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

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IMMUNOLOGY
Baxter Innovations GmbH

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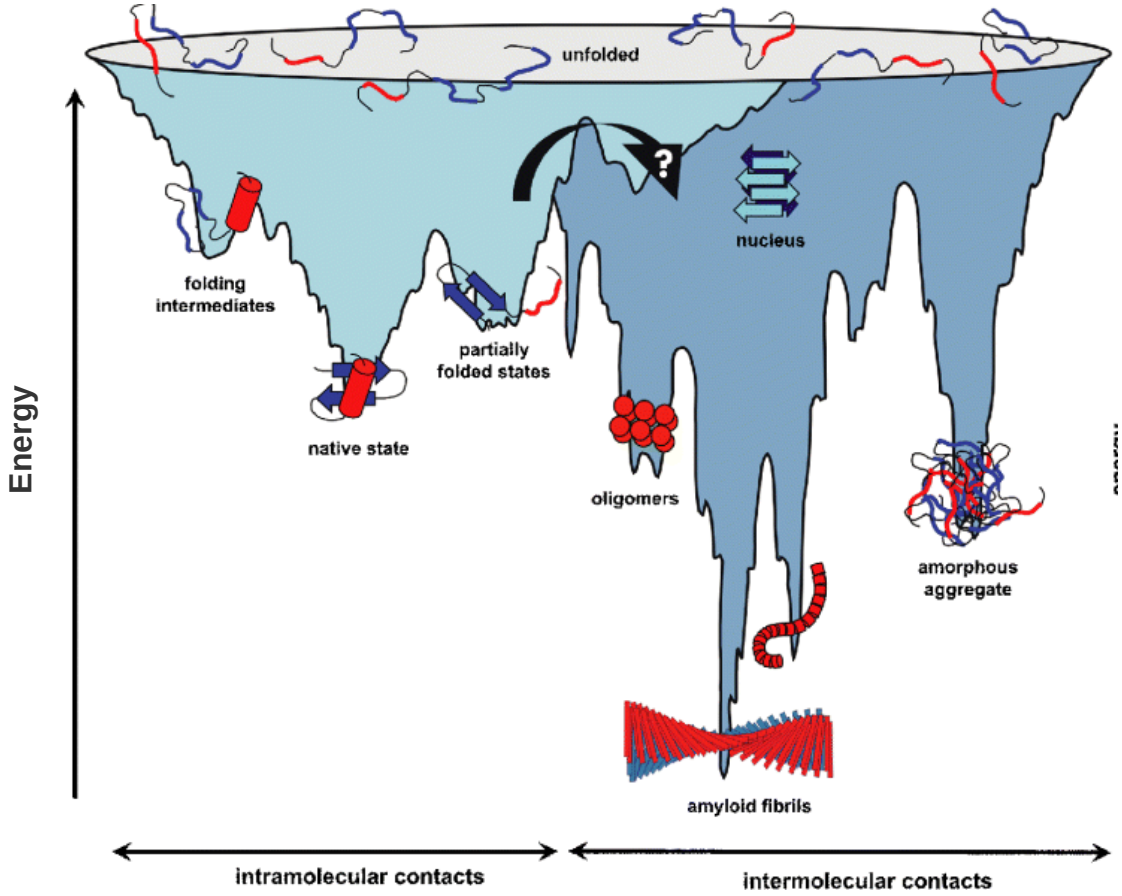
Aggregation is a generic property of a polypeptide chain



Jahn & Radford, FEBS Journal, 2005,75

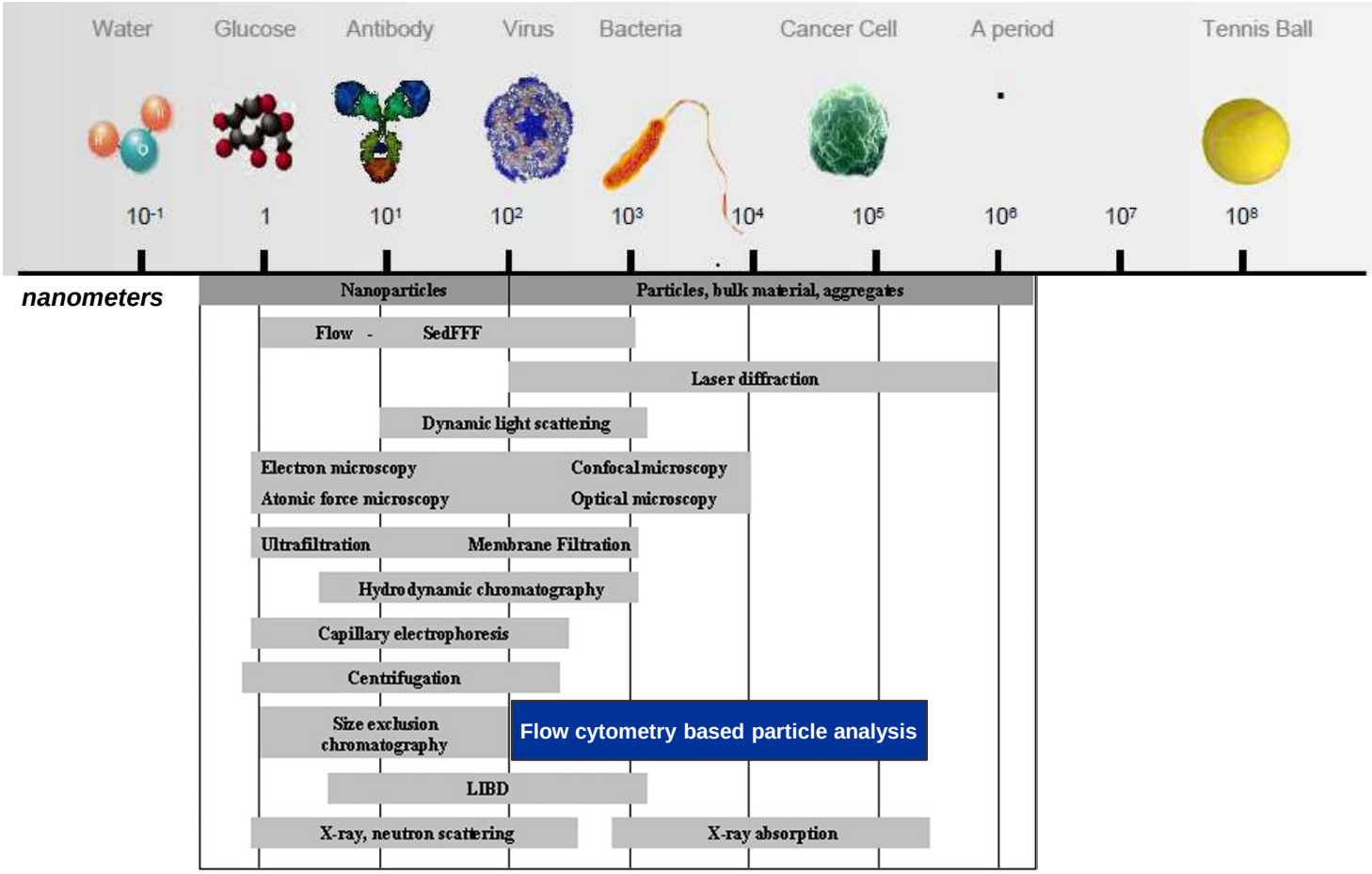
sub-visible particles

"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."

European Immunogenicity Platform (EIP),
the Protein Characterization Subcommittee (EIP-PCS)

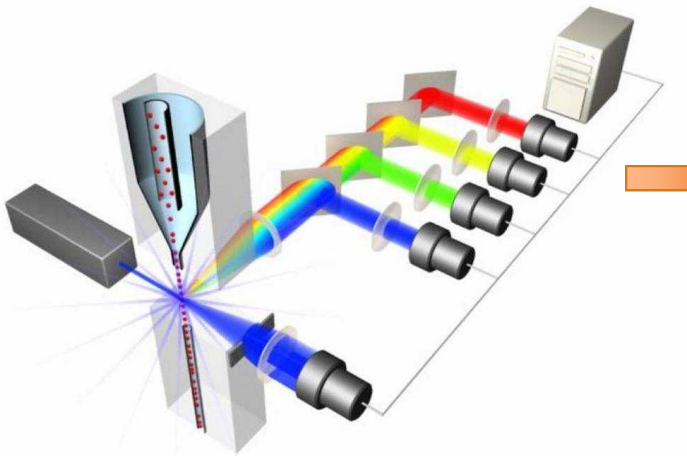
den Engelsman et al Pharm Res. 2011 Apr;28(4):920

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



Properties of sub-visible particles

Quantity and size of sub-visible particles

Quantity and properties of sub-visible particles with different sizes

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 by 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

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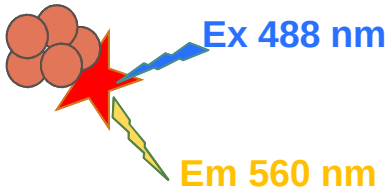
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		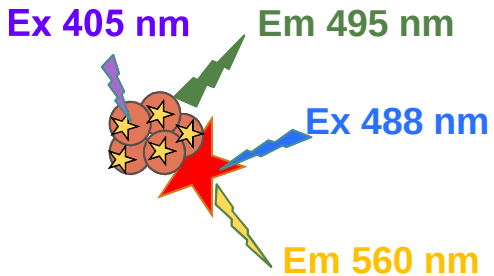
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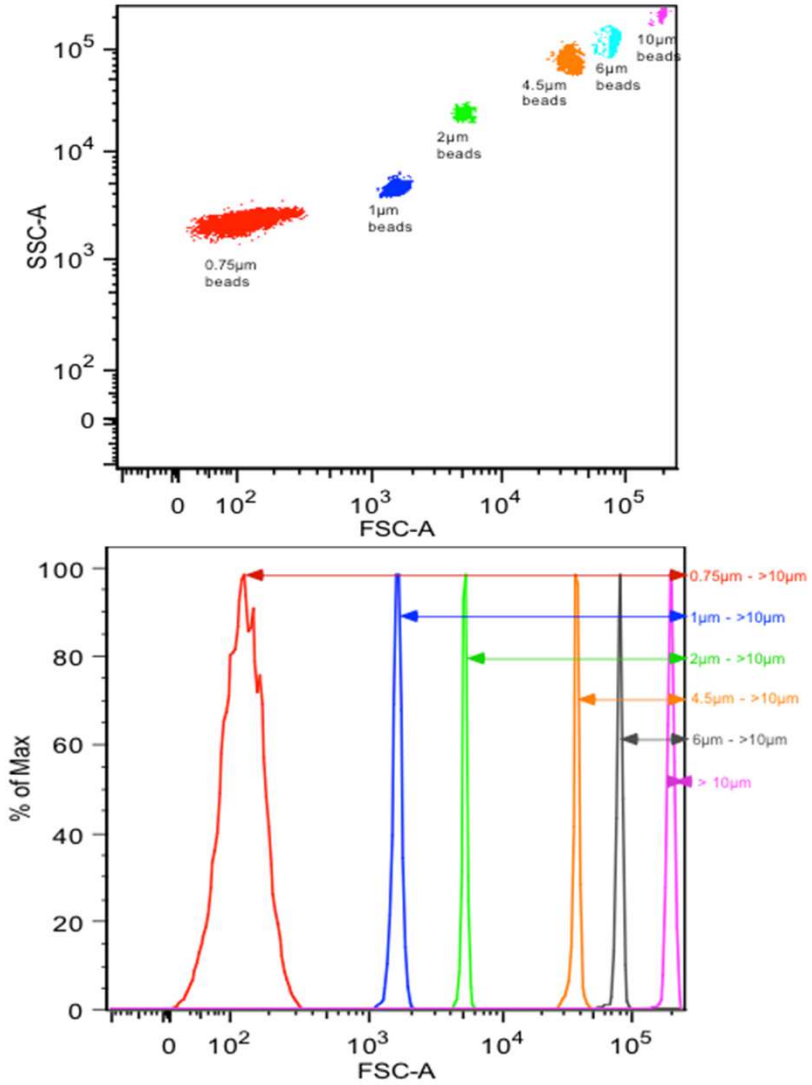
Flow cytometry based sub-visible particle detection and analysis: establishing size ranges

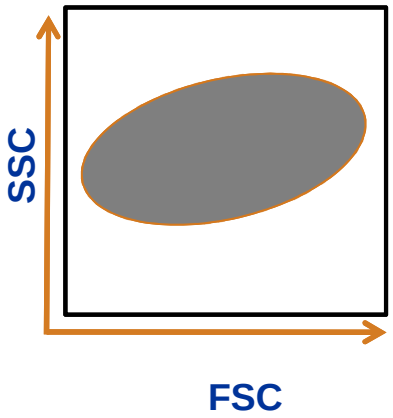


0.1 – ca 200 µm depending on available equipment

Currently using:
0.75 µm – ca 70 µm

Gating strategy

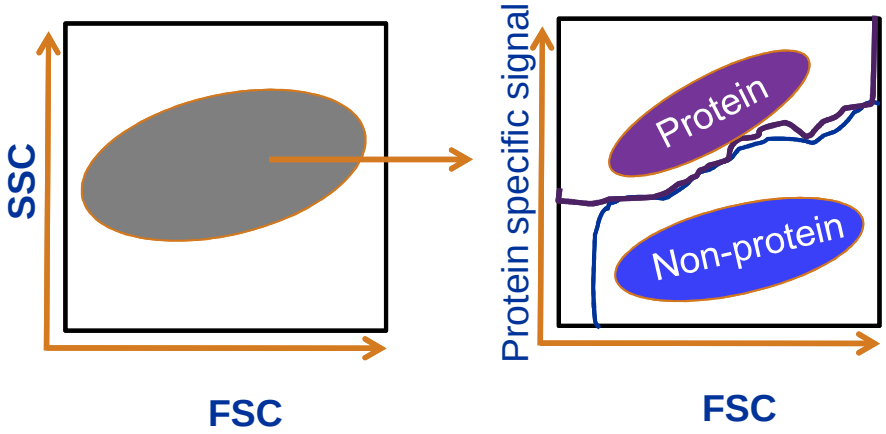
Detected sub-visible particles



Gating strategy

Detected sub-visible particles

Protein/non-protein



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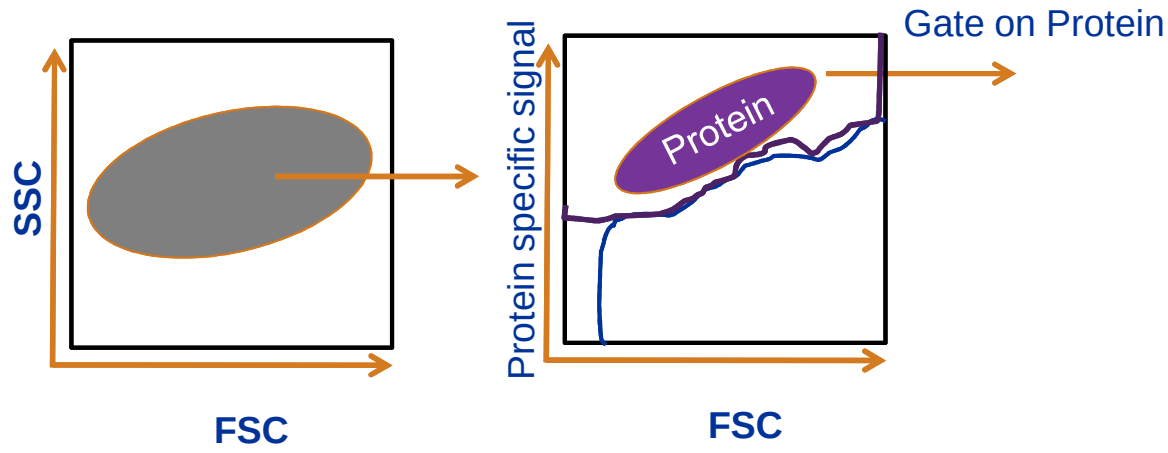

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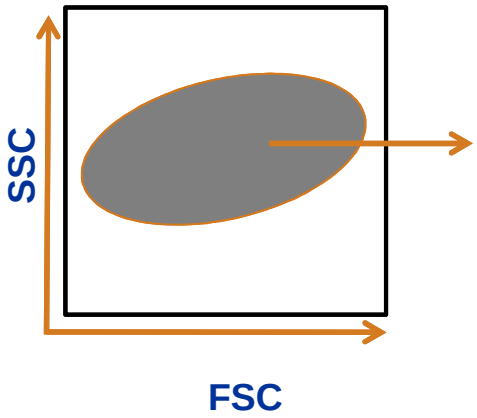

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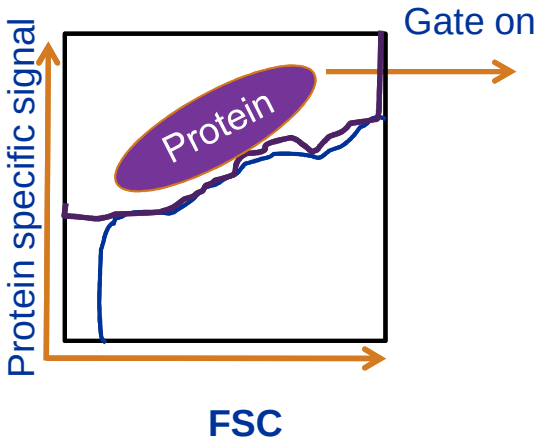
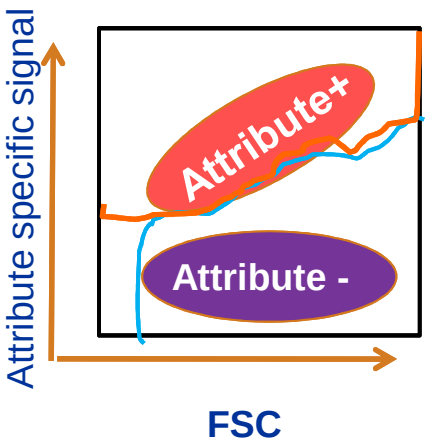


Gate on Protein

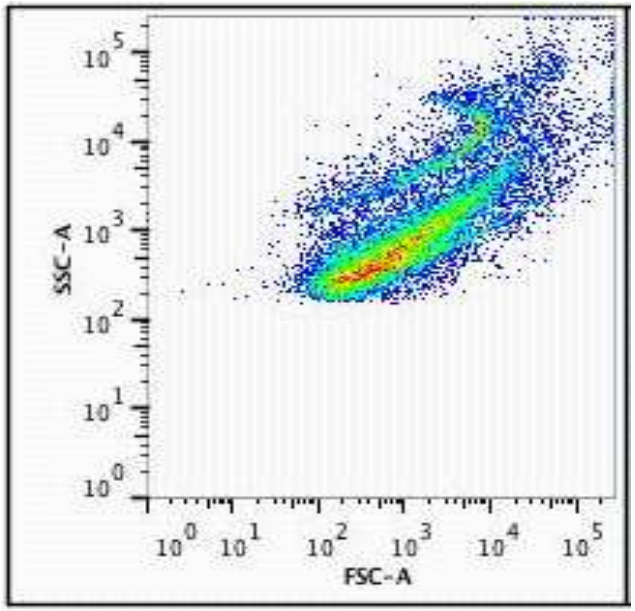
Property of protein particles



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



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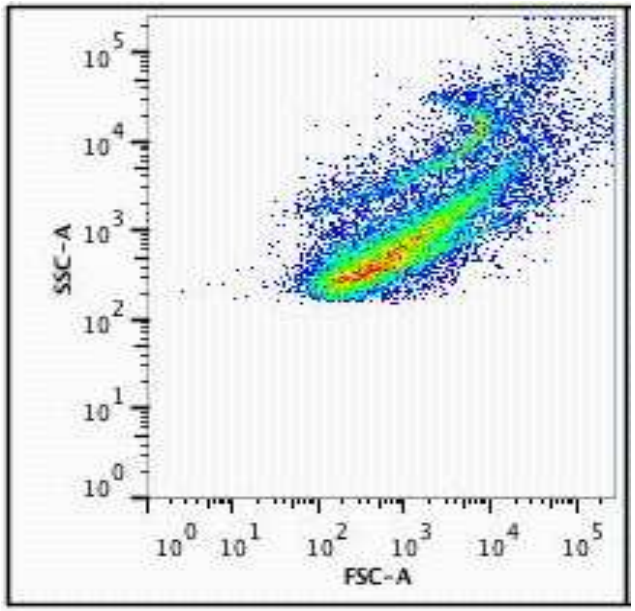
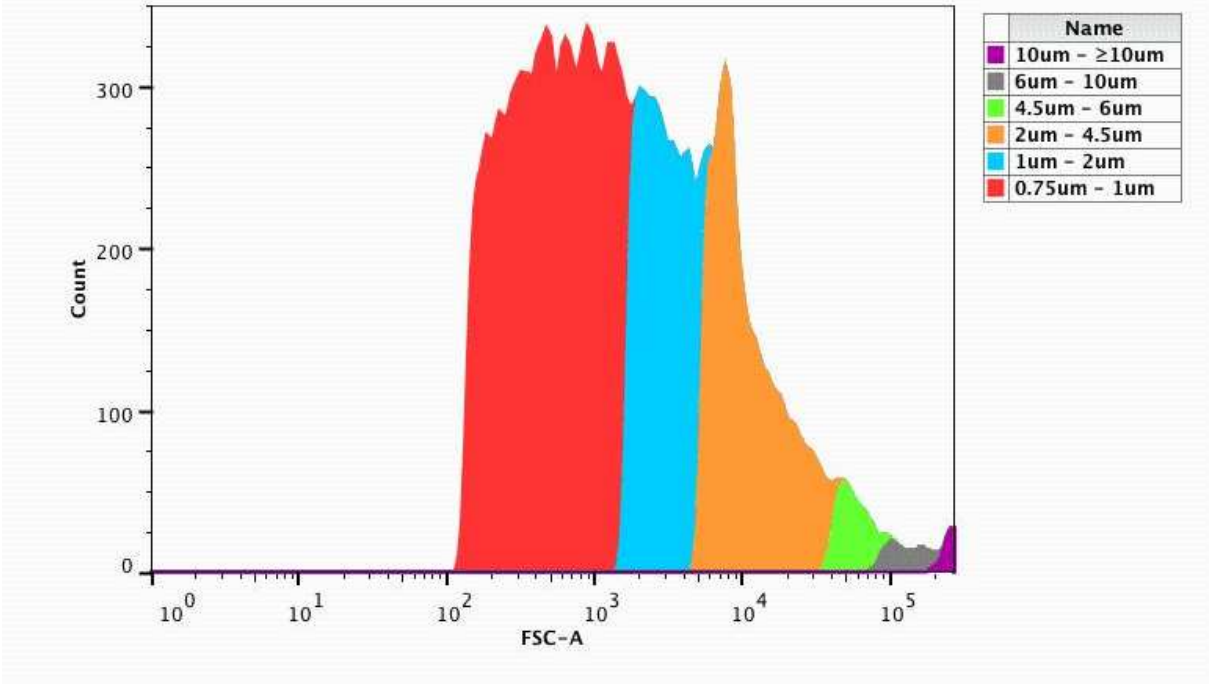
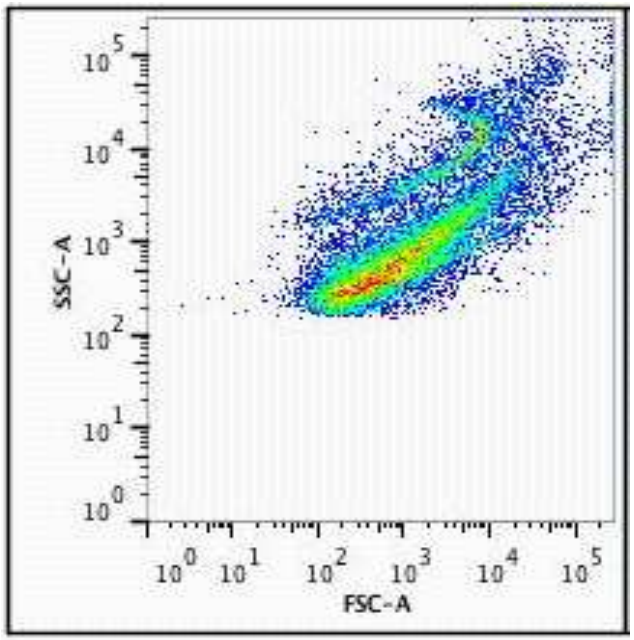


Number of detected particles in each size gate

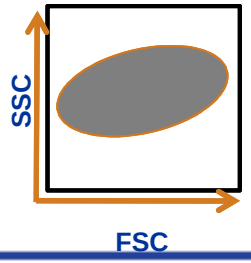


Discrimination between protein and non-protein particles

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



Detected sub-visible particles



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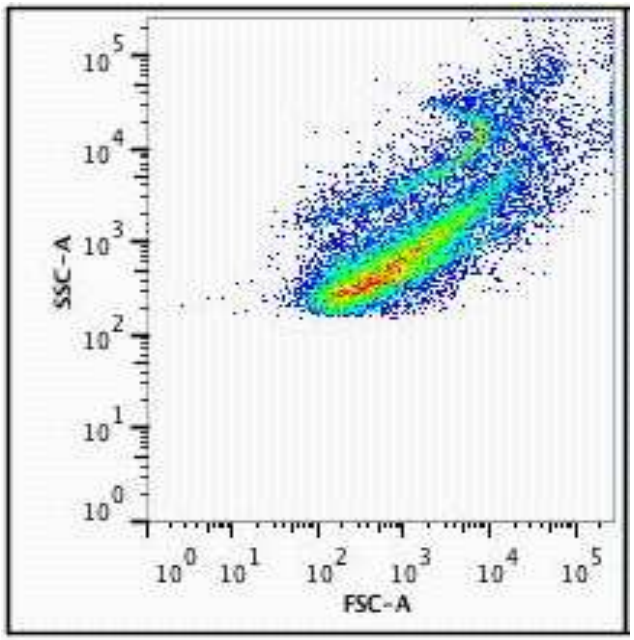
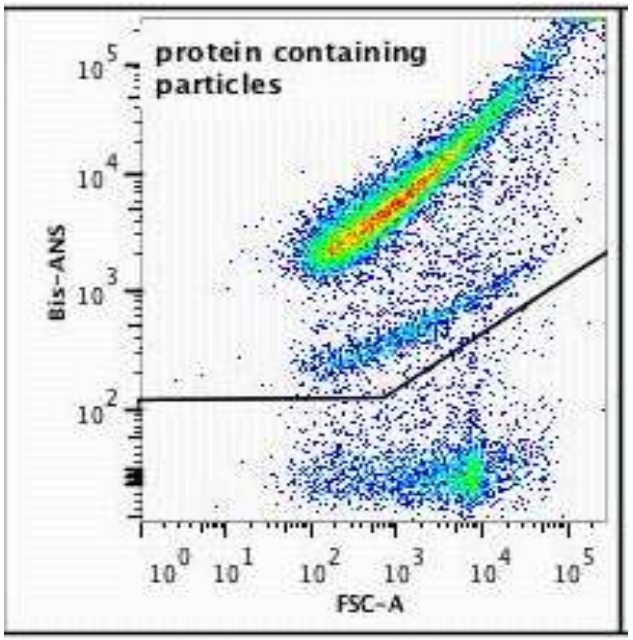


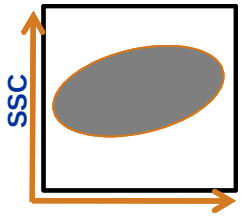
BisANS

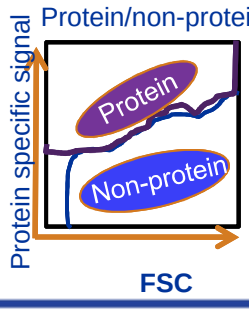


Protein

Non-protein

Detected sub-visible particles





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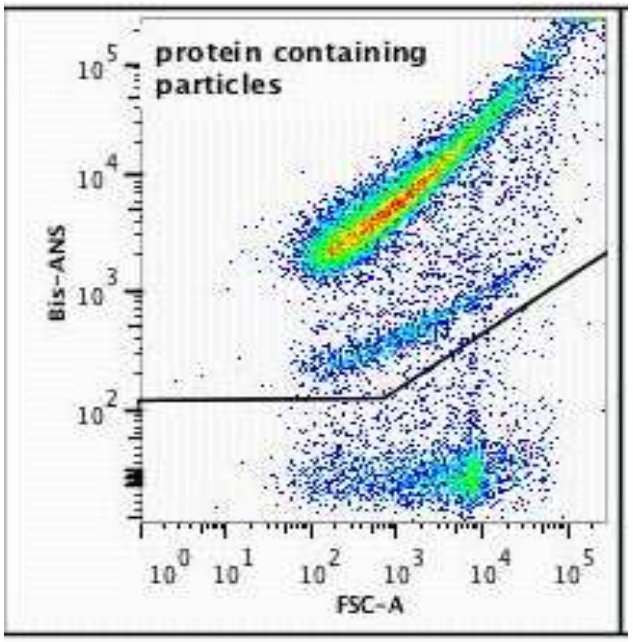

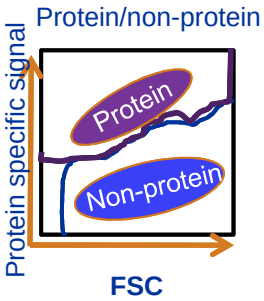

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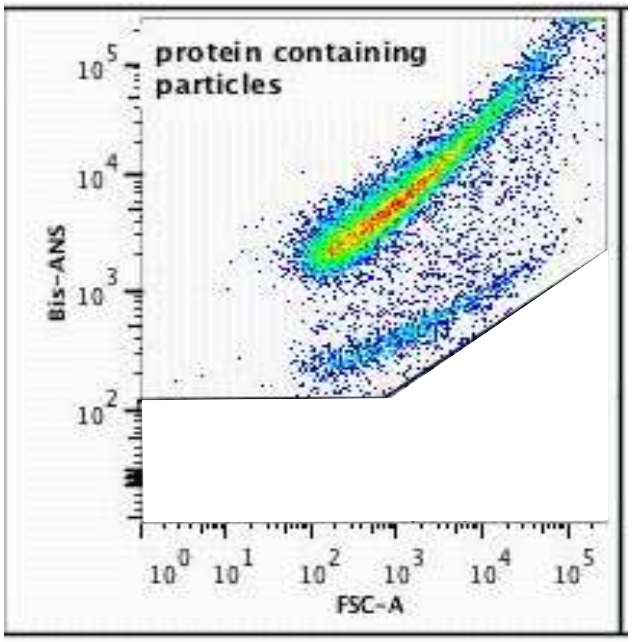

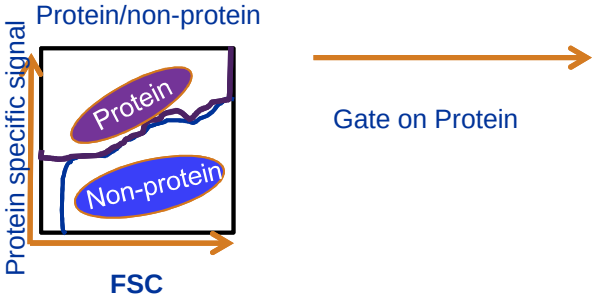

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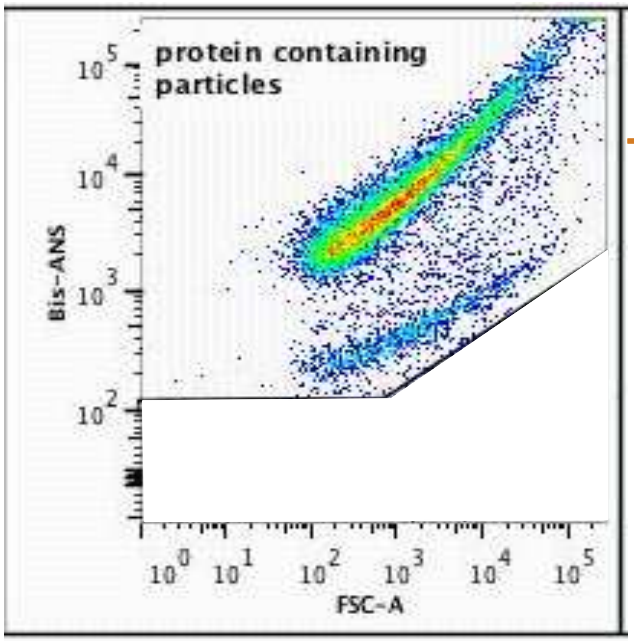
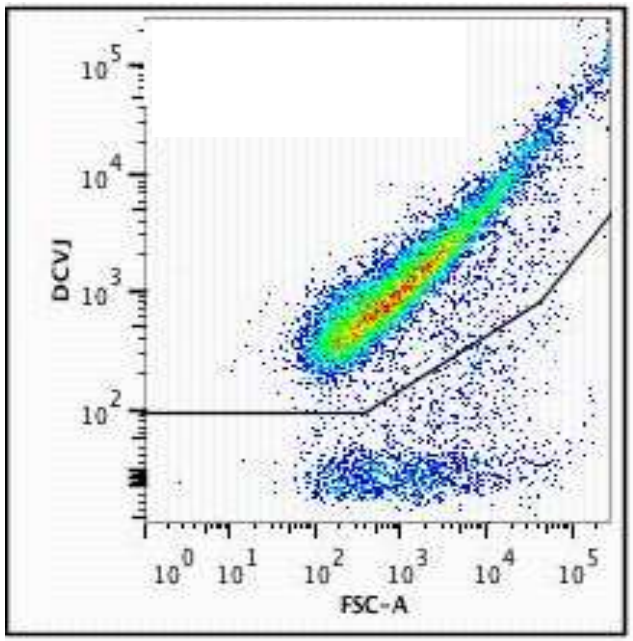
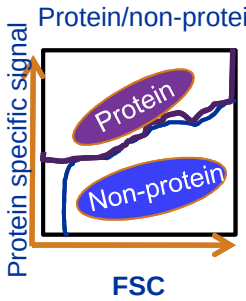


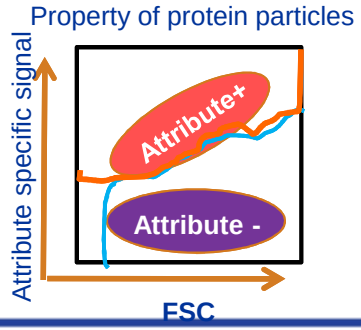
DCVJ



Cross-beta-sheets containing particles



Gate on Protein



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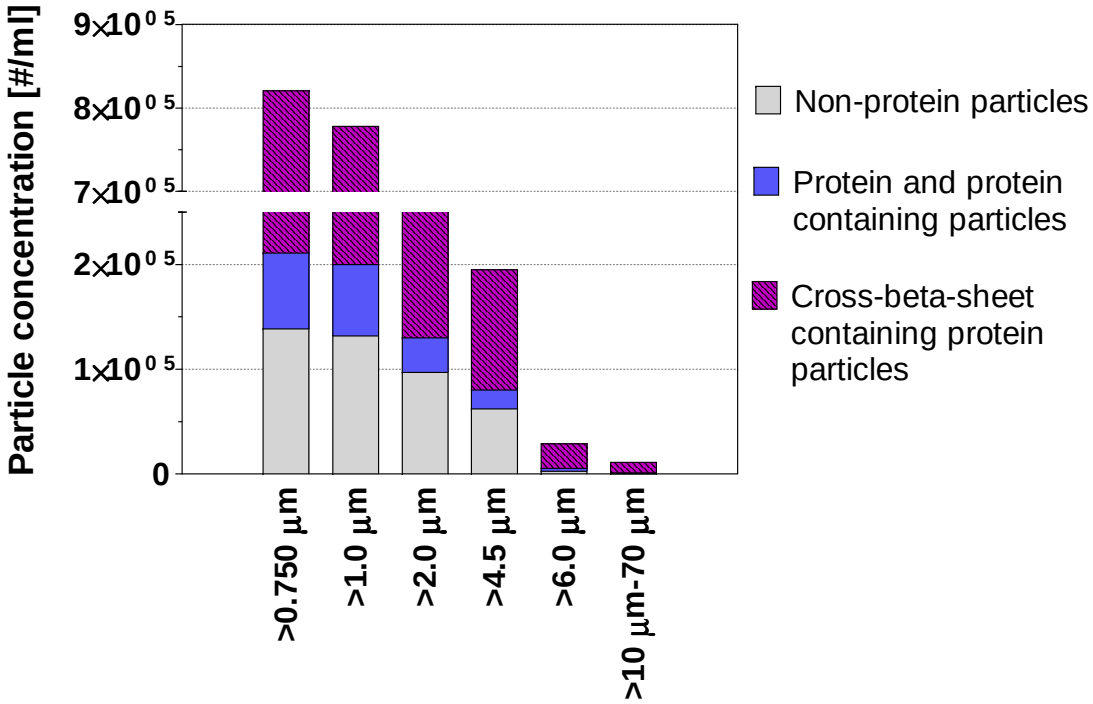

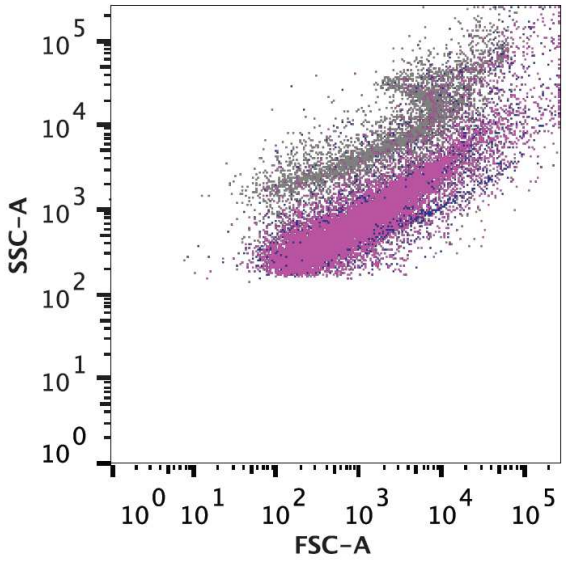

Use of flow cytometry based particle analysis for product development

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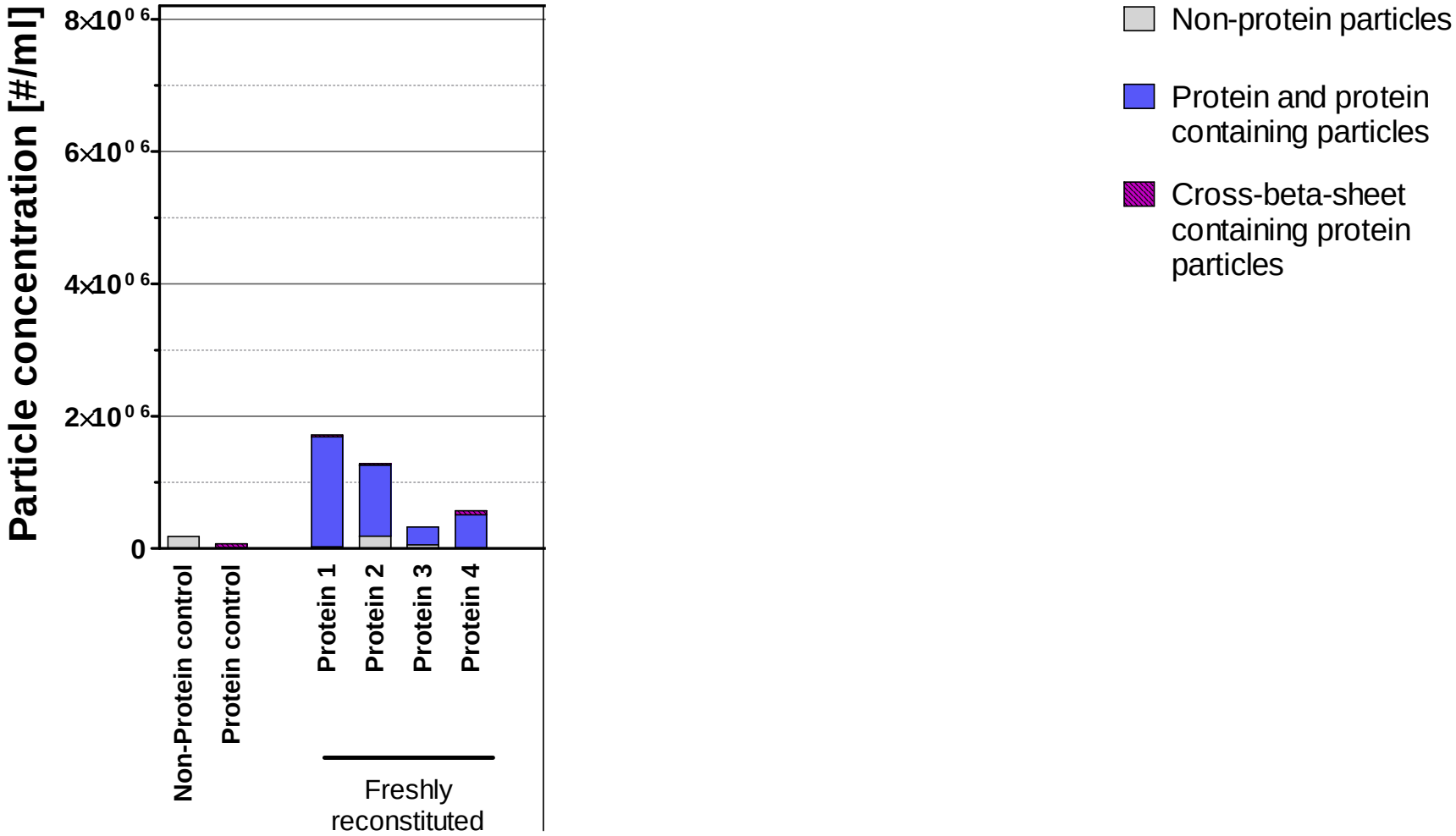

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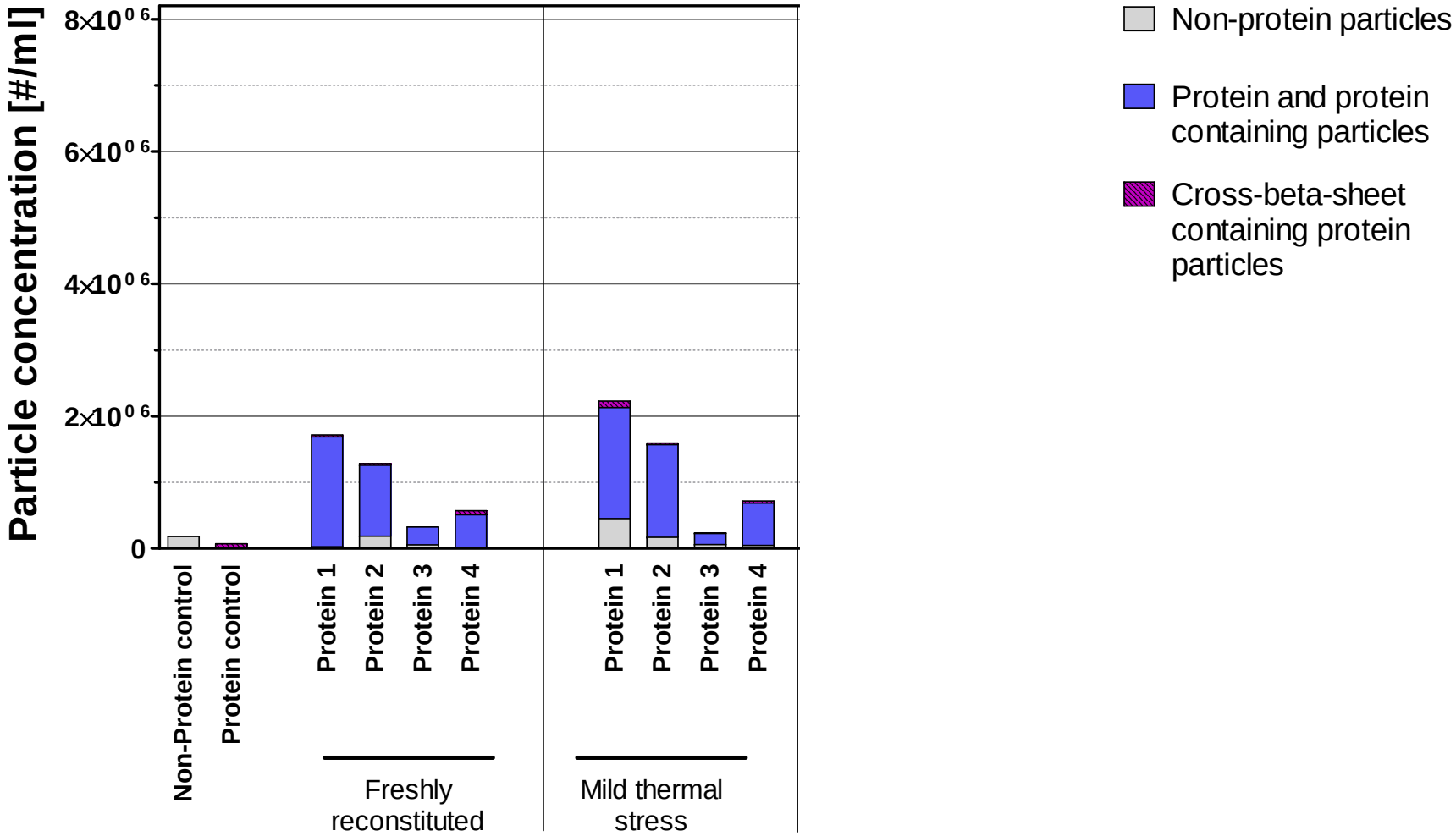

Use of flow cytometry based particle analysis for product development

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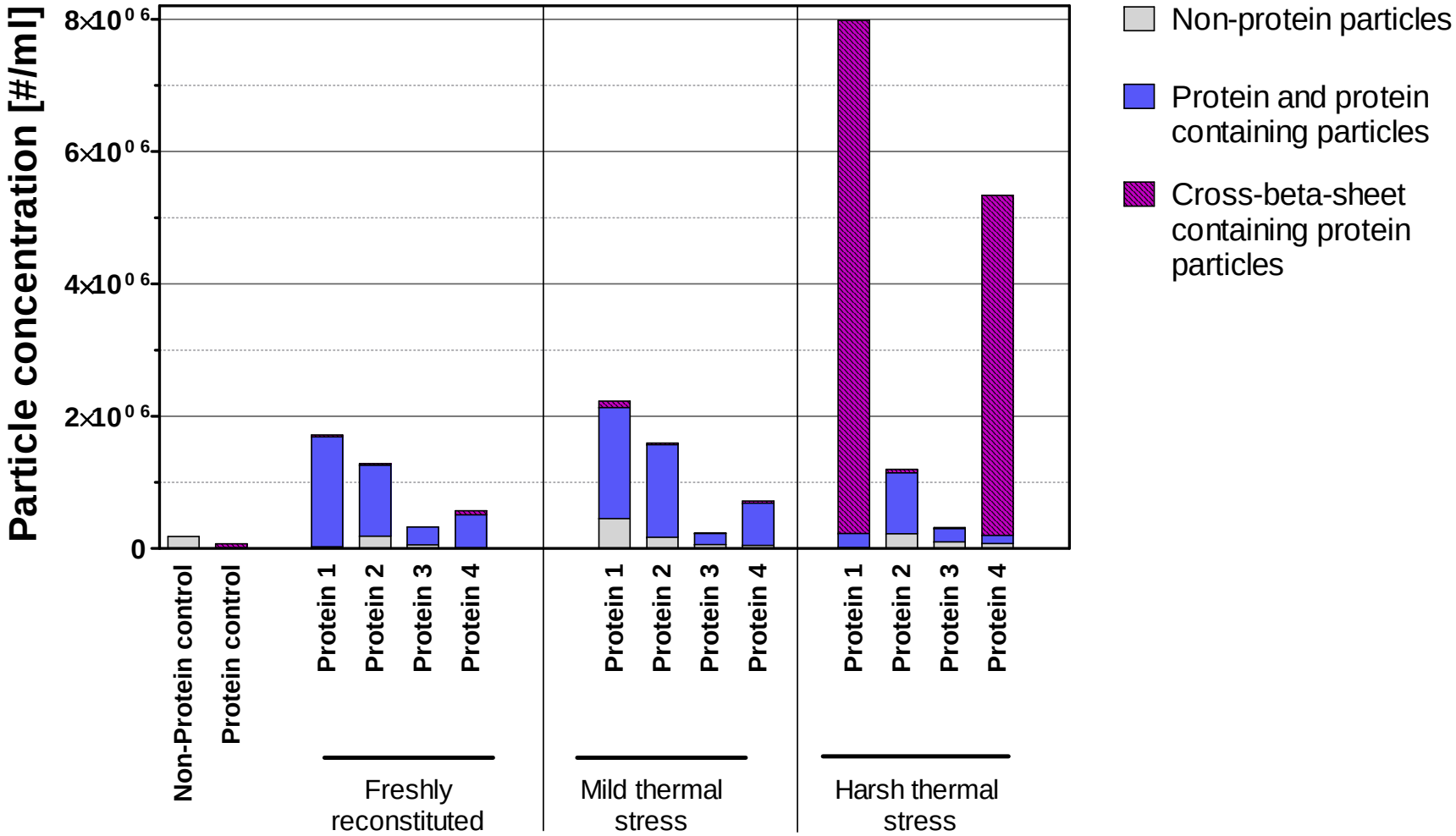
The properties of sub-visible particles may be different for all proteins



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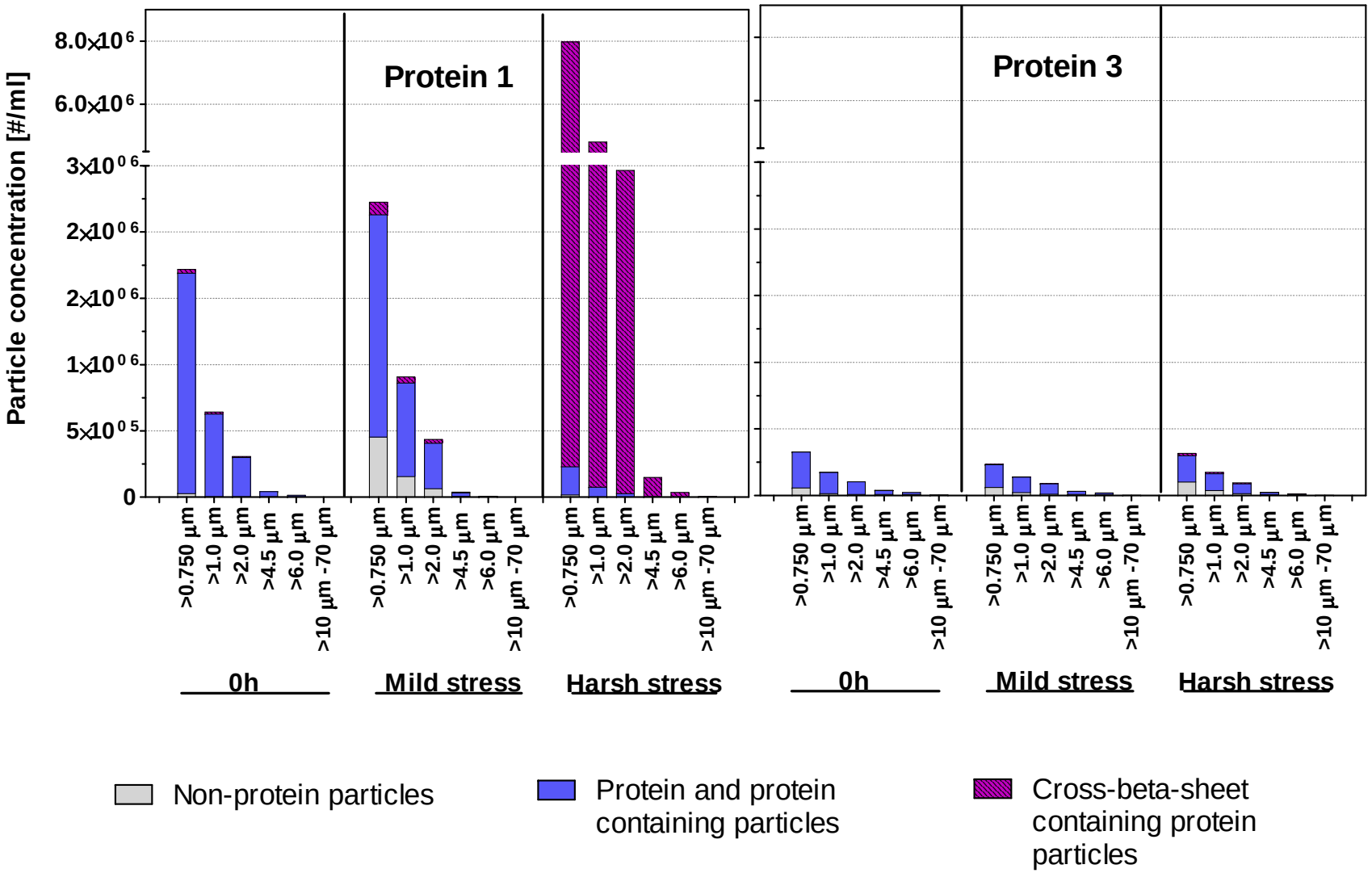

Use of flow cytometry based participle analysis to study protein-silicon interaction

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Does a native protein bind to silicon?

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

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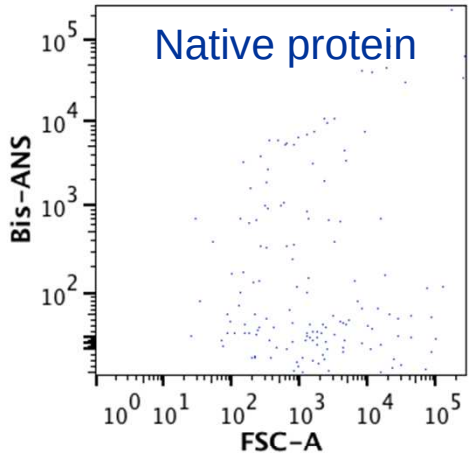
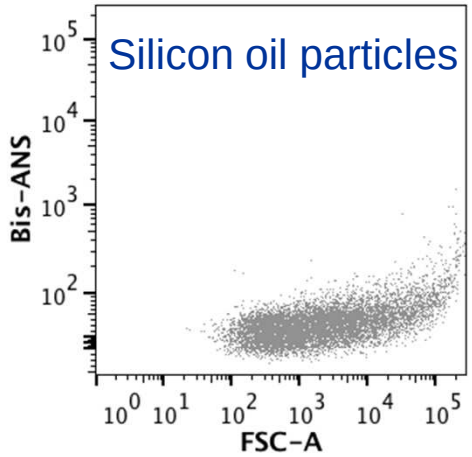
Does a native protein bind to silicon?





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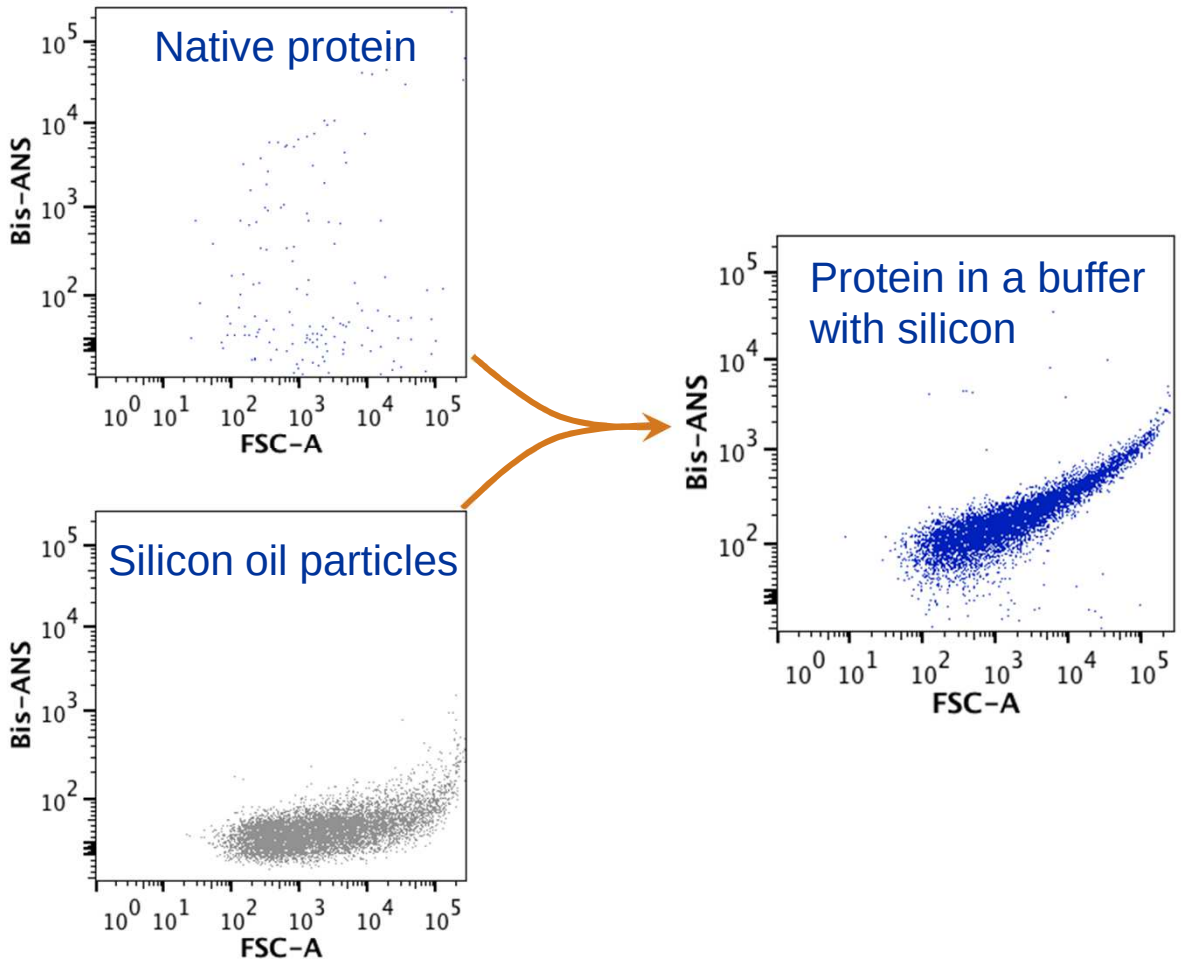
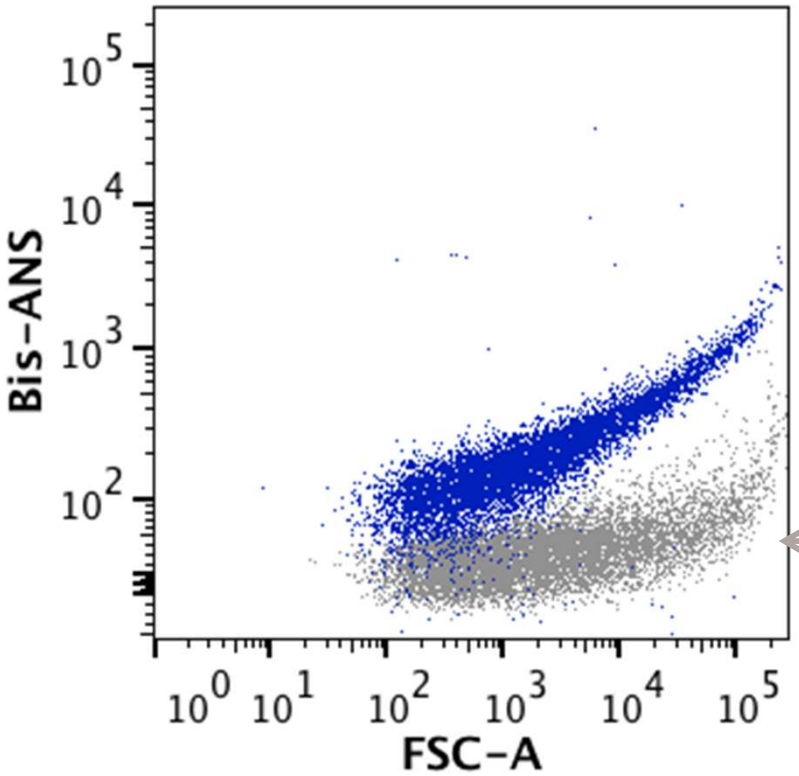


Use of flow cytometry based participle analysis to study protein-silicon interaction

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



Protein in a buffer with silicon

Silicon oil particles

Take home message

- Sub-visible particles can differ not only in size but also in their structural properties

- Flow cytometry based sub-visible paricle 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 !