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Small molecule targeting amyloid fibrils inhibits *Streptococcus mutans* biofilm formation

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Materials and Methods

Virtual screening

We used MOE v2015.1001 for structure-based virtual screening. The X-ray structure of C123 segment was obtained from RCSB Protein Data Bank (PDB ID: 3QE5), where C3 was extracted. Specs library were used as a screening library. Firstly, all compounds were dealt with the Wash module in MOE, then all compounds were ranked according to London dG scoring with high throughput rigid docking, and top 10 K compounds were enrolled in the flexible docking with the aid of “induced fit” method. Next, the force field in AMBER12: EHT as well as implicit solvation model in Reaction Field (R-field) were utilized before docking. We optimized the protonation and hydrogen state of the C3 segment with LigX module and set the situation at the pH = 7 and temperature = 300 K. At last, flexible docking were conducted where the docked poses were firstly ranked based on London dG scoring, and a force field was refined on the top 10 poses with a rescoring of GBVI/WSA dG. Finally the top 99 hits of Specs were identified.

qRT-PCR For gene expression

qRT-PCR was utilized to quantify the gene expression level according to manufactures' instruction. Reaction mixture at the volume of 20 μ L were depicted below: 10 μ L SYBR green PCR master mix (Hieff® qPCR SYBR® Green Master Mix, Yeasen Biotech CO., Ltd, China), 0.8 μ L for forward and reverse primers, 2 μ L template DNA and 6.4 μ L DEPC water. Real-time PCR were designated as follows: 95 °C for 300s, 40 repeats of 95 °C for 10 s, 60 °C for 20 s and 72 °C for 20 s. Sequences of primes were presented in Table S1.

Biofilm removal assays

The effects of D25 on *S. mutans* biofilms at distinct conditions were assessed. In brief, *S. mutans* were incubated anaerobically to form biofilms. After 4, 8, 16 and 24 h, the medium were aspirated and the biofilms were treated with D25 at the concentration of 6.25 μ g/mL and incubated for another 24 h. Thereafter, the biofilms were fixed and measured biomass using crystal violet staining as described above. Meanwhile, mature biofilms formed after 24 h were treated with D25 at the concentration of 1.56 to 25 μ g/mL as detailed in the text. The biomass was also evaluated by crystal violet staining as mentioned before. Statistical analysis of all data were performed with GraphPad Prism 7 (GraphPad Software, San Diego, USA) using Student's two-sample t-test. Significant differences were set at the 95% confident level.

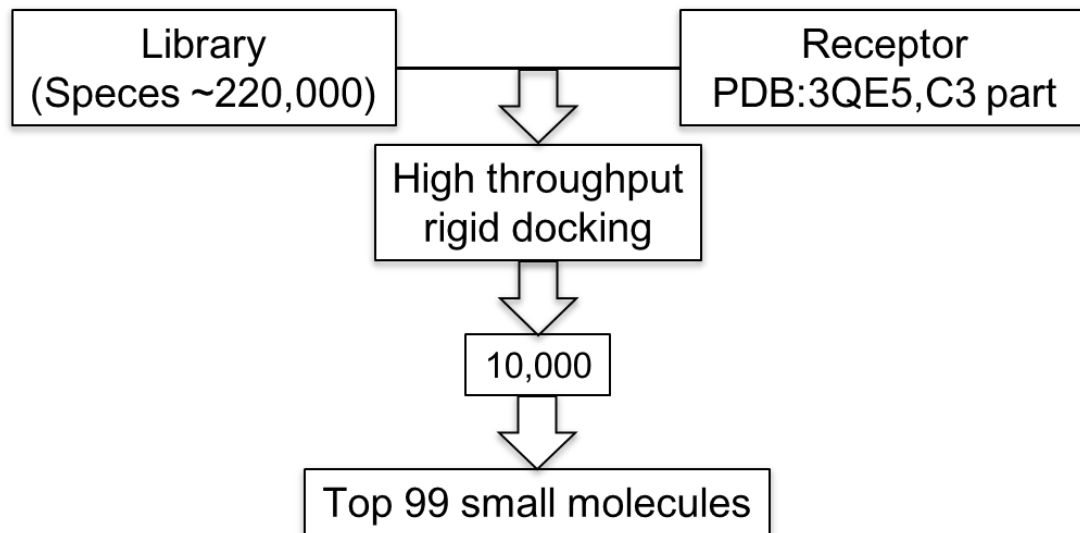


Figure S1 The flowchart of structure-based virtual screening.

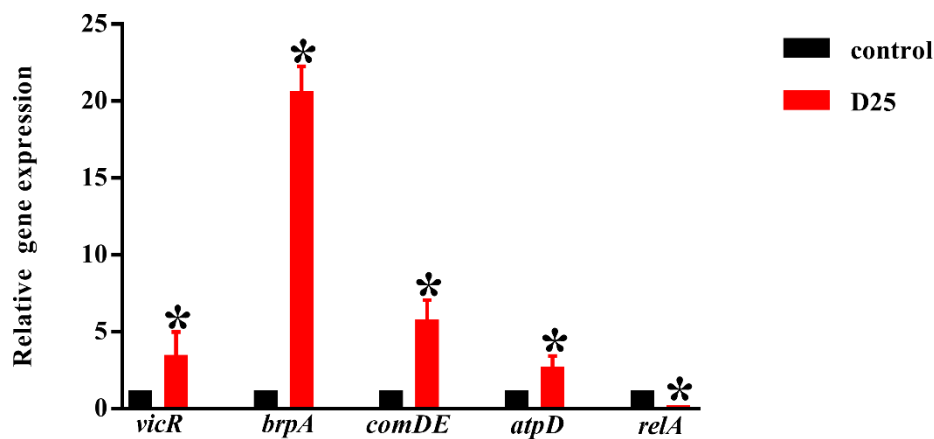


Figure S2 Effects of compound D25 on the expression of *vicR*, *brpA*, *comDE*, *atpD* and *relA* genes. The results from qRT-PCR were obtained with the $2^{-\Delta\Delta CT}$ method. All results were shown as mean $\pm$ standard deviations from three independent experiments. *, differences were statistically significant when compared with control group ($P < 0.05$).

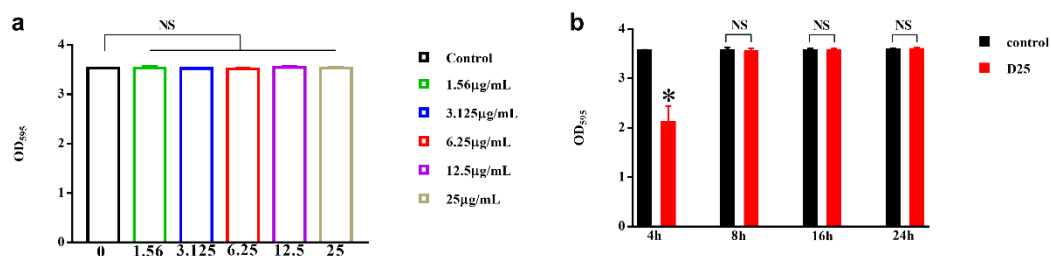


Figure S3 Effects of D25 on biofilms at different concentrations and time intervals. **a** *S. mutans* biofilms formed after 24 h were treated with different concentrations of D25. NS, differences were not statistically

significant when compared with control group. **b** Effects of D25 on *S. mutans* biofilms formed after 4, 8, 16 and 24 h. *, differences were statistically significant when compared with control group ($P < 0.05$). NS, differences were not statistically significant when compared with control group.

Table S1 Primers used in this study for qRT-PCR to quantify gene expression level.

Primes	Nucleotide Sequence (5'-3')	References
16s RNA-F	5'-CTTACCAGGTCTTGACATCCCG-3'	(Li et al. 2018)
16s RNA-R	5'-ACCCAACATCTCACGACACGAG-3'	
<i>srtA</i> -F	5'-GAAGCTTCCTGTAATTGGCG-3'	(Li et al. 2018)
<i>srtA</i> -R	5'-TTCATCGTTCCAGCACCATA-3'	
<i>pacR</i> -F	5'-GACTTTGGTAATGGTTATGCATCAA-3'	(Li et al. 2018)
<i>pacR</i> -R	5'-TTTGTATCAGCCGGATCAAGTG-3'	
<i>vicR</i> -F	5'-TGACACGATTACAGCCTTTGATG-3'	(He et al. 2019)
<i>vicR</i> -R	5'-CGTCTAGTTCTGGTAACATTAAGTCCAATA-3'	
<i>brpA</i> -F	5'-GGAGGAGCTGCATCAGGATTC-3'	(Zhao et al. 2020)
<i>brpA</i> -R	5'-AACTCCAGCACATCCAGCAAG-3'	
<i>comDE</i> -F	5'-ACAATTCCTTGAGTTCCATCCAAG-3'	(He et al. 2019)
<i>comDE</i> -R	5'-TGGTCTGCTGCCTGTTGC-3'	
<i>atpD</i> -F	5'-TGTTGATGGTCTGGGTGAAA-3'	(Xu et al. 2011)
<i>atpD</i> -R	5'-TTTGACGGTCTCCGATAACC-3'	
<i>relA</i> -F	5'-ACAAAAAGGGTATCGTCCGTACAT-3'	(He et al. 2019)
<i>relA</i> -R	5'-AATCACGCTTGGTATTGCTAATTG-3'	

Table S2 Top 99 small molecules selected by targeting C3 segment.

Name (Database ID)	Docking Score	accessible ^a	dissolved in DMSO ^b	code in this study ^c
AJ-030/12105382	-9.0334539			
AG-205/33005040	-8.7709694			
AO-763/14815004	-8.4502993	No		
AC-776/15493088	-8.4416676			

AK-968/12973943	-8.1781998		
AN-329/40922913	-8.1487646	Yes	D13
AN-329/40869136	-8.1423845	Yes	D14
AQ-405/42300267	-8.0910368	Yes	D25
AE-641/00366010	-8.0623646	Yes	D6
AG-205/33005003	-7.9195437	Yes	D1
AG-690/36946065	-7.7946362	Yes	D4
AO-295/15484020	-7.7111669	Yes	D12
AN-329/11378358	-7.7030687		
AH-487/40936667	-7.6935005	Yes	D10
AG-690/36760011	-7.4458237	No	
AP-828/40969990	-7.4327574		
AF-399/42017858	-7.3187318	Yes	D2
AK-968/41172133	-7.2762218	Yes	D3
AK-968/40337278	-7.2611494	Yes	D5
AK-968/41924813	-7.2485061		
AN-988/40787335	-7.2341418	Yes	D21
AJ-292/14241003	-7.1575971	Yes	D9
AG-690/33095035	-7.1439466		
AN-329/12244630	-7.1276588	Yes	D7
AK-968/15603038	-7.1146321		
AN-329/40614587	-7.0713944		
AG-690/13701210	-7.0665359		
AE-848/37236068	-7.0612454	Yes	D8
AG-690/33095033	-7.0505381		
AK-968/13029369	-7.0412726	Yes	D11
AN-329/11140252	-7.0232868		
AP-263/41946485	-7.0181203	No	
AO-022/43452516	-7.0050678	Yes	D31

AN-989/14465007	-6.9879174		
AN-989/15133071	-6.9779348		
AO-081/15570099	-6.9649482	Yes	D24
AG-670/41897787	-6.9485807	Yes	D27
AF-399/14944030	-6.9389381		
AN-329/12393005	-6.9308062	Yes	D19
AG-690/11241412	-6.9258189		
AK-968/37202032	-6.9115748		
AP-906/41639626	-6.8927941	Yes	D20
AM-814/41093933	-6.8766088	Yes	D15
AN-648/41666225	-6.8744097		
AK-918/41676778	-6.8597665		
AK-968/41021583	-6.8533864	Yes	D32
AG-205/32366022	-6.8522239	Yes	D29
AO-861/15351016	-6.842351	Yes	D22
AG-690/32517015	-6.8387337	Yes	D16
AJ-292/41944536	-6.8350224	Yes	D17
AN-655/14614055	-6.8202996		
AK-968/41926615	-6.809855		
AG-205/36953353	-6.793582	Yes	D23
AN-023/14771025	-6.7817311		
AE-842/31981057	-6.7753167	Yes	D26
AG-690/12889320	-6.7213387		
AS-871/43475574	-6.7142501		
AO-801/41077351	-6.7012362	Yes	D28
AN-655/13943034	-6.6954532	Yes	D18
AK-968/37156054	-6.6903319	Yes	D30
AK-968/41025568	-6.688437	Yes	D33
AP-006/41010231	-6.6830568		

AN-329/41434969	-6.6671605	Yes	D34
AK-968/11565149	-6.6249909		
AN-465/13570104	-6.6206021	Yes	D35
AF-962/32159049	-6.6044345		
AG-650/41069277	-6.6000171		
AK-777/11761035	-6.595283		
AP-653/41544882	-6.5851698		
AH-487/40716398	-6.580194	Yes	D36
AO-081/41715312	-6.5782266	Yes	D37
AT-057/43485784	-6.5480027	Yes	D38
AK-918/43446363	-6.5438714	Yes	D39
AN-584/43462284	-6.540699	No	
AE-848/42434927	-6.5384293	Yes	D40
AO-081/14336112	-6.5314841	Yes	D41
AG-690/40135004	-6.5241876		
AK-968/41923827	-6.5086575	Yes	D52
AS-871/43478157	-6.4983797	Yes	D55
AP-406/41885719	-6.4979715		
AG-690/11085024	-6.4913282	Yes	D42
AP-906/41647557	-6.4867191	Yes	D43
AK-968/15361557	-6.4785218	Yes	D44
AM-807/14146116	-6.468154	Yes	D45
AN-698/14990012	-6.4593225		
AO-022/43454978	-6.4592953		
AN-689/41741199	-6.456862		
AN-652/13064001	-6.4533682	Yes	D46
AN-919/40736967	-6.4520273	Yes	D47
AK-968/12385565	-6.444633	Yes	D48
AK-968/41924318	-6.4379086	Yes	D49

AG-670/36062016	-6.4365873		Yes	D50
AM-900/12897042	-6.4347148			
AN-465/43369895	-6.431859		Yes	D51
AH-034/32464023	-6.42485	No		
AK-820/13220141	-6.4085131			
AE-641/40789605	-6.4008918			
AK-968/41169804	-6.3960643		Yes	D54
AO-022/43453830	-6.3873978		Yes	D53

a: Small molecules were able to be purchased from Specs company with enough mass used in further study.

b: Small molecules were able to dissolved in DMSO to get 10 mg/mL stocking solution.

c: Small molecules were presented as specific codes in this article.

Reference

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- Xu X, Zhou XD, and Wu CD (2011) The tea catechin epigallocatechin gallate suppresses cariogenic virulence factors of *Streptococcus mutans*. Antimicrob Agents Ch 55:1229-1236. doi:10.1128/AAC.01016-10
- Zhao M, Qu Y, Liu J, Mai S, and Gu L (2020) A universal adhesive incorporating antimicrobial peptide nisin: Effects on *Streptococcus mutans* and saliva-derived multispecies biofilms. Odontology 108:376-385. doi:10.1007/s10266-019-00478-8