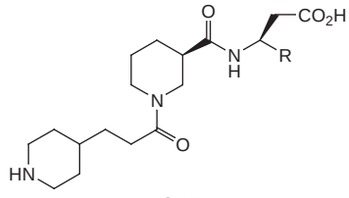
platelet aggregation, atherosclerotic plaque formation and, ultimately, thrombosis. In the process of thrombus formation, the final crucial step is the adherence of the protein fibrinogen to the activated membrane-bound glycoprotein GPIIb/IIIa. Compounds that can compete with fibrinogen for the platelet glycoprotein receptor are potential antithrombotic agents. In the search for orally active agents that have the potential to be used for chronic cardiovascular care, a group from the R.W. Johnson Pharmaceutical Research Institute have used combinatorial chemistry to optimize a prototype fibrinogen receptor antagonist[3].

Solid-phase parallel synthesis, employed in the preparation of approximately 250 analogues of compound (**vi**), led to the discovery of RWJ53308 (**vii**). This compound is an antiplatelet



vi R = H
vii R = 3-pyridyl

agent that can be administered both intravenously and orally, and has a long duration of action. The compound has been successfully progressed through Phase II clinical trials.

3 Hoekstra, W.J. *et al.* (1999) Potent, orally active GPIIb/IIIa antagonists containing a nipecotic acid subunit. Structure–activity studies leading to the discovery of RWJ-53308. *J. Med. Chem.* 42, 5254–5265

Nick Terrett
Discovery Chemistry
Pfizer Central Research
Sandwich, Kent, UK
fax: +44 1304 655419
e-mail: nick_terrett@sandwich.pfizer.com

High-throughput screening

Evolution of Homogeneous Time Resolved Fluorescence (HTRF) technology for HTS

Increasing demand for drug candidates has led pharmaceutical companies to identify alternative strategies for improving the efficiency of HTS. One of the key technologies that has assisted the impressive development of HTS since its implementation is the use of fluorescence-based assays. In addition to being relatively inexpensive, the main advantages of these detection systems include sensitivity, versatility, stability, safety and ease of disposal.

However, two key problems are associated with the detection of fluorescence signals. Firstly, the presence of quenchers in the reaction can inhibit the signal generation. Secondly, autofluorescence from free probes or contaminant components can mask the signal. These limitations have been overcome to some extent by altering specific aspects of the fluorescent measurement such as the fluorescence lifetime, the energy transfer, or the anisotropy, enabling quench or autofluorescence correction adjustment.

The Homogeneous Time Resolved Fluorescence (HTRF) technology, developed by CIS-BIO International (Bagnols/Ceze Cedex, France, is one of the four main fluorescence-based technologies that are used in HTS (Ref. 1). The selectivity of the signal detection combined with the powerful data-analysis system enables not only the development of homogeneous assays but also the generation of a readout, corrected for the non-specific effects of the media.

Principle of the HTRF technology

HTRF uses a Eu^{3+} ion caged into a polycyclic cryptate (Eu–cryptate) as a donor. Laser exitation at 337 nm transfers the energy from the Eu–cryptate complex to an allophycocyanin acceptor molecule, the APC. This results in the emission of light at 665 nm over a prolonged time (milliseconds). This light emission is recorded in a time-resolved fashion over a 400 μs period, starting 50 μs after the excitation pulse so that the autofluorescence from the media and the short-lived fluorescence of the free APC are not recorded.

The modulation of the energy transfer depends on the distance between Eu–cryptate and the APC. The signal emitted by free molecules labelled with Eu–cryptate and APC is not recorded because they are not in close proximity during the required period of time. The Eu–cryptate emission peak at 620 nm is also recorded and used as an internal control. The data are expressed as a ratio of the fluorescent signal at 665 nm (coming from energy transfer between the two bound molecules) to the signal at 620 nm. This ratio of flourescence is proportional to the quantity of biological complexes present in solution (Fig. 1)[2].

The evolution of HTRF in HTS

Since its validation and implementation in HTS, the HTRF technology has undergone constant evolution (Fig. 2). Initial applications for the technique were developed using classical biochemical formats to assess, for example, binding of epidermal growth factor (EGF) to its receptor, the JUN/JOS protein–protein interaction, and for use in various enzymatic assays[2].

The development of generic formats for HTRF biochemical assays became a necessity as the demand for higher throughput in HTS increased and there was more dependence placed on the vendor for the labelling of reagents and for expertise. A complete HTRF generic test requires:

- Generic tools such as anti-tag antibody or streptavidine linked to the cryptate or APC tracers that will recognize the tagged proteins or the biotinylated antibodies
- Generic protocols
- Generic formats for a whole family of

  PII: S1359-6446(00)01491-4

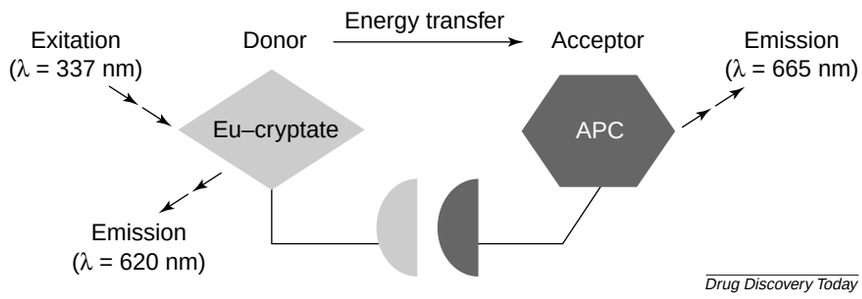


Figure 1. *Schematic representation of the principle of Homogeneous Time Resolved Fluorescence (HTRF). The time-resolved measurements significantly enhance the signal-to-noise ratios, because of a difference between the fluorescence lifetime (milliseconds) of the energy transfer and the lifetime of impurities of the media (nanoseconds). The ratiometric expression of the signal (signal at 665 nm/signal at 620 nm) reflects the specificity of the signal and is not dependent on the apparatus.*

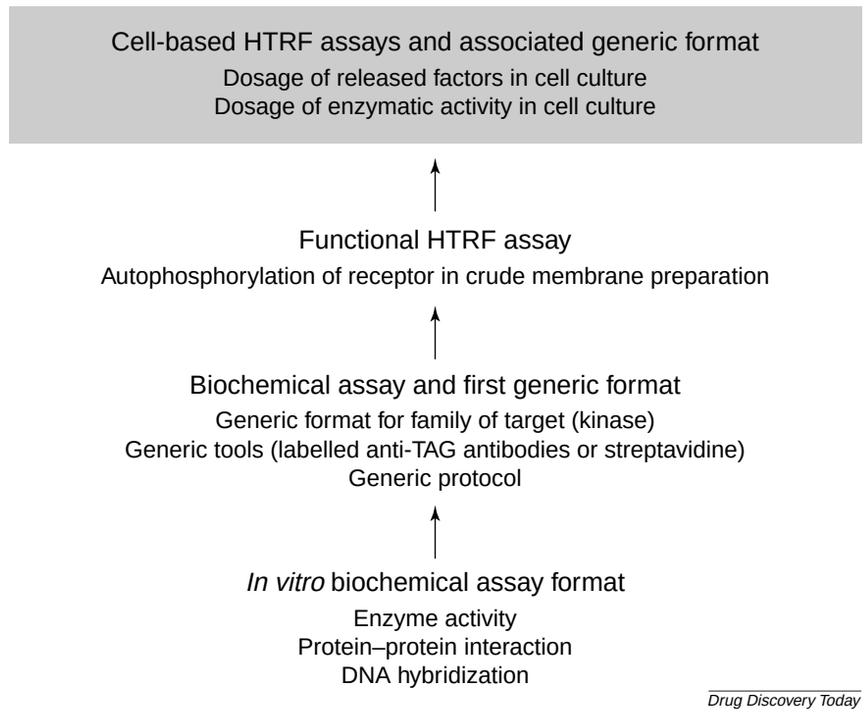


Figure 2. *Evolution of the Homogeneous Time Resolved Fluorescence (HTRF) format from biochemical assay to generic cell-based HTRF test. The flexibility and the potential of this technique enables its adaptation to continuous changes in HTS to answer the pressures to deliver more high-quality lead compounds.*

targets (kinases, phosphatases, protein–protein interactions).

The improvement of the HTRF technology by the addition of one or more generic characteristics to the tests, significantly increased the efficiency of the HTS phase without altering its cost (Pernelle, C. Protocol development: set-up, validation and optimization of procedures for HTS assays. *IBC Global Conference on Drug Discovery Technologies*. 26–28 April 1999, Amsterdam, The Netherlands).

In parallel with the race for improved throughput and miniaturization, there has been an increased demand for high-quality drug candidates. The incorporation of cell-based assays in HTS appeared as one method of isolating more physiologically relevant compounds early in the drug discovery process.

Keeping the efficiency of HTS in mind, the strategy to improve lead generation was therefore to combine the fluorescence-based detection systems with the cell-based assays to meet all qualitative and quantitative HTS criteria. In response to these needs, several companies proposed innovative specific screening platforms for fluorescent cell-based assays. However, most of these platforms lack versatility in their application and require large investments.

By contrast, the classical fluorescent-based methodologies used in HTS such as fluorescence polarization (FP) and fluorescence correlation spectroscopy (FCS) are not easily adapted to cell-based assays[3]. The HTRF technology is an exception and the literature is increasingly reporting the use of HTRF cell-based assays in HTS. For example, the release of factors in cell culture media is monitored using HTS across a broad range of therapeutic areas ranging from the CNS (amyloid peptide release) to inflammation and oncology (cytokine release)[4]. HTRF cell-based assays for the measurement of receptor autophosphorylation events, protein–protein interactions or kinase activity on cell culture have been, or are, in development (Grepin, C. Combining cell-based assay and homogenous time-resolved fluorescence (HTRF) to develop relevant and miniaturized functional tests for HTS. *IBC Global Conference on Effective Assay Development*. 7–8 July 1999, London, UK).

Future development of HTRF

The HTRF technology has been shown to be particularly flexible throughout its use in drug discovery. After its successful adaptation to miniaturization and automation thanks to its homogeneous and sensitive format, HTRF is now being adapted for use in cell-based assays at no extra cost. This technology enables the generation of high-quality data with a throughput of 50,000-100,000 samples-per-day. The future lies in the development of ultra-HTS (uHTS). The HTRF technology is already validated in 1536-well plate format for biochemical assays, and it is likely that cell-based HTRF assays will follow soon.

1 Kolb A. *et al.* (1997) A homogenous, time-resolved fluorescence method for drug discovery. In *High Throughput Screening, The Discovery of Bioactive Substance* (Devlin, J.P. and Kolb, A., eds), pp. 345–360, Marcel Dekker

2 Mathis, G. (1995) Probing molecular interactions with homogenous techniques based on rare earth cryptates and fluorescence energy transfer. *Clin. Chem.* 41, 1391–1397

3 Rogers, M.V. (1997) Light on high-throughput screening: fluorescence-based assay technologies. *Drug Discovery Today* 4, 156–160

4 Mathis, G. (1993) Rare earth cryptates and homogenous fluoroimmunoassays with human sera. *Clin. Chem.* 39, 1953–1959

Claudine Grepin and
Christine Pernelle
AVENTIS pharma
Lead Generation/HTS
13, quai Jules Guesde
94403 Vitry Sur Seine
France

tel: +33 1 55 71 32 44
fax: +33 1 55 71 30 63
e-mail: claudine.grepin@aventis.com
or christine.pernelle@aventis.com

About *Monitor*...

Monitor provides an insight into the latest developments in the fields allied to drug discovery through brief synopses of recent publications and presentations together with expert commentaries on the latest technologies. There are two sections:

Molecules summarizes the chemistry, pharmacological significance and biological relevance of new molecules reported in the literature and on the conference scene.

Profiles offers commentary on promising lines of research, new technologies, emerging molecular targets, novel strategies and legislative issues.

We welcome topical contributions for inclusion as *Profiles*. Articles of approximately 500–1000 words in length should provide an accurate summary of the essential facts together with an expert commentary to provide a perspective. Authors should be aware that articles for publication in *Monitor* are subject to peer-review, and occasionally *Monitor* articles might be rejected or, as is more likely, authors could be asked to revise their contribution. Articles might also be edited after acceptance. Proposals for articles should be directed to: Dr Andrew W. Lloyd, *Monitor* Editor, Department of Pharmacy, University of Brighton, Moulsecoomb, Brighton, UK BN2 4GJ. tel: +44 1273 642049, fax: +44 1273 679333, e-mail: A.W.Lloyd@brighton.ac.uk

In the May issue of *Pharmaceutical Science & Technology Today*...

Update – latest news and views

Therapeutic applications of colloidal drug carriers
Gillian M. Barratt

Brain delivery technology: novel approaches for transporting therapeutics
Jamal Temsamani, Jean-Michel Scherrmann, Anthony A. Rees and Michel Kaczorek

Optimal process design for the manufacture of transdermal drug delivery systems
Hans-Michael Wolff

Monitor – process technology, drug delivery, analytical methodologies, legislative issues, patents, invited profile

Products