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Nanosecond structural dynamics of intrinsically disordered β-casein micelles by neutron spectroscopy

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ABSTRACT

β-casein undergoes a reversible endothermic self-association, forming protein micelles of limited size. In its functional state, a single β-casein monomer is unfolded, which creates a high structural flexibility, supposed to play a major role in preventing the precipitation of calcium phosphate particles. We characterize the structural flexibility in terms of nano-second molecular motions, depending on the temperature by quasi-elastic neutron scattering. Our major questions are: Does the self- association reduce the chain flexibility? How does the dynamic spectrum of disordered caseins differ from a compactly globular protein? How does the dynamic spectrum of β-casein in solution differ from that of a protein in hydrated powder states? We report on two relaxation processes on a nano-second and a sub-nano-second time scale for β-casein in solution. Both processes are analyzed by Brownian Oscillator model, by which the spring constant can be defined in the isotropic parabolic potential. The slower process, which is analyzed by neutron spin echo, seems a characteristic feature of the unfolded structure. It requires bulk solvent and is not seen in hydrated protein powders. The faster process, which is analyzed by neutron backscattering, has a smaller amplitude and requires hydration water, which is also observed with folded proteins in the hydrated state. The self-association had no significant influence on internal relaxation, and thus a β-casein protein monomer flexibility is preserved in the micelle. We derive spring constants of the faster and slower motions of β-caseins in solution, and compared them with those of some proteins in various states; folded or hydrated powder.

DISCUSSION

Sub-diffusion of protein domains: the Brownian oscillator model

According to Fig.6, the NSE internal relaxation time, τ_1, varies with the temperature proportional to the solvent viscosity. In general, such a simple relation was established for the visco-elastic relaxation of liquids by Maxwell in 1867 (46):

$$\tau = \eta / G_\infty, \qquad (6)$$

where η denotes the long time-scale relaxed Newtonian viscosity, and G_∞ is the short time-scale unrelaxed elastic shear modulus. Eq. 6 applies not only to liquids but accounts also for the rates of ligand entry and exit reactions of a compact protein (47,48). The viscosity was adjusted using various glass-forming solvent mixtures, like 75% glycerol/water. At viscosities near 100 cP, the respective modulus yields, $G_\infty \sim 10^{11}$ cPs^{-1} (0.1 GPa) at a Maxwell relaxation time of $\tau \approx 1$ns. If stress is applied on a shorter time scale, the response is elastic, the liquid has turns into a glass. In low temperature studies of microscopic protein dynamics, the role of the α-relaxation of hydration water plays an important role (9,11). The NSE process of β-casein in aqueous solution, at solvent viscosities of 1 cP and $\tau_1 \approx 1$ ns, yields $G_\infty \sim 10^9$ cP/s (0.001 GPa)

about a factor of 100 less than for viscous solvents. G_∞ is closely related to the Young's modulus E by 1/3 E < G_∞ < ½ E. For dry β-casein powder, a Young's modulus of 7 GPa was determined and 9.7 GPa for the compact β-barrel protein GFP (28). The characteristic density relaxation rate will thus depend on the scale, increasing with q according to $\Gamma_D(q) = Dq^2$. Above $q = q_{max} \sim 1/d$, on the scale of the intermolecular distance (d), the diffusion rate levels off at the α-relaxation rate $\Gamma_D(q > 2\pi/d) \sim \tau^{-1}$. The liquid structural relaxation becomes q-independent and thus localized at high q. With protein structural relaxation one expects localized dynamics and thus q-independent correlation times at all q. The most popular model among neutron scatterers, analyzing protein residue motions, is the "diffusion inside a sphere" model (49). This model predicts an effective line-width, which increases with q above q_S with $q_S = 2\pi/r_S$, r_S being the radius of the sphere. Although the cross-over at q_S was never really observed, q-dependent effective rates were often explained by free diffusion with rigid boundaries. From the elastic incoherent structure factor of α-amylase, the radius of the spheres r_S = 1.2 Å (native) and 1.8 Å (unfolded) was obtained, which leaves little space to free diffusion (50). In his more recent work Volino (49) votes for a continuous phenomenological "Gaussian model" with soft boundaries. He introduces a joint Gaussian probability distribution of a particle assuming different positions at two different times. Since the positions are not independent, the distribution is "joint" by a two-time correlation coefficient. This joint probability distribution has been derived before as the solution of the Smoluchovski equation of a harmonically bound particle (51). It is known as the Ornstein-Uhlenbeck process in the overdamped case. The parabolic potential of a harmonic oscillator and its Gaussian displacement distribution seems a plausible approximation to continuous residue motions. The latter was analysed recently by the Brownian oscillator (BO) model (52). The BO model is also discussed in Grimaldo et al (32). The BO leads to a remarkably compact time-domain intermediate scattering function, which can be easily applied to evaluate experimental data. The powder averaged 3D BO correlation function reads (52,53):

$$I_{BO}(q,t) = exp\{-q^2\delta^2[1 - exp(-t/\tau_{BO})]\}, \quad (7)$$

$\delta^2 = \langle u_x^2 \rangle$ denotes the x-component of variance of the isotropic 3D displacement.

$$\delta^2 = k_B T/(m\omega_0^2) = k_B T/K, \quad (8)$$

where K is spring constant of the isotropic parabolic potential: $F = K \cdot u_r^2$ with $\langle u_r^2 \rangle/3 = \langle u_x^2 \rangle = \langle u_y^2 \rangle = \langle u_z^2 \rangle$. The BO relaxation time is given by $\tau_{BO} = \delta^2/D_L$, where D_L denotes the effective local diffusion coefficient. At short times, $t << \tau_{BO}$, the particle performs long range diffusion, $\langle \tau_{BO} \rangle^{-1} = D_L \cdot q^2$. At long times, the BO intermediate scattering function tends to a constant, the elastic fraction, EISF(q)=1–A_1(q) and A_1(q) denotes the "quasi-elastic fraction" or the dynamic amplitude:

$$I_{BO}(q, t \gg \tau_{BO}) = 1 - A_1 = exp(-q^2\delta^2), \quad (9)$$

Eq.7 deviates only slightly from an exponential function, the fits to the data in Fig. 1 thus yield within experimental error the same parameters as those shown in Figs. 5 and 6. Accordingly, the q-dependence of A_1(q) is rather well explained by a Gaussian distribution of displacements, the variance is $\delta_1^2 \approx 0.1$ nm^2. This is much larger than the diffusive displacements observed in folded proteins, such as $\delta^2 \sim 0.1$ Å^2 in hydrated myoglobin (27). Since the relaxation time varies with the solvent viscosity, $\tau_1 \sim \eta$, as for global diffusion, it appears that the NSE process reflects sub-global diffusion of entire protein domains.

Stadler et al. (31) investigated "intrinsically disordered myelin basic protein" (MBP) applying the same NSE method, covering the same time and q-range. These authors also observe a fast component (initial decay) in the intermediate scattering function. Their Fig. 3A strikingly resembles the β-casein data of Fig. 1. The β-casein data show a more pronounced fast component, possibly because the protein concentration was about 50 % larger. Moreover, the correlation time $\tau_1^{MBP} \sim 8.4$ ns overlaps with the results of Fig. 6. Fig. 5 shows that even

the dynamic amplitudes $A_1(q)$ in these unfolded proteins are nearly identical. Thus, one can assume a similar nature of these processes in spite of different amino acid sequences. Stadler et al. (31) apply the Zimm model of random polymer chains to MBP, which is a coarse-grained description of flexible beads: N beads of fixed length are connected by entropic springs. It predicts a number of relaxation modes, the slowest mode is overall rotation (31). It neglects internal friction or motions that occur at length scales shorter than the beads, such as hindered dihedral rotations, side chain interactions and hydrogen bonding. This is the range of the NBS internal processes at the time scale τ_2. The Zimm model and even more sophisticated extensions do not explain the NSE data of MBP. One has to take into account. that MBP is not fully unfolded, but retains a compact core and a folded secondary structure content of 44 %. Thus, one expects slower dynamics than for the ideal random polymer chain. This conclusion is supported by the index of compactness, the ratio of R_g/R_h, which is close to 0.91. A Gaussian chain would yield 1.5. For a compact native protein like myoglobin one obtains 0.79, close to the limit of a rigid sphere of $(3/5)^{1/2} = 0.775$. Fig. 3 shows that this ratio evolves for β-casein from 1.3 at low temperatures, mostly monomers, to 0.91 at high temperatures, after the formation of micelles is established.

Protein spring constants, in folded and unfolded states

Bicout and Zaccai proposed in 2001 to determine protein spring constants (K) from the temperature slopes of the mean squared displacements with hydrated protein powders (54). It was even proposed to explain the enhancement of displacements at the "the dynamical transition" by a softening of the protein spring constants. Above the transition, K~0.3 N/m was obtained for hydrated myoglobin (54). The effective vibrational spring constant of hydrated myoglobin was 10 N/m (9). A high temperature spring constant of hydrated myoglobin was recently derived by fitting back-scattering data to the BO model in the time domain: $\delta_2^2 = 0.11$ Å^2 and K = 3.8 N/m (27), much larger than the Zaccai results (54). Hydrated β-casein with a time constant around 100 ps yields K = 0.4 N/m (28), while for the similar NBS process in solution a nearly identical spring constant is derived: K= 0.38 N/m. The slow NSE process, ($\delta_1^2 = 0.1$ nm^2) is characterized by a spring constant K (1ns) = 0.04 N/m at 300 K. (eq. 8), ten times less than K at 100 ps obtained by NBS. Interestingly, spring constant K at 100 ps is independent of sample conditions, powder and solution. For MBP, Stadler et al. derive from the fast initial phase at 8 ns displacements similar to those of β-casein (31). The resulting spring constant of MBP in solution is K (300 K, 10 ns) = 0.08 N/m. This result was interpreted as the coupling of different protein domains. The spring constant is about a factor of two larger than K of β-casein at the same conditions. The spring constants as determined from the diffusion method for Taq-polymerase and ADH are ten times smaller and overlap with the range measured with AFM (43,55,56). The AFM method, however, probes the protein elasticity on a much longer time scale (ms) than neutron scattering (ns) (55). The small spring constants derived with neutron scattering are thus surprising. The method by Bicout and Zaccai involves the cross-over region of relaxation time and instrumental resolution time, which may explain some differences. Most striking are the nearly identical force constants determined for the NBS relaxation of β-casein, hydrated and in solution, suggesting that the relevant structural process should be decoupled from the bulk solvent. Moreover, nearly identical values were observed for NSE process of two unfolded proteins in solution: β-casein and Myelin Basic Protein. These spring constants discussed above are summarized in Table 1.

Table 1: Spring constants K determined with different instruments and methods: BZ: Bicout/Zaccai (54), BO: Brownian oscillator analysis, F: folded, UF unfolded, NSE/Diff: determined with the diffusion/structure method (43,56), BO displacements δ^2 and respective resolution time, τ.

Protein	State	Process	$\delta^2/Å^2$	τ/ps	K [N/m]	Reference
myoglobin	hydrated	vibration	0.04	1	10	9(Doster)
myoglobin	hydrated	NBS/BZ	0.04	100	0.3	54(Zaccai)
myoglobin	hydrated	NBS/BO	0.11	100	3.8	27(Doster)
MBP (F)	hydrated	NBS/BZ	1	100	0.185	30
Tau (UF)	hydrated	NBS/BZ	1	100	0.096	30
β-casein	hydrated	NBS/BO	1	100	0.4	28
β-casein	solution	NBS/BO	1	100	0.4	present
β-casein	solution	NSE/BO	10	1000	0.04	present
Myelin (UF)	solution	NSE/BO	5	8000	0.085	31
ADH (F)	solution	NSE/Diff	70	75000	5.5×10^{-3}	43
Taq (F)	solution	NSE/Diff	50	50000	8×10^{-3}	56
P-sel (F)	solution	AFM	81	ms	4×10^{-3}	55

REFERENCES

1. Warshel, A. and R . P . Bora. 2016. Perspective: Defining and quantifying the role of dynamics in enzyme catalysis, *J. Chem. Phys.* 144:180901.
2. Go, N. 1983. Theoretical studies of protein folding, *Annu. Rev. Biophys. Bioeng.* 12:183-210.
3. Bryngelson, J. D. and P. G. Wolynes. 1987. Spin glasses and the statistical mechanics of protein folding, *Proc. Natl. Acad. Sci. USA* 84: 7524-7528.
4. Go, N., T. Noguchi, and T. Nishikawa. 1983. Dynamics of a small globular protein in terms of low-frequency vibrational modes, *Proc. Natl. Acad. Sci. USA* 80: 3696-3700.
5. Holt, C., M. Wahlgren, and T. Drakenberg. 1996. Ability of a beta-casein phosphopeptide to modulate the precipitation of calcium phosphate by forming amorphous dicalcium phosphate nanoclusters, *Biochem. J.* 314:1035-1039.
6. Smith, J. C., K. Kuczera, B. Tidor., W. Doster., S. Cusack, S., and M. Karplus. 1989 Internal dynamics of globular proteins, comparison of neutron scattering measurements and theoretical models, *Physica B.*156:437-443.
7. Smith, J. C., K. Kuczera, and M. Karplus. 1990 Dynamics of myoglobin (in vacuo): comparison of simulation results with neutron scattering spectra, *Proc. Natl. Acad. Sci. USA* 87: 1601-05.
8. Loncharich, R., and B. Brooks. 1990. The temperature dependence of dynamics of hydrated myoglobin, comparison of force field calculations with neutron scattering data, *J. Mol. Biol.* 215, 439-445
9. Doster, W., W. Petry, and S. Cusack, 1989. Dynamical transition of myoglobin revealed by inelastic neutron scattering, *Nature* 337:754-756.
10. Cusack, S. and W. Doster. 1990. Temperature dependence of the low frequency dynamics of myoglobin. Measurement of the vibrational frequency distribution by inelastic neutron scattering, *Biophys. J.* 58:243-25
11. Doster, W., A. Bachleitner, R. Dunau, M. Hiebl, and E. Lüscher, 1986. Thermal properties of water in myoglobin crystals and solutions at sub-zero temperatures, *Biophys. J.* 50:213-219.

12. Gaspar, A. M., S. Busch, M. -S. Appavou, W. Haeussler, R. Georgii, Y. Su, and W. Doster. 2010. Using polarization analysis to separate the coherent and incoherent scattering from protein samples, *Biochim. Biophys. Act.* 1804:76-82.
13. Gaspar, A., M. –S. Appavou, S. Busch, T. Unruh, and W. Doster. 2008. Dynamics of well-folded and natively disordered proteins in solution: a time-of-flight neutron scattering study, *Eur. Biophys. J.* 37:573-582.
14. Farrell, H. M., E. L. Malin, E. M. Brown, P. X. Qi. 2006. Casein micelle structure: What can be learned from milk synthesis and structure biology?, *Curr. Opin. Coll. Inter. Sci.* 11:135-147.
15. Garnier, J. 1966. Conformation de la Caséine β en Solution Analyse d'une Transition Thermique entre 5 et 40°C, *J. Mol. Biol.* 19:586-590.
16. O'Connell, J. E., V. Y. Grinberg, and C. G. de Kruif. 2003. Association behavior of β- casein, *J. Colloid Interface Sci.* 258:33-39.
17. Faizullina, D. A., T. A. Konnovaa, T. Haertleb, and Y. F. Zueva. 2013. Self-Assembly and Secondary Structure of Beta-Casein, *Rus. J. Bioorg. Chem.* 39:366-372.
18. de Kruif, C. G., and V. Y. Grinberg. 2002. Micellisation of β-casein, *Colloids Surf. A Physicochem. Eng.* 210:183-190.
19. Kegeles, G. A. 1979. A Shell Model for Size Distribution in Micelles, *J. Phys. Chem.* 83:1728-1732.
20. Payens, T. A. and K. Heremans. 1969. Effect of Pressure on the Temperature-Dependent Association of β-Casein, *Biopolymers.* 8:335-345.
21. Gebhardt, R., N. Takeda, U. Kulozik, and W. Doster. 2011. Structure and Stabilizing Interactions of Casein Micelles Probed by High-Pressure Light Scattering and FTIR, *J. Phys. Chem. B.* 115:2349-2359.
22. Farrell, H.M., E.D. Wickham, J.J. Unruh, P.X. Qi, P.D. Hoagland. 2001. Secondary structural studies of bovine caseins: a temperature dependence of β-casein structure as analysed by circular dichroism and FTIR spectroscopy and correlation with micellization, *Food Hydrocolloids* 15:341-354.
23. Leclerc, E. and P. Calmettes. 1997. Interactions in Micellar Solutions of β-Casein. *Phys. Rev. Lett.* 78:150.
24. Doster, W. and M. Settles, M. 2005. Protein-water displacement distributions, *Biochem. Biophys. Acta*. 1749:173-186.
25. Doster, W., H. Nakagawa, and M. -S. Appavou. 2013. Scaling analysis of bio-molecular dynamics derived from elastic incoherent neutron scattering experiments, *J. Chem. Phys.* 139:045105.
26. Doster, W. 2019. Time domain versus energy domain neutron scattering analysis of protein dynamics, *Proc. Natl. Acad. Sci. USA* 116:8649-8650.
27. Doster, W., 2018. Are proteins dynamically heterogeneous? Neutron scattering analysis of hydrogen displacement distributions, *Int. J. Mol. Theor. Phys. 2*:1-14.
28. Perticaroli, S., J. D. Nickels, G. Ehlers, E. Mamontov, and A. P. Sokolov. 2014. Dynamics and Rigidity in an Intrinsically Disordered Protein, β-Casein, *J. Phys. Chem. B* 118:7317-7326.
29. Dhindsa, G. K., M. Tyagi and X. Chu, 2014. Temperature dependent dynamics of β-casein, studied by quasi-elastic neutron scattering, *J. Phys. Chem. B* 118: 10821-10829.
30. Gallat, F. X., A. Laganowsky, K. Wood, F. Gabel, L. van Eijk, J. Wuttke, M. Moulin, M. Härtlein, D. Eisenberg, J-P. Colletier, G. Zaccai, M. Weik. 2012. Dynamical coupling of intrinsically disordered proteins and their hydration water: comparison of with folded soluble and membrane proteins, *Biophys. J.* 103: 129-136.

31. Stadler, A. M., L. Stingaciu, A. Radulescu, O. Holderer, M. Monkenbusch, R. Biehl, and D. Richter, 2014. Internal nanosecond dynamics of the intrinsically disordered Myelin Basic Protein, *J. Am. Chem. Soc.* 136:6987-6994.
32. Grimaldo, M., F. Roosen-Runge, F. Zhang, F. Schreiber and T. Seydel. 2019. Dynamics of proteins in solution, *Q. Rev. Biophys.* 52:1-63.
33. Feoktystov, A., H. Frielinghaus, Z. Di, S. Jaksch, V. Pipich, M. -S. Appavou, E. Babcock, R. Hanslik, R. Engels, G. Kemmerling, H. Kleines, A. Ioffe, D. Richter, and T. Brückel. 2015. KWS-1 high resolution SANS instrument at JCNS: current state. *J. Appl. Cryst.* 48:61-70.
34. Hayter, J. B., and J. Penfold. 1981. An analytic structure factor for macroion solutions. *Mol. Phys.* 42:109-118.
35. Hansen, J-. P., and J. B. Hayter. 1982. A rescaled MSA structure factor for dilute charged colloidal dispersions. *Mol. Phys.* 46:651-656.
36. Pipich, V., QtiKWS: visualisation, reduction, analysis and fit framework with focus on Small Angle Scattering (http://qtisas.com/qtikws)
37. Bee, Marc 1988, Quasi-elastic neutron scattering, Adam Hilger, p.72-105
38. Holderer, O., M. Monkenbusch, R. Schätzler, H. Kleines, W. Westerhausen, and D. Richter. 2008. The JCNS neutron spin-echo spectrometer J-NSE at the FRM II, *Meas. Sci. Technol.* 19:034022.
39. Wuttke, J., A. Budwig, M. Drochner, H. Kämmerling, F. Kayser, H. Kleines, V. Ossovyi, L. C. Pardo, M. Prager, D. Richter, G. J. Schneider, H. Schneider, and S. Staringer. 2012. SPHERES, Jülich's high-flux neutron backscattering spectrometer at FRM II, *Rev. Sci. Instrum.* 83:075109.
40. Fitter, J. 2003. Conformational dynamics of a protein in the folded and the unfolded state, *Chem. Phys.* 292:405-411.
41. Perticaroli, S., G. Ehlers, C. B. Stanley, E. Mamontov, H. O'Neill, Q. Zhang, X. Cheng, D. A. A. Myles, J. Katsaras and J. D. Nickels. 2017. Description of Hydration Water in Protein (Green Fluorescent Protein) Solution, *J. Am. Chem. Soc.* 139:1098-1105.
42. Wuttke, J. FRIDA: fast reliable inelastic data analysis (http://apps.jcns.fz-juelich.de/doku/frida/start)
43. Biehl, R., B. Hoffmann, M. Monkenbusch, P. Falus, S. Préost, R. Merkel and D. Richter, 2008, Direct observation of correlated inter-domain motions in alcohol dehydrogenase, *Phys. Rev. Lett.* 101:13102.
44. Andrews, A. L., D. Atkinson, M. T. A. Evans, I. G. Finer, J. P. Green, M. C. Phillips, Robertson, R. N. 1979. The conformation and aggregation of bovine β-casein A. I. Molecular aspects of thermal aggregation. *Biopolymers*. 18:1105-1121.
45. Hofmann, H., A. Soranno, A. Borgia, K. Gast, D. Nettels, and B. Schuler. 2012. Polymer scaling laws of unfolded and intrinsically disordered proteins quantified with single-molecule spectroscopy, *Proc. Natl. Acad. Sci. USA* 109:16155-16160.
46. Maxwell, J. C. 1867. IV. On the dynamical theory of gases, *Trans. Roy. Soc. (London)* 157:49-88.
47. Kleinert, Th., W. Doster, H. Leyser, W. Petry, V. Schwarz and M. Settles, 1998. Solvent composition and viscosity effects on the kinetics of CO-binding to horse myoglobin, *Biochem.* 37: 717-733.
48. Longeville, S. and W. Doster S., 2012, Protein dynamics and function, in Dynamics of soft matter, neutron applications, Ch. 8, Ed. V.G. Sakai, Springer Science and Business Media
49. Volino, F., J. C. Perrin, S. Lyonnard, 2006, Gaussian model for localized motion,

application to incoherent neutron scattering, *J. Chem. Phys. B.* 110: 11217-11222.
50. Fitter, J. 2003. A measure of conformational entropy change during thermal protein unfolding using neutron spectroscopy, *Biophys. J.* 84:3924-3930.
51. Risken, H. 1996. The Fokker Planck equation, methods of solution and application, p. Springer Series in Synergetics, p. 96-132
52. Doster, W., 2006, Brownian oscillator analysis of molecular motions in biomolecules, in Neutron scattering in biology, techniques and applications, Eds. J. Fitter, T. Gutberlet, J. Katsaras, Springer, Biological and medical Physics p.461-482.
53. Uhlenbeck, G. E., and L. S. Ornstein. 1930. On the theory of the brownian motion, *Phys. Rev.* 36:823-841.
54. Bicout, D.J. and G. Zaccai, 2001, Protein flexibility from the dynamical transition, a force constant analysis, *Biophys. J.* 80: 1115-1123.
55. Marshall , B. T., K. K. Sarangpani, J. Wu, M.B. Lawrence, R. B. McEver and C. Zhu. 2006. Measuring molecular elasticity by atomic force cantilever fluctuations, *Biophys. J.* 90:681-692.
56. Bu, Z., R. Biehl, M. Monkenbusch, D. Richter, and D. J. E. Callaway. 2005. Coupled protein domain motion in Taq polymerase revealed by neutron spin-echo spectroscopy, *Proc. Natl. Acad. Sci. USA* 102:17646-17651.
57. Pérez, J., J. -M. Zanotti, and D. Durand. 1999. Evolution of the Internal Dynamics of Two Globular Proteins from Dry Powder to Solution, *Biophys. J.* 77:454-469.
58. Stadler, A. M., F. Demmel, J. Olivier, T. Seydel. 2016. Picosecond to nanosecond dynamics provide a source of conformational entropy for protein folding, *Phys. Chem. Chem. Phys.* 18: 21527-21538.
59. Richter, D., B. Ewen, B. Farago, and T. Wagner. 1989. Microscopic Dynamics and Topological Constraints in Polymer Melts: A Neutron-Spin-Echo Study, *Phys. Rev. Lett.* 62:2140-2143.
60. Gebhardt, R., W. Doster, W. Friedrich, and U. Kulozik. 2006. Size distribution of pressure-decomposed casein micelles studied by dynamic light scattering and AFM, *Eur. Biophys. J.* 35:503-509.
61. Bu, Z., D. A. Neumann, S. H. Lee, C. M. Brown, D. M. Engelman, and C. C. Han. 2000. A view of dynamics changes in the molten globule-native folding step by quasielastic neutron scattering, *J. Mol. Biol.* 301:525-536.
62. Grimaldo, M., F. Roosen-Runge, M. Hennig, F. Zanini, F. Zhange, N. Jalarvo, M. Zamponi, F. Schreiber, and T. Seydel. 2015. Hierarchical molecular dynamics of bovine serum albumin in concentrated aqueous solution below and above thermal denaturation, *Phys. Chem. Chem. Phys.* 17:4645-4655.
63. Ameseder, F., A. Radulescu, M. Khaneft, W. Lohstroh, and A. M. Stadler. 2018. Homogeneous and heterogeneous dynamics in native and denatured bovine serum albumin, *Phys. Chem. Chem. Phys.* 20:5128-5139.