

# CF-PPiD: A Cell-Free Human Protein Array Technology Using Proximity

## Biotinylation-Based Protein-Protein Interaction Identification

Shusei Sugiyama<sup>1</sup>, Miwako Denda<sup>1</sup>, Kodai Yamada<sup>2</sup>, Tatsuya Sawasaki<sup>2</sup>, Ryo Morishita<sup>1</sup>

(<sup>1</sup>CellFree Sciences, Co., Ltd., <sup>2</sup>Proteo-Science Center, Ehime University)



### Research Background & Purpose

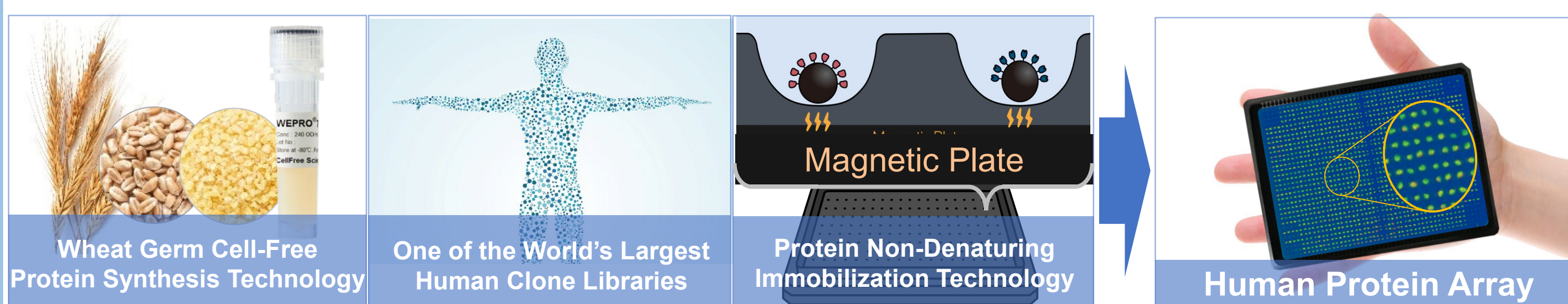
To understand drug targets and mechanisms of action, it is important to **identify and analyze the proteins that bind (interact) with drugs**. In particular, the identification of drug targets obtained by phenotypic screening is an essential technique in drug discovery and development, but it is difficult to detect such Drug-Protein Interactions (DPIs) easily and with high sensitivity.

We have previously demonstrated that the use of **Proximity-Dependent Biotinyltransferase (AirID)-Fused target proteins on cell-free Protein Bead Arrays (PBAs) enable the sensitive analysis of Protein-Protein Interactions (PPIs)** with 23,000 different proteins (CF-PPiD, *Scientific Reports*, 2022, DOI: 10.1038/s41598-022-14872-w). The system can also specifically detect interactions between **Cereblon-Thalidomide** and **neo-substrates** as compound-dependent PPIs as well as PPIs mediated by bifunctional compounds (PROTAC) on PBAs.

Furthermore, in this study, a **modified AirID specifically designed for covalent binding of drugs was successfully prepared, enabling the detection of direct interactions between drugs and target proteins**.

### Protein Beads Array Principle

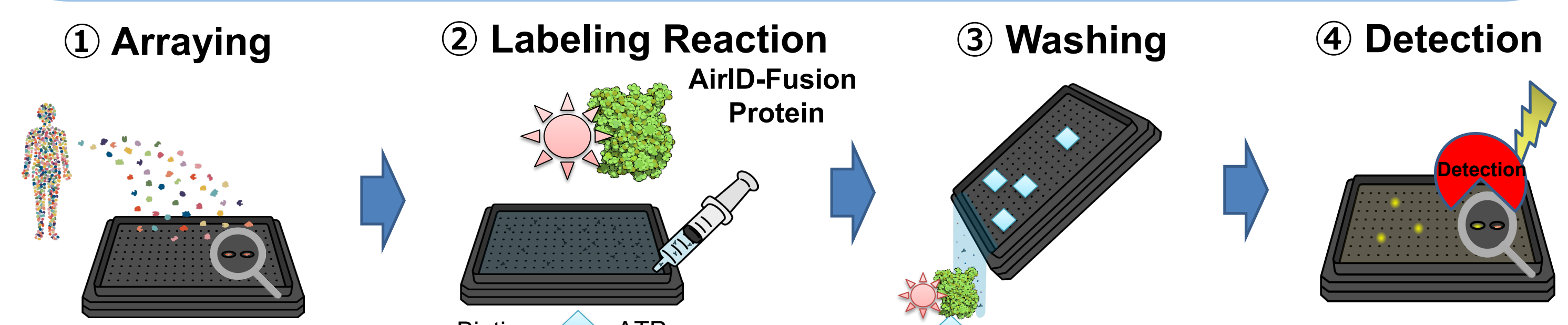
Combining three in-house technologies for our Protein Arrays



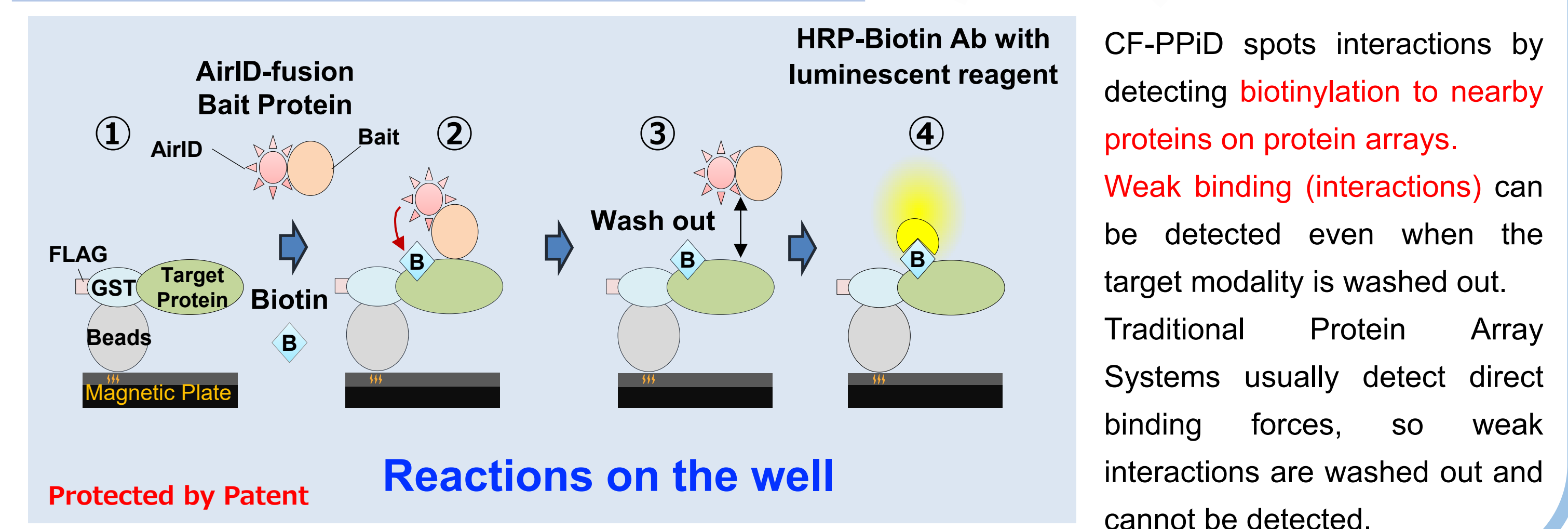
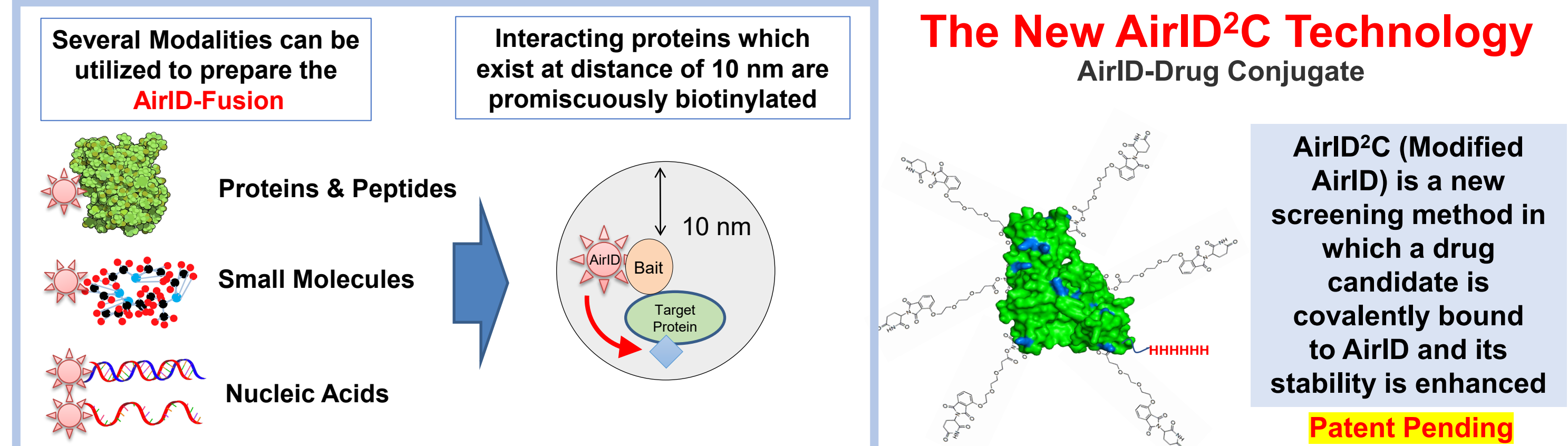
**80% of proteins are always purified in solution/retained on carrier, loaded at molar ratios within 10x**

### Flow the CF-PPiD\* Technology

\*(Cell-Free based Protein array for Protein-protein interaction identification using proximity biotinylation enzyme)



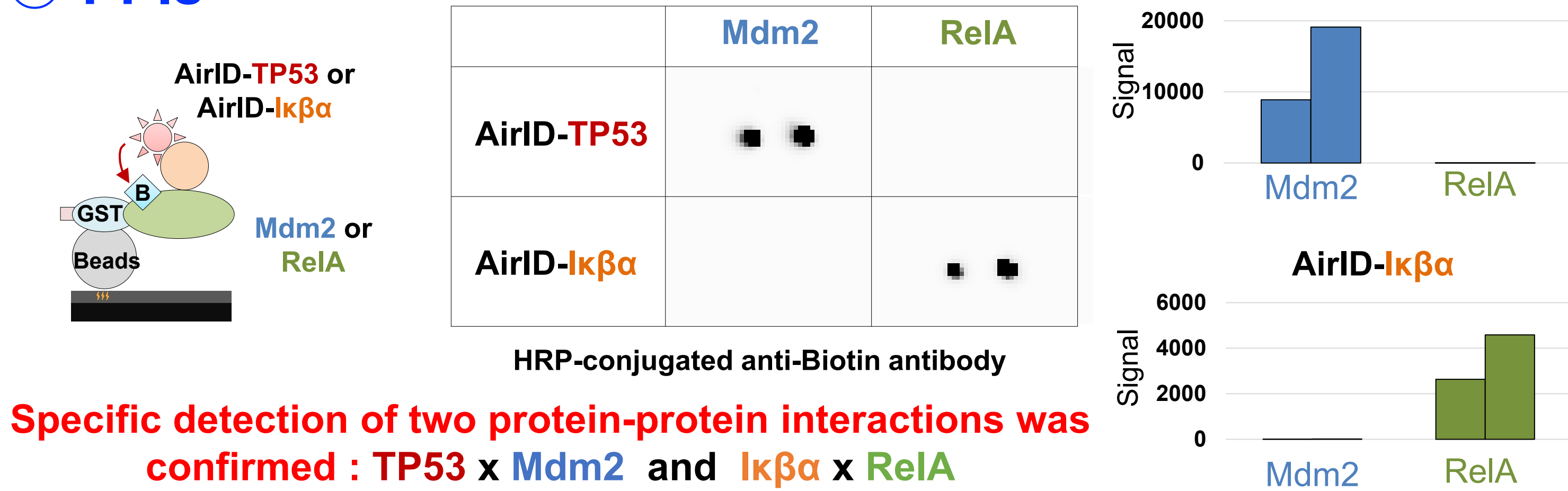
Searching is possible for proteins that bind to drugs from approximately **23,000 types of human proteins**



CF-PPiD spots interactions by detecting **biotinylation to nearby proteins on protein arrays**. **Weak binding (interactions)** can be detected even when the target modality is washed out. Traditional Protein Array Systems usually detect direct binding forces, so weak interactions are washed out and cannot be detected.

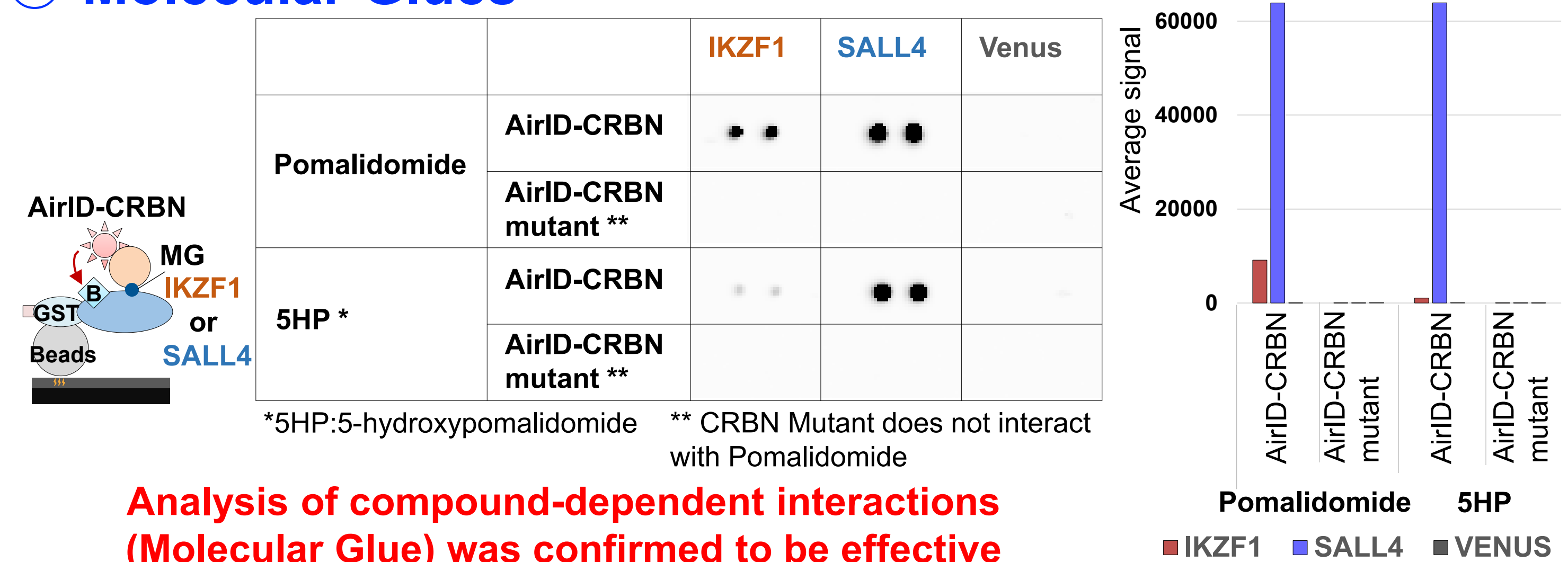
### Types of Interactions Detected by CF-PPiD

#### 1 PPIs



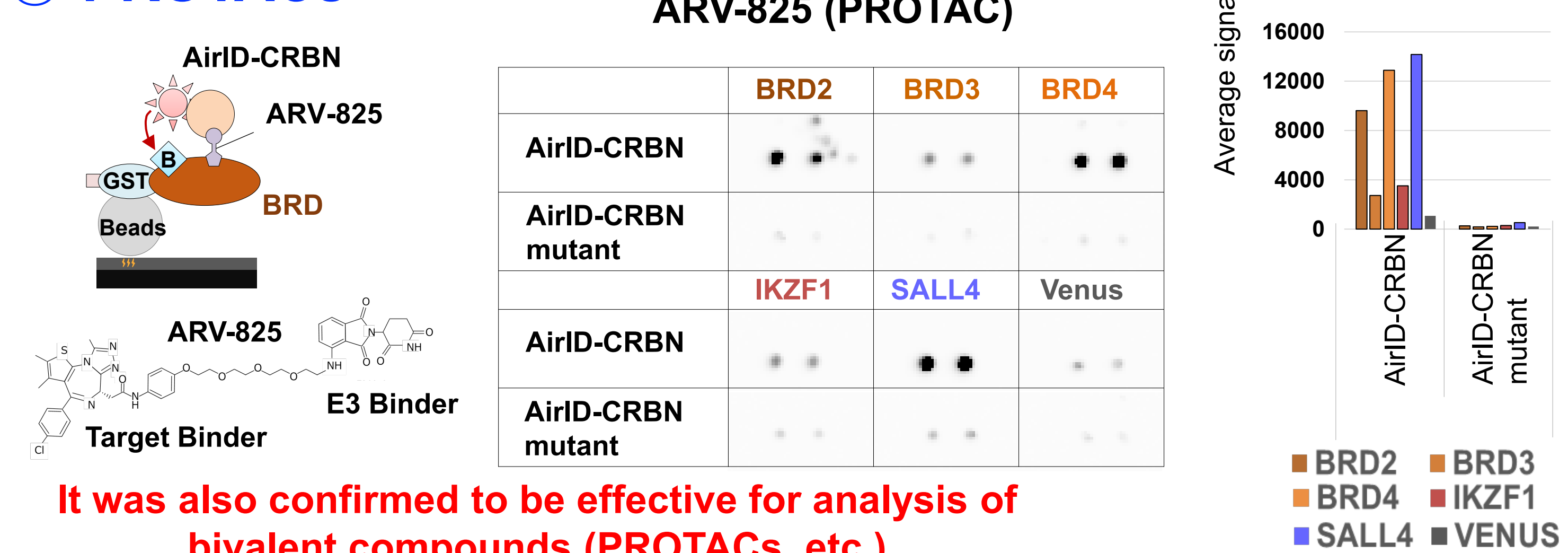
Specific detection of two protein-protein interactions was confirmed: TP53 x Mdm2 and Ikβa x RelA

#### 2 Molecular Glues



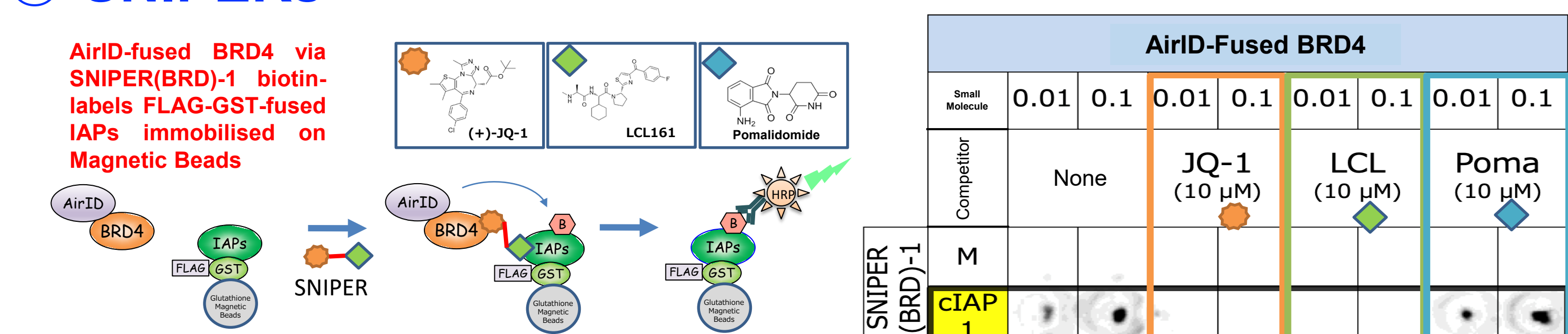
Analysis of compound-dependent interactions (Molecular Glue) was confirmed to be effective

#### 3 PROTACs



It was also confirmed to be effective for analysis of bivalent compounds (PROTACs, etc.)

#### 4 SNIPERS



### Interaction Analysis using AirID2C

#### 1 Evaluation of Thalidomide Conjugate: NHS Conjugate or Click Conjugate

	Thalidomide Modification (NHS)			No Modification	Thalidomide Modification (Click)			Azide Modification
	1	2	3		1	2	3	
Mock	—	Poma Addition	JQ1 Addition	—	—	Poma Addition	JQ1 Addition	—
FLAG-GST(FG)								
FG-BRD4								
FG-CRBN								
FG-CRBN YW/AA								

- The drug (Thalidomide)-conjugated AirID<sup>2C</sup> has biotinylated only the target protein Cereblon (CRBN).
- Pomalidomide that was added as a competitive agent inhibited the biotinylation reaction of CRBN, suggesting that **AirID<sup>2C</sup> Thalidomide specifically binds to CRBN**.

#### 2 Evaluation of JQ1 Conjugate: Click Conjugate

	JQ1 Modification (Click)			Thalidomide Modification (Click)	Azide Modification		
	1	2	3			1	2
Mock	—	Poma Addition	JQ1 Addition	—	—	Poma Addition	JQ1 Addition
FLAG-GST(FG)							
FG-BRD2							
FG-BRD3							
FG-BRD4							
FG-CRBN							
FG-CRBN YW/AA							

- The drug (JQ1)-bound AirID<sup>2C</sup> has biotinylated only the BRD family target proteins.
- JQ1 added as a competitor inhibited the biotinylation reaction of the BRD family, suggesting that **AirID<sup>2C</sup> JQ1 specifically binds to the BRD family**.
- AirID<sup>2C</sup> Drug specifically binds to its target protein and biotinylates it**

### Summary and Future Prospects

- Proteins mounted on the array are all **non-denatured** and they are as **close as possible** to their native states in cells.
- The CF-PPiD has the competitive advantage of detecting even **weak binding** (interactions).
- Several types of human protein beads arrays are commercially available including: **23K Protein Array**, **Diversity Array & Category Array**: E3 ligase (572 kinds), kinase (457 kinds), and **DNA binding protein** (1,314 kinds), other arrays are under development.
- The human protein beads array allows detecting PPIs (Protein-Protein Interactions) and can also utilize Molecular Glues, PROTACs and SNIPERS.
- We believe the CF-PPiD technology can be adapted to a **wide variety of molecules**, including **low/medium molecular weight compounds, peptides, proteins and nucleic acids**.
- We have **successfully prepared a "Drug-Binding-Specific Modified AirID = AirID<sup>2C</sup>"** that retains stability after drug binding.
- AirID<sup>2C</sup> Drugs prepared using the **NHS group/Click chemical reactions** retained their activity to **specifically bind/biotinylate target proteins** thus providing **useful results** to search for **small molecule-binding proteins**