

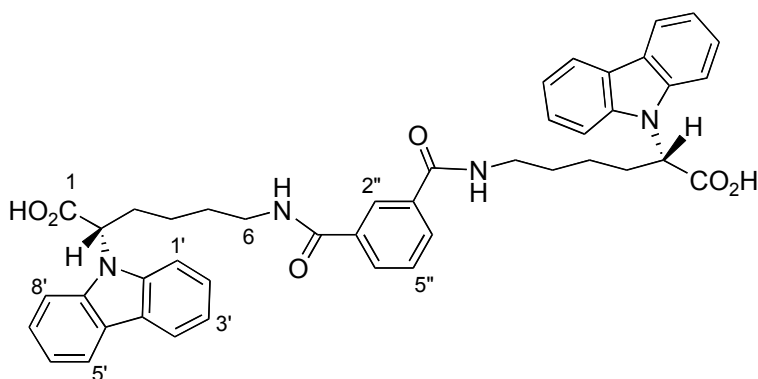
New Magnetically Responsive Polydicarbazole-Magnetite Nanoparticles

Jean-Paul Lellouche,* Nurit Perlman, Augustine Joseph, Senthil Govindaraji, Ludmila Buzhansky, Aline Yakir and Ian Bruce

Electronic Supplementary Information

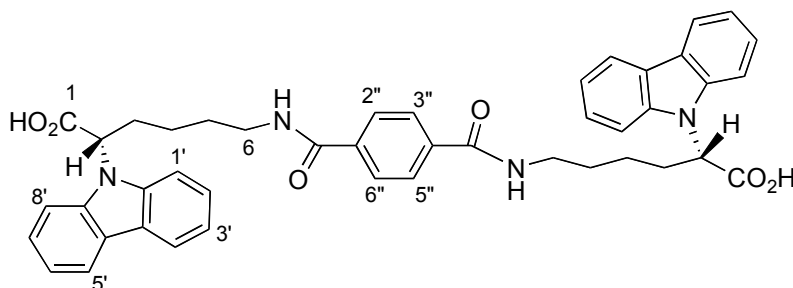
I. Selected Analytical/Spectroscopic Data for Dicarbazole Monomers (*S,S*)-4 and (*S,S*)-5:

(*S,S*)-Dicarbazole diacid 4



mp: 157-158 °C; $[\alpha]_D^{27.1} = -19^\circ$ ($c = 0.01 \text{ gmL}^{-1}$, MeOH); FT-IR (KBr pellet, cm^{-1}): 3370 ($\nu_{\text{N-H}}$), [3048, 2932, 2858] ($\nu_{\text{C-H}}$ stretch), 1732 ($\nu_{\text{C=O}}$ acid), 1627 ($\nu_{\text{C=O}}$ amide), [1545, 1452] ($\nu_{\text{C=C}}$), 1328 ($\nu_{\text{C-H}}$ bending), 1223 ($\nu_{\text{C-O}}$), 1157 ($\nu_{\text{C-N}}$), [753, 726] ($\nu_{\text{C=C-H}}$); ^1H NMR ($\text{Me}_2\text{SO-D}_6$, 300 MHz): δ 8.46 (t, $J = 5.4 \text{ Hz}$, 2H, 2 x N-H), 8.20 (bd, $J = 7.8 \text{ Hz}$, 5H, aromatic), 7.87 (dd, $J = 7.8, 1.5 \text{ Hz}$, 2H, aromatics), 7.62 (d, $J = 8.4 \text{ Hz}$, 4H, aromatics), 7.55-7.41 (m, 5H, aromatics), 7.24 (t, $J = 7.5 \text{ Hz}$, 4H, aromatics), 5.67 (t, $J = 7.8 \text{ Hz}$, 2H, 2 x $\text{C}_2\text{-H}$), 3.24-3.12 (m, 4H, 2 x $\text{C}_6\text{-H}_2$), 2.42 (q, $J = 7.5 \text{ Hz}$, 4H, 2 x $\text{C}_3\text{-H}_2$), 1.66-1.43 (m, 4H, 2 x $\text{C}_5\text{-H}_2$), 1.41-1.25 (m, 2H, 2 x $\text{HC}_4\text{-H}$), 1.03-0.87 (m, 2H, 2 x $\text{HC}_4\text{-H}$); ^{13}C NMR ($\text{Me}_2\text{SO-D}_6$, 75 MHz): δ 172.9 (C=O, acid), 166.7 (C=O, amide), 140.9 (N-C=C), 135.8 (C1" and C3"), 130.4 (C4" and C6"), 129.0 (C5"), 127.1 (C2"), 126.7 (C3' and C6'), 123.4 (C4' and C5'), 121.2 (C2' and C7'), 119.9 (N-C=C), 110.9 (C1' and C8'), 57.1 (C2), 39.9 (C6), 29.8 (C5), 29.6 (C3), 24.1 (C4); FAB-MS m/z 723.3 (96.7%, $[\text{MH}]^+$), 692.4 (22.6%), 634.3 (96.4%, $[\text{M}-2\text{COO}]^+$); High Resolution MS (DCI, CH_4) m/z calcd for $\text{C}_{42}\text{H}_{42}\text{N}_4\text{O}_2$ $[\text{M}-2\text{COO}]^+$ 634.3308. Found 634.3270.

(*S,S*)-Dicarbazole diacid 5



mp: 148-150 °C; $[\alpha]_D^{26.5} = -4^\circ$ ($c = 0.015 \text{ gmL}^{-1}$, MeOH); FT-IR (KBr pellet, cm^{-1}): 3390 ($\nu_{\text{N-H}}$, $\nu_{\text{O-H}}$), [3056, 2932, 2858] ($\nu_{\text{C-H}}$ stretch), 1724 ($\nu_{\text{C=O}}$ acid), 1619 ($\nu_{\text{C=O}}$ amide), [1541, 1483,

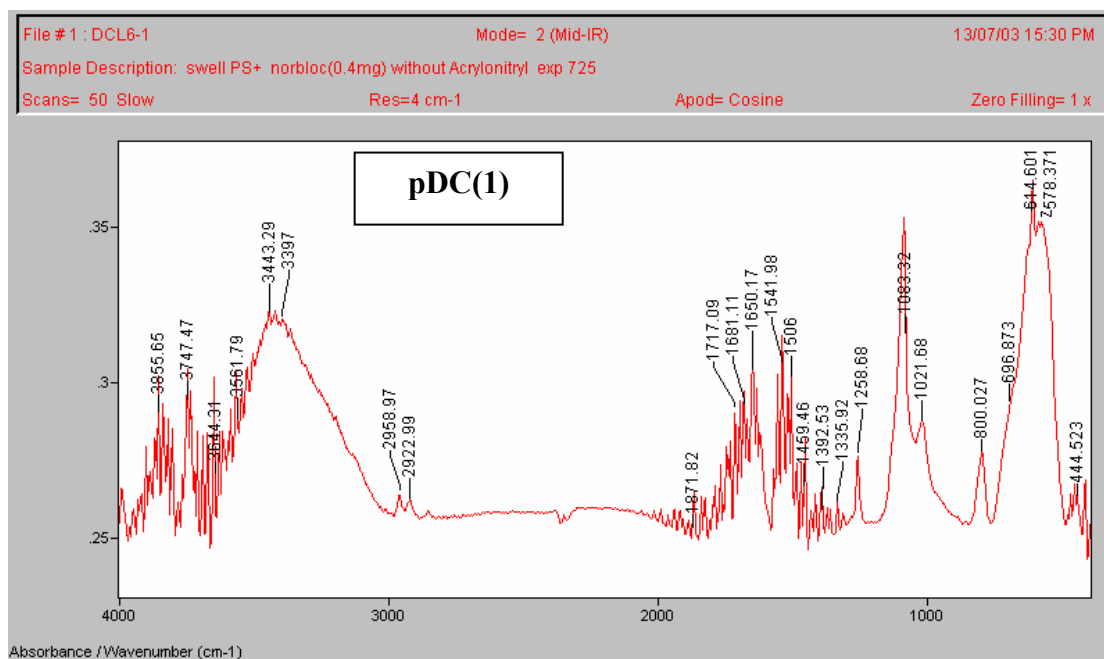
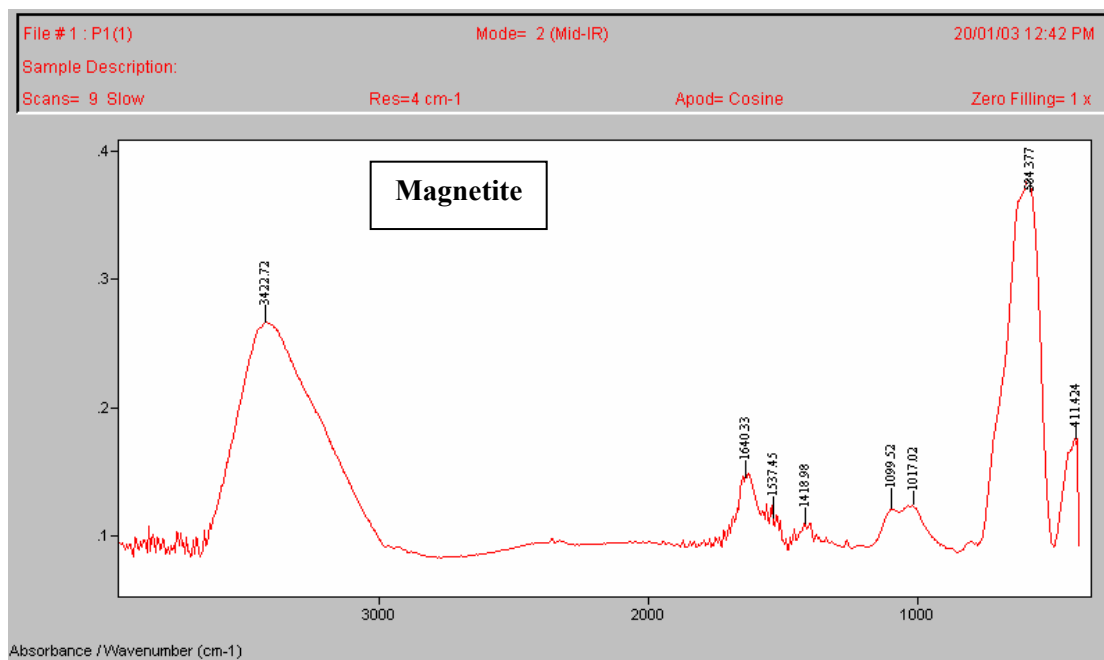
1452] ($\nu_{C=C}$), 1328 (ν_{C-H} bending), 1223 (ν_{C-O}), 1165 (ν_{C-N}), [753, 726] ($\nu_{C=C-H}$); 1H NMR (CD₃OD, 300 MHz): δ 8.02 (d, $J = 7.8$ Hz, 4H, aromatics), 7.57 (s, 4H, aromatics), 7.43-7.41 (m, 4H, aromatics), 7.34 (t, $J = 7.8$ Hz, 4H, aromatics), 7.14 (t, $J = 7.8$ Hz, 4H, aromatics), 5.39 (dd, $J = 10.2$ and 5.4 Hz, 2H, 2 x C₂-H), 3.15-3.08 (m, 4H, 2 x C₆-H₂), 2.48-2.37 (m, 4H, 2 x C₃-H₂), 1.54-1.41 (m, 4H, 2 x C₅-H₂), 1.25-1.21 (m, 2H, 2 x H-C₄H), 0.94-0.80 (m, 2H, 2 x HC₄-H); ^{13}C NMR (CD₃OD, 75 MHz): δ 173.9 (C=O, acid), 169.1 (C=O, amide), 141.4 (N-C=C), 138.1 (C1" and C4"), 128.1 (C2", C3", C5" and C6"), 126.6 (C3' and C6'), 124.3 (C4' and C5'), 121.1 (C2' and C7'), 120.2 (N-C=C), 110.8 (C1' and C8'), 57.6 (C2), 40.5 (C6), 30.3 (C5), 29.7 (C3), 24.5 (C4); FAB-MS m/z 723.3 (100%, [MH]⁺), 677.5 (51.8%, [M]⁺-COOH), 634.4 (54.2%, [M]⁺-2COO); High Resolution FAB-MS m/z calcd for C₄₄H₄₂N₄O₆ [M]⁺ 722.3104. Found 722.3100.

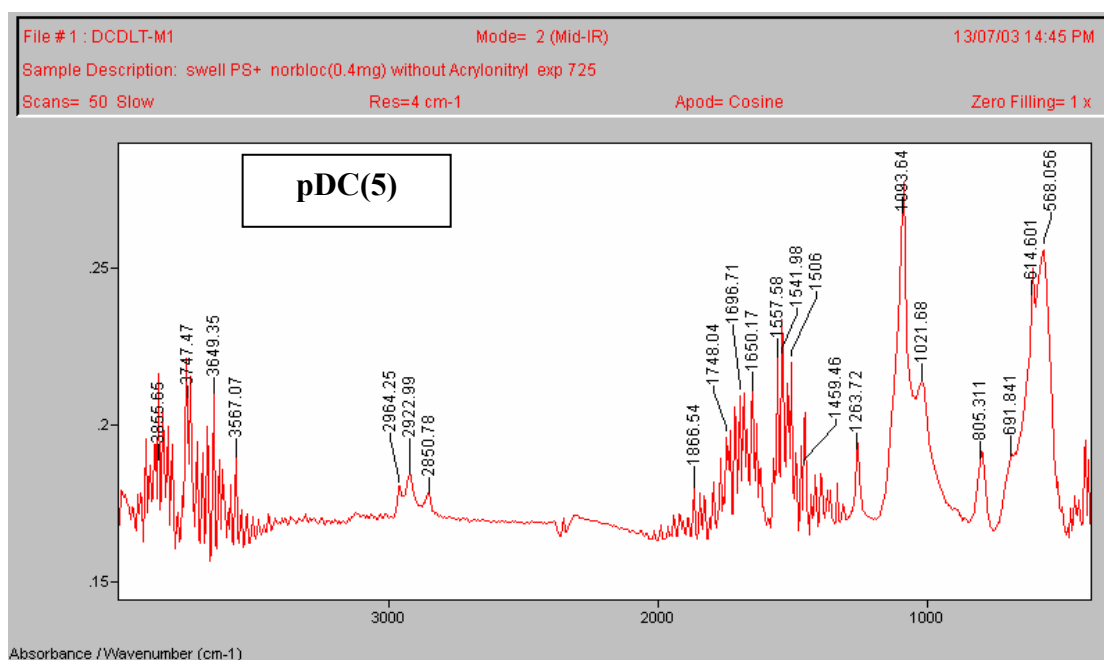
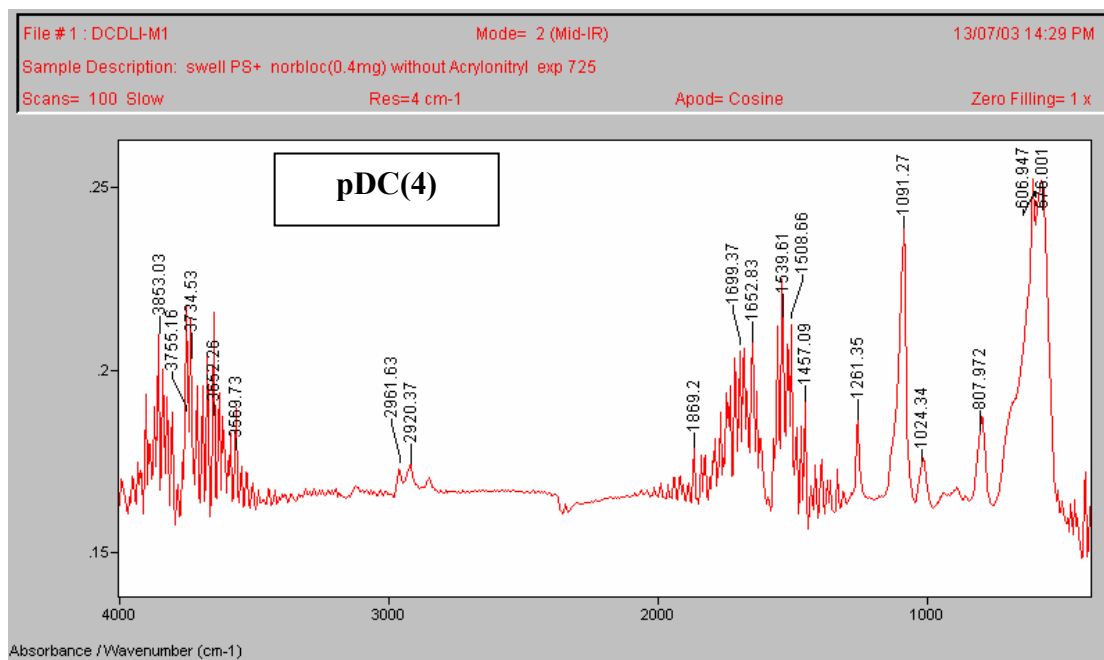
II. Elemental Analyses (CE Instruments, model EA 1110/CHNS-O)

Polydicarbazole polymers have been polymerized *in absence and in presence of magnetite* (results for each element are given in %):

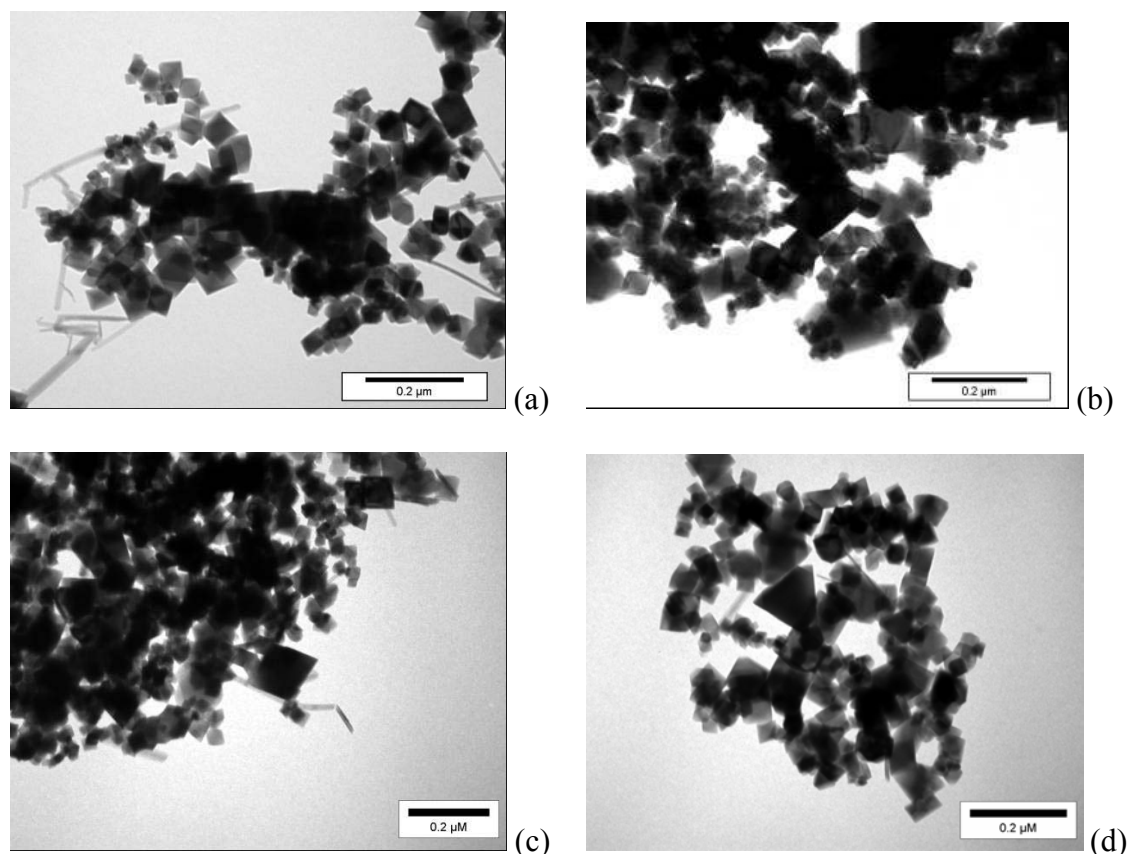
	C	H	N
pDC-(S)-1	77.7117	5.9984	7.9096
COOH-pDC-magnetite NC pDC(1)	19.5201	1.4017	2.0113
pDC-(S,S)-4	69.5175	5.7577	8.0717
COOH-pDC-magnetite NC pDC(4)	20.8723	1.7826	2.4102
pDC-(S,S)-5	65.4988	4.2545	8.9628
COOH-pDC-magnetite NC pDC(5)	21.1776	1.6701	2.3236

III. FT-IR Spectra of Magnetite and pDC-Magnetite NCs pDC(1) and pDC(4-5) (KBr pellet, ν in cm⁻¹, Bomen-Hartmann & Braun device):





IV. Transmission Electron Microscopy (TEM, JEOL-1200EX)



TEM images of starting magnetite nanoparticles (a), of magnetite nanoparticles coated by poly(1) toward pDC(1) (b), of magnetite nanoparticles coated by poly(4) toward pDC(4) (c), and of magnetite nanoparticles coated by poly(5) toward pDC(5) (d).

V. High-Resolution Transmission Electron Microscopy and Energy Dispersive X-Ray Microanalysis of Nanocomposites

The nanocomposite pDC(5) was examined using a high-resolution TEM electron microscope (HR-TEM) JEOL-JEM 2010 (Oxford Instruments; 400 mesh copper grids from SPI Supplies, West Chester, PA, USA) working at a 200 kV accelerating voltage. A CCD video camera (Gatan), operated at a spatial resolution of 1024×1024 pixels, is used to digitize micrographs, that are processed using the Oxford Digital Micrograph software. Elemental compositions for the three nanocomposites pDC(1) and pDC(4-5) were determined by energy-dispersive X-ray microanalysis (EDAX) using an Oxford Instruments X-ray detector and the INCA software (electron beam \varnothing 15 nm).

Nanocomposite pDC(5) ($\times 780000$)

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Nanocomposite pDC(5) (**×520000**)

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

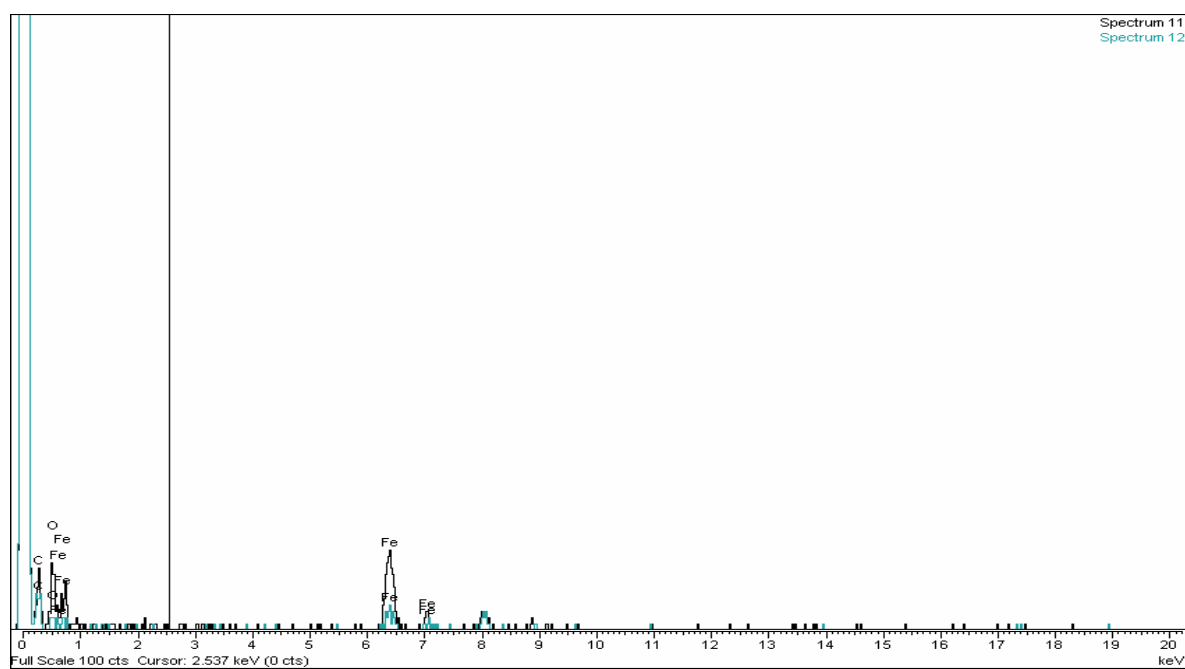
Elemental analyses of nanocomposites pDC(1) and pDC(4-5)

pDC(4)

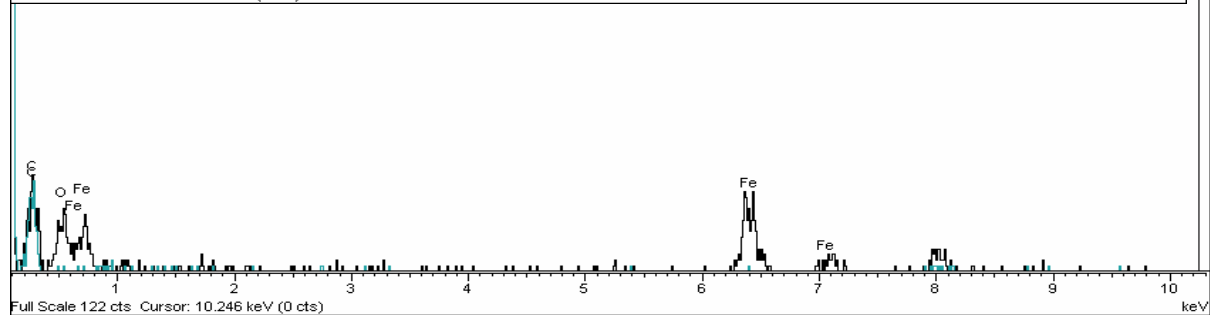
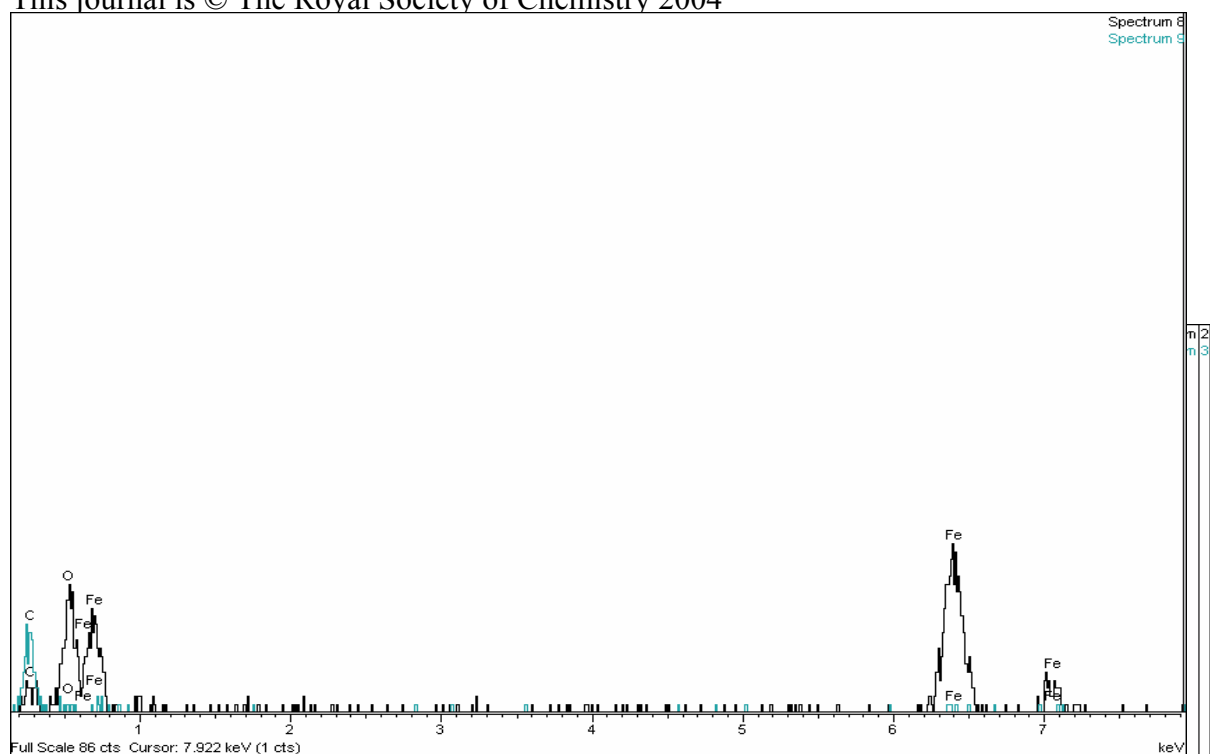
(Processing option : All elements analyzed (Normalised) and results in weight percent)

Spectrum	C	O	Fe	Total
Spectrum 4	100.00			100.00
Spectrum 5	100.00			100.00

Spectrum 6	12.10	39.75	48.16	100.00
Spectrum 7	29.49	30.36	40.15	100.00
Spectrum 8	39.61	17.97	42.43	100.00
Spectrum 9	100.00			100.00
Spectrum 10	100.00			100.00
Spectrum 11 (center)	30.44	26.79	42.77	100.00
Spectrum 12 (edge)	63.40	15.43	21.17	100.00
Spectrum 13	100.00			100.00
Max.	100.00	39.75	48.16	
Min.	12.10	15.43	21.17	

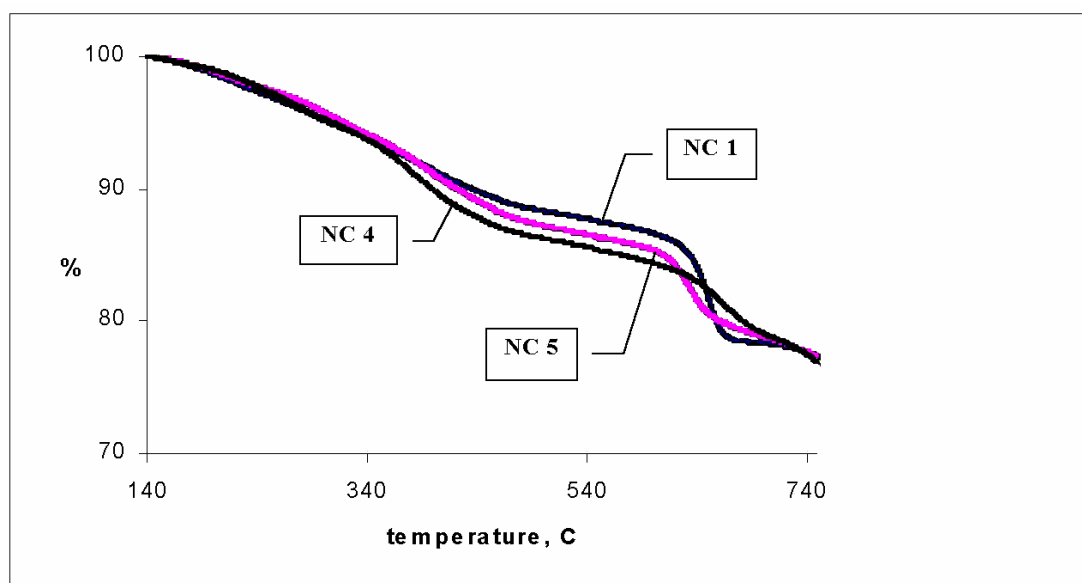


Spectrum 3 (edge)	100.00			100.00
Spectrum 5	13.50	30.14	56.36	100.00
Spectrum 6	100.00			100.00
Spectrum 7	26.77	27.22	46.01	100.00
Spectrum 8	100.00			100.00
Max.	100.00	30.14	56.36	
Min.	13.50	16.10	28.83	



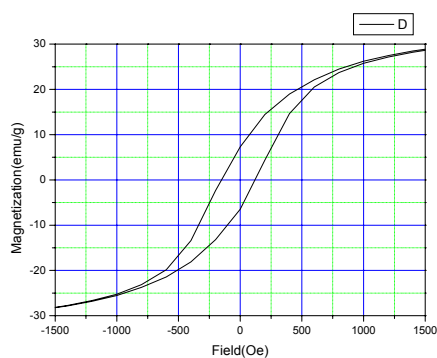
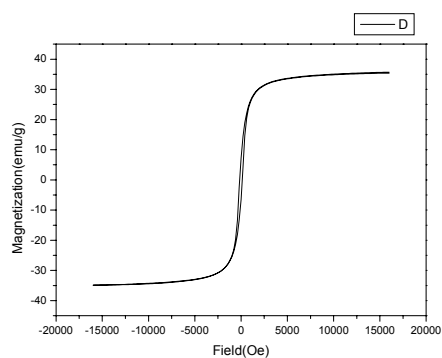
Spectrum 4	100.00			100.00
Spectrum 5	100.00			100.00
Spectrum 6	12.10	39.75	48.16	100.00
Spectrum 7	29.49	30.36	40.15	100.00
Spectrum 8	39.61	17.97	42.43	100.00
Spectrum 9	100.00			100.00
Spectrum 10	100.00			100.00
Spectrum 11 (center)	30.44	26.79	42.77	100.00
Spectrum 12 (edge)	63.40	15.43	21.17	100.00
Spectrum 13	100.00			100.00
Max.	100.00	39.75	48.16	
Min.	12.10	15.43	21.17	

VI. TGA Analyses of pDC-NCs 1 & 4-5 (obtained using a TGA-Mettler apparatus, TG-50 DSS50 model)

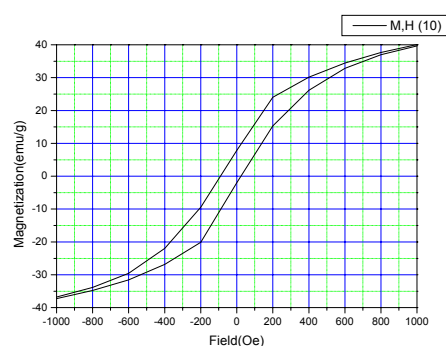
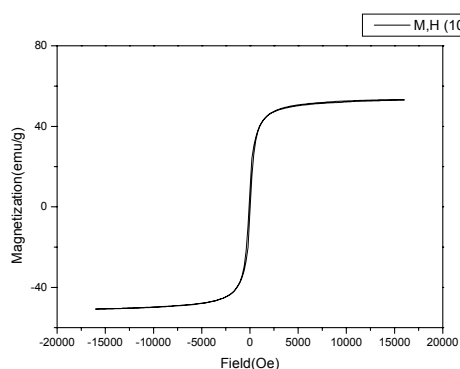


VII. Magnetism Measurements (obtained with an Oxford VSM system Magnetometer equipped with an Aerosonic VSM 3001 controller)

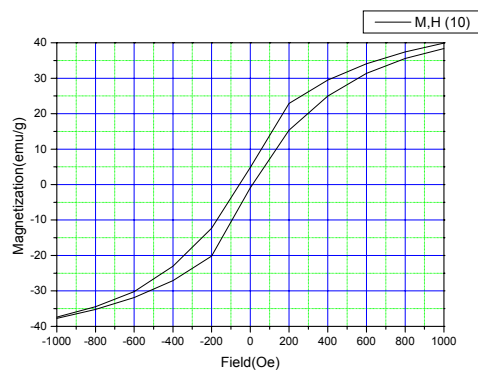
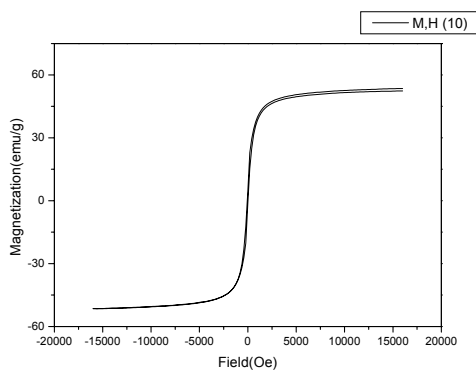
COOH-pDC-magnetite NC pDC(1)
(specific saturation magnetization M_s : 35 emu/g, coercivity H_c : 132 Oe)



COOH-pDC-magnetite NC pDC(4)
(specific saturation magnetization M_s : 52 emu/g, coercivity H_c : 35 Oe)

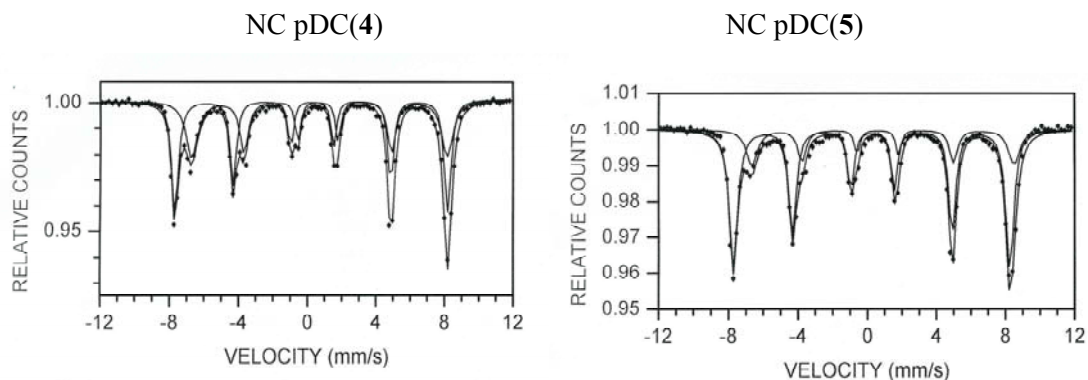


COOH-pDC-magnetite NC pDC(5)
(specific saturation magnetization M_s : 51 emu/g, coercivity H_c : 55 Oe)



VIII. ^{57}Fe Mössbauer Spectroscopy

^{57}Fe Mössbauer spectra were obtained at the Hebrew University of Jerusalem (Racah Institute of Physics, Prof. Israel Felner) using a home-made instrument. They were carried out at 300 K using a conventional constant acceleration drive and a 50 mCi ^{57}Co :Rh source. Experimental spectra were analyzed in terms of several magnetic sub-spectra, by a least square fit procedure. The ^{57}Fe isomer shift is relative to Fe measured at 300 K



NC pDC(4). There are two A/B sub-spectra (A/B ratio: 1.13 ± 0.08)

- A) line width = 0.33 ± 0.02 mm/s
Isomer shift = 0.27 ± 0.02 mm/s
Quadrupole interaction (eqQ/4) = 0.025 ± 0.003 mm/s
Magnetic hyperfine field (H_{eff}) = 49.2 ± 0.1 Tesla
53%
- B) line width = 0.27 ± 0.02 mm/s
Isomer shift = 0.67 ± 0.02 mm/s
Quadrupole interaction (eqQ/4) = 0.05 ± 0.01 mm/s
Magnetic hyperfine field (H_{eff}) = 46.2 ± 0.1 Tesla
47%

NC pDC(5). There are two A/B sub-spectra (A/B ratio: 2.84 ± 0.29)

- A) line width = 0.38 ± 0.02 mm/s
Isomer shift = 0.28 ± 0.02 mm/s
Quadrupole interaction (eqQ/4) = 0.014 ± 0.003 mm/s
Magnetic hyperfine field (H_{eff}) = 49.5 ± 0.1 Tesla
74%
- B) line width = 0.26 ± 0.02 mm/s
Isomer shift = 0.75 ± 0.02 mm/s
Quadrupole interaction (eqQ/4) = 0.16 ± 0.01 mm/s
Magnetic hyperfine field (H_{eff}) = 47.0 ± 0.1 Tesla
26%

IX. DNA Covalent Attachment and Hybridization Experiment onto pDC-Magnetite NCs

The appropriate pDC-magnetite NCs suspension (1% w/v suspension in a neutral PBS buffer, 100 μL , 1-mL Eppendorf tube) was washed by (i) the same PBS neutral buffer (3 x 100 μL), and by (ii) a 0.4M MES buffer (pH 5.0, 100 μL). After magnetically-driven decantation, the supernatant was removed, and, successively added at 20 °C with (i) EDC (0.15M EDC in 0.4M MES buffer, 200 μL , 20.0 mg) and, with (ii) the 20-mer amine-modified oligonucleotide **DNA-1** $\text{NH}_2\text{-(CH}_2\text{)}_{12}\text{-}^5\text{GACTGGGAGCATTGAGGCT}$ (1.2 nmol, 0.4 MES buffer at pH 5.0; Danyiel Biotech Ltd, Israel, chemical purity better than 98%). The mixture was incubated at room temperature for 2 h (smooth vortex agitation) for carboxylate activation.

Following completion, pDC-magnetite NCs were washed with 4 x 100 μL of PBS buffer to remove **DNA-1** in excess. DNA-coated nanocomposites can be stored for weeks at 4 °C in this same neutral PBS buffer but containing 0.02% w/v NaN_3 without any noticeable degradation.

DNA-linked nanocomposites were suspended in a TNET buffer (100 μL , pH 7.5) and distributed by 5 μL portions amongst wells of a termowell polycarbonate non-sterile 96-well microtiter plate. The microtiter plate was then contacted by a Dynal MPC[®] magnetic particle concentrator to magnetically separate the nanocomposites. Each well was added with the FITC-labeled *anti*-sense oligonucleotide **DNA-2** FITC-^{5'}AGCCTCAATGCTCCCAGTGC dissolved in a pH 7.5 TNET buffer (0.1 pmol/ μL of solution, 50 μL ; Danyiel Biotech Ltd, Israel, chemical purity better than 98%) and the 96-well microtiter plate incubated for 3 min at 60 °C for hybridization. Hybridized pDC-magnetite NCs were washed by 50 μL of a commercially available Assay Solution[®] (Savyon Diagnostics Ltd, Israel) before incubation with the reporter *anti*-FITC HRP-labeled mouse monoclonal antibody (commercially available from Hoffman la Roche Inc., 0.1 $\mu\text{g}/\text{mL}$, 10 min at 20°C, 50 μL of an Assay[®] Solution). After magnet-driven decantation and plate washings (4 x 100 μL of a Washing Solution), the TMB substrate (0.05% w/w TMB solution in de-ionized water, 100 μL , Savyon Diagnostics Ltd) is added for color development during 5 min.

Following decantation, supernatants were removed and 80 μL fractions for each well were transferred to a second PS 96 well-microplate for optical reading at 620 nm using an Elisa plate reader Anthos ht II. Washing steps in order to (i) passivate NCs surfaces toward nonspecific binding, and (ii) maximize signal/noise ratio made use of proteins such as BSA and Egg albumin (0.5-1.0% w/v concentrations), surfactants such as 1% w/v PEG₁₀₀₀/Triton X100, and polysaccharides such as dextran).

Specific Reagents, Buffers and Washing/Assay Solutions. PBS buffer (pH 7.0) prepared from Dubelcco's Phosphate Buffered Saline (Sigma), 0.4M MES (pH 5.0): prepared using 2-morpholinoethanesulfonic acid hydrate 99%, adjusted to pH 5.0 by addition of 10M NaOH and stored at 4 °C, TNET buffer (pH 7.5): prepared from a mixture of 10mM Tris-HCl, 0.5M NaCl, 1mM EDTA and 0.02% Tween-20, Washing Solution: prepared from a mixture of 3M NaCl and 2M Tris-HCl (pH 7.5). Assay solution: prepared from a mixture of 154 mM NaCl, 50 mM Tris-HCl (pH 7.8), 0.5% BSA, and 0.1% Tween 20. EDC: *N'*-(3-dimethylamino-propyl)-*N*-ethylcarbodiimide hydrochlorid, > 98% purity (Aldrich).