Good afternoon, Ma'am; good afternoon, Seniors; and good afternoon, my friend.

Today, I'll be presenting my slide on "Coronavirus membrane fusion mechanism opportunistic model for SARS-CoV-2 fusion**"**

Slide 2

Here is the Overview

In this we will firstly we look about ACES 2 then will go for Function Of TMPRSS -2 **how it's work. After that we will closely look on the basic** S protein structure, Life Cycle of SARS-CoV-2 and then close with a small summary.
so we have a better understanding how that actually work.

Slide 3

So, let' Start… with

Now, spike is the main protein that interacts with the host cell receptors. In the first step of receptor binding, spike protein is cleaved into S1 and S2 by the host cell protease, one of which is transmembrane protease serine 2 abbreviated as TMPRSS2. The main function of S1 subunit is to bind with the host cell surface receptors, while the function of S2 subunit is to mediate membrane fusion. So one of the potential therapeutic approaches is to either develop vaccine that contains 01:30antigens(**Antibodies are proteins produced by B cells (in the bone marrow), designed to bind to specific molecules (antigens), Antigens are molecules capable of stimulating an immune response. Each antigen has distinct surface features, or epitopes, resulting in specific responses. Antibodies (immunoglobins) are Y-shaped proteins produced by B cells of the immune system in response to exposure to antigens**) derived from the spike protein, which can boost recognition of the virus by the immune cell or to develop monoclonal antibodies (**These cloned or duplicated antibodies are called Monoclonal Antibodies or MABs. In contrast to MABs, a vaccine aims to boost or provoke one's immune system to generate antibodies that are effective in fighting the foreign antigen)** that bind to the coronavirus spike protein and block the interaction with human cells. Another potential target is transmembrane protease serine 2, which appears to be essential for entry and viral spread of the coronavirus. One agent that is currently being tested, called Camostat Mesylate (**Camostat is a serine protease inhibitor. Serine protease enzymes have a variety of functions in the body, and so camostat has a diverse range of uses. Camostat is approved in Japan for the treatment of chronic pancreatitis and postoperative reflux esophagitis.[1][2] The oral proteolytic enzyme inhibitor "Foipan Tablets" has been on the market since 1985. The manufacturer is Ono Pharmaceutical. The drug is used in the treatment of some forms of cancer and is also effective against some viral infections, as well as inhibiting fibrosis in liver or kidney disease or pancreatitis.**)has shown to inhibit TMPRSS2 activity and thus is being considered as a potential anticoronavirus candidate.

02:00Now, recent protein modeling studies on the spike protein suggest that SARS-CoV-2 has a strong binding affinity to human angiotensin converting enzyme 2 (ACE2) receptors and likely uses them as a mechanism for cell entry. One location where ACE2 receptors are highly expressed is type II alveolar cells, which are found in the lungs. However ACE2 receptors are also found in many extrapulmonary tissues including heart, kidney, endothelium, and intestine. which we have discussed in part 1

So another potential drug targets could be the interaction sites between ACE2 and spike protein. One compound that has already been found during recent computational study, which is predicted to bind with the binding interface of the Spike–ACE2 complex, is a natural flavonoid called Hesperidin.(**Hesperidin** is a flavanone glycoside found in citrus fruits. Its aglycone form is called hesperetin. Its name is derived from the word "hesperidium", for fruit produced by citrus trees.

Hesperidin was first isolated in 1828 by French chemist Lebreton from the white inner layer of citrus peels

) Now studies in mice demonstrated that coronavirus binding of spike protein to ACE2, downregulates the receptor, and thereby contributes to severe lung injury.
03:00This suggests that delivery of excessive soluble form of ACE2 may competitively bind with SARS-CoV-2 and not only neutralize the virus but also rescue cellular ACE2 which regulates the renin-angiotensin system to protect the lungs from injury. One small study has already found recombinant human ACE2 to be safe, with no negative hemodynamic effects in healthy subjects

Now, moving on to the next replication step.03:30So after uncoating, first the genomic RNA of coronavirus acts as an mRNA for translation of the replicase polyprotein 1a (pp1a) and 1ab (pp1ab). Autoproteolytic cleavage of these polyproteins then produces number of non-structural proteins including RNA-dependent RNA polymerase, helicase and nonstructural protein 3, 4, and 6. These nonstructural proteins 3, 4, and 6 are thought to be responsible for anchoring the coronavirus replication/transcription complex through recruitment of intracellular endoplasmic
04:00reticulum membranes to form double membrane vesicles abbreviated as DMV. RNA-dependent RNA polymerase (RdRp) and helicase localize to double membrane vesicles and drive the production of subgenomic RNAs (sgRNAs) from which the structural and accessory proteins are produced in the next phase of translation. Now RNA-dependent RNA polymerase (RdRp) is a target of investigational drugs such as Remdesivir and Favipiravir.
04:30Preliminary research has shown that both of these agents inhibit RNA-dependent RNA polymerase and thus might be useful in treatment of early or mid-stages of the coronavirus disease. Allright, going back to the next replication step, once synthesized, transmembrane structural proteins "S", "M", and "E" are inserted, and folded in the

endoplasmic reticulum (ER) and then transported to the Endoplasmic reticulum–Golgi intermediate compartment abbreviated as ERGIC.
05:00The "N" proteins on the other hand bind the viral genomic RNA in cytoplasm to form nucleocapsid. Once the final virion assembly occurs in the intermediate compartment, mature virions are released via smooth-walled vesicles by exocytosis.(**Exocytosis** (/ˌɛksoʊsaɪˈtoʊsɪs/[1][2]) is a form of active transport and bulk transport in which a cell transports molecules (e.g., neurotransmitters and proteins) out of the cell (*exo-* + *cytosis*). As an active transport mechanism, exocytosis requires the use of energy to transport material. Exocytosis and its counterpart, endocytosis, are used by all cells because most chemical substances important to them are large polar molecules that cannot pass through the hydrophobic portion of the cell membrane by passive means. Exocytosis is the process by which a large amount of molecules are released; thus it is a form of bulk transport. Exocytosis occurs via secretory portals at the cell plasma membrane called porosomes. Porosomes are permanent cup-shaped lipoprotein structure at the cell plasma membrane, where secretory vesicles transiently dock and fuse to release intra-vesicular contents from the cell.) Now, in addition to the investigational agents that we discussed thus far, there are few antiviral drugs that are already on the market that have been reported as potentially effective against coronavirus. One of them is antimalarial drug called Chloroquine, which is thought to exert its antiviral activity
05:30in part by increasing the pH in host-cell lysosomes, which in turn inhibits hydrolytic activity of protease enzymes that are required for processing of viral glycoproteins during infection. Other drugs of interest are the combination of Lopinavir and Ritonavir, as well as Darunavir and Cobicistat, which belong to class of drugs known as protease inhibitors. These drugs were originally designed to block HIV viral replication.
06:00However molecular docking (In the field of molecular modeling, **docking** is a method which predicts the preferred orientation of one molecule to a second when a ligand and a target are bound to each other to form a stable complex.[1] Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using, for example, scoring functions.) studies have shown that they may also interact with some proteins that are required for replication of coronavirus.

Slide 3

So, let' look at some of the SARC-CoV Images

a. Human coronavirus 229E virus particles, a coronavirus in the same family as SARS-CoV, as seen in a colorized electron microscopic image. Virions contain characteristic club-like projections emanating from the viral membrane.

Image source: F.A. Murphy and S. Whitfield, CDC

b. Negative stain electron microscopy shows a SARS-CoV particle with club-shaped surface projections surrounding the periphery of the particle, a characteristic feature of coronaviruses.

Image source: C.D. Humphrey, CDC

c. An electron microscopic image of a thin section of SARS-CoV within the cytoplasm of an infected cell, showing the spherical particles and cross-sections through the viral nucleocapsid.

Image source: C.S. Goldsmith, CDC

d. A SARS-CoV-infected cell with virus particles in vesicles, which appear to migrate toward the cell surface and fuse with the plasma membrane, releasing the viral particles. Many of the particles adhere to the plasma membrane, creating a characteristic knob-like appearance on the surface of the cell.

Image source: C.S. Goldsmith, CDC

So lets start the final slide about

Life cycle of SARS-CoV-2 consists of cellular invasion of virus, expression of viral genes, and formation of progeny and eventual exit. It can roughly be divided into following 5 steps.

First and foremost we look at

Attachment to Host Cell Surface how it's attached

Three monomeric S polypeptides make up the Spike protein system. Each monomeric polypeptide contains S1 and S2 subunits with multiple functional domains that go through a number of conformational changes, as we discussed in the previous presentation. SARS-CoV-2 binds to the host cell receptor ACE2 (angiotensin-converting enzyme 2) zinc-binding carboxypeptidase via the receptor binding domain (RBD) of the S1 subunit of the spike glycoprotein. (Zinc is a trace element that aids in direct antiviral and immune responses. The increased risk of viral infections (Herpesviridae, HIV, and Hepatitis C) in people with zinc deficiency supports this theory. Zinc supplementation has been recommended as a

preventive or therapeutic strategy to control viral infections) this zinc-binding carboxypeptidase which is normally involved in the regulation of blood pressure and cardiac function. Furthermore, a recent molecular simulation-based study suggests that SARS-S CoV-2's protein can bind to nicotinic acetylcholine receptors, indicating its diverse binding potential and possibly explaining multi-organ pathogenesis. Other proteases, such as transmembrane protease serine, 2 (TMPRSS2), cathepsin L, and/or other proteases, that also can cleave the S1/S2 site. Following cleavage at the S′2 site, membrane fusion is presumably triggered by irreversible conformational changes, facilitating access to the host cell.

Viral Penetration and Uncoating

After fusion of viral spike glycoprotein with ACE2 there is conformational changes, releasing the viral nucleocapsid into the cell cytosol. This process is aided by several host factors, including type II transmembrane protease serine 2 (TMPRSS2) protease and cathepsin L.

7.3. Replication-Transcription Complex (RTC) Formation

Immediately after release of viral nucleocapsid, +ssRNA serves as functional mRNA with respect to ORF1a and ORF1b encoding polyprotein pp1a (440–500 kDa) and pp1ab (740–810 kDa), respectively. However, due to differences in frameshift efficiency between ORF1a and ORF1b genes, pp1a is 2 times more expressed than pp1ab. The autoproteolytic processing of these two polyproteins yields 16 nsps, which together form the RTC for viral RNA synthesisThis functional RTC results into formation of a nested set of guide sgRNAs via discontinuous transcription.

7.4. Synthesis of Viral RNA

RTC formation initiates a molecular process that results in the production of multiple copies of viral RNA. The intermediate template is -ssRNA (negative ssRNA). Meanwhile, during -ssRNA synthesis, polymerase switches template at short motifs called transcription regulated sequences (TRS), resulting in a multiple 5′-nested set of negative sense sgRNAs that are then used as templates to make a 3′-nested set of positive sense sgRNAs. They then associate with the ribosome of the host, synthesizing a variety of structural and accessory proteins in order to construct multiple virus structures.

7.5. Molecular Assembly and Release of SARS-CoV-2

The majority of membrane-associated structural and accessory proteins, such as S, M, and E, are synthesized by endoplasmic reticulum-bound ribosomes, whereas other viral proteins, such as N protein, are translated by free cytosolic ribosomes of host cell. These structural proteins are also subjected to posttranslational modifications that affect their functions. The virion's assembly converges at the endoplasmic reticulum–Golgi intermediate compartment (ERGIC), where M protein serves as a scaffold and orchestrates virion morphogenesis through heterotypic interactions with other structural proteins like M-S and M-E, allowing for easier molecular incorporation. M-N interactions also play a role in the condensation of the nucleocapsid with the envelope, along with E protein.

At the bottom

The S protein has an extracellular N-terminus, a transmembrane (TM) domain anchored in the viral membrane, and a short intracellular C-terminal segment. When the virus interacts with the host cell, the S protein undergoes extensive structural rearrangement, allowing the virus to fuse with the host cell membrane. The spikes are coated with polysaccharide molecules to hide them from the host immune system's detection during entry. The viral particle is surrounded by a bulbous, crown-like halo formed by S protein trimers.

The bulbous head and stalk region are formed by the S1 and S2 subunits, which are based on the structure of coronavirus S protein monomers. The structure of the SARS-CoV-2 trimeric S protein has revealed the different conformations and functions of the S RBD domain in open and closed states. Which we will discuss in upcoming sildes

When the S protein adopts the prehairpin conformation, FP is a short segment of 15–20 conserved amino acids composed primarily of hydrophobic residues such as glycine (G) or alanine (A) that anchor to the target membrane. Previous research has shown that FP is required for membrane fusion by disrupting and connecting the host cell membrane's lipid bilayers.

HR1 and HR2 are composed of a repetitive heptapeptide: HPPHCPC, where H stands for hydrophobic or traditionally bulky, P for polar or hydrophilic, and C for another charged residue. HR1 and HR2 form the six-helical bundle (6-HB), which is required for the S2 subunit's viral fusion and entry function. HR1 is found on the C-terminus of a hydrophobic FP, while HR2 is found on the N-

terminus of the TM domain. The S protein's downstream TM domain anchors it to the viral membrane, and the S2 subunit has a CT tail at the end.

RBD binds to ACE2, and S2 changes conformation by inserting FP into the target cell membrane, exposing the prehairpin coiled-coil of the HR1 domain and triggering interaction between the HR2 domain and the HR1 trimer to form 6HB, allowing viral fusion and entry. HR1 forms a homotrimeric assembly on the surface with three highly conserved hydrophobic grooves that bind to HR2. To interact with the HR1 domain, the HR2 domain forms both a rigid helix and a flexible loop. There are many strong interactions between the HR1 and HR2 domains inside the helical region, which is referred to as the "fusion core region," in the postfusion hairpin conformation of CoVs (HR1core & HR2 core regions, respectively).

The S2 subunit, composed successively of a FP, HR1, HR2, TM domain, and cytoplasmic domain fusion (CT), is responsible for viral fusion and entry.