



FLUORESCENCE POLARIZATION

The Protein Company™

TECHNICAL RESOURCE GUIDE • THIRD EDITION



The Protein Company™

ABOUT PANVERA® CORPORATION

Located in Madison, Wisconsin, PanVera® Corporation, a subsidiary of Vertex Pharmaceuticals Incorporated, develops products and technologies that allow pharmaceutical companies to select and develop new drugs more rapidly and efficiently.

PanVera® has produced hundreds of recombinant proteins for commercial sale, focusing on protein families that are of broad interest from a therapeutic perspective, including nuclear receptors, protein kinases and drug metabolizing enzymes.

In addition to recombinant proteins, PanVera® provides fluorescence polarization-based assays for high-throughput screening, and contract services in protein manufacturing, assay development and drug metabolite production.

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FOREWORD

to the Third Edition

The theory of fluorescence polarization (FP) was first described by Jean Perrin in 1926 and expanded by Gregorio Weber and others in the 1950's. Surprisingly, even into the late 1980's this powerful technique was almost unknown to all but the diagnostic industry and biophysicists. Typically, the only exposure biochemists and molecular biologists have had to FP is a single chapter in Joseph Lakowicz's seminal work, "Principles of Fluorescence Spectroscopy".

In 1993, we set out to investigate FP for the study of biomolecular interactions and develop it as a core technology for PanVera®. Unfortunately, there were no instruments available that could measure FP easily. Typical FP instruments were hand-built from analytical fluorescence spectrophotometers and often required manual operation.

Realizing the need for an easy-to-use, sensitive, bench-top FP instrument we began marketing the Beacon® instrument in 1994. Two years later, we introduced the Beacon® 2000, which boasted better sensitivity than laser-based analytical instruments, used disposable glass test tubes, temperature controls, and a minimum volume requirement of only 100 μ L.

PanVera® published the First Edition of the Fluorescence Polarization Application Guide in 1995. It was filled with data generated on the Beacon® 2000 and how-to information. We knew that until FP was demystified for the at-large scientific community, the technology's full potential would not be realized. With this guide, it was our intention to empirically demonstrate the versatile nature of FP and how it could be used to observe a wide range of biomolecular interactions. FP differed dramatically from all other methods in use at that time in that it was a truly homogeneous technique, required no separation of bound and free species, no radioactivity, and allowed real-time measurements to be made directly in solution. We also highlighted some of the differences one must consider when designing FP-based assays. For example, FP differs from a traditional radioactive binding assay in that the small fluorescent ligand is held at a low concentration while the larger binding partner is titrated into it. Therefore, basic binding equations had to be discussed and modified slightly to accommodate these differences.

It did not take long for researchers in drug discovery to realize that FP is a format well suited for high throughput screening (HTS). Instrumentation is now available that can measure FP in high-density microplates very rapidly and with great precision. Assays require very few additions and no separation steps. No immobilization of reaction components is required, reducing the potential for artifacts generated by attaching molecules to solid supports. The method is non-radioactive, improving safety and reducing the costs associated with waste disposal. We continue to build on our extensive knowledge base and long history in this field to produce innovative assays for drug discovery.

It is our hope that the Third Edition of this Guide will help the novice gain a basic understanding of FP while serving as a desktop reference to the initiate.