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The properties of glycophorin A transmembrane helices in erythrocyte asymmetric membrane: a molecular dynamics study

Paruyr K. Hakobyan[a,*], Armen H. Poghosyan[a], and Aram A. Shahinyan[b]

[a] *International Scientific-Educational Center NAS RA 0019, Yerevan, Armenia*
[b] *Institute of Applied Problems of Physics NAS RA 0014, Yerevan, Armenia*

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Abstract. We have performed an 80ns molecular dynamics (MD) simulation of human red blood erythrocyte asymmetric membrane model. The NAMD code and CHARMM27 force field were used. We have estimated some features of embedded glycophorin A (GpA) protein and have discussed some important problems concerning the interaction between the protein and surrounding media. It is stated that the lipid environment and protein immediate neighboring lead to the changes in helix-helix association, as well as to the protein orientation. The interaction nature between protein and neighboring phospholipid chains are dominant forces governing to helix-helix association.

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Key words: glycophorin A (GpA) protein, erythrocyte membrane, molecular dynamics simulation, helix-helix association

1 Introduction

The glycophorin A (GpA) is the major intrinsic membrane protein of the erythrocyte, which is largely composed of hydrophobic amino acids and spans the membrane once, presenting its amino-terminal end at the extra-cellular surface of the human red blood cell. The GpA system has been studied by a variety of biochemical and biophysical techniques aimed at understanding the basis of this self-association.

The molecular dynamics simulation (MD) method, which is intensively used for bio-systems study, is a great tool to well understand the intra- and intermolecular structure of biological systems [1–5]. During last decade a lot of works were done using MD study for investigation of structure and behavior of biological membranes [4–7].

*Corresponding author. *Email address:* paruyr.hakobyan@gmail.com (P. K. Hakobyan)

It is known, that the transmembrane part of human GpA is responsible for dimerization [8]. NMR study provides us to measure and better understand the so called helix-helix contacts [9]. In parallel to real experiment, the MD study of GpA feature have been done by Braun and coworkers [9]. They have studied GpA transmembrane helices embedded in sodium dodecyl sulfate (SDS) micelles to reveal the dimerization contacts. The native structure of transmembrane domain of GpA was examined by Soumana and coworkers [10] and they claimed that GxxxG-like helix motif at the dimer interface is presumed to drive receptor oligomerization. The GxxxG sequence motif mediates the association of transmembrane helices by providing a site of close contacts between them and therefore outside the contact interface can exert a significant influence on transmembrane helix association affinity [11]. Some studies have shown that small and/or polar residues are more occur in helical interface of GpA than other amino acids [12]. Smith and coauthors report that the direct packing contacts of the GpA occur between glycire residues at position of 79 and 83 in the transmembrane sequence. The estimated distance between the above mentioned residues is about 4.9 Åbased on MAS NMR distance measurements [13, 14].

The main purpose of this work is to investigate the structure and dynamical properties of GpA in human red blood erythrocyte asymmetric membrane. We are going to explain the influence of lipid environment and protein immediate neighborhood on helix-helix contracts and protein orientation. The effect of boundary neighboring lipids on protein is still debated issues, and to the best of our knowledge, there is no computer simulation study of multicomponent complex systems with embedded protein. A few works were done on DPPC and DMPC with embedded GpA pure systems and as well GpA has been intensively studied with presence of SDS micelle [13] and in vacuum [15].

The asymmetry and more accuracy model of human red blood erythrocyte membrane lead to the real mimic of biological membrane and will help us to understand the behavior of protein and surrounding phospholipids.

According to the real experimental finding [16], the phospholipid composition is the following. 24:0 SM lingoceroyl sphingomyelin (LSM), 16:0 SM hexadecanoyl sphingomyelin (HSM), 1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphatidylserine (SAPS), 1-stearoyl-2-docosahexaenoyl-sn-glycero-3-phosphatidylserine (SDPS), 1-stearoyl-2-oleoyl-sn-glycero-3-phosphatidylethanolamine (SOPE), 1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphatidylethanolamine (SAPE), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (POPC), 1-stearoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (SOPC) and cholesterol.

2 Construction and simulation details

The construction of model membrane was implemented by using of Hyperchem (Hypercube Inc.) software and MDesigner [17, 18]. First the molecules of POPE, POPC, SAPE, SOPC, SAPS, SDPS, LSM, HSM, and Cholesterol were created and we have received a system consisting of 252 molecules of phospholipids, cholesterol, and 27 Sodium counterions by random replication making allowance for asymmetry of model membrane and final concentration of

phospholipids and cholesterol. The GpA protein transmembrane part were inserted into the system ready from previous simulation. The investigated model was solvated: inserted into water bulk with 8572 water molecules of TIP3P [19] model. The membrane model was hydrated at about 33 water per phospholipid so in order to assure that the system is at fully hydrated. The initial membrane model size was about $10.5\times9\times9$ nm^3 so the rough estimation for area per phospholipid was about 0.89 nm^2. The initial configuration of was stabilized by an energy minimization using conjugate gradient method for 15000 steps and was subjected to short (about 1000 ps) MD simulation in NVT ensemble .The Langevin dynamics [20] with 5 ps^{-1} damping coefficient was used. The constant temperature and pressure were set correspondingly to 310 K and normal 1 atm. The constant temperature and pressure were controlled by using the Langevin piston Nose-Hoover method [21]. For the non bonded full electrostatic interactions between atom pairs cutoff parameter was set to 14 Å. The coordinates and velocities were saved with 2 stages first, each 0.001 ns for calculation of MSD and diffusion coefficient values of phospholipids and cholesterol and second 0.01 ns for calculation of other parameters of the system. The visual representation performed by using VMD package. System MD simulation run (in NPT ensemble) was done with 2 fs time step, using NAMD software code with CHARMM27 all-atom force field on parallel Linux cluster. The simulation was performed for 80 ns on 12 nodes (24 Intel Xeon 3.06 GHz processors) of the cluster with the duration of about 60 days. The force field parameters of Cholesterol molecule were generated using Dundee PRODRG server. The ready for MD simulation system consists of 57640 atoms.

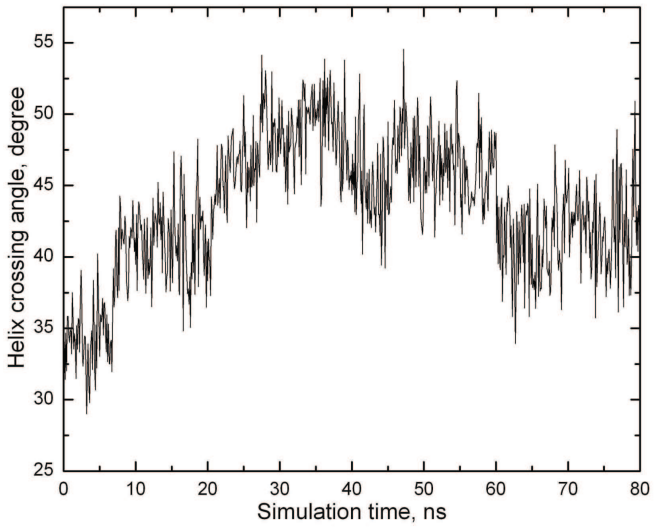
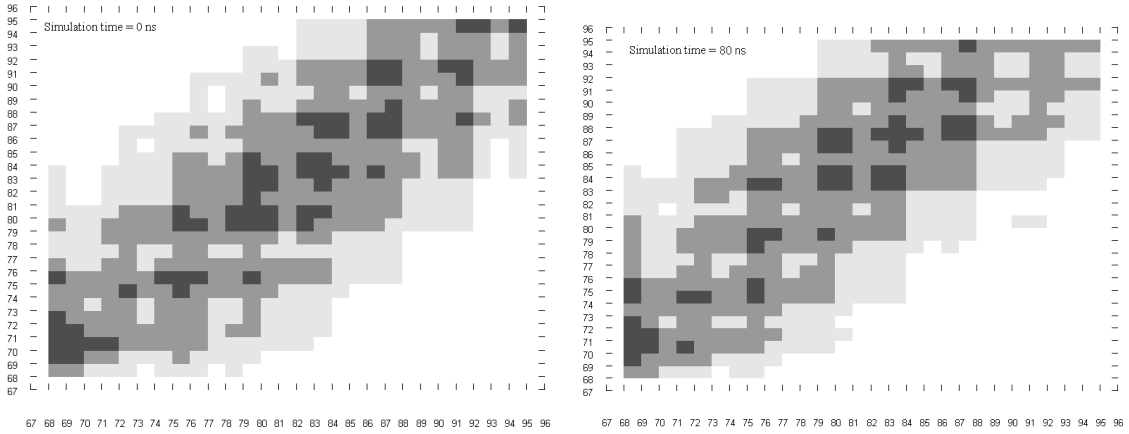


Figure 1: The helix crossing angle vs the simulation time.

3 Results and discussion

The helix crossing angle Ω, which measure the angle between two helical axes (usually known positive - a left handed and negative right-handed coiled-coil) can be derived from the real experiment. It is important parameter for comparison and analysis. In Fig. 1. the GpA helix angle (left handed coiled-coil) changes during the whole simulation time are represented. As one can see, the angle value fluctuates from $33^\circ \pm 2$ to $42^\circ \pm 2$. A change in helix-helix angle is caused by the rotation around the helix interface. The last 20ns simulation time (assuming the equilibrium of the system) the mean angle is estimated to be $40^\circ \pm 1$. A variety of crossing angle values ranged from -50° to 25° are discussed [14]. Over the 24 ns of computer experiment the authors [9] have received the GpA helix crossing angle about $44^\circ \pm 2$, where GpA transmembrane helices were embedded in sodium dodecyl sulfate (SDS) micelles. Meanwhile, Petrache and coworkers [22] claim that the crossing angle of GpA protein in phosphatidylocholine (PC) bilayers (separately DPPC, DMPC, DOPC and POPC) is in 39° – $46^\circ \pm 3$ range. They argued that the crossing and tilt angles vary depending on hydrocarbon chain lengths. In our system, the protein neighboring molecules have hydrocarbon chains of 16 to 24 lengths. In contract to pure systems, the set of various hydrocarbon chains and the degree of unsaturation of hydrocarbon tails, in fact do not alter the orientation of helix, as experimentally proved in [13].



(a) **(b)**

Figure 2: Helix contacts by residue for GpA each 0 ns and 80 ns simulation time. The distance for each pair is shown as a square colored by dark-gray between 0.0 and 10.0 Å, 10 Å to 15 Å gray, 15 Å to 20 Å light-gray and white > 20.0 Å.

In addition, it should be noted that the data from NMR and polarized IR spectroscopy shows the crossing angle less than 35° [14] in membrane structure and about 40° in the detergent structure.

It is valuable to reveal the interactions feature of helix contacts. In this regards, we de-

termine the contact map of interaction of amino acids in two opposite helices. Two maps are shown derived from 0 and 80 ns simulation points.

For clarification, we have designated the distance between the center of masses (COM) of amino acids as follows: 0 to 10 Å is dark–gray, 10–15 Å gray, 15–20 Å is light–gray and above 20 Å in white. The strongly contacted amino acids are localized in the so called "first shell" (dark–gray). From the starting point, one can notice a lot of close or strong contacts. The observed inter-helix distances are the results of intermolecular interactions and is generally governed by the immediate neighboring (helix-dipole and helix-chain) lipid environments. The

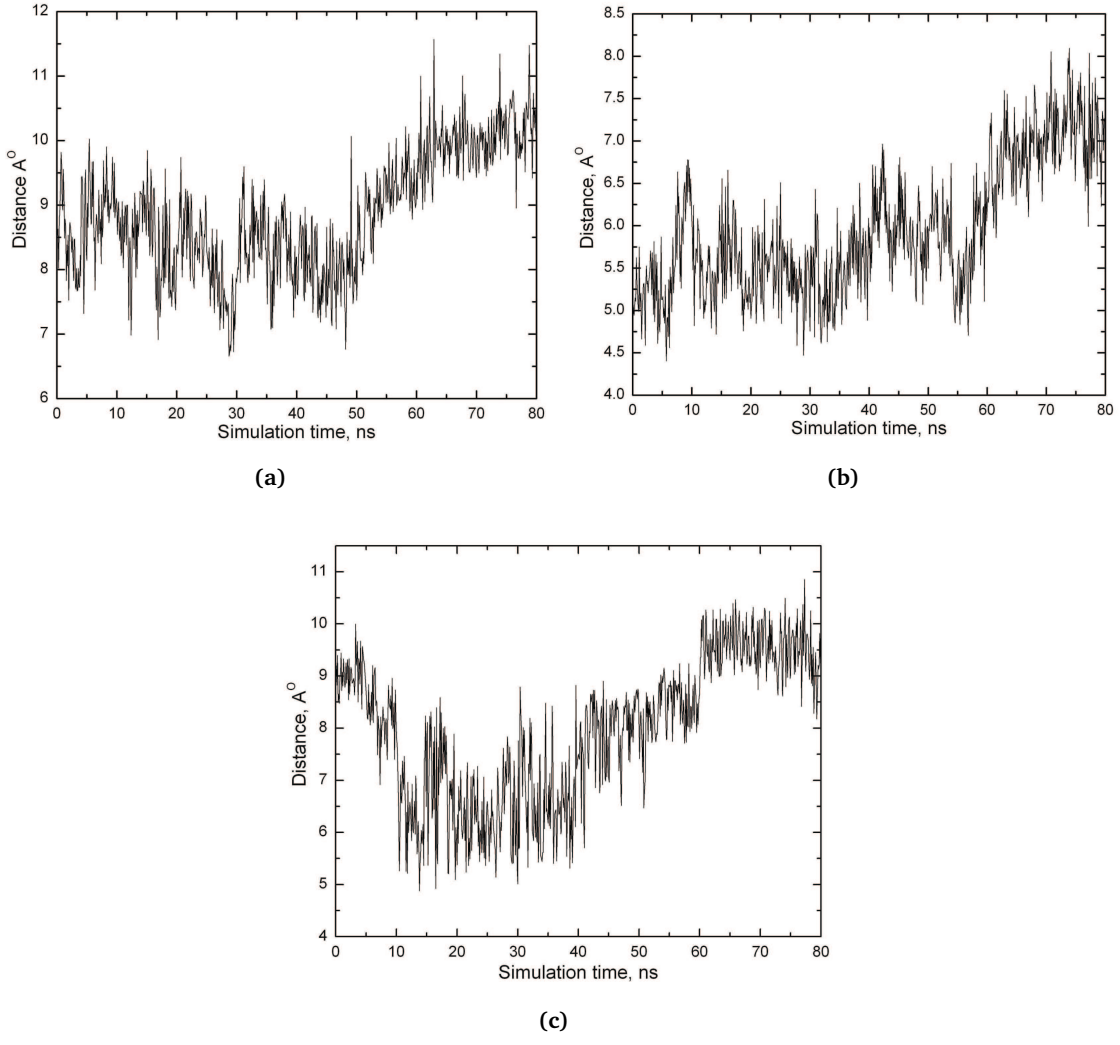


Figure 3: Helix Ieu^{75}-Ile^{76}, Gly^{79}-Val^{80} and Gly^{79}-Gly^{83} distances depending on simulation time.

starting point configuration GpA protein molecule was extracted from our previous simulated system with overall simulation time of about 100 ns (PC/PE/GpA/water system). However, after additional 80 ns of simulation some "close contacts" disappear, which means that the distance between amino acids become more than 10 Å. In Fig. 2(c). the strongly connected amino acids from helix A and B is shown.

It is known from NMR experiment that Gly^{79} plays an important role in GpA dimerization and hydrogen bonds between Gly^{79}, Val^{80}, Gly^{83} of two helixes stabilize the dimer [13]. We have estimated the distances of above mentioned amino acids and represented the distance changes during the whole simulation time (Fig. 3(a),(b),(c)). In addition we have also examined the contact distance between Leu^{75} and Ile^{76} in order to explain the effect of this contacts in dimerization. The Gly^{79} - Val^{80} distance fluctuates in the range of 5 Å$\pm$ 1 and 7 Å$\pm$ 1 during whole simulation time. Indeed, we can see the strong and close contacts over the whole simulation time. In comparison with experimental data (NMR refinements) we can see a shift (about 3 Å) of Gly^{79}–Val^{80} distance.

The NMR data of Gly^{79}–Val^{80} distance is 3.5 Å, which is almost the starting point of our simulation (Fig. 3(b)). During the 80 ns of MD simulation one can see the increasing of the distance up to 7 Å and as well equilibrium since 60 ns of the simulation. The increasing is due to presence of surrounding media, namely phospholipids and cholesterol molecules. Changing the media, for instance, the presence of surfactants (SDS micelle) [9] lead to the decrease of Gly^{79}–Val^{80} distance to the value of 3.5 Å which means that the surrounding media influences on the GpA structural parameters. For explanation, we have also visualized and presented the snapshots extracted from the last point of simulation (80 ns last point). We have measured the hydrogen – hydrogen distance. From the structural parameters which we have calculated the equilibrium state reaches after 60 ns of simulation and therefore the Gly^{79}–Val^{80} distance is estimated to be about 7 Å. The Hydrogen- hydrogen distance is about 4.5 Å as shown in Fig. 4.

Table 1: The starting configuration (0 ns) of the system: % of molecules in intra cellular and extra cellular parts of membrane model. PC-phosphatidylcholine, PE-phosphatidylethanolamine, PS-phosphatidylserine, and SM-sphingomyelin.

Phospholipids	Chol	PC	PE	PS	Sph
intra cellular (protein surrounding)	14,4%	9.5%	28,5%	47,6	0%
intra cellular (average concentration in the membrane)	22,9%	15,1%	36,6%	20,6	4,8%
extra cellular (protein surrounding)	24,8%	25,2%	10,6%	0%	39,4%
extra cellular (average concentration in the membrane)	24,1%	30,6%	4,8%	0%	40,5%

We have also calculated the percentage of phospholipids and cholesterols in the so called "first shell" of the GpA surrounding neighbors. The radius of the shell is 10 Å. In the Tables 1 and 2 the percentage amount of surrounding neighbors is shown of starting and last (80 ns) state configuration of the system respectively.

We do not observe almost any changes in neighbor molecules. The neighboring phospholipid have thermally fluctuated and stayed in first shell with the radius of 10 Å.

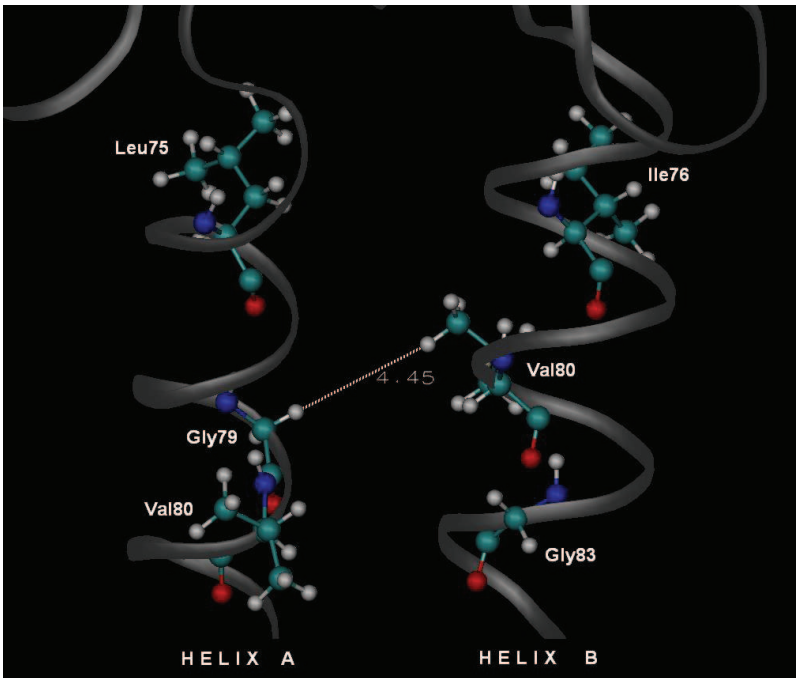


Figure 4: The last simulation point. The Gly^{79}, Gly^{83}, Val^{80}, Leu^{75}, and Ile^{76} are pointed in VDW mode.

Table 2: The configuration after 80 ns simulation of the system: % of molecules in inner and outer parts of membrane model. PC-phosphatidylcholine, PE-phosphatidylethanolamine, PS-phosphatidylserine, and SM-sphingomyelin.

Phospholipids	Chol	PC	PE	PS	Sph
intra cellular (protein surrounding)	13%	13%	21.7%	47.8%	4.5%
intra cellular (average concentration in the membrane)	22.9%	15.1%	36.6%	20.6%	4.8%
extra cellular (protein surrounding)	20%	30.5%	10%	0%	40%
extra cellular (average concentration in the membrane)	24.1%	30.6%	4.8%	0%	40.5%

Hence, on can conclude that either the starting random configuration is energetically favorable, or it should be subjected to the further computer experiment. It is natural to suppose that long chain PS molecules (about 48%) interacts with protein and these interactions go to dominant forces, both for helix orientation and helix-lipid association. In outer layer due to the absence of PS molecules, we see the connected long chain SM (about 40%) and PE (about 37%) molecules. As one can see from Tables 1 and 2, the only little change occurs and some phospholipids appear at the first shell and replaced by another one. From the trajectory movie, we see that all the surrounding molecules of first shell interact with protein amino acid units and stay close contacted (mainly the head groups of lipids) the protein during the all simulation time.

Due to the complexity and asymmetry of the system and variety of phospholipids lead to the assumption that the simulated 80 ns is probably not enough in order to well describe the nature of interactions.

References

[1] G. A.Yeghiazaryan, A. H. Poghosyan, and A. A. Shahinyan, Physica A 362 (2006) 197.
[2] D. P. Tieleman and H. J. C. Berendsen, J. Chem. Phys. 105 (1996) 4871.
[3] S. W. Chiu, M. Clark, V. Balaji, *et al.*, Biophys. J. 69 (1995) 1230.
[4] A. H. Poghosyan, H. H. Gharabekyan, and A. A. Shahinyan, Int. J. Mod. Phys. C 18 (2007) 73.
[5] K. Mori, M. Hata, S. Neya, and T. Hoshino, Chem-Bio. Inf. J. 4 (2004) 15.
[6] A. J. Robinson, W. G. Richards, P. J. Thomas, and M. M. Hann, Biophys. J. 67(6) (1994) 2345.
[7] M. L. Berkowitz, Biochimica et Biophysica Acta Biomembranes 1788 (2009) 86.
[8] S. H. White and W. C. Wimley, Annu. Rev. Biophys. Biomol. Struct. 28 (1999) 319.
[9] R. Braun, D. M. Engelman, and K. Schulten, Biophys. J. 87 (2004) 754.
[10] O. S. Soumana, N. Garnier, and M. Genest, Eur. Biophys. J. 36 (2007) 1071.
[11] J. Zhang and T. Lazaridis, Biophys. J. 96 (11) (2009) 4418.
[12] M. Eilers, A. B. Patel, W. Liu, and S. O. Smith, Biophys. J. 82 (2002) 2720.
[13] S. O. Smith, D. Song, S. Shekar, *et al.*, Biochem. 40 (22) (2001) 6553.
[14] S. O. Smith, M. Eilers, D. Song, *et al.*, Biophys. J. 82 (2002) 2476.
[15] A. J. Beevers and A. Kukol, J. Mol. Graph. Model. 25 (2) (2006) 226.
[16] E. Sackmann, Biological membranes architecture and function, in: Structure and Dynamics of Membranes, Volume 1 of Handbook of Biological Physics, eds. R. Lipowsky and E. Sackmann (Elsevier, North-Holland, 1995).
[17] Hypercube Inc. `http://www.hyper.com/`.
[18] A. A. Shahinyan, A. H. Poghosyan, G. A.Yeghiazaryan, and H. H. Gharabekyan, Elect. J. Nat. Sci. 1 (2004) 56.
[19] W. L. Jorgensen, J. Chandrasekhar, J. D. Medura, *et al.*, J. Chem. Phys. 79 (1983) 926.
[20] R. M. Levy, J. A. McCammon, and M. Karplus, Chem. Phys. Lett. 64 (1979) 4.
[21] S. E. Feller, Y. H. Zeng, R. W. Pastor, and B. R. Brooks, J. Comp. Phys.103 (1995) 4613.
[22] H. I. Petrache, A. Grossfield, K. R. MacKenzie, *et al.*, J. Mol. Biol. 302 (2000) 727.