A High-Throughput Platform to Develop Highly Potent and Functional Antibodies against G-protein Coupled Receptors

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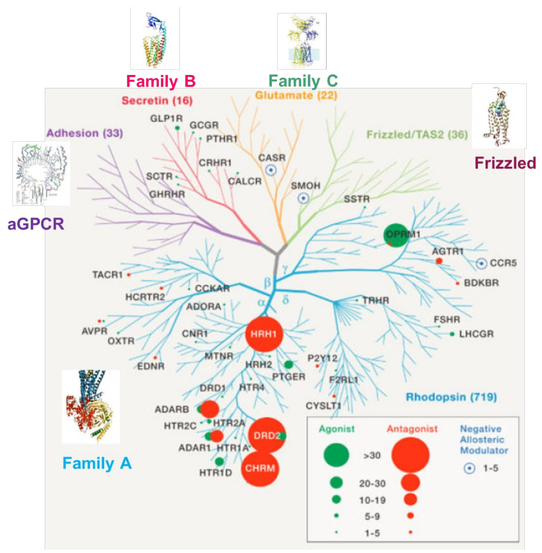
ABSTRACT

G-protein coupled receptors (GPCRs) are transmembrane proteins and the targets of a number of small-molecule drugs. Owing to their specificity, half-life, and inability to cross the blood-brain barrier, antibodies are also promising candidates for targeting these receptors. Though there are >500 GPCRs in humans, however, only two approved GPCR antibody-based drugs exist, which highlights the challenges in identifying functional antibodies against this receptor family.

GPCRs are well-studied and many ligands and peptides that bind to this family have already been identified, so one approach to tackling this challenge is to incorporate these natural binding partners into an antibody library design. We describe here the construction of a GPCR-focused phage-display antibody library. By mining GPCR binding ligands and peptides and incorporating their sequences into the heavy chain CDR3, we developed a design for identifying functional antibodies to a range of GPCR targets.

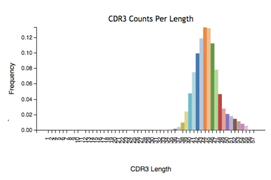
This platform also incorporates Twist's silicon-based DNA synthesis capabilities, which enable (1) precise library synthesis of high-diversity domain libraries and (2) rapid gene synthesis to dramatically shorten the time from lead identification to functional antibody validation. Here, we highlight the application of high-throughput platform to the generation of potent functional antibodies against multiple GPCR targets.

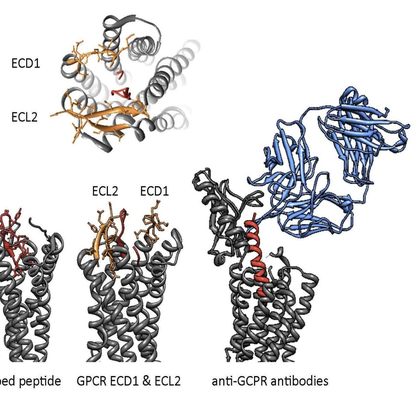


GPCR Target Profile

GPCR-FOCUSED PHAGE LIBRARY DESIGN

Leader	Heavy chain	Linker	Light chain	pIII
	IGHV1-69 IGHV3-30		IGKV1-39 IGKV3-15 IGLV1-51 IGLV2-14	



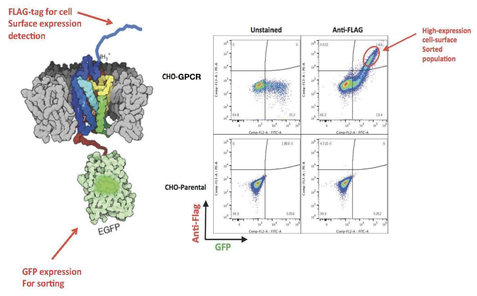


Design and Development of GPCR-Focused Libraries
Known GPCR interactions were analyzed to identify binding motifs, which were then engineered into the heavy chain CDR3 loop of antibodies. These binding partners included cytokines, peptides, external GPCR ECDs, GPCR extracellular loops, and anti-GPCR antibodies. Multiple frameworks were prescreened to tolerate these motifs and incorporated into the design for better expression, display, and stability. The CDRs were also screened to ensure they did not contain amino acid liabilities, cryptic splice sites, or nucleotide restriction sites.

Twist's long oligo synthesis technology was used to synthesize the DNA. The synthetic oligos were then assembled and electroporated into TG1 cells. We generated a final library with a diversity of 1.1 x 10^{10} that was verified by NGS to ensure good representation of our design.

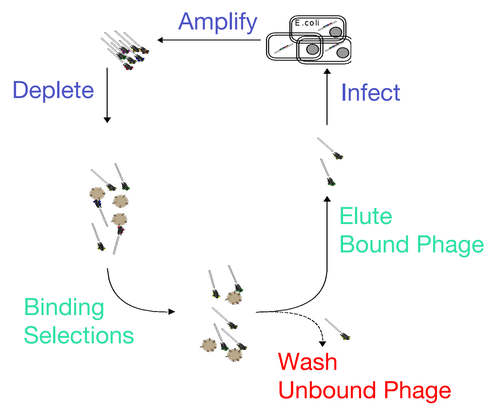
TWIST BIOPHARMA'S ANTIBODY DISCOVERY WORKFLOW

A. Stable Cell-Line Development



Stable cell lines expressing GPCR targets are generated, and target expression confirmed by FACS. Cells expressing >80% of the target are then used for cell-based selections (B).

B. Cell-Based Selections

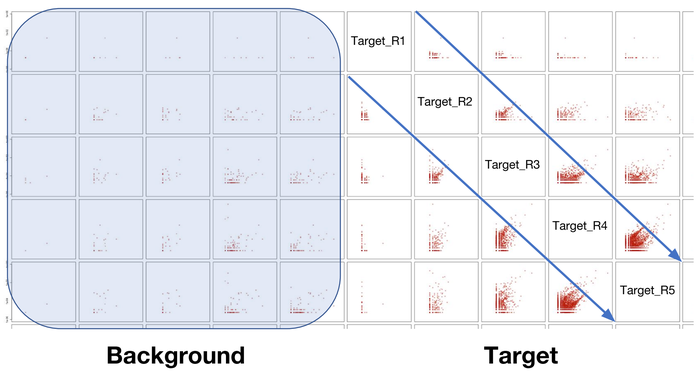


Cells overexpressing targets of interest are subjected to five rounds of selection to enrich for binding clones. For each round, 10^8 cells are used and before selection, phage from each round are first depleted on 10^8 CHO background cells. Stringency is increased in each round by increasing the number of washes. Enrichment ratios are monitored for each round of selection.

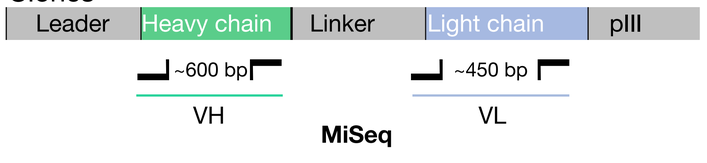
C. Next-Generation Sequencing

I. NGS Sequencing Follows Each Round of Selection

After each round of selection, phage pools are deep-sequenced by NGS to follow enrichment and identify cross-sample clones. Target-specific clones are selected after filtering out CHO background clones from the NGS data. This also offers motif enrichment information that helps identify functional binders.



II. High-Throughput Sequencing to Identify Unique Clones

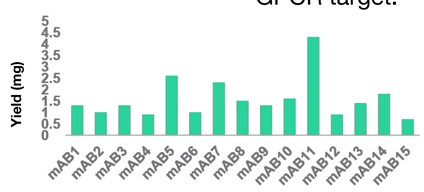


For each target, 2000 VH and VL pairs are barcoded directly from a bacterial colony and sequenced on a MiSeq to identify unique clones. In our process, the VH and VL linkage information is conserved. On average, 100-200 unique clones are identified for each target and are directly converted to IgGs for downstream characterization.

D. High-Throughput IgG Conversion and Purification

Twist Clonal Genes

Twist's DNA synthesis technology enables high-throughput gene conversion from scFv to IgG in 3 weeks. On average, the top 100-200 scFvs from phage selections are converted to full-length IgGs for any given GPCR target.

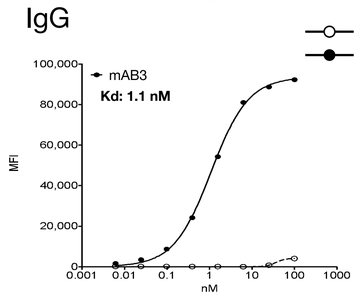


After IgG conversion, the clones are transiently transfected in ExpiCHO cells to produce IgGs. Magnetic bead separators, e.g. Kingfisher, and automation deck, are used for batch IgG purifications followed by lab-chip to collect purity data for all purified IgGs. High yields and purities are obtained from 10 ml cultures.

IgG	% Purity
mAb1	100
mAb2	100
mAb3	100
mAb4	100
mAb5	98
mAb6	100
mAb7	97
mAb8	100
mAb9	100
mAb10	100
mAb11	100
mAb12	100
mAb13	100
mAb14	100
mAb15	100

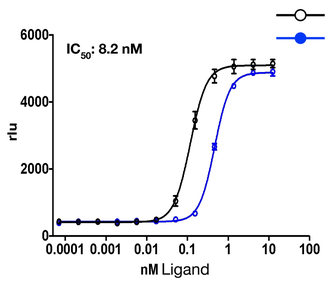
E. Cell-Based Affinity Measurements and Functional Assays with Purified IgGs

I. FACS binding with Purified IgG



Antibodies bind selectively to GPCR-expressing cells with affinities in the low nanomolar range. This example is of an antibody that selectively binds target expressing cell with an affinity of 1.1 nM.

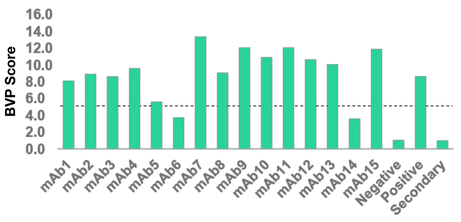
II. cAMP Assay with Purified IgG



Our cell-based assays identify functional antibodies with agonistic, antagonistic, or allosteric effects. Here is an example of an allosteric inhibitor against a GPCR.

F. Biophysical characterization of purified IgGs

Developability Test by BVP ELISA
Our BVP ELISA reveals some antibodies have BVP scores comparable to those from commercial antibodies.



Thermostability Screening
UNCLE is used for T_m and T_{agg} assessment.

SUMMARY

Our high-throughput antibody discovery platform identifies functional antibodies against GPCR targets within a few months. We have demonstrated that this robust platform can readily develop antibodies with affinities in the nanomolar range. These antibodies exhibited many modes of action to modulate GPCR activity**. In addition to this platform, for our lead antibodies, we can rapidly improve manufacturability and affinity with our Twist Antibody Optimization (TAO) process.**