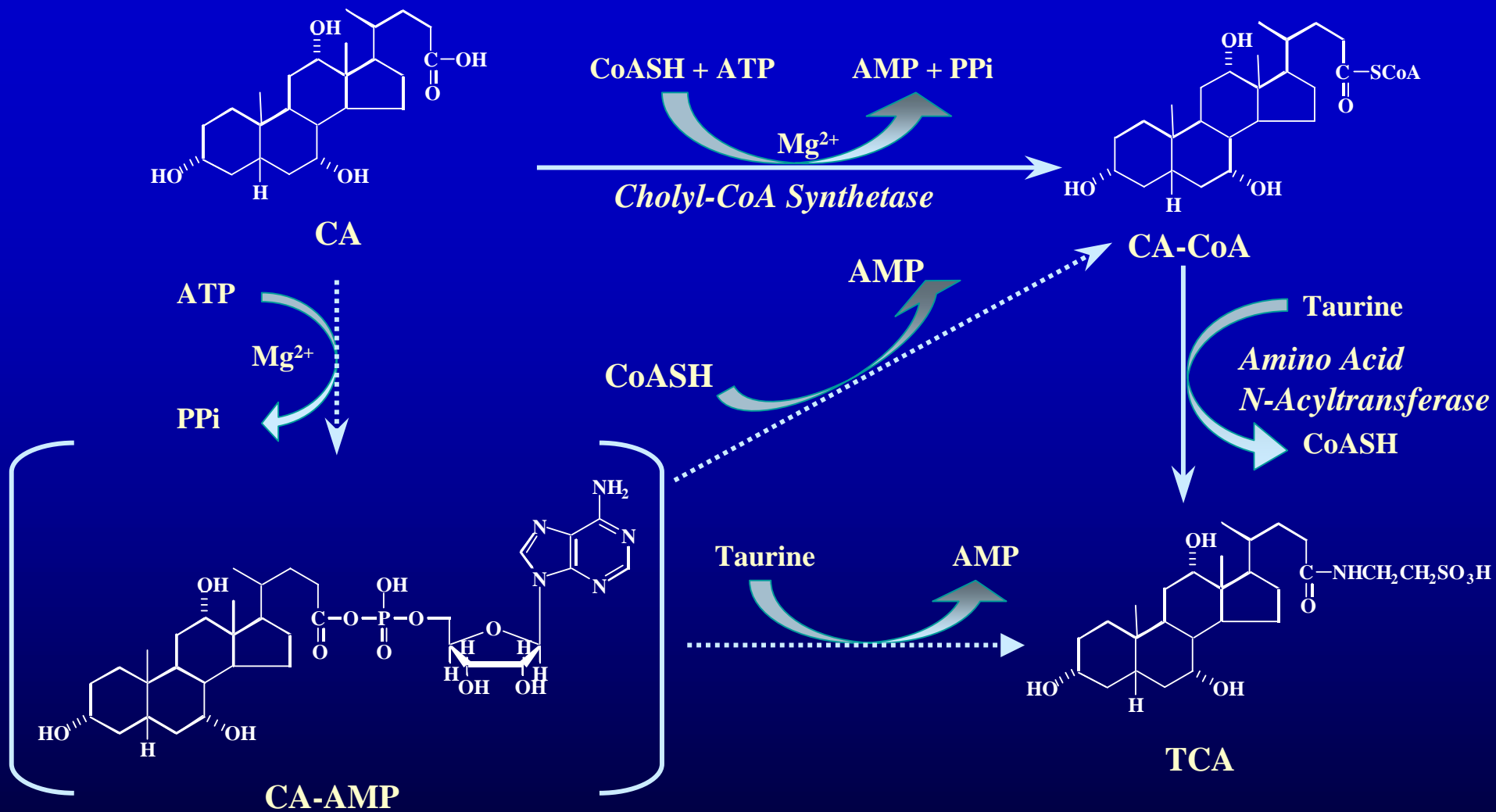


Unconjugate	: $R_1=OH$
Glycine conjugate	: $R_1=NHCH_2COOH$
Taurine conjugate	: $R_1=NHCH_2CH_2SO_3H$
CA	: $R_2=\alpha-OH, R_3=OH$
CDCA	: $R_2=\alpha-OH, R_3=H$
DCA	: $R_2=H, R_3=OH$
UDCA	: $R_2=\beta-OH, R_3=H$
LCA	: $R_2=H, R_3=H$



Bile acid 24-glucuronides





Proposed Mechanism for Biosynthesis of Taurocholic Acid



Anti-DCA monoclonal antibody (8.7 nmol)

DCA-AMP (17.4 nmol)

in 50 mM potassium phosphate buffer (pH 6.0)

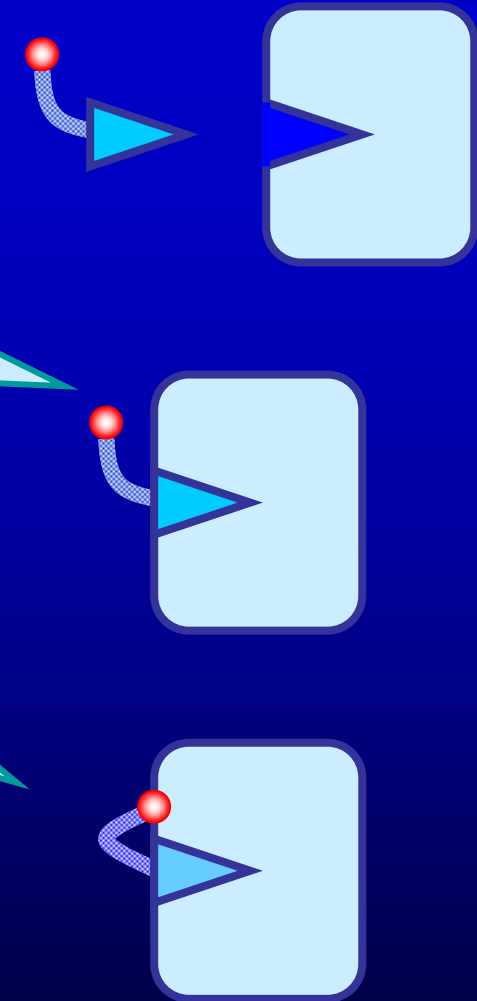
Incubate at 37°C for 2 hours

500 mM NaOH aq.

Incubate at 37°C for 24 hours (pH 7.4)

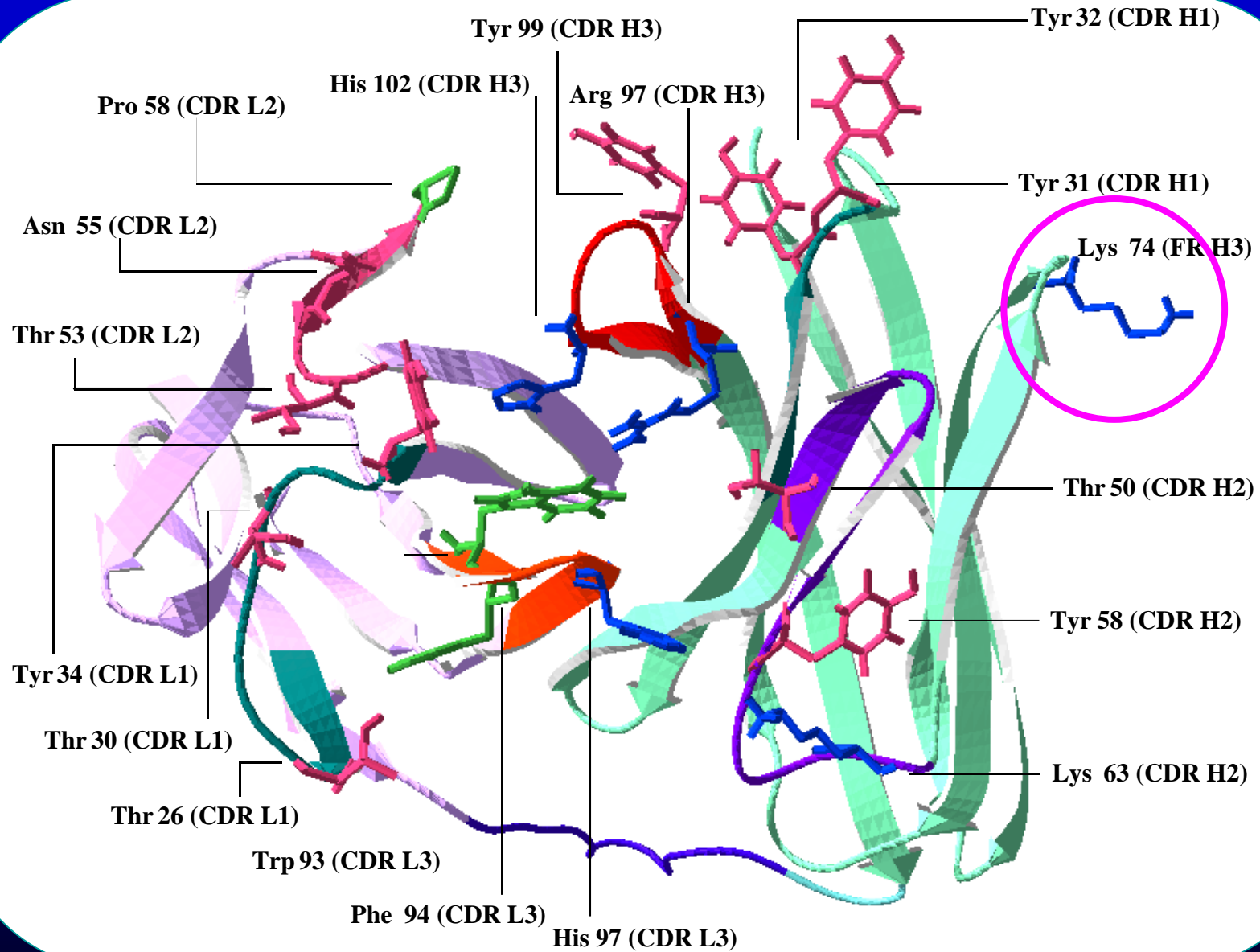
Iodocarbamidation

Trypsin digestion



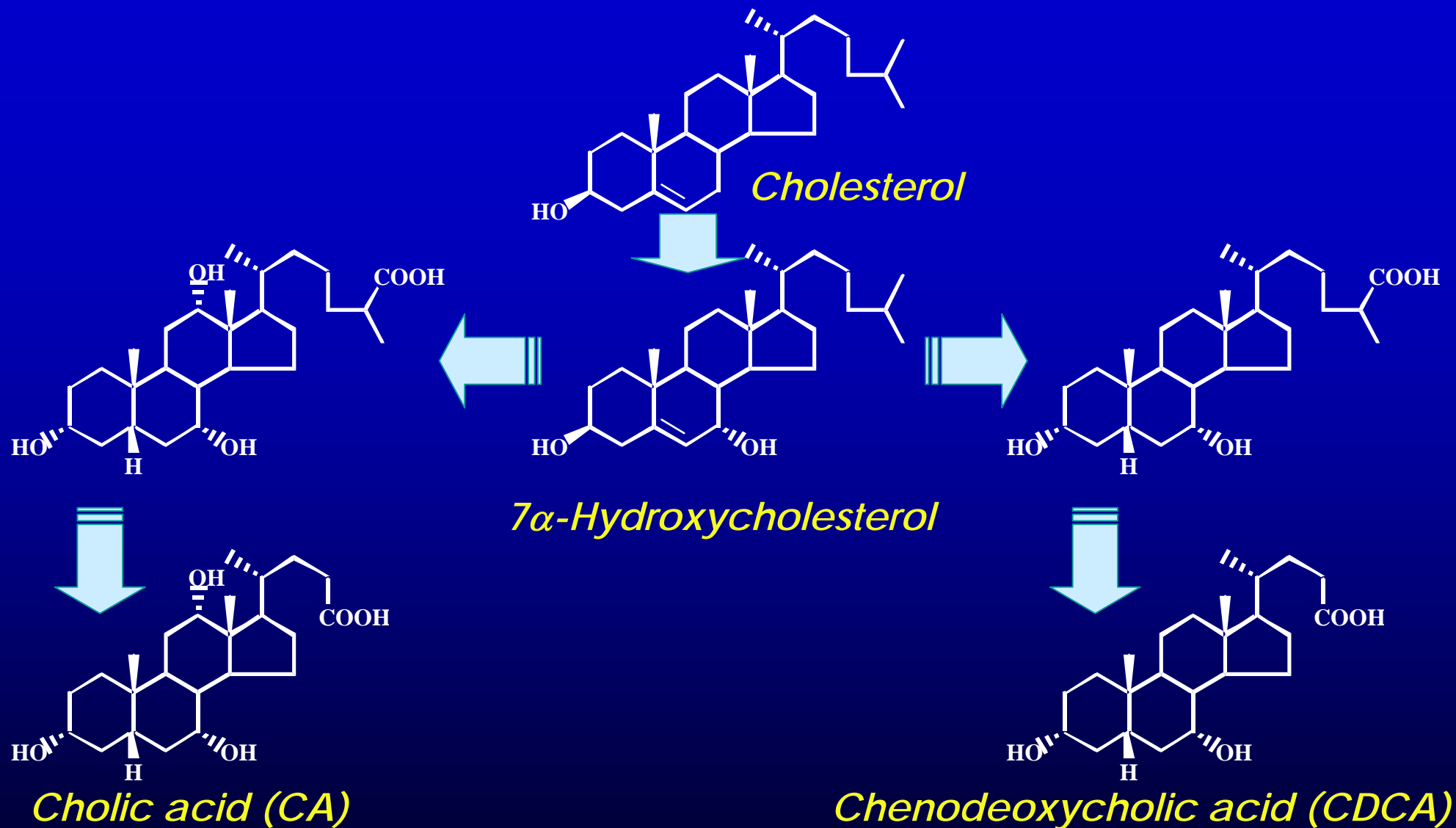
Procedure for Affinity Labeling of Deoxycholyadenylate to the Binding Site on the Anti-DCA Monoclonal Antibody





Protein Modeling of the ScFv of Anti-Deoxycholate Monoclonal Antibody with the SWISS-MODEL





Biosynthesis of Bile Acids from Cholesterol in Human Hepatocyte



Rat brain cytosolic fraction

1.65 M Tris-HCl buffer (pH 8.6)
containing 0.03 M EDTA,
7.3 M guanidine hydrochloride
300 μ L

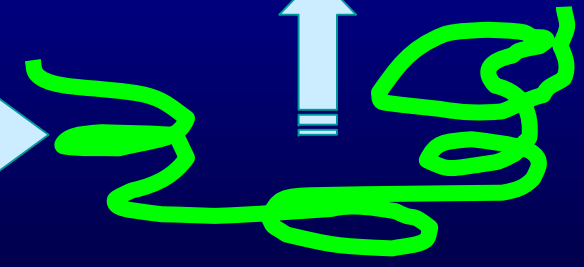
Mix for 60 min

Add hot ethanol 5 mL

Mix
sup.
3,000 rpm for 10 min

Evaporate

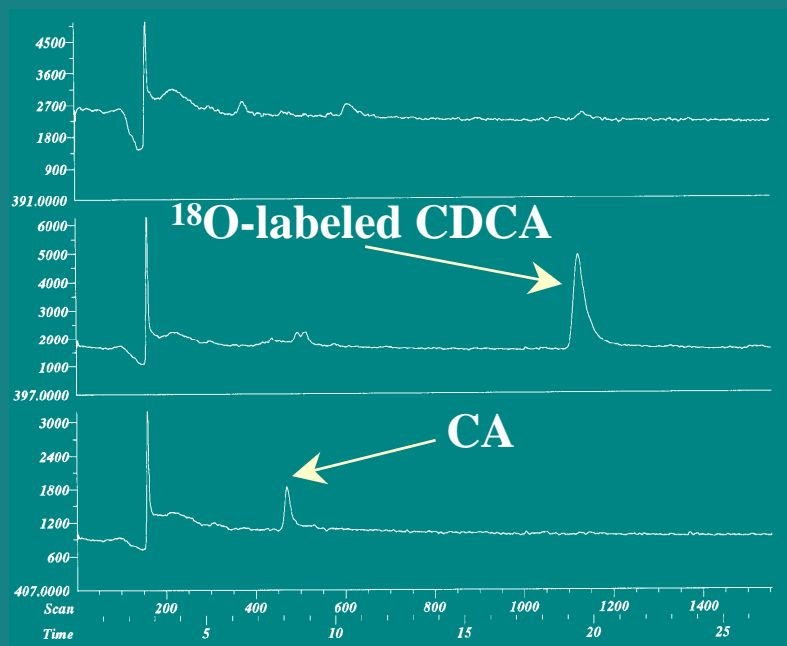
LC/ESI-MS



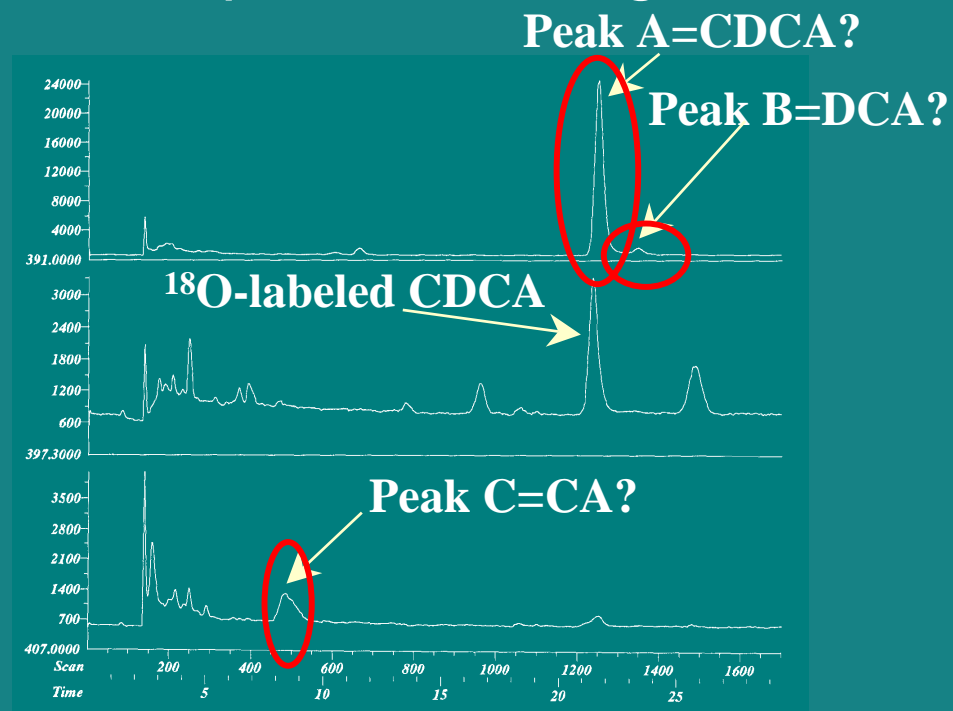
Procedure for Analysis of Bile Acids Bound with Protein in Rat Brain Cytosolic Fraction



(A) Without protein unfolding



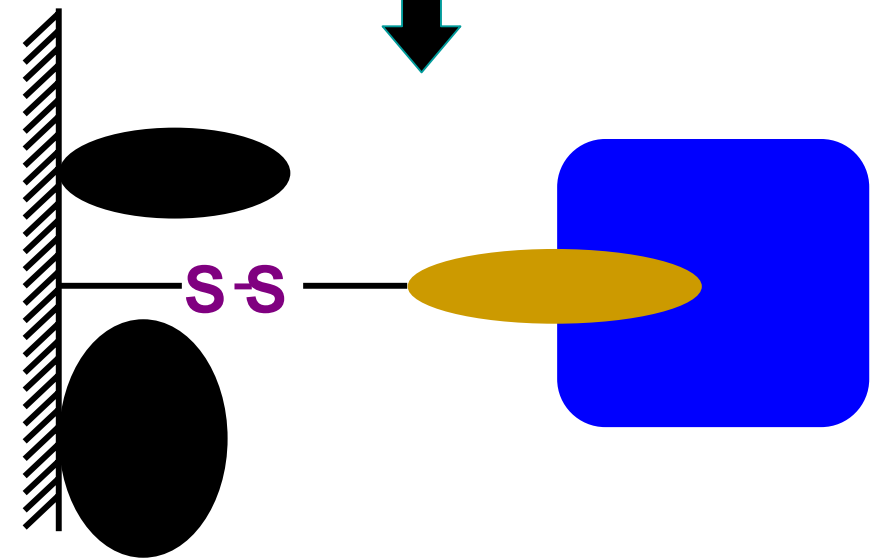
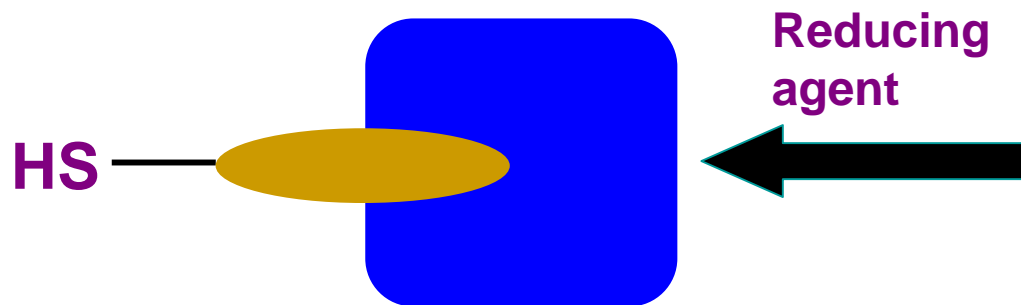
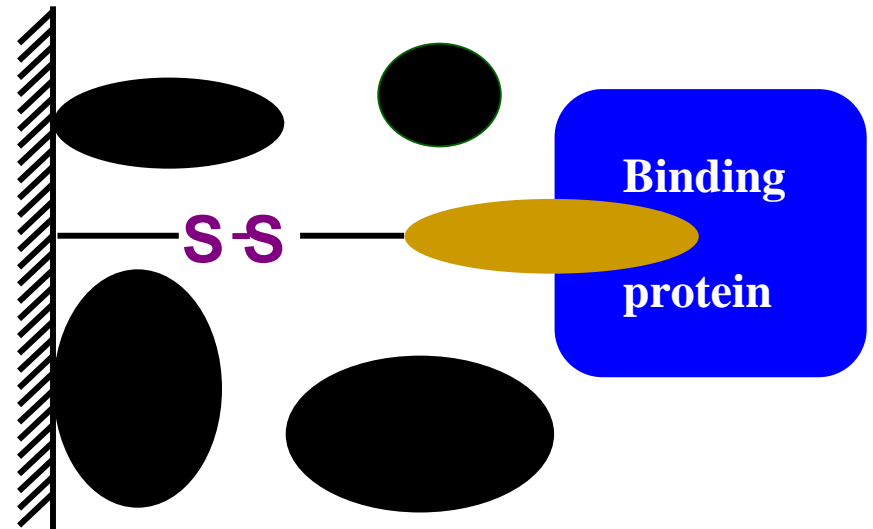
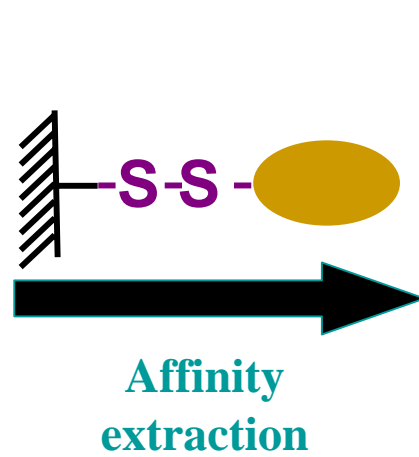
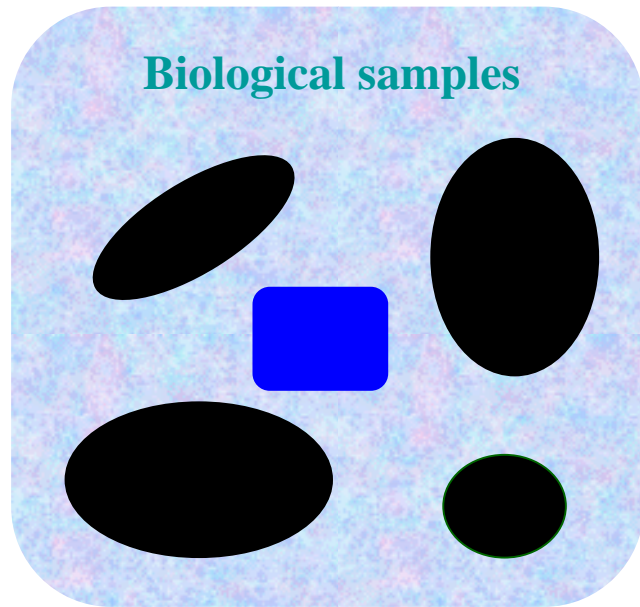
(B) With protein unfolding



LC/ESI-MS Analysis of Rat Brain Cytosolic Fraction with or without Protein Unfolding

Chromatographic conditions: column, Inertsil ODS-2 (2.1 mm i.d. x 150 mm), mobile phase; 20 mM ammonium acetate (pH 7.0)/acetonitrile (2:1), flow rate; 0.22 mL/min, electrospray voltage; -2.5 kV, orifice voltage; -20 V, ring lens voltage; -100 V.





Specific Extraction Strategy of Small Molecule-Binding Proteins by Cleavable Affinity Gel

