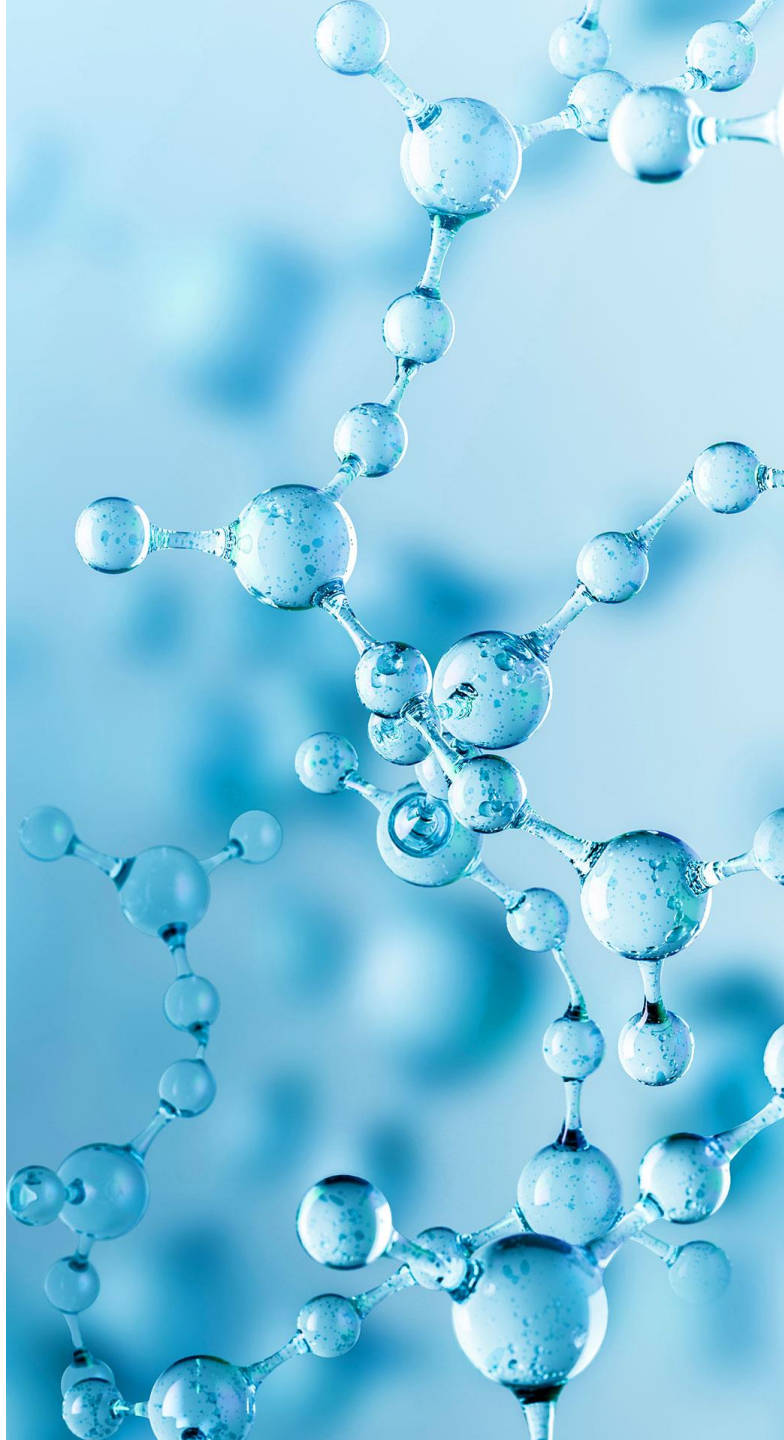
Proteinaceous Visible Particle in Liquid Monoclonal Antibody Formulations

Satoshi Saitoh
Analytical Development Department

CHUGAI PHARMACEUTICAL CO., LTD.

05 December 2022



Agenda

01 Protein & Interfacial Stresses

02 Surfactants

03 Case Studies of effectiveness of PX188

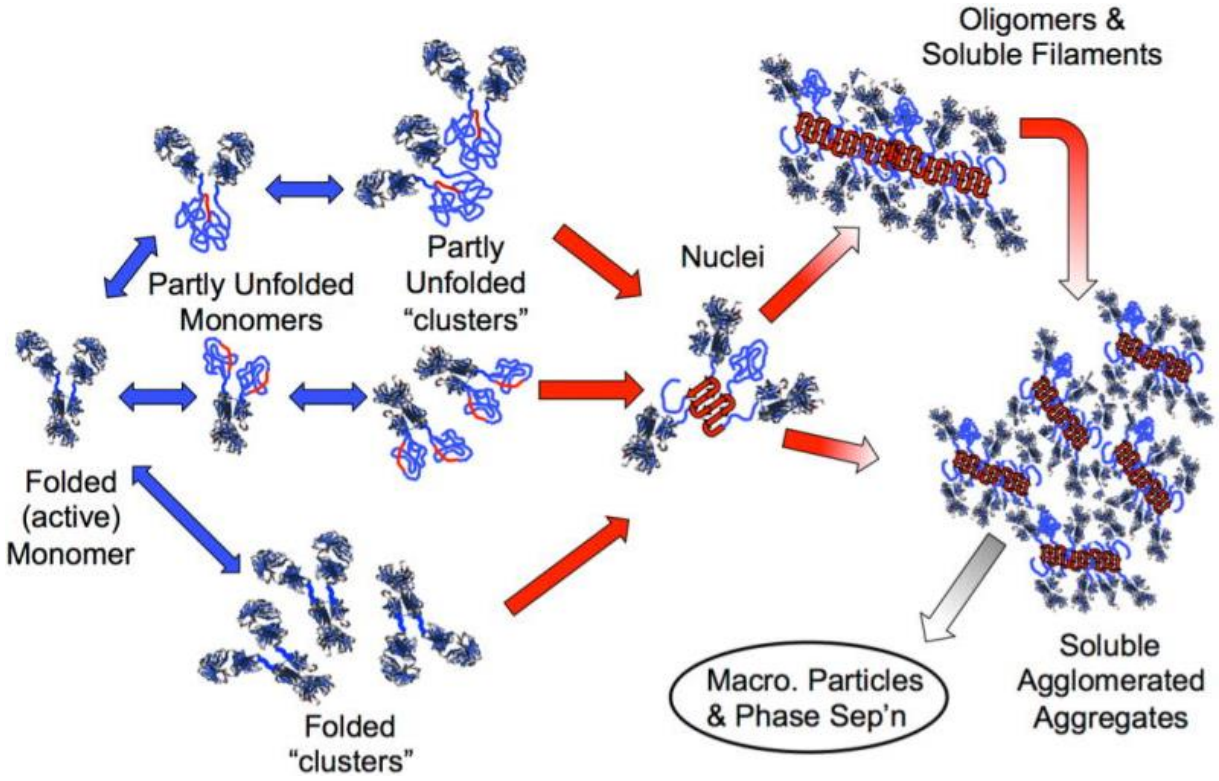
04 Message

Aggregation of Proteins

Therapeutic proteins are inherently aggregation-prone especially in their unfolded or partially unfolded states

Key Point!

- Fold vs Unfold
- Nature vs Denature

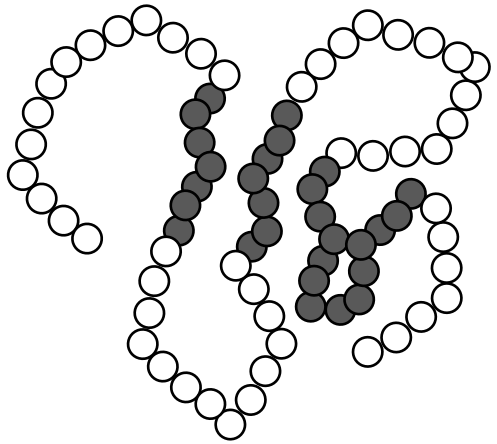


Roberts CJ. Therapeutic protein aggregation: mechanisms, design, and control. Trends Biotechnol. 2014 Jul;32(7):372-80.

Folding of Proteins

All Proteins can be UNFOLDED by any type of stresses

Native Protein/ Folded Protein

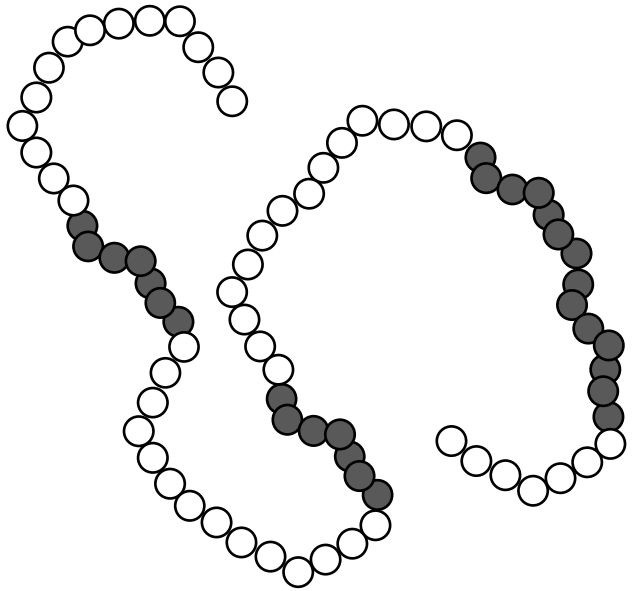


○ Hydrophilic Residues
● Hydrophobic Residues

Folding

Unfolding
(Stress)

Denatured Protein / Unfolded Protein

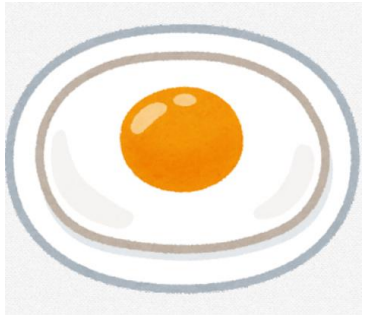


Denaturation of Proteins

All Proteins can be DENATURED by any type of stresses

- Therapeutic Proteins (e.g. Antibody) are the same as Egg Protein (e.g. Albumin)

Denatured Protein

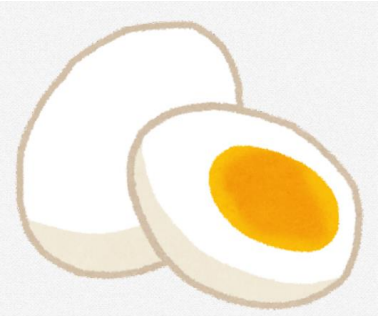


Temperature

Native Protein

Pressure

Denatured Protein



Denatured Protein

pH

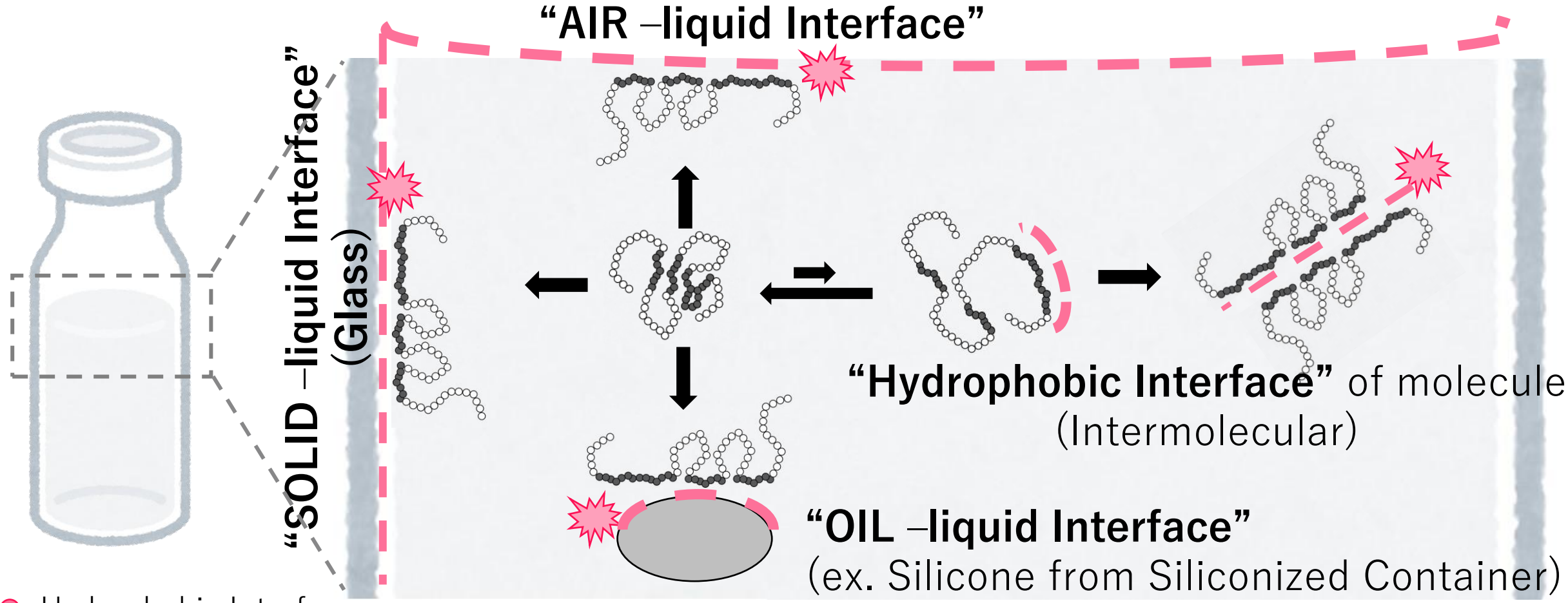
Denatured Protein

Interfaces

can be HOT SPOT for Aggregation

Hot spot / Interfaces in Therapeutic Protein Drug

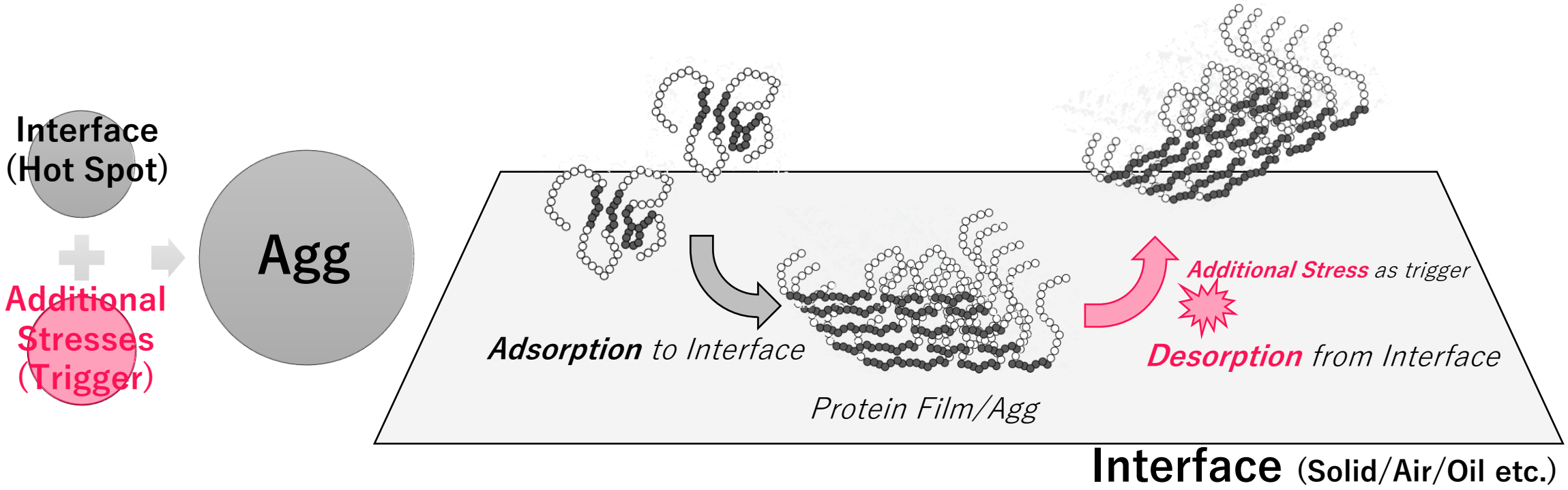
All Hydrophobic Interfaces can be “Hot spots for Aggregation”.



● Hydrophobic Interface

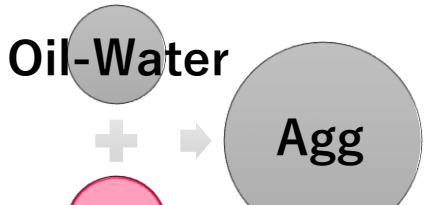
Mechanical Stresses / Trigger of particle formation

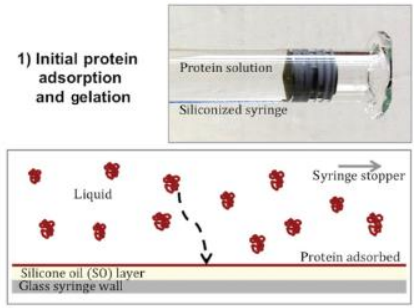
- **Importance of Desorption step and Trigger of the step are often discussed in many literatures.**

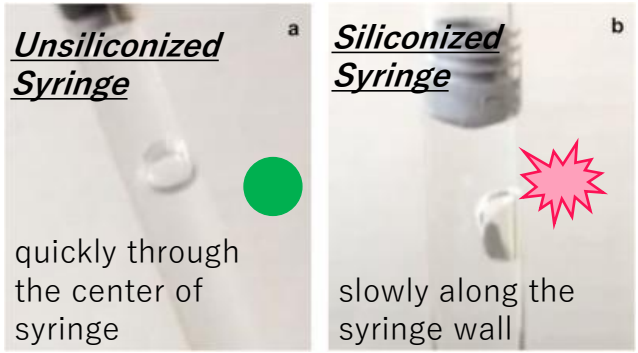


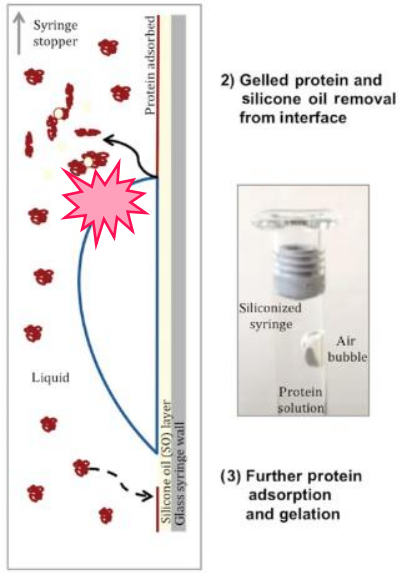
Mechanical Stresses / Trigger of particle formation

- Ex. Moving Air bubble, mechanical stress by needle

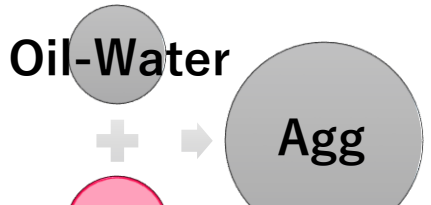








Gerhardt A, Mcgraw NR, Schwartz DK, Bee JS, Carpenter JF, Randolph TW. Protein aggregation and particle formation in prefilled glass syringes. J Pharm Sci. 2014 Jun;103(6):1601-12. doi: 10.1002/jps.23973. Epub 2014 Apr 11. PMID: 24729310.



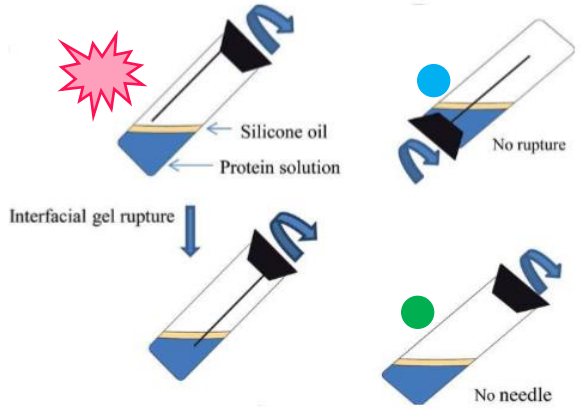
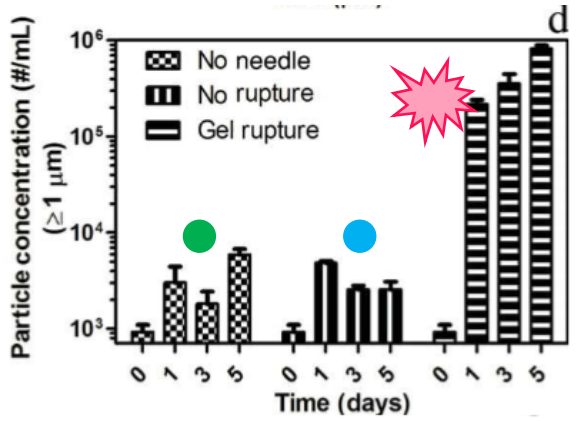


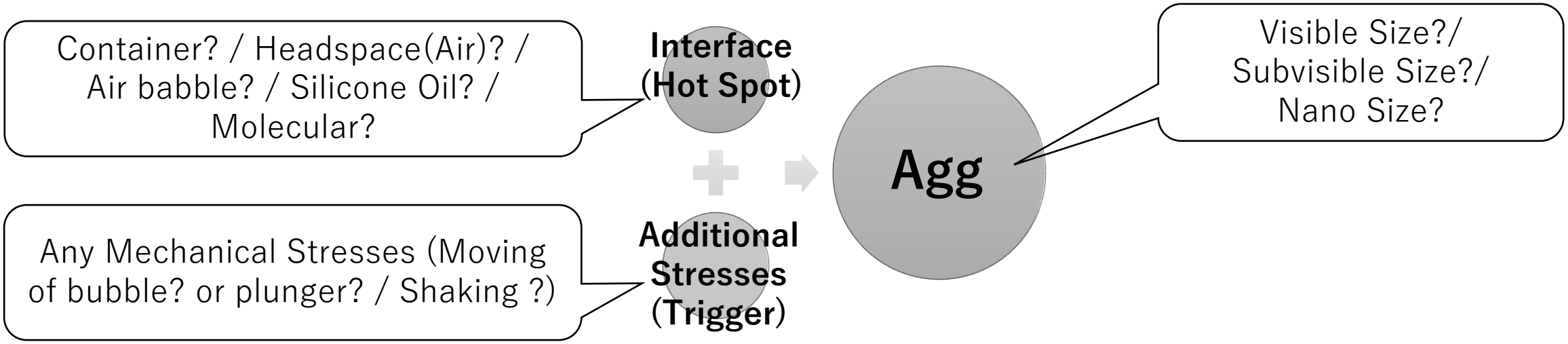
Figure 1. Interfacial gel rupture, no rupture, and no needle setup.



Mehta SB, Lewus R, Bee JS, Randolph TW, Carpenter JF. Gelation of a monoclonal antibody at the silicone oil-water interface and subsequent rupture of the interfacial gel results in aggregation and particle formation. J Pharm Sci. 2015 Apr;104(4):1282-90. doi: 10.1002/jps.24358. Epub 2015 Jan 30. PMID: 25639229.

Uncertainty/Complexity/Ambiguity of Mechanism

- **All protein can form aggregates, especially on the interface.**
- **However, a wide variety of worst cases/root causes can be considered.**



- **It is impossible to remove all interfaces & all stresses.**

➡ **Surfactants can reduce a wide variety of stresses.**

Agenda

01 Protein & Interfacial Stresses

02 Surfactants

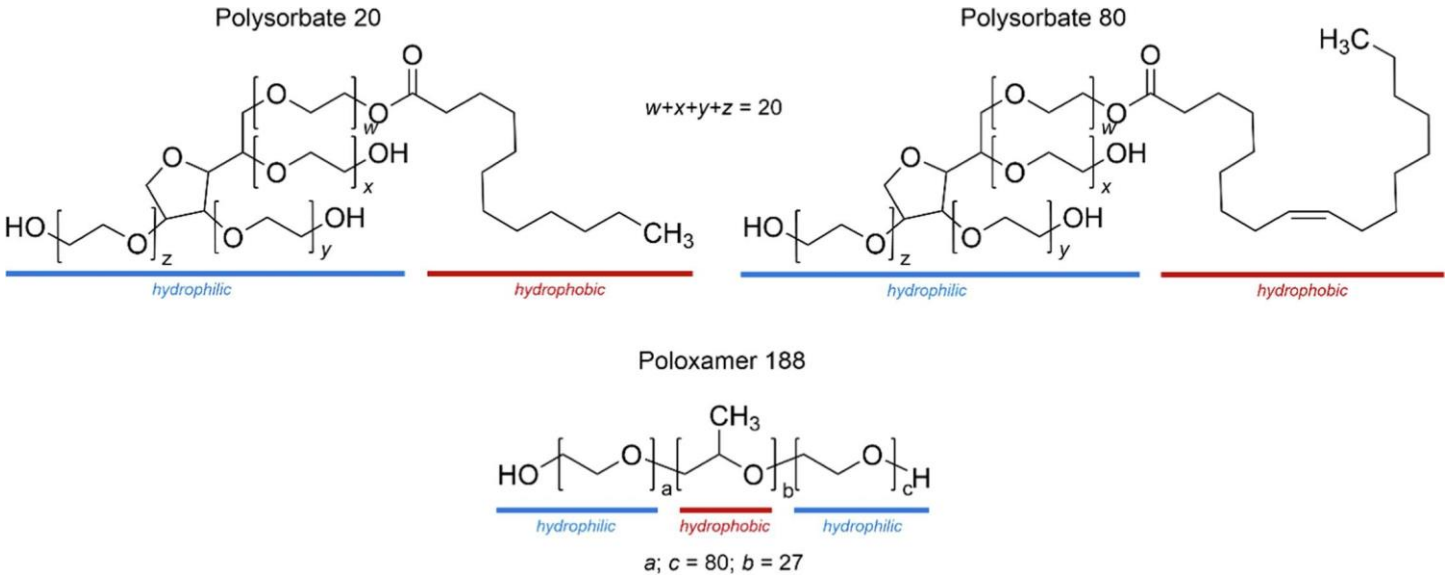
03 Case Studies of effectiveness of PX188

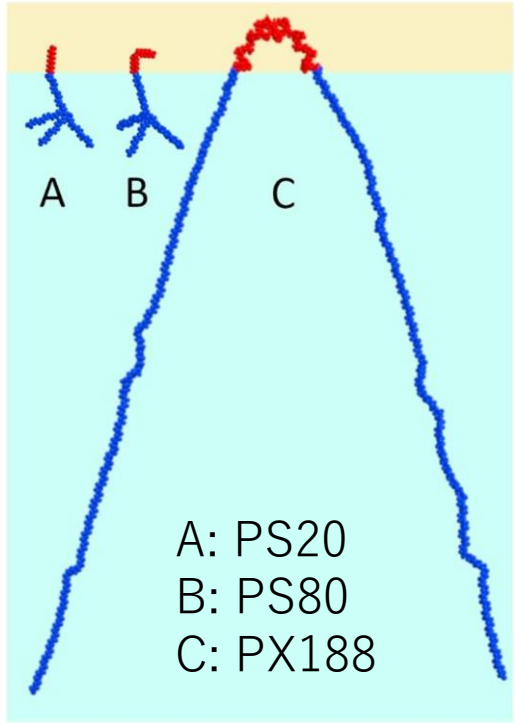
04 Message

Surfactants for Interfacial Properties

Surfactants are used to stabilize Therapeutic Proteins.

- Polysorbate 20/80, Poloxmer188

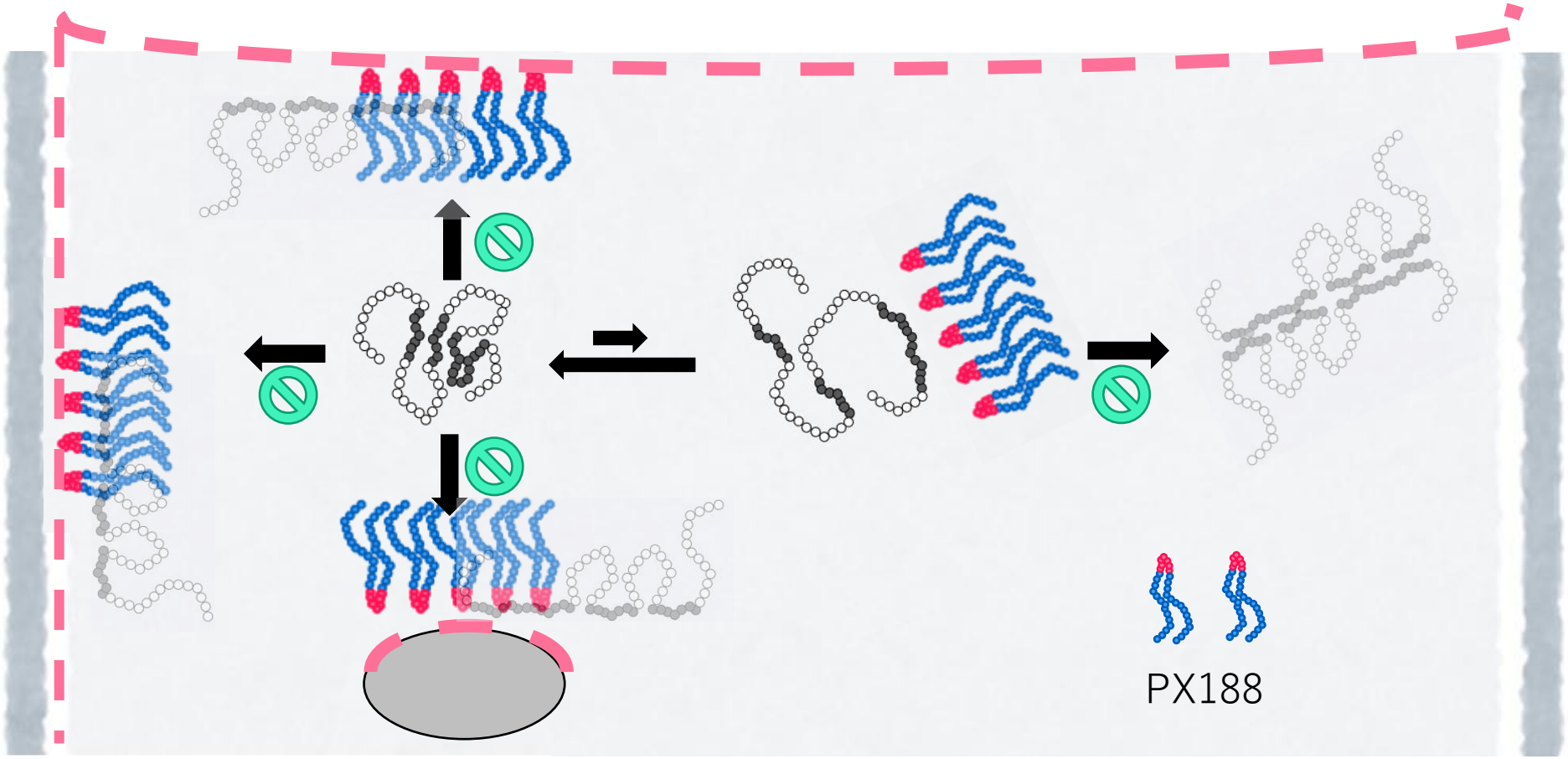




Lukas Bollenbach, Julia Buske, Karsten Mäder, Patrick Garidel,
Poloxamer 188 as surfactant in biological formulations – An alternative for polysorbate 20/80?,
International Journal of Pharmaceutics,Volume 620,2022,121706

Surfactants for Interfacial Properties

Mechanism of Stabilization by surfactants is frequently discussed.



Hydrophobic Interface

Agenda

01 Protein & Interfacial Stresses

02 Surfactants

03 **Case Studies of effectiveness of PX188**

04 **Message**

Characterization of PX188

- **A method to characterize the PPO(polypropylene oxide) block length.**

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

The development and qualification of liquid adsorption chromatography for poloxamer 188 characterization

Wei Chen, Alfred Ross, Bernd Steinhuber, Gabriel Hoffmann, Nuria Sancho Oltra, Satya Krishna Kishore Ravuri, Steven Bond, Christian Bell, Robert Kopf*

F. Hoffmann-La Roche AG, Grenzacherstrasse 124, 4070 Basel, Switzerland.

ARTICLE INFO

Article history:
Received 30 March 2021
Revised 14 June 2021
Accepted 16 June 2021
Available online 21 June 2021

Keywords:
Poloxamer
Critical point of adsorption
Liquid adsorption chromatography

ABSTRACT

Poloxamer 188 (P188) is formulated in proteinaceous therapeutics as an alternative surfactant to polysorbate because of its good chemical stability and surfactant properties, which enable interfacial protection, preventing visible and sub-visible particle formation. However, due to the nature of polymer heterogeneity and limited analytical approaches to resolve the superimposed components of P188, the impact of its quality variance on protein stability is still not well understood. In this study, we developed an analytical method to evaluate the components of P188 as a function of the length of polypropylene oxide (PPO), by maintaining polyethylene oxide (PEO) at the critical point of adsorption (CPA) to eliminate its chromatographic interference. The effectiveness of the separation was confirmed by nuclear magnetic resonance (NMR) spectroscopy and mass spectroscopy (MS) of the individual fractions corresponding to each peak. Additionally, a design of experiments (DoE) and method qualification were carried out to identify and optimize the key operation parameters, including column temperature and evaporative light scattering detector (ELSD) settings that need to be strictly controlled for reliable analytical results. In conclusion, this method is sensitive and reliable to compare the quality variance of commercial P188 and is suitable for routine quality control purposes. The application of this method could help in further understanding the Critical Material Attributes (CMA) that may affect the quality attributes of proteins in formulations.

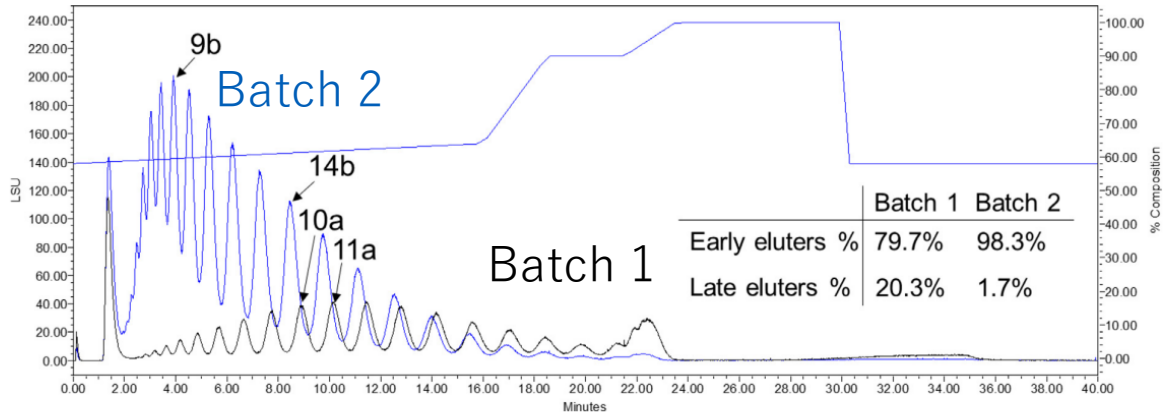
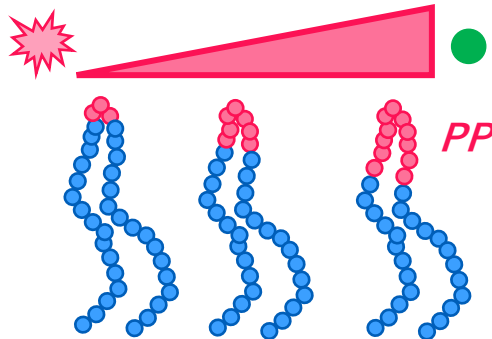


Fig. 3. The chromatograms of P188, batch 1 (black curve) and batch 2 (blue curve). Fraction numbers of the highest peak per batch are assigned with an arrow.

Peak characterization using NMR & MS



PPO block (hydrophobic)

PEO block (hydrophilic)

Characterization of PX188

- **Surface Tension depends on the Chromatographic characteristics**
- **Lot-to-lot variability was confirmed.**

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

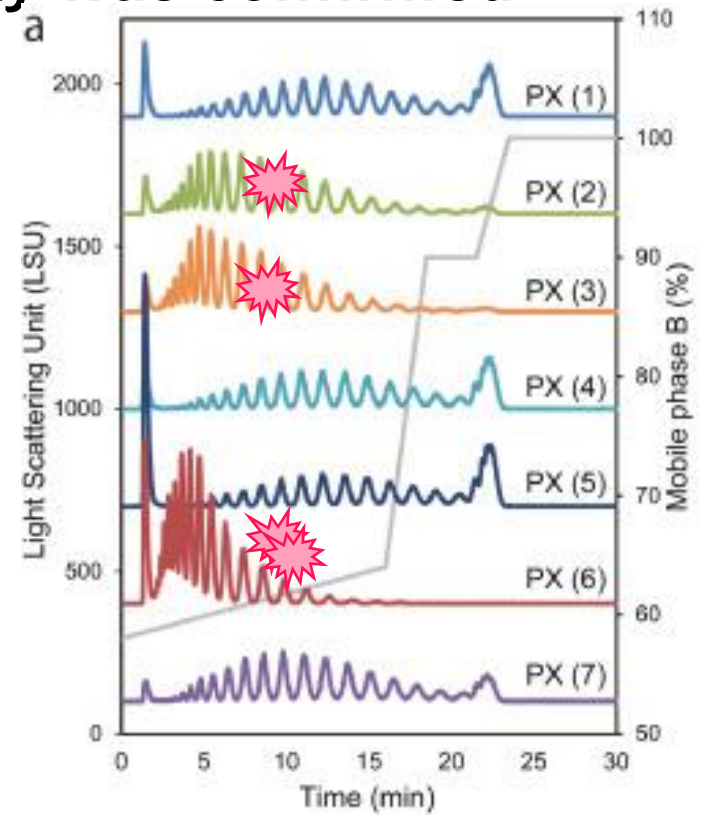
Pharmaceutical Biotechnology

Impact of Poloxamer 188 Material Attributes on Proteinaceous Visible Particle Formation in Liquid Monoclonal Antibody Formulations

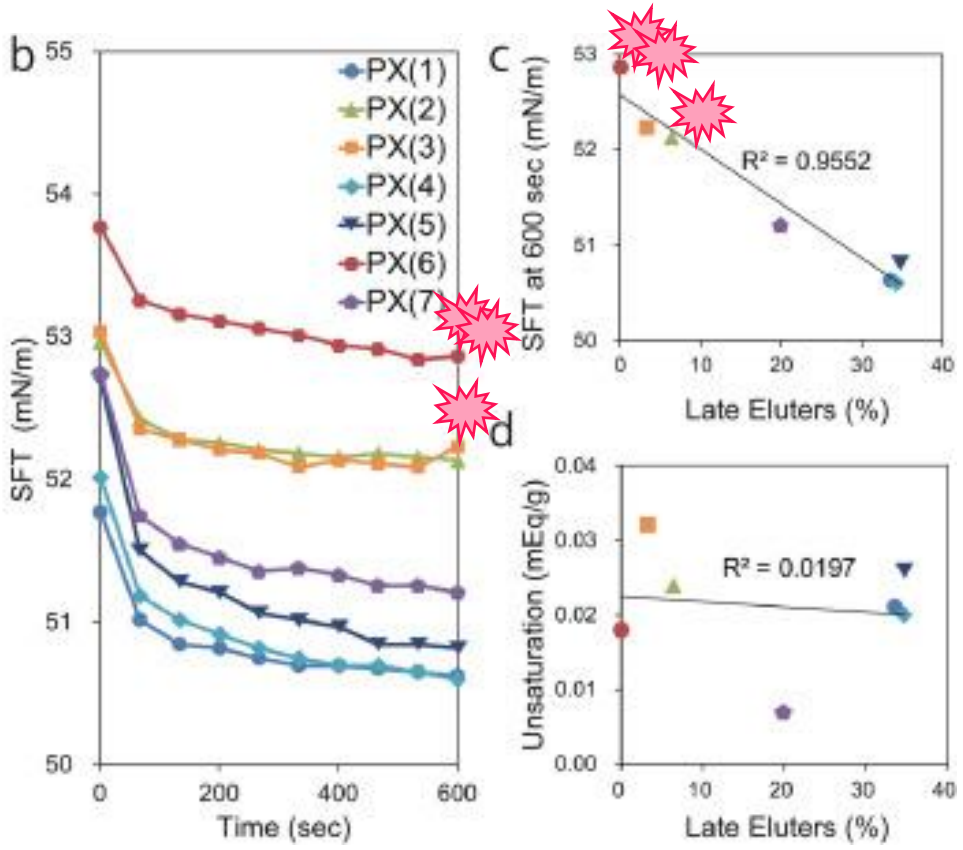
Kohei Soeda[a], Masakazu Fukuda[a,*], Masaya Takahashi[b], Hirotaka Imai[b], Kengo Arai[a], Satoshi Saitoh[b], Ravuri S.K. Kishore[c], Nuria Sancho Oltra[c], Jeremy Duboeuf[c], Daisuke Hashimoto[a], Yuji Yamanaka[a]

[a] Formulation Development Department, Chugai Pharmaceutical Co., Ltd., 5-5-1 Ukima, Kita-ku, Tokyo 115-8543, Japan
[b] Quality Development Department, Chugai Pharma Manufacturing Co., Ltd., 5-5-1 Ukima, Kita-ku, Tokyo 115-8543, Japan
[c] F. Hoffmann-La Roche Ltd., Pharmaceutical Development & Supplies, Pharma Technical Development Biologics Europe, Grenzacherstrasse 124,

ID	Product name
PX(1)	Kolliphor P188 (Lot A)
PX(2)	Kolliphor P188 (Lot B)
PX(3)	Kolliphor P188 (Lot C)
PX(4)	Kolliphor P188 Bio
PX(5)	Poloxamer 188 Emprove Expert
PX(6)	Poloxamer 188 Emprove Expert cell culture optimized
PX(7)	PLONON#188P



Length of PPO block
(Late Eluters>17min[area%])



Surface tension
(SFT)

Impact of PX188 variability for HMWS by SE-HPLC

- No clear impact for HMWS (high molecular species (e.g. dimer))

HMWS (%) evaluated by SE-HPLC.

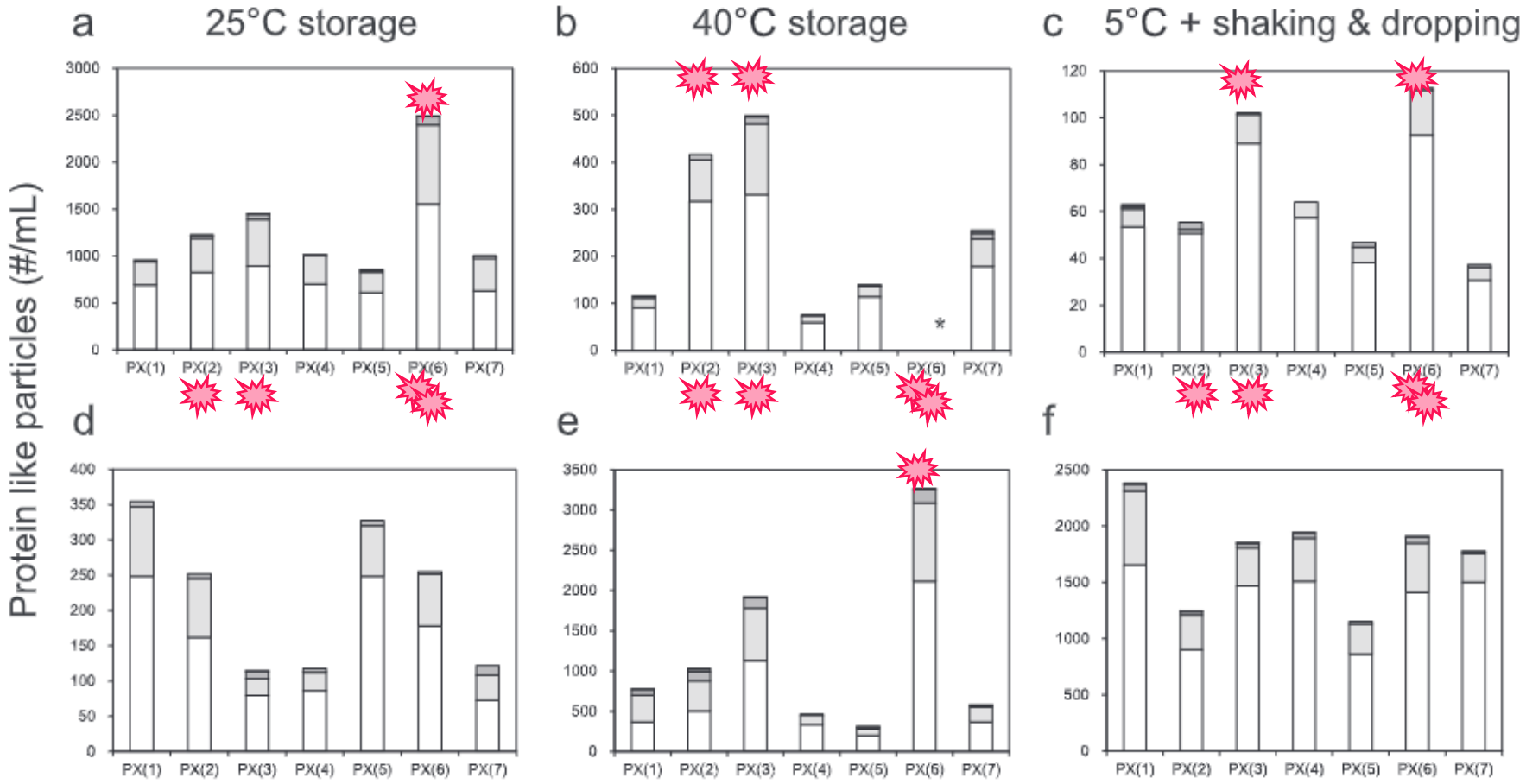
mAb	Formulation	Initial	5°C		25°C		40°C		5°C + shaking & dropping	
			3 months	6 months	3 months	6 months	3 months	6 months	3 months	6 months
mAb1	PX(1)	0.1	0.2	0.2	0.3	0.4	1.3	3.0	0.2	0.2
	PX(2)	0.0	0.2	0.2	0.3	0.4	1.4	3.3	0.2	0.2
	PX(3)	0.0	0.2	0.1	0.3	0.4	1.4	3.5	0.2	0.2
	PX(4)	0.0	0.2	0.2	0.3	0.4	1.4	3.5	0.2	0.2
	PX(5)	0.0	0.2	0.2	0.3	0.4	1.4	3.5	0.2	0.2
	PX(6)	0.0	0.2	0.2	0.3	0.4	1.3	3.5	0.2	0.2
	PX(7)	0.1	0.2	0.1	0.3	0.4	1.4	3.5	0.2	0.2
mAb2	PX(1)	0.3	0.3	0.4	0.7	0.8	2.2	4.8	0.4	-
	PX(2)	0.3	0.4	0.4	0.6	0.8	2.3	4.6	0.4	-
	PX(3)	0.3	0.4	0.4	0.6	0.8	2.1	4.9	0.4	-
	PX(4)	0.3	0.4	0.4	0.6	0.8	2.0	5.3	0.4	-
	PX(5)	0.3	0.4	0.4	0.7	0.8	2.2	5.3	0.4	-
	PX(6)	0.3	0.4	0.4	0.6	0.8	1.9	5.3	0.4	-
	PX(7)	0.3	0.3	0.4	0.6	0.8	1.8	5.3	0.4	-

Impact of PX188 variability for SvP by Flow imaging

- **A minor trend but not conclusive.**

mAb1

mAb2



＊ ＝ Data not available due to preferential use for particle identification

Protein-like images are counted by filtering (Aspect ratio <0.80, intensity mean <780)

Impact of PX188 variability for VP by visual inspection

- **VP occurrence depends on the PX188 characteristics.**
  - 5deg, 6 months: No VP observed
  - 25deg, 6 months

Formulation	mAb1			mAb2		
	Protein-only VPs (1)	Protein–PDMS VPs (2)	Proteinaceous VPs (1) + (2)	Protein-only VPs (1)	Protein–PDMS VPs (2)	Proteinaceous VPs (1) + (2)
PX(1)	0/10	0/10	0/10	0/10	0/10	0/10
PX(2)	1/10	2/10	3/10	0/10	1/10	1/10
PX(3)	2/10	2/10	4/10	0/10	0/10	0/10
PX(4)	0/10	0/10	0/10	0/10	0/10	0/10
PX(5)	1/10	0/10	1/10	0/10	0/10	0/10
PX(6)	0/10	5/10	5/10	0/10	2/10	2/10
PX(7)	0/10	1/10	1/10	0/10	0/10	0/10

  - 40deg, 6 months

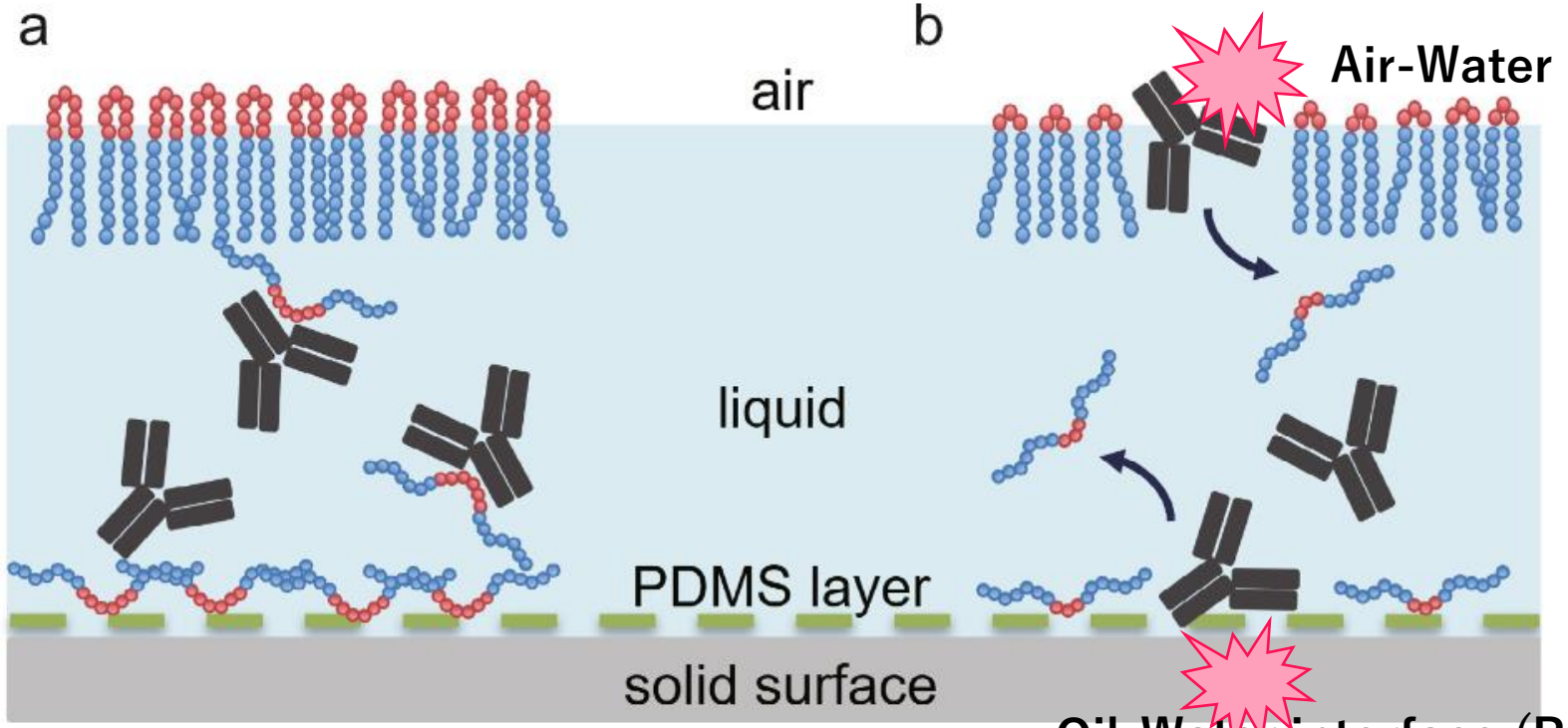
Formulation	mAb1			mAb2		
	Protein-only VPs (1)	Protein–PDMS VPs (2)	Proteinaceous VPs (1) + (2)	Protein-only VPs (1)	Protein–PDMS VPs (2)	Proteinaceous VPs (1) + (2)
PX(1)	0/10	0/10	0/10	1/10	1/10	2/10
PX(2)	1/10	0/10	1/10	1/10	4/10	5/10
PX(3)	1/10	2/10	3/10	0/10	7/10	7/10
PX(4)	0/10	0/10	0/10	0/10	1/10	1/10
PX(5)	0/10	0/10	0/10	0/10	3/10	3/10
PX(6)	0/10	9/10	9/10	1/10	7/10	8/10
PX(7)	0/10	0/10	0/10	0/10	2/10	2/10

Note = Raman spectroscopy was used for particle identification

Conclusion of the Case Study

- **Higher hydrophobic PX188 can reduce the risk of Proteinaceous Pericles.**

higher hydrophobic/longer PPO **lower hydrophobic/shorter PPO**



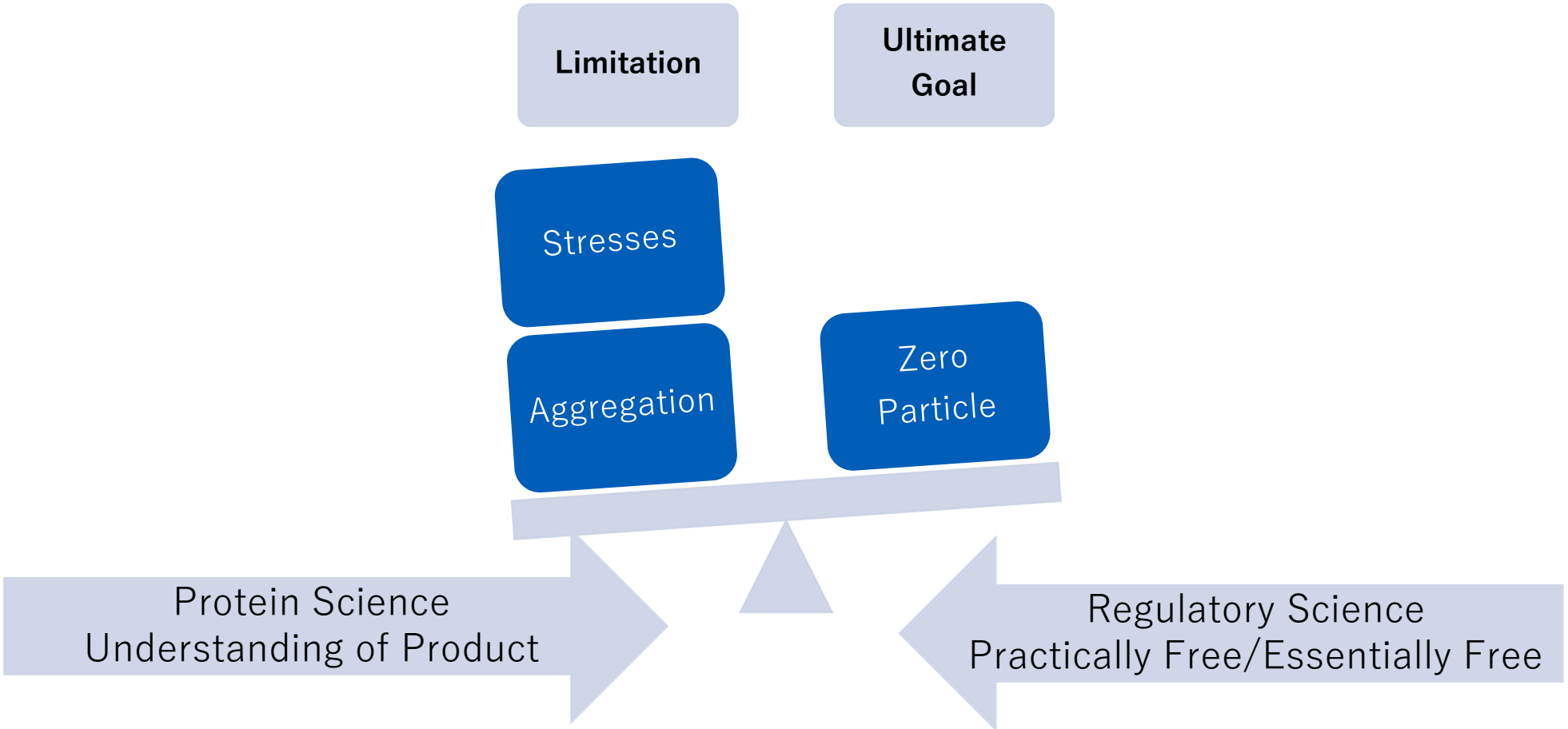
Air-Water interface

Oil-Water interface (PMDS = Silicone oil
Solid-Water interface

Agenda

Message

- **Protein unfolding/aggregates/particles might be unavoidable.**
- **However, they can be controlled by appropriate/practical strategies.**



INNOVATION BEYOND IMAGINATION