Support Information

Lu et al

Dynamic Light Scattering

As larger particles with a larger hydrodynamic radius diffuse slower in bulk solution, the autocorrelation function can thus distinguish the particle sizes by calculating the diffuse time. However, this means it can not detect the precise shape of the measured molecules. In this work, the detection angle was 173° with respect to the incoming beam. Samples analyzed were contained in a 1 cm path length quartz cell, and the data were analyzed using Malvern Instruments Dispersion Technology Software. The polymer refractive index was taken to be 1.45 with an absorbance of 0.001. The viscosity and refractive index of water were taken as 0.8872 cPa and 1.330, respectively.

Small Angle Neutron Scattering

SANS measures the differential scattering cross section which contains information about particle size, shape and interactions between particles. The scattering intensity from a sample solution can be expressed as

\[ I(q) = NV^2\Delta\rho^2P(q)S(q) + B \]  

where \( q \), \( N \), \( V \) and \( \Delta\rho \) are the momentum transfer, the number concentration of scattering particles, the volume of one scattering particle and the contrast of scattering length density between the scattering particles and the bulk solution, respectively. \( P(q) \) is the form factor that represents the interference of neutrons scattered from different parts of the same object while...
S(q) indicates the interference of neutrons scattered from different objects. B is the background signal. As S(q) is determined by interference effect between scattering particles, it is dependent on the concentrations of scattered particles in solution. In this work, the concentration of keratin solution is so small that S(q) can be regarded as unity. Thus the scattered intensity I(q) is only determined by proportion to the variable P(q). Here, an ellipsoidal model was used as a fitting model for keratin solution. The output of the 2D scattering intensity function for oriented ellipsoids is given by

\[ P(q,\alpha) = \frac{\text{scale}}{V} f^2(q) + bkg \]  

(10)

\[ f(q) = \frac{3(\Delta \rho) V (\sin[qr(R_a R_b, \alpha)] - qr\cos[qr(R_a R_b, \alpha)])}{qr(R_a R_b, \alpha)^3} \]  

(11)

\[ r(R_a R_b, \alpha) = \left[R_b^2 \sin^2 \alpha + R_a^2 \cos^2 \alpha\right]^{1/2} \]  

(12)

where \( \alpha \) is the angle between the axis of the ellipsoid and the beam; V is the volume of the ellipsoid; \( R_a \) and \( R_b \) are the lengths along and perpendicular to the rotation axis of the ellipsoid respectively; \( \Delta \rho \) is again the difference of scattering length density between the scattered particle and bulk solution.

The fitting process uses the iterated method until an acceptable fit is produced. By comparing the calculated profile from the ellipsoidal model with the measured scattering profile, the geometrical shape and size of the protein molecule in bulk solution can be obtained. To make SANS data comparable to the NR work, the measurements are conducted at pH 6 with NaCl concentration fixed at 5 mMs.

The normal ellipsoidal model did not contain the volume fraction of the scattered particles in solvent. Thus we first used a Hayter-MSA structure factor that was introduced to complement
the fitting parameters needed in this study. In general, the mean spherical approximation (MSA)\(^3\) method can describe the structure of a dispersion of charged colloidal particles interacting through a screened Coulombic potential. It was found from MSA fittings that the scattered particle in solvent was very weakly charged and could be treated as no charge under low salt concentrations. Thus the MSA method is no longer appropriate in high salt systems. In the zero charge limits, the Percus-Yevick hard sphere solution can then be recovered\(^4\). It assumes the inter-particle potential is given by

\[
U(r) = \begin{cases} 
\infty, & r < 2R \\
0, & r > 2R 
\end{cases}
\]  

(13)

The Percus-Yevick method provides very accurate approximation for particle volume fractions of up to \(\sim 0.45\)\(^5\). The hard sphere structure method combined with ellipsoidal model calculates the inter-particle structure factor for mono-disperse spherical particles that interact through hard sphere interactions. In this case, the form factor \(S(Q)\) can no longer be treated as unity but be approximated as \(^6\)

\[
S(q) = \left[1 + \frac{24\eta_{HS}G(2R_{HS}q)}{2R_{HS}q}\right]^{-1}
\]  

(14)

The two variables in the \(S(q)\) equation are the hard sphere volume fraction \(\eta_{HS}\) and the corresponding hard sphere radius \(R_{HS}\). In data analysis, the fitting parameters contained the background, the two radii of the ellipsoid, the scale factor, the volume fraction and the scattering length densities of the scattered particles and solvents. The scale factor and scattering length density of solvent were fixed at 1 and \(6.35 \times 10^{-6}\), respectively. Thus the only variables left in the fitting process were the ellipsoidal radii \(a\) and \(b\), the scattering length density and the volume fraction of the scatters.
Table SI1 Comparison of the scattering length density (SLD) values as calculated from three different keratins and BSA. This illustrates that despite different sequences their SLDs in a given solvent (H₂O, D₂O or their mixtures) are virtually the same. 7

<table>
<thead>
<tr>
<th></th>
<th>SLD in D₂O (×10⁻⁶ Å⁻²)</th>
<th>SLD in H₂O (×10⁻⁶ Å⁻²)</th>
<th>SLD in NRW (×10⁻⁶ Å⁻²)</th>
<th>Molecular Weight (×10³ g/mol)</th>
<th>Molecular Volume (×10³ Å³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Hair Keratin (a3)</td>
<td>3.46</td>
<td>1.94</td>
<td>2.07</td>
<td>46.2</td>
<td>54.9</td>
</tr>
<tr>
<td>Mouse Hair Keratin (a1)</td>
<td>3.42</td>
<td>1.94</td>
<td>2.06</td>
<td>47.9</td>
<td>56.8</td>
</tr>
<tr>
<td>Sheep Wool Keratin (47.6)</td>
<td>3.40</td>
<td>1.91</td>
<td>2.03</td>
<td>47.3</td>
<td>56.6</td>
</tr>
<tr>
<td>Bovine Serum Albumin</td>
<td>3.45</td>
<td>1.97</td>
<td>2.09</td>
<td>69.3</td>
<td>83.4</td>
</tr>
</tbody>
</table>

The sequence of the sheep wool keratin (47.6 kD) taken from the work by Wilson et al (Wilson, B.W.; Edwards, K.J.; Sleigh, M.J.; Byrne, C.R.; Ward, K.A., *Gene* 1988, 73, 21-31) and the helical forming segments were marked in red (bold) following the work by Yu et al (Yu, J.; Yu, D.-W.; Checkla, D. M.; Freedberg, I. M.; Bertolino, A. P. *Journal of investigative dermatology* 1993, 101, 56S)

MSPAPCPALSPASSCSSAPCVPSSCCGTT 29
LPGACAIASPVGSCATPCGGSPAGAGLGM 59
GPLAAALASTLGLVAGLGAGAGLGAAILG 89
ASGGGGGPLVCPATGSPATIGGLGGLLICG 119
LSGAAALVVGIAAALLASAAPATLGTGVS 149
LAGLVGAAALGLAILAGLTCLSLGAAV 179
GSLLGGLICLLGAHGGGVATLASGLGAALA 209
VGVAAAPTVALAHVLAGTAAAGTGALVGTA 239
AAVGGTTIAAGTGGLAGVSSSGGLGSCGA 269
GIIGLAAATVAALGVGLGAGHALAASLGLATL 299
TGTGAATSCGLAGVGSLIVSVGSGLAGIAS 329
ALGAGAGGTVGLVVAAALGCGIATTAGLL 359
ASGACLLPCAPCATTATCGLPICISA 387
PCVSATACGPCATPVH 403
**Table SI2** Two layer model fits to the reflectivity profiles of keratin measured with 5 mM and 500 mM NaCl under NRW.

<table>
<thead>
<tr>
<th>[NaCl] (mM)</th>
<th>layer</th>
<th>Thickness (Å) ±1</th>
<th>SLD (×10⁻⁶ Å⁻²) ±0.02</th>
<th>Volume fraction ±0.02</th>
<th>Layer mass (mg/m²) ±0.03</th>
<th>Total mass (mg/m²) ±0.03</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>1</td>
<td>19</td>
<td>1.05</td>
<td>0.52</td>
<td>1.3</td>
<td>1.65</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>25</td>
<td>0.2</td>
<td>0.1</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>25</td>
<td>1.1</td>
<td>0.55</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>26</td>
<td>0.22</td>
<td>0.11</td>
<td>0.39</td>
<td>2.29</td>
</tr>
</tbody>
</table>

**Table SI3** Ellipsoidal model fits to the scattering profiles of keratin measured with 5 mM NaCl in D₂O.

<table>
<thead>
<tr>
<th>Ellipsoid model</th>
<th>Keratin 1 mg/ml</th>
<th>Keratin 0.5 mg/ml</th>
<th>Keratin 0.25 mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLD Ell (×10⁻⁶ Å⁻²) ±0.02</td>
<td>4.48</td>
<td>4.48</td>
<td>4.48</td>
</tr>
<tr>
<td>SLD Solv (×10⁻⁶ Å⁻²) ±0.02</td>
<td>6.35</td>
<td>6.35</td>
<td>6.35</td>
</tr>
<tr>
<td>Radius a (Å) ±2</td>
<td>60</td>
<td>60</td>
<td>59</td>
</tr>
<tr>
<td>Radius b (Å) ±8</td>
<td>138</td>
<td>139</td>
<td>138</td>
</tr>
</tbody>
</table>

**Table SI4** Ellipsoidal model fits to the scattering profiles of keratin measured at 1 mg/ml, with NaCl changed from 5 mM to 0.5 M in D₂O, pD 6.3.

<table>
<thead>
<tr>
<th>Ellipsoid model</th>
<th>[NaCl] = 5mM</th>
<th>[NaCl] = 20mM</th>
<th>[NaCl] = 40mM</th>
<th>[NaCl] = 100mM</th>
<th>[NaCl] = 200mM</th>
<th>[NaCl] = 500mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLD Ellip (×10⁻⁶ Å⁻²) ±0.02</td>
<td>4.48</td>
<td>4.5</td>
<td>4.53</td>
<td>4.58</td>
<td>4.69</td>
<td>5.28</td>
</tr>
<tr>
<td>Radius a (Å) ±2</td>
<td>60</td>
<td>59</td>
<td>59</td>
<td>55</td>
<td>50</td>
<td>37</td>
</tr>
<tr>
<td>Radius b (/Å) ±8</td>
<td>138</td>
<td>168</td>
<td>201</td>
<td>260</td>
<td>280</td>
<td>350</td>
</tr>
<tr>
<td>Volume fraction</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Particle volume (×10⁴/Å³) ±0.02</td>
<td>1.94</td>
<td>2.37</td>
<td>2.55</td>
<td>3.54</td>
<td>2.93</td>
<td>2.01</td>
</tr>
<tr>
<td>Volume fraction of keratin</td>
<td>0.58</td>
<td>0.59</td>
<td>0.6</td>
<td>0.61</td>
<td>0.61</td>
<td>0.39</td>
</tr>
<tr>
<td>No. of keratin molecules in one aggregate</td>
<td>21</td>
<td>25</td>
<td>27</td>
<td>38</td>
<td>31</td>
<td>14</td>
</tr>
</tbody>
</table>
Figure S11 SDS PAGE of keratin extracted from the method utilized in this work (K1S) and that extracted by a slightly modified method without SDS (K2S).
**Figure S12** Cryo-EM image of 1 mg/ml keratin in 5 mM NaCl solution. The dashed line represented the area that was shown in Figure 4.
Figure S13 Cryo-EM image of 1 mg/ml keratin in 500 mM NaCl solution. The dashed line represented the area that was shown in Figure 7.
Figure S14 NR reflectivity profiles measured from keratin adsorption onto the D$_2$O surface at concentrations of $3 \times 10^{-3}$ (○), $1 \times 10^{-2}$ (□), $3 \times 10^{-2}$ (△), 0.3 (α) mg/ml. Solutions were prepared in 5 mM NaCl at pH 6.7±0.1. Solid lines indicate the best fits to the data measured.
Figure SI5 The effect of keratin concentration on its aggregation studied at 0.25 (○), 0.5 (△) and 1 (□) mg/ml by SANS. All solutions were prepared in buffers with 5 mM NaCl at pH 6.1. All the scattering curves were able to be fitted with the same size and shape of aggregates, indicating that they remained the nanostructure over this concentration range.
Figure S16 Keratin surface adsorbed amounts obtained from NR plotted against solution concentration, with all data measured after 60 minutes adsorption. The blue diamond dots are measured at keratin solution with 5 mM NaCl at concentrations from 0.003 to 1 mg/ml while the red square dots are keratin solution with 0.5 M NaCl at concentrations of 0.03 and 0.3 mg/ml.

References