

Chromatophores efficiently promote light-driven ATP synthesis and DNA transcription inside hybrid multicompartment artificial cells

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The construction of energetically autonomous artificial protocells is one of the most ambitious goals in bottom-up synthetic biology. Here, we show an efficient manner to build adenosine 5'-triphosphate (ATP) synthesizing hybrid multicompartment protocells. Bacterial chromatophores from *Rhodospirillum rubrum* accomplish the photophosphorylation of adenosine 5'-diphosphate (ADP) to ATP, functioning as nanosized photosynthetic organelles when encapsulated inside artificial giant phospholipid vesicles (ATP production rate up to ~ 100 ATP \cdot s⁻¹ per ATP synthase). The chromatophore morphology and the orientation of the photophosphorylation proteins were characterized by cryo-electron microscopy (cryo-EM) and time-resolved spectroscopy. The freshly synthesized ATP has been employed for sustaining the transcription of a DNA gene, following the RNA biosynthesis inside individual vesicles by confocal microscopy. The hybrid multicompartment approach here proposed is very promising for the construction of full-fledged artificial protocells because it relies on easy-to-obtain and ready-to-use chromatophores, paving the way for artificial simplified-autotroph protocells (ASAPs).

artificial protocells | artificial photosynthesis | bacterial chromatophores | light transduction | synthetic biology

Bottom-up synthetic biology foresees the construction of artificial protocells as the main ambitious goal (1–6). Following the pioneer phase (7–10), current challenges include the implementation of more complex behavior, such as primitive compartment self-assembly (11), chemical signaling (12–15), population-level dynamics (16, 17), self-reproduction (18–21), and autonomous generation of energy (22–24).

In cellular biochemistry, many anabolic reactions require fueling, and the energy is often provided by adenosine 5'-triphosphate (ATP). The latter is generated *in vivo* by 1) photosynthetic phosphorylation, 2) oxidative phosphorylation, and 3) substrate-level phosphorylation. Photosynthetic and oxidative phosphorylations share a common design that requires the establishment of a proton gradient and a difference of chemical potential across the lipid membranes of cells which, ultimately, powers the membrane-bound enzymatic complex ATP synthase (ATPsyn). In photosynthesis, the gradients are generated by the absorption of light, the energy of which needs to be transduced into that of reduced chemical species by a rather complex dedicated enzymatic apparatus.

Photosynthetic anoxygenic bacteria are ancient photosynthetic organisms that appeared on Earth long before any oxygenic species and possess a relatively simple apparatus for proton translocation. The purple nonsulfur bacterium *Rhodospirillum rubrum* contains a minimal set of well-characterized integral membrane enzymes: the reaction center (RC), the ubiquinol:cytochrome *c* oxidoreductase (bc1), and the ATPsyn. The first two complexes are engaged in a

photo-induced cyclic electron transport and transmembrane proton translocation involving coenzyme Q (Q/QH₂) and cytochrome *c*₂ (cyt^b₂/cyt^b₂) redox pools. ATPsyn eventually converts the energy of the proton gradient into that of ATP molecules, via adenosine 5'-diphosphate (ADP) phosphorylation (Fig. 1A) (25–27).

The RC reconstitution in lipid vesicles for light-driven proton pumping dates back to the 1980s (28, 29). To exploit the ability of *R. rubrum* chromatophores to produce ATP inside artificial protocells upon light irradiation, we have recently started a systematic investigation about these sorts of systems that we call photoactive "artificial simplified-autotroph protocells" (ASAPs) (see *SI Appendix, Text S1* for the used terminology). We have previously developed a single-compartment approach, consisting of giant

Significance

Chromatophores are closed vesicles extracted from photosynthetic bacteria that efficiently perform the photophosphorylation reaction (ADP + Pi → ATP) under illumination. Here we show that, when entrapped inside giant lipid vesicles, they behave as nano-biophotosynthetic organelles, allowing the construction of energetically autonomous artificial protocells. As proof of concept, DNA has been transcribed within an artificial protocell, thanks to the continuous ATP photo-production from chromatophores. Such a hybrid multicompartment approach will speed up the current efforts of constructing cell-like systems of increasing complexity, allowing the implementation of several ATP-dependent processes. Developed at the interface between synthetic biology and systems chemistry, the science of artificial protocells promises unprecedented biotechnological applications, as well as unveiling still unsolved origins-of-life questions.

Author contributions: E.A., P.S., and F. Mavelli designed research; E.A. and P.A. performed research; M.F. contributed new reagents/analytic tools; E.A., P.A., R.M., F. Milano, M.T., P.S., and F. Mavelli analyzed data; R.M. performed the cryo-electron microscopy and cryo-electron tomography analysis; F. Milano performed charge recombination kinetic experiments; M.T. supported *Rhodospirillum rubrum* cultivation; and E.A., P.S., and F. Mavelli wrote the paper.

The authors declare no competing interest.

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Microencapsulated Chromatophores For The Production Of Atp

Rachel Sandford



Microencapsulated Chromatophores For The Production Of Atp:

This book delves into Microencapsulated Chromatophores For The Production Of Atp. Microencapsulated Chromatophores For The Production Of Atp is a crucial topic that needs to be grasped by everyone, from students and scholars to the general public. This book will furnish comprehensive and in-depth insights into Microencapsulated Chromatophores For The Production Of Atp, encompassing both the fundamentals and more intricate discussions.

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