# 1 (C 1)

H L , *, 1, J , 1, , F , M z / H ,
H , F H

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A , .
H , fl , , .
C 1 A
, *Apis cerana*. fl (λ = 332 ) C 1
fi . D , $\Delta H > 0$, $\Delta S > 0$,
C 1 .
C 1 fl fl C 1 ,
. M -
.
C (CD) α- C 1
C 1 . I , C 1 fl β-
50% ($K_A$) 0.28−2.53 /

N , , 15 . A - - , 16 , fi . F , (AO ) $LD_{50}$ 72 69.68 / 17 . fi , , , ( ) 18 . , fl 19,20 . C , - *CSP8* , *Plutella xylostella*, 21 , *CSP1* *CSP2* , *Bombyx mori*, 22 , *CSP1* fl , *Bemisia tabaci*, 23 . C - , C , . A , *Apis cerana*, fl , 24 . I , C 1 , fi β- . I C 1 *A. cerana* 25 . A , , 26–28 , , - fl , , fi - C 1 C 1 . , *A. cerana*, .

## 2. Materials and methods

### 2.1. Chemicals and reagents

I (>97% ) ( F .1(A)) β- (>98% ) CI . J , ( , A) $1.0 \times 10^{-3}$ $L^{-1}$ , 4 °C . M (18.2 MΩ, M , )

### 2.2. Preparation of recombinant CSP1 protein

*CSP1* fi - C , MD18- 29 . *CSP1* E -32 (+) BL21 (DE3) C 1 . A 1 $L^{-1}$ I G, C 1 - fi fi I ™ $N^{2+}$-N A , z B ( H 7.4) 72 4 °C C 1 . fi C 1 B , fi 1 μ $L^{-1}$, −20 °C .

### 2.3. Multiple fluorescence spectra of CSP1 protein with imidacloprid

(1) F . F F-5301 fl ( z , J ). fl - 281 , fl 5 , 290–500 . C 1 1 μ $L^{-1}$ B ( H 7.4), z 1 . C 1 1 $L^{-1}$ , fl fl . 284 K 294 K, .

(2) fl ( F) . F C 1 Δλ (λ - λ ) = 15 60 , . - C 1 .

(3) ( ) . =1800 ( z , J ). C 1 C 1 190–400 .

### 2.4. Circular dichroism (CD) spectra

C 1 , CD 200–250 . 1: 0, 1: 0.5 1: 4, . C 1 CD ELCON3 30 .

### 2.5. Molecular docking analysis

z 31 . - C 1 *Mamestra brassicae* C M A6 ( DB , 1 8 ) 32 I -MODEL 33 . 3D NCBI ( : 638014). B M D (M ) 4.2 ( ), C 1 . M D O z M D , 34 .

### 2.6. Functional inhibition of CSP1 by imidacloprid

1 μ $L^{-1}$ C 1 10 $L^{-1}$ β- 2.3-(1) . fl fl

C 1 .
C 1/ (1:0, 1:1, 1:5, 1:9, 1:13 μ $L^{-1}$)
10 $L^{-1}$ β- ,
. β-
C 1 .

## 3. Results and discussion

### 3.1. Expression of recombinant CSP1 protein and fluorescence quenching spectra

E -32 (+)/C 1
BL21 (*DE3*) *E. coli*. A I G, fi
$N^{2+}$-N A , C 1
B . D - AGE
fi
F . 1, L 1 2
E 32-C 1 -
1 $L^{-1}$ I G. L 3 fi C 1
. C 1
28 KD, 95%.
C 1
, F . 2(A), fl
C 1 -
332 , -
. I
fl C 1 . ,

C 1.

### 3.2. Synchronous fluorescence spectra

I -
fl ( )
C 1 , fl
$\Delta\lambda = 15$ 60 , .
B fl -
fl ,
35 . I ,

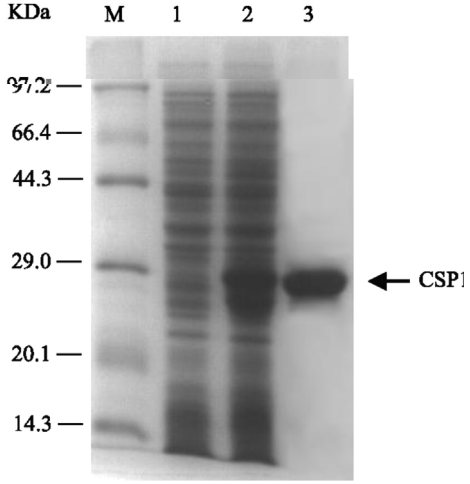


**Fig. 1. SDS-PAGE of induced and purified recombinant CSP1 protein**. M
. L 1 2
E 32-C 1
1 $L^{-1}$ I G, . L 3 fi C 1
, fi .

A F . 2(B C), fl
fi , fl -
C 1 .
, fl
,
I ,
F . 2(C),
36 . I ,
,
(F . 2(B)). -
C 1 -
,
37 , 38 , -
39 , .

### 3.3. Fluorescence quenching mechanism

F
40 . D -
, -
.
fl 41 . D
- 42 :

$$\frac{F_0}{F} = 1 + K_q\tau_o[Q] = 1 + K_{sv}[Q] \quad (1)$$

$F_0$ fl fl
; $F$ fl
fl
$Q$ ; $K_q$ fl ; (
$\tau_0 = 10^{-8}$ ); $K_{sv}$
- 43 . fl
284 294 K , . A
F . 3(A) 1, $K_{sv}$
, -
. -
, ,
44 .
,
, -
45 . A F . 3(B), -
C 1 -
(1 μ $L^{-1}$),
C 1 , fi
. I ,
C 1
-
A 2 *A. cerana* 46 , -
.

### 3.4. Thermodynamic analysis

I ,
,
, -
47 . -
:

$$\Delta G = -RT \quad K = \Delta H - T\Delta S \tag{2}$$

$$\Delta H = \frac{RT_1T_2 \quad (K_{0,2}/K_{0,1})}{T_2 - T_1} \tag{3}$$

$$\Delta S = (\Delta H - \Delta G)/T \tag{4}$$

$\Delta G, \Delta H$ $\Delta S$ G , , . $\Delta H$ <0 $\Delta S$ <0,

. $\Delta H > 0$ $\Delta S > 0$,
. $\Delta H < 0$ $\Delta S > 0$,
48 . -
C 1
1. $\Delta G < 0$, C 1
. $\Delta H > 0$ $\Delta S > 0$,
. -
A 2
46 ,
.

### 3.5. Circular dichroism (CD) spectra

CD
49 .
208 222 CD
α- 50 .
A F . 3(C), C 1
, , , C 1 .
A 2 ,
46 .

### 3.6. Molecular docking

A C M A6 ( DB ID, 1 8 )
*Mamestrabrassicae* 32 , -
C 1 I -MODEL .
C 1 C M A6 (1 8 )
F . 4(B). A M D ( 2),
C 1 -
F . 3(A). B , 8
C 1
, ( 11, 43,
44, I 62), (G 63), 2
(A 9 A 40), (H 47) (F . 3(A)). F
F . 3(A), ( 73
82) C 1,
fl
C 1 fl (F . 3(B)).

**Table 2**
I C 1 .

| | N | E |
|---|---|---|
| | 44 | −24.1035 |
| G | 63 | −16.0482 |
| A | 9 | −9.4608 |
| | 43 | −9.3838 |
| A | 40 | −8.6277 |
| H | 47 | −7.3377 |
| I | 62 | −6.9446 |
| | 11 | −4.0133 |

E :
.

### 3.7. Functional inhibition of CSP1 by imidacloprid

F
C 1 , 10 $L^{-1}$ β-
1 μ $L^{-1}$ C 1
C 1 (
C 1: 1:1, 1:5, 1:9 1:13, ).
281 , fl
fl
332 . $K_A$
L 51 :

$$\frac{F_0 - F}{F} = K_A + n\ [Q] \quad (5)$$

$F_0$ fl fl
; $F$ fl
fl
$Q$ ; $K_A$ ; $n$
. A F . 4(C) 3,

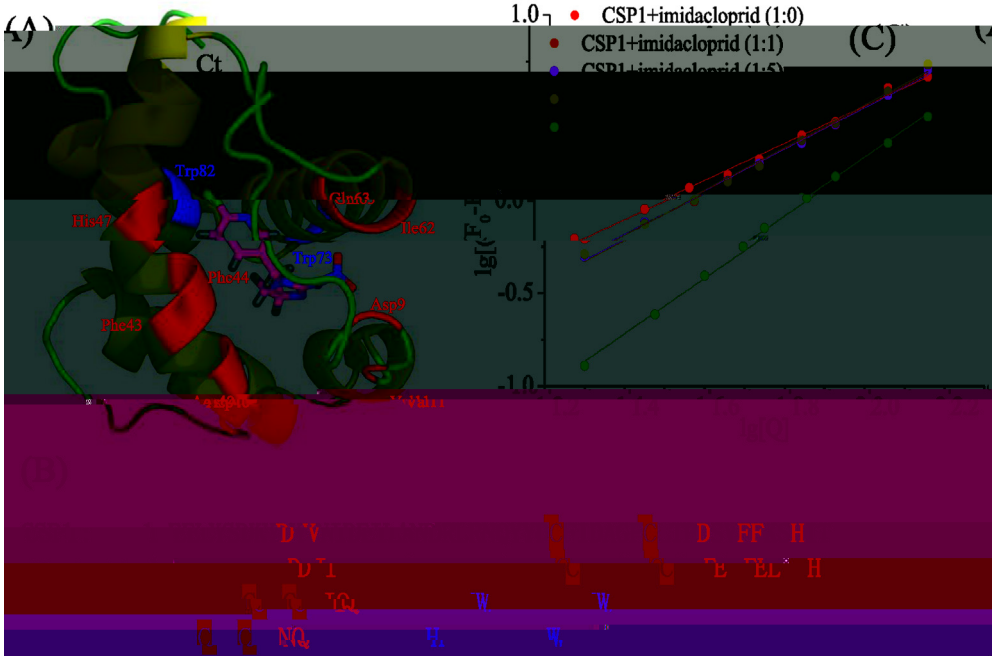


**Fig. 4. Molecular docking of CSP1 with imidacloprid (A) and the Double-Log plots of the fluorescence of the mixture of CSP1 and imidacloprid quenching by β-ionone (C).**
**(A).** I α- N- fl . B
. (B). A C 1 C M A6 (1 8 ). 8 C 1
. 4 . (C). C 1 1 μ $L^{-1}$,
β- 10 $L^{-1}$ (fi 0, 1, 5, 9, 13 μ $L^{-1}$, . fi β- 20, 30, 40, 50, 60, 76, 93, 125
157 μ $L^{-1}$, ). (F fi , .)

$K_A$ (1: 0) β

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z , 1 (*Acer-CSP1*)
C , *Apis cerana cerana* (H : A ), A
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: CON IN, ELCON, CD
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fl E
(*Apis cerana*), I . J. B . M . 56 (2013) 114–121.
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HOB A 1
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