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# Analysis of salivary biomarkers for point-of-care sensors detecting head and neck cancer and infections by SERS/MD approach

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**Abstract:** We propose a proof-of-concept study combining SERS and MD simulations, which give leave to explore the biomolecular absorption mechanism on nanoparticle surfaces, to detect salivary Interleukin-8 and Lysozyme, implicated in oropharyngeal carcinoma and oral infections. © 2024 The Author(s)

Early detection and precision medicine are fundamental paradigms that improve patient prognosis and enhance the success of rapid therapy. These aspects are crucial in the successful treatment of important and widespread diseases such as cancer, bacterial infections, cardiovascular diseases, and diabetes mellitus, thus preventing complications and boosting the probability of survival and the life quality of the patient. In this scenario, point-of-care diagnostics represents the research branch where cutting-edge technologies are tested with the aim of providing rapid, sensitive, and suitable medical information, avoiding the time delays associated with traditional protocols for collecting and transporting patient samples into centralized labs performing gold-standard methods such as high-performance liquid chromatography (HPLC)-based approaches combined with mass spectrometry. The ultimate goal is the development of portable and easy-to-use biosensors for health monitoring, early detection, and specific treatments based on the patient's characteristics. However, the challenge in point-of-care diagnostics for personalized medicine is determining which biomarkers to test and estimating their concentration using a suitable technique. Consequently, access to high-quality measurable nano-indicators of a healthy or irregular physiological status, which allow for estimating any alteration in human health conditions, is required. An important goal to pave the way for simple access to point-of-care diagnostics is related to the development of liquid biopsy diagnostic devices with the attempt to provide valid, non-invasive, and reliable assays identifying biomarkers from body fluids. Among the possible non-invasively collectible biofluids, saliva is one of the best candidates, as it is easily accessible and easy to transport and store. Further, saliva is considered the "mirror of the body" due to its continuous renovation and its proven richness in biomarkers that are dysregulated in several diseases. Recent studies investigating the use of surface-enhanced Raman scattering (SERS) using plasmonic metal nanoparticles interacting with biomarkers for biofluid characterization to enhance the molecular specific Raman fingerprint information by 4 to 8 orders of magnitude have shown promising results. The enhancement in the Raman signal overpasses the sensitivity issues that have limited the application of Raman spectroscopy in disease diagnosis so far with the attempt to identify cancer-related biomarker concentration changes in urine, serum, plasma, and saliva. Differences in the occurrence of Raman peaks and peak intensity ratios have been found between patients and healthy people, allowing for the non-invasive detection of lung cancer, cervical cancer, and other types of diseases. Moreover, by applying a portable Raman spectrometer for read-out, the SERS technique is available at the point-of-care. However, attempts to characterize and discriminate proteins in biological fluids using the SERS approach is still a challenging task. Indeed, difficulties in spectral interpretation caused by an unpredictable enhancement in components and bands overlapping are still hampering its wide application. Combining SERS biosensing techniques with molecular dynamics (MD) can be an effective strategy to deal with the complexity of the SERS dataset.

Notably, MD has proven to be successful in correlating SERS spectra with protein structural signatures and detecting the conformational modulation of protein interfaces. All-atom MD computations are in fact powerful tools for the atomistic characterization of proteins and offer a thorough understanding of the molecular drivers behind protein adsorption on the metal surfaces' highlighting structural hotspot that accounts for SERS signal differences. In this scenario, the combined SERS-MD approach will be used to disentangle specific disease-related biomarkers present in a biological fluid [1]. Within this paper, we aim to report on salivary biomarkers analyzed with such a combined SERS-MD approach to characterize two important proteins found in saliva, which were not, to the best of our knowledge, described before. Currently detected predominantly using an expensive enzymatic immunoassay (i.e., ELISA test), lysozyme (LYZ) and interleukin-8 (IL-8) have already been shown to be differentially expressed in several disease states of head and neck cancer or infections. IL-8 (1–3 ng/mL) is a non-structural protein secreted by neutrophils and macrophages, which plays an important role in the proliferation of angiogenesis and, in particular, its concentration dramatically increases in the presence of oropharyngeal squamous cell carcinoma (OSCC), which is one type of head and neck cancer. On the other hand, LYZ (0.5–4 ng/mL) is a muramidase protein that is part of the innate salivary defense mechanisms, resulting in high concentration levels in cases of low immunity conditions. Overall, this pilot study, by combining SERS and MD techniques, showcases the needed tool to address the challenging recognition and interpretation of disease-specific biomarkers in body fluids, paving the way to novel, fast, and accurate point-of-care SERS-based devices for healthcare monitoring.

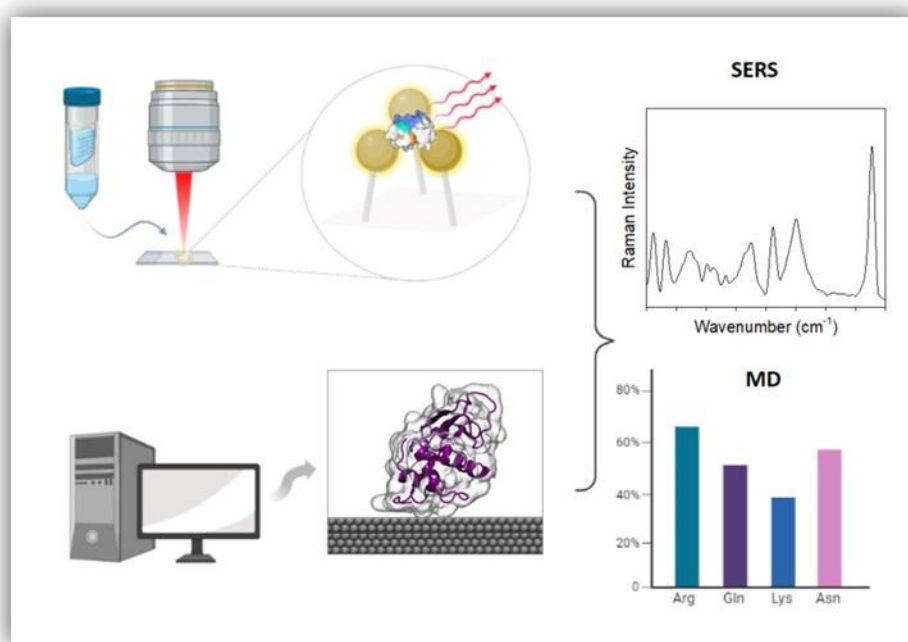


Fig. 1. SERS/Molecular dynamic (MD) simulations combined workflow for identification of salivary tumor markers.

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