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DNA detection by dye labeled oligonucleotides using surface enhanced Raman spectroscopy

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The detailed procedure for synthesis of the used dye-labeled oligonucleotides was optimized previously and was fully described.[1] For the synthesized dye-labeled oligonucleotides (Table S1) mass spectrometry (MS) was applied as the method of characterization. Purity of the oligonucleotides was controlled by LC-UV. The Ultimate 3000 chromatography with liquid chromatography quadrupole (LCQ) fleet MS detection was utilized. LC conditions were following: flow: 0.3 ml/min; temperature control: 45 °C; buffer system: A: 10 mM diisopropilamine, 15 mM HFIP[1] (100% water milliQ grade) and B: 10 mM diisopropilamine, 15 mM HFIP (20% water milliQ grade, 80% acetonitrile UHPLC grade); step elution: A: 0–1 min 100%, B: 1–3.5 min 100%; UV detection: at 260 nm; MS conditions: ESI source in negative scan mode.

Raman spectra were collected using InVia Raman confocal microscope (Renishaw, UK) for 1 μM concentrations of analytes. SERS spectra were measured with 10% power neutral density filter. A 50× short working distance objective lens with a spot size of 2 μm was used under a static single scan of 10 s. The selected measurement parameters are due to the possibility of photodegradation of the AgNPs surface. Before measurements a silicon wafer was used for calibration.

For each sample after hybridization, a 10 μl droplet of the resulting mixture was pipetted on a small square piece of SERS-active surface (4 × 4 mm^2) of AgNPs. For all the SERS measurements, the analyte was adsorbed on the silver surface in a static mode for 30 min at room temperature. We scanned each SERS sensor surface to receive minimum 20 spectra for elimination of sample outliers and estimated the reproducibility of the obtained analytical signals by measuring spectra for identical solutions on three different SERS sensors.

[1] HFIP – Hexafluoroisopropanol

Table S1 The samples used in the developed technique.

Name	Sequence
A/Rh6G	Rh6G-AAAGGGCCCGGAGGGGGCTGGGCCGGGGACCCGGGAGGGGTCGGGACGG
A/Cy3	Cy3-AAAGGGCCCGGAGGGGGCTGGGCCGGGGACCCGGGAGGGGTCGGGACGG
B/Rh6G	Rh6G-AAAGGGCCCGGAGGGGGCTGGGCCGGGGACCCGGGAG
B/Cy3	Cy3-AAAGGGCCCGGAGGGGGCTGGGCCGGGGACCCGGGAG
C/Rh6G	Rh6G-AAAGGGCCCGGAGGGGGCTGGGCCG
C/Cy3	Cy3-AAAGGGCCCGGAGGGGGCTGGGCCG
D/Rh6G	Rh6G-AAAGGGCCCGGAG
D/Cy3	Cy3-AAAGGGCCCGGAG

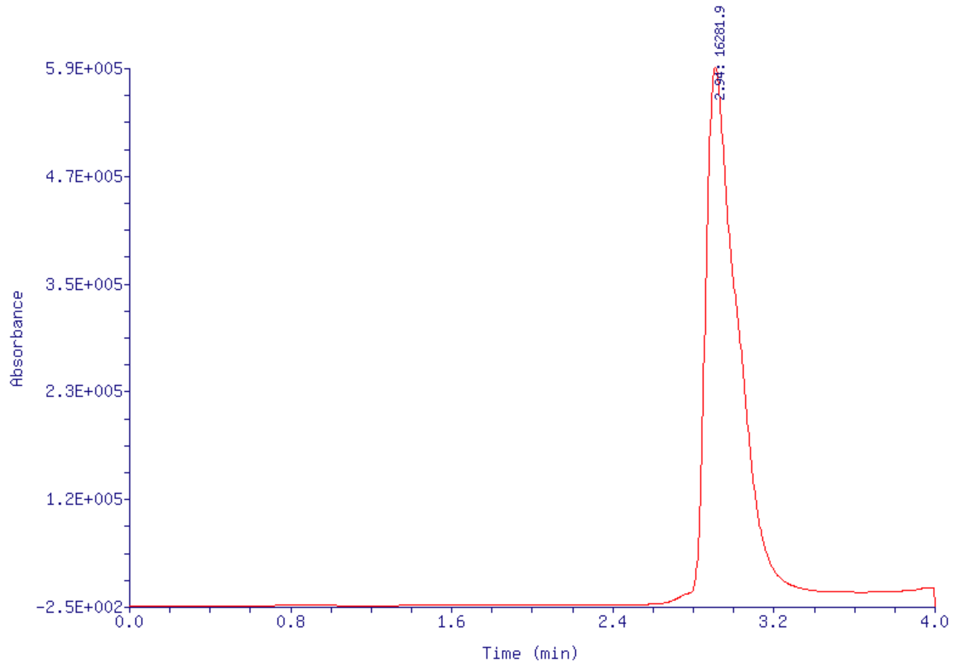

Figure S1 LC–UV chromatogram of **A/Rh6G** sample.

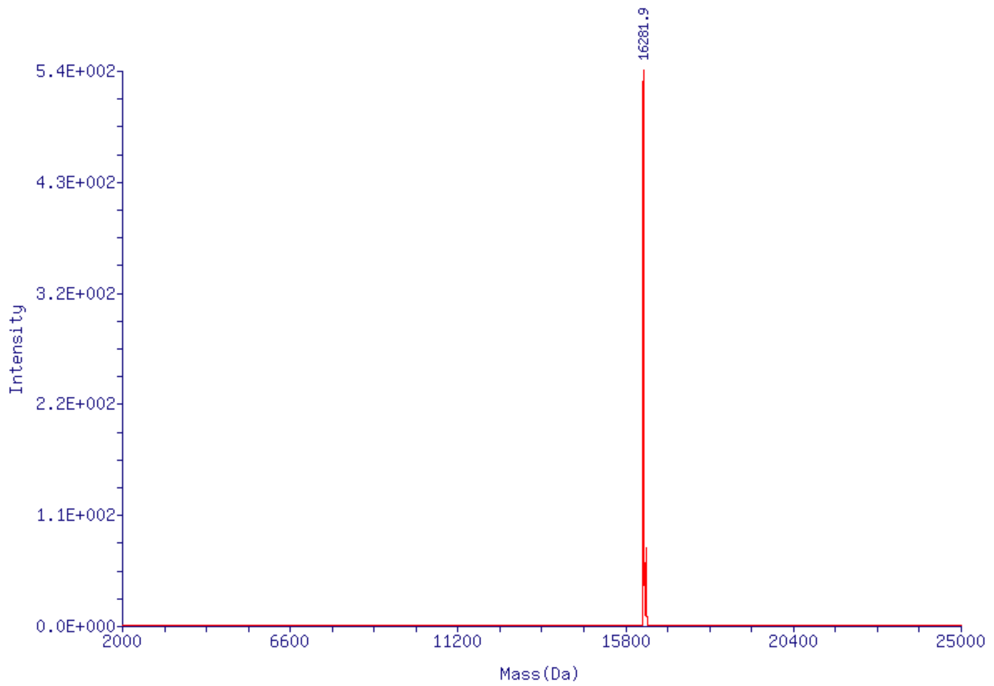

Figure S2 Deconvoluted mass-spectrum of **A/Rh6G** sample. Accurate mass: 16278.2 Da, exact mass: 16281.9 Da, mass error: 3.7 Da.

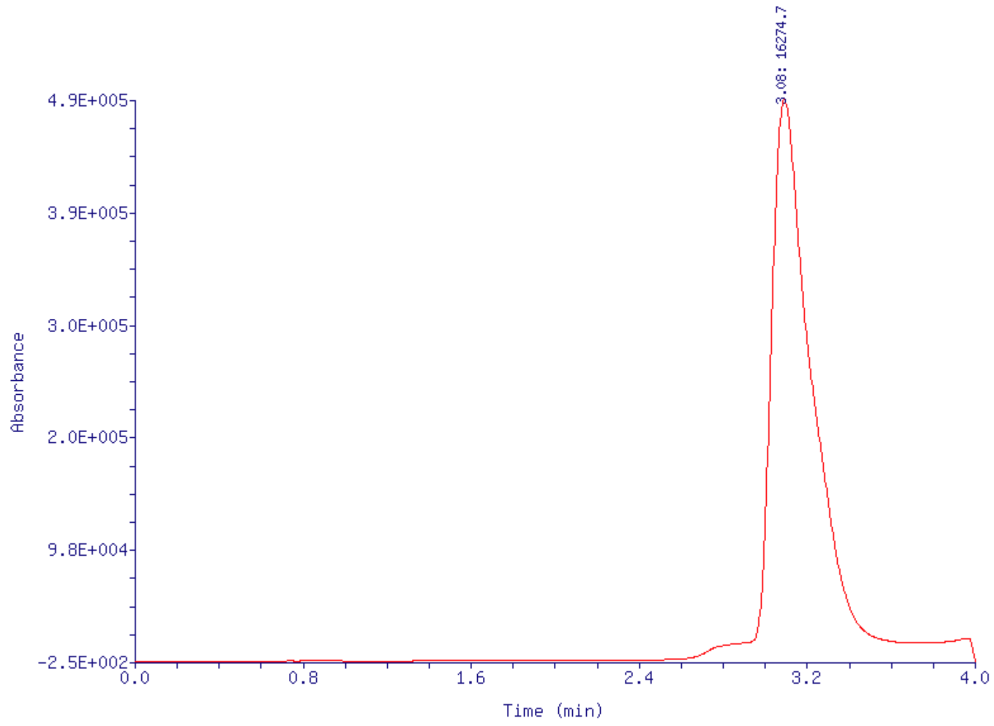

Figure S3 LC–UV chromatogram of **A/Cy3** sample.

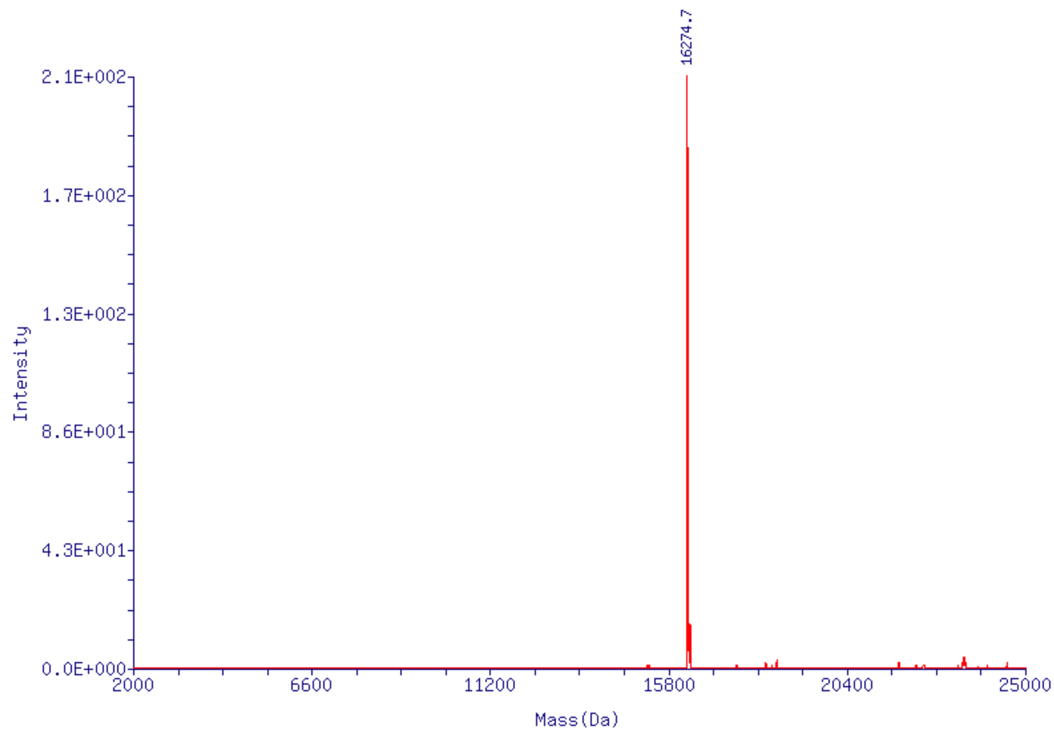

Figure S4 Deconvoluted mass-spectrum of **A/Cy3** sample. Accurate mass: 16277.2 Da, exact mass: 16274.7 Da, mass error: 2.5 Da.

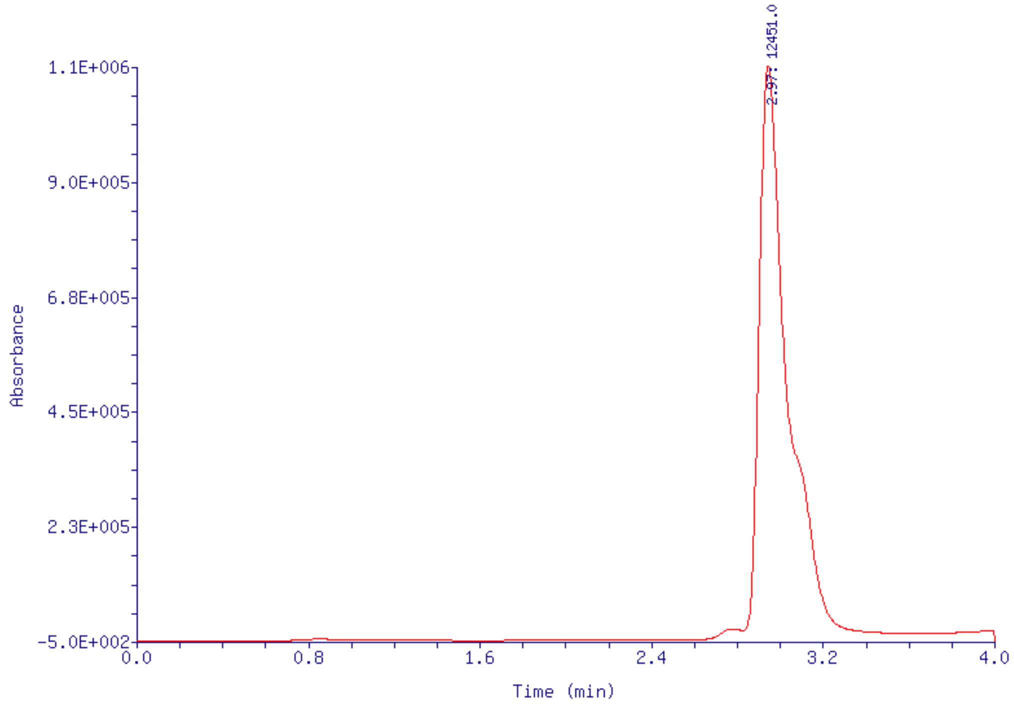

Figure S5 LC–UV chromatogram of **B/Rh6G** sample.

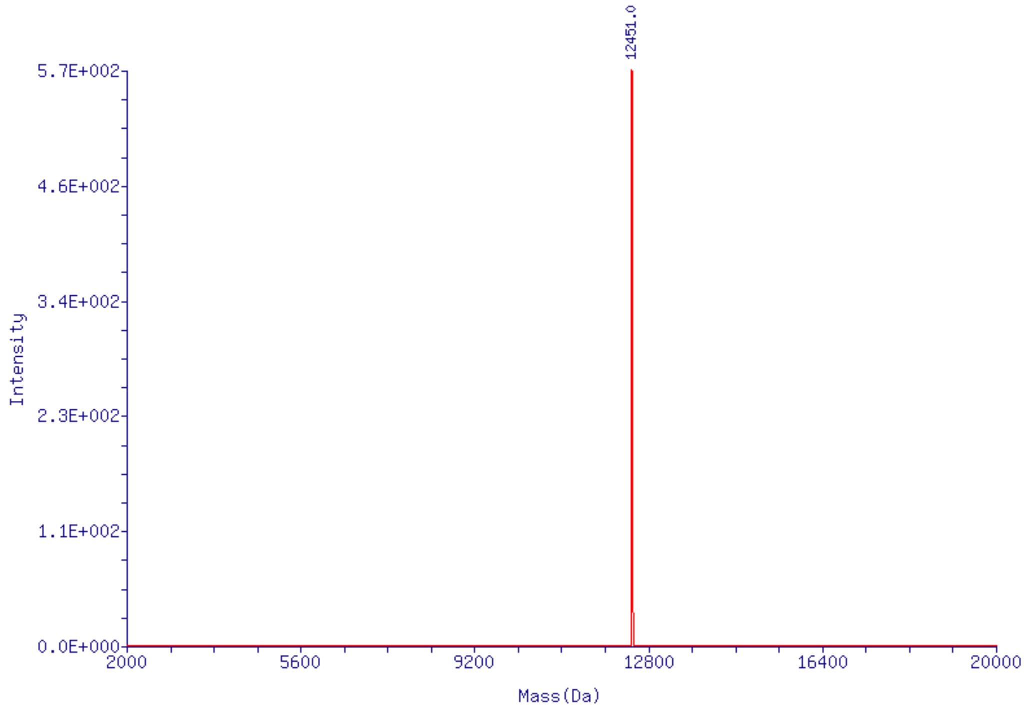

Figure S6 Deconvoluted mass-spectrum of **B/Rh6G** sample. Accurate mass: 12448.8 Da, exact mass: 12451.0 Da, mass error: 2.2 Da.

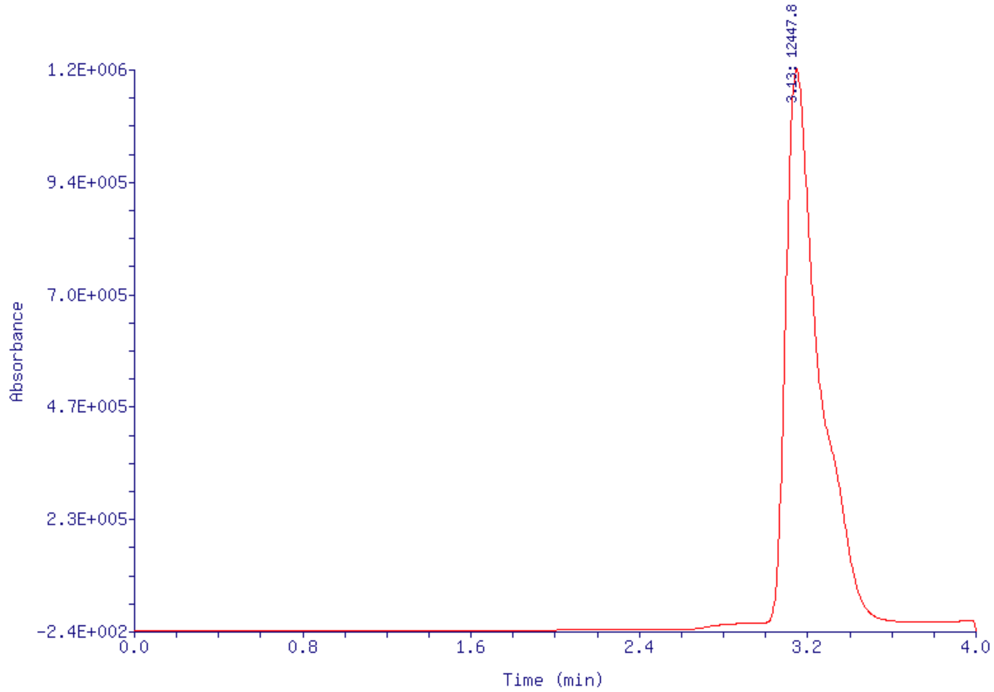

Figure S7 LC–UV chromatogram of **B/Cy3** sample.

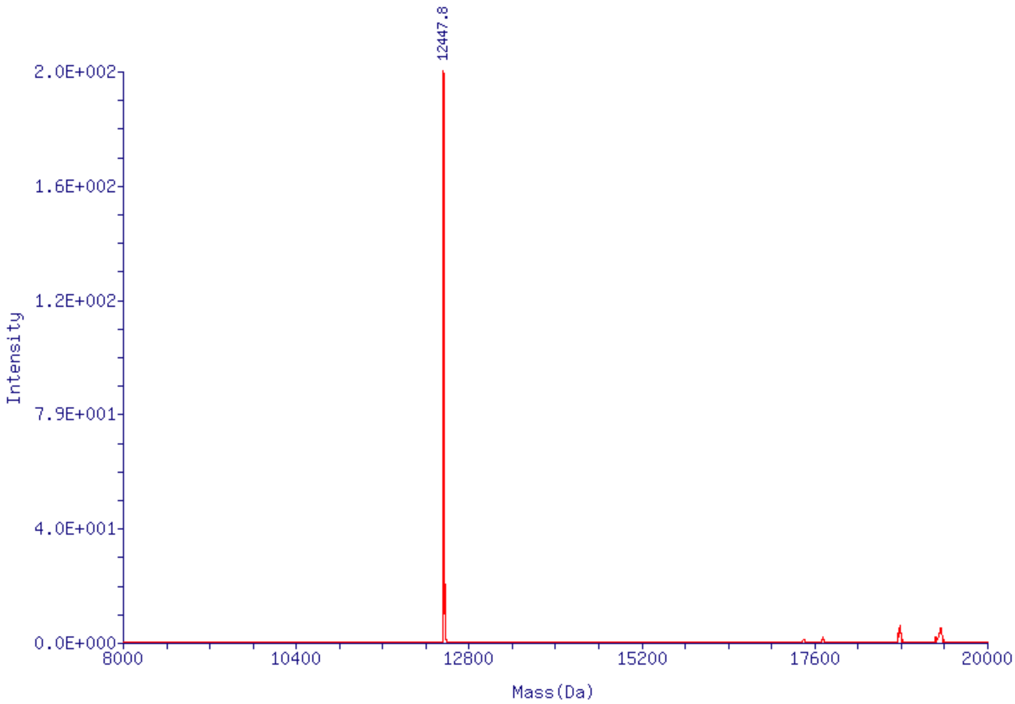

Figure S8 Deconvoluted mass-spectrum of **B/Cy3** sample. Accurate mass: 12447.8 Da, exact mass: 12447.8 Da, mass error: 0.0 Da.

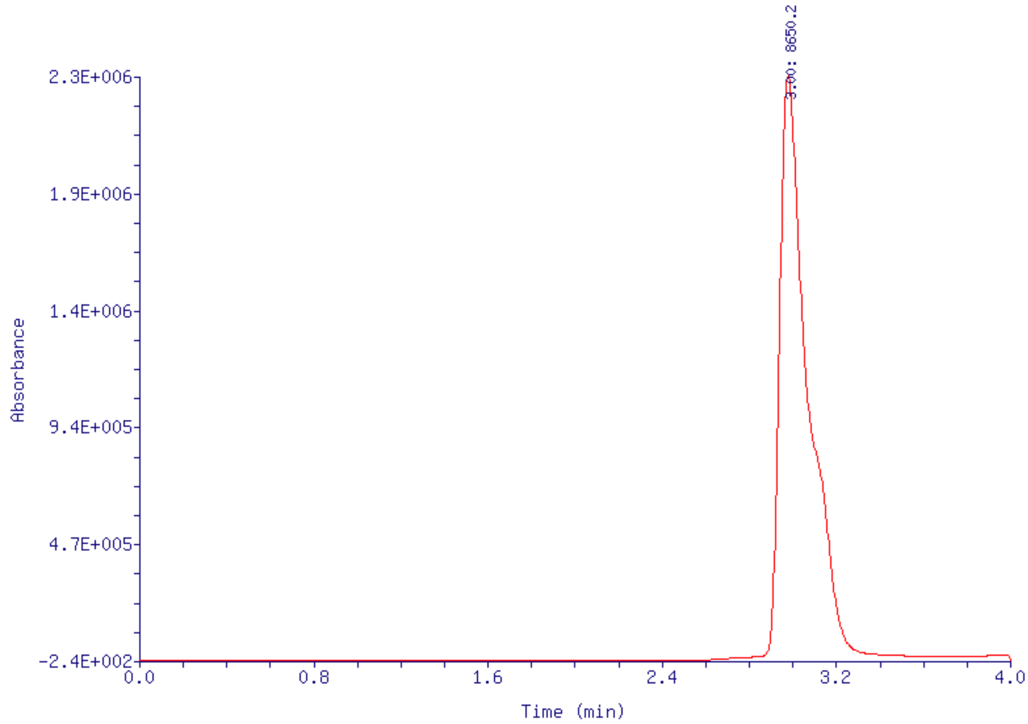

Figure S9 LC–UV chromatogram of **C/Rh6G** sample.

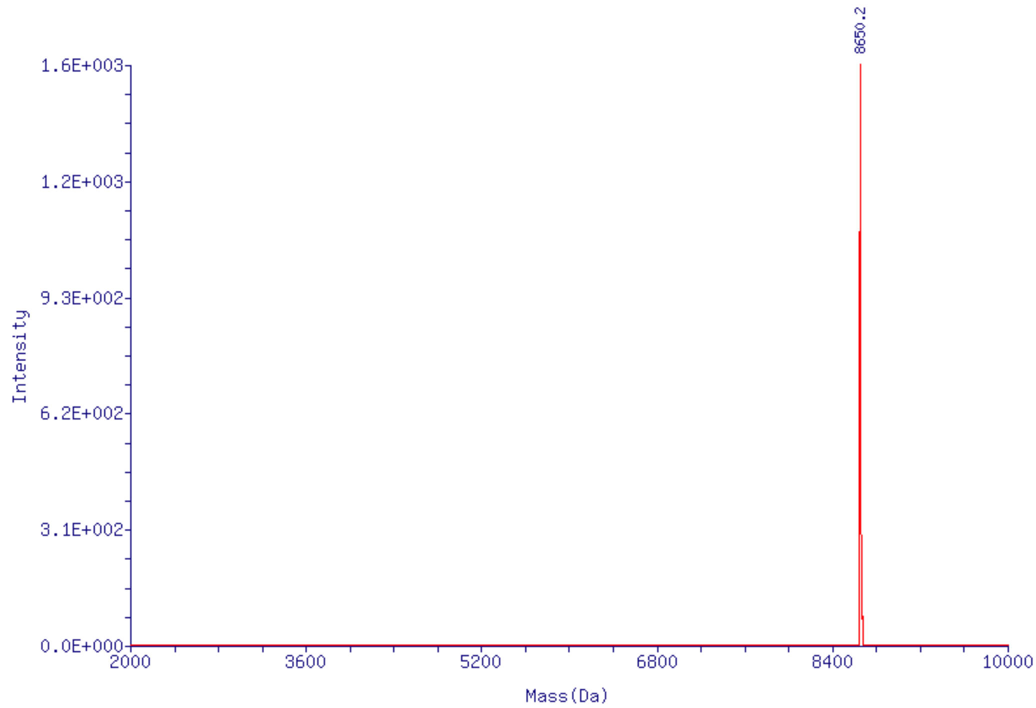

Figure S10 Deconvoluted mass-spectrum of **C/Rh6G** sample. Accurate mass: 8650.3 Da, exact mass: 8650.2 Da, mass error: 0.1 Da.

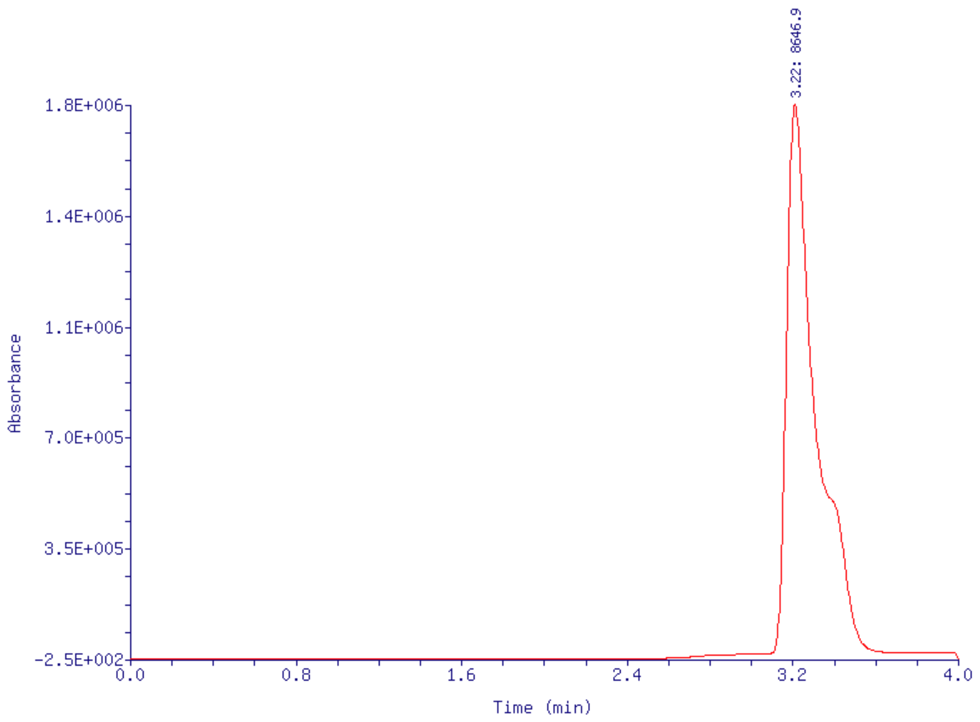

Figure S11 LC–UV chromatogram of **C/Cy3** sample.

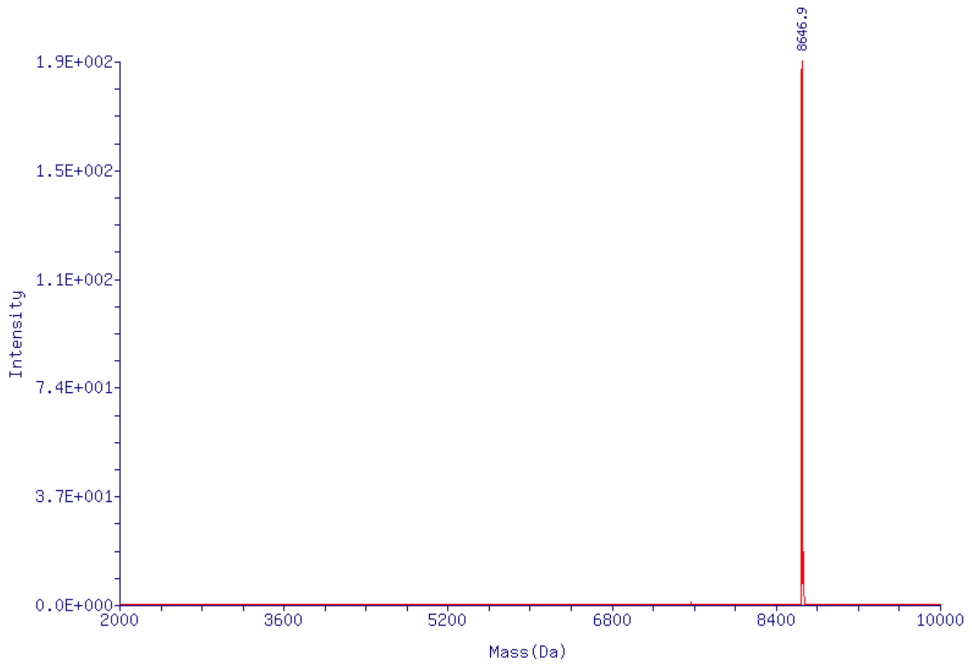

Figure S12 Deconvoluted mass-spectrum of **C/Cy3** sample. Accurate mass: 8649.3 Da, exact mass: 8646.9 Da, mass error: 2.4 Da.

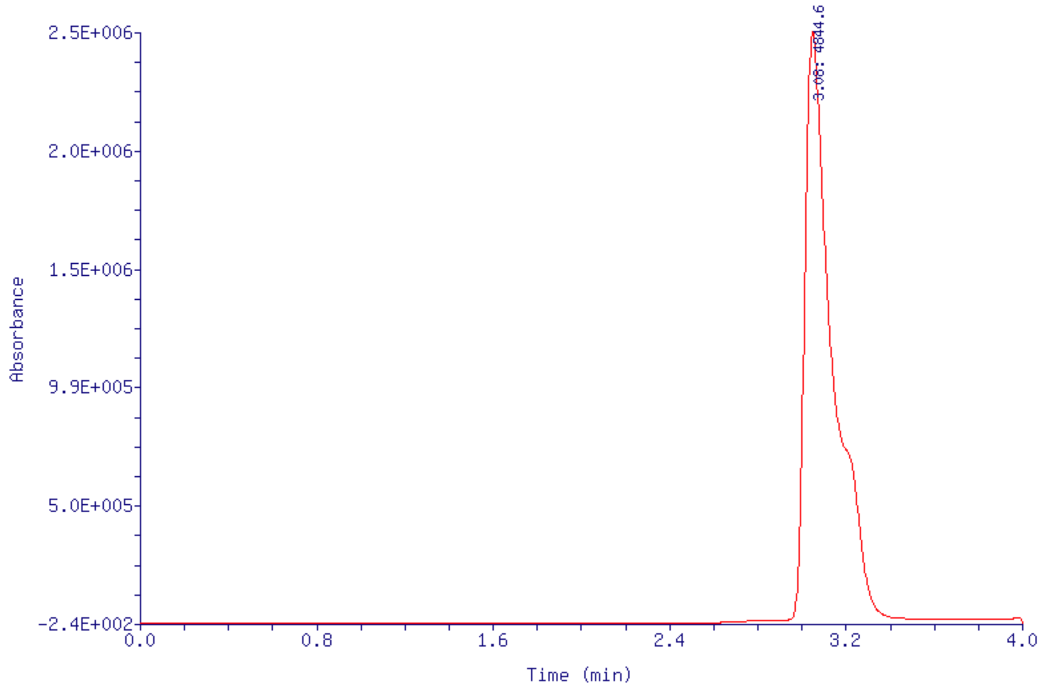

Figure S13 LC–UV chromatogram of **D/Rh6G** sample.

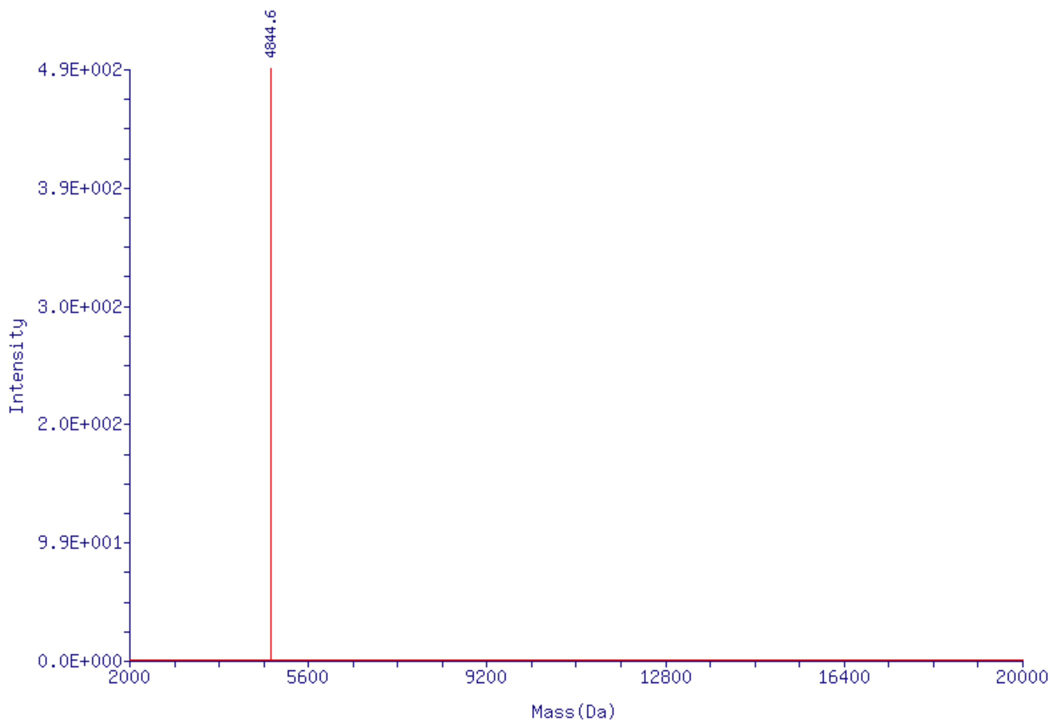

Figure S14 Deconvoluted mass-spectrum of **D/Rh6G** sample. Accurate mass: 4844.9 Da, exact mass: 4844.6 Da, mass error: 0.3 Da.

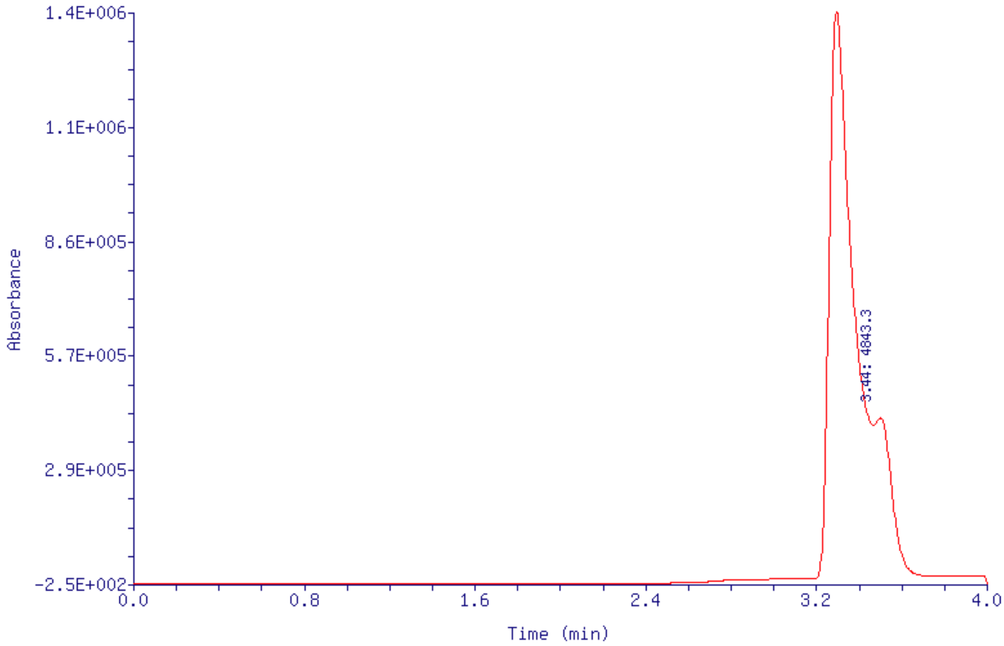

Figure S15 LC–UV chromatogram of **D/Cy3** sample.

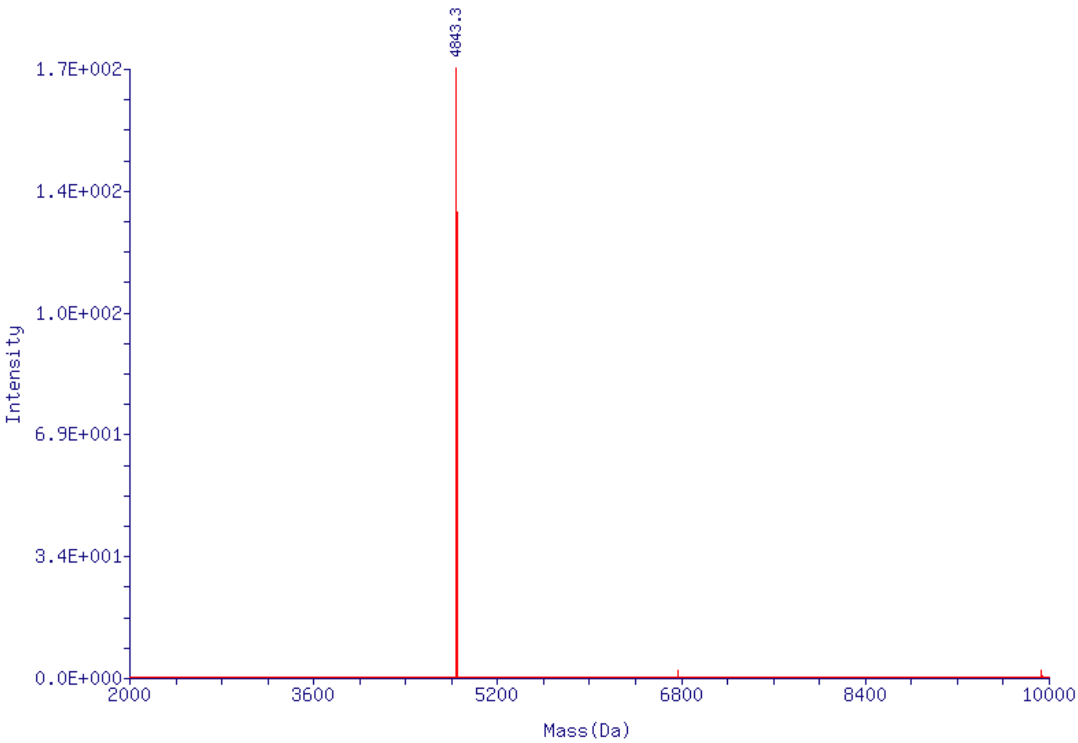

Figure S16 Deconvoluted mass-spectrum of **D/Cy3** sample. Accurate mass: 4843.9 Da, exact mass: 4843.6 Da, mass error: 0.3 Da.

Table S2 Raman shifts for the dyes and the dye-labeled oligonucleotides.

Tag	Raman shifts, cm^{-1}	
	Dye	Dye-labeled oligonucleotide
Cy3	556, 612, 796, 927, 1121, 1184, 1271, 1398, 1471, and 1590	1383, 1470, and 1590
Rh6G	613, 775, 1130, 1278, 1364, 1389, 1512, 1577, and 1651	1183, 1361, 1508, 1572, and 1650

Limit of detection LOD was determined as the lowest amount of analyte (measurand) in a sample that can be detected with (stated) probability, although perhaps not quantified as an exact value. LODs were calculated based on the standard deviation (*s*) of the response and the slope (*S*):

$$LOD = \frac{3s}{S}$$

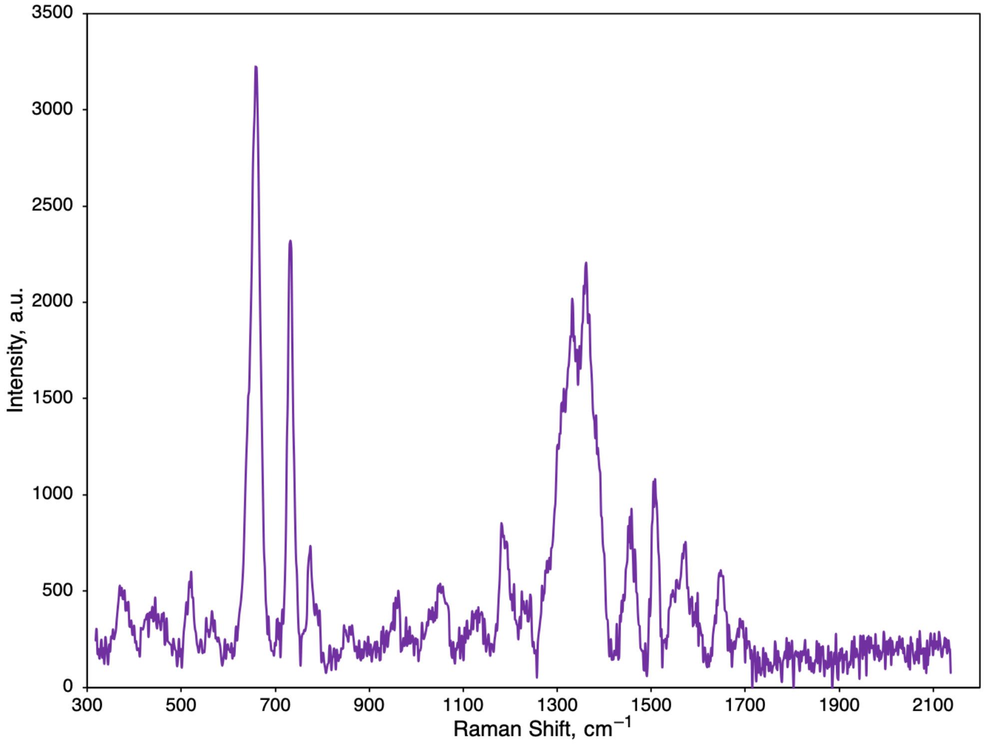

Figure S17 SERS spectrum on the USSR surface (4 × 4 mm^2) of Anchor 1–Promoter with 10 µl 10 µM A/Rh6G measured using a 20 mW 633 nm He–Ne laser (10%, 10 s).

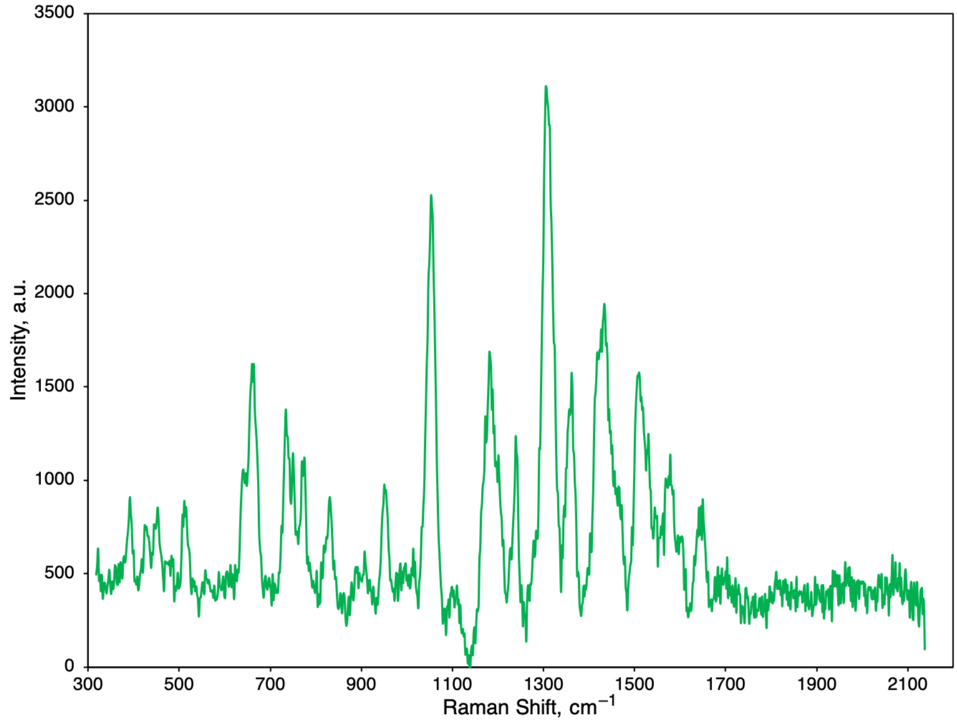

Figure S18 SERS spectrum on the USSR surface (4 × 4 mm^2) of Anchor 1–Promoter with 10 μl 10 μM B/Rh6G measured using a 20 mW 633 nm He–Ne laser (10%, 10 s).

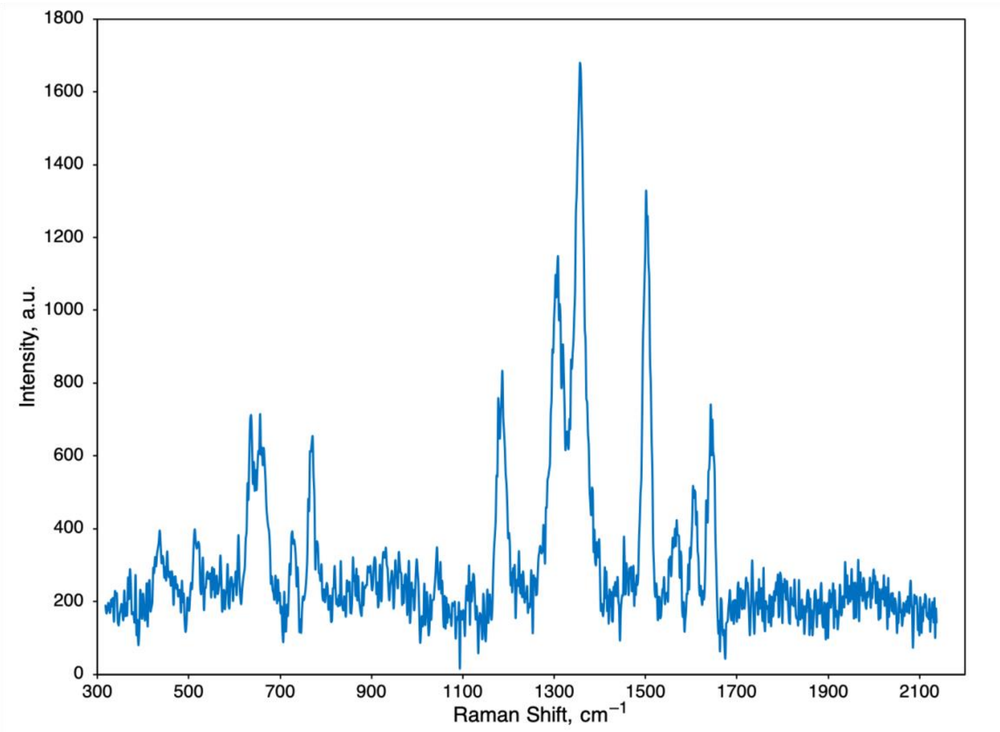

Figure S19 SERS spectrum on the USSR surface (4 × 4 mm^2) of Anchor 1–Promoter with 10 μl 10 μM C/Rh6G measured using a 20 mW 633 nm He–Ne laser (10%, 10 s).

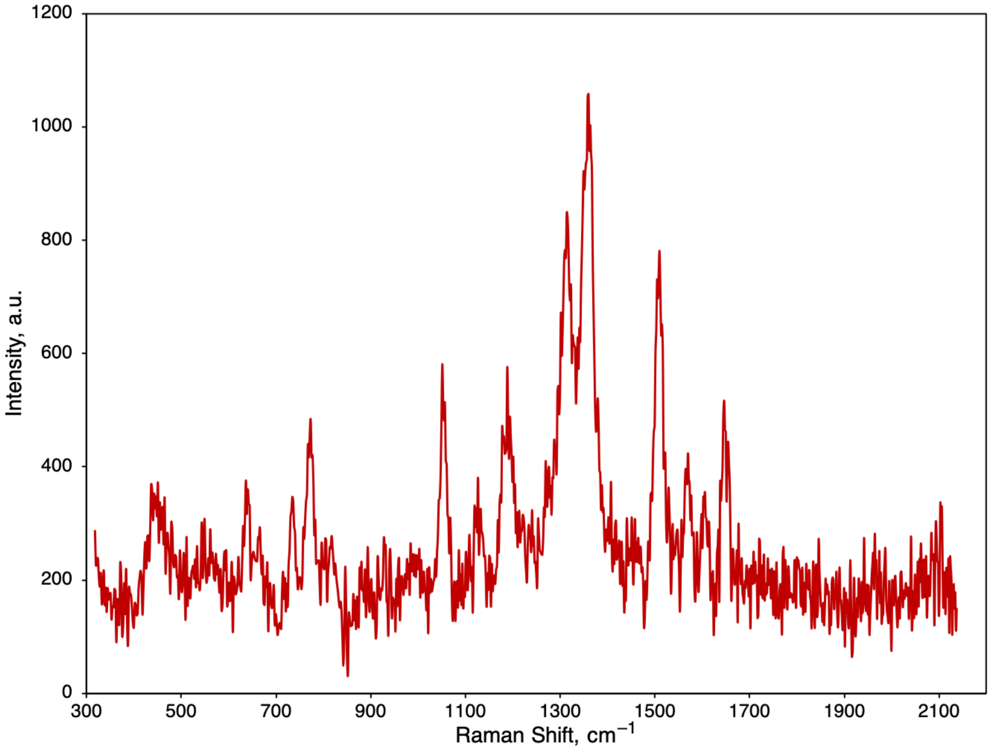

Figure S20 SERS spectrum on the USSR surface (4×4 mm^2) of Anchor 1–Promoter with 10 µl 10 µM D/Rh6G measured using a 20 mW 633 nm He–Ne laser (10%, 10 s).

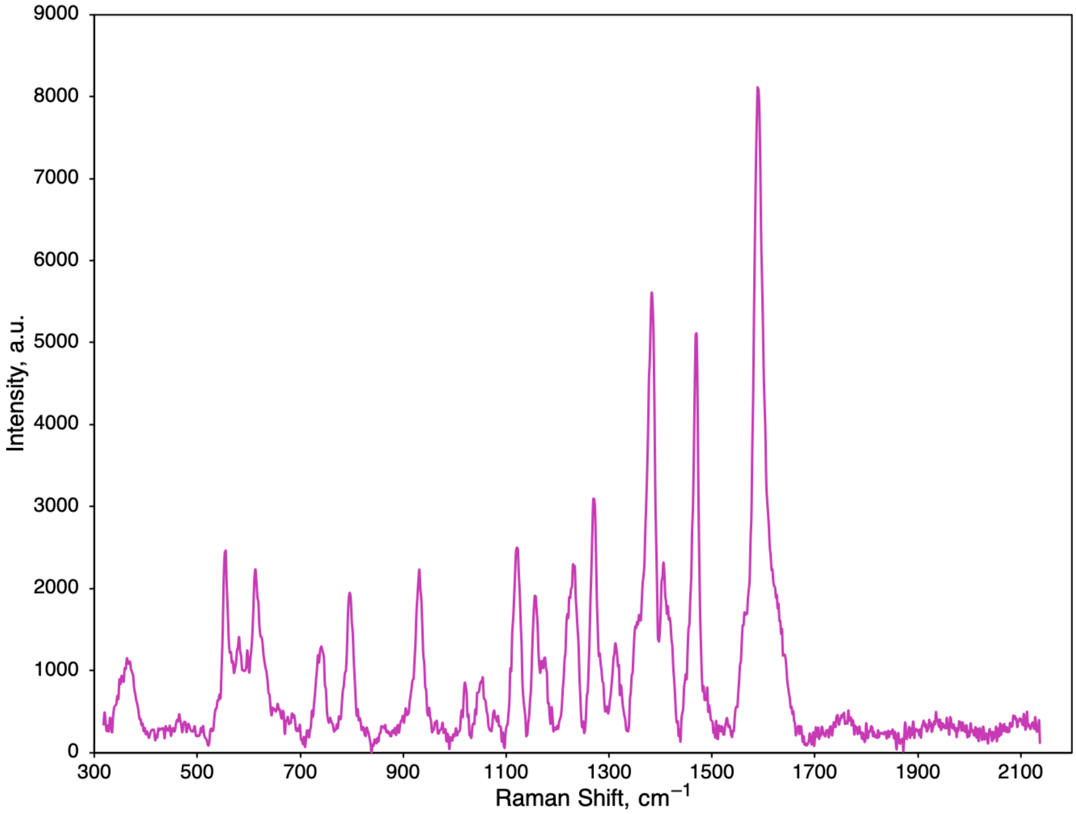

Figure S21 SERS spectrum on the USSR surface (4×4 mm^2) of Anchor 1–Promoter with 10 µl 10 µM D/Cy3 measured using a 20 mW 633 nm He–Ne laser (10%, 10 s).

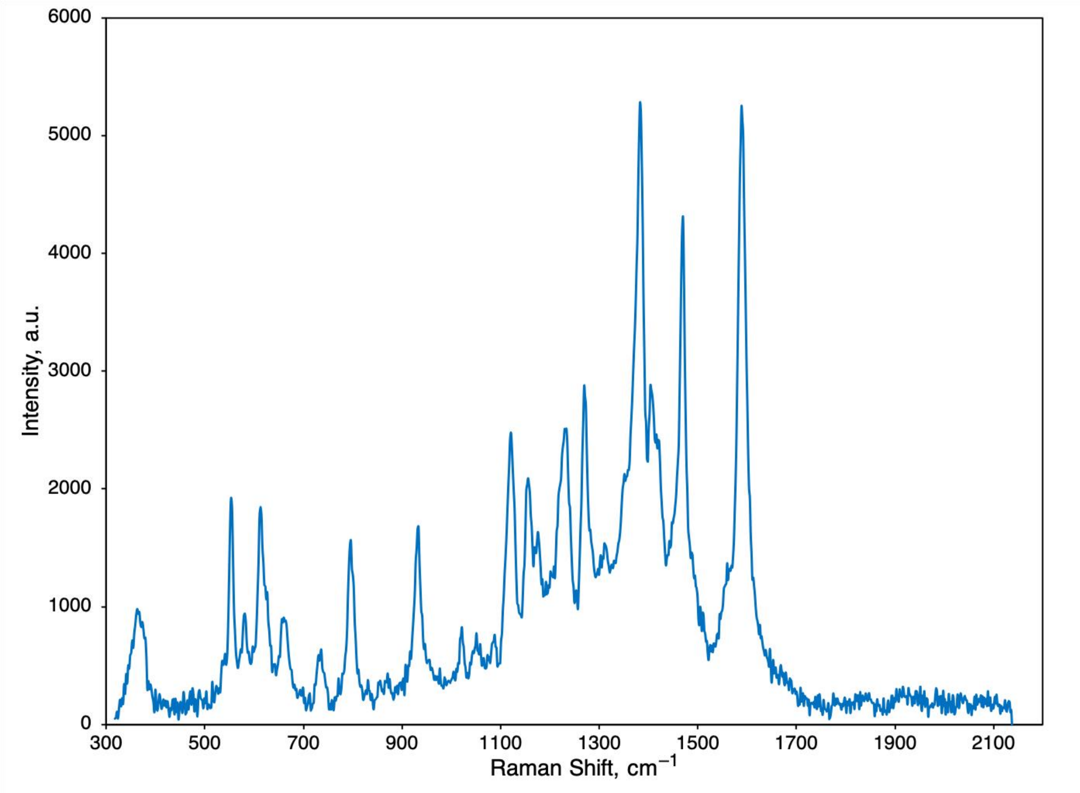

Figure S22 SERS spectrum on the USSR surface (4×4 mm^2) of Anchor 1–Promoter with 10 μl 10 μM C/Cy3 measured using a 20 mW 633 nm He–Ne laser (10%, 10 s).

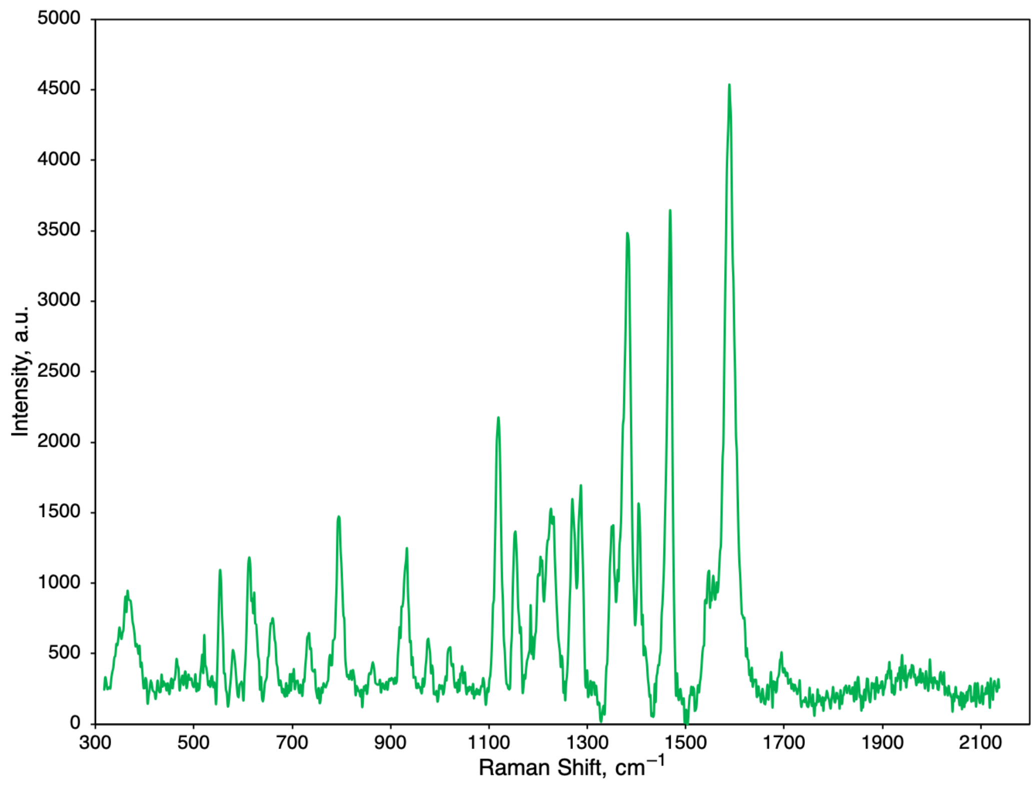

Figure S23 SERS spectrum on the USSR surface (4×4 mm^2) of Anchor 1–Promoter with 10 μl 10 μM B/Cy3 measured using a 20 mW 633 nm He–Ne laser (10%, 10 s).

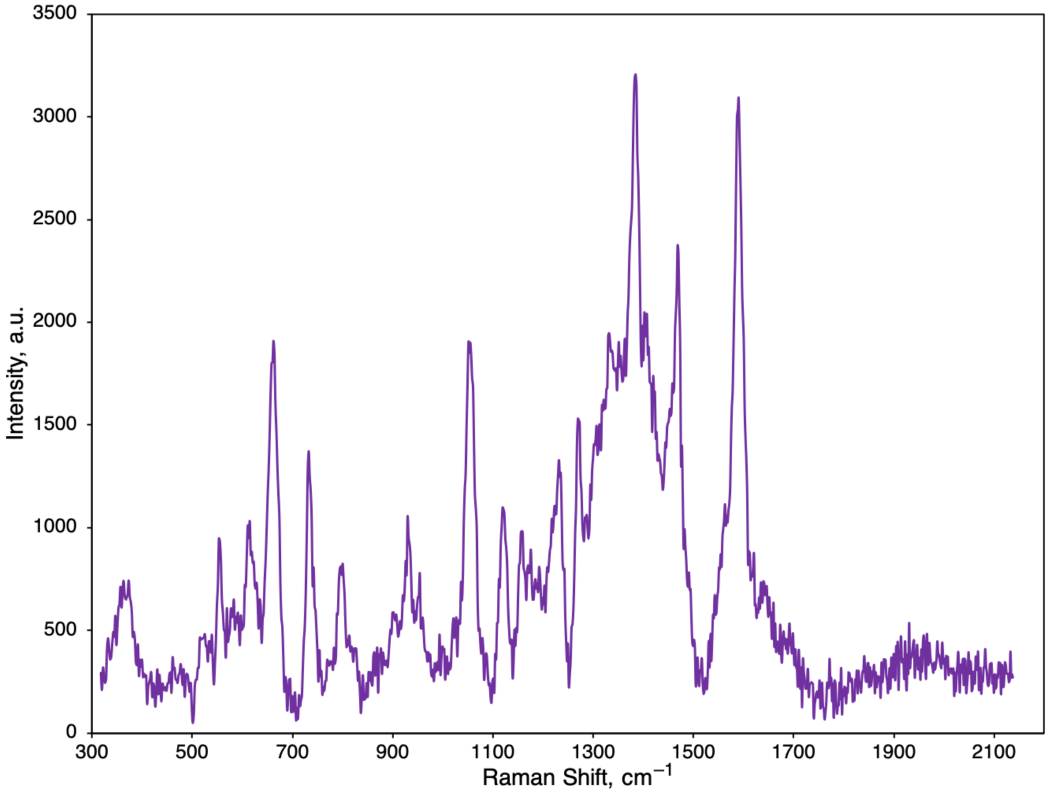

Figure S24 SERS spectrum on the USSR surface (4 × 4 mm^2) of Anchor 1–Promoter with 10 μl 10 μM A/Cy3 measured using a 20 mW 633 nm He–Ne laser (10%, 10 s).

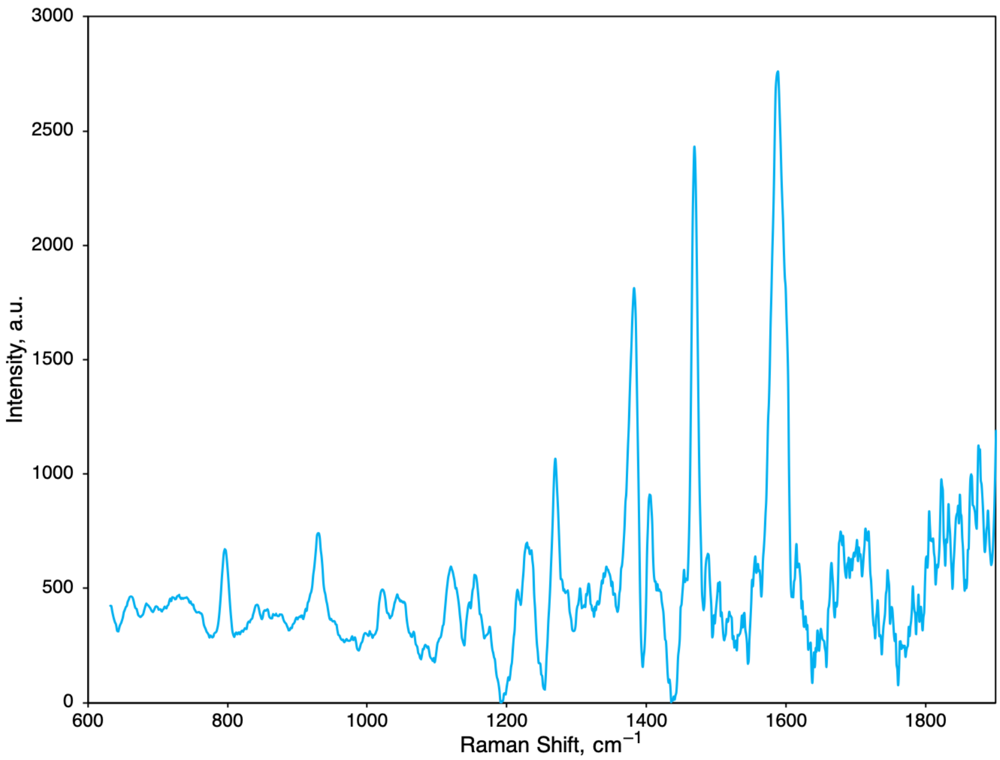

Figure S25 SERS spectrum on the USSR surface (4 × 4 mm^2) of Anchor 1–Promoter with 10 μl 10 μM A/Cy3 measured using a 17 mW 514 nm Ar^+ laser (10%, 10 s).

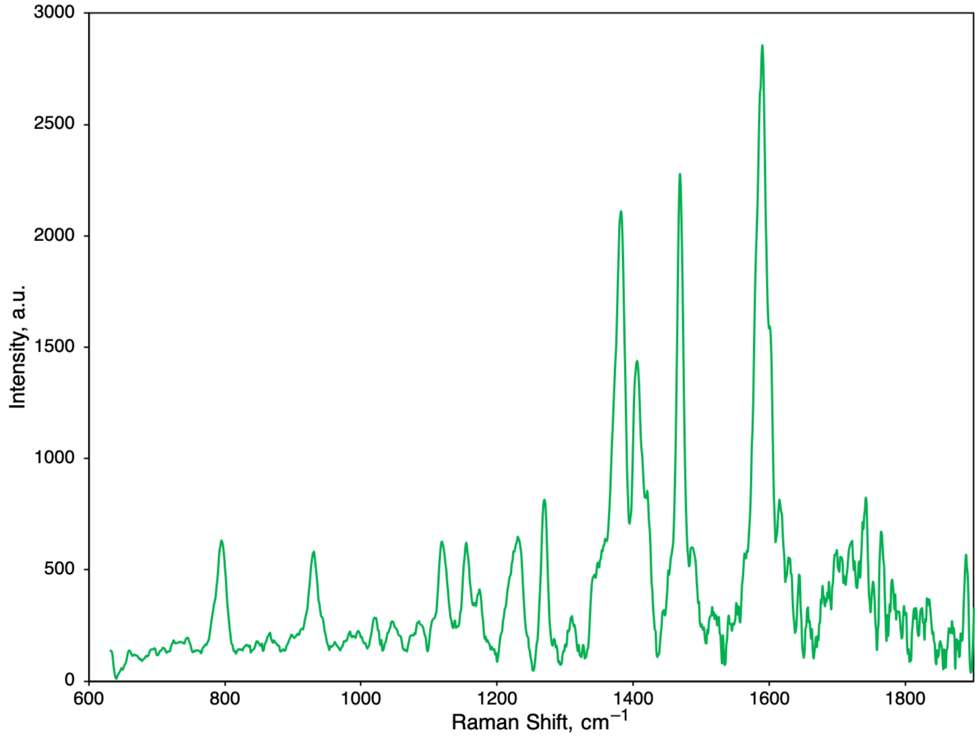

Figure S26 SERS spectrum on the USSR surface (4 × 4 mm^2) of Anchor 1–Promoter with 10 µl 10 µM B/Cy3 measured using a 17 mW 514 nm Ar^+ laser (10%, 10 s).

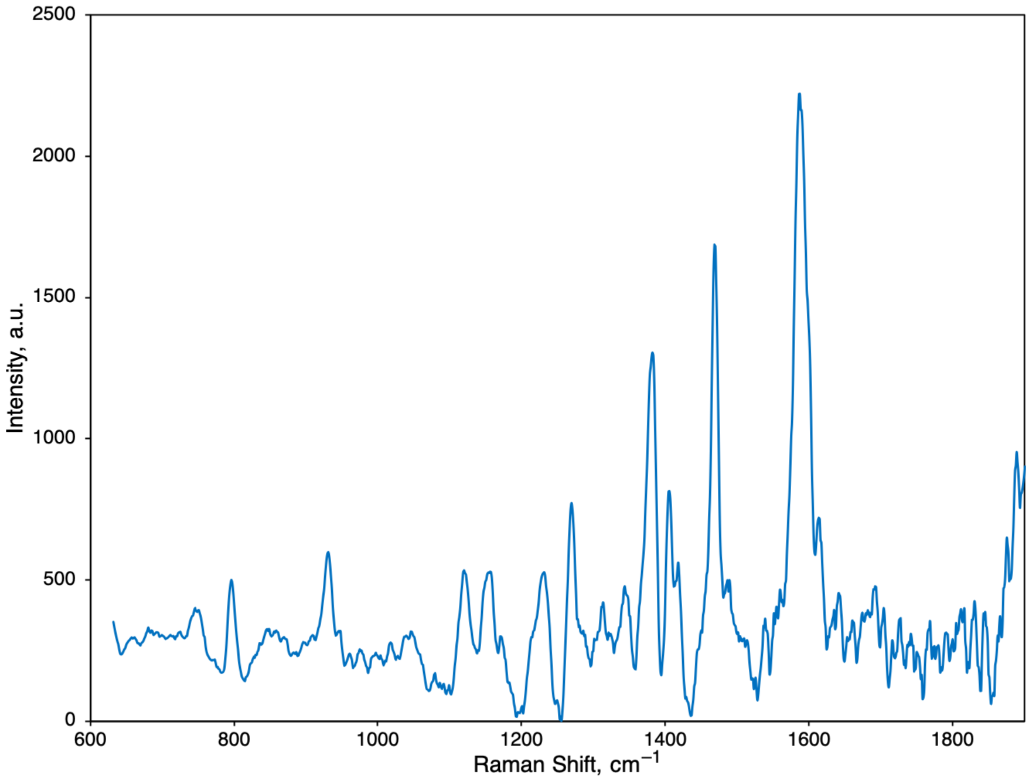

Figure S27 SERS spectrum on the USSR surface (4 × 4 mm^2) of Anchor 1–Promoter with 10 µl 10 µM C/Cy3 measured using a 17 mW 514 nm Ar^+ laser (10%, 10 s).

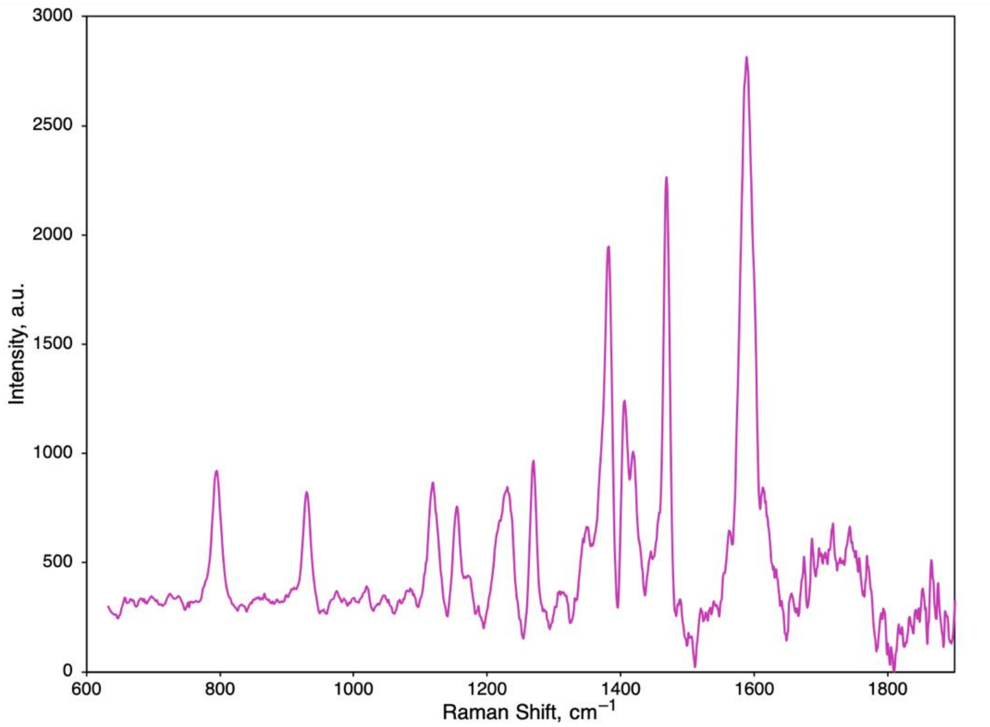

Figure S28 SERS spectrum on the USSR surface (4×4 mm^2) of Anchor 1–Promoter with 10 μl 10 μM D/Cy3 measured using a 17 mW 514 nm Ar^+ laser (10%, 10 s).

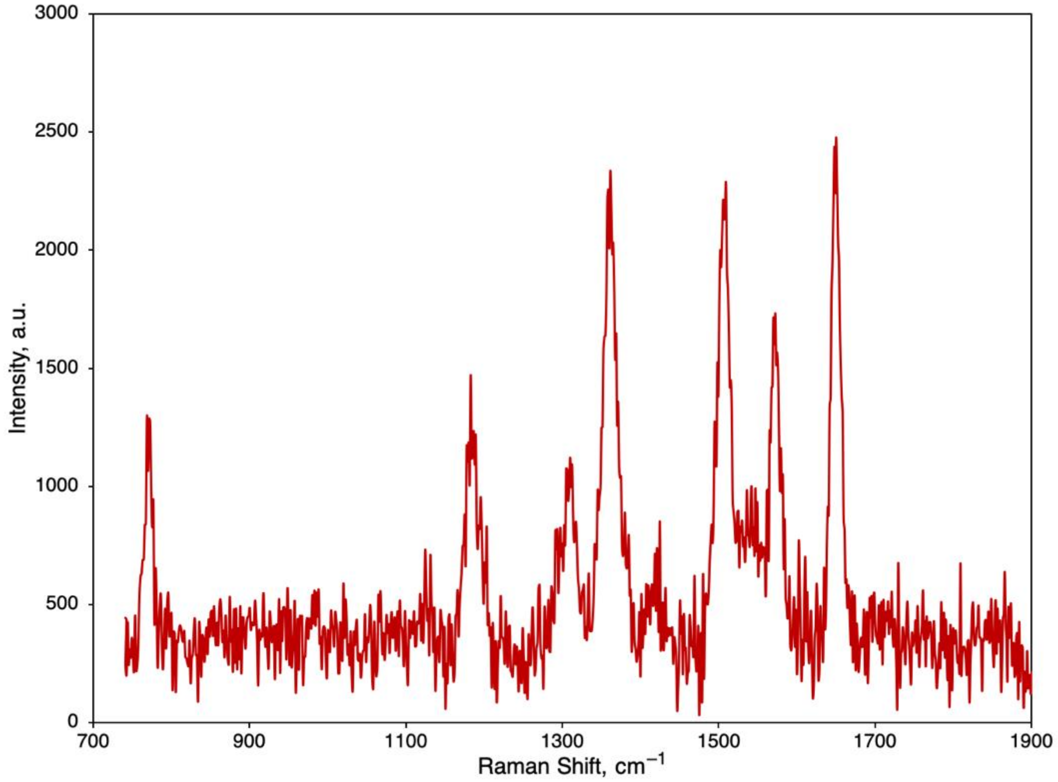

Figure S29 SERS spectrum on the USSR surface (4×4 mm^2) of Anchor 1–Promoter with 10 μl 10 μM D/Rh6G measured using a 17 mW 514 nm Ar^+ laser (10%, 10 s).

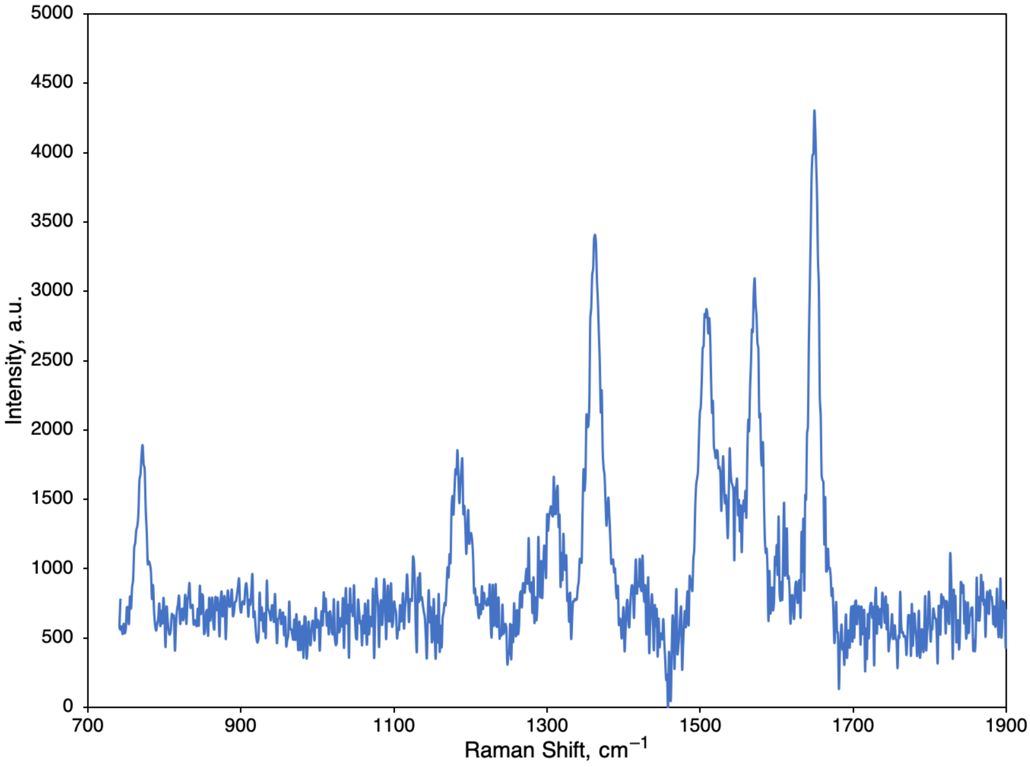

Figure S30 SERS spectrum on the USSR surface (4×4 mm^2) of Anchor 1–Promoter with 10 μl 10 μM C/Rh6G measured using a 17 mW 514 nm Ar^+ laser (10%, 10 s).

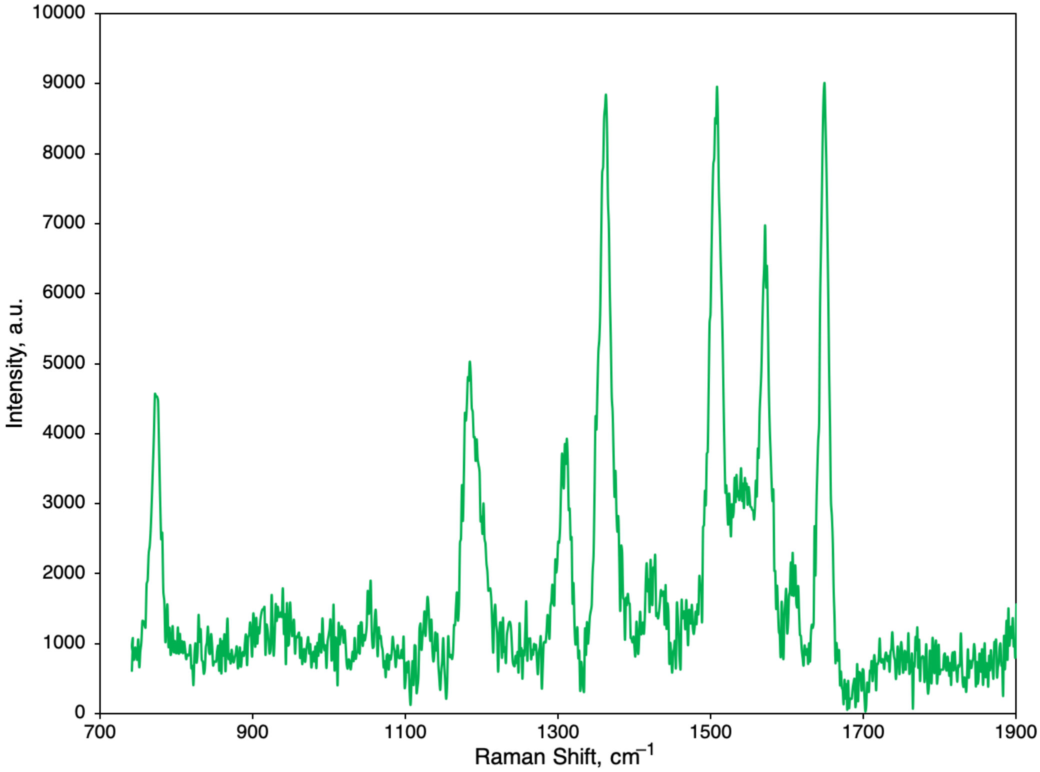

Figure S31 SERS spectrum on the USSR surface (4×4 mm^2) of Anchor 1–Promoter with 10 μl 10 μM B/Rh6G measured using a 17 mW 514 nm Ar^+ laser (10%, 10 s).

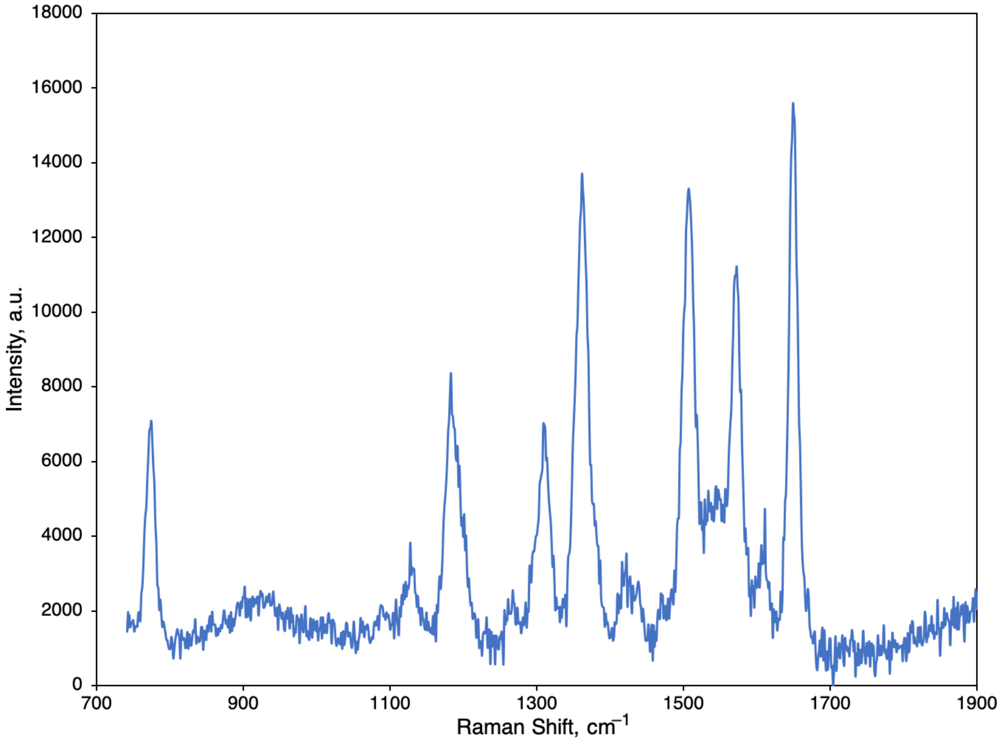

Figure S32 SERS spectrum on the USSR surface (4 × 4 mm^2) of Anchor 1–Promoter with 10 µl 10 µM A/Rh6G measured using a 17 mW 514 nm Ar^+ laser (10%, 10 s).

Before data processing using methods of mathematical statistics, errors were detected using the *Q*-criterion. Numerous studies have shown that the data of most analytical determinations in the presence of a general set of results of chemical analysis obey the law of normal distribution (Gaussian distribution). The probability density of the normal distribution law has the form:

$$\varphi(x) = \frac{1}{\sigma\sqrt{2\pi}} e^{\frac{-(x-\mu)^2}{2\sigma^2}}$$

where µ and σ^2 – mean and dispersion, respectively. Since the sample under consideration is small, we used the Student distribution (*t*-distribution). Confidence intervals were calculated according to the following algorithm:

$\bar{x}$ – average for a series of *n* measurements ($n \geq 3$)	$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$
V – dispersion	$V = \frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}$

$s(x)$ – standard deviation $$s(x) = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}}$$

s_r – relative standard deviation $$s_r = \frac{s(x)}{\bar{x}}$$

Δx – confidence interval (P = 0.95) $$\Delta x = \pm\frac{s(x) \cdot t_{P,f}}{\sqrt{n}}$$

The lower limit of the determined contents was characterized by the minimum determined concentration at a given confidence probability $P = 0.95$, $n \geq 3$, $s_r \leq 0.33$. Limit of detection (LOD) was calculated as the triple ratio of the standard deviation of the noise (background) signal to the sensitivity coefficient of the calibration dependence for the analyte in the sample, the signal of which can be minimally reliably distinguished from the background. The signal related to the desired component if it was equal to or higher than the signal level set for the software with a confidence probability of more than 99%. To process the experimental data, the batch programs Excel 2011: for mac and OriginLab v9.1 were used.

References

1. Farzan, V. M.; Ulashchik, E. A.; Martynenko-Makaev, Y. V.; Kvach, M. V.; Aparin, I. O.; Brylev, V. A.; Prikazchikova, T. A.; Maklakova, S. Y.; Majouga, A. G.; Ustinov, A. V.; Shipulin, G. A.; Shmanai, V. V.; Korshun, V. A.; Zatsepin, T. S., Automated Solid-Phase Click Synthesis of Oligonucleotide Conjugates: From Small Molecules to Diverse N-Acetylgalactosamine Clusters. *Bioconjug. Chem.* **2017,** *28* (10), 2599-2607.