Supplementary Information

Multi-Wavelength transparent microfluidic device for UV-Visible illumination and X-ray Scattering studies of Photoactive Systems

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1. Estimate of the *trans-cis* and *cis-trans* conversion using UV-Visible absorbance data

In the following, the formula used to obtain an approximate estimate of the fraction of *trans-cis* and *cis-trans* conversion is reported. It is based on the absorbance measured with UV-Visible spectroscopy under different illumination conditions. The calculation of the fraction of isomer conversion is adapted from Wang et al: [1]

$$R_{\%} = \frac{A_0 - A_{irr}}{A_0} \qquad (1)$$

$R_{\%}$	photoisomerized fraction
A_0	absorbance at λ_{max} before irradiation
A_{irr}	absorbance at λ_{max} after irradiation

Reference

1 Z. Wang, Q. Jiang, M. Reynders, C. Barwig, A. Mishra, K. Ramesh, and C. Selhuber-Unkel, *J. Phys. Photonics*, 2024, **6**, 035024.

2. SAXS data manipulation

In the following, the formulas employed to calculate relevant SAXS parameters for the present study are reported:[2]

Radius of gyration R_g:

$$I(q) = I(0)e^{-\frac{q^2 Rg^2}{3}} \qquad (2)$$

q	Scattering vector
I(q)	Scattering intensity
I(0)	Forward scattering intensity

Pair Distance Distribution Function (PDDF) p(r)

$$p(r) = \frac{1}{2\pi^2}\int_0^{\infty} I(q)qrsin\,(qr)dq \qquad (3)$$

Invariant Q:

$$Q = \int_0^{\infty} I(q)q^2 dq$$

Correlation length l_c

$$l_c = \pi \frac{\int_0^{\infty} I(q) q dq}{Q}$$

As in the present case it was not possible to integrate in the range from q= 0 to q= ∞, we integrated in the measured q range, therefore:

$$Q = \int_{q_{min}}^{q_{max}} I(q) q^2 dq \tag{4}$$

$$l_c = \pi \frac{\int_{q_{min}}^{q_{max}} I(q) q dq}{Q} \tag{5}$$

Reference

2. O. Glatter, *Scattering Methods and their Application in Colloid and Interface Science*, Elsevier, Amsterdam, Netherlands 2018.

3. Estimate of SUEX attenuation length

According to the method commonly employed in X-ray lithography,[3] the first step to estimate the transparency of a material to X-rays of a given wavelength is to determine its attenuation length, as it is independent from the material thickness. For this, the chemical composition of the material must be at least approximately known.

In the present case, SUEX is composed of a mixture of epoxy resins. The major components are Bisphenol A (BPA) and Bisphenol A diglycidyl ether (DGEBA). BPA can be described as $C_{15}H_{16}O_2$ with a density of 1.2 g/cm^3, DGEBA is $C_{21}H_{24}O_4$ with density of 1.17 g/cm^3.

In order to estimate SUEX transparency to 8 KeV X-rays, we therefore determined the attenuation length of these two components.

X-ray wavelength, chemical formula and density were inserted in the calculation sheet prepared by the Center for X-ray Optics (CXRO) of Lawrence Berkeley National Laboratory's (LBNL): https://henke.lbl.gov/optical_constants/

The resulting attenuation lengths are 1.642 mm for BPA, and 1.587 for DGEBA. The attenuation length of the mixture will be an intermediate between these two values.

For comparison, the attenuation length of quartz at 8 KeV is 0.11 mm, more than ten times shorter.

Transmission can also be calculated, considering the thickness of the material. For SUEX of 0.5 mm the value of transmission is similar for the two epoxy resins and approximately 73%.

Reference

3 M. Tormen, G. Grenci, B. Marmiroli, F. Romanato in *Nanolithography*, ed. Stefan Landis, ISTE Ltd. and John Wiley & Sons, Inc., London, 2011, 1,1, 1-79.

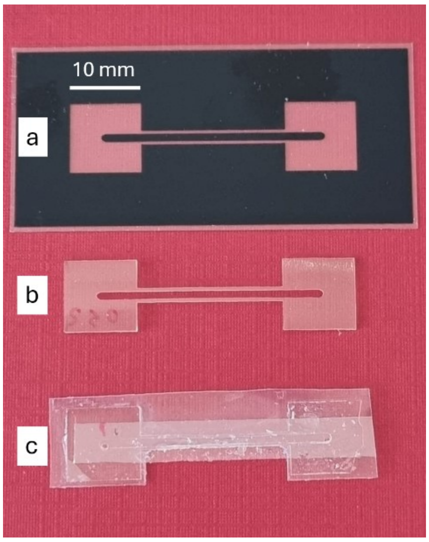


Figure S1. (a) UV lithography mask (b) 250 µm thick SUEX channel, and (c) final device after step iv of the fabrication process.

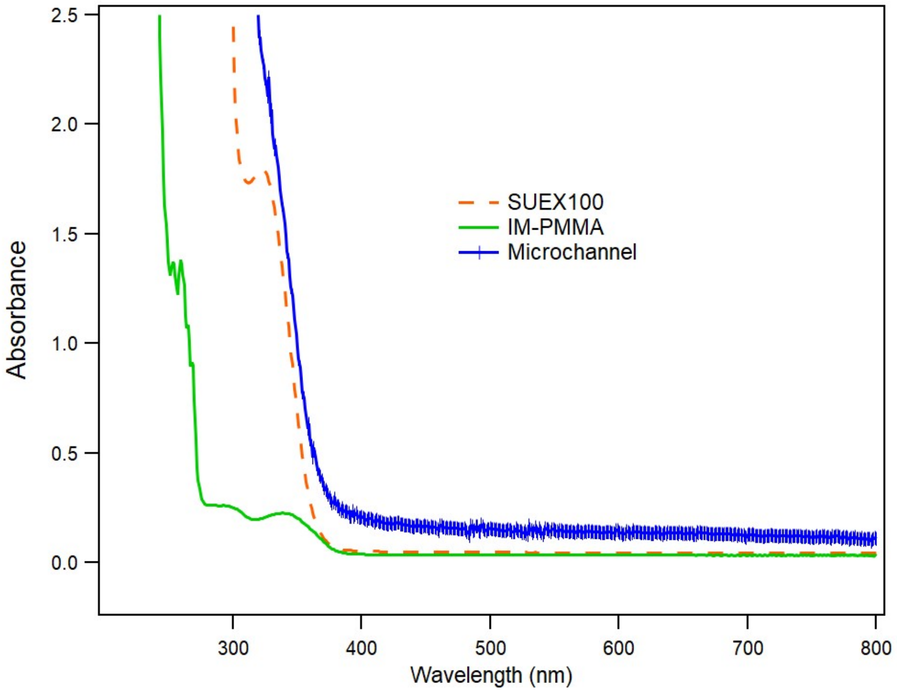


Figure S2. UV-Vis absorbance spectra of the materials employed for the fabrication of the microfluidic device, as well as spectrum of the empty channel itself. IM-PMMA has been used as top cover of the channel, as it is almost transparent to UV light down to a wavelength of 280 nm (continuous green line). The SUEX 100 slide (orange dashed line), and therefore the microchannel (blue line with vertical bars), are only transparent to visible light.

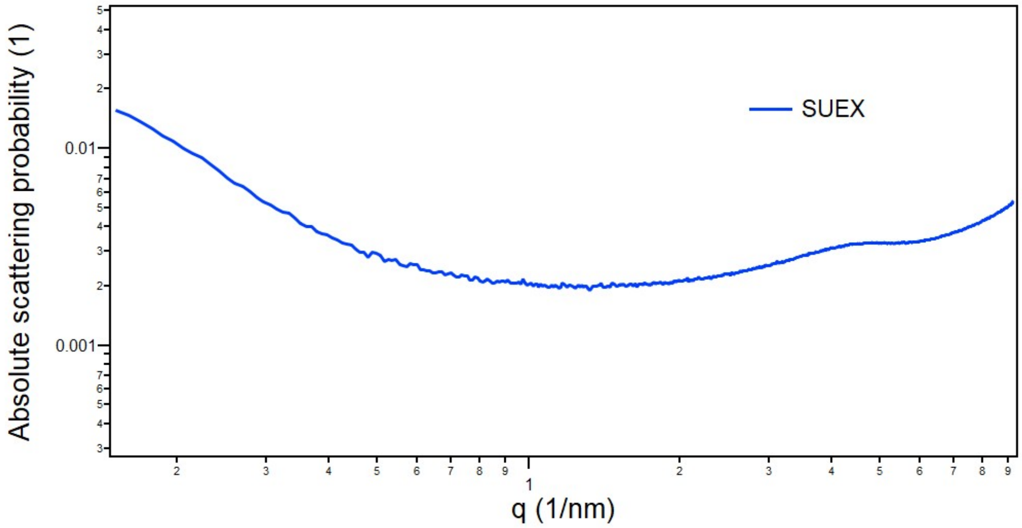
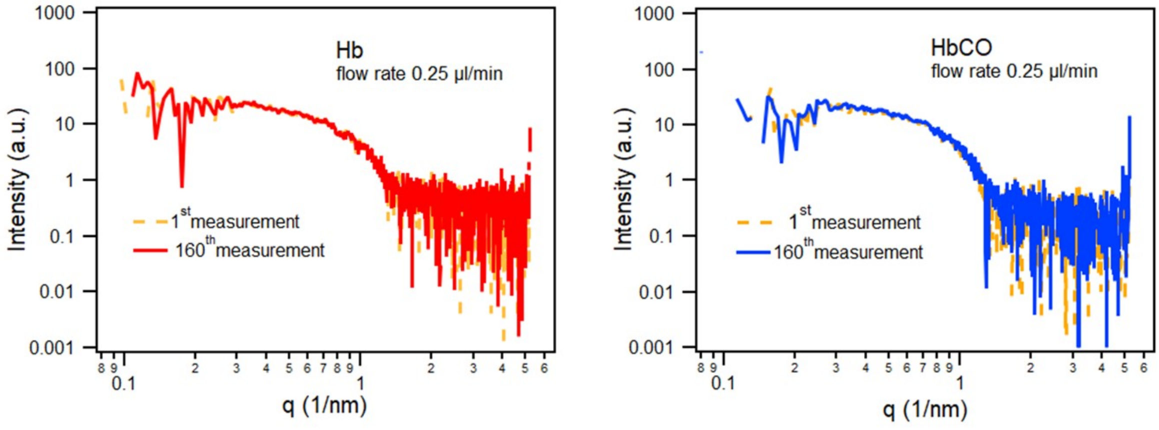


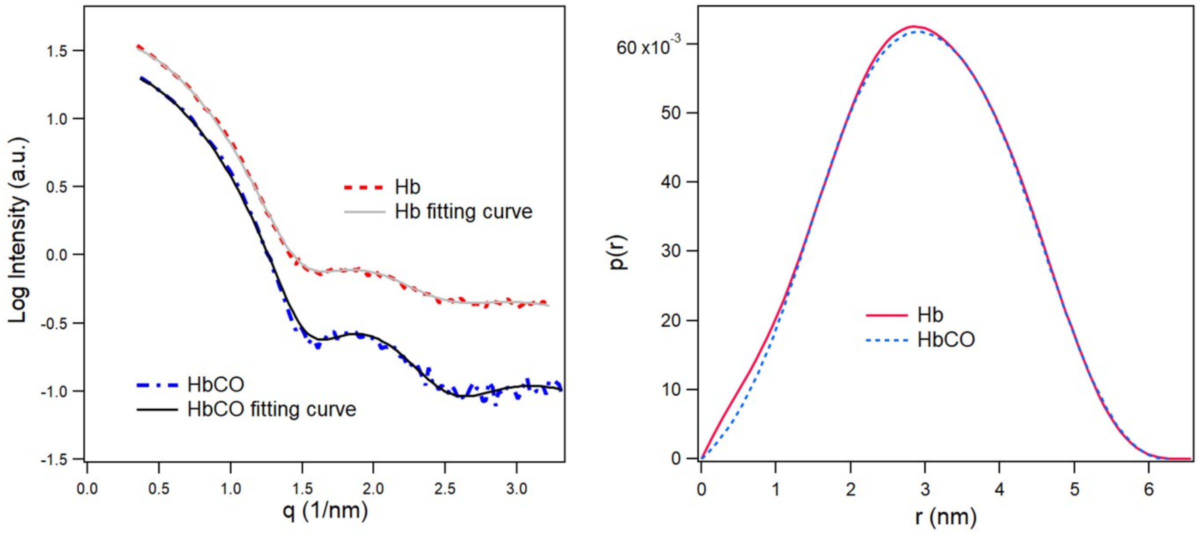
Figure S3. Absolute scattering probability of SUEX dry film resist.



a

b

Figure S4. First and last scattering curves of a radiation damage test consisting in the acquisition of 160 exposures of 10 s with a 1 s delay of the proteins flowing in the microchannel at 0.25 µl/min. (a) Hb first measurement (yellow dashed line) and last measurement (continuous red line) (b) HbCO first measurement (yellow dashed line) and last measurement (continuous blue line). In both cases the curves are almost superimposed.



a b

Figure S5. (a) Scattering curves and corresponding indirect Fourier transform fits calculated using GNOM program: Hb (red dashed line) and fitting (continuous grey line); HbCO (blue dashed with dots line) and fitting (continuous black line). (b) Resulting Pair Distance Distribution Function of Hb (red continuous line) and of HbCO (blue dashed line).

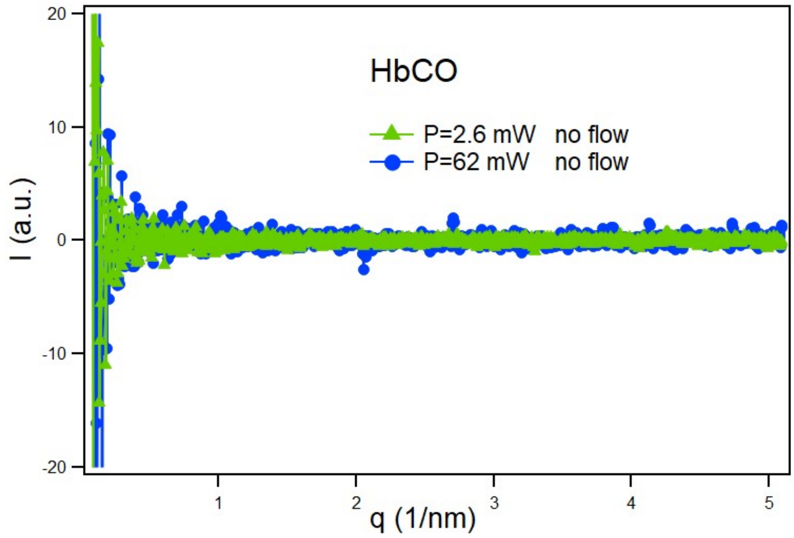
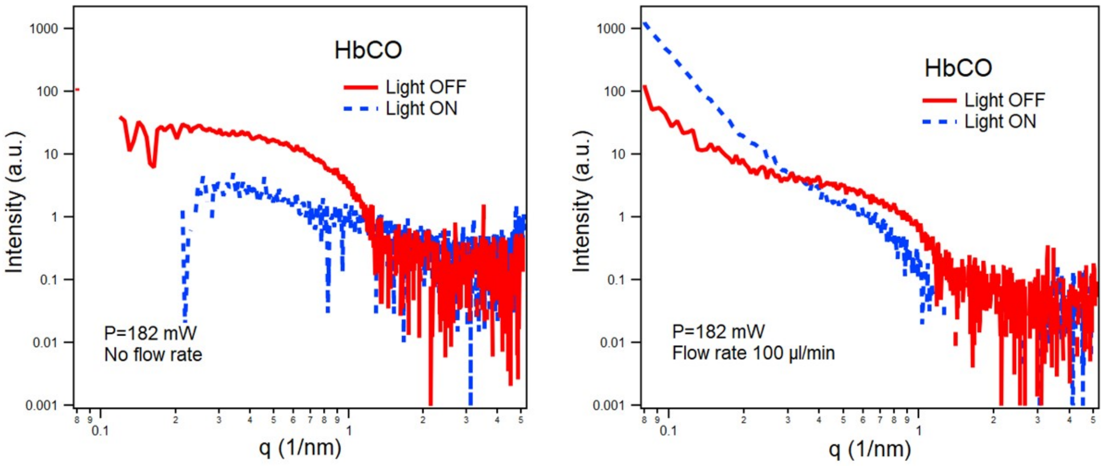
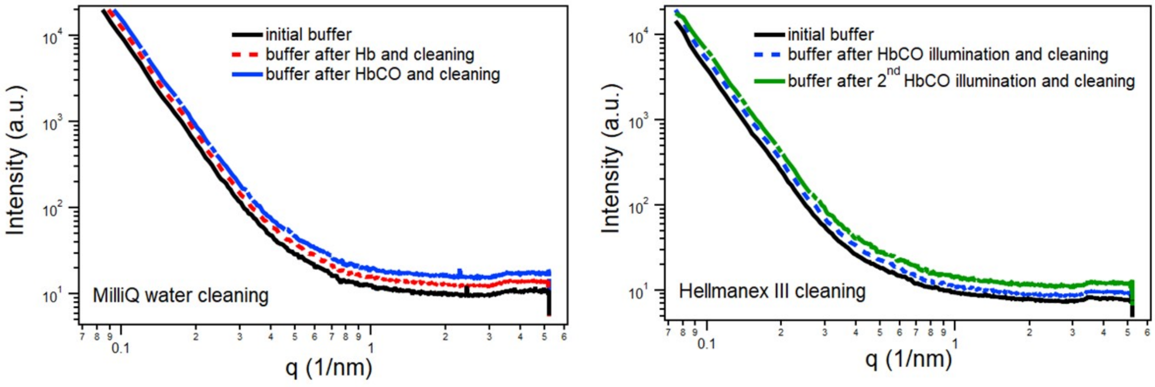


Figure S6. Scattering curve of HbCO with laser ON subtracted by the one with laser OFF. Two different transmission values of the laser have been used: T 5% corresponding to P= 2.6 mW (green curve with triangles), T 50% corresponding to P= 61.7 mW (blue curve with circular dots) with no liquid flow.



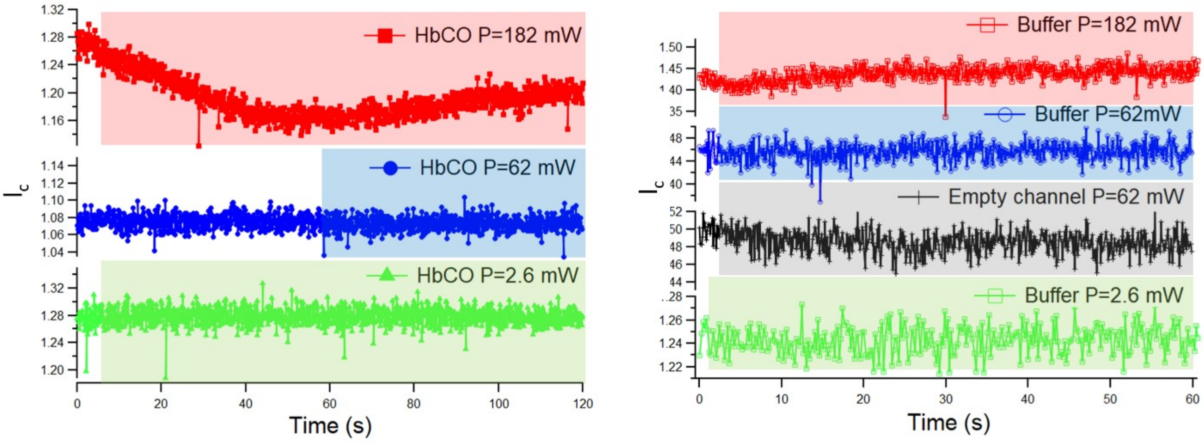
a b

Figure S7. (a) Scattering curve of HbCO in the microchannel with an exposure time of 10s without pumping. The red continuous curve corresponds to not irradiated protein, the blue dashed one to the protein exposed to laser light with a power P= 182mW **(b)** Scattering curve of HbCO in the microchannel with an exposure time of 10s while pumping with a flow rate of 100 µl/min. The red continuous curve corresponds to the protein before irradiation, the blue dashed one to the same protein exposed to laser light.



a b

Figure S8. Scattering curves of the buffer in the microchannel before and after cleaning. All buffer measurements were taken for 10 s. The curves are vertically shifted for clarity. **(a)** Cleaning with Milli-Q water. Initial buffer measurement (black continuous lower line), buffer after 160 measurements of 10 s on Hb protein while flowing at 0.25 µl/min and cleaning with water (red dashed intermediate line), buffer after 160 measurements of 10 s on HbCO protein while flowing at 0.25 µl/min and cleaning with water (blue dashed upper line). **(b)** Cleaning with a 1% v/v aqueous solution of Hellmanex III, followed by rinsing with Milli-Q water. Initial buffer measurement (black continuous lower line), buffer after 1000 images of 0.1 s on HbCO during laser irradiation with P= 182 mW and cleaning (blue dashed intermediate line), buffer after 1000 images of 0.1 s on HbCO during laser irradiation with P= 182 mW while flowing protein at 100 µl/min and cleaning (green dashed upper line).



a

b

Figure S9. (a) Calculated correlation length vs SAXS exposure time for the protein inside the microfluidic device flowing at 100 µl/min. During the SAXS measurement the light is turned ON. The shadowed area corresponds to the measurements done with laser ON. Different laser power values are shown. The laser with P=62 mW (blue shadow) was turned on around one minute after starting the measurement. **(b)** Calculated correlation length vs SAXS exposure time for the buffer inside the microfluidic device flowing at 100 µl/min and for the empty channel for different laser power values.