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Design and Synthesis of Peptide Modulators of Gαi/s Proteins

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Introduction

The stimulation of G protein-coupled receptors (GPCRs) by ligand binding initiates the shuttle of heterotrimeric G proteins (Gαβγ) between the active (Gα-GTP) and inactive (Gα-GDP) state[1]. Subsequently, the active Gα-GTP subunit and the Gβγ dimer interact with downstream effectors that trigger several intracellular pathways. These can provoke cellular alterations that account for severe diseases. Such cellular changes can be investigated through peptides targeting specifically the Gα subunits[2-3]. For instance, the discovery of the macrocyclic depsipeptides YM-254890 and FR900359, inhibitors of the Gαq subfamily, evoked curiosity in developing linear and cyclic peptides against other Gα subunits. Interestingly, procedures such as the screening of **combinatorial peptide libraries** revealed the KB-752, GIV-Girdin, and GPM-1c/d (G protein modulator) peptides, which exhibit novel GEM (guanine nucleotide exchange modulator)-like activities by inhibiting Gαi and activating the Gαs signaling cascades[3-9].

Workflow

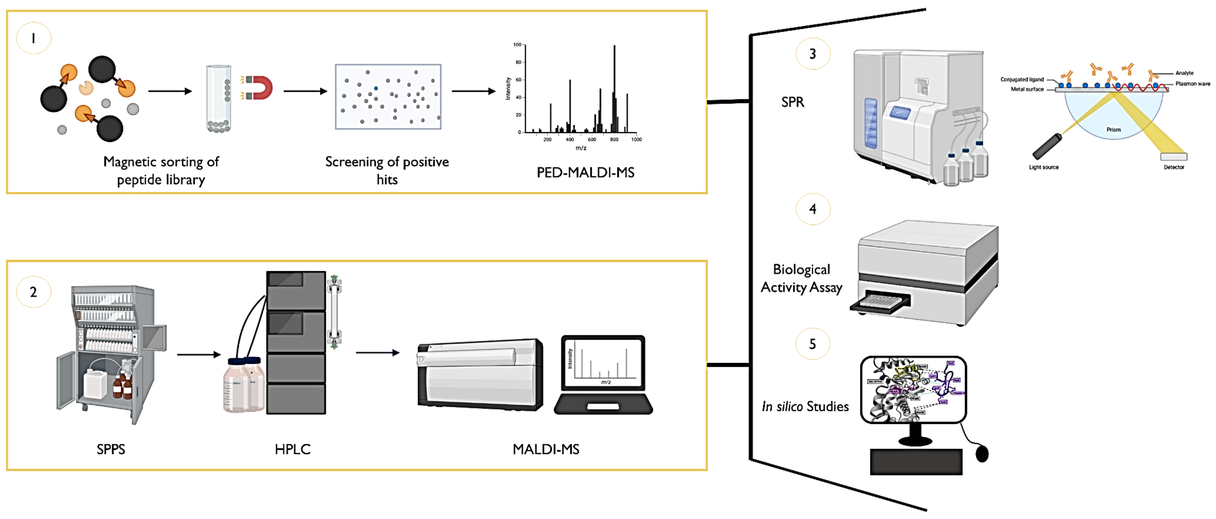
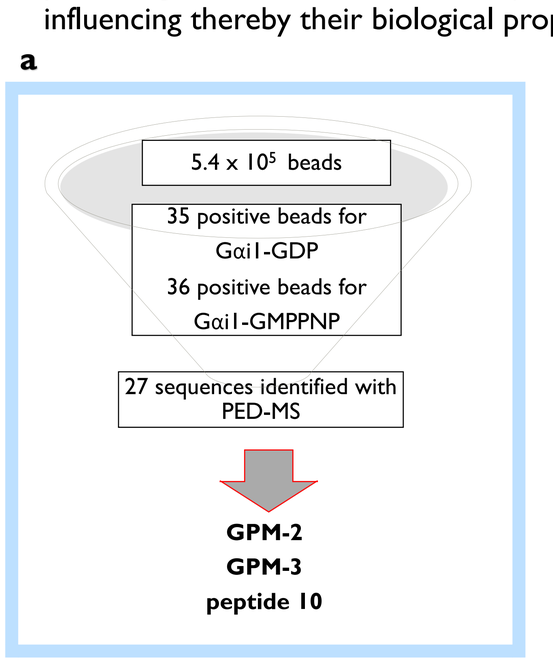
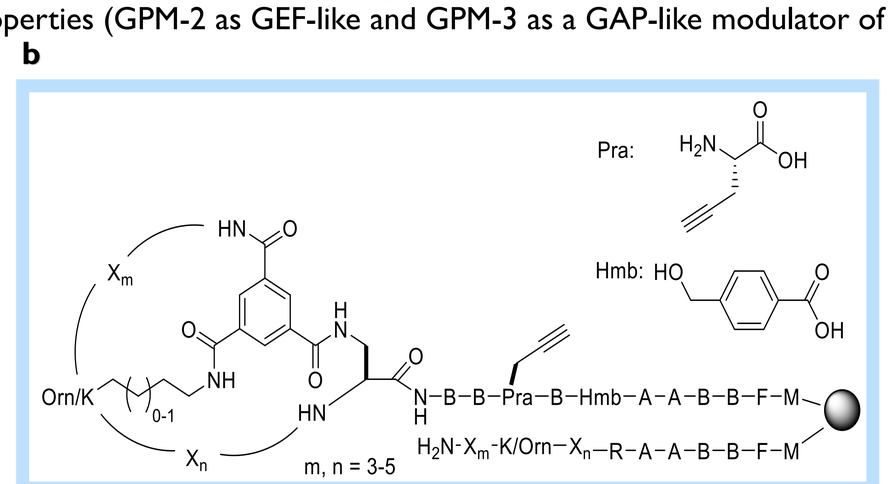


Figure 1. The methodology includes (1) peptide library screening, (2) synthesis, (3) binding analysis, (4) cellular activity assays, and (5) computational modeling and docking studies. Created with BioRender.com.

Results

- We previously identified a linear nonapeptide, GPM-1, from the screening of a one-bead-one-compound (OBOC) library against Gαi-GDP[3-5], which revealed high binding affinities to Gαi and Gαs, resulting in a bifunctional GEM-like activity. Chemical modifications of the GPM-1 lead compound, such as cyclization and conjugation with a cell-penetrating peptide (series GPM-1b/c/d), resulted in modulators with enhanced proteolytic stability and cell permeability.
- Second, an advanced **one-bead-two-compound (OBTC)** library was screened towards both activity states of the Gαi protein and revealed bicyclic peptides containing non-proteinogenic and D-amino acids (GPM-2, GPM-3, peptide 10, (*Fig. 2a-c)*). Cellular studies showed that the GPM-2/3 compounds bind to novel sites on the protein surface influencing thereby their biological properties (GPM-2 as GEF-like and GPM-3 as a GAP-like modulator of the Gαi signaling (*Fig. 2d*)).





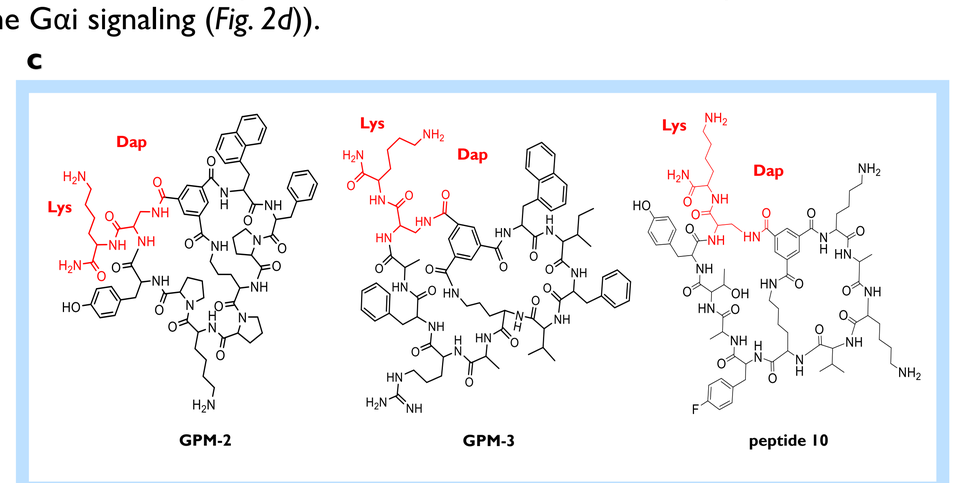
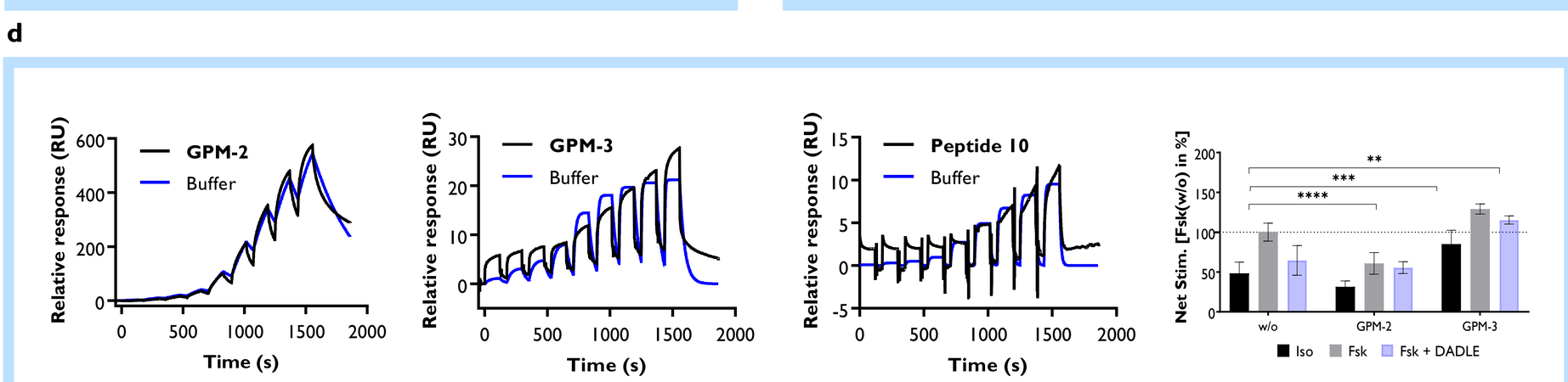
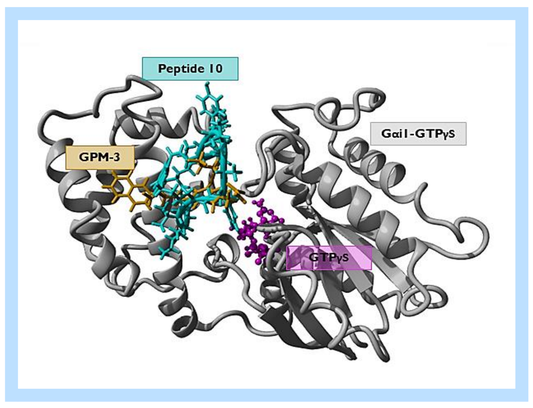
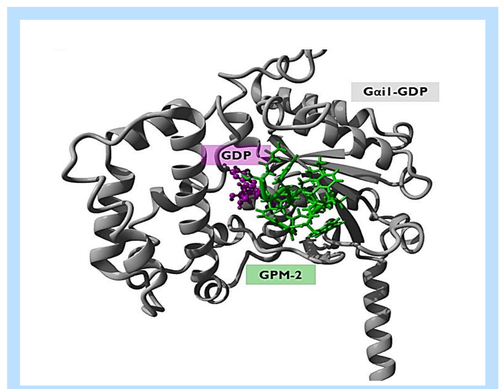


Figure 2. (a) OBTC combinatorial peptide library screening. (b) Synthesis scheme of peptides. (c) Chemical structures of bicyclic peptides GPM-2, GPM-3, and peptide 10. Amino acids occuring in all three peptides highlighted in red. (d) Membrane-based assays for GPM-2 and GPM-3 and SPR binding studies for compounds GPM-2, GPM-3 and peptide 10.



- *In silico* studies of the peptides GPM-2, GPM-3, and peptide 10 defined the structural similarities and differences between them (*Fig. 3a, b*). Protein-ligand docking studies also revealed similarities in the binding site between peptide 10 and GPM-3, which were found to interact between the α-helical and switch III region (SWIII) (*Fig. 3a*).





- Despite the binding site similarity, the K_D value (9.077 µM) was higher for peptide 10 compared to GPM-2 (5.0 µM) and GPM-3 (0.7 µM) (*Fig. 2d*). The reduced binding affinity may be a result of the positioning of the high numbers of hydrogen bond donor and acceptor residues (Lys, Thr, Tyr, Dap).
- Additionally, the interaction of both rings of peptide 10 with the protein might introduce unfavorable interactions, contributing to the overall weaker binding affinity.

Figure 3. Computational superimposition of peptides on the Gαi1 protein (a) Comparison of interaction sites of the peptides GPM-3 (yellow) and peptide 10 (cyan) on Gαi1-GTPγS (PDB: 1GIA[9]) (gray). (b) The superimposed position of GPM-2 on Gαi1-GDP[3] (gray). Nucleotides are shown in magenta.

Summary & Outlook

Our studies with different combinatorial peptide libraries revealed novel Gαi/s modulators with distinct guanine nucleotide exchange activities. Compounds GPM-2, GPM-3, and peptide 10 were identified from an OBTC peptide library with promising selectivity for Gαi. *In silico* studies unveiled new important structural features for Gαi binding, which were confirmed by comparison of peptide 10 to the Gαi modulators GPM-2 and GPM-3. These findings provide fundamental knowledge for deeper investigations of peptide 10, and optimization of the biological properties of the obtained compounds to achieve higher pharmacological potencies.

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Acknowledgment

DFG Deutsche Forschungsgemeinschaft
heinrichhertzstiftung
DAAD Deutscher Akademischer Austauschdienst German Academic Exchange Service
GDCh

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