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Exploring structural differences between antagonistic peptides for the development of orally bioavailable PCSK9 inhibitors

Bhavya Soni
Nova Southeastern University, bs1808@mynsu.nova.edu

Pritika Vemulapalli
Nova Southeastern University, pv239@mynsu.nova.edu

NSU Florida
Farquhar Honors College
NOVA SOUTHEASTERN UNIVERSITY

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3d molecular designs

Nova Southeastern University CREST Team

Bhavya Soni & Pritika Vemulapalli
Faculty Advisors: Emily Schmitt Lavin, Ph.D. and Arthur Sikora, Ph.D.
Halmos College of Arts and Sciences Nova Southeastern University, Fort Lauderdale, FL, 33314, USA

Exploring structural differences between antagonistic peptides for the development of orally bioavailable PCSK9 inhibitors

PDB File: 6U3I Chain A and B, 5VLP Chain D: LDLR antagonist peptide

Primary Citations:

Burdick, D. J., Skelton, N. J., Ultsch, M., Beresini, M. H., Eigenbrot, C., Li, W., Zhang, Y., Nguyen, H., Kong-Beltran, M., Quinn, J. G., & Kirchhofer, D. (2020). Design of organo-peptides as bipartite PCSK9 antagonists. *ACS Chemical Biology*, *15*(2), 425–436. https://doi.org/10.1021/acschembio.9b00899

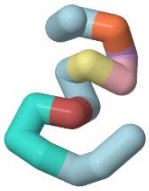
Zhang, Y., Ultsch, M., Skelton, N. J., Burdick, D. J., Beresini, M. H., Li, W., Kong-Beltran, M., Peterson, A., Quinn, J., Chiu, C., Wu, Y., Shia, S., Moran, P., Di Lello, P., Eigenbrot, C., & Kirchhofer, D. (2017). Discovery of a cryptic peptide-binding site on PCSK9 and design of antagonists. *Nature Structural & Molecular Biology*, *24*(10), 848–856. https://doi.org/10.1038/nsmb.3453

Description:

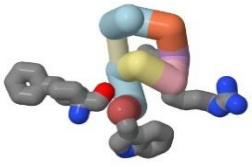
Familial hypercholesterolemia (FH) is an autosomal genetic disease which causes elevated blood levels of low-density lipoprotein (LDL). One of the main causes of FH is gain-of-function mutations in the gene coding for PCSK9 (proprotein convertase subtilisin/kexin type 9). The PCSK9 protein binds to low-density lipoprotein receptors (LDLR) on the surface of hepatocytes and promotes their degradation, preventing recycling of LDLRs and thus increasing LDL blood levels. PCSK9 inhibitors, a class of FH drugs, are injectable prescriptions containing monoclonal antibody therapies that bind to PCSK9 inhibiting LDLR binding. Orally bioavailable medications can be developed using small-molecule-peptide inhibitor strategies in a cryptic site (N-terminal groove) adjacent to the EGF(A) binding site located in the catalytic domain (Zhang et al., 2017). This groove contains a P' helix region (S153-I161) with conformational flexibility leaving the site open to small-molecule peptides. Fab7G7 acts as the crystallization chaperone (binds to the second module (M2) of the C-terminal domain) by instigating the conformational change. The peptide must contain two components: a P' helix like peptide with high binding affinity and an extension with antagonistic properties to inhibit LDLR binding. The extension must encroach upon the EGF(A) binding site's hydrophobic pocket, a significant contributor to EGF(A)'s binding energy. Bipartite inhibitors with organic moieties attached to the peptide core were found to have potent antagonistic activity and high binding affinity (Burdick et all., 2020).

Specific Model Details:

There are two peptides and one active site. 6U3I and 5VLP, each, contain a different peptide which binds to the N-terminal groove of the catalytic domain of PCSK9. The binding site (shown in Model A. View 1) is derived from 6U3I. The binding site, peptide A, and peptide B contain magnets to show the structural interactions. Peptide A and Peptide B will both be removable from the binding site.



Peptide A



Peptide B

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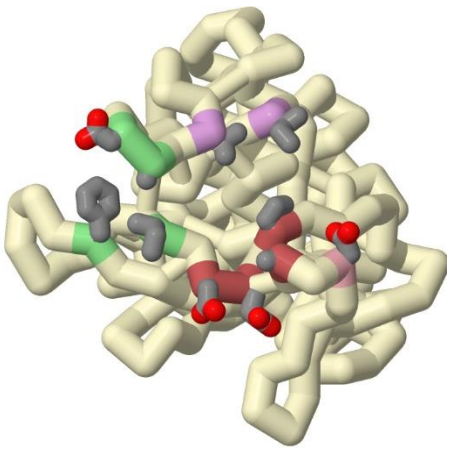
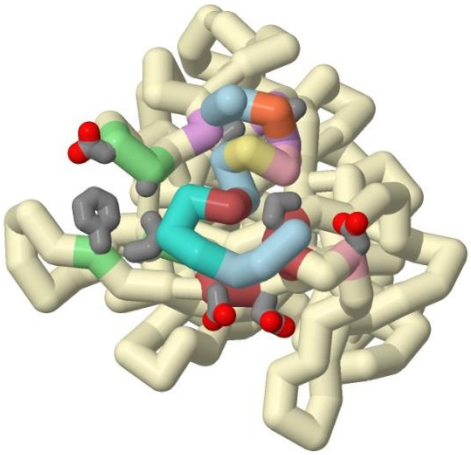
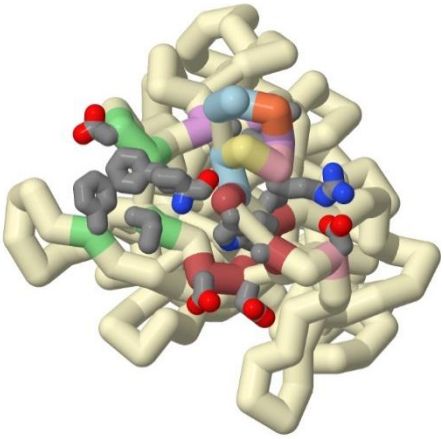


Model A. View 1: No peptide attached



Model A. View 2: Peptide A attached



Model A. View 3: Peptide B attached

Significant Interactions between PCSK9 and Peptides A and Peptide B		
Interaction (color noted on model)	**Residues**	**Explanation**
1 (lightpink)	**PCSK9:** Asp343 **Peptide A:** hArg5 **Peptide B:** hArg5	The L-homoarginine substitution from L-arginine improved binding affinity through the formation of a new salt bridge interaction by the side chain guanidium group and the Asp343 residue on PCSK9.
2 (plum/purple)	**PCSK9:** Val241, Ile395 **Peptide A:** Ile6 **Peptide B:** Ile6	The binding of isoleucine to the extremely hydrophobic region on PCSK9 (Val241 and Ile395) increased binding affinity due to van der Waals contact.
3 (indianred)	**PCSK9:** Ala341, Pro364, Glu366, Asp367 **Peptide A:** Trp1 **Peptide B:** Trp1	Integrating tryptophan as the vector choice was confirmed by 1) the van der Waals interactions with Ala341 and Pro364, and 2) the hydrogen bonding between tryptophan's indole and the backbone carbonyls of Glu366 and Asp367.
4 (lightgreen)	**PCSK9:** Ile369, Phe379, Asp238, Ala239 **Peptide A:** N/A **Peptide B:** 1-amino-phenylcyclohexane-1-carbonyl*	The PCSK9 contact residues were seen to interact with the organic moiety of peptide B, intruding in the hydrophobic pocket of the EGF(A) binding region. This pocket is a significant contributor to EGF(A)'s binding energy. This interaction led to the inhibition of PCSK9.

*Colored cpk to show hydrophobic functional group of the organic moiety

Some important residues are highlighted on the model as well. (1) In khaki/yellow, the fourth residue (val4 in peptide A and lys4 in peptide B) in the peptide core was found to be a surface-exposed residue instead of a contact residue, making lysine the best substitution with increased binding affinity. (2) In coral/orange, the gly7 (peptide A) to d-Ser7 (peptide B) substitution is highlighted. This D-serine substitution favored the required positive φ backbone dihedral angle and further assisted in positioning the lysine residues downstream. This change resulted in a 2-3-fold gain in affinity. (3) Phe-2, Pro-1, Gly0 (FPG motif) in peptide A is colored turquoise. Initially it was thought that the FPG motif and the anchor (tryp1) made a beta-turn causing the proline 5 residue to extend into the EGF(A) binding site, creating steric clash. Instead, with further modeling and experimentation, it was shown to preclude further extension.