Assembly of a machine for concurrent action at eight phosphodiester bonds

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The BcgI restriction-modification system consists of two polypeptides: BcgIA contains both endonuclease and methyltransferase motifs; BcgIB is homologous to the HsdS subunits of Type I RM systems that mediate DNA sequence recognition. The two polypeptides form an $A_2B_1$ protomer, which presumably contains one DNA recognition unit (BcgIB), two nuclease active sites and two sites for methyl transfer (both in BcgIA).

BcgI is the archetype of a subset of Type II RM systems that recognise specific DNA sequences and then cut both DNA strands on both sides of the site, leaving the site on a short fragment (32 bp for BcgI,) excised from the remainder of the DNA. BcgI thus cleaves a total of four phosphodiester bonds around each site. But it will be shown here that the enzyme is active only when bound to two copies of its site, and that it then cuts eight phosphodiester bonds, those on both sides of both sites, before dissociating from the DNA.

To address how a protomer containing two nuclease active sites is organised to cut eight target bonds in one reaction, the assembly of the BcgI protein and its complexes with DNA were characterised by analytical ultracentrifugation and by QTOF nanospray mass spectroscopy.