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The convergence of quantum-dot-mediated fluorescence resonance energy transfer and microfluidics for monitoring DNA polyplex self-assembly in real time

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Abstract

We present a novel convergence of quantum-dot-mediated fluorescence resonance energy transfer (QD-FRET) and microfluidics, through which molecular interactions were precisely controlled and monitored using highly sensitive quantum-dot-mediated FRET. We demonstrate its potential in studying the kinetics of self-assembly of DNA polyplexes under laminar flow in real time with millisecond resolution. The integration of nanophotonics and microfluidics offers a powerful tool for elucidating the formation of polyelectrolyte polyplexes, which is expected to provide better control and synthesis of uniform and customizable polyplexes for future nucleic acid-based therapeutics.

1. Introduction

Advances in genomics continue to fuel the development of future therapeutics that can target pathogenesis at the cellular and molecular level. Often functional only inside the cell, nucleic acid-based therapeutics require an efficient intracellular delivery system. One widely adopted approach is to complex these charged molecules with a carrier to form nanocomplexes via electrostatic self-assembly, facilitating cellular uptake of DNA while protecting it against degradation [1–5]. Many researchers have focused on developing new gene carriers to optimize the stability of nanocomplexes, since premature dissociation or overly stable binding would be detrimental to their cellular uptake and therapeutic efficacy. Furthermore, the chemical properties of gene carriers have been demonstrated to affect the condensation of DNA, the structure of resulting nanocomplexes, and subsequent transfection efficiency [6–9]. Previously, we have shown that the technique of quantum-dot-mediated fluorescence resonance energy transfer (QD-FRET) provides a quantitative and highly sensitive indication of polyplex stability in either extra or intracellular environments [10,11], allowing for unambiguous detection of the onset of interactions between DNA and the gene carrier. DNA polyplexes synthesized by bulk mixing showed a diverse range of intracellular unpacking and trafficking behavior, which was attributed to the heterogeneity in size and stability of polyplexes [10,11]. The heterogeneity of polyplexes resulting from bulk synthesis hinders the accurate assessment of the self-assembly kinetics and adds to the difficulty in correlating their physical properties to transfection efficiencies or bioactivities.

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Some figures in this article are in colour only in the electronic version

Currently, the dynamic process of electrostatic binding between the gene carrier and DNA is typically studied near or after equilibrium and rarely in real time [12–14]. To improve the intracellular delivery efficiency of these polyplexes, there is a critical need for new technology platforms that can facilitate better understanding and control of the synthesis process.

Microfluidics technology has been demonstrated to provide well-controlled microreactor conditions for the production of monodisperse nanocrystals, oxide particles, or metallic nanoparticles [15–18]. In a microfluidic device, diffusive mixing can be precisely controlled due to the characteristically low Reynolds number from miniscule characteristic lengths of microfluidic channels ($\sim\mu\text{m}$ scales). Further, small reaction volumes (nl to μl) result in minimal reagent consumption and prompt response to external perturbations, allowing rapid reactions to be studied at higher temporal resolutions. Previous studies have shown that microfluidics is capable of generating uniform microenvironments (microreactors, microcapillary, continuous or segmented microfluidics) [15–17,19] for monodisperse and customizable nanoparticle synthesis [20–22]. However, real time characterization of the nanoparticle synthesis process within a microfluidic device has not been exploited with the exception of growing intrinsically luminescent CdS nanocrystals [23].

In this paper, we present a novel convergence of microfluidics and the QD-FRET technique as a potentially universal platform capable of capturing the kinetic aspect of synthesis of micro- and nano-scale polyplexes and lipoplexes encapsulating DNA, RNA, or peptides. As a proof-of-concept, we demonstrate monitoring the self-assembly of chitosan/DNA polyplexes [1]. Chitosan, a biodegradable natural polysaccharide comprised of glucosamine, was selected as a model polymeric gene carrier as it is a promising vector with high positive charge density and relatively low toxicity, and has been previously shown to elicit a therapeutic response *in vivo* [24–26]. A FRET pair of 605QD (donor) and Cy5 (acceptor) [27] were labeled to plasmid DNA (pDNA) and chitosan respectively (figure 1(a)), allowing for their interactions to be monitored via FRET signals. A microfluidic T-junction (figure 1(b)) provided means to control the rapid mixing of pDNA and cationic chitosan, as well as to enable real time monitoring of noncomplex formation via QD-FRET detection. Due to the nature of laminar flow in the microfluidic channel, mixing of 605QD-pDNA and Cy5-chitosan takes place at the flow interface, facilitating precise determination of mass transport as a function of time [23,28]. Therefore, the spatial pattern of FRET signals can be transformed to resolve the temporal aspect of self-assembly kinetics. Consequently, the mixing or reaction time can be controlled and determined by the time interval, $t = y/v$, where y is the distance from the juxtaposition to the point of measurements, and v is the mean flow velocity. The flow velocity across the channel width can be approximated to be constant due to the channel's high aspect ratio (width/depth), neglecting wall effects [28]. Upon excitation of 605QD at 488 nm, the FRET-mediated Cy5 emission was detected along the interface of the two reagent streams (figure 1(b), inset). Detection of Cy5 emission provides tangible evidence of pDNA/chitosan interactions and facilitates *in situ* monitoring of polyplex formation in the microreactor. In addition, Cy5 signal was detected starting right from the juxtaposition, indicating that the electrostatic interaction of DNA and cationic carriers indeed occurs immediately.

2. Experimental details

2.1. Preparation of 605QD-pDNA and Cy5-chitosan

Plasmid DNA (pEGFP-C1, 4.9 kb, Clontech, Mountain View, CA) was labeled with streptavidin-functionalized 605QDs (Qdot 605 ITK, Invitrogen, Carlsbad, CA) via a biotin–streptavidin linkage as reported previously [10,11]. Briefly, the molar ratio of pDNA to QD was kept in excess to ensure complete conjugation of QDs to pDNA, and the incubation

time was around 15 min. Prior to this conjugation, the pDNA were biotinylated as described by the manufacturer (Label IT Biotin, Mirus Bio, Madison, WI) and scaled to have ~1–2 biotin labels per pDNA. Biotinylated pDNA were purified from unreacted biotin cross-bridges by ethanol or isopropanol precipitation and centrifugation following standard protocols. The number of QDs labeled onto each pDNA is estimated to be ~1–3 [10]. 605QD-labeled pDNA (10 μ g) were then added to 100 μ l of 50 mM of sodium sulfate solution.

The free amines on the chitosan polymer backbone (390 kDa, 83.5% deacetylated, Vanson, Redmond, WA) were labeled with Cy5 (Amersham Biosciences, Piscataway, NJ) using *N*-hydroxy-succinimide (NHS) and ethyl-3-(dimethylaminopropyl) carbodiimide (EDC) following a standard protocol. To facilitate complete conjugation of Cy5 dye, the molar ratio of Cy5 to free amines of chitosan was kept at 1:200. The reaction mixture was maintained at pH ~6.5 to keep chitosan soluble. Any remaining free dye was removed by dialysis to obtain only Cy5-chitosan. The final labeling level of Cy5 determined by comparing fluorescence intensities of Cy5-chitosan to a standard curve from Cy5 dye, resulting in ~2–6 Cy5 dyes per chitosan monomer. Cy5-chitosan (pH 5.5–5.7; 0.01–0.1% in 25 mM acetic acid solution) were then diluted with Millipore Milli-Q gradient water (>18.0 M Ω) according to wanted N/P ratio, the theoretical ratio of protonated amines in the chitosan solution to the negative phosphates in the DNA solution. The FRET pair, 605QD and Cy5, was chosen based on maximizing spectral overlap between the donor and acceptor and minimizing potential cross-talk [27]. For this pair, the Förster distance was determined to be 69.4 Å [27]. When chitosan and DNA interact electrostatically to form polyplexes, multiple Cy5 dyes come in close proximity to a QD donor and thereby increases the energy transfer efficiency within a compact polyplex. Several control experiments have been conducted separately to ensure false FRET from simply mixing donor and acceptor fluorophores alone was minimal (data not shown). The acceptor-to-donor ratio was controlled by the N/P ratio and optimized based on bulk preparation [1,10]. For the polyplexes prepared in bulk, the acceptor-to-donor ratio was estimated to be larger than 100 at the N/P ratio of 4, assuming both components (605QD-pDNA and Cy5-polymer) were consumed in the reaction [29].

2.2. Microfluidic chip fabrication and operation

This microfluidic chip was fabricated by conventional soft lithography techniques [30], casting and curing the PDMS prepolymer on a SU-8 2025 (MicroChem Corp., Newton, MA, now acquired by Nippon Kayaku Co., Ltd) master following a standard protocol, which gives the channel height at around 35 μ m. PDMS strips with through holes, as fluidic connections, were then oxygen plasma (1 min, 20 W) treated before bonded with a cover glass. The bonded PDMS chip was then left in an oven at 95 °C for overnight to enhance the bonding strength.

Prior to the reagents were loaded into the microfluidic chip, the PDMS chip was treated with oxygen plasma again to alter the surface properties to be hydrophilic temporarily. The micro-T-junction was filled with water (free of bubbles), before loading the reagents to ensure smooth flow during the experiment. Flow rate was controlled by a syringe pump (Harvard apparatus, Holliston, MA). The experiments were conducted under laminar flow conditions where the Reynolds number was calculated to be ~0.14, according to the channel geometry and applied flow rate.

2.3. Image acquisition and analysis

Epifluorescent images were captured with a fluorescence microscope (BX-51, Olympus America, Inc., Melville, NY) equipped with a 100 W mercury arc lamp and an intensified Retiga CCD (Qimaging, Burnaby, BC, Canada). Excitation light was filtered (475AF40,

Omega Optical Inc., Brattleboro, VT) and transmitted through a 10× objective (Olympus America, Inc., Melville, NY). Monocolor emission from 605QD to Cy5 were collected and filtered through appropriate filters (595AF60 and 670DF40, respectively; Omega Optical Inc., Brattleboro, VT) and dichroics (500 DRLP and 595DLPBX-04, respectively; Omega Optical Inc., Brattleboro, VT). Image processing and analysis was performed with ImageJ (v1.36b, <http://rsb.info.nih.gov/ij/>). Subsequent data processing and fitting were conducted with Origin (OriginPro8, Student Version, OriginLab, Northampton, MA).

2.4. Estimation of relative diffusivity

The apparent diffusivity [31] of free QD-pDNA was determined by conducting experiments in the T-junction with QD-pDNA and dH₂O introduced to the right and left inlets, respectively and measuring the cross-sectional fluorescent intensity of 605QD along the microchannel. The diffusion patterns, obtained from 605QD channel, were analyzed following a previously reported one-dimensional diffusion model [28,32,33],

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} \quad (1)$$

with the initial and boundary conditions, $C(x, 0) = C_0 H(x)$, $C(-\infty, t) = 0$, $C(\infty, t) = C_0$, where $H(x)$ is the Heaviside step function. The solution is in the form as

$$C(x, t) = C_0 \left[1 + \operatorname{erf} \left(\frac{x}{\sqrt{4Dt}} \right) \right] \quad (2)$$

where C_0 is the initial concentration, t is the time interval and x is the transverse distance from the channel centerline. The sample concentration was assumed to be proportional to the fluorescence intensity. Similarly, the apparent diffusivity of nucleating polyplexes was obtained with the QD-pDNA and Cy5-chitosan as illustrated in figure 1(b). The relative diffusivity of the nucleating polyplexes was then calculated by normalizing to the referenced diffusivity of free QD-pDNA.

3. Results and discussion

Figure 2 shows a series of monochrome images of Cy5 signals obtained at three time intervals. The Cy5 fluorescent band becomes brighter and wider as the time interval (t) increases, indicating the on-going process of polyplex self-assembly. Cross-sectional fluorescence intensity profiles were analyzed from the fluorescent images and plotted across the channel width (figure 3(a)). The measured profiles are well represented as Gaussian functions by least-squares fitting ($R^2 > 0.92$). Assuming that the FRET-mediated Cy5 intensity is linearly proportional to DNA polyplex concentration, this symmetric pattern implies that the initial self-assembly process of negatively charged DNA and positively charged polymer is likely diffusion-limited. The DNA and polymer rapidly interacted at the laminar interface and depleted free reagents. The resulting Cy5 signal indicates the successful nucleation and subsequent maturation into newly formed polyplexes which begin to diffuse outwards in the channel and cause widening of the Gaussian Cy5 intensity profile across the channel. This stage of the process was previously attributed to 'fast cationic binding' in the millisecond range, dominated mainly by electrostatic interactions [34,35]. At the primary stage, the integrated Cy5 intensity, which describes the growth of DNA polyplexes, was found to be linearly dependent on the square root of time interval, SQRT of

time ($R^2 = 0.995$, figure 3(b)) suggesting the process is indeed diffusion-limited [31]. However, the symmetric profile of the Cy5 intensity was found to deviate at around 1 s (figure 3(a)), signaling a change in reaction kinetics. The polyplex formation entered a secondary 'nano-assembly flocculation' stage which was non-linear (figure 3(b)), a characteristic feature of a diffusion-reaction limited process [23,31]. To our knowledge, this is the first attempt to visually and spatially resolve this fast interaction between cationic polymers and DNA, through a direct fluorescence response in real time and with high temporal resolution.

Empirical determination of molecular interactions has been previously demonstrated in microfluidic T-junction devices through the interdiffusion of analyte and indicator to produce a signal change that may be correlated to a physical parameter, such as diffusion coefficients, concentrations or reaction kinetics [32,33,36]. Herein, to qualitatively resolve the onset of self-assembly, the relative diffusivity is determined from the fluorescent profile of QD-pDNA following the previously described one-dimensional model [28,32] as discussed in section 2. The effect of velocity profile along the direction of interdiffusion is considered to be minimal at the designed aspect ratio (~ 5.7) [33]. During the measurement, the focal plane was fixed at approximately one half of the channel height (h), such that the fluorescence signal was collected as uniformly as possible [28]. Figure 4 shows representative cross-sectional intensity profiles obtained from 605QD channel for both free QD-pDNA and nucleating polyplexes at three different time intervals under the flow rate of 20 nl s^{-1} . The apparent diffusivity [31] of QD-pDNA was determined by fitting the experimental fluorescent profiles with the theoretical result of equation (2). Relative diffusivity of nucleating polyplexes, referenced by the apparent diffusivity of free QD-pDNA, was measured by conducting experiments with QD-pDNA and Cy5-chitosan, as described in section 2. Upon interacting with Cy5-chitosan (i.e. the onset of polyplex nucleation), the subsequent change of relative diffusivity was monitored at different temporal resolutions through altering mean flow velocity. The apparent diffusivity of free QD-pDNA is consistent throughout different time intervals. Whereas in figure 5, immediately after mixing with chitosan in a separate experiment (SQRT of time $< 15 \text{ ms}^{1/2}$), the relative diffusivity of nucleating polyplexes rose to $\sim 16\text{--}21$. The sudden, large increase of relative diffusivity suggests that DNA experiences a drastic conformational change, ~ 20 fold compaction, upon interacting with chitosan, presumably from the relaxed to condensed form. During polyplex formation, Cy5-chitosan condensed QD-pDNA into a compact state due to charge neutralization [34] and interchain entanglement. Prior analysis by transmission electron microscopy showed that the multiple QD-pDNA were encapsulated within the polyplex [10,11]. This result again demonstrates instantaneous interactions of DNA and the cationic carrier, which agrees with the previous observation via FRET-mediated Cy5 signals (figure 1(b), inset). Subsequently, the value of the relative diffusivity decreases progressively, suggesting a process of polyplex assembly that leads to a continuing increase in mass.

4. Conclusion

We present the first attempt to monitor DNA polyplex self-assembly kinetics in real time through QD-FRET responses within a simple microfluidic chip. QD-mediated FRET provides a highly sensitive and quantitative indication of the onset of molecular interactions and throughout the self-assembly process, whereas microfluidics offers a well-controlled microenvironment to spatially analyze the process during the polyplexes synthesis. The convergence of QD-FRET and microfluidics enables an innovative platform to monitor fundamental reactions with high sensitivity and temporal resolution ($\sim$ milliseconds). For the model system of chitosan/DNA polyplexes, two distinct stages in the self-assembly process were captured by this analytic platform. Further, customized polyplexes may be generated

through proper design of microfluidic devices, and the resulting QD-FRET DNA polyplexes could be readily applied for establishing structure-function relationships [10,11]. The kinetic aspect of the self-assembly process obtained at the microscale would be particularly valuable for microreactor-based reactions which are relevant to many micro- and nano-scale applications.

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References

1. Mao HQ, Roy K, Troung-Le VL, Janes KA, Lin KY, Wang Y, August JT, Leong KW. Chitosan-DNA nanoparticles as gene carriers: synthesis, characterization and transfection efficiency. *J Control Release* 2001;70:399–421. [PubMed: 11182210]
2. Li SD, Huang L. Gene therapy progress and prospects: non-viral gene therapy by systemic delivery. *Gene Ther* 2006;13:1313–9. [PubMed: 16953249]
3. Mastrobattista E, van der Aa MAEM, Hennink WE, Crommelin DJA. Artificial viruses: a nanotechnological approach to gene delivery. *Nat Rev Drug Discov* 2006;5:115–21. [PubMed: 16521330]
4. Niidome T, Huang L. Gene therapy progress and prospects: nonviral vectors. *Gene Ther* 2002;9:1647–52. [PubMed: 12457277]
5. Middaugh CR, Ramsey JD. Analysis of cationic-lipid-plasmid-DNA complexes. *Anal Chem* 2007;79:7240–8. [PubMed: 17972398]
6. Tang MX, Szoka FC. The influence of polymer structure on the interactions of cationic polymers with DNA and morphology of the resulting complexes. *Gene Ther* 1997;4:823–32. [PubMed: 9338011]
7. Kiang T, Wen J, Lim HW, Leong KW. The effect of the degree of chitosan deacetylation on the efficiency of gene transfection. *Biomaterials* 2004;25:5293–301. [PubMed: 15110480]
8. Erbacher P, Zou SM, Bettinger T, Steffan AM, Remy JS. Chitosan-based vector/DNA complexes for gene delivery: biophysical characteristics and transfection ability. *Pharm Res* 1998;15:1332–9. [PubMed: 9755882]
9. Prabha S, Zhou WZ, Panyam J, Labhasetwar V. Size-dependency of nanoparticle-mediated gene transfection: studies with fractionated nanoparticles. *Int J Pharm* 2002;244:105–15. [PubMed: 12204570]
10. Ho YP, Chen HH, Leong KW, Wang TH. Evaluating the intracellular stability and unpacking of DNA nanocomplexes by quantum dots-FRET. *J Control Release* 2006;116:83–9. [PubMed: 17081642]
11. Chen HH, Ho YP, Jiang X, Mao HQ, Wang TH, Leong KW. Quantitative comparison of intracellular unpacking kinetics of polyplexes by a model constructed from quantum dot-FRET. *Mol Ther* 2008;16:324–32. [PubMed: 18180773]
12. Lai E, van Zanten JH. Monitoring DNA/poly-L-lysine polyplex formation with time-resolved multiangle laser light scattering. *Biophys J* 2001;80:864–73. [PubMed: 11159453]
13. Tecle M, Preuss M, Miller AD. Kinetic study of DNA condensation by cationic peptides used in nonviral gene therapy: analogy of DNA condensation to protein folding. *Biochemistry* 2003;42:10343–7. [PubMed: 12950160]
14. Braun CS, Fisher MT, Tomalia DA, Koe GS, Koe JG, Middaugh CR. A stopped-flow kinetic study of the assembly of nonviral gene delivery complexes. *Biophys J* 2005;88:4146–58. [PubMed: 15805171]
15. Chan EM, Mathies RA, Alivisatos AP. Size-controlled growth of CdSe nanocrystals in microfluidic reactors. *Nano Lett* 2003;3:199–201.
16. Wagner J, Kohler JM. Continuous synthesis of gold nanoparticles in a microreactor. *Nano Lett* 2005;5:685–91. [PubMed: 15826109]

17. Edel JB, Fortt R, DeMello JC, DeMello AJ. Microfluidic routes to the controlled production of nanoparticles. *Chem Commun* 2002;1136–7.
18. Abou Hassan A, Sandre O, Cabuil V, Tabeling P. Synthesis of iron oxide nanoparticles in a microfluidic device: preliminary results in a coaxial flow millichannel. *Chem Commun* 2008;1783–5.
19. Song H, Chen DL, Ismagilov RF. Reactions in droplets in microfluidic channels. *Angew Chem Int Edn* 2006;45:7336–56.
20. Jahn A, Vreeland WN, Gaitan M, Locascio LE. Controlled vesicle self-assembly in microfluidic channels with hydrodynamic focusing. *J Am Chem Soc* 2004;126:2674–5. [PubMed: 14995164]
21. Martin-Banderas L, Flores-Mosquera M, Riesco-Chueca P, Rodriguez-Gil A, Cebolla A, Chavez S, Ganan-Calvo AM. Flow focusing: a versatile technology to produce size-controlled and specific-morphology microparticles. *Small* 2005;1:688–92. [PubMed: 17193506]
22. Song YJ, Hormes J, Kumar C. Microfluidic synthesis of nanomaterials. *Small* 2008;4:698–711. [PubMed: 18535993]
23. Sounart TL, Safier PA, Voigt JA, Hoyt J, Tallant DR, Matzke CM, Michalske TA. Spatially-resolved analysis of nanoparticle nucleation and growth in a microfluidic reactor. *Lab Chip* 2007;7:908–15. [PubMed: 17594011]
24. Huang M, Fong CW, Khor E, Lim LY. Transfection efficiency of chitosan vectors: Effect of polymer molecular weight and degree of deacetylation. *J Control Release* 2005;106:391–406. [PubMed: 15967533]
25. Koping-Hoggard M, Mel'nikova YS, Varum KM, Lindman B, Artursson P. Relationship between the physical shape and the efficiency of oligomeric chitosan as a gene delivery system *in vitro* and *in vivo*. *J Gene Med* 2003;5:130–41. [PubMed: 12539151]
26. Roy K, Mao HQ, Huang SK, Leong KW. Oral gene delivery with chitosan–DNA nanoparticles generates immunologic protection in a murine model of peanut allergy. *Nat Med* 1999;5:387–91. [PubMed: 10202926]
27. Zhang CY, Yeh HC, Kuroki MT, Wang TH. Single-quantum-dot-based DNA nanosensor. *Nat Mater* 2005;4:826–31. [PubMed: 16379073]
28. Kamholz AE, Weigl BH, Finlayson BA, Yager P. Quantitative analysis of molecular interaction in a microfluidic channel: the T-sensor. *Anal Chem* 1999;71:5340–7. [PubMed: 10596213]
29. Clamme JP, Azoulay J, Mely Y. Monitoring of the formation and dissociation of polyethylenimine/DNA complexes by two photon fluorescence correlation spectroscopy. *Biophys J* 2003;84:1960–8. [PubMed: 12609898]
30. Xia YN, Whitesides GM. Soft lithography. *Angew Chem Int Edn* 1998;37:551–75.
31. Truskey, GA.; Yuan, F.; Katz, DF. *Transport Phenomena in Biological Systems*. Upper Saddle River: Prentice-Hall; 2004.
32. Culbertson CT, Jacobson SC, Ramsey JM. Diffusion coefficient measurements in microfluidic devices. *Talanta* 2002;56:365–73. [PubMed: 18968508]
33. Kamholz AE, Yager P. Theoretical analysis of molecular diffusion in pressure-driven laminar flow in microfluidic channels. *Biophys J* 2001;80:155–60. [PubMed: 11159391]
34. Bloomfield VA. DNA condensation. *Curr Opin Struct Biol* 1996;6:334–41. [PubMed: 8804837]
35. Porschke D. Dynamics of DNA Condensation. *Biochemistry* 1984;23:4821–8. [PubMed: 6238622]
36. Ristenpart WD, Wan JD, Stone HA. Enzymatic reactions in microfluidic devices: Michaelis–Menten kinetics. *Anal Chem* 2008;80:3270–6. [PubMed: 18355085]

Figure 1.

(a) Pictorial schematic of the self-assembly of QD-FRET DNA polyplexes. Plasmid DNA (pDNA) and the cationic polymeric gene carrier were labeled with 605QD (donor) and Cy5 (acceptor), respectively. Polyplexes were formed through electrostatic complex coacervation. Upon QD excitation at 488 nm, Cy5 emission via FRET indicates a compact and intact polyplex. (b) Integration of QD-FRET and microfluidics to monitor polyplex self-assembly kinetics in a simple microfluidic T-junction. 605QD-labeled pDNA (right inlet) and Cy5-labeled polymer (left inlet) were loaded at equal volume flow rates. Self-assembly of the polyplexes was initiated only at the mixing interface due to the laminar flow condition within the microchannel. (Inset) overlay epifluorescent image measured at both the 605 nm (QD/pseudo-red) and 670 nm (Cy5/pseudo-green) detection channels. QD signal was observed in the right stream while no signal was seen in the left stream. QD-FRET signal (combined QD and Cy5 signal/yellow) was observed along the interface of the streams, demonstrating the formation of polyplexes. The dashed lines outline the channel boundaries. Channel width is 200 μm and height is 35 μm .

Figure 2. Monochrome fluorescent images ($285\ \mu\text{m} \times 200\ \mu\text{m}$) of FRET-mediated Cy5 signal (670 nm) at increasing time intervals obtained from their corresponding axial positions under the volume flow rate of $\sim 20\ \text{nl s}^{-1}$. Time interval was calculated as described in the text, with a distance (y) from the juxtaposition to the point of interest.

Figure 3.

(a) Cross-sectional intensity profiles of FRET-mediated Cy5 signals. The experimentally measured profiles (symbols) exhibit a Gaussian distribution (solid lines). With increasing time interval, both the peak height and width increase due to the on-going self-assembly process and diffusion of formed polyplexes outward from the mixing interface. The Gaussian-like profile deviates slightly at $t > 1000$ ms, signaling changes in the reaction kinetics.

(b) Integrated FRET-mediated Cy5 intensity is linearly dependent on the square root of time interval (SQRT of time) during the primary stage. This linear relationship suggests that the polyplex self-assembly is a diffusion-limited process at the primary stage, which can be attributed to an efficient DNA condensation by the polymer. The secondary nano-assembly flocculation stage displays a non-linear function, which suggests a diffusion-reaction limited process.

Figure 4.

Representative 605QD intensity profiles obtained from (a) free QD-pDNA and (b) nucleating polyplexes, measured at various time intervals under a mean flow velocity of 20 nl s^{-1} . Relative diffusivities were determined following previously described one-dimensional model as shown in equation (2). The sigmoidal curve for the nucleating polyplexes is slightly off from the theoretical prediction, probably due to different reaction rates occurring at DNA-excess or Chitosan-excess side.

Figure 5. Relative diffusivity of nucleating polyplexes as determined by analysis of QD intensity profile. QD-pDNA and Cy5-chitosan were mixed in a microfluidic T-junction at mean flow velocity of 12 and 20 nl s⁻¹