

## ABSTRACT

Dimers and small aggregates as well as compact aggregates of Ag nanoparticles (NPs) were assembled and chemically anchored to supporting surfaces. The supporting surfaces were either glass slides or SiO<sub>2</sub> – coated Cu or Au grids for TEM, both chemically functionalized by 3-aminopropyltrimethoxysilane (APTMS). Compact aggregates of Ag NPs incorporating protoporphyrin IX (PPIX) molecules were prepared by adsorption of chlorides in the presence of PPIX. Dimers and small aggregates of Ag NPs were assembled by selected molecular linkers: 4,4'-diaminoazobenzene (DAAB), 4,4'-diaminoterphenyl (DATP) and 5,10,15,20-tetrakis(4-aminophenyl)porphine (TAPP). The most efficient strategy of dimers and small aggregates preparation has been their assembling by a three – step procedure involving (i) attachment of isolated Ag NPs to the NH<sub>2</sub> groups of APTMS functionalized TEM grid, (ii) attachment of molecular linker (with two functional NH<sub>2</sub> groups in para position) to Ag NPs by a one terminal NH<sub>2</sub> group, and (iii) attachment of Ag NPs to the second, free terminal NH<sub>2</sub> group of the linker. In this procedure, the control over the perpendicular orientation of the bifunctional linker and its attachment by one terminal group to Ag NP surface has been accomplished by functionalization of Ag NPs by adsorbed citrate which acted as the adsorbate pre – orienting matrix. The control over the resulting dimer (small aggregate) separation from other dimers and small aggregates has been achieved by a sufficiently wide spacing of Ag NPs in the first step (i) of the three – step procedure. Furthermore, the strategy for obtaining SERS signal of molecular linker from a particular, selected single dimer and/or small aggregate of Ag NPs visualized by TEM has been developed. An unequivocal correspondence between the TEM – imaged dimer (and/or small aggregate) and the SERS signal obtained from it has been accomplished by employment of SiO<sub>2</sub> coated Au finder grids for TEM. An accurate establishment of the positional coordinates of the nanoobject (dimer or aggregate) with respect to the marks (letter or number) on the finder grid enabled to find the same nanoobject both in TEM (for its visualisation) and in the optical microscope which focused exciting laser beam onto it and enabled to obtain SERS signal of the molecular linker from it. The SERS signal of molecular linkers exhibits temporal fluctuations associated with achievement of a single molecule level of detection. Stability of the molecular linkers in strong optical fields appears to be conditioned by the perpendicular orientation of the linker with respect to Ag NP surface, and is further affected by the actual structure of the linker as well as by the nanoobject morphology. Finally, the single molecularly – bridged Ag NP dimer was found to represent an optimal system in which the single molecule dynamics can be followed via time – evolution of the SERS signal of the molecular linker obtained from it. The advantage of a dimer over a small aggregate is, that the SERS signal of the dimer originates from a single hot spot, while, in case of a small aggregate, the SERS signal is a superposition of molecular dynamic events occurring in several hot spots. Furthermore, the molecularly – bridged Ag NP dimer is an exact experimental realization of a model system of Ag NP dimer with a single molecule located in a single hot spot at the interconnect between the two NPs. According to the theoretical model calculations, a dimer of Ag NPs with the molecule located between them is the most efficient light amplification system providing largest enhancement of SERS by the EM mechanism of SERS.