

Class: SARS-CoV S-protein

**Attributes:**

**Accession #:** NP\_828851

**Synonyms:** Orf 2, spike protein

**Molecular weight:** 150 kDa

**Number of amino acids:** 1255

**Structure:**

**1) Homology:**

- similar structural motifs in HIV-1 gp41 and SARS-CoV S2 proteins suggest an analogous membrane fusion mechanism (4)
- S2 and gp41 share a hairpin structure (4)
- N-term heptad repeat on residues 913-1000 of CUHK-W1 isolate (4)
- C-term heptad repeat on residues 1151-1185 (4)
  - the N-heptad repeat of S2 is longer than gp41 (4)
  - while the C-heptad repeat of gp41 shows barely any repeat signal the C-heptad repeat for S2 has a perfect Leucine / Isoleucine heptad repeat in its "d" positions (4)
- a tryptophan / tyrosine-rich motif is located between the C-heptad repeat and the transmembrane domain (4)

**2) Domains:**

- type I membrane glycoprotein with the N-term and majority of protein on outside of virus (aa 14-1195) (1)
- form trimers
- residues 12-50, 426-456, 478-494, 541-564, and 922-1118 display highest antigenic potential (1)
- N-terminal contains short type I signal peptide (amino acids 1-13) composed of hydrophobic amino (1)
- C-terminal (aa 1196-1255) consists of a transmembrane domain and cytoplasmic tail rich in cysteine residues (1)
- domain I** (aa 727-845) with a coiled coil fold contains a set of five anti-parallel helices,
- domain II** (aa 864-1048) with a sandwich fold-like conacanavalin A is characterized by 12-14 strands in two sheets
- domain III** (aa 1049-1195) is a six-stranded anti-parallel beta barrel
  
- S1 subunit** (2)
  - hydrophobic residues Phe187, Phe253, Phe334, Trp340, Trp423, Tyr677 contribute to extensive hydrophobic cluster formation (2)
  - 20 cysteines (2)
  - disulphide bridges predicted between (2)
    - C19-C128, C133-C467, C159-C288, C278-C474, C323-C657, C348-C511, C366-C378, C419-C603, C524-C576 and C635-C648 (2)
- S2 subunit**
  - two putative binding sites in the Phe850-Phe870 and the Phe1077-Phe1079 regions are located in the putative receptor binding sites (2)
  - disulphide bridges predicted between: C731-C833, C742-C822, C1014-C1025, C1064-C1108 (2)

**3) Regions of S1 and ACE2 Receptor Association**

- a 193 aa fragment of the S protein (aa 318-510) bound ACE2 more efficiently than did the full S1 domain (6)
- residues 503-510 are unnecessary for binding (7)
- fragment contains seven cysteine, five of which are essential for expression of ACE2 association (6)
  - mutation of cys 323 and 378 decreased ability to bind ACE2 (6)
  - alteration of 355 or 419 substantially impaired expression of the 193 aa fragment (6)
  - alteration of cys 348, 467 and 474 prevented efficient precipitation of ACE2 without major effect on expression (6)
    - determinants between 318 and 326 other than cysteine 323 contribute directly or indirectly to ACE2 association (6)
- 193 fragment blocked S protein-mediated infection with an IC50 of less than 10 nM whereas the full S1 domain blocked S protein-mediated infection with an IC50 of ~50 nM (6)
  - higher affinity for 193 residue fragment raises possibility the S protein partially masks this receptor-binding domain as compared to full length S1 (6)
  - or the receptor-binding domain may be more soluble or better folded than the S1 protein (6)
- a point mutation at aspartic acid 454 or glutamic acid 452 abolished association of the full S1 domain and of the 193-residue fragment with ACE2 (6)
- the ability to mediate cell-cell fusion is dependent of the presence of ACE2 (5)

**4) pH requirements:**

- no apparent pH requirement for S glycoprotein-mediated cell-cell fusion (3)
- little or no cell-cell fusion occurred at either pH 7.5 or pH 5.0 (3)
- lysosomotropic agents inhibited SARS-CoV S-mediated transduction in multiple cell lines suggesting SARS-CoV requires acidification of endosomes for entry (3)
- lack of sensitivity of S-protein to low pH but sensitivity to lysosomotropic agents explained by 4 possibilities (3)
  1. pH-induced conformational changes in S are reversible in a manner similar to VSV-G protein (5)
  2. triggering of S by receptor interactions is required before low pH can induce further rearrangements such as for avian sarcoma/ leucosis virus Env glycoprotein (6)

3. the S-protein undergoes processing event (cleavage) in endosomes that is prerequisite for acid activation such as for influenza infection of Madin-Darby bovine kidney cells (7)
4. low pH is not required for infection with sensitivity to lysosomotropic agents resulting from a mechanism other than ablation of intracellular pH gradients

**5) Inhibitor Design:**

- peptide (ISGINASVVNIQKEIDRLNEVAKNLNESLIDLQEL) might inhibit virus-induced membrane fusion blocking SARS-CoV infection (4)
- single chain variable region fragment antibody with epitope within the N-term 261-672 aa of S protein inhibited syncytia formation and is not glycosylation-dependent (8)
  - finding that monovalent scFv (80R) has potent neutralizing activity shows that SARS neutralization does not require bivalent binding (8)
  - this antibody bound with high affinity  $K_d = 32.3 \text{ nM}$  (8)
- human IgG1 form of the antibody (80R) bound with  $K_d$  of 1.59 nM comparable to ACE2 ( $K_d = 1.70 \text{ nM}$ ) (8)
  - primary mechanism of the neutralizing activity of 80R is through blocking of S1 binding to ACE2 (8)

**Processing:**

- short type I signal peptide (amino acids 1-13) composed of hydrophobic amino acids that are presumably removed during cotranslational transport through ER (1)
- twenty-three potential N-linked glycosylation sites (1)
- assembled into virions through non-covalent interactions with the M protein
- furin-like protease cleavage site is absent but includes two single basic amino acids as potential targets for trypsin-like cleavage (3)
  - trypsin cleavage was a prerequisite for S protein-mediated cell-cell fusion (3)
  - SARS-CoV S may be cleaved after virion release in the cell supernatant or by the target cell (3)
  - cleavage before infection reduced infectivity (3)

**Cellular Location:**

- Cell surface
- Golgi apparatus

**Functions:**

- initiate infection by binding to the ACE2 receptor
- cause syncytia formation by residing on a cells surface and facilitating the fusion of neighboring cells
- S-protein may mediate infection in a pH-dependent manner w/o cleavage, but trypsin mediated cleavage may reduce threshold for conformational changes so cell-cell fusion can occur at neutral pH (3)
  - differing requirements for cleavage might reflect differences in available receptor type or receptor density (low receptor density needs cleavage, high density does not)

<b>Responsibilities:</b>	<b>Collaborators:</b>
Bind receptor to initiate infection	ACE2 receptor
Fusion to allow entry of virus into cell, cell-cell fusion	Membrane
Membrane anchor to secure S protein to virus	Membrane
Interact with M to retain M protein and prevent it from escaping to the Golgi apparatus	M_protein
Trimerization, which is important for cell-cell fusion and syncytia formation	S_protein
Functional consequence unknown	U274 (8)
May serve as a receptor for S protein	caveolin-1 (9)

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- 3) Simmons, G., et al., (2004) Characterization of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) spike glycoprotein-mediated viral entry, *Proc Natl Acad Sci U S A*, **101(12)**, 4240-5.
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