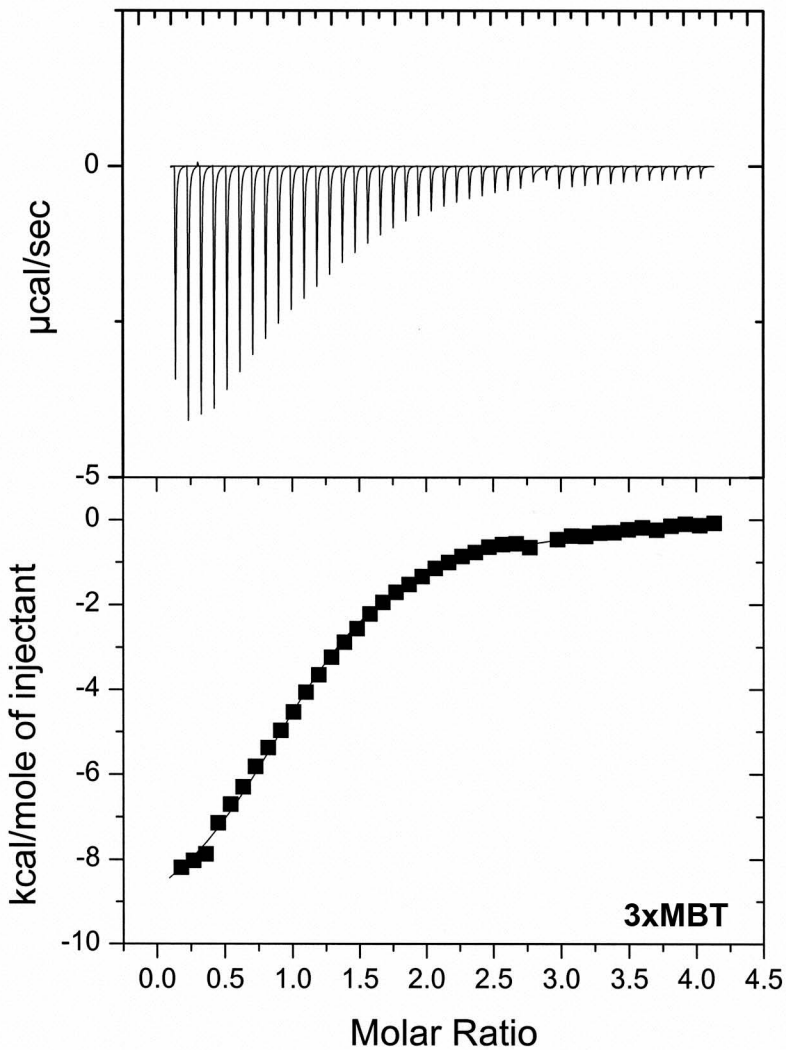


Time (min)

-100 1020304050607080901001020304050607080

H4K20me1

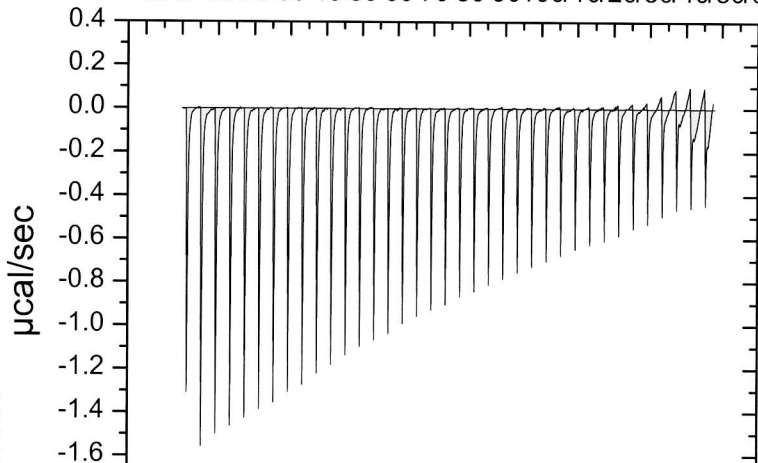


3xMBT

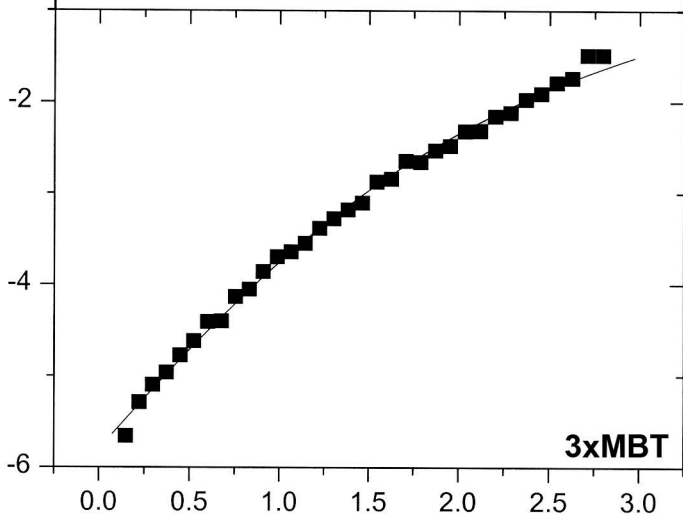
Time (min)

-10 0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160

H3K9me1



kcal/mole of injectant



Molar Ratio

	H4K20me1	H3K9me1
n	0.98	1.08
ΔH (kcal/mol)	12.9	12.1
TΔS (kcal/mol)	6.4	6.0
ΔG (kcal/mol)	6.5	6.1
K_D (μM)	17	32

Isothermal titration calorimetry (ITC) was performed to confirm binding to (A) an H4K20me1 peptide and (B) an H3K9me1 peptide. Injection curves from representative experiments are shown. ITC experiments were performed using a Microcal-VP-ITC instrument (Microcal Inc., Northampton, MA). The concentration of protein in the reaction cell was 110 μM (stirred at 310 rpm); peptides were injected at 1.2 mM. Measurements were performed at 27°C with 50 injections (7 μl each over 14 seconds with a spacing delay of 240 secs). Data analysis was performed using the Microcal program package. The single site-binding constant (K_D), the heat of binding (ΔH), and the number of binding sites (n) were measured. The free energy (ΔG) and entropy (ΔS) changes of binding were calculated using the following equations: $\Delta G = -RT \ln K_D$ and $\Delta G = \Delta H - T\Delta S$, respectively.