

第19回日本薬物動態学会年会（金沢）
2004.11.18

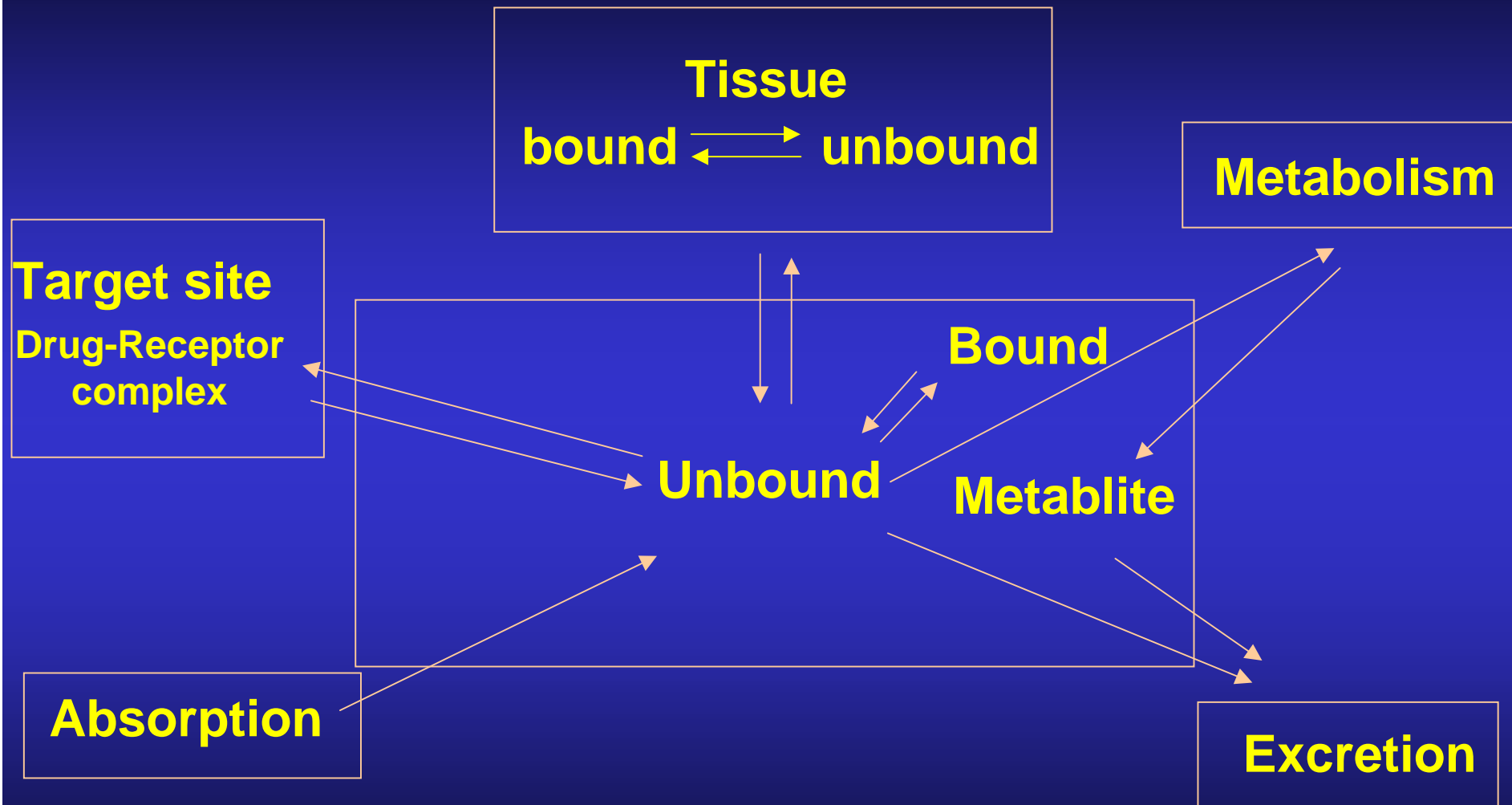
薬物と血清蛋白質との相互作用に関する 分子機能的な研究



熊本大学大学院医学薬学研究部
薬物動態制御学分野

小田切 優樹

Protein Binding and Distribution of Drug



Binding parameter, Binding Site

Approaches for structural biology

High-resolution
at atomic level

X-線結晶構造解析

Spectrometry

Genetics

Short cut
via gene

部位特異的変異法

Organic
Chemistry

Analysis through
Covalent bonds

光アフィニティラベル法

Structure of Fluorescent Probes



7-Anilino-4-Methylcoumarin
-3-Acetic Acid (ACMA)



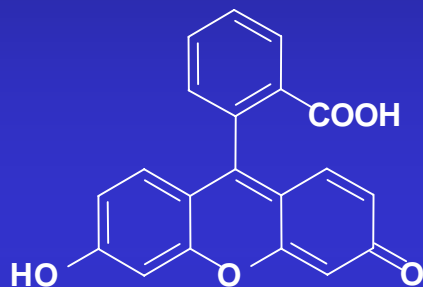
Dansyl-DL-Norleucine (DNSL)



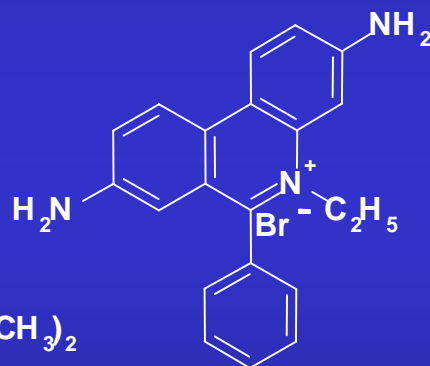
Pamaquine (PQ)



Primaquine (PR)



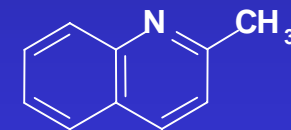
Fluorescein (Flu)



Ethidium Bromide (EB)



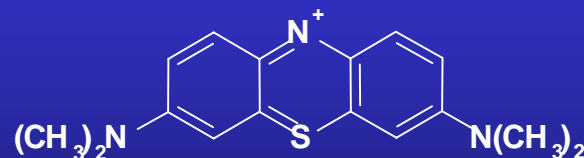
Acridine Orange-10-Dodesyl
Bromide (AODB)



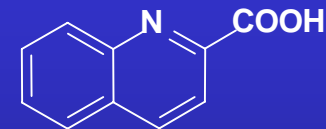
Quinaldine (QD)



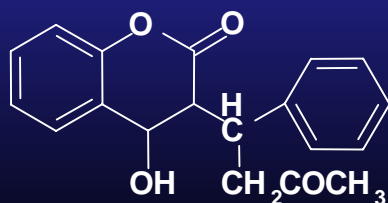
Auramin (AO)



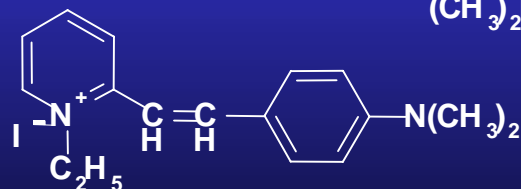
Methylene Blue (MB)



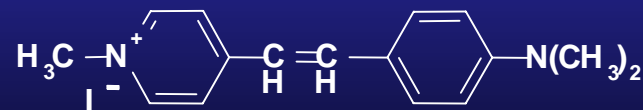
Quinaldic Acid (QA)



Warfarin (WF)



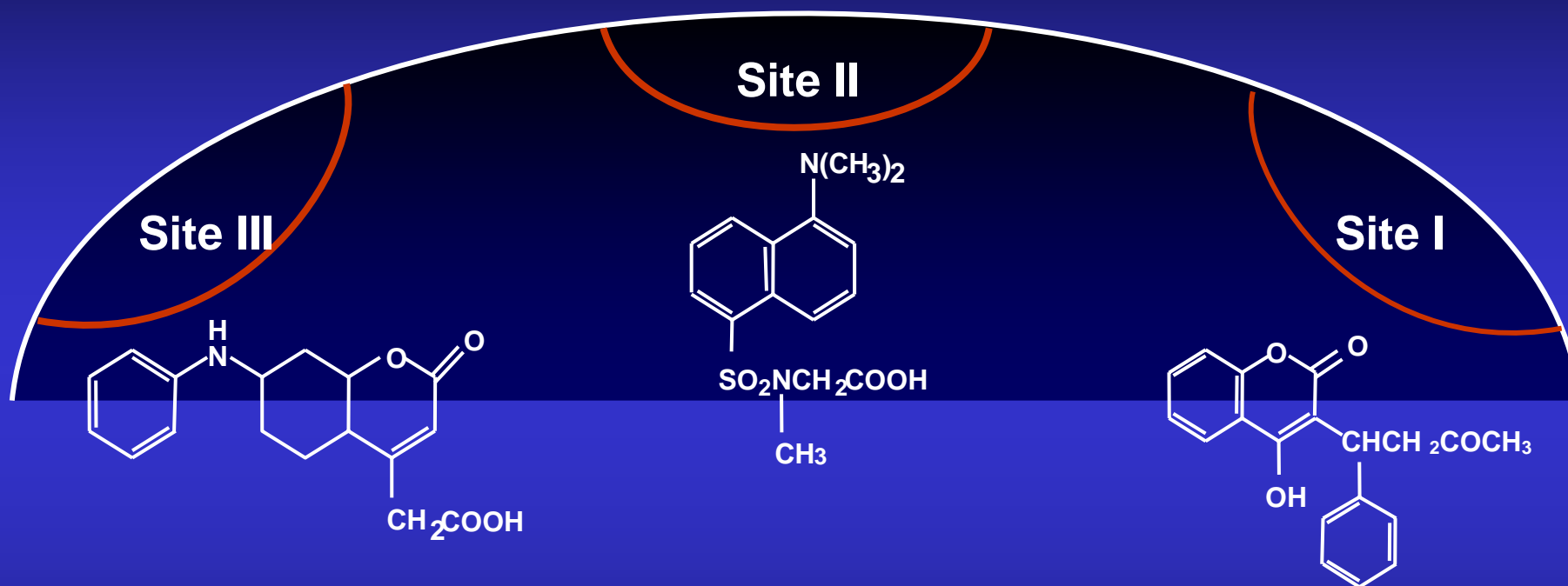
2-(4-Dimethylaminostyryl)-n
-ethylpyridinium Iodide (DASP)



4-(4-Diethylaminostyryl)-n
-ethylpyridinium Iodide (4-DASP)

Drug binding sites on HSA

G.Sudlow. et al: The characterization of two specific drug binding sites on human serum albumin. *Mol. Pharmacol.* **11**, 824-832. (1975)



Z value
(疎水性)

82

67

74

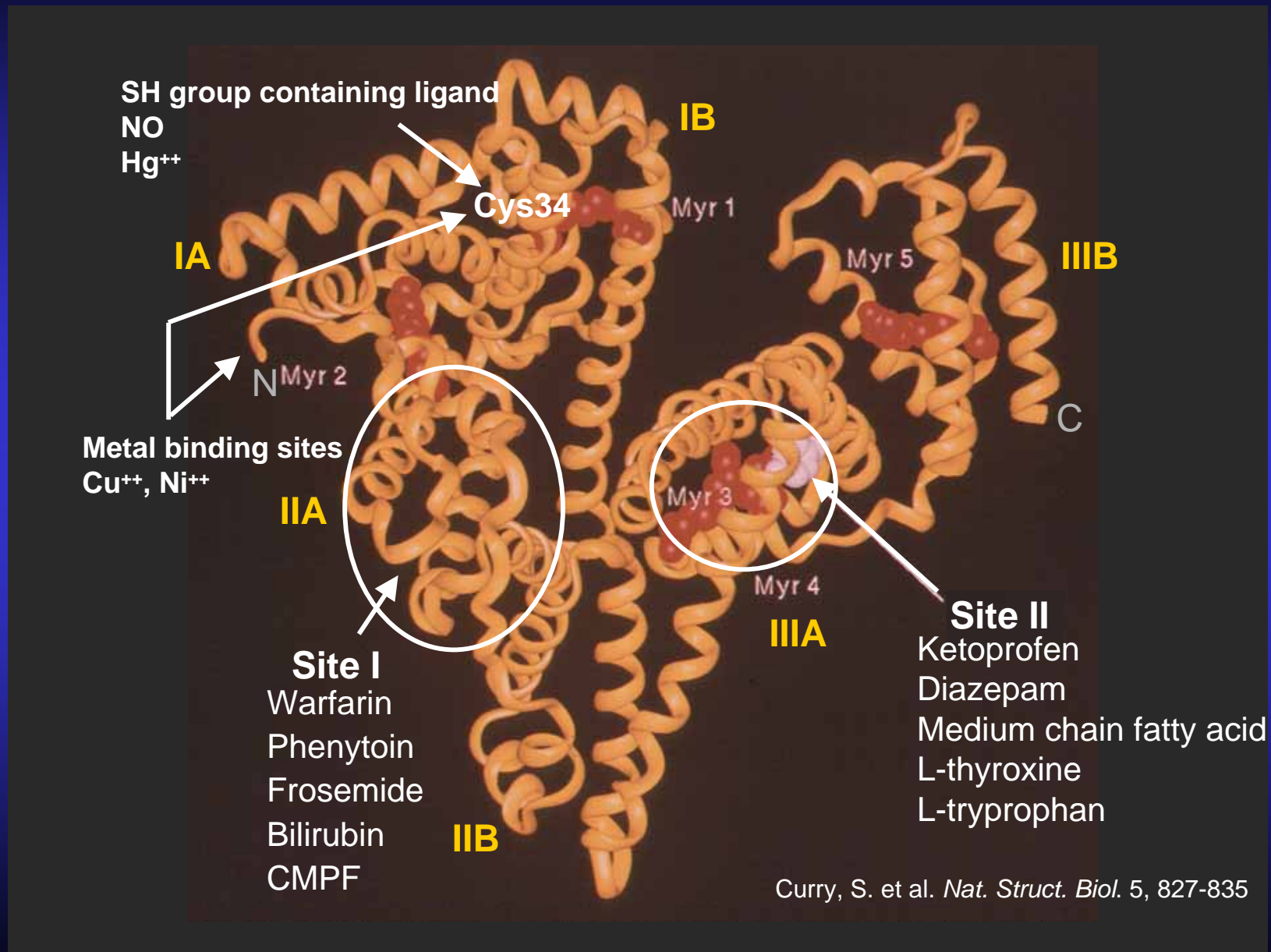
Depth

14Å

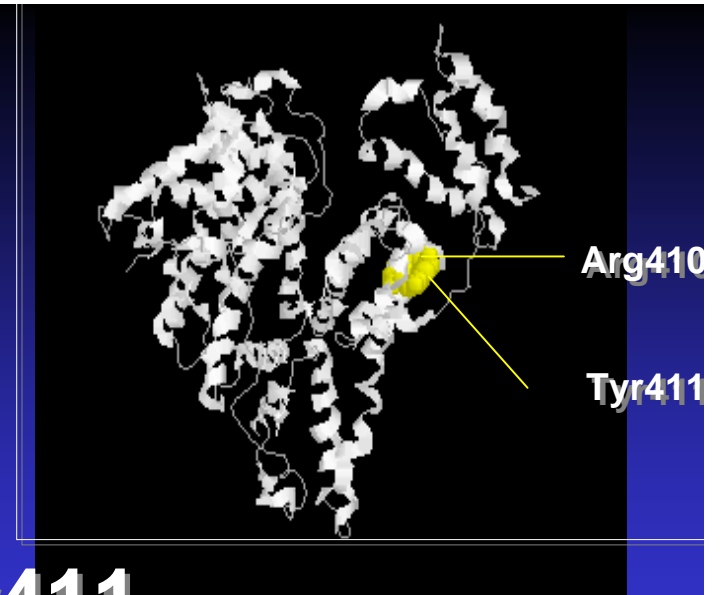
16Å

19Å

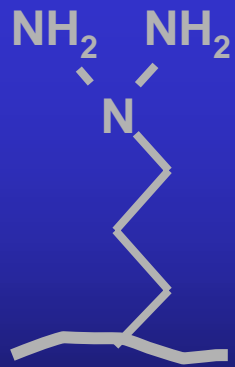
Location of the ligand binding sites on HSA



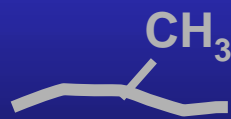
Type of mutations



Arg410



Wild-type

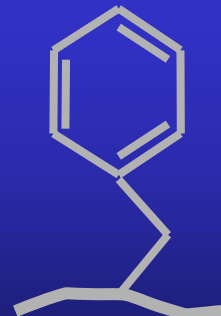


R410A

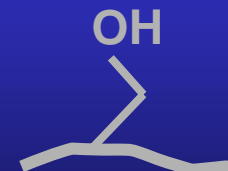
Tyr411



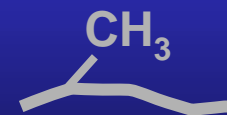
Wild-type



Y411F

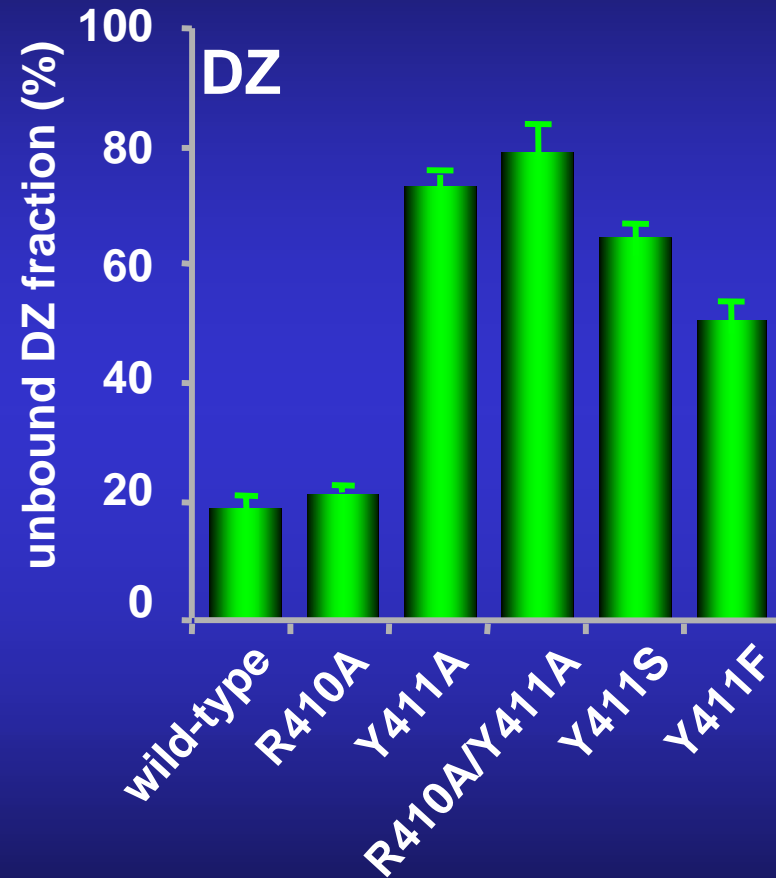
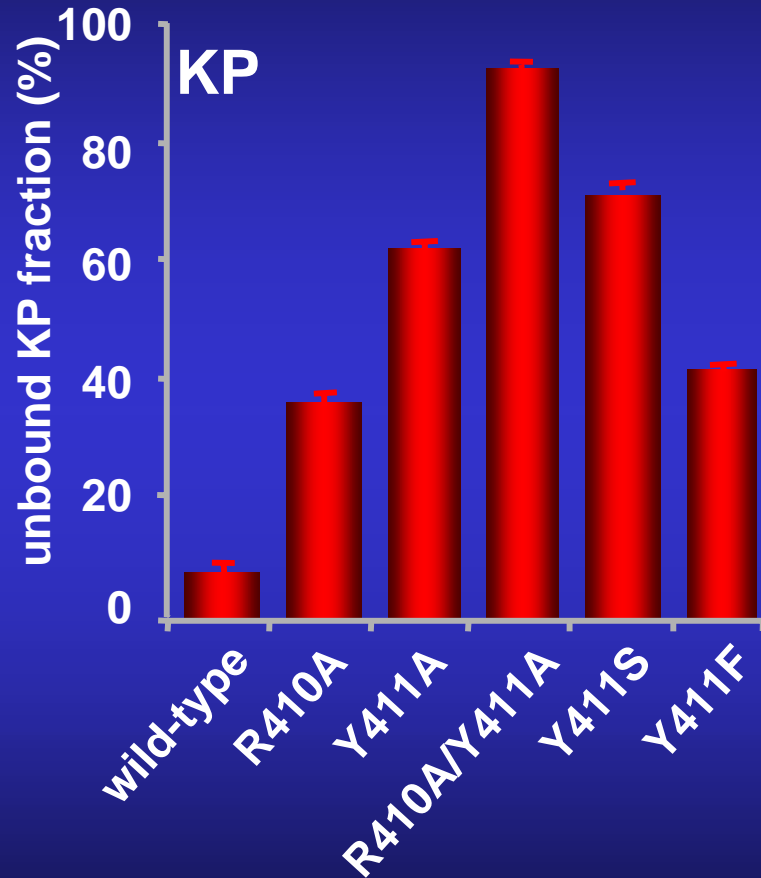


Y411S



Y411A

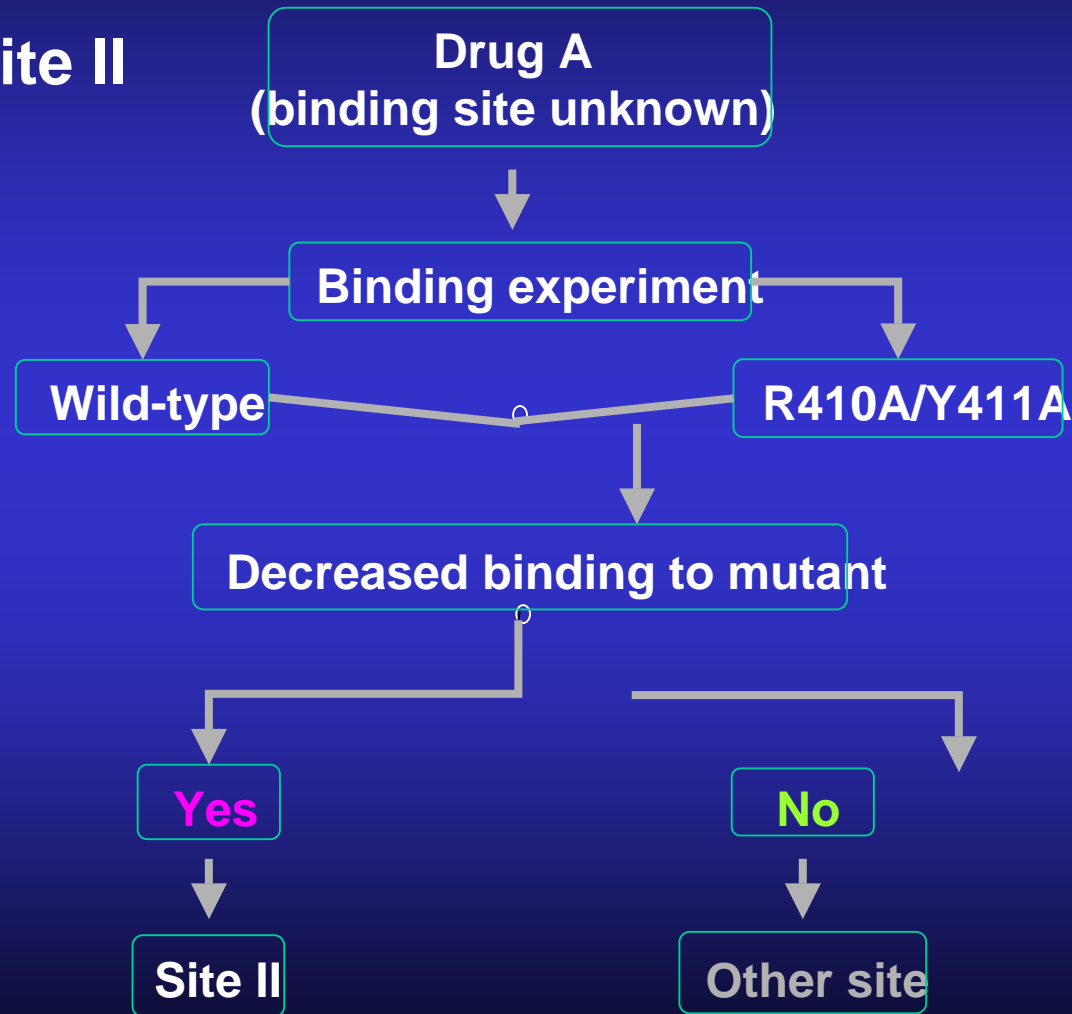
Ketoprofen (KP) と Diazepam (DZ) の各変異体への結合



The sample solutions contained 5 μ M KP or DZ and 10 μ M wild type or mutant HSA in 67mM sodium phosphate buffer (pH7.4).

HSA変異体を利用した薬物結合部位予測

e.g.) Site II

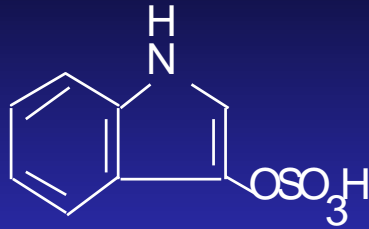


各種病態下での血清蛋白結合の変動とその要因

	薬物結合		蛋白濃度		内因性蛋白結合阻害物質の蓄積
	酸性薬物	塩基性薬物	HSA	AGP	
腎疾患	↓	↑	↓	↑	尿毒症物質 遊離脂肪酸 (透析時) グリコシル化HSA カルバミル化HSA
ネフローゼ症候群	↓	↓	↓	↓	
肝疾患	↓	↓	↓	↓	ビリルビン 胆汁酸
糖尿病	↓		↓		グリコシル化HSA
心不全	↓		↓		
心筋梗塞		↑		↑	遊離脂肪酸
外傷	↓	↑	↓	↑	
炎症性疾患 ¹⁾		↑		↑	
妊娠	↓	↑	↓	↑	遊離脂肪酸 その他?

1) 関節リウマチ、潰瘍性大腸炎、クローン病

HSA bound uremic toxins

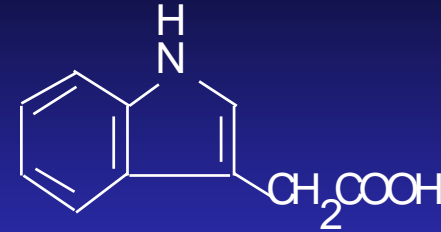


indoxyl sulfate

(IS)

$K_a = 9.1 \times 10^5$ (Site II)

血中濃度 ; 104.3 ± 49.4 (4.1 μM)

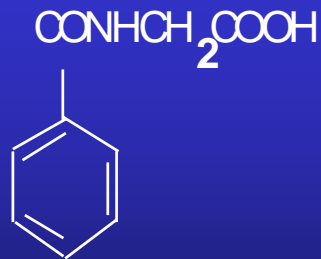


indoleacetic acid

(IA)

$K_a = 2.1 \times 10^5$ (Site II)

血中濃度 ; 26.3 ± 11.7 (1.45 μM)

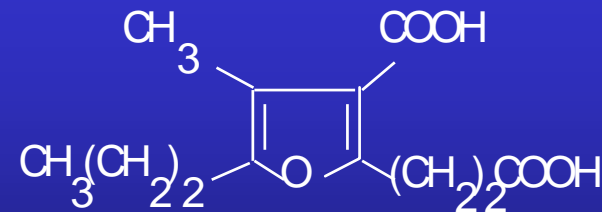


hippuric acid

(HA)

$K_a = 0.1 \times 10^5$ (Site II)

血中濃度 ; 270.9 ± 141.2 (13.3 μM)



3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid

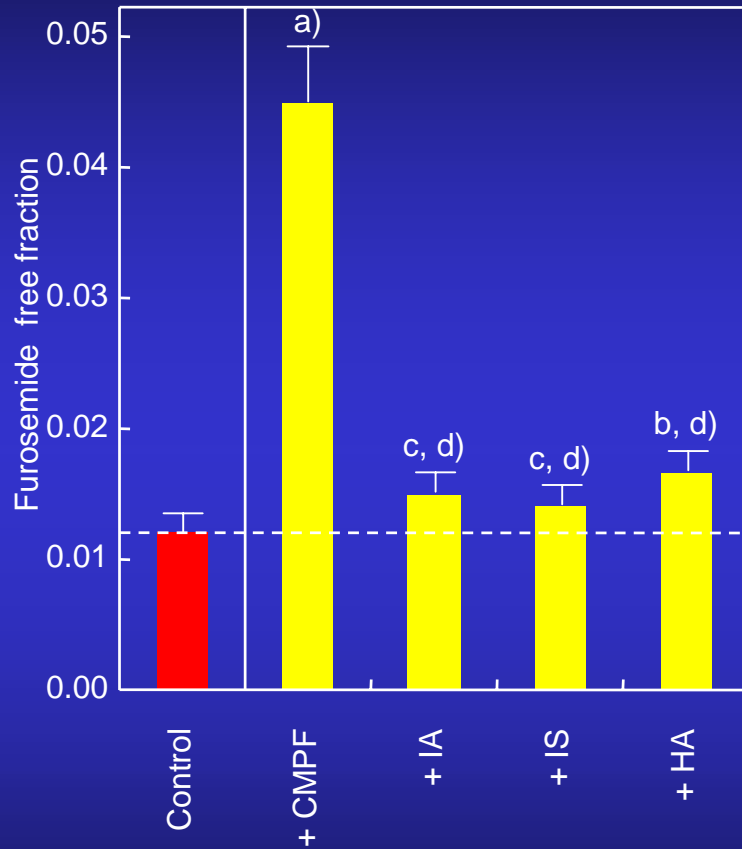
(CMPF)

$K_a = 130.5 \times 10^5$ (Site I)

血中濃度 ; 183.0 ± 38.0 (15.1 μM)

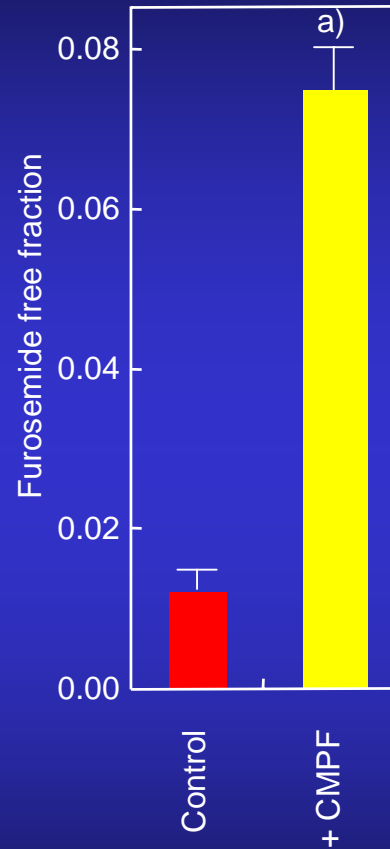
Drug-uremic toxin-fatty acid interaction

FR-uremic toxins



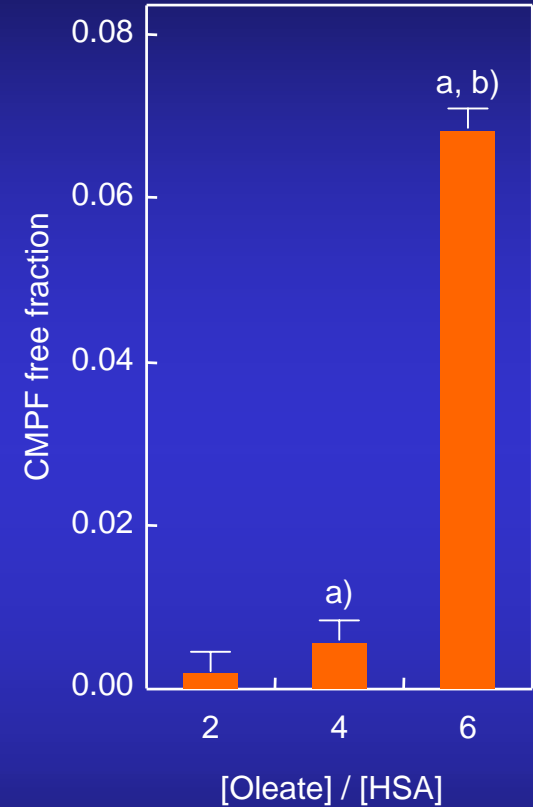
a: $P < 0.001$ vs Control
b: $P < 0.01$ vs Control
c: $P < 0.05$ vs Control
d: $P < 0.01$ vs + CMPF

FR-CMPF-oleate



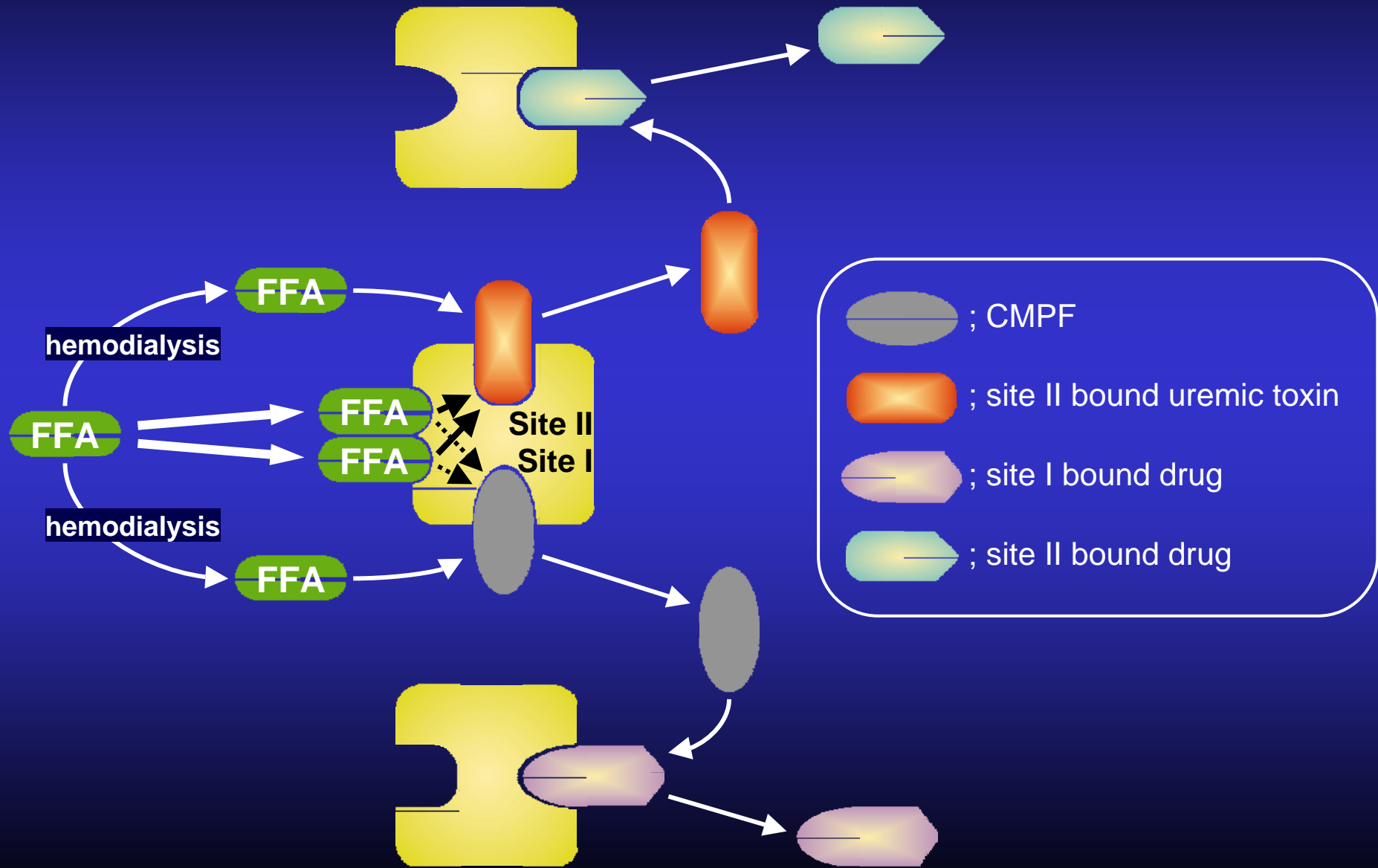
[oleate]/[HSA] = 6
a: $P < 0.01$ vs Control

CMPF-oleate

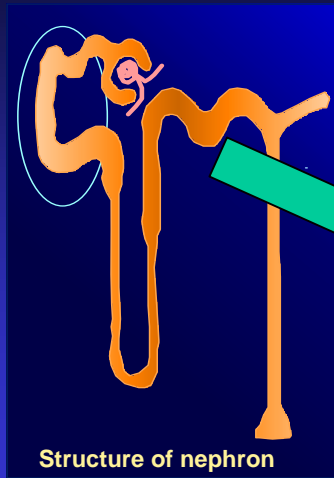


a: $P < 0.001$ vs 2
b: $P < 0.001$ vs 4

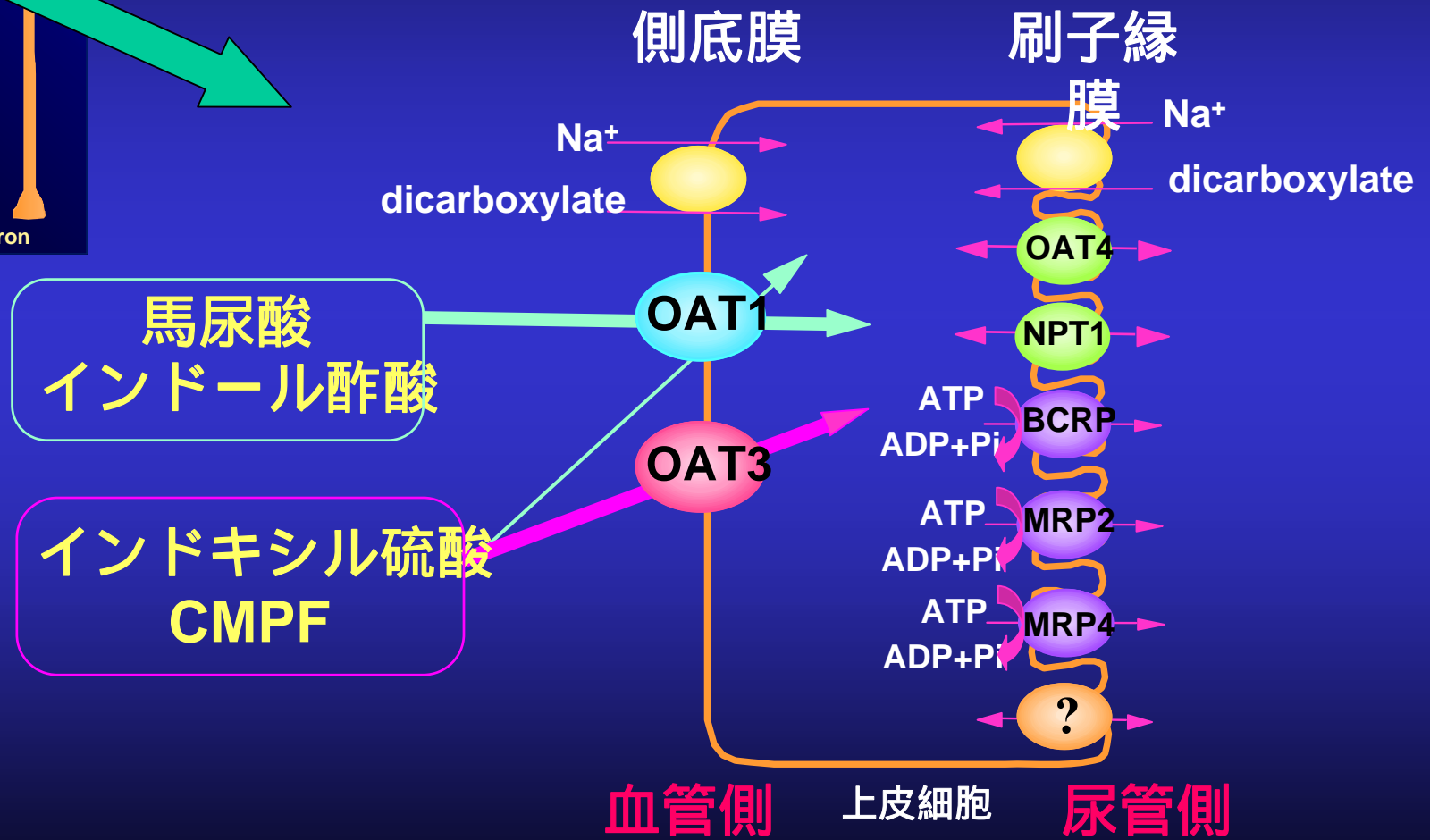
Possible cascade displacement model in fatty acid-uremic toxin-drug system



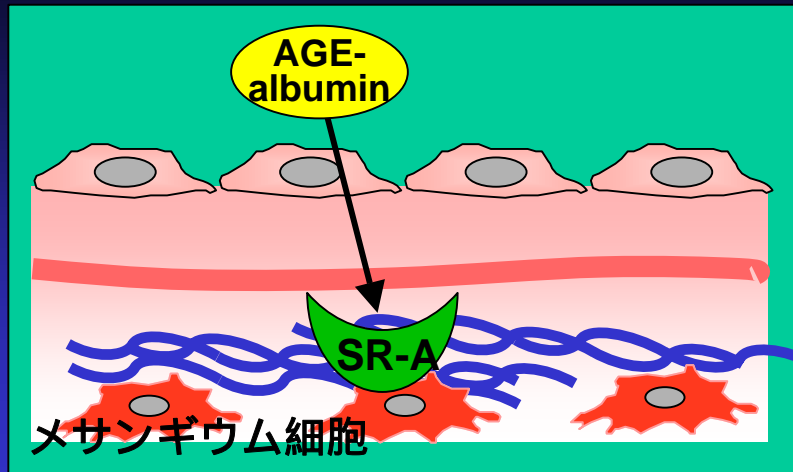
尿毒症物質の腎取り込みに関わる トランスポーター



近位尿細管



AGE-albuminの体内動態特性

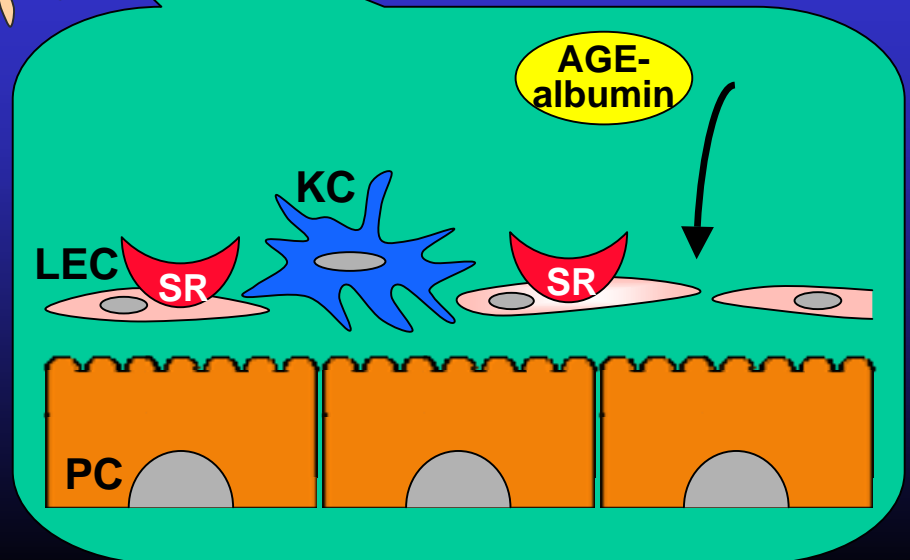


◀ 腎臓による取り込みにはメサンギウム細胞のSR-Aを介したエンドサイトーシスが関与する。

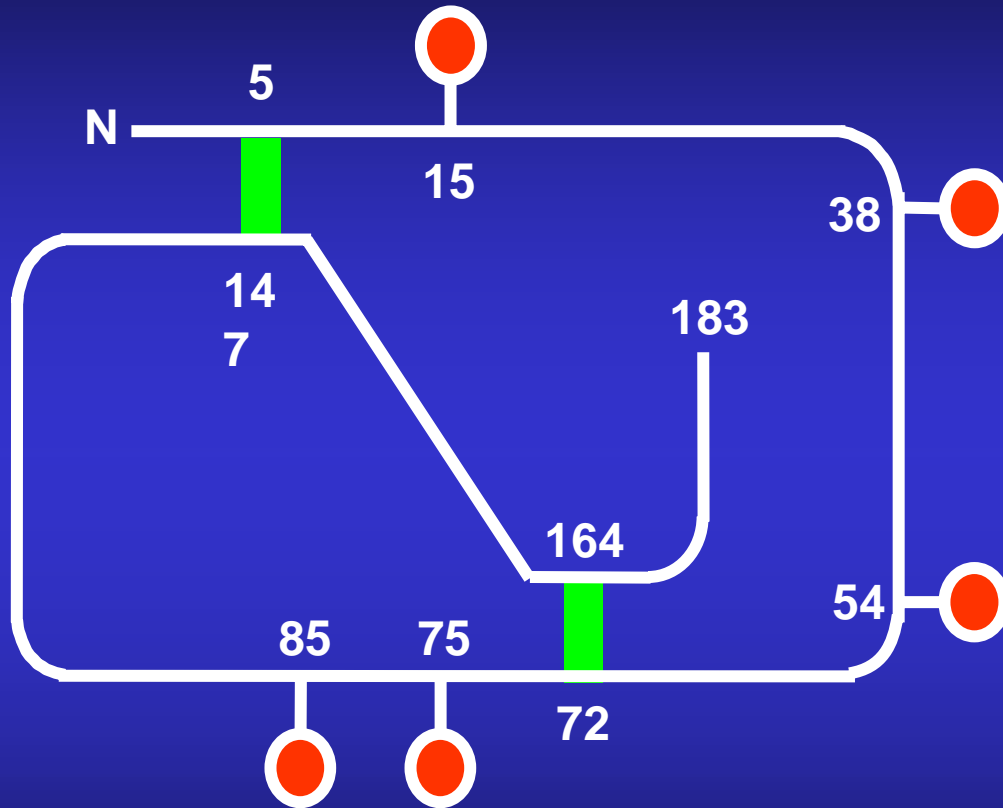
▼ 肝臓による取り込みには肝内皮細胞のスカーベンジャー受容体を介したエンドサイトーシスが関与する。

AGE-albumin

SR; スカーベンジャー受容体
PC; 肝実質細胞
LEC; 肝内皮細胞
KC; クッパー細胞



Structural Characteristics of AGP



■ 分子量：40-45kDa

■ 糖鎖含量：45%



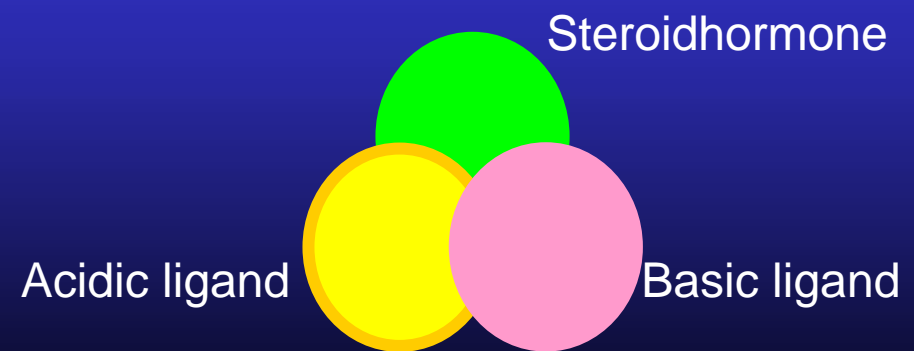
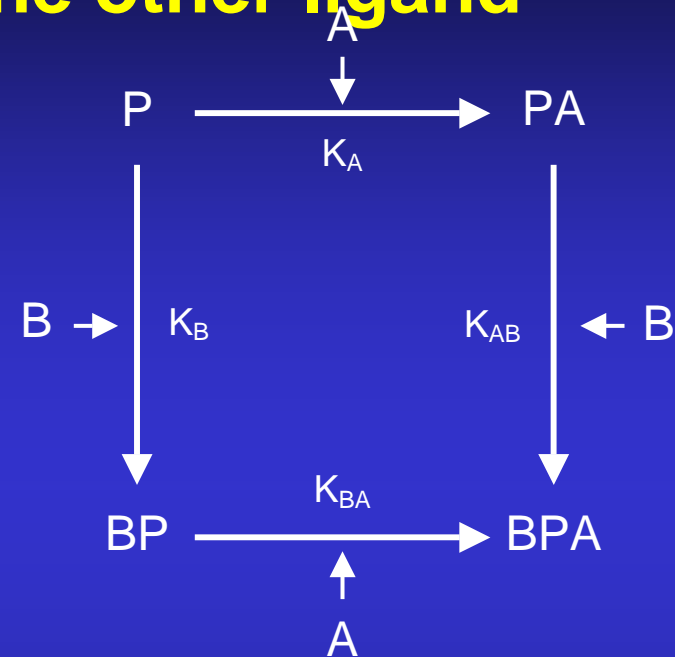
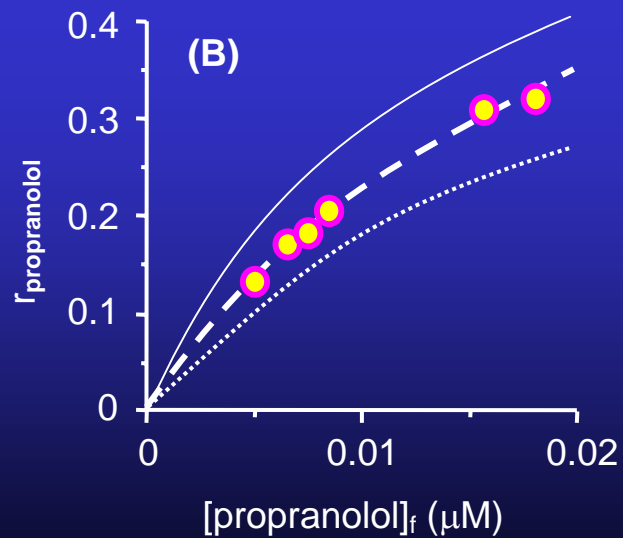
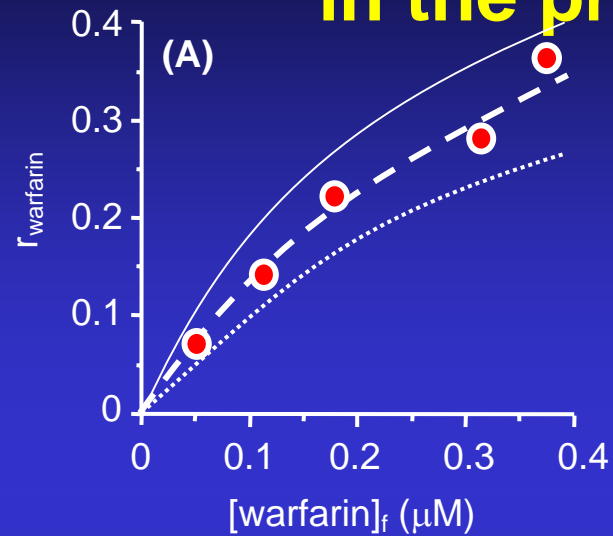
詳細な立体構造は不明

■ β -sheet 構造

■ 等電点：3.0
(シアル酸含量:16%)

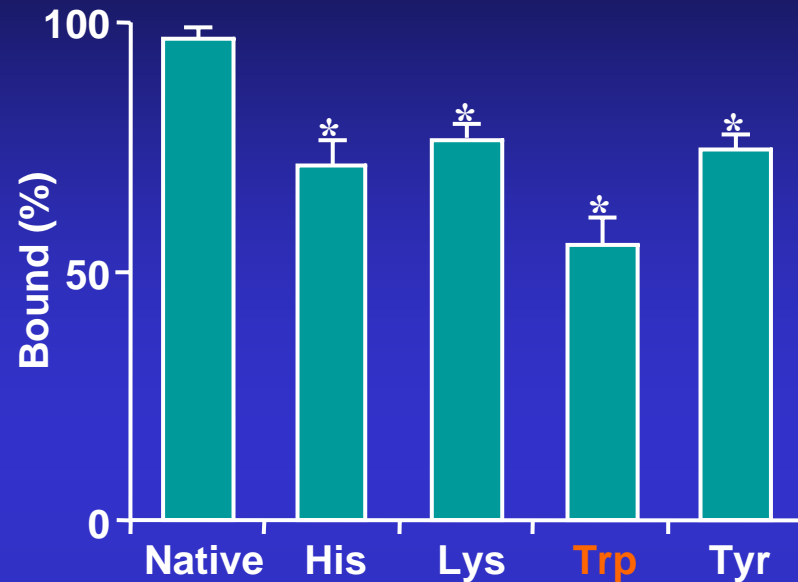
Binding of warfarin (A) and propranolol (B) to AGP

in the presence of the other ligand



————— independent binding, - - - - anti-cooperative interaction, competitive binding

Binding of UCN-01 to AGP



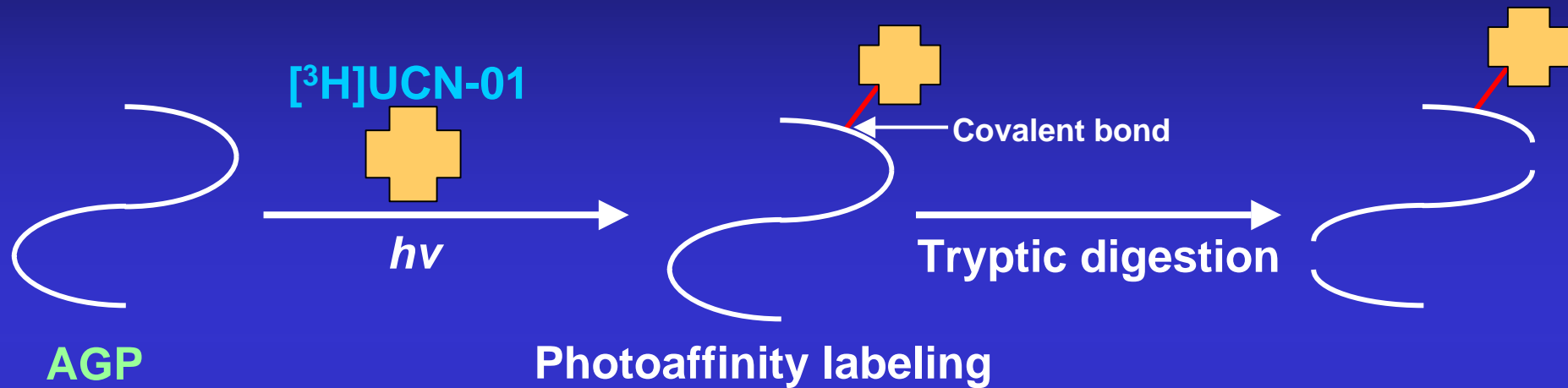
*Statistically significant compared with native AGP; $p < 0.01$.

Parameters	Cancer patients
$t_{1/2}$ (hr)	855~1660
Vd_{ss} (mL/kg)	79.6~158
CL_{tot} (mL/h/kg)	0.0407~0.102

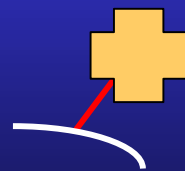
Ligands	R	K_a ($\times 10^6 M^{-1}$)
UCN-01	OH (β)	288 ± 75
Staurosporine	H	11.3 ± 5.74
UCN-02	OH (α)	1.48 ± 0.11

Katsuki M *et al* (2004) *Pharm Res.*, 21:1648-1655

Experimental process of photoaffinity labeling techniques

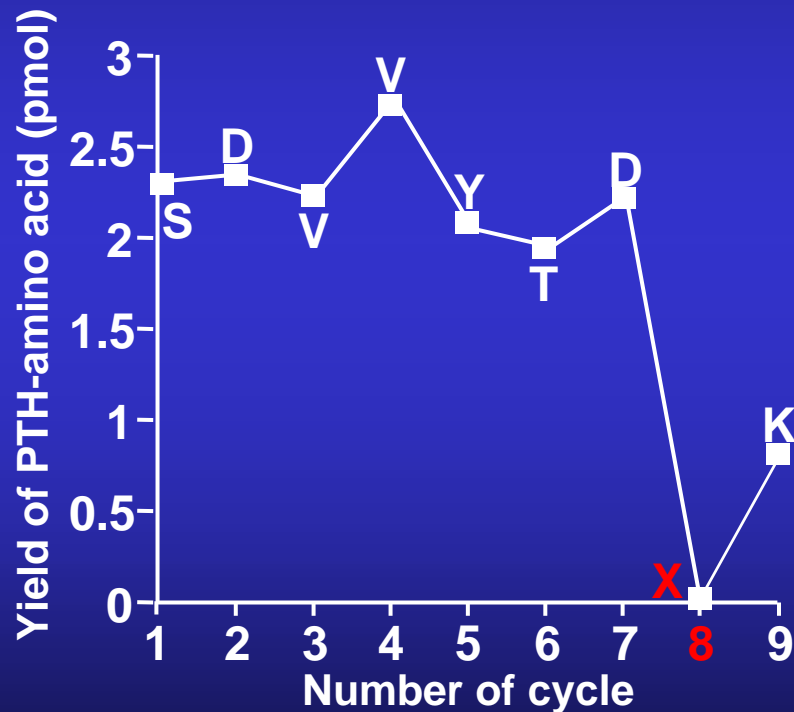


Purification of peptidic fragments by rHPLC
Separation of peptidic fragments by cHPLC



Amino acid analysis by Edman degradation method

N-terminal amino acid sequence analysis by the Edman degradation method



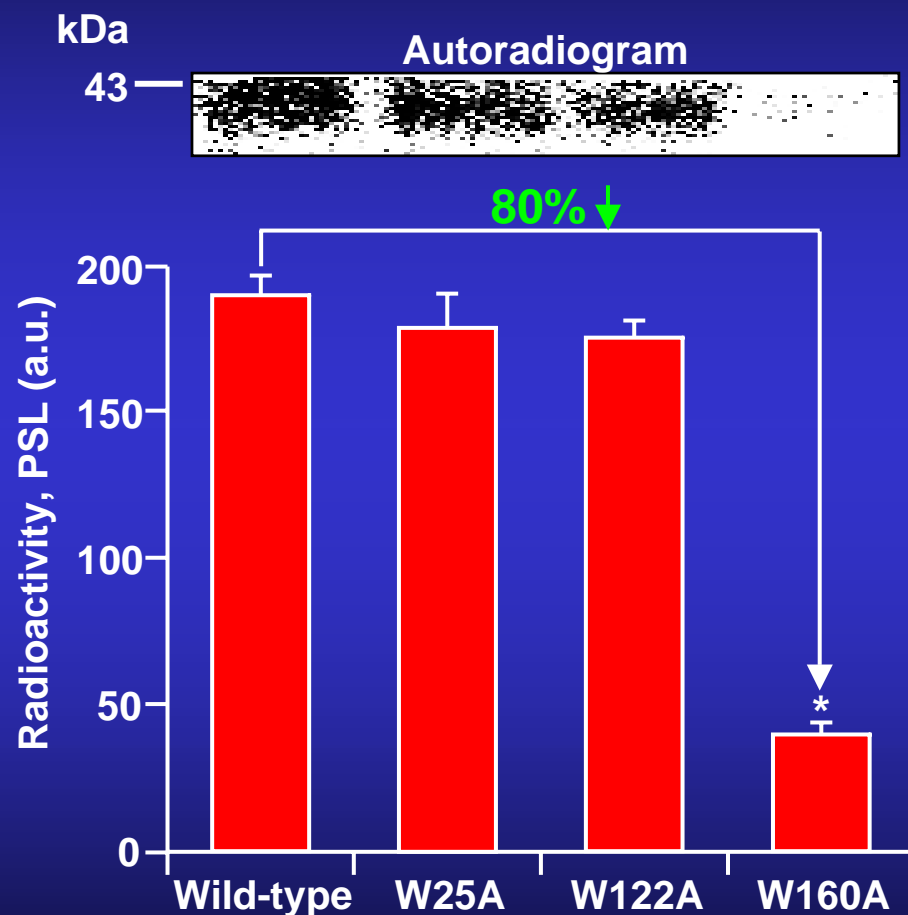
Amino acid sequence of AGP

10	20	30
QIPLCANLVP	VPITNATLDQ	ITGKWFYIAS
40	50	60
AFRNEEYNKS	VQEIQATFFY	FTPKNKTEDTI
70	80	90
FLREYQTRQD	QCIYNTTYLN	VQRENGTISR
100	110	120
YVGGQEHF FAH	LLILRDTKTY	MLAFDVNDEK
130	140	150
NWGLSVYADK	PETTKEQLGE	FYEALDCLRI
160	170	180
PKSDVVYTDW	KKDKCEPLEK	QHEKERKQEE
GES	UCN-01	

PITC: phenylisothiocyanate

PTH : phenylthiohydantoin

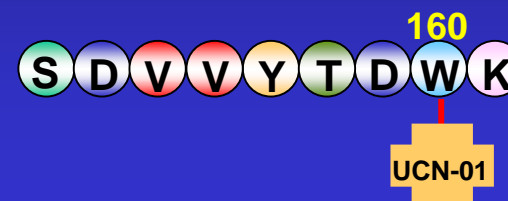
Photolabeling of wild type, W25A, W122A and W160A with [³H]UCN-01



光アフィニティラベル法



ラベルペプチドの同定



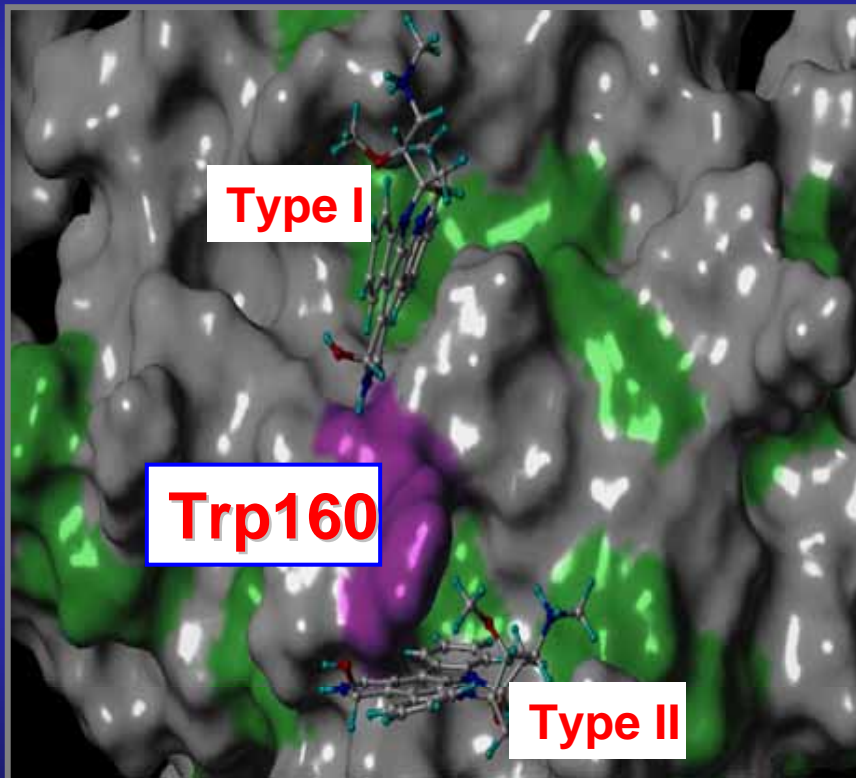
部位特異的変異法

rAGP

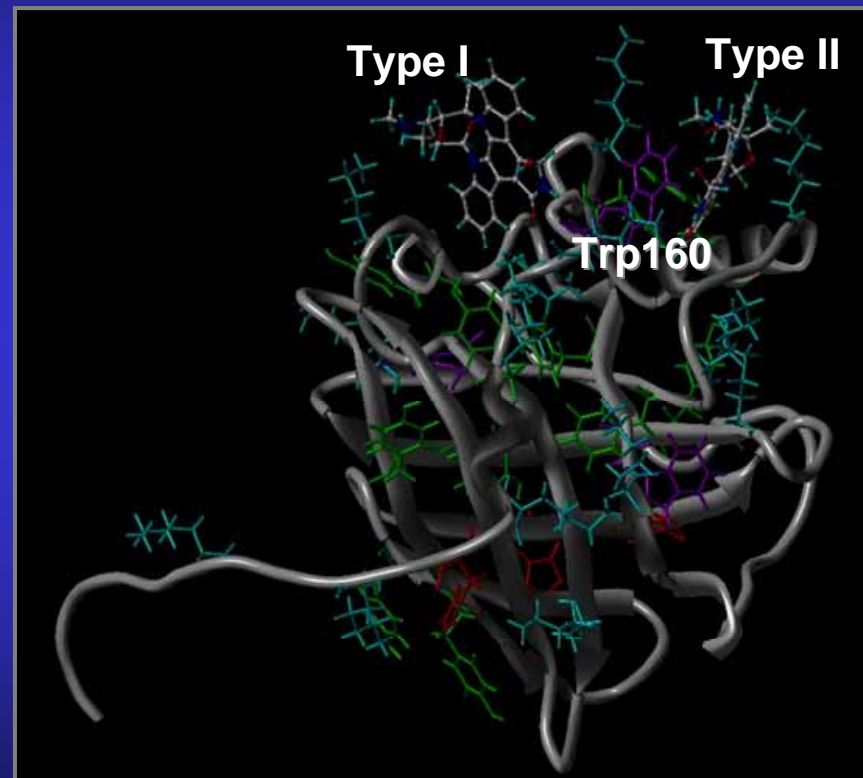
ラベル部位の同定

W160A

Type I and II docking model of UCN-01 and AGP

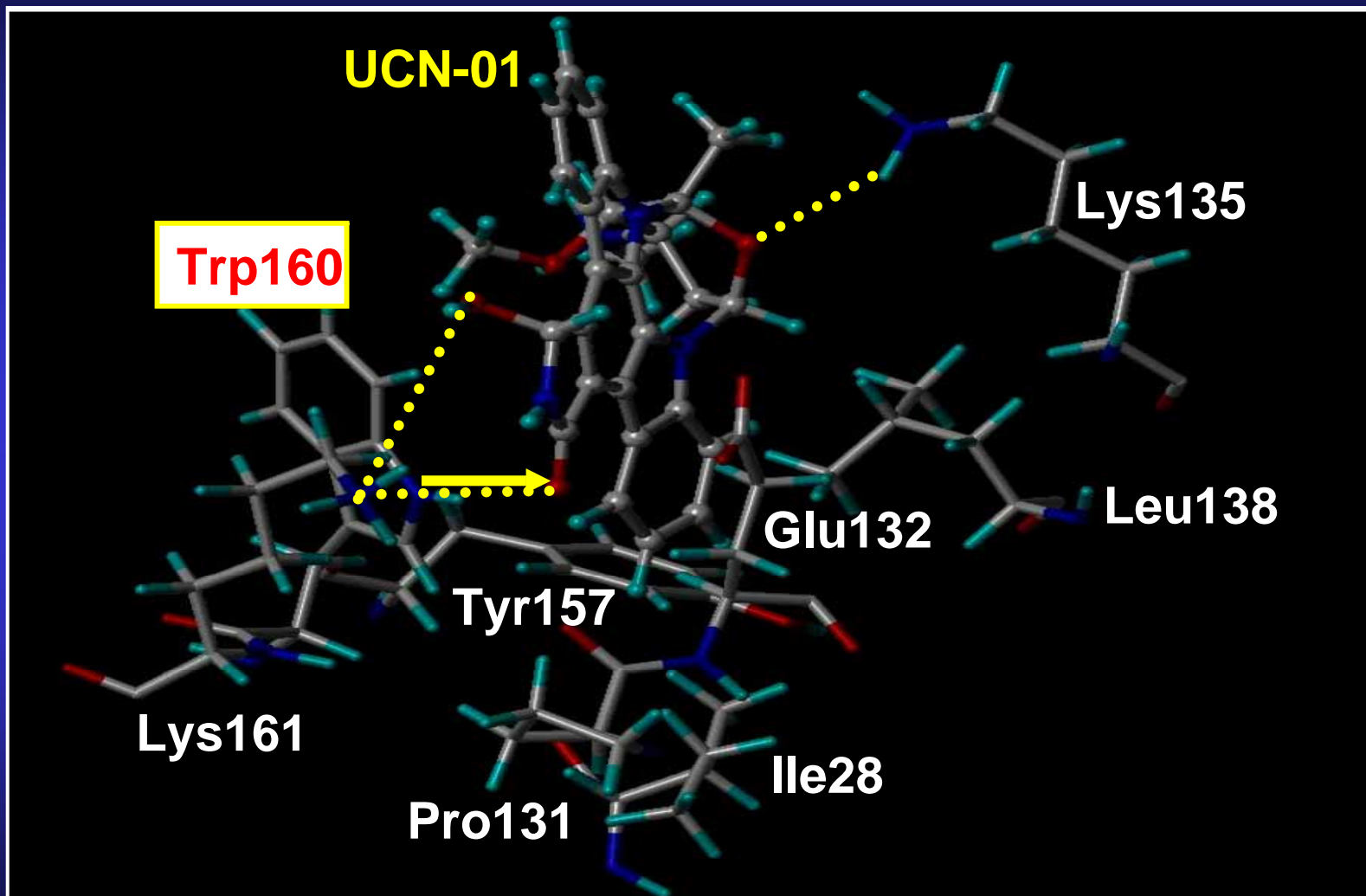


Hydrophobic amino acids are shown in green.



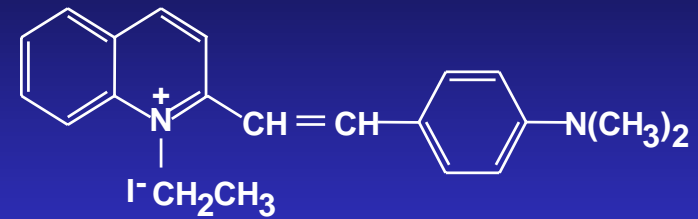
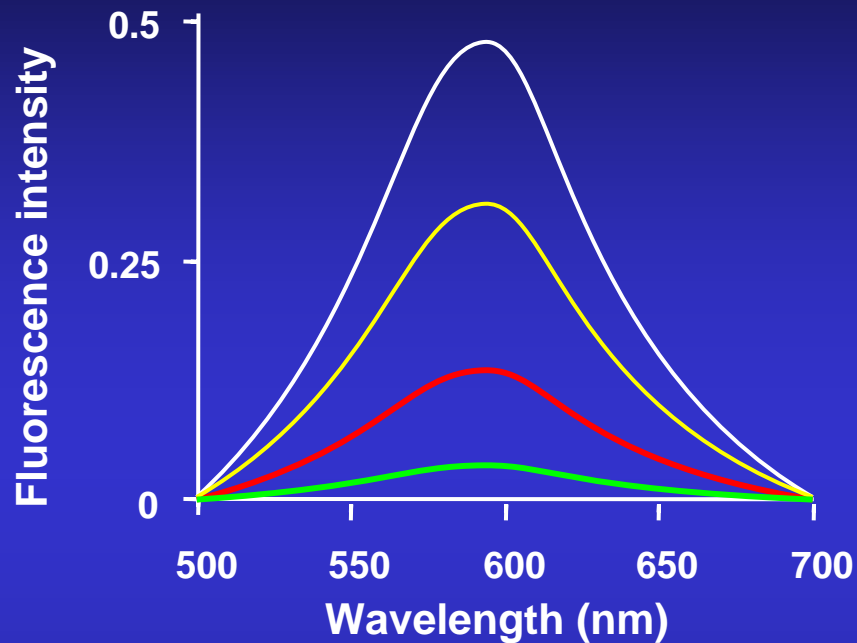
Kopecky *et al* *Biochem Biophys Res Commun* **300**, 41-6 (2003).

Amino acid residues around Trp160 that interacts with UCN-01 exhibited in type II docking model



- Electrostatic interaction
- > Hydrogen bonding

Fluorescence spectra of QR in the presence or serum protein



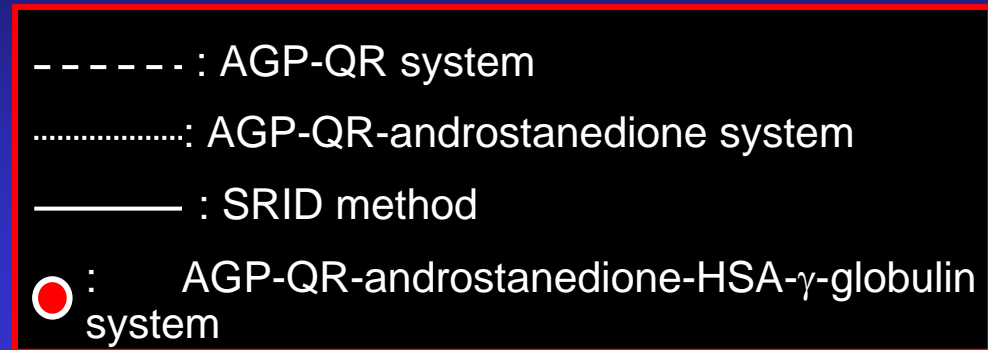
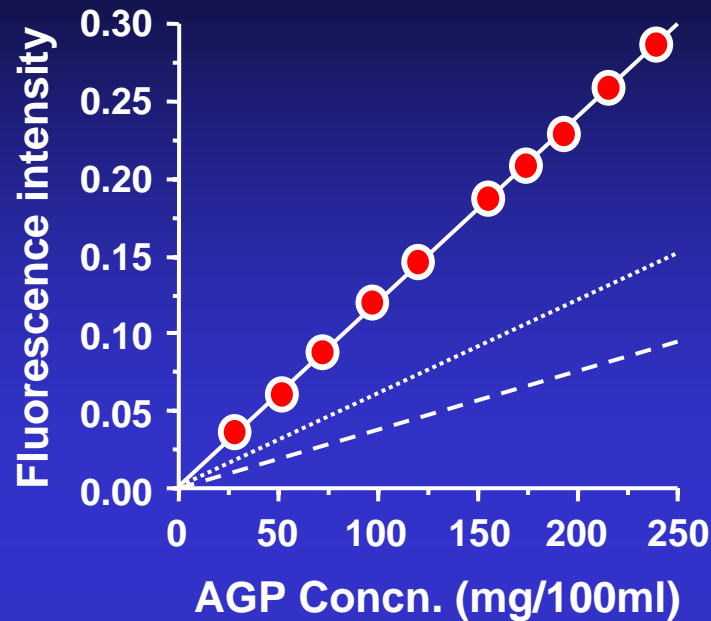
Quinaldine red (QR)

- HSA (2.0×10^{-6} M)
- AGP (0.5×10^{-6} M)
- AGP (1.0×10^{-6} M)
- AGP (2.0×10^{-6} M)

Binding of QR (%) to serum protein

AGP (2mg/100ml)	HSA (80mg/100ml)	γ -globulin (17mg/100ml)
23.0	3.4	1.2

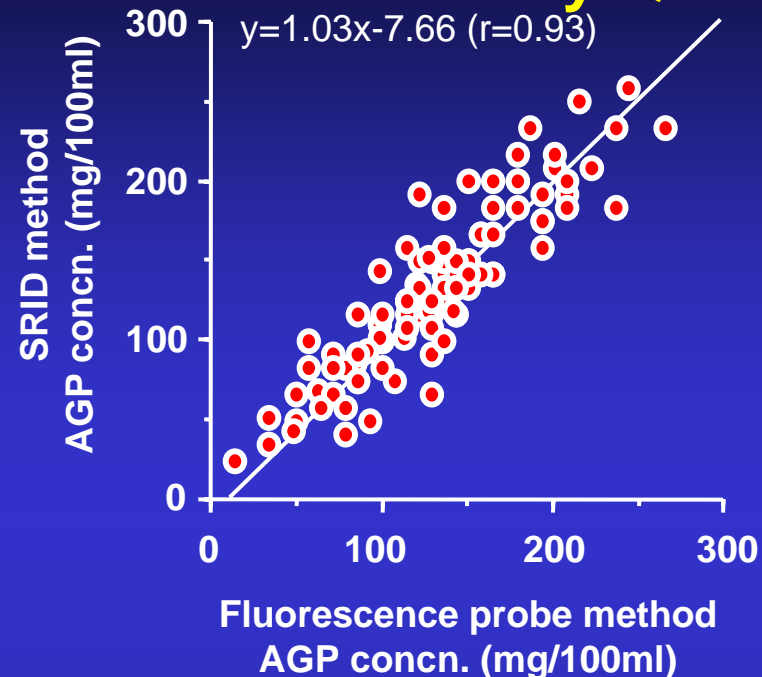
Standard curves for measurement of AGP



Effect of androstenedione of serum protein on binding percentage for QR-AGP systems

	AGP-QR system	AGP-QR-androstenedione system	AGP-QR-androstenedione-HSA- γ -globulin system
Binding (%)	23.0	5.0	24.0

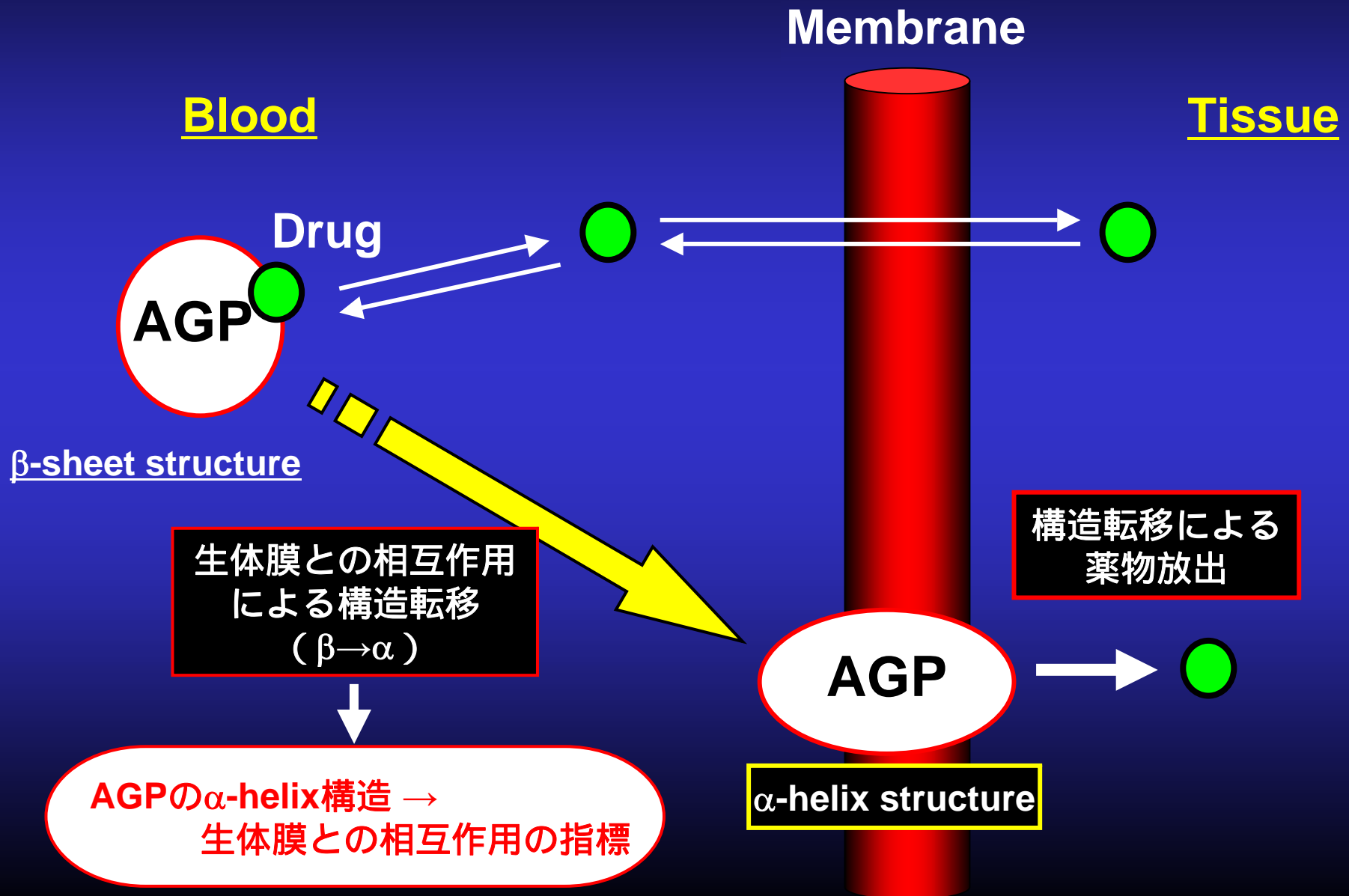
Relationship of the AGP concentrations determined by SRID method and by QR method



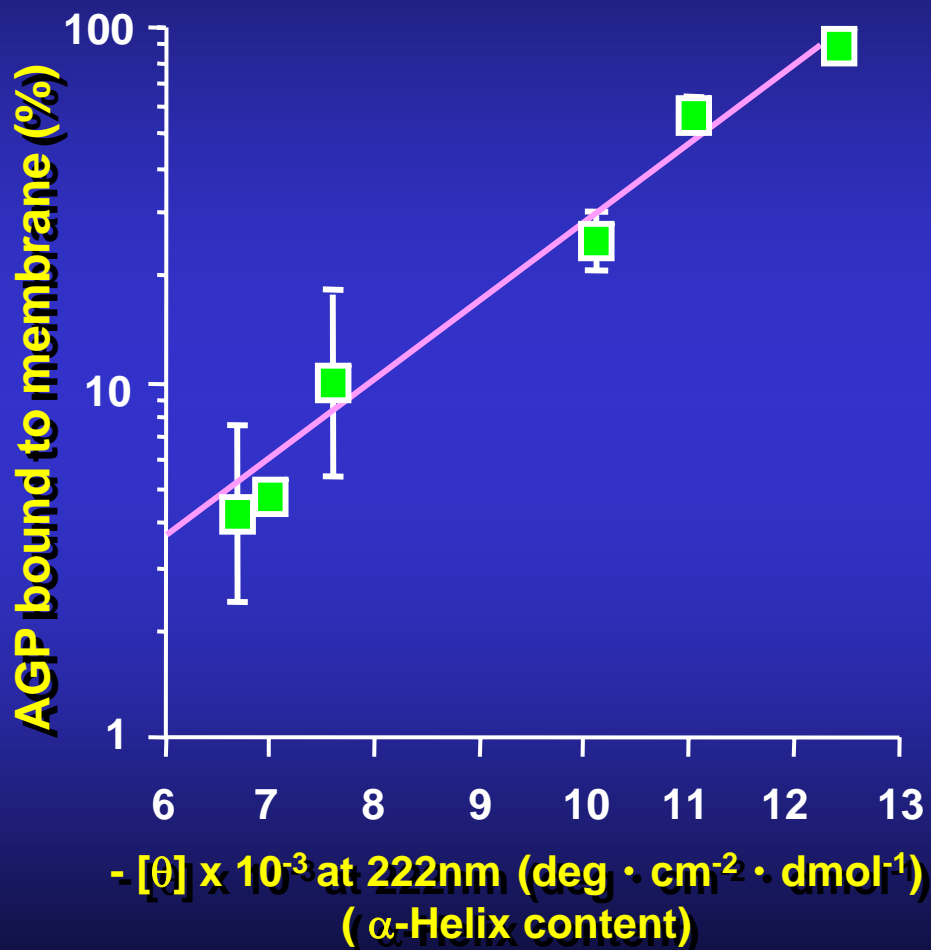
Comparison of QR method and SRID method

	QR method	SRID method
Measuring time	Short (1hr)	Long (50hr)
Operating procedure	Simple	Complex
Detection range	5-500 mg/100ml	12-199 mg/100ml
Operation cost	Inexpensive	Expensive
Coefficient of variation	< 3%	< 10%

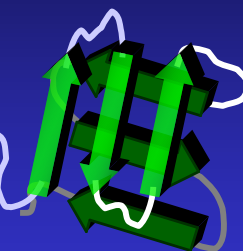
Proposed model for AGP-mediated drug transport through interaction with biomembrane



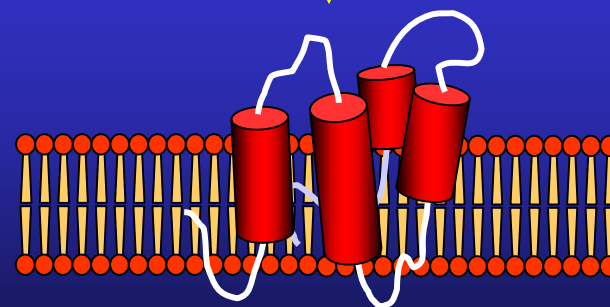
α -Helix Formation of AGP through Binding to Membrane



Native state
(β -sheet form)



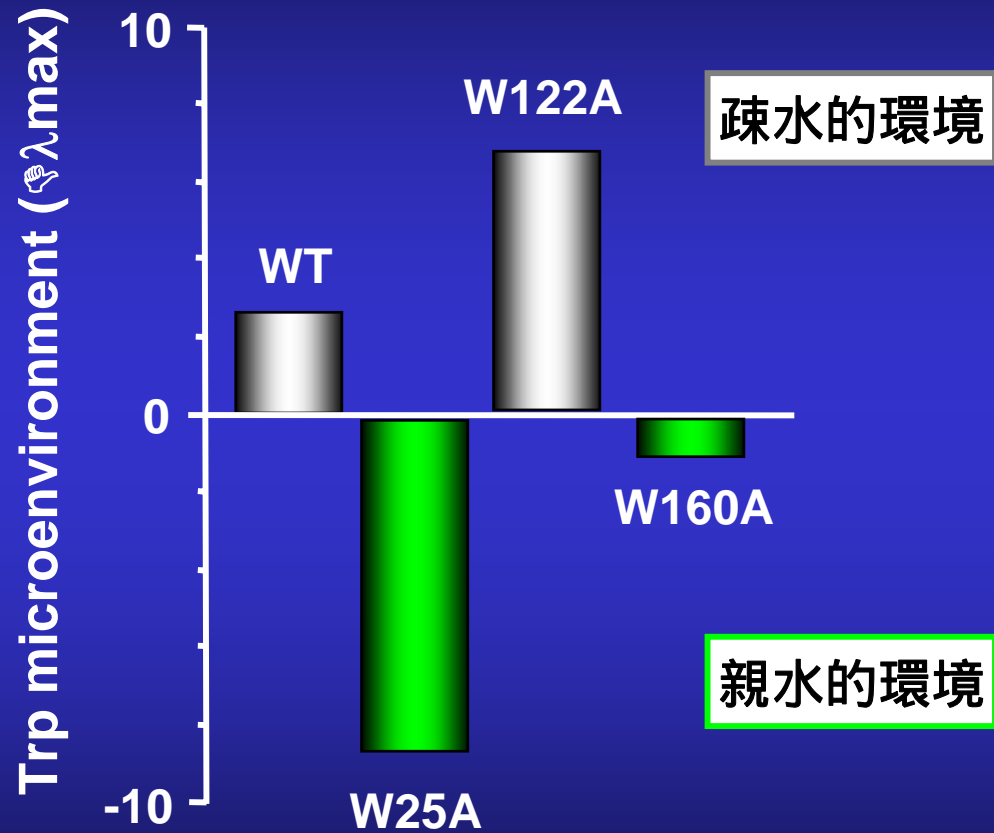
Binding to Membrane



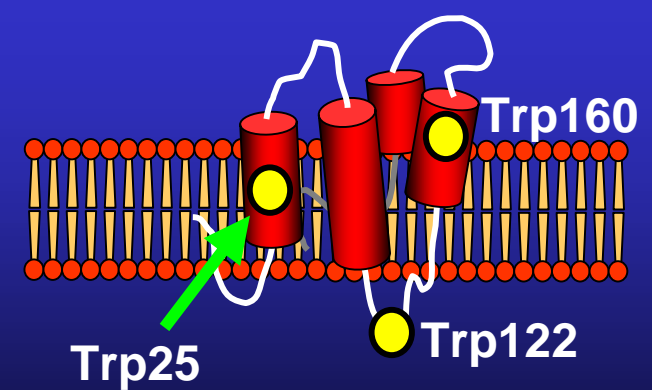
α -Helix formation

α -Helix

Trp (W25, W122, W160) Microenvironment of AGP in the Presence of PG-membrane



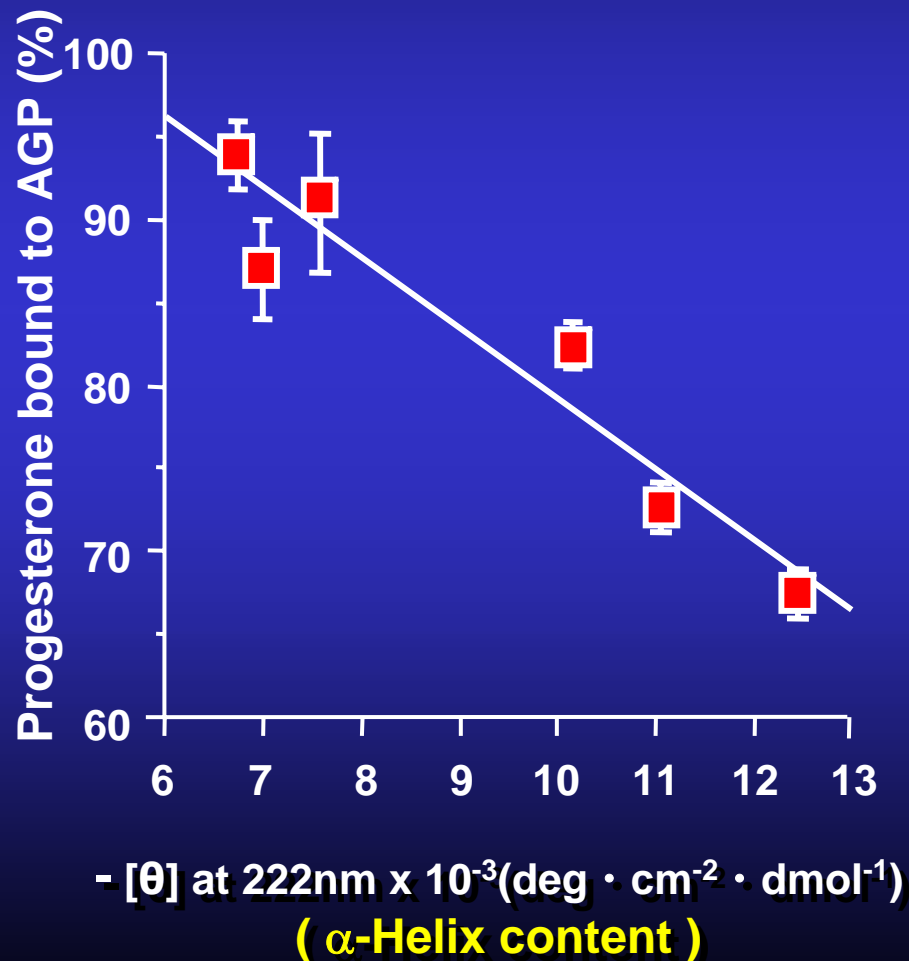
- W25 → 膜内部 (Membrane interior)
- W122 → 膜外部 (Membrane exterior)
- W160 → 膜表面近傍 (Near membrane surface)



$$\lambda_{max} = \lambda_{max}(\text{PG非存在下}) - \lambda_{max}(\text{PG存在下})$$

Correlation between Binding Capacity and α -Helix Contents of AGP

Ligand Binding vs α -Helix Contents (pH7.4 ~ 4.5)



生体膜との相互作用による α -Helix構造の形成

薬物結合能の低下
(結合型薬物の遊離)

非結合型薬物濃度の上昇
→組織へ

Proposed model for interaction of AGP with membrane



Binding to Membrane
(Electrostatic Interaction)

Mild Acidic Condition

Membrane

構造転移 ($\beta \rightarrow \alpha$)

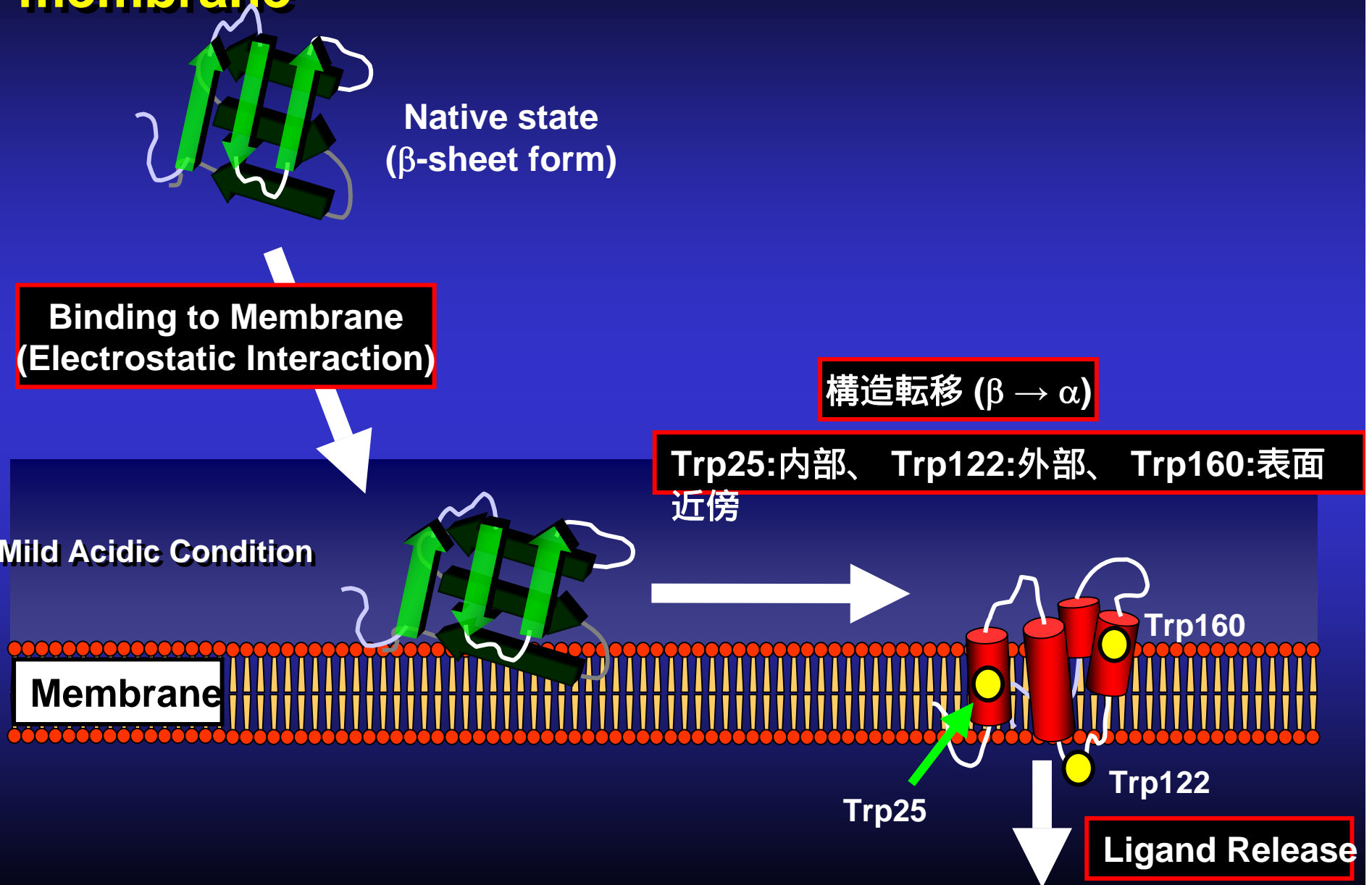
Trp25:内部、 Trp122:外部、 Trp160:表面
近傍

Trp25

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- ・ 構造活性相関解析
- ・ 光アフィニティラベル法
- ・ 部位特異的変異法
- ・ ドッキングシュミレーション

病態時での蛋白結合

- ・ 蛋白質の量的変動
- ・ 蛋白質の質的変動
(コンフォメーション変化, 翻訳後修飾)
- ・ 内因性物質の蓄積
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蛋白介在性組織取り込み

- ・ 生体膜との相互作用に伴う構造転移