

# Effect of Intrinsic Disorder and Self-Association on the Translational Diffusion of Proteins: The Case of $\alpha$ -Casein

Melnikova D., Skirda V., Nesmelova I.

Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia

## Abstract

© 2017 American Chemical Society. Translational diffusion is the major mode of macromolecular transport in living organisms, and therefore it is vital to many biological and biotechnological processes. Although translational diffusion of proteins has received considerable theoretical and experimental scrutiny, much of that attention has been directed toward the description of globular proteins. The translational diffusion of intrinsically disordered proteins (IDPs), however, is much less studied. Here, we use a pulsed-gradient nuclear magnetic resonance technique (PGF NMR) to investigate the translational diffusion of a disordered protein in a wide range of concentrations using  $\alpha$ -casein that belongs to the class of natively disordered proteins as an example.

<http://dx.doi.org/10.1021/acs.jpcc.7b00772>

## References

- [1] Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. The Protein Data Bank *Nucleic Acids Res.* 2000, 28, 235-242 10.1093/nar/28.1.235
- [2] Dyson, H. J.; Wright, P. E. Intrinsically Unstructured Proteins and Their Functions *Nat. Rev. Mol. Cell Biol.* 2005, 6, 197-208 10.1038/nrm1589
- [3] Habchi, J.; Tompa, P.; Longhi, S.; Uversky, V. N. Introducing Protein Intrinsic Disorder *Chem. Rev.* 2014, 114, 6561-88 10.1021/cr400514h
- [4] Mao, A. H.; Crick, S. L.; Vitalis, A.; Chicoine, C. L.; Pappu, R. V. Net Charge Per Residue Modulates Conformational Ensembles of Intrinsically Disordered Proteins *Proc. Natl. Acad. Sci. U. S. A.* 2010, 107, 8183-8 10.1073/pnas.0911107107
- [5] Uversky, V. N. Unusual Biophysics of Intrinsically Disordered Proteins *Biochim. Biophys. Acta, Proteins Proteomics* 2013, 1834, 932-51 10.1016/j.bbapap.2012.12.008
- [6] Dehner, A.; Kessler, H. Diffusion Nmr Spectroscopy: Folding and Aggregation of Domains in P53 *ChemBioChem* 2005, 6, 1550-65 10.1002/cbic.200500093
- [7] Jones, J. A.; Wilkins, D. K.; Smith, L. J.; Dobson, C. M. Characterisation of Protein Unfolding by NMR Diffusion Measurements *J. Biomol. NMR* 1997, 10, 199-203 10.1023/A:1018304117895
- [8] Leighton, G. O.; Konnova, T. A.; Idiyatullin, B.; Hurr, S. H.; Zuev, Y. F.; Nesmelova, I. V. The Folding of the Specific DNA Recognition Subdomain of the Sleeping Beauty Transposase is Temperature-Dependent and is Required for its Binding to the Transposon DNA *PLoS One* 2014, 9, e112114 10.1371/journal.pone.0112114
- [9] Pan, H.; Barany, G.; Woodward, C. Reduced Bpti Is Collapsed. A Pulsed Field Gradient NMR Study of Unfolded and Partially Folded Bovine Pancreatic Trypsin Inhibitor Protein *Sci.* 1997, 6, 1985-92 10.1002/pro.5560060919
- [10] Penkett, C. J.; Redfield, C.; Jones, J. A.; Dodd, I.; Hubbard, J.; Smith, R. A.; Smith, L. J.; Dobson, C. M. Structural and Dynamical Characterization of a Biologically Active Unfolded Fibronectin-Binding Protein from *Staphylococcus Aureus* *Biochemistry* 1998, 37, 17054-67 10.1021/bi9814080
- [11] Wang, Y.; Benton, L. A.; Singh, V.; Pielak, G. J. Disordered Protein Diffusion under Crowded Conditions *J. Phys. Chem. Lett.* 2012, 3, 2703-2706 10.1021/jz3010915

- [12] Skirda, V. D.; Sundukov, V. I.; Maklakov, A. I.; Zgzdzai, O. E.; Gafurov, I. R.; Vasiljev, G. I. On the Generalized Concentration and Molecular Mass Dependencies of Macromolecular Self-Diffusion in Polymer-Solutions *Polymer* 1988, 29, 1294-1300 10.1016/0032-3861(88)90059-6
- [13] Nesmelova, I. V.; Skirda, V. D.; Fedotov, V. D. Generalized Concentration Dependence of Globular Protein Self-Diffusion Coefficients in Aqueous Solutions *Biopolymers* 2002, 63, 132-40 10.1002/bip.10023
- [14] Tokuyama, M.; Oppenheim, I. Dynamics of Hard-Sphere Suspensions *Phys. Rev. E: Stat. Phys., Plasmas, Fluids, Relat. Interdiscip. Top.* 1994, 50, R16-R19 10.1103/PhysRevE.50.R16
- [15] Kunz, C.; Lonnerdal, B. Human-Milk Proteins: Analysis of Casein and Casein Subunits by Anion-Exchange Chromatography, Gel Electrophoresis, and Specific Staining Methods *Am. J. Clin. Nutr.* 1990, 51, 37-46
- [16] Holt, C.; Sawyer, L. Primary and Predicted Secondary Structures of the Caseins in Relation to Their Biological Functions *Protein Eng., Des. Sel.* 1988, 2, 251-9 10.1093/protein/2.4.251
- [17] Sawyer, L.; Holt, C. The Secondary Structure of Milk Proteins and Their Biological Function *J. Dairy Sci.* 1993, 76, 3062-78 10.3168/jds.S0022-0302(93)77646-8
- [18] Syme, C. D.; Blanch, E. W.; Holt, C.; Jakes, R.; Goedert, M.; Hecht, L.; Barron, L. D. A Raman Optical Activity Study of Rheomorphism in Caseins, Synucleins and Tau. New Insight into the Structure and Behaviour of Natively Unfolded Proteins *Eur. J. Biochem.* 2002, 269, 148-56 10.1046/j.0014-2956.2001.02633.x
- [19] Redwan, E. M.; Xue, B.; Almehdar, H. A.; Uversky, V. N. Disorder in Milk Proteins: Caseins, Intrinsically Disordered Colloids *Curr. Protein Pept. Sci.* 2015, 16, 228-42 10.2174/1389203716666150224145900
- [20] Byler, D. M.; Farrell, H. M.; Susi, H. Raman-Spectroscopic Study of Casein Structure *J. Dairy Sci.* 1988, 71, 2622-2629 10.3168/jds.S0022-0302(88)79855-0
- [21] Creamer, L. K.; Richardson, T.; Parry, D. A. Secondary Structure of Bovine Alpha S1- and Beta-Casein in Solution *Arch. Biochem. Biophys.* 1981, 211, 689-96 10.1016/0003-9861(81)90505-1
- [22] Haga, M.; Yamauchi, K.; Aoyagi, S. Conformation and Some Properties of Bovine Alpha-S2-Group-Casein *Agric. Biol. Chem.* 1983, 47, 1467-1471 10.1271/abb1961.47.1467
- [23] Malin, E. L.; Brown, E. M.; Wickham, E. D.; Farrell, H. M., Jr. Contributions of Terminal Peptides to the Associative Behavior of Alphas1-Casein *J. Dairy Sci.* 2005, 88, 2318-28 10.3168/jds.S0022-0302(05)72910-6
- [24] Byler, D. M.; Susi, H. Examination of the Secondary Structure of Proteins by Deconvolved Ftir Spectra *Biopolymers* 1986, 25, 469-87 10.1002/bip.360250307
- [25] Smyth, E.; Clegg, R. A.; Holt, C. A Biological Perspective on the Structure and Function of Caseins and Casein Micelles *Int. J. Dairy Technol.* 2004, 57, 121-126 10.1111/j.1471-0307.2004.00141.x
- [26] Holt, C. Structure and Stability of Bovine Casein Micelles *Adv. Protein Chem.* 1992, 43, 63-151 10.1016/S0065-3233(08)60554-9
- [27] Andrews, A. L.; Atkinson, D.; Evans, M. T. A.; Finer, E. G.; Green, J. P.; Phillips, M. C.; Robertson, R. N. Conformation and Aggregation of Bovine Beta-Casein-A 0.1. Molecular Aspects of Thermal Aggregation *Biopolymers* 1979, 18, 1105-1121 10.1002/bip.1979.360180507
- [28] O'Connell, J. E.; Grinberg, V. Y.; de Kruijff, C. G. Association Behavior of Beta-Casein *J. Colloid Interface Sci.* 2003, 258, 33-39 10.1016/S0021-9797(02)00066-8
- [29] Alaimo, M. H.; Farrell, H. M., Jr.; Germann, M. W. Conformational Analysis of the Hydrophobic Peptide Alphas1-Casein(136-196) *Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol.* 1999, 1431, 410-20 10.1016/S0167-4838(99)00061-8
- [30] McMeekin, T. L.; Groves, M. L.; Hipp, N. J. Apparent Specific Volume of A-Casein and B-Casein and the Relationship of Specific Volume to Amino Acid Composition *J. Am. Chem. Soc.* 1949, 71, 3298-3300 10.1021/ja01178a007
- [31] Tanner, J. E. Use of Stimulated Echo in NMR-Diffusion Studies *J. Chem. Phys.* 1970, 52, 2523-2526 10.1063/1.1673336
- [32] Maklakov, A. I.; Skirda, V. D.; Fatkullin, N. F. Self-Diffusion in Polymer Solutions and Melts; Kazan University Press: Kazan, Russia, 1987; p 224.
- [33] Gafurov, I. R.; Skirda, V. D.; Maklakov, A. I.; Perevezentseva, S. P.; Zimkin, Y. A. NMR Study of the Structure of Aqueous Gelatine Gels and the Process of Their Formation *Polym. Sci. U.S.S.R.* 1989, 31, 292-300 10.1016/0032-3950(89)90382-1
- [34] Gafurov, I. R.; Skirda, V. D.; Maklakov, A. I.; Ryskina, I. I. Self-Diffusion and Gelation in Benzyl Alcohol Solutions of Cellulose Triacetate *Polym. Sci. U.S.S.R.* 1988, 30, 1639-1644 10.1016/0032-3950(88)90458-3
- [35] Price, W. S. NMR Studies of Translational Motion; Cambridge University Press: Cambridge, U.K., 2009; p xxii, 393 pp.
- [36] Skirda, V. D.; Doroginikij, M. M.; Sundukov, V. I.; Maklakov, A. I.; Fleischer, G.; Häusler, K. G.; Straube, E. Detection of Spatial Fluctuations of Segments in Swollen Polybutadiene Networks by Nuclear Magnetic Resonance Pulsed Field Gradient Technique *Makromol. Chem., Rapid Commun.* 1988, 9 (9) 603-607 10.1002/marc.1988.030090902

- [37] Marchesseau, S.; Mani, J. C.; Martineau, P.; Roquet, F.; Cuq, J. L.; Pugnieri, M. Casein Interactions Studied by the Surface Plasmon Resonance Technique *J. Dairy Sci.* 2002, 85, 2711-21 10.3168/jds.S0022-0302(02)74358-0
- [38] Horne, D. S. Casein Interactions: Casting Light on the Black Boxes, the Structure in Dairy Products *Int. Dairy J.* 1998, 8, 171-177 10.1016/S0958-6946(98)00040-5
- [39] Euston, S. R.; Naser, M. A. Simulating the Equation of State of Model Globular Proteins Adsorbed at a Surface *Langmuir* 2005, 21, 4227-35 10.1021/la046977p
- [40] Burchard, W. Filamentous Supramolecular Structures *Macromol. Symp.* 2010, 295, 49-58 10.1002/masy.201000004
- [41] von Hippel, P. H.; Waugh, D. F. Casein: Monomers and Polymers *J. Am. Chem. Soc.* 1955, 77, 4311-4319 10.1021/ja01621a041
- [42] Aslanyan, I. Y.; Skirda, V. D.; Zaripov, A. M. The Self-Diffusion of Macromolecules in Binary Blends of Poly(Ethylene Glycol) *Polym. Adv. Technol.* 1999, 10, 157-163 10.1002/(SICI)1099-1581(199903)10:3<157::AID-PAT857>3.0.CO;2-V
- [43] Sagidullin, A. I.; Muzafarov, A. M.; Krykin, M. A.; Ozerin, A. N.; Skirda, V. D.; Ignat'eva, G. M. Generalized Concentration Dependence of Self-Diffusion Coefficients in Poly(Allylcarbosilane) Dendrimer Solutions *Macromolecules* 2002, 35, 9472-9479 10.1021/ma0213246
- [44] Doi, M.; Edwards, S. F. Dynamics of Concentrated Polymer Systems 0.2. Molecular-Motion under Flow *J. Chem. Soc., Faraday Trans. 2* 1978, 74, 1802-1817 10.1039/F29787401802
- [45] Doi, M.; Edwards, S. F. Dynamics of Concentrated Polymer Systems 0.1. Brownian-Motion in Equilibrium State *J. Chem. Soc., Faraday Trans. 2* 1978, 74, 1789-1801 10.1039/F29787401789
- [46] Doi, M.; Edwards, S. F. Dynamics of Concentrated Polymer Systems 0.3. Constitutive Equation *J. Chem. Soc., Faraday Trans. 2* 1978, 74, 1818-1832 10.1039/F29787401818
- [47] de Gennes, P. G. *Scaling Concepts in Polymer Physics*; Cornell University Press: Ithaca, NY, 1979; 324 pp.
- [48] Wright, P. E.; Dyson, H. J. Intrinsically Disordered Proteins in Cellular Signalling and Regulation *Nat. Rev. Mol. Cell Biol.* 2015, 16, 18-29 10.1038/nrm3920
- [49] Tompa, P. Intrinsically Disordered Proteins: A 10-Year Recap *Trends Biochem. Sci.* 2012, 37, 509-16 10.1016/j.tibs.2012.08.004
- [50] Uversky, V. N.; Oldfield, C. J.; Dunker, A. K. Intrinsically Disordered Proteins in Human Diseases: Introducing the D(2) Concept *Annu. Rev. Biophys.* 2008, 37, 215-246 10.1146/annurev.biophys.37.032807.125924
- [51] Nesmelova, I. V.; Fedotov, V. D. Self-Diffusion and Self-Association of Lysozyme Molecules in Solution *Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol.* 1998, 1383, 311-6 10.1016/S0167-4838(97)00224-0
- [52] Ilyina, E.; Roongta, V.; Pan, H.; Woodward, C.; Mayo, K. H. A Pulsed-Field Gradient NMR Study of Bovine Pancreatic Trypsin Inhibitor Self-Association *Biochemistry* 1997, 36, 3383-8 10.1021/bi9622229
- [53] Price, W. S.; Tsuchiya, F.; Arata, Y. Lysozyme Aggregation and Solution Properties Studied Using Pqse NMR Diffusion Measurements *J. Am. Chem. Soc.* 1999, 121, 11503-11512 10.1021/ja992265n
- [54] Wattenbarger, M. R.; Bloomfield, V. A.; Bu, Z.; Russo, P. S. Tracer Diffusion of Proteins in DNA Solutions *Macromolecules* 1992, 25, 5263-5265 10.1021/ma00046a024
- [55] Landau, L. D.; Lifshitz, E. M. *Statistical Physics*, 3rd ed.; Course of Theoretical Physics, Vol. 5; Butterworth-Heinemann Ltd.: Oxford, U.K., 1980.
- [56] Gospodarczyk, W.; Szutkowski, K.; Kozak, M. Interaction of Bovine Serum Albumin (Bsa) with Novel Gemini Surfactants Studied by Synchrotron Radiation Scattering (SR-SAXS), Circular Dichroism (CD), and Nuclear Magnetic Resonance (NMR) *J. Phys. Chem. B* 2014, 118, 8652-61 10.1021/jp5047485
- [57] Szasz, C. S.; Alexa, A.; Toth, K.; Rakacs, M.; Langowski, J.; Tompa, P. Protein Disorder Prevails under Crowded Conditions *Biochemistry* 2011, 50, 5834-44 10.1021/bi200365j
- [58] Das, R. K.; Pappu, R. V. Conformations of Intrinsically Disordered Proteins Are Influenced by Linear Sequence Distributions of Oppositely Charged Residues *Proc. Natl. Acad. Sci. U. S. A.* 2013, 110, 13392-7 10.1073/pnas.1304749110
- [59] Le Feunteun, S.; Ouethrani, M.; Mariette, F. The Rennet Coagulation Mechanisms of a Concentrated Casein Suspension as Observed by PFG-NMR Diffusion Measurements *Food Hydrocolloids* 2012, 27, 456-463 10.1016/j.foodhyd.2011.09.008
- [60] Holt, C.; Carver, J. A.; Ecroyd, H.; Thorn, D. C. Invited Review: Caseins and the Casein Micelle: Their Biological Functions, Structures, and Behavior in Foods *J. Dairy Sci.* 2013, 96, 6127-46 10.3168/jds.2013-6831
- [61] Ermolina, I. V.; Fedotov, V. D.; Feldman, Y. D. Structure and Dynamic Behavior of Protein Molecules in Solution *Phys. A* 1998, 249, 347-352 10.1016/S0378-4371(97)83870-8
- [62] Sophianopoulos, A. J.; Vanholde, K. E. Physical Studies of Muramidase (Lysozyme). II. pH-Dependent Dimerization *J. Biol. Chem.* 1964, 239, 2516-24