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# Tracking peptide–membrane interactions: Insights from in situ coupled confocal-atomic force microscopy imaging of NAP-22 peptide insertion and assembly

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**Abstract**

Elucidating the role that charged membrane proteins play in determining cell membrane structure and dynamics is an area of active study. We have applied in situ correlated atomic force and confocal microscopies to characterize the interaction of the NAP-22 peptide with model membranes prepared as supported planar bilayers containing both liquid-ordered and liquid-disordered domains. Our results demonstrated that the NAP-22 peptide interacts with membranes in a concentration-dependent manner, preferentially inserting into DOPC ($l_d$) domains. While at low peptide concentrations, the NAP-22 peptide formed aggregate-like structures within the $l_d$ domains, at high peptide concentrations, it appeared to sequester cholesterol into the $l_d$ domains and recruited phosphatidyl-*myo*-inositol 4,5-bisphosphate by inducing a blending effect that homogenizes the phase-segregated domains into one liquid-ordered domain. This study describes a possible mechanism by which the NAP-22 peptide can affect neuronal morphology.


*Keywords:* Peptide–membrane interactions; Atomic force microscopy; Confocal fluorescence microscopy; Membrane domains; Supported lipid bilayers

## 1. Introduction

NAP-22 (neuronal axonal myristoylated membrane protein of 22 kDa) is a protein localized in the synapse of neurons that is important for neuronal sprouting and plasticity (Frey et al., 2000). NAP-22 is also found in kidney, testis, and lymphoid tissue, in part as *N*-terminal fragments of this protein, with the sequence myristoyl-GG KLSKKKKGYNVNDEKAK-amide (Zakharov et al., 2003). Myristoylated proteins are commonly found in cholesterol-rich domains in membranes (Melkonian et al., 1999). In addition to detergent extraction of neuronal membranes (Maekawa et al., 1997), fluorescence microscopy studies using intact biological membranes (Laux et al., 2000; Terashita et al., 2002) as well as model membranes (Khan et al., 2003) suggest that NAP-22 associates with neuronal rafts. NAP-22 is very similar to CAP-23 (a cortical cytoskeleton-associated protein of 23 kDa) in sequence and probably also in function (Widmer and Caroni, 1990). Along with GAP-43 (a growth-associated protein of 43 kDa) and MARCKS (myristoylated alanine-rich C-kinase substrate), CAP-23 accumulates and co-localizes with phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)$P_2$) in rafts (Laux et al., 2000). Common in all three of these proteins are clusters of cationic amino acid residues. At least for MARCKS, these cationic clusters are essential for sequestering

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PtdIns(4,5)$P_2$ into domains (Gambhir et al., 2004; McLaughlin et al., 2002). NAP-22 is known to bind to phosphatidylcholine liposomes in a cholesterol-dependent manner (Epand et al., 2001; Maekawa et al., 1999).

The mechanism by which charged peripheral membrane proteins can induce domain formation has recently been proposed (Mbamala et al., 2005). Recently, it was demonstrated that NAP-22 peptide inserts into membranes, sequesters PtdIns(4,5)$P_2$ in a cholesterol-dependent manner, and induces formation of a cholesterol-depleted domain (Epand et al., 2004, 2005a). The formation of membrane domains containing both cholesterol and PtdIns(4,5)$P_2$ is also promoted by a peptide segment of caveolin (Wanaski et al., 2003). A similar caveolin peptide also promotes the sequestration of cholesterol in the absence of PtdIns(4,5)$P_2$ (Epand et al., 2005b). To date, however, the mechanism(s) of lipid recruitment into membrane rafts remains unclear. In particular, one would not expect that PtdIns(4,5)$P_2$ would be a component of a membrane domain enriched in cholesterol because of the low miscibility of PtdIns(4,5)$P_2$ with cholesterol owing to (a) its polyunsaturated acyl chains (Stillwell and Wassall, 2003) and (b) its overall negative charge (Bach and Wachtel, 2003). In the present study, we used coupled confocal and atomic force microscopy to characterize the concentration-dependent interactions of NAP-22 peptide with model membranes that phase-separate into liquid-ordered ($l_o$) and liquid-disordered ($l_d$) domains.

## 2. Materials and methods

### 2.1. Materials

*N*-Stearoyl-D-*erythro*-sphingosylphosphorylcholine (Syn-SM, 18:0), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC, 18:1), brain bovine $G_{M1}$ ganglioside ammonium salt (brain $G_{M1}$), brain porcine (2*S*,3*R*,4*E*)-2-(acylamino)octadec-4-ene-3-hydroxy-1-phosphocholine (brain SM), and brain porcine phosphatidylinositol-4,5-bisphosphate triammonium salt (brain PtdIns(4,5)$P_2$) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Cholesterol (Chol) was purchased from Sigma-Aldrich Canada (Oakville, ON, Canada). BODIPY®TMR phosphatidylinositol 4,5-bisphosphate (TMR-PtdIns(4,5)$P_2$) was purchased from Echelon Lab (Salt Lake City, Utah, USA) (see Fig. 1A for structure). Anti-PtdIns(4,5)$P_2$, mouse IgM, monoclonal 2C11 (anti-PtdIns(4,5)$P_2$), and Alexa Fluor-488 anti-mouse IgM were purchased from Invitrogen Canada (Burlington, ON, Canada). BODIPY-Chol (Fig. 1B) was synthesized as described (Li et al., 2006). The synthetic lipopeptide (NAP) with the sequence: myristoyl-GGKLSKKK KGYNVNDEKAK-amide, corresponding to the 19

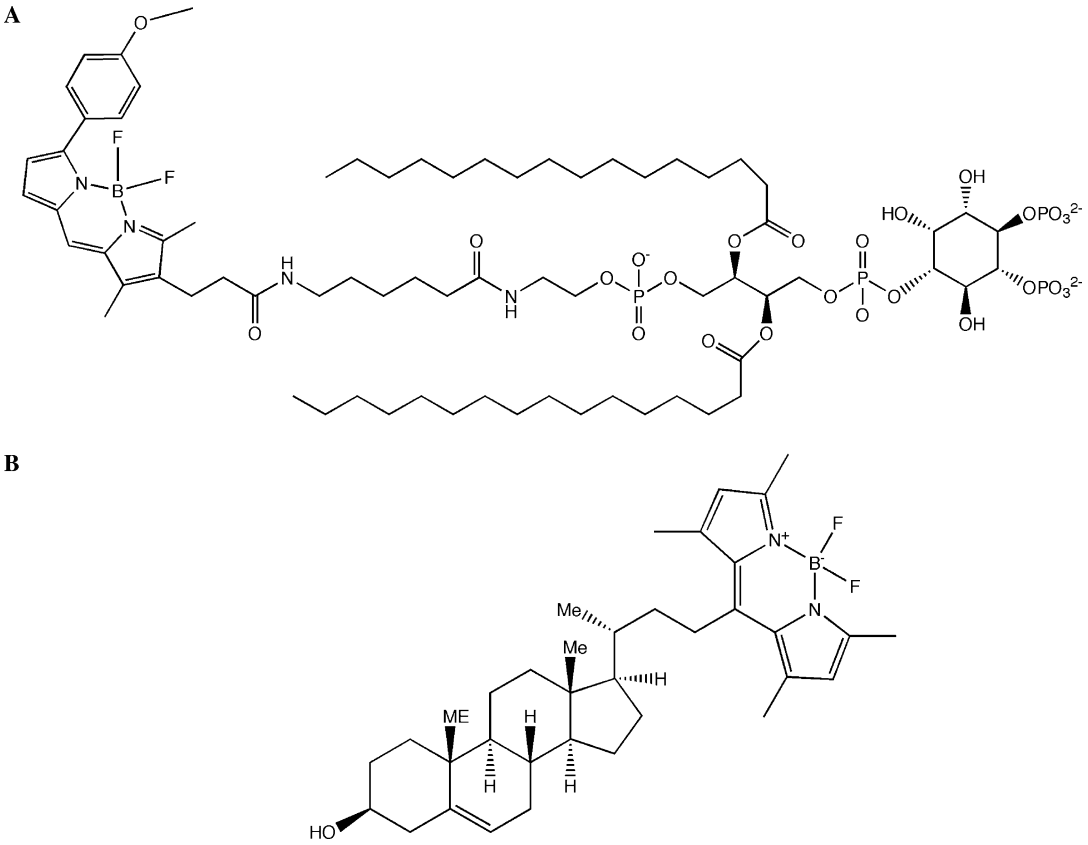


Fig. 1. Chemical structures of (A) BODIPY®TMR-PtdIns(4,5)$P_2$ and, (B) BODIPY-Chol.