Functional Coating of Porous Silica Microparticles with Native Biomembranes towards Portable Flow-Through Biochemical Microreactors

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In biological cells, various transmembrane enzymes function as highly effective chemical reactors confined in space with characteristic length scales of tens of nanometers to micrometer. However, it is still challenging to quantitatively confine membranes in compact reactor platforms without losing their biochemical functions. Here, a simple and straightforward strategy towards the fabrication of a new flow-through reactor by the functional coating of porous silica microparticles with sarcoplasmic reticulum membranes is described. After a short incubation, the membranes achieve the homogeneous, full coverage of the particle surface, spanning across pores with the diameter of about 100 nm. By using the underlying pores as cavity reservoirs, transmembrane enzyme (Ca^{2+}-ATPase) in the membrane retains their capability of ATP hydrolysis. This enables us to confine 1.1 m^2 of native membranes containing a large amount of Ca^{2+}-ATPase (approx. 10 nmol) in a column-packaged, flow-through reactor with merely 1.8 mL volume, which cannot be achieved by the reconstitution of proteins in artificial lipid membranes or condensation of membranes in suspensions. The distinct functional levels corresponding to different reaction buffers can be reproduced even after many buffer exchanges over 14 days, confirming the stability and reproducibility of the membrane-particle hybrid reactors.

1. Introduction

Biological membranes are key components of living systems, forming the outer boundary of living cells or of intracellular organelles. They consist of a lipid bilayer with various proteins and carbohydrates that facilitate communication and transport across the membrane. Membranes do not only selectively filter specific nutrients, wastes and metabolites but also confine many biochemical processes to the organelles and keep toxic substances out of the cell. Prominent examples are transmembrane enzymes that act as the catalytic centers of many important biochemical reactors. For example, transmembrane ATPase catalyzes the hydrolysis of ATP and exchanges various metabolites and wastes, while glycosyltransferase catalyzes the biosynthesis of various carbohydrates.

The complexity of biological membranes and their interactions with intra- and extracellular networks makes direct investigations difficult. For this reason, artificial model membranes have played an important role in unraveling the physical and chemical characteristics of membranes. For about 20 years now, phospholipid membranes deposited onto solid substrates (supported membranes) have been the most commonly used cell surface model systems and enable us to understand the principle of immune reactions and cell adhesion processes.[1–4] They are readily prepared by directly depositing lipid monolayers or bilayers on solid surfaces, maintaining excellent mechanical stability without loosing their fluid nature. The combination of fluidity and stability on planar surfaces offers unique advantages over freestanding black lipid membranes or spherical lipid vesicles, because it makes it possible to carry out experiments and use analytical methods, such as FTIR,[5] surface plasmon resonance,[6] and X-ray and neutron scattering.[7–9] Membranes can also be deposited on microparticles with diameters of 3–30 μm by fusion of lipid vesicles.[10] Particle-supported membranes offer unique advantages over membranes on planar supports due to the increase in surface area on microparticles. For example, the interaction of analytes with membranes or membrane-associated proteins, the larger accessible area will significantly improve the signal of spectroscopic techniques, such as NMR.[10] To date, particle-supported membranes have been functionalized by incorporating various lipids and lipid-anchored proteins to detect the enzymatic degradation of phosphatidylinositol,[11] binding of proteins to

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gangliosides,[12] and protein clustering during recruitment of synaptic vesicles.[13]

Not only artificial lipid membranes but also native membranes can be deposited on particle supports. Branton et al. reported the two-step immobilization of cell membranes onto latex beads (diameter: 30 μm) coated with poly-(L-lysine): the cell firstly adhered onto polymer-coated particles, then the adhered cells were ruptured by brief sonication.[14,15] In 2003, we reported that human erythrocyte ghosts can be deposited in one step on solid and porous silica microparticles (diameter: 3–5 μm),[16] demonstrating the possibility to quickly isolate plasma membranes from intra-cellular elements. More recently, Roizard et al.[17] deposited the membrane extracts from cells on lectin-coated silica particles, and observed interactions between the different signaling partners.

However, the applications of particle-supported membranes in material sciences were rather limited. Although several studies proposed potential applications of particle-supported phospholipid membranes in drug screening[18] and purification of proteins,[19] there has been no study utilizing the transmembrane enzymes as biochemical micro-reactors. Since major practical difficulties are to reconstitute purified transmembrane proteins into vesicles at high concentrations, the deposition of native membranes incorporating transmembrane enzymes would therefore be a straightforward strategy to retain the natural composition of protein and lipid mixtures. In our recent account, we reported the deposition of native chitosomal membranes isolated from yeast cells on solid silica microparticles, and demonstrated that chitin synthase in supported membranes can be used for the in vitro synthesis of chitin polymers.[20] Owing to the large density of silica, particle-supported membranes and materials in bulk can be separated within 1 min, which allows for the termination of the polymerization reaction without the disruption of the whole membranes. However, the calculated enzymatic activity on particle supports is found to be approximately one order of magnitude smaller than that in suspension, which can partially be attributed to the lack of water reservoir between the membranes and solid silica particles. Moreover, the detection of products (chitin polymers) with radio activity assays made the evaluation of protein functions very laborious.

In this study, we overcome these two problems by the functional coating of porous silica microparticles with rabbit sarcoplasmic reticulum (SR) membranes. SR is a smooth endoplasmic reticulum in smooth and striated muscles, which stores and pumps Ca^{2+} ions via transmembrane Ca^{2+}-ATPase. Here, pores with the diameter of 100 nm can serve as reservoirs to allow for the active transport of Ca^{2+} across the membrane. The packaging of a large amount of particle-supported SR membranes (active area: 1.1 m^2) into a compact, flow-through microreactor (volume < 2 ml) allows for the evaluation of specific function of Ca^{2+}-ATPase, ATP hydrolysis in the presence of Ca^{2+}, by monitoring the concentration of released inorganic phosphates as a function of time. The details of the obtained results were described in the following sections.

2. Results and Discussion

The homogeneity of the sarcoplasmic reticulum (SR) membranes on particle supports is monitored by confocal microscopy. Ca^{2+}-ATPase in the SR membranes are labeled with a first monoclonal mouse IgG antibody and a second TRITC-labeled polyclonal goat anti-mouse IgG antibody. **Figure 1** represents the confocal fluorescence image of three particles, taken at the plane of equator. The continuous fluorescence contour at the particle rim implies that the particle surfaces are continuously coated with fluorescently labeled native SR membranes. In fact, the three-dimensional fluorescence image of the left most particle reconstructed from the image stacks (inset) further confirms that the entire surface is homogeneously coated with sarcoplasmic membranes. It should be noted that no fluorescence signal could be detected inside the particles, suggesting that the membranes span across the pores and cover only the outer particle surface. The slight deviation of fluorescence signals can be attributed either to the artifacts through the deconvolution of image stacks (collected every 220 nm) or to the inhomogeneous labeling with primary and secondary antibodies. Experimentally, the aggregation of Ca^{2+}-ATPase can not be excluded as a number of studies reported the existence of "clusters" of proteins.[21]

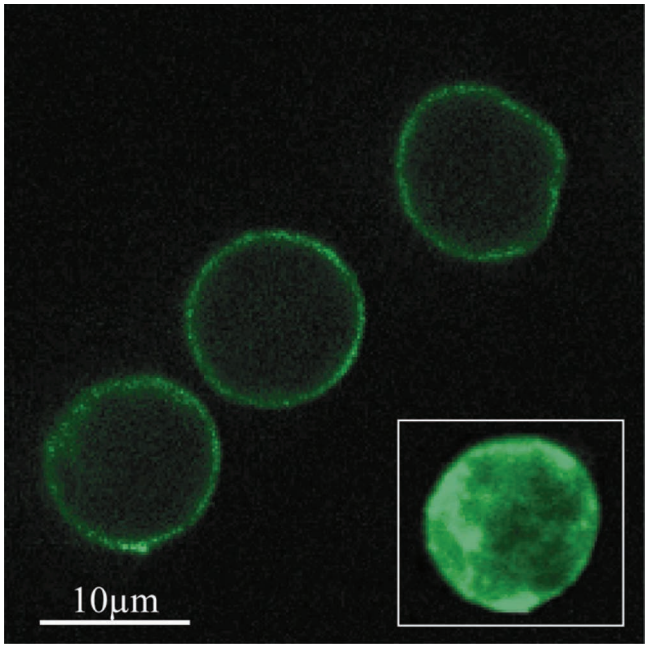

Figure 1. Confocal fluorescence microscopy images of silica beads (Ø = 10 μm) coated with sarcoplasmic reticulum (SR) membranes from rabbit muscles. The membranes on the beads were labeled with a monoclonal antibody against Ca^{2+}-ATPase which was labeled by a polyclonal antibody conjugated with FITC. The inset represents the reconstructed 3D image of the bead on the left.

In the next step, the coverage of particle surfaces with the SR membranes is preliminarily checked by comparing the phase contrast and fluorescence images of SR membranes labeled with fluorescent lipid markers (Supporting Information S1). Furthermore, the surface coverage is calculated from the amount of lipid molecules measured by phosphate analysis. After subtraction of the intrinsic phosphate contamination from bare silica particles very close to the detection limit (~1 μM), the amount of phospholipids in the SR membranes on silica

particles is calculated out of three independent measurements. Taking the bead radius (r = 5 μm) and the density of porous silica (ρ_{silica} = 0.45 g/ml), the accessible surface area of the certain weight of silica particles can be calculated to be 1.3 m^2 per 1 g bead. If one takes the mean area per one lipid molecule in a fluid phase (70 $Å^2$),[22] the calculated area of a lipid bilayer is about 110–120% of the outer surface area of silica particles. The obtained results give additional supporting evidence to the confocal image that the membranes span across the pores and coat only the outer particle surface. This finding seems plausible if one considers the fact that the membrane fragments prepared by the homogenization are much larger than the pore size (~180 nm) determined by the autocorrelation of electron microscope images (Supporting Information S2). This finding also seems to be consistent with the ESEM images of the bare silica microparticles in the presence and absence of SR membranes, suggesting that membranes span across pores (Supporting Information S3). Thus, optical microscopy, phosphate analysis, and electron microscopy consistently demonstrate the formation of continuous SR membranes on porous silica microparticles.

The compositions of membrane proteins in freely suspended SR membranes and particle-supported membranes are compared by electrophoresis in SDS polyacrylamid gels (SDS PAGE) after boiling the beads in Laemmli buffer. As presented in **Figure 2**, the fraction of Ca^{2+}-ATPase (105 kDa) and Ca^{2+} binding proteins (calsequestrin, 44 kDa) in the membranes in free suspensions (lane 2) and that on particle supports (lane 3) are almost identical, suggesting that the composition of the membrane proteins was maintained after immobilization on silica microparticles.

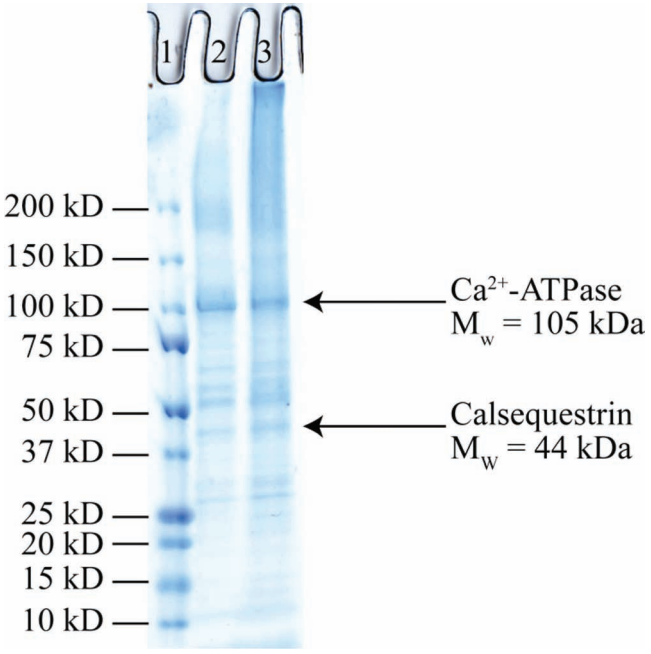

Figure 2. SDS-PAGE analysis of protein patterns in sarcoplasmic reticulum (SR) membranes stained with Coomassie blue: lane 1, molecular weight standard; lane 2, SR membranes in free suspensions; lane 3; SR membranes on particle supports. Representative proteins (Ca^{2+}-ATPase, calsequestrin) are indicated by arrows. The total amount of membranes applied to each lane is set comparable by assuming that the membrane span across pores.

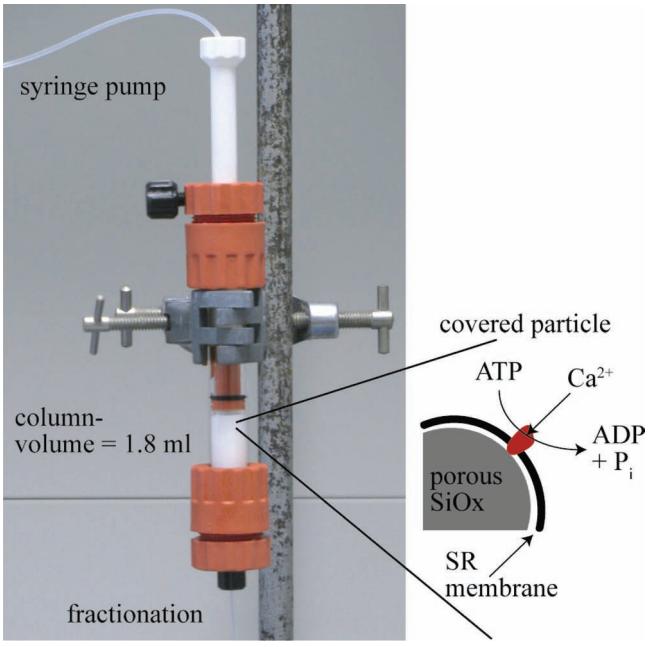

Figure 3. Packaging of silica particle coated with SR membranes (area: 1.1 m^2) into a portable flow-through reactor (volume: 1.8 ml). Reaction buffer is fed by a syringe pump, led through the column, and fractionated. The function of Ca^{2+}-ATPase in different buffers can be detected by monitoring the free phosphate concentrations in the eluate.

To examine the potential applications of native membranes on particle supports for biochemical microreactors, silica particles coated with SR membranes are packaged in a chromatography column (**Figure 3**). This enables one to concentrate 1.1 m^2 of SR membranes (containing approx. 10 nmol Ca^{2+}-ATPase) in 1.8 ml volume. The column is combined with a syringe pump and a fraction collector, which allows for the precise control of reactant feeding (flow rate: 50 μl/min) and product fractionation every 10 min (500 μl per fraction). Here, the function of Ca^{2+}-ATPase in supported membranes is evaluated by measuring the concentration of free phosphate produced by the ATP hydrolysis as a function of time. **Figure 4** represents the first demonstration of the in vitro ATP hydrolysis using SR membranes packaged in the membrane-particle reactor. Reactions with Ca^{2+}-loaded buffers with and without 30 μM ATP are indicated by shaded and non-shaded backgrounds, respectively. It should be noted that the concentration of released inorganic phosphate reaches to the constant saturation level (5.3 ± 0.3 μM) within 30–40 min. The phosphate concentration was reversibly switched back to the initial level below the detection limit (<1 μM) simply by changing the reactant to ATP-free buffer.

Indeed, the fast and reversible on-off switching of ATP hydrolysis between distinct levels demonstrated in this study offers a significant advantage of the packaged particle-supported membranes over other membrane systems. For example, the exchange of reactants for freely suspended SR membranes (with no particle supports) is extremely laborious, since the

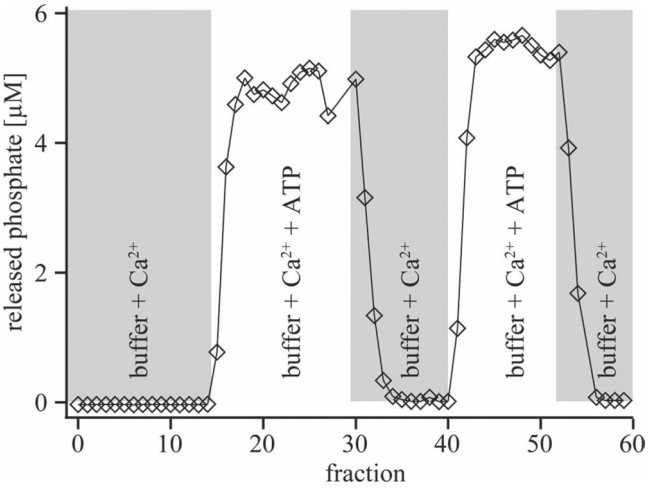

Figure 4. In vitro ATP hydrolysis by particle-supported SR membranes packaged in a column upon the exchange of Ca^{2+}-loaded buffers with and without ATP.

separation of membranes needs a high centrifugation power (>100 000 × g) due to the poor density contrast to aqueous buffer. In fact, the quantitative comparison of Ca^{2+}-ATPase activity in suspended SR membranes is still difficult because i) the reaction slows down according to the consumption of ATP and ii) the access of ATP and Ca^{2+} to the "folded" membrane stacks is hardly controllable. Although particle-supported membranes allows for an easier separation of particles from bulk materials under much milder centrifugation conditions (100 × g), the reaction kinetics was also slowed down as a function of time due to the consumption of ATP from the bulk reservoir (Supporting Information S4).

In the next step, the specificity and sensitivity of the membrane-particle hybrid reactors are further examined by monitoring the enzymatic functions in different buffers. As presented in **Figure 5**a, Ca^{2+}-ATPase showed a capability to hydrolyze ATP even in the absence of Ca^{2+} in the reaction buffer (shaded region). Although the functional level (2.4 ± 0.3 μM) was less than one half of the value in Ca^{2+}-loaded buffer, it is clearly distinguishable from the "blank" buffer that contains no Ca^{2+} or ATP. The hydrolysis in Ca^{2+}-free buffer observed here can be attributed to the presence of Ca^{2+} weakly bound to calsequestrin in SR membranes, which possesses 43 × Ca^{2+} binding pockets.[23] In fact, compared to the membrane pre-treated with the blank buffer, the same sample after the treatment with Ca^{2+}-loaded buffer shows a slightly higher function. The coexistence of different proteins in native membranes offers a unique advantage against artificial membranes to potentially utilize the interplays of membrane proteins in membrane-based biochemical reactors.

It should also be noted that no phosphate production could be detected when the buffer contains no ATP or non-hydrolyzable ATP (adenosine 5'-[γ-thio]triphosphate tetralithium salt, Sigma-Aldrich, Deisenhofen, Germany), as presented in Figure 5 (b). The observed tendency seems plausible, since there is no potential source or storage for ATP in our experimental system. The rate of ATP hydrolysis can be recovered to the functional level (~5.3 μM) as soon as the reaction buffer is exchanged to the Ca^{2+}-loaded buffer. The summary of the obtained results are presented in **Table 1** . Although intrinsic errors, such as the loss of beads during the packaging and the random orientation of the proteins, can not be excluded due to technical reasons, it is noteworthy that the distinct functional level corresponding to each buffer can be reproduced even after many buffer exchanges (>20 times in total) independently repeated over 14 days. Since a number of studies demonstrated that the enzymatic function of Ca^{2+}-ATPase strongly depends on the lipid compositions,[21] the obtained results confirms the material robustness and reproducibility of our biochemical microreactors based on membrane-particle hybrid materials.

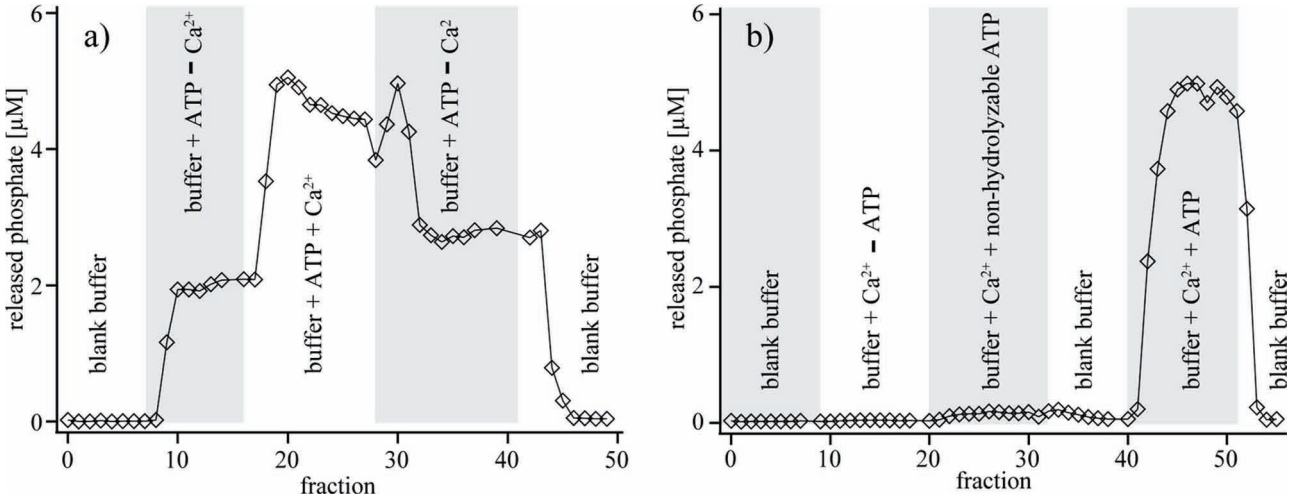

Figure 5. Comparison of ATP hydrolysis in different buffers. a) SR membranes in Ca^{2+}-free buffer with ATP have a reduced hydrolysis capability. The clearly different functional level from that in the blank buffer (no Ca^{2+}, no ATP) can be attributed to Ca^{2+} weakly bound to calsequestrin in SR membranes. b) Functions of Ca^{2+}-ATPase in buffers containing no ATP or non-hydrolyzable ATP are almost identical to that in the blank buffer even in the presence of Ca^{2+}.

Table 1. Distinct functional levels of Ca^{2+}-ATPase in different buffers.

Buffer Compositions	Released Phosphate [μM]
$-Ca^{2+}$,–ATP (blank buffer)	N.D.[a]
+ Ca^{2+}, + ATP (functional state)	5.3 ± 0.3
+ Ca^{2+},–ATP (control 1)	N.D.[a]
$-Ca^{2+}$, + ATP (control 2)	2.4 ± 0.3
+ Ca^{2+}, + nATP (control 3)[b]	N.D.[a]

[a]Not detectable. The concentration was below the detection limit of the instrument ~ 1 μM; [b]Non-hydrolyzable ATP.

The membrane-particle reactor systems proposed in this study seems to include a large potential towards the in vitro synthesis of ATP, which is a fundamental chemical energy source used in biological systems. Previously, we reported the orientation selective incorporation of F_0F_1 ATP synthase complex isolated from *Micrococcus luteus* in artificial lipid vesicles and planar supported membranes.[24] Unfortunately, the orientation of membrane proteins in SR membranes was intrinsically random, as the preparation steps include the homogenization in a blender. Therefore, the deposition of native membranes retaining the native protein composition on porous particle supports would further enable us to transfer complex protein machineries to portable biochemical reactors.

3. Conclusions

We demonstrated that the functional coating of porous silica microparticles with native membranes enables one to concentrate about 10 nmol transmembrane enzymes into a portable flow-through reactor with a volume of less than 2 ml. The phosphate analysis, the labeling of membranes with fluorescent lipids, and the immunofluorescence labeling of Ca^{2+}-ATPase all implied that sarcoplasmic reticulum (SR) membranes purified from rabbit muscles can homogeneously be deposited on the surface of silica particles. Owing to the larger density of silica compared to those of water and free membranes, 1.1 m^2 large particle-supported native membranes can be confined into <2 ml volume. The transmembrane enzyme Ca^{2+}-ATPase retains its specific functions against more than 20 reactant exchanges over 14 days, confirming the sensitivity, robustness, and reproducibility of the membrane-particle biochemical reactors. The deposition of other native membranes, such as outer membranes of cyanobacteria and thylakoid membranes, would further enable us to extend the current strategy towards portable bio-inspired reactors for the in vitro ATP synthesis.

4. Experimental Section

All chemicals were of p.A. quality, if not otherwise specified. De-ionized ultrapure water (Genpure, TKA Niederelbern, Germany), was used for preparation of all buffer solutions.

Preparation and Characterization of Sarcoplasmic Reticulum (SR) Membranes: SR membranes were isolated according to the method of Hasselbach and Makinose.[25] In brief, the hind leg muscles of a freshly slaughtered rabbit were prepared by carefully removing connective tissue and cooled to 4 °C. All subsequent manipulations were performed at 4 °C. Muscle tissue (500 g) was minced and suspended in 1.5 L of pre-cooled buffer A (2.5 mM KH_2PO_4/ 2.5 mM K_2HPO_4, pH 7.4, 100 mM KCl, 2 mM EDTA, 1 mM DTT, 10 μM leupeptin). The mixture was homogenized in a Waring blender (Waring laboratories, Torrington, USA) for 1 min. Myofibrils were removed by 15 min centrifugation at 6350 × g. The supernatant was adjusted to pH 7.4 using 7 M KOH. Mitochondria were removed by 15 min centrifugation at 8000 × g. Crude SR membranes were obtained from the supernatant by centrifugation for 60 min at 40 000 × g. The pellet was re-suspended in buffer B (1 mM triethanolamine pH 7.4, 50 mM KCl, 1 M sucrose, 1 mM DTT, 10 mM leupeptin and 0.1 μM PMSF), homogenized with 15 strokes in a Potter-Elvehjem tissue homogenizer (Bellco Glass Inc. NJ, USA), and centrifuged for 15 min at 4500 × g. The supernatant was diluted with 1.5 × volume of buffer C (1 mM triethanolamine pH 7.4, 1 M KCl, 160 mM sucrose, 3.35 mM ATP, 3.35 mM $MgCl_2$, 1 mM DTT, 10 mM leupeptin, 0.1 μM PMSF) and centrifuged for 90 min at 80 000 × g. The sedimented membranes were washed twice in buffer D (1 mM triethanolamine pH 7.4, 100 mM KCl, 96 mM sucrose, 1 mM DTT 10 mM), sedimented as described and re-suspended in buffer E (1 mM triethanolamine pH 7.4, 100 mM KCl, 0.2% NaN_3). For long term storage, 500 μl aliquots were shock-frozen in liquid nitrogen and stored at –80 °C. Experiments were performed using freshly thawed crude membranes without further purification.

Lipid concentrations were determined according to the procedure of Fiske and Subbarow.[26] Crude SR membranes (100 μl) or membrane coated silica particles (10 mg) were boiled at 180 °C for 30 min in 300 μl 70% perchloric acid. After cooling to room temperature, 1.2 ml water, 0.2 ml ammonium molybdate (2.5 wt%), 0.2 ml ascorbic acid (10 wt%) were added. The mixture was boiled for 5 min at 100 °C, cooled down to room temperature, and the absorbance of the formed complex was quantified at λ = 797 nm using a UV-Vis spectrophotometer (Genesys 10 UV, Thermo Scientific, Schwerte, Germany). Free inorganic phosphate was used for calibration based on a linear relationship. SR membrane area was calculated by multiplying the molar lipid concentration with the required area per phospholipid molecule.[22] Assuming a 1:1 w/w phosphate/lipid ratio in biological membranes, we calculated the surface area of 1 mg crude membranes to be approx. 2×10^{16} nm^2.

Analysis of Membrane Proteins: Experimental Details. The protein compositions in SR membranes before and after the deposition were analyzed by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE).[27] In brief: a) SR membranes were incubated with Laemmli buffer (65 mM Tris/HCl pH 6.8, 3.3 wt% SDS, 5 vol% mercaptoethanol and 10 vol% glycerol) at a mixing ratio of 1: 1 (v/v), boiled for 5 min at 95 °C and cooled to room temperature. A 15 μL portion sample was loaded on a 5–15% (w/v) polyacrylamide gel. b) After the incubation of particle-supported membranes with Laemmli buffer, the membrane proteins separated from particles were subjected to the gel. Following Coomassie brilliant blue staining for 10 min and de-staining (25% v/v methanol, 10% v/v acetic acid), the proteins were identified according to the molecular weight standards (200 kDa to 10 kDa, BioRad, Munich, Germany).

Deposition and Characterization of SR Membranes on Particle Supports: Porous silica microparticles (10 μm in diameter, 100 nm pore size, Machery-Nagel, Dueren, Germany) were extensively washed in buffer E prior to the membrane deposition. SR membranes (10 fold membrane area excess over the particle surface) were incubated with purified particles for 1 h in an overhead rotator at room temperature. Unbound membranes were removed by several washing steps.

For the optical characterization of particle-supported membranes with confocal microscopy, the C-terminal domain of Ca^{2+}-ATPase is labeled with a SERCA2-specific monoclonal antibody (Clone $VE12_1G9$, Dianova, Hamburg, Germany) and a secondary polyclonal antibody (goat anti-mouse IgG) conjugated with fluorescein isothiocyanete (FITC) (Sigma-Aldrich, Deisenhofen, Germany). Prior to the antibody labeling, the samples were pre-treated with 1 ml of BSA solution (30 mg/ml) to avoid nonspecific adsorption of immunochemical reagents. All incubation steps were performed for 1 h, washing steps were performed 3×, and centrifugation steps were performed for 1 min at 500 × g. Image stacks

were recorded on a laser-scanning confocal microscope (A1R, Nikon Instruments Inc.) with a 60× objective (N.A. = 1.2) at the Nikon Imaging Center (Heidelberg). The resulting confocal images were deconvoluted using Huygens software (Scientific Volume Imaging, Netherlands) to reconstruct the 3D profiles.

Particle-supported membranes were also characterized by a Quanta 400 environmental scanning electron microscope (E-SEM) (FEI, Eindhoven, Netherlands), equipped with a gaseous secondary electron detector (GSED).[28] A 10 µl portion of the particle-supported membrane suspension was deposited on a silicon wafer and imaged at 710–740 Pa. The stage and the silicon wafer were cooled to 3 °C before application of the sample, and the chamber was purged between 800 and 1200 Pa during vacuum pumping. Images were obtained at 10 keV and working distance: 6 mm to achieve the 20,000 × magnification.[29]

Packaging of Supported Membranes into Portable Flow-Through Reactors: To fabricate a membrane-based continuous flow reactor, 800 mg porous silica microparticles (corresponding to a volume of 1.8 ml, surface area of 1.1 m^2) coated with SR membranes were filled in a chromatography column (C10/10, GE Healthcare, Freiburg, Germany) of 1 cm inner diameter and 10 cm length. The column was equipped with two variable flow adaptors and connected to a precision syringe pump (Harvard apparatus, Holliston, Massachusetts, USA). Prior to the experiments, the column was adjusted with a Ca^{2+} and ATP-free buffer (10 mM Tris, 100 mM NaCl, pH 7.4). Throughout the experiments, the flow rate was adjusted to 0.05 ml/min and eluate was manually collected in 0.5 ml fractions. To evaluate the rate of ATP hydrolysis by Ca^{2+}-ATPase in particle-supported membranes, a buffer containing 30 µM ATP was used. The rate of ATP hydrolysis was compared by performing the experiments in the presence and absence of 240 µM Ca^{2+}. ATP hydrolysis reaction was monitored by measuring the concentration of released inorganic phosphate in each fraction by following the method of Kallner using KH_2PO_4 standards.[30] Fractions of 0.5 ml were mixed with a 2.0 ml portion of malachite green (0.8 mg/ml in H_2O), 3.9 mg polyviol (23.3 mg/ml in H_2O), 19 mg ammonium molybdate (57.2 mg/ml in 6 M HCl), and H_2O mixed at the ratio of 2:1:1:2 (v/v). Samples were incubated for 30 min at room temperature and the phosphor molybdate complex was colorimetrically determined at $\lambda = 630$ nm.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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