Polysaccharide based organic frameworks with embedded nanoparticles: advanced SPR study on the antiviral activity of gold composites derived from glucuronoxylomannan

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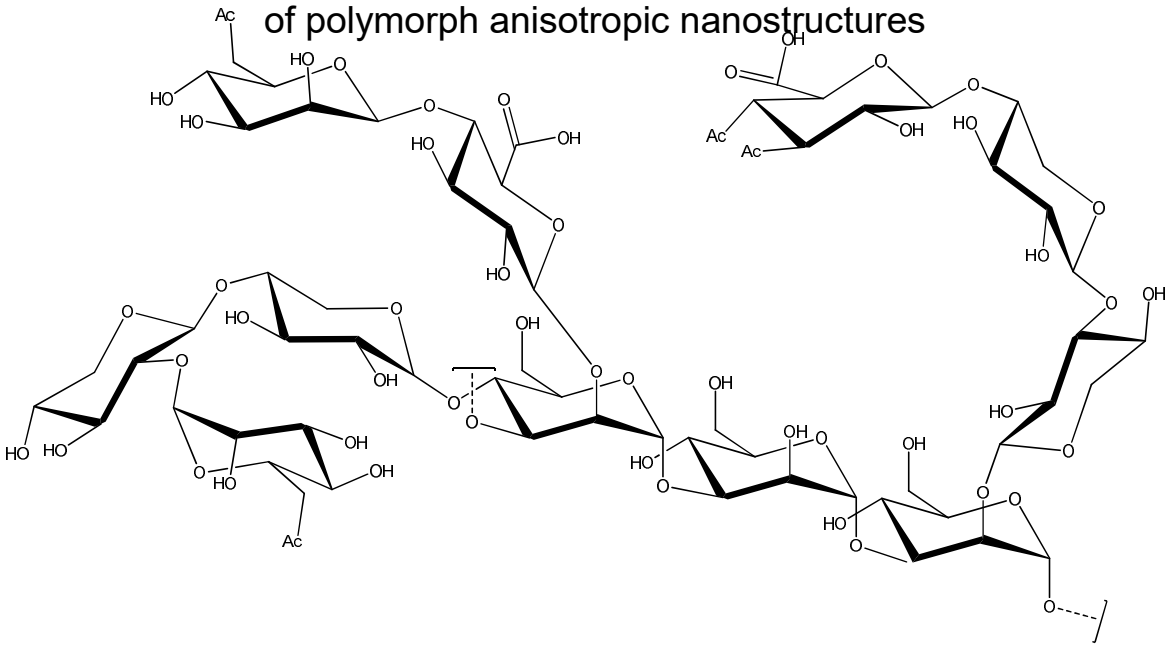
Ganoderma adspersum and structure of GXM

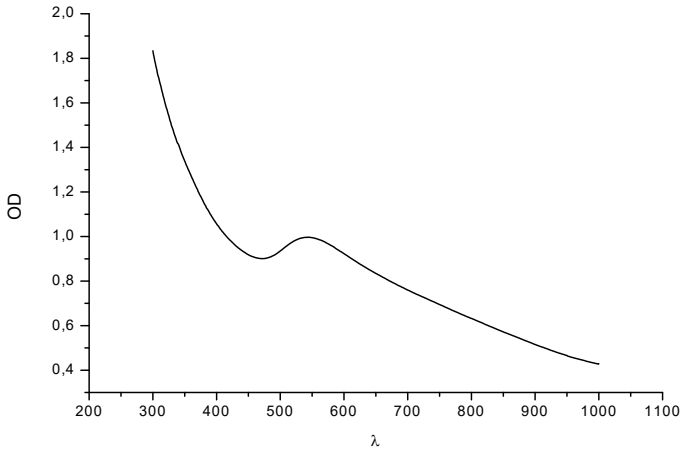
Ganoderma adspersum

Molecule of glucuronic acid contains carboxylic acid group which gives acidic properties to GXM. The same acid moiety of citric acid is the main functional group taking part in classical Turcevich's reaction for GNPs synthesis. On the other hand, carboxylic acid reveals the agglomeration property in neutral and acid solutions through hydrogen bonds formation. Therefore, carboxylic group in sugar backbone presents additional possibility for forming three-dimentional arrays determined by polysaccharide structure. For that reason, GXM may be used as biogenic precursors for synthesis of polymorph anisotropic nanostructures

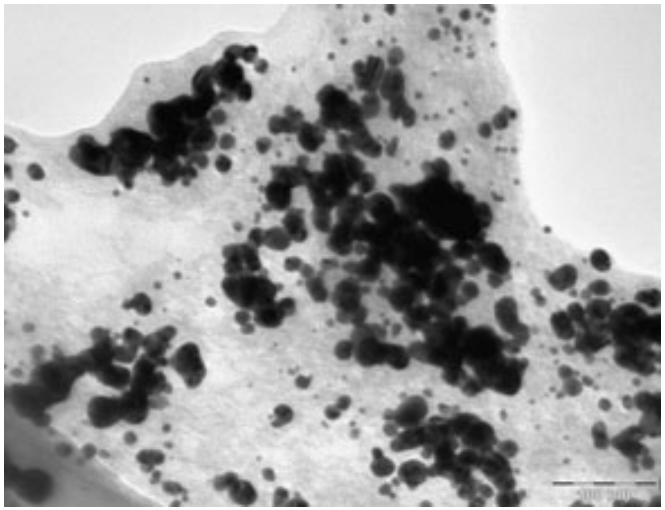


Au-GXM nanocomposite

Au-GXM nanocomposite was obtained by reduction of metal from HAuCl4 saltunder the base condition where glycane play a role of macromolecular reducer and stabilizer. Shortly, NaOH was added to glycane solution with following addition of Au salt. Initially solution was getting purple with further change of color to violet. Absorption spectra of product (Fig.2) demonstrated the presence of the wide band with the maximum near 560nm specific for local surface plasmon excitation in gold nanostructures.

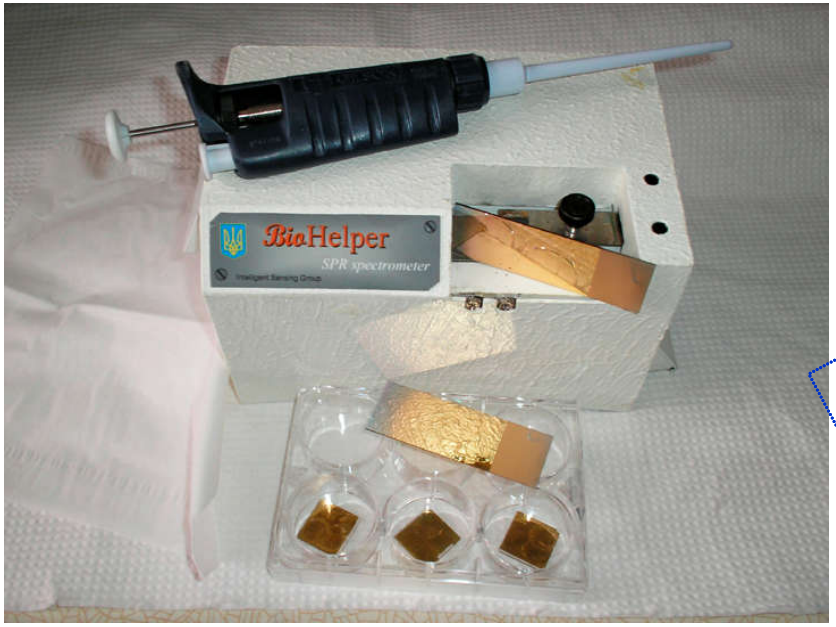


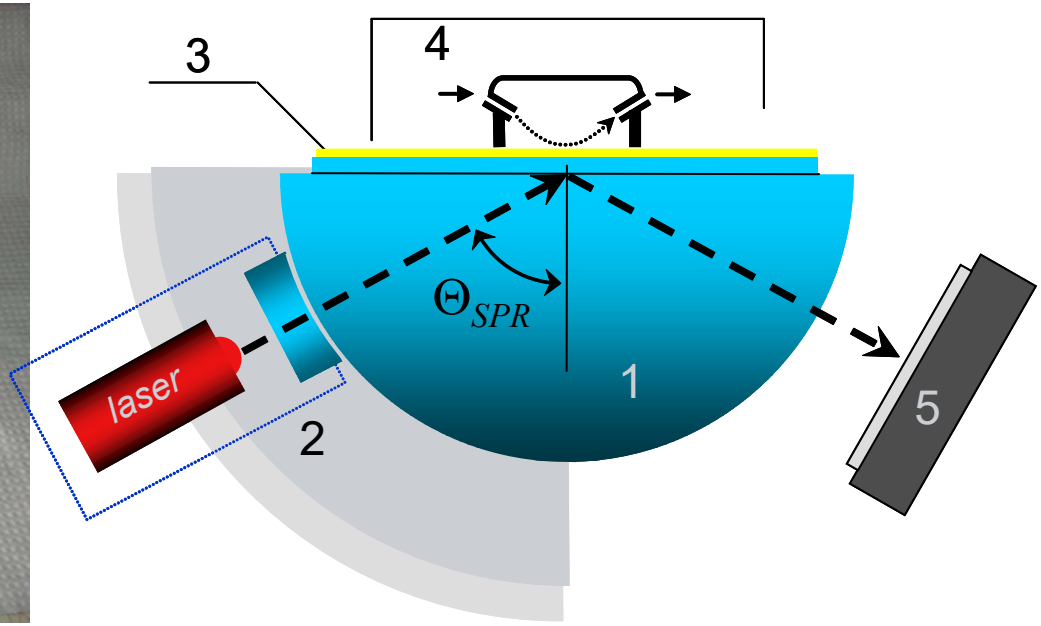
Absorption spectra of Au nanoparticles embedded in glycan matrix



TEM image of Au nanoparticles embedded in glycan matrix

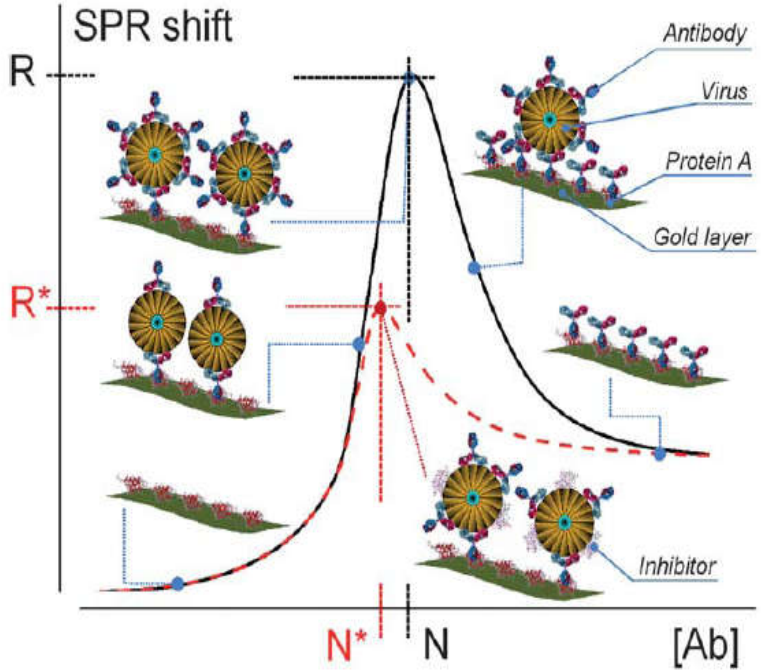
Scanning SPR spectrometer "BioHelper - 01"





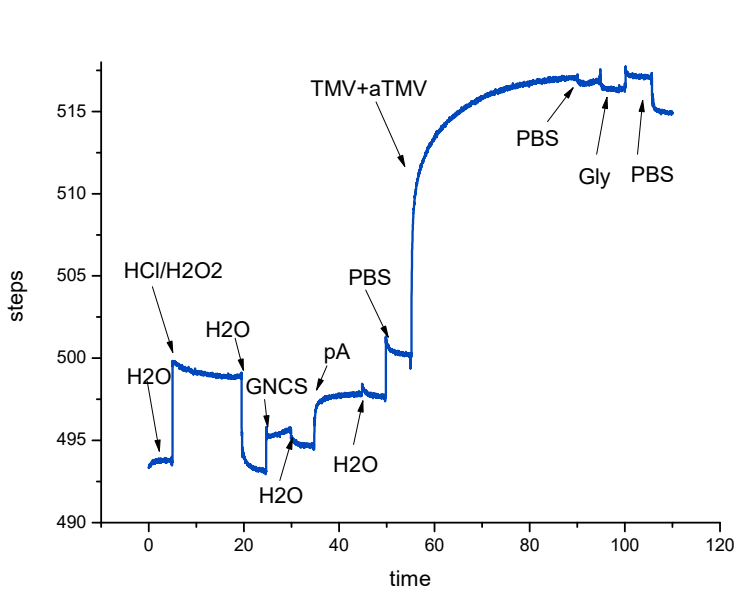
1 - glass prism
2 - mechanically scanning semiconductor laser λ=650 nm
3 - chip
4 - flow cell
5 - strip-line photodiode

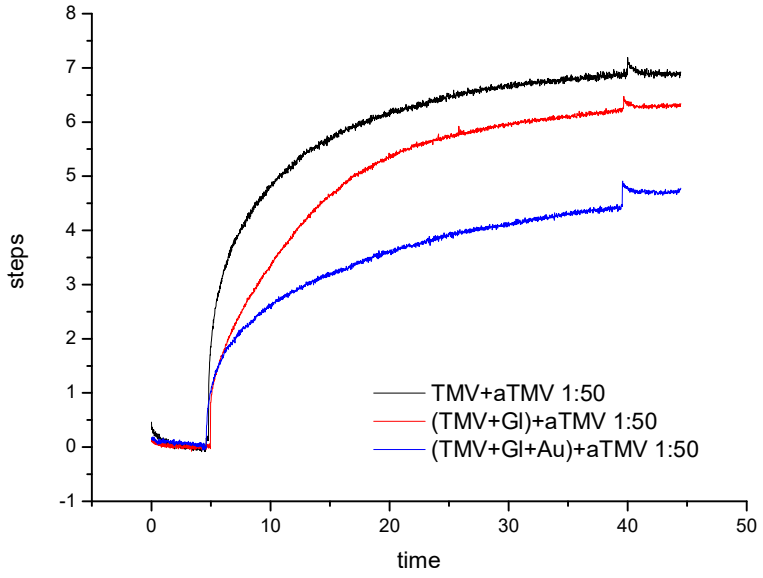
DViFA (density variations in fixed architectures) approach



In the conventional approach, the SPR response depends on the effective thickness of the analyte layer that is bound to the receptors on the surface, - the density of both layers (receptor and analyte) is uniform, - i.e. the variation of the SPR signal is due to the changes of the thickness. However, an SPR shift depends also on the change of the refractive index within the layer. Therefore, variations of the layer density can also affect the response value due to the variations of the refractive index inside the layer ("variative" refraction). One of the possible mechanisms is changing the packing of the objects of different size and shape within interfacial architectures on the surface (e.g. a virus, an antibody, a small molecule etc.). If the thickness of the surface layer is fixed due to the constant form of the biggest interacting components (e.g. virion), the SPR shift is a single-valued function of the molecular assembly compactness.

General course of the experiment

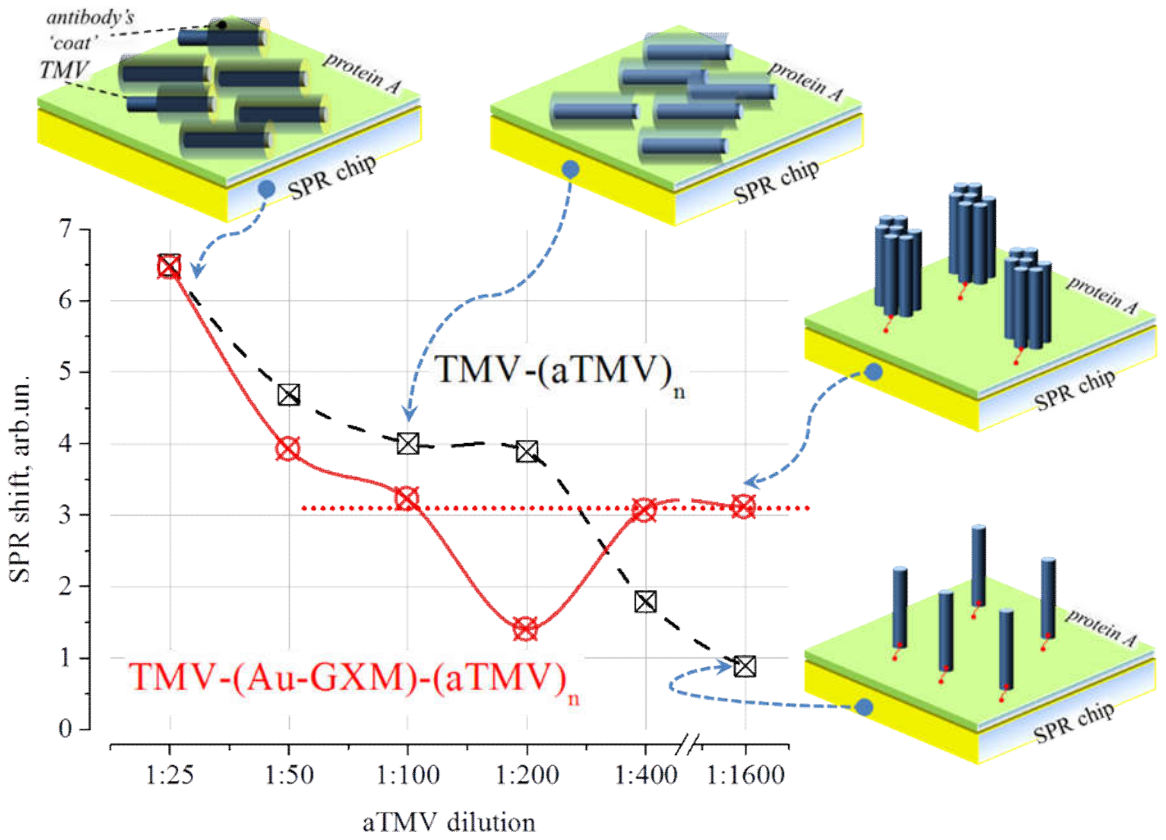




general course of the experiment

Influence of Au/Gly nanocomposite on the Ab/Ag complex adsorbtion

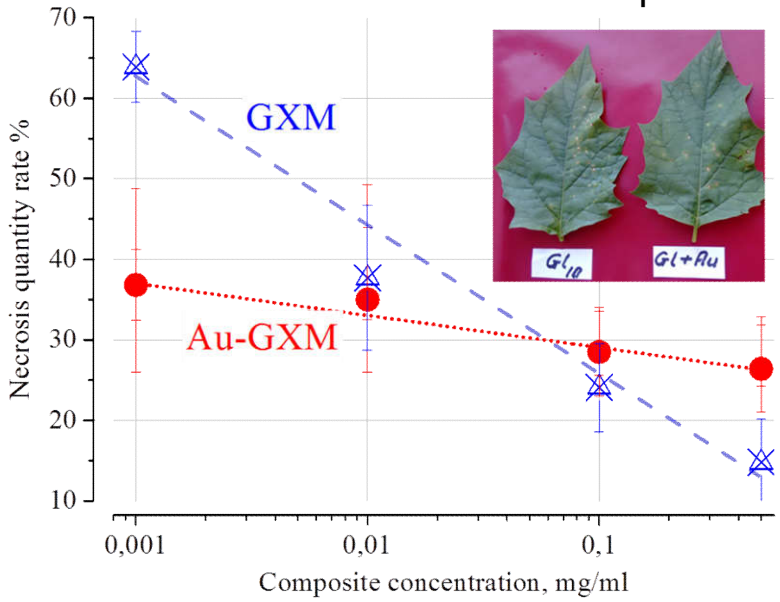
Comparasion of the immobilization of TMV-aTMV complexes in the presence and in the absence of Au-GXM nanocomposite



The figures around the graph show schematic representations of the surface orientation of viral particles "on side" (at low dilutions) and "at end" (at large dilutions of antibodies). Individual virus particles or aggregates are attached to the surface by "bridging" antibodies, the Fc fragment of which is bound by protein A immobilized on the surface.

Biological experiments in vivo

In order to compare the effectiveness of potent antiviral drugs, a concentration dependence of the percentage of necrosis on the experimental leaf halves to the number of necrosis on the control leaf halves on the drug concentration was constructed (i.e., the lower the value, the more effectively the virus is suppressed). For the preparation of native GXM, a clear linear (exponential in linear coordinates) dependence of the degree of viral infection suppression on the concentration was demonstrated. The difference in the concentration range of 0.5 mg/ml - 0.001 mg/ml is c.a. 50%. It was shown that at high concentrations (0.5 mg/ml) the activity of the GXM is higher than the activity of composite. However, the effectiveness of the GXM-Au composite is significantly less dependent on the concentration of the drug. This leads to the fact that the antiviral activity of the composite is much higher at low concentrations. In particular, for a concentration of 0.001 mg / ml, the degree of viral infection suppression more than twice better for Au composite in respect to native glucan.



Percentage of necrosis per leaf with native glucan (GXM) and Au-glucan composite (Au-GXM). Insert: *Datura stramoniumL* leaves infected with TMV in a *mixture* with native glucan GXM (labeled “Gl10”) andGXM-Au nanocomposite(labeled “Gl+Au”)

Conclusions

The results of molecular analysis and *in vivo* studies suggest that polysaccharide matrices with embedded gold nanoparticles have a stronger antiviral effectat low concentrations in comparison with natural polysaccharides. The mechanism of this action is due to the fact that the metal composite induces the aggregation of viral particles into clusters incapable of subsequent infection of plant cells.