# 2022 Best Research Poster Award

Barwon Health

DEAKIN UNIVERSITY

# Superiority of anti-HER2 Affibody molecules over monoclonal anti-HER2 antibodies in labeling and detecting HER2$^+$ extracellular vesicles

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## INTRODUCTION

Extracellular vesicles (EVs) are important intercellular communication messengers [1]. The use of bulky ligands may introduce inaccurate characterisations of EVs, especially for surface proteins that exist as dimers or clusters (e.g., HER2, a cancer biomarker), leading to the incorrect picture of EVs. By virtue of a small size, Affibody molecules would be superior to their monoclonal antibody counterparts in accessing their targets on EVs through steric advantage, and in detecting EVs through their rapid rotation in fluorescence polarisation assays (Fig. 1). Hence, the use of Affibody may provide not only a precise view on EVs but also increased sensitivities of EV detection methods. In this study, we set out to directly compare the capacity of anti-HER2 Affibody molecules with the cognate anti-HER2 antibodies in accessing and detecting HER2$^+$ EVs.

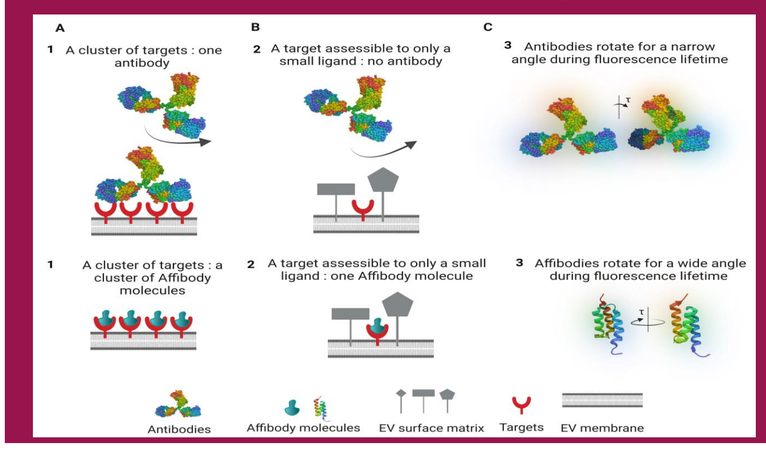


**Figure 1.** Illustrations of bindings of Affibody molecules, monoclonal antibodies to epitopes on EV surface and rotations of these ligands.

## METHOD

Two sources of HER2-positive EVs derived from two cancer cell lines (SKBR3 and HT-29) and three different HER2 antibodies in this work acted for comprehensive confirmation of the hypothesis. Anti-HER2 Affibodies (a dimeric form) and anti-HER2 antibodies were first assessed for their specificities and binding affinities using wild-type MDA-MB-231 and HER2 gene knockout MDA-MB-231 cell lines. Experimental conditions in comparison assays were thoroughly optimised before direct comparisons were performed through flow cytometry-based fluorescence intensity, colorimetry, and fluorescence polarisation assays.

## RESULTS

### 1. HER2 Affibodies and HER2 antibodies are specific to HER2

HER2 Affibody molecules and three HER2 antibodies are highly specific to HER2 (Fig. 2B). Moreover, all three HER2 antibodies have significantly higher binding affinities to HER2 as compared to that of HER2 Affibodies (Fig. 2C).

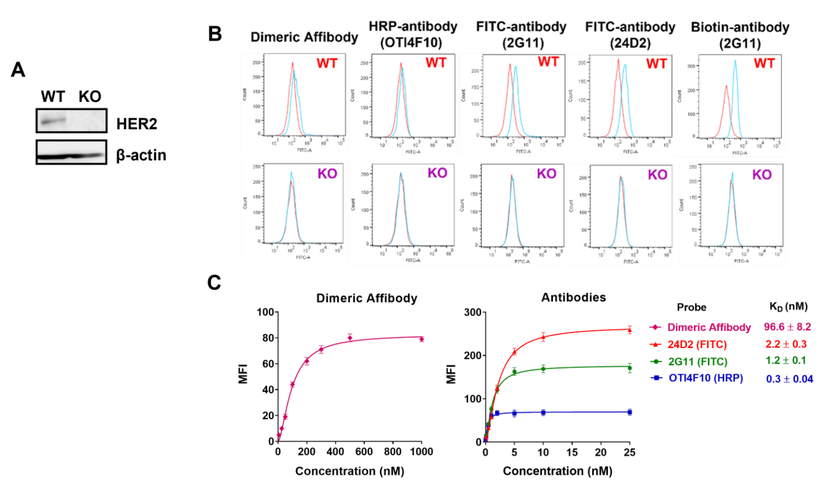


**Figure 2**. (**A**), Western blot images of wild-type MDA-MB-231 and HER2 gene knocked out MDA-MB-231 cell lysis. (**B**), Representative binding histograms of HER2 ligands against wild-type and HER2 gene knockout MDA-MB-231 cells. Red curve, positive sample; blue curve, the background fluorescence of cell only. (**C**), Fitting binding data and respective apparent dissociation constants of these ligands on SKBR3 cells. Data shown are means ±S.D., n=3.

## RESULTS

### 2. Superiority of HER2 Affibody molecules over HER2 antibodies in accessing HER2 receptors on HER2-positive EVs

The outperformance of HER2 Affibody molecules against three HER2 antibody clones in labeling HER2$^+$ EVs was demonstrated (~4-fold using FITC-labeled ligands, and ~10-fold using HRP-conjugated ligands) (Fig. 3).

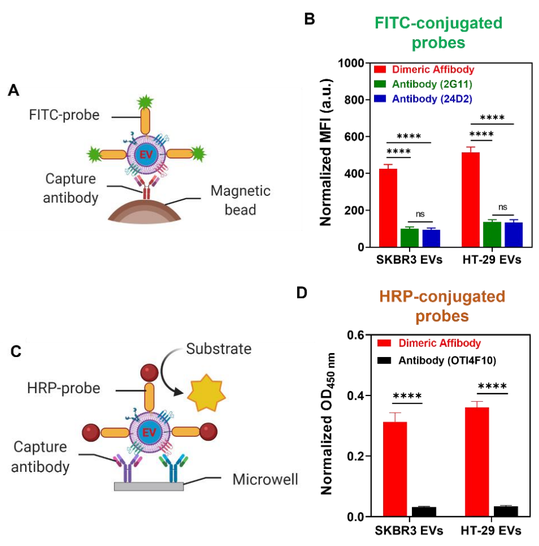


**Figure 3.** (**A**), Illustration of the interactions between FITC-conjugated detection ligands and immobilized EVs on a bead for a flow cytometry measurement. (**B**), Superiority of FITC-conjugated dimeric anti-HER2 Affibody molecules over FITC-conjugated anti-HER2 antibodies (clones 2G11 and 24D2) in binding to SKBR3 EVs and HT-29 EVs immobilized on beads. (**C**), Illustration of the interactions between HRP-conjugated detection ligands and immobilized EVs in a microwell for a measurement by a microplate reader. (**D**), Superiority of HRP-conjugated dimeric anti-HER2 Affibody molecules over HRP-conjugated anti-HER2 antibody (clone OTI4F10) in binding to SKBR3 EVs and HT-29 EVs immobilized in microwell. Data shown are means ± S.D, n=3. ****$P$<0.0001, ns: not significant.

### 3. Superiority of HRP-conjugated HER2 Affibody molecules over HRP-conjugated HER2 antibody in detecting HER2-positive EVs

Straightforwardly, HRP-Affibody molecules could detect EVs spiked in buffer and human serum with significantly higher sensitivities (1.7–2.6-fold) in comparison with the sensitivities using HER2 antibody (Table 1).

**Table 1. Summary of limit of detection (LOD) of HER2$^+$ EV detection using HRP-conjugated HER2 Affibody and HRP-conjugated HER2 antibody (n=3)**

| Method | Detection probe | LOD in PBS (EVs/mL) | LOD in plasma (EVs/mL) |
|---|---|---|---|
| SKBR3 EVs | HER2 Affibody | $9.5 \times 10^8$ | $2.1 \times 10^9$ |
| | HER2 antibody | $2.5 \times 10^9$ | $3.5 \times 10^9$ |
| HT-29 EVs | HER2 Affibody | $7.2 \times 10^8$ | $9.8 \times 10^8$ |
| | HER2 antibody | $1.9 \times 10^9$ | $2.4 \times 10^9$ |

### 4. Superiority of HER2 Affibody molecules over HER2 antibody in detecting HER2-positive EVs via fluorescence polarisation (FP)

While HER2 Affibody molecules could sensitively detect HER2$^+$ EVs, HER2 antibodies are not able to do so (Fig. 4).

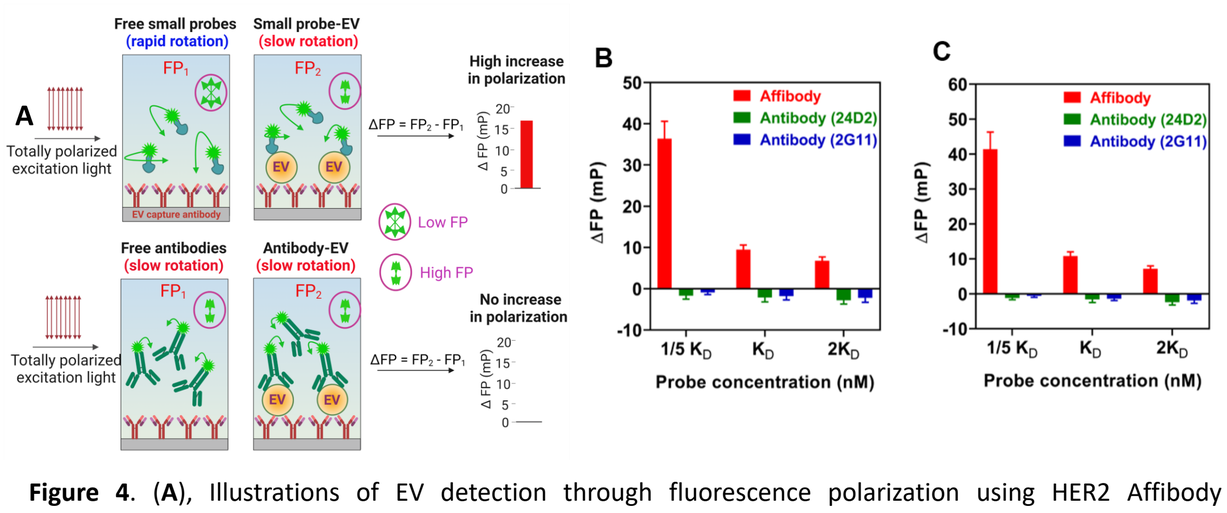


**Figure 4**. (**A**), Illustrations of EV detection through fluorescence polarization using HER2 Affibody molecules or HER2 antibody. (**B-C**), FP change of FP assays using HER2 Affibody molecules or HER2 antibodies (clones 24D2 and 2G11) at three concentrations of 1/5$K_D$, $K_D$, and 2$K_D$: (**B**), SKBR3 EVs; (**C**), HT-29 EVs. Data shown are means ± S.D, n=3.

## CONCLUSION

For the first time, the small ligands, HER2 Affibodies exhibit the size-dependent superiority over the bulky antibodies in accessing HER2 receptors on the surface of EVs. Moreover, these ligands surpass antibodies in detecting and quantifying HER2$^+$ EVs in colorimetric and fluorescence polarisation-based assays. These encouraging findings provide the way toward the utilization of Affibodies in EV characterization and EV-based liquid biopsy, especially single EV analysis in the future.

## REFERENCES

[1] Mathieu M, Martin-Jalular L, Lavieu G, Théry C. Nat Cell Biol, 21 (1) (2019), pp. 9-17.