Structural Insights into the Trimeric Assembly of the HERC5 HECT Domain through SAXS Analysis

Cansu Deniz Tozkoparan Ceylan[1] *, Çağdaş Dağ*[1,2,]*

[1]Nanofabrication and Nanocharacterization Center for Scientific and Technological Advanced Research (n[2]STAR), Koç University, İstanbul, Türkiye

[2]Koç University Işbank Center for Infectious Diseases (KUISCID), Koç University, Istanbul, Türkiye

*Corresponding author: cdag@ku.edu.tr

KEYWORDS: HERC5, ISGylation, HECT Ligase

ABSTRACT

HERC5 is an interferon-induced E3 ubiquitin ligase involved in ISGylation, a crucial post-translational modification regulating antiviral responses. The HECT domain of HERC5 plays a key role in substrate recognition and ubiquitin transfer. In this study, we employed small-angle X-ray scattering (SAXS) to investigate the molecular organization and oligomeric state of the HERC5 HECT domain in solution. The protein was expressed and purified using affinity and size-exclusion chromatography, followed by SAXS measurements at 2.0 mg/ml and 10°C. Data analysis revealed a trimeric assembly, supported by Guinier and Kratky analyses, distance distribution functions, and ab initio modeling. Structural overlays with the AlphaFold model confirmed the SAXS-derived envelope, providing insights into the conformational stability and trimeric self-assembly properties of HERC5 HECT. These findings suggest that trimerization may play a regulatory role in HERC5 function, contributing to a broader understanding of HECT-type E3 ligase mechanisms.

Keywords: HERC5, HECT domain, SAXS, ISGylation, ubiquitin ligase, protein structure

Introduction

The 15 kDa ubiquitin-like protein ISG15 (Interferon Stimulated Gene 15) covalently binds to other proteins in a process called ISGylation. This process plays a pivotal role in the control of viral infections within the mammalian innate immune system. The ISGylation process involves three multi-step enzymes : E1 activating enzyme (Uba7), E2 conjugation enzyme (UbcH8) and E3 ligase enzyme (HERC5) (Perng and Lenschow., 2018; Abe et al., 2020). E3 ubiquitin ligases are typically classified into four main classes, namely homologous to E6AP C terminus (HECT), really interesting new genes (RING), RING- between- RINGs (RBR), and U-box-type (Rotin and Kumar., 2009). The HECT E3 ligase family comprises the Cysteine 994 HECT catalytic domain located in the carboxyl terminal lobes (Wong et al., 2006; Bernassola et al., 2008). HECT families, with a flexible linker bridging the N-terminal and C-terminal lobes and containing a cysteine active site, establishes an intermediate thioester bond with cysteine prior to being transferred ISG15 from E2 to the target protein (Sluimer and Distel., 2018). Research has demonstrated that HECT ligases are capable of adopting diverse conformations, with these conformations exhibiting a direct correlation with the ubiquitin transfer mechanism. The HECT domain has been observed to exist in monomeric or oligomeric forms, with this variation potentially impacting enzymatic activity.

The E3 ligase enzyme of the ISGylation, HERC5, interacts with the UbcH8 (E2-conjugating enzyme) via its HECT domain, thereby facilitating the binding of the enzyme to the target protein. HERC5 is a 117 kDa antiviral immune protein induced by interferon-alpha/beta (IFN-α/β) signaling.HERC5 possesses the capability to interact with the polyribosome and directly alter specific proteins, distinguishing it as a standout among other E3 ligases (Takeuchi, Inoue and Yokosawa et al., 2006 ; Wong et al., 2006). HERC5 acts as a positive regulator of innate defense mechanisms against HIV-1. This process involves the modification of Gag proteins by ISG15, affecting viral assembly and budding (Woods et al., 2011). The molecular mechanism of HERC5, especially regarding the structural and dynamic characteristics of the HECT domain, is not well understood.

Small angle X-ray scattering (SAXS) is an effective structural technique, offering broad application in multiple disciplines. In protein studies, SAXS data provides valuable information on particle size and distribution, and particle morphology about the macromolecules in solution (Blanchet and Svergun, 2013). In this study, the structural organization and conformational characteristics of the HECT domain located at the C-terminus of the HERC5 protein, was investigated in solution using the SAXS technique. The collected SAXS data offer significant insights into the general structure, molecular dimensions, potential flexibility, and conformational variability of the HERC5 HECT domain in solution. Furthermore, in relation to existing literature, this may provide fresh perspectives on the varying oligomerization states of the protein in solution and the impact of these states on its structural dynamics. These results will

contribute to the understanding of the ISG15 transfer mechanism of HERC5, laying a crucial foundation for future biotechnological and pharmacological applications focused on this protein.

Material & Method

Chemicals

Lysogeny broth (LB) , kanamisin, chloramphenicol, isopropyl-1-thio-β-d-galactopyranoside (IPTG), TRIS (tris(hydroxymethyl)aminomethane), sodium chloride (NaCl), dithiothreitol (DTT), Glutathione-s-transferase, and all other common chemicals were obtained from Sigma (USA).

Protein expression

The HECT domain of HERC5 (residues 702-1024) with a glutathione-S-transferase (GST) fusion protein was expressed in *Escherichia coli* BL21 (DE3) (Thermo Fisher Scientific, USA) (Dağ et al., 2025). The cells were grown at 37 °C in lysogeny broth (LB) culture medium supplemented with the required antibiotics 50 µl/mg kanamisin and 35 µl/mg chloramphenicol. Upon reaching a bacterial cell culture density of OD595: 0.4, the temperature was cooled down to 16 °C. Following that, when the cell density attained OD595: 0.7, recombinant protein production, induced with 0.4 mM IPTG. Bacterial cultures were incubated for 19 hours at 18°C. After 19 hours of expression, the cells were harvested by centrifugation at 3000 rpm for 45 minutes. The cell pellets were stored at -45 °C for further use.

Protein purification

The frozen cell pellets were thawed and resuspended in lysis buffer consisting of 500 mM NaCl, 50 mM Tris-base, 0.1% (v/v) Triton X-100, 5% (v/v) glycerol, and 1 mM DTT at pH 7.5. The cells expressing HERC5 HECT were lysed by sonication and subsequently centrifuged at 11,500 rpm for 59 minutes. The supernatant containing the GST-HERC5 fusion protein was purified using the Glutathione Sepharose® affinity column (GSTrap) and the ÄKTA™ Start protein purification system. Following equilibration of the column with the equilibration buffer(20 mM Tris-base,150 mM NaCl, and 1 mM DTT pH: 7.4), the sample was loaded onto the column and washed with a wash buffer. Then, elution was performed with an elution buffer (20 mM Tris-base, 150 mM NaCl, 30 mM Glutathione and, 1 mM DTT pH: 7.4) to obtain GST HERC5 HECT. The eluted protein fractions were pooled and dialyzed overnight against a storage buffer (20 mM Tris-base,150 mM NaCl, and 1 mM DTT pH: 7.4) with Thrombin enzyme. Then, the sample was loaded onto the column equilibrated with an equilibration buffer and free HERC5 HECT was collected. After the wash step, an elution buffer was passed through to obtain the GST proteins bound to the column (Dağ et al., 2025).

Size-exclusion Chromatography (SEC)

The eluted free HERC5 HECT protein fractions were pooled together, concentrated to about 5 ml by 30K centrifugal ultrafilters. Size-exclusion chromatography was carried out in the ÄKTA™ start protein purification system chromatography system using the Superdex S75 16/60 Column. The column was equilibrated with an equilibration buffer (20 mM Tris-base,150 mM NaCl, and 1 mM DTT pH: 7.4) and samples were loaded onto the column. The elution fractions were collected by SEC buffer (20 mM TRIS, 150 mM NaCl and 1 mM DTT pH: 7.4)

Small Angle Scattering X-Ray (SAXS) data collection, processing, and modeling

Small- angle X-Ray scattering (SAXS) experiments were performed to determine native state of HERC5-HECT in solution. The SAXS experiment was performed using the SAXSpace instrument (Anton Paar 5.0), with a 'Multi Cuvette Holder', 'Heated/Cooled Sample Holder', and a 'Beam Stopper' utilizing a 2 mm Ni. Furthermore, the sample-to-detector distance (SDD) is set to 1550 nm, and the 6 frames are set to last 1200 seconds each. Then, SAXS analysis was initiated at 2.0 mg/ml at 10 °C using an 80 µl of protein sample. The gathered two-dimensional scattering data was processed using various steps, including Zero Point 2D by Moments, Masking, Q Transformation, Transmission, Data Reduction, and Standard Operations 1D in SAXSanalysis™ software (Göcenler et al., 2022). The generated '.dat' file was subsequently uploaded to the PRIMUS package within ATSAS 4.0 to compute the log-log plot, Guinier plot, Kratky plot, pair-wise distance distribution function P(R), radius of gyration (Rg), scattering

intensity I(0), maximum particle size in solution (Dmax), and Porod volume (Vp) values. The graphical data revealed that the protein existed in a trimeric form, prompting the use of the DAMMIF tool to produce low-resolution initial models of protein structures by analyzing.. The obtained model was fitted with crystal structure from the PDB database. The overlay was performed using the ATSAS CIFSUB plugin and the PyMol software.

Results and Discussion

X-ray small-angle scattering studies were conducted with the HERC5 HECT protein to reveal its intricate structural features and distinct morphology, providing valuable insights into the protein-protein interactions and conformational flexibility of HERC5. In this study, the SAXS data of a protein sample at 2.0 mg/ml concentration was analyzed. The parameters were calculated using the ATSAS package and are given in Table 1.

Guinier analysis was performed on the SAXS data obtained from the protein sample, as illustrated in Supplementary Figure 1A. In Guinier plots, a good linear section was observed in the low q^2 range, indicating that the data are suitable for determining the radius of gyration (Rg). The radius of gyration (Rg) values obtained from Guinier analysis are presented in Table 1. The Rg and I(0) values obtained from the Guinier analysis and the P(r) plot are found to be highly associated, thereby confirming the existence of overlap between the data in both real and reciprocal space (Jagdev, Dandapat and Vasudevan, 2021). Numerous studies in the literature provide evidence that the radius of gyration (Rg) value demonstrates a pronounced decrease with increasing protein concentration (Receveur-Bréchot and Durand, 2012; Putnam and Hammel,

2013). The findings suggest that HERC5 HECT may exhibit increased conformational flexibility or a heightened propensity for interactions when present at higher concentrations. Supplementary Figure 1B present Kratky plots derived from protein samples demonstrate an overall folded structure. The prominent peaks observed in the plot suggest that the proteins possess well-folded structures with a spherical or globular shape (Jacques and Trewhella, 2010; Skou, Gillilan, and Ando, 2014). This observation, combined with the Rg values, suggests that high concentrations may induce minor structural changes. However, it's encouraging to note that the overall folding topology of the protein remains largely intact.

Figure 1A displays the log(I(q)) versus s scatter curves for the sample. The scattering curve shows typical behavior associated with small particles. The sample (Fig. 1A) exhibits noise and fluctuations, particularly in the high q region. The increased noise in the high q region of the curve from Figure 1A imply potential effects related to the high concentration. Distance distribution function P(r) analyses (Fig. 1B) support our Kratky plot findings. The single peak and generally symmetrical shape of the P(r) plot confirm that protein has a globular structure (Jacques and Trewhella, 2010; Skou, Gillilan, and Ando, 2014). These findings can be interpreted by Guinier and Kratky analyses, which can lead to slight conformational adaptations while maintaining the protein's basic structure.

The molecular weight estimates presented in Table 1, which are almost three times higher than the theoretical molecular weight of the protein suggest that the protein forms a trimer in solution. Low-resolution bead envelopes for the HERC5 HECT protein were constructed using the DAMMIF program (Putnam et al., 2007; Svergun & Koch, 2003; Jacques and Trewhella, 2010).

The AlphaFold structure of the HERC5 HECT protein (Q9UII4) was superimposed with the DAMAVER model, which averaged the 21 DAMMIF generated images. The ab initio model was fitted using PyMOL software (Fig. 2).

The ab initio findings were compared to the known AlphaFold structure for HERC5 HECT by superimposition. No examples of trimer formation in other HECT structures were found, unlike in distinct HECT domains such as WWP1 (Verdecia et al., 2003) and Smurf2 (Ogunjimi et al., 2005), HUWE1 (Pandya et al., 2010), Nedd4-1 (Maspero et al, 2011) while E6AP has a unique feature related to trimer formation (Huang et al., 1999). The prevailing hypothesis regarding E6AP (E6-AP ubiquitin-protein ligase) suggests that it primarily functions as a monomer. However, in their influential study, Huang et al. (1999) present evidence of a trimeric configuration, a formation consisting of three interconnected E6AP molecules. They speculate that this trimeric arrangement may arise from intermolecular interactions that occur during the crystal packing process used for structural analysis (Huang et al.,1999). Further studies employing kinetic and biophysical analyses in a similar manner (Ronchi et al., 2014) revealed that the E6AP enzyme requires oligomerization to form the polyubiquitin degradation signal and suggested a trimeric structure. This was found in an ongoing process of Phe727, which was conducive to trimer formation through the provision of hydrophobic interactions between E6AP subunits.Furthermore, it has been established that mutations at the Phe727 position induce impairment in trimerization and enzymatic activity, which may result in neurodevelopmental diseases, such as Angelman Syndrome. The HPV E6 protein has been shown to accelerate the degradation of tumor suppressor proteins, such as p53, by increasing the trimerization and catalytic activity of E6AP through its ability to form homodimers. Subsequent analysis of

enzyme kinetics revealed that E6AP could bind to E2 even in the presence of a mutation, and that the Cys820 residue within the HECT domain was capable of initiating ubiquitin transfer by forming a transient bond with ubiquitin (Ronchi et al., 2014). In 2017, Ronchi et al. advanced this research by integrating in silico modeling and kinetic analyses, revealing that E6AP does not possess a single E2 binding site. Instead, it showed that polyubiquitin chain formation exhibits two functionally distinct binding sites, challenging the classical transthiolation model. Ronchi et al. (2014) experimentally verified that the catalytically active form of E6AP is a trimer, and that this oligomer structure is essential for the assembly of polyubiquitin chains. Furthermore, Ronchi et al. (2017) corroborated the trimeric structure by identifying the presence of two functional E2 binding sites, designated as Site 1 and Site 2 (Ronchi et al., 2017). These two studies suggest that two different sites in different subunits of the E6AP trimer work together to coordinate polyubiquitin chain assembly. Takade et al. revealed that the hinge loop flexibility in the E6AP HECT domain leads to a significant conformational change in the structure of the domain (Takade et al., 2022). The study identified L-shaped, inverted T, and catalytic conformations using the HS-AFM technique, and analyses exhibited monomeric and dimeric forms of the HECT domain (Takade et al., 2022). In contrast, our study revealed that the HERC5 protein exhibits a trimeric structure in the HECT domain solution, with an approximate molecular weight that is three times the protein's monomer theoretical molecular weight. This finding is further supported by the fitting of the bead model to the trimer structure. Todaro et al. (2018) demonstrated that NEDD4-2 HECT E3 ligases can modulate their enzymatic activity through the process of oligomerization. Their study elucidated that the oligomerization of the Phe823 residue, in conjunction with the structural properties of NEDD4-2, plays a crucial role in the assembly of polyubiquitin chains. Phe823 partially stabilizes the full-length NEDD4-2

protein into a trimeric structure, as revealed by structural analyses. According to this suggestion, Phe823, which has both structural and functional similarities to Phe727 in E6AP, could specifically hinder the formation of Lys63-linked polyubiquitin chains. These findings reveal that the functional activity of HECT E3 ligases can be regulated not only by monomer or dimer structures but also by oligomeric complexes (Todaro et al., 2018). Hodokowa et al. conducted a structural investigation that demonstrated UBR5 (ubiquitin protein ligase E3 component n-recognin 5) can exist in both dimeric and tetrameric forms, as evidenced by cryo-EM analysis. The results indicate that two crescent-shaped monomers of UBR5 come together end-to-end to create a dimer, which then pairs face-to-face with another dimer, resulting in a cage-like tetramer configuration that orients all catalytic HECT sites toward the central cavity.

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AUTHORS' CONTRIBUTIONS

C.D.T.C. contributed to Investigation, Formal analysis, Writing-Original draft preparation, Ç.D. contributed to Investigation, Conceptualization, Methodology, Supervision, Funding acquisition, Writing-Original draft preparation, Formal analysis, Writing−Reviewing and Editing

REFERENCES

Abe, T., Minami, N., Bawono, R. G., Matsui, C., Deng, L., Fukuhara, T., Matsuura, Y., & Shoji, I. (2020). ISGylation of Hepatitis C Virus NS5A Protein Promotes Viral RNA Replication via Recruitment of Cyclophilin A. Journal of virology, 94(20), e00532-20. https://doi.org/10.1128/JVI.00532-20

Bernassola F, Karin M, Ciechanover A, Melino G. The HECT family of E3 ubiquitin ligases: multiple players in cancer development. Cancer Cell 2008; 14: 10-21. https://doi.org/10.1016/j.ccr.2008.06.001

Blanchet, C. E., & Svergun, D. I. (2013). Small-angle X-ray scattering on biological macromolecules and nanocomposites in solution. Annual review of physical chemistry, 64, 37–54. https://doi.org/10.1146/annurev-physchem-040412-110132

Dağ, Ç., Tozkoparan Ceylan, C. D., & Cansız, C. S. (2025). Inconsistent Protein Stability Despite Pre-HECT Domain Helix: Unveiling Variability in HECT Ligases. Protein and peptide letters, 10.2174/0109298665362863250114075840. Advance online publication.

Göcenler, O., Yenici, C. M., Kahraman, K., Büyükdağ, C., et al. (2022). BIOMOLECULAR SOLUTION X-RAY SCATTERING AT n2STAR BEAMLINE. Mugla Journal of Science and Technology, 8(2), 60-69. https://doi.org/10.22531/muglajsci.1174474

Huang, L., Kinnucan, E., Wang, G., Beaudenon, S., Howley, P. M., Huibregtse, J. M., & Pavletich, N. P. (1999). Structure of an E6AP-UbcH7 complex: insights into ubiquitination by the E2-E3 enzyme cascade. Science (New York, N.Y.), 286(5443), 1321–1326. https://doi.org/10.1126/science.286.5443.1321

Jagdev, M. K., Dandapat, J., & Vasudevan, D. (2021). Recombinant expression, purification and SAXS analysis of Arabidopsis thaliana ClpC1. International journal of biological macromolecules, 167, 1273–1280. https://doi.org/10.1016/j.ijbiomac.2020.11.081

Jacques, D. A., & Trewhella, J. (2010). Small-angle scattering for structural biology--expanding the frontier while avoiding the pitfalls. Protein science : a publication of the Protein Society, 19(4), 642–657. https://doi.org/10.1002/pro.351

Maspero, E., Mari, S., Valentini, E., Musacchio, A., Fish, A., Pasqualato, S., & Polo, S. (2011). Structure of the HECT:ubiquitin complex and its role in ubiquitin chain elongation. EMBO reports, 12(4), 342–349. https://doi.org/10.1038/embor.2011.21

Ogunjimi, A. A., Briant, D. J., Pece-Barbara, N., Le Roy, C., Di Guglielmo, G. M., Kavsak, P., Rasmussen, R. K., Seet, B. T., Sicheri, F., & Wrana, J. L. (2005). Regulation of Smurf2 ubiquitin ligase activity by anchoring the E2 to the HECT domain. Molecular cell, 19(3), 297–308. https://doi.org/10.1016/j.molcel.2005.06.028

Pandya, R. K., Partridge, J. R., Love, K. R., Schwartz, T. U., & Ploegh, H. L. (2010). A structural element within the HUWE1 HECT domain modulates self-ubiquitination and substrate ubiquitination activities. The Journal of biological chemistry, 285(8), 5664–5673. https://doi.org/10.1074/jbc.M109.051805

Perng, YC., Lenschow, D.J. ISG15 in antiviral immunity and beyond. Nat Rev Microbiol 16, 423–439 (2018). https://doi.org/10.1038/s41579-018-0020-5

Putnam, C.D., Hammel, M., Hura, G.L. and Tainer, J.A., "X-ray solution scattering (SAXS) combined with crystallography and computation: Defining accurate macromolecular structures, conformations and assemblies in solution", Quarterly Reviews of Biophysics, Vol. No. 40(3), 191–285, 2007. https://doi.org/10.1017/S0033583507004635

Receveur-Brechot, V., & Durand, D. (2012). How random are intrinsically disordered proteins? A small angle scattering perspective. Current protein & peptide science, 13(1), 55–75. https://doi.org/10.2174/138920312799277901

Rotin D, Kumar S. Physiological functions of the HECT family of ubiquitin ligases. Nat Rev Mol Cell Biol. 2009;10(6):398–409. https://doi.org/10.1038/ nrm2690.

Ronchi, V. P., Klein, J. M., Edwards, D. J., & Haas, A. L. (2014). The active form of E6-associated protein (E6AP)/UBE3A ubiquitin ligase is an oligomer. The Journal of biological chemistry, 289(2), 1033–1048. https://doi.org/10.1074/jbc.M113.517805

Ronchi, V. P., Kim, E. D., Summa, C. M., Klein, J. M., & Haas, A. L. (2017). In silico modeling of the cryptic E2~ubiquitin-binding site of E6-associated protein (E6AP)/UBE3A reveals the mechanism of polyubiquitin chain assembly. The Journal of biological chemistry, 292(44), 18006–18023. https://doi.org/10.1074/jbc.M117.813477

Sluimer J, Distel B. Regulating the human HECT E3 ligases. Cell Mol Life Sci. 2018;75(17):3121-3141. https://doi.org/10.1007/s00018-018-2848-2

Skou, S., Gillilan, R.E. and Ando, N., “Synchrotron-based small-angle X-ray scattering of proteins in solution”, Nature Protocols, Vol. No. 9(7), 1727-1739, 2014. https://doi.org/10.1038/nprot.2014.116

Svergun, D. I., & Koch, M. H. J. (2003). Small-angle scattering studies of biological macromolecules in solution. Reports on Progress in Physics, 66(10), 1735–1782. https://doi.org/10.1088/0034-4885/66/10/r05

Takeuchi, T.; Inoue, S.; Yokosawa, H. Identification and Herc5-mediated ISGylation of novel target proteins. Biochem. Biophys. Res. Commun. 2006, 348, 473–477. https://doi.org/10.1016/j.bbrc.2006.07.076

Takeda, K., Flechsig, H., Muro, I., Amyot, R., Kobayashi, F., Kodera, N., Ando, T., & Konno, H. (2023). Structural Dynamics of E6AP E3 Ligase HECT Domain and Involvement of a Flexible Hinge Loop in the Ubiquitin Chain Synthesis Mechanism. Nano letters, 23(24), 11940–11948. https://doi.org/10.1021/acs.nanolett.3c04150

Todaro, D. R., Augustus-Wallace, A. C., Klein, J. M., & Haas, A. L. (2018). Oligomerization of the HECT ubiquitin ligase NEDD4-2/NEDD4L is essential for polyubiquitin chain assembly. Journal of Biological Chemistry, 293(47), 18192–18206. https://doi.org/10.1074/jbc.ra118.003716

Verdecia, M. A., Joazeiro, C. A., Wells, N. J., Ferrer, J. L., Bowman, M. E., Hunter, T., & Noel, J. P. (2003). Conformational flexibility underlies ubiquitin ligation mediated by the WWP1 HECT domain E3 ligase. Molecular cell, 11(1), 249–259. https://doi.org/10.1016/s1097-2765(02)00774-8

Yang, Q., Zhao, J., Chen, D., & Wang, Y. (2021). E3 ubiquitin ligases: styles, structures and functions. Molecular biomedicine, 2(1), 23. https://doi.org/10.1186/s43556-021-00043-2

Wang, Y.; Argiles-Castillo, D.; Kane, E.I.; Zhou, A.; Spratt, D.E. HECT E3 ubiquitin ligases-emerging insights into their biological roles and disease relevance. J. Cell. Sci. 2020, 133. https://doi.org/10.1242/jcs.228072

Wong, J.J.; Pung, Y.F.; Sze, N.S.; Chin, K.C. HERC5 is an IFN-induced HECT-type E3 protein ligase that mediates type I IFN-induced ISGylation of protein targets. Proc. Natl. Acad. Sci. USA 2006, 103, 10735–10740. https://doi.org/10.1073/pnas.0600397103

Woods, M. W., Kelly, J. N., Hattlmann, C. J., Tong, J. G., Xu, L. S., Coleman, M. D., Quest, G. R., Smiley, J. R., & Barr, S. D. (2011). Human HERC5 restricts an early stage of HIV-1 assembly by a mechanism correlating with the ISGylation of Gag. Retrovirology, 8, 95. https://doi.org/10.1186/1742-4690-8-95

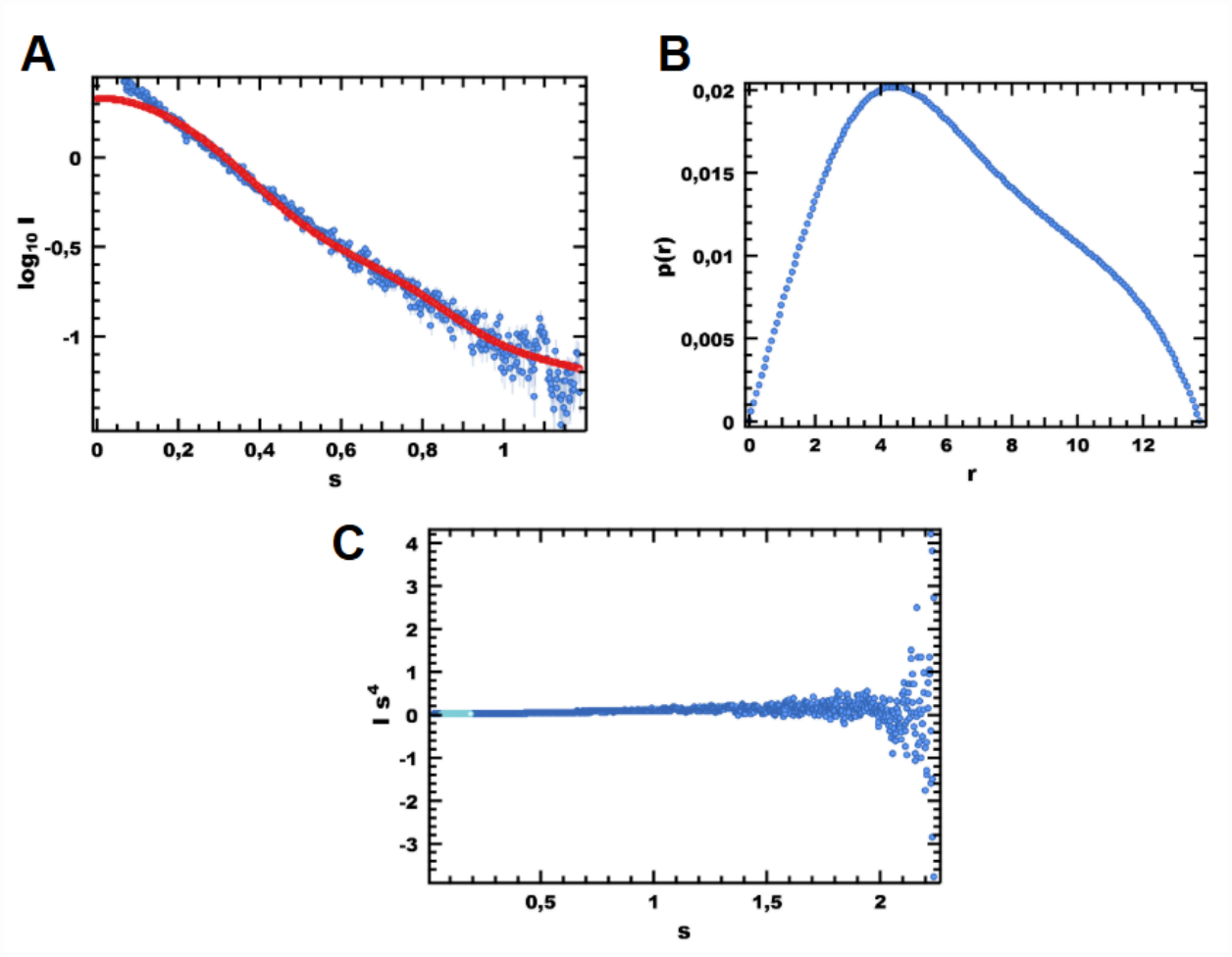


Figure 1. Small-angle X-ray scattering (SAXS) profiles. The plot of log intensity of the scattering of HERC5 HECT against the scattering vector (s) (A), the pair distance distribution function P(R) curve (B), porod volume (C).

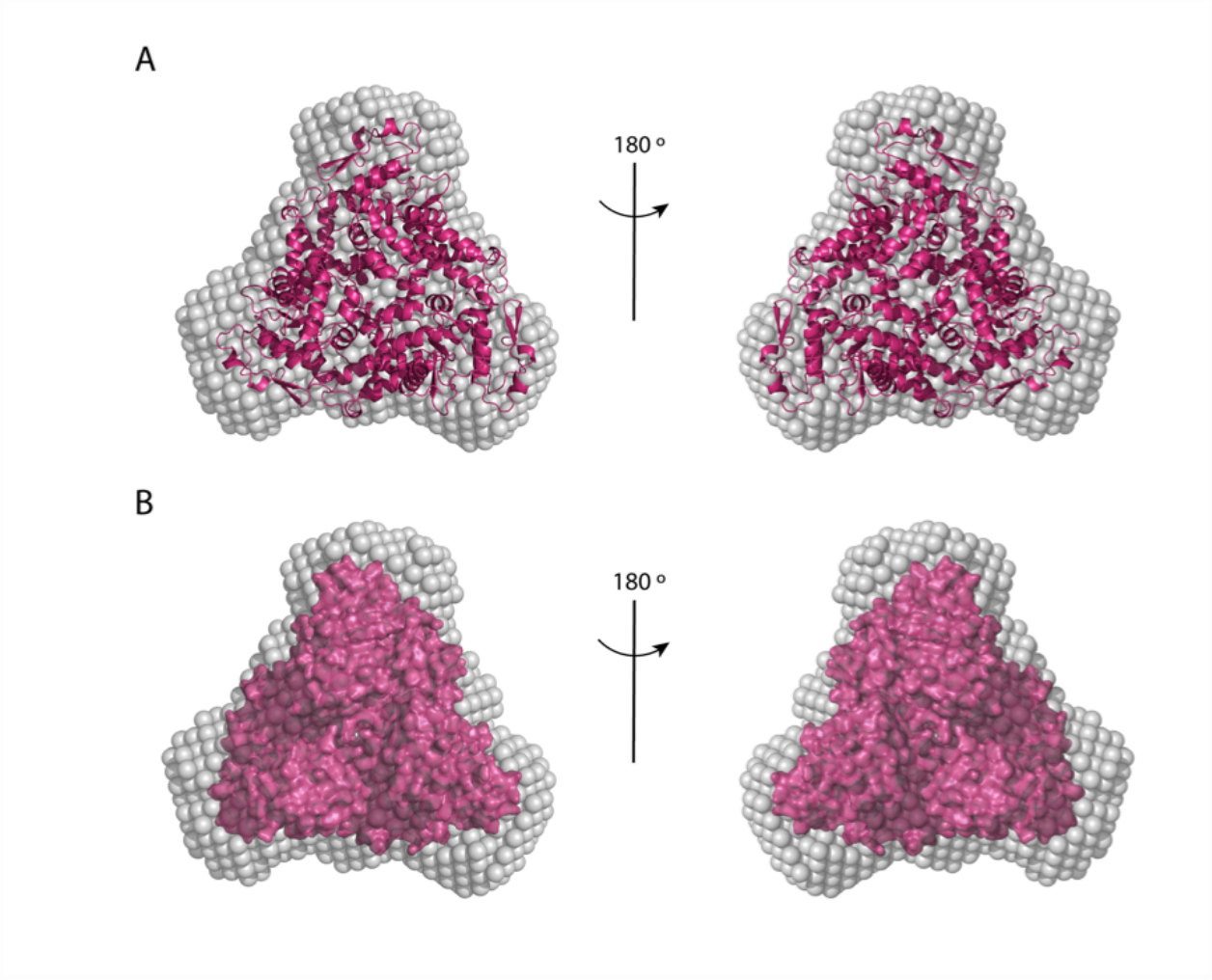


Figure 2. Cartoon (A) and Surface (B) representations of the trimeric crystal structure of HERC5 HECT (AlphaFold: Q9UII4) superimposed on SAXS ab initio envelope generated from the SAXS scattering profile using DAMMIF with P3 symmetry.

Table 1. SAXS Instrument parameters of HERC5 HECT

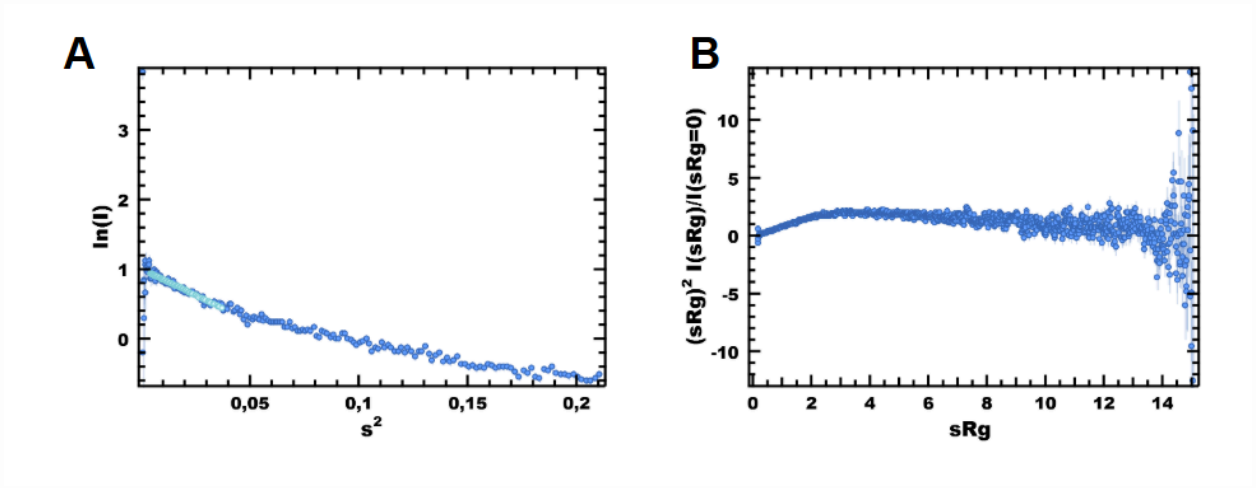
Instrument	SAXSpoint 5.0 (Anton Paar)

Q range (nm−1)	0.01 nm^{-1} to 49.3 nm^{-1}
Exposure time (min)	120
Temperature (C°)	10
Sample volume (µl)	80
Sample concentration (mg/ml)	2.0

Table 2. SAXS parameters of HERC5 HECT

I(0) [from Guinier]	2.71
*Rg (nm) [from Guinier]	5.03
I(0) [from P(r)]	2.13
Rg (nm) [from P(r)]	4.91
*Dmax (nm)	12
Porod volume estimate (Vp) (A^3)	17644
From Porod volume (Da)	274576
From SAXSMow2 (Da)	175701
Molecular mass from sequence (Da)	157050
Symmetry	P3
Primary data reduction	SAXSanalysis
Data processing	ATSAS 4.0 PRIMUS
Ab initio analysis	DAMMIF
Validation and averaging	DAMAVER
3D graphics representations	PYMOL

SUPPLEMENTAL FIGURES



Supplementary Figure 1. Figure 1. Small-angle X-ray scattering (SAXS) profiles. (A) Guinier plot analysis (B) Kratky plot analysis.