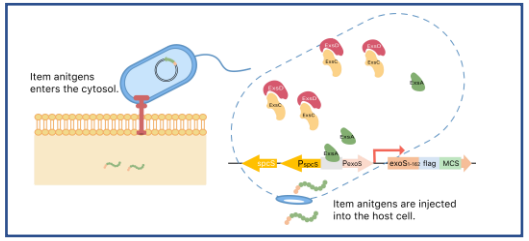
Rosetta@Common & Type three secretion system: T3SS



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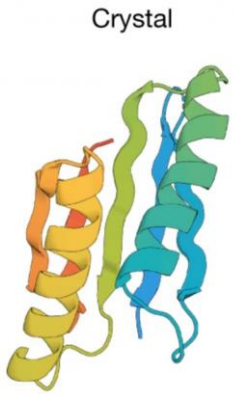
Date: January 06, 2022

Rosetta common

Rosetta@home: *distributed computing project*



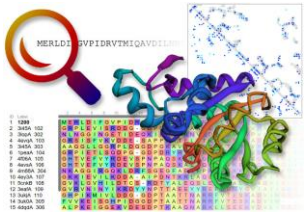
Baker Lab
UW INSTITUTE FOR PROTEIN DESIGN

UNIVERSITY OF WASHINGTON 1861

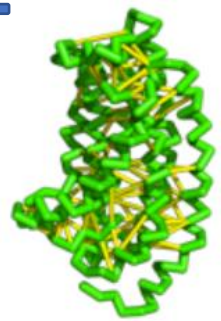
Rosetta@Home

fold it BETA
Solve Puzzles for Science

flodit



Robetta



GREMLIN

- **55000** active volunteered computers processing **487,946** GigaFLOPS; Sep 2020
- Oriented toward basic research to improve the accuracy and robustness of proteomics methods,
- Rosetta@home also does applied research on malaria, Alzheimer's disease, and other pathologies.

- Rosetta@home uses **idle computer processing**
- Assimilated into project databases.
- Individual protein structure prediction on the **Rosetta@home screensaver.**

https://www.bakerlab.org/
https://www.rosettacommons.org/

Rosetta common

Types of Biological Problems

01. Protein Structure Prediction

- *De Novo Modeling*
- *Comparative Modeling (Homology Modeling)*
- *Specialized Protocols*

02. Protein–Protein Docking

- Docking Two Partners With Known Structures
- Docking According to the Lock and Key Model
- Docking According to the Conformer Selection Model
- Docking According to the Induced Fit Model
- Docking According to the Conformer Selection and Induced Fit Model

- Docking Two Partners Where One Structure Is Unknown
- Docking Two Partners With Two Unknown Structures
- Docking Homooligomers

03. Protein–Peptide Docking

04. Protein–Ligand Docking

05. Protein Design

- Protein Redesign
- Protein Interface Design
- Enzyme Design

06. Protein Loop Modeling

- Modeling Loops in Regions of Low Electron Density
- Modeling Loops in Regions of Low Homology or with No Secondary Structure
- What if I am modeling a protein with a disordered region?

07. Nucleic Acids modeling

08. Solving Crystal Structures

09. Solving NMR structures

https://new.rosettacommons.org/docs/latest/getting_started/Getting-Started

Rosetta common

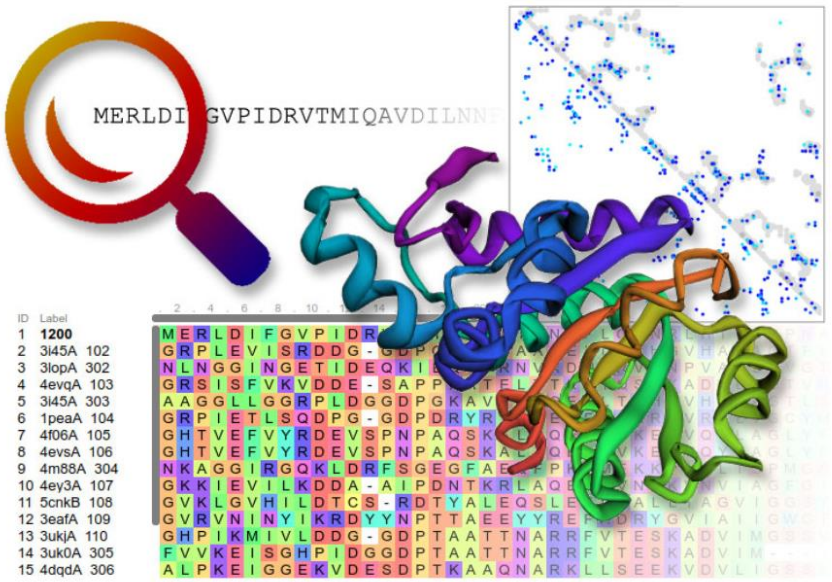
ab initio folding and structure prediction

Robetta Project Structure Prediction Register Login

Robetta is a protein structure prediction service that is continually evaluated through CAMEO

Features include relatively fast and accurate deep learning based methods, RoseTTAFold and TrRosetta, and an interactive submission interface that allows custom sequence alignments for homology modeling, constraints, local fragments, and more. It can model multi-chain complexes using RoseTTAFold (user must provide paired MSA) or comparative modeling (CM) and provides the option for large scale sampling. The CM method uses the PDB100 template database, which is updated weekly, a co-evolution based model database (MDB), and also provides the option for custom templates. Computing resources are provided by the Baker lab, HHMI's Janelia Research Campus, and by volunteers from the distributed computing project Rosetta@home. You can help this service by joining Rosetta@home.

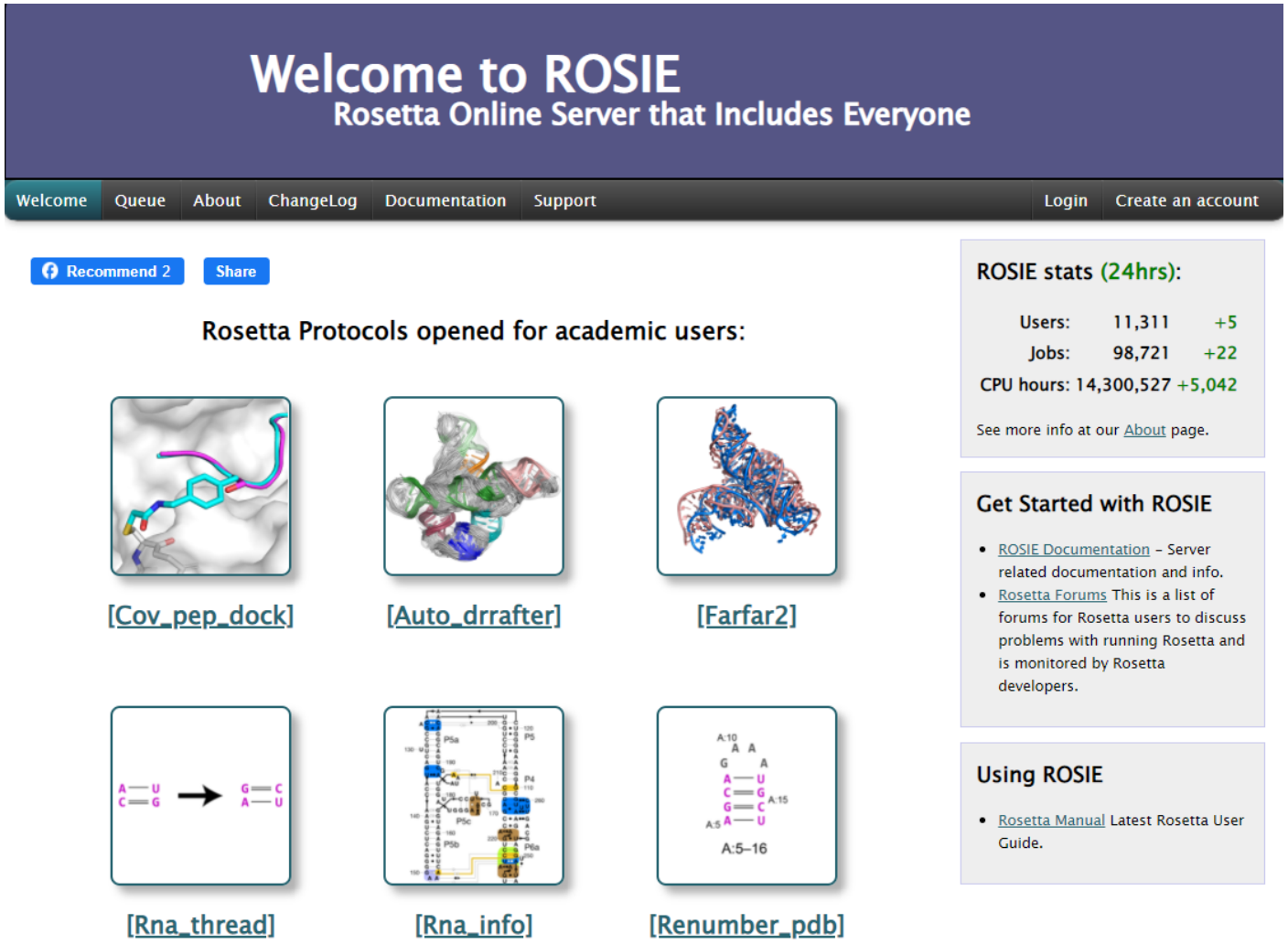
For more information please visit our Frequently Asked Questions.



https://rosie.rosettacommons.org/

Rosetta common

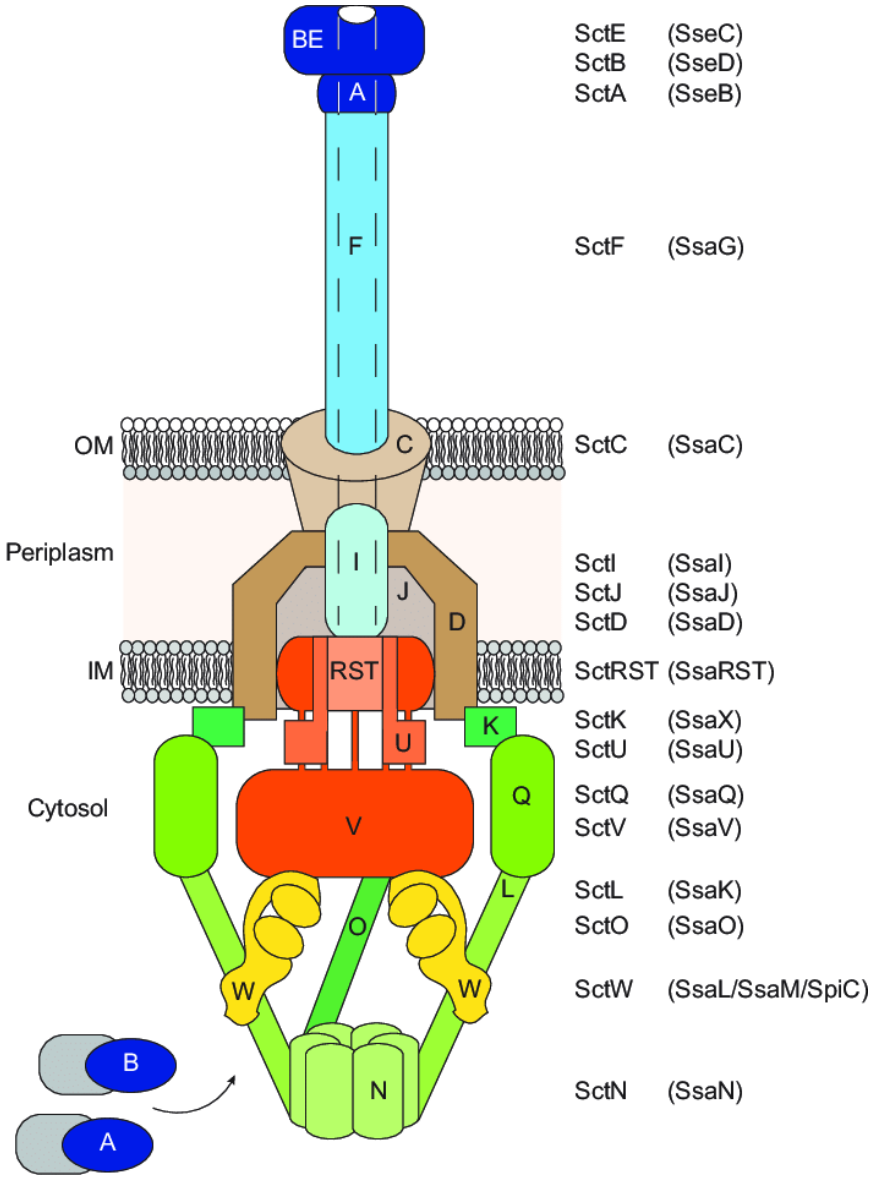
Rosetta Online Server



https://rosie.rosettacommons.org/

Type three secretion system

- **Type three secretion system** is a protein appendage found: gram – (ve) bacteria.
- Alternative names:
  - Type III secretion system; TTSS or **T3SS**
  - **Injectisome**
- **Needle-like structure** is used as a **sensory probe**;
  - **detect** the **presence of eukaryotic organisms**
  - **secrete proteins (effector)** that help bacteria **infect** them.
- **effector proteins** secreted **directly from the bacterial cell into** the **eukaryotic (host) cell**,
- where they **exert** a number of **effects**; help **pathogen to survive** & escape **immune response.**



Schematic drawing of T3SS DOI:10.1128/mBio.01149-18

Type three secretion system; Questions

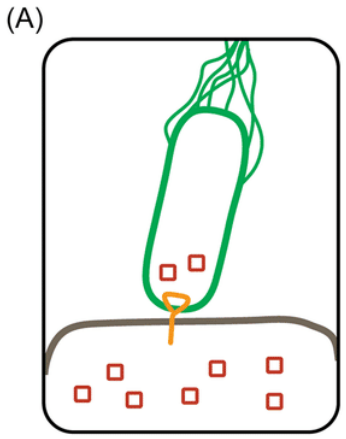
(01)The **folding process of secreted needle protomers** at the end of the growing filament is still poorly understood.

- **Tight space** inside the **needle tube** and the **specific electrostatic pattern** observed at the internal surface directly question the **nature of the secretion process** inside the needle:
  - Is **passive diffusion** of secreted molecules possible?
  - Might subtle**, local, protein–protein interactions trigger a secretion** force to export the molecules along the needle?

- biophysical characterization of the **T3SS needle filament[?]**

(02) A series of secretion signals, **docking complexes** and switch **mechanisms enable** the chronological delivery of substrates to the T3SS export gate.

- Substrate translocation across the inner membrane is achieved by a **PMF-coupled fast protein pump**,
- which permits secretion rates of more than a thousand amino acids per second



(C)

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2	SctF	PrgI
3	SctI	PrgJ
4	SctJ	PrgK
5	SctD	PrgH
6	SctK	OrgA
7	SctQ	SpaO
8	SctL	OrgB
9	SctN	InvC
10	SctO	InvI
11	SctR	SpaP
12	SctS	SpaQ
13	SctT	SpaR
14	SctU	SpaS
15	SctV	InvA
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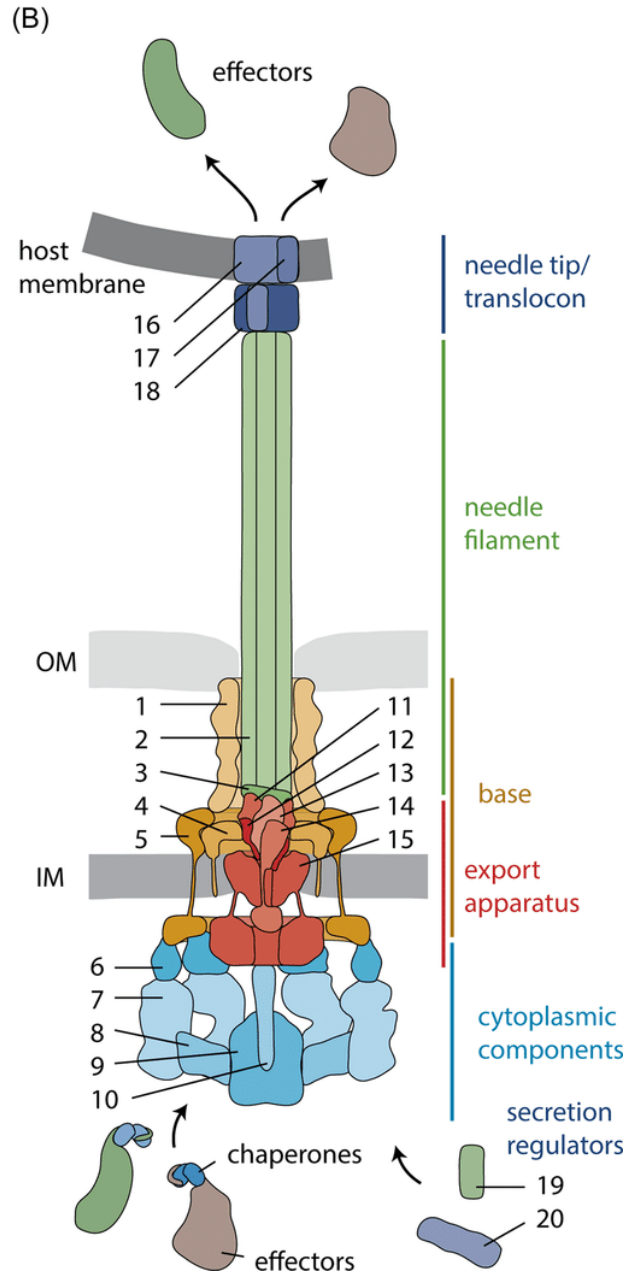
17	SctB	SipC
18	SctA	SipD
19	SctP	InvJ
20	SctW	InvE



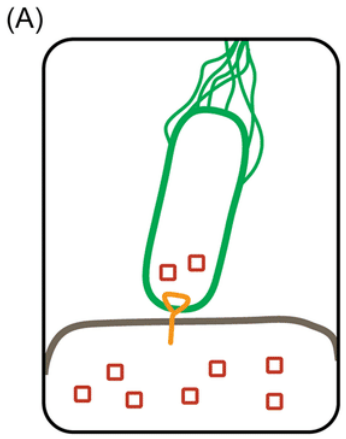
Type three secretion system; Questions

(03) Atomic structures of the **C-terminal** cytoplasmic domains of **FlhA and FlhB, FliH, FliI, FliJ, and the $FliP_5FliQ_4FliR_1$** helical assembly have been solved.

*It remains unknown **how flagellar proteins are unfolded** and **transported** by the **PMF-driven export gate complex**.*

(04) Search for **inhibitors** of the T3SS.

- Therapeutics that inhibit the **assembly or dynamics** of the T3SS



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10	SctO	InvI
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12	SctS	SpaQ
13	SctT	SpaR
14	SctU	SpaS
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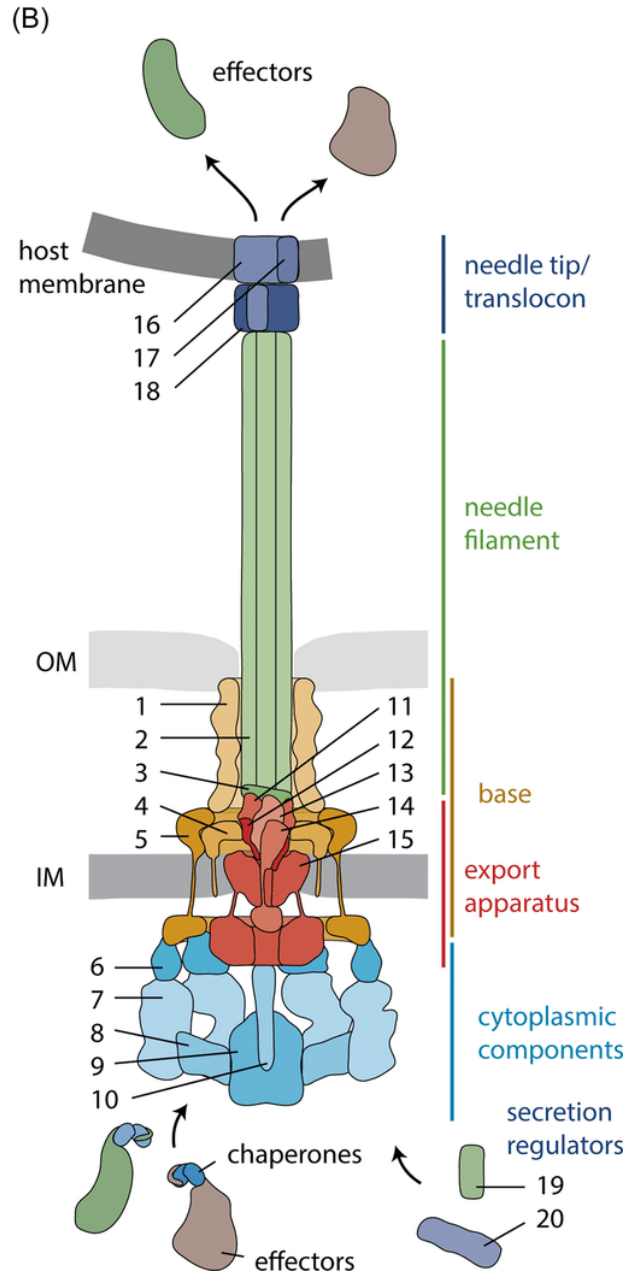
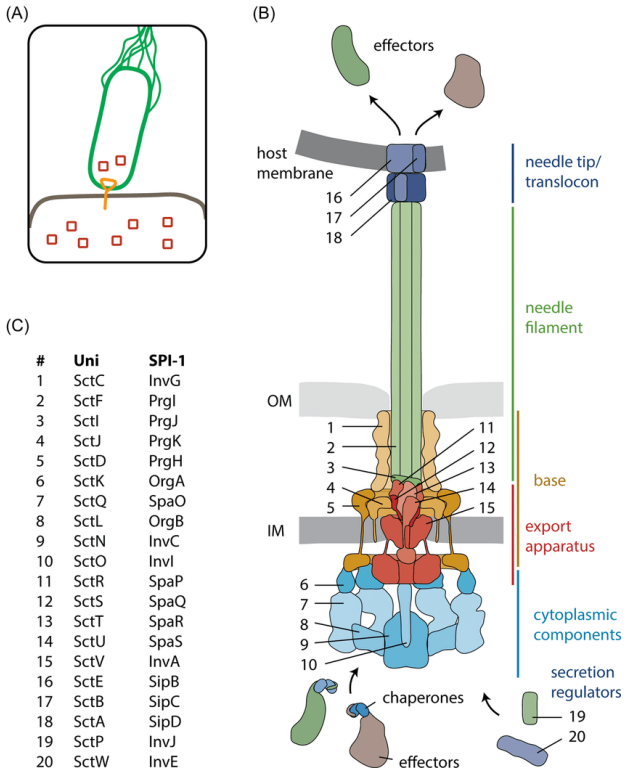
18	SctA	SipD
19	SctP	InvJ
20	SctW	InvE



Type three secretion system; Questions



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9	SctN	InvC
10	SctO	InvI
11	SctR	SpaP
12	SctS	SpaQ
13	SctT	SpaR
14	SctU	SpaS
15	SctV	InvA
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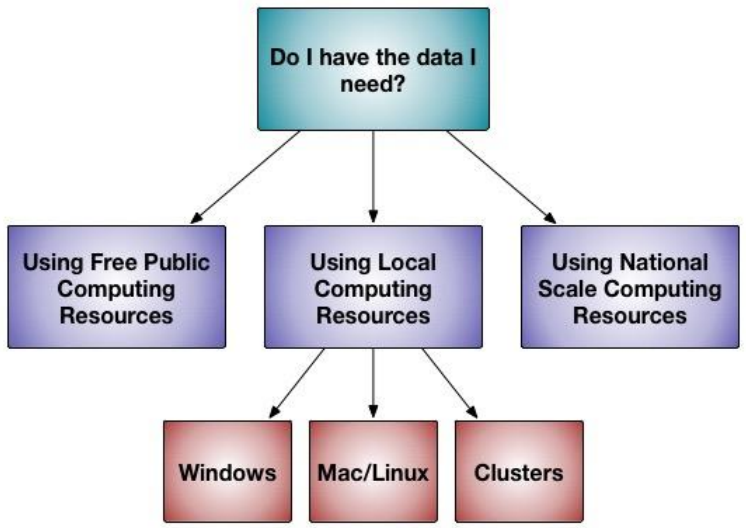
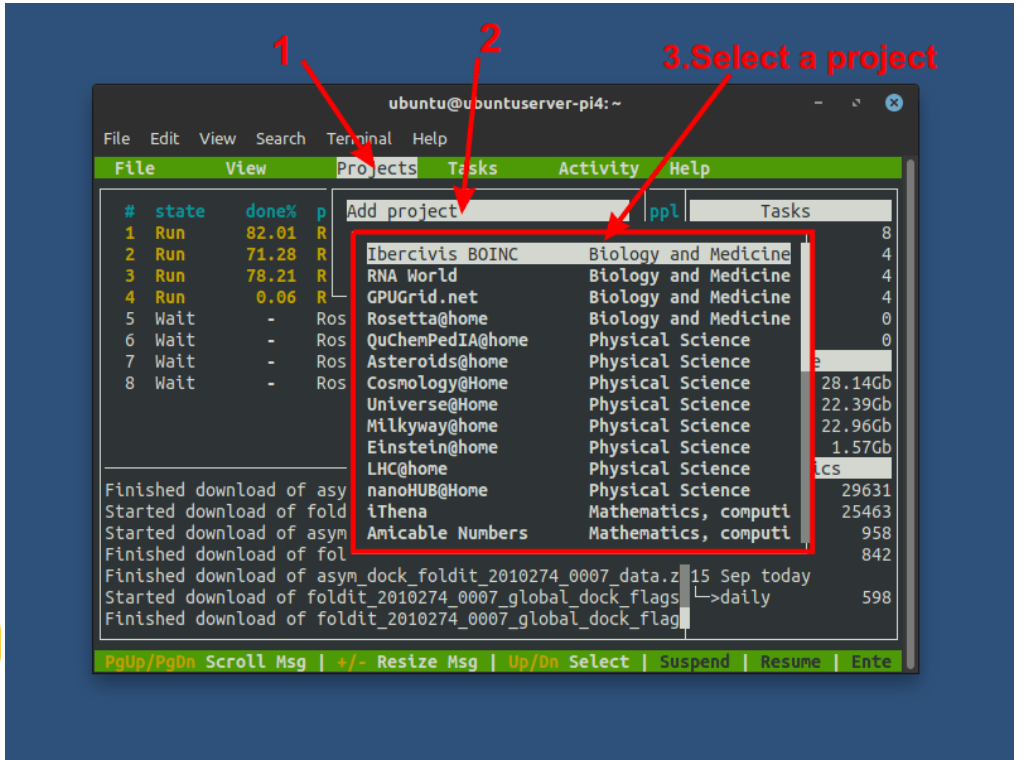
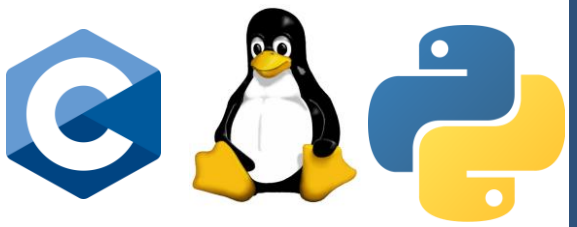
18	SctA	SipD
19	SctP	InvJ
20	SctW	InvE





Development of Rosetta/ likely programs/interfaces/servers

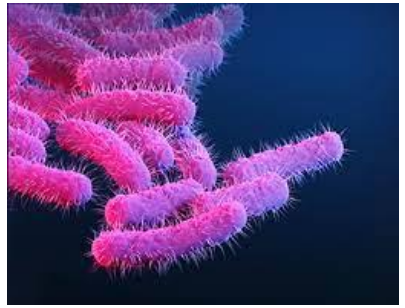
- Protein-protein docking interface
- C++ ; Python; Linux, Servers
- Improve algorithom for folding of protein



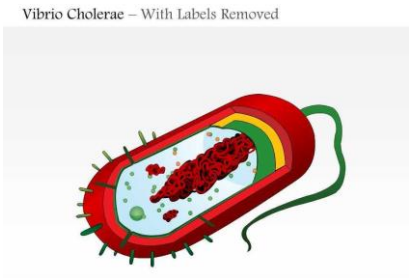
Type three secretion system

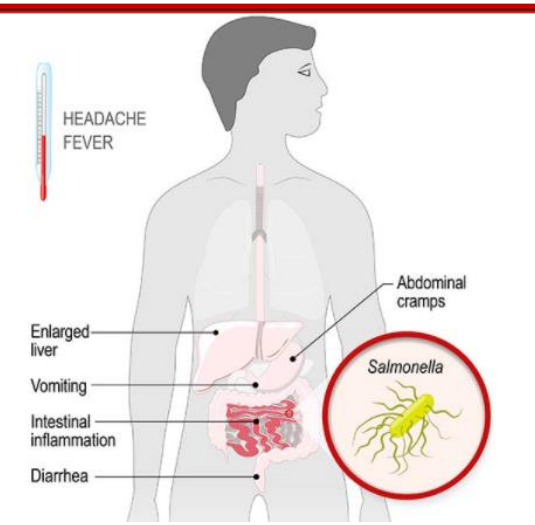
Overview

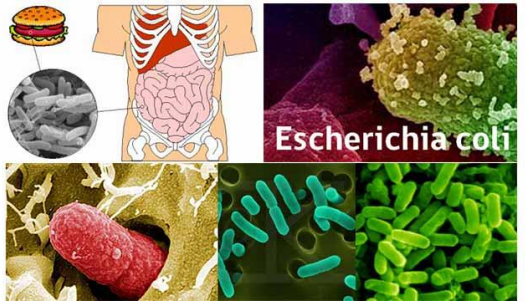
- Many animal + plant associated bacteria possess similar **T3SSs**.
- These T3SSs result of **divergent evolution** and phylogenetic analysis supports a model
- In which gram-ve bacteria transfer **T3SS gene cassette** horizontally to other species.



Shigella







The **most researched** T3SSs are from species of:

- ***Shigella*** (causes bacillary dysentery),
- ***Salmonella*** (typhoid fever),
- ***Escherichia coli*** (Gut flora, food poisoning),
- ***Vibrio*** (gastroenteritis and diarrhea),
- ***Burkholderia*** (glanders),
- ***Yersinia*** **(**plague**),**
- ***Chlamydia*** (STD),
- ***Pseudomonas*** (infects humans, animals and plants) and
- **Plant pathogens**; *Erwinia, Ralstonia and Xanthomonas, & plant symbiont Rhizobium.*

Type three secretion system

Overview

- **Type III secretion** used: both secreting **infection-related proteins** + **flagellar components**.
- T3SS composed of: **30 different proteins** (approx.)
- This made it **one of the most complex secretion systems.**
- Structure **similarities** with **bacterial flagella**
- Some **proteins** participating in T3SS share aa sequence homology to **flagellar proteins.**
- Bacteria possess **T3SS + flagella + motile** (Salmonella), only **T3SS** (Shigella)
- "type III secretion" term used mainly relating to **infection apparatus.**
- **Bacterial flagellum** shares a **common ancestor** with the **type III secretion system.**
- T3SSs essential for bacterial pathogenicity.
- **Defects T3SS** render a bacterium **non-pathogenic.**
- **Non-invasive strains** of gram –(ve) bacteria lost T3SS;
  because energetically costly system is no longer of use.
- Traditional antibiotics were effective against these bacteria **in the past**,
- **antibiotic-resistant strains constantly emerge.**
- Understanding the **way the T3SS works**
- **Developing drugs targeting**

Type three secretion system

Structures

- Hallmark of T3SS; **Needle**
  - **Needle** complex (NC) or **T3SS apparatus (T3SA)**
  - **Injectisome**; when the ATPase is excluded
- Bacterial proteins **secreted** & **pass from the bacterial cytoplasm** through the **needle** directly into the **host cytoplasm**.
- **Three membranes** separate the **two cytoplasms**:
  - **double membranes** (inner and outer membranes) of **the Gram-negative bacterium** and
  - **eukaryotic membrane.**
- **Needle** provides a **smooth passage** through those **highly selective** and almost **impermeable membranes.**
- Single bacterium have **several hundred needle complexes** spread across its membrane.
- It proposed that the **needle complex**; universal feature of all **T3SSs of pathogenic bacteria**.

Type three secretion system

Structures

- Needle complex **starts** at the **cytoplasm of the bacterium**,
- **crosses** the **two membranes** and **protrudes** from the cell.
- Part **anchored** in the **membrane** is **the base** (or basal body) of the T3SS.
- The extracellular part is the needle.
- A so-called **inner rod** connects the **needle to the base**.
- The **needle itself**, although the **biggest** and most prominent part of the **T3SS**, is made out of **many units of a single protein**.
- The majority of the **different T3SS proteins** are therefore those **that build the base** and **those** that are **secreted into the host**.

- **Needle complex:** similarities with **bacterial flagella**.
- B**ase** of the **needle** complex; structurally similar to **flagellar base**;
- **Needle** itself analogous to **flagellar hook** (structure connecting **base** to the flagellar **filament**)

Type three secretion system

Structures

- Base composed of several **circular rings**; first structure that is **built** in a **new needle complex**.
- Once **base** is completed, it **serves** as **secretion machine** for the **outer proteins** (the needle).
- Once **whole complex** is completed, system **switches** to **secreting proteins delivered** into **host.**
- **Needle** is presumed to be **built** from **bottom to top;**
  units of needle monomer protein pile upon each other,
  so that **the unit** at the **tip of the needle** is the **last one added.**
- N**eedle subunit** is one of the **smallest T3SS proteins**, around 9 kDa.
  100–150 subunits comprise **each needle.**

Type three secretion system

Structures

- **T3SS needle** ~60–80 nm in **length**; 8 nm in external **width**.
- It needs to have a minimal length
  so that **other extracellular bacterial structures** (adhesins and the lipopolysaccharide layer, for instance) **do not interfere with secretion**.
- The **hole of the needle** has a **3 nm diameter.**
- Most **folded effector proteins** are **too large to pass through** the **needle opening**,
  so **most secreted proteins** must **pass through the needle unfolded**,
  a task carried out by the **ATPase at the base of the structure**.

Thank you