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Review

Surface plasmon resonance spectroscopy for characterisation of membrane protein–ligand interactions and its potential for drug discovery ☆

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ABSTRACT

Surface plasmon resonance (SPR) spectroscopy is a rapidly developing technique for the study of ligand binding interactions with membrane proteins, which are the major molecular targets for validated drugs and for current and foreseeable drug discovery. SPR is label-free and capable of measuring real-time quantitative binding affinities and kinetics for membrane proteins interacting with ligand molecules using relatively small quantities of materials and has potential to be medium-throughput. The conventional SPR technique requires one binding component to be immobilised on a sensor chip whilst the other binding component in solution is flowed over the sensor surface; a binding interaction is detected using an optical method that measures small changes in refractive index at the sensor surface. This review first describes the basic SPR experiment and the challenges that have to be considered for performing SPR experiments that measure membrane protein–ligand binding interactions, most importantly having the membrane protein in a lipid or detergent environment that retains its native structure and activity. It then describes a wide-range of membrane protein systems for which ligand binding interactions have been characterised using SPR, including the major drug targets G protein-coupled receptors, and how challenges have been overcome for achieving this. Finally it describes some recent advances in SPR-based technology and future potential of the technique to screen ligand binding in the discovery of drugs. This article is part of a Special Issue entitled: Structural and biophysical characterisation of membrane protein–ligand binding.

Contents

Abbreviations: ABC, ATP binding cassette; ADP, Adenosine-5′-diphosphate; AMP, Adenosine-5′-monophosphate; AMPPNP, Adenosine-5′-(β,γ-imido)triphosphate; ATP, Adenosine-5′-triphosphate; BACE1, β-Site amyloid precursor protein cleaving enzyme 1; BPM, Biophysical Mapping; CHAPSO, 3-[(3-Cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate; CMC, Critical micelle concentration; DDM, *n*-Dodecyl-β-D-maltoside; EOT, Extraordinary optical transmission; EGF, Epidermal growth factor; GABA, γ-Aminobutyric acid type A (receptors); GDP, Guanosine-5′-diphosphate; GPCR, G protein-coupled receptor; GTP, Guanosine-5′-triphosphate; hOR17-4, Human olfactory receptor 17-4; HPA, Hydrophobic association (sensor chip); hPRR, Human (pro)renin receptor; HTA, ω-Hydroxy-undecanethiol; MSP, Membrane scaffold protein; N-Y4, Neuropeptide Y4; NPY, Neuropeptide Y; PDB, Protein Data Bank; POPC, 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; PP, Pancreatic polypeptide; PYY, Polypeptide YY; RU, Resonance or response units; SAM, Self-assembled monolayer; SDF-1α, Stromal cell-derived factor 1α; SLB, Supported lipid bilayer; SPR, Surface plasmon resonance; SPRM, Surface plasmon resonance microscopy; StaR, Stabilised receptor

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1. Introduction

Membrane proteins are coded by up to 30% of the open reading frames in known genomes [1–3], they have important roles in many biological processes (e.g. transport of ions and molecules, control of transmembrane potential, generation and transduction of energy, signal recognition and transduction, catalysis of chemical reactions) and mutations in membrane proteins have been linked with a number of human diseases [4–10]. The molecular targets for around 50–60% of current validated medicines are membrane proteins and they remain the principal target for new drug discovery [11–17]. Owing to the difficulties in applying the main biophysical techniques for high-resolution protein structure determination: X-ray crystallography and NMR spectroscopy, the number of structures of membrane proteins is still relatively few, contributing less than 1% of protein structures in the Protein Data Bank (PDB) [18], thus limiting the amount of information available for traditional structure-based drug design. At the time of writing, there are high-resolution structures determined for only seventeen unique G-protein-coupled receptors (GPCRs) [19], which represent the largest class of membrane protein drug target. Other membrane protein drug targets include cytokine receptors, tyrosine and histidine kinase receptors, antibody receptors, ligand- and voltage-gated ion channels and transport proteins. It is important to have a range of chemical, biochemical and biophysical techniques available for characterisation of ligand binding by membrane proteins and for screening libraries of compounds as potential drug candidates. A developing technique in this respect is surface plasmon resonance (SPR) spectroscopy, which is label-free and enables measurement of real-time quantification of ligand-binding affinities and kinetics using relatively small amounts of membrane protein in a native or native-like environment and has potential to be medium-throughput. Following a description of the SPR experiment, this review first considers the challenges associated with applying SPR-based methods to characterise ligand binding by membrane proteins and then demonstrates how some of these have been overcome with examples of its application to a range of specific membrane protein systems. In some cases, this involves combination with results from other experimental techniques and with molecular modelling. Finally it describes some recent developments in SPR-based technology and considers its future potential for drug discovery with membrane protein targets.

2. The surface plasmon resonance experiment

Surface plasmon resonance (SPR) uses an optical method to measure a change in refractive index of the medium in close vicinity of a metal surface that can be used to monitor the binding of analyte molecules to receptor molecules immobilised on the metal surface [20,21]. This exploits the phenomenon of surface plasmon generation in thin metal films and the total internal reflection of light at a surface-solution interface to produce an electromagnetic film or evanescent wave that extends a short distance (up to 300 nm) into the solution (see other reviews for a more detailed description of the theory behind surface plasmon generation [22–27] and references therein). SPR has predominantly been developed and performed using BIAcore™ technology [20,28–36] with the first commercial instrument in 1991; an illustration of the basic instrument set up is shown in Fig. 1A. The surface is typically a thin film of gold on a glass support that forms the floor of a small-volume (less than 100 nl) flow cell through which an aqueous solution is passed continuously. In order to detect the binding of an analyte molecule to a receptor molecule, the receptor molecule is usually immobilised on the sensor surface and the analyte molecule is injected in the aqueous solution through the flow cell. Polarised light from a laser source is directed through a prism to the under surface of the gold film where surface plasmons are generated at a critical angle of the incident light. This absorption of light is seen as a decrease in intensity of the reflected light. The critical angle is dependent on the refractive index of the medium within 300 nm of the gold surface and changes when molecules bind to the surface, e.g. when analyte molecules bind to immobilised receptor molecules (Fig. 1B). The real-time response of the SPR experiment is usually presented in the form of a sensorgram (Fig. 1C). If interaction between the immobilised receptor molecules and the analyte molecules occurs, the refractive index at the surface of the gold film changes and this is seen as an increase in signal intensity. Resonance or response units (RU) are used to describe the increase in the signal, where 1 RU is equal to a critical angle shift of 10^{-4} deg. At the start of the experiment all immobilised receptor molecules have not been exposed to analyte molecules and the RU value corresponds to the starting critical angle *a*. Analyte molecules are injected into the flow cell; if they bind to the immobilised receptor molecules, there is an association phase during which binding sites become occupied and the shape of this curve can be used to measure the rate of association (k_{on}). When steady-state is achieved the RU value corresponds to the changed final critical angle *b*. This maximum RU value relates to the concentrations of immobilised receptor and analyte molecules and so can be used to measure the binding affinity (K_D). When analyte molecules are removed from the continuous flow there is a dissociation phase during which binding sites become unoccupied and the shape of this curve can be used to measure the rate of dissociation (k_{off}). The surface can then be regenerated and returned to the critical angle *a* to start the experiment again. The lowest detectable concentration in the SPR experiment depends on a number of factors including the molecular weight, optical property and binding affinity of the analyte molecule