WHITE PAPER

Key considerations for automating Octet assays: ligand screening at Avitide

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Introduction

Drug development and production is challenging. Avitide, based in Lebanon, New Hampshire, provides on-demand development and supply of high-performance affinity purification resins for the manufacture of biotherapeutic drug molecules. Molecule-specific affinity purification resins simplify complex downstream manufacturing processes that can require years of development and multiple steps that often sacrifice yield for purity. Using a proprietary discovery and development platform technology, Avitide enables its partners to rapidly advance candidate molecules to the clinic and achieve faster and more predictable bioprocess development timelines by providing one-step affinity purification solutions for a range of biomolecules including enzymes, novel scaffolds, multi-specific antibodies, gene therapies and vaccines—in just three months. The speed at which Avitide serves their growing client base is a key strategic advantage for their partners but presents a unique challenge in the R&D process.

Avitide screening process

Development at Avitide begins by discovering thousands of ligands that selectively bind the drug molecule of interest, then screening the ligands to identify higher affinity binders. Throughput improvements led to production of more than 5000 ligands each week, and Avitide turned to the Octet® HTX system as a tool to maintain their aggressive project turnaround times. The Octet platform relies on Bio-Layer Interferometry (BLI), a label-free optical technique for measuring the binding interactions of biomolecules in real time. The Octet HTX system can measure up to 96 samples simultaneously, scaling to meet throughput requirements. In addition to a simple yes or no binding screen, BLI is used to characterize binding affinities via on-rates, off-rates and K_D values. Disposable biosensors with a variety of surface chemistries such as Protein A/G/L, Anti-Human Fc, Streptavidin, and Nickel-NTA simplify assay development by allowing easy coupling to antibodies and affinity tags.

Avitide used these off the shelf biosensors to transfer ligand screening to the Octet HTX system. The 384-well low volume microplate format processed by the Octet system lent itself to high throughput screening. To achieve maximum capacity, the Octet HTX system needed to be continually resupplied with microplates and biosensors between screening runs for around the clock operation.

Pairing the Octet HTX system with a Twister II™ robotic arm (PerkinElmer) and Overlord™ integration software (PAA Automation) allows users to set up several screenings to be executed in series without an operator (Figure 1). To perform an automated series of screening runs, microplates are prepared offline with a liquid handling system and loaded onto the plate stacks, sensors are placed on tray racks as needed, and the user prepares screening methods as necessary. These runs can occur overnight or over the course of the working day.

By using this automated strategy for ligand screening, Avitide has grown from supporting abbreviated affinity ligand discovery programs every two or three months to far more comprehensive and exhaustive programs in less than a month.

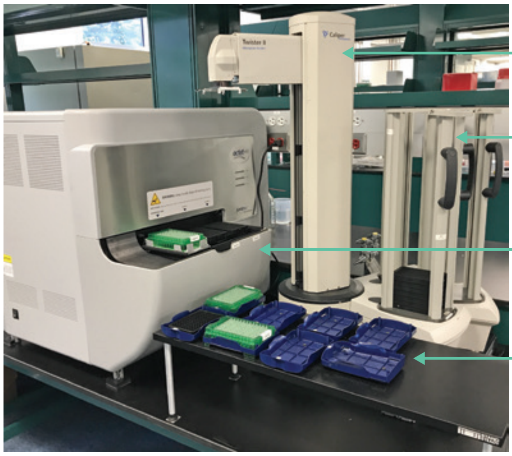

Figure 1: The Octet HTX instrument sits alongside a small table with attached sensor tray racks and a robotic arm that interfaces with the instrument, the sensor tray racks and the plate stacks.

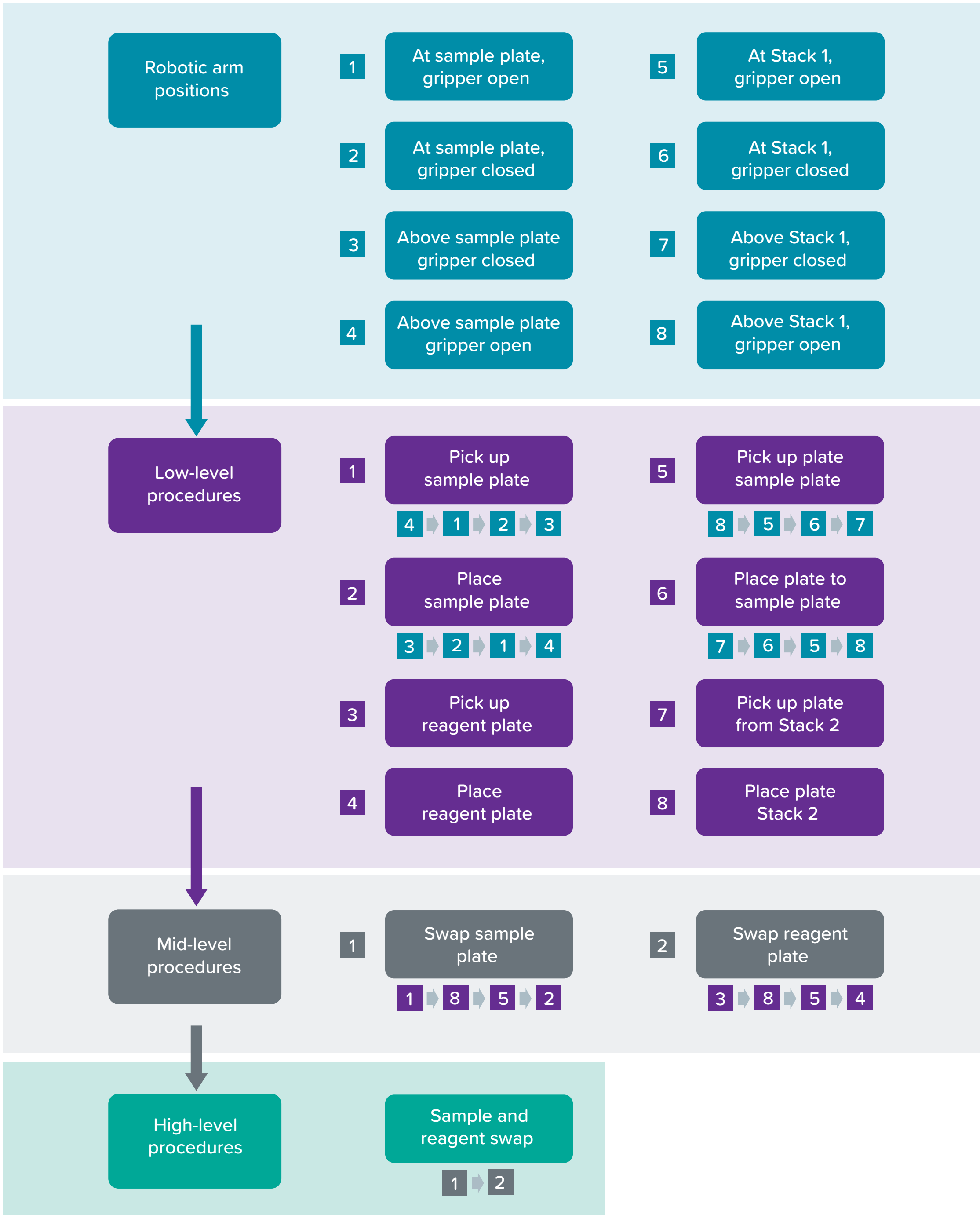

Figure 2: Complex movements are constructed by layering modular procedures to make troubleshooting and improvements easier. The numbered boxes below each procedure denote how positions or movements from the grouping above are used in that procedure. Robots are taught individual positions by hand (blue). Simple movements (orange) are implemented by moving the arm from position to position. Simple movements are performed one after the next to accomplish more complex tasks (green), which are performed one after the next in the highest level procedures.

** Stack 1 is a plate source stack and Stack 2 is a discard stack in this example. The high-level procedure "Sample and Reagent Swap" removes plates from the Sample and Reagent positions on the instrument, places the plates in Stack 2, and replaces the Sample and Reagent positions with plates from Stack 1.*

Some practical tips from Avitide's experience integrating the Octet system into an automated workflow are as follows:

GENERAL AUTOMATION GUIDELINES

1. Is it worth it to automate? Systems engineers should carefully consider the expected longevity of the workflow in question, the reason for the task's automation, financial and time investment, and available resources. For example, is the task being automated subject to error, is it because it is monotonous or because it is complex? For the latter, simplifying the workflow may provide an easier solution. In addition, finances can be recouped, but time spent automating a process cannot be regained.

2. Hardware automation solutions purchased off the shelf and, if necessary modified to suit specific workflow needs often will provide a more cost-effective solution. If this is to be done by an equipment or independent contractor, it is critical to clearly establish the development timeline, implementation strategy, and performance specifications in any work agreement or contract. It is very difficult to achieve more than two unique applications for an individual hardware implementation instance without a very cleverly thought out design.

3. Open source software automation solutions can be better than vendor-based solutions, depending on your needs. Open source software with a sufficient user base is essentially field-tested by a wide audience. Open source options also do not rely on a single vendor for troubleshooting and design implementations. However, vendor-based solutions can provide specialized support, supplementing hardware, software, and assay knowledge that may not be readily available.

4. Divide the entirety of your automation workflow into small units, or islands, that are separate and modular so you can troubleshoot and iterate each part without affecting the others (Figure 2). As such, design and implement your automation workflow in the smallest chunks possible. Ensure each piece functions properly, then string the pieces together to achieve a fully functioning solution.

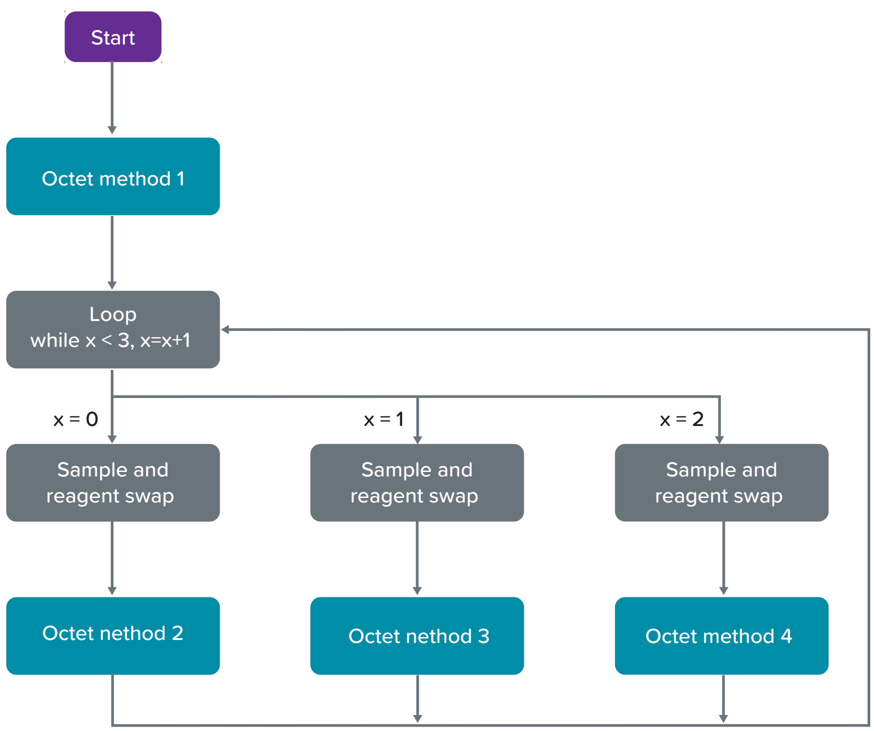

Figure 3: Full integration of the Octet HTX instrument with the robotic arm allows a user to run four Octet methods with different sample and reagent plates for each assay without operator intervention between runs. Without automation, three instances of operator intervention would be required to switch the sample and reagent plates between methods.

AUTOMATION GUIDELINES SPECIFIC TO OCTET SYSTEMS

1. If you are planning on integrating a robotic arm, the position of each piece of hardware must be fixed and reproducible. The plate handler and Octet HTX system should be well anchored to a sturdy table to ensure consistent and proper transfer of microplates and biosensor trays. Any change in position can offset the taught positions of the robotic arm. By far, the least tolerant procedure is placing sensor trays.

2. The islands of automation strategy to unitize an entire workflow into individual automated tasks can be used in managing plate and sensor tray placement on the Octet system. Avitide uses a set of absolute positions, low level procedures, and higher-level procedures to control the robotic arm and allow the user to easily run a method to remove a sample plate from the Octet system and place another plate from a plate stack into the sample plate position. The servomotor axis positions, taken together, define the position of the robotic arm in space. Procedures that move the robotic arm between absolute locations can be used to move sensor trays or plates. For example, the robotic arm can move a plate from the Sample Plate position on instrument to Stack 2 and then a plate from Stack 1 to the Sample Plate position. These procedures together define a "sample plate swap" (Figure 2). Avitide uses procedures that move the robotic arm between absolute locations to move a plate or sensor tray. Several of these low-level procedures are combined into meta-procedures that might swap reagent plates, sample plates, or perform a more complicated task. Depending on what is necessary, individual positions of the robotic arm, low-level procedures, or the meta-level procedures can be troubleshot or improved.

3. Automation-friendly APIs are documented in the ForteBio Octet Data Acquisition Software User Guide.

CONCLUSION

High throughput characterization systems like the Octet HTX system can greatly accelerate discovery programs, as evidenced by the successes at Avitide in their ligand discovery and characterization workflow. However, the speed of any assay system is limited by its inputs - namely the replenishment of samples and biosensors in the case of the Octet system. By automating the repetitive tasks in the workflow, users can achieve continuous operation of the Octet system without increasing operator time. Following some of the guidelines presented above, novices to automation can avoid some of the pitfalls in over-engineering solutions rather than successfully realizing incremental improvements.

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