# Molecular Basis of Binding between Novel Human Coronavirus MERS-CoV and its Receptor CD26

The newly-emerged Middle East respiratory syndrome coronavirus (MERS-CoV) can specifically engage CD26 via the surface spike protein. We identified the spike region (denoted as receptor binding domain (RBD)) that is responsible for receptor-binding, and solved the RBD structures in both the free and receptor-bound forms. MERS-CoV RBD is composed of a core subdomain and an external receptor binding motif that recognizes blades IV and V of CD26 β-propeller. The viral ligand locates at the membrane-distal tip of the receptor dimer, forming a "U"-shaped structure. Elucidation of the receptor-recognition mechanism for MERS-CoV would facilitate rational design of anti-viral drugs and prophylactic vaccines.

In 2012, a new coronavirus, named the Middle East respiratory syndrome coronavirus (MERS-CoV) [1], first emerged in Saudi Arabia [2], and then rapidly spread to multiple countries in the Middle East and Europe [3, 4]. The virus is highly pathogenic [2, 5]. Patients suffer severe pulmonary diseases, including fever, cough, and an acute respiratory distress syndrome. In some cases, the disease course is accompanied by renal failure, leading to exceptionally high mortality [2, 5]. As of April 10, 2014, the World Health Organization has compiled a total of 211 laboratory-confirmed infection cases globally, including 88 deaths [4]. In addition, there has been accumulating evidence showing the limited local transmission of the virus among close contacts [6]. Consequently, there is concern about a second potential coronavirus pandemic ten years after the SARS outbreak [7]. To combat this virus, there is an urgent need to identify how the virus infects humans.

MERS-CoV belongs to the *betacoronavirus* genus in the *Coronaviridae* family [8]. As with other coronaviruses [9], MERS-CoV utilizes the surface spike protein for receptor recognition [10]. The specific binding of the viral spike to the cellular receptor mediates the attachment of viruses to host cells, which is a crucial step initiating infection.

Recently, CD26 (also known as dipeptidyl peptidase 4, DDP4) was identified as the functional receptor for MERS-CoV [10]. The molecular mechanism of CD26 recognition by the novel coronavirus, however, remains elusive. This is a key question regarding the viral pathogenesis and would be useful for the development of anti-viral drugs targeting the viral entry process.

To delineate the receptor-recognition basis of MERS-CoV, we need first to identify the spike region that is responsible for receptor-engagement. By testing the interaction between CD26 and differently-truncated spike proteins through flow-cytometric assays, this receptor binding domain (RBD) was attributed to the MERS-CoV spike residues 367-606. A specific and potent binding of RBD to CD26 was further demonstrated by the surface plasmon resonance Biacore data, which revealed a dissociation constant ($K_d$) of about 16.7 nM [Fig. 1(a)].

We then successfully crystallized the free RBD protein and its complex with CD26, and managed to solve both structures at 2.5 Å and 2.7 Å, respectively. The viral RBD is composed of a core subdomain and an external receptor binding motif [Fig. 1(b)]. The former is homologous to that of the SARS-CoV spike protein [11], while the latter is a novel strand-dominated unit which recognizes blades IV and V of CD26 β-propeller. The receptor itself is a type II transmembrane protein, presented on the cell surface as homodimers [12, 13]. MERS-CoV RBD locates at the membrane-distal tip of the receptor dimer, forming an overall "U"-shaped structure [Fig. 1(c)].

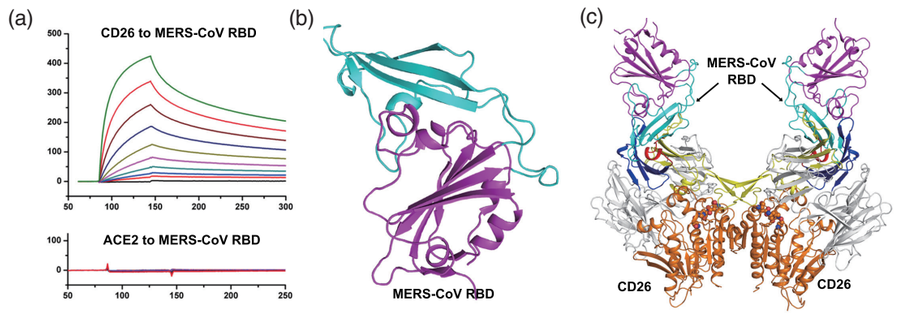


Figure 1: The high-affinity binding and complex structure between MERS-CoV RBD and CD26. (a) A surface plasmon resonance assay showing that MERS-CoV RBD can bind to CD26 but not to the SARS-CoV receptor human ACE2. (b) A schematic representation of the MERS-CoV RBD structure. The core subdomain and the external receptor binding motif are colored magenta and cyan, respectively. (c) The complex structure between MERS-CoV RBD and CD26. The CD26 dimer and its tip-located RBD molecules are shown and labeled.

Detailed analysis revealed that the RBD/CD26 interaction is mainly mediated by salt-bridges and hydrogen-bond contacts among hydrophilic residues at the binding interface. Interestingly, this featured pattern of RBD/CD26 engagement is very similar to those mediating the binding between adenosine deaminase (ADA) and CD26 [14], indicating competition between MERS-CoV and ADA for CD26 receptor. The ADA/CD26 interaction has been shown to play an important role in T cell activation [15], therefore our work indicates potential manipulation of the host immune system by MERS-CoV through competition for the ADA-recognition site. By comparing with other reported coronaviral spike-domain structures and aligning the spike sequences of representative betacoronaviruses, we assumed that members of the *betacoronavirus* genus might have evolved to reserve a similar core-domain fold in the spike protein to present external amino acids with divergent structures for viral pathogenesis, such as receptor recognition.

The emergence of MERS-CoV infections is a worldwide public health concern. The viral entry process represents one of the best drug targets. The identification of key residues, via high-resolution structures, mediating the attachment of MERS-CoV to CD26, could set the direction for future anti-viral research. Our work also indicates a potential application of the properly-folded MERS-CoV RBD proteins in prophylactic vaccination, which is worthy of future study.

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