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Monitoring drug–serum protein interactions for early ADME prediction through Surface Plasmon Resonance technology

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ABSTRACT

Many molecules fail to reach the market due to poor pharmacokinetic (PK) properties, rendering the potential drug virtually unavailable for the primary target despite efficient administration to the body. PK properties of endogenous and exogenous compounds in mammals are dependent, among other factors, on their ability to interact with serum proteins. The extent of binding can greatly influence their ADME (adsorption, distribution, metabolism and execration) profile. Reliable and cost-effective bioavailability studies, early in the drug discovery process, can lead to an improvement of the success rate for compounds entering clinical trials. Optical biosensors based on surface plasmon resonance (SPR) detection emerged as an efficient approach to obtain large amounts of information about the binding of small molecules to serum proteins. Simple, automated and fast assays provide a good throughput, versatility and highly informative data output, rendering the methodology particularly suited for early screening. The ability to provide basic information on PK can be easily coupled to structure–activity relationship analysis. In this review, features of the technology and its employment for the study of serum protein–small molecule interactions are presented and discussed.

1. Importance of ADME profiling early in the drug discovery process

The journey that leads to the commercialization of a new therapeutic agent usually goes through three different sequential steps: the discovery, the development and the launch on the market. The discovery phase involves the identification and the validation of a new target, the screening for compounds able to modulate the target functionality and the hit-to-lead optimization, with the final aim to produce a new chemical entity (NCE). The development phase, on the other hand, is represented by sequential clinical trials, where the NCE is tested on a representative number of human patients for effectiveness and safety. The time estimated to go through the complete process is 12–15 years with an associated cost of approximately 1 billion $ [1,2]. Nonetheless, out of all compounds entering clinical phase I, roughly 11% reach commercialization [3,4].

Oncology drugs account for more than 30% of the total fraction under development, however they have the lowest success rate of all NCE, with a likelihood of approval of only 6.4%. Vaccines are the most likely to advance from phase I to the commercialization, with an associated success rate of 14.9%, while small molecules represent the drug class with the lowest success rate of all with 7.6%. Although chances of reaching the market varies depending on the drug class and the disease considered, the overall picture emerged from the studies showed a general inefficiency during drugs development, which reflects in a big waste in term of money and resources of the pharmaceutical industry.

Inspection of the reasons underlying the high-attrition rate reveals two major causes: low efficacy of the NCE in humans and a poor pharmacokinetic (PK)/bioavailability profile [5]. Surprisingly, the latter is still today the third most common cause of attrition in Phase I, even though its contribution lowered from 39% to 16% over the last two decades [6,7]. While low therapeutic effects in human can depend on genotype differences, something that is difficult to assess before testing the drug on a representative number of patients, drug-likeness should be carefully evaluated during the discovery phase in order to ease the path toward commercialization. The era of combinatorial chemistry led to the construction of huge libraries containing larger and more lipophilic

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molecules, two characteristics affecting their pharmaceutical properties while reducing their drug-likeness [8]. Therefore, rapid and reliable bioavailability studies early in the drug discovery process can significantly help to discover better leads that enter clinical trials, with a balanced potency and improved drug-likeness profiles [9,10].

In vitro bioavailability studies involve the assessment of different properties of a NCE, including: cell permeability, chemical stability, potential drug–drug interaction, inhibition of Cyp 450 enzymes and interactions with active transporters [11,12]. The PK properties of a compound entering the human body are also related to its ability to interact with serum proteins, which markedly impacts its ADME profile. The two main actors in this framework are the human serum albumin (HSA) and the α_1-acid glycoprotein (AGP), which together constitute more than 55% of the total protein content in human blood plasma [13,14]. The extent of binding to serum proteins modifies the free concentration of drugs, and as a consequence its physiological activity. Although the therapeutic effect may be compromised by undesired removal of a compound from the circulation, the drug–protein complexes formed can constitute a reservoir that extends the time the compound resides within the circulatory system, potentially increasing its effect. Hence, binding to HSA and AGP can constitute a valid tool to forecast drug-likeness in the initial screening steps of drug development [15]. Poorly drug-like compounds can be rejected, and chemical moieties can be modified to improve both pharmacodynamics and pharmacokinetics at an early stage.

Traditionally, the binding of molecules to serum proteins has been analyzed using methods such as analytical ultracentrifugation [16,17], equilibrium dialysis [18,19], ultrafiltration [20], electrophoresis [21], spectroscopic techniques [22,23] and affinity chromatography [24,25]. These techniques are based either on the separation of the compound from the serum protein, or on a change in intrinsic parameters of the protein upon formation of a complex with the compound, such as its mobility or spectroscopic properties. These assays are well established; nonetheless, the sometimes-ambiguous interpretation of data, the relatively high material consumption and the low throughput does not facilitate their employment for routine binding analysis.

The last two decades have seen a constant growth in the application of surface plasmon resonance (SPR)-based optical biosensors for molecular recognition analysis in many different fields [26–31]. SPR biosensors are analytical platforms where one of the two interacting partners is anchored to the surface of a sensor chip (technically the ligand), while the other is flowing in solution within a small flow cell (the analyte). They bear some peculiar features, which allows the accurate measurement of the amount of analyte–ligand complex, its equilibrium dissociation constant and the association and dissociation rate of the reaction. The detection principle, based solely on refractivity at the surface, does not require any pre-labeling of molecules, avoiding time- and money-consuming steps, which could also potentially affect the interaction.

At present, the main application is arguably in structure–activity relationship (SAR) studies, where the highly informative data output is used to gather precious information on new drug lead candidate [32–35]. For instance, kinetic parameters of the drug–target reactions provide a direct tool to evaluate the rate of dissociation of the complex and a simple mean to extrapolate the residence time (τ). This parameter has gained increasing interest among medicinal chemists, and it has been demonstrated that in a wide range of biological systems τ, measured *in vitro*, remarkably correlates with the *in vivo* potency of a compound, better than the overall drug–complex steady state affinity [36,37]. SPR-based platforms have also become an elective technique for fragment-based drug discovery, and different approaches and protocols were developed to help improve experimental design and data analysis [38–40].

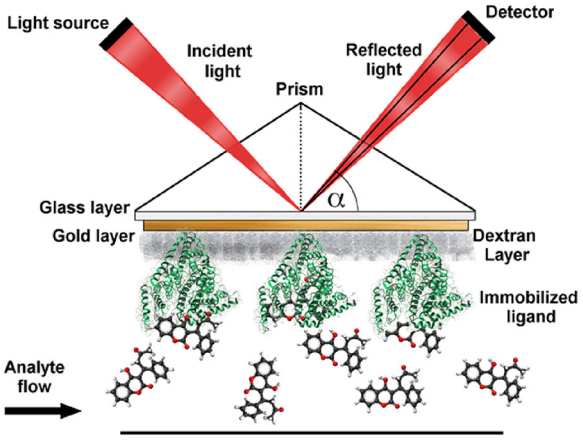

Fig. 1. Schematic representation of the SPR detection principle. The SPR angle α, when all other parameters are kept constant, is dependent only on the refractive index of the solution in proximity of the functionalized surface. Upon interaction between the analyte and the ligand, the refractivity at the surface changes, resulting in changes in α. The magnitude can be directly correlated to the amount of complex formed.

Nonetheless, optical biosensing is an intriguing approach to estimate also ADME profiles. Automated instruments minimize operator applications and increase the throughput, favoring a fast screening process, particularly useful when dealing with compound libraries. The assessment of the potency of a lead candidate can be supported with basic information on the drug-like profile. SPR technology offers the possibility to probe the binding to HSA and AGP simultaneously upon immobilization over two different spots on the same sensor chip. This provides an excellent *in vitro* mimic of the PK of a drug, whenever whole human serum is difficult to access. In this review a brief description of the technology is presented and its application for bioavailability studies is discussed.

2. SPR, description of the technique

2.1. Detection principle and instrumentation

The optical phenomenon of SPR occurs when a beam of plane-polarized light hits the surface of a thin, electron-rich, metal layer placed at the interface between two media of different refractive indices ($n_1 \neq n_2$) (Fig. 1) [41]. When the refractive index of the media n_2 is perturbed, for instance as consequence of mass accumulating at the immediate proximity of the metal layer, a signal can be detected. In a typical SPR experiment, a biological target (ligand) is tethered to the surface of a sensor chip, through chemical modification of the gold film facing the fluidic side, and the analyte is injected over the surface, free in solution. As a consequence of the ligand–analyte interaction, a response arises. This response is quantified and typically expressed as the change in refractivity (refractivity/response units, RU) over time [42].

Different optical configurations have been employed to investigate molecular binding events through SPR phenomena. The so-called Kretschmann configuration was used to develop a vast array of SPR platforms, both in the traditional and in the imaging (SPRi) format [43–46]. More recently, an alternative SPR configuration, termed localized SPR (LSPR) has emerged. With this mode, SPR is generated on the surface of a nanoparticle rater than a metal film, endowing completely new spectroscopic properties to the metal depending on nanoparticle composition, size, shape, orientation and local dielectric environment. Thanks to the miniaturization potential, LSPR-based devices are viewed as affordable, portable

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