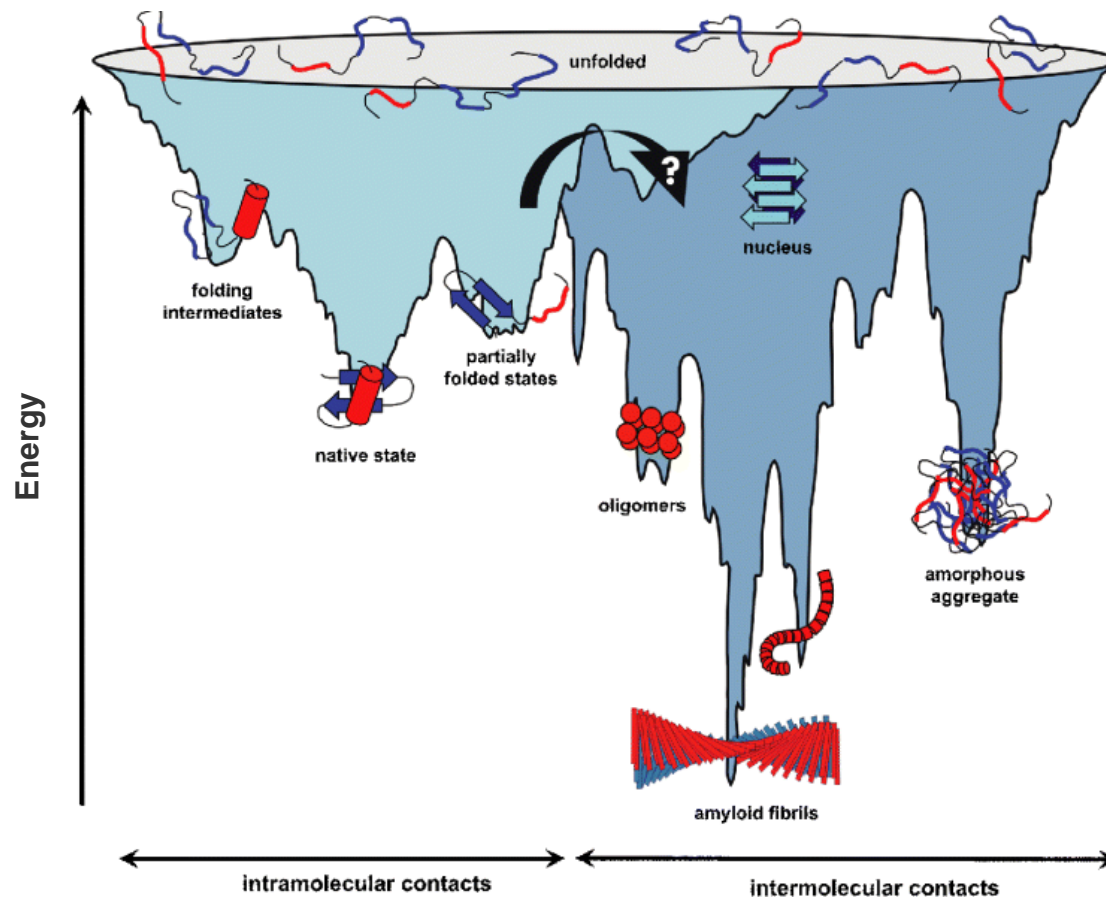


A novel flow cytometry based technology for the quantification and characterization of sub-visible particles in protein therapeutics

Mantas Malisauskas, PhD
Biologics Research & Development
IMMUNOLOGY
Baxter Innovations GmbH

Protein folding and aggregation

Aggregation is a generic property of a polypeptide chain

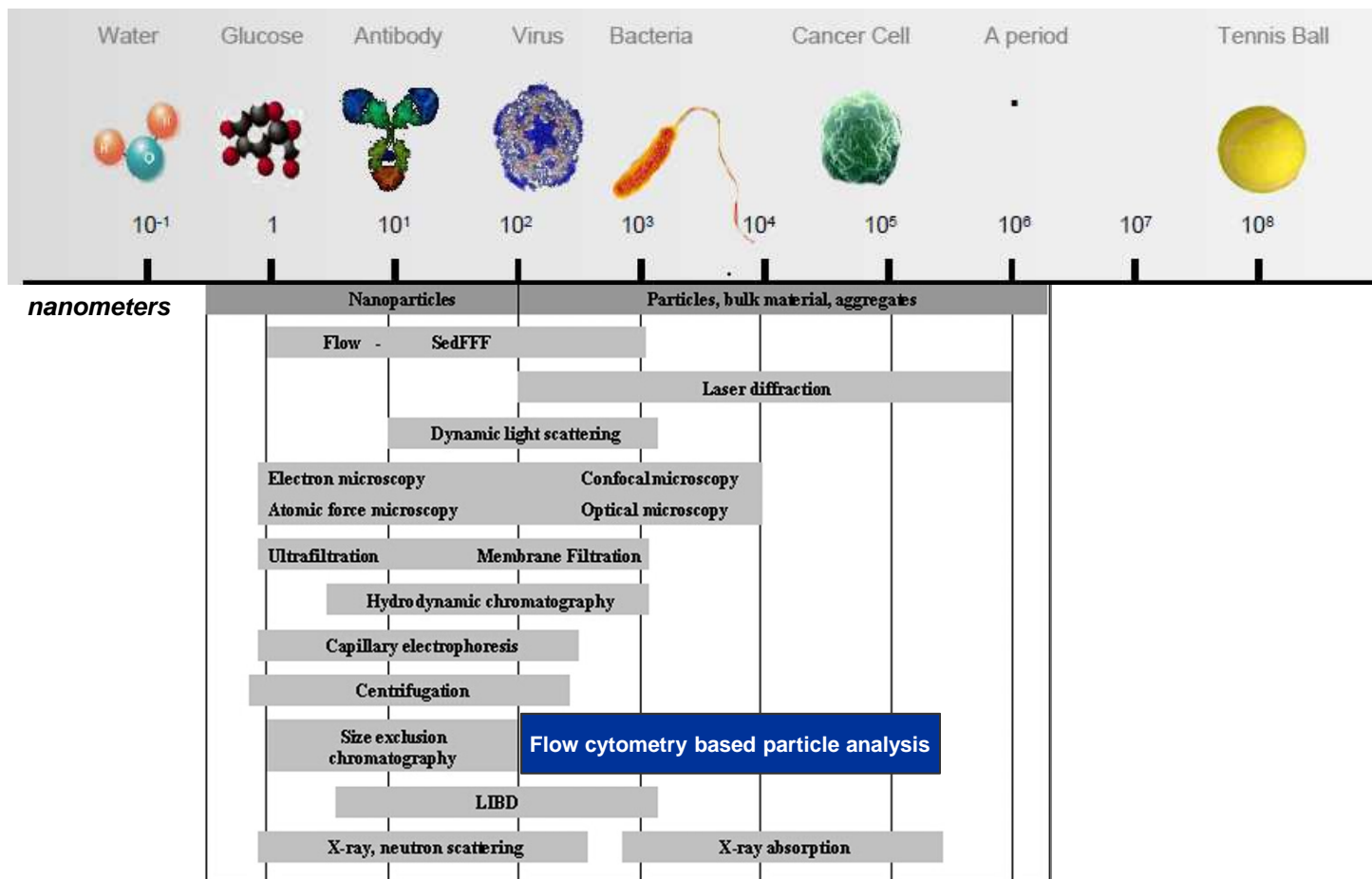


Jahn & Radford, FEBS Journal, 2005,75

sub-visible particles

“Undissolved species (other than gas bubbles or droplets) that are unintentionally present in the product. Particles can be foreign (not intrinsic to drug substance) or protein-related (i.e. large aggregates). Particles can be further categorized as visible (>ca. 50 μm) and sub-visible (between ca. 0.1–50 μm); submicron particles (between ca. 0.1–1 μm) are a subcategory of sub-visible particles.”

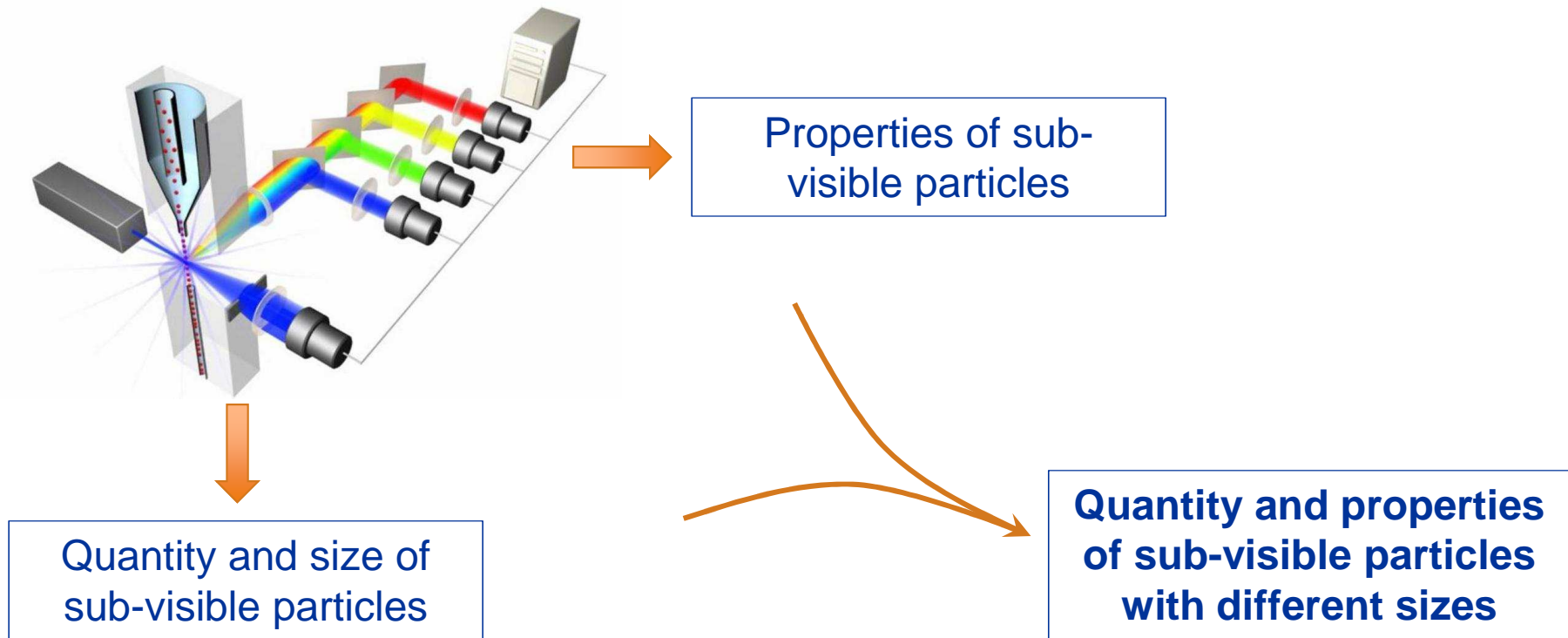
Methods for size determination of sub-visible particles and aggregates



Tiede et al, Food Additives and Contaminants, 2008, 25

Flow cytometry based sub-visible particle detection and analysis




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<http://www.invitrogen.com/site/us/en/home/support/Tutorials.reg.at.html>

Flow cytometry based sub-visible particle detection and analysis

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Not detectable 	Non-protein particles; detected 	Protein particles; detected and characterized 
< 100 nm	(0.1 μm) 0.75 μm – ca 70 μm	

- Particles larger than 100 nm can be detected
- Detected particles can be counted
- The size and properties of particles can be determined

 Protein specific dye

 Attribute specific dye



 Protein monomer

 Protein particles

 Non-protein particle

Flow cytometry based sub-visible particle detection and analysis

Baxter

Non-protein particles; detected	Protein particles; detected and characterized
	
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- **Particles larger than 100 nm can be detected**
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 Protein specific dye

 Attribute specific dye


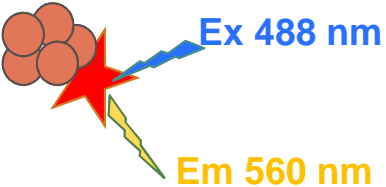
 Protein monomer

 Protein particles

 Non-protein particle

Flow cytometry based sub-visible particle detection and analysis

Baxter

Non-protein particles; detected 	Protein particles; detected and characterized 
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 Protein specific dye

 Protein monomer


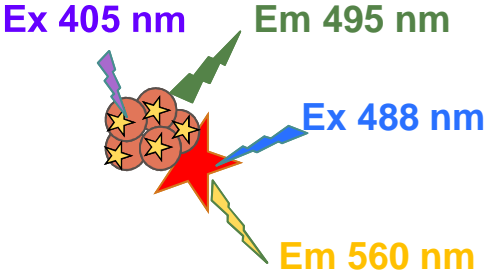
 Non-protein particle

 Attribute specific dye

 Protein particles

Flow cytometry based sub-visible particle detection and analysis

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<p>Non-protein particles; detected</p> 	<p>Protein particles; detected and characterized</p> 
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★ Protein specific dye

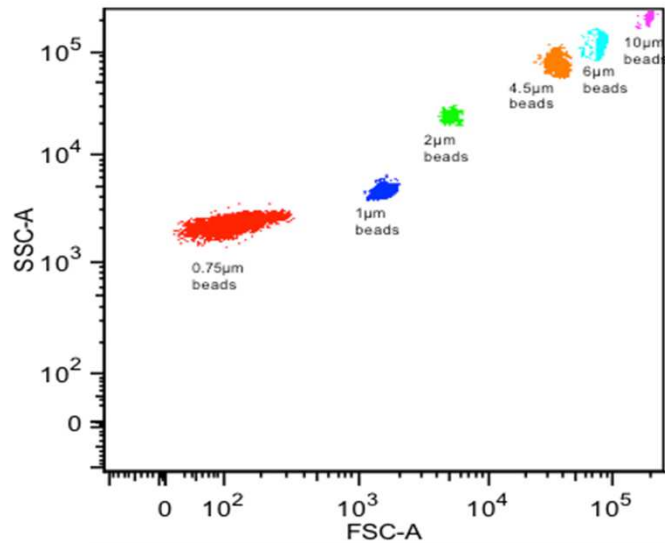
★ Attribute specific dye

● Protein monomer

● Protein particles

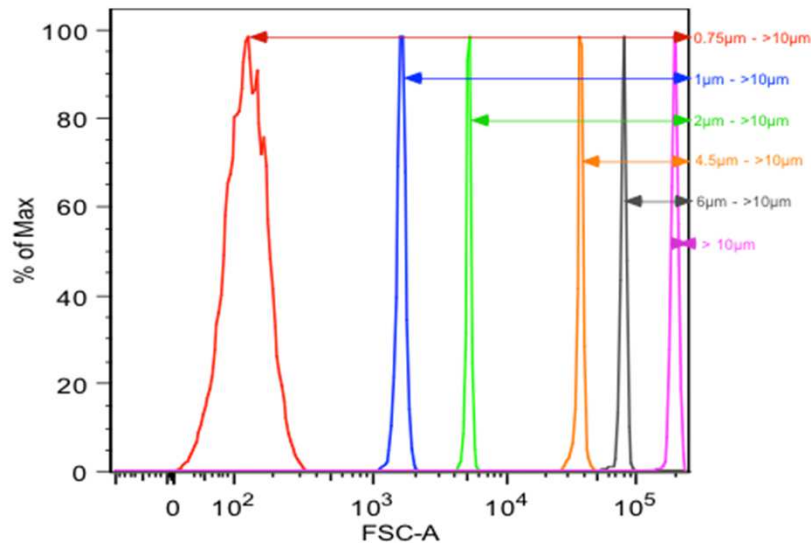
● Non-protein particle

Flow cytometry based sub-visible particle detection and analysis: establishing size ranges



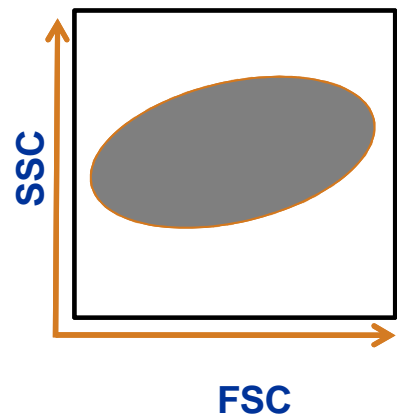
0.1 – ca 200 µm depending on available equipment

Currently using:
0.75 µm – ca 70 µm

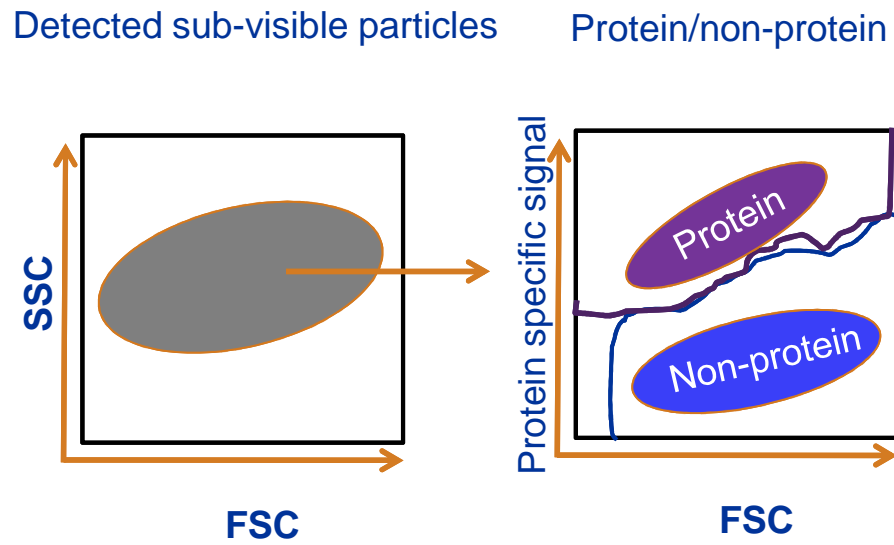


Gating strategy

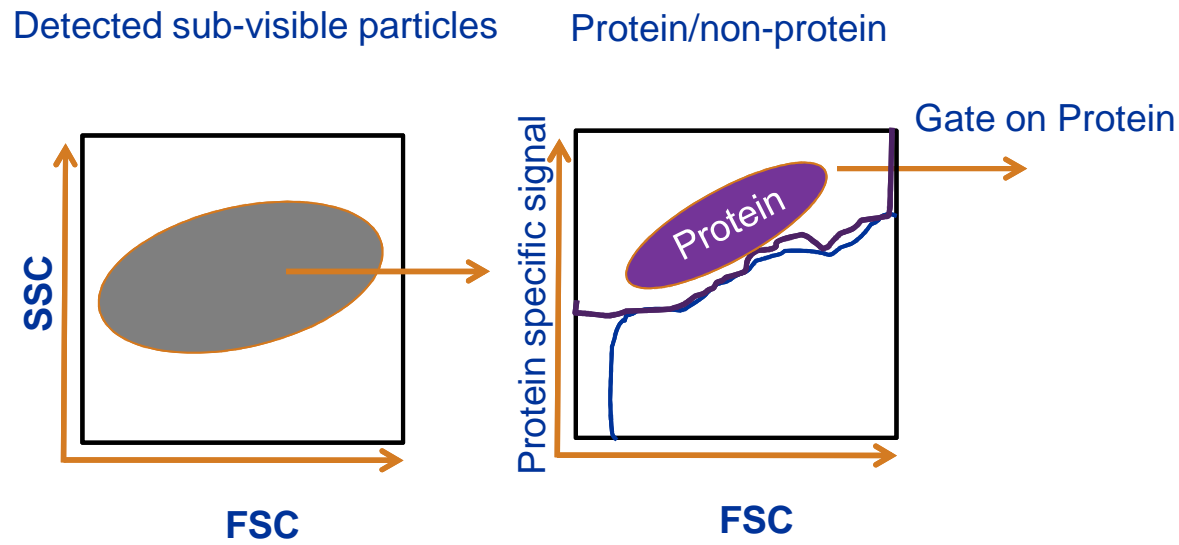
Detected sub-visible particles



Gating strategy



Gating strategy

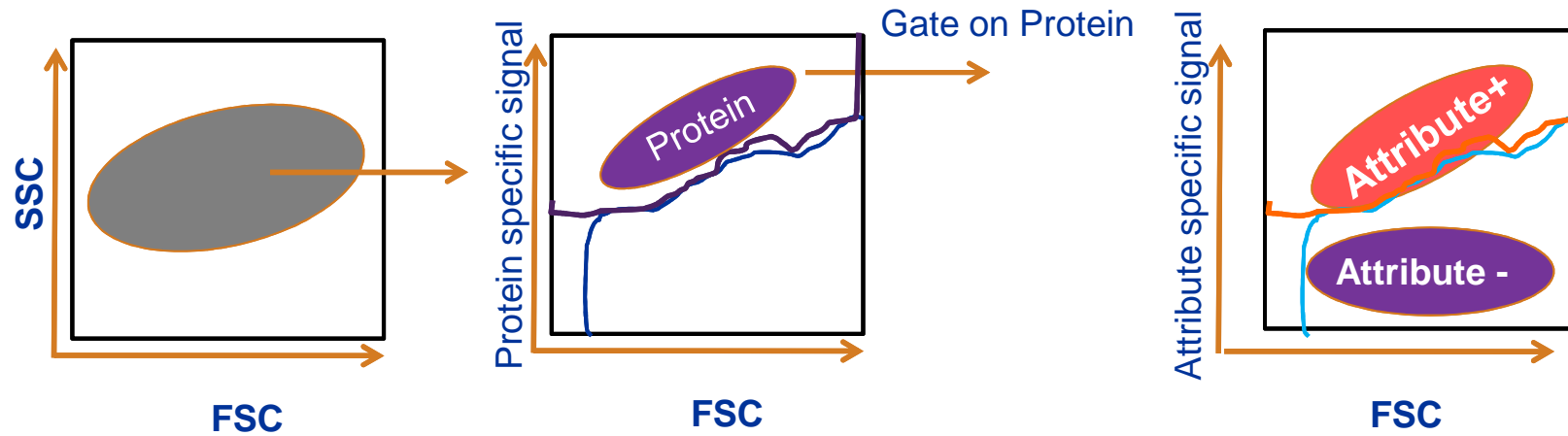


Gating strategy

Detected sub-visible particles

Protein/non-protein

Property of protein particles

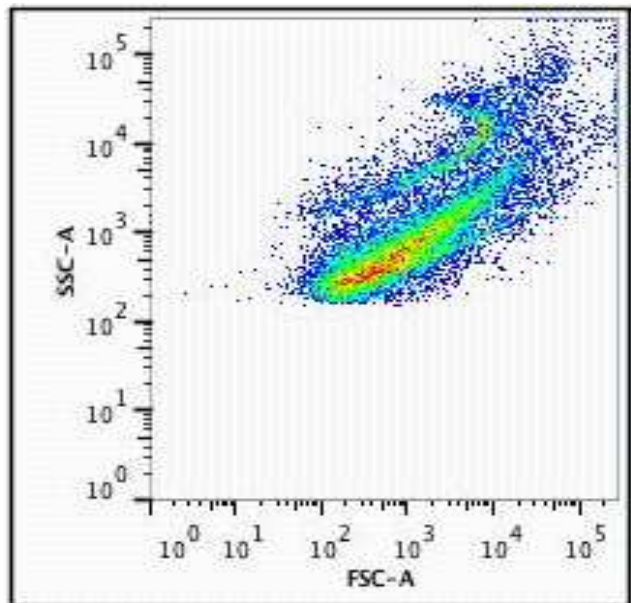


Flow cytometry based sub-visible particle detection and analysis: example

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Analysis of protein particles, cross-beta sheet containing protein particles and non-protein sub-visible particles in one sample

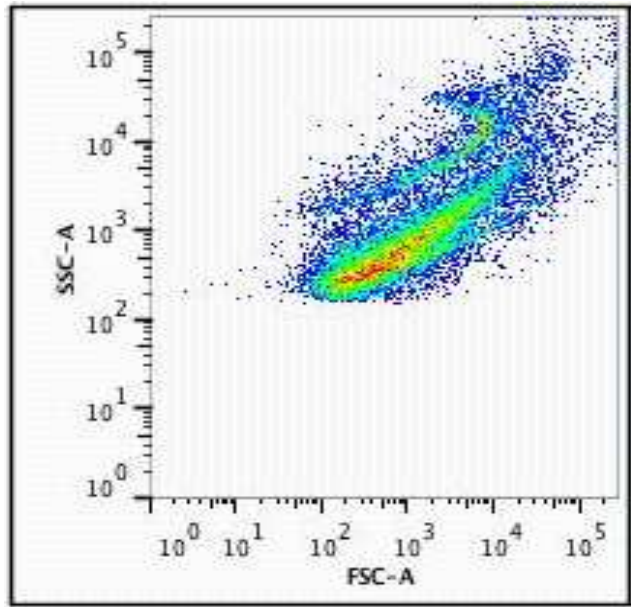
All detected particles



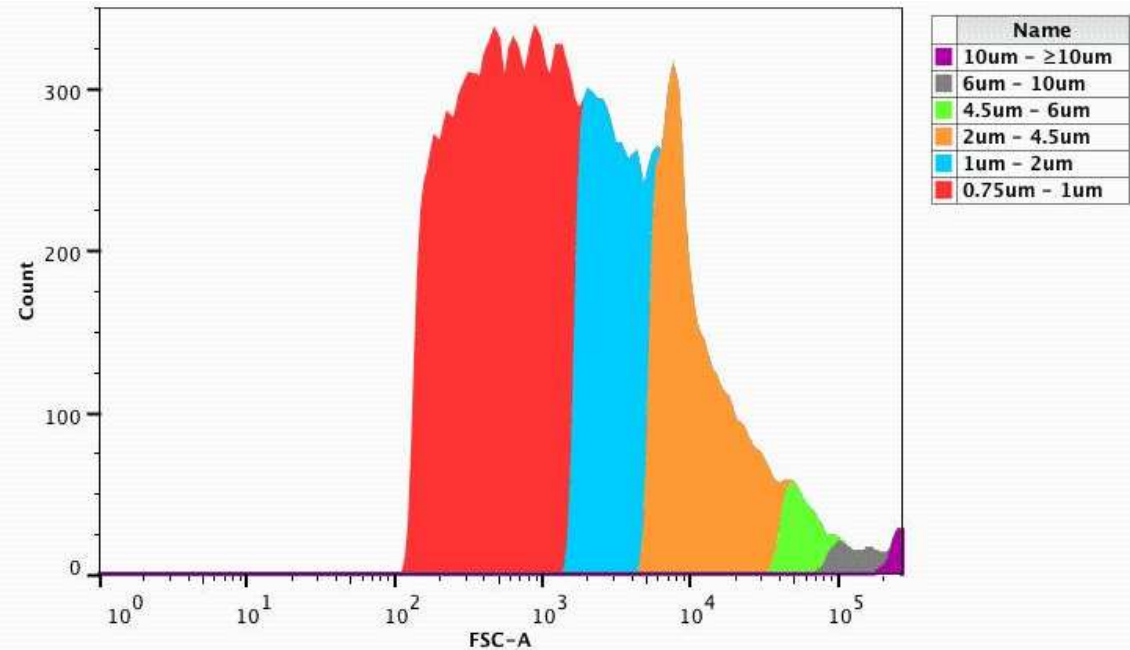
Flow cytometry based sub-visible particle detection and analysis: example

Analysis of protein particles, cross-beta sheet containing protein particles and non-protein sub-visible particles in one sample

All detected particles

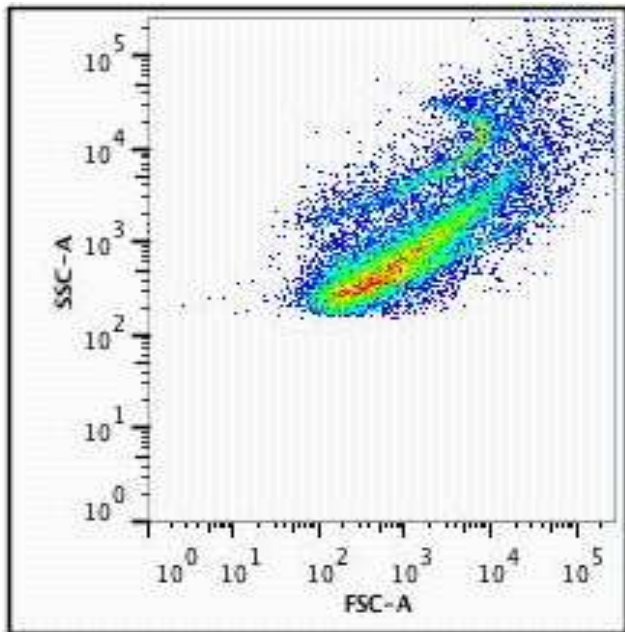


Number of detected particles in each size gate

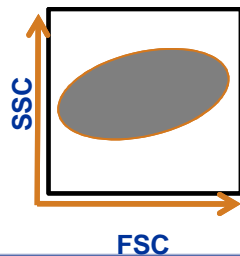


Discrimination between protein and non-protein particles

Analysis of protein particles, cross-beta sheet containing protein particles and non-protein sub-visible particles in one sample

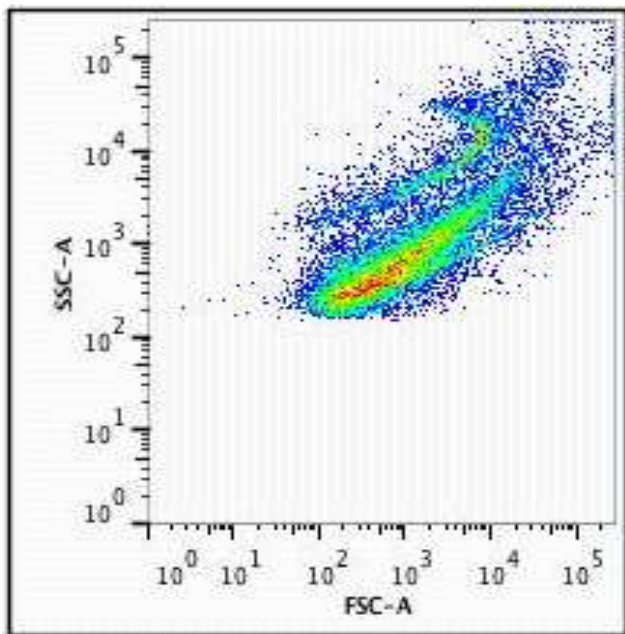


Detected sub-visible particles

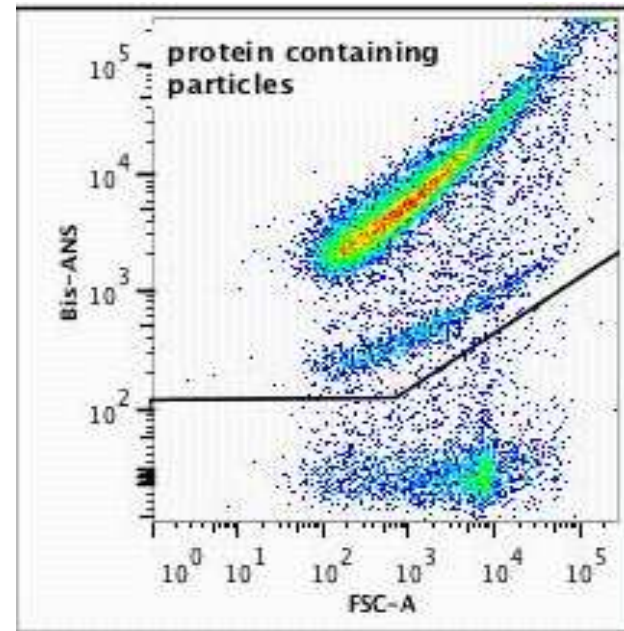


Discrimination between protein and non-protein particles

Analysis of protein particles, cross-beta sheet containing protein particles and non-protein sub-visible particles in one sample



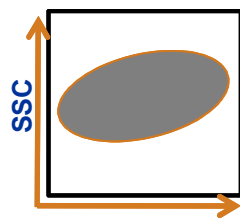
BisANS



Protein

Non-protein

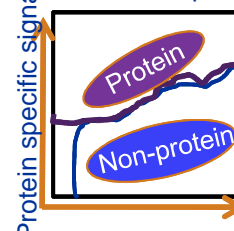
Detected sub-visible particles



FSC



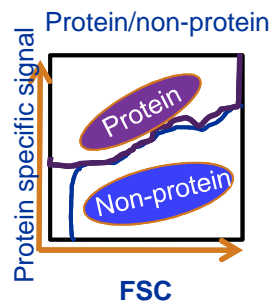
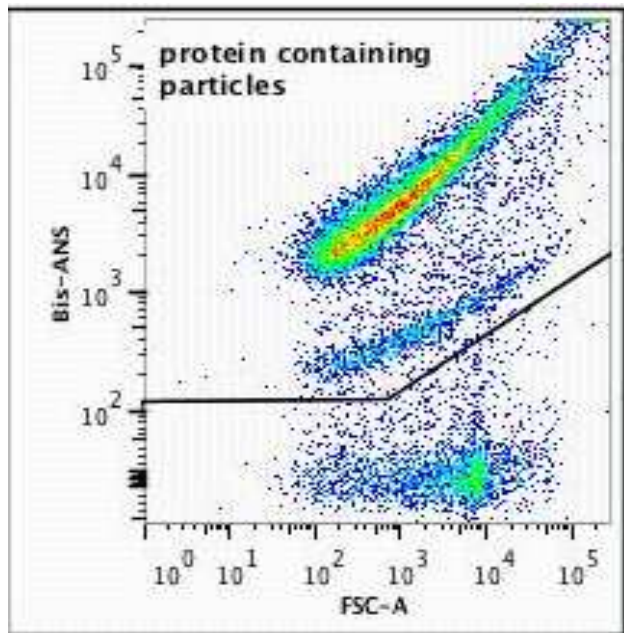
Protein/non-protein



FSC

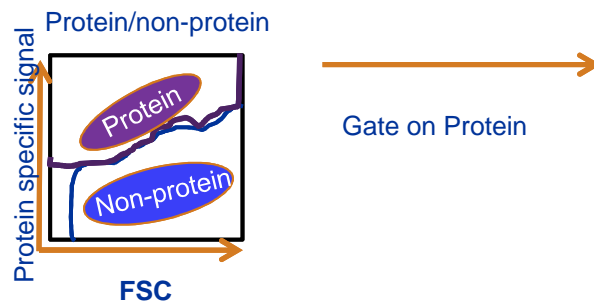
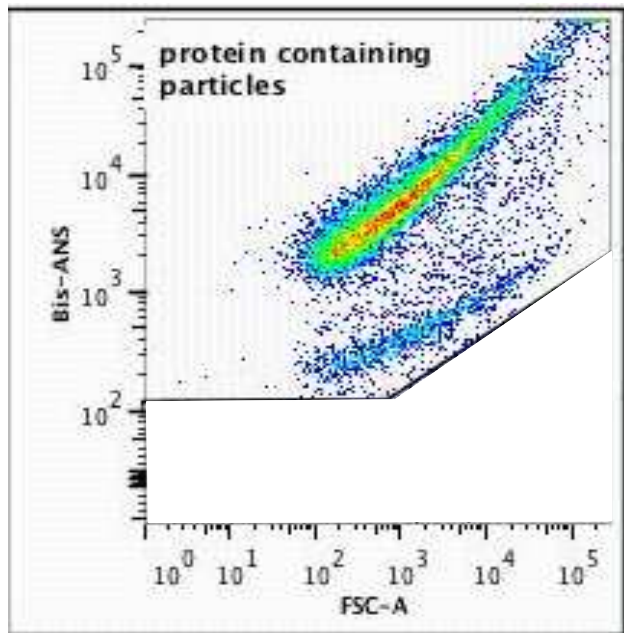
Characterization of the protein particles

Analysis of protein particles, cross-beta sheet containing protein particles and non-protein sub-visible particles in one sample



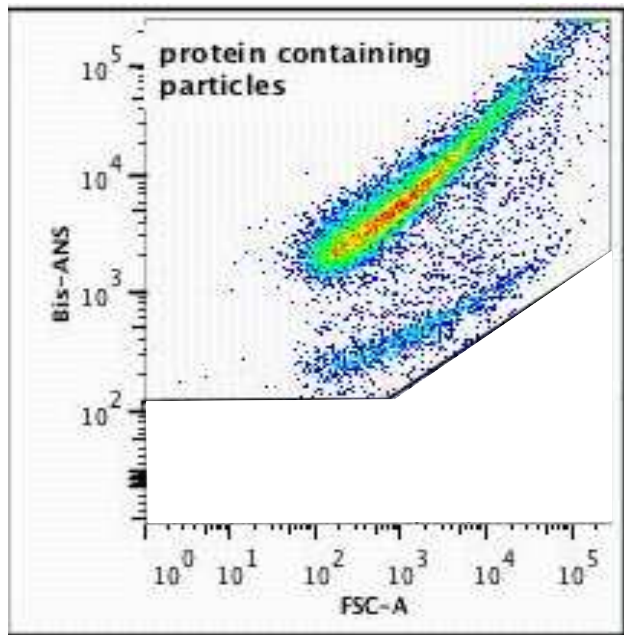
Characterization of the protein particles

Analysis of protein particles, cross-beta sheet containing protein particles and non-protein sub-visible particles in one sample

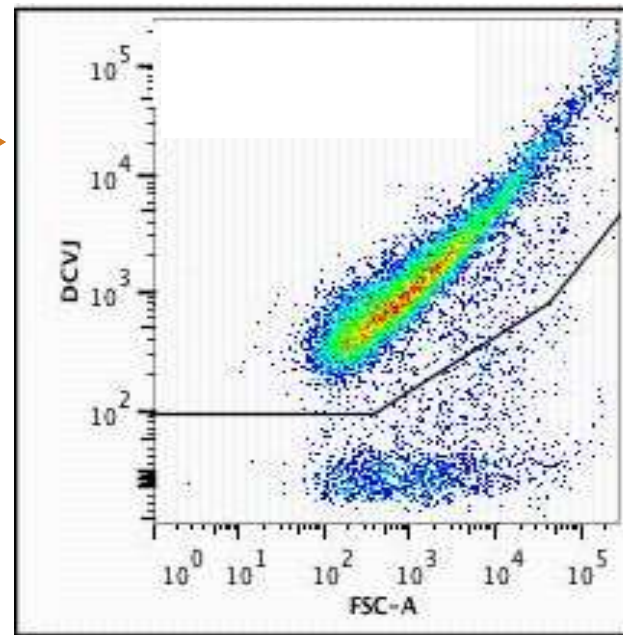


Characterization of the protein particles

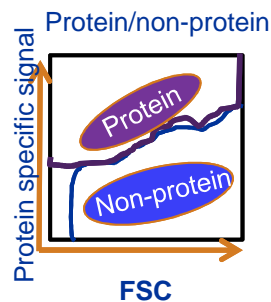
Analysis of protein particles, cross-beta sheet containing protein particles and non-protein sub-visible particles in one sample



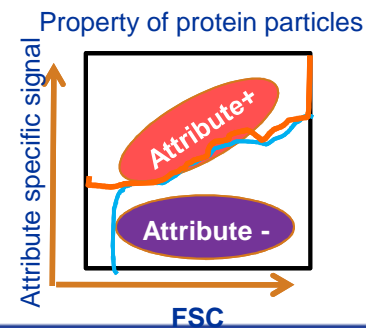
DCVJ



Cross-beta-sheets containing particles

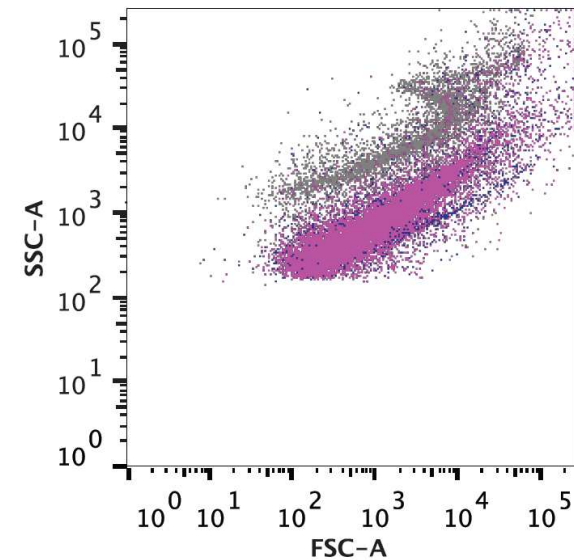
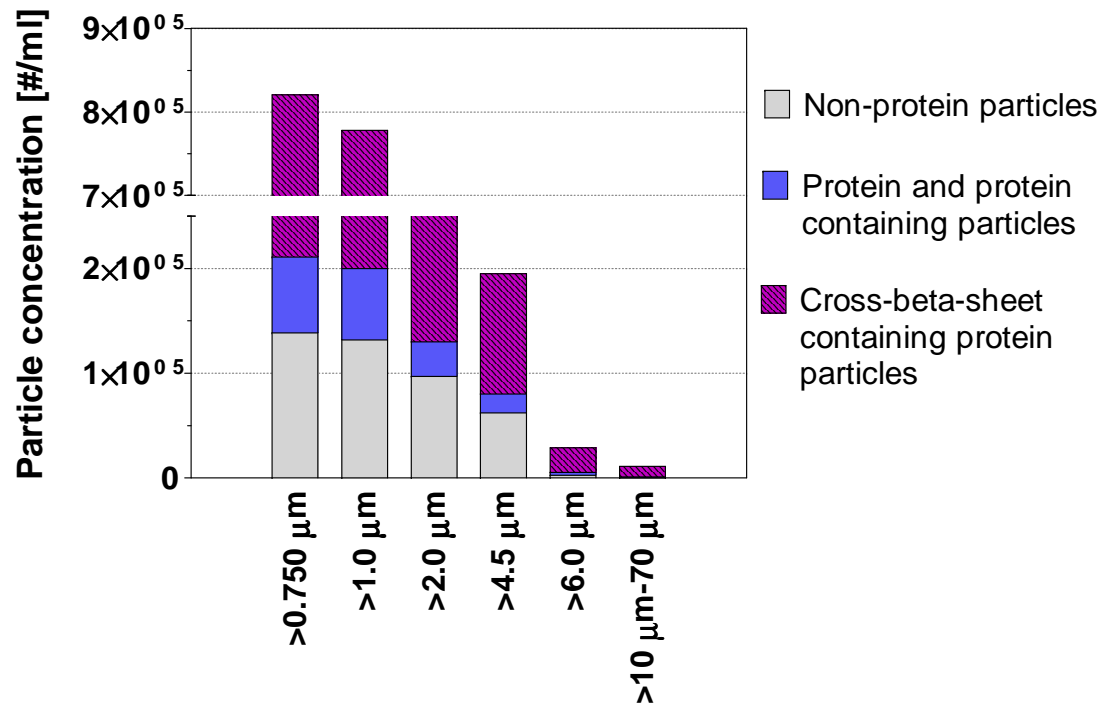


Gate on Protein



Number and properties of sub-visible particles

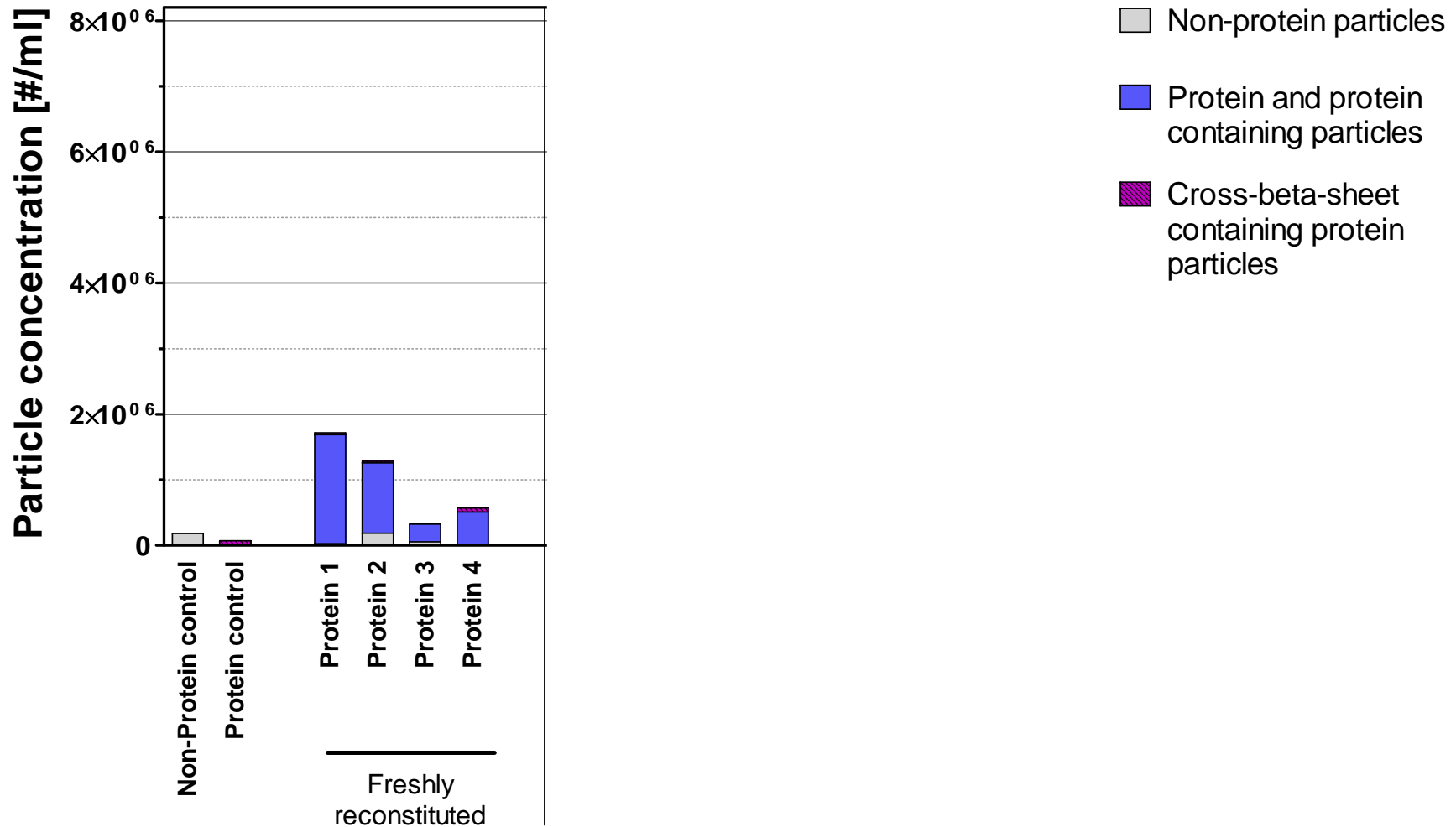
Analysis of protein particles, cross-beta sheet containing protein particles and non-protein sub-visible particles in one sample



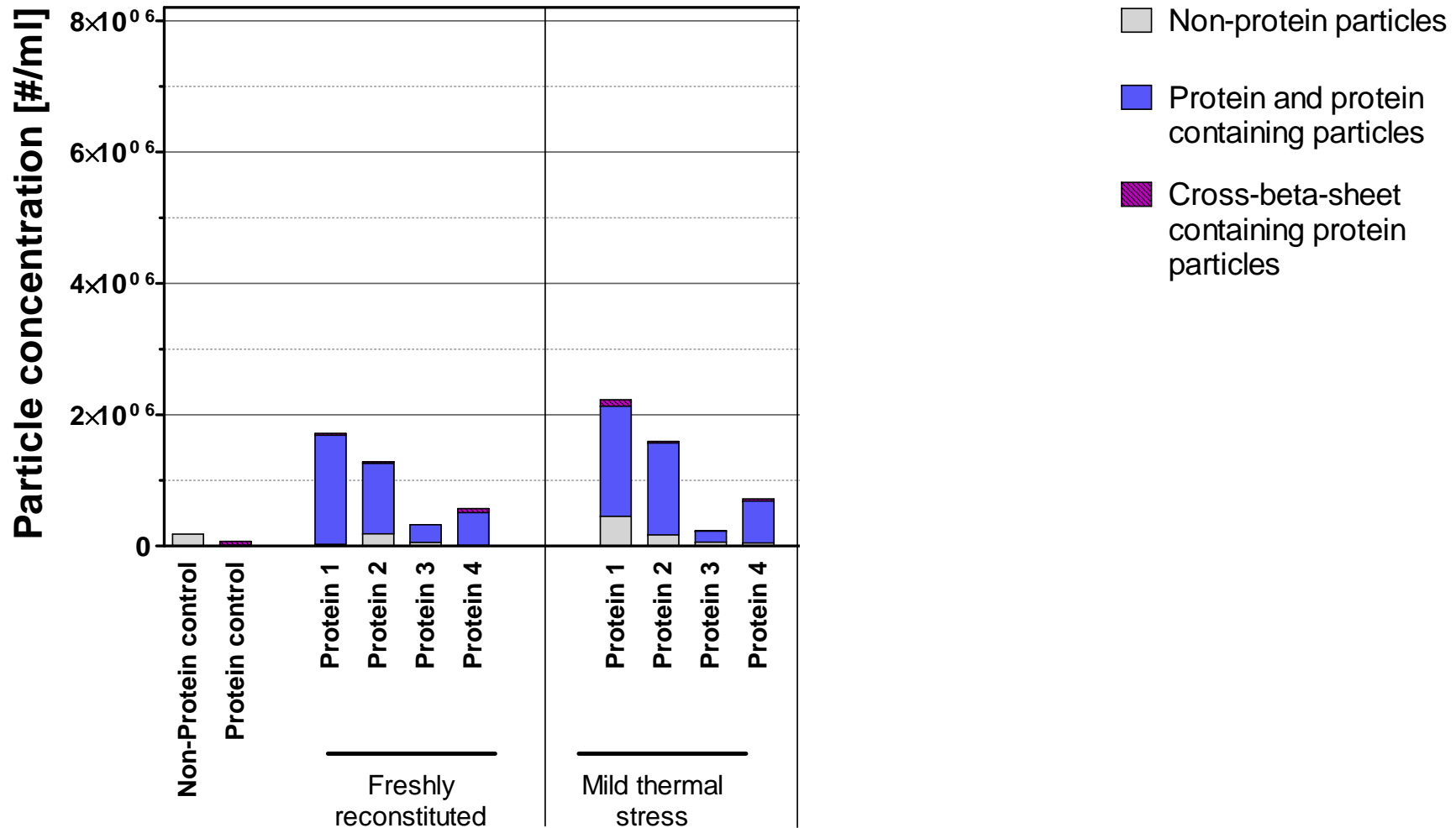
Use of flow cytometry based particle analysis for product development

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Use of flow cytometry based particle analysis for product development

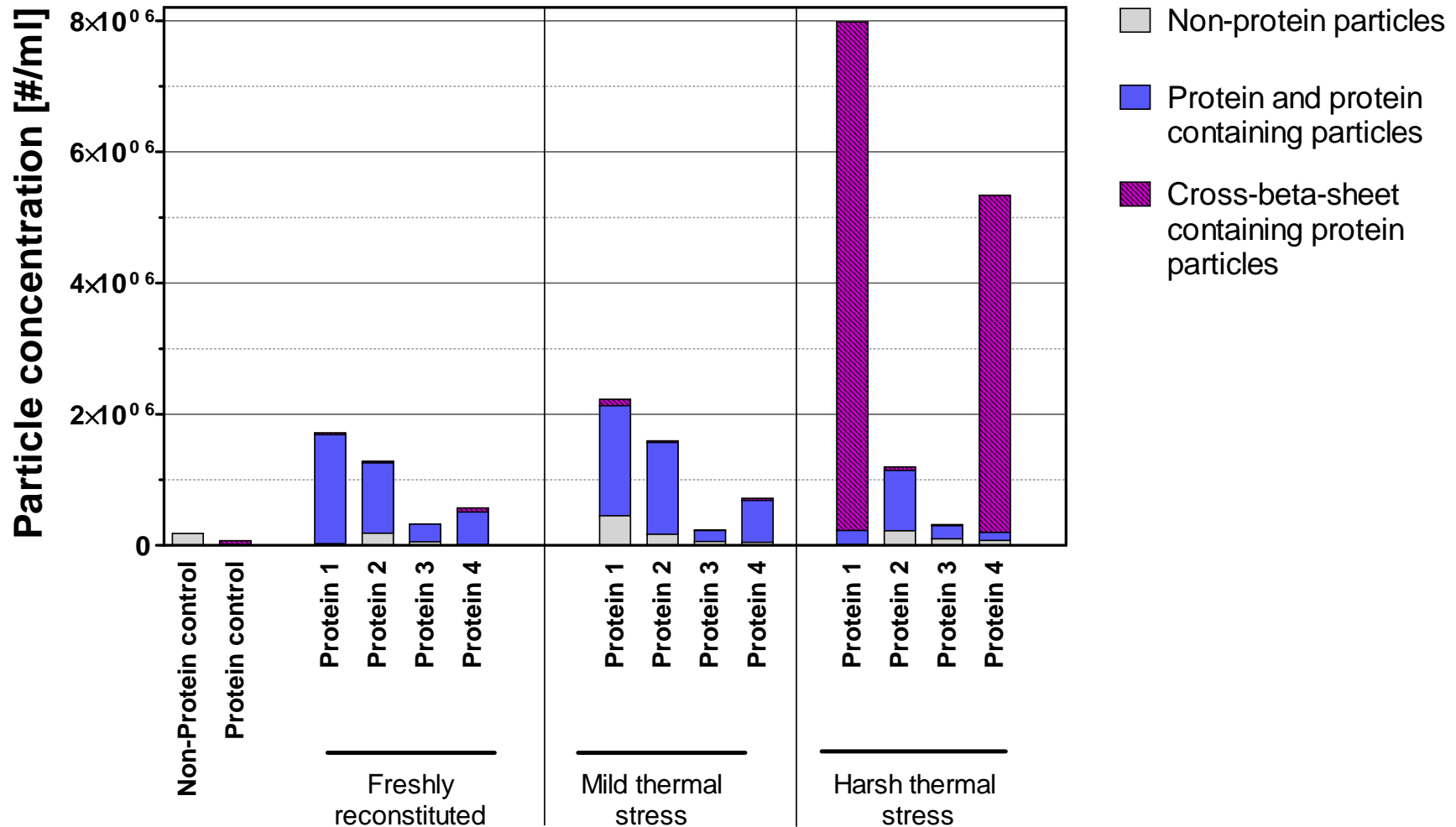


Use of flow cytometry based particle analysis for product development

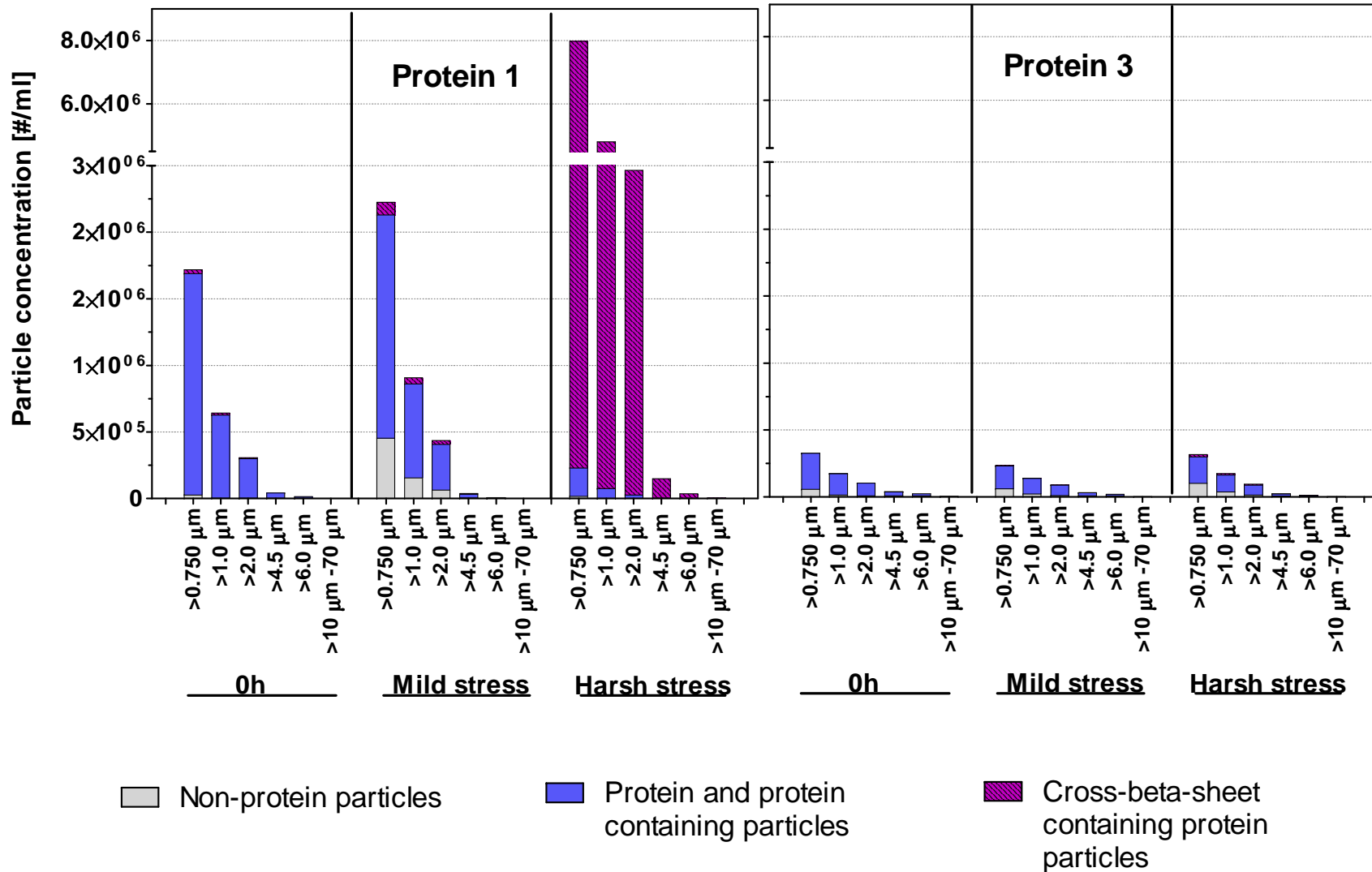


Use of flow cytometry based particle analysis for product development

The properties of sub-visible particles may be different for all proteins



Use of flow cytometry based particle analysis for product development



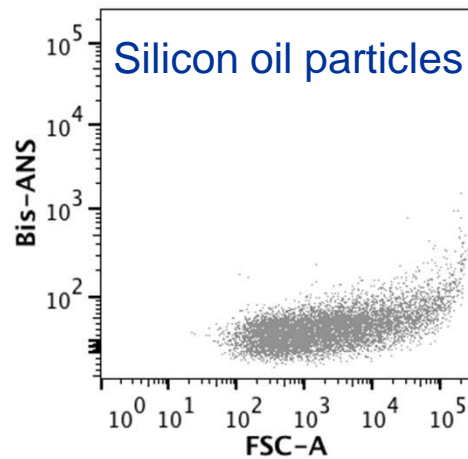
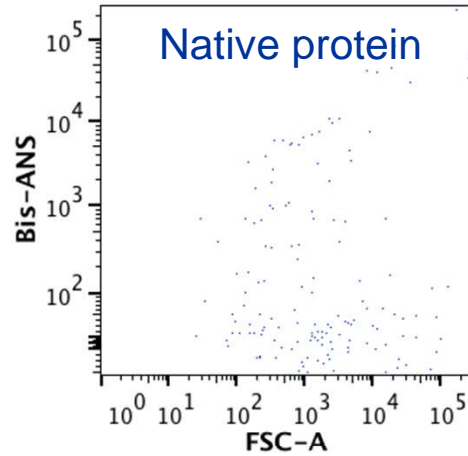
Use of flow cytometry based participle analysis to study protein-silicon interaction

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Does a native protein bind to silicon?

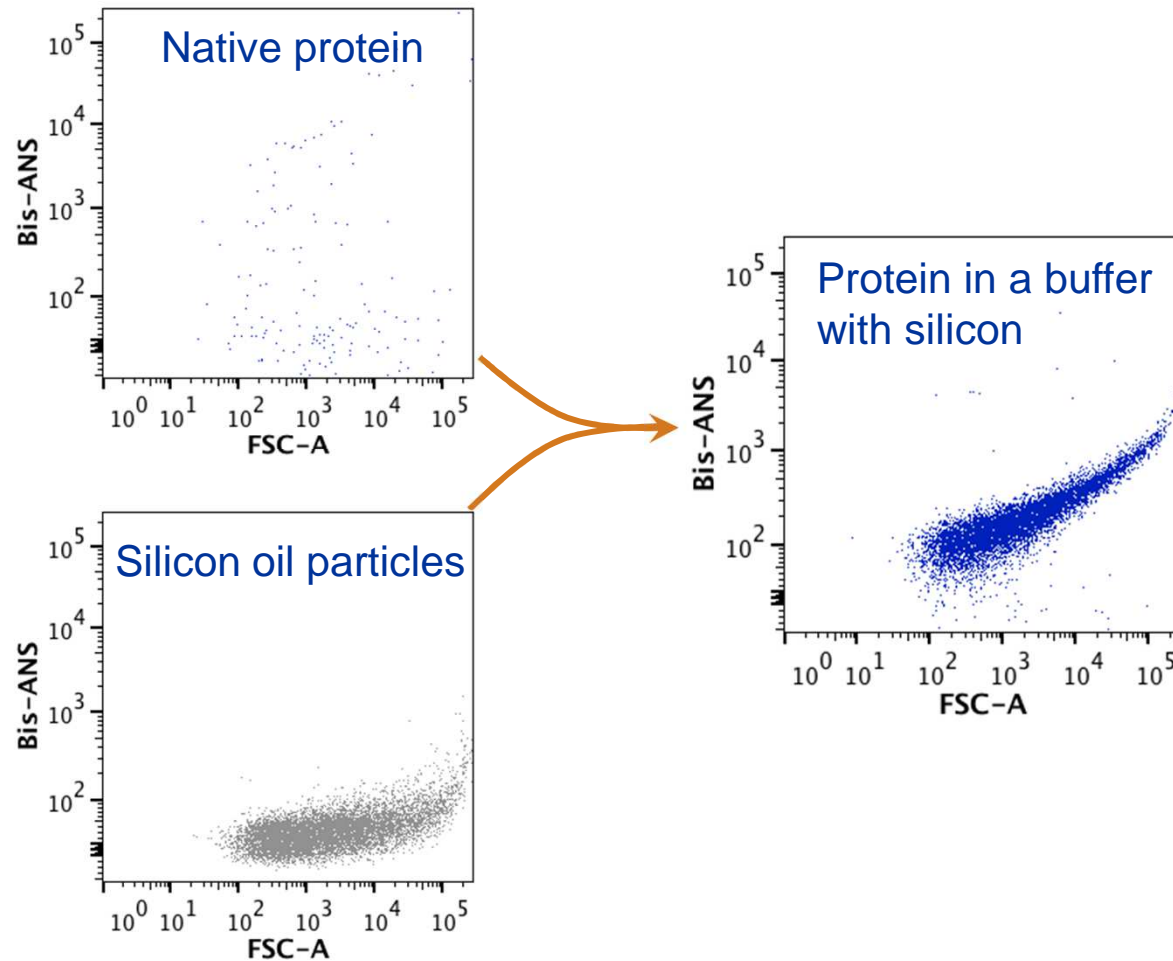
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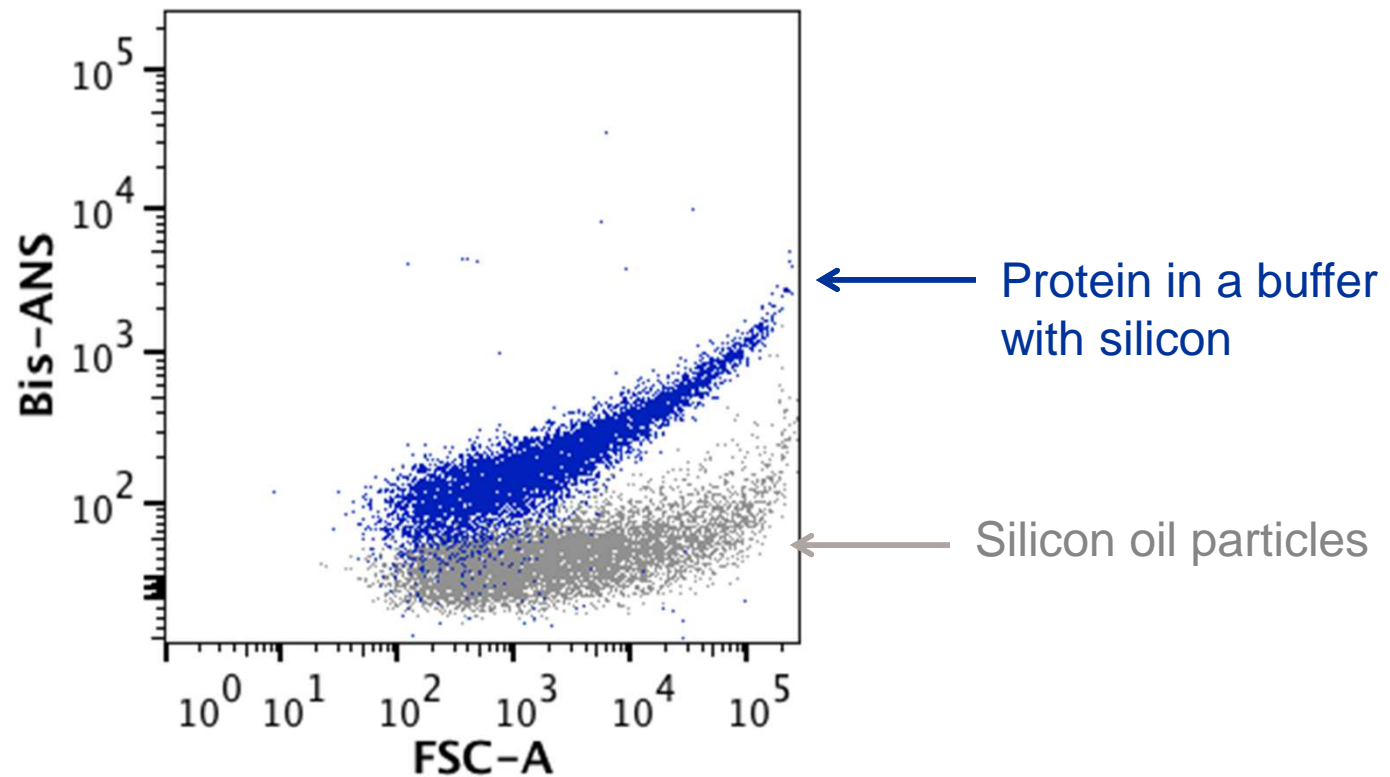
Use of flow cytometry based participle analysis to study protein-silicon interaction

Does a native protein bind to silicon?



Use of flow cytometry based participle analysis to study protein-silicon interaction

Protein binds to silicon sub-visible particles which become stained with BisANS



Take home message

- Sub-visible particles can differ not only in size but also in their structural properties
- Flow cytometry based sub-visible particle analysis is a powerful tool for detection, quantification and characterization of proteinaceous particles
- The method provides orthogonal information to currently available techniques such as Micro-flow imaging and Nanoparticle tracking analysis

Thank you for your attention !