Investigation into the relaxation dynamics of polymer-protein conjugates reveals surprising role of polymer solvation on inherent protein flexibility

Daniela Russo[1,2*], Marie Plazanet[3,4], Jose Teixeira[5], Martine Moulin[6,7] ,Michael Härtlein[6,7] Frederik R. Wurm[8], Tobias Steinbach[8]

[1] CNR-IOM (Italy), c/o Institut Laue-Langevin, Grenoble, France

[2]Institut Lumière Matière, Université de Lyon, France

[3]Physics Department, Universita degli Studi di Perugia, Italy

[4]LIPhy, CNRS and Université Grenoble Alpes, France

[5]Laboratoire Léon Brillouin, CEA and CNRS, Gif Sur Yvette, France

[6]ILL Deuteration Laboratory, Partnership for Structural Biology, Grenoble, France.

[7]Life Sciences Group, Institut Laue-Langevin, Grenoble, France

[8]Max Planck Institute for Polymer Research, Mainz, Germany

To whom correspondence should be addressed.russo@ill.fr

Abstract

Fully biodegradable protein-polymer conjugates, namely MBP-PMeEP (Maltose Binding protein-poly-methyl-ethylene phosphonate), have been investigated in order to understand the role of polymer solvation on protein flexibility. Using elastic and quasi-elastic incoherent neutron scattering, in combination with partially deuterated conjugate systems, we are able to disentangle the polymer dynamics from the protein dynamics and meaningfully address the coupling between both components. We highlight that, in the dry state, the protein-polymer conjugates lack of any dynamical transition in accordance with the generally observed behavior for dry proteins. In

addition, we observe a larger flexibility of the conjugated protein, compared to the native protein, as well as a lack of polymer-glass transition. Only upon water hydration does the conjugate recover its dynamical transition, leading to the conclusion that exclusive polymer solvation is insufficient to unfreeze fluctuations on the picosecond-nanosecond timescale in biomolecules. Our results also confirm the established coupling between polymer and protein dynamics in the conjugate.

1. Introduction

Protein-polymer conjugates combine the biological function of proteins with the convenient handling and processability of synthetic polymers. The attachment of synthetic macromolecules to molecules of biological relevance has proven to be an efficient strategy to address problems in a variety of disciplines: improvement of protein stability; reduction of immunogenicity; development of biomarkers and drug-delivery complexes; and also improvement of protein solubility. In general, the biological function of proteins can be preserved and in some cases enhanced [1, 2, 3, 4, 5]. Depending on the desired applications, polymers can be tailored for bio-compatibility, biodegradation, and other physical properties. The influence of polymer properties in conjugation with proteins can be exploited to allow more effective control of protein-polymer properties such as precipitation, solubility, unfolding, transport and resistance. Polymer-protein conjugation for biomedical application is the subject of an already large and increasing number of new scientific studies. Among a variety of water-soluble and biocompatible polymers, polyethylene glycol (PEG) is the most frequently used polymer for modification of drugs and proteins to enhance stability and drug-retention time. This is mainly attributed to the low toxicity of PEG and its robust solubility profile in both organic solvents (for the synthesis of protein-reactive PEGs) and water (for the protein conjugation reaction) [6]. Alternatives to PEG have been investigated for their ability to both increase the activity of the protein and also to selectively cleave the attached polymer. In addition, derivatives of polyglycerol and polyacrylic acid (PAA)[7] have been used to investigate the influence of molecular weight and especially polymer architecture (degree of branching) on the biological activity and thermo-resistance of a class of proteins. Polyphosphoesters (PPE)[8],[9] represent a novel and fully biodegradable alternative that is currently under active investigation.

Despite the many ongoing investigations into a number of classes of polymer-conjugated proteins, and the large body of literature available on their biochemical properties, very few studies

have been published on protein structural dynamics in conjugated systems. However, on a more fundamental level, it is vital to have a deeper understanding of the underlying molecular processes involved in modulating biological function in polymer-conjugated proteins.

On the molecular scale, the question arises as to how the polymer structurally organizes itself around the protein. The primary picture of mono-PEGylated proteins invokes the concept of the shroud, in which the polymer chains are wrapped around the protein to create an effective shield that modifies properties at the interface. Although this model may be adequate for short chain lengths at higher grafting densities[5, 10], recent works have shown that the PEGylation of a small number of chains onto the protein can lead instead to a dumbbell-type shape with the polymer chains forming random-coil structures adjacent to the protein. [11, 12, 13–15] In particular, D. Svergun and coworkers[12] show that the PEG surface acts as a shield that introduces intermolecular repulsion, which can increase with additional PEG conjugation.

The unique dynamics of proteins, which are essential to their biological function, occur on timescales from milliseconds down to picosecond timescales. Understanding how protein dynamics is affected by covalent bonding of polymers is therefore of fundamental importance. Protein diffusion is directly impacted by conjugation, as confirmed through hydrodynamics and viscosity measurements that show a slowing down of center-of-mass translational motion of the conjugate through solution. [11] On the microscopic scale, protein flexibility requires rapid dynamics on nanosecond (ns) to picosecond (ps) timescales. This has been characterized in temperature studies by the presence of a so-called dynamical transition around 200 K [16-17]. Protein dynamical fluctuations are highly environmentally dependent, with onsets that can be controlled and tuned by changing the microscopic environment around the protein through a number of mechanisms: solvation (including hydration); pressure; complexation; as well as conjugation. This is also true in the case of biological activity. The dynamical transition of proteins has been extensively studied by

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neutron scattering, due in large part to the well-suited timescale accessible with this technique. The general picture drawn by recent work performed by M. Weik and coworkers[18] focuses attention on the impact of an absence of water in protein-polymer dynamics. The group has shown that polymer dynamics is similar to hydrated-water dynamics at the protein interface, the presence of which enables necessary fluctuations on the ps-ns timescale that directly facilitate protein function. In fact, it has been shown that the protein dynamics remain undisturbed by the presence of conjugated polymer, which can act *like* water around the protein. This scenario could be applied to a protein wrapped by a surfactant layer (shroud model), but may not necessarily apply in general to behavior in conjugated systems. Indeed, the dynamical behavior may be very different in the dumbbell model in which the polymer chain protrudes as an adjacent coil, or in situations where the conjugate does not form a liquid at room temperature. Hence, there is a significant need to better understand how protein relaxation dynamics is impacted by different bio-compatible and biodegradable polymers to examine the general applicability of this behavior.

In the present paper we address the effects of polymer conjugation on protein dynamical fluctuations on the ns timescale. We analyze the dynamical properties of proteins solvated with polymer in order to better understand the relative importance of molecular mechanisms related to hydration water versus bio-compatible materials. Using neutron scattering spectroscopy we present the investigation of the dynamics of a new class of polymer-protein conjugates, i.e. protein conjugation with fully degradable and highly water-soluble polymethyl ethylene phosphonate.

The highlight of our work is presented in the experimental investigation performed with partially deuterated conjugates at two different polymer molecular weights (5 kDa and 10 kDa). Here we describe for the first time that polymers do not promote a dynamical transition in absence of hydration water and that the length of the polymers within the conjugate is important for the degree of the protein flexibility in the dry state. We discuss these findings in the context of protein

environmental dynamics.

Materials and Methods

1. Sample Preparation:

About 400 mg of completely hydrogenated Maltose-Binding Protein (MBP-H) and 300 mg of perdeuterated MBP (MBP-D) were purified at the ILL Deuteration Laboratory (Grenoble, France) and used to synthesize the polymer-protein conjugates. Completely hydrogenated and partially hydrogenated conjugates were then synthesized at the Max Plank Institute for Polymer Research (Mainz, Germany).

Controlled anionic ring-opening polymerization (AROP) of 2-methyl-1,3,2-dioxaphospholane 2-oxide(MeEP / MeEP-d_4) was conducted as described previously (for both the hydrogenated as well as the partially deuterated monomer) using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as the organocatalyst.[19] MeEP-d_4 was prepared from ethyleneglycol-d_6 and methylphosphonate dichloride, the synthesis and characterization of which is provided in Supporting Information (SI) Figures S1-S3). Benzyloxyethanol was used as an initiator, which allowed determination of the molecular weight by ^{1}H NMR spectroscopy by comparison of the integral of the aromatic protons at 7.43 – 7.20 ppm to the integral of the protons of the methylphosphonate groups of the repeating units at 1.51 ppm. The living polymerization was terminated by *N*,*N*′-disuccinimidyl carbonate to introduce an amine-reactive succinimidyl carbonate (SC) functionality (Figure S4-S6). Two sets of polymers were synthesized: PMeEP^{H}-SC (5& 10kDa); and PMeEP^{D}-SC (5 and 10 kDa). Note that we use the superscript H to denote fully hydrogenated while D is used to denote partially deuterated polymers (see Table S1). Conjugation of PMeEP-SC to MBP was performed in in borate buffer (pH 8.5, 100 mM) for 2 hours (details in SI). In this way the SC group forms a stable urethane linkage to a free-

amine residue (a surface-accessible lysine). Purification of the conjugates was accomplished by dialysis (25000 MWCO) against deionized water (see SI for more details). The degree of modification was determined by aqueous size exclusion chromatography (SEC) by applying an online ultraviolet (UV at a wavelength of 280 nm), multi-angle laser-light scattering (MALLS) and refractive index detector setup. The absolute molecular mass (M_n) of the conjugate was calculated according to the Wyatt Astra software. Subtraction of the known molecular weight of the native protein (42.5 kDa) confirmed successful conjugation and allowed calculation of the number of attached polymer chains (see SI Table S2). On average, two PMeEP-chains were attached to each protein during the conjugation process. Successful conjugation of hydrogenated MBP^H with hydrogenated $PMeEP^H$ or $PMeEP^D$ was verified by a significant decrease in elution volume of the corresponding SEC traces as the hydrodynamic radii increased for all conjugates (see Figures S7-S8). The same was observed for the conjugates of $PMeEP^H$ to MBP^D, but since the deuterated protein was prone to form high molecular-weight aggregates the elution profile was not comparable to the hydrogenated counterpart (see Figures S9-S11). Preparation of MBP-conjugate and a schematic representation of the modified protein are reported in Figure 1.

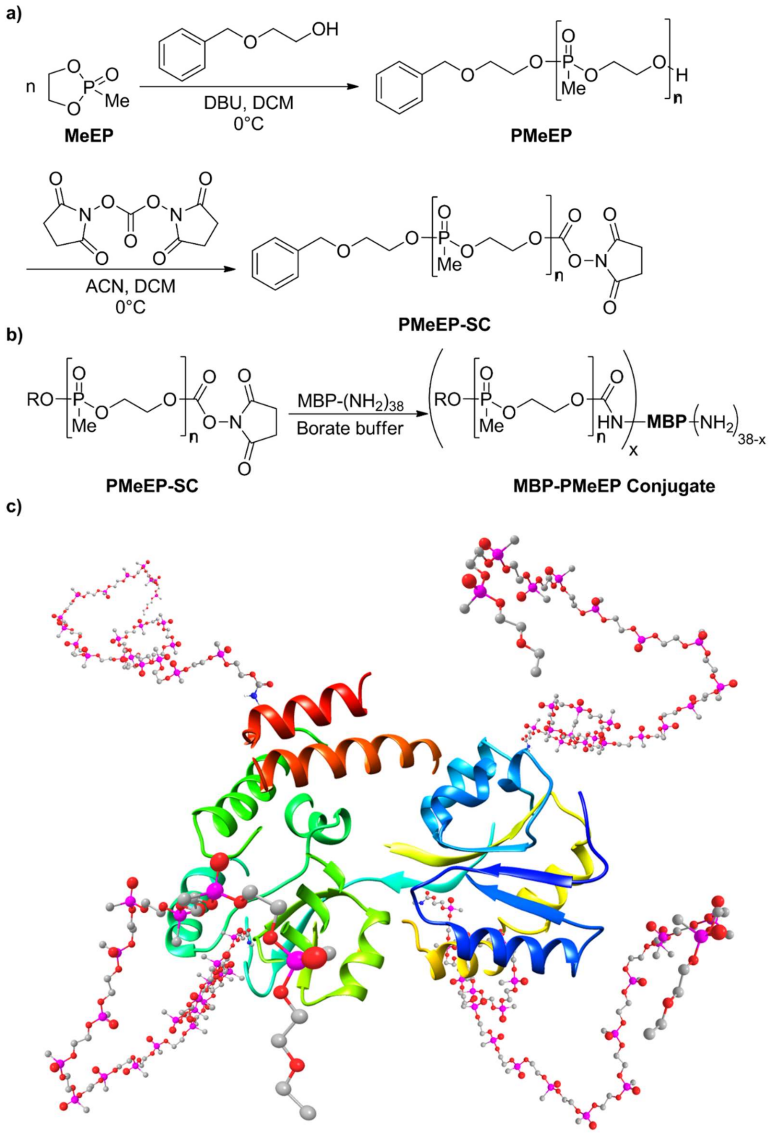

Figure 1. *Preparation of MBP-polymer conjugates: a) Anionic ring-opening polymerization of MeEP and functionalization with a succinimidyl carbonate (SC) group. b) Conjugation of amine-reactive PMeEP-SC to MBP via free lysine residues on the protein surface. c) Graphical*

illustration of a modified MBP (PDB # 1ANF). Note that the polymer arrangement shown is for illustrative purposes and is not intended to represent an actual configuration.

A significant strength of neutron scattering is its ability to differentiate the scattering cross sections between hydrogen and deuterium, which enables selective deuteration to provide unique information on highly specific (hydrogenated) portions of the compound. For this purpose, four different MBP/polymer were carried out: hydrogenated MBP/ hydrogenated polymer 5 kDa (MBP(H)-PMeEP(H)$_{5kDa}$); hydrogenated MBP/ hydrogenated polymer 10 kDa (MBP(H)-PMeEP(H)$_{10kDa}$); deuterated MBP/ hydrogenated polymer 10 kDa (MBP(D)-PMeEP(H)$_{10kDa}$); and hydrogenated MBP/ deuterated polymer 10 kDa (MBP(H)-PMeEP(D)$_{10kDa}$). Native MBP, pure polymers and previous conjugates, were investigated in both their dry and hydrated forms. All samples were first dissolved in D_2O solution and subsequently lyophilized. The obtained samples were further dried under primary vacuum for a minimum of 24 hours prior to performing the measurements. Any separable water was therefore concluded to be absent from the dry samples. All hydrated samples were exposed to D_2O through a saturated D_2O atmosphere and weighed until the desired hydration level was obtained. All hydrations of the various samples ranged between 40% and 50% (MBP native protein 40%; fully protonated conjugates (5000 et 10 000) 50%; MBP conjugated with deuterated polymer 40%).

The handling of the deuterated MBP(D)-PMeEP(H)$_{10kDa}$ conjugate in the dry form was more challenging compared to the other samples, which had a different texture. Because of technical issues during the experiment, the measurements on the MBP(D)-PMeEP(H)$_{10kDa}$ conjugate were less reliable. For this reason we have discarded these results from the main manuscript (although we do present some of the results in SI).

2. Experimental procedure

Elastic and Quasi-elastic Incoherent Neutron Scattering (EINS and QENS) experiments have been used to investigate the polymer-protein dynamics, flexibility and dynamical transition for completely hydrogenated and partially deuterated conjugates. We use elastic scattering to refer to the intensity that arises from atoms that are immobile on the timescale of the instrumental resolution, which give quantitative information about the fraction of "immobile" atoms. Elastic scans as a function of temperature therefore give access to the evolution of this population (mainly hydrogen because of its significantly larger cross section) as a function of temperature. QENS, in contrast, gives a measurement of the relaxation time of proton motions. In order to evaluate which component contributes to the signal, total and partial cross section of the polymer and the protein are given in Table S3. A detailed description of the analysis strategy is also provided in the SI.

Results and discussion

Dry samples. Hydrogenated and partially deuterated, fully biodegradable protein-polymer conjugates have been prepared. The synthesis of partially deuterated MeEP allows us to generate well-defined protein-reactive polyphosphonates for the first time. Our study of the motions and flexibility of the protein in a polymer-protein complex is initially based on a detailed interpretation of the temperature dependence of elastic scans (see SI). With the help of perdeuterated combinations, we have been able to address the contributions arising from the protein itself in the whole system using dry and hydrated samples, which provides for a more complete understanding of the dynamics.

In Figure 2 are presented the integrated elastic intensities (IEI summed up to Q= 1.8 A^{-1}) as

a function of temperature for all dry samples, such as the MBP protein, bulk PMeEP polymer, and conjugates with two different polymer chain lengths (5 kDa and 10 kDa with low molecular-weight dispersity $Đ$<1.2). Error bars are shown only for the MBP protein curve, but are representative for all curves. The corresponding mean-square displacements (MSD) are calculated from Eq. S1 for the protein, polymer and conjugate 5 kDa, as reported in Figure S13a. In Figure 2, data for all samples containing MBP exhibit the same general trend of a smooth decrease as a function of temperature. The bulk polymer shares the same behavior up to the temperature of the glass transition - 240 K, at which point there is an abrupt drop in the IEI. A noteworthy result observed in Figure 2 is the absence of dynamical transition in the polymer-protein conjugates, which is typical of dry-state biological molecules from small peptides[20] to native proteins, including MBP[21,22].

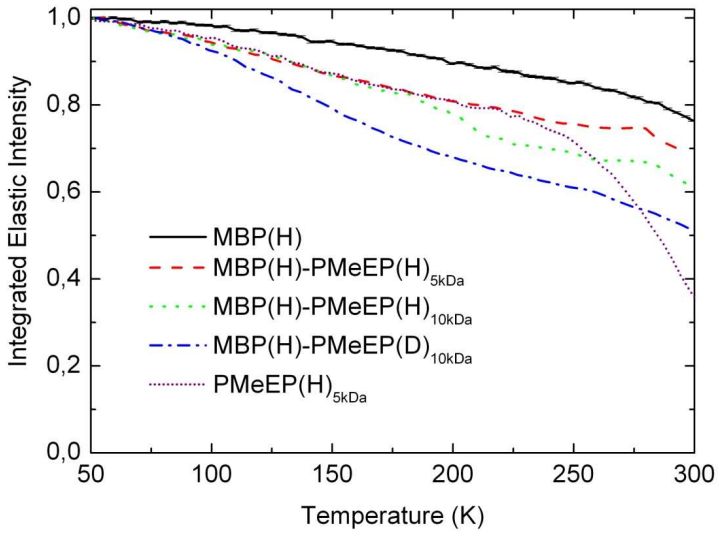

Figure 2*: Integrated elastic intensity as a function of temperature for dry conjugates and their components. The dry polymer glass transition can be observed at around 240K. A complete absence*

of abrupt change in the temperature dependence of the intensity is characteristic of Native MBP protein (solid line), MBP(H)-PMeEP(H)$_{5kDa}$ (dashed line), MBP(H)-PMeEP(H)$_{10kDa}$ (dotted line), MBP(H)-PMeEP(D)$_{10kDa}$ (dash-dotted line),

The dynamical transition would manifest itself in the data in Figure 2 as a major increase in the negative slope of intensity with temperature, similar to what is observed in bulk dry polymer. The conjugated polymer therefore does not provide a flexible enough environment to drive the unfreezing of protein fluctuations as is observed in the case of hydration water[20], or with surrounding surfactants of the solvent-free protein liquid[18]. However, the lower intensity of the conjugates, compared to the native MBP fingerprint, suggests a non-negligible increase in flexibility of the whole complex. In particular, we can compare in more detail the curves obtained for MBP(H)-PMeEP(H)$_{5\ kDa}$ and MBP(H)-PMeEP(H)$_{10\ kDa}$, as both samples are completely hydrogenated and differ only in polymer molecular weight. The IEI in Figure 2 demonstrates that the length of polymer chain plays a role in the degree of enhancement in flexibility of the whole complex. Comparing to dry polymer, PMeEP(H)$_{5kDa,}$ and the respective MBP(H)-PMeEP(H)$_{5kDa}$ conjugate, we observe that the polymer behavior dominates the measured profile up to the bulk polymer glass transition. This feature also holds true for the 10 kDa samples: it is remarkable to note the dramatic change in elastic profile that the MBP(H)-PMeEP(D)$_{10kDa}$ sample undergoes (in which the intensity arises mainly from the protein) when compared to pure MBP. The results lead to the conclusion that conjugation, in the dry state, while not enabling the dynamical transition, does serve to soften the environment of the protein sufficiently to enhance biomolecular flexibility.

One surprising aspect of this general picture is that not only does the larger overall flexibility in the conjugates increase, but also the marked absence of a signature of the polymer-glass transition. Looking at the elastic profile presented in Figure 2, it is clear that signals of the

native MBP and all conjugates have a different slope, but present profiles completely different from the bulk polymer. In support this argument, we report in Figure 3 the elastic intensity of the MBP(H)-PMeEP(H)$_{5kDa}$ and MBP(H)-PMeEP(H)$_{10kDa}$ conjugates, together with the *computed* elastic intensity resulting from the native protein and bulk polymer summed in their respective proportion in each sample. We assume for this estimation that PMeEP(H)$_{10kDa}$ has the same elastic signal as PMeEP(H)$_{5kDa}$. The glass transition of the polymer is still present in our calculation, affirming that the transition would be observed if present. The absence of glass transition in the polymer suggests a strong coupling / integration of the dynamics of the protein and polymer in the conjugate.

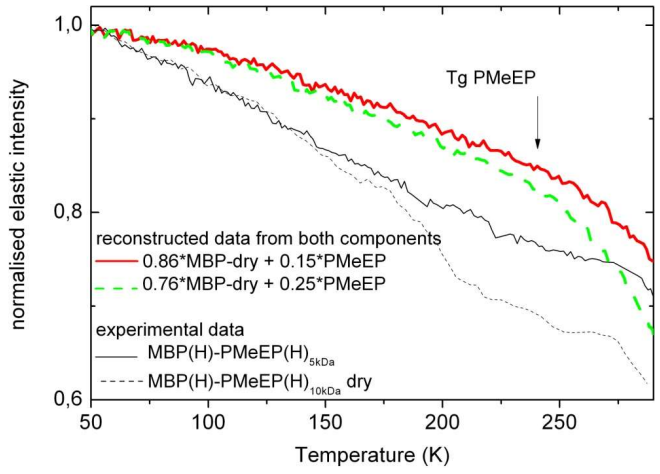

Figure 3 *Experimental and computed integrated elastic intensity as a function of temperature for dry conjugates MBP(H)-PMeEP(H)$_{5kDa}$ (solid line), MBP(H)-PMeEP(H)$_{10kDa}$ (dashed line). The glass transition of the polymer is still present in the computed spectra, demonstrating that the transition would be expected to be observed if present.*

Hydrated samples. Aware that the role of water in the dynamical behavior of biomolecules is often associated with the so-called dynamical transition, we investigate hydrated conjugates and compare results to native MBP. Figure 4 shows the integrated elastic intensities of the hydrated MBP, MBP(H)-PMeEP(H)$_{5kDa}$, MBP(H)-PMeEP(H)$_{10kDa}$ and MBP(H)-PMeEP(D)$_{10kDa}$, in comparison to the dry MBP protein. As in the case of the dry samples, all the curves show a smooth decrease at low temperature. However, upon hydration, an abrupt drop of the integrated intensity can be observed above T_d=200 K for MBP, and above T_d=225 K for all conjugates. A recovering of the dynamical transition, or polymer-glass transition, upon hydration is clearly evident.

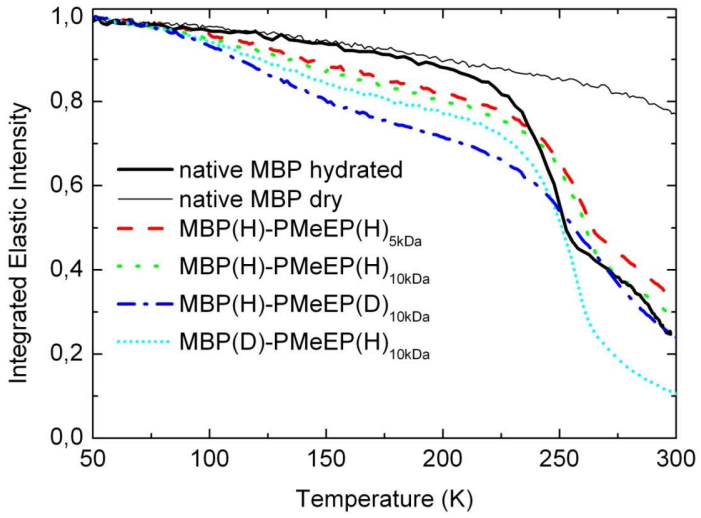

Figure 4. *Integrated elastic intensity as a function of temperature for hydrated conjugates compared to dry and hydrated native MBP. All hydrated curves show an abrupt change in the elastic intensity, which is a fingerprint of dynamical transition. That is, the larger the loss of elastic intensity the higher is the flexibility of the system. The MBP (solid line) dynamical transition is observed at T=200 K while for all other samples it is observed at T=225 K: MBP(H)-*

PMeEP(H)$_{5kDa}$ (dashed line); MBP(H)-PMeEP(H)$_{10kDa}$ (dotted line); and MBP(H)-PMeEP(D)$_{10kDa}$ (dash-dot line).

The different temperature dependence of IEI for hydrated pure MBP protein and conjugates is consistent with the IEI presented in Figure 2, and demonstrates that polymer solvation not only increases the flexibility at low temperature but also shifts the dynamical transition temperature upon hydration. In addition, while in the dry state the length of the polymers seems to have an impact on the whole-complex dynamics (for T>150K), in the hydrated state only a small difference is observed above T_d. The curve associated with the MBP(H)-PMeEP(D)$_{10kDa}$ sample shows again an important decrease, at very low temperature, of the elastic intensity, which confirms the enhanced flexibility of the MBP protein in the conjugate as compared to the native MBP protein. A closer observation of the IEI for all samples reveals a change in the slope at around 250K in addition to the common T_d of the conjugates at 225 K. This change in slope of the latter transition appears less abrupt in conjugates than it is in the native MBP. Based on this it is tempting to speculate that, at equivalent global hydration level, the protein in the conjugate is less hydrated than in the native MBP protein and that the transition is mediated by the polymer presence. In other words, a larger proportion of water molecules hydrate the polymer chains more than the protein, resulting in a lower hydration of the protein surface, a higher mobility for the polymer chains, and a shift of the Td to higher temperature. This hypothesis seems to be supported by the behavior of the sample MBP(D)-PMeEP(H)$_{10kDa}$ (see Figure S14c), in which the main observation is due to polymer dynamics. The elastic profile shows a pronounced decrease at 225 K, which highlights the importance of polymer fluctuations in the whole complex and the reciprocal dynamical influence of both components. A further comparison between respective dry and hydrated samples is reported in the SI.

As for the dry case, we compute the MSD from the Q-dependence of the elastic intensity, as shown in the Figure S13b. The MSD are the sum of three contributions: *vibrational; rotational; and translational* dynamics. The latter contribution is the main origin of the dynamical transition mediated by water dynamics [23,24,25]. The vibrational and rotational terms are responsible for the increase of MSD before the onset of the translational dynamics, which more drastically changes the temperature behavior around 225 K. An evaluation of these two terms can be made from the slope between 50 K and ~210 K, which is subtracted from the total MSD to extract the translational term to get a direct comparison of the dynamical transition temperature between the different samples. The *translational* MSD are shown in Figure 5, emphasizing that the dynamical transition occurs at higher temperature (~25 K) in the conjugates than in the native protein. It follows that pure and conjugated MBP have a different temperature dependence of their translational motion suggesting a larger hindrance to the protein in its conjugate form.

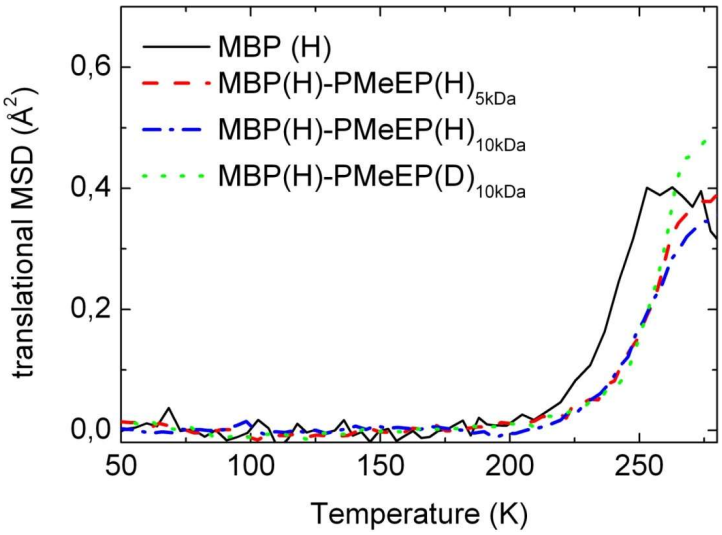

Figure 5. *Translational MSD as a function of temperature for hydrated conjugates compared to hydrated native MBP (solid line). It appears that the dynamical transition occurs at higher temperature in the polymer-protein complexes (225 K) than in MBP. The completely hydrogenated conjugates, at the two different molecular weights, have a quite similar MSD and present the same dynamical transition temperature.*

Upon hydration, it is well established that proteins undergo the dynamical transition around 200 K, a temperature that is related mainly to the solvent behavior [26,27]. Furthermore, the glass transition of a polymer usually shifts toward lower temperature due to the low glass-transition temperature of water (136 K), an effect that has been previously modeled in several ways [28]. According to the simplified Fox equation [29], we expect for the hydrated PMeEP at 40% hydration a glass transition temperature Tg~186 K. Assuming a density of 1.2 [units?] for the polymer, the correction introduced in the Gordon-Taylor/Kelley-Bueche[30,31]model leads to a glass transition temperature of 175 K, giving a lower limit to this estimation (see SI for details of the models). The transition temperature of the polymer-protein mixture should therefore fall in between those for each component, which in our case places both below 200 K. The observation of the transition temperature at 225K indicates a complex coupling of the polymer and protein dynamics, which lies far away from an ideal mixing behavior.

It is important to compare the dry conjugates to the hydrated MBP protein, in which we observe a very different mobility. While below the dynamical transition of the hydrated protein (T_d=200 K), the conjugates show a more signifcant loss of elastic intensity (higher mobility in the observed time scale), above T_d the hydrated protein is more dynamically active. This simple result then suggests that polymer solvation does not affect the protein flexibility in the same way as water molecules, as observed in surfactant-grafted Myoglobin by Weik et coworkers[18]. However, it is possible that a non-negligible presence of water molecules, together with the solvating polymer,

reproduces the elastic profile (or MSD) of highly hydrated proteins.

Relaxation time. In order to quantify the relaxation time at room temperature, we also measure the quasi-elastic spectrum around the elastic window. This type of experiment allows probing of the local dynamics and checks the degree to which is it affected by the environment. Within the time scale of 60 ps - 1 ns, we do not observe a quasi-elastic line in the dry samples. However, in the hydrated samples, a small quasi-elastic signal is observed and analyzed in terms of relaxation. The relaxation times as a function of momentum transfer are shown in Figure 6 for the hydrated samples (MBP protein and the investigated conjugates). It is important to remember that smaller relaxation times are associated with faster associated dynamics / relaxation. In general, we observe that the native protein "relaxes" faster than the conjugates. The MBP(H)-PMeEP(H)$_{10kDa}$ and MBP(D)-PMeEP(H)$_{10kDa}$ samples share the same relaxation times, while the conjugated MBP(H)-PMeEP(D)$_{10kDa}$ exhibits a slower relaxation, particularly at low Q values. In other words, MBP solvated by the polymers shows relaxation times longer than is the case for the solvating polymer and native MBP. The results as a function of polymer molecular weight do not enhance differences in the dynamics of the whole system (Figure S16). The observed behavior is consistent with previous results for the increase of dynamical transition temperature upon conjugation (Figure 5), and we conclude that the conjugated polymer slows down the protein translational dynamics. As previously discussed, it is plausible that a heterogeneous water distribution between the polymer and the protein plays a role in this different relaxation behavior: for a given total hydration, the polymer is more hydrated than the protein and thus undergoes faster dynamics, resulting in protein dynamics that correspond to a less hydrated sample. Due to the fact that what is measured concerns local motions, it is important to keep in mind that the MBP protein, in the conjugate, is geometrically confined by the presence of the polymer. This will result in more localized and slower motions compared to pure native proteins. Changing the protein hydration level and

geometrical constraints are then two key parameters for further consideration.

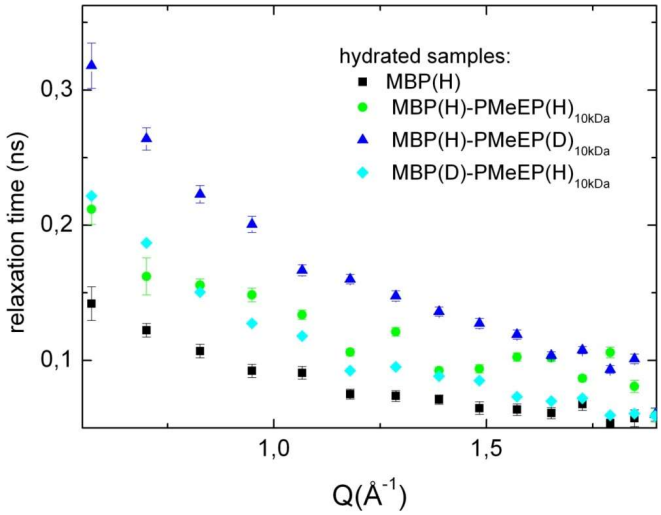

Figure 6. *Polymer-protein relaxation time, inferred from Eq. S4, as a function of Q, for hydrated native MBP (full square), completely hydrogenated (full circle) and partially deuterated conjugates 10 kDa (deuterated MBP: full diamond; deuterated polymer: full triangle). Relaxation time associated with the conjugated MBP (full triangle) is slower by a factor 7 compared to the native MBP.*

Conclusions

We investigate the dynamics of polymer-protein conjugates using new biodegradable and highly water soluble polymers with two different chain lengths in conjugation with MBP. The conjugation with MBP presents a different case with respect to previous studies of dynamics in protein-polymer conjugates. It is representative of a large family of conjugates in which the polymer protrudes from the protein, modifying both steric hindrance and surface-accessible

properties. Thanks to the combination of neutron scattering and specific deuteration, we also investigate the effect of several factors, namely polymer chain length and hydration, on the fast fluctuations, from which we are able to disentangle the dynamics of each component, polymer and protein, which occurr on relatively close timescales.

We show that polymer solvation, in the absence of water, enhances protein fluctuations, but does not enable the protein to undergo the dynamical transition as previously observed in native proteins or in solvent-free liquid proteins. We also observe that the polymer loses its glass properties, providing a signature of a reciprocal and complex influence of both components on their respective dynamics. Upon hydration, while larger fluctuations are observed at lower temperatures in the conjugated-protein, the dynamical transition occurs at higher temperature than in the single MBP protein, and the polymer-glass transition is not recovered. As in native proteins, water is necessary to recover this transition. However, the recovered transition occurs at ~25 K higher than in the native protein at similar hydration, showing an important effect of the environment on the dynamics of the protein. This still provides evidence for the strong coupling between polymer and protein dynamics.

Concerning more specific interactions between the polymer and protein, we show that increasing the polymer chain length (from 5 kDa to 10 kDa) enhances the protein flexibility in the dry system, but that this influence is lost upon hydration. The protein, confined within the polymer, has therefore a larger flexibility, but its dynamical transition from small to large amplitude motions occurs at higher temperature, as if water is held by the polymer, leading to a drier protein surface than in fully hydrated proteins. The fluctuations of the whole systems are then mediated by the polymer. Eventually, we find that the coupling is mutual: the polymer, once conjugated with the protein, has a surprising loss of vitreous properties.

The effects of dynamical coupling between macromolecules and proteins are particularly relevant in drug design, but also for understanding the properties of biological systems, in which the crowding is very large and may lead to similar effects as those observed in this study.

Electronic Supplementary Material:

Supplementary material (MBP deuteration, purification and Characterization, Synthesis and characterization of polymer, Polymer grafting on Maltose Binding Protein, Elastic and quasi-elastic neutron scattering experiment) is available

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Figure Captions

Figure 1.: *Preparation of MBP-polymer conjugates: a) Anionic ring-opening polymerization of MeEP and functionalization with a succinimidyl carbonate (SC) group. b) Conjugation of amine-reactive PMeEP-SC to MBP via free lysine residues on the protein surface. c) Graphical illustration of a modified MBP (PDB # 1ANF). Polymer arrangement is not representative of the real configuration.*

Figure 2*: Integrated elastic intensity as a function of temperature for dry conjugates and their components. Dry polymer glass transition can be observed at around 240K. A complete absence of abrupt change in the temperature dependence of the intensity is characteristic of native MBP protein (solid line), MBP(H)-PMeEP(H)$_{5kDa}$ (dashed line), MBP(H)-PMeEP(H)$_{10kDa}$ (dotted line), MBP(H)-PMeEP(D)$_{10kDa}$ (dash-dott line),*

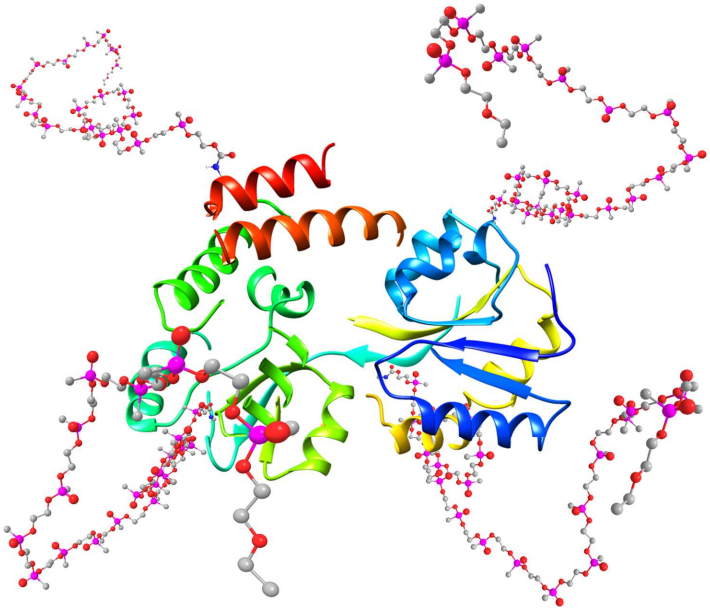
Figure 3 *Experimental and computed integrated elastic intensity as a function of temperature for dry conjugates MBP(H)-PMeEP(H)$_{5kDa}$ (solid line), MBP(H)-PMeEP(H)$_{10kDa}$ (dashed line). The glass transition of the polymer is still present in the computed spectra, proving that transition would be observed if occurring.*

Figure 4. . *Integrated elastic intensity as a function of temperature for hydrated conjugates compared to dry and hydrated native MBP. All hydrated curves present an abrupt change in the elastic intensity as a fingerprint of dynamical transition. Larger the loss of elastic intensity and higher is the dynamical activity of the system. MBP(solid line) dynamics transition is observed at T= 200 K while for all the others samples it is observed at T=225 K (MBP(H)-PMeEP(H)$_{5kDa}$ (dashed line), MBP(H)-PMeEP(H)$_{10kDa}$ (dotted line), MBP(H)-PMeEP(D)$_{10kDa}$ (dash-dot line), MBP(D)-PMeEP(H)$_{10kDa}$ (dotted line))*

Figure 5. *Translational MSD as a function of temperature for hydrated conjugates compared to hydrated native MBP(solid line). It appear that the temperature of dynamical transition occur at higher temperature in the polymer-protein complexes (225 K) than in MBP. The completely hydrogenated conjugates, at the two different molecular weights, have a quite close MSD and share the same dynamical transition temperature.*

Figure 6. *Polymer-protein relaxation time, as inferred from eq S4, as a function of Q, for hydrated native MBP (full square), completely hydrogenated (full circle) and partially deuterated conjugates 10 kDa (deuterated MBP: full diamond; deuterated polymer: full triangle). Relaxation time associated to the conjugated MBP (full triangle) is slower of a factor 7 compared to the native MBP.*

TOC



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