Supplementary Material

Microwave-assisted Synthesis, Characterization, Cell Imaging of Fluorescent Carbon Dots Using L-Asparagine as Precursor

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1. **Theoretical calculated result of relative content of N, C, O in the 20 amino acids.**

**Table S1 Relative contents of C, O, N in the 20 amino acids.**

<table>
<thead>
<tr>
<th>Atomic content/%</th>
<th>N</th>
<th>C</th>
<th>O</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine ((C_3H_7NO_2))</td>
<td>17.18</td>
<td>43.93</td>
<td>38.99</td>
<td></td>
</tr>
<tr>
<td>Arginine ((C_6H_{14}N_4O_2))</td>
<td>35.00</td>
<td>45.01</td>
<td>19.99</td>
<td></td>
</tr>
<tr>
<td>Asparagine ((C_4H_8N_2O_3))</td>
<td>22.73</td>
<td>38.73</td>
<td>38.70</td>
<td></td>
</tr>
<tr>
<td>Aspartic acid ((C_4H_7NO_4))</td>
<td>11.12</td>
<td>38.12</td>
<td>50.76</td>
<td></td>
</tr>
<tr>
<td>Cysteine ((C_3H_7NO_2S))</td>
<td>12.29</td>
<td>31.62</td>
<td>14.04</td>
<td>28.14</td>
</tr>
<tr>
<td>Glutamic acid ((C_3H_3NO_4))</td>
<td>10.15</td>
<td>43.50</td>
<td>46.35</td>
<td></td>
</tr>
<tr>
<td>Glutamine ((C_4H_{10}N_2O_3))</td>
<td>20.60</td>
<td>44.14</td>
<td>35.26</td>
<td></td>
</tr>
<tr>
<td>Glycine ((C_2H_3NO_2))</td>
<td>20.01</td>
<td>34.30</td>
<td>45.69</td>
<td></td>
</tr>
<tr>
<td>Histidine ((C_6H_{12}N_3O_2))</td>
<td>28.77</td>
<td>49.33</td>
<td>21.90</td>
<td></td>
</tr>
<tr>
<td>Isoleucine ((C_6H_{13}NO_2))</td>
<td>11.87</td>
<td>61.05</td>
<td>27.08</td>
<td></td>
</tr>
<tr>
<td>Leucine ((C_6H_{13}NO_2))</td>
<td>11.87</td>
<td>61.05</td>
<td>27.08</td>
<td></td>
</tr>
<tr>
<td>Lysine ((C_6H_{14}N_2O_2))</td>
<td>21.21</td>
<td>54.56</td>
<td>24.23</td>
<td></td>
</tr>
<tr>
<td>Methionine ((C_3H_{11}O_2NS))</td>
<td>10.14</td>
<td>43.48</td>
<td>23.17</td>
<td>23.21</td>
</tr>
<tr>
<td>Phenylalanine ((C_9H_{11}NO_2))</td>
<td>9.09</td>
<td>70.15</td>
<td>20.76</td>
<td></td>
</tr>
<tr>
<td>Proline ((C_5H_9NO_2))</td>
<td>13.21</td>
<td>56.63</td>
<td>30.16</td>
<td></td>
</tr>
<tr>
<td>Serine ((C_3H_7NO_3))</td>
<td>14.29</td>
<td>36.75</td>
<td>48.96</td>
<td></td>
</tr>
<tr>
<td>Threonine ((C_4H_9NO_3))</td>
<td>12.73</td>
<td>43.66</td>
<td>43.61</td>
<td></td>
</tr>
</tbody>
</table>
Tryptophan (C\textsubscript{11}H\textsubscript{12}N\textsubscript{2}O\textsubscript{2}) 14.58 68.76 16.66
Tyrosine (C\textsubscript{9}H\textsubscript{11}NO\textsubscript{3}) 8.24 63.55 18.81
Valine (C\textsubscript{5}H\textsubscript{11}NO\textsubscript{2}) 13.21 56.63 30.16

2. Photos of A-CDs under powder and solution condition.

Yellow powders of A-CDs were got by freeze drying (Figure S1a). And dissolving these yellow powders into deionized water, a light yellow solution was obtained (Figure S1b). Put the A-CDs solution under a 365 nm UV irradiation lamp, it emits a bright blue photoluminescence.

Fig. S1 Photos of A-CDs. (a) The powder of A-CDs after vacuum freezing drying. (b) The A-CDs solution in white light. (c) The A-C solution under 365 nm excitation.

3. Dynamic Light Scattering result of A-CDs
Figure S2 The Dynamic Light Scattering result of A-CDs.

4. Elemental content of A-CDs.

The relative elemental contents of A-CDs were determined by XPS. The relative contents of C, O, N were 58.03%, 32.53%, 9.44%, indicating the doping of nitrogen atoms.

Table S2 Relative contents of C, O, N.

<table>
<thead>
<tr>
<th>Atomic content/%</th>
<th>C</th>
<th>O</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>58.03</td>
<td>32.53</td>
<td>9.44</td>
</tr>
</tbody>
</table>

5. Possible structure of A-CDs.

Fig. S3 Possible structure of A-CDs.
6. PL lifetime of A-CDs.

The average lifetimes were calculated using the equation below:

\[
\tau_{\text{average}} = \frac{\sum_{i=1}^{n} a_i \cdot \tau_i^2}{\sum_{i=1}^{n} \tau_i}
\] (1)

In the equation, \(\tau\) means the decay lifetime, \(a\) is the fractional contribution of decay lifetime, \(n\) stands for total number of fractions, and \(i\) represents each fraction.

![Fig. S4 The time-correlated single-photon counting (TCSPC) of C-dots (360 nm excitation, decay time at 450 nm).](image)

7. Quantum yield calculation of A-CDs.

The quantum yield of A-CDs was measured by reference method, using quinine sulfate (QS) in 0.1 M \(\text{H}_2\text{SO}_4\) as reference. The quantum yield calculation of A-CDs was according to the equation below:

\[
Q = Q_R \cdot \frac{I}{I_R} \cdot \frac{A_R}{A} \cdot \frac{n^2}{n_R^2}
\] (2)

In the equation, \(Q\) represents the quantum yield, \(I\) means the integrated emission intensity measured by FL emission spectroscopy, \(A\) is the UV-vis absorbance at PL
excitation wavelength, $n$ stands for the refractive index, and $R$ is the reference.

Fig. S5 Liner fitting of integrated emission intensity and absorbance of Quinine Sulfate (a) and A-CDs (b).

Table S3 Calculation of the quantum yield of A-CDs.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Integrated emission Intensity ($I$)/Absorbance ($A$)</th>
<th>Refractive index of solvent ($n$)</th>
<th>Quantum yield ($Q$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinine Sulfate</td>
<td>2975870</td>
<td>1.34</td>
<td>55.7%</td>
</tr>
<tr>
<td>A-CDs</td>
<td>419330</td>
<td>1.34</td>
<td>7.8%</td>
</tr>
</tbody>
</table>

More than four groups of data were used to obtain the liner fitting of each sample (Figure S3). Results were shown in Table S2, the quantum yield of A-CDs was 7.8%, using Quinine Sulfate as standard.

8. Photos and PL emission spectrum of A-CDs after stored for over 6 months, and antiphotobleaching property of A-CDs.

The A-CDs could still emit bright blue photoluminescence, as shown in Figure S5 inset photograph. And the fluorescent intensity of A-CDs remains over 80%, after being irradiated under 365 nm UV lamp for 2 hours.

There was no obvious PL intensity change of A-CDs under various conditions including of persistent excitation, different pH solutions, different ionic strengths, and different incubation time in DMEM culture.
Fig. S7 Stability of A-CDs. (a) Dependence of fluorescence intensity on persistent excitation times for the A-CDs. (b) Effect of pH on the fluorescence intensity of the A-CDs. (c) Effect of ionic strengths on the fluorescence intensity of the A-CDs (ionic strengths were controlled by various concentrations of NaCl). (d) Dependence of A-CDs PL emission intensity on incubation time in DMEM culture.

10. Influence of various ions on A-CDs in PBS buffer.

As illustrated in Figure S8, there is nearly no influence on the PL intensity of the A-CDs when the A-CDs’ solution mix with various ions (up to 400 μM) in PBS buffer.
Fig. S8 Normalized PL emission intensity of A-CDs in PBS buffer with 400 μM ions.

References
