THE INVESTIGATION OF AMPHIPHILIC PEPTIDES V_6K_2, V_6K_3 AND PEPTIDE MIXTURE SYSTEMS

By

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ABSTRACT OF THE THESIS

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Peptide self-assembly is a wide disciplinary study. There are 20 amino acids that provide miscellaneous combinations of peptide which based on different amino acid sequences. Different amino acid sequences peptides can self-assemble into different supramolecular nanostructures via self-assembly process. These nanostructures can be applied to many fields such as tissue engineering, drug delivery and electronic industry. There are many types of research have been focused on amphiphilic peptides mainly based on experimental studies. The goal of this study is to investigate two amphiphilic peptides: V_6K_2 (valine-valine-valine-valine-valine-valine-lysine-lysine), V_6K_3(valine-valine-valine-valine-valine-valine-lysine-lysine-lysine) and their mixture systems based on Molecular Dynamics Study. The mixture systems of these two peptides provide the in-depth scope of the physical, chemical and thermodynamic properties of the peptide system.

ACKNOWLEDGMENT

I would like to express my gratitude to my advisor Dr. Meenakshi Dutt for her support and encouragement for the past two years of my graduate program study. Before joined to the Computational Hybrid Soft Materials Laboratory, I do not have any computational background in Molecular Dynamics. Under her guidance for two years, I have found out and explored another beautiful world in computation. I truly thank her for leading and training me to be a qualified scientist.

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Table of Contents

List of Figures

List of Tables

Chapter 1 Introduction and Motivation

Molecular self-assembly has become a powerful and popular tool to synthesize advanced materials. Molecular self-assembly can be used in many fields such as advanced biomaterials design and synthesis [1-6], neurodegenerative disease treatments[7-10], tissue engineering[11-19] and electronics[20-25]. For example, biomaterials are designed for specific medical purposes like the regenerative treatment for injury and transplantation. In electronics, self-assembly nanostructures of phenylalanine peptide can be used in organic electronics. Molecular self-assembly of the amphiphilic peptide is the one we focus on in this work. There are 20 amino acids that provide many combinations of amphiphilic peptides and building blocks for design biomaterials. The amphiphiles can self-assemble into a high degree of controllable nanostructures such as nanorod, nanotube, nanovesicle, micelle, film and lamella.[26] Based on different sequences of the peptide, the creation of different nanomaterial morphologies can be found via a self-assembly process. Each sequence and their molecular characteristics can generate the interplay relationships.

In this work, we will focus on two amphiphilic peptides which are V_6K_2 (valine-valine-valine-valine-valine-valine-lysine-lysine), V_6K_3 (valine-valine-valine-valine-valine-valine-lysine-lysine-lysine) and mixture systems of both. Many studies have found out that V_6K_2 (valine-valine-valine-valine-valine-valine-lysine-lysine) will self-assemble into a nanotube, nanovesicle (based on different pH environments) or rod-like fibril based on experimental results. On the other hand, V_6K_3 (valine-valine-valine-valine-valine-valine-lysine-lysine-lysine) will self-assemble into nanovesicle. Currently, there is no any study

focus on these two peptides based on the computational method. Hence, the validation based on the computational model for these two peptides against existing experimental results is presented. Here, we will use Molecular Dynamics in conjunction with a MARTINI coarse-grained force field to study the morphology of each peptide via the self-assembly process.

The study of the mixture systems of V_6K_2 (valine-valine-valine-valine-valine-valine-lysine-lysine) and V_6K_3 (valine-valine-valine-valine-valine-valine-lysine-lysine-lysine) is another important topic in this work. In the mixture systems, we can observe the interactions (like or unlike interactions), line tension, solvent accessible surface area (SASA) and clusters. The way to investigate the mixture system is by varying the molecular composition through the total concentration of the peptides and the relative concentration of the peptides. The nanostructures of the hybrid materials can be classified into several categories. Later, the analysis part will demonstrate how the analysis codes work out to analyze the physical characteristics of the nanostructures for V_6K_2 (valine-valine-valine-valine-valine-valine-lysine-lysine) and V_6K_3 (valine-valine-valine-valine-valine-valine-lysine-lysine-lysine).

In Chapter 2, the MARTINI Coarse-Grained Molecular Dynamics simulations are introduced as a tool for building peptide models and the peptide systems. The methodology, detailed parameters are provided in this chapter for both polarizable and non-polarizable water models.

In Chapter 3, the mixture systems of V_6K_2 (valine-valine-valine-valine-valine-valine-lysine-lysine) and V_6K_3 are investigated through one total peptide concentration with 11

relative peptide concentration (ranging from 0% to 100%). The results are based upon 10 independent particle trajectories. The analysis will be discussed in this chapter based upon the mixture systems.

In Chapter 4, we make conclusions of our investigation of the V_6K_2 (valine − valine − valine − valine − valine − valine − lysine − lysine) peptide, V_6K_3 (valine-valine-valine-valine-valine-valine-lysine-lysine-lysine) peptide and peptide mixture systems of both.

Chapter 2 Modeling V_6K_2 and V_6K_3 peptides using Molecular Dynamics

2.1 Molecular Dynamic and Martini force field

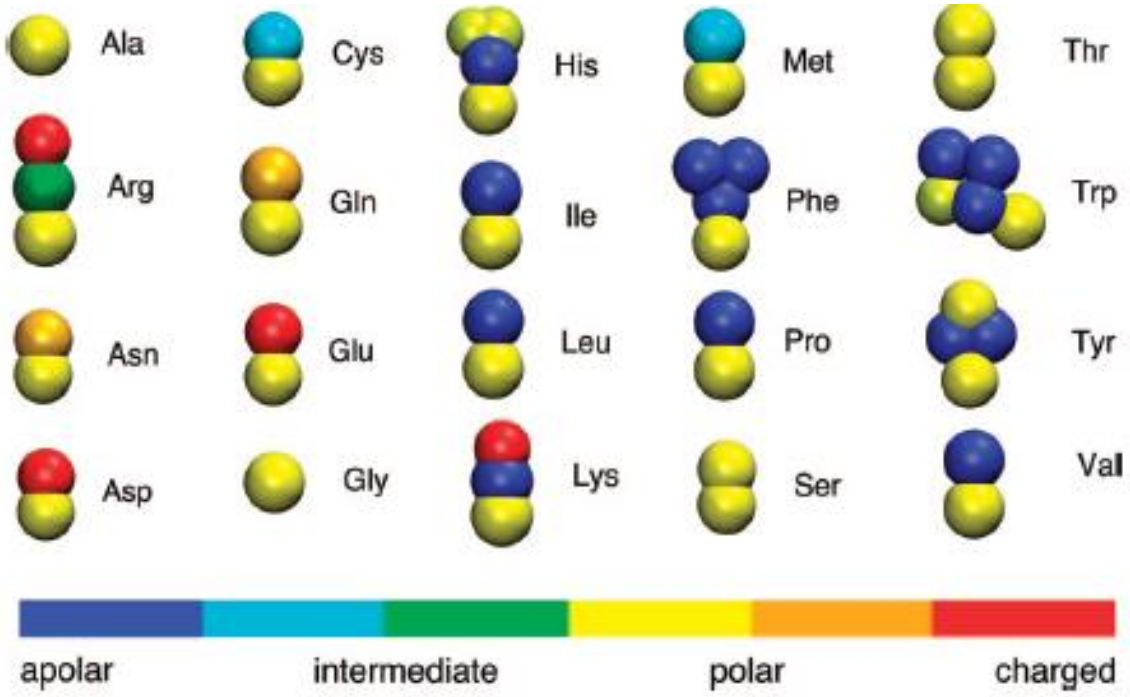
The Molecular Dynamic simulation technique computes the particle trajectory of systems by integrating Newton's second law of motion. The particle trajectories of these systems can be further analyzed to determine equilibrium and transport properties. The result is a trajectory that explicitly states how the positions, velocities and accelerations of the particles in the system vary with time. The trajectory is obtained by solving the differential equations in Newton's second law (F=ma):

$$\frac{d^2x_i}{dt^2} = \frac{F_{xi}}{m_i} \qquad (1)$$

The Equation (1) represents the motion of a particle of mass m_i along the coordinate x_i with force F_{xi} being on the particle in the specific direction. The MD simulations are run using the MD package called GROMACS. GROMACS is a molecular dynamics package for simulating proteins, lipids and nucleic acids and the Martini force field is implemented in GROMACS. In our work, we perform simulations and analyses by using GROMACS package. Although quantum mechanics simulation represents the most accurate results, it is difficult for the existing computational tools to simulate the complex systems as we have in our work.

The Martini v2.2 coarse-grained (CG) force field is used for the non-polarizable water model system.[27-29] The coarse-grained molecular modeling is a technique enables people to study larger time magnitude and scale (like 2-3 orders) than atomistic modeling simulation. Four main types of interaction sites are considered in the model which are polar (P), nonpolar (N), apolar (C), and charged (Q).[27] Subtypes are either showed as a letter to describe the hydrogen-bond capabilities (d= donor, a= acceptor, da= both, 0= none) or by using a number to denote the degree of polarity (from 1 to 5, the lowest to the highest).[27] The mapping scheme of all protein amino acids is shown as (Fig 1) below.[27]

Figure (1) *Mapping scheme of all protein amino acids.*



The nonbonded interactions for particles interact via Lennard-Jones (LJ) potential:

$$V_{LJ}(r) = 4\varepsilon\left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}\right] \qquad (2)$$

Where the value of well depth ε ranges from 5.6 kJ/mol to 2.0 kJ/mol for which the interactions with strongly polar groups to polar and apolar groups.[27] The σ is the effective size for all normal particle types which is 0.47 nm.[27] For the special class of particles used for ring-like molecules the effective size is 0.43 nm.[27] And r is set to 1.2nm, which is the cut-off distance.[27]

In addition to the LJ interaction, charged groups (type Q) with a charge q_{ij}interact via a Coulombic energy function

$$V_{el} = \frac{q_i q_j}{4\pi\varepsilon_0\varepsilon_{rel} r_{ij}} \qquad (3)$$

The relative dielectric constant ε_{rel}= 15 for explicit screening.[27] The dielectric constant was set as 20 with reduced charges.[29] When dielectric constant at 20, the screening of the electrostatic interaction of the lipid heads groups well reproduce bilayer properties as a test example.[29] Now, the increase hydration strength of ions which in combination with reduced dielectric constant counteracts the effect. If the dielectric constant is too high, then the ions must have a very low charge to compensate for the effect. The cutoff distance $r_{cut}$is set at 1.2 nm.

The level of interactions between the different Coarse-Grained sites is summarized in Table (1) below.[27] Level of interaction indicates the well depth in the Leonard-Jones potential: O, ε = 5.6 kJ/mol; I, ε = 5.0 kJ/mol; II, ε = 4.5 kJ/mol; III, ε = 4.0 kJ/mol; IV, ε = 3.5 kJ/mol; V, ε = 3.1 kJ/mol; VI, ε = 2.7 kJ/mol; VII, ε = 2.3 kJ/mol; VIII, ε = 2.0 kJ/mol; IX, ε = 2.0 kJ/mol; Four different Coarse-Grained sites are considered: charged (Q), polar (P), nonpolar (N), apolar (C).[27] Subscripts are used to describe the chemical

nature where 0: no hydrogen-bonding capabilities; d, groups acting as hydrogen bond donor; a, groups acting as both donor and acceptor. 1-5 indicated the increasing polarity affinity.[27]

Table (1) *Level of interactions between the different Coarse-Grained sites.*

		Q				P					N				C				
	sub	da	d	a	0	5	4	3	2	1	da	d	a	0	5	4	3	2	1
Q	da	O	O	O	II	O	O	O	I	I	I	I	I	IV	V	VI	VII	IX	IX
	d	O	I	O	II	O	O	O	I	I	I	III	I	IV	V	VI	VII	IX	IX
	a	O	O	I	II	O	O	O	I	I	I	I	III	IV	V	VI	VII	IX	IX
	0	II	II	II	IV	I	O	I	II	III	III	III	III	IV	V	VI	VII	IX	IX
P	5	O	O	O	I	O	O	O	O	O	I	I	I	IV	V	VI	VI	VII	VIII
	4	O	O	O	O	O	I	I	II	II	III	III	III	IV	V	VI	VI	VII	VIII
	3	O	O	O	I	O	I	I	II	II	II	II	II	IV	IV	V	V	VI	VII
	2	I	I	I	II	O	II	II	II	II	II	II	II	III	IV	IV	V	VI	VII
	1	I	I	I	III	O	II	II	II	II	II	II	II	III	IV	IV	IV	V	VI
N	da	I	I	I	III	I	III	II	II	II	II	II	II	IV	IV	V	VI	VI	VI
	d	I	III	I	III	I	III	II	II	II	II	III	II	IV	IV	V	VI	VI	VI
	a	I	I	III	III	I	III	II	II	II	II	II	III	IV	IV	V	VI	VI	VI
	0	IV	IV	IV	IV	IV	IV	IV	III	III	IV	IV	IV	IV	IV	IV	IV	V	VI
C	5	V	V	V	V	V	V	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	V	V
	4	VI	VI	VI	VI	VI	VI	V	IV	IV	V	V	V	IV	IV	IV	IV	V	V
	3	VII	VII	VII	VII	VI	VI	V	V	IV	VI	VI	VI	IV	IV	IV	IV	IV	IV
	2	IX	IX	IX	IX	VII	VII	VI	VI	V	VI	VI	VI	V	V	V	IV	IV	IV
	1	IX	IX	IX	IX	VIII	VIII	VII	VII	VI	VI	VI	VI	VI	V	V	IV	IV	IV

The bonded interactions for particles are interacted by the following set of potential functions:[27]

$$V_b = \frac{1}{2}K_b(d - d_b)^2 \qquad (4)$$

$$V_a = \frac{1}{2}K_a[\cos(\varphi) - \cos(\varphi_a)^2] \qquad (5)$$

$$V_d = K_d[1 + cos(n\psi - \psi_d)] \qquad (6)$$

$$V_{id} = K_{id}(\psi - \psi_{id})^2 \qquad (7)$$

Where V_b is bonded potential which is used for chemical bonded sites. K_b=1250 kJ/mol/ nm^{-2} and d_b=0.47 nm.[27] The angle potential V_a represents chain stiffness.[27] K_a=25 kJ/mol with an equilibrium bond angle φ_a=180^o.[27] V_{id} is used to prevent out-of-plane distortions of planar groups.[27] ψ is the angle between the planes constituted between atoms i,j,k and

j,k,l, with equilibrium angle $\psi_{id}$and force constant K_{id}. V_d stands for the proper dihedrals which are used to impose the secondary structure of the peptide backbone.[27] The force constants K are generally weak.[27] In Equation (4) where d stands for the distance between bonded sites and d_b denotes as equilibrium distance.[27] φ, φ_a are the angles and ψ, ψ_d, ψ_{id} are the dihedral angles.[27]

All the twenty amino mapping schemes are shown in Figure (1). Both V6K2 and V6K3 are amphiphiles, where there is a hydrophilic part (lysine) of the peptide and the other is hydrophobic (valine). The VK combination is based on the existing computational result.[31]Valine is one of the most hydrophobic amino acids residues and lysine is one of the most hydrophilic amino acids residue. Hence, this combination can lead to a faster self-assembly process. The strategy of building CG scheme is based on Fig (1). The apolar amino acids (Leu, Pro, Ile, Val, Cys and Met) are represented as C-type particles.[27] The positively charged amino acids Arg and Lys are modeled by a combination of a Q-type particle.[27] The parameterizations of bond length, angles, improper dihedral angels are also included in Table (2)~(6).[27] Simulations were performed by using the GROMACS package (v5.1.4).

Table (2) *Mapping of amino acids*

side chain	CG representation	mapping scheme[a]	free energy (kJ/mol) CG	free energy (kJ/mol) exptl.
Leu	C1[b]		22	22
Ile	C1[b]		22	22
Val	C2[b]		20	17
Pro	C2[b]		20	
Met	C5		9	10
Cys	C5		9	5
Ser	P1		−11	−14
Thr	P1		−11	−11
Asn	P5		< −25	−28
Gln	P4		−23	−25
Asp	Qa		< −25	
Asp (uncharged)	P3		−18	−19
Glu	Qa		< −25	
Glu (uncharged)	P1		−11	−11
Arg	N0—Qd	N0: Cβ—Cγ—Cδ—Nε	< −25	
Arg (uncharged)	N0—P4	Qd/P4: Cζ—Nω1—Nω2	−23	−25
Lys	C3—Qd	C3: Cβ—Cγ—Cδ	< −25	
Lys (uncharged)	C3—P1	Qd/P1: Cε—Nω	−1	−2
His	SC4—SP1—SP1	SC4: Cβ—Cγ SP1: Cδ—Nε SP1: Nδ—Cε	−19	−20
Phe	SC4—SC4—SC4	SC4: Cβ—Cγ—Cδ1 SC4: Cδ2—Cε2 SC4: Cε1—Cζ	19	17
Tyr	SC4—SC4—SP1	SC4: Cβ—Cγ—Cδ1 SC4: Cδ2—Cε2 SP1: Cε1—Cζ—OH	−1	−2
Trp	SC4—SP1—SC4—SC4	SC4: Cβ—Cγ—Cδ2 SP1: Cδ1—Nε—Cε1 SC4: Cε2—Cζ2 SC4: Cε1—Cω	12	9

Table (3) *Parameters for different types of backbone particle*

backbone	coil bend free	helix	helix (N-terminus/C-terminus)	β-strand turn
backbone	P5	N0	Nd/Na	Nda
Gly	P5	N0	Nd/Na	Nda
Ala	P4	C5	N0	N0
Pro	Na	C5	N0/Na	N0

Table (4) *Parameters for backbond bonded*

backbone	d_{BB} (nm)	K_{BB} (kJ nm^{-2} mol^{-1})	θ_{BBB} (deg)	K_{BBB} (kJ mol^{-1})	ψ_{BBBB} (deg)	K_{BBBB} (kJ mol^{-1})
helix	0.35	1250	96*	700	60	400
coil	0.35	200	127	25		
extended	0.35	1250	134	25	180	10
turn	0.35	500	100	25		
bend	0.35	400	130	25		

Table (5) *Equilibrium bond length and force constants for each amino acid side chain*

side chain	d (nm)	K (kJ nm^{-2} mol^{-1})
Leu	0.33	7500
Ile	0.31	constraint
Val	0.265	constraint
Pro	0.30	7500
Met	0.40	2500
Cys	0.31	7500
Ser	0.25	7500
Thr	0.26	constraint
Asn	0.32	5000
Gln	0.4	5000
Asp	0.32	7500
Glu	0.4	5000
Arg d_{BS}	0.33	5000
Arg d_{SS}	0.34	5000
Lys d_{BS}	0.33	5000
Lys d_{SS}	0.28	5000
His d_{BS}	0.32	7500
His d_{SS}	0.27	constraint
Phe d_{BS}	0.31	7500
Phe d_{SS}	0.27	constraint
Tyr d_{BS}	0.32	5000
Tyr d_{SS}	0.27	constraint
Trp d_{BS}	0.3	5000
Trp d_{SS}	0.27	constraint
Cys–Cys d_{S-S}	0.39	5000

Table (6) *Equilibrium angles, improper dihedral angles and force constants for side chains*

side chain	θ (deg)	K (kJ mol^{-1})
θ_{BSS} (all)	100	25
θ_{BSS} (Lys, Arg)	180	25
θ_{BSS} (His, Tyr, Phe)	150	50
θ_{BSS} (Trp)	90, 210	50, 50
side chain	**ψ (deg)**	**K (kJ rad^{-2} mol^{-1})**
ψ_{SSSS} (His, Tyr, Phe)	0	50
ψ_{SSSS} (Trp)	0, 0	50, 200

For the polarizable water model, the Martini v2.2p coarse-grained (CG) force field is used for the polarizable water model system.[31-32] Both of the models (V_6K_2, V_6K_3) do not change when the polarizable water is used. Figure (2-a) shows the model of $V_6K_2$and (2-b) shows V_6K_3. Based on Martini mapping scheme Fig (1), each valine is represented by two coarse-grained beads and lysine is represented by three coarse-grained beads. Hence, for V_6K_2, there are 18 beads and 21 beads for V_6K_3. Fig (3-a) shows the chemical structure of valine and (3-b) shows the chemical structure of lysine.[33-34]

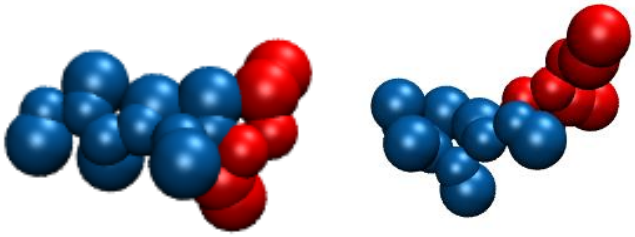


Fig 2-a V_6K_2 ; ***Fig 2-b*** V_6K_3

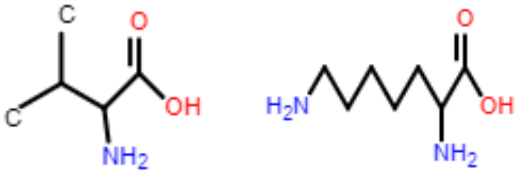


Fig 3-a Valine ; ***Fig 3-b Lysine***

2.2 Self-assembly of V_6K_2 and V_6K_3

To investigate the products of V_6K_2 and V_6K_3 peptides via the self-assembly process, we need to pick up a suitable box size and a number of peptides to achieve the critical-micelle-concentration (CMC).[35] For V_6K_2, we use the $(11\ \text{nm})^3$ box and with 200 V_6K_2 peptides for our simulation. The CMC for V_6K_2 is 0.33mM,[35] however, the system we used has been much higher concentration than the given value to accelerate the self-assembly process and decrease the cost and time spent in simulation.

2.3 Modeling and parametrization of the system (polarizable)

For the polarizable water model, there are 200 capped V_6K_2 peptides and 11300 water molecules. Since V_6K_2 has two charged groups (Lys), the counterions are needed. In this case, it needs 400 counterions (CL-) to neutralize the system. The system is then energy minimized by using a steep descent integrator to remove inappropriate geometry (overlap) between the particles. Equilibration is performed for 1 ns after energy minimization where the timestep is 10 fs. The production simulation is performed after the system is well-equilibrated and ran for 8 μs with a timestep of 8 fs.

The simulation is carried out in NPT ensemble (isothermal-isobaric). The temperature is maintained at 310K using velocity rescaling and the barostat is Berendsen.[36]

The constraint algorithm is LINCS. Figure (4-a) and (4-b) show the model of V_6K_2using polarizable water model.

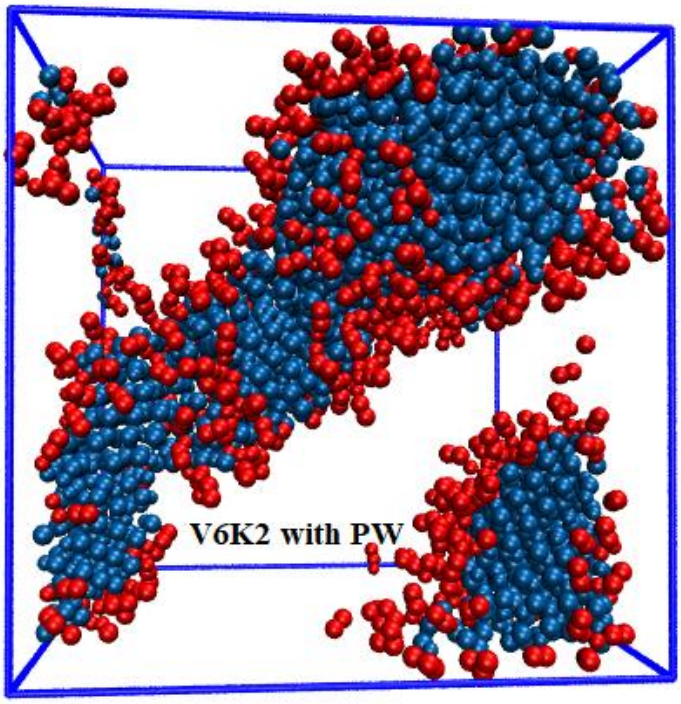


Fig 4-a *200 V_6K_2peptides with polarizable water model (front)*

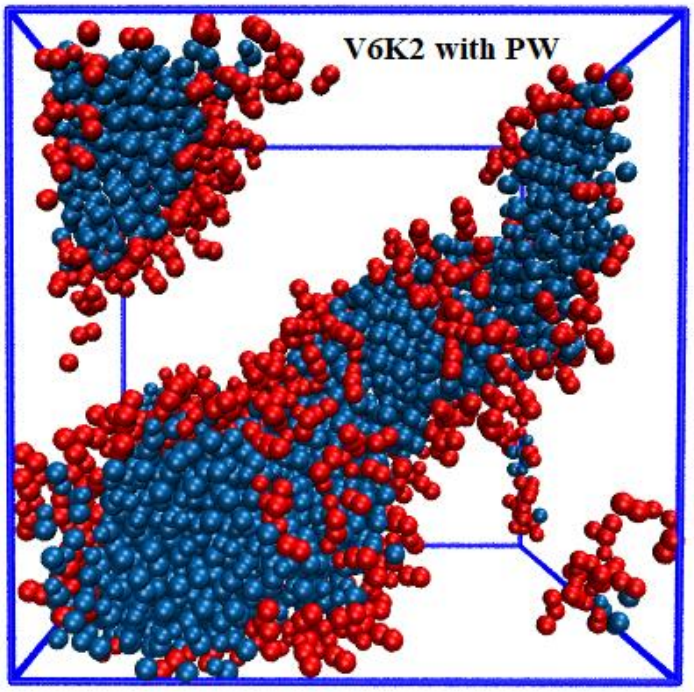


Fig 4-b *200 V_6K_2peptides with polarizable water model (back)*

2.3-1 Modeling and parametrization of the system (Non-polarizable)

For the non-polarizable water model, there are 200 capped V_6K_2 peptides and 9106 water molecules. For this water model, the antifreeze particles should be added into the system to prevent the freezing phenomena in our system.[29] The anti-freeze particle is called Big P_4, which it disrupts the lattice packing of the normal P_4 water beads. The Lenard Jones parameter σ of Big P_4 is 0.57 nm.[29] Since V_6K_2 has two charged groups (Lys), the counterions are needed. In this case, it needs 400 counterions (CL-) to neutralize the system. The ion particles are needed based on system dependent. There are several types of counterions such as sodium ion, chloride ion and choline ion. The ions in Martini Coarse Grained model are represented by Q type particles. However, the sodium ion is represented by Qd, chloride ion is represented by Qa and choline ion is represented by Q0 based on Table (1). In Martini Coarse Grained mapping scheme all beads have the same size.

The system is then energy minimized by using a steep descent integrator to remove inappropriate geometry (overlap) between the particles. Equilibration is performed for 1 ns after energy minimization where the timestep is 10 fs. The production simulation is performed after the system is well-equilibrated and ran for 8 μs with a timestep of 10 fs.

The simulation is carried out in NPT ensemble (isothermal-isobaric). The temperature is maintained at 310K. The barostat is Parrinello-Rahman.[37-39]Figure (5-a) and (5-b) show the model of V_6K_2using non-polarizable water model.

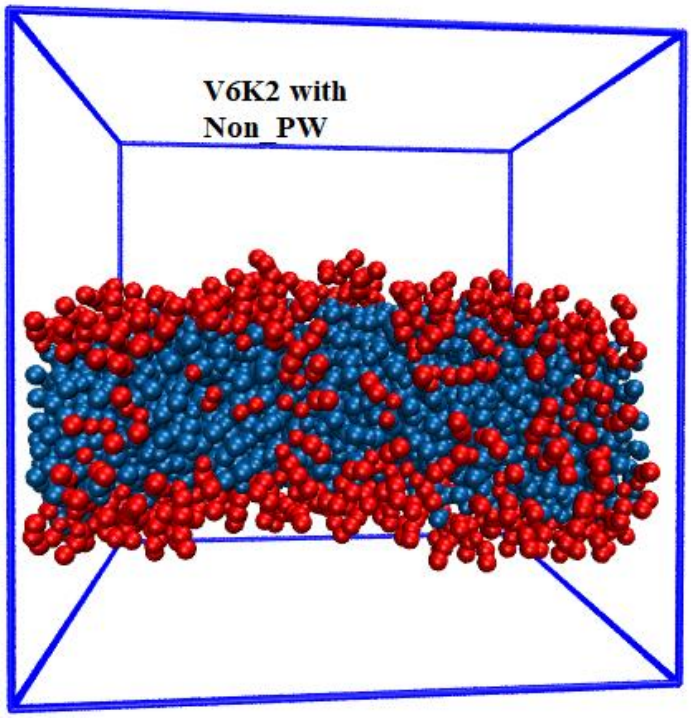


Fig 5-a *200 V_6K_2peptides with non-polarizable water model*

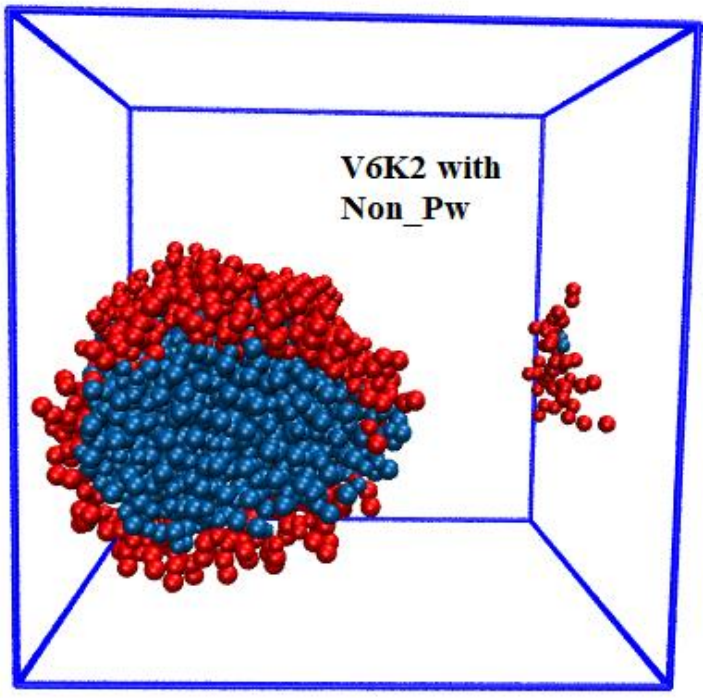


Fig 5-b *200 V_6K_2peptides with non-polarizable water model*

The non-polarizable water model clearly shows that there is only one solid-cored rod-like fibril.[40-44] Baumann et al[40-42] have revealed the secondary structure of V_6K_2 by using circular dichroism spectroscopy and dimensions of the rod-like fibril which includes: radius, width and length by using AFM and TEM. Figure (6-c) and (6-d) shows the experimental results of V_6K_2peptides. Table (7) provides the dimension of the nanofibrils. Our simulation results are validated along with experimental results. We use Visual Molecular Dynamics (VMD) to calculate those criteria (radius, height and width). Each coarse-grained bead is located with a position (x,y,z) in the hypothetical coordinate system in VMD. By applying the distance formula, we can obtain the radius, height and width of the nanofibril structure. Calculating the magnitude of the length of our simulation result is not under consideration because the periodic imagine will repeat itself.

Fig 6-c,6-d *experimental results for V_6K_2peptides*

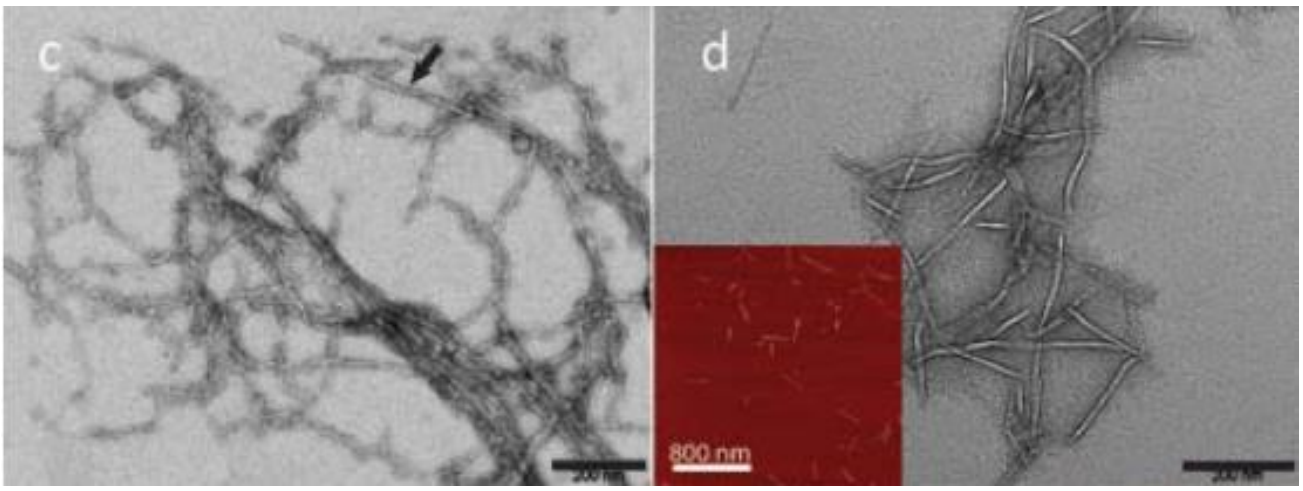


Table (7) *Dimensions of V_6K_2peptides formed nanofibrils*

peptide amphiphile	measured width (nm)	measured height (nm)	bulk radius (nm)
V6K2	13	2.2	3.0

The reproducibility of the non-polarizable water model is much better than the polarizable water model. Nine of the ten independent seeds simulations show that the rod-like fibril can be found in the non-polarizable water model. Also, the non-polarizable water model is much cheaper than the polarizable water model in simulation cost. Hence, the non-polarizable water model is the better choice than the polarizable water model in this project. The secondary structure of the peptide has two versions: beta-sheet and coil. We choose to use the beta-sheet version because the coil version of the peptide doesn't show any favorable result in this work.[44]

Chapter 3 Modeling Mixture Systems

3.1 Introduction for V_6K_2 And V_6K_3 Mixture Systems

As mentioned in the previous chapter, we use the non-polarizable water model for further investigation due to the reproducibility and computational cost. In this chapter, we will investigate the V_6K_2 and V_6K_3 peptides with the non-polarizable water model in the selected box size for the mixture systems.

3.1.1 Modeling Mixture Systems for V_6K_2 And V_6K_3 in One Total Peptide Concentration

The MARTINI Coarse Grained Forcefield (v2.2) is used to study the mixture systems. We use only one total peptide concentration that we are investigated in this chapter. Firstly, we scale up a system that has the same peptide concentration in analogy to the $(11\ \mathrm{nm})^3$ cubic box system with 200 V_6K_2 peptides ($0.150\ peptides/nm^3$). We examined several box sizes with a range from $(15\ \mathrm{nm})^3$ to $(17\ \mathrm{nm})^3$ with three independents particle trajectories of each box size.

In $(15\ \mathrm{nm})^3$box, for example, we firstly insert 520 capped at C terminus with and N terminus of V_6K_2 peptides into the box and then the system is solvated with 25248 water molecules. For this water model, 2681 antifreeze particles should be added into the system to prevent the freezing phenomena in our system.[27] SinceV_6K_2 has two charged groups

(Lys), 1040 counterions (CL-) are needed to neutralize the system. The system is then energy minimized by using a steep descent integrator to remove inappropriate geometry (overlap) between the particles. Equilibration is performed for 1 ns after energy minimization where the timestep is 10 fs. The production simulation is performed after the system is well-equilibrated and ran for 8 μs with a timestep of 8 fs. The simulation is carried out in NPT ensemble (isothermal-isobaric). The temperature is maintained at 310K. The barostat is Parrinello-Rahman.[37-39]

In $(16\ \mathrm{nm})^3$box, for example, we firstly insert 650 capped at C terminus with and N terminus of V_6K_2 peptides into the box and then the system is solvated with 30468 water molecules. For this water model, 3242 antifreeze particles should be added into the system to prevent the freezing phenomena in our system.[27] SinceV_6K_2 has two charged groups (Lys), 1300 counterions (CL-) are needed to neutralize the system. The system is then energy minimized by using a steep descent integrator to remove inappropriate geometry (overlap) between the particles. Equilibration is performed for 1 ns after energy minimization where the timestep is 10 fs. The production simulation is performed after the system is well-equilibrated and ran for 8 μs with a timestep of 8 fs. The simulation is carried out in NPT ensemble (isothermal-isobaric). The temperature is maintained at 310K. The barostat is Parrinello-Rahman.[37-39]

In $(16.5\ \mathrm{nm})^3$box, for example, we firstly insert 650 capped at C terminus with and N terminus of V_6K_2 peptides into the box and then solvated with 33941 water molecules. For this water model, 3589 antifreeze particles should be added into the system to prevent the freezing phenomena in our system.[27] SinceV_6K_2 has two charged groups (Lys), 1300

counterions (CL-) are needed to neutralize the system. The system is then energy minimized by using a steep descent integrator to remove inappropriate geometry (overlap) between the particles. Equilibration is performed for 1 ns after energy minimization where the timestep is 10 fs. The production simulation is performed after the system is well-equilibrated and ran for 8 μs with a timestep of 8 fs. The simulation is carried out in NPT ensemble (isothermal-isobaric). The temperature is maintained at 310K. The barostat is Parrinello-Rahman.[37-39]

In $(17\ \mathrm{nm})^3$box, for example, we firstly insert 650 capped at C terminus with and N terminus of V_6K_2 peptides into the box and then solvated with 37132 water molecules. For this water model, 3908 antifreeze particles should be added into the system to prevent the freezing phenomena in our system.[27] SinceV_6K_2 has two charged groups (Lys), 1300 counterions (CL-) are needed to neutralize the system. The system is then energy minimized by using a steep descent integrator to remove inappropriate geometry (overlap) between the particles Equilibration is performed for 1 ns after energy minimization where the timestep is 10 fs. The production simulation is performed after the system is well-equilibrated and ran for 8 μs with a timestep of 8 fs. The simulation is carried out in NPT ensemble (isothermal-isobaric). The temperature is maintained at 310K. The barostat is Parrinello-Rahman.[37-39]

To prevent periodic artifacts, the box size should be large enough for both $V_6K_2$and V_6K_3peptides in the mixture system. If the box size is too small, then the structure itself may interact with its image in a neighboring box. Therefore, the $(17\ \mathrm{nm})^3$box with 650 V_6K_2 peptides (0.132 peptides/ nm^3) system represents the most favorable result (rod-like

fibril structures) as shown in the figure below. We will build up 11 relative peptide concentrations (ranging from 0% to 100%) systems based upon this box size and total peptide concentration for further investigation and analysis.

The number of peptides, water molecules and anti-freeze particles that needed for each system are represented as a summary in Table (8) below. Figure (7-a) and (7-b) show the the nanofibril structure in the $(17\ \mathrm{nm})^3$ system.

Table (8) *Summaries of different scale-up V_6K_2peptide systems.*

Size of the System (nm^3)	**15**	**16**	**16.5**	**17**
Numbers of Peptide	520	650	650	650
Peptide Concentration	0.1540	0.1586	0.1447	0.1323
Water Molecules (W)	25248	30468	33941	37132
Anti-Freeze Particles (WF)	2681	3242	3589	3908
Counterions (CL-)	1040	1300	1300	1300

Figure (7-a) (17 nm)^3*with 650* V_6K_2*peptides system.*

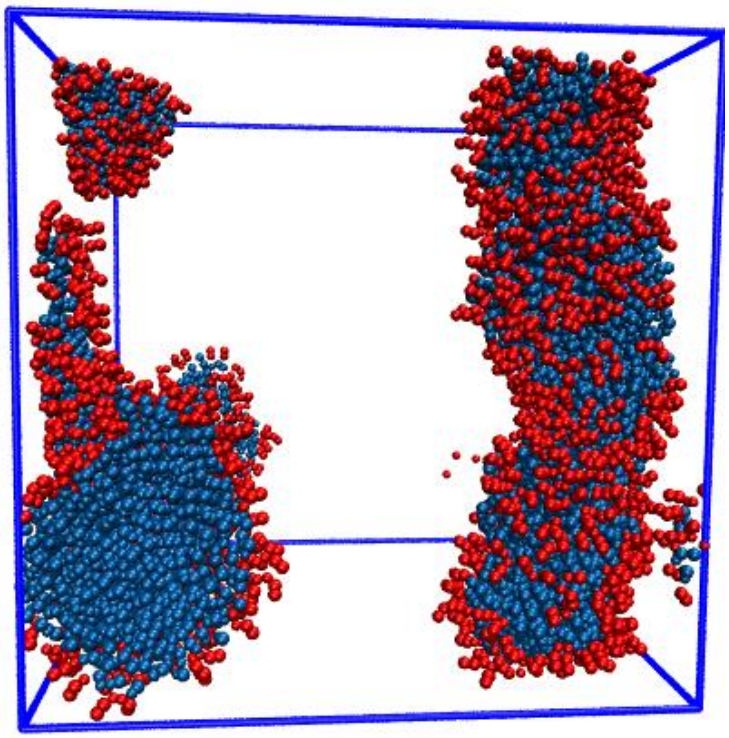
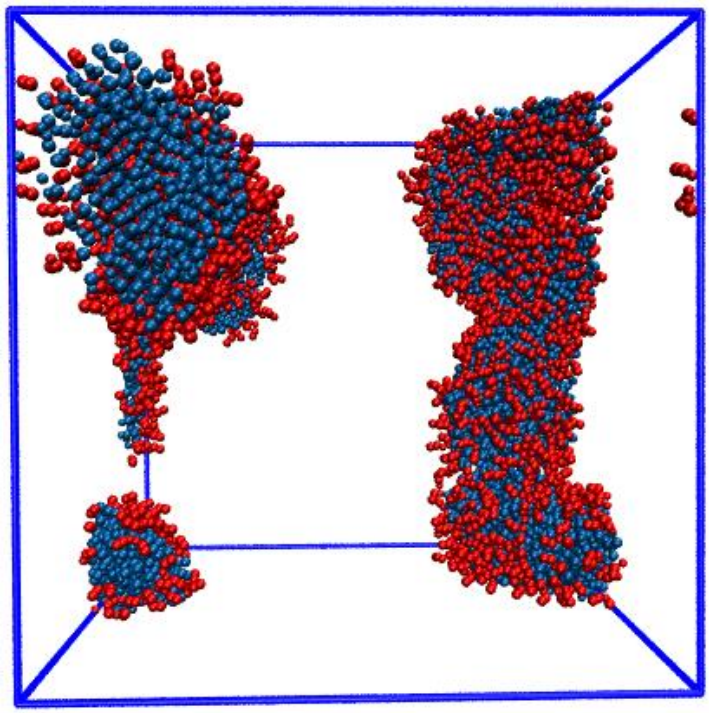


Figure (7-b) (17 nm)^3*cubic with 650* V_6K_2*peptides system.*



3.1.2 Introduction for V_6K_2 And V_6K_3 Mixture System in Relative Peptide Concentration Composition

Based upon what we discussed in the previous section, we will use this one total peptide concentration ($(17\ \text{nm})^3$ box with 650 V_6K_2 peptides system) to build up 11 relative peptide concentrations mixture systems. The composition of the relative peptide concentrations mixture systems which are ranging from 0% to 100%. Ten independent particle trajectories are performed for each of the mixture systems, therefore, 110 simulations are studied in the later chapter.

3.1.3 Modeling Each Mixture System for V_6K_2 And V_6K_3 In Relative Peptide Concentration Composition

The MARTINI Coarse Grained Forcefield (v2.2) is used to study all the mixture systems in relative peptide concentration composition. For pure V_6K_2 system, which means the system only has one composition ($(17\ \text{nm})^3$with 650 V_6K_2 peptides system). On the other hand, the pure V_6K_3 system which implies there is no any V_6K_2 peptide in the system (0% V_6K_2).

The methodology to build up each of the mixture systems is the same but based on different compositions of $V_6K_2$and V_6K_3, the number of counter ions (CL-) will be different since $V_6K_2$and V_6K_3 have the different numbers of lysine.

First, we will discuss how we build up the 10% V_6K_2 system. In the $(17\ \text{nm})^3$ box, we firstly insert 65 capped at C terminus and N terminus of V_6K_2 peptides and then insert 585 capped at C terminus and N terminus of V_6K_3 peptides into the box. This system is then solvated with 36033 water molecules. Moreover, 1885 counter ions (CL-) are needed to neutralize the system. Since we are using the non-polarizable water model, 3857 anti-freeze particles are needed to prevent freezing phenomena.[27] The system is then energy minimized by using a steep descent integrator to remove inappropriate geometry (overlap) between the particles. Equilibration is performed for 1 ns after energy minimization where the timestep is 10 fs. The production simulation is performed after the system is well-equilibrated and ran for 8 μs with a timestep of 8 fs. The simulation is carried out in NPT

ensemble (isothermal-isobaric). The temperature is maintained at 310K. The barostat is Parrinello-Rahman.[37-39]

Second, we will talk about how we build up the 20% V_6K_2 system. In the $(17\ \text{nm})^3$cubic box, we firstly insert 130 capped at C terminus and N terminus of V_6K_2 peptides and then insert 520 capped at C terminus and N terminus of V_6K_3 peptides into the box. This system is then solvated with 36271 water molecules. Moreover, 1820 counter ions (CL-) are needed to neutralize the system. Since we are using the non-polarizable water model, 3874 anti-freeze particles are needed to prevent freezing phenomena.[27] The system is then energy minimized by using a steep descent integrator to remove inappropriate geometry (overlap) between the particles. Equilibration is performed for 1 ns after energy minimized with the timestep is 10 fs. The production simulation is performed after the system is well-equilibrated and ran for 8 μs with a timestep of 8 fs. The simulation is carried out in NPT ensemble (isothermal-isobaric). The temperature is maintained at 310K. The barostat is Parrinello-Rahman.[37-39]

Third, the way to set up the 30% V_6K_2 system is that we firstly insert 195 capped at C terminus and N terminus of V_6K_2 peptides into the $(17\ \text{nm})^3$ box and then insert 455 capped at C terminus and N terminus of V_6K_3 peptides into the system. This system is then solvated with 36279 water molecules. Moreover, 1755 counter ions (CL-) are needed to neutralize the system. The non-polarizable water model is used therefore 3868 anti-freeze particles are needed in this system to prevent freezing phenomena.[27] The system is then energy minimized by using a steep descent integrator to remove inappropriate geometry

(overlap) between the particles. Equilibration is performed for 1 ns after energy minimization where the timestep is 10 fs. The production simulation is performed after the system is well-equilibrated and ran for 8 μs with a timestep of 8 fs. The simulation is carried out in NPT ensemble (isothermal-isobaric). The temperature is maintained at 310K. The barostat is Parrinello-Rahman.[37-39]

Fourth, for the 40% V_6K_2 system, we firstly insert 260 capped at C terminus and N terminus of V_6K_2 peptides into the $(17\ \text{nm})^3$box and then insert 390 capped at C terminus and N terminus of V_6K_3 peptides into the system. This system requires 36496 water molecules to solvate the system. Moreover, 1690 counter ions (CL-) are needed to neutralize the system. To prevent freezing phenomena[27], 3884 anti-freeze particles are needed. The system is then energy minimized by using a steep descent integrator to remove inappropriate geometry (overlap) between the particles. Equilibration is performed for 1 ns after energy minimization where the timestep is 10 fs. The production simulation is performed after the system is well-equilibrated and ran for 8 μs with a timestep of 8 fs. The simulation is carried out in NPT ensemble (isothermal-isobaric). The temperature is maintained at 310K. The barostat is Parrinello-Rahman.[37-39]

Fifth, for 50% V_6K_2 system, we firstly insert 325 capped at C terminus and N terminus of V_6K_2 peptides into the $(17\ \text{nm})^3$box and then we insert 325 capped at C terminus and N terminus of V_6K_3 peptides into the system. The system is solvated with 36525 water molecules. This system requires 1625 counter ions (CL-) to neutralize the system. To prevent freezing phenomena[27], 3880 anti-freeze particles are added into the system. The

system then energy minimized by using a steep descent integrator to remove inappropriate geometry (overlap) between the particles. Equilibration is performed for 1 ns after energy minimization where the timestep is 10 fs. The production simulation is performed after the system was well-equilibrated and ran for 8 μs with a timestep of 8 fs. The simulation is carried out in NPT ensemble (isothermal-isobaric). The temperature is maintained at 310K. The barostat is Parrinello-Rahman.[37-39]

Sixth, for 60% V_6K_2 system, 390 capped at C terminus and N terminus of V_6K_2 peptides are firstly inserted into the $(17\ \text{nm})^3$box and then we insert 260 capped at C terminus and N terminus of V_6K_3 peptides into the system. In this system, we will need 36765 water molecules to solvate the system and the system also needs 1560 counter ions (CL-) to neutralize the positive charge that provided from lysine. To prevent freezing phenomena in the system[27], 3898 anti-freeze particles are added into the system. The system is then energy minimized by using a steep descent integrator to remove inappropriate geometry (overlap) between the particles. Equilibration is performed for 1 ns after energy minimization where the timestep is 10 fs. The production simulation is performed after the system was well-equilibrated and ran for 8 μs with a timestep of 8 fs. The simulation is carried out in NPT ensemble (isothermal-isobaric). The temperature is maintained at 310K. The barostat is Parrinello-Rahman.[37-39]

Seventh, for 70 % V_6K_2 system, we firstly insert 455 capped at C terminus and N terminus of V_6K_2peptides into the $(17\ \text{nm})^3$box and then we insert 195 V_6K_3peptides that

capped at C terminus and N terminus of into the system. The system is solvated with 36781 water molecules. It requires 1495 counter ions (CL-) to neutralize the system. To prevent freezing phenomena in the system[27], 3893 anti-freeze particles are needed to add to the system. The system is then energy minimized by using a steep descent integrator to remove inappropriate geometry (overlap) between the particles. Equilibration is performed for 1 ns after energy minimization where the timestep is 10 fs. The production simulation is performed after the system is well-equilibrated and ran for 8 μs with a timestep of 8 fs. The simulation is carried out in NPT ensemble (isothermal-isobaric). The temperature is maintained at 310K. The barostat is Parrinello-Rahman.[37-39]

Eighth, for 80% V_6K_2 system, 520 capped at C terminus and N terminus of V_6K_2 peptides are inserted into the $(17\ \text{nm})^3$ box and then we insert 130 capped at C terminus and N terminus of V_6K_3 peptides into the system. The system is then solvated with 36973 water molecules. In this system, we will need 1430 counter ions (CL-) to neutralize the system. To prevent the freezing phenomena, 3905 anti-freeze particles are added into the system.[27] The system is then energy minimized by using a steep descent integrator to remove inappropriate geometry (overlap) between the particles. Equilibration is performed for 1 ns after energy minimization where the timestep is 10 fs. The production simulation is performed after the system is well-equilibrated and ran for 8 μs with a timestep of 8 fs. The simulation is carried out in NPT ensemble (isothermal-isobaric). The temperature is maintained at 310K. The barostat is Parrinello-Rahman.[37-39]

Ninth, for 90% V_6K_2 system, 585 capped at C terminus and N terminus of V_6K_2 peptides are inserted into the $(17\ \mathrm{nm})^3$ box and then we insert 65 capped at C terminus and N terminus of V_6K_3 peptides into the system. The system is then solvated with 37058 water molecules. In this system, we will need 1365 counter ions (CL-) to neutralize the system. To prevent the freezing phenomena, 3907 anti-freeze particles are added into the system.[27] The system is then energy minimized by using a steep descent integrator to remove inappropriate geometry (overlap) between the particles. Equilibration is performed for 1 ns after energy minimization where the timestep is 10 fs. The production simulation is performed after the system is well-equilibrated and ran for 8 μs with a timestep of 8 fs. The simulation is carried out in NPT ensemble (isothermal-isobaric). The temperature is maintained at 310K. The barostat is Parrinello-Rahman.[37-39]

Tenth, for 100% V_6K_2 system, 650 capped at C terminus and N terminus of V_6K_2 peptides are inserted into the $(17\ \mathrm{nm})^3$ box. The system is then solvated with 37132 water molecules. In this system, we will need 1300 counter ions (CL-) to neutralize the system. To prevent the freezing phenomena, 3908 anti-freeze particles are added into the system.[27] The system is then energy minimized by using a steep descent integrator to remove inappropriate geometry (overlap) between the particles. Equilibration is performed for 1 ns after energy minimization where the timestep is 10 fs. The production simulation is performed after the system is well-equilibrated and ran for 8 μs with a timestep of 8 fs. The simulation is carried out in NPT ensemble (isothermal-isobaric). The temperature is maintained at 310K. The barostat is Parrinello-Rahman.[37-39]

Eleventh, for 0% V_6K_2 system (pure V_6K_3), 650 capped at C terminus and N terminus of V_6K_3 peptides are inserted into the $(17\ \text{nm})^3$ box. The system is then solvated with 35942 water molecules. In this system, we will need 1950 counter ions (CL-) to neutralize the system. To prevent the freezing phenomena, 3854 anti-freeze particles are added into the system.[27] The system is then energy minimized by using a steep descent integrator to remove inappropriate geometry (overlap) between the particles. Equilibration is performed for 1 ns after energy minimization where the timestep is 10 fs. The production simulation is performed after the system is well-equilibrated and ran for 8 μs with a timestep of 8 fs. The simulation is carried out in NPT ensemble (isothermal-isobaric). The temperature is maintained at 310K. The barostat is Parrinello-Rahman.[37-39]

The number of peptides, water molecules and anti-freeze particles that needed for each system are represented as a summary table in the Table (9) below.

Table (9) *Summaries of different V_6K_2peptide mixture systems with one total peptide concentration.*

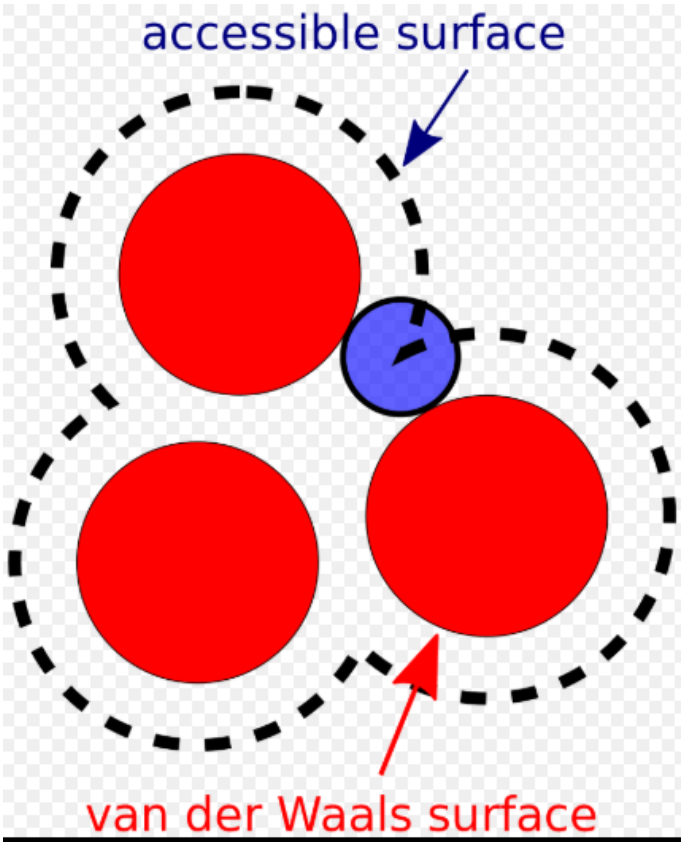
Composition Of V_6K_2	**Number of V_6K_2Peptides**	**Number of V_6K_3Peptides**	**Number of Water Molecules**	**Number of Anti-Freeze Particles**	**Number of Counter ions (CL-)**
100%V_6K_2	650	0	37132	3908	1300
90%V_6K_2	585	65	37058	3907	1365
80%V_6K_2	520	130	36973	3905	1430
70%V_6K_2	455	195	36781	3893	1495
60%V_6K_2	390	260	36765	3898	1560
50%V_6K_2	325	325	36525	3880	1625
40%V_6K_2	260	390	36496	3884	1690
30%V_6K_2	195	455	36279	3868	1755
20%V_6K_2	130	520	36271	3874	1820
10%V_6K_2	65	585	36033	3857	1885
0%V_6K_2	0	650	35942	3854	1950

Chapter 4 Results and Discussion

4.1 Introduction of Solvent Accessible Surface Area For V_6K_2 And V_6K_3 Mixture Systems

Solvent Accessible Surface Area (SASA) means the surface area of a biomolecule that can be accessible by the solvent molecule. The mechanism of the surface accessible surface area is that the orbit of the center of the solvent molecule rolls over the van der Waals surface of a biomolecule.[46] The analysis tool of solvent accessible surface area for each of the mixture system is based on using the GROMACS v5.1.4 package. The Figure (8) below shows the mechanism of solvent accessible surface area.[47]

Figure (8) *Mechanism of solvent accessible surface area.*



4.1.1 The Solvent Accessible Surface Area Analysis For V_6K_2 And V_6K_3 Mixture Systems

For analysis of the solvent accessible surface area, we will need to use the files that have been performed after 8 μs simulation. The input files which are used for this analysis are trajectory files, the final configuration file and the index files for the peptide group of the system. We will study the last 400 ns trajectories of the peptide system to analyze all the peptide mixture systems since at that time range the structures of the peptide are stable. The analysis of each group with 10 independent trajectories are performed. Hence, the average and standard deviation will also be calculated and included in the discussion which shows in the Table (10) and Figure (9) below. Based on the Table (10) and Figure (9), the surface accessible surface area increased as the composition of V_6K_3 increased since V_6K_3peptide has a larger head group than the V_6K_2 peptide. Hence, the surface accessible surface area will increase. Figure (9) and Table (10) below shows the summary of all mixture systems. In Figure (9), the error bars which parallel to the y-axis on the solvent accessible surface area bars show the variation of the solvent accessible surface area of each mixture system.

Figure (9) *Summaries of the solvent accessible surface area of each peptide system.*

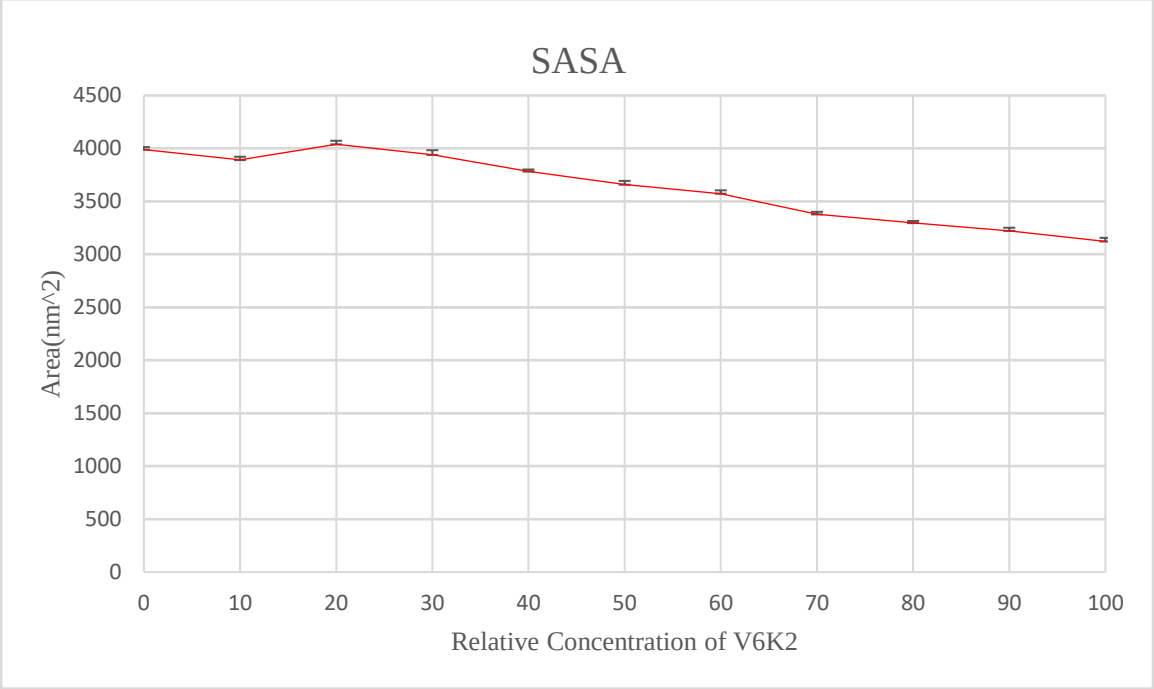


Table (10) *Summaries of the solvent accessible surface area of each peptide system.*

Relative concentration of V_6K_2	**Solvent Accessible Surface Area (nm^2)**
100%V_6K_2	3122.016
90%V_6K_2	3222.201
80%V_6K_2	3295.868
70%V_6K_2	3377.916
60%V_6K_2	3573.371
50%V_6K_2	3658.359
40%V_6K_2	3782.326
30%V_6K_2	3939.299
20%V_6K_2	4038.834
10%V_6K_2	3890.787
0%V_6K_2	3987.983

4.2 Introduction of Line Tension For V_6K_2 And V_6K_3 Mixture Systems

Line tension was first described by J.W.Gibbs in his well-known work “On the Equilibrium of Heterogeneous Substances”. Line tension is defined as the three-phase contact along a line and it may have a negative value.[48] On the other hand, surface tension is defined as the two-phase contact.[48] We will use line tension to investigate the phase separation of the peptide mixture systems.

4.2.1 Line Tension Calculation For V_6K_2 And V_6K_3 Mixture Systems

To estimate the line tension, we might consider there are two different types of molecules (A, B) in the domain boundary and it can be described by Equation (8) (9) where λ is line tension, l is the characteristic length of the molecule.[48] ΔU is defined as the difference in interaction energies between pair interactions of molecule AA, BB and AB.[48] Based on the definition of the line tension,[46] the investigations of the line tension for the pure $V_6K_2$and pure V_6K_3do not provide the physical meaning.

$$\lambda \cong \Delta U/l \qquad (8)$$

$$\Delta U = [\frac{1}{2}(U_{AA+}U_{BB}) - U_{AB}] \qquad (9)$$

To obtain the characteristic length of the molecule, we need to combine the results from the previous section where we discuss the solvent accessible surface area. Firstly, we assume all the peptides in the system are at the surface of the supramolecular structure. Since all the peptides are all at the surface of the structure, we use the solvent accessible surface area that divided by the total number of the peptides to know how much area is occupied by each of the peptide molecule. Therefore, we can obtain the characteristic length. We can use the 90% V_6K_2 and 10% V_6K_3 system as an example.

The solvent accessible surface area for 90% V_6K_2 and 10% V_6K_3system is 3222.201 nm^2. There are 585 V_6K_2 and 65 V_6K_3peptides in the system. Hence, we can do the characteristic length calculation as the followings,

$$\frac{3222.201(nm^2)}{650} = \frac{1}{4}\pi l^2$$

l which is the characteristic length can be obtained as 2.512 nm for 90% V_6K_2 system. Table (11) summarized the characteristic length of each of the mixture system.

Table (11) *Summaries the characteristic length of each peptide system.*

Mixture Systems	Characteristic length (nm)
100%V_6K_2	2.472
90%V_6K_2	2.512
80%V_6K_2	2.540
70%V_6K_2	2.572
60%V_6K_2	2.652
50%V_6K_2	2.682
40%V_6K_2	2.721
30%V_6K_2	2.777
20%V_6K_2	2.812
10%V_6K_2	2.760

Since we have the characteristic length of each mixture system, we will need the interaction energy of pair molecules AA, BB and AB. The interaction energy is obtained by using the GROMACS v5.1.4 package.

In the mixture system, there are several different types of molecules such as V_6K_2peptides, V_6K_3peptides, water molecules, anti-freeze particles and counter ions. In the

line tension calculation, V_6K_2 peptide is represented by A molecule and V_6K_3peptide is represented by B molecule.

For the 90%V_6K_2 system, when we calculate the pair interaction energy of group A (V_6K_2 peptides), we firstly should exclude the rest of the molecules (V_6K_3 peptides, counterions, water molecules and anti-freeze particles) which we will need index file of the group A, final configuration file and the trajectory file of the system. After we obtain the system with only group A (V_6K_2 peptides), we can use this system to create an energy file to obtain the interaction energy U_{AA} by using GROMACS v5.1.4. We use the same methodology to calculate the other group B (V_6K_3 peptides). Firstly, we create an index file of the group B and we also need the final configuration file and the final trajectory file of the system. Then, we can use this system which only contains group B to create an energy file and obtain the interaction energy U_{BB} by using GROMACS v5.1.4.

To calculate U_{AB}, we will follow the equation below where U_T means the total interaction energy of group A (V_6K_2 peptides), group B (V_6K_3 peptides) and the interaction energy between group A (V_6K_2 peptides) and group B (V_6K_3 peptides).

$$U_T = U_{AA} + U_{BB} + U_{AB} \qquad (10)$$

To calculate U_T, we will need to create an index file that contains group A (V_6K_2 peptides) and group B (V_6K_3 peptides). We will also need the final configuration file and final trajectory file of the system. Then, we can use this system which contains group A (V_6K_3 peptides) and group B (V_6K_3 peptides) to create an energy file to obtain the

interaction energy U_T by using GROMACS v5.1.4. By subtracting the pair interaction energy $U_{AA}$and $U_{BB}$with U_T, we will obtain U_{AB} for our line tension calculation. And we will use the same method to calculate the rest of the mixture system.

For the 80%V_6K_2 system, to calculate the pair interaction energy of group A (V_6K_2 peptides), we firstly exclude the rest of the molecules (V_6K_3 peptides, counterions, water molecules and anti-freeze particles) in the system which we will need index file of the group A, final configuration file and the trajectory file of the system. After we obtain the system with only group A (V_6K_2 peptides), we can use this system to create an energy file to obtain the interaction energy U_{AA} by using GROMACS v5.1.4. We use the same methodology to calculate the other group B (V_6K_3 peptides). Firstly, we create an index file of the group B and we also need the final configuration file and the final trajectory file of the system. Then, we can use this system which only contains group B to create an energy file and obtain the interaction energy U_{BB} by using GROMACS v5.1.4.

To calculate U_{AB}, we will follow the Equation (10). To obtain U_T, we will need to create an index file that contains group A (V_6K_2 peptides) and group B (V_6K_3 peptides). We will also need the final configuration file and final trajectory file of the system. Then, we can use this system which contains group A (V_6K_3 peptides) and group B (V_6K_3 peptides) to create an energy file to obtain the interaction energy U_T by using GROMACS v5.1.4. By subtracting the pair interaction energy $U_{AA}$and $U_{BB}$with U_T, we will obtain U_{AB} for our line tension calculation of this system.

For the 70%V_6K_2 system, to calculate the pair interaction energy of group A (V_6K_2 peptides), we firstly exclude the rest of the molecules (V_6K_3 peptides, counterions, water molecules and anti-freeze particles) in the system which we will need index file of the group A, final configuration file and the trajectory file of the system. After we obtain the system with only group A (V_6K_2 peptides), we can use this system to create an energy file to obtain the interaction energy U_{AA} by using GROMACS v5.1.4. We use the same methodology to calculate the other group B (V_6K_3 peptides). Firstly, we create an index file of the group B and we will also need the final configuration file and the final trajectory file of the system. Then, we can use this system which only contains group B to create an energy file and obtain the interaction energy U_{BB} by using GROMACS v5.1.4.

To calculate U_{AB}, we will follow the Equation (10). To obtain U_T, we will need to create an index file that contains group A (V_6K_2 peptides) and group B (V_6K_3 peptides). We will also need the final configuration file and final trajectory file of the system. Then, we can use this system which contains group A (V_6K_3 peptides) and group B (V_6K_3 peptides) to create an energy file to obtain the interaction energy U_T by using GROMACS v5.1.4. By subtracting the pair interaction energy $U_{AA}$and $U_{BB}$with U_T, we will obtain U_{AB} for our line tension calculation of this system.

For the 60%V_6K_2 system, to calculate the pair interaction energy of group A (V_6K_2 peptides), we firstly screen out the rest of the molecules (V_6K_3 peptides, counterions, water molecules and anti-freeze particles) in the system which we will need index file of the group A, final configuration file of the system and the trajectory file of the system. After we obtain the system with only group A (V_6K_2 peptides), we can use this system to create

an energy file to obtain the interaction energy U_{AA} by using GROMACS v5.1.4. We use the same methodology to calculate the other group B (V_6K_3 peptides). Firstly, we create an index file of the group B and we also need the final configuration file and the final trajectory file of the system. Then, we can use this system which only contains group B to create an energy file and obtain the interaction energy U_{BB} by using GROMACS v5.1.4.

To calculate U_{AB}, we will follow the Equation (10). To obtain U_T which we will need to create an index file that contains group A (V_6K_2 peptides) and group B (V_6K_3 peptides). The final configuration file and final trajectory file of the system are needed. Then, we can use this system which contains group A (V_6K_3 peptides) and group B (V_6K_3 peptides) to create an energy file to obtain the interaction energy U_T by using GROMACS v5.1.4. By subtracting the pair interaction energy $U_{AA}$and $U_{BB}$with U_T, we will obtain U_{AB} for our line tension calculation of this system.

For the 50%V_6K_2 system, to calculate the pair interaction energy of group A (V_6K_2 peptides), we firstly rule out the rest of the molecules (V_6K_3 peptides, counterions, water molecules and anti-freeze particles) in the system which we will need index file of the group A, final configuration file of the system and the trajectory file of the system. After we obtain the system with only group A (V_6K_2 peptides), we can use this system to create an energy file to obtain the interaction energy U_{AA} by using GROMACS v5.1.4. We use the same methodology to calculate the other group B (V_6K_3 peptides). We create an index file of the group B which we need the final configuration file and the final trajectory file

of the system. Then, we can use this system which only contains group B to create an energy file and obtain the interaction energy U_{BB} by using GROMACS v5.1.4.

To calculate U_{AB}, we will follow the Equation (10). To obtain U_T, we will need to create an index file that contains group A (V_6K_2 peptides) and group B (V_6K_3 peptides). We will also need the final configuration file and final trajectory file of the system. Then, we can use this system which contains group A (V_6K_3 peptides) and group B (V_6K_3 peptides) to create an energy file to obtain the interaction energy U_T by using GROMACS v5.1.4. By subtracting the pair interaction energy $U_{AA}$and $U_{BB}$with U_T, we will obtain U_{AB} for our line tension calculation of this system.

For the 40%V_6K_2 system, to calculate the pair interaction energy of group A (V_6K_2 peptides), we firstly screen out the rest of the molecules (V_6K_3 peptides, counterions, water molecules and anti-freeze particles) in the system which we will need index file of the group A, final configuration file and the trajectory file of the system. After we obtain the system with only group A (V_6K_2 peptides), we can use this system to create an energy file to obtain the interaction energy U_{AA} by using GROMACS v5.1.4. We use the same methodology to calculate the other group B (V_6K_3 peptides). Firstly, we create an index file of the group B and we also need the final configuration file and the final trajectory file of the system. Then, we can use this system which only contains group B to create an energy file and obtain the interaction energy U_{BB} by using GROMACS v5.1.4.

To calculate U_{AB}, we will follow the equation (10). We will need to create an index file that contains group A (V_6K_2 peptides) and group B (V_6K_3 peptides) to obtain U_T. The final configuration file and final trajectory file of the system are needed. Then, we can use this system which contains group A (V_6K_3 peptides) and group B (V_6K_3 peptides) to create an energy file to obtain the interaction energy U_T by using GROMACS v5.1.4. By subtracting the pair interaction energy $U_{AA}$and $U_{BB}$with U_T, we will obtain U_{AB} for our line tension calculation of this system.

For the 30%V_6K_2 system, to calculate the pair interaction energy of group A (V_6K_2 peptides), we firstly screen out the rest of the molecules (V_6K_3 peptides, counterions, water molecules and anti-freeze particles) in the system which we will need index file of the group A, final configuration file and the trajectory file of the system. After we obtain the system with only group A (V_6K_2 peptides), we can use this system to create an energy file to obtain the interaction energy U_{AA} by using GROMACS v5.1.4. We use the same methodology to calculate the other group B (V_6K_3 peptides). Firstly, we create an index file of the group B and we also need the final configuration file and the final trajectory file of the system. Then, we can use this system which only contains group B to create an energy file and obtain the interaction energy U_{BB} by using GROMACS v5.1.4.

To calculate U_{AB}, we will follow the Equation (10). We will need to create an index file that contains group A (V_6K_2 peptides) and group B (V_6K_3 peptides) to obtain U_T. The final configuration file and final trajectory file of the system are needed. Then, we can use this

system which contains group A (V_6K_3 peptides) and group B (V_6K_3 peptides) to create an energy file to obtain the interaction energy U_T by using GROMACS v5.1.4. By subtracting the pair interaction energy $U_{AA}$and $U_{BB}$with U_T, we will obtain U_{AB} for our line tension calculation of this system.

For the 20%V_6K_2 system, to calculate the pair interaction energy of group A (V_6K_2 peptides), we firstly exclude the rest of the molecules (V_6K_3 peptides, counterions, water molecules and anti-freeze particles) in the system which we will need index file of the group A, final configuration file and the trajectory file of the system. After we obtain the system with only group A (V_6K_2 peptides), we can use this system to create an energy file to obtain the interaction energy U_{AA} by using GROMACS v5.1.4. We use the same methodology to calculate the other group B (V_6K_3 peptides). Firstly, we create an index file of the group B and we also need the final configuration file and the final trajectory file of the system. Then, we can use this system which only contains group B to create an energy file and obtain the interaction energy U_{BB} by using GROMACS v5.1.4.

To calculate U_{AB}, we will follow the Equation (10). We will need to create an index file that contains group A (V_6K_2 peptides) and group B (V_6K_3 peptides) to obtain U_T. The final configuration file and final trajectory file of the system are needed. Then, we can use this system which contains group A (V_6K_3 peptides) and group B (V_6K_3 peptides) to create an energy file to obtain the interaction energy U_T by using GROMACS v5.1.4. By subtracting

the pair interaction energy $U_{AA}$and $U_{BB}$with U_T, we will obtain U_{AB} for our line tension calculation of this system.

For the 10%V_6K_2 system, to calculate the pair interaction energy of group A (V_6K_2 peptides), we firstly screen out the rest of the molecules (V_6K_3 peptides, counterions, water molecules and anti-freeze particles) in the system which we will need index file of the group A, final configuration file and the trajectory file of the system. After we obtain the system with only group A (V_6K_2 peptides), we can use this system to create an energy file to obtain the interaction energy U_{AA} by using GROMACS v5.1.4. We use the same methodology to calculate the other group B (V_6K_3 peptides). First, we create an index file of the group B and we also need the final configuration file and the final trajectory file of the system. Then, we can use this system which only contains group B to create an energy file and obtain the interaction energy U_{BB} by using GROMACS v5.1.4.

To calculate U_{AB}, we will follow the equation (10). We will need to create an index file that contains group A (V_6K_2 peptides) and group B (V_6K_3 peptides) to obtain U_T. The final configuration file and final trajectory file of the system are needed. Then, we can use this system which contains group A (V_6K_3 peptides) and group B (V_6K_3 peptides) to create an energy file to obtain the interaction energy U_T by using GROMACS v5.1.4. By subtracting the pair interaction energy $U_{AA}$and $U_{BB}$with U_T, we will obtain U_{AB} for our line tension calculation of this system.

4.2.2 Results and Analysis from Line Tension Calculation For V_6K_2 And V_6K_3 Mixture Systems

We investigated the line tension of each mixture system composition (pure systems are excluded). In Figure (10) below, the curve of line tension calculation is symmetric which indicates that the 90% V_6K_2 system and 90% V_6K_3 system have a lower line tension configuration than other mixture compositions. In the 40% V_6K_3 and 60% V_6K_2 mixture system, the system has a higher line tension configuration than others. The black bars are the standard deviation of each mixture system.

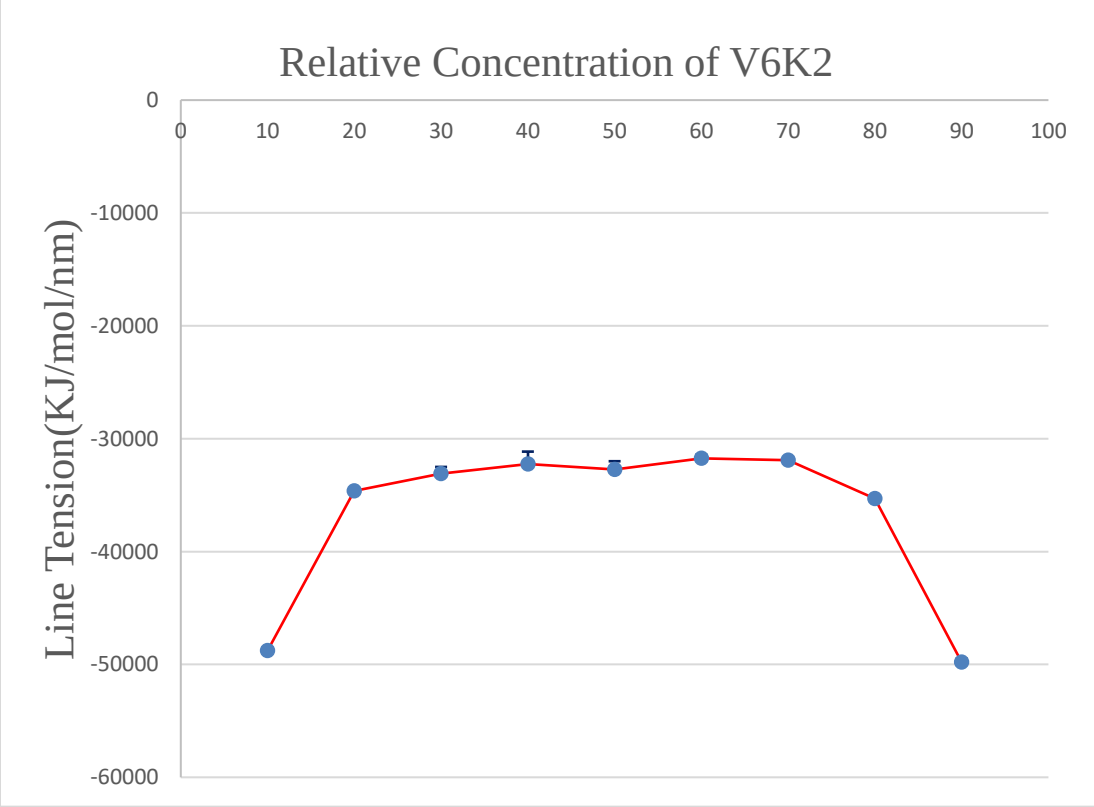


Figure (10) *Summaries the line tension of each mixture system.*

4.3 Introduction of Cluster Calculation For V_6K_2 And V_6K_3 Mixture Systems

Cluster calculation which points out the number of the clusters in the peptide system. The mechanism of peptide self-assembly is mainly because of the hydrophobic effect which leads peptide to form various nanostructures. In this section, we will discuss the cluster calculation for the $V_6K_2$and V_6K_3mixture systems.

4.3.1 Cluster Calculation For V_6K_2 And V_6K_3 Mixture Systems

The analysis tool is based on using GROMACS v5.1.4 package. The files that we need for analyzing the number of the clusters are the last 400 ns trajectory file, index files for specific peptide groups of each peptide composition and the topology file of the mixture system. Before we executed the cluster analysis command, we need to know the cut-off distance that needed for each of the peptide ($V_6K_2$and V_6K_3). If the cut-off distance is either too big or too small, the analysis result can not represent the reasonable meaning of the system. We will use the GROMACS v5.1.4 to obtain the plot of radial distribution functions and then we can determine the cut-off for our cluster calculation. The radial distribution function also called pair distribution function which is denoted as g(r). It represents the probability to find a particle within a distance of r and r+dr away from the reference particle as shown in Fig (11).

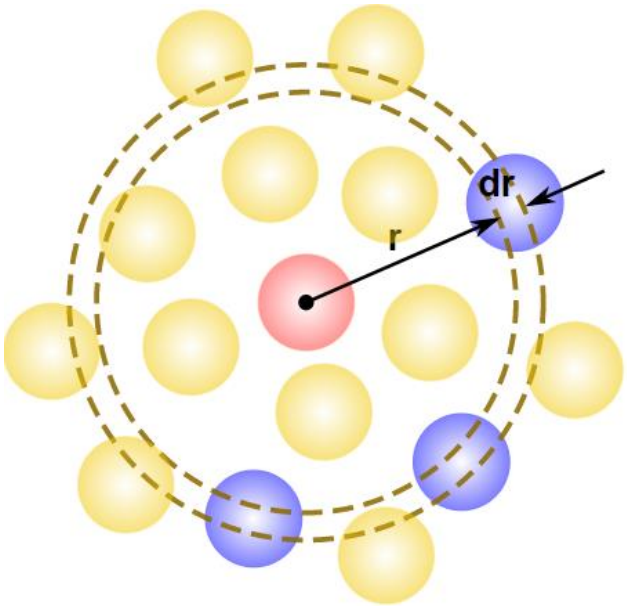


Figure (11) *Scheme of the radial distribution function.*

Firstly, we use the $(11\ \text{nm})^3$ cubic box with 200 V_6K_2peptides as a test example since this system only has one big cluster (nanofibril) in the final configuration. To get the plot of radial distribution function, we will need the trajectory file, topology file of the peptide system and the index file of the peptide group. The Figure (12) below presents the radial distribution function of the $(11\ \text{nm})^3$cubic system with 200 V_6K_2peptides.

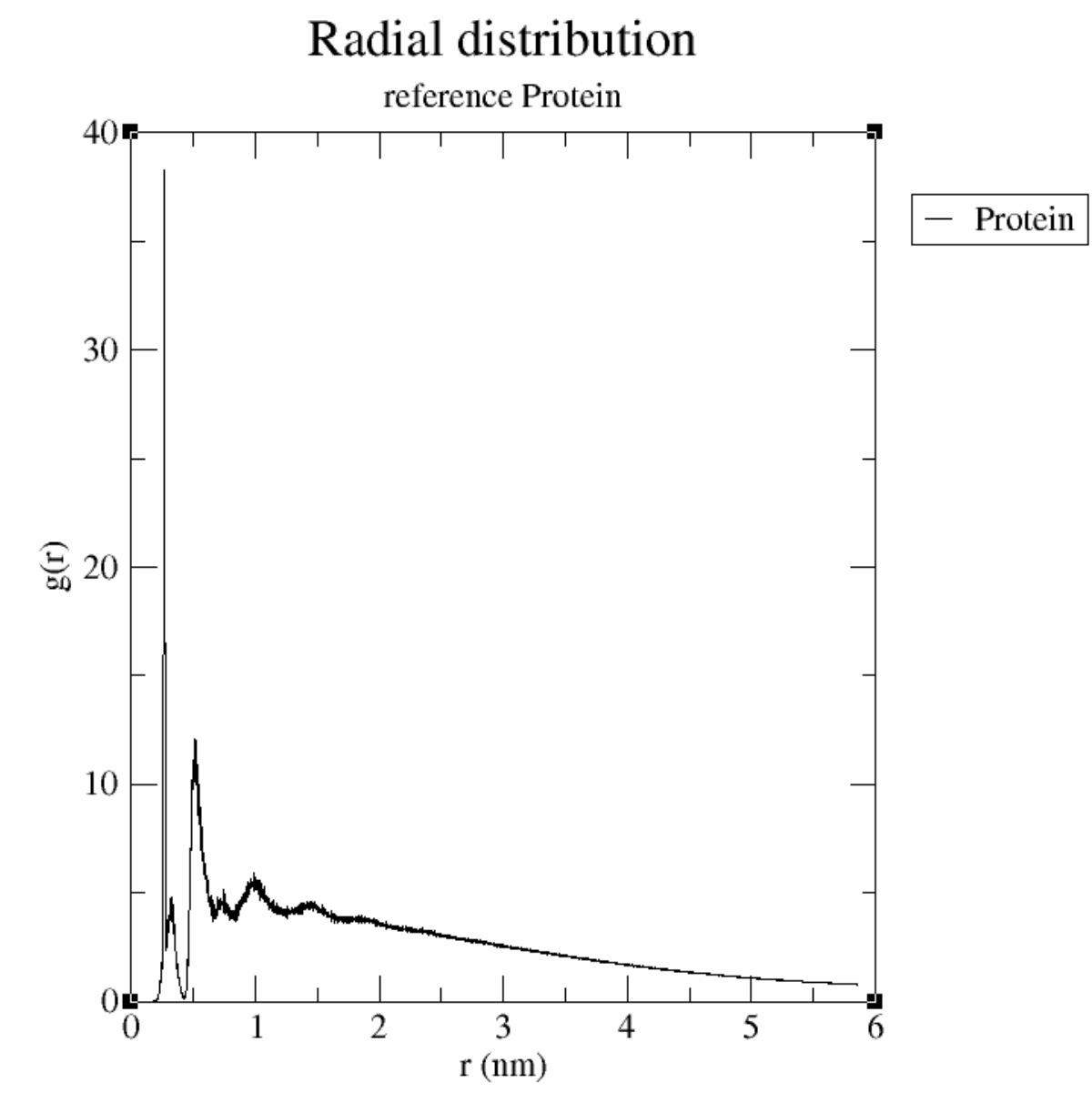


Figure (12) *The radial function distribution of 11 nanometer cubic with 200* V_6K_2*peptides.*

At the cut-off around 0.48 nm is the suitable cut-off distance for calculating the clusters for V_6K_2peptides which is the largest distance to be considered in a cluster. Based on the RDF graph, at 0.48 nm (g(r)=0) which means at this point the density as a function of distance is zero. This implies the neighboring cluster will not be double-counted at this cut-off distance. We will then use this number to calculate each mixture system for the group of V_6K_2peptides.

For the V_6K_3peptide system, we will use the $(17\ \text{nm})^3$system with 650 V_6K_3peptides as the test example.

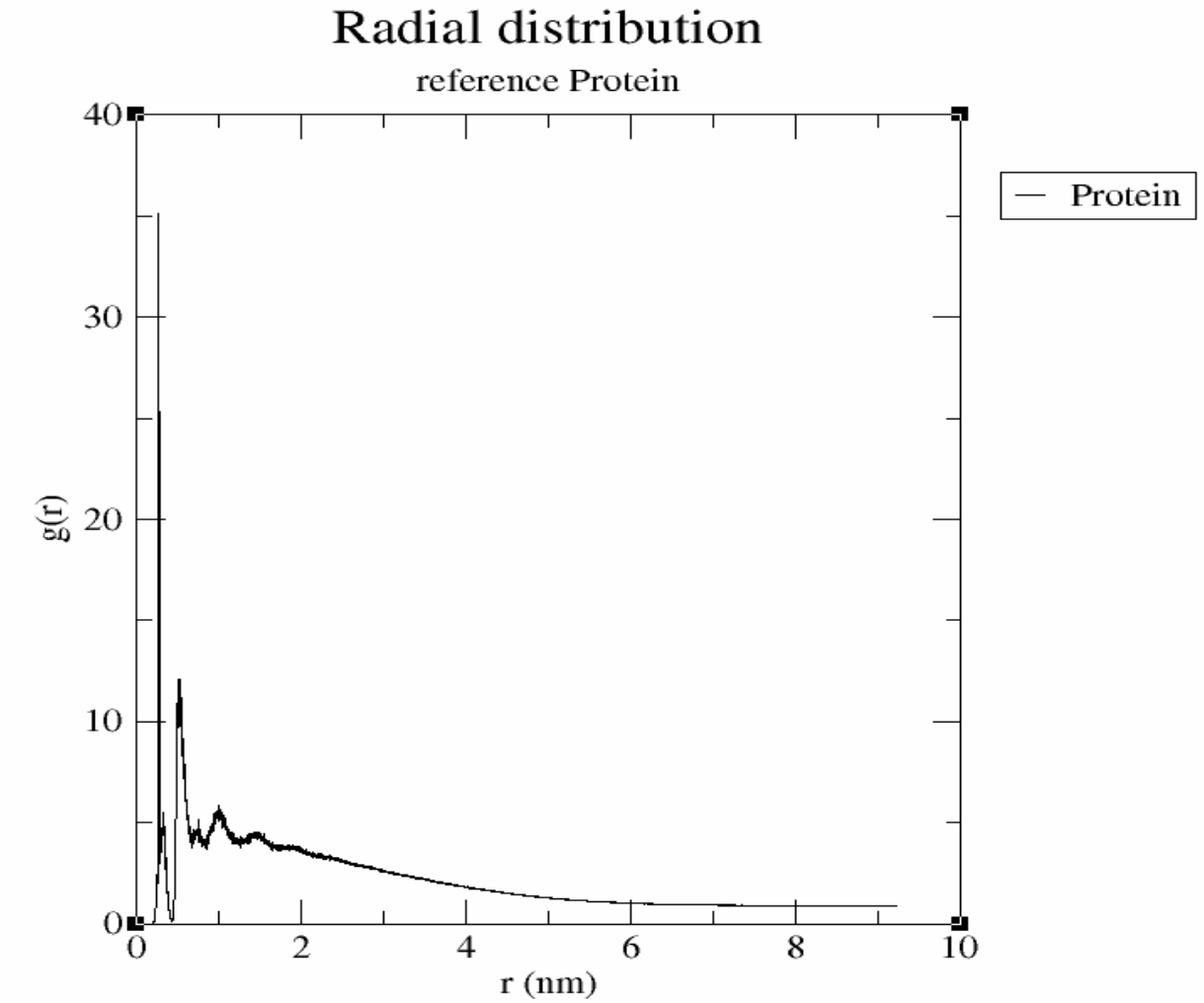


Figure (13) *The radial function distribution of 17 nanometer cubic with 650* V_6K_3*peptides.*

In Figure (13) which shows that 0.5 nm will be a suitable cut-off distance for us to do the cluster calculation for the group of V_6K_3peptides.

Now, we have the cut-off distance for each of the group of the peptide so that we can begin our cluster calculation for the mixture systems.

To begin with the cluster calculation for the pure systems (pure V_6K_2 and pure V_6K_3systems), we will need the last 400 ns trajectory files, index file for the peptide group and the topology file for each of the peptide system. We may also need to include the cut-off distance for the peptide group which is 0.48nm for the V_6K_2peptides group and 0.5nm for the V_6K_3peptides group in the analysis command.

For the 90%V_6K_2 system, we need the last 400 ns trajectory files, index file for each of the peptide groups and the topology file for each of the peptide systems. We may also need to include the cut-off distance for the peptide group which is 0.48nm for the V_6K_2peptides group and 0.5nm for the V_6K_3peptides group in the analysis command.

For the 80%V_6K_2 system, we need the last 400 ns trajectory files, index file for each of the peptide groups and the topology file for each of the peptide systems. We may also need to include the cut-off distance for the peptide group which is 0.48nm for the V_6K_2peptides group and 0.5nm for the V_6K_3peptides group in the analysis command.

For the 70%V_6K_2 system, we need the last 400 ns trajectory files, index file for each of the peptide groups and the topology file for each of the peptide systems. We may also need to include the cut-off distance for the peptide group which is 0.48nm for the V_6K_2peptides group and 0.5nm for the V_6K_3peptides group in the analysis command.

For the 60%V_6K_2 system, we need the last 400 ns trajectory files, index file for each of the peptide groups and the topology file for each of the peptide systems. We may also need

to include the cut-off distance for the peptide group which is 0.48nm for the V_6K_2peptides group and 0.5nm for the V_6K_3peptides group in the analysis command.

For the 50%V_6K_2 system, we need the last 400 ns trajectory files, index file for each of the peptide groups and the topology file for each of the peptide systems. We may also need to include the cut-off distance for the peptide group which is 0.48nm for the V_6K_2peptides group and 0.5nm for the V_6K_3peptides group in the analysis command.

For the 40%V_6K_2 system, we need the last 400 ns trajectory files, index file for each of the peptide groups and the topology file for each of the peptide systems. We may also need to include the cut-off distance for the peptide group which is 0.48nm for the V_6K_2peptides group and 0.5nm for the V_6K_3peptides group in the analysis command.

For the 30%V_6K_2 system, we need the last 400 ns trajectory files, index file for each of the peptide groups and the topology file for each of the peptide systems. We may also need to include the cut-off distance for the peptide group which is 0.48nm for the V_6K_2peptides group and 0.5nm for the V_6K_3peptides group in the analysis command.

For the 20%V_6K_2 system, we need the last 400 ns trajectory files, index file for each of the peptide groups and the topology file for each of the peptide systems. We may also need to include the cut-off distance for the peptide group which is 0.48nm for the V_6K_2peptides group and 0.5nm for the V_6K_3peptides group in the analysis command.

For the 10%V_6K_2 system, we need the last 400 ns trajectory files, index file for each of the peptide groups and the topology file for each of the peptide systems. We may also need to include the cut-off distance for the peptide group which is 0.48nm for the V_6K_2peptides group and 0.5nm for the V_6K_3peptides group in the analysis command.

4.3.2 Analysis and Results for Cluster Calculation of V_6K_2 And V_6K_3 Mixture Systems

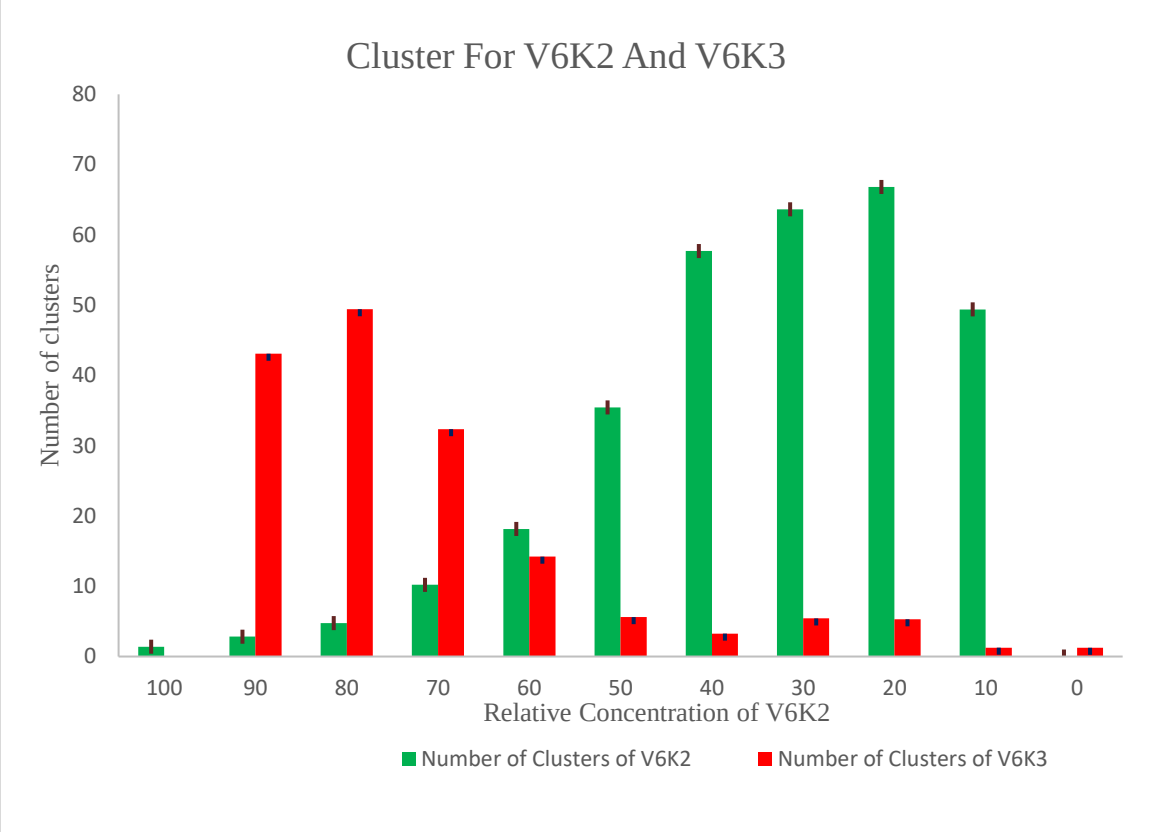
We implemented the method that discussed in the previous section for 10 independent trajectories of each mixture system and then Table (12) and Figure (14) with standard deviation represent the results from our calculation.

Table (12) *Summaries the cluster calculation of each mixture peptide system.*

V_6K_3Relative Concentration	**Average**	**V_6K_2Relative Concentration**	**Average**
0%V_6K_3	0	100%V_6K_2	1.400
10%V_6K_3	43.089	90%V_6K_2	2.823
20%V_6K_3	49.421	80%V_6K_2	4.758
30%V_6K_3	32.341	70%V_6K_2	10.165
40%V_6K_3	14.209	60%V_6K_2	18.156
50%V_6K_3	5.609	50%V_6K_2	35.447
60%V_6K_3	3.261	40%V_6K_2	57.700
70%V_6K_3	5.422	30%V_6K_2	63.640
80%V_6K_3	5.300	20%V_6K_2	66.817
90%V_6K_3	1.263	10%V_6K_2	49.401

100%V_6K_3	1.247	0%V_6K_2	0

Figure (14) *Summaries the cluster calculation of each mixture peptide system.*



Based on Fig (14), the maximum number of the clusters occurred in the 20%V_6K_2 and 20%V_6K_3systems. This happens maybe because this is the relative concentration for minor phase peptides starts to form aggregates. For example, at the 10 % V_6K_2 system, V_6K_2 is the minor phase of the peptide system, there are too few minor phase peptides to associate with each other and remain dispersed in the system. When the concentration goes above 20%, the minor phase peptides start to form aggregates. Hence, at 20% V_6K_2 and

20%V_6K_3systems, we would find the maximum cluster numbers. The minimum number of clusters occurred in the pure system since there is only one component. One interesting phenomenon is that in the 60%$V_6K_2$and 40%V_6K_3 system, the number of clusters of each peptide group has the closest number of the clusters. This happens maybe because this group has the highest line tension based on the result from the previous section. Also, in 20%$V_6K_3$and 30%V_6K_3 systems, more clusters can be found than in the 40%V_6K_3system. This can be explained by the analysis of surface accessible surface area which 20% and 30% V_6K_3 systems have more surface accessible surface area than the 40% V_6K_3. Furthermore, the V_6K_3peptide group seems to have fewer clusters than the V_6K_2peptide group even in the high relative concentration. The bars are the standard deviation.

4.4 Introduction of Radius of Gyration Analysis For V_6K_2 And V_6K_3 Mixture Systems

The radius of gyration (R_g) of a peptide is a measurement of its compactness. If a peptide is stably folded, it may maintain a steady value radius of gyration otherwise the value will change over time. We use GROMACS v5.1.4 to analyze our mixture systems for the radius of gyration.

4.4.1 Radius of Gyration Analysis For V_6K_2 And V_6K_3 Mixture Systems

To obtain the radius of gyration of the mixture systems, we will need the last 400 ns trajectory file, the index file for the lysine group of each peptide mixture system and the topology file of the mixture system. We only investigate the radius of gyration of "lysine group" of the peptides in each mixture system because we want to know how hydrophilic effect that affects the compactness of peptide's folding. We will only use one seed of each mixture system with ten independent lysine index groups.

For pure V_6K_2 and V_6K_3 systems, we will use the last 400 ns trajectory files, the specific index files for each pure peptide system and the topology file of each system. We will then average the radius of gyration based on the ten independent index groups of lysine for each system.

For the 90%V_6K_2 system, we will use the last 400 ns trajectory files, the specific index files for each peptide component and the topology file of the system. We will then average the radius of gyration based on the five independent index groups of lysine for each peptide group.

For the 80%V_6K_2 system, we will use the last 400 ns trajectory files, the specific index files for each peptide component and the topology file of the system. We will then average the radius of gyration based on the five independent index groups of lysine for each peptide group.

For the 70%V_6K_2 system, we will use the last 400 ns trajectory files, the specific index files for each peptide component and the topology file of the system. We will then average the radius of gyration based on the five independent index groups of lysine for each peptide group.

For the 60%V_6K_2 system, we will use the last 400 ns trajectory files, the specific index files for each peptide component and the topology file of the system. We will then average the radius of gyration based on the five independent index groups of lysine for each peptide group.

For the 50%V_6K_2 system, we will use the last 400 ns trajectory files, the specific index files for each peptide component and the topology file of the system. We will then average the radius of gyration based on the five independent index groups of lysine for each peptide group.

For the 40%V_6K_2 system, we will use the last 400 ns trajectory files, the specific index files for each peptide component and the topology file of the system. We will then average the radius of gyration based on the five independent index groups of lysine for each peptide group.

For the 30%V_6K_2 system, we will use the last 400 ns trajectory files, the specific index files for each peptide component and the topology file of the system. We will then average

the radius of gyration based on the five independent index groups of lysine for each peptide group.

For the 20%V_6K_2 system, we will use the last 400 ns trajectory files, the specific index files for each peptide component and the topology file of the system. We will then average the radius of gyration based on the five independent index groups of lysine for each peptide group.

For the 10%V_6K_2 system, we will use the last 400 ns trajectory files, the specific index files for each peptide component and the topology file of the system. We will then average the radius of gyration based on the five independent index groups of lysine for each peptide group.

4.4.2 Analysis and Results for Radius of Gyration of V_6K_2 And V_6K_3 Mixture Systems

Based on the method we discussed in the previous section, we can obtain the radius of gyration as figure (15) shows below. The V_6K_3peptide has a larger head group therefore the radius of gyration will be larger than the V_6K_2peptide. At 10% relative concentration of each mixture system, the radius of gyration is the largest than the rest of the relative concentration systems.

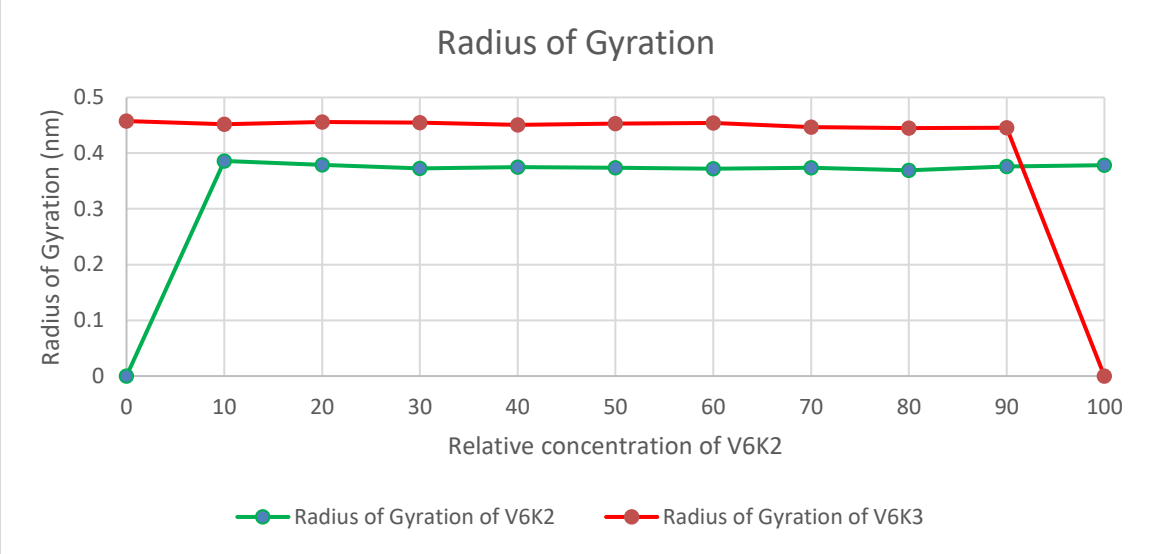


Figure (15) *Summaries the radius of gyration calculation of each mixture peptide system.*

Chapter 5 Conclusion

We study the V_6K_2 and V_6K_3 peptides based on using MARTINI Forcefield and GROMACS v5.1.4 package. Different water models (polarizable and non-polarizable) have been used to investigate the validation of V_6K_2 self-assembly supramolecular nanostructure based on experimental results.[37-41]Both of the water models validate the experimental results in the small system; however, due to computational cost and reproducibility consideration in the bigger system, we decide to use non-polarizable water model to study the mixture system of V_6K_2 and V_6K_3 peptides.

The mixture systems were built up based upon the peptide concentration in the small system. The reason to scale up the size is that we try to prevent the finite size effect for our peptide system. We will then use the one total peptide concentration with 11 relative peptide concentrations for further study.

We also investigate the 11 mixture systems based on the solvent accessible surface area, line tension, cluster calculation and the radius of gyration of lysine group. The solvent accessible surface area provides a clue about how much area of the supramolecular structure in the peptide system, line tension tells us how much excess interaction energy per characteristic length, the cluster calculation gives us an important information about the number of aggregations in the system and finally the radius of gyration shows us the compactness of the peptide structure. These examinations provide a meaningful story behind the peptide system in both chemical and physical ways.

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