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Research on Protein Thermal Condensation Detection Based on Phase Modulation SPR Imaging

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Abstract

A novel SPR imaging biomolecular interatction detection method based on time domain phase modulation is presented in this thesis. Experimental apparatus of SPR imaging biomolecular interaction detection based on TDPM is established. Biomolecular interaction is detected. 2×2 lysozyme array chip is prepared and lysozyme thermal condensation is detected by the experimental apparatus. Extracting phase information changes through the Stoilov algorithm. SPR curves of the interaction are obtained and kinetic parameters are calculated. It can sensitively acquire real-time phase change caused by biomolecular interaction based on interference imaging, and resolve related bioinformation, which is a potential tool for proteomics research.

Keywords: *surface plasmon resonance (SPR), time domain phase modulation (TDPM), biomolecular interaction, Lysozyme thermal condensation*

1. Introduction

Studies have shown that nearly 20 kinds of neurodegenerative diseases, such as alzheimer's disease, familial amyotrophic lateral sclerosis, huntington's disease, prion disease, all related to the abnormal coagulation of protein, deposit in the corresponding tissues, undermined its normal biological functions [1-4]. There are lots of people suffering from the disease every year, due to the particularity of diseases, it's difficult to cure, affected the happiness of itself and the family, also increased the burden of the society. Nowadays scientists from all over the world and relevant organizations are actively study the disease, protein condensation problem is particularly prominent.

Now mostly by studying the mechanism of protein folding or directly observed the state of protein aggregates to explain condensation phenomena of the protein, because of the real structure of protein folding is very complex, the researchers have proposed many simplified algorithm model, but these algorithms model was still proved to be NP-complete problem[5-6]. Due to the limitation of the time of protein coagulation, operation method and the instrument and so on, Direct observation method exists the problem of time-consuming, complicated operation and expensive experimental equipment. Then it can be seen by studying the mechanism of protein folding and the method of directly observed state of protein aggregates to interpret protein condensation phenomenon also has many disadvantages.

SPR (Surface Plasmon Resonance) sensing technology compared with traditional detection methods, has the advantage of real-time and fast detection, no use of tag samples, high sensitivity, able to testing in the turbid or opaque samples [7-9]. Above all, this paper is put forward the method based on SPR imaging detection. To get the change of reflected light phase caused by protein in the process of condensed through the interference imaging, analysis the relevant biological information and find the law of the condensation process of protein [10-11].

2. Materials and Methods

2.1. The Experimental Device

Experiment device is constituted by the light source, SPR interference imaging light path, SPR sensor unit, the microfluidic system, CCD image acquisition system and the computer. Light source emitted the laser go through the phase modulation and beam expander,

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reach to the prism and gold film interface of SPR sensor unit, after the reflected light intervention, the imaging lens imaging the sensor chip surface in CCD target surface. CCD image acquisition system put the image data into the computer, then processing and analysis. SPR imaging detection of biomolecular interaction experiment device is shown in Figure 1.

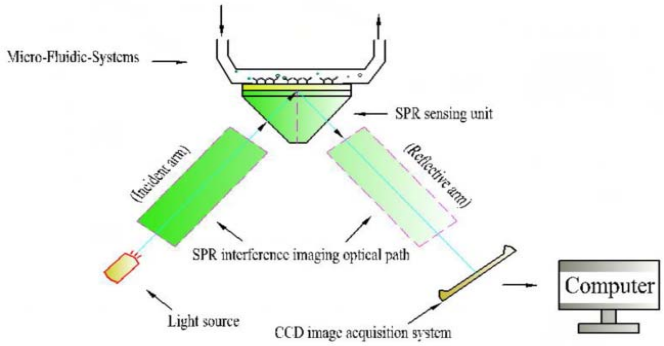

Figure 1. SPR Imaging Detection of Biomolecular Interaction Experiment Device

2.1.1. The Light Source

The light wavelength from 600 nm to 900 nm can inspire SPR. We choose the semiconductor laser as light source, because it's advantage of small size, low consumption and preheat fast. Beside, use the semiconductor refrigerator building thermostatic device to control the working temperature of semiconductor laser can significantly reduce the power noise and restrain the temperature drift caused by wavelength drift. Semiconductor laser output wavelength is 635 nm, coupled to the single mode fiber which the core diameter is 4μm, connected to the fiber optic collimator by FC optical fiber connectors. The fiber collimator output beam diameter is about 4mm, divergence Angle is less than 1mrad.

2.1.2. SPR Interference Imaging Light Path

Shown in Figure 2, laser device 1 emitted the light, polarizer 2 adjusted the light intensity between the p and s light, electro-optic crystal 3 modulated the phase, the modulated light through the beam expander lens group 4, reach to the surface between the prism and gold film of SPR sensor unit through the prism 5, the reflected light intervention through the anylyzer 6, the imaging lens 7 imaging the sensor chip surface in CCD 8 target surface. CCD image acquisition system put the image data into the computer9, then processing and analysis.

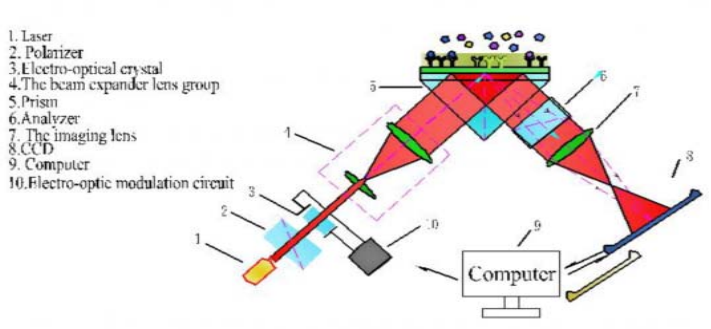

Figure 2. SPR Interference Imaging Light Path

2.1.3. SPR Sensor Unit

Function of SPR sensor unit is converted the biomolecular interaction response signal into the changes of phase or intensity of the light. Most important part in SPR sensor unit is SPR sensing chip. SPR chip is mainly composed of glass substrate, gold membrane and the probe

molecules linked on it, plating the thick of 2nm chromium film on the substrate as adhesive layer, then plating the thick of 50 nm gold film on it, thus constituted the bare gold film SPR chip. The bare gold film needs link the probe molecules when SPR chip used in biological detection. But the gold film surface is hydrophobic, needed chemical modification for bare gold film chip surface, in order to link the probe molecules, constituted the sensing chip. The probe in this paper is lysozyme molecules, taking the lysozyme solution (1mg/mL) make four sample points which diameter is 1mm in the chip surface, constituted a 2×2 array. The preparation process is shown in Figure 3.

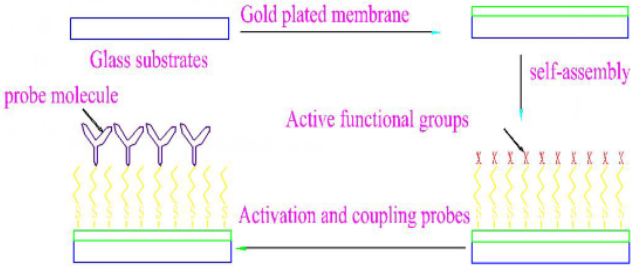

Figure 3. SPR Sensor Chips Preparation Process Schematic Diagram

The chip and prism nicely linked by refractive index oil (methyl iodide, refractive index is 1.740). Material of prism and chips is ZF5 glass (refractive index is 1.740), section of prism is isosceles trapezoid, the base angle is 60°. Microfluidic pool (volume is 30μL) installed on the chip surface, the appropriate pressure can be seal.

2.1.4. Microfluidic System

Function of microfluidic filling system is let the test sample solution, buffer and eluent respectively and continuous injected to the fluid pool. In the SPR sensor, the reflection phase is very sensitive to the change of the sensing surface dielectric refractive index, so don't allow air into the liquid pool in the process of experiment. Then using automatic injection pump input sample solution, implemented different solution of continuous injected to the fluid pool by switching the valve, to avoid air into the liquid pool.

2.2. Experimental Method

Protein thermal condensation is a common biological molecular interaction process, Before did not reach the protein denaturation temperature, protein thermal condensation, form micelles, after the temperature drop, can also be dissociated to the original state, this is a reversible process. Lysozyme is one king of common protein, easily getting from the biological products market. The probe in this paper is lysozyme molecules, taking the lysozyme solution make four sample points which diameter is 1mm in the chip surface, constituted a 2×2 array. By detecting the interaction between the probe lysozyme molecules and the lysozyme molecules in the fluid pool after heating, resolved the dynamics parameters of the interaction.

2.2.1. Receptor and Ligand Reaction Process

In order to make the problem simple, using the most simple biomolecular interaction model, the receptor and the ligand binding equation is:

$$A_1 + A_2 \underset{k_d}{\overset{k_a}{\rightleftharpoons}} A_1A_2 \quad (1)$$

A_1 is the receptor, A_2 is the ligand, A_1A_2 is the polymer formation of A_1 and A_2. k_a is the association rate constant, unit is $(mol/L)^{-1}s^{-1}$; k_d is the dissociation rate constant, unit is s^{-1}. In the SPR sensor, reactions on solid phase surface, $[A_1]$ is the molarity of A_1, $[A_2]$ and $[A_1A_2]$ is the number of molecules, shown as Figure 4.

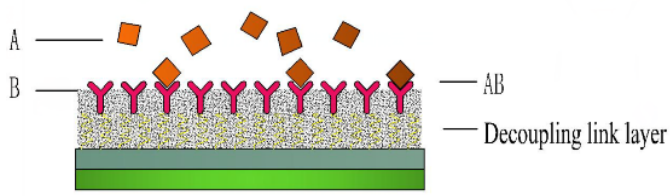

Figure 4. Solid Phase Surface Molecules Reflect Schematic Diagram

In the process of association reaction, continuously filling it with a certain temperature of A_1, $[A_1]$ is constant. At the time of t, association reaction rate is $k_a[A_1][A_2]$, dissociation reaction rate is $k_d[A_1A_2]$, the difference value is A_1 A_2 generation rate.

$$d[A_1A_2]_t / dt = k_a[A_1][A_2]_t - k_d[A_1A_2]_t \quad (2)$$

As A_1A_2 generated, the corresponding reduction in the number of A_2.

$$d[A_1A_2]_t / dt = k_a[A_1]([A_2]_0 - [A_1A_2]_t) - k_d[A_1A_2]_t \quad (3)$$

At the time of t, the number of A_1A_2 is:

$$[A_1A_2]_t = [A_2]_0 \frac{k_a[A_1]}{k_a[A_1]+k_d}[1-e^{-(k_a[A_1]+k_d)t}] \quad (4)$$

At the time of $t=\infty$, association and dissociation reached equilibrium.

$$e^{-(k_a[A_1]+k_d)t} = 0 \quad (5)$$

Now, the number of A_1A_2 is:

$$[A_1A_2]_\infty = [A_2]_0 \frac{k_a[A_1]}{k_a[A_1]+k_d} \quad (6)$$

Stop injecting A_1 at the time of $t=t_1$, to inject the buffer excluding A_1. At this time, $[A_1]=0$, reaction towards to dissociation, the rate of reaction is:

$$d[A_1A_2]'_t / dt = -k_d[A_1A_2]'_t \quad (7)$$

Then at the time of t, the number of A_1A_2 as follows:

$$[A_1A_2]'_t = [A_1A_2]_{t_1} e^{-k_d(t-t_1)} \quad (8)$$

Dissociation reaction reach to a certain extent, change into the eluent, A_1 was quickly separated from the surface of the solid phase, $[A_1A_2] = 0$.

2.2.2. The Calculation Method of Dynamic Parameters and the Equilibrium Constant

The dynamic parameters [12] ascertain reaction speed of the biological molecules association and dissociation, including the association rate constant k_a and the dissociation rate constant k_d. Equilibrium constant ascertains the relative number of molecular complexes

when biomolecules reaction reaches to balance, including the association equilibrium constant K_a and the dissociation equilibrium constant K_d. The relationship between the 4 constants is:

$$K_a = k_a / k_d$$
$$K_d = k_d / k_a \quad (9)$$

SPR sensing method can be linear and real-time detection the surface density of biological molecules reaction products, so the curve of the signal R changing with time is obtained. According to the formula 3 and formula 7:

$$dR_t / dt = k_a[\mathrm{A}_1]\mathrm{R}_{\max} - (\mathrm{k}_a[\mathrm{A}_1] + \mathrm{k}_d)\mathrm{R}_t \quad (10)$$

$$dR_t^{'} / dt = -k_d R_t^{'} \quad (11)$$

Drawing with a temperature association signal curve about dR_t / dt and R_t, getting $\mathrm{k}_a[\mathrm{A}_1] + \mathrm{k}_d$ after fitting. Drawing with dissociation signal curve about $dR_t^{'} / dt$ and $R_t^{'}$, getting k_d after fitting. Therefore, according to the complete testing curve can be calculated the association rate constant k_a, the dissociation rate constant k_d, the association equilibrium constant K_a and the dissociation equilibrium constant K_d.

2.2.3. Time Phase Modulation SPR Imaging Detection Methods

The frequency of visible light is much higher than the response frequency of the photoelectric detection device, the change of the optical phase must be converted to light intensity changes which can be measured. Using interferometry can convert the change of phase to the change of interferogram light intensity, calculating phase according to the measured light intensity. Some commonly phase detection algorithm can be used for SPR including Stoilov algorithm、three steps fixed length algorithm、four steps fixed length algorithm、four steps Carre algorithm and Hariharan algorithm.

The comparison of various performance of the algorithm is shown in Table 1, the more "√" said the stronger the ability to suppress the noise or error. Visible from the table, the more phase shift steps, stronger the ability to suppress random noise, Stoilov is the algorithm of strongest ability.

Phase error of various algorithms shown in Table 2, light intensity noise is the main source of error. In this 5 kinds of algorithms, Stoilov algorithm has the strongest ability to restrain the light intensity noise, can eliminate the linear error of phase shifter, the error of real-time calculating phase signal is smaller. Therefore, choose Stoilov algorithm to calculating phase.

Table 1. The Comparison of Various Performance of the Algorithm

Error sources	Three steps	Four steps	Carre	Hariharan	Stoilov
Light intensity noise		√	√	√√	√√
Phase-shifting noise			√	√	√√
Linear phase-shifting error			√√	√	√√
Optical power fluctuations			√	√	√
SPR signal changes over time			√√	√	√

Table 2. Phase Error of Various Algorithms(Unit °)

Error sources	Three steps	Four steps	Carre	Hariharan	Stoilov
Light intensity noise	0.035	0.03	0.03	0.025	0.025
Linear phase-shifting error	5	0.1	0	0	0
Optical power fluctuations	0.2	0.3	<0.005	<0.005	<0.005
SPR signal changes over time	0.02	0.01	<0.01	<0.01	<0.01

3. The Results and Discussion

Injected lysozyme solution with different temperatures, the distribution of refractive index change after the reaction was carried out 5 minutes is shown in Figure 5. Four elliptical area represented the four lysozyme probe area. Background represented the BSA molecular area. Oval probe area is caused by imaging system imaging rate is inconformity in two mutually perpendicular directions. It's the inherent error, will not affect the test and calculation results.Also visible from the figure, specificity polymerization is exist between the lysozyme molecules in the solution and probe lysozyme molecule, the higher the temperature of the solution, the stronger the signal, the deeper the color.

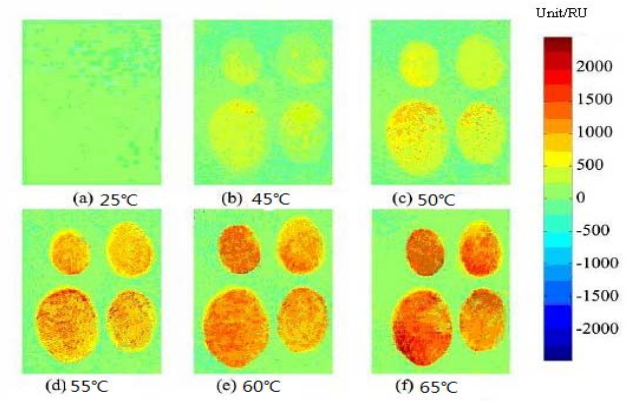

Figure 5. Distribution of Refractive Index Change after the Reaction was Carried out 5 minutes

We can know from the formula 4, signal R_t increases as the increasing of the temperature of the sample under test, but after the temperature increases to a certain value, the signal will be saturated. 5 minutes after injecting the lysozyme solution, the relationship curve between the response signal and lysozyme solution temperature is shown in Figure 6. Visible from the figure, along with the injection of lysozyme solution temperature increases, the signal is stronger and stronger, with the increase of the temperature, curve slope gradually decreases, this consistent with the results of theoretical analysis.

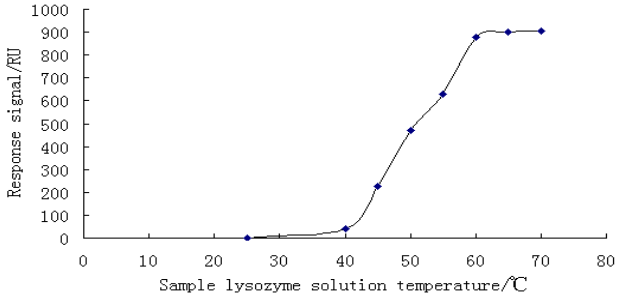

Figure 6. Relationship Curve between the Response Signal and Lysozyme Solution Temperature

There are many influence factors of biomolecular interaction experiment, need by repeated experiments, obtain the average, to accurately calculate the reaction kinetics constants. Get four real-time detection curves of different temperature lysozyme solution by four times of experiments, shown in Figure 7. Visible from the figure, when t=2min, start filling it with lysozyme, lasting for 5 minutes, at the time of t=7min stop injecting lysozyme solution, then continue to inject PBS buffer.

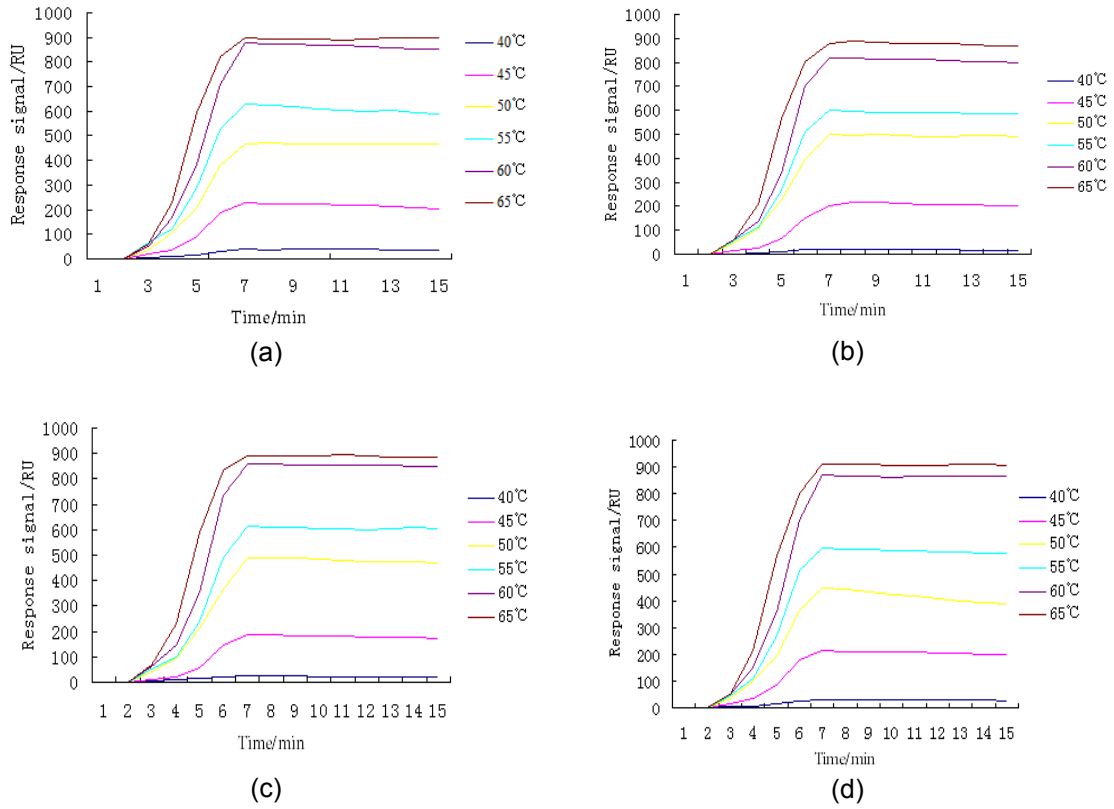

Figure 7. Real-time Detection Curves of Different Temperature Lysozyme Solution Obtain by Four Times of Experiments

Curve fitting used the methods described above, averaged the results of four times, obtained the association rate constant is 1.13×10^{3} $(mol/L)^{-1}s^{-1}$, the dissociation rate constant is 0.84×10^{-3} s^{-1}. According to the formula 9, obtained the association equilibrium constant is 1.35×10^{6} $(mol/L)^{-1}$ and the dissociation equilibrium constant is 8.4×10^{-7} mol/L.

Compared with methods of traditional biomolecular interaction analysis, it has two advantages. First, without tags, to avoid the influence on the activity of biological molecules, eliminating the complicated and time-consuming markup steps; Second, real-time detection, can be real-time and dynamically record the biomolecular interaction process, getting more information compared with the ELISA and other end signal detection methods.

4. Conclusion

Through analyzing of the model of receptor and ligand reaction, proved that the method of SPR sensing phase measurement can determine the biomolecular interactions. Put forward the detection method based on phase modulation SPR imaging, analyzed the precision of phase calculating algorithm, the results show that Stoilov algorithm has the highest accuracy. Build the experiment device of SPR imaging detection of biomolecular interaction, analyzing some error sources of the apparatus, and put forward the way to improve detection precision. Biomolecular interaction experiment was carried out in this experimental device, getting the

lysozyme thermal coagulation kinetics parameters. It is proved that this method is high sensitive, labeless, real-time and capable ofarray detection, which can meet the requirements of the research of lysozyme thermal coagulation.

Acknowledgements

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