Supplementary Information

Identification and molecular mechanism of antithrombotic peptides from *Crassostrea gigas* proteins released in simulated gastro-intestinal digestion

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Running title: The antithrombotic mechanism of peptides from oyster

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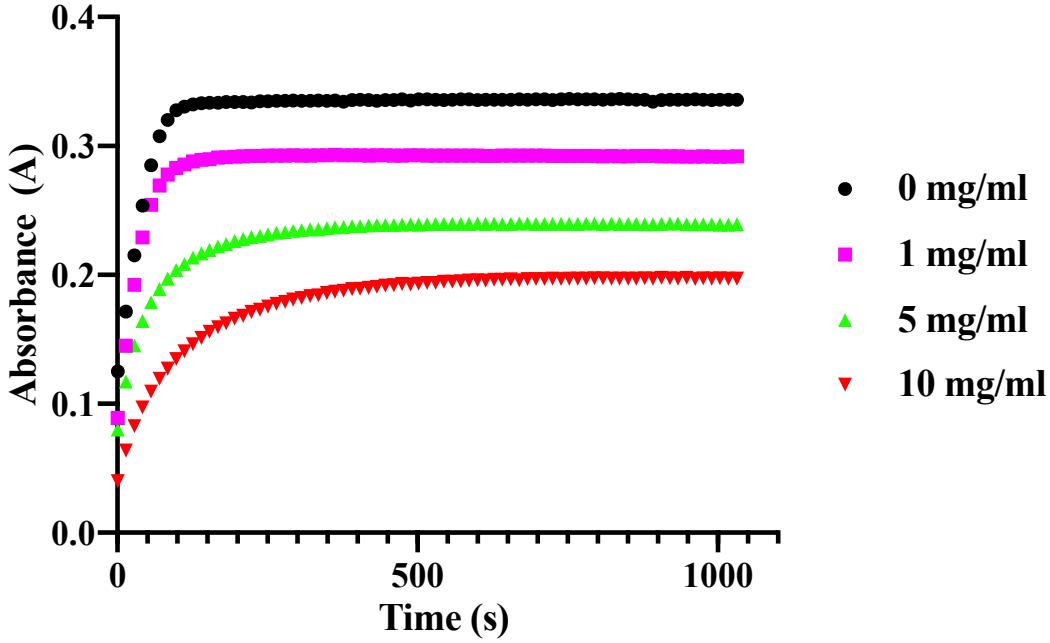
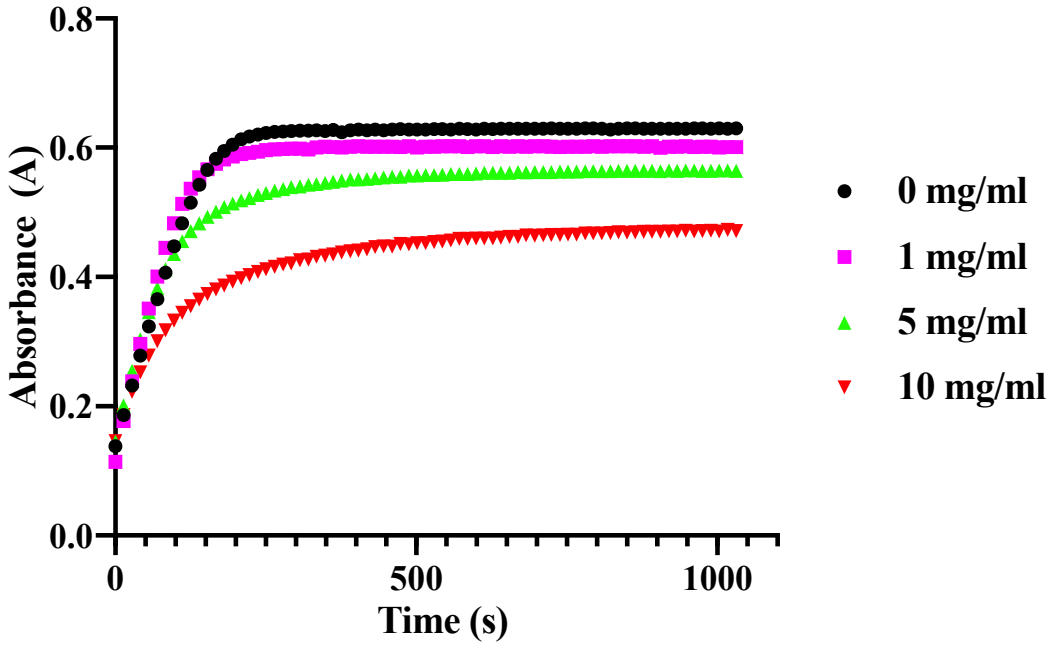
Figure S1 Fluorescence generation of S2238 hydrolyzed by thrombin with and without peptiedes in SID 4 h. (a)-(d), S2238 substrate concentration are 0.375 mM, 0.75 mM, 1.5 mM and 2 mM, against with thrombin of 5 U/ml. Black line, garnet line, green line and red line are the curve influenced by peptides concentraion in 0 mg/ml, 1 mg/ml, 5mg/ml and 10 mg/ml.

Figure S2 . The b, y ions of potential antithrombotic peptides matched by Macot engine.

Figure S1

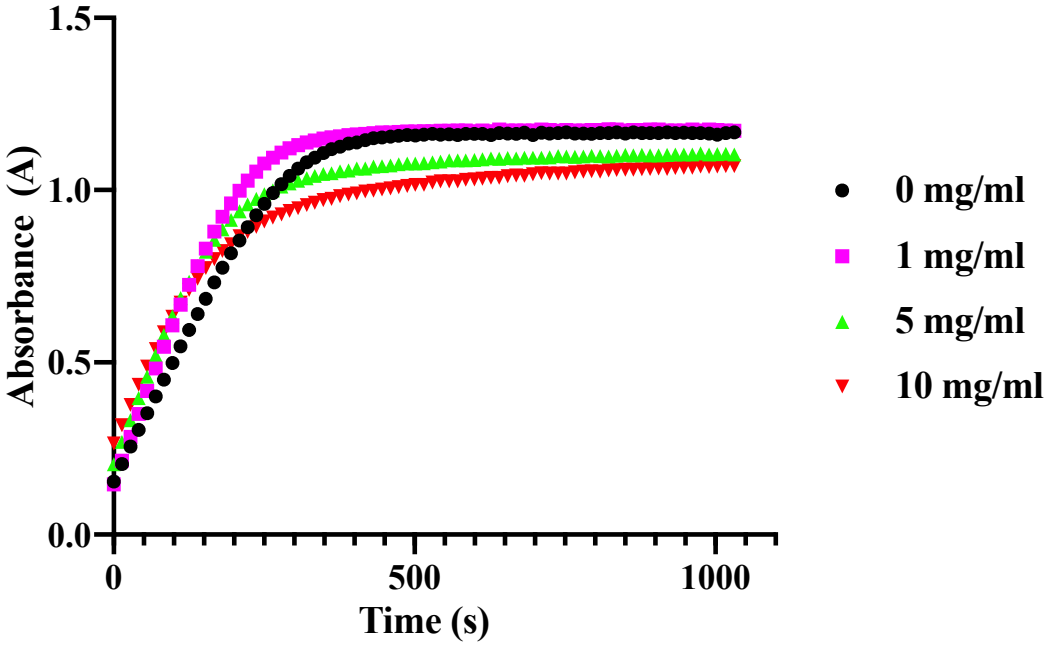

0.4
0.3
0.2
0.1
0.0
Absorbance (A)
0
500
1000
Time (s)
0 mg/ml
1 mg/ml
5 mg/ml
10 mg/ml

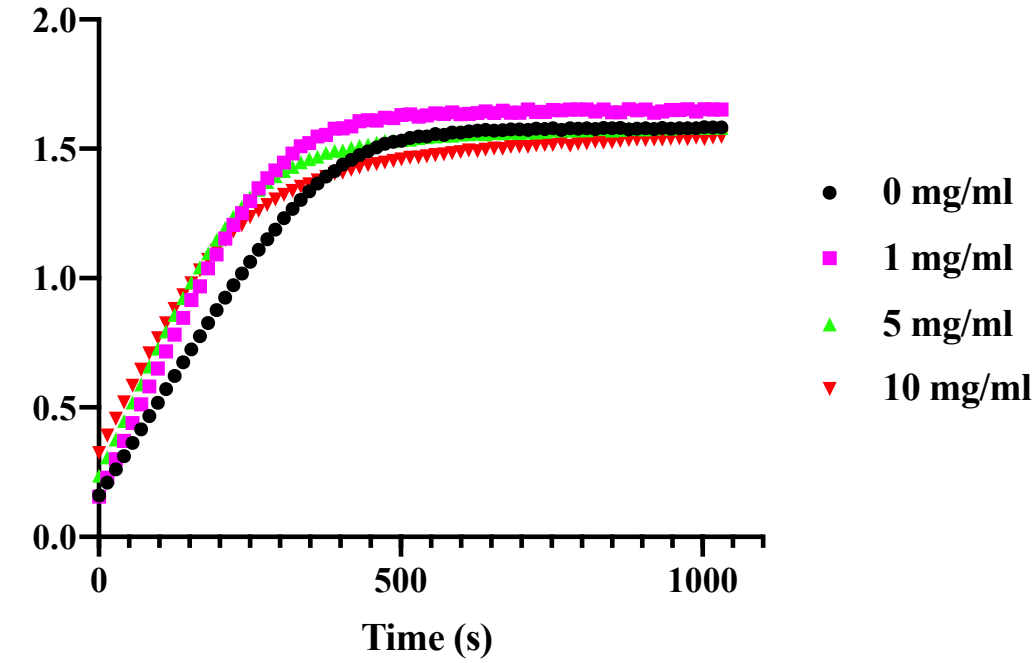

0.8
0.6
0.4
0.2
0.0
Absorbance (A)
0
500
1000
Time (s)
0 mg/ml
1 mg/ml
5 mg/ml
10 mg/ml

(a)

(b)

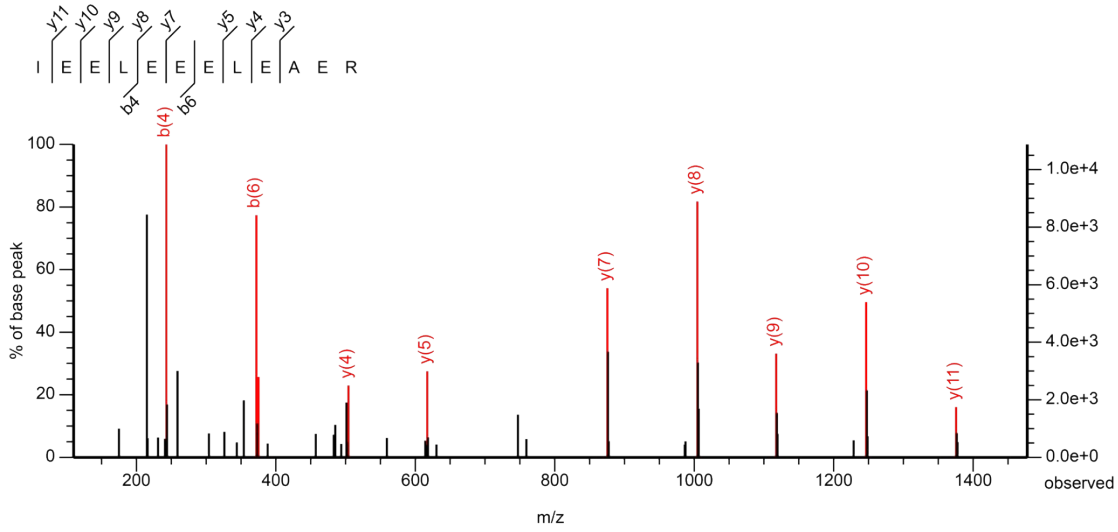
(c)


1.5
1.0
0.5
0.0
Absorbance (A)
0
500
1000
Time (s)
0 mg/ml
1 mg/ml
5 mg/ml
10 mg/ml

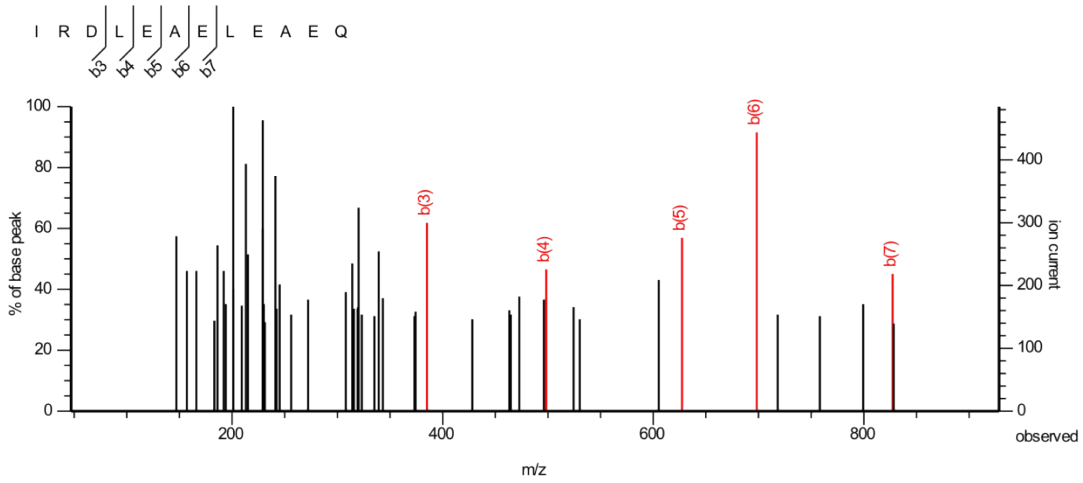

2.0
1.5
1.0
0.5
0.0
0
500
1000
Time (s)
0 mg/ml
1 mg/ml
5 mg/ml
10 mg/ml

(d)

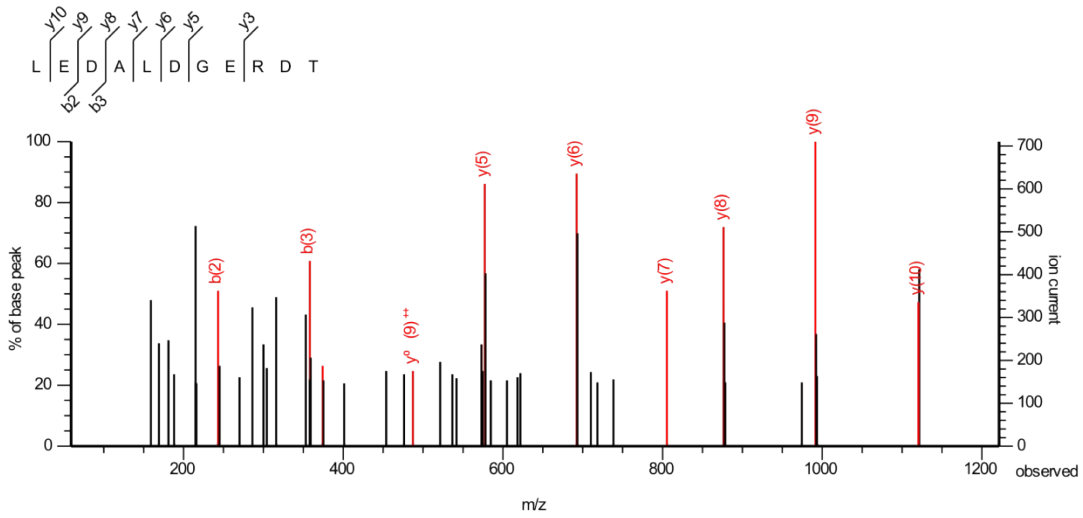
(a)


y11 y10 y9 y8 y7 y5 y4 y3
I E E L E E E L E A E R
b4 b6
b(4) b(6) y(4) y(5) y(7) y(8) y(9) y(10) y(11)
% of base peak
m/z
observed
100 80 60 40 20 0
200 400 600 800 1000 1200 1400
1.0e+4 8.0e+3 6.0e+3 4.0e+3 2.0e+3 0.0e+0

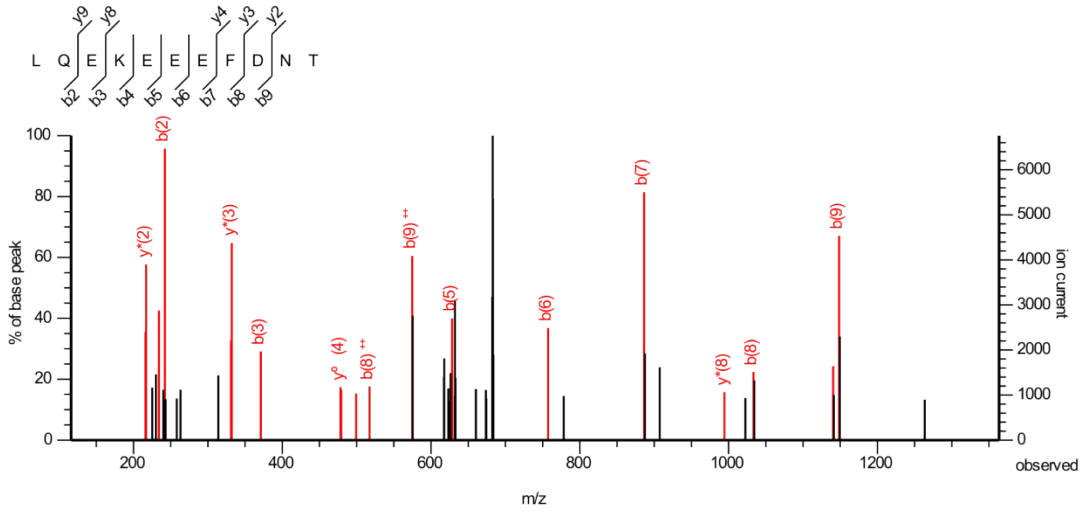
(b)


I R D L E A E L E A E Q
b3 b4 b5 b6 b7
b(3) b(4) b(5) b(6) b(7)
% of base peak
m/z
observed
ion current
100 80 60 40 20 0
200 400 600 800
400 300 200 100 0

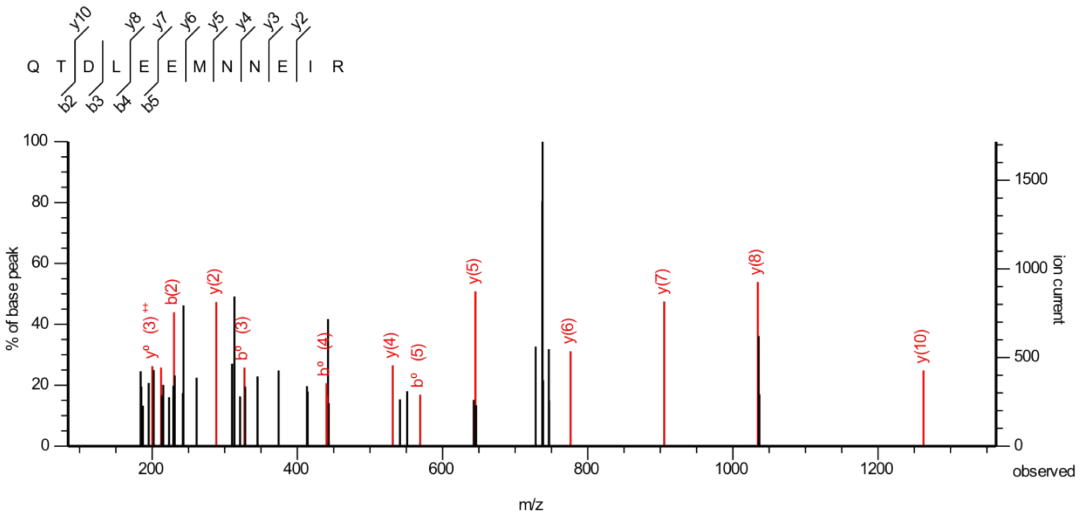
(c)


L E D A L D G E R D T
% of base peak
ion current
m/z
observed

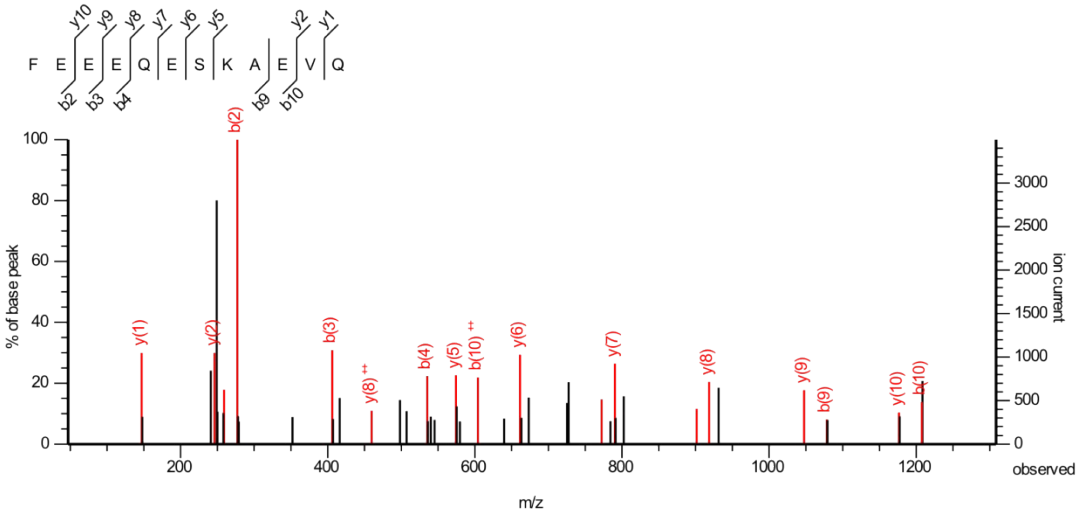
(d)


L Q E K E E E F D N T
% of base peak
ion current
m/z
observed

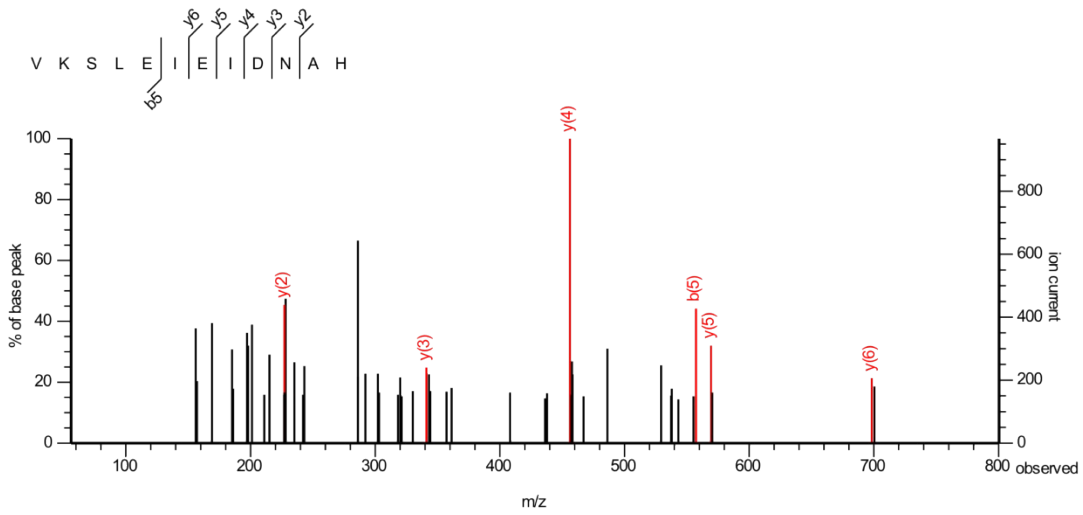
(e)


Q T D L E E M N N E I R
y10 y8 y7 y6 y5 y4 y3 y2
b2 b3 b4 b5
% of base peak
ion current
m/z
observed
y(2)
b(2)
y(5)
y(6)
y(7)
y(8)
y(10)
y(4)

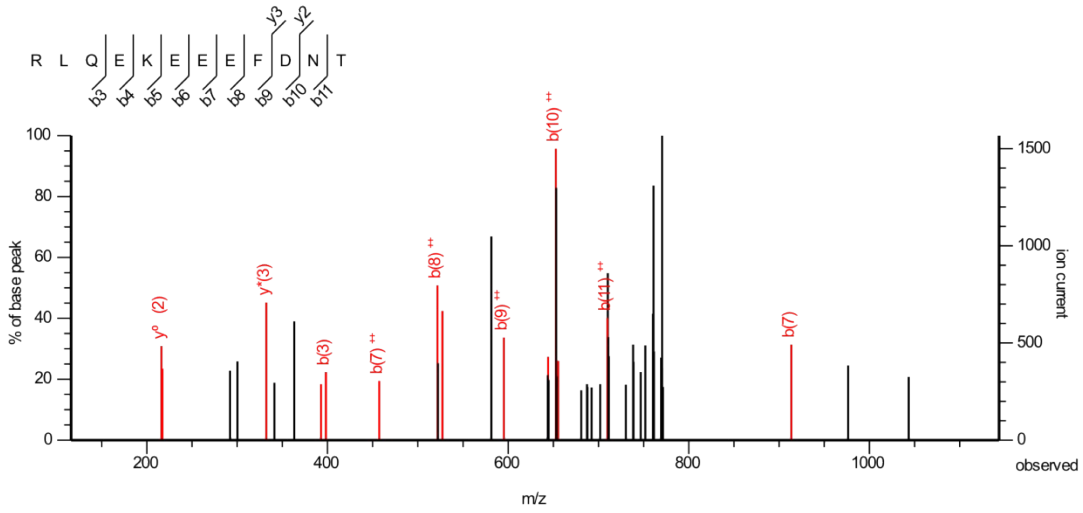
(f)


F E E E Q E S K A E V Q
y10 y9 y8 y7 y6 y5 y2 y1
b2 b3 b4 b9 b10
% of base peak
ion current
m/z
observed
b(2)
y(1)
y(2)
b(3)
b(4)
y(5)
y(6)
y(7)
y(8)
y(9)
b(9)
y(10)
b(10)

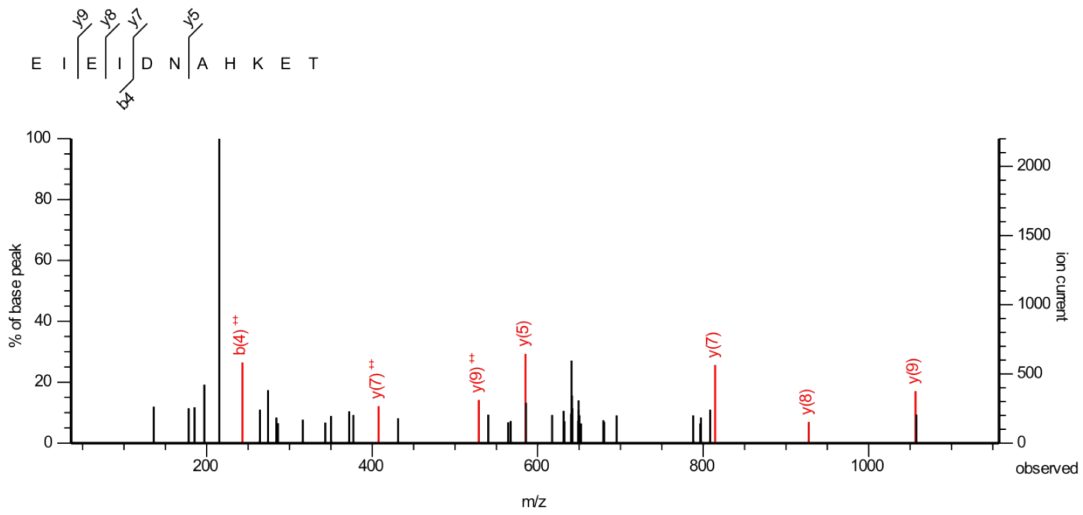
(g)


V K S L E I E I D N A H
b5
y6 y5 y4 y3 y2
% of base peak
ion current
m/z
observed
y(2)
y(3)
y(4)
y(5)
b(5)
y(6)

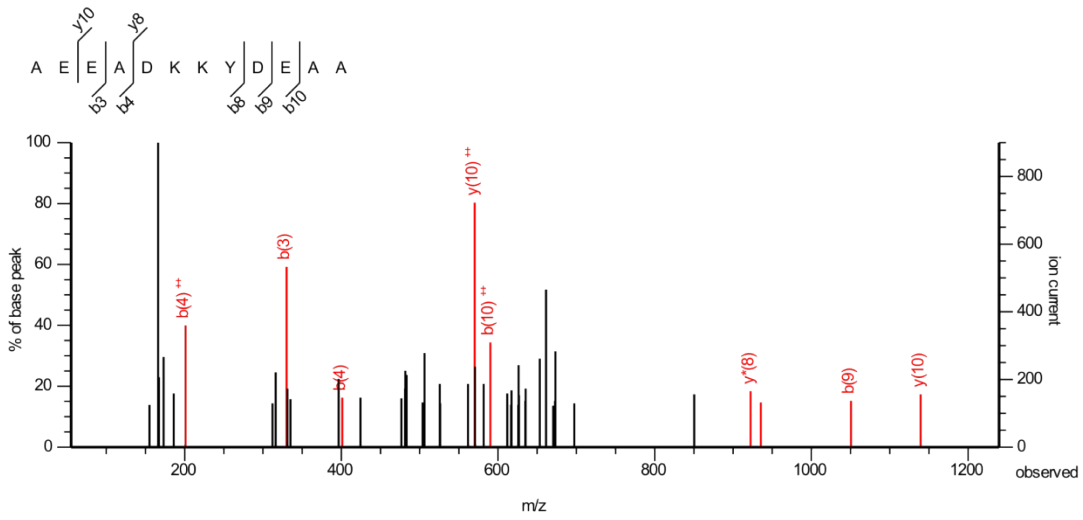
(h)


R L Q E K E E E F D N T
b3 b4 b5 b6 b7 b8 b9 b10 b11
y3 y2
% of base peak
ion current
m/z
observed
y° (2)
y*(3)
b(3)
b(7) ++
b(8) ++
b(9) ++
b(10) ++
b(11) ++
b(7)

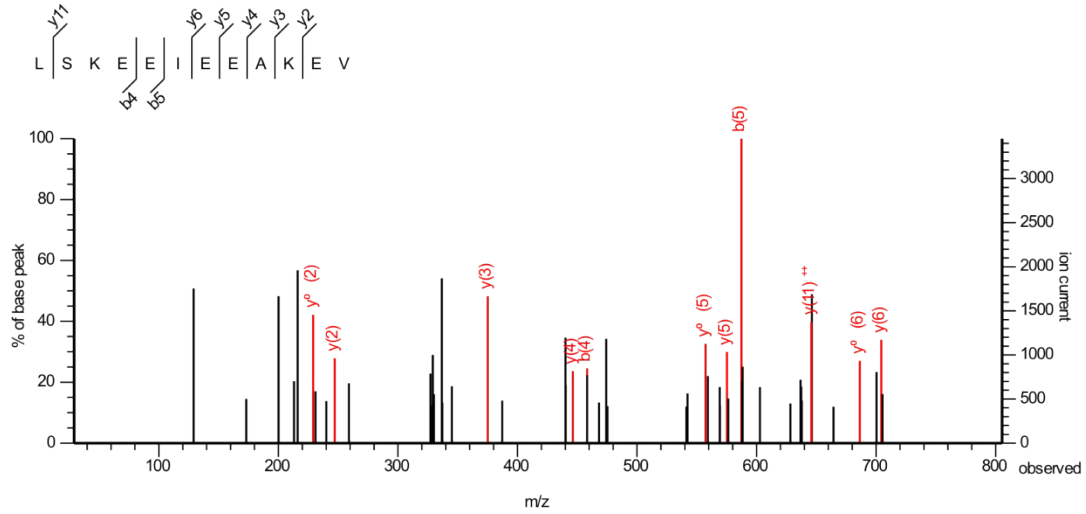
(i)


y9
y8
y7
y5
E I E I D N A H K E T
b4
% of base peak
ion current
m/z
observed
b(4) ++
y(7) ++
y(9) ++
y(5)
y(7)
y(8)
y(9)

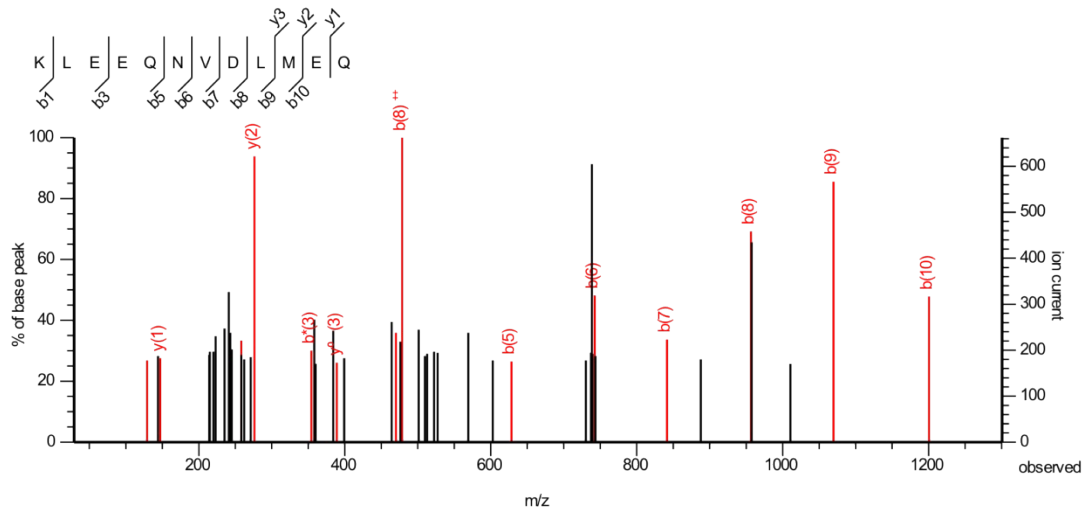
(j)


y10
y8
A E E A D K K Y D E A A
b3
b4
b8
b9
b10
% of base peak
ion current
m/z
observed
b(4) ++
b(3)
b(4)
y(10) ++
b(10) ++
y*(8)
b(9)
y(10)

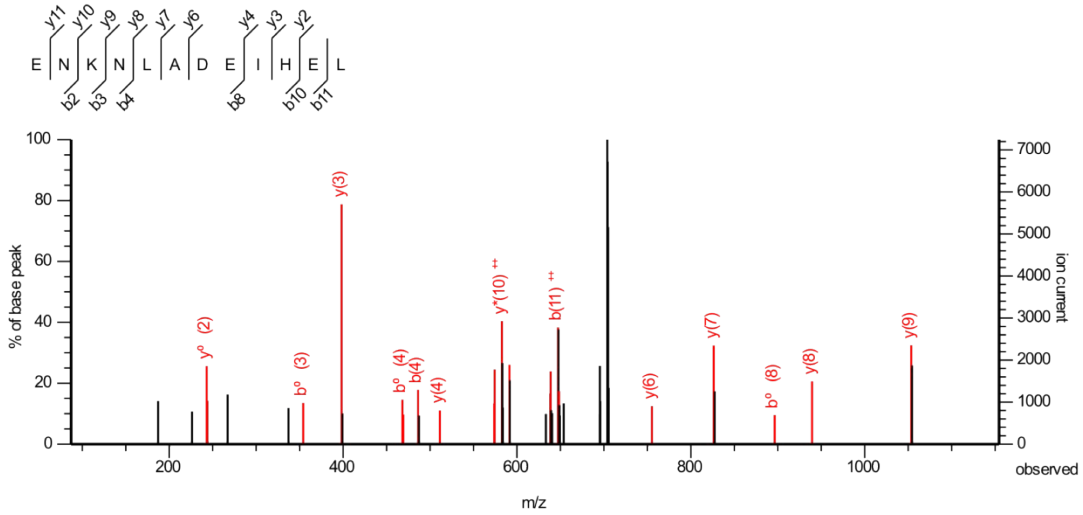
(k)


L S K E E I E E A K E V
% of base peak
ion current
m/z
observed

(l)


K L E E Q N V D L M E Q
% of base peak
ion current
m/z
observed

(m)


E N K N L A D E I H E L
% of base peak
ion current
observed
m/z

(n)

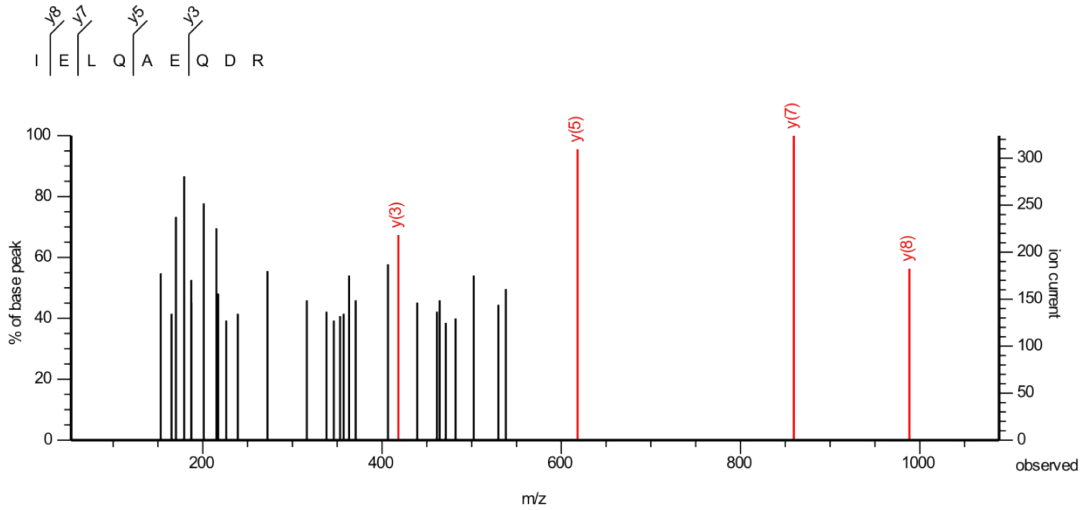

I E L Q A E Q D R
% of base peak
ion current
observed
m/z

Table S1 The non-bond catogory of inteactionsites of LSKEEIEEAKEV against thrombin.

Name	Category	Types	From	From Chemistry	To	To Chemistry
H:LYS36:HZ1 - LSKEEIEEAKEV:O131	Hydrogen Bond;Electrostatic	Salt Bridge; Attractive Charge	H:LYS36:HZ1	H-Donor; Positive	LSKEEIEEAKEV: O131	H-Acceptor; Negative
LSKEEIEEAKEV:H3 - LSKEEIEEAKEV:O67	Hydrogen Bond;Electrostatic	Salt Bridge; Attractive Charge	LSKEEIEEAKEV:H3	H-Donor; Positive	LSKEEIEEAKEV: O67	H-Acceptor; Negative
LSKEEIEEAKEV:H51 - LSKEEIEEAKEV:O82	Hydrogen Bond;Electrostatic	Salt Bridge; Attractive Charge	LSKEEIEEAKEV:H51	H-Donor; Positive	LSKEEIEEAKEV: O82	H-Acceptor; Negative
LSKEEIEEAKEV:H162 - LSKEEIEEAKEV:O197	Hydrogen Bond;Electrostatic	Salt Bridge; Attractive Charge	LSKEEIEEAKEV:H162	H-Donor; Positive	LSKEEIEEAKEV: O197	H-Acceptor; Negative
H:ARG67:NH1 - LSKEEIEEAKEV:O116	Electrostatic	Attractive Charge	H:ARG67:NH1	Positive	LSKEEIEEAKEV: O116	Negative
H:LYS110:NZ - LSKEEIEEAKEV:O178	Electrostatic	Attractive Charge	H:LYS110:NZ	Positive	LSKEEIEEAKEV: O178	Negative
LSKEEIEEAKEV:N49 - LSKEEIEEAKEV:O116	Electrostatic	Attractive Charge	LSKEEIEEAKEV:N49	Positive	LSKEEIEEAKEV: O116	Negative
LSKEEIEEAKEV:N160 - LSKEEIEEAKEV:O178	Electrostatic	Attractive Charge	LSKEEIEEAKEV:N160	Positive	LSKEEIEEAKEV: O178	Negative
H:GLN38:HN - LSKEEIEEAKEV:O66	Hydrogen Bond	Conventional Hydrogen Bond	H:GLN38:HN	H-Donor	LSKEEIEEAKEV: O66	H-Acceptor
H:TYR76:HH - LSKEEIEEAKEV:O165	Hydrogen Bond	Conventional Hydrogen Bond	H:TYR76:HH	H-Donor	LSKEEIEEAKEV: O165	H-Acceptor
H:TYR76:HH - LSKEEIEEAKEV:O196	Hydrogen Bond	Conventional Hydrogen Bond	H:TYR76:HH	H-Donor	LSKEEIEEAKEV: O196	H-Acceptor

H:LYS110:HZ3 - LSKEEIEEAKEV:O177	Hydrogen Bond	Conventional Hydrogen Bond	H:LYS110:HZ3	H-Donor	LSKEEIEEAKEV:O177	H-Acceptor
LSKEEIEEAKEV:H2 - LSKEEIEEAKEV:O54	Hydrogen Bond	Conventional Hydrogen Bond	LSKEEIEEAKEV:H2	H-Donor	LSKEEIEEAKEV:O54	H-Acceptor
LSKEEIEEAKEV:H4 - H:GLN38:O	Hydrogen Bond	Conventional Hydrogen Bond	LSKEEIEEAKEV:H4	H-Donor	H:GLN38:O	H-Acceptor
LSKEEIEEAKEV:H23 - H:GLN38:O	Hydrogen Bond	Conventional Hydrogen Bond	LSKEEIEEAKEV:H23	H-Donor	H:GLN38:O	H-Acceptor
LSKEEIEEAKEV:H50 - LSKEEIEEAKEV:O115	Hydrogen Bond	Conventional Hydrogen Bond	LSKEEIEEAKEV:H50	H-Donor	LSKEEIEEAKEV:O115	H-Acceptor
LSKEEIEEAKEV:H52 - H:GLN38:OE1	Hydrogen Bond	Conventional Hydrogen Bond	LSKEEIEEAKEV:H52	H-Donor	H:GLN38:OE1	H-Acceptor
LSKEEIEEAKEV:H52 - LSKEEIEEAKEV:O81	Hydrogen Bond	Conventional Hydrogen Bond	LSKEEIEEAKEV:H52	H-Donor	LSKEEIEEAKEV:O81	H-Acceptor
LSKEEIEEAKEV:H56 - LSKEEIEEAKEV:O81	Hydrogen Bond	Conventional Hydrogen Bond	LSKEEIEEAKEV:H56	H-Donor	LSKEEIEEAKEV:O81	H-Acceptor
LSKEEIEEAKEV:H105 - H:LYS36:O	Hydrogen Bond	Conventional Hydrogen Bond	LSKEEIEEAKEV:H105	H-Donor	H:LYS36:O	H-Acceptor
LSKEEIEEAKEV:H161 - LSKEEIEEAKEV:O180	Hydrogen Bond	Conventional Hydrogen Bond	LSKEEIEEAKEV:H161	H-Donor	LSKEEIEEAKEV:O180	H-Acceptor
LSKEEIEEAKEV:H161 - LSKEEIEEAKEV:O196	Hydrogen Bond	Conventional Hydrogen Bond	LSKEEIEEAKEV:H161	H-Donor	LSKEEIEEAKEV:O196	H-Acceptor
LSKEEIEEAKEV:H163 - LSKEEIEEAKEV:O180	Hydrogen Bond	Conventional Hydrogen Bond	LSKEEIEEAKEV:H163	H-Donor	LSKEEIEEAKEV:O180	H-Acceptor
H:LYS36:HE2 -	Hydrogen Bond	Carbon Hydrogen	H:LYS36:HE2	H-Donor	LSKEEIEEAKEV:	H-Acceptor

LSKEEIEEAKEV:O118		Bond			O118	
H:LYS36:HE2 - LSKEEIEEAKEV:O130	Hydrogen Bond	Carbon Hydrogen Bond	H:LYS36:HE2	H-Donor	LSKEEIEEAKEV:O130	H-Acceptor
H:LYS110:HE1 - LSKEEIEEAKEV:O177	Hydrogen Bond	Carbon Hydrogen Bond	H:LYS110:HE1	H-Donor	LSKEEIEEAKEV:O177	H-Acceptor
LSKEEIEEAKEV:H28 - H:THR74:O	Hydrogen Bond	Carbon Hydrogen Bond	LSKEEIEEAKEV:H28	H-Donor	H:THR74:O	H-Acceptor
LSKEEIEEAKEV:H48 - H:GLN38:OE1	Hydrogen Bond	Carbon Hydrogen Bond	LSKEEIEEAKEV:H48	H-Donor	H:GLN38:OE1	H-Acceptor
LSKEEIEEAKEV:H48 - LSKEEIEEAKEV:O116	Hydrogen Bond	Carbon Hydrogen Bond	LSKEEIEEAKEV:H48	H-Donor	LSKEEIEEAKEV:O116	H-Acceptor
LSKEEIEEAKEV:H58 - LSKEEIEEAKEV:O66	Hydrogen Bond	Carbon Hydrogen Bond	LSKEEIEEAKEV:H58	H-Donor	LSKEEIEEAKEV:O66	H-Acceptor
LSKEEIEEAKEV:H107 - LSKEEIEEAKEV:O115	Hydrogen Bond	Carbon Hydrogen Bond	LSKEEIEEAKEV:H107	H-Donor	LSKEEIEEAKEV:O115	H-Acceptor
LSKEEIEEAKEV:H147 - H:ILE82:O	Hydrogen Bond	Carbon Hydrogen Bond	LSKEEIEEAKEV:H147	H-Donor	H:ILE82:O	H-Acceptor
LSKEEIEEAKEV:H184 - H:TYR76:OH	Hydrogen Bond	Carbon Hydrogen Bond	LSKEEIEEAKEV:H184	H-Donor	H:TYR76:OH	H-Acceptor