Time-resolved fluorometry: an overview of the labels and core technologies for drug screening applications

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Time-resolved fluorometry (TRF) with lanthanide chelate labels is a well-established technology in diagnostics. Recently, TRF has attracted great interest as a tool for application in a range of assay formats in drug screening. The advent of highly fluorescent lanthanide chelates has made it possible to develop a wide variety of highly sensitive TRF-based assay technologies, both heterogeneous and homogeneous. In addition, the ability of TRF technology to combine temporal resolution with high spatial resolution opens up the possibility of assay configurations with features such as miniaturization, multilabel or multiparametric analysis and greatly improved sample throughput.

The advent of radioisotopic labels at the end of the 1950s revolutionized diagnostic routines by providing easy, rapid and quantitative assays[1,2]. During the 1970s, the major goal was to find alternative technologies, to replace radioisotopes, to develop simpler homogeneous assays, and to progress to even more sensitive and more easily automated assays. Enzyme immunoassays[3] were the first to gain wide acceptance in diagnostics. Fluorometric assays did not achieve wider acceptance (except in cellular applications and in some homogeneous assays, such as fluorescence polarization assays[4], later commercialized by Abbott) until the development of sensitive time-resolved fluorometric assays in the early 1980s (Ref. 5). The major trend in diagnostics during the 1980s and 1990s was not to find new technologies, but to develop complete automation of clinical routines, first with batch-analysing devices, and later with continuous or random-access automates. All of those systems are based on nonradioactive label technologies.

Somewhat similar trends are found in the field of drug discovery. In high-throughput screening (HTS), however, the use of radioisotopic labels still forms the core of assay methodology. The development of scintillation proximity assay (SPA) principles by Amersham researchers[6] strengthened the long-term position of radiolabels in pharmaceutical screening. The main reason for this continued popularity stems from the requirements of the targets, which are generally low molecular weight compounds (molecular weight range 300–700). Because of their binding properties, size and the existence of only a few active sites for binding, it is much more difficult to label these with a nonradioactive external label than with internal labels such as ^{3}H, ^{14}C, ^{32}P or ^{33}P. Nevertheless, efforts to find nonradioactive labels continue, not only because of the problem of radiation disposal, but also for reasons of performance, simplicity, automation, speed, assay miniaturization, multiparametric possibilities, stability of tracers and safety.

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Fluorometry uses nonbiological, stable labels. It is a very versatile technology, providing a real alternative to radioisotopes in screening and, as will be described here, it could make an important contribution to future assay development.

Assay technologies – from heterogeneous to homogeneous

The correct definition of what constitutes a heterogeneous or a homogeneous assay format is fundamental to the understanding of assay design principles. Heterogeneous or separation assays may be defined simply: after an incubation period, the free or unincorporated label and (assay) matrix are removed, thus effectively stopping the reaction at a specific time point. In homogeneous assays, all of the assay components are in the same phase (i.e. liquid) and there is no separation of the free label and matrix before measurement; in addition, the reaction is not usually stopped (i.e. the assay equilibrium is in a state of flux). The measurement can include real-time kinetics.

There is a third assay format, the nonseparation assay, where one or more of the assay components are not in the same phase (i.e. on a bead or other solid phase). Although the free labelled ligand and matrix remain in the well during measurement, unlike the homogeneous assay, the reaction is centred on or around a solid phase, thus effectively localizing the signal and essentially converting the solid phase (bead) into a partial separation step. The best-known system based on this concept is SPA (Amersham International).

In drug discovery, the primary screening – the search for 'hits' – is based on the variability of competitive binding reactions, such as protein–protein, hapten–protein, receptor–ligand and protein–nucleotide interactions, in addition to assays of secondary signalling cascades, enzyme activities (phosphorylases, kinases, proteases, peptidases), expressed genes, cellular interactions (adhesions, cytotoxicity assays), etc.

For diagnostics, a noncompetitive assay format is required, because the advantages of a homogeneous readout are outweighed by the need for a wide dynamic range and high sensitivity not achievable without steps to reduce the nonspecific background (i.e. low-affinity reactions)[7].

It was the noncompetitive, excess-reagent immunometric assays that provided the possibility to develop ultrasensitive immunoassays for cancer antigens, peptide hormones, cytokines, etc. While the separation-based noncompetitive assay is able to measure analyte concentrations within a range of four or five orders of magnitude without dilution[7], most homogeneous assays hardly achieve signal dynamics of more than one order of magnitude.

In a competitive HTS assay, however, reaction sensitivity is influenced not so much by the method of separation but by other, more important factors[7]. Thus, for HTS there are fewer restrictions preventing the conversion of quantitative heterogeneous assays to homogeneous formats.

Although almost all of the assay steps, such as dispensing and diluting, washing, harvesting and incubation, are easily automated, adoption of a simple homogeneous assay design would speed up sample throughput. The reason is that homogeneous assays require fewer steps, have more rapid binding kinetics (all in solution), and do not need tediously slow washing or harvesting or extra steps for signal production (substrate incubation). In addition, the homogeneous concept facilitates assay miniaturization, which is another important goal for HTS laboratories.

The homogeneous assay format does, however, have some drawbacks. In most of the homogeneous assay designs known today, performance is limited by factors such as the size of the analyte (ligand), the assay principle, sensitivity and suitability for different sample matrices. In homogeneous assay technologies, the dynamic range of the signal (signal-to-noise ratio) is limited and in optimization of the assay it is difficult to find enough signal; thus, less emphasis can be put on increasing the binding kinetics, use of saturating levels of binding reagents and obtaining a wide dynamic range. This means, for example, that real reagent-excess systems (i.e. noncompetitive assay formats) cannot be applied.

For signal measurement in the presence of all assay components, considerable care should be taken to adjust for any interference, such as fluorescence quenching or enhancing, or interference by coloured components. In the past, methods to eliminate interference were based on a combination of dilution, use of long-wavelength probes (>600 nm), sample pretreatment, or some form of simple algorithm to adjust the measured signal to that of a known pure standard. With new sophisticated software used in combination with multitechnology instrumentation, the problems associated with such interferences can be virtually eliminated.

Fluorescence resonance energy transfer

One of the earliest, and now one of the most widely discussed, homogeneous fluorometric assay systems is fluorescence resonance energy transfer (FRET), which was first described in 1948 (Ref. 8) and was soon recognized as a good method for enzyme kinetics as well as end-point determinations. The first working immunoassay (FETI) was described by Syva in 1976 (Ref. 9).

FRET is based on the proximity of two labels brought together by a specific binding reaction. With a suitable label pair, the excited energy of the donor label is transferred by a nonradioactive resonance mechanism to an acceptor molecule, provided that they are at a distance of less than 10 nm from one another and that they overlap energetically[8–11]. The main disadvantages of FRET are the narrower transfer distance compared with conventional organic fluorescent probe chemistries, and the fact that there is no way to separate the emission of an acceptor excited by energy transfer through the donor from the emission derived from its direct excitation[11]. In addition, the vulnerability of conventional fluorometry to the matrix and disposable derived interferences can limit the sensitivity. Only since the advent of time-resolved fluorometry (TRF) and the use of donor–acceptor pairs of differing decay times has FRET become a technique suitable for wider application in HTS.

Fluorescence polarization

Fluorescence polarization (FP) technology is based on measurement of the depolarization of emissions from fluorescent labels irradiated with polarized light. This depolarization is a function of rotational movements of molecules during their excited state lifetime (generally in the range 3–5 ns). FP has undergone a renaissance in HTS, mainly as a result of the sterling work of Jolley[12] and coworkers over the years. It has had most success in small-molecule binding experiments [originally in fluorescence polarization immunoassay (FPIA) of drugs], but with new optimized labels, such as Texas Red and Pobidy, it has also found application in protease analysis with labelled casein or peptides. As a true homogeneous assay, FP is rapid and simple, but applications are limited to analyses where there is considerable change in rotational movements – either the binding of a small ligand to a larger receptor or the dissociation of a larger complex into smaller fragments.

Fluorometry in screening

Fluorometry is a very powerful and viable nonradioactive technology for screening. All fluorescence technologies (FP, FRET and FCS – fluorescence correlation spectroscopy) are now being applied to drug discovery[13].

One of the strengths of fluorometry is its versatility. The parameters provided by fluorometry include emission intensity, the wavelengths, temporal parameters (rise and decay times), energy transfer in measuring distances, polarization for rotational movements and bleaching recovery for lateral movements, quenching caused by a variety of compounds (in oxygen sensors, pH sensors, etc.) and very high spatial resolution in two- and three-dimensional matrices. Fluorometry can also be very sensitive, and the ultimate limit of sensitivity – detection of a single molecule – has repeatedly been reported[14,15].

The inherent sensitivity of fluorometry to external interferences derived, for example, from the sample matrix has, however, prevented its use in areas requiring robust and highly sensitive analysis. Conventional fluorometry employs probes that give short Stokes' shifts (frequency of radiation used to produce the excited state minus frequency of fluorescence emission) and decay times, and is hence prone to interference from effects such as autofluorescence, scattering and backgrounds of microtitration plates and optics[16]. Interesting developments are taking place in the design of new fluorescent probes, probes with wider Stokes' shift (e.g. tandem probes)[17], near-infrared emissive probes[18] and various fluorogenic substrates applied in enzyme analysis and used in sensitive enzyme immunoassays. Also, a large number of microtitration plate fluorometers have been developed by a number of companies, although there are still few instruments available for time-resolved counting of labels such as the lanthanide chelates (Table 1).

The advent of temporal resolution in a time domain of milliseconds has been the major factor allowing the development of highly sensitive and robust fluorometric assays[19,20]. The synthesis of lanthanide chelates with long fluorescence lifetimes[21] has played an essential role in that development, assay development being paralleled by the development of new lanthanide chelates.

Time resolution

The additional feature available in fluorometric analysis – time – has become very important in modern assays, particularly in different types of TFR analysis. Traditionally, temporal resolution has been applied in phosphorometers, for example, to discriminate the generally weak phosphorescence from the bulk of prompt fluorescence[22]. There are various ways in which the temporal features of emission can be utilized. Single-photon recording at nano- and picosecond time resolution is used to analyse rapid transitions[23]. Another temporal resolution is accomplished using sinusoidally modulated excitation with different frequencies (phase-resolved fluorometry[24]) and following either demodulation or phase shift of the emission. Phase-resolved fluorometry has been applied in analysing rapid decays,

Table 1. Counting devices for lanthanide time-resolved fluorescence measurement

	Label ions	Plate format	Other label technologies	Stackers
Wallac 1234	Eu, Sm, Tb	96	None	None
Wallac 1420, Victor MLC	Eu, Sm, Tb, Dy	1 (petri dish) to 384 (864 option)	Fluorescence/ luminescence photometry	Yes
SLT, SpectoFluor	Eu	6–384	Fluorescence photometry	None
BMG and SLT, FLUOstar	Eu	6–384 (864 option)	Fluorescence photometry	None
Packard Discovery	Eu	96, 384	None	Yes
Perkin-Elmer LS-50	Eu, Sm, Tb, Dy	Tube with plate option	Luminescence	None

Information based on commercial literature.

changes in decay times and, recently, in lifetime imaging[24].

For time resolution in micro- or millisecond time domains long-lifetime fluorescent (luminescent) probes, short pulsed excitations and delayed detection are used. The primary use of this method is to provide more sensitive detection systems by avoiding background interference[16]. However, the system requires luminescent probes with decay times that are substantially longer than those of the background fluorescence. Chelates of certain of the lanthanides possess these long decay times and have made TRF a viable alternative[19].

Lanthanide chelates

The lanthanide series comprises metals with a $(4f)^n$ electronic configuration, where the unfilled 4f orbitals are largely shielded from the environment and are not involved in bonding. Lanthanides therefore possess very specific magnetic and electronic properties. The ions in the middle of the lanthanide series, mainly europium (Eu), terbium (Tb), samarium (Sm) and dysprosium (Dy), have sharp electronic transitions, are able to accept excitation energy from the surroundings (from the ligand field) and produce their typical narrow-banded emissions with exceptionally long Stokes' shift and excited-state lifetimes ranging from a few microseconds for dysprosium chelates to over 2 ms for highly fluorescent europium and terbium chelates[19].

Because of their unique fluorescent properties and their relatively small size and hydrophilicity, lanthanide chelates are able to form perfect labels (Figure 1). Lanthanides do not, however, easily form chelates that are kinetically or thermodynamically very stable, which sets high requirements on the design of suitable labels. Other concerns relate to light harvesting or energy absorption, which has to be accomplished by the organic ligand, followed by energy transfer from excited ligand to chelated ion, ion protection, avoidance of internal and external quenching, as well as demands set by the biological assay system[19].

Heterogeneous TRF assays

Starting from theoretical ideas about their potential application as labels in the early 1970s (Refs 25,26), through the development of the first working system – dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA) – in the early 1980s (Ref. 5), lanthanides have found widespread application, particularly in neonatal and prenatal screening where both immunological and hybridization recognition reactions are utilized. Today, at least half a dozen additional TRF assay technologies are available for routine diagnostic use (Wallac Oy, CyberFluor, Cis-Bio)[5,19,27–30]. The first assay system, DELFIA, is still the most widely used (Table 2). It is a technology based on two different chelates: the first, a stable hydrophilic chelate optimized for labelling the target molecules, and the second, a highly fluorescent β-diketone chelate, formed after completion of the binding assay[5]. As a heterogeneous assay performed on a 96-well microtitration plate, it has been applied in competitive and noncompetitive immunoassays[19,20], in DNA/RNA hybridization[31,32], enzyme analysis[33,34], cellular applications[35] and receptor–ligand interactions[36,37]. The assay design has the advantage that it does not include any compromises in chelate design with respect to size, hydrophilicity and charge. Different chelates can be optimized for each type of binding assay according to the individual requirements. Accordingly, chelates have been developed that are optimized for immunoassays[38], DNA hybridization assays[39], europium release in cytotoxicity

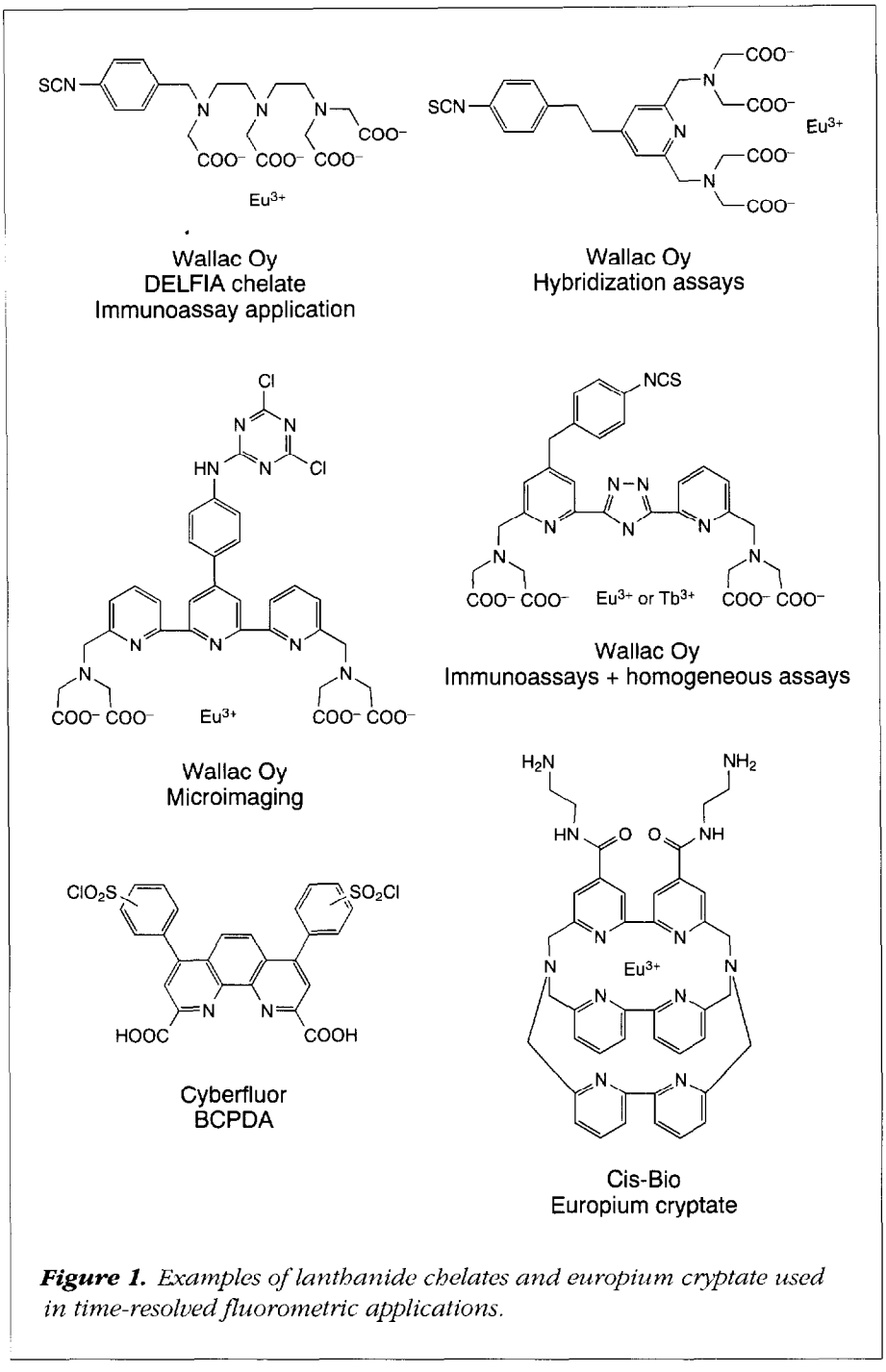

Figure 1. *Examples of lanthanide chelates and europium cryptate used in time-resolved fluorometric applications.*

assays[35] and *in vivo* experiments such as pharmacokinetics[40]. Regardless of the target, type of assay and labelling reagent, the dissociative fluorescence enhancement solution[5] creates an optimally fluorescent solution with high quantum yield (>70% with europium), long decay time (730 μs) and sensitivity down to a few attomoles (10^{-18} mol) per well. In addition, DELFIA can incorporate up to four labels in simultaneous quadruple-label assays[41], a feature that is particularly important in high-throughput genomic screening for analysing a number of point mutations simultaneously[42].

Multilabel assays have real application possibilities for HTS. Scientists from Xenova have published details of a dual-label screen[43] where the second label acted as an internal control to identify false positive wells that would otherwise be incorrectly recorded as a 'hit'. This work has led Xenova to develop screens where the quality of the 'hits' observed can be automatically checked, thus eliminating false results and unnecessary retesting.

The extensive research on lanthanide chelates[21,30,44–46] that incorporate high absorption, efficient energy transfer and hydrophilic properties into one single structure without compromising any of them has resulted in alternative assay technologies (Table 2), including assays with direct surface measurement (immunofluorescence, fluorescence *in situ* hybridization, microimaging and microfluorometry) and different forms of homogeneous assays.

Homogeneous TRF assays

Homogeneous assays in particular would benefit from time resolution because all components of the samples are present during measurements. Samples such as human plasma or serum contain a myriad of interfering fluorescent compounds in addition to their high scattering interference, which renders development of sensitive homogeneous fluoroimmunoassays based on conventional fluorometry almost impossible[16]. TRF brings new perspectives to homogeneous assays. The first homogeneous TRF assay for serum analysis was based on environmentally sensitive europium or terbium chelates, the fluorescence of which can be modulated separately when bound to antibodies or free in solution (hapten-labelled; Figure 2a and 2b and Table 3)[48,49]. This assay format is simple, rapid and easy to optimize. The most suitable assay formats for fluorescence-modulated

Table 2. Key references for heterogeneous time-resolved fluorometric assay technologies

Assay principle	Label ions	Refs
Dissociative enhancement (DELFIA)	Eu, Sm, Tb or Dy	5,19,20
Dissociative multilabel	Eu, Sm, Tb and Dy	41
Desorptive enhancement (LANFIA)	Eu or Tb	29
Ligand as label, BCPDA (FiaGen)	Eu	27
Ligand as label, TDA in cell labelling	Eu	35
Time-resolved fluorometric enzyme immunoassay, chelating substrate	Tb	28
Direct measurement of cells or tissues	Eu or Tb	47
Direct measurement of beads	Eu and Tb	32

BCPDA, see Figure 1; DELFIA, dissociation-enhanced lanthanide fluoroimmunoassay; LANFIA, lanthanide fluoroimmunoassay; TDA, terpyridine diacetate.

chelates are small haptenic antigens or small ligands, where the environment of the tracer is changed upon protein binding.

Time-resolved FRET (TR-FRET; Figure 2c) is another homogeneous TRF principle. The assay utilizes probes with different decay times to avoid the general problem of interference from directly excited acceptor emission[11]. The advent of fluorescent, long excited state lifetime lanthanide chelates makes this a fascinating alternative. The homogeneous TRF developed by Cis-Bio[30] is based on the low photon emission efficiency of europium cryptate as donor, and a signal-amplifying fluorescent phycobiliprotein (derivative) as acceptor. The donor–acceptor pair described by Mathis[30] leaves space for further improvement with regard to donor quantum yield, decay time, hydrophilicity and emission distribution (centralized emission into donating transition). Unfortunately, the use of high millimolar concentrations of fluoride ions needed to reduce water quenching of the cryptate luminescence appears to limit its application. This addition of an antiquench agent is not required for assays that use hydrophilic labels (e.g. 9-dentate lanthanide chelates).

As well as europium and allophycocyanine (A-PC), new acceptors are being published, such as rhodamine for terbium[50–53], and an infrared-emissive carbocyanine dye (Cy-5) for europium[51–53]. The great variety of europium and terbium chelates synthesized has also served as a platform for the development of time-resolved energy-transfer based assay systems for immunoassays (e.g. for prostate specific antigen and human chorionic gonadotrophin), receptors[54], protein kinases and proteolytic enzymes[55], with various organic compounds as acceptors (rhodamines, carbocyanines, phycobiliproteins). In addition, the combination of a lanthanide chelate with a quenching spin label has been exploited for enzyme kinetics studies (Figure 2d)[55].

Regardless of the robust detection of energy transfer by TRF (no matrix-related background fluorescence), the very variable matrices used in compound libraries may still cause quenching problems in some homogeneous assays (e.g. coloured compounds). The schematic energy flow diagram in Figure 3 shows the critical processes of the FRET assay and highlights the transitions potentially sensitive to matrix-related interferences. For time-resolved measurement, the most common interference relates to high UV absorbances and excitation attenuation, but in screens containing coloured extracts of natural products there is a risk that up to 20% of constituents are compounds exhibiting long wavelength absorbtion. To be able to correct for all different samples, a multiparametric analysis is required, including fluorometric and photometric measurements to adjust for all types of interference.

Table 3. Key references for homogeneous time-resolved fluorometric assay technologies

Assay principle	Label ions	Refs
Fluorescence modulation	Eu or Tb	48,49
TR-FRET, cryptate with A-PC	Eu	30
TR-FRET, chelates with various acceptors	Tb	51,53,54
Fluorescence quenching	Tb	55

A-PC, allophycocyanine; TR-FRET, time-resolved fluorescence resonance energy transfer.

Automation

In HTS, the major goal is the total automation and increase of throughput. In contrast to to diagnostics, where random-access single-tube systems have largely been adopted, the HTS

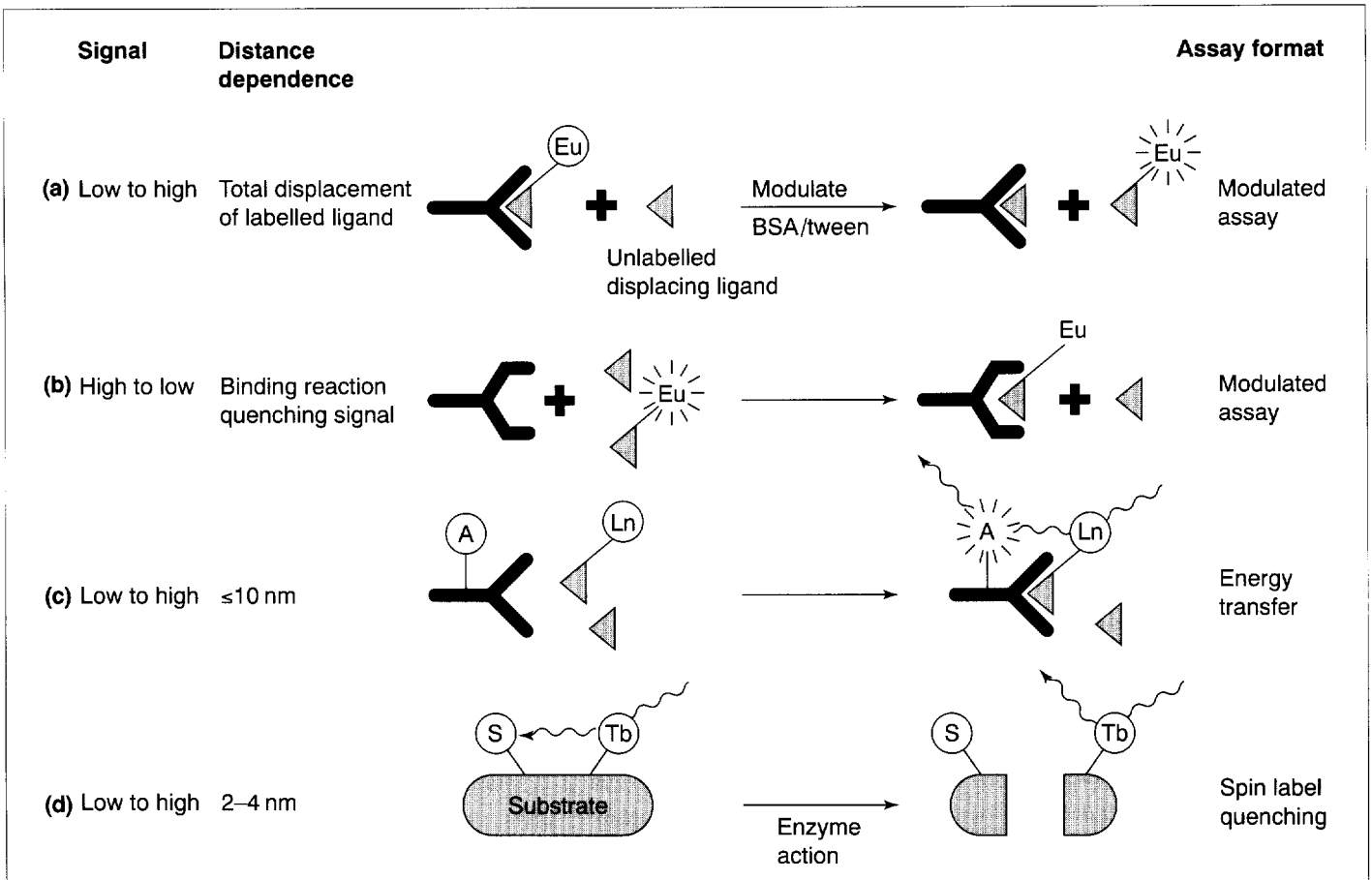

Figure 2. *Principles of time-resolved fluorometric homogeneous assays based on environmentally sensitive chelate labels or energy transfer. A, acceptor molecule (e.g. tetramethylrhodamine, A-PC or Cy-5); BSA, bovine serum albumin; Eu, europium chelate; Ln, terbium or europium chelate; S, spin label.*

applications are, by definition, high-throughput assays and accordingly development has moved towards totally automated batch-analysing systems based on the microtitration plate. The wide variation in assay designs and tailor-made protocols, however, requires an open system that can easily be adapted to different protocols; automation through robot workstations has consequently been the answer. The black-box type integrated automates have not yet found applications in HTS. The requirements in HTS are flexibility, adaptability to a large number of assay formats, and a wide range of sensitivity demands, albeit not as stringent as in some diagnostic assays. The major trend is to move away from the use of radioisotopes, not so much because of their performance, but mainly because of regulatory waste problems and assay speed.

Microtitration plates are now the standard accepted format, and all of the required apparatus, such as dispensers, washers, harvesters and measuring devices, is available for both diagnostic clinical and HTS utilization. Despite the tendency towards higher throughput densities, the microtitration plate remains the standard footprint for future assay development. Current instruments are mainly defined for microtitration plates of 6–864 wells.

Whether future development will proceed towards ever-increasing well densities based on the current footprint of the 96-well plate (a frequent discussion point at conferences) or towards more open 'free' formats remains to be seen. Interestingly, the instrument manufacturers will play a greater role in (maybe limiting some) future developments than is often considered by screening laboratories.

A major feature of the current robotic automated lines is that they can easily be used for very different technologies such as those based on homogeneous solution chemistry, a heterogeneous solid-phase format or filtration harvesting – a modular approach has therefore been the key. Similarly, the same system can usually handle many forms of signal production, from simple photometry (enzyme immunoassay or

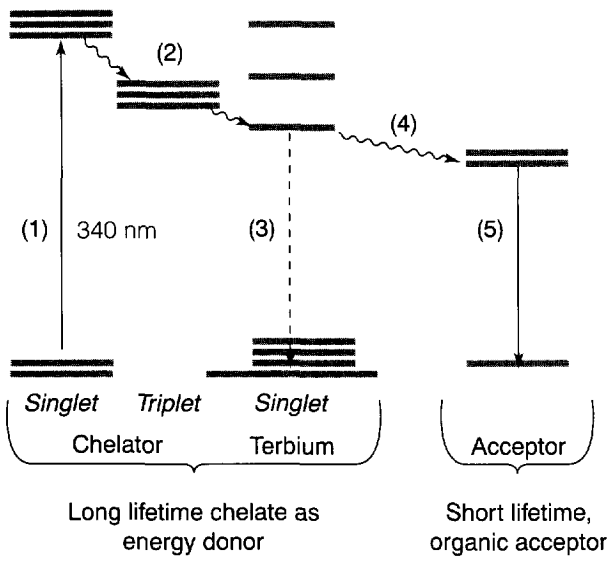

Figure 3. *Energy flow in time-resolved fluorescence resonance energy transfer. The potential interference points are highlighted by numbers: (1) excitation and its attenuation by coloured compounds; (2) energy transfer from the singlet level of the chelator through its triplet level to the chelated ion, with interference, for example, by triplet level quenchers; (3) long lifetime donor emission, with possible interference by colours or by direct quenching of the ion; (4) resonance energy transfer and competing processes; (5) energy-transfer-related acceptor emission, and possible quenching.*

ELISA), fluorometry in cellular assays, fluorescence polarization in FPIA, luminescence with luminescent substrates, reporter gene assays and time-resolved fluorescence. This is in addition to beta-counting capabilities for assays such as SPA where, because of size or coupling difficulties, alternative nonradioactive technologies do not exist.

One solution to coping with the various detection requirements is the integration of multitechnology (or multilabel) detectors within one box. Combining multiple detection modes in one instrument not only makes it more versatile and saves space, but provides the facility to run various forms of multilabel assays and to control matrix interferences in homogeneous fluorometric assays. By using simultaneous photometry, the excitation or emission attenuation by coloured samples can be monitored and corrected, and combining prompt fluorometric analysis with time-resolved analysis of decay times will give additional information about all possible interfering processes that might occur with aggressive coloured samples.

Future of TRF in HTS

Clearly, the main trend is towards increasingly rapid assays and the analysis of yet larger libraries. Presentations given at recent HTS conferences (for example the NMHCC Meeting held in January 1997 and Biochip Arrays, March 1997) reveal that, if the trend for larger sample screening is to continue, assay volumes must decrease to economically viable amounts (costs and limiting amounts of library compound dictate this conclusion). The increasing number of conferences on the newly termed 'nano' technology reflects one extreme future assay scenario. However, the converse situation could occur, whereby rational use of multilabel assays, genomics, computer modelling and neural networks would eliminate the need for many repetitive assays, thus decreasing the need for screening of large numbers. This would decrease economic pressure to reduce assay volume and give the 96-well plate a longer shelf-life than is currently predicted. However, the need to screen more samples more quickly may push the design of assays far beyond the current constraint (i.e. keeping the assay in a standard plate footprint) to new approaches – for example, conveyer systems turning screening laboratories into virtual screening factories.

Nano assays, however, are still in their infancy. The ability of the current liquid-handling systems to cope with nanolitre volumes is poor because of problems with sample solubility, variable viscosities, etc. There is a need for a new generation of dispensing and aspirating systems, albeit still based perhaps around piezoelectric dispensers (ink-jet technology). Measurement devices will need to alter dramatically in concept to cope with the high-density sample matrices predicted for the future. The most likely systems to emerge will be imaging of whole plates, or even the free format assay, assuming that the combination of homogeneous TRF assay in a 384- or 864-well plate detected by imaging does not have the required sample throughput. This leaves free format assays as a possibility.

The free format assay has been around for some years, and was utilized initially by cell biologists for quantitative pathology *in situ*. If we picture uniquely tagged individual assay droplets or beads instead of tagged cells, we can then envisage a potential HTS format for the future. If this scenario is taken further and existing multiparametric TRF assay technology is applied, each assay array could be measured in a few seconds. In real terms, this could translate to the simultaneous measurement of thousands of individual reactions in an area of a few square centimetres. Without the fixed sample format of the microplate, this type of assay

system must have a multiparametric design so that in the first phase of measurement the unique tags are identified and logged, and in the second phase all the reactions are simultaneously quantified and the results assigned to the logged samples. This format is already being developed with time-resolved label technology for future diagnostic systems[32].

However, the common trend both in diagnostics and in HTS is towards miniaturized devices able to analyse multiple parameters from submicrolitre volumes of samples. The ideas presented vary from multiparametric dual-label ratio measurement designs[56–58] and imaging of arrays (chips and beads) to multiparametric or multiplexing assays using micro or nano beads in a capillary micro time-resolved fluorometer[32]. There are now many companies in diagnostics, genomics and HTS with clear ambitions in this area of research.

Some established remote-sensing technologies will evolve to second- and third-generation systems, in particular FCS and, of course, FTIR (Fourier transform infrared) spectroscopy, which can combine spatially resolved diffuse absorbance FTIR with modern data visualization (hyperspectral analysis) software[59]. This is the first remote-sensing technique to be applied to routine screening and could potentially replace some traditional fluorescence and time-resolved fluorescence label methods.

In all of the current and future developments in label chemistry, TRF, because of its ability to combine high spectral resolution and potential sensitivity to high submicro-metre spatial resolution, is the logical detection method.

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