Electronic Supplementary Material

Appendix S1. Instrumental considerations about Gouy and Taylor techniques. The following is a very simplified description of the Gouy technique; more information can be found in the huge literature on this issue. As well known the Gouy interferometric technique allows to determine the diffusion coefficients of multicomponent systems, just following the evolution in time of a sharp boundary formed, at time $t = 0$, between two solutions of different compositions. One of the necessary conditions to run this kind of experiments is that the initial boundary is stable by gravitational point of view. This can be easily obtained stratifying, at $t = 0$, the less dense solution (Top solution) over the more dense one (Bottom solution).

In a binary system solute(i)–solvent the diffusion coefficient, D_i, can be obtained by a single experiment at the average concentration $\overline{c}_i$ forming an initial boundary between two solutions which differ in concentration for the quantity Δc_i. In general, for a binary system, the density ρ and the refractive index can be expressed, around the average composition, by the equations:

$$\rho(c_i) = \rho(\overline{c}_i) + H_i \cdot (c_i - \overline{c}_i) \tag{S1}$$

$$n(c_i) = n(\overline{c}_i) + R_i \cdot (c_i - \overline{c}_i) \tag{S2}$$

where H_i and R_i are defined by the following expressions:

$$H_i = \frac{\mathrm{d}\rho}{\mathrm{d}c_i}$$

$$R_i = \frac{\mathrm{d}n}{\mathrm{d}c_i}$$

While R_i is always positive, H_i can, in principle, be positive or negative. If H_i is positive, the density is an increasing function of the solute molar concentration, otherwise it is a decreasing function of c_i. This second possibility is not very common and then usually the bottom solution has a larger refractive index value than the top one. In this condition the typical Gouy interference fringes are formed under the apparatus optical axis coinciding with the position of the initial step boundary in the diffusion cell. As a consequence of this, the Gouy apparatus located in Naples has been set up to automatically read the position of fringes located under the apparatus optical axis.

In the case of the binary MPD(2)–water(0) system, while the refractive index is, as usual, an increasing function of the solute concentration, $R_2 > 0$, the density has a more complicated trend presenting, in a large concentration range, a minimum and then a maximum. In the concentration

range where the density is a decreasing function of the concentration H_2 is negative, and then to have initially stable diffusion boundaries it should be necessary to stratify the less concentrated solution under the more concentrated one. This gives $R_2(\text{Top}) > R_2(\text{Bottom})$ and will result in the formation of interference fringes over the Gouy apparatus optical axis. Then for using the interferometric technique for the system MPD(2)–water(0) system it would have been necessary to switch the Gouy apparatus from the present configuration to the opposite one. This is would have been very time consuming, also requiring a successive realignment of the optic of the apparatus. For this reason we preferred to use, for determining the D_2 values, the Taylor dispersion technique that is insensitive to gravitational instabilities.

For the protein the density increment R_1 was always positive and this permitted us to use the Gouy technique for the binary HSA(1)–water(0) system.

In a ternary system solute(i)–solute(j)–solvent the four diffusion coefficients are determined, at each average composition, by performing at least two independent experiments starting from with initial boundaries with different values of the ratio $\Delta c_i / \Delta c_j$. For these systems the density and the refractive index can be expressed, around the average composition $(\overline{c}_i, \overline{c}_j)$, by the following equations:

$$\rho(c_i, c_j) = \rho(\overline{c}_i, \overline{c}_j) + H_i \cdot (c_i - \overline{c}_i) + H_j \cdot (c_j - \overline{c}_j) \tag{S3}$$

$$n(c_i, c_j) = n(\overline{c}_i, \overline{c}_j) + R_i \cdot (c_i - \overline{c}_i) + R_j \cdot (c_j - \overline{c}_j) \tag{S4}$$

where:

$$H_k = \left(\frac{\partial \rho}{\partial c_k} \right)_{c_{l \neq k}}$$

$$R_k = \left(\frac{\partial n}{\partial c_k} \right)_{c_{l \neq k}}$$

are function of the concentration of both solutes. In general the H_k , and R_k values depend on the molecular size of the molecules, and therefore in our particular case it results that the density and refractive index contribution of the protein is always much larger than the correspondent MPD one. In particular H_1 is about 17.0 kg mol^{-1}, while H_2 varies between $-1.7 \cdot 10^{-3}$ kg mol^{-1} and $0.8 \cdot 10^{-3}$ kg mol^{-1}, thus indicating that, in presence of the protein, the MPD contribution to define the density value is almost negligible. Since the choice of the possible $\Delta c_i / \Delta c_j$ ratios is very wide, it is possible to run several independent experiments with a bottom solution with a refractive index

larger than the top one and this permitted to use, for this system, our Gouy apparatus in the standard configuration.

Appendix S2. HSA diffusional polydispersity. In an ideal binary system solute(i)–solvent(0) the only quantity describing the diffusion process is the diffusion coefficient D_i. In the presence of impurities, the system solute(i)–impurities($j,k,\dots$)–solvent(0) should be considered as a n-component system and then described by n^2 diffusion coefficient. If a single impurity is present the system should be completely described by four D_{ij} that could be obtained by, at least, two different independent runs at the same composition but with different values of the ratio $\Delta c_i / \Delta c_j$. Obviously, because the solute(i) and the impurity(j) are together in a fixed ratio in the impure sample, it is possible to perform, at a given average composition, only a single experiment with a value of the ratio $\Delta c_i / \Delta c_j$ proportional to the purity degree. Therefore the D_{ij} can not be obtained and the system must be regarded as a "pseudo-binary" one and it is characterized, by diffusional point of view, by the experimental diffusion coefficient D_A that represents an average value of the diffusivity of all the components in the sample and by the so called "integral of the deviation function", Q_0, that indicates how different are the diffusivities of the different species in solution. For a pure substance we have $D_1 = D_A$ and $Q_0 = 0$. If Q_0 is small enough, it is usual to suppose the presence of a single component with $D_1 \cong D_A$ and the values of Q_0 is usually not reported. If Q_0 is large, as in the case of polydisperse substances, it is preferable to talk of an average diffusion coefficient, D_A, and the Q_0 is reported as an index of the diffusional polydispersity of the sample.

A system containing an impure solute, for simplicity with only an impurity, a pure solute, and a solvent is of course a pseudo-ternary system that can be described only by four diffusion coefficients. Because in computing the D_{ij}, the D_A and the Q_0 values of each independent run are needed, it is important, to obtain meaningful D_{ij} values, that the binary Q_0 values were small enough in respect to the ternary Q_0 values. Otherwise a complex approximate procedure must be used in computing the ternary diffusion coefficients. In this case we verify that the value of Q_0 for the binary HSA(1)-water(0) is of the order of the typical error in this quantity; therefore it is not necessary to take its contribution into account in analyzing the ternary diffusion data.

FIGURES
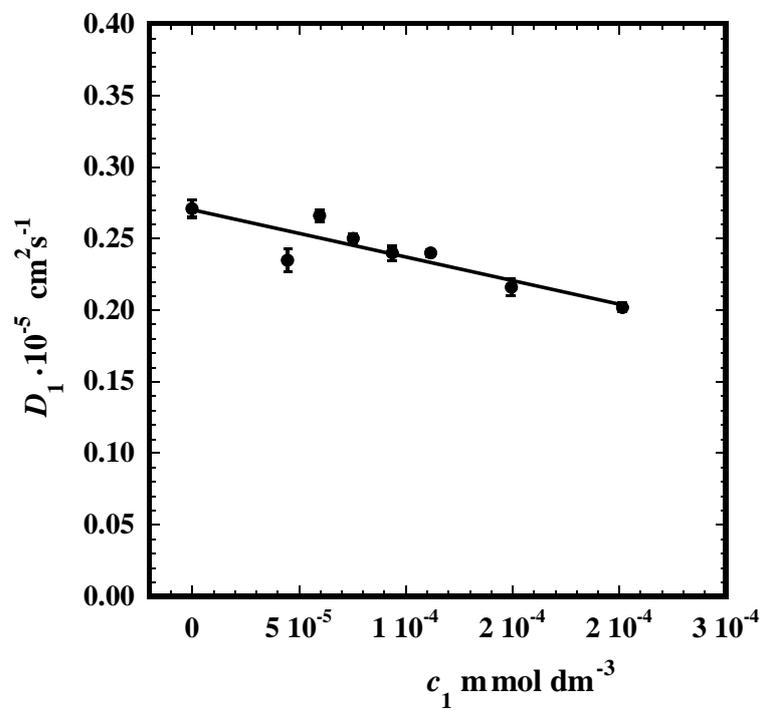
Figure S1 – HSA binary diffusion coefficient D_1 as a function of protein concentration c_1.
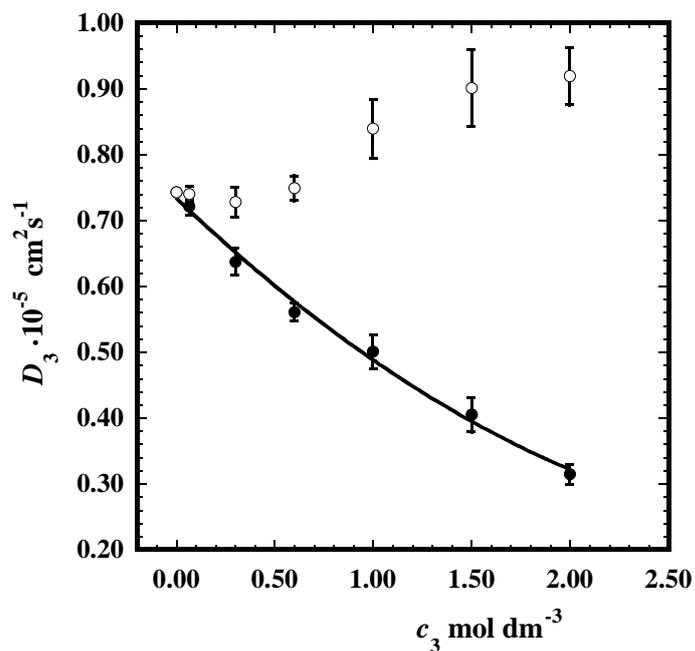
Figure S2 – MPD binary diffusion coefficient as a function of precipitant concentration. Binary system MPD(3)–water(0): ● D_3 *vs.* c_3. Product between the MPD binary diffusion coefficient and the solution relative viscosity, η_3/η_0, as a function of precipitant concentration. Binary system HSA(1) –MPD(3) –water(0): ○ $D_3 \cdot (\eta_3/\eta_o)$ *vs.* c_3. See equation 8 in the main text

TABLES

$m_1/(\text{mmol kg}^{-1})$	$\rho_1/(\text{g cm}^{-3})$	$m_1/(\text{mmol kg}^{-1})$	$\rho_1/(\text{g cm}^{-3})$
0	0.997044	0.0882	0.998494
0.00563	0.997130	0.0955	0.998611
0.00684	0.997150	0.114	0.998917
0.0159	0.997331	0.136	0.999287
0.0341	0.997611	0.140	0.999346
0.0453	0.997806	0.173	0.999876
0.0525	0.997915	0.211	1.000502
0.0773	0.998321	0.269	1.001393

Table S1 – Binary system HSA(1)-water(0). Density, ρ_1, as a function of molal concentration, m_1

$c_2/(\text{mol dm}^{-3})$	η_2/η_0
0	1
0.01500	1.704
0.02999	2.758
0.04498	4.354
0.06092	6.698

Table S2 – Binary system PEG3400(2)–water(0). Relative viscosity, η_2/η_0, as a function of molar concentration, c_2

$c_3/(\text{mol dm}^{-3})$	η_3/η_0	$c_3/(\text{mol dm}^{-3})$	η_3/η_0
0	1	1.0001	1.706
0.4445	1.212	1.2004	1.916
0.5001	1.251	1.5005	2.236
0.5715	1.307	2.3946	3.445
0.6667	1.400	2.9932	4.783
0.8001	1.513		

Table S3 – Binary system MPD(3)–water(0). Relative viscosity, η_3/η_0, as a function of molar concentration, c_3

$c_1/$(mmol dm^{-3})	$c_2/$(mol dm^{-3})	$\rho/$(g cm^{-3})	$c_1/$(mmol dm^{-3})	$c_2/$(mol dm^{-3})	$\rho/$(g cm^{-3})
0.1698	0.01340	1.007072	0.1642	0.04391	1.024297
0.1117	0.01480	1.007182	0.1231	0.04493	1.024161
0.1235	0.01451	1.007164	0.1582	0.04406	1.024050
0.1568	0.01440	1.007339	0.1115	0.04524	1.024137
0.1700	0.01630	1.009100	0.2283	0.04522	1.026183
0.2280	0.01480	1.008990	0.1817	0.04642	1.026049
0.2165	0.01512	1.009008	0.2169	0.04553	1.026159
0.1861	0.01599	1.008833	0.1700	0.04668	1.026001
			0.2049	0.04581	1.026110
0.1119	0.02991	1.015657	0.1758	0.04653	1.026023
0.1700	0.02844	1.015791			
0.1584	0.02874	1.015765	0.1119	0.06080	1.033108
0.1235	0.02961	1.015685	0.1700	0.05940	1.033179
0.2282	0.02991	1.017663	0.1584	0.05970	1.033164
0.1700	0.03137	1.017529	0.1235	0.06050	1.033122
0.1817	0.03108	1.017555	0.2281	0.06080	1.035052
0.2165	0.03020	1.017635	0.1700	0.06230	1.034981
			0.1816	0.06200	1.034996
0.1701	0.04378	1.024319	0.2165	0.06110	1.035038
0.1352	0.04465	1.024210			

Table S4 – Ternary system HSA(1)–PEG3400(2)–water(0). Density ρ as a function of molar concentrations, c_1 and c_2

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c_1/(mmol dm^{-3})	c_3/(mol dm^{-3})	ρ/(g cm^{-3})	c_1/(mmol dm^{-3})	c_3/(mol dm^{-3})	ρ/(g cm^{-3})
0.1132	0.2500	0.998675	0.1133	1.9998	0.998992
0.1244	0.2408	0.998882	0.1648	1.9624	0.99988
0.1473	0.2225	0.999306	0.1246	1.9897	0.999186
0.2265	0.2500	1.000628	0.1473	1.9722	0.999582
0.2153	0.2592	1.000421	0.1586	1.9634	0.999776
0.1921	0.2776	0.999997	0.1586	1.9634	0.999776
			0.1246	1.9903	0.999186
0.1132	0.5000	0.99859	0.1246	1.9901	0.999186
0.1246	0.4911	0.998787	0.1359	1.9812	0.999383
0.1360	0.4821	0.998983	0.2267	1.9997	1.000869
0.1529	0.4687	0.999277	0.1751	2.0359	0.999981
0.2267	0.5000	1.000536	0.2153	2.0080	1.000675
0.2153	0.5089	1.000338	0.1924	2.0263	1.000279
0.2040	0.5179	1.000143	0.1813	2.0348	1.000085
0.1870	0.5313	0.999849	0.1813	2.0349	1.000086
			0.2153	2.0080	1.000675
0.09451	0.9926	0.998418	0.2153	2.0080	1.000675
0.1322	0.9698	0.999056	0.2039	2.0169	1.000478
0.1142	0.9861	0.998755			
0.1549	0.9539	0.999435	0.1473	1.4731	0.999595
0.1398	0.9636	0.999181	0.1246	1.4909	0.999187
0.09439	0.9963	0.998419	0.1132	1.4997	0.998983
0.08682	1.0016	0.998292	0.1473	1.4729	0.999595
0.2456	0.9999	1.001041	0.1019	1.5087	0.998779
0.2077	1.0224	1.000404	0.1926	1.5266	1.000341
0.2259	1.0064	1.000705	0.2153	1.5088	1.000748
0.1851	1.0387	1.000024	0.2266	1.4997	1.000953
0.2002	1.0281	1.000278	0.1926	1.5265	1.000341
0.2456	0.9964	1.00104	0.2380	1.4908	1.001156
0.2532	0.9909	1.001167			

Table S5 – Ternary system HSA(1)–MPD(3)–water(0). Density ρ as a function of molar concentrations, c_1 and c_3.

c_1/(mmol dm^{-3})	c_2/(mol dm^{-3})	$\overline{V}_1^{(T)}$/(dm^3 mol^{-1})	$\overline{V}_2^{(T)}$/(dm^3 mol^{-1})	$\overline{V}_1^{(B)}$/(dm^3 mol^{-1})	$\overline{V}_2^{(B)}$/(dm^3 mol^{-1})
0.1700	0			49.70 ± 0.08	2.846 ± 0.004
0.1700	0.01492	48.67 ± 1.13	2.72 ± 0.04	49.70 ± 0.08	2.846 ± 0.004
0.1700	0.02991	49.28 ± 1.07	2.85 ± 0.04	49.70 ± 0.08	2.846 ± 0.004
0.1700	0.04523	48.87 ± 1.41	2.83 ± 0.05	49.70 ± 0.08	2.846 ± 0.004
0.1700	0.06083	49.65 ± 0.36	2.84 ± 0.01	49.70 ± 0.08	2.846 ± 0.004

TableS6 – Ternary system HSA(1)–PEG3400(2)–water(0). Solute partial molar volumes, $\overline{V}_1^{(T)}$ and $\overline{V}_2^{(T)}$, at constant protein concentration, $c_1 = 0.1700$ mmol dm^{-3}, as a function of precipitant concentration, c_2. For comparison the corresponding binary values, $\overline{V}_1^{(B)}$ and $\overline{V}_2^{(B)}$, at the same solute concentrations, are also reported

c_1/(mmol dm^{-3})	c_3/(mol dm^{-3})	$\overline{V}_1^{(T)}$/(dm^3 mol^{-1})	$\overline{V}_3^{(T)}$/(dm^3 mol^{-1})	$\overline{V}_1^{(B)}$/(dm^3 mol^{-1})	$\overline{V}_3^{(B)}$/(dm^3 mol^{-1})
0.1700	0			49.70 ± 0.08	0.120 ± 0.004
0.1700	0.2500	50.04 ± 2.45	0.120 ± 0.006	49.70 ± 0.08	0.119 ± 0.004
0.1700	0.5000	49.31 ± 2.74	0.119 ± 0.007	49.70 ± 0.08	0.119 ± 0.004
0.1700	0.9962	48.84 ± 0.66	0.118 ± 0.002	49.70 ± 0.08	0.118 ± 0.004
0.1700	1.4998	49.13 ± 0.68	0.118 ± 0.002	49.70 ± 0.08	0.118 ± 0.004
0.1700	1.9991	49.80 ± 1.66	0.119 ± 0.004	49.70 ± 0.08	0.119 ± 0.004

Table S7 – Ternary system HSA(1)-MPD(3)-water(0). Solute's partial molar volumes, $\overline{V}_1^{(T)}$ and $\overline{V}_3^{(T)}$, at constant protein concentration, $c_1 = 0.1700$ mmol dm^{-3}, as a function of precipitant concentration, c_3. For comparison the corresponding binary values, $\overline{V}_1^{(B)}$ and $\overline{V}_3^{(B)}$, at the same solute concentrations, are also reported

c_1/(mmol dm^{-3})	c_2/(mmol dm^{-3})	$10^5 D_{11}$/(cm^2 s^{-1})	$10^5 (D_{11})_{pred}$/(cm^2 s^{-1})
0.1700	0.0000	0.2138	
0.1700	1.492E-02	0.1375 ± 0.0067	0.133
0.1700	2.991E-02	0.0922 ± 0.0023	0.079
0.1700	4.523E-02	0.0478 ± 0.0035	0.050
0.1700	6.083E-02	0.0331 ± 0.0004	0.034

Table S8 – Ternary system HSA(1)-PEG3400(2)-water(0). Experimental and predicted (see equation (4)) D_{11} diffusion coefficients at constant protein concentration, $c_1 = 0.1700$ mmol dm^{-3}, as a function of precipitant concentration, c_2.

c_1/(mmol dm^{-3})	c_3/(mmol dm^{-3})	$10^5\, D_{11}$/(cm^2 s^{-1})	$10^5\, (D_{11})_{pred}$/(cm^2 s^{-1})
0.1700	0.0000	0.2138	
0.1700	0.2500	0.2042 ± 0.0020	0.192
0.1700	0.5000	0.1840 ± 0.0040	0.170
0.1700	0.9962	0.1535 ± 0.0016	0.130
0.1700	1.4998	0.0988 ± 0.0047	0.098
0.1700	1.9991	0.0842 ± 0.0024	0.075

Table S9 – Ternary system HSA(1)-MPD(3)-water(0). Experimental and predicted (see equation (4)) D_{11} diffusion coefficients at constant protein concentration, $c_1 = 0.1700$ mmol dm^{-3}, as a function of precipitant concentration, c_3.