Scintillation proximity assay: a versatile high-throughput screening technology

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Recent advances in generating small molecule libraries, using a wide variety of combinatorial chemistry approaches, have increased the pressure on screening methodology to enable rapid evaluation of the compound library database and, accordingly, to identify lead chemical structures. In order to screen such large numbers effectively, the high-throughput screen must be robust, automatable and precise. Scintillation proximity assay is an established high-throughput screening technology that allows the design of high-flux assays for a wide variety of biochemical and cellular targets. The method requires no separation step and relies entirely on pipetting in a 'mix and measure' format.

Scintillation proximity assay (SPA) is a radioisotopic assay technique that has been shown to be widely applicable in radioimmunoassays (RIA), receptor binding assays and enzyme assays. The technique requires only pipetting steps: there is no requirement to perform a separation step or a need to use scintillation cocktails. SPA technology is therefore ideally suited to automation by robotic pipetting stations, and, when coupled to microtitre plate scintillation counters such as the Microbeta™ (Wallac, Turku, Finland) or the TopCount™ (Packard, Meriden, USA), provides a highly versatile, precise and rapid assay technology for automated high-throughput screening (HTS)[1].

The technology has already been utilized effectively for RIA, receptor binding assays and a wide variety of enzyme assays. More recently, SPA has been applied to cellular adhesion molecule binding, protein–peptide interactions, protein–DNA interactions, and cellular biochemistry assays. Some examples of SPAs that have been reported are listed in Boxes 1–4; the lists are not comprehensive but do indicate the versatility of SPA technology.

Principle of SPA

Originally described as an 'immediate ligand binding assay'[2], the technique relies upon the observation that a β particle emitted from a radioisotope will only travel a limited distance in an aqueous environment. In order for the radioactive disintegration to be detected, the β particle must interact with a scintillant molecule, resulting in a transfer of energy and subsequent emission of light. The pathlength of the β particle is determined by its energy and varies for different isotopes. For example, electrons from ^{3}H have a range of energies leading to an average pathlength of approximately 1.5 μm, and the two monoenergetic internal conversion electrons emitted by ^{125}I have pathlengths of 1 μm and 17.5 μm, respectively. In general, these two isotopes are ideally suited to SPA.

Other isotopes of interest, such as ^{14}C, ^{35}S and ^{33}P, have pathlengths with mean ranges of approximately 58, 66 and 126 μm, respectively, which are less suited to application of the proximity principle. Although SPA has recently been adapted to utilize ^{35}S and ^{33}P, the use of ^{14}C is limited because the specific activities of ^{14}C-labelled compounds are generally too low (approximately 60 mCi/mmol), even though the pathlength of ^{14}C is comparable to that of ^{35}S.

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In SPA the target of interest is immobilized to a small scintillant-containing microsphere, approximately 5 μm in diameter. When a radioisotopically labelled molecule binds to the microsphere, the radioisotope is brought into close proximity to the scintillant and effective energy transfer from the par-ticle will take place, resulting in the emission of light. While the radioisotope remains in free solution it is too distant from the scintillant; the β particle will therefore dissipate the energy into the aqueous medium and thus remain undetected. The proximity principle is depicted in Figure 1.

Microspheres have been prepared from inorganic scintillators such as yttrium silicate[3] and hydrophobic polymers such as polyvinyltoluene. An optimized microsphere has been developed, consisting of a solid scintillant-containing polyvinyltoluene core coated with a polyhydroxy film. Coupling molecules, such as antibodies, are covalently attached to the coating, allowing generic links for assay design. These coupling formats are high-affinity biological molecule-mediated linkages, and no complex chemistry is required to achieve attachment of assay components to the SPA microspheres. The structure of commercially available polyvinyltoluene SPA microspheres is depicted in Figure 2.

The particles disperse easily in aqueous solution and are stable in the presence of organic solvents such as dimethylsulphoxide, methanol or ethanol up to 20% (v/v). With a density of 1.05 g/cm³ they are buoyant in aqueous solution and amenable to liquid-handling automation.

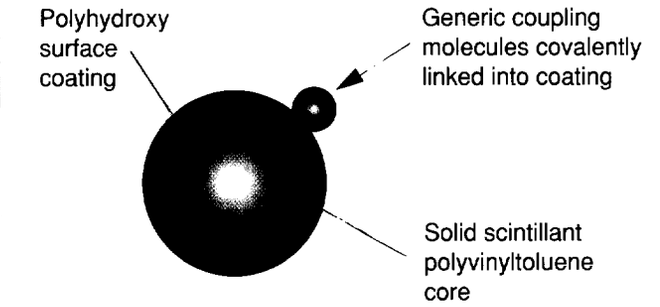


Figure 2. *Structure of scintillation proximity assay (SPA) microspheres. The polyvinyltoluene SPA microsphere is the most successful design for SPA applications. The commercially available SPA microsphere consists of a scintillant-containing solid polyvinyltoluene core. The bead is coated with a polyhydroxy film which reduces the hydrophobicity of the particle. The beads have an average diameter of 5 μm and a density of 1.05 g/cm³. Generic protein coupling molecules are chemically linked to the coating on the particle. Polyvinyltoluene SPA beads are available with sheep antimouse, donkey antirabbit, donkey antisheep, protein A, wheat germ agglutinin and streptavidin.*

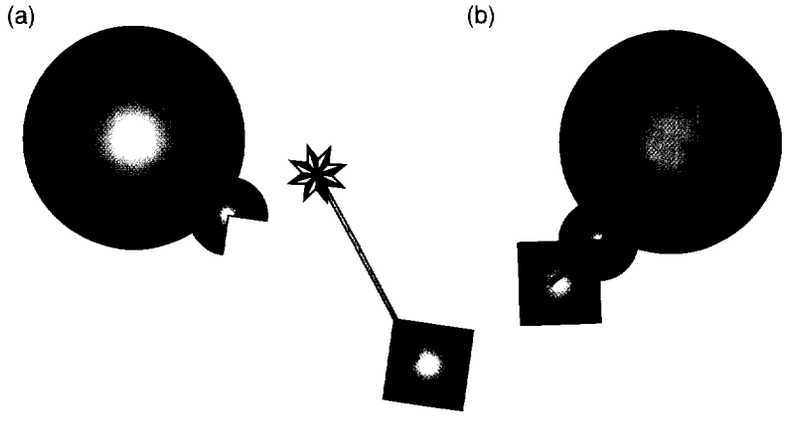


Figure 1. *The principle of scintillation proximity assay (SPA). (a) When a radioisotope, such as tritium, decays it releases a β particle which will travel a limited distance in an aqueous assay buffer. If the radioligand is free in solution, the β particle will dissipate its energy into the aqueous environment, and the disintegration will go undetected. (b) If the radioligand is bound to the SPA microsphere, the radioisotope is brought into close proximity to the scintillant and on disintegration the β particle will transfer its energy to the scintillant bead, causing it to emit light.*

Application of SPA to RIA

Radioimmunoassays (RIAs) developed by SPA are rapid and automatable. Some examples of RIA applications are listed in Box 1. The addition of microspheres containing protein A or secondary antibodies, such as donkey anti-rabbit, sheep antimouse and donkey antisheep, are used to simultaneously capture and quantitate the antibody–antigen complex. The SPA microspheres therefore replace the traditional separation steps employed in RIA, such as polyethylene glycol or charcoal precipitations.

The technology has been widely utilized with a broad spectrum of applications, including mass measurement in research, pharmacological studies and cellular function screening for analytes such as prostaglandins[4,5], steroids[6–8], second messengers[9,10], serum markers and

Box 1. Examples of radioimmunoassays developed using SPA

Acyclovir	Insulin
Ranitidine	Growth hormone
Thromboxane B_2	Platelet-activating factor
6-Ketoprostaglandin $F_{1\alpha}$	Melatonin
Bicyclic prostaglandin E_2	Substance P
Prostaglandin D_2	Cortisol
Prostaglandin $F_{2\alpha}$	1α,25-Dihydroxyvitamin D
Leukotriene B_4	11-Dehydrocorticosterone
Leukotriene C_4	Progesterone
cAMP	Aldosterone
cGMP	Testosterone
Tumour necrosis factor	Neuropeptide Y
Platelet-derived growth factor	Interleukin 1
Epidermal growth factor	Interleukin 2

Box 2. Examples of receptor–ligand binding assays developed using SPA

Endothelins	5-Hydroxytryptamine (5-HT)
Nerve growth factor	Dopamine
Neuropeptide Y	Interleukins
Platelet-derived growth factor	Insulin
Transforming growth factor (TGF-α and TGF-β)	β-Adrenoceptors
	Somatostatin
Muscarinic acetylcholine receptors	Epidermal growth factor
	Basic fibroblast growth factor
Angiotensins	

salivary samples[11–13] and drug quantitation[14]. The application of SPA to RIA is not restricted to particular analytes, and in general the technique may be applied in place of traditional separation-based RIA. Comparisons of performance between SPA and conventional methods have been presented[15–17]. In some circumstances the removal of the separation step increases the precision and reproducibility[11] of the assay, because it is often the precipitation procedure that introduces the greatest error in the overall assay performance.

Where SPA affords particular advantage is for sample throughput and automation[18] of immunoassays where, in many instances, it is the method of choice because of ease of operation or time-saving[7,8,18].

The automation of scintillation proximity RIAs (SP-RIAs) for analytes such as cAMP allows the design of high-flux cell-based screens for receptor activation and other cellular functions. Jelinek and coworkers utilized a cAMP SP-RIA to measure the stimulation of cAMP production by glucagon in transfected COS-7 cells[19]. Similarly, Hancock and coworkers used an SP-RIA to monitor the downregulation of cAMP production by α_2-adrenergic agonists[20].

Automated SP-RIA has also been used to develop high-throughput assays for drug analysis during clinical trials. Linacre and Morris applied SP-RIA to the clinical trial assays for ranitidine[18], and Tadepalli and coworkers similarly automated the clinical trial SP-RIA for acyclovir[21].

Receptor binding assays using SPA

Some examples of receptor binding assays employing SPA are listed in Box 2. Receptor binding assays were first performed by Nelson[22] using a hydrophobic polyvinyltoluene particle dispersed in Triton X-100 to measure [^{125}I]iodo-α-bungarotoxin binding to acetylcholine receptor solubilized from *Torpedo* membranes. In this application, the receptor was immobilized to the particle by non-specific hydrophobic interactions; however, while it demonstrates the principle for receptor binding by SPA, the system is neither robust nor versatile enough for HTS applications.

Hoffman and Cameron[23] first reported the development of an SPA screen for TGF utilizing commercially available yttrium silicate SPA particles coated with polylysine to immobilize A431 cellular membranes containing the epidermal growth factor (EGF) receptor. Kienhaus and coworkers extended the study of the EGF receptor by utilizing the homogeneous nature of the SPA system to measure association and dissociation rates by continuously monitoring SPA assays in real time[24].

A similar technique was utilized by Game and Cook to determine the k_{obs} in real time for the association of the ^{125}I-labelled peptide PYY to the neuropeptide Y_1 receptor in cultured SKN-MC cell membranes[25], and O'Beirne and coworkers measured the on and off rates for [^{3}H]scopolamine binding to cholinergic M_1 receptors[26]. Hurwitz and coworkers used SPA to determine the K_d of soluble EGF receptor domain with and without pretreatment with the growth factor, elegantly demonstrating an increase in the affinity of binding resulting from receptor dimerization[27].

Berry and coworkers were first to demonstrate the use of a commercially available polyvinyltoluene SPA particle coated with the lectin wheat germ agglutinin (WGA) to perform competition binding studies with porcine lung and human placental membranes containing endothelin-1 receptors[28]. The WGA coating is particularly effective in immobilizing glycosylated cellular membranes. The lectin binds directly to glycoproteins in the membrane and no chemical derivatization

is required to bind the membranes to the SPA beads. The WGA polyvinyltoluene SPA particle has become the reagent of choice for constructing SPA receptor binding assays. Jing and coworkers[29] have demonstrated the use of SPA to perform Scatchard analysis on nerve growth factor (NGF) receptors in E25-42 cell membranes, while Murray and coworkers[30,31] used SPA to screen a series of substituted pyrrolidin-2-one biphenyltetrazoles as potential angiotensin II antagonists. These researchers used the high throughput of assays to determine K_d values for the entire series as a measure of rank potency. Lee and coworkers used a similar SPA to characterize a novel angiotensin II antagonist[32], while Carrick and coworkers used WGA SPA microspheres to develop an assay for growth hormone releasing factor[33]. Recently, O'Beirne and coworkers presented data on the use of WGA SPA beads to construct assays for commercially available preparations of angiotensin II (type 1), dopamine D_2 and 5-hydroxytryptamine ($5HT_{1A}$) receptors expressed in insect cells by the baculovirus expression system[34], demonstrating the wide applicability of the WGA interaction to immobilize cellular membranes. These authors also determined kinetic parameters for the assays in comparison to filter binding methodology and found good agreement in K_d and K_i by both methods.

Banks and coworkers[35] constructed a linked SPA receptor binding assay for a soluble interleukin 5 (IL-5) receptor expressed as a protein A fusion protein. The assay was configured using antirabbit SPA beads to immobilize the receptor and ^{125}I-labelled IL-5 as the receptor ligand. Similar assay formats have been used for soluble receptors by direct biotinylation and immobilization with streptavidin SPA beads.

Kodukula and coworkers have recently reported the discovery of a tetracyclic inhibitor for the neuropeptide Y receptor using an SPA to screen microbial fermentation extracts[36]. The active compound was discovered in an extract from *Aspergillus niger*.

Other binding assays performed by SPA

SPA has been applied to a variety of non-traditional binding events in HTS formats (Box 3). In most cases, the general assay format involves the biotinylation of one of the binding partners, the other molecule being labelled with either ^{3}H or ^{125}I. The assay is configured using streptavidin SPA microspheres.

Pernelle and coworkers[37] developed a simple screening SPA for the leucine zipper domains of the transcription factors Fos and Jun using ^{125}I-labelled Fos(160–200) and biotinylated Jun(275–315). The interactive complex was captured using streptavidin SPA beads.

Box 3. Examples of other binding assays developed using SPA

Fos–Jun peptides	SH3 binding domains
Selectin adhesion assays	Ras–neurofibronin (NF1)
Integrin adhesion assays	Ras–Raf
SH2 binding domains	NF-κB

A binding assay for the adhesion molecule E-selectin was reported by Game and coworkers[38]. The assay was constructed by biotinylation of cloned E-selectin protein and ^{3}H-labelling of HL-60 membranes by *in vivo* labelling in culture with [^{3}H]leucine. Assays were constructed with both intact cells and cell membranes utilizing streptavidin SPA beads to capture the selectin–membrane complex. These authors used the homogeneous nature of the technology to monitor the rate of association of the complex and demonstrated rapid dissociation with an anti-E-selectin monoclonal antibody. The original work has been extended by Anostario and Huang[39], who further adapted the assay format to include a capture step using a biotinylated anti-E-selectin monoclonal antibody. In addition, they were able to substitute ^{125}I-labelled carcinoembryonic antigen, which contains the sialyl Lewis X ligand, in place of ^{3}H-labelled HL-60 cell membranes.

SPA has been successfully applied to the assay of SH2 and SH3 domain interactions. Mobbs and coworkers[40] demonstrated the interaction of the SH2 domain of Src and the SH3 domain of Crk using ^{3}H- or ^{125}I-labelled peptide sequences from a number of known binding proteins. Blair and coworkers[41] used a similar format to investigate the SH2 interaction of GAP (GTPase-activating protein) with a number of phosphotyrosine-containing peptide sequences. They found the interaction to be highly temperature-dependent and utilized the non-separation advantages of the assay format to temperature-cycle the assay between 4°C and 20°C, demonstrating that the shift in binding affinity was fully reversible and not caused by denaturation of the assay components. The observed effect was ascribed to temperature-dependent conformational changes in the proline-containing peptide sequences used as ligands in these assay formats.

A novel approach was adopted by Skinner and coworkers[42] to measure the binding of neurofibronin (NF1) to Leu-61 Ras. The oncogenic Ras protein was labelled by exchange with [^{3}H]GTP. The NF1 was expressed as a fusion protein with a glutathione *S*-transferase (GST) domain and captured to configure the assay using an anti-GST antibody and a protein A

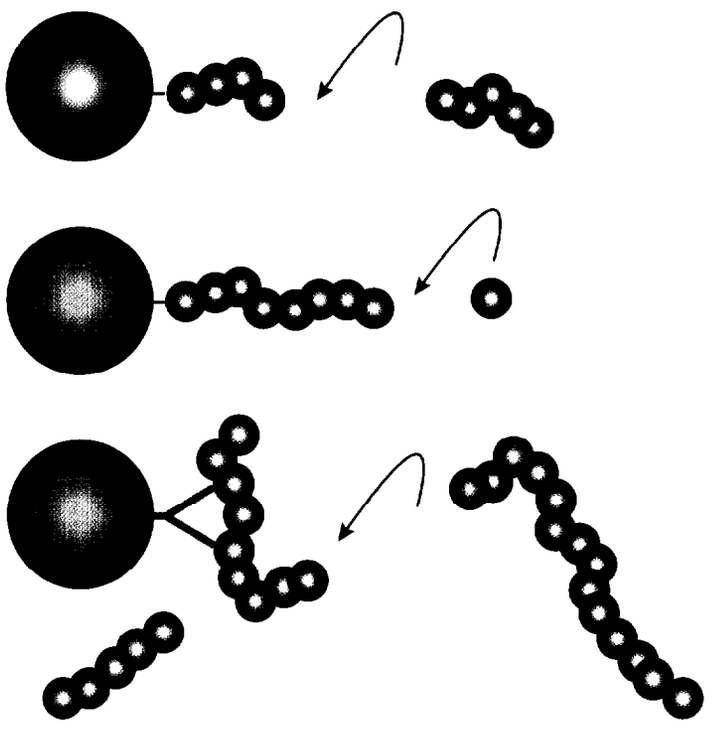
SPA bead. A similar approach was used by Pachter and coworkers to capture the solubilized $\alpha_5\beta_1$ integrin from over-expressing Chinese hamster ovary (CHO)-7 cells with a monoclonal antibody and antimouse SPA beads[43]. The assay was configured to measure ^{125}I-labelled fibronectin binding and displacement by a number of peptide sequences, including a series of novel cyclic peptides.

An adaptation of the antibody-capture method was used by Bodian and coworkers to screen for inhibitors of conformational change, which exposes the fusion peptide of influenza haemagglutinin[44]. By using a specific antibody to the fusion peptide sequence and protein A SPA beads, these researchers identified a number of potential lead compounds and determined IC_{50} values in the range 1–20 μM.

Owen and coworkers applied the antibody-capture format to measure the binding interaction between NF-κB and the DNA consensus sequence for this transcription factor[45]. The assay employed an anti-GST antibody to capture the GST-fusion transcription factor protein and a ^{3}H-labelled oligonucleotide.

Enzyme assays by SPA

Enzyme assays by SPA are generally constructed in one of three basic formats (Figure 3). Some enzyme assay applications of SPA are given in Box 4. A simple concept for hydrolase activity was first demonstrated using HIV-1 protease[46]. A peptide substrate was designed which was labelled with ^{125}I at the N-terminal tyrosine and biotinylated at the C-terminus.



(a) **Signal removal**
Hydrolase activities: e.g. proteases, nucleases, phospholipases and esterases

(b) **Signal addition**
Molecular transfer activities: e.g. transferases, polymerases and kinases

(c) **Product capture**
Utilization of product specific capture: e.g. antibodies, DNA probes, receptors or other specific binding proteins

Figure 3. *Enzyme assay formats in scintillation proximity assay (SPA). Enzyme SPAs may be grouped into three main formats. (a) Signal removal. In this format the radioisotopically labelled substrate is linked to the bead, often through biotinylation. The substrate is designed such that the action of the enzyme separates the radioisotope from the biotinylated portion of the molecule, resulting in a decrease in the signal generated. The assays are normally performed in free solution and the reaction products captured on termination of the assay. This eliminates the possibility that the presence of the microsphere may interfere with the kinetics of the assay. (b) Signal addition. In this format the acceptor substrate is biotinylated and the donor substrate is radiolabelled. The action of the enzyme causes the transfer of radiolabel to the biotinylated substrate. The radiolabelled reaction product is captured with SPA beads on termination of the assay, resulting in an increase in the signal generated. (c) Product capture. In this assay format the product of the reaction is captured by biospecific recognition (e.g. an antibody). It is important that the magnitude of discrimination between the substrate and the product is sufficient to provide an adequate signal-to-noise. The format may be used to measure appearance of product or disappearance of substrate.*

The 12 amino acid sequence utilized had a single cleavage site. The action of the protease separates the radiolabel from the biotinylated portion of the peptide. On termination of the assay, the biotinylated peptide is captured by streptavidin SPA beads and the amount of cleavage determined. This assay format results in a signal decrease, which is generally perceived as a less sensitive assay strategy because the signal window may be small relative to the high background. Despite this limitation, Fehrentz and coworkers utilized this assay to determine comparative IC_{50} values for a series of statine-based peptides as inhibitors of HIV-1 protease[47,48]. Wilkinson and coworkers further adapted the assay to perform high-volume screening of serum samples for protease inhibitor levels in pharmacological studies on rats. The signal decrease SPA format was compared with an HPLC method, a renin bioassay and a protease assay linked to immunoassay of angiotensin I. All methods showed comparable performance in measuring ditekiren levels in rat serum extracts[49].

The signal decrease format has subsequently been applied to a number of proteases for both HTS and research assays. Brown and coworkers used this approach with a tritiated β-amyloid peptide sequence to screen cathepsin G. These authors also used the SPA methodology in a signal decrease format to determine K_m and V_{max} values for the β-amyloid substrate[50]. Other assays for hydrolase activity such as RNase H, phospholipase A_2 and phospholipase D have been developed utilizing biotinylated substrates that have been strategically radiolabelled to release the radioactive moiety by the action of the enzyme of interest.

Box 4. Examples of enzyme assays developed using SPA

- HIV integrase
- HIV-1 protease
- HIV-2 protease
- CMV protease
- Endothelin converting enzyme
- Interleukin converting enzyme
- Cathepsin G
- Phospholipase A_2
- Phospholipase D
- Reverse transcriptase
- MAP (mitogen-activated protein) kinase
- Telomerase
- DNA polymerase
- Farnesyltransferase
- TNF-α (tumour necrosis factor) convertase
- Chemistry ester transfer protein
- DNA helicase
- Geranyltransferase
- Fucosyltransferase
- RNase H
- Cyclin-dependent kinases
- cdc kinases

Signal increase SPA formats have also utilized biotinylated acceptor molecules as substrates for ^{125}I- or ^{3}H-labelled donor substrates. By using biotinylated DNA primers to DNA or RNA templates and tritiated nucleotides, a number of assays have been constructed for DNA and RNA polymerases.

Reverse transcriptase (RT) activity has been measured routinely using SPA (Ref. 51). The assay has been reported to be useful in the evaluation of both chemical[52] and natural product[53] non-nucleoside inhibitors. Taylor and coworkers[53] used an RT SPA to characterize the complete kinetic profile of a new and novel class of RT inhibitors, the inophyllums, while Cannon and coworkers utilized RT SPA to measure the replication rate of mutant HIV-1 in cultured T cell lines[54].

Another transferase SPA concept was described for farnesyltransferase by Santos and Cook[55]. In this assay, [^{3}H]farnesyl pyrophosphate was used as a donor substrate to a biotinylated human lamin B peptide sequence containing a single farnesylation site. SPA has been reported to offer a rapid and more sensitive alternative to traditional trichloroacetic acid precipitation methods[56], and the simplicity of the assay technology enhances the automation capability for HTS. The assay was also used to perform detailed kinetic analysis on both of the enzyme substrates and for short peptide inhibitors.

The measurement of cholesteryl ester protein (CETP) has been assayed by SPA in two ways. Amersham International (Amersham, Bucks, UK) have produced a commercially available kit which utilizes high-density lipoprotein (HDL)-containing [^{3}H]cholesteryl ester as a donor substrate and biotinylated low-density lipoprotein (LDL) as an acceptor substrate. Following incubation with purified preparations of CETP, the biotinylated LDL is captured with streptavidin SPA beads and the transfer of [^{3}H]cholesteryl ester from HDL to LDL may be monitored. Coval and coworkers used this system in HTS to identify the natural product wiedendiol as an inhibitor of CETP (Refs 57,58). Lagros and coworkers achieved a similar result using an anti-apolipoprotein B (Apo B) antibody coupled to a donkey antisheep SPA bead[59]. This assay format allowed the measurement of CETP activity in total human serum. In addition, by using an anti-Apo B antibody, the transfer to both LDL and VLDL could be determined.

Sensitive, rapid screening assays have also been performed in signal addition formats by using product-capture assay strategies. Takahashi and coworkers[60,61] adapted a porcine lung endothelin-1 receptor assay[28] to capture and quantitate levels of endothelin for studies on the endothelin converting enzyme (ECE). Endothelin production from ^{125}I-labelled big

endothelin was monitored by capturing the product using the receptor bound to WGA SPA beads. This novel approach was successfully applied during the purification of ECE from rat lung. Kinetic parameters and pH dependence were also determined. The assay was subsequently used to monitor phosphoramidon-sensitive activity during cloning and expression of the enzyme from rat endothelial cells[61].

Another approach adopted for product capture was reported by Umekawa and coworkers[62], who used polyclonal antisera to endothelin to capture ^{125}I-labelled endothelin produced from ^{125}I-labelled big endothelin; the complex was subsequently captured using protein A SPA microspheres. This antibody-capture approach is dependent upon the specificity of the antisera available. In order to obtain adequate signal-to-noise it is important that the antisera discriminates the product of the reaction with at least three orders of magnitude greater avidity than for the substrate.

Norey and coworkers used a biotinylated polynucleotide probe to capture complementary ^{3}H-labelled oligonucleotides. This concept was successfully applied to configure a DNA helicase assay[63]. A radiolabelled oligonucleotide was bound to an M13 plasmid and the action of bacterial or viral helicases resulted in an ATP-dependent unwinding of the duplex. The unwound ^{3}H-labelled oligonucleotide was subsequently captured with the biotinylated probe and quantified by the addition of streptavidin SPA beads. Other DNA modifying enzymes have been assayed by SPA, including telomerase[64] and HIV integrase[65].

Quantification of PCR by SPA

SPA has also been applied to quantitate PCR by utilizing biotinylated PCR primers and [^{3}H]dNTPs. PCR amplification produces biotinylated [^{3}H]DNA which can subsequently be captured by streptavidin beads and quantified directly[66].

Hughes and coworkers used this method to detect and quantify the level of baculovirus infection in laboratory cultures of *Mamestra brassicae* insects. They estimated the level of infection in their insect populations to be of the order of 13–20 viral genome copies per cell. The method was reported to be rapid and reproducible and avoided the problems involved with the separation and quantification of products from agarose gels[67].

Rawal and coworkers used SPA in a quantitative nested PCR method to measure cytomegalovirus in blood specimens from bone marrow transplant recipients. They compared the SPA method with their in-house nested PCR method and found equivalent sensitivity[68].

Summary

SPA is a highly versatile technology platform for the design of high-flux automated assays for drug screening and routine sample measurement. The SPA technology has been applied to assays as diverse as RIA and transcription factor binding assays. The versatility of the technique is apparent not only in the variety of assay applications but also, importantly, in the variety of strategic assay design options that the technology offers for a specific assay target.

The technique has been routinely applied to RIA, enzyme assay, protein–peptide interactions and receptor binding assays, as well as to complex formats such as protein transferase assays and DNA modification assays. Further advances in applications are still appearing. SPA has now been successfully applied to the assay of protein kinases using [^{33}P]ATP as substrate for the reaction[69]. In addition, the SPA principle has been utilized to study *in situ* biochemical processes in living cultured cells[70]. This latest advance in the application of the SPA technique further enhances the versatility of the technology to address the complex requirements of high-throughput drug discovery screening and research programmes.

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REFERENCES

1 Cook, N.D., Sutton, J.R. and Takeuchi, K. (1992) *Pharm. Manuf. Int.* 1, 49–53
2 Bertaglio-Matte, J.H. (1986) US Patent No. 4,568,649
3 Bosworth, N. and Towers, P. (1989) *Nature* 341, 167
4 Baxendale, P.M. *et al.* (1990) in *Advances in Prostaglandins, Thromboxane and Leukotriene Research*, Vol. 21 (Samuelsson, B. *et al.*, eds), pp. 303–306, Raven Press
5 Sugatani, J., Hughes, K.T. and Saito, K. (1994) *J. Lipid Mediators* 10, 9–10
6 Fiet, J. *et al.* (1994) *Clin. Chem.* 40, 2296–2305
7 Swinkles, M.J.W., Ross, H.A. and Benraad, T.J. (1991) *Ann. Clin. Biochem.* 28, 87–90
8 Fiet, J. *et al.* (1991) *Clin. Chem.* 37, 293
9 Heath, R. *et al.* (1992) in *Cell Signalling: Experimental Strategies* (Reid, E., Cook, G.M.W. and Luzio, J.P., eds), pp. 193–194, Royal Society of Chemistry
10 Heath, R., Bryant, B. and Horton, J.K. (1992) *The Biology of Nitric Oxide. Part 2. Enzymology, Biochemistry and Immunology*, pp. 98–102, Portland Press
11 Pallesen, T., *et al.* (1992) *Glycoconjugate J.* 9, 331–335
12 Vangsted, A. *et al.* (1994) *Cancer Detect. Prev.* 18, 221–229
13 Jones, K.P. *et al.* (1994) *Thromb. Res.* 75, 503–511
14 Fenwick, S. *et al.* (1994) *Anal. Proc.* 31, 103–106
15 Hildebrand, M., Louton, T. and Schutt, A. (1992) *Eicosanoids* 5, 5–8
16 Sugatani, J. *et al.* (1990) *Life Sci.* 46, 1443–1450
17 Horton, J.K. and Baxendale, P.M. (1995) in *Methods in Molecular Biology. 41, Signal Transduction Protocols* (Kendall, D.A. and Hill, S.J., eds), pp. 91–105, Humana Press

18 Linacre, P. and Morris, S.E. (1992) in *Bioanalytical Approaches for Drugs, including Anti-asthmatics and Metabolites 22*, 2nd edn (Reid, E. and Wilson, I.D., eds), pp. 325–326 Royal Society of Chemistry
19 Jelinek, L.J. *et al.* (1993) *Science* 259, 1614–1616
20 Hancock, A.A. *et al.* (1995) *J. Receptor Signal Transduction Res.* 15, 557–579
21 Tadepalli, S.M., Topham, P.A. and Quinn, R.P. (1990) *Clin. Chem.* 36, 1104
22 Nelson, N. (1987) *Anal. Biochem.* 165, 287–293
23 Hoffman, R. and Cameron, L. (1992) *Anal. Biochem.* 203, 70–75
24 Kienhaus, C.B.M. *et al.* (1992) *J. Receptor Res.* 12, 389–399
25 Game, S. and Cook, N.D. (1992) Proceedings of Rapid Functional Screening for Drug Development, P6, International Business Communications
26 O'Beirne, G. *et al.* (1994) Proceedings XIIth International Congress of Pharmacology. *Can. J. Physiol. Pharmacol.*, 72 (Suppl.1), P23.1.18, 608
27 Hurwitz, D.R. *et al.* (1991) *J. Biol. Chem.* 266, 22035–22043
28 Berry, J.A., Burgess, A.J. and Towers, P. (1991) *J. Cardiovasc. Pharmacol.* 17 (Suppl. 7), S143–S145
29 Jing, S., Tapley, P. and Barbacid, M. (1992) *Neuron* 9, 1067–1079
30 Murray, M.V. *et al.* (1992) *Bioorg. Med. Chem. Lett.* 2, 1775–1779
31 Murray, M.V. *et al.* (1993) *Bioorg. Med. Chem. Lett.* 3, 369–374
32 Lee, D.K.H. *et al.* (1993) *FASEB J.* 7, A245
33 Carrick, T.A. *et al.* (1995) *Endocrinology* 136, 4701–4704
34 O'Beirne, G. *et al.* (1995) Proceedings 25th Annual Meeting of the Society for Neuroscience, *Society of Neuroscience Abstracts* 21, p.1844, 724.6
35 Banks, M. *et al.* (1995) *Anal. Biochem.* 230, 321–328
36 Kodukula, K. *et al.* (1995) *J. Antibiot.* 48, 1055–1059
37 Pernelle, C. *et al.* (1993) *Biochemistry* 32, 11682–11687
38 Game, S.M. *et al.* (1993) Proceedings of Cell Adhesion Molecules: Platelets, Cancer, Inflammation and Infection, P3, Cambridge Health Institute
39 Anostario, M. and Huang, K. (1995) *Anal. Biochem.* 232, 122–128
40 Mobbs, D.R. *et al.* (1995) Proceedings 2nd Annual IBC Signal Transduction Therapy. Novel Targets for Therapeutic Intervention, P5, International Business Communications
41 Blair, J. *et al.* (1995) Proceedings 2nd European Conference on High Throughput Screening, Budapest, P1
42 Skinner, R.H. *et al.* (1994) *Anal. Biochem.* 223, 259–265
43 Pachter, J.A., Zhang, R. and Mayer-Ezell, R. (1995) *Anal. Biochem.* 230, 101–107
44 Bodian, D.L. *et al.* (1993) *Biochemistry* 32, 2967–2978
45 Owen, P.J. *et al.* (1995) Proceedings 3rd Annual IBC Transcriptional Regulation Meeting, P4, International Business Communications
46 Cook, N.D. *et al.* (1991) *Structure and Function of the Aspartic Proteinases* (Dunn, B.M., ed.), pp. 525–528, Plenum Press
47 Fehrentz, J.A. *et al.* (1992) *Biochem. Biophys. Res. Commun.* 188, 865–872
48 Fehrentz, J.A. *et al.* (1992) *Biochem. Biophys. Res. Commun.* 188, 873–878
49 Wilkinson, K.F. *et al.* (1993) *Pharm. Res.* 10, 562–566
50 Brown, A.M. *et al.* (1994) *Anal. Biochem.* 217, 139–147
51 Lemaitre, M. *et al.* (1992) *Antiviral Res.* 17, 48
52 Kleim, J.P. *et al.* (1993) *Antimicrob. Agents Chemother.* 37, 1659–1664
53 Taylor, P.B. *et al.* (1994) *J. Biol. Chem.* 269, 6325–6331
54 Cannon, P.M. *et al.* (1994) *J. Virol.* 68, 4768–4775
55 Santos, A.F. and Cook, N.D. (1992) Proceedings from Rapid Functional Screens for Drug Development, P7, International Business Communications
56 Tahraoui, L. *et al.* (1993) Proceedings 1st International Conference on Advanced Pharmaceutical Screening, Vienna, P5
57 Coval, S.J. *et al.* (1995) *Bioorg. Med. Chem. Lett.* 5, 605–610
58 Chackalamannil, S. *et al.* (1995) *Bioorg. Med. Chem. Lett.* 5, 2005–2010
59 Lagros, L. *et al.* (1995) *Clin. Chem.* 41, 914–919
60 Takahashi, M. *et al.* (1993) *J. Biol. Chem.* 268, 21394–21398
61 Shimada, K., Takahashi, M. and Tanzawa, K. (1994) *J. Biol. Chem.* 269, 18275–18278
62 Umekawa, T. *et al.* (1992) *Jpn. J. Pharmacol.* 59 (Suppl. 1), 335
63 Norey, C.G. *et al.* (1994) Proceedings of International Forum on Advances in Screening Technologies and Data Management, P4
64 Savoysky, E. *et al.* (1996) *Nucleic Acids Res.* 24, 1175–1176
65 Downes, M.J. *et al.* (1994) Proceedings of International Forum on Advances in Screening Technologies and Data Management, P7
66 Cross, L., Potts, C. and Anson, J.G. (1994) in *Methods in Amplification* (Roffs, A. *et al.*, eds), pp. 19–26, Plenum Press
67 Hughes, D.S., Possee, R.D. and King, L.A. (1994) *J. Virol. Methods* 50, 21–28
68 Rawal, B.K. *et al.* (1994) *J. Virol. Methods* 47, 189–202
69 Brophy, G. *et al.* (1995) 9th International Conference on Second Messengers and Phosphoproteins, P274, Vanderbilt University
70 Cook, N.D. (1992) European Patent Application No. 92/303806.9, International Application Pct/GB9H01040