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There has been considerable interest and speculation as to why, when a lobster is cooked, it changes from a dark slate blue colour, to bright red. It is now known that the chromophore responsible for the colour of the lobster is the carotenoid astaxanthin, AXT (3,3'-dihydroxy -β,β-carotene-4,4'-dione), which is found in the protein α-crustacyanin (α-CR). The crystal structure of β-CR has been determined [1] allowing a number of theories to be put forward to explain the bathochromic shift between the unbound and protein bound forms of AXT. The crystal structures of a number of unbound carotenoids have also been determined, including that of AXT [2]. Most of these adopt the 6-s-*cis* conformation, unlike the protein bound molecule, which is 6-s-*trans*. Recently, however, we have determined the crystal structures of the 6-s-*cis* and 6-s-*trans* isomers of the diacetates of astaxanthin (AXT), **s-*cis*-1** and **s-*trans*-1,** and those of 7,8-didehydroastaxanthin ((3S,3'S)-3,3'-dihydroxy-7,8-didehydro-β,β-carotene-4,4'-dione), **(2),** and 7,8,7',8'-tetradehydroastaxanthin ((3S,3'S)-3,3'-dihydroxy-7,8,7',8'-tetradehydro-β,β-carotene-4,4'-dione), **(3)** [3]. The conformations of these four molecules vary in particular, with the angle of twist of the end rings out of the plane of the polyene chain; for **s-*cis*-1**, the end rings are bent out of the plane of the polyene chain by an angle of -49.0(5)°, and the conformation is therefore similar to that found for unesterified AXT as well as for the carotenoids, canthaxanthin and β,β-carotene. For **s-*trans*-1**, the end rings are coplanar with the polyene chain and its conformation is much more similar to that of the protein bound AXT in crustacyanin. In **(2)** and **(3),** the end rings are also almost coplanar with the polyene chain with the end rings in **(2)** in the s-*cis* conformation, and in **(3)** in the s-*trans* conformation. Thus, an extensive ensemble of the possible β end ring conformations has been determined [2,3]. These structures are compared with one another as well as unbound, unesterified AXT and protein bound AXT. Also, the effect of the end ring conformations on the colour and UV/Vis spectra of the crystals was established, and showed that the s-*trans* conformation provides part, but not the entire bathochromic shift seen in the AXT in crustacyanin.

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Structural Investigations of the Proteolytic Complexes Triggering the Complement System. Christine Gaboriaud[a], Nicole Thielens[b], Gérard Arlaud[b]. [a]*LCCP or* [b]*LEM, Institut de Biologie Structurale, Grenoble, France.*
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The complement system is a first line of defence against infection, and is also involved in immune tolerance, allograft rejection and various pathologies. Several large protein complexes (> 500 kDa) can specifically trigger this system: the classical pathway C1 complex, and the MBL-MASP and ficolin-MASP complexes involved in activation of the lectin pathway. These complexes are assembled from two kinds of subunits: an oligomeric pattern recognition protein (C1q, MBL, L- or H-ficolin), and a protease component, which is either a tetramer (C1s-C1r-C1r-C1s) or a dimer ((MASP)(2)). These modular proteases (C1r/C1s, MASPs) trigger the proteolytic cascade of the complement system as a consequence of the binding of their associated recognition protein to the target. The main objective was to decipher the main structure-function relationships involved in these complex activation processes.
We have used a dissection strategy to define the main functional protein fragments involved in this process: the protease catalytic domains, the recognition domain of C1q and ficolins, the protease interaction domains, which mediate both the protease dimerization and their association to their cognate recognition proteins. We have solved by X-ray crystallography the structure of these domains, including several complexes with ligands for the recognition domains [1-2]. Site-directed mutagenesis was also used to probe the main binding sites in the proteases interaction domains [3]. Recent functional and 3-D structural investigations have revealed that C1r/C1s and the MASPs associate through a common mechanism involving their N-terminal CUB1-EGF region. Moreover, they use homologous binding sites in their CUB module to associate to their cognate recognition protein [3]. New structures of protein-ligand complexes have shed lighton the structural bases of the various recognition properties of C1q and ficolins. Regarding the proteolytic cascade involved in C1 activation, C1r autoactivation was shown to be the first step. The structure of the C1r protease, has revealed that this first step of activation requires a mechanical stress transmitted from the C1q stems to the C1r catalytic region through the C1q–C1r–C1s interfaces [1, 4].An overview of these investigations, including the most recent structural studies, will be given.

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ββα-Me Restriction Endonuclease Hpy99I in Complex with Target DNA. Honorata Czapinska[a,b], Monika Sokolowska[a,b], Matthias Bochtler[a,b,c]. [a]*IIMCB, Trojdena 4, 02-109 Warsaw, Poland.* [b]*MPI-CBG, Pfotenhauerstr, 108, 01309 Dresden, Germany.*

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The ββα-Me type II restriction endonuclease Hpy99I from the human pathogen *Helicobacter pylori* has been overexpressed from a synthetic gene in *E. coli* and crystallized in complex with target DNA. The enzyme binds the pseudopalindromic CGWCG| target sequence as a dimer and cleaves two DNA strands with unusual stagger (five nucleotide 3'-overhangs, "|" marks the cleavage site). The Hpy99I protomer consists of an antiparallel β-barrel and two β4α2 repeats. The repeats are poorly conserved on a sequence level but are readily indentified in a structural comparison. Each repeat coordinates a structural zinc ion with four cysteine thiolates in two CXXC motifs positioned in a β-hairpin and at the N-terminal end of an α-helix. The ββα region of the second β4α2 repeat holds the catalytic metal ion (Me) via Asp148 and Asn165 and activates a water molecule with the general base His149. Hpy99I dimer forms a tight ring-like structure around the DNA. Each protomer recognizes one half of the target sequence and contacts the CG/GC base pairs on the major and minor groove side via the first and second β4α2 repeat, respectively. The enzyme interacts with the central symmetry-breaking base pair of the recognition sequence only on the minor groove side, where A:T resembles T:A and G:C is similar to C:G. The Hpy99I-DNA co-crystal structure provides the first detailed illustration of the ββα-Me active site in restriction endonucleases and complements the information on the use of this motif in other groups of enzymes such as homing endonucleases (e.g. I-PpoI, I-HmuI) and Holliday junction resolvases (e.g. T4 endonuclease VII) [1].

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Structural Basis for Rab-effector Specificity. Amir R. Khan[a], Rosario Recacha[a], Nicholas Jagoe[a]. [a]*School of Biochemistry and Immunology, Trinity College Dublin, Ireland.*
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The Rab family of small GTPases orchestrate the trafficking of vesicular cargo in eukaryotic cells. Rabs have a conserved three-dimensional fold in their active (GTP) state, yet recognize a distinct subset of effector proteins to mediate their biological effects. Unlike Rabs, effector proteins are diverse in size and composition. We have determined the crystal structures of Rab6 and Rab11 with their effectors to gain insight into specificity and subsequent biological function. Our lab and others have observed that Rabs generally bind alpha-helical determinants of cognate effectors via highly conserved residues in switch I, switch II and interswitch regions. However, conformational variability in these conserved regions plays a key role in effector specificity. The seemingly contradictory properties of specificity and promiscuity (Rab6 binding to several unrelated effectors) will be discussed in light of emerging structural data.

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