Bioengineered yeast nanofragments integrated into SERS-microfluidics for ultrasensitive dengue detection in mosquitoes

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Screening of geographical areas infested by dengue virus (DENV) infected mosquitoes facilitates vector mosquito mapping and accurate surveillance before human involvement in a dengue transmission cycle. However, this requires highly sensitive technologies to accurately detect the presence of the dengue biomarker non-structural protein 1 (NS1) in mosquitoes. Furthermore, identification of the dengue serotype, particularly DENV2, is important to inform about potential risks of severe disease when transmitted to humans. Here, we develop a surface-enhanced Raman scattering (SERS) microfluidic immunoassay for ultra-sensitive detection of DENV2 in a single infected mosquito. Our microfluidic immunoassay combined yeast affinity bio-nanofragments displaying single chain variable fragment (nanoyeast scFv) as an efficient nanocapture partner with plasmonic nanotags as the detector partner for SERS read-out. Critical to the ultra-sensitivity was the use of anisotropic Au/Ag nanoboxes to enhance the Raman signal and nanoscopic fluid mixing by alternating current electrohydrodynamics to improve NS1 capture and labelling with nanoboxes. Our platform differentiated DENV2 infected from Zika infected and uninfected single mosquito with an assay detection limit of 500 fg DENV2 NS1. Our innovative nanotechnology-based platform provides a promising strategy for early DENV2 detection in individual infected mosquito, mapping mosquito habitats and will enable early intervention about potential severe dengue disease resulting from DENV2 transmission.

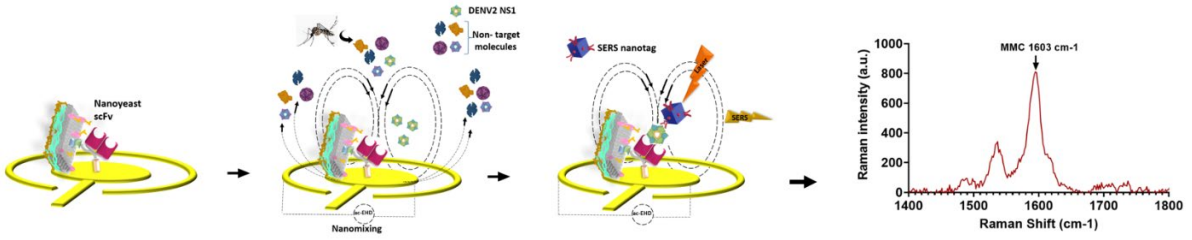

Figure 1. SERS microfluidic immunoassay using yeast affinity bionanofragments for DENV2 detection in single infected mosquito.

[1] *Farokhinejad, F, et.al. Analytical Chemistry. 2022;94(41):14177-84.*