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#### Protein corona-resistant SERS tags for live cell detection of integrin receptors

Sian Sloan-Dennison<sup>+</sup>, MaKenzie R. Bevins<sup>+</sup>, Brian T. Scarpitti, Victoria K. Sauvé, and Zachary D. Schultz<sup>\*</sup>

Department of Chemistry and Biochemistry, The Ohio State University, Columbus, OH, 43210

+ denotes equal author contributions.

\* corresponding author email: Schultz.133@osu.edu

## **Supplementary information**

#### **Table of Contents**

Supplementary Figures S1-S12 and Tables S1-S2	2
References	9

# **Supplementary Figures and Tables**



Figure S1. a) Extinction spectrum of Au NP b) SEM image of Au NP



Figure S2 a) Structure of cyclic RGDFC and b) extinction and c) SERS spectra of Au NP functionalized with different concentrations of cyclic RGDFC. Color scheme shows low concentrations in darker blue and higher concentrations in lighter blue (0, 43, 215, 430, 645, and 860 nM).

Cyclic RGDFC gives a distinct SERS signal with phenylalanine peaks at 1000 and 1205 cm<sup>-1</sup> and an arginine peak at 1030 cm<sup>-1</sup>.

Concentration of RGDFC (nM)	Size (nm)	Zeta potential (mV)
0	41.0±0.47	-41.0±3.7
43	45.0±0.9	-24±0.66
215	47±0.9	-21±3.7
430	48±4.5	-28±0.46
645	212±43	-27±1.41
860	801±37	-21±1.2

Table S1 Change in size and zeta potential with increasing concentration of RGDFC on Au NP



Figure S3 a) Structure of MBN b) extinction spectra and c) SERS spectra of Au-RGDFC NP functionalized with different concentrations of MBN. Color scheme shows low concentrations in darker blue and higher concentrations in lighter blue (0.1, 0.5, 1, 2, and 3  $\mu$ M).

Concentration of MBN ( $\mu$ M)	Size (nm)	Zeta potential (mV)
0.1	59.3±2.0	-27±0.2
0.5	49.0±2.16	-30.13±1.2
1	56±6.16	-29.6±0.7
2	67.3±2.05	-27.7±1.3
3	77.6±0.47	-20.4±0.77

Table S2 Change in size and zeta potential with increasing concentration of MBN on Au-RGDFC NP



Figure S4 a) SERS spectra of Au NP (black) and Au NP with 10% cell media (red) and b) SERS spectra of Au NP (black) and Au NP with 10% FBS supplemented cell media (red).



Figure S5 a, b, c, and d) Extinction spectra of Au, Au-MBN, Au-RGDFC, and Au-RGDFC-MBN NP, respectively, in water (blue) and in FBS supplemented media (red).



Figure S6 a) False colour images of non-MBN peaks, tryptophan (green), CH bending (blue) and amide (pink) and MBN peaks (red dashed boxes) obtained from fixed cells incubated with Au-RGDFC-MBN NP. <sup>2,3</sup> The white circles indicate an area where non-MBN peaks and MBN signal are present in both maps. The yellow circles indicate an area where non-MBN peaks are predominantly present and the red circles indicates an area where only MBN peaks are present. The spectra from the map are also shown in a) and the 3 non-MBN peaks highlighted. b) False colour image and associated spectra obtained from fixed cells incubated with Au-RGDFC NP and c) False colour image and associated spectra obtained from fixed cells incubated with Au-NP



Figure S7 a, b, c, and d) White light (WL) and false color (FC) images of Raman mapped fixed cells incubated with Au, Au-RGDFC, Au-MBN, and Au-RGDFC-MBN NP, respectively.



Figure S8 a) Schematic of FCS3 System expanded view [1]. 1 and 2 depict the FCS3 top and pressure plate, respectively. 3 is a 40 mm coverslip on which the cells are grown. 4 is the singular lower gasket, where media nourishes the cells. 5 is the microaqueduct slide, which directs the media into the gasket and provides heat to the cells. 6 is the upper gasket, which acts as a seal. 7 shows two perfusion tubes where media is flowed in and out of the chamber. Finally, 8 is the FCS3 base, cinching the system together and securing it onto the stage adapter. b) Picture of the FCS3 under confocal

microscope/Raman laser. The media is heated, which minimizes bubble formation inside the gasket. The peristaltic pump flows media overtop the cells.



Figure S9) White light image of the SW620 cells in the FCS3 Bioptechs gasket after 4.5 hours in a solution of acidic media, trypan blue, and Au-RGD-MBN NP.



Figure S10) White light image of cells as a reference for the effect of trypan blue; during this experiment, some cells died as indicated by their blue color. Flow speed and media temperature were adjusted for future experiments to ensure cell viability. The cells were observed to detach from the substrate as they died.



Figure S11 a) White light and zoomed in false color image of a living cell. The media conditions were 37 degrees Celsius and pH 7.4. Representative SERS spectrum from the cluster of red pixels in the false color image. The 1074, 1180, and 1584 cm<sup>-1</sup> peaks of MBN are boxed in red. The false color image was processed on WiRe by<sup>00</sup> analyzing the intensity of the 1584 cm<sup>-1</sup> peak of MBN for each spectrum on the map. The red pixels depict where the 1584 cm<sup>-1</sup> peak of MBN was present.



Figure S12 a) White light and zoometrin false  $c_{250}^{500}$  image of a living cell. The media conditions were 37 degrees Celsius and pH 6.5. h) Bepresentative SERS the formulation the cluster of red pixels in the false color image. The 1000 cm<sup>-1</sup> peak of RGDFC and the 1074<sup>2</sup> cm<sup>-1</sup> and 1180 cm<sup>-1</sup> peaks of MBN are boxed in red. The false color image was processed on WiRe by analyzing the intensity of the 1074 cm<sup>-1</sup> peak of MBN for each spectrum on the map. The red pixels depict where the 1074 cm<sup>-1</sup> peak of MBN was present.

### References

1. Bioptechs FCS3 System. Retrieved from http://www.bioptechs.com/product/fcs3-system/

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