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## COMMUNICATION

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## Self-powered Kinesin-Microtubule System for Smart Cargo Delivery

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**A smart self-powered cargo delivery system that composed of creatine phosphate kinase (CPK) microspheres, kinesin and microtubule was demonstrated. The CPK microsphere was not only acted as an ATP generation and buffering system, but also as a carrier for cargo transport, thus realizing the easy loading and self-powered delivery of cargos at the same time.**

Over the past two decades, advances in modern biology and nanotechnology have allowed us to develop diverse nanodevices for various applications. One important development is biomotor-based (kinesins, myosins, dyneins) vehicles, which is expected to lead to devices with multi-functions and may totally change the way we monitor, diagnose, and cure diseases. With adenosine triphosphate (ATP) as 'fuel', these biomotors could effectively and directly convert chemical energy into mechanical energy and move directionally. Due to their small size, biocompatibility and remarkable performance, these biomotors have attracted great attentions and have been successfully applied in cargo transport,<sup>1-5</sup> nanostructuring,<sup>6, 7</sup> surface imaging,<sup>8, 9</sup> sorting of protein assemblies,<sup>10</sup> biosensor<sup>11, 12</sup> and optical devices.<sup>13</sup> In the building of these nanodevices, the energy supply of ATP is essential. Many researchers have tried to control ATP supplying by ultraviolet light,<sup>1, 14</sup> electric field<sup>15, 16</sup> or microfluidics.<sup>17</sup> However, ultraviolet light and electric field may cause damage to the activity of proteins; meanwhile they require additional equipment and cannot realize continuous supply of ATP. Though microfluidic technology could continuously supply ATP to the system, repeated replacement of ATP solution is required. For most system, one problem should also be addressed that the increased concentration of adenosine diphosphate (ADP) released during the motion of biomotor, has an inhibition effect on the activity of biomotor,<sup>18</sup> accompanying with decreased both the velocity and the run length.<sup>19</sup> Therefore, an ATP-regenerated system with the ability of supplying and buffering the concentration of ATP in artificial devices was needed.

Creatine phosphate kinase (CPK), also known as creatine kinase, is an enzyme found mainly in the brain, skeletal muscles, heart and other tissues. In the tissues and cells that consume ATP rapidly, especially skeletal muscle, CPK serves as an energy generator for

production of ATP in situ, as well as energy buffering.<sup>20</sup> The strong buffering ability of CPK is essential to keep intracellular ATP constant during contraction cycles. Based on this, CPK microspheres were fabricated through co-precipitation method and employed as an ATP generation and buffering system for biomotor kinesin, as shown in Fig. 1. Enzymatically induced 'fuel' production can be achieved in mild conditions and without the use of external fields. It is thus better suited for use in biomotor systems than most other ways at small scales. In view of the advantages of co-precipitation method, various cargos could be easily encapsulated into the microspheres, thus realizing the easy loading and self-powered delivery of cargos at the same time. This method present here would have great potential in intelligent self-powered cargo delivery system for performing challenging tasks.<sup>21</sup>

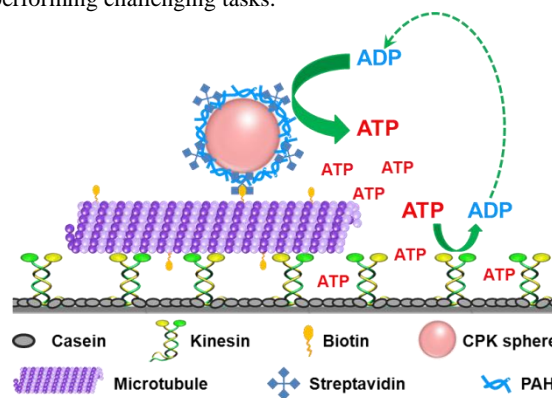


Fig. 1 Schematic illustration of self-powered MT-CPK complex ("train") gliding on kinesin-modified surface ("rail").

Spherical colloidal particles are found to be attractive candidates for cargo delivery applications as they are cost effective, easy to synthesize. Here, CPK microspheres for both energy generation and cargo delivery were prepared through one-step protein-CaCO<sub>3</sub> co-precipitation method.<sup>22, 23</sup> Briefly, 1 mL 0.33 M Na<sub>2</sub>CO<sub>3</sub> solution and 1 mL 4 mg mL<sup>-1</sup> CPK solution were first vigorously stirred in a round flask for 5 min. Then 1 mL 0.33 M CaCl<sub>2</sub> solution was added quickly to the above mixture with intense agitation on a magnetic stirrer (1200 rpm min<sup>-1</sup>). After 24 s, this system was allowed to stand for 2 min. The obtained microspheres were washed with deionized

water thoroughly for three times, and then incubated in 0.025 wt% glutaraldehyde (GA) solution for 2 h, followed by centrifugation and washing. Fig. 2a, 2b are scanning electron microscope (SEM) image and confocal laser scanning microscopy (CLSM) image of CPK microspheres excited at 559 nm. It is clear to see that the CPK microspheres were obtained with an average diameter of 3.6  $\mu\text{m}$  and displayed an intriguing autofluorescent property without conjugating to any external fluorochromes. This phenomenon can be attributed to the  $n-\pi^*$  transition of C–N bonds in the Schiff's base bonds formed during the crosslinking reaction between amino groups of CPK and aldehyde groups of GA.<sup>24, 25</sup> The autofluorescence of these CPK microspheres would facilitate in monitoring the motion of microtubule-CPK (MT-CPK) complex in the following gliding assay, avoiding the use of external fluorochromes.

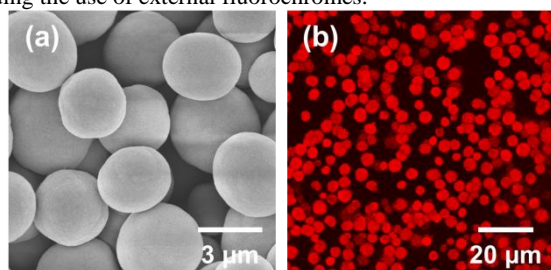


Fig.2 SEM image (a) and CLSM image (b) of CPK microspheres.

To confirm the generation of ATP from CPK microspheres, a bioluminescence luciferin- luciferase assay kit was used. Luciferin is rapidly oxidized by luciferase in the presence of ATP, producing light.<sup>26</sup> Since the luminescence intensity is proportional to the amount of ATP in the sample, we have first derived a standard ATP curve by plotting the luminescence intensity as a function of the ATP concentration. To analyse the ATP concentration, the CPK microspheres were first dispersed into BRB80 buffer supplement with ADP and creatine phosphate (CP). Then 5  $\mu\text{L}$  of the CPK microspheres dispersion (2.85  $\text{mg mL}^{-1}$ ) was added into a luminometer cuvette and mixed with 50  $\mu\text{L}$  of luciferin-luciferase reagent, immediately followed by registering the luminescence intensity on a recorder. ATP production within an 800 s reaction time was measured, as shown in Fig. 3a. It can be seen clearly that the concentration of ATP continuously increased with reaction time. The result proved that the CPK assembled in the microspheres retain their bioactivity and could catalyze the conversion of ADP and CP to ATP.

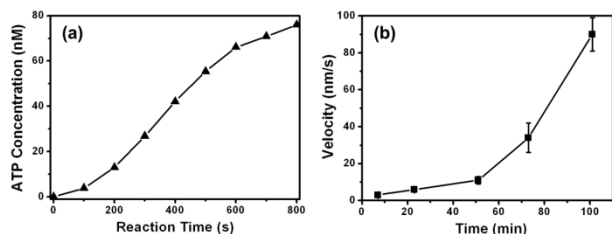


Fig.3 (a) ATP generated by CPK microspheres as a function of reaction time, (b) The velocity of MT gliding (without conjugating with microspheres) as a function of reaction time. Data represent mean values  $\pm$  standard error,  $n=5$ .

To further confirm the generation of ATP from CPK microspheres, the *in vitro* gliding assay of microtubules were conducted. The MT suspension containing CPK microspheres, ADP, CP and oxygen

scavenger was flowed into the kinesin coated chamber, and the time-lapse images of microtubules gliding were taken by CLSM. It can be seen from Fig. 3b (also see supplementary data, Mov. S1-S5) that the time-average velocity of the MT was increase exponentially with time, from  $3 \pm 1 \text{ nm s}^{-1}$  to  $90 \pm 10 \text{ nm s}^{-1}$ . This result further demonstrated that ATP was continuously generated by catalysis of the CPK microspheres, and the concentration of ATP in the chamber was increased with time. It should be mentioned that in the presence of a fixed concentration of ATP, the velocity of MT gliding was reported to be reduced exponentially due to gradual consumption of ATP and increased production of ADP.<sup>19, 27</sup> Here, our self-powered system successfully got over this problem and could maintain the ATP concentration on a nearly constant level, which maintaining the velocity of MT gliding on a constant value at equilibrium (the average velocity of MT gliding is  $92 \pm 7 \text{ nm s}^{-1}$  from 100 min to 260 min in the observation time), further confirming the CPK microspheres' ability of ATP buffering. Compared with CPK solutions, the CPK microspheres could carry chemically active zones which convert species from their surroundings,<sup>28</sup> thus they may produce concentration gradients without any extra force. For verification, the velocity of MT gliding versus different distances between MT and CPK microspheres was measured. It can be seen from Fig. S1 that the velocity of MT gliding is distance-dependent that the gliding velocity decreased with the increased distance between MT and CPK microspheres, especially obvious when over 3000  $\mu\text{m}$ . It demonstrated that the CPK microsphere may have great potential in local control of biomotors.<sup>29</sup>

For cargo delivery, glucose oxidase (GOD) loaded, fluorescent isothiocyanate-dextran (FITC-dextran, 20 KDa) loaded and doxorubicin (DOX) loaded CPK microspheres (Fig. S2-S3) were fabricated through the same procedure as those of pure CPK microspheres, respectively. In order to achieve controlled release of the cargos, layer-by-layer (LbL) assembly of CPK and GA on the cargo loaded microspheres were conducted, and (FITC-dextran-CPK)/(GA/CPK)<sub>4</sub> microspheres was obtained. Fig. S4 showed that the release of FITC-dextran exhibited pH-responsibility. This phenomenon was mainly due to the faster hydrolysis of Schiff's base bond (formed between CPK and GA) at acid conditions, which makes the crosslinked multilayer structure loosen and allows FITC-dextran to diffuse through the shells at pH 4.5.<sup>24, 30</sup> In consideration of the versatility of LbL assembly, various functional or responsive components could be easily assembled on the surface of CPK microspheres, thus rendering flexible, controllable release of cargos in a smart system.

For fabrication of self-powered kinesin-microtubule cargo delivery system, the streptavidin-biotin system is applied to link cargo-loaded CPK microspheres with microtubules.<sup>3</sup> GOD-loaded CPK microspheres were first modified with a layer of positive charged poly(allylamine hydrochloride) (PAH), and then incubated with negative charged streptavidin (SA) to form a stable complex. The GOD-CPK/PAH/SA complex microsphere was subsequently linked to the biotinylated microtubule through the strong specific interaction between streptavidin and biotin. The obtained GOD-loaded CPK-MT complex was then applied to the *in vitro* motility assay. The time-lapse images in Fig. 4 (see also Supplementary Data, Mov. S6) showed that the GOD-loaded CPK-MT complex

successfully moved on kinesin-modified surface in the presence of ADP and CP. The average velocity of the GOD-loaded CPK-MT complex is  $62 \text{ nm s}^{-1}$ . The result demonstrated that the CPK microsphere coupled to MT here could successfully act both as a 'fuel' generation system for kinesin-MT movement and as a carrier for cargo delivery. This makes it extremely attractive toward the design of self-powered biomotor-based hybrid nano- and micro-devices for intelligent cargo delivery.

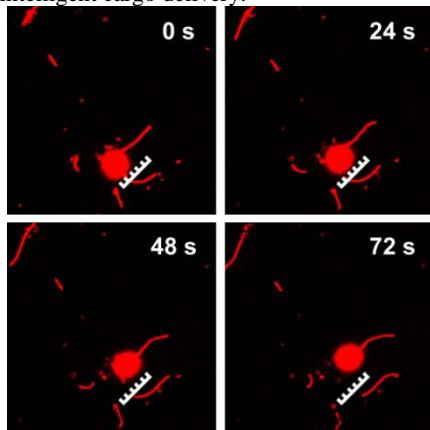


Fig.4 CLSM images of GOD-loaded CPK-MT complex gliding on kinesin-modified surface at different time using self-supplying ATP. The scale bars represent  $5 \mu\text{m}$ .

## Conclusions

We have successfully prepared a smart self-powered cargo delivery system with CPK microsphere, kinesin and microtubule. The CPK microsphere fabricated through co-precipitation method was employed both as a 'fuel' generation, buffering system for kinesin-MT movement and as a carrier for cargo delivery. CPK catalyzed 'fuel' production can be achieved in mild conditions and without the use of external fields. It is thus better suited for use in biomotor systems than most other ways at small scales. Compared with CPK solutions, the CPK microspheres are able to produce concentration gradients without any external forces; therefore they may have great potential in local control of biomotors. Considering the advantages of co-precipitation method, various cargos were easily encapsulated into the microspheres, thus realizing the easy loading and self-powered delivery of cargos at the same time. In view of the versatility of LbL assembly, various functional or responsive components could be easily assembled on the surface of CPK microspheres, thus rendering flexible, controllable release of cargos in a smart system. The method present here would pave the way to develop self-powered intelligent machines for cargo delivery, materials separation and certain biomedical applications.

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## Notes and references

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