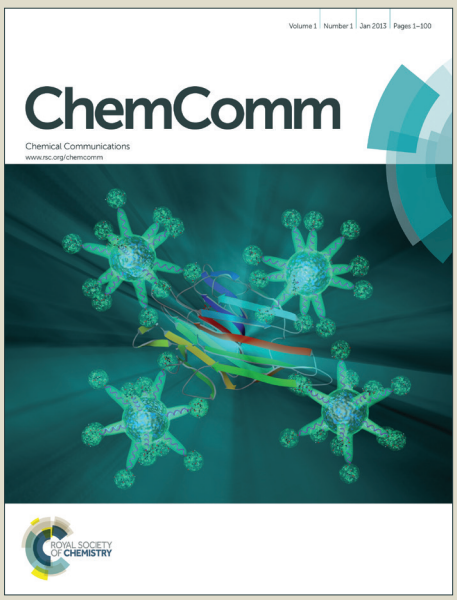
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Dynamic Assembly of DNA and Polylysine Mediated by Electric Energy

Lin Niu,[a] Xuyan Yang,[a] Xiaocui Zhu,[b] Yudan Yin, [a] Wei Qu, [a] Jihan Zhou, [a] Meiping Zhao,*[b] Dehai Liang*[a]

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

Under electric field, the complexes formed by DNA and polylysine exhibit novel features, such as selective merging of particles, ejecting of daughter vehicles, and differentiation of particles of varying mobility. The mobility of the complex could be three times faster than that of free DNA.

Introduction

The main assumption about the origin of life on earth is that life originated from inanimate matter through a spontaneous and gradual increase of molecular complexity.[1] It is also proposed that the compartmentalization is responsible for the transition from non-living to living matter.[2-3] Many protometabolic reactions, such as transcription,[4] self-replication,[5] growth and division,[6] have been conducted within the lipid vesicles with varying degree of success. However, Mann and coworkers demonstrated that the micro-droplet formed by complex of polypeptide and mononucleotide can also functioned as a protocell model,[7-8] implying that the membrane was not needed for effective compartmentalization. Trevors and Pollack[9] proposed that a hydrogel environment was a prerequisite for the assembly of the first cell. The lipid bilayer was developed later on.

The current study on protocell, with or without membrane, is based on the static assembly of molecules, in which the process is controlled by equlibirum. However, life is dynamic: every life activity occurs under a flux of energy. The cell dies if the energy flux is stopped.[10] The hydrogel environment and the compartmentalization repsonsible for the origin of life should be maintained under energized and non-equlibrium conditions. The main energy form in life is chemical energy. However, electric field, which exists extracellularly and intracellularly[11-12] and can be as high as 10^7 V/cm,[13-14] is essential in regulating appropriate cell behaviors during tissue morphogenesis and regeneration.[12, 15] It is also the key to the constituents of nucleic acids, proteins, and membrane bilayers in prebiotic conditions.[16]

The basic units of life, including DNA, RNA, and proteins, contain charged or chargible groups. They belong to the category of polyelectrolytes.[17] The conformation and the movement of polyelectrolytes, as well as their interactions, are sensitive to electric filed.[18-19] On the other hand, the functions of biopolymers are generally activated by interacting with each other to form certain assembled structures. The complexation of different biopolymers is also responsible for the formation of hydrogel envirment, such as extracellular matrix. Therefore, the assembly of the biopolymers under electric field, i.e., dynamic assembly,[10] is crucial to the orgin of life under non-equilibrium conditions.

Herein, we choose negatively charged salmon testes DNA (2000 bp) and positively charged poly-*l*-lysine (PLL, 200 repeating units) as the model molecules, and studied their behaviour of forming complex (or droplet) in the presence of electric field. Microfluidic chips are employed as the platform to monitor the whole complexation process. 80 μm wide and 25 μm height micro channel with double-cross layout is etched on a glass chip, as schematically shown in Figure 1A. Double gate-injection[20] protocol is used to drive DNA and PLL into the channels simultaneously as short plugs by applying designed voltages at the desirable positions (Figure S1). To optimize the conditions for injection, DNA and PLL are tagged with Ultrapower™ dye and FITC fluorescence probes, respectively. Both of the labelled molecuels can be observed under the illumination of blue laser. The +/- charge ratio can be tuned by the injected plug length. It is fixed at 3 in this study.

Results and discussion

Without electric field, DNA and PLL can coacervate into a large droplet, and eventually form precipitate in a vial or in the microchannel (Figure S2). The situation is quite different under electric field. Figure 1B and 1C compares the snapshots of the complex formed at 250 V/cm and 125 V/cm, respectively. To minimize the effect of dye molecules, only PLL is labelled. The movies are included in the supporting information. At 125 V/cm, a bright borderline, indicating the formation of DNA and PLL complex, is observed when the two plugs encounter (Figure 1C and Movie 1). The front moves in the same direction as free DNA under electric field, suggesting that the complex is negatively charged. The amount and size of the complex increase as the reaction proceeds. Merging, splitting, and differentiation are observed at this stage. In about 30 s, PLL consumes up. The differentiation becomes heavier and results in three different morphologies: fully neutralized droplet with zero mobility (formed at about 52s), large particles with slow mobility, and very small particle with high mobility. These small and fast moving particles are originally released from those larger particles, instead of being formed from fresh DNA and PLL. The size of these small particles (excluding those large particles trapped in the channel), after collected from the outlet and imaged by AFM, is uniform and in the order of 30-50 nm (Figure S3).

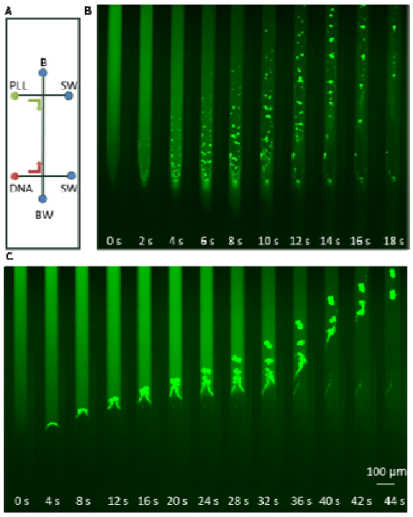


Figure 1. (A) drawing of the double-cross glass microchip for electrophoresis, with B, BW, and SW refer to buffer, buffer waste, and sample waste, respectively. Panels (B) and (C) show the snapshots of the complex in micro-channel at 250 V/cm and 125 V/cm, respectively. Charge ratio +/- = 3. FITC-PLL is monitored in the laser channel.

At 250 V/cm, a large amount of small particles are quickly generated in the encounter zone (Figure 1B and Movie 2) until all the PLLs consume up. These particles migrate in the same direction as free DNA at a relatively fast rate. Merging of the particles with different speed occurs when they collide with each other. Splitting is not prominent probably because the size is small. Differentiation also generates particles of different size and mobility. As shown in Figure 2A, a large droplet with zero mobility is able to selectively capture the negatively charged particle to desirable position when it is passing by. The merging results in a conformation adjustment, followed by a release of tiny messenger vehicles (Figure 2B). This implies that the formed droplet is also internally dynamic under electric filed. The release of daughter particles is phenomenally similar to the release of the exosomes from melanoma cells before tumour metastasis. [21]

With further increasing the electric field to 375 V/cm (Movie 3) or 495 V/cm (Movie 4), the formed particles are even smaller, as demonstrated by the reduced fluorescence intensity. The degree of mergering and differentiation is heavily suppressed at elevated electric field. Moreover, no trapped particle is observed in the channel.

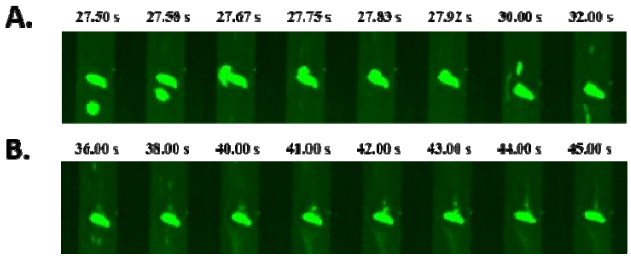


Figure 2: Capture of a pasing by vehicle (A) and the release of messagers (B) after conformation adjustment. A small particle merged into the droplet at 27.75 s, and the droplet started to release messagers at 40.00 s.

To quantitativley compare the spacial distribution of the particles at different electric fields, we set a virtual detection window about 1000 μm away from the point where the interaction begins, and transform the snapshot at the timepoint when the first particle hits the window into an electropherogram. For free DNA and PLL, the fluorescence intensity is uniform in the plug. However, the complex particle, as shown in Figure 3, exhibit a sharp peak due to the crowding of lableled PLL. Since the fluoresence intensity is roughly proportional to the concentration of the dyes at the studied conditions (No photobleaching), the area of the peak represents the degree of aggregation. At 125 V/cm, the number of the formed partilces is small, but the size is huge (Figure 3A). The splitting of the peak suggests that the particles is not uniform, in agreement with the observings that the large particle is merged from small ones. With increasing electric field strength, the size of the particles becomes smaller, but the number increases. At 495 V/cm, the intensity of the particle is not prominent, as shown in the inset in panel D.

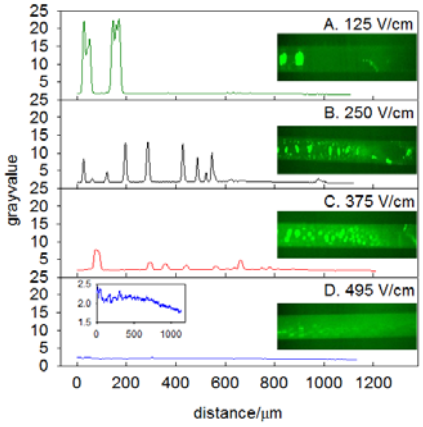


Figure 3. Electropherograms of DNA/PLL particles at different electric field strength. The snapshot in each panel is the corresponding time point for the electropherogram. The inset in Panel D is the magnification of the signals.

The complex particle differentiates with time under electric fields. The trend can be described by the changes in the mobility of the particle. Figure 4A compares the front mobility at different electric fields. It can be divided into two stages: moving inside the PLL zone in the early stage and the migration after passing the zone. The mobility of the front particles is dependent on the electric field while migrating inside the PLL zone. It decreases with time (< 25 s) at 125 V/cm, is almost constant (< 8 s) at 250 V/cm, and increases (< 4 s) at 375 V/cm (Figure 4A, Figure S4). However, the mobility always increases when migrating outside the PLL zone. The calculation on the mobility becomes difficult at 495 V/cm due to the small size and fast movement of the front particle. It is also hard to tell when the front particle migrate out of the PLL zone. But it is clear that the mobility increases with time under the electric field.

The mobility of free DNA determined in our experiment (Figure S5) is 2.2×10^{-4} $cm^2/(V.s)$, similar to the value reported in literature.[22] However, the maximum mobility of the front particle increases to 4.5×10^{-4} $cm^2/(V.s)$ in 10 s at 495 V/cm. This value is twice the mobility of free DNA. It is surprising since the neutralization of the charges on DNA should reduce its mobility.

To reveal the portion of the particles migrating faster than free DNA, we analysed the mobility of all the particles. Figure 4B shows the time dependence of the mobility distribution at 125 V/cm. Clearly, the particles gain their speed mainly at later stage

(60 s, Panel d) when no free PLL molecules are around. The heavy differentiation at this stage (Movie 1) results in a broad mobility distribution, as well as the mobility (of the small particle) higher than that of free DNA. Note that the fastest particle is not necessarily the originally formed front particle. The degree of differentiation is dependent on electric field. In 10 s at 495 V/cm, most of the particles have the mobility higher than that of free DNA (Figure 4C). The maximum value is about three times higher. The differentiation under electric field is responsible for the particles with broad distribution of mobility.

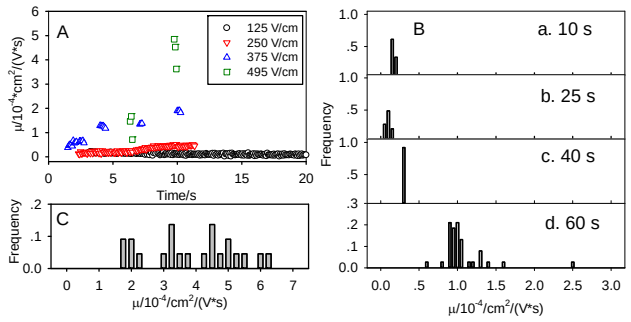


Figure 4. (A) Time dependence of the front particle mobility under different electric field. (B) the mobility distribution at different time points at 125 V/cm with the corresponding snapshot shown as the inset. (C) The mobility distribution at 10 second at 495V/cm.

The complex particle formed by DNA and PLL can be described as a physical microgel with unneutralized segments inside the core and dangling on the surface.[23] The particle is permeable to the solvents, and the permeability is related to the heterogeneous distribution of unneutralized segments. Duval and Ohshima proposed a model[24-25] to describe the mobility of the soft particles, including microgels, bacteria, and viruses, under electric field. According to their model, the mobility change of the particle in a fixed solvent is caused not only by the space charge density ρ_0 and the softness parameter λ(reflects the extent of hydrodynamic flow penetration within the particle),[25] but also by the radial density distribution *f(r)*of the charged segments.[24] It is very difficult to determine the ρ_0 , λ, and *f(r)* of each complex particle under electric field because they changes with time. A practical approach is to treat each particle before differentiation as an entity and evaluate the evolutional change of mobility without quantitatively separating the contribution from each parameter.

We choose the complex particles before differentiation and calculated their mobility. For a better comparison, the mobility is normalized by the slowest value at the same electric field. As shown in Figure 5, the inverse of the mobility decreases exponentially with time and can be fitted by the equation

$$\left(u/u_s\right)^{-1} = A \times \exp\left(-t/\tau\right) \qquad (1)$$

with A being a constant. The characteristic time τ shows the evolutional rate under electric field. The fitting results indicate that the characteristic time τ is 20s, 7.6s, and 5.7s at 125 V/cm, 250 V/cm, and 375 V/cm, respectively. They decrease with increasing electric field strength (Figure S6), indicating that the electric field not only affects the morphology of the complex particle, but also accelerates the evolutional rate.

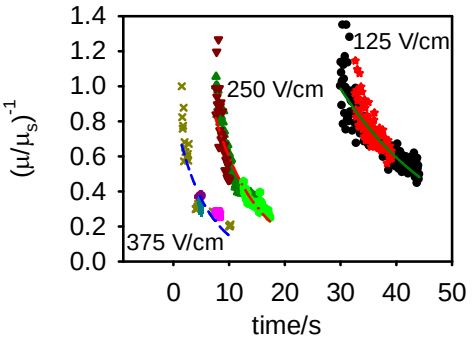


Figure 5. Time dependence of the inverse mobility normalized by that of the slowest particle.

It is established in literature that the polyelectrolyte complex is subject to a disproportionation process to generate particles close to neutralization and particles far from neutralization.[23, 26] The relaxation rate of polyelectrolyte is a key to the disproportionation process. DNA and PLL migrate in opposite directions and the dragging force is proportional to the strength of electric field. On one hand, the electric field constrains the particle to a certain size by reducing the interaction time between DNA and PLL, with higher electric field strength yielding smaller size complex. On the other hand, the elecric field mediated the disproportionation rate of the complex by accelerating the relaxation of the polyelectrolyte chains. The relaxation rate of DNA or PLL is proportional to the strength of electric field. DNA is much more rigid and longer than PLL, and its charge density is also doubled. We speculate that the disporportionation favours the complex particle containing larger amount of DNA. This is the reason that all the observed complex particle migrating in the same direction as free DNA.

Figure 6. Schematically showing the polarization of the complex and the release of the daughter particle.

As schematically shown in the Figure 6, the relaxation of DNA and PLL in opposite directions globally polarized the complex particle along the electric field, even though the local distribution of charges is heterogeneous. The increase in the mobility could result from the release of free PLL to increaseρ_0, the shrinkage and orientation of the particle along the electric field to decrease λ, or a preferred *f(r)*.[24] Since the mobility of certain particles increases by a factor of five with time (Figure 4A, 495V/cm), and no prominent change in morphology is observed, we attribute the enhancement on mobility to the cooperative effect from all these variables. As the polarization reaches certain degree, the domain in rich of DNA, whose charge density is much higher than that of the periphery, suffers from a repulsion force from the environment and a stronger dragging force from the electric field. This domain will eject from the mother particle once the attachment and entanglement interaction is overcome. The

mobility of the daughter particle could be much higher than that of free DNA, because most of the counterions on DNA have been released upon forming complex. The condensation of the counterions back to the complex particle is hindered in electric field, especially when the electric field is high. Note that the Bjerrum length is 0.7 nm in water at 25°C, indicating that over ¾ the counterions on free DNA are condensed. The higher ρ_0 and decreased softness λ renders the complex particle a mobility higher than that of free DNA under electric field.

Conclusions

The complex formed by oppositely charged polyelectrolyte, such as DNA and PLL, should be able to mimic the environment of life forms. In the presence of electric field, it exhibits some interesting features, such as merging, splitting, and differentiation, all of which are dependent on the stregnth of electric field. We attributed these live features to energy mediated disproportionation process, which generates particles or domains of different charge density, softness, and density distribtuions. The dynamic assembly of oppositely charged biopolymers in the electric field could offer a platform to explore the emergence of life and the mechanism of life processes at molecular level.

Acknowledgement

The financial support from the National Natural Science Foundation of China (21174007) and the National Basic Research Program of China (973 Program, 2012CB821500) were greatly acknowledged.

Notes and references

[a] *Beijing National Laboratory for Molecular Sciences and the Key Laboratory of Polymer Chemistry and Physics of Ministry of Education, College of Chemistry and Molecular Engineering, Peking University, Beijing, China, 100871. Fax: 86-10-62751708; Tel: 86-10-62756170; E-mail: dliang@pku.edu.cn*

[b] *Beijing National Laboratory for Molecular Sciences, MOE Key Laboratory of Bioorganic Chemistry and Molecular Engineering, College of Chemistry and Molecular Engineering, Peking University, China, 100871. Fax: 86-10-62751708; Tel: 86-10-62758153; E-mail: mpzhao@pku.edu.cn*

† Electronic Supplementary Information (ESI) available: [Experimental method, Chip layout, AFM results, and movies on particle migration are included in the supporting information.]. See DOI: 10.1039/b000000x/

‡ Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

1 P.L. Luisi, the emergence of life: From Chemical Origins to Synthetic Biology. Cambridge University Press: 2006.
2 U. J. Meierhenrich, J. J. Filippi, C. Meinert, P. Vierling and J. P. *Angew. Chem. Int. Edit.* 2010, **49** , 3738.
3 A. J. Dzieciol, S. Mann, *Chem. Soc. Rev.* 2012, **41** (1), 79.
4 V. Noireaux and A. Libchaber, *Proc. Natl. Acad. Sci. U. S. A.* 2004, **101** (51), 17669.
5 K. Adamala and J. W. Szostak, *Science* 2013, **342** (6162), 1098.
6 K. Kurihara, M. Tamura, K. Shohda, T. Toyota, K. Suzuki and T. Sugawara, *Nat. Chem.* 2011, **3** (10), 775.
7 S. Koga, D. S. Williams, A. W. Perriman and S. Mann, *Nat. Chem.* 2011, **3** (9), 720.
8 D. S. Williams, S. Koga, C. R. C. Hak, A. Majrekar, A. J. Patil, A. W. Perriman and S. Mann, *Soft Matter* 2012, **8** (22), 6004.
9 J. T. Trevors and G. H. Pollack, *Prog. Biophys. Mol. Biol.* 2005, **89** (1), 1.
10 G. M. Whitesides and B. Grzybowski, *Science* 2002, ***295*** (5564), 2418.
11 L. F. Jaffe, *Proc. Natl. Acad. Sci. U. S. A.* 1966, ***56*** (4), 1102.
12 C. D. McCaig, A. M. Rajnicek, B. Song and M. Zhao, *Physiol. Rev.* 2005, **85** (3), 943.
13 A. A. Gurtovenko and I. Vattulainen, *J. Am. Chem. Soc.* 2007, **129** (17), 5358.
14 K. M. Tyner, R. Kopelman and M. A. Philbert, *Biophys. J.* 2007, **93** (4), 1163.
15 C. O. Joseph and R. R. Isseroff, *J. Investig. Dermatol.* 2003, **121** (1), 1.
16 K. Ruiz-Mirazo, C. Briones and A. de la Escosura *Chem. Rev.* 2013, **114** (1), 285.
17 R. Cotterill, Biophysics: An Introduction. John Wiley & Sons: **2002**.
18 P. Y. Hsiao, Y. F. Wei and H. C. Chang, *Soft Matter* 2011, 7 (3), 1207.
19 R. R. Netz, *Phys. Rev. Lett.* 2003, **90**, 128101.
20 S. C. Jacobson, L. B. Koutny, R. Hergenroder, A. W. Moore and J. M. Ramsey, *Anal. Chem.* 1994, **66** (20), 3472.
21 J. L. Hood, S. S. Roman and S. A. Wickline, *Cancer Res.* 2011, **71** (11), 3792.
22 B. M. Olivera, P. Baine and N. Davidson, *Biopolymers* 1964, **2** (3), 245.
23 C. Zheng, L. Niu, W. Pan, J. Zhou, H. Lv, J. Cheng, D. Liang, *Polymer* 2014, **55** (10), 2464.
24 J. F. L. Duval and H. Ohshima, *Langmuir* 2006, **22** (8), 3533.
25 H. Ohshima, *Adv. Colloid Interface Sci.* 1995, ***62*** (2–3), 189.
26 R. Zhang and B. I. Shklovskii *Physica A: Statistical Mechanics and its Applications* 2005, **352** (1), 216.