

# 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>/cyt<sup>c</sup>) 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 reagent/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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web 2020 21 academic school calendar january amendment 2020 21 academic calendar january amendment accessible  
version 2019 20 academic school calendar 2018 19

*fresno unified school district calendar 2023 and 2024* - Feb 26 2022

web aug 14 2023 school holidays starts finishes first day of school 14 aug 2023 mon thanksgiving break 20 nov 2023 mon  
24 nov 2023 fri christmas break 18 dec 2023 mon 5 jan 2024 fri spring break 25 mar 2024 mon 1 apr 2024 mon last day of  
school 6 jun 2024 thu summer break 7 jun 2024 fri

**summer school 2023 gaston middle school fresno unified school** - Jul 02 2022

web summer bus schedule gaston summer bus routesdownload gaston middle school phone 559 457 3400 email gastonms  
fresnounified org 1100 e church ave fresno ca 93706

**summer school 202 1 faqs fresno unified school district** - Mar 10 2023

web jan 11 2021 general what are the summer school program dates for 2021 the elementary middle comprehensive high  
school and special education summer programs include 2 sessions the dates are session 1 is june 14 2021 june 28 2021  
session 2 is june 29 2021 july 14 2021 the alternative ed high school summer program

[summer programs summer school fresno unified extended](#) - Jul 14 2023

web there are three distinctive extended learning summer programs offered within fresno unified summer academy grades k  
8 summer enrichment camps credit recovery grades 9 12

**2023 24 academic school calendar fresno unified school district** - Oct 05 2022

web independence day july 4 labor day sept 4 veterans day nov 10 thanksgiving nov 20 24 winter break dec 18 jan 5 m l k  
jr day jan 15 lincoln s birthday feb 12 presidents day feb 19 spring break march 25 apr 1 memorial day may 27 independence

day july 4 2024 holidays for classified personnel

fresno unified school district - Dec 27 2021

web fresno unified school district academic calendar for the 2022 2023 school year board adopted on february 2 2022 april 10 2023 spring break school not in session may 2023 monday may 29 2023 memorial day holiday june 2023 thursday june 8 2023 last day of school july 2023 tuesday july 4 2023 independence day

**calendars about us central unified school district - Aug 03 2022**

web elementary sports schedule illuminate student login paper 24 7 tutoring service central unified school district fresno ca 93722 4605 n polk ave fresno ca 93722 559 274 4700 559 271 8200 email protected normal business hours 8 00 am to 4 30 pm district office 559 274 4700 transportation 559 275 9734 operations 559

additional service pay schedule national council on - Apr 30 2022

web for regular summer school starting with the 2014 2015 school year and subsequent years teacher summer school salaries are based on an hourly rate of 31 88 for the number of student days plus two 2 days to be used for faculty meetings preparation time and student registration 1 2 supplemental compensation for district assigned