Electronic Supplementary Material (ESI) for Analytical Methods.

Molecular beacon decorated silver nanowires for the quantitative miRNA detection by a SERS approach

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S1. Name and sequence of the different oligonucleotides tested

Table S1

Oligonucleotide name	Sequence (5'-3')
miR-183	UAUGGCACUGGUAGAAUUCACU
MB1	CGACGAGTGAATTCTACCAGTGCCATACGTCG
MB2	CGACGGAATTCTACCAGTGCCATCGTCG
MB_f	(Cy5) – CGACGGAATTCTACCAGTGCCATCTCG - (BBQ)
MB	(ROX) – CGACGGAATTCTACCAGTGCCATCXCG
Target (T)	ATGGCACTGGTAGAATTC
Random	CAGCTGGAAATCTAAAACCTATCTTGTACAA
Single mismatch	ATGGCACCGGTAGAATTC

where X=C_6-dT Thio (Thio is a functional group used for the immobilization of the MB onto surfaces that can interact with -SH groups; the C_6 sequence has been inserted as a spacer-harm of the MB which will be immobilized onto a solid support)

S2. Design of the MB for miR-183

The design of a MB requires specific rules to be followed[1]:

1. the *loop* is a single chain sequence, composed of 18-30 bases, complementary to the target sequence (the selected miRNA for COPD in this case);
2. the *stem* is a double-chain sequence composed of 5-7 bases, which are complementary to each other, located at the end of the *loop*;
3. the fluorophore and the quencher are covalently bonded to their respective ends of the sequence;

4. the *stem* energy-formation must be strong enough (approximately -1.5 to -2 kcal/mol) allowing the MB to maintain the double helix in absence of the target and not to appear fluorescent (because the fluorophore and quencher remain close together);
5. the bases used for the *stem* are therefore mainly cytosine/guanine (CG) characterized by a higher energy bond than adenine/thymine (AT). In fact, a standard configuration is given by CCGCGC-loop-GCGCGG, but the exact melting temperature of the stem also depends on the length of the MB;
6. in the presence of a perfect complementary target, the MB should hybridize in a stable way with its target;
7. a guanine next to the fluorophore should be avoided since it tends to quench the fluorescence.

Keeping in mind the mentioned rules, the following MB sequence, specific for miR-183 (5'-UAUGGCACUGGUAGAAUUCACU-3'), have been first evaluated:

MB1: 5'-*CGACG*<u>AGTGAATTCTACCAGTGCCATA</u>*CGTCG*-3'

In italics and underlined are represented the *stem* and the *loop*, respectively. The *loop* is complementary to whole miR-183 sequence.
With the use of the on-line available software UNAFold[2] the possible conformations assumed by the given sequence and the correlated energies involved were studied. Considering a high hybridization energy involved (ΔG_0 -2.96 kcal/mol) and a length unbalance of the stem as compared to the loop of the first MB sequence (MB1), a second MB, MB2, was instead considered.
In Figure S1 the folding study of MB1 is reported. The software generated only one possible conformation.

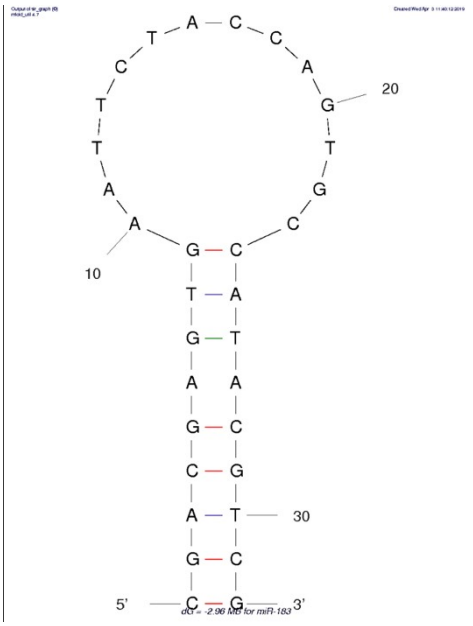

Figure S1. MB1 folding study.

S3. Fabrication of the SERS substrate and experiments of SERS reproducibility

A B

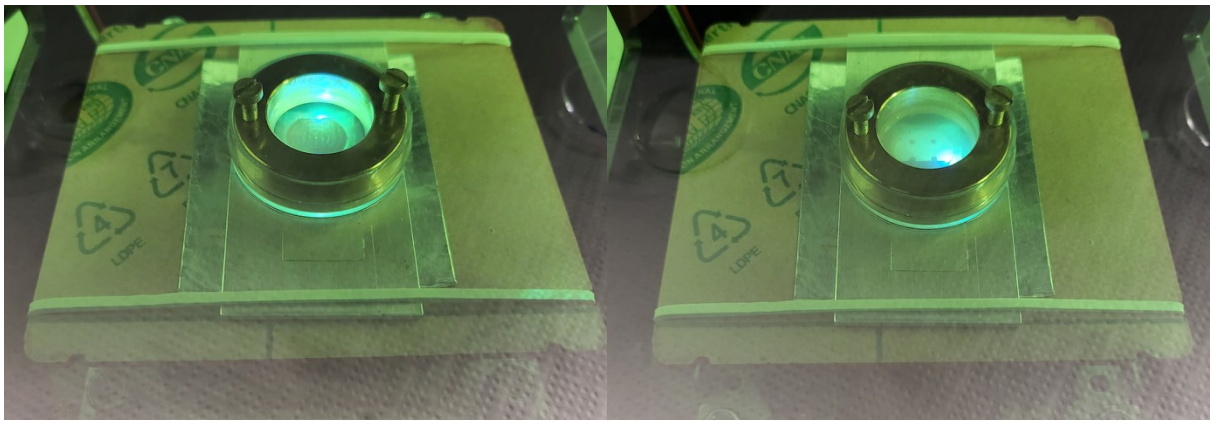

Figure S2. SERS substrate preparation. The AgNWs layer on the PTFE membrane is processed with the engraver machine in ethanol solution to remove selected areas of the silver surface and obtain 4 spots of 1 mm of diameter: (A) initial AgNWs layer engraving and (B) final AgNWs layer engraving process.

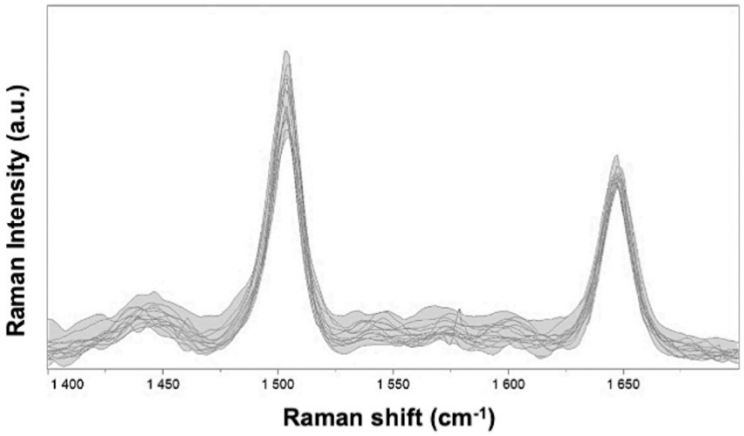

Figure S3. Raman mapping experiment of MB immobilized on the AgNWs SERS spot (25 spectra). The relative standard deviation (RSD) referred to the ROX peak intensity at 1646 cm^{-1} is < 8% showing that the SERS substrate is highly reproducible.

S4. SERS spectra of pure MB and mixed MB-MCH immobilized on the SERS substrate

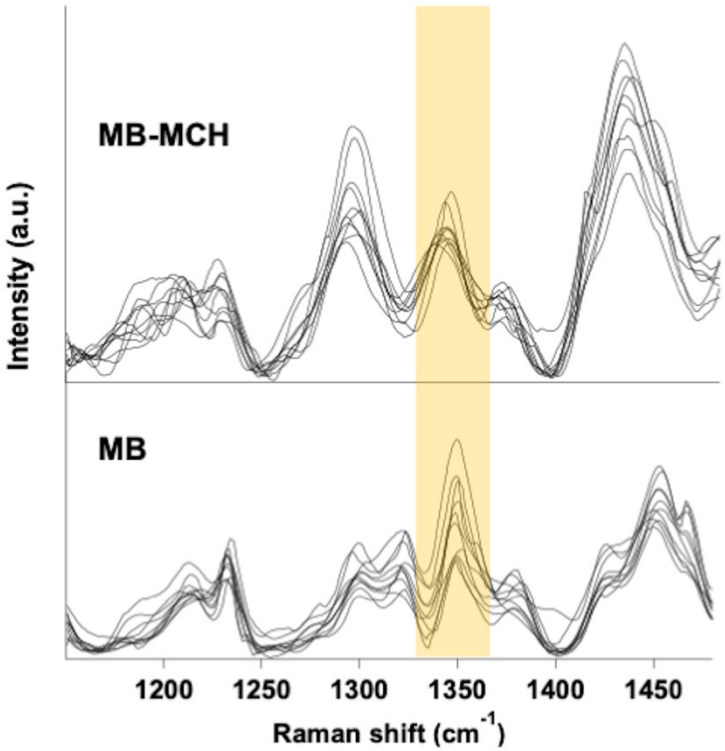

Figure S4. Raman mapping of MB and MB-MCH SERS nanoprobe before the addition of miRNA target. The adenine band region proper of the MB oligonucleotide is highlighted to show the difference in intensity variability between the pure MB and the mixed MB-MCH nanoprobe, revealing a higher homogeneity in the MB oligonucleotide conformation in the presence of MCH.

S5. SERS spectra of MB-target at different target concentration with pure MB and mixed MB-MCH nanoprobe

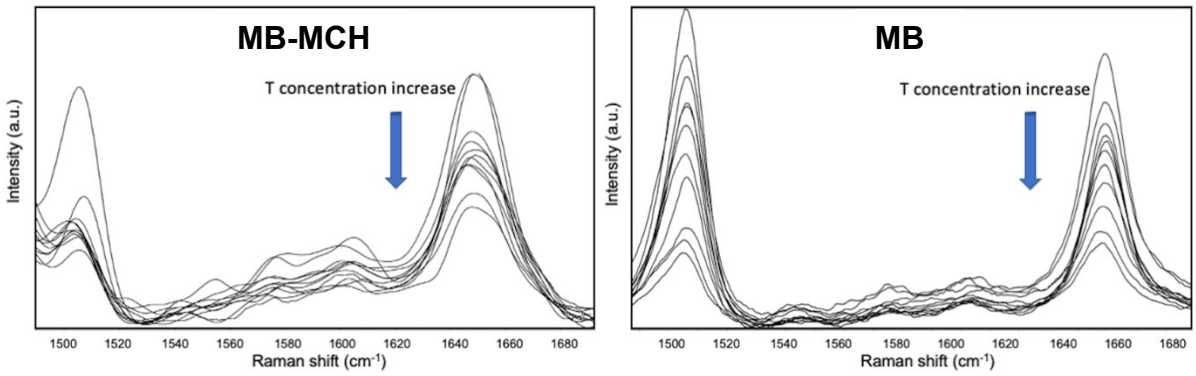

Figure S5. SERS spectra of the MB and MB-MCH biosensor as a function of target concentration, in the spectral range of prominent ROX bands.

References

1. Goel G, Kumar A, Puniya AK, Chen W, Singh K. Molecular beacon: a multitask probe. J Appl Microbiol. 2005;99(3):435-42. doi: 10.1111/j.1365-2672.2005.02663.x. PMID: 16108784.
2. http://www.unafold.org