



# Open Port Sample Injector with Electromagnetic Mixing for an Affinity Selection MS System -- WP 147



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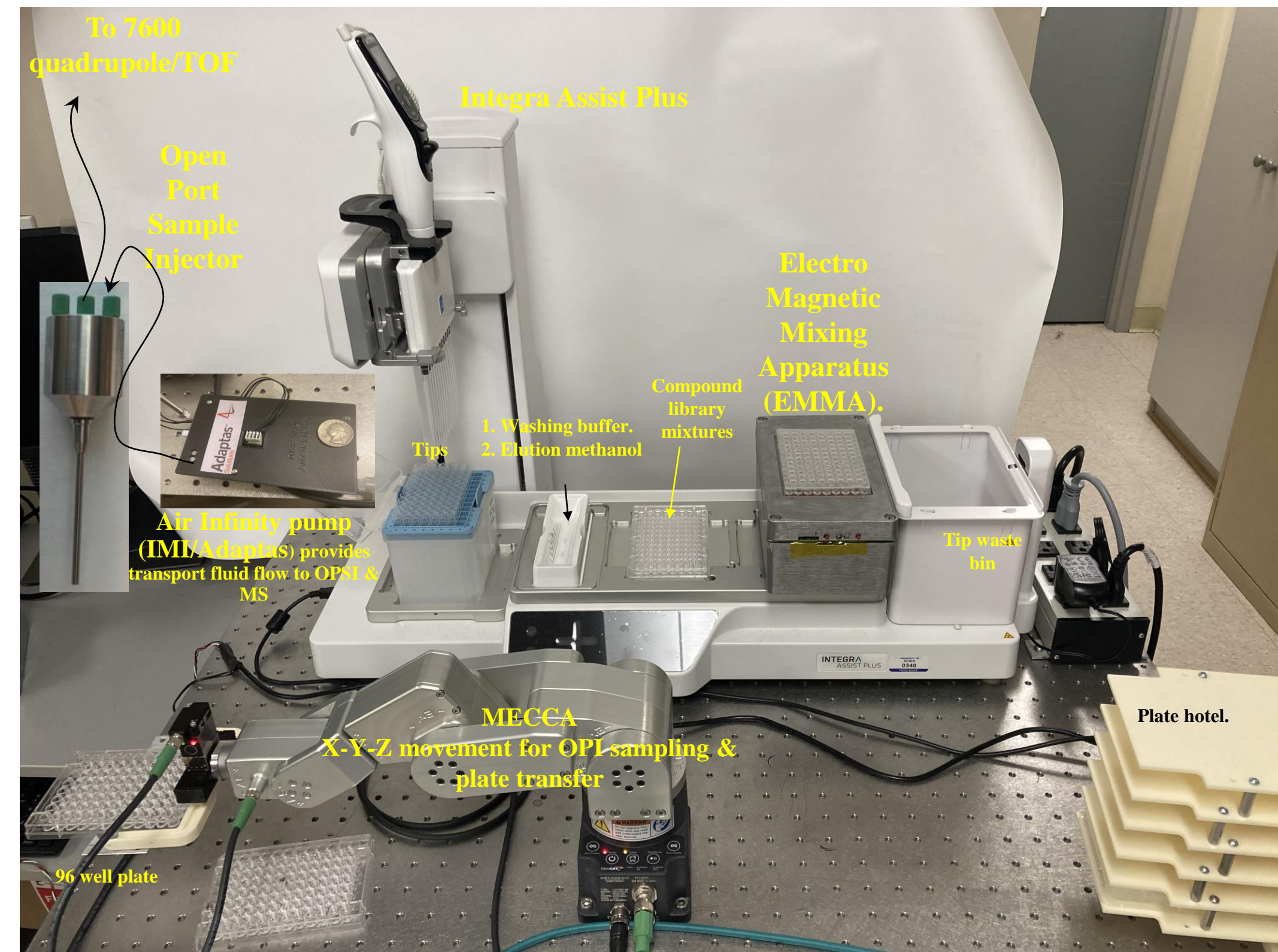
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## ABSTRACT

At the 2022 Minneapolis ASMS meeting we introduced the concept of an Affinity Selection Mass Spectrometry (AS-MS) system that implements high frequency rotating electromagnetic fields to disperse ferrimagnetic beads used to immobilize the protein targets. The use of paramagnetic beads to immobilize targets was first demonstrated by Van Breemen<sup>1</sup>. The use of ferrimagnetic beads and rotating magnetic fields offers a reaction speed advantage over paramagnetic beads. This poster reports on the progress made on two critical components of the system over this past year. (1) The first linking of a protein target to a ferrimagnetic bead and demonstration of its activity by successfully screening a compound library for known high affinity compounds. (2) development of a high-speed sample injection system that supersedes the use of a conventional HPLC autosampler for flow injection analysis. A new mode of operating the Open Port Sample Injector (OPSI) has been implemented to provide a 1 Hz sampling frequency for 2  $\mu$ L volume sample injections with high precision for quantitative applications. The goal is to sustain a throughput of 100,000 compounds screened for their affinity to specific targets per day using 100 test compounds per well in 96 well plates and analyzing each well in one minute. Adding more or fewer compounds per reaction well will increase or decrease the throughput proportionally.

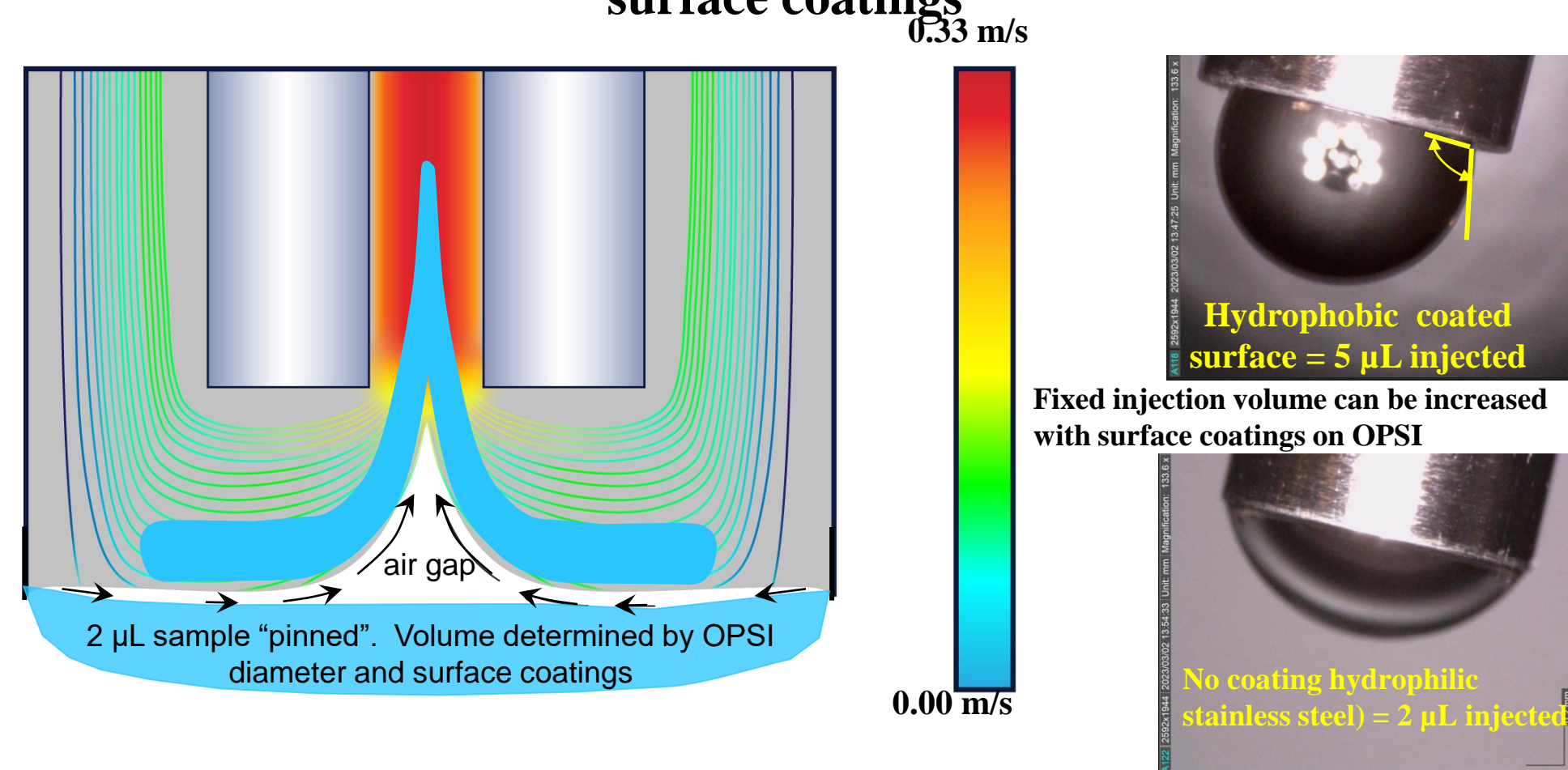
## BREADBOARD COMPONENTS

The sub-components being integrated to execute the ASMS workflow are shown below. Details regarding the operation and performance of the Open Port Sample Injector (OPSI) and the immobilization of protein targets on the ferrimagnetic beads for affinity screening using the electromagnetic mixer for bead mixing are provided. Other components of the system are commercially available and are being integrated using the IMI Adaptas All Motion software. These are the Integra Multichannel pipettor, the Mecca 6-axis laboratory robot, and the Air Infinity microprocessor driven air pressure pump to deliver the transport fluid to the OPSI sampler.

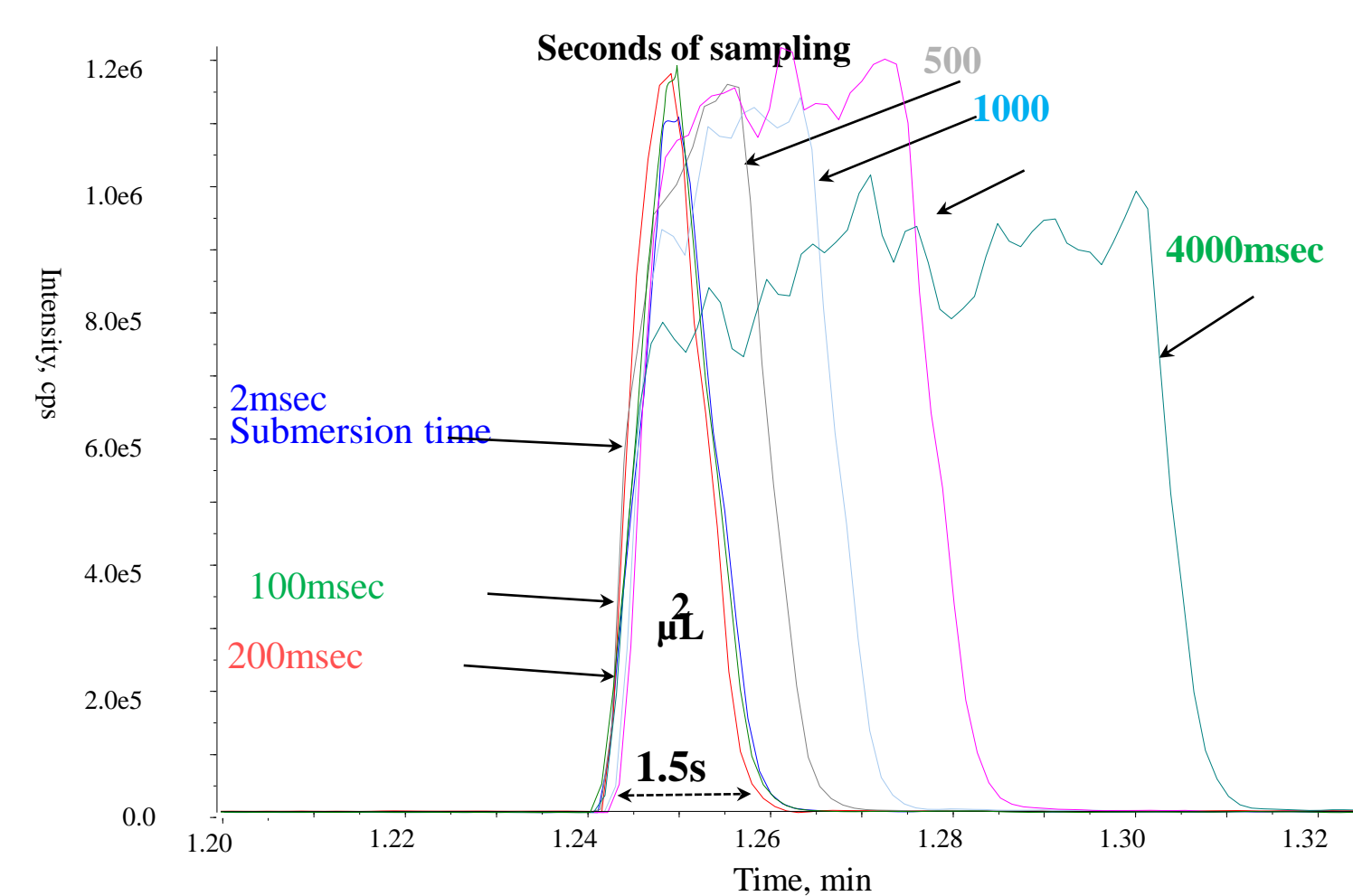
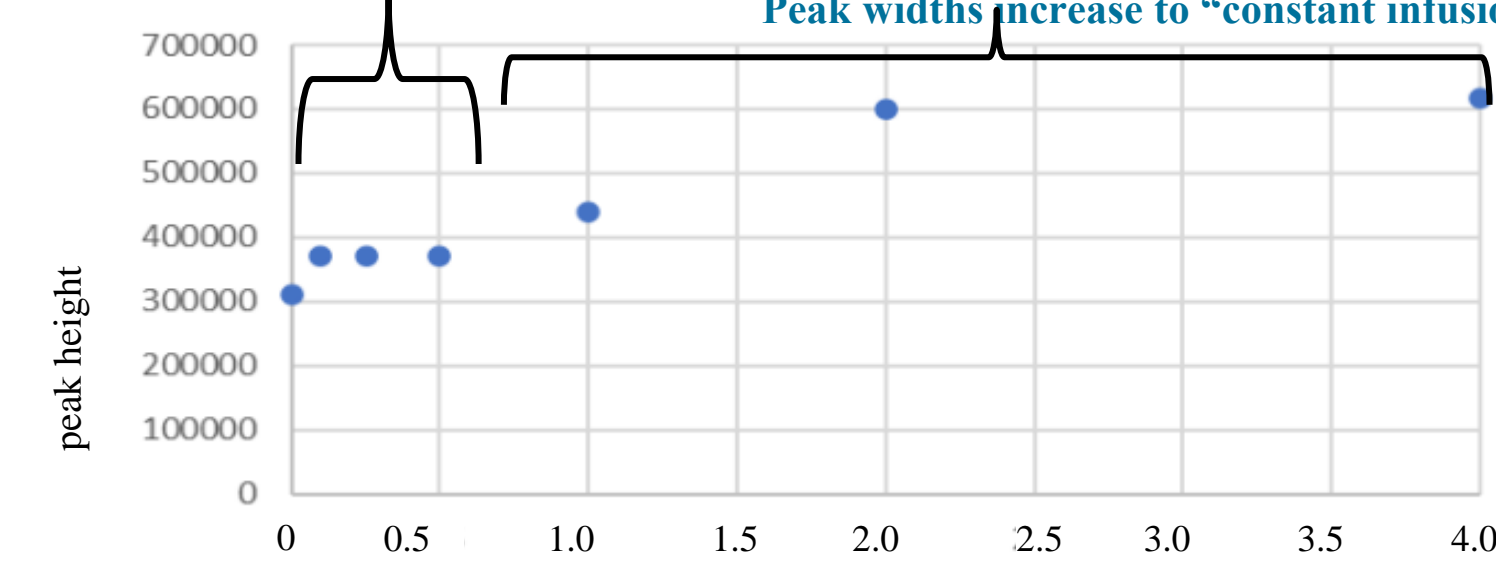


## “Open Port Sample Injector”™ (OPSI)

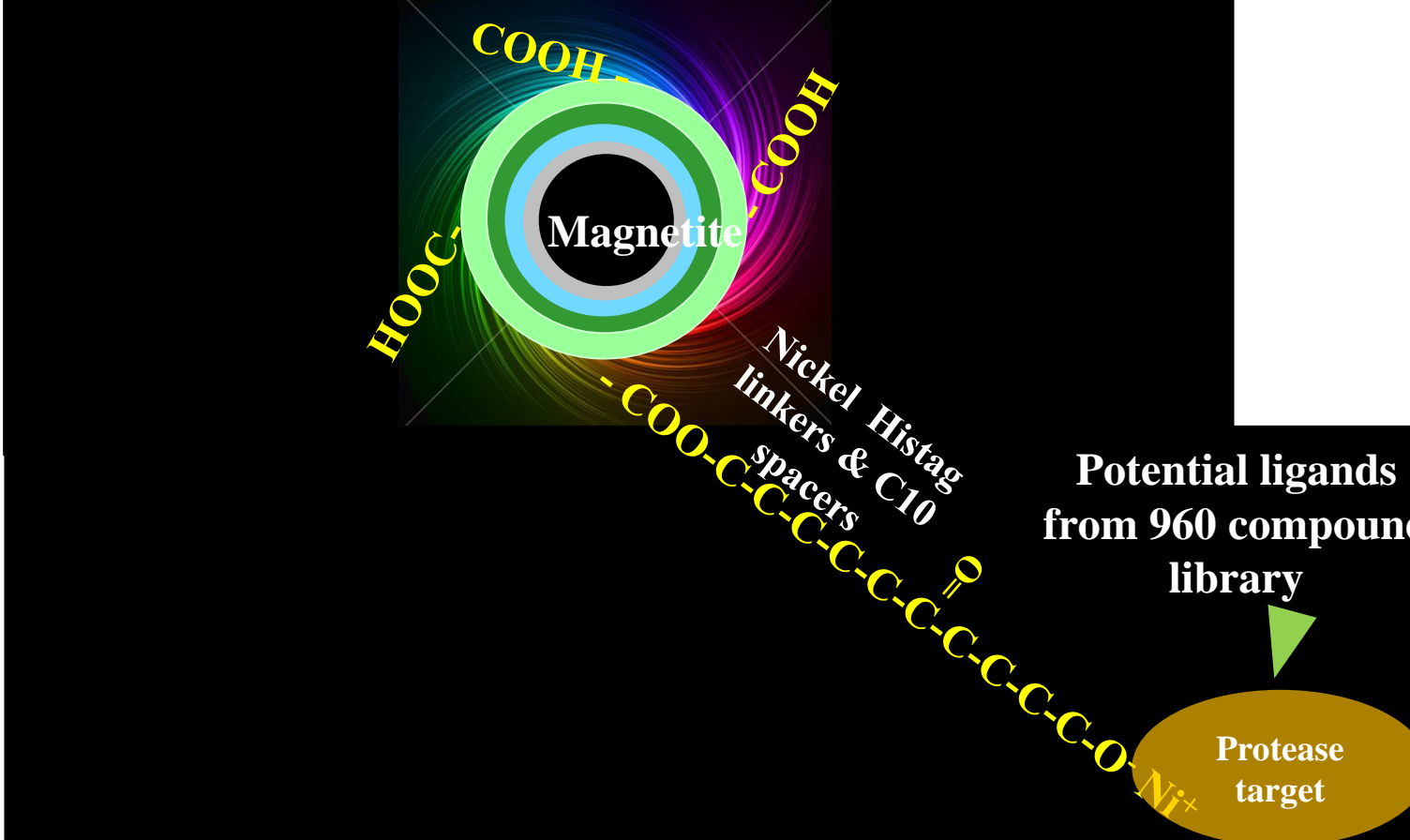
Reproducible  $\mu$ L volume injections controlled by OPSI dip time and surface coatings



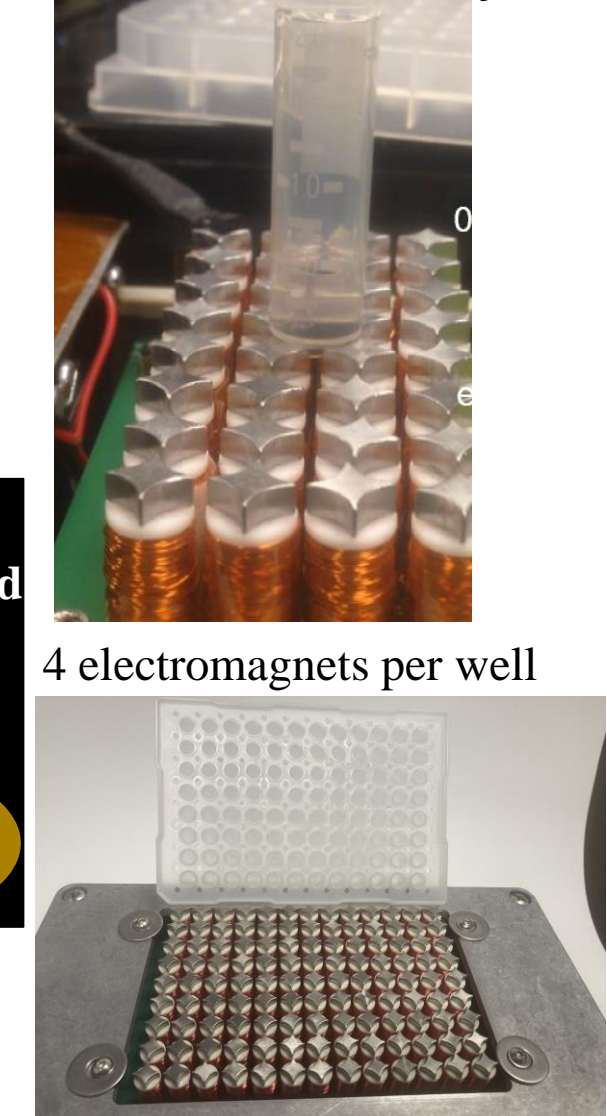
50-300 msec = Constant volume injection range  
Peak widths 1-2 sec  
>500 msec = Variable volume injection  
Peak widths increase to “constant infusion mode”



## Linking Protein Targets to Ferrimagnetic Beads



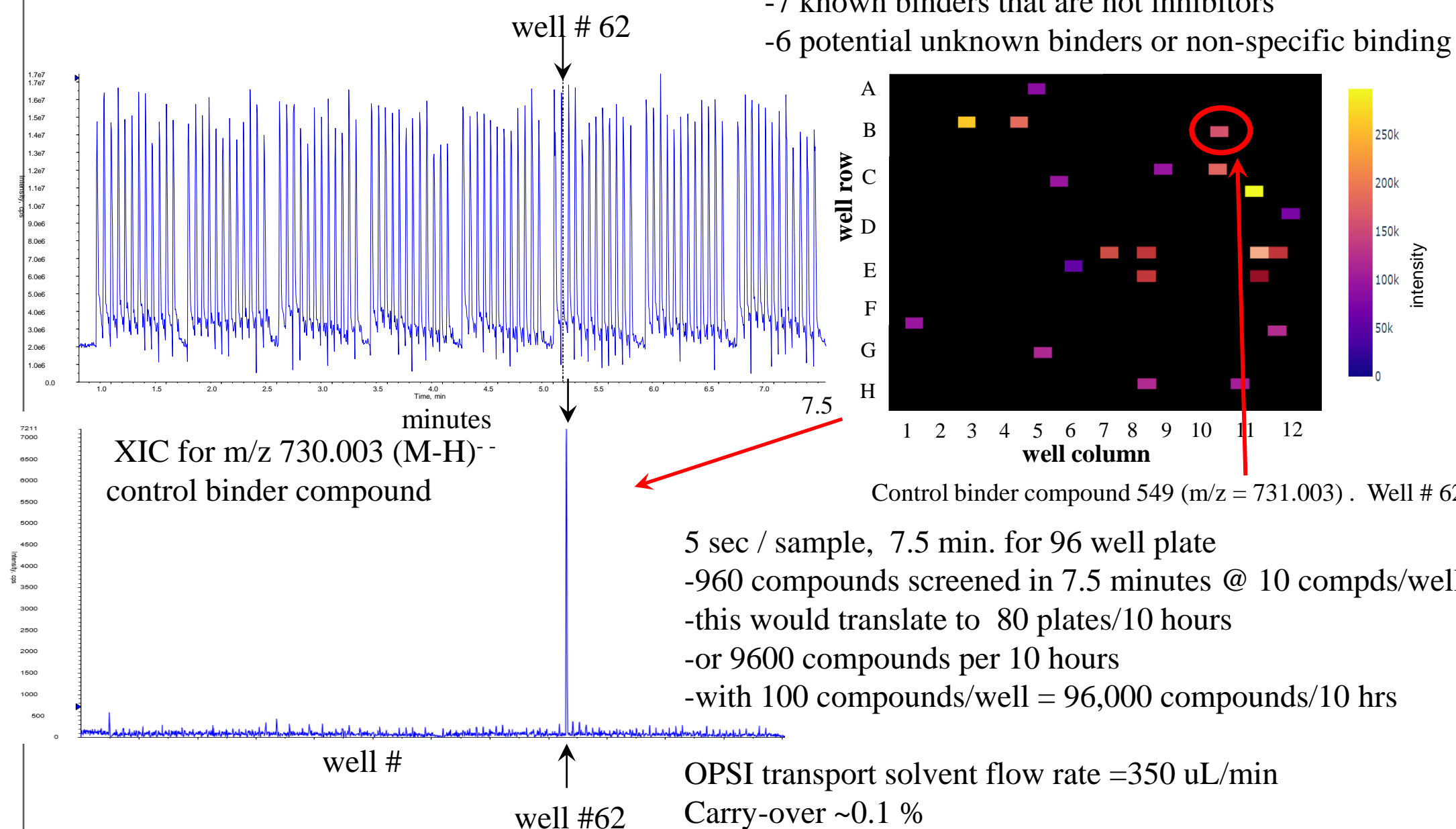
100Hz rotating Electromagnetic fields. Mixing Apparatus (EMMA) processes 96 samples simultaneously



## Target Protein #1 BBhtrA Protease

Total Ion Current Chronogram of 96 well plate  
10 library compounds/well.  
960 compound library screened in 7.5 min.

Heat map showing 20 hits from library  
-7 known inhibitors that bind  
-7 known binders that are not inhibitors  
-6 potential unknown binders or non-specific binding



5 sec / sample, 7.5 min. for 96 well plate  
-960 compounds screened in 7.5 minutes @ 10 compds/well  
-this would translate to 80 plates/10 hours  
-or 9600 compounds per 10 hours  
-with 100 compounds/well = 96,000 compounds/10 hrs

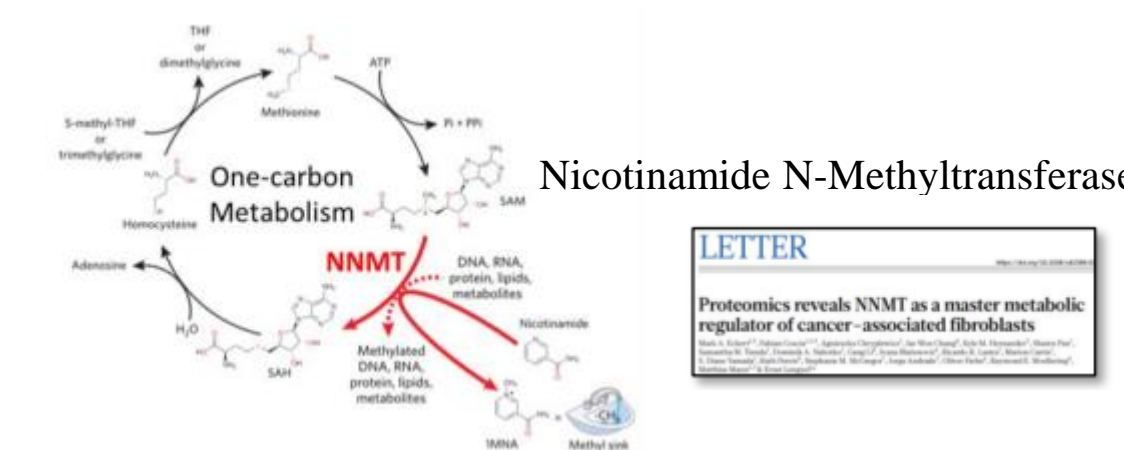
OPSI transport solvent flow rate =350  $\mu$ L/min  
Carry-over ~0.1 %

## Ongoing work on affinity selection step

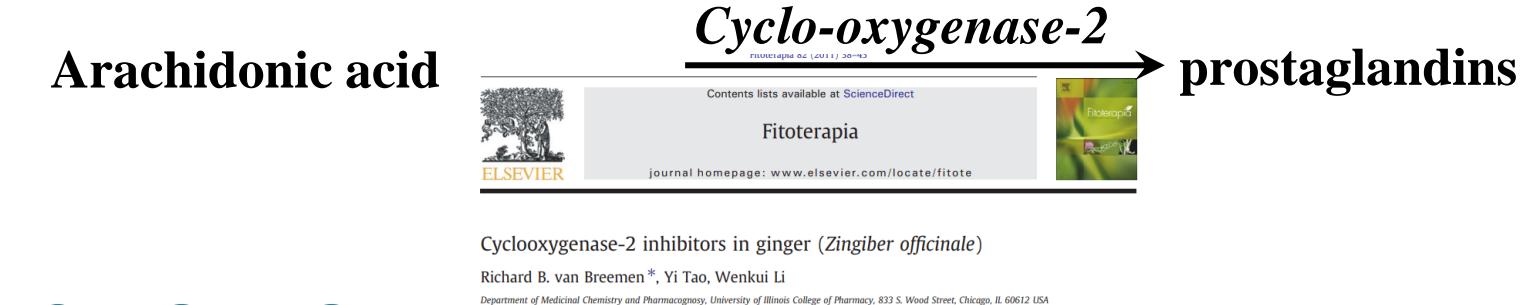
**A. BBhtr Protease Target. Ferrimagnetic bead affinity extraction step. Establish optimum affinity reaction time in a range of 30 seconds to 30 minutes. The increase in reaction speed for tryptic digestion indicates single digit minutes should be achievable with ferrimagnetic beads and rotating magnetic fields.**

\*Ronzetti MH, Baljinnayam B, Itkin Z, Jain S, Rai G, Zakharov AV, Pal U, Simeonov A. Application of temperature-responsive HIS-tag fluorophores to differential scanning fluorimetry screening of small molecule libraries. *Frontiers in Pharmacology*. 2022;13. doi: 10.3389/fphar.2022.1040039.

**B. Future target to be tested**  
**NNMT**



**C. Future target to be tested**  
**Cyclo-oxygenase-2**



Cyclooxygenase-2 inhibitors in ginger (Zingiber officinale)  
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## RESULTS & CONCLUSIONS

- A means of operating the Open Port Sampling Injector to inject low  $\mu$ L volume samples with high precision at 2-5 Hz sampling speed has been developed. < 10 min/96 well plate. Carry-over is < 0.1%.
- Application of rotating magnetic fields of 100 Hz to 100nm ferrimagnetic beads enhances the speed of the enzymatic reactions. Target for affinity reactions < 10 min incubation time per plate. Estimated daily throughput 30,000 – 90,000 compounds screened/day with 10 – 100 compounds screened per well.
- The protease target BBhtrA has successfully registered several known binders against a 960 compound library containing using this affinity selection MS method.
- Additional targets to be tested, affinity reaction speed to be optimized with ferrimagnetic beads in rotating magnetic fields, and system integration to achieve fully automated operation is in progress.

## REFERENCES

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- Integra Assist Plus, Integra Biosciences Corp, Hudson, N.H., [info@Integra-Biosciences.com](mailto:info@Integra-Biosciences.com)

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