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Slow-to-fast transition of hydrogen bonds dynamics in the acetamide hydration shell formation

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The formation of hydration shell in acetamide aqueous solution has been investigated by means of UV Raman spectroscopy. The experimental results reveal the existence of two distinct regimes of water dynamics. At high acetamide concentration water molecules show a structural and dynamical behavior consistent with the so called iceberg model. Increasing the amount of water we observe the formation of an hydration shell marked by a fastening of hydrogen-bonds dynamics. Such a behavior may help to shed light on the scientific debate on how water rearranges around the hydrophobic portions of solute molecules (iceberg vs non-iceberg models).

Introduction

The understanding of hydration dynamics in biomolecules is a challenge that scientists working in chemistry, physics and biology are facing since decades [1]. This interest is due also to the fact that several mechanisms peculiar of biomolecules, such as assembly of membranes, protein folding and protein−ligand binding processes, are strongly related to the behavior of hydration water [2-5]. Many biomolecules exhibit in their structure extended non-polar domains that are characterized by a marked hydrophobic behavior, which causes a negative change in enthalpy and an increase of the entropy [6]. It is recognized that the mechanism used from water molecules to orient and re-arranging itselves around these hydrophobic groups plays a crucial role in driving important process, like the membrane stability and protein aggregation phenomena [7,8]. More than fifty years ago Frank and Edwards introduced the so called *iceberg* model to explain the increase of heat capacity in aqueous solutions of molecules rich of apolar parts [9]. They proposed a picture where the water molecules surrounding the hydrophobic groups organize itselves in a tetrahedrally coordinated "ice-like" structure. This idea got theoretical and experimental endorsements [10-13]. In particular Rezus and co-authors [11] shown that the formation of the ice-like water structures near the hydrophobic groups is characterized by slower water dynamics with respect to bulk water one. A similar result was obtained by Petersen and co-authors [13] for aqueous solutions of tetramethylurea and tertiary butyl alcohol. They observed, below 30°C, a strong slowing-down of the water reorientation time around the hydrophobic groups.

However some recent works are in contrast with this description of the phenomenon of "hydrophobic solvation" [14-18]. For example, in Refs. [14-17] the authors show that the diffusion of water molecules surrounding the hydrophobic moieties in alcohols and oligo-peptides aqueous solutions does not involve an arrested ice-like dynamics. These experimental evidence have been interpreted on the basis of a model recently proposed by Laage at al. [19] that is in contrast with the predictions of the iceberg model. To complicate the picture nearly recent neutron diffraction experiments, carried out on alcohol/water solutions [18] demonstrated how the local structure of water near the hydrophobic group is actually closed to the bulk one.

In order to explain the discrepancy between these results and the *iceberg* model, Qviste and Halle [16] proposed a mechanism where, if the solute hydration shell is not completely surrounded by bulk water, therefore the overlapping between the hydration shells belonging to different solute molecules have a non-negligible role in the hydration shell formation. Besides, it has been demonstrated [20] that the dynamics of water molecules more closely bonded to the hydrophilic part of the solute molecule strongly affects the

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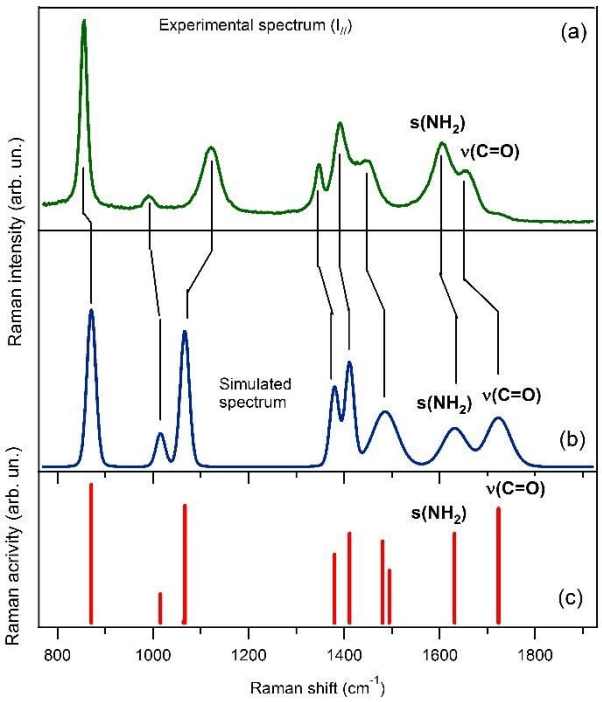

Fig. 1: Panels (a) and (b): experimental ($I_{//}$) and simulated Raman spectrum of the *n*=1.4 acetamide aqueous solution. Panel (c): values of Raman activity, as obtained from the MO simulations.

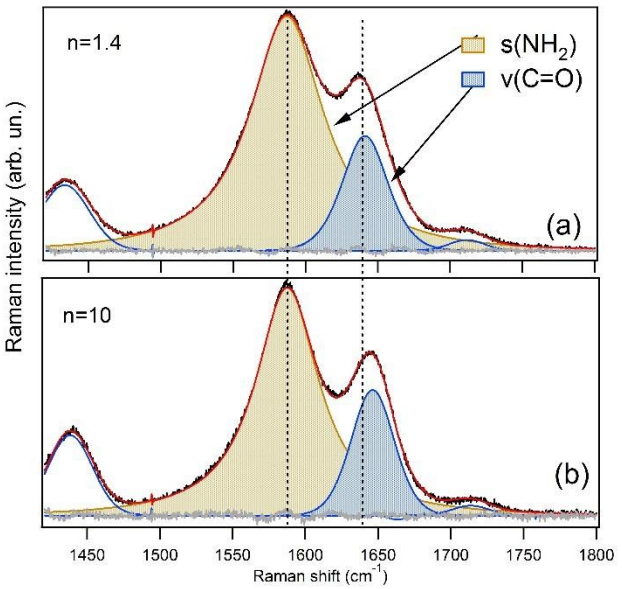

Fig. 2: Panel (a): black and red lines are the experimental lineshape of the isotropic Raman spectra of acetamide aqueous solutions at *n*=1.4 and the corresponding total best fit curve, respectively. The yellow and cyan shaded peaks are the s(NH_2) and ν(C=O) features, respectively. Panel (b): as in panel (a) for *n*=10.

conformation of the overall hydration shell [21]. It should be taken into account if the presence of such hydrophilic parts play a role even in the structural conformation and dynamics of water surrounding the hydrophobic moieties. It implies that different behaviors of the hydration shells (*iceberg* vs non-*iceberg*) may be addressed to different contributions given by the hydrophilic sites in the global rearrangement of water molecules.

A possible experimental approach to shed light on the behavior of hydration water is to investigate how the hydration process depends on the solute concentration and how the hydrophilic/hydrophobic parts contributes to the rearrangement of water molecules. In this respect, Raman spectroscopy is undoubtedly a valid tool for studying the solvation processes in molecules of biological relevance, allowing to investigate the dynamics of hydrogen-bonds (HBs) established between water and specific chemical groups. For example, we recently demonstrated that a lineshape analysis (based on the Kubo model) of the isotropic Raman profile permits to determine the mean lifetime of carboxylate-water HBs [22,23]. The great advantage of this experimental approach is that it provides information on the entire solvation process by measuring the vibrational relaxation dynamics of specific molecular probes. Furthermore, it has been demonstrated that the spectral analysis of the complex OH stretching band of water in the high frequency range of Raman spectra allows to obtain quantitative information of the different co-operativity degrees of the HB patterns of interfacial water [24]. Motivated by the above arguments we performed a Raman scattering investigation on the hydration shell formation in acetamide aqueous solutions. The choice of acetamide arises from the twofold reasons: a) it possesses both an hydrophilic (C=O) and an hydrophobic (CH_3) group, hence allowing to investigate how both functional groups can cooperate to the creation of the hydration shell; b) the high solubility in water of acetamide (1.4 water molecules per acetamide at 25° [25]) makes possible to characterize the behavior of hydration water in a wide range of concentration. Besides these arguments, acetamide is an interesting system to be studied also as model of behaviour of peptides or proteins in water. As a matter of fact, it is one of the simple smaller molecules that exhibits in its chemical structure an amide (peptide) bond, thus mimicking the basic covalent bonds in the structure of proteins.

The experimental findings here reported allowed us to characterize how water molecules rearrange by changing the concentration of acetamide in water. As main result, we find that the surrounding water molecules passes from a slow ("ice-like") behaviour, observed at high solute concentration, to a faster ("non ice-like") regime met when the water content increases.

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Experimental and data analysis

Acetamide solutions at different solute concentrations have been obtained by dissolving acetamide powder (Sigma Aldrich, purity 99%) in double-distilled water. For sake of simplicity in the following we indicate the solution concentration with the parameter *n* defined as the molar ratio between water and acetamide. The sample temperature was kept fix to 30 ± 0.1 degree in order to avoid reciprocation due to over-saturation at the maximum concentration used (*n*=1.4, when the saturation limit *n*=1.12 [25]). Raman measurements have been carried out using 266 nm of excitation source. The spectrum of the back-scattered radiation from the sample has been analysed by a triple stage spectrometer (Trivista, Princeton Instruments). The experimental resolution was set to 1.0 cm^{-1} in order to ensure enough resolving power and count-rate. A complete description of the experimental set-up can be found elsewhere [26]. By using polarizers and wave plates, we have collected the diffused inelastic signal polarized parallel ($I_{\parallel}$) and perpendicular ($I_{\perp}$) with respect to the incident radiation. According with the current scientific literature the isotropic signal has been obtained as $I_{iso}=I_{\parallel}-(4/3)I_{\perp}$[27].

The fitting procedure of the isotropic Raman spectra have been carried out through a least squares χ^2 minimization procedure, following the steps described in Ref. [23] to ensure the likelihood of the results. The elements of the correlation matrix of fitting parameters range between -0.8 and 0.8. *Ab inizio* molecular orbitals (MO) calculations have been employed, by means of the Gaussian03 program package [28], to determine the energy minimized geometry of a single acetamide molecule and to calculate its the vibrational modes. The DFT B3LYP level, with the 6-311++g(d,p) basis set have been used carry out these simulations.

In Fig. 1(a) we show the isotropic Raman spectrum of acetamide aqueous solution at *n*=1.4. Fig. 1(b) reports the corresponding simulated spectrum, obtained as the sum of several Gaussian functions centred at the wavenumbers obtained from MO calculations and with Gaussian line-width σ_G given by σ_G=28 cm^{-1} for the peaks above 1480 cm^{-1} and σ_G=10 elsewhere. The area of each peak was assumed to be proportional to the theoretical Raman Activity, also obtained from the MO calculations and shown in Fig. 1 (c). The feature labelled as s(NH_2) and ν(C=O) correspond respectively to the NH_2 scissoring and to the C=O stretching. These attributions are fully consistent with those done in other studies [29-31].

In order to quantitatively investigate the behavior of s(NH_2) and ν(C=O) as a function of *n* we have used the Kubo model to fit the isotropic Raman spectra in the wavenumber range 1400-1800 cm^{-1}.

In Fig. 2 are displayed the isotropic Raman spectra at *n*=1.4 (top panel) and at *n*=10 (bottom panel), as an example. Four Kubo functions were employed to obtain a likelihood fit. The small component found at 1720 cm^{-1} can be identified with the stretching vibration of C=O group that is non-involved in HB, consistently with what observed in the case of acetic acid aqueous solutions [23,32], while the peak at about 1450 cm^{-1} is assigned to the CH_3 rocking [29-31]. Although the study of these two features are not the subject of the present work, they have to be properly taken into account in the fitting procedure to ensure the fit likelihood.

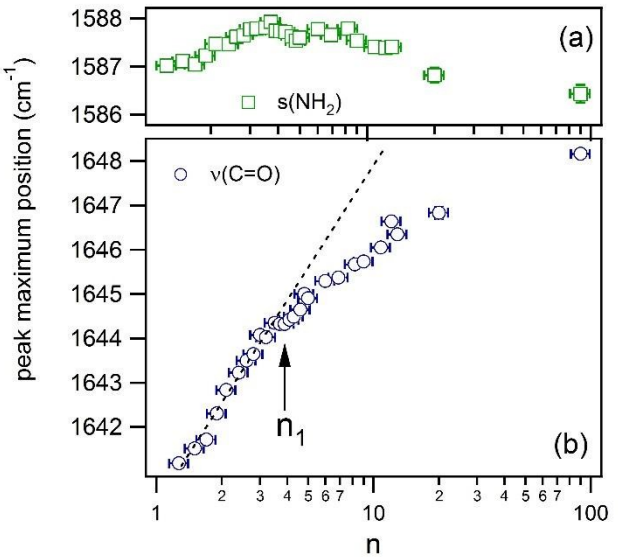

Fig. 3: *n*-dependence of the s(NH_2) (panel a) and ν(C=O) (panel b) peak frequency position. The arrow indicates the position of n_1. Dotted line is a guide for the eye.

Results and discussion

In the Kubo model the linewidth of a vibrational peak in the isotropic Raman spectrum depends on the parameter $\alpha=\sigma\tau_c$ where σ and τ_c are, respectively, the spread in the central frequency and the characteristic decay time of a generic stochastic perturbation acting of the vibrating atoms. If these vibrating groups are involved in HB's, then σ and τ_c are, respectively, the spread in the central frequency due to the stochastic variance in the HB geometry and the HB lifetime [22]. In the α=0 limit (fast modulation) the peak assumes a Lorentzian lineshape with linewidth inversely proportional to the vibrational dephasing (i.e., τ_{deph}^{-1}), while in the α>>1 limit (slow modulation) the peak has a Gaussian lineshape with linewidth proportional to σ [33-35].

In the investigated *n* range, the mode s(NH_2) is characterized by small value of α (i.e., α<0.2) and a shift in the peak frequency lower than 1 cm^{-1} (see Fig. 3 panel a).

Such a behavior recalls what observed for the CH_2 scissoring mode in glycine [22] and the CH_3 stretching mode in acetic acid aqueous solutions [23], thus suggesting a weak tendency of NH_2 group to form HB's. On the contrary, ν(C=O) shows a clear shift to higher wavenumbers upon increasing *n* (≈ 1.0 cm^{-1} per unit *n*) as pointed out in Fig. 3 panel (b).

Moreover the value of parameter α>1, obtained for this vibration, strongly suggests the presence of HB's involving

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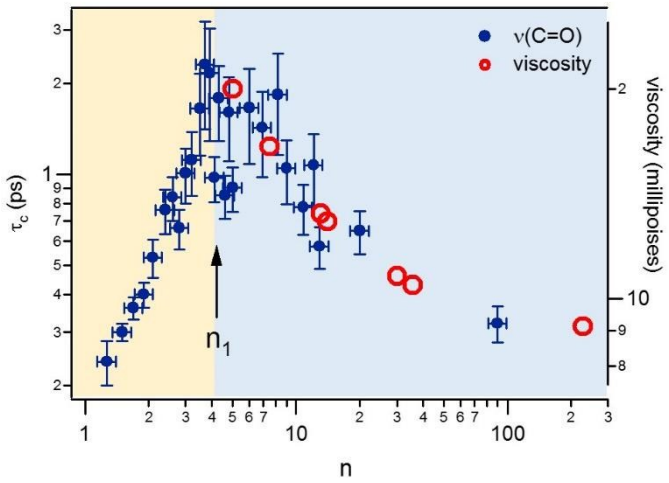

Fig. 4: *n*-dependence of τ_c, as extracted from the lineshape analysis of the ν(C=O) peak, on the same graph is also reported the viscosity of the acetamide solution (from Ref. [38]); see text for further details.

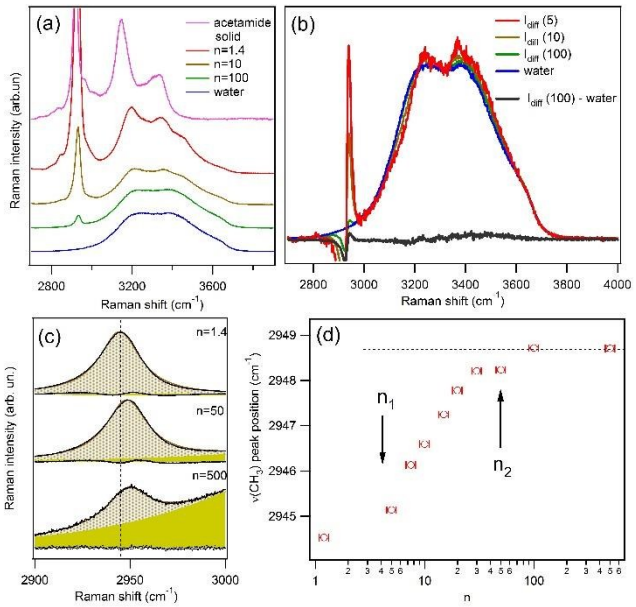

Fig. 5: Panel a: Isotropic Raman spectra of acetamide aqueous solutions collected in the 2700-3900 cm^{-1} wavenumer range. Spectra of water and acetamide solid state are also reported. Panel b: plot of I_{diff} curves for some representative n. In grey is reported the difference between I_{diff}(100) and water. Panel c: Isotropic Raman spectra of the $\nu(CH_3)$ vibrational component collected of acetamide aqueous solutions at three different concentrations, with the corresponding best fit curve (i.e. *n*=1.4, *n*=50, *n*=500). Panel d: *n*-dependence of $\nu(CH_3)$ peak frequency position. The arrows highlight the position of n_1 and n_2. Dotted line is a guide for the eye.

the carbonyl groups, as occurred, for example, in acetic acid [23] and glycine [22] aqueous solutions. The proposed lineshape analysis of isotropic Raman profiles allows us to estimate the *n*-dependence of τ_c, as displayed in Fig. 4.

In analogy with what found in acetic acid aqueous solutions [32,36,37], we expect the formation of many H-bonded (via N-H···O=C HB) acetamide oligomers near the concentration of saturation of acetamide in water (i.e. *n*=1.4). Therefore the values of τ_c obtained from the lineshape analysis of the ν(C=O) peak represent the average lifetime of breaking and reformation of the acetamide-acetamide (N-H···O=C) and acetamide-water (O-H···O=C) HB's. When *n* increases from 1.4 (saturation limit) to ≈ 4 (namely n_1), τ_c rises from 0.2 to 2 ps. This trend can be interpreted taking into account that, as decreasing the concentration of acetamide in water, the acetamide-acetamide HBs tend to break at the expense of the formation of an HB-network between water and acetamide.

Similarly to what happens for acetic acid aqueous solutions [32,36], the acetamide-acetamide HB's are expectedly weaker than to the ones established between acetamide and water. Hence the formation of such stronger (i.e., more stable) HB's in consistent with the large slowing down observed for τ_c ranging from *n*=1.4 to n_1.

For n>n_1 instead we observe a marked decrease in τ_c down to ≈ 0.3 ps at n=100. It is interesting to note how the damping of τ_c is strongly related to the decreasing of the viscosity of acetamide-warter solution, as found by Christoffers and co-authors [38] (see red circles on Fig. 4).

Indeed in this range we expect that, increasing n, the water molecules further added in the solution rearrange around the (hydrophobic) methyl group up to completely fill the hydration shell. Therefore, it is to be expected a correlation between the dynamics of water molecules in the hydration shell and the trend observed for the viscosity of the acetamide/water solution. In order to verify the correctness of this assumption we have analysed the isotropic Raman spectra of acetamide solutions collected in the wavelength range of 2900-4000 cm^{-1}. Some representative spectra (*n* = 1.4, red curve; *n* = 10, brown curve; *n* = 100, green curve) are shown in panel (a) of Fig. 5. The peak centred at ≈ 2950 cm^{-1} can be addressed to the methyl CH_3 symmetric stretching, namely $\nu(CH_3)$, while the broadband between 3000 and 3800 cm-1 appears as the superposition between the O-H stretching band of water and the NH_2 stretching of acetamide. In the same graph, the isotropic spectra of pure water (blue curve) and acetamide in solid state (purple curve) are also displayed for comparison. Fig. 5, panel c shows a zoom of the behavior of the $\nu(CH_3)$ peak in the spectral range 2900-3000 cm^{-1}, at three representative values of *n*. The experimental spectrum has been fitted with a Kubo function superposed on a polynomial background, in order to take into account the tail of the O-H stretching broadband of water. The dashed line highlights the marked shift of the peak position of $\nu(CH_3)$ observed by changing n. Such shift is analysed in detail in Fig. 5, panel d. For *n*>n_1, $\nu(CH_3)$ shifts of about 4 cm^{-1} to higher wavenumber, consistently with the decrease of the τ_c estimated for ν(C=O). The peak position rises continuously until *n* ≈ 60 (namely n_2). Above n_2 it reaches a plateau around 2948.7 cm^{-1}. A similar behavior was observed on the CH_3 symmetric

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stretching mode in acetic acid aqueous solutions [23]. It was attributed to the formation of a hydrophobic shell surrounding the methyl groups. Therefore we can assume that at n_2 the acetamide hydration shell is completely formed, and further increase of water corresponds to an increase of bulk water content.

To endorse this assumption, Figure 5 panel b displays some representative curves of $I_{diff}(n)$, as defined by the following relation: $I_{diff}(n)=I(n)-I(1.4)$; where $I(n)$ are the isotropic spectra acquired at n and $I(1.4)$ is the one collected at n=1.4. The spectra I(n) were previously normalized to the $\nu(CH_3)$ area and the $I_{diff}(n)$ curves so obtained were normalized again with respect to their total area (set equal to 1), excluding the spectral region between 2800 and 3000 cm^{-1}.

The spectrum of water (also normalized to its total area) was included for comparison. The observed changes in the I_{diff} lineshape are caused both by variations in the $\nu(NH_2)$ peak position or by modifications of the water O-H stretching band. We have seen before how the group NH_2 has a weak tendency to form HB's. Therefore we expect the formation of an hydrophobic shell surrounding the amine atoms, consistently to what observed by many authors in the terminal NH_2 of peptides systems [39-41]. The absence of changes occurring to I_{diff} above n_2, evidenced by the curve difference $n_{diff}(100)$-water of Fig. 5(b) is a further confirmation that, at these values of concentration, the hydration shell is completed.

Taking into account all these considerations, the damping of τ_c observed between n_1 and n_2 (Fig. 4) can be related both to the viscosity changes and to the hydration shell formation process. It is known that the increase of viscosity in hydrogen bonded systems is due to an average increasing of the HB strength [42]. Furthermore, Ruderman and co-authors observed a rise in the viscosity on carboxylic acids aqueous solutions, related to the solute hydration and to the decreasing HB lifetime between the solution molecules [43]. This leads us to state that the average lifetime of HB established among the water molecules in the hydration shell behaves as the water-C=O hydrogen bond.

In this framework it is possible to explain the discrepancies found between the ice-like model and many experimental evidences. Experiments carried out in solutions sufficiently diluted to complete the hydration shell formation, lead to results far from the prediction of the *iceberg* model [14,15]. On the contrary, if the experiment is performed at high concentration of solutes that exhibit in their structure also hydrophilic portions, then the gained results agree with the *iceberg* picture [11]. Anyhow both results are in agreement with our findings, which basically point towards a speed up of the water dynamics on decreasing solute concentration.

It is noteworthy to highlight that results similar to those of our experiments have been obtained by dielectric studies carried out on dioxane aqueous solutions [44,45] . In these works, an increase in the Debye relaxation time at high dioxane concentration was observed by reducing the water amount. Finally we point out how the agreement between theoretical models (as those proposed in Ref. [10] and references therein) and the *iceberg* model view can be explained by taking into account that in such models consider a number of water molecules not sufficient to completely fill the hydration shell.

Conclusions

By employing UV Raman scattering experiments on acetamide aqueous solutions, we observed the existence of two different regimes of water dynamics. For $n \approx n_1$, a slowing down of the dynamics of water molecules is found, in qualitative agreement with the so called *iceberg* model, while for $n_1 < n < n_2$ the experimental results suggest the formation of the acetamide hydration shell in which the water dynamics becomes faster.

This trend can be explained by invoking a progressive rearrangement of the water molecules in the hydration shell of solute upon increasing the water content. Such a slow-to-fast transition found in the dynamics of hydration water surrounding acetamide can reconcile some contradictory results reported in the scientific literature.

Notes and references

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