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Metal binding to the surface lipoprotein AdcAI from Streptococcus pyogenes and to the salivary antimicrobial peptide Histatin-5

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Supplementary materials

Supplementary Material 1 Sequence of *adcA* and *adcAII* used in amplification and purification

> *S. pyogenes adcA* (M5005_Spy0543)

```
1   ATGAAAAAGAAAATTCTTTTAATGATGAGTTAATCAGTGTCTTTTTTGCTTGGCAACTT 60
    M K K K I L L M M S L I S V F F A W Q L
61   ACTCAGGCAAAACAAGTCTTAGCAGAGGGTAAAGTGAAGGTGGTGACAACTTTCTATCCT 120
    T Q A K Q V L A E G K V K V V T T F Y P
121  GTTTATGAATTTACAAAAGGGGTATTGGTAATGATGGCGATGTTTTCATGCTTATGAAA 180
    V Y E F T K G V I G N D G D V F M L M K
181  GCAGGAACGGAACCTCATGATTTTGAGCCTTCTACAAAAGACATTAAAAAATCCAAGAT 240
    A G T E P H D F E P S T K D I K K I Q D
241  GCAGATGCATTTGTTTATATGGATGACAATATGGAAACTGGGTTTCTGATGTGAAAAAA 300
    A D A F V Y M D D N M E T W V S D V K K
301  TCATTGACATCTAAAAAAGTGACCATCGTCAAGGGAAGTGGTAACATGCTCTTGGTAGCA 360
    S L T S K K V T I V K G T G N M L L V A
361  GGAGCTGGACATGACCATCACCATGAGGATGCTGACAAAAAGCATGAGCATAATAAACAT 420
    G A G H D H H H E D A D K K H E H N K H
421  AGCGAAGAAGGACACAACCATGCTTTTGACCCACACGTGTGGTTGTCACCATACCGTAGC 480
    S E E G H N H A F D P H V W L S P Y R S
481  ATTACAGTCGTTGAAAATATTCGCGACAGTCTTTCAAAAGCTTACCCAGAAAAAGCAGAG 540
    I T V V E N I R D S L S K A Y P E K A E
541  AACTTCAAAGCCAATGCCGCTACTTATATTGAAAAATTTAAAAGAGCTTGACAAAGACTAT 600
    N F K A N A A T Y I E K L K E L D K D Y
601  ACGGCAGCACTTTTCAGATGCTAAGCAAAAAGAGCTTTGTGACACAACACGCAGCTTTTGGT 660
    T A A L S D A K Q K S F V T Q H A A F G
661  TATATGGCACTTGACTATGGCTTGAACCAAATTTCTATTAATGGTGTACACCAGATGCA 720
    Y M A L D Y G L N Q I S I N G V T P D A
721  GAACCATCAGCAAAAACGTATTGCTACTTTGTCAAAAATACGTTAAAAAATATGGCATCAAA 780
    E P S A K R I A T L S K Y V K K Y G I K
781  TACATTTATTTTGAGGAAAATGCGTCAAGTAAAGTCGCAAAAACCCCTAGCTAAAGAAGCA 840
    Y I Y F E E N A S S K V A K T L A K E A
841  GGAGTTAAAGCGGCTGTGCTTAGTCCGCTTGAAGGTTTACTGAAAAAGAGATGAAAGCT 900
    G V K A A V L S P L E G L T E K E M K A
901  GGCCAAGATTACTTTACGGTCATGCGTAAAAACCTTGAAACCTTACGCTTAACCACTGAT 960
    G Q D Y F T V M R K N L E T L R L T T D
961  GTGGCTGGTAAAGAAAATTTCTTCCAGAAAAAGACACGACTAAGACAGTTTACAATGGTTAT 1020
    V A G K E I L P E K D T T K T V Y N G Y
1021 TTCAAAGACAAAGAAGTCAAAGATCGTCAATTATCTGACTGGTCAGGTAGCTGGCAATCT 1080
```

F K D K E V K D R Q L S D W S G S W Q S

1081 GTTTACCCCTATCTACAAAATGGTACTTTAGACCAAGTTTGGGACTACAAGGCTAAAAAA 1140
V Y P Y L Q N G T L D Q V W D Y K A K K

1141 TCTAAAGGTAAAATGACAGCAGCCGAGTACAAAAGATTACTACACTACTGGTTATAAAACT 1200
S K G K M T A A E Y K D Y Y T T G Y K T

1201 GACGTGGAACAAATCAAAATCAATGGTAAGAAAAAGACCATGACCTTTGTTCGTAATGGT 1260
D V E Q I K I N G K K K T M T F V R N G

1261 GAAAAGAAAACCTTCACTTACACATACGCCGGCAAAGAAATCTTGACCTATCCAAAAGGA 1320
E K K T F T Y T Y A G K E I L T Y P K G

1321 AATCGCGGGTTTCGTTTTCATGTTTGAAGCTAAAAGAAGCAGATGCTGGCGAATTCAAATAC 1380
N R G V R F M F E A K E A D A G E F K Y

1381 GTTCAATTCAGTGACCATGCCATTGCTCCTGAAAAAGCAAAGCATTTCACCTGTACTGG 1440
V Q F S D **H** A I A P E K A K **H** F **H** L Y W

1441 GGTGGTGACAGCCAAGAAAAATTACATAAAGAGTTAGAACATTGGCCAACTTACTACGGT 1500
G G D S Q E K L H K E L E H W P T Y Y G

1501 TCAGACTTATCTGGTTCGTGAAATCGCCCAAGAAATCAATGCTCATTAA
S D L S G R E I A Q E I N A H *

> *S pyogenes adcAll* (M5005_Spy_1711)

1 **ATGAAAAAAGGTTTTTTTTCTCATGGCTATGGTCGTGAGTTTAGTAATGATAGCAGGGTGT** 60
M K K G F F L M A M V V S L V M I A G C

61 **GATAAGTCAGCAAACCCCAAACAGCCT**ACGCAAGGCATGTCAGTTGTAACCAGCTTTTAC 120
D K S A N P K Q P T Q G M S V V T S F Y

121 CCAATGTATGCGATGACAAAAGAAGTATCTGGAGACCTCAATGATGTGAGGATGATCCAA 180
P M Y A M T K E V S G D L N D V R M I Q

181 TCAGGTGCAGGCATTCATTCCTTTGAACCGTCTGTAAATGATGTGGCAGCTATTTATGAC 240
S G A G I **H** S F E P S V N D V A A I Y D

241 GCGGATTTGTTTGTTTACCATTACATACCTTAGAAGCTTGGGCAAGGGATCTAGACCCT 300
A D L F V Y H S H T L E A W A R D L D P

301 AATTTAAAAAATCAAAGGTTGATGTGTTTGAAGCGTCAAAACCTTTGACACTAGATAGA 360
N L K K S K V D V F E A S K P L T L D R

361 GTCAAAGGGCTAGAAGATATGGAAGTCACACAAGGCATTGATCCTGCGACACTTTATGAC 420
V K G L E D M E V T Q G I D P A T L Y D

421 CCACATACCTGGACAGATCCCGTTTTAGCTGGTGAGGAAGCTGTTAATATCGCTAAAGAG 480
P **H** T W T D P V L A G E E A V N I A K E

481 CTAGGACGTTTGGATCCTAAACACAAAGACAGTTACACTAAAAATGCTAAGGCTTTCAA 540
L G R L D P K H K D S Y T K N A K A F K

541 AAAGAAGCAGAGCAACTAACTGAAGAATACACTCAAAAATTTAAAAAGGTGCGCTCAAAA 600
K E A E Q L T E E Y T Q K F K K V R S K

601 ACATTCGTGACGCAGCACACGGCATTTCCTTATCTGGCTAAACGATTCGGCTTGAAACAA 660
T F V T Q **H** T A F S Y L A K R F G L K Q

661 CTTGGTATCTCGGGCATTTCCTCCAGAGCAAGAGCCCTCTCCTCGCCAATTGAAAGAAATT 720
L G I S G I S P E Q E P S P R Q L K E I

721 CAAGACTTTGTTAAAGAATACAACGTCAAGACTATTTTTGCAGAAGACAATGTCAATCCC 780
Q D F V K E Y N V K T I F A E D N V N P

781 AAAATTGCTCATGCTATTGCGAAATCAACAGGAGCTAAAGTAAAGACATTAAGTCCACTT 840
K I A H A I A K S T G A K V K T L S P L

841 GAAGCTGCTCCAAGCGGAAACAAGACATATCTAGAAAAATCTTAGAGCAAATTTGGAAGTG 900
E A A P S G N K T Y L E N L R A N L E V

901 CTCTATCAACAGTTGAAGTAA
L Y Q Q L K *

> Predicted signal peptide

> Predicted metal binding site

> Sequence excluded in cloning

Supplementary Material 2 Dynafit script of competition assay. Ligand and protein competing 1:1

```
; L = PAR
; P = HST
; M = Cu

[task]
task = fit
data = equilibria

[mechanism]
M + L <==> ML          : Kd1  dissociation
M + P <==> MP          : Kd2  dissociation

[concentrations] micromolar
L = 10
P = 200

[constants] micromolar
Kd1 = 1.885e-7
Kd2 = 1e-6 ?

[responses] ΔAbs420nm
L = 0.02 ?

[data]
variable      M
set           20HST_1PAR420nm

[output]
directory ./20HST_1PAR420nm/

[set:20HST_1PAR420nm]
M      ΔAbs420nm
0      0.252
50     0.145
100    0.073
150    0.039
190    0.013
200    0.003
210    0.001
220    0.001
250    0
0      0.251
20     0.199
40     0.163
60     0.119
80     0.056
100    0.048
120    0.037
150    0.004
180    -0.002
200    0.001
220    0
250    0

[end]
```

Supplementary material 3 Dynafit script of competition assay. Ligand and protein competing 1:2

```
; Q = Quin2
; P = AdcA
; M = Zn

[task]
task = fit
data = equilibria

[mechanism]
M + Q <==> MQ          : Kd1  dissociation
M + P <==> MP          : Kd2  dissociation
M + MP <==> MMP        : Kd3  dissociation

[concentrations] micromolar
Q = 7
P = 10

[constants] micromolar
Kd1 = 3.7e-6
Kd2 = 1e-7?
Kd3 = 0.00069 ?

[responses] ΔAbs260nm
Q = 0.0255 ?

[data]
variable      M
set           Quin2-AdcA

[output]
directory ./Quin2-AdcA/

[set:Quin2-AdcA]
M      ΔAbs260
0      1
6      0.87730061
8      0.82208589
9      0.81595092
10     0.54601227
12     0.34969325
14     0.15337423
15     -0.0245399
16     0.00613497
18     -0.0613497
20     -0.0429448
22     -0.0490798
25     -0.0245399
30     0
0      1
2      0.98219414
4      0.8684664
6      0.82998277
8      0.82998277
10     0.70936244
12     0.59678346
14     0.38368754
16     0.13612866
18     0.06777714
```

20	0.06662837
22	0.03044227
25	0
0	1
2	0.9884859
4	0.86470927
6	0.92976396
8	0.83822683
10	0.74553828
12	0.60967185
14	0.39205527
16	0.13356362
18	0.0662061
20	0.07426598
22	0.03914796
25	0

[end]