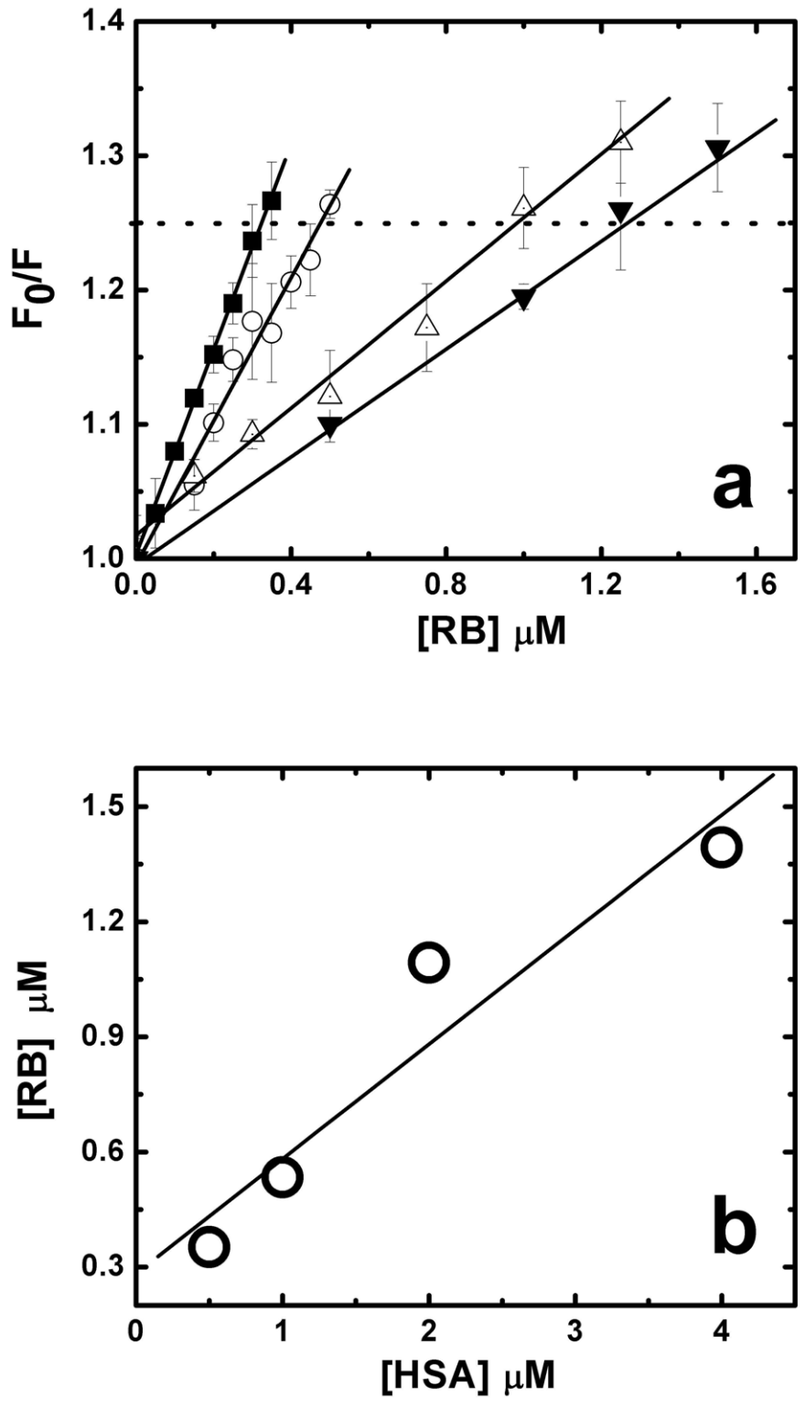
Supplementary material.

Encinas and Lissi's procedure

The methodology is based on carrying out quenching experiments at several HSA concentrations **[52]**. The Stern-Volmer plots obtained from primary data (fluorescence quenching of HSA Trp residue by different RB concentrations) are shown in the supplementary Fig.1a. RB concentrations that render Fo/F values equal to 1.25 ($RB_{1.25}$) were plotted as a function of the protein concentration (see supplementary Fig. 1b) and the association constant was calculated employing **Eq. 1.1**;

$$[RB]_{1.25} = n/K + n\,[HSA] \qquad \textbf{(Eq. 1.1)}$$

where **n** is the occupation number and K is the association constant. From the ratio between the intercept and the slope can be calculated the association constant (ca. 1.1 μM^{-1}). This value is very close to that obtained by the other methodologies.



Supplementary Figure 1. **(a)** Stern-Volmer plots for the quenching of HSA fluorescence by RB at different protein concentrations: (■) 0.5, (○) 1.0, (Δ) 2.0 and (▼) 4.0 μM. **(b)**. RB concentrations at which $F_0/F = 1.25$, plotted as a function of HSA concentration.