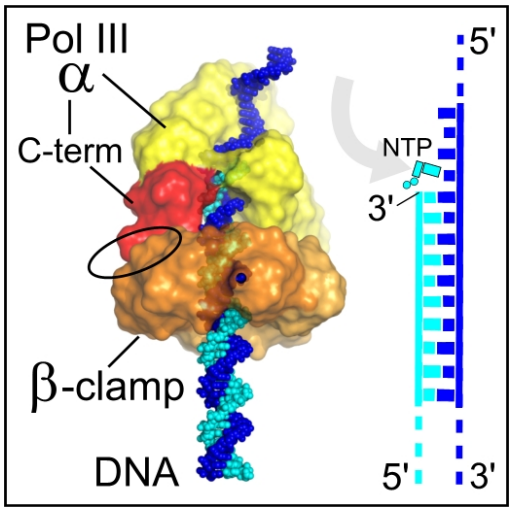
Clamping down on pathogenic bacteria – how to shut down a key DNA polymerase complex

Bacterial DNA-replication machinery

Pathogenic bacteria that are resistant to the current armoury of antibiotics are an emerging threat worldwide. One approach to developing new antibiotics is to look at structures of possible new targets such as the bacterial DNA-replication machinery (the “replisome”). This has historically been a difficult target to study due to its low abundance, but its components are now available through over-expression and reconstitution for *in vitro* assays, screening and structural analysis.

DNA replication in bacteria is carried out by a complex of proteins called DNA polymerase III (Pol III), which catalyses the addition of individual nucleotides to a growing strand of DNA. The catalytic subunit of the complex is a protein called the α-subunit. It is highly active but needs help to stay attached to the DNA for the length of time needed to add the many nucleotides to a new DNA strand. This help comes from a C-shaped protein called the β-subunit or β-clamp. Two copies of this are snapped head-to-tail around the DNA to form a toroidal (doughnut-shaped) complex [view 1]. In the cell this step is carried out by a specialised clamp-loading machine. The α-subunit then binds to the clamp using an extended region at its C-terminal end. This combination of the active α-subunit held in place by the β-clamp copies the DNA as it slides along. For this reason the β-subunit is often called a sliding clamp.



DNA polymerase III clamped onto DNA. The α-subunit (yellow) is held on to the DNA by binding with its C-terminal extension (red) to the β-clamp (orange). The interaction site is circled. The schematic (right) shows nucleoside triphosphate (NTP) addition (arrowed) at the 3’ strand end - the two small circles represent the pyrophosphate leaving group (based on ref. 1).

Can you repeat that?

Each of the β-clamp subunits contain three repeats of a domain in which two alpha helices form the inner face and two interlocking beta-sheets form the outer face [view 2]. In fact, each domain unit is also built from two internal repeats of a sub-domain, with one helix and four beta-strands in each. The three domains form the subunit by zipping together adjacent strands to create a single shared sheet. Likewise, the two subunits are held together to form the complete β-clamp by zipping together two opposed sheets.

Owing to this construction from two sets of three domains, the β-clamp has an overall hexagonal shape. It neatly fits around double-stranded DNA with enough space to allow it to slide. Eukaryotic organisms have a similar hexagonal shaped sliding clamp, but instead of two subunits with three domains each, these clamps have evolved to contain three subunits of two domains each.

PDB entry **3bep** [view 2] describes the structure of a complex prepared *in vitro* by mixing the clamp and a short DNA segment [ref. 2]. In the cell however, where the target DNA is the circular bacterial chromosome, the clamp loader must break one of the interfaces between the subunits before the clamp can embrace the DNA.

Slip sliding away...

Once it embraces the DNA, the clamp can slide along the strands [view 3]. A helical pathway matching the twist of the DNA seems a likely trajectory, like a nut turning along a bolt, but this has not been verified experimentally. [View 3] shows that the clamp is offset by 22˚ from the axis of the DNA when it reaches the end. This tilt is supported by structures with small DNA strands (PDB entry **3bep**). It may help give the α-subunit access to the clamp subunits and the DNA 3' end which is the target for the polymerase.

The full assembly of the complex shown in [view 1] is also a conceptual model [**ref. 1**] as these components have not yet been crystallised together. However, because the binding of the catalytic α-subunit to the loaded β-clamp is essential for DNA replication, designing compounds that interfere with this binding is an attractive strategy for developing new antibacterial compounds. These compounds must bind tightly as the replisome functions at low total cellular concentrations and with almost no pool of free β-clamp subunits.

Polymerase broken down by the competition

Short peptides corresponding to the C-terminal region of the α-subunit compete effectively with the whole α-subunit in binding to the β-clamp. Crystal structures of the clamp with such peptides bound have revealed their binding site. PDB entry **3d1f** [view 4] shows a nine-residue peptide (*nonapeptide*) from the Pol III α-subunit bound across the outer surface of the clamp, in the third domain of each subunit [**ref. 3**]. Owing to the way the clamp is assembled from the two identical proteins, there are two binding sites on the clamp, both on the same face of the doughnut, but positioned on opposite sides of the central hole. Although only a single site is needed to bind the catalytic α-subunit, it may be that having an extra site available is useful to the cell, for example to recruit a specialised repair polymerase to deal with damaged DNA. The other DNA polymerases that use the β-clamp share a similar C-terminal clamp binding sequence.

Small but perfectly formed - getting the most from a peptide inhibitor

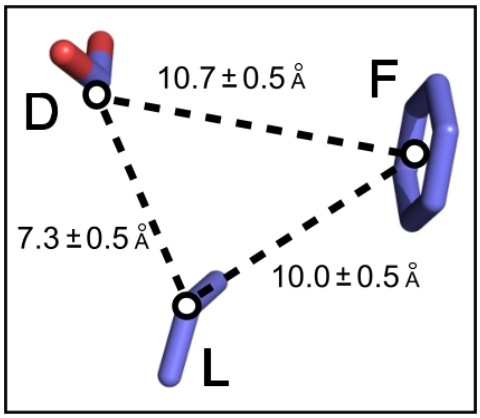
Looking at the sequences of the different clamp utilising polymerases has enabled researchers to determine a minimal binding motif. It is a five-residue consensus sequence Gln-Leu-[Ser/Asp]-Leu-Phe where the middle residue can be either

serine or aspartate. Recent attempts to develop a minimal peptide to compete for binding to the β-clamp used a modified version of this five residue motif. A synthetic *pentapeptide* containing a 3,4-chloro-phenylalanine at the C-terminus was enough to interfere with the interaction of the α and β subunits in Pol III [**ref. 4**]. The chloro-phenylalanine and the adjacent leucine have been suggested to bind in a hydrophobic pocket on the β-clamp surface [view 5] based on the structure of natural peptide-clamp complexes available in the PDB.

Although the artificial pentapeptide shows excellent binding and inhibitory activity, a small peptide is not suitable as an antibacterial drug as peptides are difficult to deliver to the interior of the bacterial cell in high enough concentrations. Instead, a small-molecule compound that can exploit the same binding site as this pentapeptide would be a better starting point for the development of new antibiotics.

Step away from the vehicle...

One way to find small-molecule drug candidates is to screen collections of thousands of chemicals called libraries. The contents of a library can been tested experimentally for the desired activity, in this case blocking the α-subunit-β-clamp interaction. A shortcut to narrow down the search is to use the known arrangement of significant atoms in the optimized pentapeptide as a starting point [**ref. 3**].The 3D arrangement of the Asp, Leu and Phe sidechains defines a chemical pattern [view 5]. This pattern, or “pharmacophore”, attempts to capture the key features that make the peptide an effective inhibitor.



Arrangement of atoms in the search fragment (pharmacophore). The key atoms and their spatial relationships derived from the pentapeptide inhibitor (view-5).

The pattern can then be used as a query for **virtual** screening (i.e., in the computer - *in silico*) of a database of small-molecule structures. Computational screening is much quicker than even highly efficient experimental work (called high-throughput screening or HTS).

Virtual screening hits pay dirt

This virtual screening approach yielded a family of candidate molecules that could then be tested experimentally for inhibitory activity [**ref. 4**]. After screening a range of variants in this family, the best of those tested, a biphenyl oxime compound, [ligand 743] was crystallised bound to the β-clamp [view 7]. This structure (PDB entry **3qsb**)

showed that the biphenyl rings occupy the hydrophobic pocket that would normally be used by the peptide's Leu and Phe residues. Comparing [view 5] with [view 6], one can see that the β-clamp surface adapts slightly to accommodate the smaller molecule which achieves tight binding without a match to the Asp side-chain.
The virtual screening suggested fewer than 200 compounds to be assayed experimentally, which led to the identification of this new lead compound [**ref 4**]. On this basis, the computational approach is over one hundred times more efficient than purely experimental approaches in which typically more than 20 000 chemicals would have been screened in the search for inhibitors.

Further exploration

You may already have realised that the Quips graphics window remains active after each view of the molecule is displayed. As a result you can get to know the molecule better by rotating the view, zooming in, or picking residues or atoms that interest you. The **mini-tutorial** associated with this Quips is a walk-through of the menus and features available to help your own explorations in Quips and the PDBe website.

References

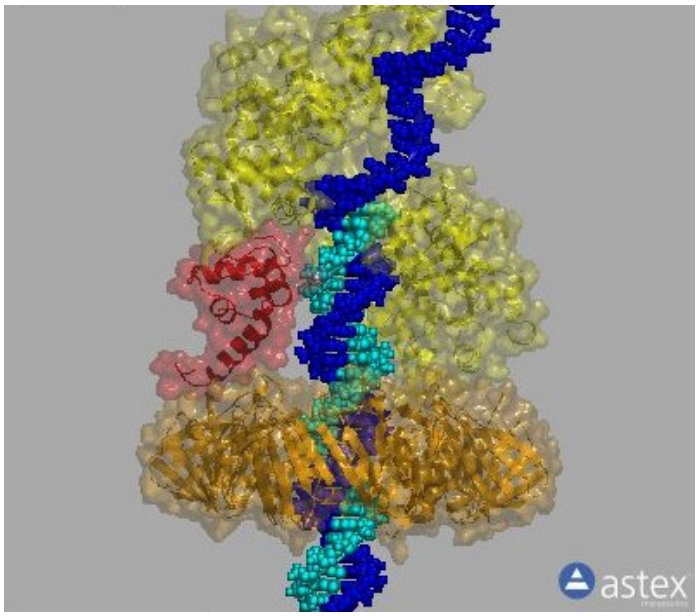
[1] Wing RA, Bailey S, and Steitz TA. J Mol Biol. 2008 Oct 17;382(4):859-69. PubMed PMID: 18691598

[2] Georgescu RE et al. Cell. 2008 Jan 11;132(1):43-54. PubMed PMID: 18191219

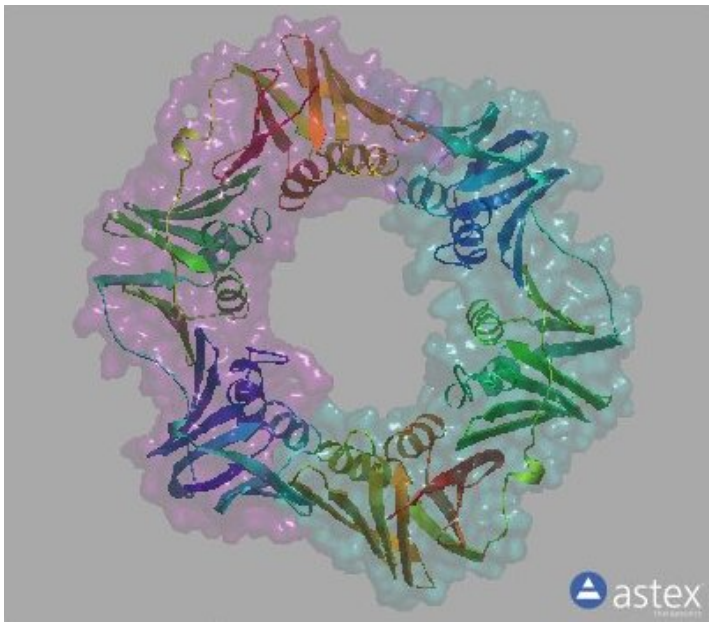
[3] Georgescu RE et al. Proc Natl Acad Sci U S A. 2008 Aug 12;105(32):11116-21. PubMed PMID: 18678908;

[4] Wijffels G et al. J Med Chem. 2011 Jul 14;54(13):4831-8. PubMed PMID: 21604761.

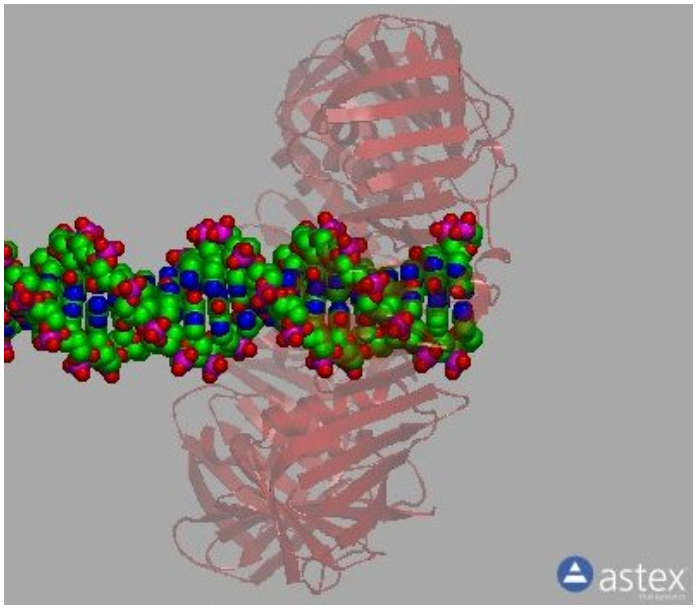
VIEWS



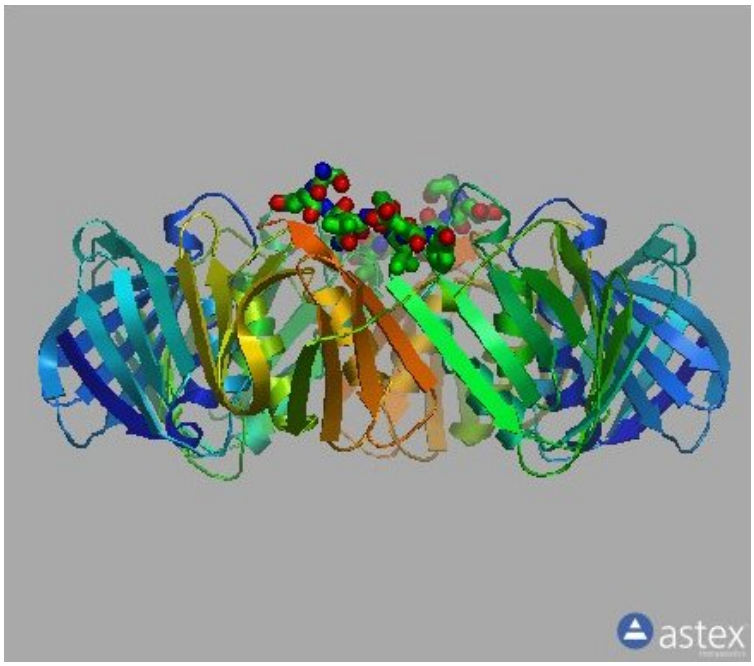
View 1 **Model of polymerase III complex bound to DNA.** The α-subunit (yellow) is held on to the DNA (blue and cyan) by binding with its C-terminal extension (red) to the β-clamp (orange)



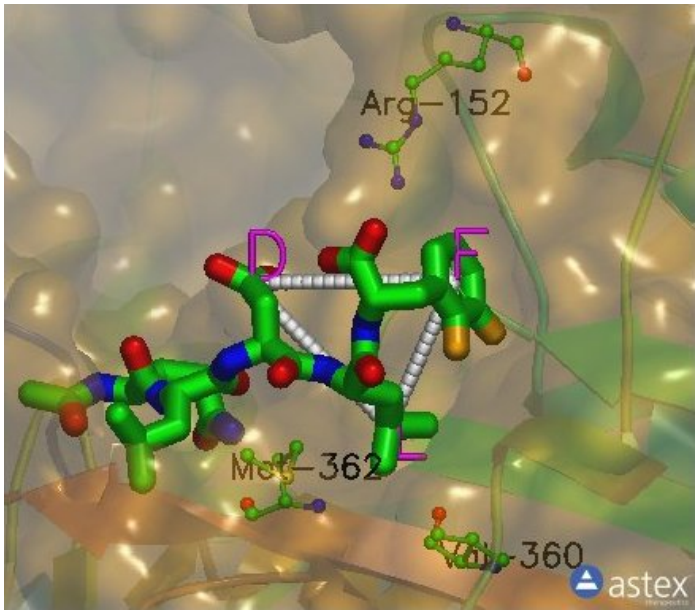
View 2 **Structural repeats in the bacterial sliding clamp.** The β-clamp is a dimer of identical subunits (cyan and magenta surfaces). Each subunit is made up of three homologous domains (coloured blue, green and red). Inter-domain and inter-subunit contacts involve a shared beta sheet.



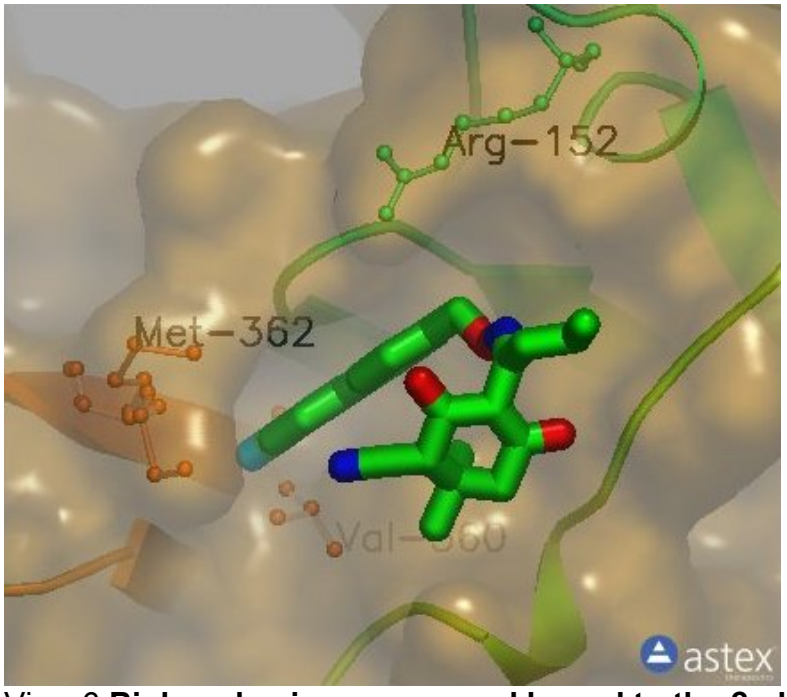
View 3 **β-clamp sliding on DNA.** The clamp dimer travels along the DNA. It undergoes a 22° tilt from the vertical (red model) when it reaches a free end of the DNA where it can commence its polymerase activity.



View 4 **Nonapeptide bound to the β-clamp.** The 9-residue peptide (shown in sticks) binds to the β-clamp, revealing the binding site for the catalytic subunit in the third domain of each clamp subunit.



View 5 **Pentapeptide bound to the β-clamp.** Based on PDB entry **3d1e**, this model shows geometrical relationships between key residues of the pentapeptide [aspartate (D), leucine (L) and 3,4-chlorophenylalanine (F)] highlighted by white dotted lines. Key residues in the binding site of the clamp are labelled.



View 6 **Biphenyl oxime compound bound to the β-clamp**. This compound occupies the same site as the leucine and chlorophenylalanine in view-5. Key residues in the binding site of the clamp are labelled as in view-5.