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Combined hydrodynamic approach to size, heterogeneity conformation and flexibility of bio-based macromolecules

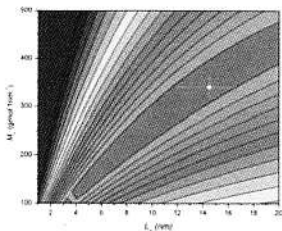
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This talk will review some of the advances in hydrodynamic methodologies for studying the size (molecular weight), heterogeneity (polydispersity and propensity to self-associate), conformation and flexibility of biomacromolecules. The grammar of enquiry depends on the type of biomacromolecule – globular proteins or linear carbohydrate based polymers for example, and I will focus mainly on the latter.

Size and heterogeneity: SEC-MALLS has been the method of choice since the early 90's but I will show the virtue of using analytical ultracentrifugation for independent verification – sedimentation equilibrium for molecular weight and sedimentation velocity for heterogeneity.

Conformation and flexibility. For linear types of macromolecule intrinsic viscosity $[\eta]$ and radius of gyration R_g measurement have been popular parameters, interpreted via the various relations describing their dependencies on molecular weight. Both the sedimentation coefficient and its concentration dependence coefficient k_s are also very useful and combinations of hydrodynamic parameter are available for interpreting the conformation type¹ of a macromolecule and conformational flexibility (persistence length L_p) unequivocally using global analysis procedures² – examples will be given. Combined approaches also help us define the domain orientation of multi-domain structures such as antibodies³.



Global hydrodynamic analysis of methyl cellulose²

¹ Pavlov, G.M.; Rowe, A.J.; Harding, S.E. *Trends. Analyt. Chem.*, 1997, 16 401-405.

² Patel, T. R.; Morris, G. A.; Garcia de la Torre, J.; Ortega, A.; Mischnick, P.; Harding, S. E. *Macromol. Bioscience*, 2008, 8, 1108-1115.

³ Lu, Y.; Longman, E.; Davis, K.; Ortega, A.; Grossmann, J.G.; Michaelsen, T.E.; Garcia de la Torre, J.; Harding, S.E. *Biophys. J.* 2006, 91, 1688-1697.