Opentrons Interfacing Turbidostat for Continuous Evolution

SBHSE | School of Biological and Health Systems Engineering

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Introduction

Nanobodies, single-domain antibody fragments, are a powerful tool in biotechnology and medicine due to factors including their small size (12-15 kDa), high stability, low structure complexity, and strong binding affinity [1]. Nanobody applications include targeted therapies, with a particularly large interest in oncology due to the success of monoclonal antibodies, diagnostics, laboratory assays, and imaging [2,3,4]. Surface display for phage assisted continuous evolution (SurPhACE) is a synthetic biology method to evolve novel nanobodies developed by the Bioprotean lab. The method leverages the bacteriophage's reproductive ability by means of a target and decoy bacteria, applying selective pressure on increased, specific binding affinity to a target binding site. SurPhACE was performed by hand utilizing 6 tubes to generate 6 evolutionary pathways, with fresh bacteria added once per day limiting the evolutionary speed and nanobody production. To improve upon this method, an Opentrons OT2 (Figure 1) is utilized to perform SurPhACE in a 96 well plate with bacteria added every 6 hours. Automation enables a 64 times increase in evolutionary speed, however a method to deliver healthy bacteria to the OT2 platform is not currently available. I have developed an open source turbidostat model to interface with an Opentrons OT2, delivering bacteria and enabling SurPhACE automation.

Figure One: *Opentrons OT2*

Methods

Key Design Parameters		
Two Independent Cultures	Turbidity Measurement	OT2 Compatible Reservoir
Microcontroller	Culture Mixing	Self Cleaning
Bidirectional Flow	5 & 12 Volt Power	Minimized Head Volume

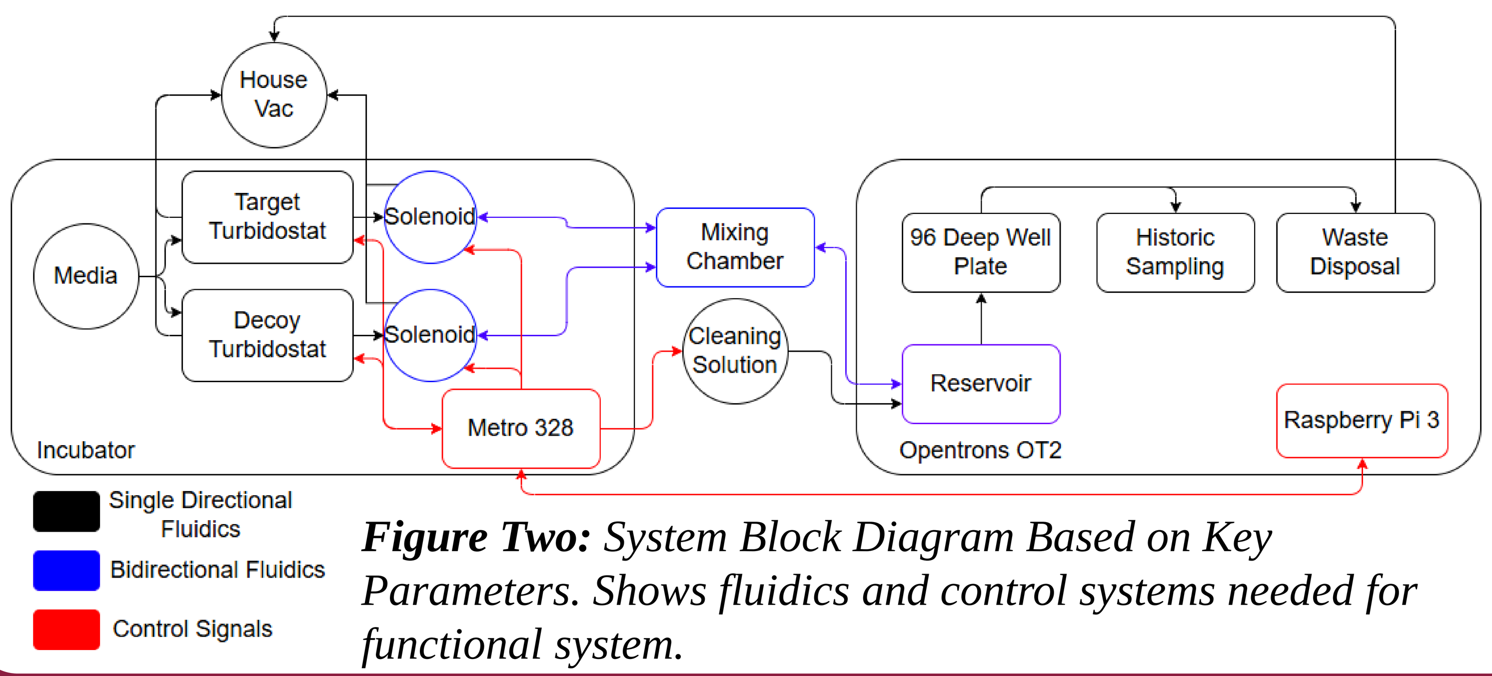

Figure Two: *System Block Diagram Based on Key Parameters. Shows fluidics and control systems needed for functional system.*

Design

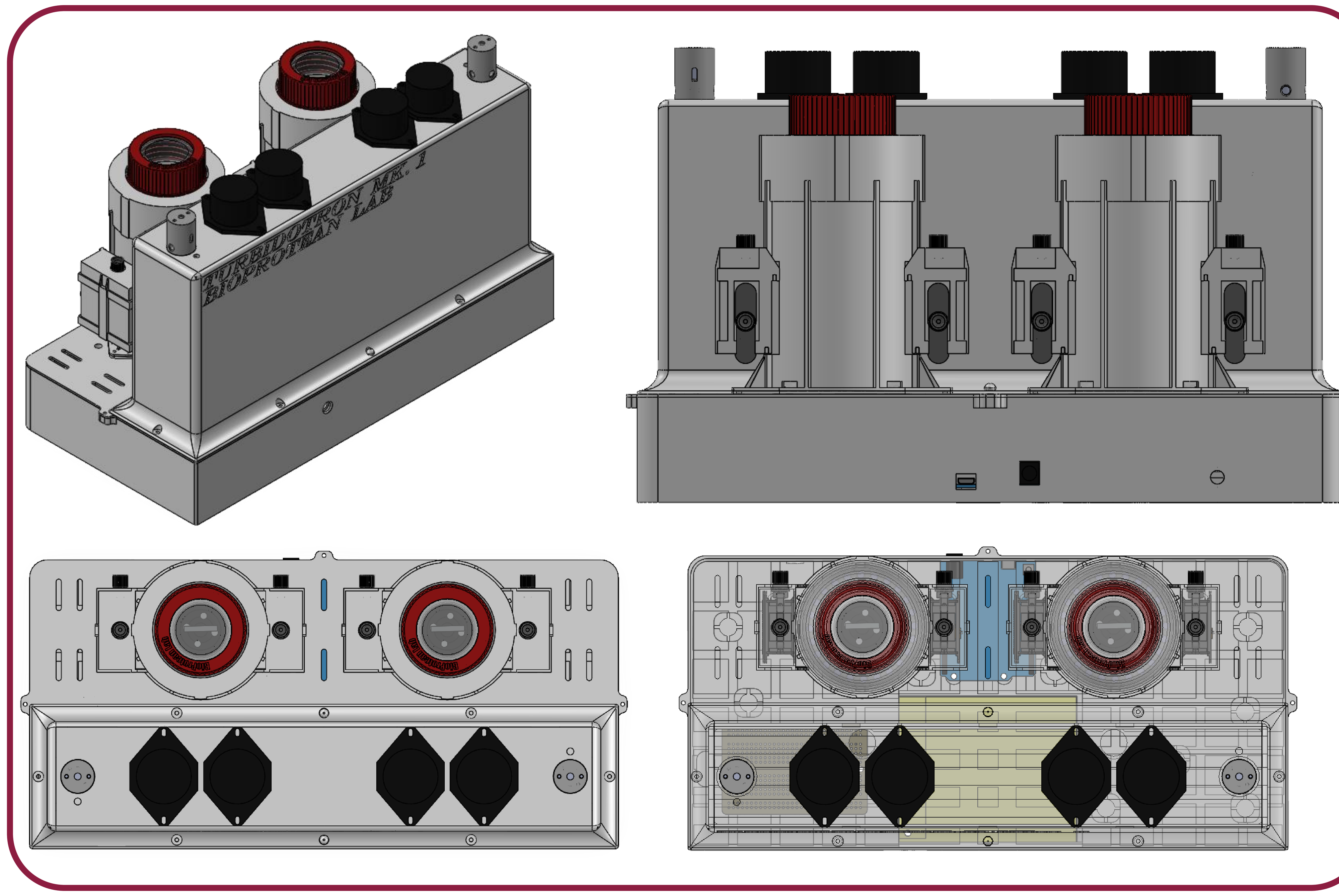

Figure Three: *Turbidostat Assembly. Isometric view (top left), rear view (top right), top view (bottom left), and transparent top view (bottom right). Designed to house all control systems and power supply.*

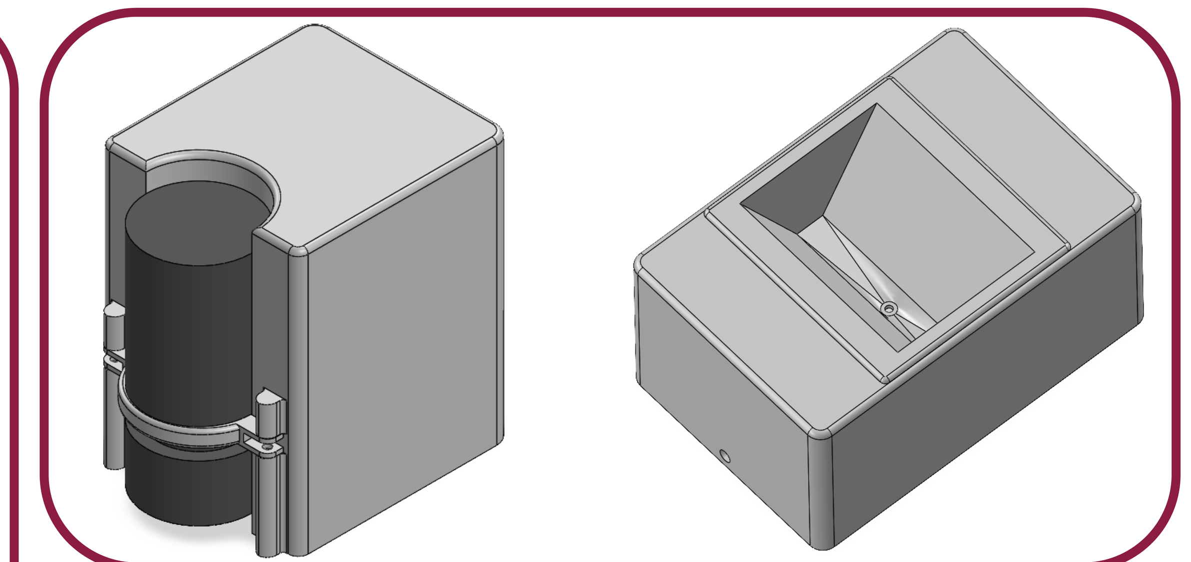

Figure Four: *Mixing Chamber Assembly (left) and Reservoir Assembly (right).*

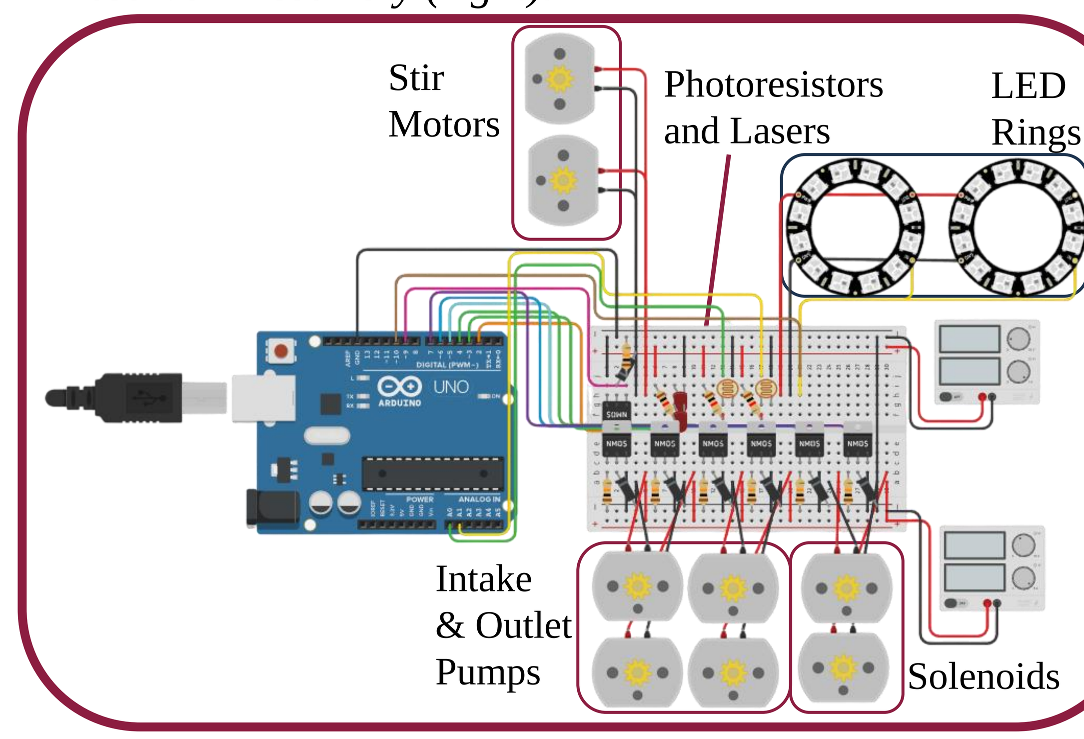

Figure Five: *Control System Schematic. Motors and solenoids actuated by MOSFETs, photoresistors in voltage divider.*

Conclusion and Future Work

Conclusion:

In this project I developed a turbidostat system to integrate with an Opentrons OT2. The system include a two culture turbidostat, in-line mixing chamber, and OT2 compatible reservoir. The turbidostat is designed to maintain controlled growth conditions in both cultures and output a specified quantity of bacteria to the reservoir upon OT2 request. This system provides a framework for accelerating nanobody engineering through SurPhACE automation.

Future Work:

The future work is focused on verifying the full functionality of the turbidostat system. Turbidity calibration must be performed to generate a growth curve and determine target turbidity. Flow rate calibrations must be performed on the pumps, ensuring an accurate volume of bacteria is delivered to the reservoir. A mixing test must be performed to ensure mixing chamber functionality and target ratios of bacteria are achieved. Finally, the Opentrons control signal output must be achieved. Signal output requires SSH login to the Opentrons Raspberry Pi, which has posed a significant challenge to date.

Acknowledgements

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Supplementary

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