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1 Molecular dynamics studies on the aggregating behaviors of cellulose  
2 molecules in NaOH/urea aqueous solution

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13 **Abstract** Aggregating behaviors of cellulose molecules in NaOH/urea aqueous solution  
14 were investigated using a molecular dynamics (MD) simulation method. All the  
15 components, including cellulose, NaOH, urea and H<sub>2</sub>O were combined into the  
16 simulation. By monitoring the transformation of cellulose aggregates, we quantitatively  
17 revealed the solvent aggregating behaviors around cellulose molecules in the whole  
18 simulation process. And the aggregation evolution of solvent components around  
19 cellulose molecules, distributions of the solvent components in cellulose ICs, and the  
20 impacts of urea and NaOH in the dissolving process were discussed. In the simulation,  
21 the aggregated structure of cellulose molecules was dramatically changed and went  
22 through a dispersion process, equilibrium process and reaggregation process. The  
23 dramatic transformation of the cellulose aggregated structure indicates that the solvent  
24 components prefer to penetrate into the cellulose molecular sheets from the direction  
25 perpendicular to the molecular sheet. In the dispersion process, sodium ions around O6  
26 and hydroxide ions around O3 and O2 play crucial roles in the dissolution of cellulose.  
27 In the reaggregation process, the sodium ions near O6 and the urea around acetalic  
28 oxygen atoms impact the stability of cellulose inclusion complexes, and obvious  
29 decrements in these solvent components were observed.

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30 **Keywords** MD simulation; cellulose; dissolution; NaOH/urea aqueous solution

## 31 **1. Introduction**

32 Cellulose, a major structural component of plant cell walls, is one of the most  
33 abundant natural polymers on earth. Due to its biodegradability and renewability,  
34 cellulose has been utilized and exploited worldwide [1-3]. However, the strong  
35 interactions between cellulose chains, especially in the crystalline cellulose region  
36 where the long cellulose chains are tightly stacked together sheet by sheet, make native  
37 cellulose hard to infiltrate and dissolve in common solvents [4-7]. This recalcitrance to  
38 dissolve has long been a key challenge for the further application of cellulose.

39 Studies of highly efficient solvent systems for cellulose have been widely carried out  
40 and a series of outstanding solvent systems **has** been developed, such as N-  
41 methylmorpholine-N-oxide [8], lithium chloride/N,N-dimethylacetamide [9], ionic  
42 liquids [10], inorganic molten salt hydrates [11] and so on. Among these established  
43 solvent systems, the alkali/urea **or** alkali/thiourea aqueous solution has received  
44 considerable attention and is a promising solvent system for the dissolution of cellulose.  
45 Due to its simplicity, low cost, low toxicity, environmental friendliness and ease of  
46 recovery, this kind of solvent system has attracted much attention. Based on in-depth  
47 studies of mercerization and the viscose process, the dissolution of cellulose was first  
48 found at certain NaOH concentrations [12-13]. However, for native cellulose, the  
49 effective dissolution only occurred in cellulose with a low degree of polymerization. It  
50 was also found that additives could improve the dissolution [14]. However, alkali  
51 solutions with additives such as urea or thiourea did not attracted attention until the  
52 1990s. In 1990, Laszkiewicz demonstrated that urea or thiourea could significantly  
53 improve cellulose solubility in alkali solution systems [15-17]. Zhang's group has also  
54 studied the dissolution of cellulose in alkali/urea or alkali/thiourea aqueous systems.  
55 And a series of alkali/urea or alkali/thiourea aqueous systems with quick dissolving  
56 power have been developed [18-21]. These studies reported that the efficient dissolution  
57 of cellulose could be achieved in precooled 7 wt% NaOH/12 wt% urea [22], 4.6 wt%  
58 LiOH/15 wt% urea [23] or 9.5 wt% NaOH/4.5 wt% thiourea [24] within several  
59 minutes. This kind of solutions is a very valid dissolution system for native cellulose.

60 Some experimental studies have been done to reveal the dissolution mechanism of  
61 cellulose in NaOH/urea aqueous solution. Zhang and his coworkers reported that Na<sup>+</sup>  
62 has a direct interaction with cellulose and urea molecules, and proposed a model based  
63 on cellulose inclusion complexes (ICs) [25-26]. They proposed that Na<sup>+</sup> occupies the  
64 area of the hydroxyl groups of cellulose and plays a crucial role in breaking the  
65 hydrogen bonds within cellulose chains. The urea is bonded to NaOH hydrates on the  
66 surface of ICs and prevents the aggregation of cellulose chains [27]. The MD simulation  
67 method has also been used to study the dissolving process of cellulose, Miyamoto found  
68 the dense areas of Na<sup>+</sup> between O2 and O3 of cellulose and claimed that Na<sup>+</sup> penetrated  
69 into cellulose sheets promoting the separation of cellulose chains [28]. We have also  
70 described the detailed structure of cellulose ICs around a single cellulose chain using  
71 the molecular dynamics method [29].

72 Although the studies mentioned above provide useful knowledge for the dissolving  
73 mechanism of cellulose, there remains much controversy regarding the impact of alkali  
74 and urea in the dissolving process of cellulose, for example. Many researchers, such as  
75 Nishimura and Takahashi, agree that Na<sup>+</sup> breaks the intermolecular hydrogen bonds  
76 within cellulose chains [30-31]. However, Xiong indicated that the OH<sup>-</sup> breaks the  
77 hydrogen bonds and that Na<sup>+</sup> hydration stabilizes the hydrophilic hydroxyl groups [32].  
78 For the interaction between urea and cellulose, Egal and Isobe concluded that urea does  
79 not interact with either NaOH or cellulose [33-34]. However, Cai and Lund believe that  
80 the urea adsorbs onto the surface of the cellulose and the hydrogen bonding between  
81 urea and cellulose directly attenuates the hydrophobic effect of the cellulose chain [35-  
82 36]. We have also confirmed that urea molecules can form hydrogen bonds with the  
83 hydroxylic and acetalic oxygen atoms of cellulose [29]. In addition, some details remain  
84 unclear regarding the dissolving process of cellulose, such as how each of the solvent  
85 components behaves in the cellulose dissolution process, how the cellulose/NaOH/urea  
86 solution becomes a gel if the temperature increases, and what the detailed structure of  
87 the cellulose ICs is, and so on.

88 In this study, to gain deeper insights into the atomic details of cellulose dissolution,  
89 an MD simulation method was employed to investigate the aggregating behavior of

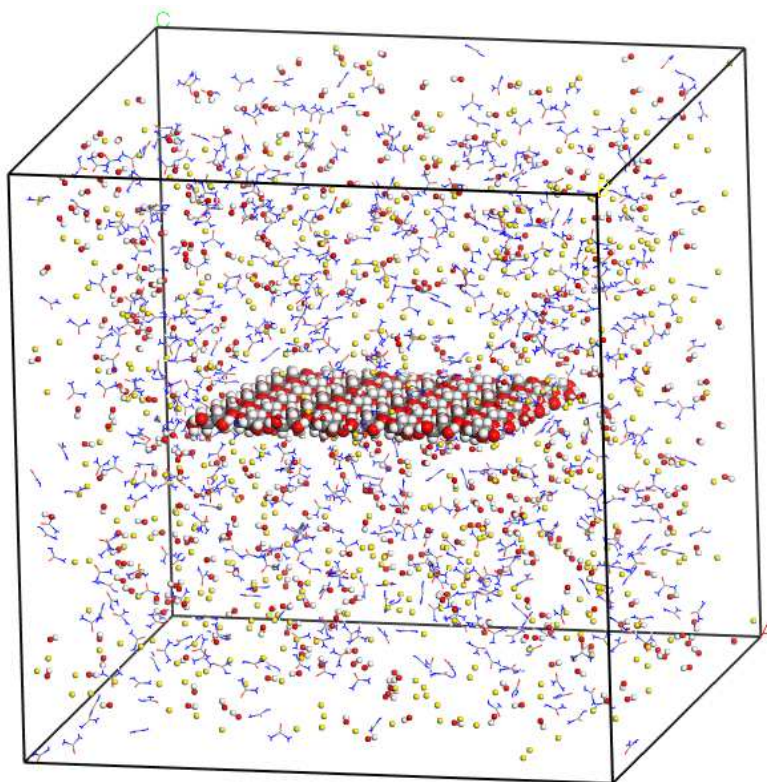
90 cellulose molecules in NaOH/urea aqueous solution. All the components, including  
91 cellulose, NaOH, urea and H<sub>2</sub>O were combined into the simulation. **Focusing on the**  
92 **complicated interaction between cellulose molecules and solvent particles, we**  
93 **investigated the transformation of cellulose aggregates in NaOH/urea aqueous solution.**  
94 **And we quantitatively revealed the aggregating behaviors of solvent particles around**  
95 **cellulose molecules in the whole simulation process. The aggregation evolution of**  
96 **solvent components around cellulose molecules, distributions of the solvent**  
97 **components in cellulose ICs, and the impacts of urea and NaOH in the dissolving**  
98 **process were also discussed.** We expect this work will give a better insight into the  
99 interaction between cellulose and solvent components, and promote the design of new  
100 cellulose aqueous solution systems.

## 101 **2. Computational procedure**

102 The MD simulation was carried out using the GROMACS free software package  
103 (version 2019.2). The cellulose was parameterized by the CHARMM36 carbohydrate  
104 force field [37-38]. The urea molecules were modeled using the same parameters used  
105 in the report by Weerashinghe and Smith [39]. The sodium ions and hydroxide ions  
106 were modeled with CHARMM36 parameters, and water was described by the TIP3P  
107 model. A sheet of cellulose with 6 cellulose molecules was extracted from an I $\beta$  crystal  
108 structure constructed using a cellulose-builder [40] and the degree of polymerization  
109 of the cellulose molecules was 8.

110 Initially, a cellulose sheet with 6 cellulose molecules, 524 sodium ions, 524  
111 hydroxide ions, 600 urea and 13437 TIP3P water molecules were randomly dispersed  
112 in a cubic box (8.12  $\times$  8.12  $\times$  8.12 nm) with periodic boundary conditions (Fig. 1). The  
113 periodic boundary conditions were applied in all directions. The initial configurations  
114 were conducted by energy minimization using the steepest descent method. Then, all  
115 the simulations were conducted in a normal pressure and temperature (NPT) ensemble  
116 for 2 ns to make the density of the system reach a reasonable value. Finally, a 120 ns  
117 MD simulation was carried out under the canonical ensemble (NVT). The time step  
118 was 2.0 fs. The temperature was kept constant at 261 K by the Berendsen thermostat  
119 algorithm [41]. The particle mesh Ewald (PME) method was used to treat the long-

120 range electrostatics [42]. All covalent bonds were constrained by the LINCS algorithm  
121 [43].



122  
123 Fig. 1. Setup of the simulation box. The urea molecules are displayed in line mode, sodium ions and  
124 hydroxide ions are displayed in ball and stick mode, and the cellulose molecules are displayed in  
125 the CPK mode. Colors for the atom scheme are O: red, C: gray, H: white and Na: yellow. The water  
126 molecules are not shown for clarity.

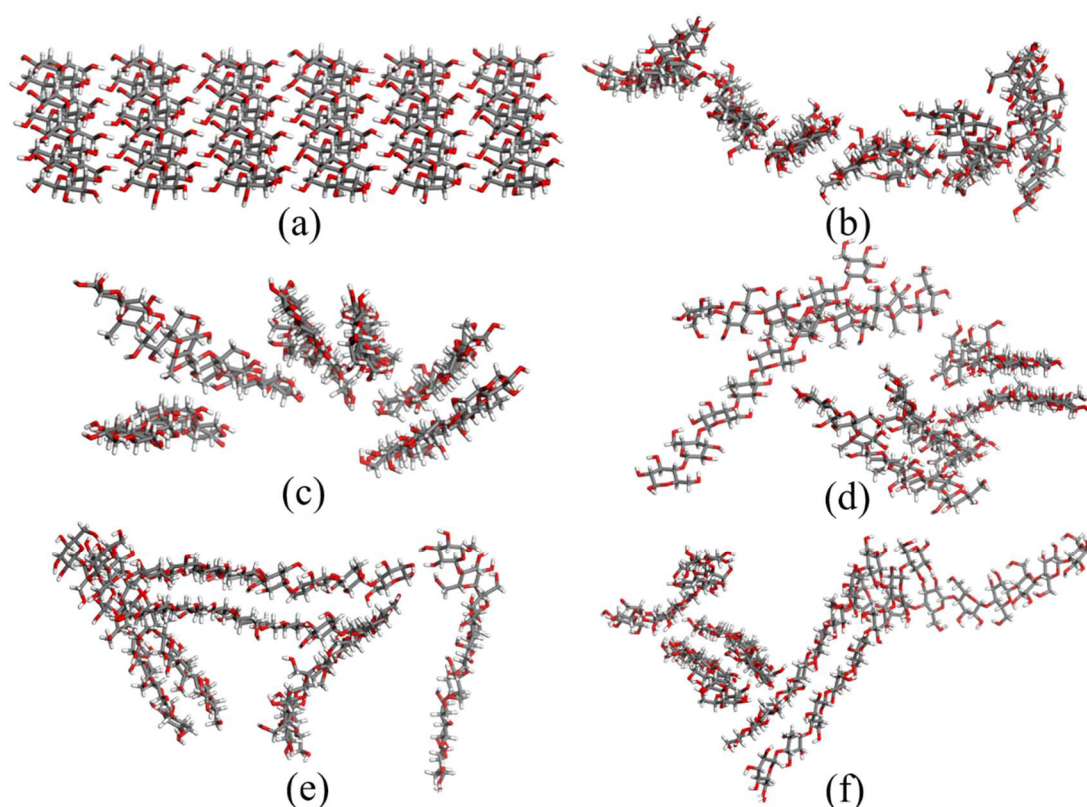
### 127 **3. Results**

#### 128 *3.1 Temporal evolution*

129 MD simulation can reveal the detailed structure at the molecular level and lead to a  
130 conclusion for the impact of alkali and urea in the dissolution process of cellulose. In  
131 this MD simulation, we were also interested in the possible transformation of the  
132 cellulose aggregated structure in NaOH/urea aqueous solution. As shown in Fig. 2, the  
133 aggregation behavior of cellulose molecules was examined. Fig. 2(a) shows the initial  
134 structure of cellulose molecules. Fig. 2(b) is the final aggregated structure of cellulose  
135 in the NPT simulation. The side-by-side cellulose sheet was rapidly disordered at the  
136 initial dissolving stage. A partial offset stacked configuration was obtained at the end  
137 of the NPT simulation. Fig. 2(c) shows the aggregated structure of cellulose molecules  
138 at 12 ns of NVT simulation. The ordered offset stacked configurations were formed.



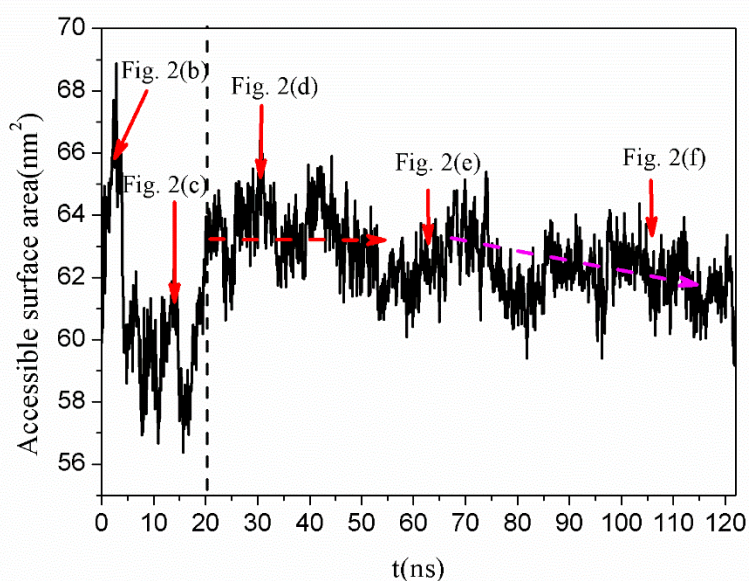
139 Following the transformation, the cellulose molecules rapidly dispersed into the  
140 precooled NaOH/urea aqueous solution. As shown in Fig. 2(d), two states of cellulose  
141 aggregates (a single molecule, bimolecular offset stacked aggregation) were obtained.  
142 In the following simulation from Fig. 2(d) to (f), the single molecule and bimolecular  
143 offset stacked configurations showed a relatively high stability. Although there were  
144 some stable junctions formed between cellulose molecules, no significant aggregating  
145 or dispersing actions were observed.



146  
147 Fig. 2. The evolution of the cellulose aggregated structure in NaOH/urea aqueous solution. (a) The  
148 initial aggregated structure at 0 ns of the NPT simulation; (b) the final aggregated structure at 2 ns  
149 of the NPT simulation; (c), (d), (e) and (f) show the cellulose aggregated structure for the NVT  
150 simulation at 12 ns, 28 ns, 60 ns and 102 ns, respectively.

151 To further confirm the aggregated structure of cellulose molecules in NaOH/urea  
152 aqueous solution, the solvent accessible surface area (SASA) of cellulose molecules  
153 was analyzed. SASA quantitatively represents the contact between cellulose molecules  
154 and solvent molecules. It can be used to understand the changes of the aggregated  
155 structure of cellulose molecules during the dissolving process. Fig. 3 shows the SASA  
156 profile. The red arrows are used to mark the aggregated morphologies of cellulose

157 molecules in Fig. 2. A significant fluctuation of SASA is observed in the NPT  
158 simulation and the initial period of NVT simulation. This is consistent with the  
159 transformation from side-by-side configuration to the offset stacked configuration of  
160 cellulose molecules. At about 3 ns, most of cellulose molecules stacked together and  
161 the SASA of the cellulose molecules decreased rapidly. At approximately 20 ns, the  
162 SASA of the cellulose molecules increases rapidly. This means that the ordered offset  
163 stacked aggregates of cellulose rapidly disperse into the NaOH/urea aqueous solution.  
164 After 20 ns, as marked by the red dashed line in Fig. 3, the fluctuation of SASA in the  
165 period from 20 ns to 70 ns is small and the average value of SASA is approximately  
166  $63.64 \text{ nm}^2$ . The fluctuation of SASA within a narrow range indicates that the dispersed  
167 structures of the cellulose molecules are in equilibrium. If the 6 cellulose molecules  
168 completely transformed into single molecular dispersion structures, the value of SASA  
169 would be  $80.88 \text{ nm}^2$ . The relatively smaller SASA obtained in the MD simulation also  
170 suggests that the cellulose molecules are partially aggregated in the NaOH/urea aqueous  
171 solution. It is also worth noting that the SASA profile shows a slight decreasing trend  
172 from 70 ns to 120 ns (marked by the magenta dashed arrow). This indicates that the  
173 cellulose molecules have a very slow aggregating trend.



174

175

Fig. 3. Solvent accessible surface area profile of cellulose during the simulation.

176

To analyze whether the rapid dispersion at about 20 ns is really due to the effect of



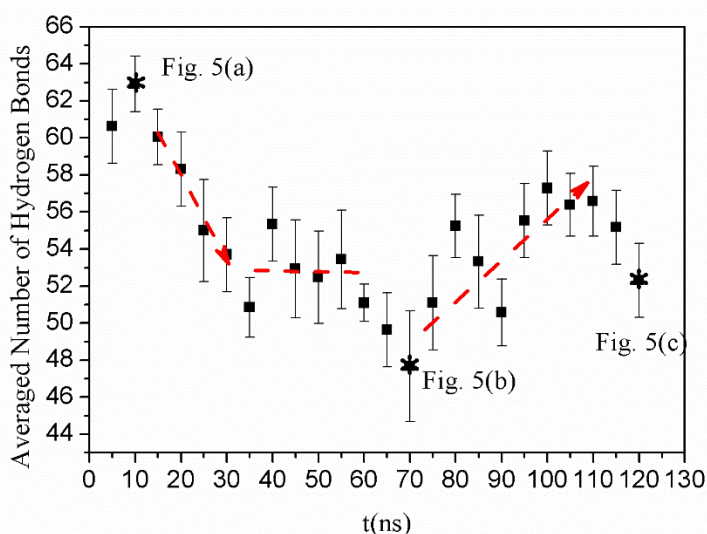
177 alkali species and urea molecules or just an artifact of the model itself, we conducted  
178 additional MD simulations of cellulose in NaOH aqueous solution and pure water. As  
179 shown in Supplementary material Fig. S1, the initial aggregation of cellulose molecules  
180 were also found in NaOH aqueous solution and pure water. That means the initial  
181 aggregation of cellulose molecules at 3 ns may be due to the effect of hydrophobic  
182 interaction of cellulose molecules. The subsequent rapid dispersion process at 20 ns  
183 was only found in NaOH/urea aqueous solution and NaOH aqueous solution. And the  
184 dispersion process in NaOH/urea aqueous solution was much earlier than that in NaOH  
185 aqueous solution. This clearly indicates that the dispersion process at about 20 ns in  
186 Fig.3 is mainly due to the effect of alkali species and urea molecules.

187 During the dissolution process, the aggregated structure of cellulose molecules  
188 changed dramatically in the NaOH/urea aqueous solution. The cellulose molecules  
189 rapidly stacked together in the initial stage within 3 ns. The intermolecular interactions  
190 within the side-by-side molecular sheet were disrupted and cellulose molecules  
191 preferred to offset stack together. With the formation of the offset stacked structure, the  
192 cellulose molecules rapidly dispersed into the solutions. An obvious dispersion process  
193 was observed at approximately 20 ns. After 20 ns, the stable dispersion configurations  
194 for the cellulose molecules, *i.e.*, single molecule and bimolecular offset stacked  
195 structure, were formed. In the following simulation, the single molecule and  
196 bimolecular offset stacked structure exhibited a relatively high stability. After 70 ns, the  
197 cellulose molecules exhibited a slowly reaggregating trend. The transformation from  
198 side-by-side molecular sheet to offset stacked structures also gives an insight for the  
199 understanding of cellulose crystal dissolution in the NaOH/urea aqueous solution. It is  
200 easy to conclude that the interactions between adjacent cellulose molecules within the  
201 side-by-side cellulose sheet are significantly weaker than those in the offset stacked  
202 structure. Then, we can predict that the disruption of the cellulose crystalline structures  
203 may first occur within the cellulose sheet in the dissolving process.

### 204 3.2 Hydrogen bonds

205 It is well known that intermolecular and intramolecular hydrogen bonds play a  
206 crucial role in the stability of cellulose. Therefore, the hydrogen bonds within the

207 cellulose network should be first broken in the dissolving process, especially for the  
 208 **intramolecular and intermolecular** hydrogen bonds. In this work, according to the  
 209 general hydrogen bonding criteria, *i.e.*, the distance between the donor and the acceptor  
 210 should be less than 0.35 nm, and the angle is less than 30 degree, the variations of the  
 211 hydrogen bonds within a cellulose sheet during the NVT simulation were also analyzed  
 212 to gain deeper insights for the dissolution of cellulose. As shown in Fig. 4, the total  
 213 number of hydrogen bonds within and between cellulose molecules decreases with the  
 214 running of the NVT simulation at the initial time. The decrement of hydrogen bonds  
 215 could be attributed to the disruption of the intermolecular hydrogen bonds. The relative  
 216 stability of hydrogen bond number from 30 ns to 60 ns in the MD simulation illustrates  
 217 a possible equilibrium for the cellulose dispersion configuration. After 70 ns, the  
 218 increment in the hydrogen bonds presents the reaggregating process for the cellulose  
 219 molecules.



220

221 Fig. 4. The average number of hydrogen bonds within and between cellulose molecules over NVT  
 222 simulation time. Every point was averaged over 5 ns of MD trajectories.

223 To further study the relationship between the amount of hydrogen bonds and the  
 224 cellulose aggregated structure, the hydrogen bonding configurations of cellulose  
 225 marked with a star in Fig. 4 are shown in Fig. 5. Fig. 5(a) shows the hydrogen bonding  
 226 structure of cellulose molecules at 12 ns. Due to the stacked configurations of cellulose  
 227 molecules, many more intermolecular hydrogen bonds were observed. At 70 ns,