Computer modelling of the interaction between *Ce*Rme-8 J domain and *Ce*Hsp70-1

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Abstract

Rme-8[1] is a J domain-containing plasma membrane protein that is required for endocytosis in various cells[2]. The J domain is a characteristic structural motif found mainly in heat shock protein 40 (Hsp40 or DnaJ) and other proteins such as Rme-8[3]. Within the J domain is a tripeptide, the HPD motif, that is required by the J-domain protein to interact with and stimulate the ATPase activity of Hsp70, a major cellular chaperone[4]. The Rme-8 protein in *C. elegans*, *Ce*Rme-8, has not been identified with a particular Hsp70 partner. *Ce*Hsp70-1 is the only cytosolic Hsp70 in *C. elegans*, therefore, we *hypothesize that CeHsp70-1 is the binding partner for the J domain of CeRme-8*. To test this hypothesis, we employed computer modelling to predict the interaction between *Ce*Rme-8 and *Ce*Hsp70-1 using the known DnaK-DnaJ protein complex as a template. In addition, we report the successful cloning and expression of the J domain of *Ce*Rme-8. AlphaFold, SwissModel, and Phyre2 modelling programs revealed that *Ce*Rme-8 possesses a J domain that contains the canonical HPD tripeptide motif. ClusPro docking program predicted similar binding interface to the DnaK-DnaJ complex, along with several non-conforming models. Complementary DNA of the J domain of *Ce*Rme-8 was cloned into the *pGEX*-Tev-KG plasmid, in-frame with the gene for glutathione-*S*-transferase (GST), to yield a GST-*Ce*Rme-8 fusion protein. IPTG-induced expression of the expected 37-kilodalton fusion protein was confirmed by both SDS-PAGE and western blotting using antibody against GST. Future work will involve purifying and testing the effect of the J domain protein on the ATPase activity of *Ce*Hsp70-1.

Modelling *Ce*Rme-8 and *Ce*Hsp70-1

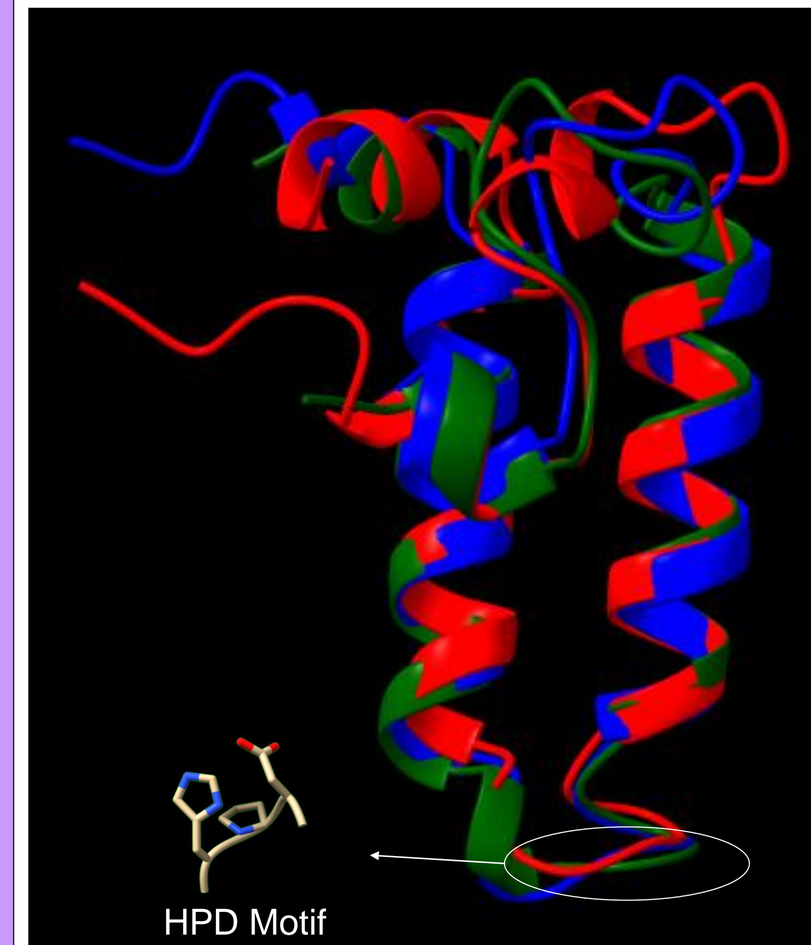

Fig. 1A: Superimposed models of *Ce*Rme-8 J-domain generated by SwissModel, AlphaFold, and Phyre2 software.

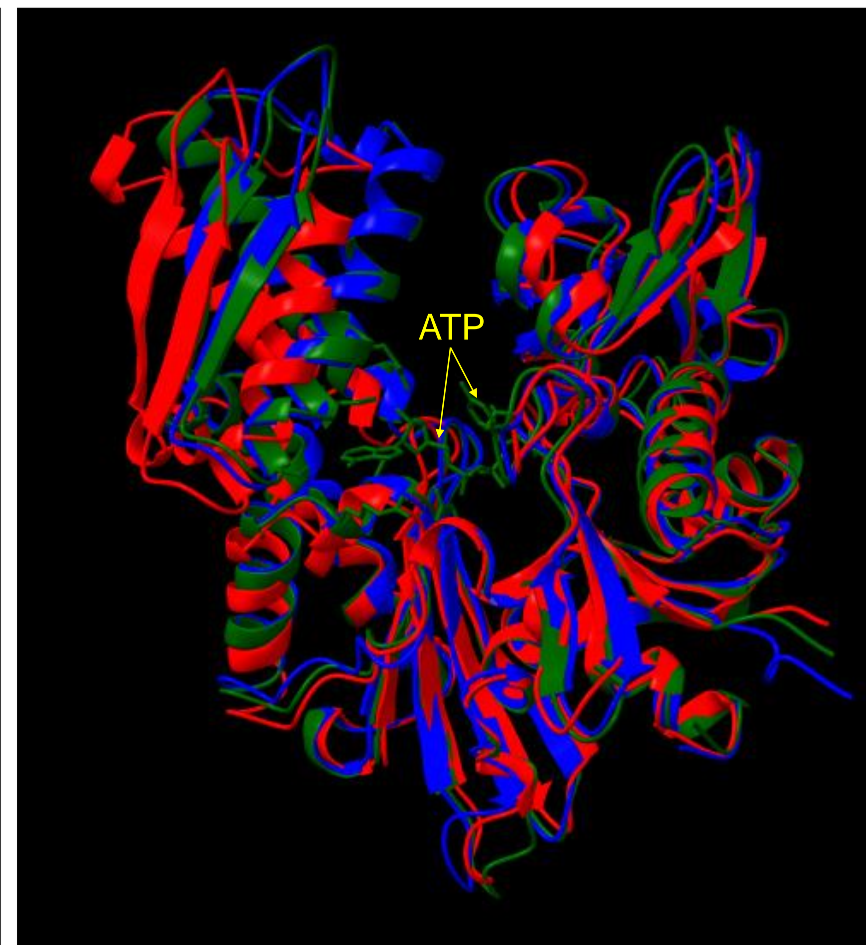

Fig. 1B: Superimposed models of *Ce*Hsp70-1 N-terminal domain. Generated by SwissModel, AlphaFold, and Phyre2 software.

Phyre 2 SwissModel AlphaFold

DNA sequences of each protein, *Ce*Rme-8's J-domain and *Ce*Hsp70-1, were translated using ExPASy translator. Protein three-dimensional structures were modelled using AlphaFold, Phyre2, and SwissModel and visualized with ChimeraX. The Matchmaker tool in ChimeraX was used to superimpose modelled protein structures to analyze and validate them. Fig. 1A shows the J-domain models were essentially similar, with the HPD motif (in white) in the expected region. Similarly, Fig. 1B shows that modelled N-terminal nucleotide-binding domain (NBD) of *Ce*Hsp70-1 were conformationally the same, except in the Phyre2 model in which the ATP cleft is wider. This widening of the ATP cleft may be due to the fact that Phyre2 software does not account for nonprotein molecules, leaving the structure in an unbound (relaxed) conformation. AlphaFold and SwissModel software modelled the protein in its bound conformation.

Docking J domain and Hsp70 Proteins

Fig. 2A: *Ce*Hsp70-1 docked with *Ce*Rme-8 J domain using ClusPro

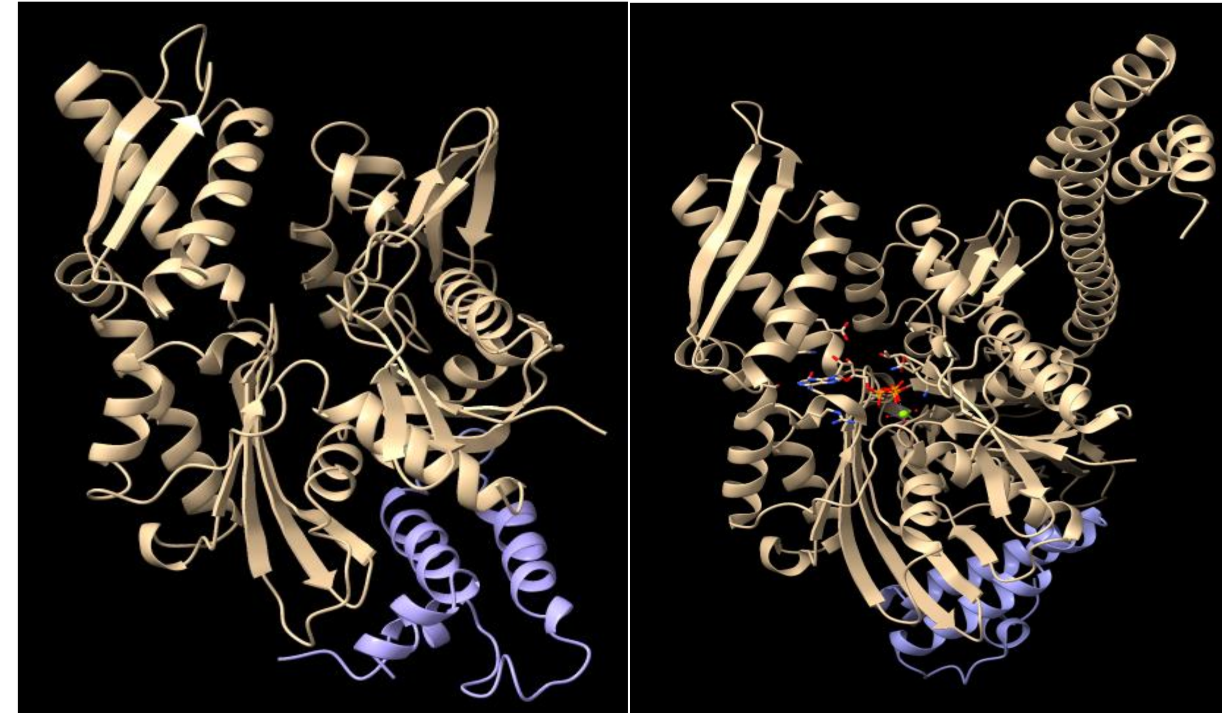

Fig. 2B: *E. coli* DnaK docked with *E. coli* DnaJ using ClusPro

HPD Motif	Contacts in DnaK	Contacts in *Ce* Hsp70-1
HIS	LEU x 3	LEU
	ARG	ARG
	ILE	ILE
	PRO	VAL
	ALA	
PRO	ALA	ILE
	ARG	ARG x 2
	PRO	ASP
ASP	LEU x 3	ILE
	VAL x 2	LYS
	ALA	ASP
	ARG	ARG x 2
	GLN	GLN

Table 1: HPD interactions of DnaK/DnaJ and *Ce*Rme-8/*Ce*Hsp70-1

ClusPro was used to generate protein-protein complexes. Structures were viewed and relabeled in PyMol. Comparison with DnaK-DnaJ interaction complex reveals that the *Ce*Rme-8 J domain binds to CeHsp70-1 at the same site and in the same orientation involving the HPD motif. Models that were non-conforming to the DnaK-DnaJ complex were discarded. Prodigy web server was used to identify and characterize the interaction interfaces in the complexes as well as obtain K_D and ΔG values. The interacting amino acid are displayed in Table 1.

Cloning *Ce*Rme-8 into an Expression Vector

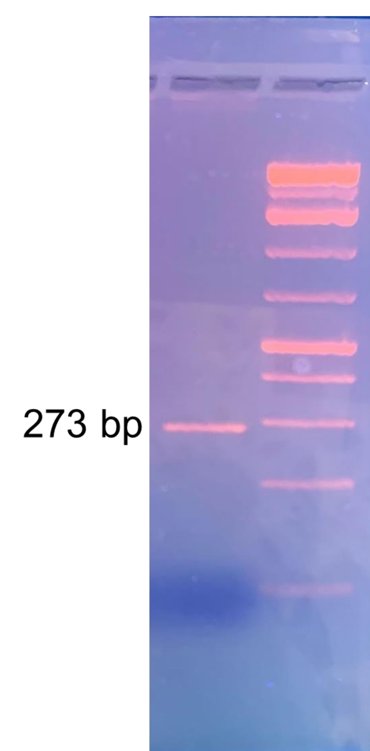

Fig 3A: CeRme-8 J PCR

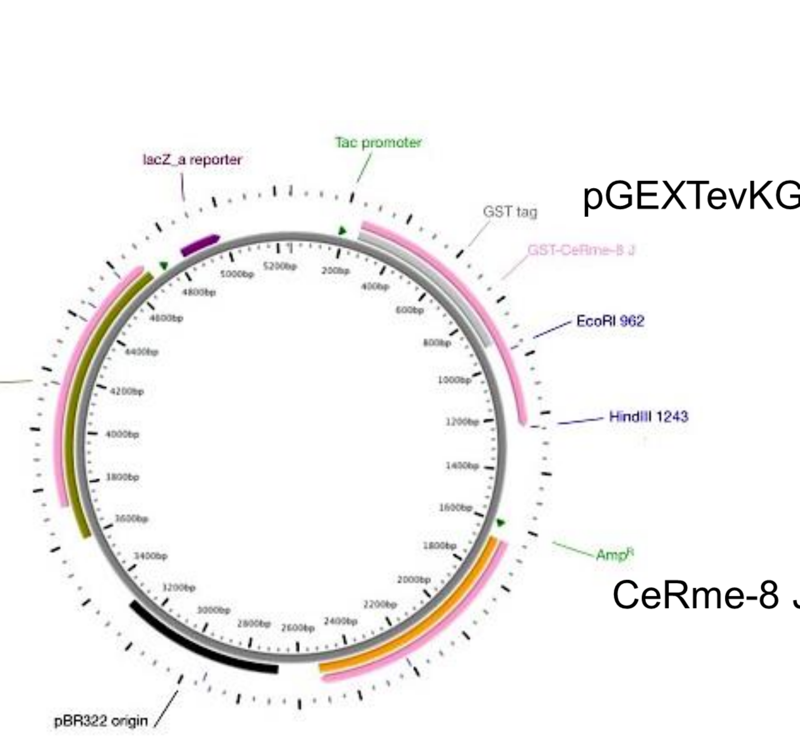

Fig 3B: Plasmid Construct

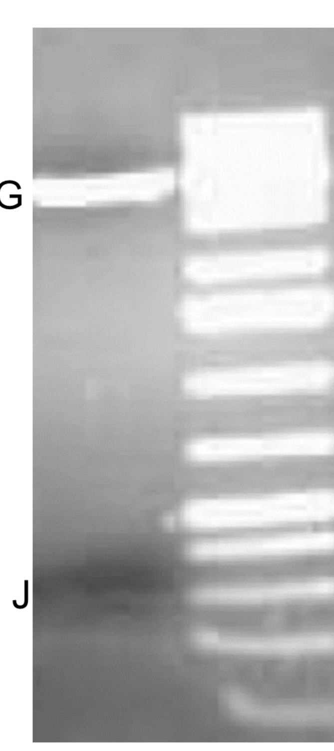

Fig 3C: CeRme-8 J Cloning

Fig 3A: The DNA sequence coding for the J domain of CeRme-8 was identified by multi-sequence alignment. The sequence was then amplified from the cDNA of full-length CeRme-8 using forward and reverse primers containing *Eco* RI and *Hind* III restriction enzyme sites respectively. Fig 3B: Amplified CeRme-8 J gene containing appropriate overhangs was ligated into the pGEXTevKG, in-frame with the gene for glutathione-*S*-transferase (GST), to generate a construct that would express a GST-CeRme-8 J fusion protein. Fig 3C: Cloning of the CeRme-8 J gene into the pGEXTevKG plasmid vector was confirmed by restriction enzyme digest using *Eco* RI and *Hind* III, as well as by DNA sequencing.

CeRme-8 J domain Expressed in Fusion with Glutathione-*S*-transferase

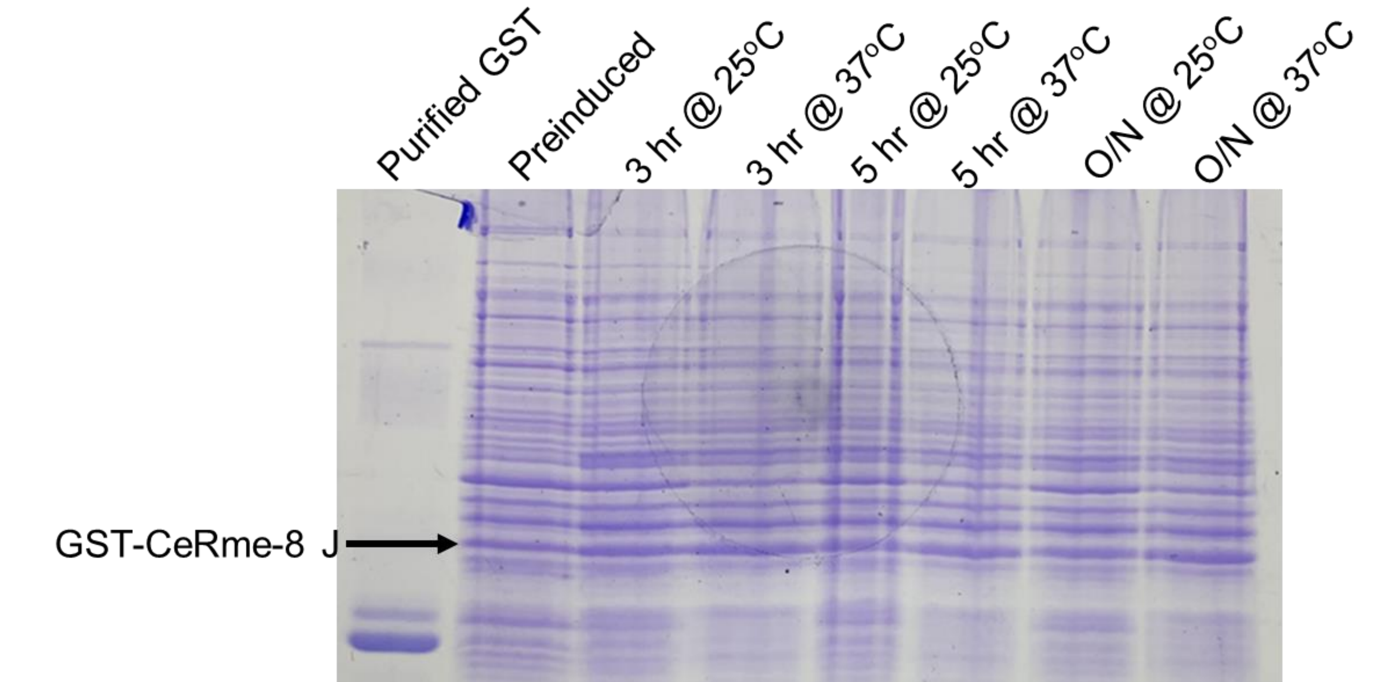

Fig 4A: Expression of GST-CeRme-8 J protein. Actively growing cultures of bacterial cells harboring plasmid construct were chemically induced to express protein at various temperatures at various times and whole-cell lysates by the addition of isopropyl ß-D-1-thiogalactopyranoside (IPTG). Samples of the expression cultures were taken analyzed on SDS-PAGE. The fusion protein was expressed at the expected size of 36 kDa.

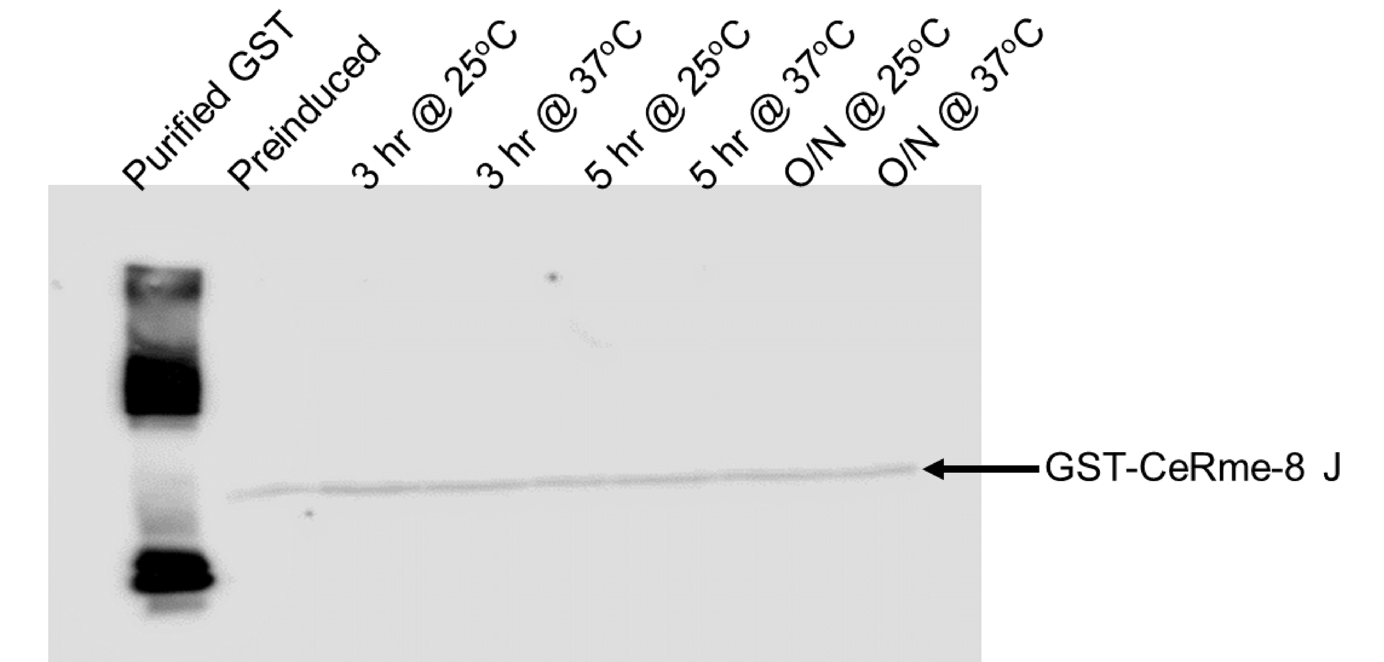

Fig 4B: Confirmation of expression of GST-CeRme-8 J. Whole-cell lysates of bacterial cells expressing GST-CeRme-8 J protein were analyzed on SDS-PAGE. Proteins were transferred onto a nitrocellulose membrane. Expression of the fusion protein was confirmed by immunoblotting using antibody against GST. Multiple bands in the first lane shows the expected oligomerization of GST.

Conclusions and Future Work

1. The J domain of CeRme-8 protein folds into expected conformation and contains the HPD motif.
2. The J domain of CeRme-8 binds to CeHsp70-1 at the predicted site.
3. The J domain of CeRme-8 was successfully cloned and expressed in fusion with GST.
4. Future work will include purification of the protein and testing its effect on the ATPase activity of CeHsp70-.

Acknowledgements

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References

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