Published in final edited form as:

J Thromb Haemost. 2011 July ; 9(Suppl 1): 162–167. doi:10.1111/j.1538-7836.2011.04300.x.

Nanoscale studies of protein-membrane interactions in blood clotting

J.H. Morrissey^{*}, E. Tajkhorshid^{*,†,§,¶}, and C.M. Rienstra^{*,‡,§}

^{*}Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL, USA

[†]Department of Pharmacology, University of Illinois at Urbana-Champaign, Urbana, IL, USA

[‡]Department of Chemistry, University of Illinois at Urbana-Champaign, Urbana, IL, USA

[§]Center for Biophysics and Computational Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA

[¶]Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Summary

Most of the steps in the blood clotting cascade require clotting proteins to bind to membrane surfaces with exposed phosphatidylserine. In spite of the importance of these protein-membrane interactions, we still lack a detailed understanding of how clotting proteins interact with membranes and how membranes contribute so profoundly to catalysis. Our laboratories are using multidisciplinary approaches to explore, at atomic-resolution, how blood clotting protein complexes assemble and function on membrane surfaces.

Keywords

phospholipids; membranes; phosphatidylserine; Gla domains

Protein-membrane interactions in blood clotting

In a typical step in the blood coagulation cascade, a soluble plasma serine protease assembles with its protein cofactor on a suitable membrane surface; the protease-cofactor complex then activates one or more membrane-bound substrates by limited proteolysis. A suitable membrane surface for blood clotting reactions is typically one with exposed phosphatidylserine (PS) [1]. Several mechanisms have been proposed to explain how PS-containing membrane surfaces promote clotting reactions, including increasing the local concentration of reactants, inducing conformational changes in clotting proteins upon binding to PS, and restricting the movement of proteins to two dimensions. Membrane-bound clotting reactions are thousands of times more efficient than the same reactions in solution; in spite of the critical importance of membrane binding to blood clotting, though, we still lack a detailed understanding of how any blood clotting protein interacts with phospholipid bilayers [1].

The most common PS-binding motifs in the blood clotting system are GLA domains, so called because they are rich in the post-translationally modified amino acid, gamma-

Correspondence: James H. Morrissey, Biochemistry Department, University of Illinois College of Medicine, 417 Med. Sci. Bldg. MC-714, 506 S. Mathews Ave., Urbana, IL 61801, USA. Tel.: +1 217 265-4036; fax: +1 217 265-5290. jhmmorris@illinois.edu.

Disclosure of conflicts of interest

The authors have no conflicts of interest to declare.

carboxyglutamate (Gla). Gla residues coordinate divalent metal ions, with GLA domains typically containing multiple tightly bound Ca^{2+} , and sometimes Mg^{2+} as well [2]. GLA domains also have a small loop near their N-terminus known as the omega loop (since it looks like the Greek letter, ω), in which three hydrophobic side chains are highly exposed. These hydrophobic side chains have been proposed to penetrate into the hydrophobic portion of the phospholipid bilayer when GLA domains bind to membranes [3].

Multiple models have been proposed to account for the interaction of GLA domains with PS-containing bilayers. In the Nelsestuen model, the GLA domain rests on, but does not penetrate into, the bilayer surface, lying at a 45° angle relative to other models (Fig. 1A) [4]. The Furie model proposes significant penetration of the hydrophobic residues in the omega loop into the bilayer (Fig. 1B), which is supported by NMR and crystallographic analyses of the bovine prothrombin GLA domain bound to lyso-PS [5] together with estimates of omega loop insertion depth based on fluorescence studies [3]. The Tajkhorshid model (Fig. 1C) is derived from molecular dynamics (MD) simulations [6, 7], in which the GLA domain's tightly bound Ca^{2+} ions are in close proximity to the phosphate groups of PS, resulting in relatively deep insertion of the omega loop into the bilayer. This agrees well the Furie model, the main difference being the depth of bilayer penetration. Interestingly, these MD simulations predict that the Nelsestuen model might be an intermediate in the pathway to full membrane insertion [6].

Some investigators have even proposed that, for factor Xa (fXa), sites outside the GLA domain are responsible for fXa's interaction with membranes. Thus, studies with short-chain, soluble PS (C6PS) have identified three C6PS binding sites on fXa, located on the EGF and protease domains, while the GLA domain failed to bind C6PS in the presence of Ca^{2+} [8]. Another laboratory proposed that fXa penetrates into PS-containing bilayers via its protease domain [9]. While neither of these studies presented a detailed model for how fXa interacts with membranes, the notion that fXa contacts the membrane via the second EGF or protease domain appears inconsistent with fluorescence resonance energy transfer studies showing that fXa is arranged more or less perpendicular to the membrane, with its active site ~ 61 Å above the membrane in the absence of its protein cofactor, factor Va (fVa), and ~ 69 Å in the presence of fVa [10]. Furthermore, models of the fVa:fXa (prothrombinase) complex from other laboratories predict that fXa interacts with the membrane solely via its GLA domain [11]. The fact that such highly disparate ideas are currently proposed for the interactions of GLA domain-containing clotting proteins with membranes underscores the need for detailed studies showing precisely how these proteins engage phospholipid bilayers. This review focuses on recent advances that are permitting new insights into the role of the phospholipid bilayer in blood clotting, with a particular emphasis on how GLA domains interact with PS-containing bilayers.

Nanodiscs offer unique advantages for studying protein-membrane interactions, including those in the blood clotting system

The Sligar laboratory has developed a composite nanoscale particle system, termed Nanodiscs, that allow the preparation of water soluble, nanometre-scale phospholipid bilayers, conferring strict control over the local composition of the membrane surface. Nanodiscs are composed of a nanoscale phospholipid bilayer (typically, about 8 nm in diameter, consisting of about 70 phospholipid molecules per leaflet) ringed and stabilized by two copies of “Membrane Scaffold Protein” (MSP), an amphipathic α -helical protein wrapped around a discoidal bilayer domain in a “belt” fashion [12, 13]. Nanodiscs self-assemble, yielding monodisperse preparations whose bilayer domain sizes are under strict control. Bilayer characteristics of the nanoscale supported membrane in Nanodiscs have been extensively characterised and have been found to recapitulate the bilayer state in

liposomes, including bilayer thickness, mean area per phospholipid, phase transition temperature, metal ion interactions, and ability to support a wide range of protein-membrane interactions [12, 14-20]. Nanodiscs provide greatly improved signal-to-noise ratios for biophysical and spectroscopic studies of protein-membrane interactions, since, unlike liposomes, both sides of the bilayer are equally accessible to solvent and therefore, to interactions with membrane-binding proteins and ligands.

We employed Nanodiscs prepared with varying proportions of PS and phosphatidylcholine (PC) to examine the effect of local PS density on the affinity of fX for membrane surfaces [21]. The K_d for fX binding to these nanoscale bilayers decreased with increasing PS content, reaching maximal binding affinities at $\geq 80\%$ PS. We also found that the number of fX binding sites per Nanodisc increased almost linearly with increasing PS content, corresponding to about 6-8 PS molecules per bound fX. This finding is consistent with the notion that a fX binding site consists of a small cluster of PS molecules; indeed, previous studies using liposomes estimated that each fX interacts with about five PS molecules [22].

Integral membrane proteins can be efficiently incorporated into the nanoscale bilayers within Nanodiscs during self-assembly reactions, embedding themselves into the bilayer just as they do in conventional liposomes [14-18, 21, 23, 24]. We showed that tissue factor (TF), the integral membrane protein responsible for triggering the blood clotting cascade, can be incorporated into Nanodiscs prepared with varying proportions of PS and PC, and we used this platform to assemble complexes of TF and factor VIIa (fVIIa) with catalytic activities very similar to those of TF:fVIIa complexes assembled on TF-liposomes [7, 21]. This also allowed us to address a long-standing question regarding the mechanism of substrate delivery to TF:fVIIa. Solution-phase fX might bind directly to TF:fVIIa on the membrane, or alternatively, membrane-bound fX molecules might laterally diffuse to encounter TF:fVIIa [1, 25-27]. TF:fVIIa complexes assembled on Nanodiscs cannot access a large pool of membrane-bound fX the way they can when assembled on much larger TF-liposomes. The limited surface area of Nanodiscs can bind at most 5 or 6 fX molecules, which should be converted into fXa by the TF:fVIIa complex within seconds. However, we observed sustained, linear rates of fX activation [21], demonstrating that the TF:fVIIa complex is not dependent on a pre-existing pool of membrane-bound fX to serve as substrate.

MD simulations of clotting factor-membrane interactions on nanoscale bilayers

Quite a few x-ray crystal structures of clotting proteins are now available. Since none of these structures include a membrane bilayer, we lack an understanding, at atomic resolution, of how any blood clotting protein actually interacts with membrane surfaces. Advances in MD methods now allow detailed simulations to be conducted of membrane proteins interacting with phospholipids, and this is allowing new insights into how blood clotting proteins bind to membrane surfaces. For example, we recently reported a large-scale MD simulation of the fVIIa GLA domain interacting with a PS bilayer [6] (see Fig. 2). This study showed that GLA domains penetrate more deeply into the membrane than was previously believed, with the tightly bound Ca^{2+} ions in the GLA domain residing at about the level of the phosphate groups of PS (Fig. 2A). In these simulations, the omega loop of the GLA domain (with its three exposed hydrophobic side chains) is fully inserted into the hydrophobic core of the bilayer [6]. Furthermore, it was found that some of the tightly bound Ca^{2+} ions stabilise GLA domain folding, while others participate in anchoring the GLA domain to the membrane.

Simulations of the fVIIa GLA domain interacting with PS molecules in bilayers indicated at least two distinct types of major phospholipid-protein interactions [6]. In particular, there was one unique binding interaction between a PS molecule and the GLA domain that involved extensive contacts between the two (Fig. 2C), in which the PS phosphate interacts with basic residues in the GLA domain while the carboxylate of the same PS molecule is involved in coordinating Ca^{2+} ions that are tightly bound to the GLA domain. In addition, there are extensive contacts between the GLA domain and the glycerol moiety of this same PS molecule. Interestingly, this PS binding mode was actually first reported in the x-ray crystal structure of the bovine prothrombin GLA domain co-crystallised with a lyso-PS molecule [5], and subsequently observed independently in these simulations of the fVIIa GLA domain binding to a PS bilayer, adding confidence to the validity of the simulations [6]. We propose that this site represents a unique, phospho-L-serine-specific binding site in each GLA domain. At least one other type of binding interaction was also consistently observed in MD simulations of GLA domain binding to PS-containing membranes. As depicted in Fig. 2D and 2E, there are multiple instances of tightly bound Ca^{2+} ions of the GLA domain participating in coordination complexes with the phosphate moieties of PS molecules, with the PS headgroups actually folding away from the GLA domain.

More recently, we have conducted detailed MD simulations of the TF:fVIIa complex assembled on PS-containing bilayers, representing the first all-atom simulation of a membrane-bound clotting protease-cofactor complex [28]. Detailed studies of the dynamics of the TF:fVIIa complex in solution and on the surface of PS bilayers revealed that fVIIa in solution undergoes large structural fluctuations, primarily due to the hinge motions between its domains (especially at the boundaries of the first EGF domain), while the isolated ectodomain of TF (sTF) was quite stable in solution. Upon complex formation, sTF was found to restrict the motion of fVIIa significantly, resulting in a more rigid complex held almost perpendicular to the membrane. Interestingly, the MD simulations also showed that the TF ectodomain interacts directly with PS headgroups even in the absence of fVIIa. When fVIIa bound to sTF, the angle of the TF ectodomain relative to the membrane surface was significantly altered [28]. These results are in excellent agreement with our previously published fluorescence resonance energy transfer studies showing that the elongated fVIIa molecule binds essentially perpendicular to the membrane surface, and that its active site is reoriented relative to the membrane when fVIIa engages TF, even when the fVIIa GLA domain had been removed [29-31]. Repositioning of fVIIa's active site relative to the membrane surface may therefore promote optimal attack on the scissile bond of its membrane-bound substrates, fIX and fX, and indeed, we showed that altering the position of fVIIa's active site above the membrane surface severely compromises its ability to activate fX [32].

Insights from solid-state NMR (ssNMR) studies of protein-membrane interactions on nanoscale bilayers

We have developed high resolution, magic-angle spinning ssNMR methods for atomic-resolution structure determination of membrane-embedded systems, most particularly for obtaining structural information on protein-membrane complexes assembled in or on Nanodiscs [33-37].

GLA domains interact with PS in bilayers in a Ca^{2+} -dependent manner. Ca^{2+} ions are known to bind to PS headgroups and have been suspected of causing PS molecules to cluster into nanodomains. However, atomic-scale details of this process have been lacking. We are now using ssNMR analyses to examine the interactions of Ca^{2+} with PS molecules to determine PS conformations both before and after GLA domains bind to membrane surfaces. To do this, we synthesise phospholipids (especially PS) with ^{13}C and ^{15}N in their

headgroups and employ these phospholipid preparations (which also have natural abundance ^{31}P and ^1H) in ssNMR studies to obtain structural information about the membrane, both in the presence and absence of membrane-binding clotting proteins. In a recent study, we combined magic-angle spinning ssNMR measurements of isotopically labelled serine headgroups in mixed lipid bilayers with MD simulations of PS lipid bilayers in the presence of different counterions, in order to provide site-resolved insights into the effects of Ca^{2+} on the structure and dynamics of lipid bilayers [37]. We observed substantial Ca^{2+} -induced conformational changes of PS headgroups in mixed bilayers using both liposomes and Nanodiscs. Site-resolved multidimensional correlation ssNMR spectra of nanobilayers containing ^{13}C , ^{15}N -labeled PS demonstrated that Ca^{2+} ions induced two major PS headgroup conformations, which were well resolved in two-dimensional ^{13}C - ^{13}C , ^{15}N - ^{13}C and ^{31}P - ^{13}C ssNMR spectra. Examining the cross peak intensity as a function of pulse sequence time yields specific ^{31}P - ^{15}N distance information (Fig. 3). Within the same study, detailed MD simulations performed on PS bilayers in the presence or absence of Ca^{2+} provided an atomic view of the conformational effects underlying our observed spectra (see the inset in Fig. 3). More recently, we have also obtained ssNMR evidence for the induction of novel PS headgroup conformations when GLA domains bind to PS-containing bilayers in Nanodiscs (manuscript in preparation).

A long-term goal of our studies is to provide atomic-resolution information regarding how blood clotting proteins interact with membrane surfaces, including the TF:fVIIa:membrane complex. As an additional step toward that goal, we have isotopically labelled sTF with ^{13}C and ^{15}N and reported the assignment of nearly all the backbone ^1H , ^{13}C and ^{15}N resonance assignments using solution NMR methods [38]. More recently, we have used ssNMR to complete most of the ^1H , ^{13}C and ^{15}N resonance assignments for nanocrystalline sTF and for membrane-anchored TF embedded Nanodisc bilayers containing ^{13}C , ^{15}N -labeled PS (unpublished observations). These studies, combined with biochemical/mutagenesis studies and detailed MD simulations, will allow detailed insights to be gained into the precise membrane-interactive regions of fVIIa and TF within the TF:fVIIa complex, and into possible conformational changes in these proteins induced upon membrane binding.

Acknowledgments

Studies in the authors' laboratories were supported by NIH grants HL47014 (J.H.M.), GM75937, GM79530, and RR025037 (C.M.R.), R01 GM086749, R01-GM067887, and P41 RR-05969 (E.T.), and HL103999 (J.H.M. and C.M.R.). All of the simulations were performed using computer time on TeraGrid resources (Grant MCA06N060).

References

1. Zwaal RFA, Comfurius P, Bevers EM. Lipid-protein interactions in blood coagulation. *Biochim Biophys Acta*. 1998; 1376:433–53. [PubMed: 9805008]
2. Bajaj SP, Schmidt AE, Agah S, Bajaj MS, Padmanabhan K. High resolution structures of paminobenzamidine- and benzamidine-VIIa/soluble tissue factor: unpredicted conformation of the 192-193 peptide bond and mapping of Ca^{2+} , Mg^{2+} , Na^{+} , and Zn^{2+} sites in factor VIIa. *J Biol Chem*. 2006; 281:24873–88. [PubMed: 16757484]
3. Falls LA, Furie BC, Jacobs M, Furie B, Rigby AC. The omega-loop region of the human prothrombin gamma-carboxyglutamic acid domain penetrates anionic phospholipid membranes. *J Biol Chem*. 2001; 276:23895–902. [PubMed: 11312259]
4. Nelsestuen GL. Enhancement of vitamin-K-dependent protein function by modification of the gamma-carboxyglutamic acid domain: Studies of protein C and factor VII. *Trends Cardiovasc Med*. 1999; 9:162–7. [PubMed: 10639722]
5. Huang M, Rigby AC, Morelli X, Grant MA, Huang GQ, Furie B, Seaton B, Furie BC. Structural basis of membrane binding by Gla domains of vitamin K-dependent proteins. *Nat Struct Biol*. 2003; 10:751–6. [PubMed: 12923575]

6. Ohkubo YZ, Tajkhorshid E. Distinct structural and adhesive roles of Ca^{2+} in membrane binding of blood coagulation factors. *Structure*. 2008; 16:72–81. [PubMed: 18184585]
7. Morrissey JH, Pureza V, Davis-Harrison RL, Sligar SG, Ohkubo YZ, Tajkhorshid E. Blood clotting reactions on nanoscale phospholipid bilayers. *Thromb Res*. 2008; 122(Suppl 1):S23–S6. [PubMed: 18691494]
8. Srivastava A, Wang J, Majumder R, Rezaie AR, Stenflo J, Esmon CT, Lentz BR. Localization of phosphatidylserine binding sites to structural domains of factor Xa. *J Biol Chem*. 2002; 277:1855–63. [PubMed: 11707438]
9. Lecompte MF. Interaction of an amphitropic protein (factor Xa) with membrane models in a complex system. *Biochim Biophys Acta*. 2005; 1724:307–14. [PubMed: 15967578]
10. Husten EJ, Esmon CT, Johnson AE. The active site of blood coagulation factor Xa. Its distance from the phospholipid surface and its conformational sensitivity to components of the prothrombinase complex. *J Biol Chem*. 1987; 262:12953–61. [PubMed: 3477541]
11. Autin L, Steen M, Dahlback B, Villoutreix BO. Proposed structural models of the prothrombinase (FXa-FVa) complex. *Proteins*. 2006; 63:440–50. [PubMed: 16437549]
12. Shaw AW, McLean MA, Sligar SG. Phospholipid phase transitions in homogeneous nanometer scale bilayer discs. *FEBS Lett*. 2004; 556:260–4. [PubMed: 14706860]
13. Denisov IG, Grinkova YV, Lazarides AA, Sligar SG. Directed self-assembly of monodisperse phospholipid bilayer Nanodiscs with controlled size. *J Am Chem Soc*. 2004; 126:3477–87. [PubMed: 15025475]
14. Bayburt TH, Carlson JW, Sligar SG. Reconstitution and imaging of a membrane protein in a nanometer-size phospholipid bilayer. *J Struct Biol*. 1998; 123:37–44. [PubMed: 9774543]
15. Bayburt TH, Sligar SG. Single-molecule height measurements on microsomal cytochrome P450 in nanometer-scale phospholipid bilayer disks. *Proc Natl Acad Sci U S A*. 2002; 99:6725–30. [PubMed: 11997441]
16. Bayburt TH, Sligar SG. Self-assembly of single integral membrane proteins into soluble nanoscale phospholipid bilayers. *Protein Sci*. 2003; 12:2476–81. [PubMed: 14573860]
17. Sligar SG. Finding a single-molecule solution for membrane proteins. *Biochem Biophys Res Commun*. 2003; 312:115–9. [PubMed: 14630028]
18. Baas BJ, Denisov IG, Sligar SG. Homotropic cooperativity of monomeric cytochrome P450 3A4 in a nanoscale native bilayer environment. *Arch Biochem Biophys*. 2004; 430:218–28. [PubMed: 15369821]
19. Shih AY, Denisov IG, Phillips JC, Sligar SG, Schulten K. Molecular dynamics simulations of discoidal bilayers assembled from truncated human lipoproteins. *Biophys J*. 2005; 88:548–56. [PubMed: 15533924]
20. Denisov IG, McLean MA, Shaw AW, Grinkova YV, Sligar SG. Thermotropic phase transition in soluble nanoscale lipid bilayers. *J Phys Chem B*. 2005; 109:15580–8. [PubMed: 16852976]
21. Shaw AW, Pureza VS, Sligar SG, Morrissey JH. The local phospholipid environment modulates the activation of blood clotting. *J Biol Chem*. 2007; 282:6556–63. [PubMed: 17200119]
22. Nelsestuen GL, Broderius M. Interaction of prothrombin and blood-clotting factor X with membranes of varying composition. *Biochemistry*. 1977; 16:4172–7. [PubMed: 901766]
23. Civjan NR, Bayburt TH, Schuler MA, Sligar SG. Direct solubilization of heterologously expressed membrane proteins by incorporation into nanoscale lipid bilayers. *BioTechniques*. 2003; 35:556–3. [PubMed: 14513561]
24. Leitz AJ, Bayburt TH, Barnakov AN, Springer BA, Sligar SG. Functional reconstitution of Beta2-adrenergic receptors utilizing self-assembling Nanodisc technology. *BioTechniques*. 2006; 40:601–2. 4, 6. passim. [PubMed: 16708760]
25. Krishnaswamy S, Field KA, Edgington TS, Morrissey JH, Mann KG. Role of the membrane surface in the activation of human coagulation factor X. *J Biol Chem*. 1992; 267:26110–20. [PubMed: 1464622]
26. Bom VJ, Bertina RM. The contributions of Ca^{2+} , phospholipids and tissue-factor apoprotein to the activation of human blood-coagulation factor X by activated factor VII. *Biochem J*. 1990; 265:327–36. [PubMed: 2302175]

27. Hathcock JJ, Rusinova E, Gentry RD, Andree H, Nemerson Y. Phospholipid regulates the activation of factor X by tissue factor/factor VIIa (TF/VIIa) via substrate and product interactions. *Biochemistry*. 2005; 44:8187–97. [PubMed: 15924438]
28. Ohkubo YZ, Morrissey JH, Tajkhorshid E. Dynamical view of membrane binding and complex formation of human factor VIIa and tissue factor. *J Thromb Haemost*. 2010; 8:1044–53. [PubMed: 20180816]
29. McCallum CD, Hapak RC, Neuenschwander PF, Morrissey JH, Johnson AE. The location of the active site of blood coagulation factor VIIa above the membrane surface and its reorientation upon association with tissue factor: A fluorescence energy transfer study. *J Biol Chem*. 1996; 271:28168–75. [PubMed: 8910432]
30. McCallum CD, Su B, Neuenschwander PF, Morrissey JH, Johnson AE. Tissue factor positions and maintains the factor VIIa active site far above the membrane surface even in the absence of the factor VIIa Gla domain - A fluorescence resonance energy transfer study. *J Biol Chem*. 1997; 272:30160–6. [PubMed: 9374497]
31. Morrissey JH, Neuenschwander PF, Huang Q, McCallum CD, Su B, Johnson AE. Factor VIIa tissue factor: Functional importance of protein- membrane interactions. *Thromb Haemost*. 1997; 78:112–6. [PubMed: 9198138]
32. Waters EK, Yegneswaran S, Morrissey JH. Raising the active site of factor VIIa above the membrane surface reduces its procoagulant activity but not factor VII autoactivation. *J Biol Chem*. 2006; 281:26062–8. [PubMed: 16835245]
33. Franks WT, Wylie BJ, Schmidt HL, Nieuwkoop AJ, Mayrhofer RM, Shah GJ, Graesser DT, Rienstra CM. Dipole tensor-based atomic-resolution structure determination of a nanocrystalline protein by solid-state NMR. *Proc Natl Acad Sci U S A*. 2008; 105:4621–6. [PubMed: 18344321]
34. Li Y, Kijac AZ, Sligar SG, Rienstra CM. Structural analysis of nanoscale self-assembled discoidal lipid bilayers by solid-state NMR spectroscopy. *Biophys J*. 2006; 91:3819–28. [PubMed: 16905610]
35. Kijac AZ, Li Y, Sligar SG, Rienstra CM. Magic-angle spinning solid-state NMR spectroscopy of nanodisc-embedded human CYP3A4. *Biochemistry*. 2007; 46:13696–703. [PubMed: 17985934]
36. Kijac A, Shih AY, Nieuwkoop AJ, Schulten K, Sligar SG, Rienstra CM. Lipid-protein correlations in nanoscale phospholipid bilayers determined by solid-state nuclear magnetic resonance. *Biochemistry*. 2010; 49:9190–8. [PubMed: 20804175]
37. Boettcher JM, Davis-Harrison RL, Clay MC, Nieuwkoop AJ, Ohkubo YZ, Tajkhorshid E, Morrissey JH, Rienstra CM. Atomic View of Calcium-Induced Clustering of Phosphatidylserine in Mixed Lipid Bilayers. *Biochemistry*. 2011 In Press.
38. Boettcher JM, Clay MC, LaHood BJ, Morrissey JH, Rienstra CM. Backbone ^1H , ^{13}C and ^{15}N resonance assignments of the extracellular domain of tissue factor. *Biomol NMR Assign*. 2010; 4:183–5. [PubMed: 20526825]
39. Banner DW, D'Arcy A, Chène C, Winkler FK, Guha A, Konigsberg WH, Nemerson Y, Kirchhofer D. The crystal structure of the complex of blood coagulation factor VIIa with soluble tissue factor. *Nature*. 1996; 380:41–6. [PubMed: 8598903]

Fig. 1.

Three models for GLA domain binding to a phospholipid bilayer. Residues 1-49 of bovine prothrombin (from PDB file 1NL2 [5]) are shown using a cartoon representation, with the side chains of Gla residues depicted in yellow and Ca^{2+} depicted as purple spheres. The omega loop of the prothrombin GLA domain (which has three exposed hydrophobic residues, Phe5, Leu6 and Val9, whose side chains are not shown in this figure) is: (A) interacting with the headgroup region, tilted $\sim 45^\circ$ relative to the other models; or (B,C) inserting to varying depths into the bilayer.

Fig. 2.

MD simulations of protein-membrane interactions of the fVIIa GLA domain [6]. (A) A snapshot from detailed MD simulations of the fVIIa GLA domain interacting with a PS bilayer. PS molecules whose carboxy and/or phosphate groups are within 5 Å of any Ca^{2+} ions are drawn with a stick representation, with other PS molecules shown as thin lines. Gla residues are coloured in yellow, while the basic side chains of the GLA domain are drawn green. The three hydrophobic residues of the omega loop of the fVIIa GLA domain (Phe4, Leu5, and Leu8) are shown with an orange surface representation. (B) A model for the membrane-associated TF:fVIIa complex built by aligning the fVIIa GLA domain of sTF:fVIIa (from PDB structure 1DAN [39]) to the structure shown in panel A. fVIIa is shown in blue with its active site in red; TF is shown in white. (C) A close up, left-side view depicting a PS molecule tightly bound to the GLA domain, with its headgroup in simultaneous contact with Arg9 and Ca^{2+} -8. (D, E) Close-ups of interactions between tightly bound Ca^{2+} ions of the GLA domain and the phosphate moieties of PS molecules. Panels A-C were reprinted from Ohkubo & Tajkhorshid, Distinct structural and adhesive roles of Ca^{2+} in membrane binding of blood coagulation factors. *Structure* 16:72-81, 2008 [6], with permission from Elsevier.

Fig. 3.

Three-dimensional ssNMR (TEDOR) trajectories of the cross-peak observed from the ^{31}P of one PS molecule and the ^{15}N of an adjacent PS molecule [37]. The cross peak intensity as a function of mixing time yields specific internuclear distance information. Also shown, as an insert, is a snapshot taken from MD simulations of Ca^{2+} interacting with PS bilayers, depicting an example of the headgroups of two adjacent PS molecules interacting with the same Ca^{2+} ion. Experimentally determined distances of this type are being used to test structural models of PS headgroup assembly. This figure was reprinted with permission from *Biochemistry* [37]; copyright 2011 American Chemical Society.