Biophysical Journal

Supporting Material

A cellular screening assay using analysis of modified fluorescence lifetime

Nicholas I. Cade, Gilbert Fruhwirth, Stephen J. Archibald, Tony Ng, David Richards

Accuracy of different lifetime fitting models

Fig. S1 shows a FLIM analysis of a region of mutant cells using different fitting models. Confocal intensity images are shown in Fig. S1 *a* and *b* when focussed on the Au film and 1 μm above the film, respectively. Fig. S1 *c* and *d* show that a monoexponential decay model is only suitable for parts of the cell with a predominant signal arising from an unmodified layer above the film (τ ~2 ns); other areas give a very poor fit.
Fig. S1 *e-g* show that a reasonable fit is achieved using a stretched exponential model with *h* fixed at the global average value; however, the longest lifetime associated with unmodified parts of the cell is skewed to a smaller value. Leaving *h* as a free parameter gives a much better fit (Fig. S1 *h-j*); those areas of the cell with an essentially unmodified EGFP lifetime have a corresponding value of *h*~1. A pixel correlation analysis of the maps in Fig. S1 *h* and *i* shows a non-trivial relationship between τ and *h*. Fig. S1 *l* and *m* show the amplitude-weighted lifetime components of a biexponential fit, with one lifetime component fixed at 2.1 ns; the average *t* value calculated from these components is shown in Fig. S1 *k* and described in the legend.

Although the FLIM maps shown in the first column of Fig. S1 show similar spatial lifetime variations, some models are more physically relevant than others and hence will report lifetime values more accurately than others. Fitting with a mono- or biexponential model is based on the erroneous assumption of only one or two discrete lifetimes; Figs. 1 and 2 show that in addition to the unmodified intrinsic fluorescence lifetime there is also a lifetime distribution that varies continuously with distance from the Au film. In this case the stretched exponential decay model is more appropriate as it can be mathematically expressed as a continuous superposition of exponential terms. Furthermore, the decay transients shown in Fig. 5 *b* and those used to calculate the data in Fig. 5 *c* and Fig. 6 were all obtained by integrating over large areas of cells, further averaging the lifetime distribution. Thus, for the purposes of the assay described here, the stretched exponential model allows a fast, convenient, and accurate data fitting with the minimum parameters required to provide a contrast mechanism.

Figure S1

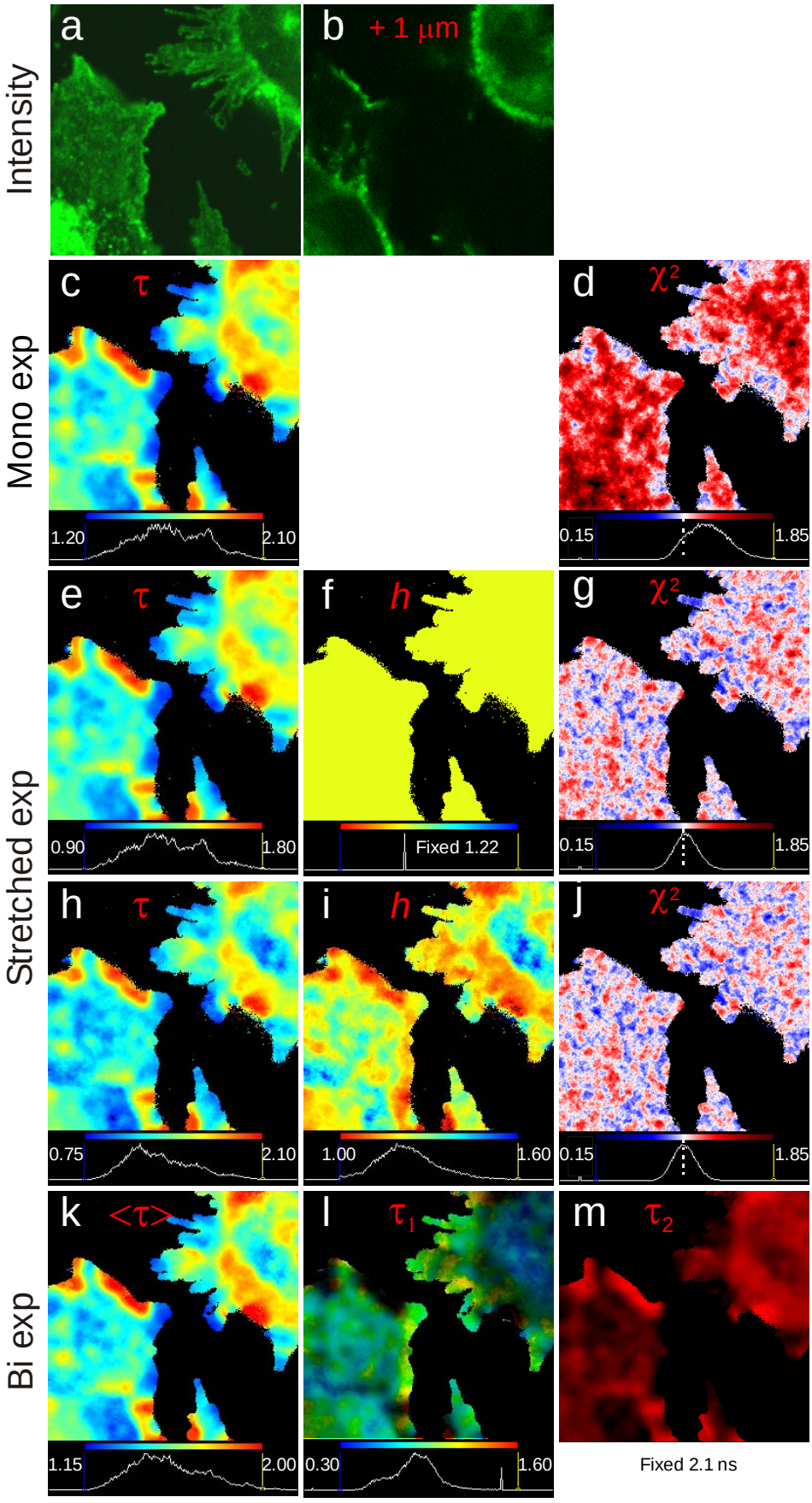
Intensity
Mono exp
Stretched exp
Bi exp
a
b + 1 μm
c τ
1.20
2.10
d χ²
0.15
1.85
e τ
0.90
1.80
f h
Fixed 1.22
g χ²
0.15
1.85
h τ
0.75
2.10
i h
1.00
1.60
j χ²
0.15
1.85
k <τ>
1.15
2.00
l τ1
0.30
1.60
m τ2
Fixed 2.1 ns

FIGURE S1 FLIM analysis of a 40 × 40 μm area of mutant cells on a 50 nm Au film. Confocal fluorescence intensity images in (*a*) the focal plane of the Au film, (*b*) 1 μm above the film. (*c*,*d*) τ and reduced χ^2 maps using a mono-exponential fit. Dashed line in the χ^2 scale indicates $\chi^2 = 1$. (*e*-*g*) τ, *h* and χ^2 maps using a stretched exponential fit, with *h* fixed at the global average of 1.22. (*h*-*j*) as *e*-*g* with *h* free. (*k*-*m*) $<\tau>$, τ_1, and τ_2 maps using a bi-exponential fit, with τ_2 fixed at 2.1 ns. Each lifetime map τ_i has been weighted with its fractional pre-exponential intensity $A_i/(A_1+A_2)$.
$<\tau> = (A_1\tau_1+A_2\tau_2)/(A_1+A_2)$.

.

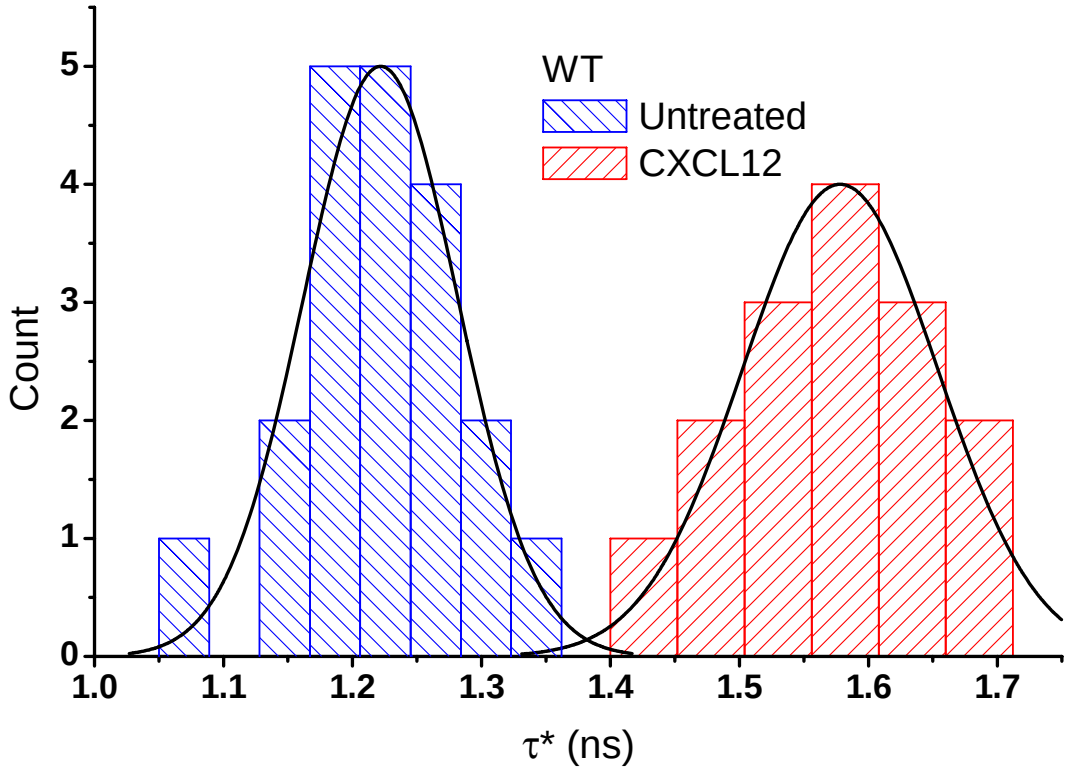

FIGURE S2 τ* histograms from Fig. 5 *c* for WT cells with (red) and without (blue) CXCL12 on a 30 nm Au film. Black curves are normal distribution fits.