Time-Resolved Imaging of Proteomics Gels

by

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Introduction

Two-dimensional gel electrophoresis is still the most widely used separation method to resolve complex mixtures of proteins in proteomic analysis

In large 2-dimensional gels a huge number of protein spots of widely differing concentration must be resolved. In addition it can be difficult to determine whether a given band is a single species or is a composite of overlapping spots.

Detection of Proteins on Electrophoresis Gels

For highest detection sensitivity it is common to stain gels with fluorescent reagents to visualise proteins

One of the most popular reagents used for this purpose is the proprietary 'Sypro Ruby' from Molecular Probes/Invitrogen

This is a reagent based on a divalent ruthenium complex that has relatively low 'fluorescence' unless bound to protein. The bound reagent emits strongly with a broad orange emission (c. 620nm peak).

The emission is a charge-transfer photoluminescence, not 'classical' fluorescence, and has a relatively long luminescence decay time (typically hundreds of nanoseconds to microseconds depending on environment).

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Environmental Sensitivity of Ruthenium Labels

Ruthenium emission is particularly sensitive to oxygen quenching and this has been used widely for oxygen sensing

At PRS we wondered whether ruthenium complexes bound to proteins might show differences in lifetime as a result of possible differences in their local environment

Experiments were therefore conducted on Sypro-Ruby stained proteins on gels (including some kindly provided by Molecular Probes Inc.)

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Time-Resolved Imaging Methodology

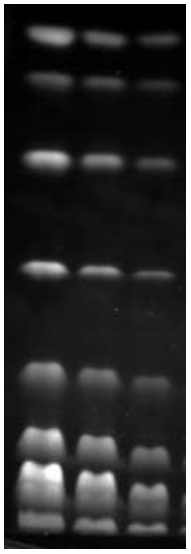
*Gels were excited with a **PRS100B** fast pulsed blue multiLED light source to excite the ruthenium complex in the Sypro Ruby.*

*They were imaged with the **Imagex-TGi** time-gated camera system to detect both prompt emission and emission delayed by a time which could be varied in the range of nanoseconds to microseconds.*

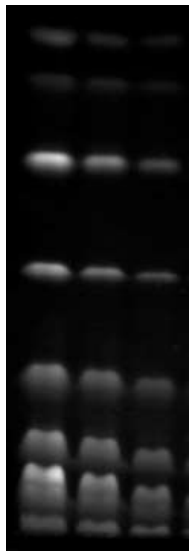
Typical results are shown in the following slide...

Fluorescence Lifetime Contrast Observed in a Sypro-Ruby Stained Protein Gel

Prompt emission



Delayed emission



Inter-image ratio operation

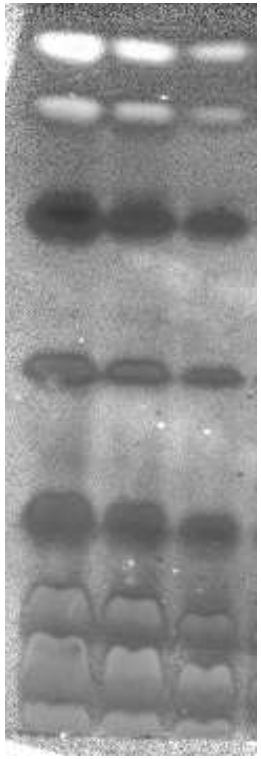


Image with Fluorescence Lifetime contrast

(lighter bands have shorter lifetimes: these change most between the prompt and delayed images)

Improving Visualisation of Fluorescence Lifetime Data

In order to show the true relative intensity of bands and to minimize the effects of 'noise' where lifetime information is calculated from regions having very little signal it is particularly convenient to encode lifetime in pseudocolour and then to multiply this image by the Intensity image.

This 'Intensity/Lifetime Matrix' image is shown on the next slide...

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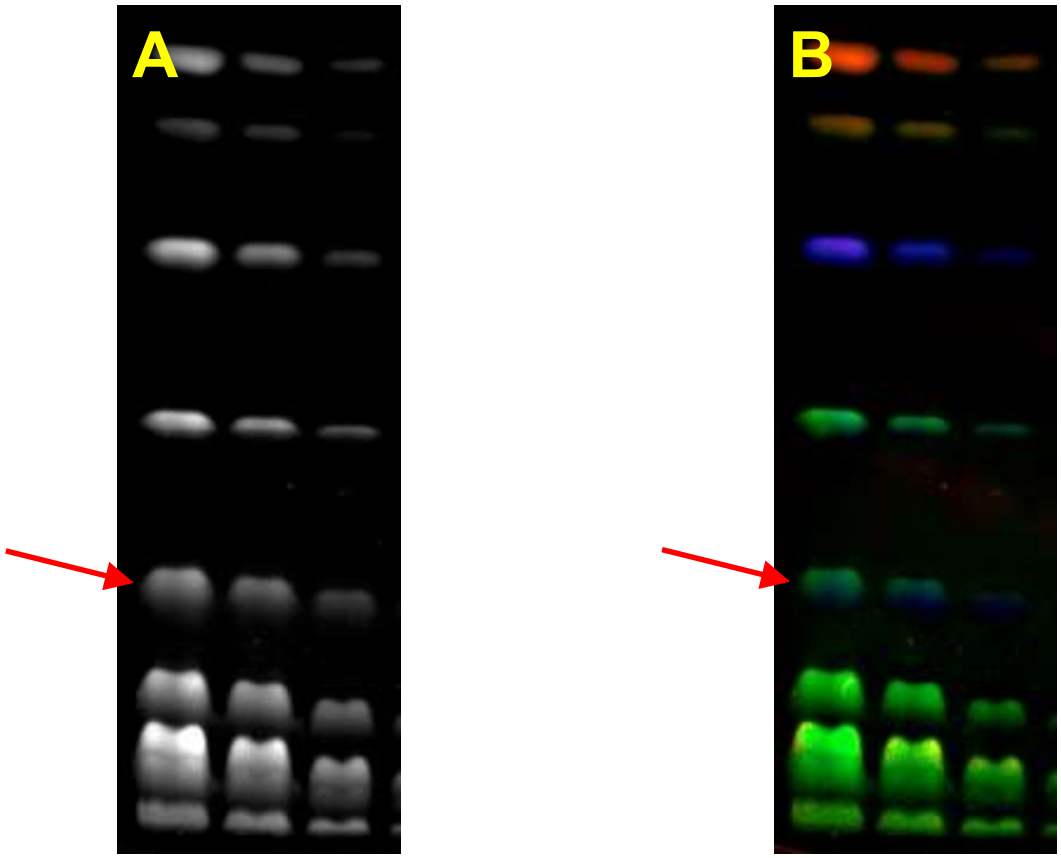
Intensity/Lifetime Matrix of Sypro-Ruby Stained Protein Gel



Intensity image (A) and a lifetime-intensity matrix (B) for protein bands stained with Ruthenium-based 'Sypro Ruby'. The intensity image is multiplied by a pseudo-coloured lifetime image to show intensity and lifetime simultaneously.

The lifetime differences may be useful to help resolve overlapping bands for Proteomics applications (e.g. see the band marked with an arrow)

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What is the Origin of Lifetime Contrast in Protein Gels?

At present we don't know what causes the contrast that we see. It may be that the local binding site for the ruthenium complex differs between proteins. One possibility is that the accessibility of label to oxygen depends on the local environment.

However variations of lifetime of stain between different proteins could in principle have consequences for quantification of protein bands. Alternatively it might provide a tool to help increase contrast between different types of protein. We have already shown that the variations in lifetime can help to show that an apparently single band is in fact a composite, as seen in the colour matrix image.