

Photosynthesis Drives the Motion of Bio-nanomotors

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Autonomous nanomotors have become the new paradigm for current research as they are expected to shift the momentum in the development of next-generation technologies. However, there is a grand challenge in gaining control over the nanomotors' motion, speed, directionality, and using biocompatible fuels to power them. Currently, light is recognized for powering micromotors with advancement in using visible light for driving motion at the nanoscale regime. In this context, micron-scaled motors are fabricated but they contain metal surfaces and fabrication is quite laborious. Herein, encapsulation of plant organelles into supramolecular assemblies for active motion is conducted to fabricate bio-nanomotors, utilizing the natural photosynthesis process for powering motion at the nanoscale. The oxygen produced by the water-splitting reaction by plant organelles in visible light and the photophoresis effect due to the transparent nature of the supramolecular assembly are the main driving forces for bio-nanomotors. The bio-nanomotors are observed to have propelled motion with speed reaching up to $120.42 \pm 12 \mu\text{m s}^{-1}$, together with on-demand reversible on/off motion and real-time control over change in directionality at the nanoscale. The observed results shift the momentum toward harnessing energy from natural processes to power nanosystems for varied applications.

explore alternative energy sources such as magnetic,^[12] electrical,^[13] ultrasound,^[14] or light^[15,16] to power the artificial systems that could provide spatial and temporal control.^[17] Light has been an attractive stimulus to power micromotors^[18,19] due to their remote propagation with precise control over energy inputs.^[20] Visible light that comprises about 43% of solar energy is currently being adopted to power micromotors.^[21] Although there have been advancements in visible light-driven micromotors, they contain metal surfaces^[22,23] and fabrication is quite laborious.^[24] We envisaged visible light to be an ideal external stimulus to power supramolecular assemblies at the nanoscale that provides soft interface to cells, is easy to fabricate, and provides opportunity for molecular-level tuning due to the "bottom-up approach." There have been limited studies that could provide the breakthrough to power nanomotors in biocompatible environments and this is critically important to expand the scope for the applications of supramolecular systems. Herein, for the first time, we

1. Introduction

Nature has always inspired researchers to fabricate artificial autonomous micro-/nanomotors that could perform complicated tasks with high specificity and efficiency.^[1,2] In this context, supramolecular assembly-based systems are gaining interest,^[3,4] which have been mainly powered by catalytic decomposition of non-biocompatible fuels such as H_2O_2 ^[5–7]/hydrazine.^[8] Presently, the motion has been achieved by biocompatible fuels^[9] such as glucose,^[10] but it is challenging to have an on-demand reversible on/off motion^[11] and gain complete control over the speed or direction of supramolecular systems with ease. To overcome aforementioned problems, it is prerequisite to

demonstrate the potential of visible light to power supramolecular bio-nanomotors using the photosynthetic process fabricated by encapsulating plant organelles. The plant organelle thylakoid produces oxygen by water-splitting reaction in the presence of visible light with the help of photosystem II (PS II) present in them. This oxygen produced can be used to propel the bio-nanomotors by the bubble propulsion mechanism. In contrast, using light source can also give rise to an interesting phenomenon called photophoresis that refers to the momentum transfer between the light and the particle due to refraction and reflection of the particle.^[25] Theoretically, it has been shown that the lower absorptivity of particle gives rise to refraction and the particle starts migrating toward the light source,^[26] but to the best of our knowledge it has not been confirmed experimentally. We envisioned that combining bubble propulsion and photophoresis to drive motors at the nanoscale would give rise to enhanced speeds with controlled motion and directionality in real time that has not been observed before. In this research, we demonstrate the self-assembly of a bio-nanomotor, with thylakoid nanoparticles-containing active PS II (TNP) encapsulated in the stomach of the bowl-shaped vesicles powered by bubble propulsion at the lower intensity of light and by synergistic effects of bubble propulsion and photophoresis at a higher light intensity. Moreover, as observed in phototactic microorganisms,^[27] the shading effect due to refractive supramolecular assemblies allows them to be "steered" toward the light propagation direction.

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2. Results and Discussion

2.1. Characterization and Encapsulation of TNP

Our quest to fabricate bio-nanomotors commenced with the extraction of TNP from spinach plants cultivated in greenhouse (Methods, Supporting Information, **Figure 1a,b**). They were characterized and studied for their oxygen evolution properties before encapsulation in bio-nanomotors. Bands corresponding to CP1 protein (65 kDa), light-harvesting complex II (LHCII) at 25 kDa,^[28] and oxygen evolving complex (OEC) at 33 and 23 kDa (Figure 1c) were observed in SDS-PAGE gel, with latter two belonging to PS II polypeptide.^[29] The extracted samples

have a spherical morphology with an average size of around 20 nm as visualized by cryo-TEM, which is closely related to the size range obtained from dynamic light scattering (DLS) measurements (Figure 1d,e). In DLS, majority of the sample population was around 15–18 nm, relatively smaller to those observed in cryo-TEM. This was most probably due to oxygen production in the presence of water, leading to a higher diffusion coefficient when exposed to 633 nm laser in built in DLS, thereby showcasing a decreased apparent size. Absorption spectroscopy analysis of the as-extracted solution had two main peaks at 675 and 436 nm, corresponding to chlorophyll a^[30] together with shoulder around 470 and 650 nm, indicating the presence of chlorophyll b and carotenoid pigments

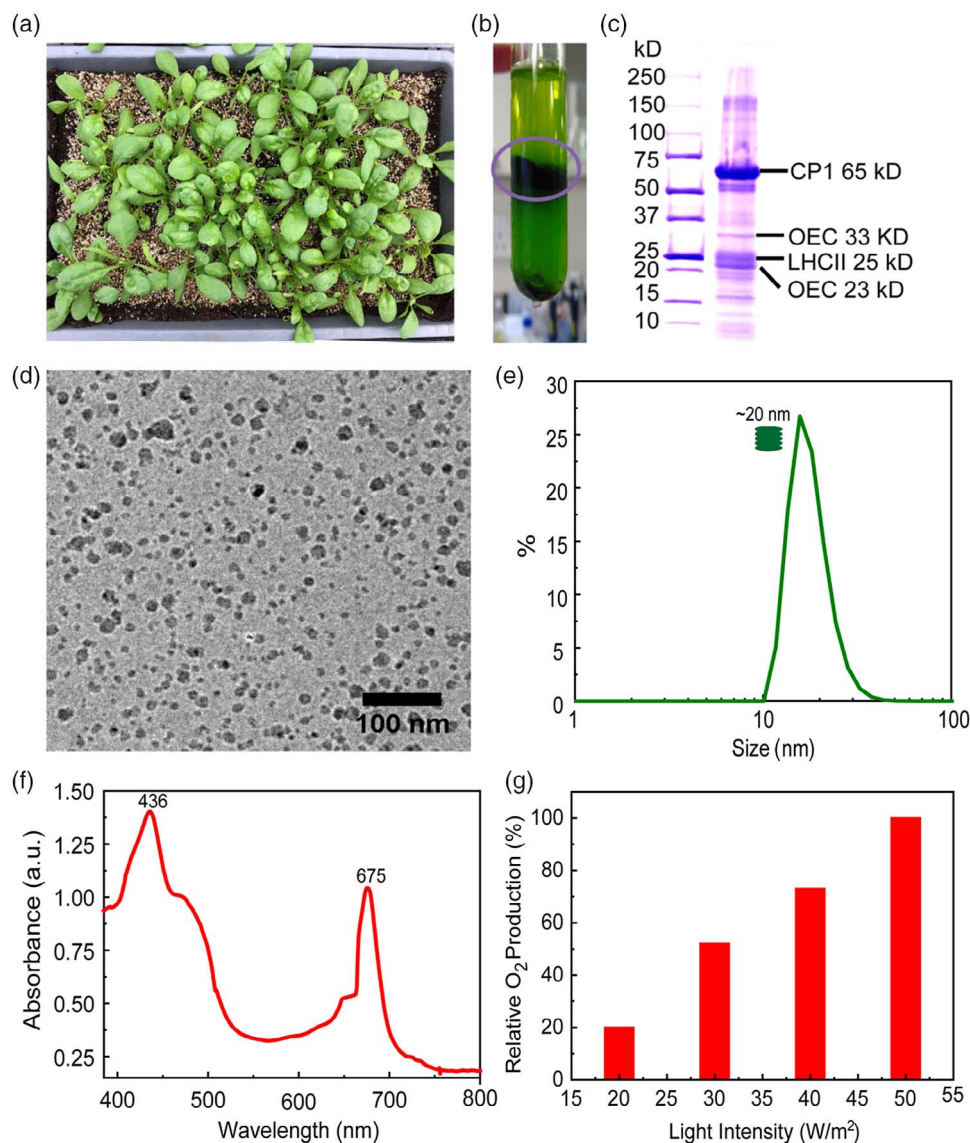


Figure 1. Extraction and characterization of TNP. Pictures of a) spinach plants grown for 3 weeks used for extraction, b) extracted TNP present in the upper percoll layer. c) SDS-PAGE for TNP showing bands for LHCII and OEC from PSII, d) cryo-TEM showcasing the spherical morphology of TNP of around 20 nm that was confirmed by e) DLS measurements. f) UV-vis spectroscopy showing strong absorbance in the visible region and presence of CAB proteins, g) oxygen evolution measurements for extracted TNP with different light intensities. Increase in light intensity showed an increased oxygen evolution rate.

(Figure 1f).^[31] These absorption peaks suggested intact LHC and OEC,^[32] showcasing its capability to conduct water-splitting reaction in the presence of light and water.^[29] To test TNP activity after isolation, the decline in the color of oxidized dichlorophenolindophenol (DCPIP) was measured over time. The thylakoid nanoparticles-containing active PS II (TNP) was observed to be active with a good oxygen evolution rate that increased with increase in light intensity (Figure 1g). Quantitatively, the rate of oxygen evolution increased from 600 to 2000 $\mu\text{mol mg chl}^{-1} \text{h}^{-1}$ for 20–50 W m^{-2} , respectively. It was also noteworthy that upon exposure to high light intensity, no oxygen evolution was observed, indicating photo-inhibition that was observed previously.^[33] After the isolation of TNP, synthetic bio-nanomotors were fabricated from the amphiphilic block copolymer PEG₄₄-b-PS₁₆₀ that was synthesized by following previously reported procedures and was characterized for purity and structural confirmation (Figure S1, Supporting Information). In brief, the diblock copolymer was dissolved in organic solvent (tetrahydrofuran (THF):dioxane)

and subsequently MilliQ water was added at a constant flow rate to form polymersomes. The polymersomes were shape transformed into bowl-shaped stomatocytes by dialysis^[34] and during this process the TNP was encapsulated, thus forming bio-nanomotors (Figure 2). In brief, during dialysis, an osmotic pressure is generated around the membrane of polymersomes that make it fold inward, hence undergoing shape transformation into stomatocytes during which particles in the vicinity are entrapped inside the stomach, thereby encapsulating them. To determine encapsulation efficiency, the bio-nanomotors were transformed back to polymersomes (Figure S2, Supporting Information) for releasing the encapsulated TNP. Inductively coupled plasma mass spectrometry (ICP-MS) was used to measure the magnesium ions content of TNP before and after encapsulation, indicating 33% encapsulation efficiency. A similar enzyme encapsulation efficiency was observed in our previous GO_x/catalase stomatocyte system fabricated by our group.^[10]

Once encapsulated, the structural morphology of both empty and bio-nanomotors was observed by TEM and cryo-TEM,

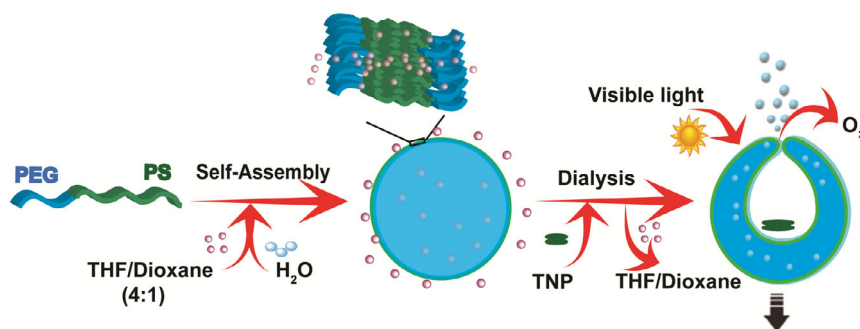


Figure 2. Schematic illustration for fabrication of TNP-encapsulated stomatocyte nanomotors (bio-nanomotors).

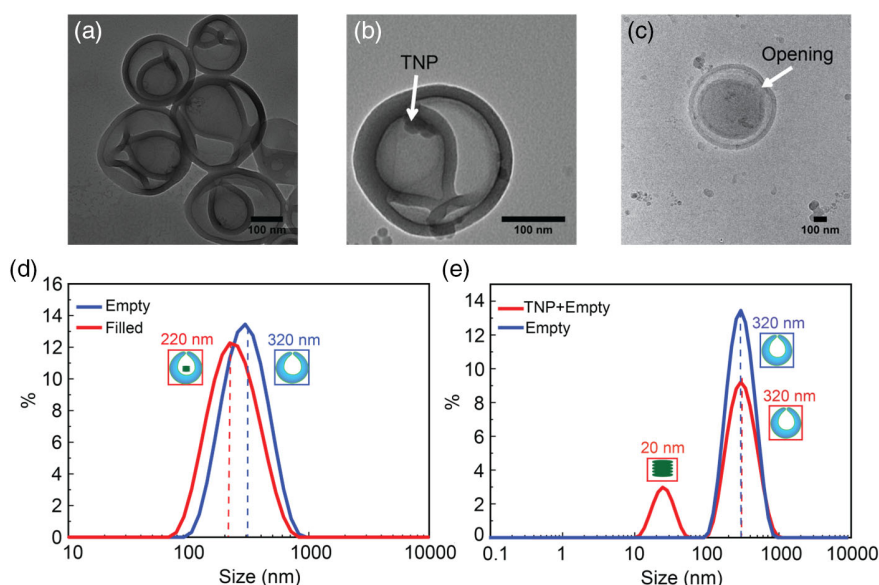


Figure 3. A comparison between empty and encapsulated (filled) stomatocytes. a,b) TEM and c) cryo-TEM images of empty and filled stomatocytes having a similar size of around 320 nm. d) DLS measurements for the mixture of free TNP and empty stomatocytes (red) and empty stomatocytes (blue) show no decrease in apparent size. e) DLS measurements for empty (blue) and filled (red) stomatocytes show a decrease in apparent size for encapsulated stomatocytes due to non-Brownian motion.

respectively (Figure 3a–c), that showed a similar average size of around 300 nm with a small opening. The size of empty stomatocytes correlated well with light-scattering techniques such as DLS (laser 633 nm) and nanoparticle tracking analysis (NTA, laser 642 nm). However, in comparison, the bio-nanomotors showed a decrease in apparent size (Figure 3d) due to the inversely proportional relation between the diffusion coefficient and apparent size of particles, as explained by Stokes–Einstein equation

$$D = \frac{TK_B}{3\pi\eta d} \quad (1)$$

where D is the particle diffusion coefficient, K_B the Boltzmann constant, η the viscosity, T the temperature, and d the hydrodynamic diameter. In our case, the bio-nanomotors diffusion coefficient increases due to non-Brownian motion caused by the water-splitting reaction in the presence of laser light equipped in both DLS and NTA, thus the observation of decreased apparent size. Further DLS measurements were carried out for two samples, one containing a mixture of TNP and stomatocytes and the other with empty stomatocytes (Figure 3e). There was no apparent change in size

that was observed when TNP was present in the vicinity of empty stomatocytes, thus asserting that encapsulation is necessary.

2.2. Motion Analysis of Bio-Nanomotors

Once characterized, the propelled motion of the bio-nanomotor was observed by calculating the average mean square displacement (MSD), following its trajectories in NTA, and the speed was extracted by the parabolic fit (Figure S3, Supporting Information) of the curve for MSD (r^2) versus time according to the following equation^[35]

$$r^2 = 4D\Delta t + (v\Delta t)^2 \quad (2)$$

Trajectories for empty and encapsulated stomatocytes (Video S1 and S2, Supporting Information, respectively) and MSD curves at 642 nm are shown in Figure S4–S6, Supporting Information, respectively. The encapsulated stomatocytes in the presence of the in-built 642 nm laser showed speed around $14.35 \pm 1.78 \mu\text{m s}^{-1}$. Once the propelled motion of bio-nanomotors was confirmed, an external light source (MAX-303 equipped from Asahi Spectra) comprising visible light

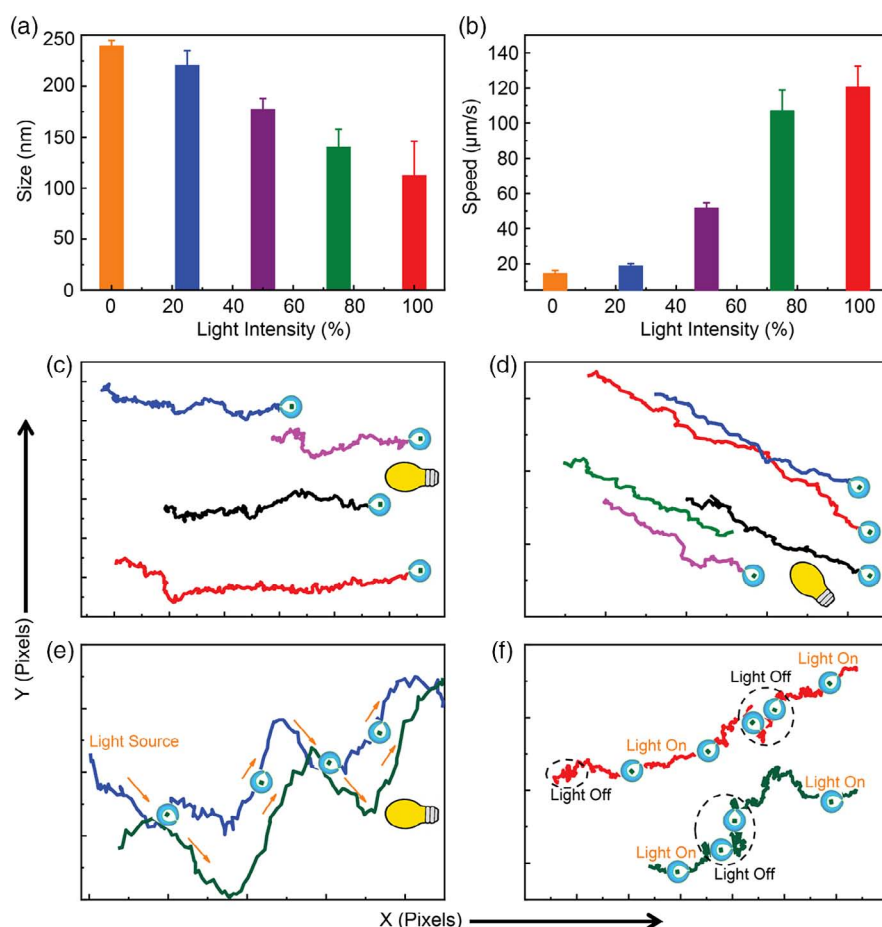


Figure 4. Maneuvering stomatocytes with complete control over speed, directionality, and motion. The light intensity is expressed in percent relative to maximum light intensity of 0.05 W cm^{-2} (100%). NTA analysis for a) size and b) speed versus intensity of light; a decrease in size and increase in speed were observed with increasing light intensity. Irradiating light from the external source from c) right, d) corner, e) fluctuating, and f) switching on/off light source. The stomatocytes were observed to move toward the light with reversible control over on/off motion.

(385–740 nm) was used for investigating its phototactic behavior with maximum light intensity of 0.05 W cm^{-2} (100%). From here on the light intensity is expressed in percent relative to maximum light intensity of 0.05 W cm^{-2} (100%). The initial light intensity of 25% from the external light source showed a small increase in MSD values. Upon further increasing the light intensity from 25% to 100%, a decrease in the size of bio-nanomotors was observed together with an increase in speed from 18.65 ± 1.49 to $120.42 \pm 12 \mu\text{m s}^{-1}$ (Figure 4a,b), with unidirectional motion toward the light source (Figure 4c–e, Video S3 and S4, Supporting Information). In the presence of high light intensity, there was no effective change observed in MSD values (Figure S7a, Supporting Information) of bio-nanomotors, suggesting that after certain extent of light intensity, there is no profound effect on the oxygen evolution of TNP. It was also observed that after one sample runs at a high light intensity, the MSD values of bio-nanomotors were similar to empty stomatocytes. This can be explained due to photoinhibition of the PSII in the presence of excess light that inactivates the electron transport and causes oxidative damage to the reaction center, resulting in no oxygen production.^[36] To establish that photophoresis is not the only working principle behind the propulsion, we conducted control experiments with empty stomatocytes that showed propelled motion but with lower MSD values compared with bio-nanomotors (Figure S7b, Supporting Information).

2.3. Possible Mechanisms of Propulsion

We believe, at a lower light intensity, the possible mechanism is bubble propulsion due to oxygen produced by the water-splitting reaction by encapsulated TNP, can escape through the opening, thus propelling bio-nanomotors. In our system, the oxygen produced is relatively less at the measured timeframe and also the observed trajectories were not perfectly linear, suggesting the motion to be not affected by flow or convection by oxygen production. Upon increasing light intensity, the propulsion mechanism shifts from bubble propulsion to the synergistic combination of bubble propulsion and photophoresis. Our stomatocyte system can be compared with that of polystyrene particles that are transparent.^[37] For transparent particles, the mechanism behind photophoresis relies on the momentum transfer between the light and the particle due to the refraction and reflection of the particle.^[25] In case of absorbing material, laser heats up the particle, resulting in convection of the medium due to surface temperature gradient that causes the photophoretic force.^[38,39] This is not possible in our case due to the absence of absorbing material, which supports the fact that convection or thermal effects are not the driving forces for our system. In case of transparent particles that have low absorptivity, the refraction is dominant and the smaller-size particles have a microlens effect, thus creating hot spots. In our system, the light is focused from one side of the chamber, thereby creating an illuminated side (exposed to light) and trailing side (not exposed to light). Previously, theoretical photophoresis studies for nonabsorbing particles have suggested that during refraction more energy is present in the trailing side of the particle, thereby causing the liquid near the trailing part to be locally heated up more than the liquid near leading part, thereby creating uneven energy

distribution, thus, causing the particle to move toward light.^[26] This indeed explains the movement of stomatocytes toward the light, thereby causing a negative photophoretic motion.

One may argue that motion observed may be due to the use of external light source that causes optical absorption within the liquid such as water in our case.^[40] This absorption can locally heat along the focal spot of the light source, thus building convective flow^[41] in the system, resulting in the collective movement of nanoparticles along the direction of flow. However, in our system, the chamber thickness is around $20 \mu\text{m}$ and broad laser focus dampens the effects of thermal convection,^[42] thus ruling out the possibility of particle drifting due to convective flow. In our system, higher speeds with increase in intensity of laser power were also observed that can be explained due to higher energy produced around the stomatocytes from higher refraction on the trailing side, together with increase in the oxygen production rate. The direction of bio-nanomotors was also observed to change instantly with the change in direction of light propagation in real time; to the best of our knowledge, this is the first report to showcase such a change at the nanoscale in real time (Video S5, Supporting Information). It can be envisioned that altering the direction of light propagation would directly affect the reflection and refraction from the particle, thus explaining the instant change in directionality of our system. Alongside, a complete control over the reversible on-demand on/off motion of the bio-nanomotor was also observed (Figure 4f, Video S6, Supporting Information) with their real-time sample trajectories shown in Figure S8, Supporting Information.

3. Conclusion

We demonstrate the fabrication of bio-nanomotors by supramolecular assembly of diblock copolymers encapsulated with active plant organelles (TNP). These systems conducted the water-splitting process to produce oxygen in nanoassemblies and thus powered the motors with complete control over speed, direction, and motion using visible light and water as fuel. We believe this system will shift the momentum for harnessing energy from natural processes to power nanosystems, thus creating a platform for smart and functional supramolecular nanomotors and facilitating powering motion in biocompatible environments.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

light, nanomotors, photophoresis, phototaxis, supramolecular chemistry

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