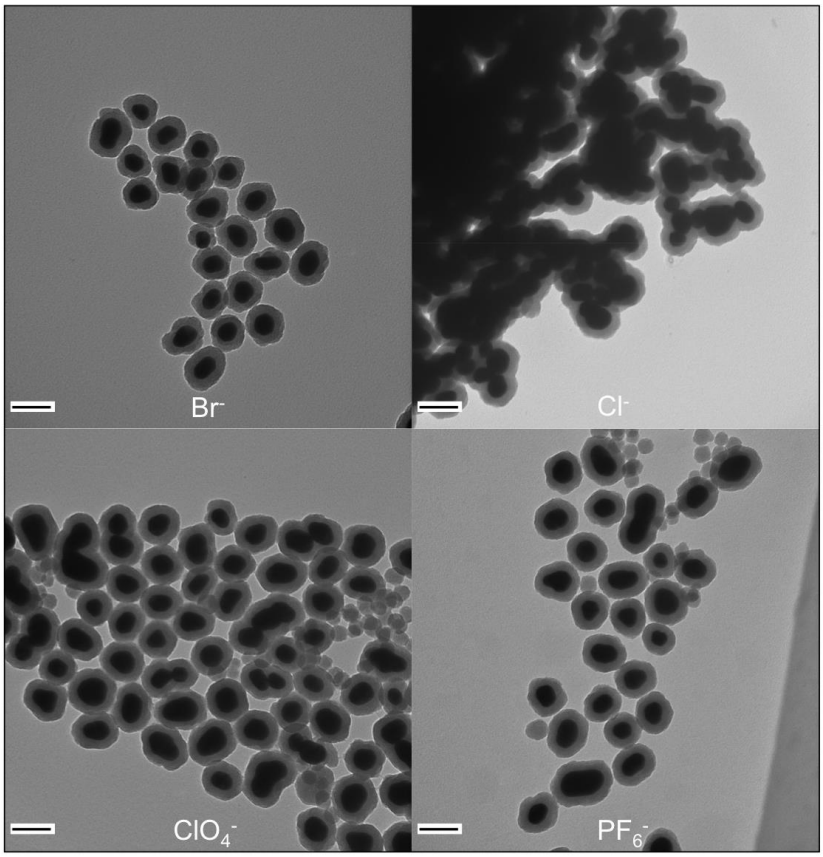
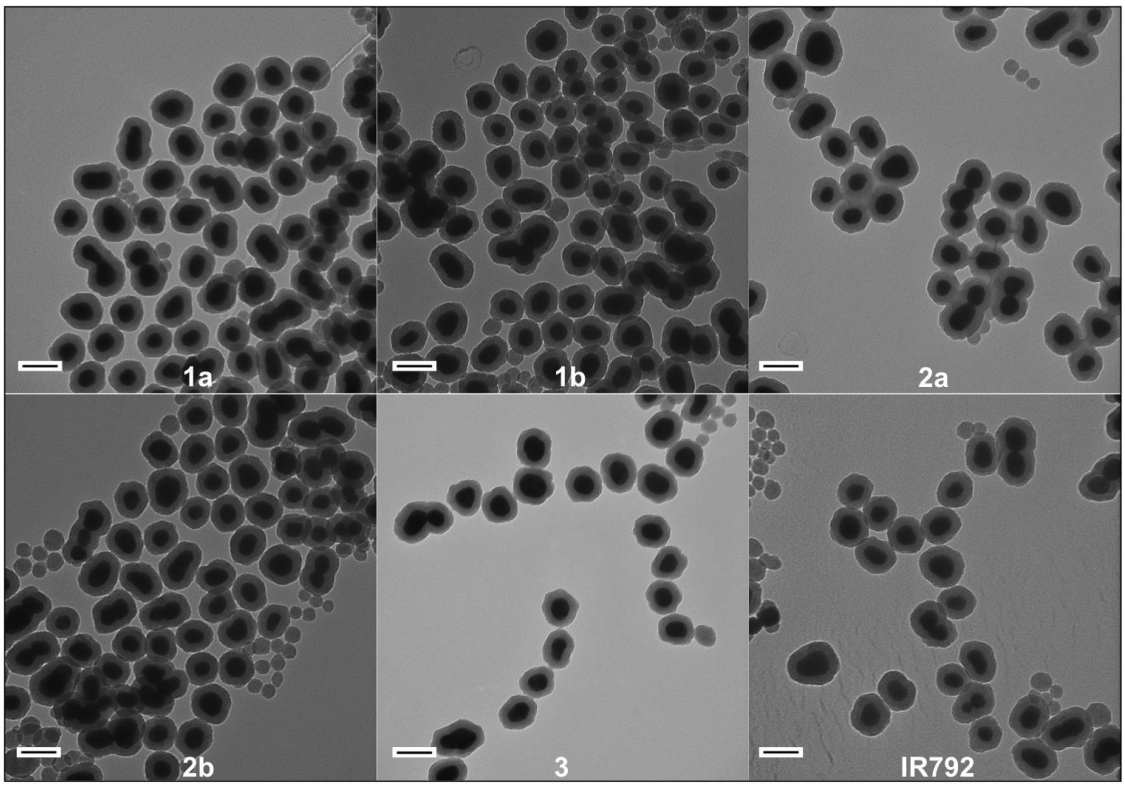
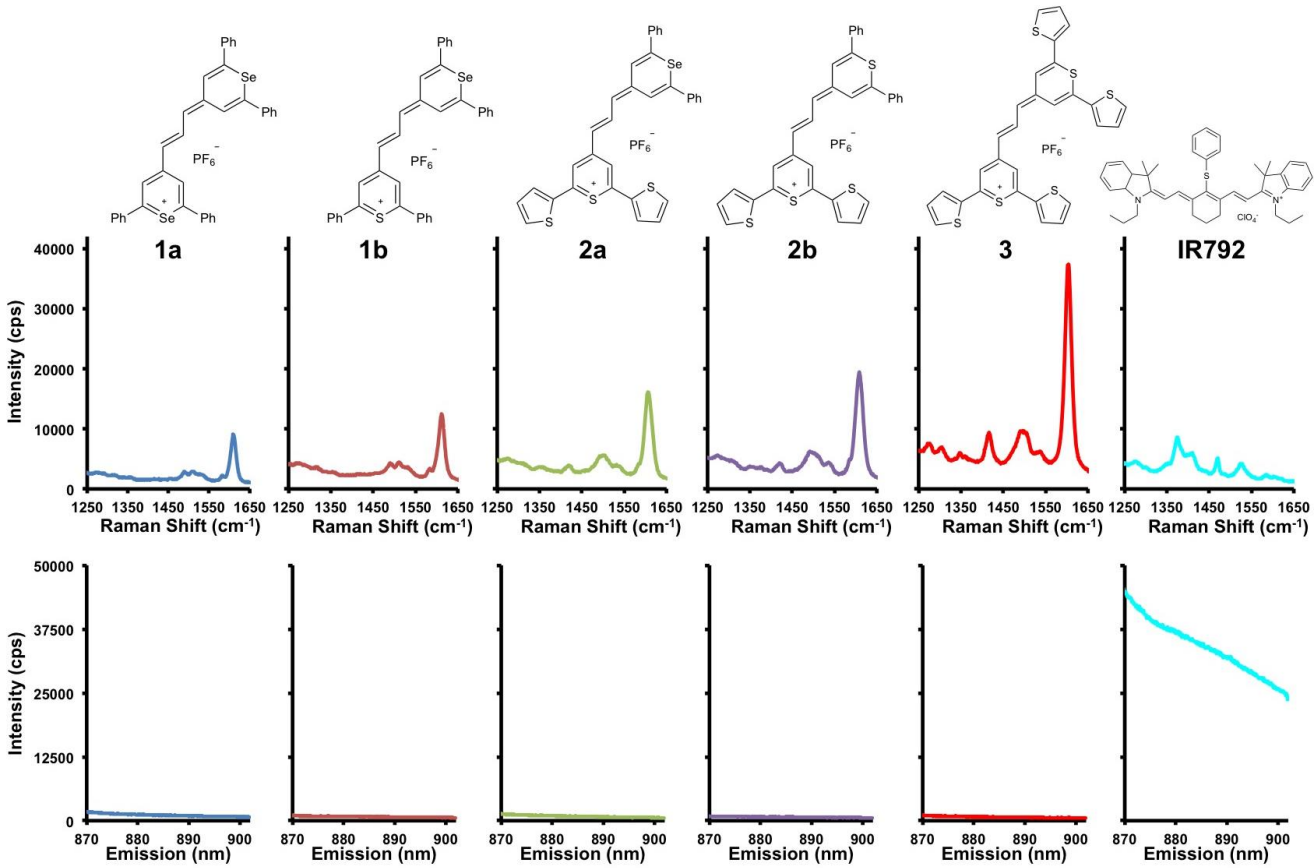


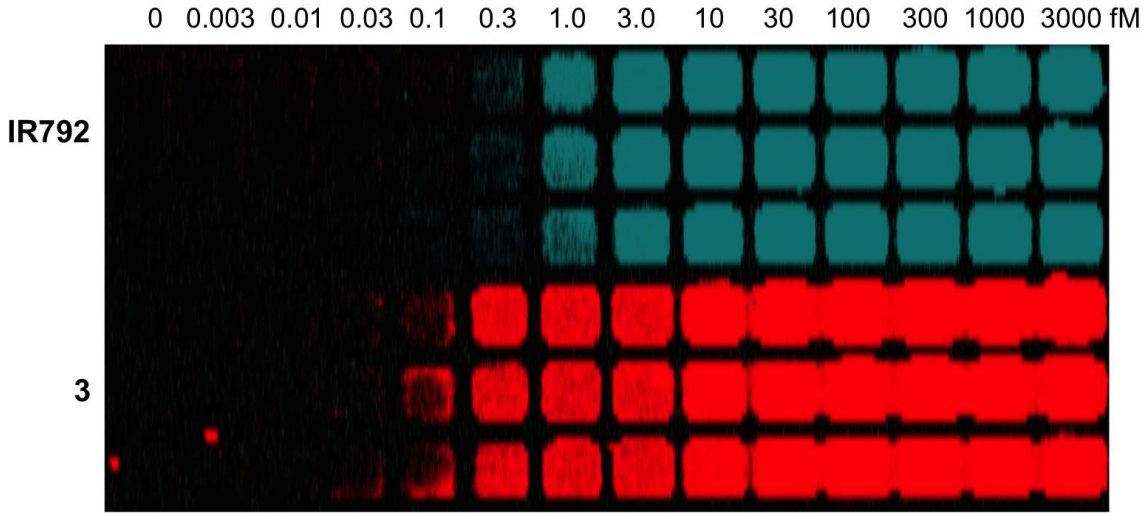
Supplementary Figure 1. Effect of the counterion on colloidal stability. Transmission Electron Micrographs of the **1a**-based SERRS-nanoprobes (Scale bar: 100 nm).



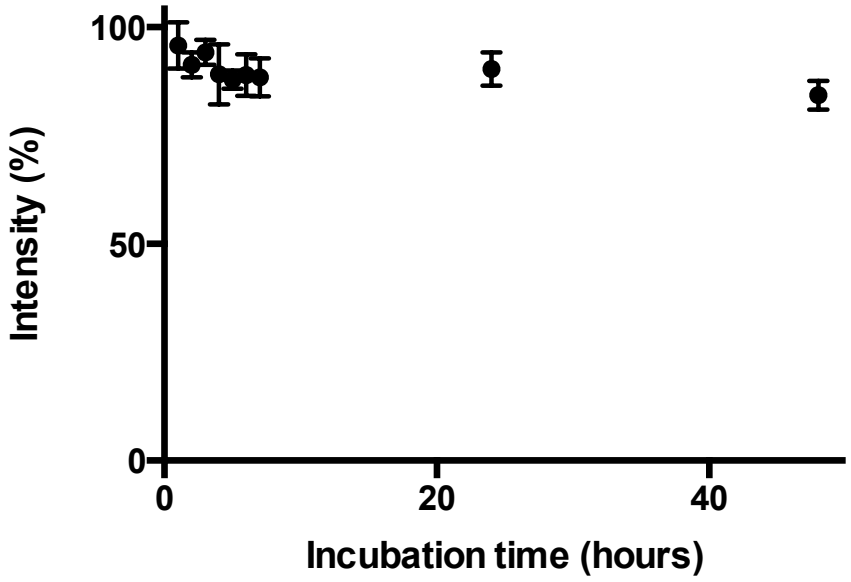
Supplementary Figure 2. Effect of increased affinity of chalcogenopyrylium dye for the gold nanoparticle surface on colloidal stability. Transmission Electron Micrographs of chalcogenopyrylium **1**−**3**-based SERRS-nanoprobes (Scale bar: 100 nm).



Supplementary Figure 3. Evaluation of fluorescence interference by the chalcogenopyrylium dyes and the cyanine-based dye IR792. Equimolar amounts of **IR792** or CP dyes **1**−**3** (Ph, phenyl) were incorporated in silica with a 60 nm gold nanocore (upper panel) and without a gold nanocore (lower panel) via a similar synthesis protocol (water was added instead of the gold nanoparticles for the latter. Reaction time was tripled). The SERRS- and fluorescence signal of each dye were measured under identical conditions (785 nm excitation laser wavelength, 50 μW, 1.0 s acquisition time, 5× objective). The SERRS spectra have not been baseline corrected.

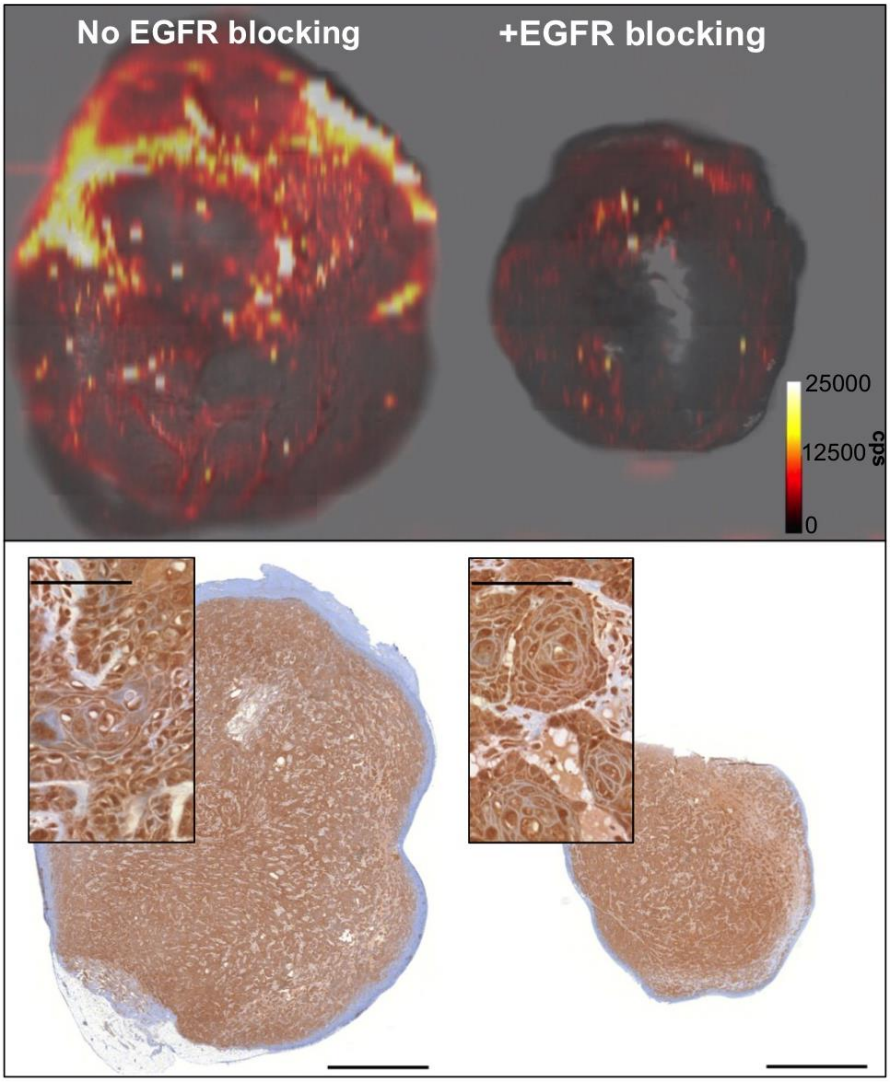


Supplementary Figure 4. Limit of detection of IR792- or 3-based SERRS-nanoprobes. SERRS imaging to determine the limit of detection was performed at 100 mW (2.0 s acquisition time) on a phantom that consisted of serial diluted chalcogenopyrylium dye **3**- and **IR792**-based SERRS-nanoprobes redispersed in 10 μL water (concentration range 3000−0.003 fM; n=3).



Supplementary Figure 5. Serum stability of 3-based SERRS-nanoprobe.

The **3**-based SERRS-nanoprobes were incubated in 50% mouse serum at 37^{o}C. At the indicated time points, a Raman spectrum was taken (50 μW; 1.0 s acquisition time; 5× objective). The intensities of the 1600 cm^{-1} peak were plotted (n=3; error bars represent standard deviations).



Supplementary Figure 6. Preblocking of EGFR reduces accumulation of EGFR-targeted SERRS-nanoprobes.

Upper panel: An excised A431 xenograft tumor of an animal that was injected with EGFR-targeted SERRS-nanoprobes (left; "No EGFR blocking") had a significantly higher Raman signal intensity than an A431 xenograft tumor of an animal that had received a cetuximab (50pmol/g) injection 3 hours prior to injection with an equimolar amount (30 fmol/g) of the EGFR-targeted SERRS-nanoprobes (right; "+EGFR blocking"). Lower panel: Immunohistochemical staining for EGFR shows that EGFR is expressed homogenously throughout both tumors (Scale bars: 2 mm). Insets: Higher magnification of EGFR-immunostaining (Scale bars: 100 μm). Together these results prove that the EGFR-targeted SERRS-nanoprobes target EGFR.

Supplementary Table 1. SERRS-nanoprobe characteristics.

CP Dye	Counterion	Conc. of CP dye (μM)	λ_{max} (CH_2Cl_2)	Log ε ($M^{-1}cm^{-1}$)	Nanoparticle aggregation (850nm/540nm)	Raman Signal Intensity ($1600cm^{-1}$) (cps)
1a	Br^-	10	806	5.36	0.27	13870±599
1a	Cl^-	10	806	5.36	0.61*	7346±1085*
1a	ClO_4^-	10	806	5.40	0.30	12685±614
1a	PF_6^-	10	806	5.40	0.28	14009±756
1a	PF_6^-	1.0	806	5.40	0.28	7101±611
1b	PF_6^-	1.0	784	5.30	0.31	9445±389
2a	PF_6^-	1.0	810	5.40	0.28	15002±772
2b	PF_6^-	1.0	789	5.34	0.28	19634±248
3	PF_6^-	1.0	813	5.45	0.35	37680±1599

*P<0.05 (significant aggregation and SERRS-signal decrease; an unpaired Student's *t*-test was performed).

Supplementary Methods

SERRS-nanoprobe serum stability

The SERRS-nanoprobes (2.0 nM) were incubated in triplicate in 50% mouse serum (Abd Serotec, Raleigh, NC) at 37 ^{o}C. At the indicated time points, a Raman spectrum was taken (50 μW; 1.0 s acquisition time; 5× objective). The intensities of the 1600 cm^{-1} were plotted in GraphPad Prism (GraphPad Software Inc., La Jolla, CA).

Immunohistochemical staining

The tissues from the imaging studies were collected and fixed in 4% paraformaldehyde, 4 ^{o}C overnight and subsequently processed to be embedded in paraffin. The Discovery XT biomarker platform (Ventana, Tucson, AZ) was used to stain the tissue sections (5 μm). Heat-induced epitope retrieval was performed using the citrate buffer (pH 6.0). The primary anti-EGFR antibody (D38B1, Cell Signaling Technology, Danvers, MA) was diluted 1:150. The biotin-labeled secondary anti-rabbit antibody (BA-1000, Vector Laboratories) was diluted 1:300.