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Streptomyces development: imaging from outside in

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Streptomyces: Nature's antibiotics factories

- ***Streptomyces* produce about 70% of the known antibiotics**
- **they also produce other valuable pharmaceuticals – examples are:**
  - **anti-tumour agents such as daunorubicin**
  - **immunosuppressants such as FK506**
- ***Streptomyces* are good at organic chemistry and evolution has provided the selection for useful compounds**

Streptomyces

- belong to the actinobacteria: gram-positive bacteria with high G+C genomes (includes *Mycobacteria & Corynebacteria*)
- exhibit a complex differentiating life-cycle

Streptomyces coelicolor as a model organism

produces pigmented antibiotics

- 8,667,507 bp chromosome
- G+C content of 72.1%
- contains 7825 orfs

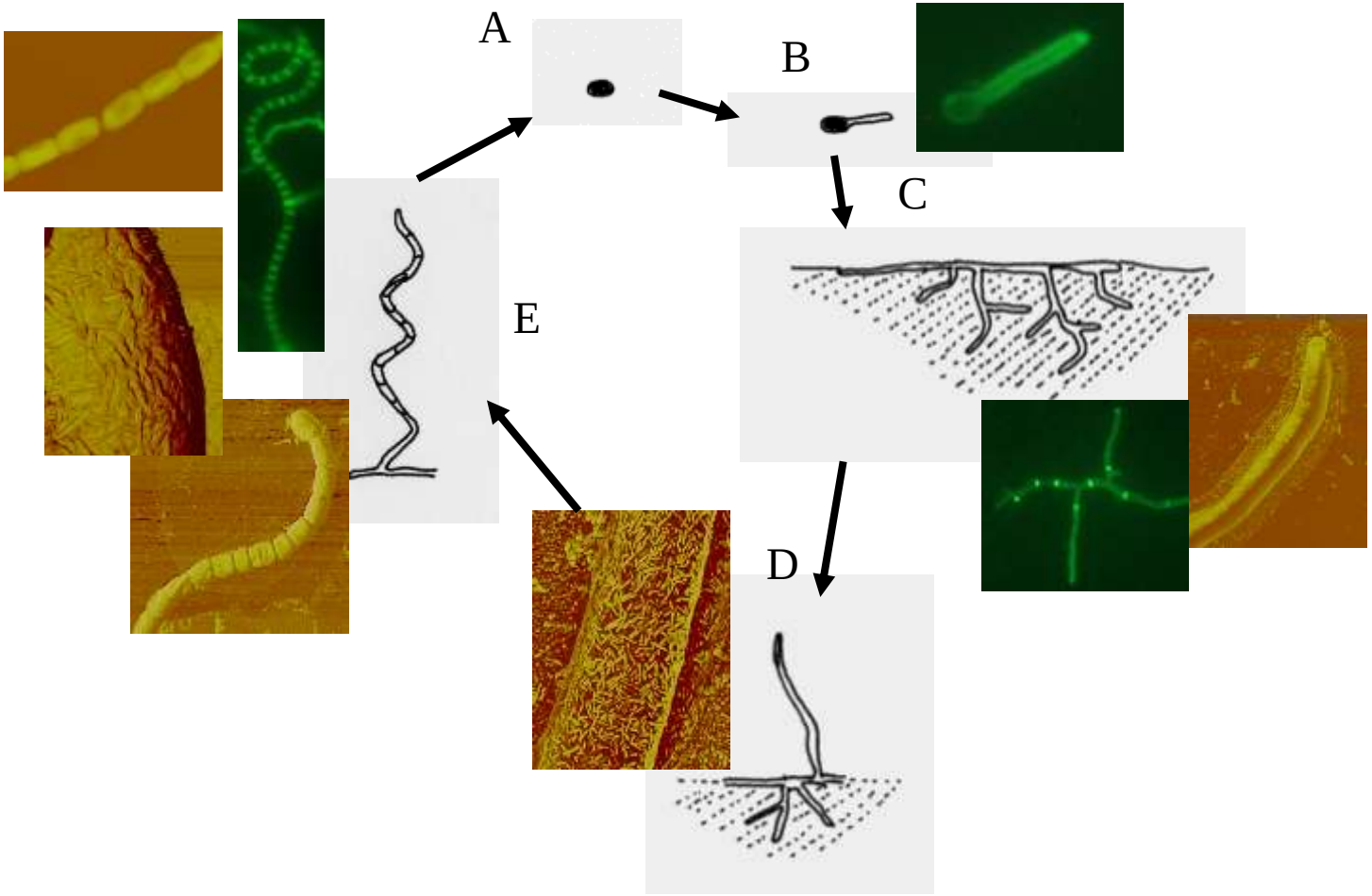
Actinorhodin

"act"

Undecyl-prodigiosin

"red"

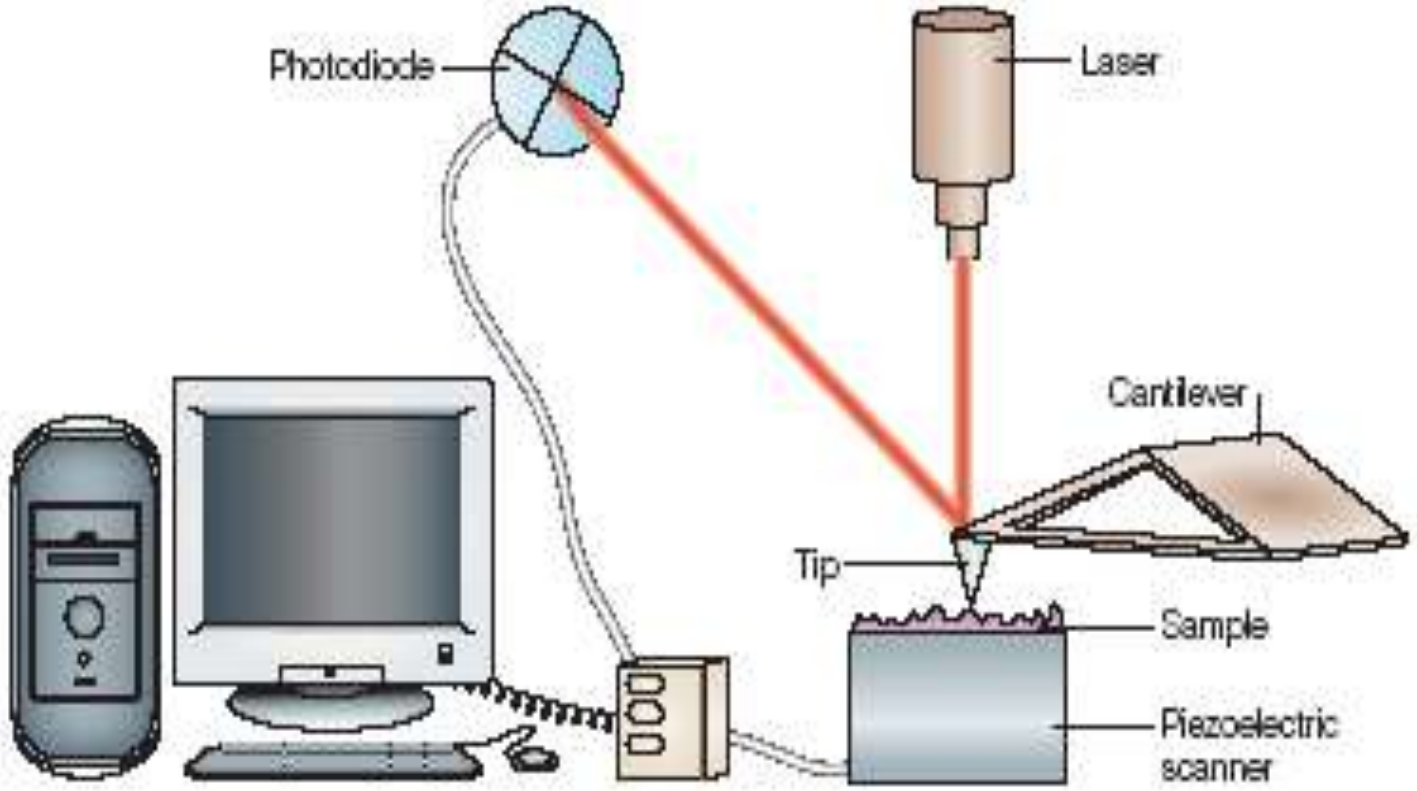
Streptomyces life-cycle



Streptomyces development: imaging from outside in

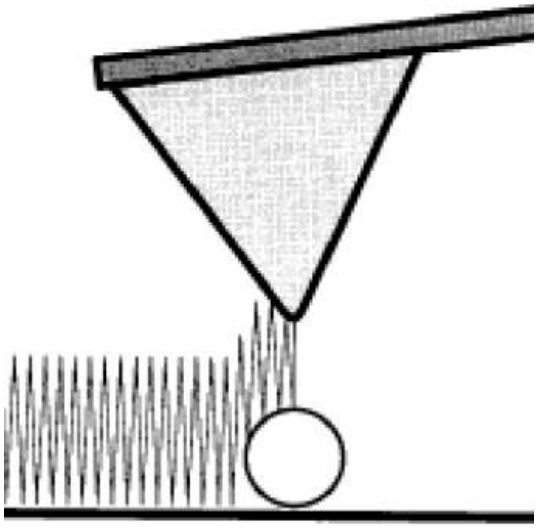
- Imaging development from the outside using Atomic Force Microscopy
- Cell division – looking inside
- Dps proteins – protein nanocage assembly

Basic elements of the Atomic Force Microscope

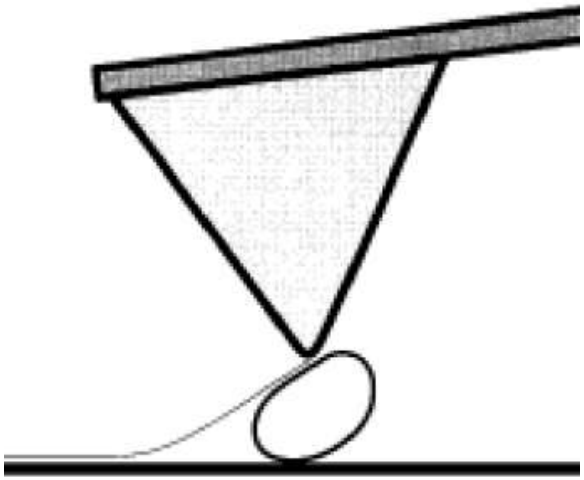


Operating modes of atomic force microscopy

Tapping mode

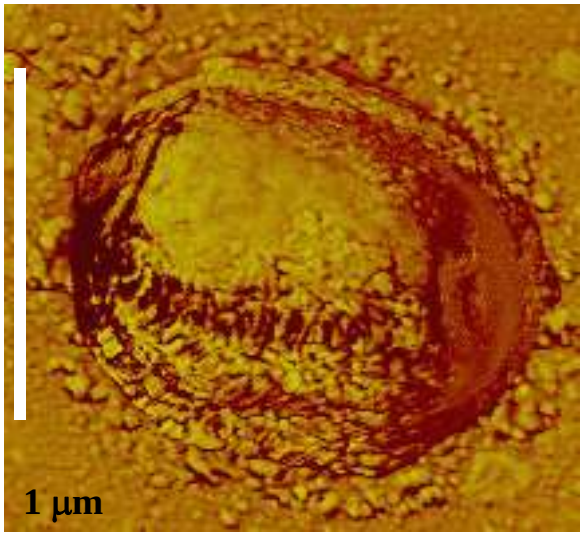


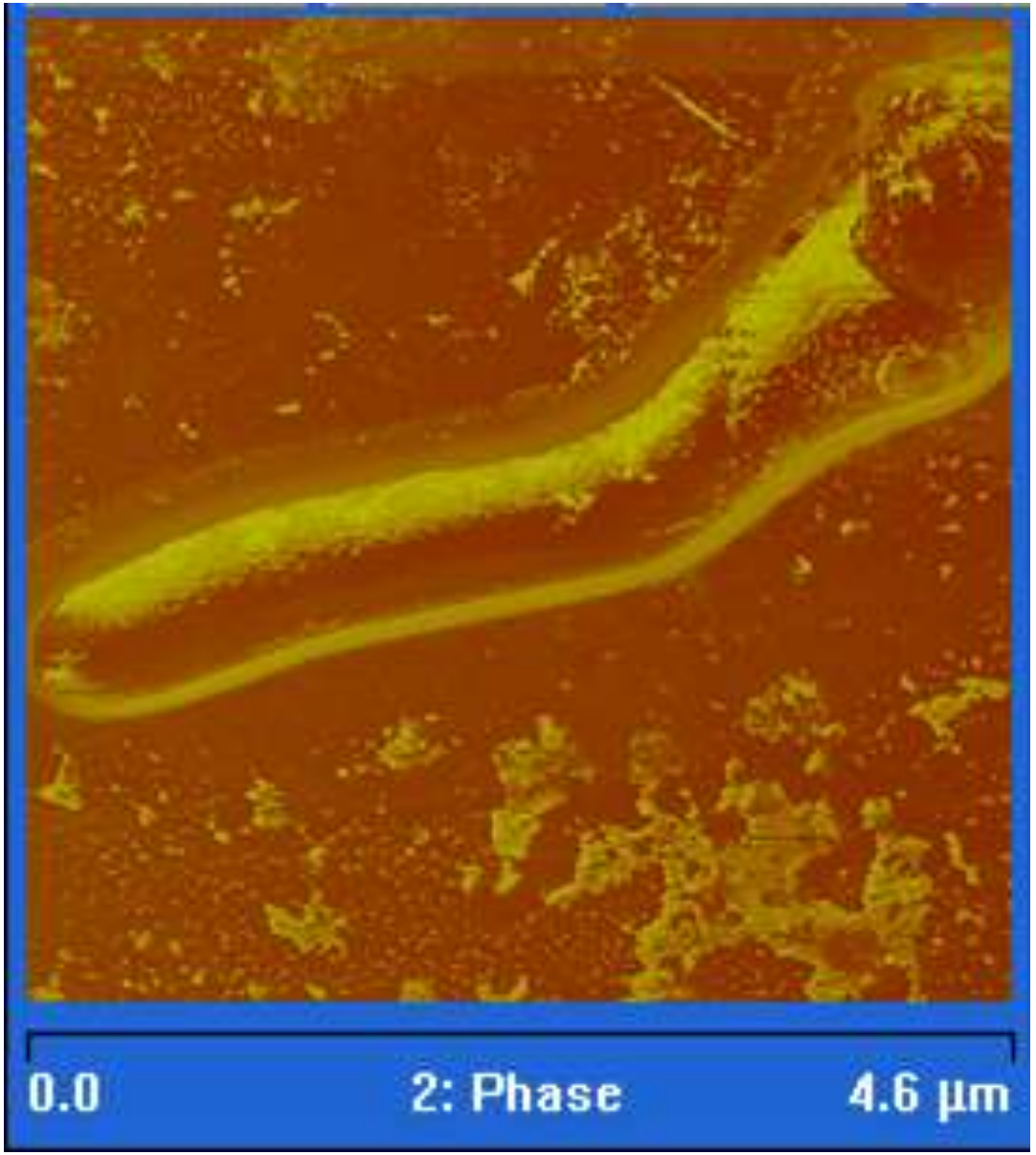
Contact mode



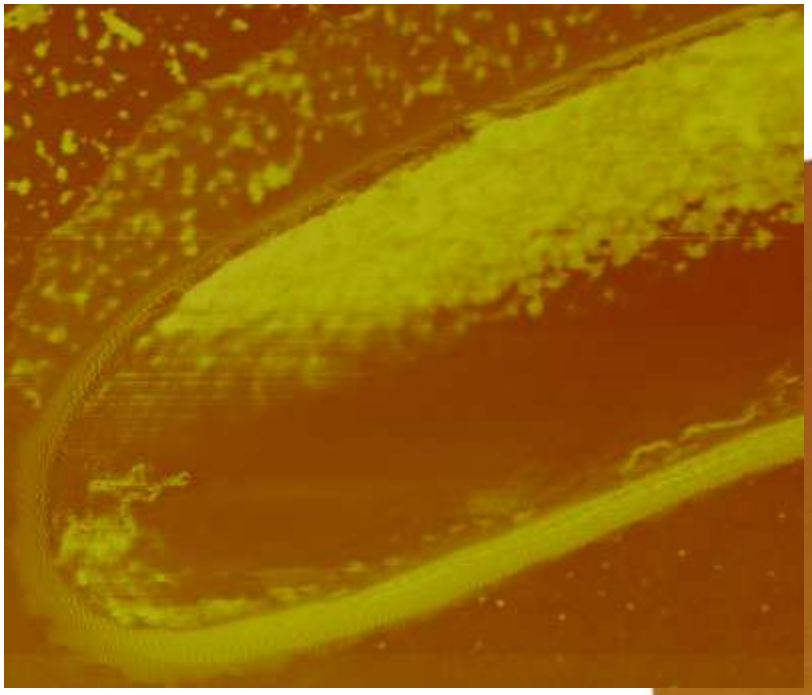
Tapping mode atomic force microscopy (TMAFM) An oscillating tip is scanned over the surface and the amplitude and phase of the cantilever are monitored near its resonance frequency. As the tip touches the sample surface only at the very end of its downward movement, lateral forces during imaging are greatly reduced, which is advantageous for imaging ‘soft’ biological samples.

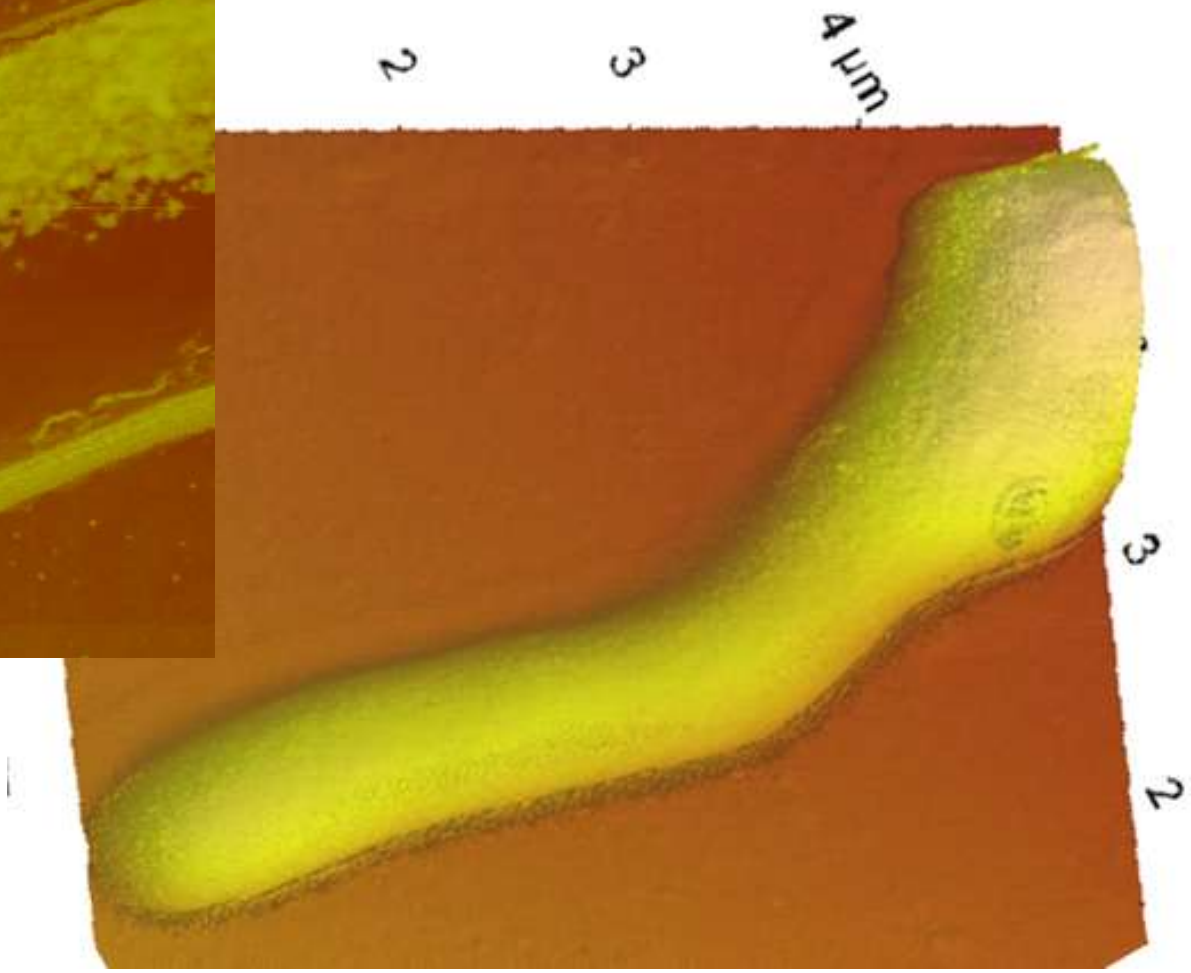
Germ tube surface

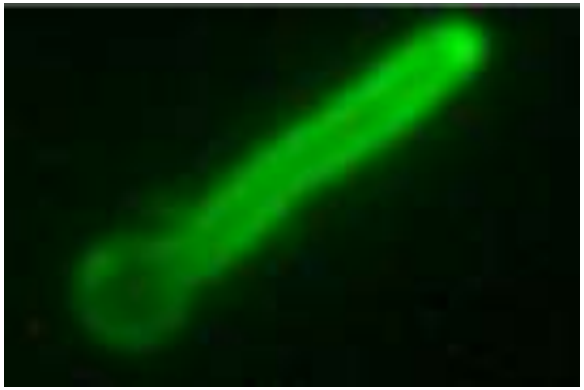




Germ tube surface is relatively smooth

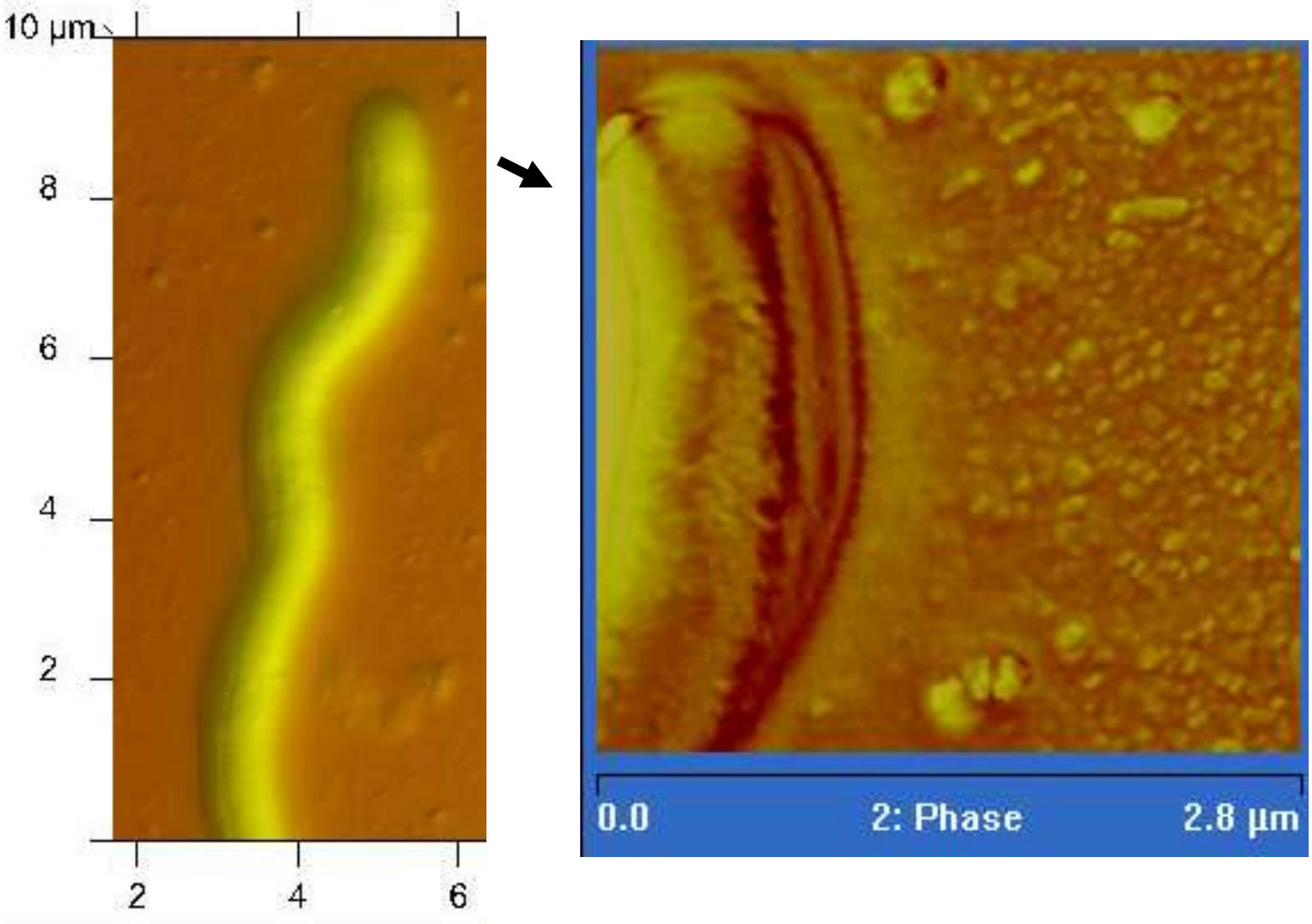


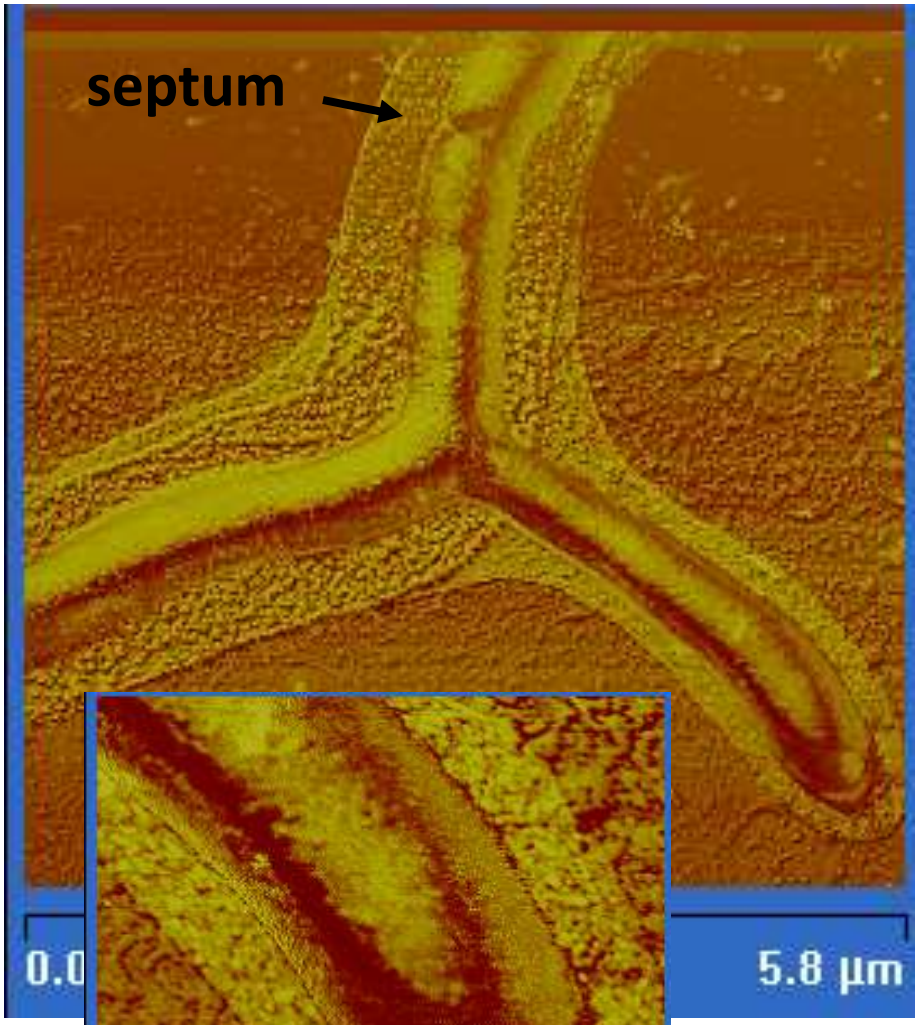


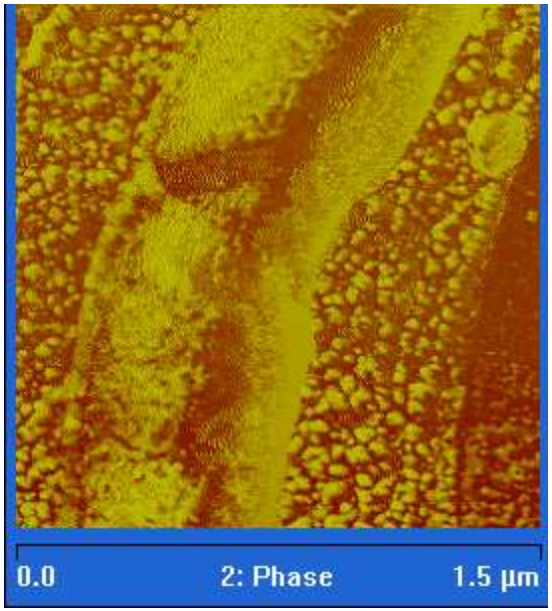


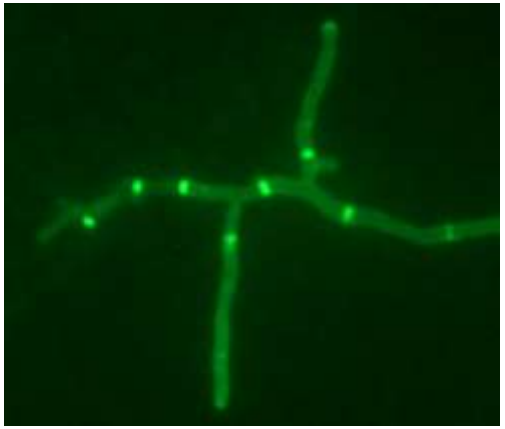
Fluo-Vancomycin staining

Vegetative hyphae grown in liquid culture



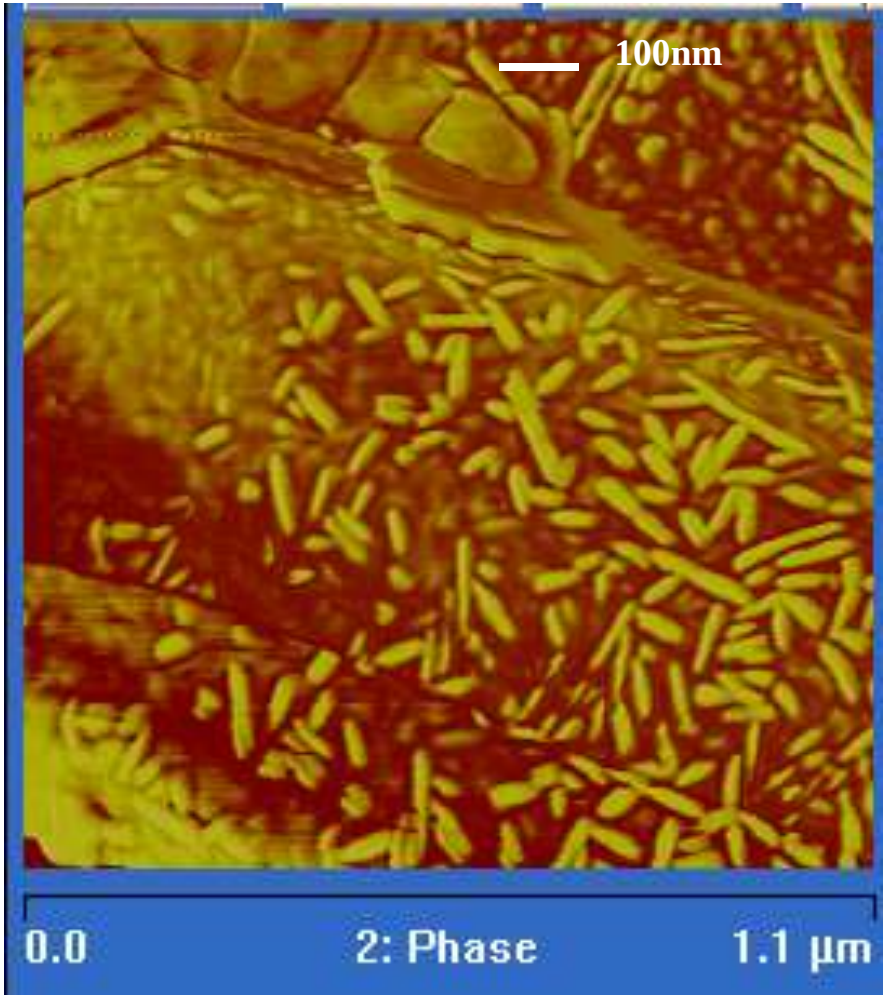


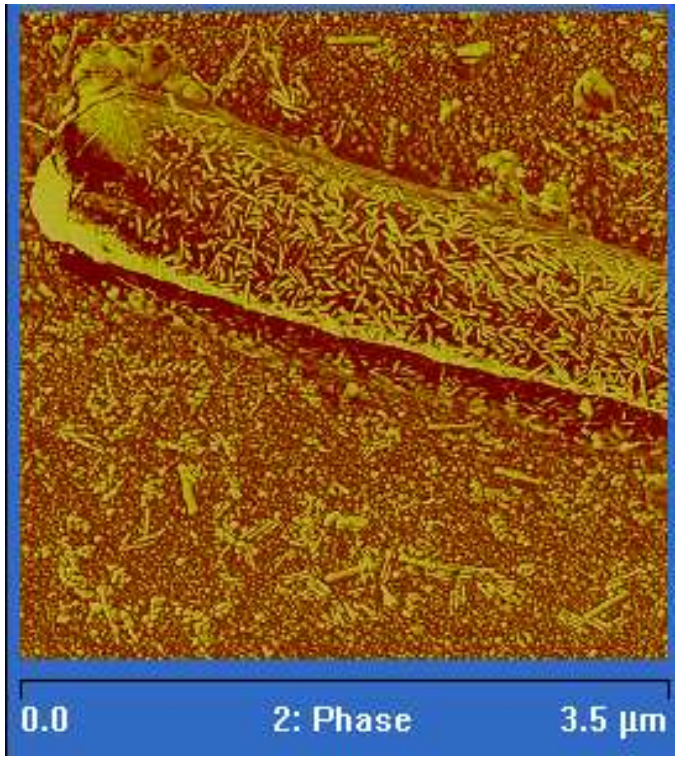




Fluo-Vancomycin staining

Vegetative hyphae growing on solid medium



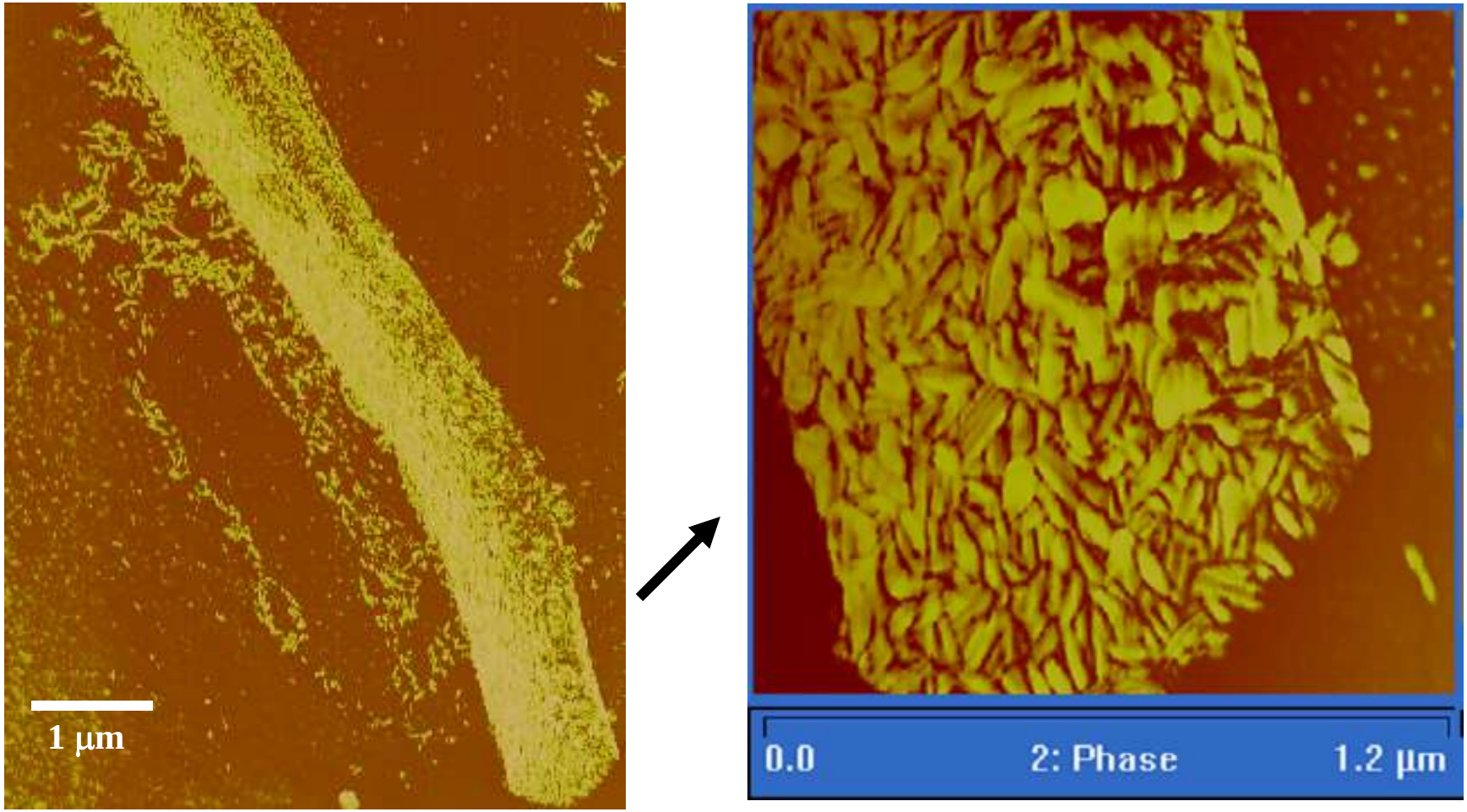


Fibrils size (approx.)

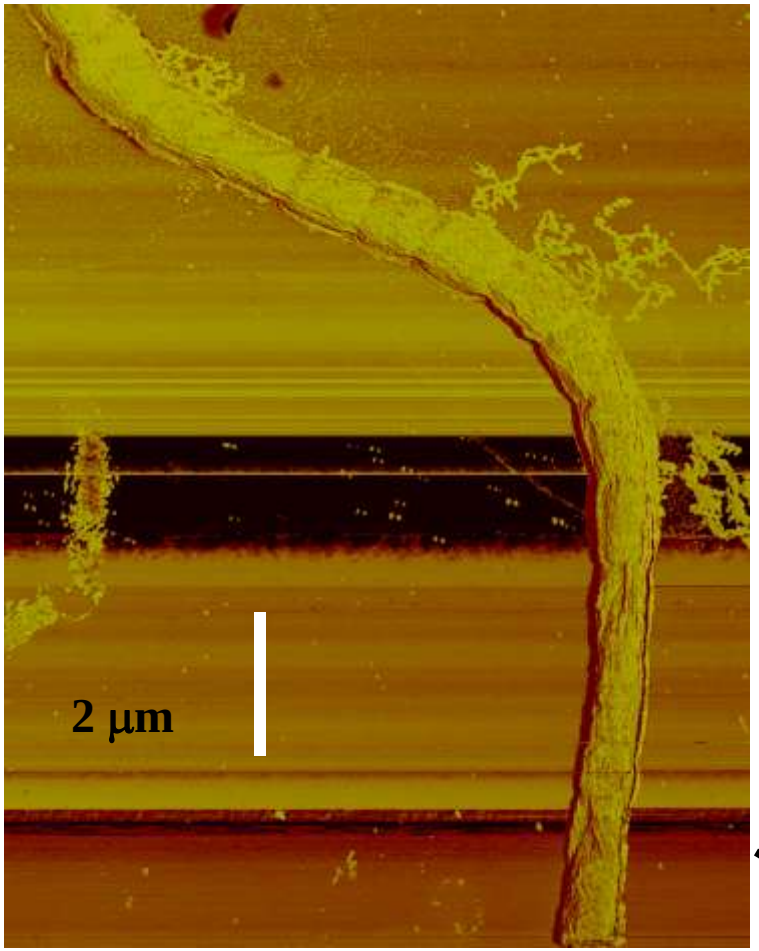
Width – 20nm

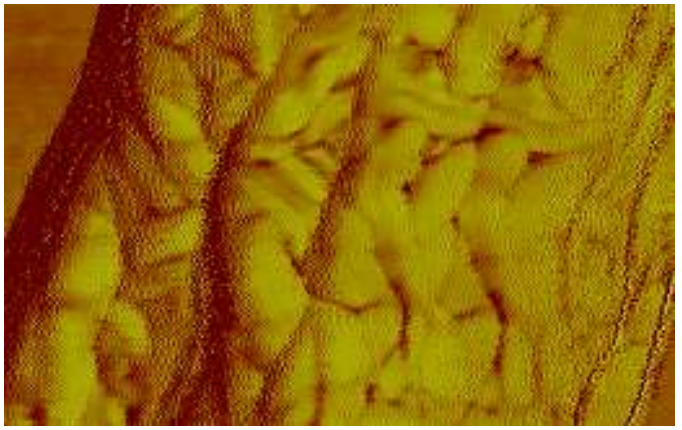
Length – 80-120nm

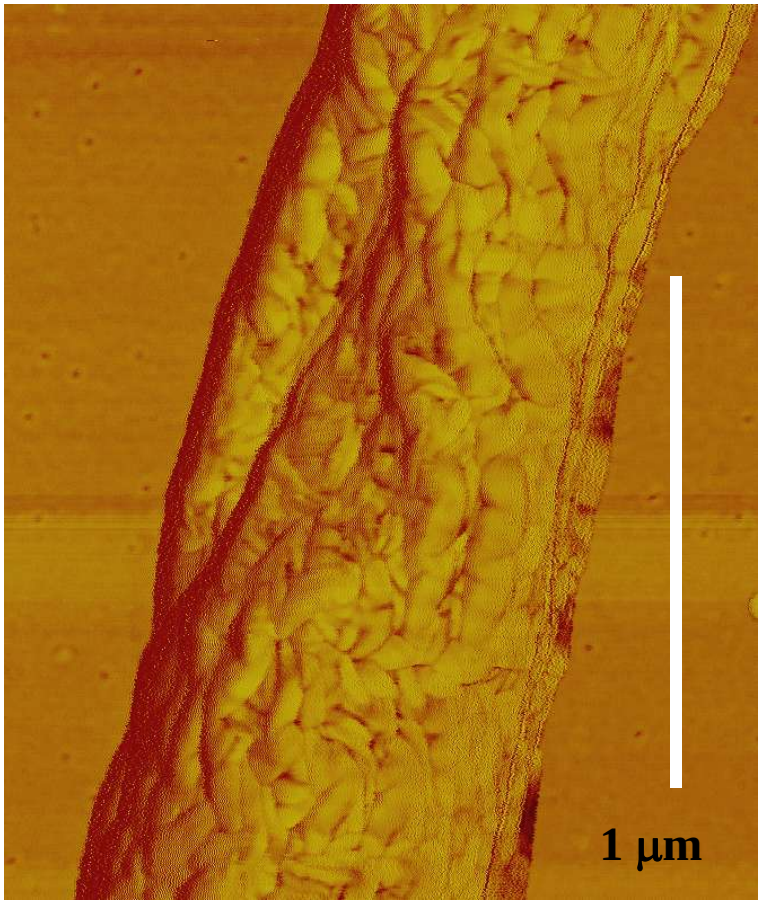
Unseptated aerial hyphae



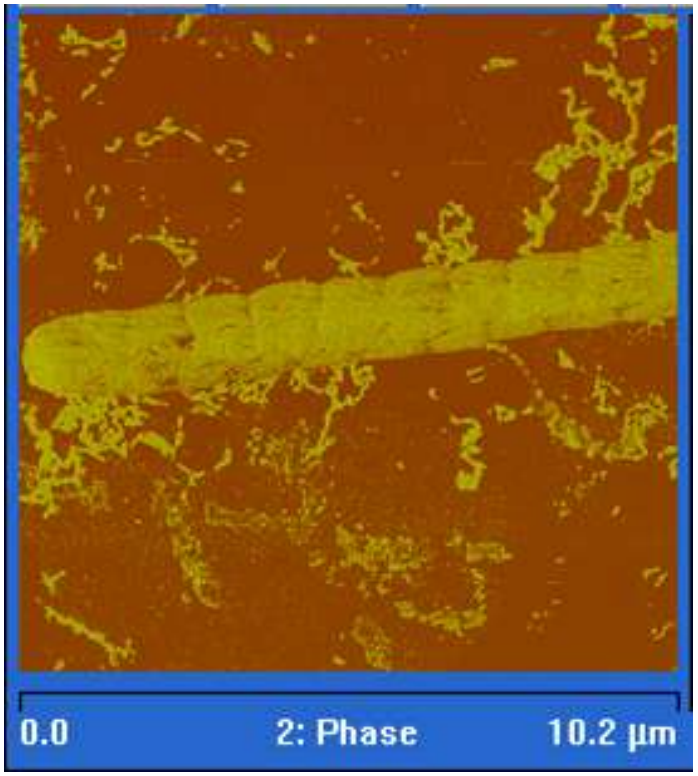
Early sporulation stage

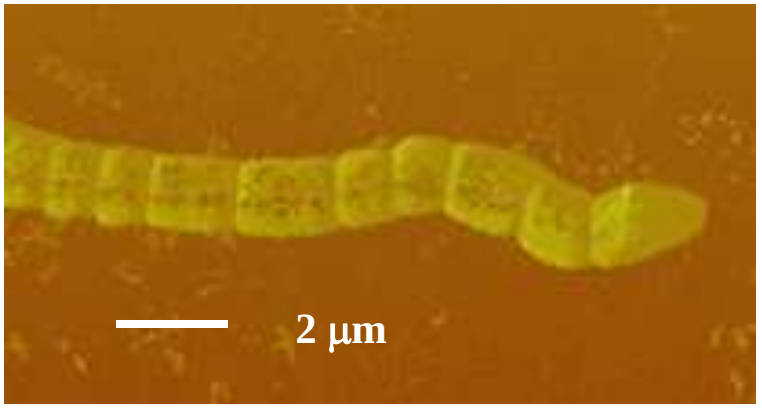


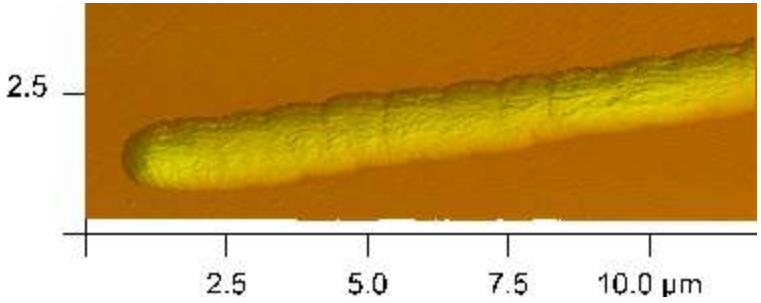


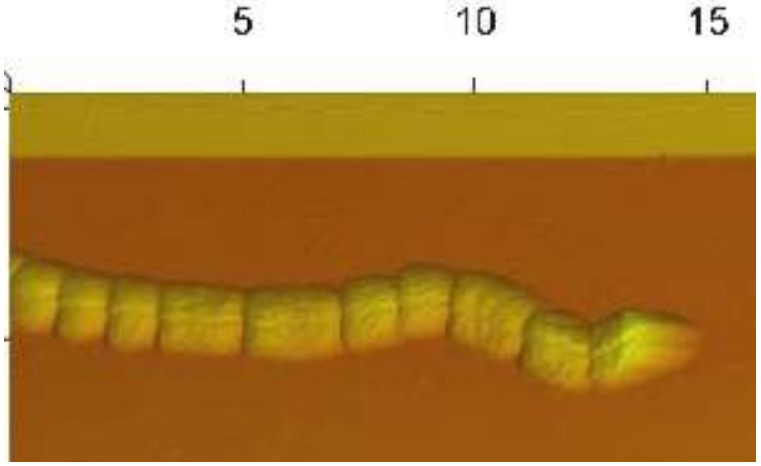


Sporulation

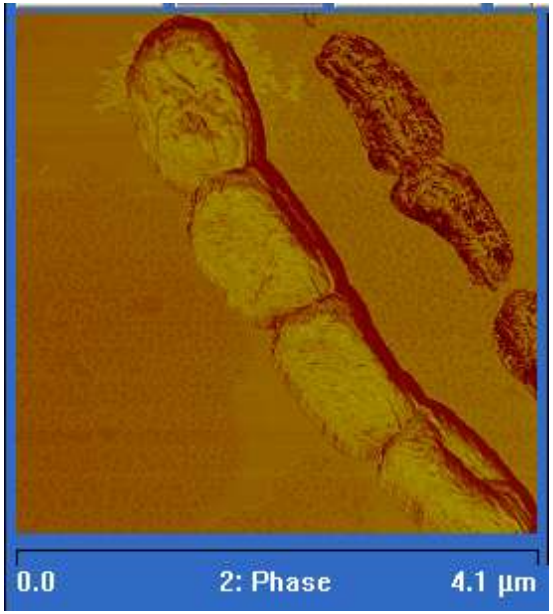


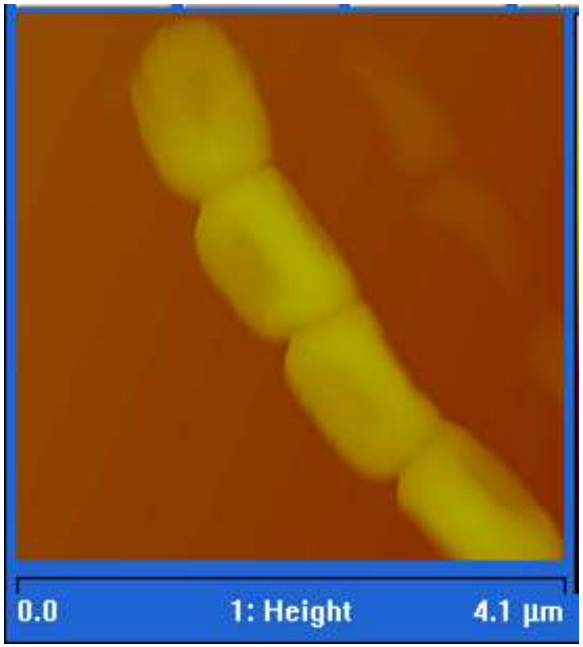


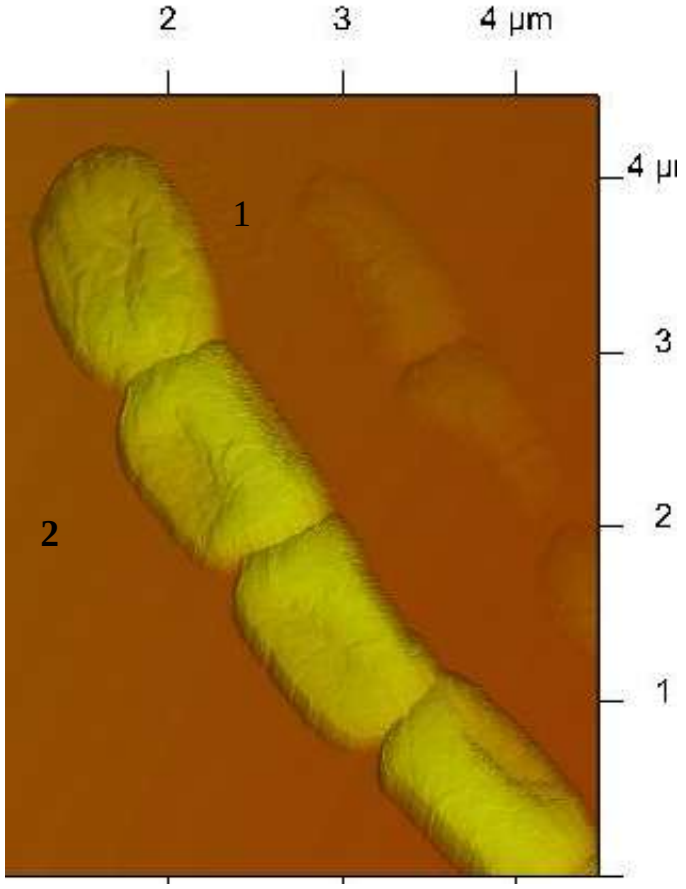




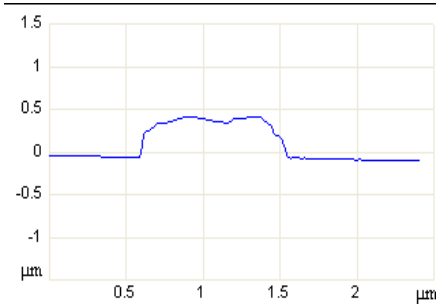
Late sporulation stage



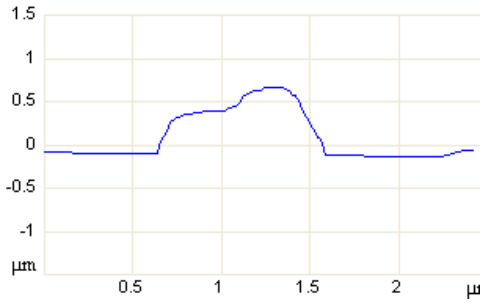




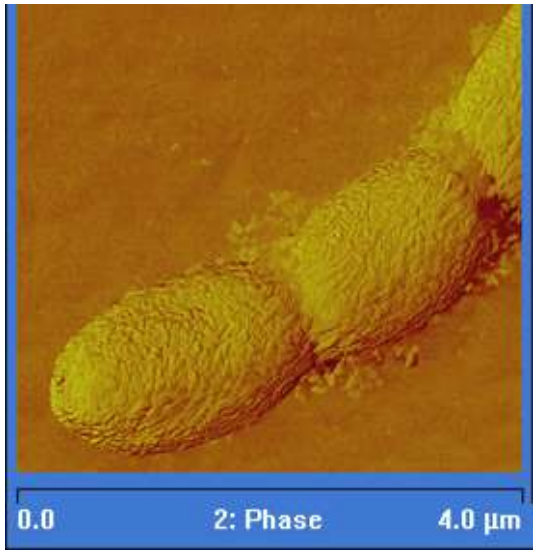
1 Cross-section

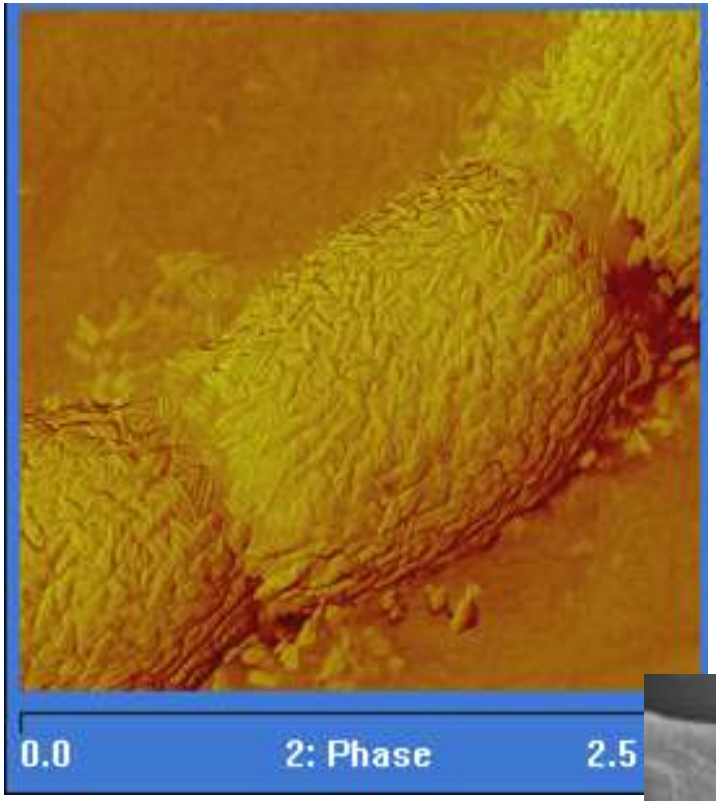


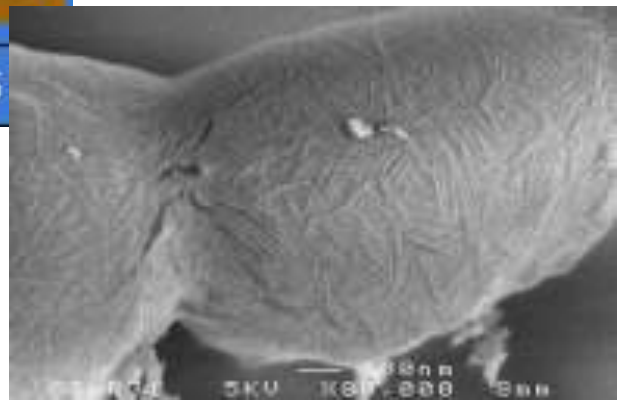
2 Cross-section



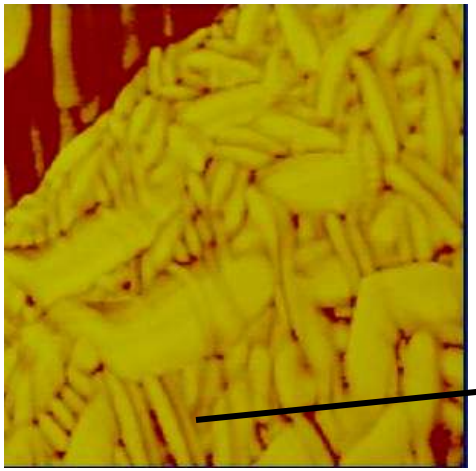
Spore surface ultrastructure





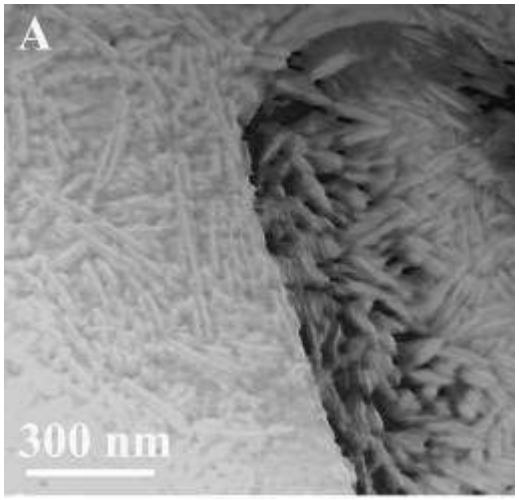


Mol. Microbiol. (2004) 53(2), 433–443



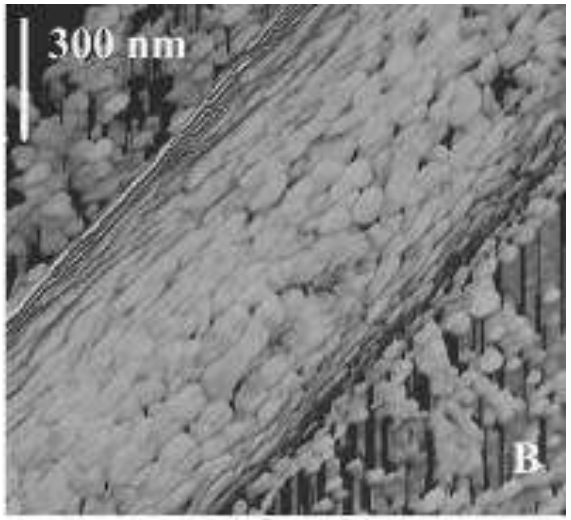
Rodlet pairs: 60-150 nm in length
12 nm monomer width

comprised of chaplin fibrils organised by rodlin proteins



Unstable fibrous layer of an aerial hypha of a Δ*chpABCDH* mutant (only expressing ChpEFG)

Chp ABC are ‘long’ chaplins that anchor to peptidoglycan



Scaly fibrous layer covering an aerial hypha of an *rdlAB* mutant

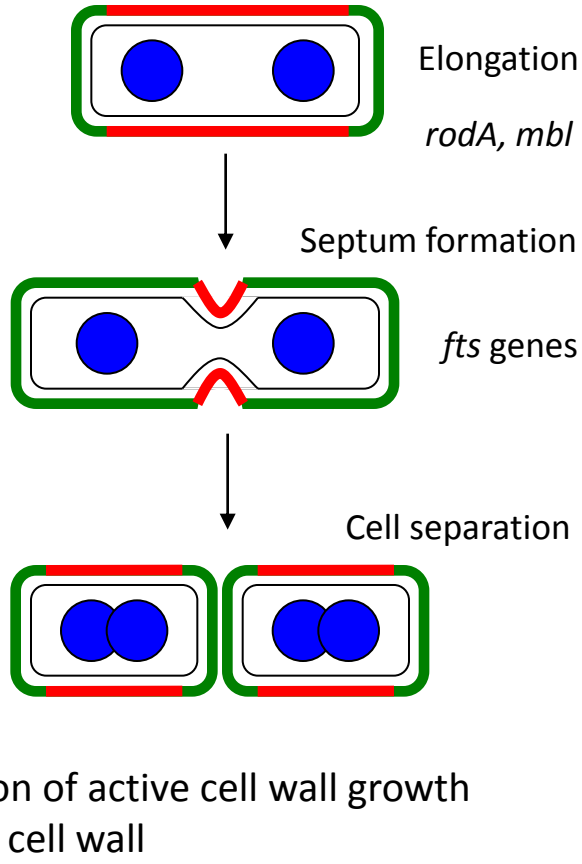
Imaging development from the outside using Atomic Force Microscopy

- High resolution imaging of living cells
- No sample preparation artefacts
- Reveals unprecedented detail
- Also useful for imaging macromolecules
  eg DNA compacted by histone-like proteins

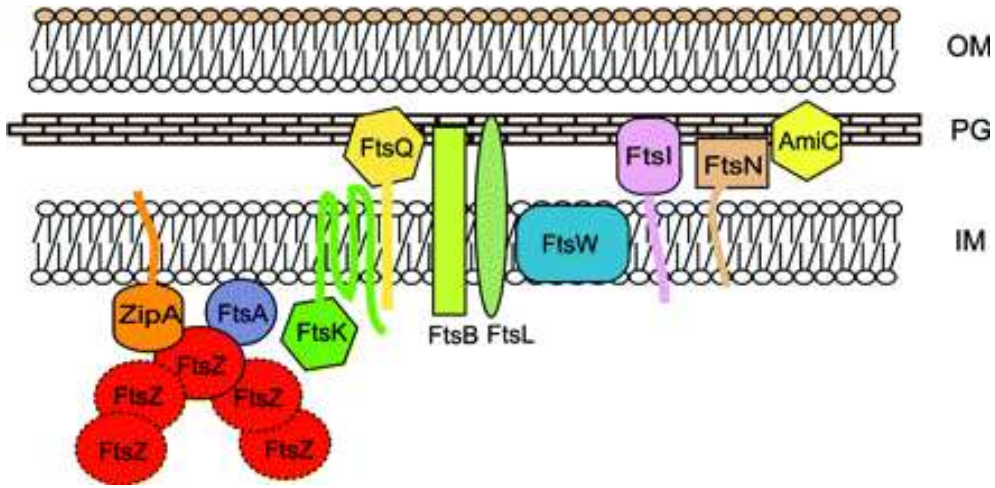
Del Sol R, Armstrong I, Wright C, Dyson P. *Journal of Bacteriology 189(6) 2219-25 2007.*

Cell division – looking inside

- Bacterial growth occurs by successive cycles of elongation and cell division
- At least 14 proteins are involved in the cell division process, among them 10 are essential
- Fts proteins play a key role in the division process



The Fts complex in *E. coli* : the ‘divisome’

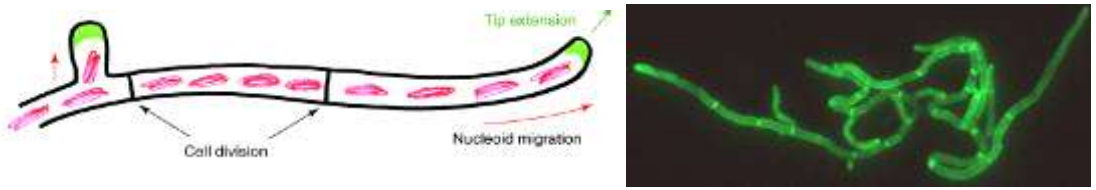


Shih and Rothfield, 2006

Cell division in *Streptomyces*

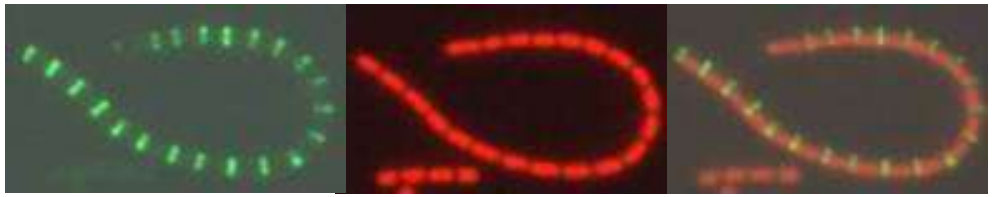
Two types of septation:

Vegetative cross-walls

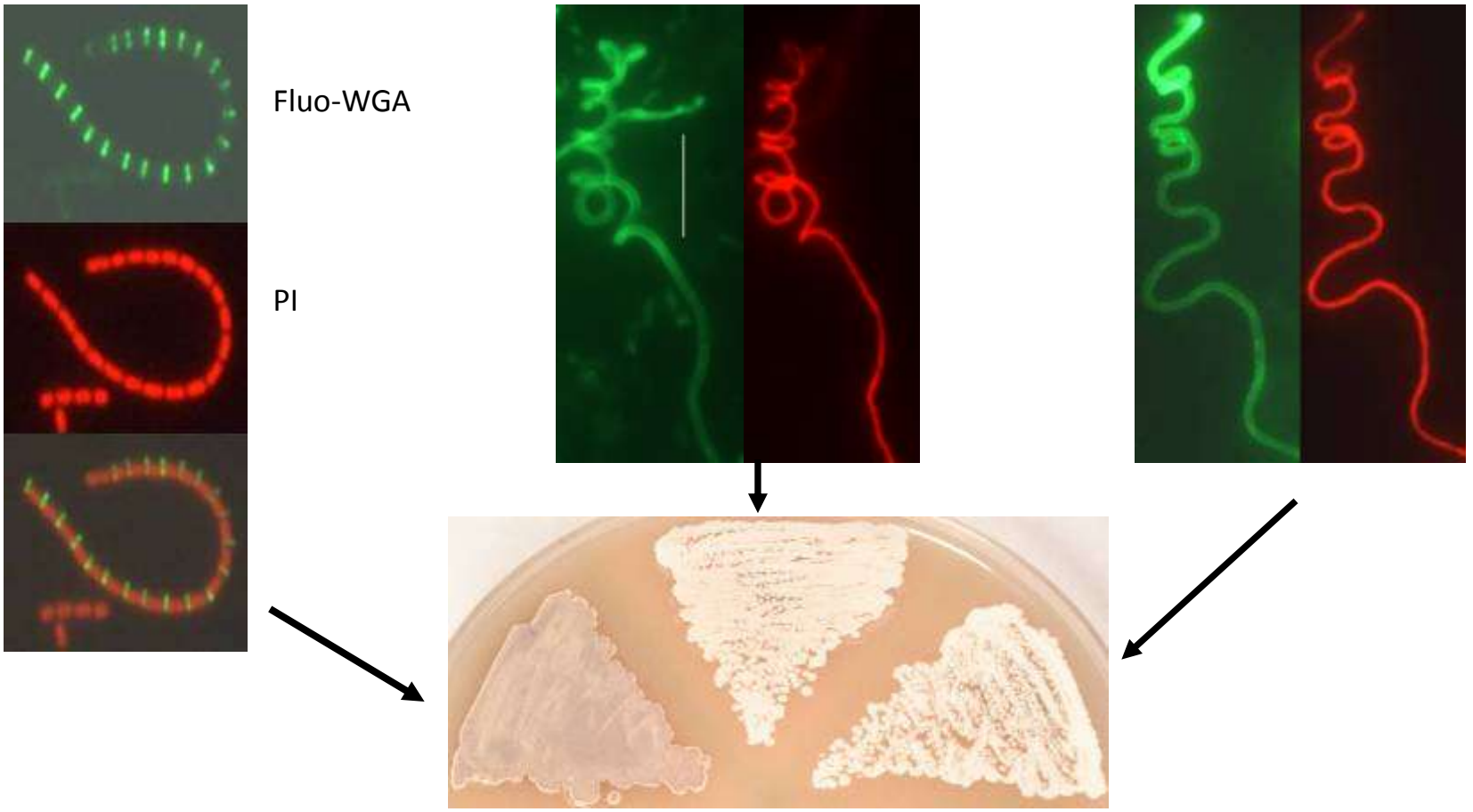


Flärdh, 2003

Sporulation septation

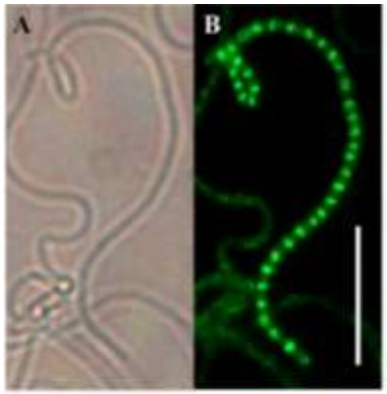


FtsW/I are required for sporulation septation

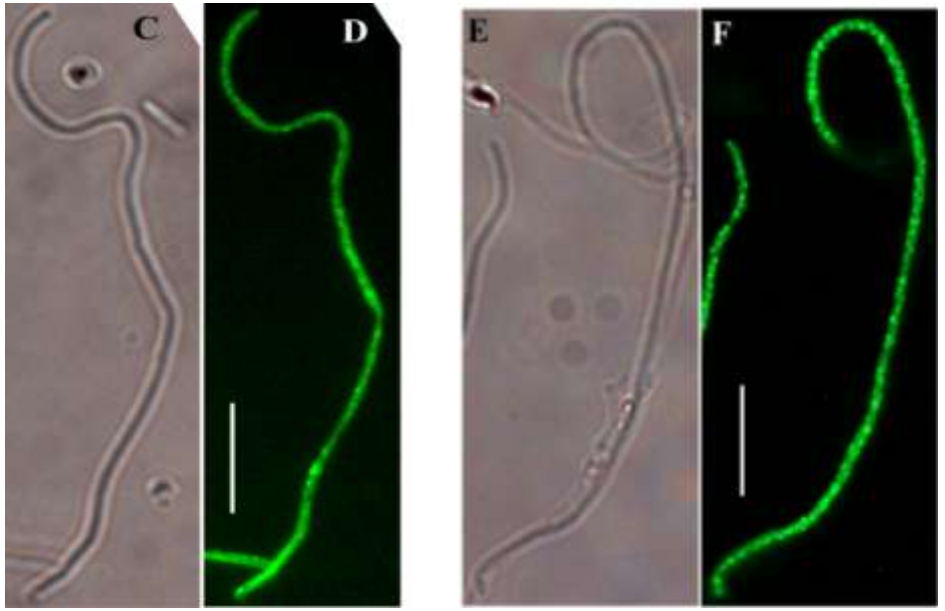


FtsW/I are required for sporulation septation

FtsZ::GFP in wild type

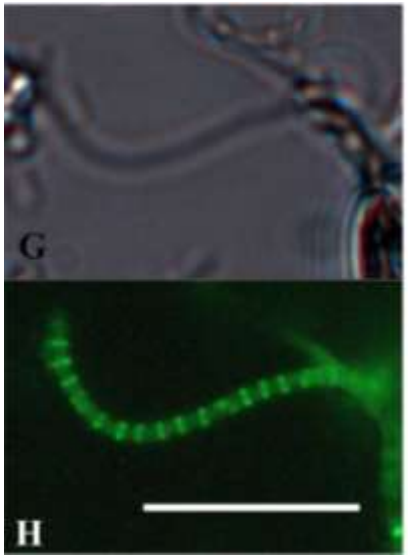


FtsZ::GFP in *ftsW* and *ftsI* mutants



- **No Z-rings formed in *ftsW* or *ftsI* mutants**

FtsQ is a late cell division protein



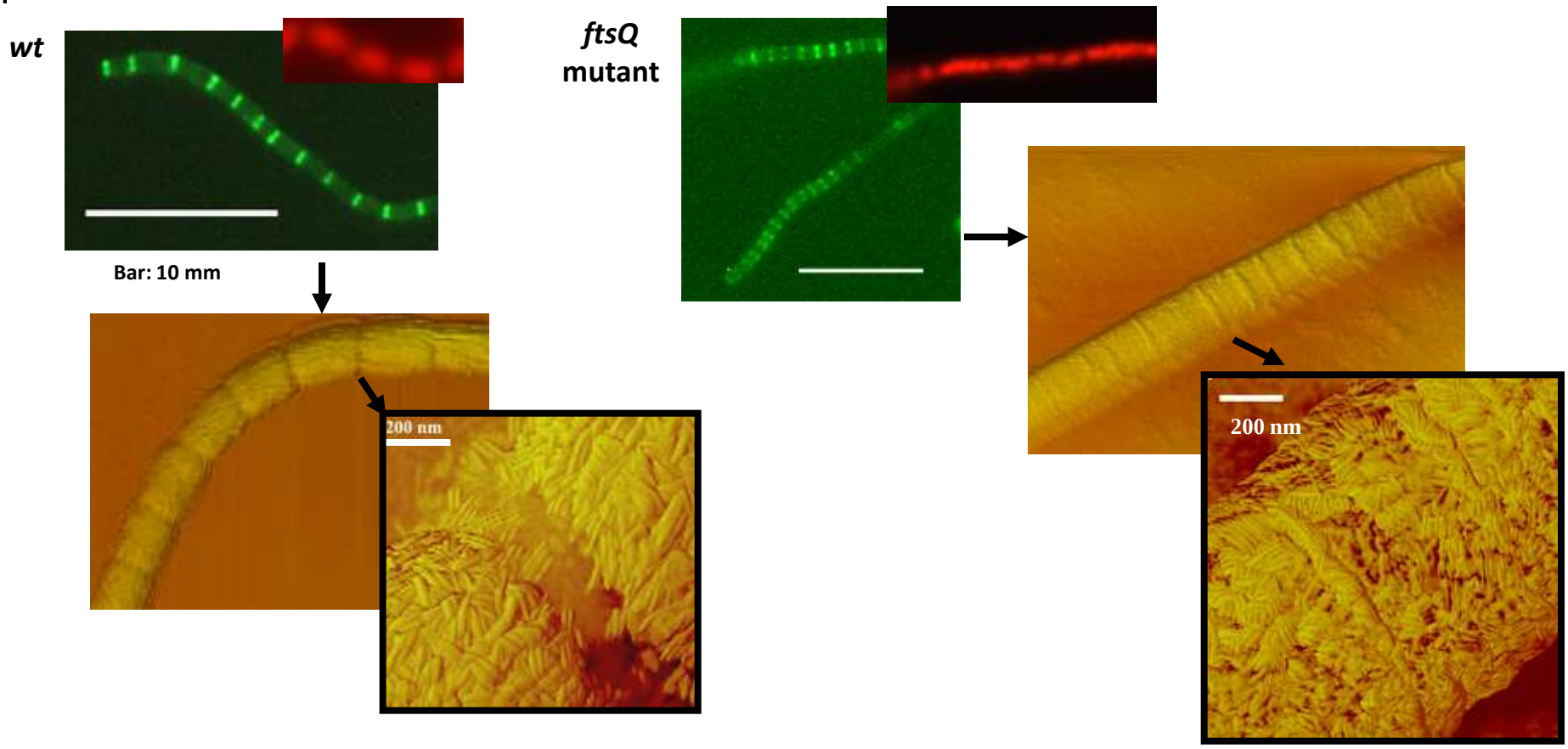
wt

ftsQ

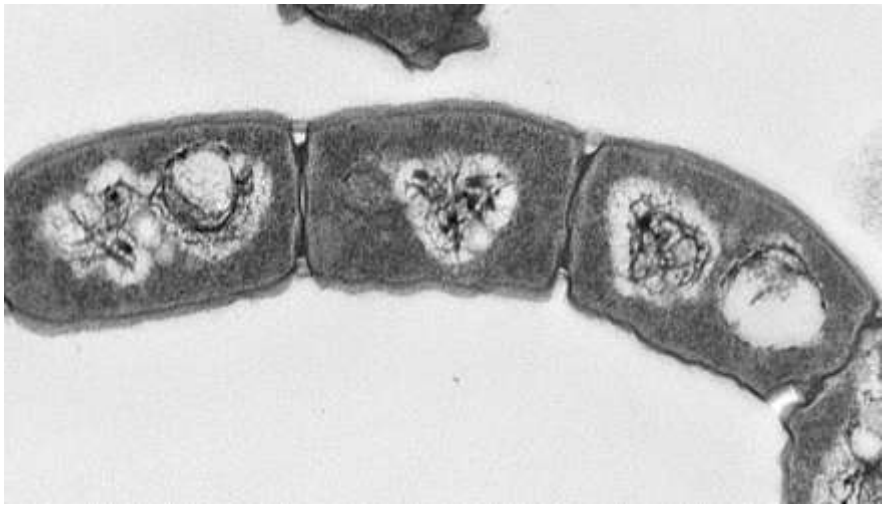
- White (defective in sporulation) phenotype
- Normal Z-ring formation

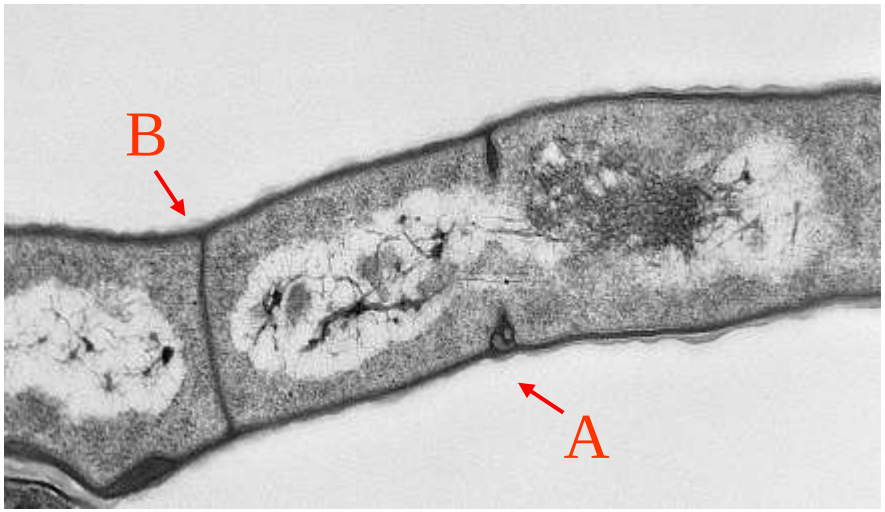
FtsQ is a late cell division protein

…but incomplete nucleoid segregation and annuli on hyphal surface indicate abnormal septation



FtsQ is a late cell division protein





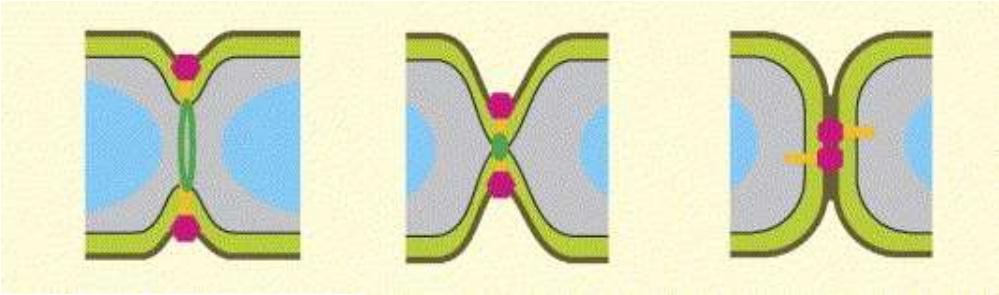
wt *ftsQ*

TEM of aerial hyphae reveals frequent aborted sporulation septa (A) and occasional complete vegetative cross-walls (B)

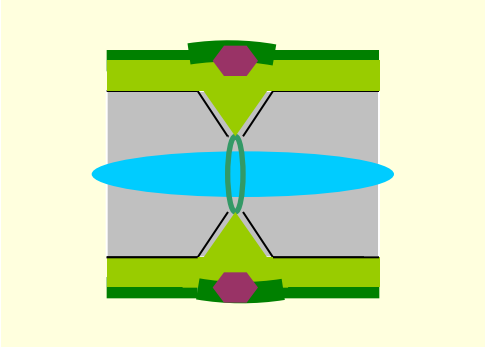
TEM courtesy of Kim Findlay, John Innes Centre, Norwich, UK

FtsQ is a late cell division protein

wt



ftsQ mutant



Septal peptidoglycan synthesis not linked with constriction of Z-ring

Inability to recruit FtsK and so uncoordinated nucleoid segregation ?

Cell division – looking inside

- Cell imaging using stains and tagged proteins can indicate functional roles of cell division proteins
- Complemented by ultrastructure imaging by AFM (external) and TEM (internal)
- FtsW/I has an ‘early’ cell division function, stabilising Z-rings
- FtsQ links septal peptidoglycan synthesis with Z-ring constriction
- Vegetative cross-walls do not require typical divisome function – sporulation is functionally equivalent to cell division in unicellular bacteria
- Sporulation septation is a good model system to study the function of ‘essential’ cell division proteins

Mistry BV, Del Sol R, Wright C, Findlay K, Dyson P. (2008) *Journal of Bacteriology* 190: 5555-66.

Dps proteins – protein nanocage assembly

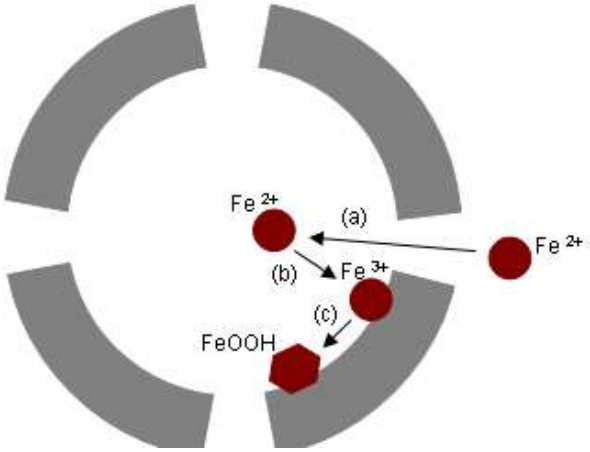
Dps proteins

- DNA-binding protein from starved cells
  - NAPs found in prokaryotes
  - Some able to bind and condense DNA
  - Classified as 'mini-ferritins'
- Structure and Function
  - Assemble into tetrahedral dodecamers with hollow cavity (12 subunits)
  - Possess ferroxidase activity and sequester iron in their cavities
    - Removes ferrous iron
    - Protects DNA and other macromolecules from hydroxyl radicals formed by Fenton reaction:

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + OH\cdot$$

Ferroxidase activity prevents the formation of oxidative radicals by sequestering Fe^{2+}

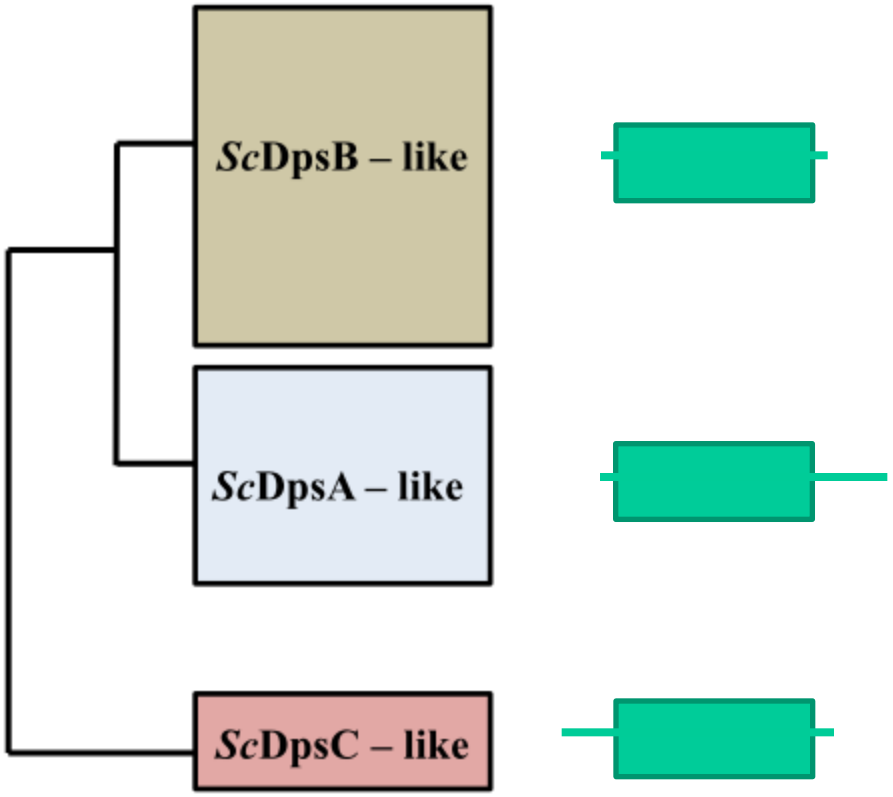
(a) Iron entry via ion pore
(b) Iron binding to ferroxidase site and oxidation
(c) Deposition for nucleation and mineralisation



Dimensions of dodecamers:

External diameter - 8 to 9 nm

Internal diameter - 4 to 5 nm



Schematic representation of phylogenetic relationship of ScDps proteins. The limited number of DpsC-like proteins and their thermophilic origin suggest a unique phylogenetic history for DpsC with mesophilic organisms acquiring a "thermophilic -adapted" gene.

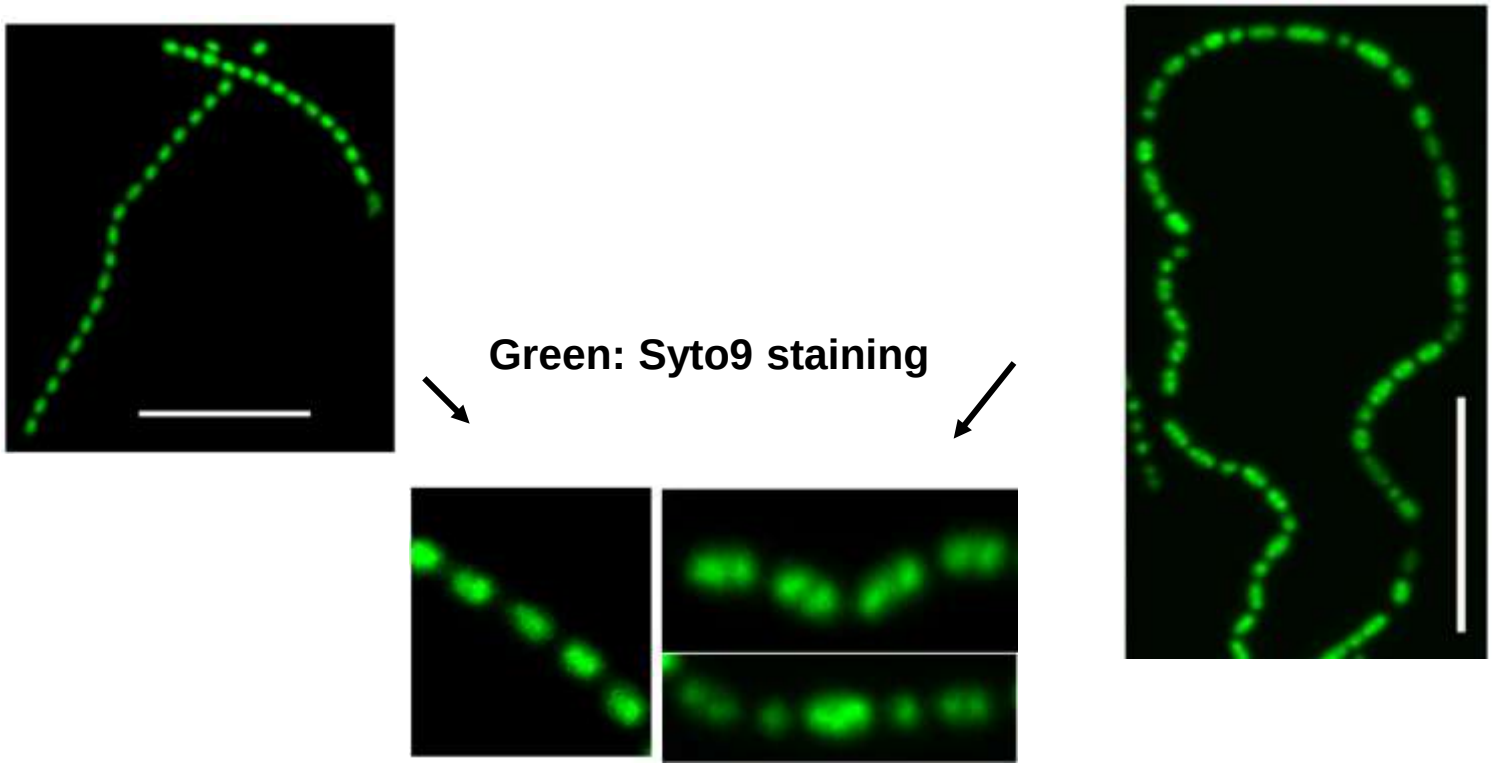
Dps clusters are congruent with tail length – even though the tails were deleted for phylogenetic analysis.

Facey PD, Hitchings MD, Williams JS, Skibinski DOF, **Dyson PJ**, and Del Sol, R (2013) The Evolution of an Osmotically Inducible dps in the Genus *Streptomyces*. PLoS ONE 8(4): e60772

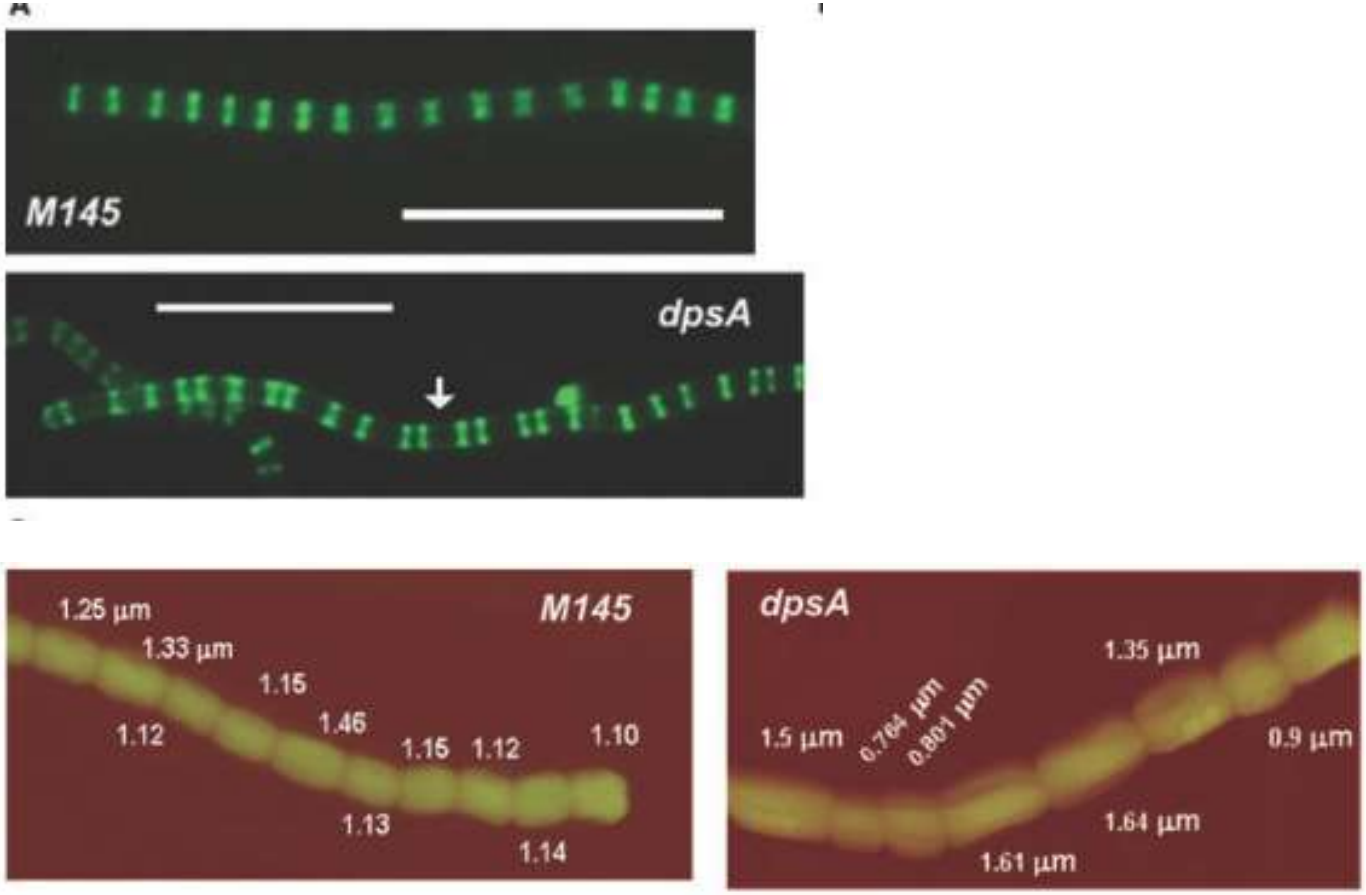
DpsA is needed for regular nucleoid condensation in *S. coelicolor*

M145

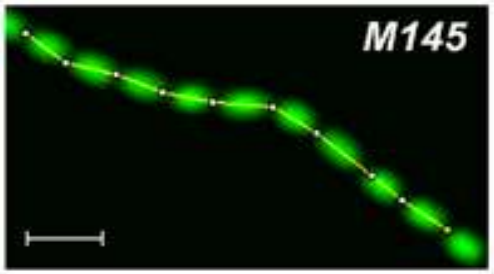
***dpsA*$^{-}$**

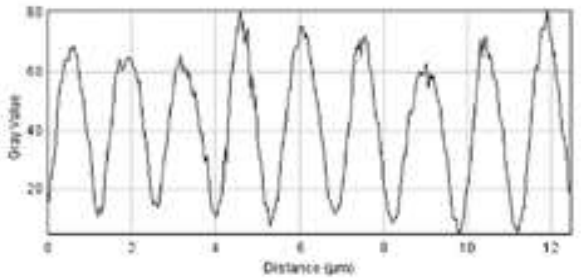


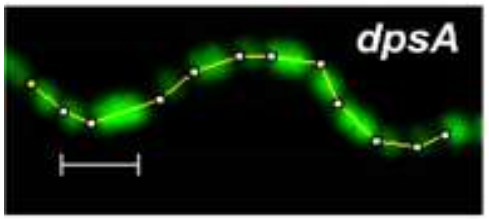
Nucleoid size is reflected in compartment size

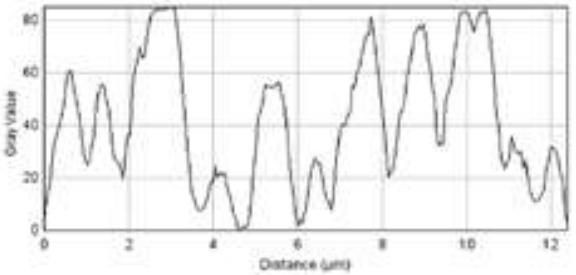


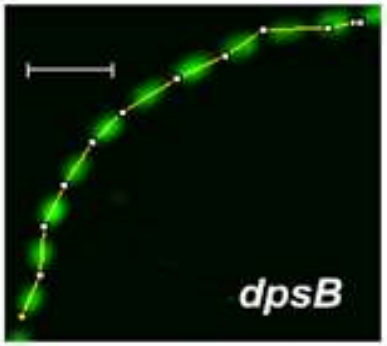
Nucleoid fluorescence profiles of *dps* mutants

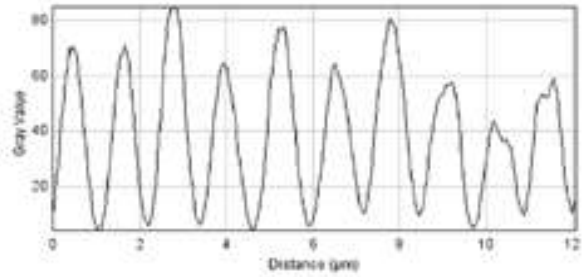


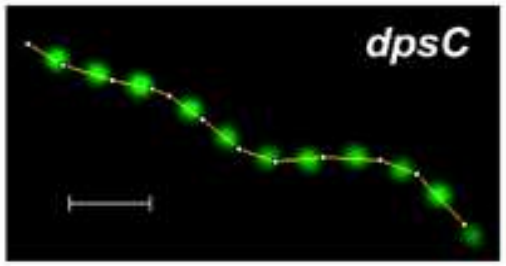


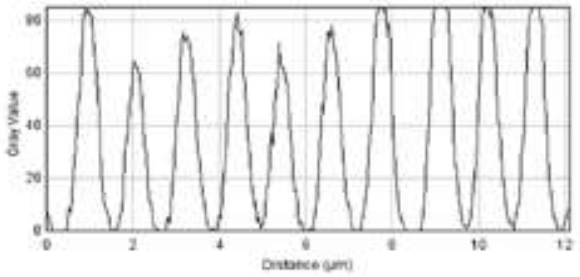




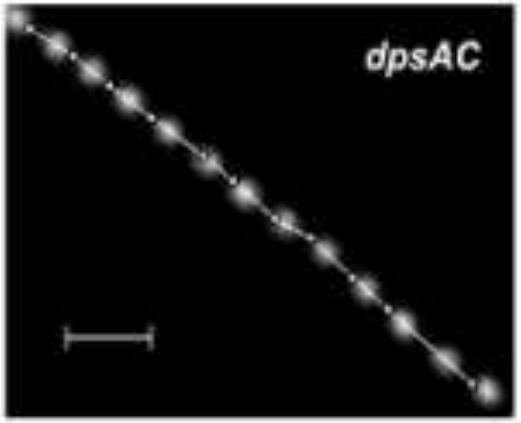


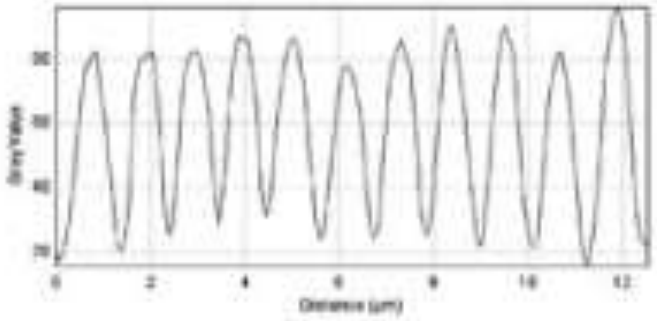


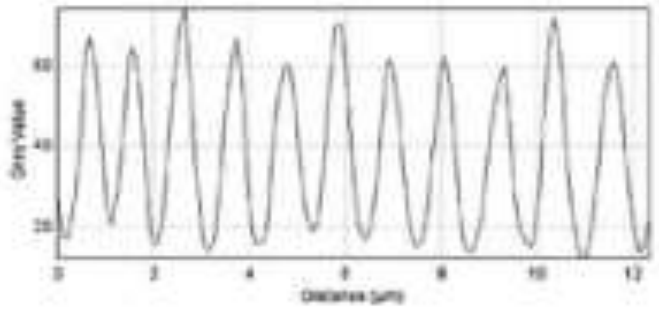




Regularity is restored in double mutants





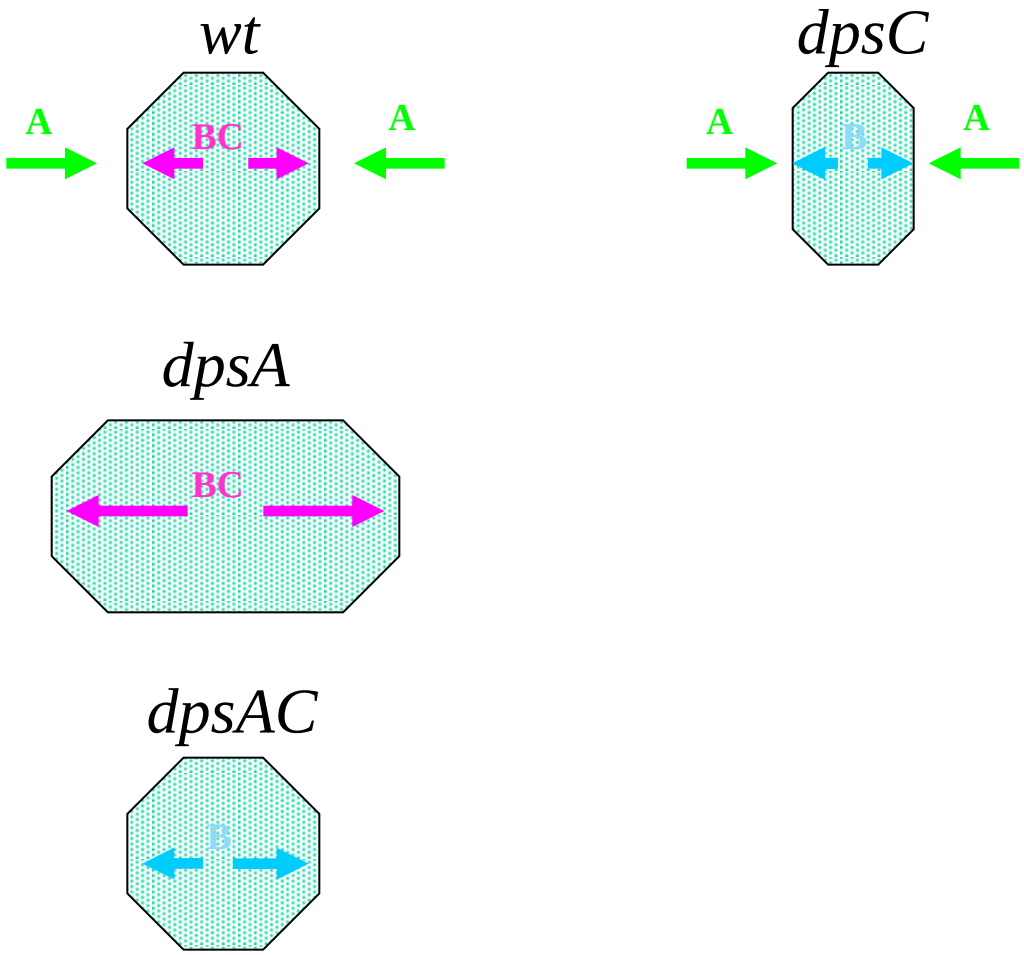


Nucleoid compaction summary

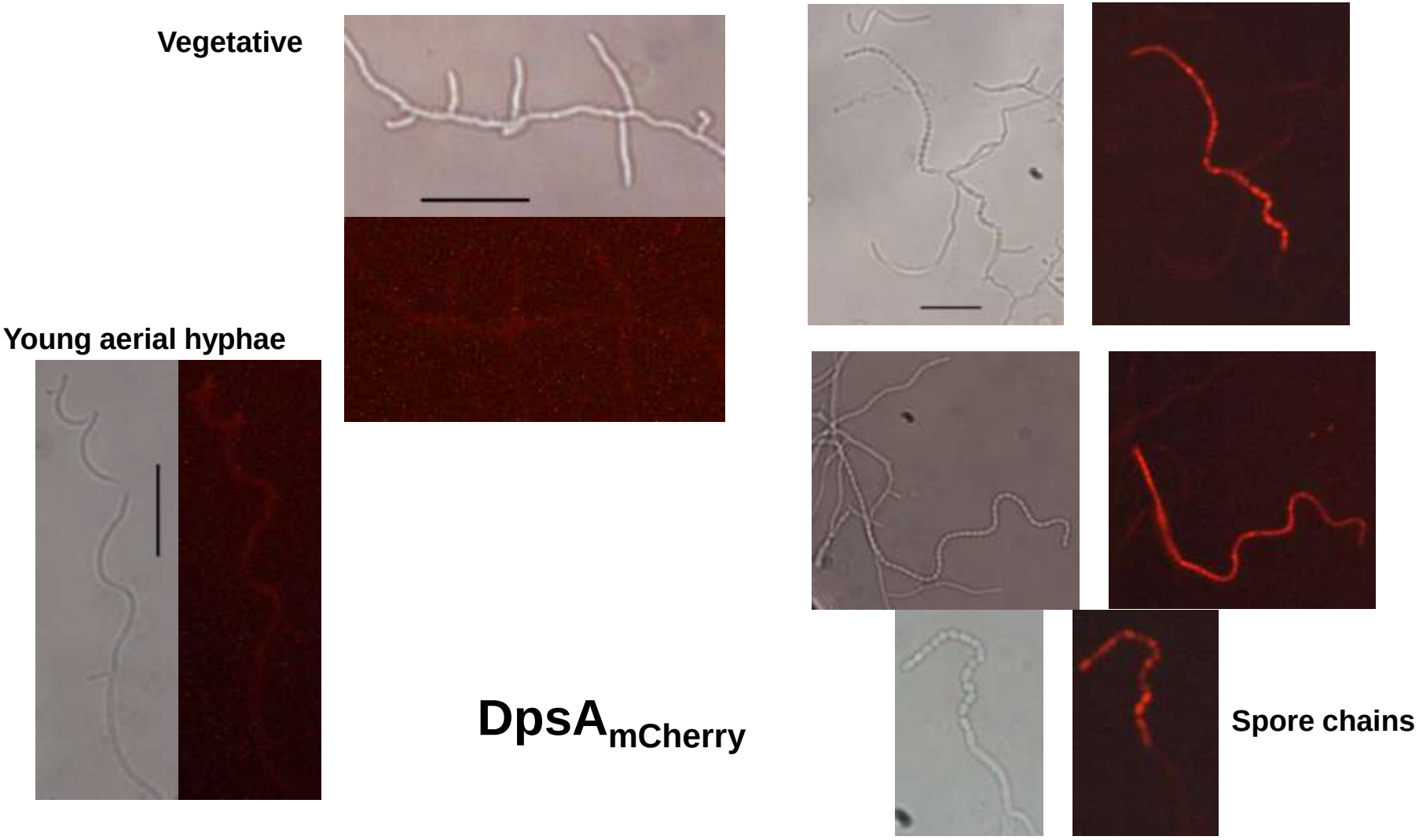
- Loss of DpsA disrupts regular nucleoid compaction, segregation and septation
- Loss of DpsC and, to some extent DpsB, results in highly compacted nucleoids
- *dpsB/dpsC* mutations are ‘dominant’ over the *dpsA* mutation

Facey PD, Hitchings MD, Saavedra-Garcia P, Fernandez-Martinez L, Dyson PJ and Del Sol R (2009) *Molecular Microbiology* 73: 1186–1202

DpsBC modulate DpsA function?

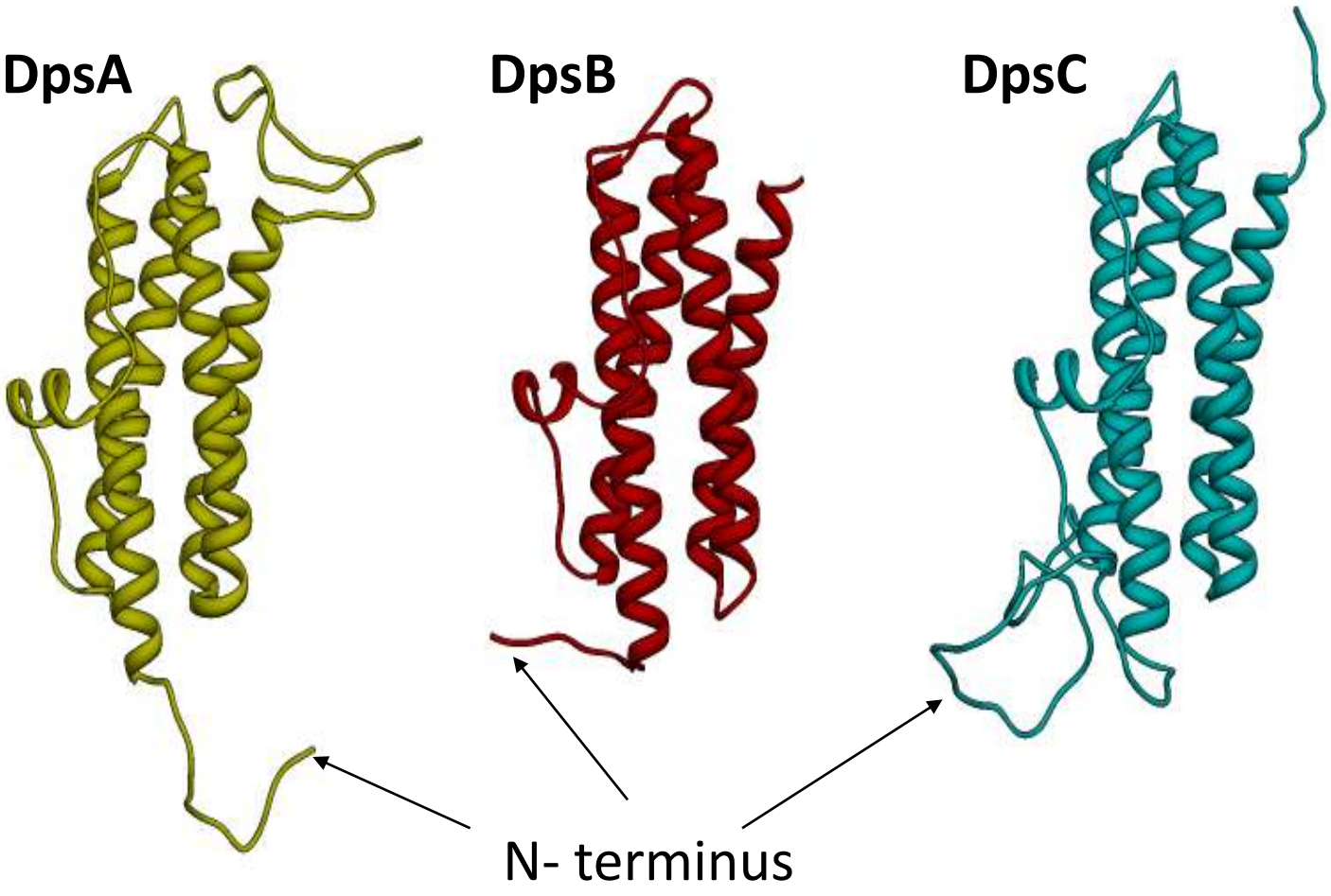


Regulation: DpsA is expressed during aerial development

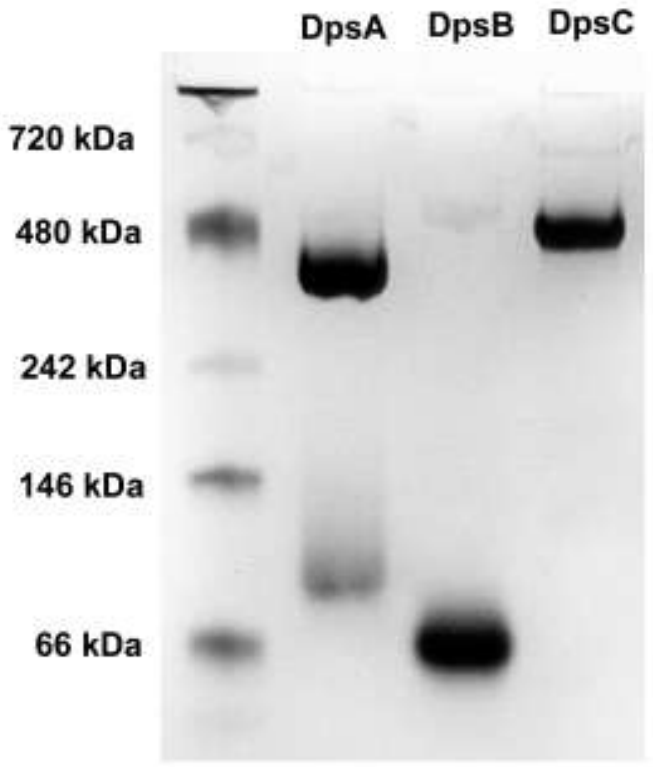


Facey PD, Sevcikova B, Novakova R, Hitchings MD, Crack J, Kormanec J, Dyson PJ, Del Sol R. (2011) The *dpsA* gene of *Streptomyces coelicolor*: induction of expression from a single promoter in response to environmental stress or during development. *PLoS ONE*, e25593

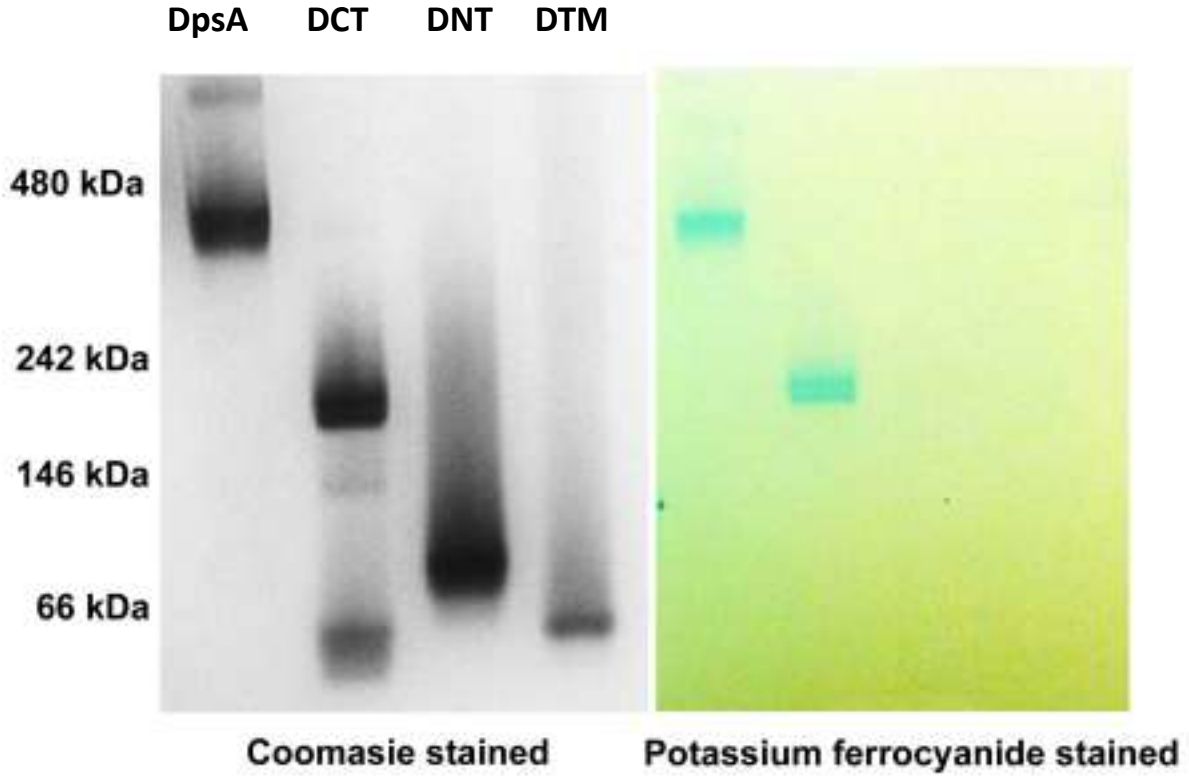
S. coelicolor monomeric Dps structures



Does the presence of ‘tails’ influence assembly of dodecamers?



Removing ‘tails’ influences assembly of dodecamers



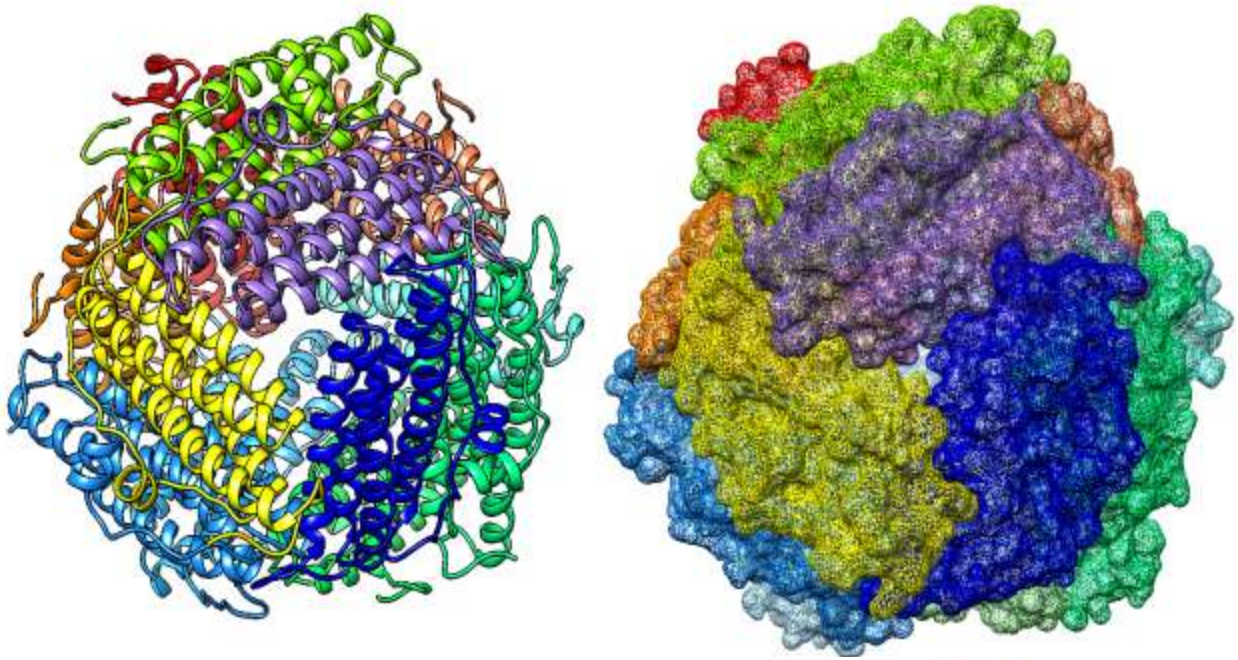
All proteins retain ferroxidase activity but only DpsA and DpsA-DCT can sequester iron as they have iron-binding cavities

Imaging the function of Dps tails – X-ray crystallography

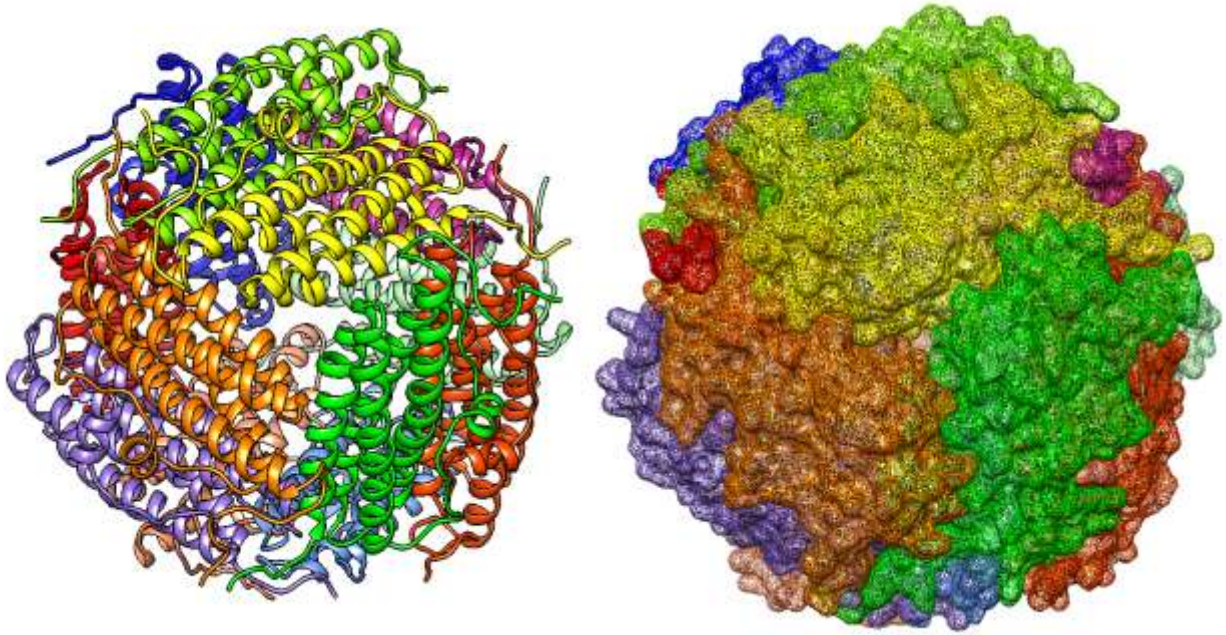
DpsA

DpsC

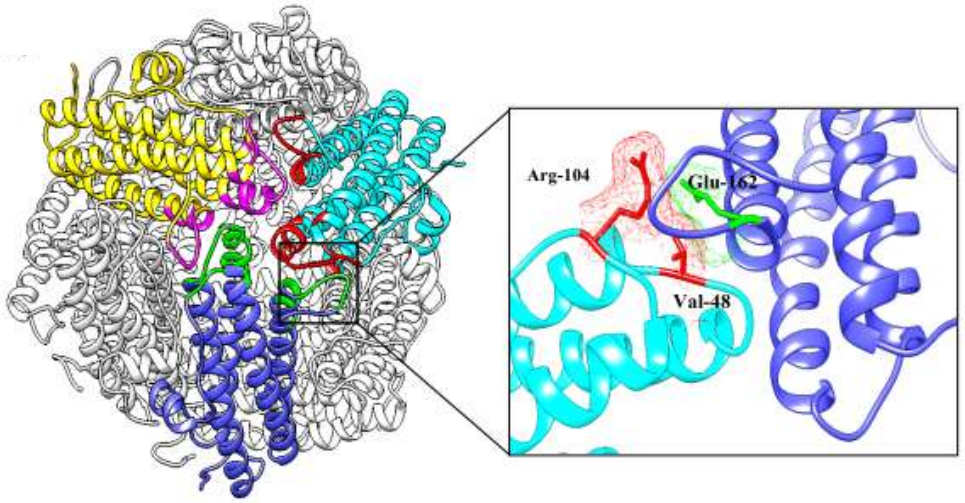
DpsA



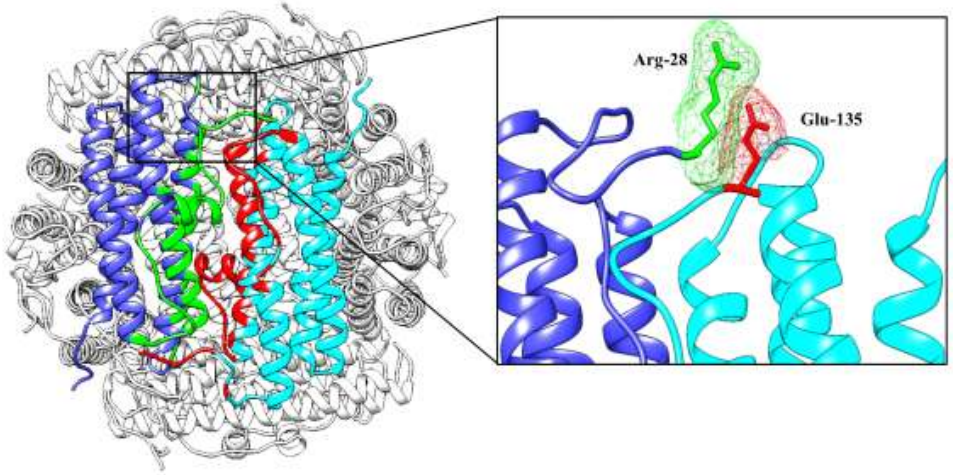
DpsC



The tails of each dimer enfold their partner, acting as braces to strengthen the interface



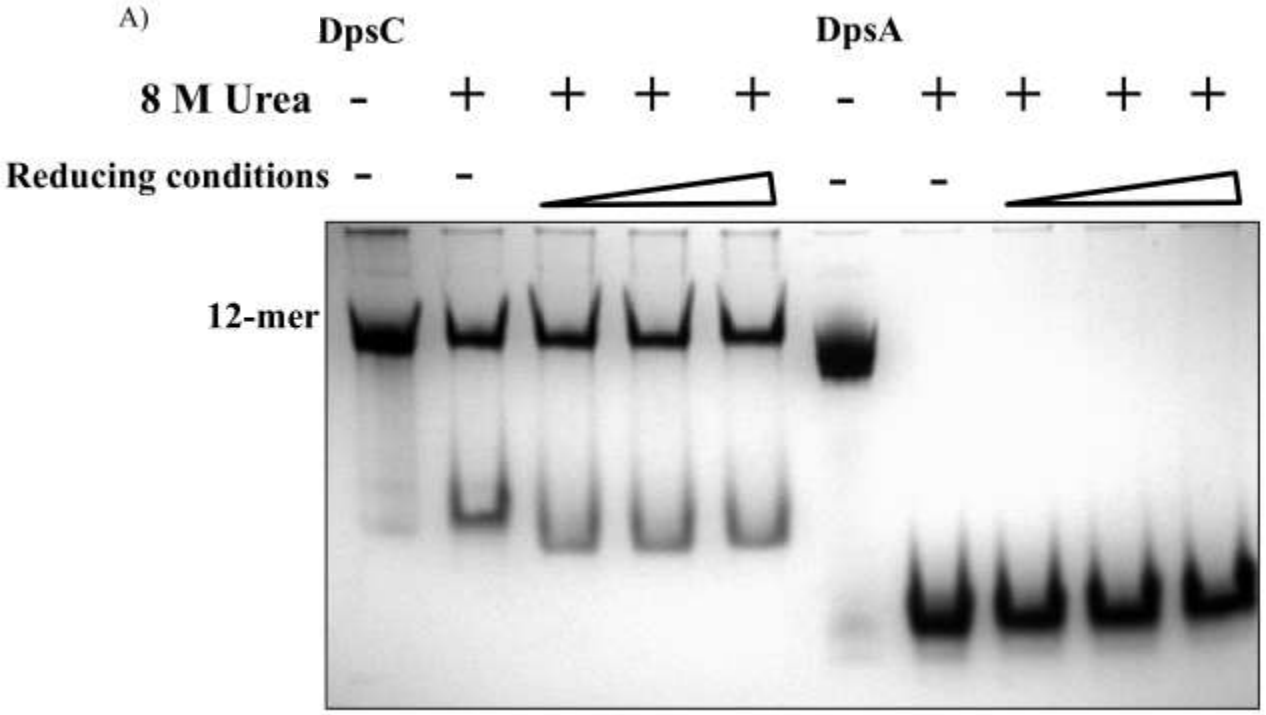
Interfacing residue of DpsA C-tail Glu-162 (green) interfacing with Val-48 and Arg-104 (red) at the C-terminal trimer interface. Hydrophilic interactions occur between Glu-162 and Arg-104 to strengthen the interface.



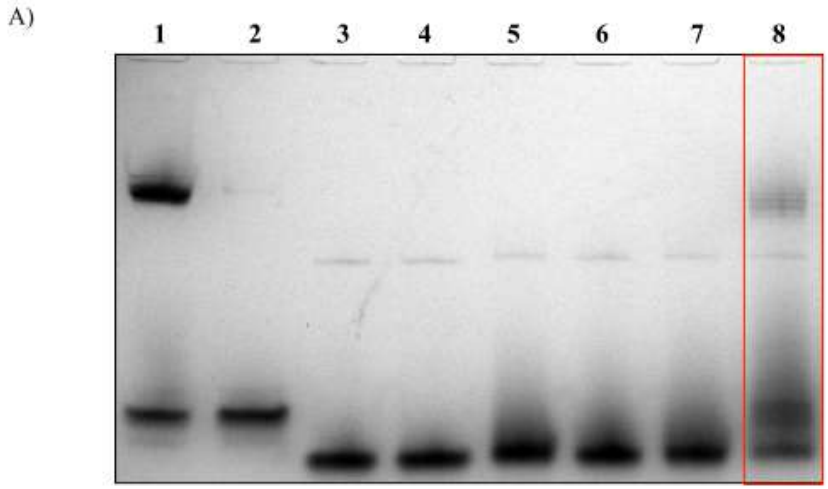
Antiparallel dimer of DpsC. Residue Arg-28 (green) of the N-tail interfaces with Glu-135 (red) of the adjacent monomer creating an environment with distances conducive to the formation of hydrophilic interactions.

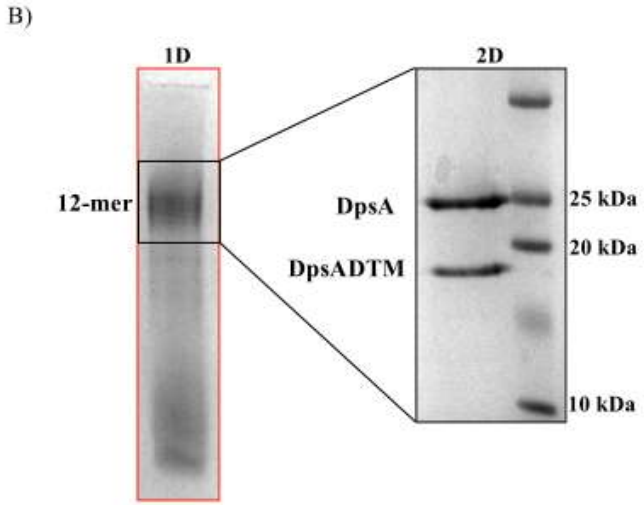
A team huddle showing unity of purpose, strength and resolve

DpsC dodecamers are braced very well!

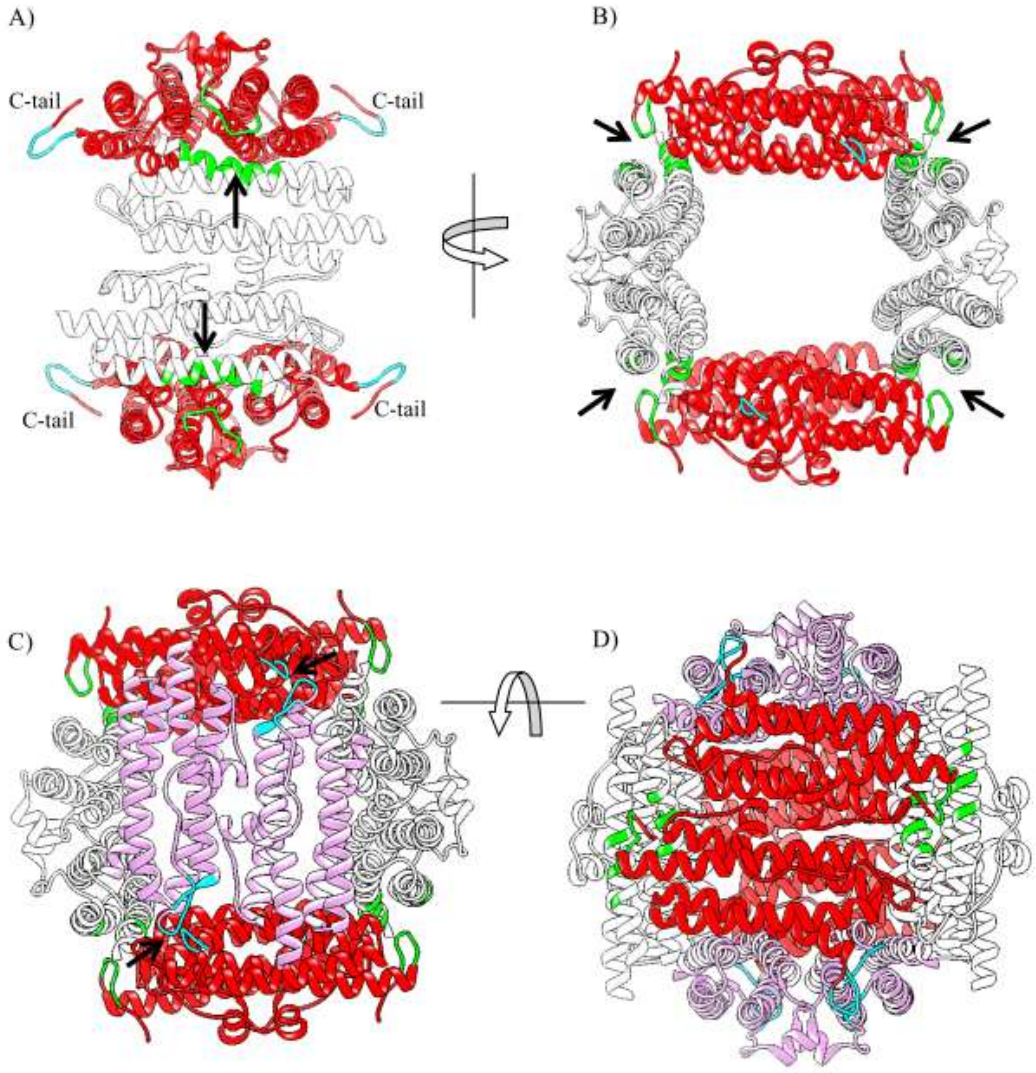


Hetero-oligomeric assembly of DpsA and DpsADTM

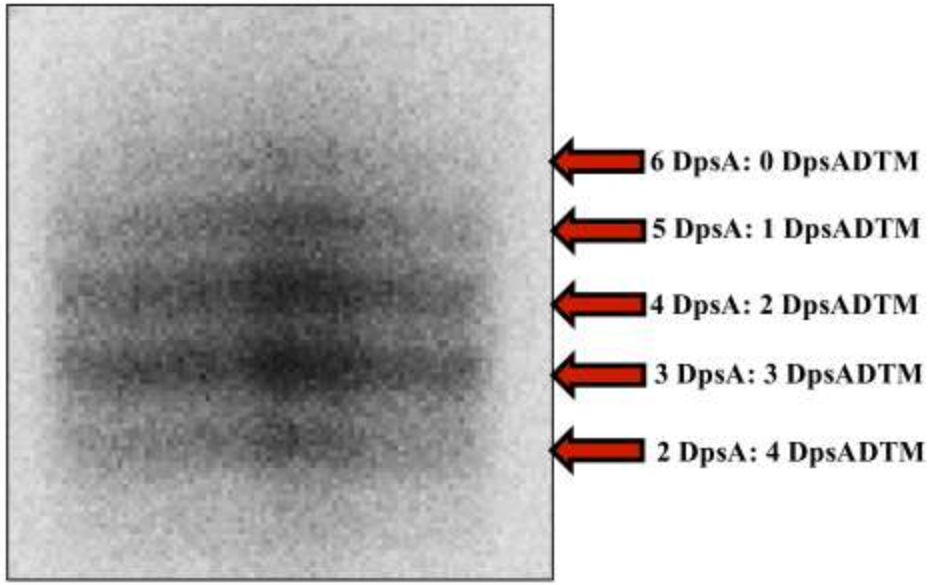




The minimum number of full length DpsA dimers required to incorporate DpsADTM into a dodecamer can be represented using a molecular model of the assembly

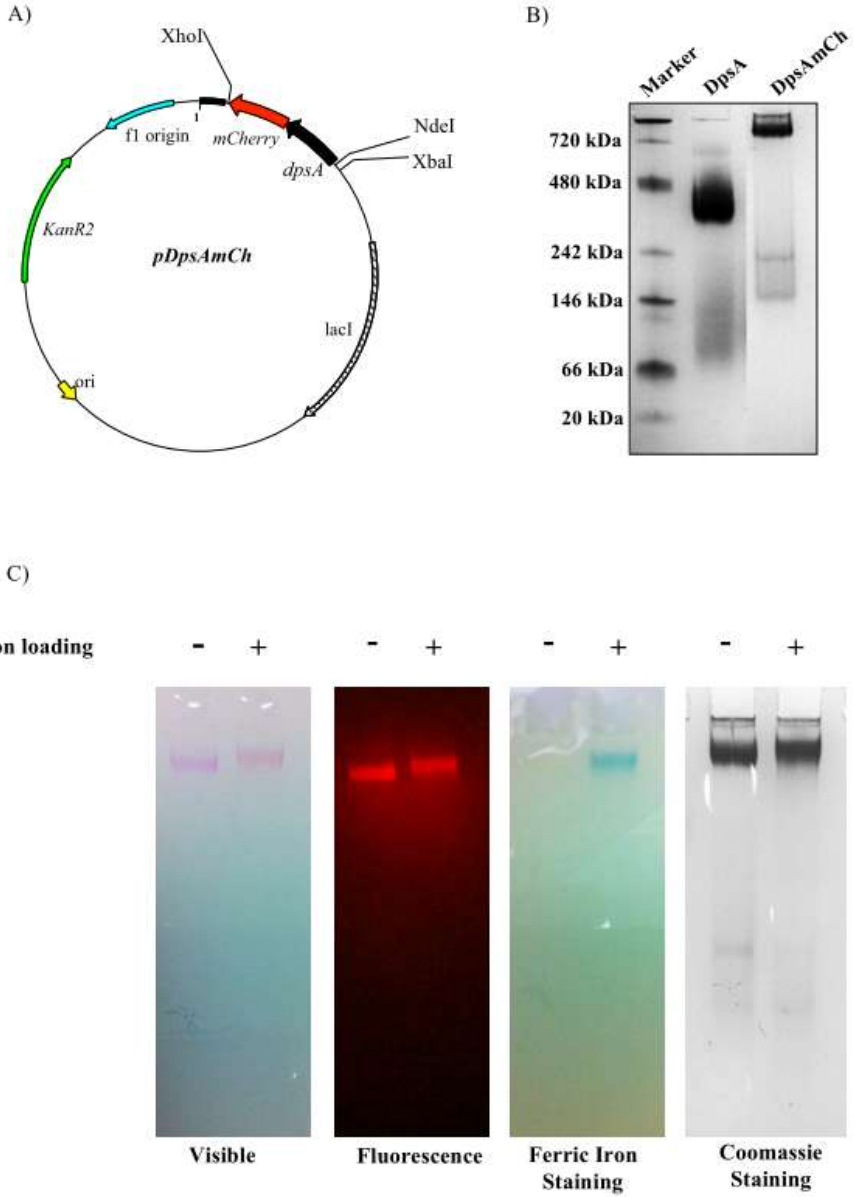


Two DpsA dimers (red) are required to provide tails, which stabilise a total of 4 DpsADTM dimers (white and pink).

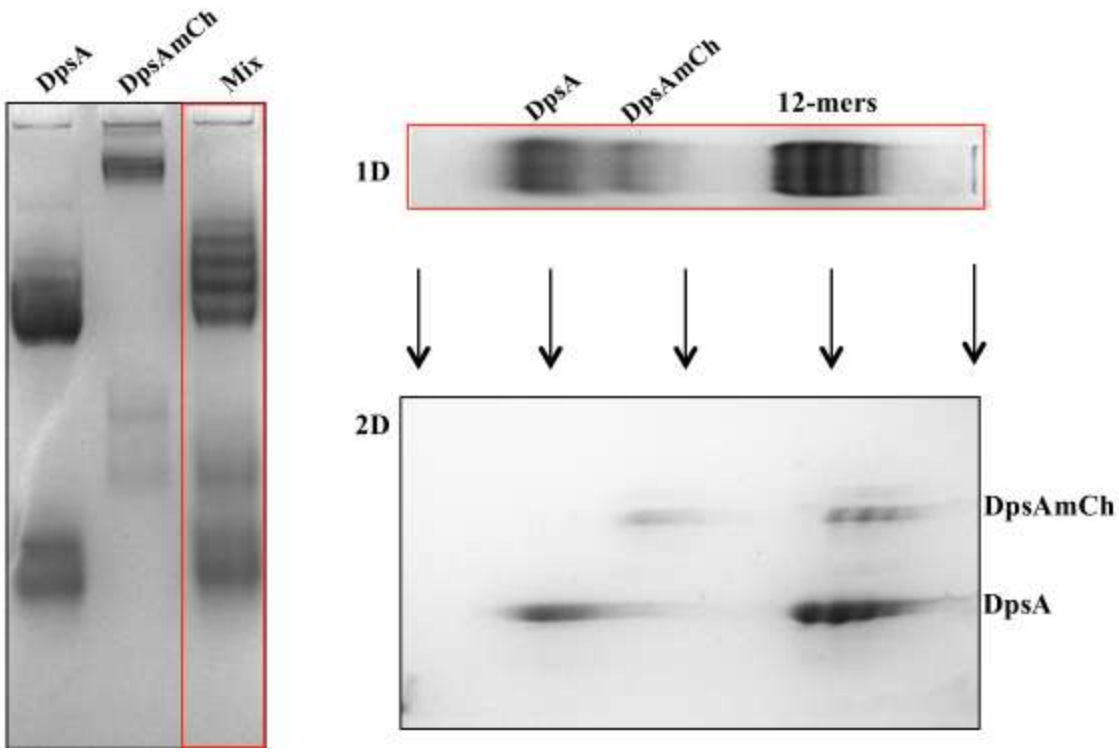


Native PAGE resolved DpsA/DpsADTM hetero-oligomeric assembly displays 5 different bands indicative of the ratios of DpsA to DpsADTM dimers within the dodecamers

Assembly and functional analysis of DpsAmCh functionalised nanocage



Hetero-oligomeric assembly of DpsA and DpsAmCh



Dps proteins – protein nanocage assembly

- *S. coelicolor* has 3 Dps proteins
- Phenotypic analysis indicates they function in concert to regulate nucleoid compaction during sporulation
- DpsA and DpsC can both self-assemble into protein nanocages
- Self-assembly and stability are determined by N- and C-terminal tails that act as molecular braces
- Exploiting the role of tails permits assembly of hetero-oligomers and functionalised nanocages

A tale of tails: deciphering the contribution of terminal tails to the biochemical properties of two Dps proteins from *Streptomyces coelicolor*.
Hitchings MD, Townsend P, Pohl E, Facey PD, Jones DH, Dyson PJ, Del Sol R (2014)
Cellular and Molecular Life Sciences DOI:10.1007/s00018-014-1658-4

diolch - thanks - hvala !

Paul Facey
Lorena Fernandez
Meirwyn Evans
Ricardo Del Sol Abascal
Bhavesh Mistry
Chris Wright
Matt Hitchings

BBSRC
bioscience for the future

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