Supporting Information

Programmable Dendritic Systems for Active Fluidics and Motility

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1. Experimental Details:

1.1 Materials:

Poly (diallyldimethylammonium chloride) solution (PDDA, Mw 200000-350000, 20wt% in water), Poly (sodium 4-styrenesulfonate) (PSS, average Mw ≈1,000,000), 4-nitrophenyl acetate (4-NPA), 4-nitrophenyl butyrate(4-NPB), 4-nitrophenyl octanoate(4-NPO), 4-nitrophenol, Polystyrene based microparticles (5 µm sizes), Polystyrene carboxylate fluorescent green beads (1 µm sizes) were purchased from Sigma Aldrich. PAMAM dendrimers (ethylenediamine core, generation 1.0 to 3.0 solution), EDC (*N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride)), NHS (*N*-Hydroxysuccinimide), were also obtained from Sigma Aldrich. HEPES buffer was purchased from Sisco Research Laboratory (SRL), India. Quartz slides and Silicon wafers were obtained from Technistro. Glass slides were purchased from Riviera. DC/GEN/184 SYLGARD (PDMS Elastomer KIT) was purchased from Kevin Electrochem. Milli-Q grade water water (18.2 MΩ•cm at 25 °C) was used in all experiments.

1.2 Characterization:

All the kinetic measurements for reaction rate determination were carried out with Agilent Cary 60 UV-Vis instrument with temperature control. Quartz cuvettes with a 1.0 cm optical path length were used for all measurements. Spectra were collected in the range of 300-600 nm, and absorbance values at 400 nm were used for quantitative analysis. The thickness of the dendrimer immobilized was determined by Spectroscopic Ellipsometer (Angstrom Sun Technology Inc. USA). All the videos and images were recorded using inverted optical microscope Olympus IX73, with 10X objective. 15-20 particles were tracked to determine speed using Tracker software (video analysis and modelling tool) for 30 seconds. The IR of dendrimer immobilized film was carried out using Thermo-Scientific Nicolet iS5 ATR-IR Spectrometer. The Zeta potential of the Polystyrene carboxylate and dendrimer modified Polystyrene carboxylate data was recorded on a Horiba Scientific Nanoparticle Analyzer SZ-100 V2.

2. Quantification of p-nitrophenolate (PNP) formation from 4-nitrophenyl acetate by UV–Vis spectroscopy:

2.1 Calibration Curve for 4-nitrophenolate (4-NP$^-$)

A p-nitrophenolate solution with concentrations ranging from 2.5 to 20 μM was prepared for kinetic experiments. The absorbance at 400 nm (A_{400}) was measured for each standard solution. A linear regression of absorbance versus concentration was performed according to the Beer–Lambert law:

$$A = \varepsilon\, l\, C$$

where

A= absorbance at 400 nm,

ε= molar extinction coefficient (M^{-1} cm^{-1}),

l= optical path length (cm),

C= concentration (M).

The molar extinction coefficient (M^{-1} cm^{-1}) was found to be 18200 M^{-1} cm^{-1}

The calibration data were fitted to the linear equation:

$$A_{400} = m\,[\mathrm{PNP}] + b$$

where m is the slope and b is the intercept.

From the linear fit, a slope of:

$$m = 0.0182\ \mu\mathrm{M}^{-1}$$

was obtained with an adjusted $R^2 = 0.99884$, indicating excellent linearity. The intercept was close to zero (0.006) and therefore neglected.

Thus, the concentration of PNP in kinetic experiments was calculated using:

$$[\mathrm{PNP}]\ (\mu\mathrm{M}) = \frac{A_{400}}{0.0182}$$

2.2 Reaction kinetics measurements:

The reaction was initiated by the addition of different generations of PAMAM dendrimers (G-3.0, G-2.0, and G-1.0) to 4-NPA at pH 8.5. The stock solution of 4-NPA was prepared by dissolving it in 50 μL of acetonitrile solution which was further diluted to 5 mL and the pH of the solution was maintained at 8.5 for further experiments. The same protocol was followed for 4-NPB and 4-NPO solutions preparation. The same stock solutions were used for fluid flow studies. Time-dependent UV–Vis spectra were recorded at fixed intervals of 1 min over a period of 50 min. The absorbance at 400 nm was extracted from each spectrum and converted to PNP concentration using the calibration equation above.

2.3 Determination of initial reaction rates:

The initial reaction rate (v_0) was obtained from the slope of the linear region at early reaction times:

$$v_0 = \frac{d[\mathrm{PNP}]}{dt}$$

2.4 Error Analysis: Each kinetic experiment was performed at least in triplicate. Reported values represent mean ± standard deviation. Linear regression fitting was carried out using Origin software.

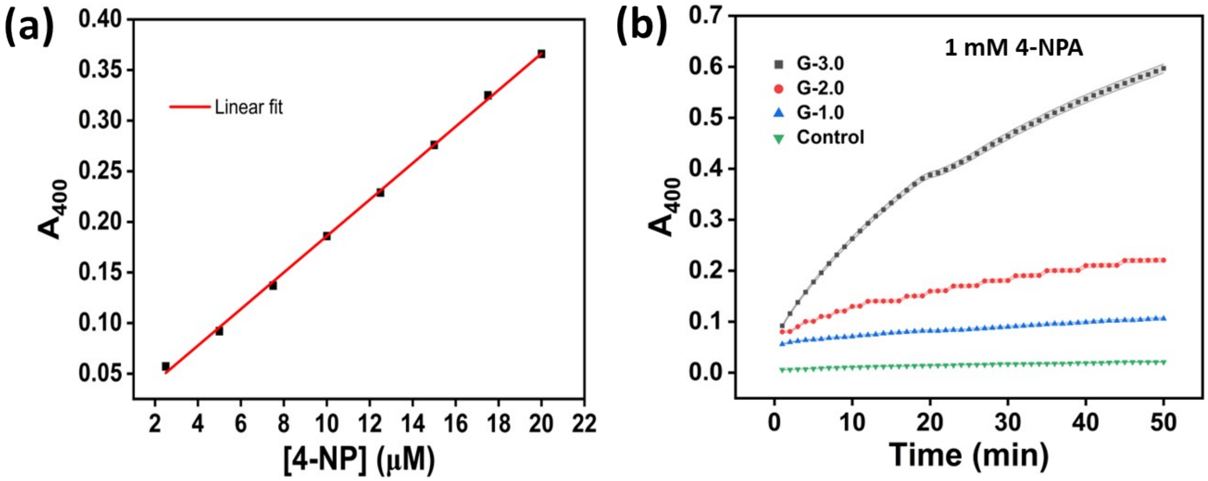


Figure S1. (a) Calibration curve for 4-nitrophenolate (b) Time-dependent change in absorbance at 400 nm (A_{400}) for 1 mM 4-NPA in the presence of different generations of PAMAM dendrimers (G-3.0, G-2.0, and G-1.0) at pH ~ 8.5. The control experiment was carried out in the absence of dendrimers.

3. Preparation of substrate:

Glass slides, silicon wafers and quartz were used as a substrate to fabricate the multilayer dendrimers functionalized films. Si wafers and quartz slides were used for determining thickness profile and IR of the dendrimer immobilized film respectively. Glass slides were used for pumping experiment. Substrates were washed with copious amount of water before and after ultrasonicating with isopropanol and acetone. Thoroughly washed substrates were then dried under hot air and treated with piranha solution (sulfuric acid: hydrogen peroxide, 7:3) for 45mins. Substrates were further washed with water and dried under Nitrogen. *Beware! Piranha solution responds violently to organic materials and requires careful handling.* For preparing PDMS elastomer, Sylgard 184 base was mixed with curing agent in 10:1 ratio in a petri dish and kept under vacuum to remove air bubbles and then kept in a hot air oven at 70^{o}C for 1hr.

4. Multilayer deposition and micropump experiments:

The layer-by-layer (LbL) assembly was carried out following a previously reported procedure. Circular multilayer film patterns were fabricated on glass slides using a PDMS thin sheet as a template. A PDMS sheet was prepared and punctured to create a circular opening of 4.6 mm in diameter, which was then affixed onto a cleaned glass slide. This assembly served as the substrate for the layer-by-layer (LbL) deposition of PDDA, PSS and different generation of PAMAM dendrimers. The coated glass slides were used after constructing the desired number of bilayers, followed by the removal of the PDMS sheet to expose the circular multilayer pattern. The resulting pattern was enclosed within an airtight imaging chamber (1 × 1 × 0.18 cm) and sealed properly. 4-nitrophenylacetate (4-NPA) solution of the desired concentration was mixed with neutral polystyrene microparticles (sizes 5 μm) and introduced into the chamber. Fluid motion was monitored at a height of 0.45 mm within the chamber under an inverted optical microscope, and videos were recorded at a 2-minute time point for all experiments. The pumping velocity was quantified by tracking the motion of 20 tracer particles for 30 seconds in each video using Tracker software.

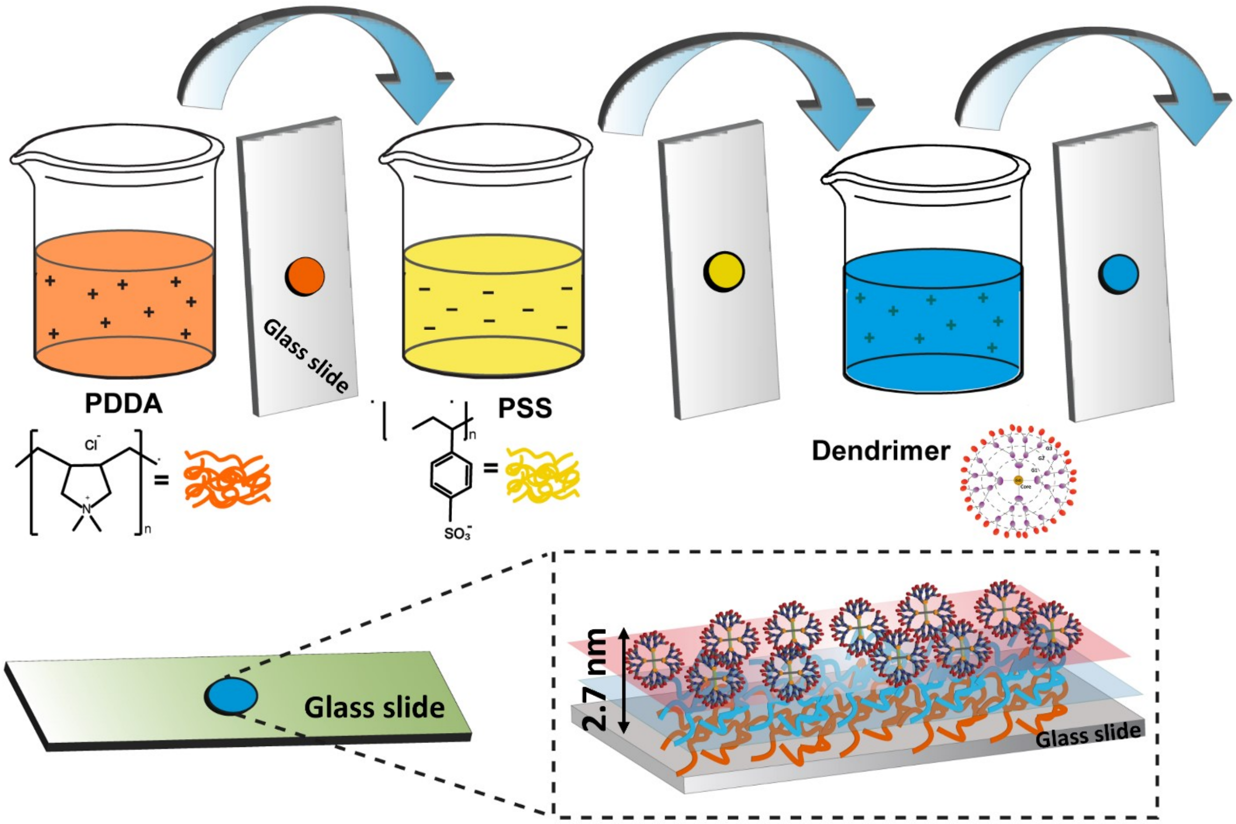


Figure S2. Schematic representation of layer-by-layer (LBL) assembly

4. IR spectra of dendrimer immobilized film grown on quartz substrate:

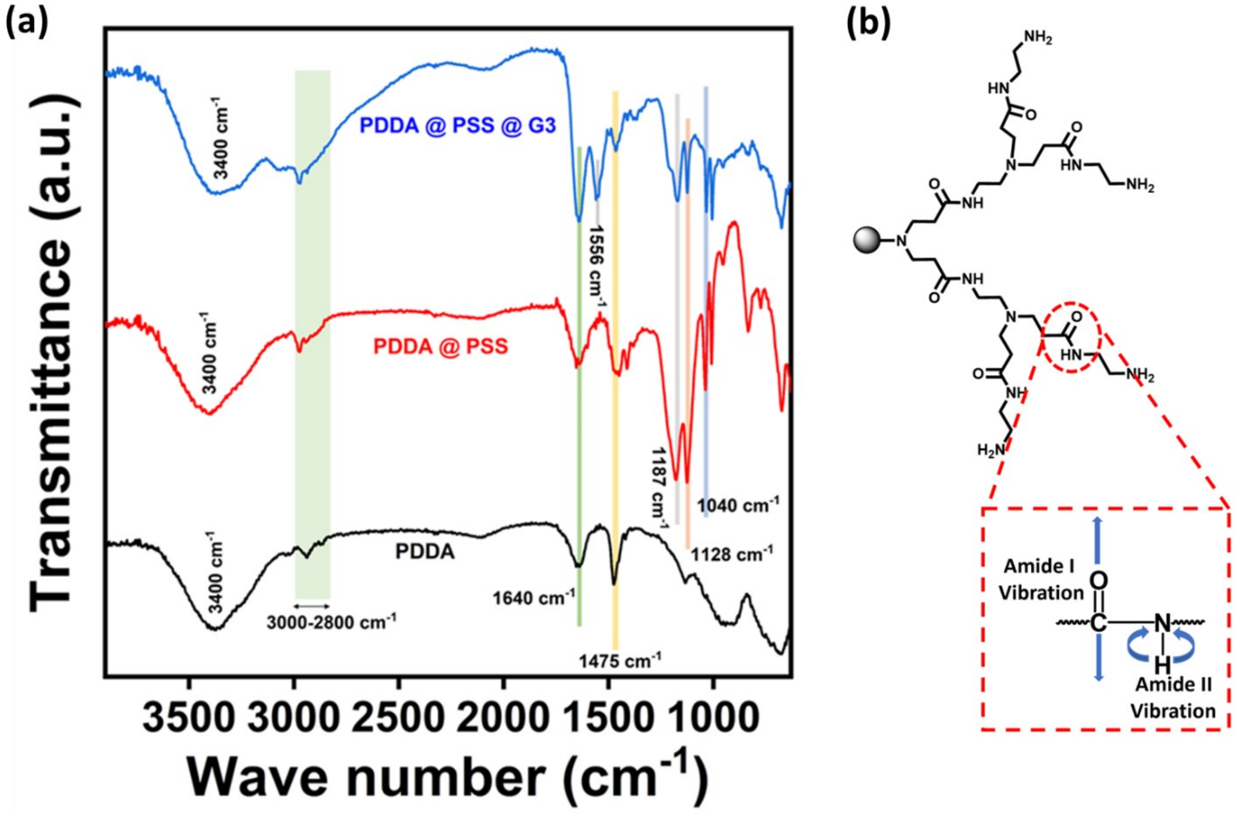


Figure S3. (a) FTIR spectra of PDDA, PDDA@PSS and PDDA@PSS@G-3.0 in LBL assembly (b) Schematic representation of amide I and amide II vibration of PAMAM dendrimers (amide-I for -C=O vibration and amide-II for N-H vibration).

For PDDA, a strong absorption band at around 3400 cm⁻¹ was attributed to the $–NR_3^+$ stretching vibration and the peaks around 3000-2800 cm⁻¹ corresponded to C–H bending. The $–NR_3^+$ deformation vibration was observed at 1640 cm⁻¹, while the peak at 1475 cm⁻¹ corresponds to C–H bending. The PDDA@PSS layer, characteristic absorption bands at 1187 cm⁻¹, 1128 cm⁻¹, and 1040 cm⁻¹ were associated with the sulfonate group vibrations of PSS. The retention of the $–NR_3^+$ deformation (1640 cm⁻¹) and $–NR_3^+$ stretching vibration (3400 cm⁻¹) bands from PDDA confirmed the successful anchoring of PSS onto the PDDA layer. For PDDA@PSS@G3, additional peaks at 1640 cm⁻¹ and 1556 cm⁻¹ corresponded to the amide-I and amide-II vibrations of the PAMAM dendrimer. There existed a possibility that the amide-I band (1640 cm⁻¹) overlaps with the $–NR_3^+$ deformation vibration of PDDA, leading to band merging in this region. The coexistence of the sulfate bands of PSS (1187, 1128, and 1040 cm⁻¹), along with the C–H bending (1475 cm⁻¹) and –

NR_3^+/amide-I feature at 1640 cm^{-1} bands implied the successful attached of the PAMAM dendrimer atop the PDDA@PSS layer through electrostatic interactions via LBL assembly process.

5. Tracer particles motion:

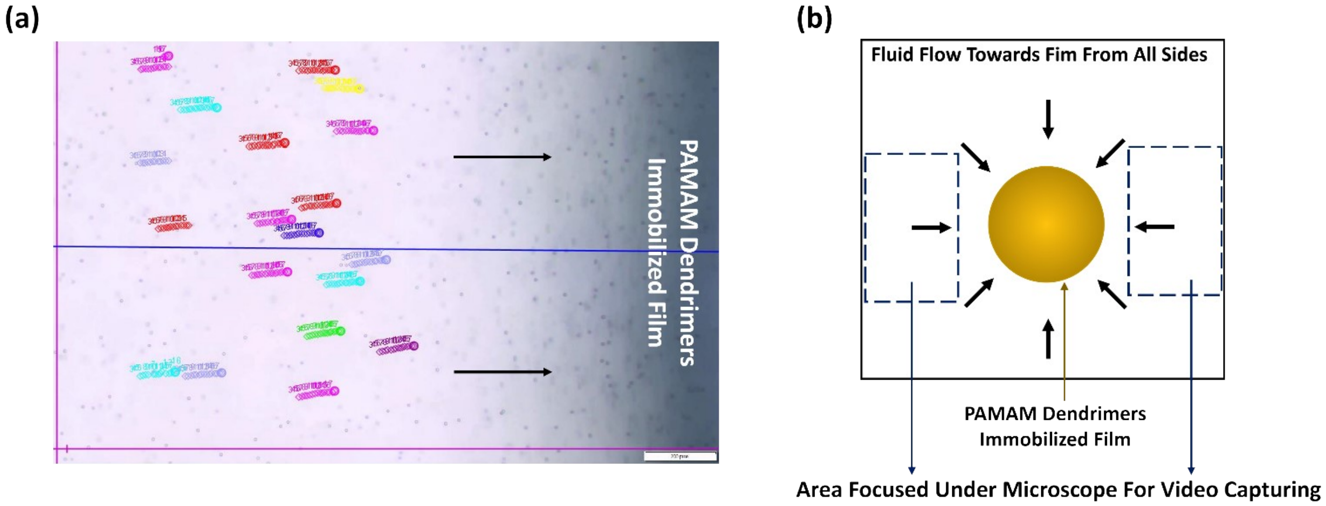
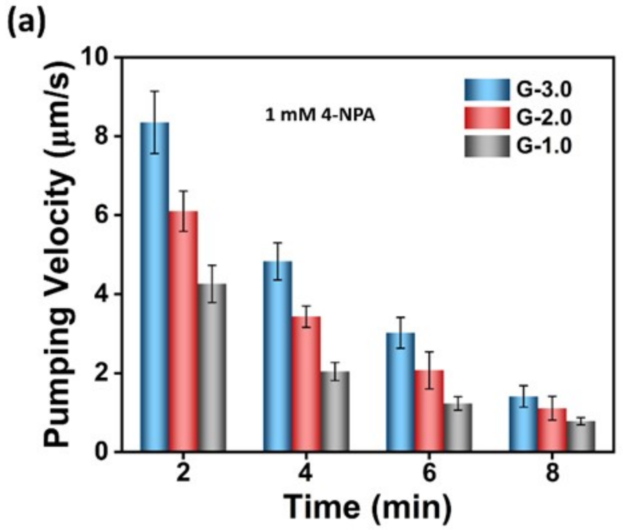


Figure S4. a) Image depicting motion of the tracer particles b) Schematic diagram of micropump chamber



6. Spatial-temporal :

Figure S5. a) Temporal decrease in pumping velocity at 1 mM 4-NPA at pH ~ 8.5 for different dendrimer generations.

7. Quantification of p-nitrophenolate (PNP) formation from 4-nitrophenyl butyrate by UV–Vis Spectroscopy:

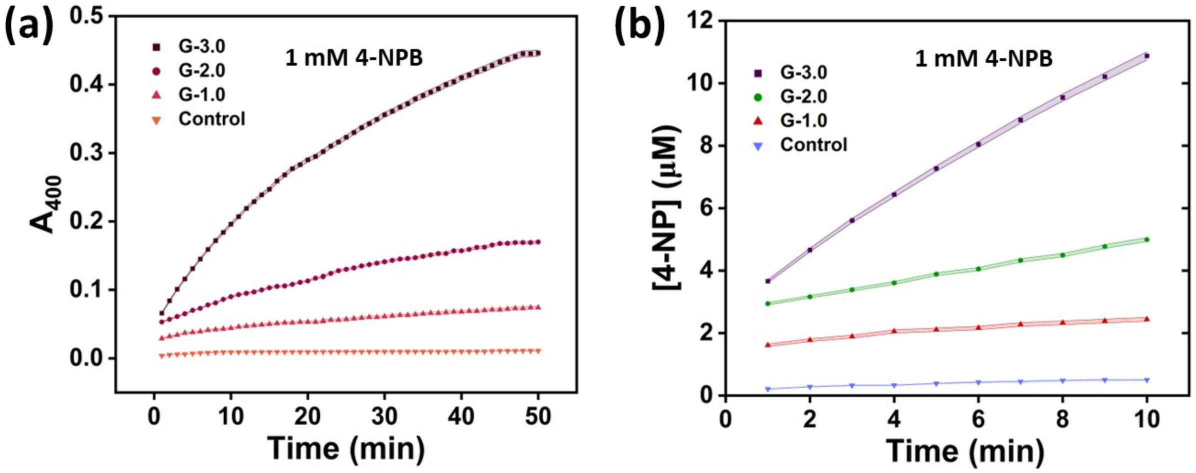


Figure S6: (a) Time-dependent change in absorbance at 400 nm (A_{400}) for 1 mM 4-NPB in the presence of different generations of PAMAM dendrimers (G-3.0, G-2.0, and G-1.0) at pH ~ 8.5. (b) Time-dependent formation of 4-nitrophenolate (4-NP^{-}) during the hydrolysis of 1 mM 4-NPB at pH ~ 8.5 in the presence of different generations of PAMAM dendrimers.

8. Fabrication of particles for enhanced diffusion:

For this purpose, carboxylate polystyrene microparticles (size 1 µm) were selected as the supporting material, as their surface charge and functional groups can be readily tuned by chemical modification. G-3.0 dendrimers were then covalently attached to the particle surface using EDC/NHS coupling, where EDC activates the carboxyl groups of the particles to form reactive intermediates that subsequently react with the primary amine groups of the dendrimers to form a stable amide linkage. In a typical procedure, NHS (78 mM) and EDC (31 mM) were first incubated with 30 µL of beads for 1 hours. After that, a 78 µM PAMAM G-3.0 in HEPES buffer at pH 7 was added to a final reaction volume of 1 mL and stirred 2 hours at room temperature. The resulting dendrimer functionalized particles were collected by centrifugation, washed three times with DI to remove unreacted components and dried. The same procedure was also used for the surface modification with G-2.0 and G-1.0 dendrimers.

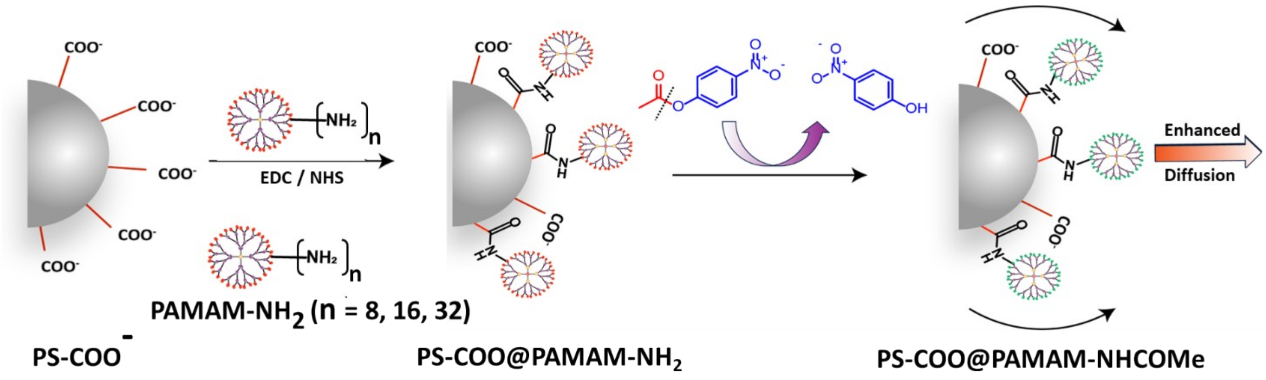


Figure S7. Schematic representation of fabrication of particles for enhanced diffusion.

9. Mean square displacements (MSD) of enhanced diffusion:

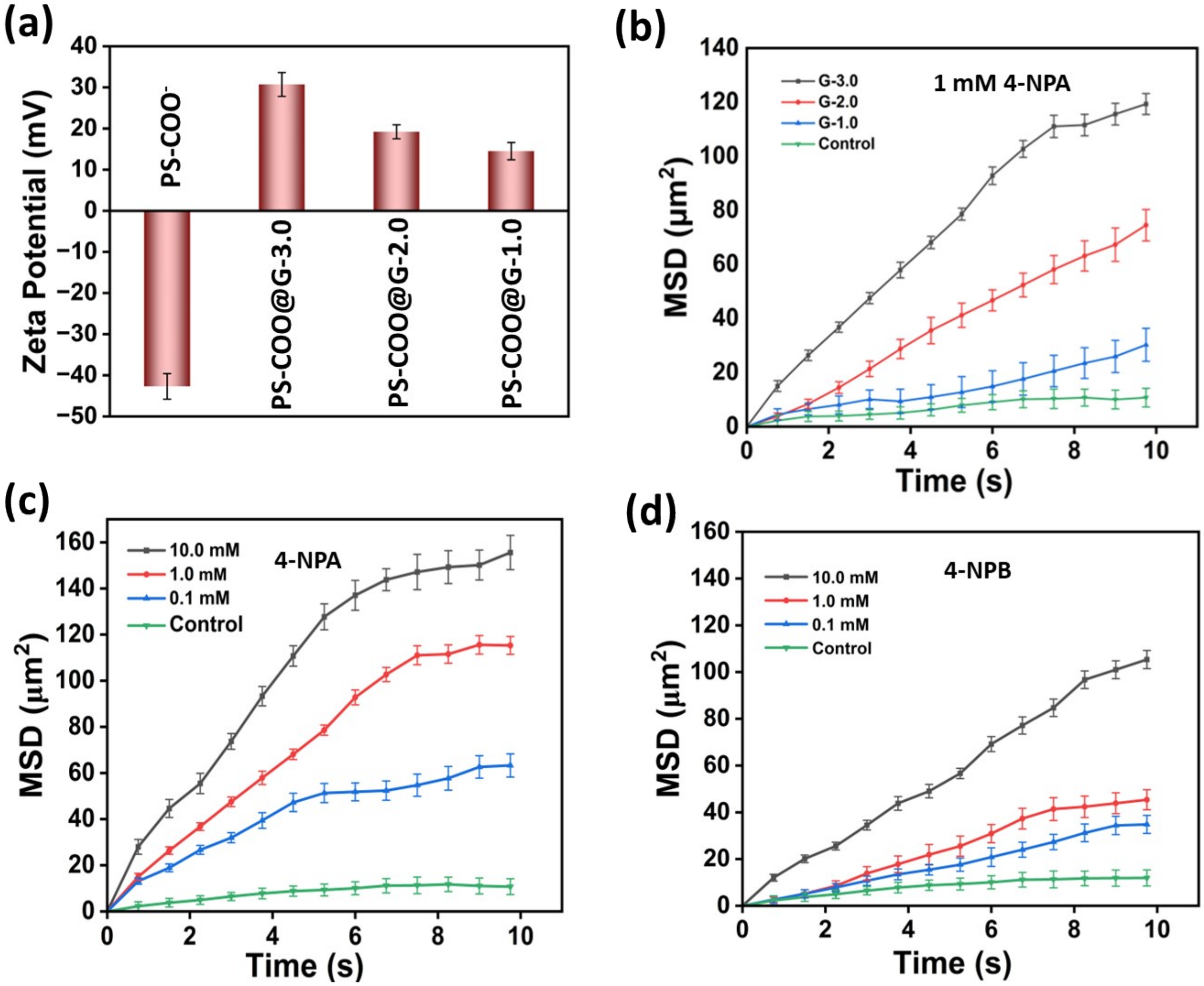


Figure S8. a) Zeta potential values before and after immobilizing different generations of dendrimers on the carboxylate polystyrene beads. b) Variation of average MSD values 1 mM 4-NPA at pH ~ 8.5 for different generation (G-3.0, G-2.0, G-1.0) of dendrimers functionalized particles. c) Variation of average MSD values with 4-NPA concentration at pH ~ 8.5 for G-3.0 functionalized particles. d) Variation of average MSD values with 4-NPB concentration at pH ~

8.5 for G-3.0 functionalized particles. (The MSD of at least eight dendrimer-conjugated particles from three independent sets of experiments was analyzed and averaged for all cases.)

10. Trajectory of the particles:

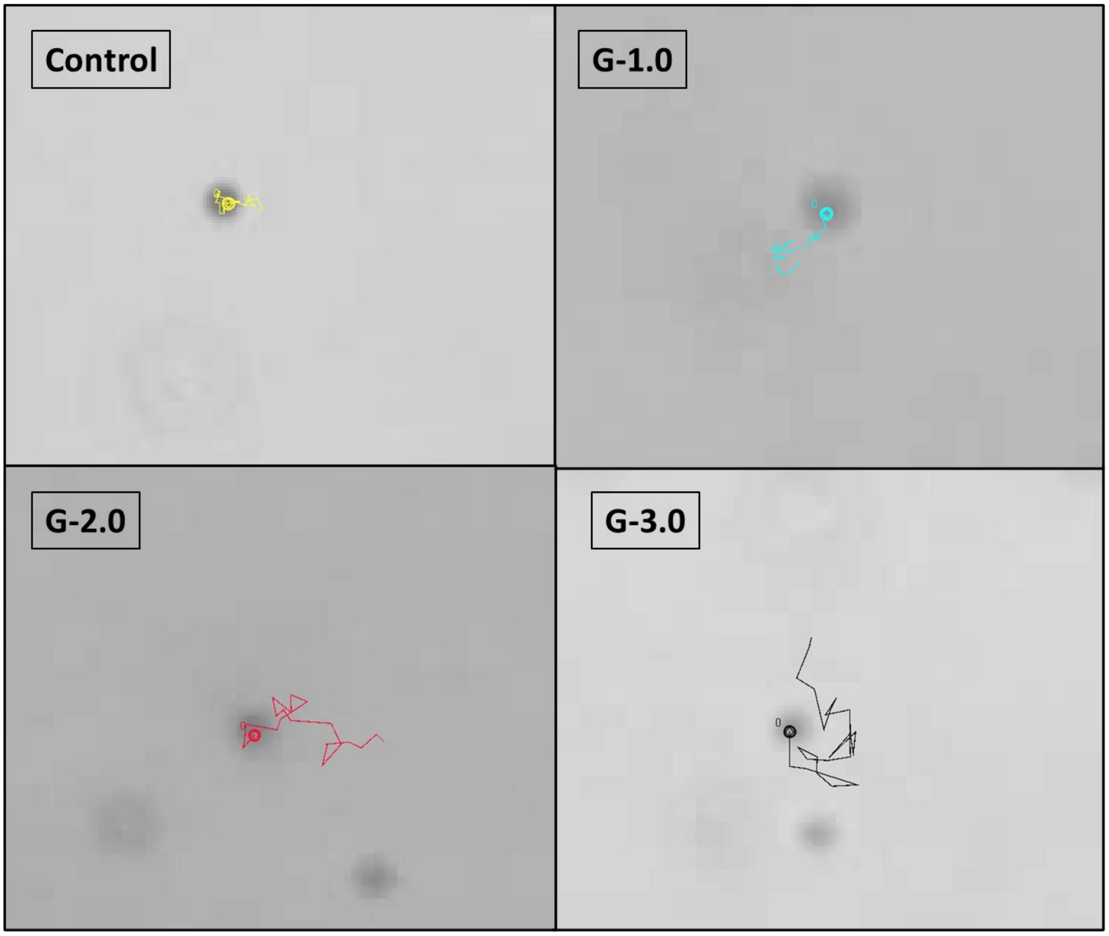


Figure S9. Trajectory of the G-3.0, G-2.0 and G-1.0 dendrimer functionalized carboxylate polystyrene beads in the presence of 1.0 mM at pH ~ 8.5 of 4-nitrophenyl acetate over 15 s in the *XY* plane observed under an optical microscope and analyzed using Tracker software.

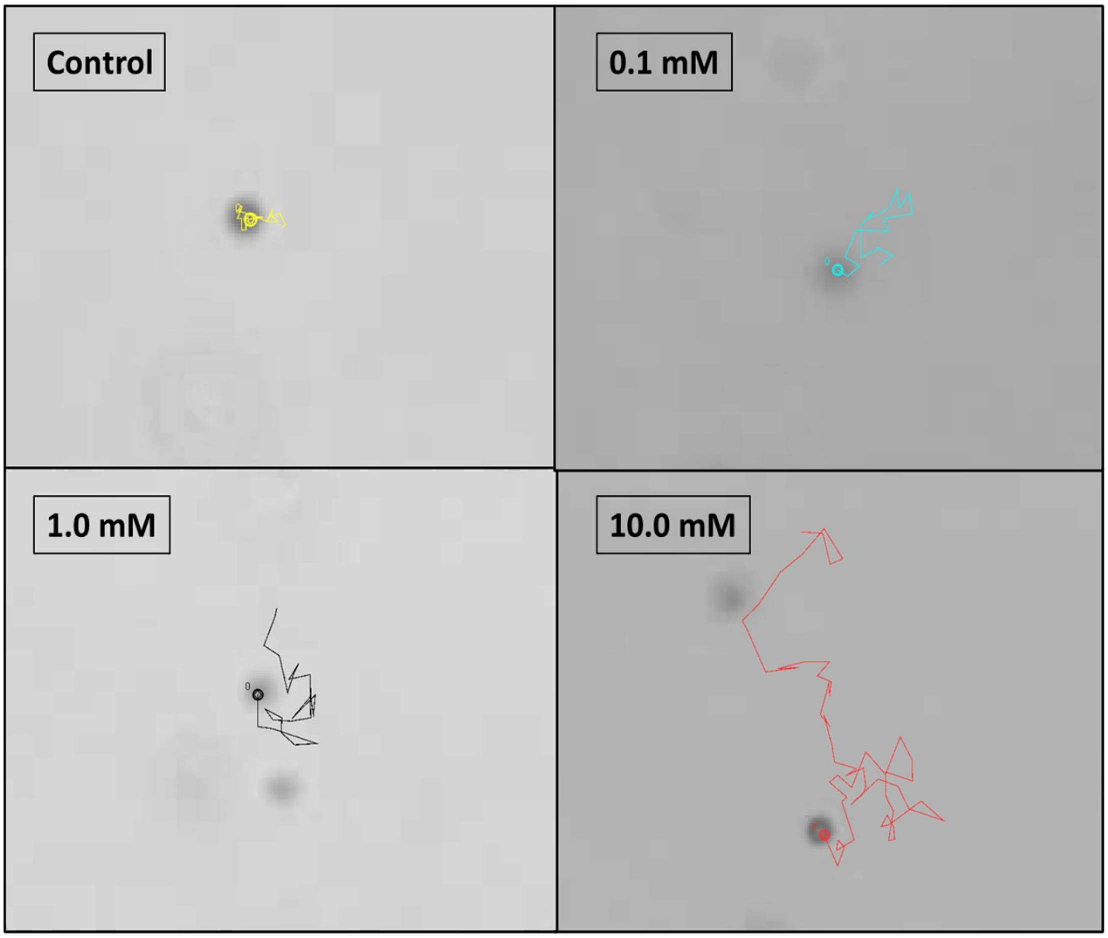


Figure S10. Trajectory of the G-3.0 dendrimer functionalized carboxylate polystyrene beads in the presence of 0.1 mM, 1.0 mM and 10.0 mM concentration of 4-nitrophenyl acetate (4-NPA) at pH ~ 8.5 over 15 s in the *XY* plane observed under an optical microscope and analyzed using Tracker software.

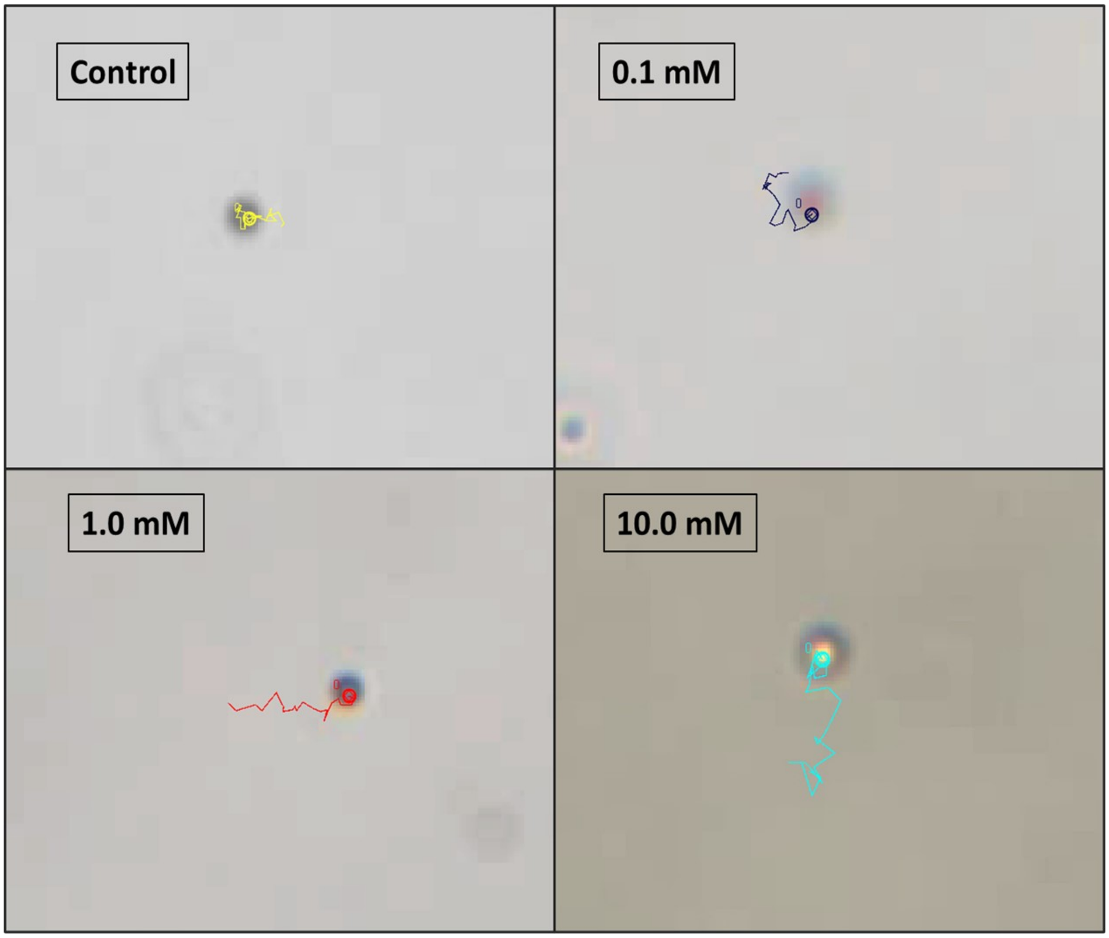

Control
0.1 mM
1.0 mM
10.0 mM

Figure S11. Trajectory of the G-3.0 dendrimer functionalized carboxylate polystyrene beads in the presence of 0.1 mM, 1.0 mM and 10.0 mM concentration of 4-nitrophenyl butyrate (4-NPB) at pH ~ 8.5 over 15 s in the *XY* plane observed under an optical microscope and analyzed using Tracker software.

11. log-log plot on MSD versus time:

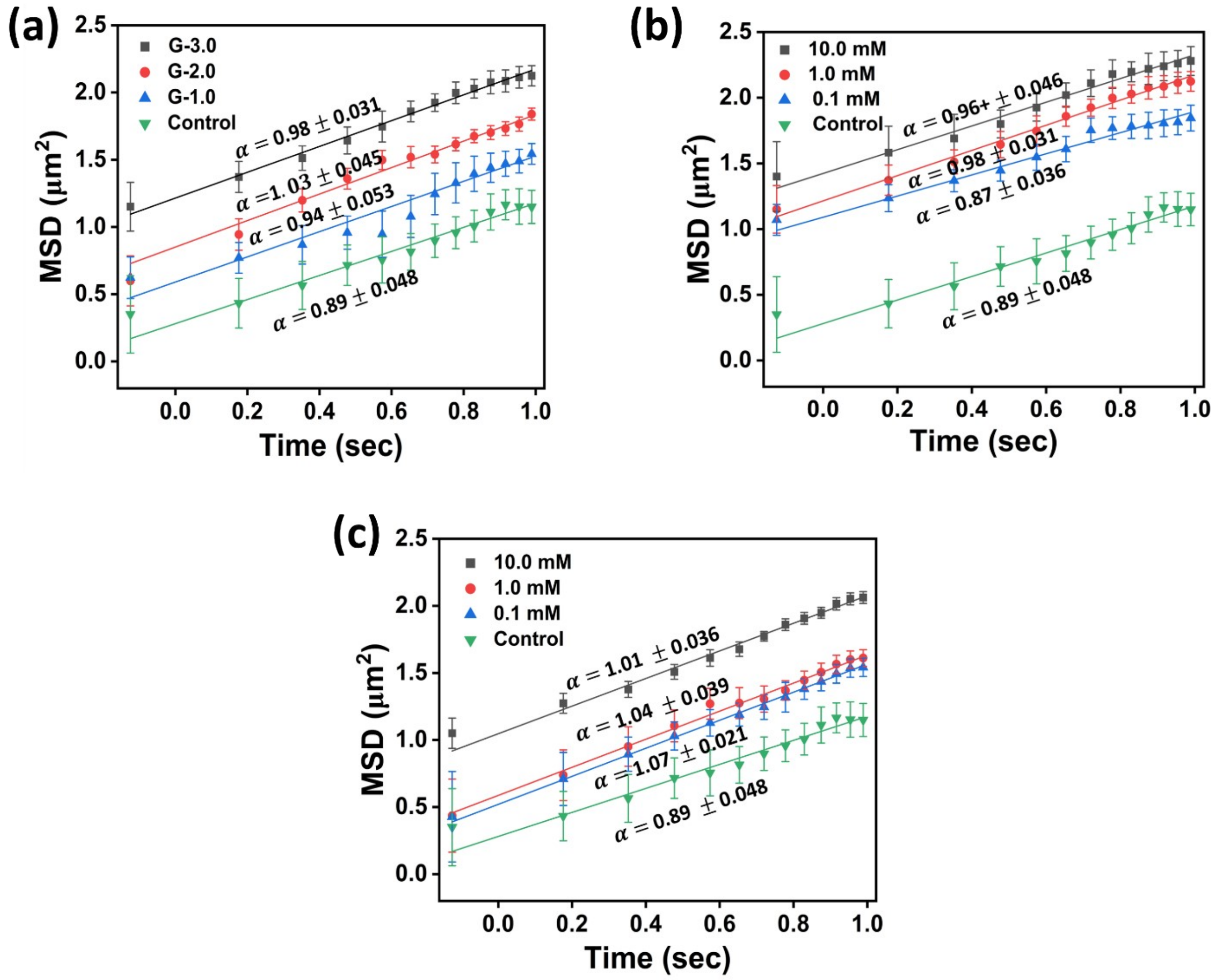


Figure S12. (a) log–log plot of mean squared displacement MSD (μm^2) versus time (sec) for particles functionalized with different generations of dendrimers in the presence of 1 mM 4-NPA at pH ~ 8.5. (b) log–log plot of mean squared displacement (MSD) (μm^2) versus time (sec) for particles functionalized with G-3.0 dendrimers in the presence of different concentration of 4-NPA (pH ~ 8.5). (c) log–log plot of mean squared displacement (MSD) (μm^2) versus time (sec) for particles functionalized with G-3.0 dendrimers in the presence of different concentration 4-NPB at pH ~ 8.5. (The MSD of at least eight dendrimer-conjugated particles from three independent sets of experiments was analyzed and averaged for all cases).

12. Calculation of diffusion coefficient and scaling factor (α) from MSD value:

The enhanced diffusion of dendrimer-conjugated particles was observed within a time interval of 30 seconds to 2 minutes after the addition of the substrate. The motion of these micron-sized particles was recorded at a rate of 15 frames per second using an inverted optical microscope (Olympus IX73) equipped with a 40× objective. In all cases, at least eight particles were tracked under each experimental condition. Each recorded video was then analyzed using Tracker software, which allowed the extraction of trajectories. From these trajectories, mean square displacement (MSD) was calculated by using the following equation for two-dimensional analysis

The MSD was defined as,

$$\langle \Delta r^2(t) \rangle = \langle [x(t) - x(0)]^2 + [y(t) - y(0)]^2 \rangle$$

where x and y are the particle coordinates in the imaging plane.

For purely diffusive motion in two dimensions the MSD scales linearly with time according to,

$$\langle \Delta r^2(t) \rangle = 4Dt$$

where D was the diffusion coefficient and t was time.

The diffusion coefficient was obtained by fitting the linear regime of the MSD vs time plot.

In addition, scaling factor (α) was calculated from the log-log plot of MSD vs time plot. This log-log representation was the standard method to determine scaling factor (α) and to distinguish between diffusive behavior and super-diffusive behavior.

For general:

$$\langle \Delta r^2(t) \rangle \; \propto \; t^{\alpha}$$

Taking logarithm on both sides:

$$\log\left(\langle \Delta r^2(t) \rangle\right) = \alpha \log(t) + constant$$

Thus, the slope of the line of the liner fitting in the log-log plot corresponded to the scaling factor α whereas $\alpha = 1$ indicates the diffusive regime and $1 < \alpha < 2$ indicated super diffusive regime.
In our analysis, linear fitting of log-log MSD data yielded a scaling factor close to 1 in most cases. This result indicated that particle motion follows diffusive behavior rather than super-diffusive dynamics.

11. Supplementary videos:

11.1 Supplementary videos 1 (SV1): Motion of polystyrene tracer particles in presence of 1 mM 4-NPA (Video 2 times faster).

11.2 Supplementary videos 2 (SV2): Random motion of polystyrene tracer particles in absence of 4-NPA (Video 2 times faster).

11.3 Supplementary videos 3 (SV3): Enhanced diffusion of different generations functionalized particles with 1 mM concentration of 4-NPA (Video 2 times faster).

11.4 Supplementary videos 4 (SV4): Enhanced diffusion of G-3.0 functionalized particles with different concentration of 4-NPA (Video 2 times faster).

11.5 Supplementary videos 5 (SV5): Enhanced diffusion of G-3.0 functionalized particles with different concentration of 4-NPB (Video 2 times faster).