Electronic Supplementary Information

Opposing enantiomers of tartaric acid anchored at a surface generate different insulin assemblies and hence contrasting cellular responses

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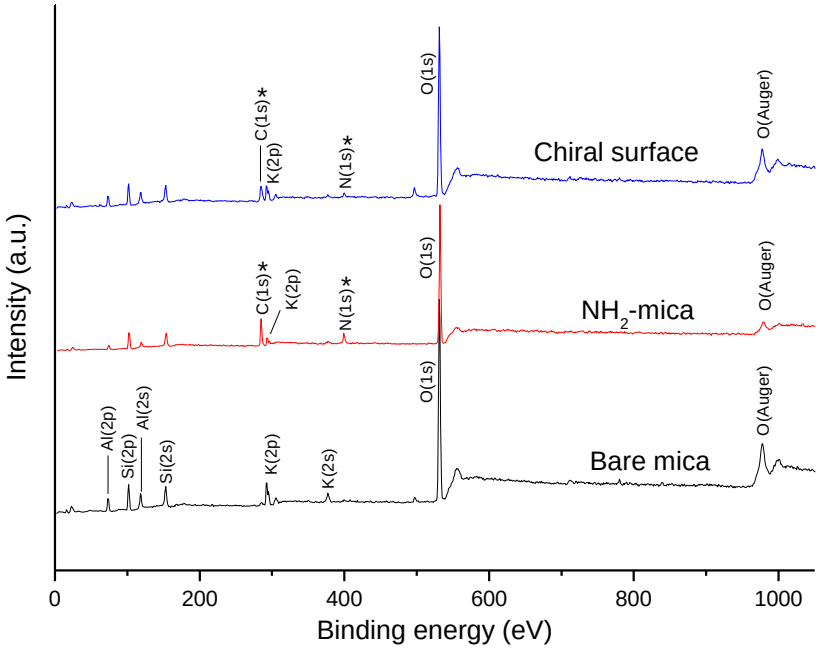


Fig. S1 XPS surveys of D-surface (blue), NH_2-mica (red) and bare mica (black).

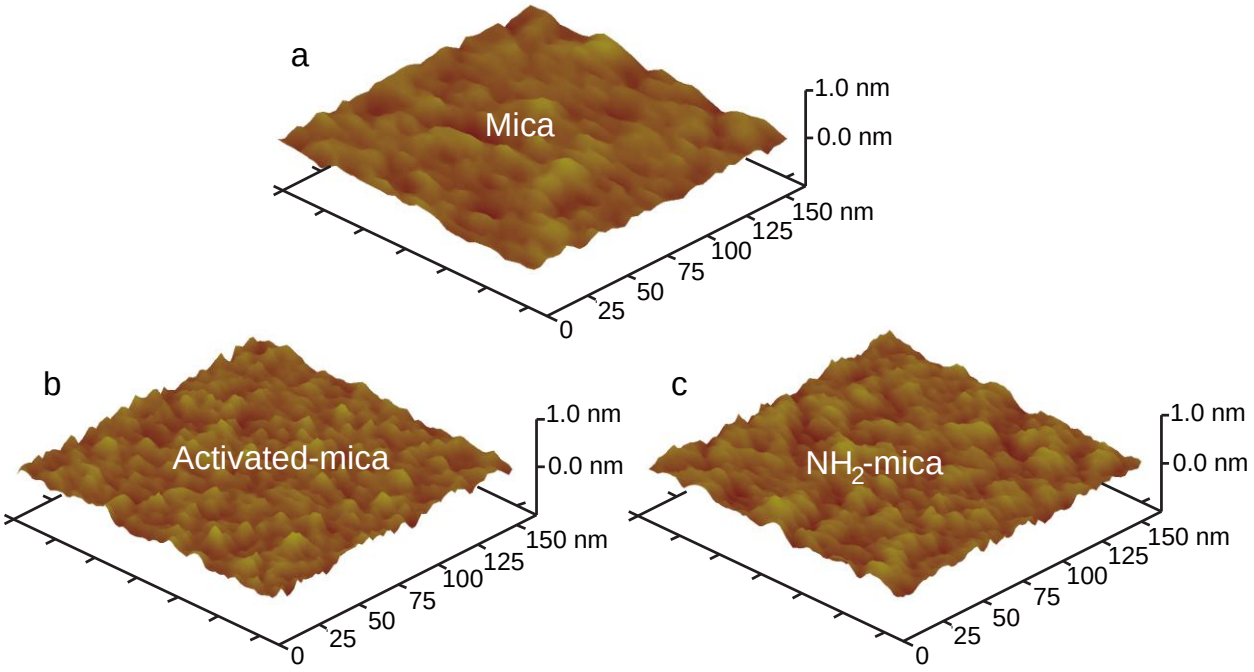


Fig. S2 AFM images of (a) cleaved mica, (b) activated mica, and (c) monolayer APTES-modified mica.

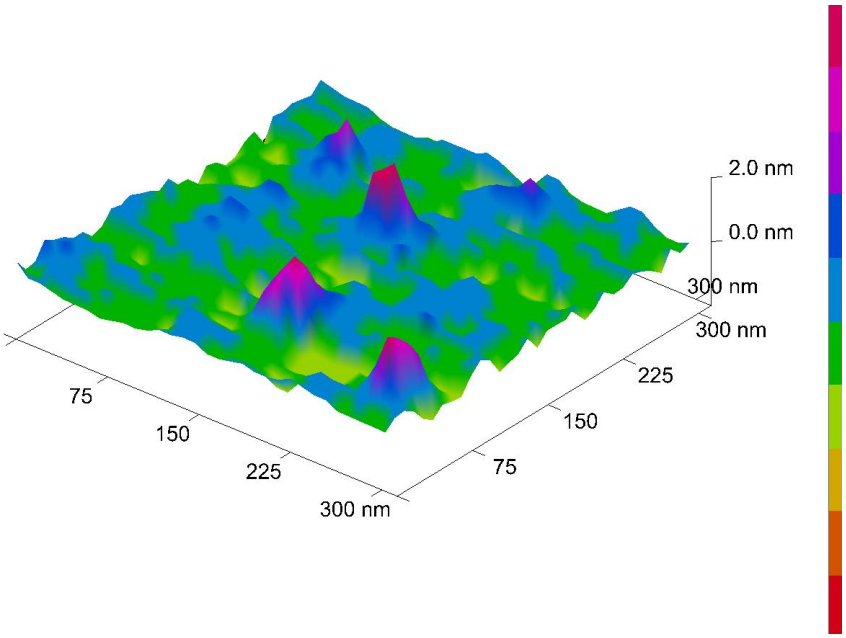


Fig. S3 Magnified AFM image of the tiny dots with height of 1-2 nm.

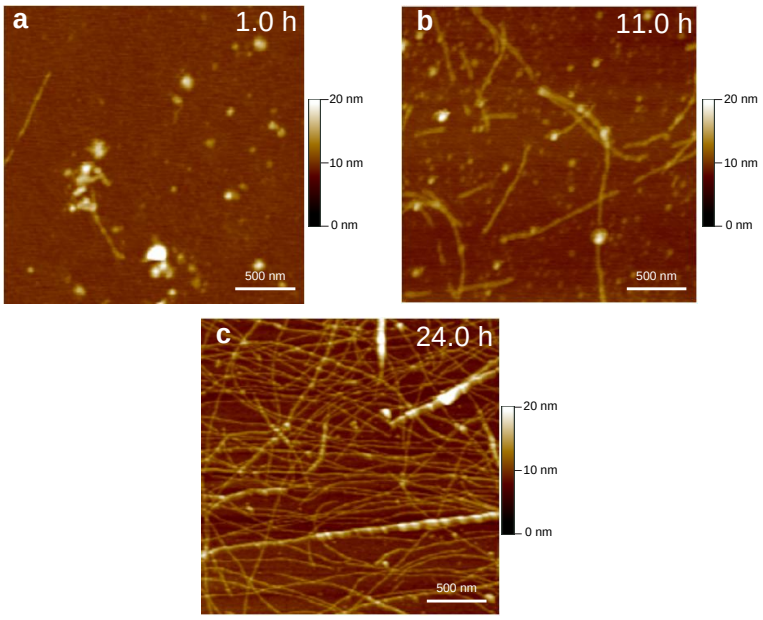


Fig. S4 AFM images of L-surface incubated with insulin solution for 1.0, 11.0 and 24.0 h, which represented the lag phase, rapid growth phase, and the final equilibrium state.

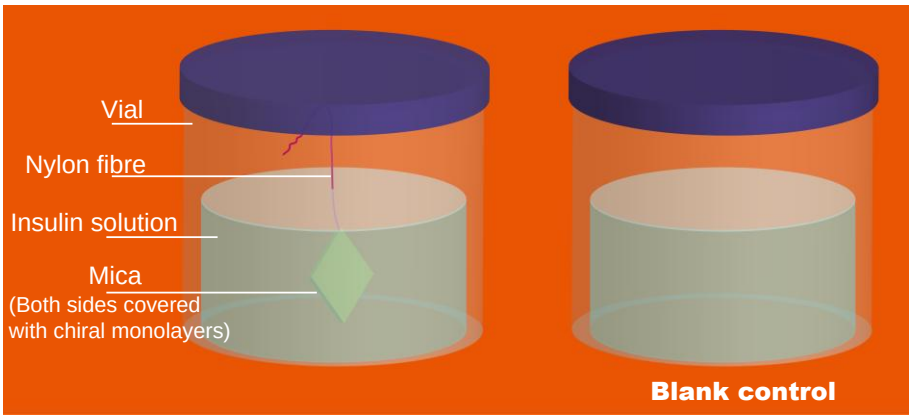


Fig. S5 Schematic showing the quantitative determination method of the chiral surfaces-adsorbed insulin. Although only one mica wafer was shown in the figure, 10 mica wafers were simultaneously suspended for better accuracy in real determinations. Five parallel samples were carried out for each determination.

Typically, a double-side modified mica wafer was suspended in insulin solution with bulk concentration of C_0 (left panel). After equilibrated for 24 h, the bulk concentration of insulin was determined by UV-vis (C_1). The adsorbed insulin should include two parts: those adsorbed at chiral surfaces (m_{chiral}) and inner sides of the vial (m_{blank}). The m_{blank} could be determined by a blank control experiment (right panel). The bulk concentration was determined as C_2 without the presence of chiral monolayer modified mica. Thus,

$$m_{blank} = (C_0 - C_2)V , \quad (1)$$

where V was the volume of insulin solution, and,

$$m_{chiral} = (C_0 - C_1)V - m_{blank} = (C_2 - C_1)V . \quad (2)$$

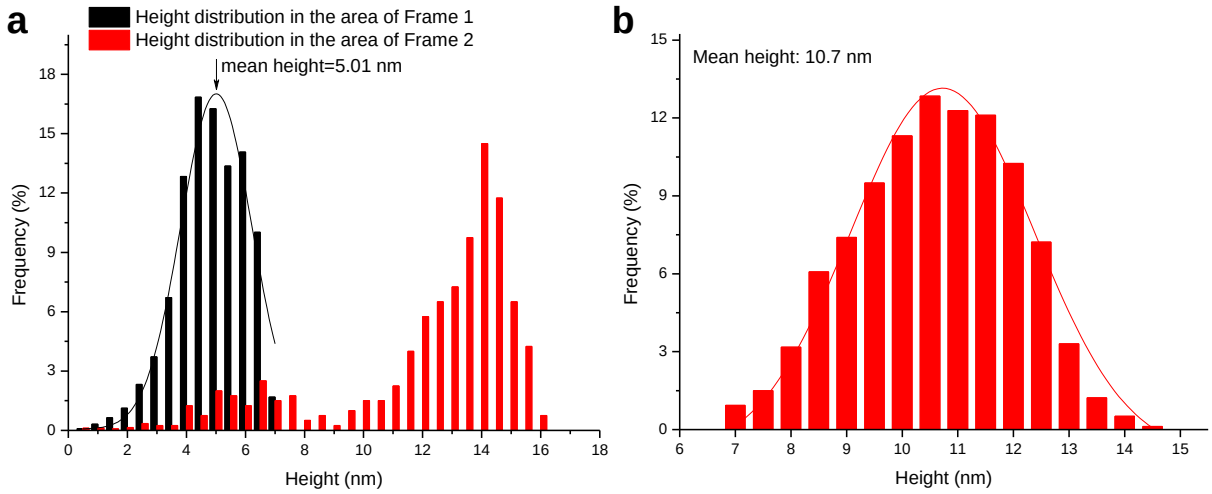


Fig. S6. Statistical height histograms of insulin adsorbed at chiral surfaces with bulk insulin concentration of 10.0 μg mL^{-1}. **a** Height distribution derived from Frame 1 (black) and Frame 2 (red) in Figure 4b. **b** Height distribution derived from Figure 4**c**.

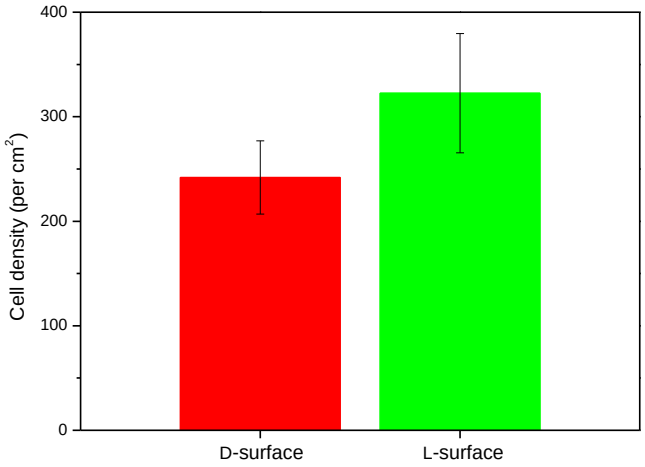


Fig. S7 The adhesive density of PC12 at D- and L-surface was 242±35 and 322±57 cells cm^{-2}, respectively.

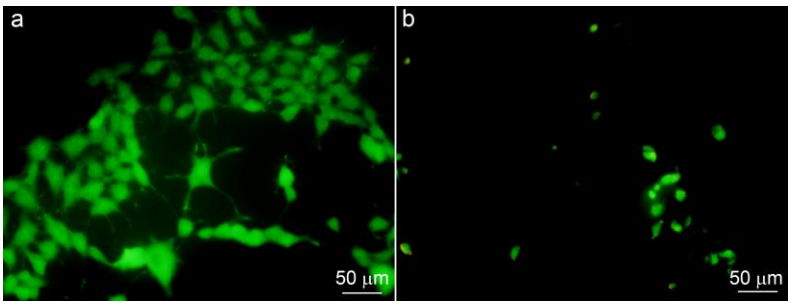


Fig. S8 Cellular behaviors of PC12 in serum free medium after cultured for 72 h on insulin-adsorbed (a) D-surface and (b) L-surface.

Experimental Section

1. General Experimental Considerations

All solvents and chemicals purchased from commercial sources (Sigma-Aldrich, Acros, Fisher Scientific or Alfa Aesar) were used without further purification.

X-ray photoelectron spectroscopy (XPS) data were obtained with an ESCALab220i-XL electron spectrometer from VG Scientific using 300W AlKα radiation. The base pressure was about 3×10^{-9} mbar. The binding energies were referenced to the C 1s line at 284.8 eV from adventitious carbon. Curve fitting of the C 1s and N 1s spectra was performed using a Gaussian-Lorentzian peak shape.

The samples dried with gentle nitrogen gas were used for AFM measurements with a Nanoscope V multimode atomic force microscope (Veeco Instruments, USA) under ambient conditions. All images were obtained by tapping mode AFM. Statistical height distribution of the adsorbed-insulin was obtained with AFM software Nanoscope 7.30.

ATR-FTIR spectra were recorded on a BRUKER Vertex 70 FTIR spectrophotometer from 4000 to 400 cm^{-1} using a resolution of 2 cm^{-1} and an accumulation of 256 scans. The insulin-adsorbed chiral surfaces were dried by lyophilization. The system was continuously purged with dry nitrogen. Background and water vapor subtractions were performed until a straight baseline was obtained between 2000 and 1750 cm^{-1}. Deconvolution and curve fitting of the amide I regions (raw spectra) was performed using GRAMS 32. 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-helical and β-sheet structures.

2. Insulin adsorption experiments.

Insulin (bovine pancreas, purchased from Sigma-Aldrich) was dissolved into 25 mM HCl (pH 1.5) to a concentration of 1 mg mL^{-1}. This insulin stock solution was kept frozen at -20 ℃ in a sealed vial. The insulin stock solution was diluted to experimental concentrations from 0.1 to 10 μg mL^{-1}. Then, the D- or L-surfaces were vertically immersed into corresponding solutions to allowing adsorption. After incubation at 60 ℃, the surfaces were taken out and successively washed by 1 mM HCl, double-distilled water gently, and dried also gently by flowing nitrogen gas for AFM measurement and ThT fluorescence imaging. As for ATR-FTIR measurement, the samples were gently wetted with D_2O and further lyophilized. Except for kinetic investigations, the chiral surfaces were incubated in insulin solutions for 24 h.

3. Cell culture and differentiation.

Rat pheochromocytoma PC12 cell lines were cultured in RPMI-1640 medium with 10% horse serum (HS), 5% fetal bovine serum (FBS), 1% penicillin-streptomycin solution, 300 mg L^{-1} L-glutamine, 25 mM sodium bicarbonate, and 25 mM 4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid (HEPES). Cells were incubated in a 5% CO_2 incubator at 37 ℃ and 100% humidity. The cells were placed in a 75T flask and maintained at between 1×10^5 and 1×10^6 cells mL^{-1} of medium. The medium was changed 24 h. For differentiation of PC12 cells, cells were seeded (5000 cells cm^{-2}) at chiral surfaces.

Cell adhesion density (cells/cm^2) on each substrate was calculated from the average number of adherent cells counted in five randomly selected, 1×1 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 α=0.01. Differentiated (neurite-bearing) cells were defined as cells with at least one neurite. The

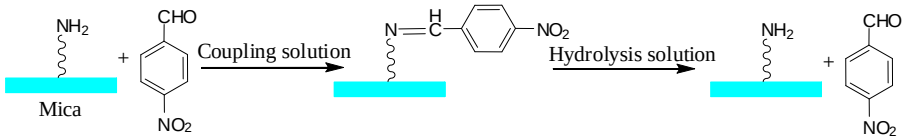
differentiation rate was determined by dividing the number of neurite-bearing cells by the total number of cells.

4. Preparation of chiral monolayers covered surfaces

The chiral surfaces were prepared stepwise as following by using molecularly smooth mica as substrate. Firstly, layered muscovite mica wafers were cut into 0.5×0.5 cm squares and several top layers were removed with careful cleaving to obtain smooth mica surface. The smooth mica surfaces were then activated with water vapor plasma to producing Si-OH groups. Subsequently, the activated mica surfaces were modified with monolayer amine groups as previously reported.[1] Specifically, the activated mica wafers were placed in a closed vessel in which the amino-containing silzanization reagent (10% v/v APTES in benzene) was kept at the bottom. Silanization reactions were performed with vaporized APTES in order to prevent deposition of silane polymers to mica surface. The vessel was warmed to facilitate vaporization of APTES and its reaction with mica surfaces. The silzanization was performed at 70 ℃ for 16 h. After the reaction, the mica wafers were modified with monolayer amino groups (NH_2-mica). Finally, the NH_2-mica were divided into two parts, and functionalized with D-TAR and L-TAR to produce D- and L-surfaces, respectively. To 50 mL water, 5 mmol (0.75 g) D- or L-TAR was added and the pH value were adjust to 6 with 4 M NaOH. Then, 4.5 mmol ethyl(dimethylaminopropyl) carbodiimide (EDC) and 4.5 mmol N-hydroxysuccinimide (NHS) were added dropwise to the TAR solution under stirring. After reacting for 5 h, mono-NHS-activated TAR ester was formed. By immersing the NH_2-mica into the above solution and adjusting the solution pH to 8.0, mono-NHS-activated TAR esters were linked to amino groups at the mica surface through forming amide bonds. As the TAR esters were far excessed, all amino groups were linked with TAR. The D- and L-surfaces were successively washed with 0.1 mM NaOH, and water, and

dried under nitrogen for further use. The successful modification was confirmed by XPS analysis.

5. Colorimetric Assay of Amine Density



Scheme S1 Schematic illustration of the determination of surface amino density by using the reversible imine ormation.

The surface density of amine groups was measured by a previously reported imine ormation reaction (Scheme S1).[2] NH_2-mica wafers (100 pieces) were placed in a 20 mL flask and washed (×4) with 10 mL of coupling solution [0.8% (v/v) glacial acetic acid in dry methanol]. Subsequently, 1 mL of 4-nitrobenzaldehyde solution (7 mg in 10 mL of coupling solution) was added to the particles and the suspension was allowed to react for 3 h with gentle end-over-end rotation. After removal of the supernatant and washing (×4 in 10 mL of coupling solution), 10 mL of hydrolysis solution (75 mL of H_2O, 75 mL of MeOH, and 0.2 mL of glacial acetic acid) was added to the particles and the tube was shaken for a further hour. The absorbance of the supernatant was measured at 282 nm. The amount of 4-nitrobenzaldehyde in the hydrolysis solution was calculated by interpolation, by use of a calibration curve constructed from a range of standard solutions of 4-nitrobenzaldehyde prepared separately.

6. ThT fluorescence studies

ThT staining: The insulin-adsorbed chiral surfaces were submerged (facing upward) in 2.5 μM ThT solution and incubated in a water bath at 37 ℃ 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 BX-51 optical system microscope (Tokyo, Japan) equipped with a DP70 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 ($\lambda \approx 470$–490 nm, U-MNB2, Olympus) 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.

The kinetics of fibril formation during insulin adsorption at chiral surfaces could be described as sigmoidal curves defined by a certain lag time where little change in ThT fluorescence intensity was observed, a sigmoidal increase in ThT fluorescence denoting the growth of fibrils, and a plateau with a constant ThT fluorescence intensity indicating the end of fibril formation. ThT fluorescence measurements were plotted as a function of time and fitted to the sigmoidal curve described by the following equation using SigmaPlot,

$$F = (F_i + m_i t) + (F_f + m_f t) / (1 + \exp - (t - t_m) / \tau) \quad (3)$$

where F is the fluorescence intensity, and t_m is the time to 50% of maximal fluorescence is reached. The initial baseline during the lag time (t_{lag}) 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 apparent rate constant, k_{app} for the growth of fibrils is given by $1/\tau$, and t_{lag} is calculated as t_m-2τ.

7. Measurement of binding constants

Binding between insulin monomer and D- / L-tartaric acid mono-*N*-methylamides was measured by equilibrium dialysis as previously reported.[3] For the dialysis experiments, Spectra/Por Biotech CE dialysis membrane tubing with a molecular weight cut-off (MWCO) of 1000 Da was used. Glass containers were filled with the sample solution (D- or L-tartaric acid mono-*N*-methylamide solution) referred to as the outside solution. In this outside solution a

dialysis tube filled with insulin monomer solution referred to as the inside solution, was immersed. To minimize disturbance of tartaric acid mono-*N*-methylamide -insulin binding equilibria in the sample as a result of tartaric acid mono-*N*-methylamide migrating into the inside solution, the volumic ratio of outside/inside solution was set at 25:1. During the experiments, the closed containers were continuously shaken at a constant room temperature of 20 ℃ for 24 h. After that, the concentration of tartaric acid mono-*N*-methylamide in the outside solution was determined as free concentration, and the bound amount of tartaric acid mono-*N*-methylamide was subsequently calculated. Accordingly, the binding constant was determined.

Supporting references

1. Okusa, H., Kurihara, K. & Kunitake, T. *Langmuir* **10**, 3577-3581 (1994).

2. Ian J. B. & Tapas S. *Langmuir* **21**, 7029-7035 (2005).

3. Anzenbacher P. & Kalous V. *Biochimica et Biophysica Acta* **386**, 603-607 (1975).