

RECENT ADVANCES IN SCINTILLATION PROXIMITY ASSAY (SPA)¹ TECHNOLOGY

R. A. JESSOP

Amersham International plc, Assay Development Group, Cardiff Laboratories
Forest Farm, Whitchurch, Cardiff, CF4 7YT, Wales, UK

ABSTRACT. Scintillation Proximity Assay (SPA) technology involves the use of scintillating microspheres coated with acceptor molecules to which ligands, radiolabeled with ³H or ¹²⁵I, can bind selectively. The low energy of the β particles emitted by ³H or the Auger electrons emitted by ¹²⁵I means that they can travel only a short distance in water. Thus, to create a scintillation event, a labeled ligand must be bound in close proximity to the microsphere. The energy from an unbound ligand is dissipated to the aqueous medium before it can interact with a microsphere. This obviates the need for a separation step in the assays, leading to considerable advantages for assay precision, "hands-on" time, safety and costs. SPA was initially applied to radioimmunoassay, but can now be used to develop receptor binding and enzyme inhibition assays. SPA technology also lends itself to automation, and is ideal for high throughput applications.

INTRODUCTION

Scintillation Proximity Assay (SPA) simplifies and speeds up radioimmunoassays, receptor binding and enzyme inhibition assays. It involves the use of scintillant beads made from either yttrium silicate or a plastic scintillator based on poly(vinyl toluene). The beads are chemically treated to impart the required surface properties and to enable coupling of molecules, such as antibodies, receptor proteins or enzyme substrates. Assays are carried out in normal aqueous buffers using radioisotopes that emit low-energy radiation easily dissipated in an aqueous environment. The beta particles emitted by ³H and the Auger electrons from ¹²⁵I have average energies of 6 and 35 keV, respectively; thus, they have very short path lengths (<1 μm and ~17 μm) in water (Nelms 1956), and are ideal for use in SPA.

If a molecule labeled with ³H or ¹²⁵I is bound to the bead surface, it is close enough to the bead for the emitted radiation to activate the scintillant and produce light. The amount of light produced, which is proportional to the amount of labeled molecules bound to the beads, can be conveniently measured with a scintillation counter. If the molecule is not attached to the bead surface, its energy is absorbed by the aqueous solvent before it reaches the bead, and no light is produced (Fig. 1).

Thus, bound ligands give a scintillation signal, but free ligands do not, and the need for a time-consuming separation step, characteristic of conventional assays, is eliminated. The technique also has other benefits. Liquid scintillation cocktail, with its associated problems of toxicity and waste disposal costs, is no longer necessary for ³H assays. The manipulations required in the assays are reduced to a few simple pipetting steps leading to better precision and reproducibility. In fact, the simplicity of the assay format, whereby all assays can be performed and counted in one tube or 96-well microtitration plate, allows almost complete automation of assays using robotic sample processors and microtitration plate scintillation counters. Consequently, SPA technology is capable of high throughput, which is particularly valuable in the case of drug- or sample-screening assays.

Receptor binding and enzyme inhibition assays are major techniques used by pharmaceutical companies to find new drugs, and often involve screening large libraries of chemicals or microbial

¹Scintillation Proximity Assay (SPA) technology is covered by U.S. Patent No. 4568649, European Patent No. 0154734 and Japanese Patent Application No. 84/52452, and is licensed exclusively to Amersham.

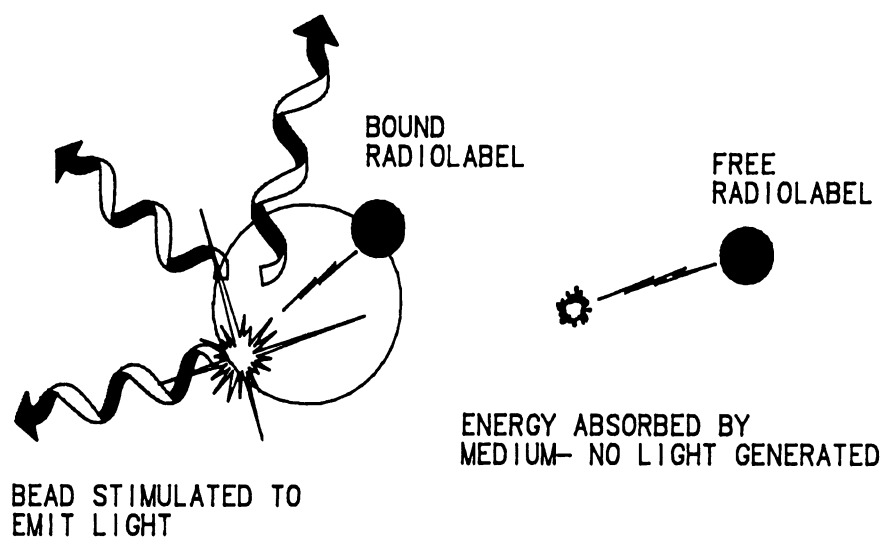


Fig. 1. Principle of SPA

broths. The number of assays per screen can exceed 100,000, which is very costly. The high throughput of samples attainable by SPA is highly desirable in such an environment. Although the earliest applications of SPA were in immunoassays (Baxendale *et al.* 1990; Fiet *et al.* 1991; Pöggeler & Huether 1992; Sugatani *et al.* 1990; Swinkels, Ross & Benraad 1991), we have recently concentrated our efforts on receptor binding and enzyme inhibition assays, for which SPA is particularly well suited.

RECEPTOR BINDING ASSAYS

In SPA receptor binding assays, a receptor is bound to or captured by the SPA bead, and an appropriate ligand is labeled with ^3H or ^{125}I . When the ligand binds to the receptor, it is held close enough to the bead to stimulate the scintillant to emit light. However, if an unlabeled ligand molecule or a competing drug replaces the radiotracer in the receptor binding site, less radioactivity is associated with the bead and, consequently, less light is emitted. Thus, at equilibrium, molecules able to compete with the radiotracer for the receptor may be detected.

SPA technology can be used to study solubilized and membrane-bound receptors. Several strategies are available for coupling the receptors to SPA beads. We have used an antireceptor monoclonal antibody to design an assay for the soluble intracellular domain of the IL-6 receptor. Another method uses beads coated with polycationic substances, such as polylysine or polyethyleneimine, to bind whole cells or membrane-bound receptors. Berry, Burgess and Towers (1991) applied this approach to the endothelin receptor, using both porcine lung and human placental membranes.

Most assays rely on the lectin-glycoprotein interaction. SPA beads coated with wheat germ agglutinin (WGA), a lectin that specifically binds to *N*-acetyl glucosamine residues in glycoproteins, can be used to couple soluble receptors, which themselves may be glycoproteins, or membrane-bound receptors *via* an interaction with other glycoproteins contained within the membrane. The neuropeptide Y (NPY) assay provides just such an example. Using this strategy, we developed an assay for the membrane-bound receptor, isolated from a cultured human neuroblastoma cell line and coupled to WGA-coated beads. The ligand of choice was ^{125}I -labeled peptide YY (PYY), a gastrointestinal peptide that gave lower non-specific binding than NPY itself.

Figure 2 shows the competitive binding curves generated for unlabeled NPY, PYY and two NPY analogs in this SPA assay. Our results compare well with published data using SK-N-MC membranes (Lobaugh & Blackshear 1990) or recombinant receptors expressed in COS1 cells (Larhammar *et al.* 1992).

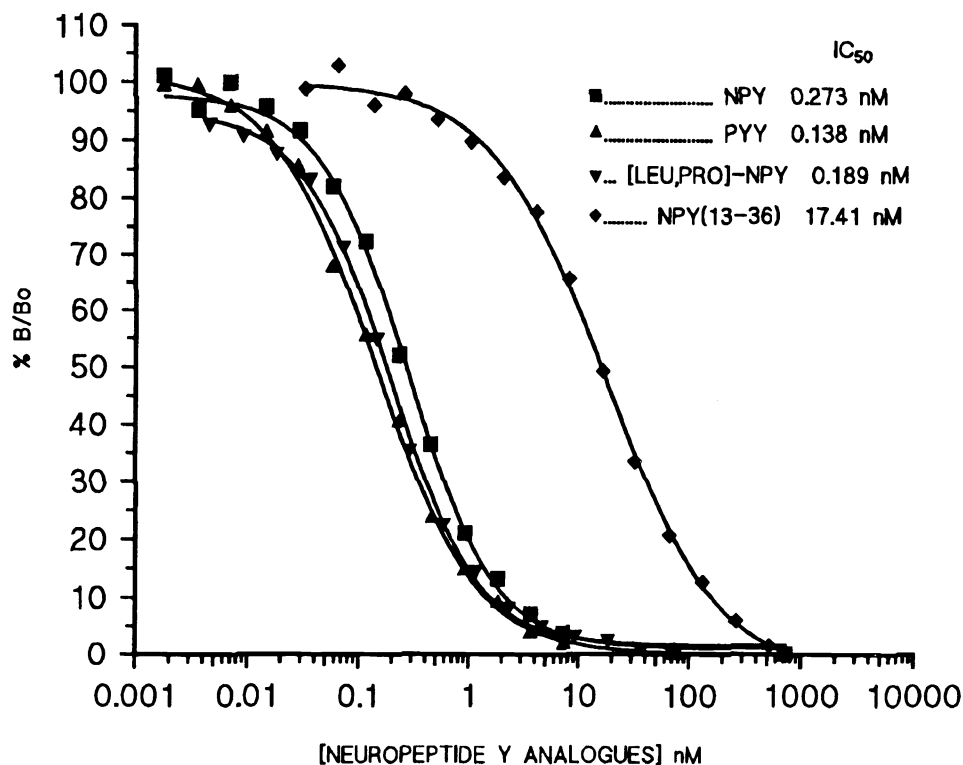


Fig. 2. Inhibition of ^{125}I -PYY binding by NPY, PYY, (LEU³¹, PRO³⁴)-NPY & NPY (13-36)

ENZYME INHIBITION ASSAYS

Enzyme inhibition assays require the separation of the enzyme reaction product from its substrate. Conventionally, this is accomplished using time-consuming techniques, such as HPLC or gel electrophoresis. SPA removes the requirement for a separation step, thereby simplifying and speeding up the assays.

The coupling strategy for this type of assay utilizes the biotin-streptavidin system. The SPA beads are coated with streptavidin, and biotinylation of the enzyme substrates allows them to be coupled to or captured by the beads. The assays were designed to produce either a decrease in signal associated with the beads or an increase in the signal, depending on the type of enzyme being studied. Assays can be carried out with the substrate precoupled to the bead or, more often, the bead may be added to capture the substrate after the reaction ends to ensure that the bead itself does not interfere with the reaction.

We performed signal decrease assays for hydrolytic enzymes, such as proteases or nucleases. One end of the enzyme substrate is radiolabeled with ^3H or ^{125}I , and the other end contains a biotin residue. The cleavage site is between the label and the biotin, so that after the reaction has taken place, the labeled portion of the substrate contains no biotin, and hence, cannot become coupled

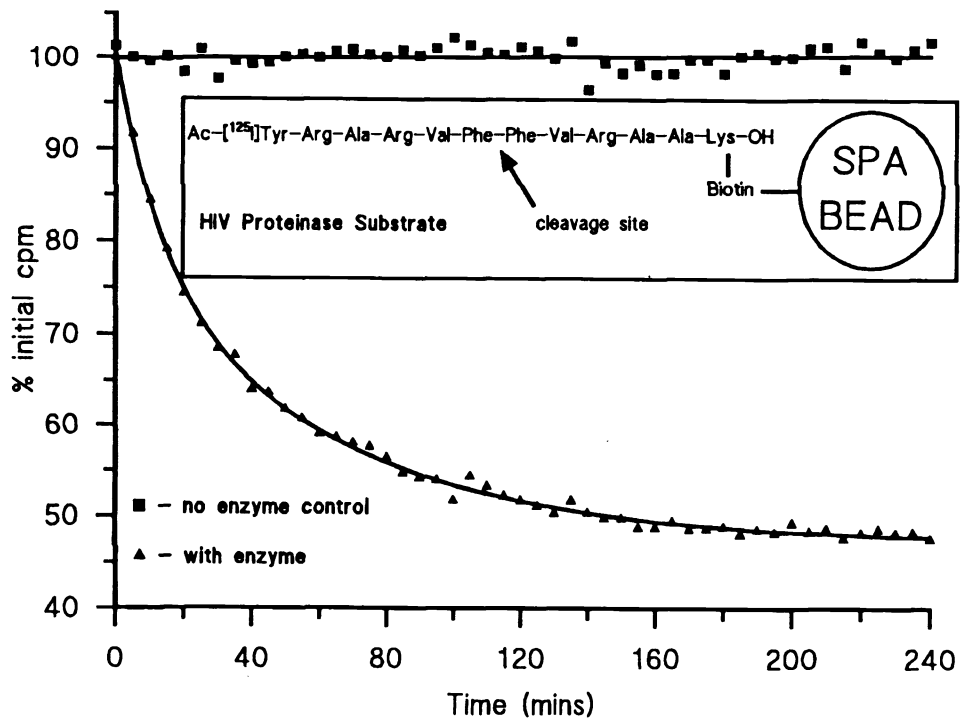


Fig. 3. Time course for HIV Proteinase enzyme assay. The insert shows the substrate and its cleavage site.

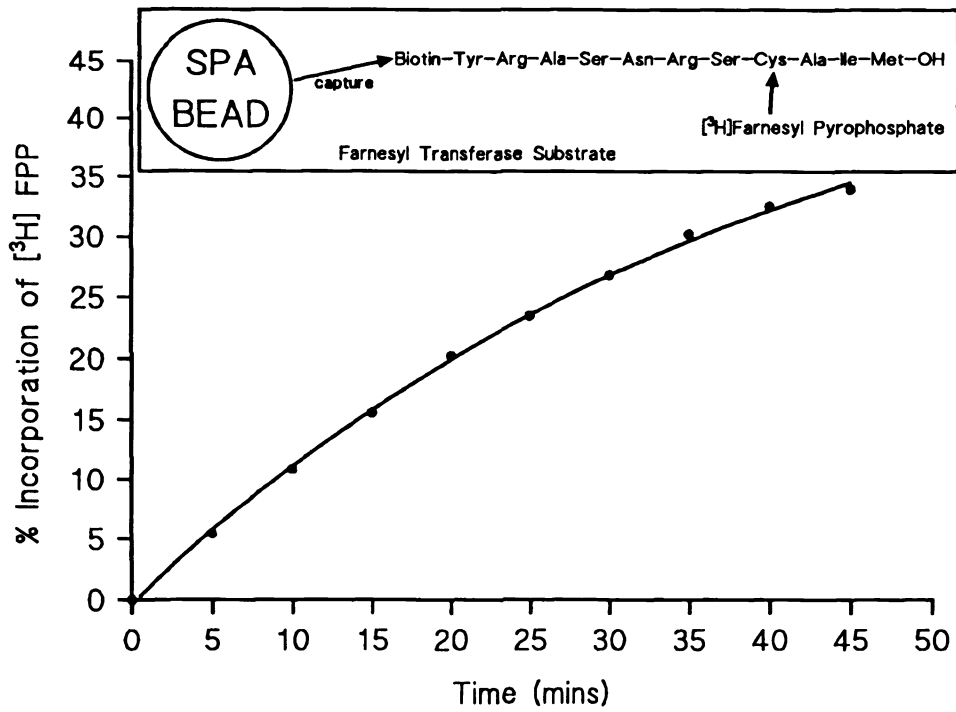


Fig. 4. Time course for Farnesyl Transferase enzyme assay. The basis of the assay is demonstrated in the insert.

to the streptavidin-coated SPA beads. Thus, we can detect a decrease in radiolabel attached to the beads as the reaction proceeds.

This process is illustrated by the HIV proteinase enzyme assay. Figure 3 shows the substrate and its cleavage site as well as a typical time course for the reaction. Although signal decrease assays are not as sensitive as signal increase assays, pseudolinear reaction profiles can be obtained from the time courses where substrate conversion is less than 30%. Consequently, dose-response and IC_{50} determinations are possible.

Signal increase assays were developed for enzymes such as DNA polymerase, reverse transcriptase and several transferases. In these assays, a donor substrate is radiolabeled such that the label is transferred by the enzyme to a biotinylated acceptor substrate. The acceptor, now labeled, is then captured by streptavidin-coated SPA beads. For example, the enzyme farnesyl transferase transfers the farnesyl moiety from farnesyl pyrophosphate to the side-chain sulphur atom of a suitable cysteine-containing peptide (Fig. 4), forming a thio-ether. The peptide is biotinylated at the amino-terminal amino-acid and the donor substrate, farnesyl pyrophosphate, labeled with 3H . Figure 4 shows the resulting time course of enzyme-catalyzed transfer to the acceptor peptide.

CONCLUSION

SPA technology has been applied to a wide range of receptor binding and enzyme inhibition assays, some of which are illustrated here and some currently in use within the pharmaceutical industry. Through a program of consultation and collaboration with pharmaceutical companies, SPA is poised to make a significant contribution to drug discovery.

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