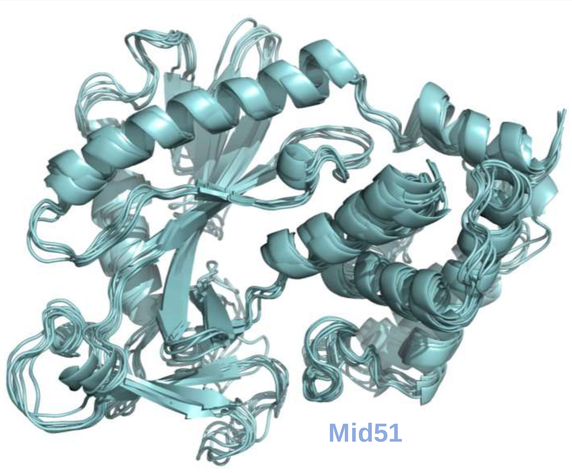


Peptide Therapeutics Targeting Drp1-Mid51 Protein-Protein Interactions to Preserve Mitochondrial Dynamics in Cardiovascular Disease

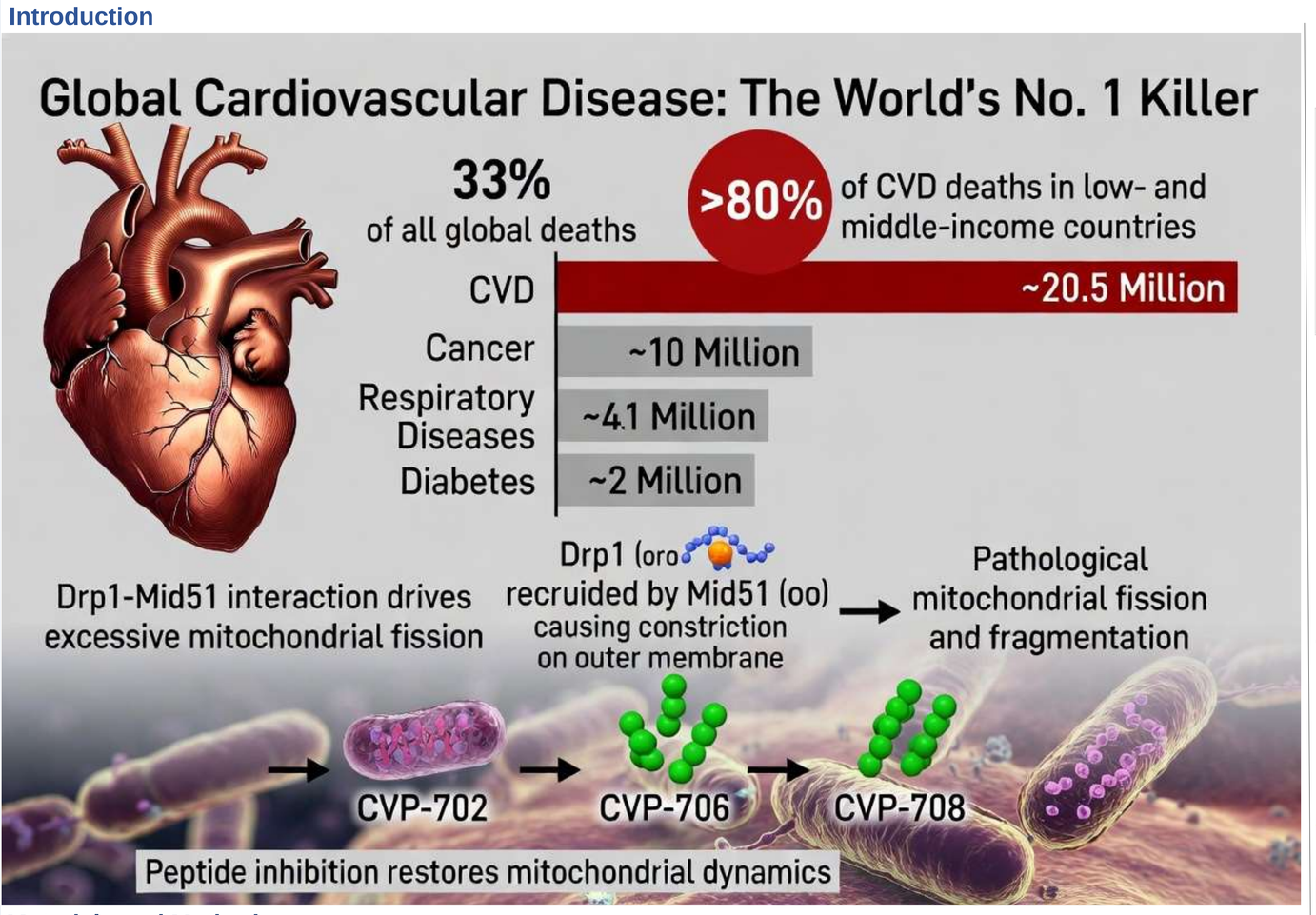
Rajkumar Khubiram, Liron Davis, Nir Qvit
The Azrieli Faculty of Medicine in the Galilee, Bar-Ilan University, Safed 1311502, Israel
Email: raj.kumar1@biu.ac.il



QVIT LAB

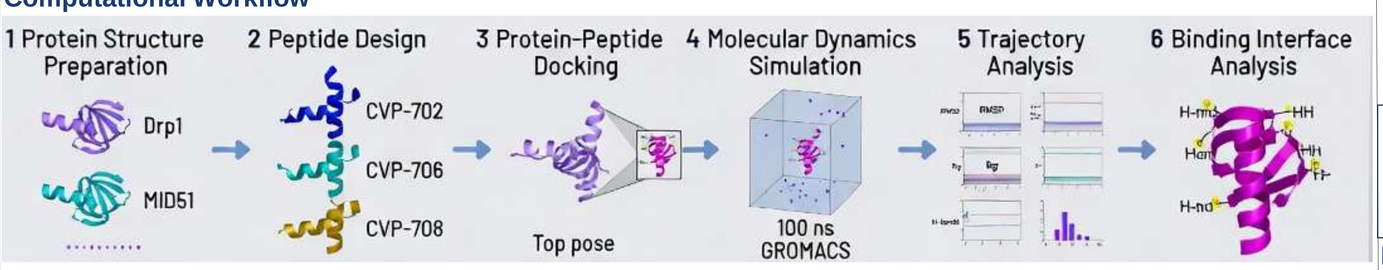
https://doi.org/10.17952/38EPS.2026.P121

Introduction



Materials and Methods

Computational Workflow



Experimental Methods and Instrumentation

Peptide designing

CVP-702	CH_3-C(O)-XXXXXXX-NH_2
CVP-706	CH_3-C(O)-XXXXXXXX-NH_2
CVP-708	CH_3-C(O)-XXXXXXXX-NH_2



Syro Peptide Synthesisers



Analytical and Preparative HPLC



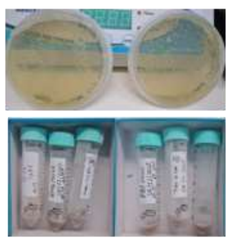
MALDI-TOF - Mass

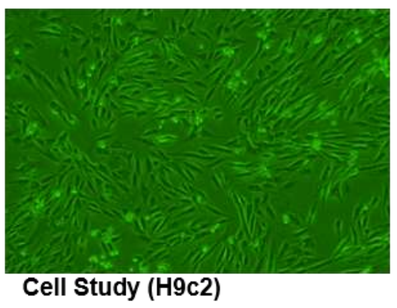


Field effect biosensing (R100)



Akta for proteins purification





Cell Study (H9c2)



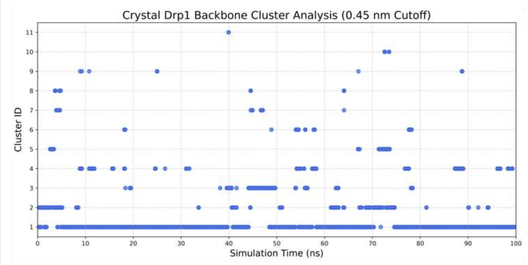
Rat model (*in Vivo*)

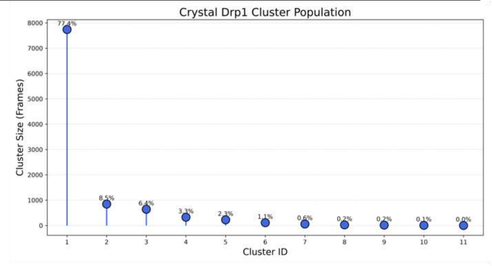
Results

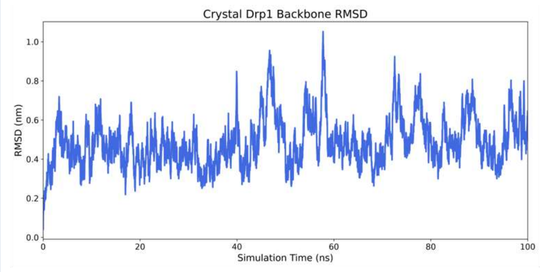
Computational Analysis

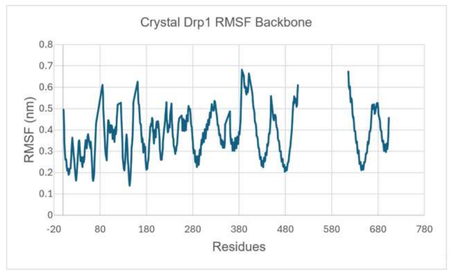
Structural Stability and Conformational Analysis of Drp1 (PDB ID: 4BEJ)

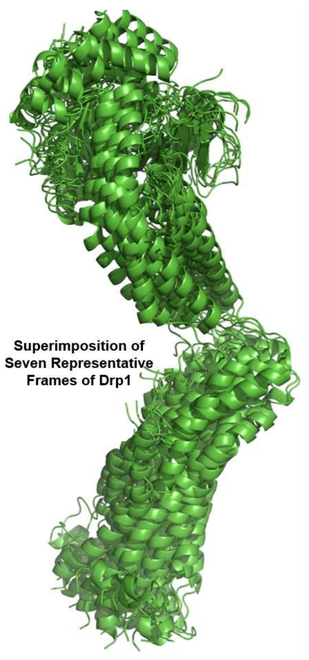
Clusters	1	2	3	4	5	6	7	8	9	10	11
Frames	7739	846	641	327	228	111	59	25	16	7	2
Size %	77	8	6	3	2	1	1	0	0	0	0







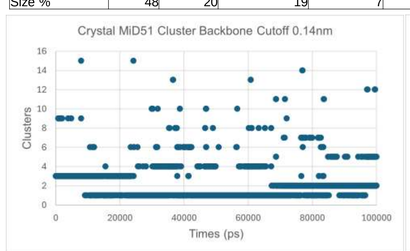


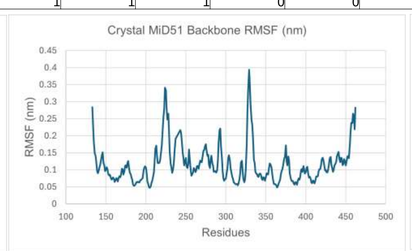


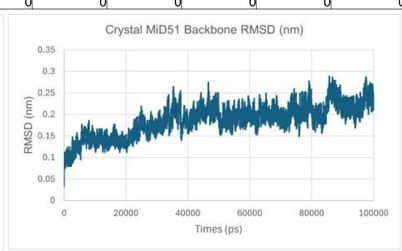
100 ns molecular dynamics simulations reveal a dynamic Crystal Drp1 framework, with backbone RMSD fluctuating between 0.2 and 0.8 nm due to localised loop and domain flexibility. Cluster analysis (0.45 nm cutoff) further shows that, despite this conformational mobility, Crystal Drp1 predominantly adopts a single dominant structural state, representing 77% of the simulation.

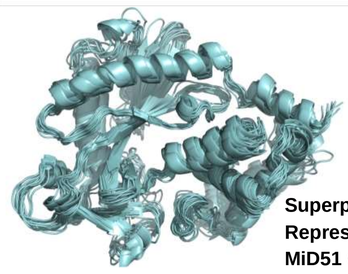
Structural and Molecular Dynamics Analysis of Crystal MiD51

Clusters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Frames	4851	2036	1960	747	147	125	70	19	17	10	6	5	4	2	2
Size %	48	20	19	7	1	1	1	0	0	0	0	0	0	0	0





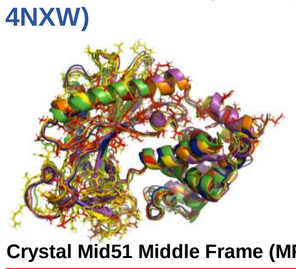




100 ns molecular dynamics simulations reveal a stable Mid51 crystal structure (RMSD: 0.15–0.25 nm), with localised loop dynamics that identify potential protein-interaction and therapeutic-target regions.

Superposition of Representative MiD51 Clusters

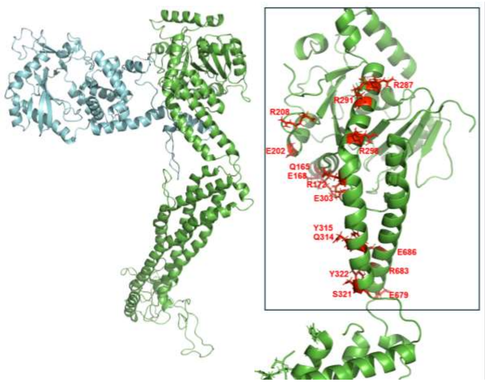
ClusPro Docking Analysis of the Interaction Between Drp1 (PDB: 4BEJ) and Mid51 (PDB: 4NXW)



Crystal Mid51 Middle Frame (MF1-MF6) Superimposition

Complexes	Docking Results
Drp1_Mid51_MF1	30
Drp1_Mid51_MF2	30
Drp1_Mid51_MF3	30
Drp1_Mid51_MF4	30
Drp1_Mid51_MF5	30
Drp1_Mid51_MF6	30
Sum	180

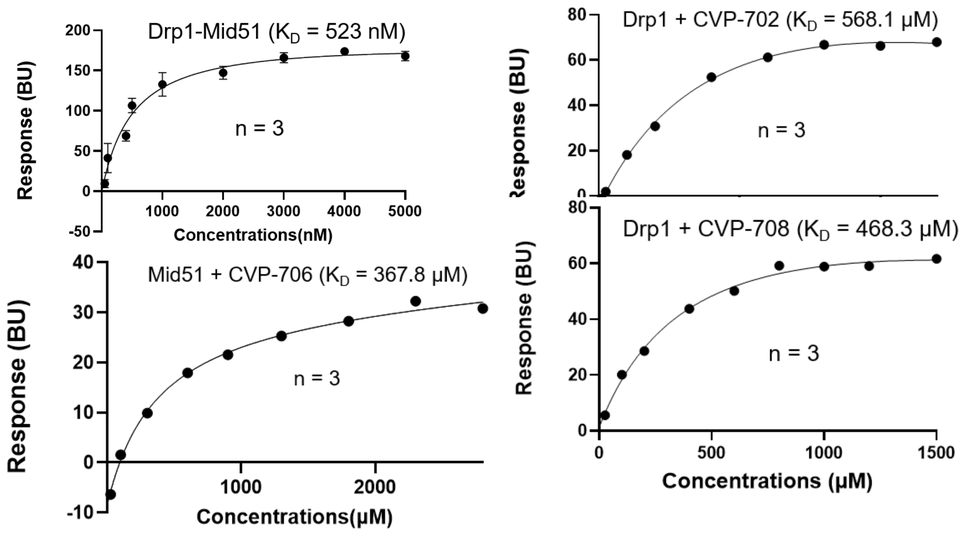
Crystal_MiD51_1MF	RMSD =	0	(4445 atoms)
Crystal_MiD51_2MF	RMSD =	1.551	(4445 atoms)
Crystal_MiD51_3MF	RMSD =	1.415	(4347 atoms)
Crystal_Mid51_4MF	RMSD =	1.530	(4616 atoms)
Crystal_Mid51_5MF	RMSD =	1.702	(4545 atoms)
Crystal_Mid51_6MF	RMSD =	1.635	(4611 atoms)



Crystal (Drp1&Mid51)	
DRP1 Count	
N448	87
R172	84
R365	82
Q314	72
Y322	65
Y315	63
R683	54
E168	52
R441	50
E202	43
D679	42
Y449	40
Q165	39
E686	39
C361	36
D21	36
R291	35
E303	33
L287	32

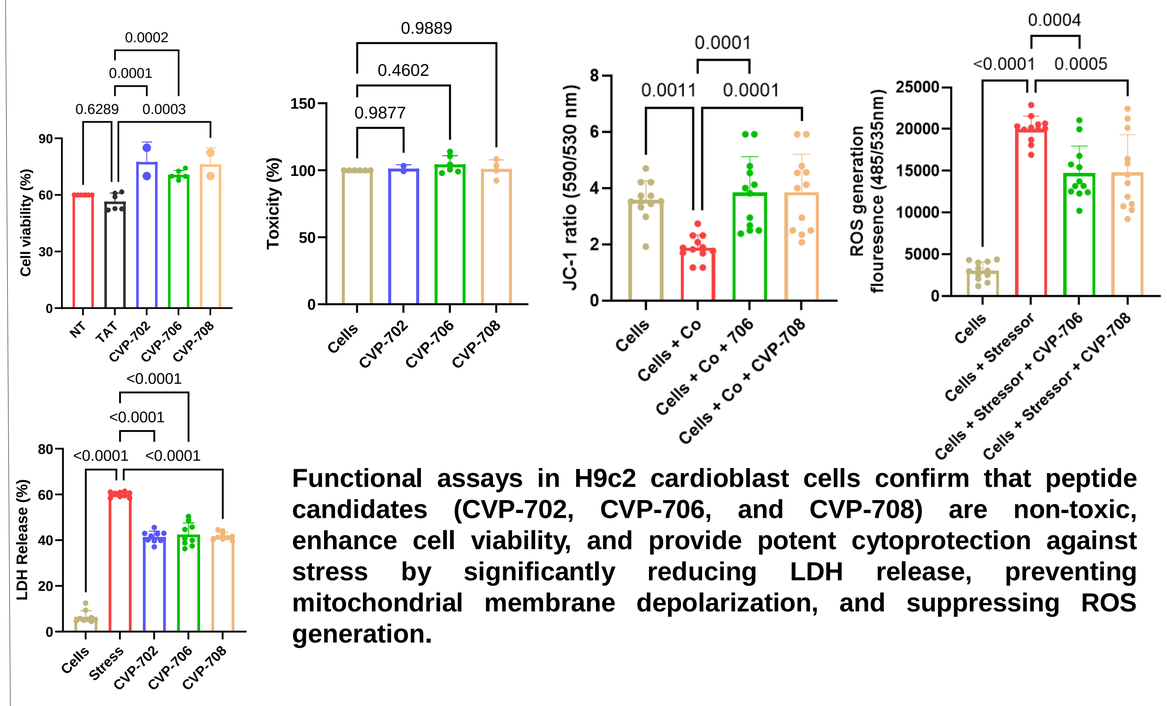
Docking representative Mid51 middle frame ensembles (RMSD range: 1.415–1.702 nm) against Drp1 identified primary interface hotspots (led by N448, R365, and Y322), revealing the core residues driving dynamic protein-protein binding across 180 total predicted complexes.

R100 Binding Affinity Analysis of CVP-702, CVP-706, and CVP-708 Peptides with Drp1, Mid51, and the Drp1-Mid51 Interaction



Using an R100 Field-Effect Biosensor (n= 3), the Drp1-Mid51 complex demonstrated strong nanomolar affinity ($K_D = 523$ nM), while individual peptide interactions displayed dose-dependent micromolar binding affinities ranging from $568.1\ \mu$M (Drp1 + CVP-702), to $367.8\ \mu$M (Mid51 + CVP-706) with Drp1 + CVP-708 exhibiting intermediate affinity ($K_D = 468.3\ \mu$M).

Peptide Activity Assessment in H9c2 Cardioblasts (XTT, JC-1, ROS, LDH Assays)



Functional assays in H9c2 cardioblast cells confirm that peptide candidates (CVP-702, CVP-706, and CVP-708) are non-toxic, enhance cell viability, and provide potent cytoprotection against stress by significantly reducing LDH release, preventing mitochondrial membrane depolarization, and suppressing ROS generation.

Conclusion

Computational protein-protein docking and molecular dynamics simulations defined the Drp1-Mid51 interaction interface and identified key interaction hotspots that provide a structural basis for peptide-mediated disruption of this protein-protein interaction. R100 binding assays validated the binding of **CVP-706 to Mid51** and **CVP-702 and CVP-708 to Drp1**, while cellular studies confirmed their cytoprotective activity. Collectively, these findings support peptide-mediated modulation of the Drp1-Mid51 interaction as a promising therapeutic strategy for preserving mitochondrial dynamics in cardiovascular disease.

PI: Prof. Nir Qvit (Email: nir.qvit@biu.ac.il)