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OPTICAL SWITCHING AND AMPLIFICATION BASED ON THE ENZYME LUCIFERASE

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Abstract Films containing luciferase and its substrates were made to act as optical memories and correlators.

Enzymatic reaction of luciferase with its substrates can be summarised[1] :

$$\begin{array}{l} \text{ATP} \quad \text{L} \quad \text{O2} \\ \downarrow \quad \downarrow \quad \downarrow \\ \text{E} \dashrightarrow \text{reaction} \dashrightarrow \text{E} + h\nu \\ \qquad\qquad\qquad\qquad\quad \downarrow \quad \downarrow \quad \downarrow \\ \qquad\qquad\qquad\qquad\quad \text{AMP} \quad \text{OL} \quad \text{CO2} \end{array} \qquad (1)$$

The symbols represent : E = enzyme, ATP = adenosine triphosphate, L = luciferin, AMP = adenosine monophosphate, OL = oxyluciferin, $h\nu$ = output photon.
The emitted light peaks at the wavelength of 560 nm with bandwidth of ~100 nm.[2] This reaction is used by the firefly *Photinus pyralis* to produce light signals. The reaction is also used for analytical purposes as an assay for ATP.

In living cells the enzymatic dephosphorylation of ATP is involved in the crucial stage of the motile mechanism where the energy given by ATP drives the mechanical motion at the molecular level. The bioluminescent reaction (1) requires 5 - 10 times greater amount of energy to produce a photon of green light, than the amount available from ATP. It is interesting to speculate how the inverse of photosynthesis involving $O_2 \rightarrow CO_2 + h\nu$ became coupled to the enzyme and ATP so that most of the energy required comes from oxygen. Oxygen under pressure gives off chemiluminescence of its own or can activate other luminophores[3]. In reaction (1) luciferin is the luminophore which is bound to the enzyme throughout the reaction and indeed influences the spectral shape of the bioluminescence. Oxygen reacts with luciferin to produce oxyluciferin which is the main site for the electronic excited state [4] . Both luciferin and oxyluciferin have very strong fluorescence with a spectral shape similar to that of bioluminescence [5] .

The practical value of this reaction lies in the fact that the principal product is light which is easy to detect and to measure quantitatively . We have measured the quantum yield with respect to luciferin as high as 80%. Therefore it is possible to detect a few thousand molecules of luciferin using a good photomultiplier tube and collecting mirror. Although the enzyme turns almost every molecule of luciferin into a photon and oxyluciferin, the reaction times are slow. Response time to the sudden increase in the

concentration of ATP is between 300 - 500 ms depending on precise experimental conditions.

The possibility for practical use of this reaction lies in the parallelism that can be obtained if the reactants are distributed in two dimensions so that each point of the plane is acting as an independent light emitting element. Mixing the reactants into a 1% agarose gel produces a desired plane film when the gel sets. Reactants remain localized if the gel is partially dried. Therefore the problem is to set the reaction 'on' or 'off' at each point in the gel. That way one would be making a memory which can record a digital pattern. One way to achieve this is by use of 'caged - ATP'. This is a derivative of ATP with diazoethane attached to the terminal phosphate group of ATP[6]. The c-ATP is not a substrate for the enzyme so that no light is produced and it is not consumed. The bond between the diazoethane and ATP is photolysed by UV light 'uncaging' the ATP which then initiates the enzyme reaction. If the gel containing c-ATP instead of ATP is exposed to UV light through a mask, the pattern of the mask selects the points where reaction (1) is turned 'on' and the luminescence can be detected from those points . An example is shown in figure 1. A 100W mercury arc lamp was used to expose a gel for 10 seconds through the mask in which the letters 'C-ATP' were cut out. The luminescence of the gel was detected by an image-intensified CCD camera. The picture from the camera was digitized by a frame grabber and the figure shows the stored image displayed on a monitor screen. The quality of the image is limited by the line structure of the monitor and the process of converting data into digital form. We have not yet endeavoured to attain the full spatial resolution available from the gel.

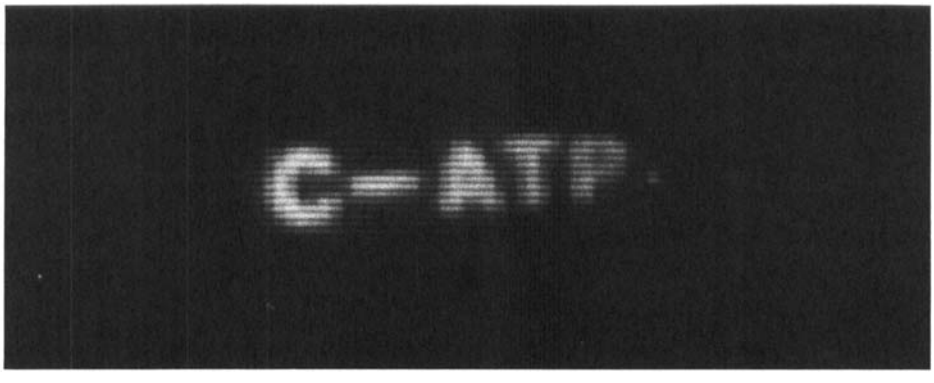

FIGURE 1 Display of the luminescent image on a monitor screen.

The light output is stable and localized to places which were irradiated with UV. The luminous intensity fades after half an hour. This time is dependent on the concentration of reactants and water content of the gel. The uncaging can be made faster with laser pulses from an excimer or a N_2 laser. A pulse of 100 mJ at wavelength of 308nm will uncage less than one percent of c-ATP. In this context we have used gels to infer the

spatial energy distribution in the laser pulse from the distribution of luminescence intensity.

The amount of light produced by a quantity of uncaged ATP can be amplified by rephosphorylating the product AMP back into ATP which continues to maintain the luminescence. The following scheme was tested :

ATP + myokinase ------> 2 ADP

ADP + phosphoenolpyruvate + pyruvate kinase ---> ATP + pyruvate. (2)

The possibility of creating a two-dimensional parallel processor exists if the enzyme can be turned 'off' in a step which is independent from the step of uncaging the ATP. Thus the light output from an element of the gel becomes a product of two patterns: STEP1(x,y) * STEP2(x,y). If the first step consists of disabling the enzyme it amounts to writing STEP1(x,y)= 0. We have used radiation damage to disable the enzyme. A beam of 10MeV protons from the University of Birmingham cyclotron was directed at the gel through a tantalum mask 0.25 mm thick with a V pattern cut in it. The beam current of 100nA for 100 seconds (400eV per enzyme molecule deposited by ionization) inscribed this pattern in the gel. Figure 2 shows the luminescent pattern obtained when STEP2(x,y)= 1 for all x and y, that is after bombardment with protons, the gel was irradiated uniformly with UV to uncage the ATP everywhere.

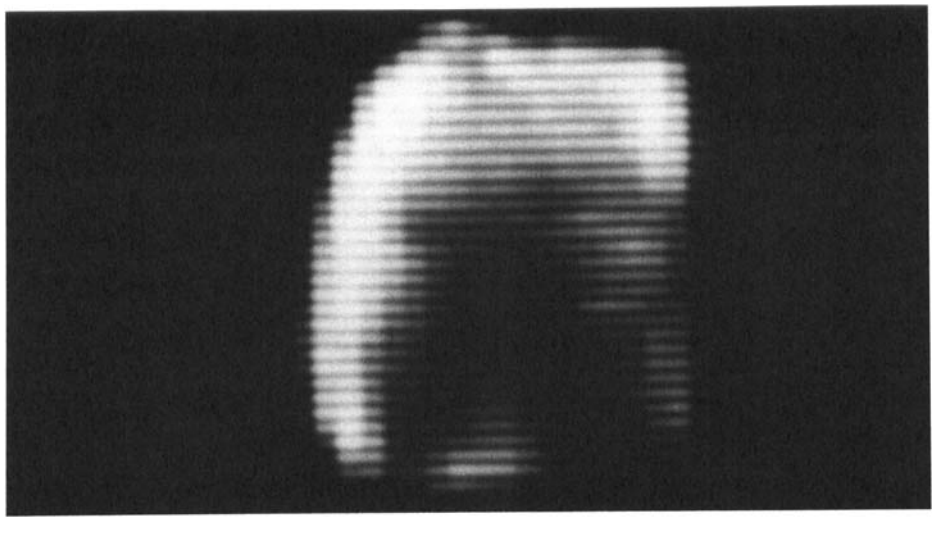

FIGURE 2 The pattern created by radiation damage in the enzyme.

In figure 3 we show an example where STEP2 also had a mask in the form of a triangle. It shows that light output is present because the V mask of STEP1 and the triangle mask of STEP2 do not coincide. When the two masks do coincide there is no light output . The processor can be used to recognise a pattern by the absence of luminescence.

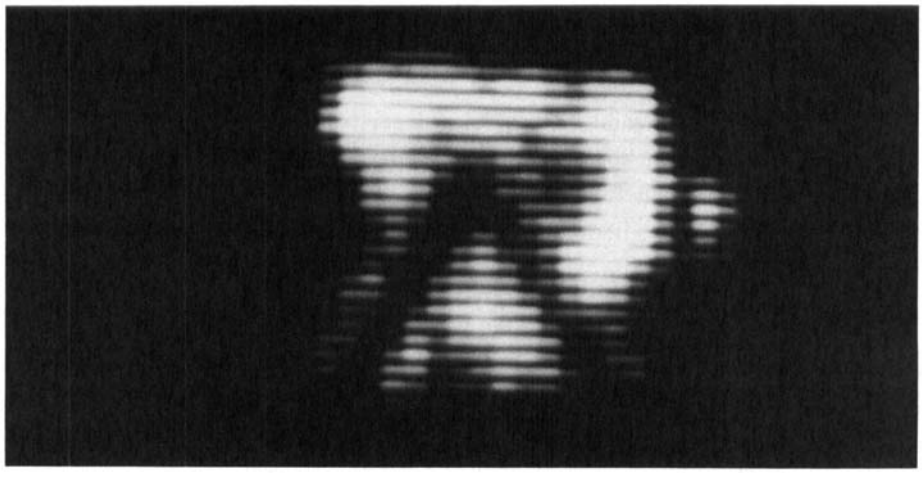

FIGURE 3 The luminescence output from two patterns in STEP1 and STEP2.

These studies show that a number of functions can be performed with naturally ocurring biomaterials. We are still a long way from producing devices which compete with semiconductors but it is interesting to see how biochemical processes can be adapted to the requirements of device technology. Of course the major advance would be a better understanding of the electronic and molecular physics of the enzyme reactions so that direct ways of control and interconnection could be found.

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