Development of 3D nanoprisms for specific detection of bacteria in complex environments

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INTRODUCTION

Unequivocal identification, localization and quantification of nanoscale probes is dependent on a combination of target specificity and affinity, signal generation, detection penetration depth and scattering events within each environment. Two-dimensional [2D] systems, e.g., single cells in flow cytometry, microarrays and affinity assays pose few obstacles to performance of nanoscale probes. The structural intricacy and physiochemical activity of in vivo systems is exponentially complex in comparison to 2-D ex vivo analytical systems, and often the functional "stumbling block" of targeted nanoparticle probes.

Precision control; directed assembly of single and mixed-metal clusters into well-defined, pre-specified nanoscale structures is therefore a primary goal of chemistry, biology and material sciences. The magnetic, optical, and electronic properties of large metal clusters can be tailored to specific applications if composition and structure could be monitored and controlled in concert during synthesis.

Here we used a hybrid low-pressure solution-phase ([LPSP)] system and protocol that produce near absolute yields of single and mixed-metal platonic solids, exhibiting synthetically-tuned quantum physical and chemical behaviour (Yaffee et al., 2010). We report the use of crystal-in-crystal nanoparticles (NPs) produced thereof and functionalised with oligonucleotides targeting specific bacterial groups. Target bacteria were localized in complex biological matrices from food (cheese) and intestinal environments (cell culture and intestinal tissue) using confocal laser scanning microscopy (CLSM) and 3D volume reconstruction.

MATERIALS AND METHODS

Biological samples

Model cheeses were manufactured from ewe's raw milk, as described by Pereira et al. (2009). Cheese milk was spiked with 1% (v/v) *Lactococcus* and *Lactobacillus* strains, and with a pathogenic consortium of *Listeria innocua, E. coli, Pseudomonas aeruginosa* and *S. aureus*.

Microbe-cell interactions were tested with human mucus-secreting intestinal colon cancer cell line HT29-MTX, as presented by Zihler et al. (2011) Interactions with *Salmonella enterica* spp. *enterica* serovar Typhimurium N-15 and bacteriocinogenic *Bifidobacterium thermophilum* RBL67 were studied with functionalized NPs and CLSM.

Oligonucleotide probes and particule conjugation

All oligonucleotides for specific bacteria FISH detection were modified at the 5' with a biotin tail and were synthesized by Microsynth (Balgach, CH).

Sample preparation for CLSM

Samples blocks were fixed with 4% (w/v) formaldehyde. Sections were then washed twice in 1x PBS and stored at 4 °C. Sections were pre-treated 3 times with a freshly prepared 0.1 %(w/v) sodium borohydride solution (15 min), washed with 1xPBS in between, 200 µg/mL Proteinase K (10 min at 37°C), lysozyme (1 or 10 mg/mL) for cell permeabilization, followed by washing with bidistilled water. Hybridization buffer containing 1/5 of DNA of interest conjugated with the respective fluorescent particle was added. When three probes were used, two hybridization steps were performed: first an overnight FISH with specific probes for lactic acid bacteria (LAB) followed by 5 h with the general EUB338 probe used to detect pathogens. The samples were incubated in a humid chamber for hybridization (42°C), washed (42°C/30 min) and added with Citifluor AF1 to prevent fading.

Confocal Laser Scanning Microscopy

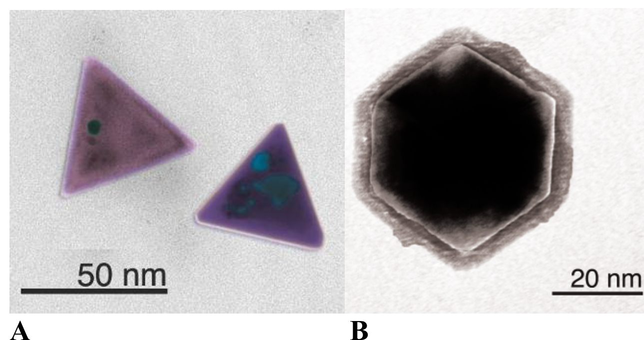
CSLM was performed using a Leica TCS SP confocal scanning laser microscope equipped with an inverted microscope (Leica DM IRBE) with an Ar /Kr laser. QDs were excited at 488 nm. Images of model cheeses and cell cultures were taken using an x 63 magnification objective and a 2.0 electronic zoom. Emission windows were set according to the maximum emission values of the fluorescent particles.

Image processing and 3D-reconstruction

CSLM equipped with a goniometric [xyz] controlled sample stage permits optical "sectioning" of samples at pre-defined nanoscale depth intervals. Acquisition of xy scanned signals is placed in individual image "slices" making up a "z-stack". Volume is reconstructed through virtualization with artificial perspectives placed at the observers' putative "point of view". Post-acquisition processing of raw data permits coloration adjustment or addition of different signals, e.g. visible interference, EM, etc.

RESULTS AND DISCUSSION

A novel class of supraatomic crystalline metal clusters has been synthesized containing one or more metals; having a uniform size of 2 to 200 nm; having a defined, three-dimensional (3D) polyhedral structure, optionally functionalized by ligands and/or embedded crystals (Fig. 1A). These tetragonal pyramidal metal particles exhibit elevated magnetic, fluorescent and visible signals in concert with a well-defined robust crystal-in-crystal geometry (Yaffee et al., 2010). Our synthetic protocol permits strict control of shape and sizing in concert producing a homogenous sol of monodisperse three-dimensional nanocrystals. We are able to stochiometrically dose interior metals, effectively doping at the atomic level concurrently capping surfaces of exterior metals with physiological antioxidants (Fig. 1B). We have ligated these derivatized nanoprisms to oligonucleotides targeting specific microbes within a consortium of pathogens and LAB growing within a model cheese system to test their efficacy and function in a complex 3D environment (Fig. 2A). Volumetric spectral scanning in multiple detection modes provided unequivocal identification, localization and quantification of individual microbes *in situ*, in complex matrices such as cheese.



A B

Figure 1 : Mixed nanocrystals: (A) shows hexagonal pyramidal FeCo inside trigonal pyramidal Au nanoparticles; (B) shows hexagonal pyramidal Fe nanoparticles coated with GSH.

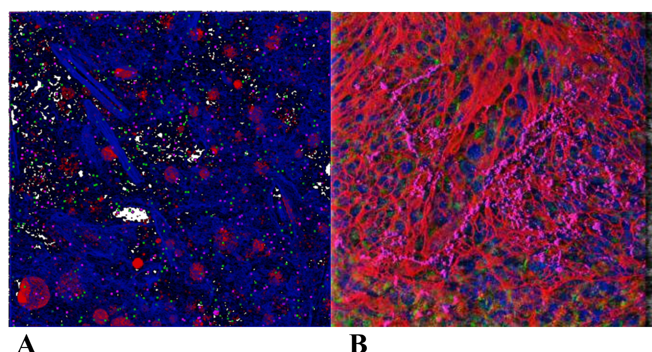


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Figure 2 : Volume reconstructions of hybridized biological samples : (a) Model cheese samples : *Lc. lactis* (green), *Lb. brevis*/fat globules (red), pathogen consortium (violet) ; (b) HT29-MTX cell monolayer after interaction with *B. thermophilum* RBL67 (pink) and *S.* Typhimurium N-15 (green); blue: cell nuclei; red: actin filaments.

A similar method allowed characterizing interactions between of a probiotic and a pathogen in mixture with an intestinal cell layer obtained by *in vitro* culture. The high and specific signals of the two NPS functionalized with a specific oligonucleotide probe lead to specific and accurate localization of probiotic bacteria at the apex of intestinal cells whereas *S.* Typhimurium N-15 was able to translocate the cell layer via two different mechanisms, internalization and extracellular transport through tight junctions. This new approach was further developed for specific detection of microbes on rat intestinal tissues. Fig. 3 illustrates the detection of *Bacteroides* interacting with intestinal wall.

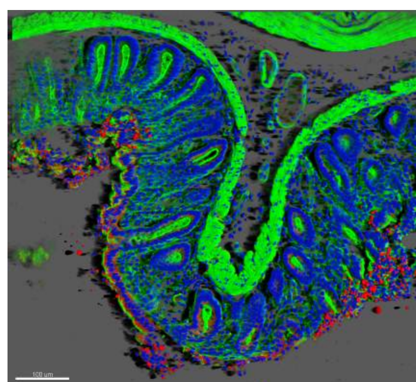

Figure 3 : Volume reconstructions of hybridized rat intestinal tissue for *Bacteroides* detection (red).

CONCLUSIONS

Our study revealed unique properties of functionalized NPs produced with LPSP system and protocol for localization and quantification of specific bacteria within complex tissue samples. Signal detection in cell and tissue cultures moves toward a near-native state in living systems and yields dynamic physiological data *in situ*.

REFERENCES

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