Development of a new method for gene mutation identification using surface-enhanced Raman spectroscopy

A sensitive, accurate identification of specific DNA fragments (usually containing a mutation) can influence clinical decisions. The standard methods routinely used for detecting specific DNA are quite complicated and time-consuming, and so many groups are trying to develop new types of DNA sensors to replace those currently in use. One technique which is considered very promising for detecting specific DNA fragments is surface–enhanced Raman scattering (SERS) spectroscopy. SERS spectroscopy takes advantage of the strong increase in the efficiency of the Raman signal generation caused by a local electric field enhancement near electromagnetic nanoresonators. The choice of SERS as a new technique for detecting DNA is mainly due to the fact that SERS is an extremely sensitive analytical tool; in some cases, it is possible to obtain a good quality SERS signal from even a single molecule. In 2019 we proposed a new strategy for gene mutation identification using SERS spectroscopy. Our preliminary *in situ* analysis of the structure of a layer formed on a gold film from capture single-stranded DNA (ssDNA) with an attached alkanethiol moiety showed that, when such structures are incubated with a sample containing analysed DNA complimentary to the immobilised capture ssDNA, the presence of the target ssDNA induces hybridization, which causes a change in the conformation of the alkanethiolate linking moiety via which the captured ssDNA is attached to the gold surface (see Figure 1). That change is indicated by a characteristic change in the measured SERS spectrum (due to the very surface-sensitive mechanism of the SERS enhancement, the SERS spectra measured for such samples are dominated by the vibrations localised in the linking layer). The analytical signal obtained is, unfortunately, quite weak, and to obtain reliable results many repetitions and the averaging of a significant amount of data are required. This means that, before the hybridization-induced structural changes in the linking monolayer we observed can be used for the construction of actual SERS DNA sensors, the mechanism of this process has to be better understood, and a method must be found for making observations of this rearrangement with SERS spectroscopy easier. The main two aims of the proposed research are to find the answers to the above two questions. In the second step, using our findings, we will construct an improved SERS DNA sensor utilising the rearrangement of the linking monolayer, and will test this sensor in an analysis of actual clinical samples. We will estimate the degree of change in the selectivity and the sensitivity of the sensor.

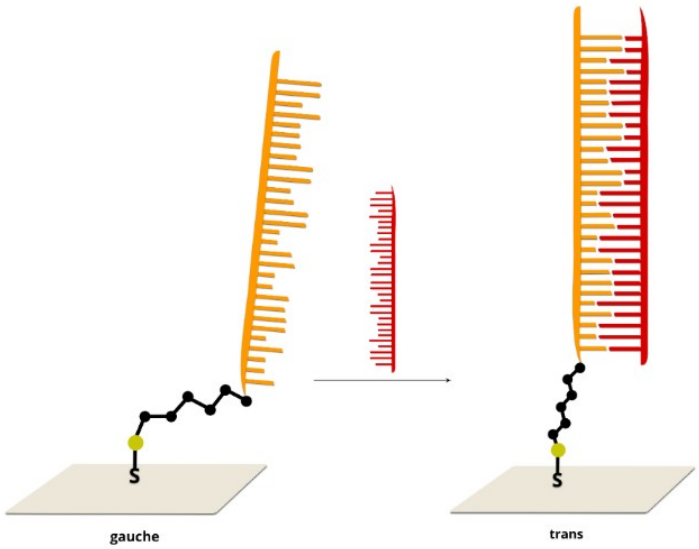

Figure 1. *Scheme of the rearrangement of the alkanethiolate linking layer caused by DNA hybridisation. Reproduced with permission from: E. Pyrak, J. Krajczewski, A. Kowalik, A Kudelski, A. Jaworska, Molecules* ***24*** *(2019) 4423.*