NMR spectroscopy to study artificial recognition of biomolecules

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NMR spectroscopy is an excellent tool to investigate the atomic details of molecular association, and to determine both the structure of the complex and the important elements required for high affinity binding. We have looked at the atomic contacts required for the design of a synthetic folded molecule that targets the specific encapsulation of fructose. A series of 1-, 2- and 3-dimensional NMR spectra have been used to characterize the binding and to provide a solution state model of the assembled complex. The use of ^{13}C-labelled sugars has also enabled high resolution study of the conformational preferences of the guest sugar molecule upon interaction with the synthetic capsule. Additional studies have focused on the use of NMR spectroscopy to characterize the assembly of oligourea molecules into defined stoichiometries. In particular we have examined the hexameric assembly of oligoureas and determined the importance in the nature of the oligourea sidechains to stabilize the assembly. We have also pursued further characterization of the oligourea hexamers and their ability to encapsulate alcohol molecules. Finally, we have taken advantage of biomolecular NMR approaches to design and characterize novel foldamers targeted for protein surface recognition. Calculation of molecular size, dynamics and protein-ligand contact surfaces are all used to help define and design improved foldamers that recognize surface features on proteins such as human carbonic anhydrase and targets relevant to health.

Molecular details of neuron and muscle-specific alternative splicing

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We have studied the atomic details of RNA binding by several proteins involved in both muscle and neuron biology in the nematode *C. elegans*. During tissue development there is selective expression of proteins that can regulate the splice patterns of specific genes. One example is the SUP-12 protein that is expressed in muscle cells of the worm and regulates the splicing pattern of genes such as the worm fibroblast growth factor receptor, *egl-15*. The solution structure and molecular details of the muscle-specific SUP-12 protein has led to a series of *in vivo* splicing assays in live worms using a fluorescent two-colour reporter system built on the isoform regulation of the EGL-15 fibroblast growth factor receptor. An extensive investigation by isothermal titration calorimetry has helped to define the key atomic properties required for binding to SUP-12 protein. Additional studies have focused on the interplay of SUP-12 with the Fox-1/RBFOX family at the molecular level, since both factors are required for splicing regulation and thus provide a model system of splicing factor co-regulation. Recent work has expanded our study to splicing factors specific to neuron development, including the MEC-8 protein and its role in mechanosensory function.