

Cell-based Phage Panning

Phage panning was performed as previously described (1) with one exception, the antagonist antibody TB-001-003 was added to the phage in molar excess to bias the population toward high affinity binders.

Antibody Reformatting and Purification

Phage with high clonal populations were reformatted from single chain variable fragments (scFv) to full length IgG and purified by protein A packed pipet tips, as previously described (1).

Binding by Flow Cytometry

Expi293 cells were transiently transfected with the same GFP- and FLAG-tagged GLP-1R used for phage panning using expifectamine 293 transfection kit (ThermoFisher). 72 hours after transfection 25,000 cells were plated into each well of a v-bottom 384 well plate and incubated in PBS + 0.5% BSA for 30 minutes at 4C. Antibodies were incubated in PBS + 0.5% BSA for one hour at 4C. Cells were subsequently washed, fixed in 4% PFA and stained with an anti-human (Jackson Immuno., catalog #109-136-098) or anti-mouse (Jackson Immuno., catalog #115-606-071) secondary antibody conjugated to APC or alexa-647 for 30 minutes at room temperature. Binding was captured using an iQue3 flow cytometer (Satorius) with appropriate laser filter settings.

cAMP Assay

HEK 293T cells were transfected using calcium phosphate as previously described (2). GLP-1R and GloSensor (Promega, catalog # E2301) was co-expressed and plated onto white 384 well

plates (Corning, catalog # 354660) 24 hours after transfection. 24 hours after plating, cells were incubated in luciferin for two hours at room temperature. Antibodies and drugs were added at a 10X concentration, and a kinetic read was captured on an M5 plate reader (Molecular Devices).

β-arrestin 2 Recruitment

A split luciferase system was developed to assess β-arrestin 2 recruitment. GLP-1R was fused to the SmBit (Promega) on the C-terminus after a short GS linker and β-arrestin 2 was fused to LgBit (Promega) on the N-terminus with a short GS linker. HEK293T cells were transiently transfected with GLP-1R-SmBit and β-arrestin 2-LgBit at a ratio of 1:2 DNA. 24 hours after transfection, cells were plated onto white 384 well plates (Corning, catalog # 354660) and 24 hours after plating, the assay was performed using the Nano-Glo furimazine mixture (Promega, catalog #N1120). Antibodies and drugs were added at a 10X concentration, and a kinetic read was captured on an M5 plate reader (Molecular Devices).

1. Liu Q, *et al.* (2021) Functional GLP-1R antibodies identified from a synthetic GPCR-focused library demonstrate potent blood glucose control. *MAbs* 13(1):1893425.
2. Peterson SM, *et al.* (2015) Elucidation of G-protein and β-arrestin functional selectivity at the dopamine D2 receptor. *Proceedings of the National Academy of Sciences* 112(22):7097-7102.