Implementation and Automation of Downstream UF/DF of AAV

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Presentation Overview

Agenda

- Background
- UF/DF System Overview and Control Loops
- Trends and Results
- Summary and Acknowledgements

Presentation Objectives

- Outline UF/DF stainless-steel system and control capabilities
- Introduce Levitronix control system w/ automatic TMP control using a second Levitronix pump in reverse direction on the retentate line
- Review trends comparing manual vs automatic TMP control

Background

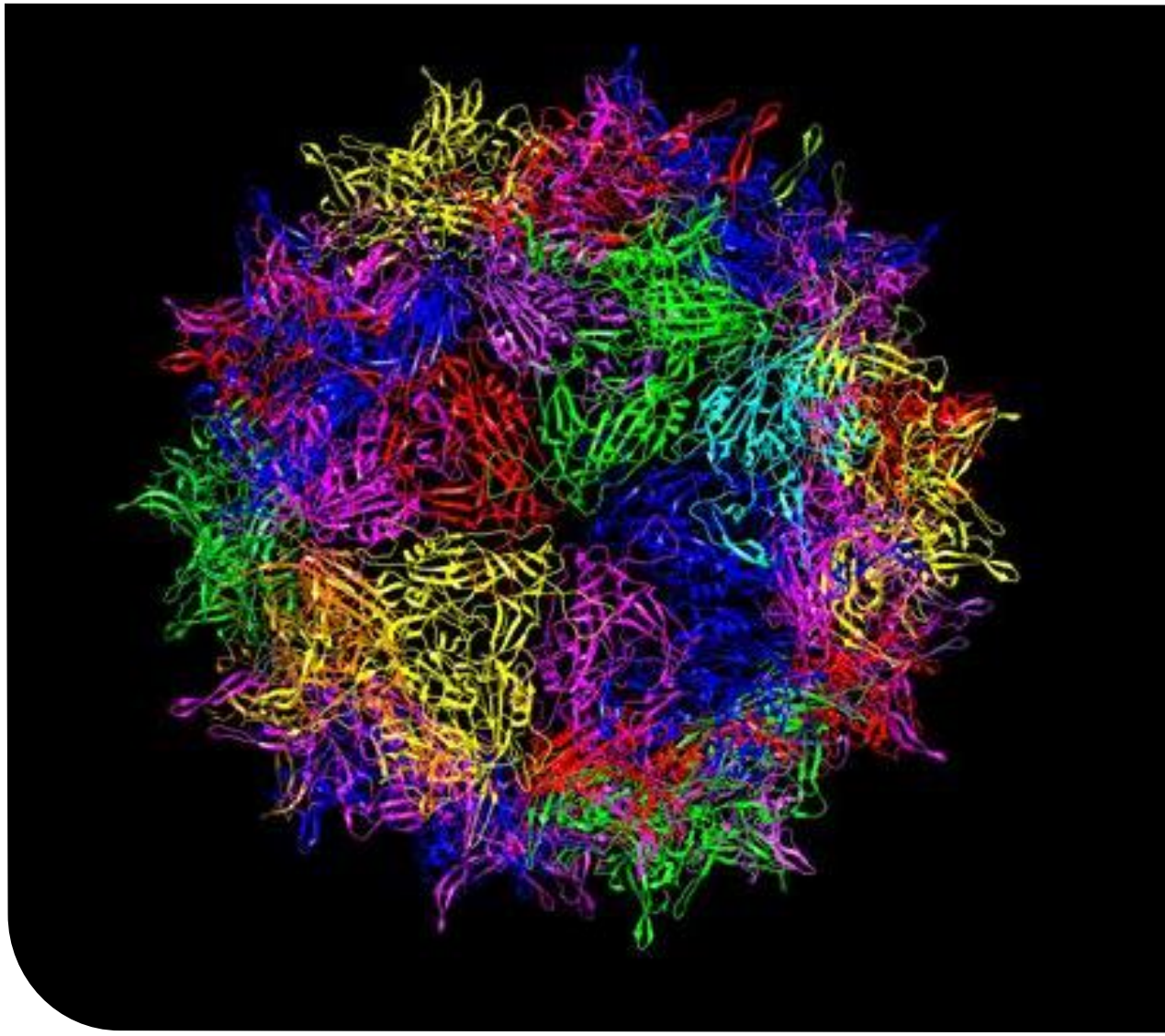
Gene Therapy (GT) and Adeno-associated Virus (AAV) Background

Gene Therapy (GT) Introduction

- GT involves delivering nucleic acids into a patient's cells to treat a life-threatening disease.
- GT applications aim to modify/replace/suppress the genes that cause the disease.
- AAVs are a common vector used in GT.

Adeno-associated virus (AAV) Structure

- Capsid
  - Icosahedral protein shell, ~22nm diameter
  - Different serotypes (e.g., AAV8, AAV9) have different capsid regions, which impact the type of cells they can infect.
- Genome
  - Composed of single-stranded DNA (ssDNA).
  - To generate the ssDNA, cells undergo transfection where a plasmid with the gene of interest (GOI) along with packaging/helper plasmids are added to the culture.



AAV- Manufacturing Process

UPSTREAM


Thawing cells


Spinner Flasks

Cell expansion


Single-use Bioreactor 50 L


Single-use Bioreactor 2 x 500 L


Cell disruption


Depth Filtration

DOWNSTREAM


UFDF


AEX


Affinity


Ultracentrifugation


Viral Inactivation


AEX


Virus Removal


DS

Focus of Today's Presentation: Ultrafiltration/Diafiltration

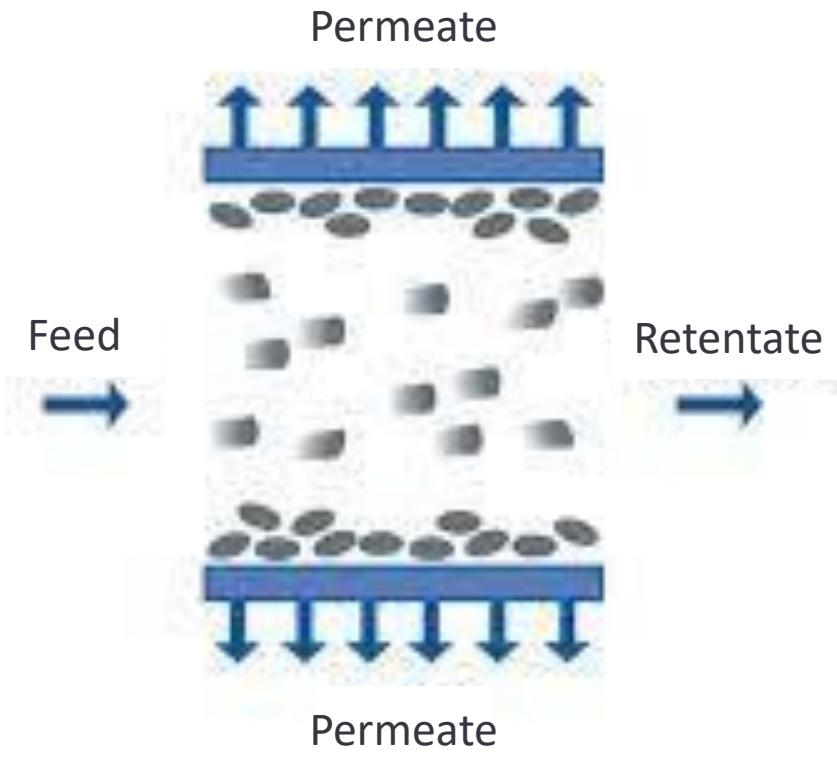
Ultrafiltration/Diafiltration Step Overview

- The UF/DF step uses a membrane with small pores that allow for size exclusion filtration
  - Molecules larger than the pore size will be retained by the membrane (retentate) and recirculated
  - Molecules smaller than the pore size can go through the membrane (permeate)

- The UF/DF step is intended to concentrate and buffer-exchange the material to the appropriate matrix for the next step.

Process Steps:

- Product Load and Concentration (UF1)
- Diafiltration (DF)
- Final Concentration (UF2)
- Recovery Wash

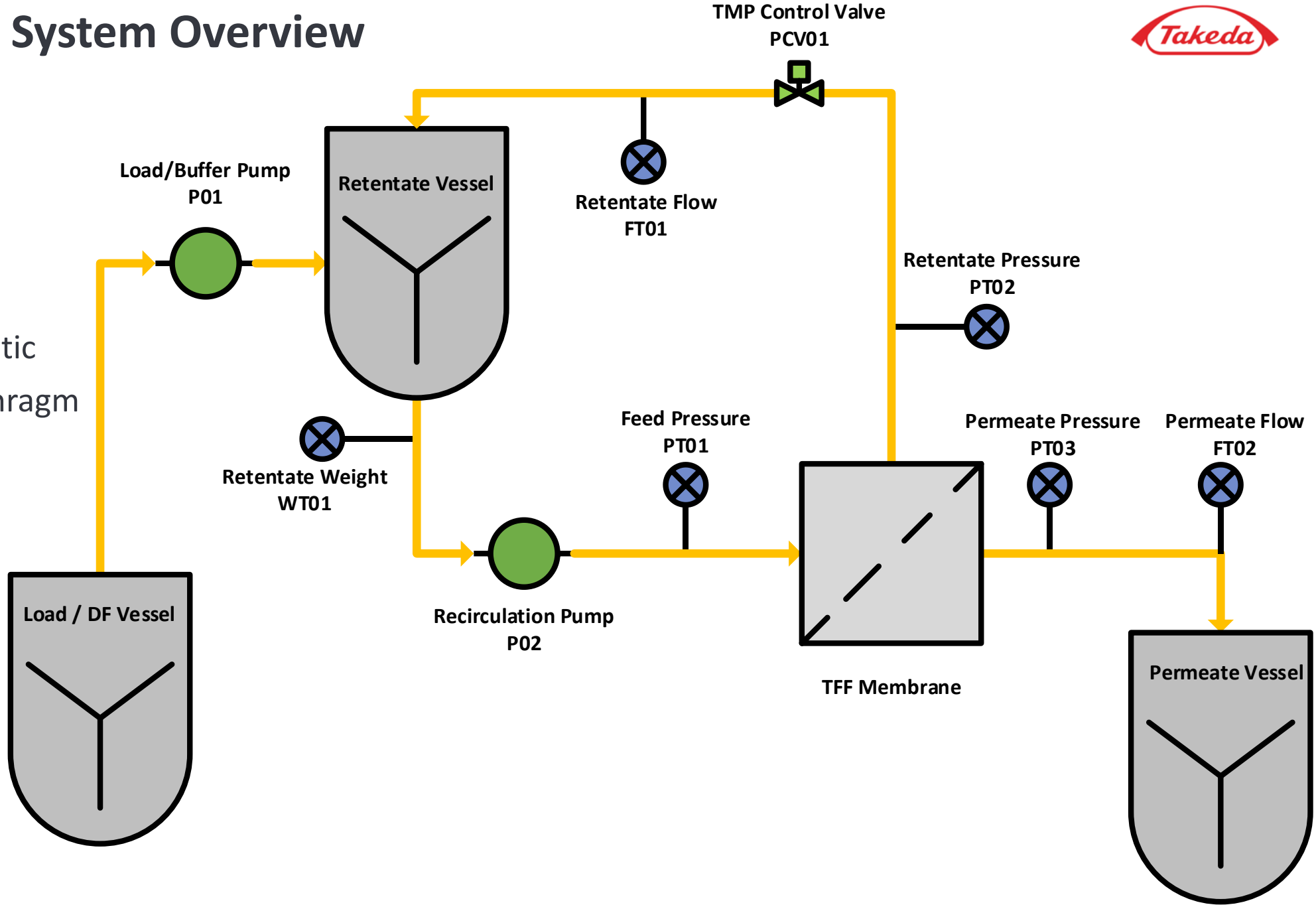


Ultrafiltration/ Diafiltration Diagrams and Control Loops

Stainless-Steel System Overview

- Stainless Steel Skid
- 0.5L-10L Retentate
- Recipe control

- Load Pump – Peristaltic
- Recirc. Pump – Diaphragm
- TMP Control Valve
- 3x Pressure Sensors
- 2x Flow Sensors
- Retentate Weight Sensor



Stainless-Steel System Control Loops

Control Loop 1:

- Retentate Flow (**FT01**) controlled by Recirculation Pump (**P02**)

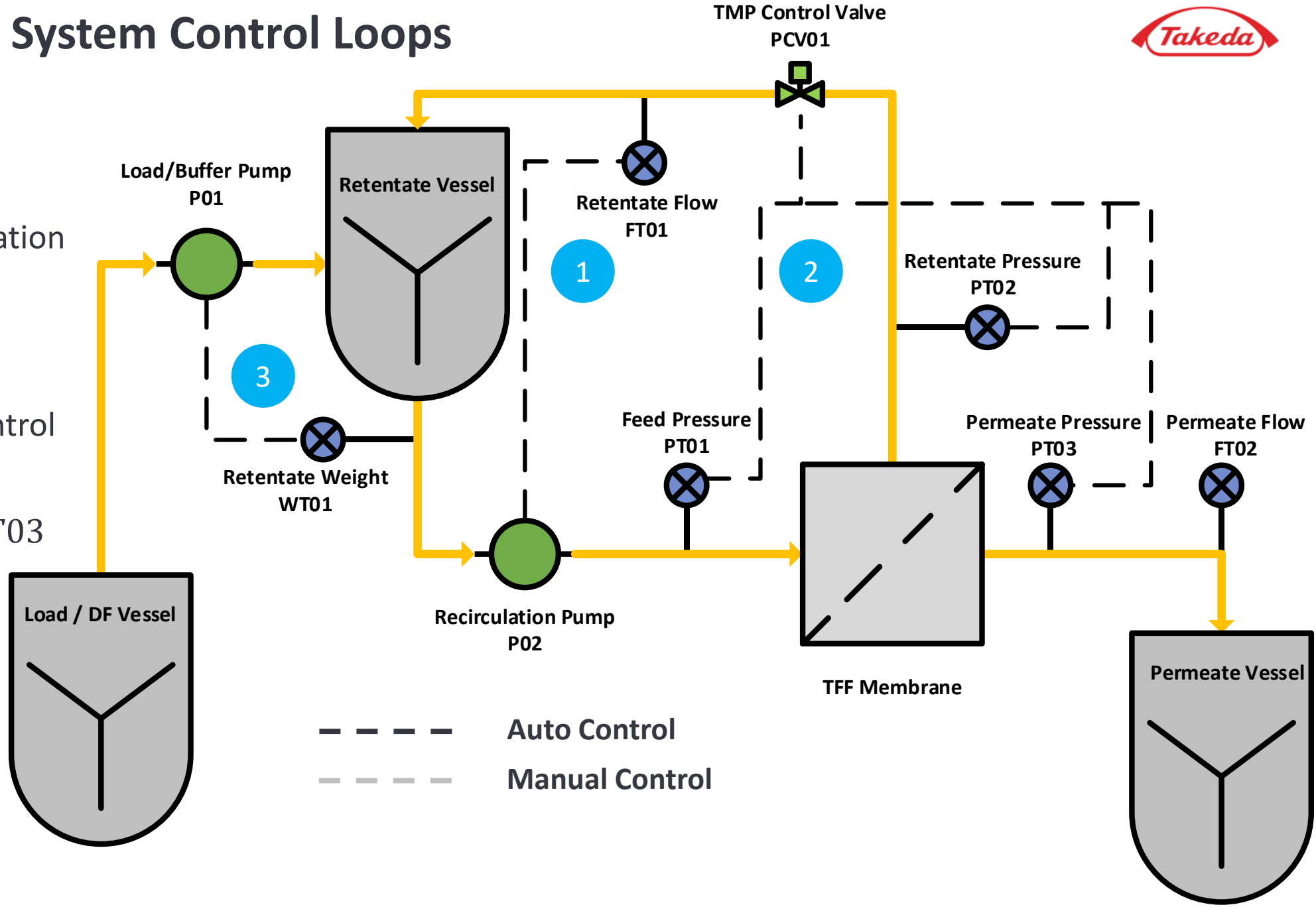
Control Loop 2:

- **TMP** controlled by control valve (**PCV01**)

$$\text{TMP} = \left(\frac{PT01+PT02}{2}\right) - PT03$$

Control Loop 3:

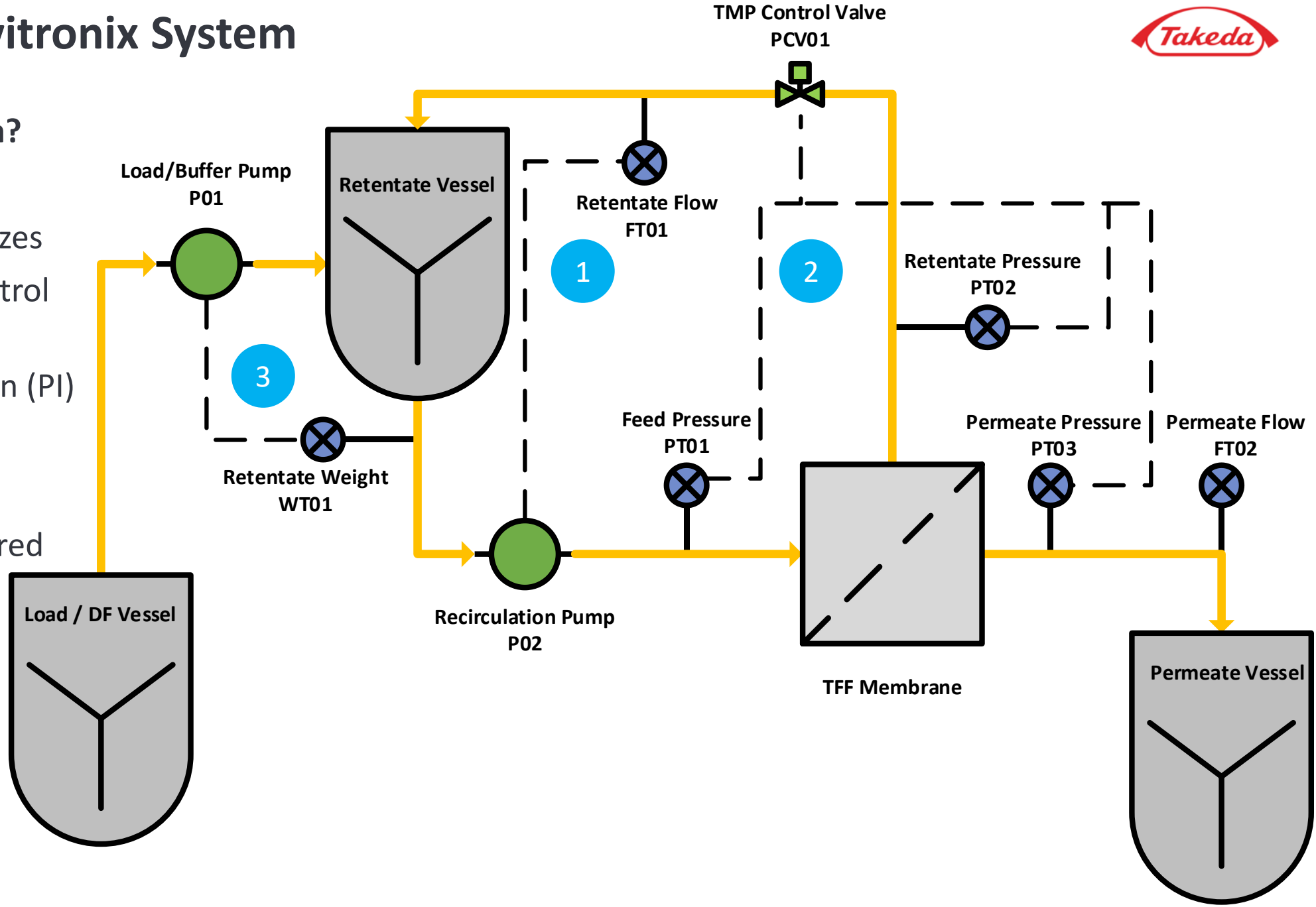
- Retentate Weight (**WT01**) controlled by Load/DF pump (**P01**)



Benefits of Levitronix System

Why Use a New System?

- Fully single-use
- Variable retentate sizes
- Integration with control system (DeltaV)
- Better data collection (PI)
- Lower shear
- Less lab space
- No annual PM required



Levitronix System – Manual TMP Control

Control Loop 1:

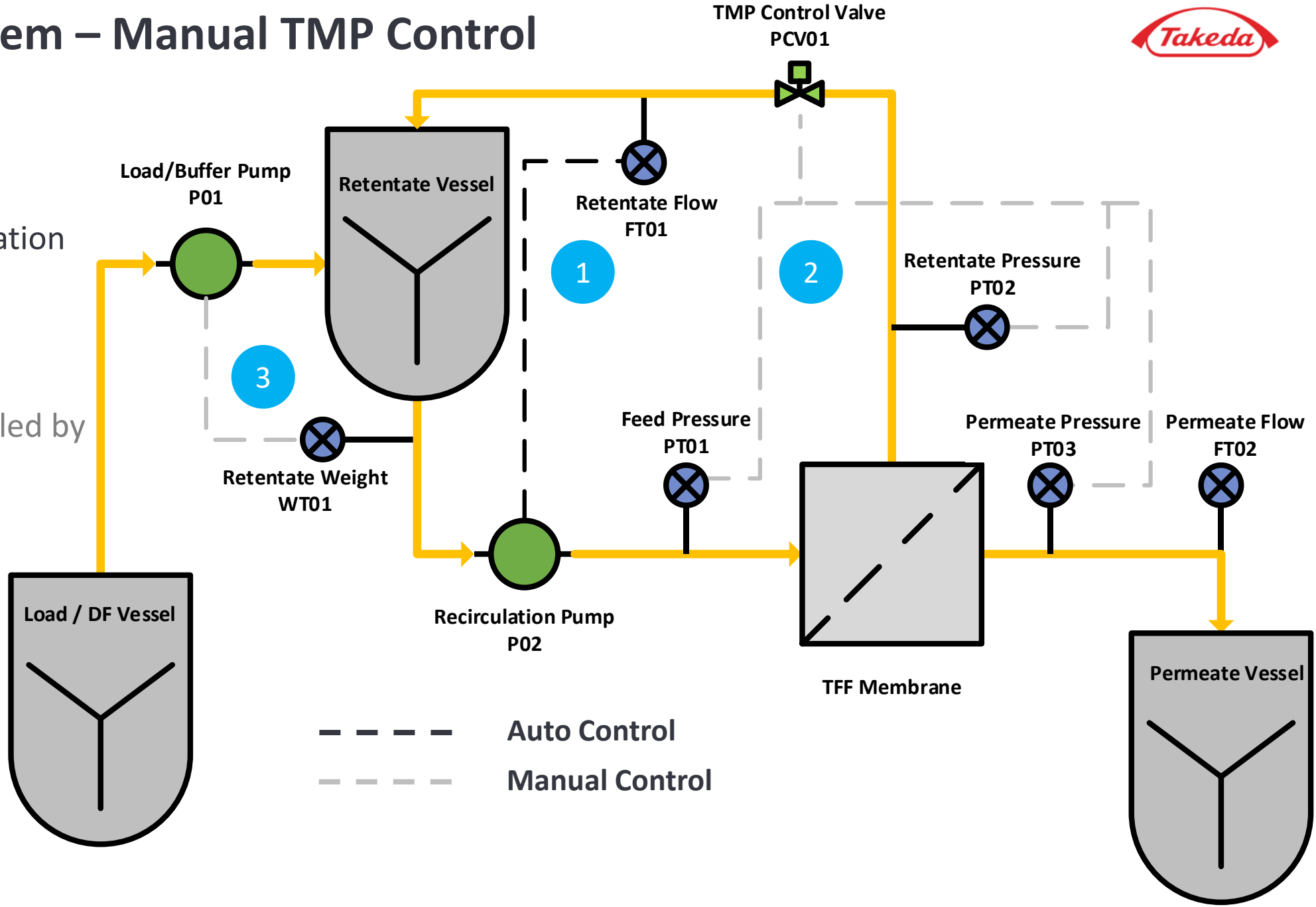
- Retentate Flow (**FT01**) controlled by Recirculation Pump (**P02**)

Control Loop 2:

- **TMP** manually controlled by control valve (**PCV01**)

Control Loop 3:

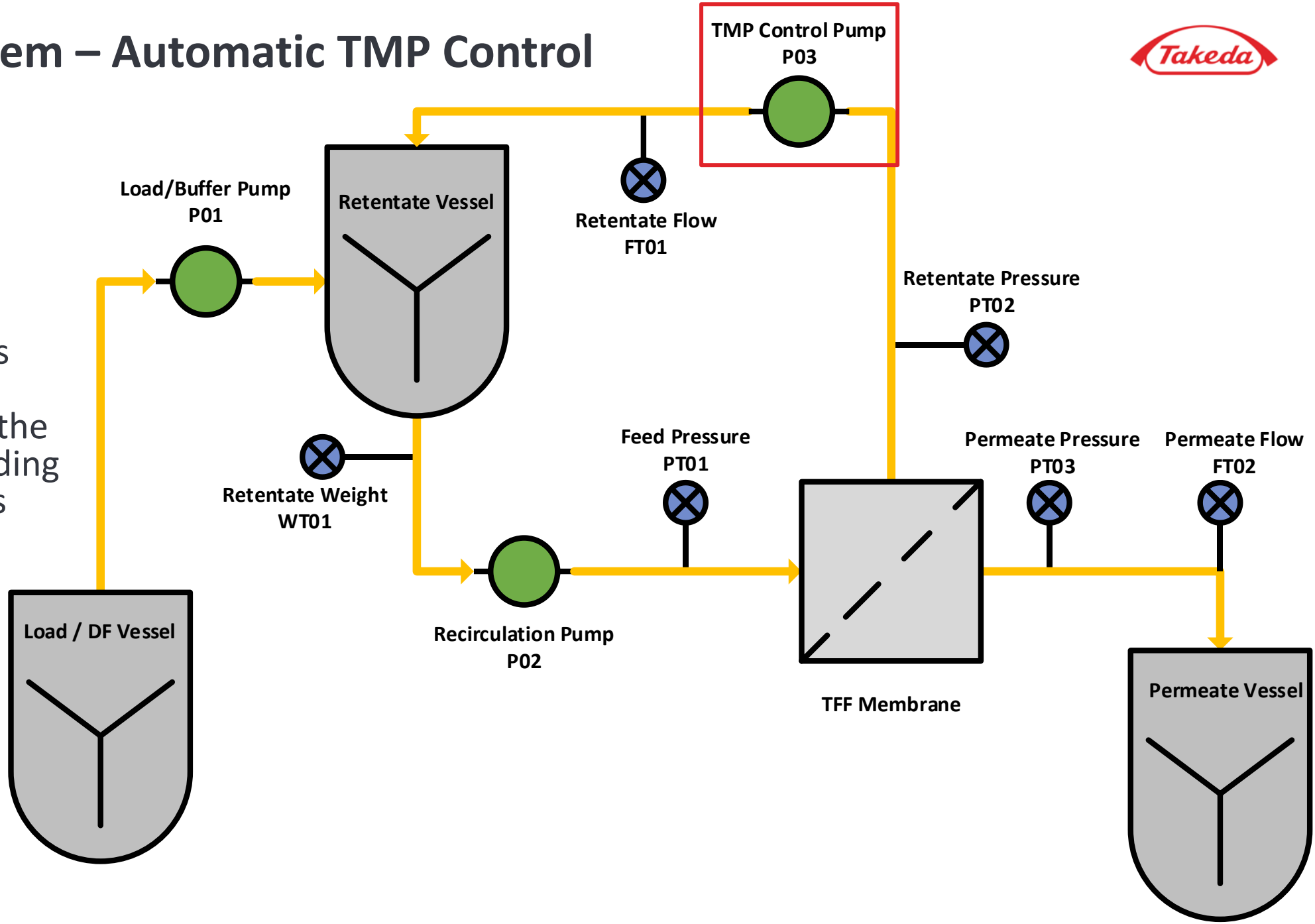
- Retentate Weight (**WT01**) manually controlled by Load/DF pump (**P01**)



Levitronix System – Automatic TMP Control

TMP Control Valve
replaced by
TMP Control Pump

The levitronix pump is backwards on the retentate line, facing the membrane and providing backpressure towards the membrane.



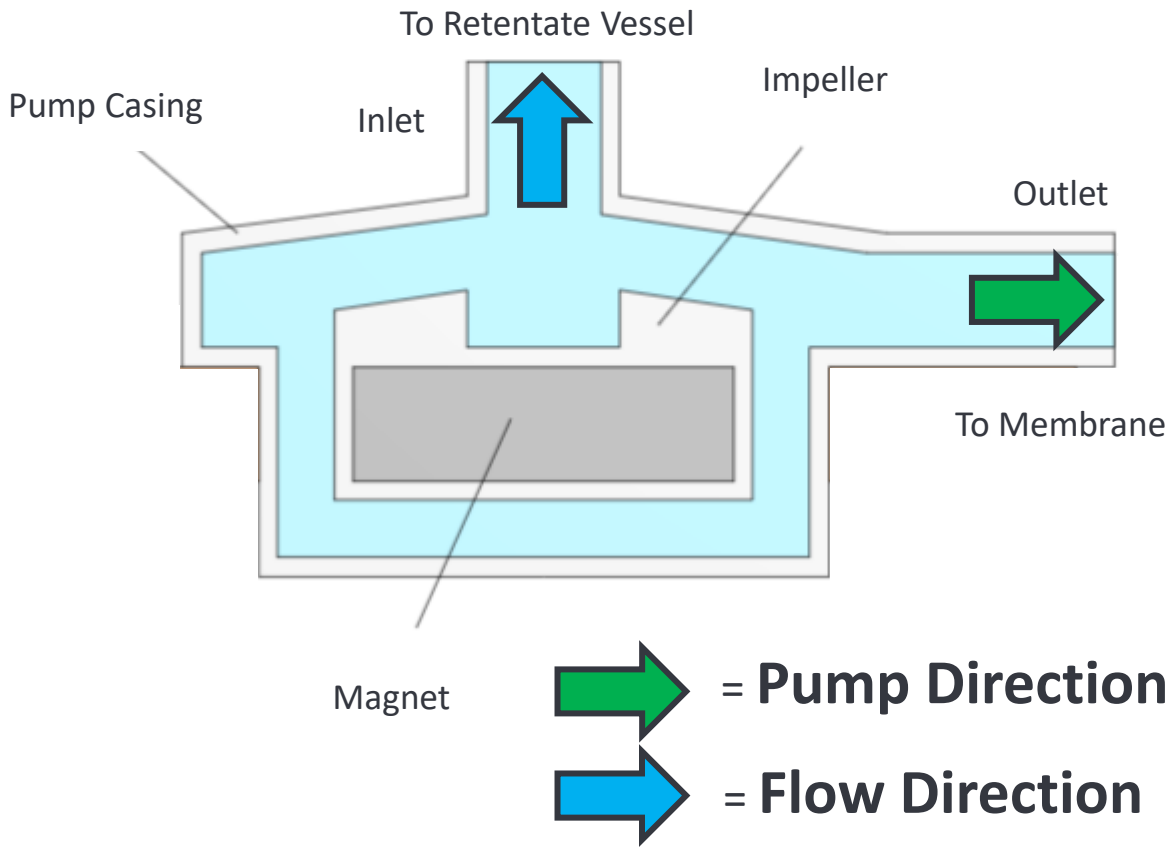
Levitronix TMP Control Pump Diagram

Levitronix pumps provide pressure, not flow

Liquid flow direction is towards the retentate vessel

Pump direction is towards the membrane

Retentate TMP Control Pump Diagram



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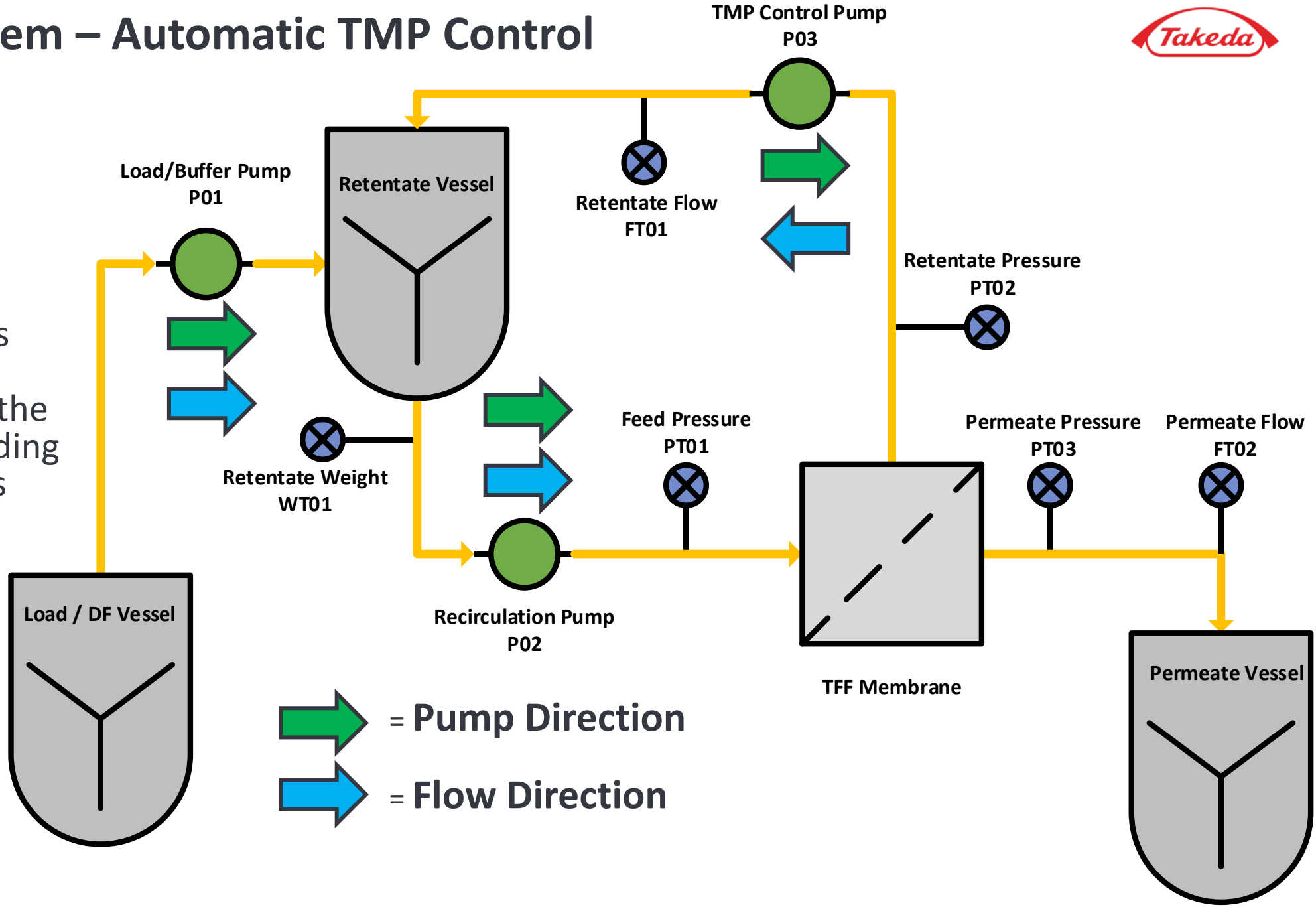


Levitronix System Control Loops

Control Loop 1:

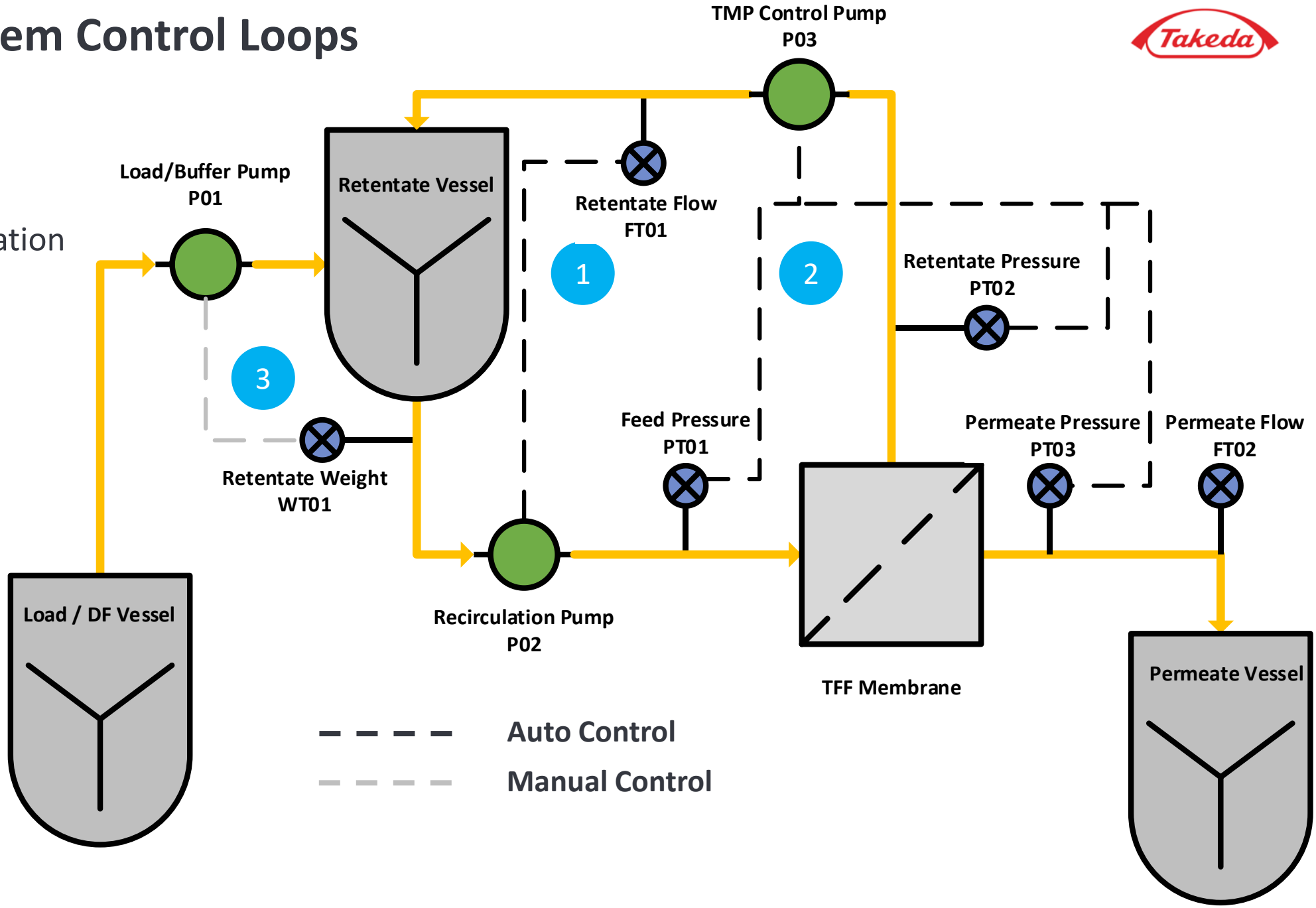
- Retentate Flow (**FT01**) controlled by Recirculation Pump (**P02**)

Control Loop 2:

- **TMP** controlled by Levitronix TMP Pump (**PCV01**)

Control Loop 3:

- Retentate Weight (**WT01**) manually controlled by Load/DF pump (**P01**)



Levitronix System Control Loops with DeltaV

Control Loop 1:

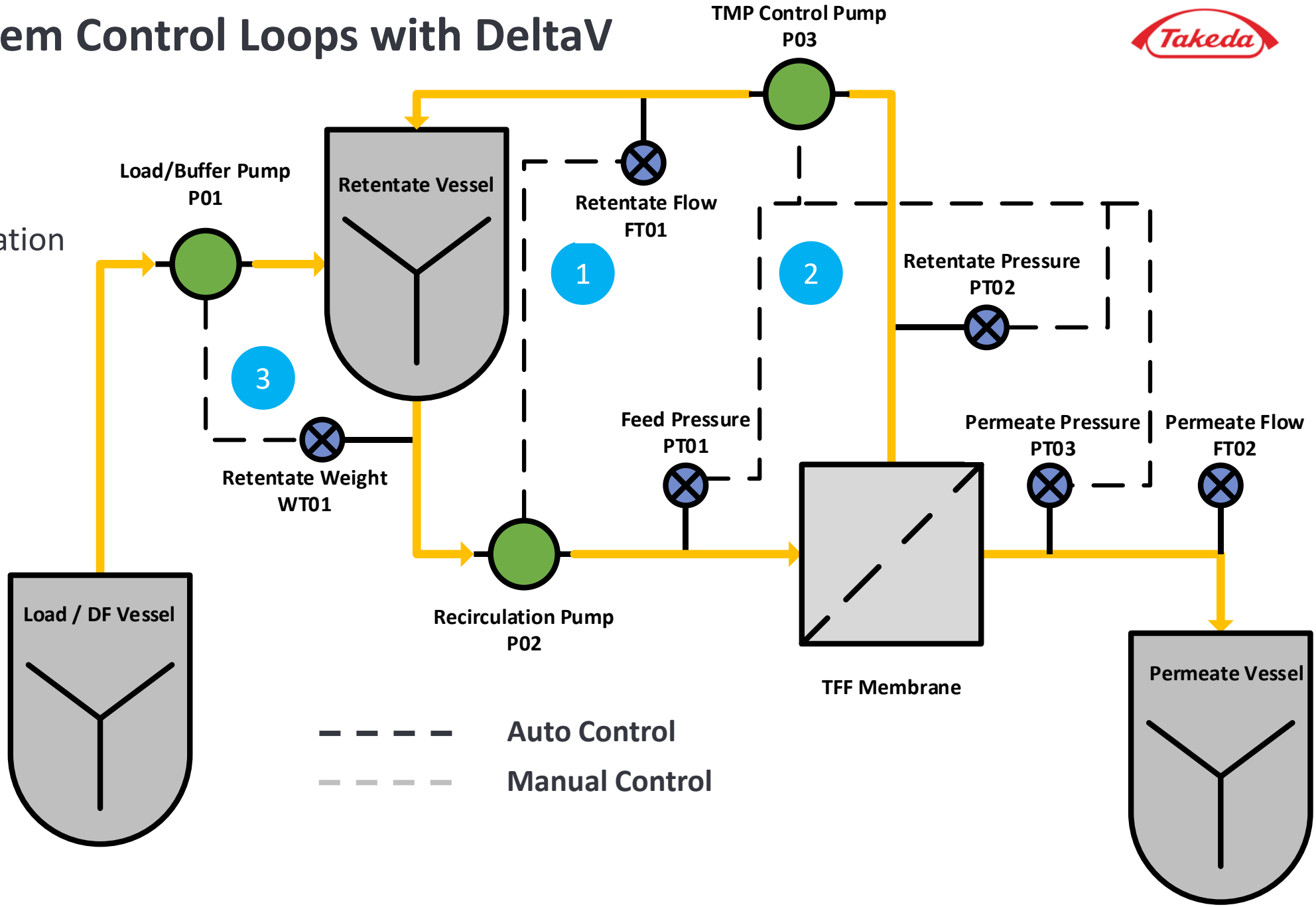
- Retentate Flow (**FT01**) controlled by Recirculation Pump (**P02**)

Control Loop 2:

- **TMP** controlled by Levitronix TMP Pump (**PCV01**)

Control Loop 3:

- Retentate Weight (**WT01**) controlled by Load/DF pump (**P01**) via DeltaV

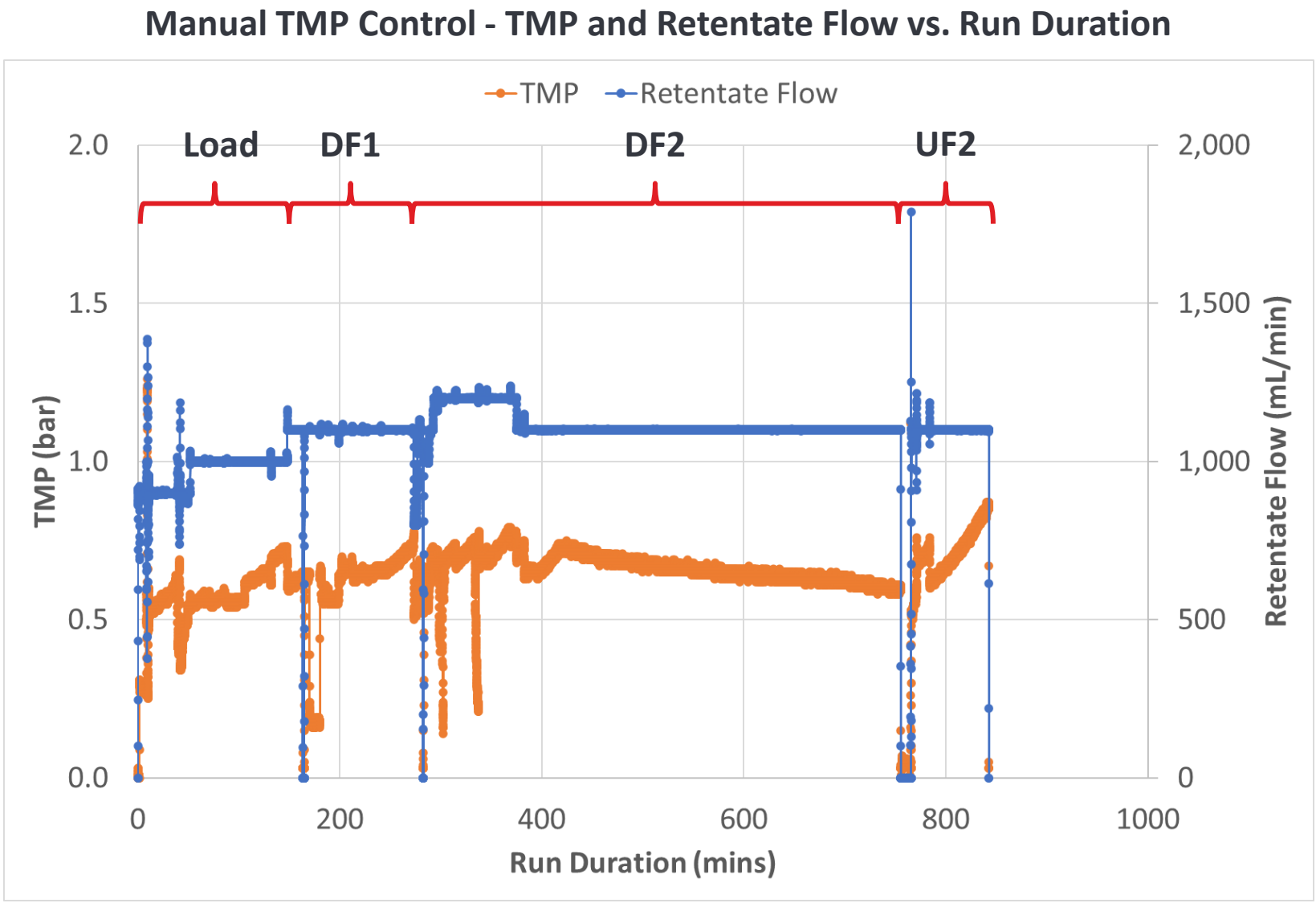


Trends and Results

Levitronix System with Manual TMP Control – Flow and TMP

Run Notes:

- 10L Load
- Retentate flow increased during run due to low permeate flow
- TMP increased during the run, and the manual clamp needed to be readjusted frequently
- Flow control was steady, but was impacted by manual clamp adjustments

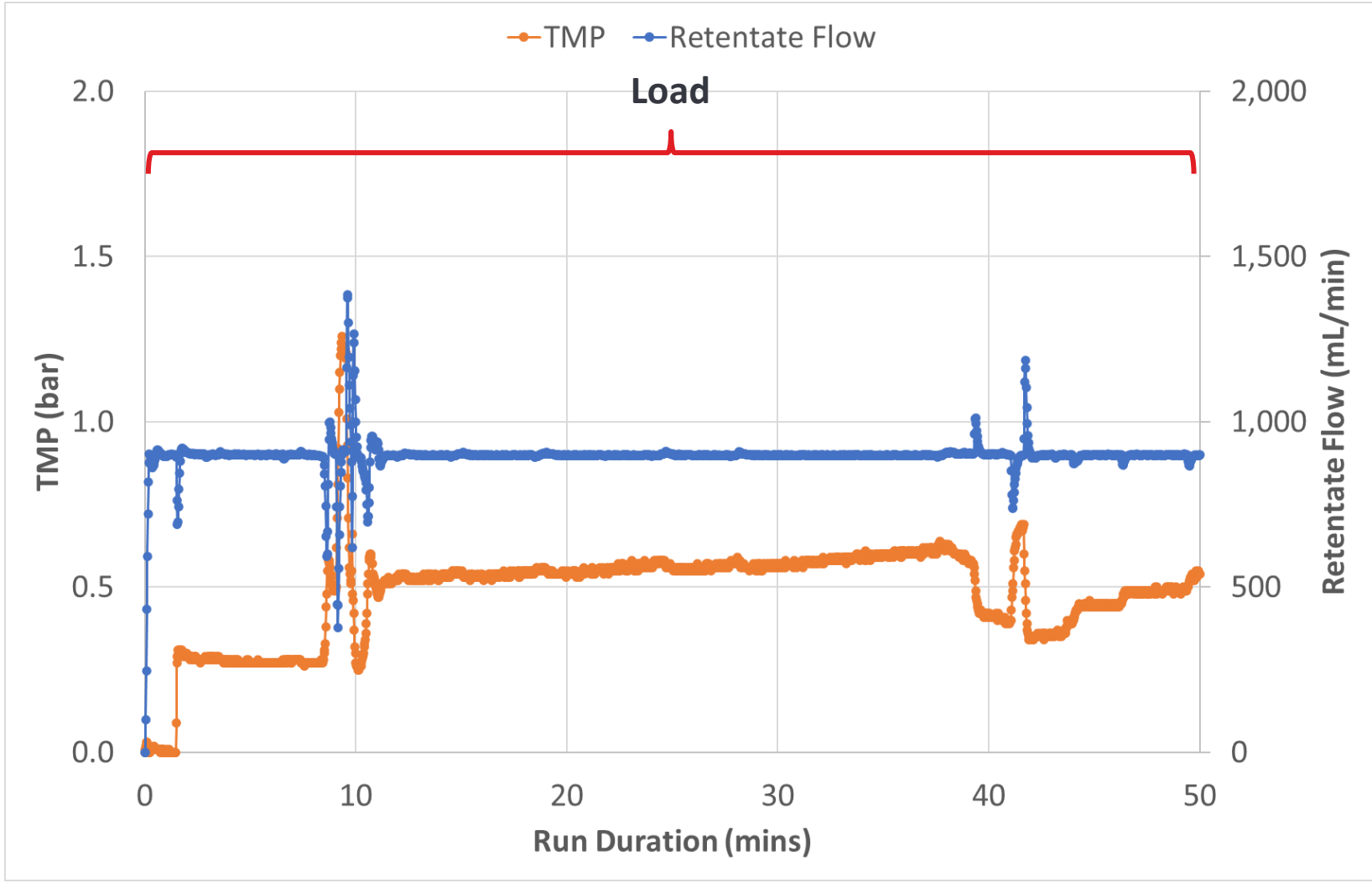


Levitronix System with Manual TMP Control – Flow and TMP

Run Notes:

- During flow start, manual clamping interfered with the flow rate, resulting in some disturbances in both pressure and flow readings
- Throughout the run, the manual clamp adjustments caused disturbances in the flow rate

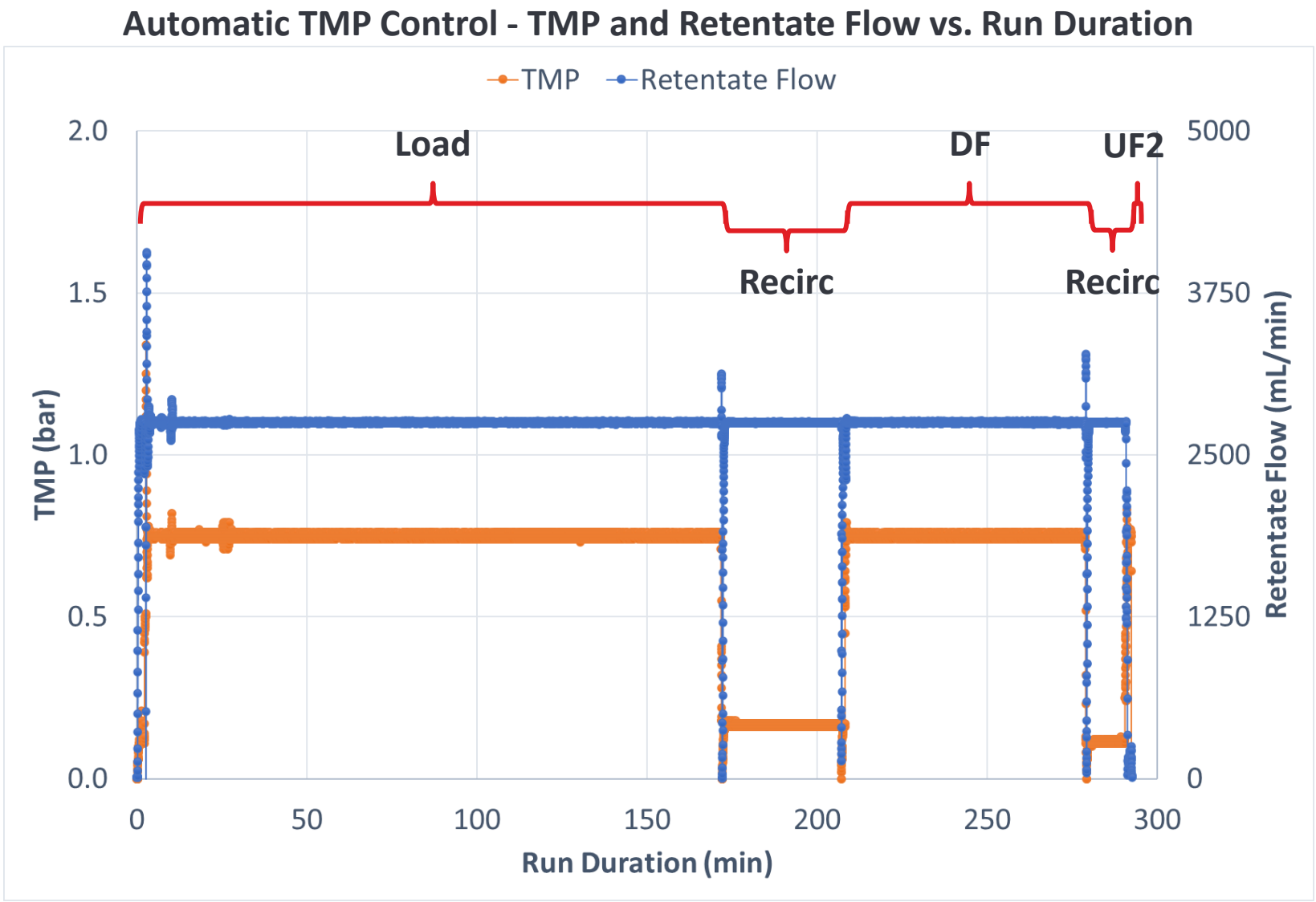
Zoomed in Load Start – TMP and Retentate Flow vs. Run Duration



Levitronix System with Automatic TMP Control – Flow and TMP

Run Notes:

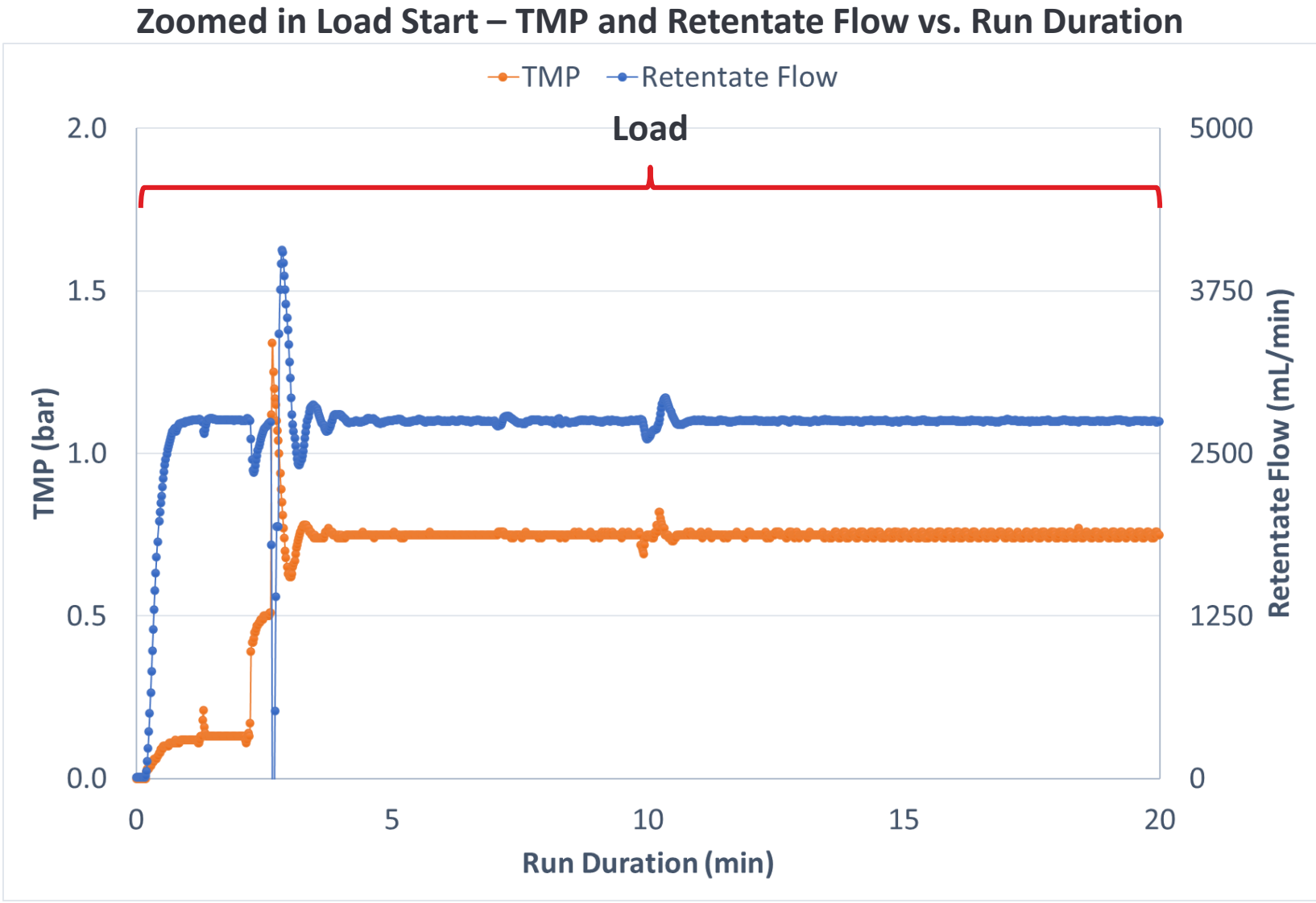
- Process changes-
  - Larger scale – 50L
  - One DF instead of two
- Automated TMP control maintained setpoint throughout run
- Recipes created on Levitronix controller for Load, DF, and UF steps
- 2x runs at 50L scale with similar performance



Levitronix System with Automatic TMP Control – Flow and TMP

Run Notes:

- Started automatic flow control first
- Started TMP control once flow was steady
- After ~10 minutes, started product loading
- Some disturbance when pressure control starts, but system quickly corrects both flow and pressure controls

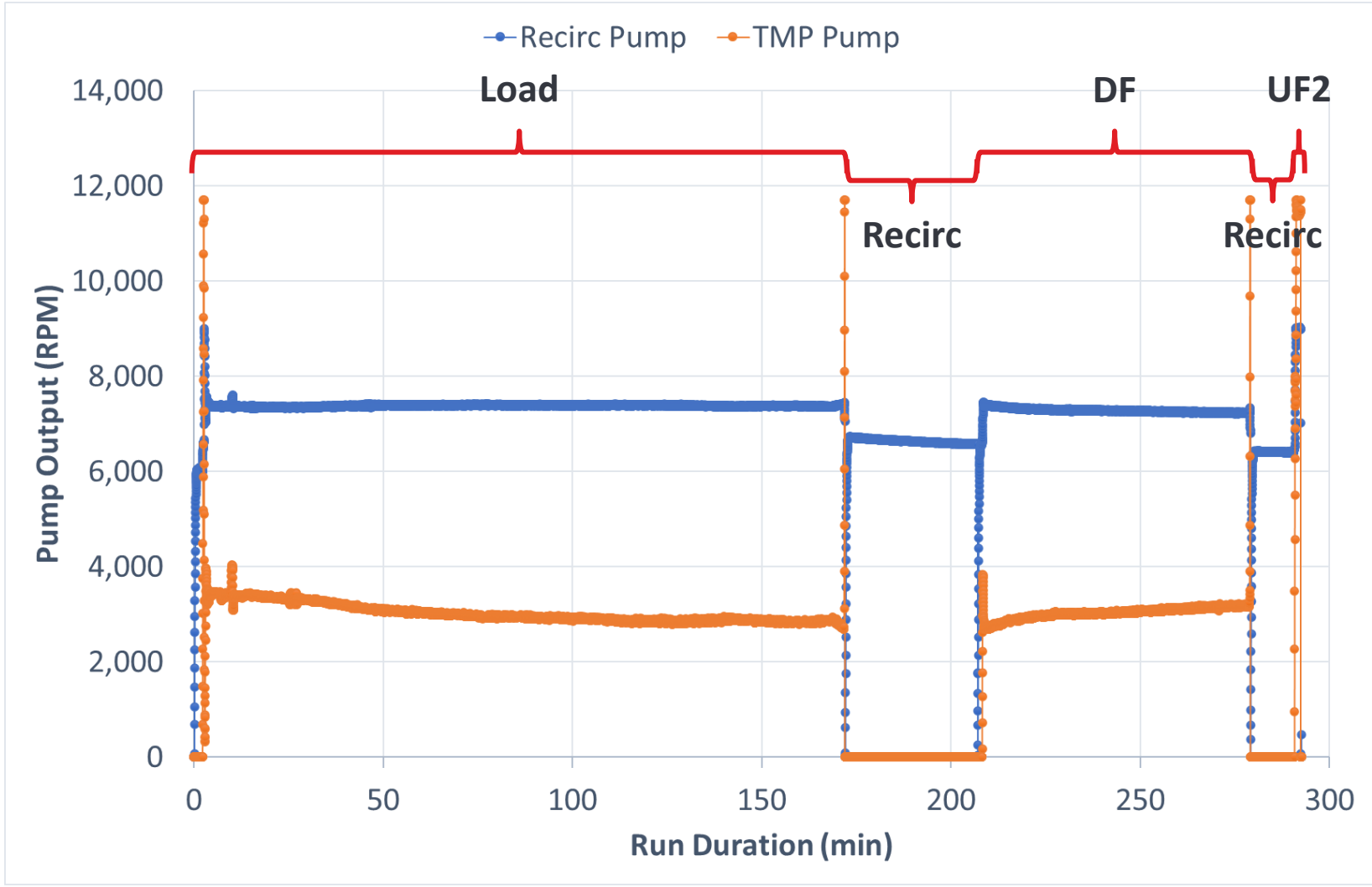


Levitronix System with Automatic TMP Control – Pump Output

Run Notes:

- Recirculation pump has a higher output (RPM) than the TMP pump
- TMP pump output (RPM) decreases during the load
  - Increased viscosity during concentration results in higher pressure in the membrane, so less pump output (RPM) is needed
- TMP pump output (RPM) increases during DF
  - Buffer exchange reduces the viscosity, so TMP pump output (RPM) increases

Automatic TMP Control – Recirculation and TMP Pump Output vs. Run Duration



Summary and Acknowledgements

Levitronix Implementation Summary

Implemented single-use Levitronix control system.

- Better TMP control
- Lower shear
- More control
- Integration with DeltaV and PI Historian
- Easily scalable for manufacturing-scale

Implemented TMP control using a second Levitronix pump in reverse direction on the retentate line.

Demonstrated system capabilities and generated comparable product quality results to historical runs.

Acknowledgements

- Ilan Goldberg, Levitronix
- Takeda GT/CT Pilot Team
- Takeda Biologics Pilot Team
- Takeda GT PD Team

Questions?