

# Controlled Fluid Transport using ATP-Powered Protein Pumps

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**Abstract.** Plants have the ability to move fluids using the chemical energy available with bio-fuels. The energy released by the cleavage of a terminal phosphate ion during the hydrolysis of a bio-fuel assists the transport of ions and fluids in cellular homeostasis. The device discussed in this paper uses protein pumps cultured from plant cells to move fluid across a membrane barrier for controllable fluid transport. This article demonstrates the ability to reconstitute a protein pump extracted from a plant cell on a supported bilayer lipid membrane (BLM) and use the pump to transport fluid expending adenosine triphosphate (ATP). The *AtSUT4* protein used in this demonstration is cultured from *Arabidopsis thaliana*. This protein transporter moves a proton and a sucrose molecule in the presence of an applied proton gradient or by using the energy released from Adenosine triphosphate's (ATP) hydrolysis reaction. The BLM supporting the *AtSUT4* is formed from 1-Palmitoyl-2-Oleoyl-sn-Glycero-3- [Phospho-L-Serine] (Sodium Salt) (POPS), 1-Palmitoyl-2-Oleoyl-sn-Glycero-3-Phosphoethanolamine (POPE) lipids supported on a porous lead silicate glass plate. The BLM is formed with the transporter and the ATP-phosphohydrolase (red beet ATPase) enzyme, and the ATP required for the reaction is added as magnesium salt on one side of the membrane. The ATP hydrolysis reaction provides the required energy for transporting a proton-sucrose molecule through the protein pump. It is observed that there is no fluid transport in the absence of the enzyme and the amount of fluid transported through the membrane is dependent on the amount of enzyme reconstituted in the BLM for a fixed sucrose concentration. This demonstrates the dependence of the fluid flux on ATP hydrolysis reaction catalyzed by ATP-ase enzyme. The dependence of fluid flux on the amount of ATP-ase provides convincing evidence that the biochemical reaction is producing the fluid transport. The fluid flux resulting from the ATP-powered transport is observed to be higher than the rates observed with a proton concentration gradient driven transport reported in our earlier work.

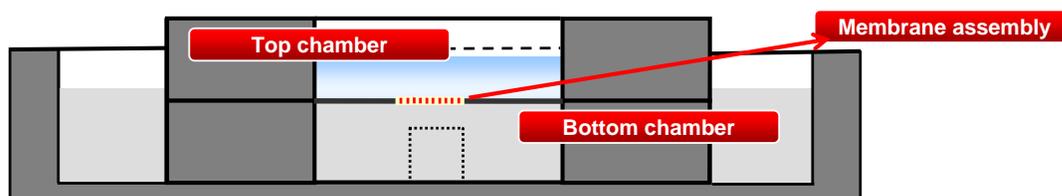
## 1. Introduction

Transport of ions and sugars are essential for life and growth in plants. Ions are absorbed in the conductive tissues of root and transported to sites where it is converted into sugars for storage. In addition, water permeates across the cell boundary together with ions to maintain ion concentration within limits to support life functions[1].

The fluid transport in plant cells results due to concentration gradients of ions and occurs at the cell wall. Ion transport in plants can be classified into two major types: passive ion transport and active ion transport. Passive ion transport in cells occurs through defined pathways in the membrane called *channels* triggered by a transmembrane potential, chemical affinity or membrane stretch [1, 2]. Active transport across the membrane occurs expending bio-chemical fuels with transport of ions against established concentration gradients. The biochemical fuel is in the form of adenosine tri-phosphate (ATP), splits at functional sites in the membrane into Adenosine di-phosphate (ADP) and a phosphate ion. A transport mechanism in which ATP hydrolysis triggers ion transport through an intermediate concentration gradient step is called secondary active transport. The hydrolysis reaction is catalyzed by an enzyme *ATP phosphohydrolase* (or) ATP-ase [3]. The passive and active transport schemes used in plants result in bulk fluid transport. The goal of this paper is to demonstrate that protein transporters harvested from plants can be used in a synthetic device for ATP-powered controlled fluid transport. In previous work [4] we have demonstrated that an existing proton gradient will produce fluid flow across AtSUT4 protein pumps supported in a BLM. In this paper we demonstrate that the fluid transport can be controlled directly by the addition of ATP to the medium surrounding the protein pumps. The relationship between the pumping rate and the quantity of ATP-ase enzyme is characterized, demonstrating that the amount of ATP-ase added to the medium can be used to control the fluid flow rate. To our knowledge this is the first demonstration of using protein pumps to create a device for controlled fluid transport.

## 2. Device for Controlled Fluid Transport

Micropumps currently available can be classified broadly into displacement pumps, dynamic pumps and aperiodic pumps as discussed by Laser and Santiago [5]. The fluid transport device developed in this work uses transporter proteins embedded in cell membranes. The active component of this electrochemical pump uses chemical stimulus to move fluid through the conductive pathway. A schematic of the device that produces fluid transport expending chemical energy through a transporter protein is shown in Figure 1. The functional component of the device is a membrane assembly which has the biological transporter proteins dispersed in a bilayer lipid membrane (BLM) and supported on a porous substrate. The membrane assembly separates the top chamber and bottom chamber (reservoir). The fluids added into the chambers subject the membrane assembly to the required chemical stimulus to move fluid into

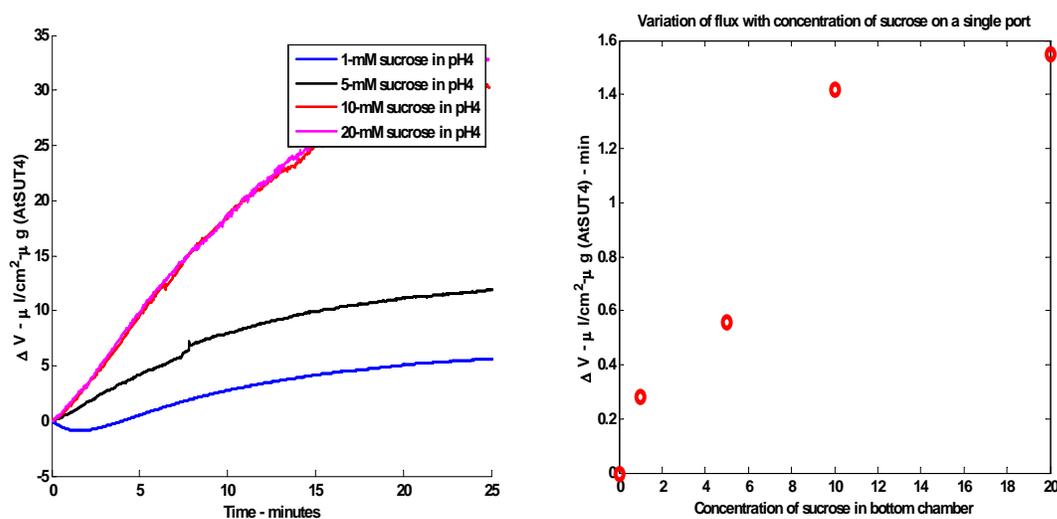


**Figure 1.** Schematic - Device to transport fluid using proteins

the top chamber against the pressure head resulting from the fluid level. The *AtSUT4* transporter protein ( $\text{H}^+$ -sucrose cotransporter) that has the ability to move sucrose and proton for an applied chemical stimulus was chosen for this study. The proton-sucrose cotransporter transports sucrose that results in an osmolar gradient across the membrane assembly which in turn results in the transport of water molecules into the top chamber.

Sucrose transport in plants is significant to plant physiologists to understand the transport of synthesized carbon. Identification of the SUT4 reported by Frommer et. al [6] and further investigation by his research group following their first report in 2000 characterized the transporter protein. The *AtSUT4* transporter protein has been reported to conduct proton and sucrose down a concentration gradient of protons. This protein can actively transport sucrose against the concentration gradient of sucrose expending ATP in the presence of the ATP-ase enzyme [7, 6, 8].

Our earlier investigation to use *AtSUT4* transporter for fluid transport (Sundaresan et. al [4]) demonstrated the ability to use the protein to transport fluid for an applied concentration gradient. The *AtSUT4* protein was reconstituted in a lipid bilayer membranes supported on 50% porous lead silicate glass plate. The BLM with *AtSUT4* transporters were subjected to sucrose gradient in addition to a proton gradient. Sucrose concentration gradient in the bottom chamber in the experimental setup served as the control parameter for fluid transport. A maximum rate of  $1.58 \mu\text{l}/\text{cm}^2\text{-}\mu\text{g}(\text{AtSUT4})\text{-min}$  was achieved through the functional membrane for pH4.0-pH7.0 concentration gradient and 10 mM sucrose in the pH4.0 medium. The results from experiments done for 1mM, 5mM, 10 mM and 20 mM sucrose concentration was reported. The flux rates extracted from this data and plotted against the concentration of sucrose predicts the increase in flux for each molar concentration increase in sucrose in the pH4.0 medium. The plot (b) in Figure 2 shows that the flux increases by  $0.0782 \mu\text{l}/\text{cm}^2\text{-}\mu\text{g}(\text{AtSUT4})\text{-min}$  for each molar increase in sucrose concentration for *AtSUT4* reconstituted on POPS:POPE BLM. Proton-sucrose co-transport through *AtSUT4* in the cell membrane resulting from ATP hydrolysis studied *in vivo* was reported by Weise et al [6]. This paper extends our previous work on fluid transport using concentration gradients, by demonstrating transport across the membrane assembly powered by a bio-chemical reaction.



**Figure 2.** Flux rate for concentration gradient driven transport [4]

### 3. Experimental Setup

The following materials and equipments were used for the experimental demonstration of ATP hydrolysis driven fluid transport.

**Substrate ‡ :** Porous lead silicate glass plate with 50% porosity

**Ion transporter & Enzymes §:** *AtSUT4* protein cultured from *Arabidopsis thaliana*, expressed in yeast, extracted and purified, suspended in pH7.0 medium (20.2 mg/ml), Red beet ATP-ase

**Fuel & Buffer ||:** Adenosine triphosphate - Magnesium Salt, Trizma base (pH7.0 buffer)

**Lipids ¶:** 1-Palmitoyl-2-Oleoyl- sn- Glycerol-3- [Phospho-L-Serine] (Sodium Salt) (POPS), 1-Palmitoyl-2-Oleoyl- sn- Glycerol-3- Phosphoethanolamine (POPE) mixed in the ratio 5:4 (w:w) and dissolved in n-decane (20 mg/ml)

**Electrodes:** Silver-Silver Chloride half-cell reference electrode <sup>+</sup>, Platinum wire <sup>\*</sup>

**Measurement equipments:** HP 4192 A impedance analyzer, Agilent E3648A digital power supply

The experiment can be divided into the following three steps:

- (i) Forming the BLM on Lead Silicate glass plate
- (ii) Reconstituting ion transporters in the BLM
- (iii) Fluid transport triggered by ATP hydrolysis

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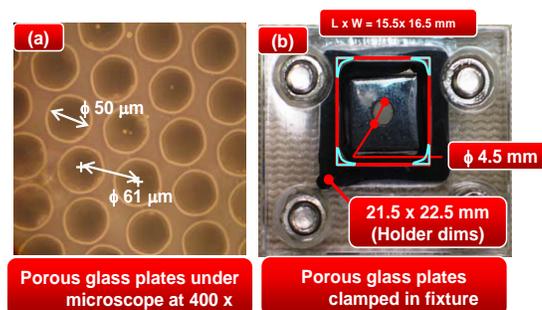
¶ Avanti Lipids, Alabaster, AL, USA

<sup>+</sup> WPI Inc., FL, USA

<sup>\*</sup> Surepure Chemetals, NJ, USA

The procedure to form a planar BLM on a lead silicate glass plate is based on the previous work in the biophysics community. The method to form a BLM on a single pore were demonstrated by Mueller et al [9], Tien's[10] and Ottova and Tien[11]. Recent studies on the formation of BLM on a surface treated substrate as in Wagner et al[12], Cheng et al [13], Saccani et al[14], Sackmann[15], Yang et al[16], Fujiwara et al[17] and Ide and Ichikawa[18] suggests a variety of methods to stabilize the BLM.

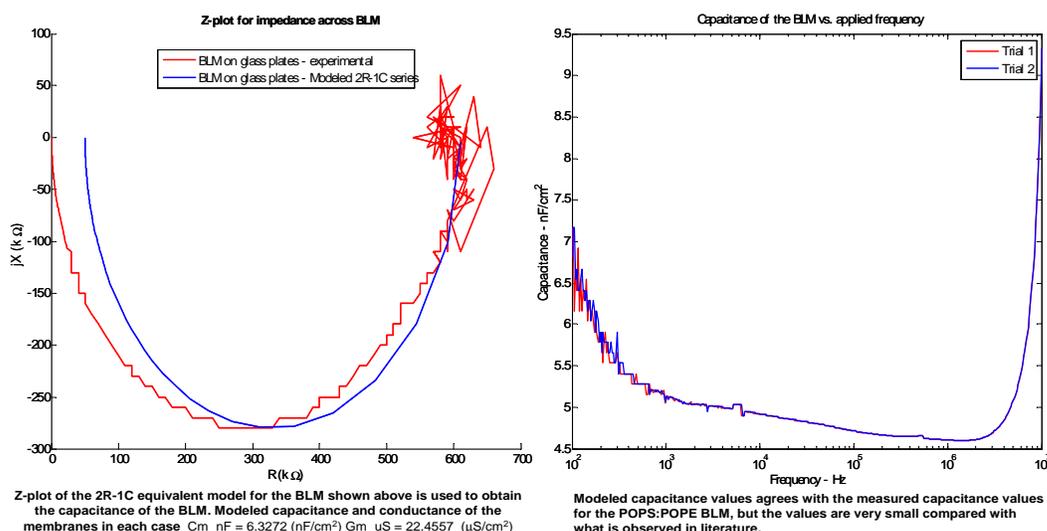
For our work, the formation of a BLM over large porous lead silicate glass plate for the purpose of creating a device for fluid transport is achieved without any chemical modification as reported in Sundaresan et. al [4]. Figure 3 (a) shows the array of circular holes seen under a microscope [Carl Zeiss Axiovert 40] at 400X magnification. The effective substrate area on which the BLM was reconstituted was restricted to a small circular area (4.5 mm diameter) by masking the glass plate with adhesive backed gasket and a transparent tape as shown in Figure 3 (b). The glass plates were thoroughly rinsed in concentrated hydrochloric acid before masking to remove any organic residue from storage. After masking, the exposed region of the substrate was thoroughly washed with 50% iso-propyl alcohol and de-ionized water. Compressed air at 150 kPa was used to blow the remaining water from pores. After this, 10  $\mu$ l of the phospholipid mixture was spread on the exposed region of the glass plate and dried under a stream of Nitrogen. The fixture with lipids was allowed to stand for 5 minutes before introducing it into the reservoir with pH7.0 buffer.



**Figure 3.** Glass plate used for demonstrating ATP-powered transport

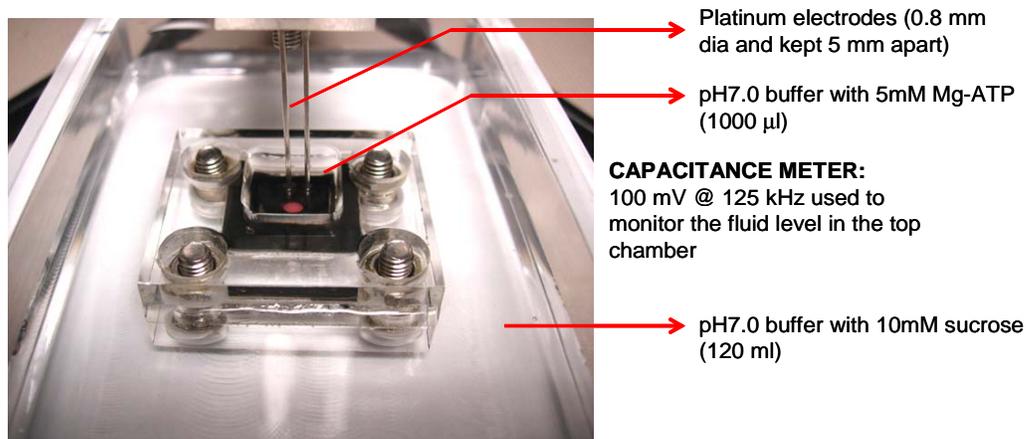
Impedance measurement is an appropriate method for confirming the presence of a BLM as discussed in Galla et al[19]. Impedance measured over a frequency range from 5 Hz to 1 MHz at 50 mV oscillator level using the HP 4192A Impedance Analyzer was used to calculate the capacitance of the self-assembled BLM. The stability of the impedance value measured over time was used as a qualitative measure to detect the ability of the BLM to hold on to the substrate. Capacitance is computed from measured real( $R$ ) and imaginary( $X$ ) parts of the complex impedance ( $Z$ ) as discussed in Sundaresan et. al [4]. The modeled impedance, resulting capacitance and measured capacitance are shown in Figure 4. Low values of capacitance can be attributed to the electrical measurements done on BLM formed across an array of pores.

The BLM with *AtSUT4* was reconstituted in the exposed circular region on the



**Figure 4.** Electrical measurements across the Bilayer lipid membrane

glass plates. After thorough cleaning with 50% iso-propyl alcohol and compressed air, 10  $\mu$ l of phospholipid mixture was added to the porous region and dried under nitrogen. On top of this 10  $\mu$ l of the *AtSUT4* transporter suspended in pH7.0 medium was added. Varying quantities of the red beet ATP-ase was added to the glass plate to quantify the effect of enzyme concentration on the fluid transport. The contents added in the pores of the plates were allowed to stand for 5-10 minutes before introducing them into the fixture. The reservoir was filled with pH7.0 buffer mixed with 10 mM sucrose. The buffer with a pH7.0 with dissolved ATP was added to the top chamber. Figure 5 shows the experimental setup for quantifying fluid transport through the BLM with ion transporters.



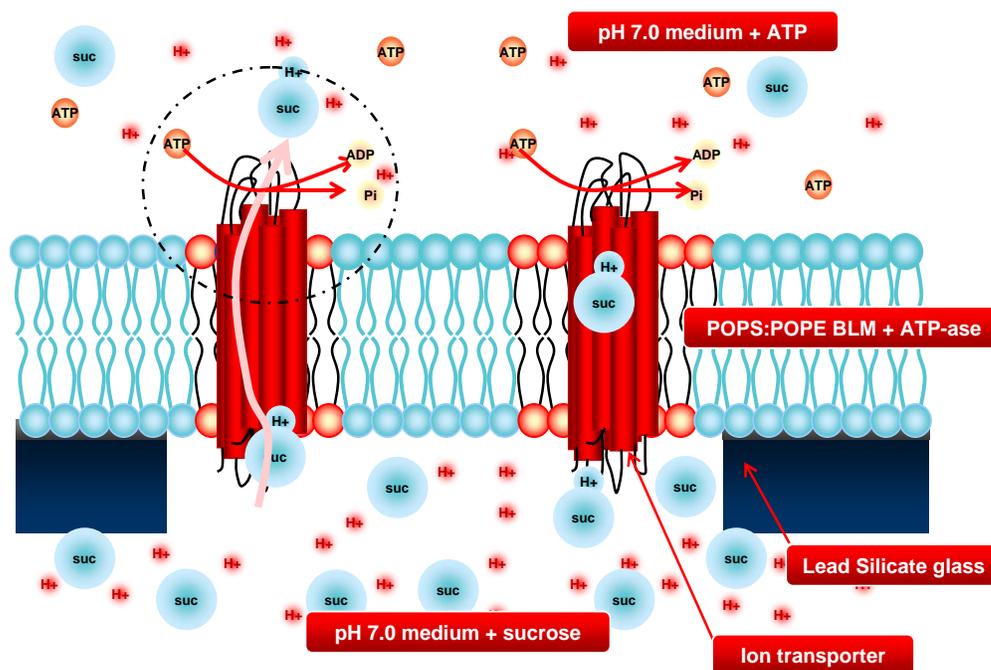
**Figure 5.** Experiment setup to demonstrate fluid transport

The change in fluid level was calculated by measuring the change in ionic conductance between the platinum electrodes (0.8 mm diameter) kept immersed in the

fluid within the top chamber. The depth of immersion of the electrodes was always kept a constant at 5 mm. We used the HP 4192A impedance analyzer for measuring the capacitance across the platinum electrodes by applying a 100 mV signal at 100 kHz. Prior to the experiment, change in conductance was correlated to the change in fluid level, producing a calibration curve for measuring the change in fluid level in the top chamber. The mean change in capacitance of the fluid between the electrodes was found to be 10.2 nF/mm change in depth of immersion of the electrodes. The change in capacitance for 1 mm change in depth of immersion of electrodes in pH7.0 buffer with Mg-ATP and 1 mM to 5 mM sucrose mixed with pH7.0 buffer was verified to be within 1% tolerance limit. This allowed monitoring the fluid level and quantifying the change in volume in the top chamber real time.

#### 4. ATP-Powered Transport through *AtSUT4* protein pump

The schematic described in Figure 6 shows the mechanism by which ATP hydrolysis establishes a pH gradient locally across the transporter to transport sucrose. ATP fuel was added to the top chamber as Mg-ATP salt dissolved in pH7.0 buffer. The sucrose

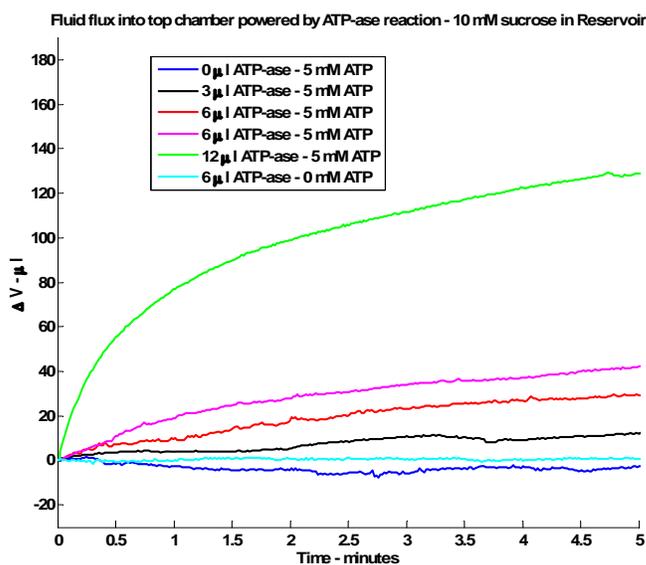


**Figure 6.** Principle of ATP-hydrolysis powered fluid transport

transporter protein was cultured in yeast cells, extracted and purified and supplied to us in pH7.0 medium in frozen state. The *AtSUT4* transporters are added onto the porous lead silicate glass plates with the ATP-ase enzyme. The *AtSUT4* proteins are available as vesicles with the yeast cell membranes. They spread on the lipids added to the substrates and form into a planar BLM, inserting the protein across the membrane as shown in the schematic in Figure 6. The enzyme helps to bind the ATP molecule

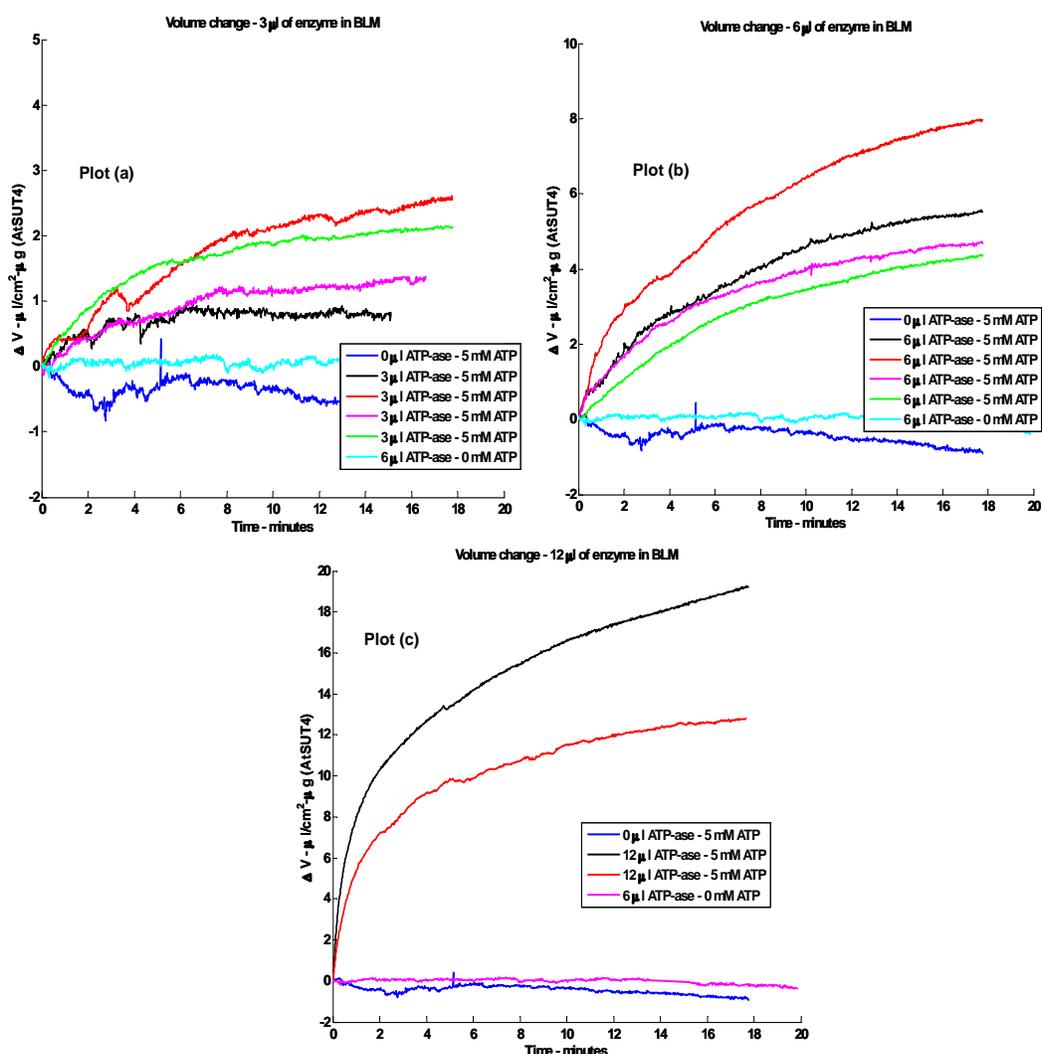
to the protein and establishes a localized proton gradient across the transporter. This serves as a trigger for proton and sucrose transport through the membrane. The sucrose transported through the protein also carries water molecules with it to result in bulk fluid transport.

The red beet ATP-ase added to the BLM was varied to study the effect of the quantity of the enzyme that catalyzes the reaction and hence fluid transport. The BLM was formed with pH7.0 buffer carrying 10 mM sucrose supporting it at the bottom and pH7.0 buffer with Mg-ATP salt in the top and dispersed with *AtSUT4* transporters. As a control study, enzyme was not added to the lipids and the flux was recorded for 10mM sucrose and 5mM ATP in the bottom chamber and top chamber respectively. In the absence of ATP-ase enzyme, hydrolysis of ATP is not initiated and hence no transport was observed.



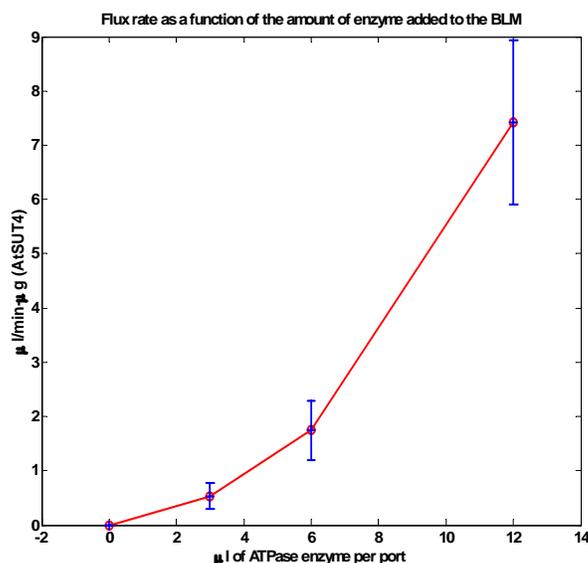
**Figure 7.** Bulk fluid flux through *AtSUT4* for varying amounts of enzyme in BLM

In the next trial, enzyme was added to the BLM. Plain pH7.0 buffer without ATP fuel was added to the top chamber. Along expected lines, no transport of fluid was observed due to the absence of ATP. In subsequent trials, the amount of the enzyme added to the plates was varied. Results in Figure 7 shows a representative data for the total flux observed for 3  $\mu$ l, 6  $\mu$ l and 12  $\mu$ l of enzyme added to the bilayer. The plots (a), (b) and (c) in Figure 8 shows the change in volume with time in top chamber normalized with respect to the area of the BLM and amount of *AtSUT4* transporters for different amounts of ATPase enzyme. Plot (d) in Figure 8 shows the normalized flux rate for different amounts of enzyme added to the BLM with 95% confidence interval obtained from t-distribution (8 trials at 3  $\mu$ l, 6  $\mu$ l and 3 trials at 12  $\mu$ l of enzyme). It is observed from this plot that ATP in the presence of ATP-ase enzyme results in fluid flux against gravity into the top chamber. Flux rate is observed to be dependent on



**Figure 8.** Effect of amount of enzyme on flux rate

the amount of enzyme added to the BLM. It is maximum in the first 5 minutes of the experiment for 6  $\mu\text{l}$  of ATP-ase enzyme in the BLM and in the first minute for 12  $\mu\text{l}$  enzyme added to the BLM. The flux rate decreases after the initial spike and settles at a steady value for the next 15 minutes under observation as shown in plots (a), (b), (c) in Figure 8. The initial rate (flux through transporter for the first minute) observed for ATP powered transport in Figure 9 is plotted from individual results shown in Figure 8. The initial rate for the ATP powered transport is approximately 8-10 times higher than the rate observed with proton concentration gradient driven transport shown in plot (b) in Figure 2. The negative flux observed in Plot(a),(b), (c) in Figure 8 are in the absence of the ATP-ase reaction in the medium. In the absence of the biochemical reaction, the experimental results indicate the flow of fluid from the top chamber to the bottom chamber. This is due to osmotic regulation resulting from higher concentration of sucrose in the bottom chamber. Since water molecules are permeable through the BLM, the top chamber drains to maintain osmolarity.



**Figure 9.** Normalized flux rate through *AtSUT4* vs. Amount of ATP-ase enzyme

## 5. Conclusion

This paper demonstrates that the fluid flow through protein pumps incorporated in a bilayer membrane can be controlled by varying the amount of ATP-ase enzyme. Bilayer lipid membranes formed using POPS:POPE lipids were supported on a lead silicate glass plate with an organized array of 50  $\mu\text{m}$  pores. This served as the host membrane for reconstituting *AtSUT4* ion transporter with the red beet ATP-ase. This article builds upon the previous work and demonstrates the ability to transport fluid using the free energy from ATP hydrolysis. It is observed that fluid transport powered by bio-fuel (Adenosine tri-phosphate) through a bilayer lipid membrane (BLM) on a porous substrate has a high initial transport rate. Initial flux rates of 6 - 9  $\mu\text{l}/\text{cm}^2\text{-}\mu\text{g}(\text{AtSUT4})\text{-min}$ , 1.25 - 2.25  $\mu\text{l}/\text{cm}^2\text{-}\mu\text{g}(\text{AtSUT4})\text{-min}$  and 0.25 - 0.68  $\mu\text{l}/\text{cm}^2\text{-}\mu\text{g}(\text{AtSUT4})\text{-min}$  were observed for 12  $\mu\text{l}$ , 6  $\mu\text{l}$  and 3  $\mu\text{l}$  of enzyme in the reconstituted BLM. ATP-powered transport is observed to be faster compared with our previous results with proton concentration gradient driven transport through *AtSUT4* co-transporter.

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