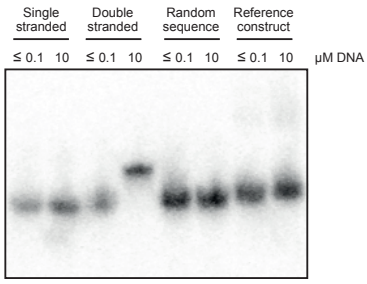
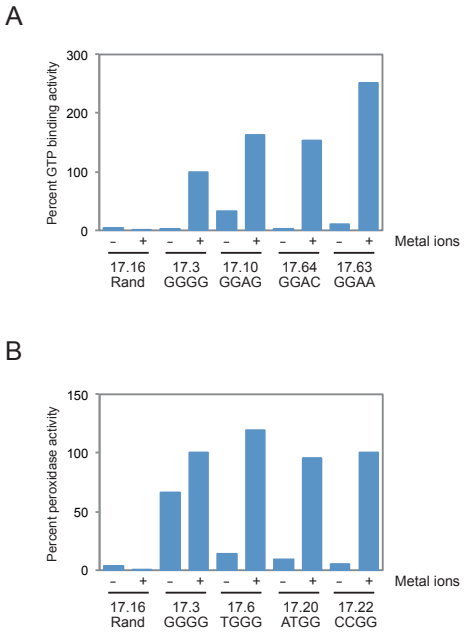
Supplementary Figure 1



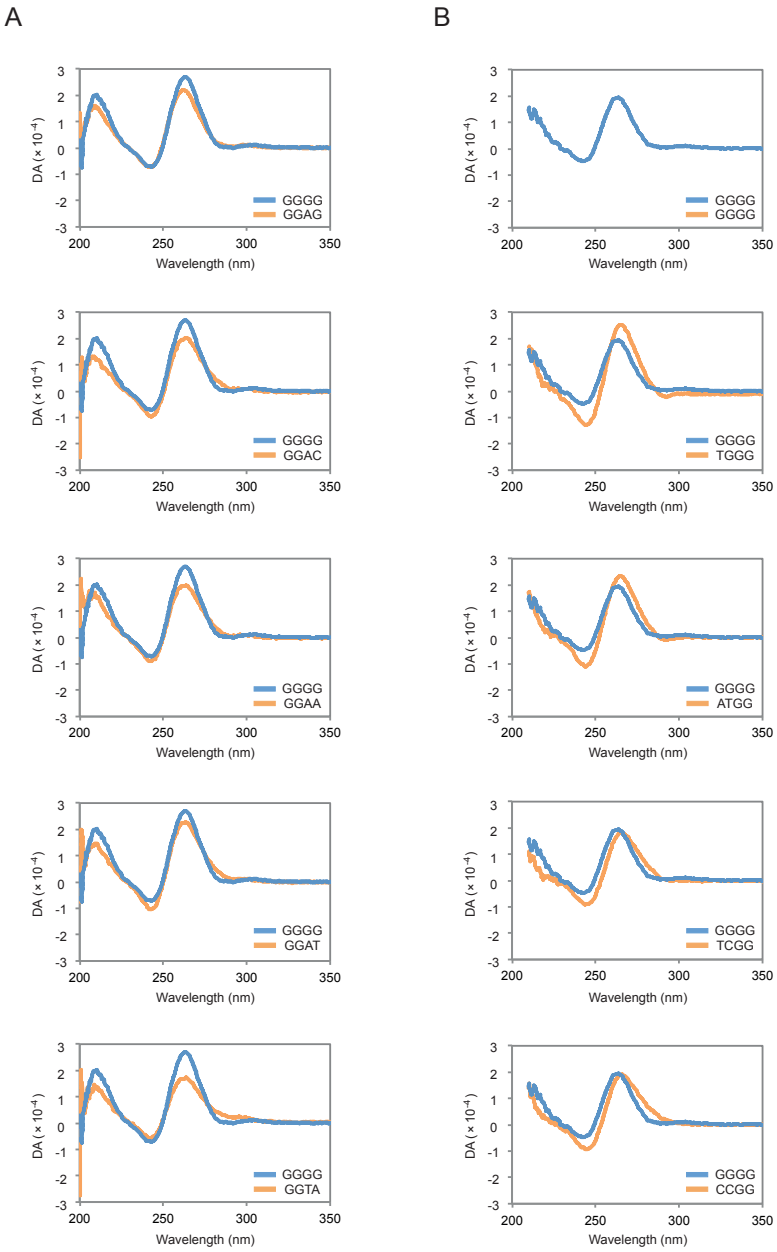

Supplementary Figure 1. Analysis of the reference construct by native PAGE. In each pair of lanes, a radiolabeled oligonucleotide (≤ 0.01 μM) was incubated for 30 minutes in either the absence or presence of 10 μM unlabeled oligonucleotide, and afterwards analyzed on a 10% native PAGE gel run in TBE buffer containing 5 mM KCl at 300 V for 30 minutes. Single stranded = a randomly generated, 17 nucleotide oligonucleotide with the sequence GACTGCCTCGTCACGAT; double stranded = a mix of the single-stranded oligonucleotide GACTGCCTCGTCACGAT and its reverse complement; random sequence = a 17 nucleotide random sequence pool; reference construct = GGGTGGGAAGGGTGGGA. Incubations were performed in a buffer containing 200 mM KCl, 1 mM $MgCl_2$, 20 mM HEPES pH 7.1, and 10 nM unlabeled GTP.

Supplementary Figure 2



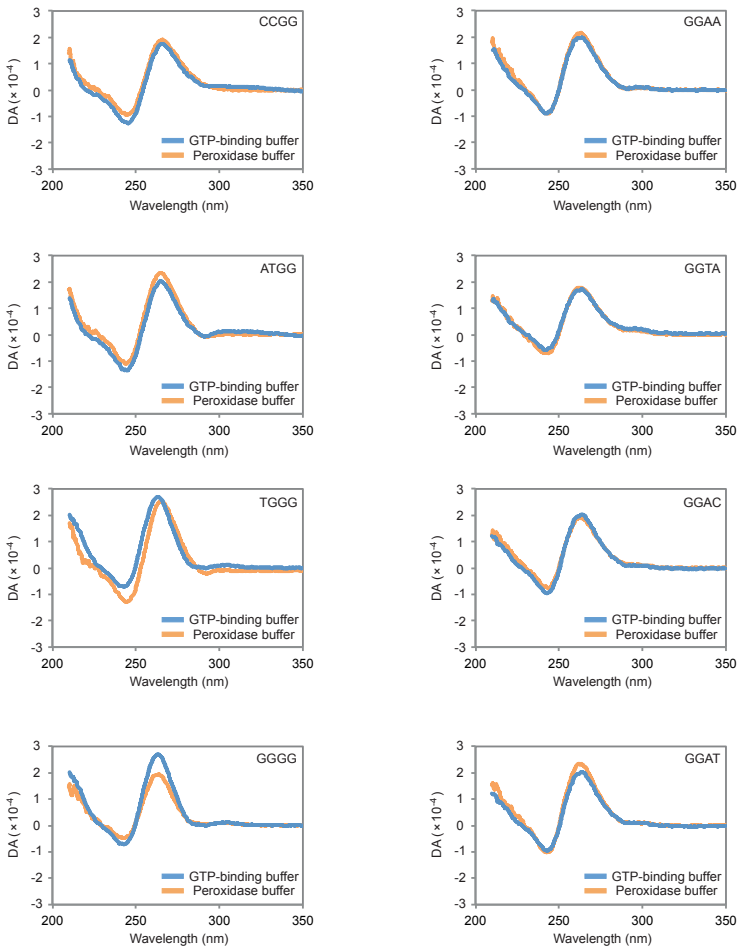

Supplementary Figure 2. Reduction of GTP-binding and peroxidase activity in the absence of metal ions. (A) GTP-binding activities of four G-quadruplex variants above our cutoff for activity in the absence and presence of metal ions. (B) Peroxidase activities of four G-quadruplex variants above our cutoff for activity in the absence and presence of metal ions. All experiments in panel (A) were performed at 10 μM DNA concentration in a buffer containing either 20 mM Trizma pH 7.5 and 10 nM $^{32}$P-γ-GTP (minus metal ions) or 200 mM KCl, 1 mM $MgCl_2$, 20 mM Trizma pH 7.5, and 10 nM $^{32}$P-γ-GTP (plus metal ions). All experiments in panel (B) were performed at 10 μM DNA concentration in a buffer containing either 20 mM Trizma pH 8, 0.05% Triton X-100, 0.5 μM hemin, 1% DMSO, 5 mM ABTS, and 600 μM $H_2O_2$ (minus metal ions) or 200 mM KCl, 1 mM $MgCl_2$, 20 mM Trizma pH 8, 0.05% Triton X-100, 0.5 μM hemin, 1% DMSO, 5 mM ABTS, and 600 μM $H_2O_2$ (plus metal ions). Reported values represent the average of two different experiments, and are expressed relative to the activity of the reference G-quadruplex (17.3) in the presence of metal ions.

Supplementary Figure 3



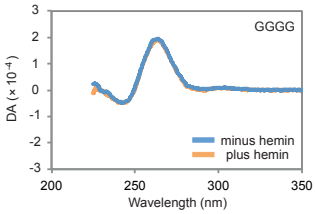

Supplementary Figure 3. The circular dichroism spectra of G-quadruplex variants with high GTP-binding activity and high peroxidase activity are similar to those of parallel strand structures. (A) Circular dichroism spectra of the five variants of the reference construct with the highest GTP-binding activity. (B) Circular dichroism spectra of the five variants of the reference construct with the highest peroxidase activity. In each graph, the blue curve represents the circular dichroism spectrum of the reference sequence, and the orange curve represents the circular dichroism spectrum of the indicated mutant. DA = differential absorption. All experiments in panel (A) were performed at 10 μM DNA concentration in a buffer containing 200 mM KCl, 1 mM $MgCl_2$, 20 mM HEPES pH 7.1, and 10 nM unlabeled GTP. All experiments in panel (B) were performed at 10 μM DNA concentration in a buffer containing 200 mM KCl, 1 mM $MgCl_2$, 20 mM HEPES pH 8, and 0.05% Triton X-100.

Supplementary Figure 4



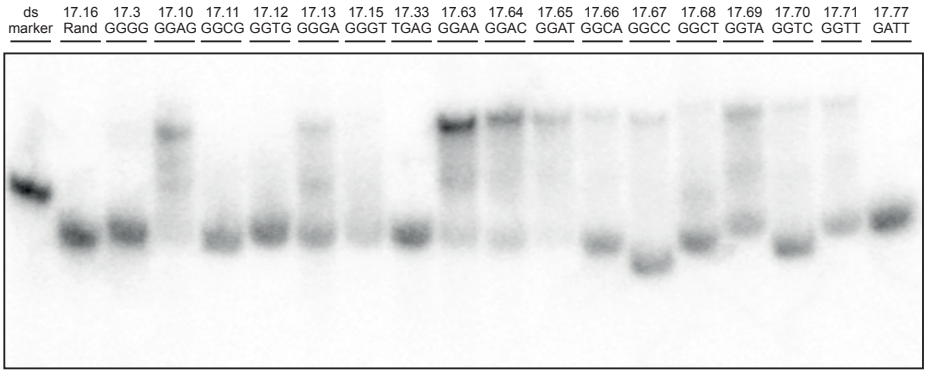

Supplementary Figure 4. Circular dichroism spectra are similar in GTP-binding buffer and peroxidase buffer. Circular dichroism spectra of selected variants of the reference construct measured in both GTP-binding buffer (blue curves) and peroxidase buffer (orange curves). DA = differential absorption. All measurements were performed at 10 μM DNA concentration. GTP-binding buffer contained 200 mM KCl, 1 mM $MgCl_2$, 20 mM HEPES pH 7.1, and 10 nM unlabeled GTP. Peroxidase buffer contained 200 mM KCl, 1 mM $MgCl_2$, 20 mM HEPES pH 8, and 0.05% Triton X-100.

Supplementary Figure 5



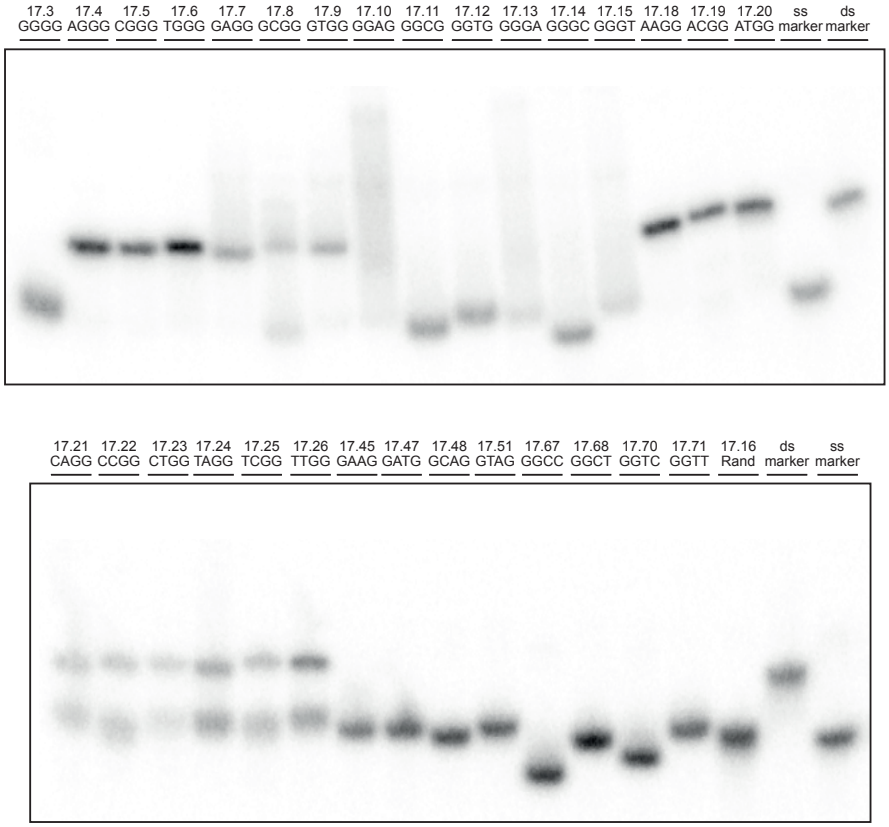

Supplementary Figure 5. Circular dichroism spectra are similar in the absence and presence of hemin. Circular dichroism spectrum of the reference construct measured in both the absence (blue curve) and presence (orange curve) of hemin. DA = differential absorption. Measurements were performed at 10 μM DNA concentration in a buffer containing 200 mM KCl, 1 mM $MgCl_2$, 20 mM HEPES pH 8, 0.05% Triton X-100, and either 0 μM hemin and 0% DMSO or 0.5 μM hemin and 1% DMSO.

Supplementary Figure 6



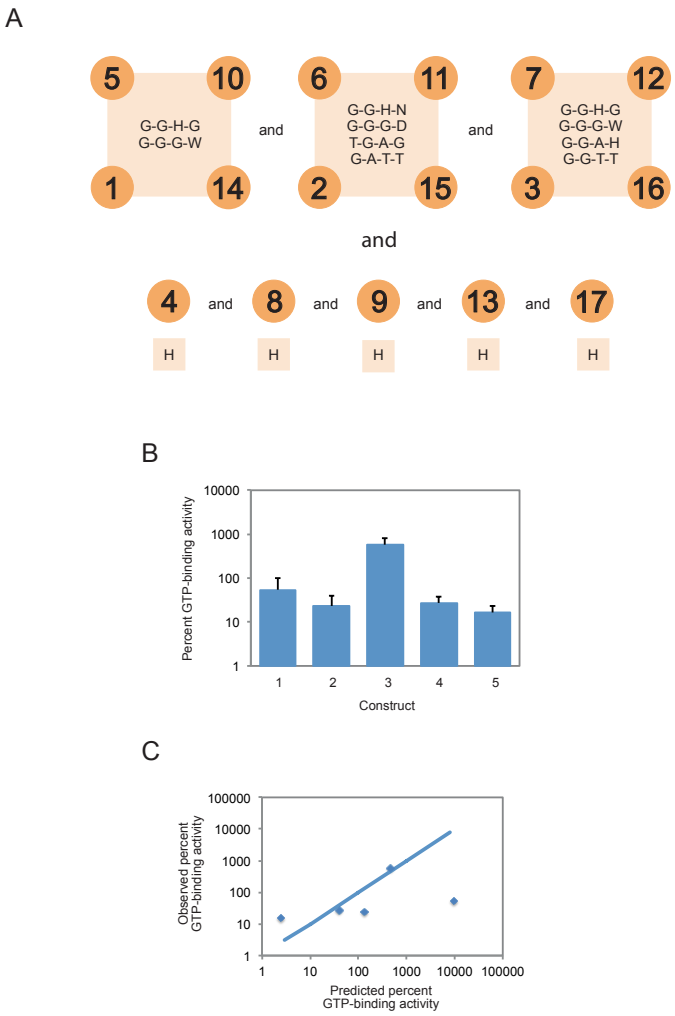

Supplementary Figure 6. Analysis of all mutants with GTP-binding activity above our cutoff for activity by native PAGE. In each case, radiolabeled mutant (≤ 0.01 μM) was incubated for 30 minutes in the presence of 10 μM unlabeled oligonucleotide, and afterwards analyzed on a 10% native PAGE gel run in TBE buffer containing 5 mM KCl at 300 V for 30 minutes. Single stranded = a randomly generated, 17 nucleotide oligonucleotide with the sequence GACTGCCTCGTCACGAT; double stranded = a mix of the single-stranded oligonucleotide GACTGCCTCGTCACGAT and its reverse complement; random sequence = a 17 nucleotide random sequence pool; reference construct = GGGTGGGAAGGGTGGGA. Incubations were performed in a buffer containing 200 mM KCl, 1 mM $MgCl_2$, 20 mM HEPES pH 7.1, and 10 nM unlabeled GTP.

Supplementary Figure 7



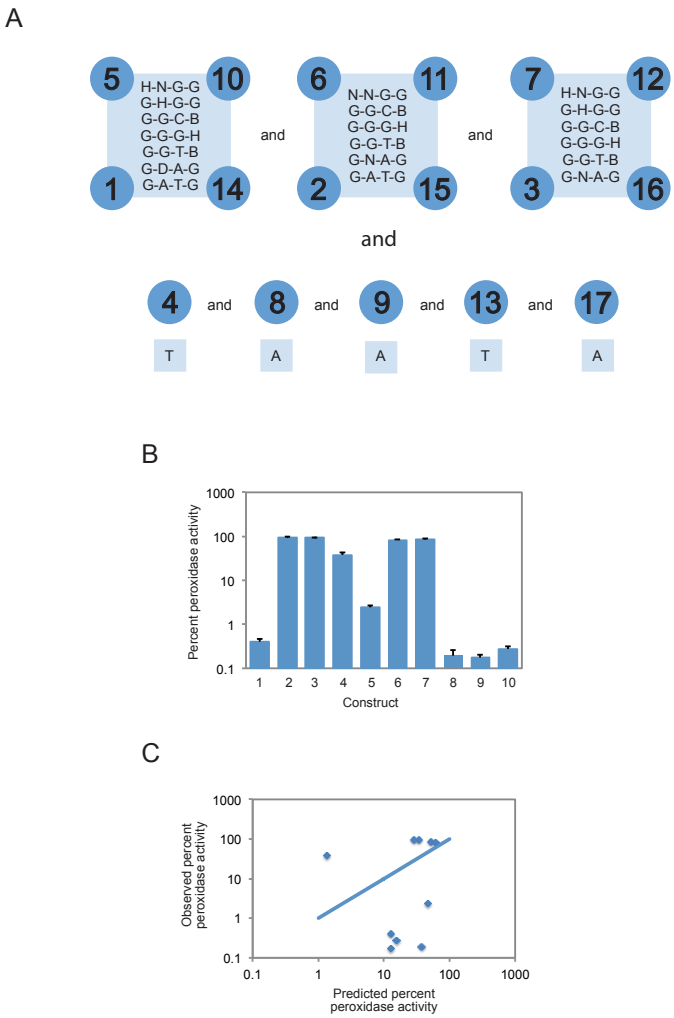

Supplementary Figure 7. Analysis of all mutants with peroxidase activity above our cutoff for activity by native PAGE. In each case, radiolabeled mutant (≤ 0.01 μM) was incubated for 30 minutes in the presence of 10 μM unlabeled oligonucleotide, and afterwards analyzed on a 10% native PAGE gel run in TBE buffer containing 5 mM KCl at 300 V for 30 minutes. Single stranded = a randomly generated, 17 nucleotide oligonucleotide with the sequence GACTGCCTCGTCACGAT; double stranded = a mix of the single-stranded oligonucleotide GACTGCCTCGTCACGAT and its reverse complement; random sequence = a 17 nucleotide random sequence pool; reference construct = GGGTGGGAAGGGTGGGA. Incubations were performed in a buffer containing 200 mM KCl, 1 mM $MgCl_2$, 20 mM HEPES pH 8, 0.05% Triton X-100, 0.5 μM hemin and 1% DMSO.

Supplementary Figure 8



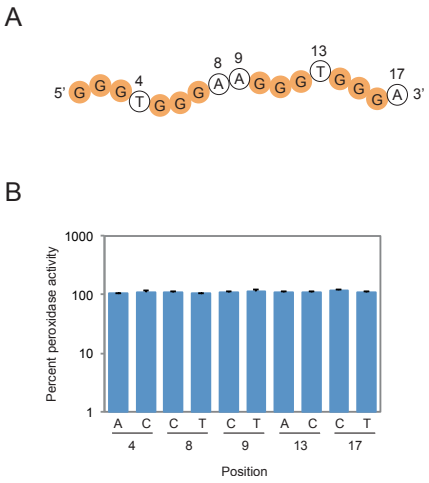

Supplementary Figure 8. GTP-binding activity of G-quadruplex variants with multiple mutated tetrads and mutated spacers. (A) Sequence model tested in this experiment. 5' tetrad = positions 1, 5, 10 and 14; central tetrad = positions 2, 6, 11 and 15; 3' tetrad = positions 3, 7, 12 and 16; spacer nucleotides = 4, 8, 9, 13 and 17. (B) GTP-binding activity of five randomly chosen constructs that satisfy the requirements of this model. GTP-binding activity is expressed relative to that of the reference construct. Reported values represent the average of three experiments, and error bars indicate one standard deviation. Construct 1 = GGGCGGGCTTATTGCGA; construct 2 = GGGAGGGAAACAAGCAA; construct 3 = GGGTGGGCAGAGATTGA; construct 4 = GGGCGGGACCGACGTTT; construct 5 = GGGCGGGATGCTCATTT. (C) Relationship between predicted and observed GTP-binding activity for these five sequences. Predicted GTP-binding activity was calculated using a model in which mutational effects in different tetrads are independent, and spacer sequence has no effect on activity. The blue line indicates the expected relationship if predicted and observed activities were identical. Measurements were performed at 10 μM DNA concentration in a buffer containing 200 mM KCl, 1 mM $MgCl_2$, 20 mM HEPES pH 7.1, and 10 nM $^{32}$P-γ-GTP. In panel (A), the IUPAC nucleotide code was used to indicate the nucleotides that are permitted to occur at variable positions in each sequence model. W = A or T; H = A, C or T; D = A, G or T; B = C, G or T; N = A, C, G or T.

Supplementary Figure 9



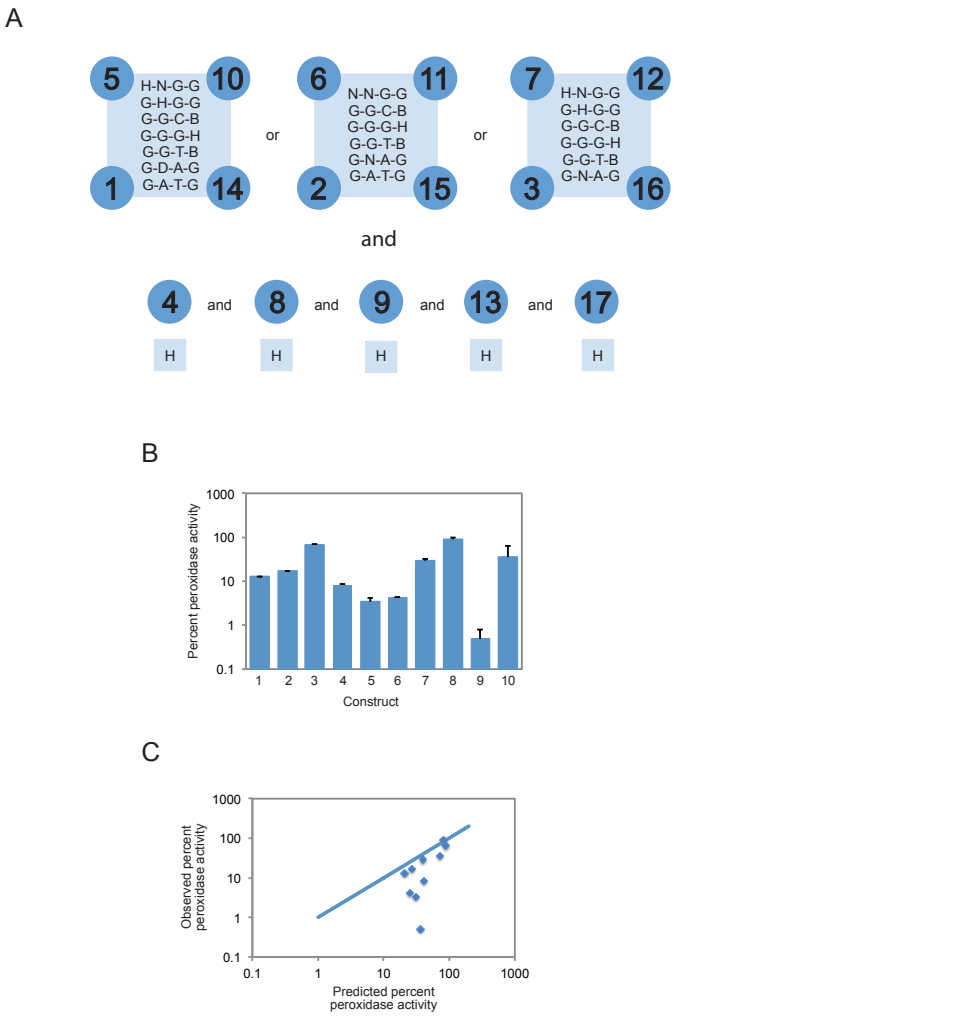

Supplementary Figure 9. Peroxidase activity of G-quadruplex variants with multiple mutated tetrads. (A) Sequence model tested in this experiment. 5' tetrad = positions 1, 5, 10 and 14; central tetrad = positions 2, 6, 11 and 15; 3' tetrad = positions 3, 7, 12 and 16; spacer nucleotides = 4, 8, 9, 13 and 17. (B) Peroxidase activity of ten randomly chosen constructs that satisfy the requirements of this model. Peroxidase activity is expressed relative to that of the reference construct. Reported values represent the average of three experiments, and error bars indicate one standard deviation. Construct 1 = GCTTGCTAAAGGTGGGA; construct 2 = GGATTCGAAGGGTGGGA; construct 3 = AAGTAATAAGGGTGGGA; construct 4 = GGGTAGAAATATTGGGA; construct 5 = GTGTGAAAAGGGTTGGA; construct 6 = CCATTGAAAGGGTGGGA; construct 7 = CGGTCTGAAGGGTGGGA; construct 8 = TGGTCGGAAGCCTGCGA; construct 9 = TGTTAGAAAGGGTGTGA; construct 10 = TGGTTGGAAGGCTGATA. (C) Relationship between predicted and observed peroxidase activity for these ten sequences. Predicted peroxidase activity was calculated using a model in which mutational effects in different tetrads are independent. The blue line indicates the expected relationship if predicted and observed activities were identical. Measurements were performed at 10 μM DNA concentration in a buffer containing 200 mM KCl, 1 mM $MgCl_2$, 20 mM HEPES pH 8, 0.05% Triton X-100, 0.5 μM hemin, 1% DMSO, 5 mM ABTS, and 600 μM $H_2O_2$. In panel (A), the IUPAC nucleotide code was used to indicate the nucleotides that are permitted to occur at variable positions in each sequence model. W = A or T; H = A, C or T; D = A, G or T; B = C, G or T; N = A, C, G or T.

Supplementary Figure 10




Supplementary Figure 10. Peroxidase activity of G-quadruplex variants with point mutations in spacers. (A) Primary sequence of the reference construct, with mutated positions numbered. (B) Peroxidase activity of variants of the reference sequence with point mutations in spacers. Peroxidase activity is expressed relative to that of the reference construct. Reported values represent the average of three experiments, and error bars indicate one standard deviation. Measurements were performed at 10 μM DNA concentration in a buffer containing 200 mM KCl, 1 mM $MgCl_2$, 20 mM HEPES pH 8, 0.05% Triton X-100, 0.5 μM hemin, 1% DMSO, 5 mM ABTS, and 600 μM $H_2O_2$.

Supplementary Figure 11




Supplementary Figure 11. Peroxidase activity of G-quadruplex variants with one mutated tetrad and multiple mutations in spacers. (A) Sequence model tested in this experiment. 5' tetrad = positions 1, 5, 10 and 14; central tetrad = positions 2, 6, 11 and 15; 3' tetrad = positions 3, 7, 12 and 16; spacer nucleotides = 4, 8, 9, 13 and 17. (B) Peroxidase activity of ten randomly chosen constructs that satisfy the requirements of this model. Peroxidase activity is expressed relative to that of the reference construct. Reported values represent the average of three experiments, and error bars indicate one standard deviation. Construct 1 = GGTTGGCACGGGAGGGA; construct 2 = GGGTGAGTCGTGAGGGT; construct 3 = GGGAGGGTTGGGAAGGA; construct 4 = GGGCGGATCGGACGGGC; construct 5 = GGGAGGGACCGGACGGA; construct 6 = GGGCGGGTAGCGCGTGT; construct 7 = GGGATGGAAAGGAGGGC; construct 8 = TGGCTGGTAGGGTGGGT; construct 9 = GGGCGCGATGAGTGGGC; construct 10 = GGGAGGGCTTGGAGGGA. (C) Relationship between predicted and observed peroxidase activity for these ten sequences. Predicted GTP-binding activity was calculated using a model in which spacer sequence has no effect on activity. The blue line indicates the expected relationship if predicted and observed activities were identical. Measurements were performed at 10 μM DNA concentration in a buffer containing 200 mM KCl, 1 mM $MgCl_2$, 20 mM HEPES pH 8, 0.05% Triton X-100, 0.5 μM hemin, 1% DMSO, 5 mM ABTS, and 600 μM $H_2O_2$. In panel (A), the IUPAC nucleotide code was used to indicate the nucleotides that are permitted to occur at variable positions in each sequence model. W = A or T; H = A, C or T; D = A, G or T; B = C, G or T; N = A, C, G or T.