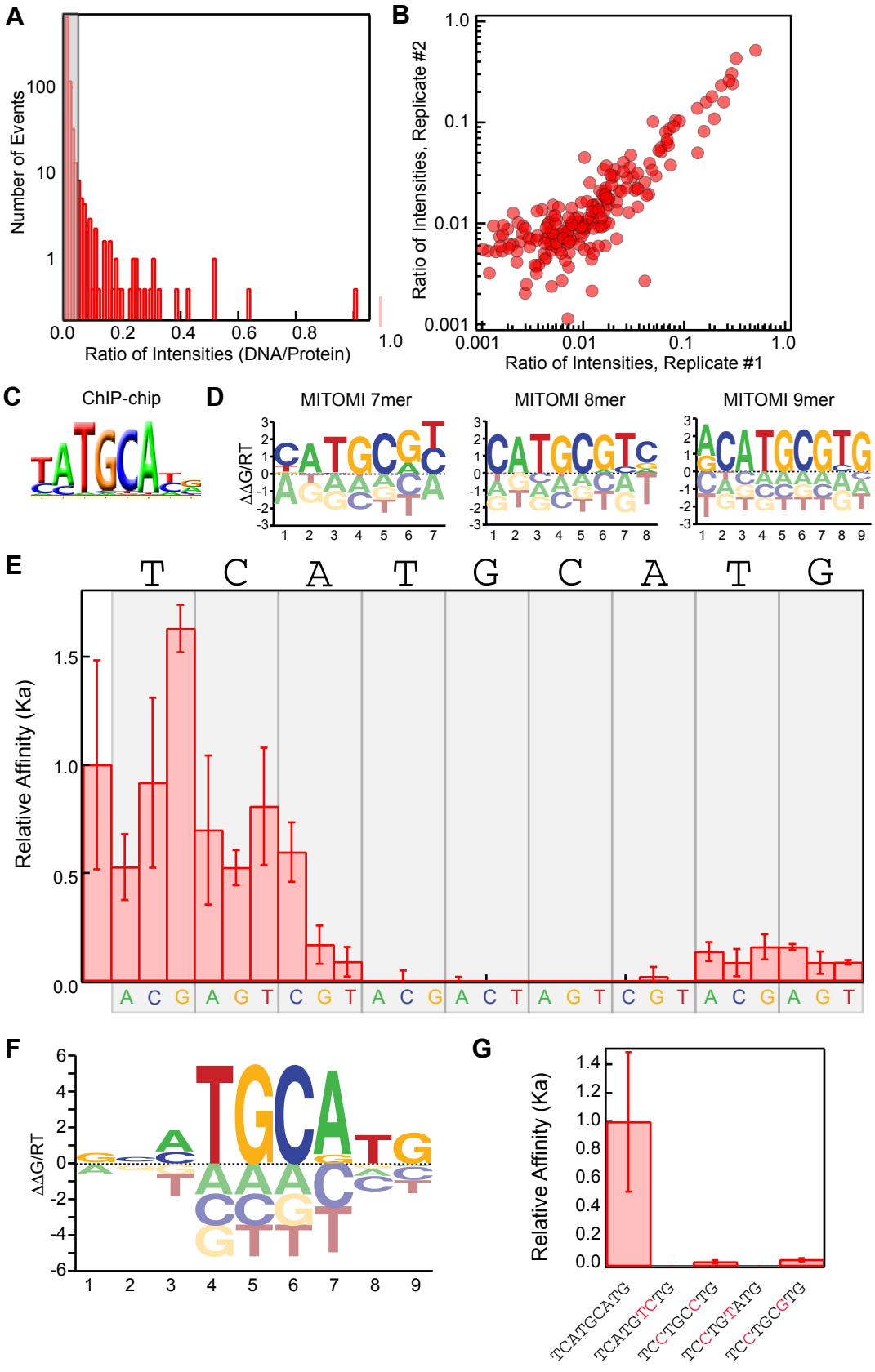
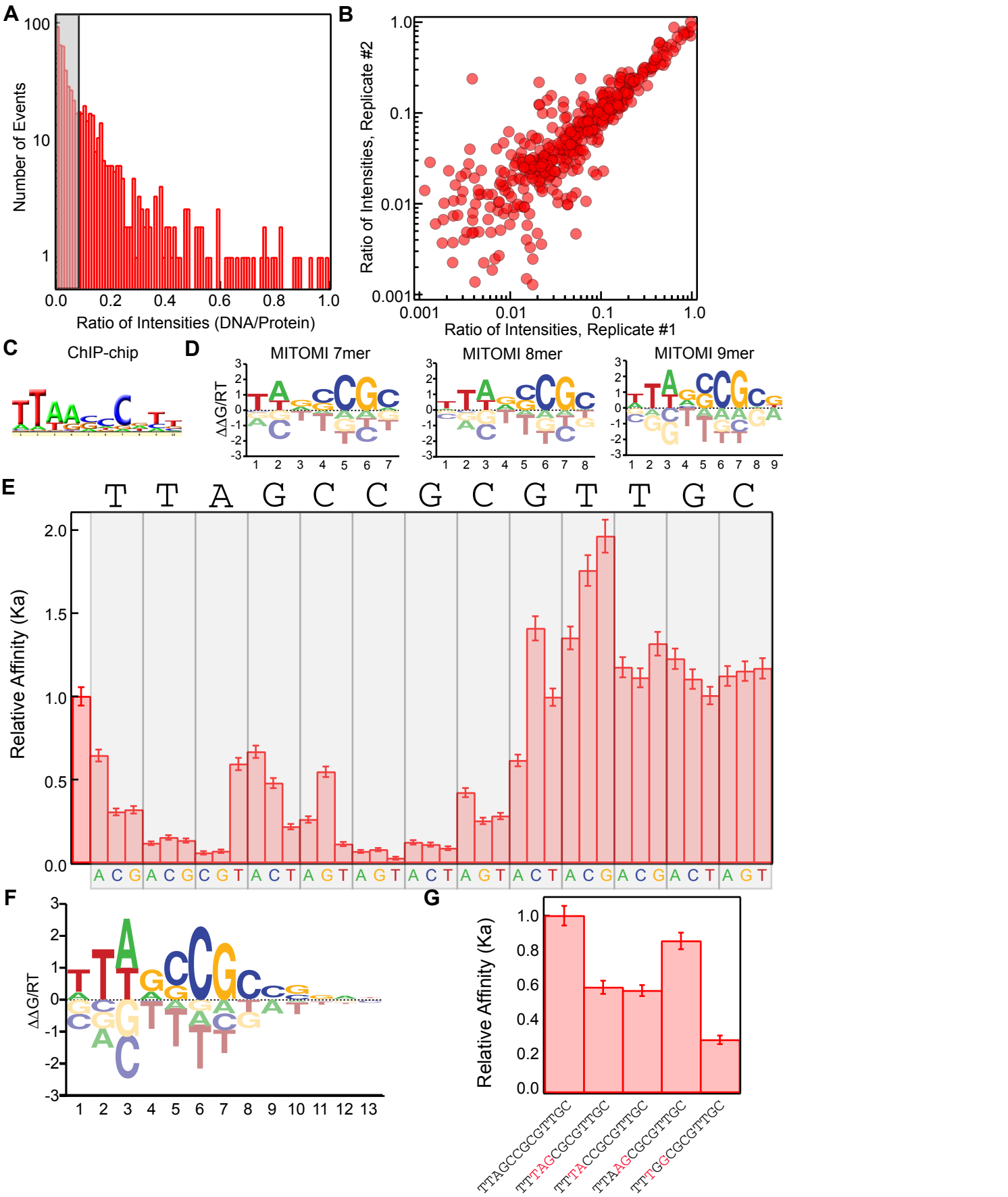
Efg1 DNA binding preferences determined via microfluidic affinity analysis.

(A) Raw data from a MITOMI 2.0 experiment assessing binding of Efg1 to a pseudorandom 8mer oligonucleotide library. Distribution of measured fluorescence intensity ratios (DNA/Protein); grey bar shows four standard deviations from the mean as determined by a Gaussian fit to the distribution centered around zero. (B) Scatter plot showing measured intensity ratios for two printed replicates of each oligonucleotide. (C) ChIP-chip developed Efg1 motif. (D) Highest scoring 7-, 8-, and 9-bp PSAMs from MITOMI 2.0 analysis of Efg1 binding to a pseudorandom 8mer library. Each motif is represented as an AffinityLogo, with the relative height of each letter denoting the contribution to overall binding affinity. (E) Measured binding affinities (K_a) relative to the "consensus" site affinity (5'-TCATGCATG) for systematic substitutions of alternate nucleotides at each position. Error bars represent the standard error on the mean. (F) AffinityLogo representation of the PSAM derived from relative affinities shown in (E). (G) Measured binding affinities (K_a) relative to the "consensus" site affinity for systematic substitutions of pairs of nucleotides.


A
Number of Events
100
10
1
0.0
0.2
0.4
0.6
0.8
1.0
Ratio of Intensities (DNA/Protein)
B
Ratio of Intensities, Replicate #2
1.0
0.1
0.01
0.001
0.001
0.01
0.1
1.0
Ratio of Intensities, Replicate #1
C
ChIP-chip
D
MITOMI 7mer
MITOMI 8mer
MITOMI 9mer
ΔΔG/RT
E
T C A T G C A T G
Relative Affinity (Ka)
1.5
1.0
0.5
0.0
A C G A G T C G T A C G A C T A G T C G T A C G A G T
F
ΔΔG/RT
G
Relative Affinity (Ka)
TCATGCATG
TCATGTCTG
TCCTGCCTG
TCCTGTATG
TCCTGCGTG

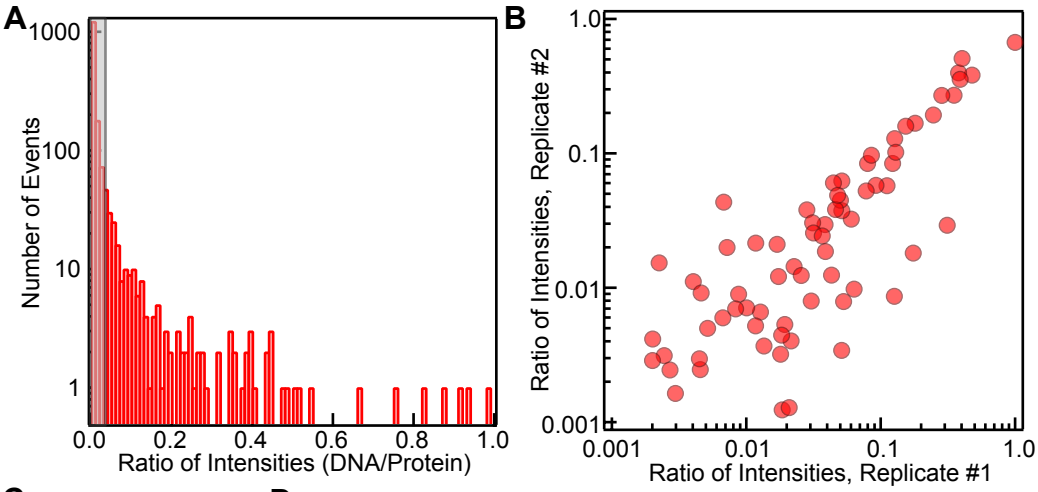
Czf1 DNA binding preferences determined via microfluidic affinity analysis.

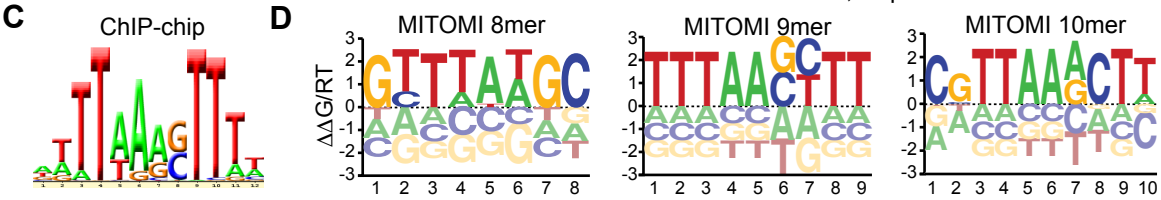
(A) Raw data from a MITOMI 2.0 experiment assessing binding of Czf1 to a pseudorandom 8mer oligonucleotide library. Distribution of measured fluorescence intensity ratios (DNA/Protein); grey bar shows four standard deviations from the mean as determined by a Gaussian fit to the distribution centered around zero. (B) Scatter plot showing measured intensity ratios for two printed replicates of each oligonucleotide. (C) ChIP-chip developed Czf1 motif. (D) Highest scoring 7-, 8-, and 9-bp PSAMs from MITOMI 2.0 analysis of Czf1 binding to a pseudorandom 8mer library. (E) Measured binding affinities (K_a) relative to the “consensus” site affinity (5’-TTAGCCGCGTTGC) for systematic substitutions of alternate nucleotides at each position. Relative affinities were determined via global fits of measured concentration-dependent binding to a single-site binding model. Error bars represent the standard error of the mean. (F) AffinityLogo representation of the PSAM derived from relative affinities shown in (E). (G) Measured binding affinities (K_a) relative to the “consensus” site affinity for systematic substitutions of pairs of nucleotides.

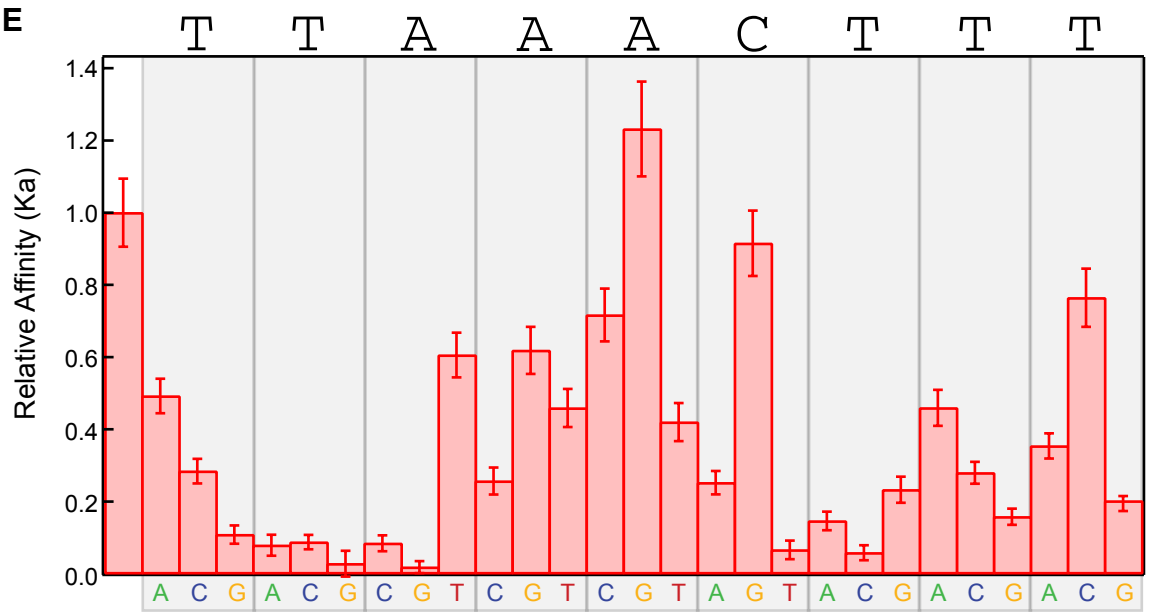

A
Number of Events
Ratio of Intensities (DNA/Protein)
B
Ratio of Intensities, Replicate #2
Ratio of Intensities, Replicate #1
C
ChIP-chip
D
MITOMI 7mer
MITOMI 8mer
MITOMI 9mer
ΔΔG/RT
E
T T A G C C G C G T T G C
Relative Affinity (Ka)
A C G A C G C G T A C T A G T A G T A C T A G T A C T A C G A C G A C T A G T
F
ΔΔG/RT
G
Relative Affinity (Ka)
TTAGCCGCGTTGC
TTTAGCGCGTTGC
TTTACCGCGTTGC
TTAAGCGCGTTGC
TTTGGCGCGTTGC

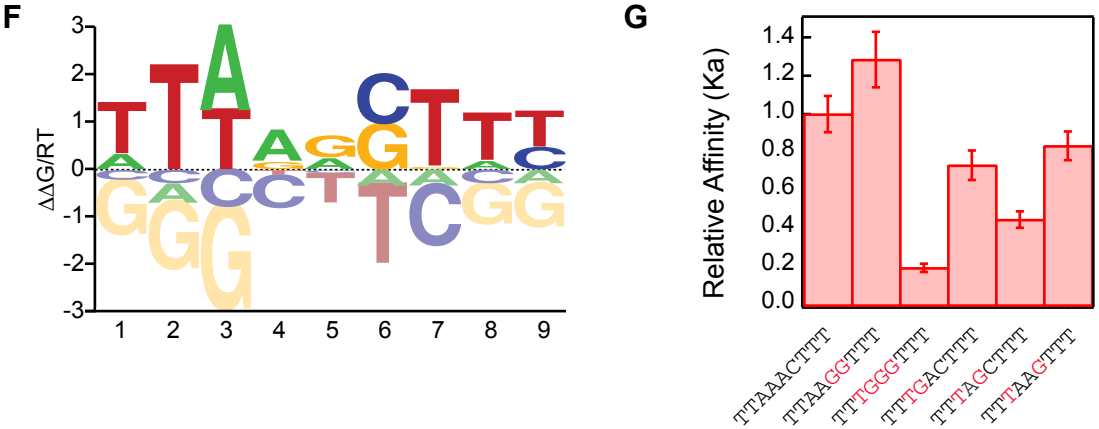
Wor1 DNA binding preferences determined via microfluidic affinity analysis.

(A) Raw data from a MITOMI 2.0 experiment assessing binding of Wor1 to a pseudorandom 8mer oligonucleotide library. Distribution of measured fluorescence intensity ratios (DNA/Protein); grey bar shows four standard deviations from the mean as determined by a Gaussian fit to the distribution centered around zero. (B) Scatter plot showing measured intensity ratios for two printed replicates of each oligonucleotide. (C) ChIP-chip developed Wor1 motif. (D) Highest scoring 8-, 9-, and 10-bp PSAMs from MITOMI 2.0 analysis of Wor1 binding to a pseudorandom 8mer library. (E) Measured binding affinities (K_a) relative to the “consensus” site affinity (5’-TTAAACTTT) for systematic substitutions of alternate nucleotides at each position. Relative affinities were determined via global fits of measured concentration-dependent binding to a single-site binding model. Error bars represent the standard error of the mean. (F) AffinityLogo representation of the PSAM derived from relative affinities shown in (E). (G) Measured binding affinities (K_a) relative to the “consensus” site affinity for systematic substitutions of pairs of nucleotides.


A
Number of Events
Ratio of Intensities (DNA/Protein)
B
Ratio of Intensities, Replicate #2
Ratio of Intensities, Replicate #1

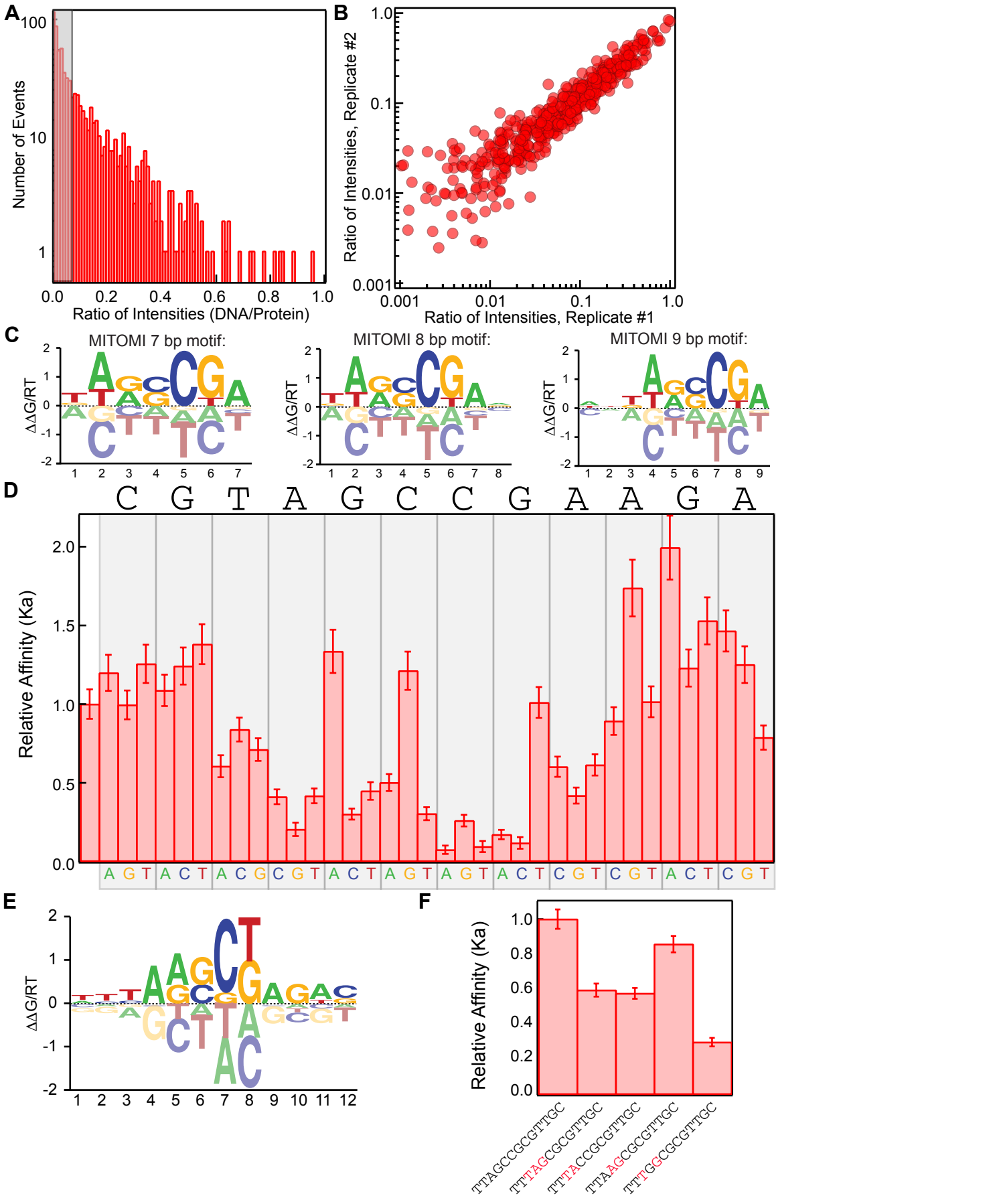

C
ChIP-chip
D
MITOMI 8mer
MITOMI 9mer
MITOMI 10mer
ΔΔG/RT


E
T T A A A C T T T
Relative Affinity (Ka)
A C G A C G C G T C G T C G T A G T A C G A C G A C G


F
ΔΔG/RT
G
Relative Affinity (Ka)
TTAAACTTT
TTAAGGTTT
TTTGGGTTT
TTTGACTTT
TTTAGCTTT
TTTAAGTTT

Wor2 DNA binding preferences determined via microfluidic affinity analysis.

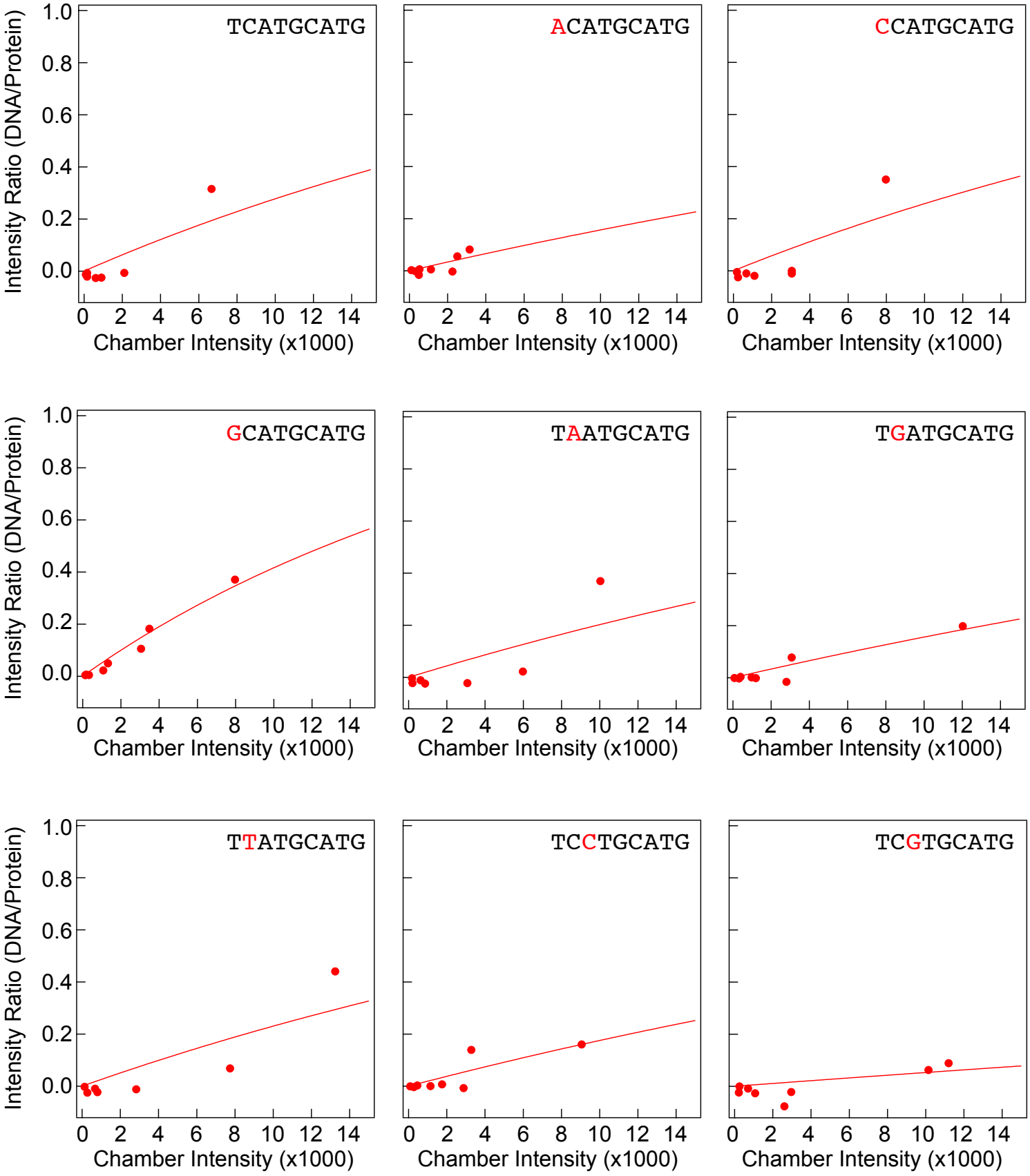
(A) Raw data from a MITOMI 2.0 experiment assessing binding of Wor2 to a pseudorandom 8mer oligonucleotide library. Distribution of measured fluorescence intensity ratios (DNA/Protein); grey bar shows four standard deviations from the mean as determined by a Gaussian fit to the distribution centered around zero. (B) Scatter plot showing measured intensity ratios for two printed replicates of each oligonucleotide. (C) Highest scoring 7-, 8-, and 9-bp PSAMs from MITOMI 2.0 analysis of Wor2 binding to a pseudorandom 8mer library. (D) Measured binding affinities (K_a) relative to the "consensus" site affinity (5'-CGTAGCCGAAGA) for systematic substitutions of alternate nucleotides at each position. Relative affinities were determined via global fits of measured concentration-dependent binding to a single-site binding model. Error bars represent the standard error of the mean. (E) AffinityLogo representation of the PSAM derived from relative affinities shown in (D). (F) Measured binding affinities (K_a) relative to the "consensus" site affinity for systematic substitutions of pairs of nucleotides.


A
Number of Events
Ratio of Intensities (DNA/Protein)
B
Ratio of Intensities, Replicate #2
Ratio of Intensities, Replicate #1
C
MITOMI 7 bp motif:
MITOMI 8 bp motif:
MITOMI 9 bp motif:
ΔΔG/RT
D
C G T A G C C G A A G A
Relative Affinity (Ka)
A G T A C T A C G C G T A C T A G T A G T A C T C G T C G T A C T C G T
E
ΔΔG/RT
F
Relative Affinity (Ka)
TTAGCCGCGTTGC
TTTAGCGCGTTGC
TTTACCGCGTTGC
TTAAGCGCGTTGC
TTTGGCGCGTTGC

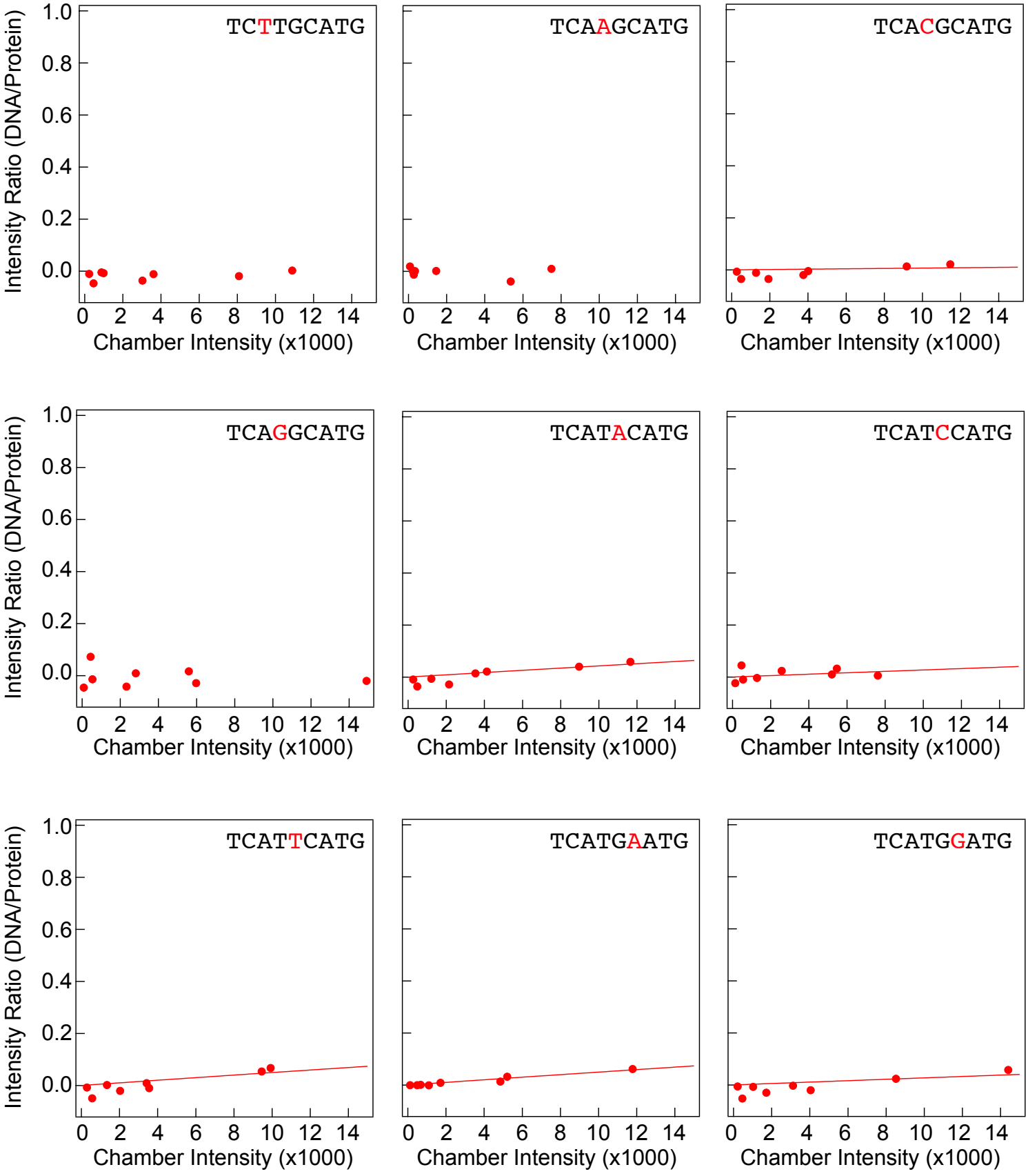
Series of concentration-dependent binding curves for a set of all possible single systematic mutations within the Efg1, Czf1, Wor1, and Wor2 *cis*-regulatory sequence and against sequences for the other regulators.

For each nucleotide, the measured fluorescence intensity ratios (y-axis, expressed as DNA/Protein, red circles) are plotted as a function of soluble DNA concentration (x-axis). For Wor1, Wor2, and Czf1 the solid red lines reflect global fits to a single-site binding model. Experiments assessing concentration-dependent binding for the Efg1 protein did not adequately capture both the linear and saturated binding regimes; therefore, we chose an arbitrary but reasonable constant maximum value for measured fluorescence intensity ratios and determined relative K_a values from individual single-site binding model fits.

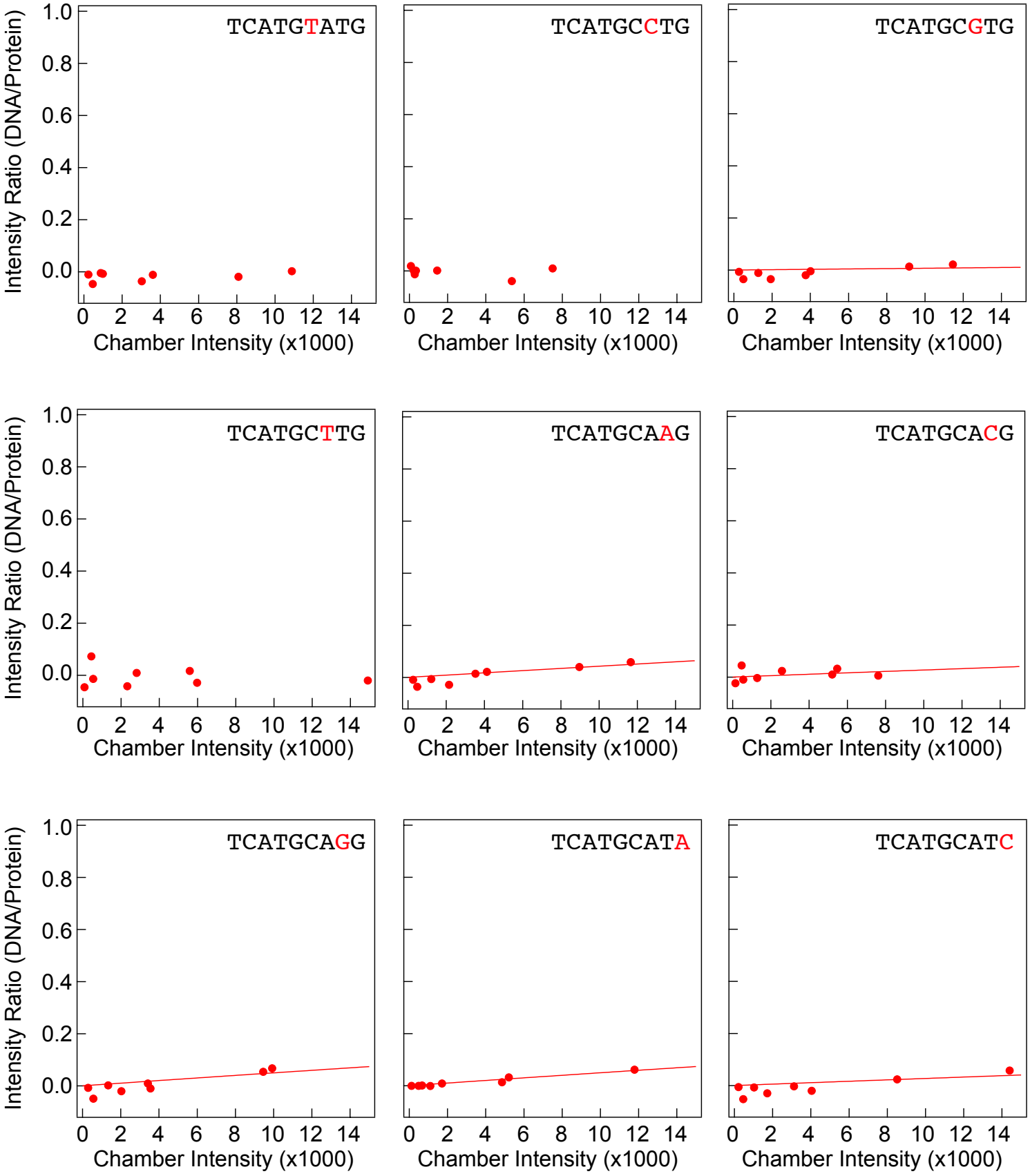
Efg1 Binding Curves, page 1



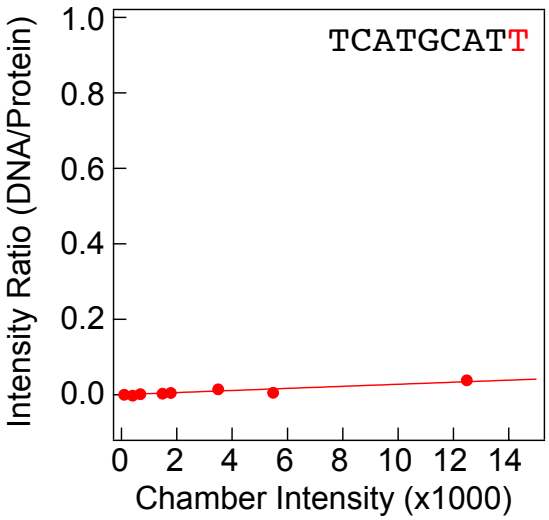
Efg1 Binding Curves, page 2

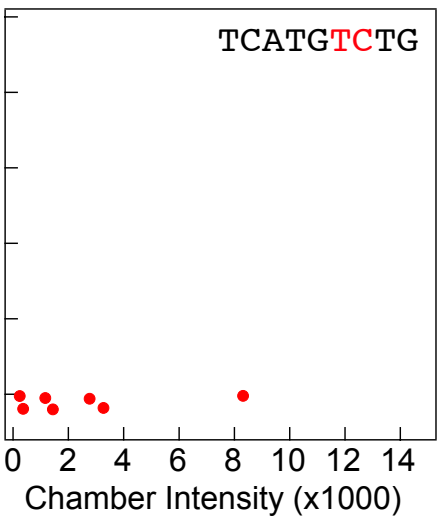


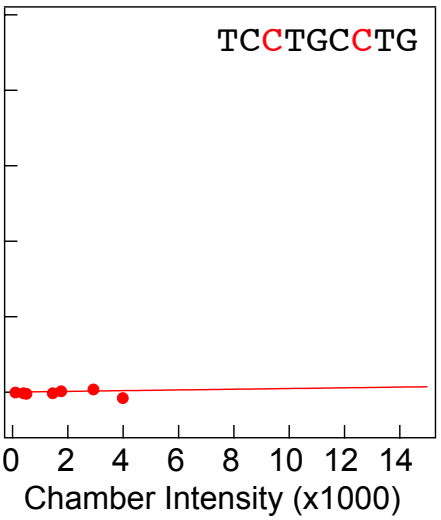
Efg1 Binding Curves, page 3

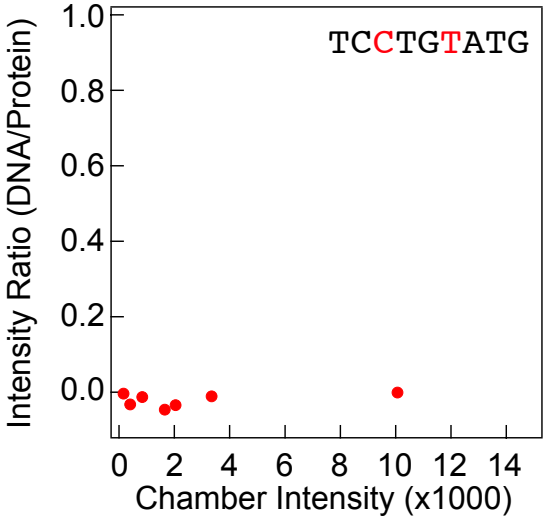


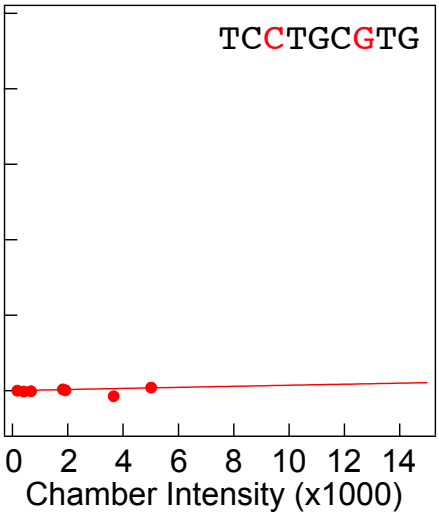
Efg1 Binding Curves, page 4



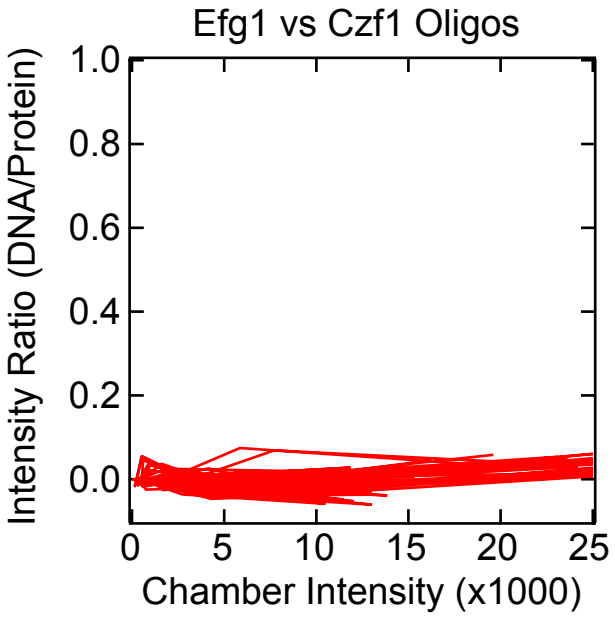


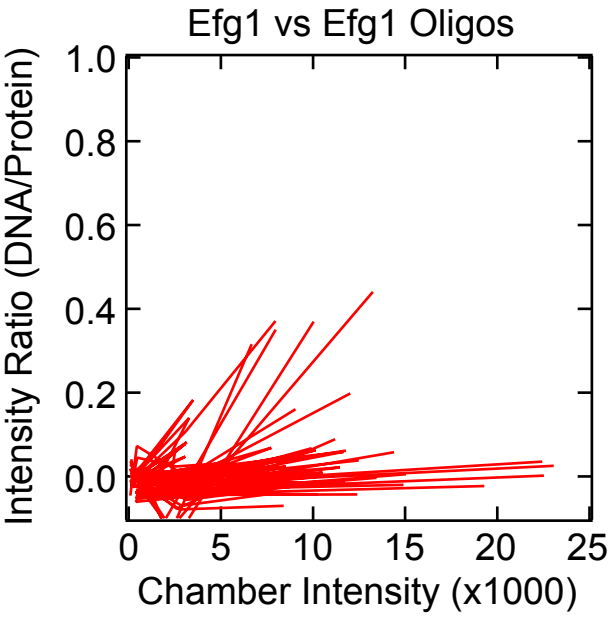


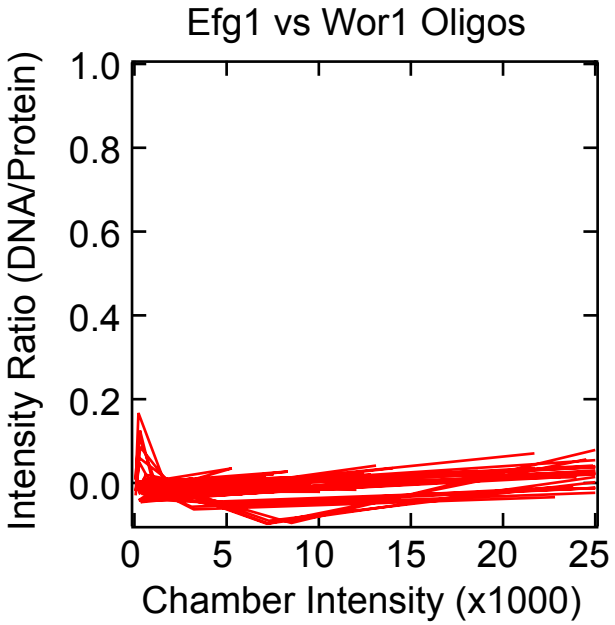


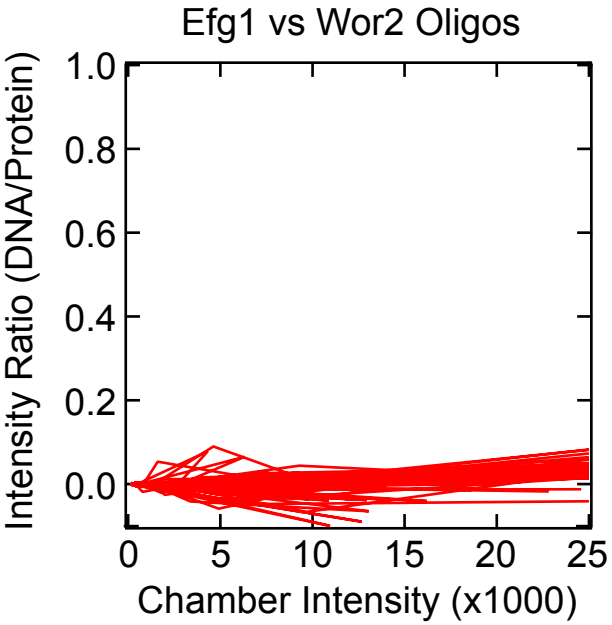


Efg1 Binding Curves, Page 5

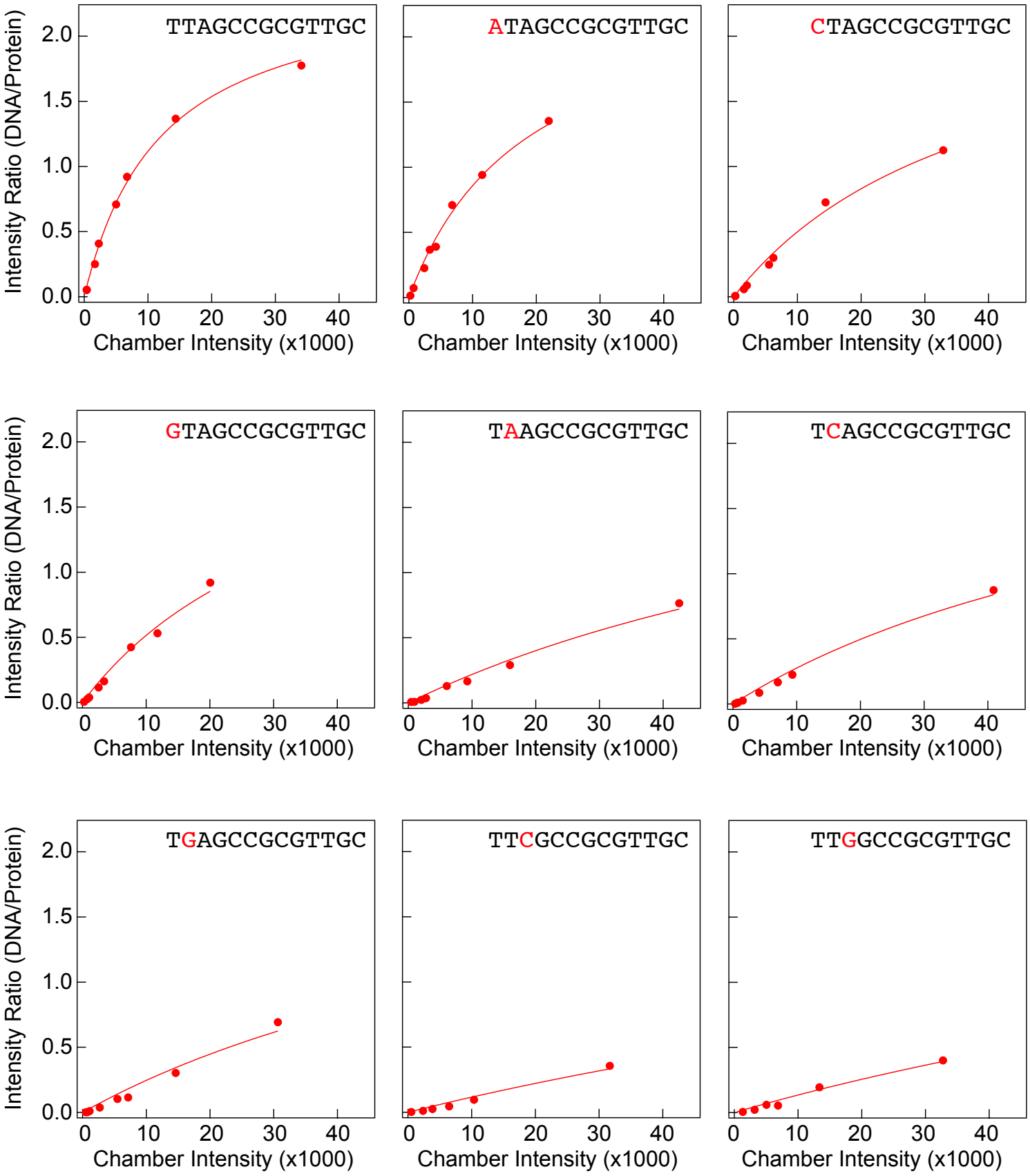




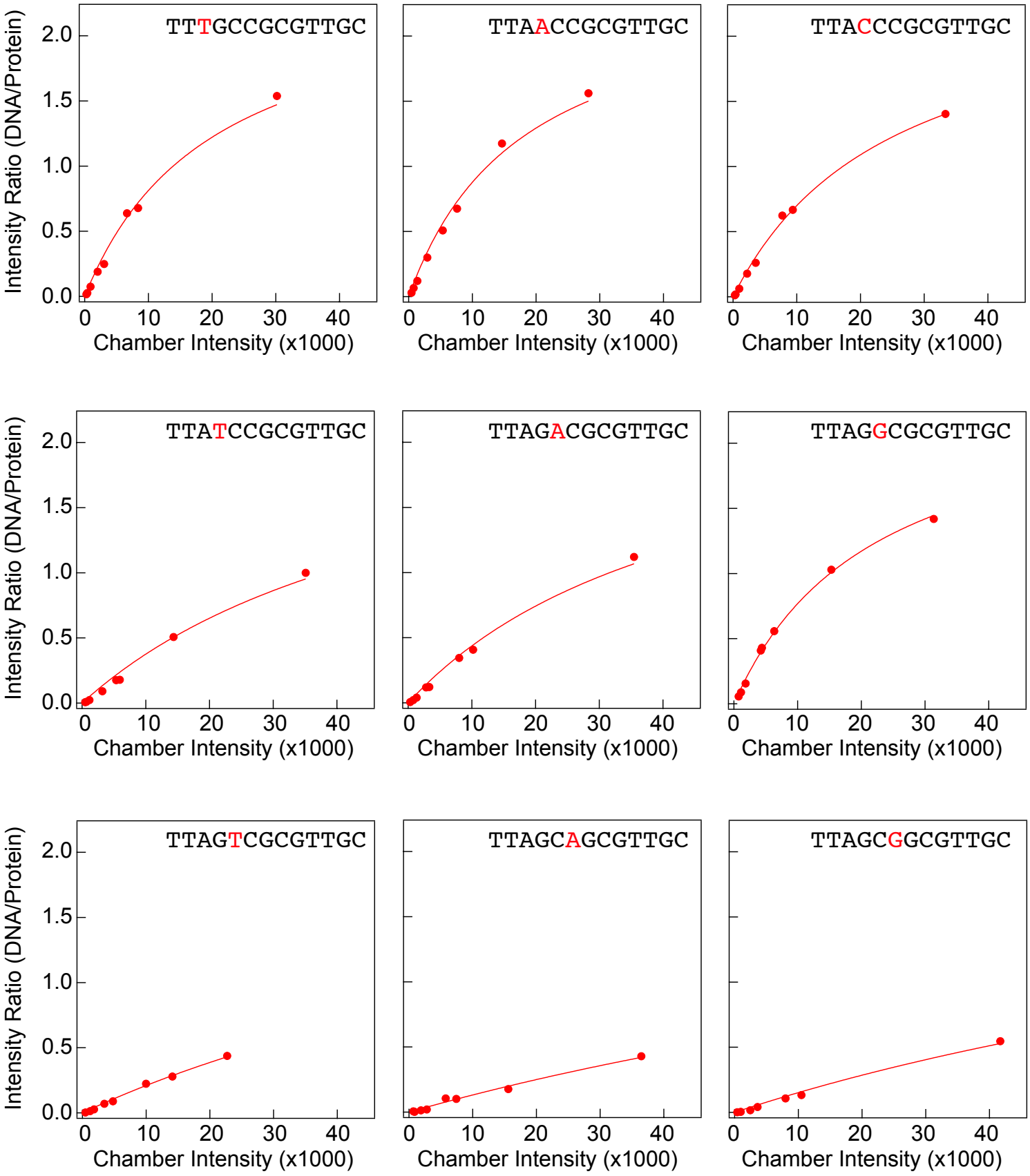




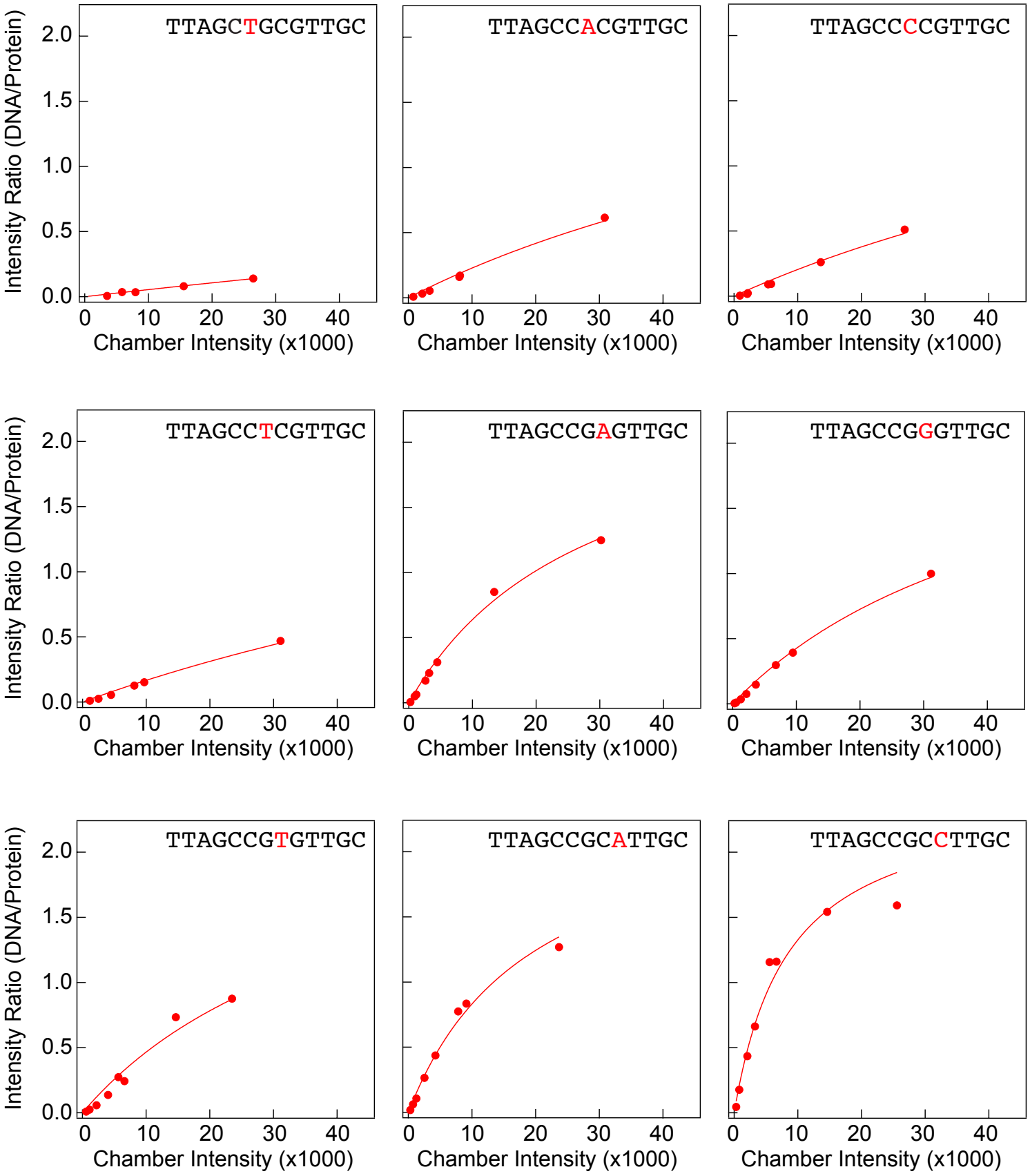
Czf1 Binding Curves, page 1



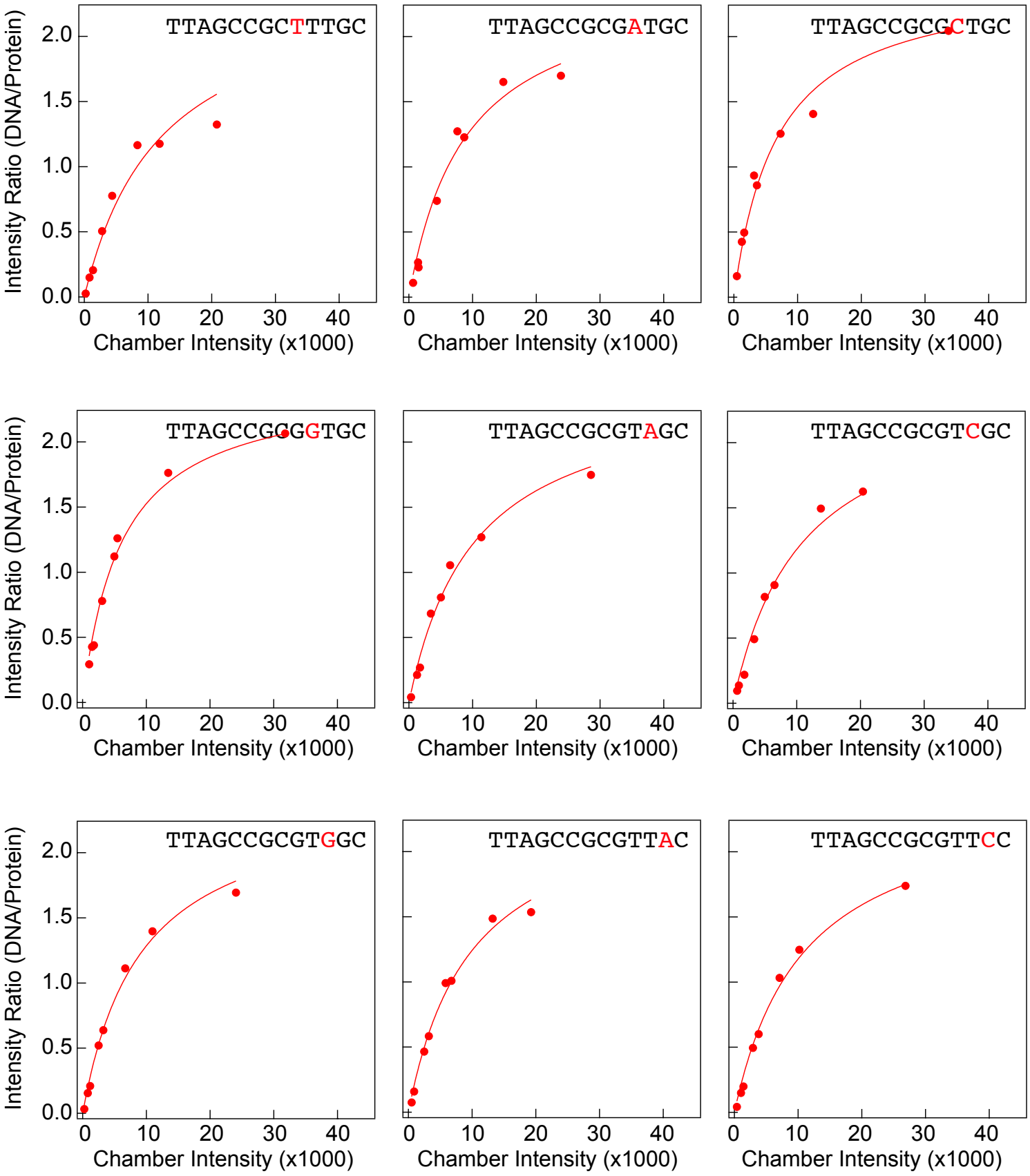
Czf1 Binding Curves, page 2



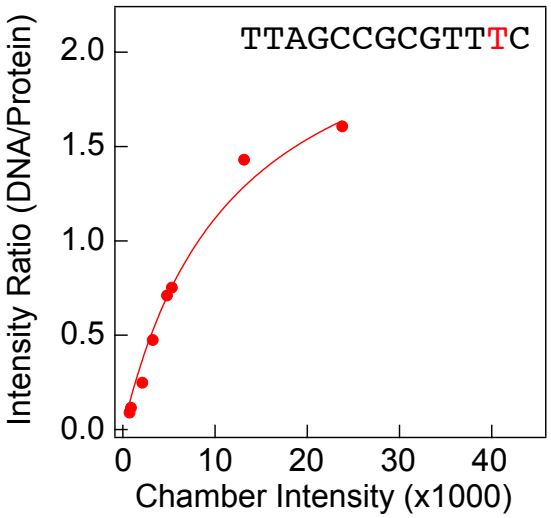
Czf1 Binding Curves, page 3

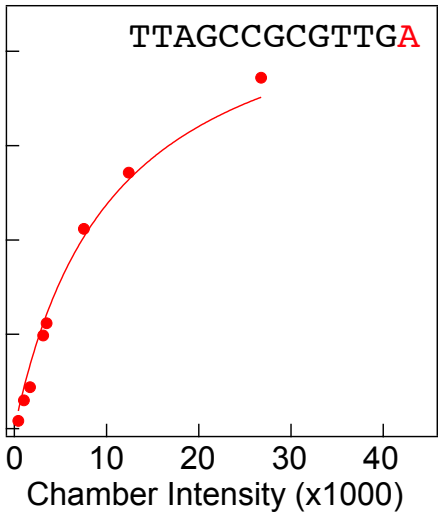


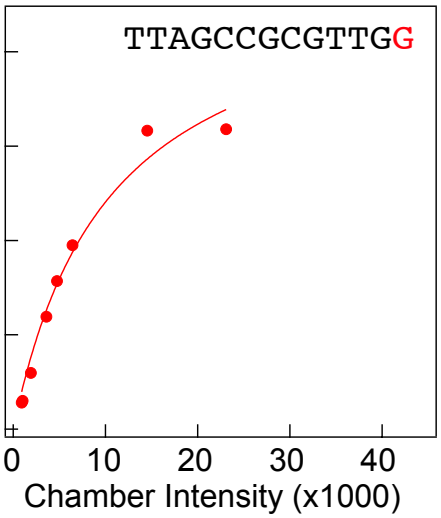
Czf1 Binding Curves, page 4

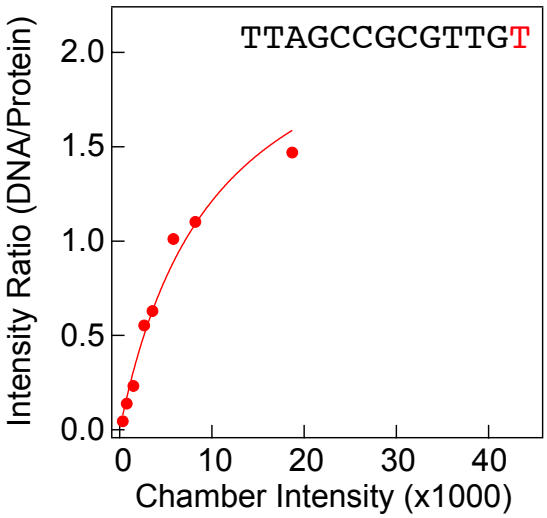


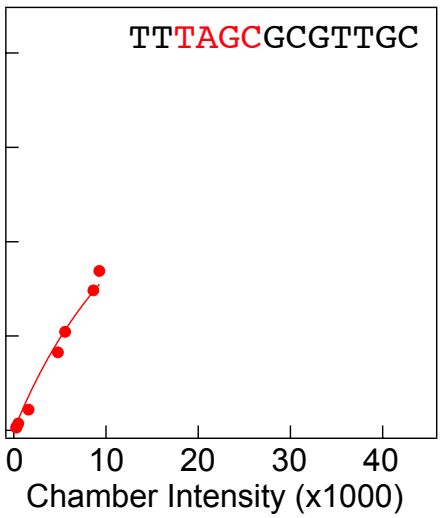
Czf1 Binding Curves, page 5

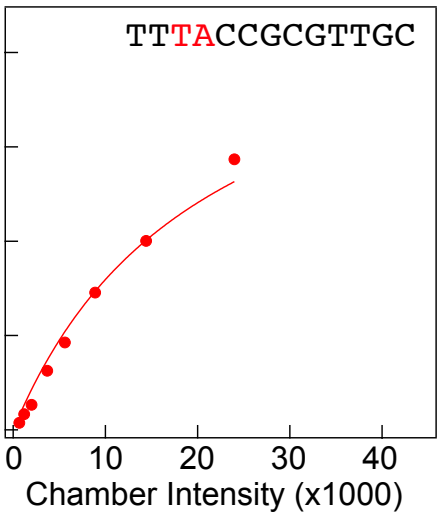


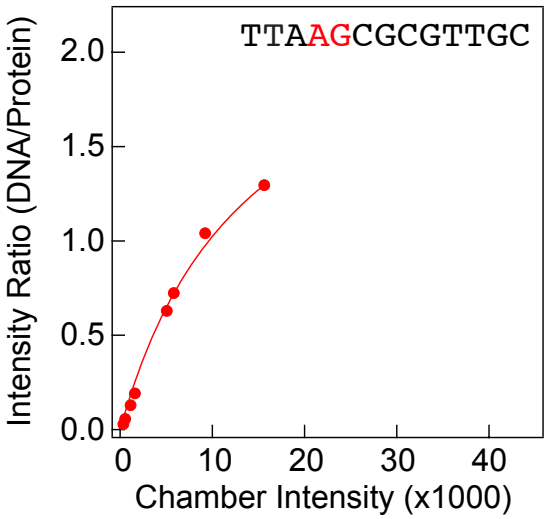


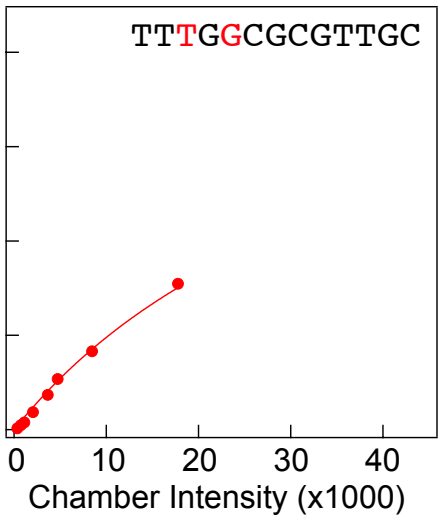




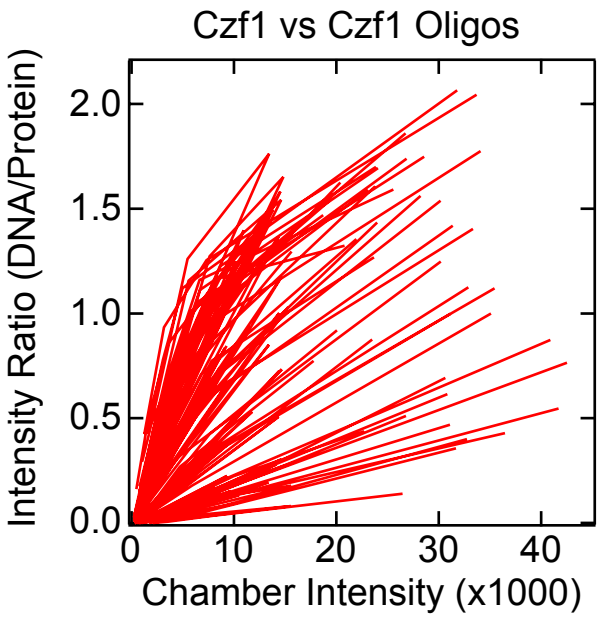


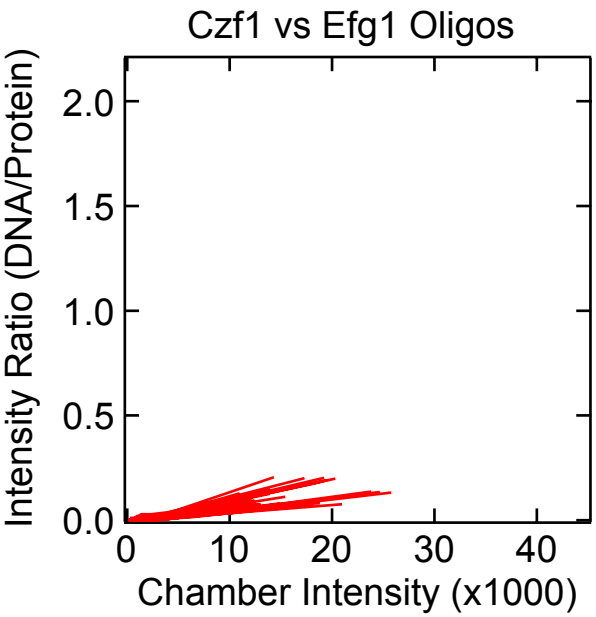


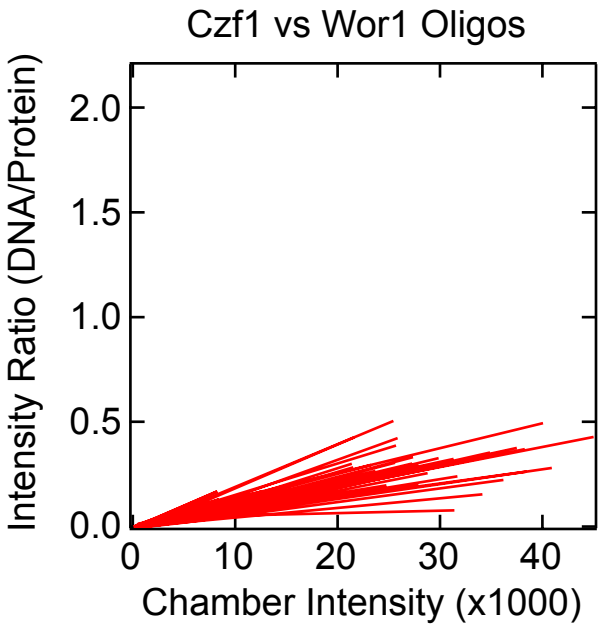


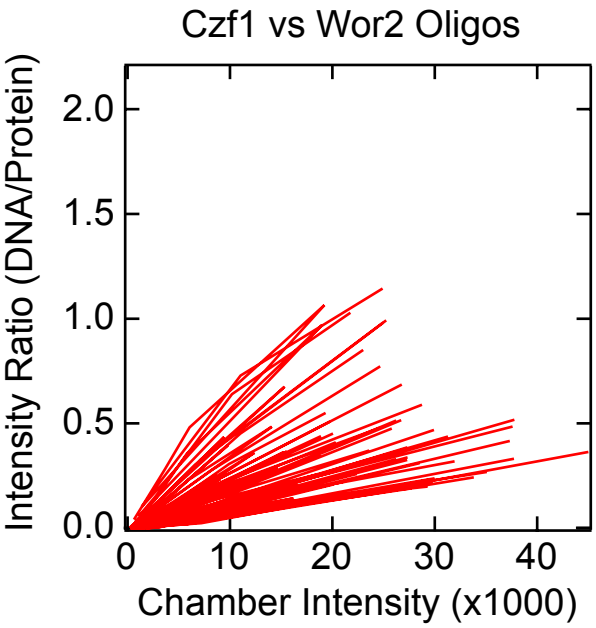


Czf1 Binding Curves, Page 6

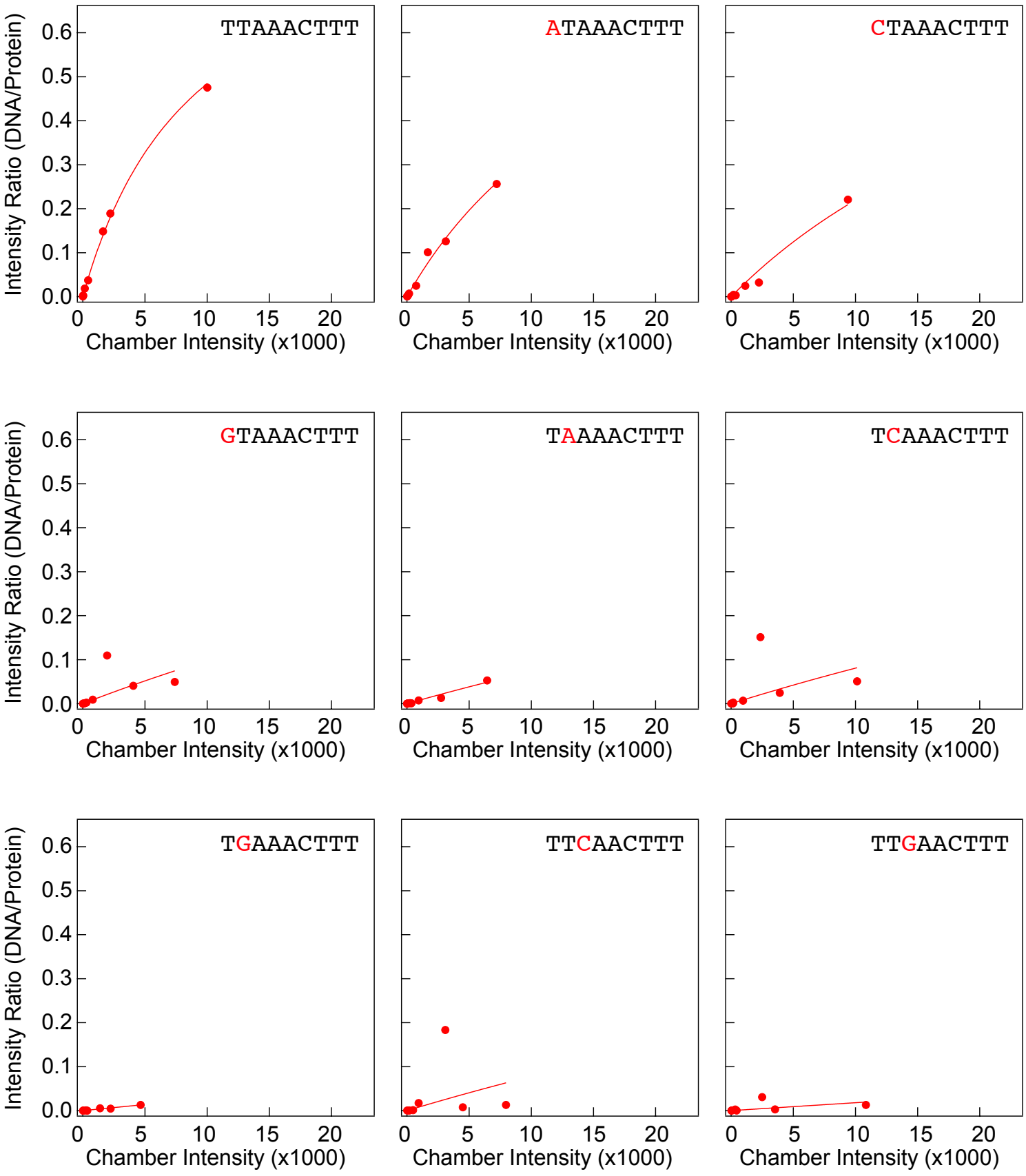




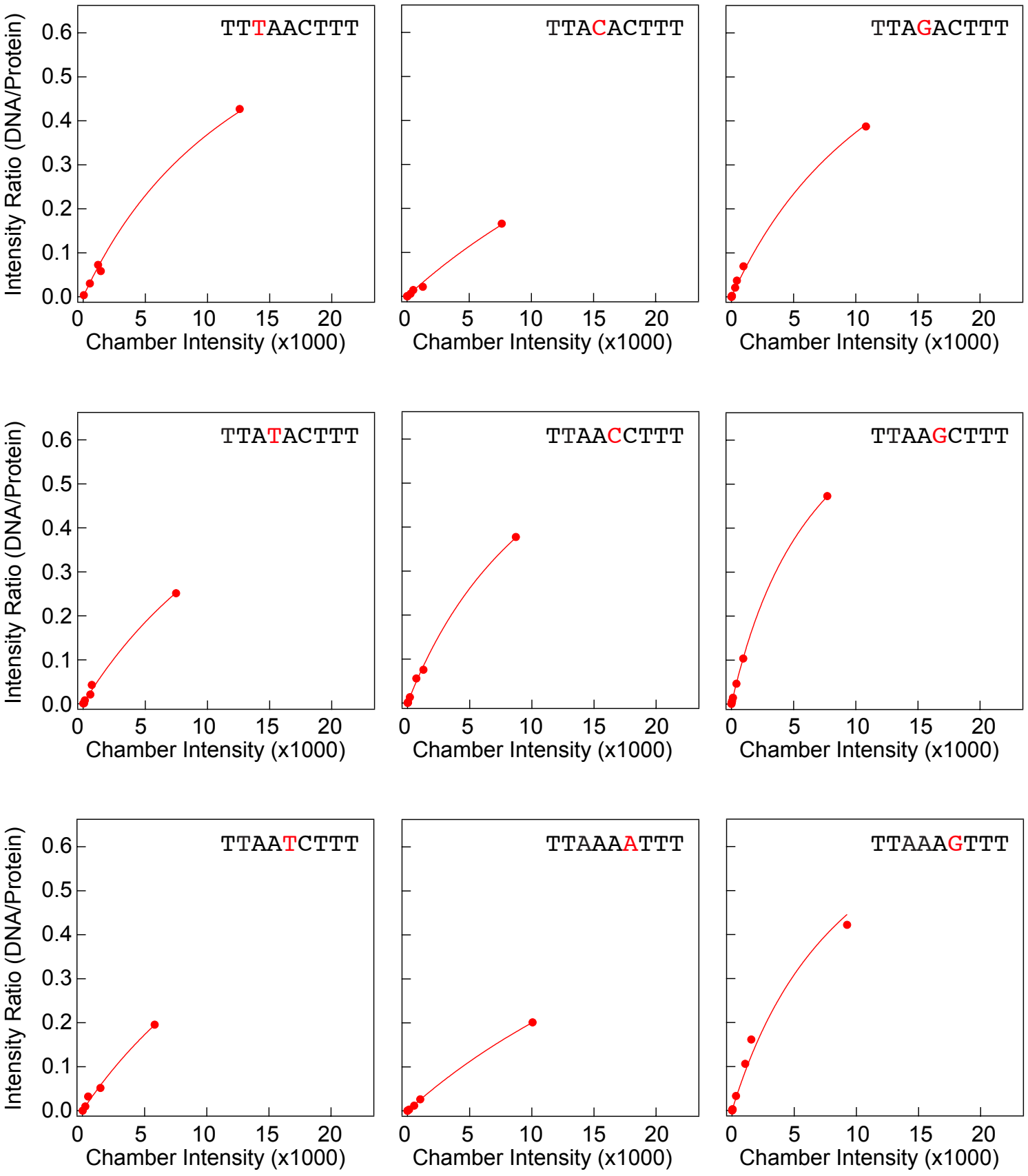




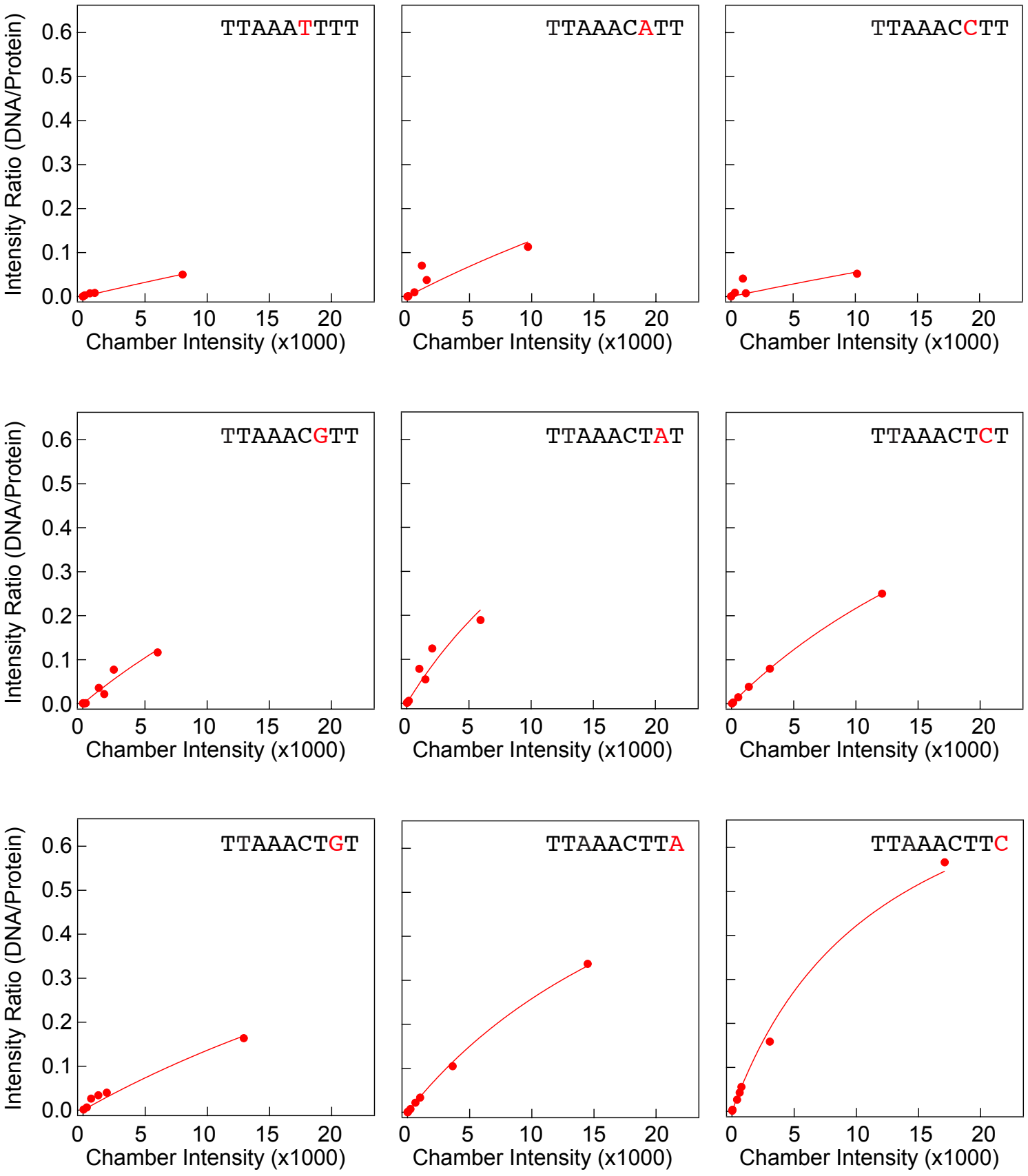
Wor1 Binding Curves, page 1



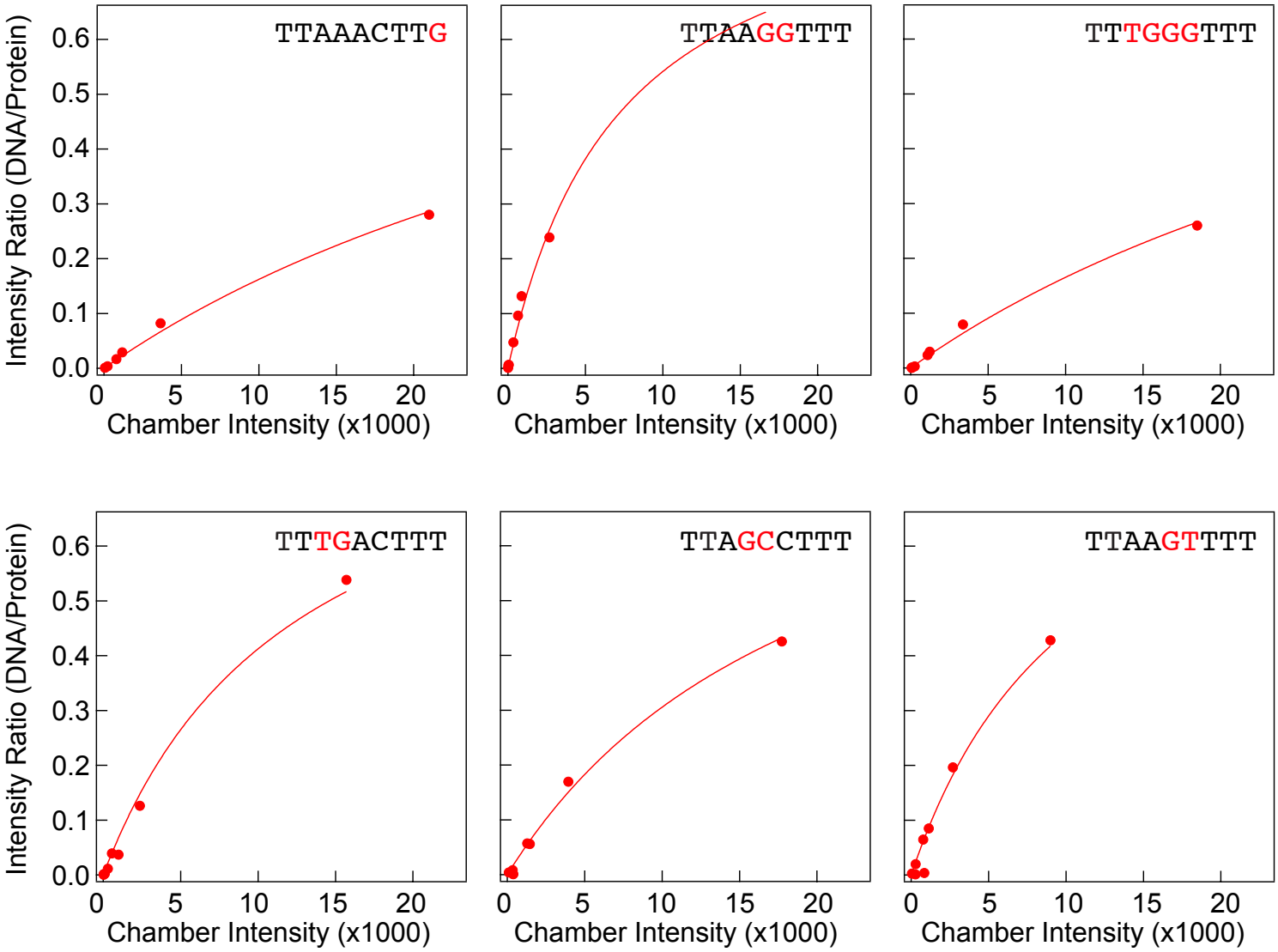
Wor1 Binding Curves, page 2



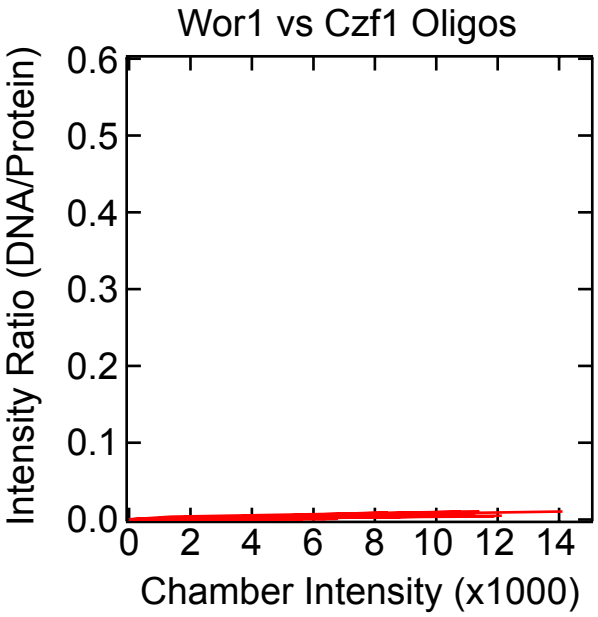
Wor1 Binding Curves, page 3

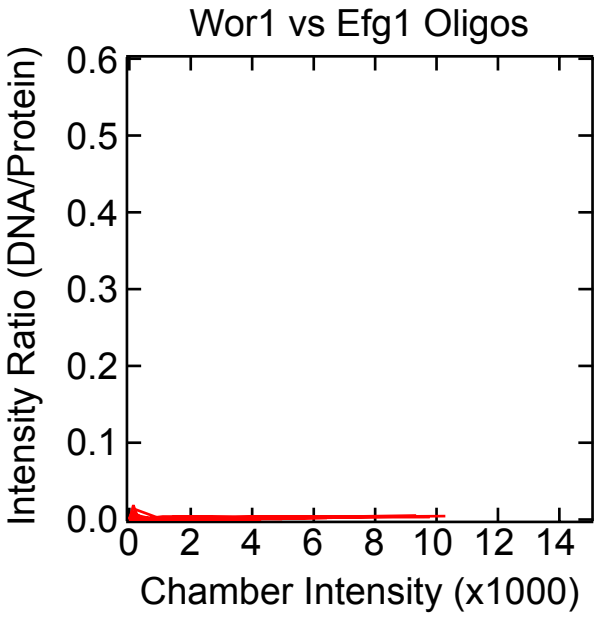


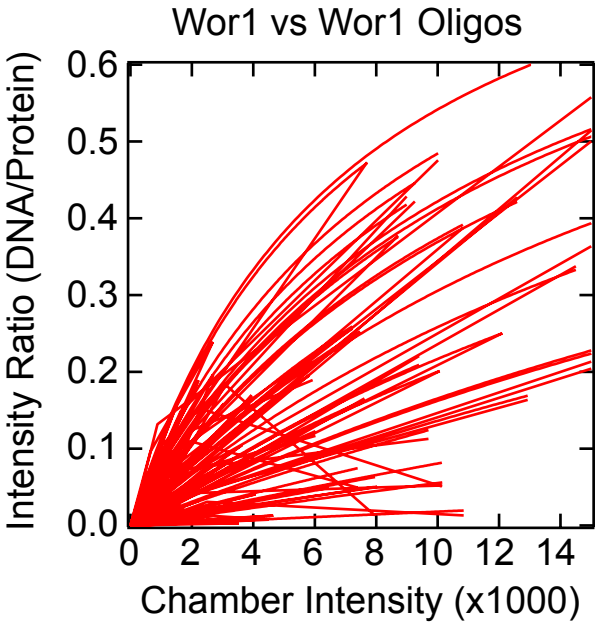
Wor1 Binding Curves, page 4

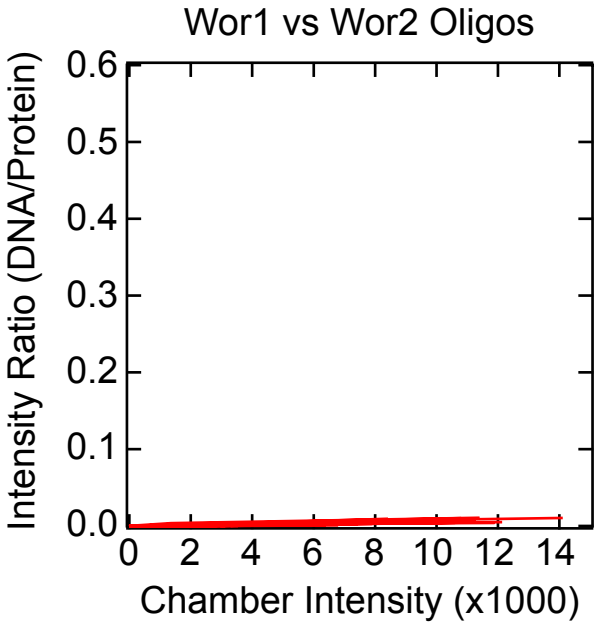


Wor1 Binding Curves, Page 5

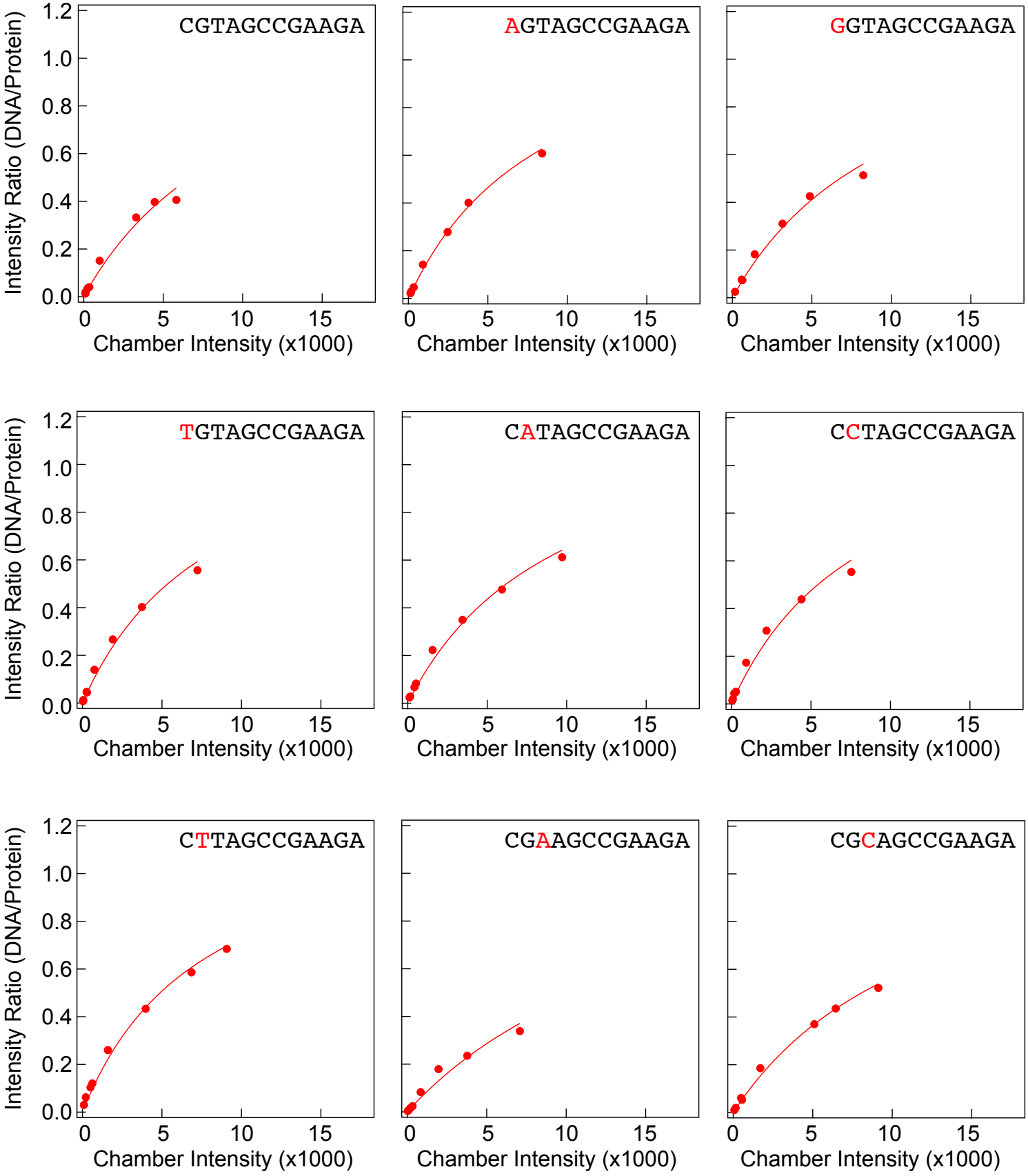




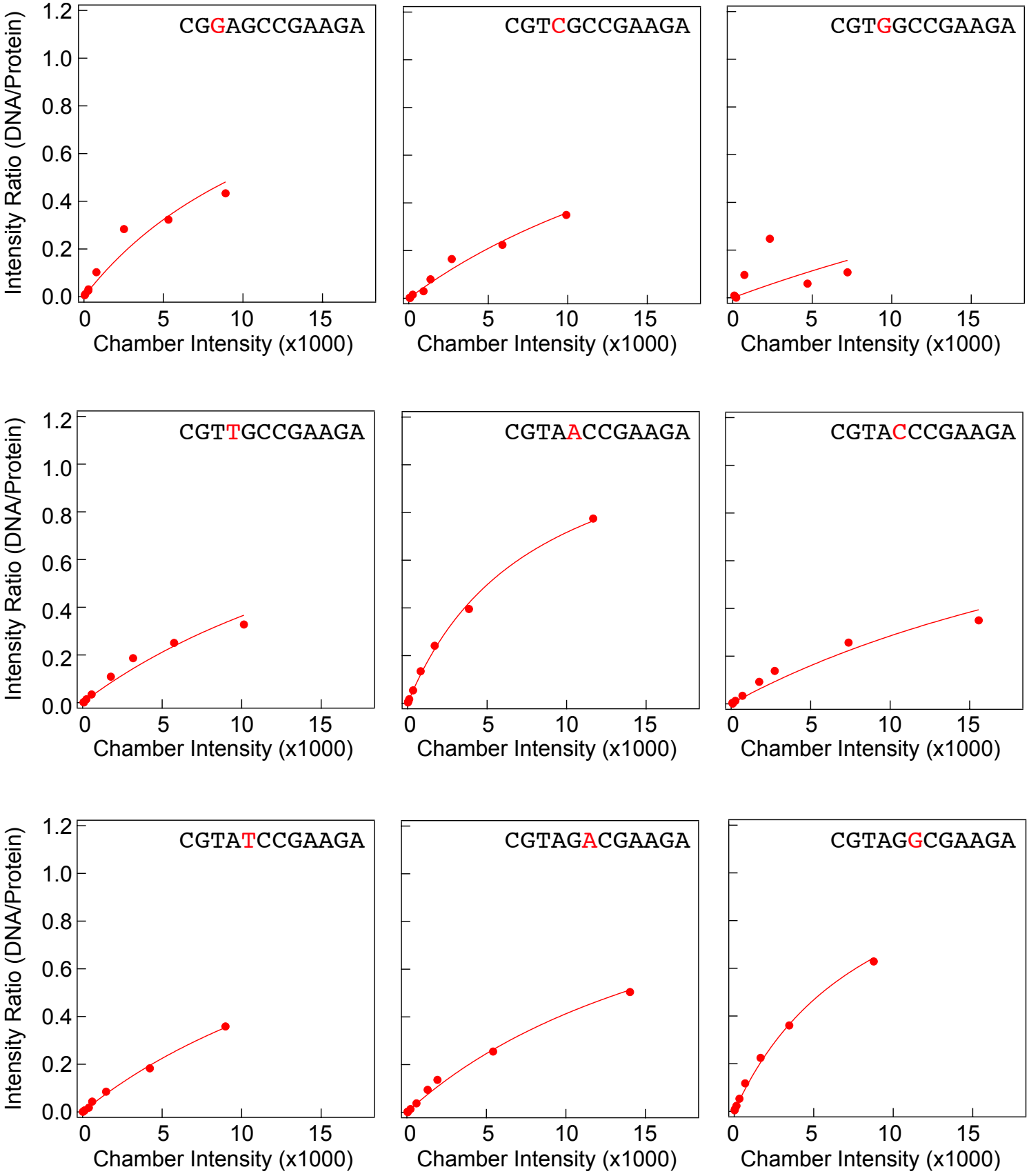




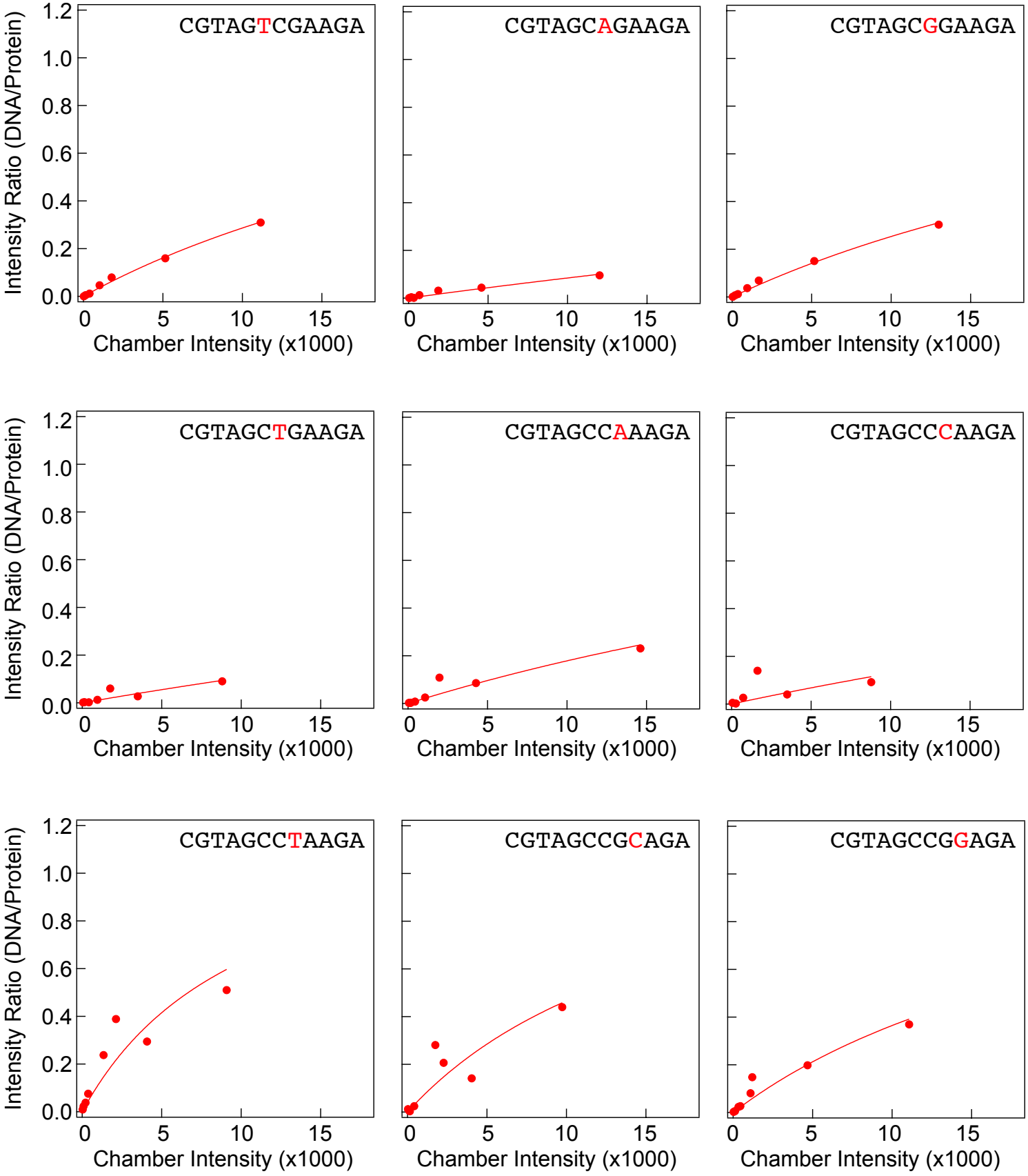
Wor2 Binding Curves, page 1



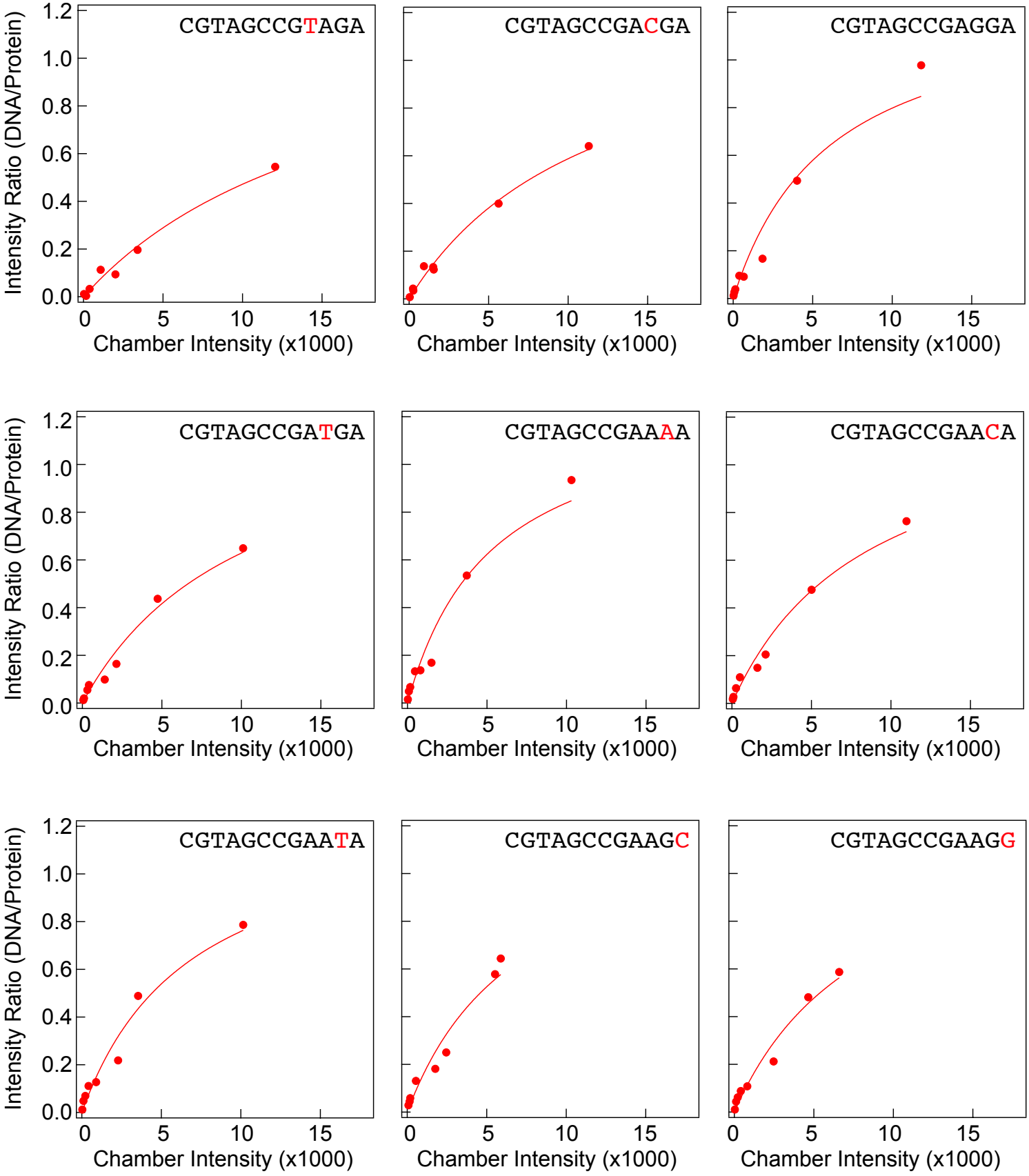
Wor2 Binding Curves, page 2



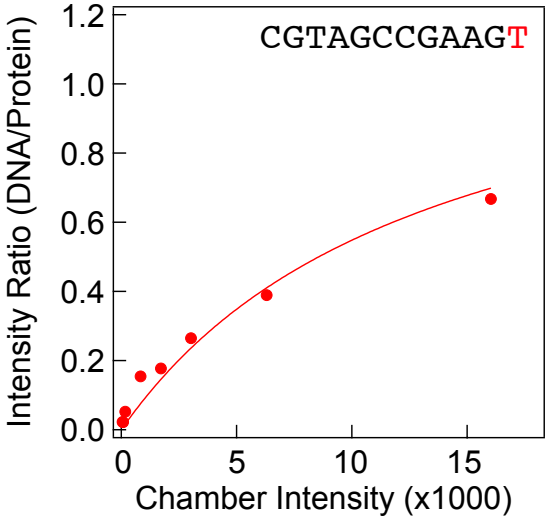
Wor2 Binding Curves, page 3

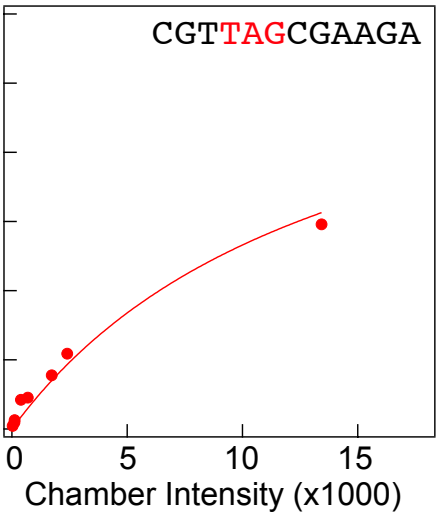


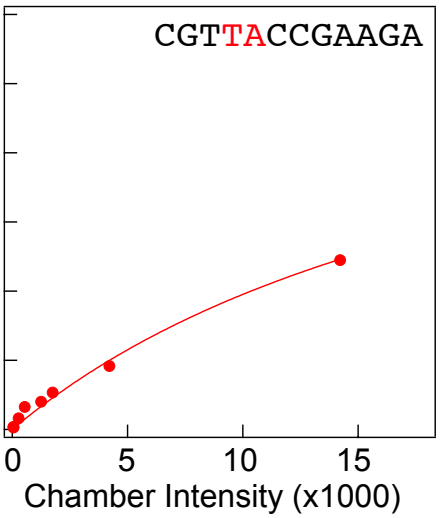
Wor2 Binding Curves, page 4

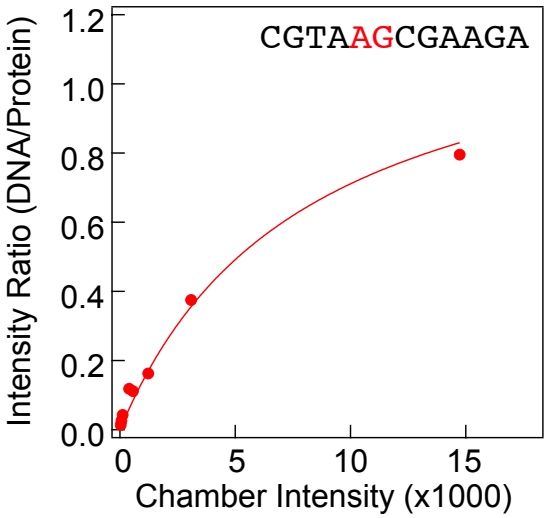


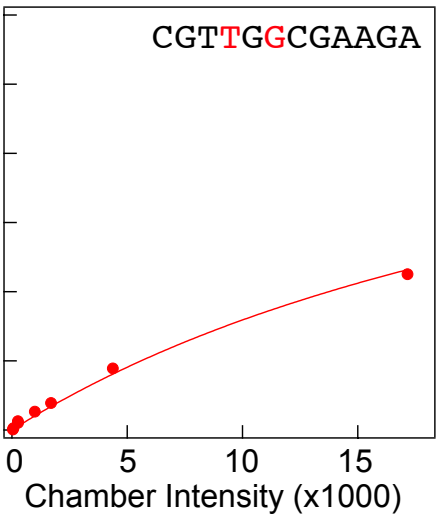
Wor2 Binding Curves, page 5

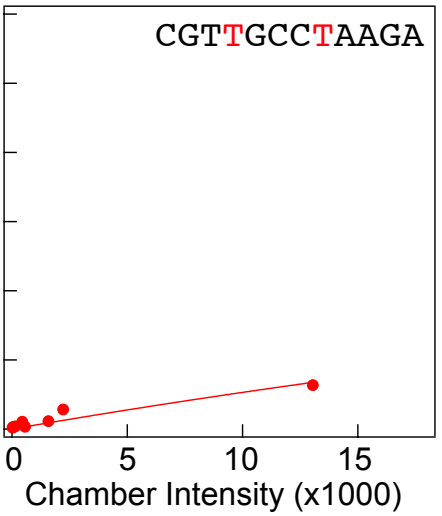


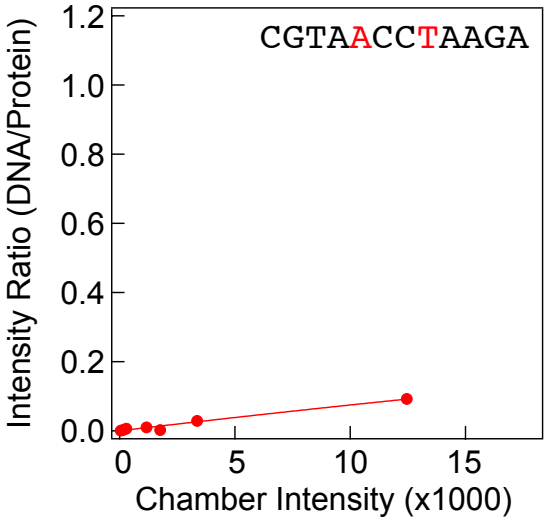


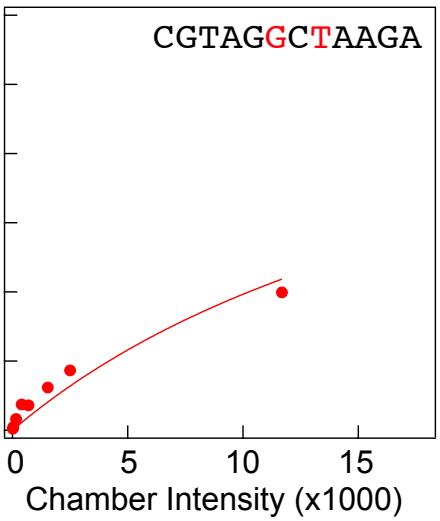












Wor2 Binding Curves, Page 6