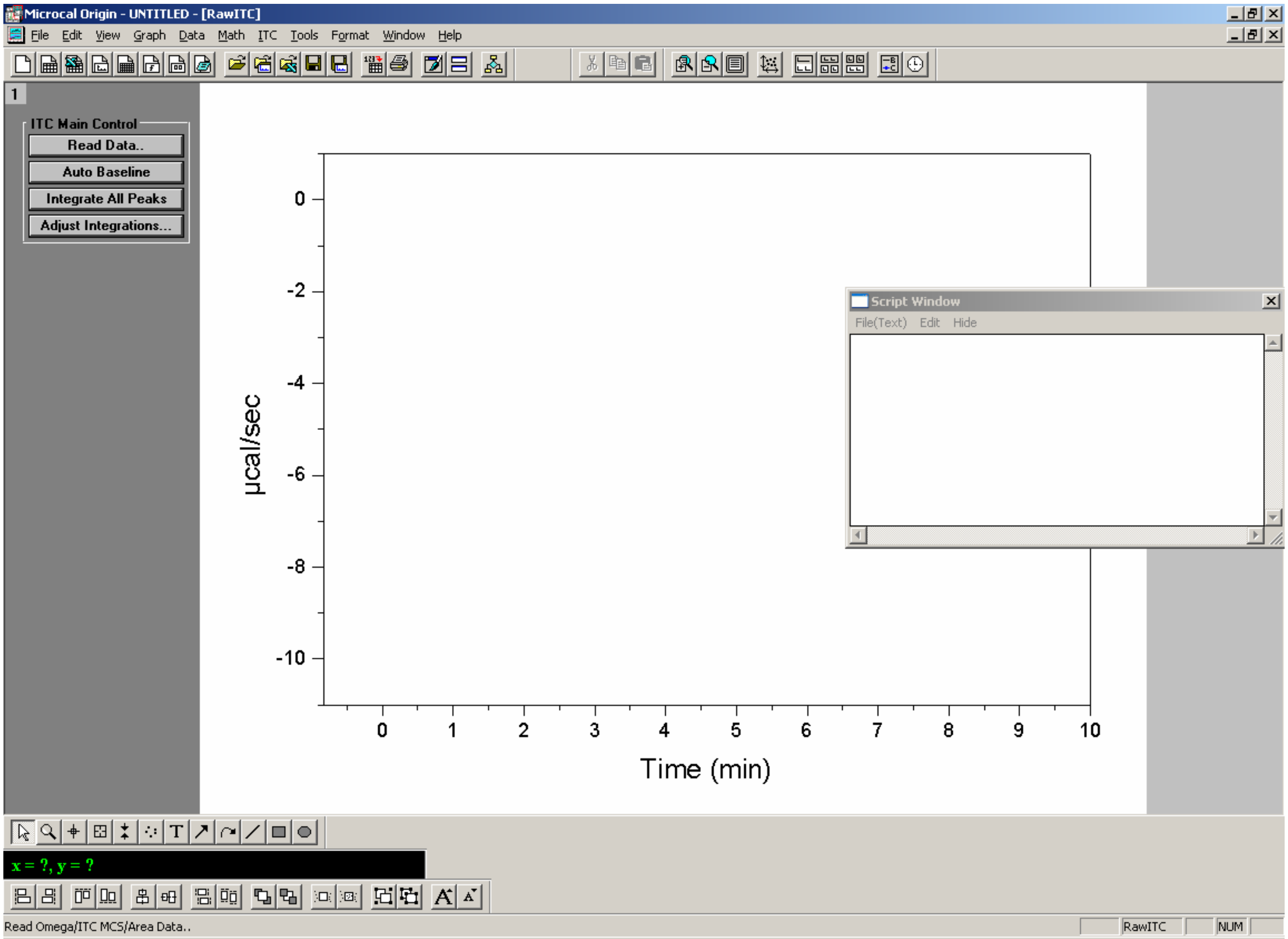
HHMI Biopolymer – Keck Foundation
Biotechnology Resource Laboratory

Biophysical Resource

Yale University

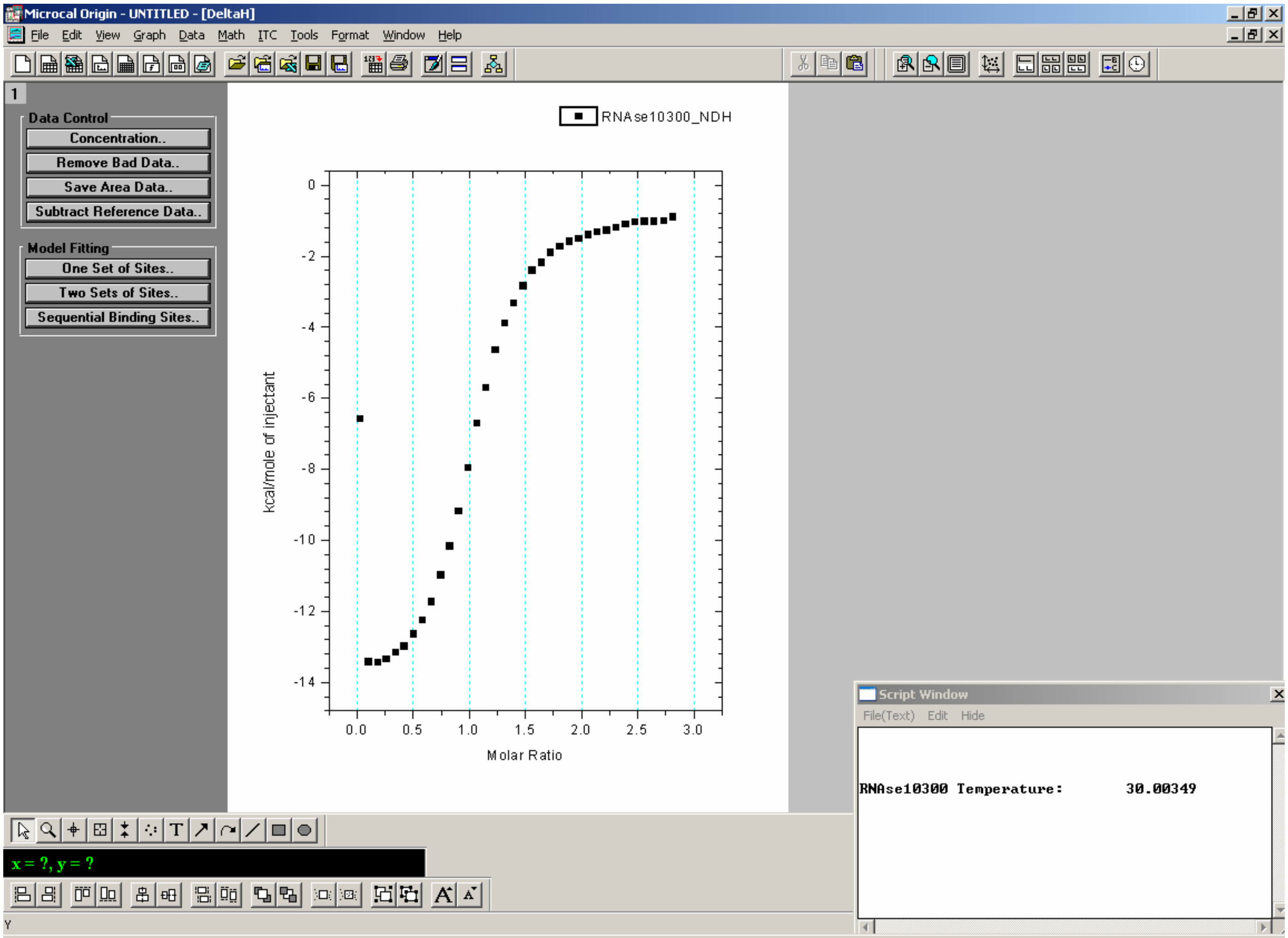
Processing ITC data using ORIGIN 5.0

1. Launch the MicroCal processing software (Origin 5.0 with Microcal ITC feature) Microcal Inc. ITC.lnk icon on a desktop.
2. The following window appears:



3. Click "Read Data" and open the data file : "*.itc" in this case "RNAse****.itc"

4. Once the data file is loaded, the following window appears:



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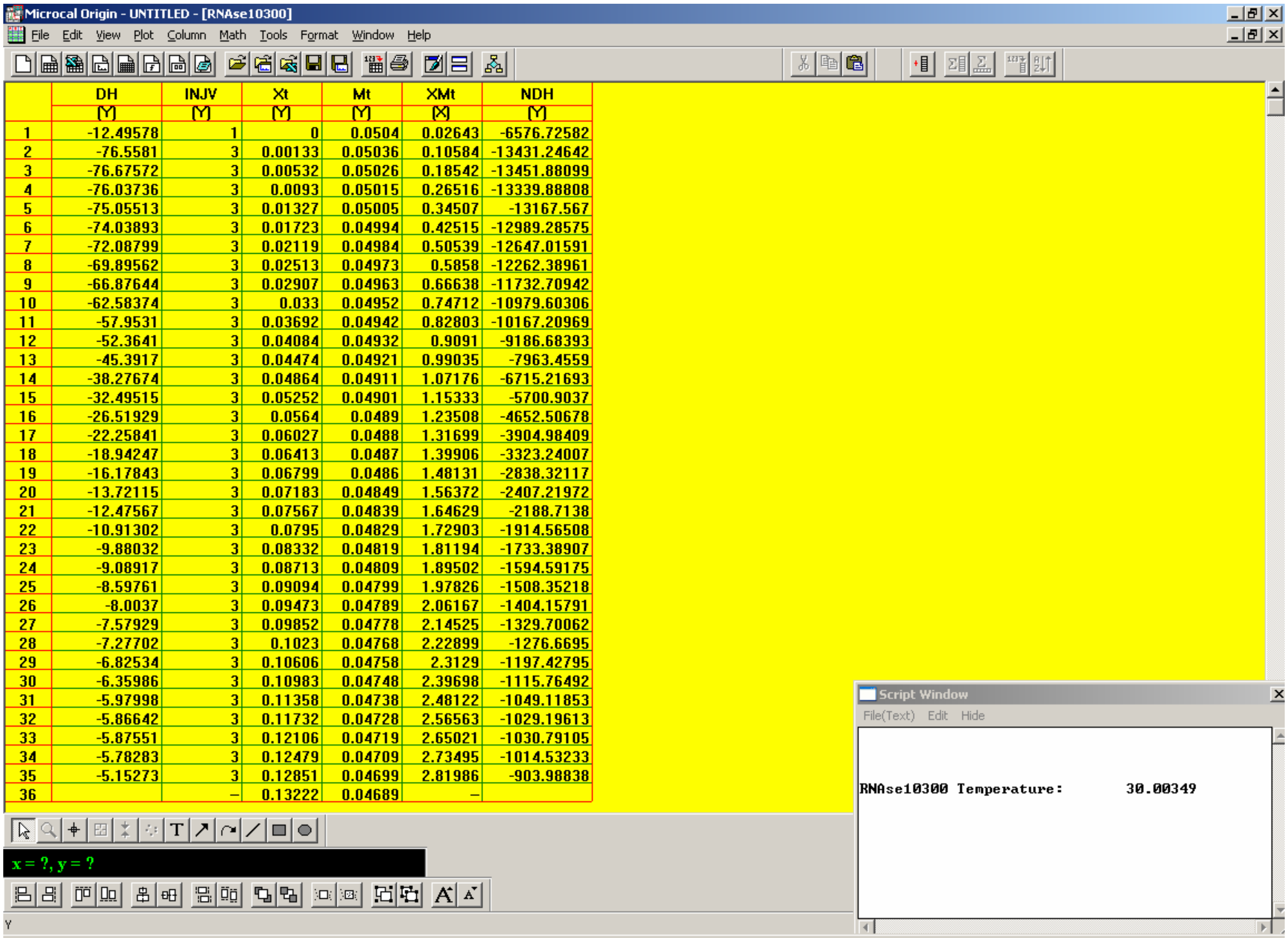
RNAse10300 Temperature:        30.00349
```

5. At this point the data can be corrected for heat of dilution of the ligand (which are the heats observed at the end of titration, where no binding events are happening and majority of heat observed are from dilution of titrant).

6. Go to "Window" (on the main tab) "Name of the data file" selection (in this case "window" →"RNAse103007")

7. The following window appears:



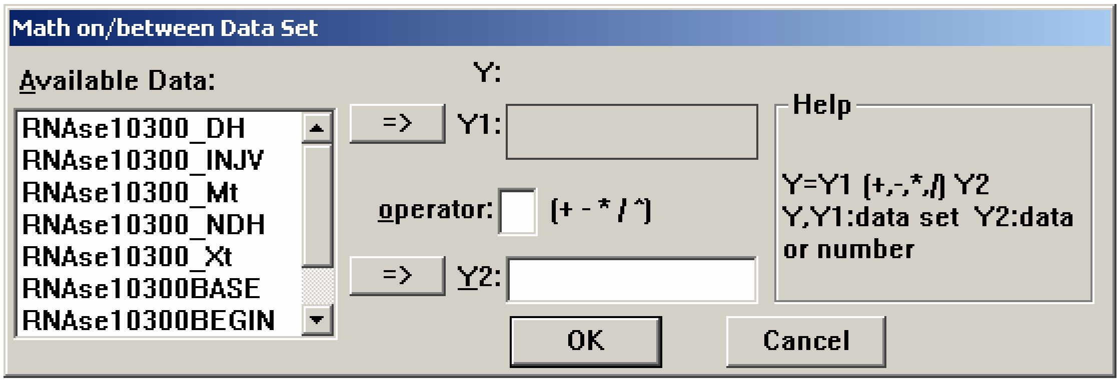
	DH	INJV	Xt	Mt	XMt	NDH
	(Y)	(Y)	(Y)	(Y)	(X)	(Y)
1	-12.49578	1	0	0.0504	0.02643	-6576.72582
2	-76.5581	3	0.00133	0.05036	0.10584	-13431.24642
3	-76.67572	3	0.00532	0.05026	0.18542	-13451.88099
4	-76.03736	3	0.0093	0.05015	0.26516	-13339.88808
5	-75.05513	3	0.01327	0.05005	0.34507	-13167.567
6	-74.03893	3	0.01723	0.04994	0.42515	-12989.28575
7	-72.08799	3	0.02119	0.04984	0.50539	-12647.01591
8	-69.89562	3	0.02513	0.04973	0.5858	-12262.38961
9	-66.87644	3	0.02907	0.04963	0.66638	-11732.70942
10	-62.58374	3	0.033	0.04952	0.74712	-10979.60306
11	-57.9531	3	0.03692	0.04942	0.82803	-10167.20969
12	-52.3641	3	0.04084	0.04932	0.9091	-9186.68393
13	-45.3917	3	0.04474	0.04921	0.99035	-7963.4559
14	-38.27674	3	0.04864	0.04911	1.07176	-6715.21693
15	-32.49515	3	0.05252	0.04901	1.15333	-5700.9037
16	-26.51929	3	0.0564	0.0489	1.23508	-4652.50678
17	-22.25841	3	0.06027	0.0488	1.31699	-3904.98409
18	-18.94247	3	0.06413	0.0487	1.39906	-3323.24007
19	-16.17843	3	0.06799	0.0486	1.48131	-2838.32117
20	-13.72115	3	0.07183	0.04849	1.56372	-2407.21972
21	-12.47567	3	0.07567	0.04839	1.64629	-2188.7138
22	-10.91302	3	0.0795	0.04829	1.72903	-1914.56508
23	-9.88032	3	0.08332	0.04819	1.81194	-1733.38907
24	-9.08917	3	0.08713	0.04809	1.89502	-1594.59175
25	-8.59761	3	0.09094	0.04799	1.97826	-1508.35218
26	-8.0037	3	0.09473	0.04789	2.06167	-1404.15791
27	-7.57929	3	0.09852	0.04778	2.14525	-1329.70062
28	-7.27702	3	0.1023	0.04768	2.22899	-1276.6695
29	-6.82534	3	0.10606	0.04758	2.3129	-1197.42795
30	-6.35986	3	0.10983	0.04748	2.39698	-1115.76492
31	-5.97998	3	0.11358	0.04738	2.48122	-1049.11853
32	-5.86642	3	0.11732	0.04728	2.56563	-1029.19613
33	-5.87551	3	0.12106	0.04719	2.65021	-1030.79105
34	-5.78283	3	0.12479	0.04709	2.73495	-1014.53233
35	-5.15273	3	0.12851	0.04699	2.81986	-903.98838
36		–	0.13222	0.04689	–	

```
RNAse10300 Temperature:       30.00349
```

8. Scroll down to the last injections and compute an average value for NDH for the last 5-15 injections; make sure these injections are really of similar heats and represent pure dilution effects; for the RNase103007 file, this value is ~-1073.

9. Go to “Math” →“Simple Math” on the main tab.

10. The following window appears:

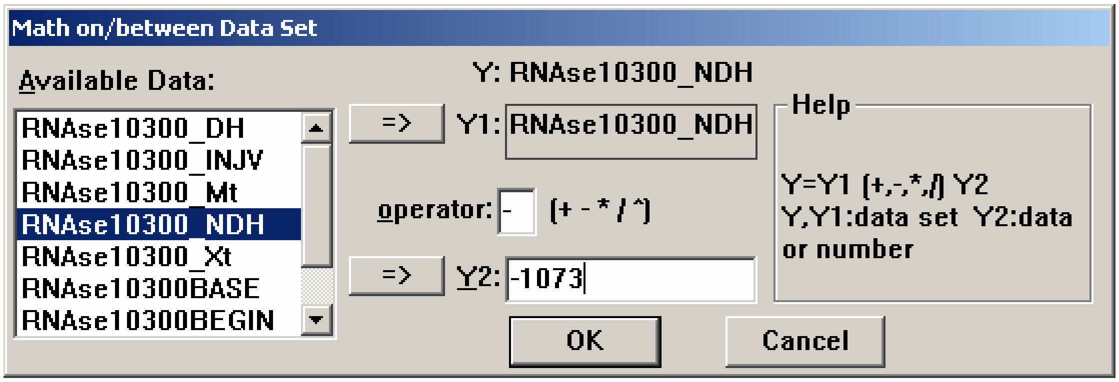


RNAse10300_DH
RNAse10300_INJV
RNAse10300_Mt
RNAse10300_NDH
RNAse10300_Xt
RNAse10300BASE
RNAse10300BEGIN

Y=Y1 (+,-,*,/) Y2
Y,Y1:data set Y2:data or number

11. Select “RNase***_NDH” press “=>” button and populated the Y1 window

12. Choose the “operator” as “-“ and populate the “Y2” window with the average heat of dilution computed in step 8 (for RNase10300, the value is “-1073”).



RNAse10300_DH
RNAse10300_INJV
RNAse10300_Mt
RNAse10300_NDH
RNAse10300_Xt
RNAse10300BASE
RNAse10300BEGIN

Y: RNAse10300_NDH
Y1: RNAse10300_NDH
operator: - (+ - * / ^)
Y2: -1073

Y=Y1 (+,-,*,/) Y2
Y,Y1:data set Y2:data or number

13. Click “OK” to subtract the heats of dilution from all measured heats.
14. Change display window to “DeltaH” (“window” → “DeltaH”).

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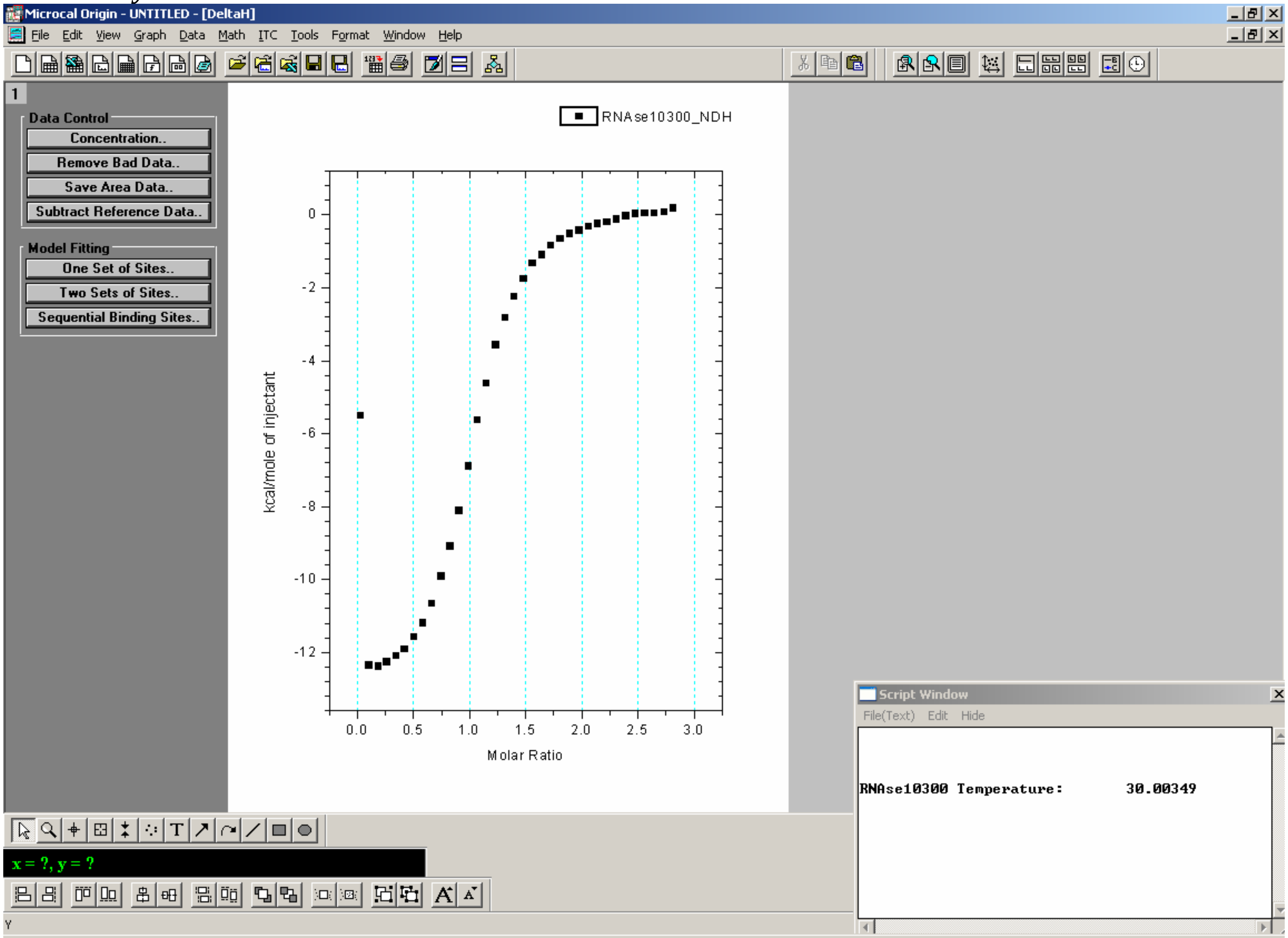
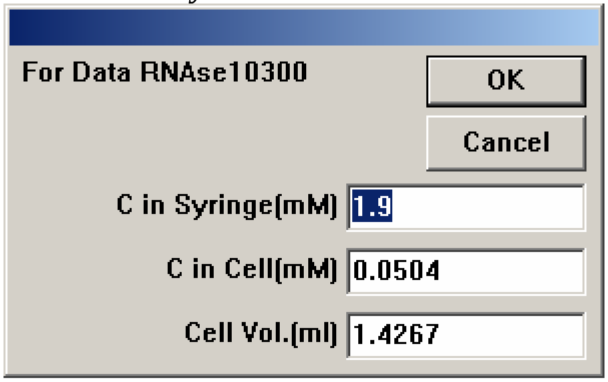
the heats should be corrected for heat of dilution as illustrated:



```
RNAse10300 Temperature:        30.00349
```

15. You may correct the concentrations at this point if needed, but choosing “Concentration” and changing the values for cell and ligand.



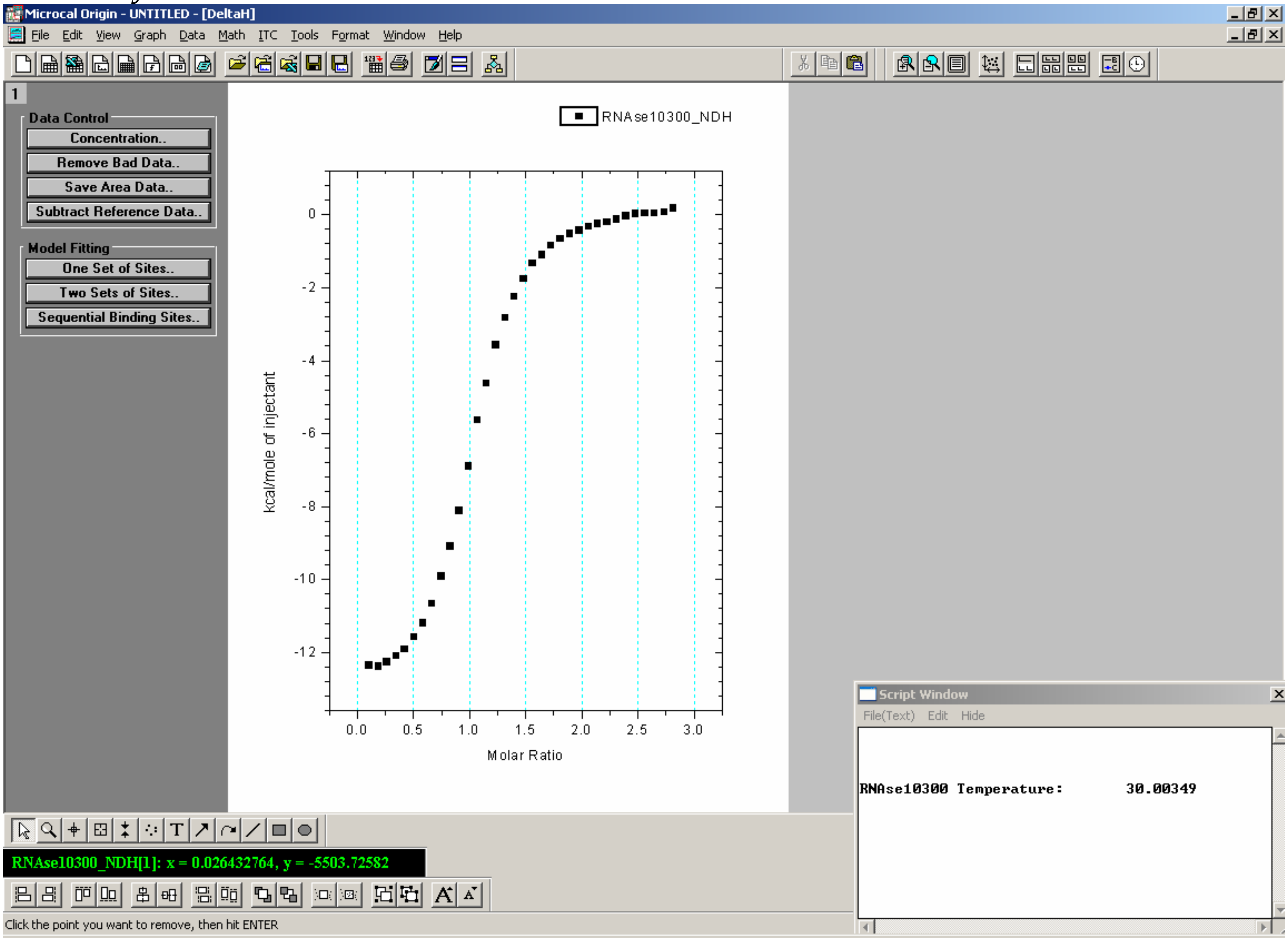
For Data RNAse10300

C in Syringe(mM) 1.9

C in Cell(mM) 0.0504

Cell Vol.(ml) 1.4267

16. Remove the results for first injection by choosing “Remove Bad Data” (under “Data Control” section on left hand side); select the data point for the first injection and hit “enter” key, which should delete the result for first injection:

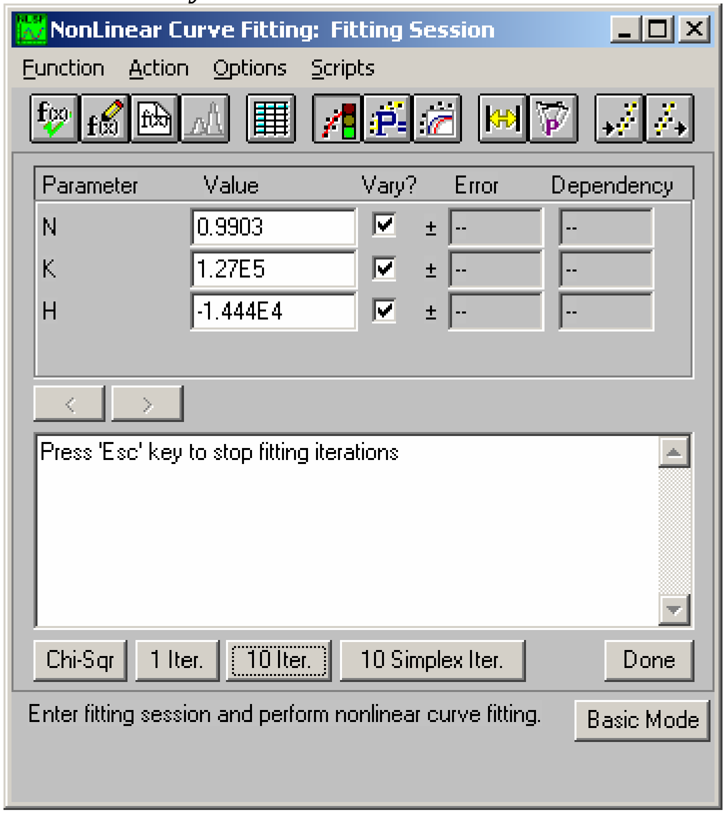


```
RNAse10300 Temperature:       30.00349
```

```
RNAse10300_NDH[1]: x = 0.026432764, y = -5503.72582
```

```
Click the point you want to remove, then hit ENTER
```

17. Now you are ready to choose the model for fitting the data from the “Model Fitting” section.
18. For single sets of sites: choose “One Set of sites” and the following window appear:

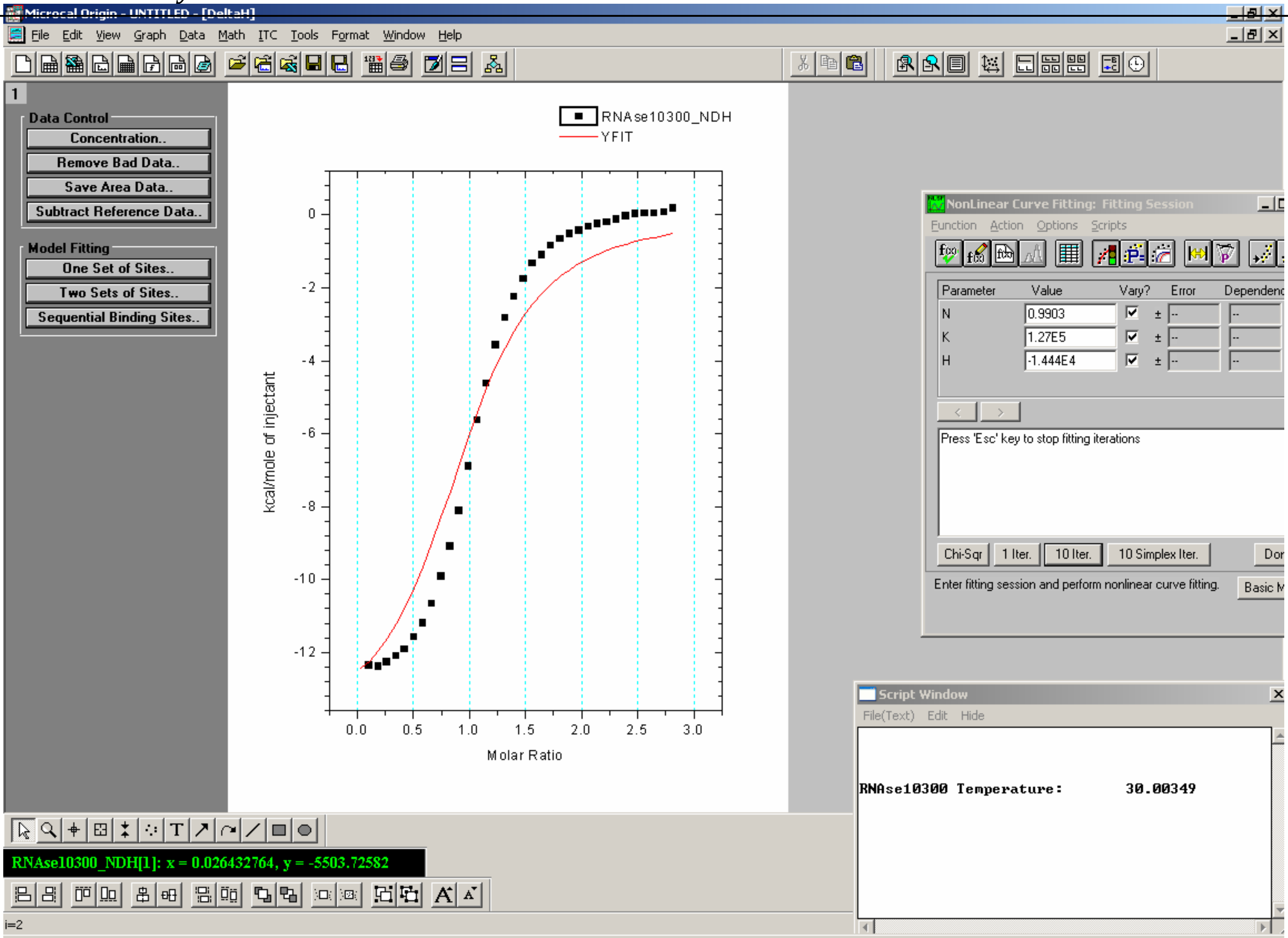


Parameter	Value	Vary?		Error	Dependency
N	0.9903	☑	±	--	--
K	1.27E5	☑	±	--	--
H	-1.444E4	☑	±	--	--

Press 'Esc' key to stop fitting iterations

Enter fitting session and perform nonlinear curve fitting.

with the initial guesses computed by the software and the red curve for fitted values displayed on the “DeltaH” window:



Parameter	Value	Vary?	Error	Dependenc
N	0.9903	☑	± --	--
K	1.27E5	☑	± --	--
H	-1.444E4	☑	± --	--

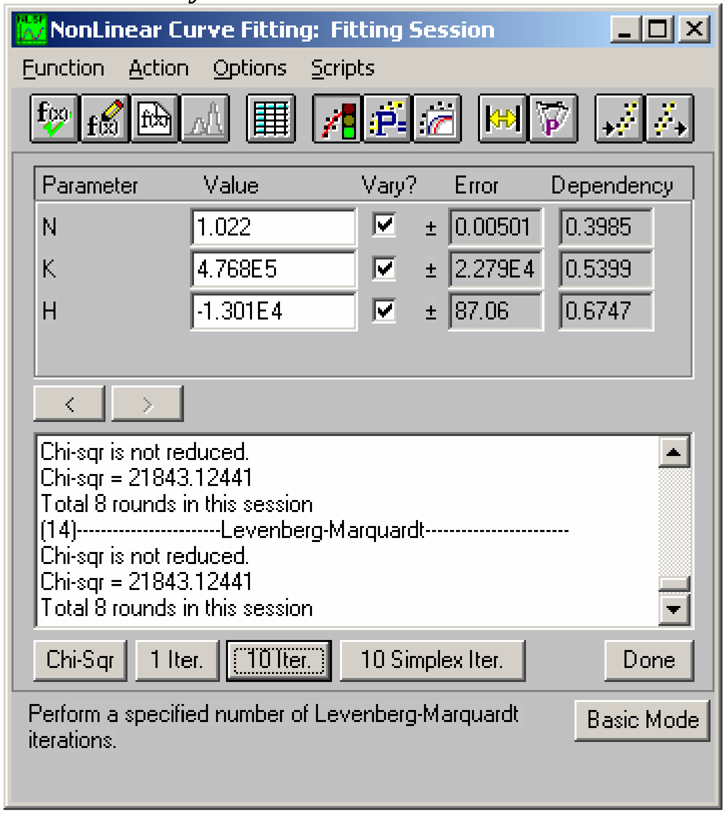
Press 'Esc' key to stop fitting iterations

Enter fitting session and perform nonlinear curve fitting.

```
RNAse10300 Temperature:       30.00349
```

RNAse10300_NDH[1]: x = 0.026432764, y = -5503.72582

19. Choose "1 Iter." (single iteration and monitor the progression to make sure the fit is converging properly and eventually do "10 Iter." until the Chi-Sqr value does not change with subsequent iterations.

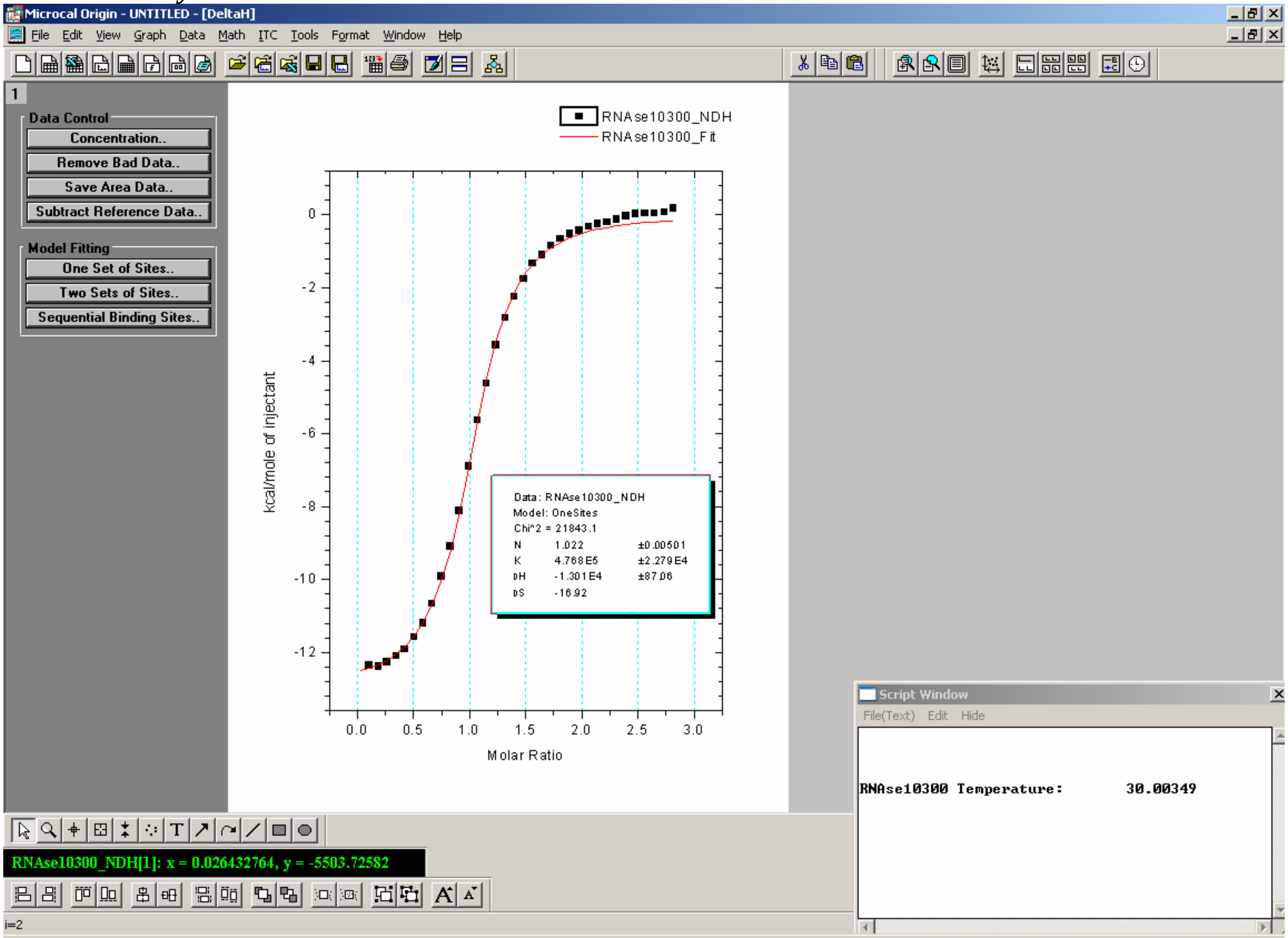


Parameter	Value	Vary?		Error	Dependency
N	1.022	☑	±	0.00501	0.3985
K	4.768E5	☑	±	2.279E4	0.5399
H	-1.301E4	☑	±	87.06	0.6747

```
Chi-sqr is not reduced.
Chi-sqr = 21843.12441
Total 8 rounds in this session
(14)----------------------Levenberg-Marquardt----------------------
Chi-sqr is not reduced.
Chi-sqr = 21843.12441
Total 8 rounds in this session
```

Perform a specified number of Levenberg-Marquardt iterations.

20. Choose “Done” and the results will be displayed on the DelatH page:



```
Data: RNAse10300_NDH
Model: OneSites
Chi^2 = 21843.1
N       1.022       ±0.00501
K       4.768E5     ±2.279E4
ΔH      -1.301E4    ±87.06
ΔS      -16.92
```

```
RNAse10300_NDH[1]: x = 0.026432764, y = -5503.72582
```

```
i=2
```

```
RNAse10300 Temperature:      30.00349
```