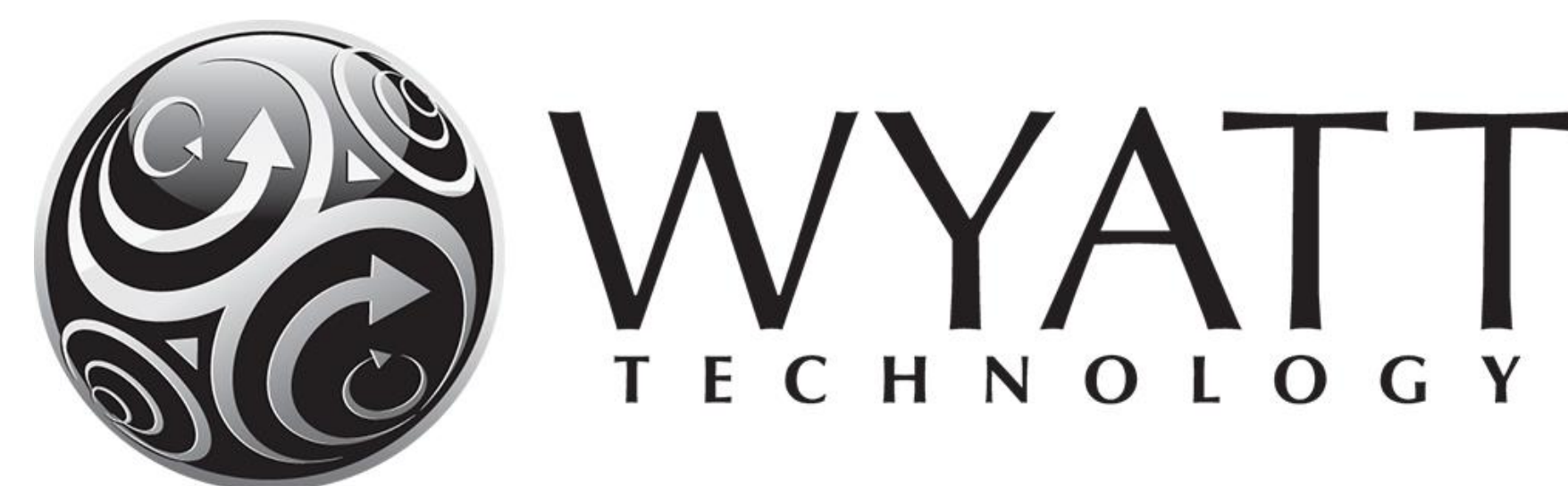




RT-MALS: Real-Time Process Analytics and Control for Vaccine Nanoparticles and Macromolecules

Daniel Some – Wyatt Technology Corporation

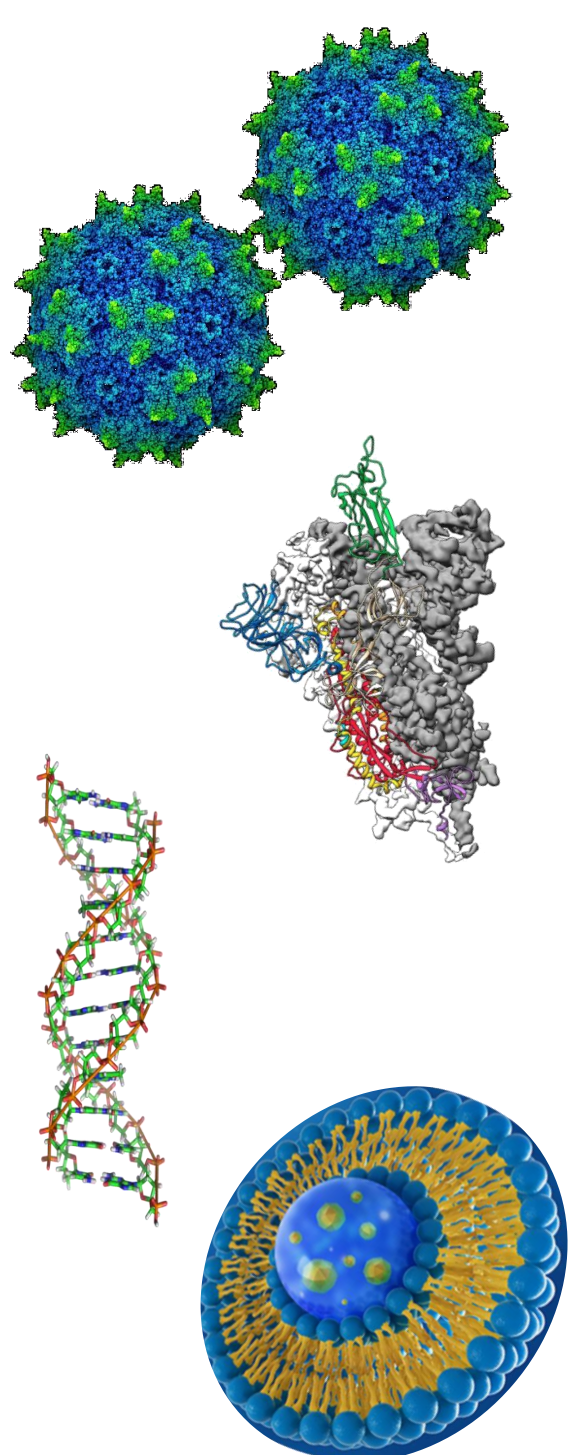
dsome@wyatt.com



Abstract

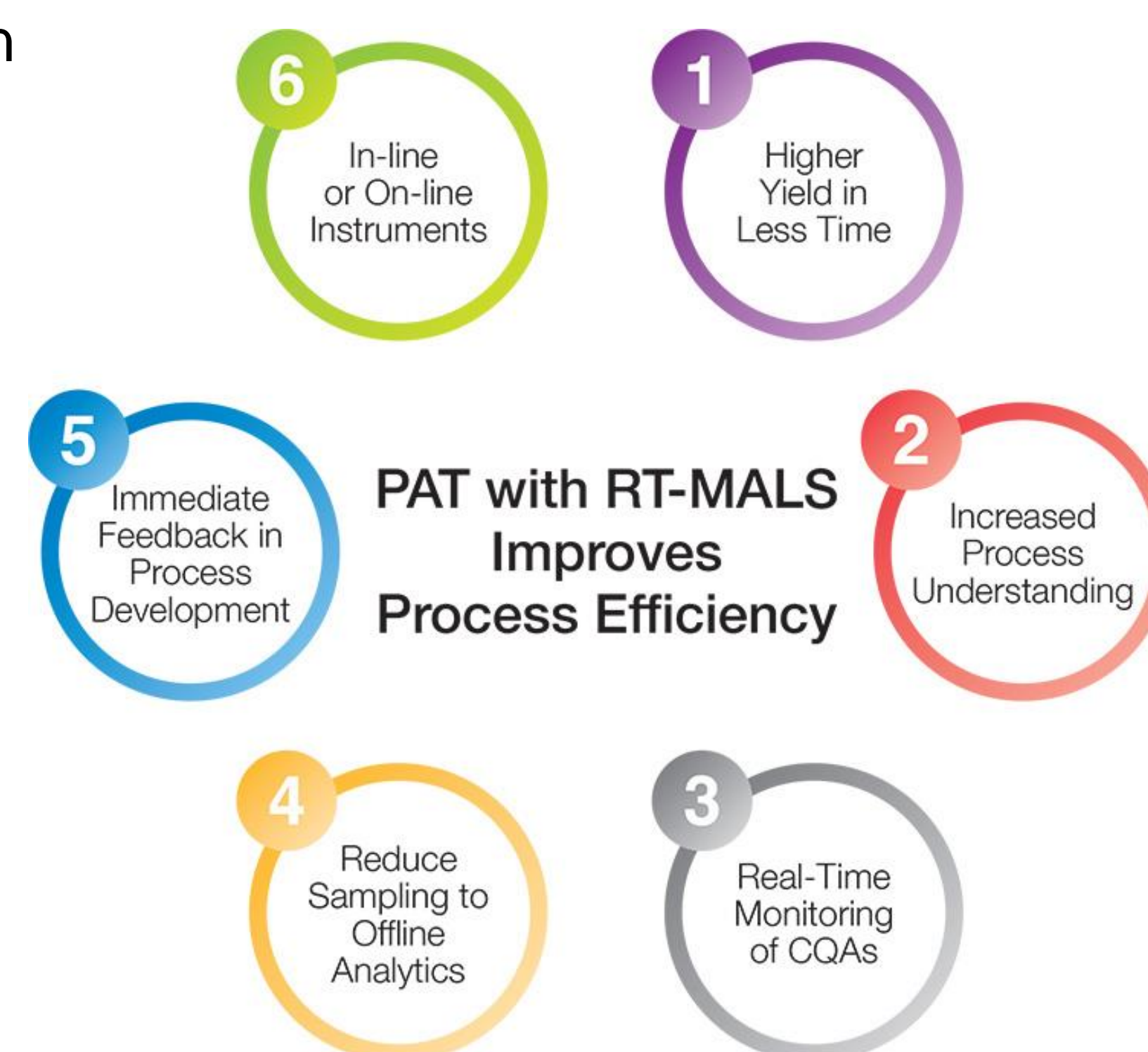
Analytical techniques combining multi-angle light scattering with size-exclusion chromatography (SEC-MALS) or field-flow fractionation (FFF-MALS) are widely applied in product and process development of many vaccine classes, including the recently developed mRNA-based vaccines. These techniques quantify essential product attributes.

Real-time MALS (RT-MALS) represents a new paradigm in PAT: instead of process parameters, it directly monitors product attributes. Implementation of RT-MALS promises to speed up process development and scale-up, increase yield during production and enhance end-product quality.



Conclusions

- RT-MALS provides a continuation to PD and Mfg. of detailed analytical characterization by SEC- or FFF-MALS
- Directly monitoring relevant product attributes offers many benefits over traditional PAT (or no PAT)
- Utilize ultraDAWN to increase productivity, yield and quality of multiple vaccine modalities.



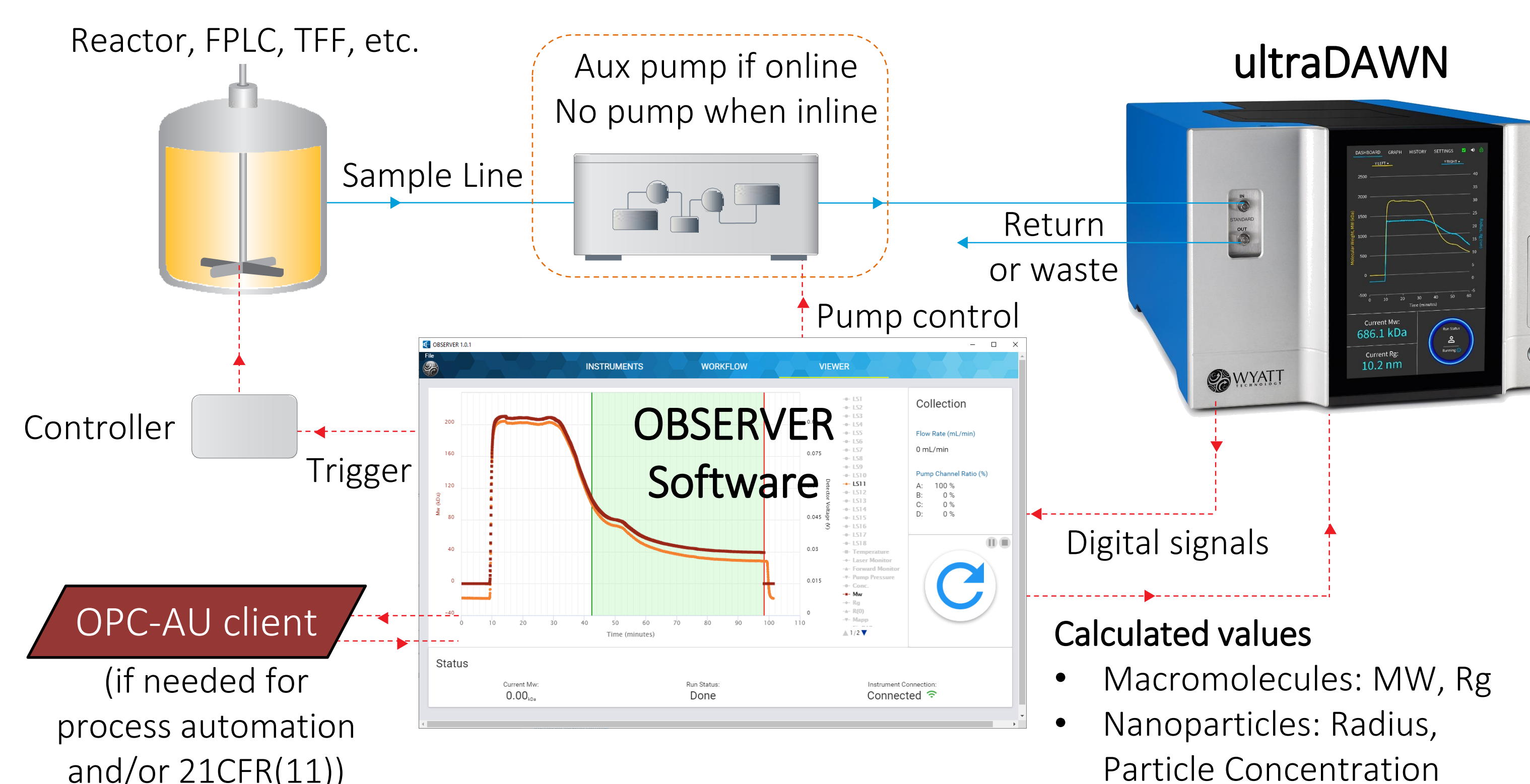
MALS measures... analytical and PAT

Vaccine type	Analytical: SEC-MALS, FFF-MALS					
	M_w	R_g	R	PC	%agg	Conjugation
Polysaccharides	✓	✓				
Glyco-conjugates	✓	✓				✓ (protein-Ps)
Protein antigens	✓				✓	✓ (glycosylation)
VLPs	✓		✓	✓	✓	✓ (DNA content)
Viruses and viral vectors			✓	✓	✓	
Lipid nanoparticles			✓	✓	✓	✓ (mRNA content)
PAT: RT-MALS						

Analytical SEC- and FFF-MALS provide detailed distributions of molar mass, size, particle concentration, %aggregate and conjugation or encapsulated/encapsulated nucleic acids. Typical analysis time: 30 – 60 minutes.

For process monitoring and control, RT-MALS determines average values every 2 seconds

RT-MALS basics: ultraDAWN™ and OBSERVER™



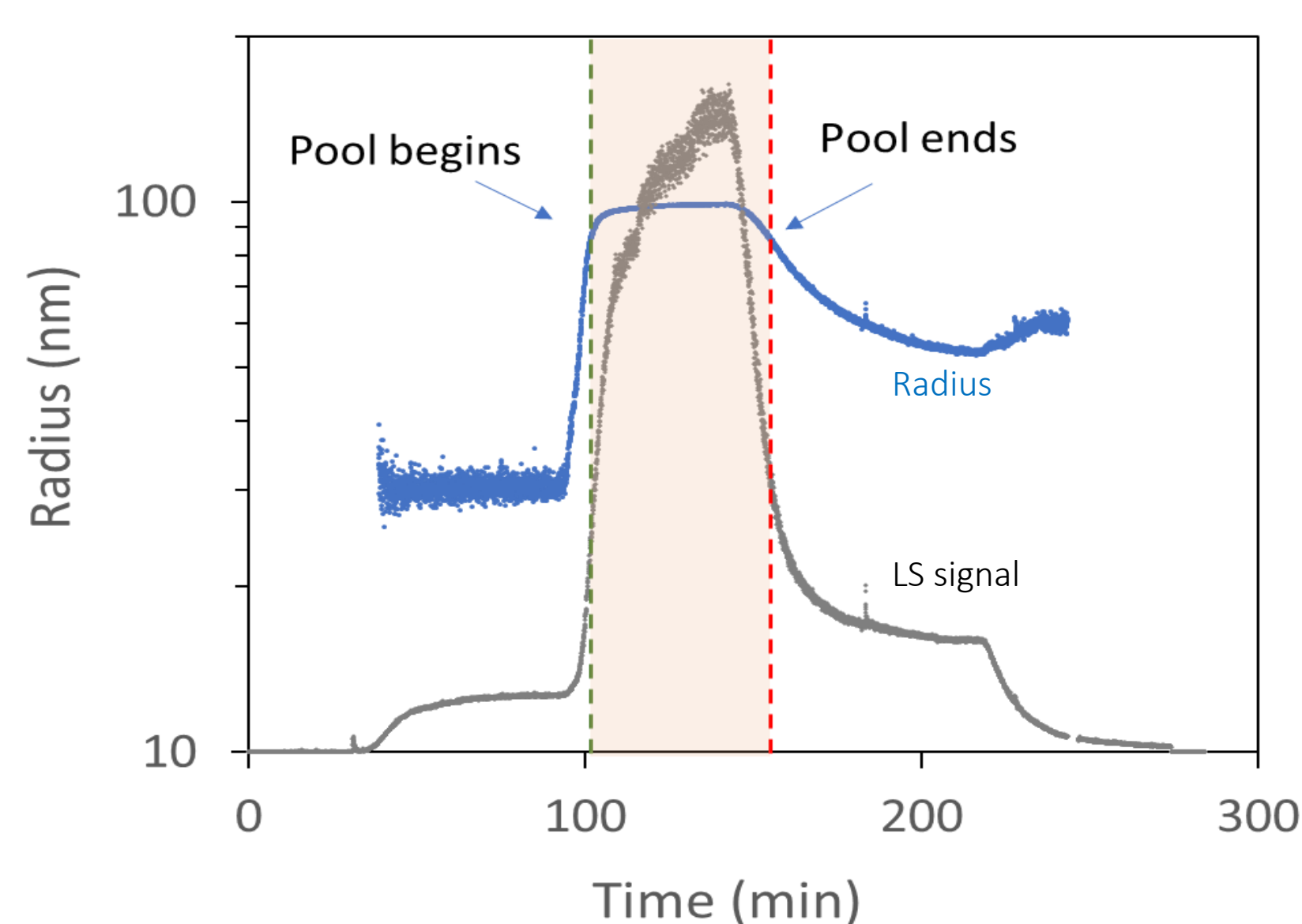
Case study: Viral vector purification

Ion-exchange exchange chromatography removes proteins, nucleic acids and other impurities

- FPLC's UV signal does not differentiate virus from impurities
- Viruses are primarily differentiated by their size → light scattering

Solution: in-line RT-MALS

- Trigger condition: $85 \text{ nm} < R < 120 \text{ nm}$
- Immediate response
- Also calculates physical titer in pool: concentration and total number of virions



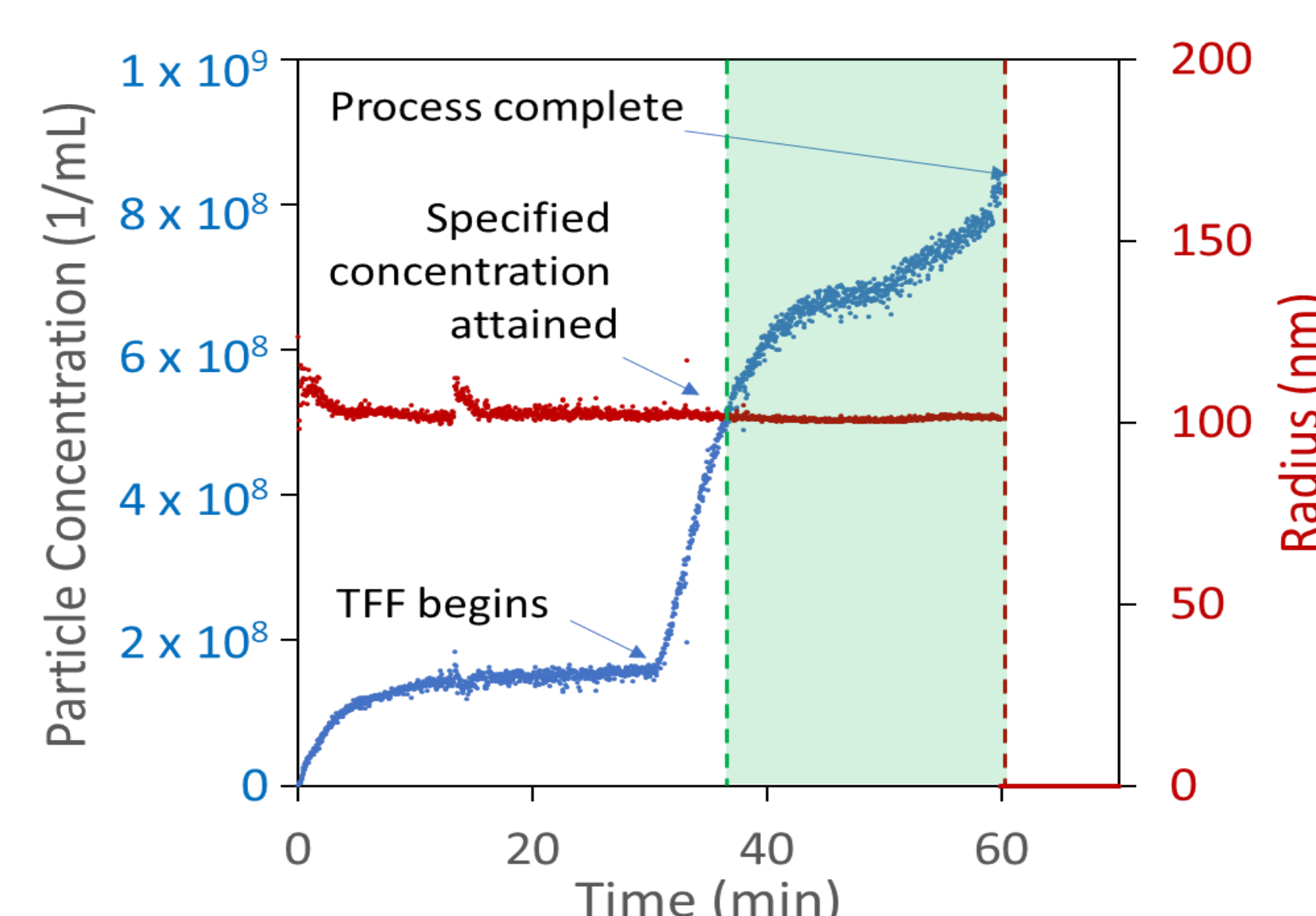
Case study: Viral vector concentration by TFF

Ultrafiltration by TFF for buffer exchange + increased concentration

- TFF goes through multiple cycles until final concentration
- Need to verify no degradation during the process

Solution: on-line RT-MALS

- Trigger condition: $PC > 5 \times 10^8 \text{ mL}^{-1}$
- RTD ~ 12 seconds does not delay processing
- Monitors average virion size to ensure no degradation



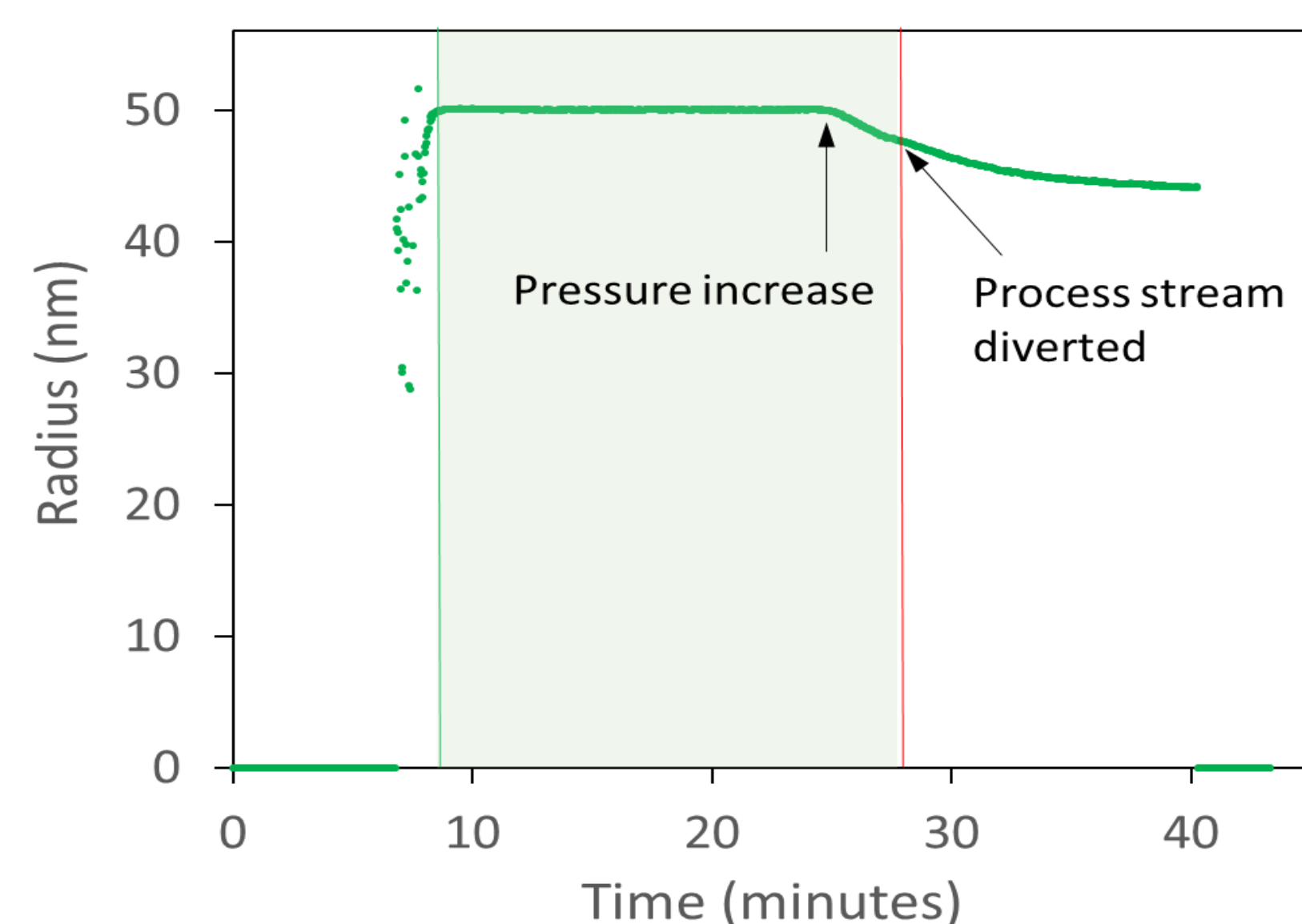
Case study: Liposome homogenization

Reduce heterogeneous liposomes from 180 – 800 nm to $100 \pm 4 \text{ nm}$

- Process is sensitive to deviations in press. and temp.
- Standard analytics: sample aliquots to at-line DLS. RTD ~ 3 minutes.
- Low flow rate during process development suitable for in-line

Solution: in-line RT-MALS

- Trigger condition: $48 \text{ nm} < \text{Radius} < 52 \text{ nm}$
- Immediate trigger; RTD = 0 relative to downstream collection/diversion valve



Case study: Polysaccharide depolymerization

Reduce $M_w < 350 \text{ kDa}$ by hydrolysis

- Standard analytics: at-line SEC-MALS, 30 min. run
- RT-MALS challenges: high concentration
 - Static process
 - Viscosity
 - Intermolecular interactions

Solution: on-line RT-MALS

- trigger condition: $M_w < 350 \text{ kDa}$
- 10x dilution by HPLC quaternary pump
- Trigger provided within 3 minutes of achieving endpoint

