

## REFERENCES

- Aadland, K., & Kolaczkowski, B. (2020). Alignment-Integrated Reconstruction of Ancestral Sequences Improves Accuracy. *Genome Biology and Evolution*, 12(9), 1549-1565. doi:10.1093/gbe/evaa164
- Ahmed, M. A., Chu, K., & Quan, F. (2018). The Plasmodium knowlesi Pk41 surface protein diversity, natural selection, subpopulation, and geographical clustering: A 6-cysteine protein family member. *PeerJ*, 6. doi:10.7717/peerj.6141
- Alonso-Morales, A., González-López, L., Cázares-Raga, F. E., Cortés-Martínez, L., Torres-Monzón, J. A., Gallegos-Pérez, J. L., ... Hernández-Hernández, F. de la C. (2015). Protein phosphorylation during Plasmodium berghei gametogenesis. *Experimental Parasitology*.  
<https://doi.org/10.1016/j.exppara.2015.05.010>
- Anasir, M. I., & Poh, C. L. (2019). Structural Vaccinology for Viral Vaccine Design. *Frontiers in Microbiology*, 10. doi: 10.3389/fmicb.2019.00738
- Ansari, H., & Raghava, G. P. (2010). Identification of conformational B-cell Epitopes in an antigen from its primary sequence. *Immunome Research*, 6(1), 6. doi: 10.1186/1745-7580-6-6
- Arora, D. K., Berka, R. M., & Singh, G. B. (2006). *Applied mycology and biotechnology: volume 6 Bioinformatics*. Amsterdam: Elsevier.
- Arredondo, S. A., & Kappe, S. H. (2017). The s48/45 six-cysteine proteins: Mediators of interaction throughout the Plasmodium life cycle. *International Journal for Parasitology*, 47(7), 409-423. doi:10.1016/j.ijpara.2016.10.002
- Audagnotto, M., & Peraro, M. D. (2017). Protein post-translational modifications: In silico prediction tools and molecular modeling. *Computational and Structural Biotechnology Journal*, 15, 307–319. doi: 10.1016/j.csbj.2017.03.004
- Bakhiet, A. M., Abdelraheem, M. H., Kheir, A., Omer, S., Gismelseed, et al. (2019). Evolution of Plasmodium falciparum drug resistance genes following artemisinin combination therapy in

Sudan. *Transactions of the Royal Society of Tropical Medicine and Hygiene*.

doi:10.1093/trstmh/trz059

Beeson, J. G., Kurtovic, L., Dobaño, C., Opi, D. H., Chan, J.-A., Feng, G., ... Boyle, M. J. (2019). Challenges

and strategies for developing efficacious and long-lasting malaria vaccines. *Science Translational Medicine*, 11(474). <https://doi.org/10.1126/scitranslmed.aau1458>

Bhasin, M., & Raghava, G. (2004). Prediction of CTL epitopes using QM, SVM and ANN techniques. *Vaccine*, 22(23-24), 3195–3204. doi: 10.1016/j.vaccine.2004.02.005

Bonetta, R., Ebejer, J.-P., Seychell, B., Vella, M., Hunter, T., & Hunter, G. J. (2016). Role of Protein Structure in Drug Discovery. *Journal of The Malta Chamber of Scientists*, 4, 126–130. doi: 10.7423/XJENZA.2016.2.03

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., ... Drummond, A. J. (2014). BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. *PLoS Computational Biology*, 10(4). doi: 10.1371/journal.pcbi.1003537

Buchan, D. W. A., & Jones, D. T. (2019). The PSIPRED Protein Analysis Workbench: 20 years on. *Nucleic Acids Research*, 47(W1). doi: 10.1093/nar/gkz297

Cao, Y., Bansal, G. P., Merino, K., & Kumar, N. (2016). Immunological Cross-Reactivity between Malaria Vaccine Target Antigen P48/45 in Plasmodium vivax and P. falciparum and Cross-Boosting of Immune Responses. *Plos One*, 11(7). doi:10.1371/journal.pone.0158212

Choudhuri, S., & Kotewicz, M. (2016). *Bioinformatics for beginners: genes, genomes, molecular evolution, databases and analytical tools*. Amsterdam: Elsevier.

Coelho, C. H., Doritchamou, J. Y. A., Zaidi, I., & Duffy, P. E. (2017). Advances in malaria vaccine development: report from the 2017 malaria vaccine symposium. *Npj Vaccines*, 2(1). doi: 10.1038/s41541-017-0035-3

Cowman, A. F., Healer, J., Marapana, D., & Marsh, K. (2016). Malaria: Biology and Disease. *Cell*, 167(3), 610-624. doi:10.1016/j.cell.2016.07.055

- Crompton, P. D., Moebius, J., Portugal, S., Waisberg, M., Hart, G., Garver, L. S., ... Pierce, S. K. (2014). Malaria Immunity in Man and Mosquito: Insights into Unsolved Mysteries of a Deadly Infectious Disease. *Annual Review of Immunology*, 32(1), 157–187. <https://doi.org/10.1146/annurev-immunol-032713-120220>
- Cummings, M. P. (2004). FigTree. *Dictionary of Bioinformatics and Computational Biology*. doi: 10.1002/9780471650126.dob0904
- Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2011). ProtTest 3: fast selection of best-fit models of protein evolution. *Bioinformatics*, 27(8), 1164–1165. doi: 10.1093/bioinformatics/btr088
- de Jong, R. M., Tebeje, S. K., Meerstein-Kessel, L., Tadesse, F. G., Jore, M. M., Stone, W., & Bousema, T. (2020). Immunity against sexual stage Plasmodium falciparum and Plasmodium vivax parasites. *Immunological Reviews*. <https://doi.org/10.1111/imr.12828>
- Delano, W. L. (2002). Pymol: An open-source molecular graphics tool. CCP4 Newsletter on Protein Crystallography, 40, 82-92.
- Deller, M. C., Kong, L., & Rupp, B. (2016). Protein stability: A crystallographer's perspective. *Acta Crystallographica Section F Structural Biology Communications*, 72(2), 72-95. doi:10.1107/s2053230x15024619
- Dhanda, S. K., Karosiene, E., Edwards, L., Grifoni, A., Paul, S., Andreatta, M., ... Sette, A. (2018). Predicting HLA CD4 Immunogenicity in Human Populations. *Frontiers in Immunology*, 9. doi: 10.3389/fimmu.2018.01369
- Dijk, M. R., Schaijk, B. C., Khan, S. M., Dooren, M. W., Ramesar, J., Kaczanowski, S., . . . Janse, C. J. (2010). Three Members of the 6-cys Protein Family of Plasmodium Play a Role in Gamete Fertility. *PLoS Pathogens*, 6(4). doi:10.1371/journal.ppat.1000853
- Doerig, C., Rayner, J. C., Scherf, A., & Tobin, A. B. (2015). Post-translational protein modifications in malaria parasites. *Nature Reviews Microbiology*, 13(3), 160-172. doi:10.1038/nrmicro3402
- Ducatez, M. F., Bahl, J., Griffin, Y., Stigger-Rosser, E., Franks, J., Barman, S., ... Webby, R. J. (2011). Feasibility of reconstructed ancestral H5N1 influenza viruses for cross-clade protective vaccine

development. *Proceedings of the National Academy of Sciences of the United States of America*.

<https://doi.org/10.1073/pnas.1012457108>

Foley, G., Mora, A., Ross, C. M., Bottoms, S., Sützl, L., Lamprecht, M. L., ... Bodén, M. (n.d.). Identifying and engineering ancient variants of enzymes using Graphical Representation of Ancestral Sequence Predictions (GRASP). doi: 10.1101/2019.12.30.891457

Frénal, K., Tay, C. L., Mueller, C., Bushell, E. S., Jia, Y., Graindorge, A., ... Soldati-Favre, D. (2013). Global Analysis of Apicomplexan Protein S-Acyl Transferases Reveals an Enzyme Essential for Invasion. *Traffic*, 14(8), 895–911. doi: 10.1111/tra.12081

Frimpong, A., Kusi, K. A., Ofori, M. F., & Ndifon, W. (2018). Novel Strategies for Malaria Vaccine Design. *Frontiers in Immunology*, 9. doi: 10.3389/fimmu.2018.02769

Garboczi, D., Singh, K., & Gittis, A. (2020). Structure of Plasmodium falciparum vaccine candidate Pfs230D1M in complex with the Fab of a transmission blocking antibody. doi:10.2210/pdb6ohg/pdb

Giles, B. M., & Ross, T. M. (2011). A computationally optimized broadly reactive antigen (COBRA) based H5N1 VLP vaccine elicits broadly reactive antibodies in mice and ferrets. *Vaccine*, 29(16), 3043-3054. doi:10.1016/j.vaccine.2011.01.100

Gromiha, M. M., & Penrose, D. (2014). *Protein Bioinformatics: From Sequence to Function*. Saint Louis: Elsevier Science.

Guinovart, C., & Alonso, P. L. (2007). Methods for Determining Vaccine Efficacy and Effectiveness and the Main Barriers to Developing a Fully Deployable Malaria Vaccine. *The American Journal of Tropical Medicine and Hygiene*, 77(6\_Suppl), 276–281. doi: 10.4269/ajtmh.2007.77.276

Gupta, R. C. (2011). *Reproductive and developmental toxicology*. Amsterdam: Elsevier/Academic Press. H. Noedl, Y. Se, K. Schaecher, B. L. Smith, D. Socheat, and M. M. Fukuda, (2008) “Evidence of artemisinin-resistant malaria in Western Cambodia,” *N. Engl. J. Med.*, 359(24), 2619–2620.

- Heath, T. A., Huelsenbeck, J. P., & Stadler, T. (2014). The fossilized birth-death process for coherent calibration of divergence-time estimates. *Proceedings of the National Academy of Sciences*, 111(29). doi: 10.1073/pnas.1319091111
- Hedges, S. B., & Kumar, S. (2009). *The Timetree of Life*. Oxford University Press.
- Huang, S.-Y. (2014). Search strategies and evaluation in protein–protein docking: principles, advances and challenges. *Drug Discovery Today*, 19(8), 1081–1096.
- Janeway, C. A. J., Travers, P., & Walport, M. (2001). The major histocompatibility complex and its functions. In *Immunobiology: The Immune System in Health and Disease*.
- Jahangiri, F., Jalallou, N., & Ebrahimi, M. (2019). Analysis of Apical Membrane Antigen (AMA)-1 characteristics using bioinformatics tools in order to vaccine design against Plasmodium vivax. *Infection, Genetics and Evolution*, 71, 224–231. doi: 10.1016/j.meegid.2019.04.001
- Jensen, K. K., Andreatta, M., Marcatili, P., Buus, S., Greenbaum, J. A., Yan, Z., ... Nielsen, M. (2018). Improved methods for predicting peptide binding affinity to MHC class II molecules. *Immunology*, 154(3), 394–406. doi: 10.1111/imm.12889
- Jespersen, M. C., Mahajan, S., Peters, B., Nielsen, M., & Marcatili, P. (2019). Antibody Specific B-Cell Epitope Predictions: Leveraging Information From Antibody-Antigen Protein Complexes. *Frontiers in Immunology*, 10. doi: 10.3389/fimmu.2019.00298
- Joy, J. B., Liang, R. H., McCloskey, R. M., Nguyen, T., & Poon, A. F. Y. (2016). Ancestral Reconstruction. *PLoS Computational Biology*, 12(7), 1–20.
- Katoh, K. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, 30(14), 3059–3066. doi: 10.1093/nar/gkf436
- Karosiene, E., Lundegaard, C., Lund, O., & Nielsen, M. (2011). NetMHCcons: a consensus method for the major histocompatibility complex class I predictions. *Immunogenetics*, 64(3), 177–186. doi: 10.1007/s00251-011-0579-8
- Kesheri, M., Kanchan, S., Chowdhury, S., & Sinha, R. P. (2014). Secondary and Tertiary Structure Prediction of Proteins: A Bioinformatic Approach. *Complex System Modelling and Control*

*Through Intelligent Soft Computations Studies in Fuzziness and Soft Computing*, 541–569. doi: 10.1007/978-3-319-12883-2\_19

Kyte, J., & Doolittle, R. F. (1982). A simple method for displaying the hydropathic character of a protein. *Journal of Molecular Biology*, 157(1), 105–132. doi: 10.1016/0022-2836(82)90515-0

Larson, B. (2019). Origin of Two Most Virulent Agents of Human Malaria: *Plasmodium falciparum* and *Plasmodium vivax*. *Malaria*. doi:10.5772/intechopen.84481

Laskowski, R. A., Macarthur, M. W., Moss, D. S., & Thornton, J. M. (1993). PROCHECK: A program to check the stereochemical quality of protein structures. *Journal of Applied Crystallography*, 26(2), 283-291. doi:10.1107/s0021889892009944

Laskowski, R. A., Jabłońska, J., Pravda, L., Vařeková, R. S., & Thornton, J. M. (2017). PDBsum: Structural summaries of PDB entries. *Protein Science*, 27(1), 129-134. doi:10.1002/pro.3289

Li, Y., Liu, X., Zhu, Y., Zhou, X., Cao, C., Hu, X., . . . Ding, J. (2013). Bioinformatic prediction of epitopes in the Emy162 antigen of *Echinococcus multilocularis*. *Experimental and Therapeutic Medicine*, 6(2), 335-340. doi:10.3892/etm.2013.1142

Liljeroos, L., Malito, E., Ferlenghi, I., & Bottomley, M. J. (2015). Structural and Computational Biology in the Design of Immunogenic Vaccine Antigens. *Journal of Immunology Research*, 2015, 1-17. doi:10.1155/2015/156241

Luckheeram, R. V., Zhou, R., Verma, A. D., & Xia, B. (2012). CD4 +T cells: Differentiation and functions. *Clinical and Developmental Immunology*. <https://doi.org/10.1155/2012/925135>

Macdonald, N. J., Nguyen, V., Shimp, R., Reiter, K., Herrera, R., Burkhardt, M., . . . Narum, D. L. (2016). Structural and Immunological Characterization of Recombinant 6-Cysteine Domains of the *plasmodium falciparum* sexual Stage Protein Pfs230. *Journal of Biological*

Mahanty, S., Prigent, A., & Garraud, O. (2015). Immunogenicity of infectious pathogens and vaccine antigens. *BMC Immunology*, 16(1). doi: 10.1186/s12865-015-0095-y

Mello, B. (2018). Estimating TimeTrees with MEGA and the TimeTree Resource. *Molecular Biology and Evolution*, 35(9), 2334–2342. doi: 10.1093/molbev/msy133

- Misra, A. (2011). *Challenges in delivery of therapeutic genomics and proteomics*. Amsterdam: Elsevier.
- Mitran, C. J., & Yanow, S. K. (2020). The Case for Exploiting Cross-Species Epitopes in Malaria Vaccine Design. *Frontiers in Immunology*, 11. doi:10.3389/fimmu.2020.00335
- Muh, F., Kim, N., Nyunt, M. H., Firdaus, E. R., Han, J., Hoque, M. R., . . . Han, E. (2020). Cross-species reactivity of antibodies against *Plasmodium vivax* blood-stage antigens to *Plasmodium knowlesi*. *PLOS Neglected Tropical Diseases*, 14(6). doi:10.1371/journal.pntd.0008323
- Müller-Sienerth, N., Shilts, J., Kadir, K. A., Yman, V., Homann, M. V., Asghar, M., . . . Wright, G. J. (2020). A panel of recombinant proteins from human-infective *Plasmodium* species for serological surveillance. *Malaria Journal*, 19(1). doi:10.1186/s12936-020-3111-5
- Nero, T. L., Parker, M. W., & Morton, C. J. (2018). Protein structure and computational drug discovery. *Biochemical Society Transactions*, 46(5), 1367–1379. doi: 10.1042/bst20180202
- Nurdiansyah, R., & Kemal, R. A. (2020). Computational Design Of Ancestral And Consensus Sequence Of Apical Membrane Antigen 1 (AMA1) of *Plasmodium spp*. *Acta Biochimica Indonesiana*, 2(2). doi: 10.32889/actabioina.v2i2.40
- Ogilvie, H. A., Heled, J., Xie, D., & Drummond, A. J. (2016). Computational Performance and Statistical Accuracy of \*BEAST and Comparisons with Other Methods. *Systematic Biology*, 65(3), 381–396. doi: 10.1093/sysbio/syv118
- Okonechnikov, K., Golosova, O., & Fursov, M. (2012). Unipro UGENE: a unified bioinformatics toolkit. *Bioinformatics*, 28(8), 1166–1167. doi: 10.1093/bioinformatics/bts091
- Ollomo, B., Durand, P., Prugnolle, F., Douzery, E., Arnathau, C., Nkoghe, D., . . . Renaud, F. (2009). A New Malaria Agent in African Hominids. *PLoS Pathogens*, 5(5). doi:10.1371/journal.ppat.1000446
- Osii, R. S., Otto, T. D., Garside, P., Ndungu, F. M., & Brewer, J. M. (2020). The Impact of Malaria Parasites on Dendritic Cell-T Cell Interaction. *Frontiers in Immunology*. <https://doi.org/10.3389/fimmu.2020.01597>

- Othman, A. S., Lin, J., Franke-Fayard, B. M., Kroeze, H., Pul, F. J., Chevalley-Maurel, S., . . . Khan, S. M. (2018). Expression of full-length Plasmodium falciparum P48/45 in P. berghei blood stages: A method to express and evaluate vaccine antigens. *Molecular and Biochemical Parasitology*, 224, 44-49. doi:10.1016/j.molbiopara.2018.07.009
- Ovchinnikov S, Kinch L, Park H, Liao Y, Pei J, Kim DE, et al. Large-scale determination of previously unsolved protein structures using evolutionary information. *eLife*. 2015;4.
- Panawala, L. (2017, September 25). Difference Between T Cells and B Cells: Definition, Characteristics, Function. Retrieved February 11, 2020, from <https://pediaa.com/difference-between-t-cells-and-b-cells/>
- Pradeu, T., & Carosella, E. D. (2006). On the definition of a criterion of immunogenicity. *Proceedings of the National Academy of Sciences*, 103(47), 17858–17861. doi: 10.1073/pnas.0608683103
- Pringle, J. C., Carpi, G., Almagro-Garcia, J., Zhu, S. J., Kobayashi, T., Mulenga, M., ... Norris, D. E. (2018). RTS,S/AS01 malaria vaccine mismatch observed among Plasmodium falciparum isolates from southern and central Africa and globally. *Scientific Reports*, 8(1). <https://doi.org/10.1038/s41598-018-24585-8>
- Pejaver, V., Hsu, W.-L., Xin, F., Dunker, A. K., Uversky, V. N., & Radivojac, P. (2014). The structural and functional signatures of proteins that undergo multiple events of post-translational modification. *Protein Science*, 23(8), 1077–1093. doi: 10.1002/pro.2494
- Potocnakova, L., Bhide, M., & Pulzova, L. B. (2016). An Introduction to B-Cell Epitope Mapping and In Silico Epitope Prediction. *Journal of Immunology Research*, 2016, 1–11. doi: 10.1155/2016/6760830
- Qiu, T., Xiao, H., Zhang, Q., Qiu, J., Yang, Y., Wu, D., ... Zhu, R. (2015). Proteochemometric Modeling of the Antigen-Antibody Interaction: New Fingerprints for Antigen, Antibody and Epitope-Paratope Interaction. *Plos One*, 10(4). doi: 10.1371/journal.pone.0122416
- Qiu, X., Duvvuri, V. R., & Bahl, J. (2019). Computational Approaches and Challenges to Developing Universal Influenza Vaccines. *Vaccines*, 7(2), 45. doi:10.3390/vaccines7020045

Ramanto, K. N., Nurdiansyah, R., & Jessica, P. (2020). The Evolution Study Of 6-Cysteine Family Member Protein of Plasmodium sp. As a Potential Drug Candidate Against Malaria Infection. *KnE Life Sciences*. doi: 10.18502/kls.v5i2.6442

Rénia, L., & Goh, Y. S. (2016). Malaria Parasites: The Great Escape. *Frontiers in Immunology*, 7. doi:10.3389/fimmu.2016.00463

Reis, M. D., Donoghue, P. C. J., & Yang, Z. (2015). Bayesian molecular clock dating of species divergences in the genomics era. *Nature Reviews Genetics*, 17(2), 71–80. doi: 10.1038/nrg.2015.8

Saha, S., & Raghava, G. P. S. (2006). Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. *Proteins: Structure, Function, and Bioinformatics*, 65(1), 40–48. doi: 10.1002/prot.21078

Salaun, C., Greaves, J., & Chamberlain, L. H. (2010). The intracellular dynamic of protein palmitoylation. *The Journal of Cell Biology*, 191(7), 1229-1238. doi:10.1083/jcb.201008160

Sanchez-Trincado, J. L., Gomez-Perez, M., & Reche, P. A. (2017). Fundamentals and Methods for T- and B-Cell Epitope Prediction. *Journal of Immunology Research*, 2017, 1–14. doi: 10.1155/2017/2680160

Santos, J. M., Kehrer, J., Franke-Fayard, B., Frischknecht, F., Janse, C. J., & Mair, G. R. (2015). The Plasmodium palmitoyl-S-acyl-transferase DHHC2 is essential for ookinete morphogenesis and malaria transmission. *Scientific Reports*.

Senior, A. W., Evans, R., Jumper, J., Kirkpatrick, J., Sifre, L., Green, T., . . . Hassabis, D. (2020). Improved protein structure prediction using potentials from deep learning. *Nature*, 577(7792), 706-710. doi:10.1038/s41586-019-1923-7

Scheiblhofer, S., Laimer, J., Machado, Y., Weiss, R., & Thalhamer, J. (2017). Influence of protein fold stability on immunogenicity and its implications for vaccine design. *Expert Review of Vaccines*, 16(5), 479-489. doi:10.1080/14760584.2017.1306441

Schneidman-Duhovny, D., Inbar, Y., Nussinov, R., & Wolfson, H. J. (2005). PatchDock and SymmDock: servers for rigid and symmetric docking. *Nucleic Acids Research*, 33(Web Server). doi: 10.1093/nar/gki481

Srisutham, S., Saralamba, N., Sripawat, K., Mayxay, M., Smithuis, F., Nosten, F., ... Imwong, M. (2018). Genetic diversity of three surface protein genes in *Plasmodium malariae* from three Asian countries. *Malaria Journal*, 17(1). doi:10.1186/s12936-018-2176-x

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30(9), 1312–1313. doi: 10.1093/bioinformatics/btu033

Sternke, M., Tripp, K. W., & Barrick, D. (2018). Consensus sequence design as a general strategy to create hyperstable, biologically active proteins. doi: 10.1101/466391

Takala, S. L., & Plowe, C. V. (2009). Genetic diversity and malaria vaccine design, testing and efficacy: Preventing and overcoming ‘vaccine resistant malaria’. *Parasite Immunology*, 31(9), 560-573. doi:10.1111/j.1365-3024.2009.01138.x

Taechalertpaisarn, T., Crosnier, C., Bartholdson, S. J., Hodder, A. N., Thompson, J., Bustamante, L. Y., ... Crabb, B. S. (2012). Biochemical and Functional Analysis of Two *Plasmodium falciparum* Blood-Stage 6-Cys Proteins: P12 and P41. *PLoS ONE*, 7(7). doi: 10.1371/journal.pone.0041937

The Minister of Health of Indonesia. (2018). *Laporan Nasional Risikesdas 2018*.

Thomas, S., & Luxon, B. A. (2013). Vaccines based on structure-based design provide protection against infectious diseases. *Expert Review of Vaccines*, 12(11), 1301–1311. doi: 10.1586/14760584.2013.840092

Trebeck, M., Zacherl, S., Herrmann, S., Cabrera, A., Kono, M., Struck, N. S., ... Gilberger, T. W. (2009). Functional Analysis of the Leading Malaria Vaccine Candidate AMA-1 Reveals an Essential Role for the Cytoplasmic Domain in the Invasion Process. *PLoS Pathogens*, 5(3). doi: 10.1371/journal.ppat.1000322

Wang, G., Yin, R., Zhou, P., & Ding, Z. (2017). Combination of the immunization with the sequence close to the consensus sequence and two DNA prime plus one VLP boost generate H5 hemagglutinin

specific broad neutralizing antibodies. *PLoS ONE*.

<https://doi.org/10.1371/journal.pone.0176854>

Wilkins, M. R., Gasteiger, E., Bairoch, A., Sanchez, J.-C., Williams, K. L., Appel, R. D., & Hochstrasser, D. F. (2005). Protein Identification and Analysis Tools in the ExPASy Server. *2-D Proteome Analysis Protocols*, 531–552. doi: 10.1385/1-59259-584-7:531

World Health Organization, (2018) “Artemisinin resistance and artemisinin-based combination therapy efficacy.”.

World Health Organization, *World Malaria Report 2019*. (2019).

Wu, Y., Nelson, M. M., Quaile, A., Xia, D., Wastling, J. M., & Craig, A. (2009). Identification of phosphorylated proteins in erythrocytes infected by the human malaria parasite *Plasmodium falciparum*. *Malaria Journal*, 8(1). doi:10.1186/1475-2875-8-105

Yang, J., Anishchenko, I., Park, H., Peng, Z., Ovchinnikov, S., & Baker, D. (2020). Improved protein structure prediction using predicted interresidue orientations. *Proceedings of the National Academy of Sciences*, 117(3), 1496–1503. doi:10.1073/pnas.1914677117

Zhang, C., Vasmatzis, G., Cornette, J. L., & Delisi, C. (1997). Determination of atomic desolvation energies from the structures of crystallized proteins 1 Edited by B. Honig. *Journal of Molecular Biology*, 267(3), 707–726. doi: 10.1006/jmbi.1996.0859

Zhou, X., Shen, X.-X., Hittinger, C. T., & Rokas, A. (2017). Evaluating Fast Maximum Likelihood-Based Phylogenetic Programs Using Empirical Phylogenomic Data Sets. *Molecular Biology and Evolution*, 35(2), 486–503. doi: 10.1093/molbev/msx302

## APPENDICES

### RAXML Command Line

#### P41 first tree:

```
./raxml -s P41_complete_align.fasta -m PROTGAMMAIWAG -x 1000 -n p41_test_result -T 2 -N 10 -p 10
```

#### P41 second tree:

```
./raxml -s P41_second_align.fasta -m PROTGAMMAWAG -x 1000 -n p41_test_second_result -T 2 -N 10  
-p 10
```

#### P48/45 first tree:

```
./raxml -s P4845_complete_align.fasta -m PROTGAMMAILG -x 1000 -n p4845_test_result -T 2 -N 10 -p  
10
```

#### P48/45 second tree:

```
./raxml -s P4845_second_align.fasta -m PROTCATIWAG -x 1000 -n p4845_test_result_second -T 2 -N  
10 -p 10
```

#### P230 first tree:

```
./raxml -s P230_complete_align.fasta -m PROTGAMMAIJTF -x 1000 -n P230_test_result -T 2 -N 10 -p  
10
```

#### P12 first tree:

```
./raxml -s P12_complete_align.fasta -m PROTGAMMAJTT -x 1000 -n P12_test_result -T 2 -N 10 -p 10
```

#### P12 second tree:

```
./raxml -s P12_second_align.fasta -m PROTGAMMAIWAG -x 1000 -n p12_test_second_result -T 2 -N  
10 -p 10
```

**A.1** Accession number of P41 and P12 retrieved from the GenBank

P41		P12	
Accession number	Species	Accession number	Species
Q8I1Y0	Falciparum 3D7	CAG25366	falciparum 3D7
CAD49141	Falciparum 3D7	C6KSX0	falciparum 3D7
XP_001351361	falciparum 3D7	P19259	falciparum 3D7
OTN68424	Knowlesi malayan strain pk1	CAA9989198	knowlesi strain H
VVS75932	Knowlesi strain H	VVS78672	knowlesi strain H
CAA9986458	Knowlesi strain H	OTN64663	knowlesi Malayan Strain Pk1 (A+)
XP_028859370	malariae UG01	XP_028862689	malariae UG01
SBT70336	malariae GA01	SCO93252	malariae UG01
SBT86198	malariae UG01	SBS87333	malariae
SCA48345	Ovale curtisi GH01	SCP05137	ovale curtisi GH01
SBT75421	Ovale walikeri CR01	SBT77792	ovale walikeri CR01
VUZ93490	Vivax P01	SBS95004	ovale curtisi
AHF49864	Vivax 82	SBT39338	ovale walikeri
AHF49865	Viva <del>k</del> 83	SBT38756	ovale walikeri
AHF49866	Vivax 84	SBS85464	ovale curtisi
AHF49867	Vivax 85	AHJ80925	vivax 32
AHF49868	Vivax 87	AHJ80924	vivax 31
AHF49869	Vivax 88	VUZ97110	vivax
AHF49870	Vivax 89	AHF49843	vivax 124
AHF49871	Vivax 90	AHF49842	vivax 123
AHF49872	Vivax 92	AHF49841	vivax 120
AHF49873	Vivax 93	AHF49840	vivax 108
AHF49874	Vivax 94	AHF49839	vivax 105
AHF49875	Vivax 95	AHF49838	vivax 100
AHF49876	Vivax 96	AHF49837	vivax 98
AHF49877	Vivax 97	AHF49836	vivax 96
AHF49878	Vivax 98	AHF49835	vivax 94
AHF49879	Vivax 99	AHF49834	vivax 91
AHF49880	Vivax 102	AHF49833	vivax 88
AHF49881	Vivax 104	AHF49832	vivax 87
AHF49882	Vivax 105	AHF49831	vivax 85
AHF49883	Vivax 107	AHF49830	vivax 84
AHF49884	Vivax 108	AHF49829	vivax 83
AHF49885	Vivax 109	AHF49828	vivax 16
AHF49886	Vivax 111	AHF49827	vivax 11
AHF49887	Vivax 112	AHF49826	vivax 8
AHF49888	Vivax 115	AHF49825	vivax 7
AHF49889	Vivax 117	AHF49824	vivax 6
AHF49890	Vivax 121	AHF49823	vivax 1
AHF49891	Vivax 124		
AIT39660	Vivax Colombian 18		
AIT39661	Vivax Colombian 28		
AIT39662	Vivax Colombian 32		
AIT39663	Vivax Colombian 38		
AIT39664	Vivax Colombian 45		
AIT39666	Vivax Colombian 72		
AIT39667	Vivax Colombian 84		

**A.2 Accession number of P48/45 retrieved from the Genbank**

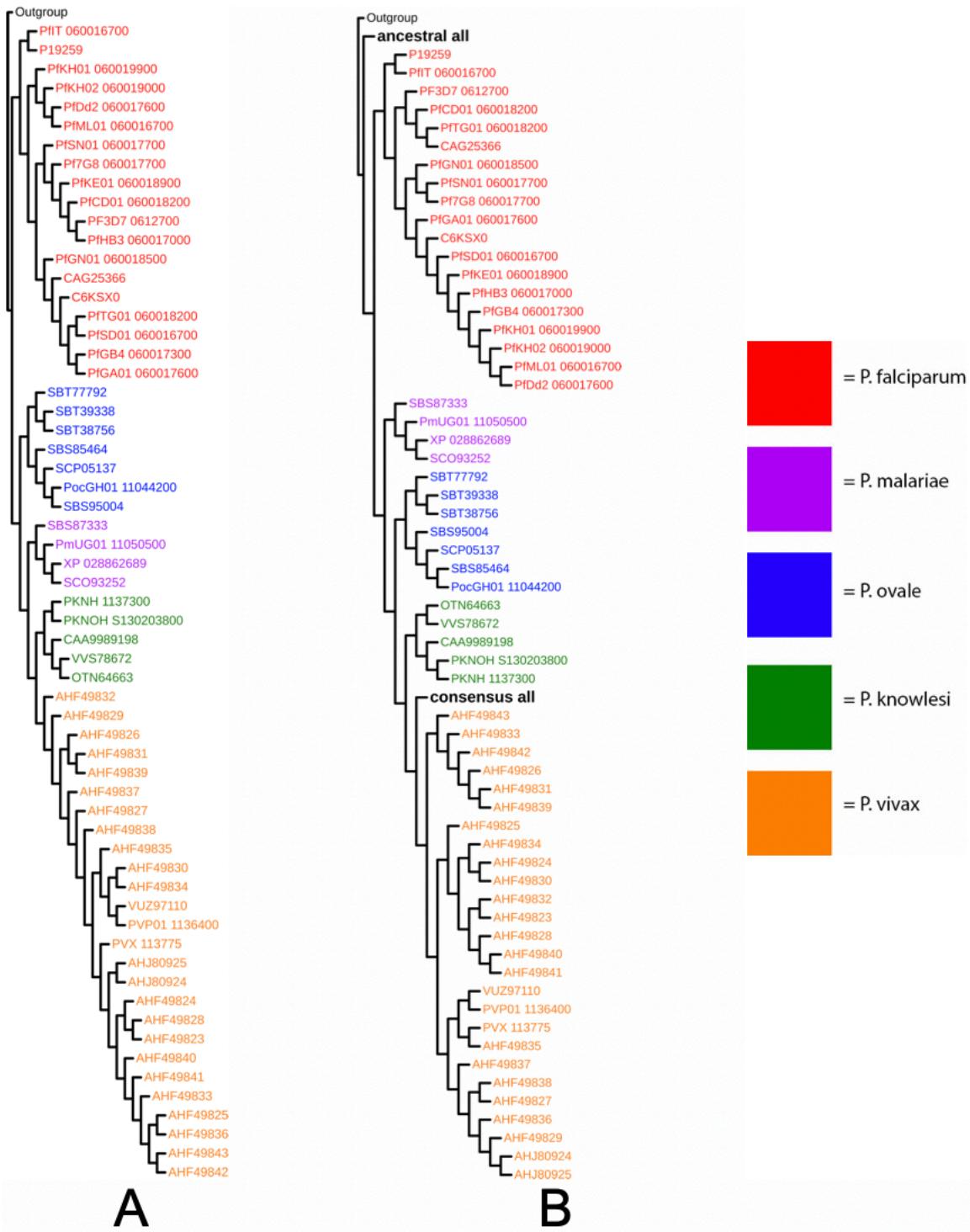
Accession number	species	Accession number	species
XP_001350181	falciparum 3D7	VUZ97357	vivax P01
VVP77780	falciparum 3D7	AFC37909	vivax 2006-12
ABO41494	falciparum Dd2	AFC37907	vivax 2006-46
ABO41491	falciparum FCB	AFC37934	vivax 2006-59
ABO41489	falciparum HB3	AFC37908	vivax 2006-66
ABO41495	falciparum K1	AFC37906	vivax 2006-67
ABO41496	falciparum Mad02	AFC37905	vivax 2006-73
ABO41497	falciparum Palo Alto	AFC37930	vivax 2007-10
ABO41492	falciparum RO33	AFC37918	vivax 2007-24
ABO41490	falciparum T9-102	AFC37911	vivax 2007-27
ABO41493	falciparum Wellcome	AFC37935	vivax 2007-32
OTN66276	knowlesi malayan strain Pk1	AFC37923	vivax 2007-34
XP_002259885	knowlesi strain H	AFC37910	vivax 2007-35
CAA9989869	knowlesi strain H	AFC37914	vivax 2007-37
WS79343	knowlesi strain H	AFC37915	vivax 2007-38
XP_028862876	malariae UG01	AFC37936	vivax 2007-40
SCO93594	malariae UG01	AFC37924	vivax 2007-43
SBS87718	malariae ALA	AFC37916	vivax 2007-45
SBT79956	malariae GA01	AFC37913	vivax 2007-47
SBS96099	ovale curtisi	AFC37937	vivax 2007-48
SBS85830	ovale curtisi	AFC37912	vivax 2007-49
SCP05306	ovale curtisi GH01	AFC37921	vivax 2008-08
SBT40475	ovale wallikeri	AFC37931	vivax 2008-10
SBT40052	ovale wallikeri	AFC37917	vivax 2008-11
SBT77972	ovale wallikeri CRO1	AFC37925	vivax 2008-19
AFB76627	vivax 2010-6	AFC37919	vivax 2008-20
AMQ67080	vivax Br02	AFC37929	vivax 2008-23
AMQ67081	vivax Br03	AFC37928	vivax 2008-24
AMQ67082	vivax Br04	AFC37927	vivax 2008-25
AMQ67083	vivax Br05	AFC37920	vivax 2010-03
AMQ67084	vivax Br06	AFC37938	vivax 2010-09
AMQ67085	vivax Br07	AFC37922	vivax 2010-10
AMQ67086	vivax Br09	AFC37926	vivax 2010-15
AMQ67087	vivax Br12	AFC37932	vivax 2010-17
AMQ67088	vivax Br14	AFC37933	vivax 2010-24
AMQ67089	vivax Br16	AFC37939	vivax India1
AMQ67090	vivax Br17	AFC37940	vivax India2
AMQ67091	vivax Br19	AFC37941	vivax India3
AMQ67092	vivax Br20	AFC37942	vivax Indonesia1
AMQ67093	vivax Co01	AFC37943	vivax Indonesia2
AMQ67094	vivax Co02	AFC37944	vivax Indonesia3
AMQ67095	vivax Co03		
AMQ67096	vivax Co04		
AMQ67097	vivax Co05		
AMQ67098	vivax Co06		
AMQ67099	vivax Co07		
AMQ67100	vivax Co08		
AMQ67101	vivax Co09		
AMQ67102	vivax Co10		
AMQ67103	vivax Co11		
AMQ67104	vivax Co12		
AMQ67105	vivax Co13		
AMQ67106	vivax Co14		
AMQ67107	vivax Co15		
AMQ67108	vivax Co16		
AMQ67109	vivax Co17		

**A.3 Accession number of P230 retrieved from the GenBank**

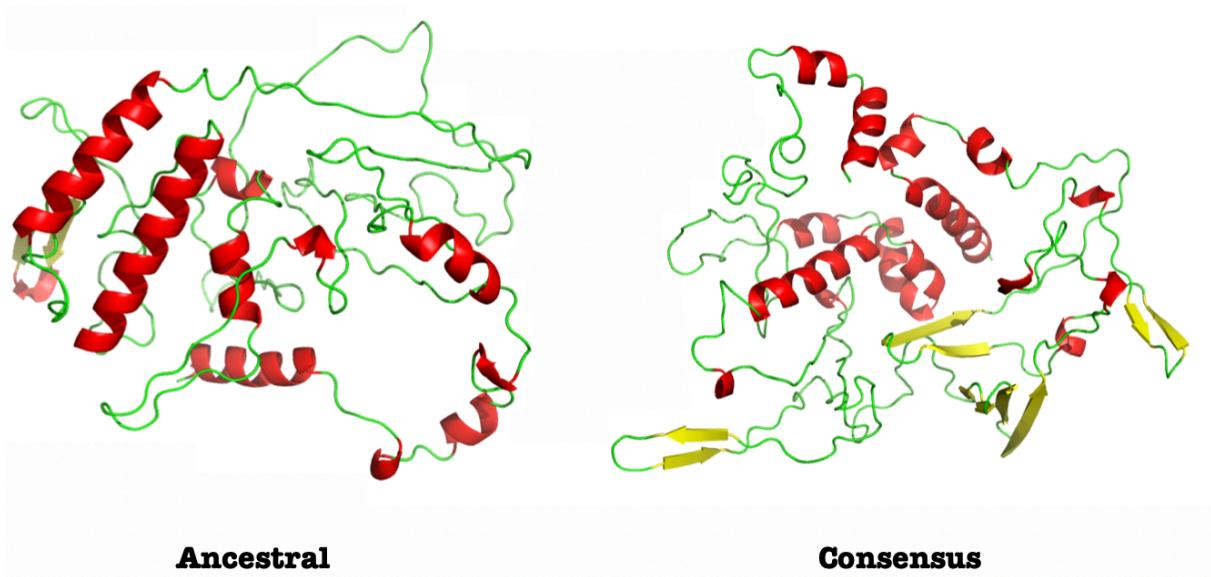
Accession number	Species	Accession number	Species	Accession number	Species
AAG12332	falciparum FCC1/HN	BAL44538	vivax T106	BAL44596	vivax 903-91
CZT98104	falciparum 3D7	BAL44539	vivax 234SHV	BAL44597	vivax 903-144
P68874	falciparum 3D7	BAL44540	vivax 62014	BAL44598	vivax 903-173
XP_001349600	falciparum 3D7	BAL44541	vivax 62415	BAL44599	vivax 904-73
P68875	falciparum NF54	BAL44542	vivax 9827106	BAL44600	vivax 904-171
AAA29734	falciparum NF54	BAL44543	vivax G29576	BAL44601	vivax 906-71
OTN66806	knowlesi Malayan strain Pk1	BAL44544	vivax 236SHV	BAL44602	vivax 906-114
CAA9986761	knowlesi strain H	BAL44545	vivax 160SHV	BAL44603	vivax 829-85
VVS76235	knowlesi strain H	BAL44546	vivax B5	BAL44604	vivax 829-109
SBT86536	malariae UG01	BAL44547	vivax B8	BAL44605	vivax 830-10
SBS91837	malariae	BAL44548	vivax A1	BAL44606	vivax Chesson
SBT70600	malariae GA01	BAL44549	vivax 692	BAL44607	vivax v102
SCN43131	ovale curtisi GH01	BAL44550	vivax 1123	BAL44608	vivax v137
SBT75700	ovale walikeri CR01	BAL44551	vivax B1	BAL44609	vivax Nicaragua
VUZ93809	vivax P01	BAL44552	vivax B3	BAL44610	vivax Panama
BAL44499	vivax 372M2	BAL44553	vivax A2	KMZ88429	vivax Brazil I
BAL44500	vivax 419M1	BAL44554	vivax A3	KMZ82130	vivax India VII
BAL44501	vivax 21ECS	BAL44555	vivax A4	KMZ94794	vivax Mauritania I
BAL44502	vivax 23PLS	BAL44556	vivax B6	KNA01442	vivax North Korean
BAL44503	vivax 30MNO	BAL44557	vivax B7	XP_001613020	vivax salvador I
BAL44504	vivax 31MTO	BAL44558	vivax B9	EDL43293	vivax salvador I
BAL44505	vivax 32MIS	BAL44559	vivax B11		
BAL44506	vivax 33ACS	BAL44560	vivax B12		
BAL44507	vivax 34LCR	BAL44561	vivax B13		
BAL44508	vivax 35RNSA	BAL44562	vivax 245		
BAL44509	vivax 36MV	BAL44563	vivax 1089		
BAL44510	vivax 37ESS	BAL44564	vivax 1094		
BAL44511	vivax 38FSN	BAL44565	vivax 1114		
BAL44512	vivax 39LRS	BAL44566	vivax KS385		
BAL44513	vivax 40CBA	BAL44567	vivax KS399		
BAL44514	vivax 42CMS	BAL44568	vivax KS434		
BAL44515	vivax 43SSD	BAL44569	vivax MS592		
BAL44516	vivax 44RPA	BAL44570	vivax MS607		
BAL44517	vivax 45FHPM	BAL44571	vivax MS614		
BAL44518	vivax 46ILF	BAL44572	vivax MS628		
BAL44519	vivax 5	BAL44573	vivax MS647		
BAL44520	vivax 6	BAL44574	vivax MS669		
BAL44521	vivax 9	BAL44575	vivax MS718		
BAL44522	vivax 15	BAL44576	vivax MS719		
BAL44523	vivax 16	BAL44577	vivax MS721		
BAL44524	vivax 17	BAL44578	vivax MS722		
BAL44525	vivax 20	BAL44579	vivax MS726		
BAL44526	vivax 23	BAL44580	vivax KS289		
BAL44527	vivax 24	BAL44581	vivax KS290		
BAL44528	vivax 27	BAL44582	vivax KS320		
BAL44529	vivax 29	BAL44583	vivax MS433		
BAL44530	vivax 33	BAL44584	vivax MS476		
BAL44531	vivax 34	BAL44585	vivax MS477		
BAL44532	vivax T58	BAL44586	vivax 827-31		
BAL44533	vivax T101	BAL44587	vivax 827-32		
BAL44534	vivax T102	BAL44588	vivax 827-47		
BAL44535	vivax T103	BAL44589	vivax 827-52		
BAL44536	vivax T104	BAL44590	vivax 827-53		
BAL44537	vivax T105	BAL44591	vivax 828-5		

**A.4 Accession number of P41, P12, P48/45, and P230 retrieved from PlasmoDB**

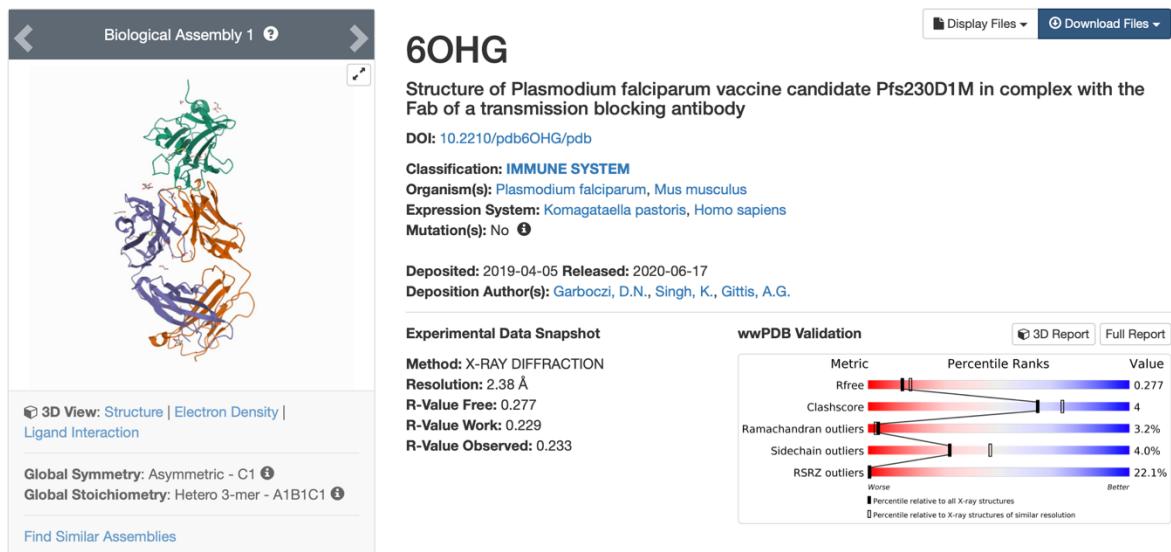
P41		P12	
Accession number	Species	Accession number	Species
PBANKA_1002600	<i>P. berghei</i> ANKA	PBANKA_0111000	<i>P. berghei</i> ANKA
PF3D7_0404900	<i>P. falciparum</i> 3D7	PF3D7_0612700	<i>P. falciparum</i> 3D7
Pf7G8_040010200	<i>P. falciparum</i> 7G8	Pf7G8_060017700	<i>P. falciparum</i> 7G8
PfCD01_040010000	<i>P. falciparum</i> CD01	PfCD01_060018200	<i>P. falciparum</i> CD01
PfDd2_040010300	<i>P. falciparum</i> Dd2	PfDd2_060017600	<i>P. falciparum</i> Dd2
PfGA01_040009900	<i>P. falciparum</i> GA01	PfGA01_060017600	<i>P. falciparum</i> GA01
PfGB4_040010400	<i>P. falciparum</i> GB4	PfGB4_060017300	<i>P. falciparum</i> GB4
PfGN01_040010300	<i>P. falciparum</i> GN01	PfGN01_060018500	<i>P. falciparum</i> GN01
PfHB3_040009100	<i>P. falciparum</i> HB3	PfHB3_060017000	<i>P. falciparum</i> HB3
PfIT_040009600	<i>P. falciparum</i> IT	PfIT_060016700	<i>P. falciparum</i> IT
PfKE01_040011600	<i>P. falciparum</i> KE01	PfKE01_060018900	<i>P. falciparum</i> KE01
PfKH01_040010000	<i>P. falciparum</i> KH01	PfKH01_060019900	<i>P. falciparum</i> KH01
PfKH02_040009800	<i>P. falciparum</i> KH02	PfKH02_060019000	<i>P. falciparum</i> KH02
PfML01_040010800	<i>P. falciparum</i> ML01	PfML01_060016700	<i>P. falciparum</i> ML01
PfSD01_070020500	<i>P. falciparum</i> SD01	PfSD01_060016700	<i>P. falciparum</i> SD01
PfSN01_040010000	<i>P. falciparum</i> SN01	PfSN01_060017700	<i>P. falciparum</i> SN01
PFTG01_040010300	<i>P. falciparum</i> TG01	PFTG01_060018200	<i>P. falciparum</i> TG01
PKNH_0303000	<i>P. knowlesi</i> strain H <i>P. knowlesi</i> strain Malayan Strain	PKNH_1137300	<i>P. knowlesi</i> strain H <i>P. knowlesi</i> strain Malayan Strain
PKNOH_S02295300	Strain Pk1 A	PKNOH_S130203800	Malayan Strain Pk1 A
PmUG01_03015000	<i>P. malariae</i> UG01	PmUG01_11050500	<i>P. malariae</i> UG01
PocGH01_03012400	<i>P. ovale</i> curtisi GH01	PocGH01_11044200	<i>P. ovale</i> curtisi GH01
PVP01_0304300	<i>P. vivax</i> P01	PVP01_1136400	<i>P. vivax</i> P01
PVX_000995	<i>P. vivax</i> Sal-1	PVX_113775	<i>P. vivax</i> Sal-1
P48/45		P230	
Accession number	Species	Accession number	Species
PBANKA_1359600	<i>P. berghei</i> ANKA	PBANKA_0306100	<i>P. berghei</i> ANKA
PF3D7_1346700	<i>P. falciparum</i> 3D7	PF3D7_0209000	<i>P. falciparum</i> 3D7
Pf7G8_130051100	<i>P. falciparum</i> 7G8	Pf7G8_020013700	<i>P. falciparum</i> 7G8
PfCD01_130052300	<i>P. falciparum</i> CD01	PfCD01_020013900	<i>P. falciparum</i> CD01
PfDd2_130052500	<i>P. falciparum</i> Dd2	PfDd2_020011800	<i>P. falciparum</i> Dd2
PfGA01_130052800	<i>P. falciparum</i> GA01	PfGA01_020012000	<i>P. falciparum</i> GA01
PfGB4_130052600	<i>P. falciparum</i> GB4	PfGB4_020012000	<i>P. falciparum</i> GB4
PfGN01_130053400	<i>P. falciparum</i> GN01	PfGN01_020014300	<i>P. falciparum</i> GN01
PfHB3_130053000	<i>P. falciparum</i> HB3	PfHB3_020013700	<i>P. falciparum</i> HB3
PfIT_130052000	<i>P. falciparum</i> IT	PfIT_020013700	<i>P. falciparum</i> IT
PfKE01_130052300	<i>P. falciparum</i> KE01	PfKE01_020011500	<i>P. falciparum</i> KE01
PfKH01_130050700	<i>P. falciparum</i> KH01	PfKH01_020014200	<i>P. falciparum</i> KH01
PfKH02_130049600	<i>P. falciparum</i> KH02	PfKH02_020013000	<i>P. falciparum</i> KH02
PfML01_130050800	<i>P. falciparum</i> ML01	PfML01_020011900	<i>P. falciparum</i> ML01
PfSD01_130053400	<i>P. falciparum</i> SD01	PfSD01_020014500	<i>P. falciparum</i> SD01
PfSN01_130049700	<i>P. falciparum</i> SN01	PfSN01_020012000	<i>P. falciparum</i> SN01
PFTG01_130052400	<i>P. falciparum</i> TG01	PFTG01_020014000	<i>P. falciparum</i> TG01
PKNH_1254400	<i>P. knowlesi</i> strain H <i>P. knowlesi</i> strain Malayan Strain	PKNH_0412100	<i>P. knowlesi</i> strain H <i>P. knowlesi</i> strain Malayan Strain
PKNOH_S09544400	Strain Pk1 A	PKNOH_S08497000	Strain Pk1 A
PmUG01_12018200	<i>P. malariae</i> UG01	PmUG01_04023100	<i>P. malariae</i> UG01
PocGH01_12016400	<i>P. ovale</i> curtisi GH01	PocGH01_04021000	<i>P. ovale</i> curtisi GH01
PVP01_1208100	<i>P. vivax</i> P01	PVP01_0415800	<i>P. vivax</i> P01



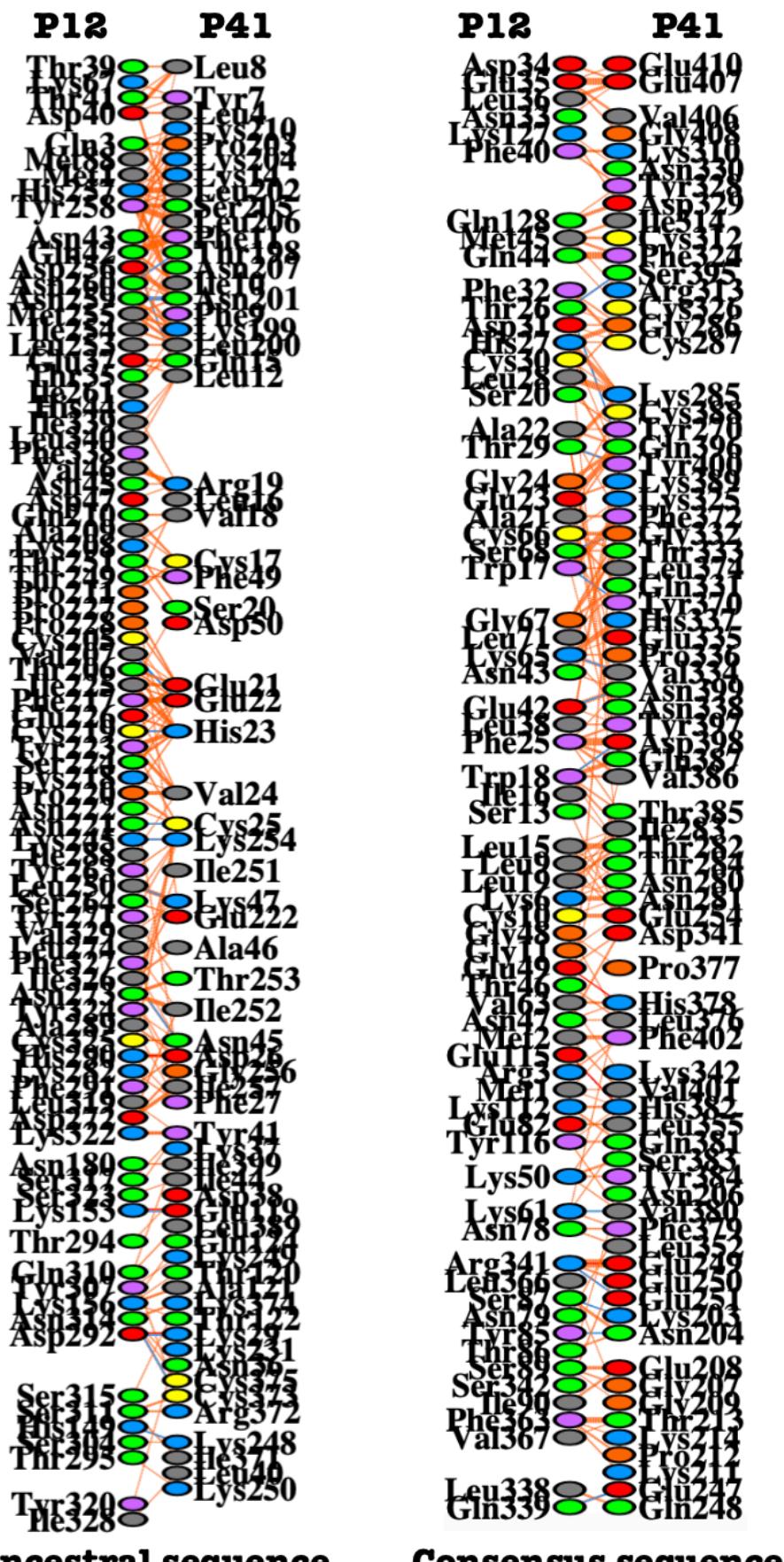
**A.5 Phylogenetic tree of P12 protein sequences. (A) Natural sequences and (B) Natural sequence with ancestral and consensus sequences.**

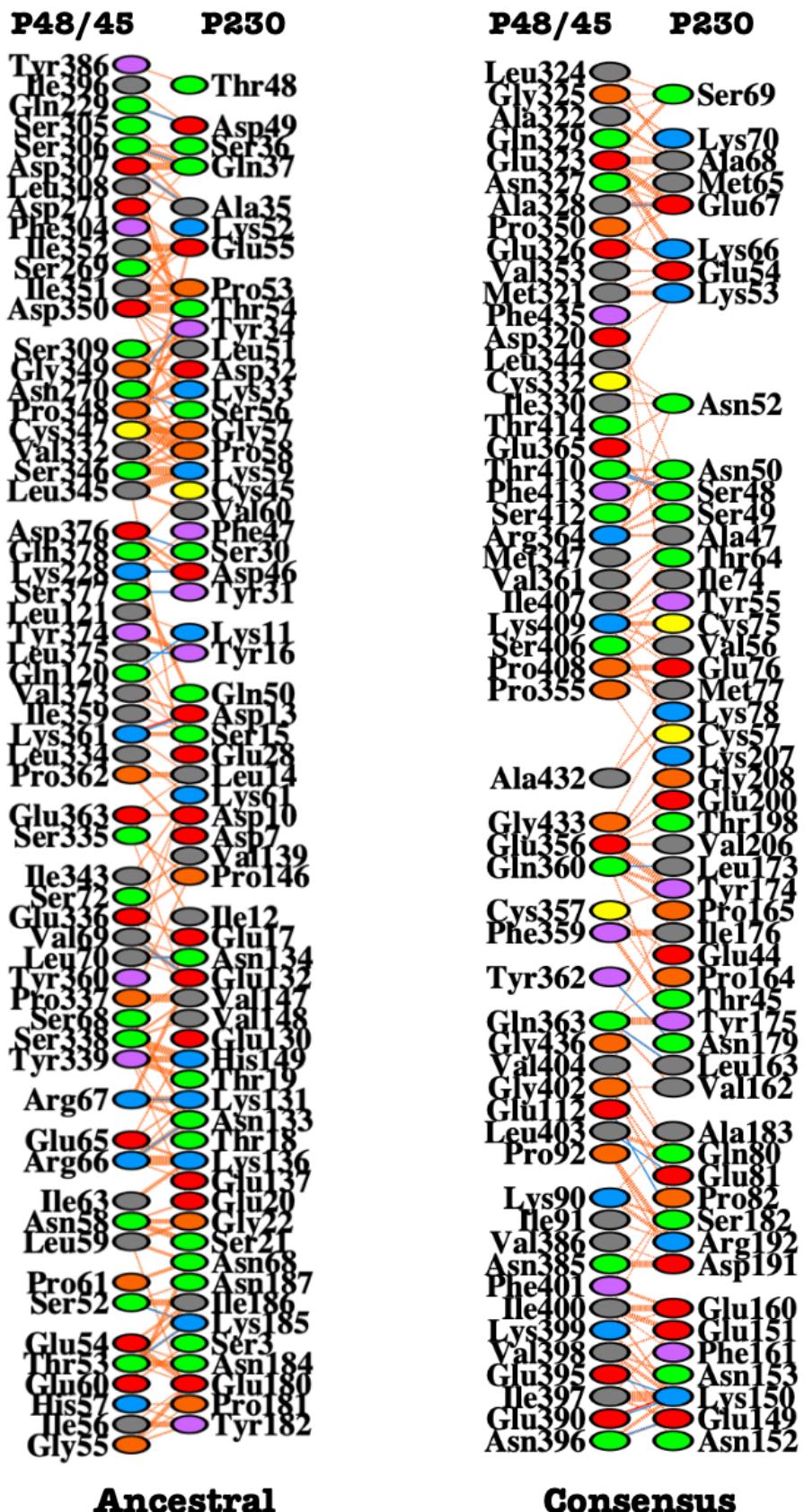


A.6 The 3D protein structure of P12 ancestral and consensus sequence.



A.7 The reference structure of P230 retrieved from RSCB PDB with accession number 6OHG.





A.9 The detail of molecular interactions of P48/45-P230 protein complexes.

**A.10 Prediction of MHC-I binding epitopes of P41ancestral and consensus**

Allele	Ancestral sequence				Consensus sequence			
	Peptide	IC50 (nM)	% Rank	Binding Level	Peptide	IC50 (nM)	% Rank	Binding Level
HLA-A01:01	YSNKRGSKY	160.76	0.25	SB	KLSIDVLLY	1109.00	0.80	WB
	LLDNKDKLY	168.78	0.25	SB	STVSQYDNY	2157.34	1.00	WB
	QISINDLLY	207.30	0.25	SB	ETDLQTLMP	2169.05	1.00	WB
HLA-A02:01	KLDEHLSRA	24.07	1.00	SB	LLLALLHLV	4.83	0.12	SB
	LLIYLFIFL	24.20	1.00	SB	HLVRALYRV	20.35	0.80	SB
	YLLDNKDKL	30.87	1.50	SB	TLYGDTLLI	36.51	1.50	SB
HLA-A03:01	KLYCEINAK	20.35	0.05	SB	TLLISPTVK	55.98	0.40	SB
	ALYRLKKYK	20.69	0.05	SB	KLSIDVLLY	77.03	0.50	SB
	GIMKVVVQK	47.34	0.25	SB	YLKKRRLTK	82.19	0.50	SB
HLA-A26:01	TLIPGYTSY	192.18	0.40	SB	STVSQYDNY	137.41	0.30	SB
	ELVGFKCIY	282.17	0.50	SB	TVKQSTTFY	288.34	0.50	SB
	DLKTLIPGY	326.55	0.50	SB	DLQTLMPGY	337.32	0.50	SB
HLA-B07:02	LPHFITKPY	819.15	2.00	WB	RVKKIRNII	1033.69	2.00	WB
	KPQKFEGGA	1804.62	3.00	WB	KPFDSVTFI	1376.93	3.00	WB
	KPFDKITFI	2430.01	3.00	WB	SPTVKQSTT	1391.91	3.00	WB
HLA-B08:01	LIKKRKSVV	17.21	0.05	SB	YLKKRRLTK	125.34	0.50	SB
	KLIKKRKSV	89.14	0.40	SB	LLHLVRQQL	311.03	1.00	WB
	YRLKKYKDL	128.78	0.50	SB	RALYRVKKI	484.69	1.50	WB
HLA-B39:01	SQISINDLL	113.71	0.80	WB	KRLVLLAL	293.06	1.50	WB
	STNYFSNPL	570.09	2.00	WB	TKLNLPKSL	649.13	2.00	WB
	SKYAFYLKL	618.28	2.00	WB	SKLSIDVLL	902.93	3.00	WB
HLA-B40:01	KEVKLDEHL	62.04	0.50	SB	KETDLQTLM	83.09	0.80	WB
	VESGESDVI	130.88	0.80	WB	GEFVGFKCI	107.14	0.80	WB
	GEVTVGKEV	274.64	1.00	WB	MEVVVPKHL	209.55	1.00	WB
HLA-B58:01	ITFICPNKI	659.75	3.00	WB	VSQYDNYVF	128.08	1.00	WB
	RGSKYAFYL	663.33	3.00	WB	KSLNIPNDI	350.34	2.00	WB
	VTNKVKPKI	922.68	3.00	WB	KLSIDVLLY	601.78	3.00	WB
HLA-B15:01	TLIPGYTSY	24.20	0.15	SB	TLMPGYISY	28.93	0.25	SB
	KQNTTFYCF	30.71	0.25	SB	KQSTTFYCF	30.71	0.25	SB
	YSNKRGSKY	126.70	1.50	WB	LQMEDLKYY	66.56	0.80	WB

**A.11 Prediction of MHC-I binding epitopes of P48/45 ancestral and consensus**

Allele	Ancestral sequence				Consensus sequence			
	Peptide	IC50 (nM)	% Rank	Binding Level	Peptide	IC50 (nM)	% Rank	Binding Level
HLA-A01:01	FSNNLFFFY	8.07	0.01	SB	CSIYSYFIY	398.91	0.40	SB
	CSIHSYFIY	299.47	0.40	SB	YTLSLKPVY	1022.56	0.80	WB
	SSDLSSHTF	307.68	0.40	SB	LVQVNVLKY	1255.95	0.80	WB
HLA-A02:01	FLAKLFILL	7.01	0.25	SB	VLPECFFQV	5.04	0.12	SB
	MLYFFSNNL	14.79	0.80	SB	FLAKIFILL	7.77	0.25	SB
	AIFKAPIYV	17.03	0.80	SB	SIFKAPVYV	19.39	0.80	SB
HLA-A03:01	SAYYGFLAK	26.25	0.10	SB	AGYFGFLAK	37.92	0.17	SB
	ASFTCICKKK	39.38	0.20	SB	STIYTLSK	54.19	0.30	SB
	ALVHVRVLK	50.24	0.30	SB	TSFTCICRK	58.14	0.40	SB
HLA-A26:01	MTIKIGSAY	5.47	0.01	SB	MSVKIAAGY	78.29	0.20	SB
	ELNTEVSGF	382.02	0.50	SB	ELNKDVSGF	236.04	0.40	SB
	SVLCSIHSY	759.40	0.80	WB	EKIENMGLV	771.82	0.80	WB
HLA-B07:02	SPSKFKLL	110.68	0.80	WB	IPGSKFKML	131.59	1.00	WB
	KPKYEKKVI	917.70	2.00	WB	LPEKCFQTV	207.30	1.50	WB
	SALVHVRVL	1033.69	2.00	WB	KPVYTKKLI	614.95	2.00	WB
HLA-B08:01	SPSKFKLL	17.40	0.05	SB	MLKRQLANL	18.97	0.07	SB
	SALVHVRVL	193.22	0.80	WB	IPGSKFKML	123.99	0.50	SB
	YMTIKIGSA	340.99	1.00	WB	NVKGRVALV	677.84	1.50	WB
HLA-B39:01	AHINCNVEL	89.14	0.80	WB	LQDGELVVL	68.75	0.50	SB
	SNIDGRSAL	302.73	1.50	WB	AQITCSIEL	182.06	1.00	WB
	NKDIEFFCL	311.03	1.50	WB	SNVKGRVAL	487.32	2.00	WB
HLA-B40:01	IELKEGELL	60.71	0.50	SB	AELGENAQI	110.68	0.80	WB
	NEYVSPDEL	121.34	0.80	WB	GEVLPECFF	209.55	1.00	WB
	LEPSEIVYL	245.15	1.00	WB	AQITCSIEL	446.91	1.50	WB
HLA-B58:01	FSNNLFFFY	49.97	0.80	SB	MSVKIAAGY	41.35	0.50	SB
	GSIPQTASF	51.89	0.80	WB	KSNKLDLEL	55.08	0.80	WB
	CSIHSYFIY	56.59	0.80	WB	CSIYSYFIY	67.28	0.80	WB
HLA-B15:01	MTIKIGSAY	27.86	0.20	SB	SLVCSIHSY	40.90	0.40	SB
	GSIPQTASF	29.09	0.25	SB	GSIPKTTSF	47.85	0.50	SB
	FSNNLFFFY	97.73	1.00	WB	MSVKIAAGY	59.41	0.80	WB

**A.12** Prediction of MHC-I binding epitopes of P230 ancestral and consensus

Allele	Ancestral sequence				Consensus sequence			
	Peptide	IC50 (nM)	% Rank	Binding Level	Peptide	IC50 (nM)	% Rank	Binding Level
HLA-A01:01	ELDKIDLSY	25.69	0.07	SB	ASSNTNKEY	1834.15	1.00	WB
	VSEDSYDKY	77.03	0.15	SB	ASDGVFDFKV	2469.77	1.00	WB
	IVEVYVEPY	421.09	0.40	SB	ASDPEVETF	3289.87	1.50	WB
HLA-A02:01	KLYDNIEYV	2.33	0.01	SB	RRAALIHGV	5.32	0.15	SB
	LLSKLIYGL	8.71	0.30	SB	VLQASDPEV	26.53	1.00	SB
	YVPKKSPYV	26.97	1.00	SB	VIEFLPPV	103.72	3.00	WB
HLA-A03:01	KLKEKLLSK	33.85	0.15	SB	ITMKKEASKK	68.75	0.40	SB
	KVKIICPLK	76.20	0.50	SB	GSMEFFPKK	75.38	0.50	SB
	KSPYVVLTK	151.47	0.80	WB	KVKIICPTK	145.05	0.80	WB
HLA-A26:01	DTAVSEDSY	1079.41	1.00	WB	EVNEKENNF	1006.10	1.00	WB
	VVHKATVFY	2430.01	2.00	WB	TTIKGDGNV	1765.99	1.50	WB
	ELDKIDLSY	4335.14	3.00	WB	EFFPKKAPY	1794.89	1.50	WB
HLA-B07:02	LPSVGVDDEL	153.95	1.00	WB	FPKKAPYVT	595.31	2.00	WB
	VPKKSPYVV	184.04	1.00	WB	CPTKYADVI	763.52	2.00	WB
	KPTESGPKV	394.62	1.50	WB	APYVTLVNA	1904.94	3.00	WB
HLA-B08:01	GPKVKKCEV	267.31	0.80	WB	SLKEKRLAA	15.20	0.05	SB
	EPLIKVKII	273.16	0.80	WB	LKEKRLAAL	194.27	0.80	WB
	VPKKSPYVV	860.02	2.00	WB	EPLVKVKII	356.07	1.00	WB
HLA-B39:01	IKVKIICPL	356.07	1.50	WB	CEMKIQEPL	792.98	3.00	WB
	HKATVFYFI	621.64	2.00	WB	NHGDRGVA	1121.07	3.00	WB
	CEVKVNEPL	2180.81	4.00	WB	LKEKRLAAL	1318.61	3.00	WB
HLA-B40:01	CEVKVNEPL	10.07	0.10	SB	CEMKIQEPL	11.60	0.10	SB
	KEGVIEFTL	22.68	0.25	SB	IEFVLPPVL	17.12	0.17	SB
	IEFTLPPVV	146.63	0.80	WB	KEASKKVVI	301.10	1.00	WB
HLA-B58:01	VVHKATVFY	3236.91	5.00	WB	VAAITIEPY	323.04	2.00	WB
	KATVFYFIC	3646.02	6.00	WB	ASDPEVETF	361.90	2.00	WB
	LSYETTESG	4752.74	6.00	WB	KVVICEMKI	1269.61	3.00	WB
HLA-B15:01	VVHKATVFY	147.43	1.50	WB	VIKYGSMEF	47.34	0.50	SB
	ASQNTNKEY	638.68	4.00	WB	VAAITIEPY	113.71	1.50	WB
	GNRGIVEVY	792.98	4.00	WB	ASSNTNKEY	525.66	4.00	WB

**A.13 Prediction of MHC-II binding epitopes of P41 ancestral and consensus**

Allele	Ancestral sequence				Consensus sequence			
	Peptide	IC50 (nM)	% Rank	Binding Level	Peptide	IC50 (nM)	% Rank	Binding Level
DRB1_0301	GKEVKLDEHLSRALY	97.13	0.03	SB	VEKAQIDEHLVRALY	60.48	1.44	SB
	VGKEVKLDEHLSRAL	96.93	0.03	SB	NVEKAQIDEHLVRA	58.03	1.57	SB
	TVGKEVKLDEHLSRA	96.56	0.04	SB	GNVEKAQIDEHLVRA	54.97	1.74	SB
DRB3_0101	FTKEKYLLDNKDKLY	58.43	0.67	SB	LDDISADKMESSKLS	11.54	4.43	WB
	KEKYLLDNKDKLYCE	56.40	0.72	SB	IMKVVVSTNNNTITKG	10.61	4.70	WB
	TKEKYLLDNKDKLYC	50.95	0.88	SB	TLDDISADKMESSKL	10.10	4.86	WB
DRB5_0101	EPEIVTNKVKPKIDG	56.06	1.45	SB	VKKIRNIIEREKNKN	68.99	0.77	SB
	LKKYKDLMEREGKVE	51.45	1.77	SB	MPGYISYGNKQKGKY	62.55	1.06	SB
	DEPEIVTNKVKPKID	49.52	1.93	SB	RVKKIRNIIEREKNK	62.00	1.09	SB
HLA-DPA10201-	VCDFTKEKYLLDNKD	19.80	0.57	SB	CPKKIGAQCFQNVNT	14.58	1.30	SB
DPB10101	HVCDFTKEKYLLDNK	15.82	1.06	SB	PKKIGAQCFQNVNTL	13.04	1.69	SB
	GSTIYGDTLLISPKV	15.35	1.14	SB	EGGESDIVAKSFQES	13.02	1.69	SB
HLA-DPA10301-	VCDFTKEKYLLDNKD	31.58	0.47	SB	CPKKIGAQCFQNVNT	26.04	0.82	SB
DPB10402	GSTIYGDTLLISPKV	28.30	0.66	SB	EGGESDIVAKSFQES	23.37	1.08	SB
HLA-DPB11401	YGSTIYGDTLLISPK	26.97	0.75	SB	PKKIGAQCFQNVNTL	20.35	1.10	SB
HLA-DPA10201-	LIKKRKSVVVDDDEN	13.24	1.99	SB	LHLVRQQQLPAHAEEH	14.04	1.75	SB
DPB11401	IKKRKSVVVDDDEN	13.09	2.04	WB	HLVRQQQLPAHAEEHI	13.48	1.92	SB
	KPKIDGTEPAKDEVV	10.18	3.18	WB	ENAKKKSTVNVDLK	11.17	2.72	WB
HLA-DQA10301-	PKDILDVDPEPEPED	7.99	0.01	SB	ILNYDVYTNPEDEEE	2.63	0.70	SB
DQB10302	IPKDILDVDPEPEPE	5.77	0.06	SB	PEDEEELVIEEEQEE	2.56	0.75	SB
	NIPKDILDVDPEPEP	4.04	0.21	SB	EDEEEELVIEEEQEEE	2.41	0.86	SB
HLA-DQA10101-	LYRLKKYKDLMEREK	2.89	1.15	SB	CFQNVNTLDDISADK	3.35	0.84	SB
DQB10501	IPGYTSYSNKRGSKY	2.11	2.10	WB	FQNVNTLDDISADKM	3.21	0.93	SB
	ESSQISINDLLYGST	2.05	2.22	WB	ILNYDVYTNPEDEEE	3.17	0.95	SB
HLA-DQA10501-	ELNVESGESDVKNS	61.43	0.92	SB	PKHLGGNVEKAQID	91.20	0.02	SB
DQB10301	FELNVESGESDVKN	58.32	1.10	SB	VPKHLGGNVEKAQI	86.72	0.08	SB
	IFELNVESGESDVK	44.78	2.12	WB	KHLGGNVEKAQIDE	84.66	0.12	SB
HLA-DRB1*07:01	FDKITFICPNKIGAH	50.28	1.77	SB	NEKEYCVVNAKPFDS	66.16	0.86	SB
	PFDKITFICPNKIGA	45.45	2.13	WB	EKEYCVVNAKPFDSV	65.26	0.90	SB
	EKLIKKRKSVVVDDD	39.41	2.66	WB	IMKVVVSTNNNTITKG	64.79	1.33	SB

**A.14 Prediction of MHC-II binding epitopes of P48/45 ancestral and consensus**

Allele	Ancestral sequence				Consensus sequence			
	Peptide	IC50 (nM)	% Rank	Binding Level	Peptide	IC50 (nM)	% Rank	Binding Level
DRB1_0301	GIHNLEPDIVERRSV	94.75	0.10	SB	PSKIVYLDALQLNIGN	62.83	1.32	SB
	YYEDVHGDNKIKLFG	93.91	0.12	SB	SKIVYLDALQLNIGNV	60.61	1.43	SB
	YEDVHGDNKIKLFGI	93.72	0.12	SB	PLSLYKSNKIVYHKN	56.97	1.63	SB
DRB3_0101	HSYFIYDKIKLIIPK	84.51	0.15	SB	PEELNKDVSGFFGFK	58.15	0.67	SB
	IHSYFIYDKIKLIIIP	78.03	0.24	SB	GCFNFSSDKSTKHNF	51.21	0.87	SB
	SYFIYDKIKLIIPKK	65.98	0.49	SB	HGCNFSSDKSTKHNF	49.52	0.93	SB
DRB5_0101	HKDFAIFKAPIYVKS	77.04	0.46	SB	VKIFGLVGSIPIKTT	79.98	0.35	SB
	YHKDFAIFKAPIYVK	71.83	0.65	SB	IVKIFGLVGSIPIKTT	75.05	0.53	SB
	KDFAIFKAPIYVKS	62.04	1.08	SB	NIVKIFGLVGSIPIKT	62.37	1.07	SB
HLA-DPA10201-	HSYFIYDKIKLIIPK	29.07	0.18	SB	GDEVKYVPPEELNKD	25.79	0.27	SB
DPB10101	NKIIYHKDFAIFKAP	25.83	0.27	SB	KGDEVKYVPPEELNK	21.14	0.48	SB
HLA-DPA10301-	SNKIIYHKDFAIFKA	25.02	0.30	SB	YSYFIYDKIKLTIPK	18.84	0.65	SB
HLA-DPA10201-	HSYFIYDKIKLIIPK	40.33	0.20	SB	YSYFIYDKIKLTIPK	30.88	0.51	SB
DPB10402	IHSYFIYDKIKLIIIP	32.16	0.44	SB	GDEVKYVPPEELNKD	26.81	0.76	SB
HLA-DPB11401	NKIIYHKDFAIFKAP	30.80	0.51	SB	IYSYFIYDKIKLTIP	25.22	0.89	SB
HLA-DPA10201-	YSERIITISPNNKD	27.75	0.29	SB	GDEVKYVPPEELNKD	16.71	1.20	SB
DQA10301-	DYSERIITISPNNK	25.40	0.41	SB	KGDEVKYVPPEELNK	13.07	2.05	WB
DPB11401	SERIITISPNNKDI	18.31	0.98	SB	PSKIVYLDALQLNIGN	10.22	3.16	WB
HLA-DQA10301-	PHKIVSVNLTDKYP	4.11	0.19	SB	EKCFQTVYTYEKRT	3.46	0.34	SB
DQB10302	IGNIEYYEDVHGDNK	3.36	0.36	SB	PEKCFQTVYTYEKR	3.40	0.35	SB
HLA-DQB10302	TEGIHNLEPDIVERR	3.22	0.40	SB	GDEVKYVPPEELNKD	3.00	0.49	SB
HLA-DQA10101-	IGNIEYYEDVHGDN	23.72	0.00	SB	NIGNVEYFEDSKGEN	11.80	0.03	SB
DQA10501-	NIGNIEYYEDVHGDN	19.50	0.00	SB	IGNVEYFEDSKGENI	11.23	0.04	SB
DQB10501	GNIEYYEDVHGDNKI	16.85	0.00	SB	LNIGNVEYFEDSKGE	7.46	0.13	SB
HLA-DQA10501-	LFGIVGSIPQTASFT	64.80	0.73	SB	GDEVKYVPPEELNKD	31.14	4.02	WB
DQB10301	KLFGIVGSIPQTAS	62.44	0.86	SB	KIGYMSVKIAAGYFG	26.95	4.97	WB
HLA-DQB10301	FGIVGSIPQTASFTC	57.76	1.12	SB	APVYVKSADVTAECS	24.96	5.52	WB
HLA-DRB1*07:01	FKAPIYVKSNDVNAE	61.85	1.07	SB	YDKIKLTIPKKIPGS	92.29	0.11	SB
DRB1*07:01	HKDFAIFKAPIYVKS	55.54	1.42	SB	IYDKIKLTIPKKIPG	91.84	0.12	SB
	SNKIIYHKDFAIFKA	54.10	1.50	SB	FIYDKIKLTIPKKIP	87.74	0.21	SB

**A.15 Prediction of MHC-II binding epitopes of P230 ancestral and consensus**

Allele	Ancestral sequence				Consensus sequence			
	Peptide	IC50 (nM)	% Rank	Binding Level	Peptide	IC50 (nM)	% Rank	Binding Level
DRB1_0301	NKEYVCDFTDQLKPT	38.38	2.94	WB	ETTIKGDG NVLQASD	92.16	0.18	SB
	TNKEYVCDFTDQLKP	35.22	3.23	WB	FETTIKGDG NVLQAS	89.35	0.28	SB
	NRGIVEVYVEPYGNK	32.86	3.45	WB	AFETTIKGDG NVLQA	82.98	0.47	SB
DRB3_0101	NKEYVCDFTDQLKPT	81.41	0.19	SB	NKEYVCD FVKHITMK	70.56	0.38	SB
	TNKEYVCDFTDQLKP	79.94	0.21	SB	TNKEYVCD FVKHITM	61.64	0.59	SB
	NTNKEYVCDFTDQLK	66.78	0.47	SB	NTNKEYVCD FVKHIT	58.32	0.67	SB
DRB5_0101	TDQLKPTESGPKVKK	61.98	1.09	SB	KKKLYYICDNGKSAD	57.05	1.38	SB
	KSPYVVLTKETKLK	52.06	1.72	SB	FVKHITMKEASKKVV	56.27	1.44	SB
	PYVVLTKETKLKEK	51.55	1.77	SB	VKHITMKEASKKVV	53.99	1.60	SB
HLA-DPA10201-	NRGIVEVYVEPYGNK	16.79	0.90	SB	DRGVAAITIEPYGQS	17.60	0.80	SB
DPB10101	SVEKLYDNI EYVPKK	15.56	1.10	SB	GDRGVAAITIEPYGQ	16.27	0.99	SB
HLA-DPA10301-	GNRGIVEVYVEPYGN	13.65	1.51	SB	HGDRGVAAITIEPYG	12.12	1.99	SB
HLA-DPA10301-	KSPYVVLTKETKLK	19.53	1.68	SB	DRGVAAITIEPYGQS	18.49	1.91	SB
DPB10402	SVEKLYDNI EYVPKK	17.27	2.24	WB	VAAITIEPYGQSVKG	16.50	2.45	WB
DPB11401	KEGVIEFTLPPVVHK	16.48	2.45	WB	GVAAITIEPYGQSVK	15.48	2.75	WB
HLA-DPA10201-	EGVIEFTLPPVVHK	11.56	2.55	WB	KEKRLAALI HGVIIT	18.70	0.94	SB
DPA10301-	SVEKLYDNI EYVPKK	10.33	3.10	WB	LKEKRLAALI HGVI	18.31	0.98	SB
DPB11401	EGVIEFTLPPVVHK	10.21	3.17	WB	SLKEKRLAALI HGVI	16.45	1.24	SB
HLA-DQA10301-	DSYDKYASQNTNKEYV	3.31	0.38	SB	DRGVAAITIEPYGQS	5.68	0.06	SB
DQA10301-	NRGIVEVYVEPYGNK	3.02	0.48	SB	GDRGVAAITIEPYGQ	5.32	0.08	SB
DQB10302	NRGIVEVYVEPYGN	2.39	0.88	SB	HGDRGVAAITIEPYG	3.80	0.25	SB
HLA-DQA10101-	NRGIVEVYVEPYGNK	4.41	0.46	SB	DRGVAAITIEPYGQS	2.88	1.16	SB
DQA10101-	RGIVEVYVEPYGNKI	3.24	0.91	SB	EKGVIEFVLPPVLKE	2.63	1.38	SB
DQB10501	NRGIVEVYVEPYGN	30.07	1.06	SB	DPEVETFASSNTNKE	2.56	1.43	SB
HLA-DQA10501-	ENNFKEGVIEFTLPP	78.89	0.22	SB	ENNFKEGVIEFVLPP	64.55	0.75	SB
DQA10501-	KENNFKEGVIEFTLP	69.01	0.56	SB	KENNFEKG VIEFVLP	52.03	1.48	SB
DQB10301	NNFKEGVIEFTLPPV	56.99	1.16	SB	DRGVAAITIEPYGQS	51.36	1.53	SB
HLA-DRB1*07:01	EGVIEFTLPPVVHK	73.83	0.58	SB	KGVIEFVLPPVLKEK	46.42	2.05	WB
DRB1*07:01	KEGVIEFTLPPVVHK	70.12	0.71	SB	EVETFASSNTNKEYV	36.76	2.94	WB
	FKEGVIEFTLPPV VH	51.79	1.66	SB	EKGVIEFVLPPVLKE	36.10	3.01	WB

**A.16** The detail of hydrogen bond interaction of P12-P41 protein complexes where some of the P41 residue were part of predicted epitope.

Ancestral				Consensus			
P12 residue	P41 residue	Epitope? (Y/N)	Distance	P12 residue	P41 residue	Epitope? (Y/N)	Distance
GLN42	LEU12	Y	2,87	TRP17	TYR370	Y	3,21
HIS149	LYS248	Y	2,32	TRP17	ASP398	N	2,8
LYS153	GL119	N	2,42	THR26	GLN396	N	2,99
VAL207	GLU22	Y	3,34	THR26	SER395	N	2,49
VAL207	GLU22	Y	2,98	THR29	TYR400	N	3,12
CYS219	HIS23	Y	2,42	GLU42	ASN399	N	2,01
ASN221	CYS25	Y	2,01	LYS61	VAL380	Y	1,61
LYS245	LYS254	Y	2,75	LYS65	VAL334	Y	2,3
LEU250	LYS47	Y	1,69	SER68	GLY332	Y	3,1
ASN259	ASN201	Y	1,73	TYR85	ASN204	N	2,91
ASN259	ASN201	Y	3,2	SER87	LYS203	N	3,23
ASN260	THR198	Y	3,04	GLN339	GLU247	Y	2,74
ASP292	LYS29	N	2,38	ARG341	GLU251	Y	2,15
ASN314	CYS373	Y	3,2				
ILE324	ASN45	N	1,86				

**A.17** The detail of hydrogen bond interaction of P48/45-P230 protein complexes where some of the P48/45 and P230 residue were part of predicted epitope.

Ancestral					Consensus				
P48/45 residue	Epitope? (Y/N)	P230 residue	Epitope? (Y/N)	Distance	P12 residue	Epitope? (Y/N)	P41 residue	Epitope? (Y/N)	Distance
SER52	N	LYS185	N	2,28	GLU112	Y	SER182	N	2,9
THR53	N	LYS185	N	2,9	ALA328	Y	GLU67	N	2,68
ARG66	Y	ASN133	Y	2,86	GLN360	N	LEU173	N	2,22
ARG67	Y	LYS131	Y	1,84	TYR362	Y	ASN179	N	3,1
VAL69	Y	GLU132	Y	2,87	GLN363	Y	LEU163	N	3,19
LEU70	Y	ASN134	Y	3,18	GLU390	N	LYS150	Y	2,97
GLN120	N	LYS11	N	3,32	GLU395	Y	LYS150	Y	2,93
LYS228	N	ASP46	Y	2,77	ASN396	Y	GLU149	Y	1,9
GLN229	N	ASP49	N	3,23	LEU403	N	GLU81	Y	3,17
ASN270	N	THR54	N	2,34	THR410	Y	SER48	N	1,43
ASN270	N	SER56	N	2,97	THR410	Y	SER48	N	1,43
SER306	N	GLN37	Y	2,73					
ASP307	N	ALA35	Y	2,38					
LYS361	Y	ASP13	N	1,91					
LEU375	N	TYR16	N	3,16					
ASP376	N	SER30	N	2,63					
SER377	N	TYR31	Y	2,99					