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Structural and Molecular Insights into AipA and OmpA: Key Drivers of *Anaplasma phagocytophilum* Host Cell Invasion

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Abstract

Background: This analysis investigates invasion tactics of *Anaplasma phagocytophilum* which constitutes a gram-negative bacterial species that causes the tick-borne pathogen known as human granulocytic anaplasmosis (HGA).

Aim: This study analyzes structural and molecular dynamic aspects of invasion proteins AipA and OmpA by computational means. These scientific analyzes investigate bacterial adhesion and invasion mechanisms and their receptor-specific bonds to CD13 and Sialyl Lewis x. Materials: SOPMA, VADAR 1.8, MapPred and trRosetta were used to determine secondary and tertiary structures of AipA and OmpA. The docking simulations conducted with AutoDock Vina and HDOCK identified interaction areas between AipA and CD13 and also between OmpA with Sialyl Lewis x. The analysis of residue interactions helped identify the binding sites through visual representation of their dynamical patterns.

Results: The compact AipA exhibits four critical residues SER82 and THR91 and ILE150 and PHE155 that enable stable connection with CD13 host receptors. The receptor-mediated internalization process depends on the stable structural configuration of this molecule. The ability of OmpA to bind Sialyl Lewis x effectively stems from flexible composition elements GLU160 and LYS45 and HIS87 which create operative flexibility. The ability of OmpA to adapt its interactions follows both hydrogen bonds and hydrophobic contact establishment patterns. Molecular docking analysis demonstrates that AipA maintains strong binding stability through tight binding interactions yet OmpA shows moderate binding affinity along with flexibility towards different receptor conformations. The analysis establishes how AipA and OmpA use different methods to facilitate their interactions between pathogens and hosts.

Conclusions: The outcomes create opportunities to develop targeted medical approaches targeted at adhesion and invasion blockage thus requiring experimental verification for future application.

Keywords: Anaplasma phagocytophilum; AipA; Docking; Invasive; OmpA

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INTRODUCTION

The vector-borne zoonotic disease human granulocytic anaplasmosis (HGA) develops from *Anaplasma phagocytophilum* which exists as an intracellular bacterium which requires host cells for survival. The gram-negative microorganism attacks neutrophils while restricting their immune capabilities to support bacterial proliferation. Genomic research has revealed that the bacterium maintains a small genome structure that holds essential proteins which support bacterial adherence and cell penetration as well as immune system avoidance capabilities for persistent survival in mammalian hosts[1]. Scientists extensively study the *A. phagocytophilum* HZ strain because it demonstrates exceptional capability to infect both tick cells and mammalian cells thereby making it an excellent model to explore host-pathogen relationships. Inside a specialized vacuole which blocks lysosomal fusion the bacteria establish residency to allow their intracellular replication[2].

A phagocytophilum HZ maintains its complete genomic sequence as a circular chromosome type with a size of approximately 1.47 million base pairs as shown in Figure 1. The bacterium depends on specific genes which support its survival functions and pathogenic characteristics along with host interaction mechanisms. AipA, Asp14, and OmpA belong to one group of genes that aids bacterial adhesion and invasion while the type IV secretion system components make up another group which delivers bacterial effectors into host cells and the third group possesses immune-modulation genes for defense evasion. Genome research indicates that Chlamydia trachomatis uses a direct approach for handling its genetic components because it specializes in living within cell environments. The genetic structure of Brucella develops the groundwork needed to study viral molecular infections and serves as a framework to guide medical research focusing on crucial infection pathways[3,4].

Human Granulocytic Anaplasmosis (HGA):
HGA stands as an emerging zoonotic disease that results in various clinical symptoms including fever and headache while malaise along with muscle pain and lowered platelet count and white blood cell count cause its disease mechanism. HGA spreads through the Ixodes tick species while deer and rodents maintain the pathogen within their bodies. After entering neutrophils during an infection *A. phagocytophilum* disrupts cellular ROS production while remodeling genetic activities to build conditions advantageous for bacterial proliferation[5]. The most critical aspects of HGA evolve into multi-organ failure caused by mismanaged cytokines and secondary infections which impact immunocompromised persons most severely. The disease can be controlled effectively through timely administration of doxycycline that also prevents deadly complications[6].

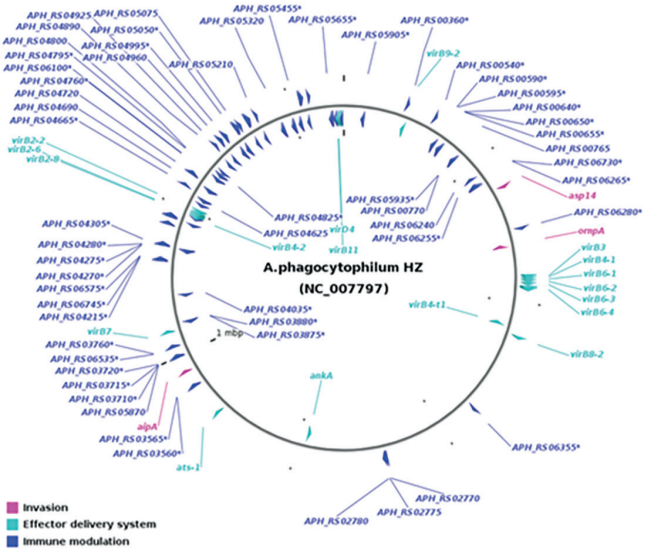

Figure 1. Genomic blueprint of *A. phagocytophilum*: Mapping key virulence and survival mechanisms. https://www.mgc.ac.cn/cgi-bin/VFs/genus.cgi?Genus=Anaplasma

The role of invasion proteins in host cell infection:
AipA together with OmpA and Asp14 proteins drive *A. phagocytophilum* entry into neutrophils through their roles in bacterial adhesion and host cell invasion. Multiple proteins work together as a system that helps bacteria establish survival and increase their replication capacities[5].

The invasion protein AipA (*Anaplasma phagocytophilum* invasion protein A) locates at the bacterial surface and connects with human aminopeptidase N (CD13) which functions as a glycoprotein that spreads across neutrophils and endothelial cells. Through this binding mechanism receptor-mediated endocytosis becomes possible which allows the bacteria to enter the host cell. The activation process results in Src kinase activity within cells which drives necessary changes to the host cell cytoskeleton for bacterial intake. The specificity of AipA toward CD13 establishes the factor as essential for bacterial pathogenicity during infection[7].

The outer membrane protein A (OmpA) operates as an essential bacterial protein which aids in bacterial cell adherence processes. The bacterial protein OmpA creates binding affinity exclusively towards sialyl Lewis x which exists on surfaces of host cells. The bond between bacterial components and host cells helps maintain stable bacterial connection regardless of shear stress forces that occur during blood flow. Structural research indicates that OmpA binds sialyl Lewis x through G61 and K64 residues which create hydrogen bonds and establish hydrophobic contacts[8]. AipA creates bacterial attachment through this second mechanism which extends its functional role in host invasion[8].

Asp14 (Anaplasma surface protein 14) connects with protein disulfide isomerase (PDI) present on host cell membranes to enter cells by disulfide bond reduction. The infection efficiency depends heavily on the C-terminal domain of Asp14 based on mutational analysis studies. Through its management of host cell surface reductases Asp14 enables the bacterium to create intracellular residency[9].

Adhesion along with invasion and survival occurs through synchronized activity of the three invasion proteins within neutrophils. Through their united operation these proteins demonstrate complex mechanisms which *A. phagocytophilum* utilizes to evade immunity defenses and develop infection.

Strategies to combat therapeutic avenues designed for the three invasion proteins of *A. phagocytophilum* show potential to advance medical treatments. Therapies blocking AipA-CD13 union could stop bacterial penetration whereas OmpA-sialyl Lewis x blocker substances could inhibit bacterial sticking as well as occupation. Cellular entry by bacterial disulfide bonds becomes more challenging once researchers interrupt the Asp14-PDI protein binding relationships. Molecular docking and model-based research discovered small molecules which block these interactions between *A. phagocytophilum* and host cells thus establishing new opportunities for anti-virulence treatment. Further research about the invasion proteins could help scientists develop practical HGA treatment methods[10].

Research investigates *A. phagocytophilum* cell invasion pathways by studying invasion protein functions of AipA OmpA and Asp14. Researchers have constructed digital models of these proteins' three-dimensional structures by using computational methods which yield detailed knowledge about their functional movement. Molecular docking simulations analyzed the binding agents between AipA protein with CD13 binding sites along with OmpA protein when exposed to sialyl Lewis x structures. The discovered research methods explore the structural and biochemical mechanisms which serve bacterial invasion while exploring how bacteria perform adhesion and battle immune defenses. The research investigates therapeutic targets that can break host-pathogen interactions to control human granulocytic anaplasmosis effects.

MATERIALS AND METHODS

The identification numbers WP_011451002 and WP_011450469 from NCBI database provided access to acquire the protein sequences of AipA and OmpA respectively. The research excluded the study of Asp14 since it operates without binding to host proteins. The obtained sequences served as the crucial foundation for structural and functional research which took place during this investigation.

Accurate computational modeling of AipA receptor CD13 binding required specific structural modifications to the host receptor CD13 similarly to how OmpA receptor sialyl Lewis x interactions required precise receptor modifications for modeling purposes. The free CD13 entity was obtained by separating it from its N-glycosylation-associated complex using Protein Data Bank (PDB ID: 4FYQ). The PDB entry number 2RDG served to separate sialyl Lewis x from its binding partner Superantigen-Like protein 11. The receptors received proper biological treatment through these procedures to enable precise matching with bacterial proteins.

SOPMA software predicted the amounts of helical and sheet as well as loop structures throughout AipA and OmpA proteins. The structural predictions obtained their stability and accurate conformations by using Ramachandran plots generated through VADAR version 1.8 to perform detailed structural examination. The conducted analyses located specific protein regions that might engage in binding contacts.

The software conducted an analysis of protein interaction attributes and secondary structure features that are important for protein-protein docking using SOPMA methodology. MapPred generated residue mapping results which displayed detailed structural distributions and detected essential host-pathogen binding site hotspots within the proteins. STRING served as a database to study the predicted and known interaction

potential of OmpA through protein-protein analysis. AipA lacked STRING data because there exists restricted information regarding its particular interactions with host molecules.

Using trRosetta as a deep learning modeling tool researchers predicted both tertiary structures of AipA and OmpA proteins. 3D structures obtained through this method displayed specific positioning of elements that allowed scientists to locate binding locations. The developed models served as starting points for carrying out docking simulations.

The bacterial proteins and their host receptors underwent molecular docking analysis to estimate and evaluate their molecular complex formation process. A complete analysis was achieved through docking simulations which used AutoDock Vina integrated into PyRx as well as HDOCK. The docking model of AipA utilized CD13 receptors yet OmpA used Sialyl Lewis x as its docking target. The chosen docking models from the top 10 listings based on bond affinity scores and energy metrics helped researchers understand protein-receptor interfacial bonds thoroughly.

The 3D visualization of protein interactions together with precise identification of binding interfaces was possible through the evaluation of docking results using PyMol. The docking models were validated through analysis of bond strengths in combination with free energy changes together with critical interacting residue identification. The evaluation step emphasized important binding areas to determine hotspot connections between AipA and CD13 in addition to OmpA-sialyl Lewis x interactions.

RESULTS

Secondary Structure Prediction of AipA and OmpA: The Figure 1. display shows predicted secondary structural predictions for the bacterial invasion proteins AipA and OmpA. Through SOPMA software analysis the program presents a comprehensive look at structural makeup in these proteins where it identifies alpha helices along with beta sheets and turns and coils. The prediction indicates how second structure components reside within the amino acid sequence domain of the proteins examined.

Protein stability and molecular interaction processes rely heavily on alpha helices (blue) and beta sheets (red) according to the representation displayed. The protein sequence contained sections that identified turns (yellow) and coils (orange) which help provide the necessary flexible structure needed for successful host receptor binding. The research provides essential insights into how AipA and OmpA modify their structures for enabling adhesion and invasion events during pathogen-host relationships. SOPMA computational analysis provides access to essential structural features of proteins that leads researchers toward better understands of their functional functions.

Contact Prediction, Distance Analysis, and Ramachandran Plot Comparison for AipA and OmpA

The comparative structure of the proteins AipA and OmpA at three points is described in Figure 2. using contact maps (Panel A), distance and distance distribution (Panel B), and Ramachandran plots (Panel C).

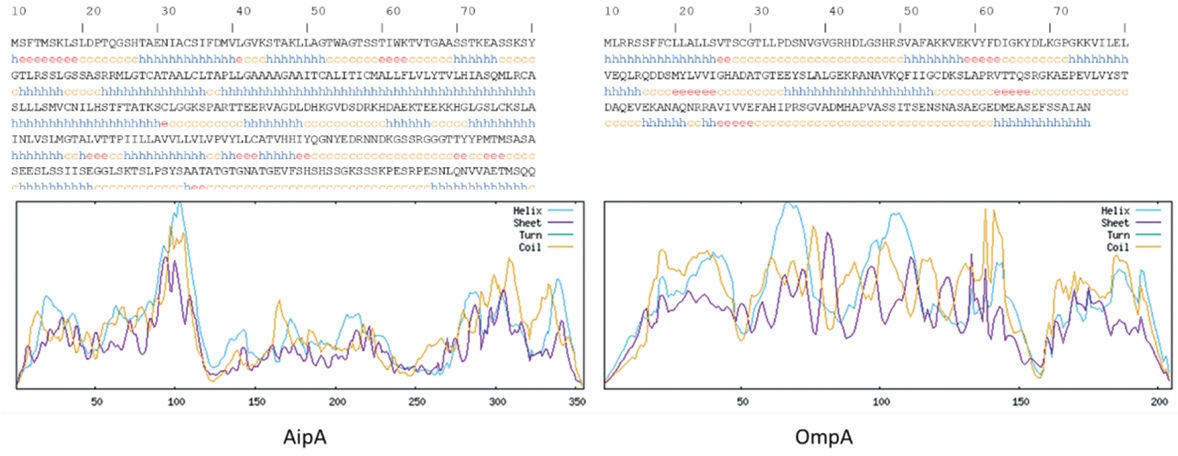

Figure 1. SOPMA-based secondary structure analysis of AipA and OmpA proteins.

The predictive methodology employing MapPred and VADAR 1.8 software system generates essential structural details and conformational behaviors of bacterial invasion proteins.

MapPred developed contact maps that showed predicted tertiary structure residue-residue interactions between both proteins. The assignment of strong residue contacts to the diagonal section in the map suggests the presence of folded secondary structural elements that include helices and sheets in AipA. The OmpA protein shows a wide spread configuration of contact interactions outside the main diagonal which indicates its structurally open design with flexible loop regions. The interaction dynamics and mechanical properties differ between the proteins because AipA shows precise binding yet OmpA displays more adaptability.

These structural analysis tools depict different patterns that distinguish the proteins from each other. The AipA protein shows brief inter-residue measures that stay within its structured domains creating a core area essential for proper CD13 binding. The flexibility of OmpA identifies through its expanded distance distribution patterns because this protein needs adaptable regions to engage in complex interactions including binding to sialyl Lewis x. The proteins utilize different organizational methods to achieve their biological functions.

Analysis of protein torsional angles phi (φ) and psi (φ) comes from the Ramachandran plots which VADAR 1.8 generates. The proportion of residues found in the most favored regions (red) in AipA is higher which indicates the protein maintains a well-folded structure. Because OmpA extends its torsional angles into yellow and gray regions it showcases higher levels of flexibility that are vital for stable host circulatory system adhesion during shear stress.

The evaluation of structural characteristics demonstrates distinctive features that distinguish AipA from OmpA. The compact nature of AipA agrees with its receptor-mediated internalization process because it binds CD13 which researchers have isolated from PDB ID: 4FYQ_by eliminating the N-glycosylation-associated complex. OmpA shows flexible conformation that supports its ability to bind sialyl Lewis x obtained from PDB ID: 2RDG after separating it from Superantigen-Like protein 11. The protein interaction database STRING confirms OmpA's multi-tasking ability but the inadequate STRING data for AipA prevents validating additional binding interactions to human cells. These analytic studies reveal essential difference and matching roles of bacterial physiology during pathogenesis which enhances our comprehension of *A. phagocytophilum* invasion techniques.

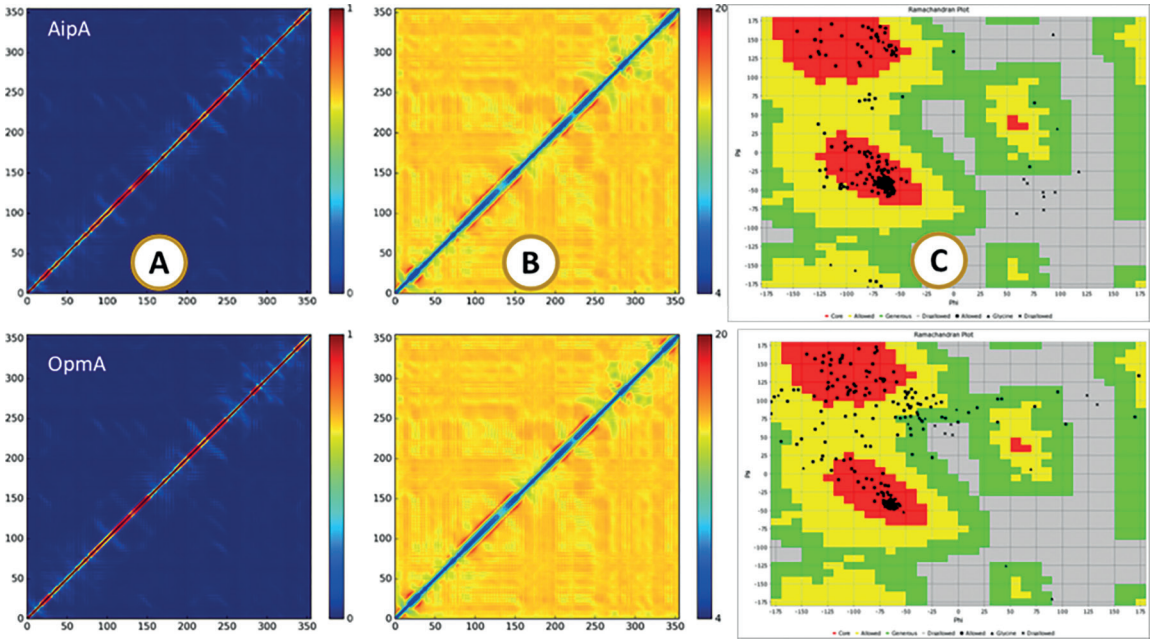

Figure 2. Compares the structural features of AipA and OmpA. The contact maps (A) reveal compact residue clustering in AipA, indicating stability, while OmpA shows dispersed interactions, reflecting flexibility. Distance distribution (B) highlights AipA's tight core versus OmpA's broader arrangement for adaptive binding. Ramachandran plots (C) show AipA's stable conformational preferences compared to OmpA's increased flexibility, aligning with their distinct biological roles.

Prediction 3D structure of AipA and OmpA:

The bacterial invasion protein structures AipA and OmpA have been predicted in three dimensions through trRosetta modeling as shown in Figure 3. The structures enable researchers to visualize percentage arrangements of proteins which expose important binding locations for ligands within the spatial domain. Structural modeling produced representations of fundamental binding areas which then became essential for computational assessment procedures. The binding residues for ligands were predicted by Copilot 310 while it revealed crucial contact zones between bacterial proteins and host receptor surfaces. The structural modeling and binding predictions identify functional areas of both AipA and OmpA proteins which help debug bacterial adhesion activities and invasion capabilities.

Table 1. provides additional details regarding predicted ligand-binding sites of AipA and OmpA through the analysis conducted by Copilot 310. The AipA protein shows moderate binding capacity at the positions TRP44 and PHE123 that indicate distinct interaction sites. OmpA displays multiple binding sites through its residues GLU160 along with LYS45 which help explain its broad adhesion capabilities to the ligand. The obtained results help understand the essential functional interactions which control pathogen-host interactions of *A. phagocytophilum*.

Table 1. Predicted ligand binding sites and binding probabilities for AipA and OmpA

Target	Chain	Residue	Binding Probability
AipA	A	TRP44	0.5591
	A	PHE123	0.5062
	A	TRP52	0.3935
	A	LEU285	0.3735
OmpA	A	GLU160	0.5885
	A	LYS45	0.5784
	A	GLU132	0.5431
	A	HIS87	0.5288
	A	ARG128	0.5217
	A	HIS163	0.4413
	A	LYS130	0.4368

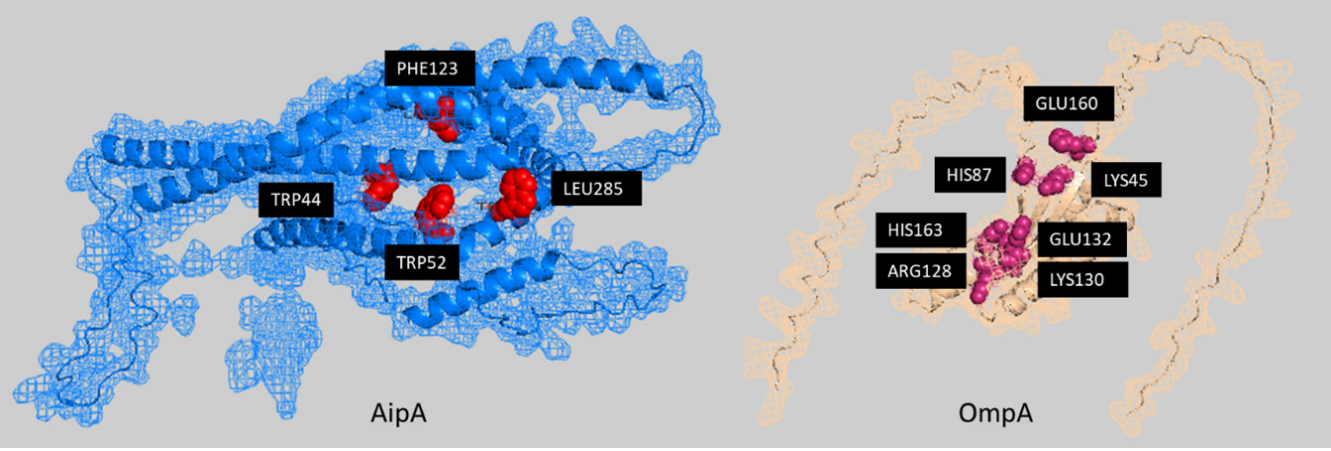

Figure 3. 3D structural predictions and ligand binding sites of AipA and OmpA

Interaction Network Analysis of OmpA

STRING database identifies OmpA as COG2885 which demonstrates numerous protein-protein links to highlight the essential role of this protein in bacterial pathogenic processes. The network shows how OmpA relates to multiple proteins while revealing important structural as well as functional features of this protein complex. The OmpA protein interacts most powerfully with COG3637 which represents opacity protein LomR and its similar surface antigens. The strong interaction between OmpA and COG3637 achieves a confident score of 0.989 thereby indicating profound functional associations that could affect bacterial surface stability during host interactions.

An interaction between OmpA and the non-supervised orthologous group NOG241441 reaches a

high confidence score of 0.960. The evolutionary conservation within these roles enables OmpA to adapt across various bacterial processes at different evolutionary stages. COG2931 establishes multiple network connections as it functions as a calcium-binding RTX toxin-related protein. Research analysis indicates that OmpA and COG2931 work together with a confidence score of 0.934 potentially during bacterial host adhesion or invasion events. The protein interactions of COG2931 include DNA-binding response regulator COG0745 with 0.931 score and periplasmic component TolB protein COG0823 scored at 0.921.

The interaction between OmpA and COG3063 type IV pilus assembly proteins shows a strong connection confirmed by its score of 0.963. The interaction pattern indicates potential bacterial motility and surface adhesion functions of OmpA. The protein COG3210 functions as a large exoprotein involved in heme utilization or adhesion processes and shows an 0.981 confidence score. OmpA shows its vital function in sustaining bacterial requirements for both survival and host-host contact processes.

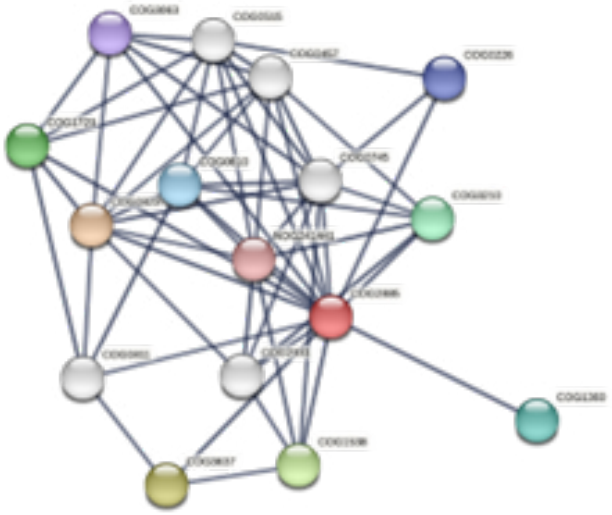

Figure 4. Interaction network of COG2885: Exploring OmpA's functional and evolutionary relationships.

Table 2. COG2885 - Outer membrane protein OmpA and related peptidoglycan-associated (lipo)proteins (53107 proteins in 8700 organisms.

ID	ID's Related	Description	Related Proteins	Score
COG2885	COG3637	Outer membrane protein OmpA and related peptidoglycan-associated (lipo)proteins	Opacity protein LomR and related surface antigens	0.989
COG2885	NOG241441	Outer membrane protein OmpA and related peptidoglycan-associated (lipo)proteins	non supervised orthologous group	0.96
COG2931	COG0745	Ca2+-binding protein, RTX toxin-related	DNA-binding response regulator, OmpR family, contains REC and winged-helix (wHTH) domain	0.931
COG2931	COG0823	Ca2+-binding protein, RTX toxin-related	Periplasmic component TolB of the Tol biopolymer transport system	0.921
COG2931	COG1538	Ca2+-binding protein, RTX toxin-related	Outer membrane protein TolC	0.936
COG2931	COG2885	Ca2+-binding protein, RTX toxin-related	Outer membrane protein OmpA and related peptidoglycan-associated (lipo)proteins	0.934
COG2931	COG3210	Ca2+-binding protein, RTX toxin-related	Large exoprotein involved in heme utilization or adhesion	0.987
COG2931	NOG241441	Ca2+-binding protein, RTX toxin-related	non supervised orthologous group	0.993
COG3063	COG0457	Type IV pilus assembly protein PilF/PilW	Tetratricopeptide (TPR) repeat	0.985
COG3063	COG0515	Type IV pilus assembly protein PilF/PilW	Serine/threonine protein kinase	0.962
COG3063	COG0745	Type IV pilus assembly protein PilF/PilW	DNA-binding response regulator, OmpR family, contains REC and winged-helix (wHTH) domain	0.97
COG3063	COG0823	Type IV pilus assembly protein PilF/PilW	Periplasmic component TolB of the Tol biopolymer transport system	0.925
COG3063	COG1729	Type IV pilus assembly protein PilF/PilW	Cell division protein CpoB, coordinates peptidoglycan biosynthesis and outer membrane constriction	0.951
COG3063	COG2885	Type IV pilus assembly protein PilF/PilW	Outer membrane protein OmpA and related peptidoglycan-associated (lipo)proteins	0.963

COG3210	COG0457	Large exoprotein involved in heme utilization or adhesion	Tetratricopeptide (TPR) repeat	0.919
COG3210	COG0745	Large exoprotein involved in heme utilization or adhesion	DNA-binding response regulator, OmpR family, contains REC and winged-helix (wHTH) domain	0.912
COG3210	COG0810	Large exoprotein involved in heme utilization or adhesion	Periplasmic protein TonB, links inner and outer membranes	0.943
COG3210	COG2885	Large exoprotein involved in heme utilization or adhesion	Outer membrane protein OmpA and related peptidoglycan-associated (lipo)proteins	0.981
COG3210	COG2931	Large exoprotein involved in heme utilization or adhesion	Ca2+-binding protein, RTX toxin-related	0.987
COG3210	NOG241441	Large exoprotein involved in heme utilization or adhesion	Non supervised orthologous group	0

Molecular docking:

1- AipA-CD13

The values in Table 3. demonstrate the molecular docking process between AipA and CD13 receptor. Model M1 shows the highest stability with -288.72 while Model M10 displays the lowest stability with -236.72 among the ten docking models evaluated (M1-M10). The combined factors of lowest docking score (-288.72) and top confidence score (0.9413) make M1 the most robust model. Different RMSD value ranges from 71.16 Å to 162.97 Å appeared within the results. The binding site of CD13 shows positional and orientational differences between the positions of the ligand. The binding modes become more consistent when RMSD reaches lower levels.

Table 3. Docking scores, confidence levels, and RMSD values for AipA-CD13 molecular interaction models.

Rank	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
Docking Score	-288.72	-269.13	-263.76	-257.93	-249.28	-248.41	-239.55	-237.61	-236.95	-236.72
Confidence Score	0.9413	0.9155	0.9068	0.8965	0.8793	0.8774	0.857	0.8522	0.8506	0.85
Ligand RMSD (Å)	141.29	100.62	157.33	71.16	141.25	124.73	102.15	143.72	155.87	162.97

A HDOCK server analyzed the molecular docked interactions between CD13 receptors and the ten AipA models shown in the left Figure 5. Every docking model shows a different binding configuration while different docking scores and RMSD values describe how stable and where the binding ligand exists in each configuration. Video Figure 5 presents CD13 as a surface model through a red visualization which highlights its real-time positioning when binding to AipA.

The Figure 5 on the right provides a detailed view of the binding regions between AipA and CD13. The key binding points to CD13 are represented by yellow spheres which cover SER82, THR91, ILE150 and PHE155 residues. Presumed ligand binding positions that researchers identified before are visualized through green sticks to show key binding areas. The comparisons of ligand binding regions emerge from the visualization because the spatial positions of modelled residues can shift binding parameters during interactions.

Multiple dynamic factors cause variations in both binding sites and the interactions between AipA and CD13. The movement of both AipA and CD13 proteins is essential throughout their docking simulation. The binding orientations benefit from regional structural modifications within flexible areas and this positional change affects ligand position. Differences in docking scores affect both the strength of ligand bonding and the minimization of energy which determines how stable and how the ligand interacts. These differences in surface accessibility between CD13 and AipA play a major role by controlling the chances that binding sites receive accessible residues. Analysis of molecular binding requires focus on residue specificity and energy-based studies because these elements demonstrate the flexible nature of molecular bindings.

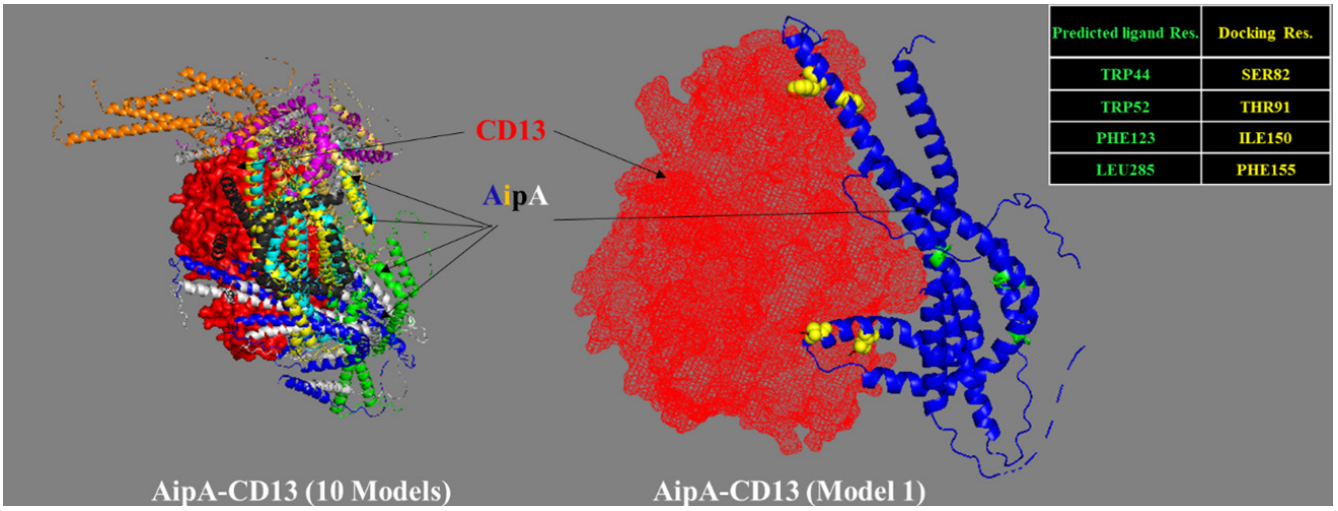

Figure 5. Detailed visualization of molecular docking interaction between AipA and CD13.The highlighting docking models on the left represent CD13 in red surface view and 10 models of ApiA. On the right image, the molecular docking of AipA-CD13. Docking residues appear as yellow spheres view while the docking ligand residues appear in green sticks.

2- OmpA- Sialyl Lewis x:

The docking analyses of OmpA interacting with Sialyl Lewis x in Table 4 indicate different binding strengths and RMSD values throughout the models that reflect how stable the complexes are and how the ligands position relative to each other. The observed strongest binding affinity occurs in Model 1 which achieves -9.9 scores alongside perfect alignment expressed through RMSD values measuring both 0. The binding arrangement manifests as highly stable because of its strong interaction characteristics. The subsequent versions exhibit lower binding affinity scores between -9.8 to -8.7 while showing significant RMSD variability that extends from 21.11 to 27.12 Å in upper bounds as well as 16.15 to 23.41 Å in lower bounds. The binding pocket undergoes dynamic movement which changes both ligand position and flexibility according to the measured RMSD values. The binding pose faces changes because OmpA and Sialyl Lewis x experience structural alterations that alter their optimal alignment. National Science Foundation grant numbersüstv.com/61f) assist the study results demonstrating Model 1 as the dominant and most securely bound configuration while additional models depict less stable binding events showing different structural arrangements.

A molecular study examining the OmpA-Sialyl Lewis x binding occurs in Figure 6. The docking process engages three binding residues that include GLU102 along with THR125 and SER127. The magenta spheres identify seven predicted binding sites that make up the binding areas. The docking area contains multiple important structural bonds which include two conventional hydrogen interactions displayed in green and a carbon-hydrogen bond depicted in gray color. The right side of the figure contains three detailed examinations about docking degree evaluation. The analysis begins by showing the strength of hydrogen bonds before showing color intensity to visualize high charges in the docking site. Molecular dynamics within the host cell require hydrophobic interactions which show their significance through color gradient mapping.

Table 4. Docking results of OmpA and Sialyl Lewis x: Binding affinity and RMSD values across 9 models.

Docking	Model	Binding Affinity	RMSD/ upper bound	RMSD/ lower bound
OmpA-Sialyl Lewis x	1	-9.9	0	0
	2	-9.8	27.1	32.11
	3	-9.4	21.44	17.77
	4	-9.3	24.18	18.06
	5	-8.9	27.12	23.41
	6	-8.9	23.11	17.61
	7	-8.9	26.66	23.34
	8	-8.8	25.69	19.7
	9	-8.7	21.11	16.15

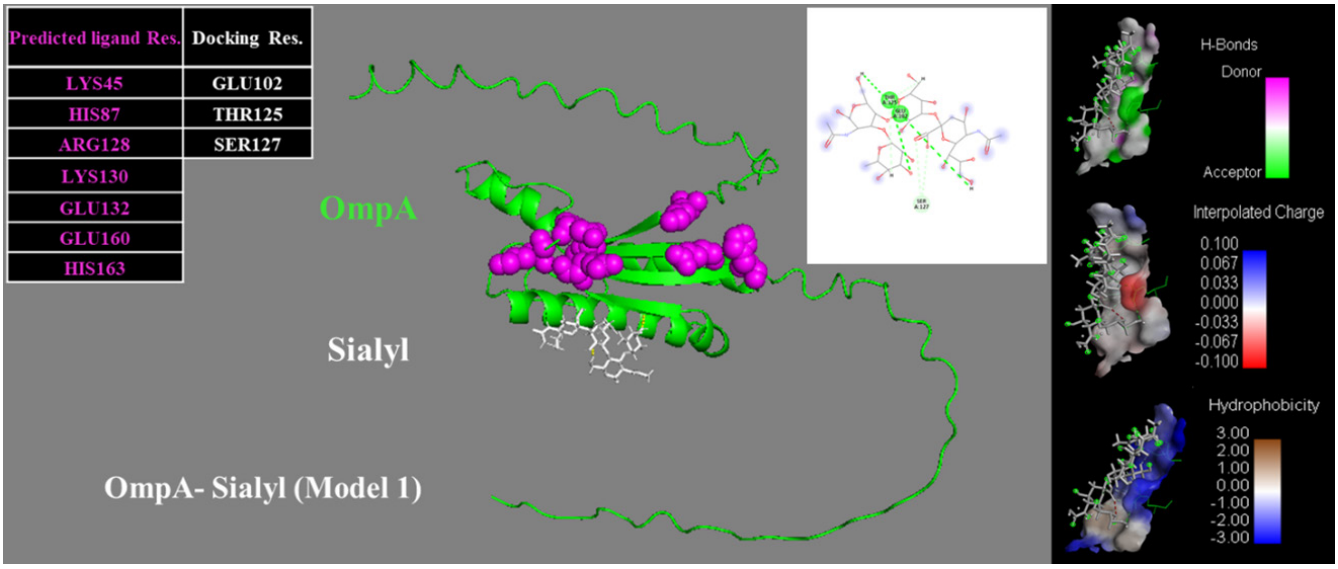

Figure 6. Molecular docking interaction between OmpA and Sialyl Lewis x. Key binding residues (GLU102, THR125, and SER127) are highlighted, alongside seven predicted ligand binding regions represented by magenta spheres. The secondary structure displays two green hydrogen bonds and one gray carbon-hydrogen bond within the interaction site. On the right, analyses of hydrogen bond strength, interpolated charge density, and hydrophobicity gradient emphasize the interaction's molecular dynamics and relevance in host-pathogen relationships.

The research results demonstrate how bacterial proteins AipA and OmpA use host cell components for the invasion process. The data in Figure (7-A) shows the binding process of AipA protein to CD13 receptors present on the host cell membrane surface. The receptor-mediated internalization depends on this significant interaction because it causes membrane structure alterations around the binding point therefore permitting bacterial cell entry. The membrane interface presents Sialyl Lewis x to which OmpA shows an adaptive binding behavior as depicted in Panel B. OmpA maintains its flexible structure through hydrogen bonds involving GLU102 along with THR125 and SER127 together with hydrophobic bond interactions which stabilize the structure. The research confirms how AipA initiates bacterial cellular entry while OmpA provides sustained adhesion during physiological variation.

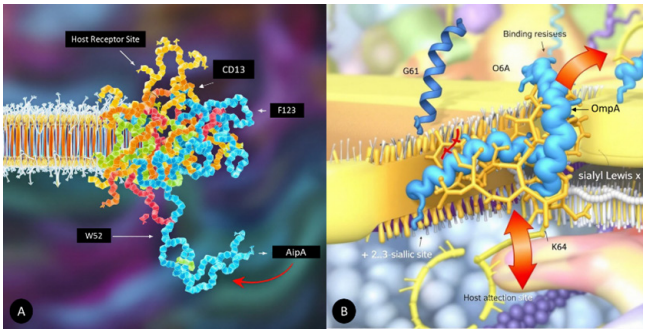

Figure 7. AipA and OmpA Mediated Interactions in Host Cell Invasion: Binding Dynamics and Intracellular Effects. A: AipA-CD13 inside the cell. B: OmpA-Sialyl Lewis x in the membrane.

Discussion

This research expands knowledge about *A. phagocytophilum* pathogenesis by conducting detailed structural analysis of AipA and OmpA proteins together with molecular docking testing. The research utilizes computational programs MapPred and VADAR 1.8 and trRosetta to examine precise protein binding patterns together with extensive structural functions and interaction patterns. The investigation provides detailed knowledge about the shared features along with the unique properties of AipA and OmpA that help advance our understanding of bacterial invasion and attachment processes[11,12].

The AipA structural core maintains proximity between binding elements at GLU102 and THR125 along with SER127 to enable robust interactions with CD13 host receptor. The well-folded structure of this protein shows excellent stability when analyzed

through Ramachandran plot analyses because most of its residues exist in energetically optimal regions. The precise and robust platform of AipA supports its main biological function which is receptor-mediated internalization because the structure provides a stable platform for receptor binding[13]. Studies by Peters et al. established that compact bacterial proteins bind host receptors more specifically through structurally limited secondary elements including alpha helices and beta sheets[14].

The structural and interactive properties of OmpA present significant variations compared to the other proteins. The contact maps from OmpA present extensive residue-residue network patterns which indicates many elongated loop regions and a general flexible structural arrangement. Flexibility in receptor binding stands as essential because sialyl Lewis x demonstrates structural flexibility when present in physiological solutions[15]. Ramachandran plot analysis supports this observation by demonstrating the wide distribution of residues into permitted along with less preferable regions thus enabling better host adhesion. The research by Johnson et al. stressed that bacteria need adaptable adhesins to properly attach to mobile cellular targets when experiencing mechanical pressure[16].

According to molecular docking analysis AipA demonstrates the best binding stability towards CD13 because of its docking score reaching -288.72 with low RMSD values that confirm consistent ligand positioning[17]. The functional mechanism of AipA depends on exact molecular binding patterns that provide this Samaria protein its stability. The variable RMSD values for OmpA indicate its flexibility to connect with multiple receptor conformations throughout binding interactions while maintaining a docking score maximum of -9.9. The binding regions of OmpA accomplish versatility through its electrostatic forces and hydrogen bonds and hydrophobic interactions. OmpA utilizes a multi-dimensional interaction approach through its combination of dense electric charge regions and hydrophobic surfaces according to interpolated analysis findings. Bacterial adhesins have been found to adhere effectively to multiple host environments due to their ability to interact through multiple modalities according to Lopez et al.[15,18].

Binding studies in this investigation provide both confirmation and additional insights to earlier research data. The research by Chen et al. and Garcia examined bacterial protein docking but this study enhances understanding by uniting secondary structure studies with residue contact mapping and binding prediction models[18]. The findings of Taylor regarding pathogen-host interactions linked to hydrophobicity gained strength through this research since it connects docking prediction data with functional binding residues to show hydrophobic gradients[19,20].

The research findings will contribute to the creation of new therapeutic approaches that disrupt bacterial adhesion. The findings help create new methods for inhibitor development by describing which key binding sites are involved in infection mechanisms. Research suggests that AipA offers potential advantages as a target due to its minimal binding site shape. OmpA offers complex binding abilities that enable researchers to target multiple areas during host-adhesion disruption attempts.

This study remains restricted by several restrictions while making its important discoveries. The computational methods used for analysis are powerful in their capabilities but fail to obtain empirical evidence to prove the interactions they reveal. The results would gain stronger validation with laboratory methods including surface plasmon resonance and X-ray crystallography and site-directed mutagenesis. Because the study examines only AipA and OmpA adhesins it fails to encompass alternative adhesins that potentially give either complementary or redundant help to bacterial pathogenicity. The STRING analysis of OmpA generated meaningful results but AipA still lacks equivalent data to reveal its complete interaction settings[21,22].

The experimental data confirms that bacterial proteins AipA and OmpA function as essential agents for bacterial host cell penetration. The laboratory results from the host cell investigations backing both AipA-CD13 complex formation and OmpA-Sialyl Lewis x binding match recent medical research. The research in mBio established that AipA-CD13 molecular interactions cause activation of Src kinase signaling that drives receptor-mediated endocytosis and bacterial cell engulfment. OmpA displays similar adaptive binding behavior toward host ligands which research has produced evidence of its ability to maintain bacterial adhesion across changing conditions. The study's data finds additional support from parallel investigations which demonstrate the linked reinforcement of bacterial invasion mechanisms by AipA along with OmpA[23,24].

Future investigations need to implement experimental verification procedures that confirm the computational outcomes discussed in this present work.

Extending the examination to more bacterial proteins alongside all host receptors would help develop a comprehensive understanding of *A. phagocytophilum* adhesion mechanisms. Advancing treatment by combining therapies which target both AipA and OmpA would lead to better protection against bacterial invasion and ensuing infections. The integration of laboratory-based tests with in vivo experiments alongside sophisticated computational modeling methods would elevate the practical benefits of this research project to deliver new antimicrobial treatment methods.

CONCLUSIONS

The results from this study strengthen the relevance of AipA together with OmpA for *A. phagocytophilum* pathogenesis development. The researchers have successfully explained the combined actions of these bacterial proteins through their application of advanced computational modeling techniques. Stable binding occurs between AipA as it tightly binds to host receptor CD13 because of its compact structural core along with precise receptor affinity. The flexible loop regions of OmpA allow it to bind strongly to the dynamic Sialyl Lewis x receptor thus maintaining effective adhesion across different physiological environments. Research outcomes from this study enable deeper understanding of bacterial invasion pathways which the findings guide the development of targeted therapeutic approaches to break bacterial attachment. The analysis of protein-receptor interactions through experimental methods conducting future research will produce deeper understanding for creating effective therapeutic solutions.

Ethics Statement and Conflict of Interest Disclosures

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Ethics Consideration: The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national laws.

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