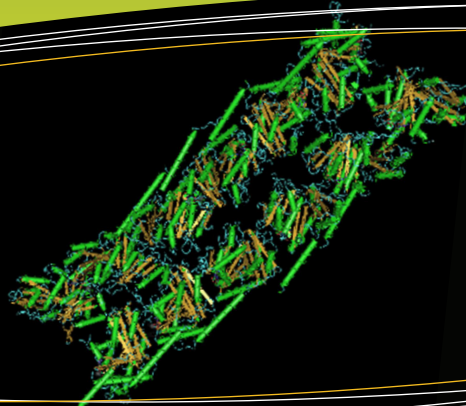




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FtsZ: a tubulin homolog and novel antibiotic target

The tubulin homolog FtsZ protein (Filamenting temperature-sensitive mutant Z) is essential for bacterial cell division and an ideal target for novel anti-microbials. Mutants lacking this protein do not divide, but continue to elongate into filaments. FtsZ is a GTPase that polymerizes in a nucleotide-dependent manner head-to-tail to form single-stranded filaments that assemble into a contractile ring. The ring is called the Z-ring and forms on the inside of the cytoplasmic membrane where it marks the future site of the septum of a dividing bacterial cell. Although FtsZ polymerization rapidly reaches steady state, the Z-ring is dynamically maintained through the course of cell division by continuous and rapid turnover of FtsZ polymers, likely fueled by FtsZ's GTP hydrolysis (1-3). FtsZ is the first protein to localize at the division site and recruits other proteins involved in bacterial cell division. Besides serving as a scaffold for the other cell division proteins, FtsZ itself may exert cytokinetic forces that lead to cell division (1-6).

FtsZ is considered a prokaryotic homolog to the eukaryotic protein tubulin (5-11). Both tubulin and FtsZ contain a GTP-binding domain (7-11), have GTPase activity (1-3,12), assemble into protofilaments, two-dimensional sheets, and protofilament rings (1-3,5,6) and share substantial structural identities (5-11). Despite these parallels, FtsZ and tubulin only share 10-18% sequence similarity (1,13,14) and the basic subunit of FtsZ is a monomer whereas the tubulin subunit is an alpha and beta heterodimer (1-3). Also, the necessity of GTP hydrolysis for FtsZ assembly *in vitro* varies with experimental conditions (5,14-16); a FtsZ mutant that assembles, but lacks GTPase activity, leads to altered cellular phenotype and function *in vivo* (17).

Recently, FtsZ has become the focus of antibiotic research as a novel target for new anti-microbials (18-23). The unprecedented increase in antibiotic-resistant pathogens and lack of new antibiotic development highlights the need for new anti-microbials active against novel targets such as bacterial cell division proteins (20,24). The amino-acid sequence identity between different FtsZ species is 35 to 99%, and most commonly 40 to 60% (see www.cytoskeleton.com/new-products for an homology database). This level of identity affects drug discovery in two main ways: 1. It is unlikely

that a broad spectrum anti-bacterial FtsZ ligand can be identified; and, 2. Using one FtsZ protein target will likely generate a highly specific drug to that species. Indeed, Haydon et al (25,26) reported that PC190723, a FtsZ inhibitor, affected FtsZ from *Staphylococcus aureus* and *Bacillus subtilis* (70% identity to each other), but not from *Escherichia coli* (51% and 47% identity, respectively; Fig. 1).

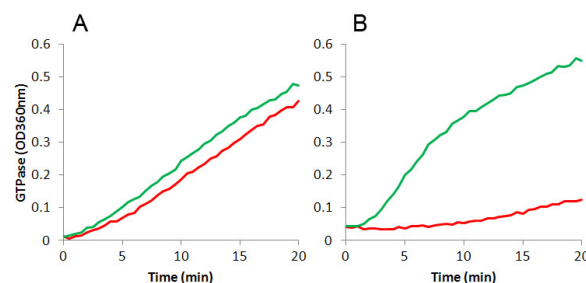


Figure 1 - Polymerization of 0.5 mg/ml FtsZ protein from *E. coli* (panel A) and *S. aureus* (panel B) in the presence of 15 μ M FtsZ inhibitor PC190723 (red line) or HEPES250 buffer (green line). GTPase activity was measured with a MESG-based assay (Cat. # BK052). See references (25-27) for additional details.

Several types of assays have been used to measure FtsZ polymerization, including GTPase, fluorescence quenching, FRET, sedimentation and light scatter assays. The requirements for drug screening applications are best served by the GTPase assay and fluorescence quenching formats (for more information, see www.cytoskeleton.com/new_products). Protein interaction studies utilize sedimentation and GTPase assays.

Despite the many recent advances in our understanding of FtsZ, many questions remain, including: (i) is the Z-ring a force-generating structure or simply a scaffold to localize the proteins involved in cell division; (ii) what is the role of nucleotide hydrolysis in cell division; (iii) where does hydrolysis occur within the filament;

Top Center Image:

FtsZ polymer adapted from: Aylett et. al. (2010). *PNAS*, 107, 19766-19771.

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(Cont'd from Pg 1)

(iv) does nucleotide exchange occur in polymers, monomers or both; and (v) what is the nature of the lateral interactions between FtsZ filaments (3)? Additionally, reports of treadmilling (2) and dynamic instability (28) in FtsZ polymerization warrant further study.

For additional information about FtsZ, a table of FtsZ-relevant assays and sequence homologies between different FtsZ proteins, please see www.cytoskeleton.com/new-products.

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New FtsZ Protein Tools

NEW

FtsZ proteins are the bacterial homologs of tubulin and are essential for bacterial cell division. FtsZ inactivation inhibits cell division, making them attractive targets for novel anti-microbial drugs. Although FtsZ proteins exhibit a degree of homology, inhibitors of the proteins show differential affinities and efficacies. Thus, improved targeting can be achieved by screening several FtsZ proteins from different species. To improve drug screening of species-selective anti-microbials, Cytoskeleton is introducing FtsZ proteins from *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Enterococcus faecalis*. Other species' FtsZ proteins are available upon request. Please e-mail tservice@cytoskeleton.com for more information.

Crystal structure of FtsZ protein
Adapted from: Lowe & Amos (1998). *Nature*, 391, 203-206.



FtsZ species	% Homology to <i>E. coli</i>	Cat. #
<i>Escherichia coli</i>	100	FTZ01, FTZ05
<i>Salmonella enterica</i>	99	NA
<i>Staphylococcus aureus</i>	47	FTZ02
<i>Streptococcus pneumoniae</i>	50	FTZ03
<i>Enterococcus faecalis</i>	49	FTZ04

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FtsZ Proteins

FtsZ Proteins	Source	Purity	Cat. #	Amount
FtsZ Protein	<i>E. coli</i> , recombinant untagged	>85%	FTZ01-A FTZ01-B	1 x 1 mg 5 x 1 mg
NEW FtsZ Protein	<i>S. aureus</i> , recombinant 6xHis-tagged	>90%	FTZ02-A FTZ02-B FTZ02-DX	1 x 1 mg 5 x 1 mg 1 x 10 mg
NEW FtsZ Protein	<i>S. pneumoniae</i> , recombinant 6xHis-tagged	>90%	FTZ03-A FTZ03-B FTZ03-DX	1 x 1 mg 5 x 1 mg 1 x 10 mg
NEW FtsZ Protein	<i>E. faecalis</i> , recombinant 6xHis-tagged	>90%	FTZ04-A FTZ04-B FTZ04-DX	1 x 1 mg 5 x 1 mg 1 x 10 mg
NEW FtsZ Protein	<i>E. coli</i> , recombinant 6xHis-tagged	>90%	FTZ05-A FTZ05-B FTZ05-DX	1 x 1 mg 5 x 1 mg 1 x 10 mg