# †Electronic Supporting Information

# Enantiomeric helical TiO2 nanofibers modulate different peptide assembling and subsequent cellular behaviors

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## Experimental Section

### 1. Reagents.

D- and L- glutamic acid (D-, L-Glu) and 3-Chloropropyltrimethoxysilane (CP-TMS, 98%) were purchased from Aladding Reagent Co., Ltd (Shanghai, China). Dopamine hydrochloride was obtained from Best Reagent Co., Ltd (Chengdu, China). Stearoyl chloride was purchased from Admas Reagent Co., Ltd (Shanghai, China). Titanium diisopropoxide bis(acetylacetonate) (75% in isopropanol, TDA) was purchased from TCI. Thioflavine-T (ThT) was obtained from Jianglai Reagent Co., Ltd (Shanghai, China). Ethanol, methyl, acetone, carbon disulfide, triethylamine (TEA), sodium hydroxide, hydrochloric acid, sulfuric acid, petroleum ether and hydrogen peroxide were purchased from KeLong Chemical (Chengdu, China). All chemicals were used as received without further purification. Distilled water (18.2 MΩ·cm) was used in the experiment.

### 2. Characterization.

$^{1}H$ and $^{13}C$ NMR spectra were collected on an Agilent 400MR DD2 400-MHz spectrometer. The ATR-FTIR was performed with a Thermo Fisher Scientific Nicolet iS 50 FT-IR spectrometer. X-ray photoelectron spectroscopy (XPS) data were obtained with an ESCALab250 250 Xi electron spectrometer from Thermo Fisher Scientific using 300W AlKα radiation. Circular dichroism (CD) spectra were recorded on a Chirascan CD spectrometer (Applied Photophysics, UK). Dynamic insulin adsorption experiment was conducted on a Quartz Crystal Microbalance (QCM) (Stanford Research System QCM 200). Solution MST binding studies were performed using standard protocols on a Monolith NT.115 (Nanotemper Technologies). Atomic Force Microscopy (AFM) investigation was conducted on a flat mica substrate using an Asylum Research MFP-3D AFM (Oxford Instruments Company) in a tapping mode. The morphology of the chiral $TiO_2$ nanofibers was observed with SEM (JEOL JSM-7800F) with an accelerating voltage of 1.0 kV. HRTEM images were collected with a Zeiss LIBRA 200 FEG TEM microscope operating at 200 kV. Powder X-ray diffraction patterns were recorded on a

PANalytical X'Pert Powder (Spectris Pte.Ltd) equipped with Cu K α radiation (60 kV, 55 mA). The ThT stained insulin-adsorbed chiral surfaces and cells were observed on IX71-F22PH inverted system microscope (OLYMPUS, Japan).

**3. Synthesis of chiral anionic surfactant.**

Each enantiomer of chiral anionic surfactants, *N*-stearoyl-L/D-glutamic acid ($C_{18}$-L/D-Glu), was synthesized by acylation of L/D-glutamic acid with stearoyl chloride. In a typical synthesis of *N*-stearoyl-L-glutamic acid ($C_{18}$-L-Glu),[1] L-glutamic (0.7056 g, 4.8 mmol) was dissolved in a mixture of distilled water (14 mL), dry acetone (12 mL), and NaOH (0.384 g, 9.6 mmol). The 1.212 g (4.0 mmol) stearoyl chloride was added dropwise to this mixture with stirring at 30 °C over a period of 20 min and synchronously, an aqueous solution (1.75 mL) of 4 M of NaOH was used to keep pH = 12. The reaction mixture was stirred for one additional 1 hour, cooled and then acidified to pH = 1 with HCl. Then the mixture was filtered off, washed with distilled water to neutralize in order to remove the unreacted amino acid. The precipitate of crude crystals of *N*-stearoyl-L-glutamic acid was washed with petroleum ether to remove the unreacted stearoyl chloride. The surfactant was filtered off and evaporated to dryness in vacuum to obtain the pure crystals. The method for the synthesis of the *N*-stearoyl-D-glutamic acid is the same as the above.

**4. Preparation of chiral and achiral $TiO_2$ nanofibers.**

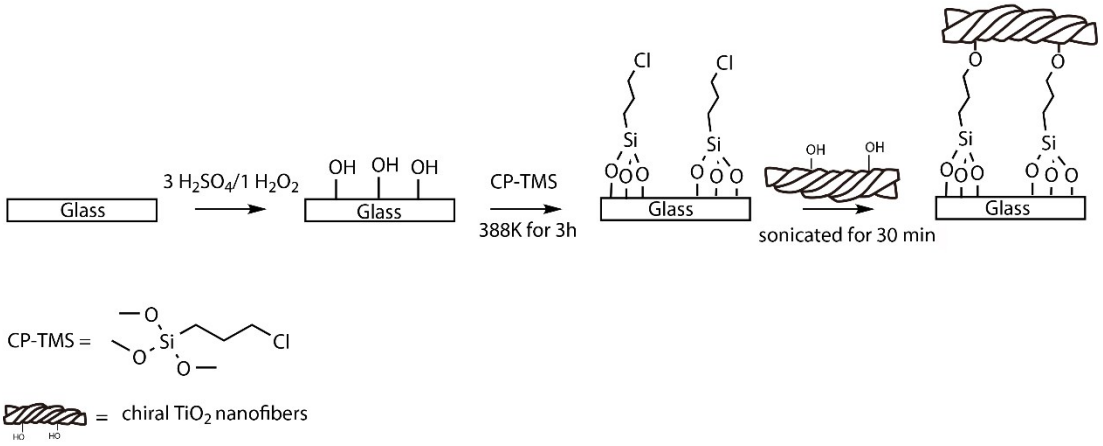
The helical $TiO_2$ was prepared with a reported method,[2] $C_{18}$-L-Glu (0.03 g, 0.08 mmol) was dissolved in a mixture of methanol (13.6 g) and distilled water (80 mL) while stirring at room temperature. After the mixture was stirred for 10 min, TDA (0.29 g, 75% in isopropanol) was added to the mixture with stirring at 55 °C. The mixture was allowed to react at 55 °C and stirring for 2 h. The products were collected by centrifugal separation and dried by freeze drying, which resulted in a pale yellow powder. All organics in this product were removed by calcination at 550 °C, and white crystalline powder was obtained. The method for the synthesis of the helical $TiO_2$ nanofibers using $C_{18}$-D-Glu is the same as the above.

For achiral $TiO_2$ nanofibers, the preparation process is accordance with chiral $TiO_2$, expects the template molecules is consisted of 0.015 g $C_{18}$-L-Glu and 0.015 g $C_{18}$-D-

Glu.

**5. Preparation of chiral and achiral $TiO_2$ nanofibers modified glass substrate.**

The glass substrates were modified according to previously reported method with modification.[3] Specifically, glass plates were placed on a comb-shaped Teflon support to avoid any surface overlap and the system was placed in a 50 mL Teflon-equipped stainless-steel autoclave charged with "piranha solution" ($H_2SO_4/H_2O_2$ = 3/1, v/v) and then heated at 100 °C for 1 hour, the plates were washed with large amounts of distilled water following with ethanol and finally dried at 80 °C. After cleaned with "piranha solution" the fixed substrates were then placed in the 50 mL autoclave containing 20 mL of toluene. The volume of toluene was large enough to keep the glass plates fully immersed within the solvent. 0.8 mL CP-TMS was added and the silanization was performed at 115 ºC for 3 hours. Subsequently, plates were washed with toluene, dried under nitrogen and placed one glass plate in 1 mg $mL^{-1}$ suspension of $TiO_2$ in toluene. Following a solution of $TiO_2$ with glass plates was sonicated for 30 minutes, the plates were taken out, washed with toluene and dried in air for further use. The achiral $TiO_2$, R-and L-$TiO_2$ functionalized glass substrates denoted as $TiO_2$ surafce, R-surfaces and L-surfaces, respectively.

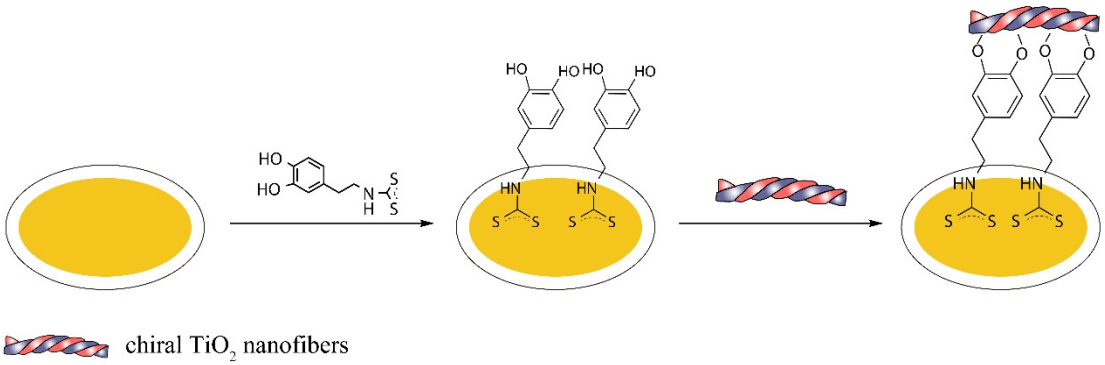



**Scheme S1. Modification process of chiral $TiO_2$ nanofibers on glass substrates.**

**6. Preparation of chiral $TiO_2$ nanofibers modified QCM sensors.**

The immobilization of chiral $TiO_2$ nanofibers on QCM sensors was achieved using a two-step process. First, in order to form a monolayer of dopamine, carbon disulfide (40 μL, 5.5 mmol), dopamine (5.5 mmol) and 5 μL of triethylamine were dissolved in 100 mL ethanol and sonicated for 5 min at room temperature to acquire the ideal

assembly of dopamine dithiocarbamate.[4] After that, the QCM resonator was immersed in the above solution with 150 rpm of oscillation for 12 hours to allow the conjugation of dopamine dithiocarbamate on Au surface by Au-S covalent bonds. Subsequently, the chemically modified QCM resonator was washed with ethanol and dried by nitrogen gas. Second, 0.8 mL of distilled water suspension (1 mg $mL^{-1}$) of L- or R-$TiO_2$ was coated on the surface of Au electrode after that the system was keeping static overnight. Following the sensors were washed by distilled water and dried by nitrogen gas.




**Scheme S2. Modification of chiral $TiO_2$ nanofibers on Au surface of QCM sensor.**

## 7. ThT fluorescence studies.

Insulin (bovine pancreas, purchased from Sigma-Aldrich) was dissolved into 25 mM HCl (pH 1.6) to a concentration of 1 mg $mL^{-1}$. This insulin stock solution was kept frozen at -20 °C in sealed vials. The working solution of insulin was prepared by diluting with HCl solution (25 mM, pH 1.6). To investigate the influence of chiral $TiO_2$ nanofibers modified substrates on the kinetics of insulin fibrillation, the insulin stock solution was diluted to experimental concentrations (0.1 mg $mL^{-1}$) followed the solution was pipetted into a flat bottom plate with 24 wells holding 1 mL each, then the R- or L-surfaces were immersed into the insulin solutions to allowing adsorption. After incubation at 60 °C, the surfaces were taken out and successively washed by 1 mM HCl and distilled water gently, then dried by weak flowing nitrogen gas. The insulin-adsorbed chiral surfaces were submerged (facing upward) in 10 mM ThT solution and incubated in a water bath at 37 °C for 10 min. After that, the surfaces were washed with distilled water for subsequent fluorescence imaging. The ThT

stained insulin-adsorbed chiral surfaces were observed with Olympus IX71-F22PH inverted system microscope (Tokyo, Japan) equipped with a Q-imaging MicroPublisher 5.0 RTV CCD camera and connected to a PC running Image Pro Plus software (Media Cybernetics). For the microscope fluorescence imaging, the excited fluorescence measured through a filter (λ = 470–490 nm) using the CCD camera. The Image Pro Plus software was then used to determine the average fluorescence intensity obtained from the optical micrographs as a function of time. A background fluorescence spectrum obtained by running a ThT solution was subtracted from each sample fluorescence spectrum. Each experiment was run in triplicated.

The kinetics of insulin fibril formation on chiral inorganic surfaces could be described as sigmoidal curves defined by a certain lag phase where little change in ThT fluorescence intensity was observed, an exponential increase phase in ThT fluorescence denoting the growth of fibrils, and an equilibrium phase with a constant ThT fluorescence intensity indicating the end of fibril formation. To fit the sigmoidal nature of the curves, the following empirical equation was used,

$$F = \left(F_i + m_i t\right) + \left(F_f + m_f t\right) / \left(1 + \exp - \left(t - t_m\right) / \tau\right) \qquad \text{Equation (S1)}$$

where $F$ is the fluorescence intensity, $t$ is time, and $t_m$ is the time to 50% of maximal $F$. Here, the initial baseline during the lag time is described by $F_i + m_i t$. The final baseline after the growth phase has ended is described by $F_f + m_f t$. The lag time is given by $t_m - 2\tau$.

**8. ATR-RTIR measurements.**

ATR-FTIR spectra were performed with a Nicolet IS 50 FTIR Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The chiral surfaces were immerged in insulin solutions and incubated for 24 h at 60 °C. Then the insulin-adsorbed chiral surfaces were dried by nitrogen. Samples were analyzed at ambient conditions in the mid-IR region (400–4000 $cm^{-1}$). Nicolet IS 50 FT-IR Spectrometer was operating in the ATR mode and measuring resolution was 4 $cm^{-1}$ with 32 scans. Deconvolution and curve fitting of the amide I regions (raw spectra) was performed using the program OriginPro 8.5 (OriginLab Corporation, USA). Second derivative and Fourier self-

deconvolved spectra were used as a peak position guide for the curve fitting procedure. The area ratios of the corresponding fitted curves to the spectra of amide I region were used for the quantitative analysis of the content of α-helix and β-sheet structures.

**9. Atomic force microscopy imaging.**

AFM measurements were performed using an Asylum Research MFP-3D AFM (Oxford Instruments Company). Fresh cleaved mica was immersed into 0.01 M NaOH solution for five minutes then rinsed by distilled water and dried under nitrogen gas to created negative charged surfaces. To overcome the problem that the chiral surfaces are too rough to use in AFM measurement, the insulin-adsorbed R- or L-surfaces (incubation for 24 h at 60 °C) were resuspended in 8 M urea, then a 2 μL aliquot dripped on mica after the system incubating for two minutes the surface was washed gently by distilled water then dried with weak nitrogen gas. Then AFM images were acquired in a tapping mode under ambient conditions.

**10. Adsorption experiments of insulin monomers and oligomers.**

The insulin oligomers were prepared through dissolving insulin in HCl solution (25 mM, pH = 1.6) at a concentration of 0.1 mg $mL^{-1}$ and cultured at 60 °C for 5 h. All QCM measurements were performed at 25 ºC using a Stanford Research Systems (SRS) QCM200 apparatus. The QCM200 System is a stand-alone instrument with a built-in frequency counter and resistance meter. It includes controller, crystal oscillator electronics, crystal holder, and quartz crystals. In this study, a 5 MHz AT-cut quartz crystal with gold electrodes from SRS was used for each measurement in a flow injection mode. Prior to binding assays between $TiO_2$ nanofibers modified sensor and insulin, QCM channels and tubes were washed with distilled water carefully and in the beginning of each experiment, distilled water was passed through the chamber until a stable baseline ($\Delta F < 0.05$/min) was achieved for ~20 min. Insulin (0.1 mg $mL^{-1}$ in 25 mM HCl) monomers or oligomers were injected into the channel at a flow rate of 50 $\mu L \cdot min^{-1}$.

**11. Microscale Thermophoresis (MST).**

Insulin was labelled with NT-647 using the RED-NHS (Amine Reactive) Protein Labelling Kit (Nanotemper Technologies). A serial dilution of R- or L-$TiO_2$ in 25 mM HCl were set up, starting with a concentration of 2.5 ng $mL^{-1}$ to 10240 ng $mL^{-1}$. Separately, a stock solution containing 10 nM labeled insulin was mixed with titration solutions. The samples were loaded into hydrophobic capillaries and heated at 40% laser power for 30 sec, followed by 5 sec cooling. All experiments were performed with a minimum of 3 replicates.

**12. Cell culture and differentiation.**

Culturing method has been previously described.[5] Briefly, Rat pheochromocytoma PC12 cell lines were cultured in high-glucose DMEM supplemented with 10% (v/v) fetal bovine serum (FBS), 1% penicillin-streptomycin solution at 37 °C in a 5% $CO_2$ incubator. The cells were placed in Polystyrene Petri dishes with a normal density maintained at between $1\times10^5$ and $1\times10^6$ cells $mL^{-1}$ of medium. The medium was changed 24 h. After sterilization, the glass substrates (~13-mm-diameter) with L- or R- $TiO_2$ modified were transferred into 24 well plates. For proliferation and differentiation of PC12 cells, cells were seeded 8000 cells $cm^{-2}$ and 4000 cells $cm^{-2}$ at chiral surfaces, respectively. After the incubation period the adhered cells on glass substrates were washed twice with PBS and fixed with 4% PFA solution. The staining of the cells was done with Rhodamine B. Cell adhesion density (cells/$cm^2$) on each substrate was calculated from the average number of adherent cells counted in five randomly selected, $1\times1$ $mm^2$ fields per substrate. The cell adhesion experiments were run in duplicate at three separate times. These data were analyzed for statistical significance using Duncan's multiple range test with a protection level of $\alpha = 0.01$. Differentiated (neurite-bearing) cells were defined as cells with at least one neurite. The differentiation rate was determined by dividing the number of neurite-bearing cells by the total number of cells.

# Supporting Figures.

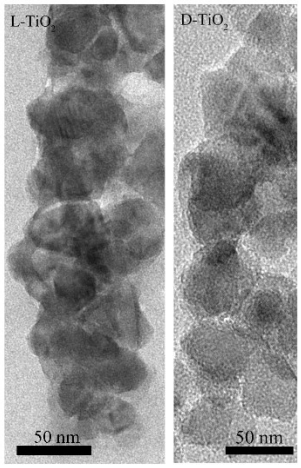



**Fig. S1 TEM images of L- and R-$TiO_2$ chiral nanofibers.**

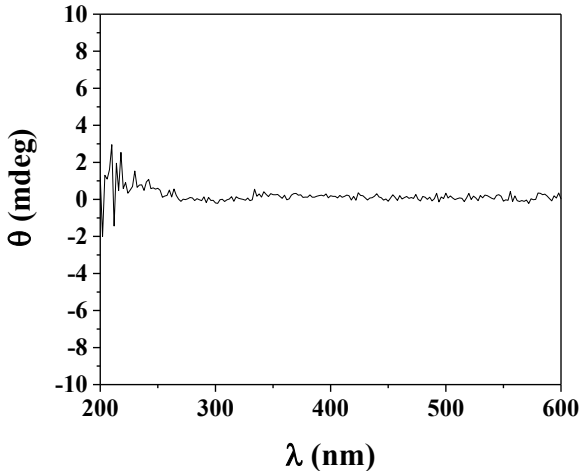



**Fig. S2. The DRCD spectrum of prepared achiral TiO2 nanofibers.**

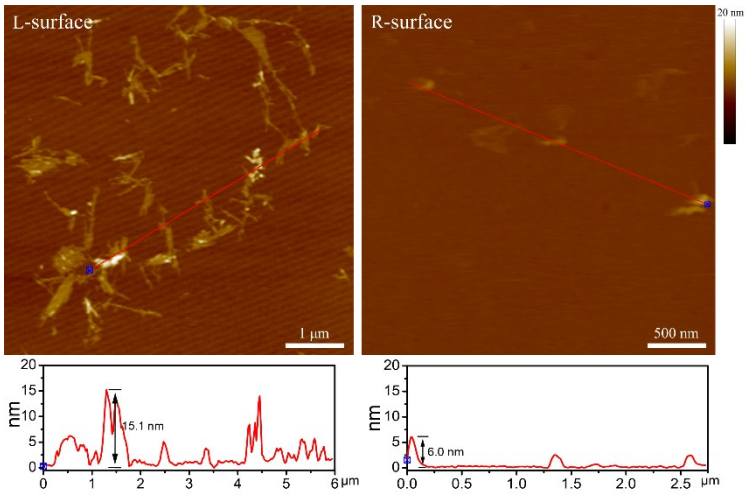



**Fig. S3 AFM images of insulin incubated with L- or R-surfaces at 60 °C for 24 h.**

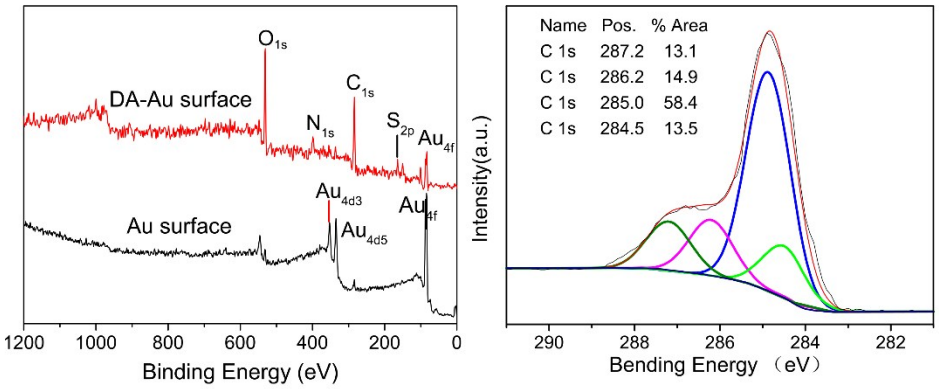



**Fig. S4 XPS of DOPA modified Au surface of QCM sensor.**

As shown in C 1s core levels, the binding energy at 287.2 eV confirms the presence of C=S on the modified films. We assign the 286.2 eV peak to C-N groups which indicates the bond formation between $CS_2$ and dopamine.

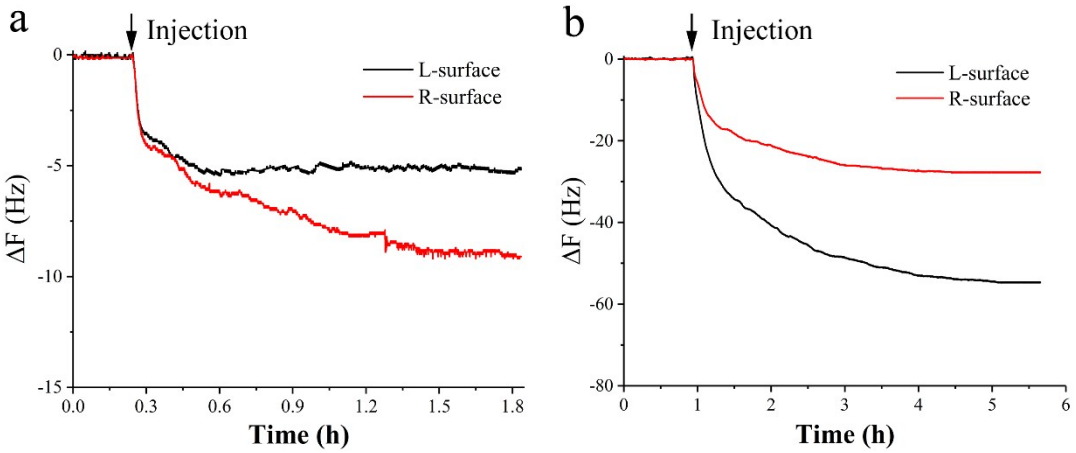



**Fig. S5** Time-dependent frequency change of insulin monomers (a) and oligomers (b) adsorbed by L- and R- $TiO_2$ chiral nanofibers modified QCM sensors. The adsorption quantities (Δm) were calculated according to the equation: $\Delta m = -\Delta F/C_f$, ng·cm$^{-2}$, where $C_f$ is the sensitivity factor for the crystal (0.0566Hz ng$^{-1}$ cm$^2$ for a 5 MHz AT-cut quartz crystal at room temperature). The concentrations of monomers and oligomers were 0.1 mg mL$^{-1}$.

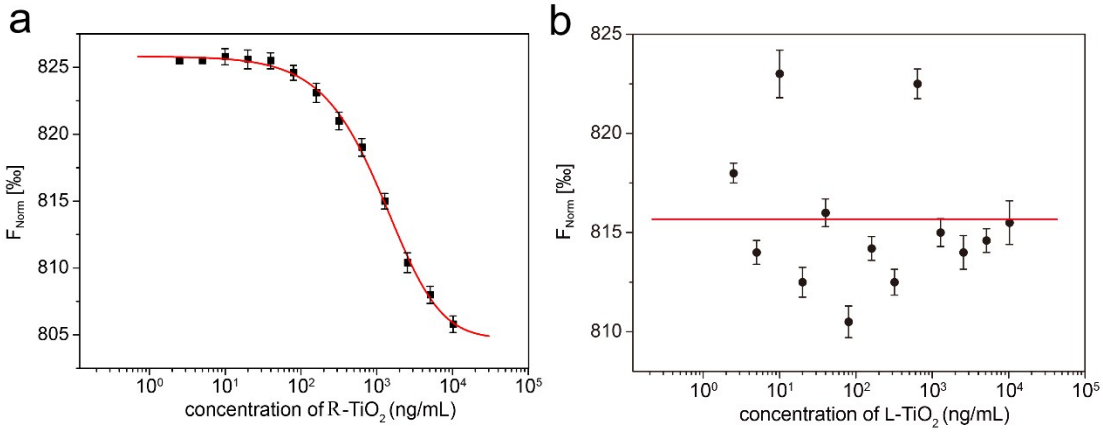


**Fig. S6 MST determinates the binding of insulin monomers to R-$TiO_2$ (a) and L-$TiO_2$ (b) in 25 mM HCl solution.**

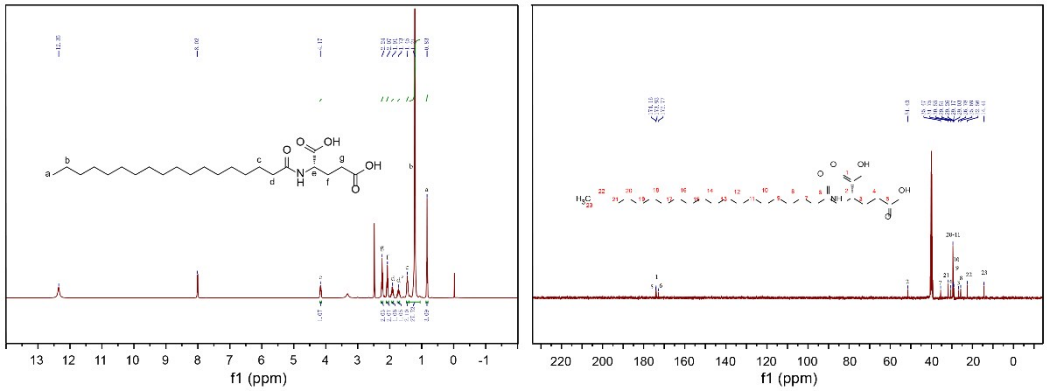



**Fig. S7 $^{1}$H NMR and $^{13}$C NMR of *N*-stearoyl-L/D-glutamic acid.**

$^{1}$H NMR (400 MHz, $CD_3SOCD_3$) $\delta$: 0.83 (t, 3H, $CH_3$), 1.21 (m, 28H, -$CH_2$-), 1.45 (t, 2H, -$CH_2$-), 1.74, 1.91 (m, 2H, -$CH_2$-, attached to the chiral center), 2.08 (m, 2H, -$CH_2$-) and 2.24 (m, 2H, -$CH_2$-, linked to carboxyl group), 4.17 (m, 1H, -CH-, chiral center), 8.03 (d, 1H, NH), 12.33 (s, 2H, carboxyl group). $^{13}$C NMR (400 MHz, $CD_3SOCD_3$) $\delta$: 14.41, 22.56, 25.69, 26.78, 29.03, 29.17, 29.26, 29.51, 30.55, 31.75, 35.47, 51.43, 172.77, 173.93 and 174.16.

# References.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