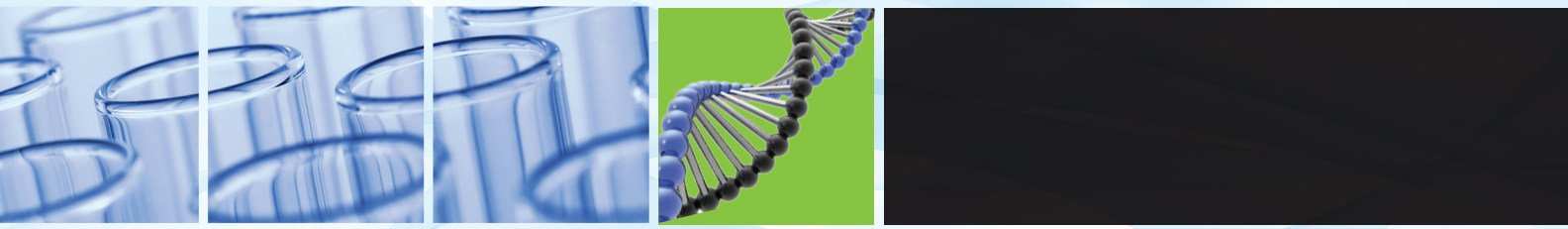
HORIBA Scientific

Detecting Conformational Rotamers via TCSPC

FL-22

ELEMENTAL ANALYSIS
FLUORESCENCE
GRATINGS & OEM SPECTROMETERS
OPTICAL COMPONENTS
FORENSICS
PARTICLE CHARACTERIZATION
RAMAN
SPECTROSCOPIC ELLIPSOMETRY
SPR IMAGING



Introduction

Among the possible fluorescence biosensors for medical and biochemical monitoring and imaging are the flavonoids, compounds that occur in many plants and their products, such as tea, chocolate, and red wine. Flavonoids recently have intrigued biologists, for they act as antioxidants in cancer and other diseases related to free radicals. Flavonoids bind to nucleic acids and proteins. Many flavonoids, like proteins, are fluorescent, thus setting the stage for experiments involving Förster resonance energy-transfer (FRET) between the two types of compounds when they bind together. Dr. Olaf Rolinski and Professor David Birch, at the University of Strathclyde in Scotland, have investigated complexation of the protein human serum albumin (HSA, Fig. 1) with the flavonoid quercetin (Q, Fig. 2), using time-domain fluorescence spectroscopy.[1]

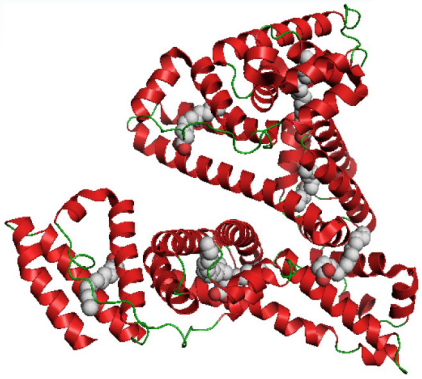


Fig. 1. Structure of HSA.

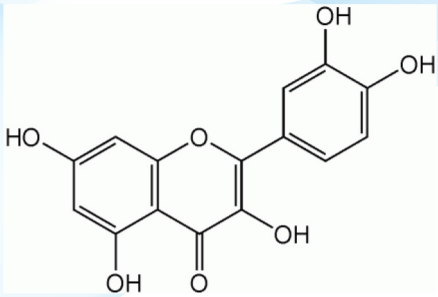


Fig. 2. Structure of quercetin.

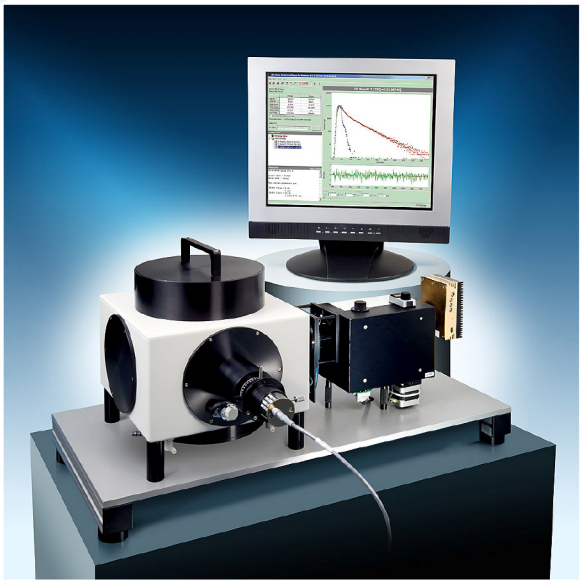


Fig. 3. 5000U lifetime spectrofluorometer system.

[1] O.J. Rolinski, *et al.,* "Human Serum Albumin and quercetin interactions monitored by time-resolved fluorescence: evidence for enhanced discrete rotamer conformations," *J. Biomed. Optics*, 12(3), 034013, 2007.

[2] C.D. McGuinness, et al., "Selective excitation of tryptophan fluorescence decay in proteins using a sub-nanosecond 295 nm light-emitting diode and time-correlated single-photon counting," *Appl. Phys. Lett.* 86 (2005), 261911–3.

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Experimental method

Aqueous 0.01-*M* phosphate-buffered solutions to pH 7.4 of HSA (30 µ*M*) mixed with Q dihydrate (0–60 µ*M*) were used. Both HSA and Q dihydrate were obtained from Sigma Aldrich.

Time-correlated single-photon counting (TCSPC) spectroscopy was performed using HORIBA Jobin Yvon's 5000U fluorescence lifetime system (Fig. 3). The excitation source was our NanoLED (λ = 295 nm, pulsewidth ~ 0.6 ns), run at 1 MHz, with a time per channel = 7.06 ps (Fig. 4).[2] This wavelength corresponds to the absorption of the sole occurrence of tryptophan in HSA (position 214).
The relation of the fluorescence decay to the observed data

Fig. 4. NanoLED pulsed excitation source used in HORIBA Jobin Yvon TCSPC spectrofluorometers.

[F(t), a convolution of the complex's fluorescence, I(t), and the excitation pulse's profile, L(t)] is found from the integral

$$F(t) = \int_0^t L(t')I(t-t')dt$$

Deconvolution was achieved with our exclusive DAS6 software, giving best fits via minimizing the goodness of fit, X^2.

Tri-exponential analysis

Fluorescence was recorded from the samples at 340 nm, the emission maximum from tryptophan. Data were fit using the least-squares method to a triexponential function with and without added Q to the solution. Results for HSA alone are given in Table 1.

Lifetime Component	Lifetime (ns)	Contribution (%)
1	0.793	2.05
2	2 4.089	44.59
3	3 7.145	53.35

Table 1. Data from a tri-exponential fit to tryptophan fluorescence decay in pure HSA.

These components are consistent with a widely accepted model of three rotamers, i.e., three conformations of tryptophan in HSA.[3]

As the amount of Q was increased in the solution, the lifetime components and their relative contributions changed (Fig. 5).

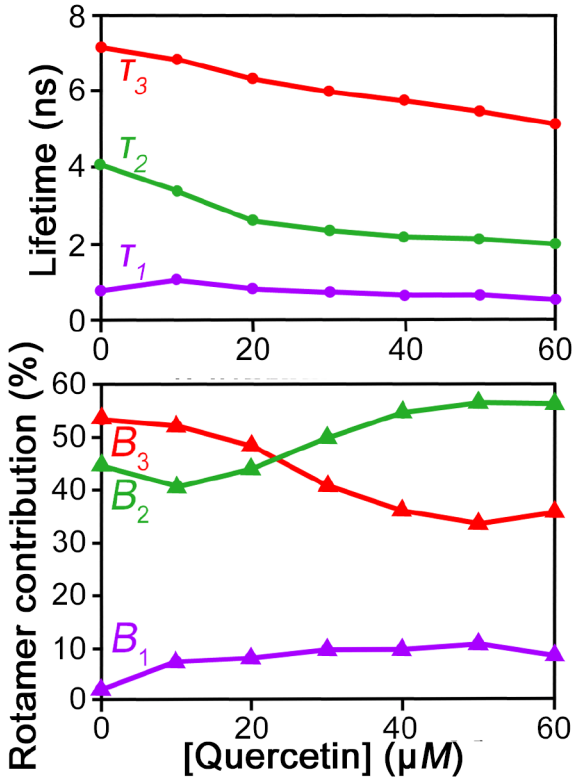

Fig. 5. Top: Change in each HSA lifetime as [Q] increases; bottom: change in relative contribution for each corresponding rotamer.

Statistically, X^2 was kept to 1.00 ± 0.05 for all cases, and the residuals appeared random.

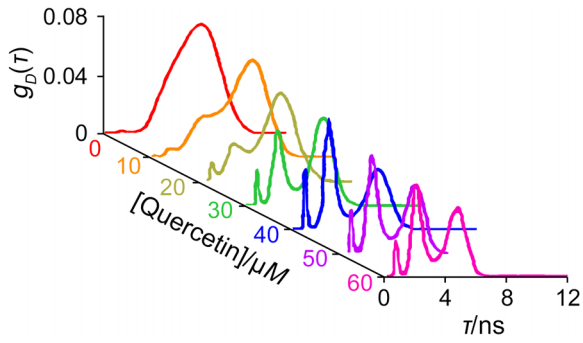

Fig. 6. Lifetime distribution function for HSA, with varying Q concentration (color-coded). λexc = 295 nm.

[3] A.G. Szabo and D.M. Rayner, "Fluorescence decay of tryptophan conformers in aqueous solutions," *J. Am. Chem. Soc.* 102, 2 (1980).

MEM analysis

Given the seemingly complicated kinetics, the data were re-analyzed using the less-restrictive Maximum Entropy Method (MEM)[4]. Here the data are considered without a predetermined superposition of components:

$$I(t) = \int_0^\infty \exp\left(-\frac{t}{\tau}\right) g_D(\tau) d\tau$$

where $g_D(\tau)$ is the fluorescence lifetime distribution function. This model, however, requires that $g_D(\tau)$ cannot be negative, thus the decay must have no rise time. In contrast, with a triexponential model analyzed via MEM, the three lifetimes would appear as sharp peaks with no half-width on a lifetime distribution versus lifetime graph. Actual data are shown in Fig. 6, which show a broad distribution of tryptophan conformations in the absence of Q. Rather than having fixed conformations, evidently the tryptophan can move fairly freely within pure HSA.

With the addition of Q, three definite lifetime peaks suddenly appear at 1.25, 3.34, and 6.25 ns. This is because the Q causes a change in the HSA's structure, fixing the tryptophan in certain rotamer conformations. As the concentration of Q rises, the lifetimes become more separated and shorter (Fig. 7), resulting from restricted tryptophan movements and more quenching through FRET. With the highest Q concentrations, lifetimes decrease further. Perhaps here quenching predominates over structural changes Rather than having fixed conformations, evidently the tryptophan can move fairly freely within pure HSA. With the addition of Q, three definite lifetime peaks suddenly appear at 1.25, 3.34, and 6.25 ns. This is because the Q causes a change in the HSA's structure, fixing the tryptophan in certain rotamer conformations. As the concentration of Q rises, the lifetimes become more separated and shorter (Fig. 7), resulting from restricted tryptophan movements and more quenching through FRET. With the highest Q concentrations, lifetimes decrease further. Perhaps here quenching predominates over structural changes.

Conclusions

Comparison of the simple triexponential deconvolution with the freeform MEM shows that they are qualitatively similar for HSA-Q binding. The MEM is more sensitive to environmental and structural changes in the complex. The HORIBA Jobin Yvon 5000U lifetime spectrofluorometer with TCSPC is an integral part of researching structure-property relations during protein-binding studies for the biochemical and medical fields.

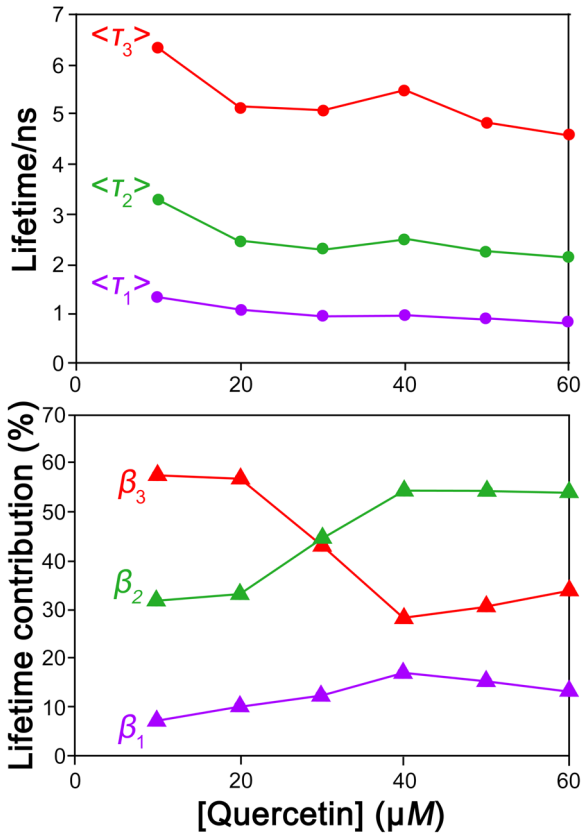

Fig. 7. Change in HSA tryptophan lifetimes (upper plot) and their relative contributions (lower plot), as Q concentration increases, via the MEM analysis.

[4] J.C. Brochon, "Maximum entropy method of data analysis in time-resolved spectroscopy", Chap. 13 in *Methods Enzymol*. **240** (1994), 262–311.

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