Supplementary Information

Measurement of Enzyme Activity of Insoluble Substrates Based on Ordered Porous Layer Interferometry and the Application in Evaluation of Thrombolytic Drugs

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1.Preparation of the SCC Films

An appropriate quantity of ~190 nm SiO_2 colloidal aqueous solution was placed into a centrifuge tube and was washed with ethanol by repeated centrifugation and ultrasonic dispersion cycles in order to remove the original solvent and impurities. The aqueous-dispersed silica particle suspensions were therefore transformed into silica alcosols. The particle volume fraction of the resultant silica alcosol were determined by drying 1 ml of the silica alcosol in an oven and then weighing the residual solid. A measured amount of the SiO_2 alcoholic solution was then dried in an oven overnight at 60℃ to determine the mass-volume fraction. The solution was diluted to the desired mass-volume fraction and poured into a glass trough. Clean glass slides were inserted vertically into the solution and left undisturbed on a benchtop for 5-7 days to allow for natural evaporation of the ethanol, leading to the spontaneous formation of a three-dimensional, ordered array of SiO_2 colloidal particles on the glass slide.

2.Assessment of Chymotrypsin Activity Utilizing Ultraviolet Spectrophotometry

By designating the micromole of tyrosine introduced into each standard solution as the x-axis and the corresponding absorbance as the y-axis, a standard curve was plotted, as depicted in Figure S1.

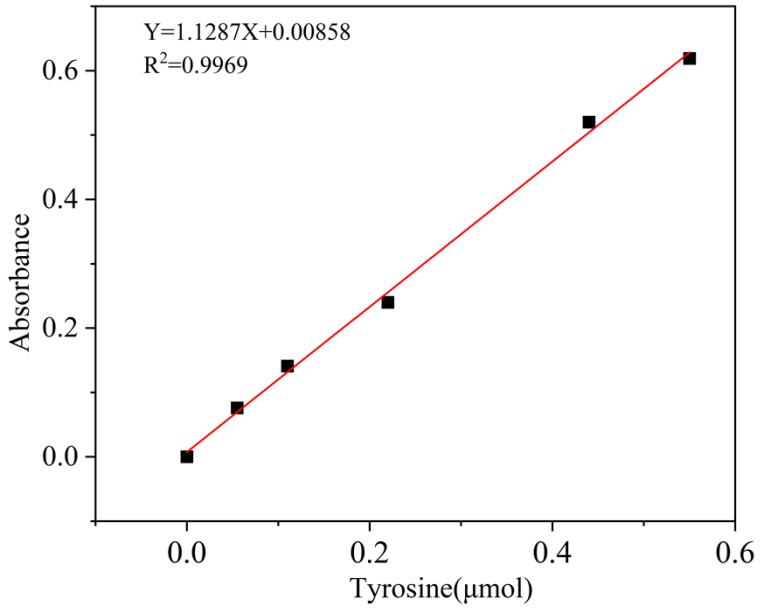

Figure S1 Standard curve illustrating the correlation between variations in absorbance and the corresponding quantity of tyrosine introduced.

3.Monitoring the Digestive Process of Caseins by Chymotrypsin using OPLI

The Figure S2 shown the raw reflectance data for the film containing immobilized caseins, and the raw reflectance spectrum after the caseins has been digested with chymotrypsin.

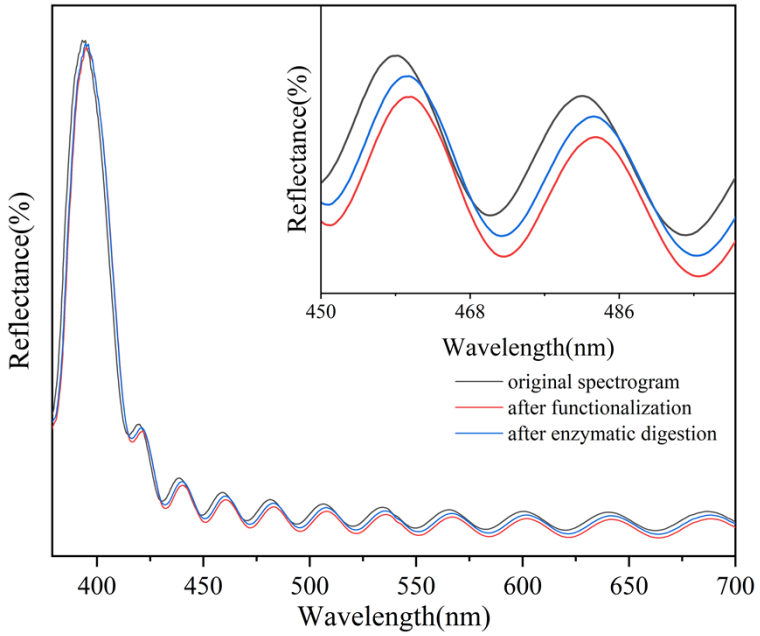

Figure S2 In situ reflectometric interference spectra showing the immobilizing of casein and the subsequent casein digestion by chymotrypsin.The inset is an enlarged view of the interference peaks with wavelengths between 450 and 500 nm.

As shown in Figure 3, following casein functionalization, the introduction of PBS solution into the sample pool resulted in a slight initial decrease in OT which then remained constant. This indicates that within the casein layer, which was rapidly bound to the silica sphere surface at high

concentration, there were non-tightly bound interactions. Portions of the directly adsorbed casein layer exhibited weaker binding and could be eluted with PBS solution. Thus, it was essential to ascertain whether prolonged washing could facilitate further removal of the adsorbed casein layer. Figure S3 illustrates that after casein molecules were adsorbed onto the silica sphere surface, PBS solution was continuously washed through the sample pool with real-time recording of changes in optical thickness. The data from Figure S3 reveal that, apart from the initial decline, OT remained stable throughout the extended washing, signifying the relative stability of the directly adsorbed casein layer.

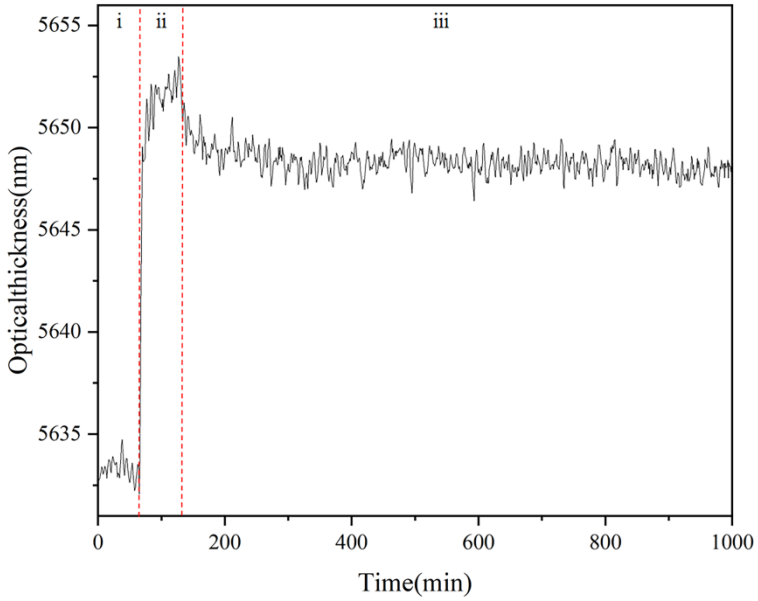

Figure S3 Stability of the casein functional layer formed by adsorption: (i) stabilizing the baseline with PBS solution, (ii) functionalization of silica surface with casein, (iii) rinsing with PBS. The change in optical thickness was recorded in real time.

4.Manufacturing of Optical Interference Fibrin.

Thrombin enzymatically cleaves fibrinogen into fibrin and induces its transformation into a clot via non-covalent interactions, effectively converting the water-soluble fibrinogen into an insoluble fibrin mesh. As depicted in Figure S4, upon introduction of a fibrinogen-thrombin mixture, there is a rapid increment in OT, which subsequently plateaus, indicating infiltration of the mixture into the SCC film and consequent enhancement of OT. A deceleration in the rate of OT increase is then observed until a steady state is achieved. No further alterations in the OT are discernible following a PBS wash, leading to the inference that the fibrin gel has been successfully affixed within the SCC lattice. Infrared spectroscopy (Figure S5) confirms the presence of amide linkages within the SCC film and subsequent digestion experiments with plasmin showed significant decrease in OT, substantiating the successful functionalization of fibrin onto the SCC substrate. This evidence points to a process transcending mere adsorption of fibrinogen or thrombin.

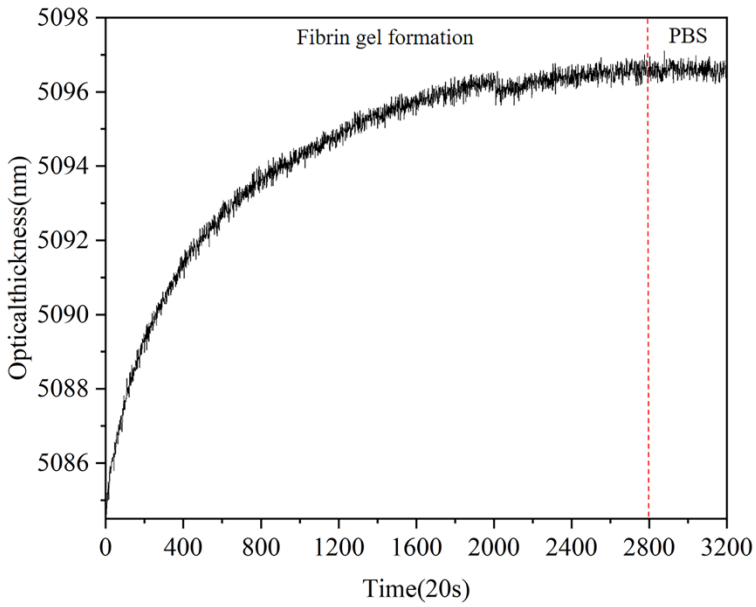

Figure S4 Real-time tracking of alterations in the OT of the SCC film following the introduction of the fibrinogen and thrombin mixture into the sample reservoir.

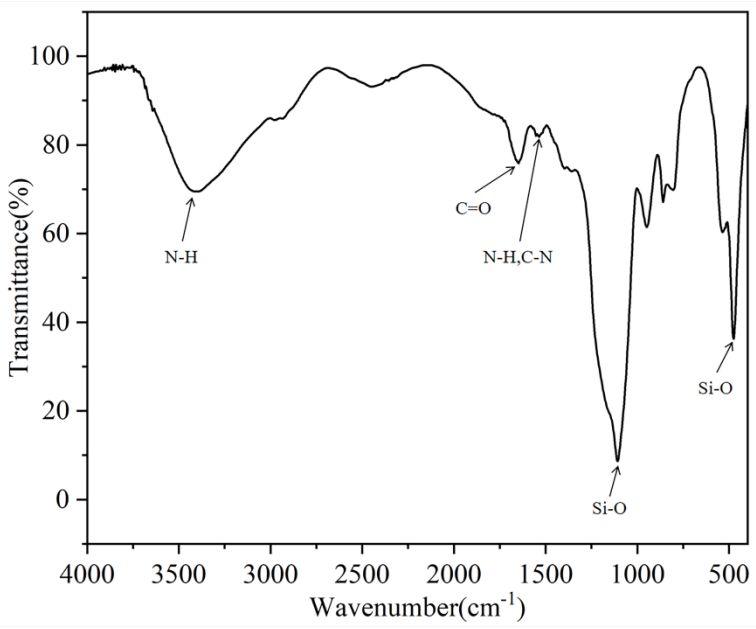

Figure S5 Infrared Spectroscopy of Fibrin via Optical Interference. In this spectrum, the amide A band is observed between 3600-3100 cm^{-1}, predominantly arising from N-H stretching vibrations and interactions within the hydrogen-bonding network. The amide I band, indicative of strong absorption, is located in the 1700-1600 cm^{-1} range, attributed to the C=O stretching vibrations. Lastly, the amide II band, detected between 1580-1500 cm^{-1}, results from the coupling of N-H bending and C-N stretching vibrations.

5.Monitoring the Digestive Process of Caseins by Chymotrypsin using OPLI

During the preparation process, we inserted a glass slide into a diluted alcohol solution of SiO_2. After 5-7 days of natural evaporation, a three-dimensional ordered SCC film formed on the

surface of the glass slide. Throughout the evaporation process, minor variations in film thickness occurred. In our experiments, we used enzymes of the same concentration to digest substrates of the same concentration. When processing the data, we normalized the varying film thicknesses to the same value. We found that the normalized ΔOT values were essentially consistent.

Table S1 Casein digested by chymotrypsin at uniform concentrations results in a normalized ΔOT that is observed to be substantially consistent.

	ΔOT(nm)	Normalization ΔOT(nm)	Initial thickness(nm)	After functionalization and PBS rinsing(nm)	After enzymatic digestion(nm)
1	3	2.9872	5021.5	5036	5033
2	3	2.7543	5446	5457	5454
3	3.5	2.9734	5885.5	5891.5	5888
4	3	2.9194	5138	5152	5149
5	3	2.9946	5009	5024	5021