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Molecular docking of an insect-derived defensin with the Plasmodium antigens Pfs25, TRAP and AMA-1 reveals a predicted preference for AMA-1

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Abstract
Malaria remains a leading cause of global morbidity and mortality, and the World Health Organization estimates 282 million cases and 610,000 deaths in 2024 alone, against the emerging partial artemisinin resistance and persistent insecticide resistance. Antimicrobial peptide (AMPs) native to the mosquito innate immune system are increasingly being explored as scaffolds for next-generation antiplasmodial agents, because their membrane and structure-disruptive mechanisms are considered less prone to rapid resistance selection than conventional small-molecule antimalarials. This study used structure-based in silico docking to evaluate the interaction of an insect defensin peptide (Defensin A; PDB: 2E3E) with two *Plasmodium falciparum* antigens and one *Plasmodium vivax* antigen that together span distinct, biologically critical stages of the parasite life cycle: the transmission-blocking waxing antigen Pfs25 (PDB: 8EZK), sporozoite motility and hepatocyte-invasion protein TRAP containing Thrombospondin Type-1 Repeat (TSR) domain (PDB: 2BBX), and the apical membrane antigen 1 (AMA-1) structural template (PDB: 1W8K), selected as a representative AMA-1 orthologue because of the conserved PAN-fold architecture among Plasmodium species. Structures were retrieved from the RCSB Protein Data Bank and docked pairwise using the Frontier-transform-based HexServer platform. The resulting complexes were visualized and inspected for interfacial contacts in PyMol. Defense A engaged all three targets, but with markedly different predicted energetics: the Defensin A-AMA-1(-595.61) produced the most favorable predicted docking score among the tested targets than entire Defensin A-Pfs25 (-394.44) or Defensin A-TRAP (-393.25), and involved a broader predicted interface (Tyr33-Leu384 and Lys35-Val382) relative to the single contact pair identified for each of the other two complexes. These preliminary findings suggest that defensin-class peptides may preferentially engage AMA-1 and support prioritization of this interaction for downstream experimental (*in vitro* and *in vivo*) antiplasmodial validation.

Keywords: Antimicrobial peptide, defensin, *Plasmodium*, AMA-1, Pfs25, TRAP, HexServer

Introduction

Malaria remains a persistent global health challenge. According to the most recent World Health Organization estimates, 2024 saw 282 million malaria cases and 610,000 malaria deaths worldwide, with the WHO African Region accounting for roughly 94-95% of both figures and children under five bearing a disproportionate share of mortality. Sustained control efforts have averted an estimated 2.3 billion cases and 14 million deaths since 2000, and two vaccines, RTS, S/AS01 and R21/Matrix-M, are now used in routine immunization programs across dozens of endemic countries. Nonetheless, progress has slowed, and the emergence of partial resistance in *Anopheles* vector populations, threatens to erode these gains. This underscores the continued need for antimalarial candidates and targets that act through mechanisms distinct from those of currently deployed drugs.

Antimicrobial peptides (AMPs) have attracted increasing attention as an alternative class of anti-parasitic agents. Because most AMPs act by disrupting membranes or engaging conserved structural features rather than a single catalytic site, they are considered comparatively resistant to the rapid target-based resistance observed with conventional antimalarials (Vale *et al*., 2014) []. Mosquito-derived AMPs are a particularly attractive starting point, since they form part of the natural innate immune repertoire that *Anopheles* and other vector species mount against ingested Plasmodium parasites. For example, cecropins isolated from *Anopheles* species, have recently been shown to possess direct antiplasmodial activity against blood-stage *P. falciparum in vitro* and *P. berghei in vivo* (Lou *et al*., 2024) [7], and a related *Anopheles gambiae* cecropin has been reported to be potently active against *P. falciparum* sporozoites (Barreto *et al*., 2024) [4]. Defensins are a second major class of mosquito AMP, *Anopheles gambiae* defensin transcription is measurably induced in the midgut and fat body following an infected blood meal, directly implicating this peptide family in the vector's response to Plasmodium infection (Richman *et al*., 1997; Vizioli *et al*., 2001) [10, 16].

Structure-based in silico docking offers a rapid, low-cost first step for prioritizing candidate AMP-target combinations, adding more resources, and cancelling organism assays. In this study, we selected three *P. falciparum* antigens that together represent distinct, biologically essential stages of the parasite life cycle: Pfs25, a leading transmission-blocking vaccine candidate expressed on the zygote/ookinete surface within the mosquito midgut (Shukla *et al*., 2023) [12]; TRAP, a sporozoite surface protein whose thrombospondin-related (TSR) domain is required for hepatocyte adhesion and invasion (Tossavainen *et al*., 2006; Sultan *et al*.,1997) [13, 14]; and AMP-1, a merozoite surface antigen essential for

erythrocyte invasion and a long-standing blood-stage vaccine candidate (Pizarro *et al*., 2005) [9]. We evaluated the in-silico docking interaction of an insect-derived Defensin A (defensin A) against representative structures of each target, to generate a preliminary structural rationale for prioritizing defensin-class peptides for downstream antiplasmodial evaluation.

Results

1. Retrieved Structures

The three-dimensional structures of Defensin A three target antigens, as rendered in RasMol, are shown in Fig. 1. Defensin A (Fig. 1a) is a compact, disulfide-stabilized peptide fold that is typical of insect defensins. Pfs25 (Fig. 1b) and AMA-1 (Fig. 1d) are shown as deposited, whereas the TRAP TSR domain (Fig. 1c) is the smallest of the three targets, consistent with its role as a single structural module within the larger TRAP ectodomain.

2. Docking Energetics

Table 1 summarizes the HexServer docking outputs for each Defensin A-target complex. The total docking energy (E-total) ranged from -393.25 to -595.61. The Defensin A-Pfs25 and Defensin A-TRAP complexes returned closely matched values (-394.44 and -393.25, respectively), whereas the Defensin A-AMA-1 complex returned a total energy approximately 1.5-fold more favorable than either (-595.61). Across all three complexes, the reported shape-complementarity term (E-shape) was numerically identical to E-total, while the force-field, air (vacuum electrostatics), and steric-clash returned to zero, which should therefore be interpreted as reflecting the shape and steric complementarity between the docked partners rather than an explicit electrostatic or all-atom force field energy.

Table 1: HexServer docking energies and PyMOL-identified interfacial contacts for Defensin A (PDB: 2E3E) docked against three Plasmodium target antigens. E-shape was numerically identical to E-total for all three complexes.

Target Protein	PDB ID	Total Docking Energy (E-total)	Defensin A Residue (Atom)	Target Residue (Atom)
Pfs25	8EZK	-394.44	Arg42 (NE)	Gln90 (O)
TRAP (TSR domain)	2BBX	-393.25	Arg42 (NE)	Ser4 (O)
AMA-1	1W8K	-595.61	Tyr33 (OH)	Leu384 (N)
			Lys35 (HZ1)	Val382 (O)

3. Interface Contacts

The interfacial contacts identified by visual inspection of the top-ranked docking poses in PyMOL are summarized in Table 1 and illustrated in Fig. 1. The Defensin A-Pfs25 interface was anchored by a single identified contact pair, Arg42 (NE) of Defensin A with Gln90 (O) of Pfs25 (Fig. 1a).

The Defensin A-TRAP interface was centered on the same Defensin A residue, Arg42 (NE), paired with ser4(O) of the TRAP TSR domain (Fig. 1b). The Defensin A_AMA-1 interface, consistent with its more favorable total energy, involved two identified compact pairs: Tyr33 (OH) of Defensin A with Leu384 (N) of AMA-1, and Lys35 (HZI) of Defensin A with Val382 (O) of AMA-1 (Fig. c).

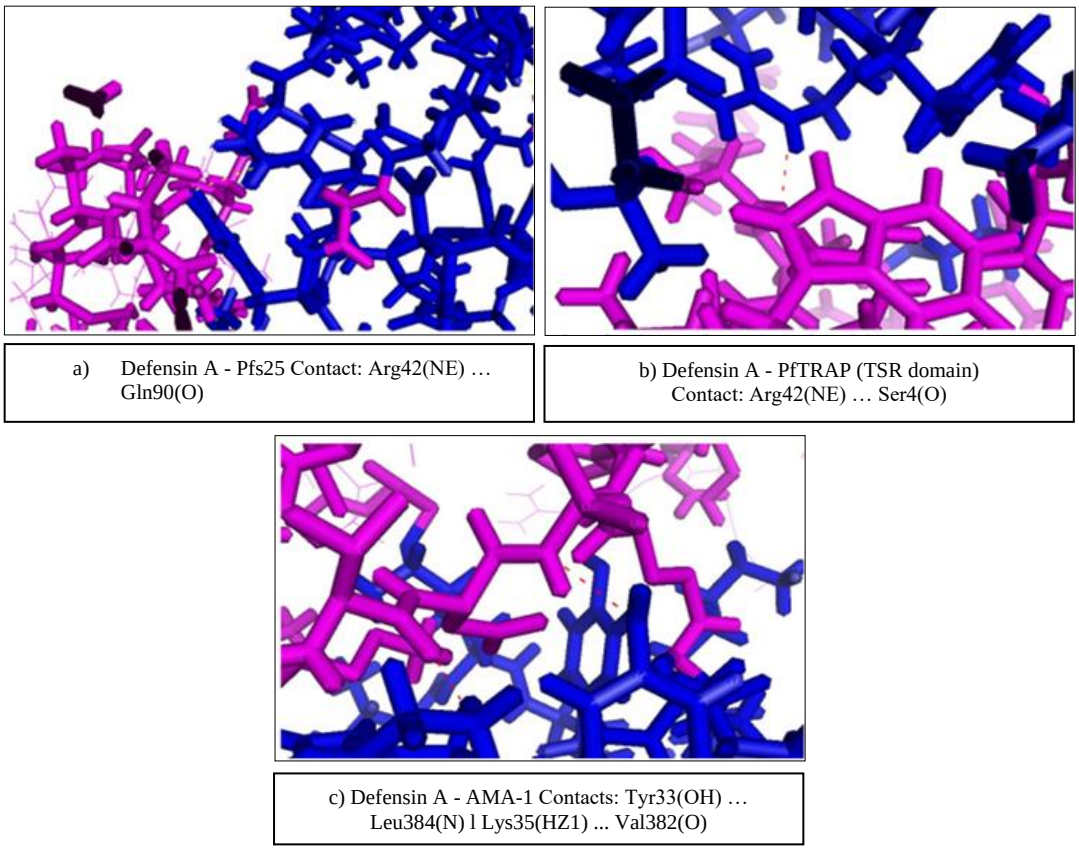


Fig. 1 Close-up views of the docked peptide-peptide interfaces, visualized in PyMOL (Defensin A, magenta; target antigen, blue; identified close contacts, dashed lines). (a) Defensin A-Pfs25. (b) Defensin A-TRAP (TSR domain). (c) Defensin A-AMP-1.

Discussion

The central finding of this preliminary in silico screen is that Defensin A engages all three *Plasmodium spp.* antigens tested, but with clearly differentiated predicted energetics. Docking agent AMA-1 returned a total energy approximately 1.5-fold more favorable than docking against either Pfs25 or TRAP, together with a broader two-contact interface compared with the single contact pair identified for the other two targets. Because AMA-1 mediates a well-characterized, druggable protein-protein interaction, this result is worth placing in context. AMA-1 forms part of the moving-junction complex that anchors the merozoite to the erythrocyte membrane during invasion, an interaction mediated through a conserved hydrophobic cleft on the AMA-1 surface that normally engages the parasite's own rhoptry neck protein 2 (RON2) (Vulliez-Le *et al*., 2012) [17]. This cleft has already been validated as a target for invasion-blocking peptides in previous work, most notably the phage-display-derived peptide R1, which competes with RON2 for the same binding groove and blocks merozoite invasion *in vitro* (Vulliez-Le *et al*., 2012) [17]. Whether the Defensin A-AMA-1 interface identified here overlapped with this specific RON2-binding cleft has not been established in the present study and would require explicit mapping of the docked contact residues onto the known hot-spot residues in future work; nonetheless, the existence of a validated, peptide-druggable pocket on AMA-1 lends biological plausibility to the comparatively strong docking signal observed here.

The weaker, near-identical energies obtained for Pfs25 and TRAP did not necessarily indicate a lack of biological relevance. Pfs25 is displayed on the extracellular surface of zygotes and ookinetes within the mosquito midgut, and antibody engagement at multiple distinct epitopes on this antigen is already known to reduce parasite transmission (Shukla *et al*., 2023) [12]; A peptide-mediated interaction, even a modest one, occurring at this exposed, midgut-localized life-cycle stage could, in principle, contribute to transmission-blocking activity, particularly for a peptide that is endogenous to the mosquito vector. Similarly, the TRAP TSR domain mediates adhesion to hepatocyte-surface heparan sulfate proteoglycans as a prerequisite for hepatocyte invasion (Tossavainen *et al*., 2006; Sultan *et al*.,1997) [13, 14]; interference at this domain, even partial, is consistent with a broader strategy of targeting multiple sequential life-cycle bottlenecks with a single peptide scaffold.

These results are consistent with, and extend, a small but growing body of evidence that *Anopheles*-derived AMPs possess genuine, measurable antiplasmodial activity rather than acting solely as antibacterial effectors. *Anopheles*-derived anti-blood-stage *P. falciparum in vitro and P. berghei in vivo* (Lou *et al*., 2024) [7], and a related *Anopheles gambiae* cecropin are potently active against *P. falciparum* sporozoites (Barreto *et al*., 2024) [4]. The present docking data extend this precedent to the defensin class and, notably, implicate a specific, druggable parasite target (AMA-1) rather than a purely membranolytic mechanism, which may help to explain how a mosquito-derived peptide could exert stage-specific antiplasmodial effects beyond simple membrane disruption.

Materials and Methods

1. Retrieval of Protein and Peptide Structures

Three-dimensional coordinate files were retrieved from the RCSB Protein Data Bank (PDB), a global open-access archive for experimentally determined macromolecular structures (Berman *et al*., 2000) [5]. The peptide analyzed in this study, referred to as Defensin A, corresponds to PDB entry 2E3E, a solution NMR structure of an engineered variant (DEF-BBB) of the native antimicrobial defensin DEF-AAA from *Anopheles gambiae* (Landon *et al*., 2008) [6]. The three target structures were: Pfs25 in complex with a human transmission-reducing monoclonal antibody (PDB: 8EZK) (Shukla *et al*., 2023) [12]; the TSR domain of TRAP, solved by solution NMR (PDB: 2BBX) (Tossavainen *et al*., 2006) [14]; and the three-domain ectodomain of AMA-1, solved by X-ray crystallography at 1.8 Å resolution (PDB: 1W8K) (Pizarro *et al*., 2005) [9]. This AMA-1 structure was originally solved from *Plasmodium vivax*; it was used here as a structural template for the *P. falciparum* orthologue on the basis of the high conservation of the PAN-fold domains I and II across *Plasmodium vivax* AMA-1 orthologues, a substitution we return to under limitations.

2. Structure Visualization

The retrieved structures were inspected and rendered in RasMol v2.6 (Sayle *et al*., 1995) [11] before docking (Fig. 2).

3. Protein-Peptide Docking

Defensin A was docked independently against each of the three target structures using HexServer (http://hexserver.loria.fr/) (Macindoe *et al*., 2010) [10], a Fourier-transform (FFT)-based rigid-body docking platform. No active-site restraints were applied to any complex; therefore, docking was unbiased (blind) with respect to the interaction site. The top-ranked solutions (Cluster 1, Solution 1) from each run were used for visualization and interface analysis.

4. Docked-Complex Visualization and Interface Analysis

Top-ranked docked complexes were visualized in PyMOL (Schrödinger, version 2.5), and residue-level contacts at each protein-peptide interface were identified by visual inspection of close inter-atomic distances.

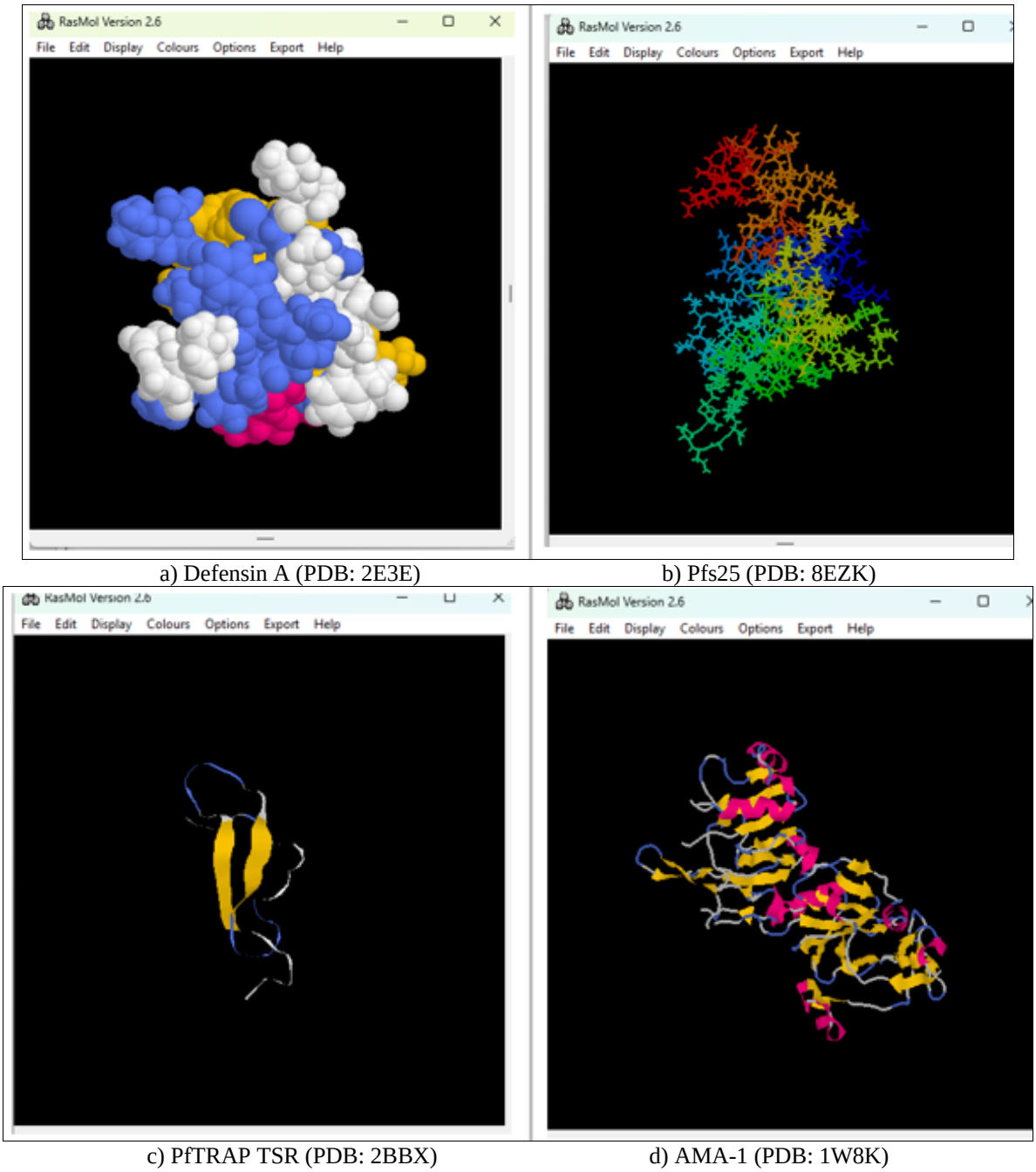


Fig 2: Three-dimensional structures used in this study, rendered in RasMol v2.6. (a) Defensin A (PDB: 2E3E), space-filling representation. (b) Pfs25 (PDB: 8EZK), showed in complex with a human antibody fragment (rainbow stick representation, N-to C-terminus). (c) The thrombospondin-related (TSR) DOMAIN OF TRAP (PBD: 2BBX) (d) AMA-1 (PDB: 1W8K), ribbon representation.

Limitations

There are several limitations in the interpretation of these findings. First, the peptide structure used here (PDB: 2E3E) is an engineered chimeric variant (DEF-BBB) of the native *Anopheles gambiae* defensin DEF-AAA, originally designed to enhance activity against *Staphylococcus aureus* (Landon *et al*., 2008) [6], rather than the wild-type peptide or a defensin native to *Aedes aegypti*, the species of primary interest to the broader research programme this work forms part of. Because no experimentally resolved *Aedes aegypti* defensin structure is currently available in the PDB, this *Anopheles*-derived template was used as a structural proxy; results obtained with the native *Aedes aegypti* sequence, once a validated structure or homology model becomes available, may differ. Second, the AMA-1 structure used (PDB: 1W8K) was solved from *Plasmodium vivax*. Although the PAN-fold architecture of domains I and II are highly conserved across *Plasmodium vivax* AMA-1 orthologs, sequence, and conformational differences may influence peptide-binding predictions. Therefore, Validation against an authentic *P. falciparum* AMA-1 structure, such as PDB: 1Z40 (Bai *et al*., 2005) [3] is recommended before the AMA-1 finding is treated as *P. falciparum*-specific. Third, docking was performed in a blind docking mode (without active-site restraints) using a single algorithm and a single top-ranked pose per complex, without cross-validation against alternative docking engines, molecular dynamics-based refinement, or binding free-energy re-scoring (e.g., MM-GBSA/MM-PBSA). The reported energies are shape/steric-complementarity scores rather than validated binding affinities. Finally, all the findings reported herein are computational and require experimental confirmation.

Conclusions

This preliminary in silico screen showed that an insect-derived Defensin A, engages three structurally and functionally distinct Plasmodium antigens with differentiated predicted energies, most notably, the most favorable predicted docking interaction with the invasion antigen AMA-1 relative to Pfs25 and TRAP. Given the recognized importance of the AMA-1 invasion interface as a therapeutic target, these finding provides a computational rationale for prioritizing the Defensin A-AMA-1 interaction and defensin-class peptides more broadly for further experimental evaluation. The predicted interactions with Pfs25 and TRAP likewise suggest potential avenues for exploring transmission-blocking and hepatocyte-invasion-blocking activities. Future integration of these computational observations with *in vitro* assays, such as SYBR Green I-based growth inhibition studies, and *in vivo* antiplasmodial evaluations, including Peters' four-day suppressive test, will be essential to determine the biological relevance and translational potential of this peptide.

Declarations

Author Contributions

Conceptualization, methodology, formal analysis, investigation, writing-original draft, by Arokia Ahino Jessie A; supervision by M. Raja; writing-review and editing by Jisha Hannie Selson and Alex Yagoo. All the authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

Not applicable; this study used only publicly available, previously deposited macromolecular structures and involved no new human, animal, or clinical data.

Data Availability Statement

The structures analyzed are publicly available in the RCSB Protein Data Bank under accession codes 2E3E, 8EZK, 2BBX, and 1W8K. The datasets generated during the current study, including docking output files and associated analyses, are available in the Dryad Digital Repository at https://doi.org/10.5061/dryad.zkh1893sr and supplemental information are available on Zenodo at https://doi.org/10.5281/zenodo.23404437.

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Conflict of Interest

The authors declare no conflict of interest.

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