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## Molecular docking simulation reveals ACE2 polymorphisms that may increase the affinity of ACE2 with the SARS-CoV-2 Spike protein

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2

3 **Molecular docking simulation reveals ACE2 polymorphisms that may increase the affinity of**  
4 **ACE2 with the SARS-CoV-2 Spike protein**

5

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27

28 **Running title:** ACE2 polymorphism and interaction with SARS-CoV-2 Spike protein

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31

32 **ABSTRACT**

33 There is increasing evidence that ACE2 gene polymorphism can modulate the interaction between  
34 ACE2 and the SARS-CoV-2 spike protein affecting the viral entry into the host cell, and / or  
35 contribute to lung and systemic damage in COVID-19. Here we used *in silico* molecular docking to  
36 predict the effects of ACE2 missense variants on the interaction with the spike protein of SARS-  
37 CoV-2. HDOCK and FireDock simulations identified 6 ACE2 missense variants (I21T, A25T,  
38 K26R, E37K, T55A, E75G) with higher affinity for SARS-CoV-2 Spike protein receptor binding  
39 domain (RBD) with respect to wild type ACE2, and 11 variants (I21V, E23K, K26E, T27A, E35K,  
40 S43R, Y50F, N51D, N58H, K68E, M82I) with lower affinity. This result supports the hypothesis  
41 that ACE2 genetic background may represent the first “genetic gateway” during the disease  
42 progression.

43

44

45 **Keywords:** COVID-19; SARS-CoV-2 Spike protein; ACE2 polymorphism; *In silico* modeling;  
46 SARS-CoV-2 Infectiousness; SARS-CoV-2 severity of infection

47

48

49 **1. Introduction**

50 Different phases can be distinguished during the progression of COVID-19 [1, 2]. During the first  
51 phase, after the incubation period lasting 6 days in average, the onset of disease may be  
52 characterized by influenza-like symptoms, from mild to moderate. The second phase, which is  
53 known as the pulmonary phase and involves ~30% of all SARS-CoV-2 infected subjects, is  
54 characterized by progressive respiratory involvement with onset of pneumonia-like symptoms. The  
55 third phase, which develops in ~15% of all patients, is known as the pro-inflammatory phase, and is  
56 characterized by severe interstitial pneumonia with focal and systemic iper-inflammation, which  
57 may lead to acute respiratory distress syndrome, and systemic inflammatory response syndrome.  
58 The fourth phase of COVID-19, which is known as the pro-thrombotic phase, develops in ~5% of  
59 patients, and is characterized by the onset of microvascular and macrovascular thrombosis possibly  
60 promoted by strong focal and/or systemic inflammation. During this phase patients require medical  
61 treatment in intensive care units, and most of them do not survive.

62  
63 SARS-CoV-2 infection susceptibility and severity seem to be influenced by environmental factors  
64 (climate, pollution, cultural, social and economic inequalities, climate, health care system  
65 organizations), co-morbidities (high blood pressure, cardiovascular disease, other heart and lung  
66 conditions, diabetes, cancer, or compromised immune system), and inter-individual genetic  
67 differences [3-6]. Inter-individual genetic differences may affect the **spatial transmission dynamics  
68 of COVID-19, the susceptibility and severity of disease, and the inflammatory and immune  
69 response**, and three “genetic gateways” have been proposed accounting for disease progression [7].

70  
71 Specifically, **there is evidence that angiotensin-converting enzyme 2 (ACE2) is the human cell  
72 receptor of SARS-CoV-2 [8-10], and it was speculated [5,7,11-17] that ACE2 gene polymorphism  
73 may modulate the interaction between ACE2 and the Spike protein of SARS-CoV-2 during the  
74 virus entry into the host cell. In particular, differential affinity of a number of ACE2 missense  
75 variants for Spike protein was predicted using different computational approaches [12,18-21].  
76 Moreover, since ACE2 regulates the renin-angiotensin-aldosterone system [22], ACE2 missense  
77 variants or expression quantitative trait loci (eQTL) variants may contribute to pulmonary and  
78 systemic injury by fostering vasoconstriction, inflammation, oxidation and fibrosis, thereby  
79 affecting the clinical outcome [4,11,15, 23-25]. The possible association between specific ACE2  
80 gene variants and COVID-19 susceptibility, severity, and clinical outcomes is supported by massive  
81 genomic data from general population [26], while large-scale genome-wide association studies are  
82 urgently needed to firmly establish the causal link [27].**

83

84 In this study we have used *in silico* molecular docking to analyze the possible effects of ACE2  
85 single nucleotide polymorphisms (SNPs) leading to missense variants on the interaction between  
86 ACE2 and SARS-CoV-2 Spike protein. Molecular docking was performed with HDOCK, a  
87 powerful pipeline for integrated protein-protein docking, which is based on hybrid docking  
88 algorithm of template-based modeling and *ab initio* free docking to optimize the adjustment of  
89 ligand [28-31]. The HDOCK pipeline differs from other molecular docking platforms in its ability  
90 to support amino acid sequences as inputs, and in its hybrid docking strategy in which experimental  
91 information on the protein-protein binding site and small-angle X-ray scattering are incorporated  
92 during the docking and post-docking processes [28].

93

94 With respect to the other pipelines that were previously used to model the interaction between  
95 SARS-CoV-2 Spike protein and ACE2 missense variants [5,7,11,13-17], HDOCK has the  
96 advantage of integrating two approaches with the same software, together with a remarkable  
97 simplicity of use, and it is completely automated with consequent high reproducibility.

98

99

## 100 **2. Materials and methods**

### 101 *2.1. Databases*

102 3D structures of proteins were downloaded from Research Collaboratory for Structural  
103 Bioinformatics Protein Data Bank (RCSB PDB) (<http://www.rcsb.org/>) [324]. We focused our  
104 analysis on structures of SARS-COV-2 Spike Receptor Binding Domain (RBD)/ACE2 complexes  
105 6M17 (10.2210/pdb6M17/pdb) [2233] 6LZG (10.2210/pdb6LZG/pdb) [2334], and 6M0J  
106 (10.2210/pdb6M0J/pdb) [2435] models. The Single Nucleotide Polymorphism Database (dbSNP)  
107 [25, 26 36,37] was used to identify the ACE2 SNPs leading to missense variants. Functional  
108 information of ACE2 was acquired by UniProt database (Q9BYF1, ACE2\_HUMAN) [2738].  
109 ACE2 SNP frequencies were obtained by the database GnomAD-Exomes  
110 (<https://gnomad.broadinstitute.org/>). ACE2 bat sequences were downloaded from NCBI database.  
111 Multiple alignments of human and bat sequences were carried out by Clustal Omega [39].

112

### 113 *2.2. Molecular docking and statistical analysis*

114 HDOCK server (<http://hdock.phys.hust.edu.cn/>) was used to carry out molecular docking between  
115 receptor binding domain (RBD) of SARS-CoV-2 Spike protein and ACE2 wild type or missense  
116 variants from the dbSNP. We focused our analysis on two ACE2 N-terminal alpha helices that form

117 the major binding interface with SARS-CoV-2 Spike protein RBD based on X-ray crystallography  
118 [33-35]. In our analysis we used, as a receptor, the amino acid sequence of ACE2 wild type or  
119 missense variants, and, as a ligand, the SARS-CoV-2 Spike protein RBD models (6LZG chain B,  
120 6M0J chain E or 6M17 chain E) downloaded from RCSB-PDB database. 6M17 was the most  
121 complete structure because it contains the ACE2 collectrin-like domain [33]. Although this domain  
122 is far from the binding interface, it could still affect the geometry of the protein. Since HDOCK  
123 only provides score for *ab initio* free docking, to compare the complex scores obtained by *ab initio*  
124 free docking and template-based modeling we used FireDock  
125 (<http://bioinfo3d.cs.tau.ac.il/FireDock/>) [~~32,33~~ 40,41].

126  
127 Results of HDOCK/FireDock simulations were confirmed by submitting HDOCK-generated ACE2/  
128 Spike protein RBD complexes to PRODIGY [42]. Furthermore, wild type ACE2 and K26R ACE2  
129 models were also built by using MODELLER 9.25 [43] via Chimera [44], and these models were  
130 used as receptors in SwarmDock simulations [45]. HDOCK/FireDock pipeline was also used to  
131 evaluate the impact of SARS-CoV-2 Spike protein RBD variants on binding to wild type or K26R  
132 ACE2. QMEANDisCo (SwissDock) [46] and MolProbity [47] were used for bad bonds and angles  
133 metrics. A detailed protocol of the computational workflow is depicted in Supplementary Figure S1.

134  
135

### 136 3. Results

137 3.1. Molecular docking of SARS-CoV-2 Spike protein RBD and ACE2 wild type or missense  
138 variants.

139 Two ACE2 N-terminal alpha helices form the major binding interface with SARS-CoV-2 Spike  
140 protein RBD based on X-ray crystallography [~~22-24~~33-35]. In this region 25 SNPs causing leading  
141 to ACE2 missense variants are listed in the dbSNP.

142

143 HDOCK and FireDock pipelines were used for molecular docking. For each ACE2 missense  
144 variant, three docking simulations were carried, each with a different PDB model (6M17, 6LZG,  
145 6M0J), and the results obtained with the two methods (template-based modeling and *ab initio* free  
146 docking) were analyzed separately.

147

148 Before proceeding with the simulations, the quality of the models generated by HDOCK was  
149 analyzed and compared with the quality of the corresponding models generated by MODELLER.  
150 The analysis with QMEANDisCo demonstrated good quality of all models with global scores

151 similar to those of the control PDB models (6LZG, 6M0J and 6M17). Moreover, percentage of bad  
152 angles according to MolProbity was slightly lower with HDOCK (wild type ACE2 = 0.8%; K26R  
153 ACE2 = 0.77) compared to MODELLER (1.24% for both wild type and K26R ACE2)  
154 (Supplementary Figure S2)

155

156 Overall, HDOCK/FireDock results with the different PDB models and methods were concordant in  
157 92% of cases (Table 1; Supplementary Table S1; Fig. S34). For only two polymorphisms (N58K  
158 and M62V), the template-based method produced results that differed from those produced by *ab*  
159 *initio* docking.

160

161 We performed 156 docking simulations (i.e., 26 ACE2 SNPs, S19AQP ACE2, wild type ACE2, all  
162 multiplied by three ligands and two methods). Global energy score (GES) average with all  
163 simulations was -47.20 Kcal/mol (Fig. 1A, gray line), total standard deviation was 6.39 Kcal/mol  
164 and confidence interval was  $\pm 1.0035$  Kcal/mol (Fig. 1A, dotted line). The highest GES was -37.94  
165 Kcal/mol (M82I), while the lowest one was -56.24 (T55A). We used total GES average, GES value  
166 with wild type ACE2 (Fig. 1A, red line), and confidence interval as a threshold to screen the SNPs,  
167 considering as relevant only the SNPs that affected significantly the binding with SARS-CoV-2  
168 Spike protein RBD. By using this approach, we found 6 out of the 25 ACE2 missense variants  
169 (24%) (I21T, A25T, K26R, E37K, T55A, E75G) that showed higher affinity for SARS-CoV-2  
170 Spike protein RBD with respect to wild type ACE2, and 11 variants (44%) (I21V, E23K, K26E,  
171 T27A, E35K, S43R, Y50F, N51D, N58H, K68E, M82I) that exhibited lower affinity *in silico* (Fig.  
172 1A)

173

### 174 3.2. Geographical distribution of ACE2 SNPs affecting binding to SARS-CoV-2 spike protein RBD

175 GnomAD-Exomes database was used to gain information about frequencies of the examined ACE2  
176 SNPs worldwide (Table 1; Supplementary Table S2). K26R is the most diffused one with a global  
177 frequency of 0.3971%. The large diffusion of this SNP is also confirmed by others database:  
178 0.4579% in TOPMED; 0.595% in 4ALFA Project; 0.368% in ExAC; 0.315% in GnomAD; 0.511%  
179 in GO-ESP; 0.21% in 1000G; 0.62% in TWINSUK; 0.93% in ALSPAC. In particular, the K26R  
180 occurs with highest frequency in European (0.503%) and American (0.329%) populations with  
181 maximum value in Ashkenazi Jewish (1.2%), while it is less common in both African (0.099%) and  
182 Asian (0.079%) populations.

183

184 Cumulative frequency analysis of ACE SNPs demonstrated that ACE2 missense variants exhibiting  
185 increased affinity for SARS-CoV-2 Spike protein were more common in European and American  
186 populations (Fig. 1B), while those exhibiting reduced affinity were more common in African and  
187 Asian populations (Fig. 1C). The frequencies of each ACE2 missense variants were plotted  
188 individually in Supplementary Figure S4.

189

### 190 *3.3 Impact of SARS-CoV-2 Spike protein RBD variants on binding to ACE2*

191 A number of missense variants affecting the SARS-CoV-2 Spike protein have recently been  
192 identified worldwide, and listed in a comprehensive database [48]. In particular, some of these  
193 variants including N439K, L455F, F456L, A475V, Q493R, Q493L and N501Y, fall into the  
194 interfaces of binding of RBD. The impact of these RBD variants on binding to wild type ACE2 or  
195 K26R ACE2 was then evaluated. We focused on K26R missense variant because of its high  
196 frequency in the general population.

197

198 Preliminary, PRODIGY was used to confirm the effect of ACE2 K26R missense on wild type Spike  
199 protein RBD binding as predicted by FireDock with HDock complexes. Moreover, PRODIGY  
200 calculated dissociation constants ( $K_d$ ) that were  $8.8E^{-10}$  for the ACE2 K26R and  $4.610E^{-9}$  for wild  
201 type ACE2. The effect of K26R missense was further confirmed using models that were generated  
202 by MODELLER, and then submitted to SwarmDock obtaining an energy of -39.88 Kcal/mol for  
203 wild type ACE2 and of -46.13 Kcal/mol for K26R ACE2.

204

205 HDock/FireDock analysis was performed using the wild type or K26R ACE2 receptor and  
206 missense variants of the Spike protein RBD listed above. Results demonstrated that 5 of the 7 RBD  
207 mutations increased binding affinity for wild type ACE2 (Fig1D), while 5 of the 7 RBD mutations  
208 decreased the binding affinity for K26R ACE2 compared to wild type RBD (Fig1E).

209

210

## 211 **4. Discussion and conclusions**

212 The present study supports the hypothesis that ACE2 gene polymorphism may contribute to the  
213 genetic susceptibility to COVID-19 affecting the SARS-CoV-2 entry into the host cells, thus  
214 representing the first “genetic gateways” during disease progression [7]. Our results broaden the list  
215 of ACE2 missense variants that can affect the interaction with the SARS-CoV-2 spike protein [7, ~~9-~~  
216 ~~12~~, 168, 189, 1921]. Specifically, we focused our attention on ACE2 SNPs affecting two N-terminal  
217 alpha helices that form the major binding interface with SARS-CoV-2 Spike protein [22-24 33-35].



218

219 We did not include in our analysis the S19P variant because it falls into the cleavage site of ACE2  
220 precursor, and it may affect the N-terminal sequence of the mature protein. Besides, there is  
221 evidence that the S19P may reduce the affinity for SARS-CoV-2 Spike protein [189]. Results about  
222 K26R that is expected to increase the affinity for Spike protein is noteworthy because this variant is  
223 relatively frequent in European people with a frequency about 0.5%, which would correspond to a  
224 potential target population of 2,230,000 people at the European Union level [912]. In this study we  
225 confirmed the results of K26R by using HDOCK that also allowed us to identify additional  
226 missense variants (I21T, A25T, E37K, T55A, E75G) with higher affinity for SARS-CoV-2 Spike  
227 protein, and 11 variants (I21V, E23K, K26E, T27A, E35K, S43R, Y50F, N51D, N58H, K68E,  
228 M82I) with lower affinity.

229

230 It is worth of noticing the K26R variant of ACE2 was identified in a COVID-19 patient but not in  
231 control subjects in Italy, in a recent genome-wide association study enrolling a cohort of 131  
232 patients and 258 controls [27], further reinforcing the hypothesis that this missense variant may be  
233 associated with clinical susceptibility to disease.

234

235 Beside, it may be also relevant to note is rather common in different families of bats including  
236 Vespertilionidae and Phyllostomidae (Supplementary Fig. S5). Specifically, Phyllostomidae are  
237 diffused in South America (*Desmodus rotundus* XP\_024425698.1, *Phyllostomus discolor*  
238 XP\_028378317.1), while Phyllostomidae are very common in China (*Pipistrellus abramus*  
239 ACT66266.1) and Indochina (*Kerivoula pellucida* QJF77795.1), and the presence of *P. abramus*  
240 was confirmed in the Wuhan area [49].

241

242 It is conceivable that the polymorphisms responsible for a higher affinity may be responsible for a  
243 greater severity of the disease in humans, especially when very high affinity receptors are  
244 overexpressed due to the environmental and pharmacological factors. Of course, underlying  
245 diseases would contribute to an even more severe course of the disease, with an intense viral  
246 replication capable of infecting in turn a large number of persons, including some individuals with  
247 similar ACE2 polymorphisms, and so on. Another aspect to consider is the co-evolution of Spike  
248 protein. Indeed, missense mutations in the Spike RBD may have conflicting effects on binding  
249 affinity for wild type and K26R ACE2 (Fig.1D and Fig.1E).

250

251 Polymorphisms in genes coding for proteases from the respiratory tract belonging to the  
252 transmembrane protease/serine subfamily (TMPRSS) may also contribute to inter-individual  
253 differences in susceptibility and severity of disease [26, 50]. Indeed, there is evidence that TMPRSS  
254 proteolytic activity induces SARS-CoV Spike protein fusogenic activity, and, notably, SARS-CoV-  
255 2 cell entry is dependent on TMPRSS2, and blocked by protease inhibitors [51].

256

257 Obviously, the impact of these polymorphisms on severity of outcome should be weighted by  
258 appropriate demographic and clinical factors. If this difference were confirmed, this would pave the  
259 way for the identification, on a population scale, of healthy individuals whose molecular  
260 phenotypes would be responsible for disease that is more serious. Apart from the usual social  
261 distancing measures, targeted drug prevention strategies could be evaluated. It could be logical to  
262 assess pharmacological prophylactic interventions, as proposed in categories of healthy people at  
263 particular risk of exposure such as care-givers. The serine protease inhibitor camostat mesylate,  
264 approved in Japan to treat unrelated diseases, has been shown to block TMPRSS2 activity [52,53],  
265 and is thus an interesting candidate. Conversely, the identification of broader categories of people  
266 with lower risk of developing severe disease, could allow a safer exit from the lock-down phases,  
267 while facilitating the establishment of a faster herd immunity, and waiting reliable serological tests  
268 and effective vaccine.

269

270

271 **Conflict of interest**

272 The authors declare no conflict of interest.

273

274 **Authors' contribution**

275 M.C. contributed to experimental set-up, pipeline development, *in silico* analysis; P.F., A.I., and  
276 C.B. contributed to study designing and data providing; M.A. and P.A. contributed to coordination,  
277 conception, designing and writing. M.A. and P.A. contributed equally to the work. All authors  
278 critically revised draft versions of the manuscript and approved the final version.

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286 **References**

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509 **Legends to Figure**

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511 **Figure 1. Molecular docking simulations and frequencies of ACE2 SNPs that either enhance**  
512 **or the affinity with SARS-Cov-2 Spike protein. A)** Global Energy Score (GES) (Kcal/mol) of the  
513 interaction between wild type ACE2 or ACE2 missense variants and SARS-Cov-2 Spike protein.  
514 Molecular docking simulation were carried out by using HDOCK, which is based on two methods:  
515 template-based modeling and *ab initio* free docking. GES here shown are an average of the GES  
516 with the two methods. As reference values are shown: the average GES obtained with all  
517 simulations (solid line), the confidence interval (dotted line), and the GES obtained with wild type  
518 ACE (red line). **B-C)** Frequencies of ACE2 SNPs that either enhance (I21T, K26R, E37K, T55A)  
519 (**B**), or reduce (I21V, E23K, K26E, T27A, E35K, S43R, Y50F, N51D, N58H, K68E, M82I) (**C**) the  
520 affinity with SARS-Cov-2 Spike protein, based on GES.

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524 **Table**

525

526 **Table 1.** ACE2 SNPs analyzed in this study, their frequencies, and Global Energy Score (GES,  
 527 Kcal/mol) of the interaction between wild type ACE2 or ACE2 missense variants and SARS-Cov-2  
 528 Spike protein.

ACE2 wild type or missense variant	dbSNP ID	Frequency	Template-based modeling GES (Kcal/mol) <sup>a</sup>		Ab initio docking GES (Kcal/mol) <sup>a</sup>		Total GES (Kcal/mol) <sup>b</sup>	
		GnomAD Exomes	Average	SD	Average	SD	Average	SD
Wild type	-	-	-48.15	1.83	-50.45	0	-49.3	1.71
I21T	<a href="#">rs1244687367</a>	0.000005	-53.83	4.35	-53.96	3.93	-53.9	3.71
I21V	<a href="#">rs778030746</a>	0.000011	-42.76	0.43	-41.17	0.65	-41.97	1
E23K	<a href="#">rs756231991</a>	0.000005	-37.66	5.38	-45.68	0.43	-41.67	5.56
A25T	<a href="#">rs1434130600</a>	-	-55.89	0.54	-54.43	0.62	-55.16	0.95
K26R	<a href="#">rs4646116</a>	0.003971	-55.2	0.36	-54.51	1.43	-54.86	1.01
K26E	<a href="#">rs1299103394</a>	0.000005	-40.94	2.48	-40.24	0.69	-40.59	1.67
T27A	<a href="#">rs781255386</a>	0.000011	-47.51	1.01	-40.99	2.07	-44.25	3.86
E35D	<a href="#">rs778500138</a>	-	-47.87	1.24	-44.42	3.63	-46.14	3.08
E35K	<a href="#">rs1348114695</a>	0.000016	-36.68	4.85	-42.19	1.03	-39.43	4.35
E37K	<a href="#">rs146676783</a>	0.000033	-54.65	2.38	-53.92	1.33	-54.29	1.77
F40L	<a href="#">rs924799658</a>	0.000016	-52.76	0.44	-50.75	3.34	-51.76	2.4
S43R	<a href="#">rs1447927937</a>	0.000005	-42.82	0.99	-45.28	0.39	-44.05	1.5
Y50F	<a href="#">rs1192192618</a>	0.000005	-45.33	0.32	-44.12	0.61	-44.72	0.79
N51D	<a href="#">rs760159085</a>	0.000005	-39.55	0.72	-38.45	2.67	-39	1.85
T55A	<a href="#">rs775273812</a>	0.000006	-57.8	4.06	-54.67	2.7	-56.24	3.53
N58K	<a href="#">rs771621249</a>	0.000011	-45.68	3.24	-54	0.84	-49.84	5.03
N58H	<a href="#">rs1222417695</a>	0.000011	-46.26	0.06	-40.53	2.16	-43.39	3.42
Q60R	<a href="#">rs759162332</a>	0.000011	-51.12	4.87	-54.81	0.12	-52.96	3.68
M62V	<a href="#">rs1325542104</a>	0.000006	-53.77	0.03	-47.37	3.07	-50.57	4.01
N64K	<a href="#">rs1199100713</a>	0.000005	-45.51	3.81	-48.97	1.46	-47.24	3.2
K68E	<a href="#">rs755691167</a>	0.000011	-39.34	0.63	-37.75	0.45	-38.54	1
F72V	<a href="#">rs1256007252</a>	0.000005	-46.91	1.51	-50.35	0.88	-48.63	2.18
E75G	<a href="#">rs867318181</a>	-	-52.47	1.29	-54.27	0.44	-53.37	1.31
S77F	<a href="#">rs1234981462</a>	N.D.	-44.85	4.78	-49.97	0.24	-47.41	4.13
M82I	<a href="#">rs766996587</a>	0.000011	-33.49	2.7	-42.39	1.05	-37.94	5.21

529 <sup>a</sup>Values are averages of GES obtained with the three different 3D PDB SRARS-CoV-2 Spike  
 530 protein RDB structures (6LZG chain B, 6M0J chain E or 6M17 chain E), which were used as  
 531 ligands. SD, standard deviation. <sup>b</sup>Values are averages of GES obtained with the two methods  
 532 (template-based modeling or *ab initio* docking) and the three different 3D PDB structures.

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