Electronic Supplementary Material (ESI) for Nanoscale.

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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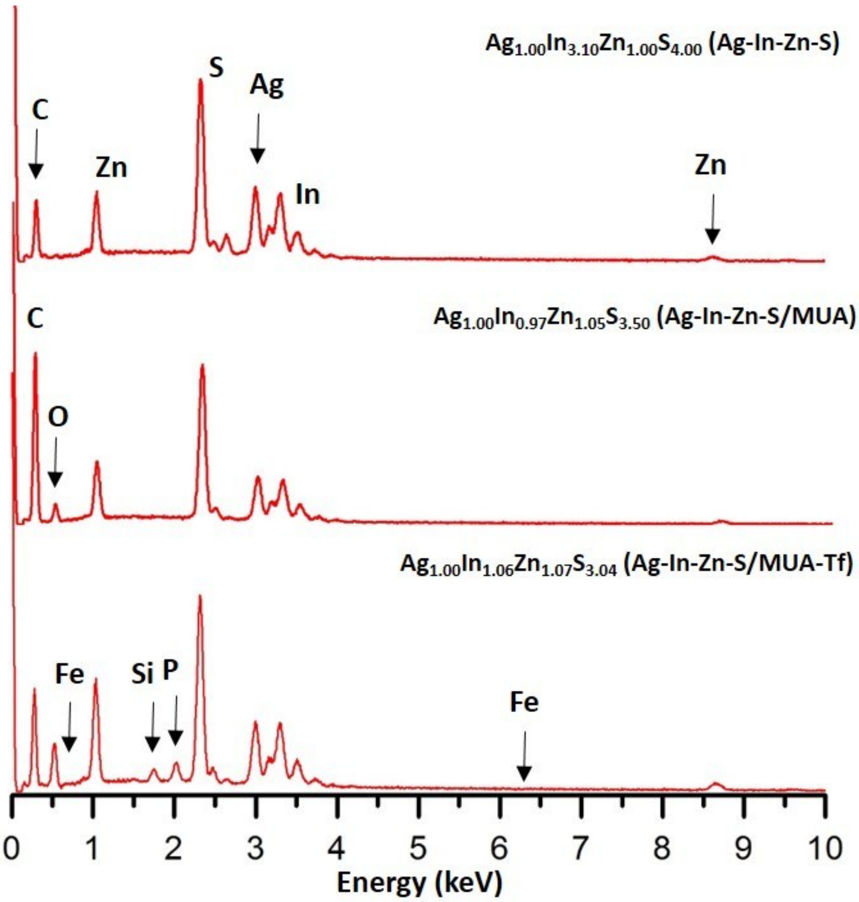

Fig. S1. Energy-dispersive spectra of Ag-In-Zn-S, Ag-In-Zn-S/MUA and Ag-In-Zn-S/MUA-Tf nanocrystals.

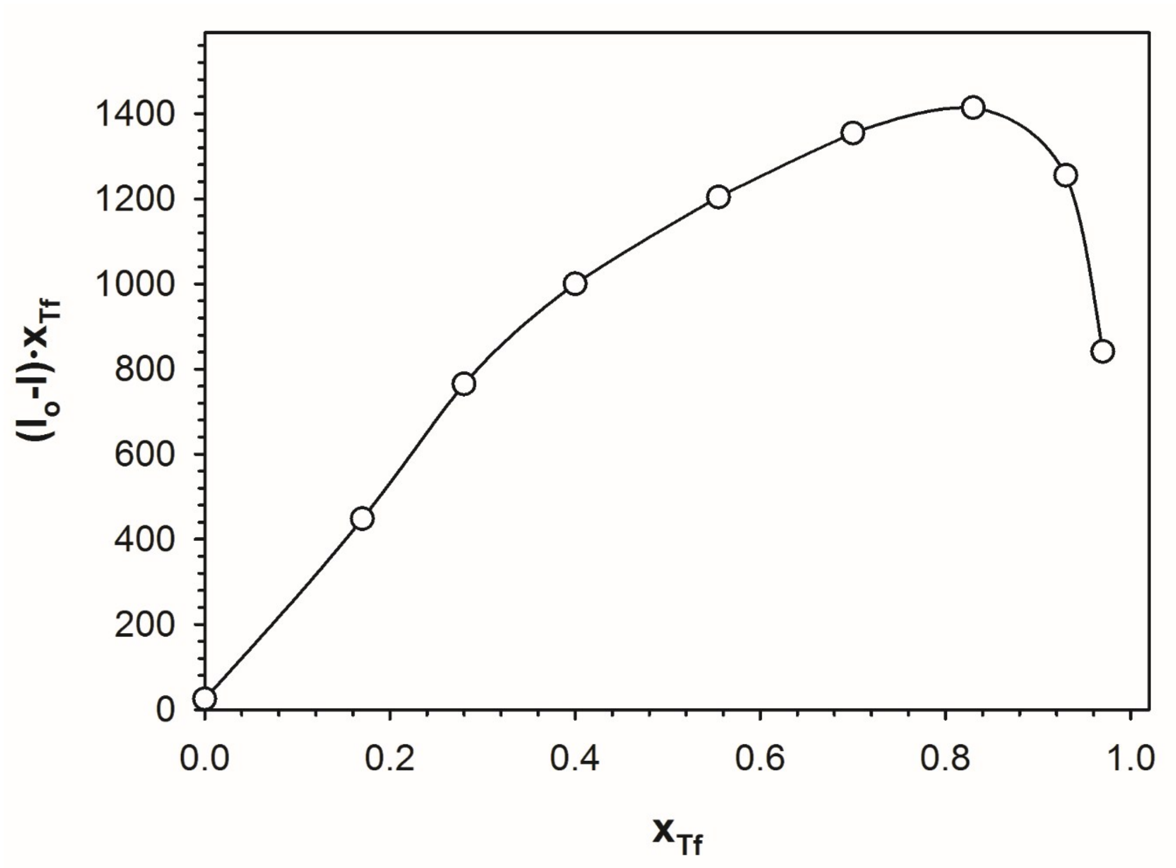

Fig. S2. Job plot for coordination of Tf to QD as measured by fluorescence spectroscopy.

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:

$$V_1 = \frac{4}{3} \times \pi \times (r)^3 = 1.00\times10^{-25}\ m^3,\ V_2 = a^3 = 1.57\times10^{-28}\ m^3$$

$$V_1/V_2 = 635,\ CN = 4,\ N = 2540$$

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 $Ag_{1.0}In_{1.0}Zn_{1.0}S_{3.0}$ 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 $Ag_{423}In_{423}Zn_{423}S_{1269}$ is 162554 g/mol. The total molecular mass of Ag-In-Zn-S/MUA nanocrystals calculated as follows: (162554×100)/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

Element	Atomic%
Ag L	3.95 ± 0.47
In L	4.19 ± 0.51
Zn K	4.23 ± 0.39
S K	12.02 ± 0.47
Fe K	0.10 ± 0.04
C K	52.39 ± 2.80
O K	21.31 ± 1.66
Si K	0.68 ± 0.06

P K	1.13 ± 0.07

Calculations of maximal mass of DOX per 1 g of QD and 1 g of nanoconjugate Tf-QD:

Diameters determined from dynamic light scattering measurements		
QD	**DOX**	**Tf-QD**
$\phi_{QD} = 9.1 \cdot 10^{-9}$ m	$\phi_{QD}^{*} = 1.5 \cdot 10^{-9}$ m	$\phi_{QD} = 12.8 \cdot 10^{-9}$ m
$r_{QD} = 4.55 \cdot 10^{-9}$ m	$r_{QD} = 0.75 \cdot 10^{-9}$ m	$r_{QD} = 6.4 \cdot 10^{-9}$ m
*the diameter of doxorubicin was determined with the help of the ChemSketch program where it was assumed that the molecules are circular		

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

$S_{DOX} = \pi r_{DOX}^2 = 3.14 \cdot (0.75 \cdot 10^{-9})^2 = 1.77 \cdot 10^{-18}$ 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 $6.023 \cdot 10^{23}$ molecules, 144 molecules of DOX correspond to the mass $1.39 \cdot 10^{-19}$ 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 $3.94 \cdot 10^{-19}$ cm^3; thus, the number of QD in 1 g is $4.87 \cdot 10^{17}$.

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

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

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

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

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

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

Since the 1 mol DOX (579.98 g·mol^{-1}) contains $6.023 \cdot 10^{23}$ molecules, 290 molecules of DOX correspond to the mass $2.80 \cdot 10^{-19}$ 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.