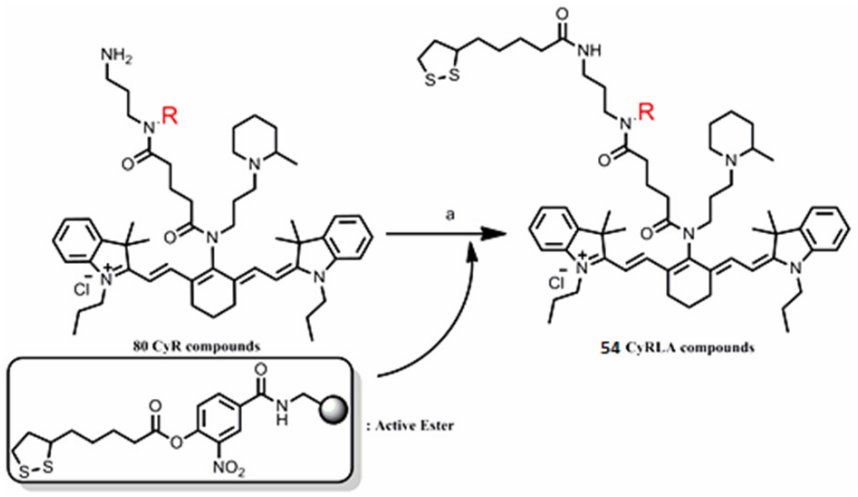
# Supporting information

**Scheme S1.** Synthesis of CyRLA library from CyR compounds.




**Reagents and conditions:** (a) DCM/ACN (7:1), $NaHCO_3$, r.t., 6 h.

**Chart S1.** CyRLA compound structures.

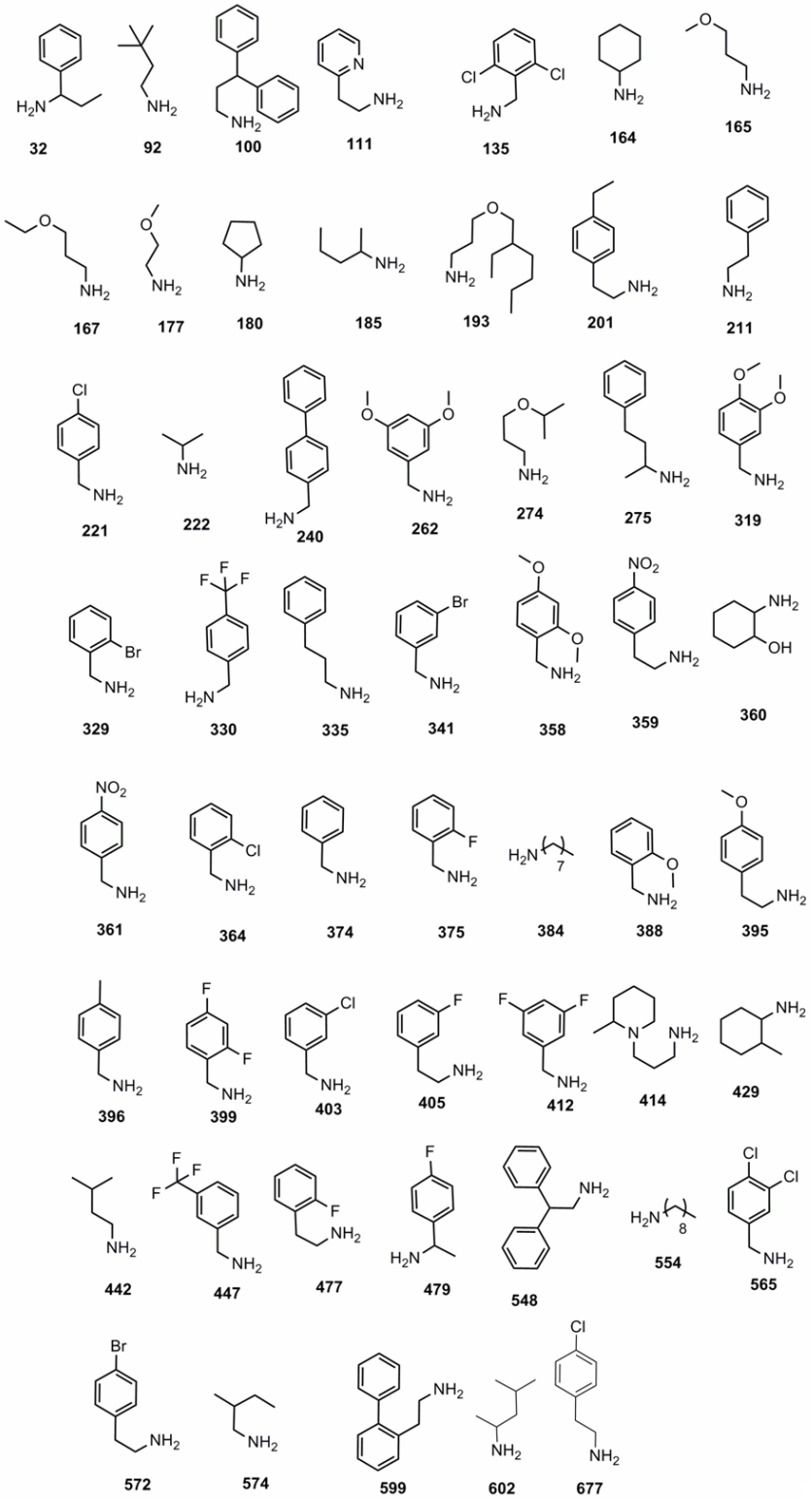


**Table S1.** Characterization by HPLC-MS and photophysical property of CyRLA library

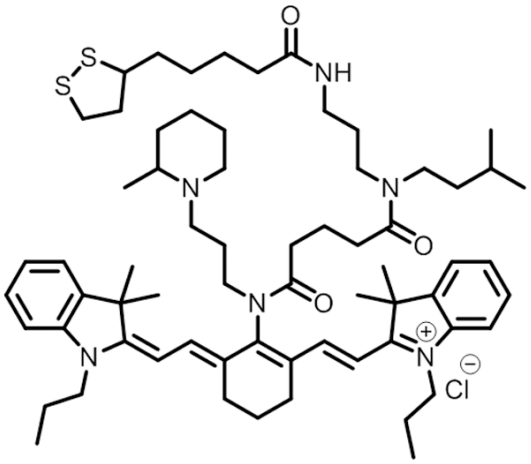
| Compound Code | $M^+$ (calc.) | $M^+$ (exp.)[a] | Purity (%)[b] | $\lambda_{abs}$/nm | $\lambda_{em}$/nm |
|---|---|---|---|---|---|
| CyRLA-677 | 1155.7 | 1155.6 | 90 | 803 | 820 |
| CyRLA-599 | 1197.7 | 1197.6 | 94 | 803 | 820 |
| CyRLA-396 | 1121.7 | 1121.6 | 82 | 803 | 821 |
| CyRLA-221 | 1141.7 | 1141.6 | 84 | 805 | 820 |
| CyRLA-358 | 1137.7 | 1137.6 | 93 | 804 | 822 |
| CyRLA-574 | 1087.7 | 1087.6 | 90 | 802 | 820 |
| CyRLA-262 | 1167.7 | 1167.6 | 86 | 803 | 820 |
| CyRLA-565 | 1175.6 | 1175.5 | 93 | 801 | 819 |
| CyRLA-330 | 1175.7 | 1175.5 | 92 | 804 | 820 |
| CyRLA-329 | 1185.6 | 1185.7 | 89 | 802 | 823 |
| CyRLA-335 | 1135.7 | 1135.6 | 93 | 801 | 820 |
| CyRLA-388 | 1137.7 | 1137.6 | 92 | 802 | 819 |
| CyRLA-399 | 1143.7 | 1143.6 | 92 | 803 | 820 |
| CyRLA-479 | 1139.7 | 1139.6 | 93 | 804 | 823 |
| CyRLA-548 | 1197.7 | 1197.6 | 82 | 803 | 820 |
| CyRLA-222 | 1059.7 | 1059.6 | 90 | 802 | 821 |
| CyRLA-447 | 1175.7 | 1175.6 | 81 | 803 | 822 |
| CyRLA-360 | 1129.7 | 1129.6 | 91 | 804 | 823 |
| CyRLA-319 | 1167.7 | 1167.6 | 89 | 801 | 820 |
| CyRLA-240 | 1183.7 | 1183.6 | 92 | 802 | 820 |
| CyRLA-193 | 1187.8 | 1187.7 | 93 | 803 | 819 |
| CyRLA-384 | 1129.8 | 1129.7 | 83 | 802 | 820 |
| CyRLA-275 | 1149.7 | 1149.6 | 84 | 804 | 820 |
| CyRLA-92 | 1101.7 | 1101.7 | 89 | 801 | 820 |
| CyRLA-111 | 1122.7 | 1122.6 | 72 | 803 | 821 |
| CyRLA-165 | 1089.7 | 1089.6 | 89 | 802 | 820 |
| CyRLA-167 | 1103.7 | 1103.6 | 92 | 803 | 822 |
| CyRLA-405 | 1139.7 | 1139.6 | 93 | 801 | 820 |
| CyRLA-341 | 1185.6 | 1185.5 | 92 | 803 | 820 |
| CyRLA-414 | 1156.8 | 1156.7 | 93 | 801 | 819 |
| CyRLA-477 | 1139.7 | 1139.6 | 92 | 803 | 820 |
| CyRLA-554 | 1143.8 | 1143.7 | 93 | 802 | 823 |
| CyRLA-572 | 1199.6 | 1199.5 | 92 | 805 | 820 |
| CyRLA-100 | 1211.8 | 1211.7 | 91 | 803 | 819 |
| CyRLA-180 | 1085.7 | 1085.6 | 92 | 804 | 820 |
| CyRLA-211 | 1121.7 | 1121.6 | 93 | 803 | 823 |
| CyRLA-274 | 1117.7 | 1117.6 | 92 | 802 | 820 |
| CyRLA-359 | 1166.7 | 1166.6 | 90 | 803 | 821 |
| CyRLA-403 | 1141.7 | 1041.6 | 91 | 802 | 822 |
| CyRLA-442 | 1087.7 | 11087.7 | 92 | 803 | 823 |
| CyRLA-602 | 1101.7 | 1101. 6 | 85 | 804 | 820 |
| CyRLA-32 | 1121.7 | 1121.6 | 89 | 802 | 820 |
| CyRLA-177 | 1075.7 | 1075.6 | 81 | 803 | 821 |
| CyRLA-361 | 1152.7 | 1152.6 | 82 | 802 | 820 |
| CyRLA-375 | 1125.7 | 1125.6 | 75 | 804 | 822 |
| CyRLA-412 | 1143.7 | 1143.6 | 85 | 803 | 820 |
| CyRLA-429 | 1113.7 | 1113.6 | 90 | 803 | 820 |

| | | | | | |
|---|---|---|---|---|---|
| CyRLA-135 | 1175.6 | 1175.5 | 92 | 801 | 819 |
| CyRLA-201 | 1149.7 | 1149.6 | 92 | 804 | 820 |
| CyRLA-374 | 1107.7 | 1107.6 | 91 | 805 | 823 |
| CyRLA-364 | 1141.7 | 1141.6 | 92 | 802 | 820 |
| CyRLA-185 | 1087.7 | 1087.6 | 92 | 801 | 819 |
| CyRLA-164 | 1099.7 | 1099.6 | 89 | 802 | 820 |
| CyRLA-395 | 1151.7 | 1151.6 | 85 | 803 | 823 |

[a]ESI-MS m/z corresponding to [M$^+$] values. [b]Purities were determined by integration of the UV absorbance signal at 780 nm. 54-member CyRLA compounds were characterized by HPLC-MS analysis. The purities of the whole library were determined by integration of the UV absorbance signal at 780 nm. The spectra of 10 μM in DMSO solution was recorded in SpectraMax M2 plate reader.

*Characterization data of representative CyRLA compounds*

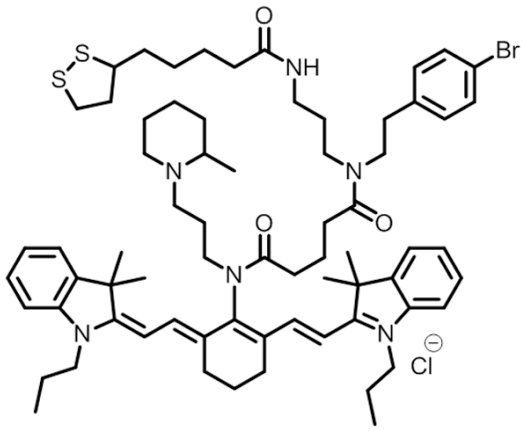
**CyRLA-442** (7 mg, 20%)




$^1$H-NMR (500 MHz, $CDCl_3$): δ 0.87-1.60 (m, 18 H), 1.65 (s, 6 H), 1.66 (s, 6 H), 1.7-1.92 (m, 14 H), 2.14-2.72 (m, 12 H), 3.03-3.28 (m, 11 H), 3.48-3.72 (m, 13 H), 4.0-4.1 (m, 6 H), 6.12 (d, 1 H, *J*=13.5 Hz), 6.24 (d, 1 H, *J*=13.5 Hz), 7.04-7.44 (m, 8 H), 7.52 (d, 1H, *J*=13 Hz), 7.55 (d, 1H, *J*=13 Hz).

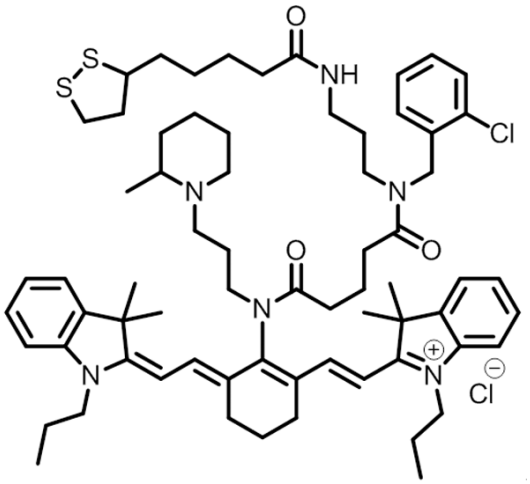
ESI-MS *m/z* (M$^+$), calc'd: 1087.7, found 1087.4.

**CyRLA-572** (9 mg, 17%)



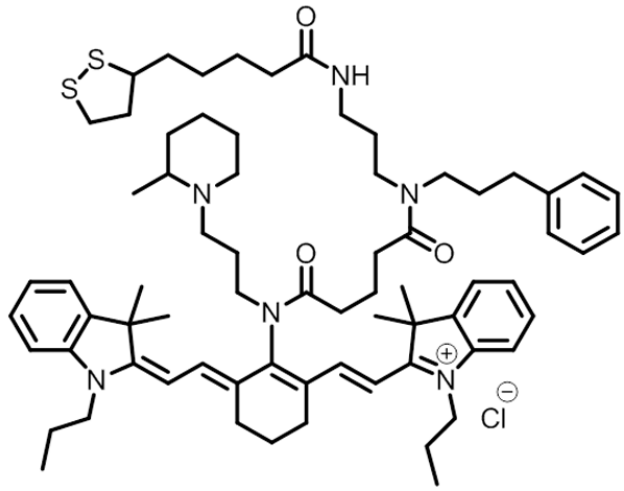
$^1$H-NMR (500 MHz, $CDCl_3$): δ 1.06-1.62 (m, 18 H), 1.66 (s, 6 H), 1.68 (s, 6 H), 1.69-2.06 (m, 9 H), 2.18-2.78 (m, 10 H), 3.04 -3.31 (m, 11 H), 3.44-3.73 (m, 13 H), 3.93-4.06 (m, 6 H), 6.14 (d, 1 H, *J*=14.5 Hz), 6.17 (d, 1 H, *J*=14.5 Hz), 6.99-7.43 (m, 12 H), 7.53 (d, 1 H, *J*=14.5 Hz), 7.56 (d, 1 H, *J*=14.5 Hz). ESI-MS *m/z* ($M^+$), calc'd: 1199.6, found 1201.1 (due to bromine isotope).

**CyRLA-364** (7.5 mg, 15%)



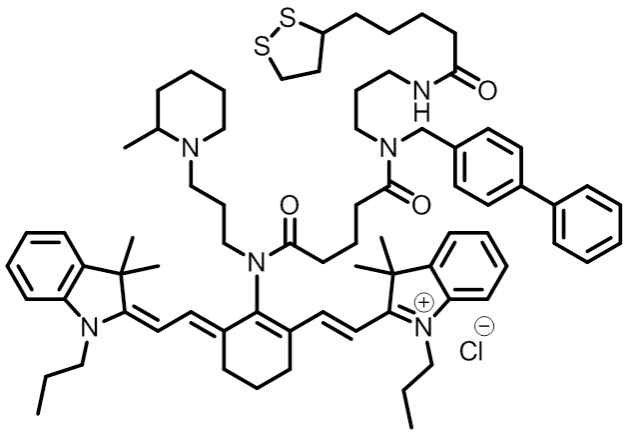
$^1$H-NMR (500 MHz, $CDCl_3$): δ 1.06-1.62 (m, 18 H), 1.67 (s, 6 H), 1.68 (s, 6 H), 1.70-1.92 (m, 9 H), 1.99-2.46 (m, 10 H), 2.52-2.62 (m, 6 H), 2.72 (m, 2 H), 3.0-3.72 (m, 15 H), 4.0-4.12 (m, 6 H), 4.53 (s, 2 H), 6.10 (d, 1 H, *J*=14.5 Hz), 6.16 (d, 1 H, *J*=14.5 Hz), 7.07-7.49 (m, 12 H), 7.52 (d, 1 H, *J*=15 Hz), 7.55 (d, 1 H, *J*=15 Hz). ESI-MS *m/z* ($M^+$), calc'd: 1141.7, found 1141.4.

**CyRLA-335** (8.2 mg, 18%)



$^1$H-NMR (500 MHz, $CDCl_3$): δ 1.09-1.57 (m, 18 H), 1.61 (s, 6 H), 1.63 (s, 6 H), 1.71-2.03 (m, 11 H), 2.16-2.80 (m, 10 H), 3.05 -3.33 (m, 11 H), 3.54-3.76 (m, 13 H), 3.96-4.17 (m, 6 H), 6.14 (d, 1 H, *J*=13.5 Hz), 6.17 (d, 1 H, *J*=13.5 Hz), 7.09-7.45 (m, 13 H), 7.56 (d, 1 H, *J*=13.5 Hz), 7.59 (d, 1 H, *J*=13.5 Hz). ESI-MS *m/z* ($M^+$), calc'd: 1135.7, found 1135.6.

**CyRLA-240** (6 mg, 12%)



$^1$H-NMR (500 MHz, $CDCl_3$): δ 1.04-1.51 (m, 16 H), 1.62 (s, 6 H), 1.64 (s, 6 H), 1.69-1.90 (m, 8 H), 1.99-2.43 (m, 10 H), 2.50-2.61 (m, 6 H), 2.76 (m, 2 H), 3.05-3.74 (m, 15 H), 3.97-4.13 (m, 6 H), 4.64 (s, 2 H), 6.10 (d, 1 H, *J*=14 Hz), 6.16 (d, 1 H, *J*=14 Hz), 7.05-7.53 (m, 17 H), 7.54 (d, 1 H, *J*=13.5 Hz), 7.57 (d, 1 H, *J*=13.5 Hz). ESI-MS *m/z* ($M^+$), calc'd: 1183.7, found 1183.6.

*SERS spectra of different Raman reporters*

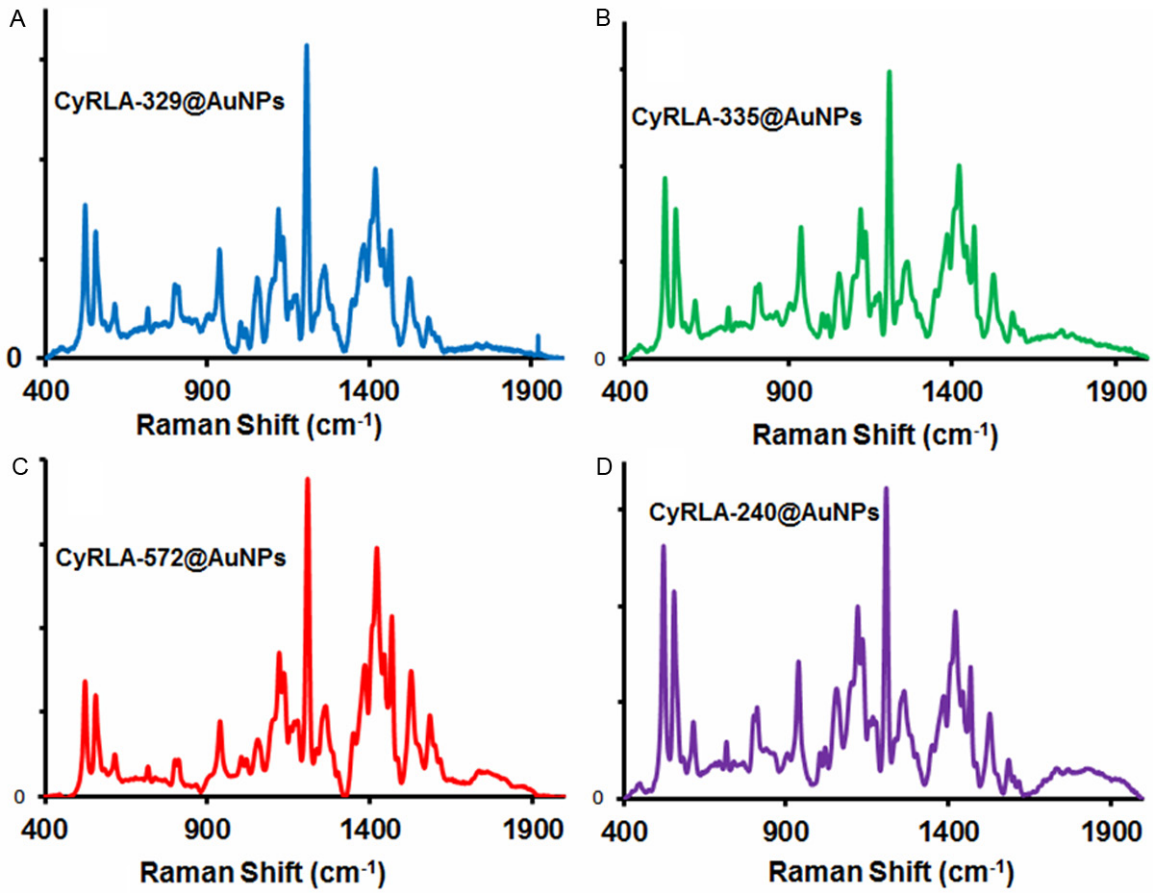



**Figure S1.** Normalized SERS spectra of CyRLA-329, CyRLA-335, CyRLA-240 and CyRLA-572 after chemisorption on 60 nm gold nanoparticle. Spectra were measured in a Raman microscope (785 nm laser excitation, 1.2 mW laser power, and acquisition time: 10 s.

*SERS measurement of three different Raman reporters*

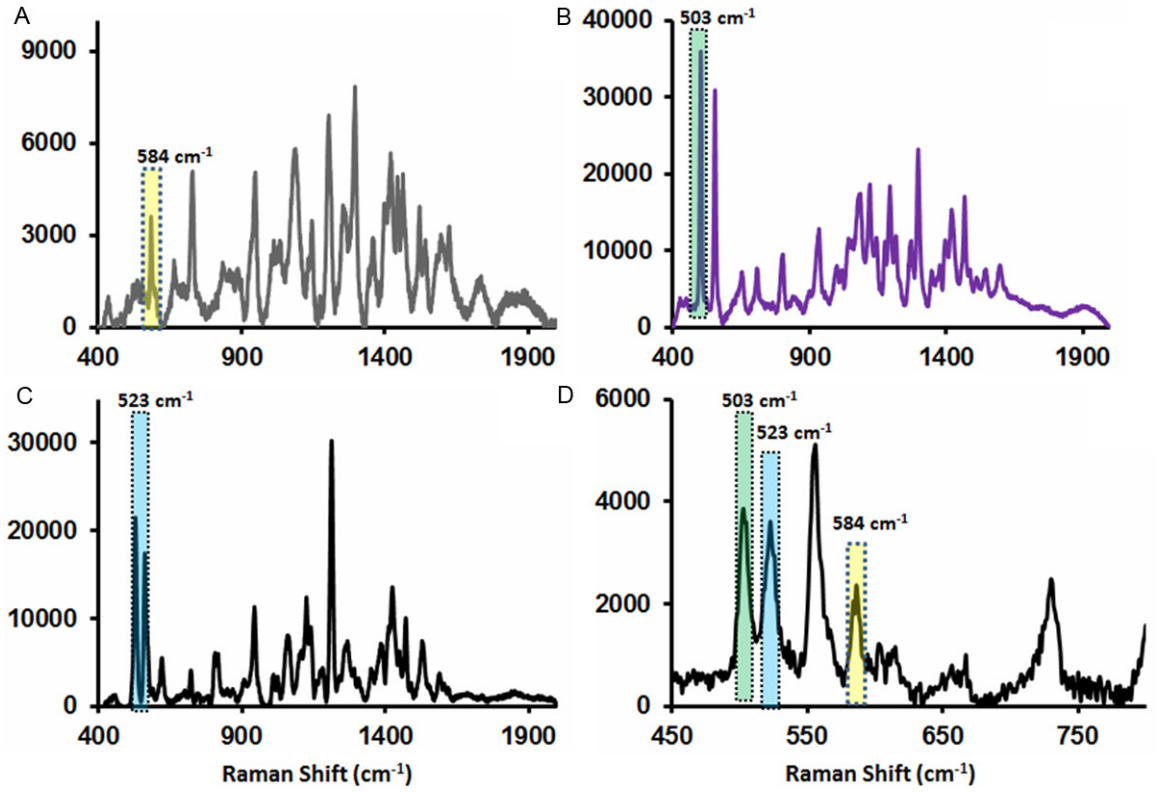



**Figure S2.** Normalized SERS spectra of (A) Cy7.5LA, (B) Cy7LA, (C) CyRLA-572 and (D) mixture of three (Cy7.5LA, Cy7LA, CyRLA-572) after chemisorption on 60 nm gold nanoparticle. Spectra were measured in a Raman microscope (785 nm laser excitation, 1.2 mW laser power, and acquisition time: 10 s. SERS spectra was measured in low concentration when all three AuNPs-Reporters were mixed.

*UV-Vis spectroscopy*

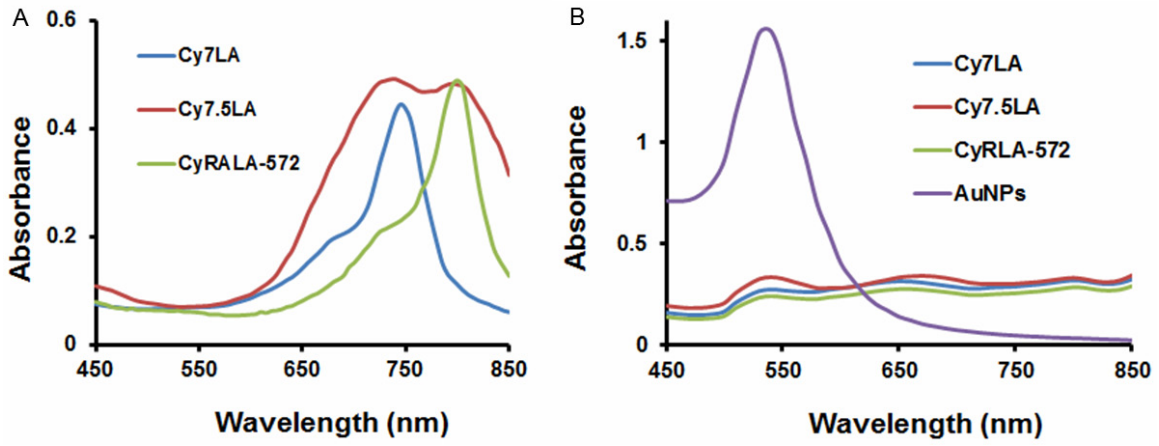



**Figure S3.** A: UV-Vis-NIR electronic absorbance of Raman reporters (Cy7LA, Cy7.5LA and CyRLA-572; 20 μM in deionized water. B: UV-Vis-NIR surface Plasmon resonance (SPR) of citrate stabilized gold nanoparticles with NIR or without Raman reporters.

*Mixed PEG coating of SERS nanoparticles and stability study*

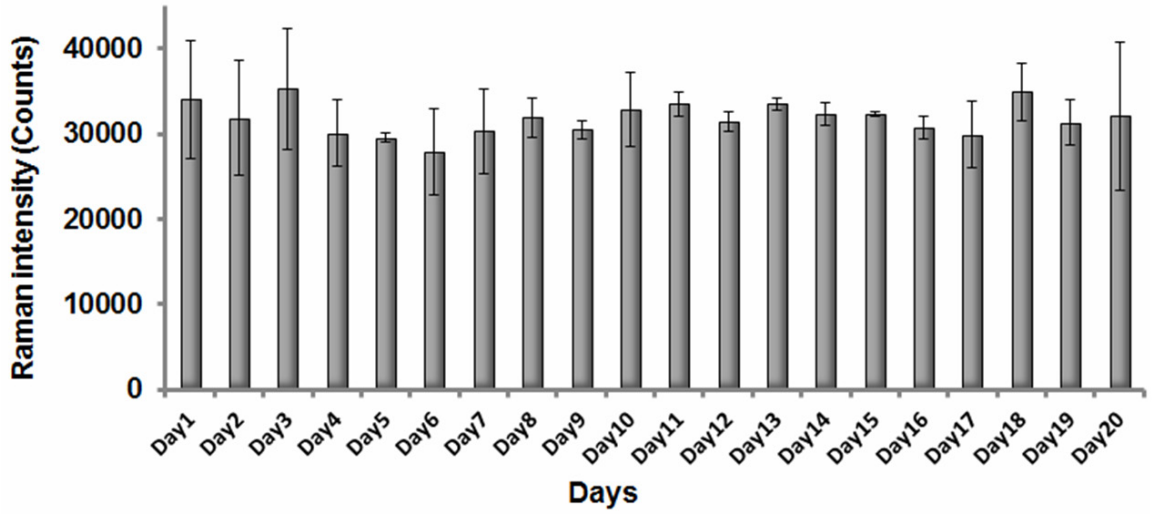



**Figure S4.** Time course SERS measurement of CyRLA-572@AuNPs@PEG in deionized water. SERS intensities of the highest Raman peaks (i.e. 523 $cm^{-1}$) are plotted as means ± standard deviation of 5 independent measurements taken from the same sample at different time points.

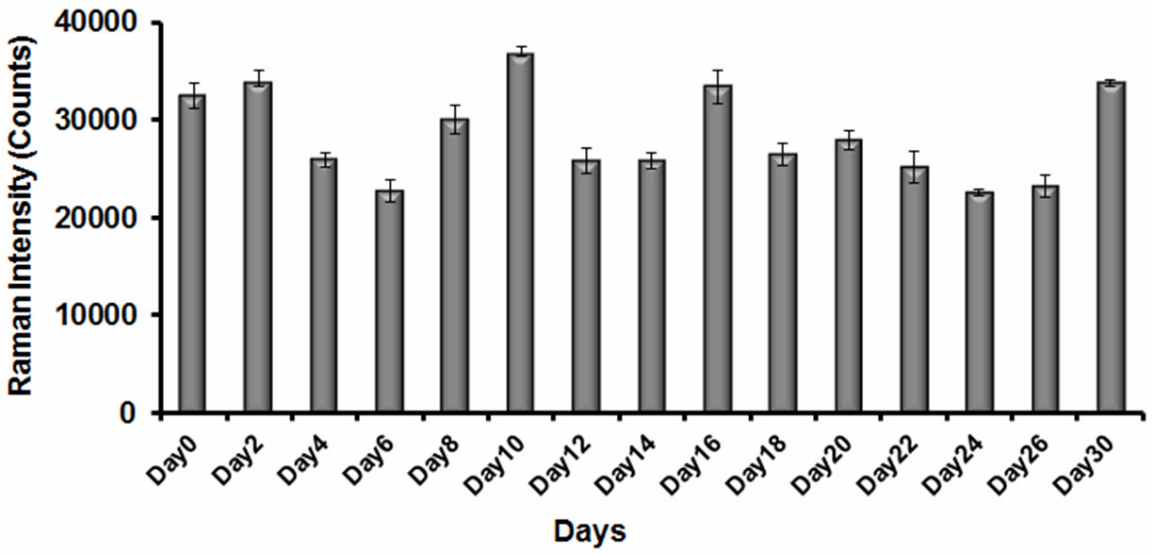



**Figure S5.** Time course SERS measurement of CyRLA-240@AuNPs@PEG in deionized water. SERS intensities of the highest Raman peaks (i.e. 523 $cm^{-1}$) are plotted as means ± standard deviation of 3 independent measurements taken from the same sample at different time points.

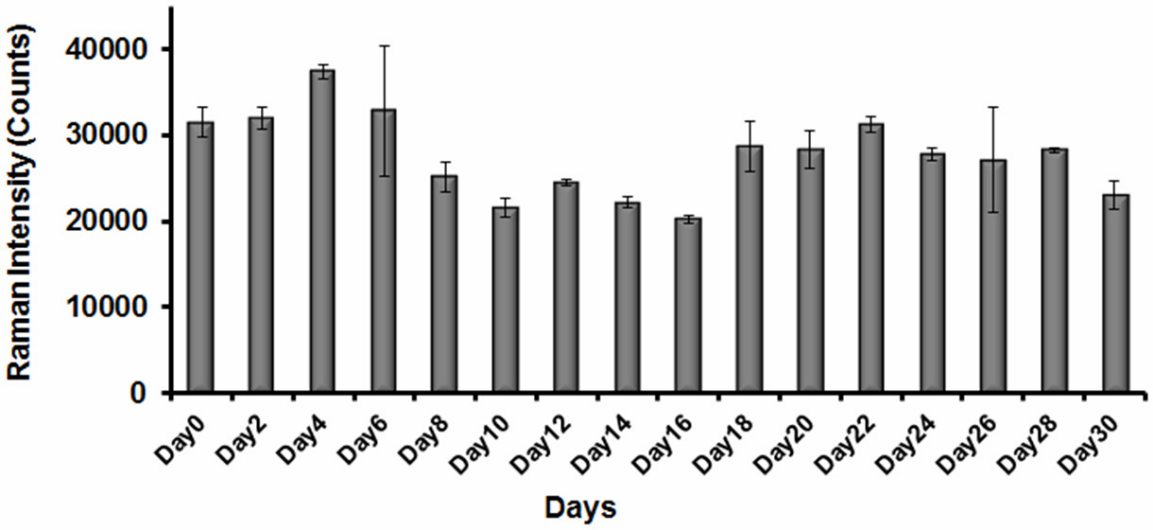



**Figure S6.** Time course SERS measurement of CyRLA-364@AuNPs@PEG in deionized water. SERS intensities of the highest Raman peaks (i.e. 523 $cm^{-1}$) are plotted as means ± standard deviation of 3 independent measurements taken from the same sample at different time points.

*Transmission electron microscopy*

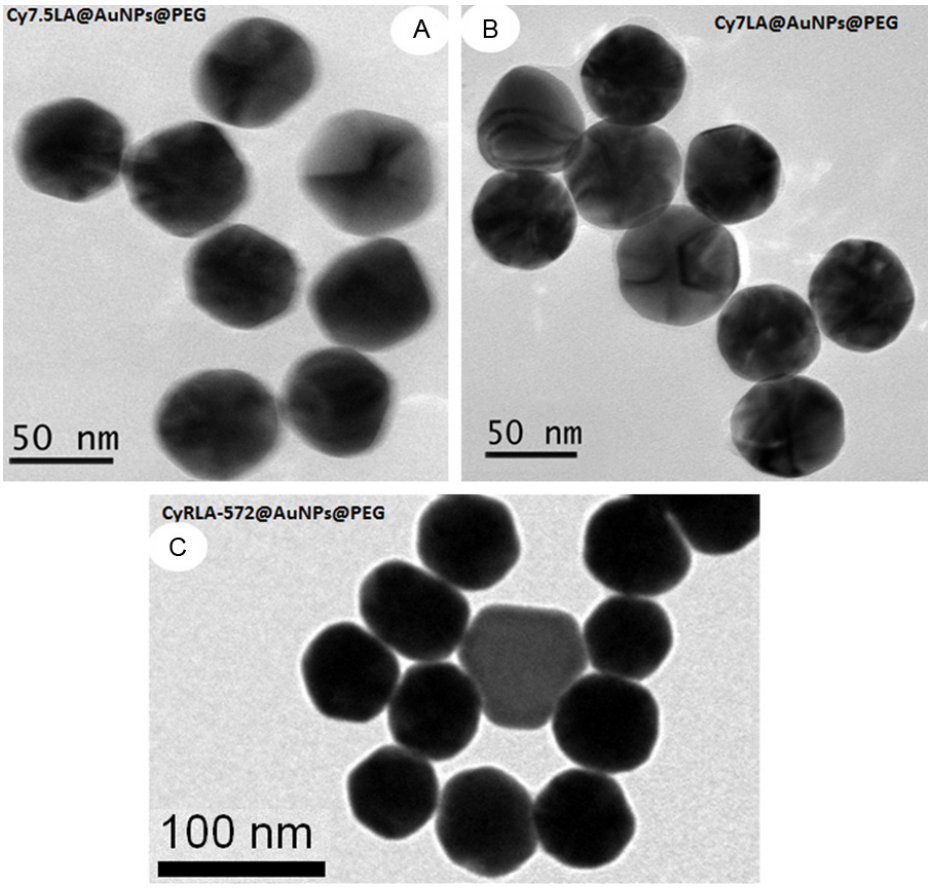



**Figure S7.** Transmission electron microscopy (TEM) images of: A: PEG-coated Cy7.5LA@AuNPs@PEG; B: Cy7LA; C: CyRLA-572 nanotag; Scale bar: 50 nm for Cy7 and Cy7.5.

*Antibody conjugation to SERS nanotags*

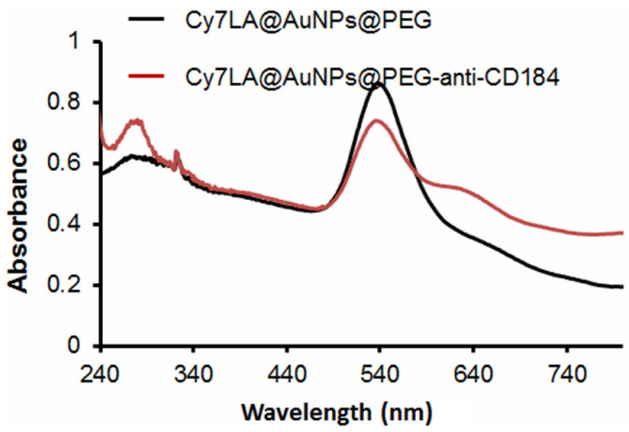



**Figure S8.** Surface plasmon resonance spectra of Cy7LA@AuNPs@PEG nanotags before and after conjugation of antibody.

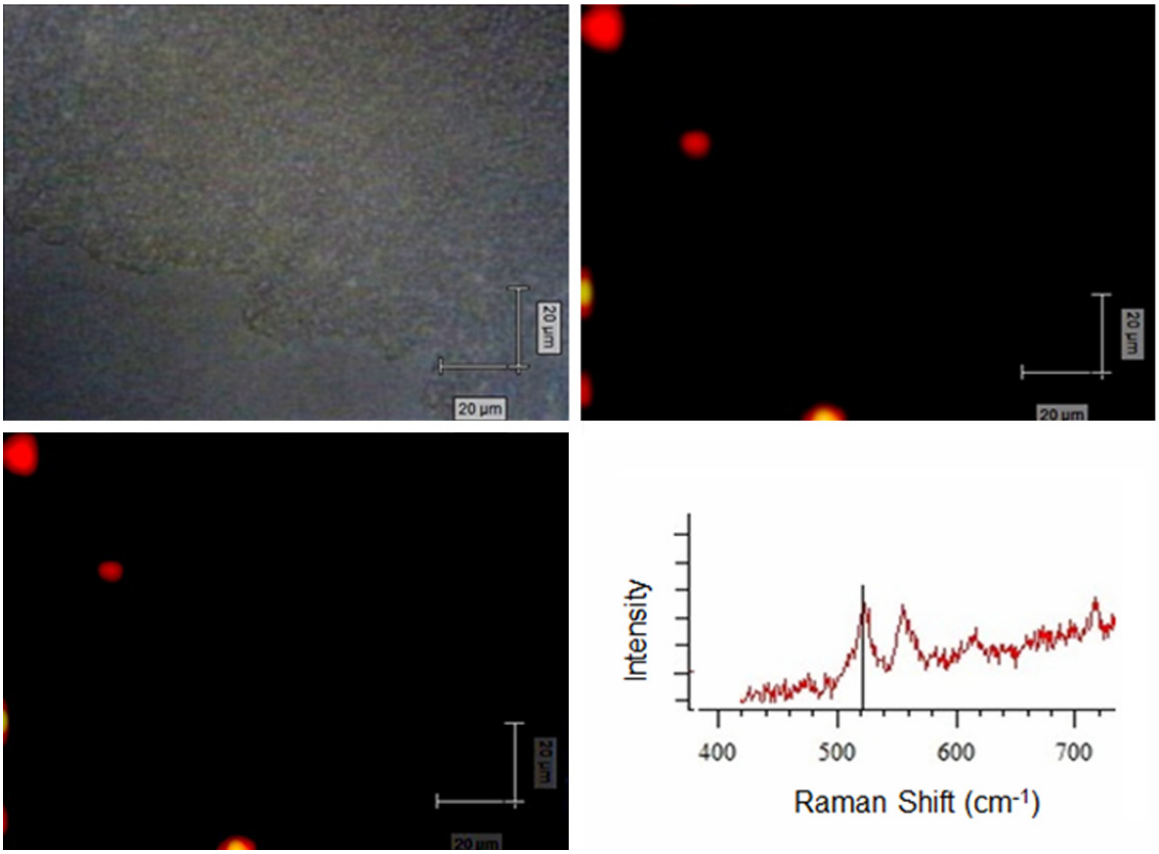



**Figure S9.** Bright field, SERS mapping images, merged images and complementary SERS spectra of differentiated mouse embryonic stem cells (mESC) treated with CyRLA-572@AuNPs@PEG. The mapping experiments were scanned at multiplex peaks of corresponding nanotags (523 $cm^{-1}$) at an interval of 2 μm (785 nm excitation) and the intensities were normalized between the lowest (0) and the highest color (1) values. Scale bar: 20 μm.

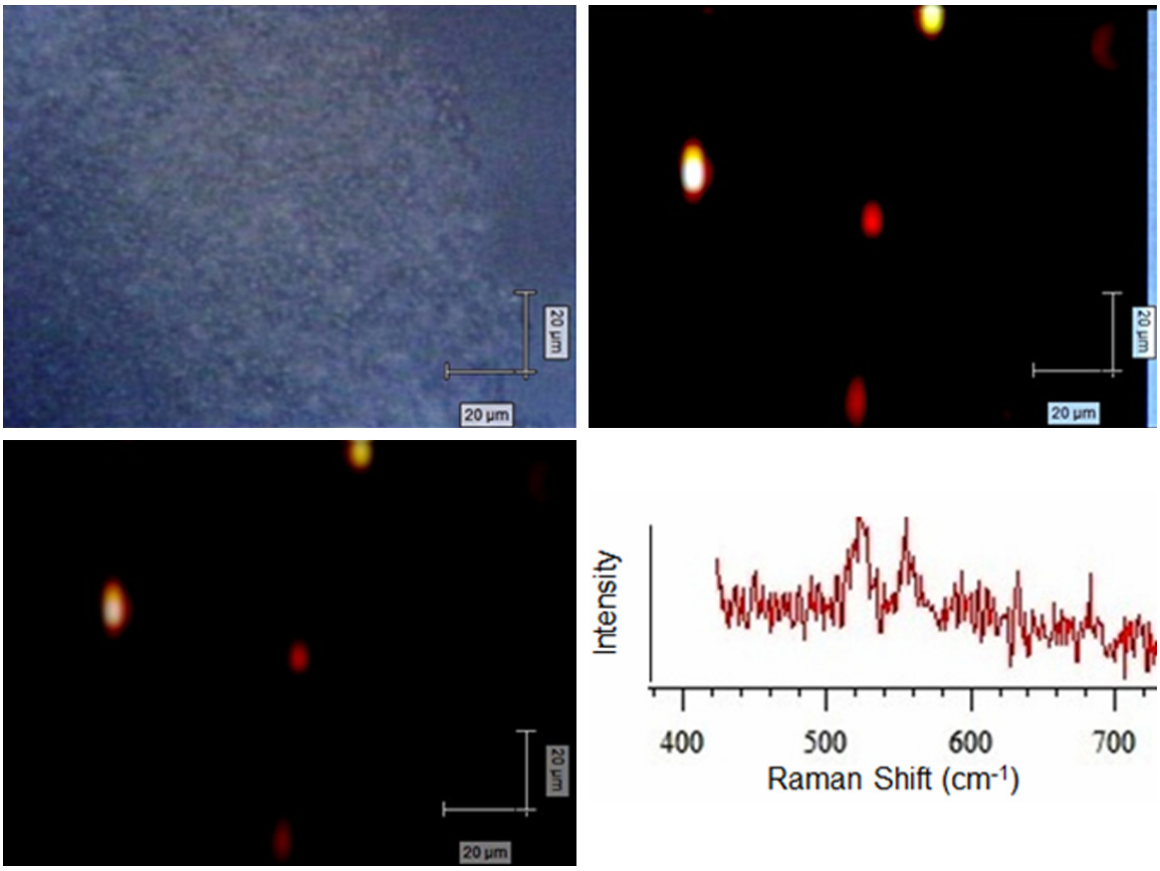



**Figure S10.** Bright field, SERS mapping images, merged images and complementary SERS spectra of differentiated mouse embryonic stem cells (mESC) treated with AuNPs@CyRLA@PEG-anti-HER2. The mapping experiments were scanned at multiplex peaks of corresponding nanotags (523 $cm^{-1}$) at an interval of 2 µm (785 nm excitation) and the intensities were normalized between the lowest (0) and the highest color (1) values. Scale bar: 20 µm.

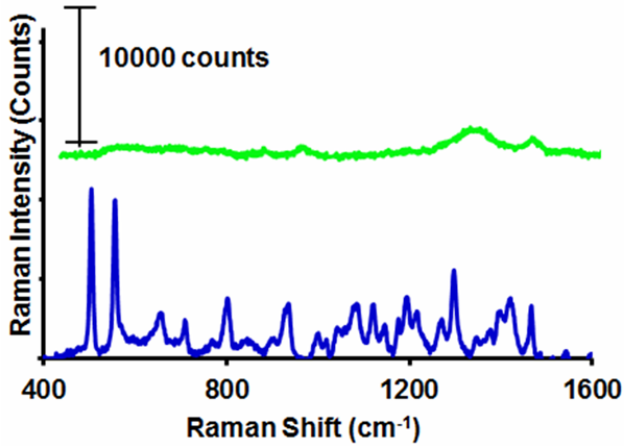



**Figure S11.** *In vivo* spectra from liver site after injection of pegylated AUNPs i.e Cy7LA@AuNPs@PEG. The spectra were scanned at an interval of 2 µm (785 nm excitation wavelength) and the intensities were normalized after baseline corrections; acquisition time: 30 s.

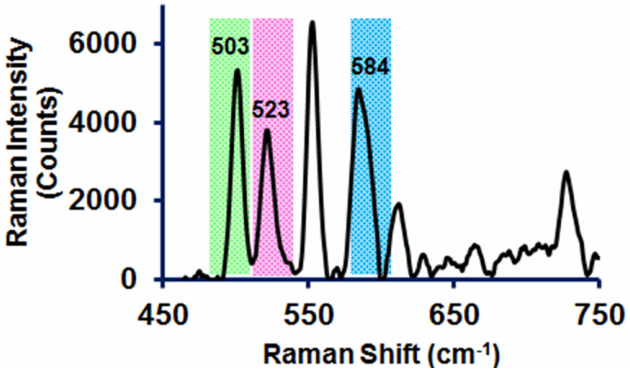



**Figure S12.** *In vivo* spectra from liver site after injection of mixture of three nanotags i.e Cy7LA@AuNPs@PEG-anti-CD184, Cy7.5LA@AuNPs@PEG-anti-Notch1 and CyRLA-572@AuNPs@PEG-anti-CD34 nanotgas. The spectra were scanned at an interval of 2 µm (785 nm excitation wavelength) and the intensities were normalized after baseline corrections; acquisition time: 30 s.

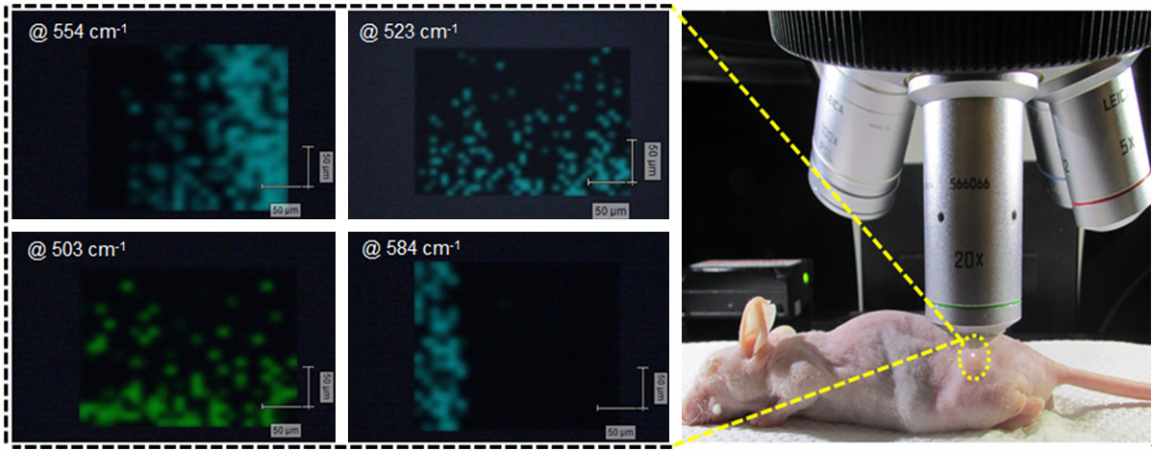



**Figure S13.** *In vivo* imaging of teratoma in animal model model. Three different multplexed peaks i.e 503 $cm^{-1}$, 523 $cm^{-1}$, and 584 $cm^{-1}$ and one common peak 554 $cm^{-1}$ for Cy7LA and CyRLA-572 were applied to scan the teratoma location for getting the images.