Supporting Information

Conjugation of antibodies to gold nanorods through Fc portion: synthesis and molecular specific imaging

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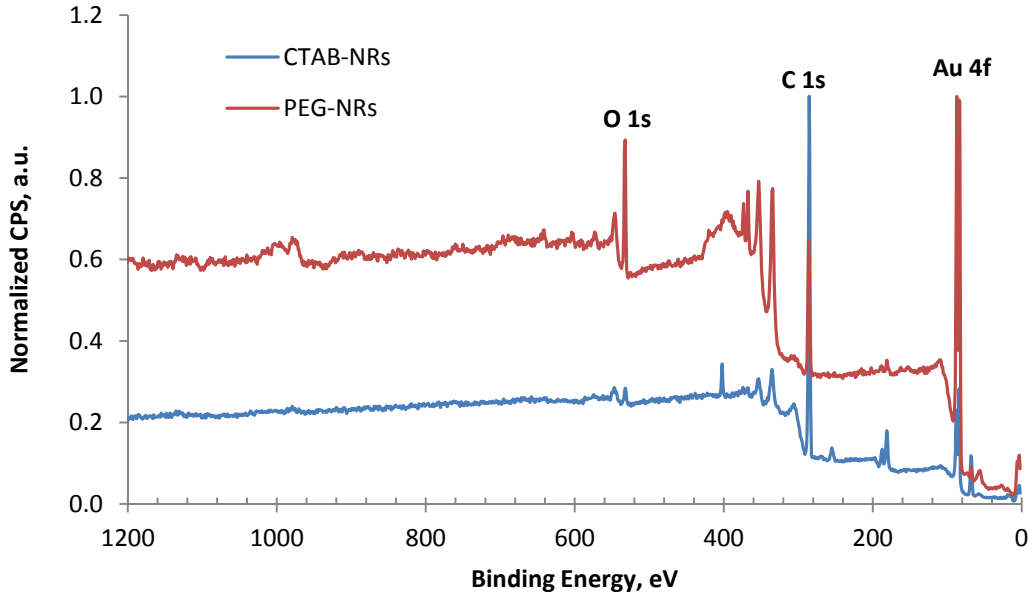
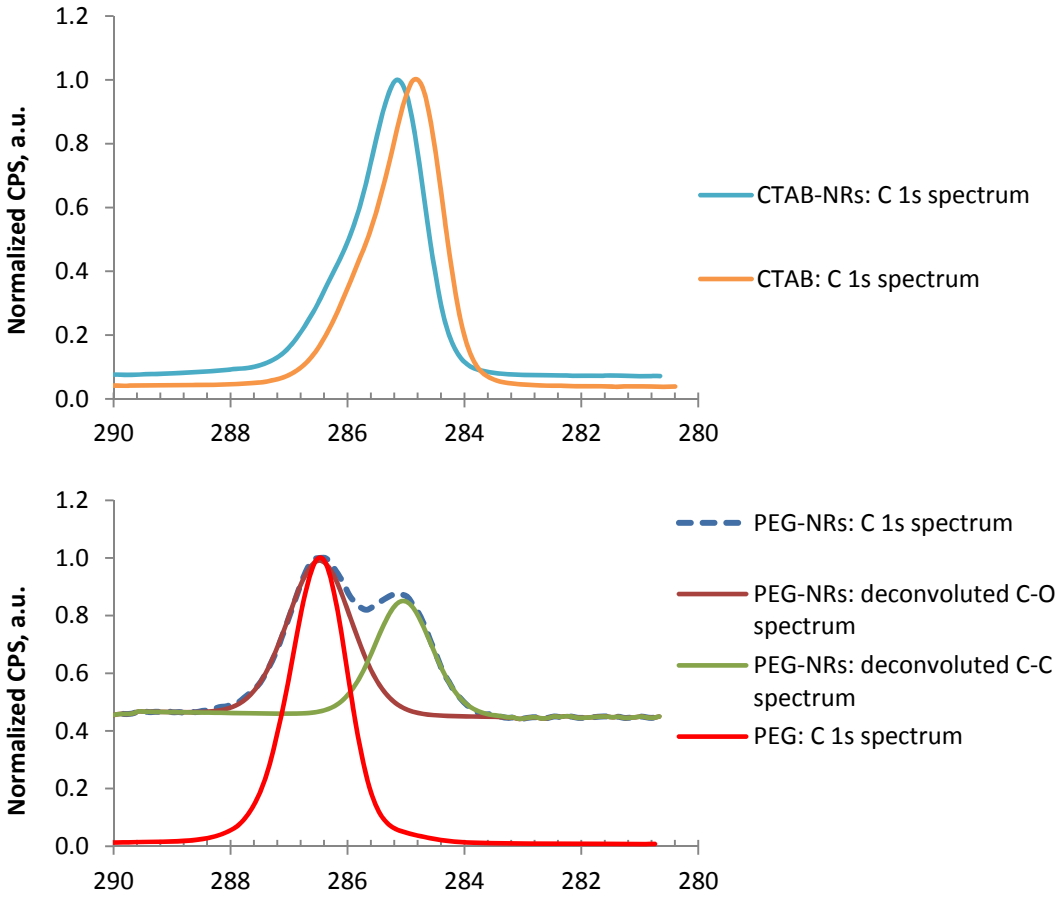
A)


CTAB-NRs
PEG-NRs
O 1s
C 1s
Au 4f
Normalized CPS, a.u.
Binding Energy, eV
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1200
1000
800
600
400
200
0

B)


Normalized CPS, a.u.
CTAB-NRs: C 1s spectrum
CTAB: C 1s spectrum
PEG-NRs: C 1s spectrum
PEG-NRs: deconvoluted C-O spectrum
PEG-NRs: deconvoluted C-C spectrum
PEG: C 1s spectrum
290
288
286
284
282
280

C)

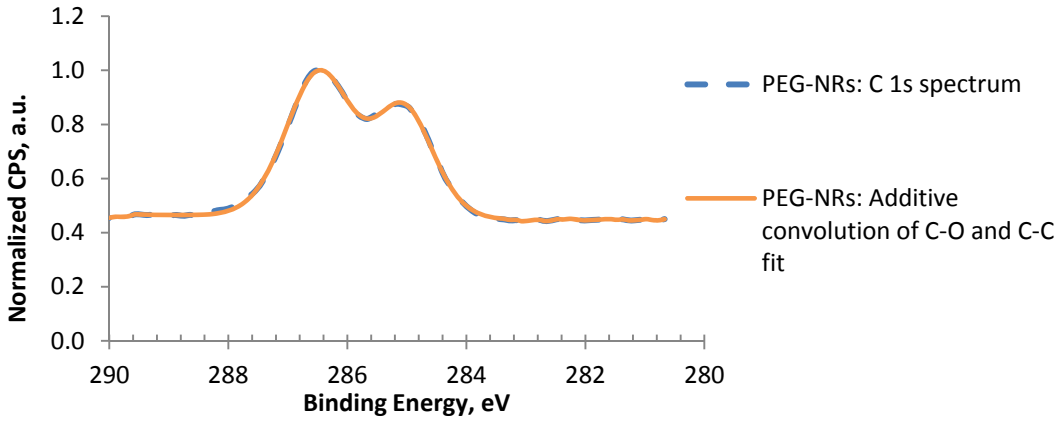


Figure S1: A) Survey scans of CTAB-NRs and PEG-NRs from XPS measurements; B) C 1s core spectra for CTAB, CTAB-NRs, PEG-SH and PEG-NRs. The PEG-NR spectrum shows contributions of C-O and C-C bonds. Electronic shift observed for CTAB-NRs as compared to pure CTAB is likely due to interactions between Au and CTAB whereby gold is more electron-withdrawing. Such shifts are characteristic of oxidation, indicating that the CTAB ligand on the nanorods is partially oxidized. C) Overlay of C 1s spectrum for PEG-NRs and additive convolution of C-O and C-C spectrum to show degree of agreement between the XPS collected spectra and the deconvolution.

Determination of CTAB/PEG ratio and CTAB surface coating by XPS

The CTAB to PEG ratio on the surface of PEG-coated gold nanorods was determined from the integration of the N 1s and O 1s peaks in the XPS spectrum. CTAB molecule contains one N and no O atoms, while 2 KDa mPEG-SH has no N and 46 O atoms. As a result we have the following relationship between molar fractions of N (y_N), O (y_O), CTAB (y_{CTAB}) and PEG (y_{PEG}) on the PEG-coated nanorods.

$$y_N = y_{CTAB} \quad \text{(I)}$$

$$y_O = 46*y_{PEG} \quad \text{(II)}$$

Integration of the N 1s and O 1s peaks in XPS spectrum of PEG-nanorods yields N/O ratio (y_N/ y_O) of 0.105. Using this ratio and the above relationships we determined that the molar ratio of CTAB to PEG (y_{CTAB}/y_{PEG}) on the surface of PEG-coated nanorods is 4.8. Then, the following equation was used to find the number of surface ligands:

$$N_{CTAB}*f_{CTAB} + N_{PEG}*f_{PEG} = A*0.9 \quad \text{(III)}$$

where, N_{CTAB} is the number of CTAB molecules on the nanorod surface divided by 2 because CTAB molecules are present on the surface as a bilayer; N_{PEG} is the number of PEG molecules on the nanorod surface; f_{CTAB} and f_{PEG} are footprints of CTAB and PEG molecules, respectively; and A is the surface area of a nanorod. Recent report showed that the footprint of CTAB on gold nanorods (f_{CTAB}) is *ca.* 0.27 nm^2.(*1*) It was shown that mPEG molecules can have a high density packing with f_{PEG} = 12 nm^2 and a low density packing with f_{PEG} = 38 nm^2.(*2*) The factor of 0.9 arises from the approximation of ligand attachment as close-packed circles on a planar surface.(*3*) Considering the 15nmx40nm gold nanorods used in this study, the calculated amount of residual CTAB on gold nanorods after replacement with PEG is 2.3% for low density PEG and 7.1% for high density PEG; the CTAB coverage on initial CTAB nanorods is considered to be 100%.

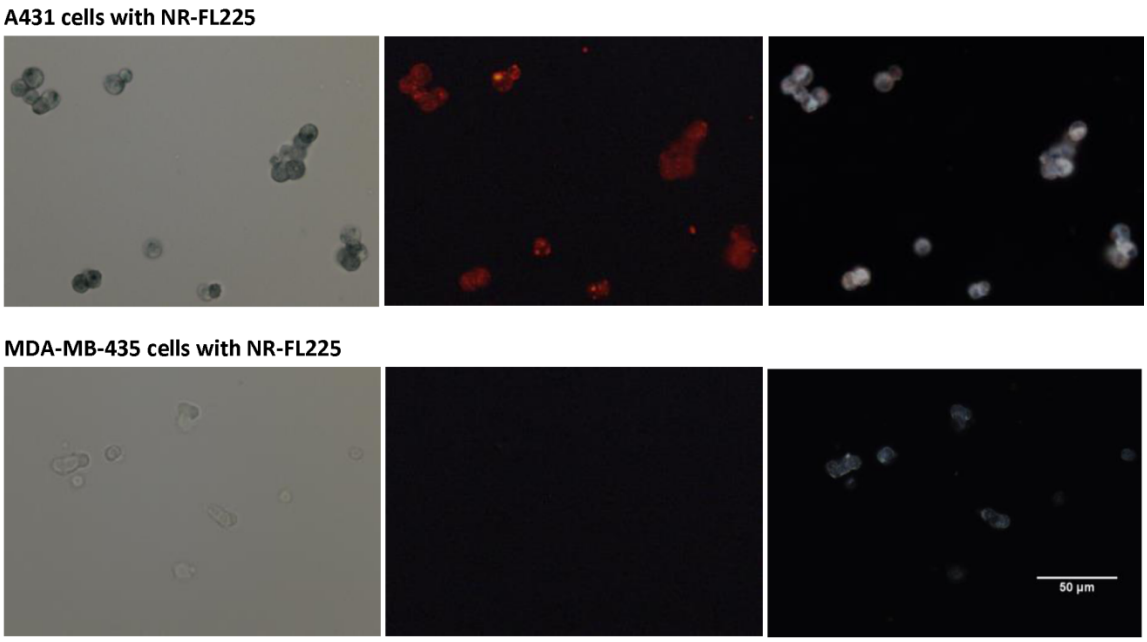


Figure S2: Right – Bright field images; middle – fluorescent images; left – dark-field images of A431 EGFR(+) (top row) and MDA-MB-435 EGFR(-) (bottom row) cells labeled using nanorods conjugated with fluorescently-labeled clone 225 (anti-EGFR) antibody. Scale bar = 50 µm.

References

(1) Vigderman, L., Manna, P., and Zubarev, E. R. (2012) Quantitative Replacement of Cetyl Trimethylammonium Bromide by Cationic Thiol Ligands on the Surface of Gold Nanorods and Their Extremely Large Uptake by Cancer Cells. *Angewandte Chemie Int. Ed. 51*, 636-641.

(2) Perry, J. L., Reuter, K. G., Kai, M. P., Herlihy, K. P., Jones, S. W., Luft, J. C., Napier, M., Bear, J. E., and DeSimone, J. M. (2012) PEGylated PRINT Nanoparticles: The Impact of PEG Density on Protein Binding, Macrophage Association, Biodistribution, and Pharmacokinetics. *Nano Letters 12*, 5304-5310.

(3) Bezdek, K. (1986) Circle Packings into Convex Domains of the Euclidean and Hyperbolic Plane and the Sphere. *Geometriae Dedicata 21*, 249-255.