
# 創薬研究におけるハイスループット技術の役割

29th January 2014

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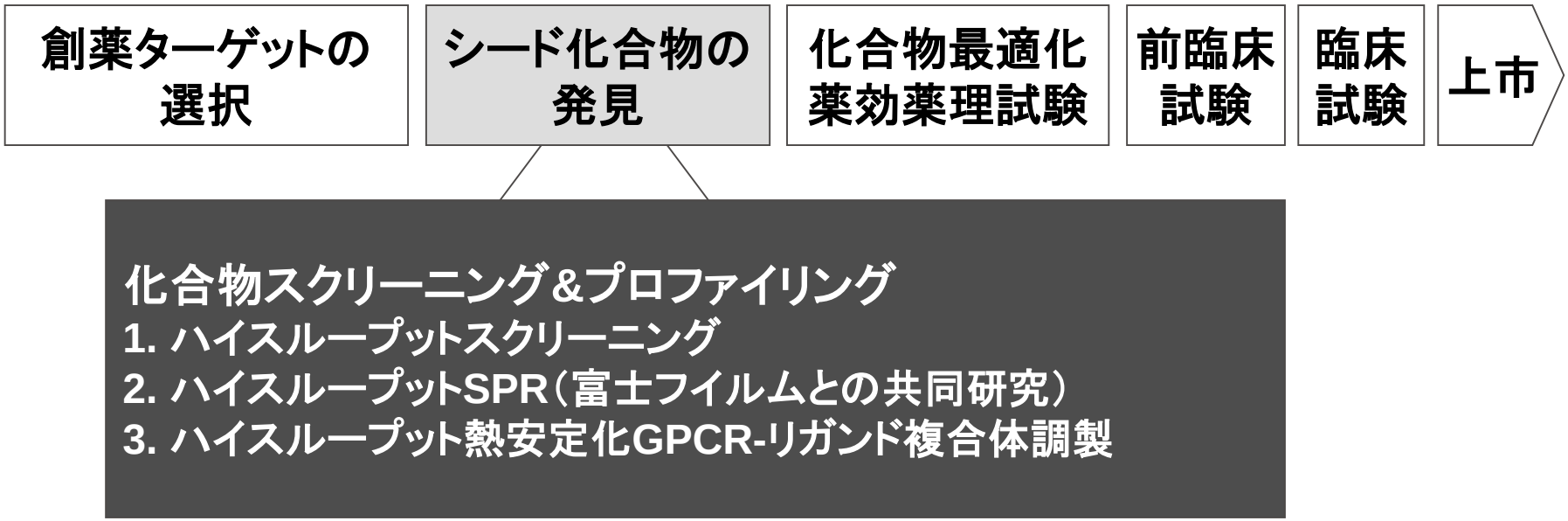
**樽井直樹**

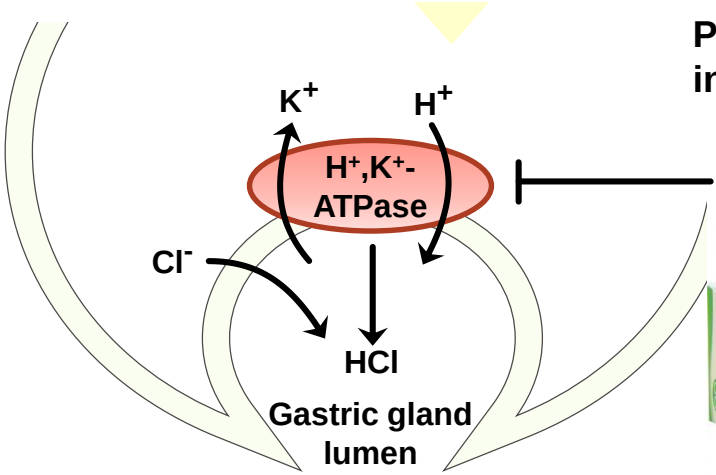
# 本日の話題

## 創薬研究の流れ

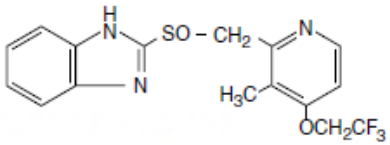


# ハイスループットスクリーニング

## PCAB (Potassium-competitive acid blocker)のスクリーニング




**Proton pump inhibitor**





**酸関連疾患**
**（胃食道逆流症、消化性潰瘍等）**

**薬効がより早く、強く発揮する化合物**
- ✓ $K^+$ と競合する化合物
- ✓ 非共有結合性化合物

# スクリーニング戦略

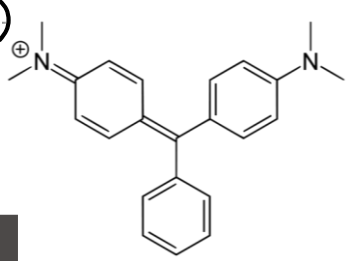
プロトンポンプの調製（ブタ胃壁細胞）

↓

アッセイ系構築

$H^+K^+$-ATPase　+ATP ⟶ ADP+Pi

Piをマラカイトグリーン法で定量
（吸光度620nm）



↓

HTS

↓ ⇦ ライブラリー化合物（56万）

↓

化合物選択

↓

濃度依存性
選択性（$Na^+K^+$-ATPase）
構造活性相関

↓

化合物選択

↓

$K^+$拮抗性・可逆性
ADMET・物性

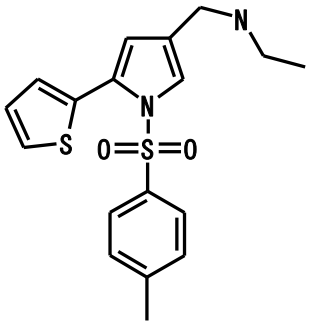
↓

化合物選択

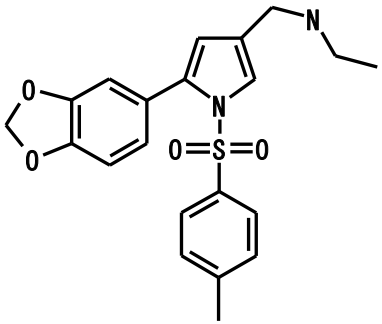
↓

ヒット化合物

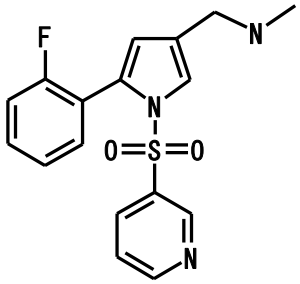
# 濃度依存性と選択性



Compound 1



Compound 2



TAK-438

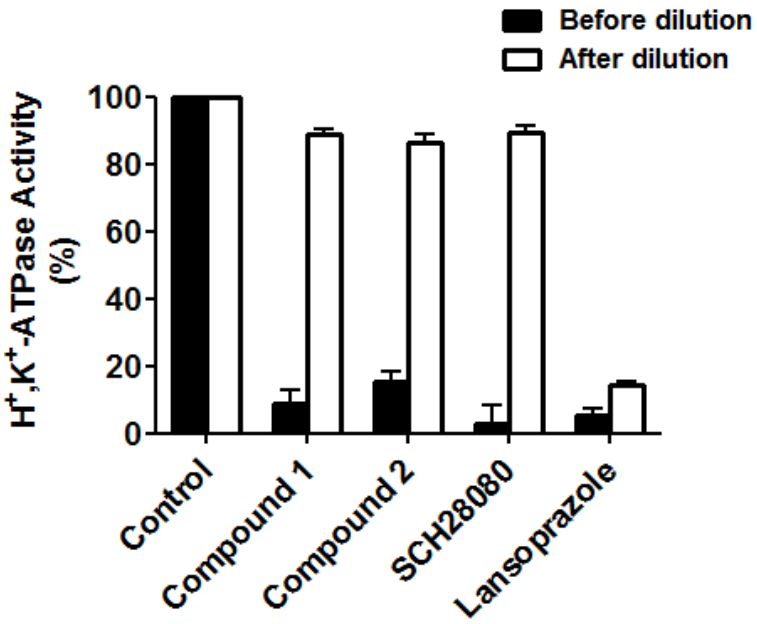
vonoprazan

Strucutes of Compound1, 2 and TAK-438

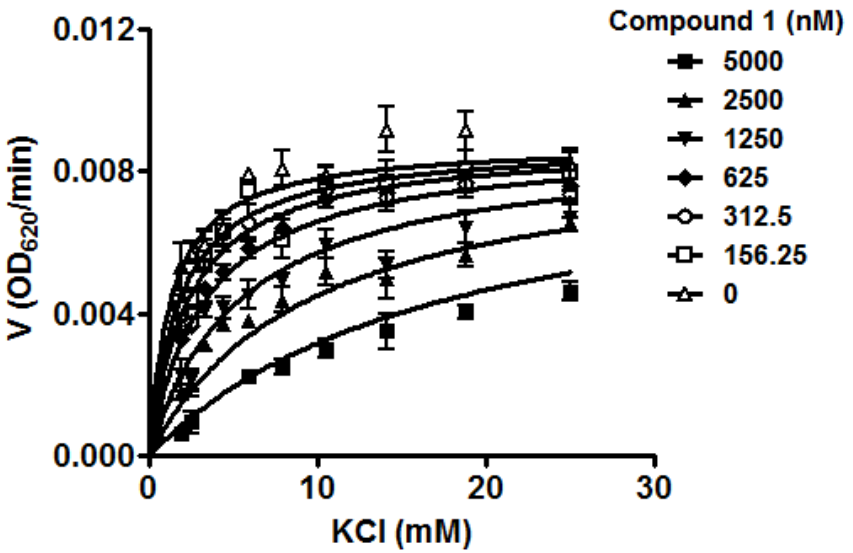
$IC_{50}$ values for the inhibition of $H^+,K^+$-ATPase and $Na^+,K^+$-ATPase

| Compound | $H^+,K^+$-ATPase | | $Na^+,K^+$-ATPase |
|---|---|---|---|
| | $IC_{50}$ (pH 6.5) μM | $IC_{50}$ (pH 7.4) μM | $IC_{50}$ (pH 7.4) μM |
| 1 | 0.31 | 1.5 | 5.7 |
| 2 | 0.54 | 1.0 | 2.5 |
| SCH28080 | 0.17 | 3.2 | >10 |
| Lansoprazole | 6.7 | 62 | >10 |

# 可逆性とカリウム拮抗性

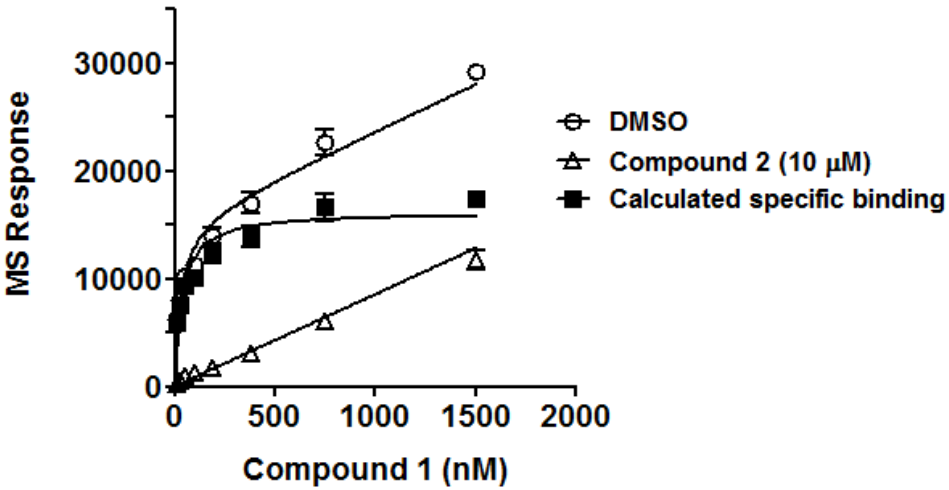



Reversibility of $H^+,K^+$-ATPase inhibition.
The effect of compound1 (10 μM), 2 (10 μM), lansoprazole (20 μM), and SCH-28080 (3 μM) on $H^+,K^+$-ATPase activity was measured after (open columns) and before (closed columns) 200-fold dilution.

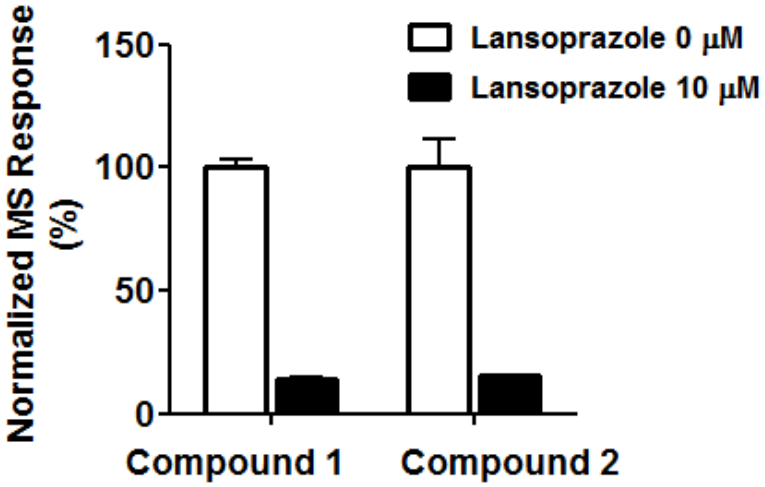



Determination of inhibition mechanism.
Global fitting of velocity versus potassium concentration data in absence or presence of compound 1. The fitting results are consistent with a competitive mode.

# 結合性と結合位置（ランソプラゾールとの競合）



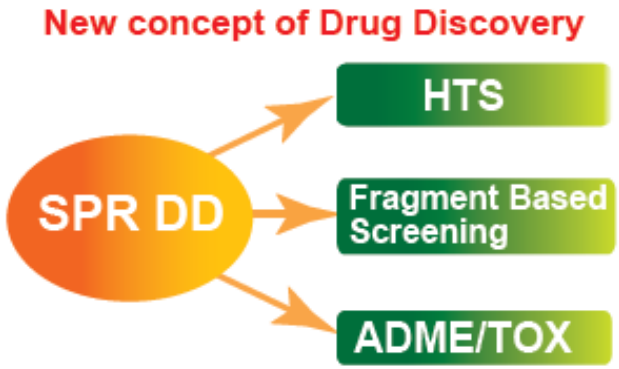

$H^+,K^+$-ATPase binding affinity of compound 1.
Apparent $K_d$ values were determined by saturation binding to $H^+,K^+$-ATPase (porcine gastric vesicles) using ASMS.



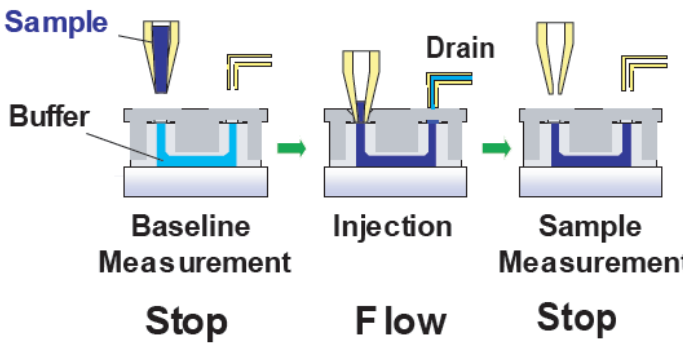

Effect of lansoprazole on binding of compound 1 and 2 in affinity selection mass spectrometry binding experiments.
Lansoprazole was added to porcine gastric vesicles and preincubated at room temperature for 30 minutes. Then compound 1 or 2 was added, and the mixture was incubated for 60 minutes before ASMS analysis.

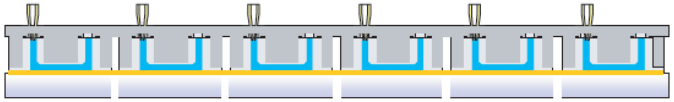
# ハイスループットSPR

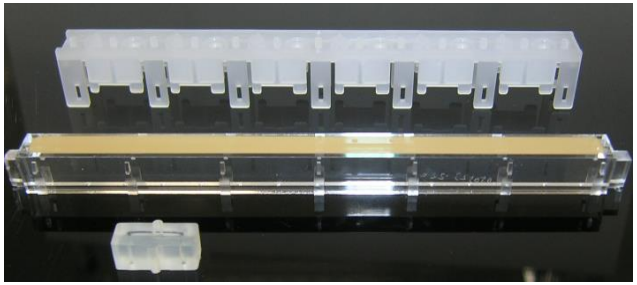
共同研究
富士写真フイルム株式会社　R&D統括本部　先進コア技術研究所
山田孝之,、江副利秀、来馬浩二、都築博彦



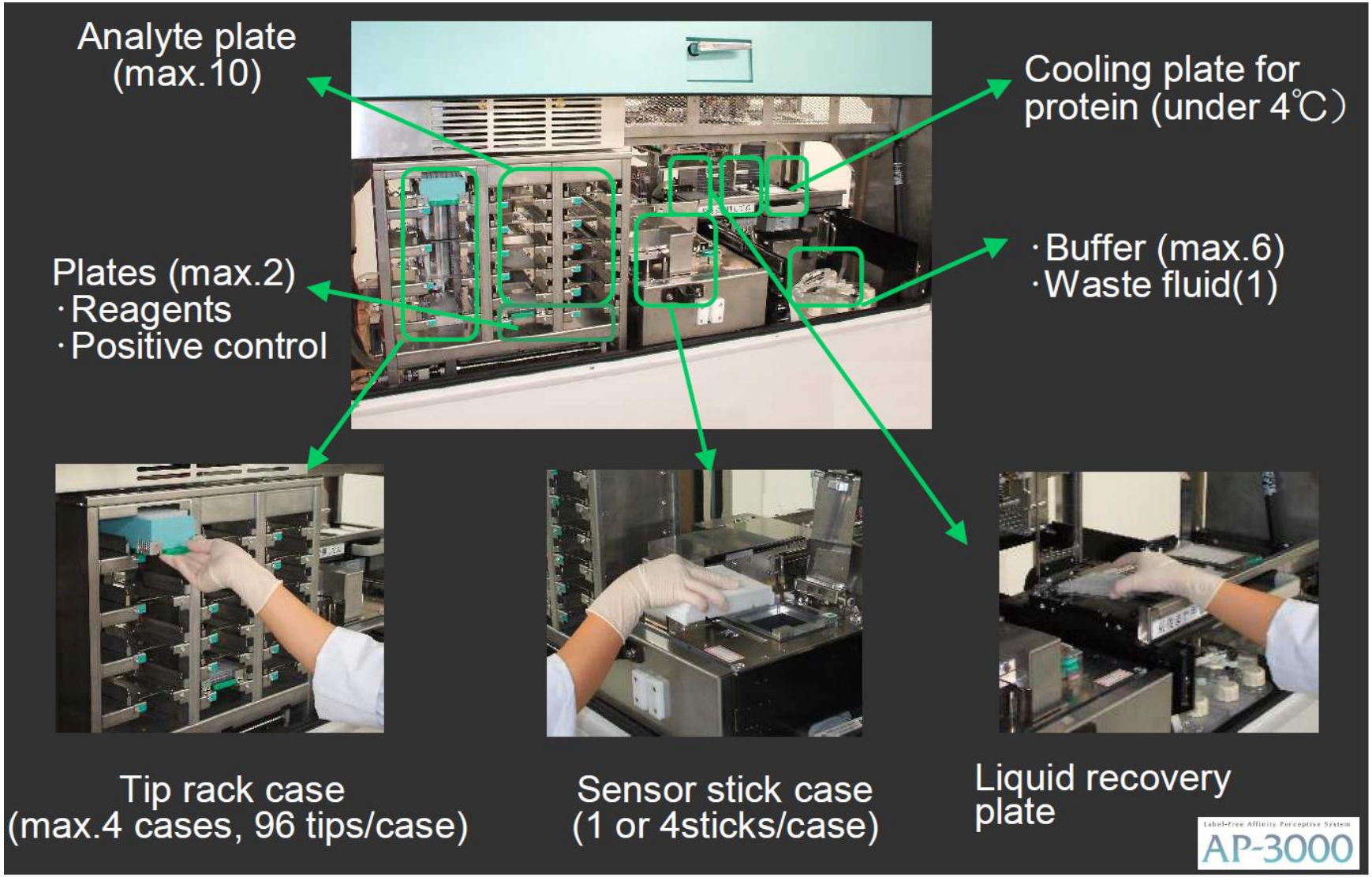

**Label-Free Affinty Perceptive System AP-3000**



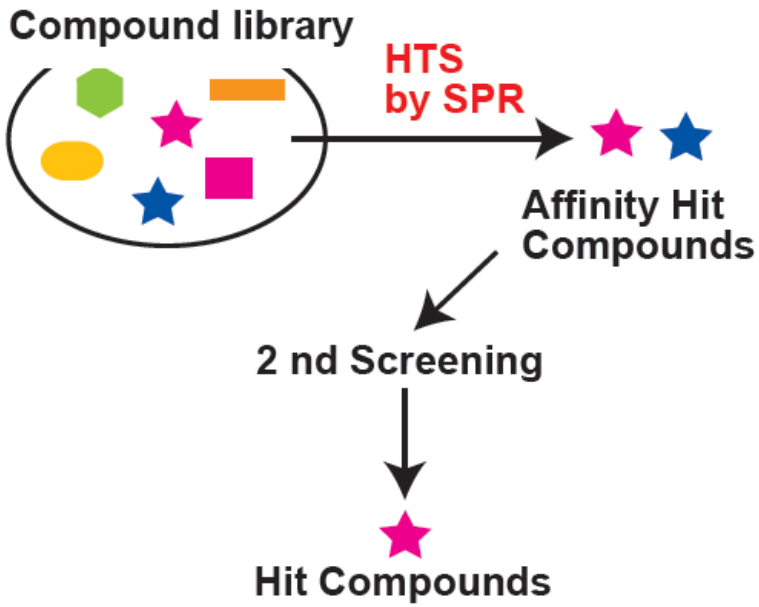


# AP-3000の構造

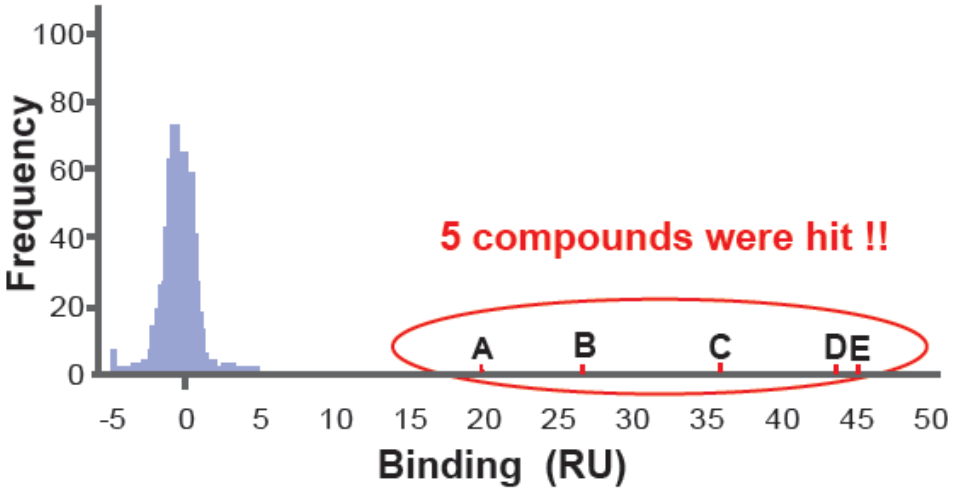

Analyte plate
(max.10)
Cooling plate for
protein (under 4℃)
Plates (max.2)
・Reagents
・Positive control
・Buffer (max.6)
・Waste fluid(1)
Tip rack case
(max.4 cases, 96 tips/case)
Sensor stick case
(1 or 4sticks/case)
Liquid recovery
plate
AP-3000

# 一次スクリーニングへの応用

## HTS for 1st screening




## Result of the screening

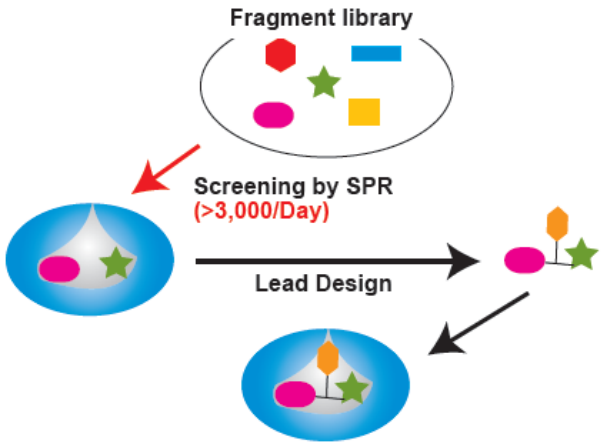



- 5 compounds were completely identical to hits that were discovered by enzymatic assay.

**Throughput: 3840 analytes/ 24hrs**

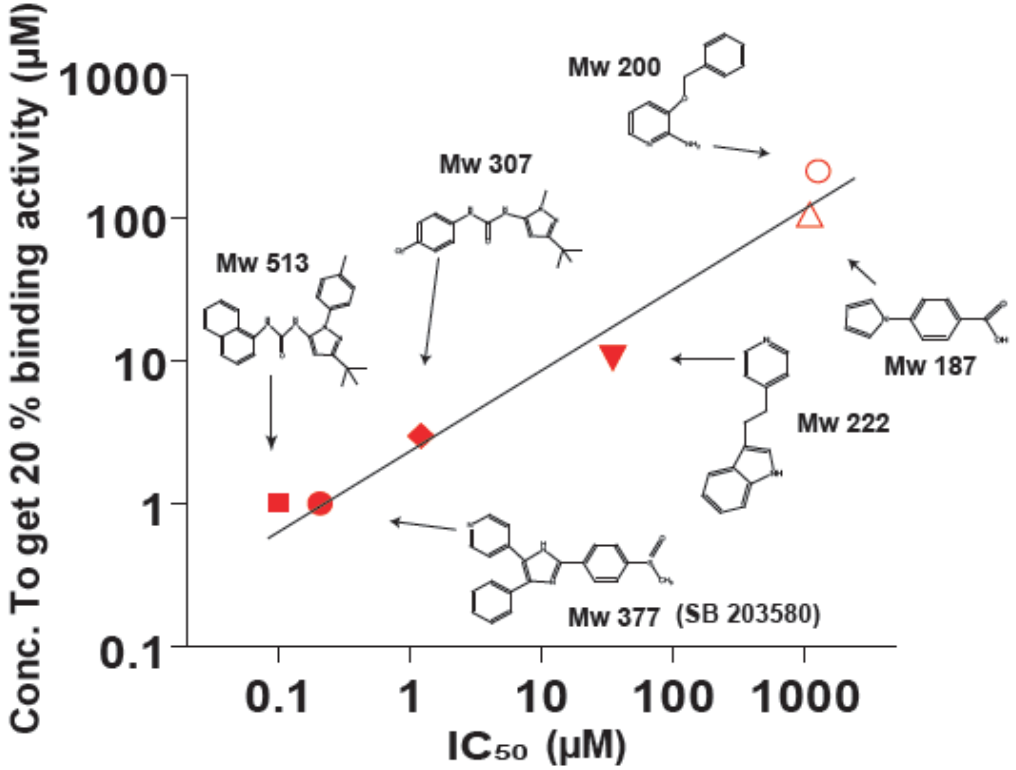
# フラグメントスクリーニングへの応用

## Fragment-based screening by SPR



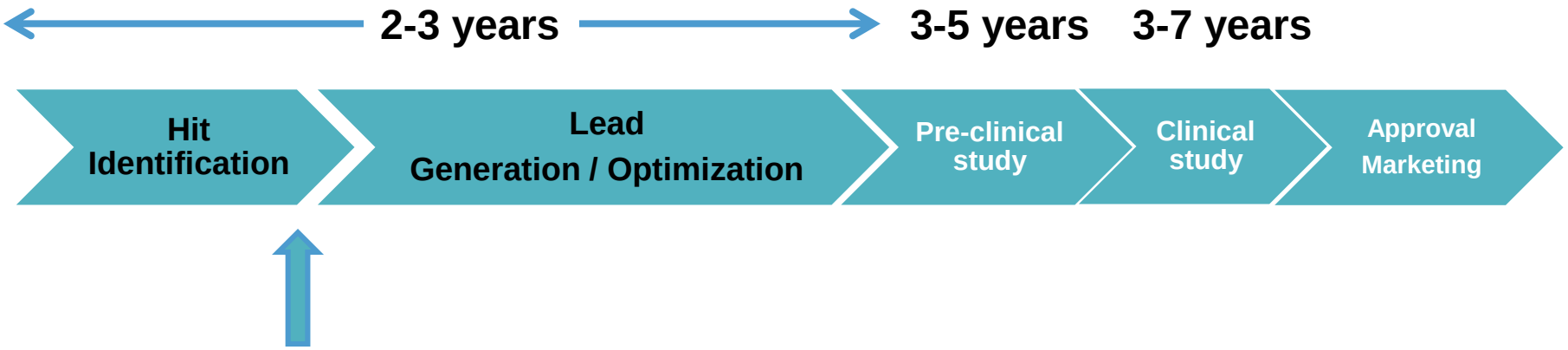

1. Small consumption of protein
   (Cf. μg order for SPR, while mg for others)
2. Filltering fragment library by SPR before low throughput screening (Cf. X-ray crystallography)

## Correlation of binding activity and $IC_{50}$




- Good correlation between binding activity and $IC_{50}$ from fragments to lead compounds (ref 1, 2, 3).

# ハイスループット熱安定化GPCR･リガンド複合体調製




**Ideally, crystal structure information available at this stage**

**Efficient SBDD for lead generation/optimization requires crystal structure information on the target GPCR earlier at this stage as with general soluble protein targets.**

# GPCR調製の問題点と対策

***Issues that should be addressed for early success***

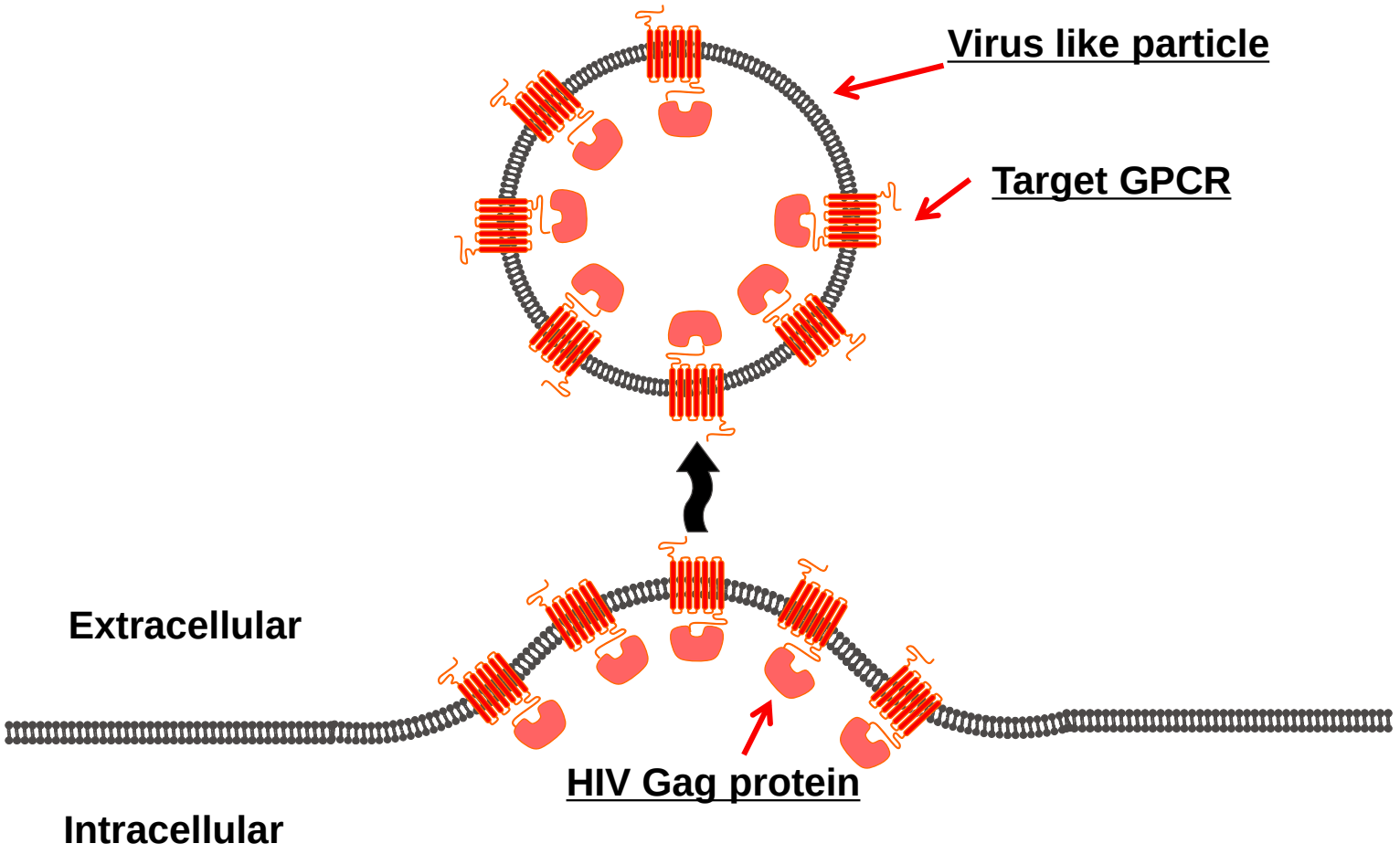
1) Sufficient stability of GPCR required for crystallization
2) Quite a few combinations between the target GPCRs and ligands for evaluation

***Our strategy***

**Development of high-throughput and versatile platform to identify the thermostabilized mutant GPCR**

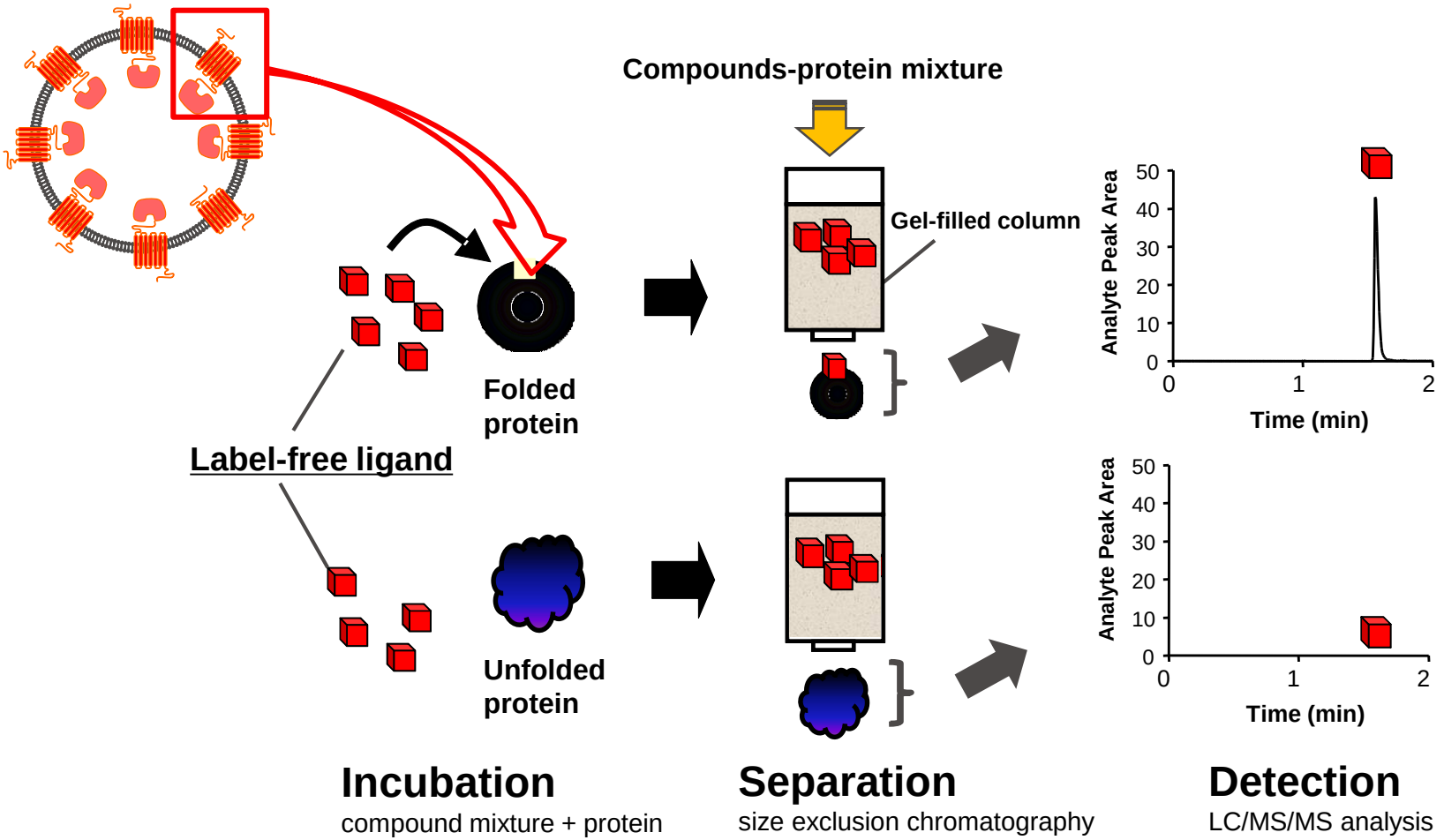
1) GPCR sample preparation as vesicle form
2) Binding assay development with label-free ligand

# Virus like particle（VLP）を用いたGPCR調製




**Observed high expression for some GPCRs**

# LC/MS/MSとゲル濾過を用いた結合試験

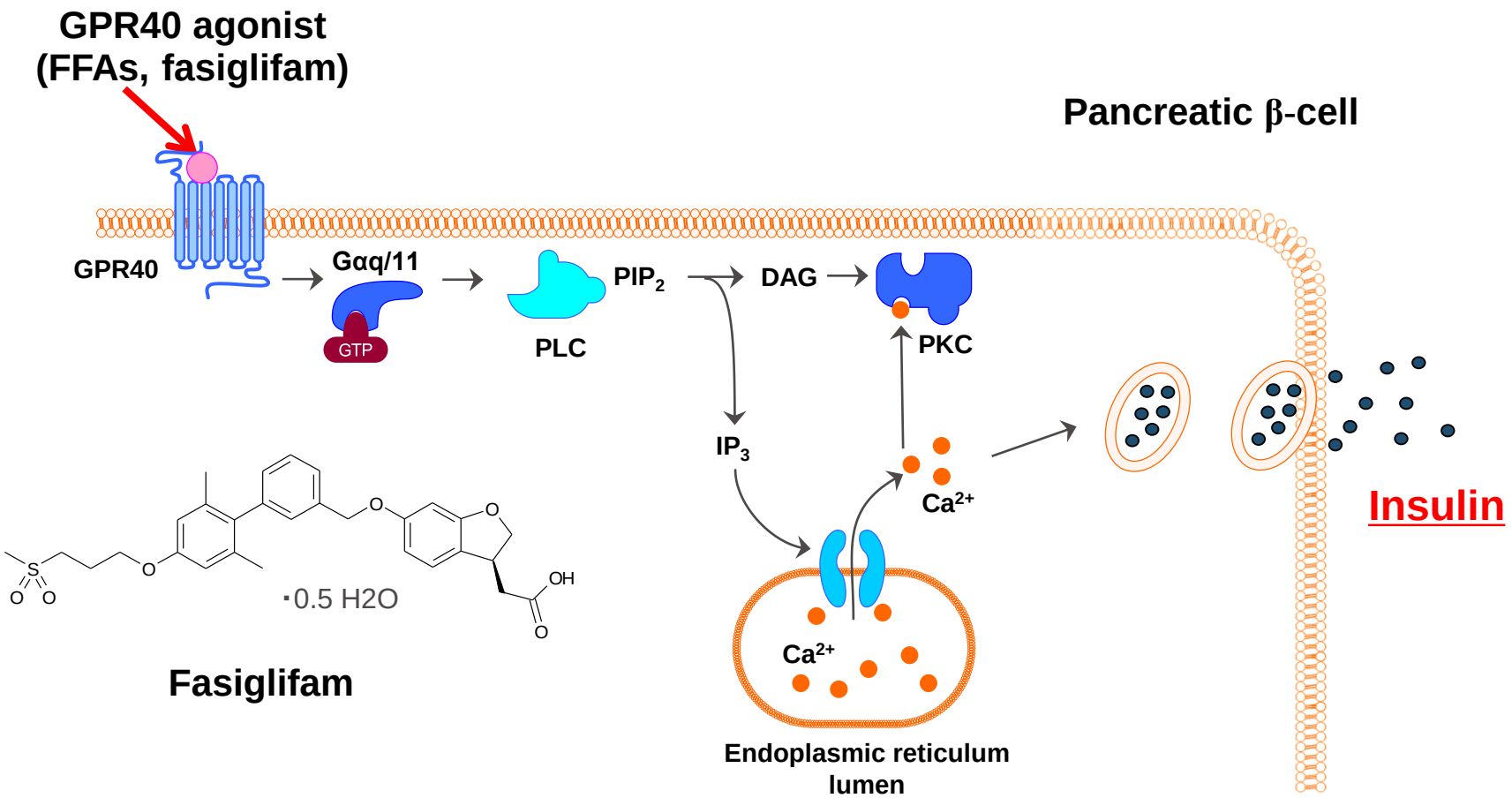



**Incubation**
compound mixture + protein

**Separation**
size exclusion chromatography

**Detection**
LC/MS/MS analysis

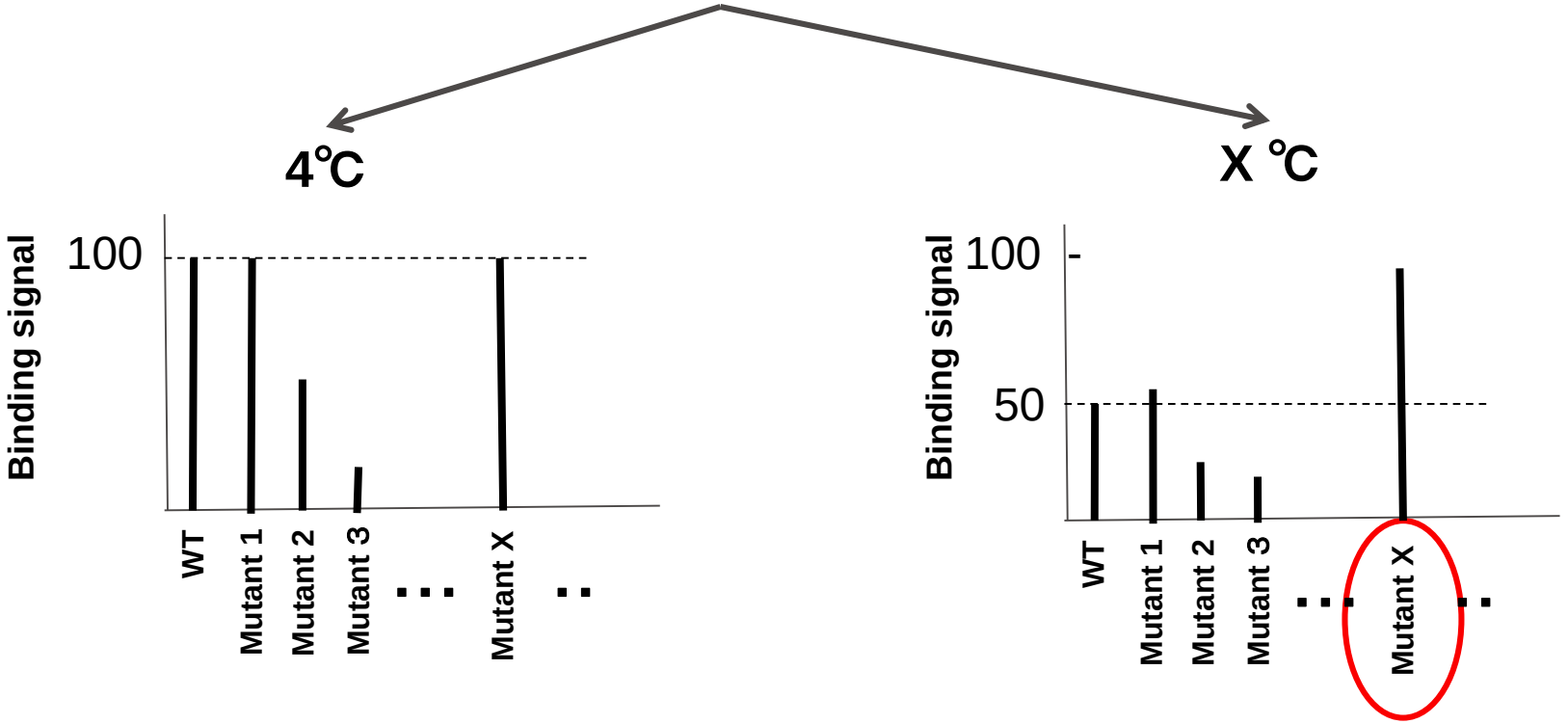
# GPR40 agonist, Fasiglifam



# 熱安定化GPCRとFasiglifam複合体の調製

***Thermostabilized mutant screening***

**4℃ and heating condition (X℃) binding assay**

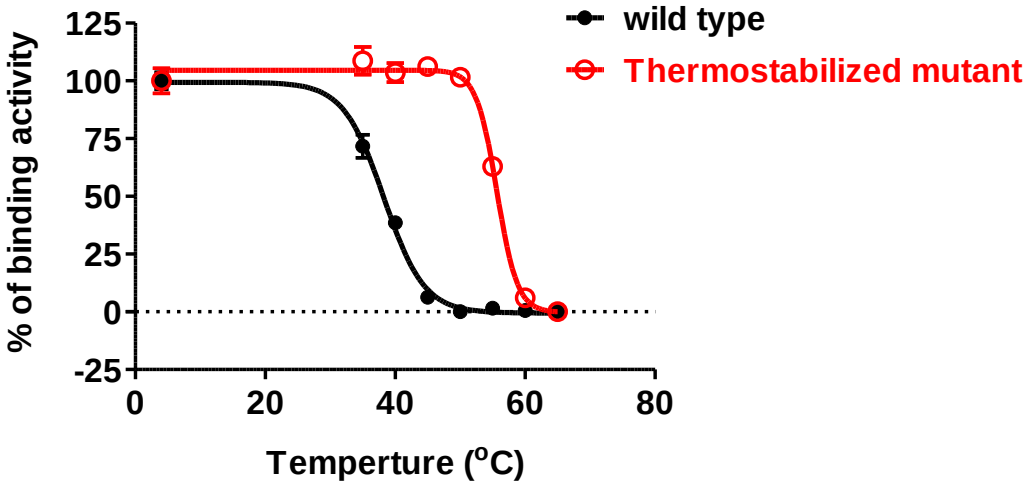



**Evaluate the effect of protein expression and ligand affinity caused by mutation**

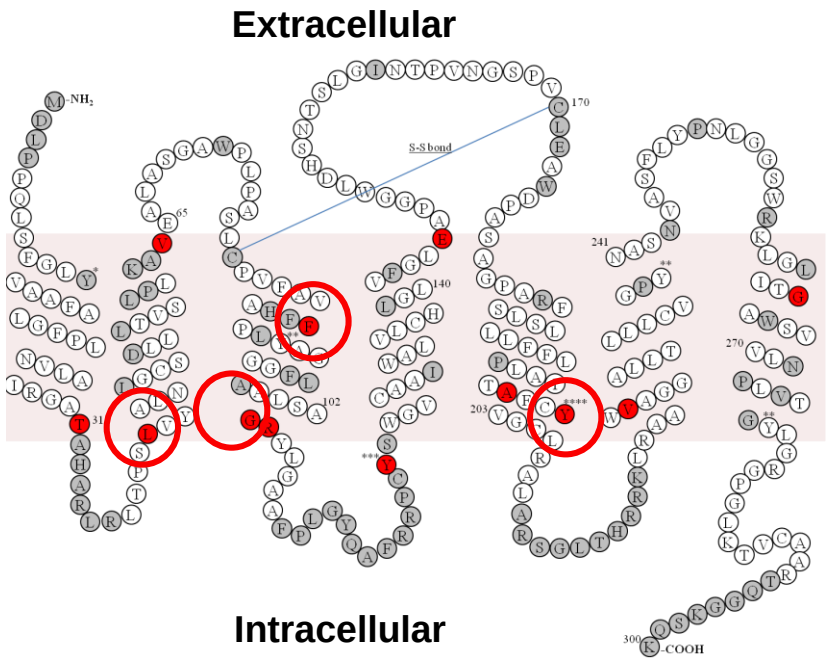
**Evaluate the effect of thermostability caused by mutation**

# 熱安定化GPCRとFasiglifam複合体の調製

## *Thermostability of GPR40*




| | Apparent $T_m$ value (℃) |
|---|---|
| Wild type | 38.3 |
| Thermostabilized mutant | 55.7 |



# ハイスループット技術の変遷と将来

| | | |
|---|---|---|
| 1991年 | HTSが初めてPubMedに登場 | |
| | インフラ整備開始 | |
| | プレート・分注器・検出器・ロボット | |
| | ライブラリー倉庫 | |
| 2000年前半 | 微量化・高速化・低コスト化の加速 | ヒトゲノム（2001） |
| | High content microscopy | |
| | イオンチャネル | NIH Road Map（2004） |
| 2000年後半 | マイクロ流路 | Chemical biology |
| | 1536プレートの汎用化 | |
| | 質量分析 | |
| | ラベルフリー検出 | |
| | ターゲット探索 （RNAiスクリーニング） | |

「ハイスループット」という強力な技術が新たな分野を切り開く