# Multi-dimensional high-throughput molecular glue screening via gas phase affinity selection native mass spectrometry and cryo-EM analysis

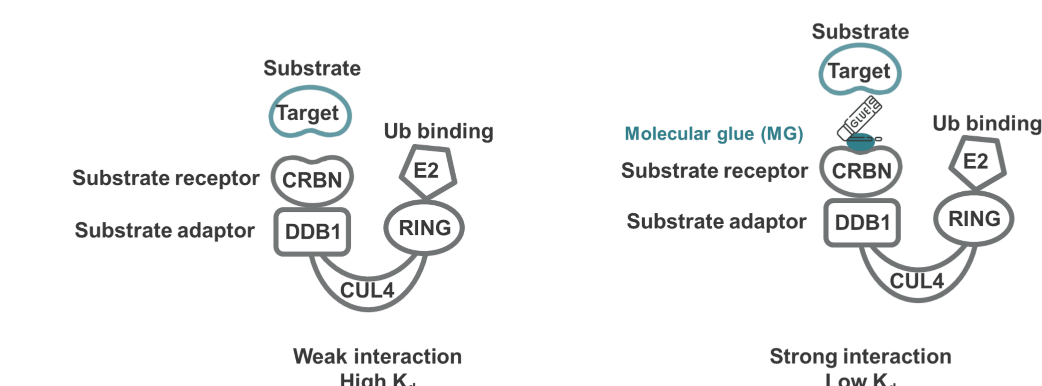
Weijing Liu[1], Kheewoong Baek[2,3], Albert Konijnenberg[4], Wenfei Song[4], Christopher Mullen[1], Yuan Xiong[2,3], Ken Durbin[5], Shane Bechler[6], Eric Fischer[2,3], Rosa Viner[1], Thomas Moehring[7]

[1]Thermo Fisher Scientific, San Jose, CA [2]Dana-Farber Cancer Cancer Institute, Boston, MA, [3]Harvard Medical School, Boston, MA, [4]Thermo Fisher Scientific, Eindhoven, Netherlands [5]Proteinaceous, Evanston, IL [6]Thermo Fisher Scientific, Sunnyvale, CA [7]Thermo Fisher Scientific, Bremen, Germany

ThermoFisher SCIENTIFIC

## Introduction

Targeted protein degradation targeting conventionally undruggable proteins is a transformative approach in drug discovery. Molecular glues (MGs) achieve this by enhancing weak intrinsic interactions between targets and E3 ligase, enabling ubiquitin-proteasome-mediated degradation. While online affinity selection mass spectrometry (MS) struggles to identify weak binders due to on-column dissociation and lack of ternary complex interrogation, native MS facilitates the direct identification of E3-MG-target complexes. However, manual sample preparation and direct infusion limit its throughput.




This study demonstrates high-throughput MG screening using native MS for WEE1 binding to CRBN-DDB1. It enables multiplex screening and analysis of over 2,500 compounds per day. Gas-phase ligand release and fragmentation support identification of unknown binders, and cryo-EM analysis further characterizes ligand-bound complexes, advancing MG discovery and validation.

## Materials and methods

### Sample Preparation

Compound library, CRBN-DDB1 and WEE1 (target) were received from Dana-Farber Cancer Institute. CRBN-DDB1 and WEE1 were mixed at 1:1 volume ratio (1:2 molar ratio). Compounds in 10 mM stock solutions were diluted with 200 mM ammonium acetate (AmAc) to the desired concentration.

### Methods

1.Prefill 96 well plate with one or more binders in each well
2.Set fraction collector temperature at desired binding temp
3.Place protein vial into autosampler
4.Autosampler → Injection Valve 1 → OBE → UV → FC
5.Transfer the sample plate from fraction collector to autosampler
6.Autosampler → Injection Valve 2 → Easy-spray

**Thermo Scientific™ Orbitrap™ Ascend Structural Biology Tribrid™ mass spectrometer**

- m/z 50-16,000
- Quad up to m/z 8,000
- Native MS
- Native top-down
- PTCR/HCD/CID/ETD/UVPD

ProSight Native

## Results

### 1. Multiplexed-compound screening

Presence of non-volatiles prevents direct native MS measurement of complexes and pre-column binding suffering from on-column dissociation limits weak binder identification. Post-column binding is the workaround to preserve weak binders.

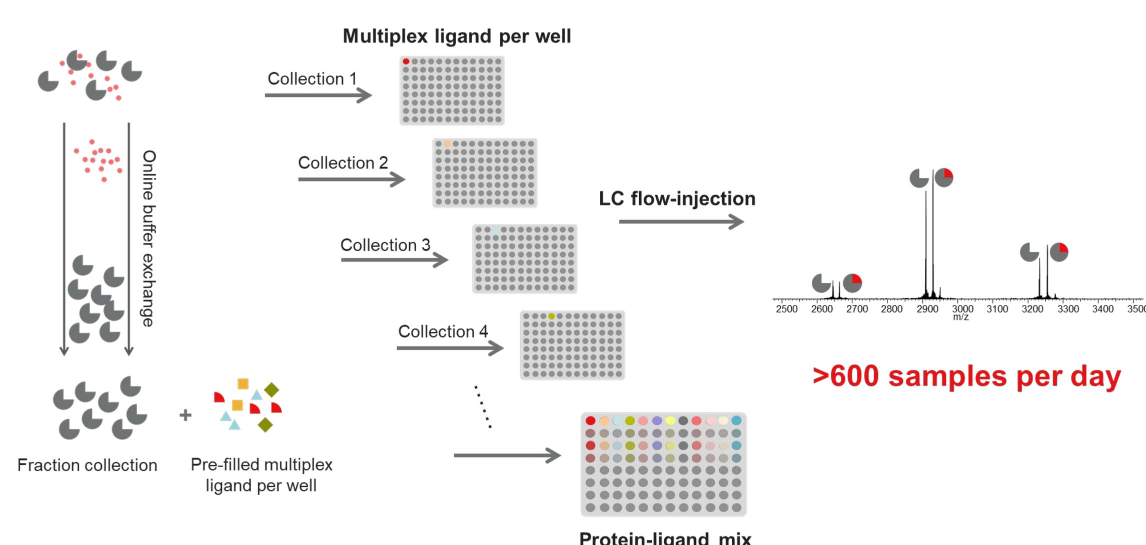
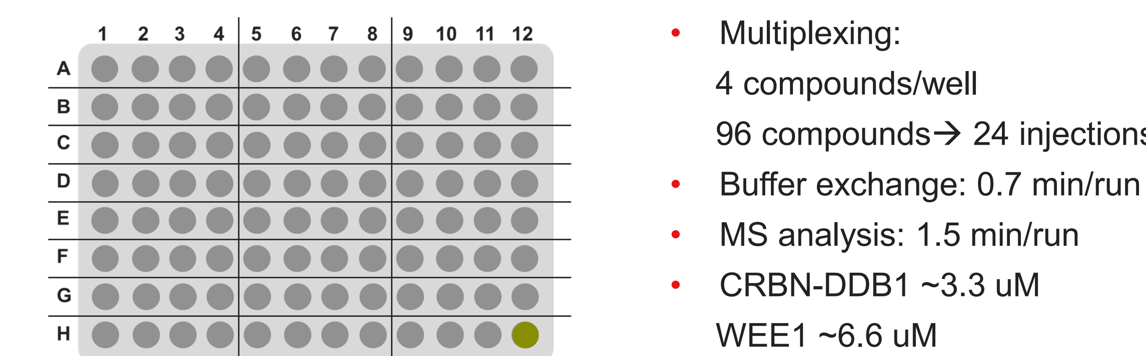
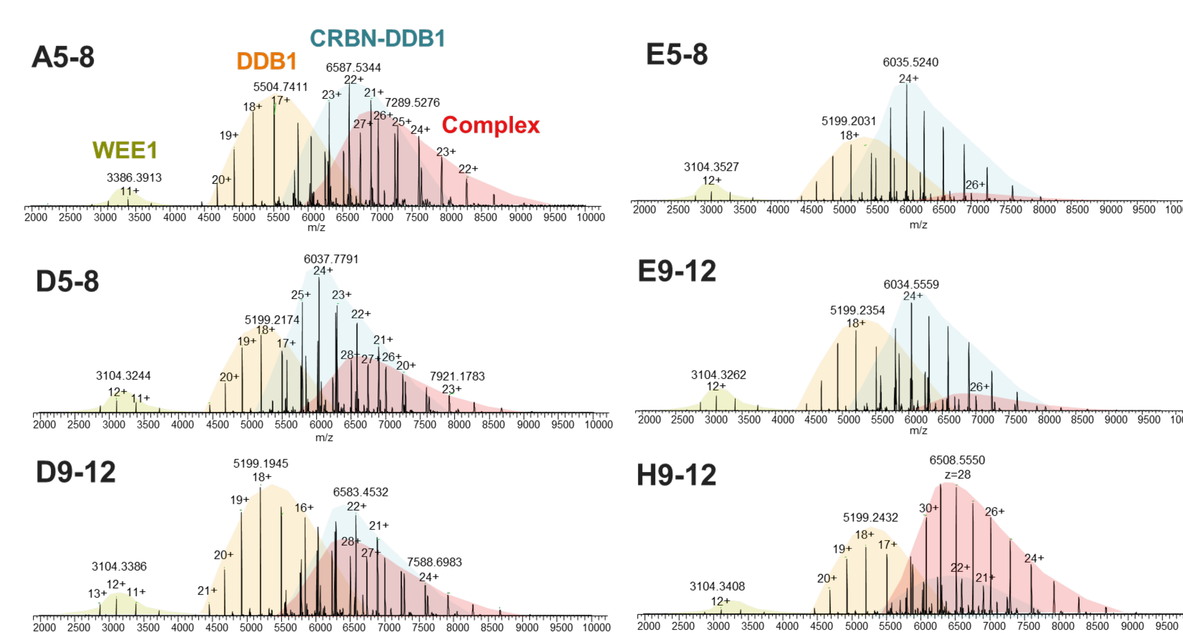



Figure 1. Multiplexed-compound screening



- Multiplexing:
  4 compounds/well
  96 compounds→ 24 injections
- Buffer exchange: 0.7 min/run
- MS analysis: 1.5 min/run
- CRBN-DDB1 ~3.3 uM
  WEE1 ~6.6 uM
  Compound ~5 uM of each

Figure 2. Example of wells showing complex formation




- Wells containing A5-A8, D5-D8, D9-D12, and H9-H12 show strong complex formation.
- Wells containing E5-E8 and E9-E12 show moderate complex formation.
- Wells containing B9-B12, C1-C4, F5-F8, F9-F12, G5-G8, and G9-G12 show weak complex formation.

## Results

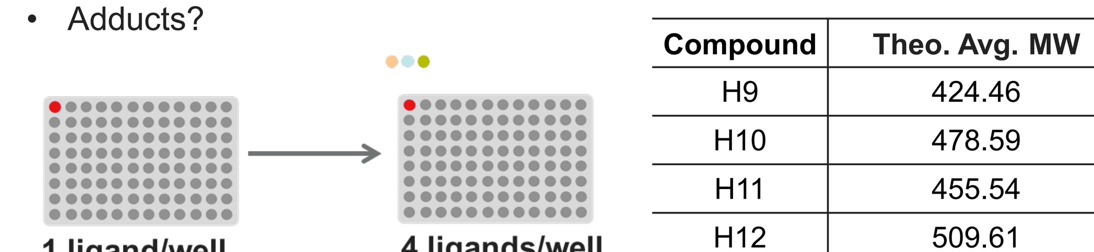
### 2. Gas phase affinity selection MS to pinpoint binding compounds

**Goal:** Increasing throughput by mixing multiple compounds per well

**Challenge:**

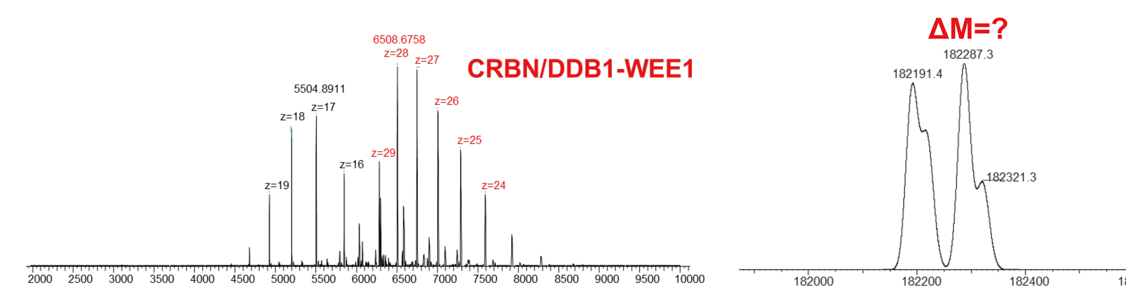
Ambiguity of determining bound compound due to:

- Compound MW similarity?
- Cofactor?
- Adducts?




| Compound | Theo. Avg. MW |
|---|---|
| H9 | 424.46 |
| H10 | 478.59 |
| H11 | 455.54 |
| H12 | 509.61 |

Figure 3. Deconvolution showing ambiguity of bound compounds




**Gas phase affinity selection MS to identify bound compounds:**

1. Full OTMS injection into OT
2. Target complex isolation in Q1
3. Target complex dissociation in FHCD
4. Dissociated complex and ligands detection in OT
5. Dissociated ligands fragmentation in IT for structure information

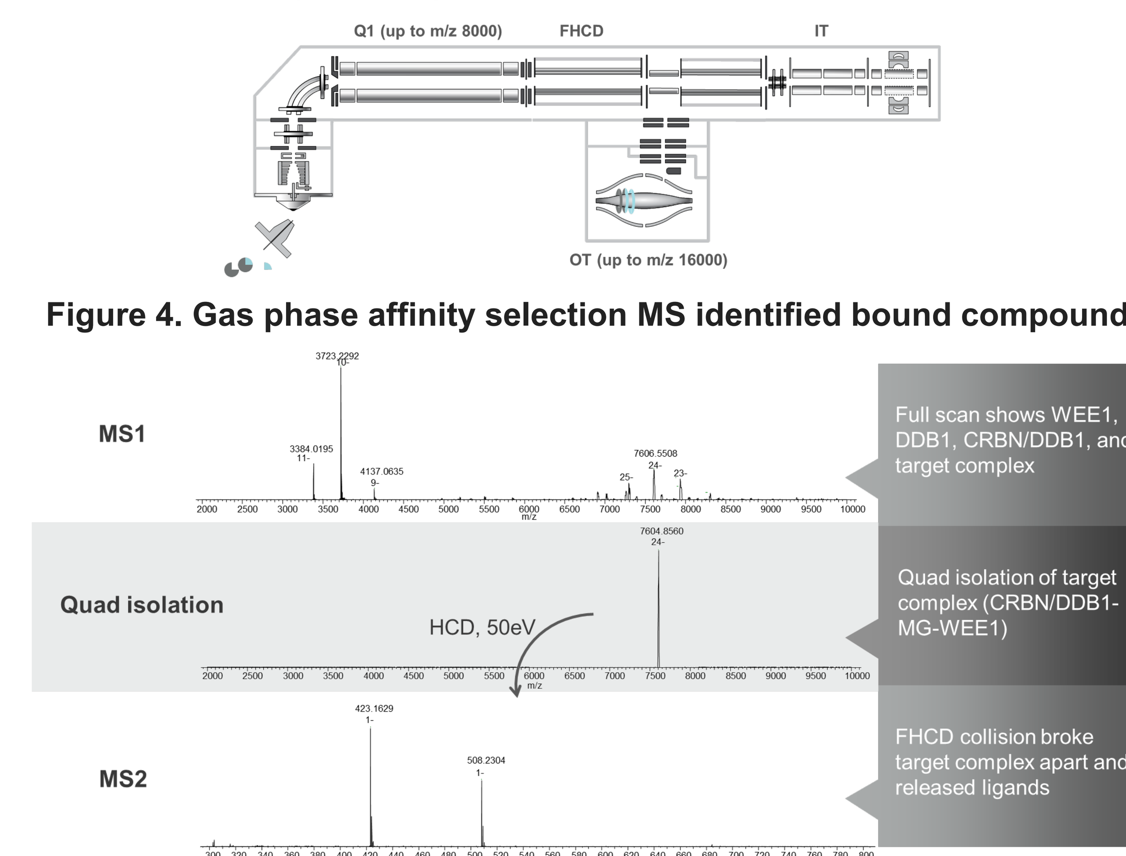



Figure 4. Gas phase affinity selection MS identified bound compounds

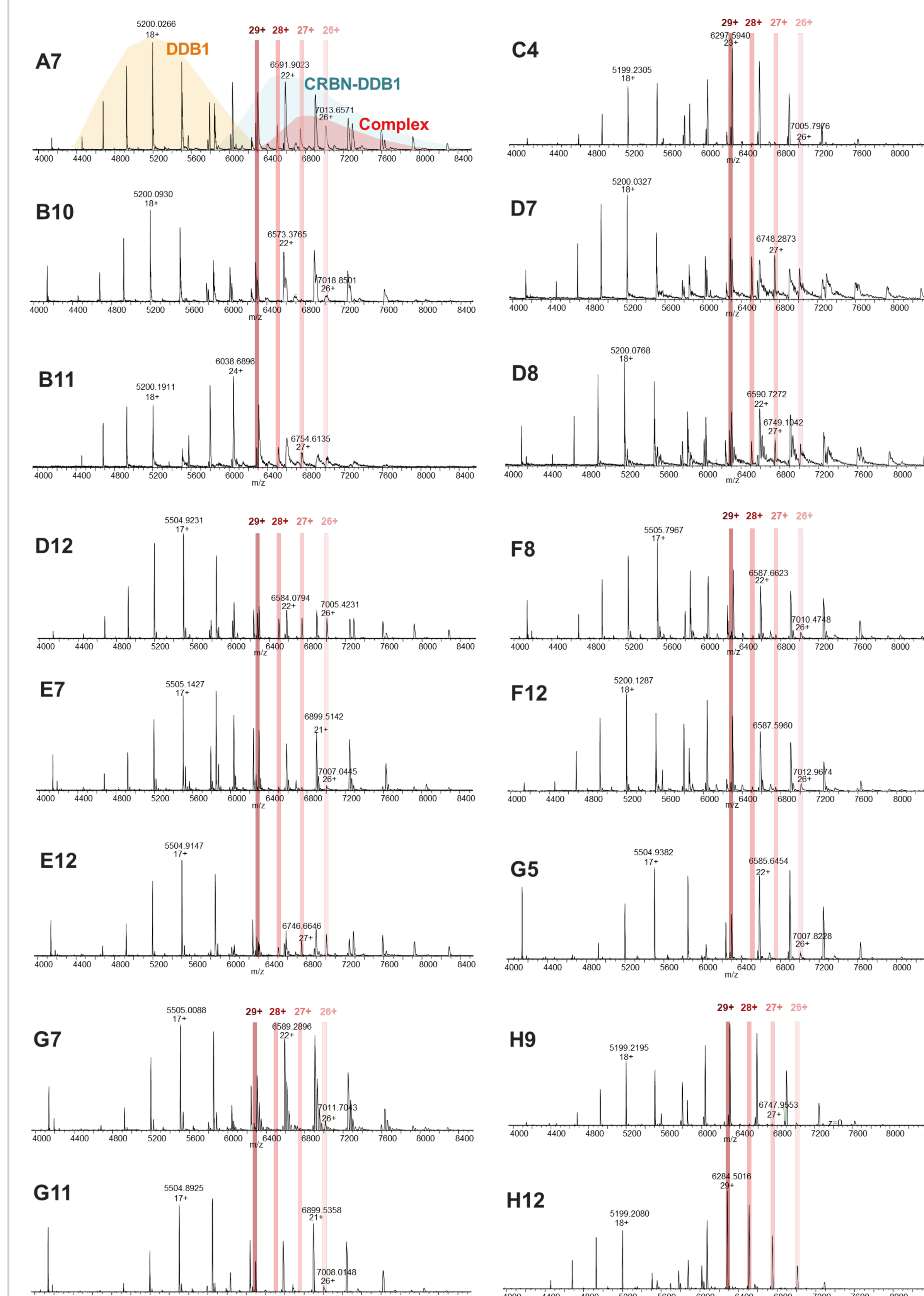
## Results

### 3. Single-compound screening

To eliminate compound competition and ensure an excess molar ratio of each compound to target complexes, we conducted the screening using one compound per well.

- One compound/well: 96 compounds→ 96 injections
- Buffer exchange: 0.7 min/run
- MS analysis: 1.5 min/run
- CRBN-DDB1: WEE1: compound = ~3.3 uM: ~6.6 uM: ~16 uM

Figure 5. Hits identified by incubating samples at 25 C for 1 hour




To increase throughput and minimize AmAc buffer effect to complexes, we also performed incubation for 5-min.

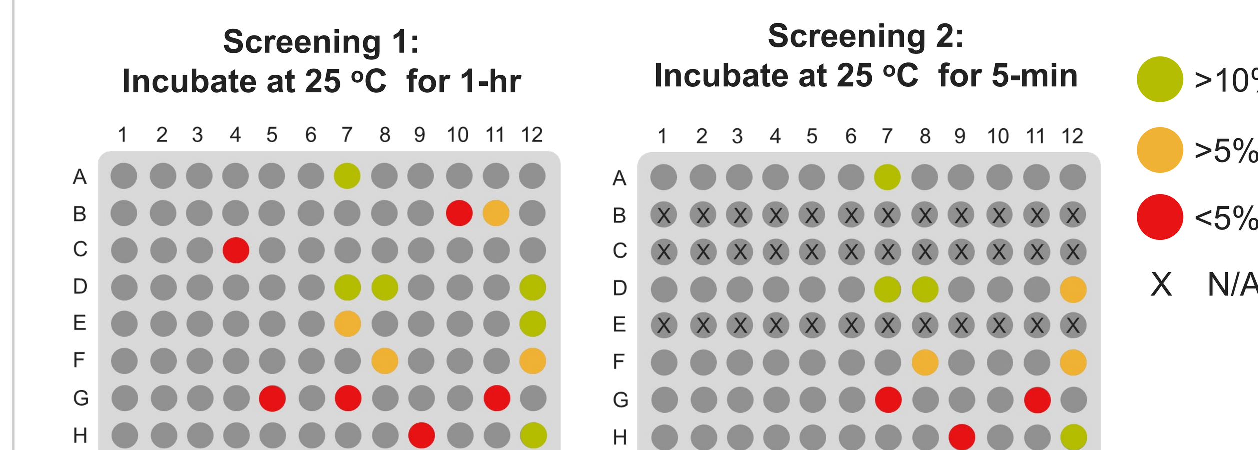



Table 2. Correlation of two screenings

| No. | Ligand | Screening 1: 25C for 1-hr | Screening 2: 25C for 5-min |
|---|---|---|---|
| 1 | A7 | >10% | >10% |
| 2 | B10 | <5% | X |
| 3 | B11 | >5% | X |
| 4 | C4 | <5% | X |
| 5 | D7 | >10% | >10% |
| 6 | D8 | >10% | >10% |
| 7 | D12 | >10% | >5% |
| 8 | E7 | >5% | X |
| 9 | E12 | >10% | X |
| 10 | F8 | >5% | >5% |
| 11 | F12 | >5% | >5% |
| 12 | G5 | <5% | <5% |
| 13 | G7 | <5% | <5% |
| 14 | G11 | <5% | <5% |
| 15 | H9 | <5% | <5% |
| 16 | H12 | >10% | >10% |

- Hits identified at 25 °C for 1hr and 4 °C for 5-min are comparable.

## Conclusions

- An integrated LC-native MS system streamlines the rapid online buffer exchange, parallel compound binding, and direct analysis of MG-bound complexes.
- Gas phase affinity selection MS increases the throughput, enables bound MGs identification, and unknown structure elucidation.
- Completed screening of 96 compounds at 1 compound/well and hits identified at 25 °C for 1hr and 25 °C for 5-min are comparable indicating rapid binding.
- Identified 16 out of 96 compounds as potential molecular glues.

## Trademarks/licensing

© 2025 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. This information is not intended to encourage use of these products in any manner that might infringe the intellectual property rights of others. PO397-2025-EN

Learn more at thermofisher.com/tribrid