Reaction coupling in ADH1A alcohol dehydrogenase enzyme by exciplex formation with adenosine diphosphate moderated by low-energy electronic excited states

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Two commonly accepted theories about enzymes were revisited. The first states that adenosine triphosphate (ATP)–stored energy is only released when the substrate is in place, because the substrate changes the enzyme structure when it is bound to the enzyme. In fact, as demonstrated and discussed presently, no structural changes are required, and ATP-stored energy is released when it can be used. The second states that ATP-released energy moves along the enzyme molecule in the form of molecular vibrations (Davydov's vibrational solitons). In fact, as reported presently, energy released upon ATP hydrolysis moves in the form of excited-state electrons (excitons), with no molecular vibrations involved. The relevant experimental evidence was obtained for the human ADH1A alcohol dehydrogenase enzyme. Spontaneous ATP hydrolysis in the absence of substrate was apparently prevented by electronically excited enzyme + adenosine diphosphate (ADP) + inorganic phosphate (P) complex (exciplex) formed upon ATP hydrolysis. This exciplex kept ADP + P bound and in place for the inverse reaction, until the excess energy was dissipated in the enzyme-catalyzed reaction or by energy transfer to a suitable acceptor. Additionally, and contrary to textbooks, ADH1A has required ATP, working orders of magnitude faster in its presence.

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I. INTRODUCTION

A possible role of quantum phenomena in biology was discussed by Davies [1], including energy transport in live systems. Recently protein-based intermediate filaments approximately 12 nm in diameter extracted from porcine retina transported energy as electronic excited states (excitons) over 100 μm distances without losses [2,3], which led to a hypothesis that excitons may be acting in many other protein-based systems, including enzymes. On the other hand, excitons propagate with low losses over macroscopic distances in very thin metal films, around 10 nm in thickness [4–6]. These results inspired the current experimental design, where the enzymatic hydrolysis of adenosine triphosphate (ATP) took place in one cell compartment, and the substrate reaction in another compartment. The enzyme molecules were chemically attached to the common bottom plate of both compartments, which were interconnected by a thin Co metal film, embedded into the bottom plate, and exchanging energy between the compartments in the form of electronic excited states (excitons).

The enzyme chosen for this study was human ADH1A alcohol dehydrogenase, for the simplicity of the chemical reaction it accomplishes—oxidation of alcohol to the respective aldehyde. Enzymatic oxidation of ethanol by ADH1A has been studied earlier quite extensively [7–12]. It was found that oxidation requires the presence of nicotinamide adenine dinucleotide (NAD^+), and proceeds in absence of the adenosine triphosphate (ATP), with turnover numbers of about 40 min^{-1} [7–9]. Apparently, the role of ATP in the functioning of alcohol dehydrogenases has been overlooked altogether, whereas in fact these enzymes function much faster with ATP available, as reported here. Acetaldehyde is the primary product of this enzymatic reaction [7–10]. The ADH1A-NAD^+ complex also absorbs ultraviolet light, with the tail of the absorption band extending to 400 nm [11,12].

Davydov [13] has proposed that energy within enzyme molecules moves from the ATP hydrolysis site to the substrate reaction site in the form of molecular vibrations (vibrational solitons). This hypothesis has difficulties in explaining the apparent lack of energy losses in enzymes, as vibrational energy should readily dissipate from the enzyme backbone to hydrogen-bonded water molecules, which have matching vibrational frequencies. Moreover, no such solitons were ever observed experimentally in proteins. On the other hand, several differing mechanisms were proposed for coupling of the ATP hydrolysis to the substrate reaction [14–16]. However, these mechanisms are all debatable, as they were deduced based on thermodynamic-equilibrium steady-state experiments, while real enzymatic reactions occur at high excess energies supplied by ATP hydrolysis, and are therefore nonequilibrium in their very nature. Note that detailed exploration of the enzymatic reaction mechanism of ADH1A in presence of ATP was not the task of the present study, focused on the reaction coupling mechanism.

The presently proposed mechanism that ensures efficient coupling of the ATP hydrolysis with the enzymatic reaction (ethanol oxidation by ADH1H) involves energy conservation within the ⟨ADH1A-NAD^+-ADP-P⟩* excited-state complex (exciplex), with energy transfer from the exciplex to the alcohol oxidation site moderated by electronic excited states

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(excitons). Indeed, the presently reported experiments show that ADH1A-NAD^+ complexes can donate and receive energy in the form of excitons. It is also reported that once the exciplex could donate its extra energy to the Co metal film, ATP hydrolysis proceeded even without the presence of the substrate (ethanol), confirming the energy conservation requirement for the exciplex. Similarly, the presence of ATP bound to ADH1A was not required for the ethanol oxidation to proceed, once the necessary energy was supplied in the form of excitons. Note that the exciplex could not lose its exciton to the surrounding small molecules, and only large enzyme-sized molecules have low-energy electronic excitations compatible with the energy released upon ATP hydrolysis (30.7 kJ/mol), as explained below. Spectroscopic evidence for the exciplex formation may be elusive, as also explained below. On the other hand, there is abundant x-ray structural evidence showing that an enzyme bound to a nonreactive ATP-model molecule adopts a structure different from that of the free enzyme [14]. Until now, it was believed that such structural changes couple the two reactions, by affecting both the substrate and the ATP. However, as demonstrated presently, it is the energy conservation in the exciplex that couples ATP hydrolysis to the substrate reaction, while any structural changes are not conditioning the chemical reactions, being only indicative of the exciplex formation. We thus argue that the exciplex structure should be very similar to that of the enzyme-ATP complex, as the fast formation of the very short-lived $\langle$ADH1A-NAD^+-ADP-P$\rangle^*$ exciplex cannot depend on structural changes in the large and heavy enzyme molecule.

II. MATERIALS AND METHODS

A. Materials and samples

Aqueous human alcohol dehydrogenase (ADH1A) at 46.7 mg/ml mixed with NAD^+ coenzyme at 25 mg/ml, and NAD^+ solution at 25 mg/ml, all from Sigma-Aldrich Inc., were used as received. Ethanol, acetaldehyde, monosodium salt of ATP trihydrate (ATP; MW = 563) and monosodium salt of ADP trihydrate (ADP; MW = 483) were also used as received from Sigma-Aldrich. Before attaching ADH1A to the solid substrate, 11.5-nm-thick Co metal layer and 10.3-nm-thick BN dielectric layer functionalized with hydroxyl groups were sequentially deposited on transparent fluorite (CaF_2) substrates sized $25.0 \times 12.5 \times 1.0\,mm^3$ [3]. Next, Sigma-Aldrich attached ADH1A at $12.17\,\mu g/cm^2$ to the BN layer, and the resulting CaF_2 substrate with the Co-BN-ADH1A trilayer sandwich structure was mounted as the bottom plate of the two-compartment cell, ADH1A layer facing the solution, with each of the two compartments sized $12 \times 12 \times 15\,mm^3$, and taking 1.5 ml of sample volume, as shown in Fig. 1.

B. Optical measurements

Photoinduced enzymatic response measurements were performed using a high-pressure Xe lamp (1000 W, Ariel Corporation, Model 66023), a monochromator (Thermo Jarrell Ash, Mono Spec/50), a DET10A Biased Si detector (THORLABS), a model 2182A nanovoltmeter (Keithley Instruments) connected to a computer by a GPIB interface, and home-made software running in the LabView programming environment (National Instruments). The light beam of the Xe lamp passed through the monochromator, to isolate light at 519 nm wavelength, used to excite the 11.5-nm Co nanolayer, or light at 290 nm wavelength used to excite the ADH1A-NAD^+ complex in solution.

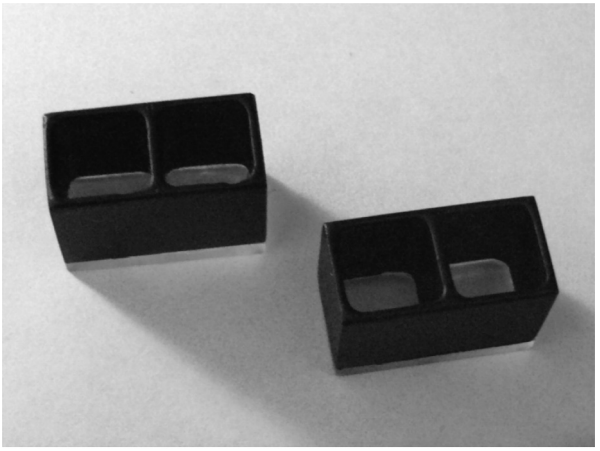

FIG. 1. Two-compartment cell with Co-BN-ADH1A sandwich structure on optically transparent fluorite (CaF_2) substrate as its bottom plate. The bottom of each compartment was covered with ADH1A molecules, and a continuous Co metal film protected by BN dielectric linked the two compartments, providing for the energy exchange between them, in the form of excitons.

C. Concentration measurements by HPLC

Enzymatic oxidation of ethanol (EtOH) to acetaldehyde (AA) was monitored by HPLC (Agilent 1260 Series HPLC System 3, from LabX Inc.), and two different columns were used to quantify the reagents and the products. One column (PRP-C18, Hamilton Inc.) with $1\,cm^3$ volume was used to quantify EtOH and AA, with the elution times 2 min for AA and 6 min for EtOH. The second column [iHILIC®-Fusion(P) HILIC Column, PEEK, 100 × 2.1 mm, 5 μm, 200 Å, Helicon Inc.] with $1\,cm^3$ volume was used to quantify ATP and ADP, with elution times 3 min for ADP and 7 min for ATP. Both columns were stabilized at 300 K. Bidistilled-deionized water was used as carrier fluid, its flow rate varied at $0.2–2.0\,cm^3$/min to optimize the measurements.

D. IR emission spectra

An optical system from a commercial PF2000 FTIR spectrometer (Perkin Elmer) was used to record IR emission spectra. Pulsed laser excitation at 290 nm was provided by a dye laser. An LN_2-cooled photodetector (Kolmar Technology) was used to detect the emission, and the signal was recorded by a digital oscilloscope (LeCroy, Wavesurfer-400). A CaF_2 optical cell sized $2.0 \times 2.0\,cm^2$ with 0.10 mm optical path length contained the sample solution.

E. Experimental concentrations

The concentrations used in the experiments for each of the reagents are listed in Table I.

TABLE I. Reagent concentrations used in the experiments.

Reagent	Concentration
Ethanol	0.217 mol/l
ADH1A	either deposited on the bottom plate at 12.17 μg/cm^2 or in suspension at 4.67 mg/ml
ATP	1.5 mg/ml
NAD$^+$	2.5 mg/ml

III. RESULTS AND DISCUSSION

Exciton involvement was explored in ADH1A human alcohol dehydrogenase enzyme, which has been studied quite well [7–12]. A nanofilm of Co metal was used as the medium transmitting excitons over centimeter distances with low losses [4–6]. The Co film thickness was chosen so as to obtain the smallest exciton energy of 30.7 kJ/mol, equal to the energy liberated upon ATP hydrolysis. The schematic diagram of the experimental cell is shown in Fig. 2(a).

The left compartment of the cell was filled with ATP + NAD$^+$ solution, the right compartment with EtOH + NAD$^+$ solution, and the cell maintained at 36.6 °C. The reactions that occurred in the left and right cells may be described by

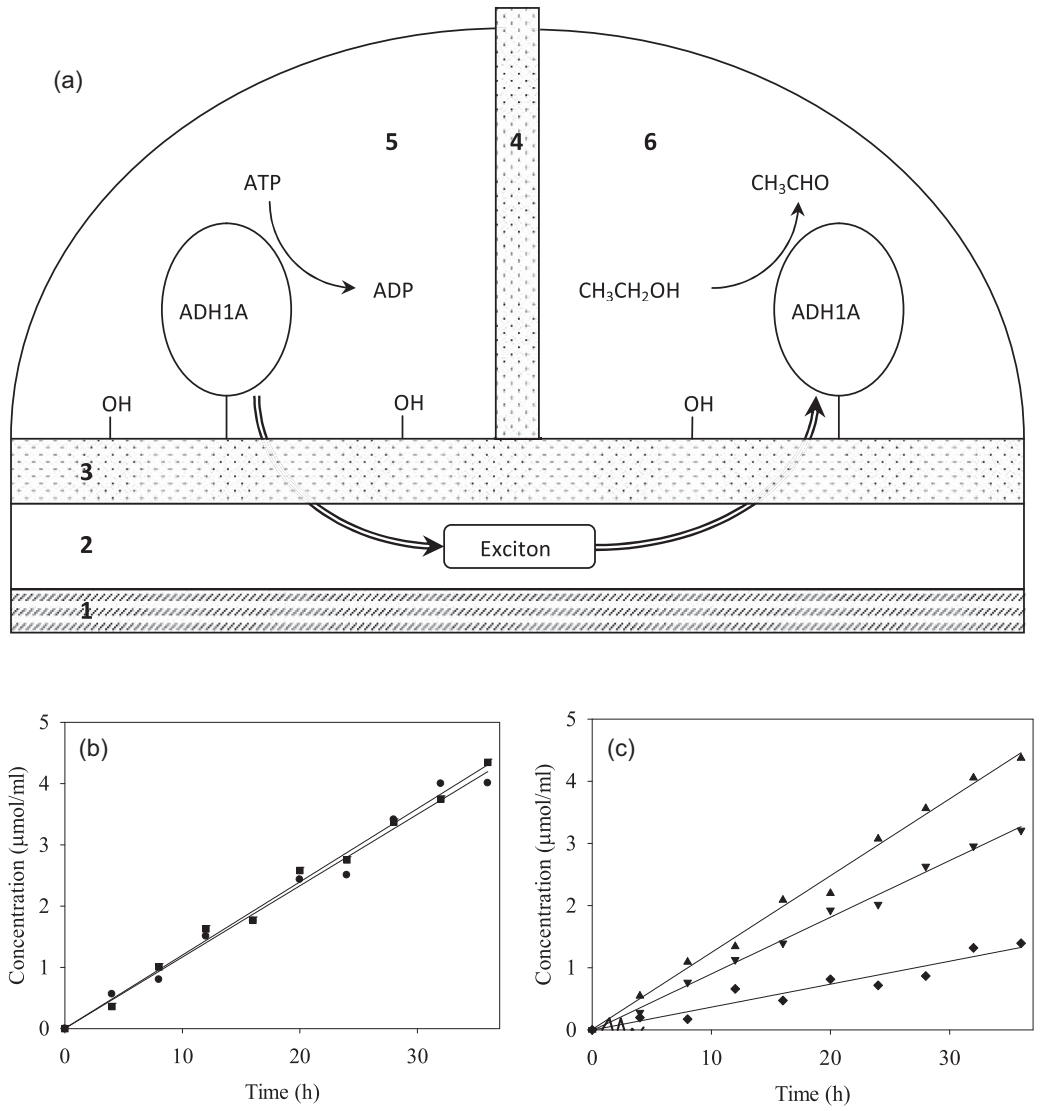

FIG. 2. Oxidation of EtOH by ADH1A, enabled by exciton transfer between adjacent cell compartments. (a) 1: CaF_2 substrate 1.0 mm thick; 2: Co metal film 11.5 nm thick; 3: BN insulator film 10.3 nm thick; 4: central separation wall of the two-compartment cell; 5: ATP + NAD$^+$ solution; 6: EtOH + NAD$^+$ solution. Excitons were produced upon hydrolysis of ATP at the left side, transmitted via the Co film to the right side, and drove oxidation of EtOH there. (b) Formation rates of ADP (circles) and AA (squares) in the single-compartment experiment (experiment 3 of Table II). (c) Formation rates of ADP and AA in double-compartment experiments. Experiment 1: triangles up, ADP; triangles down, AA. Experiment 2: diamonds, ADP (no alcohol present). Lines in (b) and (c) show the linear fits that were used to estimate the turnover numbers. EtOH oxidation in absence of ATP in the baseline experiment was much slower (experiment 4 of Table II, results not plotted). The experiments lasted 36 h and the reagents and products were monitored by HPLC.

TABLE II. Turnover rates per ADH1A molecule for acetaldehyde (AA) formation by ethanol (EtOH) oxidation and simultaneous ADP formation by ATP hydrolysis in double-compartment experiments 1 and 2, and single-compartment control experiment 3 (only the left compartment was used). Experiment 4 is the baseline experiment—oxidation of alcohol without ATP. The ADH1A enzyme was immobilized at the bottom plate of the double-compartment cell.

Experiment number	Left compartment contained in solution	Right compartment contained in solution	ADP formation turnover, min^{-1}	AA formation turnover, min^{-1}	Type of experiment
1	$ATP + NAD^+$	$EtOH + NAD^+$	3000 ± 150(0.1243 $\pm 0.0021\ \mu mol\ ml^{-1}\ h^{-1}$)	2200 ± 150(0.0912 $\pm 0.0021\ \mu mol\ ml^{-1}\ h^{-1}$)	double compartment
2	$ATP + NAD^+$	NAD^+	870 ± 120(0.0361 $\pm 0.0054\ \mu mol\ ml^{-1}\ h^{-1}$)		double compartment
3	$ATP + NAD^+ + EtOH$		2800 ± 200(0.1162 $\pm 0.0081\ \mu mol\ ml^{-1}\ h^{-1}$)	2900 ± 200(0.1202 $\pm 0.0081\ \mu mol\ ml^{-1}\ h^{-1}$)	single compartment
4	$NAD^+ + EtOH$			200 ± 15(0.0082 $\pm 0.0007\ \mu mol\ ml^{-1}\ h^{-1}$)	single compartment

Eqs. (1) and (2), respectively:

$$\text{ADH1A-NAD}^+\text{-ATP+Co} \rightarrow \text{Co}^*\text{+ADH1A-NAD}^+\text{+ADP+P}, \quad (1)$$

$$\text{ADH1A-NAD}^+\text{-EtOH} + \text{Co}^* \rightarrow \text{ADH1A-NADH} + \text{AA} + \text{Co}, \quad (2)$$

where Co* is an exciton (electronic excited state) in the Co metal film, which traveled from the left to the right half of the cell bottom plate, and P the inorganic phosphate.

Figure 2(b) shows the formation kinetics of ADP and product AA, quantified chromatographically in their respective compartments. We observe production of ADP and AA, with about 1.4 ATP molecules hydrolyzed for each ethanol molecule oxidized (experiment 1, Table II). This is different from the results of control experiments, with $ATP + NAD^+ +$ EtOH in a single-compartment experiment, where the reaction proceeded faster, with only one ATP molecule used for each ethanol [experiment 3 of Table II; see also Fig. 2(b)]. We explain this difference by the decay of some excitons in the Co metal film, with emission of IR radiation. This decay was blocked when both ATP and EtOH were present in the same cell and the (slower) exciton transfer to the Co metal film could not compete with the fast intramolecular exciton transfer to the substrate site. This explanation was tested in additional experiments where no EtOH was present in the second compartment, while the ATP hydrolysis still proceeded, although slower, as the liberated energy was dissipated through light emission by the Co metal film (experiment 2, Table II). All of these results are summarized in Table II.

The effect of Co film thickness upon the ATP hydrolysis energy transfer between the cell compartments was tested by varying the film thickness in the 7.3–13.2-nm range (Appendix A). The ethanol oxidation rate in the second compartment was significantly reduced for the 7.3-nm film, due to the absence of quasiresonant electronic states close to $2500\ cm^{-1}$ (ATP hydrolysis energy). Using Co films in the 11.5–13.2-nm range, the oxidation rates were all quite similar and weakly dependent on the film thickness, as all those films had quasiresonant excited states close to $2500\ cm^{-1}$. Note that a transverse gap 20 μm wide between the two halves of the film completely eliminated energy transfer, as no acetaldehyde formation could be detected in the second compartment (Appendix C).

Note that experiments 1 and 2 of Table II are completely consistent with each other, as the AA formation turnover rate of experiment 1 plus the ADP formation turnover rate of experiment 2 reproduced the ADP formation turnover rate in experiment 1. On the other hand, experiment 3 resulted in equal formation turnover rates for AA and ADP, with no ATP lost due to energy transfer to the Co metal layer and subsequent exciton decay there. This result indicates that intramolecular energy transfer within the ADH1A molecule was much faster than the energy transfer to the Co metal layer.

We conclude that excitons may be produced and sent or received and used by ADH1A molecules *in vitro*, therefore they should also be transporting the energy within the ADH1A molecules *in vivo*, from the ATP-binding site to the alcohol-binding site. Based on lower ATP requirements in control experiment 3, we also conclude that the internal exciton transfer within the ADH1A molecule is much faster than that to the Co nanofilm and/or neighboring ADH1A molecules, as would be expected due to much shorter distances involved in the internal energy transfer.

Importantly, the only requirement for the ATP hydrolysis occurring was the possibility to dump the electronic excitation energy produced: once such a possibility was made available in experiment 3, ATP molecules were hydrolyzed with rates determined by exciton decay in the Co metal film. However, the generally accepted theory considers conformational change of the enzyme molecule due to substrate binding at its reaction site as the necessary condition enabling ATP hydrolysis and coupling the two reactions occurring on the enzyme [17–19], which contradicts our experiments. Indeed, no conformational change was possible in experiment 3, where no alcohol was present anywhere, while ATP was still hydrolyzed. Similarly, in experiment 1 no alcohol was present in the first compartment where ATP was hydrolyzed, which happened as long as the liberated excitons could either decay in the Co firm, or be consumed in the second compartment, generating the product AA. In the same way, no conformational changes due to ATP could be possible in the second compartment, where EtOH was nevertheless oxidized, as long as the ATP hydrolysis energy reached the respective ADH1A

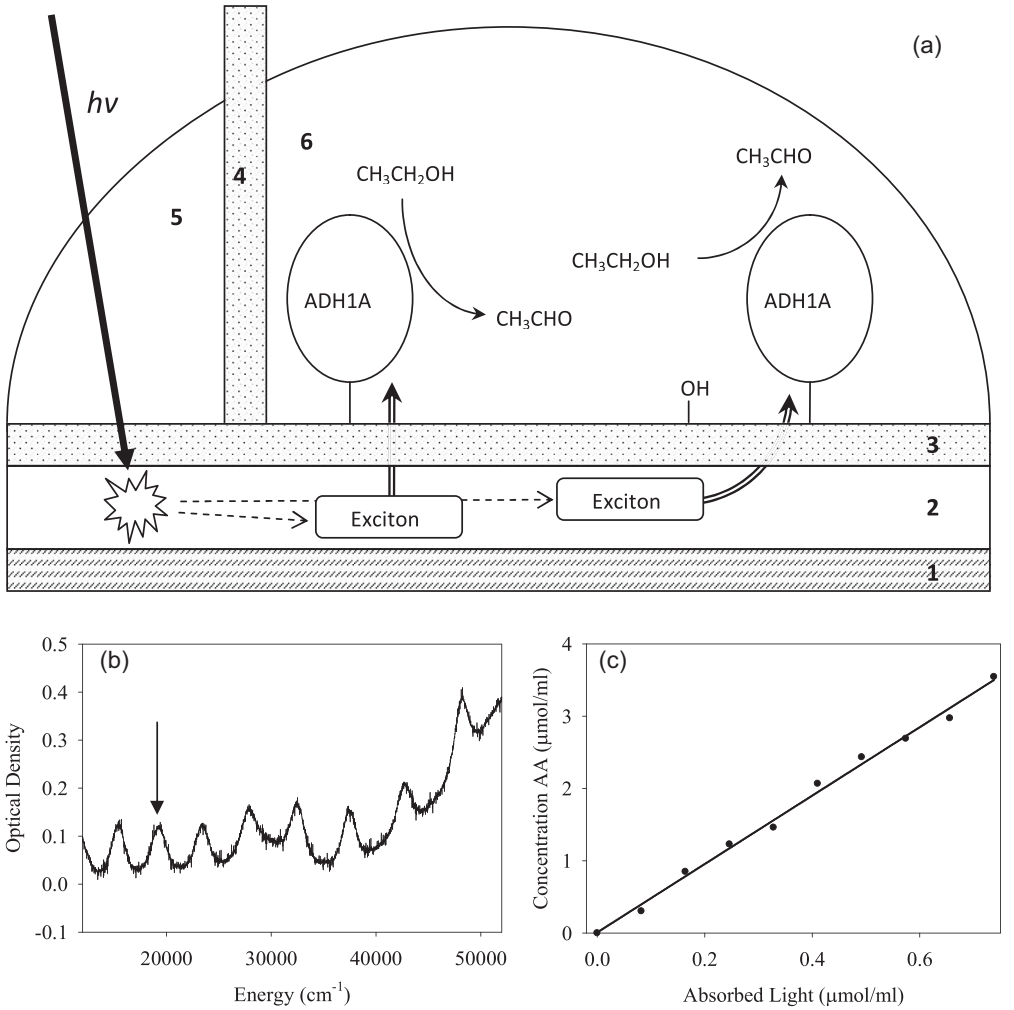

FIG. 3. Enzymatic oxidation of EtOH driven by excitons produced upon absorption of light. Multiple excitons were generated by each photon absorbed in the Co metal film in the left compartment, with EtOH oxidation occurring when these excitons got to the right compartment. (a) 1: CaF_2 substrate 1.0 mm thick; 2: Co film 11.5 nm thick; 3: BN film 10.3 nm thick; 4: central separation of the cell; 5: NAD^+ solution; 6: ethanol + NAD^+ solution. (b) Absorption spectrum of the Co film, excitation wavelength indicated by an arrow. (c) Formation of AA vs the amount of photons absorbed, with line showing the linear fit. The experiment continued for 36 h.

molecules by way of excitons transported by the Co metal film. We therefore conclude that it is the energy conservation rather than conformational change that couples ATP hydrolysis with the substrate reaction.

The energy conservation was apparently maintained by the formation of an electronic excited complex (exciplex) [20] upon hydrolysis of ATP, with ADP + P kept bound and in place for the inverse reaction, which occurred unless the excess energy was dissipated, with angle brackets denoting the enzyme-coenzyme-ADP-P exciplex:

$$\text{ADH1A-NAD}^+\text{-ATP} \leftrightarrow \langle\text{ADH1A-NAD}^+\text{-ADP-P}\rangle^* \quad (3)$$

Thus, the exciplex transitional state has to decay back to the initial state of the ADH1A-NAD^+-ATP complex, unless it dissipates the ATP hydrolysis energy in the substrate reaction (alcohol dehydrogenation by ADH1A), or transfers it to the Co metal film in our *in vitro* experiments. Once the excess energy is dissipated, the exciplex disappears and ADP + P are free to dissociate. The excitation energy of the exciplex, equal to the ATP hydrolysis energy, cannot be transferred to water or other nearby small molecules, as their electronic excitation energies are far too large. Thus, the only possibility for the exciplex to dissipate the excess energy *in vivo* is the substrate reaction, explaining high energy efficiency of the enzymes and the lack of spontaneous ATP hydrolysis *in vivo*. Spectroscopic detection of such exciplexes should be difficult, due to their short lifetimes and their eventual spectral features appearing in the IR spectral range, where they are obscured by the solvent.

We next tested whether ADH1A would transform the substrate on excitons produced by ways other than ATP hydrolysis. Thus, in the second series of experiments the left compartment was filled with NAD^+ solution (no ATP was added), while the solution in the right compartment still contained EtOH and NAD^+ [Fig. 3(a)].

The excitons were produced in the Co film in the left compartment upon absorption of photons at 519 nm (19 268 cm^{-1}; cyan-green light), exciting one of the film absorption bands, with the film optical absorption spectrum shown in Fig. 3(b). Note that none of the compounds used in our experiments

have any absorption at 519 nm, apart from the Co metal film. Figure 3(c) shows the formation kinetics of AA and the number of photons absorbed by the film, with 4.7 AA molecules produced for each photon absorbed by the film. This is explained by the high-energy excitons initially formed upon light absorption, next dividing into smaller 30.7 kJ/mol excitons, with some energy lost for exciton decay, producing visible and IR light emitted by the Co metal film. Note that similar phenomena were observed earlier in metal films [3–5]. Indeed, it was found that excitons generated in thin Co film propagate along the film across macroscopic distances [5]. This information provides additional proof to the correctness of the current interpretation of the experimental results. We therefore conclude that the source of excitons is not critical for the functioning of ADH1A, which successfully oxidizes EtOH on excitons generated by light absorbed by the Co nanofilm, instead of ATP hydrolysis.

As for the effect of Co film thickness on the photoactivation of the enzymatic reaction, similar experiments were performed using Co films with thickness in the 7.3–13.2-nm range (Appendix B). A significantly lower photoinduced oxidation rate was measured for the 7.3-nm film, while similar values of the oxidation rate were measured at larger thicknesses. Such behavior was explained by the absence of the 2500-cm^{-1} excited state in the 7.3-nm Co film, as the lowest transverse-quantized excited state appears at 5644 cm^{-1}. On the other hand, excited states as approximately 2500 cm^{-1} appear in thicker films, allowing the photon energy of 19 268 cm^{-1} to be exchanged into several excitons (excited states) at about 2500 cm^{-1}. The yield of such energy exchange was estimated at 0.62. Once more, a transverse gap 20 μm wide, separating the film into two halves, completely excluded the energy transfer from the photoexcited Co film patch to the neighboring compartment (Appendix C).

Noting that the ADH1A-NAD^+ complex has an absorption band at 290 nm (see Fig. 4), we further tested whether alcohol oxidation would function on excitons produced by 290-nm UV photons, in the absence of ATP. To test for the eventual effects of NAD^+ on the photooxidation of EtOH, we irradiated the same mixture containing EtOH and NAD^+, but with ADH1A absent. No AA formation was detected in these test experiments during photolysis. Therefore, the eventual photoactivity of NAD^+ is disregarded in the following discussion. Figure 4 shows the scheme of the experiment and the results for the number of photons absorbed and AA formation.

The turnover rate in absence of light (results not shown) was a factor of 12 lower than that of the photosensitized reaction, and the quantum yield of AA under light was close to 1.5. Apparently the excited state of the ADH1A-NAD^+ complex lives long enough for a second EtOH molecule to be oxidized by the energy obtained from only one photon, in about 50% of all cases. Alternatively, about 50% of the naturally dimeric ADH1A molecules have a second EtOH molecule complexed, with two oxidations accomplished by one photon. We therefore conclude that ADH1A can function on the energy of ultraviolet photons that it absorbs, producing high-energy electronic excitations (excitons). Due to high photon energy, several ATP-scale excitons are formed, some of which oxidize a second EtOH molecule per one UV photon.

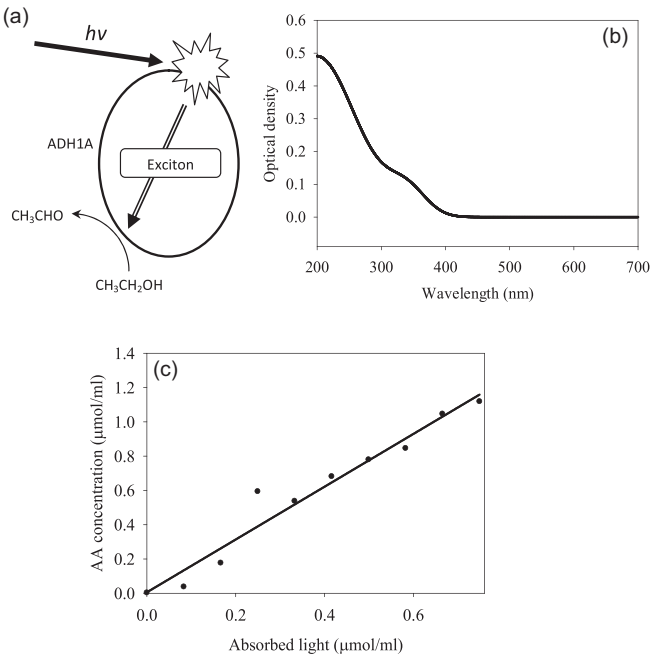

FIG. 4. Photosensitized oxidation of EtOH by ADH1A. (a) Ultraviolet light at 290 nm excited ADH1A in suspension containing also NAD^+ and EtOH. (b) ADH1A-NAD^+ ultraviolet and visible absorption spectrum in suspension. (c) AA formation vs the number of photons absorbed by ADH1A (circles), line shows the linear fit. The experiment continued for 36 h. The turnover rate was a factor of 12 lower in the baseline experiment (no light; results not shown).

We therefore believe that excitons are universally created, consumed and transferred within and between all of the enzymes, structural proteins, and other macromolecules, as all of these molecules are sufficiently large to accommodate delocalized low-energy electronic excited states with energy comparable to that of ATP hydrolysis. Such states were first identified in recent quantum chemical calculations of polypeptides, where the gap between the highest occupied and the lowest excited states was as low as 0.43 eV (41 kJ/mol) [21]. Therefore, low-energy excited states must be present in all enzymes and polypeptides in general, provided they are at least several hundred aminoacids long. Spectroscopic identification of these states in the absorption spectra should be difficult, as their bands are masked by the bands of IR vibrational transitions. However, they should be identifiable in the emission spectra, by the emission bands appearing in the IR spectral range upon excitation. These ideas were indeed confirmed by additional measurements, as shown in Fig. 5.

Note that the 2541-cm^{-1} band in Fig. 5 has the energy virtually coincident with that of ATP hydrolysis (30.4 vs 30.7 kJ/mol), and should therefore belong to the very electronic excited state of the ADH1A-NAD^+ complex that transfers the energy from the ATP hydrolysis site to the alcohol dehydrogenation site of the enzyme. This agreement provides additional confirmation for our theory. Thus, the existence of low-energy excited states in polypeptides and the ATP hydrolysis energy transfer via such states explain both the reaction coupling and the extremely high energy efficiency of biological

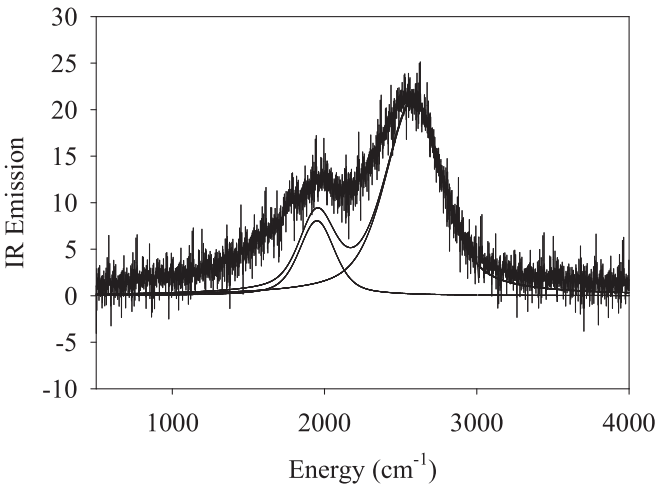

FIG. 5. IR emission spectrum of ADH1A-NAD$^+$ complex in suspension excited at 290 nm (UV light). Deconvoluted bands and their superposition are also shown (thin lines). The deconvoluted band maxima appear at 2541 cm^{-1} (30.4 kJ/mol, bandwidth 438 cm^{-1}) and 1958 cm^{-1} (23.4 kJ/mol, bandwidth 251 cm^{-1}). This IR emission is apparently originating from the low-energy long-living electronic excited states of the ADH1A-NAD$^+$ complex. No added ATP or ethanol.

processes, including those happening in the muscles. The energy conservation is apparently secured by exciplex formation upon ATP hydrolysis, preventing dissociation of ADP+P from the complex before the excess energy is used for the substrate reaction. Electronic excitations (excitons) are apparently active in many protein-based biological structures, as they were conclusively demonstrated to transport energy over macroscopic distances and from one cell to another within vertebrate retina [2]; as was already noted, these facts have motivated the present study. Macroscopic-range energy transfer by excitons indicates that, in addition to focused energy transfer, unrecognized communication paths may exist within and between cells, complementing the already known signaling paths.

The presently reported results allow a different look at many cellular structures. For instance, the enzymes forming dimers or tetramers may be doing so for summing the energy of several ATP molecules, and thus obtaining energy sufficient to drive a certain difficult reaction. Another possibility is the increased reaction rate in a polymeric enzyme. Indeed, in a tetramer (such as ADH1A in yeast) the average number of bound ATP molecules would be a factor of 4 larger, and the average number of bound substrate molecules would also be a factor of 4 larger. This will accelerate the enzymatic reaction in a tetramer by a factor of 4, compared to the four monomeric enzymes. Indeed, the ATP hydrolysis energy may be promptly and efficiently exchanged between the bound monomers, coupling ATP hydrolysis at any of the four ATP binding sites to the substrate reaction at any of the four substrate binding sites. Also cellular structures such as filaments and microtubules may be transporting energy in the form of excitons between certain locations within the cell, instead of the (slower and defocused) energy transport in the form of ATP molecules. The excitons in macromolecules with quantized nonequilibrium energy exchange also provide a ubiquitous example of nontrivial quantum effects in biological systems [1].

On a lighter note, the presently demonstrated capacity of enzymes to function on sunlight was certainly important during the pre-ATP evolution of life on Earth. Additionally, we now have a solid theory explaining why lying on the beach naked is good for our health, as enzymatic function is directly stimulated by sunlight. The success of low-level laser (light) therapy (LLLT) or photobiomodulation (PBM) therapy [22,23] is also explained by enzymes capable of using light for their substrate reaction, instead of draining the ATP stock. Note that the currently proposed explanation differs from the previously discussed mechanisms of PBM [23].

Note that the presently reported studies were performed *in vitro*, using a Co metal film to transmit excitons (electronic excited states) between different enzyme molecules; the respective properties of Co films have been reported earlier [5]. We presume that similar exciton transfer processes may occur *in vivo*, and could be used by nature for energy and information exchange between cells. Intermediate filaments are a good candidate for the role of exciton guides *in vivo*, and a follow-up study will be looking for new functions of these protein nanofibers.

IV. CONCLUSIONS

The experiments reported allow reaching several important conclusions:

(i) Energy exchange within and between enzyme molecules is mediated by low-energy electronic excited states (excitons), and not by Davydov's vibrational solitons. These phenomena should be present in all large polypeptides, due to the existence of low-energy excited states with energies comparable to that liberated upon ATP hydrolysis.

(ii) The existence of low-energy excited states in proteins and enzymes may be detected by IR emission, as was confirmed upon UV excitation of the ADH1A-NAD$^+$ complex. Such states were also identified in quantum chemical calculations.

(iii) ATP hydrolysis is coupled with the substrate reaction by energy conservation, mediated by excitons, and not by conformational changes occurring in the enzyme molecule upon binding the ATP and the substrate. These excitons are quite stable, incapable of losing energy to the surrounding small molecules that have no low-energy electronic excited states.

(iv) Spontaneous ATP hydrolysis is apparently prevented by exciplex formation, Eq. (3), which maintains ADP + P bound and in place for the inverse reaction reconstituting ATP, until the ATP hydrolysis energy in dissipated in the substrate reaction, with the exciplex dissociating and liberating ADP and the phosphate.

(v) High exciton stability explains high energetic efficiency of enzymatic reactions and the possibility of exciton transfer along protein fibers (intermediate filaments), as reported earlier *in vitro* and apparently occurring in vertebrate eyes *in vivo* [2].

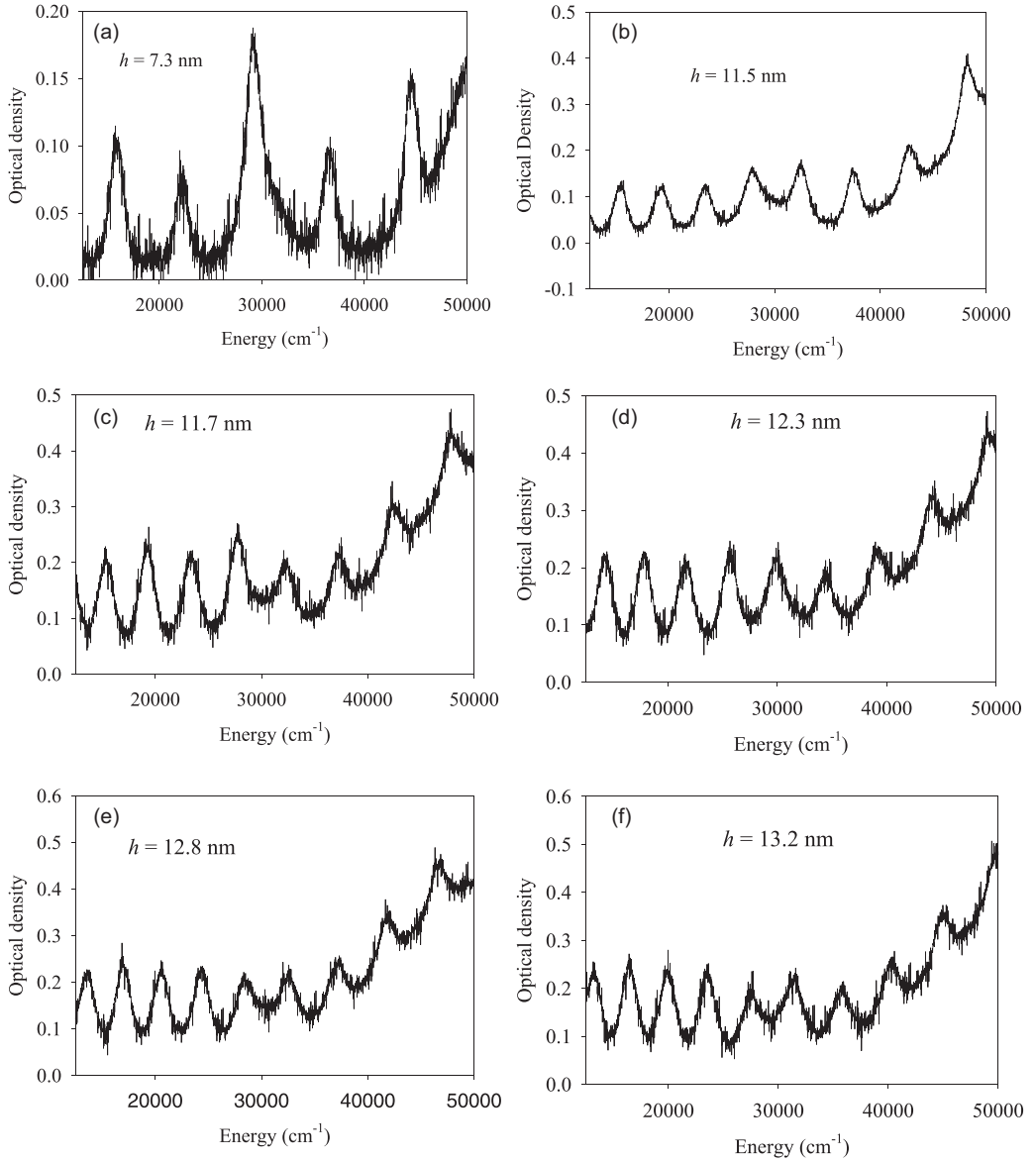

FIG. 6. Absorption spectra of the Co-BN(10.3 nm) sandwich films with different thickness of the Co layer: 7.3 nm (a); 11.5 nm (b); 11.7 nm (c); 12.3 nm (d); 12.8 nm (e); 13.2 nm (f).

(vi) Alcohol dehydrogenases (ADH) work much faster when ATP is available (e.g., by a factor of 15 to 20, as measured here), despite the common assumption that these enzymes do not need ATP, as had been stated implicitly or explicitly in about 25 thousand research papers and all biochemistry textbooks. This misapprehension probably resulted from ATP not known at the time of discovery of the first ADH enzyme, which was also the first enzyme ever discovered.

(vii) The currently reported experimental tools may be used to investigate mechanisms of enzymatic reactions, by physically decoupling the ATP hydrolysis or energy production from the substrate reaction. They may also be used to measure reaction energy requirements, by providing excitons of different energies to immobilized enzymes and measuring turnover rates.

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APPENDIX A: CONTROL EXPERIMENTS WITH VARIABLE Co THIN FILM THICKNESS

Control measurements were performed using Co metal thin films with different thickness, with the respective absorption spectra shown in Fig. 6. Thin films were fabricated by depositing Co metal film and 10.3-nm BN dielectric film on CaF_2 substrate (see the main text).

It was shown earlier [5] that the observed spectra are due to 1D quantum confinement, with the peaks described by the

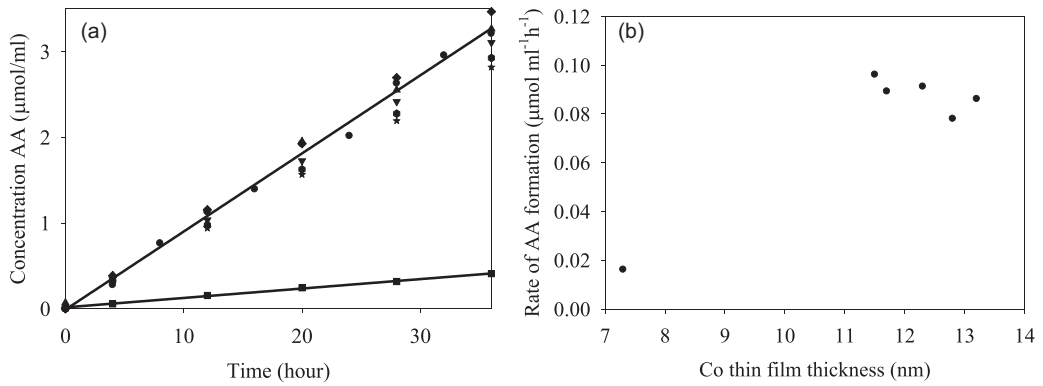

FIG. 7. (a) Experimental AA production in the two-compartment cell using Co-BN(10.3 nm) sandwich film on the cell bottom plate with different Co film thickness: 7.3 nm, squares; 11.5 nm, circles; 11.7 nm, triangles up; 12.3 nm, triangles down; 12.8 nm, diamonds; 13.2 nm, stars. (b) Rate of AA formation vs Co film thickness.

particle-in-a-box formula:

$$E_{\text{line}} = \frac{A}{fa^2}[2mn + m^2], \tag{A1}$$

where $A = 3029.2\,\text{cm}^{-1}\,\text{nm}^2$, a is the Co film thickness in nm, and $f = m_{\text{eff}}/m_e$, m_{eff} is the electron mass in Co film and m_e is the electron mass in vacuum, n is the quantum number of the highest occupied level (HO), which is dependent on the Co film thickness (8, 9, 9, 10, 10, and 10 for the respective films) and m is the quantum number increment for the respective transition.

Acetaldehyde (AA) production rates were measured for different Co films [the same experiment as in Fig. 2(c) of the main text] in the same initial conditions. The respective results are shown in Fig. 7.

To understand these results, note that the 7.3-nm-thick Co film has no excited states close to $2500\,\text{cm}^{-1}$ in energy, as its lowest excited state is located at $5644\,\text{cm}^{-1}$ above the ground state. The latter value is very far from the ATP hydrolysis energy of $2500\,\text{cm}^{-1}$, corresponding to the respective excited state of the enzyme. Therefore, energy transfer from the enzyme to the film is more difficult, requiring simultaneous hydrolysis of two ATP molecules. On the other hand, every one of the thicker Co films has excited states at energies compatible with the ATP hydrolysis energy of $2500\,\text{cm}^{-1}$, and the energy transfer operates much easier resulting in higher AA production rates.

APPENDIX B: CONTROL PHOTOACTIVATION EXPERIMENTS WITH VARIABLE Co FILM THICKNESS

We also tested dependence of the photoinduced reaction rate [Fig. 3(c)] on the Co film thickness. These experiments used 7.3-nm Co film excited by light at 628 and 451 nm, with the results averaged over the two wavelengths. Experiments with 11.5, 11.7, 12.8, and 13.2 nm Co films used excitation at 519 nm, and those with 12.3-nm Co films used excitation at 559 nm. Note that the wavelengths were always chosen to coincide with the one of the absorption peaks of the respective film, closest to the 519-nm peak used in the main experiments with the 11.5-nm film. The respective results are shown in Fig. 8.

Large differences between the results obtained for 7.3-nm Co film and other film thickness values may be explained

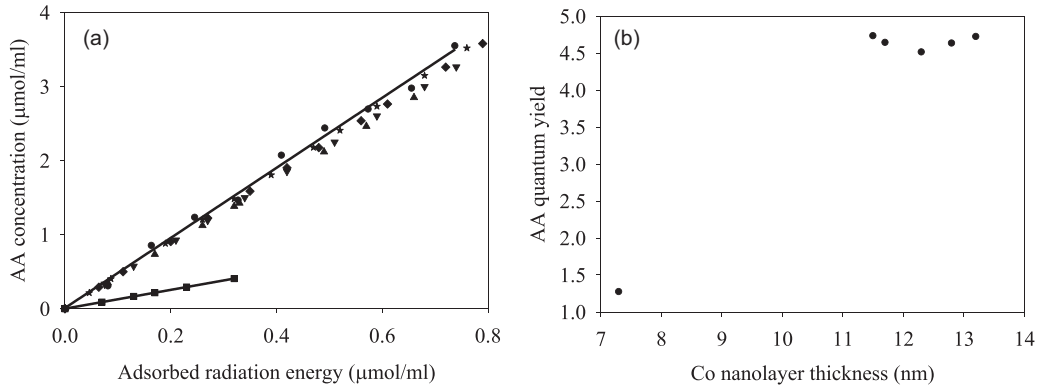

FIG. 8. (a) Measured dependencies of AA photoinduced production in the two-compartment system for Co-BN(10.3 nm) sandwich structure, with Co film thickness: 7.3 nm, squares (628- and 451-nm excitation); 11.5 nm, circles (519-nm excitation); 11.7 nm, triangles up (519-nm excitation); 12.3 nm, triangles down (559-nm excitation); 12.8 nm, diamonds (519-nm excitation); 13.2 nm, stars (519-nm excitation). (b) Number of AA molecules formed per photon absorbed by the Co-BN sandwich film in function of the Co film thickness.

by the same mechanism considered above, as the lowest energy level existing in the 7.3-nm film appears at 5644 cm^{-1} above the ground state, therefore the initially produced large excitons corresponding to visible-light photons are exchanged into a lower number of excitons, reducing the quantum yield of ethanol oxidation by the ADH1A enzyme, which requires 2500 cm^{-1} of energy for each ethanol molecule. For comparison, thicker films all have excited states at energies close to 2500 cm^{-1}, resulting in the quantum yields of ethanol oxidation of about 4.5 [Fig. 8(b)], and not more, apparently due to energy losses.

APPENDIX C: EXPERIMENTS WITH ISOLATED COMPARTMENTS

These experiments were performed in a cell where the Co(11.5 nm)-BN(10.3 nm) sandwich layer on the common bottom plate of the two compartments was separated in two, by a 20-μm-wide gap. In these experiments no additional ethanol oxidation occurred in the second compartment when excitons were produced in the first compartment, either by ATP hydrolysis or by direct excitation of the Co film with visible light. The slow formation of AA that was detected proceeded via enzymatic oxidation of EtOH by ADH1A, occurring even in absence of ATP (Fig. 9). Note that the AA formation rate in Fig. 9 is about a factor of 20 lower than that in Fig. 7(a).

Note that the oxidation rate of EtOH in these experiments was about $(4.6 \pm 0.3) \times 10^{-3}$ μmol ml^{-1} h^{-1}, the same as measured in a single cell without ATP or light. This rate is attributable to oxidation EtOH by ADH1A in the absence of ATP—alcohol dehydrogenases in general are believed to be (erroneously, as explained in the main text) the classic example of enzymes that do not require ATP. This demonstrates that excitons generated in one compartment either by ATP hydrolysis or irradiation, when transferred along the Co-BN sandwich film to another compartment, accelerate the enzymatic oxidation of EtOH present there.

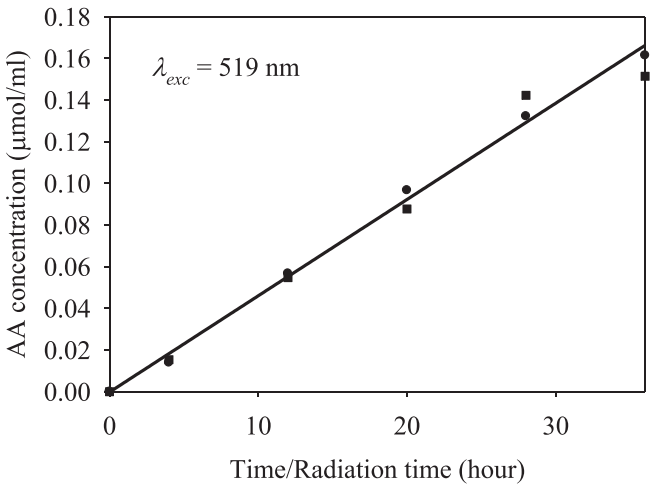

FIG. 9. EtOH oxidation dynamics in separate compartments, with the Co-BN sandwich film cut in two halves by a 20-μm-wide gap. The formation of AA was measured in the second compartment containing ADH1A . . . NAD^+ complex bound to the Co(11.5 nm)/BN(10.3 nm) sandwich film in presence of EtOH solution. The first compartment had the same ADH1A . . . NAD^+ complex bound to the Co(11.5 nm)-BN(10.3 nm) sandwich film on its bottom plate. Excitons were produced in the first compartment by ATP hydrolysis (circles), or by light at 519 nm, at the same intensity as used in the main experiment (squares), but could not affect the oxidation rate in the second compartment.

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