

Supplementary Materials for

**Recognition of Flexible Peptides in Water by Transition Metal
Complexes**

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Characterization data for the metal complexes and the peptides:

For C: Anal. Calcd. for $C_{11}H_{11}CuNO_4$: C, 46.39; H, 3.89; N, 4.92. Found: C, 46.06; H, 3.58; N, 4.62.

For 1: Anal. Calcd. for $C_{21}H_{21}Cu_3N_3O_{12} \cdot 3H_2O$: C, 33.54; H, 3.62; N, 5.59. Found: C, 33.53; H, 3.59; N, 5.54.

For 2: Anal. Calcd. for $C_{27}H_{30}Cu_3N_6O_{15} \cdot 3H_2O$: C, 35.12; H, 3.93; N, 9.10. Found: C, 35.23; H, 3.81; N, 8.35.

For m H: 1H NMR (500 MHz, D_2O): δ 7.34 (s, 1H), 7.09 – 7.05 (m, 3H), 6.81 (d, 2H, $J = 5$ Hz), 6.49 (s, 1H), 4.10 (d, 1H, $J = 15$ Hz), 4.93 (d, 1H, $J = 15$ Hz), 3.42 (t, 1H, $J = 5$ Hz), 2.62 (d, 2H, $J = 5$ Hz); ^{13}C NMR (125 MHz, D_2O): δ 175.9, 137.7, 135.8, 133.0, 128.7, 127.4, 127.3, 117.0, 55.1, 42.8, 31.9. HRMS Calcd. for $C_{13}H_{16}N_4O$: 244.1324. Found: 244.1329.

For t H: 1H NMR (500 MHz, D_2O) δ 7.35 (s, 3H), 6.60 (s, 3H), 6.49 (s, 3H), 4.09 (d, 3H, $J = 15.1$ Hz), 3.96 (d, 3H, $J = 15.1$ Hz), 3.45 (t, 3H, $J = 6.9$ Hz), 2.37 (d, 6H, $J = 6.9$ Hz); ^{13}C NMR (125 MHz, D_2O) δ 176.0, 138.5, 135.8, 133.0, 125.3, 117.1, 55.0, 42.7, 32.0. HRMS Calcd. for $C_{27}H_{36}N_{12}O_3$: 576.3033. Found: 576.3035.

For t GH: 1H NMR (500 MHz, D_2O) δ 7.19 (s, 3H), 6.80 (s, 3H), 6.64 (s, 3H), 4.16 (d, 3H, $J = 15.6$ Hz, AB), 4.13 (d, 3H, $J = 15.6$ Hz), 3.67 (s, 6H), 3.47 (t, 3H, $J = 6.4$ Hz), 2.67 (d, 6H, $J = 6.4$ Hz); ^{13}C NMR (125 MHz, D_2O) δ 177.3, 171.6, 138.7, 136.0, 133.2, 124.4, 117.1, 54.6, 42.7, 42.6, 31.8. HRMS Calcd. for $C_{33}H_{45}N_{15}O_6$: 747.3677. Found: 747.3670.

For t GGH: 1H NMR (500 MHz, D_2O) δ 7.35 (s, 3H), 6.79 (s, 3H), 6.66 (s, 3H), 4.10 (d, 3H, $J = 15.8$ Hz), 4.07 (d, 3H, $J = 15.8$ Hz), 3.74 (s, 6H), 3.68 (s, 6H), 3.42 (t, 3H, $J = 6.4$ Hz), 2.60 (d, 6H, $J = 6.4$ Hz); ^{13}C NMR (125 MHz, D_2O) δ 177.8, 172.3, 171.3, 138.6, 136.0, 133.2, 125.0, 117.2, 54.4, 42.8, 42.7, 42.5, 31.8. HRMS Calcd. for $C_{39}H_{54}N_{18}O_9$: 918.4321. Found: 918.4328.

For t OH: 1H NMR (500 MHz, D_2O) δ 7.22 (s, 3H), 6.83 (s, 3H), 6.60 (s, 3H), 4.11 (s, 6H), 4.07 (m, 3H), 3.49 (t, 3H, $J = 6.4$ Hz), 2.66 (d, 6H, $J = 6.4$ Hz); 2.04 (t, 6H, $J = 8.2$ Hz), 1.84 (m, 3H), 1.71 (m, 3H); ^{13}C NMR (125 MHz, D_2O) δ 177.8, 176.3, 173.1, 138.8, 136.0, 133.0, 125.0, 117.1, 54.5, 53.4, 42.8, 31.8, 31.1, 26.9. HRMS Calcd. for $C_{39}H_{54}N_{18}O_9$: 918.4321. Found: 918.4315.

For t AH: 1H NMR (500 MHz, D_2O) δ 7.31 (s, 3H), 6.85 (s, 3H), 6.73 (s, 3H), 4.20 – 4.10 (m, 9H), 3.20 (t, 3H, $J = 6.5$ Hz), 2.71 (d, 6H, $J = 6.5$ Hz); 1.22 (d, 9H, $J = 7.1$ Hz). ^{13}C NMR (125 MHz, D_2O) δ 174.8, 167.9, 138.8, 134.5, 125.9, 124.7, 118.6, 52.3, 51.8, 43.0, 29.8, 16.9. HRMS Calcd. for $C_{36}H_{51}N_{15}O_6$: 789.4147. Found: 789.4152.

Cu (II) Estimation of C, 1 and 2 by UV-VIS Spectrophotometry :

Cu (II) was estimated by complexation with EDTA. Measured amount of Cu (II) solution (CuCl₂·2H₂O in 250 mM HEPES Buffer, pH=7.0) was added to the EDTA disodium salt solution (50 mM in 250 mM HEPES, pH=7.0). The mixture was shaken well and kept for 5 minutes for complete complexation. The reagent blank was prepared in the same way but without addition of Cu (II) solution. A calibration curve was made for Cu (II)-EDTA complex within the linear dynamic range (LDR) followed by UV-VIS at 730 nm against the respective reagent blanks (Temperature = 25 °C)

In the next step, the synthetic Cu (II) complexes (**C**, **1** and **2**) were subjected to the same process and the Cu (II) content was determined directly from the calibration curve.

Comparison of Results:

Synthetic Cu (II) Complexes	Cu (II) Found (ppm)	Cu (II) Present (ppm)*	Difference (ppm)
C	175.59	174.99	-0.6
1	531.99	533.93	1.94
2	474.67	475.87	1.2

*Amount of Cu is calculated assuming one Cu (II) and three Cu (II) per molecule for the control and complexes **1** and **2** respectively.

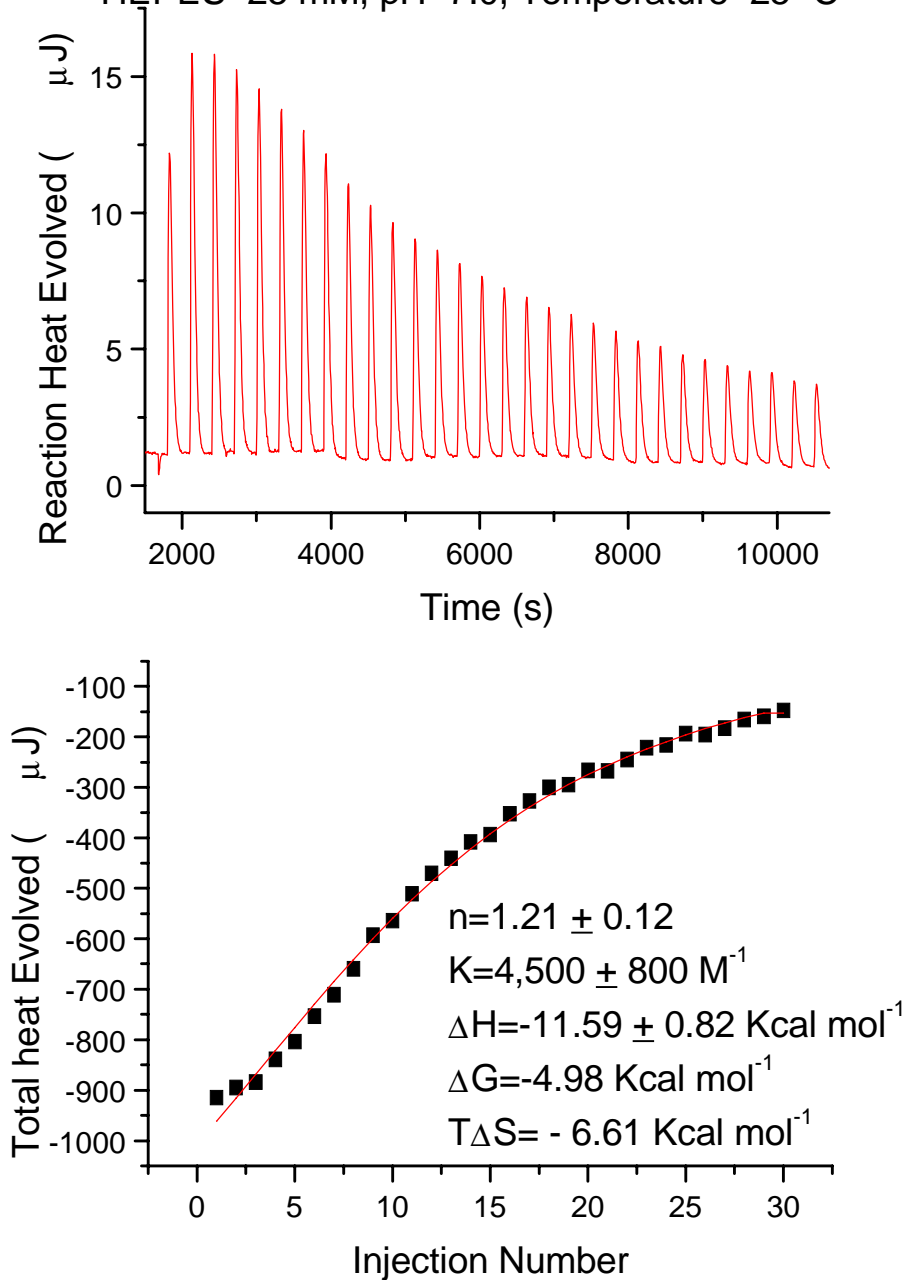
ITC Titration Data (Raw and Processed):

C-m H Titration:

Mono Cu Receptor and Mono histidine Titration

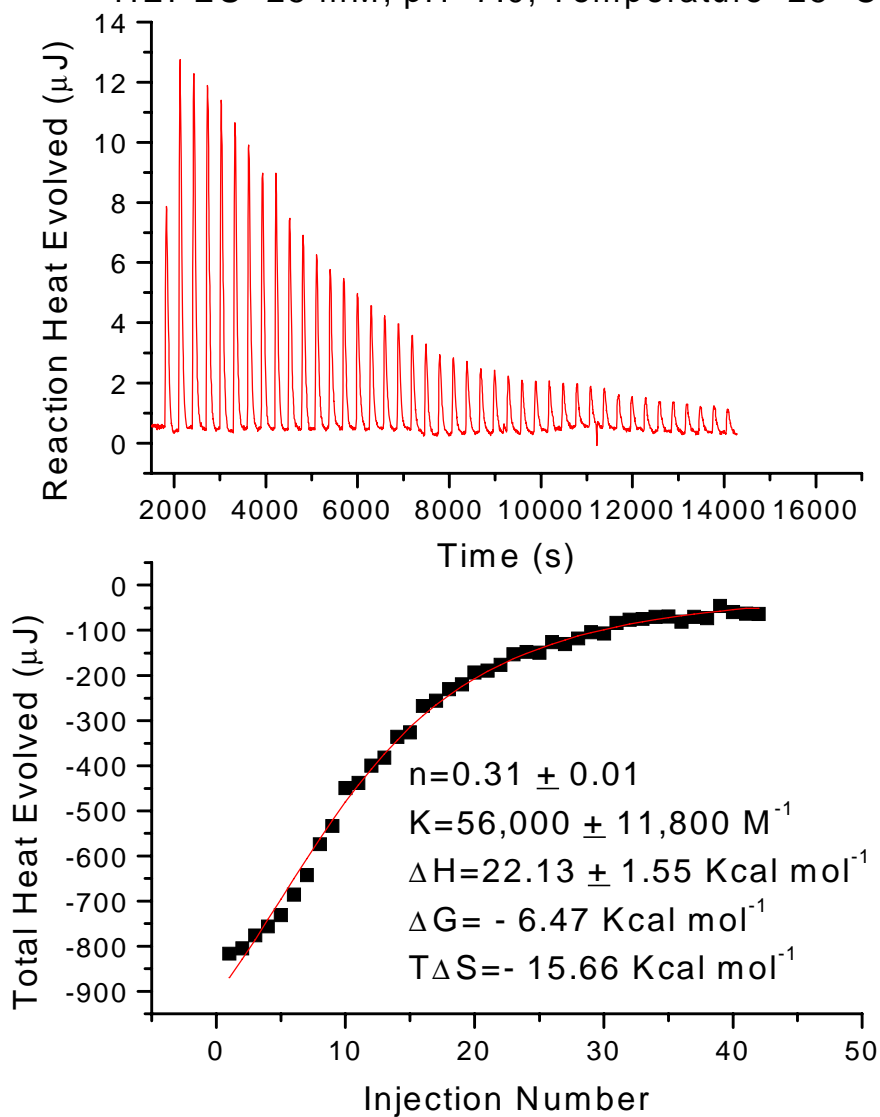
[M-Cu]=0.2 mM, [M-His]=7.0 mM

HEPES=25 mM, pH=7.0, Temperature=25 °C



C-t H Titration:

Mono Cu Receptor and Tris Histidine Titration
[m-Cu]=0.3 mM. [TH]=2.0 mM
HEPES=25 mM, pH=7.0, Temperature=25 °C

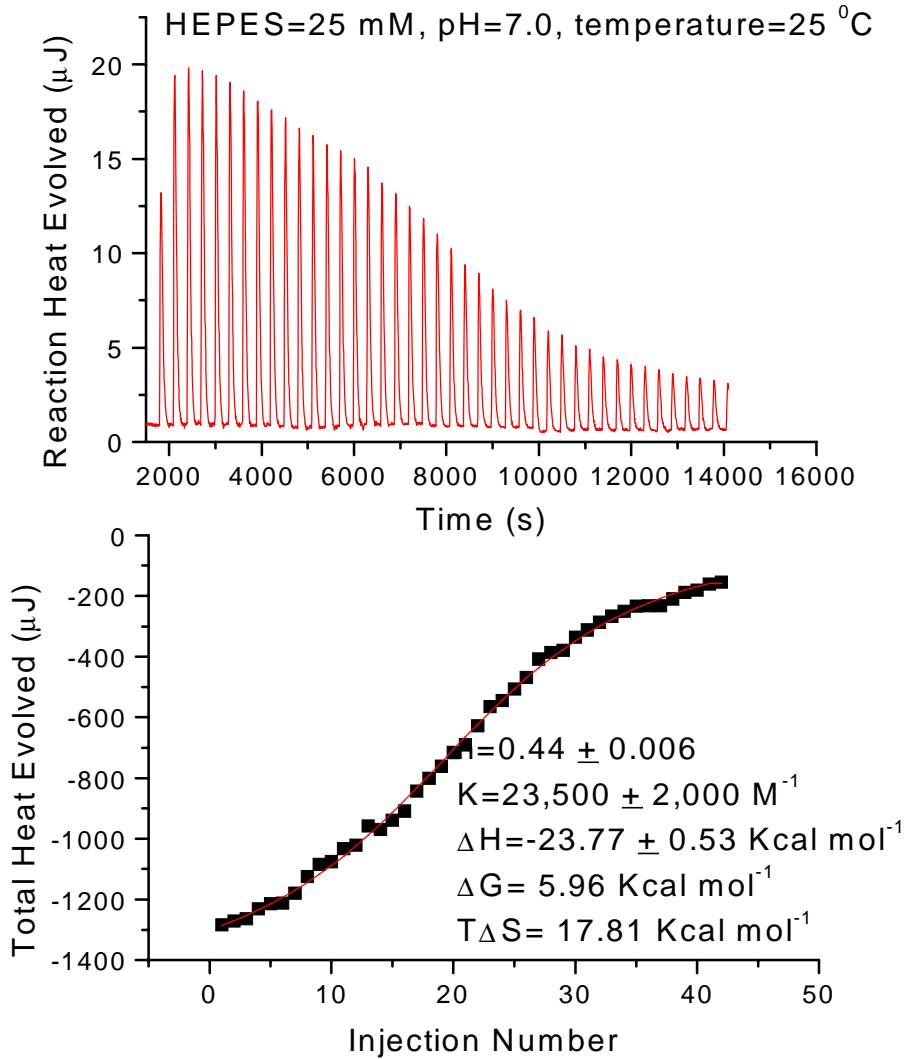


C-t GH Titration:

Mono Cu Receptor and Tris Gly Histidine

[m-Cu]=0.6 mM, [TGH]=3.0 mM

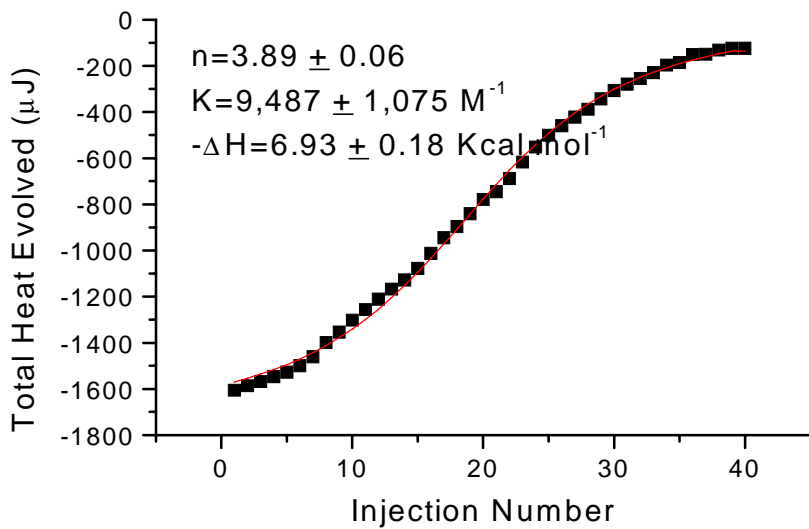
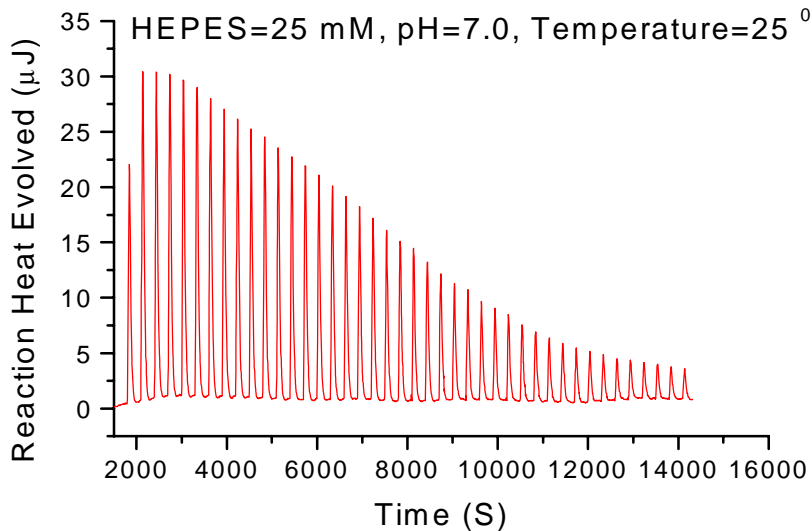
HEPES=25 mM, pH=7.0, temperature=25 °C



1-m H Titration:

Tris Cu Receptor and Mono Histidine Titration
[TCu]=0.25 mM, [THis]=12.0 mM

HEPES=25 mM, pH=7.0, Temperature=25 °C

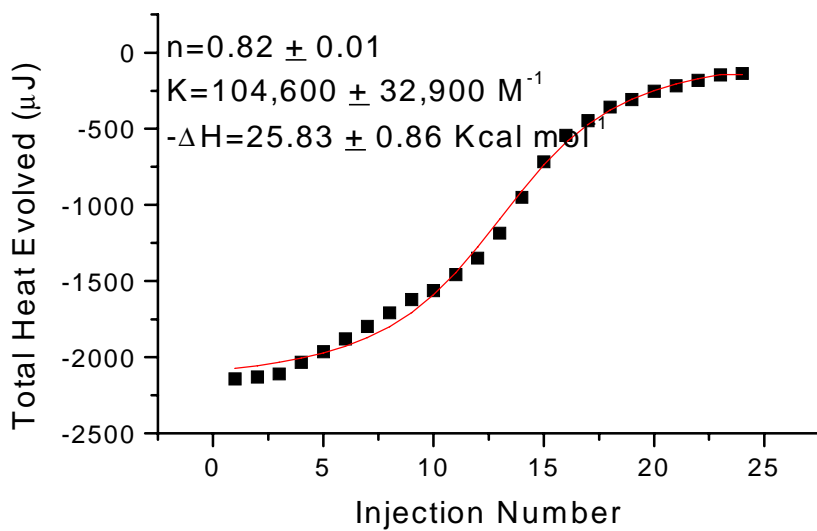
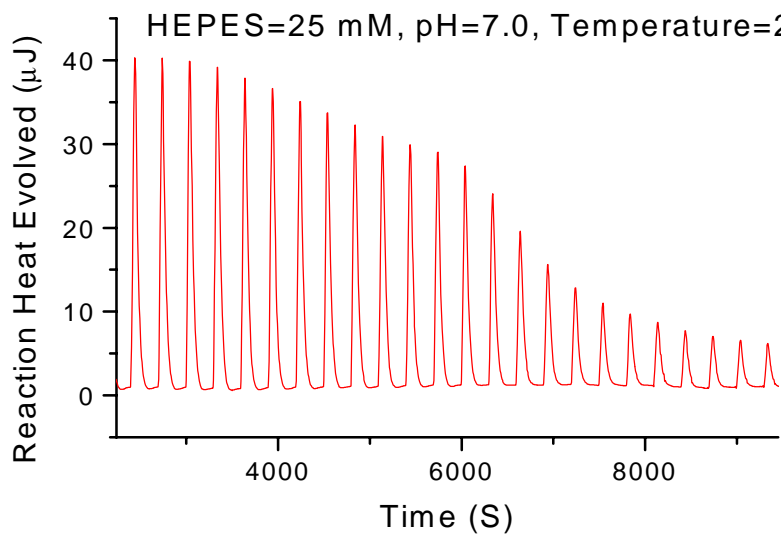


1-t H Titration:

Tris Cu Receptor and Tris Histidine Titration

[TCu]=0.25 mM, [THis]=4.0 mM

HEPES=25 mM, pH=7.0, Temperature=25 °C

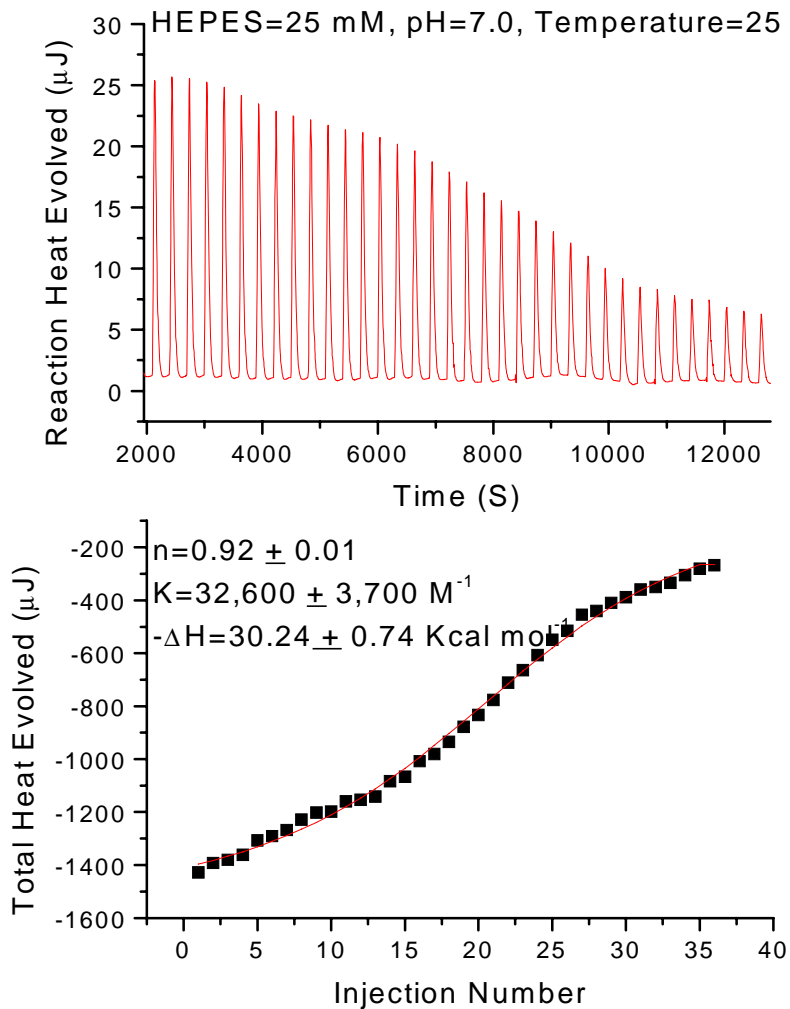


1-t GH Titration:

Tris Cu Receptor and Tris Glycine Histidine Titration

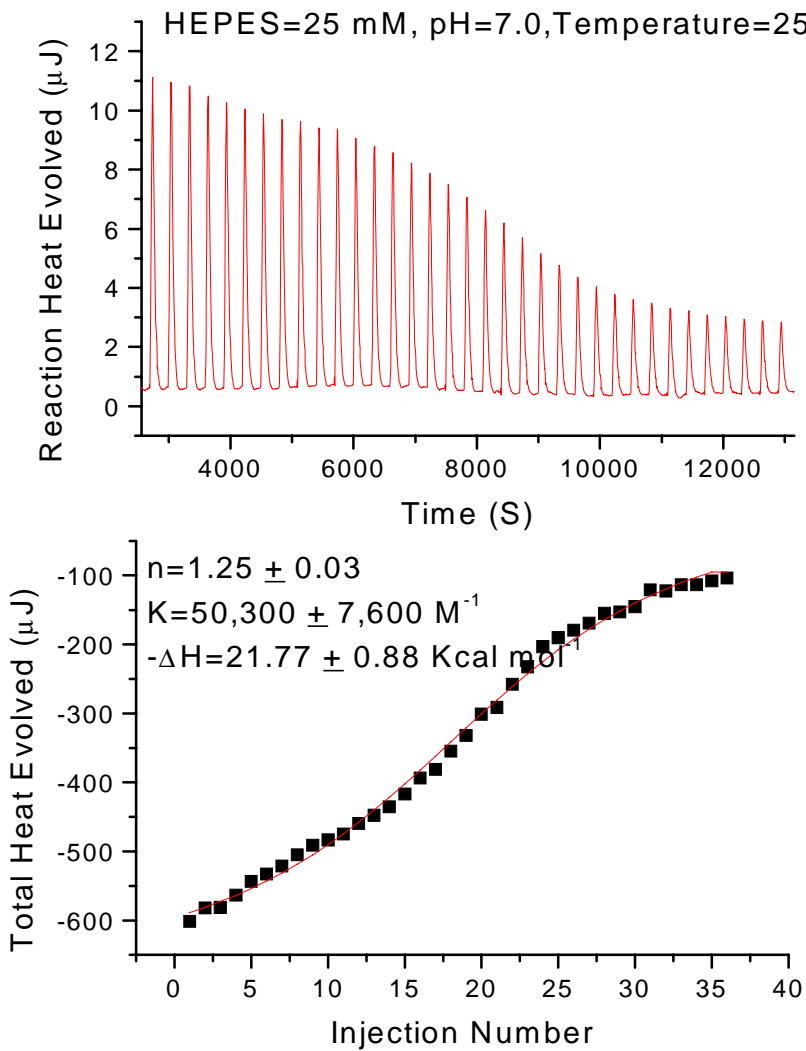
[TCu]=0.25 mM, [TGHis]=2.5 mM

HEPES=25 mM, pH=7.0, Temperature=25 °C

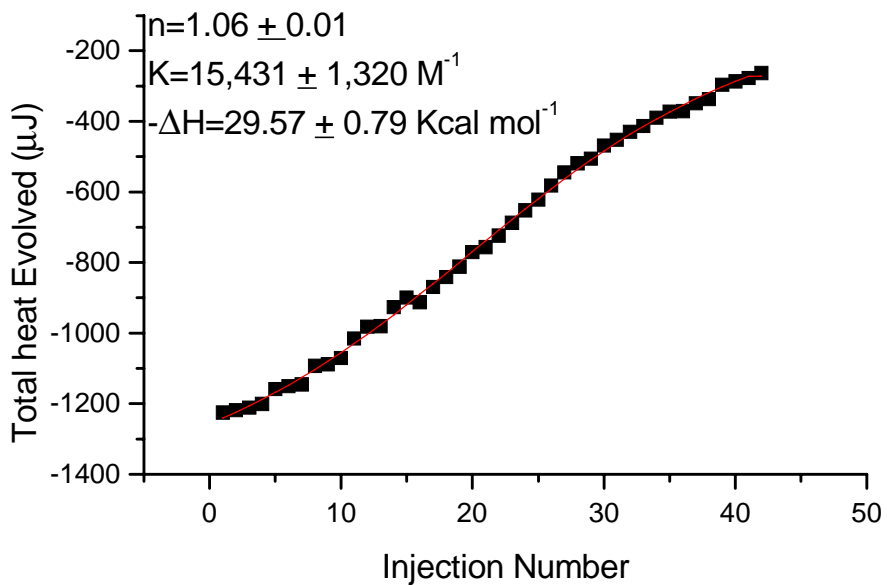
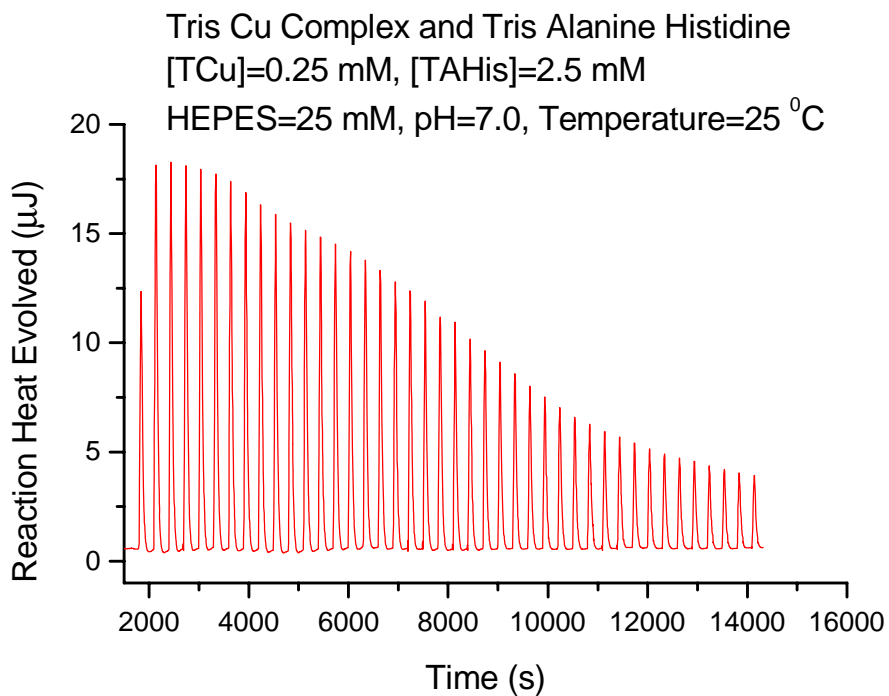


1-t GGH Titration:

Tris Cu Receptor and
Tris Glycine Glycine Histidine Titration
[TCu]=0.1 mM, [TGGHis]=1.5 mM
HEPES=25 mM, pH=7.0, Temperature=25 °C



1-t AH Titration:

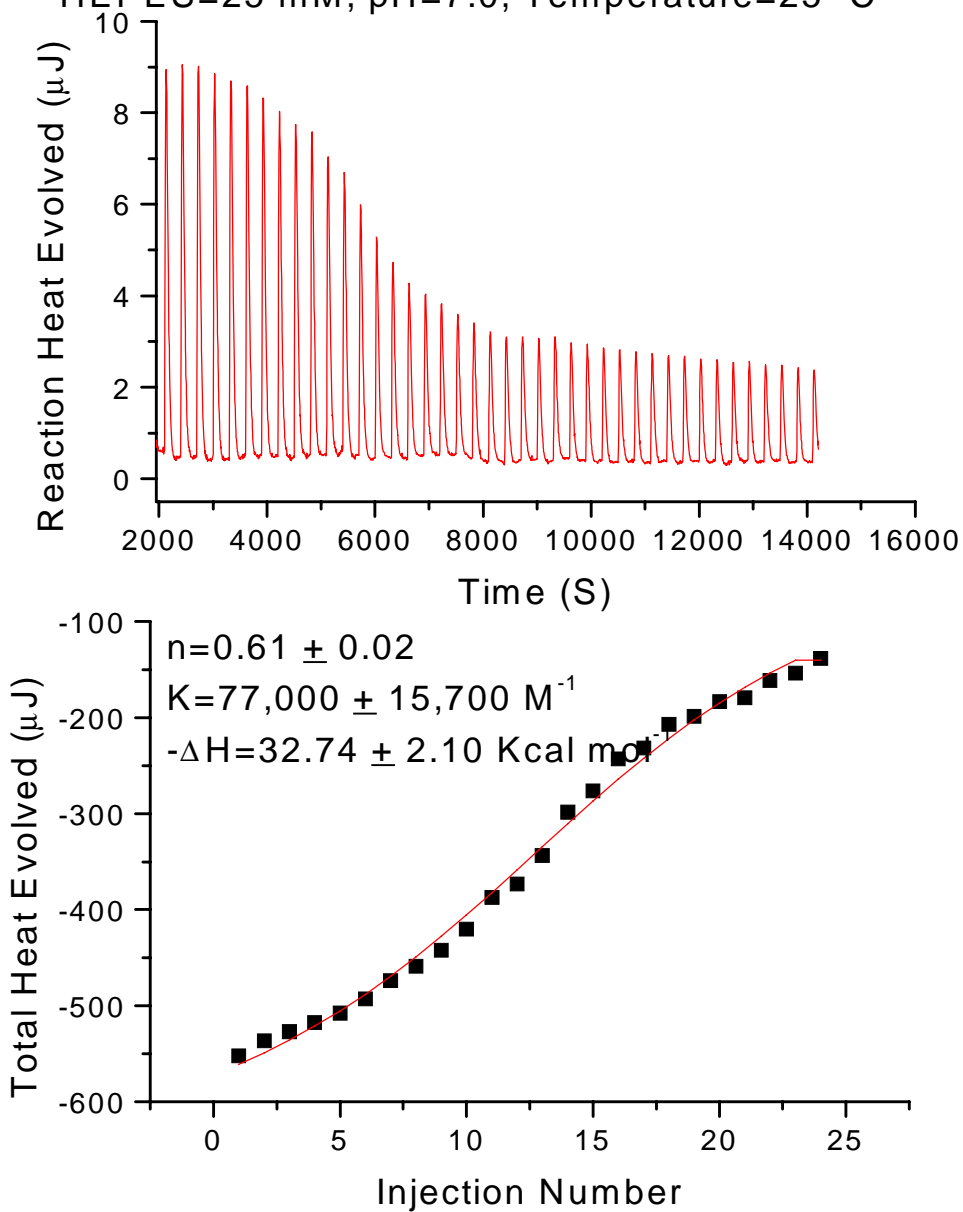


1-t QH Titration:

Tris Cu Receptor and Tris Glutamin Histidine Titration

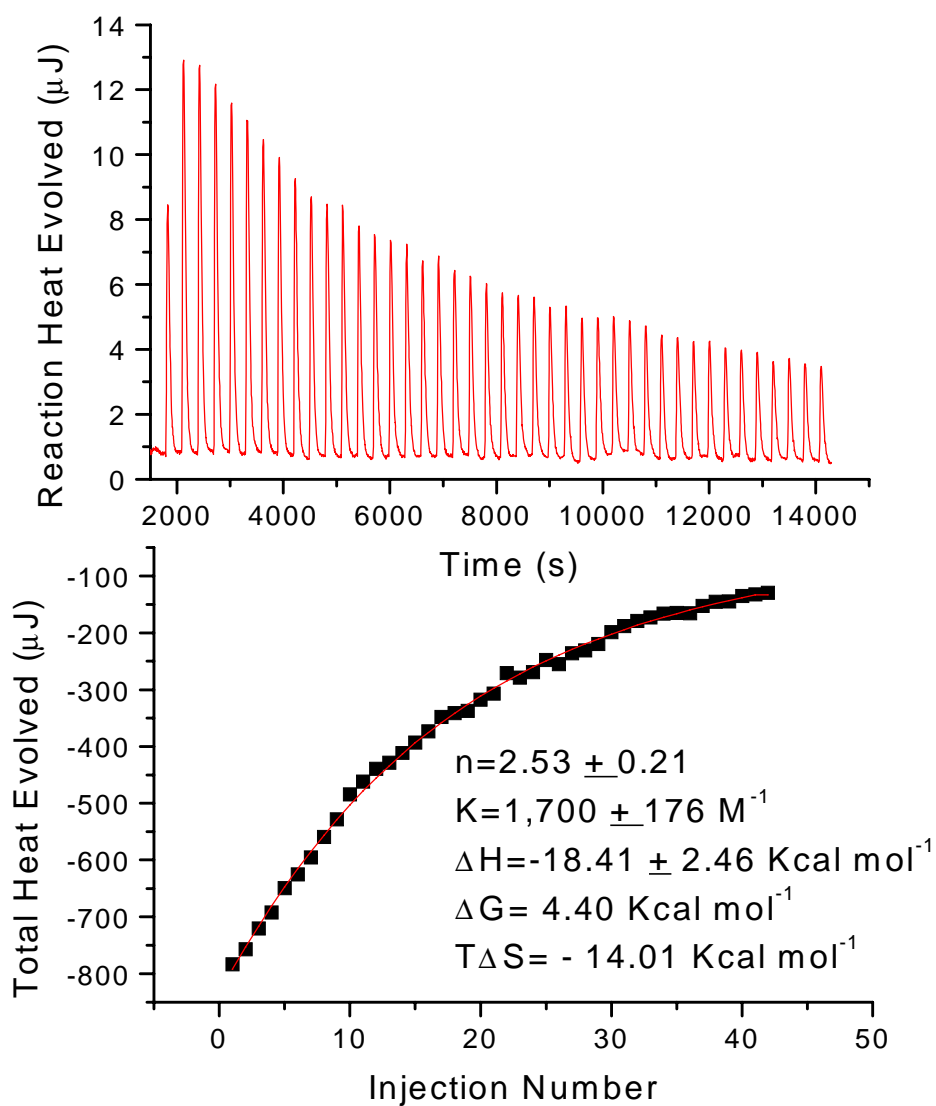
[TCu]=0.1 mM, [TGLHis]=1.0 mM

HEPES=25 mM, pH=7.0, Temperature=25 °C



2-m H Titration:

Tris Amide Cu Receptor and Mono Histidine Titration
[TA-Cu]=0.1 mM, [Mono His]=7.0 mM
HEPES=25 mM, pH=7.0, Temperature=25 °C

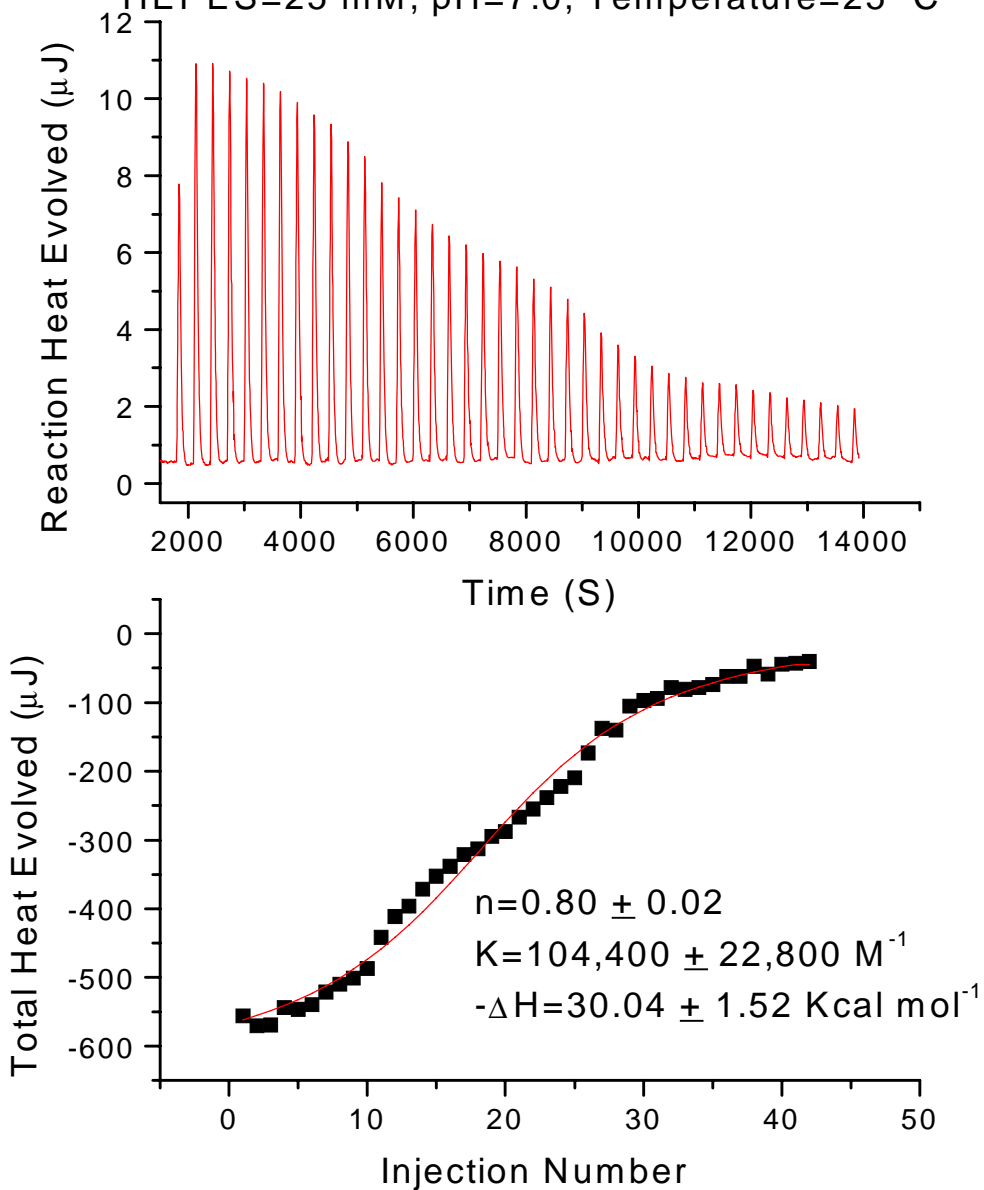


2-t H Titration:

Tris Amide Cu Receptor and Tris Histidine Titration

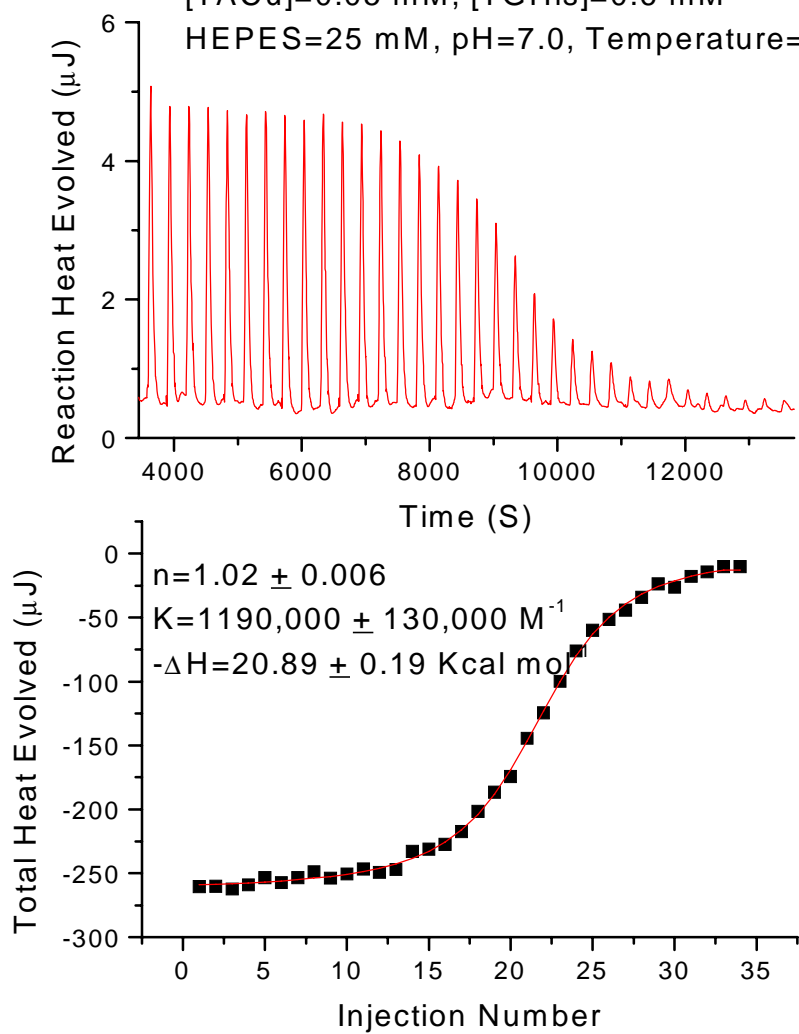
[TACu]=0.1 mM, [THis]=1.0 mM

HEPES=25 mM, pH=7.0, Temperature=25 °C



2-t GH Titration:

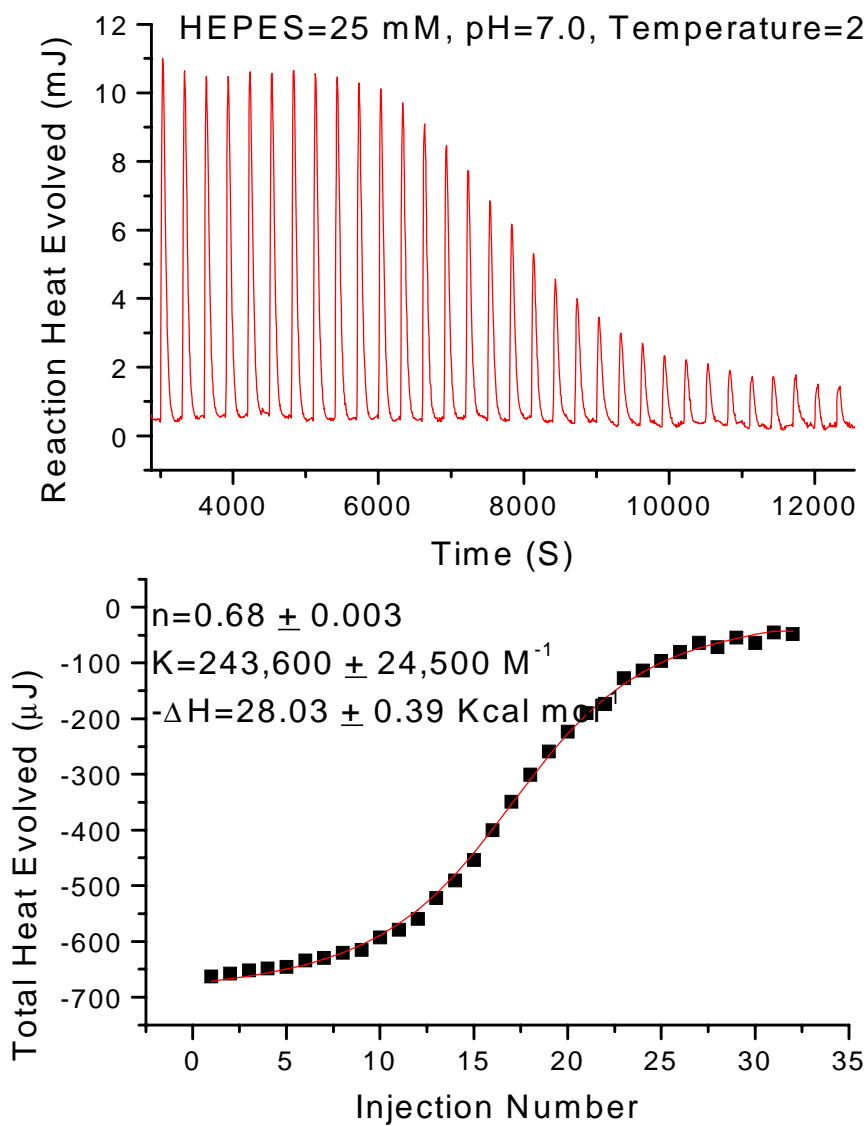
Tris Amide Cu Receptor and
Tris Glycine Histidine Titration
[TACu]=0.05 mM, [TGHis]=0.6 mM
HEPES=25 mM, pH=7.0, Temperature=25 °C



2-t GGH Titration:

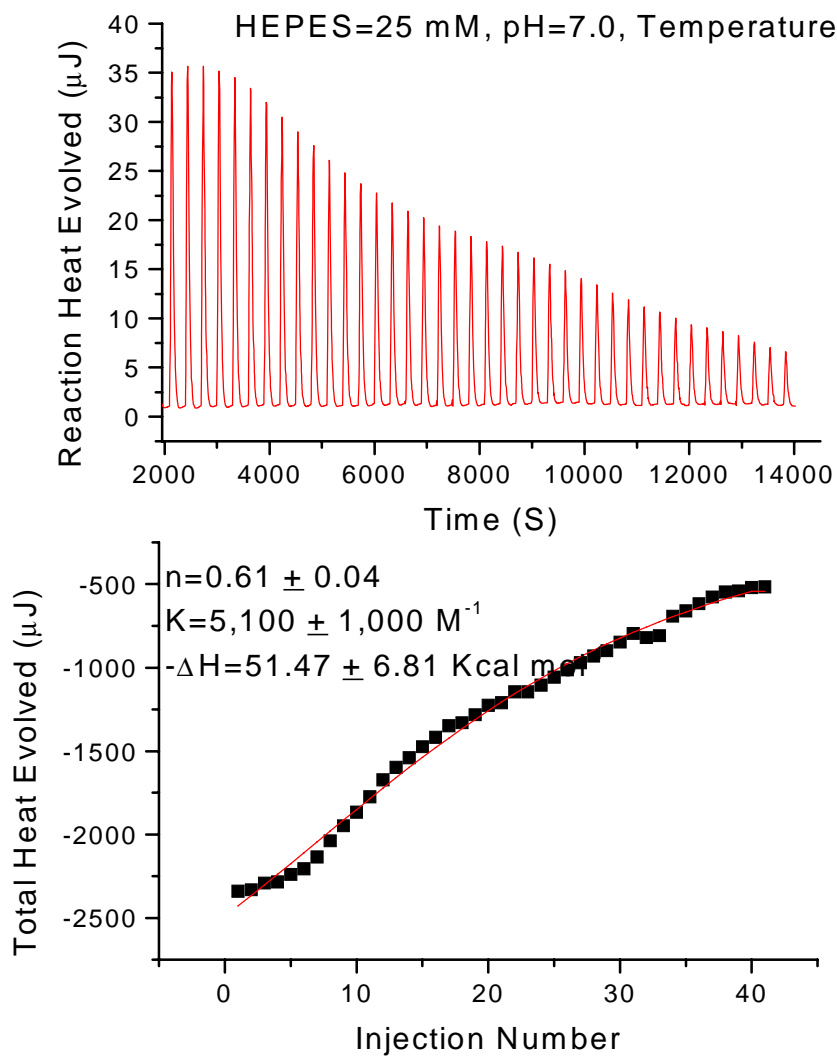
Tris Amide Cu Receptor and
Tris Glycine Glycine Histidine Titration
[TACu]=0.12 mM, [TGGHis]=1.2 mM

HEPES=25 mM, pH=7.0, Temperature=25 °C



2-t AH Titration:

Tris Amide Cu Receptor and
Tris Alanine Histidine Titration
[TAGu]=0.5 mM, [TAHis]=3.75 mM
HEPES=25 mM, pH=7.0, Temperature=25 °C



2-t OH Titration:

Tris Amide Cu Receptor and
Tris Glutamin Histidine Titration
[TACu]=0.1 mM, [TGLHis]=0.75 mM
HEPES=25 mM, pH=7.0, Temperature=25 °C

