Supplementary Materials

Specific and sensitive determination of folic acid by label-free chemosensors with microscope glass slips as single-use consumables

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S1. SEM-based morphological characterization of the sensor chip surface at different functionalization stages

Figure S1 shows the results of the SEM-based morphological characterization of the sensor chip surface at different functionalization stages: aminated surface, carboxilated surface, and surface after FA-gelatin conjugate immobilization.

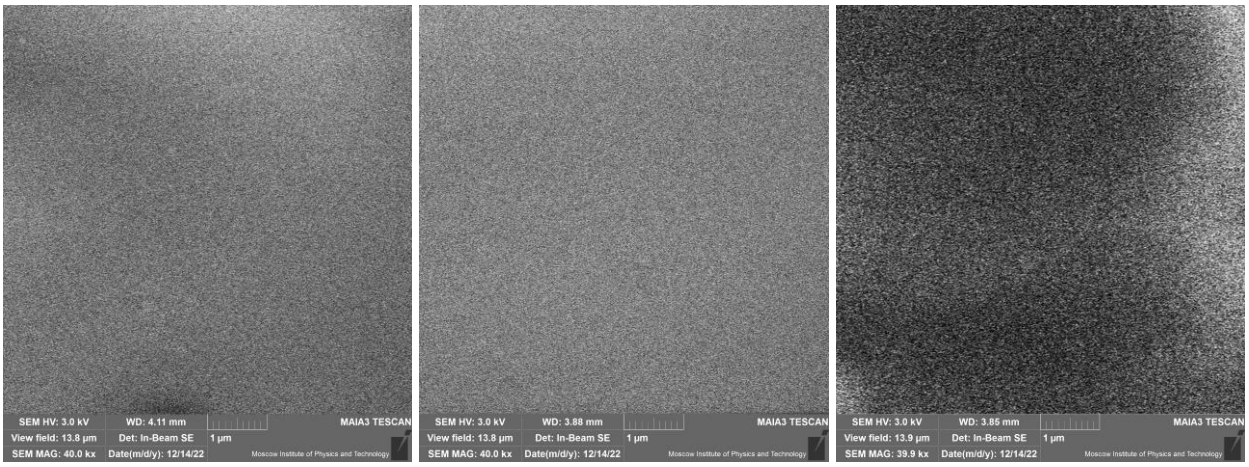

Figure S1. SEM-based morphological characterizations of the sensor chip surface at different functionalization stages: aminated surface (*left*), carboxilated surface (*center*), and surface after FA-gelatin conjugate immobilization (*right*)

S2. Characterization of wettability of the sensor chip surface by contact angle measurements

The results of characterization of wettability of the sensor chip surface by contact angle measurement are shown in Table S1. Six surface types have been studied: (1) before modification; (2) after cleaning with piranha solution; (3) after amination with APTES; (4) after carboxylation with succinic anhydride; (5) after immobilization of FA-gelatin conjugate; (6) after binding of FA antibodies with FA-gelatin conjugate. The water contact angles for all surfaces were measured using a goniometer at the Moscow Institute of Physics and Technology (Moscow, Russia). In the experiments, 5-μL droplets of mQ water were used at room temperature. The mean and standard deviation of five measurements in different areas of the surface are presented in the table. Meaningful changes of the water contact angle can be seen according to the expected variation in the surface hydrophilicity. Besides, small deviations of the angle values in different areas of the sensor chip indicate homogeneous functionalization over the whole its surface.

Table S1. Results of wettability characterization of the sensor chip surface by contact angle measurement

Step	Contact angle
1. Before modification	69.1 ± 3.9
2. After cleaning with piranha solution	28.6 ± 2.8
3. After amination with APTES	70.2 ± 1.6
4. After carboxylation with succinic anhydride	61.4 ± 2.1
5. After immobilizaion of FA-gelatin conjugate	68.5 ± 1.9
6. After binding of FA antibodies with FA-gelatin conjugate	76.3 ± 3.2

S3. Absence of FA antibody binding with the surface if the step of FA-gelatin conjugate immobilization is omitted (verified by the label-free spectral correlation interferometry)

Figure S2 shows the sensorgrams registered in real time by the label-free method of spectral correlation interferometry. It can be seen that FA antibody binding with the surface is absent in the case of omitted step of FA-gelatin conjugate immobilization in

contrast to the case of FA antibody binding with the surface having immobilized FA-gelatin conjugates.

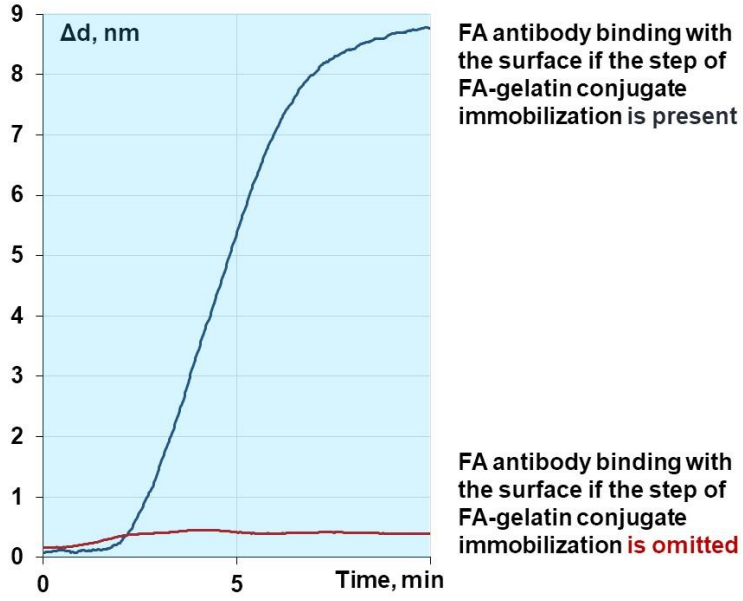

Figure S2. Absence of FA antibody binding with the surface if the step of FA-gelatin conjugate immobilization is omitted (verified by the label-free spectral correlation interferometry)

S4. Investigation with an ELISA-based technique of the effect of presence of FA-gelatin conjugates on the glass sensor chip surface onto immune complexes formation

Figure S3 exhibits the results of experimental study with an ELISA-based technique implemented on a surface of the glass sensor chip on the effect of presence of FA-gelatin conjugates on formation of "FA-gelatin conjugates – anti-FA antibodies – HRP-linked anti-mouse antibodies – TMB" complex.

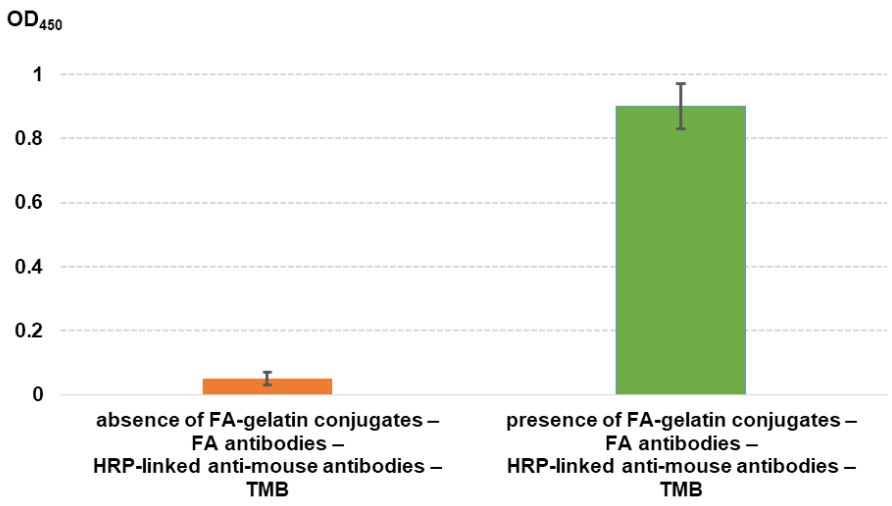

Figure S3. Effect of presence of FA-gelatin conjugates on the glass sensor chip surface on immune complexes formation studied with an ELISA-based technique

S5. Absence of binding of anti-mouse antibody with the surface if the step of FA-antibody immobilization is omitted (verified by the label-free technique of spectral correlation interferometry)

Figure S4 shows the sensorgrams registered in real time by the label-free method of spectral correlation interferometry. It can be seen that binding of anti-mouse antibody with the surface is absent in the case of omitted step of FA antibody immobilization in contrast to the case of FA antibody binding with the surface having immobilized FA-antibodies.

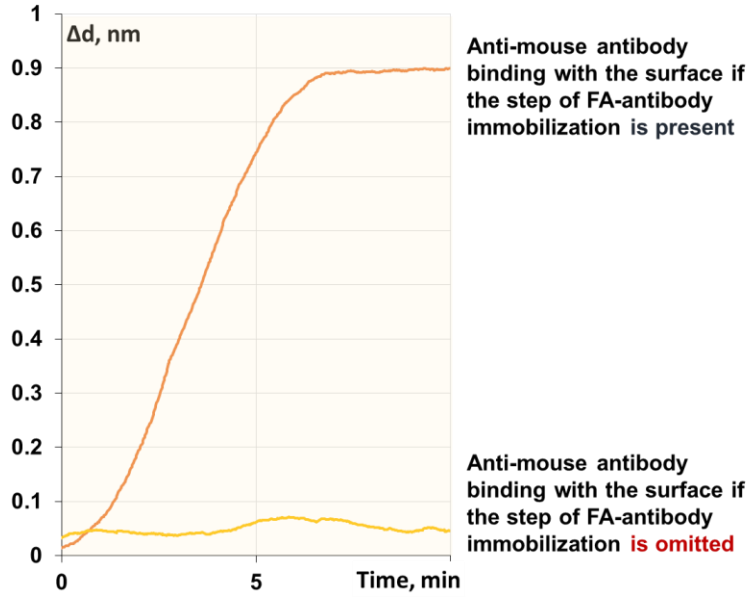

Figure S4. Absence of binding of anti-mouse antibody with the surface if the step of FA-antibody immobilization is omitted (verified by the label-free technique of spectral correlation interferometry)

S6. Study of the effect of FA-antibody presence on formation of complexes on the surface of a glass sensor chip with an ELISA-based technique

Figure S5 presents the experimental results of employment of an ELISA-based technique on the surface of a glass sensor chip for investigation of the effect of FA-antibody presence on formation of "FA-gelatin conjugates – anti-FA antibodies – HRP-linked anti-mouse antibodies – TMB" complex.

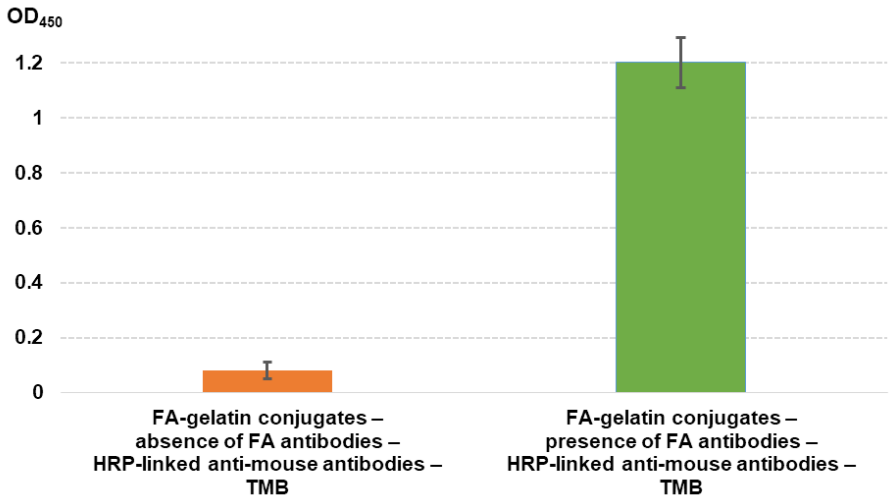

Figure S5. Study of the effect of FA-antibody presence on formation of complexes on the surface of a glass sensor chip with an ELISA-based technique

S7. Absence of FA-gelatin conjugate binding with the surface, which was not pre-activated by carbodiimide

Figure S6 shows the results of experiments that indicate chemical bondage of FA-gelatin conjugates with COOH-surface: the efficiencies are compared of FA-gelatine conjugate immobilization onto the surfaces with and without pre-activation by carbodiimide.

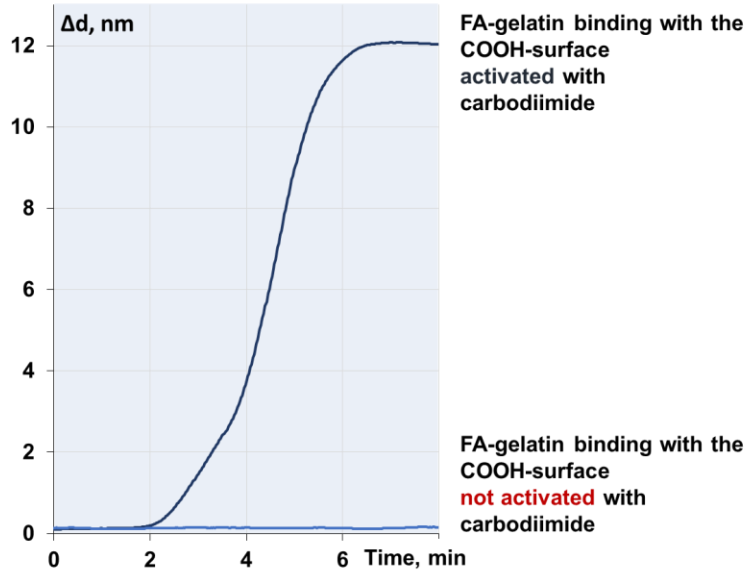

Figure S6. Demonstration of the absence of FA-gelatin conjugate binding with the sensor chip surface that was not pre-activated by carbodiimide

S8. Studies on selectivity of the developed chemosensor in the presence of non-target analytes

Figure S7 shows the results of the studies on selectivity of the developed chemosensor in the presence of non-target analytes

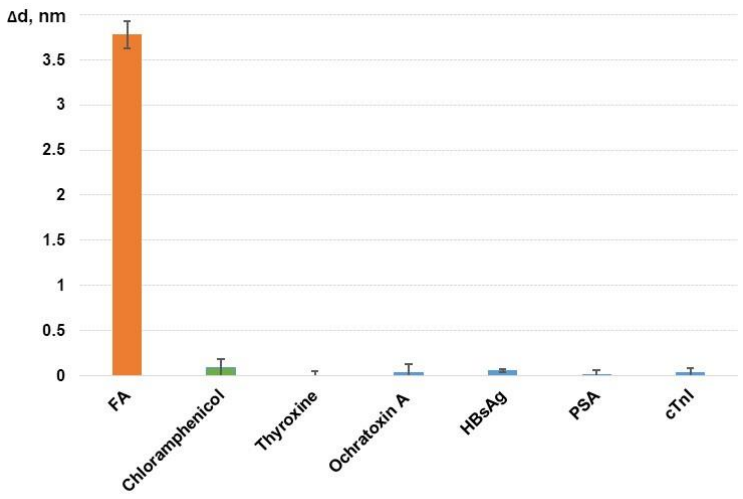

Figure S7. Studies on selectivity of the developed chemosensor in the presence of non-target analytes