Slide 1.

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's Start… with an Introduction

The SARS-CoV-2 virus can infect a wide range of cells and systems of our body. COVID-19 is most known for affecting the upper respiratory tract e.g. nose, and throat) and the lower respiratory tract e.g. (windpipe and lungs) The lungs are the organs which is most affected by COVID-19 because the virus accesses host cells through the receptor for the enzyme angiotensin-converting enzyme 2 which is also knows as (ACE2), which is most abundant on the surface of type II alveolar cells of the lungs Type II alveolar epithelial cells are a heterogeneous population that have critical secretory and regenerative roles in the alveolus to maintain lung. The virus uses a special surface glycoprotein that is "spike" to connect to the ACE2 receptor and enter the host cell basically Coronaviruses binds to a cell surface receptor and is activated for membrane fusion and cell entry by proteolytic cleavage of its spike protein by a host protease. The primary receptor for SARS-CoV-2 binding, is ACE-2.

Here in this slide

{(ACE2) is not only an enzyme but also a functional receptor on cell surfaces through which SARS-CoV-2 enters the host cells and is highly expressed in the heart, kidneys, and lungs and shed into the plasma or in a soluble form. So

What is angiotensin converting enzyme 2 (ACE2)?

ACE2 is a nothing but a protein on the surface of many cell types. It is an enzyme that generates small proteins – by cutting up the larger protein angiotensinogen – that then go on to regulate functions in the cell. Using the spike-like protein on its surface, the SARS-CoV-2 virus binds to ACE2 – like a key being inserted into a lock – prior to entry and infection of cells. Hence, ACE2 acts as a cellular doorway – a receptor – for the virus that causes COVID-19 SO here to note that **The primary receptor for SARS-CoV-2 binding is ACE-2. This is a common, but not universal, binding receptor**. now let's move to the TMPRSS2 on the next slide

Slide 4

What is the normal role ACE2 plays in the body?

ACE2 is a vital element in a biochemical pathway that is critical to regulating processes such as blood pressure, wound healing and inflammation, called the **renin-angiotensin-aldosterone** system (RAAS) pathway.

ACE2 helps modulate the many activities of a protein called angiotensin II (ANG II) that increases blood pressure and inflammation, increasing damage to blood vessel linings and various types of tissue injury. ACE2 converts ANG II to other molecules that counteract the effects **of ANG II.**

Of greatest relevance to COVID-19, ANG II can increase inflammation and the death of cells in the alveoli which are critical for bringing oxygen into the body; these harmful effects of ANG II are reduced by ACE2.

When the SARS-CoV-2 virus binds to ACE2, it prevents ACE2 from performing its normal function to regulate ANG II signaling. Thus, ACE2 action is "inhibited," removing the brakes from ANG II signaling and making more ANG II available to injure tissues. This "decreased braking" likely contributes to injury, especially to the lungs and heart, in COVID-19 patients.}

What is TMPRSS2?

Transmembrane serine protease 2 (TMPRSS2) is a cell surface protein primarily expressed by endothelial cells (Definition Endothelial cells **form the barrier between vessels and tissues**. That control the flow of substances and fluid into and out of a tissue. An impaired function can lead to serious health issues throughout the body. Helps in keeping blood vessels healthy) across the respiratory and digestive tracts. As a serine protease, it is involved in the cleaving peptide bonds of proteins that have serine as the nucleophilic amino acid within the active site. SO basically, this cleave that site

The exact biological function of TMPRSS2 is largely unknown, although research has shown that it is involved in certain pathologies

Both ACE2 and TMPRSS2 are expressed in nasal, bronchial, and gastrointestinal epithelium. TMPRSS2 activates; or primes, the spike protein domain (a key glycoprotein found on coronaviruses) which leads to the virus fusing to the respiratory epithelia on the cell surface through binding to ACE2. As TMPRSS2 is a serine protease, it primes the spike-domain (S) of SARS-CoV-2 by cleaving as the S1/S2 sites.

please wait we will look into this sites in the upcoming slide.

TMPRSS2 is expressed less in Type II alveolar cells and alveolar macrophages than in bronchial epithelial cells; additionally, no TMPRSS2 protein was found in Type I alveolar cells of the respiratory surface in this study. Another important factor to consider in this context is the modification of lung TMPRSS2 expression caused by viral infections, as suggested by previous findings on SARS-CoV and the ACE2 receptor.

Now lets move on next silde

Side 5

In this slide we will look Coronavirus spike (S) protein. **A.** Cartoon figure of the CoV particle on the top here and complete CoV viral genome at the bottom.

So, Let's start with viral particle here CoVs have a lipid envelope with three structural transmembrane proteins:

1. (S) is a spike,
2. (M) is a membrane,
3. and (E) is the envelope.

Where (n) nucleocapsid

The single-stranded RNA (ssRNA) genomes of severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) encode **ORFs. This orfs is nothing but (open reading frames) in biological terms that is** two large polyproteins, pp1a and pp1b with name ORF1a and ORF1b respectively SARS-CoV and MERS-CoV belong to the Coronavirus genus in the family *Coronaviridae* and have large, positive-sense RNA genomes e.g. is 27.9 Kilobase pairs and 30.1 Kilobase pairs, respectively for sars and mers. Similarly to all viruses in the order SARS-CoV and MERS-CoV have a unique coding strategy: two-thirds of the viral RNA is translated into two large polyproteins, and the remainder of the viral genome is transcribed into a nested set of subgenomic mRNAs. The two polyproteins, pp1a and pp1ab, encode 16 non-structural proteins that make up the viral replicase–transcriptase complex. The polyproteins are cleaved by two proteases, papain-like protease and a main protease, 3C-like protease. The nonstructural protein rearrange membranes that are derived from the rough endoplasmic reticulum (RER) into double-membrane vesicles, in which viral replication and transcription occur. Which we see in next slide. **One unique feature of coronaviruses is the exoribonuclease (ExoN) function which provides the proofreading capability required to maintain a large RNA genome without the accumulation of detrimental mutations.** SARS-CoV and MERS-CoV transcribe 12 and 9 subgenomic RNAs, respectively, and these encode the four structural proteins spike (S), envelope (E), membrane (M) and nucleocapsid (N), as well as several accessory proteins that are not involved in viral replication but interfere with the host innate immune response or are of unknown or poorly understood function.

SARS-CoV-2 is a single-stranded RNA-enveloped virus [5]. An RNA-based metagenomic next-generation sequencing approach has been applied to characterize its entire genome, which is 29,881 bp in length, encoding 9860

amino acids. Gene fragments express structural and nonstructural proteins. The S, E, M, and N genes encode structural proteins, whereas nonstructural proteins, such as 3-C-like protease, papain-like protease, and RNA-dependent RNA polymerase, are encoded by the ORF region

A large number of glycosylated S proteins cover the surface of SARS-CoV-2 and bind to the host cell receptor angiotensin-converting enzyme 2 (ACE2), mediating viral cell entry. When the S protein binds to the receptor, TM protease serine 2 (TMPRSS2), a type 2 TM serine protease located on the host cell membrane, promotes virus entry into the cell by activating the S protein. Once the virus enters the cell, the viral RNA is released, polyproteins are translated from the RNA genome, and replication and transcription of the viral RNA genome occur via protein cleavage and assembly of the replicase–transcriptase complex. Viral RNA is replicated, and structural proteins are synthesized, assembled, and packaged in the host cell, after which viral particles are released

Slide 6

Let's us move to the

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 we look at

Attachment to Host Cell Surface how it's attached

The Spike protein system consisting of three monomeric S polypeptides. Each monomeric polypeptide contains S1 and S2 subunits with multiple functional domains which undergo several conformational changes. SARS-CoV-2 via its **receptor binding domain** (RBD)of S1 subunit of spike glycoprotein binds host cell receptor ACE2 (angiotensin-converting enzyme 2) zinc-binding **carboxypeptidase** (Zinc is an essential trace element that plays an important role in direct antiviral and immune responses. Such evidence can be confirmed by the higher risk of viral infections (Herpesviridae, HIV and Hepatitis C) in individuals with zinc deficiency. In vitro demonstration of the multiple mechanisms of antiviral actions of zinc has led to the indication of its supplementation as a preventive or therapeutic strategy to control viral infections), which is normally involved in cardiac function and blood pressure regulation. In addition, a recent

molecular simulation-based work has proposed that SARS-CoV-2′s S protein can also bind to **nicotinic acetylcholine receptors** indicative of its diverse binding potential and may be one of the underlying reasons for multi-organ pathogenesis The S1/S2 site are also subjected to cleavage by other proteases such as transmembrane protease serine, **2 (TMPRSS2), cathepsin L and/or other protease**s. Subsequent cleavage at the S′2 site presumably triggers membrane fusion via irreversible, conformational changes and thereby facilitates access to 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, pp1a is **2 folds more expressed compared to pp1ab** owing to differential efficiency of frameshift between ORF1a and ORF1b genes. These two polyproteins undergo autoproteolytic processing yielding 16 nsps, which together form the RTC for viral RNA synthesis. This functional RTC results into formation of a nested set of guide sgRNAs via discontinuous transcription.

7.4. Synthesis of Viral RNA

The formation of RTC sets molecular process in motion leading to synthesis of multiple copies of viral RNA. These -ssRNA (negative ssRNA) serves as intermediate template. Meanwhile, polymerase switches template at short motifs, transcription regulated sequences (TRS) during -ssRNA synthesis, thereby producing a multiple 5′-nested set of negative sense sgRNAs which, in turn, used as templates to form a 3′-nested set of positive sense sgRNAs. Thereafter, they associate with host ribosome, synthesizing various structural and accessory proteins building multiple virus structure .

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

Most of the structural and accessory proteins associated with membrane such as S, M, and E are synthesized by endoplasmic reticulum-bound ribosomes, whereas other viral proteins, including N protein, are translated by free cytosolic ribosomes of host cells. In addition, these structural proteins also undergo posttranslational modification that modulate their functions. The assembly of virion converges at site of endoplasmic reticulum–Golgi intermediate compartment (ERGIC), wherein M protein provides scaffold and orchestrate virion morphogenesis by heterotypic interaction with other structural proteins, such as M-S and M-E, thereby facilitating

molecular incorporation. Furthermore, M-N interactions mediates condensation of the nucleocapsid with the envelope along with E protein . Post molecular assembly, progeny virions are transported in smooth-wall vesicle and using secretory pathway they are trafficked to plasma membrane and eventually exit though exocytosis and spread to other parts of body.

At the bottom

With a size of 180–200 kDa, the S protein consists of an extracellular N-terminus, a transmembrane (TM) domain anchored in the viral membrane, and a short intracellular C-terminal segment once the virus interacts with the host cell, extensive structural rearrangement of the S protein occurs, allowing the virus to fuse with the host cell membrane. The spikes are coated with polysaccharide molecules to camouflage them, evading surveillance of the host immune system during entry. S protein trimers visually form a characteristic bulbous, crown-like halo surrounding the viral particle.

Based on the structure of coronavirus S protein monomers, the S1 and S2 subunits form the bulbous head and stalk region. The structure of the SARS-CoV-2 trimeric S protein has revealing different conformations of the S RBD domain in opened and closed states and its corresponding functions

FP is a short segment of 15–20 conserved amino acids of the viral family, composed mainly of hydrophobic residues, such as glycine (G) or alanine (A), which anchor to the target membrane when the S protein adopts the prehairpin conformation. Previous research has shown that FP plays an essential role in mediating membrane fusion by disrupting and connecting lipid bilayers of the host cell membrane [23].

HR1 and HR2 are composed of a repetitive heptapeptide: HPPHCPC, where H is a hydrophobic or traditionally bulky residue, P is a polar or hydrophilic residue, and C is another charged residue [24]. HR1 and HR2 form the six-helical bundle (6-HB) (Fig. 2e), which is essential for the viral fusion and entry function of the S2 subunit [13]. HR1 is located at the C-terminus of a hydrophobic FP, and HR2 is located at the N-terminus of the TM domain [25]. The downstream TM domain anchors the S protein to the viral membrane, and the S2 subunit ends in a CT tail

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 HR1 trimer to form 6-HB, thus bringing the viral envelope and cell membrane into proximity for viral fusion and entry [26]. HR1 forms a homotrimeric assembly in which three highly conserved hydrophobic grooves on the surface that bind to HR2 are exposed. The HR2 domain forms both a rigid helix and a flexible loop to interact with the HR1 domain. In the postfusion hairpin conformation of CoVs, there are many strong interactions between the HR1 and HR2 domains inside the helical region, which is designated the "fusion core region" (HR1core and HR2core 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.

Now move let's Summarize

Model of coronavirus dual entry pathway. This model depicts the two methods of viral entry: early pathway and late pathway. As the virus binds to its receptor (*1*),

it can achieve entry via two routes: plasma membrane or endosome. For SARS-CoV: The presence of exogeneous and membrane bound proteases, such as trypsin and TMPRSS2, triggers the early fusion pathway (*2a*).

Otherwise, it will be endocytosed (*2b*, *3*).

For MERS-CoV: If furin cleaved the S protein at S1/S2 during biosynthesis, exogeneous and membrane bound proteases, such as trypsin and TMPRSS2, will trigger early entry (*2a*).

Otherwise, it will be cleaved at the S1/S2 site (*2b*) causing the virus to be endocytosed (*3*).

For both: Within the endosome, the low pH activates cathepsin L (*4*),

cleaving S2′ site, triggering the fusion pathway and releasing the CoV genome. Upon viral entry, copies of the genome are made in the cytoplasm (*5*),

where components of the spike protein are synthesized in the rough endoplasmic reticulum (ER) (*6*)

. The structural proteins are assembled in the ER-Golgi intermediate compartment (ERGIC), where the spike protein can be pre-cleaved by furin, depending on cell type (*7*),

followed by release of the virus from the cell (*8*, *9*).

For SARS-CoV-2: Studies currently show that SARS-CoV-2 can utilize membrane bound TMPRSS2 or endosomal cathepsin L for entry and that the S protein is processed during biosynthesis. Other factors that can influence the viral entry pathway are calcium and cholesterol (*not shown*).

Thank you