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Molecular investigation on peptide regulated Mfn1 and Sirt1 protein interactions in mitochondrial dynamics

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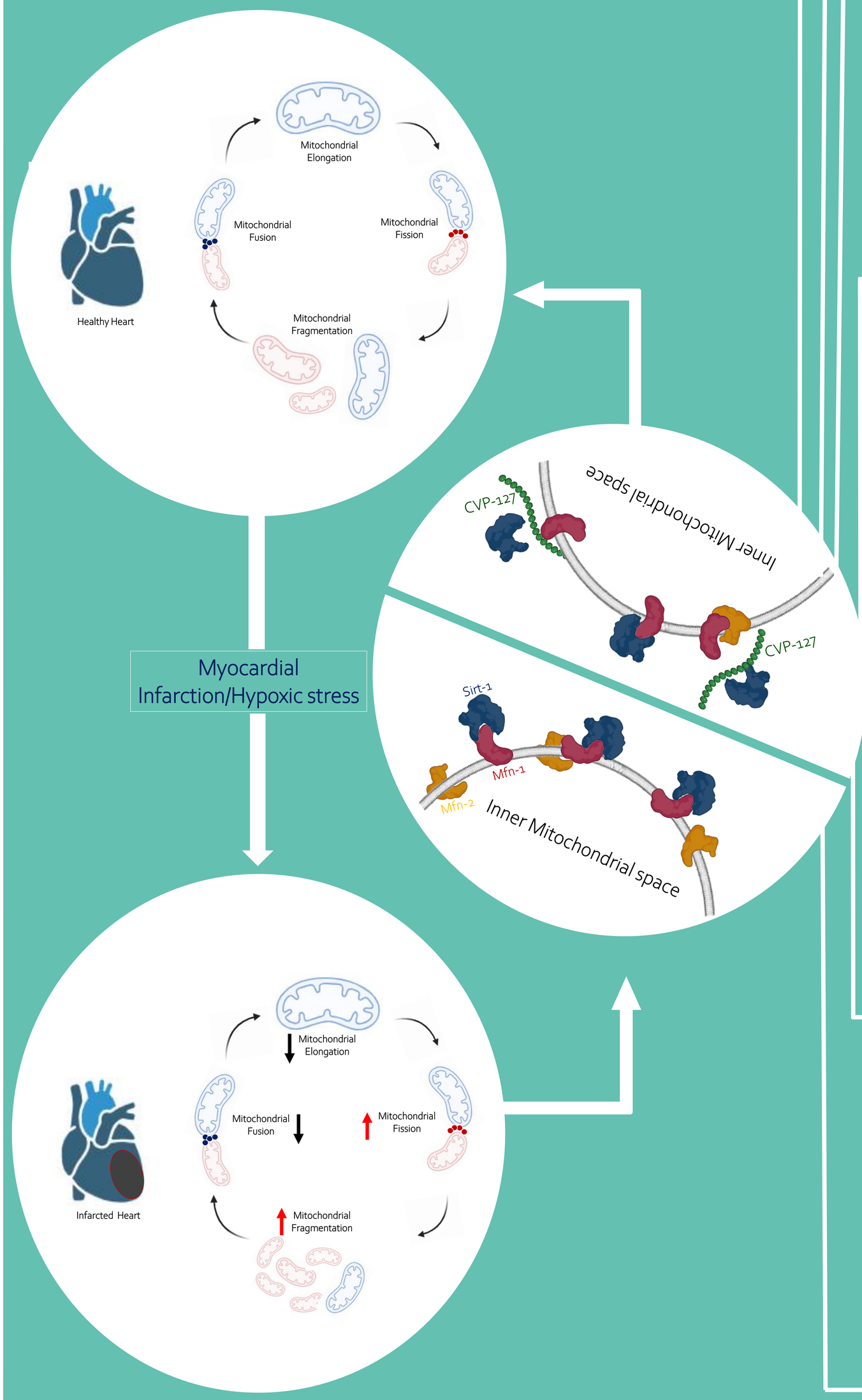
Aim and Abstract

Our study explores mitochondrial dynamics in cardiac cells with respect to myocardial infarction, focusing on the role of Mitofusin1 (**Mfn1**) and Sirtuin 1 (**Sirt1**) under hypoxia. We initially observed that Mfn1 interacts with Sirt1 to stabilize mitochondrial function during stress. We developed a peptide, **CVP-127**, designed to modulate this interaction. CVP-127 inhibited Mfn1-Sirt1 binding and promoted cell survival, enhanced mitochondrial potential, and protected against reactive oxygen species under hypoxic conditions. To understand this paradox, we are investigating how the inhibition of Mfn1/Sirt1 interaction by CVP-127 contributes to cell survival and identifying alternative mechanisms involved.

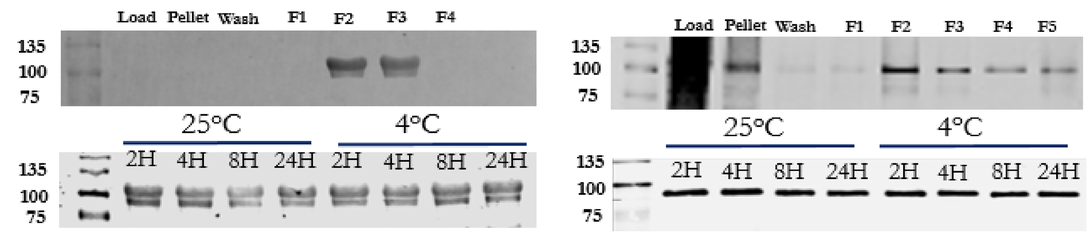
Our approach includes biochemical assays, gene expression analysis, and proteomics to uncover the underlying pathways that might compensate for the disrupted Mfn1/Sirt1 interaction and ensure cellular resilience under stress.

Graphical Representation



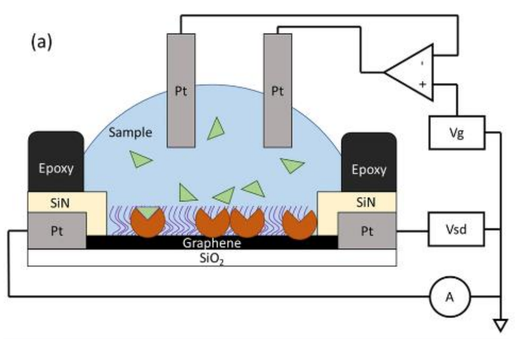
Results

1. Expression and purification of Sirt1 and Mfn1

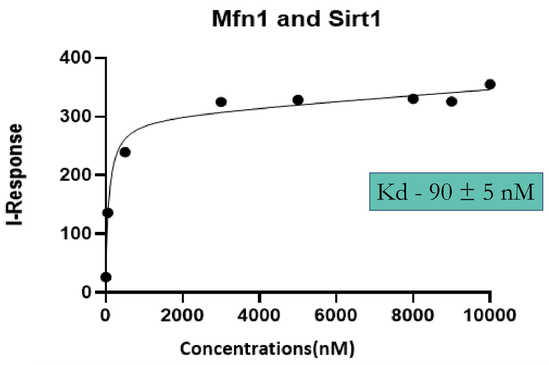


The proteins Sirt1 and Mfn1 were successfully expressed and purified. There was also found to be stable at 25° C and 4° C for about 24 hours

2. Protein-protein interaction between Sirt1 and Mfn1

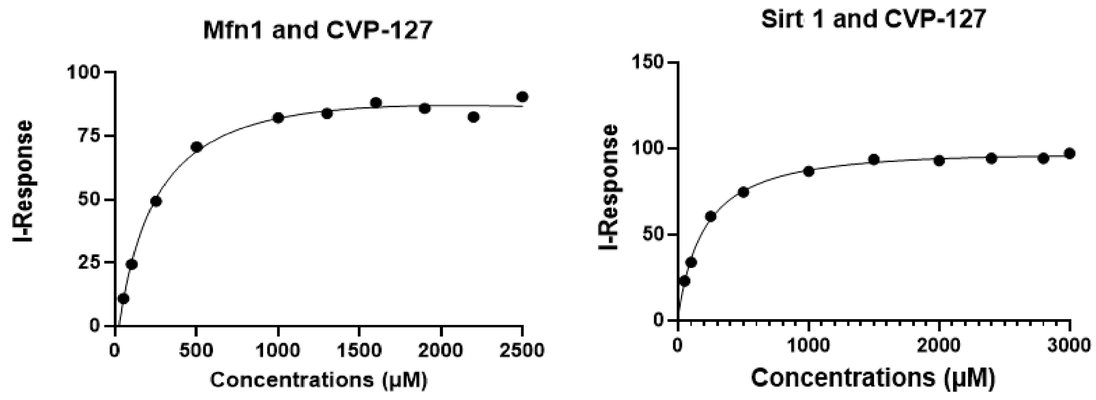


Protein is immobilized on the graphene bed. The analyte protein is made to react with the immobilized protein. Changes in the current during the interaction is measured.



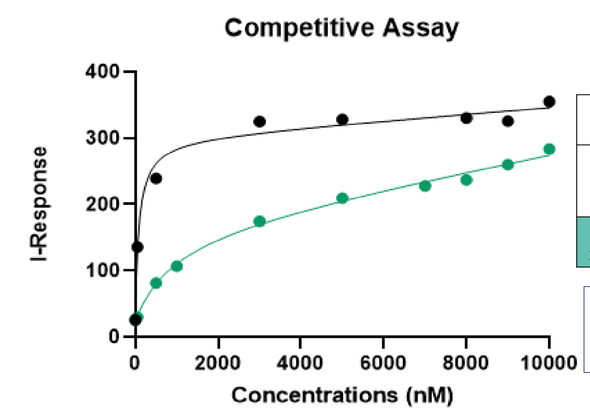
We observed good interaction between Sirt1 and Mfn1 with K_D of 90 ± 5 nM using field emission Biosensing technique.

3. Protein-peptide interaction with Sirt1, Mfn1



The peptide CVP-127 showed good interaction with the proteins Sirt1 and Mfn1 with K_D of 236.96 and 209.5 µM respectively

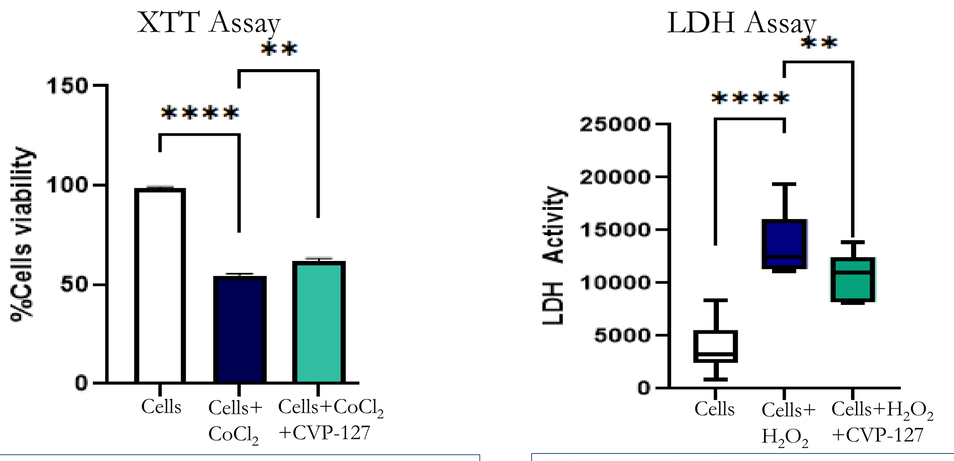
4. Protein-protein competitive interaction with peptide



Experiment Name	Immobilized protein	Analyte	Peptide	K_D
Mfn1 and Sirt1 interaction (n=3)	Mfn1	Sirt1	-	90 ± 5 nM
Competitive interaction (n=3)	Mfn1	Sirt1	CVP-127 250 µM	938.07 ± 6 nM

The interaction between Sirt1 and Mfn1 was successfully inhibited by the peptide CVP-127 with K_D 938.07 (± 6 nM) higher than that of Sirt1 and Mfn1 interaction K_D 90 (± 5 nM)

5. Cell Viability assays

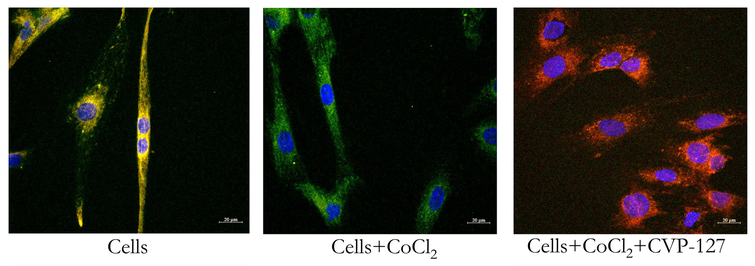


Under cobalt induced hypoxic stress the peptide was observed to significantly increase the H9c2 cell viability.

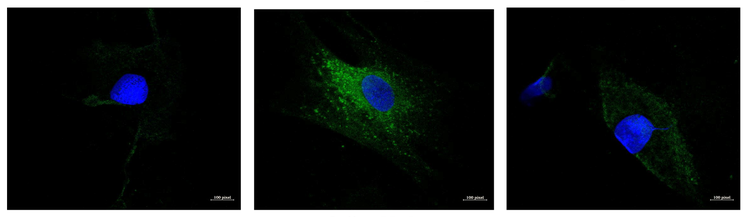
Under H_2O_2 induced hypoxic stress the peptide was observed to significantly decrease the release of LDH from H9c2 cells

6. Microscopic Assessment

Mitochondrial Membrane potential Assay



Reactive Oxygen Species release Assay



Conclusion

We observed potential interactions between Sirt1 and Mfn1 using FEB technology. We developed a peptide that successfully inhibited the interaction between Sirt1 and Mfn1. The cells showed high viability and reduced release of LDH when treated with CVP-127 under hypoxic stress. Also, under stress when treated with peptide CVP-127 we observed high mitochondrial membrane potential and reduction in the release of Reactive Oxygen Species. These results prove that CVP-127 rescues H9c2 cells from hypoxic stress. Further we are evaluating the action of peptide on myocardial infarction in rats.

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