

# **Supporting Information for publication**

## **Macromolecular and solution properties of the recombinant fusion protein HUG**

Paola Sist<sup>1</sup>, Antonella Bandiera<sup>1</sup>, Ranieri Urbani<sup>2\*</sup>, Sabina Passamonti<sup>1</sup>

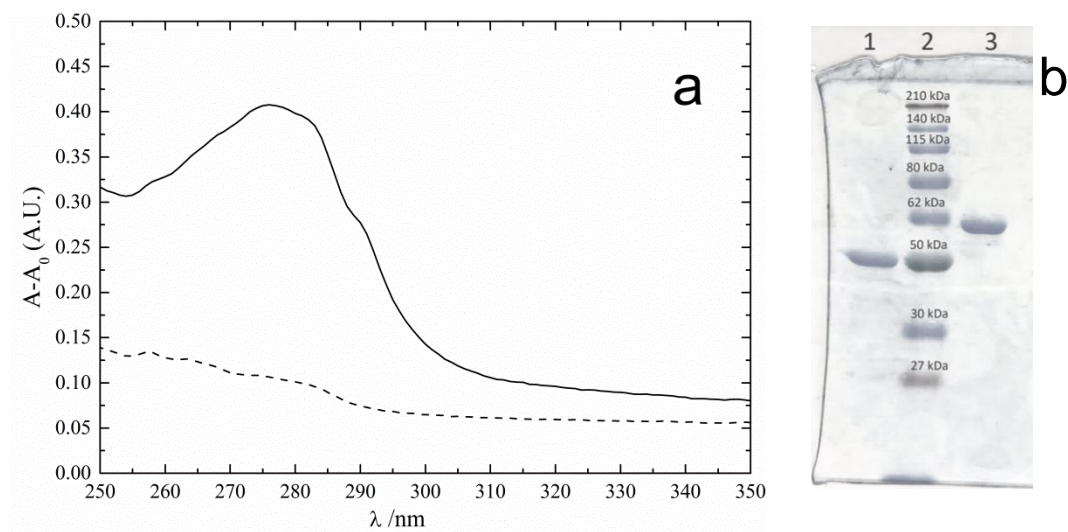
<sup>1</sup>Department of Life Sciences, University of Trieste, via Giorgieri 1, I-34127, Trieste, Italy.

<sup>2</sup>Department of Chemical and Pharmaceutical Sciences, University of Trieste, via Giorgieri 1, I-34127, Trieste, Italy.

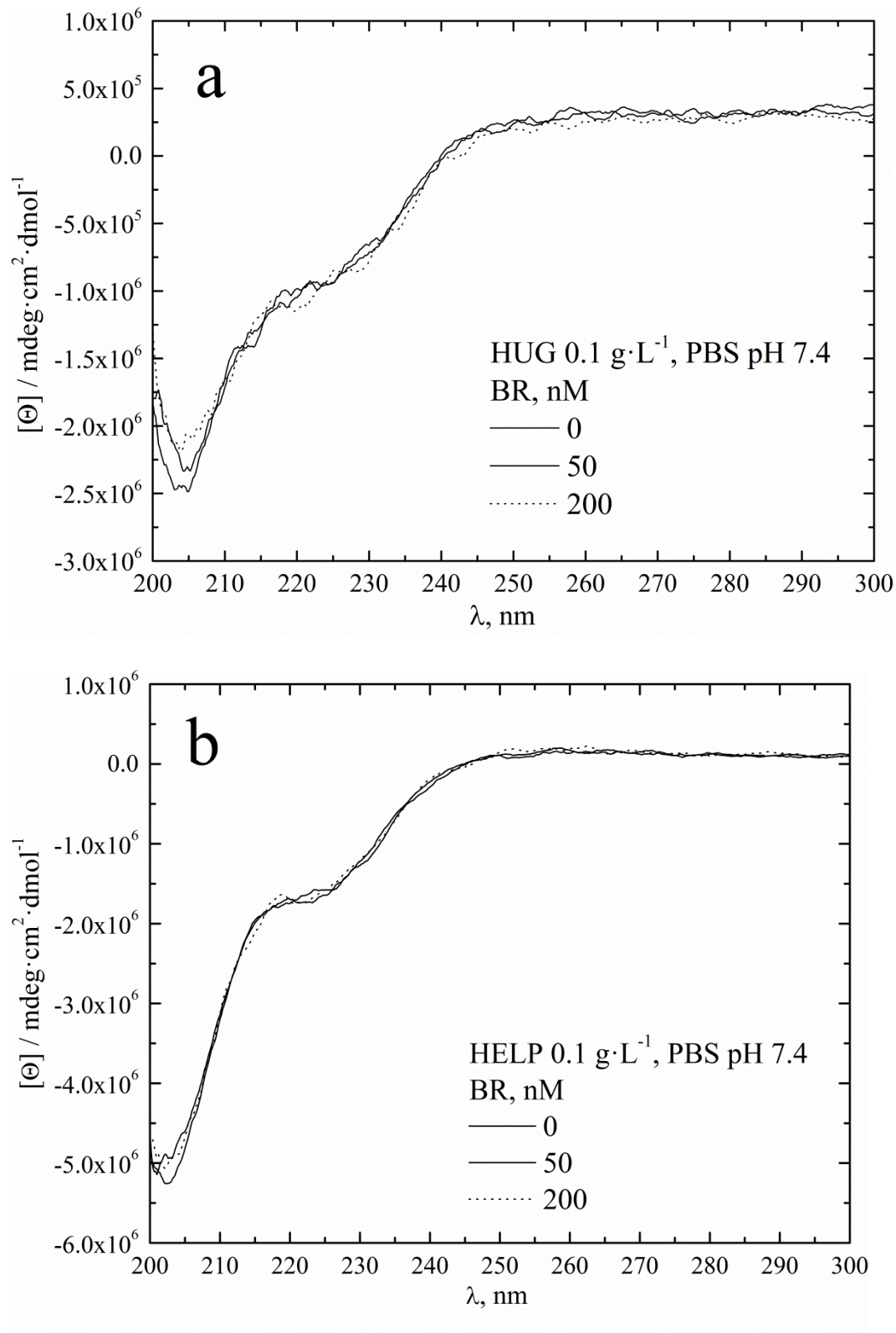
**Figure S1.** Characteristics of the HUG lot used in this study.

(a) Absorption spectra (ABS) of HUG (—) and HELP (---) solutions ( $2 \text{ g}\cdot\text{L}^{-1}$ ) were recorded in quartz cells ( $d = 1 \text{ cm}$ ) using a double-beam spectrophotometer (CARY-4E UV-visible spectrophotometer) at room temperature.

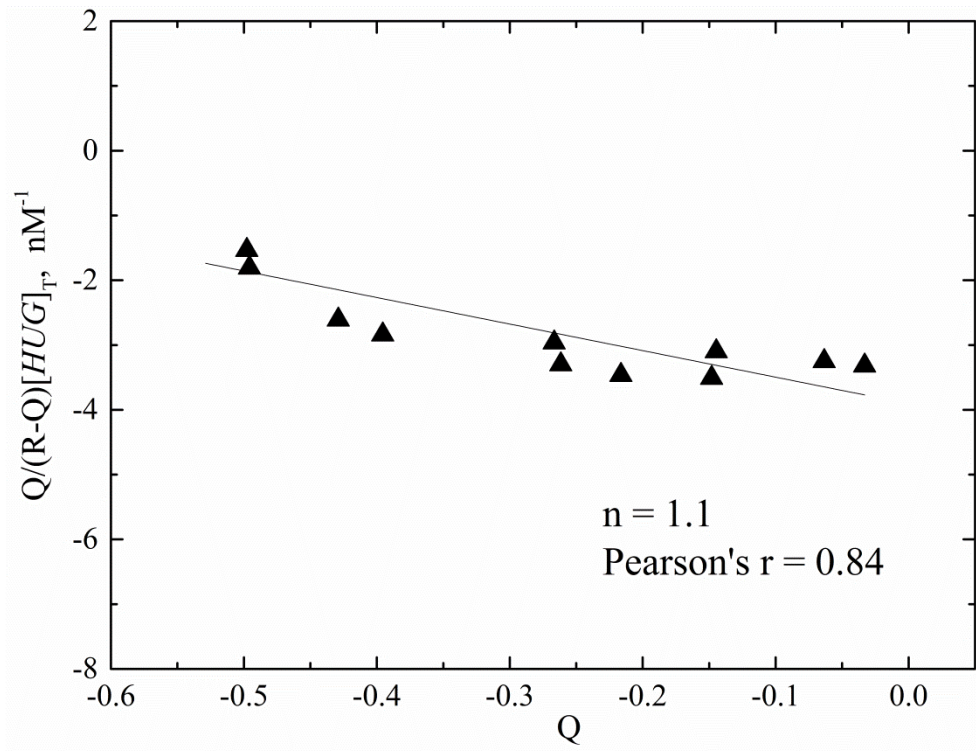
(b) SDS-PAGE separation of HELP (lane 1) and HUG (lane 3) ( $4 \mu\text{g}$  in each lane). The standard molecular weight is loaded in lane 2.



**Figure S2.** CD spectra of HUG (a) and HELP (b) without and with BR in the nanomolar range.



**Figure S3.** Scatchard plot of HUG-BR complex.



The Scatchard plot was obtained by using equation (Levine, 1977):

$$\frac{Q}{(R - Q)[HUG]_T} = nK_a - K_a Q$$

where:

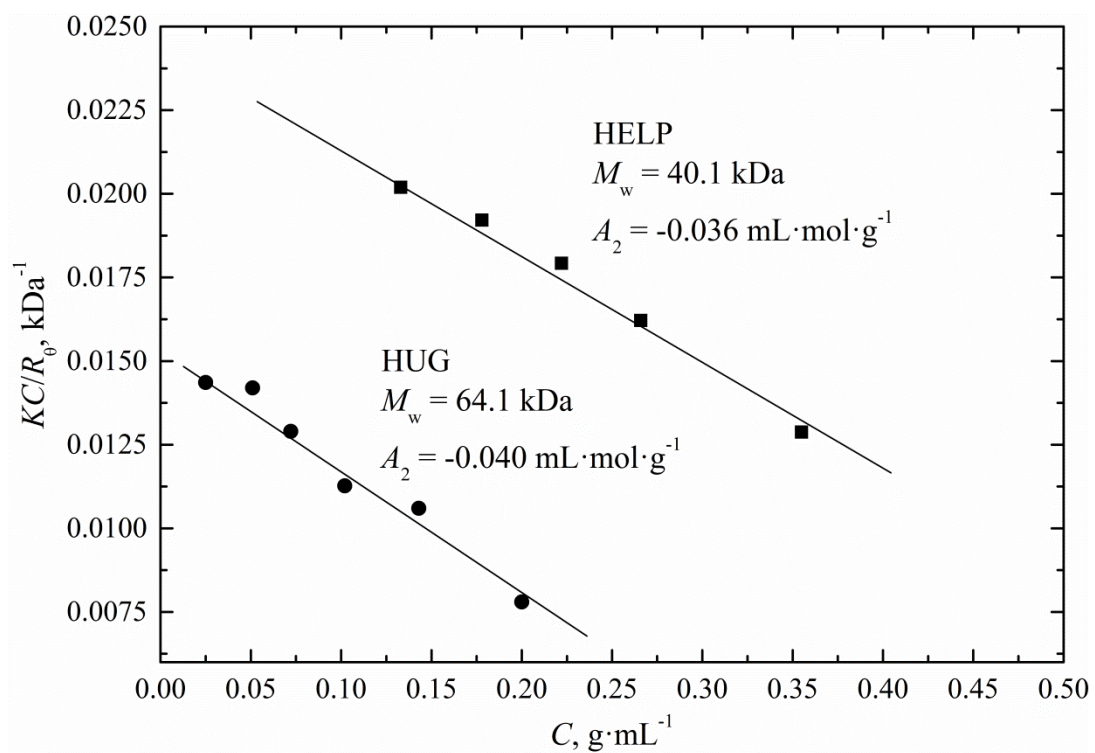
$$Q = \frac{[BR-HUG]}{[HUG]_T}$$

$$R = \frac{[BR]_T}{[HUG]_T}$$

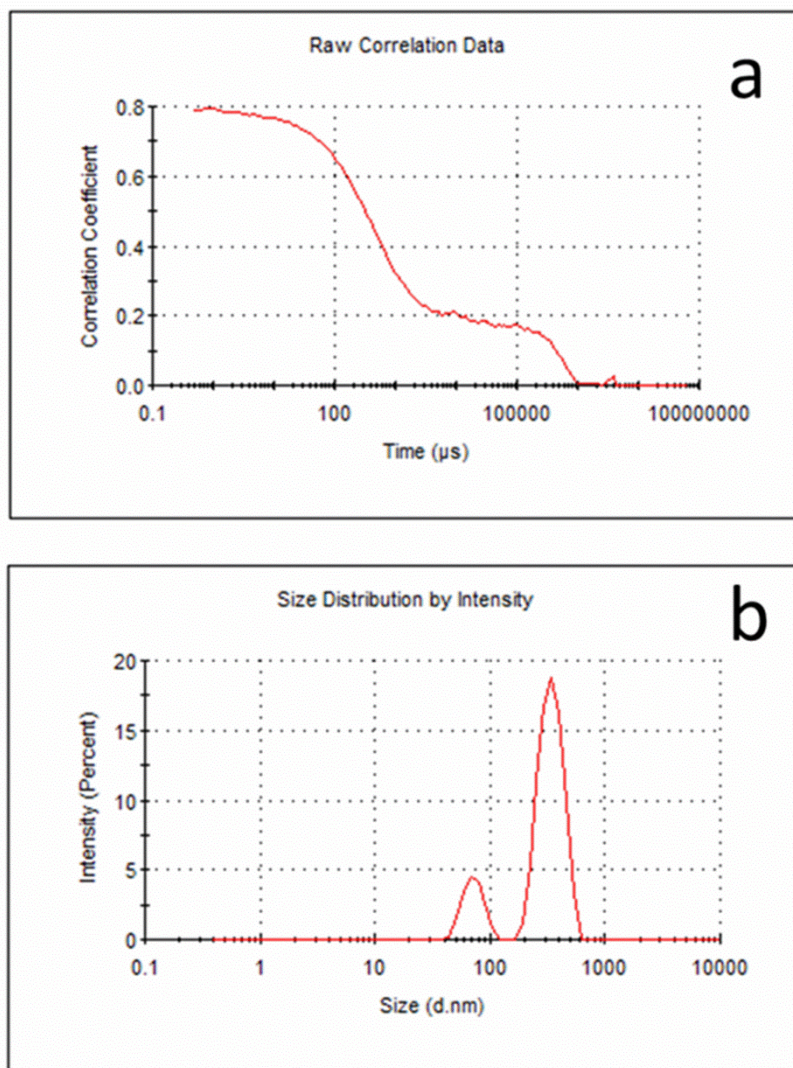
with  $[HUG]_T$  and  $[BR]_T$  the total concentration of HUG and BR, respectively.

Following Levine (1977),  $Q$  is related to the fractional saturation  $Y=F/F_0$  as defined above. The linear fitting of data in Figure S3 gives  $n = 1.1$ .

**Figure S4.** One representative Debye plot of HUG and HELP in solution from Static Light Scattering measurements.



**Figure S5.** DLS Correlation coefficient (a) and size distribution (by intensities) (b) of HELP protein at T=30°C, C=0.1 g·L<sup>-1</sup>.





**Figure S6.** DLS Correlation coefficient (a) and size distribution (by intensities) (b) of HUG protein at T=30°C, C=0.1 g·L<sup>-1</sup>.

