Enhancing Affinity of Peptide and Peptoid Ligands Using Boronic Acid-Catechol Reversible Dimerization

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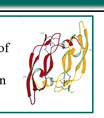
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The Abboud Lab

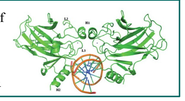
Overview

Dimerization of small peptides and peptoids enhances drug design by improving binding affinity and specificity, particularly for homodimeric drug targets. Bivalent ligands can simultaneously engage two binding sites, boosting efficacy, selectivity, and reducing side effects. Traditional dimerization often relies on irreversible reactions, increasing molecular weight and limiting bioavailability. In contrast, reversible dimerization uses chemoselective reactions to dynamically assemble monomers into dimers under specific conditions. This preserves pharmacokinetic properties like solubility and bioavailability while achieving high binding affinity and specificity at the target site

Dimer ribbon representation of the receptor-binding domain of VEGF

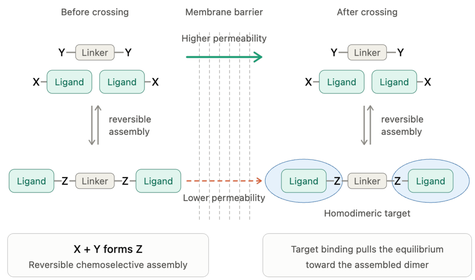


The structure of the p53 core domain in dimeric form bound to DNA



Reversible Chemistry for Increased Dimer Permeability

To exploit these benefits, we have developed a novel strategy for generating dimers using reversible chemoselective reactions. Our approach centers on a streamlined synthetic design: a single monomer is functionalized with a chemoselective handle, which then reacts with a symmetrical, difunctionalized linker to form the dimer. This modular assembly minimizes synthetic steps, avoids the need to prepare two different peptide arms, and allows for easy tuning of dimer geometry and spacing.



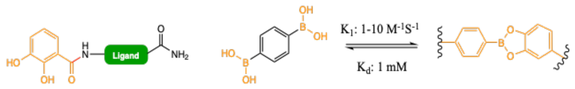
Why Boronic Acid–Catechol Chemistry?

Reversible Covalent Bonding:
Forms boronate esters through dynamic covalent interactions that remain reversible under physiological (pH 7) conditions due to equilibrium between free and bound states.

Highly Chemoselective:
Boronic acids are selective for catechol over natural amino acid side chains, enabling clean conjugation in complex mixtures.

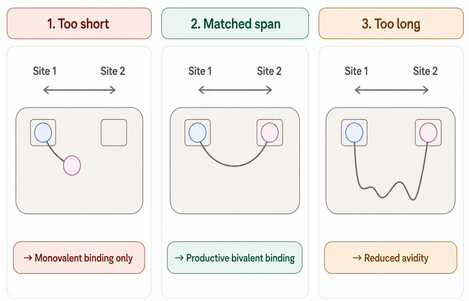
No Catalyst Required:
Proceeds efficiently in aqueous solution without the need for metal catalysts.

Arylboronic Acid Diol Complexation



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Why Linker Design Matters

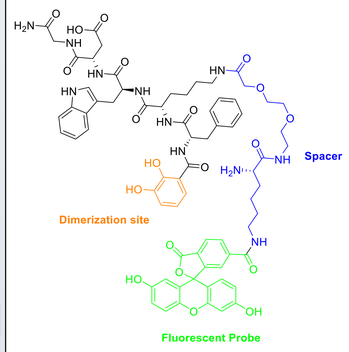


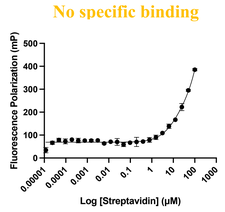
Bivalent binding depends on matching linker length and flexibility to the spacing between target binding sites. Matched linkers favor productive bivalent engagement, whereas poorly matched linkers can limit avidity.

Streptavidin – Proof of Concept

Streptavidin (SA), from *Streptomyces avidinii*, has an exceptionally high affinity for biotin (K_d ~10^{-15} M), making it a key tool in biotechnology and diagnostics. Beyond biotin, streptavidin can bind peptides with the GDWXF-like motif, which mimics biotin's structure. The motif interacts with streptavidin's binding pocket through hydrophobic/π–π contacts; binding is specific but weak, with Kd typically in the tens–hundreds of μM (sometimes up to mM)

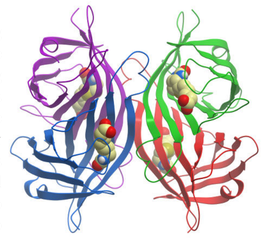
Monomer SA Binding Peptide





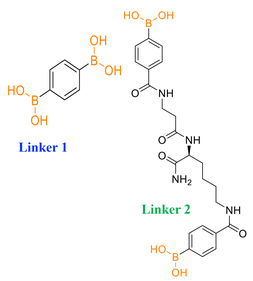
Binding isotherm of the monomer peptide against SA derived from fluorescence polarization experiments

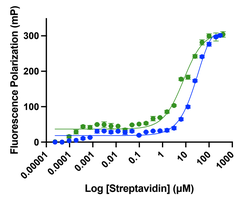
SA is a tetrameric protein with four binding sites, allowing it to interact with multiple ligands simultaneously. To enhance the binding strength of the monomer, a promising strategy is to dimerize the peptide, enabling it to engage two binding sites on the streptavidin tetramer simultaneously. However, the success of this approach depends on factors like spacing and flexibility of the linker between the two monomers. The linker must be designed to allow both monomers to access streptavidin's binding sites without steric hindrance, ensuring optimal binding efficiency.



Ligand bound Streptavidin homotetramer showing each unit binding to individual biotin molecules

Reversibly Dimerized SA Binding Peptide





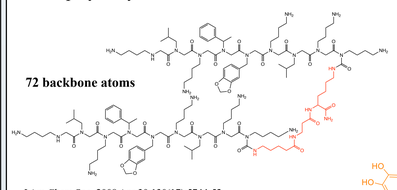
Binding isotherm of the reversibly dimerized peptide with linker 1 and linker 2 against SA derived from fluorescence polarization experiments

Dimerization enhances apparent affinity via avidity; linker length shifts the curve

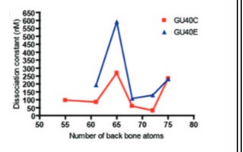
Successful application of this strategy to a SA binding peptide, demonstrating the feasibility and effectiveness of our approach.

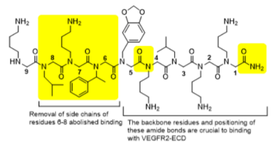
VEGFR2 / GU40C – Affinity Enhancement + Cellular Validation

The Kodadek group identified a peptoid (GU40C) antagonist of the cell surface Vascular Endothelial Growth Factor Receptor 2 (VEGFR2) with extremely high selectivity for this receptor, but modest affinity. To get a higher affinity, they took advantage of the fact that VEGFR2 functions as a dimer and synthesized dimers of the peptoid which resulted in an improvement of about 90-fold potency relative to the monomeric parent compound. Homodimerization was achieved irreversibly with a central Lys linker from which branch additional linker arms would separate the N-termini of each monomeric unit by approximately 45–85 Å. The best binding affinity was displayed by GU40C4 72 backbone atoms.

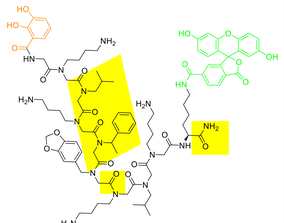


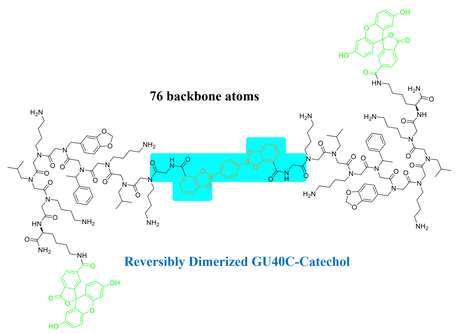
J Am Chem Soc. 2008 Apr 30;130(17):5744-52.



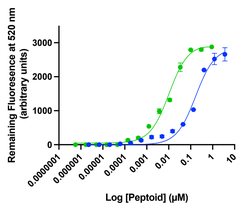


Bioorg Med Chem Lett. 2008 Nov 15; 18(22): 5892–5894.



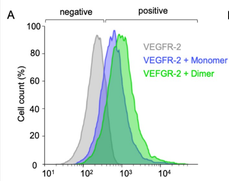


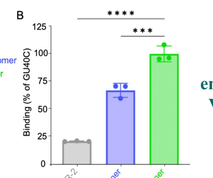
Binding Isotherm of GU40C Peptoids Against VEGFR2, Derived from ELISA-Like Experiments



≈ 100-fold increase in binding between monomer and dimer

Flow Cytometry Analysis of GU40C Cellular Uptake and Binding in VEGFR2 Expressing Cells



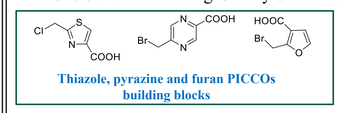


Reversible dimerization enhances GU40C binding to VEGFR2 expressing cells

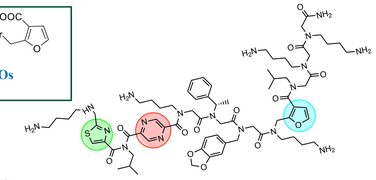
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VEGFR2 / GU40C-Th9Py8-Fr4 – SAR-Guided Optimization

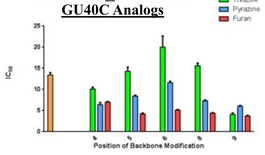
Peptoids generally lack conformational constraints due to their homogenous backbone. To enhance potency, we systematically altered backbone positions 4-9 with more rigid, structurally distinct analogs, also called PICCOs (Peptoid-Inspired Conformationally Constrained Oligomers). These substitutions increased spacing, with the aim of overcoming the conformational disorder of peptoids and enabling side chains to better match the geometry of the target.



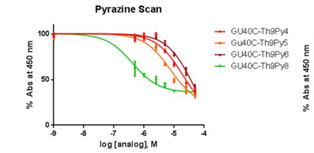
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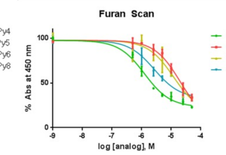


Relative IC_{50} of Modified GU40C Analogs

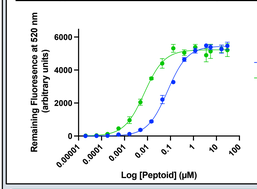


Thiazole is ineffective at positions 4–8 but improves potency at position 9, suggesting it should be used only there. In contrast, furan and pyrazine shows stronger activity across multiple positions, making them better suited for further optimization.





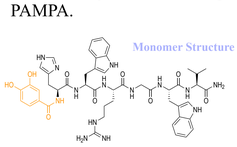
Binding Isotherm of GU40C Peptoids Against VEGFR2, Derived from ELISA-Like Experiments

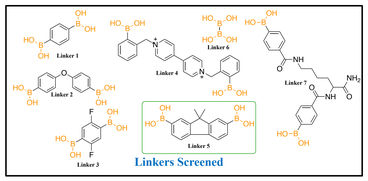


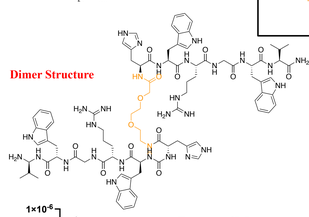
≈ 12-fold increase in binding between monomer and dimer

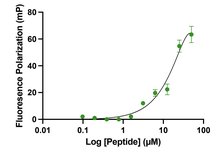
IgG1-Fc / HWRGWV – Linker Screening + Permeability

We extended this strategy to HWRGWV, a hexapeptide that binds human IgG1-Fc with ~10 μM affinity. Diboronic acid linkers were screened by FP, and a selected reversible system was compared with the monomer and irreversible dimer by PAMPA.

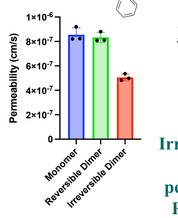






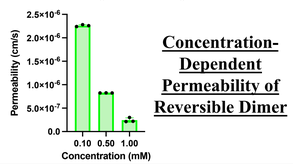


Binding isotherm of the reversibly dimerized peptide with linker 5 against IgG1-Fc derived from fluorescence polarization experiments



Reversible vs. Irreversible Dimer Permeability

Irreversible Dimer is about 38% less permeable than the Reversible Dimer



Concentration-Dependent Permeability of Reversible Dimer