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Measuring translational diffusion coefficients of peptides and proteins by PFG-NMR using band-selective RF pulses

Shenggen Yao[1,*], Daniel K. Weber[1,2], Frances Separovic[1,2], David W. Keizer[1]

[1]*Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Victoria 3010, Australia*

[2]*School of Chemistry, The University of Melbourne, Victoria 3010, Australia*

* Corresponding author:

Shenggen Yao

Bio21 Molecular Science and Biotechnology Institute,

The University of Melbourne

Victoria 3010

Australia

Phone: +61 (3) 83442203

Facsimile: +61 (3) 93478265

E-mail: shyao@unimelb.edu.au

Abstract

Molecular translational self-diffusion, a measure of diffusive motion, provides information on the effective molecular hydrodynamic radius as well as information on the properties of media or solution through which the molecule diffuses. Protein translational diffusion measured by PFG-NMR has seen increased application in structure and interaction studies as structural changes or protein-protein interactions are often accompanied by alteration of their effective hydrodynamic radii. Unlike the analysis of complex mixtures by PFG-NMR, for monitoring changes of protein translational diffusion under various conditions, such as different stages of folding/unfolding, a partial region of the spectrum or even a single resonance is sufficient. We report translational diffusion coefficients measured by PFG-NMR with a modified *ST*imulated *E*cho (STE) sequence where band-selective pulses are employed for all three ^{1}H RF pulses. Compared with conventional non-selective sequence, e.g. the BPP-LED sequence, the advantage of this modified *B*and-selective *E*xcitation *S*hort *T*ransient (BEST) version of STE (BEST-STE) sequence is multi-fold, namely: (1) potential sensitivity gain as in generalized BEST-based sequences, (2) water suppression is no longer required as the magnetization of solvent water is not perturbed during the measurement, and (3) dynamic range problems due to the presence of intense resonances from molecules other than the protein or peptide of interest, such as non-deuterated detergent micelles, are avoided.

Keywords: BEST; dynamics range; PFG-NMR; peptides and proteins; translational diffusion measurement; water suppression

Introduction

As described by the well-known Stokes-Einstein equation, the molecular translational diffusion coefficient (D, a measure of diffusive motion) is inversely proportional to the effective hydrodynamic radius (R_h) of the molecule and the viscosity (η) of the solution through which diffuses, i.e., $D = k_B T/(6\pi\eta R_h)$ with k_B and T being the Boltzmann constant and the absolute temperature, respectively. Experimentally measured molecular translational self-diffusion coefficients provide information on molecular effective hydrodynamic radii as well as the properties of the bulk media or solution. In addition to the analysis of complex mixtures, *P*ulsed-*F*ield *G*radient NMR (PFG-NMR) based methods have seen increased application in following the changes of protein/peptide effective hydrodynamic radius due to self-association and aggregation {Dingley, 1995 #75}{Yao, 2000 #7}{Yao, 2004 #26}{Ali, 2006 #80}{Bocian, 2008 #53}{Jansma, 2010 #55}{Wahlstrom, 2012 #52}, folding and unfolding processes {Buevich, 2002 #51}{Dehner, 2005 #9}, ligand binding and protein-protein interactions {Yan, 2002 #54}{Sillerud, 2012 #76}, and structural characterization of drug metabolites in mixtures {Khera, 2010 #74}. Since the pioneering work of measuring translational diffusion coefficient of haemoglobin in intact red blood cells by Kuchel and Chapman {Kuchel, 1991 #81}, protein translational diffusion measurements for studies of proteins under crowded conditions that mimic the intracellular environment have been reported recently {Wang, 2010 #60}. Compared with alternative methods capable of evaluating molecular mass, such as analytical ultracentrifugation or dynamic light scattering, translational diffusion measurements by PFG-NMR allow changes of protein size/mass and shape/conformation to be evaluated via their effective hydrodynamic radii under experimental conditions identical to those optimized for structural and interaction studies in solution.

A focus in the further development of PFG-NMR based translational diffusion measurements is to reduce the overall acquisition time thus facilitating the study of dynamic processes with an improved temporal resolution. Various versions of single transient or single shot sequences have been developed, including the single transient sequence proposed by Pelta *et al*. {Pelta, 2002 #42} and the most representative single shot sequence, *difftrain*, first reported in 2001 by Stamps *et al*. {Stamps, 2001 #34}. A more general approach for reducing the acquisition time of translational diffusion measurements is the *M*ultiple-*M*odulation-*M*ultiple-*E*cho scheme (MMME) {Sigmund, 2007 #36}, which can be considered analogous to the epic echo planar imaging technique {Mansfield, 1977 #44}. With the latest development of single shot or single transient experiments, a DOSY (*D*iffusion *O*rdered *S*pectroscop*Y*) or translational diffusion measurement can be recorded within a matter of minutes if not seconds. These fast acquisition schemes are, however, of limited application in the study of proteins due mainly to the presence of extended spin coupling networks and short spin transverse relaxation time, T_2. The presence of extended spin coupling networks potentially results in severe artifacts due to imperfect multiple de-phasing or re-phasing during MMME whereas the short T_2 values of proteins ultimately limit the number of modulations that can be carried out before the magnetization decays away completely. Furthermore, diffusion measurements or DOSY of chemical compounds are commonly performed or acquired in deuterated solvents where solvent suppression is not required. Protein samples, however, are usually studied in aqueous solution with the protein or peptide concentration being an order of magnitude lower than that used in the analysis of chemical compounds. Water suppression is, therefore, a prerequisite for measuring translational self-diffusion coefficients of proteins in aqueous solution. Water (or solvent) suppression in NMR is well established with a variety of methods available {Zheng, 2010 #56} including very robust pulsed-field gradient based methods, such as WATERGATE {Piotto, 1992 #39}

{Sklenar, 1993 #59} and excitation sculpting {Hwang, 1995 #38}. Pulse sequences for translational diffusion measurements by PFG-NMR incorporating either WATERGATE or excitation sculpting for water suppression have been reported {Price, 2002 #83}{Momot, 2004 #82}. Optimal field gradient strength (at set values) for both water suppression and the entire range of pulsed-field gradient strengths needed for measuring the translational diffusion coefficients is, however, not readily achievable, particularly when only a single axis of B_0 field gradient is present, as is the case for most modern spectrometers equipped with cryoprobes for biomolecular studies.

Since the first introduction of a heteronuclear-detected diffusion ordered sequence for the simplification of spectral overlap in DOSY {Wu, 1996 #86}, a variety of sequences involving the use of heteronuclear editing for translational diffusion measurements have been reported. In addition to sequences such as HSQC-iDOSY for the analysis of small chemical compounds {McLachlan, 2009 #91}, a number of ^{15}N-HSQC based pulse sequences have been proposed primarily for isotope enriched protein studies {Andrec, 1997 #90}{Chou, 2004 #84}{Brand, 2007 #8}{Didenko, 2011 #88}{Horst, 2011 #89}. While these ^{15}N-edited sequences may also be used to eliminate unwanted solvent signals, they have largely been used for quantification of amide solvent exchange of individual backbone amides of proteins via their apparent translational diffusion coefficients. Unlike band selective isotropic edited sequences (see below), a solvent exchange-free translational diffusion coefficient may not readily be obtainable from apparent translational diffusion coefficients measured from backbone amides due to the inherent nature of protein amide solvent exchange in solution.

In multi-transient one-dimensional and multi-dimensional NMR spectroscopy, inter-transient and/or inter-increment relaxation delay, T_R, takes up a large portion of the acquisition time. The recently developed BEST, *B*and-selective *E*xcitation *S*hort *T*ransient, scheme significantly reduces this T_R through the use of band-selective pulses for ^{1}H nuclear

spins to achieve longitudinal relaxation optimization for a predefined section of the entire chemical shift range or a selected group of nuclear spins, e.g. downfield amide protons, allowing short inter-transient relaxation delay, T_R, between transients without saturating proton magnetization of the selected subgroup {Schanda, 2005 #40}{Schanda, 2009 #41}. As a consequence of water magnetization not being perturbed in BEST-based sequences, labile backbone amides may gain additional sensitivity due to their chemical exchange with the solvent water {Yao, 2011 #77} and this additional sensitivity gain may be further improved in the presence of paramagnetic agents {Theillet, 2011 #78}{Sibille, 2012 #79}. Furthermore, making use of nutation frequency modulation arising under the BEST conditions for a quick identification of backbone amides in exchange with solvent water has recently been reported {Yao, 2014 #72}. Finally, solvent exchange induced modulation on experimentally measured translational diffusion coefficients using conventional non-selective PFG-NMR sequences for labile nuclei, e.g. amide protons, are removed when BEST-based sequences are used as the solvent magnetization is not perturbed.

In the analysis of complex mixtures of chemical compounds by PFG-NMR or DOSY, the acquisition of an entire range of (^{1}H, for example) chemical shifts may be necessary as different compounds may give rise to resonances across different regions of the spectrum. In contrast, for measuring protein translational diffusion coefficients under various conditions, such as different stages of folding/unfolding, a sub-spectrum or even a single peak will be sufficient for monitoring the change in translational diffusion (and thus effective hydrodynamic radii of the protein/protein complex under investigation). In this study, translational diffusion measurements using BEST-STE are reported and the results compared with values obtained from the conventional non-selective *B*ipolar *P*ulse *P*air *L*ongitudinal *E*ddy-current *D*elay (BPP-LED) sequence {Wu, 1995 #46}. The BEST-STE sequence was subsequently used to measure translational diffusion coefficients of Aβ42 in the presence of

144 significant molar excess of detergent (non-deuterated) in micelles. The dynamic range
145 problems caused by intense resonances arising from the micelles, as experienced in
146 conventional BPP-LED sequence, are avoided and the translational diffusion coefficients of
147 Aβ42 in the presence of zwitterionic surfactant (molar ratio 1:200) across the temperature
148 range of 278 – 313 K are reported.
149
150 **Materials and methods**
151 *Sample preparations*
152 For the demonstration of BEST-STE for translational diffusion measurements and a
153 comparison with results from conventional BPP-LED, NMR samples of BaxΔC and Aβ42 in
154 the absence and presence of dodecylphosphocholine (DPC) were used. The expression and
155 purification of ^{15}N-labelled BaxΔC have been described previously {Czabotar, 2013 #61}.
156 The ^{15}N-labelled BaxΔC sample used in the present study was the same as used previously for
157 backbone chemical shifts assignments and interaction studies {Czabotar, 2013 #61} {Yao,
158 2014 #63}: 0.8 mM of ^{15}N-labelled BaxΔC in phosphate buffer (50 mM sodium phosphate
159 containing 50 mM sodium chloride and 0.02% (w/v) sodium azide at pH 6.7). Recombinant,
160 uniformly ^{15}N-labelled Aβ42 (containing residues 1-42 of native amyloid beta peptide) was
161 expressed with an N-terminal SUMO partner {Malakhov, 2004 #64} following the protocols
162 developed by Weber *et al*. (unpublished). The fusion construct was cleaved using
163 *Saccharomyces cerevisiae* Ulp1(403-621) {Lee, 2008 #65} to generate native Aβ42, which
164 was subsequently purified by RP-HPLC. The NMR sample used in the present study was
165 pretreated by dissolution into 10% (v/v) ammonia {Ryan, 2013 #66} at 4 mg mL^{-1} and
166 lyophilized to remove pre-formed aggregates, which may seed fibril formation and cause a
167 dramatic reduction in the lifetimes observable in solution NMR of the monomeric species.
168 The Aβ42 peptide (2 mg) was then dissolved into 50 mM sodium hydroxide (60 μL), diluted

~9x with water and adjusted to pH 7.5 by careful addition of 1 M phosphoric acid. The final sample consisted of 15 mM sodium phosphate, 50 mM sodium chloride, 1 mM TSP-d4 and 10% $^{2}H_2O$. Aggregated material was removed by centrifugation (22000 g, 10 min, 4 °C) and the concentration of the peptide in the supernatant was determined by 214 nm absorbance using a theoretical extinction coefficient of 76,848 M^{-1} cm^{-1} {Kuipers, 2007 #67}. The final sample contained 0.5 mM Aβ42. The sample of Aβ42 in DPC was prepared by adding DPC stock (1 M, prepared in the same buffer solution as Aβ42, Avanti Polar Lipid) into the NMR tube to a final concentration of 100 mM. The final molar ratio of DPC:Aβ42 was 200:1, which ensured that the micelles were in molar excess of Aβ42 assuming an aggregation number of 44 DPC monomers per micelle {Kallick, 1995 #68}.

PFG-NMR diffusion measurements

All spectra were recorded on a Bruker Avance500 spectrometer using a TXI cryoprobe equipped with a single gradient (*G*z). The field gradient strength of *G*z was calibrated by measuring the self-diffusion coefficient of residual H_2O in a 100% $^{2}H_2O$ sample at 298.13 K {Yao, 2008 #25}. A diffusion coefficient of 1.9 x 10^{-9} m^2 s^{-1} for the residual H_2O signal was then used for the back calculation of the field gradient strength {Callaghan, 1983 #14}. Spectra were processed in TOPSPIN (Version 3.0, Bruker). The BEST-STE and the conventional BPP-LED with WATERGATE and presaturation incorporated for water suppression are depicted in Figure 1. The BEST-STE sequence was modified from the standard STE sequence with all three hard 90° pulses replaced with shaped-pulses. In the present study, the first and third 90° pulses are polychromatic PC9 shapes of 3 ms (corresponding to an excitation bandwidth of about ~ 1.9 kHz). The second 90° pulse is EBURP-2 with a width of 2.42 ms (corresponding to an excitation bandwidth of about ~ 2.0

kHz). Offsets for all shaped-pulses were placed either near the central region of the amide and aromatic protons (ca 8.2 ppm) or at the upfield region (1.0 ppm) for aliphatic protons.

Analysis of translational diffusion data

Diffusion coefficient, D, was determined by fitting diffusion weighted intensities of selected peaks or integrals over a chosen range to the following equation:

$$I = I_0 \exp\left\{-\gamma^2 s^2 g^2 \delta^2 \left(\Delta - \frac{\delta}{3}\right) D\right\} = I_0 \exp\left(-K^2 D\right) \qquad (1)$$

where γ is the gyromagnetic ratio of proton and s, g, δ and Δ are the shape factor, amplitude, duration and separation of the single pair of gradient pulses, respectively (see Figure 1A). For the conventional non-selective BPP-STE sequence, Δ is replaced by (Δ-τ_1/2) with τ_1 being the time interval between the bipolar gradient pulses within the bipolar gradient encoding or decoding segment (see Figure 1B). Fittings to Eq. 1 were performed using the *T_1/T_2 relaxation module* in TOPSPIN (Version 3.0, Bruker) and reported errors in D are RSS (residual sum of squares) rounded to two digits after the decimal point.

Results and discussion

The sensitivity of BEST-STE (Figure 1A) and subsequently translational diffusion coefficient measured for BaxΔC, a 19 kDa C-terminal truncated pro-apoptotic Bcl-2 family protein {Czabotar, 2013 #61}{Yao, 2014 #63}, were compared with those obtained from the conventional BPP-LED (Figure 1B). The intensity of the methyl peak (0.82 ppm) and integral of resonances over the amide/aromatic region (6.50 – 9.00 ppm), respectively, as a function of the recovery delay, T_R, between consecutive transients are shown in Figure 2B. Both peak height and integral were extracted from slices of the pseudo-2D diffusion encoded datasets recorded using BPP-LED and BEST-STE corresponding to a nearly identical diffusion weighting of $K^2 = 3.8 \times 10^9$ sm^{-2}. The total acquisition time remained constant for every

individual T_R (24 minutes for each diffusion dataset that stepped through a total of 16 diffusion encoding values) with longer T_R values corresponding to a lesser number of transients averaged. Both peak height and integral were normalized after division by the square root of the number of transients for a given T_R. The sensitivity improvements for the BEST-STE sequence (Figure 1A) over the conventional BPP-LED (Figure 1B) was comparable to that observed for selective ^{1}H-^{15}N HSQC-edited diffusion sequence over non-selective ones where triple-axis pulsed-field gradients were used {Augustyniak, 2011 #48}. As seen in Figure 2C, diffusion data acquired with BEST-STE fitted to Eq. 1 equally well as data measured by the conventional BPP-LED sequence. A further comparison of translational diffusion measurements by BEST-STE and BPP-LED was carried out on Aβ42 and the results are summarized in Figure 3B and Table 1. A correlation plot of translational diffusion coefficients measured using BEST-STE and BPP-LED for BaxΔC (Figure 2C) and Aβ42 and TSP (Figure 3B) are shown in Figure 3C.

In order to minimize the effects of non-uniform pulse field gradient and RF pulses over the sample, band-selective (shaped) pulses have been incorporated previously into the translational diffusion measurements used for spatial selection {Park, 2006 #28}{Zhang, 2006 #27}. The primary difference between the BEST scheme and well-known spectral selective schemes, including spectral editing, chemical shift selective imaging sequences, is that all non-detected ^{1}H spins remain at equilibrium throughout the sequences. As a result, their magnetization is preserved for the subsequent magnetization transfer to excited/detected spins via spin diffusion mechanism in order to achieve significant sensitivity gain. A ^{13}C-edited BEST-DOSY experiment was previously reported and applied to resolve overlaps in DOSY {Shukla, 2011 #49}. Although not a single shot experiment, the BEST-STE sequence as described here offers improved sensitivity over the conventional non-selective sequence, such as BPP-LED. Hence BEST-STE suits measurements of larger proteins where the spin

diffusion pathway is far more efficient than in smaller molecules, in particular for proteins where the concentration of proteins of interest might be low due to limited yield or solubility. In cases where the single particle self-diffusion coefficient (i.e., that of a given species at infinite dilution) is of interest, measurements of translational diffusion coefficient as a function of concentration are required {Chou, 2004 #84}. Consequently, this makes the inherent sensitivity improvement in BEST-STE even more attractive when compared with conventional non-selective sequence.

A prerequisite for obtaining high-quality NMR spectra of proteins in solution is sufficient suppression of a strong solvent signal, usually water. The same applies to quantitative translational diffusion measurements of proteins in aqueous solution. While a variety of methods have been developed, including pulsed-field gradient based schemes such as WATERGATE {Piotto, 1992 #39} {Sklenar, 1993 #59} and excitation sculpting {Hwang, 1995 #38} a single set of gradient values used for water suppression, however, may not be optimal for the entire range of field gradient strengths needed for measurements of translational diffusion coefficients. This is particularly true for weaker gradients as used for the diffusion encoding/decoding where the water signal is still intense. Inserting the WATERGATE segment into a diffusion measurement sequence may not achieve satisfactory water suppression for the entire range of field gradient strengths needed for the diffusion measurement, as observed previously {Augustyniak, 2011 #48}. In particular, when only a single gradient (*G*z) is available, as is the case for most modern cryoprobe-equipped spectrometers, one set of optimized gradient values may not work for all combinations of gradient settings. Overall, the band-selective pulses are a superior choice for measuring translational diffusion coefficients of proteins without the need for water suppression.

Effects of chemical exchange on experimentally measured translational diffusion coefficients are well understood {Johnson, 1999 #73}. For a two-site exchange, for instance,

the measured translational diffusion coefficient will be the population weighted average of diffusion coefficients of those two states in the absence of exchange if the exchange rate is fast compared with the time interval between diffusion encoding and decoding. As a result, carbon attached protons, such as methyl protons are superior to amide proton resonances as the latter may be affected by underlying chemical exchange with the solvent. In certain situations, accurate determination of protein translational diffusion coefficients from the preferred methyl protons may not be readily measurable due to the proximity of strong signals in the methyl region, as was the case for Aβ42 in the presence of DPC micelles (see Figure 4A). Note that translational diffusion coefficients determined from amide protons are not affected by underlying exchange with water as the water signal is not excited, and thus not affected by the diffusion gradients, in the band-selective sequence,

Translational diffusion coefficients obtained from PFG-NMR have previously been applied to evaluate the hydrodynamic size of peptide-micelle complexes {Barhoum, 2013 #50}. Using as an example the complex of the cationic antimicrobial peptide GAD-2 and a sodium dodecyl sulfate (SDS) micelle, the methyl region in the ^{1}H spectra was clearly overwhelmed by SDS signals, which limited hydrodynamic information to be drawn only from the acyl chain resonances of the SDS micelle {Barhoum, 2013 #50}. Whilst in this case the ratio of surfactant to peptide was sufficiently low for SDS signals to be representative of the peptide-micelle complex (i.e., negligible complications due to presence of peptide-freemicelles), most studies require that micelles are in vast excess to minimize sample inhomogeneity and avoid reduction in spectral resolution arising from formation of assemblies with multiple peptides/proteins in a single micelle. Furthermore, peptide-to-surfactant ratios are especially important for the study of amyloidogenic peptides, Aβ, in which surface-bound monomeric α-helices {Coles, 1998 #92}{Mandal, 2004 #93} or soluble β-sheet oligomers {Mandal, 2004 #93}{Wahlstrom, 2008 #95}{Yu, 2009 #96} are

induced, depending on whether SDS or DPC micelle concentrations are in excess or deficit, respectively. While micelle systems composed of either the ganglioside GM1 {Williamson, 2006 #97}{Mandal, 2004 #94} or DHPC {Dahse, 2010 #98} also have been reported, and may better represent lipid components of the cellular membrane environment, their role in inducing formation of insoluble fibril structures, however, would have impeded diffusion measurements.

The ^{1}H spectrum of Aβ42 in the presence of DPC (200:1) shown in Figure 4A clearly illustrates the dominance of DPC signals over the entire spectral range. As a consequence, satisfactory fits for the translational diffusion coefficient of Aβ42 are impossible to obtain from the conventional BPP-LED sequence; and the peaks corresponding to either the polar choline headgroup or acyl chain protons of DPC (Fig. 4) cannot be used to measure the diffusion coefficient of the Aβ42-micelle complex since the micelles were in at least four-fold molar excess (assuming an aggregation number of 44 {Kallick, 1995 #68} for a DPC micelle). Instead, with the use of the BEST-STE sequence, which excites/detects only resonances in the downfield region of the ^{1}H spectrum of Aβ42 and DPC (Figure 4A), satisfactory measurements of translational diffusion coefficients of Aβ42 were readily obtained. From the values determined using the BEST-STE sequence (Table 1), the diffusion coefficient of free Aβ42 was 0.74 (± 0.01) × 10^{-10} m^2 s^{-1} and reduced to 0.39 (± 0.01) × 10^{-10} m^2 s^{-1} upon addition of DPC, which is most likely due to association of Aβ42 with DPC micelles. Furthermore, the higher diffusion coefficient of 0.49 (± 0.01) × 10^{-10} m^2 s^{-1} measured for DPC using the conventional BPP-LED sequence exemplifies a situation with a surplus of DPC micelles not in association with Aβ42. Interestingly, upon heating the Aβ42 and DPC sample from 278 to 313 K, the diffusion coefficients summarized in Table 1 (fits depicted in Fig. 4B) obey Arrhenius behaviour (Fig. 4C), indicating that the hydrodynamic properties of the

association between Aβ42 and DPC remains constant over this temperature range, and that this amyloid peptide associates with the zwitterionic micelles.

Conclusion

In this paper a BEST-STE sequence suitable for measuring translational diffusion coefficients of peptides or proteins is described. The sequence was tested using results obtained for the BaxΔC, a 19 kDa apoptosis protein, in aqueous solution as well as the Aβ42 peptide (4.5 kDa) in the absence and presence of non-deuterated DPC micelles. The BEST-STE sequence provides: (1) improved sensitivity over the conventional non-selective sequences, such as BPP-LED, (2) translational diffusion coefficients of proteins in aqueous solution that can be readily measured without the need of water suppression, and (3) measurements of translational diffusion coefficients of proteins or peptides in the presence of non-deuterated detergent micelles, otherwise difficult to measure due to the dynamic range problems. We foresee the BEST-STE sequence to be of particular use for measuring the association of membrane-active peptides with model membrane systems.

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References

Table 1 Translational diffusion coefficients of BaxΔC, Aβ42 and DPC micelles measured by BEST-STE and/or BPP-LED

		D (10^{-10} m^2/s)	
	T (K)	BEST-STE	BPP-LED
BaxΔC [a]			
Peak (0.82ppm)	305	1.22 ± 0.01	1.16 ± 0.01
Region (6.5-9.0ppm)	305	1.18 ± 0.01	1.21 ± 0.01
Aβ42 in the absence of DPC [b]			
Peak (*)	278	0.74 ± 0.01	0.71 ± 0.01
Peak (#)	278	0.74 ± 0.01	0.74 ± 0.01
TSP [c]	278	3.41 ± 0.03	3.35 ± 0.02
Aβ42 in the presence of DPC [d]			
Aβ42	278	0.39 ± 0.01	
Aβ42	283	0.47 ± 0.01	
Aβ42	293	0.58 ± 0.01	
Aβ42	303	0.70 ± 0.01	
Aβ42	313	0.91 ± 0.01	
DPC, peak (*)	278		0.49 ± 0.01
DPC, peak (#)	278		0.48 ± 0.01
DPC, peak (*)	283		0.58 ± 0.01
DPC, peak (#)	283		0.57 ± 0.01
DPC, peak (*)	293		0.77 ± 0.01
DPC, peak (#)	293		0.78 ± 0.01
DPC, peak (*)	303		1.03 ± 0.01
DPC, peak (#)	303		1.03 ± 0.01
DPC, peak (*)	313		1.31 ± 0.01
DPC, peak (#)	313		1.32 ± 0.01

[a] Corresponding diffusion induced signal decays are depicted in Figure 2C

[b] Corresponding diffusion induced signal decays are depicted in Figure 3B

[c] TSP in the Aβ42 sample used as chemical shift reference, see Figure 3B for diffusion induced signal decays

[d] Corresponding diffusion induced signal decays are depicted in Figure 4B

Figure Captions

Figure 1 Pulse sequences for the measurements of protein translational diffusion coefficients in aqueous solution: (A) BEST-STE, and (B) non-selective BPP-LED with water suppression. In the present study, for BEST-STE (A), the first and third 90° pulses are a polychromatic PC9 shape of 3 ms (corresponding to an excitation bandwidth of about ~ 2.5 kHz). The second 90° pulse used EBURP-2 with a width of 2.42 ms (corresponding to an excitation bandwidth of about ~ 2.0 kHz). Phases for both RF pulses and the receiver were as indicated. The non-selective BPP-LED sequence (B) used in the present study was modified from the conventional BPP-LED sequence {Wu, 1995 #46} by the additions of a WATERGATE segment before the acquisition and a weak presaturation (γB_1 = 25.7 Hz) during the recycle delay for water suppression. Narrow and wide rectangles represent 90° and 180° pulses, respectively. Phases for both RF pulses and the receiver were adopted from *ledbpgp2s* (Bruker pulse sequences library): ϕ_1=(x); ϕ_2=2(x), 2(-x); ϕ_3=4(x), 4(-x), 4(y), 4(-y); ϕ_4=2(x,-x), 2(-x,x), 2(y,-y), 2(-y,y); ϕ_7=8(x), 8(y); ϕ_8=8(-x), 8(-y); ϕ_{rec}=(x,-x,-x,x,-x,x,x,-x,-y,y,y,-y,y,-y,-y,y). In both BEST-STE (A) and BPP-LED (B), gradient pulses (sinusoidal-shaped), used for diffusion encoding and decoding, are marked with horizontal lines.

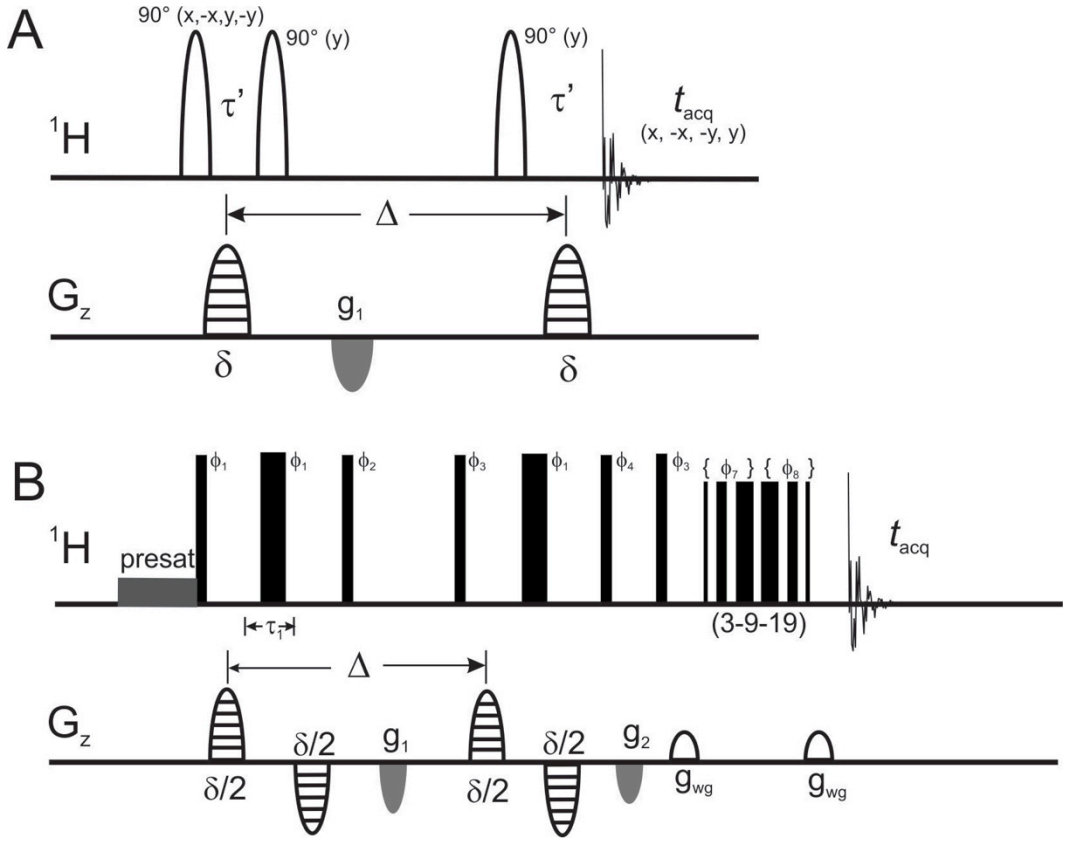
Figure 2 Comparison of relative sensitivity and translational self-diffusion coefficient of BaxΔC measured using BEST- STE (Figure 1A) and BPP-LED (Figure 1B). (A) One-dimensional ^{1}H spectrum of BaxΔC acquired at 305 K with the peak (0.82 ppm) in the upfield aliphatic region and the range (6.50 – 9.00 ppm) of the downfield amide/aromatic region used in the analysis indicated; (B) Peak height (0.82 ppm) and signal integrals over the amide/aromatic region (6.50 – 9.00 ppm) as a function of the recovery delay T_R between consecutive transients. A total acquisition time of 24 minutes was used for the collection of individual pseudo-2D diffusion dataset (stepped through 16 diffusion encoding values). As T_R

increased the total number of transients (NS) decreased. Both intensities and integrals were normalized after dividing by the square root of the number of transients for a given T_R; and (C) Translational diffusion induced signal attenuation shown as relative intensity versus the strength of diffusion encoding, $K^2=\gamma^2 s^2 g^2 \delta^2(\Delta-\delta/3)$ for BEST-STE or $K^2=\gamma^2 s^2 g^2 \delta^2(\Delta-\delta/3-\tau_1/2)$ for BPP-LED. Data acquired with T_R values of 50.8 ms (NS = 320) and 1.992 s (NS = 32) for the BEST-STE and BPP-LED, respectively, are shown. All lines represent the results of nonlinear regression to Eq. 1 and fitted values are summarized in Table 1.

Figure 3 Comparison of translation diffusion coefficients measured by BEST-STE and BPP-LED. (A) One-dimensional ^{1}H spectrum of Aβ42 in the absence of DPC at 278 K with peaks involved in the analysis indicated; (B) Translational diffusion induced signal attenuation for Aβ42, as Figure 2C, but for clarity, only lines fitted for peaks of methyl region (~ 0.82 ppm, marked with * in Figure 3A) are shown And results are are summarized in Table 1; (C) Plot of translational diffusion coefficients of BaxΔC (Figure 2C), Aβ42 and TSP (Figure 3B) measured by BEST-STE versus those by BPP-LED. Line represents the result of linear regression with a slop = 1.015 and R^2=0.999.

Figure 4 Translational diffusion coefficients of Aβ42 in the presence of DPC measured by BEST-STE. (A) One-dimensional ^{1}H spectrum of Aβ42 in the presence of DPC (molar ratio of DPC:Aβ42 is 200:1) at 278 K with DPC peaks involved in the analysis indicated. Resonance assignments of DPC were adopted from those reported previously {Manzo, 2013 #62}. The left inset displays a partial spectrum of the amide and aromatic region with the peak used for the translational diffusion measurements using BEST-STE marked; (B) Translational diffusion signal attenuation shown as relative intensities versus the strength of diffusion, from 278 – 313 K, for Aβ42 measured by the BEST-STE sequence and for DPC

measured by BPP-LED sequence. All lines represent the results of nonlinear regression to Eq.1. All fitted translational diffusion coefficients are summarized in Table 1; (C) Arrhenius plot of experimentally measured translational diffusion coefficients of Aβ42 (measured by BEST-STE) and DPC micelles (measured by BPP-LED) from 278 – 313 K. Lines represent nonlinear regression to the Arrhenius equation $D = D_0 \exp(-E_a/RT)$. Clearly, translational diffusion of both Aβ42 and DPC exhibit Arrhenius behaviour over the temperature range measured.


A
90° (x,-x,y,-y)
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g_{wg}
g_{wg}
δ/2

Figure 1

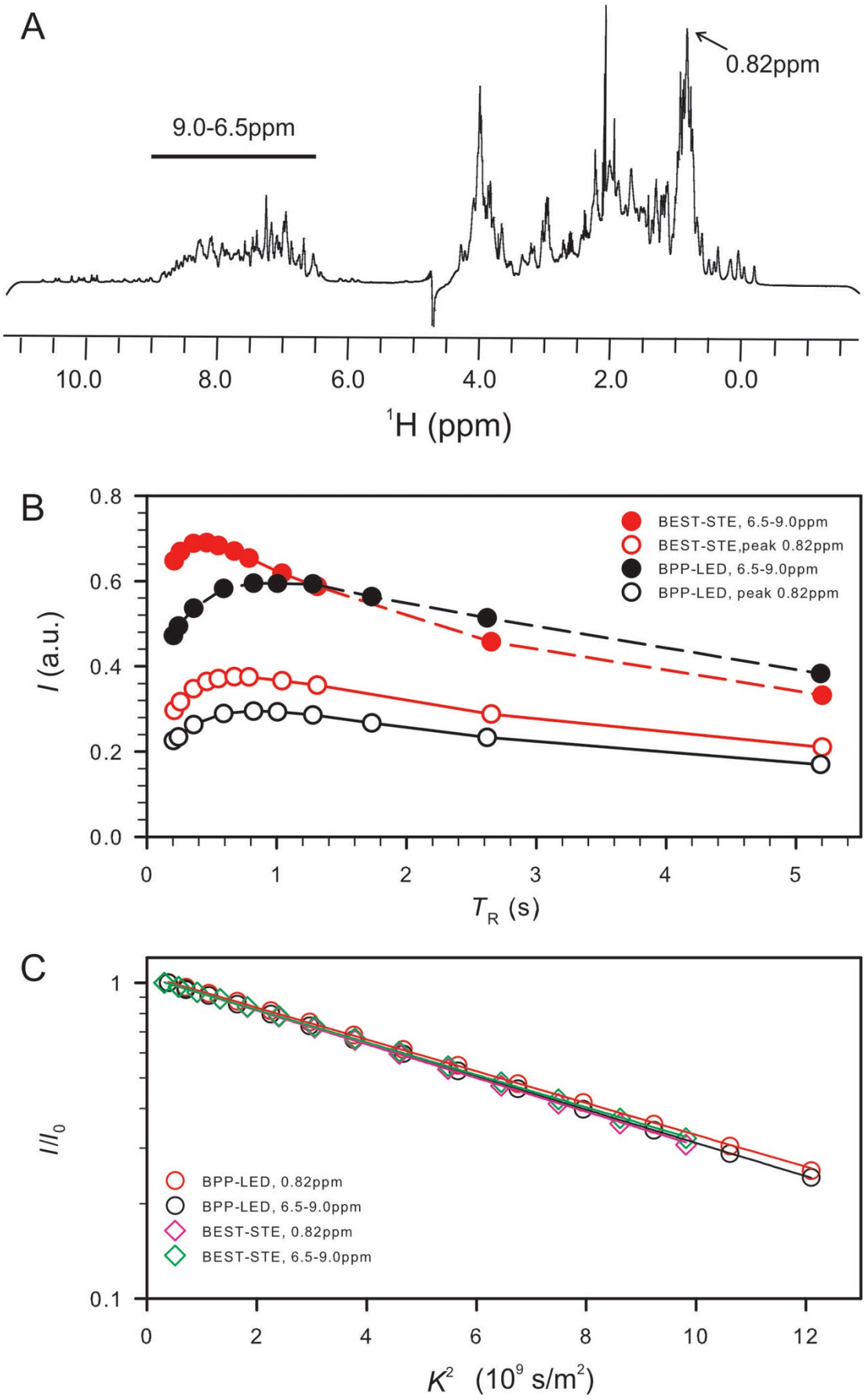
A
0.82ppm
9.0-6.5ppm
10.0
8.0
6.0
4.0
2.0
0.0
^{1}H (ppm)
B
BEST-STE, 6.5-9.0ppm
BEST-STE,peak 0.82ppm
BPP-LED, 6.5-9.0ppm
BPP-LED, peak 0.82ppm
I (a.u.)
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
T_R (s)
C
BPP-LED, 0.82ppm
BPP-LED, 6.5-9.0ppm
BEST-STE, 0.82ppm
BEST-STE, 6.5-9.0ppm
I/I_0
1
0.1
0
2
4
6
8
10
12
K^2 (10^9 s/m^2)

Figure 2

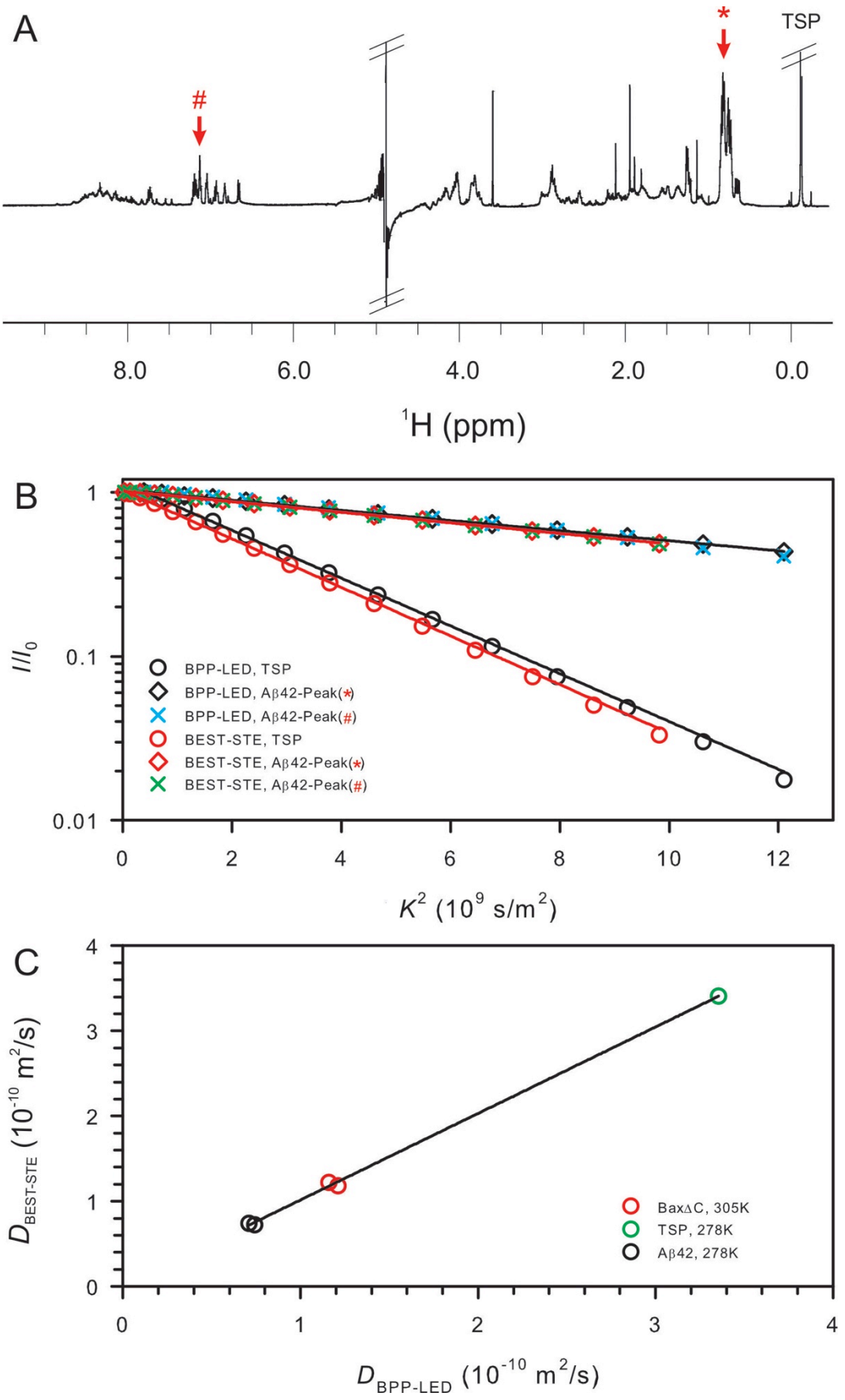
A
#
*
TSP
8.0
6.0
4.0
2.0
0.0
^{1}H (ppm)
B
1
0.1
0.01
I/I_0
BPP-LED, TSP
BPP-LED, Aβ42-Peak(*)
BPP-LED, Aβ42-Peak(#)
BEST-STE, TSP
BEST-STE, Aβ42-Peak(*)
BEST-STE, Aβ42-Peak(#)
0
2
4
6
8
10
12
K^2 (10^9 s/m^2)
C
4
3
2
1
0
$D_{BEST-STE}$ (10^{-10} m^2/s)
BaxΔC, 305K
TSP, 278K
Aβ42, 278K
0
1
2
3
4
$D_{BPP-LED}$ (10^{-10} m^2/s)

Figure 3

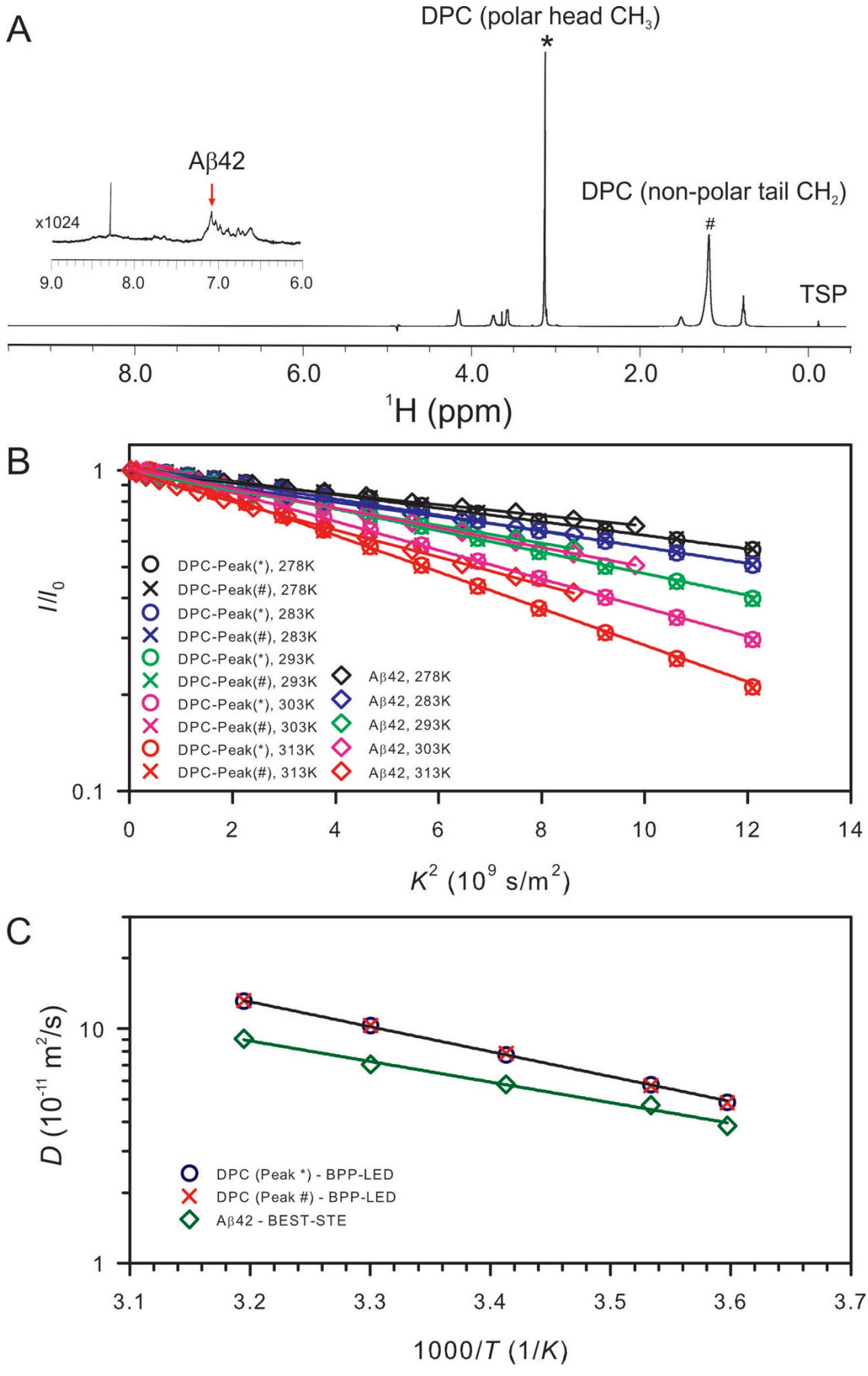
A
DPC (polar head CH3)
*
Aβ42
x1024
9.0
8.0
7.0
6.0
DPC (non-polar tail CH2)
#
TSP
8.0
6.0
4.0
2.0
0.0
1H (ppm)
B
1
0.1
I/I0
DPC-Peak(*), 278K
DPC-Peak(#), 278K
DPC-Peak(*), 283K
DPC-Peak(#), 283K
DPC-Peak(*), 293K
DPC-Peak(#), 293K
DPC-Peak(*), 303K
DPC-Peak(#), 303K
DPC-Peak(*), 313K
DPC-Peak(#), 313K
Aβ42, 278K
Aβ42, 283K
Aβ42, 293K
Aβ42, 303K
Aβ42, 313K
0
2
4
6
8
10
12
14
K2 (10^9 s/m^2)
C
10
1
D (10^-11 m^2/s)
DPC (Peak *) - BPP-LED
DPC (Peak #) - BPP-LED
Aβ42 - BEST-STE
3.1
3.2
3.3
3.4
3.5
3.6
3.7
1000/T (1/K)

Figure 4