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PREDICTION OF PROTEIN-PROTEIN INTERACTION NETWORK IN MALARIA BIOMARKERS AND IMPLICATION AS THERAPEUTIC TARGET

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Abstract

Malaria is a major global health concern, claiming thousands of lives each year. Numerous proteins are involved in the parasitic infection of the host body by malaria. Several of these proteins, including mucin 13 protein (MUC13), *Plasmodium falciparum* lactate dehydrogenase (PfLDH), plasmodium glutamate dehydrogenase (GDH), and liver-derived glutamate dehydrogenase (GDH), have been implicated as biomarkers. These proteins interact with other proteins throughout the liver and blood stages of the plasmodium life cycle. We used computational analysis to uncover protein-protein interactions (PPIs) that might be used to discover new therapeutic targets. Bioinformatics analysis utilizing the stringDB webserver was used to gather PPIs data. The PPIs data set contains the interaction of biomarkers with many proteins as well as the false discovery rate (FDR) for each biological process. Data is provided in the form of an interactive graphic and a table of PPIs. MUC13, PfLDH, Plasmodium GDH, and LISP2 were co-expressed with several proteins in 12 biological processes. In the homeostatic process, the interaction of MUC13 with MUC4, MUC17, and MUC6 has the lowest FDR value of 0.0299. Furthermore, we relate our findings to previous research and predict the implications of these proteins' inhibition.

INTRODUCTION

Malaria is still a burden in the tropical and subtropical regions—caused by coccidian protozoa from genus *Plasmodium*. Five *Plasmodium* species caused human malaria, including *Plasmodium falciparum, Plasmodium vivax, Plasmodium knowlesi, Plasmodium malariae,* and *Plasmodium ovale*. *Plasmodium* entry to human host during bites of female *Anopheles sp* [1–3]. Because the parasite requires a warm environment to thrive, malaria transmission occurs optimally between 25 and 31°C [4,5]. The primary reported case of malaria come from Africa

(93%), South East Asia (3,4%), and the Eastern Mediterranean (2,1%) [6]. Indonesia is one of the countries in Southeast Asia with a high prevalence rate of malaria [7,8]. Indonesia is also home to around 20 anopheline malaria vectors, transmitting at least four *Plasmodium* species into the human host [7]. According to the previous study, the top three provinces with the highest malaria case are Papua, West Papua, and Nusa Tenggara Timur. Various species of *Plasmodium* cause Malaria in Indonesia, but the dominant species is *P. falciparum*. In Eastern Indonesia, 62% of the case were caused by *P. falciparum*, and 33% were caused by *P.vivax* [8].

The malaria parasite has a complex life cycle [9]. Following vector injection, the sporozoite enters the liver through the circulation. Sporozoite enters the hepatocyte through the kupffer cell and develops into two stages: trophozoite and schizont [10–12]. The trophozoite stage is the main stage of parasitism, during which the parasite develops by taking nutrients from host cells through the L-FABP-UIS3 complex [13]. The schizont stage is the proliferation stage, which generates thousands of single multinucleated schizont that are ready to invade red blood cells-called merozoite. In *Plasmodium falciparum*, the pre-erythrocyte stage occurs in 10 Days [14]. For about 6-7 days, the multinucleated schizont begins to grow into merozoites [12]. In the blood-stage, merozoite develops into trophozoite and schizont as well. In this stage occur gametocyte development. The gametocytes then enter the mosquito's body during a blood meal, where they grow into the sexual stage.

An impediment to malaria eradication attempts, particularly in Indonesia and Southeast Asia, is the evolution of *Plasmodium falciparum* resistance variants resistant to the majority of antimalarial drugs, particularly chloroquine and sulfadoxine-pyrimethamine. Additionally, some studies suggest that strains of *Plasmodium vivax* are resistant to chloroquine and/or primaquine [15,16]. Since the late 1980s, this issue has existed along the Thai border and has expanded to other areas of Southeast Asia and even Africa [17–19]. The development of drug-resistant variations or multidrug-resistant (MDR) strains results in treatment failure, which prolongs the patient's recovery period and may result in a greater degree of severity [15]. Treatment with quinine, mefloquine, and artesunate is used in regions with a high MDR ratio, such as Southeast Asia [19,20]. These therapies have so far been competing with the pace of *Plasmodium* resistance, making it essential to do research on effective new medicines, particularly for MDR variants.

Several proteins are identified as a biomarker of parasite development in the human body involved mucin-13 (MUC13), liver-specific protein 2 (LISP2), *Plasmodium falciparum* lactate dehydrogenase (PfLDH), and Plasmodium's glutamate dehydrogenase (GDH) [21–23]. These proteins function as biomarkers by interacting with other proteins throughout different biological processes. Protein-protein interaction (PPI) network analysis is a technique for multiple disease molecular assays based on mathematical representations that are commonly modeled as graphs, with nodes representing proteins and edges connecting pairs of interacting proteins that are undirected and possibly weighted [24–26]. Several studies use computational analysis to understand malaria diseases' protein-protein interaction mechanism to develop novel drug development, leading to malaria eradication [27–29]. The STRING DB is a critical component of this PPI study because it is the main protein interaction network database that integrates protein interactions from various scientific sources with a computational prediction [30]. This research provides early information on the protein-protein interactions of the anticipated biomarkers.

MATERIALS AND METHODS

Selecting Biomarkers in Malaria Disease

We use protein-protein interaction (PPI) network analysis to understand and predict each biomarker's interaction. Based on our literature studies, we analyze four biomarkers in this study. They are mucin-13 protein (MUC13), liver-specific protein 2 (LISP2), *Plasmodium falciparum* lactate dehydrogenase (PfLDH), and Plasmodium's glutamate dehydrogenase (GDH).

The Protein-Protein Interaction (PPI) Analysis

Each predicted biomarker analyzed using the STRING DB v11 (https://string-db.org/) to get their PPI information. The minimum required interaction score is set to 0.700, and 10 shells are the maximum interactors. The data obtained are the interactive graphic between proteins on various biological processes and the false discovery rate (FDR) value for each interaction.

RESULTS

We identified four important proteins that may play a critical role in malaria parasite infection based on prior research, namely MUC13 (human), LISP2 (*Plasmodium*), GDH (*Plasmodium*), and PfLDH (*Plasmodium falciparum*) [21,23,31]. The STRING DB was used to evaluate these biomarkers since it is frequently used to predict protein-protein interactions among key biomarkers.

Human Mucin-13 Protein

Several nodes integrated with human mucin-13 protein (MUC13) in three biological processes. They are the homeostatic process (FDR 0.0299), positive regulation of multi-organism process (FDR 1.83e-15), and stimulatory C-type lectin receptor signaling pathway (FDR 2.50e-22). In

the homeostatic process, MUC13 interacts with MUC4, MUC17, and MUC6. While in the positive regulation of multi-organic processes, MUC13 interacts with MUC4, MUC17, MUC6, MUC16, MUC20, MUC12, MUC15, MUC3A, and MUC1. The stimulatory C-type lectin receptor signaling pathway includes MUC4, MUC17, MUC6, MUC16, MUC20, MUC12, MUC15, MUC3A, and MUC1. In general, MUC4, MUC6, and MUC17 are involved in three terms related to cell signalling (Table 1).

Table 1. The PPI network information of MUC13

No	Description	False Discovery Rate	Genes Involved
1	Homeostatic process	0.0299 (RED)	MUC4, MUC17, MUC6
2	Positive regulation of the multi-organism process	1.83e-15 (BLUE)	MUC4, MUC17, MUC6, MUC16, MUC20, MUC12, MUC15, MUC3A, MUC1
3	Stimulatory C-type lectin receptor signaling pathway	2.50e-22 (GREEN)	MUC4, MUC17, MUC6, MUC16, MUC20, MUC12, MUC15, MUC3A, MUC1

Liver-Specific Protein 2

We found the interaction between the LISP2 gene (PKH_030930) with two distinct nodes. These two nodes interacting with PKH_030930 are PKH_120710 (Pf47-like protein, putative) and PKH_142580 (6-Cysteine Protein, putative).

Table 2. The PPI network information of LISP2

No	Description	False Discovery Rate	Gene Involved
1	Sexual Stage Antigen s48/45 Domain	2.90e-05 (RED)	PKH_120710
2	Mixed, incl Sexual Stage Antigen s48/45 Domain, GTP Binding Protein OBG, C-Terminal	2.49e-06 (BLUE)	PKH_120710, PKH_142580

***Plasmodium falciparum* Lactate Dehydrogenase**

We predicted the interaction between PfLDH with several nodes. The glycolysis enolase process was marked by red color with FDR 1.51e-14. The nitrogen and carbon metabolism have FDR value 4.18e-14. In the glycolysis process, PfLDH interacts with PF14_0425 (Fructose-biphosphate aldolase), PF11_0208 (Phosphoglycerate mutase), PF10_0155 (Enolase), PF14_0378 (Triphosphate isomerase), PGK (Phosphoglycerate kinase), and GDPH (Glyceraldehyde-3-phosphate dehydrogenase). PF14_0425 (Fructose-biphosphate aldolase), PF11_0208 (Phosphoglycerate mutase), PF10_0155 (Enolase), PF14_0378 (Triphosphate isomerase), PGK (Phosphoglycerate Kinase), GDPH (Glyceraldehyde-3-phosphate dehydrogenase), and PFB0200c (Aspartate aminotransferase) integrated with pfLDH in nitrogen and carbon metabolism.

Table 3. PPI network information of PfLDH

No	Description	False Discovery Rate	Genes Involved
1	Glycolysis, enolase	1.51e-14(RED)	PF14_0378, PF10_0155, PGK, GDPH, PF14_0425, PF11_0208
2	Nitrogen and Carbon Metabolism	4.18e-14(YELLOW)	PFB0200c, PF10_0155, PF14_0378, PF14_0425, PGK, GAPDH, PF11_0208

Plasmodium Glutamate Dehydrogenase

The plasmodium GDH (PF14_0164) interacted with several nodes in several biological processes. In mixed interaction including carbon and nitrogen metabolism (FDR 1.54e-14), Plasmodium GDH interacts with PFI1110w (Glutamine synthetase, putative), PF08_0132 (Uncharacterized protein; Glutamate dehydrogenase, putative), PF14_0334 (Uncharacterized protein; NAD(P)H-dependent glutamate synthase, putative), PFB200c (Aspartate aminotransferase), PF10_0218 (Citrate synthase;), PF13_0242 (Isocitrate dehydrogenase [NADP];), PEPCK (Phosphoenolpyruvate carboxykinase). In Citrate cycle (TCA cycle) and nitrogen metabolism, plasmodium GDH interacted with PFI1110w (Glutamine synthetase, putative), PF08_0132 (Uncharacterized protein; Glutamate dehydrogenase, putative), PF14_0334 Uncharacterized protein; NAD(P)H-dependent glutamate synthase, putative), PFB200c (Aspartate aminotransferase), PF10_0218 (Citrate synthase;), PF13_0242 (Isocitrate dehydrogenase

[NADP];). In the biosynthesis of antibiotics and metabolic pathway, Plasmodium GDH integrated with PFI1110w (Glutamine synthetase, putative), PF08_0132(Uncharacterized protein; Glutamate dehydrogenase, putative), PF14_0334 Uncharacterized protein; NAD(P)H-dependent glutamate synthase, putative), PFB200c (Aspartate aminotransferase), PF10_0218 (Citrate synthase;), PF13_0242 (Isocitrate dehydrogenase [NADP]), PEPCK (Phosphoenolpyruvate carboxykinase), PF08_0045(2-oxoglutarate dehydrogenase E1 component), PF08_0066 (Uncharacterized protein; Lipoamide dehydrogenase,). In Alanine, aspartate, and glutamate metabolism pathway, Plasmodium GDH interact with PFI1110w (Glutamine synthetase, putative), PF08_0132 (Uncharacterized protein; Glutamate dehydrogenase, putative), PF14_0334 (Uncharacterized protein; NAD(P)H-dependent glutamate synthase, putative), PFB200c (Aspartate aminotransferase). PFB200c (Aspartate aminotransferase), PFI1110w (Glutamine synthetase, putative), and PF08_0132 (Uncharacterized protein; Glutamate dehydrogenase, putative) correlated with plasmodium GDH in arginine biosynthesis.

Table 4. PPI network information of Plasmodium GDH

No	Description	False Discovery Rate	Genes Involved
1	Carbon and nitrogen metabolism	1.54e-14(RED)	PFI1110w, PF08_0132, PF14_0334, PFB200c, PF10_0218, PF13_0242, PEPCK
2	Citrate cycle (TCA) and nitrogen metabolism	1.54e-14(BLUE)	PFI1110w, PF08_0132, PF14_0334, PFB200c, PF10_0218, PF13_0242
3	Biosynthesis of antibiotics and metabolic pathway	1.34e-16(GREEN)	PFI1110w, PF08_0132, PF14_0334, PFB200c, PF10_0218, PF13_0242, PEPCK, PF08_0045, PF08_0066
4	Arginine biosynthesis	5.12e-09(YELLOW)	PFB200c, PFI1110w, PF08_0132,
5	Alanine, aspartate and glutamate metabolism	5.89e-10(PURPLE)	PFI1110w, PF08_0132, PF14_0334, PFB200c

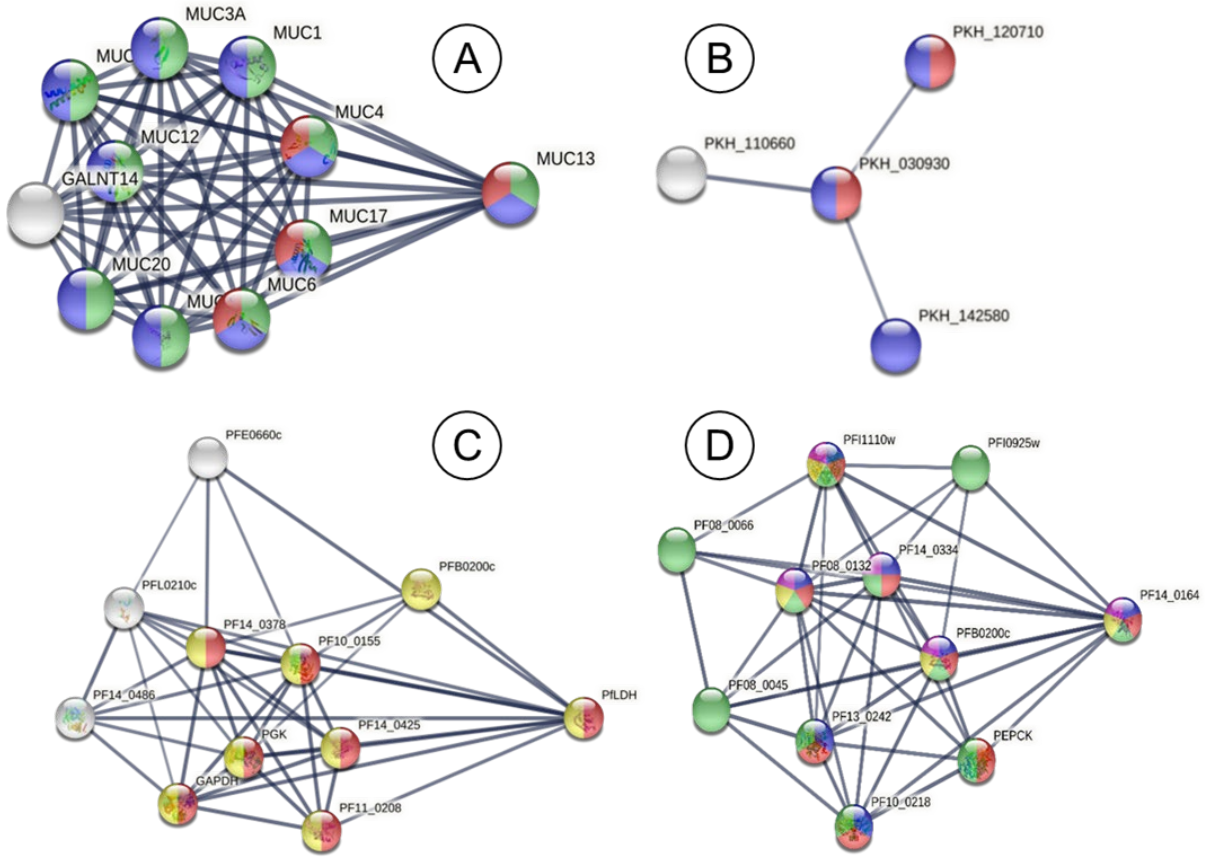

Figure 1. The protein-protein interaction network of malaria biomarkers generated using STRING DB, The thick dark blue line represents the strong interaction and white nodes mean the proteins are not interact in query biological processes. Protein interacts with several nodes and colour show the kind of biological processes involved. A) PPI network of MUC13 protein, red nodes represent hemoestatic process, blue nodes represent positive regulation of the multi-organism process, and green nodes represent Stimulatory C-type lectin receptor signaling pathway. B) PPI network of LISP2 (PKH_030930), red and blue nodes indicate Sexual Stage Antigen s48/45 Domain and mixed (incl Sexual Stage Antigen s48/45 Domain, GTP Binding Protein OBG, C-Terminal), respectively. C) PPI network of PfLDH, Red nodes indicate glycolysis processes, and yellow nodes represent nitrogen/carbon metabolism. D) PPI network of Plasmodium GDH (PF14_0164), red nodes indicate carbon and nitrogen metabolism, blue nodes indicate TCA and nitrogen metabolism, green nodes indicate biosynthesis of antibiotics and metabolic pathway, yellow nodes indicate arginine biosynthesis, purple nodes indicate alanine/aspartate and glutamate metabolism

DISCUSSION

In the present study, malaria biomarkers interact with several proteins, possibly being used for novel therapeutic targets. These proteins have a role in a different stage in the plasmodium life cycle. LISP2 interact with two sexual antigen s4/45 domain. MUC13 integrated with other mucin proteins that maybe have the same role in supporting parasite development in hepatocytes. PfLDH, as a metabolic enzyme, interacts with seven proteins related to plasmodium metabolism during red blood cell invasion. Plasmodium GDH interacts with ten proteins in nitrogen and carbon and amino acid metabolism.

The LISP2 protein has a crucial role in supporting merozoite development in the liver stage. This protein is localized on the parasitophorous vacuole membrane (PVM) and expressed by the *Plasmodium* sp [32]. The upregulated LISP2 gene was found in the trophozoite and schizogony stage [31]. The protein on PVM is exported into the hepatocyte cytoplasm. Interaction between the parasite and the human host involves LIPS2 to support merozoite development. The specific function of LISP2 is still unknown, but the previous study reported that the deletion of LISP2 inhibits merozoite development [32]. The existence of LISP2 on hepatocyte cytoplasm can cause LISP2 migration into the nucleus and downregulate cytokine expression and increase viability during their development [31]. LISP2 suppressed de novo protein synthesis and causing an increased nutrition flow from the host cell to the parasite [13]. We report two protein interactions with LISP2, namely PKH_120710 (Expressing Pf47-like protein, putative) and PKH_142580 (Expressing 6-Cysteine Protein, putative). This protein has a role as sexual stage antigen s48/45 domain. The s48/45 domain is a protein located in a parasite surface in different stages [33]. Pfs47 is involved in sexual stage gamet fusion in mosquito midgut [34]. 6-Cysteinine protein is also involved as s48/45 domain. This protein localizes in the parasite membrane or interfaces with the host cell [35].

The mucin-13 protein is highly expressed in the liver's mucosal epithelial cell during *Plasmodium* infection in the liver stage [36]. The role of Mucin-13 in malaria pathogenesis is still mostly unclear. The mucin-13 protein is expressed in normal conditions and has a role as a transmembrane protein to protect the cell from infection [21]. This study reported several mucin proteins integrated with MUC13 in three biological processes in normal conditions, including homeostatic process (FDR 0.0299), positive regulation of the multi organism process (FDR 1.83e-15), and stimulatory C-type lectin receptor signaling pathway (2.50e-22) (Table 1). When the patient got malaria, the mucin-13 exists on PVM primarily identified during merozoite development [21]. A study explains that the deletion of MUC13 does not affect merozoite development. On plasmodium infection, a mucin-13 protein is highly suggested that has a function to avoid immune cells [21] because this protein surrounds PVM during parasite liver stage development. Mucin complex is located in hepatocyte epithelial cells. All mucin protein may also be surrounding PVM during an invasion. They suggest that other mucin proteins involving MUC4, MUC17, MUC6, MUC16, MUC20, MUC12, MUC15, MUC3A, MUC1 have the same role MUC13. Knockdown of this protein may prevent parasite development through enhancing effective immune response during liver stage infection.

Plasmodium consumes sugar from the host cell to survive. Plasmodium breaks down the red blood cell in the erythrocyte stage, especially the trophozoite stage, causing increased glucose consumption up to 100 folds [23]. The existence of sugar on the *Plasmodium* cell can induce the glycolysis mechanism. The glycolysis process involves several enzymes, including PfLDH. This enzyme has a role as a metabolic enzyme that converts pyruvate to lactate in *Plasmodium falciparum*. In the present study, We predict that PfLDH enzymes are integrated with several proteins involved in glycolysis (FDR 1.51e-14), including PF14_0378, PF10_0155, PGK, GDPH, PF14_0425, PF11_0208. In another study, several proteins are integrated within the plasmodium glycolysis mechanism and potential as a drug target in plasmodium glycolysis pathway modeling, including hexokinase, *Plasmodium*'s fructose 1,6-phosphate aldolase, *Plasmodium* triosephosphate isomerase, and *Plasmodium* Glyceraldehyde-3-phosphate dehydrogenase [37]. This enzyme becomes one of the potential biomarkers due to high expression in 20-30 hours after infection during the erythrocyte stage [23]. When they are going into the schizont stage, they stop producing this enzyme [38]. Several compounds potentially as PfLDH inhibitors, including itraconazole, atorvastatin, and posaconazole [39].

Inhibition of these proteins may prevent plasmodium development through inhibition of energy generation. PfLDH is also involved in nitrogen and carbon metabolism (FDR 4.18e-14) (Table 3). The protein interacts with PFB0200c, PF10_0155, PF14_0378, PF14_0425, PGK, GAPDH, and PF11_0208. These proteins may be drug targets because targeting this protein may inhibit metabolism during plasmodium development in the blood stage.

The GDH enzyme was identified as a new biomarker by malaria rapid diagnostic test (RDT) [40]. The GDH as a biomarker has a different structure from other GDH enzymes [23], and there is no GDH enzyme on red blood cells. The GDH enzyme involving Krebs cycle on *Plasmodium* sp. We also reported that GDH interacts with PFI1110w, PF08_0132, PF14_0334, PFB200c, PF10_0218, and PF13_0242 citric cycle process. They use this enzyme to get energy using the Krebs cycle; GDH oxidizes glutamate into alfa-ketoglutarate and releases NADPH, which has the enzyme's role cofactor infection to red blood cells [40]. *Plasmodium*'s GDH is considered a potential biomarker and drug target of *Plasmodium falciparum* infection [40,41]. The present study predicted ten proteins integrated with*Plasmodium's* GDH in various biological processes, including citrate cycle (TCA), metabolic pathway, carbon and nitrogen metabolism, etc. (Table 4). Three proteins involved in the arginine biosynthesis process, including PFB200c, PFI1110w, and PF08_0132 (Table.4). Arginine is metabolized by Plasmodium and requires NO production to reduce red blood cell deformability [42]. On the other hand, Plasmodium GDH is also related to Alanine, aspartate, and glutamate metabolism. In this process, Plasmodium GDH interacts with PFI1110w, PF08_0132, PF14_0334, and PFB200c. Plasmodium requires amino acid metabolism to support parasite development during the invasion, such as glycoprotein formation, adenylosuccinate production, and critical vitamin production [43]. These proteins are considered as a potential drug target to inhibit Plasmodium's metabolism during blood-stage infection.

We found LISP2, Mucin-13 Protein, PfLDH, and Plasmodium's GDH are biomarkers in malaria that interact with several proteins on many biological processes. This study performed protein-protein interaction analysis of these malaria biomarkers. Targeting LISP2, Mucin-13 protein and their interaction partners possibly prevent parasite development in the liver stage. It can be used for a novel drug target in chemoprevention therapies strategy against malaria disease. We also predict targeting PfLDH, Plasmodium GDH and their interaction partner as a metabolic enzyme in *Plasmodium falciparum* suggested can inhibit energy generation and parasite development during blood-stage infection.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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