A series of reviews on the "application of modern instruments and technologies in drug research"

Research progress of surface plasmon resonance technology in drug discovery

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Abstract: Surface plasmon resonance (SPR) technology is a powerful and sensitive tool for investigating molecular interactions. Technical improvements in recent years have brought new functionality to SPR apparatuses that can be widely applied at multiple stages of the drug discovery process. The technology allows users to conduct ligand fishing and offers rich and in-depth information regarding the affinity, specificity, kinetics, concentration and identification of covalent/allosteric/competitive binding behaviors. The present review highlights the principle, sample types, detection ranges, experimental methods, strengths, limitations, and the latest research progress on the application of SPR technology in the drug discovery process.

Keywords: Surface plasmon resonance; SPR; Drug discovery; Molecular interaction

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Contents

1. Introduction

Molecular interactions exist in all living organisms. Studying these interactions can help researchers better understand the mechanisms of disease occurrence and development, as well as discover therapeutic targets and effective drugs. A variety of biophysical techniques are currently employed to characterize molecular interactions[1,2]. Among them, surface plasmon resonance (SPR) technology is one of the more powerful and informative tools for investigating the presence of biomolecular interactions

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and for detecting the specificity, binding affinity and kinetic parameters of an interaction[3]. It has become an important evaluation tool in drug discovery and was included in *the United States Pharmacopeia (2016)*, *the Japanese pharmacopoeia (2016)*, and *the Chinese Pharmacopoeia (2020)*[4–6].

The world's first commercially available SPR instrument was created by Biacore® in 1990 for the purpose of studying protein-protein interactions[7]. Since 2005, SPR technology has improved significantly to include optimized sensitivity, automatic injection and easy-to-use software[8]. Biacore T200 is a classic and widely used SPR device model that allows for the detection of small molecules and fragment-based drugs. In 2016, an upgraded configuration of the T200, called Biacore S200, and a higher throughput configuration named Biacore 8K, were launched onto the market. Both of the new apparatuses have higher sensitivities, lower sample consumptions, faster detection speeds, innovative A-B-A injection modes and no detection limitations on sample molecular weight. Moreover, Biacore 8K possesses a wider affinity detection range, making it more accurate in distinguishing subtle differences among ultra-high affinity samples, such as antibody drugs. These technological improvements have accelerated research progress in drug discovery.

The main purpose of this review is to help researchers comprehend the principle, sample types, detection ranges, experimental methods, strengths, limitations, and the latest research applications of SPR technology in the drug discovery process.

2. The SPR technology

2.1. The principle of SPR technology

SPR technology uses a spectroscopic method that monitors the mass change at the gold surface of a sensor chip. When analyte molecules bind to the ligands that are immobilized on the sensor chip, the mass increase at the sensor surface will cause a change in refraction index which gives rise to an SPR angle shift. By monitoring the angle shift over time during the binding process, the machine can generate a real-time sensorgram (Fig. 1). Response units (RUs) are employed to describe the angle shifts where 1 RU is equivalent to an angle shift of 10^{-4} degrees. More detailed descriptions of the principle of SPR technology can be found in other reviews[9,10].

SPR experiments can be used to determine the association rate constant (k_{on}), the dissociation rate constant (k_{off}), and the equilibrium dissociation constant (K_d). The smaller the K_d value, the stronger the binding ability. So far, the Biacore 8K device has been shown to have the widest K_d detection limits ranging from 10^{-3} to 10^{-15} M. Typically, k_{on} can be characterized from 10^3 to 10^9 $M^{-1}s^{-1}$, while k_{off} can be detected from 10^{-6} to 0.5 s^{-1}.

2.2. Sample types and affinity ranges

SPR technology is well validated on studying a diverse range of biomolecules, consisting of proteins, nucleic acids, polysaccharides, peptides, lipids, small molecules, ions, viruses, cells, bacteria and so on[11]. Measured interactions in common cases range from low to high-affinity values such as for fragments (K_d: $10^{-6} – 10^{-3}$ M), compounds (K_d: $10^{-7} – 10^{-4}$ M), antibodies (K_d: $10^{-12} – 10^{-7}$ M), and aptamers (K_d: $10^{-11} – 10^{-7}$ M)[8,12].

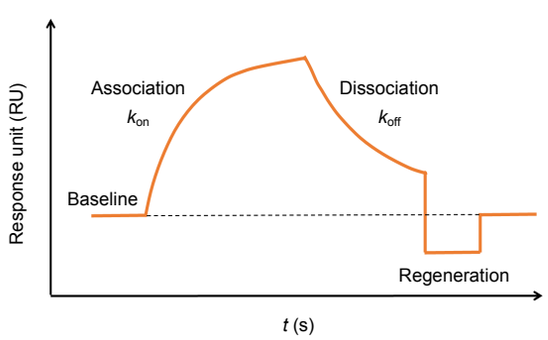

Figure 1. Representative SPR sensorgram.

2.3. Experimental methods of SPR

A typical SPR assay includes five steps: (1) sample and buffer preparation; (2) ligand immobilization on chip surface; (3) analyte measurement; (4) surface regeneration, and (5) data analysis. The experimental methods and requirements are summarized in Figure 2.

2.4. Strengths and limitations

Compared with other traditional analytical assays for molecular interactions such as enzyme linked immunosorbent assay (ELISA) and co-immunoprecipitation (Co-IP), SPR technology has many unique advantages including label-free detection, accurate affinity quantification, real-time kinetic measurements, and ultra-high sensitivity[1]. It also possesses higher sensitivity, a wider detection range, smaller sample requirements, and better data quality than other biophysical techniques such as biolayer interferometry (BLI) and isothermal titration calorimetry (ITC)[2].

However, there are several limitations of SPR technology as well. First, ligand immobilization on the chip surface might disrupt ligand activity or form an unsuitable orientation for analyte binding[2]. Second, nonspecific or undesirable bindings may form[13]. These two problems may be resolved by modifying ligand immobilization methods, optimizing the components of the running buffer, improving the sample quality, or using unbinding ligands as a deducted reference.

3. Recent research applications of SPR technology in drug discovery

SPR technology can serve as a powerful and multifunctional platform that meets the research requirements for multiple stages of the drug discovery process (Fig. 3). The progress of research on SPR technology in recent years is discussed in detail below.

3.1. Drug target structure and discovery

Drug target discovery and identification are important in studying drug mechanisms. The Biacore T200 device offers an injection and recovery program that allows for ligand fishing. The main procedure involves: (1) immobilizing a kind of molecule on a chip; (2) flowing the mixture (e.g. cell lysate) across the chip surface to allow for binding of the target components; (3) disassociating the target components from the immobilized molecules on the chip surface; (4) identifying the target components by mass spectroscopy (MS) or nuclear magnetic resonance (NMR). This process was used by Yang's and Li's groups for the discovery of a receptor for lamprey immune protein (LIP), a protein that can selectively kill tumor cells[14]. They immobilized LIP as bait on the chip of a Biacore T200 device, and flowed an N-glycosidase-treated cell lysate across the chip surface. The LIP-bound compounds were then collected. By virtue of MS analysis, they found that N-linked glycans on tumor cells were LIP targets.

Understanding the structural basis of a specific drug target is an essential prerequisite for the drug design and screening process. In 2020, a highly pathogenic coronavirus (severe acute respiratory syndrome coronavirus-2, SARS-CoV-2) caused a serious global public health emergency[15,16]. The cell receptor, angiotensin-converting enzyme 2 (ACE2), was reported as the target in mediating entry of SARS-CoV-2. This was the same cell receptor as that for SARS-CoV in 2003[17]. Zhang and Wang et al. presented the crystal structure of the receptor-binding domain (RBD) of SARS-CoV-2 for ACE2 and used SPR technology to demonstrate that the binding between SARS-CoV-2 and ACE2 is stronger than the binding between SARS-CoV and ACE2, explaining the higher infection rates of SARS-CoV-2[18]. These studies provided vital structural information for the development of therapeutic drugs to block the interaction between the RBD of SARS-CoV-2 and ACE2.

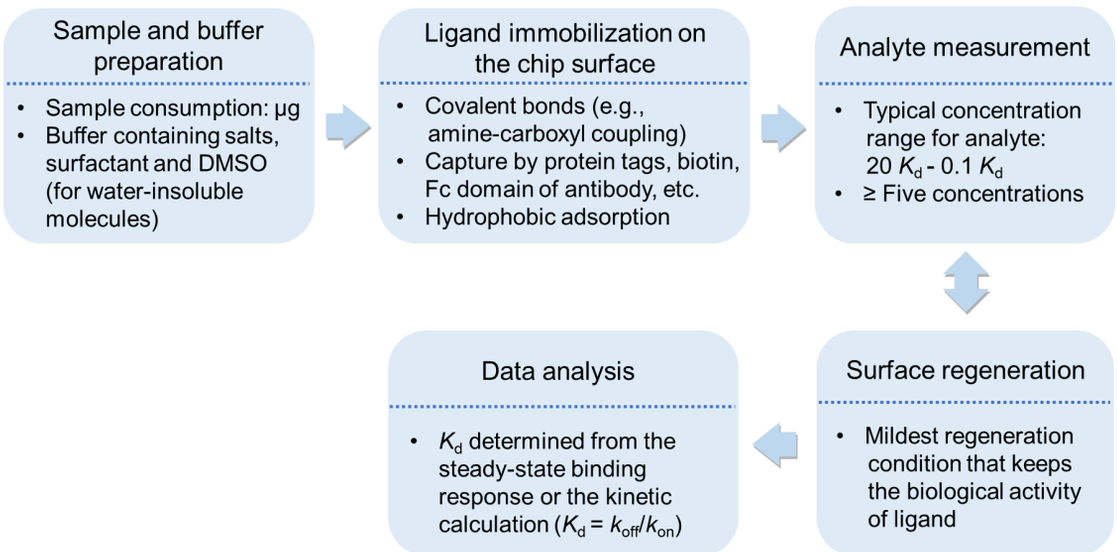

Figure 2. Typical SPR experimental methods and requirements. DMSO, dimethyl sulfoxide; Fc, fragment crystallizable.

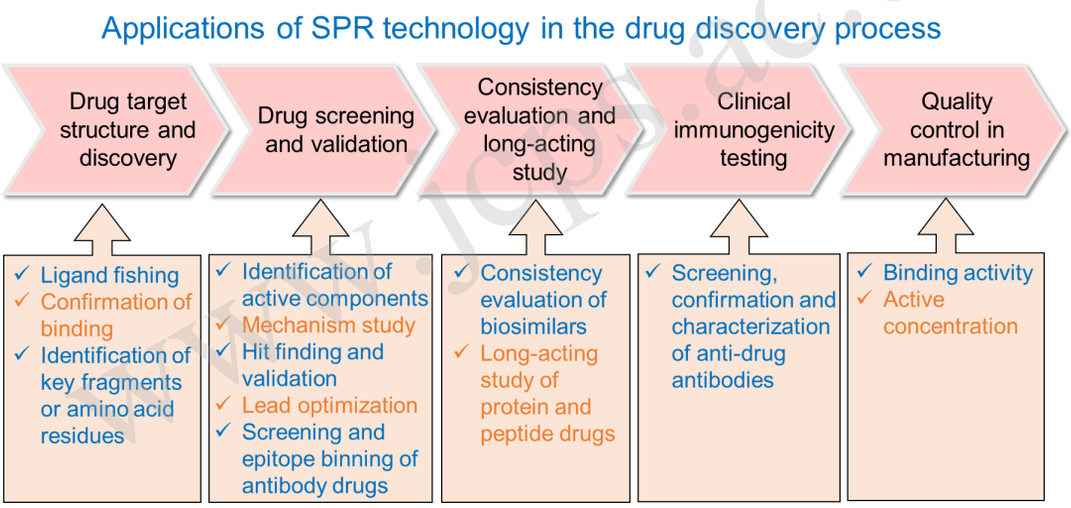

Figure 3. A multifunctional and powerful platform based on SPR technology in the drug discovery process.

Synaptic adhesion molecule 5 (SALM5) is an important molecule involved in autism spectrum disorders (ASDs) that can cause presynaptic differentiation by binding to leukocyte antigen-related receptor protein tyrosine phosphatases (LAR-RPTPs)[19]. Liu's group first reported the crystal structures of SALM5 and the SALM5/PTPδ complex and found potential amino acid residues of SALM5 that might be critical for the formation of the complex[20]. They used SPR technology to confirm the key amino acid sites of SALM5 by examining the interactions between PTPδ and wild-type SALM5 compared to variant SALM5. They also utilized SPR assays to investigate the interaction between SALM5 and PTPδ with splice code variants and discovered that MeB splice inserts in PTPδ have a significant influence on SALM5 binding. These results laid the foundation for further drug target research for autism using the SALM5/PTPδ complex.

3.2. Drug screening and validation

Chinese herbs have been applied as a unique form of traditional Chinese medicine for over two thousand years. A key research topic on Chinese herbs is the identification of active components that display significant pharmacological activities[21]. The injection and recovery program of a Biacore T200 apparatus provides a sensitive, efficient and convenient approach for screening active ingredients in Chinese herbs. For example, Chai and Zhang et al. immobilized tumor necrosis factor receptor type 1 (TNF-R1) on an SPR chip and observed remarkable binding activity when Rheum officinale extract was flowed across the chip surface[22]. The TNF-R1-bound compounds were recovered and analyzed using ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF/MS). Consequently, physcion-8-O-β-D-monoglucoside (PMG) was identified as the bioactive compound. The authors also used SPR technology to validate the binding affinity of PMG to TNF-R1 with a K_d value of 376 nM.

Mechanistic studies are another important research topic for the modernization of Chinese herbal medicine. Zhou's group utilized SPR technology to reveal the antiviral activity of pentacyclic triterpene-cyclodextrin conjugates. They showed that the conjugates could disrupt the entry of influenza virus by tightly binding with hemagglutinin proteins on the virus surface, thus blocking the attachment of the virus to the host cell[23].

SPR technology has emerged as a powerful tool for 'hit finding', 'hit validation' and 'lead optimization'[24]. 'Hit finding' means screening for compounds which can bind and modulate the activity of a target. 'Hit validation' refers to the process of verifying the compound-target interaction and characterizing the interaction by its affinity and other properties. 'Lead optimization' involves comparing hit compounds with their substituents to optimize target modulatory efficacy. A typical workflow for drug screening and validation by SPR assay is illustrated in Figure 4. First, the process of clean screening uses a blank chip to screen for any compounds that have strong non-specific adsorptions with the chip itself and then remove those compounds. Then a ligand immobilized chip is employed to conduct binding level screening and affinity determination. For instance, Gorgulla's group recently conducted a binding level screening at one specific concentration to discover 125 hits from 590 compounds with an RU>4. They then used binding level screening at five different concentrations to investigate the dose-response behavior of the 125 hits[25]. They identified 69 hits with a concentration-dependent SPR response and an RU>4 at a concentration of 20 μM. Then they determined the binding affinity of 23 of the hits and found 12 compounds with submicromolar K_d values.

Lead optimization studies usually require in-depth binding characterizations, especially the measurement of kinetic parameters (k_{on} and k_{off})[26]. Analytes with identical affinities for a target may display considerably different k_{on} and k_{off}. The k_{on} value represents target recognition, while the k_{off} value reflects binding stability. Notably, k_{off} measures the residence time of an analyte on the target, which may influence the therapeutic performance *in vivo*. A representative example was a k_{on}/k_{off} map of the binding activity between 259 compounds of five chemical series (A–E)[24]. The k_{on}/k_{off} map plotted log(k_{on}) against log(k_{off}) and underlined the significance of the kinetic data. The series E compounds were found to have only moderate-to-low affinities but very slow dissociation rates (low k_{off} values) compared to that of other series compounds. The series E compounds were thus identified as a therapeutically advantageous group that would probably be overlooked by traditional end-point assays. Danielson et al. studied the relationship

between structure and binding activity using SPR kinetic analysis[27]. They examined the interactions between human immunodeficiency virus (HIV) protease and 58 structurally diverse analogue inhibitors, revealing distinct and structurally dependent behaviors for binding recognition and complex stability.

SPR technology also enables the identification of covalent or allosteric binders, and is suitable for studying competitive inhibition as well[13]. Covalent bindings can be directly identified by a lack of dissociation behavior from the SPR sensorgram. One approach for characterizing allosteric or competitive binders is to saturate the SPR system with the competitive molecule by adding it into the running buffer and the test molecule solution (Fig. 5). The allosteric molecule is then distinguished by observing the binding response to the target while the orthosteric site is saturated by the competitive molecule. The competitive molecule can be recognized by a lack of a binding response to the target while the site is saturated. Another convenient method is offered by the new Biacore devices (Biacore S200 and 8K) with A-B-A injection modes (Fig. 6). This innovative functionality removes the need to saturate the system with competitive molecules and allows for the comparison of the test molecule binding response in the presence and absence of competitive molecules[13].

Due to its high throughout and low k_{off} detection limit (down to 10^{-6} s^{-1}), the Biacore 8K instrument is well-suited for antibody drug screening and affinity measurements[28]. It is worthy to note that adding epitope binning and mapping during early antibody drug discovery will enhance the quality of leads and the selection speed for potential clinical candidates. The experimental methods of epitope binning and mapping based on SPR technology are discussed at length in this review[29].

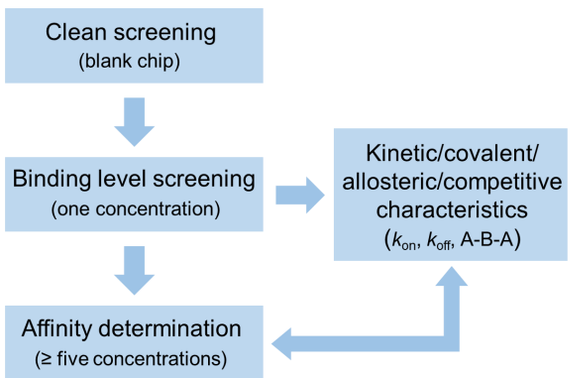

Figure 4. A typical drug screening and validation workflow by SPR assay.

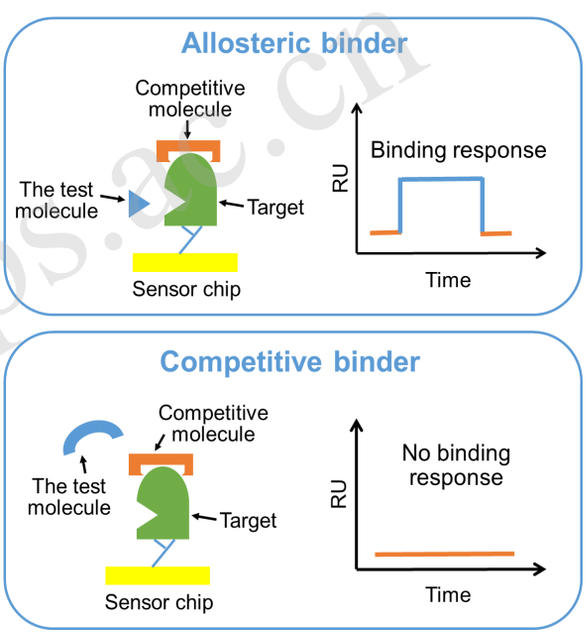

Figure 5. A scheme of allosteric and competitive binding studies using SPR analysis.

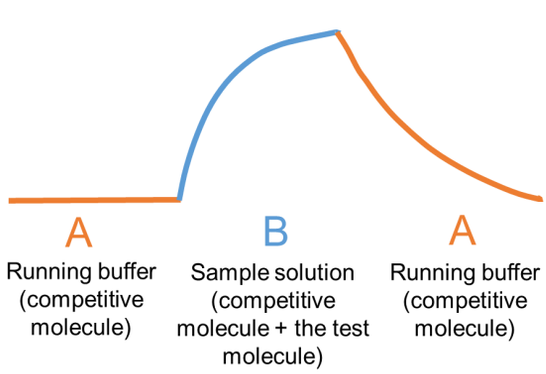

Figure 6. Innovative A-B-A injection mode based on the new Biacore devices.

3.3. Consistency evaluation and long-acting study

Recently, developing biosimilars with lower manufacturing costs has attracted tremendous attention in the biopharmaceutical industry[30]. A key indicator for assessing biosimilars is their consistency evaluation compared with innovator drugs. The Biacore instruments possess a unique function called 'sensorgram comparison' in their Data Evaluation Software that juxtaposes the sensorgrams of a biosimilar and an innovator for accurate fingerprint comparison and offers a similarity score. Chen and Gao et al. employed the SPR comparison function to investigate the binding activities of innovator Trastuzumab, biosimilar Trastuzumab with linker conjugation, innovator antibody-drug conjugate (ADC) and biosimilar ADC[31]. The results showed that the 4 samples had quite similar sensorgrams and K_d values, indicating a minimal impact of modification and demonstrating the similarity between the innovator drugs and the biosimilars.

Peptides are regarded as potent therapeutic drugs due to their high efficiency and target selectivity[32]. Nevertheless, the clinical applications of peptide drugs were severely restricted by poor *in vivo* lifespans[33]. Therefore, it is of great importance to develop a long-acting strategy to extend the half-life of peptides. Recently, Shang and co-workers constructed two new long-lasting glucagon-like peptide-1 (GLP-1) analogs which can associate with serum albumins to avoid kidney elimination and enzyme metabolism, thereby prolonging their half-lives in the body[34]. They used SPR assays to assess the binding affinities of peptides with serum albumins.

Polyethylene glycol (PEG) modification is also a classic method for extending the half-life of peptides[35]. However, the molecular weight of PEG is relatively high, which may affect the binding of peptides to the target after PEGylation. Therefore, it is essential to compare the changes in binding activity of the peptides before and after PEGylation. SPR technology can act as a powerful tool for detecting PEG impact on binding activity.

3.4. Clinical immunogenicity testing

Biotherapeutic drugs can cause an immune response in the body, which may affect its pharmacokinetic profile, decrease the drug efficacy, or cause an adverse reaction[36]. Consequently, the pharmacopoeias have listed immunogenicity as a mandatory test item in clinical trials[4]. Assessing immunogenicity by detecting anti-drug antibodies (ADAs) is important for ensuring the effectivity and safety of a drug. Using SPR technology as a real-time and sensitive assay has been recommended for clinical immunogenicity testing in pharmacopoeias. The Biacore devices have specialized modules for immunogenicity screening, confirmation and characterization. For example, Hock's group established an SPR immunoassay of D-amino acid peptide therapeutic etelcalcetide[36]. Ishii-Watabe et al. compared different immunoassay methods for detecting ADAs[37]. They found that the SPR assay was capable of detecting ADAs with low-affinities or fast dissociation rates that cannot be identified by traditional methods such as electrochemiluminescence (ECL) and ELISA.

3.5. Quality control in manufacturing

In the manufacturing process, it is necessary and significant to conduct quality control of drugs. SPR technology can help determine critical quality attributes including binding activity and active concentration of drugs for judging product quality and deciding whether or not to release a production batch. For instance, Gassner et al. established the linear dose-response curves of bispecific antibodies based on SPR assays for concentration analysis and quality assessment[38].

4. Summary and perspective

In conclusion, SPR technology serves as a sensitive and versatile platform from drug discovery to clinical testing and quality control. It allows researchers to conduct ligand fishing and offers rich and in-depth binding information including affinity, specificity, target recognition ability, complex stability, and identification of covalent/ allosteric/competitive binding behaviors, details that cannot be determined *via* traditional end-point assays.

It is worth noting that reasonable sample activity and sensible experimental designs have significant influences on SPR results. Except for mainstream microfluidic surface-based Biacore devices, some new technologies such as SPR microscopy (SPRm) and SPR imaging (SPRi) have emerged, further expanding the applications of SPR assays[39,40].

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表面等离子共振技术在药物研发领域的应用进展

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摘要: 表面等离子共振技术是用来研究分子相互作用有力而灵敏的工具。近年来随着技术的发展, 表面等离子共振仪出现一些新的功能和应用, 广泛应用于药物研发的多个阶段。表面等离子共振仪可以采集回收钓靶的成分, 提供丰富而深入的结合信息, 包括亲和力、特异性、动力学、浓度和识别共价/变构/竞争的结合行为。这篇综述重点介绍了表面等离子共振技术的原理、样品类型、检测范围、实验方法、优势、局限性、以及药物研发过程中最新的应用进展。

关键词: 表面等离子共振仪; 表面等离子共振技术; 药物研发; 分子相互作用