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;

$$[RB]_{1.25} = \frac{n}{K} + n \cdot [HSA]$$  \hspace{1cm} (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 \( \mu \)M\(^{-1}\)). 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.