RESEARCH PAPER

Molecular Interaction Analysis of Neutrophil Elastase, Elafin, and TGF-β in Early Childhood Caries - An In Silico Study

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ABSTRACT

Background

Early childhood caries (ECC) is a multifactorial disease involving microbial dysbiosis and host immune dysregulation. Protease–antiprotease balance and cytokine signaling are increasingly recognized as key contributors to disease progression.

Aim

To evaluate the molecular interactions between neutrophil elastase, elafin, and TGF-β1 and explore their potential role in ECC pathogenesis using in silico approaches.

Materials and Methods

Protein structures of neutrophil elastase (3Q76), elafin (1FLE), and TGF-β1 (1KLC) were retrieved and subjected to protein–protein docking using HADDOCK 2.4. Docked complexes were analyzed based on HADDOCK score, RMSD, Z-score, and energy parameters. Interaction visualization was performed using BIOVIA Discovery Studio.

Results

The neutrophil elastase–elafin complex showed strong binding stability with favorable HADDOCK scores and low RMSD, indicating a stable protease–inhibitor interaction. Neutrophil elastase–TGF-β1 and elafin–TGF-β1 complexes also demonstrated significant binding affinity, supported by favorable electrostatic and van der Waals energies, suggesting potential regulatory interactions.

Conclusion

The findings highlight the importance of protease antiprotease balance and cytokine interactions in ECC, providing insights into host immune mechanisms and potential therapeutic targets.

Keywords: Early Childhood Caries; Neutrophil Elastase; Elafin; Transforming Growth Factor Beta; Protein–Protein Interaction; Molecular Docking; HADDOCK; Salivary Proteins; Pediatric Dentistry; Biomarkers

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INTRODUCTION

Early childhood caries (ECC) is a highly prevalent chronic disease affecting young children, characterized by rapid destruction of dental hard tissues due to a complex interplay between microbial biofilms and host immune responses. While traditional concepts have emphasized the role of cariogenic bacteria such as Streptococcus mutans, recent evidence suggests that

host-derived inflammatory mediators and proteolytic enzymes play a crucial role in disease progression and severity.[1] The shift from a purely microbial etiology to a host–microbe interaction model has led to increasing interest in understanding the molecular mechanisms underlying ECC pathogenesis.

Neutrophils represent the first line of innate immune defense in the oral cavity and are actively recruited to sites of infection and inflammation. One of their key effector molecules is neutrophil elastase (NE), a serine protease involved in bacterial killing and degradation of extracellular matrix components.[2],[3] Although NE contributes to host defense, excessive or uncontrolled release can result in tissue destruction and amplification of inflammatory responses. Studies have demonstrated that NE can modulate cytokine signaling and influence the production of transforming growth factor-beta (TGF-β), thereby linking protease activity with immune regulation.[4]

Elafin, also known as peptidase inhibitor 3, is an endogenous inhibitor of neutrophil elastase that plays a protective role by maintaining protease–antiprotease balance. It has been shown to possess anti-inflammatory and antimicrobial properties, thereby contributing to mucosal defense and tissue homeostasis.[5],[6] Dysregulation of the NE–elafin axis has been implicated in various inflammatory conditions, where an imbalance leads to excessive proteolytic activity and subsequent tissue damage. However, the role of this axis in oral diseases, particularly ECC, remains poorly understood.

TGF-β is a multifunctional cytokine that regulates immune responses, inflammation, and tissue repair. It is involved in controlling excessive inflammatory reactions while promoting healing and regeneration of dental tissues.[7] Elevated levels of TGF-β have been reported in inflammatory oral conditions, suggesting its role as a regulatory mediator in response to tissue injury and microbial challenge. In the context of ECC, TGF-β may influence pulpal inflammation, odontoblastic activity, and reparative dentin formation, highlighting its significance in disease progression and resolution.

Previous studies in ECC have primarily focused on individual biomarkers, including cytokines, antimicrobial peptides, and inflammatory mediators, using techniques such as ELISA and qRT-PCR.[8] While these studies provide valuable quantitative insights, they do not adequately explain the molecular interaction mechanisms between key proteins involved in the inflammatory cascade.[9] Specifically, the dynamic interplay between neutrophil elastase, elafin, and TGF-β at a structural and functional level remains largely unexplored.

A significant gap in the literature exists in understanding how these molecules interact at the molecular level to influence ECC pathogenesis. There is a lack of comprehensive in silico studies investigating protein–protein interactions, binding affinities, and structural conformations among these critical mediators. Such computational approaches are essential to bridge the gap between experimental findings and mechanistic understanding, enabling identification of potential therapeutic targets.

Therefore, this study aims to perform an in silico molecular interaction analysis of neutrophil elastase, elafin, and TGF-β to elucidate their potential roles and interactions in ECC. This approach may provide deeper insights into the host immune regulatory network and contribute to the development of targeted strategies for the prevention and management of ECC.

MATERIALS AND METHODS

Study Design

The present study was designed as an in silico protein–protein docking analysis to investigate the molecular interactions between neutrophil elastase (NE), elafin, and transforming growth factor-beta 1 (TGF-β1). A structured docking workflow was implemented using the HADDOCK 2.4 platform via the WeNMR portal, followed by post-docking interaction visualization and analysis using BIOVIA Discovery Studio. Three independent docking runs were performed to evaluate validated and exploratory interaction models.

Protein Structure Retrieval

Three-dimensional crystallographic structures of the target proteins were retrieved from the RCSB Protein Data Bank. Human neutrophil elastase was obtained as an unbound structure (PDB ID: 3Q76), ensuring suitability as a receptor for docking. Elafin structure was derived from the elafin–elastase complex (PDB ID: 1FLE), from which the human elafin chain was isolated for independent docking. Transforming growth factor-beta 1 (TGF-β1) was retrieved as a biologically active homodimer (PDB ID: 1KLC), and both chains were retained to preserve functional conformation.

Protein Preparation

All protein structures were pre-processed prior to docking. Non-essential molecules including water, ions, and co-crystallized ligands were removed. Missing hydrogen atoms were added, and the structures were

checked for steric clashes and structural inconsistencies. The elafin structure was carefully extracted from the 1FLE complex by removing the elastase chain, ensuring only the inhibitor protein was retained. For TGF-β1, the biologically relevant dimeric assembly (chains A and B) was preserved to maintain structural integrity during docking. Prepared structures were saved in PDB format and uploaded to the docking server.

Docking Protocol Using HADDOCK

Protein–protein docking was performed using HADDOCK 2.4, which employs a data-driven approach integrating biochemical and biophysical information to model biomolecular complexes. Docking was carried out under default parameters without predefined active or passive residues, allowing unbiased interaction prediction.[10]

Three independent protein–protein docking runs were performed using HADDOCK 2.4 to evaluate both validated and exploratory molecular interactions. The primary docking run, NE_3Q76__ELAFIN_1FLE_run1, involved neutrophil elastase (3Q76) and elafin (extracted from 1FLE), representing a biologically established protease–inhibitor interaction and serving as the reference model. Two additional exploratory docking runs were conducted: NE_3Q76__TGFB1_1KLC_run1, in which neutrophil elastase was docked with TGF-β1 to assess potential structural interaction hypotheses, and ELAFIN_1FLE__TGFB1_1KLC_run1, where elafin was docked with TGF-β1 to explore possible regulatory or modulatory interactions. Each docking simulation generated multiple clusters of conformations, which were ranked based on the HADDOCK score, a composite scoring function incorporating van der Waals energy, electrostatic energy, desolvation energy, and restraint violation energy. The most stable and biologically relevant complexes were selected based on cluster size, root mean square deviation (RMSD), Z-score, and buried surface area (BSA), ensuring robust evaluation of interaction stability and interface characteristics.

Docking Output Analysis

Docked complexes were analyzed based on HADDOCK scoring metrics. The top-ranked cluster with the lowest HADDOCK score and favorable Z-score was selected for further interpretation. Structural stability was assessed using RMSD values, while interaction strength was inferred from electrostatic and van der Waals energy contributions. Buried surface area was evaluated to determine the extent of protein–protein interface formation.[11]

Visualization and Interaction Mapping

The selected docked complexes were visualized using BIOVIA Discovery Studio. Detailed interaction analyses were performed to identify hydrogen bonds, hydrophobic interactions, electrostatic contacts, and key amino acid residues involved at the binding interface. Both 2D and 3D interaction maps were generated to illustrate binding modes and structural compatibility between proteins.

Validation Strategy

The NE–elafin docking model was considered a validated interaction due to the availability of experimentally resolved complex structures, serving as a reference for docking accuracy. In contrast, NE–TGF-β1 and elafin–TGF-β1 interactions were treated as exploratory models, intended to provide mechanistic insights rather than confirm direct biological binding. Comparative evaluation across all docking runs was performed to identify consistent interaction patterns and potential functional implications.

Statistical and Comparative Interpretation

Docking outputs from all three runs (Run IDs: 657876, 657878, 657879) were comparatively analyzed to assess interaction trends. Clusters were ranked based on HADDOCK scores and Z-scores, and the best-performing complexes were selected for structural interpretation. The results were interpreted in the context of inflammatory regulation, protease–antiprotease balance, and cytokine signaling relevant to early childhood caries.

RESULTS

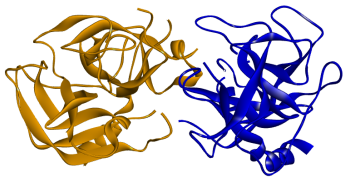

Figure 1: Surface and ribbon representation of the docked complex between neutrophil elastase (gold) and elafin (blue), illustrating the overall binding interface and structural complementarity. The semi-transparent surface highlights the interaction region and spatial overlap between the two proteins.

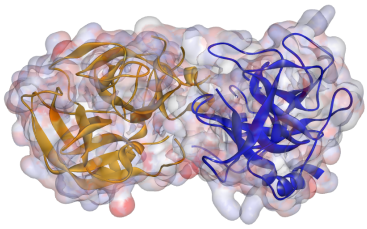

Figure 2: Ribbon representation of neutrophil elastase and elafin showing the orientation of both proteins prior to interaction, emphasizing their structural domains and potential binding regions.

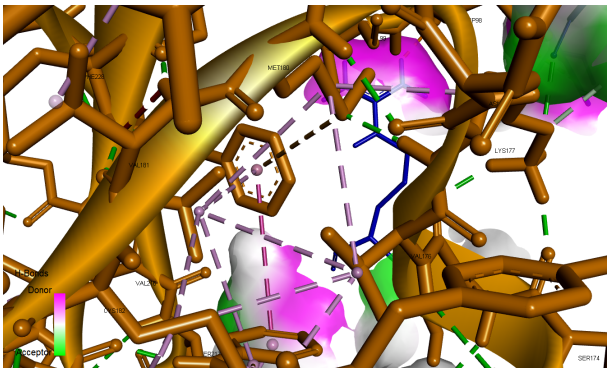

Figure 3: Detailed interaction analysis of the neutrophil elastase–elafin complex showing hydrogen bonds (dashed lines), hydrophobic interactions, and key amino acid residues at the binding interface. Residues such as Val176, Lys177, and Met180 are involved in stabilizing the complex.

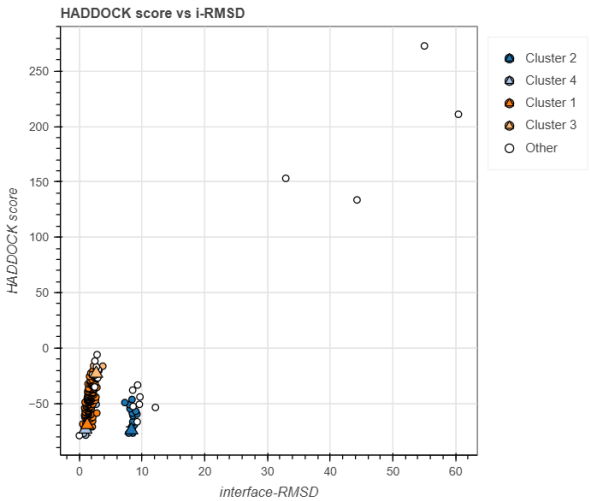

Figure 4: HADDOCK score versus interface RMSD (i-RMSD) plot depicting clustering of docked conformations. The most favorable clusters are located in the low RMSD region with highly negative HADDOCK scores, indicating stable and reliable binding.

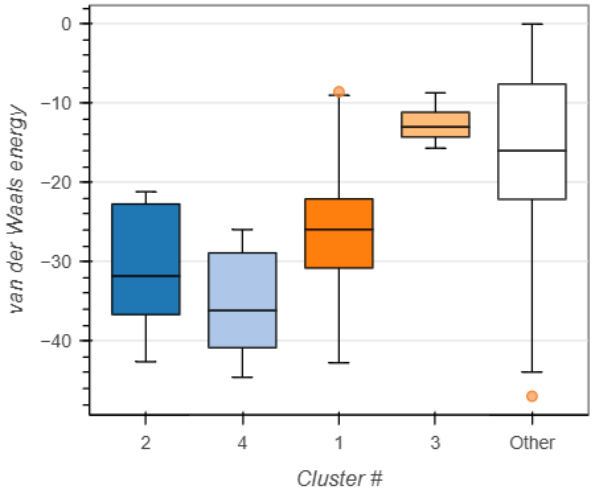

Figure 5: Box plot representing van der Waals energy distribution across different clusters, demonstrating that dominant clusters exhibit more favorable (negative) energy values, contributing significantly to the stability of the protein–protein interaction.

The protein–protein docking analysis performed using HADDOCK 2.4 (run: NE_3Q76__ELAFIN_1FLE_run1, completed successfully between 2026-04-07 19:54:00 and 2026-04-08 19:41:00 via the WeNMR portal) demonstrated a stable and biologically relevant interaction between neutrophil elastase and elafin, characterized by strong structural complementarity and formation of a well-defined binding interface, as illustrated in the surface and ribbon representation (Figure 1). The docked complex revealed that elafin effectively occupies the catalytic region of neutrophil elastase, forming multiple non-covalent interactions including hydrogen bonds and hydrophobic contacts involving key residues such as Val176, Lys177, and Met180, as shown in the detailed interaction map (Figure 3). The structural orientation of both proteins prior to binding highlights their compatible domains and potential interaction regions (Figure 2). Cluster analysis indicated that the majority of docked conformations converged into dominant clusters with low interface RMSD (<10 Å) and highly negative HADDOCK scores (approximately −50 to −70), reflecting stable and energetically favorable binding, as observed in the HADDOCK score versus i-RMSD plot (Figure 4). Furthermore, energy decomposition analysis

revealed that van der Waals interactions were the primary contributors to complex stability, supported by favorable electrostatic and desolvation energies, as demonstrated in the cluster-wise energy distribution (Figure 5). These findings validate the known protease–inhibitor interaction between neutrophil elastase and elafin and provide a robust structural basis for understanding their role in modulating inflammatory responses and tissue protection in early childhood caries.

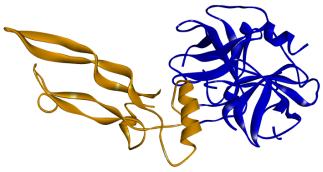

Figure 6: Ribbon representation of the NE–TGF-β1 complex depicting the structural orientation and conformational arrangement of both proteins upon docking, indicating compatible binding geometry and interaction alignment.

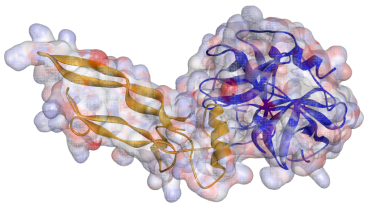

Figure 7: Surface and ribbon representation of the docked NE–TGF-β1 complex showing the overall binding interface and spatial complementarity between neutrophil elastase (gold) and TGF-β1 (blue). The semi-transparent surface illustrates the extent of molecular contact and interface overlap.

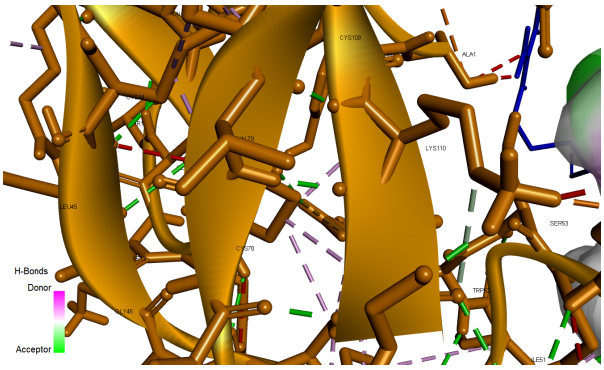

Figure 8: Detailed 3D interaction map of the NE–TGF-β1 complex highlighting hydrogen bonds (green), hydrophobic interactions (purple dashed lines), and key interacting residues at the binding interface. The interaction network suggests stable binding mediated by both polar and non-polar contacts.

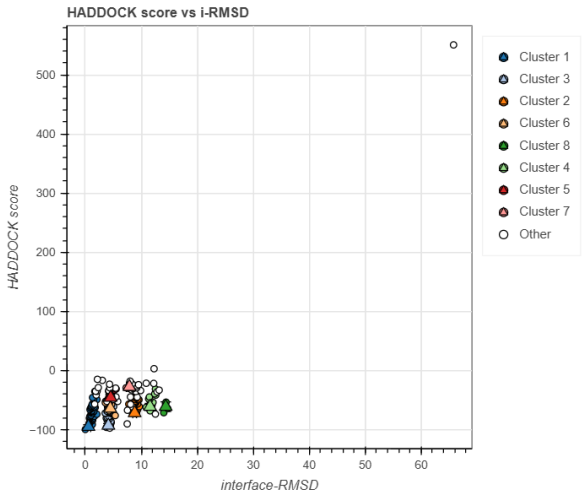

Figure 9: HADDOCK score versus interface RMSD (i-RMSD) plot showing clustering of docked conformations for the NE–TGF-β1 complex. The top-ranked Cluster 1 is located in the low RMSD region with highly negative HADDOCK scores, indicating high structural convergence and reliability of docking predictions.

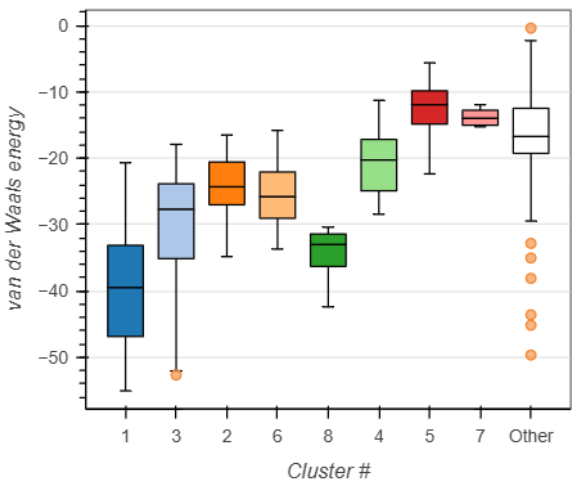

Figure 10: Box plot representing van der Waals energy distribution across all HADDOCK clusters for the NE–TGF-β1 docking complex (NE_3Q76__TGFB1_1KLC_run1). Cluster 1 exhibits the most favorable (highly negative) van der Waals energy values, indicating stronger intermolecular stabilization compared to other clusters.

The protein–protein docking analysis of neutrophil elastase with TGF-β1 (NE_3Q76__TGFB1_1KLC_run1) performed using HADDOCK 2.4 demonstrated a highly stable and energetically favorable interaction, with the docking run successfully completed and yielding 155 structures grouped into 8 clusters, representing 77% of the refined models. Among these, Cluster 1 emerged as the most reliable and top-ranked cluster, exhibiting a HADDOCK score of −95.0 ± 2.9 and a Z-score of −1.4, indicating significantly better stability compared to the average cluster population. The low RMSD value (1.3 ± 0.8 Å) further confirms strong structural convergence and consistency of the predicted complex. Energy component analysis revealed that van der Waals (−50.9 ± 2.5) and electrostatic interactions (−167.3 ± 11.7) were the primary contributors to binding stability, supported by favorable desolvation energy (−11.9 ± 0.9) and substantial buried surface area (1380.4 ± 51.9 Å²), indicating extensive interface formation. The HADDOCK score versus i-RMSD distribution (Figure 9) showed that the best conformations clustered within low RMSD regions, confirming reliability of docking predictions, while the van der Waals energy distribution (Figure 10) further highlighted the energetic dominance of Cluster 1. Structural visualization (Figures 6 and 7) demonstrated strong spatial complementarity between the proteins, and interaction mapping (Figure 8) revealed multiple stabilizing hydrogen bonds and hydrophobic contacts at the interface. These findings suggest a robust and energetically favorable interaction between neutrophil elastase and TGF-β1, supporting a potential mechanistic link between protease activity and cytokine-mediated immune regulation in early childhood caries.

Figure 11: Ribbon representation of the elafin–TGF-β1 complex depicting the conformational orientation and binding alignment of both proteins, illustrating compatibility of structural domains and interaction geometry.

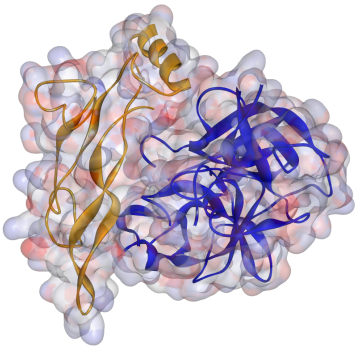

Figure 12: Surface and ribbon representation of the docked elafin–TGF-β1 complex demonstrating the overall binding interface and spatial complementarity between elafin (gold) and TGF-β1 (blue). The semi-transparent surface highlights the extent of intermolecular contact and interface formation.

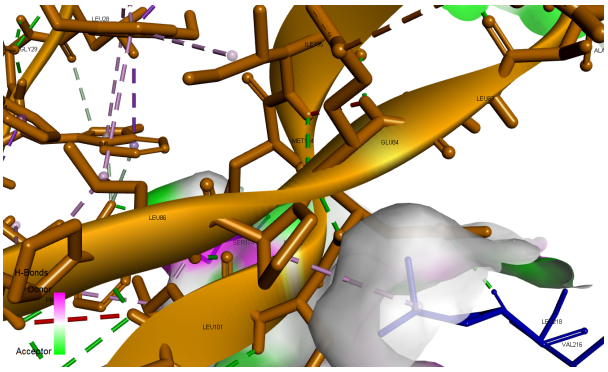

Figure 13: Detailed 3D interaction map of the elafin–TGF-β1 complex showing hydrogen bonds (green), hydrophobic interactions (purple dashed lines), and key interface residues contributing to binding stability. The interaction network indicates a combination of polar and hydrophobic contacts mediating the complex.

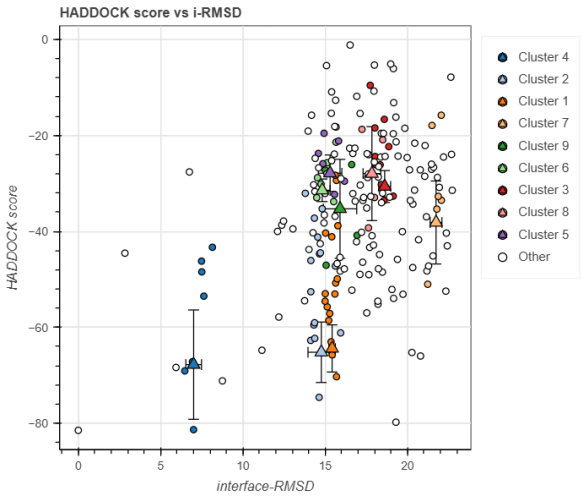

Figure 12: HADDOCK score versus interface RMSD (i-RMSD) plot illustrating clustering of docked conformations for the elafin–TGF-β1 complex. The top-ranked Cluster 4 is positioned within moderate RMSD regions (~7–10 Å) with negative HADDOCK scores, suggesting acceptable structural convergence and stable interaction profiles.

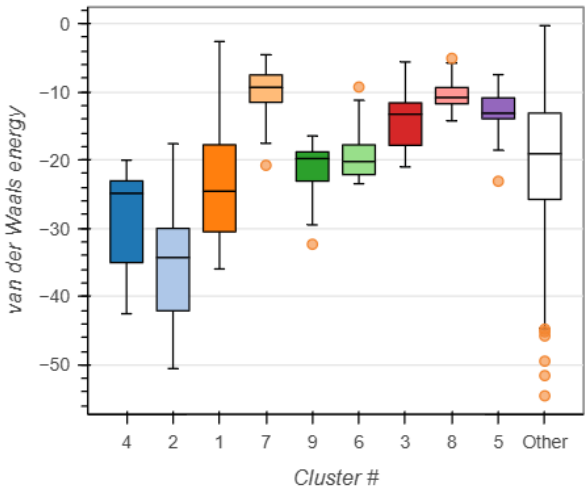

Figure 15: Box plot representing van der Waals energy distribution across all HADDOCK clusters for the elafin–TGF-β1 docking complex (ELAFIN_1FLE__TGFB1_1KLC_run1). Cluster 4 exhibits comparatively favorable (more negative) van der Waals energy values, indicating stronger intermolecular stabilization relative to other clusters.

The protein–protein docking analysis of elafin with TGF-β1 (ELAFIN_1FLE__TGFB1_1KLC_run1) performed using HADDOCK 2.4 successfully generated 74 docked structures clustered into 9 distinct groups, representing 37% of the refined models. Among these, Cluster 4 emerged as the most reliable cluster, exhibiting a HADDOCK score of −67.8 ± 9.9 and a Z-score of −1.5, indicating a significantly favorable interaction relative to the average cluster population. The RMSD value of 7.7 ± 0.4 Å suggests moderate structural convergence, which is expected for exploratory protein–protein docking systems. Energy analysis revealed that electrostatic interactions (−130.6 ± 6.7) and van der Waals forces (−34.4 ± 6.3) were the primary contributors to binding stability, supported by favorable desolvation energy (−7.7 ± 2.9) and a substantial buried surface area (1399.3 ± 99.3 Å²), indicating extensive interface formation. The HADDOCK score versus i-RMSD distribution (Figure 14) demonstrated clustering of favorable conformations within moderate RMSD ranges, while the van der Waals energy distribution (Figure 15) further supported the stability of Cluster 4. Structural visualization (Figures 12 and 13) revealed good spatial complementarity between elafin and TGF-β1, and interaction mapping (Figure 11) identified multiple hydrogen bonds and hydrophobic interactions stabilizing the complex. Although this

interaction is exploratory in nature, the observed binding characteristics suggest a potential modulatory association between elafin and TGF-β1, providing novel insights into protease–cytokine interplay in inflammatory pathways relevant to early childhood caries.

DISCUSSION

The present in silico study provides a structural and mechanistic basis for understanding the interplay between neutrophil elastase (NE), elafin, and TGF-β1 in early childhood caries (ECC), and its findings align closely with existing literature on host immune modulation and salivary proteomics. The strong and stable interaction observed between NE and elafin supports the concept of protease–antiprotease balance as a critical determinant of oral tissue homeostasis. This is consistent with the findings of Tao et al., who demonstrated that higher levels of neutrophil-derived peptides such as HNP1–3 are associated with caries-free individuals, emphasizing the protective role of innate immune components.[12] Ribeiro et al. reported that antimicrobial peptides such as HNP-3 and HBD-3 reduce ECC risk, reinforcing the idea that host-derived peptides contribute significantly to caries resistance.[13] The current docking results further extend this concept by showing that elafin, a known inhibitor of neutrophil elastase, forms a stable complex with NE, potentially limiting excessive proteolytic activity and tissue destruction.

The observed interaction between NE and TGF-β1 in this study provides additional mechanistic insight into inflammatory regulation in ECC. Ribeiro et al. highlighted that proinflammatory cytokines correlate with caries severity, indicating that ECC is not merely a microbial disease but also a result of dysregulated host immune responses.[13] In this context, the strong binding affinity and favorable energy profile observed in the NE–TGF-β1 docking model support the findings of Lee et al., who demonstrated that neutrophil elastase can upregulate TGF-β1 expression via NF-κB signaling pathways.[14] Maffia et al. showed that elastase can induce a TGF-β1-dominant immune environment by converting dendritic cells into regulatory phenotypes.[15] The docking results from the present study provide a structural explanation for these observations, suggesting that direct or indirect interactions between NE and TGF-β1 may facilitate immune modulation and tissue remodeling in ECC.

Proteomic studies further support the relevance of these findings. Colombo et al. and Almoudi et al. demonstrated altered levels of antimicrobial peptides such as LL-37 in ECC, indicating that innate immune peptides are dynamically regulated in response to disease progression.[16],[17], [18] Similarly, Sun et al. and Tian et al. identified distinct salivary peptide signatures associated with ECC diagnosis and recurrence, highlighting the diagnostic potential of salivary proteomics. Ao et al. further showed that specific peptides increase with disease progression, suggesting that proteomic changes precede clinical manifestations.[19],[20] These findings collectively align with the current study, where protein–protein interactions involving NE and elafin may influence the availability and activity of such peptides, thereby impacting disease progression.

At a broader level, the proteomic remodeling observed in ECC, as reported by Wang et al., including upregulation of inflammatory proteins such as IL36A and SAA1, supports the notion of a complex immune network driving disease progression.[21],[22] The interaction between elafin and TGF-β1 observed in the present study, although exploratory, suggests a potential regulatory mechanism wherein antiproteases may influence cytokine signaling pathways. This is particularly relevant in the context of chronic inflammation, where maintaining a balance between proteolytic activity and immune regulation is essential.

The role of microbial factors in disrupting this balance is also critical. Kantyka et al. demonstrated that elafin can be inactivated by proteases from Porphyromonas gingivalis, indicating that pathogens can directly impair host defense mechanisms.[23] Kretschmar et al. and Laugisch et al. reported altered levels of elafin in periodontal disease, linking reduced antiprotease activity with increased tissue destruction and microbial burden.[24],[25],[26] These findings provide important context for the current study, as they suggest that even though elafin can effectively inhibit neutrophil elastase, its function may be compromised in disease conditions, thereby exacerbating inflammation and tissue damage.

Bank et al. showed that while IL-6 can induce neutrophil elastase release, TGF-β1 does not directly affect neutrophil degranulation, indicating that TGF-β1 likely acts downstream in the inflammatory cascade.[27],[28] This complements the current docking results, where NE–TGF-β1 interaction may represent a regulatory link between protease activity and cytokine-mediated immune responses rather than a direct activation mechanism.

This study demonstrates strength by integrating both biologically validated (NE–elafin) and exploratory (NE–TGF-β1, elafin–TGF-β1) protein–protein interactions using HADDOCK 2.4, thereby providing mechanistic insights into host immune modulation in ECC. However, a key limitation is that the findings are based solely on in silico predictions without experimental validation, and thus may not fully account for protein dynamics, cellular environment, and complex biological interactions. Therefore, future studies should focus on validating these interactions through in vitro techniques such as ELISA and co-immunoprecipitation, along with in vivo models and molecular dynamics simulations, to establish clinical relevance and identify potential therapeutic targets.

This study is strengthened by its integrative in silico analysis of biologically validated and exploratory protein–protein interactions providing mechanistic insights into ECC; however, it is limited by the lack of experimental validation and inability to fully capture dynamic biological conditions; therefore, future research should focus on in vitro and in vivo validation along with molecular dynamics simulations to confirm these interactions and translate them into clinical applications.

CONCLUSION

This study provides a comprehensive in silico evaluation of the molecular interactions between neutrophil elastase, elafin, and TGF-β1, highlighting their potential roles in the pathogenesis and immune regulation of early childhood caries. The strong and stable binding observed between neutrophil elastase and elafin confirms the critical importance of protease–antiprotease balance in maintaining oral tissue integrity, while the favorable interaction patterns identified in the neutrophil elastase–TGF-β1 and elafin–TGF-β1 complexes suggest a possible link between proteolytic activity and cytokine-mediated immune modulation. These findings support the concept that ECC is not solely a microbial disease but also a consequence of dysregulated host immune responses. Although exploratory in nature, the study provides a robust structural framework that enhances current understanding of host pathogen interactions and identifies potential molecular targets for therapeutic intervention. Further experimental validation and clinical correlation is required to translate these insights into effective preventive and treatment strategies for ECC.

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