Supplementary Information

for

Stable nanoconjugate of transferrin with alloyed quaternary nanocrystals Ag-In-Zn-S as biological entity for tumor recognition

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Fig. S1. Energy-dispersive spectra of Ag-In-Zn-S, Ag-In-Zn-S/MUA and Ag-In-Zn-S/MUA-Tf nanocrystals.
Fig. S2. Job plot for coordination of Tf to QD as measured by fluorescence spectroscopy.
The number of transferrin units in Ag-In-Zn-S/MUA-Tf

The total number of atoms (N) in the average-radius ($r = 2.88$ nm) Ag-In-Zn-S/MUA-Tf calculated as follows:

$$V_1 = \frac{4}{3} \pi r^3 = 1.00 \times 10^{-25} \text{ m}^3, \quad V_2 = a^3 = 1.57 \times 10^{-28} \text{ m}^3$$

$$V_1/V_2 = 635, \quad \text{CN} = 4, \quad N = 2540$$

where $V_1$ is the volume of the nanocrystals, $V_2$ is the volume of the elementary cell ($a = 5.4$ Å is the lattice constant) and CN is the coordination number.

To calculate the number of iron-saturated transferrin units in Ag-In-Zn-S/MUA-Tf the relationship between the molar ratio of elements (Ag/Fe = 1.000/0.025) in the hybrid (from EDS) was used, taking into account the total number of Ag atoms in an individual nanocrystal i.e.:

An average nanocrystal of the following stoichiometry $\text{Ag}_{1.0}\text{In}_{1.0}\text{Zn}_{1.0}\text{S}_{3.0}$ contains 2540 atoms. The Ag share is therefore $2540/(1+1+1+3) = 423$ (Ag$_{423}$In$_{423}$Zn$_{423}$S$_{1269}$), Ag/Fe = 1.000/0.025, the number of iron atoms in Ag-In-Zn-S/MUA-Tf = 423 × 0.025 ≈ 10, the number of transferrin units in Ag-In-Zn-S/MUA-Tf = 10/2 = 5.

The molecular mass of Ag-In-Zn-S/MUA nanocrystals

The molecular mass of inorganic core $\text{Ag}_{423}\text{In}_{423}\text{Zn}_{423}\text{S}_{1269}$ is 162554 g/mol. The total molecular mass of Ag-In-Zn-S/MUA nanocrystals calculated as follows: $(162554 \times 100)/24.8 = 653878$ g/mol where 24.8 is the mole percent of Ag+In+Zn+S in nanocrystals (from EDS).

**EDS analysis of transferrin-functionalized Ag-In-Zn-S nanocrystals**

<table>
<thead>
<tr>
<th>Element</th>
<th>Atomic%</th>
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<tbody>
<tr>
<td>Ag L</td>
<td>3.95 ± 0.47</td>
</tr>
<tr>
<td>In L</td>
<td>4.19 ± 0.51</td>
</tr>
<tr>
<td>Zn K</td>
<td>4.23 ± 0.39</td>
</tr>
<tr>
<td>S K</td>
<td>12.02 ± 0.47</td>
</tr>
<tr>
<td>Fe K</td>
<td>0.10 ± 0.04</td>
</tr>
<tr>
<td>C K</td>
<td>52.39 ± 2.80</td>
</tr>
<tr>
<td>O K</td>
<td>21.31 ± 1.66</td>
</tr>
<tr>
<td>Si K</td>
<td>0.68 ± 0.06</td>
</tr>
</tbody>
</table>
Calculations of maximal mass of DOX per 1 g of QD and 1 g of nanoconjugate Tf-QD:

<table>
<thead>
<tr>
<th></th>
<th>QD</th>
<th>DOX</th>
<th>Tf-QD</th>
</tr>
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<tbody>
<tr>
<td>( \phi_{QD} )</td>
<td>9.1 \times 10^{-9} m</td>
<td>1.5 \times 10^{-9} m</td>
<td>12.8 \times 10^{-9} m</td>
</tr>
<tr>
<td>( r_{QD} )</td>
<td>4.55 \times 10^{-9} m</td>
<td>0.75 \times 10^{-9} m</td>
<td>6.4 \times 10^{-9} m</td>
</tr>
</tbody>
</table>

\( ^* \) the diameter of doxorubicin was determined with the help of the ChemSketch program where it was assumed that the molecules are circular.

\[ S_{QD} = 4 \pi r_{QD}^2 = 4 \cdot 3.14 \cdot (4.55 \times 10^{-9})^2 = 2.60 \times 10^{-16} \text{ m}^2 \]

\[ S_{DOX} = \pi r_{DOX}^2 = 3.14 \cdot (0.75 \times 10^{-9})^2 = 1.77 \times 10^{-18} \text{ m}^2 \]

It was assumed that DOX molecules on the surface of QD are 2D close packed, and its maximal number per one QD molecule is:

\[ \frac{S_{QD}}{S_{DOX}} = 144 \text{ molecules} \]

Since the 1 mol of doxorubicin (579.98 g·mol\(^{-1}\)) contains 6.023 \times 10^{23} molecules, 144 molecules of DOX correspond to the mass 1.39 \times 10^{-19} g DOX per one QD molecule. Taking into account the composition of QD: Ag\(_{1.00}\)In\(_{3.10}\)Zn\(_{1.00}\)S\(_{4.00}\), the density of QD determined on the basis of density of its components is 5.2 g·cm\(^{-3}\), so the volume of 1 g of QD equals 0.192 cm\(^3\) and the volume of a single QD molecule (\(V_{QD}\)) is 3.94 \times 10^{-19} cm\(^3\); thus, the number of QD in 1 g is 4.87 \times 10^{17}.

\[ V_{QD} = \frac{4}{3} \pi r^3 = \frac{4}{3} \cdot 3.14 \cdot (4.55 \times 10^{-9})^3 = 3.94 \times 10^{-25} \text{ m}^3 = 3.94 \times 10^{-19} \text{ cm}^3 \]

So maximal mass of DOX per 1 g of QD equals: 1.39 \times 10^{-19} \cdot 4.87 \times 10^{17} = 68 mg DOX.

In the case of attachment of DOX to nanoconjugate Tf-QD the maximal mass of DOX anchored to 1 g is 0.136 g, according to the following calculations:

\[ S_{Tf-QD} = 4 \pi r_{QD}^2 = 4 \cdot 3.14 \cdot (6.4 \times 10^{-9})^2 = 5.14 \times 10^{-16} \text{ m}^2 \]

\[ S_{DOX} = \pi r_{DOX}^2 = 3.14 \cdot (0.75 \times 10^{-9})^2 = 1.77 \times 10^{-18} \text{ m}^2 \]

The maximal number of DOX per one molecule of nanoconjugate Tf-QD is:

\[ \frac{S_{Tf-QD}}{S_{DOX}} = 290 \text{ molecules} \]

Since the 1 mol DOX (579.98 g·mol\(^{-1}\)) contains 6.023 \times 10^{23} molecules, 290 molecules of DOX correspond to the mass 2.80 \times 10^{-19} g DOX per one molecule of nanoconjugate Tf-QD. Including
the number of Tf-QD in 1 g the maximal mass of DOX per 1 g of nanoconjugate Tf-QD equals: 136 mg DOX.