Investigation of molecular transport through multipore into giant unilamellar vesicles using COMSOL simulation

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Abstract

Now a days many applications such as gene transfection into cells, cancer chemotherapy, insert foreign proteins into cells and transdermal drug delivery can be possible if the molecules can transport through the nanopore in cell membrane with a tractable manner [1]. Extensively investigation on Antimicrobial peptides (AMPs) magainin 2 [2] reported that magainin 2 interacts directly with the lipid bilayer rather than with a specific protein target within the cell membrane [3]. through the pores [4].However, kinetics of molecular transport such as the leakage of water-soluble fluorescent probes from the inner side to the outer side (buffer area) of GUVs through nanopores is very important to unearth the details mechanisms of pore formation and nature of its effect on molecular transport has not been investigated in detail yet. Recently, theoretical study [4] investigated the molecular transport into the GUVs through a single nanopore for the various sizes of fluorescent probe using artificially designed GUVs and nanopore on the membrane based on the experimental parameters [5]. However, kinetics of molecular transport through multipore in the membrane of GUVs has not been clearly understood. In this simulation, we have designed a group of pores in the membrane of GUVs using computer-aided design software (AutoCAD) and performed simulation to see the kinetics of molecular transport, from the inside to the outside of GUV through the nanopores. Here we used various water soluble fluorescent probes such as calcein,Texas-Red Dextran 3000 (TRD-3K), TRD-10K and TRD-40K. The diffusion constant observed by our simulation agrees reasonably consistent with experimental results.

Computational Method

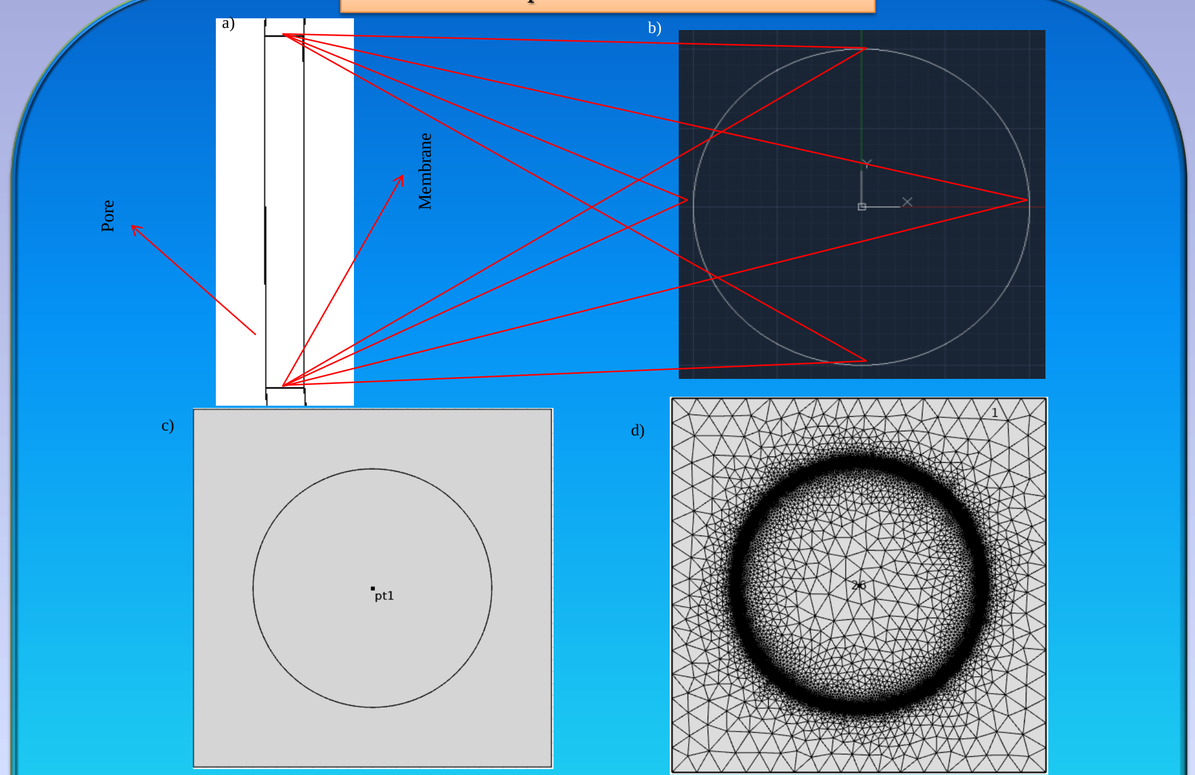

Fig. 1: Multi nanopores in the membrane of a GUV. a) Zoomed view of a single nanopore in the membrane b of a GUV designed in AutoCAD, c) 2D geometry of the membrane in COMSOL simulation and d) normal mesh of a single GUV with multi nanopores in COMSOL.

Table1: Materials assigned for each domain (M.A.S Karal et al. 2019)

Domain	Material	Relative Permittivity	Diffusion Coefficient(m^2s)
4(Cytoplasm)	Custom	80	According to table 2
3(Membrane)	Custom	80	-
2(Aqueous Pore)	Custom	80	-
1(Buffer Solution)	Custom	80	-

Table 2: Fluorescent probes, Stokes–Einstein radii and difusion coeffcients (Tamba et al. 2010; calculated according to Venturoli and Rippe 2005)

Fluorescent Probes	R_{SE} (nm)	D_i (m^2/s)×10^{-10}
TRD-3k	1.4	1.75
TRD-10k	2.7	0.91
TRD-40k	5.0	0.49

Comparison of simulation results with reported experimental results

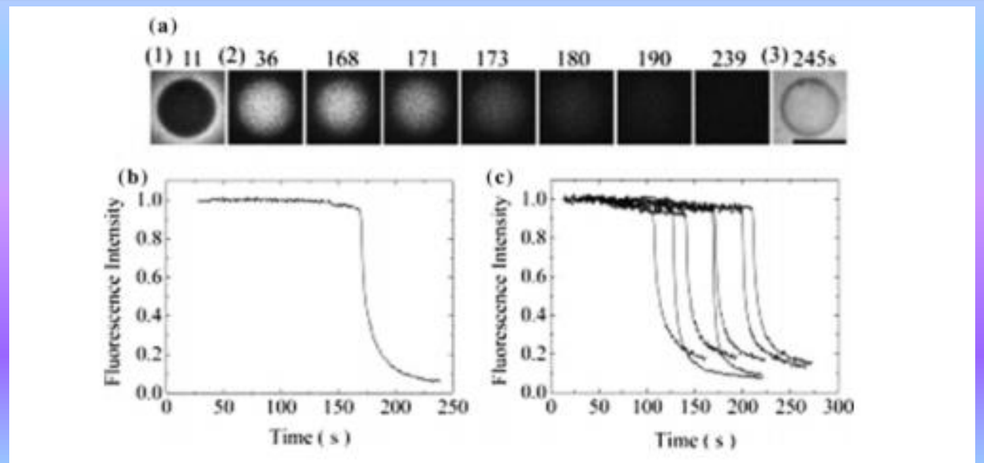

Fig. 8: Leakage of TRD-3k from DOPG/DOPC(5/5)-GUVs induced by 7 μM magainin 2. a Fluorescence images (2) show the decrease of TRD-3k concentration from the inside of a GUV due to pore formation in the membrane. The numbers above each image show the time in seconds after the addition of magainin 2. Phase contrast images are of the GUV at time 11 s (1) and 245 s (3). The bar corresponds to 10 μm. b Time course of the change in normalized fluorescence intensity of the GUV is shown in a. c Under the same conditions as in a the time course of the change in normalized fluorescence intensity of several single GUVs (Tamba et al. 2010)

Molecular transport of various fluorescent probes through multi nanopores in the membrane of a GUV

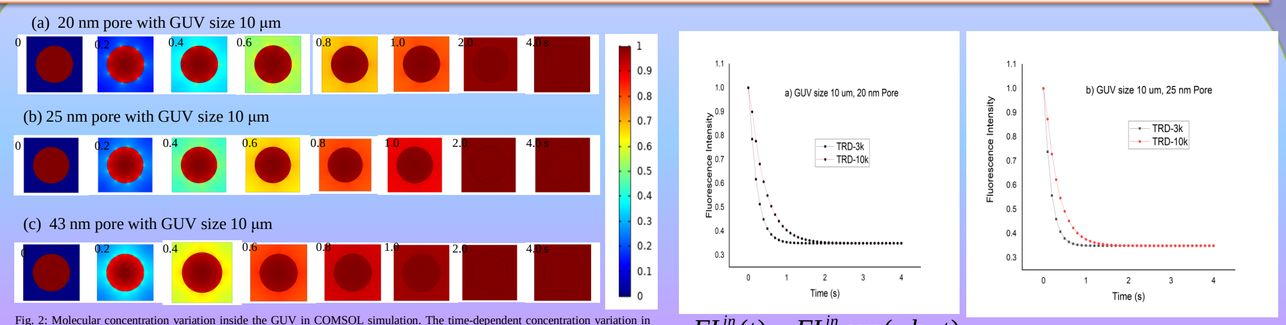

Fig. 2: Molecular concentration variation inside the GUV in COMSOL simulation. The time-dependent concentration variation in the inside of GUV for (a) 20 nm pores (b) 25 nm pores (c) 43 nm pores. The numbers at the corner of each image show the time in seconds after the simulation was started.

$$FI^{in}(t) = FI_0^{in}\exp(-k_{mt}t)$$

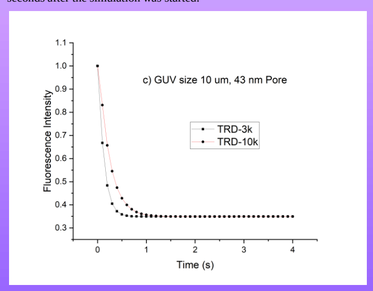

Fig. 3: The time course of the change in normalized fluorescence intensity in the GUV for various fluorescent probes including TRD-3k (black), TRD-10k (red) is shown in a,b,c respectively.

a)GUV 10 μm, Pore 20 nm

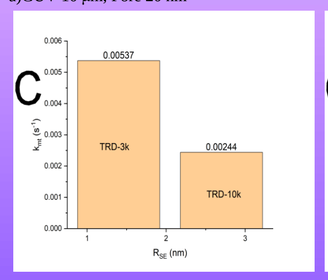

Fig. 4(a):The bar diagrams for the fluorescent probe (RSE) dependent rate constant of molecular transport though 20 nm pore with GUV size 10 μm

b) GUV 10 μm, Pore 25 nm

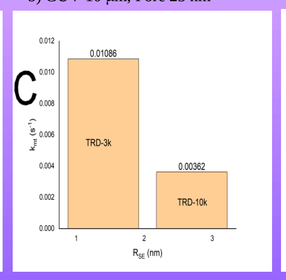

Fig. 4(b):The bar diagrams for the fluorescent probe (RSE) dependent rate constant of molecular transport though 25 nm pore with GUV size 10 μm

c) GUV 10 μm, Pore 43 nm

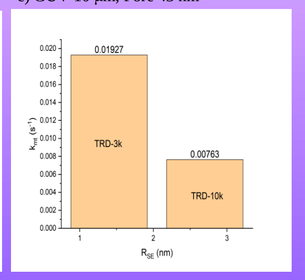

Fig. 4(c):The bar diagrams for the fluorescent probe (RSE) dependent rate constant of molecular transport though 43 nm pore with GUV size 10 μm

Table 3: Experimental data of the rate constant of magainin 2-induced leakage of various fluorescent probes and the radius of magainin 2-induced pores in single DOPG/DOPC (5/5)-GUVs (Tamba et al. 2010) and Rate constant of molecular transport in simulation for various fluorescent probes

Suspension Area *l* (um)	*d (μm)*	*r (nm)*	Probes	R_{SE} (nm)	D_i (m^2/s)×10^{-10}	Pore		Experiment	Simulation
						N	Size (nm)	k_{mt}	k_{leak}
15	10	20	TRD-3k	1.4	1.75	7	18 (2.8)	0.0045±0.0004	0.00537±
			TRD-10k	2.7	0.91		20(2.8)	0.0022±0.0005	0.00244±
		25	TRD-3k	1.4	1.75	9	26(2.8)	0.0100±0.0010	0.01086±
			TRD-10k	2.7	0.91		25(2.8)	0.0033±0.0004	0.00362±
		43	TRD-3k	1.4	1.75	12	46(3.6)	0.0200±0.0060	0.01927±
			TRD-10k	2.7	0.91		40(3.6)	0.0076±0.0009	0.00763±

Molecular transport through multi nanopores for various sizes of pores of a GUV

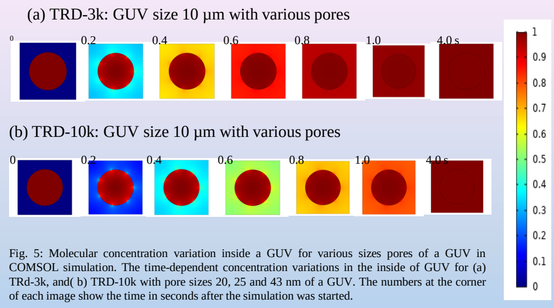

Fig. 5: Molecular concentration variation inside a GUV for various sizes pores of a GUV in COMSOL simulation. The time-dependent concentration variations in the inside of GUV for (a) TRd-3k, and(b) TRD-10k with pore sizes 20, 25 and 43 nm of a GUV. The numbers at the corner of each image show the time in seconds after the simulation was started.

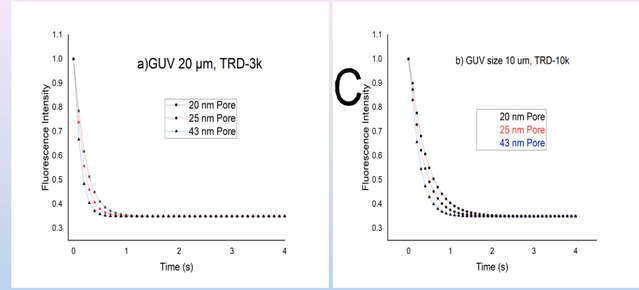

Fig. 6: The time course of the change in normalized fluorescence intensity in the GUV for different sizes of pores of a GUV as shown in a–b.

Table 4: Experimental data of the rate constant of magainin 2-induced leakage of various fluorescent probes and the radius of magainin 2-induced pores in single DOPG/DOPC (5/5)-GUVs (Tamba et al. 2010) and Table 5: Rate constant of molecular transport in simulation for various sizes of GUVs.

Suspension Area *l* (um)	*d (μm)*	Probes	R_{SE} (nm)	D_i (m^2/s)×10^{-10}	*r (nm)*	Pore		Experiment	Simulation
						N	Size (nm)	k_{mt}	k_{leak}
15	10	TRD-3k	1.4	1.75	20	7	18 (2.8)	0.0045±0.0004	0.00537±
					25	9	26(2.8)	0.0100±0.0010	0.01086±
					43	12	46(3.6)	0.0200±0.0060	0.01927±
		TRD-10k	2.7	0.91	20	7	20(2.8)	0.0022±0.0005	0.00244±
					25	9	25(2.8)	0.0033±0.0004	0.00362±
					43	12	40(3.6)	0.0076±0.0009	0.00763±

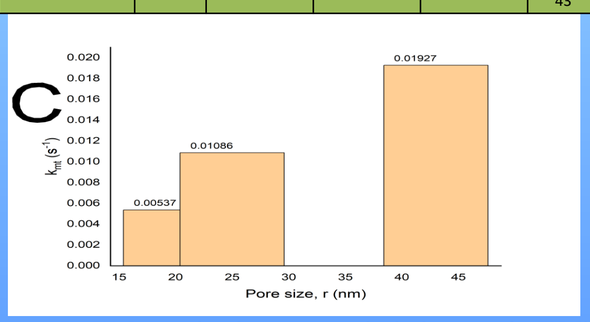

Fig. 7(a): Pore size-dependent rate constant of molecular transport for TRD-3k using 20 nm, 25 nm and 43 nm pores of GUV.

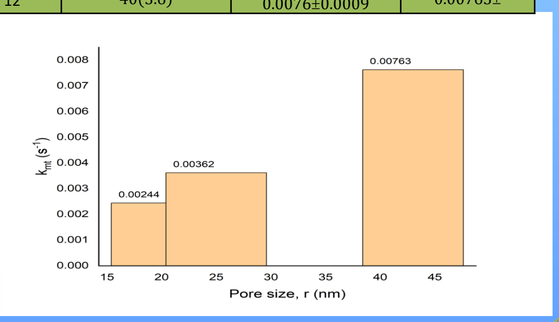

Fig. 7(b): Pore size-dependent rate constant of molecular transport for TRD-10k using 20 nm, 25 nm and 43 nm pores of GUV.

Conclusion

1. The molecular transport through multi nanopores in lipid membrane of GUVs has been studied by COMSOL simulation.
2. The molecular transport was investigated by varying the sizes of fluorescent probe (such as TRD-3k, TRD-10k, and TRD-40k) and also by varying the sizes of pores of GUV.
3. In all cases, the fluorescent probes follow a single exponential decay function to transport the molecules from the inside to the outside of the GUV through the multi nanopores.
4. The rate constant of molecular transport decreases with increasing the sizes of fluorescent probes. In addition, the rate constant of molecular transport decreases with increasing the sizes of pores of GUV.