bioRxiv preprint doi: https://doi.org/10.1101/2022.01.10.475746; this version posted January 17, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

1
Tuftsin: a natural molecule against SARS-CoV-2 infection

2
3 **Jiahao Huang[1], Jing Wang[2], Ziyuan Wang[1], Ming Chu[1]*, Yuedan Wang[1]***
4
5 [1]Department of Immunology, School of Basic Medical Sciences, Peking University.
6 NHC Key Laboratory of Medical Immunology (Peking University). Beijing, China.
7
8 [2]State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical
9 Sciences, Peking University, Beijing, China.
10
11 ***Correspondence:**
12 Corresponding Author
13 wangyuedan@bjmu.edu.cn;
14 famous@bjmu.edu.cn
15
16 **Keywords: Tuftsin, SARS-CoV-2, ACE2, NRP1, natural peptide, COVID-19**
17
18 **Abstract**
19 Coronavirus disease 2019 (COVID-19) continuously proceeds despite the application
20 of a variety of vaccines. It is still urgent to find effective ways to treat COVID-19.
21 Recent studies indicate that NRP1, an important receptor of the natural peptide tuftsin,
22 facilitates SARS-CoV-2 infection. Importantly, tuftsin is a natural human molecule
23 released from IgG. Here, we found 91 overlapping genes between tuftsin targets and
24 COVID-19-associated genes. Bioinformatics analyses indicated that tuftsin could also
25 target ACE2 and exert some immune-related functions to treat COVID-19. Using
26 surface plasmon resonance (SPR) analysis, we confirmed that tuftsin can bind ACE2
27 and NRP1 directly. Moreover, tuftsin effectively impairs the binding of SARS-CoV-2
28 S1 to ACE2. Thus, tuftsin is an attractive drug against COVID-19. And tuftsin as
29 natural immunostimulating peptide in human, we speculate that tuftsin may has crucial
30 roles in asymptomatic carriers or mild cases of COVID-19.
31
32 **Introduction**
33 Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome
34 coronavirus 2 (SARS-CoV-2) results in high morbidity and mortality [1,2]. It is known
35 that the spike (S) protein binding to angiotensin-converting enzyme 2 (ACE2) is the
36 core mechanism of SARS-CoV-2 infecting host cells. Through persistent efforts,
37 COVID-19 vaccines have been approved for human use in most countries. However,
38 the quantity of neutralizing antibodies induced by vaccines still needs to be verified in
39 humans. At present, another hopeful intervention is neutralizing monoclonal antibodies
40 (mAbs) [3]. Unfortunately, producing safe and effective mAbs is complicated, and the
41 duration of effective protection remains to be determined [4,5]. Moreover, continuous
42 mutations of SARS-CoV-2 during the pandemic may lead to escape from antibody
43 recognition and reduce the neutralizing activity of mAbs [6]. Hence, discovering a broad-

bioRxiv preprint doi: https://doi.org/10.1101/2022.01.10.475746; this version posted January 17, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

44 spectrum and effective method for treating COVIV-19 is urgent.
45
46 Recently, neuropilin 1 (NRP1) has been found to be a host factor for SARS-CoV-2
47 infection [7]. It has been reported that NRP1 facilitates the entry of SARS-CoV-2 into
48 cells in the presence of ACE2 [8]. It is worth noting that NRP1 is an important receptor
49 of tuftsin [9,10]. Tuftsin, a natural phagocytosis-stimulating peptide, was found by Victor
50 Najjar et al. in 1970 [11]. Tuftsin is released from the Fc fragment of IgG by an
51 endocarboxy-peptidase in the spleen and a leukokininase on the outer membrane of
52 neutrophilic leukocytes [11,12]. Furthermore, tuftsin is a tetrapeptide that consists of Thr-
53 Lys-Pro-Arg, located at amino acid residues 289 to 292 of the heavy chain of IgG.
54 Tuftsin has a broad spectrum of activities mainly associated with immune system
55 functions and exerts effects on phagocytic cells, especially macrophages. These
56 functions of Tuftsin briefly include cell phagocytosis, motility, immunogenic response,
57 and bactericidal and tumoricidal activities [13,14]. It was reported that tuftsin activity is
58 inversely correlated with splenectomy function and is significantly lower in patients
59 with AIDS, cirrhosis, intestinal failure and some infectious diseases [12,15,16]. Moreover,
60 it was demonstrated that tuftsin has stability and low toxicity in vitro and in vivo [14,17,18].
61 As a natural immune stimulating peptide, tuftsin is an attractive candidate for
62 immunotherapy. Thus, we hypothesized that tuftsin could inhibit SARS-CoV-2
63 infection by interacting with NRP1. We subsequently performed experiments to verify
64 our conceptions.
65
66 **Materials and Methods**
67 Compound profiling and disease-related gene identification
68 The structure of tuftsin was found in PubChem (https://pubchem.ncbi.nlm.nih.gov/).
69 The 3D structure of tuftsin was built using Chem3D. Afterward, the target proteins
70 corresponding to tuftsin screened from the Pharmmapper database and PubMed
71 database were standardized in UniProt (http://www.uniprot.org/). Finally, Cytoscape
72 3.8.2 was used to determine the drug-target network. COVID-19-related genes were
73 mined from the GeneCards database. All of the disease gene targets were normalized
74 with R software using the Bioconductor package when redundancy was deleted [19].
75
76 Network establishment
77 Screening for drug-disease crossover genes was performed. Based on previous steps,
78 two sets of target lists were prepared: drug targets and disease-related genes. The
79 crossover genes were filtered with R software using the Venn Diagram package. The
80 STRING 11.5 database (http://string-db.org/) was used to analyse the intersecting
81 protein–protein interactions (PPIs), and the common targets were counted with R
82 software.
83
84 Enrichment analysis
85 The proteins with overlapping expression patterns were evaluated by bioinformatics
86 annotation with R software using the Bioconductor package, including a panther
87 classification system (http://www.pantherdb.org/), a gene ontology (GO) annotation

bioRxiv preprint doi: https://doi.org/10.1101/2022.01.10.475746; this version posted January 17, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

88 database website (http://www.geneontology.org), and Kyoto Encyclopedia of Genes
89 and Genomes (KEGG) pathway enrichment analysis (http://www.genome.jp/kegg/). A
90 $p < 0.05$ was considered statistically significant.
91
92 Molecular docking analysis
93 The flexible docking process between tuftsin and target proteins was conducted by
94 softwere Discovery Studio 2021 (DS). Briefly, the crystallographic structures of human
95 ACE2 (PDB ID: 1R42) and human NRP1 (PDB ID: 2QQ1) with high resolution were
96 prepared using the Prepare Protein and Minimization module of DS. The active binding
97 site of each protein was defined based on the most representative features of the SARS-
98 CoV-2 interface. Tuftsin was docked into the active binding site of ACE2 and NRP1
99 using the molecular docking module in DTS.
100
101 Surface plasmon resonance analysis
102 The recombinant human ACE2 protein (Novoprotein, Beijing, China) and recombinant
103 human NRP1 protein were used for surface plasmon resonance (SPR) analysis using a
104 Biacore 8K instrument (Biacore, Uppsala, Sweden). Each target was immobilized onto
105 flow cells in a CM5 sensor chip (GE Healthcare) via the amine-coupling method.
106 Briefly, ACE2 and NRP1 were diluted in 10 mM pH 4.5 acetate to 20 μg/mL. Then, the
107 protein solutions were injected individually on the carboxyl-modified sensor surface to
108 form amine bonds. Both ACE2 and NRP1 immobilized levels were approximately
109 10000 RU. Binding analyses were carried out at 25°C and a flow rate of 10 μl/min.
110 Tuftsin diluted in running buffer (1×PBS, 0.05% Tween 20 and 5% dimethyl sulfoxide,
111 pH 7.4) was run over each target at gradient concentrations. An empty flow cell without
112 any immobilized protein was used as a deducted reference. The binding curves were
113 analysed using a kinetic binding model supplied with Biacore Evaluation Software (GE
114 Healthcare).
115
116 Competition binding experiment
117 For the competition binding experiment, the SARS-CoV-2 S1 protein was immobilized
118 on the CM5 sensor chip via the amine-coupling method. 5 nM ACE2 was injected for
119 negative control. Tuftsin was diluted into a series of solutions with gradient
120 concentrations and fixed with 5 nM ACE2, and then the solutions were injected into the
121 chip. The blocking efficacy was evaluated by comparison of response units with and
122 without tuftsin incubation.
123
124 Statistical analysis
125 The results were analysed using Student's *t* test with SPSS software and R 4.1.0.
126
127 **Results**
128 **Bioinformatics analyses revealed the connection between tuftsin and COVID-19**
129 The 2D structure of tuftsin was obtained from the PubChem database (Compound CID:
130 156080), and the most stable 3D structure was built based on the 2D structure through

bioRxiv preprint doi: https://doi.org/10.1101/2022.01.10.475746; this version posted January 17, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

131 a molecular simulation assay (Fig. 1A). In addition to the reported receptors of tuftsin,
132 the potential targets of tuftsin in humans were also predicted through the PharmMapper
133 database. Together, 284 targets of tuftsin were collected (Fig. 1B and data S1).
134 Furthermore, we collected 2572 disease-associated genes of COVID-19 from the
135 GeneCards database (data S2). We surprisingly found 91 intersecting proteins of tuftsin
136 targets and COVID-19-associated genes through intersection analysis (Fig. 1C). It is
137 intriguing that the overlapping proteins account for nearly one-third of tuftsin targets.
138 Moreover, the protein–protein interaction network of the overlapping proteins was
139 established, and it showed that JAK2, STAT1 and AKT1 are core molecules in the
140 network (Fig. 1D). Furthermore, we performed enrichment analysis for the 91
141 intersecting genes. GO annotation revealed that the expressed tuftsin-COVID-19
142 crossover proteins were mainly associated with immune functions such as neutrophil
143 activation, neutrophil-mediated immunity and cytokine receptor binding. Moreover, the
144 KEGG pathway enrichment analysis showed that the COVID-19 pathway was the most
145 significantly enriched. In addition, many target genes were strongly associated with
146 some immunologic pathways, such as Th17 cell differentiation, the IL-17 signaling
147 pathway and the immune checkpoint pathway (Fig. 1E). In the COVID-19 pathway,
148 the SARS-CoV-2 receptors ACE2 and NRP1 were targets of tuftsin. Moreover, IL-2,
149 STAT1 and some complement molecules in the COVID-19 pathway were targets of
150 tuftsin (Fig. 1F). Together, these results suggest that tuftsin is a promising candidate
151 against COVID-19, owing to its multifaceted pharmacological activities.
152

153 **The interaction of tuftsin with ACE2 and NRP1 analysed by molecular docking**
154 It is novel that ACE2 is a potential target of tuftsin, as mentioned above. Thus,
155 molecular docking was performed to determine the potential binding sites and binding
156 affinity between tuftsin and the SARS-CoV-2 receptors ACE2 and NRP1. First, we
157 defined the interaction interface of SARS-CoV-2 S1-RBD with ACE2 as the active sites
158 of ACE2. These interface sites in ACE2 include Q24, M82, N330, and R393, which are
159 mainly located in the N-terminal peptidase domain of ACE2 [20]. Then, the docking
160 region was a sphere containing the defined ACE2 active sites (Fig. S1A). The results
161 showed that the affinity of tuftsin and ACE2 was -6.9 kcal/mol, demonstrating that they
162 could combine spontaneously (Fig. 2A). Furthermore, tuftsin could form strong
163 hydrogen bonds to Ser47 and Asp67, carbon hydrogen bonds to His345, Asp67 and
164 Asn51, and salt bridges to Asp67 of ACE2 (Fig. 2A). It is worth mentioning that the
165 binding sites were adjacent to the interactional sites of S1-RBD and ACE2, indicating
166 that tuftsin could inhibit S1 binding to ACE2 by covering their binding sites.
167 Meanwhile, the b1b2 domain of NRP1 was prepared, as previous studies showed that
168 the extracellular b1b2 domain of NRP1 mediates binding to CendR peptides [21]. Then,
169 the active sites of NRP1 b1b2 were defined according to the interactional sites of S1-
170 RBD and NRP1 b1b2, including D320, E348, Y353 and so on [7]. The docking region
171 was a sphere containing the defined NRP1 b1b2 active sites (Fig. S1B). The docking
172 results showed that tuftsin and NRP1 b1b2 have a high binding affinity of -8.1 kcal/mol.
173 In addition, tuftsin solidly fits into a binding pocket on NRP1 b1b2 (Fig. 2B).
174 Furthermore, tuftsin could form a salt bridge to Lys 397 and a carbon hydrogen bond

bioRxiv preprint doi: https://doi.org/10.1101/2022.01.10.475746; this version posted January 17, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

175 to Pro398, which are near the interactional sites of S1-RBD and NRP1 b1b2. Moreover,
176 the binding region of tuftsin and NRP1 overlapped with the binding area of NRP1 and
177 S1-RBD in space (Fig. 2B). Collectively, these results demonstrated that tuftsin could
178 bind ACE2 and NRP1 and inhibit the SARS-CoV-2 S1 binding of ACE2 and NRP1 by
179 covering their interactional sites.
180

181 **Tuftsin binds ACE2 and NPR1 directly, as confirmed by surface plasmon**
182 **resonance (SPR) analyses**

183 The interactions of tuftsin with ACE2 and NRP1 were further evaluated by real-time
184 biomolecular interaction analysis with SPR. The kinetics of the binding reaction were
185 determined by injecting different concentrations of tuftsin over recombinant human
186 ACE2 immobilized on one half of the chip surface and over recombinant human NRP1
187 immobilized on another half of the chip surface. The results showed that tuftsin can
188 bind ACE2 with an equilibrium dissociation constant (K_D) of 460 μmol/L, according
189 to the obtained association and dissociation rates (Fig. 3A). Moreover, the K_D fitting
190 curves of tuftsin and ACE2 became gentle with higher concentrations of tuftsin,
191 indicating that the interaction of tuftsin and ACE2 is specific. (Fig. 3A). Tuftsin can
192 also bind NRP1 specifically with a higher binding affinity of K_D = 10.65 μmol/L. The
193 sensorgrams and K_D fitting curves of tuftsin and NRP1 are shown in Fig. 3B. As SPR
194 is the gold standard for detecting drug-target interactions, these results demonstrate that
195 tuftsin binds ACE2 and NRP1 directly and specifically, validating the accuracy of the
196 above results of bioinformatics analyses and molecular docking assays.
197

198 **Tuftsin impairs the binding of SARS-CoV-2 S1 to ACE2**

199 An SPR-based competition assay was employed to determine whether tuftsin could
200 affect the binding of S1 protein with ACE2. We first determined the binding affinity of
201 the S1 protein with ACE2 by SPR assay, which unsurprisingly showed a high affinity.
202 A suitable concentration ACE2 solution was injected over the immobilized SARS-
203 CoV-2 S1 protein as a control. Then, a series of gradient concentrations of tuftsin
204 solutions containing equal concentrations of ACE2 were injected over the immobilized
205 SARS-CoV-2 S1 protein for comparison. We observed that 9 μmol/L tuftsin had a mild
206 inhibitory effect. It is worth noting that the addition of 156 μmol/L tuftsin significantly
207 attenuated the response signal by approximately two-thirds compared to that of ACE2
208 alone over the immobilized S1. Notably, a substantial decrease in the response signal
209 was observed with increasing concentrations of tuftsin. The response single was close
210 to zero when the added concentration of tuftsin was 625 μmol/L. This result indicates
211 that the interaction between S1 and ACE2 was almost completely blocked in the
212 presence of 625 μmol/L tuftsin (Fig. 4). The experiment was repeated three times

bioRxiv preprint doi: https://doi.org/10.1101/2022.01.10.475746; this version posted January 17, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

213 independently. In conclusion, the competition binding experiment revealed that tuftsin
214 effectively impairs the binding of SARS-CoV-2 S1 to ACE2 in a dose-dependent
215 manner.
216

217 **Discussion**

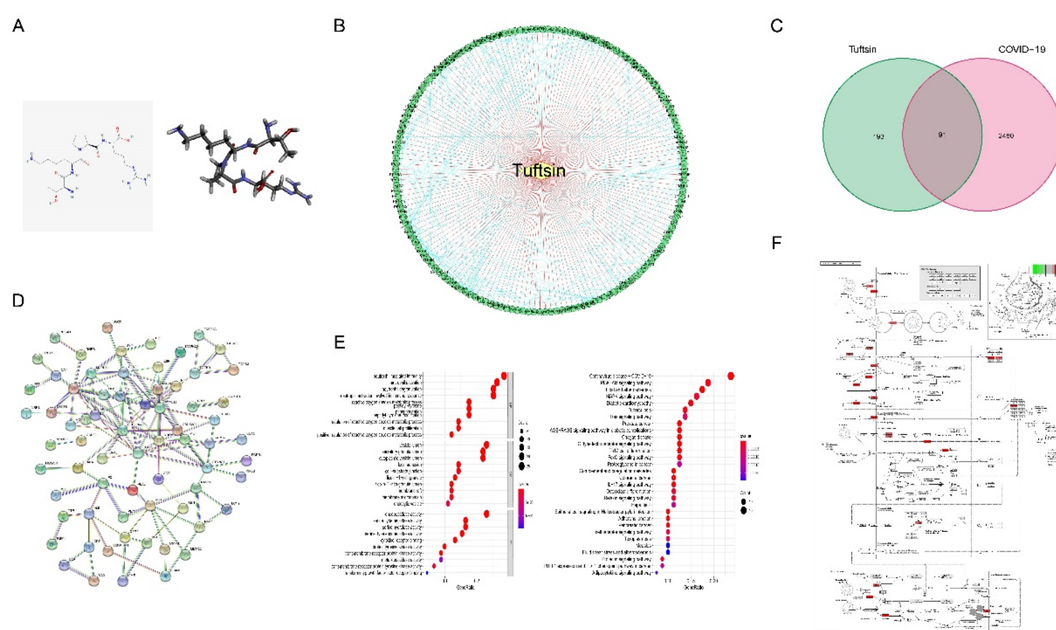
218 At present, vaccination is the most general way to prevent COVID-19; however, the
219 notable problem is the uneven distribution of vaccine resources worldwide [22]. The cost
220 of producing vaccines and neutralizing antibodies is relatively high. It has been reported
221 that the effectiveness of the SARS-CoV-2 vaccine declines significantly during 2021
222 [23]. Here, we report that an immune-stimulating peptide, tuftsin, is a potential effective
223 drug for COVID-19. Tuftsin, as a natural tetrapeptide that exists in humans, originates
224 from a special fraction of the parent carrier IgG through enzymatic processing.
225 Accordingly, tuftsin has lower toxicity and fewer side effects than other drugs [24]. There
226 are many marked drugs, such as oral liquid of spleen aminopeptide, which mainly
227 contains tuftsin, and some drugs, which are derivatives of tuftsin, which all have
228 satisfactory clinical efficacy [25]. Importantly, tuftsin can be produced on a large scale at
229 a lower cost [26]. This allows tuftsin to be widely applied for the prevention and treatment
230 of COVID-19 infection. The general existence of tuftsin in species allows wide
231 protection of animals. It is worth noting that the mutant sequence of tuftsin turns
232 inactive or inhibitory analogs [27].
233

234 In this research, 9 μM tuftsin had slight inhibitory activity. We observed that when the
235 concentration of tuftsin was 156 μM, the binding affinity of SARS-CoV-2 S1 and
236 ACE2 was reduced significantly. When the concentration reached 625 μM, the
237 combination of SARS-CoV-2 S1 and ACE2 was completely blocked. It has been
238 confirmed that a 156 μM concentration of tuftsin can exist at a high concentration in
239 the internal environment after intravenous injection [28]. We conceive that tuftsin can be
240 designed as an oral or nasal spray. In this case, the local concentration of tuftsin reached
241 625 μM. It has been reported that the amount of IgG induced by vaccines is mainly
242 focused on the lower respiratory tract. Consequently, the upper respiratory tract, which
243 mainly suffers from viral infection, lacks sterilizing immune protection [29]. Importantly,
244 the spray form of tuftsin could protect the upper respiratory tract, which the antibodies
245 induced by vaccines cannot effectively protect. The molecular docking assay showed
246 that tuftsin binds at the N-terminus of ACE2, which is the area of S1 protein binding.
247 This indicated that tuftsin can block the binding of S1 protein and ACE2 directly.
248

249 It is worth noting that there were many asymptomatic and mild infectors during the
250 pandemic. It is clear that innate and adaptive immunity functions during asymptomatic
251 infection; however, the mechanism of the T cell and antibody response is unclear [30,31].

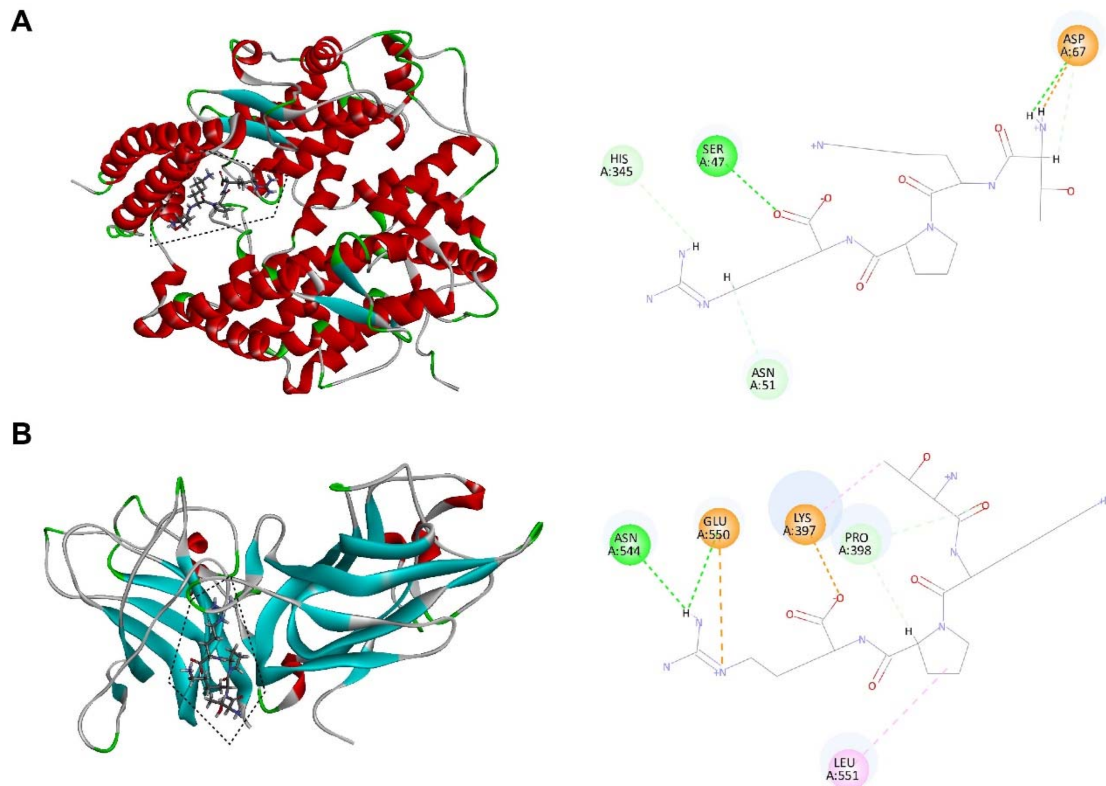
bioRxiv preprint doi: https://doi.org/10.1101/2022.01.10.475746; this version posted January 17, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

252 Asymptomatic people seem to clear the virus faster [32]. Tuftsin, a human natural
253 immunostimulating peptide released from IgG, certainly has significant roles related to
254 innate immunity. We reported that tuftsin can target the important receptors of SARS-
255 CoV-2 S1, which is similar to adaptive immunity. Thus, we speculated that tuftsin has
256 crucial roles in asymptomatic or mild infection. It is likely that the activity of tuftsin is
257 higher in asymptomatic individuals than in symptomatic individuals.
258
259 **Figures**



260
261 **Fig. 1. The connection between tuftsin and COVID-19.** (**A**) (Left) The 2D chemical
262 structure of tuftsin downloaded from the PubChem database. (Right) The 3D chemical
263 structure of tuftsin established by software based on the 2D structure. (**B**) The 'drug-
264 target' network of tuftsin. Red links represent the interactions between tuftsin and target
265 nodes. Each node is a protein target. Green points represent the targeted proteins in
266 humans. Blue links represent the interactions between the targets. (**C**) A Venn diagram
267 of tuftsin and COVID-19 cotargeted genes. (**D**) Protein–protein interaction (PPI)
268 network of the intersected targets. The interactions with a high confidence of 0.95. (**E**)
269 (Left) Gene ontology enrichment results in bubble plot. (Right) The KEGG enrichment
270 results in bubble plot. (**F**) Detailed targets of tuftsin in the COVID-19 pathway. Red
271 points represent the tuftsin targets. The intensity of the color represents the possibility
272 of tuftsin targeting. Deeper color indicates higher possibility.
273

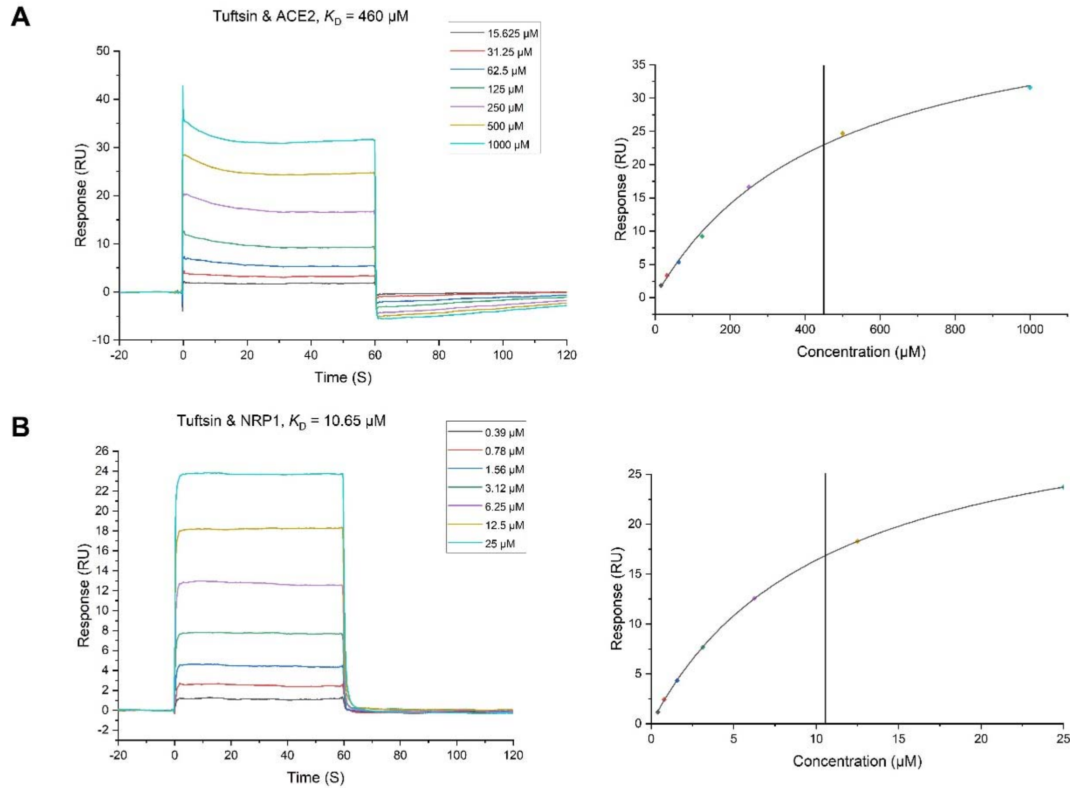
bioRxiv preprint doi: https://doi.org/10.1101/2022.01.10.475746; this version posted January 17, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



274

275 **Fig. 2. Molecular interaction of tuftsin with ACE2 and NRP1.** (**A**) (Left) The
276 binding pattern of tuftsin with ACE2. Binding area was circled by black dotted line.
277 Secondary structural elements are depicted as ribbons (coils, α-helices, arrows, β-
278 sheets). Color is based on secondary structures (α-helices, red; β-sheets, skyblue; loops,
279 green). (Right) Molecular interaction schemes of tuftsin with the relative residues of
280 ACE2. Green lines represent conventional hydrogen bonds; light green lines represent
281 carbon hydrogen bonds; orange lines represent salt bridges; and pink lines represent
282 alkyl bonds. (**B**) (Left) The binding pattern of tuftsin with NRP1. Binding area was
283 circled by black dotted line. (Right) Molecular interaction schemes of tuftsin with the
284 relative residues of NRP1. Other interpretations are the same as above.
285

bioRxiv preprint doi: https://doi.org/10.1101/2022.01.10.475746; this version posted January 17, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

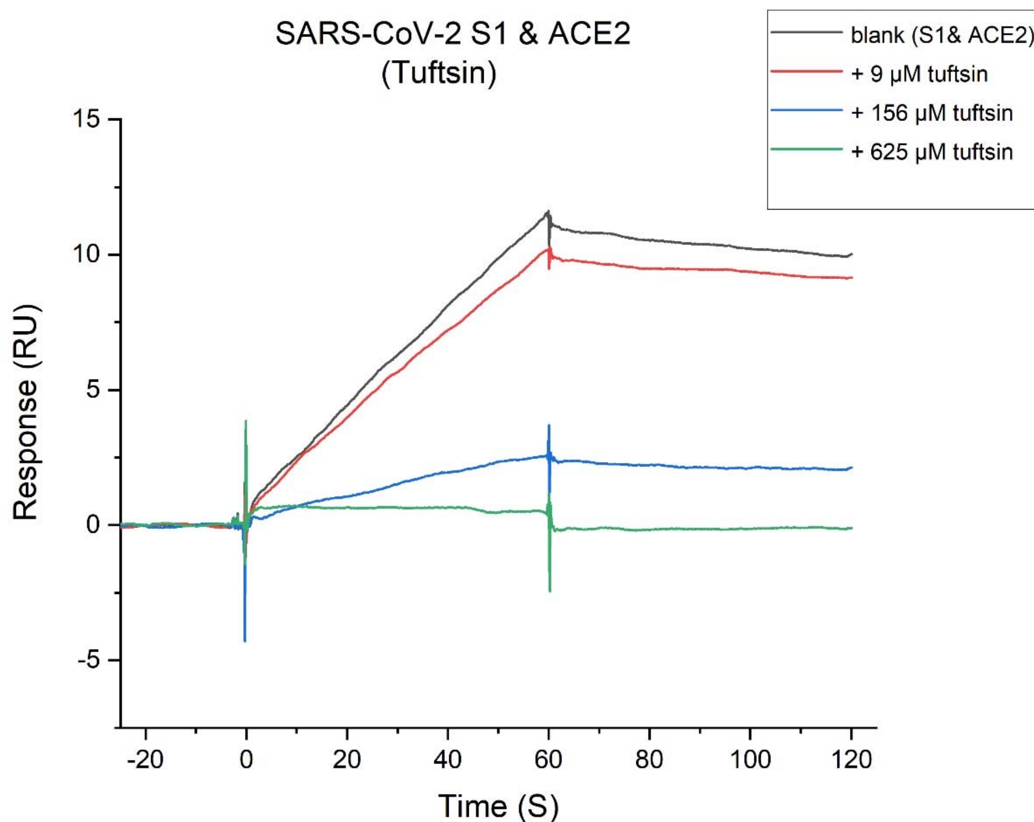


286

287 **Fig. 3. The binding of tuftsin to ACE2 and NRP1 was determined by SPR assay.**
288 (**A**) (Left) Binding curves of tuftsin with ACE2. The K_D of the ACE2 protein with a
289 series of concentrations of tuftsin was calculated by using a 1:1 binding model. Data
290 are presented as response units (RU) over time (S). (Right) The fitting carve of tuftsin
291 with ACE2. (**B**) (Left) Binding curves of tuftsin with NRP1. The K_D of the NRP1
292 protein with a series of concentrations of tuftsin was calculated by using a 1:1 binding
293 model. Other interpretations are the same as above. (Right) The fitting carve of tuftsin
294 with NRP1.

295

bioRxiv preprint doi: https://doi.org/10.1101/2022.01.10.475746; this version posted January 17, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



296
297 **Fig. 4. Tuftsin inhibits the SARS-CoV-2 S1 binding to ACE2.** The binding activity
298 of SARA-CoV-2 S1 to ACE2 in the presence of increasing concentrations of tuftsin.
299 Intensive concentrations of tuftin showed enhanced inhibitory effects.
300
301 **Conflict of Interest**
302 The authors declare that the research was conducted in the absence of any commercial
303 or financial relationships that could be construed as a potential conflict of interest.
304
305 **Author contributions**
306 Y.W., J.H. and C.M. conceptualized and designed this study. J.H. performed the
307 bioinformatic analysis. J.H. and Z.W. performed the molecular docking. J.H. performed
308 the SPR experiments with assistance from J.W. J.H. processed the data. J.H. drafted,
309 edited the manuscript. Y.W., C.M. administered the project. All authors have read and
310 agreed to the published version of this manuscript.
311
312 **Funding**
313 National Natural Science Foundation of China (81603119) and Natural Science
314 Foundation of Beijing Municipality (7174316).
315
316 **Acknowledgements**
317 We thank the State Key Laboratory of Natural and Biomimetic Drugs (Peking
318 University) for their assistance in performing the surface plasmon resonance
319 assay.

bioRxiv preprint doi: https://doi.org/10.1101/2022.01.10.475746; this version posted January 17, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

320
321 **Supplementary Material**
322 Figs. S1
323 Data S1 and S2
324
325 **Data Availability Statement**
326 All data are available in the main text or the supplementary information.
327
328 **References and Notes**
329

330 1 Zhu, N. *et al.* A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J*
331 *Med* **382**, 727-733, doi:10.1056/NEJMoa2001017 (2020).
332 2 Dai, L. & Gao, G. F. Viral targets for vaccines against COVID-19. *Nat Rev Immunol* **21**, 73-
333 82, doi:10.1038/s41577-020-00480-0 (2021).
334 3 Renn, A., Fu, Y., Hu, X., Hall, M. D. & Simeonov, A. Fruitful Neutralizing Antibody Pipeline
335 Brings Hope To Defeat SARS-Cov-2. *Trends Pharmacol Sci* **41**, 815-829,
336 doi:10.1016/j.tips.2020.07.004 (2020).
337 4 Su, W. *et al.* Neutralizing Monoclonal Antibodies That Target the Spike Receptor Binding
338 Domain Confer Fc Receptor-Independent Protection against SARS-CoV-2 Infection in
339 Syrian Hamsters. *mBio* **12**, e0239521, doi:10.1128/mBio.02395-21 (2021).
340 5 Taylor, P. C. *et al.* Neutralizing monoclonal antibodies for treatment of COVID-19. *Nat*
341 *Rev Immunol* **21**, 382-393, doi:10.1038/s41577-021-00542-x (2021).
342 6 Du, L., Yang, Y. & Zhang, X. Neutralizing antibodies for the prevention and treatment of
343 COVID-19. *Cell Mol Immunol* **18**, 2293-2306, doi:10.1038/s41423-021-00752-2 (2021).
344 7 Daly, J. L. *et al.* Neuropilin-1 is a host factor for SARS-CoV-2 infection. *Science* **370**, 861-
345 865, doi:10.1126/science.abd3072 (2020).
346 8 Cantuti-Castelvetri, L. *et al.* Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity.
347 *Science* **370**, 856-860, doi:10.1126/science.abd2985 (2020).
348 9 Vander Kooi, C. W. *et al.* Structural basis for ligand and heparin binding to neuropilin B
349 domains. *Proc Natl Acad Sci U S A* **104**, 6152-6157, doi:10.1073/pnas.0700043104 (2007).
350 10 von Wronski, M. A. *et al.* Tuftsin binds neuropilin-1 through a sequence similar to that
351 encoded by exon 8 of vascular endothelial growth factor. *J Biol Chem* **281**, 5702-5710,
352 doi:10.1074/jbc.M511941200 (2006).
353 11 Najjar, V. A. & Nishioka, K. "Tuftsin": a natural phagocytosis stimulating peptide. *Nature*
354 **228**, 672-673, doi:10.1038/228672a0 (1970).
355 12 Corazza, G. R. *et al.* Tuftsin deficiency in AIDS. *Lancet* **337**, 12-13, doi:10.1016/0140-
356 6736(91)93331-3 (1991).
357 13 Najjar, V. A. Tuftsin, a natural activator of phagocyte cells: an overview. *Ann N Y Acad Sci*
358 **419**, 1-11, doi:10.1111/j.1749-6632.1983.tb37086.x (1983).
359 14 Fridkin, M. & Najjar, V. A. Tuftsin: its chemistry, biology, and clinical potential. *Crit Rev*
360 *Biochem Mol Biol* **24**, 1-40, doi:10.3109/10409238909082550 (1989).
361 15 Zoli, G. *et al.* Impaired splenic function and tuftsin deficiency in patients with intestinal
362 failure on long term intravenous nutrition. *Gut* **43**, 759-762, doi:10.1136/gut.43.6.759

bioRxiv preprint doi: https://doi.org/10.1101/2022.01.10.475746; this version posted January 17, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

363 (1998).
364 16 Trevisani, F. *et al.* Impaired tuftsin activity in cirrhosis: relationship with splenic function
365 and clinical outcome. *Gut* **50**, 707-712, doi:10.1136/gut.50.5.707 (2002).
366 17 Siemion, I. Z. & Kluczyk, A. Tuftsin: on the 30-year anniversary of Victor Najjar's discovery.
367 *Peptides* **20**, 645-674, doi:10.1016/s0196-9781(99)00019-4 (1999).
368 18 Amoscato, A. A., Davies, P. J., Babcock, G. F. & Nishioka, K. Receptor-mediated
369 internalization of tuftsin by human polymorphonuclear leukocytes. *J Reticuloendothel Soc*
370 **34**, 53-67 (1983).
371 19 Yu, G., Wang, L. G., Han, Y. & He, Q. Y. clusterProfiler: an R package for comparing
372 biological themes among gene clusters. *Omics* **16**, 284-287, doi:10.1089/omi.2011.0118
373 (2012).
374 20 Lan, J. *et al.* Structure of the SARS-CoV-2 spike receptor-binding domain bound to the
375 ACE2 receptor. *Nature* **581**, 215-220, doi:10.1038/s41586-020-2180-5 (2020).
376 21 Plein, A., Fantin, A. & Ruhrberg, C. Neuropilin regulation of angiogenesis, arteriogenesis,
377 and vascular permeability. *Microcirculation* **21**, 315-323, doi:10.1111/micc.12124 (2014).
378 22 Forni, G. & Mantovani, A. COVID-19 vaccines: where we stand and challenges ahead. *Cell*
379 *Death Differ* **28**, 626-639, doi:10.1038/s41418-020-00720-9 (2021).
380 23 Cohn, B. A., Cirillo, P. M., Murphy, C. C., Krigbaum, N. Y. & Wallace, A. W. SARS-CoV-2
381 vaccine protection and deaths among US veterans during 2021. *Science* **0**, eabm0620,
382 doi:doi:10.1126/science.abm0620.
383 24 Catane, R. *et al.* Toxicology and antitumor activity of tuftsin. *Ann N Y Acad Sci* **419**, 251-
384 260, doi:10.1111/j.1749-6632.1983.tb37111.x (1983).
385 25 Shakya, N., Sane, S. A., Haq, W. & Gupta, S. Augmentation of antileishmanial efficacy of
386 miltefosine in combination with tuftsin against experimental visceral leishmaniasis.
387 *Parasitol Res* **111**, 563-570, doi:10.1007/s00436-012-2868-z (2012).
388 26 Siebert, A., Gensicka-Kowalewska, M., Cholewinski, G. & Dzierzbicka, K. Tuftsin -
389 Properties and Analogs. *Curr Med Chem* **24**, 3711-3727,
390 doi:10.2174/0929867324666170725140826 (2017).
391 27 Najjar, V. A. Biochemical aspects of tuftsin deficiency syndrome. *Med Biol* **59**, 134-138
392 (1981).
393 28 Blok-Perkowska, D., Muzalewski, F. & Konopińska, D. Antibacterial properties of tuftsin
394 and its analogs. *Antimicrob Agents Chemother* **25**, 134-136, doi:10.1128/aac.25.1.134
395 (1984).
396 29 Krammer, F. SARS-CoV-2 vaccines in development. *Nature* **586**, 516-527,
397 doi:10.1038/s41586-020-2798-3 (2020).
398 30 Boyton, R. J. & Altmann, D. M. The immunology of asymptomatic SARS-CoV-2 infection:
399 what are the key questions? *Nat Rev Immunol*, 1-7, doi:10.1038/s41577-021-00631-x
400 (2021).
401 31 Schijns, V. & Lavelle, E. C. Prevention and treatment of COVID-19 disease by controlled
402 modulation of innate immunity. *Eur J Immunol* **50**, 932-938, doi:10.1002/eji.202048693
403 (2020).
404 32 Nogrady, B. What the data say about asymptomatic COVID infections. *Nature* **587**, 534-
405 535, doi:10.1038/d41586-020-03141-3 (2020).
406