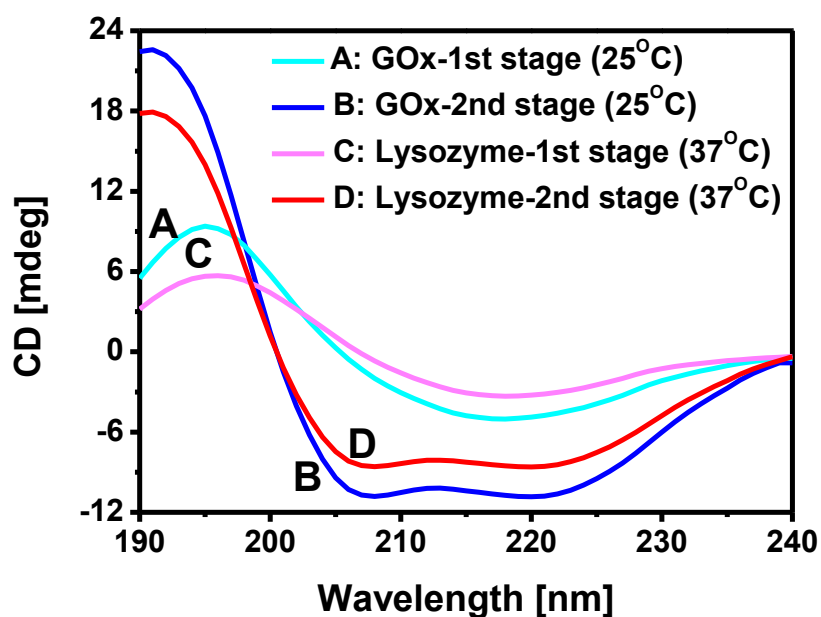


## Supplementary data



**Fig. S1.** CD spectra of GOx LB films (template-free system/25 °C) and Lysozyme LB films (SA/37 °C). The LB films were prepared by transferring 60 monolayers onto the quartz plates (for both adsorption stages) and then, characterized by a circular dichroism (CD) spectroscopy in the wavelength range of 190-240 nm. In general, the negative bands near 222 and 210 nm and the positive band at 192 nm represent the presence of helical structures. The negative bands at 215 and 180 nm and the positive band at 196 nm represent the presence of  $\beta$ -sheet structures.

**Table S1.** Relative standard deviations (RSD) for data in Table 1.

Protein	Template	Temp. (°C)	1st stage		2nd stage	
			$\alpha$ -helix (%)	$\beta$ -sheet (%)	$\alpha$ -helix (%)	$\beta$ -sheet (%)
GOx	Bare	25	1.9± 0.05	49.0± 0.07	54.8± 0.09	6.0± 0.06
	SA		2.1 ± 0.02	51.8± 0.05	56.8± 0.06	5.6± 0.08
	ODA		4.2± 0.04	48.8± 0.02	61.5± 0.10	4.8± 0.08
BSA	ODA	25	49.8± 0.02	24.0± 0.02	64.2± 0.03	10.4± 0.04
		37	7.3 ± 0.07	65.0± 0.08	72.4± 0.11	7.1± 0.09
Lysozyme	SA	25	19.3± 0.03	29.5± 0.02	44.6± 0.06	5.7± 0.04
		37	3.2± 0.09	39.5± 0.08	55.7± 0.12	5.6± 0.08

**Table S2.** Surface roughness measured from the AFM images of GOx LB films.

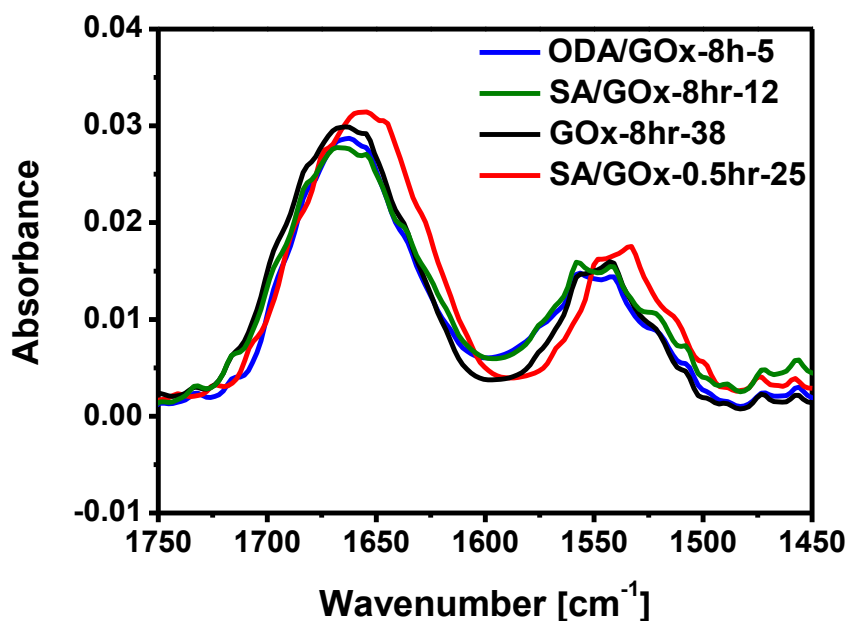
	RMS roughness (nm)	
	1 <sup>st</sup> stage	2 <sup>nd</sup> stage
GOx	0.19	0.66
SA/GOx	0.17	0.68
ODA/GOx	0.18	0.90

**Table S3.** Conformations of spreading GOx monolayers.

Protein	Surface pressure (mN/m)	Conformation		
		$\alpha$ -helix (%)	$\beta$ -sheet (%)	$\alpha/\beta$
GOx	8	1.4	49.4	0.03
	15	4.2	47.0	0.09

Note: GOx stock solution (1 mg/mL in 0.1M/pH 6.8 phosphate buffer solution) was spread directly on a pure water surface. By increasing the spreading amount of the GOx solution, an ESP (ca. 8 mN/m) identical to that of the adsorption GOx monolayer is approached. In the absence of GOx in the subphase, the surface pressure cannot increase high above 8 mN/m by further spreading of the GOx solution on the water surface, suggesting that the GOx molecules are presented as a single molecular monolayer. After transferring this GOx monolayer onto a quartz substrate, the conformation ratio of the GOx film was measured to be  $\alpha/\beta = 0.03$ . The result indicates that the GOx film also have a major conformation of  $\beta$ -sheet, which also confirm the role of the air-liquid interaction on the structure organization of adsorbed proteins.

To prepare a multilayer GOx monolayer by spreading method, a large volume of GOx stock solution was spread on an aqueous solution containing GOx (ca. 0.83mg/L in concentration). At the presence of GOx in the bulk phase, the surface pressure can be elevated up above the ESP (ca. 8 mN/m) when sufficient GOx molecules were spread from the gas phase. The GOx film on the liquid surface was transferred onto a quartz substrate when the surface pressure was increased to ca.15 mN/m. Although this surface pressure is equivalent to the value in the second adsorption stage of GOx, the measured conformation ratio ( $\alpha/\beta=0.09$ ) is much smaller than the film prepared by adsorption (ca. 9-10), which indicates the importance of GOx adsorption in undergoing the conformation transfer from  $\beta$ -sheet to  $\alpha$ -helix.



**Fig. S2.** FTIR spectra of GOx LB films. All the GOx monolayers were transferred onto Au substrate after 8 h (ODA, SA, and template free) or 0.5 h (SA) adsorption of GOx and then, characterized by an attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR). The presence of the GOx in the films was confirmed by the peaks corresponding to amide I (around 1650 cm<sup>-1</sup>) and amide II (around 1550 cm<sup>-1</sup>). The similar absorbance measured for various films indicates a similar amount of GOx incorporated in these films. The required layers for 8 h-adsorption monolayers to achieve this similar absorbance are 5 for ODA, 12 for SA, and 38 for a template-free interface. For the 0.5 h-adsorption monolayer, a higher layer number is required (25 for SA). It is noteworthy that the films prepared after 8 h adsorption have identical peak positions, which is consistent with the same conformation ( $\alpha$ -helix) of these GOx films. On the contrary, the peaks of the SA/GOx film transferred after 0.5 h adsorption right shift slightly with respect to the others, attributed to the different conformation ( $\beta$ -sheet) of this film.