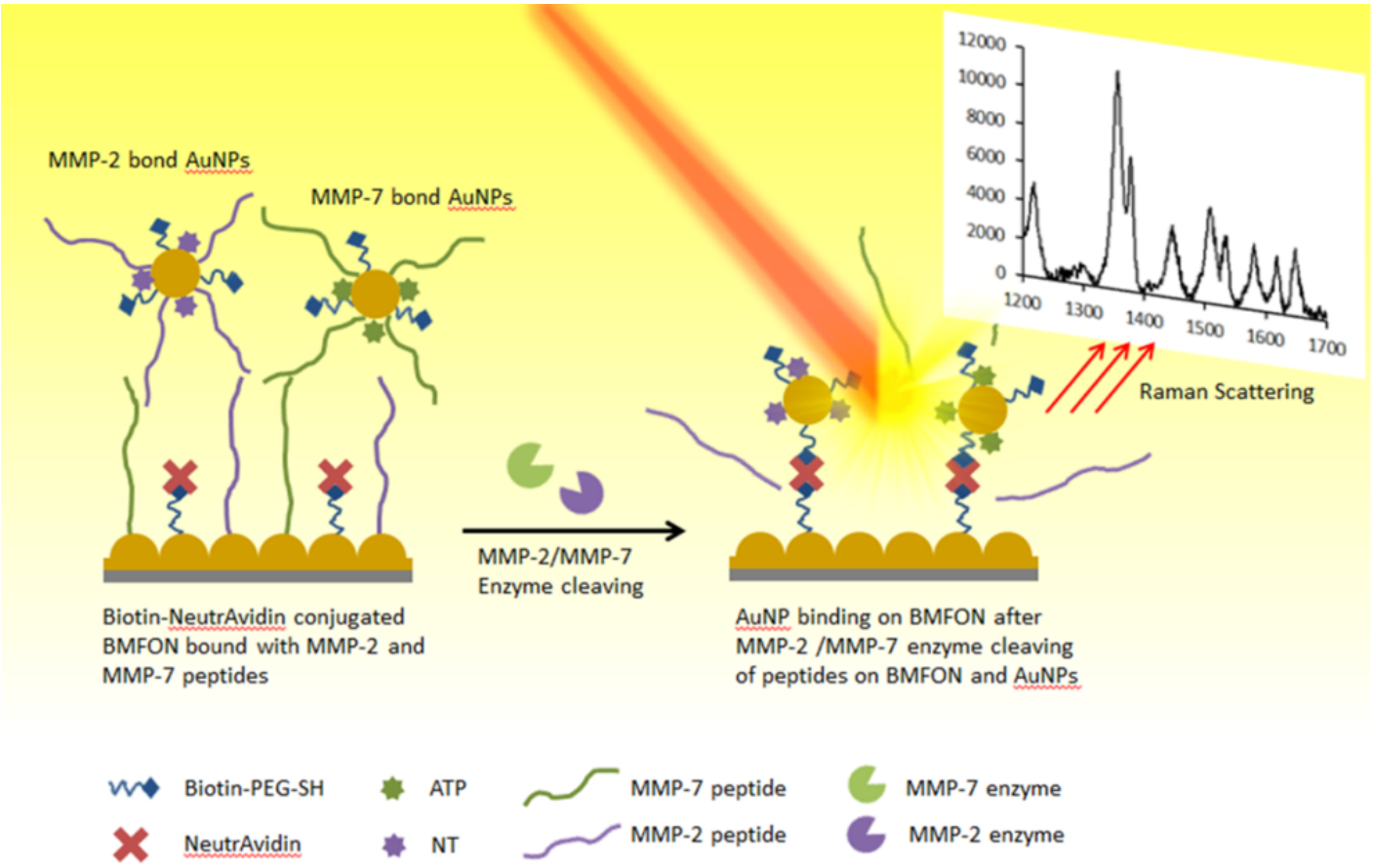
Spectroscopic platform to cut time and resources needed to quantify cancer cell biomarker concentrations

Survivability of a living cell, tissue and ultimately a being is influenced by the state of proteolytic degradation of extracellular matrix (ECM) components; if the dynamics of the degradation is perturbed, regulation of many biological processes such as cell differentiation, angiogenesis and wound repair are impaired. This perturbation cascades into formation of cancerous cells, growth of tumors and metastasis (spread of cancerous cells leading to more tumor growths). Presence of matrix metalloprotenases (MMPs), which are specialized endopeptidases (enzymes that break down proteins), is found to play a part in proteolytic degradation. Research has found more than 26 species of MMPs and established their link to the existence of cancers and tumors. For example, levels of MMP2 and MMP7 are markedly higher in cancer of the pancreas as opposed to the healthy one. Current techniques in measuring MMP levels such as ELISA (use of enzymes to capture MMP molecules), gel zymography (an electrophoretic technique to detect hydrolytic enzymes) and fluorescent dye tagging of target enzymes require hours of preparation and specialized chemicals, and lack in wide measurement range. Furthermore, current diagnostic kits can test for only one type of MMP at a single time, impeding throughput. All these problems translate to costly, arduous tests that involve large invasive biopsies and often unreliable results.



In light of the limitations faced by today's technology, we had developed a robust diagnostic platform that utilizes extremely little biological sample amount of only 0.5 µL, reduces preparation and testing time dramatically, and allows for ultra-sensitive detection of our biomarkers or targets, MMP2 and MMP7, simultaneously (from 1 ng/mL to 40,000 ng/mL) which is otherwise unachievable by other established methods. The platform comprises i) the target molecules bound to gold nanoparticles (AuNPs) that are functionalized with thiolated PEG and labelled with either 4-aminothiophenol (ATP) or 2-naphthalenethiol (NT), ii) a glass chip known as Bimetallic-Film-Over-Nanosphere (BMFON) substrate that is specially treated to have NeutrAvidin external coating, iii) enzymes of the targets and iv) an optical technique known as surface-enhanced Raman spectroscopy (SERS). The external surfaces of AuNPs and the substrate were covered with the obstructive target molecules. SERS involves the excitation of a region of interest on the substrate with low power laser and receiving signals containing characteristic vibrational information of chemical bonds of the labels (1,584 cm^{-1} for ATP and 1,377 cm^{-1} for NT).

The concept of the platform is that when the thiolated PEG of the AuNPs with the targets attached has unobstructed binding access to the NeutrAvidin on the substrate, the characteristic signature of the labels becomes present and can be detected by SERS. However, this detection is possible only if the obstructive target molecules are cleaved by their corresponding enzymes, a mechanism we

termed as “cleave-and-bind”. In other words, if signatures of the labels are not detected, then this implies that the sample contains neither of the MMPs. Furthermore, the substrate can be designed to allow testing for multiple targets or biomarkers at once, a system known as multiplexing.

In conclusion, our proof-of-concept diagnostic platform demonstrates the potential to help doctors reduce biopsy footprints required of patients, provide accurate diagnosis and reduce cost and time of the test.

Publication

Sensitive surface enhanced Raman scattering multiplexed detection of matrix metalloproteinase 2 and 7 cancer markers.
Gong T, Kong KV, Goh D, Olivo M, Yong KT
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