# Detection of single biomolecules using a solid-state nanopore SiN/Si sensor

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Developed a technique for immobilizing single molecules of the 26S proteasome in the region of a solid-state SiN nanopore under the action of an applied electric field. The process of immobilization of a single molecule of the 26S proteasome leads to a change in the ion current through the nanopore by 20%. Further development of this technique may allow highly efficient immobilization of single biomolecules in order to study their conformational activity.

**Keywords:** Nanopore, solid-state nanopore, nanopore sensor, single molecule detection, 26S proteasome.

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## 1. Introduction

The development of methods for the synthesis of solid-state nanostructures, electrical and optical detection methods, as well as molecular biology technologies has led to the emergence of a new class of highly sensitive biosensors, the principle of which is based on the interaction of biomolecules with nanoscale structures. The technologies of two-electrode electrochemical sensors [1,2], sensors based on field-effect transistors [3] and CRISPR devices [4] are widely used. Nanopore detection technologies, which allow for qualitative and quantitative analysis of single biomolecules, are a promising area for the development of biosensors.

In general, a nanopore sensor is a thin, impermeable membrane with an integrated single nanoscale pore that connects two independent volumes of an electrolyte buffer solution. The detection process consists in modulating the electrical conductivity of the pore by molecules of the analyte passing through its internal volume. The magnitude and duration of the change in pore conductivity (translocation process) depends on the size, charge, stiffness, and conformational properties of the studied molecule. Thus, the measurement of the ion current flowing through the nanopore makes it possible to detect, identify and analyze molecular compounds in ultra-low concentrations [5,6].

Nanopore sensors based on solid-state membranes include a wide range of materials, synthesis technologies, and application methods. For example, solid-state nanopores are actively used to study the mechanisms of ion transport, study the physical, chemical and conformational properties of proteins and nucleic acids without the need for preliminary modification of the analyzed substances [7–9]. The possibility of studying the conformational activity of a single molecule immobilized in the nanopore region under the action of an electric field is promising. This work is devoted to the development of a technique for the immobilization of protein compounds of — 26S components of the proteasome in the field of solid-state nanopores.

## 2. Materials and methods

26S proteasome components dissociated into 20S components and low molecular weight protein subunits were used as the studied objects in the paper. 20S proteasome components are highly stable cylindrical protein molecules with a diameter of 11.5 nm and a height of 15 nm. There is a through cavity of variable diameter inside the cylinder. The isoelectric point of the molecule is 5.5 pH. The proteasome molecules have a negative electric charge in the buffer solution used.

The nanopore sample used in the work is a freely suspended SiN membrane with an integrated single nanoscale pore. The synthesis of the membrane consists of several stages, the first of which forms a SiN layer with a thickness of 30 nm on the annealed surface of the Si substrate. SiN is grown by gas-phase epitaxy at reduced pressure (LP-CVD). Next, the Si substrate is removed by selective chemical and plasma chemical etching, leaving a freely suspended SiN membrane with a thickness of 30 nm and sides of $130 \times 130\,\mu$m. A single oval-shaped nanopore $10 \times 20$ nm is created in the resulting membrane by lithography using a focused electron beam (Figure 1, *a*). The size of the nanopore is determined by the linear dimensions of the

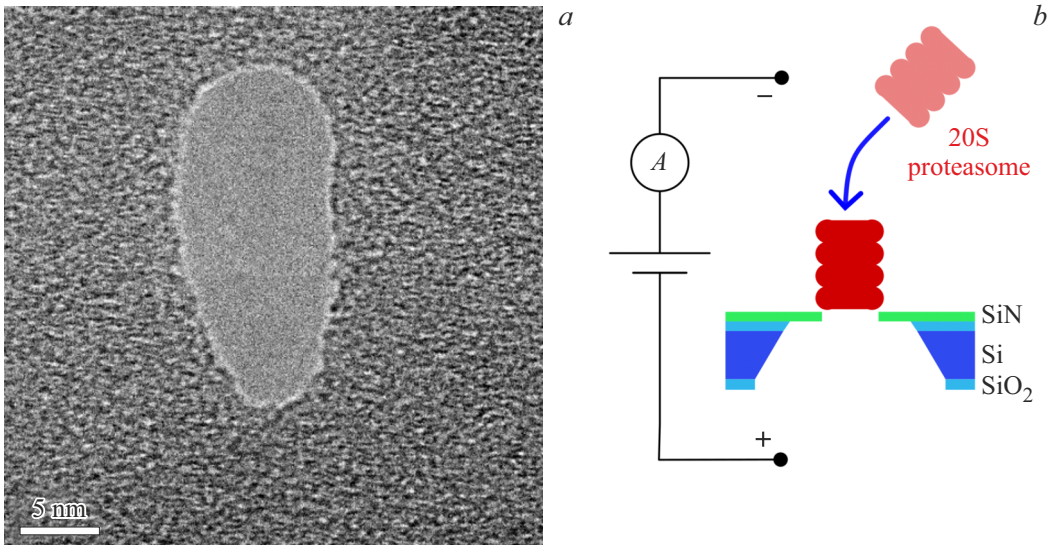


**Figure 1.** *a* — TEM image of the nanopore used in the work; *b* — scheme of the experiment on immobilization of a single molecule of the 20S proteasome. (A color version of the figure is provided in the online version of the paper).

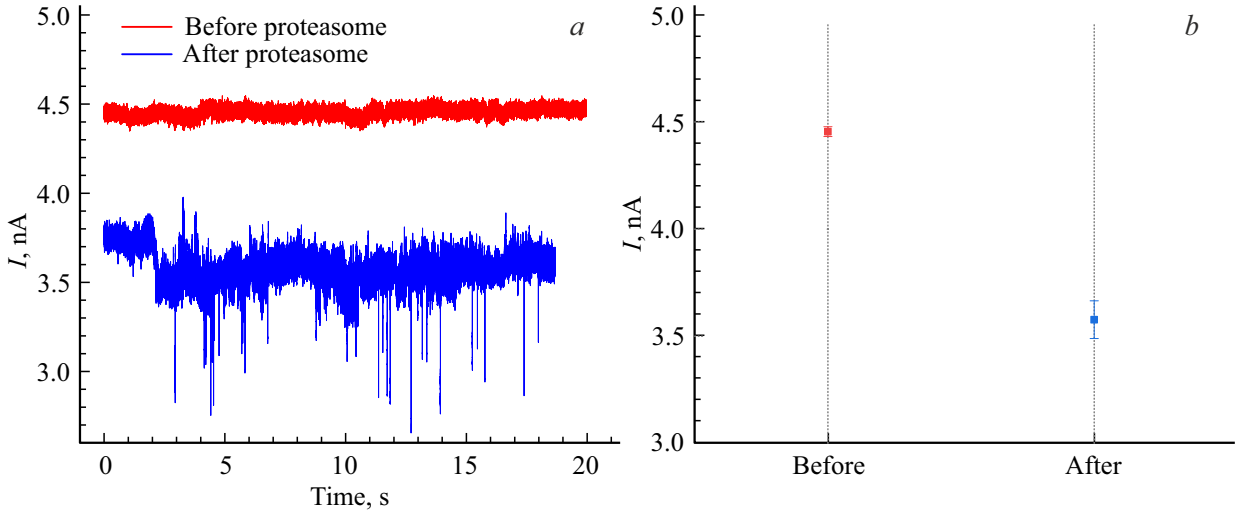


**Figure 2.** *a* — examples of 20-second current curves before and after adding proteasome molecules $U = 0.3$ V, measurement frequency — 50 kHz; *b* — equilibrium ion current levels up to and after adding the proteasome molecules $U = 0.3$ V.

studied molecules — proteins should be immobilized in the region of the pore, but should not pass through its internal volume.

A measuring cell was developed for conducting experiments on the immobilization of proteasome molecules. The cell consisted of two volumes (halves), each of which contains measuring Ag/AgCl electrodes and capillaries for supplying/discharging a buffered electrolyte solution. The nanopore sample is fixed between the cell volumes using polymer pads during assembly, after which the cell is filled with a buffer solution. The electrolyte buffer solution used in the paper is a mixture of 0.1 M KCl and 1 X PBS (1 : 1), pH = 7.4. An improvised source meter of voltage and current that allows measurements with a frequency of up to 50 kHz is used for measuring the ion current.

## 3. Experiment

At the first stage, the magnitude of the ion current through the nanopore was measured in a pure buffer solution without the addition of proteasome molecules. The resulting current curve shown in Figure 2, *a* (blue curve) shows no abrupt changes in the current level, which can be interpreted as blocking of the pore and (or) passage of large molecules through it. The value of the ion current was 4.46 nA ($U = 0.3$ V). After that, proteasome molecules with a concentration of 5 nm were added to the negatively charged volume of the cell. Negatively charged molecules move to the positively charged cell volume through the nanopore under the action of an applied electric field. Since the small size of the nanopore will not allow proteasome

molecules to pass through its internal volume, we expect that one of the molecules will be immobilized in the pore area. The immobilization process should be accompanied by a change in the configuration of the double electrical layers at the entrance to the nanopore, which will affect the transport characteristics of the entire system. After adding proteasome molecules to the volume of the measuring cell, the ion current through the nanopore decreased by 20% and amounted to 3.58 nA (Figure 2, *b*). Moreover, the resulting current curve shows sharp abrupt changes in the ion current level (see Figure 1, *a*).

We assume that the overall change in the ion current level is due to a partial overlap of the inner volume of the pore by the immobilized proteasome molecule. At the same time, ion and molecular transport through the nanopore is preserved, as evidenced by abrupt changes in the current level. The appearance of such short-term current changes may be due to the passage through the inner volume of the pore of low-molecular-weight protein fragments remaining after dissociation of part of the proteasome molecules.

## 4. Conclusion

A technique was developed in this study for immobilizing single molecules of the 20S proteasome in the region of a solid-state SiN nanopore under the action of an applied electric field. Further development of this technique may make it possible to efficiently immobilize single biomolecules in order to study their conformational activity.

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### Conflict of interest

The authors declare that they have no conflict of interest.

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