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Potency Revolution: MoA-Reflective Bioassays for Next-Gen QC Testing

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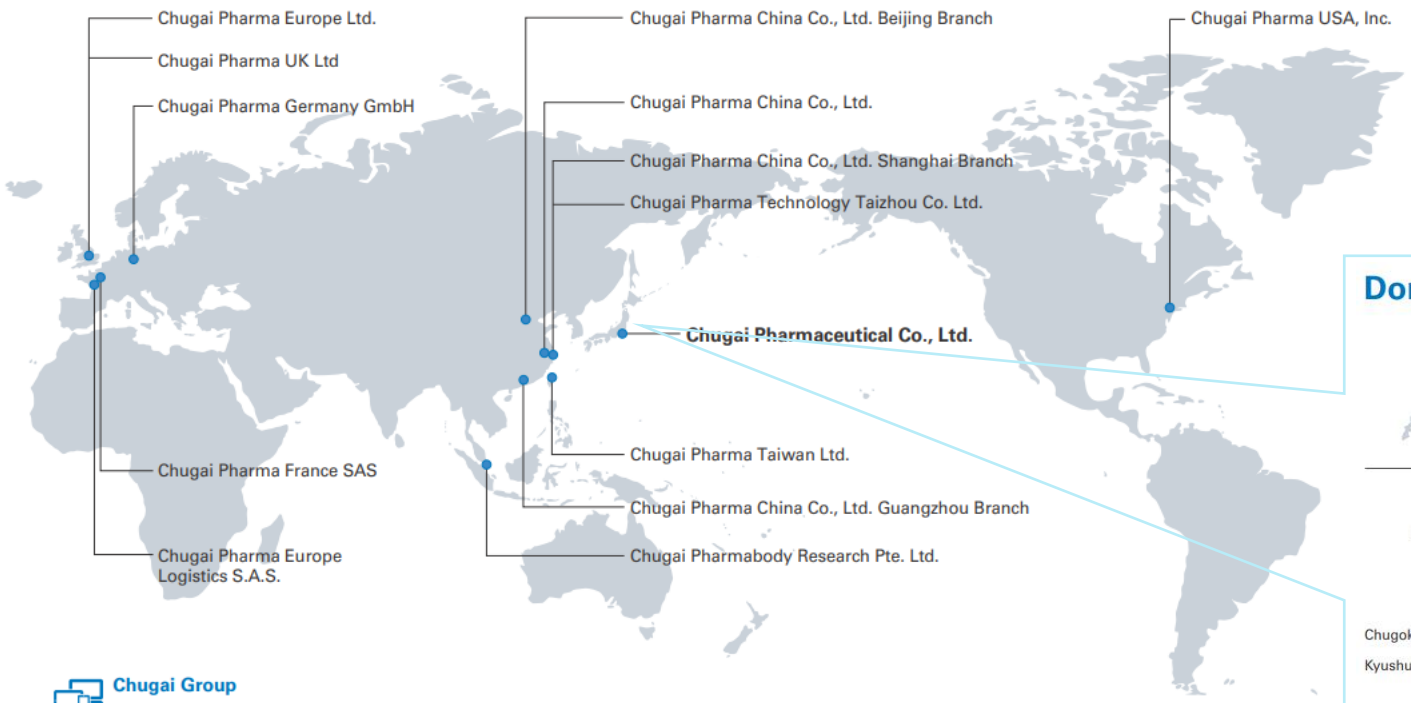
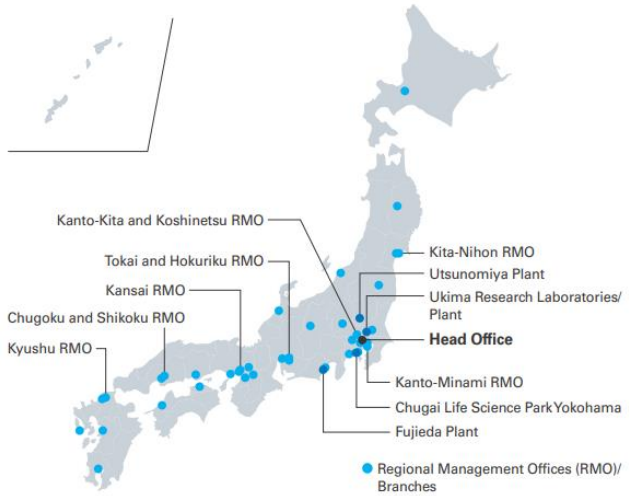
Chugai Group

Overseas Network (As of April 1, 2023)



Chugai Group
https://www.chugai-pharm.co.jp/english/profile/group/

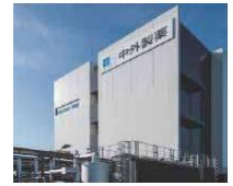
Domestic Network (As of April 1, 2023)




Head Office


Ukima Research Laboratories/ Plant


Utsunomiya Plant


Fujieda Plant

Chugai's New Research Center: Chugai Life Science Park Yokohama

By consolidating the functions of existing domestic research centers Fuji Gotemba and Kamakura Research Laboratories in one location, Chugai has built the foundation to maximize its drug discovery capabilities. In addition to the drug discovery research function, Chugai Life Science Park Yokohama also includes a facility that will mainly focus on the formulation of mid-size molecule compounds.


Chugai Life Science Park Yokohama

Agenda

01 What is CFA?

02 CFA implementation to QC testing

03 Investigation on Hyper potency phenomenon

Agenda

01 **What is CFA?**

02 CFA implementation to QC testing

03 Investigation on Hyper potency phenomenon

What is CFA?

Phase appropriate approach in potency assay

Bioassay development strategy in Chugai

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	P1	P2a	P2b	P3	Commercial
Methods	**Binding method** **・SPR method** **・ELISA** **etc…** Easy-to-develop Good precision and robustness		**Mode of action (MoA) reflective method** **・Cell proliferation assay** **・Cytotoxicity assay** **・Reporter gene assay** **Cell based assay (CBA)** **etc…** More complex and mimics MoA Inherently variable and often lacks precision Required skilled laboratory technique **⇒Cell free assay (CFA) is a MoA-reflective assay while addressing these challenges of later-stage testing methods.**		

What is CFA?

CFA is a cell-free assay system that covers the MoA of Product A

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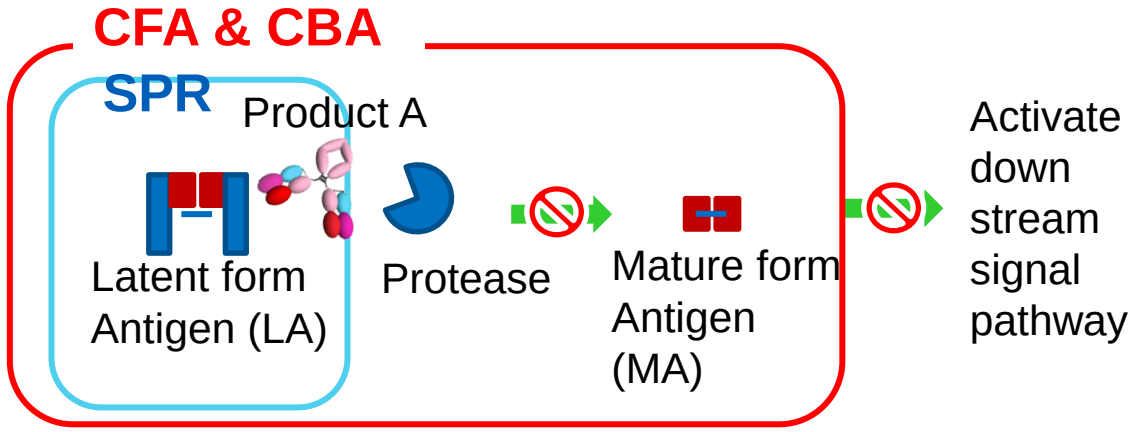
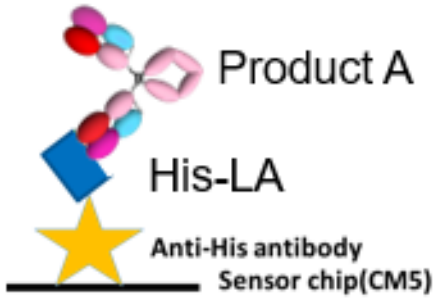
MoA of Product A



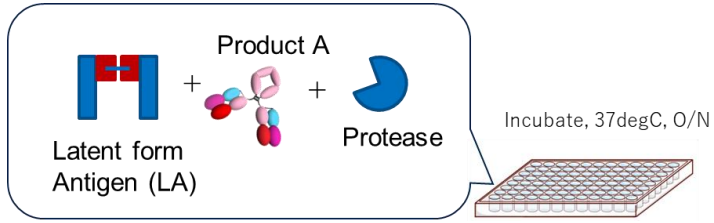
Assay principles of SPR



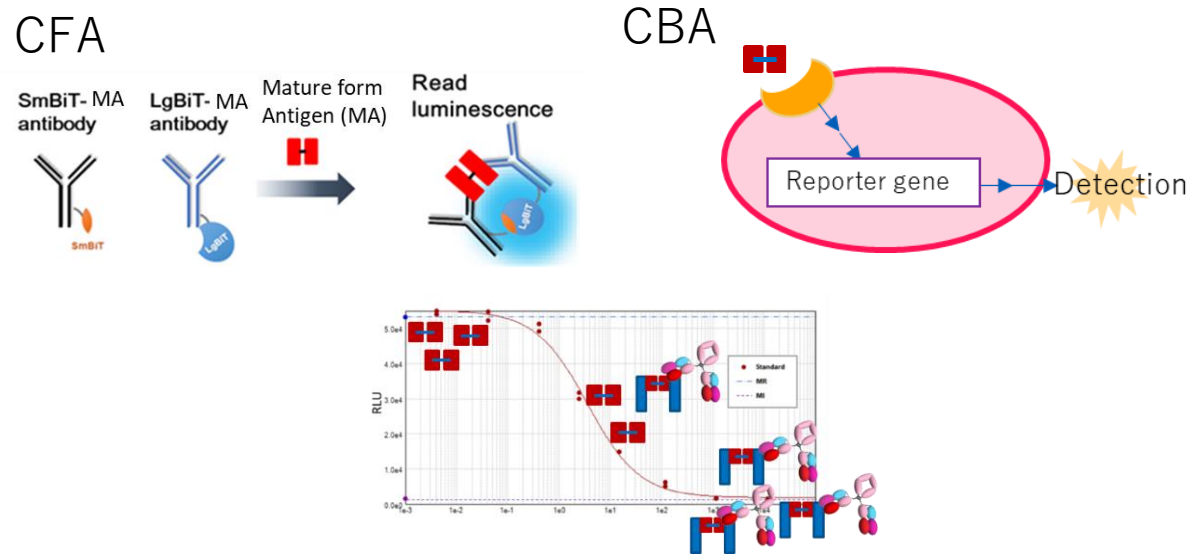
In SPR, Product A's dose-dependent binding activity to LA is evaluated as a readout.

Assay principle of CFA and CBA

Step 1. Cleavage reaction



Step 2. Mature form Antigen quantification



In both CFA and CBA, product A's dose-dependent inhibitory activity on MA production in competitive with the protease is evaluated as a readout.

What is CFA?

CFA is a QC-friendly testing method

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CFA was chosen when switching from SPR to later stage testing methods due to its suitability for QC testing, which stems from its simple procedures and robust stability throughout the lifecycle.

		Cell-based Assay	Cell-free Assay
Biologically relevant		CBA mimics vivo conditions	CFA can be applied to limited kind of MoAs
Risks during life cycle of assays	Variable factors	Many variable factors and unclear causal relationship	Less variable factors and clear causal relationship
	Materials to be controlled	Many critical reagents: Antigen, protease, growth factors, fetal calf serum, cell bank	Fewer critical reagents: Antigen, protease, detection antibody
	Lab to lab difference	Low reproducibility	Good reproducibility
	Analysts to analysts difference	Sensitive to analyst's technique	Robust to analyst's technique
Assay operability	Assay days	3 days (pre-culture + assay)	2 days
	Assay simplicity	Complicated a lot of procedure including cell passaging and cell suspension preparation	More convenient and less time-consuming due to its fewer steps (w/o plate wash!)
	Flexibility in testing dates	Less flexibility due to pre-culturing of the cells	Greater flexibility

Agenda

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01 What is CFA?

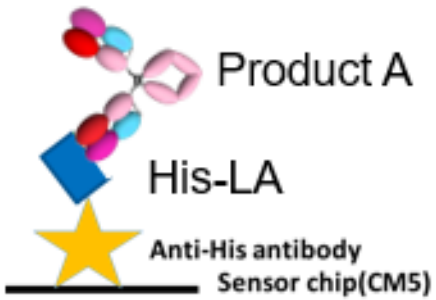
02 **CFA implementation to QC testing**

03 Investigation on Hyper potency phenomenon

Assay implementation strategy

- **Method qualification**
  - Specificity, Stability indicating
  - Accuracy, precision (IP, Repeatability), linearity
- **Method comparability to the previous method (SPR)**
  - Batch analysis, equivalence assessment via TOST analysis
  - Comparison of stability indicating samples

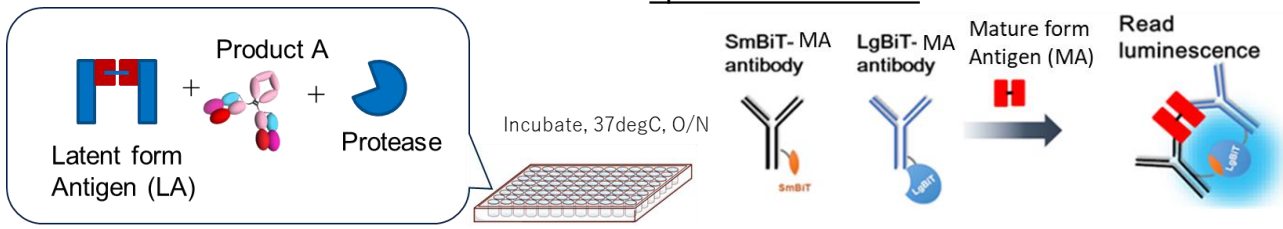
SPR



CFA

Step 1. Cleavage reaction

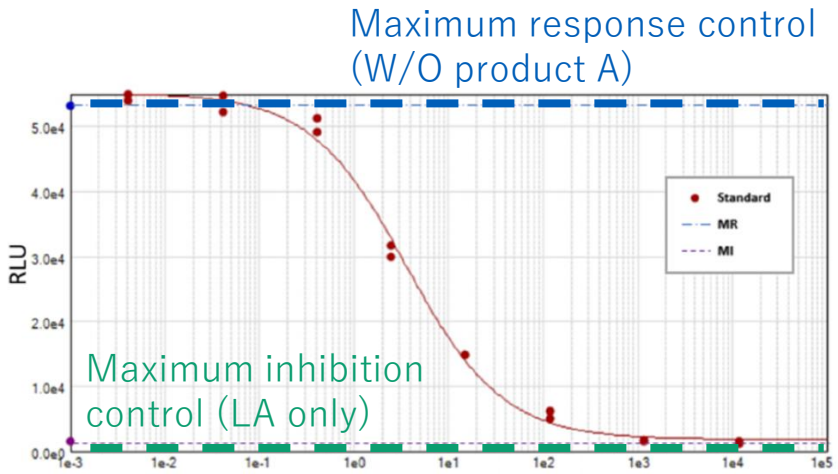
Step 2. Mature form Antigen quantification



Method qualification

CFA displayed good accuracy between 93% to 97% for each target level.

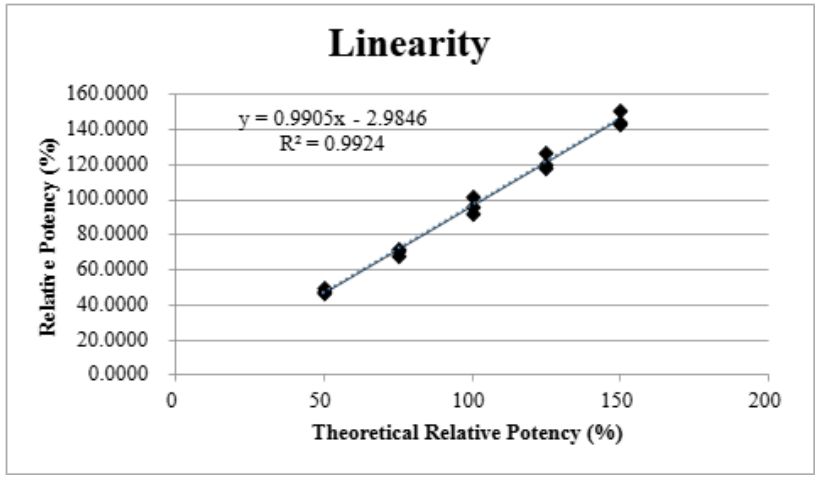
Characteristics	Acceptance criteria	Results	Acceptance
SPECIFICITY			
Product A RM/DS/DP	Dose dependent response is observed with Product A sample.	Dose dependent response was observed with Product A sample.	**passed**
Formulation buffer	Dose dependent response is not observed with formulation buffer.	Dose dependent response was not observed with formulation buffer.	**passed**
ACCURACY			
Mean recovery of relative potencies per level (N=3 per level)	70% – 130%	95% (50% of the target level) 93% (75% of the target level) 96% (100% of the target level) 97% (125% of the target level) 97% (150% of the target level)	**passed**
Mean recovery of overall [%]	Report	96%	**N/A**
95% Confidence interval of mean recovery	Report	90% - 100% (50% of the target level) 87% - 100% (75% of the target level) 85% - 107% (100% of the target level) 88% - 106% (125% of the target level) 90% - 104% (150% of the target level) 94% - 97% (overall)	**N/A**

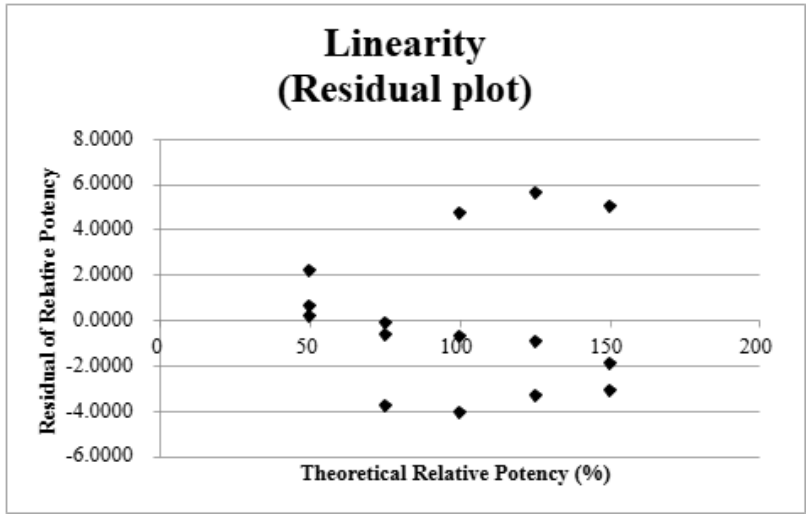


Method qualification (cont.)

CFA displayed good precision (RSD = 2% to 5% for each target level) and linearity (r = 1.00).

Characteristics	Acceptance criteria	Results	Acceptance
PRECISION (Repeatability)			
RSD of relative potencies (N=6, 100%)	RSD ≤ 15%	3%	**passed**
SD	Report	3	**N/A**
90% Confidence interval of SD	Report	2 – 6	**N/A**
PRECISION (Intermediate precision)			
RSD of relative potencies per level (50, 100 and 150%, N=6) [%]	RSD ≤ 20%	5% (50% of the target level) 4% (100% of the target level) 2% (150% of the target level)	**passed**
SD	Report	2 (50% of the target level) 4 (100% of the target level) 4 (150% of the target level)	**N/A**
90% Confidence interval of SD	Report	1 -10 (50% of the target level) 2 - 17 (100% of the target level) 2 - 16 (150% of the target level)	**N/A**
LINEARITY			
Correlation coefficient	r ≥ 0.97	1.00	**passed**
Slope	Report	0.991	**N/A**
Y-Intercept	Report	-2.985	**N/A**
Residual sum of squares	Report	141.773	**N/A**

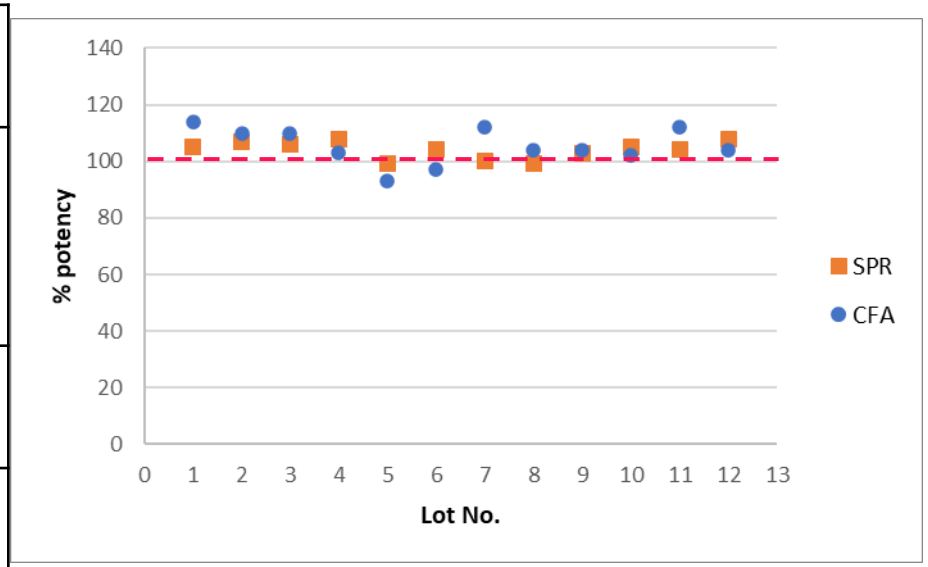




Method comparability between SPR vs CFA

- **Batch analysis**
  - **Two One-Sided Tests (TOST) was conducted to evaluate method comparability.**
  - **The 90% Confidence Interval of %Ratio was sufficiently narrow compared to the predefined maximum allowable difference (MAD), confirming the equivalence of the results.**

Evaluation Characteristics	Items	Acceptance Criteria	Results	Pass/Fail
Batch analysis (N=12)	90% Confidence Interval of %Ratio (CFA/SPR)	Within 100% ± 10%	98%—104%	Pass
	RSD%	Report	SPR: 3% CFA: 6%	not applicable
	Mean % relative potency	Report	SPR: 104% CFA: 105%	not applicable

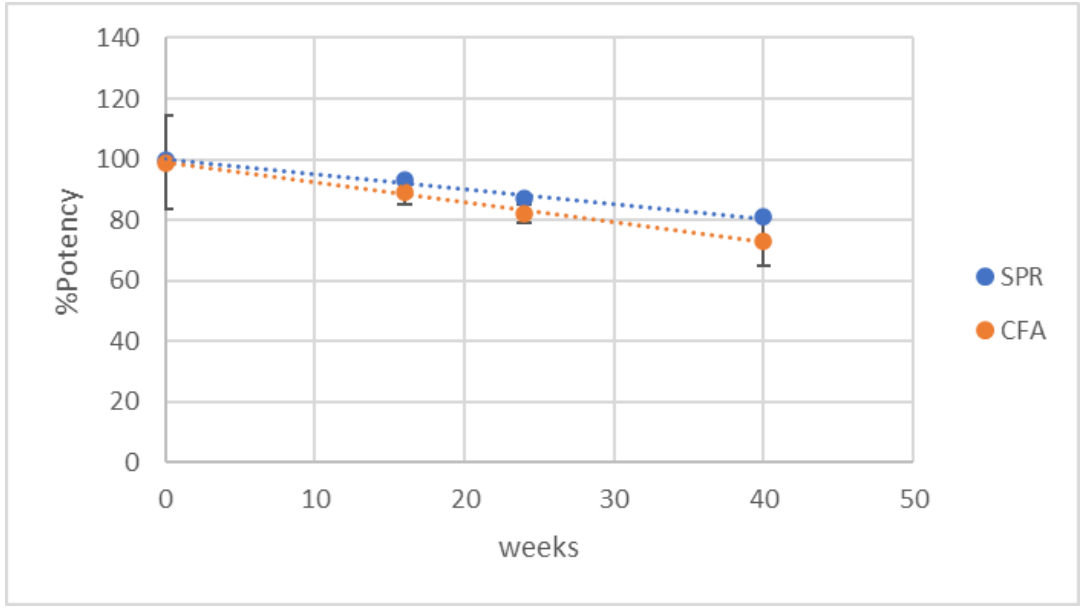


Method comparability between SPR vs CFA(cont.)

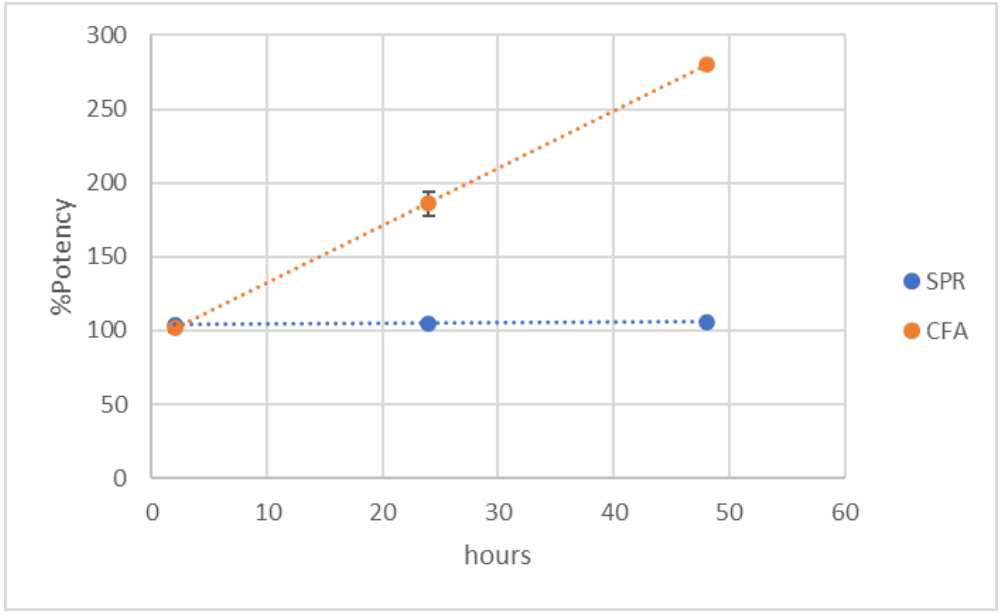
- **Comparison of stability indicating samples**
  - **For 40°C stress samples, degradation in the potency was observed in both the CFA and the SPR.**
  - **For low pH stressed samples, no change in potency was observed in the SPR, whereas the CFA assay an increase in potency.**

The stability indicating property of the CFA are equal to or greater than those of the SPR.

40°C Thermal stress sample



Low pH stress sample



Summary of CFA properties

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The assay demonstrated good properties and stability-indicating characteristics, confirming its appropriateness for determining product potency in release and stability testing.

Items	Results
Accuracy	Recovery = 93% to 97% for each target level.
Precision	RSD = 2% to 5% for each target level
Linearity	r= 1.00
Stability-indicating	Change in the potency was observed in 40°C thermally stressed sample and Low pH stressed samples.

Agenda

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01 What is CFA?

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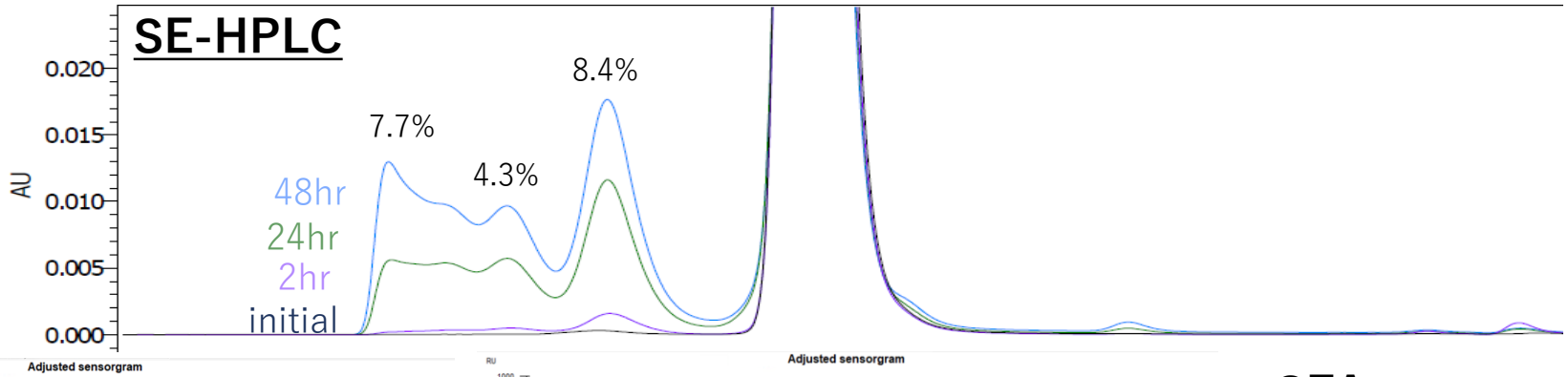
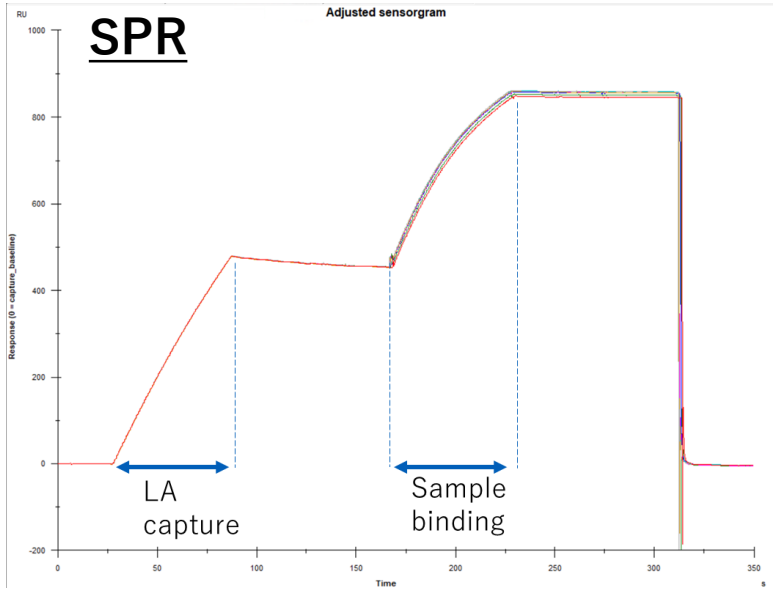
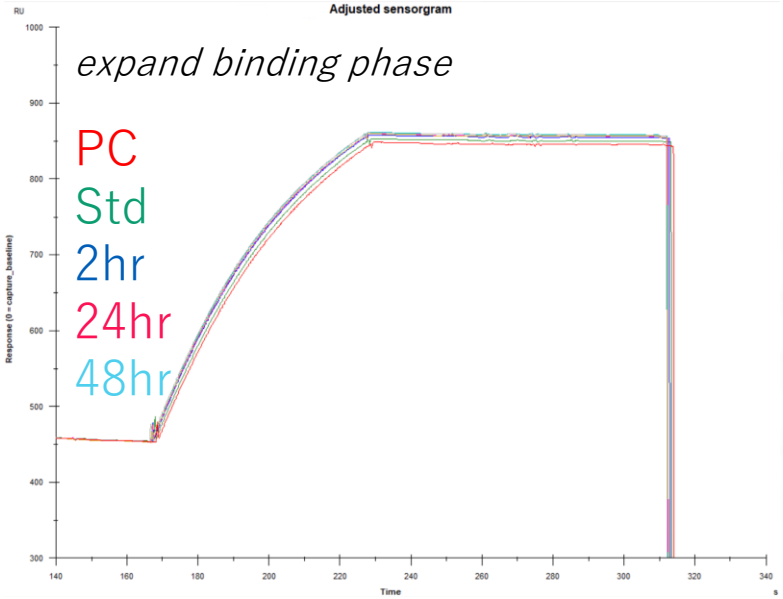
03 **Investigation on hyper potency phenomenon**

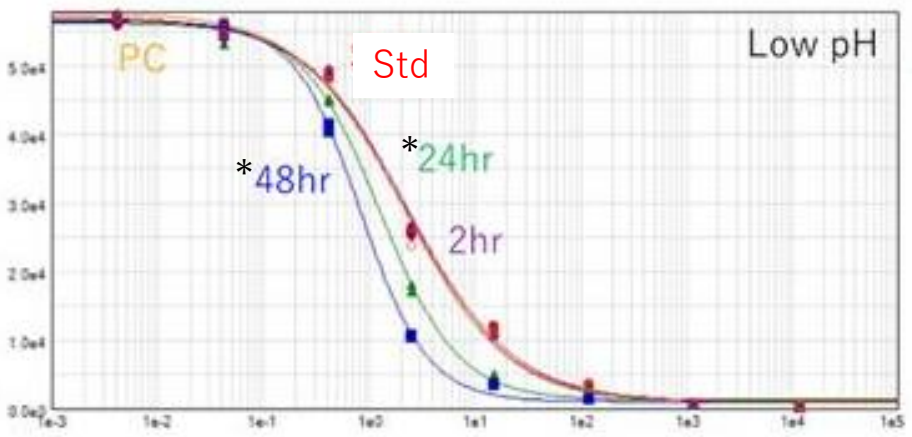
Time-dependent HMWs and CFA potency increase under low pH stress

- Low pH samples represent extreme conditions, and changes are unlikely to occur under normal manufacturing and storage processes.









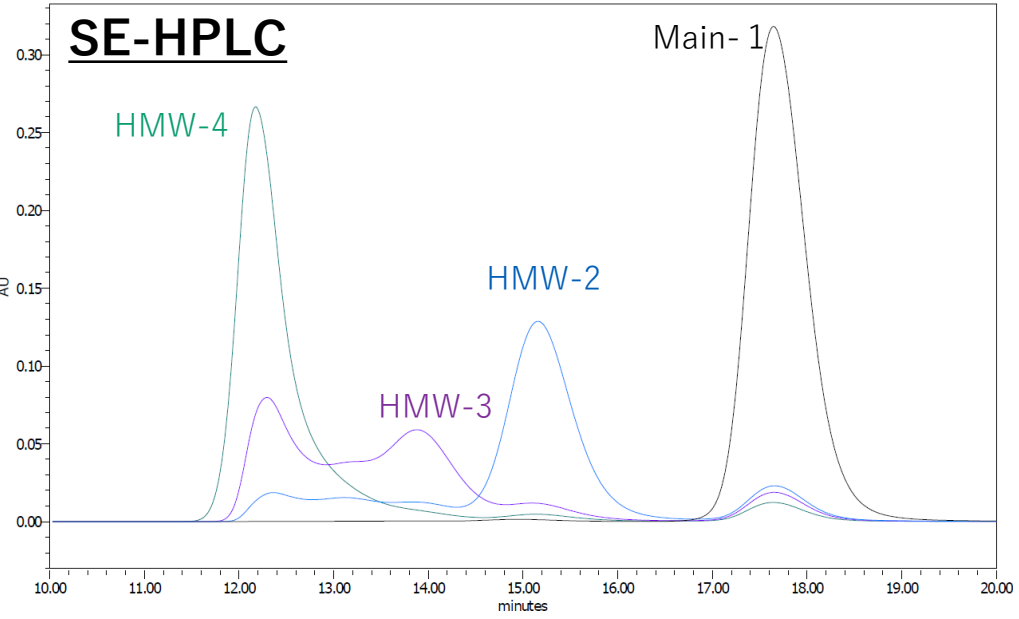
* Hill slope ratio% SST failure

HMWs lead to Hyper potency in CFA

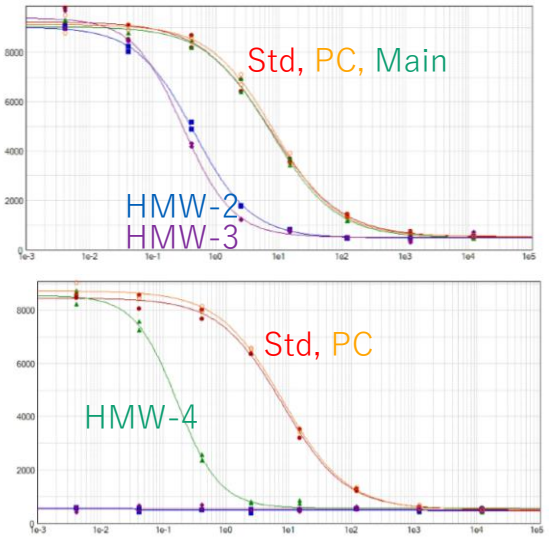
- **Results of SE-HPLC fractionated samples**

Stress condition	Result of re-chromatogram by SE-HPLC [%]				CFA	SPR
Fraction name	HMW-4	HMW-3	HMW-2	Main		
Low pH 48hr	Main-1	-	0.2	0.6	**99.2**	106%	113%
	HMW-2	7.2	12.5	**68.6**	11.6	1783%	81%
	HMW-3	34.9	**48.5**	6.7	9.9	2320%	75%
	HMW-4	**92.6**	-	2.5	5.0	4574%	76%

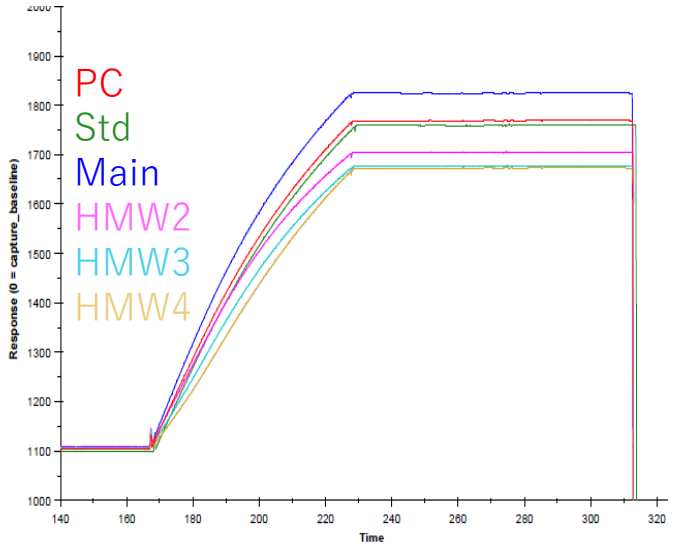
SE-HPLC



CFA



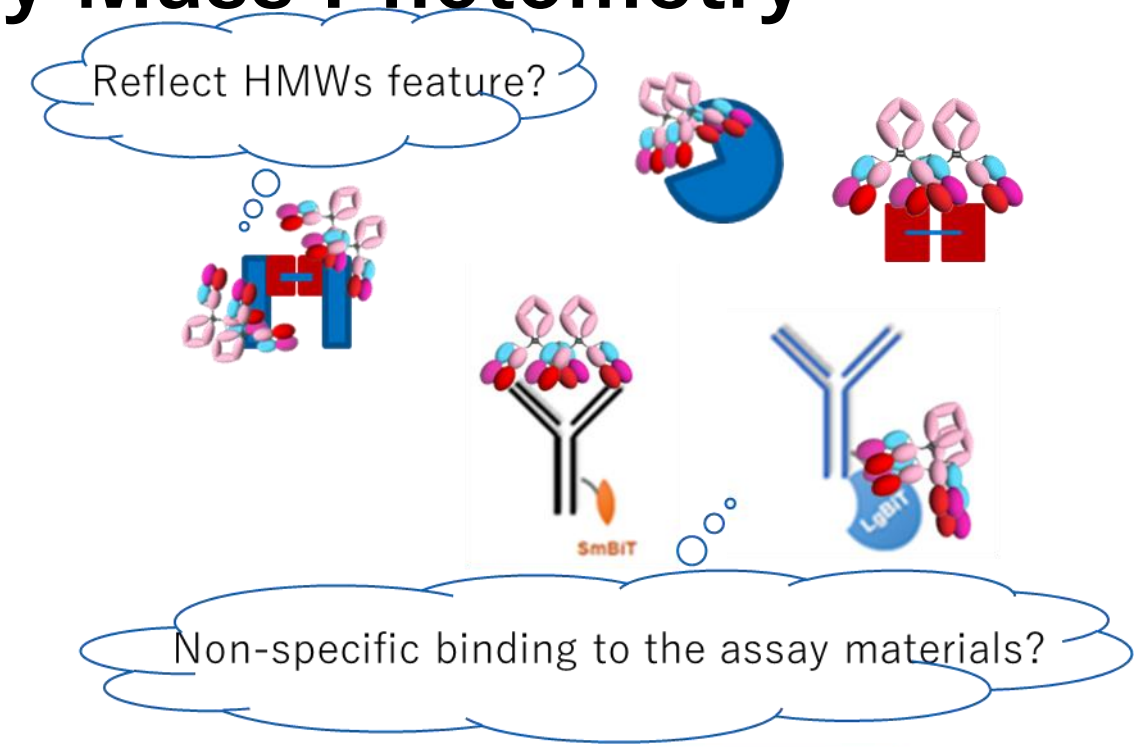
SPR(expand binding phase)



Molecular weighing by Mass Photometry

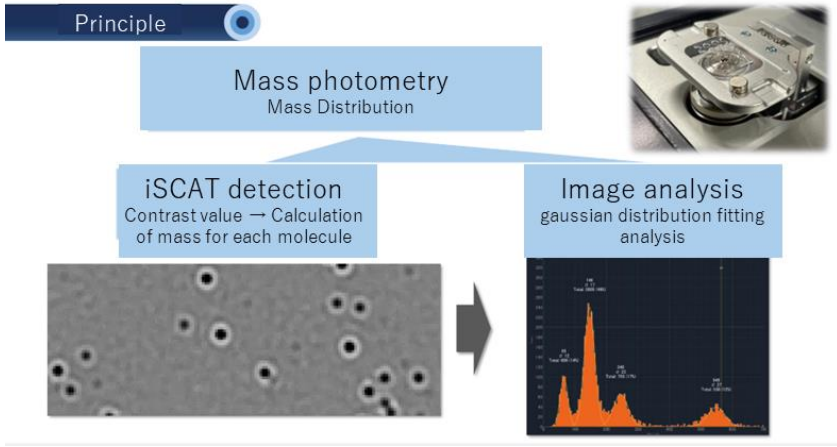
Possible hypothesis of Hyper potency

Hyper potency =



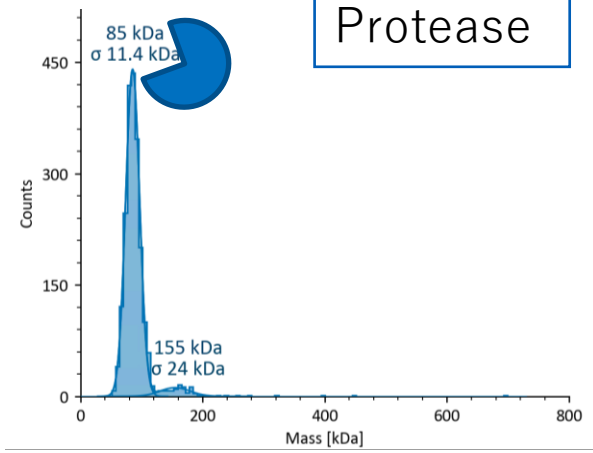
What's Mass Photometry (MP)

Principle: Measures molecular weight by analyzing scattered light from samples on glass
Key feature: Enables label-free, solution-based measurements of biomolecules. Low sample consumption(< 1 ng), quick measurement (5 min/sample) .

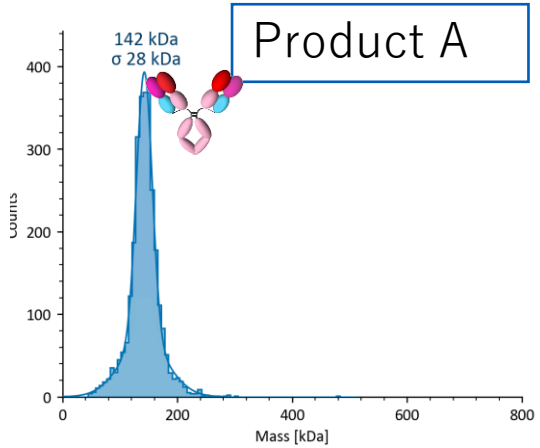


HMW's non-specific binding to Protease was not observed

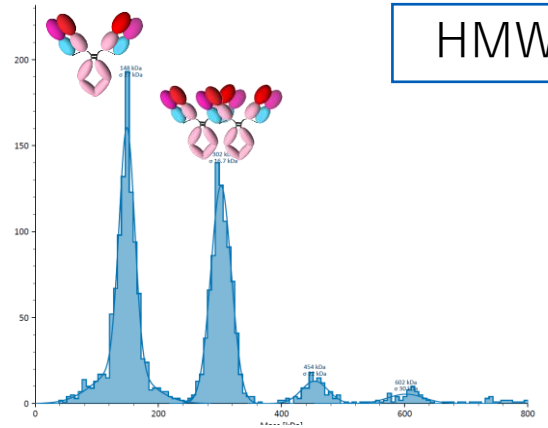
Protease



Product A

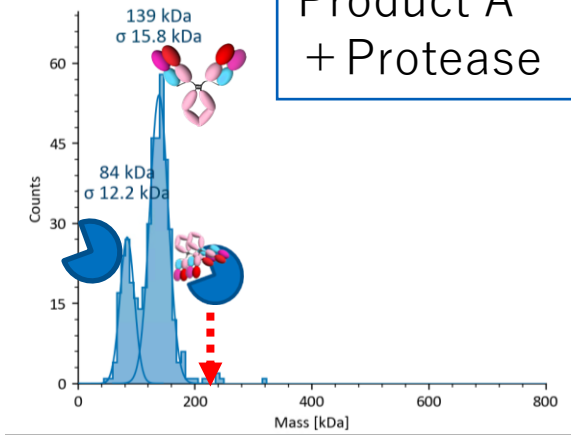


HMW-2

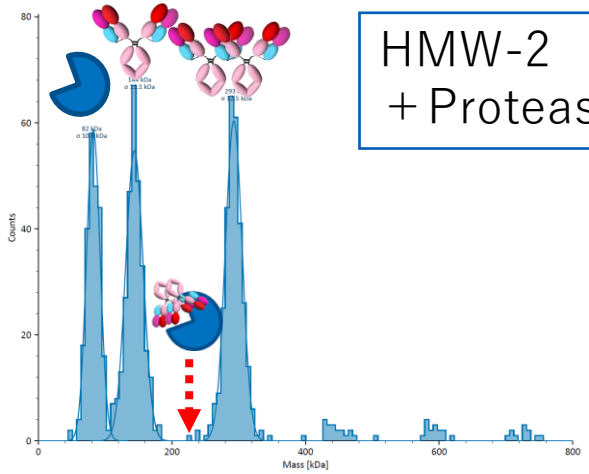


Confirmed by molecular weighing by **Mass photometory**

Product A ＋Protease



HMW-2 ＋Protease



It is suggested that HMW's non-specific binding to Protease is not the cause of Hyper potency. Considering the results of SE-HPLC fractionation as well, it was suggested that high molecular weight species is responsible for the hyper-potency. Further elucidation may help to address the risk of potency offset and justify the validity of the CFA.

Summary

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The CFA represents a significant advancement in QC testing based on the following key points:

- Cell-free assay (CFA) is implemented as a MoA-reflective testing method for a late-stage product.
- CFA is a QC-friendly testing method, particularly in terms of simplicity and robustness in life cycle management.
- The assay demonstrated good properties and stability-indicating characteristics, confirming its appropriateness for determining product potency in release and stability testing.