Available online at www.sciencedirect.com
ScienceDirect
Computational Biology and Chemistry
Computational Biology and Chemistry 31 (2007) 129–133
www.elsevier.com/locate/compbiolchem

Brief communication

Computational characterization and design of SARS coronavirus receptor recognition and antibody neutralization

Yuan Zhang [a,*], Nan Zheng [b], Yang Zhong [a]

[a] School of Life Sciences, Fudan University, Shanghai 200433, China
[b] Division of Viral Immunology, Center for AIDS Research, Kumamoto University, 2-2-1 Honjo, Kumamoto 860-0811, Japan

Received 9 December 2006; accepted 13 February 2007

Abstract

The sequential determination of crystal structures of the SARS coronavirus spike receptor-binding domain (RBD) in complex with its cellular receptor or neutralizing antibody opened a door for the design and development of antiviral competitive inhibitors. Based on those complex structures, we conduct computational characterization and design of RBD-mediated receptor recognition and antibody neutralization. The comparisons between computational predictions and experimental evidences validate our structural bioinformatics protocols. And the calculations predict a number of single substitutions on RBD, receptor or antibody that could remarkably elevate the binding affinities of those complexes. It is reasonable to anticipate our structure-based computation-derived hypotheses could be informative to the future biochemical and immunological tests.

Keywords: SARS coronavirus; Spike protein; Receptor recognition; Antibody neutralization; Protein design

1. Introduction

As an envelope glycoprotein, the spike protein of severe acute respiratory syndrome coronavirus (SARS-CoV) plays a key role in the viral entry and neutralization (Bartlam et al., 2005; Denison, 2004; Lau and Peiris, 2005; Xu and Gao, 2004; Zhu, 2004). This structural protein consists of two functional regions: the outer globular S1 region responsible for the initial attachment to cellular receptor and the inner stalk S2 region contributing to the subsequent fusion between viral envelope and cellular membrane (Beniac et al., 2006; Hofmann and Pohlmann, 2004; Lin et al., 2005; Xiao and Dimitrov, 2004). A membrane-associated zinc metallopeptidase, angiotensin-converting enzyme 2 (ACE2), has been identified as the functional receptor for SARS-CoV (Li et al., 2003). And a soluble form of ACE2 could block the association of S1 region with the permissive Vero E6 cells (Li et al., 2003; Moore et al., 2004). In addition, a 193-amino acid fragment (residues 318–510), located within the S1 region, was demonstrated as an independently folded receptor-binding domain (RBD) capable of attaching ACE2 more efficiently ($IC_{50} < 10$ nM) compared with the full S1 region ($IC_{50} \approx 50$ nM)(Wong et al., 2004). Besides, this RBD was able to elicit highly potent neutralizing antibodies in the immunized animals, which conferred those animals significant protection from the challenge of pathogenic SARS-CoV (Du et al., 2006; He et al., 2004, 2005a,b, 2006a,b,c,d; Zakhartchouk et al., 2006; Zhao et al., 2006). Moreover, a human monoclonal antibody 80R, isolated from a nonimmune human antibody library, was shown to potently neutralize SARS-CoV through targeting the RBD and blocking receptor recognition (Sui et al., 2004). The epitope mapping illustrated a 180-amino acid conformationally sensitive fragment (residues 324–503) within the RBD was the neutralizing epitope of 80R (Sui et al., 2005). Furthermore, another human monoclonal antibody m396 also exhibited potent neutralization of SARS-CoV by competition with ACE2 for binding to RBD (Prabakaran et al., 2006). Together those data suggest the receptor association process of SARS-CoV is an attractive opportunity for therapeutic intervention (De Clercq, 2006; He and Jiang, 2005; Hofmann and Pohlmann, 2004; Jiang et al., 2005; Kuhn et al., 2004; Yeung et al., 2006). The peptide or peptidomimetic antagonist leads, including the SARS-CoV spike RBD, the soluble form of ACE2 and the neutralizing antibodies 80R plus m396, should be able to potently abolish viral attachment to host cells. In this study, we conducted structural bioinformatics analyses on the crystal structures of the SARS-CoV RBD complexed with functional

* Corresponding author. Tel.: +86 21 65642957; fax: +86 21 65642468.
E-mail address: yuanzhang5@fudan.edu.cn (Y. Zhang).

1476-9271/$ – see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.compbiolchem.2007.02.005

receptor or neutralizing antibody (Hwang et al., 2006; Li et al., 2005a,2006; Prabakaran et al., 2006) to predict single substitutions on spike RBD, receptor or antibodies possibly causing remarkable elevation in the binding affinities of complexes for the design and development of anti-SARS agents.

2. Materials and methods

Three coordinates files were retrieved from the Protein Data Bank (PDB) (Berman et al., 2000). One file is the ACE2-bound RBD (PDB code: 2AJF) (Li et al., 2005a), while the others are the RBD complexed with 80R (PDB code: 2GHW) (Hwang et al., 2006) or m396 (PDB code: 2DD8)(Prabakaran et al., 2006). Both the first and second files harbor a pair of sister complexes. And in the third file, either the heavy chain or the light chain of m396 makes its own contacts with the RBD. Thus, a total of six complex structures (AE/BF for ACE2-RBD, AB/CD for 80R-RBD and HS/LS for m396-RBD) are subject to computational simulations, respectively. Firstly, the program FoldX (Schymkowitz et al., 2005), based on an empirical effective energy function, was employed for calculation of the binding free energy values of wild type complexes. Then, a computational alanine scanning on the protein-protein interfaces was performed for evaluation of energetical contribution from single binding sites to the complex formation. Those positions yielding a calculated increase in association energy of more than 1 kcal/mol on alanine substitution were defined as energetic hot (important) spots according to previous criteria (Guerois et al., 2002; Guerois and Serrano, 2000; Kiel and Serrano, 2006; Kiel et al., 2004, 2005). The next step was to redesign the interactions between RBD and its binding partners through the software DeepView (Arnold et al., 2006; Guex and Peitsch, 1997). Each of the binding sites on the RBD, receptor or antibody was saturated with virtual substitutions, i.e., replaced with all the 20 natural amino acid residues except the original one. Finally, the reconstructed models were feed to the program FoldX to compute their binding energies. Here, only the variants rewarded a value of at least 1 kcal/mol lower than that of the wild type were taken into consideration.

3. Results and discussion

The calculated binding energy values and hot spots of the wild type complexes are shown in Table 1. The complexes ACE2-RBD (AE/BF) and 80R-RBD (AB/CD) show a close correlation between their interaction energies (−15.78/−14.82 kcal/mol versus −18.36/−16.53 kcal/mol) and buried surface area (1700 Å^2 versus 2200 Å^2), gap volume (7000 Å^3 versus 4000 Å^3), or binding affinity (1.70 nM versus 1.59 nM)(Hwang et al., 2006). Those obvious associations indicate that the higher geometric complementarity, corresponding to the larger buried surface area and the smaller gap volume, offers the complex 80R-RBD rather than the complex ACE2-RBD the lower interaction energy and consequently the stronger binding affinity. Similarly, the correlation of binding energy with buried surface area is also found for the complex m396-RBD in which the heavy chain and the light chain contribute 66% and 34% to the total buried surface (Prabakaran et al., 2006). And the RBD association energy of the former chain (−8.64 kcal/mol) is remarkably lower than that of the latter (−5.42 kcal/mol). The perfect agreements of computational predictions with structural observations or biochemical evidences strongly suggest the reliability of our protocols.

As to the hot spots of complexes, the consistency between computational predictions and experimental evidences is clearly detected for RBD and receptor. In ACE2-RBD complexes, three receptor residues (GLU37, ASP38 and TYR41 on the chain A of complex AE or GLU37, TYR41 and LYS353 on the chain B of complex BF) form one hot spot cluster interacting with another hot cluster formed by five or six RBD residues (ARG426, TYR436, TYR475, TYR484 and TYR491 on the chain E of complex AE and the chain F of complex BF, whereas ASN473 only on the chain F). The interactions between the two hot clusters make the major contribution to the binding free energy of ACE2-RBD complexes. Notably, our predictions are in agreement with previous experimental alanine mutagenesis, which identified two hot spots on RBD (ARG426 and ASN473) (Chakraborti et al., 2005) and another two on receptor (TYR41 and LYS353) (Li et al., 2005b). In addition, computational alanine scanning on the sister complexes AB and CD successfully identify a RBD hot spot (ASP480) revealed in mutational binding analyses (Sui et al., 2005). In sharp contrast to ACE2, the antibody 80R possesses four hot residues (TYR102, ASN164, ARG223 and TRP226) being scattered on the binding surface rather than centralized into a cluster. The difference in the number and distribution of hot spots might account for the large gap between the interaction energies of 80R-RBD (−18.3627 and −16.5309 kcal/mol) and those of ACE2-RBD (−15.7765 and

Table 1
Calculated binding free energies and hot spots of wild type complexes

Complex	Binding free energy (kcal/mol)	Hot spots
AE	−15.78	Chain A: GLU37, ASP38, TYR41; chain E: ARG426, TYR436, TYR475, TYR484, TYR491
BF	−14.82	Chain B: GLU37, TYR41, LYS353; chain F: ARG426, TYR436, ASN473, TYR475, TYR484, TYR491
AB	−18.36	Chain A: TYR436, PRO470, LEU472, ASP480, TYR484, TYR491; chain B: TYR102, ASN164, ARG223, TRP226
CD	−16.53	Chain C: TYR436, PRO470, ASP480, TYR484; chain D: TYR102, ASN164, ARG223, TRP226
HS	−8.64	Chain H: none; chain S: TYR491, GLN492
LS	−5.42	Chain L: TRP91, ASP92; chain S: ILE489

The complexes AE and BF with the chains A, B for ACE2 and the chains E, F for RBD; the complexes AB and CD with the chains A, C for RBD and the chains B, D for 80R; the complexes HS and LS with the chains H, L and S for heavy and light chains of m396 plus RBD.

Table 2
Predicted replacements with significant increase in binding affinity

Protein	Residue	Replacement
RBD	LEU443	ARG/TRP
	LEU472	PHE
	ASN479	PHE/TRP/TYR
	TYR484	TRP
	GLN492	ARG
ACE2	THR27	ILE/MET
	LYS31	ILE
	HIS34	PHE/TRP
80R	SER101	ILE/MET/VAL
	ARG162	ASN/HIS
	SER163	HIS/PHE/THR/VAL
	ASP182	CYS/GLU/TRP
	SER184	VAL
	THR185	ARG/ASN/LEU/MET
	SER195	CYS/ILE/TRP/VAL
	SER199	MET/TYR
	THR206	PHE/TRP
m396 (H chain)	SER31	GLU/ILE/LEU/MET/PHE/VAL
	TYR32	HIS/PHE
	THR52	CYS
	ASN58	ARG/CYS/HIS/ILE/LEU/MET/SER/THR/TRP/TYR/VAL
	THR96	GLU
	VAL97	ASP
m396 (L chain)	SER30	HIS/ILE/MET/TYR
	SER93	HIS/ILE/LEU/TYR/VAL

−14.8160 kcal/mol), the stronger competence of 80R with soluble ACE2 for association with RBD, or the higher spike-binding affinity of 80R compared to that of receptor. Finally, only two neighboring hot spots (TRP91 and ASP92) are found on the light chain of m396 while none on the heavy chain. Thus, an interesting discovery is the fact that among the five or six ACE2-binding hot spots of RBD, three (TYR436, TYR484 and TYR491) are simultaneously 80R-neutralizing hot spots whereas only one (TYR491) is important for m396 neutralization. This finding indicates that 80R might have the greater potential than m396 for inhibition of spike-mediated infection. In summary, the consistency of calculations with experiments mentioned above further validates our approaches to characterize protein–protein interactions.

The predicted replacements on spike RBD, cellular receptor or neutralizing antibody with significant increase in binding affinity are listed in Table 2. The comparisons between virtual mutants derived from sister complexes of ACE2-RBD or 80R-RBD consistently identify a number of substitutions worth of biochemical and immunological experimental tests. For instance, recent experimental evidences revealed the great potential of ACE2 in the protection of several animal models from SARS-CoV-induced lung injury or severe acute lung failure (Imai et al., 2005; Kuba et al., 2005, 2006). Simultaneously, the crystal structures of the native and inhibitor-bound forms of ACE2 (Kuhn et al., 2004; Towler et al., 2004; Turner et al., 2004) successfully laid a solid foundation for the discovery of novel small-molecule inhibitors of its enzymatic activity or spike-mediated virus entry by chemical genetics (Huentelman et al., 2004; Kao et al., 2004) and the identification of its crucial active-site residues by site-directed mutagenesis (Guy et al., 2005a,b). Very recently, a modest anti-SARS activity ($IC_{50} \approx 0.1$ mM) was observed for an ACE2-derived peptide containing two segments of receptor (residues 22–44 and 351–357) linked by glycine (Han et al., 2006). It should be pointed out that both the experimentally confirmed hot spots (TYR41 and LYS353) and the predicted sites for replacements (THR27, LYS31 and HIS34) are nested in those two segments. Similarly, a small peptide derived from spike protein (residues 483–493) also block viral receptor recognition with IC_{50} of 6.99 nM (Ho et al., 2006). And our calculated two hot spots (TYR484 and TYR491) in combination with two target positions (TYR484 and GLN492) are located in this short fragment, too. Consequently, it is reasonable to anticipate that our blueprint could effectively increase the binding affinity of the two novel peptides to disrupt SARS-CoV infection.

References

Arnold, K., Bordoli, L., Kopp, J., Schwede, T., 2006. The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. Bioinformatics 22, 195–201.

Bartlam, M., Yang, H., Rao, Z., 2005. Structural insights into SARS coronavirus proteins. Curr. Opin. Struct. Biol. 15, 664–672.

Beniac, D.R., Andonov, A., Grudeski, E., Booth, T.F., 2006. Architecture of the SARS coronavirus prefusion spike. Nat. Struct. Mol. Biol. 13, 751–752.

Berman, H.M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T.N., Weissig, H., Shindyalov, I.N., Bourne, P.E., 2000. The protein data bank. Nucleic Acids Res 28, 235–242.

Chakraborti, S., Prabakaran, P., Xiao, X., Dimitrov, D.S., 2005. The SARS coronavirus S glycoprotein receptor binding domain: fine mapping and functional characterization. Virol. J. 2, 73.

De Clercq, E., 2006. Potential antivirals and antiviral strategies against SARS coronavirus infections. Expert. Rev. Anti. Infect. Ther. 4, 291–302.

Denison, M.R., 2004. Severe acute respiratory syndrome coronavirus pathogenesis, disease and vaccines: an update. Pediatr. Infect. Dis. J. 23, S207–S214.

Du, L., Zhao, G., He, Y., Guo, Y., Zheng, B.J., Jiang, S., Zhou, Y., 2006. Receptor-binding domain of SARS-CoV spike protein induces long-term protective immunity in an animal model. Vaccine.

Guerois, R., Serrano, L., 2000. The SH3-fold family: experimental evidence and prediction of variations in the folding pathways. J. Mol. Biol. 304, 967–982.

Guerois, R., Nielsen, J.E., Serrano, L., 2002. Predicting changes in the stability of proteins and protein complexes: a study of more than 1000 mutations. J. Mol. Biol. 320, 369–387.

Guex, N., Peitsch, M.C., 1997. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. Electrophoresis 18, 2714–2723.

Guy, J.L., Jackson, R.M., Jensen, H.A., Hooper, N.M., Turner, A.J., 2005a. Identification of critical active-site residues in angiotensin-converting enzyme-2 (ACE2) by site-directed mutagenesis. FEBS J. 272, 3512–3520.

Guy, J.L., Lambert, D.W., Warner, F.J., Hooper, N.M., Turner, A.J., 2005b. Membrane-associated zinc peptidase families: comparing ACE and ACE2. Biochim. Biophys. Acta 1751, 2–8.

Han, D.P., Penn-Nicholson, A., Cho, M.W., 2006. Identification of critical determinants on ACE2 for SARS-CoV entry and development of a potent entry inhibitor. Virology.

He, Y., Jiang, S., 2005. Vaccine design for severe acute respiratory syndrome coronavirus. Viral. Immunol. 18, 327–332.

He, Y., Zhou, Y., Liu, S., Kou, Z., Li, W., Farzan, M., Jiang, S., 2004. Receptor-binding domain of SARS-CoV spike protein induces highly potent neutralizing antibodies: implication for developing subunit vaccine. Biochem. Biophys. Res. Commun. 324, 773–781.

He, Y., Lu, H., Siddiqui, P., Zhou, Y., Jiang, S., 2005a. Receptor-binding domain of severe acute respiratory syndrome coronavirus spike protein contains multiple conformation-dependent epitopes that induce highly potent neutralizing antibodies. J. Immunol. 174, 4908–4915.

He, Y., Zhu, Q., Liu, S., Zhou, Y., Yang, B., Li, J., Jiang, S., 2005b. Identification of a critical neutralization determinant of severe acute respiratory syndrome (SARS)-associated coronavirus: importance for designing SARS vaccines. Virology 334, 74–82.

He, Y., Li, J., Du, L., Yan, X., Hu, G., Zhou, Y., Jiang, S., 2006a. Identification and characterization of novel neutralizing epitopes in the receptor-binding domain of SARS-CoV spike protein: revealing the critical antigenic determinants in inactivated SARS-CoV vaccine. Vaccine 24, 5498–5508.

He, Y., Li, J., Heck, S., Lustigman, S., Jiang, S., 2006b. Antigenic and immunogenic characterization of recombinant baculovirus-expressed severe acute respiratory syndrome coronavirus spike protein: implication for vaccine design. J. Virol. 80, 5757–5767.

He, Y., Li, J., Jiang, S., 2006c. A single amino acid substitution (R441A) in the receptor-binding domain of SARS coronavirus spike protein disrupts the antigenic structure and binding activity. Biochem. Biophys. Res. Commun. 344, 106–113.

He, Y., Li, J., Li, W., Lustigman, S., Farzan, M., Jiang, S., 2006d. Cross-neutralization of human and palm civet severe acute respiratory syndrome coronaviruses by antibodies targeting the receptor-binding domain of spike protein. J. Immunol. 176, 6085–6092.

Ho, T.Y., Wu, S.L., Chen, J.C., Wei, Y.C., Cheng, S.E., Chang, Y.H., Liu, H.J., Hsiang, C.Y., 2006. Design and biological activities of novel inhibitory peptides for SARS-CoV spike protein and angiotensin-converting enzyme 2 interaction. Antiviral Res. 69, 70–76.

Hofmann, H., Pohlmann, S., 2004. Cellular entry of the SARS coronavirus. Trends Microbiol. 12, 466–472.

Huentelman, M.J., Zubcevic, J., Hernandez Prada, J.A., Xiao, X., Dimitrov, D.S., Raizada, M.K., Ostrov, D.A., 2004. Structure-based discovery of a novel angiotensin-converting enzyme 2 inhibitor. Hypertension 44, 903–906.

Hwang, W.C., Lin, Y., Santelli, E., Sui, J., Jaroszewski, L., Stec, B., Farzan, M., Marasco, W.A., Liddington, R.C., 2006. Structural basis of neutralization by a human anti-severe acute respiratory syndrome spike protein antibody, 80R. J. Biol. Chem. 281, 34610–34616.

Imai, Y., Kuba, K., Rao, S., Huan, Y., Guo, F., Guan, B., Yang, P., Sarao, R., Wada, T., Leong-Poi, H., Crackower, M.A., Fukamizu, A., Hui, C.C., Hein, L., Uhlig, S., Slutsky, A.S., Jiang, C., Penninger, J.M., 2005. Angiotensin-converting enzyme 2 protects from severe acute lung failure. Nature 436, 112–116.

Jiang, S., He, Y., Liu, S., 2005. SARS vaccine development. Emerg. Infect. Dis. 11, 1016–1020.

Kao, R.Y., Tsui, W.H., Lee, T.S., Tanner, J.A., Watt, R.M., Huang, J.D., Hu, L., Chen, G., Chen, Z., Zhang, L., He, T., Chan, K.H., Tse, H., To, A.P., Ng, L.W., Wong, B.C., Tsoi, H.W., Yang, D., Ho, D.D., Yuen, K.Y., 2004. Identification of novel small-molecule inhibitors of severe acute respiratory syndrome-associated coronavirus by chemical genetics. Chem. Biol. 11, 1293–1299.

Kiel, C., Serrano, L., 2006. The ubiquitin domain superfold: structure-based sequence alignments and characterization of binding epitopes. J. Mol. Biol. 355, 821–844.

Kiel, C., Serrano, L., Herrmann, C., 2004. A detailed thermodynamic analysis of ras/effector complex interfaces. J. Mol. Biol. 340, 1039–1058.

Kiel, C., Wohlgemuth, S., Rousseau, F., Schymkowitz, J., Ferkinghoff-Borg, J., Wittinghofer, F., Serrano, L., 2005. Recognizing and defining true Ras binding domains. II. In silico prediction based on homology modelling and energy calculations. J. Mol. Biol. 348, 759–775.

Kuba, K., Imai, Y., Rao, S., Gao, H., Guo, F., Guan, B., Huan, Y., Yang, P., Zhang, Y., Deng, W., Bao, L., Zhang, B., Liu, G., Wang, Z., Chappell, M., Liu, Y., Zheng, D., Leibbrandt, A., Wada, T., Slutsky, A.S., Liu, D., Qin, C., Jiang, C., Penninger, J.M., 2005. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. Nat. Med. 11, 875–879.

Kuba, K., Imai, Y., Penninger, J.M., 2006. Angiotensin-converting enzyme 2 in lung diseases. Curr. Opin. Pharmacol. 6, 271–276.

Kuhn, J.H., Li, W., Choe, H., Farzan, M., 2004. Angiotensin-converting enzyme 2: a functional receptor for SARS coronavirus. Cell. Mol. Life Sci. 61, 2738–2743.

Lau, Y.L., Peiris, J.S., 2005. Pathogenesis of severe acute respiratory syndrome. Curr. Opin. Immunol. 17, 404–410.

Li, W., Moore, M.J., Vasilieva, N., Sui, J., Wong, S.K., Berne, M.A., Somasundaran, M., Sullivan, J.L., Luzuriaga, K., Greenough, T.C., Choe, H., Farzan, M., 2003. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 426, 450–454.

Li, F., Li, W., Farzan, M., Harrison, S.C., 2005a. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science 309, 1864–1868.

Li, W., Zhang, C., Sui, J., Kuhn, J.H., Moore, M.J., Luo, S., Wong, S.K., Huang, I.C., Xu, K., Vasilieva, N., Murakami, A., He, Y., Marasco, W.A., Guan, Y., Choe, H., Farzan, M., 2005b. Receptor and viral determinants of SARS-coronavirus adaptation to human ACE2. EMBO J. 24, 1634–1643.

Li, F., Berardi, M., Li, W., Farzan, M., Dormitzer, P.R., Harrison, S.C., 2006. Conformational states of the severe acute respiratory syndrome coronavirus spike protein ectodomain. J. Virol. 80, 6794–6800.

Lin, S., Lee, C.K., Lee, S.Y., Kao, C.L., Lin, C.W., Wang, A.B., Hsu, S.M., Huang, L.S., 2005. Surface ultrastructure of SARS coronavirus revealed by atomic force microscopy. Cell. Microbiol. 7, 1763–1770.

Moore, M.J., Dorfman, T., Li, W., Wong, S.K., Li, Y., Kuhn, J.H., Coderre, J., Vasilieva, N., Han, Z., Greenough, T.C., Farzan, M., Choe, H., 2004. Retroviruses pseudotyped with the severe acute respiratory syndrome coronavirus spike protein efficiently infect cells expressing angiotensin-converting enzyme 2. J. Virol. 78, 10628–10635.

Prabakaran, P., Gan, J., Feng, Y., Zhu, Z., Choudhry, V., Xiao, X., Ji, X., Dimitrov, D.S., 2006. Structure of severe acute respiratory syndrome coronavirus receptor-binding domain complexed with neutralizing antibody. J. Biol. Chem. 281, 15829–15836.

Schymkowitz, J., Borg, J., Stricher, F., Nys, R., Rousseau, F., Serrano, L., 2005. The FoldX web server: an online force field 10.1093/nar/gki387. Nucl. Acids Res. 33, W382–W388.

Sui, J., Li, W., Murakami, A., Tamin, A., Matthews, L.J., Wong, S.K., Moore, M.J., Tallarico, A.S., Olurinde, M., Choe, H., Anderson, L.J., Bellini, W.J., Farzan, M., Marasco, W.A., 2004. Potent neutralization of severe acute respiratory syndrome (SARS) coronavirus by a human mAb to S1 protein that blocks receptor association. Proc. Natl. Acad. Sci. U.S.A. 101, 2536–2541.

Sui, J., Li, W., Roberts, A., Matthews, L.J., Murakami, A., Vogel, L., Wong, S.K., Subbarao, K., Farzan, M., Marasco, W.A., 2005. Evaluation of human monoclonal antibody 80R for immunoprophylaxis of severe acute respiratory syndrome by an animal study, epitope mapping, and analysis of spike variants. J. Virol. 79, 5900–5906.

Towler, P., Staker, B., Prasad, S.G., Menon, S., Tang, J., Parsons, T., Ryan, D., Fisher, M., Williams, D., Dales, N.A., Patane, M.A., Pantoliano, M.W., 2004. ACE2 X-ray structures reveal a large hinge-bending motion important for inhibitor binding and catalysis. J. Biol. Chem. 279, 17996–18007.

Turner, A.J., Hiscox, J.A., Hooper, N.M., 2004. ACE2: from vasopeptidase to SARS virus receptor. Trends Pharmacol. Sci. 25, 291–294.

Wong, S.K., Li, W., Moore, M.J., Choe, H., Farzan, M., 2004. A 193-amino acid fragment of the SARS coronavirus S protein efficiently binds angiotensin-converting enzyme 2. J. Biol. Chem. 279, 3197–3201.

Xiao, X., Dimitrov, D.S., 2004. The SARS-CoV S glycoprotein. Cell. Mol. Life Sci. 61, 2428–2430.

Xu, X., Gao, X., 2004. Immunological responses against SARS-coronavirus infection in humans. Cell. Mol. Immunol. 1, 119–122.

Yeung, K.S., Yamanaka, G.A., Meanwell, N.A., 2006. Severe acute respiratory syndrome coronavirus entry into host cells: opportunities for therapeutic intervention. Med. Res. Rev..

Zakhartchouk, A.N., Sharon, C., Satkunarajah, M., Auperin, T., Viswanathan, S., Mutwiri, G., Petric, M., See, R.H., Brunham, R.C., Finlay, B.B., Cameron, C., Kelvin, D.J., Cochrane, A., Rini, J.M., Babiuk, L.A., 2006. Immunogenicity of a receptor-binding domain of SARS coronavirus spike protein in mice: Implications for a subunit vaccine. Vaccine.

Zhao, J., Wang, W., Yuan, Z., Jia, R., Zhao, Z., Xu, X., Lv, P., Zhang, Y., Jiang, C., Gao, X.M., 2006. A study on antigenicity and receptor-binding ability of fragment 450–650 of the spike protein of SARS coronavirus. Virology.

Zhu, M., 2004. SARS immunity and vaccination. Cell. Mol. Immunol. 1, 193–198.