Modeling adsorption, conformation, and orientation of the Fis1 tail anchor at the mitochondrial outer membrane

Beytullah Ozgur,[1] Cory D. Dunn,[2,*] and Mehmet Sayar[3,4,*]

[1] *College of Engineering, Koç University, Sarıyer-İstanbul, 34450, Turkey*[†]
[2] *Institute of Biotechnology, University of Helsinki, Helsinki, 00014, Finland*
[3] *Chemical & Biological Eng. Dept., College of Engineering, Koç University, Sarıyer-İstanbul, 34450, Turkey*
[4] *Mechanical Eng. Dept., College of Engineering, Koç University, Sarıyer-İstanbul, 34450, Turkey*

Proteins can be targeted to organellar membranes using a tail anchor (TA), a stretch of hydrophobic amino acids found at the polypeptide carboxyl-terminus. The Fis1 protein (Fis1p), which promotes mitochondrial and peroxisomal division in the yeast *Saccharomyces cerevisiae*, is targeted to those organelles by its TA. Substantial evidence suggests that Fis1p insertion into the mitochondrial outer membrane can occur without the need for a translocation machinery. However, recent findings raise the possibility that Fis1p insertion into mitochondria might be promoted by a proteinaceous complex. Here, we have performed atomistic and coarse-grained simulations to analyze the adsorption, conformation and orientation of the Fis1(TA). Our results support stable insertion at the mitochondrial outer membrane in a monotopic, rather than a bitopic (transmembrane), configuration. Once inserted in the monotopic orientation, unassisted transition to the bitopic orientation is expected to be blocked by the highly charged nature of the TA carboxyl-terminus and by the Fis1p cytosolic domain. Our results are consistent with a model in which Fis1p does not require a translocation machinery for insertion at mitochondria.

I. INTRODUCTION

Biochemical, genetic, and computational approaches have significantly contributed to our understanding of the signals that direct proteins to membranes. A number of polypeptides are directed to organelles with the assistance of a carboxyl-terminal, hydrophobic 'tail anchor' (TA). Nearly 5% of proteins are targeted to a destination membrane by a TA [1], and new tail-anchored proteins continue to be identified [2]. However, the mechanisms by which TAs are targeted to and inserted at specific subcellular locations remain poorly defined [3, 4].

The Fis1 protein (Fis1p) recruits additional factors to the surface of mitochondria and peroxisomes to promote their division in *Saccharomyces cerevisiae* [5–8]. Fis1p consists of a cytosolic domain that binds to its partner proteins, as well as a TA required for tethering this polypeptide to organelle surfaces [9–11]. Fis1p has served as a valuable substrate for investigating the principles governing TA trafficking [12–16]. The Fis1(TA) consists of a hydrophobic stretch of amino acids, predicted to form an alpha-helix, followed by positive charges that promote both insertion and organelle specificity [12, 15, 17]. While cytosolic chaperones and other factors may promote the delivery of Fis1p to organelles [18], most evidence suggests that the ultimate insertion of Fis1p into membranes, as well as the insertion of several other tail-anchored proteins at mitochondria, need not be catalyzed by any translocation machinery [13, 14, 19, 20]. However, a recent report has suggested that robust Fis1p insertion at the mitochondrial surface occurs with the help of the MIM import complex [21], calling into question a model in which Fis1p can spontaneously insert at its destination membranes.

Integral membrane proteins can be classified into three distinct classes: bitopic (transmembrane), monotopic (associated with membrane, but not passing through the lipid bilayer), and polytopic (passing through the membrane several times) [22]. The topology of Fis1(TA) at lipid bilayers should be informative regarding whether a translocon might be required for organellar insertion.

In this study, we have used atomistic and coarse-grained molecular dynamics simulations to investigate the behavior of the Fis1(TA) at a lipid bilayer mimicking the surface of mitochondria. Our results suggest that the Fis1(TA) can be inserted in a stable, monotopic conformation, supporting the scenario in which TA proteins are anchored at membranes without the use of a translocation machinery.

II. RESULTS

We began our analysis by performing simulated annealing to observe the interaction between Fis1(TA) and a model bilayer mimicking the *S. cerevisiae* mitochondrial outer membrane [23]. We define the Fis1(TA) peptide as residues 129-155 of the full-length polypeptide. This 27 residue long region (-LKGVVVAGGVLAGAVAVASFFLRNKRR-COOH) is necessary and sufficient for mitochondrial targeting [6, 24]. The peptide, initially placed in the aqueous environment near the membrane, spontaneously adsorbs onto the membrane via five highly charged/polar residues at the carboxyl-terminus (Fig 1a). Next, as the peptide slowly inserts itself into the membrane, the amino-

* Corresponding authors: cory.dunn@helsinki.fi & msayar@ku.edu.tr
† Current address: TUBITAK Marmara Research Center, Genetic Engineering and Biotechnology Institute, Gebze, Kocaeli, Turkey

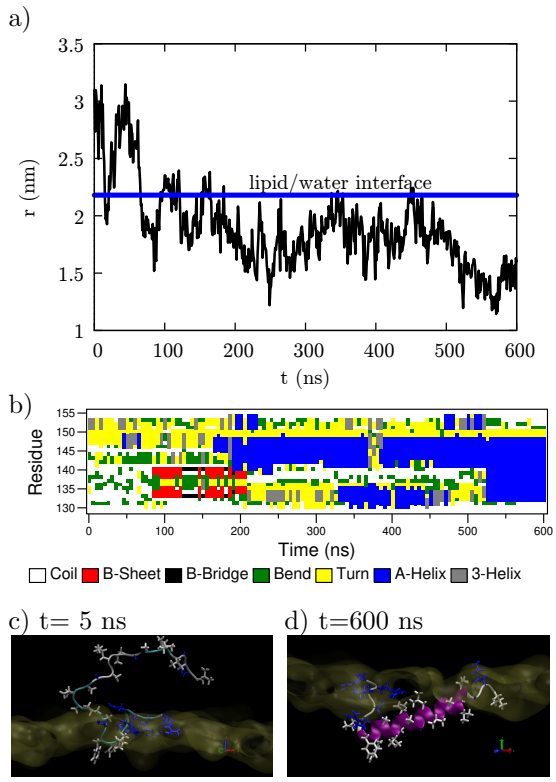

FIG. 1. Fis1(TA) spontaneously adsorbs into the lipid membrane via its carboxyl-terminus and folds into an alpha-helical structure inside the membrane in a monotopic orientation. a) Distance of the center-of-mass of the peptide to the membrane centerline, and b) secondary structure evolution during insertion into the membrane. c) Snapshots for the initial contact and the partial helical structure in monotopic orientation.

terminus of the Fis1(TA) also adsorbs. Being attached from both termini to the membrane, the peptide slowly penetrates the lipid environment. After 520 ns, the peptide folds into an extended α-helix inside the membrane in a monotopic orientation, as determined by the secondary structure analysis of the molecule (Fig. 1b). Representative snapshots for the initial contact via the carboxyl-terminus and for the folded structure inside the membrane are provided in Fig. 1c. For clarity, the charged head groups of the lipids in the upper leaflet are shown as an olive-green colored surface to denote the lipid/water interface; the space above represents the aqueous cytosol, and the space below represents the interior of the membrane above the centerline.

Inside the membrane, the conformational dynamics of the peptide slow significantly. As a result, our simulated annealing approach is not sufficient for obtaining the equilibrium secondary structure of the peptide and its preferred orientation inside the membrane. Even though the monotopic orientation was stable following 600 ns of simulation, we could not, in this analysis, rule out the possibility of a bitopic configuration of lower energy.

Consequently, we performed a μs-long atomistic replica exchange molecular dynamics (AA-REX) simulation [25, 26] on the Fis1(TA) peptide. The AA-REX approach enhances the sampling of the conformational and orientational sample space of the target system [27, 28]. Specifically, local energy barriers are overcome by frequent swaps between replicas that are run in parallel at different temperatures. We have used 80 replicas spanning a temperature range of 298-471 K for our AA-REX simulation.

For the AA-REX simulation, the Fis1(TA) was initially placed inside the lipid bilayer as a fully extended alpha-helix in the monotopic orientation, 1.2 nm below the lipid/water interface. At the target temperature of 298 K, the peptide retained a predominantly alpha-helical conformation and a monotopic orientation, as depicted in the representative snapshot in Fig. 2a. Residue-based secondary structure propensity analysis of the 298 K replica's trajectory revealed that, except for the five charged and/or polar residues at the carboxyl-terminus, the peptide displays a strong tendency towards alpha-helicity (Fig. 2b). The helical, hydrophobic portion of the peptide is submerged approximately 0.7 nm below the lipid/water interface, while the non-helical, charged carboxyl-terminus extends towards this interface (Fig. 2c). The tilt angle, defined here as the angle between the helix-axis and the membrane normal, clearly indicates (Fig. 2d) that the peptide maintains a monotopic orientation. Even though the AA-REX simulation is effective in revealing the equilibrium secondary structural preference of the peptide, we did not observe any transitions from the monotopic to bitopic orientation during the μs-long simulation (please see Supplemental Fig. S1 for secondary structure analysis of the peptide during the AA-REX simulation, and Supplemental Movie 1, a representative movie demonstrating the conformational dynamics).

The size of the AA-REX system again hampered a thorough exploration of the possible transitions from monotopic to bitopic orientation and any associated free energy changes. Therefore, to further investigate the insertion free energy and preferred orientation of the Fis1(TA) in the membrane, we performed metadynamics simulations using the coarse-grained MARTINI force field [29, 30]. MARTINI force field has been widely used for studying the conformational dynamics of proteins with the lipid bilayer [31–33]. As the MARTINI force field does not allow conformational changes, the secondary structure of the peptide was assigned according to the AA-REX results provided in Fig. 2b. The helical tilt angle (θ) and the vertical distance of the center-of-mass of the peptide from the center of the bilayer (r) were used as collective variables for the metadynamics simulation. During the metadynamics simulation, the peptide inserts itself from water to the membrane only in the monotopic orientation. Four distinct minima are observed in the resulting free energy landscape (Fig. 3a). These minima correspond to the two monotopic orientations (labeled 1 and 3) and the two bitopic orientations (labeled 2 and 4)

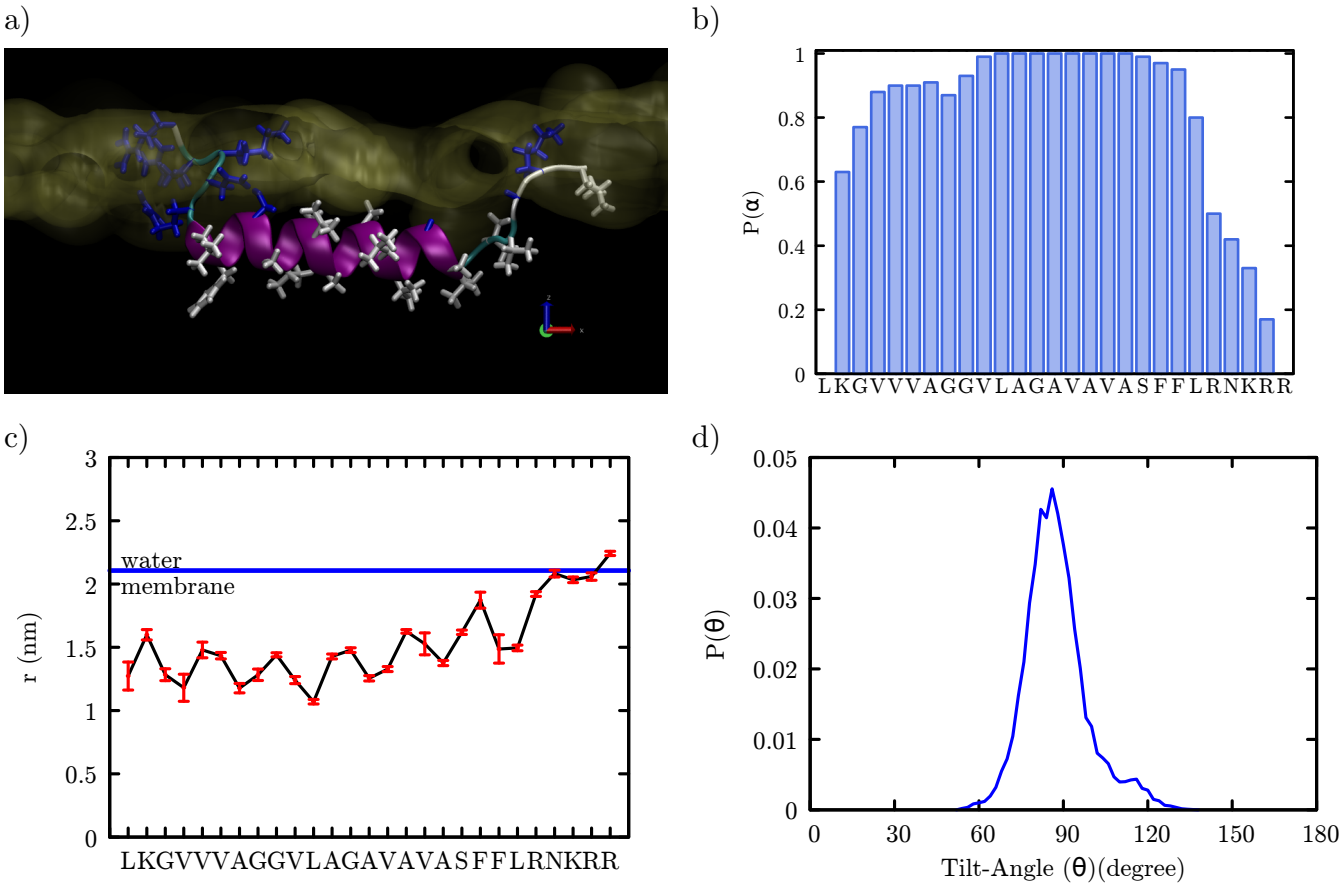

FIG. 2. Analysis of AA-REX results: a) Fis1 TA adopts a dominantly α-helical structure within the membrane. b) Sequence specific α-helix propensity. c) Average depth of the residues with respect to membrane/water interface. d) Angle between the helix axis and the membrane normal.

on either side of the membrane (Fig. 3b).

The metadynamics simulation yields an insertion free energy of 50 kJ/mol, which is in agreement with the atomistic simulated annealing result that demonstrates spontaneous insertion of the peptide from bulk water to the membrane. By both the simulated annealing and metadynamics approaches, the initial insertion starts with the peptide attaching to the membrane surface via its carboxyl-terminus. Next, Fis1(TA) rapidly adopts a monotopic orientation. When compared to the AA-REX simulation, the peptide's center-of-mass in the monotopic orientation is closer to the lipid/water interface (≈ 0.25 nm below the interface) in the MARTINI simulation.

The pathway from 1-to-2 (or 3-to-4, considering the symmetric membrane model) represents a monotopic-to-bitopic transition via the amino-terminus, whereas the pathway from 1-to-4 (3-to-2) represents a transition via the carboxyl-terminus. As revealed by the free-energy surface, transition to the bitopic orientation is only possible via the amino-terminal pathway, and with a high energy barrier (40 kJ/mol). Moreover, the monotopic orientation is 5-10 kJ/mol more favorable compared to the bitopic orientation. An unassisted transition from the carboxyl-terminus is completely blocked, as the four positive charges at the carboxyl-terminus makes this pathway highly unfavorable energetically. Since an unassisted transition pathway via the Fis1(TA) amino-terminus would also be impossible in the context of a full-length Fis1p, a Fis1(TA) in monotopic orientation should be expected to remain in that position. Importantly, when the Fis1(TA) peptide is encountered in the bitopic orientation following amino-terminal transition, the peptide must adapt by stretching the five residues at the carboxyl-terminus. However, this still leaves some of the charged carboxyl-terminal residues buried inside the hydrophobic core of the membrane, clearly making this position less favorable when compared to the monotopic orientation.

III. CONCLUSIONS

Previous results suggest that the Fis1(TA) does not require any specific translocation machinery for its membrane insertion [13, 14]. Indeed, Fis1p is integrated at

mitochondrial outer membranes treated with protease to destroy receptors and translocation machinery, and Fis1p can even be inserted into bare liposomes. Moreover, Fis1p plays a role in the division of both peroxisomes and mitochondria [5–8], although no translocation machinery is known to be shared between these two organelles, further suggesting a translocon-independent mode of insertion. However, a heavily charged region follows the hydrophobic stretch found within the Fis1(TA) that is predicted to drive membrane association, raising questions regarding how such a charged region might reach the mitochondrial intermembrane space without the assistance of a translocon if Fis1p were a transmembrane protein. Our atomistic and coarse-grained simulations, performed using a model mitochondrial outer membrane, suggest that the Fis1(TA) can be stably inserted monotopically into only one leaflet of a lipid bilayer in the absence of a translocation machinery. We speculate that the dual localization of Fis1p at mitochondria and peroxisomes is likely to be driven by the lipid environments found at those membranes, and specific lipids that might promote Fis1p association with its target organelles are a subject of current investigation.

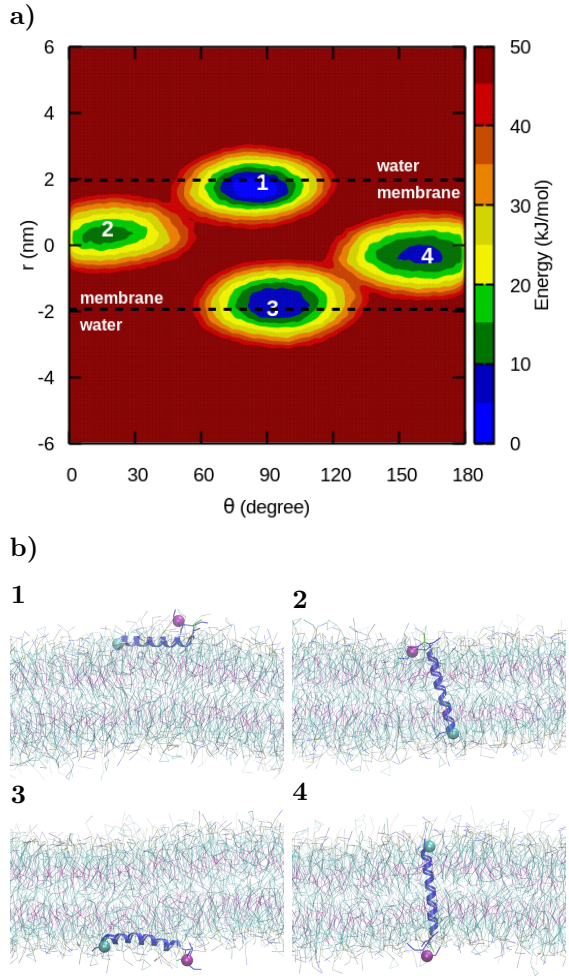

FIG. 3. a) The free-energy surface for the Fis1(TA) peptide's insertion into the membrane. The color scheme represents the relative free energy with respect to the minimum as indicated by the colorbar. Four distinct minima represent the monotopic (1 and 3) and bitopic (2 and 4) orientations of the peptide in the membrane. b) Representative snapshots of the peptide in each case.

IV. METHODS

A. Atomistic simulations

All simulations were performed with the GROMACS 2018 simulation package [34]. The CHARMM36m force field [35, 36] in combination with TIP3P water model [37] was used for the atomistic system.

Temperature was controlled by velocity-rescaling algorithm [38] with a time constant of 0.1 ps for coupling. Semi-isotropic pressure coupling was achieved by Berendsen barostat [39] for equilibration and Parrinello-Rahman [40] for production runs. Verlet cutoff-scheme with $rvdw = rcoulomb = 1.2\ nm$, as well as force switching to zero from 1.0 to $1.2nm$, were deployed. The particle-mesh Ewald method [41] with a Fourier spacing of 0.1 nm was used for electrostatic interactions.

For the atomistic simulations, CHARMM input generator v1.7 [42] was used to place the peptide and the lipid membrane system inside a hexagonal simulation box. Lipid composition of the leaflets were as listed in Table I, and in total, the system contained 200 lipids. For every lipid molecule, 50 water molecules were added to the simulation box. The salt concentration was adjusted to 0.15 M by adding Na+ and Cl- ions, as well as the neutralizing counterions.

Within a given cycle during the simulated annealing experiment, the temperature was raised from 298 to 800 K in 2.5 ns, kept at 800 K for 2.5 ns, reduced back in 2.5 ns and kept at 298 K for another 12.5 ns. The system was simulated for 600 ns in total. The Fis1(TA) peptide was initially placed in a random conformation above the membrane in water. Further details of the simulated annealing procedure are provided in Supplemental Fig. S2 and Supplemental Fig. S3.

The atomistic replica exchange simulation (AA-REX), was initialized with the Fis1(TA) peptide buried close to the upper leaflet, with the center-of-mass of the peptide vertically 1.2 nm below the phosphate groups of the upper leaflet of the membrane. Eighty replicas were used to cover a temperature range of 298 to 471.05 K in AA-REX simulations, and the temperature of each replica was determined using the web server of Patriksson and van der Spoel [43]. An exchange between neighboring replicas was attempted every 2 ps according to the Metropolis criterion. An average exchange probability of 0.185 was observed.

TABLE I. Composition and charge of the lipids in the membrane, obtained from [23].

Lipid	Percentage	Charge
DOPC (PC)	46%	0
DOPE (PE)	33%	0
POPI (PI)	10%	-1
Cardiolipin	6%	-2
DOPA (PA)	4%	-1
DOPS (PS)	1%	-1

B. MARTINI Coarse-grained simulations

For coarse-grained simulations, the MARTINI force field [29] was used. The secondary structure of Fis1(TA) was assigned inline with the AA-REX results, with the residues spanning G131 through L150 fixed as helix, and the rest left as random-coil. MARTINI scripts "martinize.py" and "insane.py" were used to prepare the system, and we used the recommended configuration file that is available in MARTINI website [44].

The number and composition of the lipid molecules were chosen identical to the atomistic composition. For every lipid molecule, 25 MARTINI water molecules were added to the simulation box. The total charge of the system (peptide and membrane) was neutralized by an equal number of counterions. Additionally, a salt concentration of 0.15 M was added to the system by adding Na+ and Cl- ions.

For facilitating the transitions between the monotopic and bitopic orientations, we carried out metadynamics simulations to obtain the free energy surface of the Fis1(TA) peptide. Two collective variables (CV) were used with the metadynamics simulation: tilt-angle (θ) and the vertical distance of the peptide from the membrane center (r). Fis1(TA) residues 132-134 and 147-149 were used to mark the start and end of the helix when representing the helical axis. Tilt-angle was measured as the angle between the helical axis and the z-axis, while r was calculated as the vertical distance of the center-of-mass of the residues 132-134 and 147-149 from the center of mass of the membrane. The metadynamics approach implemented in PLUMED 2 [45] deposits n-dimensional Gaussians, where n is the number of CVs used, with an aim to allow the molecule of interest to overcome the high energy barriers.

In both atomistic simulations and trial runs with MARTINI, we observed that the monotopic and bitopic orientations of Fis1(TA) are separated by rather high energy barriers, and even the added bias provided by metadynamics could not promote transitions. Therefore, we combined metadynamics with a Hamiltonian replica exchange based approach. The wild-type Fis1(TA) was mutated in twelve steps to a highly hydrophobic sequence that would allow transitions from monotopic to bitopic orientation. A total of 78 runs (twelve mutants plus the wild-type and six parallel runs for each) were used to facilitate the sampling of the free-energy surface. For each of the six parallel runs initial condition of the peptide was altered between six configurations: bitopic with amino-terminal in upper/lower leaflet; monotopic in upper/lower leaflet; above and below the membrane in bulk water. Our aim was to enhance transitions between monotopic and bitopic orientations in replicas using the hydrophobic sequence, where both termini lose their charged/polar character gradually, and then 'walk' these transitions towards the replica with the wild-type Fis1(TA). The analysis provided in Fig. 3 is based on the replica that corresponds to the wild-type Fis1(TA).

The Dictionary of Protein Secondary Structure [46] was used for secondary structure assignment. Molecular graphics were produced by VMD [47] and plots were produced with the Gnuplot 5.2 package [48].

V. ACKNOWLEDGMENTS

C.D.D. gratefully acknowledges funding from the Sigrid Jusélius Foundation, the Academy of Finland (project 331556), and the Jane and Aatos Erkko Foundation. C.D.D. and M.S. appreciate the excellent computational support from the TUBITAK ULAKBIM High Performance and Grid Computing Center (TRUBA resources) and from the Center for Scientific Computing, Finland (Puhti supercomputer).

[1] Ramanujan S Hegde and Robert J Keenan, "Tail-anchored membrane protein insertion into the endoplasmic reticulum," Nat. Rev. Mol. Cell Biol. **12**, 787–798 (2011).

[2] Uri Weill, Nir Cohen, Amir Fadel, Shifra Ben-Dor, and Maya Schuldiner, "Protein topology prediction algorithms systematically investigated in the yeast saccharomyces cerevisiae," Bioessays **41**, e1800252 (2019).

[3] Nica Borgese, Javier Coy-Vergara, Sara Francesca Colombo, and Blanche Schwappach, "The ways of tails: The GET pathway and more," Protein J. **38**, 289–305 (2019).

[4] Layla Drwesh and Doron Rapaport, "Biogenesis pathways of α-helical mitochondrial outer membrane proteins," Biol. Chem. **401**, 677–686 (2020).

[5] P Fekkes, K A Shepard, and M P Yaffe, "Gag3p, an outer membrane protein required for fission of mitochondrial tubules," J. Cell Biol. **151**, 333–340 (2000).

[6] A D Mozdy, J M McCaffery, and J M Shaw, "Dnm1p GTPase-mediated mitochondrial fission is a multi-step process requiring the novel integral membrane component Fis1p," J. Cell Biol. **151**, 367–380 (2000).

[7] Q Tieu and J Nunnari, "Mdv1p is a WD repeat protein that interacts with the dynamin-related GTPase, Dnm1p, to trigger mitochondrial division," J. Cell Biol. **151**, 353–366 (2000).

[8] Alison M Motley, Gemma P Ward, and Ewald H Hettema, "Dnm1p-dependent peroxisome fission requires Caf4p, Mdv1p and Fis1p," J. Cell Sci. **121**, 1633–1640 (2008).

[9] Kara L. Cerveny and Robert E. Jensen, "The WD-repeats of Net2p Interact with Dnm1p and Fis1p to Regulate Division of Mitochondria," MBoC **14**, 4126–4139 (2003).

[10] Quinton Tieu, Voytek Okreglak, Kari Naylor, and Jodi Nunnari, "The WD repeat protein, Mdv1p, functions as a molecular adaptor by interacting with Dnm1p and Fis1p during mitochondrial fission," Journal of Cell Biology **158**, 445–452 (2002).

[11] Mary Anne Karren, Emily M. Coonrod, Teresa K. Anderson, and Janet M. Shaw, "The role of Fis1p–Mdv1p interactions in mitochondrial fission complex assembly," Journal of Cell Biology **171**, 291–301 (2005).

[12] Shukry J Habib, Andreja Vasiljev, Walter Neupert, and Doron Rapaport, "Multiple functions of tail-anchor domains of mitochondrial outer membrane proteins," FEBS Lett. **555**, 511–515 (2003).

[13] Christian Kemper, Shukry J Habib, Gertraud Engl, Petra Heckmeyer, Kai S Dimmer, and Doron Rapaport, "Integration of tail-anchored proteins into the mitochondrial outer membrane does not require any known import components," J. Cell Sci. **121**, 1990–1998 (2008).

[14] Katrin Krumpe, Idan Frumkin, Yonatan Herzig, Nitzan Rimon, Cagakan Özbalci, Britta Brügger, Doron Rapaport, and Maya Schuldiner, "Ergosterol content specifies targeting of tail-anchored proteins to mitochondrial outer membranes," Mol. Biol. Cell **23**, 3927–3935 (2012).

[15] Abdurrahman Keskin, Emel Akdoğan, and Cory D. Dunn, "Evidence for Amino Acid Snorkeling from a High-Resolution, In Vivo Analysis of Fis1 Tail-Anchor Insertion at the Mitochondrial Outer Membrane," Genetics **205**, 691–705 (2017).

[16] Daniela G Vitali, Layla Drwesh, Bogdan A Cichocki, Antonia Kolb, and Doron Rapaport, "The biogenesis of mitochondrial outer membrane proteins show variable dependence on import factors," iScience **23**, 100779 (2020).

[17] Chika Horie, Hiroyuki Suzuki, Masao Sakaguchi, and Katsuyoshi Mihara, "Targeting and assembly of mitochondrial tail-anchored protein Tom5 to the TOM complex depend on a signal distinct from that of tail-anchored proteins dispersed in the membrane," J. Biol. Chem. **278**, 41462–41471 (2003).

[18] Bogdan A Cichocki, Katrin Krumpe, Daniela G Vitali, and Doron Rapaport, "Pex19 is involved in importing dually targeted tail-anchored proteins to both mitochondria and peroxisomes," Traffic **19**, 770–785 (2018).

[19] Kiyoko Setoguchi, Hidenori Otera, and Katsuyoshi Mihara, "Cytosolic factor- and TOM-independent import of C-tail-anchored mitochondrial outer membrane proteins," EMBO J. **25**, 5635–5647 (2006).

[20] Nebibe Mutlu, Görkem Garipler, Emel Akdoğan, and Cory D Dunn, "Activation of the pleiotropic drug resistance pathway can promote mitochondrial DNA retention by fusion-defective mitochondria in Saccharomyces cerevisiae," G3 **4**, 1247–1258 (2014).

[21] Kim Nguyen Doan, Alexander Grevel, Christoph U Mårtensson, Lars Ellenrieder, Nicolas Thornton, Lena-Sophie Wenz, Łukasz Opaliński, Bernard Guiard, Nikolaus Pfanner, and Thomas Becker, "The mitochondrial import complex MIM functions as main translocase for α-Helical outer membrane proteins," Cell Rep. **31**, 107567 (2020).

[22] Günter Blobel, "Intracellular protein topogenesis," PNAS **77**, 1496–1500 (1980).

[23] R Simbeni, L Pon, E Zinser, F Paltauf, and G Daum, "Mitochondrial membrane contact sites of yeast. Characterization of lipid components and possible involvement in intramitochondrial translocation of phospholipids," J. Biol. Chem. **266**, 10047–10049 (1991).

[24] Traude Beilharz, Billie Egan, Pamela A Silver, Kay Hofmann, and Trevor Lithgow, "Bipartite signals mediate subcellular targeting of tail-anchored membrane proteins in Saccharomyces cerevisiae," J. Biol. Chem. **278**, 8219–8223 (2003).

[25] Yuji Sugita and Yuko Okamoto, "Replica-exchange molecular dynamics method for protein folding," Chem. Phys. Lett. **314**, 141–151 (1999).

[26] Angel E García and Kevin Y Sanbonmatsu, "Alpha-helical stabilization by side chain shielding of backbone hydrogen bonds," Proc. Natl. Acad. Sci. U. S. A. **99**, 2782–2787 (2002).

[27] Edina Rosta and Gerhard Hummer, "Error and efficiency of replica exchange molecular dynamics simulations," J. Chem. Phys. **131**, 165102 (2009).

[28] Lukas S Stelzl and Gerhard Hummer, "Kinetics from replica exchange molecular dynamics simulations," J. Chem. Theory Comput. **13**, 3927–3935 (2017).

[29] Siewert J Marrink, H Jelger Risselada, Serge Yefimov, D Peter Tieleman, and Alex H de Vries, "The MARTINI force field: Coarse grained model for biomolecular simulations," J. Phys. Chem. B **111**, 7812–7824 (2007).

[30] Luca Monticelli, Senthil K. Kandasamy, Xavier Periole, Ronald G. Larson, D. Peter Tieleman, and Siewert-Jan Marrink, "The MARTINI Coarse-Grained Force Field: Extension to Proteins," J. Chem. Theory Comput. **4**, 819–834 (2008).

[31] Alan Chetwynd, Chze Ling Wee, Benjamin A. Hall, and Mark S. P. Sansom, "The Energetics of Transmembrane Helix Insertion into a Lipid Bilayer," Biophysical Journal **99**, 2534–2540 (2010).

[32] Benjamin A. Hall, Alan P. Chetwynd, and Mark S. P. Sansom, "Exploring Peptide-Membrane Interactions with Coarse-Grained MD Simulations," Biophysical Journal **100**, 1940–1948 (2011).

[33] Ghulam Mustafa, Prajwal P. Nandekar, Xiaofeng Yu, and Rebecca C. Wade, "On the application of the MARTINI coarse-grained model to immersion of a protein in a phospholipid bilayer," J. Chem. Phys. **143**, 243139 (2015).

[34] Mark James Abraham, Teemu Murtola, Roland Schulz, Szilárd Páll, Jeremy C Smith, Berk Hess, and Erik Lindahl, "GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers," SoftwareX **1-2**, 19–25 (2015).

[35] Jeffery B Klauda, Richard M Venable, J Alfredo Freites, Joseph W O'Connor, Douglas J Tobias, Carlos Mondragon-Ramirez, Igor Vorobyov, Alexander D MacKerell, Jr, and Richard W Pastor, "Update of the CHARMM all-atom additive force field for lipids: Valida-

tion on six lipid types," J. Phys. Chem. B **114**, 7830–7843 (2010).

[36] Jing Huang, Sarah Rauscher, Grzegorz Nawrocki, Ting Ran, Michael Feig, Bert L de Groot, Helmut Grubmüller, and Alexander D MacKerell, Jr, "CHARMM36m: An improved force field for folded and intrinsically disordered proteins," Nat. Methods **14**, 71–73 (2017).

[37] William L. Jorgensen, Jayaraman Chandrasekhar, Jeffry D. Madura, Roger W. Impey, and Michael L. Klein, "Comparison of simple potential functions for simulating liquid water," J. Chem. Phys. **79**, 926–935 (1983).

[38] Giovanni Bussi, Davide Donadio, and Michele Parrinello, "Canonical sampling through velocity rescaling," J. Chem. Phys. **126**, 014101 (2007).

[39] H J C Berendsen, J P M Postma, W F van Gunsteren, A DiNola, and J R Haak, "Molecular dynamics with coupling to an external bath," J. Chem. Phys. **81**, 3684–3690 (1984).

[40] M Parrinello and A Rahman, "Polymorphic transitions in single crystals: A new molecular dynamics method," J. Appl. Phys. **52**, 7182–7190 (1981).

[41] Tom Darden, Darrin York, and Lee Pedersen, "Particle mesh Ewald: An N·log(N) method for Ewald sums in large systems," J. Chem. Phys. **98**, 10089–10092 (1993).

[42] Sunhwan Jo, Taehoon Kim, Vidyashankara G Iyer, and Wonpil Im, "CHARMM-GUI: A web-based graphical user interface for CHARMM," J. Comput. Chem. **29**, 1859–1865 (2008).

[43] Alexandra Patriksson and David van der Spoel, "A temperature predictor for parallel tempering simulations," Phys. Chem. Chem. Phys. **10**, 2073–2077 (2008).

[44] "MARTINI website,".

[45] Gareth A Tribello, Massimiliano Bonomi, Davide Branduardi, Carlo Camilloni, and Giovanni Bussi, "PLUMED 2: New feathers for an old bird," Comput. Phys. Commun. **185**, 604–613 (2014).

[46] W Kabsch and C Sander, "Dictionary of protein secondary structure: Pattern recognition of hydrogen-bonded and geometrical features," Biopolymers **22**, 2577–2637 (1983).

[47] William Humphrey, Andrew Dalke, and Klaus Schulten, "VMD: Visual molecular dynamics," Journal of Molecular Graphics **14**, 33–38 (1996).

[48] Thomas Williams, Colin Kelley, and many others, "Gnuplot 5.2: An interactive plotting program," (2013).