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چالش های طراحی واکسن برای کروناویروس ها

کاربردهای ایمونوانفورماتیک

ابراهیم برزگری

مهر ۱۳۹۹

واکسن: تعریف و انواع آن

- **The goal of vaccination**
  - Immunization for healthy subjects (prophylaxis)
  - Avoiding off-target effects
- **Immunogen development options**
  - Live-attenuated vaccines
  - inactivated whole virus (IWV),
  - self-assembling virus-like particle vaccines
  - Vaccines based on chemically inactivated CoV virions
  - DNA vaccines
  - vectored vaccines
  - Subunit vaccines
  - ...

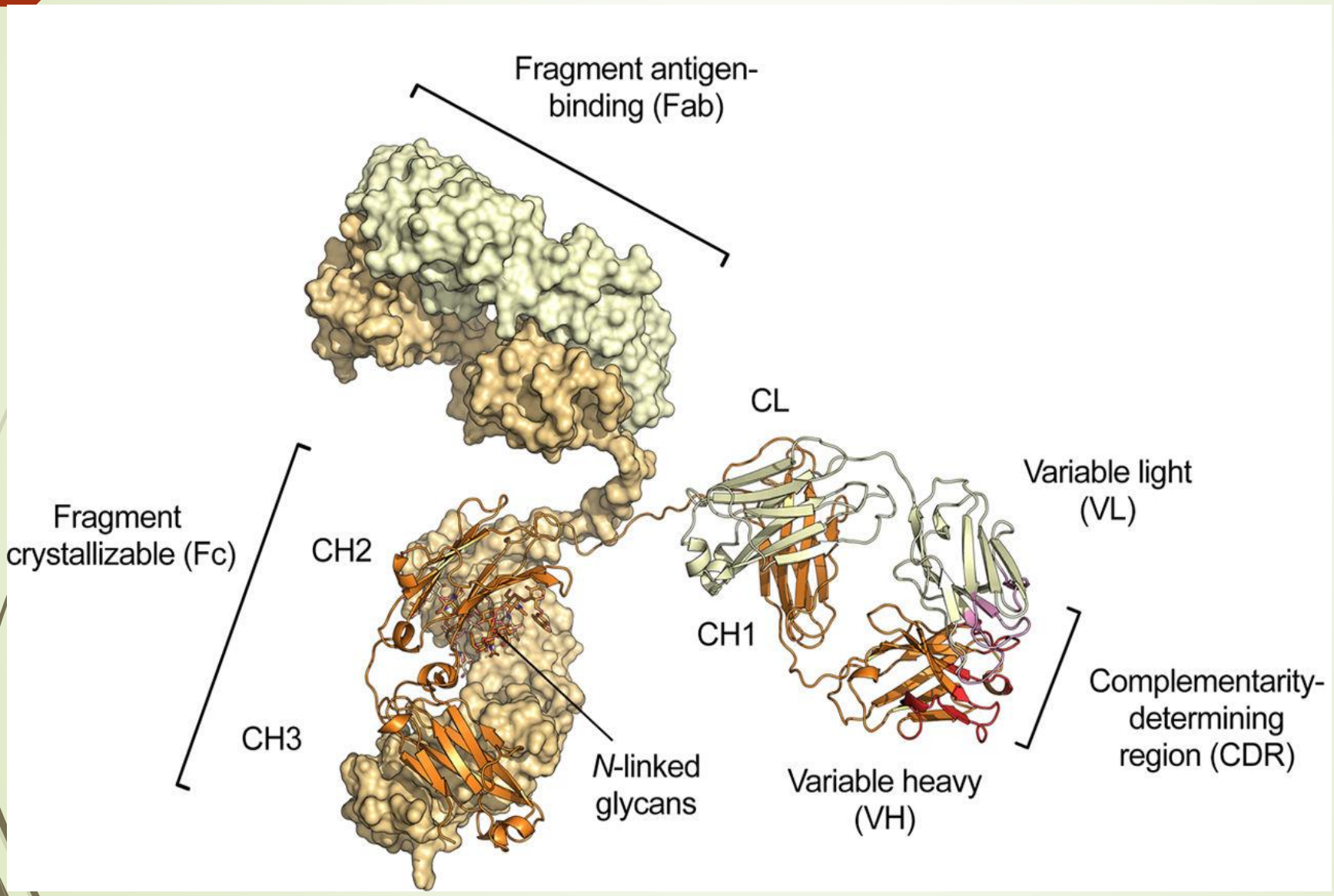
طراحی واکسن

- Vaccines for viral pathogens are usually designed to **induce antibody responses that target viral proteins** involved in cellular entry to achieve neutralizing activity in serum and mucosal secretions.
- Innate and adaptive immune mechanisms have an opportunity **to clear virus-infected cells** before viral spread and antigen load are sufficient to cause clinical symptoms.
- Therefore, in its simplest form, vaccine development for viral diseases involves the **delivery of antigens** that will **induce virus-specific neutralizing antibodies** and **avoid the induction of any off-target antibodies**.

Complexities

- Generating an antibody response after vaccination is a complex biological process;
- **Bench-to-market period of > 10 years**
- Antibodies without classical neutralizing activity can sometimes provide effective **immunity through Fc-mediated effector functions**, but would still need to bind viral proteins with high specificity.
- Additional immunological responses may be needed for effective vaccine-induced immunity, including **CD4 and CD8 T cells with particular properties and localization**

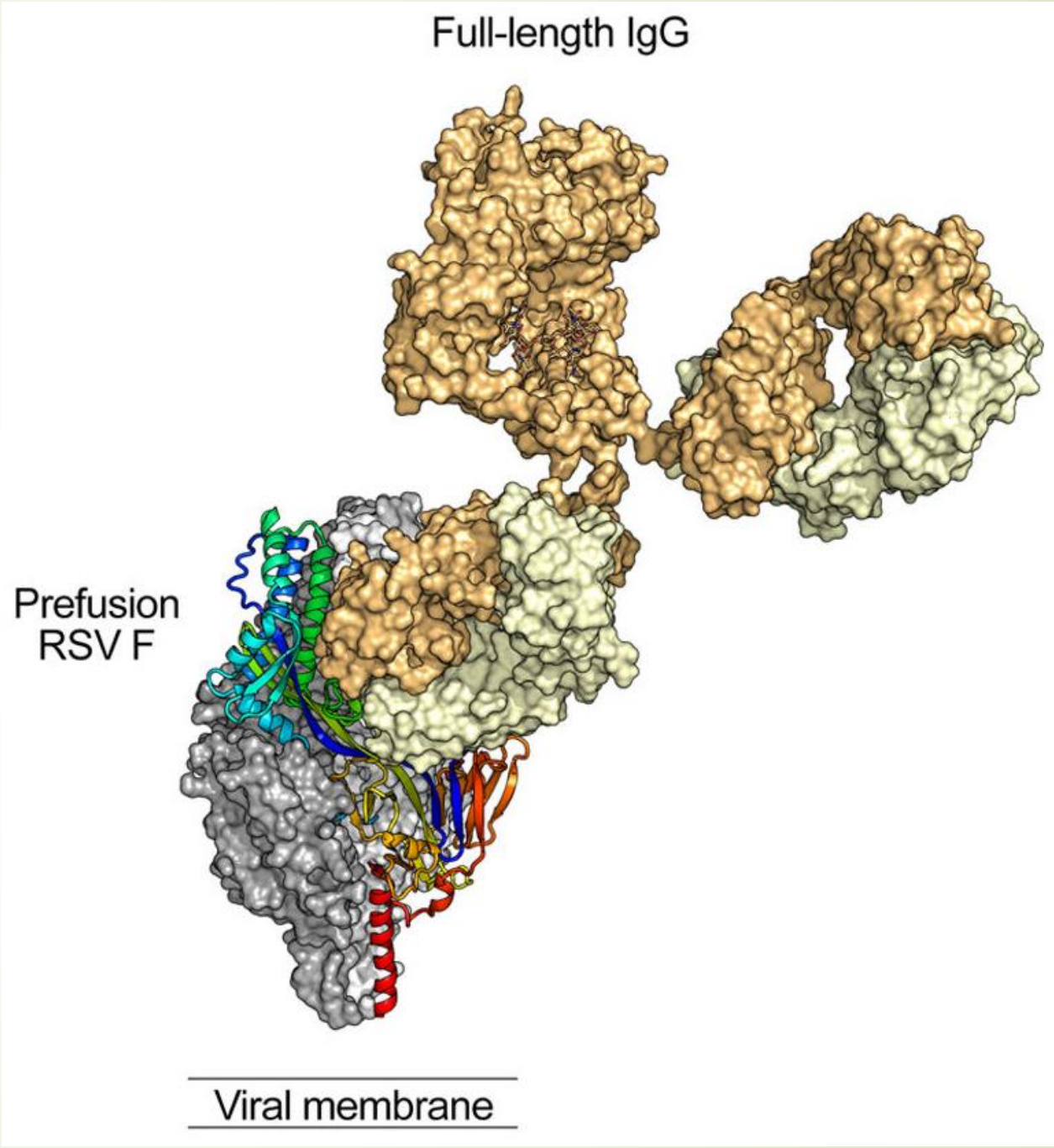
Antibodies



- **Antibodies are produced by B lymphocytes and plasma cells**

Antibodies

- **Topological complementarity between antibody variable domain and the antigen**



Structure-based vaccine design

- Structure-based vaccine design seeks to **create surfaces on immunogens that will elicit protective antibody responses against the target pathogen**
- Defining the **atomic-level details of key surfaces** on antigens accessible on pathogens is a primary requirement for structure-based vaccine design.
- Knowing **which proteins to attack**, and **which specific sites on those proteins** to target with antibodies, is fundamental to initiating a structure-based vaccine project.
- Regardless of the mechanism, antibody **binding** to the viral protein is essential.
- The strength or potency of neutralization is determined by many factors, but is often linked to **specificity or site of protein binding**, strength of binding (**affinity and avidity**), **accessibility of the binding site** on the virus, and **extent of occupancy** on the available sites on the virus.
- **The potency of neutralization is influenced by *chemistry* and affected by the *physical constraints* that determine the surface area of interaction.**

Structure-based vaccine design

- **2000: Reverse vaccinology**
  - a process by which complete sequencing of pathogen genomes could be used to identify and down-select surface-expressed or secreted proteins to arrive at new candidate vaccine antigens
- **2002: Reverse vaccinology 2.0**
  - a process by which identifying antibodies with desirable properties could be used to select or design antigens that elicited the target antibodies
- The realization is just now happening with **successes in Respiratory Syncitial Virus (RSV)** and other viral diseases (Later slides)

Importance of structural data

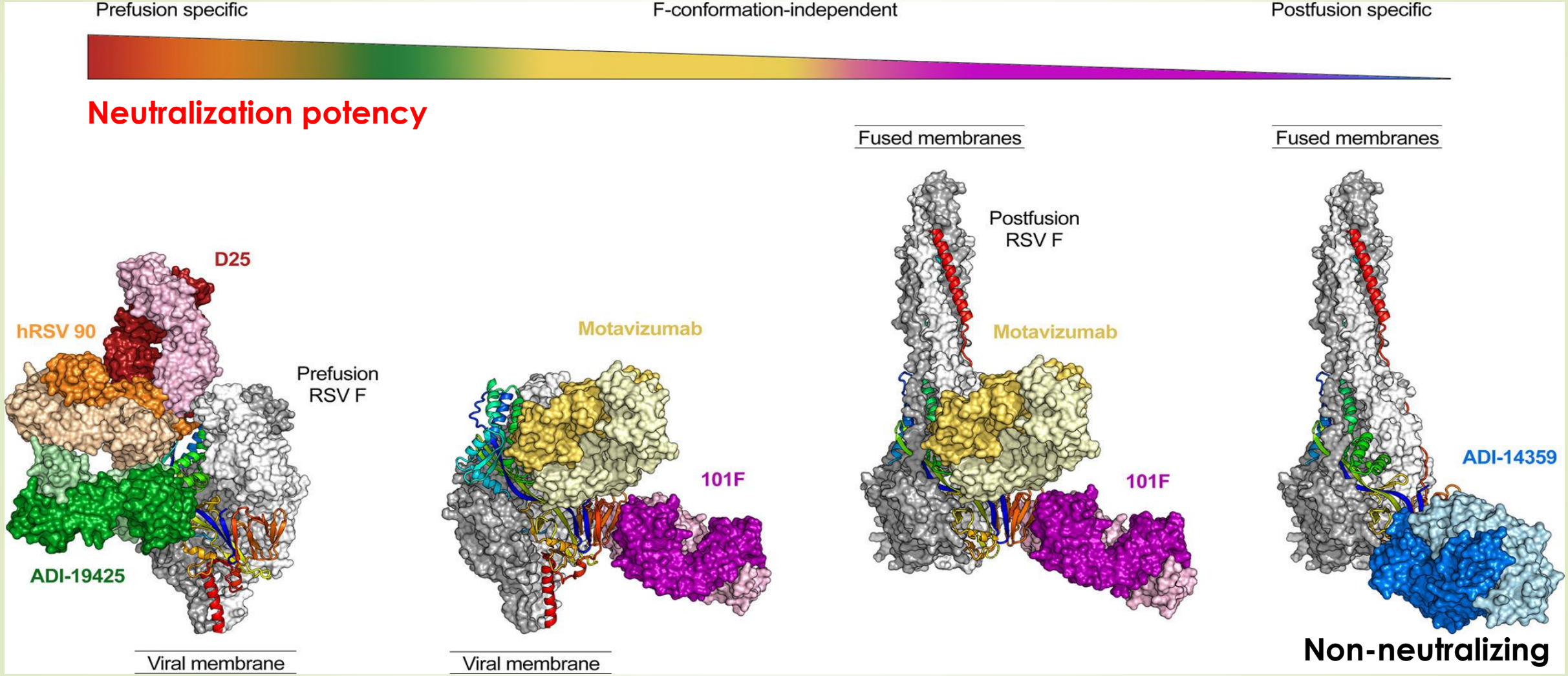
- The use of **whole viruses and VLPs** reduces the need for a detailed structural understanding of antibody binding
- Recently, a **subunit protein vaccine** based on glycoprotein E (gE) was licensed for use against herpes varicella zoster (HVZ) and is **highly effective** against shingles or reactivation. There is no structural data published for gE, and although it does induce robust antibody responses, it is thought that much of the efficacy is derived from the CD4 T-cell response.

- Therefore, **it is possible** to develop anti-viral vaccines without structural data and detailed understanding of the mechanisms of antibody neutralization.
- **Nevertheless**, **for the viral targets remaining**, the use of traditional vaccine technology will be challenging, in some cases because **it has already failed**.
  - Examples of vaccine failure and subsequent success thanks to structural knowledge comes in the following slides.

چالش ۱: ایمنی‌گریزی ویروس

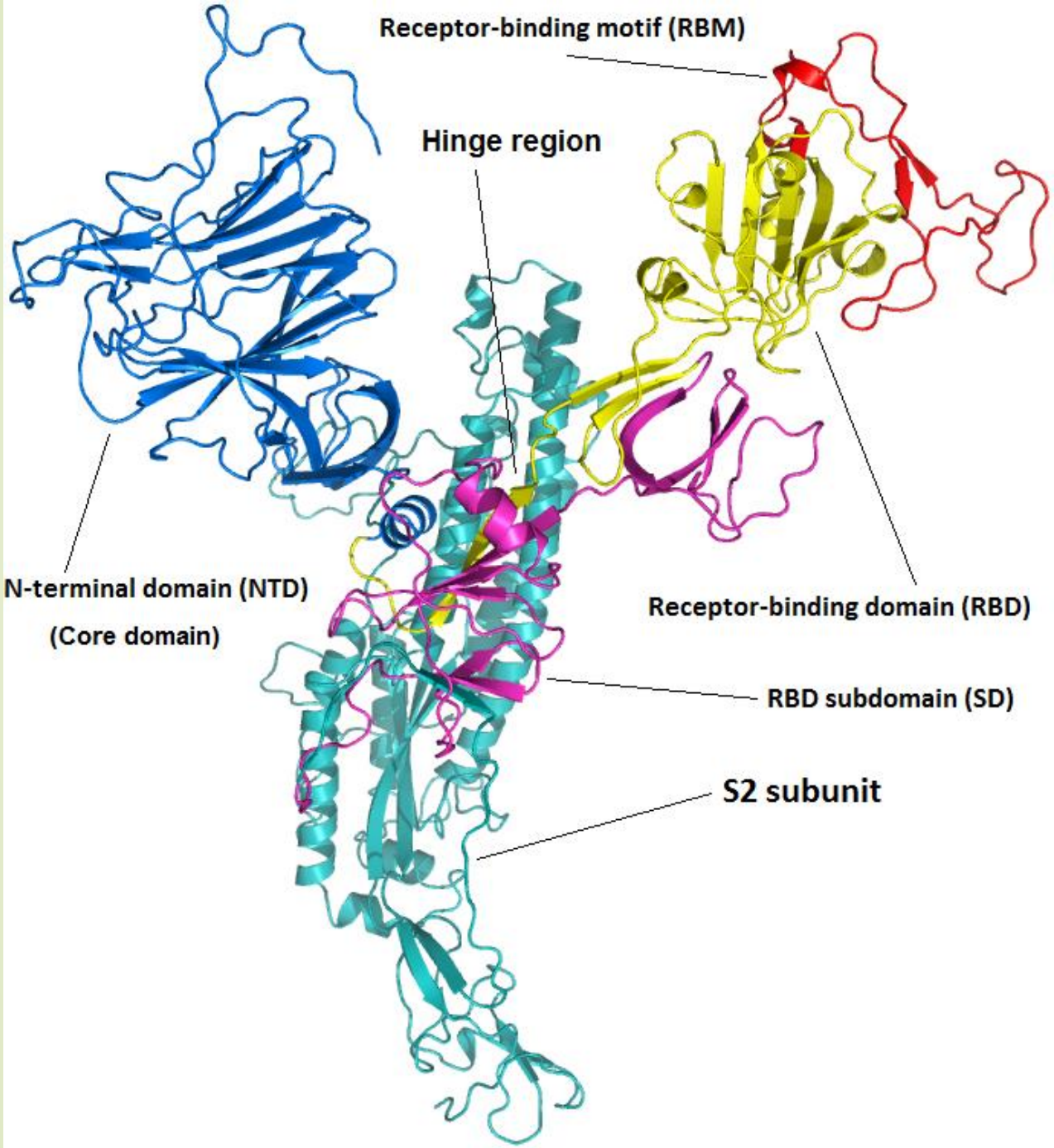
- Virus protecting its **sites of vulnerability**; i.e. epitopes or antigenic sites on proteins that can be bound by antibodies
- **Immune-evasion mechanisms**
  - Critical antigenic targets that require an antibody to **approach at a particular angle or rotation**. 'Super-sites' do not require this.
  - frequently **mutating** surrounding surfaces (genetic plasticity / antigenic variability): Limited in CoVs
  - adding or removing **glycan** groups, making epitopes neutralization-insensitive
  - Infection and virion **release site** is relatively isolated from systemic immune responses
  - initial infection during a time of **immunological immaturity** (in young children)
  - **inhibiting** both induction and effector functions of type I interferon
  - **Altering the signaling pathways** in dendritic cells
  - Non-triggered neutralization-susceptible conformation of the fusion glycoprotein trimer is unstable and rearranges spontaneously into a post-fusion conformation (**conformational heterogeneity and altering epitope exposure**).

Structure-based vaccine concepts: An example



Structure-based vaccine concepts : An example

- An RSV vaccine which was tested in the 1960s, resulted in a **vaccine-enhanced illness** syndrome upon natural RSV infection the following season.
- Protein engineering efforts resulted in authentic **stabilized pre-F trimers**, preserving the key epitopes
- Stabilizing class I fusion proteins to preserve neutralization-sensitive epitopes: **A strategy**
- It also improves the immunogenicity of live chimeric viruses, live-attenuated virus, virus-like particles and gene-based vectors
- **Same challenge and same strategy for HIV-1**



- S protein of CoVs is the major target of neutralizing antibodies produced for protection in vivo.
- The RBD is a more promising candidate in the development of CoV vaccines over the full-length S protein.
- Several residues in the hinge region of the S2 subunit were substituted with proline residues, to achieve conformational homogeneity

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- The use of prefusion ectodomains as immunogens should result in a polyclonal response directed against many neutralization-sensitive epitopes located across the large surface of the S glycoprotein, which is preferred to an RBD-exclusive immune response that could be evaded by the antigenic drift of only a few residues.
- Nanoparticle antigen display and self-assembling virus-like particles (VLPs)
- Antigen display to address: 1- some epitopes are not easily recognized by B cells, which may be addressed by antigen display approaches or masking of distracting antigenic sites. 2- antigenic diversity, particularly if multiple related but distinct antigens can be presented simultaneously.
- Structure-based vaccine design to: achieving the correct angle and rotation of approach for optimal neutralizing activity, and induction of antibodies that interact with or avoid glycans in critical locations.

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- Special adjuvant formulations may help regulate the innate antiviral immunity and also potentiate the induction of antigen-specific (adaptive) immunity.
- The magnitude and localization of antibody may be critical for protecting against some pathogens, so recognizing the optimal structurally defined epitope may have to occur in the right place, making route-of-delivery a key determinant of success.
- Going forward, successful vaccine development will require structure-guided antigen design, but also advances in antigen display, delivery, and formulation, in addition to improved understanding of lymph node and B-cell biology and more precision in our understanding of viral pathogenesis.
- Animal models infectable with CoVs and with most closeness to human (non-human primates) are extremely costly. Funding supports are essential.

چالش ۲ و ۳

- **Immunodominance** refers to the restricted peptide specificity of T cells that are detectable after an adaptive immune response. A few specific peptides are selected as representative epitopes of a given protein antigen to the immune system.
- **Short-lived response by antibodies** (from B-lymphocytes):

  Solutions: Virus-specific memory CD8+ T cells; inclusion of appropriate adjuvants
- Memory CD8+ T cells produced effector cytokines (interferon gamma; TNF-a; and interleukin 2) and cytolytic molecules. **However, dysregulation** of these inflammatory mediators (by immunomodulatory genes) can cause lethality, and should be considered during vaccine design to minimize immunopathology.
- SARS-CoV and MERS-CoV-specific **CD4+ T cell** responses are likely necessary for complete and effective protection.
- **CoV vaccines should elicit antibody responses as well as specific memory CD4+ and CD8+ T cells.**

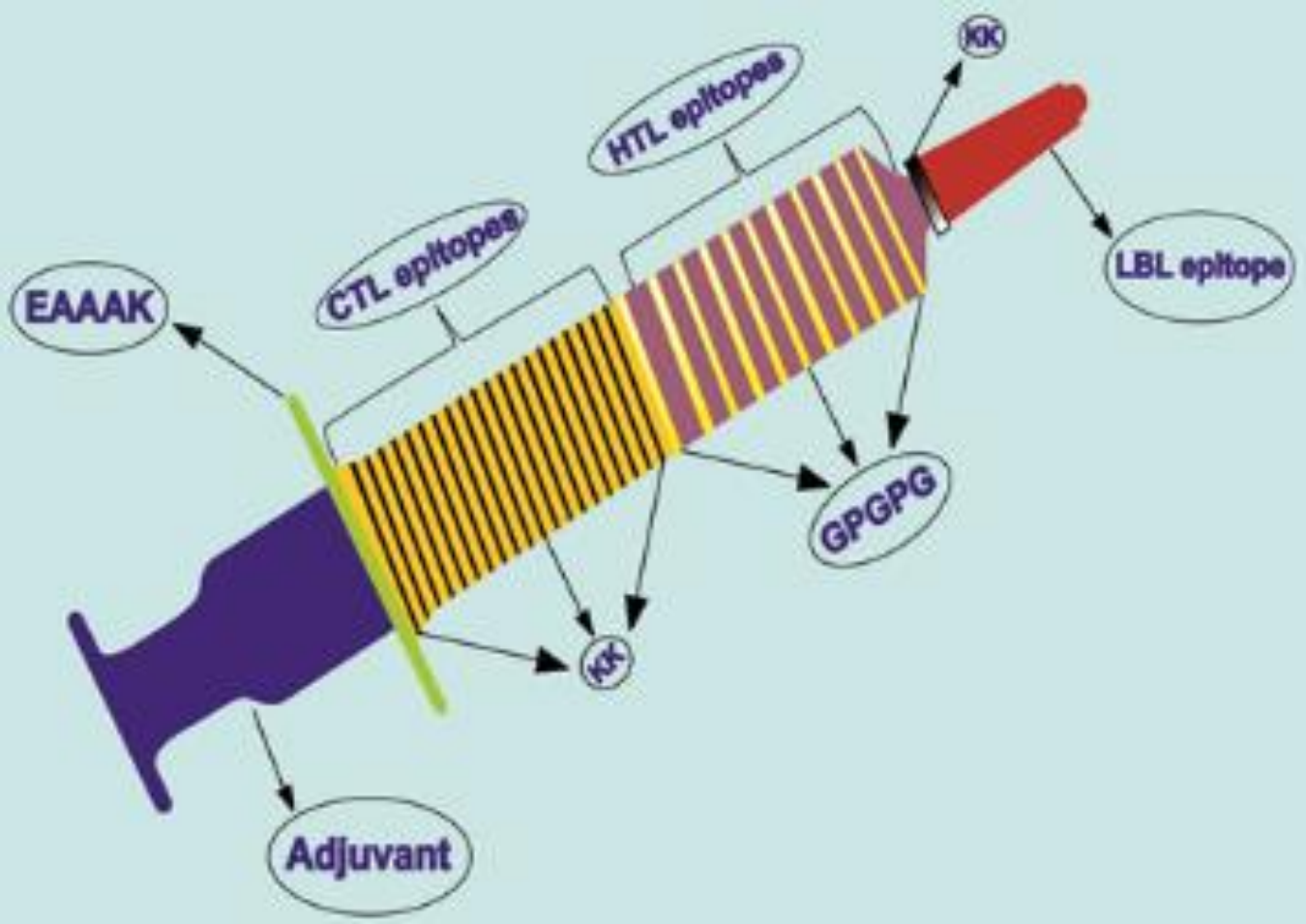
Subunit vaccines (peptide vaccines)

- **The use of peptides as immunogens attempts to remove epitopes from viral proteins and present them to the immune system to elicit a focused antibody response.**
- Epitopes are mostly contained within a linear stretch of amino acids
  - **cytotoxic T lymphocyte (CTL) epitopes**
  - **helper T lymphocyte (HTL) epitopes**
  - **linear B-lymphocyte (LBL) epitopes**
  - **conformational B-lymphocyte epitopes**
- *Advantages:*
  - Inducing **specific** antibody responses, especially by linear epitopes
  - **Diversity** in antigenic epitopes (reducing immunodominance)
  - Have highest **safety** profile
  - Avoid difficulties with **synthesis** and manufacture
- *Disadvantages*
  - Weak immune response

Design steps

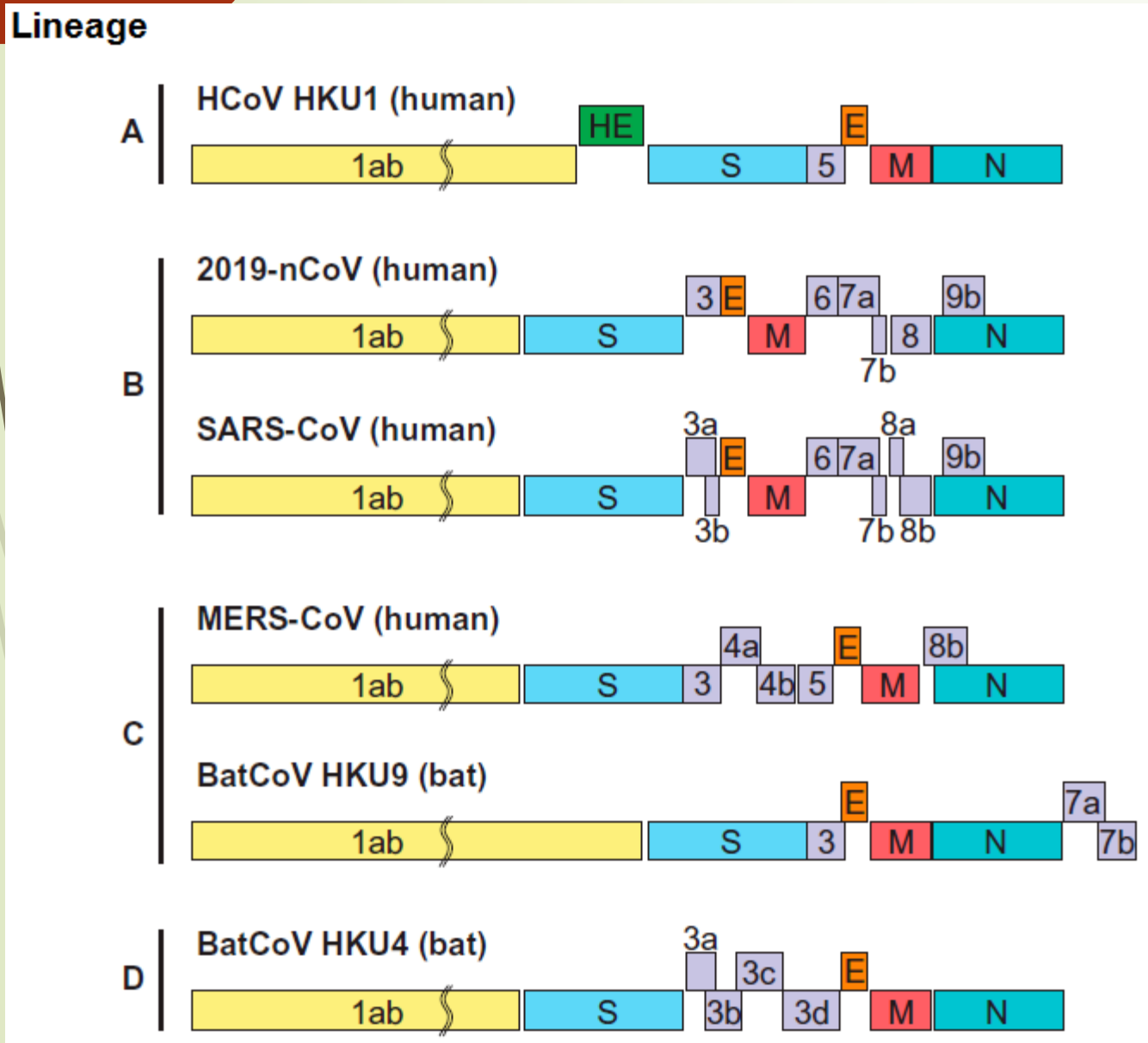
- Sequence retrieval
- Identifying cytotoxic T lymphocyte (CTL) epitopes
- Identifying helper T lymphocyte (HTL) epitopes
- Linear B-cell epitopes prediction
- Conformational B-cell epitopes prediction
- Assessment of identified epitopes for antigenicity, allergenicity, and toxicity
- Assembly to a multi-epitope vaccine construct by linkers
- Immunogenic, allergenic and physiochemical evaluation of vaccine construct
- Assay the vaccine binding to immune receptor TLR-3

Multi-epitope vaccine concept



- GPGPG linker: Enhanced immunogenicity
- KK linker: Independent epitopes
- Adjuvant = beta-defensin

کروناویروس ها



- Bat coronaviruses are at the root of the phylogenetic tree of CoVs.

SARS-CoV-2 proteins

- Nucleotide identity:
- SARS-CoV-2 with bat SARS-like-CoVZXC21: 89%
- SARS-CoV-2 with human SARS-CoV : 82%

Table 3. Amino acid identity between the 2019 novel coronavirus and bat SARS-like coronavirus or human SARS-CoV.

Amino acid identity (%)	2019-nCoV vs. bat-SL-CoVZXC21	2019-nCoV vs. SARS-CoV
NSP1	96	84
NSP2	96	68
NSP3	93	76
NSP4	96	80
NSP5	99	96
NSP6	98	88
NSP7	99	99
NSP8	96	97
NSP9	96	97
NSP10	98	97
NSP11	85	85
NSP12	96	96
NSP13	99	100
NSP14	95	95
NSP15	88	89
NSP16	98	93
Spike	80	76
Orf3a	92	72
Orf3b	32	32
Envelope	100	95
Membrane	99	91
Orf6	94	69
Orf7a	89	85
Orf7b	93	81
Orf8/Orf8b	94	40
Nucleoprotein	94	94
Orf9b	73	73

چالش ها و راهکارهای مرتبط با کروناویروس ها

- Coronaviral S glycoprotein is **highly glycosylated** and **antigenically variable**
- **Antibody-dependent enhancement of infectivity (ADEI)** is a condition whereby non-neutralizing antibodies are produced following an infection or a vaccination, which enhance the infectivity of the subsequent infection.
  Solutions:
  - **Glycosylation** to shield the non-neutralizing epitopes
  - **immunofocusing**, i.e. to direct the adaptive immune responses to target only the critical neutralizing epitope
  - inclusion of **structural proteins** (high epitope conservancy)
  - Use of **optimal combinations** of antigen and adjuvant
  - Highly **concentrated** antibody
- Eosinophilia:
  - **Solution:** TLR agonist adjuvants

چالش ها و راهکارهای مرتبط با واکسن پپتیدی

- In case of peptide vaccines, the isolated peptides are generally **flexible and adopt many conformations**, only one or a few of which resemble the conformation of the epitope as it exists in the antigen.
- Consequently, the resulting antibody response from peptide-based immunizations tend to elicit high titers of peptide-directed antibodies, but **low titers of antibodies that recognize** the native antigen.
  - **Solution**: linking the fragment to human Fc; using an adjuvant
- **Scaffolded or chimeric proteins**: One structure-based-design approach to address the flexibility of peptide immunogens is referred to as **epitope scaffolding or epitope transplantation**. Here the goal is to "transplant" an epitope onto **a heterologous protein "scaffold"** that preserves the conformation of the epitope as it exists in the native antigen.

چالش ها و راهکارهای مرتبط با واکسن پپتیدی

- Subunit vaccines only include subviral components that **do not represent the full antigenic complexity** of the virus, resulting in limited protective efficacy or unbalanced immune responses that may lead to immunopathology.
  - **Multivalent (multi-epitope) vaccines** designed using in silico methods which contain the B cell and T cell epitopes of S, E, M, N and NSPs have been proposed.

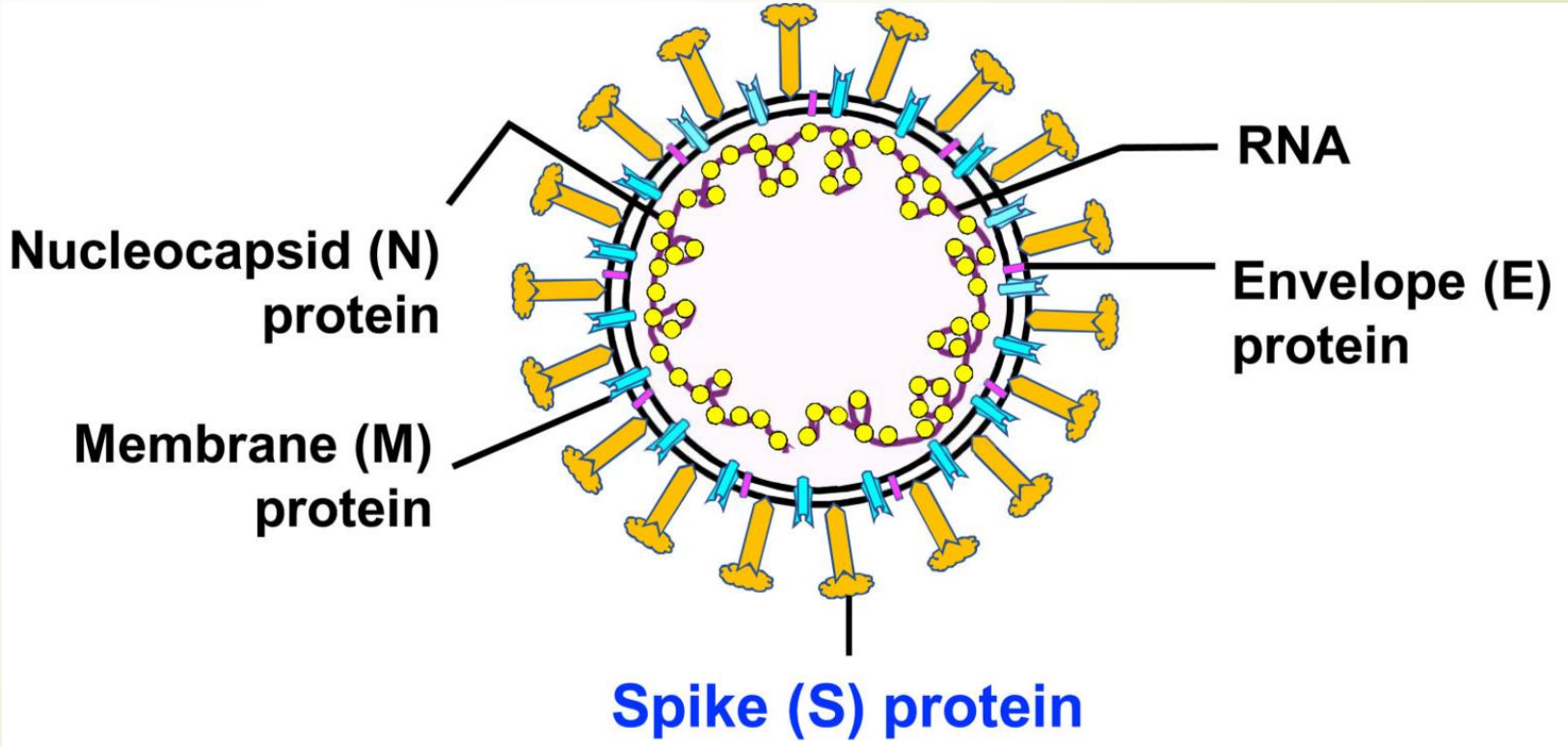
مطالعات مرتبط با کووید-۱۹

- Specific epitope regions in SARS-CoV-2 with high homology to SARS-CoV were identified (10.1016/j.chom.2020.03.002; 10.1007/s13337-020-00571-5; 10.3390/v12030254).
- Antigenic properties of spike glycoprotein were more focused by theory and experimental researchers (10.1002/jmv.25698; 10.1038/s41423-020-0377-z; 10.1016/j.cell.2020.02.058).
- Peptide-based vaccine designs integrating epitopes from a single or few viral protein(s) (10.1002/jmv.25736; 10.1038/s41598-020-67749-1; 10.7717/peerj.9572; 10.1016/j.micpath.2020.104236; 10.1080/07391102.2020.1780944)
- Designs integrating epitopes from the whole proteome of SARS-CoV-2 (10.1038/s41598-020-70864-8)

Structural proteins as epitope source

- ample evidence on their augmented immunogenicity
- a highly-conserved epitope set
- overlap with similar studies (high epitope conservancy among geographical strains)
- Coping with ADEI issue

- Appending an adjuvant to potentiate the immune response elicitation



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