Supporting figures.

Fig. S1 Fluorescence spectra coming from regions of the same sample treated in different ways. The black lines are related to an incubation time of 1 hour, while the red lines are related to an incubation time of 2 hours. A fluoresceine-labelled glucose-binding protein solution was employed. The dependence of the amount of bound proteins on the incubation time is demonstrated.

Fig. S2 Comparison of the fluorescence spectra coming from two equivalent areas of the same sample, one irradiated by the scanning beam filling submicrometer lines (500 nm wide, 1 µm spaced), and the other fully filled. It can be seen that the fluorescence intensity is almost proportional to the surface fraction directly irradiated by the electron beam (1/3 in the case of lines), confirming the specific binding of proteins and the submicrometer resolution of the technique. The spectrum of a non-irradiated region is shown as a reference.

Fig. S3 Fluorescence spectra obtained from regions irradiated at two different electron beam energy values. After the irradiation, the sample was exposed to a fluoresceine-labelled glucose-binding protein solution for 1 hour at 37°C. The fluorescence intensity increases with the energy, confirming that the internal surface is involved, and the protein nanopatterns can be controlled in 3 dimensions. The spectrum of a non-irradiated region is shown for comparison.

Fig. S4 Dependence of the bound proteins amount on the irradiation dose. Fluorescence spectra obtained from regions of the same sample irradiated with two different electronic dose values, after exposure to a rhodamine-labelled glutamine-binding protein solution for 1 hour at 37°C. It can be seen that the amount of bound proteins increases with the dose.

Fig. S5 Fluorescence spectra acquired from two regions irradiated by the electron beam and a non-irradiated one, after exposure to a fluoresceine-labelled glucose-binding protein solution for 5 minutes at room temperature. Dose 1 and dose 2 were 70 mC/cm² and 140 mC/cm², respectively, while the electron energy was 15 keV. This result demonstrates that an incubation of a few minutes is sufficient for protein selective binding onto the porous silicon surface. Furthermore, the incubation time can be used as a variable parameter in order to choose the concentration of proteins to be bound on the 3D nanopatterns.
Figure S1

E = 15 keV; Dose = 140 mC/cm²

- non-irradiated, 1h dipping
- irradiated, 1h dipping
- non-irradiated, 2h dipping
- irradiated, 2h dipping

Intensity (a.u.)

Wavelength (nm)
Fig. S2

- $E = 15$ keV
- Dose = 140 mC/cm$^2$
- Incubation time: 2 h

Graph showing intensity versus wavelength (nm) for fully irradiated, irradiated lines, and non-irradiated samples.
Fig. S3
Fig. S4

- \( E_{\text{beam}} = 20 \text{ keV} \)
- Varying the dose:
  - \( D1 = 70 \text{ mC/cm}^2 \)
  - \( D2 = 140 \text{ mC/cm}^2 \)
Fig. S5

Intensity (a.u.)

Wavelength (nm)

Incubation: 5 min @ RT

- non-irradiated
- irradiated @ dose 1
- irradiated @ dose 2