

Characterization and epitope prediction of phosphopyruvate hydratase from *Penaeus monodon* (black tiger shrimp)

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Abstract: Shellfish allergies constitute an important cause of food-induced anaphylactic reactions, which pose challenges to food safety and human health worldwide. In the present study, the specific IgE (sIgE) binding characteristics of different shrimp proteins of black tiger shrimp (*Penaeus monodon*) to the sera of eight shrimp-allergic patients from China were studied by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) and nanoliquid chromatography time-of-flight mass spectrometry. According to the PLGS scores (>2000) and the sequence coverage (>40%), eight proteins with sIgE binding activity were identified, including myosin heavy chain type 1 (K4Q4N8), hemocyanin (G1AP69 and Q95V28), phosphopyruvate hydratase (O96656), arginine kinase (C7E3T4), tropomyosin (A1KYZ2), sarcoplasmic calcium binding protein (H7CHW2) and glyceraldehyde-3-phosphate dehydrogenase (A0A097BQP2). Among these eight proteins, phosphopyruvate hydratase was a prevalent IgE-binding protein among these Chinese patients with binding observed in 100% of sera. Moreover, 13 peptides were predicted as epitopes of phosphopyruvate hydratase. These new details help us to understand the crustacean IgE-binding proteins especially *Penaeus monodon* IgE-binding proteins, that would cause allergic reaction to Chinese patients. And our findings may provide essential information to improve allergy prevention and clinical treatment to shrimp allergy in China.

Practical Application: This research may have diagnostic and therapeutic value for shrimp allergies in China.

KEYWORDS

allergen, epitope, *Penaeus monodon*, phosphopyruvate hydratase

1 | INTRODUCTION

Food allergies are a pathological disorder of the immune system with adverse reactions, including skin irritation, diarrhea, respiratory disease and even syncope, and affect approximately 1%–5% of adults and at least 4%–8% of children worldwide (Monaci et al., 2018;

Wang et al., 2009). In recent years, the prevalence of food allergies has increased and thus has aroused new public concern for food safety.

Although hundreds of foods are known to cause food allergies, eight food groups, including eggs, fish, milk, peanuts, crustacean shellfish, soy, tree nuts, and wheat, are considered priority allergens and account for over 90%

of all food allergies (Ho et al., 2014). Food allergies mediated by IgE are the most serious type of allergy and occur with almost all kinds of food, including the “big eight” allergens. However, among the “big eight” allergens, crustacean shellfish are one of the most common causes of IgE-mediated food allergies. In fact, crustacean shellfish are a common trigger for some acute allergies and life-threatening anaphylactic reactions (Ruethers et al., 2018). It has been estimated that the prevalence of food allergies induced by shellfish is 0.5%–2.5%, and this percentage may be even higher in coastal countries with higher dietary consumption (Khora, 2016). In South China, crustacean shellfish (prevalence rate: 3.2%–4.4%) was found to be the leading cause of adverse reactions from food allergies among preschool children based on an epidemiological survey by self-reported food allergy diagnosis (Zeng et al., 2015).

In 1993, an original study from the United States identified tropomyosin as the major shrimp allergen (Shanti et al., 1993). And arginine kinase was the second allergen characterized in shrimp with clinical significance (Yu et al., 2003). Out of 53 patients that were allergic to shrimp from Spain and the United States, 81% showed IgE binding activity to tropomyosin and 51% of these patients had IgE binding activity to arginine kinase (Ayuso et al., 2010). To date, tropomyosin (Pen m 1), arginine kinase (Pen m 2), myosin light chain 2 (Pen m 3), sarcoplasmic calcium-binding protein (Pen m 4), and troponin C (Pen m 6) have been confirmed as the allergens in black tiger shrimp by the WHO/IUIS Allergen Nomenclature Sub-Committee (<http://www.allergen.org>). However, most of the aforementioned studies of crustacean allergens were conducted in Europe or the United States. In contrast, knowledge of crustacean allergies in China remains limited (Lee et al., 2018). Furthermore, the pattern of food allergies may vary between populations, suggesting that a genotype-by-environment interaction is involved. For example, the prevalence of peanut and tree nut allergies is relatively low in Asia, whereas shellfish allergies are predominated in comparison with Western populations. These investigations highlight important differences of food allergens between the East and West and even within Asian regions. Therefore, more in-depth studies of allergens from crustacean shellfish are one noteworthy issue in China to supply more evidence for clinical research on food allergies.

Black tiger shrimp (*Penaeus monodon*) is one of the most popular farming shrimp species in Latin America and Asia, especially in South China, where its production is increasing every year (Duan et al., 2016; Sengprasert et al., 2015). Although there have been several studies about allergens in black tiger shrimp, works on the activity of allergens in different populations (ethnic or geographical) are limited. And studies identifying unknown aller-

gens in black tiger shrimp are also rare. The diversity of crustacean species and the limited number of confirmed allergens and pose a challenge for diagnostics and therapeutic treatments of shrimp allergic patients. This study aimed to comprehensively elucidate the potential cause of food allergies in black tiger shrimp to Chinese allergic patients. In the present study, after separation by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE), allergens in black tiger shrimp were determined using IgE immunoblotting with sera from shrimp-allergic patients. IgE-binding proteins were identified by nanoliquid chromatography time-of-flight mass spectrometry (nano-LC-TOF-MS). The binding of an epitope to a receptor on the surface of an immune cell is the first step in an allergic reaction. Considering the importance of epitopes in sensitization and in the treatment of allergies, the sIgE binding activities of identified allergens of black tiger shrimp were further investigated, and the epitopes of phosphopyruvate hydratase were predicted.

2 | MATERIALS AND METHODS

2.1 | Sera of patients

Sera of eight patients with histories of allergic reactions after shrimp ingestion were collected from Qingdao Traditional Chinese and Western Medicine Hospital (Shandong, China) and Guangdong Women's and Children's Hospital (Guangdong, China). Sera from four healthy subjects without an allergic history were used as negative controls. Written informed consent was required for each patient and healthy volunteer. All serum samples were stored at -80°C before use.

Four female patients and four male patients, two healthy female control subjects and two healthy male control subjects were enrolled. The patients had a mean age of 6.9 years (range: 3–11 years) and allergies elicited by shrimp. In addition to the history of shrimp allergies, four of eight patients had an additional history of allergic reactions to crabs, milk, eggs, or tree nuts. All eight patients had a history of typical IgE-mediated symptoms after ingesting shrimp. All patients underwent a serum-specific IgE test using the Mediwiss Allergy Screen System (Mediwiss Analytic GmbH, Germany) (Miao et al., 2018). The concentrations of total IgE in the sera of all eight patients were greater than 200 IU/ml, and the concentrations of sIgE_{shrimp} in the sera of five patients were higher than 100 IU/ml. A synopsis of historical details and allergy diagnostic tests for patients is listed in Table 1.

2.2 | Sample extraction

Fresh black tiger shrimp samples were purchased from a local market in Beijing, China. The protein extraction

TABLE 1 Clinical characteristics of patients with shrimp allergy

No.	Age	Sex	Total-IgE [IU/ml]	sIgE _{shrimp} [IU/ml]	sIgE _{crab} [IU/ml]	sIgE _{dust} [IU/ml]	History for shrimp allergy	History for other allergies	symptoms
1	11	F	>200	>100	>100	13.9	+	Crab allergy	AD
2	7	F	>200	>100	>100	6.5	+	Crab allergy	AD
3	8	M	>200	>100	0	41.8	+	-	Ab, AD, Dy
4	11	M	>200	>100	>100	>100	+	-	Dy
5	3	F	>200	>100	>100	15.2	+	Milk, egg, and tree nut allergy	AD
6	4	M	>200	74.6	>100	20.0	+	-	AD
7	7	F	>200	43.1	35.9	48.6	+	Crab allergy	Dy
8	4	M	>200	29.4	30.9	>100	+	-	Ab, AD, Dy
9	7	M	<100	0	0	0	-	-	None
10	1	M	<100	0	0	0	-	-	None
11	4	F	<100	0	0	0	-	-	None
12	3	F	<100	0	0	0	-	-	None

Abbreviations: Ab, abdominal symptoms; AD, atopic dermatitis; Dy, dyspnoea; F, female; M, male.

method was adopted from previous reports with minor modifications (Qi et al., 2019; Abdel Rahman et al., 2013). The details of the sample extraction process were as follows. First, 200 mg of homogenized shrimp sample and 1 ml of buffer (1 M KCl, 25 mM Tris-HCl, pH 8.0, 0.25 M DTT, and 0.5 mM EDTA) were added to the homogenizing tube containing 1.0 mm silica spheres (MP, Santa Ana, USA). The proteins were extracted on an ice bath for 20 s by a homogenizer (MP, Santa Ana, USA). Then, the homogenates were centrifuged at 10,000 rpm for 15 min at 4°C. The concentration of total protein in the extract was quantified using a Bradford Protein Assay Kit (Bio-Rad, Hercules, USA). The crude extract was used for the further characterization of IgE-binding proteins. The protein extracts were stored at -80°C before use.

2.3 | SDS-PAGE and western blot analysis

Ten microliters of extract (containing 5 µg of protein) diluted with lithium dodecyl sulfate (LDS) sample buffer (4 ×) (Life Technologies, Carlsbad, CA, USA) and the appropriate amount of distilled water were mixed and heated at 70°C for 10 min. Then, the mixture was added to a sample well. The reference marker was added to the left and right wells of each gel. The different protein fractions were separated by 4%–12% SDS-PAGE at 150 V for 90 min. After electrophoresis, the proteins in the gel were stained using Coomassie brilliant blue stain (Bio-Rad, Hercules, CA, USA) for 1 hr and washed with distilled water.

Otherwise, the separated proteins were electrotransferred onto polyvinylidene fluoride membranes (PVDF, Millipore Corp., Burlington, MA, USA) at 120 V for 1.5 hr. After being blocked with bovine serum albumin (10% in PBS) (4°C, 1 hr), the PVDF membranes were incubated with patient sera diluted with PBS (1:200) at 4°C overnight. Then, the PVDF membranes were washed with Tris-buffered saline with 0.1% Tween 20 (TBST) (15 min × 3) three times, incubated with antihuman IgE antibody (1:5000) (Sigma, St. Louis, MO, USA) for 2 hr at 4°C and washed with TBST buffer (15 min × 3). Finally, the PVDF membranes were imaged with a C-Digit blot scanner (Li-COR, Germany) after treated with Pierce ECL Western Blotting Substrate (Thermo Fisher Scientific, Waltham, MA, USA).

2.4 | Chromatographic and mass spectrometric analysis

According to the Western blot analysis results, the interesting bands were marked in the PVDF membrane. The protein and marker bands in SDS-PAGE

stained with Coomassie brilliant blue were carefully compared to PVDF membranes by a Bio-5000 gel scanner (MICROTEK, Shanghai, China). The interesting protein band in SDS-PAGE was traced according to marker bands both on SDS-PAGE and Polyvinylidene Fluoride (PVDF) membranes. Then, each interesting protein band in SDS-PAGE was completely excised from the gel. Each excised band was further cut into small pieces (1 mm³), and all of the small pieces were added into a 1.5 ml Eppendorf tube. The protein was digested according to a published in-gel digestion procedure (Gu et al., 2007). The digested peptides were extracted and separated by a nano-UPLC system (nano ACQUITY UPLC, Waters, USA) in dilution mode, which was coupled to a Q-tof Synapt HDMS system (Waters, Corp., Milford, USA) as a detector.

In the following separation procedure, a Symmetry C18 (5 μ m, 180 μ m $\times$ 20 mm) was selected as the trap column, and a BEH130 C18 (1.7 μ m, 75 μ m $\times$ 250 mm) (Waters Corp., Milford, MA, USA) was selected as the analytical column. The trap column is used to desalt the sample by the trapping procedure. In the desalt procedure, 0.1% formic acid in water was used as the mobile phase, and the flow rate was set at 10 μ l/min for 5 min. After trapping, an analysis procedure was triggered. In the analysis procedure, 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) were used as the mobile phases, and the flow rate was 400 nl/min. The linear gradient was as follows: the gradient schedule of the mobile phase was initiated with 95% A, followed by a linear gradient to 60% A over 120 min, and mobile phase A was set to 15% at 121 min, which was then held for 4 min.

The mass spectrometry data were acquired from 100 Da to 2000 Da in positive ion mode using independent alternative scanning (MS^E) mode. The scan time was set as 1.5 s, and the collision energy voltage was set as 20–45 eV with a gradient. [Glu1]-fibrinopeptide B ([M + 2H]²⁺; *m/z* 785.84206) (Waters Corp.) was used as a lock mass correction reference.

2.5 | Identification of sIgE binding protein bands

ProteinLynx Global SERVER (PLGS) v3.0 (Waters Corp.) was used for data analysis (Hummel et al., 2015). In this study, the general parameters were set as automatic, such as the MS resolution and peak width. The detection thresholds were 300 and 50 counts for the low- and high-energy channels, respectively. The identification intensity threshold was set as 500 counts.

In this study, the UniProt database was used to identify the target proteins. In order to improve the match ratio between the target proteins and known proteins in

the UniProt database, the fixed posttranslational modifications of carbamidomethylation (C) and oxidation (M) were included. Two possible trypsin missed cleavages were allowed. For good accuracy, it was necessary that more than three fragment ions were matched per peptide and more than two peptides were matched per protein. The false discovery rate (FDR) of protein was set as 4%.

2.6 | Prediction of the epitope

In order to predict the epitopes of the allergens, an immunoinformatic method-based computational approach, BepiPred-2.0 (<http://www.cbs.edu.dk/services/BepiPred>), was used. The complete AA sequence of phosphopyruvate hydratase (O96656_PENMO) in fasta format was downloaded from UniProt, and then sequence information was submitted to BepiPred-2.0 to predict epitopes of allergy.

3 | RESULTS AND DISCUSSION

3.1 | sIgE reactivity profiling of the sera of patients with shrimp allergy

The typical protein profile of the extracts of black tiger shrimp following SDS-PAGE and Coomassie blue staining is shown in Figure 1a. The typical profile of sIgE reactive proteins in black tiger shrimp to patient sera is illustrated in Figure 1b. Eight different proteins from black tiger shrimp were shown to bind specifically to the sera IgE from one or more of the shrimp-allergic individuals. The protein bands were observed by SDS-PAGE in the molecular weight range of 20 to 180 kDa. IgE-binding proteins were noted at apparent molecular weights of approximately 20, 25, 35, 40, 50, 60, 75, and 170 kDa (B-P8–B-P1 on SDS-PAGE).

3.2 | Mapping of the sIgE binding protein bands

All of the sIgE protein bands (B-P1 to B-P8) were excised and digested in-gel by trypsin and identified by nano-LC-TOF-MS using the bottom-up approach of peptide fragment fingerprinting. The generated peptides were identified by nano-LC-TOF-MS in MS^E scan mode. The precursor ion and fragmentation spectra were compared to the nonredundant UniProt protein database by PLGS software.

Generally, the ProteinLynx Global Server (PLGS) score and the calculated sequence coverage are important

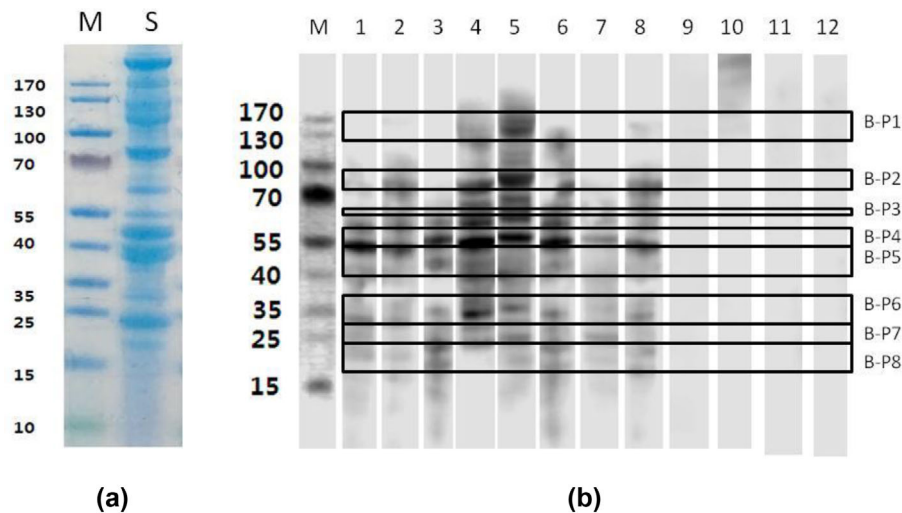


FIGURE 1 SDS-PAGE pattern of extract of *Penaeus Monodon* (a) and immunoreactive bands for allergens in *Penaeus Monodon* with specific-IgEs in patients' sera (b). M: protein marker, S: extract of *Penaeus Monodon*, 1–8: patients' sera, 9–12: sera of control subject

TABLE 2 Table of identified allergenic proteins in *Penaeus monodon* extract

Band No.	Protein name	Accession number	Allergen code	Molecular size (kDa)	PLGS score	Sequence coverage (%)
B-P1	Myosin heavy chain type 1	K4Q4N8	/	219	3103	55.8
B-P2	Hemocyanin	G1AP69	/	78	2045	40.7
B-P3	Hemocyanin	Q95V28	/	51	2406	64.0
B-P4	Phosphopyruvate hydratase	O96656	/	47	24995	64.7
B-P5	Arginine kinase	C7E3T4	Pen m 2	40	2580	64.0
B-P6	Tropomyosin	A1KYZ2	Pen m 1	38	3659	47.4
B-P7	Sarcoplasmic calcium binding protein	H7CHW2	Pen m 4	22	2439	64.8
B-P8	Glyceraldehyde-3-phosphate dehydrogenase	A0A097BQP2		19	22690	76.2

parameters to measure the reliability of identification results. A PLGS score higher than 2000 is considered credible. It was suggested that the identification result was receivable when sequence coverage was greater than 30% (Tables S1–S8). According to the reliability criterion mentioned above, eight protein bands for black tiger shrimp were identified as IgE-binding proteins, as shown in Table 2. These eight IgE-binding proteins were myosin heavy chain type 1 (K4Q4N8, B-P1), hemocyanin (G1AP69, B-P2), hemocyanin (Q95V28, B-P3), phosphopyruvate hydratase (O96656_PENMO, B-P4), arginine kinase (C7E3T4, Pen m 2, B-P5), tropomyosin (A1KYZ2, Pen m 1, B-P6), sarcoplasmic calcium-binding protein (H7CHW2, Pen m 4, B-P7) and glyceraldehyde-3-phosphate dehydrogenase (A0A097BQP2, B-P8).

Among the eight proteins, three have been identified as allergens by the WHO/IUIS Allergen Nomenclature Sub-Committee, which were arginine kinase, tropomyosin, and sarcoplasmic calcium-binding protein. Myosin

heavy chain type 1, hemocyanin, and glyceraldehyde-3-phosphate dehydrogenase have also been shown as allergens by other researchers (Faber et al., 2017). However, phosphopyruvate hydratase (O96656_PENMO), an IgE-binding protein and a putative allergen, has not been well studied yet. In 2013, phosphopyruvate hydratase was tentatively identified as a potential allergen in *Mar-supenaeus japonicus* (Tomm et al., 2013). In that report, there was insufficient evidence that the potential allergen identified was phosphopyruvate hydratase since its amino acids sequence was not confirmed until April 2015. In the current study, the protein B-P4 was identified as phosphopyruvate hydratase (O96656_PENMO) with strong evidence. All of the detected *m/z* values of protein band B-P4 are shown in Figure 2(a). Furthermore, according to the match results of the peptide spectrum (shown in Table S4) with the the UniProt database using data analysis software PLGS, the protein B-P4 was identified as phosphopyruvate hydratase (O96656, 47 kDa) with

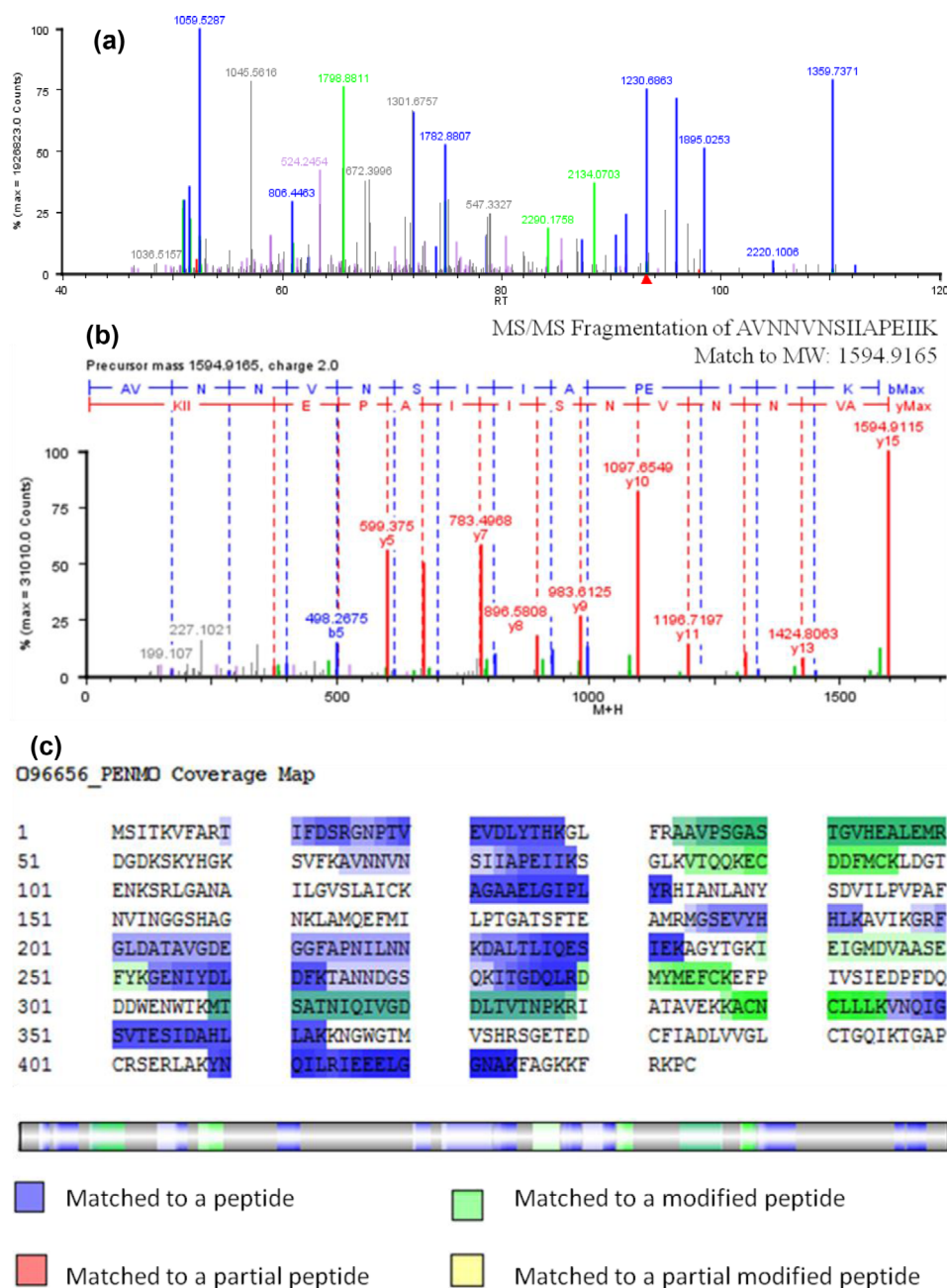


FIGURE 2 The full MS spectra and MS/MS spectra of band B-P4 after in-gel digestion and the coverage map result after search and match using uniprot data base. (a) The MS spectra of all detected precursor ions of band B-P4; (b) an example of significant match (product ion scan data against UniProt database). Peaks matched as b and y ions of the peptide sequence are labeled; and (c) the coverage map

the highest sequence coverage of 64.7% and the highest score of 24,995. Twenty-eight digested peptides of protein B-P4 successfully matched to phosphopyruvate hydratase, allowing two possible missed trypsin cleavages. As shown in Table S4, the error between the m/z of observed $[M+H]^+$ and the m/z of theoretical $[M+H]^+$ ranged from -3.21 ppm to 3.72 ppm, which were all lower than 5 ppm. Therefore, the shrimp allergic protein band B-P4 most likely represents phosphopyruvate hydratase. A typical MS/MS fragmentation and peptide sequence

match spectrum of protein B-P4 are shown in Figure 2(b), and the coverage map result after search and matching using the UniProt database is shown in Figure 2(c).

Further, Gene Ontology (GO) annotation with QuikGO (<https://www.ebi.ac.uk/QuickGO>) was used to investigate the molecular functions of phosphopyruvate hydratase. The GO annotation results demonstrated that phosphopyruvate hydratase activity (GO: 0004634), which catalyzes the synthesis of phosphoenolpyruvate from 2-phospho-D-glycerate, was the main molecular function

TABLE 3 The reactivity of allergens in *Penaeus Monodon* with sIgE in patients' serum

Band No.	Protein name	Number of patients' serum								Reactivity
		1	2	3	4	5	6	7	8	
B-P1	Myosin heavy chain type 1	–	–	–	–	+	+	–	–	2/8
B-P2	Hemocyanin	–	+	–	+	+	+	–	+	5/8
B-P3	Hemocyanin	+	+	+	+	+	+	–	+	7/8
B-P4	Phosphopyruvate hydratase	+	+	+	+	+	+	+	+	8/8
B-P5	Arginine kinase	–	–	+	+	+	+	–	–	4/8
B-P6	Tropomyosin	+	+	+	+	+	+	–	+	7/8
B-P7	Sarcoplasmic calcium binding protein	–	–	+	–	+	+	+	–	4/8
B-P8	Glyceraldehyde-3-phosphate dehydrogenase	–	–	+	–	–	+	–	–	2/8

“–”: The sIgE-binding protein band not be observed.

“+”: The sIgE-binding protein band be observed.

of phosphopyruvate hydratase. The GO annotation also indicated that phosphopyruvate hydratase is involved in glycolysis. Enolase, another reported allergen in fish, was annotated into the same functional category as phosphopyruvate hydratase, indicating their similarity in molecular functions (Liu et al., 2011). Moreover, enolase (G3GDU2_PENVA), with 117 amino acid residues, has the same sequence as residues 178–294 of phosphopyruvate hydratase (O96656_PENVA). Homologous amino acid sequences are one of the important factors in predicting allergic activity (Abdelmoteleb et al., 2021). Therefore, phosphopyruvate hydratase was predicted to be a potential allergen, and it may have the same domain for allergic reactions with enolase.

3.3 | The sIgE binding activities of shrimp IgE-binding proteins

The reactivity of sera from allergic patients with black tiger shrimp was investigated, and the results are summarized in Table 3. The intensity of reactivity of proteins in black tiger shrimp was arranged in descending order as follows: B-P4 (phosphopyruvate hydratase) (8/8) > B-P3 (hemocyanin) (7/8), B-P6 (tropomyosin) (7/8) > B-P2 (hemocyanin) (5/8) > B-P5 (arginine kinase) (4/8), and B-P7 (sarcoplasmic calcium binding protein) (4/8) > B-P1 (myosin heavy chain type 1) (2/8), B-P8 (glyceraldehyde-3-phosphate dehydrogenase) (2/8). Tropomyosin and arginine kinase were reported as important allergens in shrimp. In previous study, 72%–98% of patients showed positive IgE binding to tropomyosin and 10%–51% of patients showed positive IgE binding to arginine kinase (Ruethers et al., 2018). Our study in Chinese child patients showed similar result. However, all eight child patients showed positive IgE binding to phosphopyruvate hydratase, with higher IgE binding activ-

ity than tropomyosin and arginine. Phosphoenolpyruvate hydratase has not been previously shown to be an IgE-binding protein or allergen in shrimp or any species of crustacean shellfish. Previous studies have suggested that allergic sensitization may be induced by different allergens across ethnicities (Scheurer et al., 2001; Schocker et al., 2004). Therefore, our study suggested that allergic activity of phosphopyruvate hydratase in shrimp has been seriously underestimated in Chinese child patients. On the other hand, only sera from children were investigated in this study. Allergic sensitization would be different between children and adults, therefore, further investigation is needed to confirm whether phosphopyruvate hydratase is a novel shrimp allergen to Chinese adults.

3.4 | Prediction of the IgE-binding epitopes of phosphopyruvate hydratase

The IgE-binding epitopes of phosphopyruvate hydratase in black tiger shrimp were predicted using BepiPred-2.0 (<http://www.cbs.dtu.dk/services/BepiPred/>), which is an open platform used to predict B-cell epitopes. The method of prediction was based on a random forest algorithm. The epitopes were predicted according to training on epitopes annotated from antibody–antigen protein structures (Jespersen et al., 2017). As shown in Figure 3(a) and Table S9, the epitope probability value of each amino acid residue was calculated as the result of the combination of position exposure, relative surface accessibility, helix probability, sheet probability and coil probability. Here, the amino acid residues with epitope probability values higher than 0.5 were predicted as epitopes.

Thirteen peptides were predicted as epitopes (Figure 3b), including T1 (ATGVHEA, 40–46), T2 (EMRDGDKSKY-HGK, 48–61), T3 (LKVTQQKE, 82–89), T4 (DGTENKS, 98–104), T5 (NLANYSDV, 136–143), T6 (HAGNKL, 158–163),

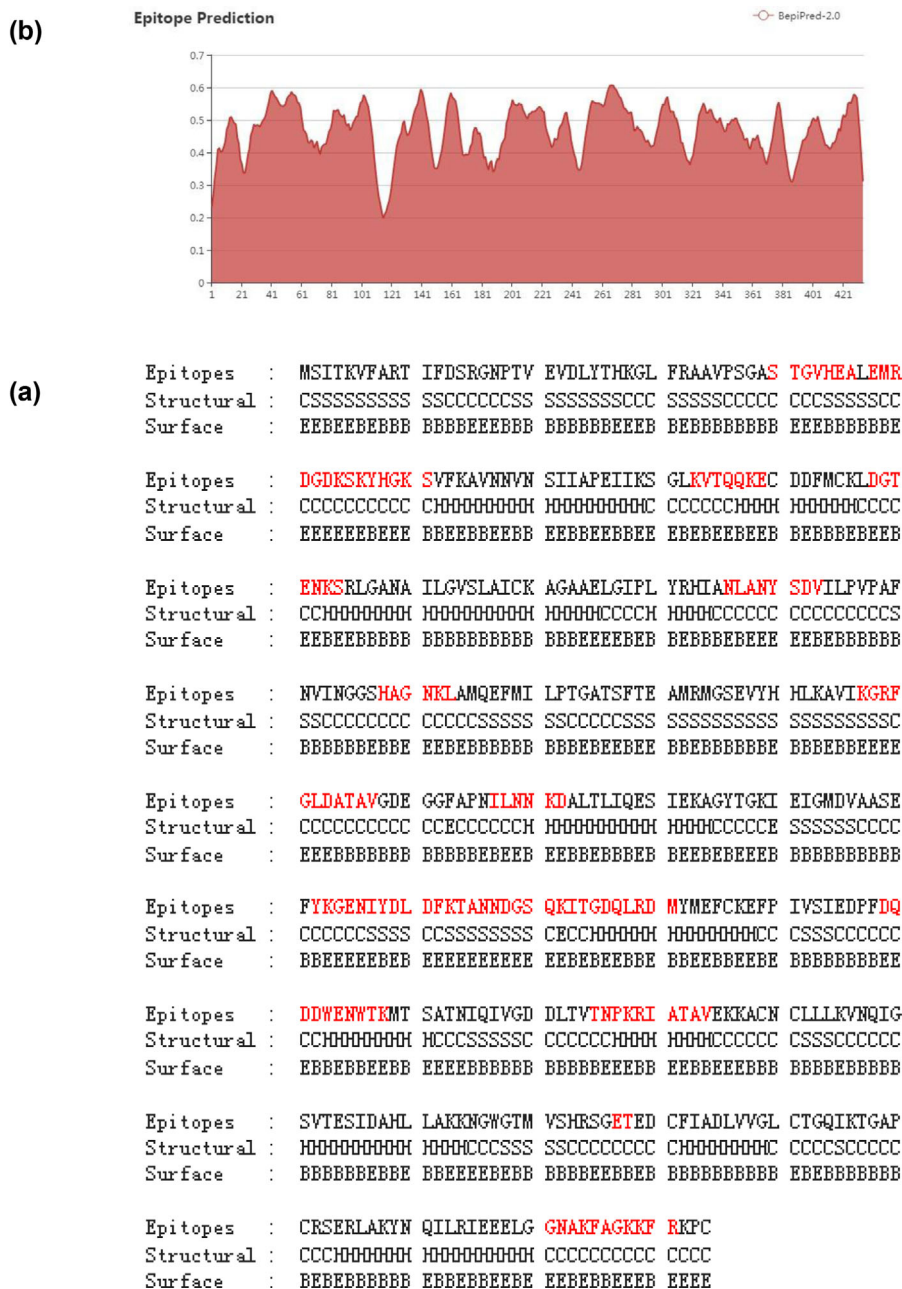


FIGURE 3 The predicted epitopes of phosphopyruvate hydratase in *Penaeus Monodon*. The value of epitope prediction of each amino acid was shown in figure (a), and predicted epitopes were labeled by red color in figure (b). Epitopes: positions of epitopes; Structural: H = Helix, S = Sheet, C = Coil (predicted using NetsurfP); Surface: B = Buried, E = Exposed (NetsurfP's default)

T7 (KGRFGLDATAV, 197–207), T8 (ILNNKD, 217–222), T9 (YKGENIYDLDFKTANNDGSQKITGDQLRDM, 252–281), T10 (DODDWENWTK, 299–308), T11 (TNPKRIATAV, 325–334), T12 (ET, 377–378), and T13 (GNAKFAGKKFR, 421–431).

There have been very few studies on the IgE binding epitopes of phosphopyruvate hydratase. Enolase, a protein that is highly related to phosphopyruvate hydratase, is a minor allergen in certain fish species and an important fungal allergen (Liu et al., 2011; Simon-Nobbe et al., 2000).

Enolase from black tiger shrimp is highly homologous to phosphopyruvate hydratase. Birgit demonstrated that a peptide with a length of 69 amino acids (120–189) in enolase (Cal h 6) was the region of IgE binding. And Ala124–Leu130 (AEKRVPL) and Arg163 (R) are the most likely epitopes of IgE binding (Simon-Nobbe et al., 2000). However, we compared the cross-reaction of epitopes of phosphopyruvate hydratase and enolase (Cal h 6) and some other allergens, such as tropomyosin in shrimp, there was also no cross-reactivity found (Zhang et al., 2020). The exact epitopes of

phosphopyruvate hydratase for IgE binding need to be further investigated.

4 | CONCLUSION

In summary, eight IgE-binding proteins from black tiger shrimp were identified using sera from shrimp-allergic Chinese children. Among the identified IgE-binding proteins, phosphopyruvate hydratase was identified as a novel, putative shrimp allergen, and the epitope of phosphopyruvate hydratase was predicted by a bioinformatics method. Further studies are needed to demonstrate the biological activity of this protein to confirm its importance. Although the number of patients was limit and only child patients were involved, the identified protein may have diagnostic and therapeutic value for shrimp allergies in Chinese population.

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AUTHOR CONTRIBUTIONS

Yi Yang: Conceptualization; Funding acquisition; Investigation; Project administration; Writing-original draft; Writing-review & editing. Huiying Liu: Data curation; Investigation. Wen Zeng: Data curation; Investigation. Yunjia Yang: Formal analysis; Methodology; Writing-original draft; Writing-review & editing. Jing Zhang: Conceptualization; Supervision; Validation. Jie Yin: Writing-original draft. Jieliang Wu: Formal analysis. Kefeng Lai: Formal analysis

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Supporting Information

Characterization and epitope prediction of phosphopyruvate hydratase from *Penaeus monodon* (black tiger shrimp)

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Supporting Tables

Table S1. The identified tryptic peptide fragments matched for myosin heavy chain type 1

Position	z	Precursor MH ⁺ (Da)	Theoretical MH ⁺ (Da)	Error (ppm)	Sequence	Modifications	Peptide Type
8-23	2	1794.8582	1794.8545	2.08	(K)STGPDPTPEYLFISR(E)	/	/
174-188	2	1520.7302	1520.7373	-4.69	(R)QNQSMILITGESGAGK(T)	/	/
174-188	2	1536.7420	1536.7322	6.36	(R)QNQSMILITGESGAGK(T)	M	/
194-207	2	1512.8145	1512.8057	5.84	(K)KVLSYFANVGASEK(K)	/	MC
195-207	2	1384.7107	1384.7107	-0.01	(K)VLSYFANVGASEK(K)	/	/
209-234	3	2902.4463	2902.4429	1.18	(K)EGESKQNLEDQIIQTNPILAYGNAK(T)	/	MC
214-234	2	2372.2097	2372.2092	0.20	(K)QNLEDQIIQTNPILAYGNAK(T)	/	/
258-270	2	1449.7820	1449.7834	-1.06	(K)LSGADIEVYLLEK(A)	/	/
352-365	2	1586.7921	1586.7930	-0.58	(K)VTASVMHHGNMKFK(Q)	/	MC
387-397	2	1277.6766	1277.6736	2.35	(K)LLGVDAEELYN(N)	/	/
387-403	3	2052.0679	2052.0583	4.67	(K)LLGVDAEELYNFCKPK(I)	C	MC
414-429	2	1724.8151	1724.8094	3.27	(K)GMNVDQVNYNIGAMAK(G)	/	/
414-429	2	1707.7910	1707.7830	4.74	(K)GMNVDQVNYNIGAMAK(G)	/	NL
571-599	6	3244.6213	3244.6174	1.20	(K)AGQPENHFAIVHYAGTVSYNLTGWLEK NK(D)	/	MC
600-611	2	1356.6888	1356.7004	-8.65	(K)DPLNDTVVDQLK(K)	/	/
647-661	2	1654.8458	1654.8469	-0.65	(K)TVSSGYKDQLANLMK(T)	/	/
647-661	3	1670.8531	1670.8418	6.77	(K)TVSSGYKDQLANLMK(T)	M	/
662-672	3	1306.7050	1306.7015	2.69	(K)TLNATHPHFIR(C)	/	/
724-730	1	792.4559	792.4614	-6.96	(R)YNILAAK(E)	/	/
724-730	2	775.4341	775.4349	-0.99	(R)YNILAAK(E)	/	NL
741-748	1	925.4134	925.4197	-6.77	(K)AATACFER(A)	C	/
749-757	2	1032.5518	1032.5472	4.39	(R)AGLNPELYR(T)	/	/
767-777	2	1143.6392	1143.6368	2.00	(R)AGVLGTLEEV(R)	/	/
767-780	2	1529.7983	1529.7917	4.31	(R)AGVLGTLEEV(RDDR)	/	/
781-793	3	1629.9304	1629.9298	0.39	(R)IMKLVSQWLAQV(R)	/	MC
809-816	2	947.5331	947.5342	-1.21	(R)TALIVMQ(R)	M	/
809-816	1	931.5317	931.5393	-8.21	(R)TALIVMQ(R)	/	/
845-858	2	1545.7979	1545.8007	-1.78	(R)GEELEKLEATAVK(A)	/	MC
869-884	2	1944.9724	1944.9807	-4.29	(K)VREELEAQNALLMQ(R)	M	/
869-884	3	1928.9927	1928.9858	3.56	(K)VREELEAQNALLMQ(R)	/	/
871-884	2	1673.8217	1673.8163	3.22	(R)EELEAQNALLMQ(R)	/	/
871-884	2	1689.8237	1689.8112	7.39	(R)EELEAQNALLMQ(R)	M	/
885-895	2	1160.6180	1160.6157	2.03	(R)NELLAQVDSK(G)	/	/
896-904	1	999.4511	999.4452	5.93	(K)GGMSEYLDK(Q)	/	/
913-925	2	1487.7296	1487.7336	-2.71	(K)AELEGQLNDTL(R)	/	/
953-963	2	1316.6882	1316.6945	-4.70	(K)EELEELSVQK(G)	/	/
953-963	2	1298.6906	1298.6838	5.14	(K)EELEELSVQK(G)	/	NL
969-991	4	2739.3677	2739.3794	-4.32	(K)QTKDQQLTNLNEEISHQEELITK(V)	/	/
972-991	3	2382.1877	2382.1782	3.94	(K)DQQLTNLNEEISHQEELITK(V)	/	/

1006-1022	3	1947.9261	1947.9077	9.46	(K)TAEDLQGVEDKCNLNK(V)	C	MC
1006-1016	2	1204.5707	1204.5692	1.26	(K)TAEDLQGVEDK(C)	/	/
1025-1040	3	1877.9342	1877.9338	0.19	(K)TKLESSLDELEDTLER(E)	/	MC
1025-1040	3	1859.9258	1859.9233	1.36	(K)TKLESSLDELEDTLER(E)	/	NL
1027-1042	3	1905.9327	1905.9288	2.12	(K)LESSLDELEDTLEREK(K)	/	/
1027-1040	2	1648.7935	1648.7913	1.33	(K)LESSLDELEDTLER(E)	/	/
1061-1071	2	1260.6464	1260.6430	2.68	(R)LTQEAVSDLER(N)	/	/
1072-1082	2	1271.6942	1271.6954	-0.94	(R)NLKELEVAAER(K)	/	/
1075-1082	1	916.4687	916.4734	-5.17	(K)ELEVAAER(K)	/	/
1094-1103	2	1235.6239	1235.6266	-2.22	(K)IEDEQALVYR(D)	/	/
1094-1106	3	1634.8153	1634.8134	1.24	(K)IEDEQALVYRDQR(Q)	/	/
1115-1126	3	1526.7078	1526.6969	7.13	(R)LEELEEDVEHER(Q)	/	/
1142-1149	1	932.4661	932.4684	-2.41	(R)ELSELGER(L)	/	/
1142-1165	3	2514.2856	2514.2683	6.96	(R)ELSELGERLDEAGGATAAQIEINK(K)	/	MC
1150-1165	2	1600.8147	1600.8177	-1.87	(R)LDEAGGATAAQIEINK(K)	/	/
1150-1166	3	1728.9216	1728.9127	5.17	(R)LDEAGGATAAQIEINKK(R)	/	/
1176-1193	2	2066.0642	2066.0625	0.77	(R)RDIEESNLQHEAALATLR(K)	/	/
1177-1193	3	1909.9591	1909.9614	-1.20	(R)DIEESNLQHEAALATLR(K)	/	/
1196-1212	2	2006.9252	2006.9124	6.39	(K)HNDVVAEMSEQVDYLNK(M)	M	/
1233-1240	1	850.4068	850.4087	-2.27	(K)ASMDSLAR(D)	/	/
1248-1262	2	1804.8914	1804.9011	-5.42	(K)TTKQLQHGYGELCAK(L)	C	MC
1251-1262	1	1474.7060	1474.7108	-3.16	(K)QLQHGYGELCAK(L)	C	/
1290-1303	2	1630.8145	1630.8031	6.99	(R)QLEEAENQVSQLSR(V)	/	/
1290-1303	2	1612.8048	1612.7925	7.60	(R)QLEEAENQVSQLSR(V)	/	NL
1304-1316	2	1502.8295	1502.8173	8.12	(R)VKLSLTNQLDDTR(K)	/	MC
1306-1317	2	1403.7526	1403.7489	2.65	(K)LSLTNQLDDTRK(M)	/	/
1335-1344	2	1208.6399	1208.6382	1.40	(R)NLEHDIQALR(D)	/	/
1335-1354	3	2339.1157	2339.1110	2.06	(R)NLEHDIQALRDQLDEESDAK(G)	/	/
1345-1359	2	1689.7920	1689.7926	-0.35	(R)DQLDEESDAKGDVLR(M)	/	MC
1355-1372	3	2004.0317	2004.0154	8.15	(K)GDVLRMLSAANAEALMWR(S)	/	MC
1355-1372	2	2019.9951	2020.0103	-7.51	(K)GDVLRMLSAANAEALMWR(S)	M	/
1360-1372	2	1463.7200	1463.7134	4.53	(R)MLSAANAEALMWR(S)	/	/
1360-1372	2	1495.7133	1495.7032	6.77	(R)MLSAANAEALMWR(S)	M	/
1360-1372	2	1479.7196	1479.7083	7.66	(R)MLSAANAEALMWR(S)	M	/
1360-1372	2	1445.6971	1445.7028	-3.88	(R)MLSAANAEALMWR(S)	/	NL
1397-1410	2	1660.8339	1660.8210	7.73	(R)LEEAEMQIESLNVR(N)	/	/
1397-1410	2	1676.8209	1676.8160	2.95	(R)LEEAEMQIESLNVR(N)	M	/
1419-1434	2	1755.8538	1755.8444	5.38	(K)MRAAAELDDLHASAER(A)	/	MC
1421-1434	2	1468.7008	1468.7028	-1.27	(R)AAAELDDLHASAER(A)	/	/
1421-1444	3	2409.2050	2409.2004	1.89	(R)AAAELDDLHASAERAQALASAAEK(K)	/	MC
1460-1472	2	1360.6692	1360.6590	7.45	(K)VDDLAAEVDASQK(E)	/	/
1476-1483	2	1053.4762	1053.4749	1.30	(R)NYSTEHR(L)	/	/
1484-1499	2	1828.9423	1828.9400	1.24	(R)LKAANDENIEQLDSIR(R)	/	MC
1484-1499	3	1810.9348	1810.9294	3.00	(R)LKAANDENIEQLDSIR(R)	/	NL

1486-1499	2	1587.7622	1587.7609	0.82	(K)AANDENIEQLDSIR(R)	/	/
1486-1500	3	1743.8630	1743.8620	0.57	(K)AANDENIEQLDSIRR(E)	/	/
1501-1510	2	1217.6207	1217.6120	7.10	(R)ENKNLSDEIR(D)	/	MC
1504-1521	3	2033.9587	2033.9556	1.55	(K)NLSDEIRDLMQDLGEGGR(A)	M	/
1504-1510	1	846.4316	846.4316	0.03	(K)NLSDEIR(D)	/	/
1511-1521	2	1190.5521	1190.5470	4.27	(R)DLMDQLGEGGR(A)	/	/
1561-1569	2	1073.5956	1073.5948	0.61	(R)TQLELSQVR(Q)	/	/
1561-1569	2	1055.5819	1055.5844	-2.35	(R)TQLELSQVR(Q)	/	NL
1576-1587	2	1523.6998	1523.6971	1.68	(R)VQKEEEFDNTR(K)	/	/
1593-1606	2	1491.7354	1491.7358	-0.43	(R)AIDSMQASLEVEAK(G)	/	/
1593-1606	2	1507.7386	1507.7308	5.22	(R)AIDSMQASLEVEAK(G)	M	/
1593-1606	1	1489.7291	1489.7203	5.93	(R)AIDSMQASLEVEAK(G)	M	NL
1609-1615	1	818.4552	818.4553	-0.10	(K)AEALRMK(K)	/	MC
1617-1634	3	2052.0706	2052.0608	4.74	(K)KLESDINELEIALDHANK(A)	/	MC
1618-1634	3	1923.9691	1923.9658	1.71	(K)LESDINELEIALDHANK(A)	/	/
1618-1641	4	2689.3557	2689.3428	4.85	(K)LESDINELEIALDHANKANSDLHK(H)	/	MC
1646-1656	2	1312.6469	1312.6492	-1.80	(R)VHDEIKDAETR(V)	/	/
1669-1677	2	1121.5717	1121.5698	1.70	(R)EQYGAERR(F)	/	/
1669-1676	1	965.4663	965.4687	-2.47	(R)EQYGAER(R)	/	/
1677-1689	3	1557.7731	1557.7769	-2.39	(R)RFNALHGELEESR(T)	/	MC
1678-1689	2	1401.6877	1401.6757	8.55	(R)FNALHGELEESR(T)	/	/
1678-1697	3	2344.1610	2344.1528	3.47	(R)FNALHGELEESRTLLEQSDR(G)	/	MC
1690-1697	2	961.4931	961.4949	-1.87	(R)TLLEQSDR(G)	/	/
1690-1699	2	1174.6060	1174.6176	-9.85	(R)TLLEQSDRGR(R)	/	MC
1690-1697	2	943.4769	943.4843	-7.88	(R)TLLEQSDR(G)	/	NL
1701-1727	3	3030.4617	3030.4512	3.48	(R)HAETELNDAREQINNFTNQNTALTASK(R)	/	/
1711-1727	2	1893.9220	1893.9300	-4.27	(R)EQINNFTNQNTALTASK(R)	/	/
1711-1728	3	2050.0425	2050.0312	5.51	(R)EQINNFTNQNTALTASKR(K)	/	/
1729-1749	3	2395.1362	2395.1367	-0.17	(R)KLEGEMSTLQADLEEMLNEAK(N)	M	/
1758-1770	3	1444.7530	1444.7577	-3.24	(K)AMLDAAARLADLR(S)	/	MC
1765-1781	3	1997.9497	1997.9523	-1.29	(R)LADELRSQEHAQSQEK(M)	/	MC
1765-1770	1	716.3921	716.3937	-2.28	(R)LADEL(R)	/	/
1797-1806	2	1094.5056	1094.5034	2.00	(R)LEESESAAMK(A)	/	/
1811-1818	1	877.4206	877.4196	1.11	(K)AVSNMEAR(I)	/	/
1864-1874	2	1345.7062	1345.7144	-6.10	(R)MQDLVDKLQK(I)	/	MC
1864-1874	2	1361.7119	1361.7094	1.90	(R)MQDLVDKLQK(I)	M	/
1864-1870	2	848.4202	848.4182	2.32	(R)MQDLVDK(L)	/	/
1880-1895	3	1797.9742	1797.9705	2.11	(K)RQIEEAEIEAALNLAK(F)	/	MC
1881-1895	2	1641.8712	1641.8694	1.10	(R)QIEEAEIEAALNLAK(F)	/	/
1898-1914	2	2019.9908	2019.9869	1.91	(R)KTQQUEESETVTVVHY(-)	/	MC
1899-1914	2	1891.9045	1891.8920	6.62	(K)TQQUEESETVTVVHY(-)	/	/

M: oxidation; C: carbamidomethyl; MC: missed cleavage; NL: Neutral Loss

Table S2. The identified tryptic peptide fragments matched for hemocyanin (G1AP69)

Position	z	Precursor MH ⁺ (Da)	Theoretical MH ⁺ (Da)	Error (ppm)	Sequence	Modifications	Peptide Type
39-48	2	1250.6656	1250.6640	1.25	(K)QHDVNFLHKK(I)	/	/
49-59	2	1293.6781	1293.6797	-1.27	(K)IYGDIRDSNLK(G)	/	/
62-79	3	1909.8248	1909.8200	2.58	(K)ADSFDPKANLSHYSDGGK(A)	/	/
244-258	3	1897.9701	1897.9820	-6.28	(R)KGENFFVHHQLTVR(F)	/	MC
292-313	3	2440.1287	2440.1165	5.00	(K)YGGQFSPRPDNVDFEDVDGVAR(I)	/	/
314-323	2	1213.7263	1213.7263	0.00	(R)IRDLLIVESR(I)	/	/
316-326	1	944.5391	944.5411	-2.14	(R)DLLIVESR(I)	/	/
324-336	3	1498.8150	1498.8125	1.68	(R)IRDAIAHGYIVDR(A)	/	/
326-336	2	1229.6349	1229.6273	6.17	(R)DAIAHGYIVDR(A)	/	/
337-347	2	1269.6050	1269.6003	3.59	(R)AGNHIDIMNER(G)	/	/
389-404	3	1844.9158	1844.9177	-1.05	(K)YDLPPGVLEHFETATR(D)	/	/
405-410	1	768.3707	768.3675	4.15	(R)DPSFFR(L)	/	/
414-420	2	930.4378	930.4390	-1.25	(K)YMDNIFK(E)	/	/
490-498	2	1030.5922	1030.5891	2.97	(K)IKIDVSNNK(G)	/	MC
492-507	3	1742.9456	1742.9395	3.48	(K)IDVSNNKGQEVLATVR(I)	/	MC
499-507	2	972.5490	972.5473	1.78	(K)GQEVLATVR(I)	/	/
540-549	2	1164.5923	1164.5910	1.23	(K)LPAGTHHFER(K)	/	/
550-570	3	2239.1436	2239.1494	-2.53	(R)KSSESAVTPDVPSFATLFK(T)	/	MC
571-594	3	2421.1853	2421.1892	-1.62	(K)TKEALAGADSGLTDFESATGIPNR(F)	/	/
573-594	2	2192.0527	2192.0466	2.84	(K)EALAGADSGLTDFESATGIPNR(F)	/	/
640-652	3	1599.7734	1599.7664	4.46	(K)YPDNRPHGYPLDR(K)	/	/
673-683	2	1358.6172	1358.6124	3.54	(K)VFNHGEYIQHD(-)	/	/

M: oxidation; C: carbamidomethyl; MC: missed cleavage; NL: Neutral Loss

Table S3. The identified tryptic peptide fragments matched for hemocyanin fragment (Q95V28)

Position	z	Precursor MH ⁺ (Da)	Theoretical MH ⁺ (Da)	Error (ppm)	Sequence	Modifications	Peptide Type
10-24	3	1897.9701	1897.9820	-6.28	(R)KGENFFVHHQLTVR(F)	/	MC
58-79	3	2439.1190	2439.1323	-5.57	(K)YGGQFPSPDNVNFEDVDGVAR(I)	/	/
80-89	2	1213.7263	1213.7263	0.00	(R)IRDLLIVESR(I)	/	/
82-89	1	944.5391	944.5411	-2.14	(R)DLLIVESR(I)	/	/
90-102	3	1498.8150	1498.8125	1.68	(R)IRDAIAHGYIVDR(A)	/	/
92-102	2	1229.6349	1229.6273	6.17	(R)DAIAHGYIVDR(A)	/	/
103-113	2	1269.6050	1269.6003	3.59	(R)AGNHIDIMNER(G)	/	/
155-170	3	1844.9158	1844.9177	-1.05	(K)YDLPPGVLEHFETATR(D)	/	/
171-176	1	768.3707	768.3675	4.15	(R)DPSFFR(L)	/	/
180-186	2	930.4378	930.4390	-1.25	(K)YMDNIFK(E)	/	/
256-264	2	1030.5922	1030.5891	2.97	(K)IKIDVSNNK(G)	/	MC
258-273	3	1742.9456	1742.9395	3.48	(K)IDVSNNKGQEVLATVR(I)	/	MC
265-273	2	972.5490	972.5473	1.78	(K)GQEVLATVR(I)	/	/
294-301	2	988.5286	988.5098	19.00	(R)WNAIELDK(F)	/	/
306-315	2	1164.5923	1164.5910	1.23	(K)LPAGTHHFER(K)	/	/
316-336	3	2239.1436	2239.1494	-2.53	(R)KSSESAVTPDVPSFATLFEK(T)	/	MC
337-360	3	2407.1853	2407.1736	4.83	(K)TKEALGGADSGLTDFESATGIPNR(F)	/	/
406-418	3	1599.7734	1599.7664	4.46	(K)YPDNRPHGYPLDR(K)	/	/

M: oxidation; C: carbamidomethyl; MC: missed cleavage; NL: Neutral Loss

Table S4. The identified tryptic peptide fragments matched for Phosphopyruvate hydratase.

Position	z	Precursor MH ⁺ (Da)	Theoretical MH ⁺ (Da)	Error (ppm)	Sequence	Modifications	Peptide Type
10-15	1	738.3771	738.3781	1.18	(R)TIFDSR(G)		
16-28	2	1472.7408	1472.7380	1.18	(R)GNPTVEVDLYTHK(G)		
33-50	3	1782.8789	1782.8802	-0.79	(R)AAVPSGASTGVHEALEMR(D)		
33-50	3	1798.8750	1798.8752	-0.12	(R)AAVPSGASTGVHEALEMR(D)	M	NL
33-50	3	1780.8607	1780.8646	-2.21	(R)AAVPSGASTGVHEALEMR(D)	M	
65-79	2	1594.9165	1594.9163	-1.01	(K)AVNNVNSIIAPEIK(S)		
84-96	2	1704.7028	1704.7028	-1.43	(K)VTQQKECDDFMCK(L)	C, M	
84-96	2	1688.7100	1688.7078	-0.14	(K)VTQQKECDDFMCK(L)	C	
89-96	2	1104.3807	1104.3796	1.28	(K)ECDDFMCK(L)	C	
89-96	2	1086.3676	1086.3690	-0.99	(K)ECDDFMCK(L)	C	NL
97-105	2	1019.5152	1019.5116	3.53	(K)LDGTENKSR(L)		MC
121-132	2	1230.6850	1230.6841	0.69	(K)AGAAELGIPLYR(H)		
133-162	3	3102.6086	3102.6118	-1.05	(R)HIANLANYSVDILPVPAFNVINGGSHAGNK(L)		
184-193	2	1200.5815	1200.5830	-1.29	(R)MGSEVYHHLK(A)		
198-221	3	2433.2217	2433.2158	2.46	(K)GRFGLDATAVGDEGGFAPNILNNK(D)		MC
200-221	3	2220.0952	2220.0930	-1.92	(R)FGLDATAVGDEGGFAPNILNNK(D)		
222-233	2	1359.7357	1359.7366	-1.13	(K)DALTIQESIEK(A)		
234-253	3	2166.0469	2166.0422	2.11	(K)AGYTGKIEIGMDVAASEFYK(G)	M	
234-239	1	596.3054	596.3039	2.52	(K)AGYTGK(I)		
240-253	2	1588.7585	1588.7563	0.31	(K)IEIGMDVAASEFYK(G)	M	
254-263	2	1213.5731	1213.5735	-0.44	(K)GENIYDLDFK(T)		
264-279	3	1717.8500	1717.8464	0.63	(K)TANNDGSQKITGDQLR(D)		MC
273-279	1	802.4387	802.4417	-1.33	(K)ITGDQLR(D)		
280-287	2	1123.4260	1123.4257	0.42	(R)DMYMEFCK(E)	C	
280-287	2	1139.4235	1139.4207	2.65	(R)DMYMEFCK(E)	M, C	
309-328	2	2118.0823	2118.0747	0.93	(K)MTSATNIQIVGDDLTVTNPK(R)		
309-328	2	2134.0786	2134.0696	1.51	(K)MTSATNIQIVGDDLTVTNPK(R)	M	
309-329	3	2274.1814	2274.1758	2.50	(K)MTSATNIQIVGDDLTVTNPKR(I)		
309-329	3	2290.1743	2290.1707	1.56	(K)MTSATNIQIVGDDLTVTNPKR(I)	M	
309-329	3	2272.1675	2272.1602	3.23	(K)MTSATNIQIVGDDLTVTNPKR(I)	M	NL
337-345	2	1119.5996	1119.6014	-1.39	(K)KACNCLLLK(V)	C	MC
338-345	2	991.5075	991.5064	1.66	(K)ACNCLLLK(V)	C	
346-363	3	1895.0281	1895.0233	2.55	(K)VNQIGSVTESIDAHLLAK(K)		
409-414	2	806.4504	806.4519	0.58	(K)YNQILR(I)		
415-424	2	1059.5280	1059.5317	-3.21	(R)IEEELGGNAK(F)		

M: oxidation; C: carbamidomethyl; MC: missed cleavage; N: no missed cleavage; NL: Neutral Loss

Table S5. The identified tryptic peptide fragments matched for arginine kinase

Position	z	Precursor MH ⁺ (Da)	Theoretical MH ⁺ (Da)	Error (ppm)	Sequence	Modifications	Peptide Type
10-51	1	664.3640	664.3665	-1.59	(K)LEAGFK(K)	/	/
16-24	2	1035.5164	1035.5140	3.11	(K)KLEAATDCK(S)	C	MC
34-42	2	1077.5934	1077.5939	0.24	(K)AVFDQLKEK(K)	/	/
34-40	1	820.4571	820.4563	2.81	(K)AVFDQLK(E)	/	/
103-118	2	1752.8185	1752.8075	4.56	(K)DFGVDNTFVNVDPEGK(Y)	/	/
119-124	1	738.4143	738.4145	1.77	(K)YVISTR(V)	/	/
130-146	2	2034.8955	2034.8936	-1.77	(R)SMEGYPFNPCLTEAQYK(E)	C	/
130-146	2	2050.8945	2050.8884	0.21	(R)SMEGYPFNPCLTEAQYK(E)	M,C	/
130-151	3	2623.1600	2623.1514	3.22	(R)SMEGYPFNPCLTEAQYKEMEAK(V)	C	/
130-151	3	2639.1570	2639.1462	4.07	(R)SMEGYPFNPCLTEAQYKEMEAK(V)	C, M	/
130-151	3	2655.1530	2655.1410	4.46	(R)SMEGYPFNPCLTEAQYKEMEAK(V)	M, C	/
147-164	2	1937.9778	1937.9736	-0.19	(K)EMEAKVSSTLSSLEGELK(G)	/	MC
152-175	3	2564.2760	2564.2800	-1.56	(K)VSSTLSSLEGELKGTYYPLTGMSK(E)	M	/
152-175	3	2548.2961	2548.2852	4.32	(K)VSSTLSSLEGELKGTYYPLTGMSK(E)	/	MC
152-164	2	1349.7217	1349.7160	4.06	(K)VSSTLSSLEGELK(G)	/	/
165-175	2	1233.5839	1233.5820	1.69	(K)GTYYPLTGMSK(E)	M	/
165-175	2	1217.5912	1217.5872	3.58	(K)GTYYPLTGMSK(E)	/	/
165-180	3	1829.9193	1829.9102	4.97	(K)GTYYPLTGMSKEVQQK(L)	/	/
165-175	2	1199.5784	1199.5765	1.83	(K)GTYYPLTGMSK(E)	/	NL
165-175	2	1215.5726	1215.5714	1.17	(K)GTYYPLTGMSK(E)	M	NL
181-189	2	1147.6145	1147.6146	0.34	(K)LIDDHFLFK(E)	/	/
181-193	3	1604.8174	1604.8066	6.65	(K)LIDDHFLFKEGDR(F)	/	/
190-202	2	1507.7102	1507.7072	1.24	(K)EGDRFLQAANACR(Y)	C	MC
190-202	3	1489.6956	1489.6965	-0.61	(K)EGDRFLQAANACR(Y)	C	NL
194-202	2	1050.5182	1050.5150	3.87	(R)FLQAANACR(Y)	C	/
194-202	2	1033.4920	1033.4884	4.23	(R)FLQAANACR(Y)	C	NL
203-208	1	749.3714	749.3729	-0.07	(R)YWPAGR(G)	/	/
209-216	2	960.4530	960.4534	0.72	(R)GIYHNDNK(T)	/	/
217-229	3	1657.8380	1657.8333	2.85	(K)TFLVWVNEEDHLR(I)	/	/
230-244	2	1683.8232	1683.8193	0.88	(R)IISMQMGGDLGQVFR(R)	M	/
230-244	2	1667.8250	1667.8243	-1.08	(R)IISMQMGGDLGQVFR(R)	M	/
230-244	2	1651.8333	1651.8295	0.98	(R)IISMQMGGDLGQVFR(R)	/	/
230-245	3	1839.9302	1839.9204	5.34	(R)IISMQMGGDLGQVFRR(L)	M	/
230-245	3	1823.9319	1823.9255	3.53	(R)IISMQMGGDLGQVFRR(L)	M	/
230-245	3	1807.9340	1807.9305	1.90	(R)IISMQMGGDLGQVFRR(L)	/	/
230-245	3	1805.9167	1805.9149	0.99	(R)IISMQMGGDLGQVFRR(L)	M	NL
245-255	2	1259.6980	1259.6954	2.12	(R)RLTSAVNEIEK(R)	/	MC
246-255	2	1103.5938	1103.5944	0.08	(R)LTSAVNEIEK(R)	/	/
246-256	2	1259.6985	1259.6954	2.52	(R)LTSAVNEIEKR(I)	/	/
246-255	2	1085.5860	1085.5837	2.86	(R)LTSAVNEIEK(R)	/	NL
246-256	3	1241.6860	1241.6848	0.95	(R)LTSAVNEIEKR(I)	/	NL

257-264	2	1008.5018	1008.5010	1.74	(R)IPFSHHDR(L)	/	/
265-280	2	1796.9373	1796.9364	-1.41	(R)LGFLTFCPTNLGTTVR(A)	C	/
281-289	2	992.6298	992.6251	5.70	(R)ASVHIKLPK(L)	/	MC
295-303	2	1002.5446	1002.5466	-1.04	(R)EKLEEVAGK(Y)	/	MC
295-303	2	984.5337	984.5360	-1.35	(R)EKLEEVAGK(Y)	/	NL
297-309	3	1518.8347	1518.8275	4.76	(K)LEEVAGKYNLQVR(G)	/	MC
297-303	1	745.4111	745.4090	4.74	(K)LEEVAGK(Y)	/	/
310-328	3	2033.9537	2033.9523	0.69	(R)GTRGEHTAEGGIYDISNK(R)	/	MC
310-328	4	2015.9672	2015.9417	12.64	(R)GTRGEHTAEGGIYDISNK(R)	/	NL
313-329	3	1875.8893	1875.8830	3.28	(R)GEHTAEGGIYDISNKR(R)	/	/
313-328	2	1719.7924	1719.7820	4.38	(R)GEHTAEGGIYDISNK(R)	/	/
330-340	2	1279.6850	1279.6827	1.76	(R)RMGLTEFQAVK(E)	/	MC
330-340	2	1295.6788	1295.6776	0.83	(R)RMGLTEFQAVK(E)	M	
330-340	3	1261.6737	1261.6722	1.23	(R)RMGLTEFQAVK(E)	/	NL
330-340	3	1277.6672	1277.6671	0.11	(R)RMGLTEFQAVK(E)	M	NL
331-340	2	1123.5828	1123.5815	1.59	(R)MGLTEFQAVK(E)	/	/
331-340	2	1105.5714	1105.5710	0.92	(R)MGLTEFQAVK(E)	/	NL
341-351	2	1288.6823	1288.6816	0.40	(K)EMQDGILELIK(M)	/	/
341-351	2	1304.6753	1304.6766	-1.12	(K)EMQDGILELIK(M)	M	/
341-354	3	1692.8604	1692.8547	3.39	(K)EMQDGILELIKMEK(E)	M	/
341-351	2	1270.6718	1270.6711	0.52	(K)EMQDGILELIK(M)	/	NL
341-351	2	1286.6650	1286.6661	-0.79	(K)EMQDGILELIK(M)	M	NL
341-354	3	1674.8446	1674.8441	0.30	(K)EMQDGILELIKMEK(E)	M	NL

M: oxidation; C: carbamidomethyl; MC: missed cleavage; NL: Neutral Loss

Table S6. The identified tryptic peptide fragments matched for tropomyosin

Position	z	Precursor MH ⁺ (Da)	Theoretical MH ⁺ (Da)	Error (ppm)	Sequence	Modifications	Peptide Type
13-21	2	1091.5586	1091.5150	9.96	(K)LEKDNAMDR(A)	/	/
16-30	2	1748.8044	1748.7867	6.12	(K)DNAMDRADTLEQQNK(E)	/	MC
16-30	3	1764.7957	1764.7817	7.93	(K)DNAMDRADTLEQQNK(E)	M	/
22-35	3	1630.7764	1630.7780	-0.95	(R)ADTLEQQNKEANNR(A)	/	/
36-48	3	1540.7595	1540.7603	-0.44	(R)AEKSEEEVHNLQK(R)	/	MC
39-49	2	1368.6853	1368.6866	-0.96	(K)SEEEVHNLQKR(M)	/	MC
39-48	2	1212.5876	1212.5854	1.73	(K)SEEEVHNLQK(R)	/	/
49-66	3	2203.1216	2203.1023	4.76	(K)RMQQLENDLDQVQESLLK(A)	M	/
49-66	3	2187.1287	2187.1074	5.69	(K)RMQQLENDLDQVQESLLK(A)	/	MC
50-66	2	2047.0030	2047.0012	0.88	(R)MQQLENDLDQVQESLLK(A)	M	/
50-66	3	2031.0037	2031.0063	-1.28	(R)MQQLENDLDQVQESLLK(A)	/	/
67-76	2	1157.6528	1157.6525	0.29	(K)ANIQLVEKDK(A)	/	/
67-74	2	914.5356	914.5306	5.51	(K)ANIQLVEK(D)	/	/
75-90	2	1657.8658	1657.8503	4.30	(K)DKALSNAEGEVAALNR(R)	/	MC
77-91	3	1570.8381	1570.8296	5.42	(K)ALSNAEGEVAALNRR(I)	/	/
77-90	2	1414.7426	1414.7285	6.98	(K)ALSNAEGEVAALNR(R)	/	/
91-101	2	1413.7885	1413.7697	3.28	(R)RIQLLEEDLER(S)	/	MC
92-101	2	1257.6298	1257.6685	-3.86	(R)IQLLEEDLER(S)	/	/
92-105	2	1758.9037	1758.8868	6.59	(R)IQLLEEDLERSEER(L)	/	MC
106-112	2	748.4264	748.4199	4.63	(R)LNTATTK(L)	/	/
113-125	2	1376.6312	1376.6288	1.72	(K)LAEASQAADSEER(M)	/	/
113-127	3	1679.7770	1679.7653	6.95	(K)LAEASQAADSEERM(R)	M	/
134-149	2	1877.9190	1877.8909	14.89	(R)SLSDDEERMDALENQLK(E)	/	MC
141-152	2	1417.7210	1417.7104	7.48	(R)MDALENQLKEAR(F)	/	/
141-152	2	1061.5400	1061.5295	6.92	(R)MDALENQLK(E)	/	/
153-160	2	950.4678	950.4578	6.53	(R)FLAEEADR(K)	/	/
153-161	2	1078.5406	1078.5529	-1.17	(R)FLAEEADRK(Y)	/	MC
168-178	2	1274.6962	1274.6772	4.83	(R)KLAMVEADLER(A)	/	MC
168-178	2	1290.6793	1290.6722	5.50	(R)KLAMVEADLER(A)	M	/
169-178	2	1146.5936	1146.5823	4.82	(K)LAMVEADLER(A)	/	/
169-182	2	1631.8210	1631.8057	5.41	(K)LAMVEADLERAEER(A)	/	MC
169-178	2	1162.5709	1162.5771	-5.45	(K)LAMVEADLER(A)	M	/
169-182	2	1647.8114	1647.8007	6.53	(K)LAMVEADLERAEER(A)	M	/
183-198	3	1831.9333	1831.9283	2.75	(R)AETGESKIVELEEELR(V)	/	MC
183-189	2	721.3444	721.3363	5.27	(R)AETGESK(I)	/	/
190-205	2	1854.0060	1854.0331	-4.62	(K)IVELEEELRVVGNLK(S)	/	MC
190-198	1	1129.6104	1129.6099	0.42	(K)IVELEEELR(V)	/	/
206-217	2	1389.7057	1389.6968	6.37	(K)SLEVSEKANQR(E)	/	MC
206-213	1	920.4559	920.4571	-1.32	(K)SLEVSEK(A)	/	/
214-226	3	1606.8176	1606.8184	-0.47	(K)ANQREEAYKEQIK(T)	/	/
239-244	1	722.3467	722.3468	-0.11	(R)AEFAER(S)	/	/

252-264	2	1587.7994	1587.7861	5.40	(K)EVDRLEDELVNEK(E)	/	MC
256-266	2	1345.6971	1345.6846	4.33	(R)LEDELVNEKEK(Y)	/	/
256-264	2	1088.5363	1088.5470	-5.92	(R)LEDELVNEK(E)	/	/
267-284	3	2095.9990	2095.9707	3.59	(K)YKSITDELDQTFSELSGY(-)	/	MC

M: oxidation; C: carbamidomethyl; MC: missed cleavage; NL: Neutral Loss

Table S7. The identified tryptic peptide fragments matched for Scrcoplasmic calcium binding protein

Position	z	Precursor MH ⁺ (Da)	Theoretical MH ⁺ (Da)	Error (ppm)	Sequence	Modifications	Peptide Type
15-27	2	1623.7046	1623.6995	3.12	(R)YMYDIDNNGFLDK(N)	M	
15-27	2	1607.7095	1607.7046	3.03	(R)YMYDIDNNGFLDK(N)		
37-43	1	802.4416	802.4417	-0.18	(R)NTLIEGR(G)		
44-56	2	1414.6268	1414.6233	2.44	(R)GEFSADAYANNQK(I)		
60-73	3	1676.8156	1676.8279	-7.31	(R)NLWNEIAELADFNK(D)		
74-83	2	1138.5331	1138.5262	6.01	(K)DGEVTVDEFK(Q)		
74-88	2	1692.8372	1692.8439	-4.02	(K)DGEVTVDEFKQAVQK(H)		MC
95-103	2	1015.4901	1015.4884	1.71	(K)YGEFPGAFK(V)		
95-103	2	997.4725	997.4778	-5.31	(K)YGEFPGAFK(V)		NL
104-111	2	966.5405	966.5407	-0.24	(K)VFIANQFK(A)		
104-120	3	1835.9713	1835.9650	3.42	(K)VFIANQFKAIDVNGDGK(V)		MC
112-127	2	1720.8410	1720.8501	-5.26	(K)AIDVNGDGKVGLEDEYR(L)		MC
121-127	2	851.4256	851.4258	-0.19	(K)VGLEDEYR(L)		
128-138	1	777.3908	777.3924	-2.04	(R)LDCITR(S)	C	
134-148	2	1713.8344	1713.8330	0.75	(R)SAFAEVKEIDEAYNK(L)		
134-140	1	751.4032	751.3985	6.28	(R)SAFAEVK(E)		
134-148	3	1695.8315	1695.8224	5.40	(R)SAFAEVKEIDEAYNK(L)		NL
141-148	1	981.4504	981.4524	-2.00	(K)EIDEAYNK(L)		
141-148	2	963.4430	963.4418	1.24	(K)EIDEAYNK(L)		NL
156-164	2	944.5553	944.5524	3.12	(R)KAGGLTLER(Y)		MC
157-164	1	816.4580	816.4574	0.74	(K)AGGLTLER(Y)		
157-164	2	798.4418	798.4468	-6.30	(K)AGGLTLER(Y)		NL

M: oxidation; C: carbamidomethyl; MC: missed cleavage; NL: Neutral Loss

Table S8. The identified tryptic peptide fragments matched for Glyceraldehyde-3-phosphate dehydrogenase

Position	z	Precursor MH ⁺ (Da)	Theoretical MH ⁺ (Da)	Error (ppm)	Sequence	Modifications	Peptide Type
1-9	2	984.4971	984.4932	4.00	(-)GCLVVDGHK(I)	C	/
10-25	3	1932.9937	1932.9888	2.54	(K)ITVFNEMKPENIPWSK(A)	/	/
26-43	2	1914.9713	1914.9695	0.94	(K)AGAEYIVESTGVFTTIEK(A)	/	/
26-43	3	1896.9648	1896.9589	3.09	(K)AGAEYIVESTGVFTTIEK(A)	/	NL
54-75	3	2364.1804	2364.1760	1.79	(K)KVIISAPSADAPMFVCGVNMEK(Y)	C	MC
82-98	2	1791.8698	1791.8729	-1.70	(K)VVSNASCTTNCLAPVAK(V)	C	/

M: oxidation; C: carbamidomethyl; MC: missed cleavage; NL: Neutral Loss

Table S9. The predicted epitopes results of Phosphopyruvate hydratase by BepiPred 2.0

Position	Amino Acid	Exposed /Buried	Relative Surface Accessibility	Helix Probability	Sheet Probability	Coil Probability	Epitope Probability
1	M	E	0.476	0.005	0.015	0.979	0.228
2	S	E	0.513	0.003	0.718	0.279	0.263
3	I	B	0.081	0.001	0.900	0.099	0.314
4	T	E	0.387	0.001	0.900	0.099	0.351
5	K	E	0.447	0.006	0.962	0.032	0.397
6	V	B	0.059	0.006	0.962	0.032	0.403
7	F	E	0.308	0.011	0.918	0.071	0.388
8	A	B	0.071	0.018	0.846	0.136	0.391
9	R	B	0.232	0.018	0.846	0.136	0.401
10	T	B	0.213	0.011	0.918	0.071	0.415
11	I	B	0.071	0.018	0.846	0.136	0.453
12	F	B	0.109	0.021	0.756	0.223	0.461
13	D	B	0.210	0.022	0.359	0.619	0.482
14	S	B	0.291	0.018	0.047	0.935	0.489
15	R	E	0.410	0.018	0.019	0.964	0.480
16	G	E	0.311	0.005	0.015	0.979	0.473
17	N	E	0.375	0.005	0.045	0.951	0.475
18	P	B	0.172	0.005	0.336	0.660	0.436
19	T	B	0.112	0.002	0.816	0.182	0.421
20	V	B	0.045	0.001	0.959	0.040	0.377
21	E	B	0.190	0.001	0.959	0.040	0.367
22	V	B	0.027	0.001	0.959	0.040	0.342
23	D	B	0.150	0	0.983	0.017	0.338
24	L	B	0.026	0	0.983	0.017	0.361
25	Y	B	0.141	0	0.983	0.017	0.393
26	T	B	0.031	0.001	0.959	0.040	0.402
27	H	E	0.408	0.004	0.514	0.481	0.440
28	K	E	0.455	0.018	0.088	0.893	0.450
29	G	E	0.371	0.020	0.205	0.775	0.457
30	L	B	0.252	0.004	0.420	0.576	0.449
31	F	B	0.190	0.004	0.616	0.381	0.442
32	R	E	0.354	0.023	0.655	0.322	0.440
33	A	B	0.094	0.021	0.756	0.223	0.426
34	A	B	0.171	0.023	0.655	0.322	0.425
35	V	B	0.072	0.022	0.552	0.426	0.429
36	P	B	0.117	0.021	0.279	0.699	0.431
37	S	B	0.189	0.021	0.279	0.699	0.444
38	G	B	0.260	0.066	0.296	0.638	0.475
39	A	B	0.249	0.064	0.216	0.721	0.491
40	S	B	0.240	0.064	0.216	0.721	0.520

41	T	E	0.434	0.056	0.142	0.802	0.531
42	G	E	0.352	0.056	0.142	0.802	0.523
43	V	E	0.305	0.064	0.216	0.721	0.523
44	H	B	0.295	0.074	0.484	0.442	0.522
45	E	B	0.142	0.087	0.683	0.230	0.513
46	A	B	0.078	0.087	0.683	0.230	0.501
47	L	B	0.182	0.087	0.683	0.230	0.496
48	E	B	0.229	0.087	0.683	0.230	0.503
49	M	B	0.152	0.069	0.386	0.545	0.514
50	R	E	0.288	0.064	0.216	0.721	0.528
51	D	E	0.408	0.052	0.084	0.864	0.554
52	G	E	0.578	0.018	0.047	0.935	0.570
53	D	E	0.627	0.018	0.019	0.964	0.582
54	K	E	0.568	0.053	0.043	0.903	0.605
55	S	E	0.504	0.053	0.043	0.903	0.604
56	K	E	0.338	0.053	0.043	0.903	0.603
57	Y	B	0.162	0.113	0.043	0.844	0.594
58	H	E	0.368	0.058	0.017	0.925	0.572
59	G	E	0.455	0.115	0.016	0.868	0.562
60	K	E	0.470	0.181	0.016	0.803	0.548
61	S	B	0.236	0.257	0.016	0.727	0.506
62	V	B	0.026	0.802	0.014	0.185	0.477
63	F	E	0.347	0.938	0.007	0.055	0.467
64	K	E	0.438	0.970	0.001	0.030	0.458
65	A	B	0.021	0.970	0.001	0.030	0.436
66	V	B	0.036	0.970	0.001	0.030	0.436
67	N	E	0.541	0.970	0.001	0.030	0.445
68	N	E	0.306	0.970	0.001	0.030	0.444
69	V	B	0.025	0.970	0.001	0.030	0.420
70	N	B	0.236	0.970	0.001	0.030	0.429
71	S	E	0.564	0.970	0.001	0.030	0.438
72	I	E	0.283	0.970	0.001	0.030	0.415
73	I	B	0.032	0.923	0.002	0.076	0.397
74	A	B	0.090	0.923	0.002	0.076	0.417
75	P	E	0.472	0.970	0.001	0.030	0.420
76	E	E	0.233	0.970	0.001	0.030	0.423
77	I	B	0.037	0.923	0.002	0.076	0.418
78	I	B	0.186	0.879	0.010	0.111	0.435
79	K	E	0.584	0.717	0.014	0.269	0.459
80	S	E	0.522	0.176	0.004	0.820	0.465
81	G	E	0.751	0.016	0.005	0.979	0.480
82	L	B	0.112	0.018	0.019	0.964	0.512
83	K	E	0.428	0.018	0.019	0.964	0.511
84	V	B	0.061	0.268	0.043	0.689	0.518

85	T	E	0.439	0.268	0.043	0.689	0.520
86	Q	E	0.376	0.268	0.043	0.689	0.509
87	Q	B	0.091	0.923	0.002	0.076	0.511
88	K	E	0.503	0.970	0.001	0.030	0.506
89	E	E	0.420	0.970	0.001	0.030	0.510
90	C	B	0.023	0.970	0.001	0.030	0.486
91	D	B	0.093	0.970	0.001	0.030	0.482
92	D	E	0.521	0.970	0.001	0.030	0.485
93	F	B	0.124	0.970	0.001	0.030	0.458
94	M	B	0.024	0.923	0.002	0.076	0.465
95	C	B	0.234	0.923	0.002	0.076	0.482
96	K	E	0.547	0.782	0.003	0.216	0.494
97	L	B	0.074	0.321	0.003	0.675	0.498
98	D	E	0.367	0.109	0.005	0.886	0.503
99	G	E	0.585	0.016	0.005	0.979	0.536
100	T	B	0.242	0.016	0.005	0.979	0.545
101	E	E	0.776	0.053	0.005	0.942	0.549
102	N	E	0.473	0.109	0.005	0.886	0.568
103	K	B	0.270	0.502	0.002	0.495	0.545
104	S	E	0.390	0.600	0.003	0.397	0.514
105	R	E	0.423	0.782	0.003	0.216	0.482
106	L	B	0.178	0.802	0.014	0.185	0.451
107	G	B	0.143	0.858	0.002	0.139	0.415
108	A	B	0.053	0.923	0.002	0.076	0.369
109	N	B	0.172	0.923	0.002	0.076	0.323
110	A	B	0.081	0.970	0.001	0.030	0.279
111	I	B	0.031	0.970	0.001	0.030	0.247
112	L	B	0.051	0.970	0.001	0.030	0.229
113	G	B	0.036	0.970	0.001	0.030	0.225
114	V	B	0.029	0.970	0.001	0.030	0.213
115	S	B	0.036	0.970	0.001	0.030	0.200
116	L	B	0.066	0.988	0	0.012	0.218
117	A	B	0.022	0.988	0	0.012	0.229
118	I	B	0.030	0.988	0	0.012	0.244
119	C	B	0.031	0.970	0.001	0.030	0.256
120	K	B	0.206	0.970	0.001	0.030	0.278
121	A	B	0.053	0.970	0.001	0.030	0.311
122	G	B	0.064	0.970	0.001	0.030	0.346
123	A	B	0.038	0.970	0.001	0.030	0.379
124	A	E	0.499	0.923	0.002	0.076	0.407
125	E	E	0.541	0.858	0.002	0.139	0.422
126	L	E	0.417	0.406	0.004	0.590	0.435
127	G	E	0.782	0.016	0.005	0.979	0.445
128	I	B	0.103	0.005	0.015	0.979	0.473

129	P	E	0.297	0.058	0.017	0.925	0.482
130	L	B	0.067	0.502	0.102	0.396	0.454
131	Y	B	0.093	0.605	0.105	0.290	0.440
132	R	E	0.358	0.751	0.050	0.199	0.443
133	H	B	0.159	0.779	0.100	0.120	0.457
134	I	B	0.045	0.660	0.049	0.291	0.469
135	A	B	0.169	0.455	0.046	0.498	0.493
136	N	E	0.461	0.354	0.048	0.598	0.514
137	L	B	0.178	0.354	0.048	0.598	0.524
138	A	E	0.358	0.115	0.016	0.868	0.531
139	N	E	0.498	0.018	0.019	0.964	0.552
140	Y	E	0.534	0.018	0.019	0.964	0.562
141	S	E	0.650	0.018	0.047	0.935	0.546
142	D	E	0.596	0.019	0.141	0.840	0.530
143	V	B	0.224	0.022	0.359	0.619	0.502
144	I	E	0.261	0.022	0.359	0.619	0.478
145	L	B	0.117	0.022	0.359	0.619	0.452
146	P	B	0.099	0.064	0.216	0.721	0.413
147	V	B	0.103	0.118	0.150	0.732	0.378
148	P	B	0.049	0.118	0.150	0.732	0.354
149	A	B	0.038	0.148	0.418	0.435	0.338
150	F	B	0.035	0.087	0.683	0.230	0.336
151	N	B	0.029	0.087	0.683	0.230	0.332
152	V	B	0.024	0.079	0.592	0.329	0.350
153	I	B	0.047	0.069	0.386	0.545	0.368
154	N	B	0.140	0.020	0.205	0.775	0.390
155	G	B	0.103	0.018	0.088	0.893	0.419
156	G	B	0.181	0.018	0.088	0.893	0.453
157	S	E	0.388	0.019	0.141	0.840	0.491
158	H	B	0.233	0.018	0.088	0.893	0.517
159	A	B	0.252	0.018	0.047	0.935	0.537
160	G	E	0.658	0.018	0.019	0.964	0.551
161	N	E	0.378	0.018	0.019	0.964	0.533
162	K	E	0.567	0.018	0.019	0.964	0.519
163	L	B	0.181	0.018	0.047	0.935	0.505
164	A	E	0.431	0.004	0.085	0.910	0.471
165	M	B	0.137	0.005	0.336	0.660	0.430
166	Q	B	0.153	0.004	0.616	0.381	0.390
167	E	B	0.084	0.001	0.900	0.099	0.362
168	F	B	0.029	0.001	0.959	0.040	0.331
169	M	B	0.026	0.001	0.959	0.040	0.334
170	I	B	0.015	0.001	0.959	0.040	0.338
171	L	B	0.028	0.001	0.900	0.099	0.346
172	P	B	0.030	0.004	0.514	0.481	0.364

173	T	B	0.179	0.004	0.085	0.910	0.402
174	G	E	0.395	0.005	0.015	0.979	0.419
175	A	B	0.222	0.018	0.019	0.964	0.449
176	T	E	0.466	0.058	0.017	0.925	0.462
177	S	E	0.241	0.053	0.005	0.942	0.469
178	F	B	0.045	0.970	0.001	0.030	0.454
179	T	E	0.395	0.970	0.001	0.030	0.462
180	E	E	0.204	0.988	0	0.012	0.447
181	A	B	0.021	0.988	0	0.012	0.410
182	M	B	0.032	0.988	0	0.012	0.377
183	R	E	0.297	0.988	0	0.012	0.378
184	M	B	0.040	0.988	0	0.012	0.357
185	G	B	0.019	0.988	0	0.012	0.344
186	S	B	0.042	0.988	0	0.012	0.362
187	E	B	0.179	0.988	0	0.012	0.367
188	V	B	0.021	0.988	0	0.012	0.332
189	Y	B	0.070	0.988	0	0.012	0.346
190	H	E	0.298	0.970	0.001	0.030	0.369
191	H	B	0.165	0.988	0	0.012	0.386
192	L	B	0.025	0.988	0	0.012	0.381
193	K	B	0.257	0.970	0.001	0.030	0.415
194	A	E	0.517	0.970	0.001	0.030	0.429
195	V	B	0.162	0.970	0.001	0.030	0.440
196	I	B	0.035	0.970	0.001	0.030	0.454
197	K	E	0.504	0.923	0.002	0.076	0.501
198	G	E	0.693	0.879	0.010	0.111	0.505
199	R	E	0.454	0.600	0.003	0.397	0.528
200	F	E	0.364	0.339	0.016	0.645	0.532
201	G	E	0.648	0.053	0.005	0.942	0.549
202	L	E	0.581	0.018	0.019	0.964	0.535
203	D	E	0.433	0.018	0.088	0.893	0.534
204	A	B	0.165	0.019	0.141	0.840	0.526
205	T	B	0.242	0.020	0.205	0.775	0.527
206	A	B	0.164	0.022	0.359	0.619	0.521
207	V	B	0.085	0.021	0.279	0.699	0.518
208	G	B	0.08	0.020	0.205	0.775	0.495
209	D	B	0.206	0.018	0.088	0.893	0.485
210	E	B	0.232	0.018	0.088	0.893	0.476
211	G	B	0.196	0.019	0.141	0.840	0.470
212	G	B	0.104	0.022	0.359	0.619	0.482
213	F	B	0.096	0.022	0.552	0.426	0.477
214	A	B	0.050	0.005	0.336	0.660	0.484
215	P	B	0.091	0.004	0.197	0.799	0.488
216	N	E	0.453	0.004	0.085	0.910	0.490

217	I	B	0.094	0.005	0.045	0.951	0.501
218	L	E	0.618	0.005	0.015	0.979	0.510
219	N	E	0.432	0.018	0.019	0.964	0.516
220	N	B	0.099	0.782	0.003	0.216	0.512
221	K	E	0.547	0.970	0.001	0.030	0.505
222	D	E	0.423	0.970	0.001	0.030	0.506
223	A	B	0.030	0.970	0.001	0.030	0.464
224	L	B	0.039	0.970	0.001	0.030	0.444
225	T	E	0.446	0.970	0.001	0.030	0.433
226	L	B	0.096	0.970	0.001	0.030	0.419
227	I	B	0.014	0.988	0	0.012	0.403
228	Q	B	0.173	0.988	0	0.012	0.411
229	E	E	0.416	0.988	0	0.012	0.432
230	S	B	0.020	0.988	0	0.012	0.424
231	I	B	0.030	0.970	0.001	0.030	0.432
232	E	E	0.509	0.970	0.001	0.030	0.465
232	K	E	0.488	0.858	0.002	0.139	0.479
234	A	B	0.057	0.502	0.002	0.495	0.483
235	G	E	0.335	0.176	0.004	0.820	0.494
236	Y	B	0.223	0.115	0.016	0.868	0.503
237	T	E	0.441	0.053	0.043	0.903	0.501
238	G	E	0.604	0.018	0.047	0.935	0.464
239	K	E	0.457	0.018	0.047	0.935	0.445
240	I	B	0.050	0.023	0.655	0.322	0.405
241	E	B	0.188	0.011	0.918	0.071	0.374
242	I	B	0.052	0.011	0.918	0.071	0.343
243	G	B	0.029	0.011	0.918	0.071	0.326
244	M	B	0.048	0.018	0.846	0.136	0.300
245	D	B	0.066	0.021	0.756	0.223	0.300
246	V	B	0.048	0.079	0.592	0.329	0.301
247	A	B	0.046	0.148	0.418	0.435	0.323
248	A	B	0.040	0.135	0.317	0.548	0.357
249	S	B	0.097	0.231	0.330	0.439	0.398
250	E	B	0.214	0.135	0.317	0.548	0.435
251	F	B	0.106	0.135	0.317	0.548	0.475
252	Y	B	0.139	0.069	0.386	0.545	0.505
253	K	E	0.404	0.066	0.296	0.638	0.531
254	G	E	0.642	0.052	0.084	0.864	0.548
255	E	E	0.788	0.018	0.019	0.964	0.548
256	N	E	0.392	0.019	0.141	0.840	0.553
257	I	E	0.397	0.022	0.552	0.426	0.549
258	Y	B	0.159	0.021	0.756	0.223	0.548
259	D	E	0.345	0.021	0.756	0.223	0.546
260	L	B	0.114	0.022	0.552	0.426	0.545

261	D	E	0.583	0.022	0.359	0.619	0.543
262	F	E	0.387	0.022	0.359	0.619	0.549
263	K	E	0.452	0.022	0.552	0.426	0.564
264	T	E	0.361	0.022	0.552	0.426	0.581
265	A	E	0.378	0.021	0.451	0.528	0.589
266	N	E	0.502	0.022	0.359	0.619	0.600
267	N	E	0.379	0.064	0.216	0.721	0.605
268	D	E	0.627	0.052	0.084	0.864	0.607
269	G	E	0.644	0.018	0.047	0.935	0.597
270	S	E	0.544	0.052	0.084	0.864	0.592
271	Q	E	0.402	0.021	0.451	0.528	0.580
272	K	E	0.408	0.004	0.514	0.481	0.572
273	I	B	0.126	0.004	0.420	0.576	0.569
274	T	E	0.282	0.004	0.138	0.858	0.558
275	G	B	0.196	0.879	0.010	0.111	0.544
276	D	E	0.655	0.970	0.001	0.030	0.540
277	Q	E	0.337	0.970	0.001	0.030	0.532
278	L	B	0.037	0.970	0.001	0.030	0.524
279	R	B	0.085	0.970	0.001	0.030	0.527
280	D	E	0.524	0.970	0.001	0.030	0.527
281	M	B	0.132	0.970	0.001	0.030	0.502
282	Y	B	0.032	0.988	0	0.012	0.467
283	M	E	0.323	0.970	0.001	0.030	0.473
284	E	E	0.551	0.970	0.001	0.030	0.479
285	F	B	0.066	0.970	0.001	0.030	0.464
286	C	B	0.053	0.923	0.002	0.076	0.460
287	K	E	0.655	0.782	0.003	0.216	0.453
288	E	E	0.649	0.600	0.003	0.397	0.430
289	F	B	0.105	0.109	0.005	0.886	0.412
290	P	E	0.302	0.005	0.045	0.951	0.402
291	I	B	0.047	0.005	0.336	0.660	0.404
292	V	B	0.086	0.003	0.718	0.279	0.390
293	S	B	0.044	0.002	0.816	0.182	0.379
294	I	B	0.033	0.003	0.718	0.279	0.386
295	E	B	0.103	0.021	0.451	0.528	0.392
296	D	B	0.099	0.019	0.141	0.840	0.415
297	P	B	0.093	0.018	0.019	0.964	0.444
298	F	B	0.086	0.018	0.019	0.964	0.469
299	D	E	0.342	0.018	0.019	0.964	0.506
300	Q	E	0.557	0.115	0.016	0.868	0.534
301	D	E	0.432	0.058	0.017	0.925	0.552
302	D	B	0.147	0.053	0.005	0.942	0.554
303	W	B	0.185	0.858	0.002	0.139	0.566
304	E	E	0.566	0.858	0.002	0.139	0.571

305	N	B	0.172	0.858	0.002	0.139	0.547
306	W	B	0.045	0.923	0.002	0.076	0.531
307	T	E	0.427	0.970	0.001	0.030	0.523
308	K	E	0.484	0.923	0.002	0.076	0.517
309	M	B	0.051	0.923	0.002	0.076	0.494
310	T	B	0.185	0.858	0.002	0.139	0.485
311	S	E	0.695	0.502	0.002	0.495	0.461
312	A	E	0.570	0.109	0.005	0.886	0.443
313	T	E	0.289	0.018	0.019	0.964	0.403
314	N	E	0.463	0.004	0.085	0.910	0.402
315	I	B	0.034	0.004	0.514	0.481	0.381
316	Q	B	0.149	0.001	0.900	0.099	0.375
317	I	B	0.019	0.001	0.959	0.040	0.366
318	V	B	0.031	0.001	0.900	0.099	0.368
319	G	B	0.024	0.023	0.655	0.322	0.367
320	D	B	0.122	0.019	0.141	0.840	0.392
321	D	B	0.153	0.052	0.084	0.864	0.401
322	L	B	0.092	0.118	0.150	0.732	0.437
323	T	B	0.170	0.056	0.142	0.802	0.456
324	V	B	0.190	0.052	0.084	0.864	0.490
325	T	B	0.284	0.053	0.043	0.903	0.517
326	N	E	0.282	0.115	0.016	0.868	0.522
327	P	E	0.337	0.923	0.002	0.076	0.531
328	K	E	0.567	0.923	0.002	0.076	0.535
329	R	B	0.226	0.970	0.001	0.030	0.522
330	I	B	0.034	0.970	0.001	0.030	0.508
331	A	E	0.456	0.923	0.002	0.076	0.515
332	T	E	0.460	0.858	0.002	0.139	0.523
333	A	B	0.059	0.782	0.003	0.216	0.524
334	V	B	0.134	0.622	0.015	0.363	0.504
335	E	E	0.663	0.339	0.016	0.645	0.489
336	K	E	0.469	0.181	0.016	0.803	0.482
337	K	E	0.460	0.115	0.016	0.868	0.468
338	A	B	0.158	0.113	0.087	0.80	0.468
339	C	B	0.055	0.125	0.227	0.648	0.468
340	N	B	0.227	0.125	0.227	0.648	0.437
341	C	B	0.067	0.148	0.418	0.435	0.423
342	L	B	0.095	0.079	0.592	0.329	0.406
343	L	B	0.139	0.079	0.592	0.329	0.407
343	L	B	0.104	0.159	0.516	0.325	0.424
345	K	E	0.272	0.125	0.227	0.648	0.416
346	V	B	0.134	0.191	0.086	0.723	0.427
347	N	B	0.196	0.268	0.043	0.689	0.436
348	Q	B	0.181	0.354	0.048	0.598	0.427

349	I	B	0.083	0.268	0.043	0.689	0.433
350	G	B	0.141	0.354	0.048	0.598	0.432
351	S	B	0.111	0.622	0.015	0.363	0.420
352	V	B	0.043	0.970	0.001	0.030	0.414
353	T	B	0.156	0.970	0.001	0.030	0.409
354	E	B	0.211	0.988	0	0.012	0.408
355	S	B	0.021	0.988	0	0.012	0.393
356	I	B	0.106	0.988	0	0.012	0.398
357	D	E	0.400	0.988	0	0.012	0.415
358	A	B	0.030	0.988	0	0.012	0.399
359	H	B	0.031	0.970	0.001	0.030	0.394
360	L	E	0.394	0.970	0.001	0.030	0.419
361	L	B	0.120	0.970	0.001	0.030	0.437
362	A	B	0.019	0.970	0.001	0.030	0.430
363	K	E	0.479	0.923	0.002	0.076	0.436
364	K	E	0.688	0.782	0.003	0.216	0.448
365	N	E	0.361	0.109	0.005	0.886	0.425
366	G	E	0.709	0.003	0.003	0.994	0.403
367	W	B	0.131	0.005	0.045	0.951	0.403
368	G	E	0.281	0.004	0.616	0.381	0.384
369	T	B	0.054	0.001	0.959	0.040	0.362
370	M	B	0.030	0.001	0.959	0.040	0.351
371	V	B	0.034	0.001	0.959	0.040	0.366
372	S	B	0.025	0.002	0.816	0.182	0.377
373	H	B	0.092	0.004	0.420	0.576	0.391
374	R	B	0.174	0.019	0.141	0.840	0.414
375	S	E	0.305	0.018	0.088	0.893	0.451
376	G	E	0.282	0.018	0.047	0.935	0.479
377	E	B	0.203	0.018	0.047	0.935	0.509
378	T	B	0.215	0.053	0.043	0.903	0.515
379	E	E	0.378	0.113	0.043	0.844	0.495
380	D	B	0.215	0.181	0.016	0.803	0.458
381	C	B	0.178	0.430	0.016	0.555	0.435
382	F	B	0.115	0.660	0.049	0.291	0.398
383	I	B	0.047	0.751	0.050	0.199	0.376
384	A	B	0.034	0.751	0.050	0.199	0.343
385	D	B	0.116	0.701	0.107	0.192	0.318
386	L	B	0.034	0.725	0.163	0.112	0.301
387	V	B	0.032	0.725	0.163	0.112	0.304
388	V	B	0.044	0.662	0.225	0.113	0.316
389	G	B	0.030	0.578	0.229	0.194	0.337
390	L	B	0.070	0.199	0.152	0.649	0.358
391	C	E	0.383	0.018	0.019	0.964	0.376
392	T	B	0.040	0.018	0.019	0.964	0.395

393	G	E	0.225	0.018	0.047	0.935	0.401
394	Q	B	0.148	0.022	0.359	0.619	0.424
395	I	B	0.030	0.022	0.552	0.426	0.421
396	K	B	0.156	0.021	0.451	0.528	0.424
397	T	B	0.122	0.022	0.359	0.619	0.431
398	G	B	0.100	0.020	0.205	0.775	0.444
399	A	B	0.107	0.019	0.141	0.840	0.444
400	P	B	0.100	0.018	0.088	0.893	0.456
401	C	B	0.115	0.018	0.088	0.893	0.467
402	R	E	0.295	0.113	0.043	0.844	0.465
403	S	B	0.212	0.339	0.016	0.645	0.453
404	E	E	0.343	0.522	0.016	0.462	0.472
405	R	B	0.170	0.802	0.014	0.185	0.455
406	L	B	0.039	0.879	0.010	0.111	0.444
407	A	B	0.122	0.879	0.010	0.111	0.439
408	K	B	0.203	0.938	0.007	0.055	0.420
409	Y	B	0.060	0.938	0.007	0.055	0.404
410	N	B	0.116	0.923	0.002	0.076	0.404
411	Q	E	0.255	0.923	0.002	0.076	0.403
412	I	B	0.045	0.970	0.001	0.030	0.402
413	L	B	0.055	0.938	0.007	0.055	0.408
414	R	E	0.314	0.938	0.007	0.055	0.425
415	I	B	0.034	0.975	0.003	0.022	0.422
416	E	B	0.083	0.938	0.007	0.055	0.430
417	E	E	0.452	0.938	0.007	0.055	0.449
418	E	E	0.343	0.879	0.010	0.111	0.468
419	L	B	0.055	0.802	0.014	0.185	0.457
420	G	E	0.362	0.502	0.002	0.495	0.487
421	G	E	0.695	0.406	0.004	0.590	0.508
422	N	E	0.447	0.430	0.016	0.555	0.502
423	A	B	0.114	0.268	0.043	0.689	0.511
424	K	E	0.421	0.307	0.165	0.527	0.545
425	F	B	0.137	0.278	0.093	0.628	0.553
426	A	B	0.272	0.268	0.043	0.689	0.551
427	G	E	0.306	0.257	0.016	0.727	0.562
428	K	E	0.530	0.339	0.016	0.645	0.579
429	K	E	0.582	0.430	0.016	0.555	0.576
430	F	B	0.220	0.257	0.016	0.727	0.569
431	R	E	0.368	0.115	0.016	0.868	0.512
432	K	E	0.527	0.058	0.017	0.925	0.453
433	P	E	0.487	0.018	0.019	0.964	0.379
434	C	E	0.715	0.003	0.003	0.994	0.310