Supporting Information

Carbon Dot mediated G-quadruplex Nano-Network Formation for Enhanced DNAzyme Activity and Easy Catalyst Reclamation

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Table S1: DNA sequences used in the study. All DNA have 5'- phosphate and 3'-OH

1. For CD-G quadruplex network formation[1] 5-AAATTGGGGTTGGGGTATTC-3'
2. For intramolecular CD- G quadruplex formation[2] 5' AAAAAGGTGGTGGTGGTTGTGGTGGTGGTGG 3'
3. Control non G- rich sequence[3] 5' TCGGATAGTGCGGCTGTTGACTGA 3'

1. B. Tuesuwan, J. T. Kern, P. W. Thomas, M. Rodriguez, J. Li, W. M. David and S. M. Kerwin, *Biochemistry*, 2008, **47**, 1896-1909.

2. a. Haq, I.; Trent, J.O.; Chowdhry, B.Z.; Jenkins, T.C. Intercalative G-Tetraplex Stabilization of Telomeric DNA by a Cationic Porphyrin. *J. Am. Chem. Soc.* **1999**, *121*, 1768-1779.

b. Fan, X., Sun, L., Wu, Y., Zhang, L., & Yang, Z. (2016). Bioactivity of 2′-deoxyinosine-incorporated aptamer AS1411. *Scientific reports*, 2016, *6*, 25799.

3. Kumari, S., Solanki, A., Mandal, S., Subramanyam, D., & Das, P., Creation of Linear Carbon Dot Array with Improved Optical Properties through Controlled Covalent Conjugation with DNA. *Bioconjugate chemistry*, 2018, *29*(5), 1500-1504.

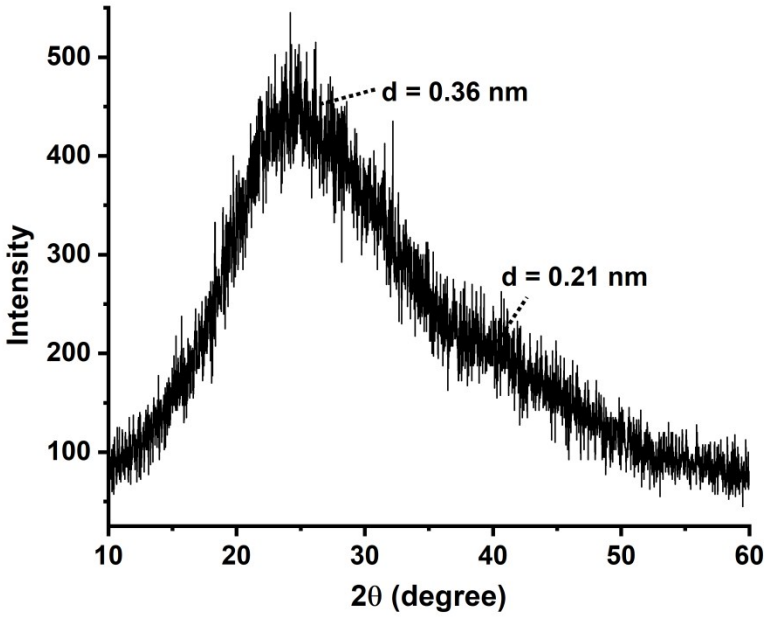

Figure S1: Powered XRD of CD

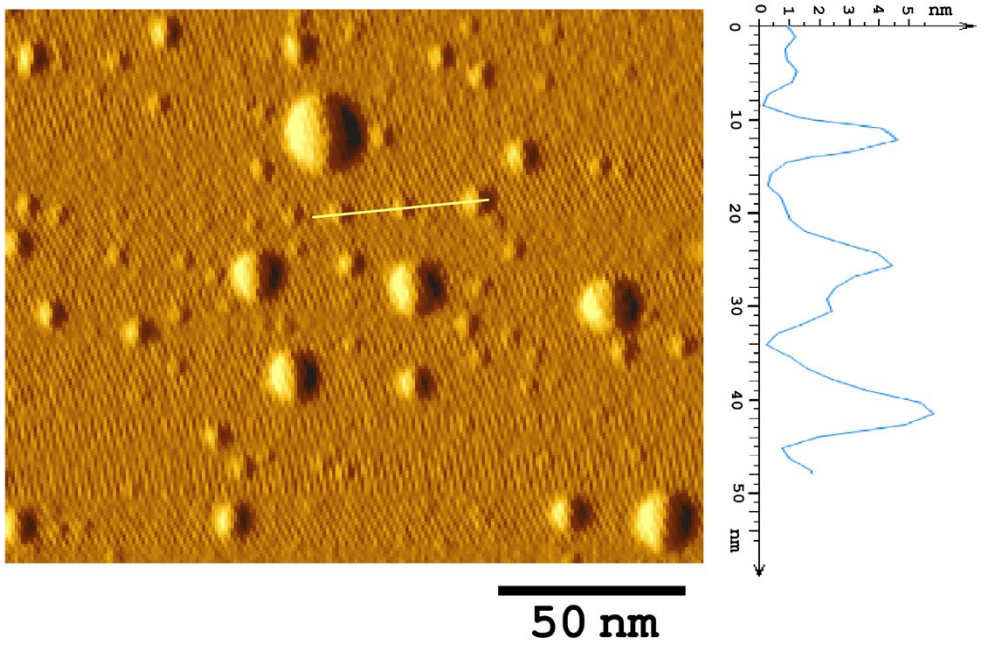

Figure S2: AFM image of CD

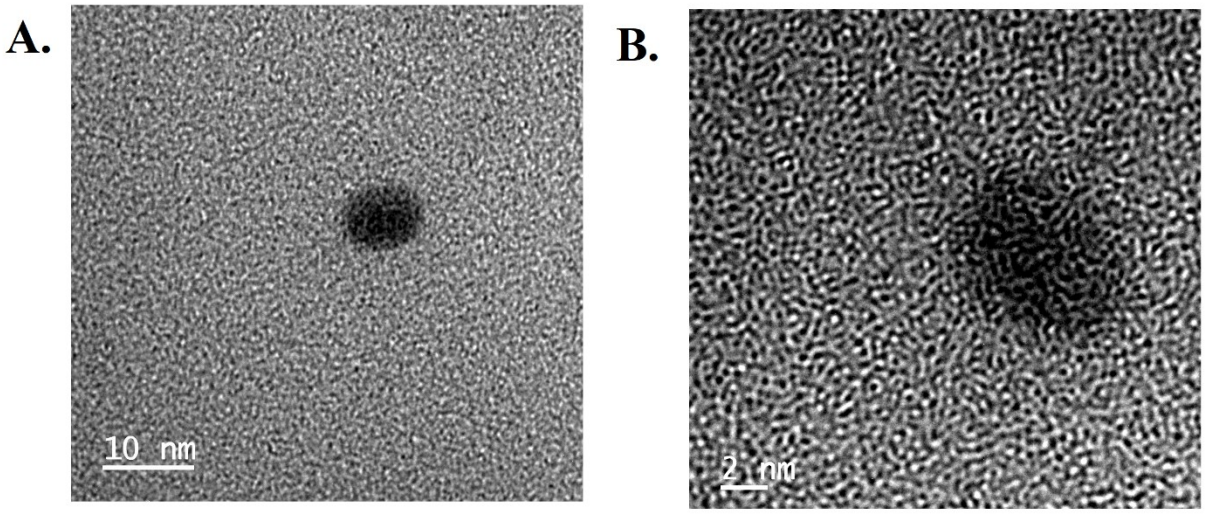

Figure S3: TEM images of CD

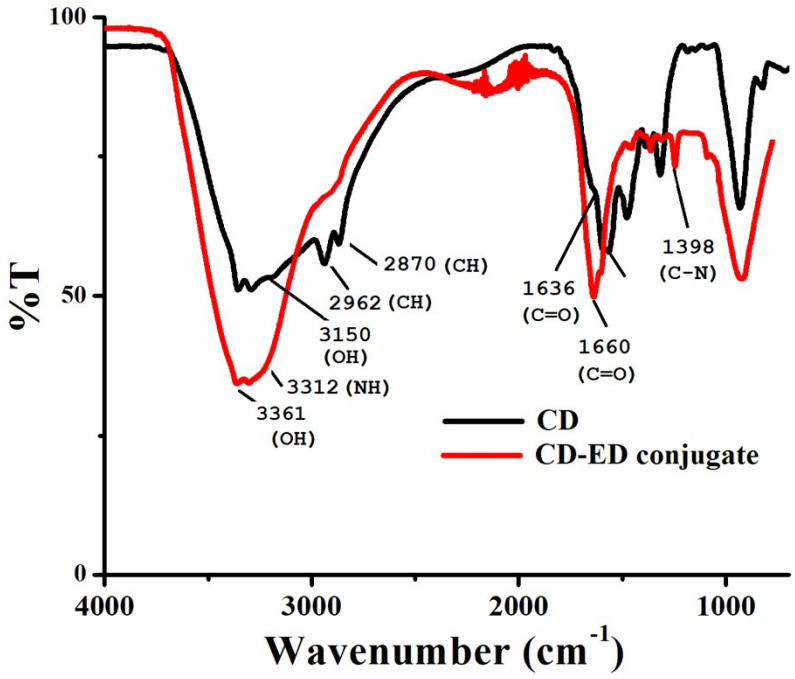

Figure S4: FTIR spectra of CD before and after conjugation with ED

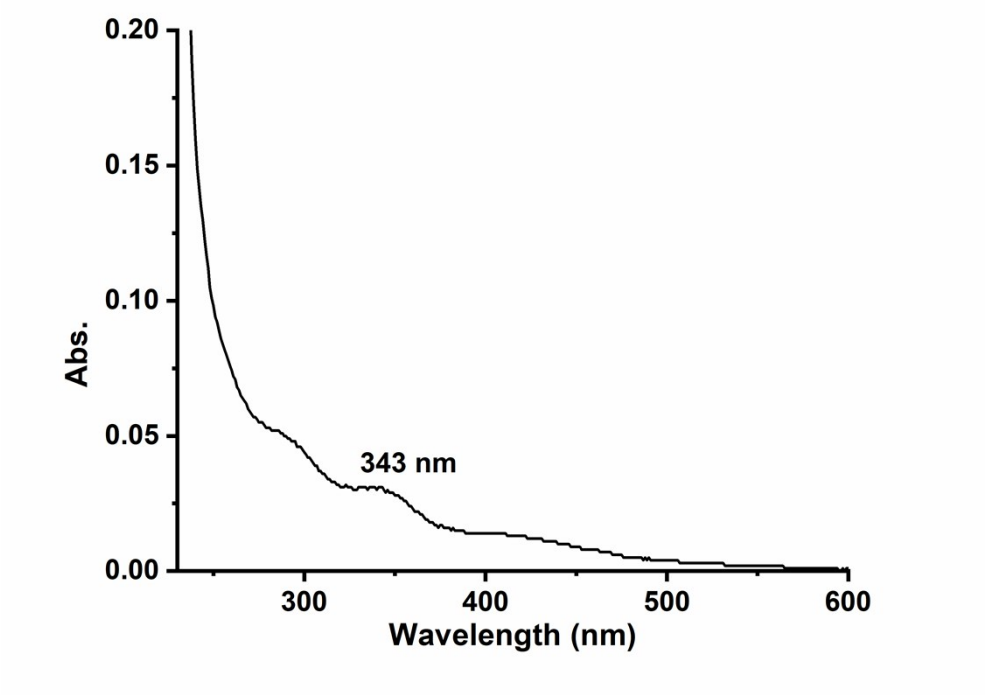

Figure S5: UV-Vis spectra of CD

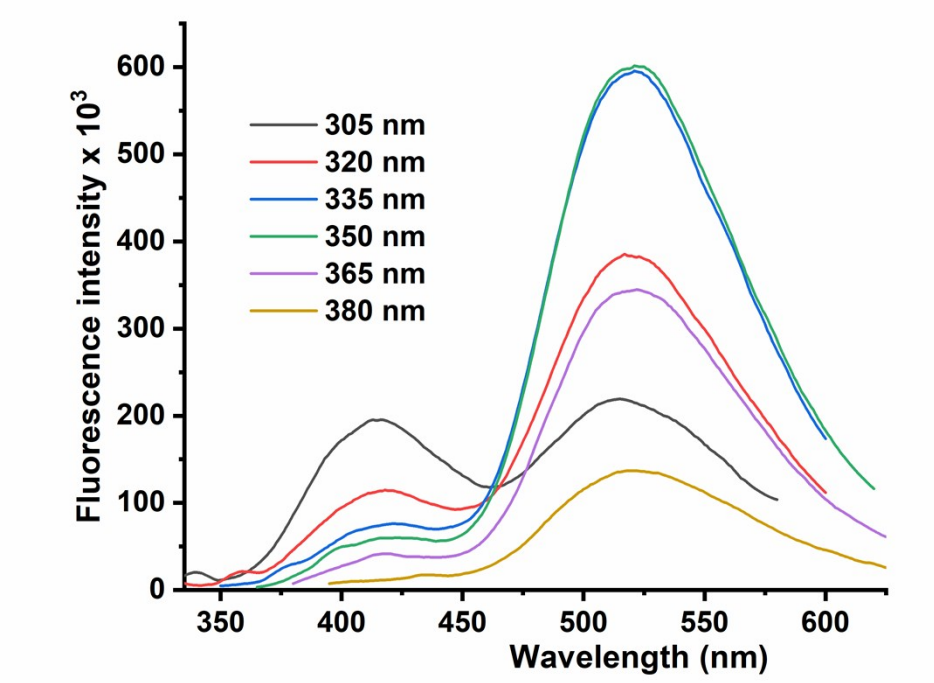

Figure S6: Steady state of CD when excited with wavelength 308 to 350 nm.

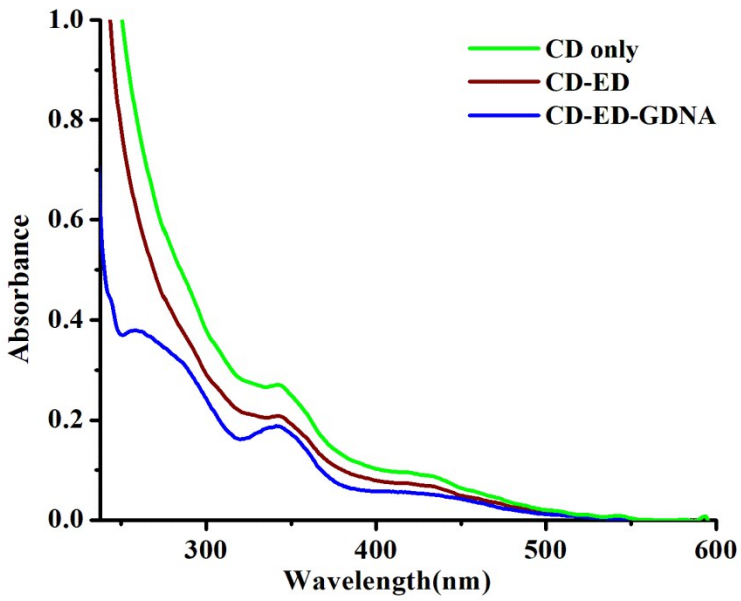

Figure S7: UV-Vis spectra of CD, CD-ED conjugate and CD-DNA conjugate

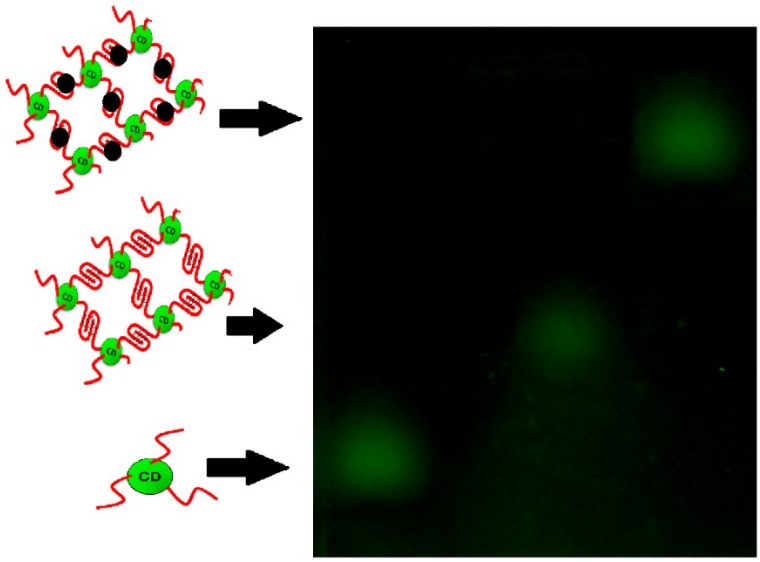

Figure S8: Agarose gel electrophoresis showing restricted mobility of CD-G quadruplex network after Hemin complexation. Black sphere in top network depicts Hemin

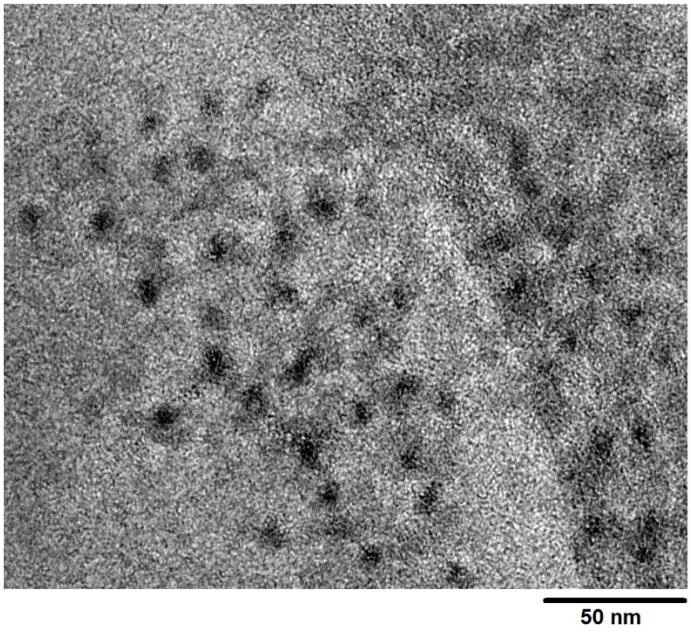

Figure S9: TEM image of CD-G-quadruplex network

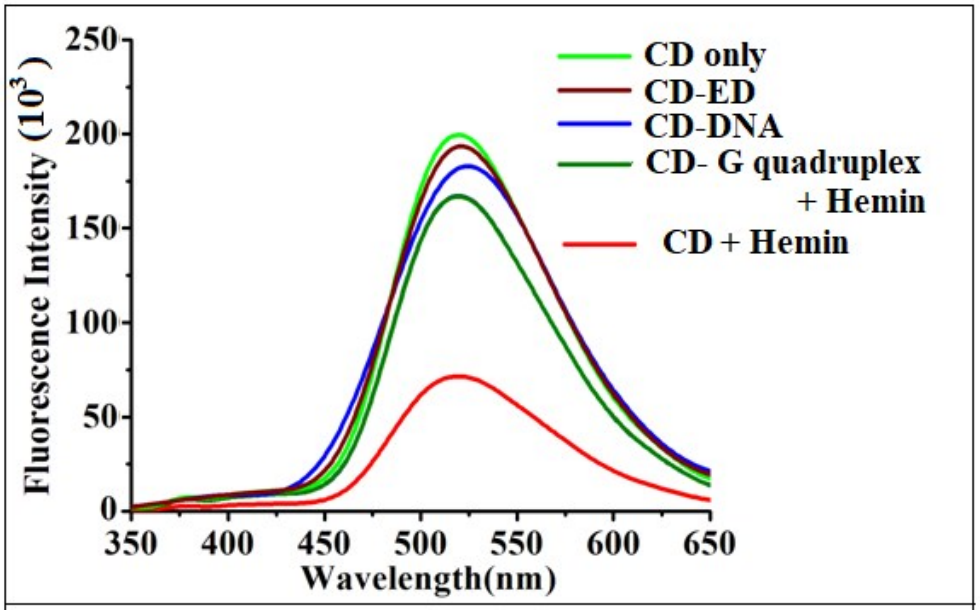

Figure S10: Steady State Spectra (excitation at 330nm) of CD, CD-DNA conjugate and CD-G quadruplex-Hemin nanonetwork. (The concentration of DNA and hemin was maintained at 1:1 at 2μM)

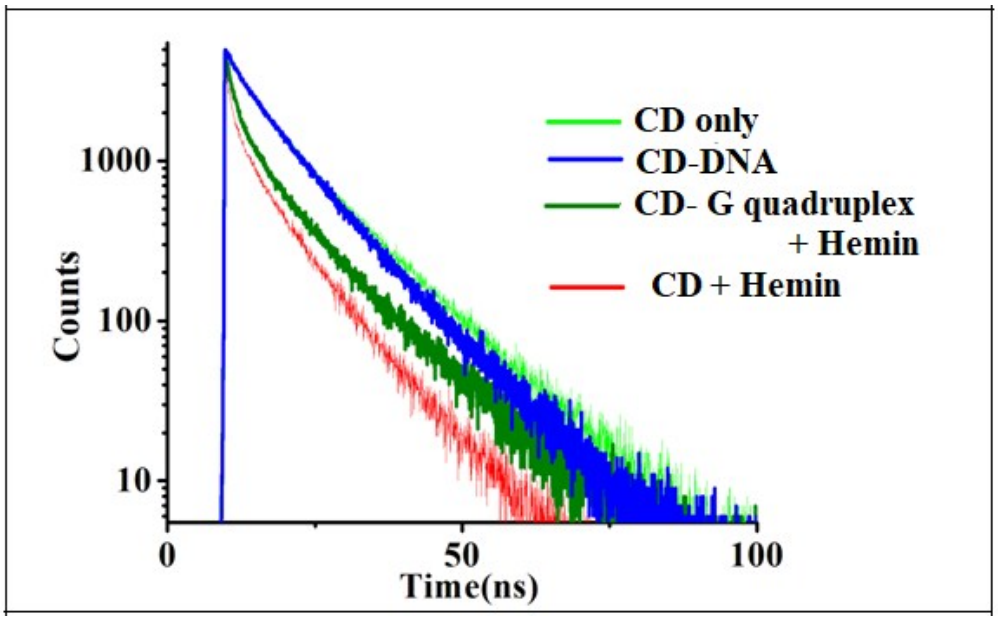

Figure S11: Fluorescence Lifetime spectra of CD, CD-DNA conjugate and CD-G quadruplex-Hemin nanonetwork. (The concentration of DNA and hemin was maintained at 1:1 at 2μM)

Sample	τ1(ns)	Relative %	τ2 (ns)	Relative %	τ 3 (ns)	Relative %	χ 2	Average Lifetime (ns)
CD	0.58	2.61	4.31	20.47	10.01	76.93	1.056	8.59809
CD-ED-ssDNA	0.45	4.39	2.45	27.28	11.53	68.33	1.39	8.56656
CD-ED-Hemin	0.18	9.12	2.42	30.28	10.3	60.6	1.212	6.99099
CD-G Quadruplex-Hemin	0.55	2.65	4.31	20.49	10.11	76.87	1.056	8.66925

Table S2: Data for Fluorescence lifetime study

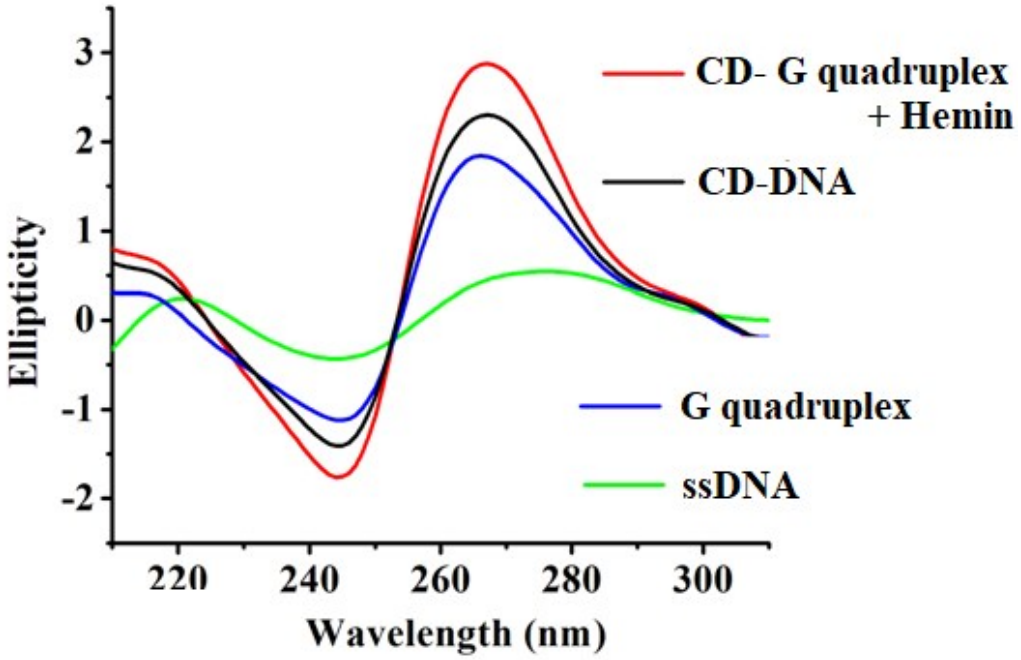

Fig. S12: Circular Dichroism spectra of CD, CD-DNA conjugate and CD-G quadruplex-Hemin nanonetwork. (The concentration of DNA and Hemin was maintained at 1:1 at 2μM)

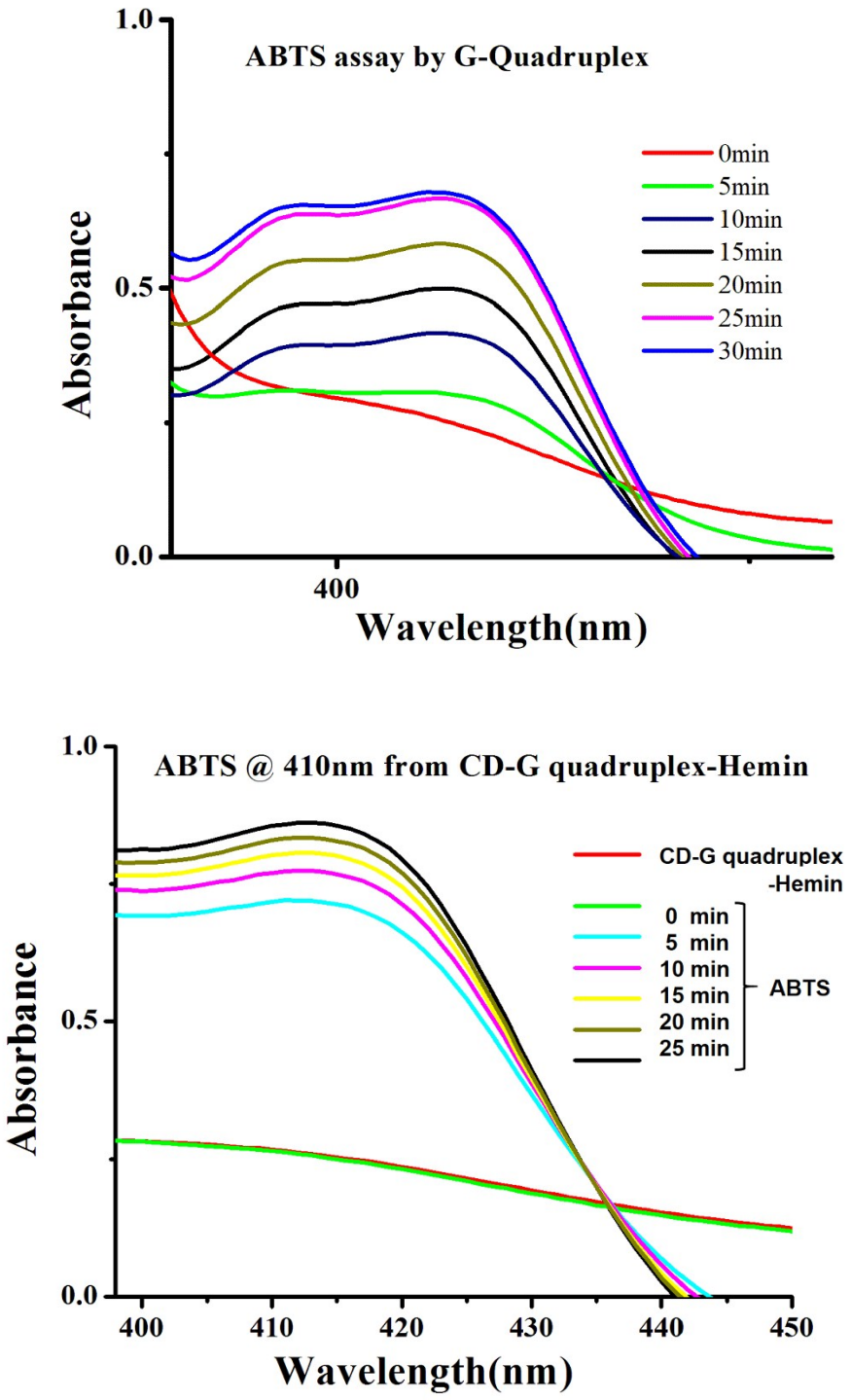

Figure S13: UV spectrophotometric scan for reaction of ABTS in presence of Hydrogen peroxide with control G-quadruplex-Hemin complex (top) and CD-G quadruplex-Hemin Nanonetwork (bottom). (Concentration of DNA is maintained at 2μM, ABTS and H_2O_2 at100 μM).

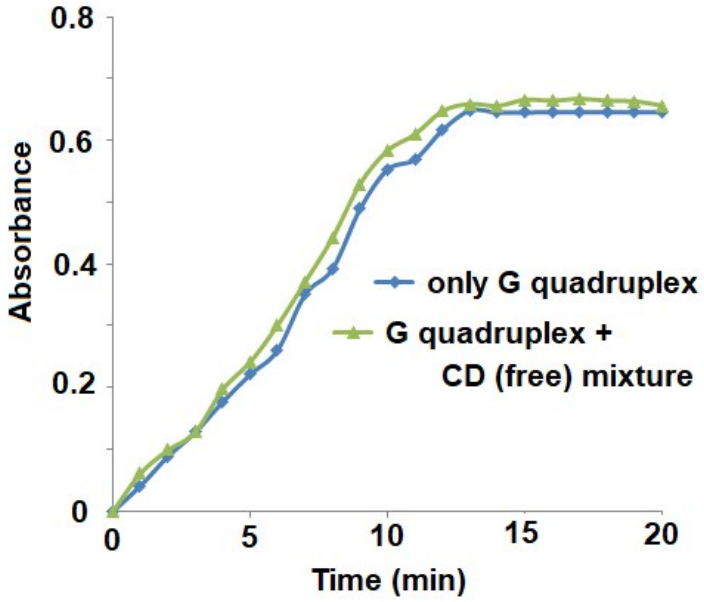

Figure S14: Comparison of peroxidase activity of G quadruplex-Hemin and G quadruplex-Hemin mixed with free CD without any covalent conjugation using ABTS reaction (average of 3 points)

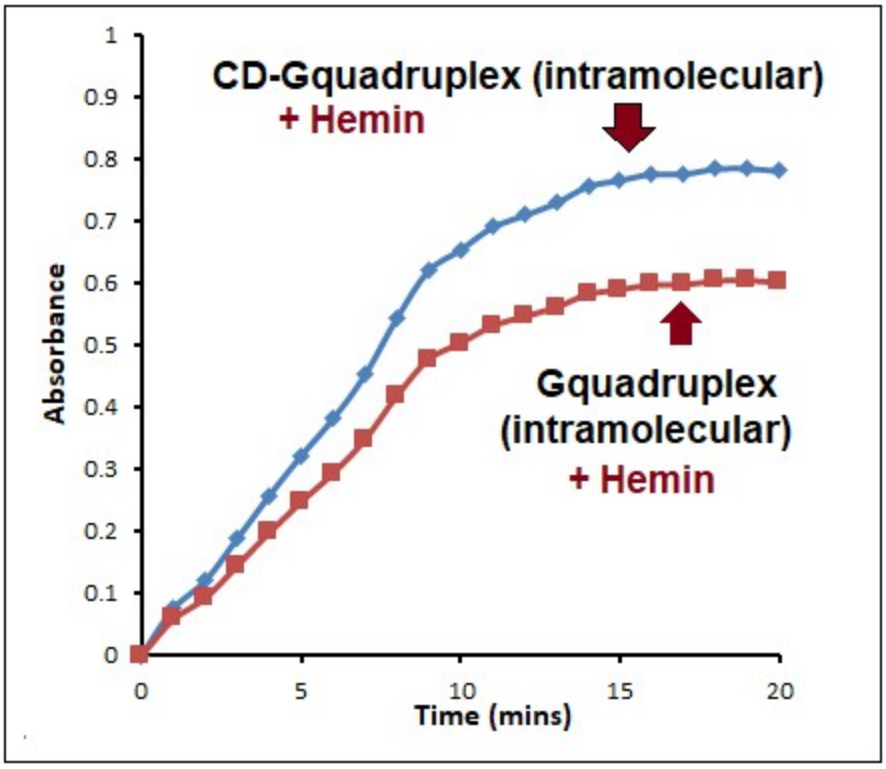

Figure S15: Comparison of peroxidase activity of **intramolecular** G quadruplex-Hemin and CD- **intramolecular** G quadruplex-Hemin using ABTS reaction (average of 3 points) (top). (Concentration of DNA is maintained at 2µM, ABTS and H_2O_2 at100 µM). Digital image of ABTS reaction of the same solution after 15 min ABTS reaction (left: CD-intramolecular Gquadruplex, right: only intramolecular G quadruplex

Table S3: ITC revelations of G quadruplex +Hemin and CD-G quadruplex network +Hemin.

Sample	No of binding sites	$K_{1:1}$ (M^{-1})	ΔG^0 (kcal/mol)
G quadruplex +hemin	0.93	6.05×10^4	-6.52
CD-G quadruplex+hemin	1.64	4.54×10^5	-7.71

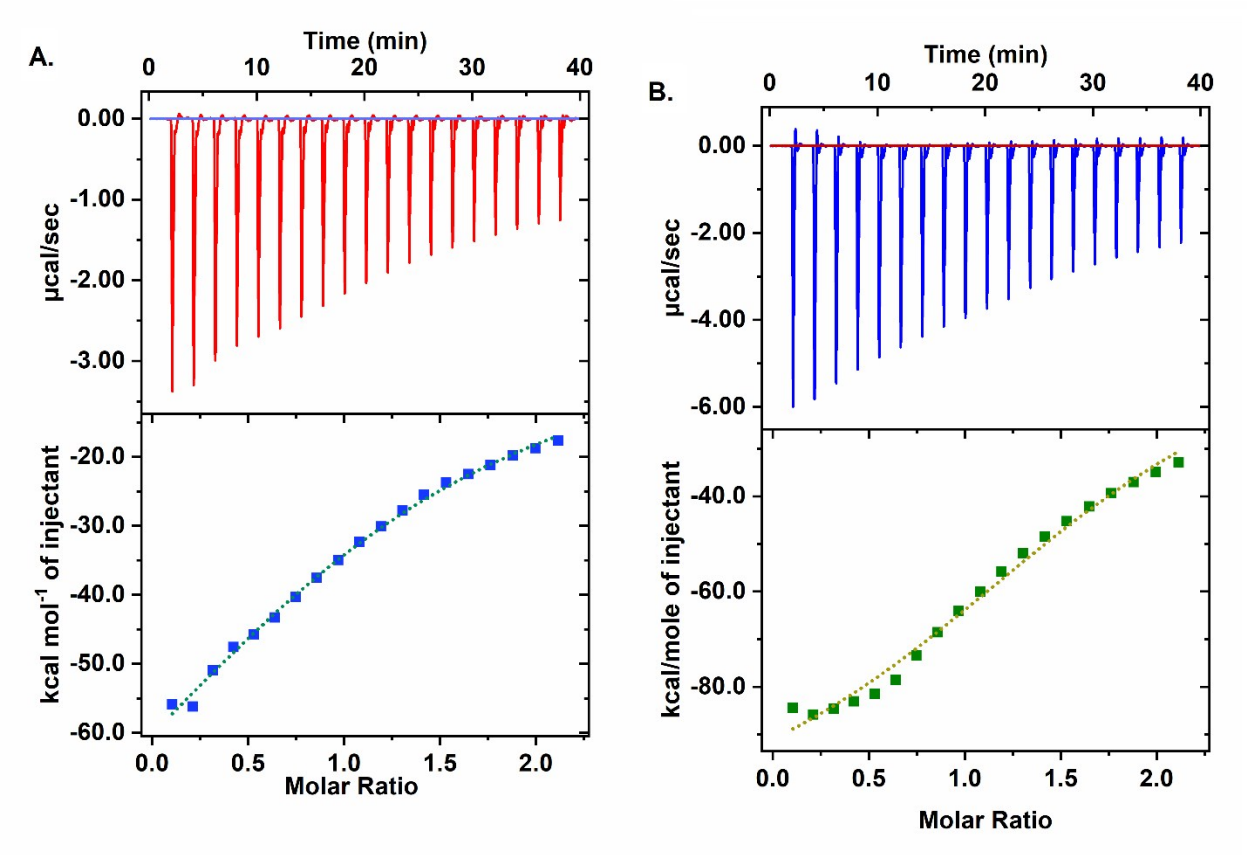

Figure S16: ITC data revealing the higher binding constant of Hemin with the CD-**intramolecular** G quadruplex. A. Intramolecular G quadruplex + Hemin B. CD-intramolecular G-quadruplex +Hemin.

Table S4: ITC revelations of **<u>intramolecular</u>** G quadruplex +Hemin and CD-intramolecular G quadruplex + Hemin (no network).

Sample	No of binding sites	$k_{1:1}$ (M^{-1})	ΔG^0 (kcal/mol)
G quadruplex + Hemin	1.18	5.02×10^4	-5.55
CD-G quadruplex+ Hemin	1.69	1.10×10^5	-6.87

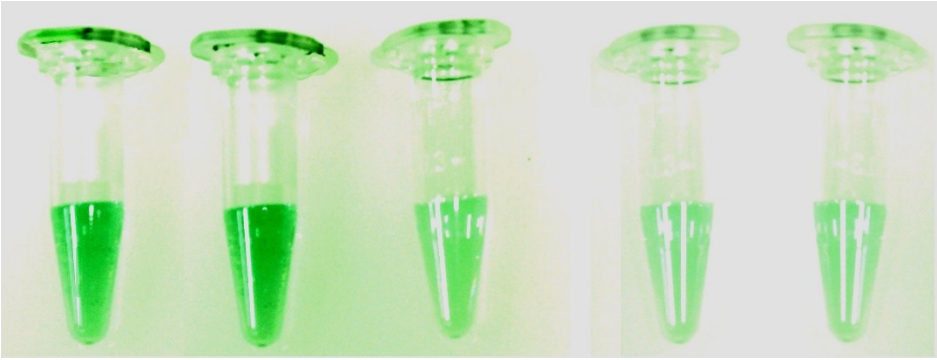

Figure S17: ABTS assay results with recovered DNAzyme upto five cycles.