

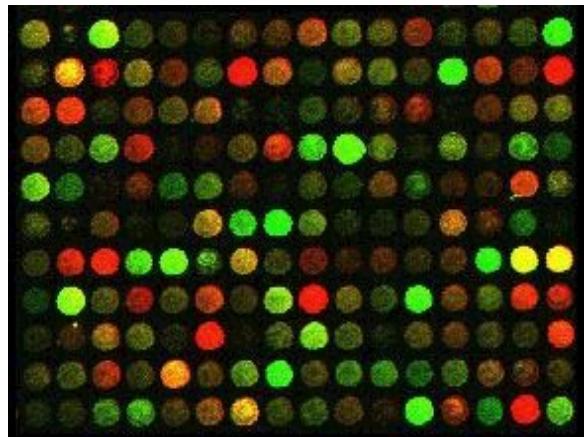
Combining **surface chemistry** with affinity selection mass spectrometry accelerates drug discovery for protein and oligonucleotide targets

Zack Gurard-Levin, PhD
SAMDI Tech Inc.
A Charles River Company

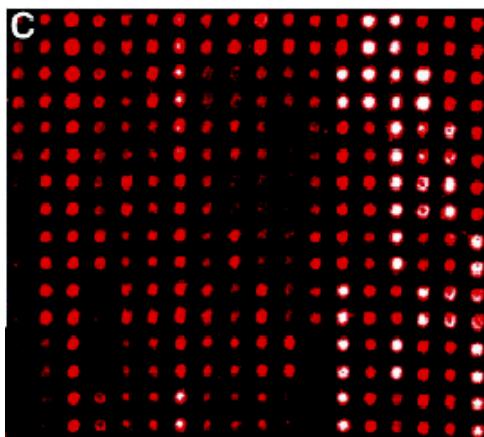
BETTER. FASTER. LABEL-FREE.



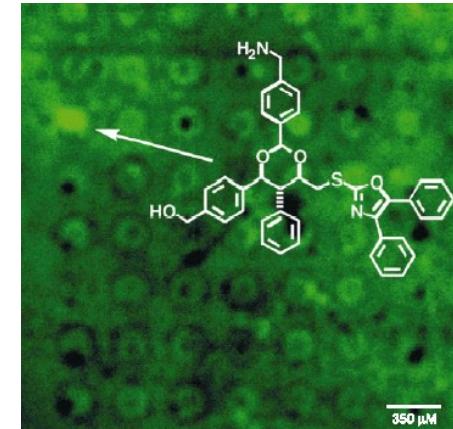
Biochips: Successes and Limitations



DNA Array



Protein Array



Molecule Array

Successes

- Mapping kinase substrates
- Identify lipid binding proteins
- Adaptor protein specificities
- Antibody arrays

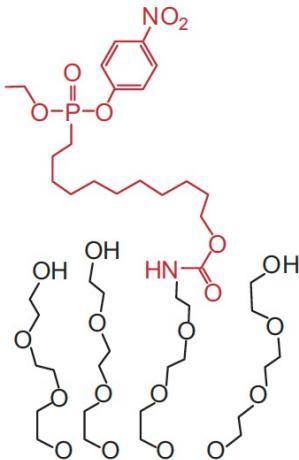
Limitations

- Non-specific interactions
- Inconsistent activity of immobilized protein
- Labels restrict assay types
- Cannot discover unanticipated activities

A surface engineering approach to biochips

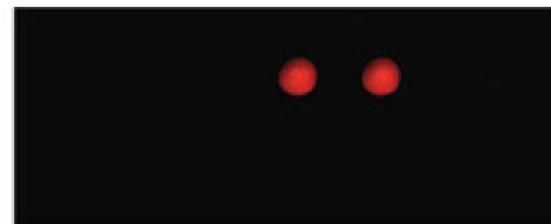
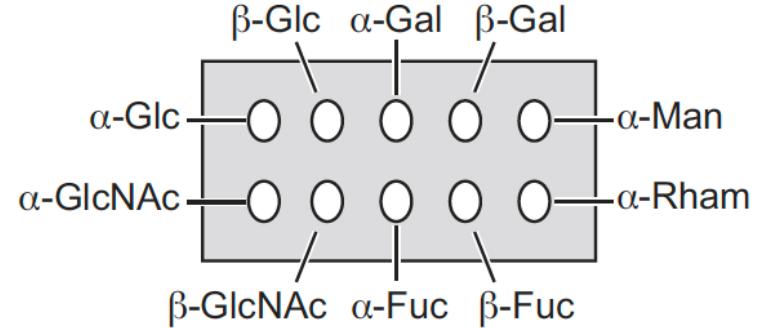
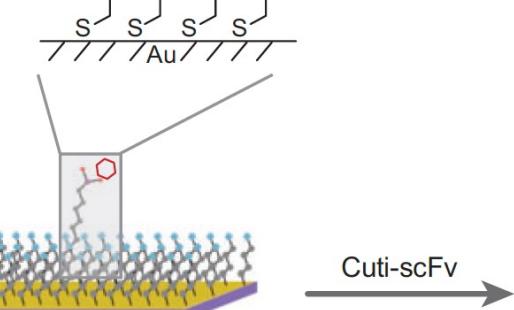
Surface Chemistry

**Binding molecules to surface
Providing an inert environment**



Immobilization Chemistries

**homogeneous environment for ligands
control density of ligands**

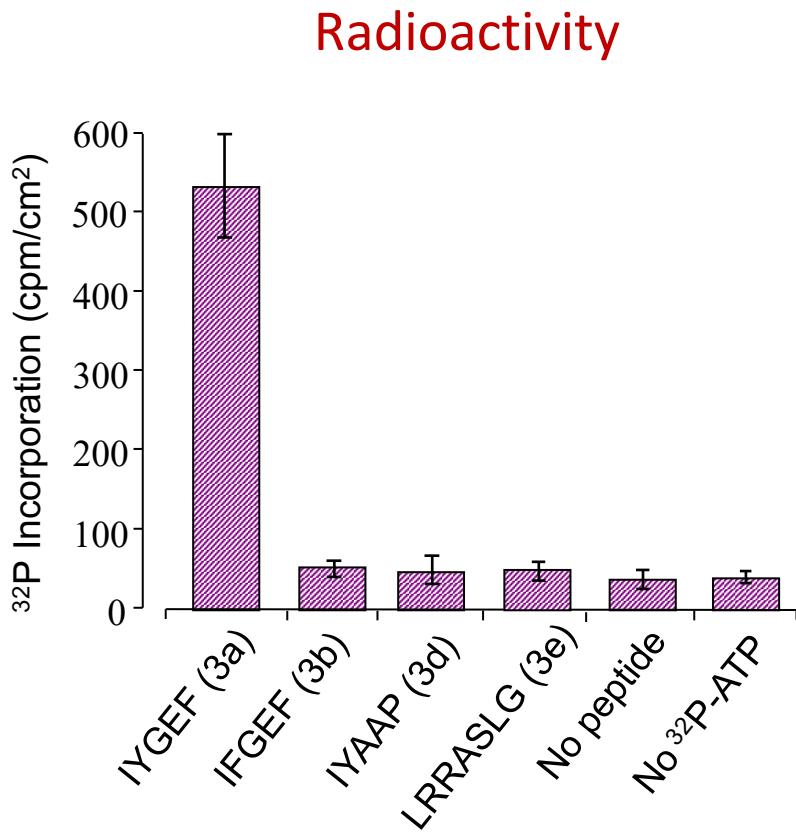


E. cristagalli

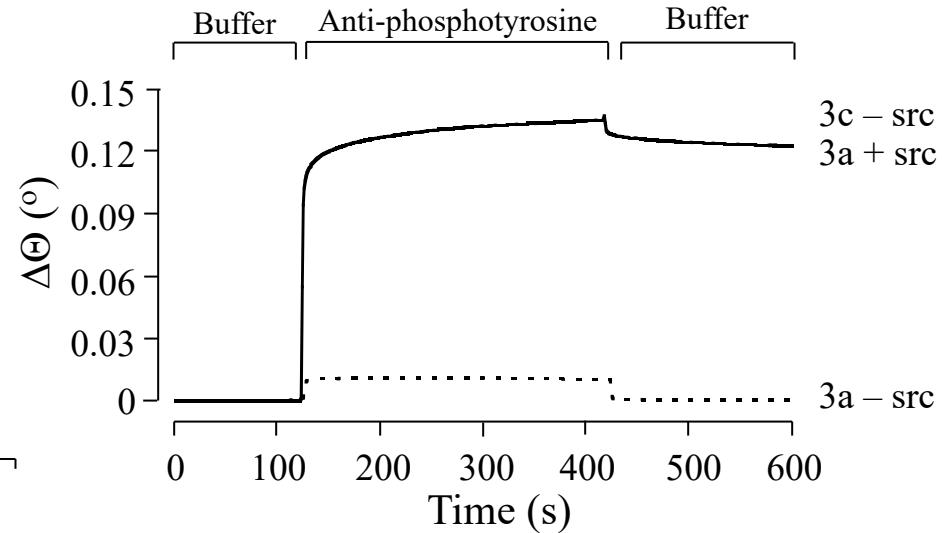
Detection Methods

Consistent activity

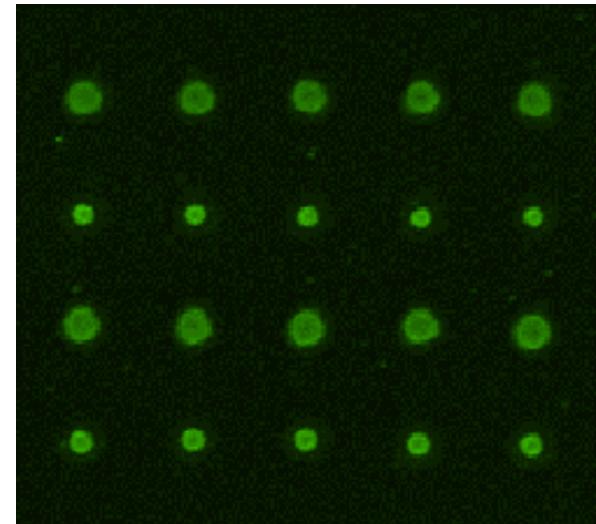
Quantitative detection methods



Surface Plasmon Resonance (SPR)



Immunofluorescence



- Monolayers provide an environment inert to non-specific adsorption
- Enable uniform ligand density and orientation (consistent data)
- Detection methods largely still require labels

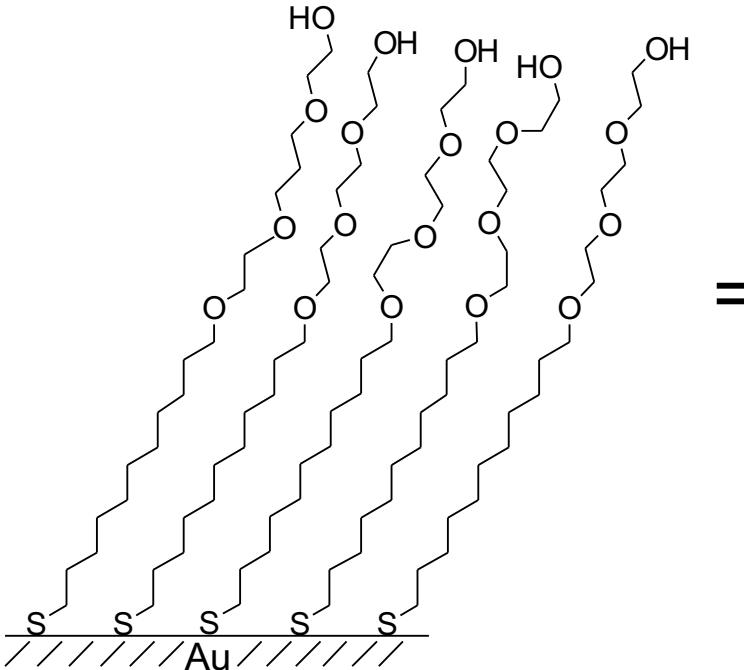
Goat anti-mouse Alexa 488
 Antiphosphotyrosine
 Immobilized phosphotyrosine



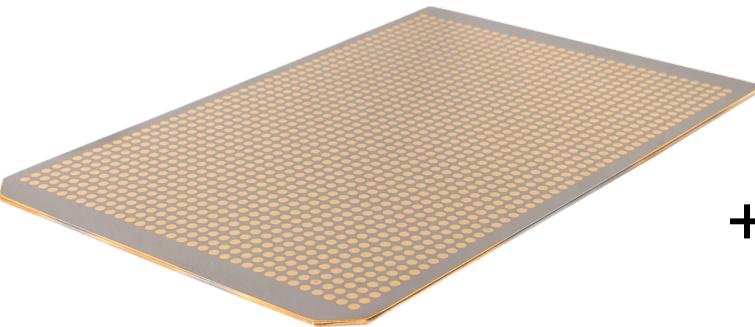
A biochip breakthrough!



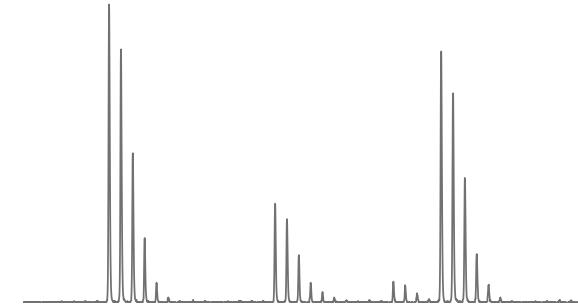
Self-Assembled Monolayer Desorption Ionization



=



+



**High Density Biochip Arrays
With Engineered Surfaces**

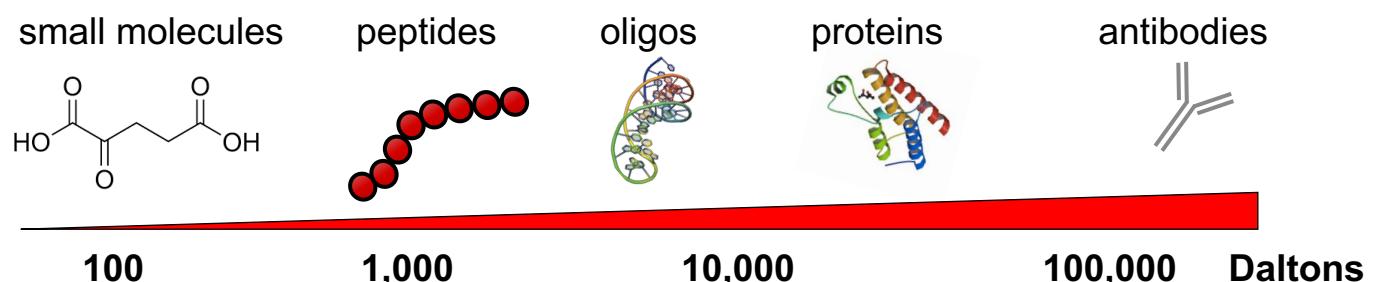
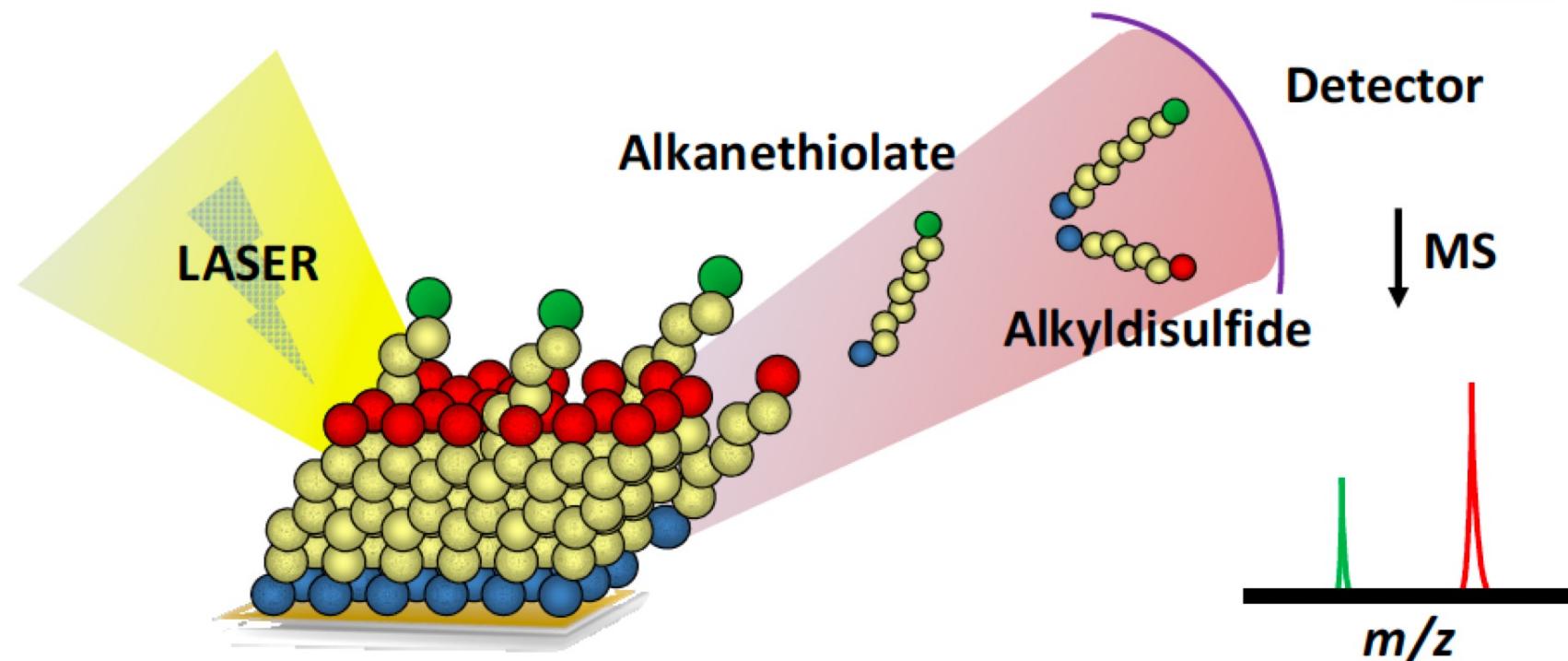
**MALDI TOF
Mass Spectrometry**

Hanley...*Langmuir*, **1998**, 14, 1664; Wilkins...*JACS*, **2001**, 123, 769

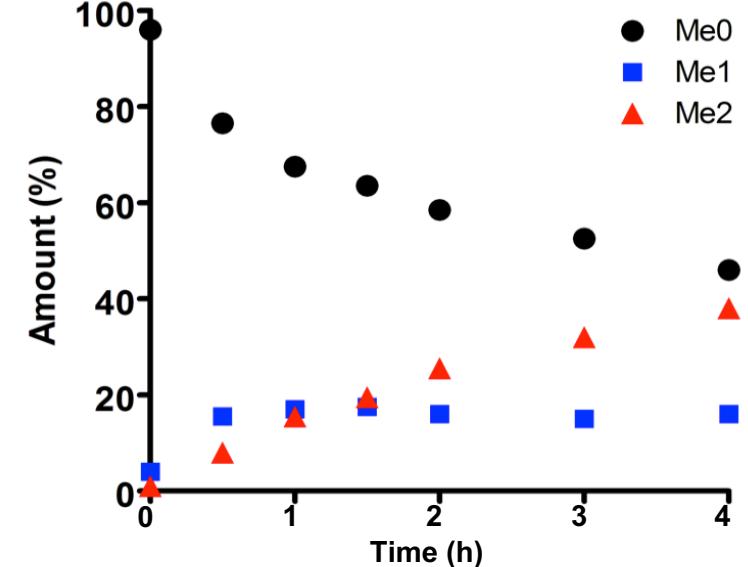
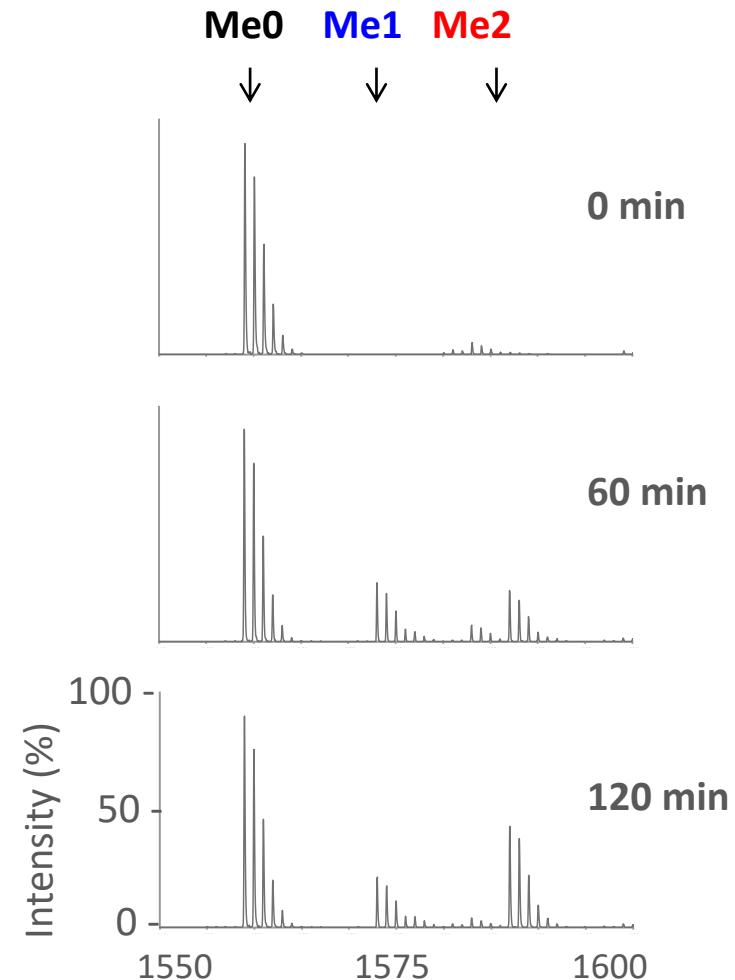
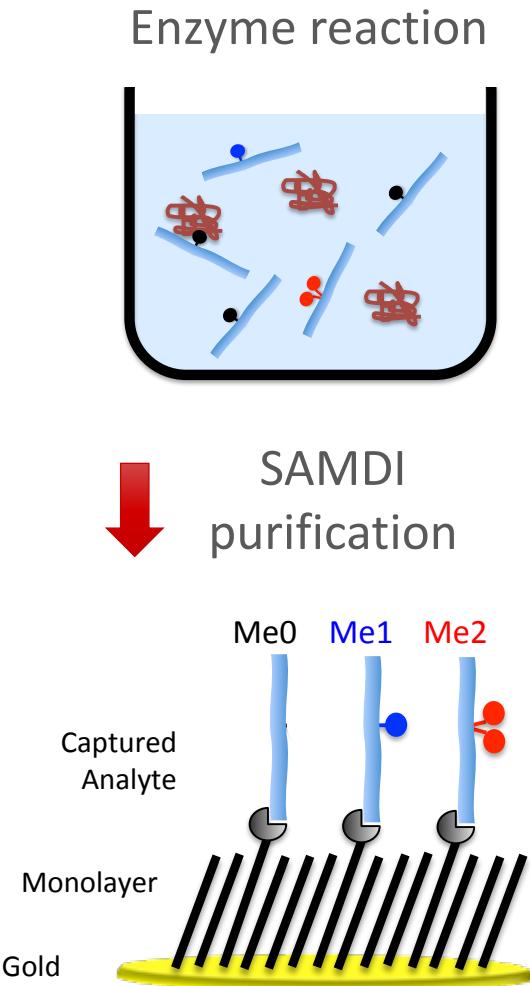
Houseman & Mrksich, **2002**, *Nat Biotechnol*, 20, 270-274. Gurard-Levin et al., **2011**, *ACS Comb Chem*

For reviews: Gurard-Levin & Mrksich, **2008**, *Annu Rev Biochem* 1, 767-800; Mrksich, **2008**, *ACS Nano*, 2, 7-18,

SAMs for MALDI TOF MS Analysis: SAMDI



How to run a SAMDI assay?



SAMDI-MS driving discovery for > 20 years



Biochem. J. (2013) 453, 241–247 (Printed in Great Britain) doi:10.1042/BJ20130439

Convergent evolution of chromatin enzymes: comparative enzymology of human polycomb repressive complex 2

Brooke Robert Epizyme Inc.

Dual inhibition of histone deacetylase inhibitors in clinical development

BIO including

Char

Complex with ATF7IP

Aravind Basavapathruni,[†] Jodi Gureasko,^{†,‡} Margaret Porter Scott,^{†,‡} William Hermans,[‡] Adarsh Godbole,[§] Peter A. Leland,[§] P. Ann Boriack-Sjodin,[†] Tim J. Wigle,^{†,¶} Robert A. Copeland,[†] and Thomas V. Riera,^{*,†}

A High-Throughput Assay Coupled with SAMDI Reduces Artifact Lysine Demethylation

A Mass Spectrometric Assay of METTL14 Methyltransferase Activity

Shane M. Baker¹, Zachary A. Gurard-Levin², Benjamin D. Wheeler^{3,4}, Michael D. Scholle², April W. Case¹, Jeffrey L. Hirsch³, Scott Ribich¹, Robert A. Copeland¹, and P. Ann Boriack-Sjodin¹

Tim J. Wigle¹, Kerren K. Swinger¹, John E. Campbell¹, Michael D. Scholle², John Sherrill², Elizabeth A. Admirand¹, P. Ann Boriack-Sjodin¹, Kevin W. Kuntz¹, Richard Chesworth¹, Mikel P. Moyer¹, Margaret Porter Scott¹, and Robert A. Copeland¹

Development of a Novel Label-Free and High-Throughput Arginase-I Assay Using Self-Assembled Monolayer Desorption Ionization Mass Spectrometry

Cheng Liu^{a,1}, Sandro Boland^{b,1}, Michael D. Scholle^{c,1}, Dorothee Bardiot^b, Arnaud Marchand^b, Patrick Chaltin^{b,e}, Lawrence M. Blatt^a, Leonid Beigelman^a, Pierre Raboisson^d, Zachary A. Gurard-Levin^c, Koen Vandyc

Original Research

SLAS Discovery
1–8
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DOI: 10.1177/24725552211000677
journals.sagepub.com/home/jbx

LETTER
pubs.acs.org/acscbmsci

Small Molecule Libraries using SAMDI

Sam H. Eisenberg, and Milan Mrksich*

Identification of Small-Molecule Noncovalent Binders Utilizing SAMDI Technology

Erica C. VanderPorten¹, Michael D. Scholle², John Sherrill², John C. Tran¹, and Yichin Liu¹

chemical
biology

Reaction Coupling between Wild-Type and Disease-Associated Variant EZH2

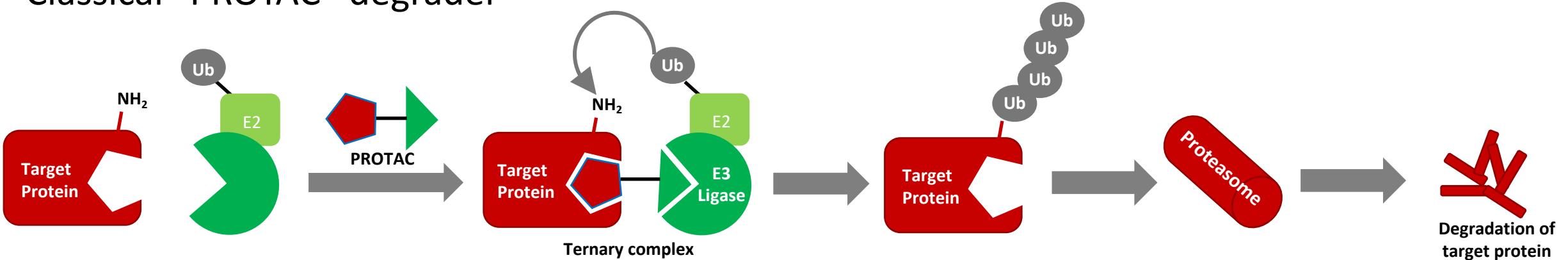
Brooke M. Swalm, Sarah K. Knutson, Natalie M. Warholic, Lei Jin, Kevin W. Kuntz, Heike Keilhack, Jesse J. Smith, Roy M. Pollock, Mikel P. Moyer, Margaret Porter Scott, Robert A. Copeland, and Tim J. Wigle*

SLAS Discovery
1–7
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DOI: 10.1177/2472555217712761
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Letters
pubs.acs.org/acscbmsci

Chemically induced proximity (CIPs)

Classical “PROTAC” degrader



AUTAC
DUBTAC
KineTAC
Molecular Glue
LYTAC
PhosTAC
SERD
PROTAC
PhoRC
AceTAG
RIBOTAC
MeTAC
DeMeTAC
RIPTAC

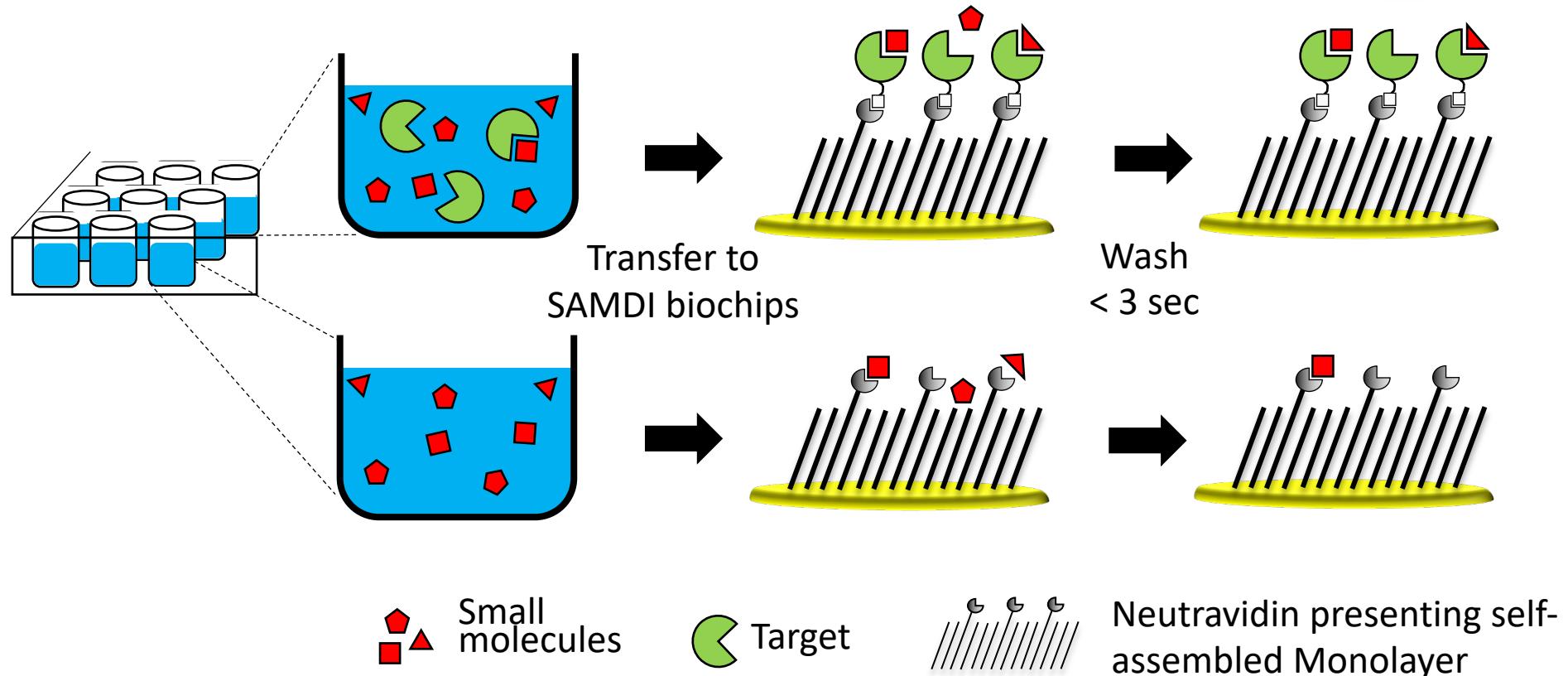
How to identify novel CIPs for diverse targets?

SAMDI ASMS: *Target agnostic approach to drug discovery*

BETTER. FASTER. LABEL-FREE.

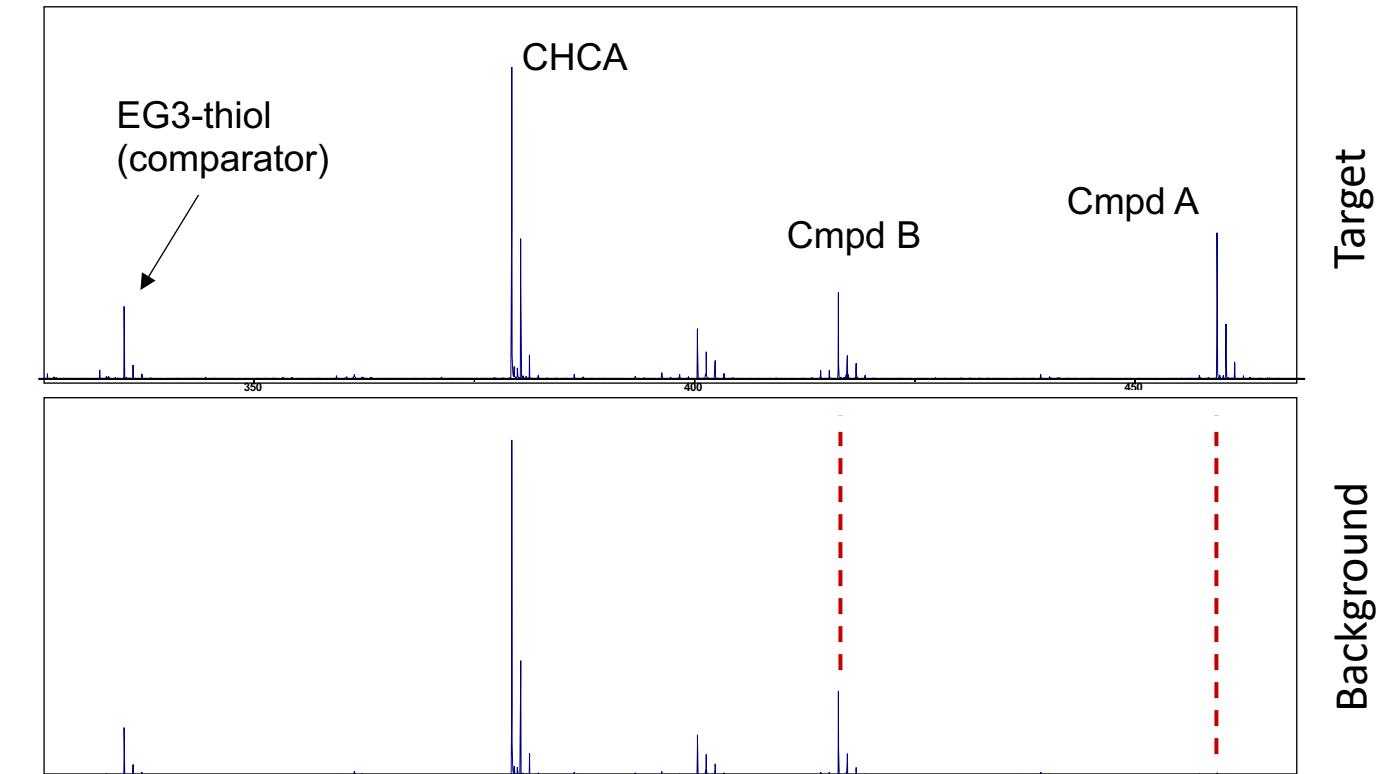


SAMDI ASMS workflow



- Eliminate chromatography steps (faster workflow)
- Minimal compound compression (8 cmpds per reaction)
- Up to 50x less target concentrations (~100-400 nM)

SAMDI ASMS: representative spectra



- **Relative Signal Value (RSV):**

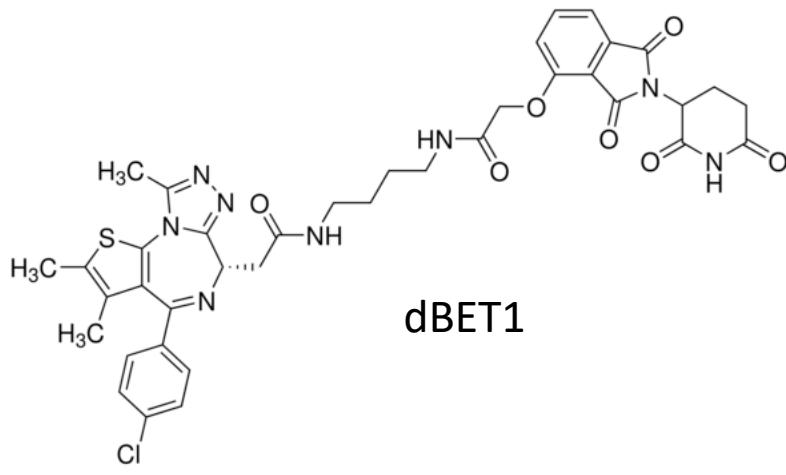
$$\text{RSV}_{\text{Compound}} = \frac{\text{AUC}_{\text{Compound}}}{(\text{AUC}_{\text{Compound}} + \text{AUC}_{\text{EG3}})}$$

- **Signal / Background (S/B):**

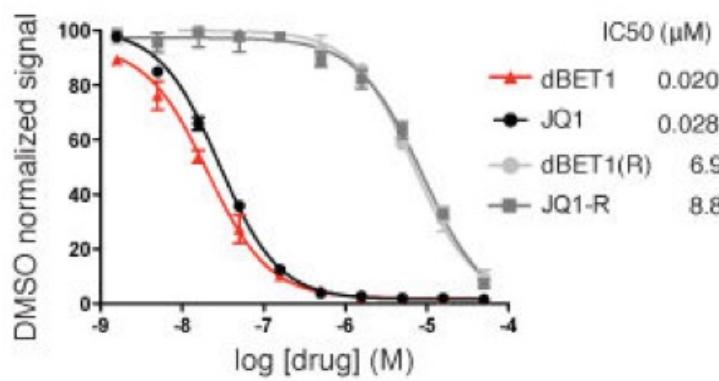
$$\text{S/B} = \frac{\text{RSV}_{\text{Target}}}{\text{RSV}_{\text{Background}}}$$

- Monolayer prevents non-specific adsorption of proteins to the surface and acts as an internal comparator to rank order binding molecules
- Compound A is a target-specific binder, compound B is a non-selective binder

SAMDI ASMS for PROTAC Targets



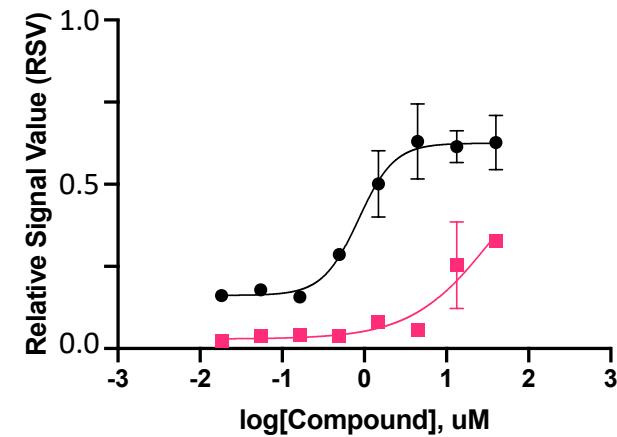
AlphaLisa



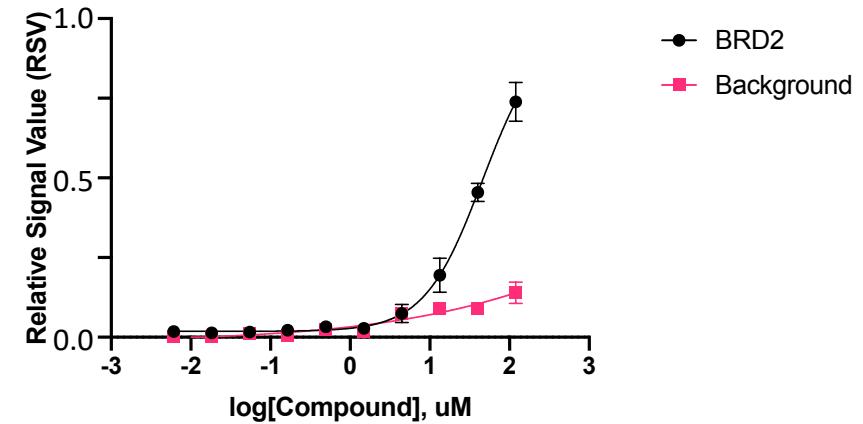
Winter et al., 2015 *Science*

SAMDI ASMS Analysis of PROTACs

dBET1



Compound "A"

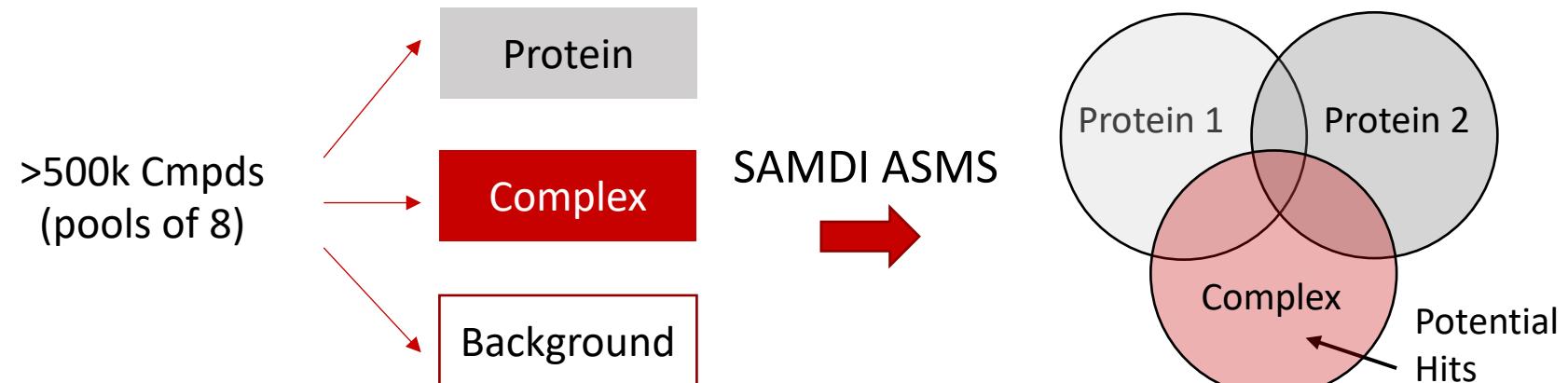
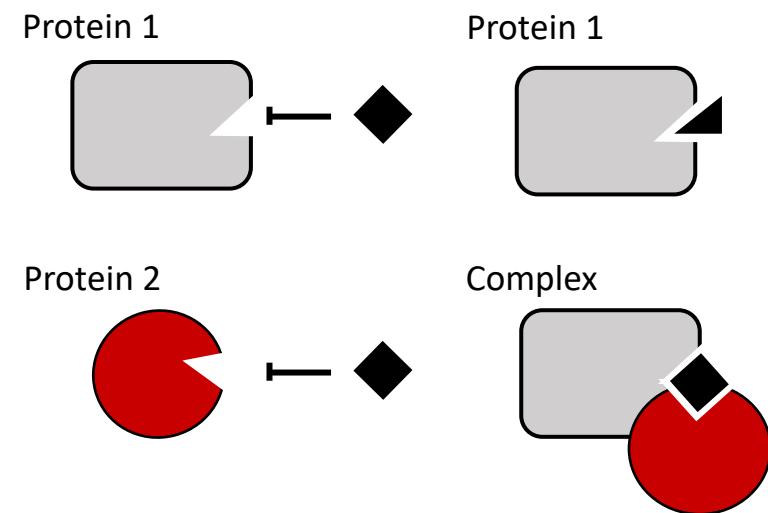


→ dBET1 shows potent binding to BRD2 by SAMDI ASMS

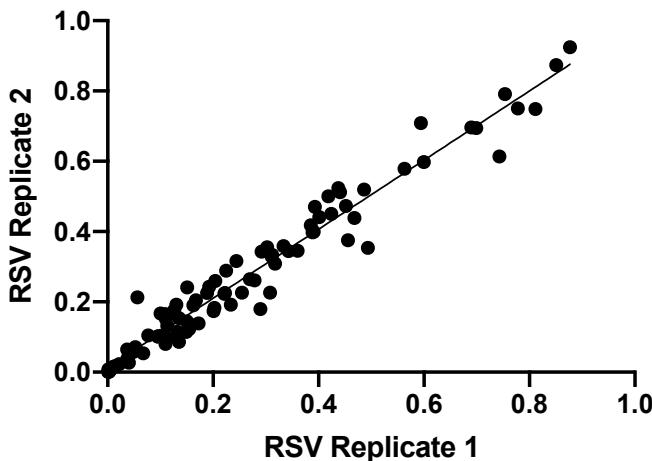
→ Compound A exhibits selective binding to BRD2, weaker affinity (~20 - 40 μM)

→ Compounds detected with off rates ~ 5 seconds

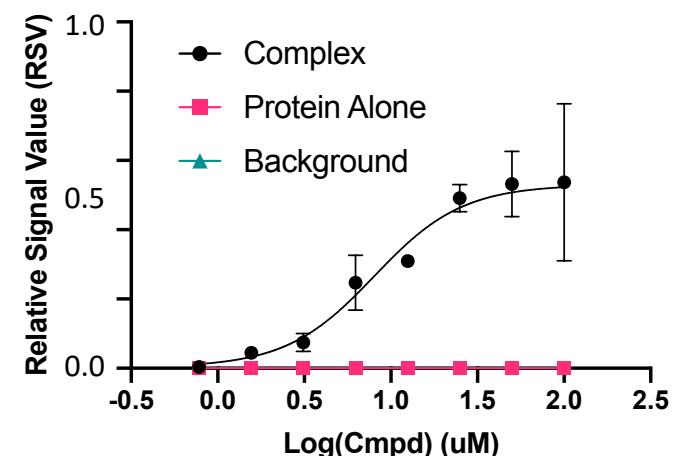
SAMDI ASMS for Molecular Glues



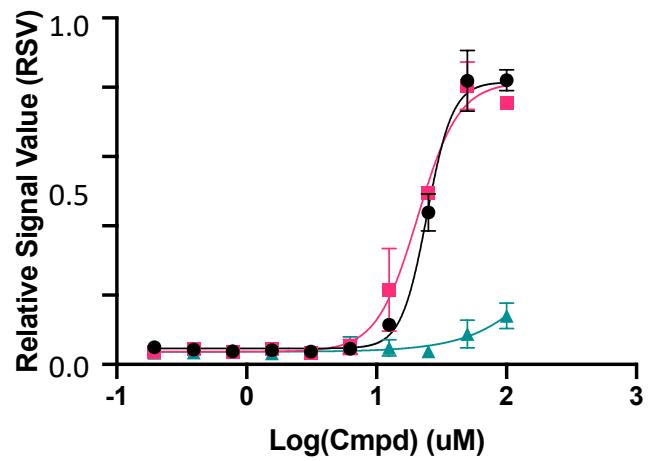
Hit Confirmation



Molecular Glue Candidate



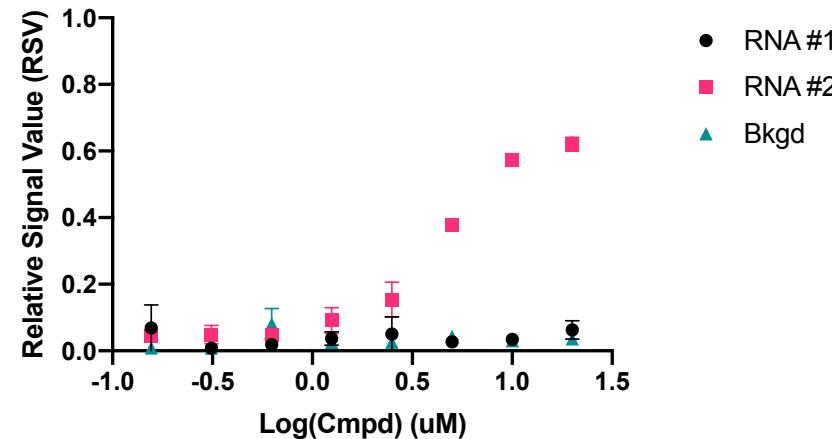
Non-Glue Candidate



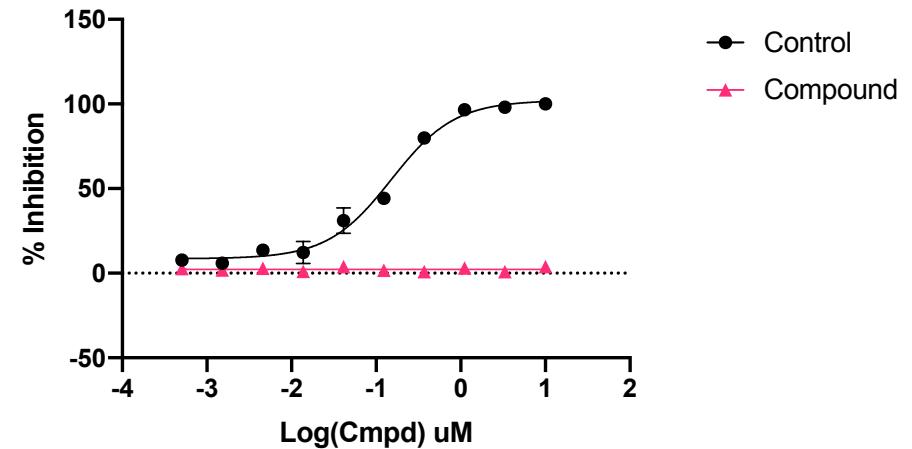
SAMDI ASMS with RNA



SAMDI ASMS Dose Response



Thiazole Orange Intercalation Assay

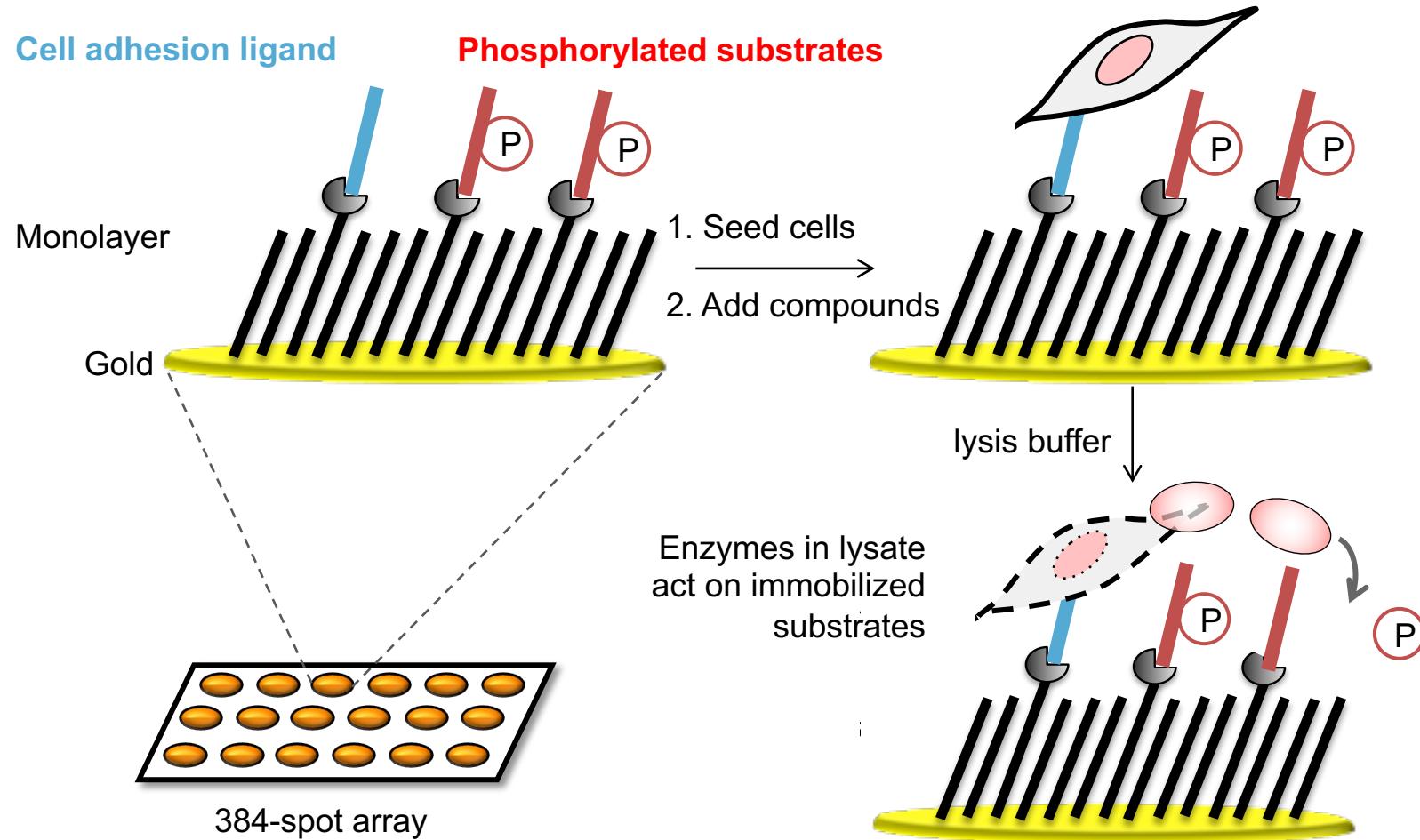


- Biotinylated RNA or biotinylated handle to immobilize any RNA
- Fold RNA in optimal buffer (no buffer limitations with SAMDI)
- Validate selectivity with scrambled RNA sequences and intercalation assays

- Monolayer chemistry provides inherent quality control and internal comparator for every experiment
- Rapid workflow and readout amenable for any target (protein, oligonucleotide, complexes, competition studies)
- Minimal compound compression alleviates challenges with compound misbehavior
- Hit rates 0.3-0.5%, confirmation rates on par with biochemical assays,
- Affinity limit 20-40 μM and off rates > 5 seconds.

Tandem Culture and Lysis (TCAL)-SAMDI MS

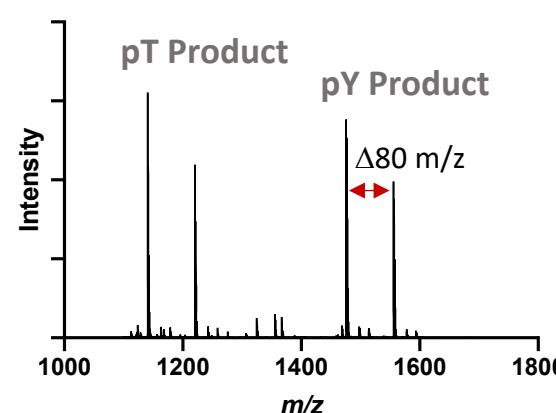
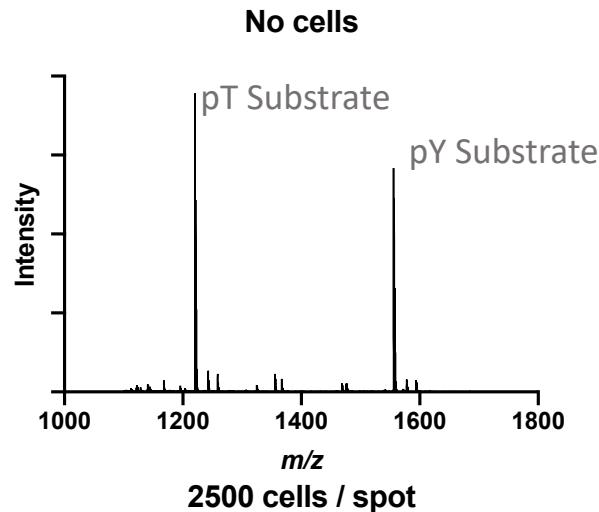
- SAMDI measured distinct endogenous activities from cell lysates
- SAMs presenting RGD ligands mediate cellular adhesion



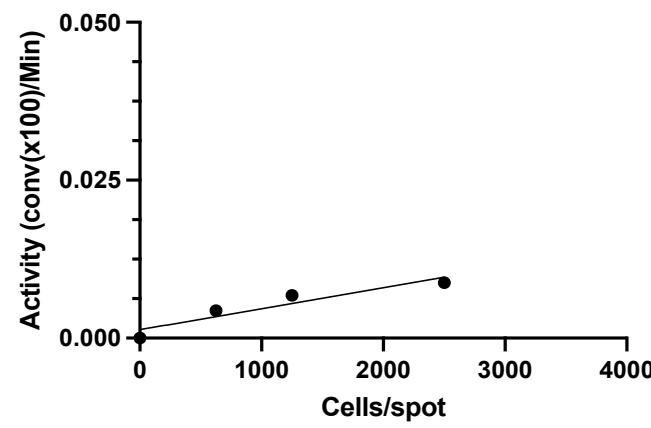
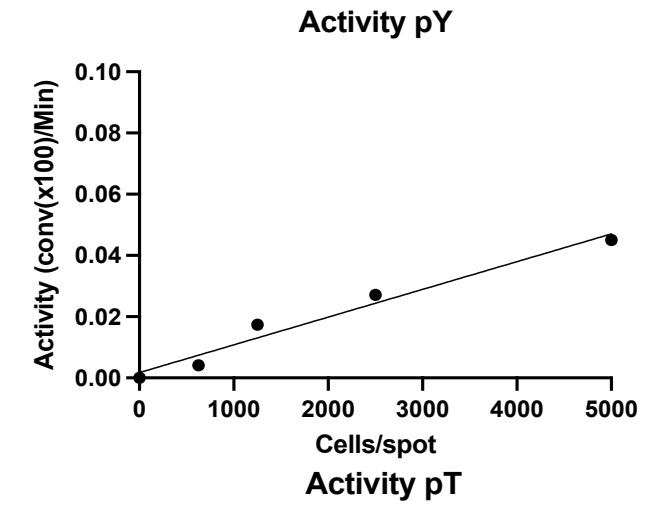
Development of a duplexed TCAL-SAMDI assay

- Co-immobilize two peptide substrates (phosphothreonine and phosphotyrosine) to inform on specific activity

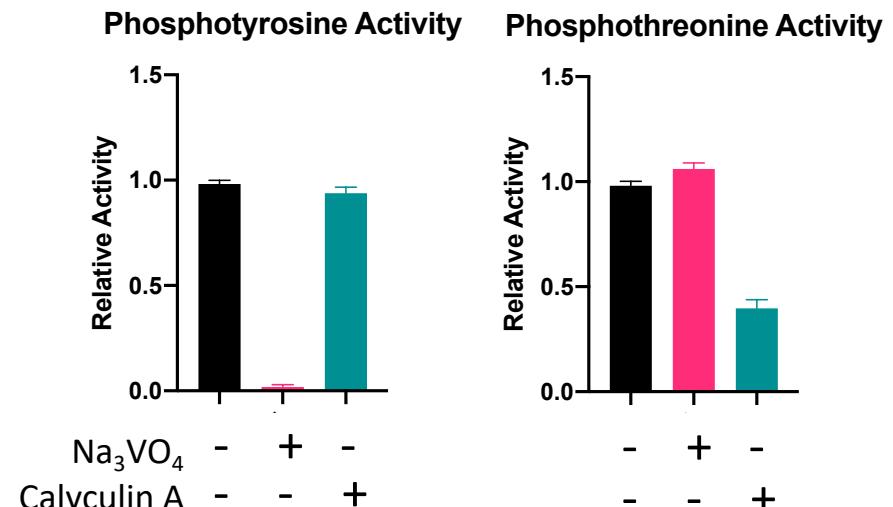
Duplexed TCAL-SAMDI Spectra



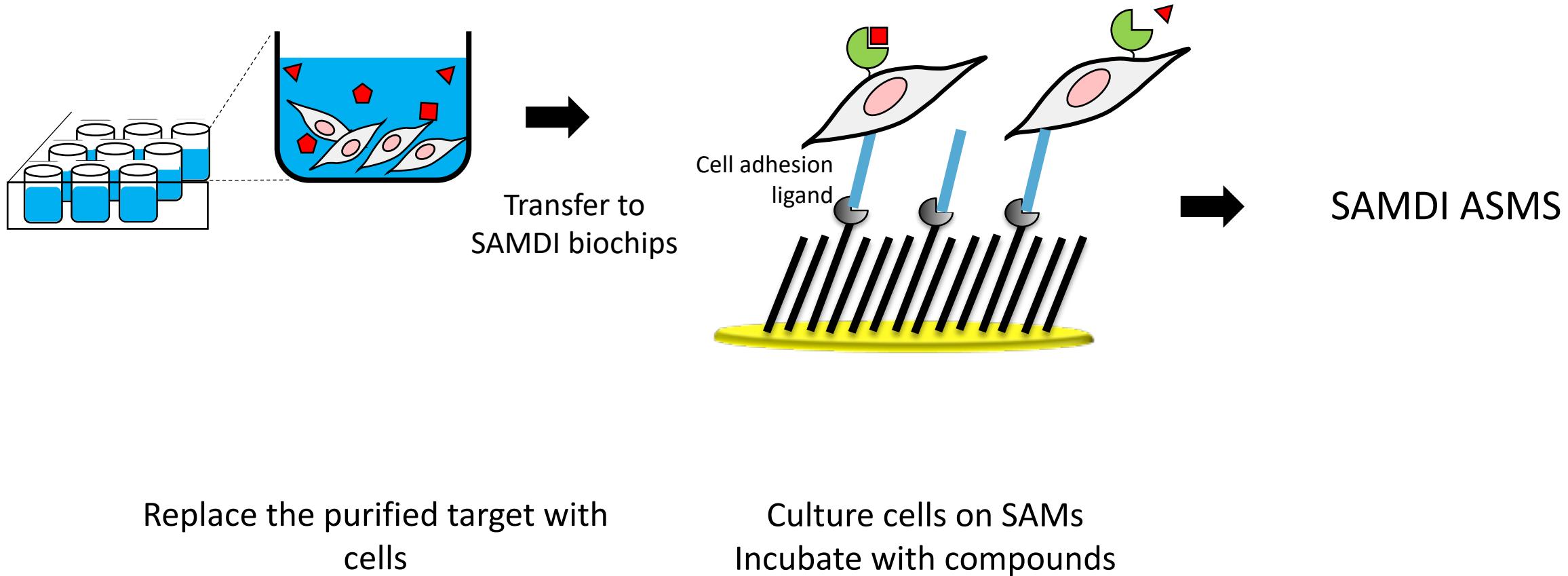
V vs. [Cells] in duplex format



Distinguish activity with specific inhibitors

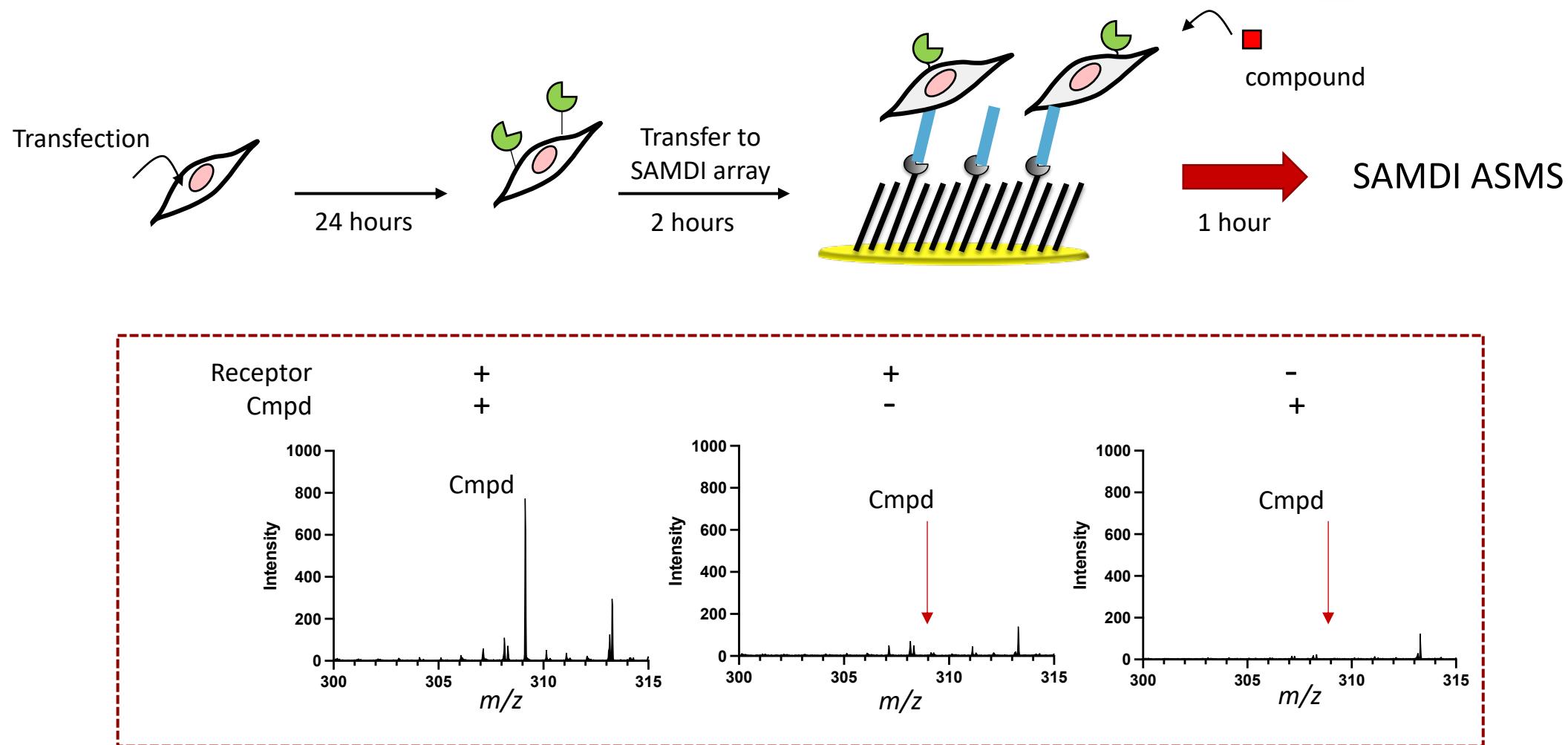


Teaser: can we combine these two innovations?



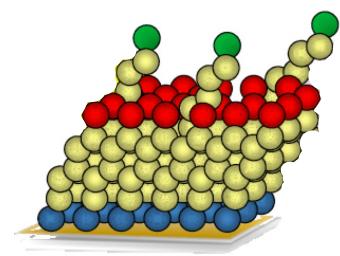
- High-throughput screening of cellular receptors, membrane bound proteins
- Selectivity profiling of lead candidates

Can SAMDI ASMS measure binding to cell receptors?

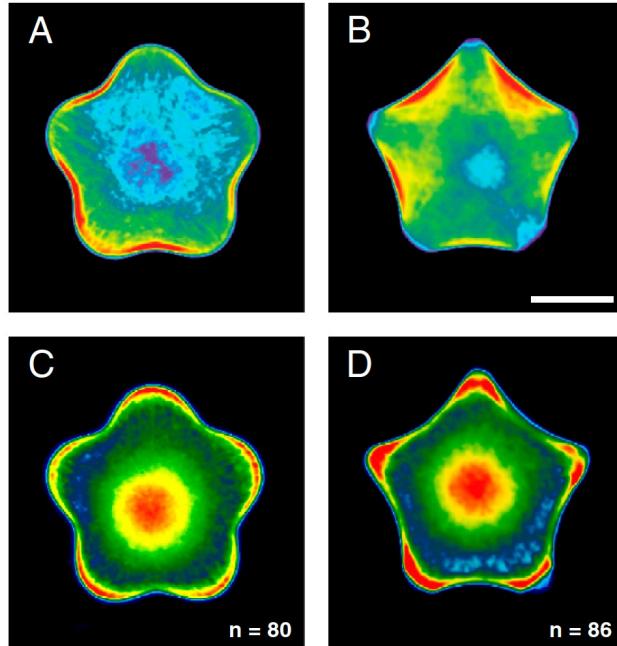


→ Cmpd detected by SAMDI ASMS on cells expressing *GENE* and not non-transfected cells

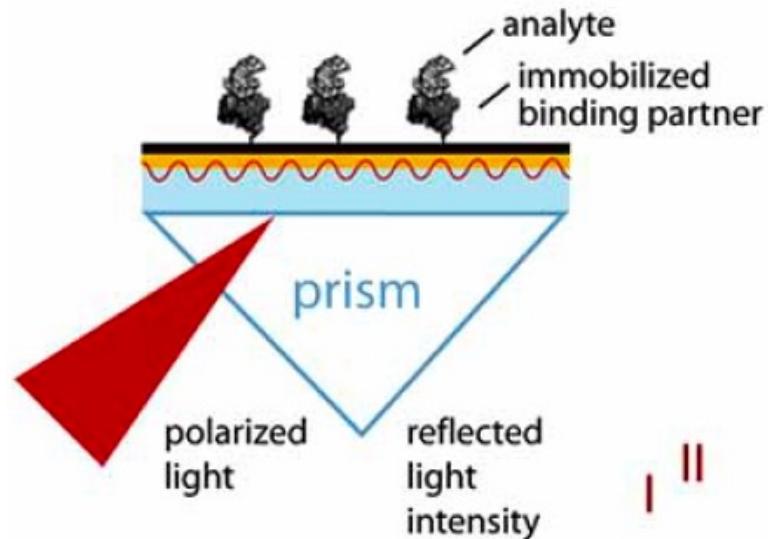
Flexible Surface Engineering: Drug Discovery Solutions



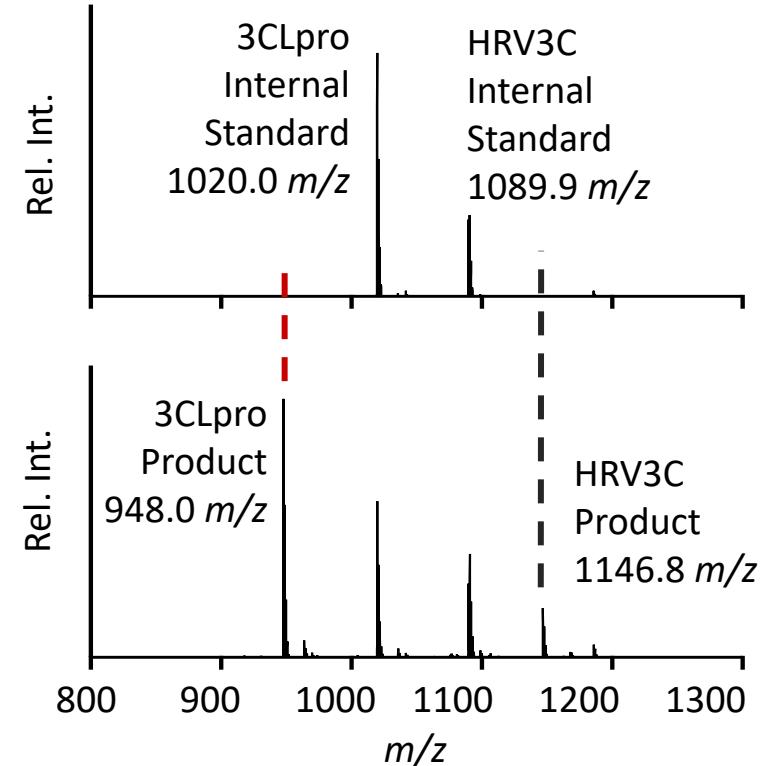
**Cellular behavior
(Microscopy)**



**Binding interactions
(SPR)**



**Biochemical, binding, cellular activities
(MALDI MS)**



Thank you for your attention!

Zack.Gurard-Levin@crl.com

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