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Investigation of the role of Mfn2 and Drp1 protein-protein interactions in mitochondrial dynamics and its biological implications in cardiovascular diseases

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1. Introduction and Aim

Mitochondrial dynamics is a potential target for cardiovascular diseases (**CVDs**). Deregulation of dynamicity causes mitochondrial dysfunction, impacting on cellular functions and leading to disease.
Protein-protein interactions (**PPIs**) are promising therapeutic targets toward the embarrassment of medical conditions.
The interaction between **Drp1** and **Mfn2** suggests a regulatory mechanism where Mfn2 can influence the balance between fusion and fission. Specifically, Mfn2's interaction with Drp1 may promote mitochondrial elongation which can affect mitochondrial morphology. Thus, Drp1/Mfn2 PPI can lead to mitochondrial dysfunction and quality controls associated with various diseases.
Therefore, inhibiting Drp1/Mfn2 interactions may reduce excessive mitochondrial fusion, improving mitochondrial dynamics, morphology, and quality control.

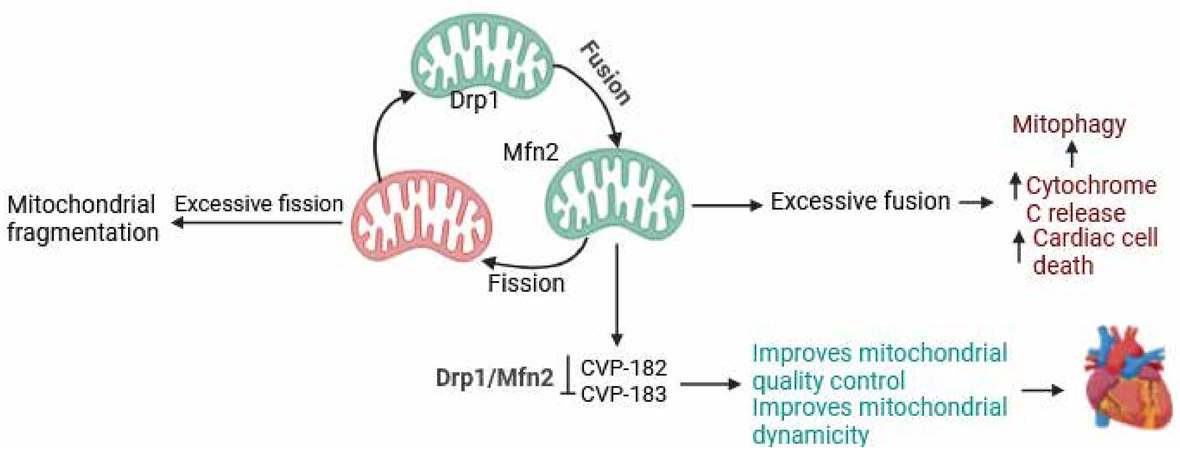
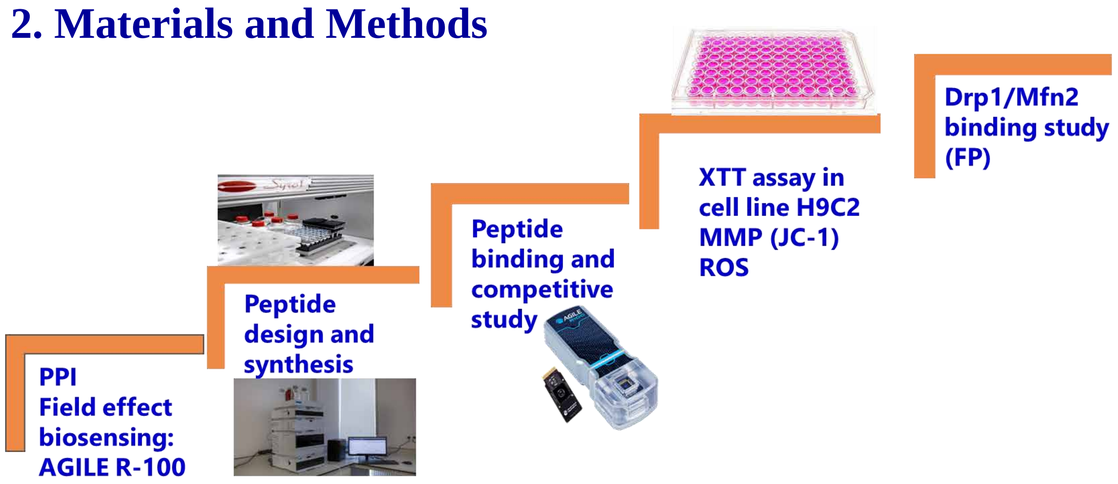


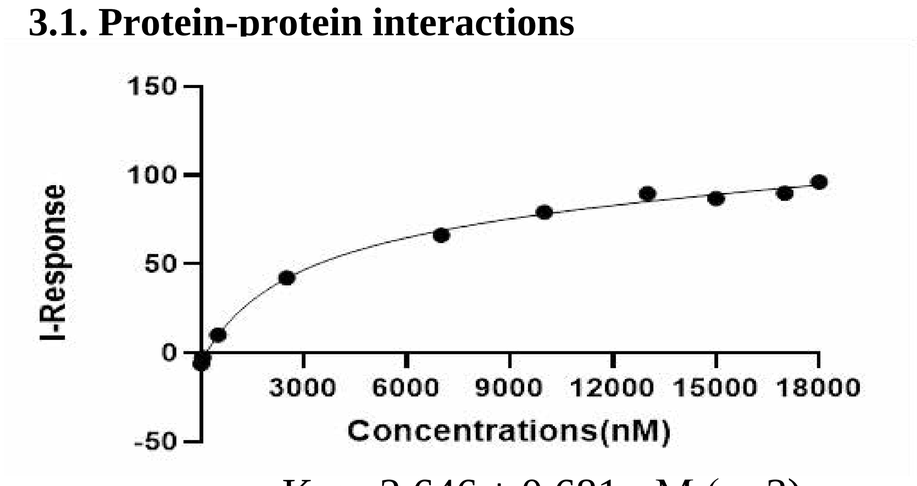
Figure 1. Schematic representation of excessive mitochondrial fusion affects mitochondrial dynamics and mitochondrial quality controls.

2. Materials and Methods



3. Result and Discussion

3.1. Protein-protein interactions



K_D: 2.646 ± 0.681 µM (n=3)

Figure 1. Drp1 and Mfn2 protein-protein interactions.

3.3. In vitro competitive study

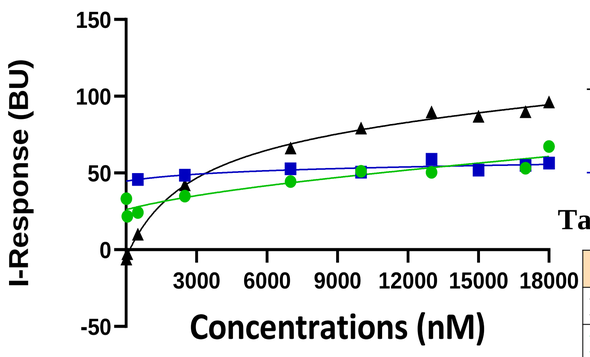
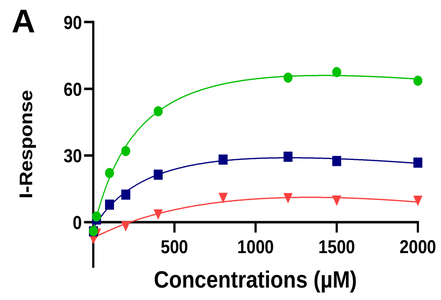


Table 1: K_D values of the competitive assay

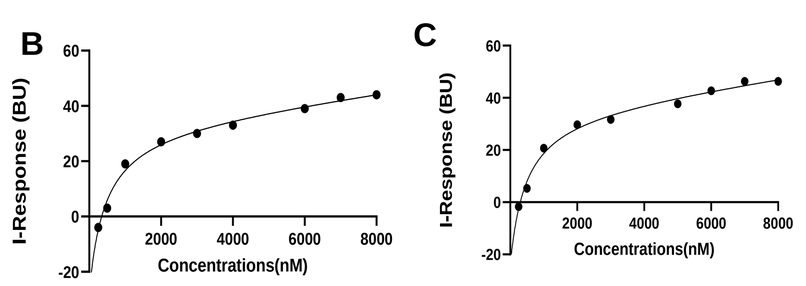
Competitive assay	kD (µm)	R^2
Drp1/Mfn2	2.646 ± 0.681	0.99
Drp1/Mfn2-CVP-182	6.201 ± 0.945	0.99
Drp1/Mfn2-CVP-183	4.710 ± 0.902	0.99

Figure 3: Drp1/Mfn2 PPI inhibition curves of Drp1/CVP-182 and Mfn2/CVP-183, *in vitro* study.

3.4. Protein-peptide binding using FEB and fluorescence polarizations



Peptide ID	K_D (µM)	R^2
Drp1/CVP-182	325.90 ± 28.13	0.99
Mfn2/CVP-183	454.50 ± 12.27	0.99



Peptide ID	Drp1/CVP-188 (B)	Mfn2/CVP-189 (C)
K_D (µM)	0.460 ± 0.684	0.439 ± 0.539
R^2	0.99	0.99

D. K_D values of the protein-peptides bindings

Figure 4: Protein-peptide binding curves. (**A**): protein-peptide binding curve using FEB. (**B & C**): Protein-peptide binding curves using fluorescence polarization. (**D**): Summary of K_D values of protein-peptide binding curves.

3.5. Cell viability study

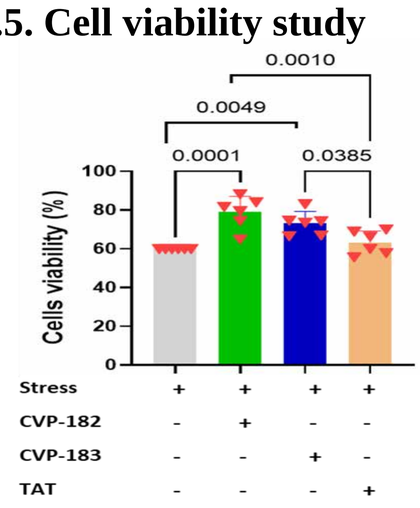


Figure 5: Cell viability study of CVP-182 and CVP-183 peptides using H9c2 cells.

3.6. Measurement of mitochondrial membrane potential (JC-1)

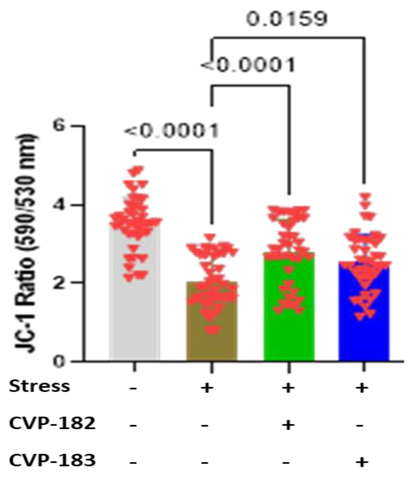


Figure 6: Measurement of mitochondrial membrane potential of H9c2 cells using CVP-182 and CVP-183 peptides.

3.7. Measurement of Reactive Oxygen Species (ROS) potential

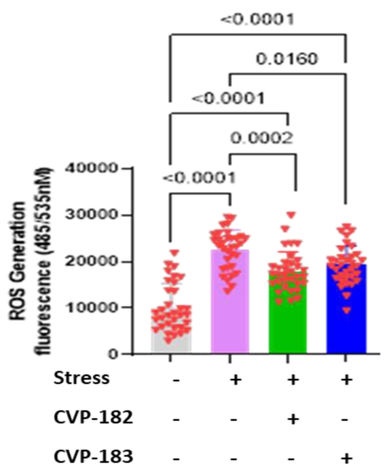


Figure 7. Measurement of Reactive Oxygen Species (ROS) for H9c2 cells using CVP-182 and CVP-183 peptides

Conclusion

Drp1 and Mfn2 proteins exhibit strong interactions. The peptides CVP-182 and CVP-183 have been synthesized with 100% purity. These peptides demonstrate high binding activities with Drp1 and Mfn2 proteins when assessed using field effect biosensor technology and fluorescence polarization (CVP-188 & CVP-189), respectively. Notably, CVP-182 and CVP-183 are biologically active in cultured cardiomyocyte (H9c2) cells, and they inhibit the interaction between Drp1 and Mfn2. These peptides enhance mitochondrial membrane potential and reduce reactive oxygen species (ROS) levels in H9c2 cells.

Future directions

- ✓ A study of CVP-182 and CVP-183 is necessary to advance therapeutic interventions in diseases characterized by mitochondrial dysfunction.
- ✓ The candidate peptides required studies of the optimal dosing and treatment intervals for cardio-protection in cardio-myocytes and other models.