Fig. 1

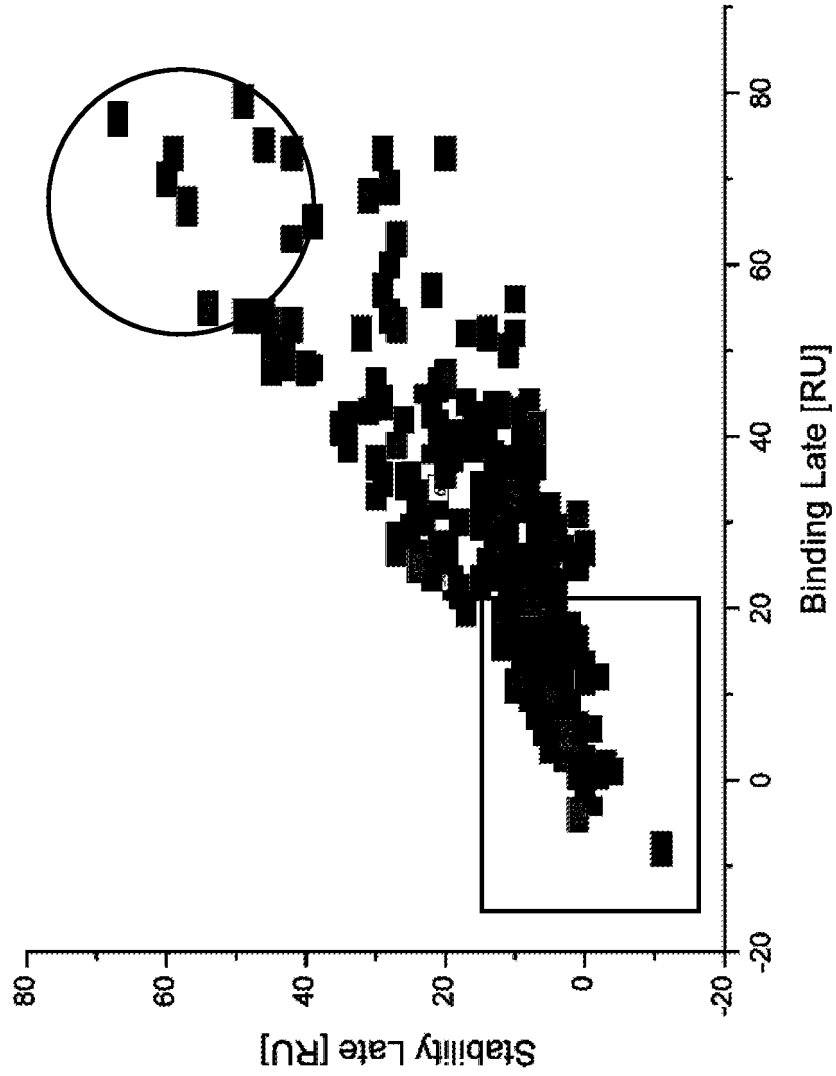

Stability Late [RU]
80
60
40
20
0
-20
Binding Late [RU]
-20
0
20
40
60
80

Fig. 2

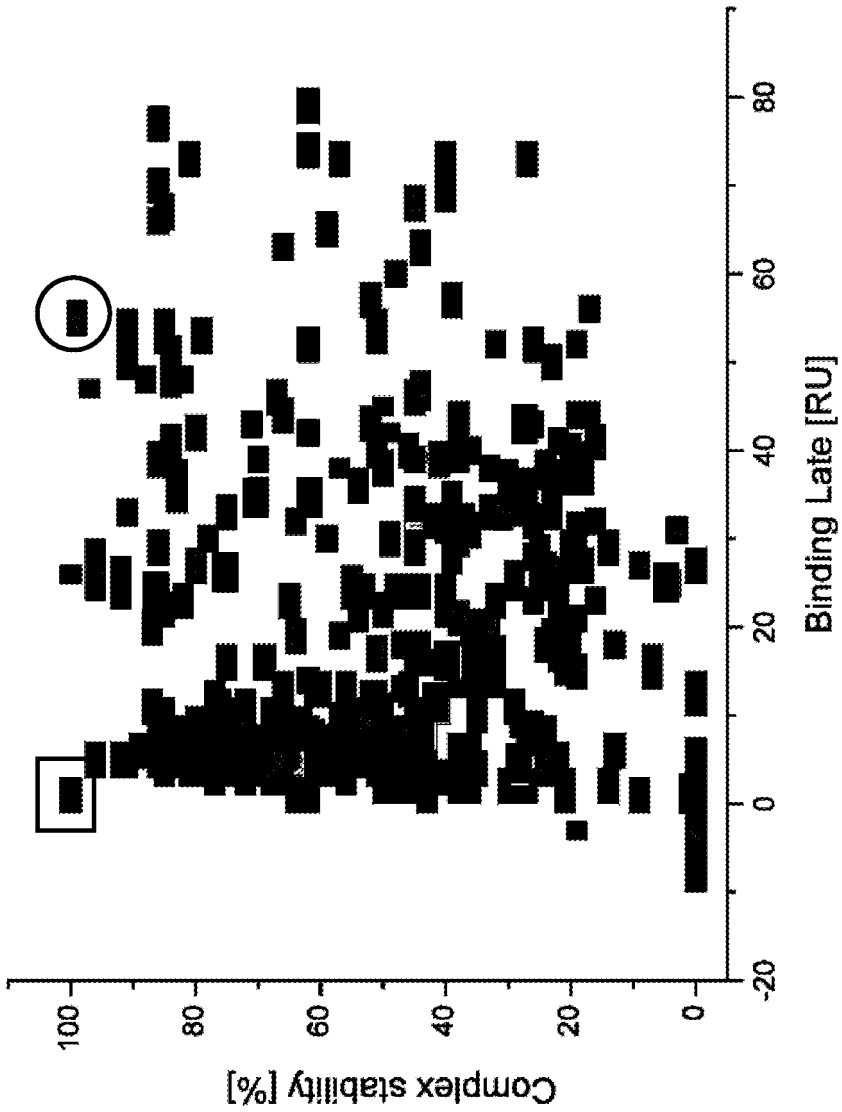

Complex stability [%]
100
80
60
40
20
0
-20
0
20
40
60
80
Binding Late [RU]

Fig. 3

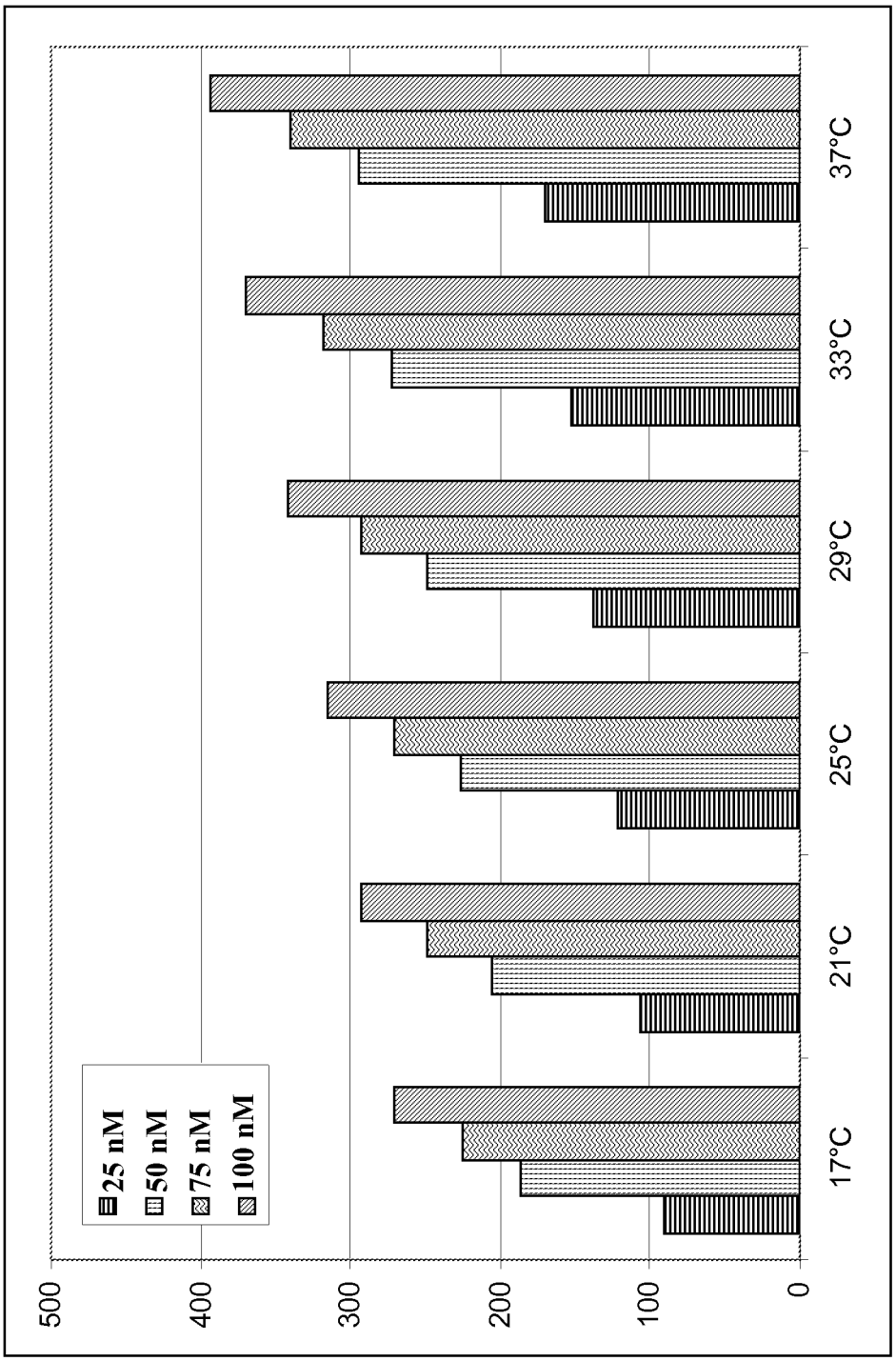

500
400
300
200
100
0
17°C
21°C
25°C
29°C
33°C
37°C
25 nM
50 nM
75 nM
100 nM

**Fig. 4a**

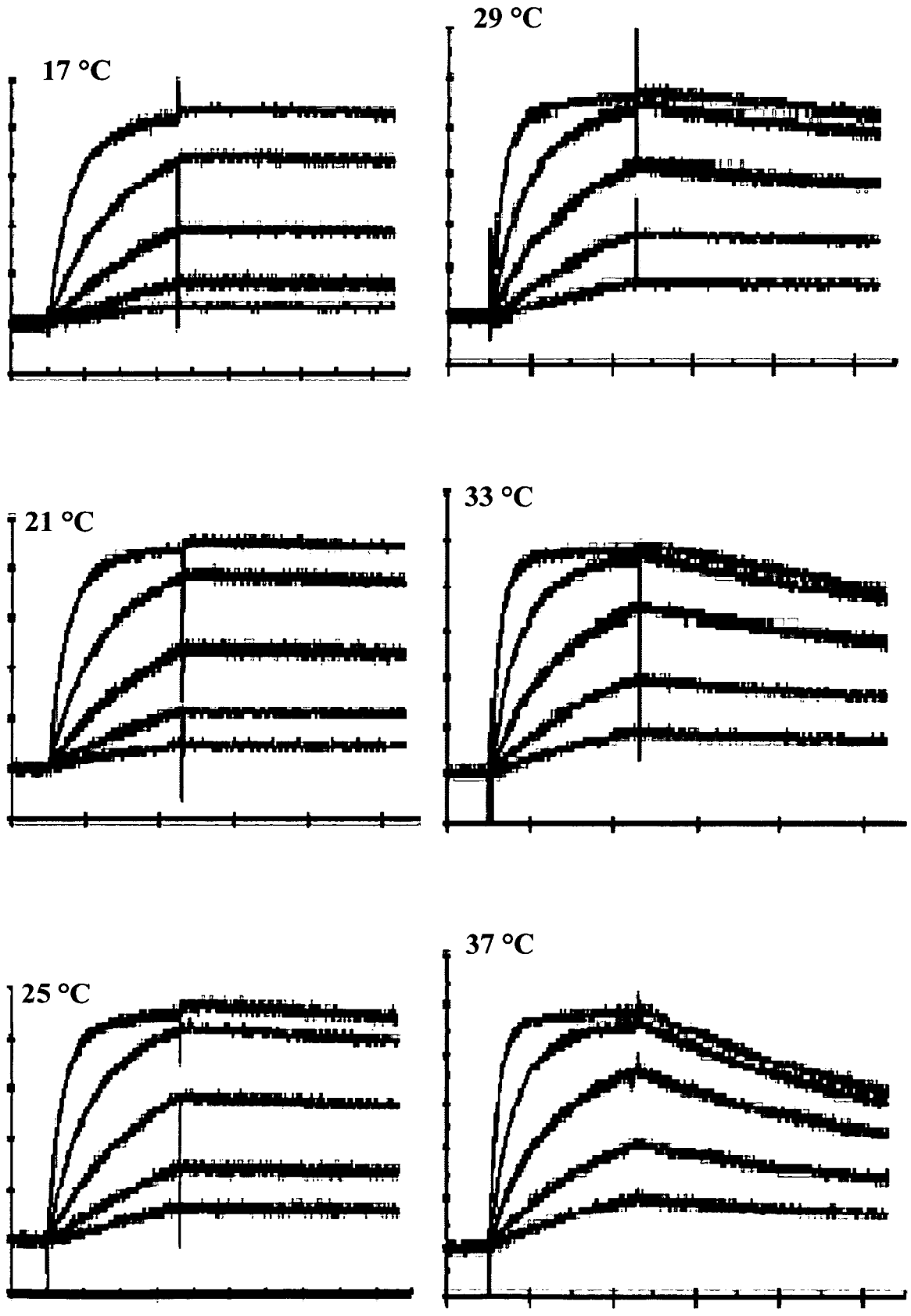


Fig. 4b

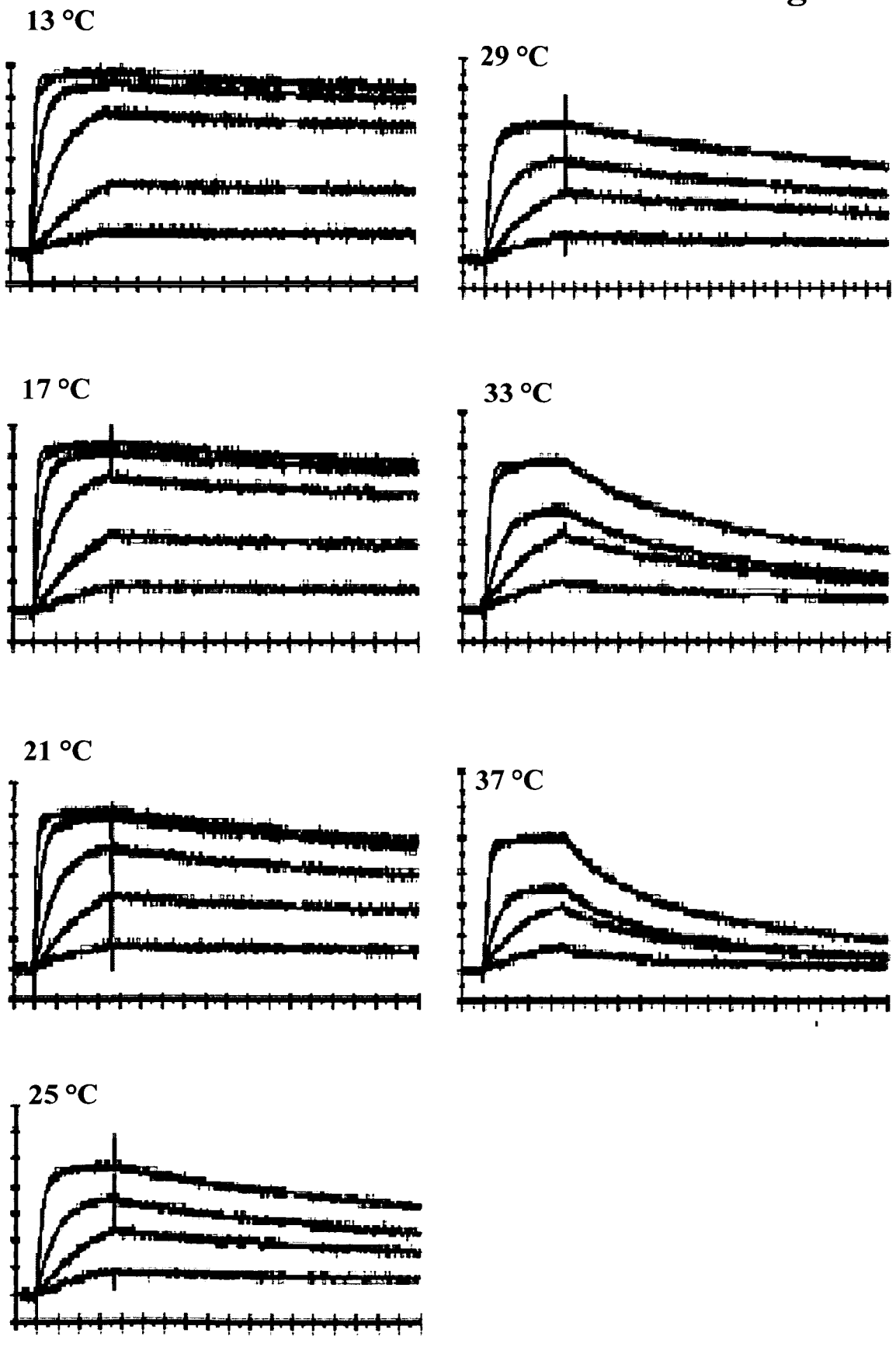


**Fig. 5a**

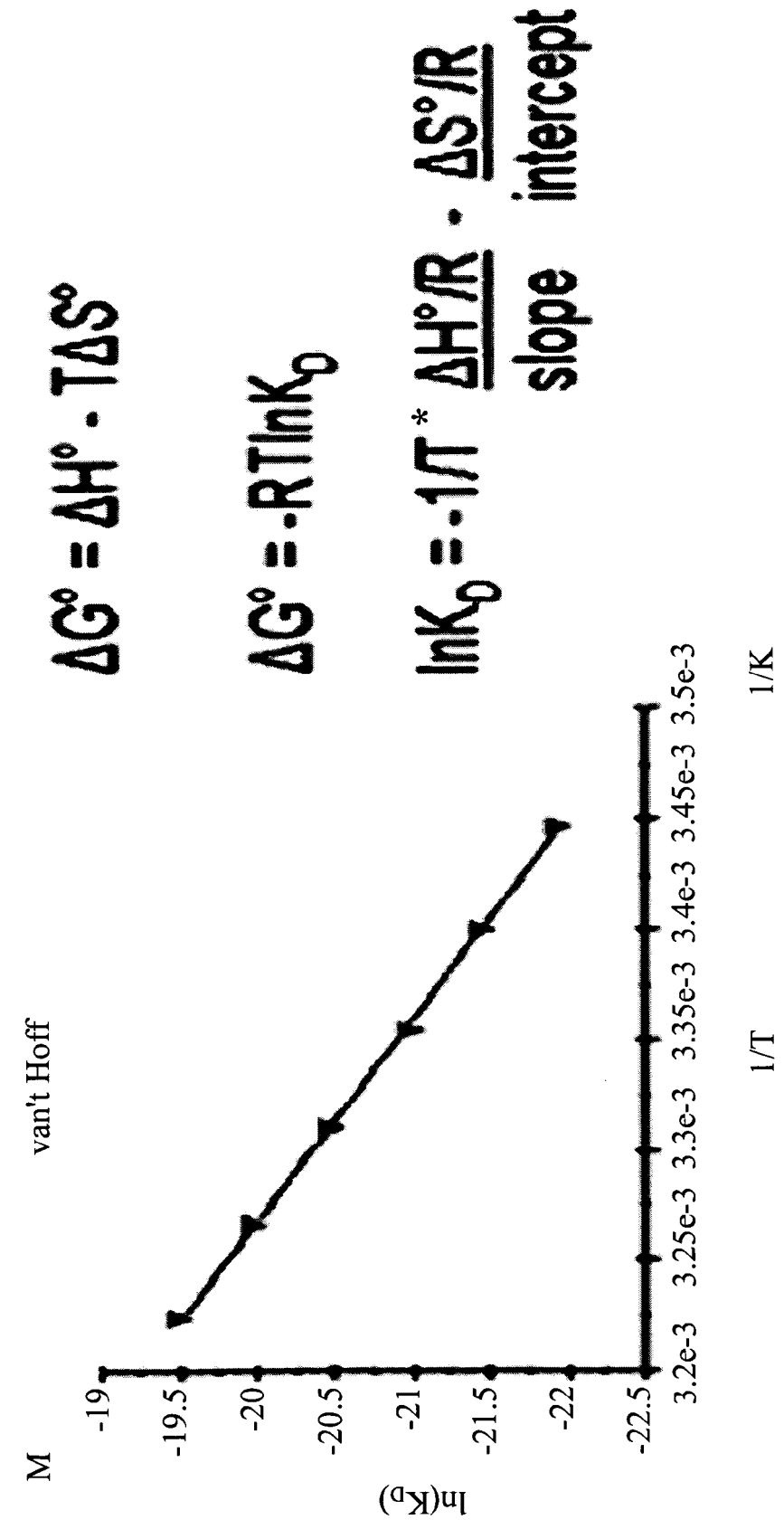

ΔG° = ΔH° - TΔS°
ΔG° = -RTlnK_D
lnK_D = -1/T * ΔH°/R + ΔS°/R
slope
intercept
van't Hoff
M
ln(K_D)
-19
-19.5
-20
-20.5
-21
-21.5
-22
-22.5
3.2e-3
3.25e-3
3.3e-3
3.35e-3
3.4e-3
3.45e-3
3.5e-3
1/T
1/K

**Fig. 5b**

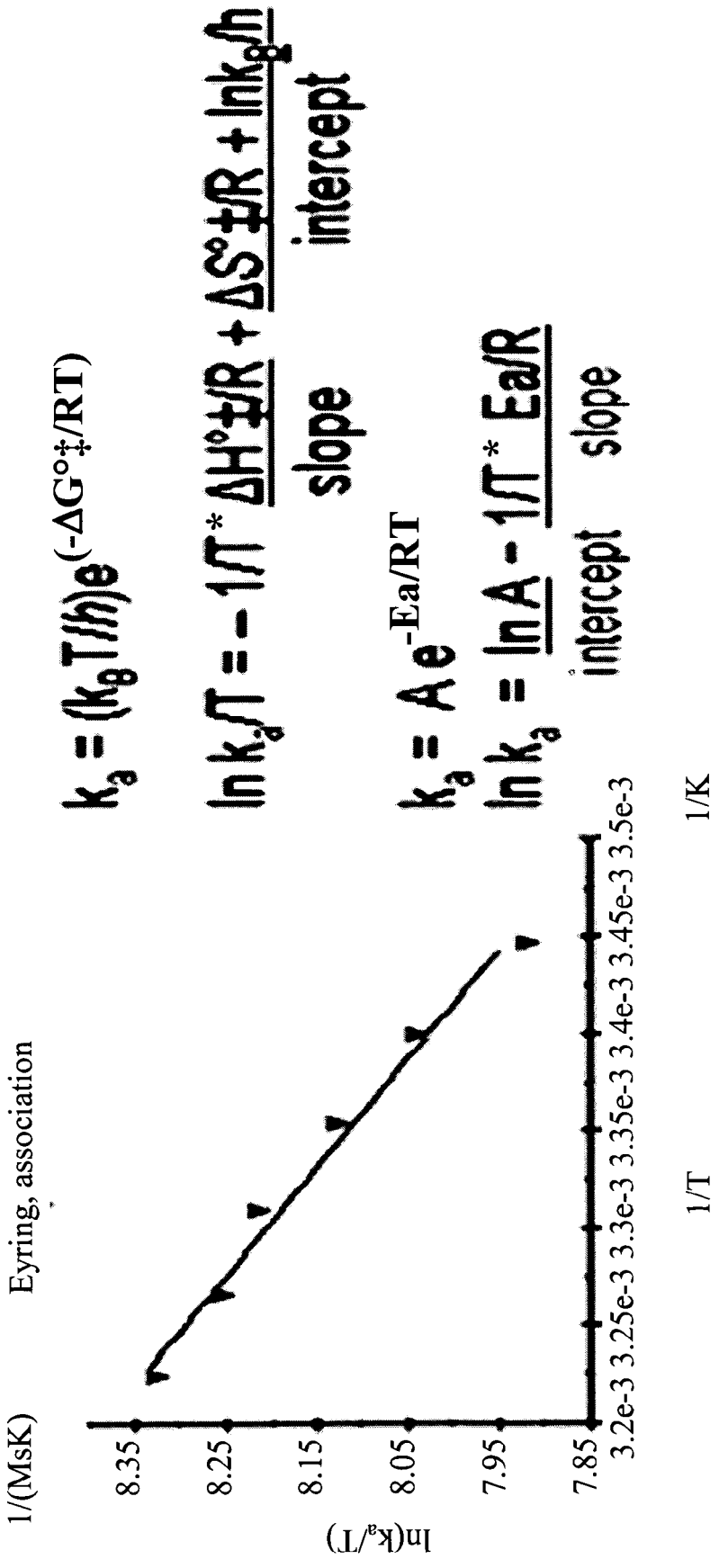

$k_a = (k_B T/h) e^{(-\Delta G^{\circ\ddagger}/RT)}$
$\ln k_a/T = -1/T * \underbrace{\Delta H^{\circ\ddagger}/R}_{\text{slope}} + \underbrace{\Delta S^{\circ\ddagger}/R + \ln k_B/h}_{\text{intercept}}$
$k_a = A e^{-Ea/RT}$
$\ln k_a = \underbrace{\ln A}_{\text{intercept}} - 1/T * \underbrace{Ea/R}_{\text{slope}}$
Eyring, association
1/(MsK)
ln(k_a/T)
8.35
8.25
8.15
8.05
7.95
7.85
3.2e-3 3.25e-3 3.3e-3 3.35e-3 3.4e-3 3.45e-3 3.5e-3
1/T
1/K

Fig. 5c

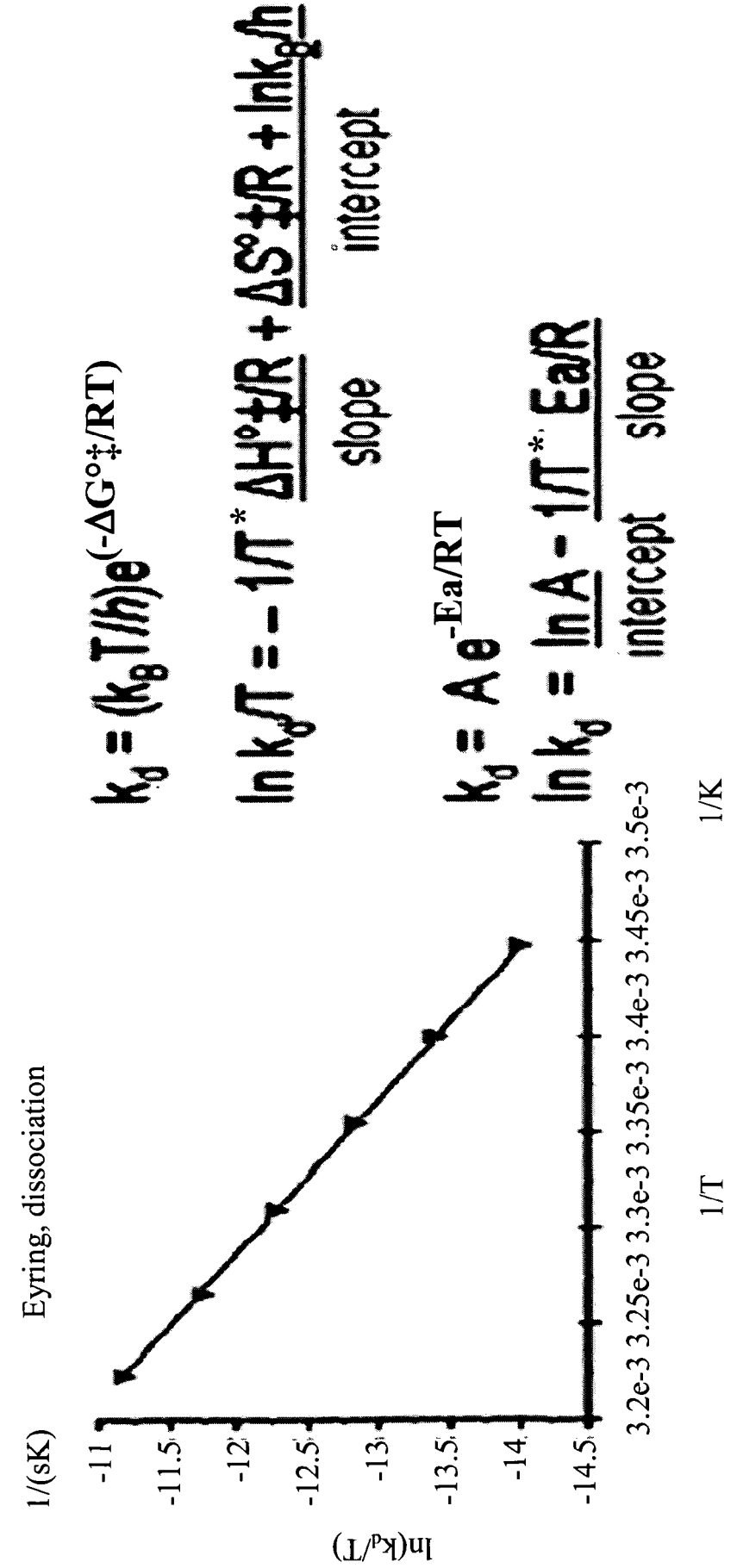

Eyring, dissociation
1/(sK)
-11
-11.5
-12
-12.5
-13
-13.5
-14
-14.5
ln(kd/T)
3.2e-3 3.25e-3 3.3e-3 3.35e-3 3.4e-3 3.45e-3 3.5e-3
1/T
1/K
kd = (kBT/h)e(-ΔG°‡/RT)
ln kd/T = - 1/T * ΔH°‡/R + ΔS°‡/R + lnkB/h
slope
intercept
kd = A e-Ea/RT
ln kd = ln A - 1/T * Ea/R
intercept
slope

**Fig. 6a**

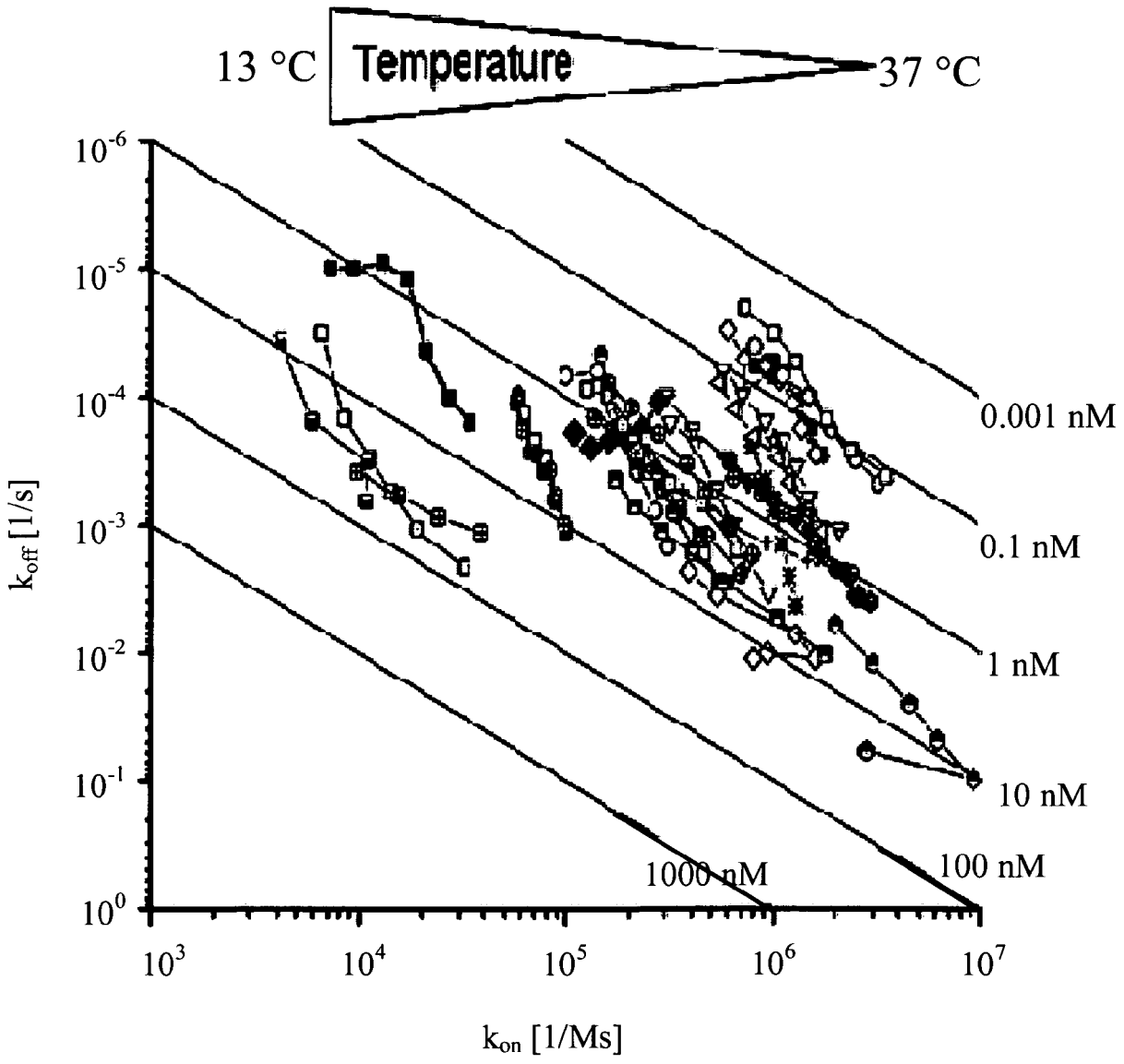

13 °C
Temperature
37 °C
$10^{-6}$
$10^{-5}$
$10^{-4}$
$10^{-3}$
$10^{-2}$
$10^{-1}$
$10^{0}$
$k_{off}$ [1/s]
0.001 nM
0.1 nM
1 nM
10 nM
100 nM
1000 nM
$10^{3}$
$10^{4}$
$10^{5}$
$10^{6}$
$10^{7}$
$k_{on}$ [1/Ms]

**Fig. 6b**

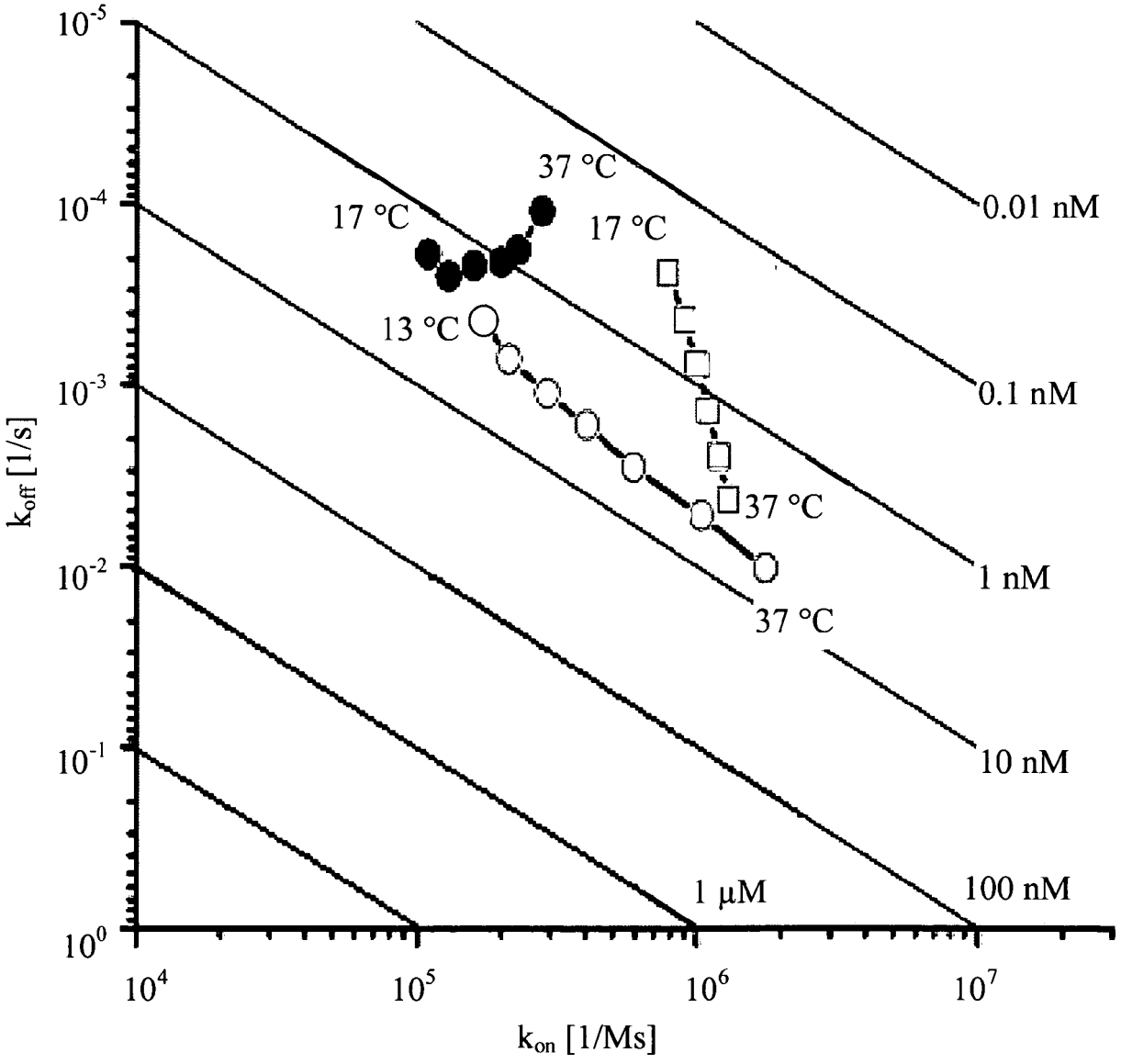

$10^{-5}$
$10^{-4}$
$10^{-3}$
$10^{-2}$
$10^{-1}$
$10^{0}$
$k_{off}$ [1/s]
$10^{4}$
$10^{5}$
$10^{6}$
$10^{7}$
$k_{on}$ [1/Ms]
37 °C
17 °C
17 °C
13 °C
37 °C
37 °C
0.01 nM
0.1 nM
1 nM
10 nM
100 nM
1 µM

Fig. 7

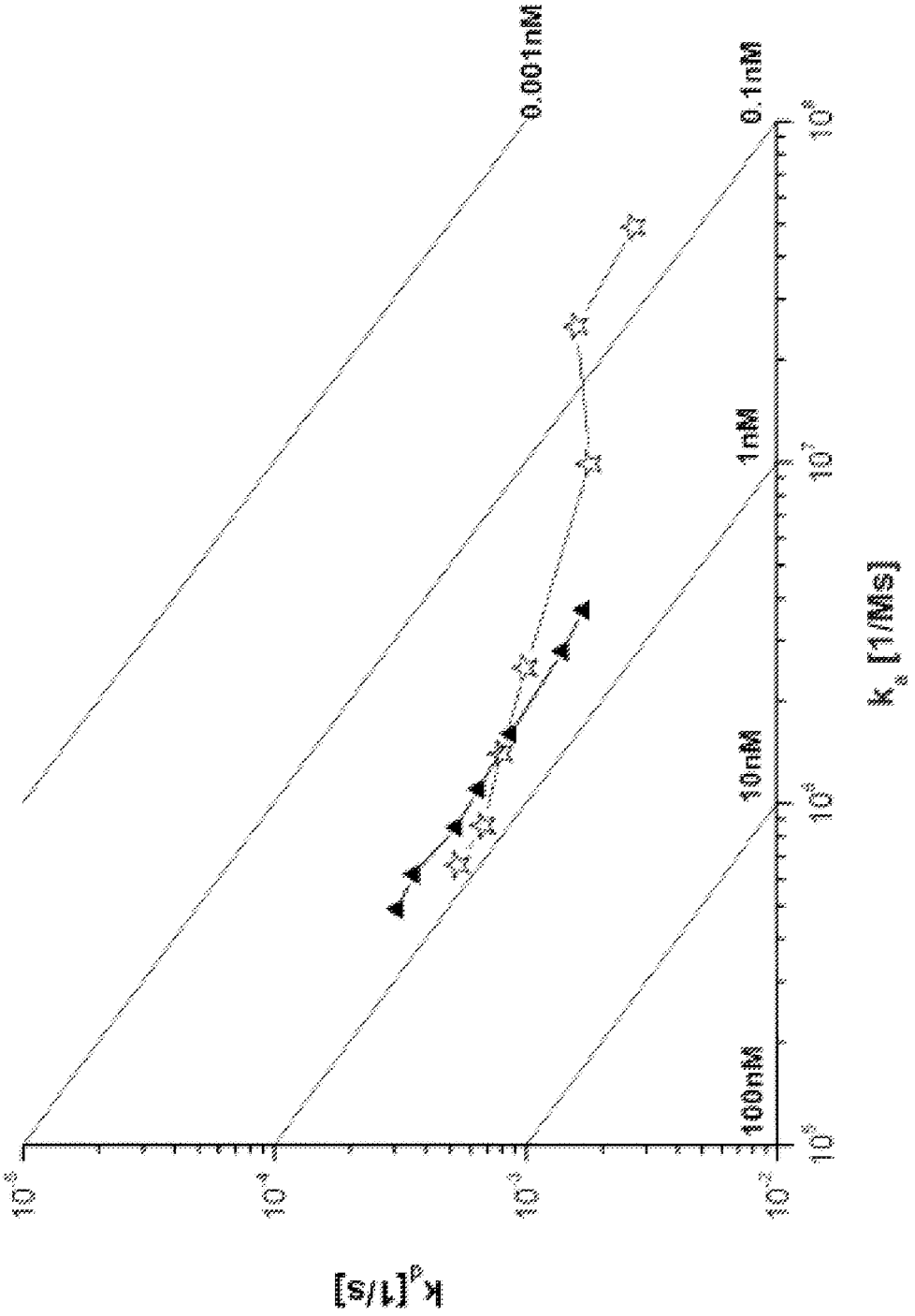

0.001nM
0.1nM
1nM
10nM
100nM
$10^{-2}$
$10^{-3}$
$10^{-4}$
$10^{-5}$
$10^{6}$
$10^{7}$
$10^{8}$
$k_d$ [1/s]
$k_a$ [1/Ms]

Fig. 8

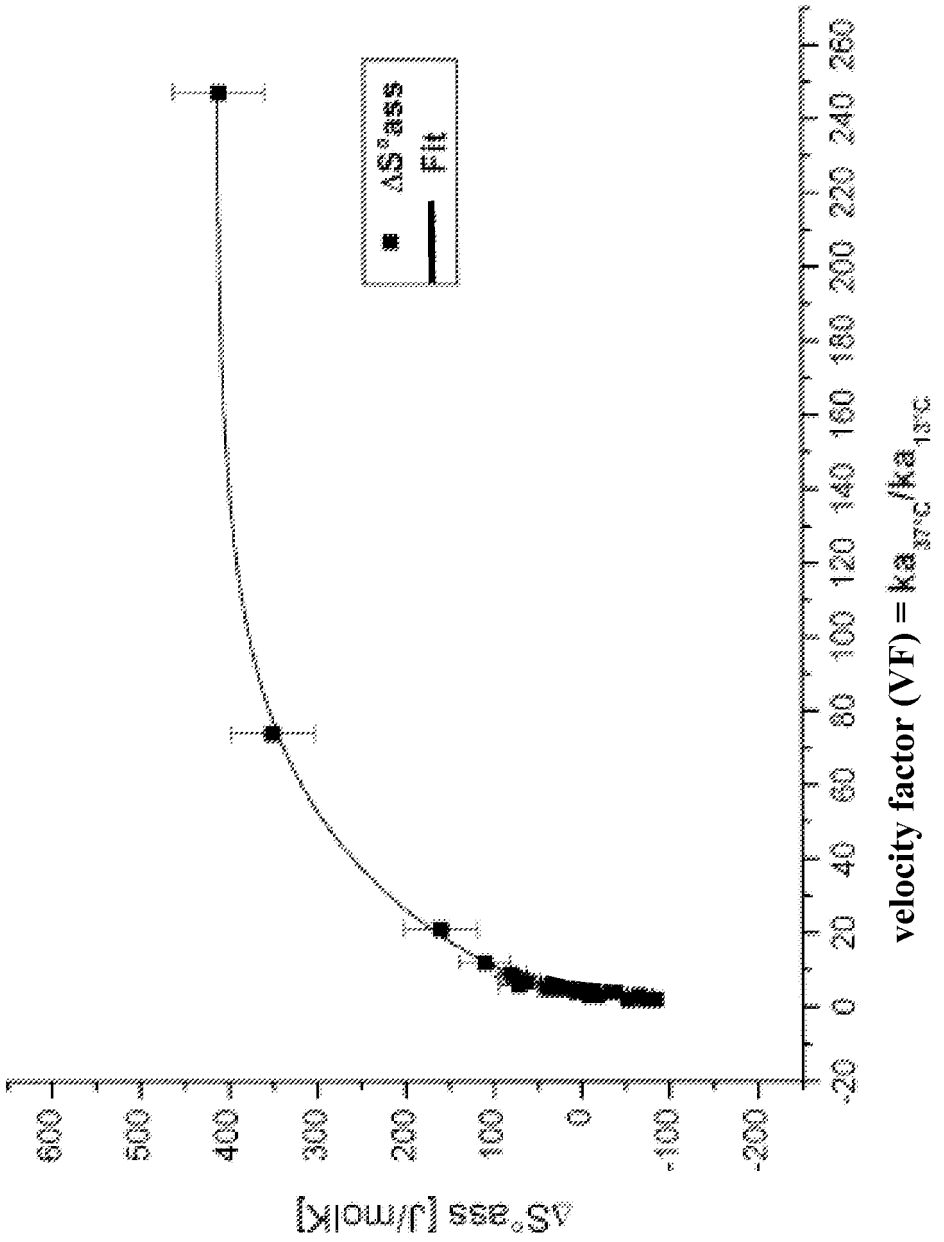
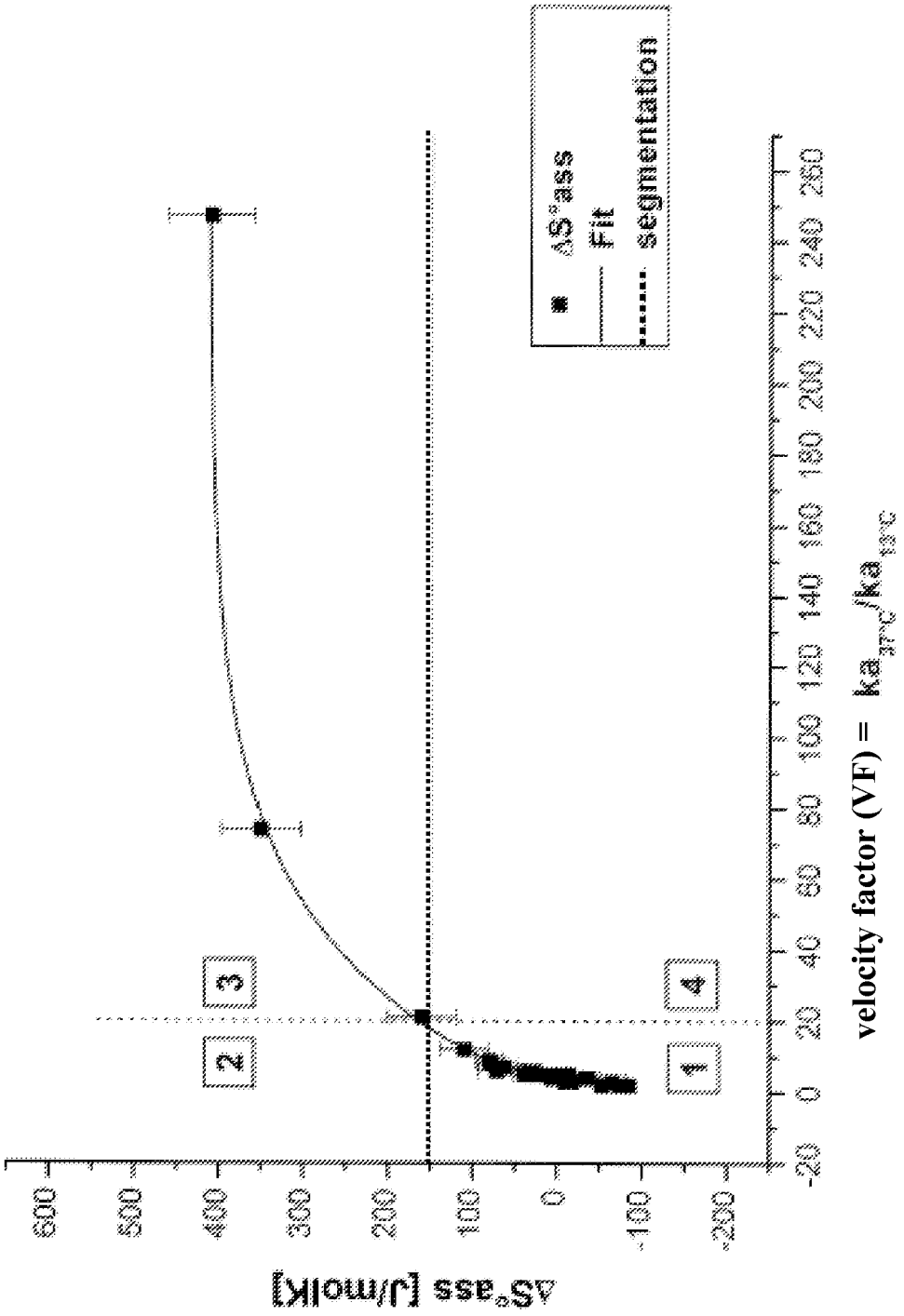

ΔS°ass
Fit
ΔS°ass [J/mol/K]
600
500
400
300
200
100
0
-100
-200
-20
0
20
40
60
80
100
120
140
160
180
200
220
240
260
velocity factor (VF) = ka_src/ka_1src

Fig. 9


ΔS°ass [J/molK]
velocity factor (VF) = ka_src/ka_trc
ΔS°ass
Fit
segmentation
1
2
3
4