Available online on 15.02.2025 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Full Text Article

Check for updates

Review Article

Protein Engineering: A Novel Approach in Vaccine Development

Diwas Adhikari *, Sharada Pokhrel

M. Pharm, Veer Madho Singh Bhandari Uttarakhand Technical University, Dehradun, India

Article Info:

Article History:

Received 21 Nov 2024
Reviewed 07 Jan 2025
Accepted 01 Feb 2025
Published 15 Feb 2025

Cite this article as:

Adhikari D, Pokhrel S, Protein Engineering: A Novel Approach in Vaccine Development, Journal of Drug Delivery and Therapeutics. 2025; 15(2):137-142 *DOI: http://dx.doi.org/10.22270/jddt.v15i2.7003*

*Address for Correspondence:

Diwas Adhikari, M. Pharm, Veer Madho Singh Bhandari Uttarakhand Technical University, Dehradun, India

Abstract

Due to advances in biotechnology, immunology and molecular biology, protein engineering has been an innovative technique for the development, optimization and production of vaccine. Deliberate alternation in the protein structure causes the improvement or change in the properties of the protein and due to this approach modification of the antigens for inducing the strong immune response is possible thus creating a benchmark in vaccine development. Recombinant DNA Technology, Epitope Mapping, Molecular Display Systems, Fusion Proteins and Designing of virus like particles are some of the key techniques in the protein engineering. Development of the various vaccines such as Hepatitis B vaccine, HPV Vaccine and Covid-19 Vaccine are some of the successes of protein engineering approach. However, there are some challenges associated with the techniques such as Antigen Stability, Immune Evasion and High production cost. Study of structure function relationship is a crucial part of the vaccine development.

Keywords: Protein Engineering, Antigens, Recombinant DNA Technology, Epitope Mapping, Immune Evasion.

Background:

The past few decades have been an incredible era for vaccine development due to advances in biotechnology, immunology, molecular biology, and other scientific technologies. Protein engineering is one of the most innovative techniques which help to optimize the process of vaccines development, optimization, and production.[1] Researchers are working closer with protein engineering for developing better, more specific, safer and stable vaccines to meet the challenges of emerging infectious disease.[2] Thus there is great contribution of protein engineering to the development of vaccines. Development of the vaccine is connected with the various platforms as in figure 1.

Protein engineering is the deliberate alteration of protein structures for improvement or change in their properties.[4] Protein engineering in vaccine development usually refers to the process of designing and producing antigens to induce a strong and protective immune response. The conventional method of vaccine development was often based on inactivated or attenuated pathogens, or their subunits. However, the future of vaccines lies within protein engineering, which enables scientists to create recombinant proteins similar to the surface structures of viruses or bacteria that, can elicit a strong immune response without requiring whole pathogens.

Key Techniques in Protein Engineering for Vaccine Development

Advanced antibody/protein engineering has played a key role in vaccine development. These include:

Recombinant DNA technology: The ability to make a clone and express those foreign genes in a host cell is enabled by the recombinant DNA Technology. This method enables the large-scale production of specific proteins from specific pathogens that can be subsequently used as antigens in vaccines. One of the earliest successes of this technique is the recombinant hepatitis B vaccine.[5]

Molecular Display Systems: Molecular display systems are utilized to present a large diversity of proteins on the surface of viral or yeast particles (phage display and yeast display, respectively). This system facilitates for screening of the ideal and optimal protein variants that bind immune receptors effectively, thus allowing researchers to screen protein candidates for best immunogenic potential. These types of technology drastically reduce the cost of vaccine development.[6]

Epitope Mapping: Epitope mapping refers to determining the precise locations (epitopes) on a pathogen's protein recognized by the immune system. Such epitopes can then be engineered using protein engineering techniques to enhance immunogenicity or develop more targeted vaccines. This is particularly crucial for diseases caused by viruses that change rapidly due to mutation, such as influenza. Epitope mapping comprises of integrated network of immunochemical, computational and structural analysis as indicated in Figure 2.[7]

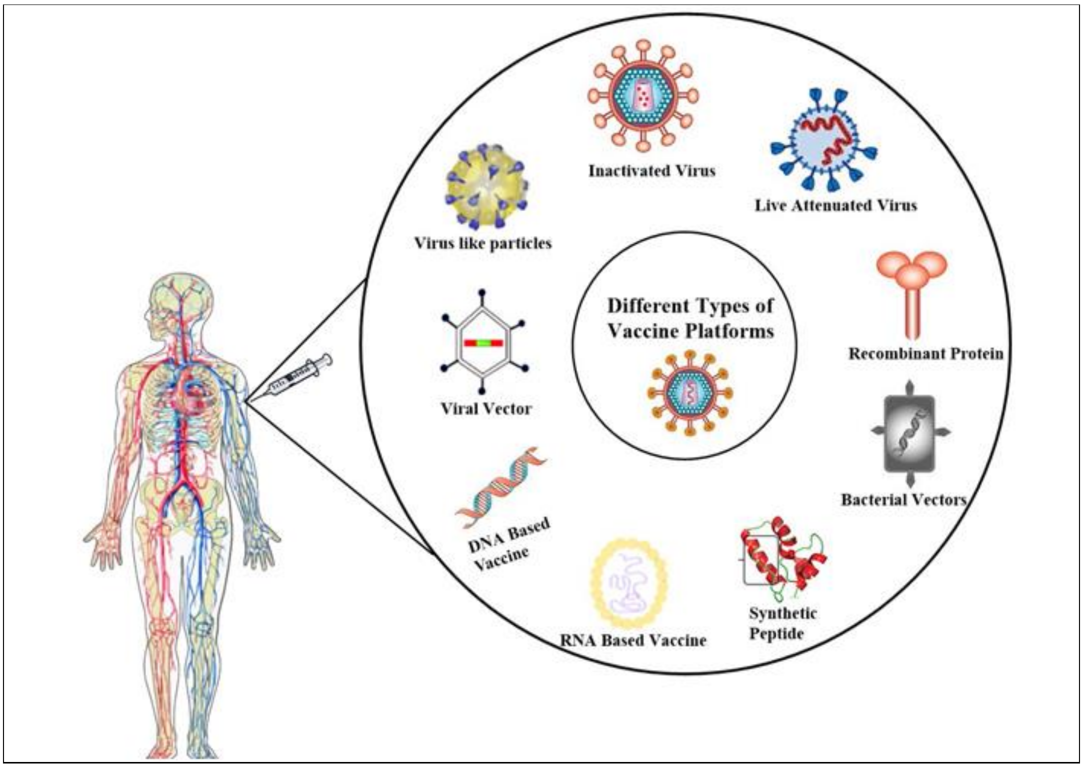

Figure 1: Various forms of vaccine platforms[3]

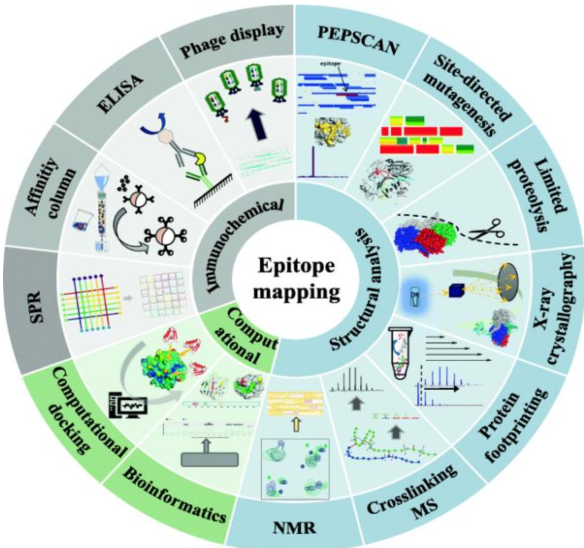

Figure 2: Epitope mapping network[8]

Fusion Proteins: A fusion is created in some instances where an antigen, derived from the pathogen, is fused with an immunostimulatory molecule. It works by making the antigen more stable or active, thus improving the efficiency of the immune response.[9]

Designing of Virus-Like Particles (VLPs): VLPs are a specialized engineered particle that resemble viruses in terms of their structural appearance but do not contain an actual viral genome. These complex ultra-structures not only protect the proteins from natural degradation, they can also mimic the effects of natural infections by triggering potent immune responses without the risk of disease.[10]

Protein Engineering in Development of Multiple Vaccines

Protein engineering through structure and function design played a role in the development of multiple successful vaccines, including for hepatitis B, human papillomavirus (HPV) and more recently, COVID-19.

Hepatitis B vaccine: The first recombinant protein vaccine was based on the hepatitis B surface antigen (HBsAg) developed in the 1980s. The HBsAg was subsequently expressed in yeast cells using recombinant DNA technology, and the yeast could be purified and used as a vaccine. This strategy was very successful in preventing hepatitis B infection.[11]

HPV Vaccines: The most well-known application of Virus like particles (VLPs) are for two HPV Vaccines (Gardasil, Cervarix), which consist of VLPs made up of the L1 protein of the human papillomavirus. These VLPs mimics the surface of the virus, are highly immunogenic, and provide protection against cervical cancer and additional cancers caused by high-risk HPVs.[12]

COVID-19 Vaccines: Another great significant achievement in protein engineering is development of COVID-19 vaccines. The spike protein of the SARS-CoV-2 virus was the target of the developed Pfizer-BioNTech and Moderna mRNA vaccines. These vaccines encode the spike protein in mRNA, which promptly enhance the body's immune system to recognize and defeat the virus effectively. Protein engineering was also key to developing protein subunit vaccines, such as Novavax's vaccine, which uses a recombinant spike protein to stimulate immunity.[13]

Advantages of Protein Engineering on Vaccine Development

Using protein engineering helps to design vaccines which offer several major benefits.

Precision and Specificity: Protein engineering enables the targeted design of antigens that stimulate a specific immune response, minimizing the likelihood of side effects and enhancing the efficacy of the vaccine[14].

Safety: Protein engineering has the advantage of using recombinant proteins or engineered virus-like particles rather than live pathogens that can minimize the adverse effect seen with classical/traditional vaccines, such as infection from live-attenuated viruses.[15]

Versatility: Development of the vaccine against the wide range of pathogens such as parasites, bacteria and viruses is possible though the protein engineering. Vaccine development against the various new and several infectious diseases is possible through the genetic engineering.[16]

Rapid development of the vaccine: As recombinant proteins and synthetic biology do not require culturing of entire pathogens, their use can lead to shorter timelines of vaccine production.[17]

Challenges in Protein Engineering on Vaccine Development

Though the utilization of protein engineering in vaccine development holds great potential, there are many challenges such as:

Antigen stability: One of the continuing friction points in protein engineering is making sure engineered proteins are stable enough for production and/or storage for vaccine purposes. Instability can result in diminished vaccine potency or shelf life.[18]

Immune Evasion: Some pathogens have the ability to mutate quickly to escape attack by the immune system (for example, HIV and the flu virus). So, protein engineering has to consider the possibility of mutations and create vaccines that will confer immunity to whatever strain may come.[19]

Production Cost: As the recombinant protein production becomes efficient, there appears a significant cost for the scale up and large scale manufacturing. So the cost appears to be the challenging issue which needs to be solved.[20]

Structure Function Relationship in Vaccine Design:

Structure-function relationship (SFR) is a basic principle of vaccine design when the engineered proteins, antigens are considered. It is about how the 3D shape of a molecule (such as protein, or antigen, etc.) determines its biological activity, including its capacity to induce an immune response. Efficient, safe, and specific vaccines rely on understanding the structure-function relationship[21]

Key aspects of structure function relationship during vaccine design:

Antigen structure and immune recognition: Antigens are the molecules that are recognized as foreign bodies by immune system and trigger an immune response. Antigens are usually pieces of pathogens (like viral or bacterial proteins) that are designed as part of a vaccine to elicit an immune response.

Structural Features:

Epitope mapping: The immune system recognizes specific parts of an antigen called epitopes. It is the structural organization of the antigen that makes specific sites accessible and thus able to be recognized by antibodies (humoral immune response) or T-cells (cell mediated immune response).[21]

Linear Epitope: Linear sequences of amino acids in the protein structure that are recognized by antibodies.[22]

Conformational Epitopes – 3D shapes that are formed by non-contiguous sequences of amino acids that fold in space and which are also recognized by the immune system.[22]

Immune Function: The physical arrangement of the antigen in 3D space affects the strength of the interactions between immune receptors (i.e. B-cell receptors for antibodies or T-cell receptors).

In order to improve how bodily immune systems recognise and respond to the pathogen depends upon the modification of the antigen structure for exposure of more immunogenic epitopes.[23]

Stability of Antigens and Folding

Protein Folding: Antigens must fold in the correct 3D conformation to successfully stimulate an immune response. For the immune system to properly recognize the structure, it must be stable and retain its functional structure.

Unfolded or incorrectly folded proteins may not be able to mount an effective immune response or may be less immunogenic.[24]

Stability Engineering: Antigens can be subjected to structural modifications to increase stability (e.g. addition of disulphide bonds or specific mutations that enhance thermal or pH stability) to deliver them intact during vaccine production, storage, and administration.[15]

Engineering Protein Based Vaccines:

Subunit Vaccines: In these vaccines, only specific and non-infectious parts of the pathogen are used (ex: a protein or protein fragment which is not infectious). Structures of these proteins are subjected to engineering for retaining the immunogenic epitopes that elicit robust immune responses.

For instance, the HPV vaccine uses a virus-like particle (VLP) technology: the capsid protein is engineered to assemble into VLPs that resemble the entire virus so that the immune system recognizes the VLPs as if they were the actual virus.[25]

Virus like Particles (VLPs): These mimic virus structures without genetic material. The structure is important for generating a robust immune response.

The self-assembly of the engineered protein subunits into a structure that mimics the natural virus (e.g. the Hepatitis B VLP vaccine)

For the immune cell to detect the virus, the outer protein shell has to imitate the native state of the virus.[26]

Enhancement of the Immunogenicity through structural modifications:

Conformational Modifications — Protein engineering enables modification of the structure of antigens to enhance interaction with immune system.[27] This could involve:

Protein glycosylation: modification the carbohydrate chains on proteins to improve immune recognition.[28]

Epitope Exposure: Change in folding and conformation pattern of the causes the more exposure of epitopes and then they are recognised by antibodies.[29]

Adjuvants: Proteins can be engineered to have adjuvants (immunostimulatory molecules) that potentiate the immune response, typically by enhancing the presentation of the antigen to immune cells or activating specific pathways. This includes multimerization (for example, some antigens can be created in multimeric forms, including dimers, trimers, or other higher order structures). This can amplify the level of immune activation, because the immune system may recognize several copies of the same antigen at the same time.[30]

Future Directions of Protein Engineering

Personalized and Précised Vaccines

Development of the personalized vaccine is the prominent future direction of the protein engineering approach. Protein engineering can be a key in generating vaccines tuned to immune response with the higher efficacy and lower side effects.

Development of the cancer vaccine is the most prominent field where protein engineering could be applied to build custom antigens matching patient's tumor profile, which may enhance the efficacy of immunotherapy.

Furthermore, protein engineering might enable the identification of genetic variants that modulate responses to public health vaccines and pave the way for personalized vaccination strategies across individuals with different genetic backgrounds, underlying disease states, and age groups.[31]

Broad Spectrum and Universal Vaccines: There are various challenges associated with the protein engineering due to the rapid mutation of the pathogens. So, creation of the broad-spectrum vaccine is the exciting field which is possible through the genetic engineering and it helps in providing the protection against the varieties of the multiple pathogens like corona virus, influenza, HIV etc. Designing of the more stable and antigens with the cross reactive behaviour can be facilitated by the protein engineering which helps in triggering the immune response which is capable of recognizing and neutralization of the diverse variants.[32]

Development of the next generation mRNA and DNA Vaccines:

The success of mRNA vaccines against COVID-19 paved the way for a new vaccine development. Protein engineering will also play an important role in advancing mRNA and DNA vaccine technology. For example, next-generation mRNA vaccines could employ engineered proteins that elicit more potent immune responses or offer greater protection against multiple variants of a virus.

Furthermore, DNA vaccine which are analogous to mRNA vaccine platforms but makes the utilization of plasmid DNA may also get benefitted from protein engineering by enhancing the stability and delivery efficiency of the encoded antigens. For example, protein engineering can be applied to modify the proteins which are encoded to improve their immunogenicity or for the optimization of immune cells interaction.[33]

Improvement in Delivery System and Nanoparticles based Vaccines:

Nanotechnology, in particular nanoparticle delivery systems, is already being studied to enhance the efficacy and safety of protein-based vaccines. In the future, there might be addition of engineered proteins into the nanoparticles for increasing and improving the vaccine delivery. Such nanoparticles may aid in stabilizing antigens, safeguarding them against degradation and selectively targeting specific cells to elicit an immune response precisely.

For instance, lipid nanoparticles, which are used in mRNA vaccines, could be protein-engineered to enhance their capacity to deliver the genetic material into cells. Similarly, protein-engineered nanoparticle vaccines would mimic the virus itself, boosting the immune response and avoiding the risk associated. [34]

Combination Vaccines: Combination vaccines, which protect against more than one disease with a single shot, may also be part of the future of vaccine development. Thus, protein engineering will be critical for designing multi-target antigens that can prevent infection from multiple pathogens at once.[35]

For instance, a mix of vaccine for both influenza and COVID-19 or multiple respiratory viruses could become more realistic through the use of engineered proteins that induce immune responses against many pathogens. This could make it easier to schedule vaccines and increase the coverage of vaccination.[36]

Vaccines for complex pathogens like parasites and multistage pathogens:

Protein engineering could open the door for new vaccines against complex pathogens such as malaria or tuberculosis, which undergo a multi-stage life cycle or complex immune evasion strategies. Other protein-based vaccines target different stages of these pathogens to offer wider protection.

Engineered proteins, for example, could enable vaccines that block different stages of a parasite's lifecycle, like we see with malaria. Protein engineering might allow researchers to design antigens expressed during both the mosquito and human phases of malaria's lifecycle, a two-pronged strategy to prevent infection.[37]

Vaccine stability and cold chain independence:

A significant hurdle in distributing vaccines around the world is the requirement for cold storage and refrigeration. The problem can be addressed in part by protein engineering techniques that enable the design of thermo stable vaccines that do not need to be frozen or refrigerated, making them easier and cheaper to roll out in low-resource settings.[38]

Self-replicating vaccines:

Synthetic biology based vaccines use engineered living organisms which is capable of producing the antigens or even allowing the vaccine to be directly delivered to body. These could include self-replicating vaccines or bacteria that are designed to generate and deliver vaccine components at the site of an infection, potentially bypassing the need for mass production and distribution.

This kind of living vaccine could be designed to generate particular proteins when triggered by the surrounding environment, allowing for incredible adaptability and efficiency in fighting off infectious diseases as they arise. [39]

Conclusion:

Protein engineering is a novel approach for the development, optimization and production of the vaccine delivery system using the various techniques such as Recombinant DNA Technology, Epitope Mapping, Molecular Display systems, Fusion proteins and designing of virus like particles (VLPs). It enhances the targeted and precise delivery of the antigens ensuring the safety and versatility. However, some challenges are associated with the approach such as stability of antigens, immune evasion and high cost of production. A lot of innovations and reformations can be expected in the vaccine delivery through protein engineering in future.

Funding: There is no funding involve

Conflict of Interest: None

Author Contribution: Both Authors have contributed equally for gathering information, writing a review paper for publication.

Ethical Statement: Not Applicable

Inform Consent: Not Applicable

References

1. Fetzer S. Optimized vaccine development and manufacturing: a technology overview. Biopharm International. 2008;2008(1). https://doi.org/10.1142/9789812790958_0001

2. Adhikari D, Pokhrel S. An Overview on Protein and Peptide Drug Delivery System: Advances and Strategies. Journal of Drug Discovery and Health Sciences. 2024;1(04):239-43.

3. Ahire ED, Kshirsagar SJ. Immune responses induced by different vaccine platforms against coronavirus disease-19. Exploration of Immunology. 2021;1(4):243-57. https://doi.org/10.37349/ei.2021.00016

4. H. Tobin P, H. Richards D, A. Callender R, J. Wilson C. Protein engineering: a new frontier for biological therapeutics. Current drug metabolism. 2014;15(7):743-56. https://doi.org/10.2174/1389200216666141208151524 PMid:25495737 PMCid:PMC4931902

5. Zahradnik JM, Couch RB, Gerin JL. Safety and immunogenicity of a purified hepatitis B virus vaccine prepared by using recombinant DNA technology. Journal of Infectious Diseases. 1987;155(5):903-8. https://doi.org/10.1093/infdis/155.5.903 PMid:2951449

6. Levin A, Weiss G. Optimizing the affinity and specificity of proteins with molecular display. Molecular Biosystems. 2006;2(1):49-57. https://doi.org/10.1039/B511782H PMid:16880922

7. Deem MW, Pan K. The epitope regions of H1-subtype influenza A, with application to vaccine efficacy. Protein Engineering, Design & Selection. 2009;22(9):543-6. https://doi.org/10.1093/protein/gzp027 PMid:19578121 PMCid:PMC3307478

8. Sun H, Ma L, Wang L, Xiao P, Li H, Zhou M, et al. Research advances in hydrogen-deuterium exchange mass spectrometry for protein epitope mapping. Analytical and bioanalytical chemistry. 2021;413:2345-59. https://doi.org/10.1007/s00216-020-03091-9 PMid:33404742

9. Wu L, Barry MA. Fusion protein vectors to increase protein production and evaluate the immunogenicity of genetic vaccines. Molecular Therapy. 2000;2(3):288-97. https://doi.org/10.1006/mthe.2000.0126 PMid:10985959

10. Roldão A, Mellado MCM, Castilho LR, Carrondo MJ, Alves PM. Virus-like particles in vaccine development. Expert review of vaccines. 2010;9(10):1149-76. https://doi.org/10.1586/erv.10.115 PMid:20923267

11. Whitacre D, Peters C, Sureau C, Nio K, Li F, Su L, et al. Designing a therapeutic hepatitis B vaccine to circumvent immune tolerance. Human vaccines & immunotherapeutics. 2020;16(2):251-68. https://doi.org/10.1080/21645515.2019.1689745 PMid:31809638 PMCid:PMC7062423

12. Bai C, Wang R, Yang Q, Hao J, Zhong Q, Fan R, et al. Design and antiviral assessment of a panel of fusion proteins targeting human papillomavirus type 16. Plos one. 2024;19(10):e0311137. https://doi.org/10.1371/journal.pone.0311137 PMid:39453911 PMCid:PMC11508125

13. Li M, Wang H, Tian L, Pang Z, Yang Q, Huang T, et al. COVID-19 vaccine development: milestones, lessons and prospects. Signal transduction and targeted therapy. 2022;7(1):146. https://doi.org/10.1038/s41392-022-00996-y PMid:35504917 PMCid:PMC9062866

14. Kanekiyo M, Ellis D, King NP. New vaccine design and delivery technologies. The Journal of Infectious Diseases. 2019;219(Supplement_1):S88-S96. https://doi.org/10.1093/infdis/jiy745 PMid:30715361 PMCid:PMC6452296

15. Vishweshwaraiah YL, Dokholyan NV. Toward rational vaccine engineering. Advanced drug delivery reviews. 2022;183:114142. https://doi.org/10.1016/j.addr.2022.114142 PMid:35150769 PMCid:PMC8931536

16. Frietze KM, Peabody DS, Chackerian B. Engineering virus-like particles as vaccine platforms. Current opinion in virology. 2016;18:44-9. https://doi.org/10.1016/j.coviro.2016.03.001 PMid:27039982 PMCid:PMC4983494

17. Lestari C, Novientri G, editors. Advantages of yeast-based recombinant protein technology as vaccine products against infectious diseases. IOP Conference Series: Earth and Environmental Science; 2021: IOP Publishing. https://doi.org/10.1088/1755-1315/913/1/012099

18. Akbarian M, Chen S-H. Instability challenges and stabilization strategies of pharmaceutical proteins. Pharmaceutics. 2022;14(11):2533. https://doi.org/10.3390/pharmaceutics14112533 PMid:36432723 PMCid:PMC9699111

19. Ernst JD. Mechanisms of M. tuberculosis immune evasion as challenges to TB vaccine design. Cell host & microbe. 2018;24(1):34-42. https://doi.org/10.1016/j.chom.2018.06.004 PMid:30001523 PMCid:PMC6482466

20. Ulmer JB, Valley U, Rappuoli R. Vaccine manufacturing: challenges and solutions. Nature biotechnology. 2006;24(11):1377-83. https://doi.org/10.1038/nbt1261 PMid:17093488

21. Zolla-Pazner S, Cardozo T. Structure-function relationships of HIV-1 envelope sequence-variable regions refocus vaccine design. Nature reviews Immunology. 2010;10(7):527-35. https://doi.org/10.1038/nri2801 PMid:20577269 PMCid:PMC3167078

22. Patronov A, Doytchinova I. T-cell epitope vaccine design by immunoinformatics. Open biology. 2013;3(1):120139. https://doi.org/10.1098/rsob.120139 PMid:23303307 PMCid:PMC3603454

23. Dariushnejad H, Ghorbanzadeh V, Akbari S, Hashemzadeh P. Design of a novel recombinant multi-epitope vaccine against triple-negative breast cancer. Iranian Biomedical Journal. 2022;26(2):160.

24. Feige MJ, Buchner J. Principles and engineering of antibody folding and assembly. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics. 2014;1844(11):2024-31. https://doi.org/10.1016/j.bbapap.2014.06.004 PMid:24931831

25. Tsoras AN, Champion JA. Protein and peptide biomaterials for engineered subunit vaccines and immunotherapeutic applications. Annual Review of Chemical and Biomolecular Engineering. 2019;10(1):337-59. https://doi.org/10.1146/annurev-chembioeng-060718-030347 PMid:31173518

26. Lua LH, Connors NK, Sainsbury F, Chuan YP, Wibowo N, Middelberg AP. Bioengineering virus-like particles as vaccines. Biotechnology and bioengineering. 2014;111(3):425-40. https://doi.org/10.1002/bit.25159 PMid:24347238

27. Borbulevych OY, Baxter TK, Yu Z, Restifo NP, Baker BM. Increased immunogenicity of an anchor-modified tumor-associated antigen is due to the enhanced stability of the peptide/MHC complex: implications for vaccine design. The Journal of Immunology. 2005;174(8):4812-20. https://doi.org/10.4049/jimmunol.174.8.4812 PMid:15814707 PMCid:PMC2241749

28. Kuriakose A, Chirmule N, Nair P. Immunogenicity of biotherapeutics: causes and association with posttranslational modifications. Journal of immunology research. 2016;2016(1):1298473. https://doi.org/10.1155/2016/1298473 PMid:27437405 PMCid:PMC4942633

29. Van Beers MM, Bardor M. Minimizing immunogenicity of biopharmaceuticals by controlling critical quality attributes of proteins. Biotechnology journal. 2012;7(12):1473-84. https://doi.org/10.1002/biot.201200065 PMid:23027660

30. Bonam SR, Partidos CD, Halmuthur SKM, Muller S. An overview of novel adjuvants designed for improving vaccine efficacy. Trends in pharmacological sciences. 2017;38(9):771-93. https://doi.org/10.1016/j.tips.2017.06.002 PMid:28668223

31. De Groot AS, Moise L, Terry F, Gutierrez AH, Hindocha P, Richard G, et al. Better epitope discovery, precision immune engineering, and accelerated vaccine design using immunoinformatics tools. Frontiers in immunology. 2020;11:442. https://doi.org/10.3389/fimmu.2020.00442 PMid:32318055 PMCid:PMC7154102

32. Xue W, Li T, Gu Y, Li S, Xia N. Molecular engineering tools for the development of vaccines against infectious diseases: current status and future directions. Expert Review of Vaccines. 2023;22(1):563-78. https://doi.org/10.1080/14760584.2023.2227699 PMid:37339445

33. Harisa GI, Faris TM, Sherif AY, Alzhrani RF, Alanazi SA, Kohaf NA, et al. Coding therapeutic nucleic acids from recombinant proteins to next-generation vaccines: Current uses, limitations, and future horizons. Molecular Biotechnology. 2024;66(8):1853-71. https://doi.org/10.1007/s12033-023-00821-z PMid:37578574

34. Cao P, Xu ZP, Li L. Tailoring functional nanoparticles for oral vaccine delivery: recent advances and future perspectives. Composites Part B: Engineering. 2022;236:109826. https://doi.org/10.1016/j.compositesb.2022.109826

35. Ellis RW. Development of combination vaccines. Vaccine. 1999;17(13-14):1635-42. https://doi.org/10.1016/S0264-410X(98)00424-1 PMid:10194816

36. Tzenios N, TAZANIOS ME, Chahine M. Combining Influenza and COVID-19 Booster Vaccination Strategy: A Systematic Review and Meta-Analysis. Available at SSRN 4276608. 2022. https://doi.org/10.2139/ssrn.4276608

37. Doolan DL, Hoffman SL. Multi-gene vaccination against malaria: a multistage, multi-immune response approach. Parasitology Today. 1997;13(5):171-8. https://doi.org/10.1016/S0169-4758(97)01040-5 PMid:15275087

38. Zhang J, Pritchard E, Hu X, Valentin T, Panilaitis B, Omenetto FG, et al. Stabilization of vaccines and antibiotics in silk and eliminating the cold chain. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(30):11981. https://doi.org/10.1073/pnas.1206210109 PMid:22778443 PMCid:PMC3409735

39. Aliahmad P, Miyake-Stoner SJ, Geall AJ, Wang NS. Next generation self-replicating RNA vectors for vaccines and immunotherapies. Cancer Gene Therapy. 2023;30(6):785-93. https://doi.org/10.1038/s41417-022-00435-8 PMid:35194198 PMCid:PMC8861484