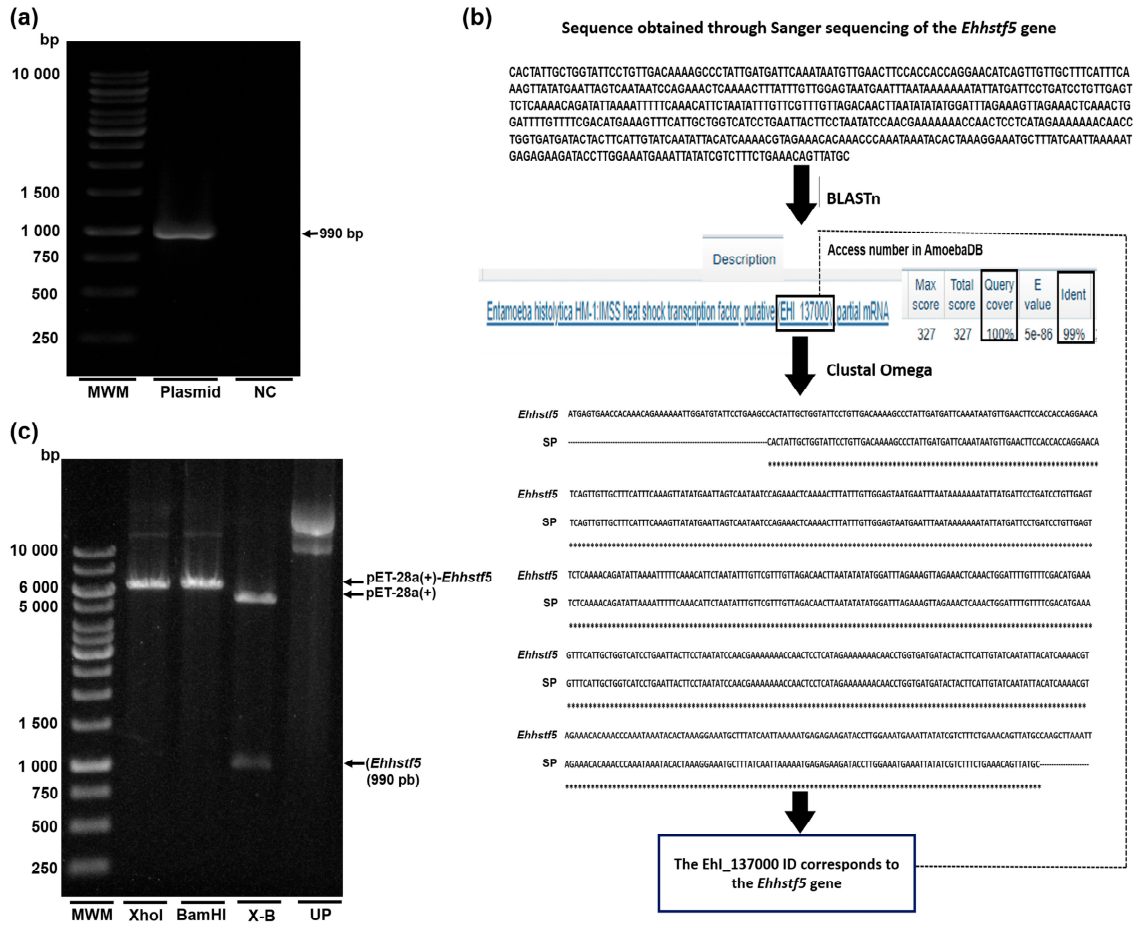
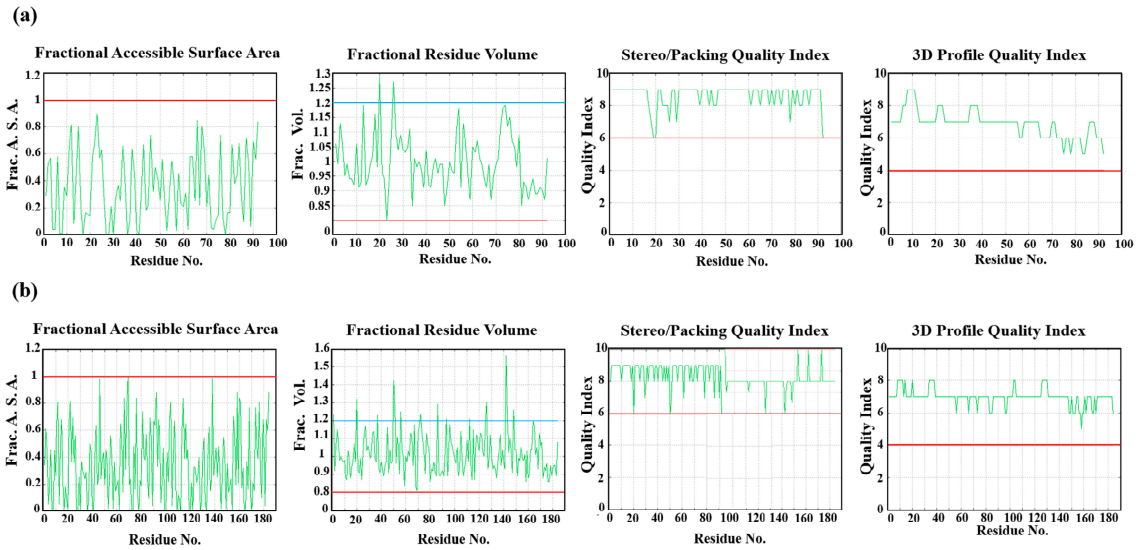
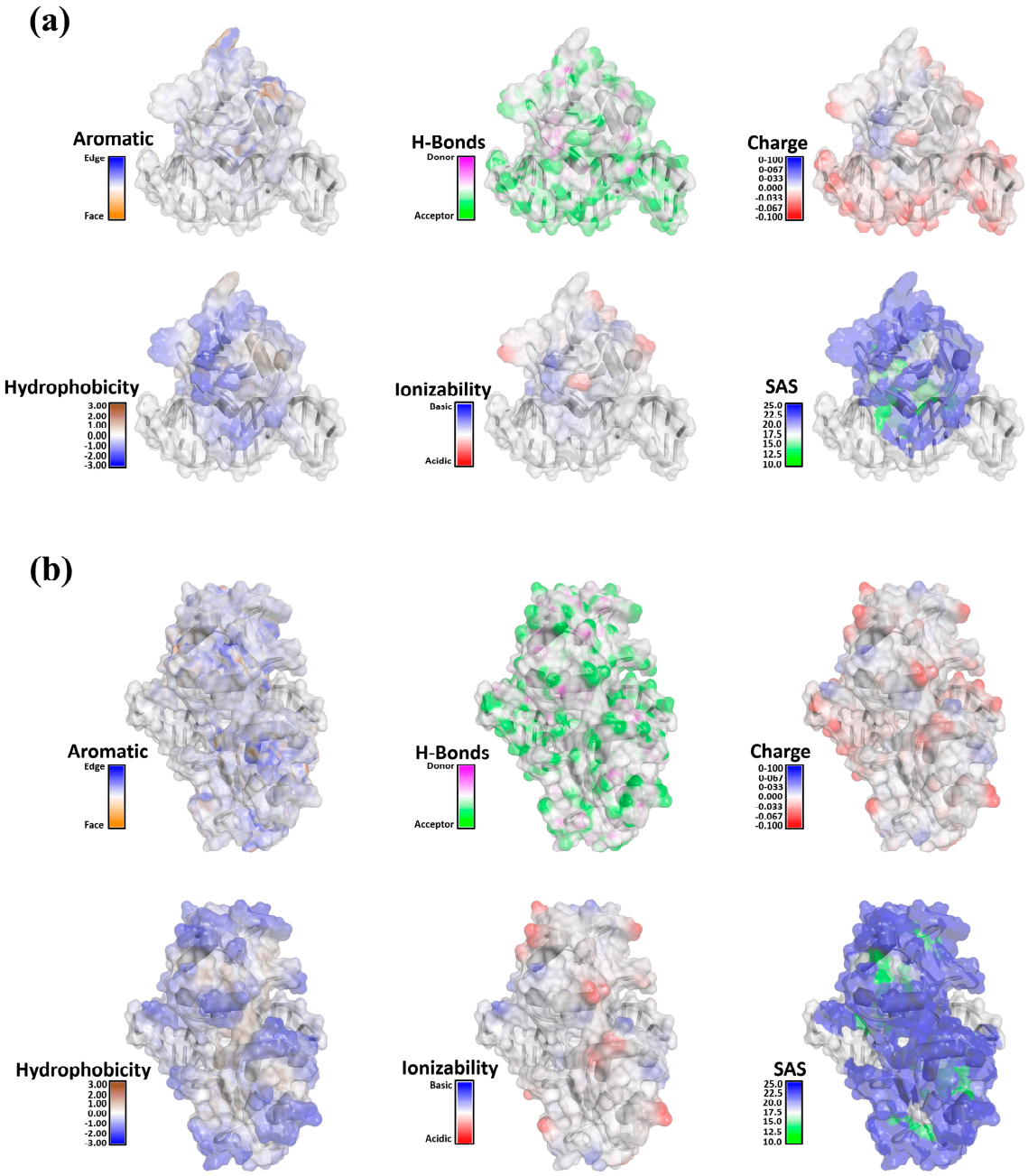


Supplementary Figure S1. The insertion of the *Ehhstf5* gene into the pET-28a(+) plasmid was confirmed by PCR, obtaining a product of approximately 990 base pairs (bp) (a). This product was subsequently sequenced using Sanger sequencing. The sequenced product (SP) was analyzed using BLASTn, showing alignment with the *Ehhstf5* gene (ID EHI_137000). Clustal Omega analysis comparing the sequenced product with the AmoebaDB-derived sequence showed consistent results, with 99% identity and 100% homology. (b). Enzymatic restriction assays were additionally conducted using the plasmid. When XhoI or BamHI enzymes were applied separately, the plasmid linearized, revealing an approximate size of 6000 bp. Simultaneous application of both enzymes (X-B) resulted in the release of the Ehhstf5 gene, with a size close to 1000 bp, and the plasmid reduced to approximately 5000 bp. As a negative control, the unrestricted plasmid (UP) was used, showing a size greater than 6000 bp (c). MWP; molecular weight marker, NC; negative control. In the alignment with the Clustal Omega server, asterisks (*) indicate amino acids that are identical across all sequences.



Supplementary Figure S2. Prediction of physicochemical properties and geometric characteristics of mEhDBD5 and dEhDBD5 Models: The VADAR server criteria indicate desirable scores below 1 for Fractional Accessible Surface Area analysis, between 0.8 and 1.2 for Fractional Residue Volume, and above 6 for the Stereo/Packing Quality Index, as well as exceeding 4 for the 3D Profile Quality Index. Notably, both mEhDBD5 (a) and dEhDBD5 (b) models achieved these target scores in all categories, underscoring their structural integrity and quality.



Supplementary Figure S3. The physicochemical properties of the dEhDBD5-HSE_*EhPgp5* complexes, both in the monomeric (a) and dimeric (b) conformations, were comprehensively analyzed using BIOVIA Discovery Studio. This analysis encompassed a range of characteristics including intra- and intermolecular aromatic interactions, the potential for hydrogen bonding, the distribution of molecular charges, degrees of

hydrophobicity, ionizability, and the solvent-accessible surface area (SAS). These properties are essential for understanding the stability of the complexes, reactivity, and potential biological interactions.

Supplementary Table S1. Intermolecular interaction of the mEhDBD5-HSE_*EhPgp5* and dEhDBD5-HSE_*EhPgp5* complexes

mEhDBD5 *EhPgp5* (string 5'-3')

Hydrogen Bonds

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	55 (1)	ARG	2.88	3.67	137.95	Yes	Yes	459 [Ng+]	890 [Npl]
2	55 (1)	ARG	3.41	3.91	113.72	No	Yes	890 [Npl]	457 [Ng+]
3	55 (1)	ARG	2.72	3.33	120.61	No	Yes	911 [Npl]	457 [Ng+]
4	58 (1)	ASN	3.09	3.75	126.85	No	Yes	894 [O3]	484 [O2]
5	58 (1)	ASN	2.78	3.7	156.54	Yes	Yes	485 [Nam]	881 [O3]

Salt Bridges

Index	Residue	AA	Distance	Protein positive?	Ligand Group	Ligand Atoms
1	92 (1)	LYS	4.13	yes	Phosphate	896, 896, 881, 898, 915, 916

mEhDBD5_*EhPgp5* (string 3'-5')

Hydrogen Bonds

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	1 (1)	ILE	3.6	4.1	112.66	Yes	No	1 [N3]	1202 [O3]
2	55 (1)	ARG	3.11	3.9	137.32	Yes	Yes	460 [Ng+]	1279 [Nar]
3	55 (1)	ARG	3.41	3.96	117.4	No	Yes	1278 [Npl]	457 [Ng+]
4	56 (1)	GLN	2.39	3.33	158.59	Yes	Yes	469 [Nam]	1257 [Npl]
5	60 (1)	TYR	2.09	2.55	109.25	Yes	Yes	505 [O3]	1220 [O2]
6	91 (1)	ARG	1.85	2.79	158.78	Yes	Yes	760 [Ng+]	1236 [Npl]
7	91 (1)	ARG	2.3	3.11	139.27	Yes	Yes	761 [Ng+]	1236 [Npl]

Salt Bridges

Index	Residue	AA	Distance	Protein positive?	Ligand Group	Ligand Atoms
1	47 (1)	HIS	4.21	Yes	Phosphate	1242, 1242, 1227, 1244, 1261, 1262

dEhDBD5_*EhPgp5* (string 5'-3')

Hydrogen Bonds

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	48 (1)	SER	3.48	4.04	118.53	Yes	No	1170 [Nam]	1666 [O3]
2	49D (1)	ASN	2.49	3.37	149.61	Yes	Yes	1183 [Nam]	1649 [O3]
3	49D (1)	ASN	3.15	3.73	119.28	Yes	No	1176 [Nam]	1666 [O3]
4	52D (1)	SER	2.1	2.69	117.71	Yes	Yes	1203 [O3]	1666 [O3]
5	55 (2)	ARG	3.1	3.44	101.78	Yes	Yes	460 [Ng+]	1743 [O2]
6	56D (1)	GLN	2.14	2.6	106.83	Yes	Yes	1241 [Nam]	1646 [O3]
7	58B (2)	ASN	2.05	2.95	150.48	Yes	Yes	485 [Nam]	1708 [O3]
8	64 (2)	LYS	1.42	2.38	163.28	Yes	No	532 [Nam]	1707 [O2]

Salt Bridges

Index	Residue	AA	Distance	Protein positive?	Ligand Group	Ligand Atoms
1	47 (1)	HIS	3.79	Yes	Phosphate	1647, 1647, 1666, 1632, 1649
2	63 (2)	ARG	5.03	Yes	Phosphate	1709, 1709, 1728, 1711, 1695, 1727
3	64 (2)	LYS	3.29	Yes	Phosphate	1668, 1668, 1653, 1670, 1687, 1688
4	92 (2)	LYS	3.83	Yes	Phosphate	1709, 1709, 1728, 1711, 1695, 1727

dEhDBD5 *EhPgp5* (string 3'-5')

Hydrogen Bonds

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	52 (2)	SER	2.7	3.41	130.78	Yes	Yes	431 [O3]	1970 [O3]
2	52 (2)	SER	2.68	3.41	132.26	No	Yes	1970 [O3]	431 [O3]
3	55 (2)	ARG	3.23	3.89	125.7	No	Yes	1987 [Npl]	457 [Ng+]
4	55 (1)	ARG	1.84	2.51	121.89	Yes	Yes	1232 [Ng+]	2051 [Nar]
5	55 (1)	ARG	2.5	3.41	152.63	No	Yes	2050 [Npl]	1229 [Ng+]
6	56 (2)	GLN	2.01	2.55	111.79	Yes	Yes	469 [Nam]	1950 [O2]
7	58 (1)	ASN	2.52	3.5	171.33	Yes	Yes	1257 [Nam]	2013 [O2]
8	64 (1)	LYS	2.27	3.11	143.41	Yes	No	1304 [Nam]	2012 [O3]
9	66 (2)	GLU	2.99	3.39	106.89	Yes	Yes	557 [O3]	2133 [O3]

Salt Bridges

Index	Residue	AA	Distance	Protein positive?	Ligand Group	Ligand Atoms

1	46 (1)	LYS	5.17	Yes	Phosphate	2176, 2176, 2195, 2178, 2161, 2194
2	47 (2)	HIS	4.84	Yes	Phosphate	1951, 1951, 1971, 1935, 1953, 1970
3	63 (1)	ARG	4.8	Yes	Phosphate	2014, 2014, 1999, 2016, 2033, 2034
4	64 (1)	LYS	4.85	Yes	Phosphate	1972, 1972, 1957, 1974, 1991, 1992
5	91 (2)	ARG	4.21	Yes	Phosphate	1909, 1909, 1911, 1894, 1927, 1928
6	92 (1)	LYS	4.92	Yes	Phosphate	2014, 2014, 1999, 2016, 2033, 2034

The amino acids marked with numbers (1) or (2) correspond to those involved in the interaction with HSE_*EhPgp5* of monomer 1 or 2, respectively.