

Computational investigation of myoferlin-C2D-targeting potent peptides

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Abstract: Myoferlin (MYOF) is a multidomain protein involved in cellular membrane dynamics and vesicular processes. Its C2D domain has emerged as a region of interest for molecular targeting of MYOF. However, the systematic design and molecular-level characterization of peptide candidates targeting C2D remain limited. Here, we aimed to computationally design short peptide candidates targeting the MYOF-C2D domain and evaluate their interactions with C2D. *De novo* peptide candidates were generated using RFdiffusion, followed by docking analysis, molecular dynamics simulations, and binding free energy calculations. Among 10,000 designs, 100 candidates were selected for docking, and the 10 candidates identified from the docking results were further evaluated using 100-ns MD simulations. Eight candidates maintained their interactions with C2D throughout the simulation, whereas two failed to form stable complexes. Among the peptide candidates, SIENIAAY exhibited the most favorable calculated MM/PBSA binding free energy and maintained its interaction with C2D throughout the simulation, supported by multiple polar and hydrophobic contacts. However, while SIENIAAY showed a predicted negative The blood–brain barrier (BBB) permeability, the second-ranked peptide, DLVAELYRKLLE, showed a positive BBB permeability prediction. Therefore, C2D-binding potential and predicted BBB accessibility were considered together, and DLVAELYRKLLE was selected as a potential peptide candidate targeting the MYOF-C2D domain.

Keywords: Myoferlin, peptides, molecular dynamics simulation

1 Introduction

Cancer-related mortality is largely associated not only with the local growth of the primary tumor but also with the development of invasion and metastasis.¹ Metastatic progression encompasses a multistep process involving the invasion of surrounding tissues by tumor cells, their entry into the circulation, and colonization of distant organs.² Throughout these processes, membrane dynamics, vesicular trafficking, and the regulation of cell-surface receptors play critical roles.³

MYOF, a type II transmembrane protein belonging to the Ferlin family, is involved in processes such as membrane fusion, membrane repair, endocytosis, and vesicular trafficking.^{4,5} Initially associated primarily with muscle development and membrane repair, MYOF has now been implicated in tumor progression across various malignancies, including breast, pancreatic, colorectal, gastric, and lung cancer. In addition to supporting tumor cell proliferation,

migration, and invasion. Furthermore, MYOF contributes to critical processes involved in cancer progression, including angiogenesis, epithelial–mesenchymal transition (EMT), metabolic reprogramming, and exosome-mediated intercellular communication.^{6–9} Therefore, MYOF has emerged as a potential therapeutic target for cancer treatment.

MYOF is a multidomain protein composed of seven C2 domains (C2A–C2G), together with FerA, FerB, DysF, and a C-terminal transmembrane region.⁴ While the C2 domains have distinct roles in membrane and protein interactions, the C2D domain has emerged as a region through which MYOF-associated functions in tumor progression can be pharmacologically modulated.^{10,11} In particular, targeting MYOF C2D with WJ460, a known MYOF–C2D inhibitor, has been shown to disrupt the MYOF–Rab7 interaction and associated endosomal functions in breast cancer cells, thereby altering receptor tyrosine kinase trafficking and reducing EGFR degradation. In addition, several studies have demonstrated that the MYOF–C2D axis can be targeted by different small molecules and that this approach may affect tumor cell invasion, migration, and proliferation.^{12–14}

As an alternative to small molecules, peptides have attracted considerable attention in cancer research as molecular tools due to their high targeting specificity, their

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amenability to rational sequence design, and their ability to be readily conjugated with imaging agents. Recent studies have also highlighted the utility of peptide-based compounds combined with molecular docking approaches for investigating potential target interactions.^{15,16} Targeting peptides can be used to recognize tumor-associated molecular targets and can also be conjugated with fluorescent or radionuclide labels for molecular imaging and diagnostic applications. They may further be conjugated with drugs, toxins, or nanoparticles for targeted delivery and therapeutic applications. Therefore, the development of peptides capable of recognizing MYOF-C2D may not only provide new candidates for the functional targeting of MYOF but also offer the potential to develop molecular tools that could, at later stages, be adapted for imaging or targeted delivery platforms. Nevertheless, current studies targeting MYOF-C2D have predominantly focused on small molecules, while the systematic development of C2D-targeting peptide candidates and the molecular- and dynamic-level characterization of these interactions remain limited.

Molecular docking and molecular dynamics simulations have also been applied to investigate the structural basis of protein–protein interactions and to characterize interaction interfaces.¹⁷ In this study, short peptide candidates targeting the MYOF-C2D domain were designed *de novo*, and their interactions with C2D were investigated using molecular docking and molecular dynamics approaches.

2 Experimental

2.1 RFDiffusion-Based Peptide Design and Molecular Docking

Peptide candidates targeting the MYOF-C2D domain were designed *de novo* using the RFDiffusion algorithm.¹⁸ The cryo-EM structure of the MYOF complex (PDB ID: 9H6X), obtained from the Protein Data Bank, was used as the design template. The C2D domain was defined as residues 1135–1273 according to the domain architecture reported by Dominguez et al.¹⁰ and was extracted from the full-length MYOF structure using Discovery Studio.¹⁹ A total of 10,000 peptide models ranging from 6 to 8 amino acids in length were generated. Among these models, 100 peptide candidates meeting the pAE_interaction <10 criterion were selected for molecular docking analysis (<https://github.com/RosettaCommons/RFDiffusion>). Following molecular docking, the 10 peptide candidates with the lowest HPEPDOCK ITScore values were selected for molecular dynamics simulations and binding free energy analyses. Twelve 12-amino-acid peptide sequences were selected from a previously generated RFDiffusion-derived peptide pool. MYOF was not specified as the target during the initial generation of the peptide pool.

Blind molecular docking analyses were performed using the HPEPDOCK web server (<http://huanglab.phys.hust.edu.cn/hpepdock/>) to identify the potential binding sites of the designed peptides on the extracted C2D domain.^{20,21} No pre-defined binding site was specified during docking, allowing the peptides to explore the entire surface of C2D for the most favorable binding conformations. HPEPDOCK generated 10

representative clusters, and the resulting docking poses were ranked according to their ITScore values (Table S1). For each peptide, the top-ranked cluster based on the ITScore was selected as the representative docking pose for subsequent molecular dynamics simulations and binding free energy analyses.

2.2 MD Simulations

MD simulations were performed using GROMACS.²² The protein–peptide complexes were parameterized with the CHARMM36m force field,²³ and the TIP3P water model was used for solvation. Each system was placed in a dodecahedral simulation box with a minimum distance of 1.0 nm between the protein and the box boundaries. For each protein–peptide complex, three independent 100-ns molecular dynamics simulations were performed. System neutrality was achieved by adding appropriate numbers of Na⁺ and Cl[−] ions, followed by the addition of NaCl to a final concentration of 0.15 M to mimic physiological ionic strength.

2.3 Molecular Dynamics Simulation Analyses

All simulation trajectories were analyzed using a standardized analysis workflow. Prior to analysis, water molecules and ions were removed from the trajectories. Root means square deviation (RMSD) and root means square fluctuation (RMSF) analyses were performed using VMD version 1.9.4,²⁴ and the resulting graphs were generated with XMGrace (Grace plotting tool). Three-dimensional molecular visualizations were prepared using UCSF ChimeraX.²⁵ Free energy landscape (FEL) analyses were performed using Python 3.9. Binding free energy calculations were carried out using the gmxMMPBSA,²⁶ while protein–peptide interactions were analyzed using Discovery Studio Visualizer 2024.²⁷ Detailed simulation analysis and non-covalent interaction for all peptides are provided in Supplementary Information (Figure S1). BBB permeability of the designed peptides was predicted using the B3BPFN web server (<https://ycclab.cuhk.edu.cn/b3bpfn/index.php>) based on ESM2 embeddings, iFeature-Omega descriptors, feature fusion, and a TabPFN classifier.

2.4 Solid Phase Peptide Synthesis and Characterization

BBB-penetrating candidate peptides were synthesized by Fmoc-based solid-phase peptide synthesis (SPPS) on 100 mg ring amid resin. Fmoc deprotection was performed using 20% piperidine/DMF, while HBTU and DIPEA were used for amino acid activation and coupling, respectively. Following chain assembly, peptides were cleaved from the resin using TFA/TIPS/H₂O (95:2.5:2.5, v/v/v) and lyophilized.²⁸ Peptide identities were confirmed by LC-HRMS/MS. Samples showing the expected molecular masses were purified by preparative RP-HPLC using a Hypersil GOLD C18 column and analyzed using an Inertsil ODS-3 C18 column. Samples were prepared in acetonitrile/water (1:1, v/v) at 0.3–1.3 mg/mL for HPLC analysis (Figure S2).

2.5 MTT Assay

The cytotoxic effects of the selected peptides on human brain microvascular endothelial cells (HBMECs) were evaluated

using the MTT assay. Peptides were dissolved in 2.5% DMSO and subsequently diluted in cell culture medium to the desired concentrations (25, 50, 100, and 500 μM). Cell viability was determined by measuring the reduction of MTT to formazan, with absorbance recorded at 570 nm (Figure S3). Each experiment was performed in triplicate.

2.6 Statistical Analysis

Statistical analyses were performed using two-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test in GraphPad Prism 9. All experiments were performed in three independent replicates ($n = 3$). A p -value < 0.05 was considered statistically significant.

3 Results and Discussion

MYOF is a member of the Ferlin family of proteins found in eukaryotes and contains multiple C2 domains involved in Ca^{2+} signaling.²⁹ It is highly expressed particularly in skeletal and cardiac muscle cells, which are susceptible to membrane damage during contraction. In addition, recent studies have shown that MYOF expression is elevated in various human cancers and that alterations in MYOF-mediated vesicular trafficking and cellular functions are associated with cancer cell proliferation, metastatic behavior, and chemoresistance.^{10,12} These properties make MYOF a promising therapeutic target and biomarker in cancer biology. Accordingly, in this study, short peptide candidates capable of targeting the MYOF-C2D domain were designed, and the molecular behavior of the complexes formed between these candidates and the target domain was evaluated using MD simulations. Among the peptides designed de novo using RFdiffusion, 8 of the 10 candidates subjected to MD simulations maintained their interactions with C2D across the three independent simulations, whereas the remaining 2 candidates failed to form stable complexes (Figure S1). These findings indicate that the designed candidates exhibit distinct interaction potentials toward C2D and support the prioritization of candidates that form stable complexes for further analysis.

Among the peptide candidates that maintained their interaction with C2D throughout the MD simulations, SIENIAAY exhibited the most negative mean MM/PBSA binding free energy ($\Delta G = -40.81 \pm 3.19$ kcal/mol) (Table 1). However, considering the associated uncertainties, this value was not clearly distinguishable from that of GMEYVEQAIKEI ($\Delta G = -38.80 \pm 5.20$ kcal/mol). Therefore, SIENIAAY was selected as a computationally prioritized candidate based on its numerically lower mean MM/PBSA binding free energy. The differences in the energy components among the candidates indicate that the overall binding energy should not be evaluated based on a single energy term. For example, although SSTSGLSR exhibited a highly favorable electrostatic contribution ($\Delta E^{\text{ele}} = -108.20$ kcal/mol), this contribution was largely offset by the high positive polar solvation energy ($\Delta E^{\text{GB}} = +111.29$ kcal/mol), resulting in a ΔG_{MMPBSA} value of -20.37 kcal/mol. In contrast, SIENIAAY exhibited a more balanced energy profile resulting from the combined contributions of electrostatic (-55.12 kcal/mol), van der Waals (-35.97 kcal/mol), and favorable non-polar solvation (-4.39 kcal/mol) terms, leading to the most favorable overall ΔG_{MMPBSA} value among the evaluated candidates (Table 1). Therefore, the prioritization of SIENIAAY was based not on the dominance of a single energy component, but on the overall energetic balance reflected in the combined contributions to the total binding energy. However, considering that the MM/PBSA approach does not directly measure experimental binding affinity and is an approximate method based on several assumptions, the obtained value of -40.81 kcal/mol should be interpreted not as an absolute binding free energy, but as a computational metric supporting the relative prioritization of candidates.

When the interactions of the peptide candidates with C2D were examined, the overall structural integrity of the target domain was largely preserved, and the peptides were observed to maintain their positions on the C2D surface throughout the simulation (Figure S1 and Figure 1). While the RMSD profiles of C2D and the SIENIAAY-C2D complex remained stable within the range of 0.1–0.2 nm over 100 ns, the higher ligand RMSD values, ranging from 0.4–0.6 nm,

Table 1. MM/PBSA binding free energy analysis over the 100ns MD simulation. ΔE^{ele} (ΔEEL)-Electrostatic Energy, ΔE^{vdw} ($\Delta\text{VDWAALS}$)-Van der Waals Energy, $\Delta G^{\text{PB}}/\Delta E^{\text{GB}}$ -Polar Solvation Energy, $\Delta G^{\text{SA}}/\Delta E^{\text{SURF}}$ -Non-polar Solvation Energy

Target-Peptide Complex	ΔE^{ele} (kcal/mol)	ΔE^{vdw} (kcal/mol)	$\Delta G^{\text{PB}}/\Delta E^{\text{GB}}$ (kcal/mol)	$\Delta G^{\text{SA}}/\Delta E^{\text{SURF}}$ (kcal/mol)	ΔG_{MMPBSA} (kcal/mol)	BBB permeability
C2D-EELELLFE	46.54	-27.40	-41.78	-3.23	-25.88 (± 6.01)	5.0%
C2D-GLLLYKEE	-49.80	-33.88	52.25	-4.02	-35.46 (± 5.19)	11.1%
C2D-GMEYVEQAIKEI	-34.41	-41.49	+41.98	-4.88	-38.80 (± 5.20)	17.6%
C2D-GPLLYIAE	-60.07	-30.54	+63.18	-3.60	-31.03 (± 3.50)	19.5%
C2D-PPLLYIEE	-26.63	-28.73	+31.97	-3.40	-26.79 (± 8.09)	39.6%
C2D-SIENIAAY	-55.12	-35.97	+54.67	-4.39	-40.81 (± 3.19)	21.6%
C2D-SLEEYLEL	+26.28	-26.82	-20.15	-3.13	-23.82 (± 6.44)	6.7%
C2D-SSTSGLSR	-108.20	-20.78	+111.29	-2.68	-20.37 (± 4.93)	58.3%
C2D-DLVAELYRKLE	-20.66	-35.04	+19.96	-4.20	-39.94 (± 5.32)	75.2%

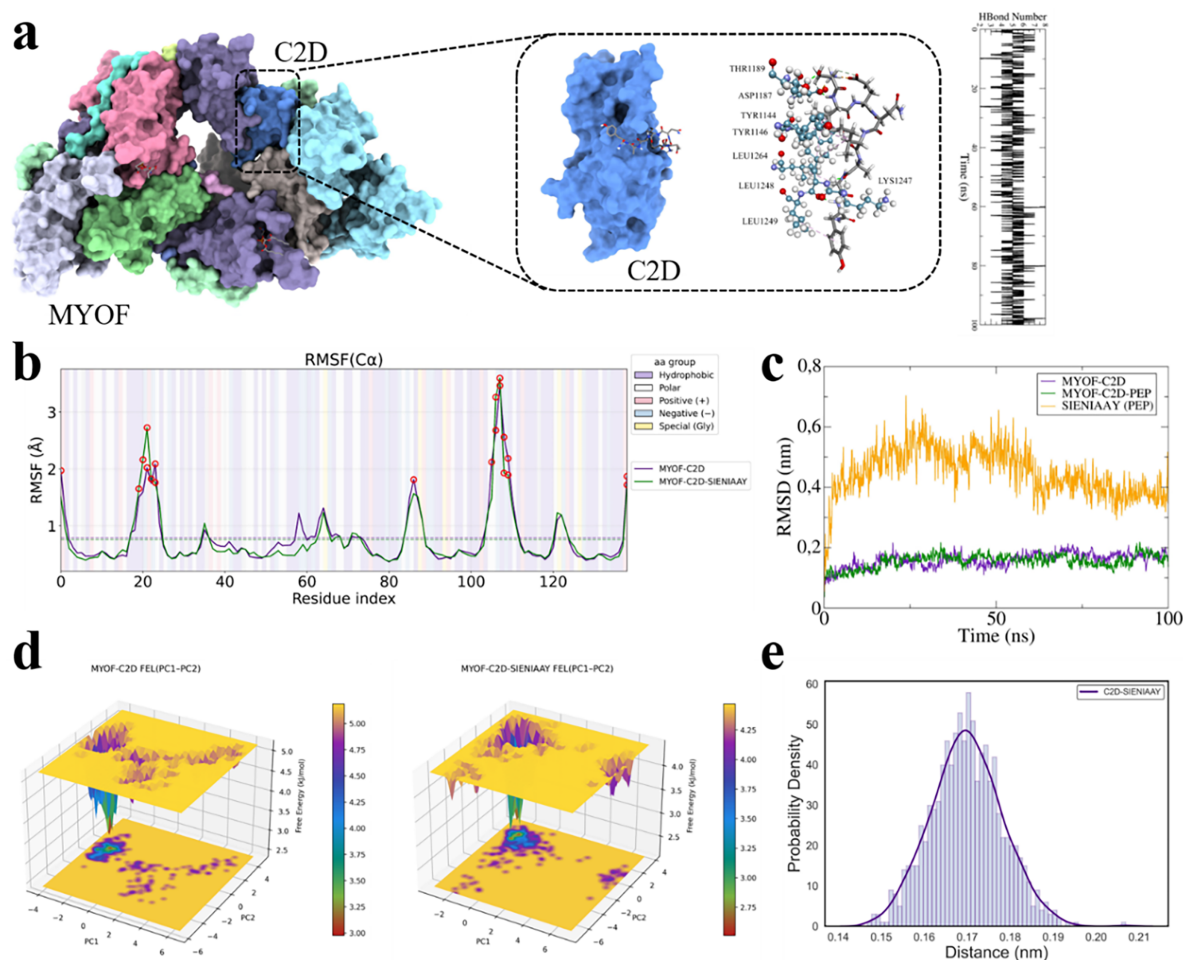


Figure 1. Molecular dynamics analysis of the SIENIAAY-MYOF-C2D complex. (a) Location of the C2D domain within the MYOF structure and the binding model of the SIENIAAY peptide on the C2D surface, together with the time-dependent distribution of hydrogen-bond interactions within the complex. (b) RMSF profiles of the Cα atoms of free C2D and SIENIAAY-bound C2D systems. (c) RMSD profiles of C2D, the SIENIAAY-C2D complex, and the SIENIAAY peptide over 100 ns. (d) Free energy landscape (FEL) analyses of free C2D and the SIENIAAY-C2D complex calculated based on principal components. (e) Probability density distribution of contact distances observed in the SIENIAAY-C2D complex

indicate that SIENIAAY exhibited a more dynamic conformational behavior compared with C2D (Figure 1c). This observation is consistent with the ability of short peptides to exhibit greater conformational flexibility upon binding to protein targets. Similarly, the preservation of similar fluctuation patterns in the RMSF profiles of ligand-bound and unbound C2D, together with the pronounced peaks observed at the same amino acid residues, supports the notion that peptide binding does not induce large-scale structural destabilization of C2D (Figure 1b). In a recent cryo-EM study of human MYOF, the C2C-C2D region was identified as part of the relatively rigid Fer-core module of MYOF, and this region was reported to exhibit low conformational variability across different functional states.¹¹ Therefore, these findings suggest that C2D provides a suitable target surface for peptide interactions while preserving its overall structural integrity, and that this property may be considered in the future development of peptides for diagnostic and imaging applications.

The interaction analysis of SIENIAAY with C2D indicates that complex stability is maintained through the combined contribution of multiple polar and hydrophobic interactions rather than a single contact point (Figure 1a and Table 2). In particular, the close contacts between ASP1187 and the SER1 and ILE2 residues of the peptide suggest that this region may represent an important polar interaction site for the positioning of the peptide on the C2D surface. In addition, the hydrogen bonds between TYR8-LEU1248, THR1189-SER1, and LEU1248-ALA6, together with the hydrophobic contacts between ILE2-LEU1248, ILE5-LEU1248, TYR8-LEU1249, and TYR1144-ILE5, indicate that the peptide is retained on the target surface through multiple contact points.

The contacts observed around LEU1248 and LEU1249 are particularly noteworthy in the context of previous MYOF-C2D ligand studies (Table 2). Previous studies have shown that the WJ460 molecule directly binds to MYOF-C2D and that residues including Leu1249, His1251, Phe1232, Ser1230, and Glu1241 are involved in ligand interactions based on

Table 2. The non-covalent molecular associations defining the C2D-SIENIAAY interaction surface. “-” denotes electrostatic interactions and interactions with distances exceeding 4.00 Å

MYOF-C2D	Residue involved in hydrogen bonds and positions	X...H distance (Å)	Bond type
SIENIAAY	SER1:HT2-ASP1187:OD1	1.63	Salt Bridge
	ILE2:HN-ASP1187:OD1	1.72	Hydrogen Bond
	TYR8:HN-LEU1248:O	1.96	Conventional Hydrogen Bond
	THR1189:HG1-SER1:OG	1.93	Hydrogen Bond
	LEU1248:HN-ALA6:O	1.91	Hydrogen Bond
	SER1:HB1-ASP1187:OD1	2.64	Carbon Hydrogen Bond
	ALA7:HA-LEU1248:O	2.85	Carbon Hydrogen Bond
	LYS1247:HA-ALA6:O	2.86	Carbon Hydrogen Bond
	ILE2-LEU1248	–	Hydrophobic-Alkyl
	ILE5-LEU1248	–	Hydrophobic-Alkyl
	TYR8-LEU1249	–	Hydrophobic-Pi-Alkyl
	TYR1144-ILE5	–	Hydrophobic-Pi-Alkyl

initial docking analyses.¹² Accordingly, the contacts observed around LEU1248–LEU1249 with SIENIAAY point to a preferred interaction region of the peptide on C2D. Importantly, the binding site of SIENIAAY was identified using a blind docking approach performed across the entire C2D domain, without targeting a previously defined ligand-binding site. The contacts observed around Leu1248–Leu1249 in our study suggest that this region may be favorable for interactions with different molecular ligands. However, a recent cryo-EM study reported that the C2D binding site previously proposed for WJ460 is not accessible in either the lipid-free or lipid-bound conformations of full-length MYOF,¹¹ highlighting the need for further studies to determine whether this region represents the cellular target site of WJ460. Because the present docking calculations were performed using the extracted C2D domain rather than the complete MYOF structure, the potential influence of neighboring domains on the accessibility of the predicted peptide-binding surface cannot be fully excluded. Therefore, the interaction of SIENIAAY with Leu1248–Leu1249 should be interpreted as a domain-level computational observation, and its accessibility in the native full-length MYOF conformation remains to be experimentally established.

The concentration of the contact distance distribution around approximately 0.17 nM in Figure 1e indicates that short-range contact geometries were predominantly maintained within the complex. Similarly, the sampling of distinct low-energy conformational regions by the SIENIAAY-bound system in the FEL analysis shown in Figure 1d supports the presence of preferred conformational states throughout the MD simulation. Taken together, the RMSD, RMSE, FEL, and contact analyses provide a consistent picture of the structural stability of the SIENIAAY–C2D complex throughout the simulation. However, it should not be assumed that the 100-ns MD simulation captured all slow conformational transitions; recent studies have reported that MM/PBSA results are sensitive to sampling adequacy and the force field used, and that short simulations may exhibit apparent artificial convergence in some systems.³⁰ Therefore, the present findings support

SIENIAAY as a computationally prioritized C2D-targeting peptide candidate, but do not establish its experimental binding affinity or biological activity. However, target-binding potential alone may not be sufficient for selecting peptide candidates intended for brain-targeted applications.

BBB represents a major physiological obstacle to the delivery of peptides and other macromolecular agents to the central nervous system due to the restrictive properties of brain microvascular endothelial cells, tight junctions, limited vesicular transport, and active efflux mechanisms.^{31,32} Since the C2D domain of MYOF is located on the cytoplasmic side, it may not be appropriate to consider this domain as a target directly accessible from the vascular lumen. Nevertheless, the potential of the peptides developed in this study to reach the brain environment was additionally evaluated in terms of their potential applicability in brain-related applications.^{33,34} Following the MD simulation, SIENIAAY, which exhibited the most favorable binding energy for MYOF, was predicted to have negative BBB permeability (21.6%), whereas the DLVAELYRKLL peptide, which showed the second most favorable binding energy, was predicted to have positive BBB permeability, with a score of 75.2% (Table 1). This difference indicated that not only C2D-binding potential but also predicted BBB accessibility could be considered when selecting candidates for experimental evaluation. However, computational prediction of BBB permeability has certain limitations, as BBB transport is not determined solely by passive physicochemical properties and may also involve mechanisms such as receptor-mediated or adsorptive-mediated transcytosis. Therefore, predicted BBB permeability was not considered definitive evidence of experimental BBB penetration but rather as a complementary criterion to the binding properties in candidate selection. Accordingly, DLVAELYRKLL was selected and synthesized for experimental evaluation based on the combined consideration of its C2D-binding potential and predicted BBB accessibility.

Although DLVAELYRKLL exhibited greater conformational fluctuations than SIENIAAY, its higher peptide RMSD did not translate into pronounced destabilization of the MYOF–C2D–peptide complex (Figure 2c). The

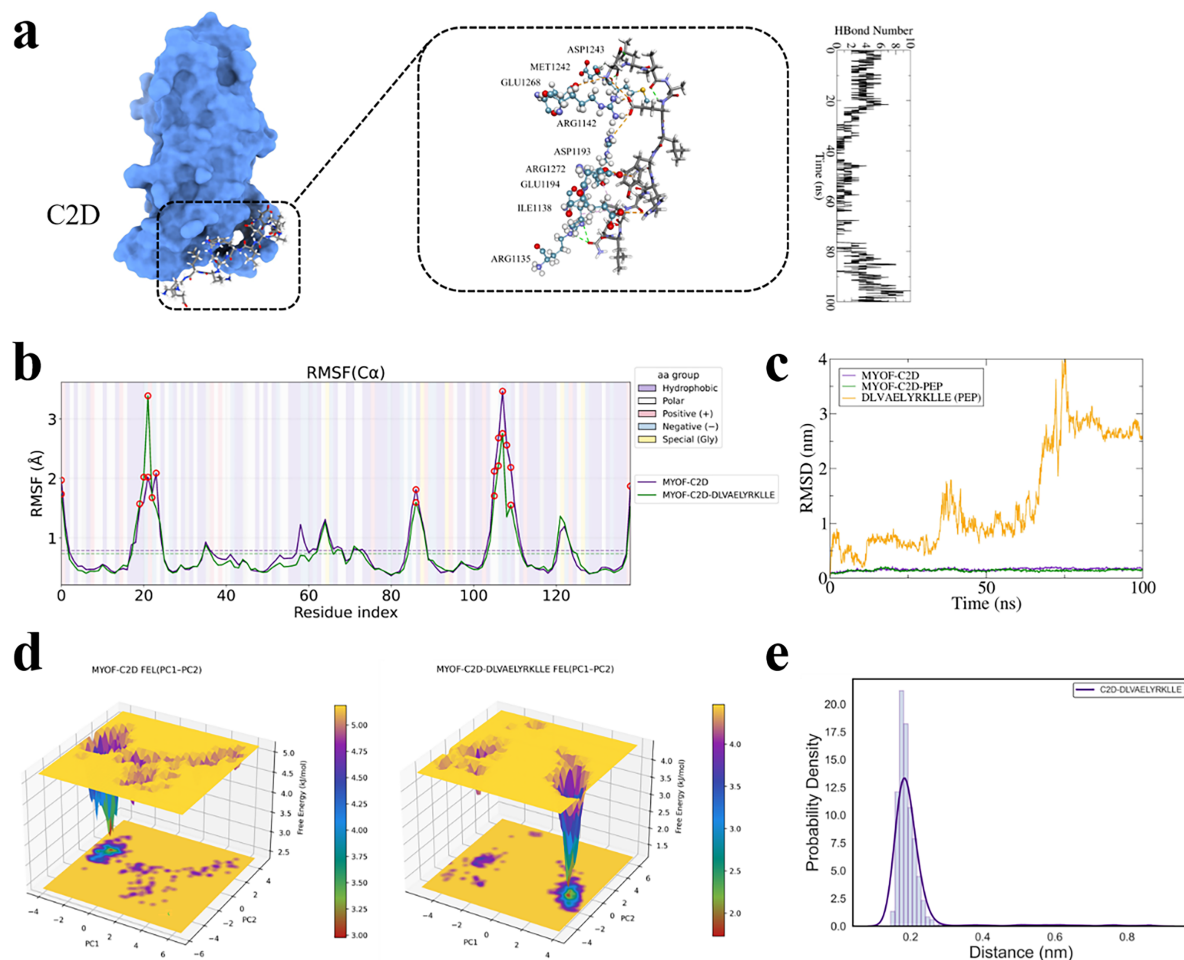


Figure 2. Molecular dynamics analysis of the DLVAELYRKLE-MYOF-C2D complex. (a) Location of the C2D domain within the MYOF structure and the binding model of the DLVAELYRKLE peptide on the C2D surface, together with the time-dependent distribution of hydrogen-bond interactions within the complex. (b) RMSF profiles of the Cα atoms of free C2D and DLVAELYRKLE-bound C2D systems. (c) RMSD profiles of C2D, the DLVAELYRKLE-C2D complex, and the DLVAELYRKLE peptide over 100 ns. (d) Free energy landscape (FEL) analyses of free C2D and the DLVAELYRKLE-C2D complex calculated based on principal components. (e) Probability density distribution of contact distances observed in the DLVAELYRKLE-C2D complex

RMSF profiles of apo C2D and the peptide-bound system remained largely comparable, indicating that peptide binding did not substantially perturb the overall flexibility of the C2D domain (Figure 2b). Interaction analysis further showed that DLVAELYRKLE maintained multiple contacts with C2D throughout the simulation (Figure 2e). In particular, Glu5 of DLVAELYRKLE formed short-range salt-bridge and attractive-charge interactions with Arg1142 of C2D, with X...H distances of 2.15 and 1.98 Å. Additional hydrogen bonding involving Arg1135-Glu12, together with hydrophobic interactions such as Leu10-Ile1138 and Leu10-Arg1272 and a Tyr7-Arg1272 hydrophobic- π -alkyl interaction, further contributed to the interaction network (Figure 1a and Table 3).

Thus, although the peptide displayed substantial conformational mobility, this flexibility was accompanied by the maintenance of multiple electrostatic, hydrogen-bonding, and hydrophobic contacts with C2D. Consistent with these observations, the FEL analysis revealed a distinct low-energy

basin for the MYOF-C2D-DLVAELYRKLE complex, suggesting the preferential population of specific conformational states during the simulation (Figure 2d). Moreover, the pronounced contact-distance distribution around approximately 0.18 nm further supported the persistence of short-range interactions within the complex. Although the calculated MM/PBSA binding free energy of DLVAELYRKLE was less favorable than that of SIENIAAY, the difference did not preclude DLVAELYRKLE from exhibiting a favorable interaction with C2D. Importantly, DLVAELYRKLE showed a positive predicted BBB permeability, whereas SIENIAAY was predicted to be BBB-negative. Therefore, rather than relying solely on the calculated binding free energy, C2D-binding behavior, conformational characteristics, and predicted BBB accessibility were considered together, supporting the selection of DLVAELYRKLE for experimental validation.

In addition, treatment with DLVAELYRKLE did not significantly affect HBMEC viability at either 24 or 48 h across

Table 3. The non-covalent molecular associations defining the C2D-DLVAELYRKLLE interaction surface. “-” denotes electrostatic interactions and interactions with distances exceeding 4.00 Å

MYOF-C2D	Residue involved in hydrogen bonds and positions	X...H distance (Å)	Bond type
DLVAELYRKLLE	ARG1142:HH12-GLU5:OE2	2.15	Salt Bridge; Attractive Charge
	ARG1142:HH22-GLU5:OE1	1.98	Salt Bridge; Attractive Charge
	ASP1:N-GLU5:OE2	–	Electrostatic
	ASP1:N-GLU1268:OE2	–	Electrostatic
DLVAELYRKLLE	ARG8:NH2-GLU1194:OE1	–	Electrostatic
	ARG1272:NH2-GLU5:OE1	–	Electrostatic
	ASP1:HT3-ASP1243:O	2.59	Hydrogen Bond
	LEU2:HN-ASP1:OD2	1.80	Hydrogen Bond
	GLU5:HN-VAL3:O	2.16	Hydrogen Bond
	ARG1135:HE-GLU12:O	2.67	Hydrogen Bond
	ARG1135:HH21-GLU12:O	3.06	Hydrogen Bond
	ASP1243:HN-ASP1:O	2.60	Hydrogen Bond
	ASP1:HA-ASP1243:O	2.54	Carbon Hydrogen Bond
	MET1242:HA-ASP1:O	3.08	Carbon Hydrogen Bond
	ASP1193:OD2-TYR7	–	Electrostatic
	LEU10-ILE1138	–	Hydrophobic-Alkyl
	ARG1272-LEU10	–	Hydrophobic-Alkyl
	TYR7-ARG1272	–	Hydrophobic-Pi-Alkyl

the tested concentrations (25–500 μ M). Although the overall two-way ANOVA indicated a statistically significant effect ($p < 0.001$), none of the pairwise comparisons reached statistical significance following Tukey’s multiple comparisons test (Figure S3). A slight upward trend in cell viability was observed at some concentrations; however, these differences were not statistically significant. Overall, these findings indicate that DLVAELYRKLLE did not exert a significant cytotoxic effect on HBMECs under the tested conditions, supporting its cytocompatibility and further experimental evaluation.

4 Conclusion

Our study demonstrates that the MYOF-C2D region represents a structure that can be considered for peptide-based molecular targeting and that computational approaches can be used to prioritize candidate peptides targeting this region. This approach provides a preliminary screening strategy that may reduce time and resource requirements by enabling the identification of suitable candidates before proceeding to experimental studies. Given the multifaceted role of MYOF in cancer biology, peptides targeting C2D may have potential for future investigation in targeted molecular imaging, delivery systems, or approaches aimed at modulating MYOF-mediated processes.

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Author Contributions

Özgür Yılmaz and Fulya Çağlar contributed equally to the study and manuscript preparation. Both authors approved the final manuscript.

Availability of Data and Materials

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Artificial Intelligence (AI) Statement

The authors utilized ChatGPT (OpenAI) for enhancing language and correcting grammatical errors, while ChatGPT (OpenAI) was employed to aid in the creation of the graphical abstract and figures. All content generated with the assistance of AI was thoroughly reviewed, verified, and sanctioned by the authors, who assume complete responsibility for the precision and integrity of the final manuscript.

Conflict of Interest

The authors declare that they do not have any conflict of interest.

Supporting Information

Supporting information accompanies this paper on <http://www.acgpubs.org/journals/organic-communications>.

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