The table of contents entry We have developed a dendrimer-based capture strategy for improved biomarker detection, and have validated it by using it as a solid phase for an ELISA assay for interleukin-6 and IL-1β in human serum. The dendrimer modified plate provided assays with enhanced sensitivity, specificity, and reproducibility, with an interleukin-6 detection limit of 0.13 pg ml^{-1}. This improved capture approach could be expanded for the detection of a wide range of biomarkers.

Keyword (biosensors, dendrimers, antibody conjugation, cytokine, Interleukin-6, IL-1β)

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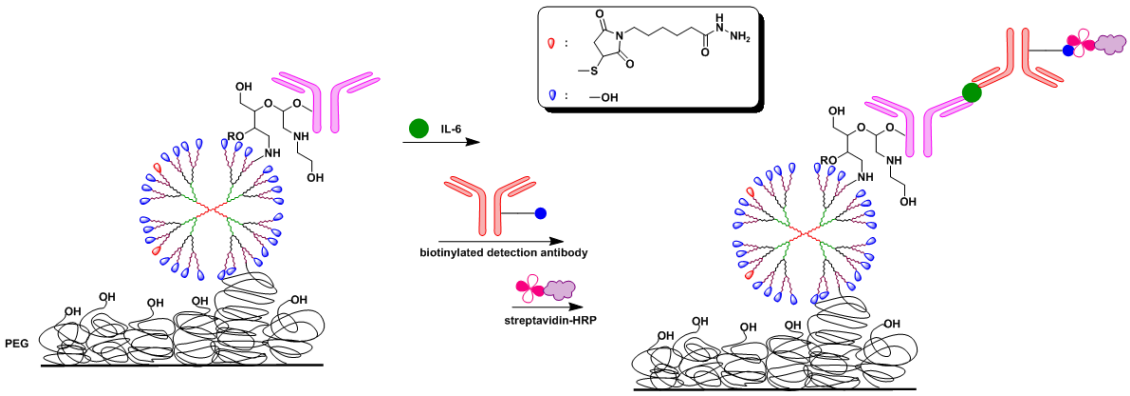
Multifunctional Dendrimer-templated Antibody Presentation on Biosensor Surfaces for Improved Biomarker Detection



Column Title: *Hye Jung Han et al.*/Dendrimer-based Diagnostic Nanodevice

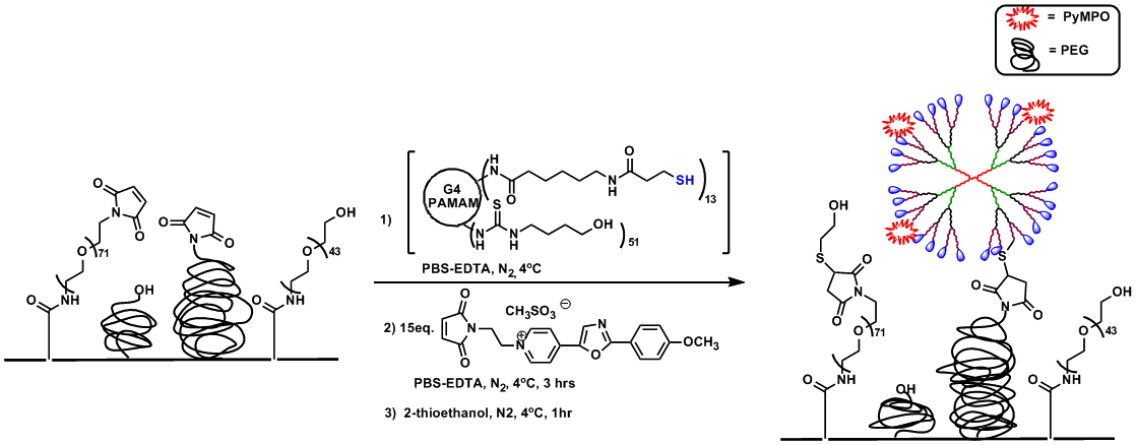
Supporting Information

HPLC Characterization The purity of the conjugate was characterized by RP-HPLC. HPLC characterization was carried out with Waters HPLC instrument equipped with dual pump, an autosampler and dual UV detector interfaced to Breeze software. Symmetry300 C5 RP-HPLC column was used for characterization of the conjugate. The mobile phase used was acetonitrile/water (0.14% TFA) and gradient method used for analysis was (100:0)

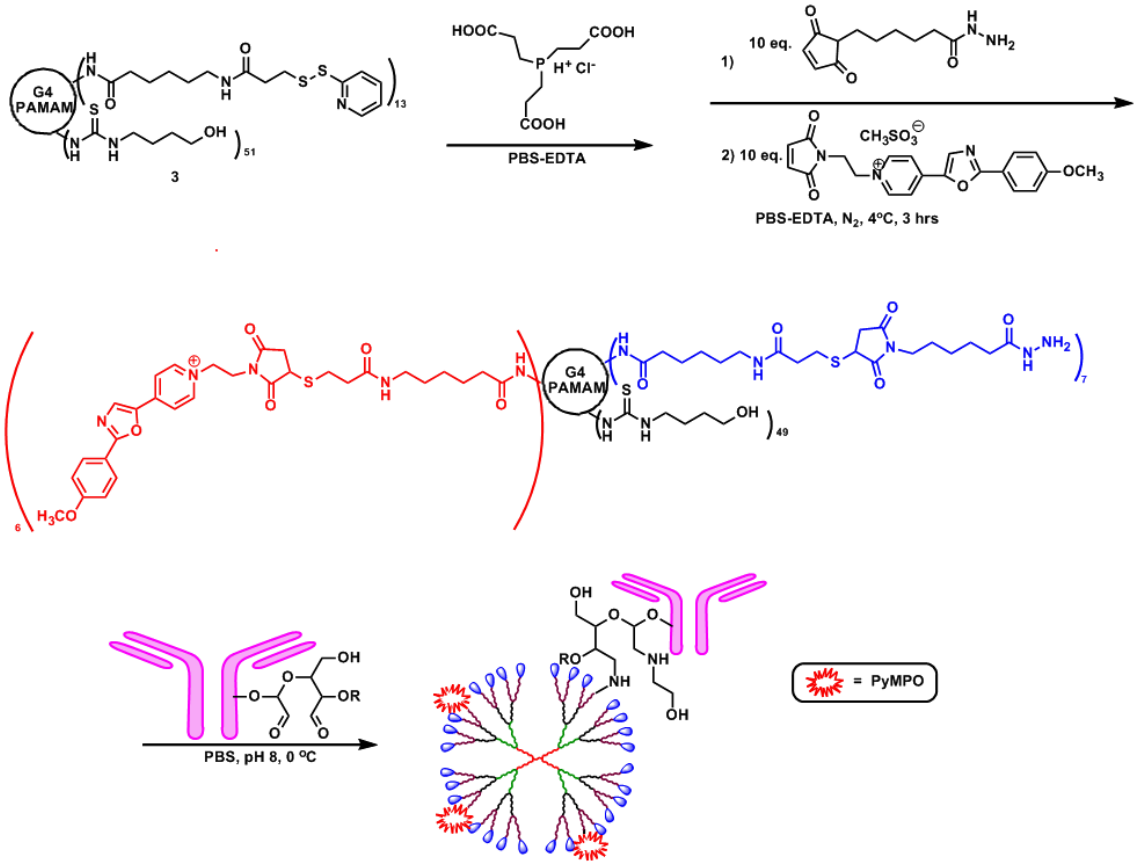
(water:acetonitrile) to (50:50) in 40 min. The flow rate used was 1 ml min^{-1} and HPLC chromatogram was monitored at 210 nm.

Synthesis of hydroxyl/EMCH functionalized G4 PAMAM dendrimer 5 2.2 mg of dendrimer conjugate **3** (98 nmole) was dissolved in 1ml of PBS/EDTA buffer (pH 7.4, 1mmole EDTA). To this solution, 0.84 mg of TCEP (2.94 µmole, 30 equiv.) was added. The reaction mixture was evacuated, flushed with N_2 and the reaction mixture was stirred at room temperature under N_2 until all the conjugate **3** was dissolved and converted to free thiolated conjugate **4**. The reaction mixture was cool down to 4°C and 0.99 mg of EMCH (2.94 µmole, 30 equiv.) in I ml of PBS buffer was added. The reaction mixture was reacted for 3 hrs at 4 °C under N_2. 5 µl of 2-mercaptoethanol was added and reacted for 1 hr. The reaction mixture was dialyzed against D.I. water and lyophilized to give conjugate **5** as a pale yellow oily solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.95 (s, 1H, hydrazide amide N*H*), 8.03 (bs, amide N*H*s), 7.92 (bs, amide N*H*s), 7.82-7.72 (bs, amide N*H*s) 7.55 and 7.43 (bs, bs, 2.9H, $CH_2NHC(S)NHCH_2$), 3.95 (dd, $SCH(C{=}O)CH_2$, dd, J = 12 and 4.8 Hz), 3.36 (m), 3.15 and 3.05 (bs, bs), 2.63 (bs, 11.3H), 2.41 (bs), 2.18(bs, 11.4H), 2.01(bt, 3.7H), 1.46-1.32 (m, 16.8 H), 1.18 (m, 9.87H) ppm. MALDI-TOF (pos) *m/z* 26325



Scheme S1. Schematic representation of immobilization thiol/hydroxyl G4-PAMAM **4** and PyMPO onto ELISA plate.



Scheme S2. Schematic representation of conjugation reaction of hydrazide/PyMPO functionalized dendrimer **6** with oxidized antibody.

Synthesis of PyMPO/EMCH functionalized G4 PAMAM dendrimer 6 22 mg of dendrimer conjugate **3** (980 nmole) was dissolved in 3 ml of PBS/EDTA buffer (pH 7.4, 1mmole EDTA). To this solution, 8.4 mg of TCEP (29.4 µmole, 30 equiv.) was added. The reaction mixture was evacuated, flushed with N_2 and the reaction mixture was stirred at room temperature under N_2 until all the conjugate **3** was dissolved and converted to free thiolated conjugate **4**. The reaction mixture was cool down to 4°C and 3.3 mg of EMCH (9.8 µmole, 10 equiv.) in 1 ml of PBS buffer and 4.6 mg of PyMPO (9.8 µmole, 10 equiv.) in 2 ml of ethanol were added. The reaction mixture was reacted for 3 hrs at 4 °C under N_2. 10 µl of 2-mercaptoethanol was added and reacted for 1 hr. The reaction mixture was dialyzed against D.I. water in the dark and lyophilized to give conjugate **6** as a orange-yellow oily solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.21 (bs, 2H, PyMPO pyridine ring H α to N), 8.95 (bs, 1H, hydrazide amide N*H*), 8.61 (bs, 2H, PyMPO pyridine ring H), 8.03 (bs, bs, amide N*H*s and PyMPO benzene ring H), 7.92 (bs, amide N*H*s), 7.82-7.72 (bs, amide N*H*s) 7.55 and 7.43 (bs, bs, 2.9H, $CH_2NHC(S)NHCH_2$), 7.10 (bs, 2H, pyMPO benzene ring H), 4.05 and 3.95 (dd, dd, SC*H*(C=O)CH_2, 2H), 3.92 (s, 3H, OCH_3), 3.36 (m), 3.15 and 3.05 (bs, bs), 2.63 (bs, 11.3H), 2.41 (bs), 2.18(bs, 11.4H), 2.01(bt, 3.7H), 1.46-1.32 (m, 16.8 H), 1.18 (m, 9.87H) ppm. MALDI-TOF (pos) *m/z* 26616.0

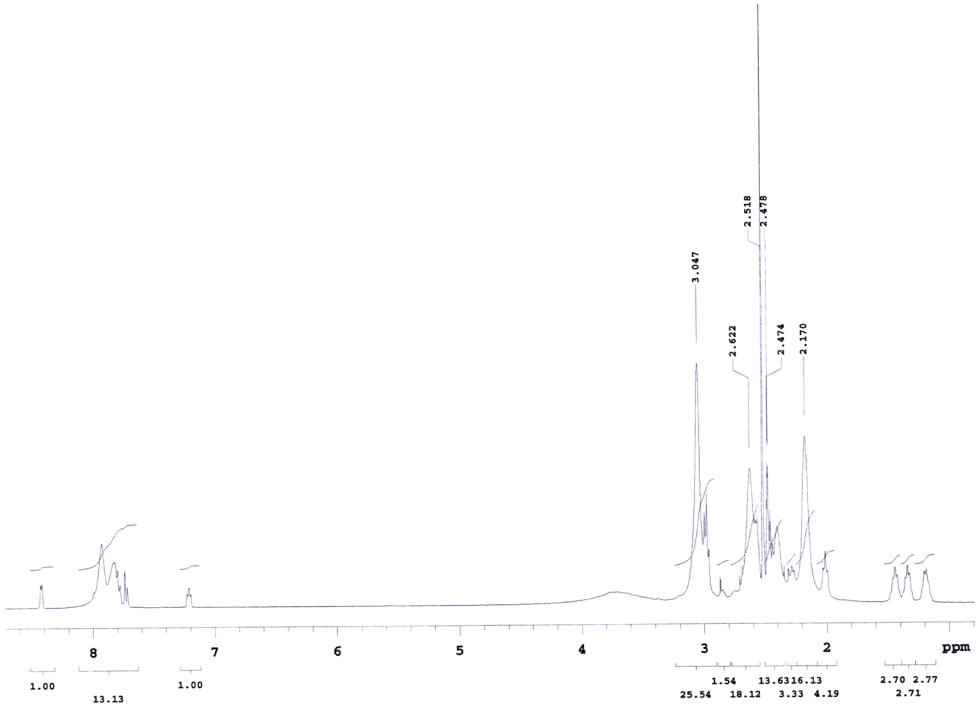


Figure S1. ^{1}H NMR of PDP functionalized G4 PAMAM dendrimer **2**.

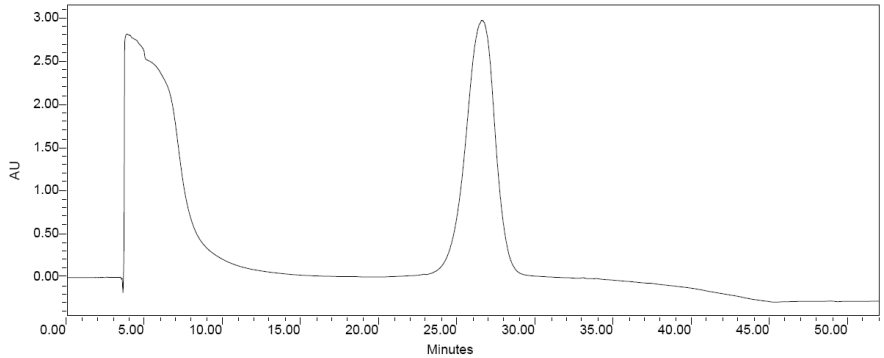


Figure S2. Elution profile of PDP functionalized G4 PAMAM dendrimer **2** monitored at 210 nm.

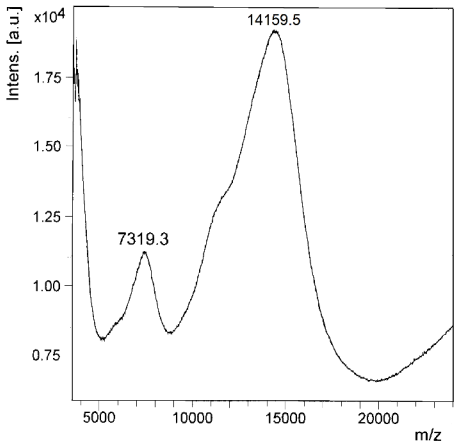


Figure S3. MALDI MS of G4-PAMAM-NH_2.

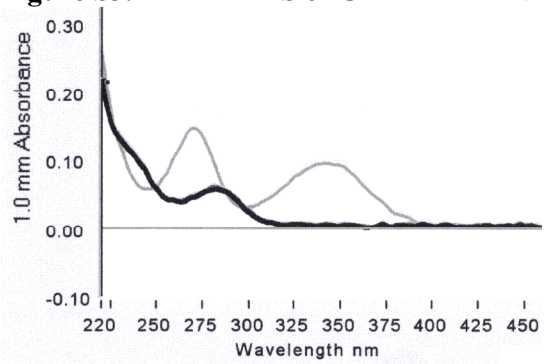


Figure S4. UV-Vis spectra of LC-PDP functionalized G4-PAMAM dendrimer **2** (black) and reduced **2** after addition of DTT (gray). UV-Vis spectra was taken using NanoDrop spectrophotometer ND-1000 (NanoDrop Technologies) and 1mm absorbance at 343 nm was multiplied by 10 in order to convert 1cm absorbance.

$$\frac{\Delta A}{8080} \times \frac{\text{Mw of Conjugate}}{\text{mg/ml of Conjugate}} = \text{Moles of PDP per Moles of Conjugate}$$

$$\frac{0.98}{8080} \times \frac{18545}{1\text{mg}/6\text{ml}} = 13$$

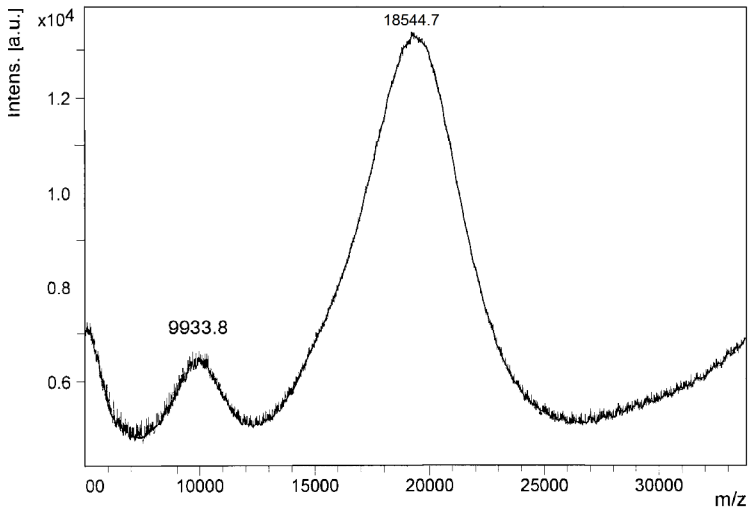
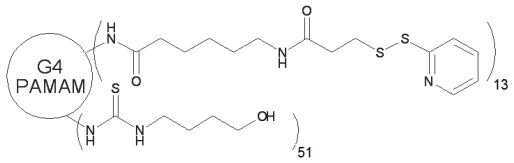


Figure S5. MALDI MS of LC-PDP functionalized G4-PAMAM dendrimer **2**.



3

Figure S6. ^{1}H NMR of hydroxyl/PDP functionalized G4-PAMAM dendrimer **3**.

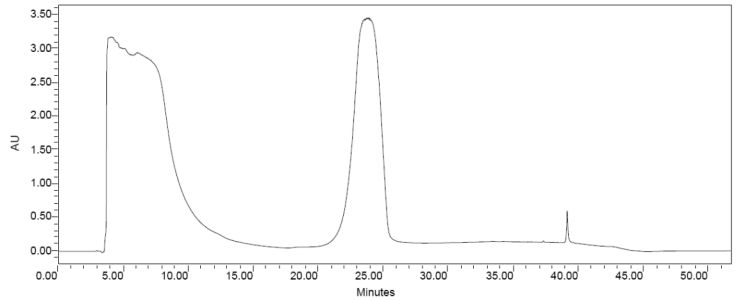


Figure S7. HPLC elution profile of hydroxyl/PDP functionalized G4-PAMAM dendrimer **3** monitored at 210 nm.

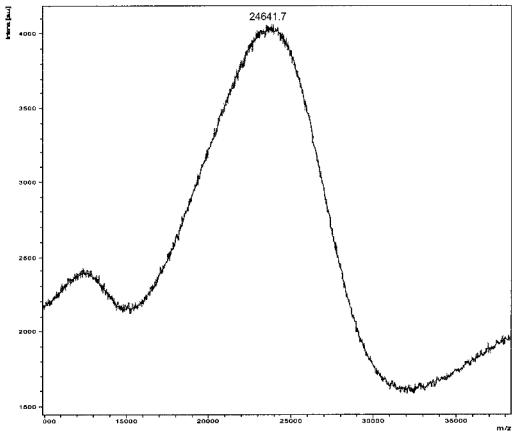


Figure S8. MALDI MS of hydroxyl/PDP functionalized G4-PAMAM dendrimer **3**.

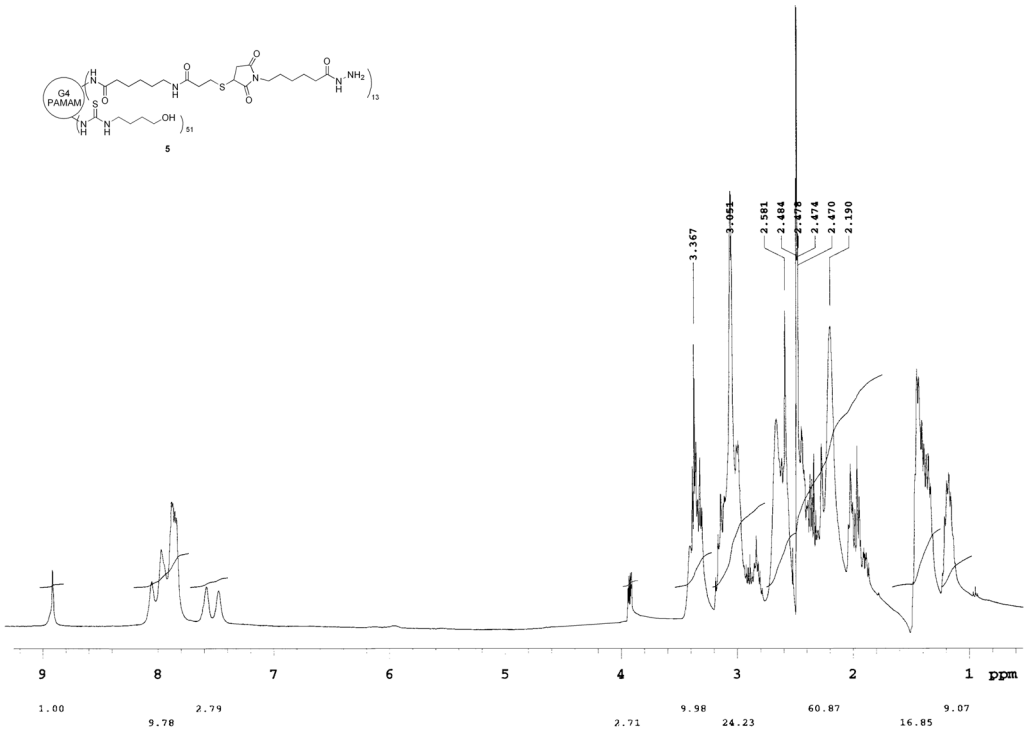


Figure S9. ^{1}H NMR of hydroxyl/EMCH functionalized G4 PAMAM dendrimer **5**.

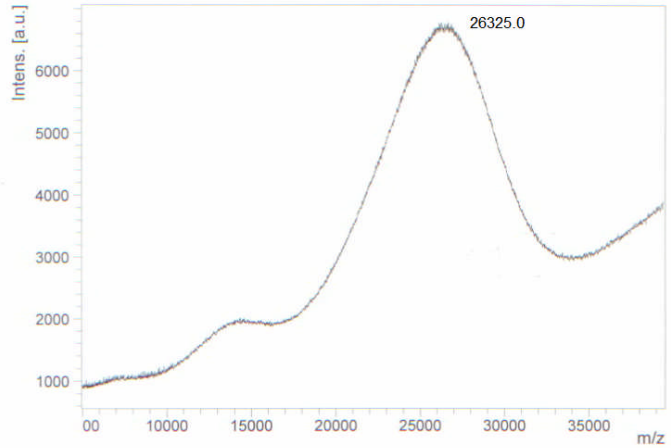


Figure S10. MALDI MS of hydroxyl/EMCH functionalized G4 PAMAM dendrimer **5**.

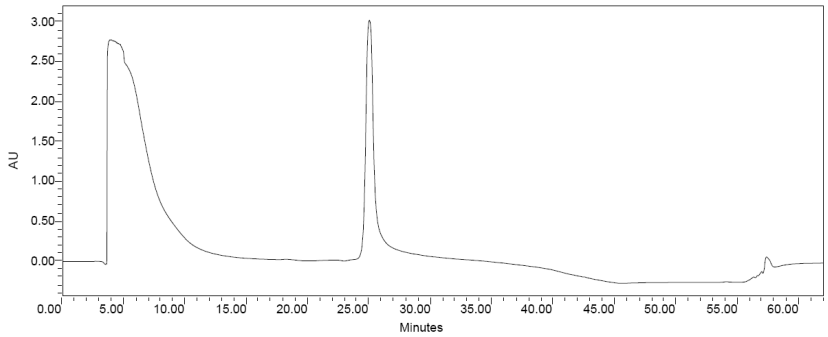


Figure S11. HPLC elution profile of hydroxyl/EMCH functionalized G4 PAMAM dendrimer **5**.

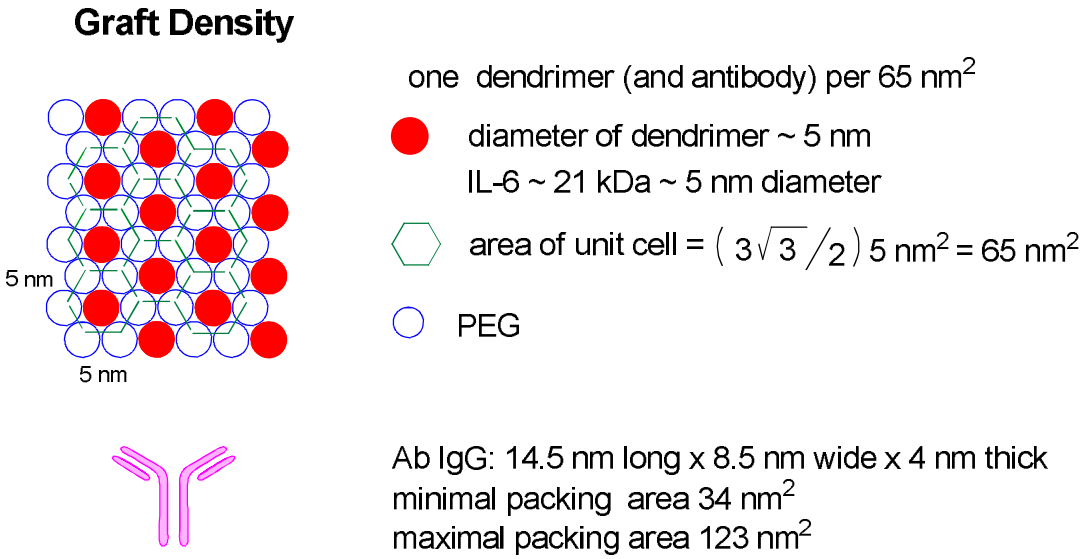


Figure S12. Grafting density of Mal-PEG-NH_2 (dendrimer/antibody).

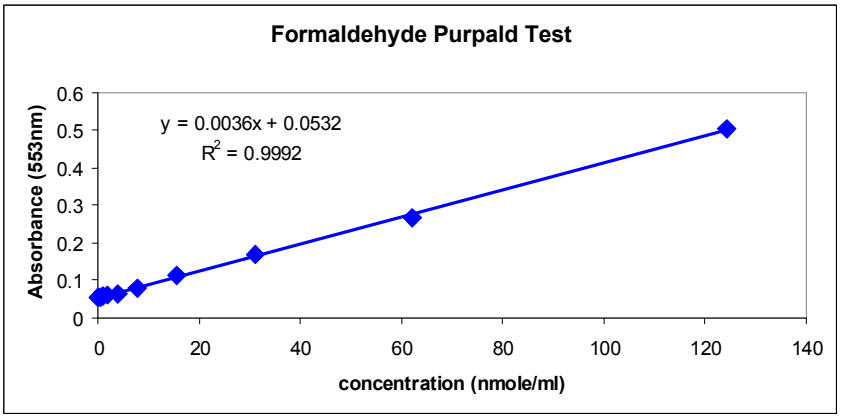


Figure S13. Standard curve of formaldehyde purpald test.

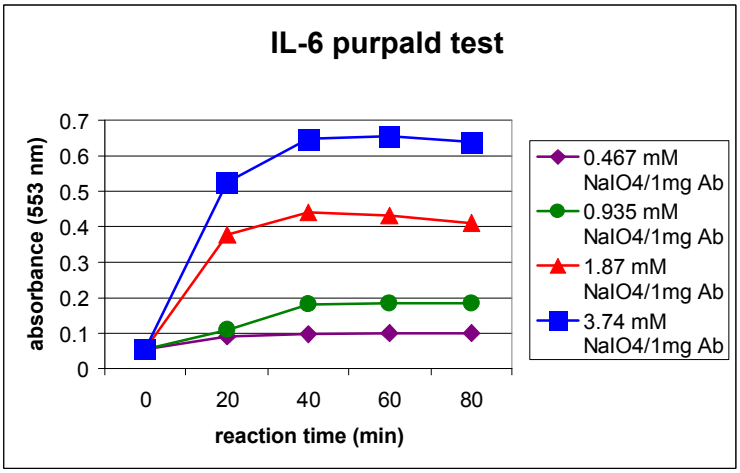


Figure S14. Anti-human IL-6 antibody purpald test.

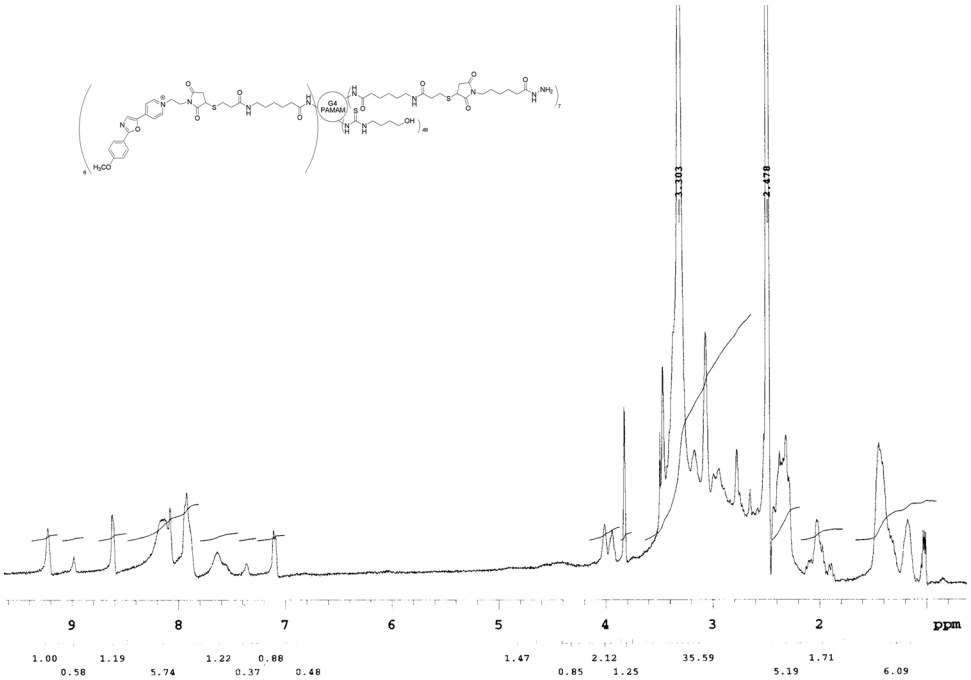


Figure S15. 1H NMR spectra of PyMPO/EMCH functionalized G4 PAMAM dendrimer **6.**

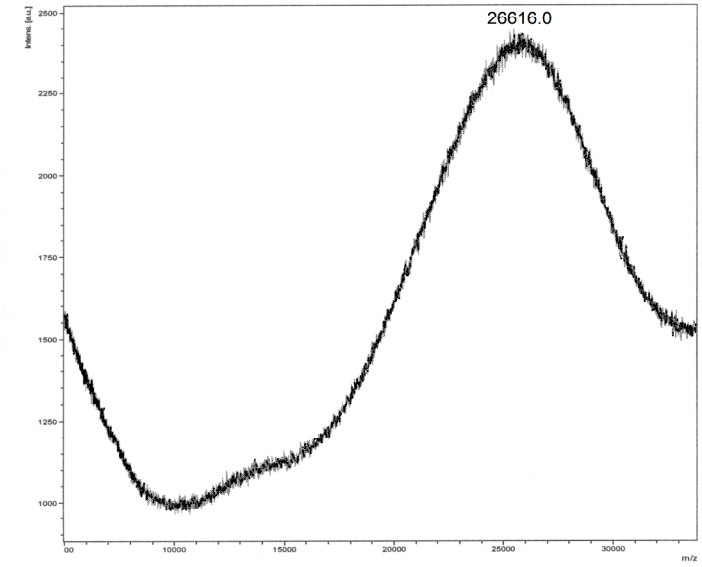


Figure S16. MALDI-MS of PyMPO/EMCH functionalized G4 PAMAM dendrimer **6.**