Supplementary Information (SI) for Analytical Methods.

SUPPLEMENTARY INFORMATION

Lyophilizing SERS Biosensors to Enable Translation into Easy-to-Use Assay

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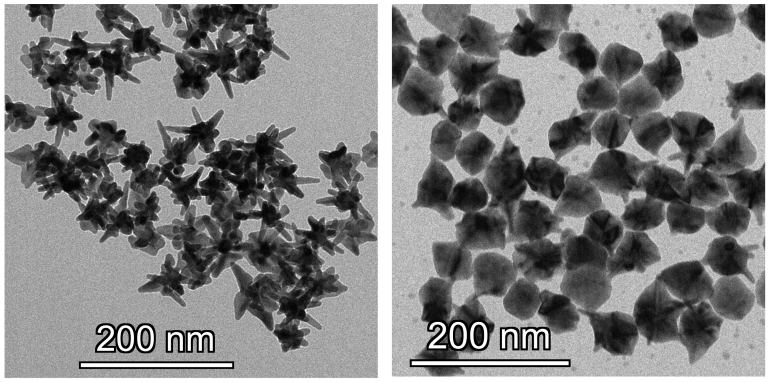

Figure S1. TEM images of gold nanostars (a), silver-coated gold nanostars.

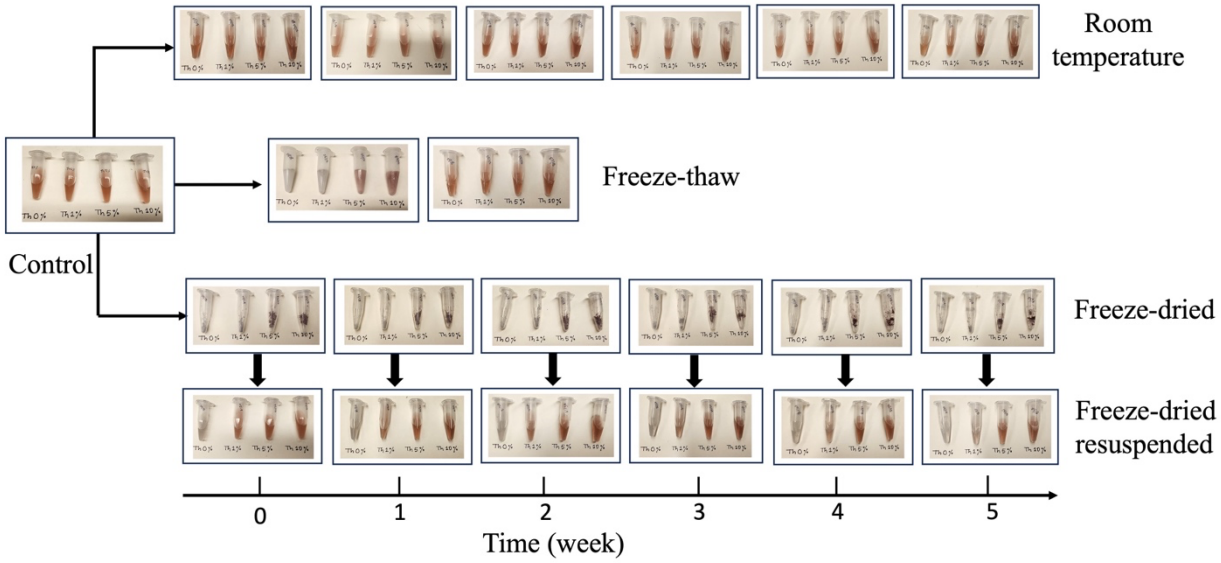

Figure S2. Physical appearance of sensors during storage over time in solution (C), freeze-thaw (FT) and freeze-dried (FD). The last one both in dry form and resuspended. All sensors are represented as a function of storage time in weeks. FT sensors were only pictured for one cycle on day 2.

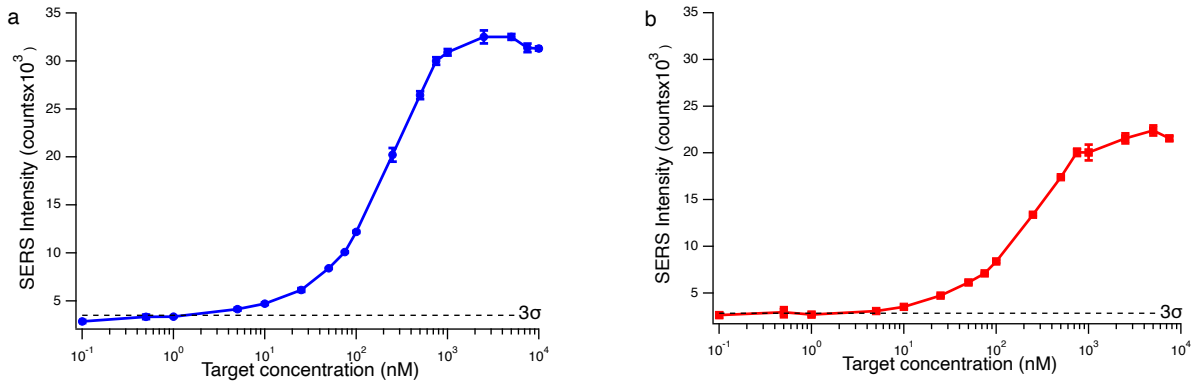

Figure S3. Calibration curves of sensors in solution (a) and after the freeze-drying process (b) as a function of increasing concentrations of synthetic target DNA. 3σ dashed line represent the LOD level (blank signal + 3×standard deviation of the blank). While the signal in the freeze-dried sensors is decreased, the LOD falls for both calibration between 1 and 5 nM.

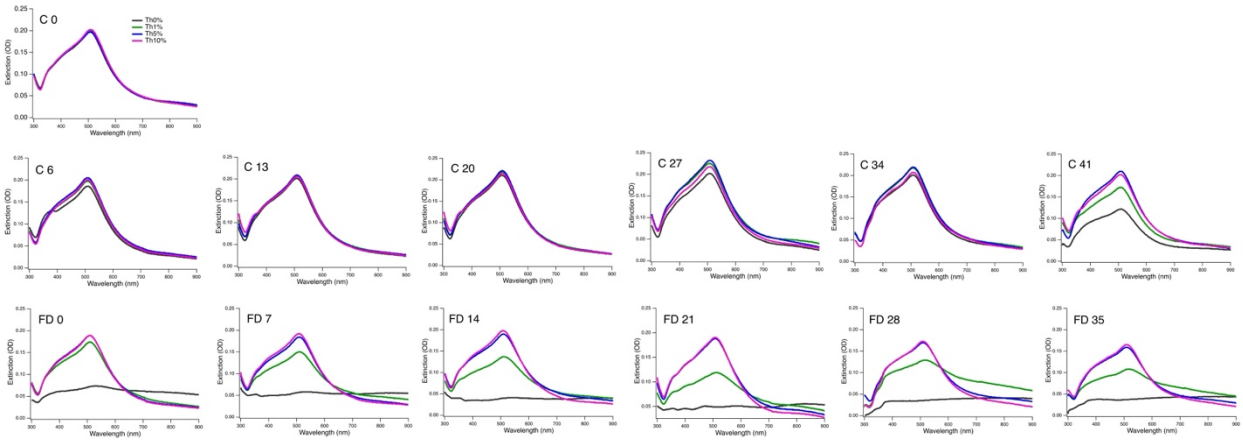

Figure S4. The extinction spectra of sensors at different time intervals of storage at room temperature in solution (C) and freeze-dried (FD) after resuspension.

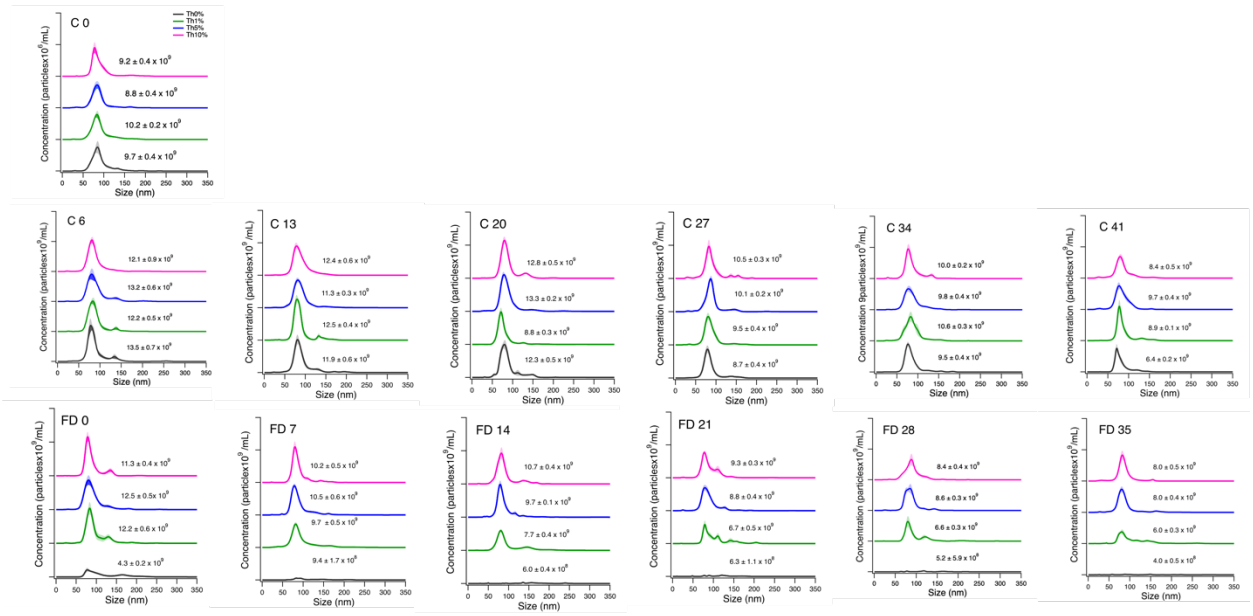

Figure S5. NTA shows size and concentration of functionalized sensors at different time intervals of storage at room temperature in solution (C) and freeze-dried (FD) after resuspension (y-offset for clarity). Each figure reports the calculated concentration. All graphs report different concentrations of trehalose as different colors.

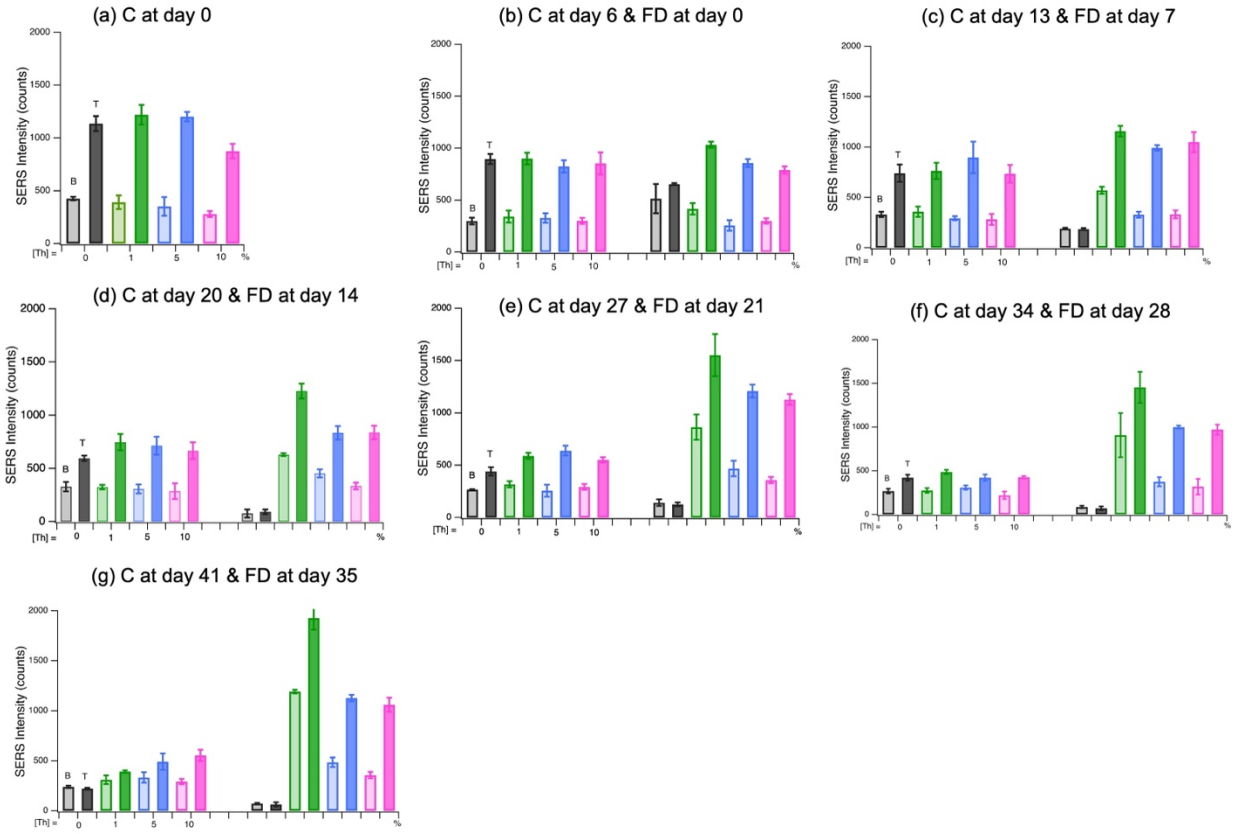

Figure S6. SERS sensor intensity for blank and target samples. All graphs show sensors stored in solution (C) and freeze-dried (FD), respectively left and right clusters in each graph. (a) Represent day 0 that is the initial state of the sensors as synthesized and has no freeze-dried correspondence. Different color lines represent concentrations of trehalose as reported in the x-axis ([Th] = % w/w of trehalose).

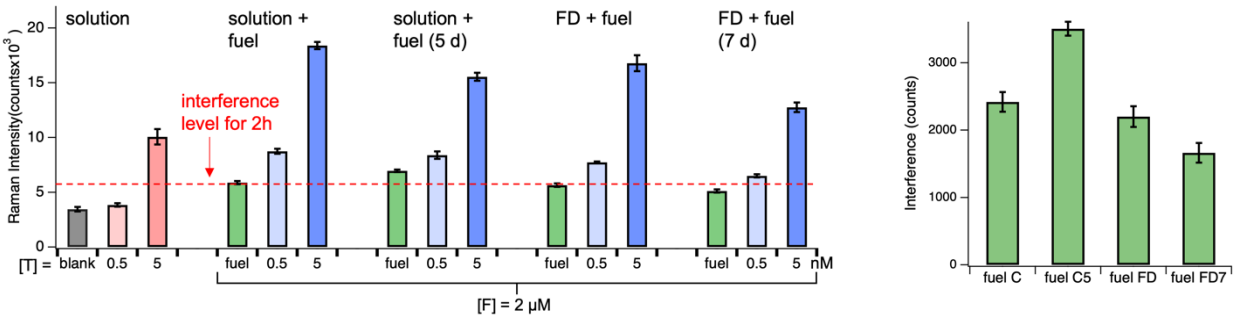

Figure S7. Left panel: Sensors response as a function of target concentration ([T]) with and without fuel (+ fuel) for sensors stored in different conditions (solution or lyophilized (FD)) and for different time (0 and 5 or 7 days for solution and FD, respectively). Right panel: Interference for the different storing condition and time calculated as fuel - blank. Fuel concentration ([F]) used is 2 µM. Red dashed line reports the benchmark interference for catalytic sensing.

Table S1. DNA sequences used in the experiments.

Probe	HS-TTTTTTATAGCATTTAGAAAATGTTACTTGGTTCCATGCTATA-Cy5
PH	GACATATATAGCATGGAACCAAGTAACATTTTCTAAA
Fuel	TTTAGAAAATGTTACTTGGTTCCATGCTATATTTTTTAT
COVID Target (Omicron):	AATGTTACTTGGTTCCATGCTATATATGTC
COVID Target (Delta):	AATGTTACTTGGTTCCATGCTATACATGTC
Probe	HS- AAAAACTCTATAACTTGATGGTGGTGTAGGGATTATAGAG-Cy5
PH	GAAAGCGACTCTATAATCCCTACACCACCATCAAGTTAT
Fuel	AAAGCTGAGGAGGTGGTGTAGGGATTATAGAGTCGCTTTCAAGATAAATT
RSAD2 Target	ATAACTTGATGGTGGTGTAGGGATTATAGAGTTTTTCTCTAT

Table S2. Nanoparticle average sizes during the lyophilization process.

	Particle size (nm)			
Sensors	Peak	C	FT	FD
Th0%	Major	85	81	78
	Minor	191	193	165
Th1%	Major	84	83	84
	Minor	222	133	189
Th5%	Major	79	82	81
	Minor	187	180	181
Th10%	Major	81	79	79
	Minor	161	199	135