A FASCINATING EXAMPLE OF CONVERGENT EVOLUTION INVOLVES STAPHYLOCOCCUS AUREUS PHAGE ENCODED DIMERIC AND TRIMERIC dUTPases IN SIGNALLING

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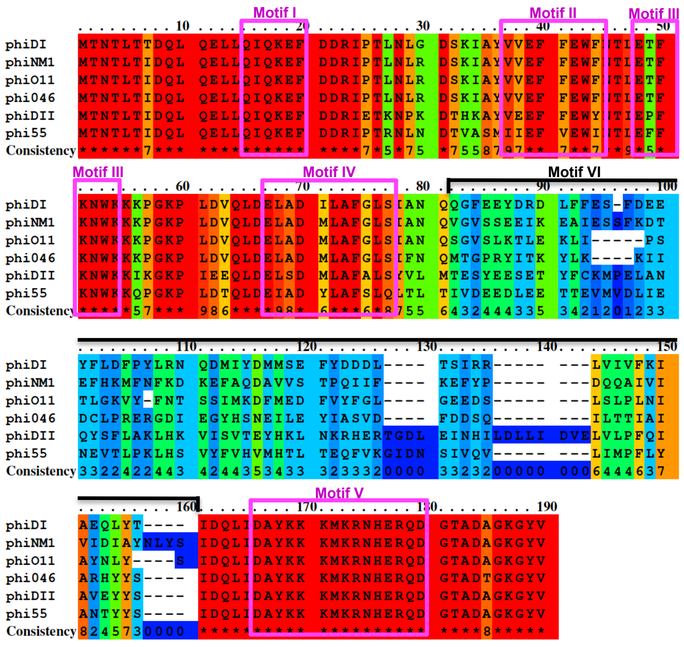
Background

The dUTPase (Dut) enzymes prevent the misincorporation of uracil into the DNA and are encoded by almost all free-living organisms and some viruses. We have previously showed that phage-encoded trimeric Duts mediates the *Staphylococcus aureus* pathogenicity island (SaPIs) transfer by interacting to the SaPI-encoded repressor Stl, proposing that these Duts are regulatory proteins. Some *S. aureus* phages encode structurally unrelated dimeric Duts instead trimeric Duts. Surprisingly, a recent work, has involved one of these predicted dimeric Duts in the transfer of SaPIs by interacting with the same Stl repressor.

Results



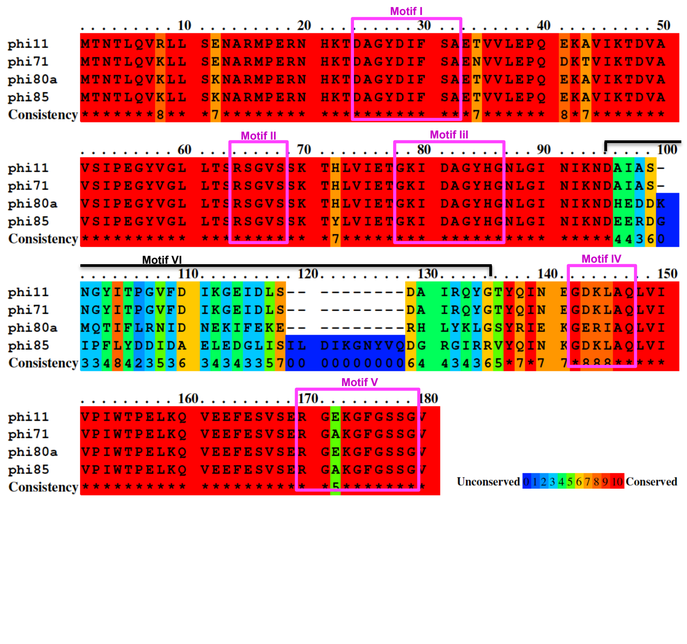
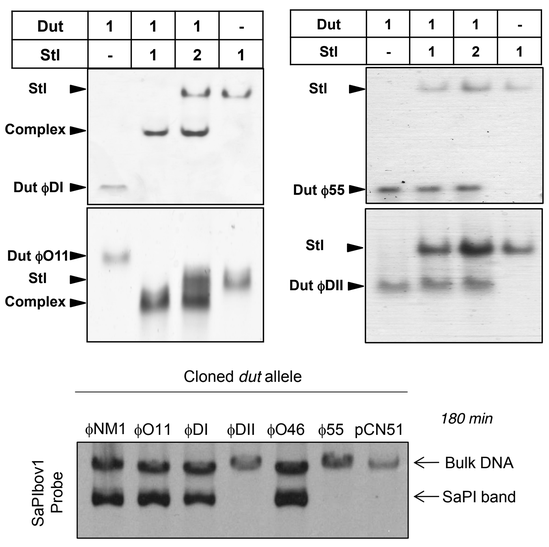
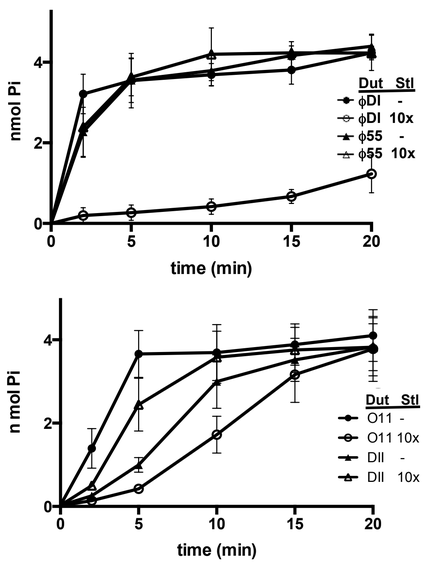


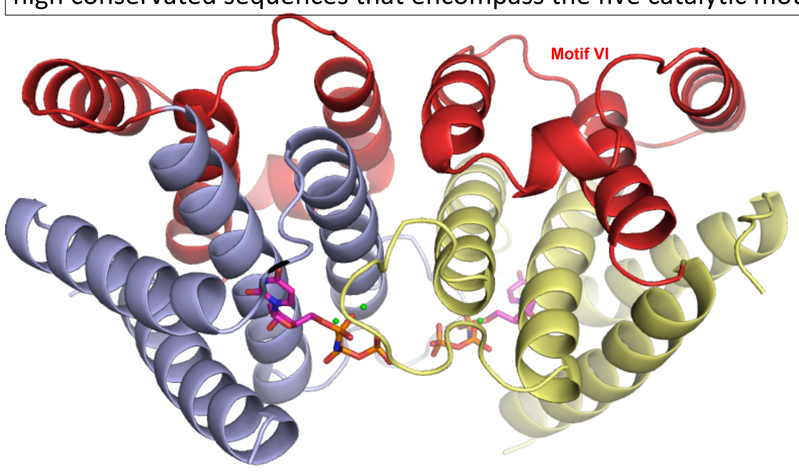
Figure1. Sequence comparision of staphylococcal Duts confirms the presence of multiple allelic variants for both dimeric (left) and trimeric (right). Variability is accounted by a central region, named motif VI, and flanked by two high conservated sequences that encompass the five catalytic motifs.



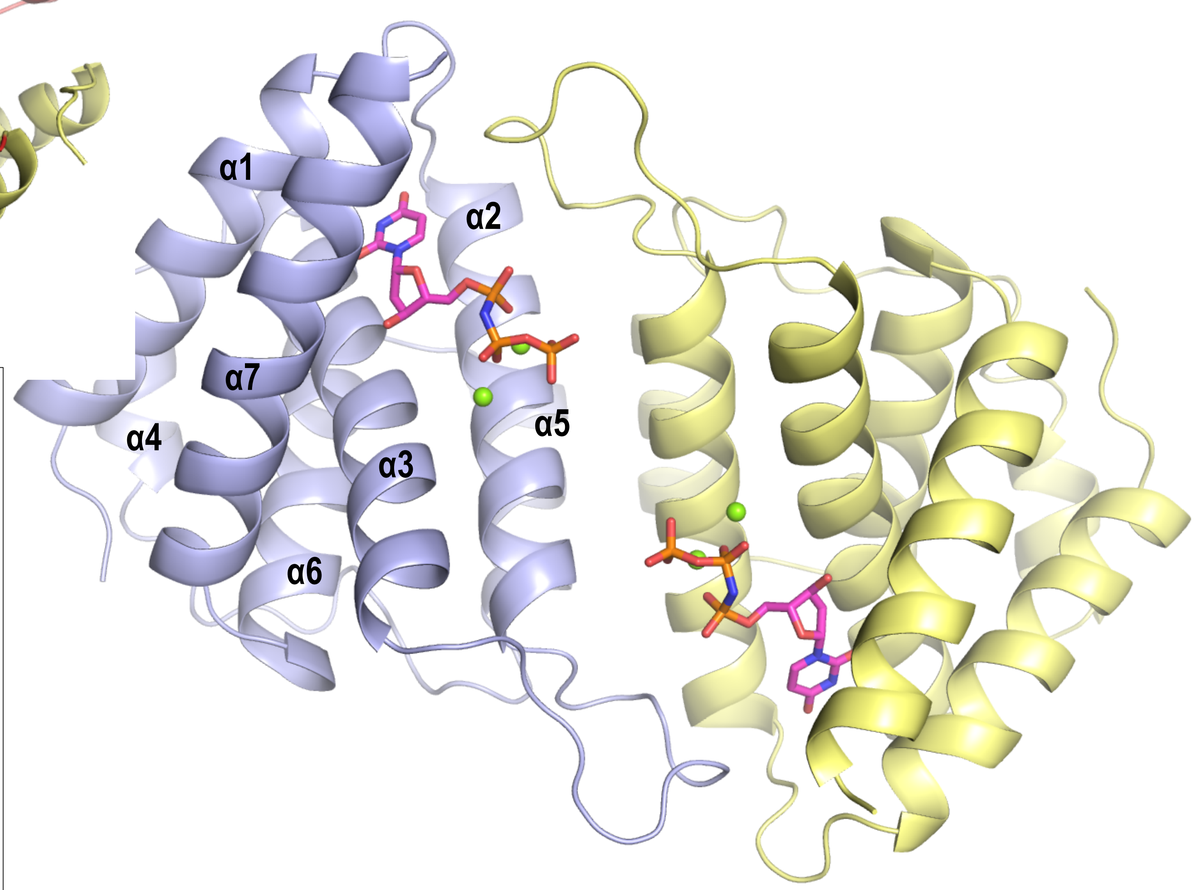
Native-PAGE (a) shows that induction of SaPI mobilization by the allelic variants of dimeric Duts is mediated by a direct interaction with Stl . Contrary, all trimeric Duts induce SaPI.

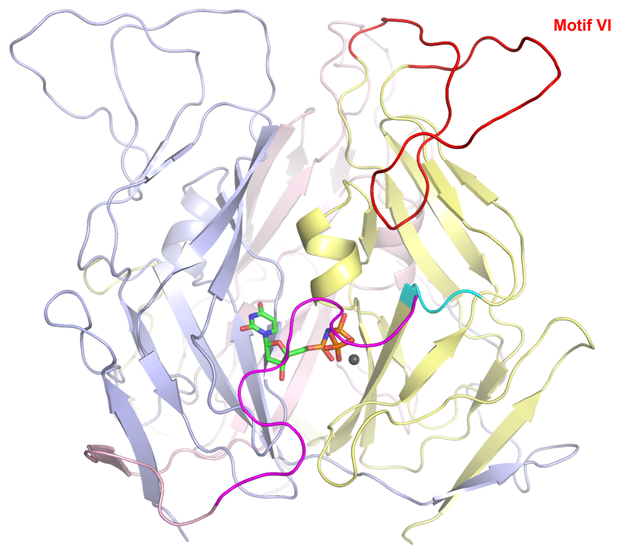


Stl inhibits the dUTPase activity of the variants that induce the SaPI, as reported for trimeric Duts,

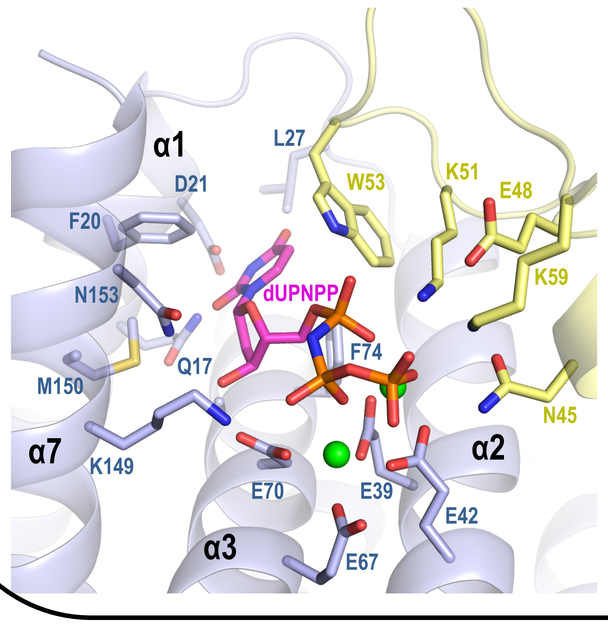


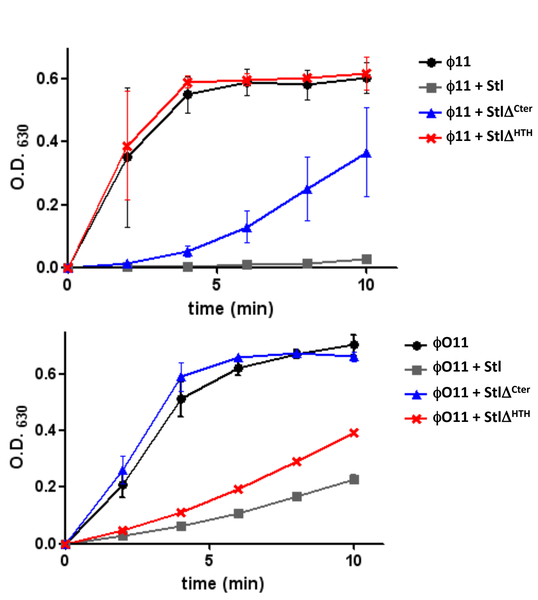
φO11 dimeric Dut presents an all-helix structure. Each protomer is made of 8 helices with the characteristic structural core of dimeric Duts composed of four helices (α1-α3 and α7) that conform the active centre. Dimerization interface is mainly provided by the helices α2 and α5. The motif VI (red), composed by the helices α4-α6, is placed in one face of the molecule, whereas both active centres of the dimer are oriented towards the same molecule face, forming a long channel that accommodates two molecules of dUPNPP.
The dimer has two active centers whth each one occupied by a molecule of dUPNPP. β- and γ-phosphates chelate two divalent metals (Mg), whereas trimeric requires one.



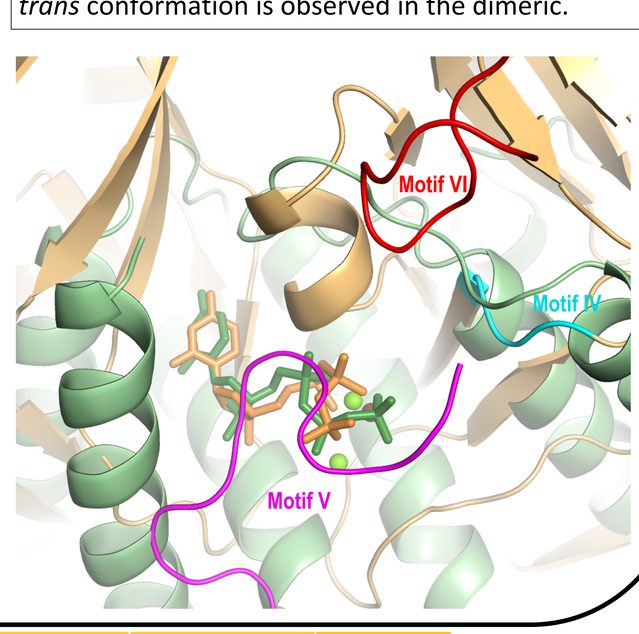


80α trimeric Dut (up) show the difference in folding between dimeric (all-alpha) and trimeric (all-beta). The structural motifs implicated in Stl recognition for trimeric Duts are labelled and coloured in cyan (motif IV), magenta (motif V) and red (motifVI).
At the bottom, the superimposition of dUPNPP molecules shows both active centres, dimeric (green) and trimeric (orange), are completely different. Orientation of the plane of the uracil moiety and the disposition of the phosphates is different. In trimeric Dut, α-phosphates acquire a *gauche* catalytic-competent geometry meanwhile a *trans* conformation is observed in the dimeric.





Stl repressor full-lenght inhibits the dUPase activity of both dimeric (φO11) and trimeric (φ11). Stl Δ^{HTH} inhibits dUTPase activity of dimeric but not trimeric, while StlΔ^{Cter} has the opposite capacity, being this specific domain interaction a fascinating example of convergent evolution of Stl repressor by targeting with structurally unrelated proteins (antirepressors) performing the same function, and allowing thus to the SaPI to hijack the helper phage cycle by exploiting conserved phage processes.



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