

Review

Activity assay of membrane transport proteins

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Membrane transport proteins are integral membrane proteins and considered as potential drug targets. Activity assay of transport proteins is essential for developing drugs to target these proteins. Major issues related to activity assessment of transport proteins include availability of transporters, transport activity of transporters, and interactions between ligands and transporters. Researchers need to consider the physiological status of proteins (bound in lipid membranes or purified), availability and specificity of substrates, and the purpose of the activity assay (screening, identifying, or comparing substrates and inhibitors) before choosing appropriate assay strategies and techniques. Transport proteins bound in vesicular membranes can be assayed for transporting substrate across membranes by means of uptake assay or entrance counterflow assay. Alternatively, transport proteins can be assayed for interactions with ligands by using techniques such as isothermal titration calorimetry, nuclear magnetic resonance spectroscopy, or surface plasmon resonance. Other methods and techniques such as fluorometry, scintillation proximity assay, electrophysiological assay, or stopped-flow assay could also be used for activity assay of transport proteins. In this paper the major strategies and techniques for activity assessment of membrane transport proteins are reviewed.

Keywords membrane transport protein; activity assay; protein-ligand interaction

Statistical analysis of available genome-wide sequences from eubacterial, archaean, and eukaryotic organisms revealed that 20%–30% of all open reading frames are predicted to encode integral membrane proteins (IMPs) [1]. A recent report using different computational methods

predicted that approximately 15%–39% of human genes encoded IMPs [2]. These IMPs locate in cell membranes and are involved in many biological processes, such as mediating the flow of materials and information between the cytosol and the extracellular environment, and energy generation and transformation. Receptors and transporters play key roles in regulating the physiological state of cells and are often the primary targets for pharmaceutical drugs. Actually more than half of today's drugs are targeted to these two IMP classes [3,4]. Research on receptors and transport proteins is one of the hottest biological areas. To date, many techniques and theories have been developed to facilitate structural and functional investigations of the two IMP classes. For screening potential drugs, techniques for assaying interaction between ligands and target proteins are very important. For transport proteins, the main focus is on transport assays of substrates and inhibitors.

All living cells are able to obtain nutrients and other essential substrates for metabolism and excrete metabolic waste and other products. This process is either energy-dependent or energy-independent and carried out by different kinds of transport proteins. As recommended by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB), the term “transport protein” refers to all membrane proteins directly involved in different transport mechanisms (<http://www.chem.qmul.ac.uk/iubmb/mtp/>). These proteins are responsible for non-specific permeation or specific transport of materials [5] (**Table 1**). In bacteria, 3%–15% of genes are predicted to encode membrane transport proteins [6–9].

Lipid membranes are essential for maintaining the structure and functions of transport proteins and always among the important factors to be considered in activity assay experiments. Other factors include energy supply, protein expression, physiological form and state of proteins, and specificity of substrates and inhibitors. There have been many publications about membrane transport proteins and their

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Table 1 Cellular transport systems and mechanisms [5]

Types of mechanisms	Transport systems/transported solutes
Non-specific permeation	Hydrophobic domains of membranes/various small, medium-sized and large lipophilic molecules
	Water-filled pores/virtually all solutes up to a certain size
	True pores in the lipid bilayer/all solutes, including macromolecules
	Non-receptor endocytosis/all solutes present in extracellular aqueous medium
Specific transport	Selective channels/cations, anions, water, also non-electrolytes
	Specific carriers
	Mediated (or facilitated) diffusion/monosaccharides in animal, non-insulin-dependent tissues, some yeast cells
	Primary active transport/cations, anions, amino acids, sugars, xenobiotics
	Secondary active transport/various non-electrolytes (mainly nutrients), anions cations (often in combination), ADP/ATP
	Group translocation/monosaccharides and disaccharides, amino acids
	Receptor-mediated pinocytosis/ferritransferrin, hormones

structure, functions, and applications in medicine [10–12].

The emphasis of this paper is the major strategies and techniques in assaying the activity of transport proteins including pores, channels, transporters, carriers, and translocators. Transport involving endocytosis and exocytosis will not be discussed.

Availability of Transport Proteins for Activity Assay

In nature, many membrane proteins are expressed at very low levels. Expression and isolation is always the bottleneck for research in membrane transport proteins, especially for structural investigation [13]. Some common problems for the recombinant production of membrane proteins are: low overall yields due to a lack of intrinsic stability or folding efficiency; susceptibility to proteolytic degradation; toxicity of the recombinant product; low translational efficiency caused by different codon usage; wrong or missing post-translational modifications; and mistargeting, such as accumulation of plasma membrane proteins in internal membrane.

In transport assays, only a small amount of proteins show detectable activity. Thus, the challenge is to avoid interference from irrelevant proteins. Homogeneity and quality are more important than the quantity of proteins. For example, if one needs to examine the nucleoside transport in *Escherichia coli*, researchers have to avoid interference from nucleoside transporters such as NupC, NupG, and XapB. This can be achieved by using a transporter-deficient strain [14] or purifying and assaying target transport proteins [15].

Expression of transport proteins can be naturally occurring or artificially controlled. Development of modern molecular biology has made possible deliberate gene expression. Nowadays, the following strategy is most effective for recombinant production of membrane transport proteins [13].

(1) Select the gene of interest, transfer it into an expression vector, and construct a recombinant form of target protein containing an affinity tag. The affinity tag can be used for monitoring the expression and purification of the target protein. However, researchers need to test whether the affinity tag affects the expression, structure, and activity of transporters by changing and comparing the types and position of the affinity tag. For example, Mohanty and Wiener analyzed the effects of polyhistidine tag length and position on the expression of *E. coli* water channel AqpZ [16]. They placed 6- or 10-histidine tags at the N- or C-termini of AqpZ and found that expression levels were similar and tag length had a greater effect than tag position upon yield.

(2) Select the expression host and optimize growth conditions to induce the expression of the target transport protein. When using a eukaryotic expression system, researchers need to identify the membrane localization of target transporters. Green fluorescent protein fusions are useful to visualize whether transporters are expressed in plasma membranes or internal membranes. Using green fluorescent protein-tagged transporters, Bai *et al* showed that human sodium-dependent dicarboxylate co-transporter protein 1 predominantly locates on the plasma membrane, consistent with the results predicted by a bioinformatics approach [17]. Proteins localized to different membranes

might also require different assays for activity. If protein expression is detected in plasma membranes, the transport assay can be carried out in intact cells. However, for proteins located at internal membranes, vesicles or purified protein/proteoliposomes would need to be used. In a different expression strategy, the *Xenopus* oocyte expression system is used and the researcher needs to inject mRNA or DNA into the eggs to initiate protein expression [18].

(3) Following the expression of target proteins, membrane vesicles containing expressed transport proteins can be isolated. Different types of membrane vesicles can be obtained at this stage. For plasma membranes, researchers can use the French press technique to obtain reversed membrane vesicles [inside out membrane vesicles (IOVs)] [19]. Or researchers can use the method described by Hugenholtz *et al* [20] to isolate right side out membrane vesicles (RSOVs). Internal membrane vesicles such as mitochondria and chloroplasts can be isolated by disrupting cells and subsequent centrifugation [21,22].

(4) With proper detergents, membrane proteins can be solubilized and affinity purified. Detergents have two major roles in membrane protein purification: disrupting lipid membranes and releasing lipid-bound membrane proteins; and maintaining the native and functional structure of lipid-free membrane proteins [23]. Detergents vary in their chemical structures and physical properties. Researchers need to screen and find the most appropriate detergent for the protein of interest. Purification of lipid-free membrane proteins is similar to that of soluble ones. Detergents have to be included at a concentration above critical micelle concentration in buffers to maintain the native structure of membrane proteins. Purified membrane transporters can be assayed for interactions with substrates. Alternatively, these purified transporters could be reconstituted into liposomes for transport assay [13].

The remainder of this paper will give more information regarding activity assays for transport proteins. To monitor substrate transport across lipid membranes, transporters should be bound in vesicular membranes. In the assay, researchers need to examine changes of substrates in the vesicle lumen. To monitor interaction between ligands and transport proteins, the protein can be in purified form or bound in lipid membranes. Various techniques can be applied to investigate ligand-protein interactions.

Assaying Transport Activity of Membrane Proteins

Assay strategies and principles

The basic procedure for transport assay (uptake assay) is

as follows: (1) prepare membrane vesicles bearing transport protein of interest; (2) initiate uptake by supplying with substrate and/or energy; and (3) terminate uptake at the desired time and monitor the intake of substrate [24]. The conventional method to index the intake of substrate is by using isotopically-labeled substrates [24]. Alternatively, movement of fluorescent substrates or fluorescence change in indicators related to a transport process can be measured [25,26]. Changes in pH and the volume of vesicles can also be used as transport assay indexes [25,27].

In transport assays, the intake of labeled substrate mediated by transport proteins is described by **Equation 1**:

$$V_{in} = \frac{V_{max} [S]_{out}}{K_m + [S]_{out}} \quad 1$$

where V_{in} refers to the substrate intake rate, V_{max} refers to the maximal substrate intake rate, $[S]_{out}$ refers to the environmental substrate concentration, and K_m refers to the Michaelis-Menten constant.

There are three destinations of intake substrate.

(1) The substrate can be metabolized and will result in the decrease of substrate concentration within intact cells. If this happens, researchers can eliminate the metabolism system by either using enzyme-deficient mutants or using membrane vesicles instead of intact cells. Or researchers could select a non-metabolizable analog. For example, 3-O-methylglucose is a non-metabolizable glucose analog and can be used as a marker to assess transport by evaluating its uptake within various cells [27–29].

(2) If the substrate is not metabolized and does not exit, it will remain in the cell. This is the ideal situation and the change of substrate within the cell or vesicles can be monitored. For determination of Michaelis-Menten kinetics, researchers have to measure the linear initial transport rate. This is impossible because the reaction occurs too quickly. Practically, a short transport period is used to approximate initial velocities of transport. For example, researchers monitored the 15 s or 1 min uptake for estimation of the apparent V_{max} and K_m values for nucleoside transport in *E. coli* or putrescine transport in the cyanobacterium *Synechocystis* sp. PCC 6803 [15,30].

(3) The substrate can exit from the cell or closed membrane vesicle. One example is the transport mediated by transport systems such as pores or channels. Another example is artificial proteoliposomes where the same amounts of reconstituted proteins are facing both sides of the lipid membranes. In both cases the passage of substrates takes place in two directions. The intake rate (V_{in}) is determined by **Equation 1** and the outflow rate (V_{out}) is given by **Equa-**

tion 2:

$$V_{\text{out}} = \frac{V_{\text{max}} [S]_{\text{in}}}{K_m + [S]_{\text{in}}} \quad 2$$

where V_{max} refers to the maximal substrate transport rate, $[S]_{\text{in}}$ refers to the substrate concentration in the lumen, and K_m refers to the Michaelis-Menten constant.

If intake and outflow rates are similar and the environmental substrate concentration is low, there is a difficulty with this technique in monitoring the change of substrate concentration between the lumen and the environment. To solve this problem, the entrance counterflow assay was developed [13] that relies on two types of substrates, isotope-labeled and unlabeled. Membrane vesicles are preloaded with unlabeled substrate at a high concentration. Transport assay is initiated by dilution of preloaded vesicles into large volumes of buffers containing isotope-labeled substrate at a low concentration. Both labeled and unlabeled substrates can be transported in dual directions (towards the outside or the inside of membrane vesicles). It can be defined that $[S^U]_{\text{in}}$ and $[S^R]_{\text{in}}$ are the concentrations of unlabeled and radiolabeled substrates, respectively, in vesicle lumen. $[S^U]_{\text{out}}$ and $[S^R]_{\text{out}}$ are environmental concentrations of unlabeled and radiolabeled substrates, respectively. The intake and outflow rates of radiolabeled substrate (V_{in}^R and V_{out}^R) and unlabeled substrate (V_{in}^U and V_{out}^U) are determined by the following **Equations**:

$$V_{\text{in}}^R = \frac{V_{\text{in}} \times [S^R]_{\text{out}}}{[S^U]_{\text{out}} + [S^R]_{\text{out}}} \quad 3$$

$$V_{\text{out}}^R = \frac{V_{\text{out}} \times [S^R]_{\text{in}}}{[S^U]_{\text{in}} + [S^R]_{\text{in}}} \quad 4$$

$$V_{\text{in}}^U = \frac{V_{\text{in}} \times [S^U]_{\text{out}}}{[S^U]_{\text{out}} + [S^R]_{\text{out}}} \quad 5$$

$$V_{\text{out}}^U = \frac{V_{\text{out}} \times [S^U]_{\text{in}}}{[S^U]_{\text{in}} + [S^R]_{\text{in}}} \quad 6$$

The different stages of the entrance counterflow assay are shown in **Fig. 1**. This assay is extremely useful in assaying different types of substrates. Practically, it is difficult and expensive to radiolabel all types of substrates. However, with the entrance counterflow method, researchers can assay different substrates by changing the types of loaded unlabeled substrate in the vesicle lumen and comparing resultant changes in radiolabeled substrate uptake. Using the entrance counterflow assay, transporters such as galactose transporter GalP and nucleoside transporter

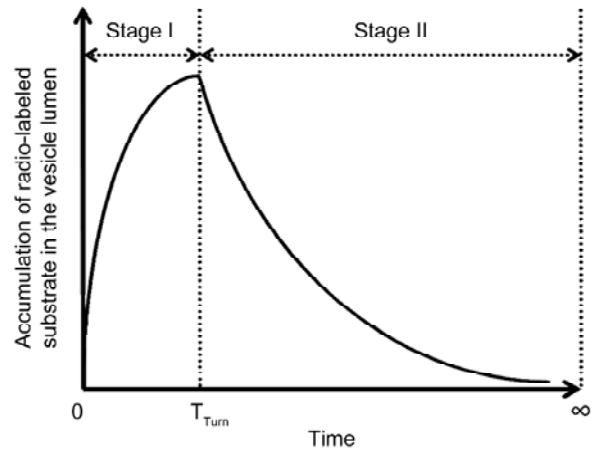


Fig. 1 Illustration of different stages in an entrance counterflow transport assay (1) At initial time $t=0$, the concentration of radiolabeled substrate in the lumen is 0, and the concentration gradient drives the radiolabeled substrate into the vesicle. (2) At stage I, the accumulated radiolabeled substrate in the lumen will be transported out together with the unlabeled substrate. However, the outflow rate of the radiolabeled substrate is lower than its intake rate. The radiolabeled substrate will still be accumulated. (3) At time $t=T_{\text{Turn}}$, the radiolabeled substrate reaches its maximum concentration in the lumen. (4) At stage II, accumulated radiolabeled substrate will be transported out faster than it will be transported in, and the concentration of radiolabeled substrates in the vesicle lumen is decreasing. (5) At time $t=\infty$, both unlabeled and radiolabeled substrates are equilibrated between the environment and the lumen, and the radiolabeled substrate will enter and exit the vesicle at the same rate.

NupG were investigated for their activity in proteoliposomes [13,15].

Transport assay in intact cells

There is at least one disadvantage of the uptake assay in intact cells, in that live cells have a metabolic mechanism to modify or degrade substrates and will affect the subsequent measurement. However, there are some advantages, including the fact that the transport protein is in its natural status, and intact cells have a complete system to support transport. To monitor substrate transport in intact cells, researchers must first mix intact cells with labeled substrates, and initiate uptake with energy and oxygen at the appropriate temperature. Then for different expression systems, researchers will choose different methods, such as centrifugation or filtration, to stop the uptake interval by separating cells from the environmental labeled substrate [24]. Other methods can also be used, such as reducing the temperature or adding inhibitors [24]. **Table 2** compares different procedures for ending the uptake interval. A combination of the above methods can also be

Table 2 Comparison of procedures for ending uptake interval

Procedures for ending uptake interval	Expression system and culturing methods	Examples of assayed transporters
Vacuum filtration	Yeast and bacteria cultured in shaking flask	Polyamine transporters DUR3 and SAM3 (<i>Saccharomyces cerevisiae</i>) [31], nucleoside transporter NupG (<i>Escherichia coli</i>) [15], putative drug transporter (<i>E. coli</i>) [32], glucose transporter GlcP (<i>Bifidobacterium longum</i>) [33]
Centrifugation	Cells cultured in shaking flask	Major tripartite multidrug efflux pumps (<i>E. coli</i>) [34]
Rinsing the monolayers of cultured cells	Cells cultured on surface of cell-culture plates	Sodium-ascorbic acid transporter SVCT1 [35], S-Nitroso-L-cysteine stereoselective transporters [36], renal urate-anion exchanger URAT1 [37]
Simple wash	<i>Xenopus</i> oocyte	Human organic anion transporter [38], <i>Arabidopsis</i> sugar alcohol permease homolog AtPLT5 [39], aquaporin-8 (rat, human, mouse) [40]

considered. The amount of proteins or cells involved in assays can be determined by a variety of methods, such as the bicinchoninic acid protein assay or spectrometry. More detailed protocols for transport assay in intact cells have been described by Jarvis [24].

Transport assay in RSOVs and IOVs

RSOVs are obtained by removing cell plasma and keeping the original orientation of plasma membranes [20]. These vesicles can be assayed for uptake by supplying an appropriate substrate and establishing a driving force, such as proton motive force, for secondary transporters. The advantage of using RSOVs for transport assay is significant: the intrinsic metabolic system is removed so that the intake substrate will not be degraded or metabolized. Sometimes researchers might also obtain reversed membrane vesicles (IOVs) [19]. The IOVs are broadly used for assaying substrate transport in primary transport systems. Researchers can easily supply ATP to the transport system, which is exofacial in IOVs, and establish the driving force for substrate transport.

Lots of transport proteins have been assayed using RSOVs and IOVs. For example, Xie *et al* [15] observed and assayed nucleoside transport in RSOVs of *E. coli*. Ames *et al* [41] examined histidine transport in IOVs of *E. coli* and found that ATP induced efflux of histidine from IOVs. Thanassi *et al* [42] investigated bile salts transport in IOVs of *E. coli* and found that everted membrane vesicles accumulated bile salts in an energy-dependent manner. Soksawatmaekhin *et al* [43,44] also compared transport properties of transporter CadB in RSOVs and IOVs.

However, there are still disadvantages for activity assay

in RSOVs or IOVs. As well as target transport proteins, membrane vesicles still contain other irrelevant membrane proteins, such as pores or enzymes that might affect transport assays.

Transport assay in artificial proteoliposomes

Purified transporters can be reconstituted into liposomes by dilution of a ternary mixture containing proteins, lipids, and detergents [13,45]. Or researchers can use BioBeads (Bio-Rad, Hercules, USA) to absorb and remove detergents [13]. Once the free detergent concentration in the mixture is lower than the critical micellar concentration, detergent is recruited from the bound detergent pool, and the association of proteins and lipids is initiated. The reconstituted proteoliposomes contain a single type of transporter facing both sides of the lipid membrane and can be assayed for transport activity. For example, Xie *et al* [15] purified and reconstituted nucleoside transporter NupG into liposomes and observed nucleoside transport in proteoliposomes. Juge *et al* [46] co-reconstituted vesicular glutamate transporter VGLUT1 and bacterial F-ATPase into liposomes and found that ATP induced L-glutamate uptake in proteoliposomes. Bowsher *et al* reconstituted amyloplast envelope membrane proteins from spring wheat and assayed ADP, AMP, and ADP-glucose transport in these proteoliposomes [47]. Eytan *et al* reconstituted P-glycoprotein from cultured multidrug-resistant Chinese hamster ovary cells and observed ATP-driven, valinomycin-dependent uptake of rubidium in these proteoliposomes [48,49].

Assaying Interactions between Substrate and Transport Proteins

Sometimes transport efficiency is low (for example, use of inhibitors) and it results in technique difficulties for activity assay. An alternative choice is to measure the interaction between substrates (inhibitors) and transport proteins. Both purified transport proteins and membrane vesicles can be used for ligand-protein interaction assays. Although lipid membranes still play important roles in maintaining the functional structure of transport proteins, they are not essential for ligand-protein interaction assay. Most assaying techniques for ligand-protein interactions can be used for transport proteins. The concentration change of substrate after ligand-protein interaction can be directly measured. Research examples include investigation into the interaction between galactose or glucose transporters and inhibitors such as forskolin and cytochalasin B using the equilibrium dialysis method [50–52].

The interaction between ligands and proteins can change the conformation and energy status of proteins. Scientists take advantage of these changes and have developed techniques such as isothermal titration calorimetry (ITC) and nuclear magnetic resonance (NMR) spectroscopy to monitor interaction between ligands and proteins. ITC is a biophysical quantitative technique used to determine the thermodynamic parameters (binding affinity, enthalpy changes, and binding stoichiometry) of biochemical interactions. Using ITC, tungstate transport protein A was observed to bind tungstate and molybdate and the dissociation constant for binding was also determined [53]. Wei and Fu also observed selective metal binding to a membrane-embedded aspartate in the *E. coli* metal transporter YiiP [54].

NMR spectroscopy is a powerful technique used to obtain physical, chemical, electronic, and structural information about molecules. NMR spectroscopy depends on the splitting of nuclear energy levels in a magnetic field and the transition induced between the levels. Interaction between many transporters and substrates has been assayed using this technique [6]. For example, Patching *et al* [55] assayed the interaction between methyl- β -D-glucuronide with glucuronide transporter GusB and revealed the dissociation constant K_D is higher than the Michaelis-Menten constant K_m for energized transport.

Other Techniques for Functional Assay of Transport Proteins

Fluorometry

Fluorometry is an analytical technique for identifying and characterizing minute amounts of a substance by excitation of the substance with a beam of ultraviolet light and detection and measurement of the characteristic wave-

length of fluorescent light emitted. Fluorometry is often a technique used to monitor substrate transport by transporters. For example, Woebking *et al* [26] assayed ethidium transport mediated by ATP-binding cassette transporter MsbA expressed in intact *Lactococcus lactis* cells. In transport assay, cells were preloaded with the fluorescent substrate until a steady-state level was reached. Then glucose was added to the cells as a source of metabolic energy, after which the ethidium fluorescence was monitored. With this method, Woebking *et al* [26] showed that MsbA-mediated efflux of ethidium is affected by the protein expression level. They also investigated the Michaelis-Menten kinetic of MsbA-mediated ethidium transport.

Scintillation proximity assay (SPA)

SPA is a technique for carrying out binding assays without separation of bound and unbound radiotracers. In SPA, the scintillant is incorporated into small fluomicrospheres (beads). If a radioactive molecule is bound to the bead, it can stimulate the scintillant to emit light. The unbound radioactivity is too distant from the scintillant and the energy released is dissipated before reaching the bead, therefore these beads do not produce a signal. Quick and Javitch [56] described a direct scintillation proximity-based isotope-binding assay for determining transport protein functions in crude cell extracts and in purified form. The copper chelate SPA scintillation beads were used to immobilize His-tagged Na⁺/tyrosine transporter Tyt1, which binds radiolabeled tyrosine. The bound radiolabeled tyrosine stimulated SPA beads and produced a signal as an index for the binding assay. With SPA, the activity of the Na⁺/tyrosine transporter Tyt1 has been investigated and confirmed.

Surface plasmon resonance (SPR)

SPR is a technique to measure biomolecular interactions in real time in a label-free environment. SPR-based instruments use an optical method to measure the refractive index near the sensor surface. Using SPR, Benabdelhak *et al* [57] characterized a specific interaction between the nucleotide-binding domain of the ATP-binding cassette transporter HlyB and a C-terminal fragment of its transport substrate haemolysin A. The C-terminal fragment of haemolysin A was expressed and immobilized on the sensor surface. The specific interaction between this peptide and the nucleotide-binding domain of HlyB results in the change of reflected light and is used as an index for binding assays.

Electrophysiological assays

For any transporter that transports a net charge, there are

electrophysiological assays, including patch clamping and two-electrode voltage clamping, for recording membrane potential [58]. The assays can be applied to both cultured cells and *Xenopus* oocytes. For example, Glaaser *et al* [59] and Romanenko *et al* [60] assayed activity of sodium channels and potassium channels expressed in cultured cells, and Reinders *et al* [39] and Vicente *et al* [61] investigated the transport activity of *Arabidopsis* sugar alcohol permease homolog AtPLT5 and potassium channels expressed in *Xenopus* oocytes.

Spectrometric assays

Heuberger *et al* [62,63] developed a spectroscopic carbohydrate-transport assay that does not require isotopically-labeled substrates. They constructed a membrane system (hybrid membranes or proteoliposomes) bearing the transport system of interest, and soluble glucose dehydrogenase and the electron acceptor 2,6-dichloroindophenol enclosed in the vesicle lumen. After transport across the vesicular membrane, the sugar is oxidized by soluble glucose dehydrogenase. The accompanying reduction of 2,6-dichloroindophenol results in a decrease in A_{600} and is used as the index for sugar transport. With this method both solute/ H^+ symport and exchange types of transport can be measured with high sensitivity in crude membranes as well as in proteoliposomes.

Stopped-flow assays

Stopped-flow is one of the most frequently used rapid kinetics techniques. Small volumes of solutions are driven through a mixer and pass through a measurement flow cell. Using appropriate techniques such as fluorescence spectrometry, the kinetics of the reaction in the solutions can be measured in the cell. For example, for activity measurement of metal transporters YiiP and ZitB of *E. coli* [25,54,64], researchers preloaded proteoliposomes with metal-sensitive fluorescence indicator fluozin-1 mixed with a buffer containing Zn^{2+} or Cd^{2+} . The uptake of metal into proteoliposomes resulted in fluorescence change of the indicator and was recorded in a stopped-flow apparatus. Water permeabilities in liposomes can also be measured by detecting the light scattering. Proteoliposomes are preloaded with sorbitol, sucrose, or mannitol at low concentrations, and mixed with assay buffers containing a high concentration of these chemicals in an assay. Because these chemicals are impermeant for proteoliposomes, the osmotic gradient drives the water efflux, and the consequent reduction in liposome volume is measured as an increase in the intensity of scattered light at 600 nm. The rate constant of the normalized light intensity increase in-

dicates the rate constant of water efflux, which is proportional to the water permeability coefficient [65,66]. Using this method, transport activity of water transporters like aquaporin-8 from rat, human, and mouse were assayed in proteoliposomes [40]. Mallo and Ashby showed that this method can also be used for assaying water permeability mediated by water transporter AqpZ in intact *E. coli* cells [67].

Conclusions

The unique properties and functions of membrane transport proteins make it possible to take advantage of various assay strategies and techniques. Although the conventional isotope-based technique is still the most reliable method, novel techniques such as ITC and SPA provide more options in assaying activity. Sometimes a combination of these methods will be more applicable for a specific transport protein. Researchers need to consider the physiological status of proteins (bound in lipid membranes or purified) and the purpose of the transport assay (screening, identifying, or comparing substrates and inhibitors) before choosing the most suitable assay strategies and techniques.

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