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REVIEW

Proteoliposomes in nanobiotechnology

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Abstract Proteoliposomes are systems that mimic lipid membranes (liposomes) to which a protein has been incorporated or inserted. During the last decade, these systems have gained prominence as tools for biophysical studies on lipid–protein interactions as well as for their biotechnological applications. Proteoliposomes have a major advantage when compared with natural membrane systems, since they can be obtained with a smaller number of lipidic (and protein) components, facilitating the design and interpretation of certain experiments. However, they have the disadvantage of requiring methodological standardization for incorporation of each specific protein, and the need to verify that the reconstitution procedure has yielded the correct orientation of the protein in the proteoliposome system with recovery of its functional activity. In this review, we chose two proteins under study in our laboratory to exemplify the steps necessary for the standardization of the reconstitution of membrane proteins in liposome systems: (1) alkaline phosphatase, a protein with a glycosylphosphatidylinositol anchor, and (2) Na,K-ATPase, an integral membrane protein. In these examples, we focus on the production of the specific proteoliposomes, as well as on their biochemical and biophysical characterization, with emphasis on studies of lipid–protein interactions. We conclude the chapter by highlighting current prospects of this technology for biotechnological applications, including the construction of nanosensors and of a multiprotein nanovesicular biomimetic to study the processes of initiation of skeletal mineralization.

Keywords Proteoliposomes · Biotechnology · Alkaline phosphatase · Na,K-ATPase · Leishmaniasis · Nanosensor · Biomineralization

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Purification or expression of the membrane protein

Proteins are the biochemically most active components present in biological membranes and may contribute from 20 to 80% of the weight of a particular membrane. The more specialized the membrane, the bigger the proportion of proteins in its composition. Generally, a membrane protein presents one or more coiled-coil peptidic chains, in order to present an apolar surface that can interact hydrophobically with the apolar region of the fatty acid chains (tails), while the polar or charged regions of the protein interact through electrostatic hydrogen bonds with the polar groups (heads) of the lipids on the lipidic bilayer (Yeagle 1993).

Many of the so-called integral or transmembrane proteins completely cross the lipidic bilayer and, due to the strong hydrophobic interaction with the phospholipids, can only be removed with the use of detergents–tensoactive compounds

which have the ability to decrease the lipid–lipid and lipid–protein interactions during the protein solubilization process (Gennis 1989; Le Maire et al. 2000; Santos and Ciancaglini 2000; Santos et al. 2002; Magalhães et al. 2003; Schuck et al. 2003; Heerklotz 2008). On the other hand, peripheral or extrinsic proteins have a superficial interaction with the polar heads of the membrane lipids and can be removed simply by a change in ionic strength or pH. There is still another group of proteins that present the characteristics of totally soluble proteins, but are found on the membrane surface because they establish a covalent bond with a lipid present on the lipidic bilayer; these proteins are called anchored proteins (Pizauro et al. 1987, 1994; Ciancaglini et al. 1990; Camolezi et al. 1999, 2002).

If one chooses to purify a membrane-bound protein, the procedures usually require the isolation of membrane fragments from the cell of interest or specific organelles, which can be done by methods such as differential centrifugation (Fig. 1). The lysis (to isolate the membrane from the contents of the cell or organelle) is the next step and is usually done via osmotic shock or the use of detergents. The removal of the different types of lipids is based on their solubilization in organic solvents (chloroform, methanol, acetone, among others) in which they are soluble, followed by their separation/characterization by analytical methods such as thin layer chromatography (Prasad 1996). The removal of the proteins from the isolated membranes is the more toilsome process, because proteins tend to lose their structures if removed from the membrane into an aqueous medium (Yeagle 1993; Gennis 1989; Rigaud et al. 1995; Le Maire et al. 2000; White et al. 2001; Magalhães et al. 2003; Seddon et al. 2004; Kalipatnapu and Chattopadhyay 2005). These laborious procedures may be needed for complex, multimeric, multi-domain proteins, not easily obtainable in recombinant form, e.g. Na,K-ATPase. For simpler proteins, the preferred source is the expression and purification of recombinant proteins (Simão et al. 2007, 2010a).

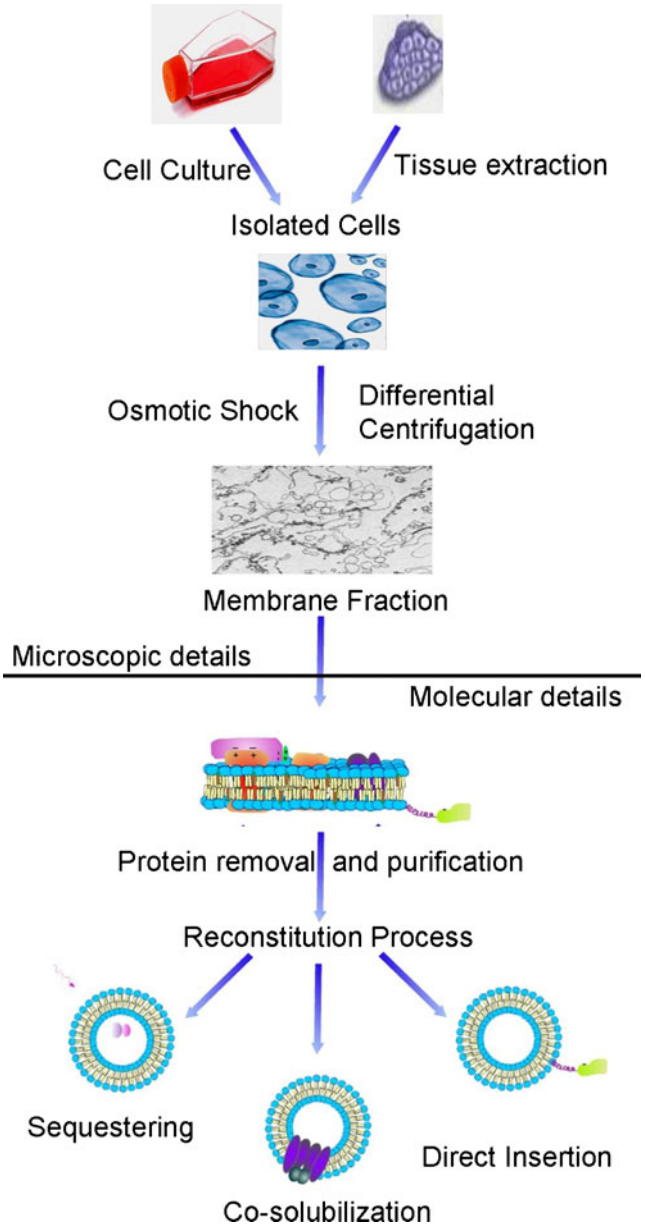

Fig. 1 Scheme for the obtention of a biomimetic system. Microscopic details: tissues or cell cultures can be used for the obtention of cells and, after an osmotic process, organelles or membrane fractions can be isolated by differential centrifugation. Molecular details: each protein can be solubilized and purified by a different method depending on its interaction with the lipid membrane (for details, see text). After solubilization and purification, the protein can be reconstituted in the membrane using direct insertion or co-solubilization processes (for details, see text). Alternatively, the protein can also be sequestered into the liposome

Constructions of lipid mimetic systems

Since biological membranes are very complex systems, it is not easy to understand the physicochemical behavior of the lipidic bilayer, or to explain many of its properties. Thus, researchers have developed "models" of biological membranes that consist of simplified lipidic mono- or bilayers. These mimetic systems constituted of a pure lipid (mono-components), mixtures of more than one type of lipid, and reconstituted mixtures of lipids and a single or multiple proteins can be prepared (Silvius 1992; Rigaud et al. 1995; Singer 2004).

The monolayers, although they mimic a half-part of a natural biological membrane, can be easily obtained by the dispersion of an amphiphilic compound (such as a lipid) in a water–air interface. The system thus formed is a film of molecular thickness where the polar head is in contact with the water and the hydrophobic tail extends itself in the direction of the air (monolayers of Langmuir) These models of monolayers are very useful for the study of lipid–lipid

interactions, but have little applicability in the study of interactions with integral proteins. From monolayers, bilayer model systems can be easily prepared by, for example, immersing solid substrates in previously formed monolayers, and obtaining what are called the Langmuir–Blodgett (LB) films (Dynarowicz-Latka et al. 2001; Colhone et al. 2009).

On the other hand, the most studied membrane model systems are the liposomes or vesicles (Fig. 1), which can be obtained in solution and not in a solid support as are the LB films. In an aqueous suspension, most lipids (generally phospholipids) organize spontaneously, forming systems with many (multilamellar) bilayers. This organization is driven by the entropy of the water molecules and by the interaction between the lipid hydrophobic chains (constituted by fatty acids). These multilamellar systems can be sonicated or forced to go through (extrusion) pores of nanometric dimensions, to form unilamellar systems (with a single concentric bilayer), very similar to the natural membranes. The sonication as well as the extrusion process supplies the necessary energy to break the different lamellae and to re-organize the bilayers in uniform and control-sized vesicles (New 1990; Camolezi et al. 1999, 2002; Ierardi et al. 2002; Daghastanli et al. 2004; Santos et al. 2002, 2005; Rigos et al. 2008; dos Santos et al. 2010).

When the protein to be reconstructed in a membrane system presents a hydrophobic domain that is too large, it is difficult to force it to dislocate from the micellar system into the liposomes space, even at low concentrations of detergent. In this case, the co-solubilization technique is the most recommended. This methodology takes advantage of the presence of the detergent in the protein that was removed and purified from the other components of the natural biomembrane. Next, a lipid suspension (or a mixture of more than one lipid), also in the presence of the same detergent (therefore the name co-solubilization), is added to this solution. After an adequate time of incubation of this ternary system, the detergent is removed using an appropriate technique (for example, dialysis, gel filtration or the use of hydrophobic resins). After detergent removal, the lipid molecules tend to organize themselves so as to form a bilayer structure in which their hydrophobic tails are isolated from the aqueous medium guided by the entropy of the water molecules. In addition, proteins tend to accommodate among the lipid groups creating lipid–protein domains and forming the vesicles. These systems are called "proteoliposomes" (Fig. 1). By changing the type and proportion of the lipids, incubation time, method used for detergent removal, or even the velocity by which the detergent is removed, different vesicular systems can be obtained, varying in the type and quantity of proteins that can be reconstituted (Camolezi et al. 2002; Ierardi et al. 2002; Daghastanli et al. 2004; Santos et al. 2005, 2006b; Rigos et al. 2008, 2010; Simão et al. 2010a; Bolean et al. 2010, 2011).

Applications for the study of lipid–protein interactions

Anchored proteins: an example using alkaline phosphatase

Anchored membrane proteins are a class of proteins with a covalently bound lipid or alkyl chain, which provides a hydrophobic moiety that allows attachment to the membrane and play specific functions in cells (Gennis 1989; Yeagle 1993). They can be solubilized from the membrane either by treatment with detergents or proteases or released enzymatically by using phospholipases (Furth et al. 1984; Santos and Ciancaglini 2000). It is known that tissue-nonspecific alkaline phosphatase (TNAP) from cartilage and bone is a glycosylphosphatidylinositol (GPI)-anchored membrane ectoprotein (Leone et al. 1997; Pizauro et al. 1995; Simão et al. 2007) in contact with extracellular cartilage fluid, in which natural substrates (ATP, ADP, PP_i) are present at nanomolar or micromolar concentrations. The phosphatidylinositol structure is a phosphatidylinositol–glycolipid anchor, which is covalently attached to the carboxyl terminus (C-terminus) of the protein through an amide linkage. This anchor structure of alkaline phosphatase results in mobility in the membrane (Camolezi et al. 2002; Ierardi et al. 2002).

The techniques used to solubilize proteins from the membrane usually destroy the native membrane structure, and care must be taken during the solubilization and purification procedures so that the protein function is preserved. Ciancaglini et al. (1990) improved the method for solubilization of membrane-bound TNAP using the detergent polyoxyethylene 9-lauryl ether. The principal action of the detergent during the solubilization of a protein is to break lipid–lipid and lipid–protein interactions present in biomembranes, often competing with the lipids for the occupation of the hydrophobic sites of the protein. High yields of solubilized TNAP have been reported for a series of detergents (Pizauro et al. 1987; Ciancaglini et al. 1990). In addition, Pizauro et al. (1994) reported the biochemical characterization of rat osseous plate TNAP selectively released from the membrane by a phosphatidylinositol-specific phospholipase C from *Bacilus thuringiensis*. This treatment results in a soluble enzyme form without the 1,2-diacylglycerol moieties. The C-terminus in this preparation retains one glycan and one inositol–phosphate group. Enzyme activity was released up to 100% from the membrane by using 0.1 units of phospholipase C. This solubilization procedure did not destroy the ability of the enzyme to hydrolyze different substrates. Treatment of the enzyme reconstituted into liposome system with phospholipase C confirmed that TNAP interacts with the liposome membrane through the GPI-anchor (Camolezi et al. 2002). Similar results were obtained by Kihn et al. (1990) for placenta and liver alkaline phosphatase. Solubilization of TNAP with protease (bromelain or tripsin) results in

a soluble enzyme without the GPI-anchor. This process breaks the polypeptide chain nonspecifically and yields an enzyme with reduced stability (Camolezi et al. 2002).

A large number of studies have been carried out using liposomes as systems to mimic biomembranes (Daghastanli et al. 2004; Camolezi et al. 2002; Ciancaglini et al. 2006; Benatti et al. 2007; Sesana et al. 2008; Simão et al. 2010a, b; Ciancaglini et al. 2010; Bolean et al. 2010, 2011). Experiments confirmed that the interaction between detergent-solubilized TNAP, free of detergent excess, and the lipid bilayer of liposomes occurs via the GPI-anchor of the enzyme and our method of incorporation via direct insertion guarantees that the enzyme is associated with the external leaflet of the liposome membrane (Camolezi et al. 2002). Enzyme incorporation is time-dependent and the incorporation yields depend of the lipid composition used to form the vesicles (Simão et al. 2010a). An important point to be observed is that the enzyme bound to liposomes retains the ability to hydrolyze the different substrates, such as ATP, ADP, AMP, PP_i, and p-nitrophenylphosphate (pNPP) (Ciancaglini et al. 2006, 2010; Simão et al. 2010a, b; Bolean et al. 2010).

To investigate the impact of the liposome phospholipid composition on the activity of liposome-reconstituted TNAP, the kinetic parameters for the hydrolysis of known major TNAP substrates (ATP and PP_i) were determined (Simão et al. 2010a). Figures 2 and 3 illustrate the results for the catalysis by osteoblast-derived TNAP, analyzed as native membrane-bound enzyme and as reconstituted enzyme in the various proteoliposomes, showing how the activity depends on substrate concentration.

During hydrolysis of ATP by liposome-reconstituted TNAP (Fig. 2), different saturation curves were obtained for the different proteoliposomes, with variable V_{max} and up to a 6-fold lower $K_{0.5}$ in DPPC:DODAB proteoliposomes (Fig. 2b) than for cell membrane-bound TNAP (Fig. 2a) or DPPC:DPPS (8:2) proteoliposomes (Fig. 2d). Correspondingly, catalytic efficiencies were up to 10-fold different between DPPC:DODAB (9:1) (Fig. 2b) and DPPC:DPPS (8:2) (Fig. 2d) proteoliposomes. Inhibition of ATPase activity was observed for ATP of >8 mM, in the case of the DPPC proteoliposomes, >5 mM for the DODAB proteoliposomes and >7 mM for DPPS proteoliposomes. Hence, the specific phospholipid microenvironment was a co-determinant of the TNAP activity during hydrolysis of ATP (Simão et al. 2010a).

For the hydrolysis of PP_i by reconstituted TNAP (Fig. 3), $K_{0.5}$ values were similar and the maximal rates of substrate conversion (V_{max}) differed more than 3-fold between proteoliposomes. Inhibition of the enzyme pyrophosphatase activity occurred for PP_i of >5 mM with DPPC-reconstituted TNAP and >4 mM with DODAB- and DPPS-reconstituted TNAP (i.e. at concentrations just above those saturating TNAP, at V_{max}) (Simão et al. 2010a). These experiments illustrate that

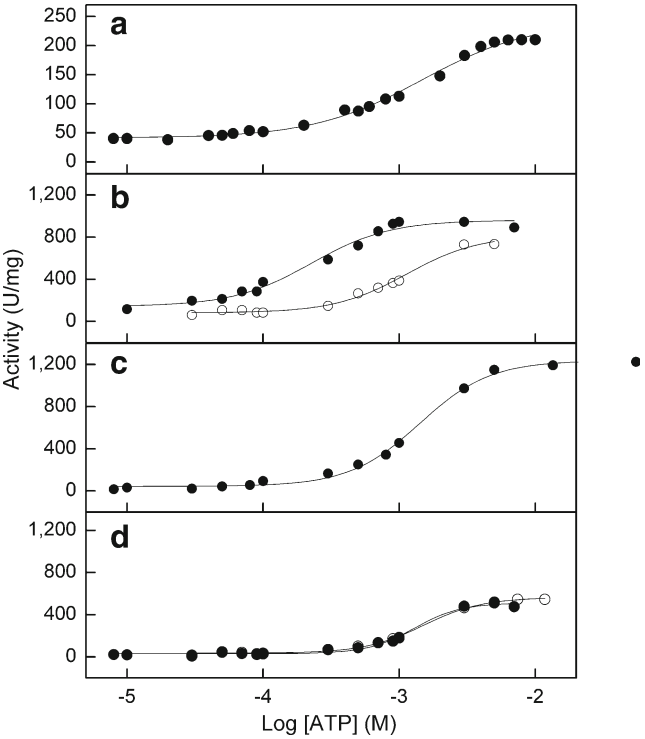

Fig. 2 Effect of increasing concentrations of ATP on the P_i-generating activity of osteoblast-derived TNAP: **a** membrane-bound; **b** reconstituted in DPPC:DODAB liposomes (8:2, molar ratio, o) and (9:1, molar ratio, ●); **c** reconstituted in DPPC liposomes and **d** reconstituted in DPPC:DPPS liposomes (8:2, molar ratio, o) and (9:1, molar ratio, ●). Assays were done at 37°C in 50 mM AMPOL buffer, pH 9.5, containing substrate and 2 mM $MgCl_2$ and released P_i measured

the phospholipid microenvironment influenced enzyme catalysis by TNAP differentially for the various substrates.

In plasma membranes, most GPI-anchored proteins are associated with "lipid rafts" (Simons and Ikonen 1997; Simons and Toomre 2000; Giocondi et al. 2007; Lingwood and Simons 2010), ordered microdomains enriched in sphingolipids, glycosphingolipids, and cholesterol (Simons and Toomre 2000; Damek-Poprawa et al. 2006). In order to better understand the role of the lipids present in rafts and their interactions with GPI-anchored proteins, the insertion of TNAP into different lipid raft models was studied using dipalmitoylphosphatidylcholine (DPPC), cholesterol (Chol), sphingomyelin (SM), and ganglioside (GM1) (Bolean et al. 2010, 2011). Changes in lipid critical transition temperature (Tc) and enthalpy variation (ΔH) were monitored using differential scanning calorimetry (DSC). Calorimetry analysis of the liposomes and proteoliposomes indicated that lateral phase segregation could be noted only in the presence of cholesterol, with the formation of cholesterol-rich microdomains centered above T_c=41.5°C. The presence of GM1 and SM into DPPC-liposomes influenced mainly ΔH and $\Delta t_{1/2}$ values. The gradual increase in the complexity of the systems decreased the activity of the enzyme incorporated.

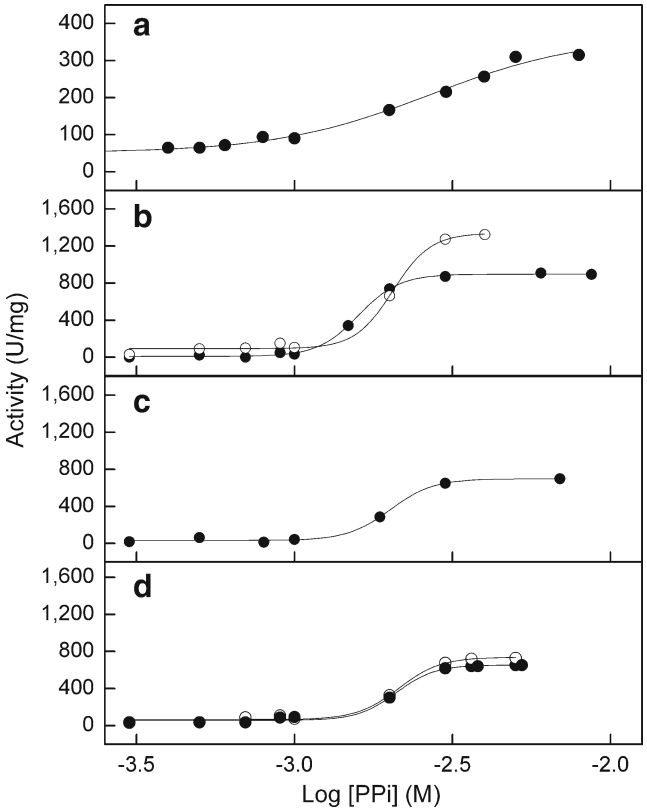

Fig. 3 Effect of increasing concentrations of PP_i on the P_i-generating activity of osteoblast-derived TNAP: **a** membrane-bound; **b** reconstituted in DPPC:DODAB liposomes (8:2, molar ratio, o) and (9:1, molar ratio, •); **c** reconstituted in DPPC liposomes and **d** reconstituted in DPPC:DPPS liposomes (8:2, molar ratio, o) and (9:1, molar ratio, •). Assays were done at 37°C in 50 mM AMPOL buffer, pH 9, containing substrate and 2 mM $MgCl_2$ and released P_i measured

The presence of the enzyme also fluidified the systems, as seen by the intense reduction in ΔH values, but did not alter T_c values significantly (Bolean et al. 2010, 2011).

Membrane protein: an example using NaK-ATPase

Na,K-ATPase or sodium pump, originally described in 1957, is a member of the P-type ion transport ATPases present in animal plasma membrane. This enzyme carries three Na^+ ions out of and two K^+ ions into the cell, across the plasma membrane, against their respective electrochemical gradients, fueled by the free energy of ATP hydrolysis (Lingrel and Kuntzweiler 1994; Kaplan 2002; Rajasekaran et al. 2005). The action of the pump, together with differential permeability through gated and ion-specific channels, leads to a resting membrane potential that is typically in the range of −30 to −70 mV (negative on the inside of the membrane) in most living mammalian cells (Morth et al. 2011). Sodium pump is ubiquitous because of the need to regulate the volume of animal cells and organelles bounded by flexible lipid bilayers, to cellular uptake of ions, nutrients and neurotransmitters. Probably because the pump is essential for cell viability, Na,K-ATPase has been indirectly implicated in the etiology of diseases like essential hypertension and diabetes (Morth et al. 2007; Faller 2008; Lingrel 2010).

The Na,K-ATPase consists of α and β subunits and a regulatory FXYD protein (γ subunit in kidney outer medulla) (Shull et al. 1986; Béguin et al. 1997). The α subunit has 10 transmembrane segments, a molecular weight of approximately 112 kDa, based on amino acid composition, transports the cations and is represented by four isoforms (Martin 2005; Blanco 2005; Geering 2008). The β subunit is a type II glycoprotein, represented by three isoforms, and it has an essential function as a molecular chaperone (Sweadner 1989; Geering 2001; Blanco 2005). FXYD2 or γ subunit has two alternative splice variants (γa and γb) and it is mainly expressed in kidney (Geering 2005; Dempski et al. 2008). FXYD2 was shown to increase the apparent K^+ affinity of Na,K-ATPase at high negative membrane potentials in both presence and absence of extracellular Na^+ (Béguin et al. 2001; Geering 2006). It has been demonstrated that the αβ form of the Na,K-ATPase is capable of both ATP hydrolysis and active ion transport (Ward and Cavieres 1993, 1996, Martin et al. 2000; Miles et al. 2011). However, accumulating evidence suggests that the enzyme normally self associates as $(\alpha\beta)_2$ dimers (Santos et al. 2002, 2006b; Thoenges and Schoner 1997; Antolovic et al. 1999; Costa et al. 2003; Santos and Ciancaglini 2003) or as $(\alpha\beta)_4$ tetramers (Mahaney et al. 1990; Donnet et al. 2001; Taniguchi et al. 2001).

The E1–E2 reaction cycle model is based on the pioneering work of Albers (1967) and Post et al. (1972). They describe the transport accomplished by protein conformational changes between two states, E1 and E2, that bind three Na^+ and two K^+ ions, respectively. The crystal structure at 3.5 Å resolution was described by Morth et al. (2007). The authors have provided clear evidence of the presence of a state in which the two counter-transported Rb^+/K^+ ions are occluded, as originally proposed on the basis of kinetic measurements and a high similarity between the α subunits of the Na,K-ATPase and Ca-ATPase. In a 2.4-Å resolution structure of the Na,K-ATPase, only a single water molecule is observed with the coordination of the two K^+ ions (Shinoda et al. 2009). The Na,K-ATPase crystal form was also soaked with cardiotonic steroid ouabain, which provided insight into a low-affinity cardiotonic steroid-binding site of Na,K-ATPase between helices M1, M2 and M4–M6 (Ogawa et al. 2009).

The activity of a membrane-bound enzyme can be influenced by the physical state and/or properties of membrane as well as the function of others neighboring intrinsic membrane proteins (Cuevas et al. 2006). It is known that the plasma membrane is compartmentalized into structurally and functionally different microdomains. These microdomains may sequester specific proteins and lipids, while excluding

others, to form a dynamic center for regulating cellular processes such as signal transduction, vesicular transport, and cargo delivery (Tian and Xie 2008). The very complex lipid composition of the plasma bilayer and a formation of a specific lipid domain is likely important in the regulation of Na,K-ATPase (Cornelius et al. 2003). Its activity is directly related to membrane fluidity (Sotomayor et al. 2000).

The Na,K-ATPase has been solubilized by non-ionic detergents, and reconstituted in a liposome system which keeps the natural environment of the lipid membrane. For example, good results for Na,K-ATPase reconstitution were obtained using a 1:1 (w/w) DPPC:DPPE mixture with a lipid:protein 1:3 (w/w) ratio resulting in proteoliposomes containing 89% of protein with average diameter of 140 nm and 79% recovered ATPase activity. Integrity studies of the Na,K-ATPase in this liposomes system have demonstrated that the enzyme is reconstituted with the ATP hydrolysis site located at the external side of the lipid bilayer vesicle, called inside–out orientation (Santos et al. 2002, 2006b). These inside–out proteoliposomes enable a series of biophysical studies on the stability and structure of the enzyme. Thus, circular dichroism spectroscopy (CD) studies suggest that the α and β subunits present different thermal stability, which may be modulated by the nature of the co-solvent (detergent or lipid) used in the preparations of the Na,K-ATPase. Furthermore, a protective role of the phospholipid bilayer was observed for the reconstituted enzyme compared to the detergent solubilized enzyme (Rigos et al. 2006, 2008, 2010; Barbosa et al. 2010; Miles et al. 2011). Infrared studies indicated that some segments of the Na,K-ATPase keep their secondary structure while others participate in intra-molecular, self-aggregation at high temperature (Rigos et al. 2010).

Several authors described that the Na,K-ATPase denaturation process above 50°C leads to an α–α form aggregation, without the participation of the β subunit, and that heating above 60°C leads to a complete loss of the enzymatic activity (Donnet et al. 2001; Rigos et al. 2006, 2008, 2010; Barbosa et al. 2010; Miles et al. 2011). The infrared spectra of trypsinized proteoliposomes are in agreement with this behavior, indicating that the aggregation occurs through extra-liposomal α subunit domains.

The activity of membrane proteins can be regulated by several physical properties of the lipid membrane including composition, phase state, and length of the hydrocarbon chains. Theoretical models explain this regulation considering the thermodynamics of the interactions of lipid hydrocarbon chains with the hydrophobic transmembrane segment of the protein (Jensen and Mouritsen 2004) and the heterogeneous profile of lateral pressure exerted by the membrane on the transmembrane segment (Cantor 1997). As described by these models, mutual interactions between the protein and the lipids produce both of the following: shifts of the temperature of the lipid phase transition and shifts in the equilibrium between different conformations (activities) of the protein (Marsh 2008). The observation that Na,K-ATPase shifts the lipid phase transition suggests the existence of this kind of mutual interaction and that the biological activity could be regulated by lipid properties within the natural membranes in the liquid crystalline phase.

The enzyme is initially in the $(\alpha\beta)_2$ form, and can lose the activity when subunit separation occurs (Santos et al. 2002, 2006b; Santos and Ciancaglini 2003). This triggers the aggregation process through the α-subunits, according to what was revealed by the infrared spectroscopy. Besides that, kinetic studies strongly suggest that, in the DPPC:DPPE-liposome systems, the Na,K-ATPase is found in the same $(\alpha\beta)_2$ form. The latter observation derives from a comparative study on the solubilized enzyme, in which it was found that the effect of ions (Na^+ and K^+) and the substrates ATP and pNPP showed the same behavior (Santos et al. 2002, 2006b; Santos and Ciancaglini 2003). Therefore, according to these present results, a similar mechanism may be occurring in both $C_{12}E_8$-solubilized Na,K-ATPase and that reconstituted in DPPC:DPPE-liposomes.

New perspectives for applications of proteoliposomes

Multiple proteins carrier systems to study the biomineralization process

Mineralization of cartilage and bone occurs by physicochemical and biochemical processes that facilitate the deposition of hydroxyapatite (HA) in specific areas of the extracellular matrix (ECM). Experimental evidence has pointed to the presence of HA crystals along collagen fibrils in the ECM (Glimcher 2006) and also within the lumen of chondroblast- and osteoblast-derived matrix vesicles (MVs) (Anderson et al. 2005; Golub 2009; Simão et al. 2007, 2010b).

PP_i, derived both from ectoplasmic PC-1/nucleotide pyrophosphatase/ phosphodiesterase-1 (NPP1)-catalyzed production from extracellular nucleoside triphosphates (Terkeltaub 2001) and by intracellular export via the ankylosis protein (ANK) transporter, inhibits matrix mineralization (Ho et al. 2000). This inhibition is released through the action of TNAP, which hydrolyzes PP_i, thus simultaneously removing the inhibitor and providing additional P_i for mineral formation (Hessle et al. 2002; Harmey et al. 2004). Mineralization is said to proceed in two phases: an initial formation of apatite within MVs, and a subsequent propagation phase in the matrix (Anderson et al. 2005; Golub 2009). According to this mechanism, Ca^{2+} enters MVs via an annexin channel and phosphate enters via a type III Na^+-dependent phosphate transporter (PiT), and possibly others, to form apatite within MVs (Arispe et al. 1996; Wu et al.

2003). Acidic phospholipids and other MV components are thought to nucleate these intravesicular nanocrystals (Wuthier et al. 1992; Kirsch et al. 1994; Wu et al. 1996). Subsequently, the intravesicular mineral grows beyond the confines of MVs onto a collagenous matrix aided by a number of promoters and inhibitors of calcification (Giachelli 2005). HA crystals are still present in TNAP-deficient MVs, and it has been proposed that the soluble MV phosphatase PHOSPHO1 might be involved in increasing the local intravesicular concentration of P_i to change the P_i/PP_i ratio to favor precipitation of HA seed crystals (Roberts et al. 2007). Recent data evaluating the skeletal mineralization defects in PHOSPHO1 knockout mice and the dramatic complete absence of skeletal mineralization in the TNAP/PHOSPHO1 double knockout mice confirm an important role for PHOSPHO1 function in the initiation of skeletal mineralization (Yadav et al. 2011; Huesa et al. 2011).

In order to understand the physiological interplay between important MV-associated enzymes in the initiation of biomineralization, it is important to keep in mind the microenvironment in which these enzymes function, which can have a profound effect on their biological properties, since phospholipids play an important role in the initiation of the biomineralization process (Anderson et al. 1997; Wu et al. 2002). Early studies of MV lipid composition have determined that there are significant differences between the lipid content of MVs and the plasma membranes from which they arose. Wuthier (1976) reported these differences and hypothesized that the increase in acidic phospholipids in MVs was somehow associated with MV calcification. Further studies from his group, along with those of Boyan et al. (1989) have proposed that MV lipids could act as a nucleation site for HA formation (Skrtic and Eanes 1992; Genge et al. 1989, 2008). This premise has been extensively developed under the idea of a "nucleational core complex", which describes the interactions of MV phospholipids, Ca^{2+}, PO_4^{3-}, and some MV proteins to form a molecular architecture that nucleates apatite crystallization (Genge et al. 2007, 2008). The acidic phospholipids can bind Ca^{2+} but, more importantly, it has been shown that they facilitate calcium-dependent annexin binding, and are permissive for annexins to form calcium channels through the membrane (Wuthier et al. 1992; Kirsch et al. 1997). Several lines of evidence suggest that glycosylphosphatidylinositol (GPI)-anchored proteins are commonly found to be enriched within putative cholesterol- and (glyco)sphingolipid-enriched platforms called "rafts", and that such association is required for the expression of their biological function (Hooper 1999; Brown and London 2000).

Recent data (Ciancaglini et al. 2006) suggest that the location of TNAP on the membrane of MVs plays a role in determining substrate selectivity in this micro-compartment. These data suggest that assays of TNAP bound to MVs or to liposome-based systems might be more biologically relevant than assays done with solubilized enzyme preparations, particularly when studying the hydrolysis of organophosphate substrates. The ability of synthetic or natural vesicles (Camolezi et al. 2002; Ierardi et al. 2002) to mimic the organizational structure and function of biomembranes makes these structures an advantageous and convenient experimental model to help in advancing our understanding of MV-mediated calcification. Proteoliposomes are easy to prepare and can be manufactured using different methods and with controlled lipid and protein composition, electrolytes, and sizes, representing a convenient experimental model to mimic the organizational structure and function of natural biomembranes and to reproduce some essential features of the biomineralization process (Blandford et al. 2003; Simão et al. 2010a). But since proteoliposomes are artificial systems, it is important to also consider the stability of the vesicles, which may affect the structure and function of the reconstituted enzymes. An important experimental consideration is the need to establish the appropriate lipid composition, especially the cholesterol content, to be used in the construction of the proteoliposomes, since the amount of cholesterol modulates the fluidity of the bilayer and influences membrane features, such as curvature, that can affect the activity of the enzymes in different ways, depending on the substrates used (Camolezi et al. 2002; Bolean et al. 2010). Moreover, the lipid membrane plays an important role as a nucleation agent in the biomineralization process (Eanes and Hailer 1985; Eanes 1989), as a protective and/or activation agent (Carruthers and Melchior 1986).

Two of the main lipids found in the MV membranes are dipalmitoylphosphatidylserine and dipalmitoylphosphatidylcholine (DPPC), and many studies have revealed that they play a crucial role in the biomineralization process, regulating both calcium entry into the MVs and formation of HA crystals (Kirsch et al. 1997; Wu et al. 2002). Using the direct insertion method and DPPC liposomes, Simão et al. (2010a) described the production and characterization of proteoliposomes harboring TNAP alone, NPP1 alone, and TNAP + NPP1 together as MV biomimetics to further understand the interplay between these enzymes in the utilization of physiological substrates as a means of understanding the more complex interplay of these enzymes in intact MVs, crucial during early events of skeletal mineralization. These studies were validated by simultaneous studies on isolated MVs. Thus, Ciancaglini et al. (2010) examined substrate utilization by isolated osteoblast-derived MVs, where these and other enzymes are present together in a physiological–biological compartment, reporting the relative ability of wild-type MVs, as well as MVs deficient in TNAP, NPP1, or PHOSPHO1 to utilize the substrates ATP, ADP, and PP_i under physiological conditions. TNAP- and PHOSPHO1-deficient MVs showed reduced calcification ability, while NPP1-deficient MVs hypercalcified, demonstrating that the cooperativity as well as the competition of TNAP,

NPP1, and PHOSPHO1 for the biomineralization substrates provides an additional level of regulation of metabolite flow for the control of the calcification process.

The size of natural osteoblast- and chondrocyte-derived MVs varies from 20 to 300 nm in diameter and it is not known if a single cell produces multiple sub-classes of MVs or only one class at a time (Sela et al. 2000; Ciancaglini et al. 2006; Simão et al. 2010b). The size of TNAP proteoliposomes reconstituted from DPPC was 300 nm, as determined by dynamic light scattering (Simão et al. 2010a), and thus is comparable to the median size of natural MVs (Ciancaglini et al. 2006; Simão et al. 2010b), and can adequately serve as a vesicular mimetic system to examine TNAP function in the context of a lipid membrane environment that mimics the MV environment. Electron microscopy of empty DPPC liposomes and TNAP-proteoliposomes showed that enzyme reconstitution does not affect the morphology of the liposomes.

Simão et al. (2010a, b) showed that the specific phospholipid microenvironment of MVs strongly influences the catabolism of the various physiological substrates of biomineralization by TNAP and NPP1, as well as their relative weight in catabolism. It further suggests that the mechanistic study of these enzymes and their pathological mutants in biomineralization would, therefore, be better conducted in relevant liposomes rather than in solution. Most previous work on MVs has focused on their disassembly to determine chemical composition, and on assays to elucidate how they function in mineralization.

The proteoliposome system provides a means of reconstituting lipid vesicles that will function like MVs. There have been previous attempts to simulate MV mineralization using multilamellar liposomal systems (Eanes et al. 1984; Eanes and Hailer 1985, 1987; Eanes 1989). In addition, liposomes containing various proportions of anionic and neutral phospholipids and cholesterol were used to study the ability of annexin V to facilitate Ca^{2+} uptake (Kirsch et al. 1997). However, an accurate model of MVs must entail a unilamellar system closely approximating the size, lipid, electrolyte, and protein composition of native MVs that is capable of inducing mineralization.

Such an MV biomimetic proteoliposome system would be useful for many important translational applications. The enzymatic defects associated with disease-causing mutations in the TNAP molecule, such as those found in hypophosphatasia (Di Mauro et al. 2002), could be further elucidated in a membrane vesicle that better mimics their in vivo biological environment. Since this artificial vesicular system adequately mimics the kinetic behavior of the enzymes in the natural vesicular MV environment (Simão et al. 2010a, b), this proteoliposome system can be used for the screening of small molecule compounds able to modulate (inhibit or activate) the activity of MV enzymes for potential therapeutic uses (Sergienko et al. 2009; Lanier et al. 2010). Such an approach seems indicated, especially when these compounds bear organic moieties capable of interacting with membrane phospholipids, directly or indirectly via Ca^{2+} ions present in the mineralizing microenvironment. A liposome environment will mimic the phospholipid-modified availability of organic substrates, inhibitors, and modulators to membrane-bound enzymes, thus allowing the study of enzyme catalysis in a more physiological manner than with solubilized enzymes.

This nascent in vitro experimental system will also allow the construction of progressively more complex proteoliposomes containing TNAP, NPP1, PHOSPHO1, PiT1/2, ANK, annexins, etc. that closely simulate the lipid, size, electrolyte, and protein composition of native MVs, and that also reproduce the kinetic properties of native MV in the formation of calcium phosphate minerals, with the ultimate goal of replicating in vitro the key events leading to the initiation of HA crystal formation in chondrocyte- and osteoblast-derived MVs. Once the proteoliposomes have been built and characterized, these vesicles can be added to fixed amounts of MVs, wild-type or deficient in specific enzymes, as a way of modulating their in vitro calcification properties. Those experiments would validate the use of these nanovesicles in promoting or delaying calcification, and such an artificial nanovesicular system could also potentially prove useful for the repair/treatment of craniofacial and other skeletal defects and to facilitate the mineralization of titanium-based tooth implants. In addition, the property of liposome to carrying in aqueous or lipid bilayers could be used as a system for delivery of drug or dyes with many applications (Santos et al. 2005, 2007; Saddi et al. 2008; Alves et al. 2009).

Carrier for antigenic protein: vaccines and nanosensors for neglected diseases

Efficient vaccination strategies have been desired for overcoming new pathogens and evolution of resistance by microorganisms (Yuba et al. 2010). However, effective vaccines to eliminate pathogens entrenched in the human population, such as malaria, leishmaniasis, measles, acute respiratory infections, and HIV, are still to be developed (Kalkanidis et al. 2006; Nagill and Kaur 2010). Traditional vaccines consist of either whole-inactivated or live-attenuated microorganisms. However, these can be unsafe and can produce some side effects, such as inflammation (Harrison et al. 1999). On the other hand, vaccines that include purified or recombinant macromolecules of pathogens, such as surface proteins or polysaccharides, seem to have minimal side effects (Kwon et al. 2005).

Leishmania spp. is a protozoan parasite, necessarily intracellular, that infects skin macrophages and viscera,

causing an illness in the vertebrate hosts known as leishmaniasis (Bhowmick et al. 2007). Leishmaniasis is a serious and increasing worldwide public health problem. Approximately 300 million people live in or travel to tropical and subtropical risk areas. Moreover, human leishmaniasis is endemic in more than 80 countries, and its prevalence exceeds 12 million cases worldwide, i.e. 1.5–2.0 million new cases may occur annually (http://www.who.int/en/).

Leishmaniasis is a disease that is most likely to be controlled by a successful vaccination program. The relatively uncomplicated leishmanial life cycle, and the fact that recovery from a primary infection renders the host resistant to subsequent infections, indicate that a vaccine is feasible (Kedzierski 2010).

The ultimate goal of vaccination is to generate a strong immune response that will confer long-lasting protection against leishmaniasis. Since purified proteins or protein fragments alone are poor immunogens (Titus et al. 1995), they require the addition of an adjuvant or antigen-carrier system to be effective. The adjuvant should be selected according to the antigens to be used, animals to be vaccinated, route of administration, and side effects (Nagill and Kaur 2010). Several adjuvants, such as alum, antigen encapsulation in positively charged liposomes, monophosphoryl lipid A, IL-12, BCG, saponin, and lipid microspheres, have been tested against leishmaniasis (Santos et al. 2009; Nagill and Kaur 2010). Lipid microspheres are excellent drug delivery or vaccine adjuvant systems and are relatively stable (Santos et al. 2009).

Liposomes have been recognized as potent immunostimulators and are able to induce humoral as well as cell-mediated immune response when used along with an antigen (Khalil et al. 2006; Badiee et al. 2007). Recently, technologies that incorporate adjuvants to increase the immunogenicity of antigens have been demonstrated to be one of the top ten technologies that will significantly impact global health (Mutwiri et al. 2004). The nanotechnologies processes are much used as new tools for vaccine development in several areas of immunogenicity improvement such as specific professional cell antigen delivery.

Several publications have demonstrated how liposomes are being used as appropriate antigen carriers for the generation of antibody response in vivo or in vitro (Daghastanli et al. 2004; Santos et al. 2006a; Migliaccio et al. 2008) and as effective vehicles for peptides and proteins, thus enhancing their immunogenicity in leishmaniasis infection models (Afrin et al. 2002; Jaafari et al. 2006; Badiee et al. 2007; Santos et al. 2006a; Migliaccio et al. 2008). Depending on the nature of the antigen, however, the procedure for the preparation of the liposomes may vary considerably. The aqueous space in most liposomal structures allows the incorporation of hydrophilic antigens. Thus, preparation of liposomes by addition of aqueous solution of antigens to dried lipid films resulting in multilamellar vesicles (MLV) is the conventional and most favored method of liposome preparation for vaccine formulations (Afrin and Ali 1997; Yang et al. 2008). Another method used for insertion of antigens in liposomes is through direct insertion of proteins anchored by GPI of biological membrane into vesicles already formed and with high efficiency of incorporation (Colhone et al. 2009). These processes allow the preservation of the structural characteristics desirable for its antigenicity without the danger of pathogenicity. Reconstitution of membrane proteins into liposomes has proved a viable alternative for the preparation of antigenic components to induce immunogenicity and humoral protection in animal models (Leserman 2004). In addition to preserving the native structure of proteins, liposomes have the advantage of giving adjuvant activity in vaccines against various pathogens (Daghastanli et al. 2004; Santos et al. 2006a; Migliaccio et al. 2008; Colhone et al. 2009).

It is expected that an ideal vaccine against leishmaniasis will likely combine more than one antigen, and that antigens will preferentially be conserved among *Leishmania* species and present in both the amastigote and promastigote stages of the parasite (de Oliveira et al. 2009; Grenfell et al. 2010). The ideal candidates for vaccine prototype are the proteins required for parasite survival, which have low mutation rates and conserved epitopes (Nagill and Kaur 2010).

Several *Leishmania* proteins have been identified, and the most comprehensively studied antigens include glycoprotein 63 (gp63) (Xu et al. 1995; Bhowmick et al. 2008), glycoprotein 46 (gp46) or parasite surface antigen 2 (PSA-2) (McMahon-Pratt et al. 1992, 1993), *Leishmania* homologue of receptors for activated C kinase (LACK) (Coelho et al. 2003; Pinto et al. 2004), and focused mannose ligand (FML) (Palatnik de Sousa et al. 1994; Santos et al. 2003).

Now we are testing a pool of proteins anchored by GPI to the surface promastigotes of *Leishmania amazonensis* as a source of antigens, incorporated into liposomes, for the preparation of a prototype vaccine against leishmaniasis. Other authors have already observed that immunization with gp63 encapsulated in neutral (Jaafari et al. 2006) and cationic liposomes (Bhowmick et al. 2008) and native PSA-2 antigen along with *Cryptosporidium parvum* (Handman et al. 1995; Sjolander et al. 1998) protected mice through Th1 cell-mediated immune response; however, recombinant PSA-2 failed to confer protection despite the induction of Th1 response (Sjolander et al. 1998).

Many challenges must be met before new classes of vaccines become available. Ideally, an adjuvant or delivery vehicle will have the ability to stimulate humoral, cellular, and mucosal immune responses concurrently or discretely, depending upon the treatment strategy desired. The time of response should be long and the vaccine components should be easily metabolized (Peek et al. 2008). An additional challenge includes developing alternative, less invasive

approaches for the administration of vaccinations. Perhaps most importantly, the cost of producing and distributing new vaccines should at risk including persons in less developed parts of the world. As these challenges are met, the prevention and therapy of many previously untreatable diseases should become increasingly possible (Peek et al. 2008).

Therefore, nanotechnology has offered enormous improvement in field of therapeutics by designing drug delivery systems and opened the possibility of controlling infections at the molecular level (Calderon et al. 2009, 2011; Salay et al. 2011). Nanoparticle-based systems, such as liposomes, have significant potential not only for treatments and vaccines as well as for possible diagnostics systems against various diseases.

Many research groups have focused on novel strategies for treatment and for low-cost, effective diagnosis systems (Vega-López 2003; Singh and Sivakumar 2003). Despite such efforts, diagnosis methods are still ineffective, with limitations regarding cost, sensitivity, difficulty in using under field conditions, and specificity (Romero et al. 2009). The limitation found in immunoassays and molecular diagnosis has motivated the development of integrated electronic biosensors systems, which in most cases employ nanostructured biomaterials. These systems are capable of detecting analytes via specific recognition based upon interaction between protein and ligands or antigens and antibodies (Zucolotto et al. 2007). Integrated biosensors may exhibit high sensitivity and specificity, as exemplified by the detection of Pasteurellosis. Detection is performed via electrical measurements, following the concept of a taste sensor, in which the bioreceptor material is immobilized on the gaps of an interdigitated electrode and immersed in aqueous solutions containing different concentration of the analyte (Zucolotto et al. 2007). Differences in the electrical capacitance of the electrodes are correlated to the type and concentration of the analytes using equivalent electric circuits (Perinoto et al. 2010). The latter biodetection systems are user-friendly and are capable of providing in-field diagnosis within a short period of time. These technical characteristics are advantageous for the high throughput needs inherent in developing new drugs and devising diagnosis tests.

We present a nanostructured biosensor system to detect specific anti-*L. amazonensis* antibodies using capacitance measurements as the detection method (Perinoto et al. 2010; Paulovich et al. 2011). The system is comprised of liposomes incorporating membrane antigenic proteins as the immobilized phase, which had been anchored to the surface of interdigitated electrodes. The electrodes containing antigenic proteins were used to detect antibodies, in which the biological reaction was converted into variations in the electrical response (capacitance). These biosensors showed that efficient distinction between cutaneous Leishmaniasis and Chagas' disease can be obtained with a low-cost biosensors system made with nanostructured films containing specific *L. amazonensis* and *T. cruzi* antigens and employing impedance spectroscopy as the detection method. This is undoubtedly one of the great advantages of these biosensors, since the immunological diagnosis used for Leishmaniasis are often not specific, since cross-reactions may occur frequently with Chagas' disease by generating false positives (Perinoto et al. 2010; Paulovich et al. 2011).

This unprecedented selectivity was afforded by antigen–antibody molecular recognition processes inherent in the detection with the immobilized antigens, and by statistically correlating the electrical impedance data, which allowed distinction between real samples that tested positive for Chagas' disease and Leishmaniasis. Distinction could be made of blood serum samples containing 10^{-5} mg/mL of the antibody solution in a few minutes (Perinoto et al. 2010; Paulovich et al. 2011). The methods used are generic and can be extended to any type of biosensor, which is important for an effective diagnosis of many other diseases.

In conclusion, these proteoliposomes systems are gaining prominence in the literature as very useful tools, not only for the study of interactions between proteins or peptides (dos Santos et al. 2010; Barbosa et al. 2011; Salay et al. 2011) with lipid membrane but as systems to produce more complex vesicular systems with great potential for biotechnological applications mimicking natural vesicles, and in the area of vaccines or nanosensors (Fig. 4).

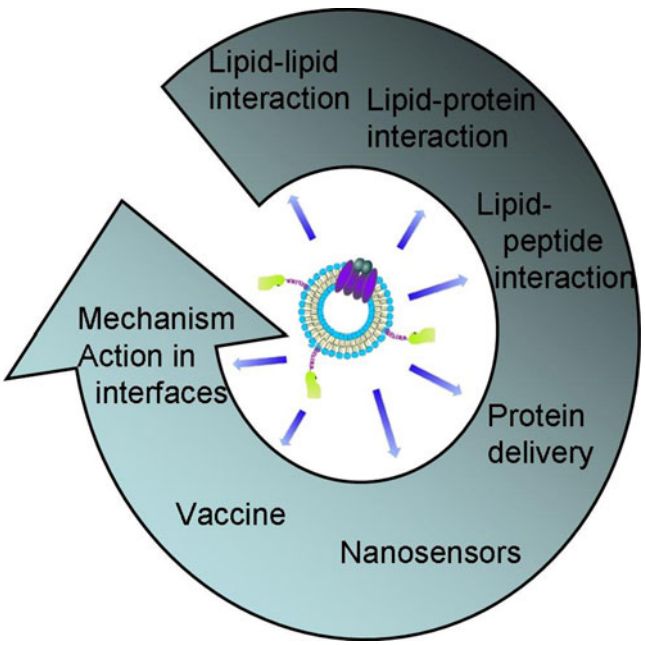

Fig. 4 Scheme of the different approaches to use proteoliposomes: to study lipid–lipid, lipid–protein, and lipid-peptide interactions as delivery systems, as vesicular mimetic systems, as nanosensors or vaccines, and as a system to study interfaces

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Conflict of interest None

References

Albers RW (1967) Biochemical aspects of active transport. Ann Biochem 36:727–756

Afrin F, Ali N (1997) Adjuvanticity and protective immunity elicited by Leishmania donovani antigens encapsulated in positively charged liposomes. Infect Immun 65:2371–2377

Afrin F, Rajesh R, Anam K, Gopinath M, Pal S, Ali N (2002) Characterization of Leishmania donovani antigens encapsulated in liposomes that induce protective immunity in BALB/c mice. Infect Immun 70:6697–6706

Alves JB, Ferreira CL, Moreira AF, Silva GA, Alves GD, Paulino TP, Ciancaglini P, Thedei G Jr, Napimoga MH (2009) Local delivery of EGF-liposome mediated bone modeling in orthodontic tooth movement by increasing RANKL expression. Life Sci 85(19–20):693–699

Anderson HC, Hsu HH, Morris DC, Fedde KN, Whyte MP (1997) Matrix vesicles in osteomalacic hypophosphatasia bone contain apatite-like mineral crystals. Am J Pathol 151:1555–1561

Anderson HC, Garimella R, Tague SE (2005) The role of matrix vesicles in growth plate development and biomineralization. Front Biosci 10:822–837

Antolovic R, Hamer E, Serpersu EH, Kost H, Linnertz H, Kovarik Z, Schoner W (1999) Affinity labelling with MgATP analogues reveals coexisting Na + and K + forms of he alpha-subunits of Na+/K + −ATPase. Eur J Biochem 261:181–189

Arispe N, Rojas E, Genge BR, Wu LN, Wuthier RE (1996) Similarity in calcium channel activity of annexin V and matrix vesicles in planar lipid bilayers. Biophys J 71:1764–1775

Badiee A, Jaafari MR, Khamesipour A (2007) Leishmania major: immune response in BALB/c mice immunized with stress-inducible protein 1 encapsulated in liposomes. Exp Parasitol 115:127–134

Barbosa LR, Rigos CF, Yoneda JS, Itri R, Ciancaglini P (2010) Unraveling the Na, K-ATPase alpha(4) subunit assembling induced by large amounts of C(12)E(8) by means of small-angle X-ray scattering. J Phys Chem B 114(35):11371–11376

Barbosa SC, Cilli EM, Dias LG, Stabeli RG, Ciancaglini P (2011) Labaditin, a cyclic peptide with rich biotechnological potential: preliminary toxicological studies and structural changes in water and lipid membrane environment. Amino Acids 40:135–144

Béguin P, Wang X, Firsov D, Puoti A, Claeys D, Horisberger JD, Geering K (1997) The γ subunit is a specific component of the Na, K-ATPase and modulates its transport function. EMBO J 16:4250–4260

Béguin P, Crambert G, Guennoun S, Garty H, Horisberger JD, Geering K (2001) CHIF, a member of the FXYD protein family, is a regulator of Na, K-ATPase distinct from the gamma-subunit. EMBO J 20(15):3993–4002

Benatti CR, Epand RM, Lamy MT (2007) Low cholesterol solubility in DODAB liposomes. Chem Phys Lipids 145(1):27–36

Bhowmick S, Ravindram R, Ali N (2007) Leishmanial antigens in liposomes promote protective immunity and provide immunotherapy against visceral leishmaniasis via polarized Th1 response. Vaccine 25:6544–6556

Bhowmick S, Ravindran R, Ali N (2008) Gp63 in stable cationic liposomes confers sustained vaccine immunity to susceptible BALB/c mice infected with Leishmania donovani. Infect Immun 76:1003–1015

Blanco G (2005) Na, K-ATPase subunit heterogeneity as a mechanism for tissue-specific ion regulation. Semin Nephrol 25:292–303

Blandford NR, Sauer GR, Genge BR, Wu LN, Wuthier RE (2003) Modeling of matrix vesicle biomineralization using large unilamellar vesicles. J Inorg Biochem 94:14–27

Bolean M, Simão AM, Favarin BZ, Millán JL, Ciancaglini P (2010) The effect of cholesterol on the reconstitution of alkaline phosphatase into liposomes. Biophys Chem 152:74–79

Bolean M, Simão AM, Favarin BZ, Millán JL, Ciancaglini P (2011) Thermodynamic properties and characterization of proteoliposomes rich in microdomains carrying alkaline phosphatase. Biophys Chem 158(2–3):111–118

Boyan BD, Schwartz Z, Swain LD, Khare A (1989) Role of lipids in calcification of cartilage. Anat Rec 224:211–219

Brown DA, London E (2000) Structure and function of sphingolipidand cholesterol-rich membrane rafts. J Biol Chem 275:17221–17224

Calderon LA, Silva-Jardim I, Zuliani JP, Silva AA, Ciancaglini P, Pereira da Silva LH, Stabeli RG (2009) Amazonian biodiversity: a view of drug development for leishmaniasis and malaria. J Braz Chem Soc 20(6):1011–1023

Calderon LA, Silva AA, Ciancaglini P, Stabeli RG (2011) Antimicrobial peptides from Phyllomedusa frogs: from biomolecular diversity to potential nanotechnologic medical applications. Amino Acids 40:29–49

Cantor RS (1997) The lateral pressure profile in membranes: a physical mechanism of general anesthesia. Biochemistry 36:2339–2344

Camolezi FL, Pizauro JM, Leone FA, Ciancaglini P (1999) A simple laboratory experiment to demonstrate the interaction of proteins bearing glycosylphosphatidyl-inositol anchors with liposomes. Biochem Educ 27:37–40

Camolezi FL, Daghastanli KR, Magalhaes PP, Pizauro JM, Ciancaglini P (2002) Construction of an alkaline phosphataseliposome system: a tool for biomineralization study. Int J Biochem Cell Biol 34:1091–1101

Carruthers A, Melchior DL (1986) How bilayer lipids affect membrane protein activity. Trends Biochem Sci 11:331–335

Ciancaglini P, Pizauro JM, Rezende AA, Rezende LA, Leone FA (1990) Solubilization of membrane-bound matrix-induced alkaline phosphatase with polyoxyetylene 9- lauryl ether (polidocanol): purification and metalloenzyme properties. Int J Biochem 22:385–392

Ciancaglini P, Simao AM, Camolezi FL, Millan JL, Pizauro JM (2006) Contribution of matrix vesicles and alkaline phosphatase to ectopic bone formation. Braz J Med Biol Res 39(5):603–610

Ciancaglini P, Yadav MC, Simão AM, Narisawa S, Pizauro JM, Farquharson C, Hoylaerts MF, Millán JL (2010) Kinetic analysis of substrate utilization by native and TNAP-, NPP1-, or PHOSPHO1-deficient matrix vesicles. J Bone Miner Res 25(4):716–723

Coelho EA, Tavares CA, Carvalho FA, Chaves KF, Texeira KN, Rodriguez RC, Charest H, Matlashewski G, Gazzinelli RT, Fernandes AP (2003) Immune response induced by the Leishmania (Leishmania) donovani A2 antigen, but not by the LACK antigen, are protective against experimental Leishmania (Leishmania) amazonensis infection. Infect Immun 71:3988–3994

Colhone MC, Nobre TM, Zaniquelli MED, Stabeli RG, Ciancaglini P (2009) Incorporation of antigenic GPI-protein from Leishmania amazonensis to membrane mimetic systems: Influence of DPPC/cholesterol ratio. J Colloid Interface Sci 333:373–379

Cornelius F, Turner N, Christensen HR (2003) Modulation of Na, K-ATPase by phospholipids and cholesterol. II. Steady-state and presteady-state kinetics. Biochemistry 42(28):8541–8549

Costa CJ, Gatto C, Kaplan JH (2003) Interactions between Na, K-ATPase alpha-subunit TP-binding domains. J Biol Chem 278:9176–9184

Cuevas FJ, Jameson DM, Sotomayor CP (2006) Modulation of reconstituted pig kidney Na/K-ATPase activity by cholesterol in endodenous lipid vesicles: role of lipid domains. Biochemistry 45:13855–13868

Daghastanli KR, Ferreira RB, Thedei G Jr, Maggio B, Ciancaglini P (2004) Lipid composition-dependent incorporation of multiple membrane proteins into liposomes. Colloids Surf B Biointerfaces 36(3–4):127–137

Damek-Poprawa M, Golub E, Otis L, Harrison G, Phillips C, Boesze-Battaglia K (2006) Chondrocytes utilize a cholesterol-dependent lipid translocator to externalize phosphatidylserine. Biochemistry 45:3325–3336

De Oliveira CI, Nascimento IP, Barral A, Soto M, Barral-Netto M (2009) Challenges and perspectives in vaccination against leishmaniasis. Parasitol Int 58:319–324

Dempski RE, Lustig J, Friedrich T, Bamberg E (2008) Structural arrangement and conformational dynamics of the gamma subunit of the Na+/K+−ATPase. Biochemistry 47(1):257–266

Di Mauro S, Manes T, Hessle L, Kozlenkov A, Pizauro JM, Hoylaerts MF, Millán JL (2002) Kinetic characterization of hypophosphatasia mutations with physiological substrates. J Bone Miner Res 17(8):1383–1391

Donnet C, Aristarkhova E, Sweadner KJ (2001) Thermal denaturation of the Na, K-ATPase provides evidence for alpha–alpha oligomeric interaction and gamma subunit association with the C-terminal domain. J Biol Chem 276:7357–7365

dos Santos GA, Thomé CH, Ferreira GA, Yoneda JS, Nobre TM, Daghastanli KRP, Scheucher PS, Teixeira HL, Constantino MG, de Oliveira KT, Faça VM, Falcão RP, Greene LJ, Rego EM, Ciancaglini P (2010) Interaction of 10-(octyloxy) decyl-2-(trimethylammonium) ethyl phosphate with mimetic membranes and cytotoxic effect on leukemiccells. Biochim Biophy Acta (Biomem) 1798(9):1714–1723

Dynarowicz-Latka P, Dhanabalanb A, Oliveira ON Jr (2001) Modern physicochemical research on Langmuir monolayers. Adv Colloid Interf Sci 91:221–293

Eanes ED (1989) Biophysical aspects of lipid interaction with mineral: liposome model studies. Anat Rec 224:220–225

Eanes ED, Hailer AW (1985) Liposome-mediated calcium phosphate formation in metastable solutions. Calcif Tissue Int 37:390–394

Eanes ED, Hailer AW (1987) Calcium phosphate precipitation in aqueous suspensions of phosphatidylserine-containing anionic liposomes. Calcif Tissue Int 40:43–48

Eanes ED, Hailer AW, Costa JL (1984) Calcium phosphate formation in aqueous suspensions of multilamellar liposomes. Calcif Tissue Int 36:421–430

Faller LD (2008) Mechanistic studies of sodium pump. Arch Biochem Biophys 476(1):12–21

Furth AJ, Bolton H, Potter J, Priddle JD (1984) Separating detergent from proteins. Methods Enzymol 104:318–328

Geering K (2001) The functional role of beta subunits in oligomeric P-type ATPases. J Bioenerg Biomembr 33(5):425–438

Geering K (2005) Function of FXYD proteins, regulators of Na, K-ATPase. J Bioenerg Biomembr 37(6):387–392

Geering K (2006) FXYD proteins: new regulators of Na-K-ATPase. Semin Nephrol 25:304–311

Geering K (2008) Functional roles of Na, K-ATPase subunits. Curr Opin Nephrol Hypertens 17(5):526–532

Genge BR, Wu LN, Wuthier RE (1989) Identification of phospholipid-dependent calcium-binding proteins as constituents of matrix vesicles. J Biol Chem 264:10917–10921

Genge BR, Wu LN, Wuthier RE (2007) Kinetic analysis of mineral formation during in vitro modeling of matrix vesicle mineralization: effect of annexin A5, phosphatidylserine, and type II collagen. Anal Biochem 367:159–166

Genge BR, Wu LN, Wuthier RE (2008) Mineralization of annexin-5-containing lipid-calcium-phosphate complexes: modulation by varying lipid composition and incubation with cartilage collagens. J Biol Chem 283:9737–9748

Gennis RB (1989) Biomembranes: molecular structure and function. Springer, Berlin

Giachelli CM (2005) Inducers and inhibitors of biomineralization: lessons from pathological calcification. Orthod Craniofac Res 8:229–231

Giocondi MC, Besson F, Dosset P, Milhiet PE, Grimellec CL (2007) Remodeling of ordered membrane domains by GPI-anchored intestinal alkaline phosphatase. Langmuir 23:9358–9364

Glimcher MJ (2006) Bone: nature of the calcium phosphate crystals and cellular, structural, and physical chemical mechanisms in their formation. In: Sahai N, Schoonen MAA (eds) Medical mineralogy and geochemistry. Reviews in Mineralogy & Geochemistry, vol 64. pp 223–282

Golub EE (2009) Role of matrix vesicles in biomineralization. Biochim Biophys Acta 1790:1592–1598

Grenfell RFQ, Marques-da-Silva EA, Souza-Testasicca MC, Coelho EAF, Fernandes AP, Afonso LCC, Rezende SA (2010) Antigenic extracts of Leishmania braziliensis and Leishmania amazonensis associated with saponin partially protects BALB/c mice against Leishmania chagasi infection by suppressing IL-10 and IL-4 prodution. Mem Inst Oswaldo Cruz 105:818–822

Handman E, Symons FM, Baldwin TM, Curtis JM, Scheerlinck JPY (1995) Protective vaccination with promastigote surface antigen 2 from Leishmania major is mediated by a Th1 type of immune response. Infect Immun 63:4261–4267

Harmey D, Hessle L, Narisawa S, Johnson KA, Terkeltaub R, Millán JL (2004) Concerted regulation of inorganic pyrophosphate and osteopontin by akp2, enpp 1, and ANK: an integrated model of the pathogenesis of mineralization disorders. Am J Pathol 164:1199–1209

Harrison GB, Shakes TR, Robinson CM, Lawrence SB, Heath DD, Dempster RP, Lightowlers MW, Rickard MD (1999) Duration of immunity, efficacy and safety in sheep of a recombinant Taenia ovis vaccine formulated with saponin or selected adjuvants. Vet Immunol Immunopathol 70:161–172

Heerklotz H (2008) Interactions of surfactants with lipid membranes. Q Rev Biophys 41(3–4):205–264

Hessle L, Johnson KA, Anderson HC, Narisawa S, Sali A, Goding JW, Terkeltaub R, Millan JL (2002) Tissue-nonspecific alkaline phosphatase and plasma cell membrane glycoprotein-1 are central antagonistic regulators of bone mineralization. Proc Natl Acad Sci USA 99(14):9445–9449

Ho AM, Johnson MD, Kingsley DM (2000) Role of the mouse ank gene in control of tissue calcification and arthritis. Science 289:265–270

Hooper NM (1999) Detergent-insoluble glycosphingolipid/cholesterol-rich membrane domains, lipid rafts and caveolae. Mol Membr Biol 16:145–156

Huesa C, Yadav MC, Finnilä MJ, Goodyear SR, Robins SP, Tanner KE, Aspden RM, Millán JL, Farquharson C (2011) PHOSPHO1 is essential for mechanically competent mineralization and the avoidance of spontaneous fractures. Bone 48:1066–1074

Ierardi DF, Pizauro JM, Ciancaglini P (2002) Erythrocyte ghost cell-alkaline phosphatase: construction and characterization of a vesicular system for use in biomineralization studies. Biochim Biophys Acta 1567:183–192

Jaafari MR, Ghafarian A, Farrokh-Gisour A, Samiei A, Kheiri MT, Mahboudi F, Barkhordari F, Khamesipour A, McMaster WR (2006) Immune response and protection assay of recombinant major surface glycoprotein of Leishmania (rgp63) reconstituted with liposomes in BALB/c mice. Vaccine 24:5708–5717

Jensen MØ, Mouritsen OG (2004) Lipids do influence protein function-the hydrophobic matching hypothesis revisited. Biochim Biophys Acta 1666(1–2):205–226

Kalipatnapu S, Chattopadhyay A (2005) Membrane protein solubilization: recent advances and challenges in solubilization of serotonin1a receptors. IUBMB Life 57(7):505–512

Kalkanidis M, Pietersz GA, Xiang SD, Mottram PL, Crimeen-Irwin B, Ardipradja K, Plebanski M (2006) Methods for nano-particle based vaccine formulation and evaluation of their immunogenicity. Methods 40:20–29

Kaplan JH (2002) Biochemistry of Na, K-ATPase. Annu Rev Biochem 71:511–535

Kedzierski L (2010) Leishmaniasis Vaccine: Where are we today? J Glob Infec Dis 2(2):177–185

Khalil EA, Musa AM, Modabber F, El-Hassan AM (2006) Safety and immunogenicity of a candidate vaccine for visceral leishmaniasis (Alum-precipated autoclaved Leishmania major + BCG) in children: an extended phase II study. Ann Trop Paediatr 26:357–361

Kihn L, Rutkowski D, Stinson RA (1990) Incorporation of human and placental alkaline phosphatase into liposomes and membranes is via phosphatidylinositol. Biochem Cell Biol 68:1112–1118

Kirsch T, Ishikawa Y, Mwale F, Wuthier RE (1994) Roles of the nucleational core complex and collagens (types II and X) in calcification of growth plate cartilage matrix vesicles. J Biol Chem 269:20103–20109

Kirsch T, Nah HD, Demuth DR, Harrison G, Golub EE, Adams SL, Pacifici M (1997) Annexin V-mediated calcium flux across membranes is dependent on the lipid composition: implications for cartilage mineralization. Biochemistry 36(11):3359–3367

Kwon YJ, Standley SM, Goh SL, Fréchet JM (2005) Enhanced antigen presentation and immunostimulation of dendritic cells using acid-degradable cationic nanoparticles. J Control Release 105:199–212

Lanier M, Sergienko S, Simao AMS, Chung T, Millán JL, Cashman JR (2010) Design and synthesis of selective inhibitors of placental alkaline phosphatase. Bioorg Med Chem 18:573–579

Le Maire M, Champeil P, Moller JV (2000) Interaction of membrane proteins and lipids with solubilizing detergents. Biochim Biophys Acta 1508(1–2):86–111

Leone FA, Pizauro JM, Ciancaglini P (1997) Rat osseous plate alkaline phosphatase: a search for its role in biomineralization. Trends Comp Biochem Physiol 3:57–73

Leserman L (2004) Liposomes as protein carriers in immunology. J Liposome Res 14:175–189

Lingrel JB (2010) The physiological significance of the cardiotonic steroid/ouabain-binding site of the Na+, K(+)-ATPase. Annu Rev Physiol 72:395–412

Lingrel JB, Kuntzweiler T (1994) Na+, K(+)-ATPase. J Biol Chem 269:19659–19662

Lingwood D, Simons K (2010) Lipid rafts as a membrane-organizing principle. Science 327:46–50

Magalhães PP, Paulino TP, Thedei G Jr, Larson RE, Ciancaglini P (2003) A 100 kDa vanadate and lanzoprazole-sensitive ATPase from streptococcus mutans membrane. Arch Oral Biol 48 (12):815–824

Mahaney JE, Girard JP, Grisham CM (1990) Saturation transfer EPR measurements of the rotational motion of a strongly immobilized ouabain spin label on renal Na, K-ATPase. FEBS Lett 260:160–164

Martin DW (2005) Structure-function relationships in the Na+, K(+)-pump. Semin Nephrol 25:282–291

Martin DW, Marecek J, Scarlata S, Sachs JR (2000) Protomers of Na+, K(+)-ATPase from microsomes of duck salt gland aremostlymonomeric: formation of higher oligomers does notmodifymolecular activity. Proc Natl Acad Sci USA 97:3195–3200

Marsh D (2008) Protein modulation of lipids, and vice-versa, in membranes. Biochim Biophys Acta 1778:1545–1575

McMahon-Pratt D, Traub-Cseko Y, Lohman KL, Rogers DD, Beverley SM (1992) Loss of the GP46/M-2 surface membrane glycoprotein gene family in the Leishmania braziliensis complex. Mol Biochem Parasitol 50:151–160

McMahon-Pratt D, Rodriguez D, Rodriguez JR, Zhang Y, Manson K, Bergman C, Rivas L, Rodriguez JF, Lohman KL, Ruddle NH, Esteban M (1993) Recombinant vaccínia viruses expressing gp46/M-2 protect against Leishmania infection. Infect Immun 61:3351–3359

Migliaccio V, Santos FR, Ciancaglini P, Ramalho-Pinto FJ (2008) Use of proteoliposome as a vaccine against Trypanosoma cruzi in mice. Chem Phys Lipids 152(2):86–94

Miles AJ, Wallace BA, Esmann M (2011) Correlation of structural and functional thermal stability of the integral membrane protein Na, K-ATPase. Biochem Biophys Acta (Biomem) 1808(10):2573–2580

Morth JP, Pedersen BP, Toustrup-Jensen MS, Sørensen TLM, Petersen J, Andersen JP, Vilsen B, Nissen P (2007) Cristal structure of the sodium-potassium pump. Nature 150:1043–1050

Morth JP, Pedersen BP, Buch-Pedersen MJ, Andersen JP, Vilsen B, Palmgren MG, Nissen P (2011) A structural overview of the plasma membrane Na+, K(+)-ATPase and H(+)-ATPase ion pumps. Nat Rev Mol Cell Biol 12(1):60–70

Mutwiri GK, Nichani AK, Baiuk S, Baiuk LA (2004) Strategies for enhancing the immunostimulatory effects of CpG oligodeoxynucleotides. J Control Releas 97:1–17

Nagill R, Kaur S (2010) Enhanced efficacy and immunogenicity of 78 kDa antigen formulated in various adjuvants against murine visceral leishmaniasis. Vaccine 28:4002–4012

New RRC (1990) Liposomes: a practical approach. Oxford University Press, New York

Ogawa H, Shinoda T, Cornelius F, Toyoshima C (2009) Crystal structure of the sodium-potassium pump (Na, K)-ATPase with bound potassium and ouabain. Proc Natl Acad Sci USA 106(33):13742–13747

Palatnik de Sousa CB, Paraguai de Souza E, Gomes EM, Borojevic R (1994) Experimental murine Leishmania donovani infection: immunoprotection by the fucose-manose ligand (FML). Braz J Med Biol Res 27:547–551

Paulovich FV, Maki RM, de Oliveira MC, Colhone MC, Santos FR, Migliaccio V, Ciancaglini P, Perez KR, Stabeli RG, Perinoto AC, Oliveira ON Jr, Zucolotto V (2011) Using multidimensional projection techniques for reaching a high distinguishing ability in biosensing. Anal Bioanal Chem 400:1153–1159

Peek LJ, Middaugh RC, Berkland C (2008) Nanotecnology in vaccine delivery. Adv Drug Deliv Rev 60:915–928

Perinoto AC, Maki RM, Colhone MC, Santos FR, Migliaccio V, Daghastanli KR, Stabeli RG, Ciancaglini P, Paulovich FV, de Oliveira MCF, Oliveira OR Jr, Zucolotto V (2010) Biosensors for efficient diagnosis of leishmaniasis: innovations in bioanalytics for a neglected disease. Anal Chem 82:9763–9768

Pinto EF, Pinheiro RO, Rayol A, Larraga V, Rossi-Bergmann B (2004) Intranasal vaccination against cutaneous leishmaniasis with a particulated leishmanial antigen or DNA encoding LACK. Infect Immun 72:4521–4527

Pizauro JM, Curti C, Ciancaglini P, Leone FA (1987) Triton X-100 solubilized bone matrix-induced alkaline phosphatase. Comp Biochem Physiol 87B:921–926

Pizauro JM, Ciancaglini P, Leone FA (1994) Osseous plate alkaline phosphatase is anchored by GPI. Braz J Med Biol Res 27:453–456

Pizauro JM, Ciancaglini P, Leone FA (1995) Characterization of the phosphatidylinositol-specific phospholipase C-released form of rat osseous plate alkaline phosphatase and its possible significance on endochondral ossification. Mol Cel Biochem 152:121–129

Post RL, Hegyvary C, Kume S (1972) Activation by adenosine triphosphate in the phosphorylation kinetics of sodium and potassium ion transport adenosine triphosphatase. J Biol Chem 247 (20):6530–6540

Prasad R (1996) Manual of membrane lipids. Springer, Berlin

Rajasekaran SA, Barwe SP, Rajasekaran AK (2005) Multiple functions of Na, K-ATPase in epithelial cells. Semin Nephrol 25(5):328–334

Rigaud JL, Pitard B, Levy D (1995) Reconstituition of membrane proteins into liposomes: aplication to energy-transducing membrane proteins. Biochim Biophys Acta 1231:223–246

Rigos CF, De Lima SH, Ward RJ, Ciancaglini P (2006) Lipid bilayer stabilization of the Na, K-ATPase reconstituted in DPPC/DPPE liposomes. Cell Biochem Biophys 44(3):438–445

Rigos CF, Nobre TM, Zaniquelli MED, Ward RJ, Ciancaglini P (2008) Circular dichroism associated with surface tension and dilatational elasticity to study the association of Na, K-ATPase subunits. J Coll Interf Sci 325(2):478–484

Rigos CF, Santos HL, Yoneda JS, Montich G, Maggio B, Ciancaglini P (2010) Cytoplasmatic domain of Na, K-ATPase alpha-subunit is responsible for the aggregation of the enzyme in proteoliposomes. Biophys Chem 146(1):36–41

Roberts S, Narisawa S, Harmey D, Millan JL, Farquharson C (2007) Functional involvement of PHOSPHO1 in matrix vesiclemediated skeletal mineralization. J Bone Miner Res 22:617–627

Romero GAS, Noronha EF, Pirmez C, Pires FESS, Fernandes O, Nehme NS, Cupolillo E, Firoozmand L, da Graça GC, Volpini A, Santos SL, Romanha A (2009) Sensitivity and reproducibility of a PCR assay for Leishmania detection using skin biopsy imprints on filter paper. Acta Trop 109:74–77

Salay LC, Nobre TM, Colhone MC, Zaniquelli MED, Ciancaglini P, Stabeli RG, Leite JRSA, Zucolotto V (2011) Dermaseptin 01 as antimicrobial peptide with rich biotechnological potential: study of peptide interaction with membranes containing Leishmania amazonensis lipid-rich extract and membrane models. J Peptide Sci 17(10):700–707

Saddi KRGC, Alves GD, Paulino TP, Ciancaglini P, Alves JB (2008) Epidermal growth factor in liposomes may enhance osteoclast recruitment during tooth movement in rats. Angle Orthod 789 (4):604–609

Santos HL, Ciancaglini P (2000) A practical approach to the choice of a suitable detergent and optimal conditions to solubilize a membrane protein. Biochem Educ 28:178–182

Santos HL, Ciancaglini P (2003) Kinetic characterization of Na, K-ATPase from rabbit outer renal medulla: properties of $(\alpha\beta)2$ dimer. Comp Biochem Physiol B 135(3):539–549

Santos HL, Lamas RP, Ciancaglini P (2002) Solubilization of Na, K-ATPase from rabbit kidney outer medulla using only C12E8. Braz J Med Biol Res 35:277–288

Santos WR, Aguiar IA, De Sousa EP, De Lima VMF, Palatnik M, Palatnik de Sousa CB (2003) Immunotherapy against murine experimental visceral leishmaniasis with the FML-vaccine. Vaccine 21:4668–4676

Santos HL, Lopes ML, Maggio B, Ciancaglini P (2005) Na, K-ATPase reconstituted in liposomes: effects of lipid composition on hydrolytic activity and enzyme orientation. Coll Surf B: Biointerf 419 (4):239–248

Santos FR, Ferraz DB, Daghastanli KRP, Ramalho-Pinto FJ, Ciancaglini P (2006a) Mimetic membrane system to carry multiple antigenic proteins from Leishmania amazonensis. J Membr Biol 210:173–181

Santos HL, Rigos CF, Ciancaglini P (2006b) Kinetics behaviors of Na, K-ATPase: comparison of solubilized and DPPC:DPPE-liposome reconstituted enzyme. Comp Biochem Physiol C Toxicol Pharmacol 142(3–4):309–316

Santos HL, Rigos CF, Tedesco AC, Ciancaglini P (2007) Biostimulation of Na, K-ATPase by low-energy laser irradiation (685 nm, 35 mW): comparative effects in membrane, solubilized and DPPC:DPPE-liposome reconstituted enzyme. J Photochem Photobiol B 89:22–28

Santos LE, Colhone MC, Daghastanli KR, Stabeli RG, Silva-Jardim I, Ciancaglini P (2009) Lipid microspheres loaded with antigenic membrane proteins of the Leishmania amazonensis as a potential biotechnology application. J Colloid Interface Sci 340:112–118

Schuck S, Honsho M, Ekroos K, Shevchoenko A, Simons K (2003) Resistence of cell membranes to different detergents. Proc Natl Acad Sci USA 100(10):5795–5800

Seddon AM, Curnow P, Booth PJ (2004) Membrane proteins, lipids and detergents: not just a soap opera. Biochim Biophys Acta 1666 (1–2):105–117

Sela J, Gross UM, Kohavi D, Shani J, Dean DD, Boyan BD, Schwartz Z (2000) Primary mineralization at the surfaces of implants. Crit Rev Oral Biol Med 11(4):423–436

Sergienko E, Su Y, Chan X, Brown B, Hurder A, Narisawa S, Millán JL (2009) Identification and characterization of novel tissuenonspecific alkaline phosphatase inhibitors with diverse modes of action. J Biomol Screen 14(7):824–837

Sesana S, Re F, Bulbarelli A, Salerno D, Cazzaniga E, Masserini M (2008) Membrane features and activity of GPI-anchored enzymes: alkaline phosphatase reconstituted in model membranes. Biochemistry 47:5433–5440

Shinoda T, Ogawa H, Cornelius F, Toyoshima C (2009) Crystal structure of the sodium-potassium pump at 2.4 A resolution. Nature 459(7245):446–450

Shull GE, Lane LK, Lingrel JB (1986) Amino-acid sequence of the beta-subunit of the (Na + K+) ATPase deduced from a cDNA. Nature 321:429–431

Silvius JR (1992) Solubilization and functional reconstitution of biomembrane components. Annu Rev Biophys Biomol Struct 21:323–348

Simão AMS, Beloti MM, Cezarino RM, Rosa AL, Pizauro JM, Ciancaglini P (2007) Membrane-bound alkaline phosphatase from ectopic mineralization and rat bone marrow cell culture. Comp Biochem Physiol A 146:679–687

Simão AMS, Yadav MC, Narisawa S, Bolean M, Pizauro JM, Hoylaerts MF, Ciancaglini P, Millán JL (2010a) Proteoliposomes harboring alkaline phosphatase and nucleotide pyrophosphatase as matrix vesicles biomimetics. J Biol Chem 285(10):7598–7609

Simão AMS, Yadav MC, Ciancaglini P, Millán JL (2010b) Proteoliposomes as matrix vesicles' biomimetics to study the initiation of skeletal mineralization. Braz J Med Biol Res 43(3):234–241

Simons K, Ikonen E (1997) Functional rafts in cell membranes. Nature 387:569–572

Simons K, Toomre D (2000) Lipid rafts and signal transduction. Nat Rev Mol Cell Biol 1(1):31–39

Singer SJ (2004) Some early history of membrane molecular biology. Annu Rev Physiol 66:1–27

Singh S, Sivakumar R (2003) Recent advances in the diagnosis of leishmaniasis. J Postgrad Med 49:55–60

Sjolander A, Baldwin TM, Curtis JM, Bengtsson KI, Handman E (1998) Vaccination with recombinant parasite surface antigen 2 from Leishmania major induce a Th1 type of immune response but does not protect against infection. Vaccine 16:2077–2084

Skrtic D, Eanes ED (1992) Membrane-mediated precipitation of calcium phosphate in model liposomes with matrix vesicle like lipid composition. Bone Miner 16:109–119

Sotomayor CP, Aguilar LF, Cuevas FJ, Helms MK, Jameson DM (2000) Modulation of pig kidney Na+/K + −ATPase activity by cholesterol: role of hydration. Biochemistry 39(35):10928–10935

Sweadner KJ (1989) Isozymes of the Na+/K + −ATPase. Biochim Biophys Acta 988(2):185–220

Taniguchi K, Kaya S, Abe K, Mardh S (2001) The oligomeric nature of Na/K-transport ATPase. J Biochem 129:335–342

Terkeltaub RA (2001) Inorganic pyrophosphate generation and disposition in pathophysiology. Am J Physiol Cell Physiol 281:C1–C11

Tian J, Xie ZJ (2008) The Na-K-ATPase and calcium-signaling microdomains. Physiology (Bethesda) 23:205–211

Titus RG, Gueiros-Filho FJ, de Freitas LA, Beverley SM (1995) Development of a safe live Leishmania vaccine line by gene replacement. Proc Natl Acad Sci USA 92:10267–10271

Thoenges D, Schoner W (1997) 2′-O-Dansyl analogs of ATP bind with high affinity to the low affinity ATP site of Na+/K + −ATPase and reveal the interaction of two ATP sites during catalysis. J Biol Chem 272:16315–16321

Vega-López F (2003) Diagnosis of cutaneous leishmaniasis. Curr Opin Infect Dis 16:97–101

Ward DG, Cavieres JD (1993) Solubilized Na, K-ATPase remains protomeric during turnover yet shows apparent negative cooperativity toward ATP. Proc Natl Acad Sci USA 96:5332–5336

Ward DG, Cavieres JD (1996) Binding of 2′(3′)-O-(2, 4, 6-trinitrophenyl)ADP to soluble alpha beta protomers of Na, K-ATPase modified with fluorescein isothiocyanate: Evidence for two distinct sites. J Biol Chem 271:12317–12321

White SH, Ladokhin AS, Jayasinghe S, Hristova K (2001) How membranes shape protein structure. J Biol Chem 27:32395–32398

Wu LN, Genge BR, Sauer GR, Wuthier RE (1996) Characterization and reconstitution of the nucleational complex responsible for mineral formation by growth plate cartilage matrix vesicles. Connect Tissue Res 35:309–315

Wu LN, Genge BR, Kang MW, Arsenault AL, Wuthier RE (2002) Changes in phospholipid extractability and composition accompany mineralization of chicken growth plate cartilage matrix vesicles. J Biol Chem 277:5126–5133

Wu LN, Sauer GR, Genge BR, Valhmu WB, Wuthier RE (2003) Effects of analogues of inorganic phosphate and sodium ion on mineralization of matrix vesicles isolated from growth plate cartilage of normal rapidly growing chickens. J Inorg Biochem 94:221–235

Wuthier RE (1976) Lipids of matrix vesicles. Fed Proc 35:117–121

Wuthier RE, Wu LN, Sauer GR, Genge BR, Yoshimori T, Ishikawa Y (1992) Mechanism of matrix vesicle calcification: characterization of ion channels and the nucleational core of growth plate vesicles. Bone Miner 17:290–295

Xu D, McSorley SJ, Chatfield SN, Dougan G, Liew FY (1995) Protection against Leishmania major infection in genetically susceptible BALB/c mice by gp63 delivered orally in attenuated Salmonella typhimurium (Aro-AroD-). Immunology 85 (1):1–7

Yadav MC, Simão AMS, Narisawa S, Huesa C, McKee MD, Farquharson C, Millán JL (2011) Loss of skeletal mineralization by the simultaneous ablation of PHOSPHO1 and alkaline phosphatase function - A unified model of the mechanisms of initiation of skeletal calcification. J Bone Miner Res 26:286–297

Yang K, Whalen BJ, Tirabassi RS, Selin LK, Levchenko TS, Torchilin VP, Kislauskis EH, Guberski DL (2008) A DNA vaccine prime followed by a liposome encapsulated protein boost confers enhanced mucosal immune responses and protection. J Immunol 180:6159–6167

Yeagle PL (1993) Phosphorus-31 nuclear magnetic resonance in membrane fusion studies. Methods Enzymol 220:68–79

Yuba E, Kojima C, Harada A, Tana WS, Kono K (2010) pH-Sensitive fusogenic polymer-modified liposomes as a carrier of antigenic proteins for activation of cellular immunity. Biomaterials 31 (5):943–951

Zucolotto V, Daghastanli KRP, Hayasaka CO, Riul A Jr, Ciancaglini P, Oliveira ON Jr (2007) Using capacitance measurements as the detection method in antigen-containing layer-by-layer films for biosensing. Anal Chem 79:2163–2167