

# 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  $\sim 100$  ATP  $\cdot$  s<sup>-1</sup> 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/QH<sub>2</sub>) and cytochrome *c*<sub>2</sub> (cyt<sup>b</sup>/*c*<sub>2</sub>) 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* 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

**J. Tampion, M. D. Tampion**



## **Microencapsulated Chromatophores For The Production Of Atp:**

**Biocatalysis**, 1988      *Immobilized Cells and Organelles* Mattiasson, 2018-01-18 Cells and organelles are small units for biochemical synthetic purposes often the smallest practically feasible unit since they contain coenzyme regenerating system ordered enzyme sequences etc These volumes besides giving some insight into basic technology immobilisation procedures etc also sum up the current know how in this subject area and try to predict some future trends The term immobilized cells covers everything from dead cells with a single active enzyme species to cells proliferating on or within a three dimensional polymer matrix The practical handling of these structures make them useful in various applications e g large scale production of biomolecules biodegradation analysis etc      *Immobilization of Cells* Colin R. Phillips, Yiu Cheong Poon, 2012-12-06 Growth of immobilized cells can be viewed as an alternative to growth of free cells in many instances In others immobilization confers a precision of control over the process not possible in free growth Immobilization of cells can sometimes be considered to be a lower cost alternative to immobilization of enzymes In this volume immobilization procedures based on mechanical means and bonding of various types are examined with detailed application examples These applications include microorganisms plant and animal cells sub cellular organelles and multiple enzyme systems Particular attention is devoted to enzyme properties in immobilized cells and the properties of the carrier The volume should provide the reader with a comprehensive overview of the subject together with copious references As well as serving as a research monograph it could be used to provide reference material for a graduate course Special thanks are due Mrs JENNIFER KERBY for her dedicated work in the preparation of the manuscript and IT CHIN HSIEH for bibliographical assistance COLIN R PHILLIPS Toronto July 1988 YIU C POON v Table of Contents 1 Introduction 1 References 9 2 Methods of Cell Immobilization 11 2 1 Mechanical Immobilization 11 2 1 1 Mycelial Pellet and Mat 11 2 1 2 Encapsulation 48 2 1 3 Dialysis Culture 49 2 1 4 Entrapment 50 2 2 Covalent Attachment 61 2 3 Ionic Attachment 62 2 3 1 Flocculation 62 2 3 2 Adsorption 64 References 66 3 Special Problems and Extended Applications 75 3 1 Special Problems and Techniques      **Immobilized Cells** J. Tampion, M. D. Tampion, 1987-12-10 This 1987 book gives a coherent overview of preparation and uses of immobilized enzymes      Bibliography of Agriculture, 1975      **Cell-free synthetic biology, volume II** Jian Li, Yong-Chan Kwon, Yuan Lu, Simon J. Moore, 2023-06-21      Cumulated Index Medicus, 1981      Photosynthetic Regeneration of ATP Using Native and Immobilized Bacterial Chromatophores Ho Seung Yang, 1976

## **Microencapsulated Chromatophores For The Production Of Atp** Book Review: Unveiling the Magic of Language

In an electronic era where connections and knowledge reign supreme, the enchanting power of language has become more apparent than ever. Its capability to stir emotions, provoke thought, and instigate transformation is really remarkable. This extraordinary book, aptly titled "**Microencapsulated Chromatophores For The Production Of Atp**," published by a very acclaimed author, immerses readers in a captivating exploration of the significance of language and its profound impact on our existence. Throughout this critique, we shall delve into the book's central themes, evaluate its unique writing style, and assess its overall influence on its readership.

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