STUDI *IN SILICO* DESAIN VAKSIN SUBUNIT PROTEIN DALAM UPAYA PENGEMBANGAN VAKSIN *MONKEYPOX* (MPOX)

SKRIPSI

REGINA YASMINE HERMAN
A211030

SEKOLAH TINGGI FARMASI INDONESIA
YAYASAN HAZANAH
BANDUNG
2025

STUDI *IN SILICO* DESAIN VAKSIN SUBUNIT PROTEIN DALAM UPAYA PENGEMBANGAN VAKSIN *MONKEYPOX* (MPOX)

SKRIPSI

Sebagai salah satu syarat untuk memperoleh gelar Sarjana Farmasi

REGINA YASMINE HERMAN
A211030

SEKOLAH TINGGI FARMASI INDONESIA
YAYASAN HAZANAH
BANDUNG
2025

STUDI *IN SILICO* DESAIN VAKSIN SUBUNIT PROTEIN DALAM UPAYA PENGEMBANGAN VAKSIN *MONKEYPOX* (MPOX)

REGINA YASMINE HERMAN
A211030

Juli, 2025

Disetujui oleh:

Pembimbing	**Pembimbing**
Umi Baroroh, S.Si., M.Biotek	**apt. Khairunnisa Sy., M.S.Farm.**

Terimakasih kepada Ibu tercinta, keluarga, dan teman, yang selalu memberi dukungan dalam proses penyelesaian tulisan dan penelitian ini. Serta kepada seluruh karakter One Piece yang menemani jatuh bangun selama proses penelitian. Semoga tulisan ini bisa menjadi bagian dari kemajuan ilmu pengetahuan.

ABSTRAK

Monkeypox (MPOX) merupakan penyakit zoonotik yang disebabkan oleh *monkeypox virus* (MPXV) dan masih menjadi ancaman kesehatan global. Pengembangan vaksin subunit dipandang sebagai pendekatan yang lebih aman dan efisien. Penelitian ini bertujuan merancang vaksin subunit protein fusi A35R-M1R menggunakan dua variasi *linker* fleksibel, yaitu $(GGGGS)_2$ dan $(GGGGS)_3$. Desain dilakukan secara *in silico* melalui pemodelan struktur 3D menggunakan AlphaFold, prediksi epitop sel B dengan Discotope 3.0, serta simulasi dinamika molekuler dengan perangkat lunak AMBER. Hasil menunjukkan bahwa desain vaksin dengan *linker* $(GGGGS)_3$ memiliki kestabilan struktural lebih tinggi dengan nilai RMSD dan RMSF yang lebih rendah dibandingkan varian $(GGGGS)_2$. Selain itu, prediksi epitop sel B menunjukkan distribusi yang lebih tersebar pada varian $(GGGGS)_3$. Simpulan dari penelitian ini adalah bahwa panjang *linker* berpengaruh terhadap kestabilan dan penyajian epitop dalam desain vaksin fusi, dan linker $(GGGGS)_3$ memberikan hasil yang lebih optimal.

Kata kunci : *In silico*, MPOX, vaksin subunit, linker $(GGGGS)_n$

ABSTRACT

Monkeypox (mpox) is a zoonotic disease caused by the monkeypox virus (MPXV) and remains a global health threat. Subunit vaccine development is considered a safer and more efficient approach. This study aimed to design a subunit vaccine based on the fusion protein A35R-M1R using two variations of flexible linkers, namely $(GGGGS)_2$ and $(GGGGS)_3$. The design was performed in silico through 3D structural modeling using AlphaFold, B-cell epitope prediction via Discotope 3.0, and molecular dynamics simulation using the AMBER software. The results showed that the vaccine design with the $(GGGGS)_3$ linker demonstrated higher structural stability, indicated by lower RMSD and RMSF values compared to the $(GGGGS)_2$ variant. In addition, B-cell epitope prediction showed a broader epitope distribution in the $(GGGGS)_3$ variant. It was concluded that the linker length significantly affected the structural stability and epitope presentation of the vaccine construct, with $(GGGGS)_3$ yielding a more optimal outcome.

Keywords : In silico, MPOX, subunit vaccine, linker $(GGGGS)_n$

KATA PENGANTAR

Bismillahirrahmanirrahim,

Puji dan syukur penulis panjatkan ke hadirat Allah SWT atas segala berkah rahmat dan ridho-Nya penulis dapat menyelesaikan penelitian dan penulisan skripsi yang berjudul "**Studi *In Silico* Desain Vaksin Subunit Protein dalam Upaya Pengembangan Vaksin *Monkeypox* (MPOX)**".

Penelitian dan penulisan skripsi ini dilakukan untuk memenuhi salah satu syarat untuk mendapatkan gelar sarjana pada Program Studi Sarjana Farmasi Sekolah Tinggi Farmasi Indonesia.

Penulis mengucapkan terima kasih kepada dosen pembimbing Umi Baroroh, S.Si., M.Biotek., dan apt. Khairunnisa Sy, M.S.Farm., atas bimbingan, nasihat, dukungan, serta pengorbanan yang diberikan. Pada kesempatan ini, tidak lupa penulis mengucapkan terima kasih yang sebesar–besarnya kepada:

1. Dr. apt. Adang Firmansyah, M.Si., selaku Ketua Sekolah Tinggi Farmasi Indonesia,
2. Dr. apt. Diki Prayugo, M.Si., selaku Wakil Ketua I Bidang Akademik,
3. Dr. apt. Wiwin Winingsih, M.Si., selaku Ketua Program Studi Sarjana Farmasi,
4. Nur Asni Setiani, M.Si., selaku Dosen Wali yang telah banyak memberikan bimbingan dan arahan kepada penulis,
5. Seluruh staf dosen, asisten laboratorium, staf administrasi, serta jajaran karyawan Sekolah Tinggi Farmasi Indonesia, terima kasih atas ilmu, pengalaman dan bantuan yang telah diberikan selama perkuliahan,
6. Kepada teman-teman reguler sore angkatan 2021 yang sama-sama berjuang menyelesaikan studi di Sekolah Tinggi Farmasi Indonesia.

Dalam penyusunan skripsi ini masih banyak kesalahan dan kekurangan karena pengetahuan yang masih terbatas. Oleh karena itu, dengan segala kerendahan hati diharapkan masukan berupa kritik dan saran yang bersifat membangun untuk perbaikan di masa yang akan datang. Penulis berharap semoga tugas akhir ini akan memberikan manfaat bagi penulis sendiri dan juga pihak lain yang berkepentingan.

Bandung, Juli 2025

Penulis

DAFTAR ISI

DAFTAR TABEL

DAFTAR GAMBAR

DAFTAR LAMPIRAN

DAFTAR PUSTAKA

Abramson, J., Adler, J., Dunger, J., Evans, R., Green, T., Pritzel, A., Ronneberger, O., Willmore, L., Ballard, A. J., Bambrick, J., Bodenstein, S. W., Evans, D. A., Hung, C. C., O'Neill, M., Reiman, D., Tunyasuvunakool, K., Wu, Z., Žemgulytė, A., Arvaniti, E., … Jumper, J. M. (2024). Accurate Structure Prediction of Biomolecular Interactions with AlphaFold 3. *Nature*, *630*(8016), 493–500.

Apostolopoulos, V., & Chavda, V. P. (2024). Subunit Protein-Based Vaccines. In V. P. Chavda, L. K. Vora, & V. Apostolopoulos (Eds.), *Developments in Immunology Series: Advanced Vaccination Technologies for Infectious and Chronic Diseases A Guide to Vaccinology*. Academic Press.

Arya, H., & Bhatt, T. K. (2021). Role of Bioinformatics in Subunit Vaccine Design. In *Molecular Docking for Computer-Aided Drug Design Fundamentals, Techniques, Resources and Applications* (pp. 425–439). Academic Press.

Begum, J. P. S., Ngangom, L., Semwal, P., Painuli, S., Sharma, R., & Gupta, A. (2023). Emergence of Monkeypox: A Worldwide Public Health Crisis. *Human Cell*, *36*(3), 877–893.

Bhattacharya, M., Dhama, K., & Chakraborty, C. (2022). A Call for A Novel and Next-Generation Vaccine Against Monkeypox Disease. In *Annals of Medicine and Surgery* (Vol. 84). Elsevier Ltd.

Boora, S., Yadav, S., Soniya, K., Kaushik, S., Yadav, J. P., Seth, M., & Kaushik, S. (2023). Monkeypox Virus is Nature's Wake-up Call: A Bird's-eye View. *VirusDisease*, *34*(2), 191–203.

Can, H., Köseoğlu, A. E., Erkunt Alak, S., Güvendi, M., Döşkaya, M., Karakavuk, M., Gürüz, A. Y., & Ün, C. (2020). In Silico Discovery of Antigenic Proteins and Epitopes of SARS-CoV-2 for the Development of a Vaccine or a Diagnostic Approach for COVID-19. *Scientific Reports*, *10*(1).

Case *et al*. (2023). *Amber 2023*. University of California.

Chavda, V. P., Balar, P. C., & Apostolopoulos, V. (2024). History of Vaccination. In V. P. Chavda, L. K. Vora, & V. Apostolopoulos (Eds.), *Development in Immunology Series: Advanced Vaccination Technologies for Infectious and Chronic Diseases A Guide to Vaccinology*. Academic Press.

Chen, H., Chen, Z., Wu, B., Ullah, J., Zhang, T., Jia, J., Wang, H., & Tan, T. (2017). Influences of Various Peptide Linkers on the Thermotoga maritima MSB8 Nitrilase Displayed on the Spore Surface of Bacillus subtilis. *Journal of Molecular Microbiology and Biotechnology*, *27*(1), 64–71.

Elshafei, S. O., Mahmoud, N. A., & Almofti, Y. A. (2024). Immunoinformatics, Molecular Docking and Dynamics Simulation Approaches Unveil A Multi Epitope-based Potent Peptide Vaccine Candidate Against Avian Leukosis Virus. *Scientific Reports*, *14*(1).

Erdős, G., & Dosztányi, Z. (2024). Deep Learning for Intrinsically Disordered Proteins: From Improved Predictions to Deciphering Conformational Ensembles. In *Current Opinion in Structural Biology* (Vol. 89). Elsevier Ltd.

Fang, Y., Chen, X., Sun, Z., Yan, X., Shi, L., & Jin, C. (2025). Discovery and Investigation of the Truncation of the (GGGGS)n Linker and its Effect on the Productivity of Bispecific Antibodies Expressed in Mammalian Cells. *Bioprocess and Biosystems Engineering*, *48*(1), 159–170.

Firmansyah, R. P., Alimah, S. N., Artika, I. M., & Kurniatin, P. A. (2024). In Silico Phylogenetic, Physicochemical, and Structural Characteristics of Phytase Enzyme from Ten Aspergillus Species. *E-Journal Menara Perkebunan*, *92*(1).

Freyn, A. W., Atyeo, C., Earl, P. L., Americo, J. L., Chuang, G.-Y., Natarajan, H., Frey, T., Gall, J., Moliva, J. I., Hunegnaw, R., Arunkumar, G. A., Ogega, C., Nasir, A., Bennett, H., Johnson, J., Durney, M. A., Stewart-Jones, G., Hooper, J. W., Colpitts, T., … Moss, B. (2022). A Monkeypox mRNA-lipid Nanoparticle Vaccine Targeting Virus Binding, Entry, and Transmission Drives Protection Against Lethal Orthopoxviral Challenge. *BioRvix*.

Heidary, M., Kaviar, V. H., Shirani, M., Ghanavati, R., Motahar, M., Sholeh, M., Ghahramanpour, H., & Khoshnood, S. (2022). A Comprehensive Review of the Protein Subunit Vaccines Against COVID-19. In *Frontiers in Microbiology* (Vol. 13). Frontiers Media S.A.

Høie, M. H., Gade, F. S., Johansen, J. M., Würtzen, C., Winther, O., Nielsen, M., & Marcatili, P. (2024). DiscoTope-3.0: Improved B-cell Epitope Prediction Using Inverse Folding Latent Representations. *Frontiers in Immunology*, *15*.

Hollingsworth, S. A., & Dror, R. O. (2018). Molecular Dynamics Simulation for All. In *Neuron* (Vol. 99, Issue 6, pp. 1129–1143). Cell Press.

Hou, F., Zhang, Y., Liu, X., Murad, Y., Xu, J., Yu, Z., Hua, X., Song, Y., Ding, J., Huang, H., Zhao, R., Jia, W., & Yang, X. (2022). Novel mRNA Vaccines Encoding Monkeypox Virus M1R and A35R Protect Mice from A Lethal Virus Challenge. *BioRxiv*.

Jiang, L., Xu, A., Guan, L., Tang, Y., Chai, G., Feng, J., Wu, Y., Li, M., Zhang, C., Liu, X., Xu, X., Liu, Q., Song, L., Tong, Y., Blundell, R., & Fan, H. (2024). A Review of Mpox: Biological Characteristics, Epidemiology, Clinical Features, Diagnosis, Treatment, and Prevention Strategies. *Exploration*, 1–26.

Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., Tunyasuvunakool, K., Bates, R., Žídek, A., Potapenko, A., Bridgland, A., Meyer, C., Kohl, S. A. A., Ballard, A. J., Cowie, A., Romera-Paredes, B., Nikolov, S., Jain, R., Adler, J., … Hassabis, D. (2021). Highly Accurate Protein Structure Prediction with AlphaFold. *Nature*, *596*(7873), 583–589.

Kardani, K., Bolhassani, A., & Namvar, A. (2020). An Overview of In Silico Vaccine Design Against Different Pathogens and Cancer. In *Expert Review of Vaccines*. Taylor and Francis Ltd.

Kemenkes RI. (2024, October 7). *Apa Itu Mpox? Gejala, Penyebab, dan Pencegahan Cacar Monyet*. https://ayosehat.kemkes.go.id/apa-itu-mpox

Lee, T., Lee, D., Jung, E., Son, M., Koo, K., & Choi, J. (2025). Engineering of Long-acting Human Growth Hormone-Fc Fusion Proteins: Effects of Valency, Fusion Position, and Linker Design on Pharmacokinetics and Efficacy. *PLoS ONE*, *20*(5 May).

Merkx, M. (Ed.). (2021). *Linkers in Biomacromolecules* (Vol. 647). Elsevier Science.

Olive, D., Fleri, W., Paul, S., Dhanda, K., Mahajan, S., Xu, X., Peters, B., & Sette, A. (2017). The Immune Epitope Database and Analysis Resource in Epitope Discovery and Synthetic Vaccine Design. *Front. Immunol*, *8*, 278.

Oyewusi, H. A., Wahab, R. A., Akinyede, K. A., Albadrani, G. M., Al-Ghadi, M. Q., Abdel-Daim, M. M., Ajiboye, B. O., & Huyop, F. (2024). Bioinformatics Analysis and Molecular Dynamics Simulations of Azoreductases (AzrBmH2) from Bacillus megaterium H2 for the Decolorization of Commercial Dyes. *Environmental Sciences Europe*, *36*(1).

Patel, D. K., Menon, D. V., Patel, D. H., & Dave, G. (2022). Linkers: A Synergistic Way for The Synthesis of Chimeric Proteins. *Protein Expression and Purification*, *191*(106012).

Petersen, B. W., Harms, T. J., Reynolds, M. G., Lee, ;, & Harrison, H. (2016). *Use of Vaccinia Virus Smallpox Vaccine in Laboratory and Health Care Personnel at Risk for Occupational Exposure to Orthopoxviruses-Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2015* (Vol. 65, Issue 10). http://www.cdc.gov/vaccines/acip.

Saldanha, L., Langel, Ü., & Vale, N. (2023). In Silico Studies to Support Vaccine Development. In *Pharmaceutics* (Vol. 15, Issue 2). MDPI.

Sandoval, C., Guerrero, D., Muñoz, J., Godoy, K., Souza-Mello, V., & Farías, J. (2023). Effectiveness of mRNA, Protein Subunit Vaccine and Viral Vectors Vaccines Against SARS-CoV-2 in People Over 18 Years Old: A Systematic Review. In *Expert Review of Vaccines* (Vol. 22, Issue 1, pp. 35–53). Taylor and Francis Ltd.

Shamriz, S., Ofoghi, H., & Moazami, N. (2016). Effect of Linker Length and Residues on the Structure and Stability of a Fusion Protein with Malaria Vaccine Application. *Computers in Biology and Medicine*, *76*, 24–29.

Sklenovská, N., & Van Ranst, M. (2018). Emergence of Monkeypox as the Most Important Orthopoxvirus Infection in Humans. *Frontiers in Public Health*, *6*.

Soegiarto, G., & Purnomosari, D. (2023). Challenges in the Vaccination of the Elderly and Strategies for Improvement. In *Pathophysiology* (Vol. 30, Issue 2, pp. 155–173). Multidisciplinary Digital Publishing Institute (MDPI).

Soria-Guerra, R. E., Nieto-Gomez, R., Govea-Alonso, D. O., & Rosales-Mendoza, S. (2015). An Overview of Bioinformatics Tools for Epitope Prediction: Implications on Vaccine Development. In *Journal of Biomedical Informatics* (Vol. 53, pp. 405–414). Academic Press Inc.

Sternke, M., Tripp, K. W., & Barrick, D. (2019). Consensus Sequence Design as A General Strategy to Create Hyperstable, Biologically Active Proteins. *Proceedings of the National Academy of Sciences of the United States of America*, *166*(23), 11275–11284.

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, *38*(7), 3022–3027.

Tan, M., Zhang, R., Shen, T., Li, A., Hou, X., Zhang, Y., Wang, T., Zhang, B., Sun, P., Gong, X., Li, L., Wu, J., Wu, J., Zhang, R., & Liu, B. (2024). Systematic Evaluation of The Induction of Efficient Neutralizing Antibodies by Recombinant Multicomponent Subunit Vaccines Against Monkeypox Virus. *Vaccine*, *42*(126384).

Teli, D., Balar, P., Patel, K., Sharma, A., Chavda, V., & Vora, L. (2023). Molnupiravir: A Versatile Prodrug against SARS-CoV-2 Variants. In *Metabolites* (Vol. 13, Issue 2). MDPI.

Thornhill, J. P., Barkati, S., Walmsley, S., Rockstroh, J., Antinori, A., Harrison, L. B., Palich, R., Nori, A., Reeves, I., Habibi, M. S., Apea, V., Boesecke, C., Vandekerckhove, L., Yakubovsky, M., Sendagorta, E., Blanco, J. L., Florence, E., Moschese, D., Maltez, F. M., … Orkin, C. M. (2022). Monkeypox Virus Infection in Humans across 16 Countries — April–June 2022. *New England Journal of Medicine*, *387*(8), 679–691.

Vij, S., Thakur, R., & Rishi, P. (2022). Reverse Engineering Approach: A Step Towards A New Era of Vaccinology with Special Reference to Salmonella. *Expert Review of Vaccines*, *21*(12), 1763–1785.

Wang, Y., Yang, K., & Zhou, H. (2023). Immunogenic Proteins and Potential Delivery Platforms for Mpox Virus Vaccine Development: A Rapid Review. *International Journal of Biological Macromolecules*, *245*(125515), 1–12.

WHO. (2024a). *Mpox*. World Health Organization.

WHO. (2024b). *Public Health Advice on Understanding, Preventing and Addressing Stigma and Discrimination Related to Mpox*.

WHO. (2024c, October 3). *Mpox Outbreak*. https://www.who.int/emergencies/situations/mpox-outbreak

Wu, Q., Xu, C., Shi, W., Li, L., Zhang, H., Liu, T., Fan, J., Cui, L., & Li, J. (2023). Suitable Carrier Protein and Linker Peptide Significantly Increase the Secretory Expression of Human Lysozyme in Aspergillus niger. *Acta Biochimica et Biophysica Sinica*, *55*(10), 1677–1680.

Yu, K., Liu, C., Kim, B.-G., & Lee, D.-Y. (2015). Synthetic Fusion Protein Design and Applications. *Biotechnology Advances*, *33*(1), 155–164.

Zhang, O., Liu, Z. H., Forman-Kay, J. D., & Head-Gordon, T. (2025). *Deep Learning of Proteins with Local and Global Regions of Disorder*.

Zubair, M. S., Maulana, S., & Mukaddas, A. (2020). Penambatan Molekuler dan Simulasi Dinamika Molekuler Senyawa Dari Genus Nigella Terhadap Penghambatan Aktivitas Enzim Protease HIV-1. *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy) (e-Journal)*, *6*(1), 132–140.