N/N	Conjugate* 5'→ 3'	Mr (calculated)	Mr (experimental)
1	Hemin-T <sub>15</sub> -EAD2	11075.9	11067.4
2	Hemin-T <sub>10</sub> -EAD2	9554.9	9548.1
3	Hemin-T <sub>5</sub> -EAD2	8033.9	8027.8
4	Hemin-EAD2	6512.9	6507.5
5	EAD2-T <sub>15</sub> -Hemin	11105.8	11097.3
6	EAD2-T <sub>10</sub> -Hemin	9584.9	9578.1
7	EAD2-T <sub>5</sub> -Hemin	8063.9	8057.8
8	EAD2-Hemin	6542.9	6537.5
9	Hemin-Prd <sub>10</sub> -EAD2	7893.5	7887.6
10	EAD2-Prd <sub>10</sub> -Hemin	7893.5	7887.7

Table 1S. Characterization of covalent hemin-EAD2 conjugates by mass-spectrometry.

\*Symbol d in the notation of oligothymidilate spacers is omitted.



Figure S1. Purification of PMDNAzyme( $T_{10}$ ) by gel electrophoresis in 20% PAAG, 7 M urea with UV visualization. Lanes: *1* - xylene cyanol marker; *2* – random 40-mer DNA as a length control; *3* - initial NH<sub>2</sub>-T10-EAD2; *4*,*5* - products of hemin conjugation to NH<sub>2</sub>-T<sub>10</sub>-EAD2. Only conjugate hemin-T<sub>10</sub>-EAD2 was isolated from the gel.



Figure 2S. Analysis of the electrophoretic mobility of EAD2-T<sub>10</sub>-Hemin and EAD2-T10-NH<sub>2</sub> in the presence of 200 mM CH<sub>3</sub>COONa (**A**) or 200 mM CH<sub>3</sub>COONa and 20 mM CH<sub>3</sub>COOK (**B**). Lanes: *1* - control random DNA 25-mer, *2* - 28-mer, *3* - 35-mer, *4* - EAD2-dT<sub>10</sub>-NH<sub>2</sub>, *5* - EAD2-dT<sub>10</sub>-Hemin. All oligonucleotides were 5'-end <sup>32</sup>P-labelled. A blurred band in the lane 5 (A) may result from a partial destruction of the quadruplex structure under nonequilibrium electrophoresis conditions. Electrophoresis was performed in 20% acrylamide native gels at 4°C in 100 mM TBE (Tris/boric acid/EDTA) buffer, pH 8.0.

Optimization of experimental conditions for chemiluminescent determination of PMDNAzyme(T<sub>10</sub>) measured towards luminol.



Figure S3. pH-Dependence of activity of PMDNAzyme( $T_{10}$ ) measured towards luminol. Experimental conditions: 25 mM Tris buffer with 20 mM KCl, 200 mM NaCl, and 0.1% Triton X100; [H<sub>2</sub>O<sub>2</sub>] = 1.3 mM; [luminol] = 5 $\mu$ M; [PMDNAzyme( $T_{10}$ )] = 6 x 10<sup>-8</sup> M.



Figure S4. Effect of luminol concentration on chemiluminescence intensity produced upon PMDNAzyme( $T_{10}$ ) -catalyzed oxidation of luminol. Experimental conditions: 25 mM Tris buffer, pH 8.6 with 20 mM KCl, 200 mM NaCl, and 0.1% Triton X100; [H<sub>2</sub>O<sub>2</sub>] = 1.3 mM; [PMDNAzyme( $T_{10}$ )] = 6 x 10<sup>-8</sup> M.



Figure S5. Effect of hydrogen peroxide concentration on chemiluminescence intensity produced upon PMDNAzyme( $T_{10}$ )-catalyzed oxidation of luminol. Experimental conditions: 25 mM Tris buffer, pH 8.6 with 20 mM KCl, 200 mM NaCl, and 0.1% Triton X100; [luminol] = 5  $\mu$ M; [PMDNAzyme( $T_{10}$ )] = 4 x 10<sup>-8</sup> M.

Optimization of experimental conditions for colorimetric determination of PMDNAzyme(T<sub>10</sub>) measured towards ABTS.



Figure S6. Dependence of initial rate of PMDNAzyme( $T_{10}$ )-catalyzed oxidation of ABTS as a function of pH. Experimental conditions: 25 mM citrate-phosphate buffer with 20 mM KCl, 200 mM NaCl, and 0.1% Triton X100; [ $H_2O_2$ ] = 3 mM; [ABTS] = 3mM; [PMDNAzyme( $T_{10}$ )] = 3 x 10<sup>-8</sup> M.



Figure S7. Dependence of initial rate of PMDNAzyme( $T_{10}$ )-catalyzed oxidation of ABTS on concentration of hydrogen peroxide. Experimental conditions: 25 mM citrate-phosphate buffer, pH 6.0 with 20 mM KCl, 200 mM NaCl, and 0.1% Triton X100; [ABTS] = 3mM; [PMDNAzyme( $T_{10}$ )] = 1 x 10<sup>-7</sup> M.



Figure S8. Kinetics of PMDNAzyme( $T_{10}$ )-catalyzed oxidation of ABTS in the presence of different ABTS concentration. Experimental conditions: 25 mM citrate-phosphate buffer, pH 6.0 with 20 mM KCl, 200 mM NaCl, and 0.1% Triton X100; [ $H_2O_2$ ] = 12 mM; [PMDNAzyme( $T_{10}$ )] = 1x10<sup>-7</sup> M.