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Macromolecular and nanoscale investigation of intermolecular interactions driving the self-assembly of collagen

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Abstract

Collagens have remarkable ability to self-assemble into ordered fibrils. Assembly of collagens on 2-dimensional surfaces serves as a model system to study the dynamics of assembly process and the resulting fibrillar structure has potential biotechnological and biomedical applications. However, intermolecular forces driving the self-assembly of collagen are not well understood. Here, we apply the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory to investigate the interactions between collagens and between collagen and the mica surface. Interactions are found to be attractive at all distances, which is consistent with literature. To further investigate the sequence-dependent organization of collagen molecules, we examined interaction near contact distances when collagen molecules are staggered to different degrees. We compared electrostatic, hydrophobic, and polar hydration interaction, and found that hydrophilic interaction plays a significant role on the molecular assembly by protecting the molecule from random collapse with a consistent repulsion barrier. Electrostatic interaction, on the other hand, exhibits local energy maxima and minima on D-periodic arrangements throughout the molecule leading to an oscillation effect on the axial self-assembly. Our approaches, in both macromolecular and nanoscale, provide insights into the factors that determine interactions among collagens and between collagen and mica.

1. Introduction

Collagen is the most abundant protein in the human body (30% by protein mass) and it is the most abundant component of the extracellular matrix (ECM) providing strength, stability, and elasticity to tissues [1]. Among different collagen types, type-I collagen is the largest in fraction (70%), and it is found in many tissues such as tendon, ligament, skin, cornea, and blood vessel wall [1]. A type-I collagen molecule is 300 nm in length and 1.5 nm in diameter, which is a heterotrimer of two $\alpha_1(I)$ and one $\alpha_2(I)$ polypeptide chains.

Collagen molecules self-assemble into structures ranging from molecular to macroscopic length scale with D-periodic axial staggering both *in vitro* and

in vivo [2]. Self-assembly of collagen is an important phenomena in various aspects of biotechnology applications such as tissue engineering [3], cellular processing [4], bio-compatibility [5], and surface functionalization [6]. Different forms of collagen have been utilized in different clinical applications. Collagen gels were used as a delivery system to transport cells, DNA, or proteins to target structure [7, 8]. Collagen shields have been used to deliver hydrophobic and hydrophilic drugs to cornea [9]. As a drug delivery system, collagen film has been found to be relatively more effective with increased duration of therapeutic effect of drug at the target site [10]. Also, type-I and type-III collagen matrix and membrane have been used for guided tissue and bone regeneration [11]. For such applications, it is important to understand and

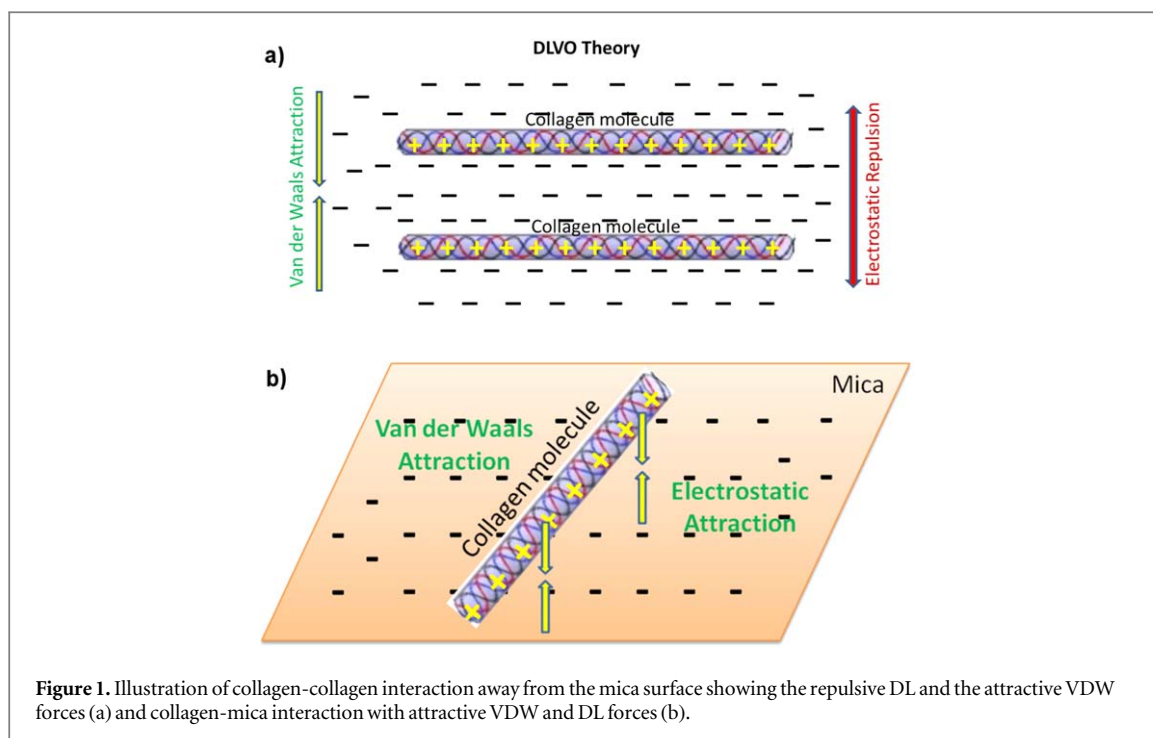


Figure 1. Illustration of collagen-collagen interaction away from the mica surface showing the repulsive DL and the attractive VDW forces (a) and collagen-mica interaction with attractive VDW and DL forces (b).

manipulate the interactions between collagens and between collagens and other materials. To this end, we investigated collagen self-assembly on a commonly used substrate, mica [12–14].

We theoretically examined the interactions between collagen and mica first using the DLVO theory. When surfaces interact within a liquid medium, three main types of interactions exist: van der Waals (VDW), electrostatic double layer (DL) and hydration forces (hydrophobic and polar hydration). The VDW and DL interactions were accounted for by the DLVO theory [15]. It has been widely successful for studying interactions between various types of surfaces including hydrophobic, hydrophilic, or with different surface treatments [16–18]. Although non-DLVO effects have also been observed in certain cases including the mixture between water and other polar solvents [17], or between titania surfaces [19], we expect that DLVO theory still provides an approximate description of the interactions involving collagens that occur in purely aqueous environment.

The DLVO theory has been applied to biological systems such as bacteria, yeast cells and viruses [20], but to our knowledge, it has not been used to investigate collagen assembly. We find that collagen molecules experience stronger attraction from the negatively charged mica surface than from the neighbouring collagen molecules. Also, variations in the surface potential and the electrolyte concentration are not significant enough to change the nature of the interaction from attractive to repulsive. Thus, during the early stages of assembly, the mica surface will be first covered by randomly adsorbed collagen molecules, after which interactions between collagen

molecules drive them into more ordered fibrils. The diffusion of collagen molecules on mica surface is likely due to hydration shells that surround the collagen and mica surfaces. To gain a detailed picture of the sliding between collagen molecules, we align collagen sequences at different D-periodic positions and use a sequence-dependent pseudo-energy scoring scheme originally developed by Hulmes *et al* [21]. We find an oscillatory behaviour of the electrostatic interaction between collagen molecules, whereas hydrophobic and hydrophilic interactions are distributed more uniformly and besides hydrophilic repulsion exhibits a consistent barrier along the molecule that could prevent molecules from random collapse during self-assembly. While the DLVO approach addresses long-range interactions, the sequence analysis reveals more detailed picture of the interactions between collagen molecules at close distances. Our approach of combining meso-scale interactions and sequence-specific interactions may provide insights into the self-assembly of bio-macromolecules on solid surfaces.

2. Methods

2.1. Collagen—collagen interaction

In a collagen fibril, collagen molecules assemble in parallel along the axial direction. We thus modelled collagen molecules as cylindrical rods and mica as a flat surface (figure 1). Let R be the radius of collagen and D_c be the distance between the axes of cylinders of two collagen molecules. The DLVO interaction energy per unit length of two collagen molecules in parallel represented in figure 1(a) is [22]

$$\begin{aligned}
E_{cc}^{DLVO} &= E_{cc}^{VDW}(D_c) + E_{cc}^{DL}(D_c) \\
&= -\frac{A}{12\sqrt{2}}D_c^{-\frac{3}{2}}\sqrt{\frac{R}{2}} + \kappa\frac{1}{2}Z_{cc}\sqrt{\frac{R}{4\pi}}e^{-\kappa D_c},
\end{aligned}
\tag{1}$$

where $A = 1.0 \times 10^{-20}$ J is the Hamaker constant [23], κ is the inverse of the Debye length, and Z_{cc} is the interaction constant of the electrostatic DL force, which depends on the surface potential of collagen molecules. These parameters are further explained below. The corresponding DLVO force is given as

$$F_{cc} = -\frac{\partial}{\partial D_c}E_{cc}^{DLVO}(D_c). \tag{2}$$

To investigate to what level the repulsive electrostatic DL force is affected by the external system parameters, we calculated $F_{cc}^{DL}(D)$, between collagen molecules with varying concentration of KCl and surface potential of collagen using the following formula whose parameters will be explained in the following subsections.

$$\begin{aligned}
F_{cc}^{DL}(D) &= \frac{\kappa^{\frac{3}{2}}Z_{ss}}{2}\sqrt{\frac{R}{\pi}}e^{-\kappa D} \\
&= \kappa^{\frac{3}{2}}(6.32 \times 10^{-19})e^{-\kappa D}.
\end{aligned}
\tag{3}$$

2.1.1. Debye length

In a buffer containing different ions, the Debye length κ^{-1} is

$$1/\kappa = \left(\varepsilon_0 \varepsilon k_B T / \sum_i c_i e^2 z_i^2 \right)^{1/2} \tag{4}$$

where ε_0 is the permittivity of vacuum, ε is the dielectric constant of the solution, k_B is Boltzmann constant, $T = 300$ K is the temperature, c_i is the concentration of the i -th ion, $e = 1.6 \times 10^{-19}$ C is the magnitude of the charge of an electron, and z_i is the valency of the i -th ion. For ions, we use the buffer composition in our previous experimental study [14]; 30 mM Na_2HPO_4 , 10 mM KH_2PO_4 , and 200 mM KCl. These yield $\kappa^{-1} = 5.6\text{\AA}$.

2.1.2. Surface potential

Between two identical surfaces, Z_{cc} is given as [22]

$$Z_{cc} = 64\pi\varepsilon_0\varepsilon\left(\frac{kT}{e}\right)^2 \tanh^2\left(\frac{ze\Psi_0}{4kT}\right). \tag{5}$$

Here, Ψ_0 is the surface potential of collagen, which is related to the surface charge density σ via the Graham equation

$$\Psi_0 = \sigma/(\varepsilon_0\varepsilon\kappa). \tag{6}$$

A collagen molecule carries a net charge of 34e (table 1(b)). Dividing by its surface area yields the surface potential $\Psi_0 = 3.07$ mV, which is consistent with experimentally measured zeta potential of collagen which is 2.5 mV in pure monovalent electrolyte solution [24]. The measured zeta potential of mica surface is about -100 mV [25], although it depends on

Table 1. Amino acid composition of type-I collagen of rat. (a) Counts for individual α chains. Type-I collagen consists of two α_1 and one α_2 chains. Numbers in parentheses are counts for telopeptides. COL1A2 does not have telopeptides marked in the UniProt Database. (b) Total counts [14].

Gene	COL1A1	COL1A2	
(a)	UniProt ID	P02454	P02466
	Length (AA)	1056	1040
	Telopeptide	16(N) + 26(C)	—
	Nonpolar	176 + (8)	208
	Polar	99 + (10)	89
	Acidic	78 + (5)	68
	Basic	87 + (4)	86
	Pro+Hyp	232 + (4)	203
Tot. count	Type-I		
(b)	Net Charge	34	
	Nonpolar	560 + (16)	
	Polar	287 + (20)	
	Pro+Hyp	667 + (8)	

the types of electrolyte and their concentrations. In this study, we used the zeta potential of mica as the surface potential because of the low ionic strength of our buffer solution.

2.2. Collagen—mica interaction

We calculated the net force between a collagen molecule and mica at contact distance (figure 1(b)). If we consider the contact distance as 3\AA , a single hydration layer, the DLVO interaction force between a collagen molecule and mica is;

$$\begin{aligned}
F_{cm}^{DLVO}(D_0) &= F_{cm}^{VDW}(D_0) + F_{cm}^{DL}(D_0) \\
&= \frac{-A}{8\sqrt{2}}\sqrt{R}D_0^{-\frac{5}{2}} - \kappa\frac{3}{2}Z_{as}\sqrt{\frac{R}{2\pi}}e^{-\kappa D_0}.
\end{aligned}
\tag{7}$$

For the surface potential of the asymmetric system of collagen-mica, the reported value of -100 mV of mica [25] was used in equation (7). The net surface potential for the interaction, however, must be lower, since the collagen molecule has much less potential, 3.07 mV, compared to mica.

2.3. Computational details

The DLVO theory applies for the case when collagen molecules are not in contact with each other. At closer distances, contribution of individual amino acid side chains to the interaction must be considered, for which we apply a sequence-level analysis. To evaluate the effect of each amino acid to the total interaction between collagen molecules, their amphiphilicity and hydrophathy values [26] were used (figure 5). Here, we investigated the electrostatic, hydrophobic, and polar characteristics of the molecule.

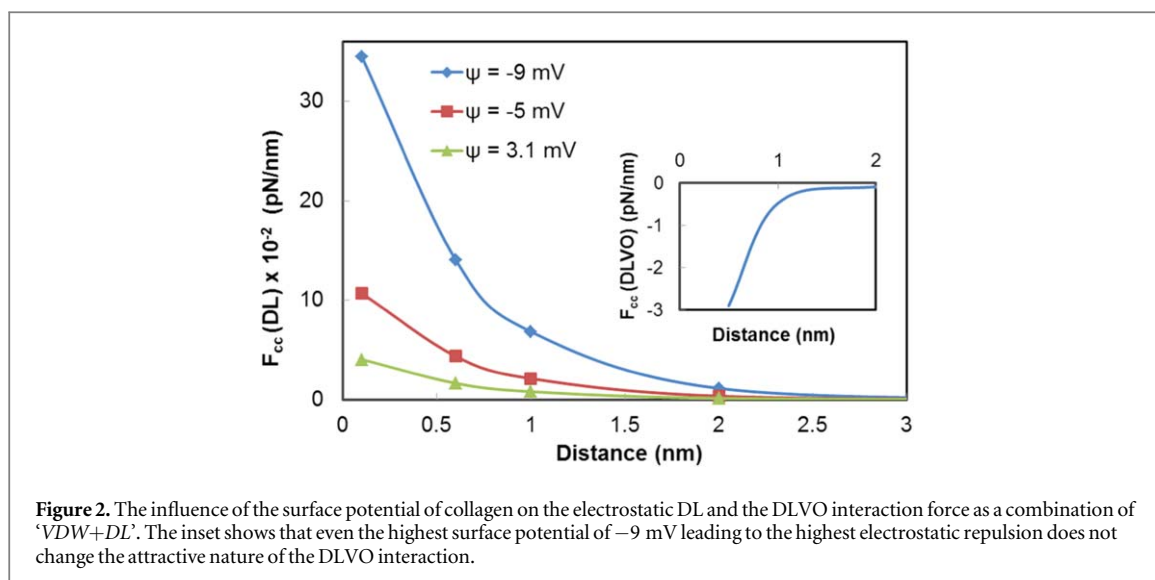


Figure 2. The influence of the surface potential of collagen on the electrostatic DL and the DLVO interaction force as a combination of ‘VDW+DL’. The inset shows that even the highest surface potential of -9 mV leading to the highest electrostatic repulsion does not change the attractive nature of the DLVO interaction.

Table 2. Charge for each D periodic segment. The second column shows the residue range of α_1 and α_2 chains for each segment. The third and the fourth column shows the total charge, $(2\alpha_1 + \alpha_2)$, for the corresponding ranges. $N \rightarrow C$ means that residue counts starts from N terminal, while $C \rightarrow N$ is that from C terminal end.

Segment	Residue range for α_1 and α_2	(Charge $N \rightarrow C$)	(Charge $C \rightarrow N$)
D1	[0:240], [0:236]	10	6
D2	[240:480], [236:472]	11	10
D3	[480:720], [472:708]	1	2
D4	[720:960], [708:944]	9	14
D4.4	[960:1056], [944:1040]	3	2

2.4. Application of DLVO theory to D-periodic segments

We also analysed the contribution of individual D-periodic segments to the total electrostatic DL interaction. We plotted the DL force between two perfectly aligned collagen molecules, $F_{cc}(DL)$, shown in figure 6 depending on the total charge of the each fragment summarized in table 2, second column. We first calculated the net charge for each D-periodic segment (table 2) and calculated the electrostatic DL force using equation (3). The net charge calculations were performed for both parallel and antiparallel fibril positions as shown in figure 7.

2.4.1. Contribution of electrostatic interaction to D-periodic arrangements of collagen fibrils

We investigated the charge density of conventional 0D, 1D, 2D, 3D, and 4D arrangements of collagen molecules showed in figure 8 by calculating the normalized charge of each arrangement. Here, 0D represents the perfect alignment of the two molecule, while 4D represents the interaction of the last parts of the two molecule showed as D4.4 in figure 6(b). The range of interacting residues for each of the five positions were provided in table 3. For the calculation of normalized charge, we used the following formula:

$$NC = \left(\frac{\text{Total charge of whole collagen}}{\text{Length of interaction (nm)}} \right) \times 300 \text{ nm}$$

where NC represents normalized net charge in table 3. The calculations were performed for both parallel and anti-parallel arrangements (figure 7), molecules pointing in the same direction throughout the fibril, and anti-parallel molecular arrangement, possessing one polarization switch on the fibril as illustrated in figure 7.

3. Results and discussion

3.1. Collagen—collagen interaction

The DLVO interaction between two collagen molecules illustrated in figure 1(a) consists of attractive VDW and repulsive DL force, unlike the interaction between collagen and mica consisting of attractive DL interaction. To understand to what extent the repulsive interaction can be manipulated via conditions such as surface potential and ionic strength, we examined the DLVO interaction between the two parallel collagen molecules.

3.1.1. Effect of surface potential

A previous study measured the dependence of zeta potential of collagen molecules on solution pH [24]. Using the experimental measurement in [24] which was taken in the electrolyte conditions of 12 mM NaCl and 10 mM Na_2HPO_4 , and 12 mM NaCl and our electrolyte condition of 30 mM Na_2HPO_4 , 10 mM KH_2PO_4 , and 200 mM KCl at pH 7 [14], we calculated the DLVO force (figure 2). Although the presence of

Table 3. The normalized charge of each D periodic arrangement of two collagen molecules which consist of two α_1 and one α_2 chains. Molecule 1 and Molecule 2 represent the upper and the bottom one, respectively, as illustrated in figure 7. There are 96 amino acids interacting at 4D, while 1056 amino acids interact at 0D arrangement.

Arrangement	α_1, α_2 chains	Molecule 1	Molecule 2 (Parallel)	Molecule 2 (Anti-parallel)
0D	Mol 1 [0:1056], [0:1040] Mol 2 [0:1056], [0:1040]	34	34	34
1D	Mol 1 [240:1056], [236:1040] Mol 2 [0:816], [0:804]	30.9	36	30.9
2D	Mol 1 [480:1056], [472:1040] Mol 2 [0:576], [0:568]	23.4	32.5	23.4
3D	Mol 1 [720:1056], [708:1040] Mol 2 [0:336], [0:332]	36.3	39.3	42.4
4D	Mol 1 [960:1056], [944:1040] Mol 2 [0:96], [0:96]	28.1	18.7	37.5

different electrolytes shifts the isoelectric point of collagen [24], both experimental conditions can be used to provide an idea of how the surface potential affects the electrostatic interaction between collagen molecules. We tabulated the solution pH and the surface potential of collagen in table 4 in which the first row represented our experimental condition and the last two were [24]. Figure 2 exhibits a clear influence of the surface potential on the DL interaction between collagen molecules away from the surface. However, the inset shows that even for the highest surface potential of 9 mV, the DLVO theory as a combination of 'VDW+DL' results in a pure attraction at all separations.

3.1.2. Effect of KCl concentration and residue level interactions

Among various electrolytes, KCl is particularly important for surface assembly of collagen on mica. The mica lattice has K layers which are partially taken out upon cleaving and mica surface is negatively charged. The open K^+ pockets on the surface are partially filled with ions from KCl in the buffer. A previous study investigated the relative affinity of various monovalent cations such as K^+ , Na^+ , Li^+ to mica and found that K^+ has a higher affinity [27], and it was found as a critical component for creating an ordered film [28]. As the concentration of KCl increases, the repulsive DL interaction also increases as shown in figure 3. However, even a high concentration of KCl such as 2 M could not affect the resulting interaction significantly because of the relatively low magnitude of the electrostatic interaction compared with VDW in collagen—collagen system. Note that for the calculation of DLVO theory we take the net charge of the whole molecule and the electrostatic interaction shows not a strong global effect. However, when we look at the charge distribution of the molecule shown in figure 5, it is seen that the negative and positive charges are located in close proximity preferentially within a few amino acid distances and in a periodic manner. This shows the

Table 4. Zeta potential of collagen in solution.

Zeta potential	pH	Source
$\psi = 3.07$ mV	pH $\cong 7$	This study
$\psi = -5$ mV	pH $\cong 8$	[24]
$\psi = -9$ mV	pH $\cong 10$	[24]

importance of the electrostatic interaction on local (nanoscale) environment rather than global macromolecular scale.

3.2. Collagen—mica interaction

Collagen self-assembly onto mica involves collagen-collagen and collagen-mica interactions at different stages [12, 29]. Collagen molecules randomly adsorb on mica upon deposition, and if the adsorption is strong enough, the molecules are pinned at their first positions, since collagen-collagen interaction is relatively weak at this stage as can be understood in equation (7) [12, 29]. Electrostatic DL interaction between collagen and mica, at this stage, is stronger due to the higher surface potential of mica compared to collagen in Z_{as} equation (7) and attractive. However, DL interaction is repulsive between collagen molecules. After adsorption, collagen molecules carry out surface diffusion and then nucleate into fibrils of D-periodic arrangement where collagen-collagen interaction plays an important role.

The DLVO interaction depends on the characteristics of the surfaces. VDW force is always attractive except the case that the dielectric property of the intervening medium is in between the two of the interacting surfaces, which does not apply to our system. The dielectric constant of water is much higher than those collagen [30] and mica. Dielectric constant of mica is reported between 5.4 and 7.0 [22]. So, the VDW force between a collagen and mica has an attractive character. The electrostatic DL force between negatively charged mica and positively charged collagen is also

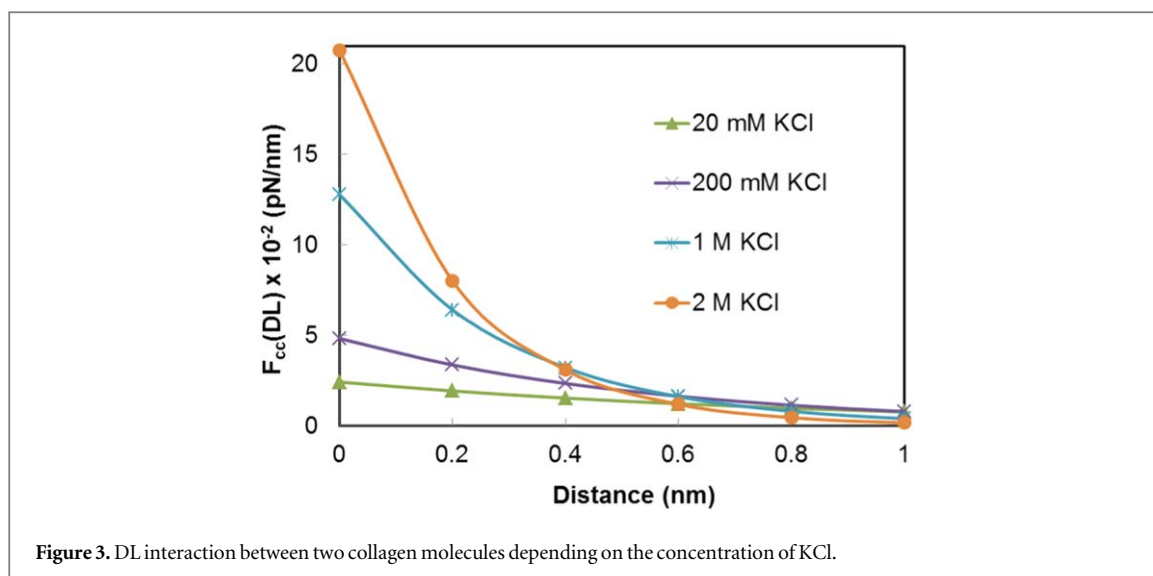


Figure 3. DL interaction between two collagen molecules depending on the concentration of KCl.

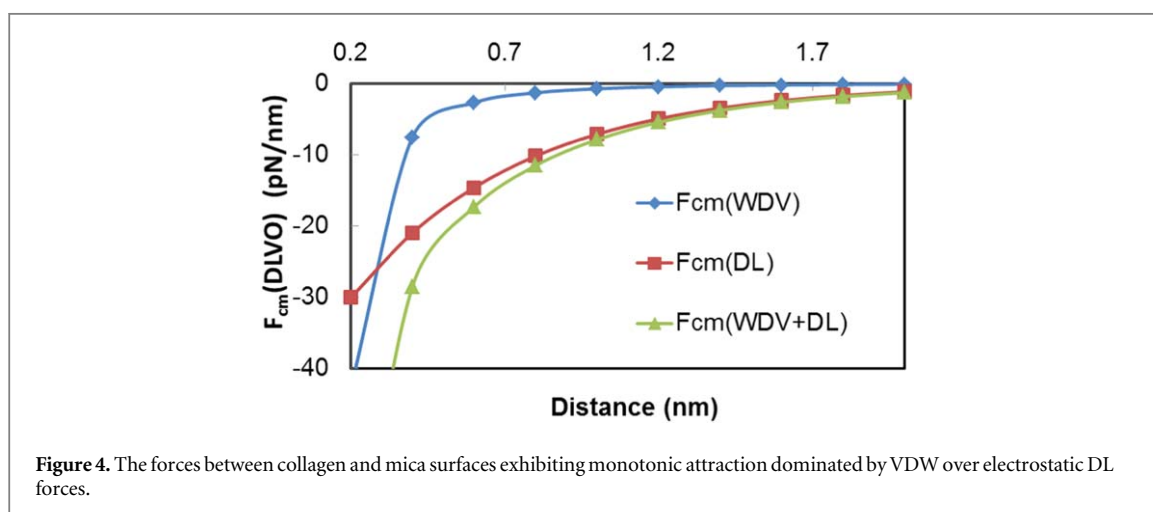


Figure 4. The forces between collagen and mica surfaces exhibiting monotonic attraction dominated by VDW over electrostatic DL forces.

attractive. Therefore, the theoretical DLVO force between a collagen and mica is a pure attraction and has a stronger magnitude compared to collagen-collagen interaction as shown in Figure 4. On the other hand, at contact distances, there should be additional interactions depending on detailed arrangements of amino acids, to drive assembly into D-periodic collagen fibrils, as explained below.

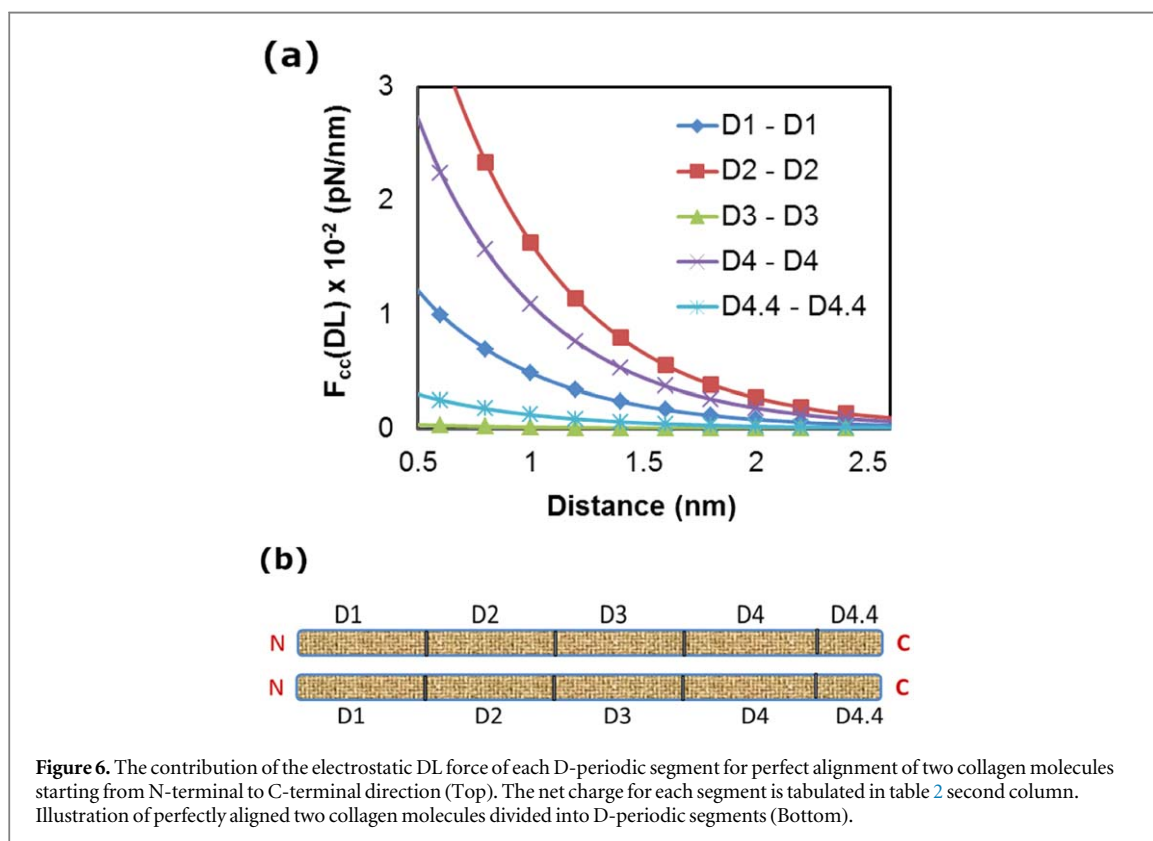
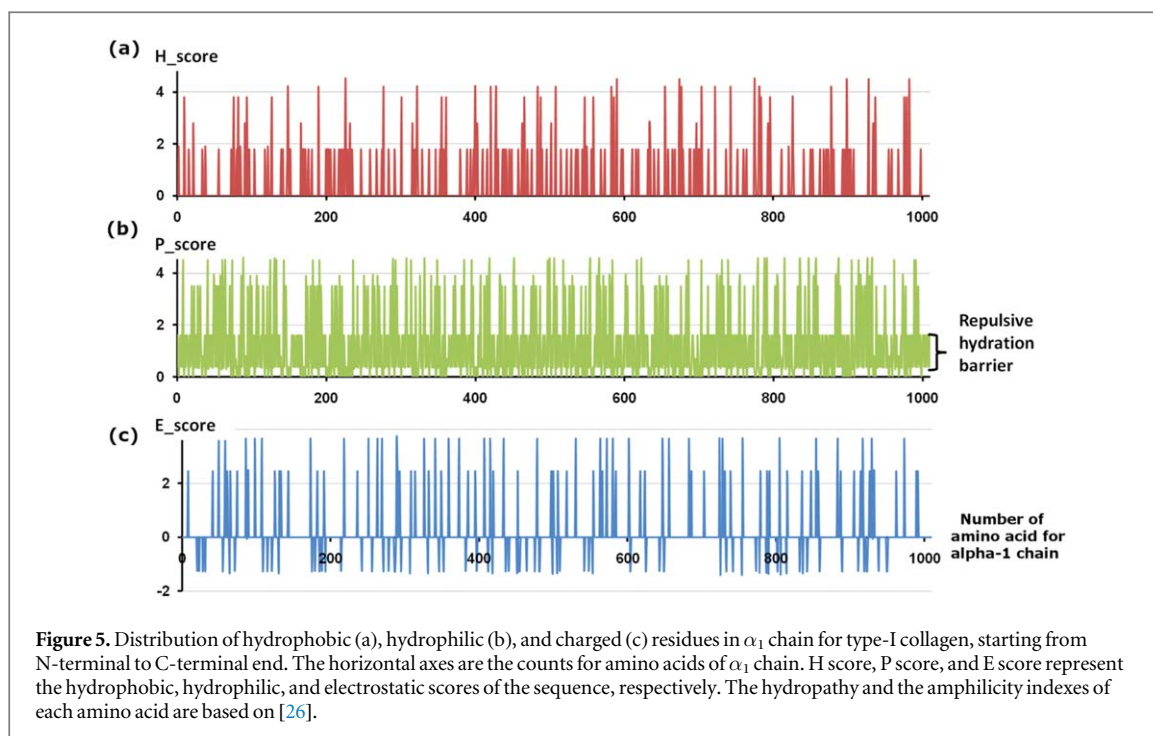
3.3. Computational analysis of collagen primary sequence

By considering linear alignment of collagen molecules in a fibril, we investigated the degree of electrostatic, hydrophobic, and polar hydrophilic interaction using collagen primary sequences.

3.3.1. Electrostatic interaction

Dependence of collagen interactions on amino acid sequence has been mostly investigated by experimental methods using synthetic collagen peptides.

The stabilizing effect of the electrostatic interactions has been studied by using host-guest peptides, and the importance of charged residues on the triple helix stability was revealed [31]. Kramer's experimental crystallization study of collagen-like peptide EKG, representing the sequence of $(PHG)_4EKG(PHG)_5$ in which P, H, G, E, and K represent Pro, Hyp, Gly, Glu, and Lys amino acids, respectively, reported that the presence of polar side-chains and adjacent opposite (intra-chain) charges are important for the staggered banding of collagen molecules [32]. Here, we investigate the distribution of charged, hydrophobic, and hydrophilic residues on α_1 chain of type-I collagen with respect to their amphiphilicity and hydrophathy indexes by using a script custom-written in Python (figure 5). We found that the positively and negatively charged amino acids are preferentially closely located. In addition, the charge distribution of the chain was nearly periodic. This indicates the role of the electrostatic interactions for the alignment of collagen molecules.



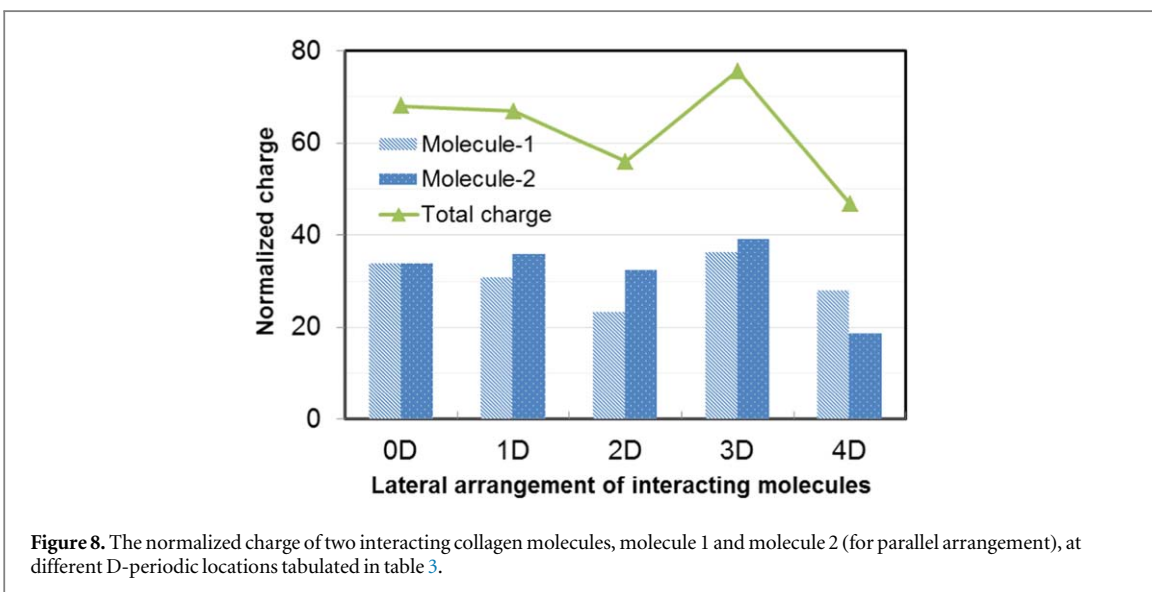
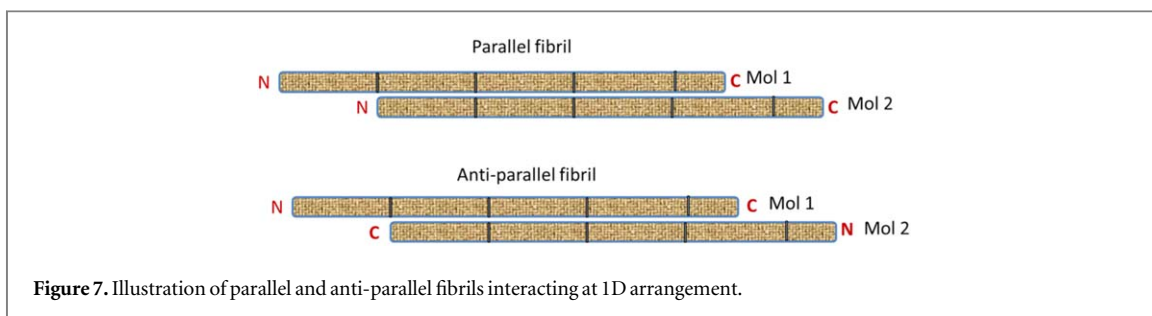
3.3.2. Hydrophobic/hydrophilic interaction

The most interesting result of our computational analysis in figure 5 is the arrangement of polar residues on the alpha chain. While the total non-polar index of the residues on the molecule corresponds to 17.35%, the polar index of residues constitutes 82.64% of the total number of residues in the whole chain. This provides a uniformly distributed repulsive hydration barrier across

the whole molecule. This may provide guidance in axial diffusion and ordering of collagen molecules after they encounter via the DLVO attraction.

3.3.3. D-periodic analysis of interactions

D-periodic segmental analysis of collagen molecules reveals an oscillatory nature of electrostatic repulsion throughout the molecule. The segments possessed



either a local minimum or a maximum of the net electrical charge. The periodic behaviour of the electrostatic interaction could help the molecule to find the most appropriate position by axial diffusion supported by hydration shell.

4. Conclusion

The main objective of this study is to present an analysis including macromolecular and sequence based analysis of the interactions between collagens and mica surface. Our macromolecular analysis using the DLVO theory elucidates the relative magnitudes of the VDW and DL forces. The stronger collagen-mica interaction than collagen-collagen interaction explains why the concentration of KCl has a significant and direct effect. Since KCl can alter the surface charge of mica, the adhesion is directly affected at the beginning of the adsorption of collagens [12, 33].

The calculated adhesion force of collagen molecules, -40.55 pN/nm, gives insights into the attraction of collagen molecules onto mica which is stronger than that between collagens, -10.95 pN/nm at contact distance. The calculated DLVO force between two collagen molecules $F_{cc}(DLVO)$ is on the same order of previous measurement [34]. In comparison, the repulsive hydration force is about 90 pN/nm.

Sequence level analysis of the primary structure of collagen molecules, provides more specific information regarding the arrangement of the molecules. The analysis showed that hydrophilic interaction between polar residues plays a significant role for the assembly, which explains why the long range attraction of the DLVO theory does not result in a random collapse of molecules. At contact distances, hydration force allows collagen molecules to slide and find energetically the most stable arrangement.

Although we can compare the relative contribution of the interactions, for further theoretical investigation of the D-period formation, additional analysis of the interactions incorporating the 3-dimensional structure of collagen molecules would be needed.

Conflict of Interest

EE, WT, and WH declare that they have no conflicts of interest to declare.

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