Engineering mRNA display for GPCR drug discovery – de novo discovery of cyclic peptide GLP1R binders.

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Background & Aim

mRNA display enables screening of large (>10^{13}) randomized *in vitro* translated peptide libraries against a target of interest. Through several rounds of screening there will be an enrichment of sequences of highly potent and selective peptide binders of the target. These peptides can further be cyclised by disulphide bond formation during the translation step, which is advantageous for the potential for higher binding affinity.

The aim of this study was to explore the use of mRNA display for discovering disulphide-cyclised peptides that act against a family B GPCR, which naturally binds a linear helical peptide. We show how mRNA display can be used for screening against the GLP1R extracellular domain (ECD). By using clustering analyses, we aim to select sequences from distinct sequence families to better cover the scope of sequences for further functional screenings.

Methods

The mRNA library was transcribed from a DNA library encoding 4-15 randomised amino acids flanked by cysteines. Peptides were transcribed using the PURE system (Protein synthesis Using Recombinant Elements) and cyclised through disulphide bonds. Cyclisation was confirmed by UPLC-HR-MS. Five selection rounds of mRNA display were performed against the GLP1R ECD; positive and negative binders were sequenced using NGS.

Sequences of binders from round 5 were clustered based on their Levenshtein distances. Top peptides from each cluster were selected for preliminary affinity screenings, and selected peptides were tested in functional assays for inhibition of cAMP accumulation.

Conclusion

+ mRNA display allows for screening of large libraries of randomised disulphide-bonded cyclic peptides.
+ Sequences selected based on clustering criteria provided basis for further functional testing.
+ Unexpectedly, linear isoforms showed increased function with µM potencies in cAMP inhibition assays.

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1 mRNA display protocol outline

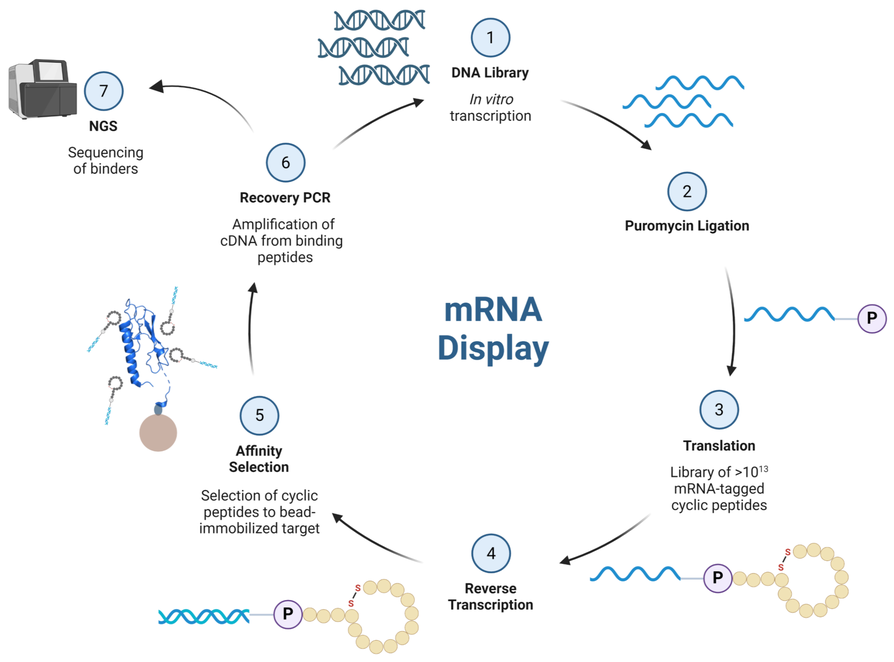


Figure 1. Protocol outline for mRNA display.

2 In vitro translation of disulphide-bonded peptides

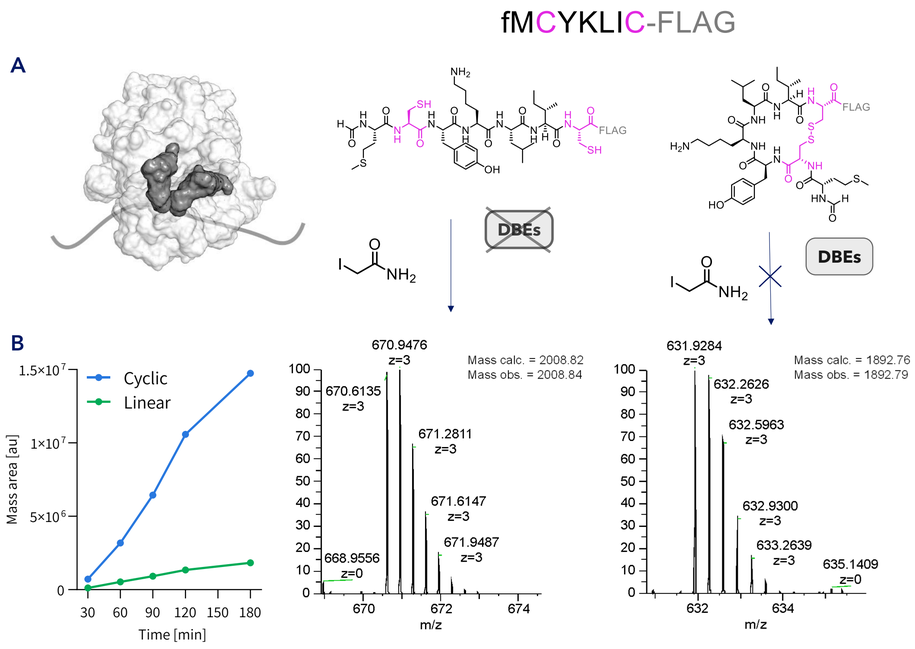


Figure 2. *In vitro* translation and cyclisation. (A) Translation of test-peptide fMCYKLIC using the PURE cell free translation system with and without disulphide-bond enhancers (DBEs). Free cysteines could react with iodoacetamide, and mass difference was measured using UPLC-HR-MS. Cyclised peptide was observed with addition of DBEs. **(B)** Amount of cyclic and linear peptide detected over time of fMCYKLIC translated in the presence of DBEs.

3 mRNA display selections were performed against hGLP1R ECD

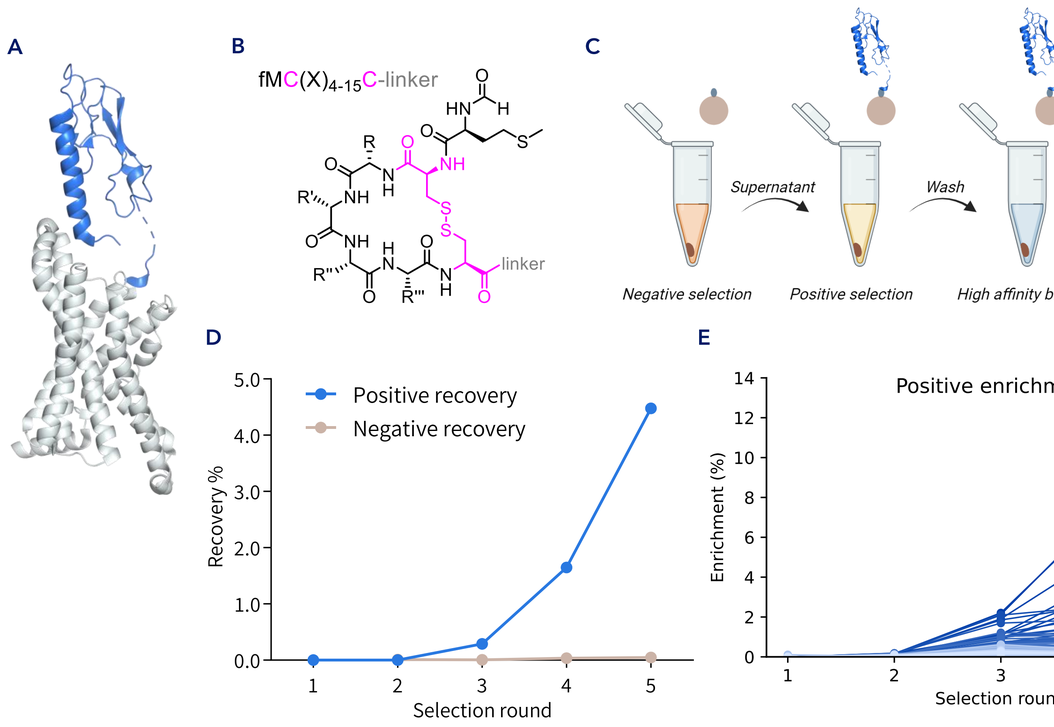
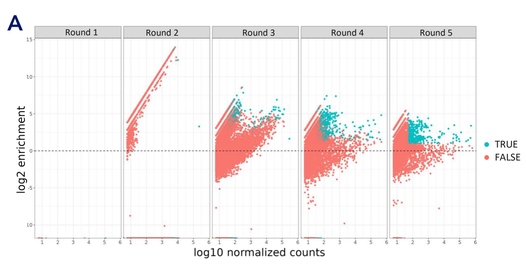


Figure 3. mRNA display screening for de novo binders of hGLP1R ECD. (A) ECD of hGLP1R shown in blue (PDB: 5VAI). **(B)** The starting peptide library consisted of unique sequences of 4-15 randomized amino acids flanked by cysteines and cyclised through disulphide-bonds. **(C)** The mRNA-tagged peptide library is first panned through a negative selection against empty streptavidin-coated beads. Non-binders in the supernatant are transferred and screened on target of interest bound to streptavidin-coated beads and supernatant is washed away. **(D)** Recovery percentage of positive and negative samples for each round of selections determined by qPCR analysis. **(E)** Enrichments of sequences found in positive and **(F)** negative samples for each round. Enrichment percentage of top 100 peptide sequences depicted.

4 Clustering analysis of binding sequences



Selection criteria for clustering analysis:

+ ≥ 2-fold positive/negative enrichment
+ ≥ 50 counts in round 5
+ ≥ 10 residues in common
+ ≥ 10 sequences per cluster

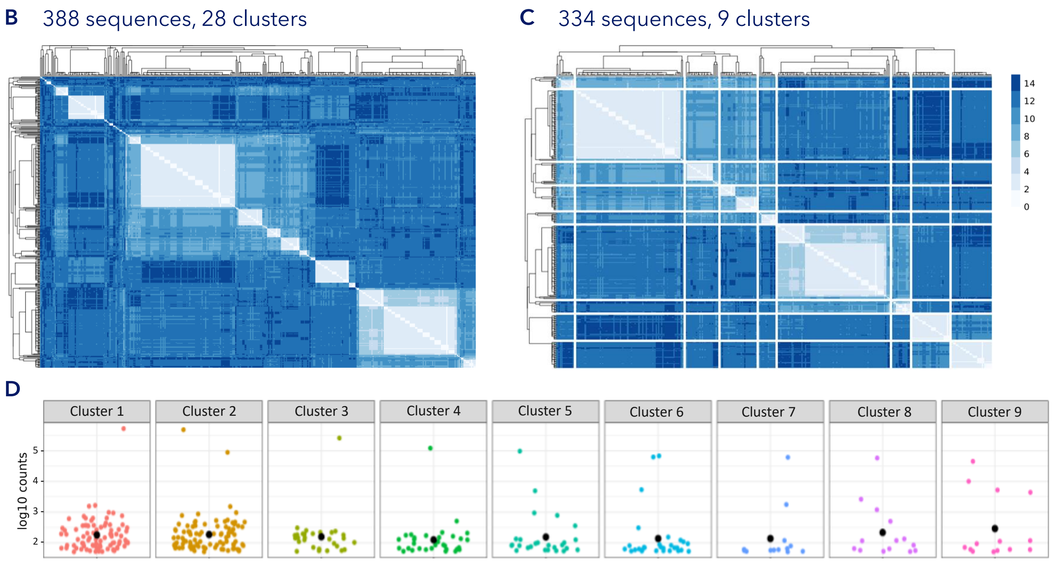


Figure 4. Clustering analysis of binding peptides. (A) Sequences with ≥ 50 counts found in positive selection round 5, and ≥ 2-fold positive/negative enrichment in round 5 were included for clustering analysis. **(B)** Heatmap of clustering based on Levenshtein distanced with ≥ 10 residues in common resulted in 388 sequences in 28 clusters. **(C)** Excluding clusters with < 10 sequences resulted in 334 sequences in 9 clusters. **(D)** Count in positive selection round 5 for each sequence per cluster. Black dot denotes average count per cluster. Top 5 ranking sequences from each cluster selected for preliminary crude screenings and from these, selected peptide sequences were chosen for further evaluations.

5 Functional inhibition of hGLP1R

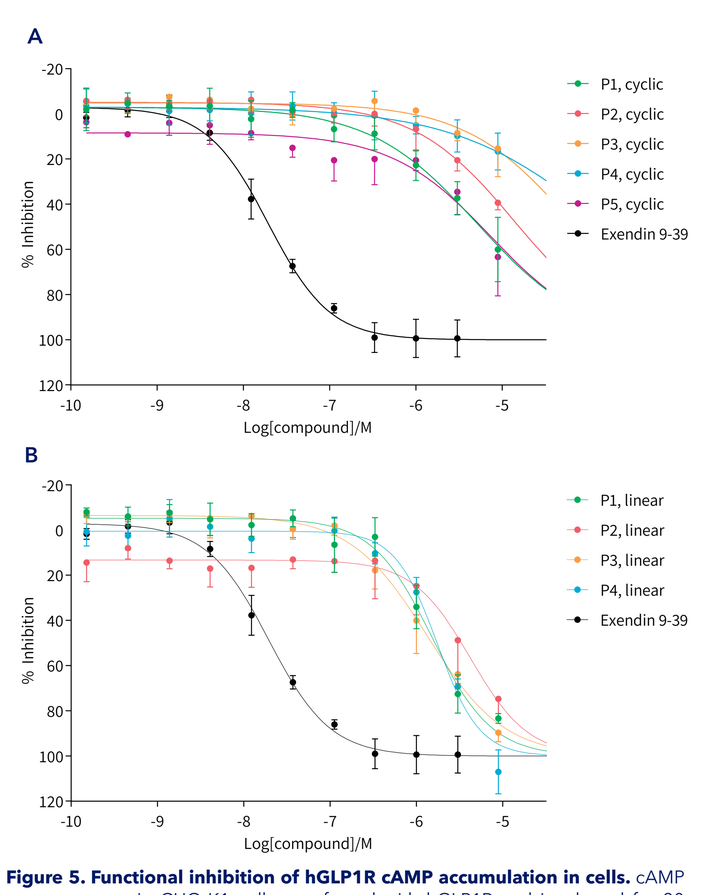


Figure 5. Functional inhibition of hGLP1R cAMP accumulation in cells. cAMP measurements in CHO-K1 cells transfected with hGLP1R and incubated for 30 minutes at room temperature with a concentration gradient of peptides 1-5 in purified cyclic **(A)** or linear **(B)** form along with a fixed concentration of 0.3 µM GLP1. Exendin 9-39 is a known hGLP1R antagonist. n=4.