

Molecular Machines & Molecular Microscopy & Infection Biology

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HOST - PATHOGEN

Host: Organism > Tissue > Cells

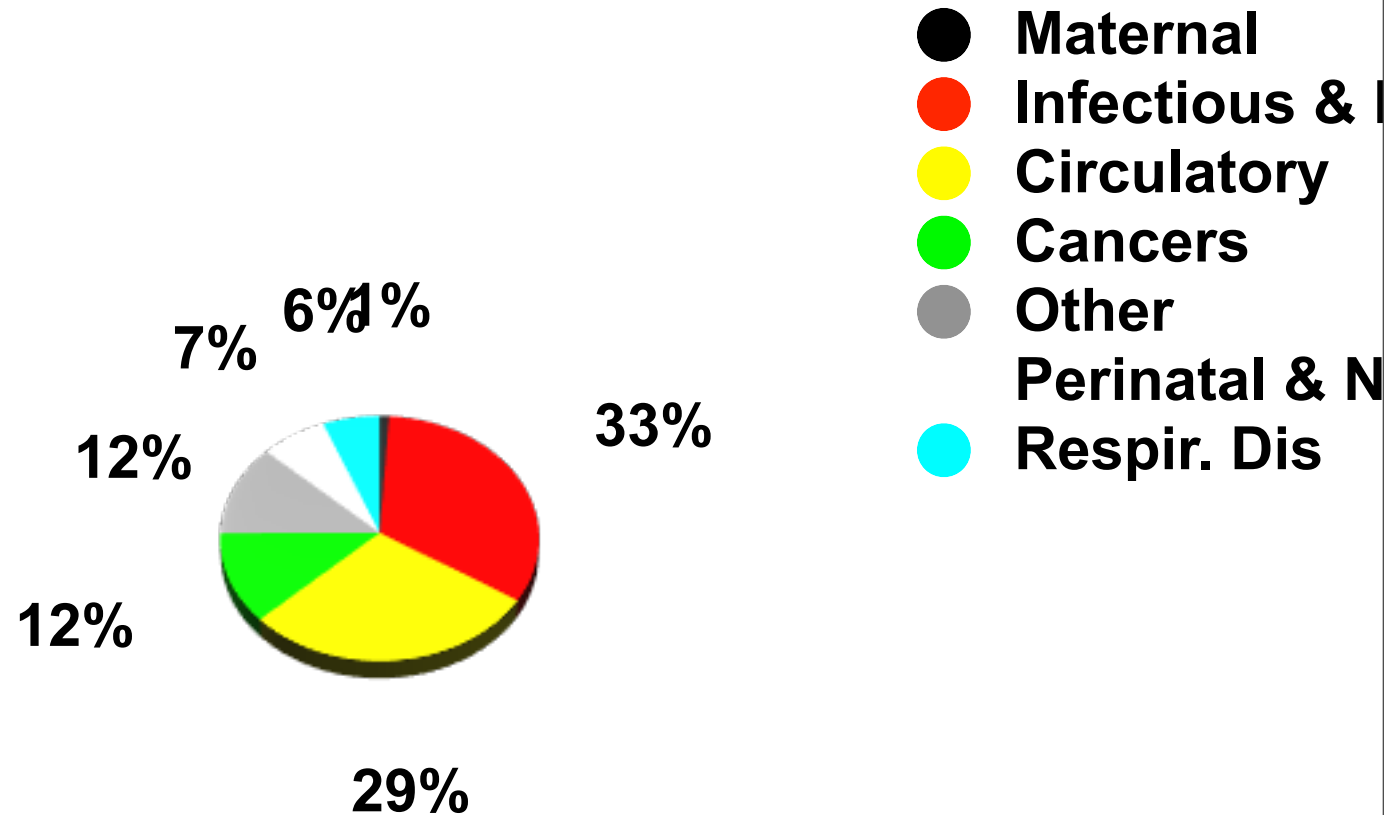
Pathogen: Virus, Bacteria, Fungi

HOST - PATHOGEN INTERACTION

Our objective:

- Why should we care ?
- Who are the players and what is common ?
- How can we investigate ?

Global Causes of Death 1997

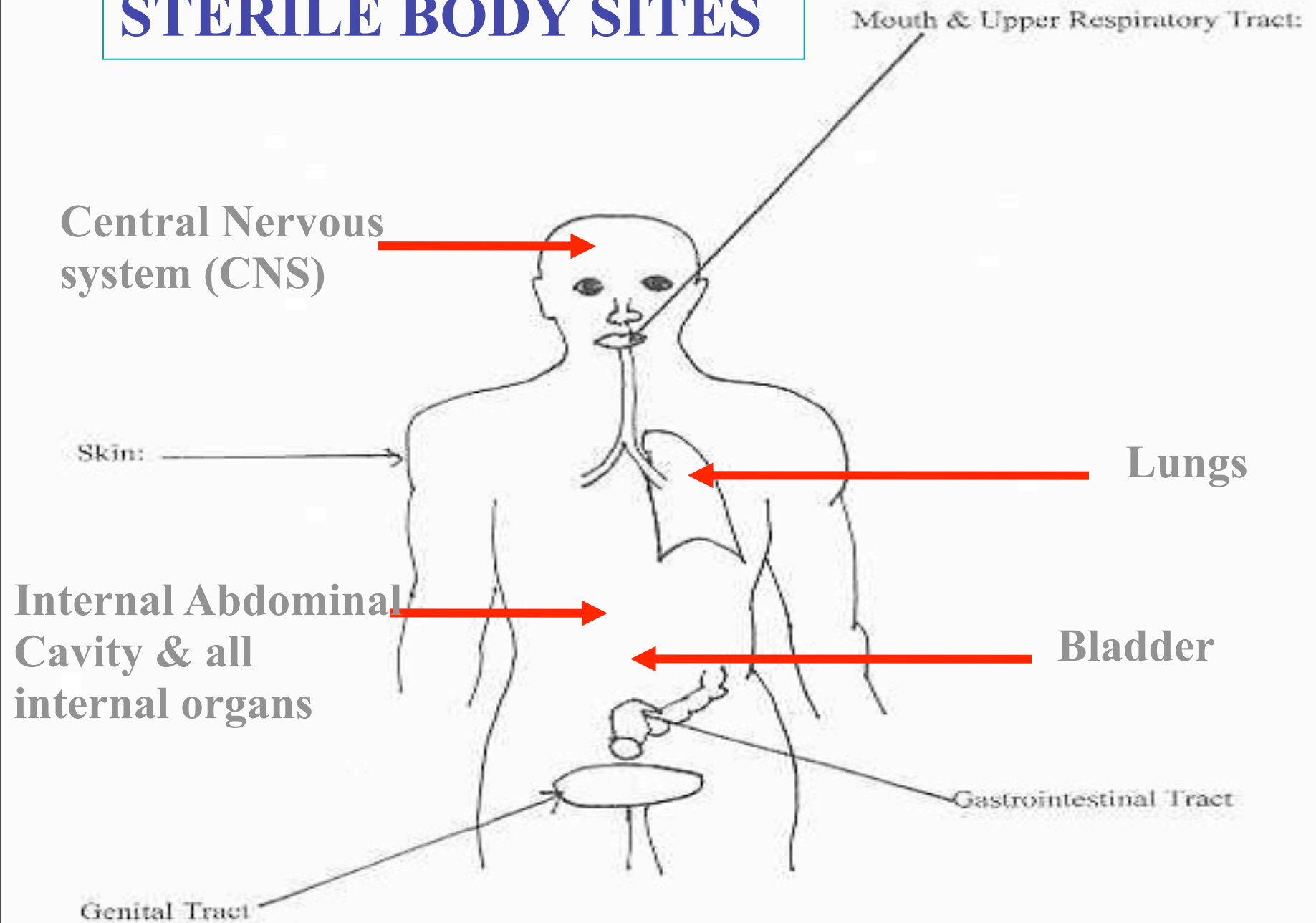


Disease Burden in Humans

Infection:	# Deaths	% of all Deaths
Lower Resp	3,745,000	7.2
Tuberculosis	2,910,000	5.6
Diarrheal	2,455,000	4.7
HIV/AIDS	2,300,000	4.4
Malaria	2,700,000	2.9-5.2
Measles	960,000	1.8
Hepatitis B	605,000	1.2
Pertussis	410,000	0.8
Dengue fever	140,000	0.3

Principles & Practice of Infectious Diseases 5th Ed 2000, Mandell (ed)

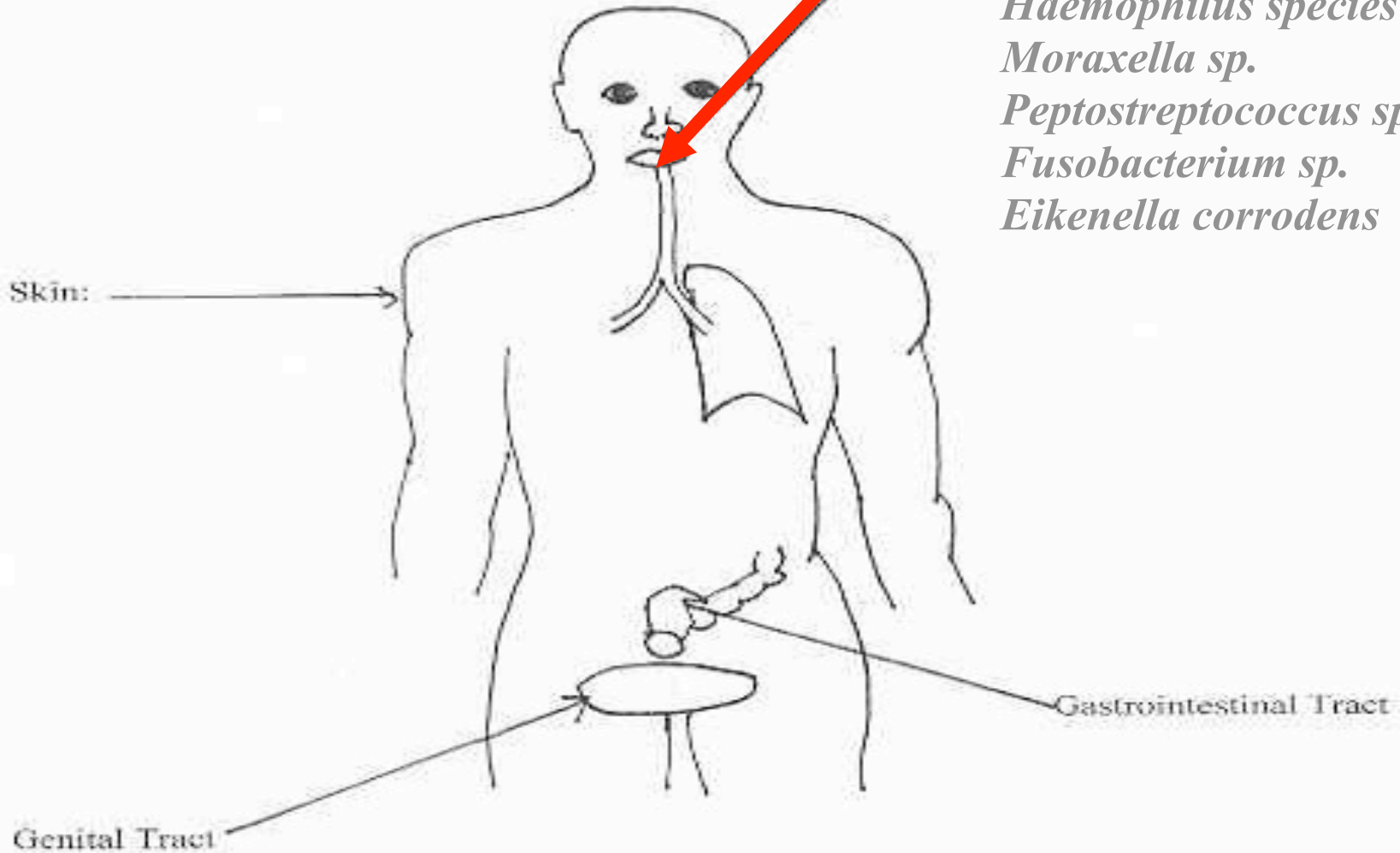
STERILE BODY SITES



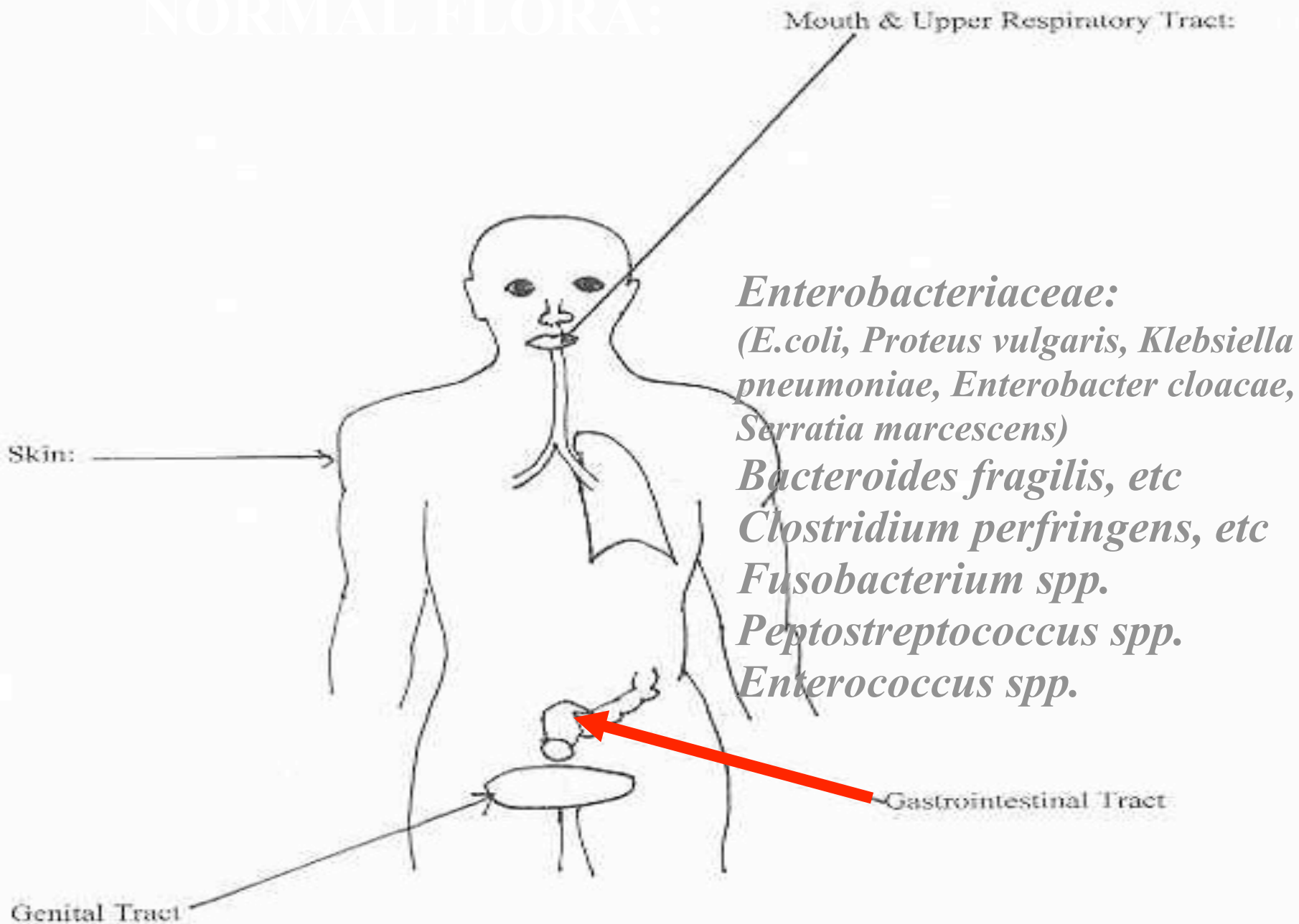
NORMAL FLORA:

Mouth & Upper Respiratory Tract:

Streptococcus salivarius
Streptococcus mitis
Staphylococcus epidermidis
Haemophilus species
Moraxella sp.
Peptostreptococcus sp.
Fusobacterium sp.
Eikenella corrodens



NORMAL FLORA:

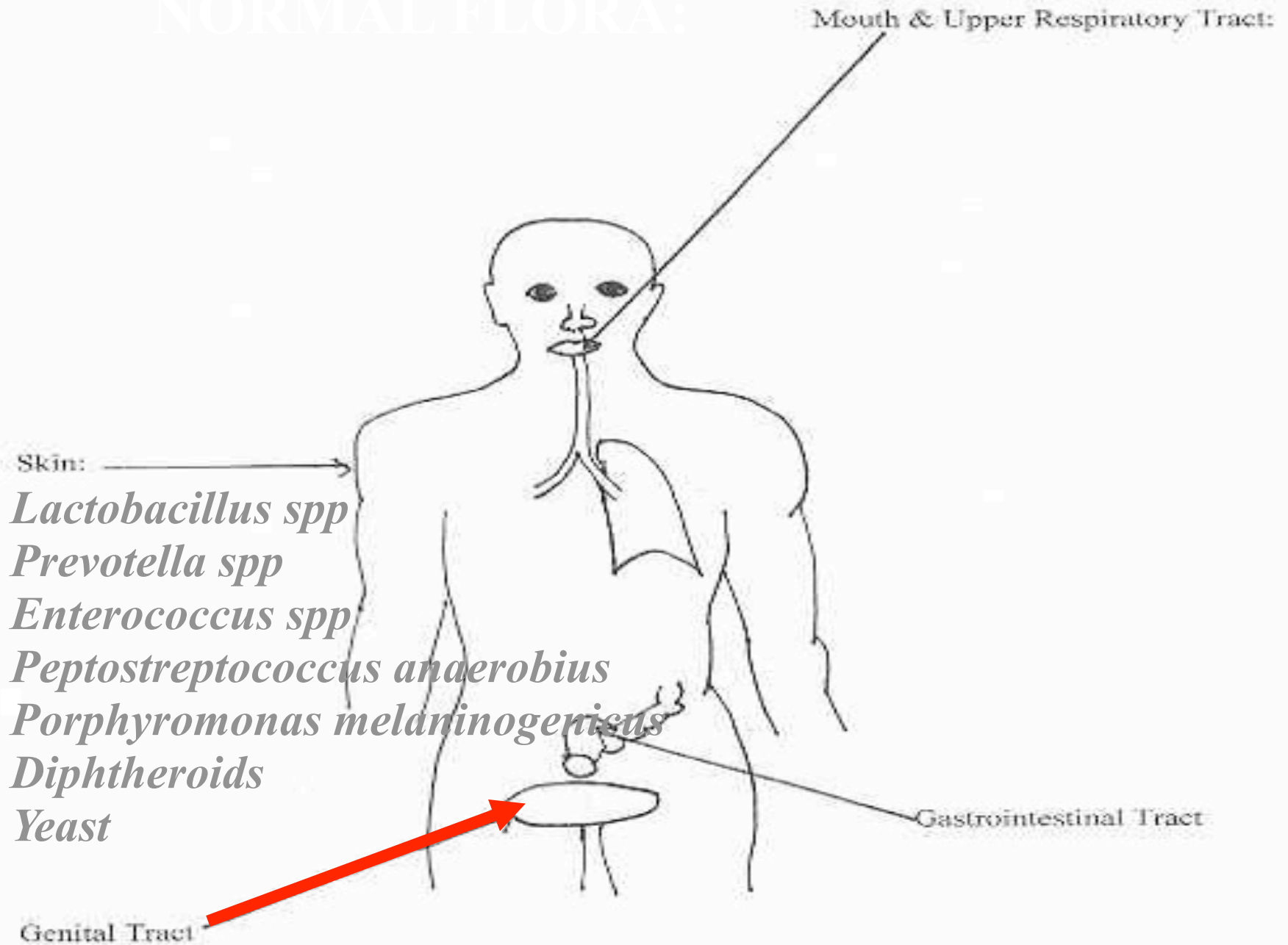


Human Gut Microbiome

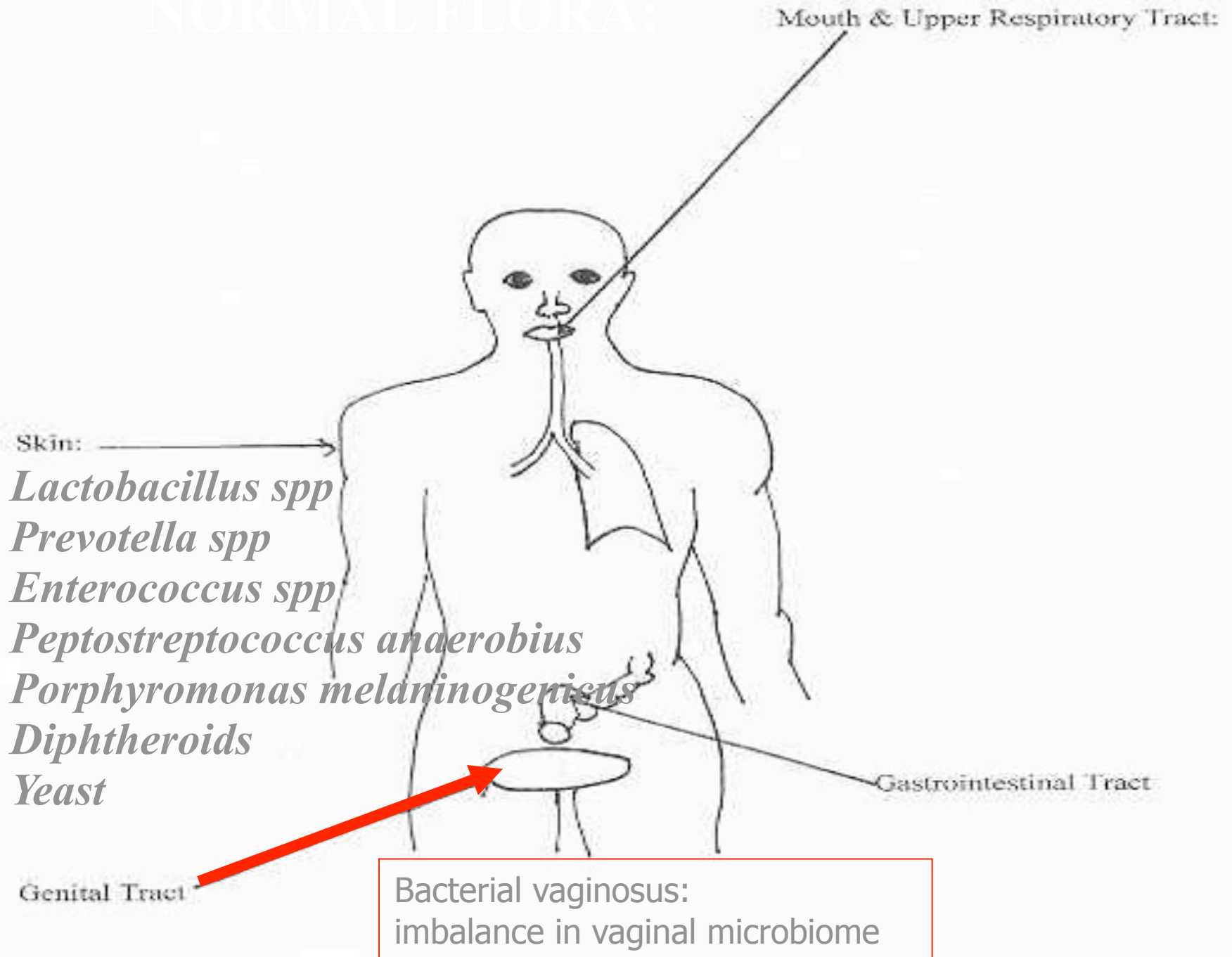
- Majority of bacteria in gut are uncharacterized
- Short Chain Fatty Acids (propionate, acetate, butyrate) from microbial fermentation supply ~10% of host energy requirements)
- Imbalance in microbiome can lead to disease (e.g. *C.difficile*, Crohn's, etc)
- Microbiome sequencing expanding our understanding

Duncan S, et al Cultivable bacterial diversity from the human colon.
Letters in Applied Microbiology 2007;44:343-350

NORMAL FLORA:



NORMAL FLORA:



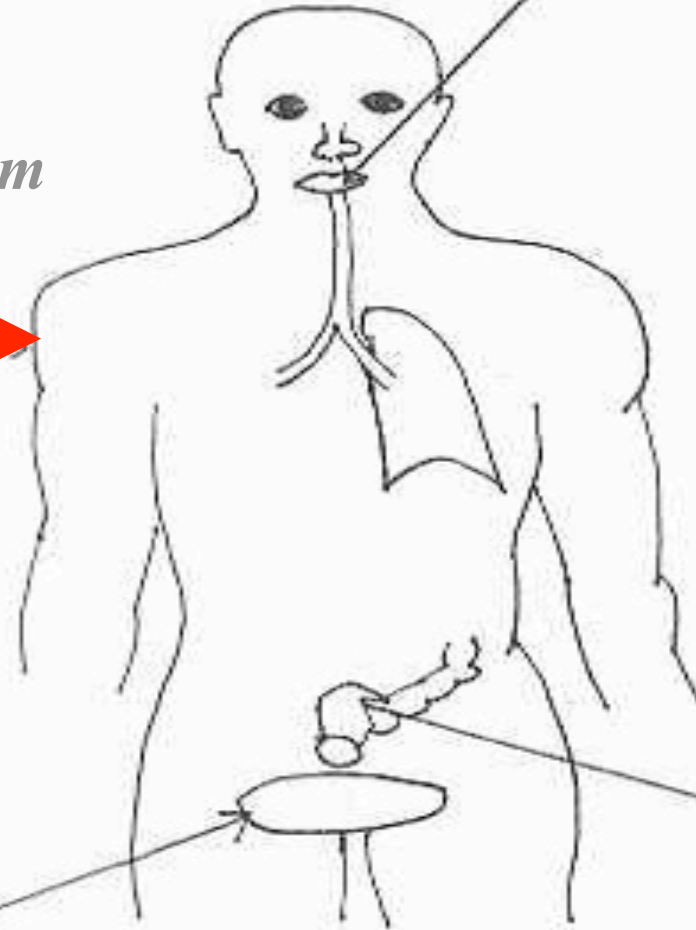
NORMAL FLORA:

S. epidermidis
Diphtheroids
Micrococcus sp.
Propionibacterium

Skin:



Mouth & Upper Respiratory Tract:



Gastrointestinal Tract

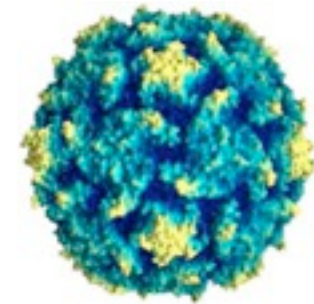
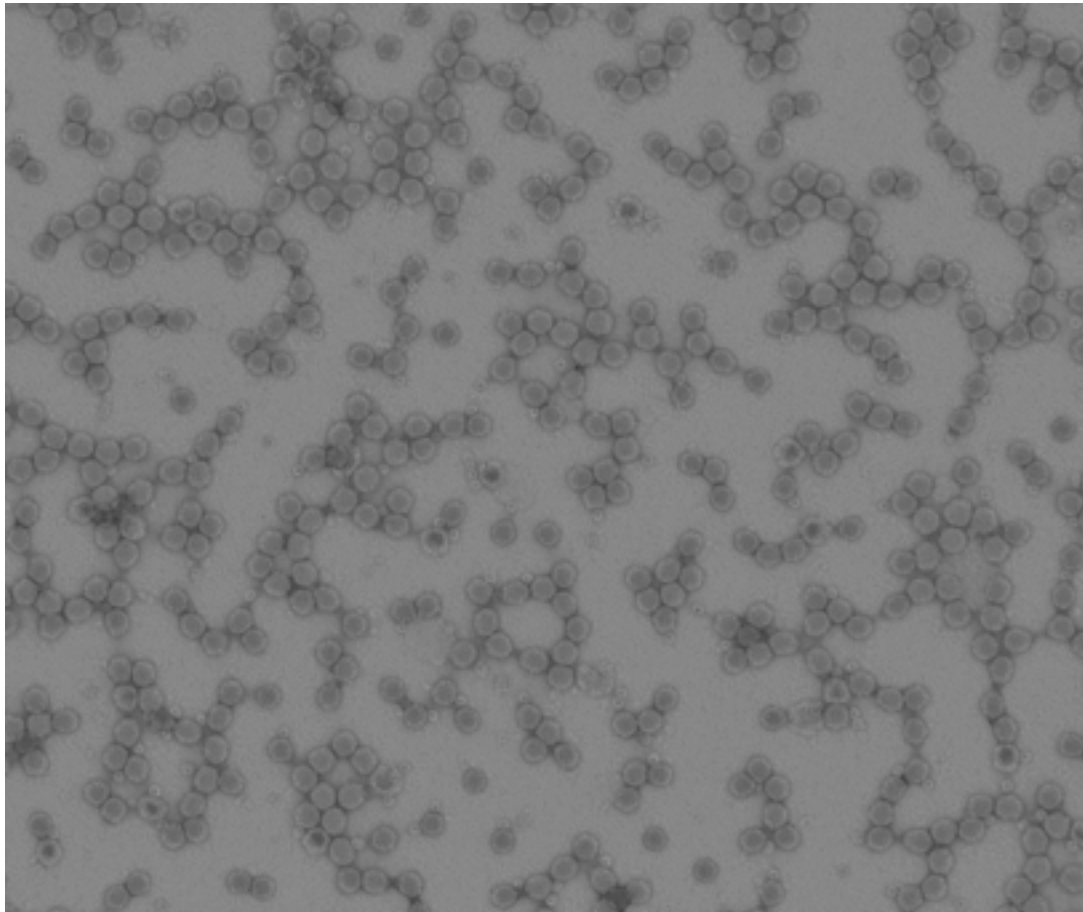
Genital Tract

Reportable Organisms

AIDS/HIV	Encephalitis	Mumps	Shigella
Anthrax	Food poisoning	Parapertussis	MRSA
Bacillus cereus	Gonorrhea	S.pneumoniae	Vanc R-S.aureus
Botulism	Hantavirus	Pen Resistant	S.pyogenes (invasive disease)
Brucellosis	H.influenza B invasive disease	Pertussis	Syphilis
Campylobacter	E.coli O157:H7	Plague	Tetanus
Chancroid	Hepatitis A,B,C,other	Polio	Toxoplasmosis
Chlamydia	Legionellosis	Psittacosis	Tuberculosis
Cholera	Leprosy	Q fever	Tularemia
C.perfringens (not wounds)	Listeriosis	Rabies	Typhus
Rubella (congenital)	Lyme Disease	Relapsing fever	Vibrio parahemolyticus
CJD	Measles	Rickettsial disease	Yellow fever
Dengue fever	Meningitis (bacterial)	Rocky Mountain Spotted fever	Yersinia
Diphtheria	N.meningitidis (invasive)	Rubella	Parasites-various
Viral hemorrhagic fever	WEE	Salmonella	

Rhinoviruses ... cause of the running nose

Human rhinovirus serotype 2 (d=30nm)

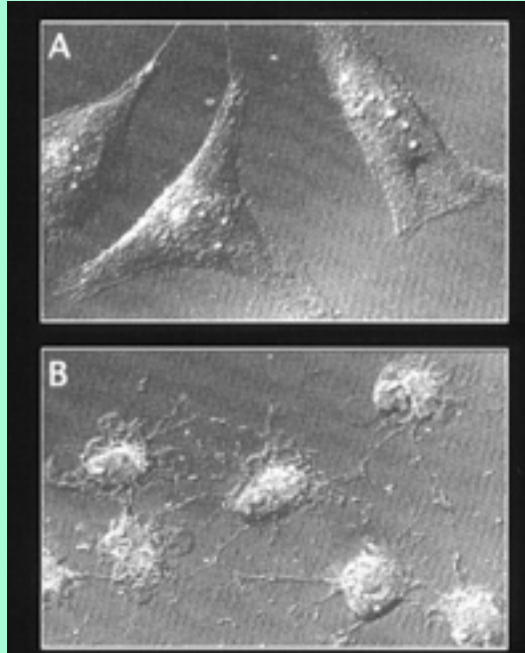


Poliovirus
(d=30nm)

-(+) ssRNA virus
-Non-enveloped

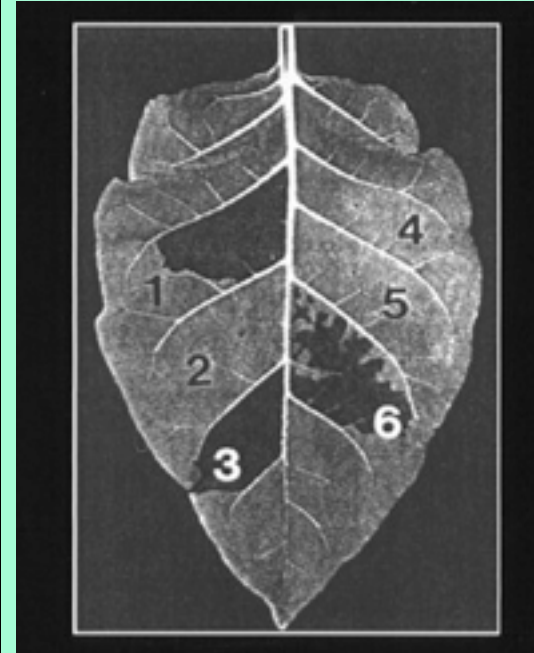
Effect of a virulence system on hosts

Yersinia cytotoxicity



Translocation of YopE leads to a collapse of the cytoskeleton

Erwinia hypersensitive response induction



Induction of localized tissue necrosis in a tobacco leaf at sites of infiltration with *Erwinia* spp. (area 1), buffer alone (area 4), type III secretion mutants (areas 2 and 5), and complemented mutants (3, 6).

Salmonella (**typhoid fever**)
Shigella (**bacillary dysentery**)
Escherichia coli (**food poisoning, neonatal diarrhea**)
Burkholderia (**meloidosis**)
Yersinia (**plague**)
Chlamydia (**sexually transmitted disease**)

modified from Hueck (1998) Microbiology and Molecular Biology Reviews 62:379-433

Erwinia, *Ralstonia*, *Rhizobium* and *Xanthomonas*.

Vaccinia Virus

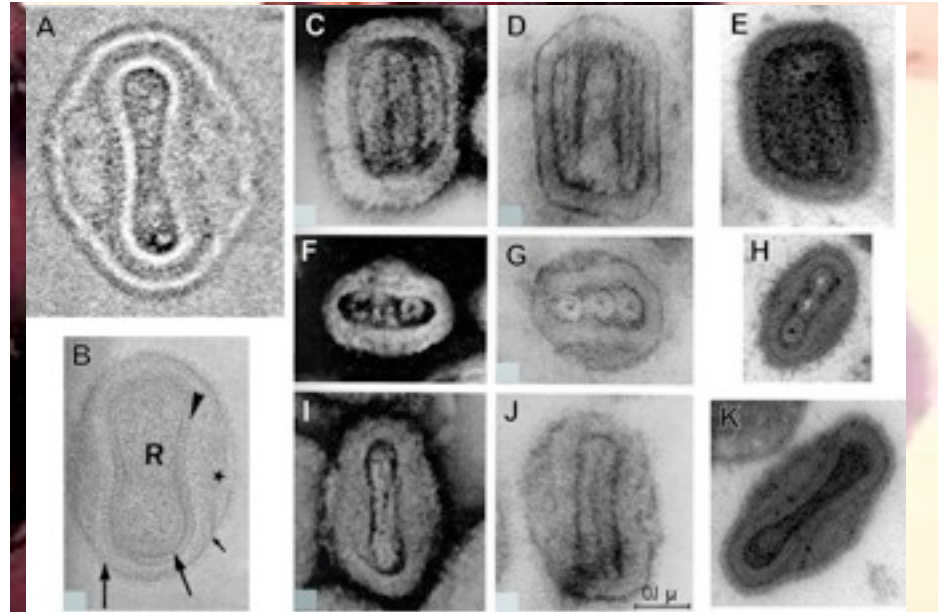
- model system for small pox
- Large double stranded DNA virus
- enveloped



Vaccinia virus internal features. (A) Cryosection (Hollinshead et al., 1999). (B) Whole mount cryo electron micrograph using uranyl acetate staining (Griffiths et al., 2001). (C, F, I) Three different projections of a whole mount preparation of virus, phosphotungstic acid stained (Peters & Mueller, 1963). (D, G, J) Sections of purified virus, uranyl acetate stained, in three different perpendicular planes (Peters & Mueller, 1963). (E, H, K) Sections of MV in three different perpendicular planes [(Kato et al., 2004) and Condit, unpublished]. (E) and (H) are virions in infected cells; (K) was from a preparation of purified virus.

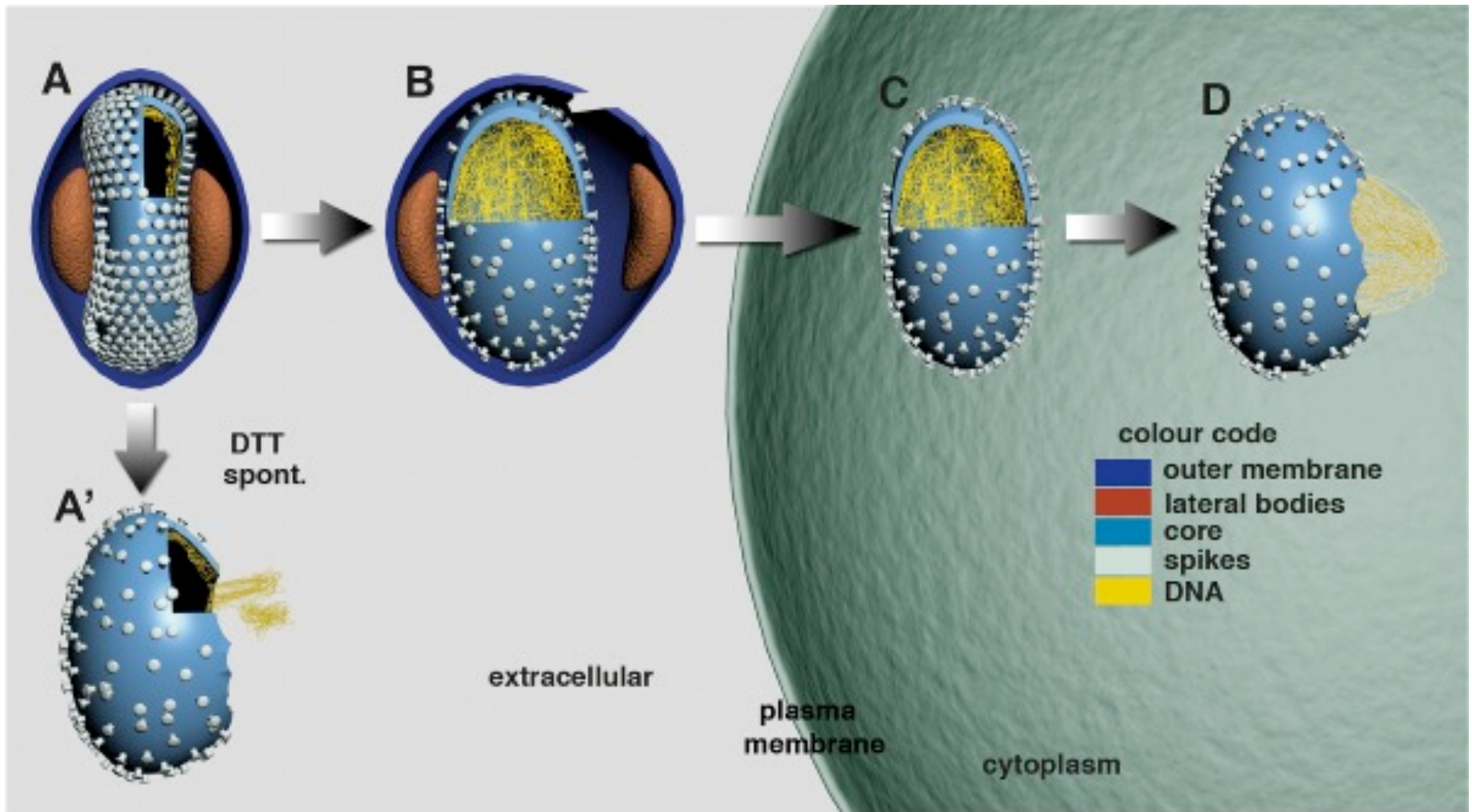
Vaccinia Virus

- model system for small pox
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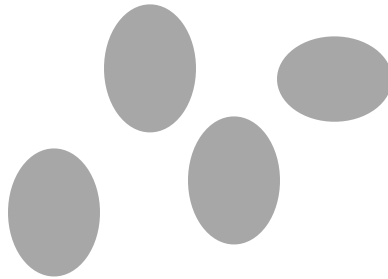
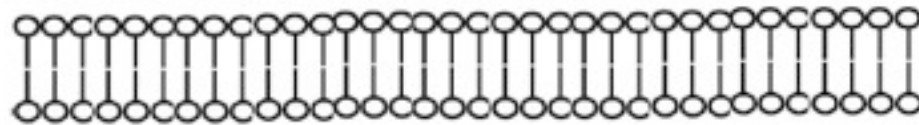
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Vaccinia Virus Entry

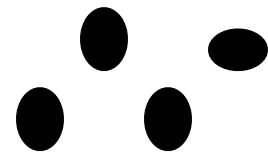
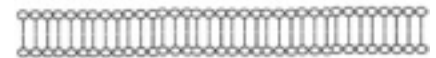
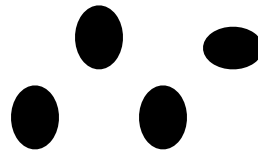
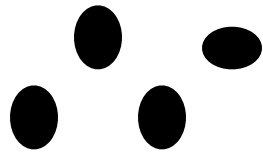
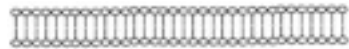


Cyrklaff et al 2007

The fundamental problem



The fundamental problem and some additional variations

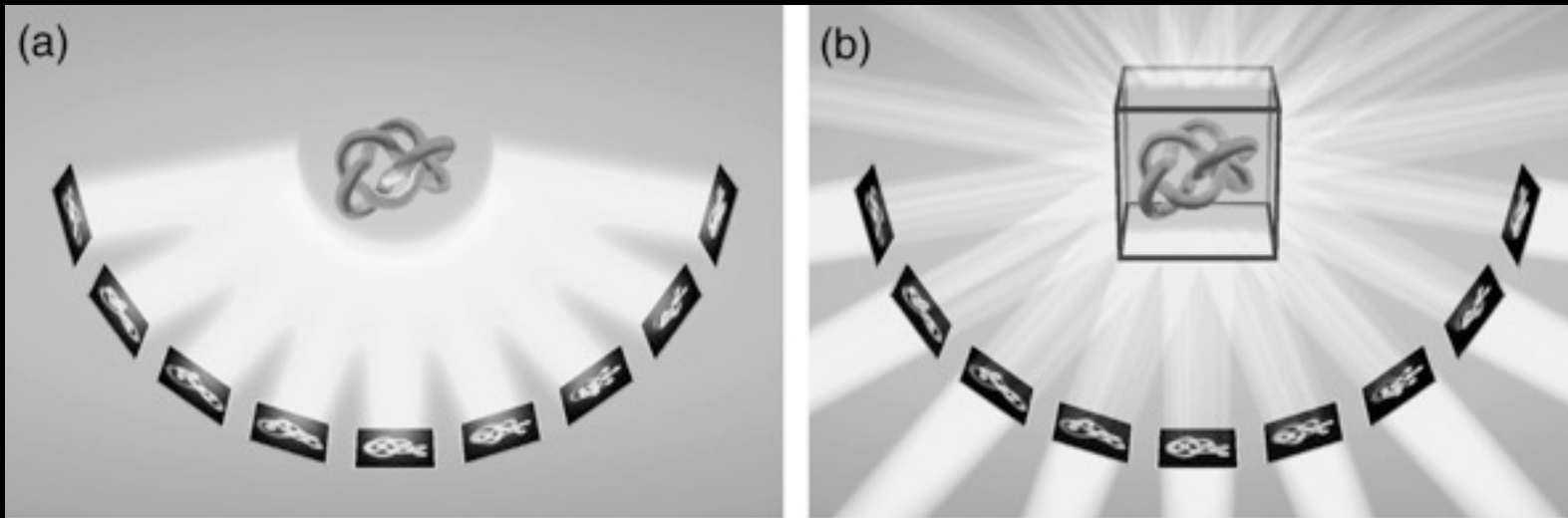


Cryo Electron Tomography (CryoET)

- single, small cell
- 40-50 Angstroem

Electron tomography

Exploring the three-dimensional structure of organelles and cells at nanometer resolution
Reconstruction of 3d volumes from a series of 2d projections

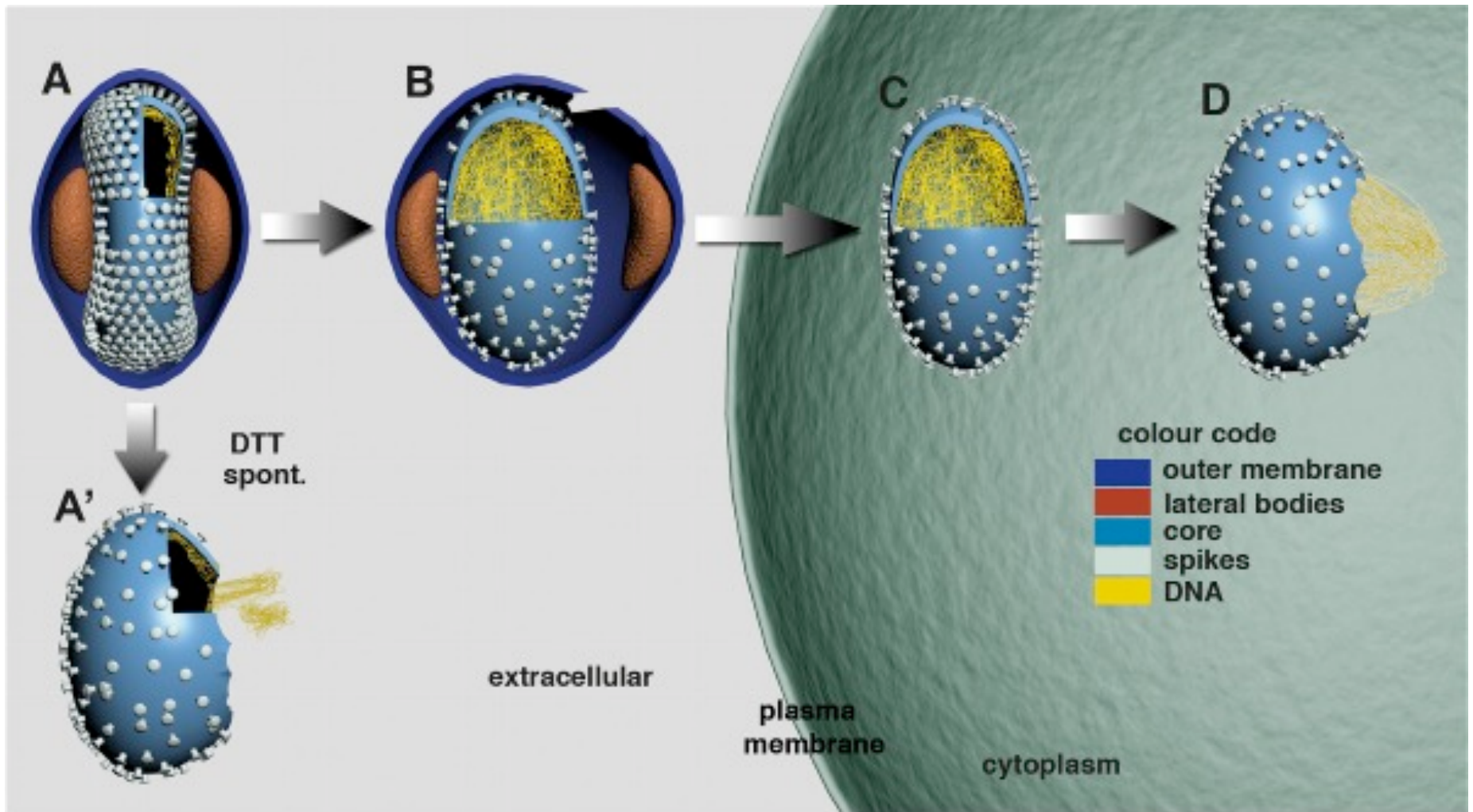


Baumeister et al., 1999

Hardware prerequisites for ET:

1. Intermediate to high acceleration voltage for penetration of thick samples
2. Stable (cryo)stage for reproducible tilting
3. Computer control for efficient data acquisition

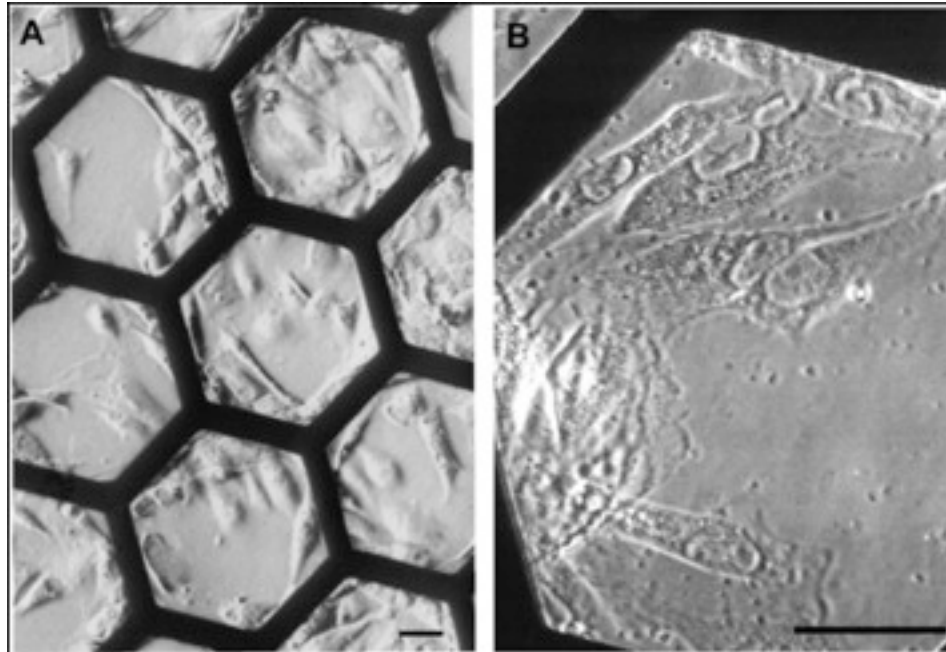
Vaccinia Virus Entry



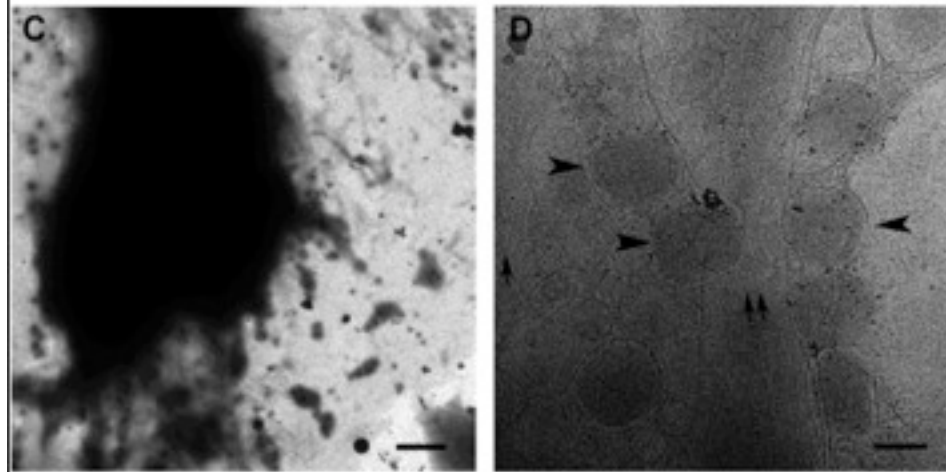
Cyrklaff et al 2007

Vaccinia Virus Entry: Sample preparation for CryoET

Light microscopy

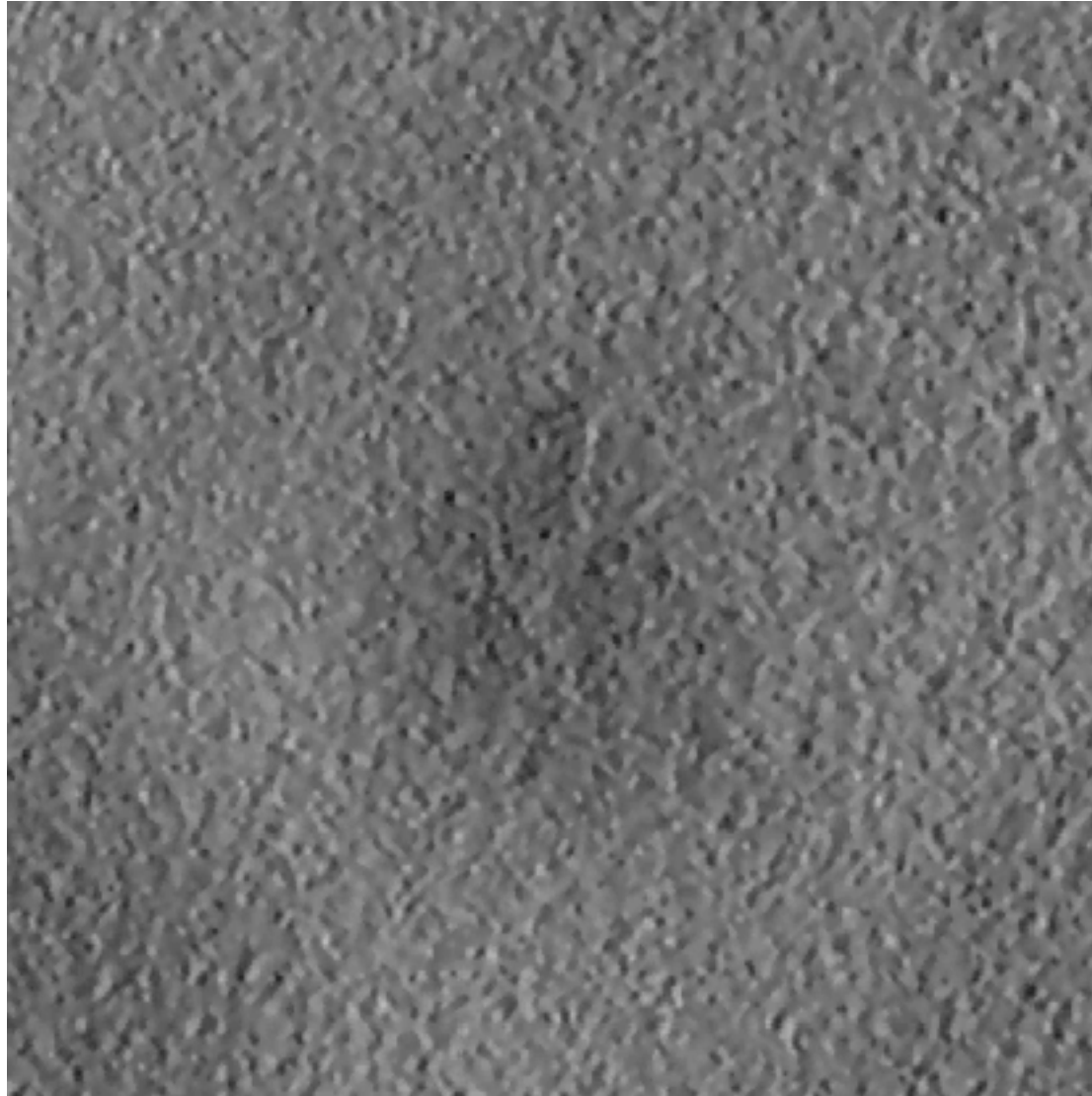


Electron microscopy

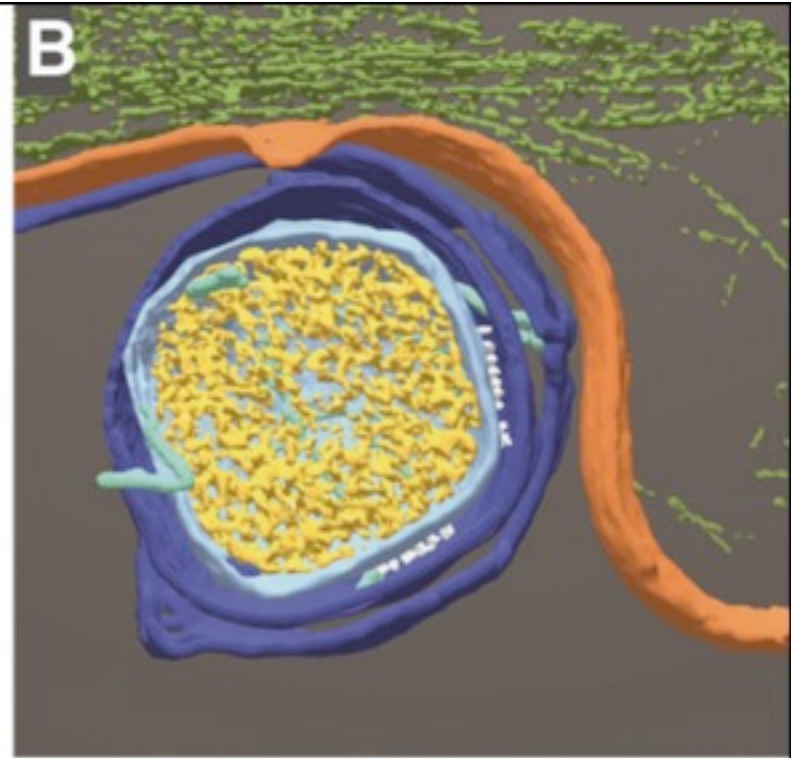
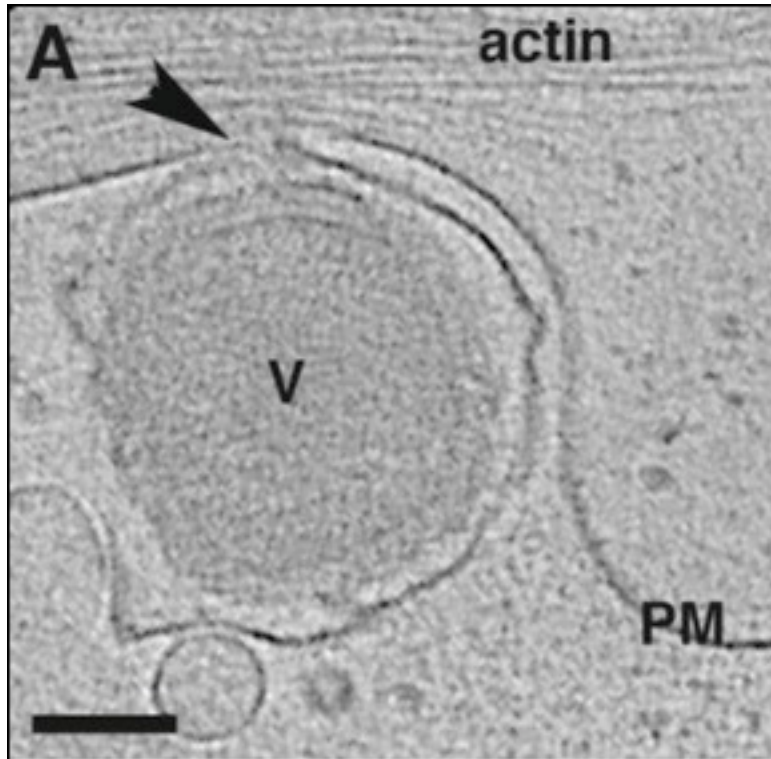


Cyrklaff et al 2007

CryoET of vaccinia virus entry

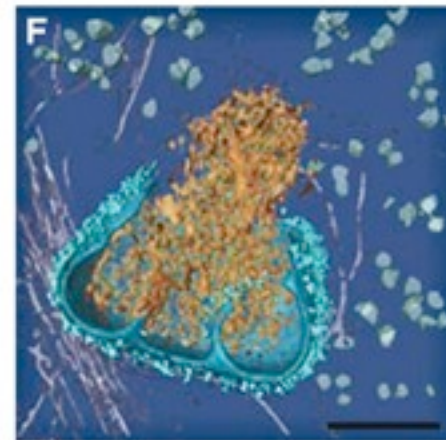
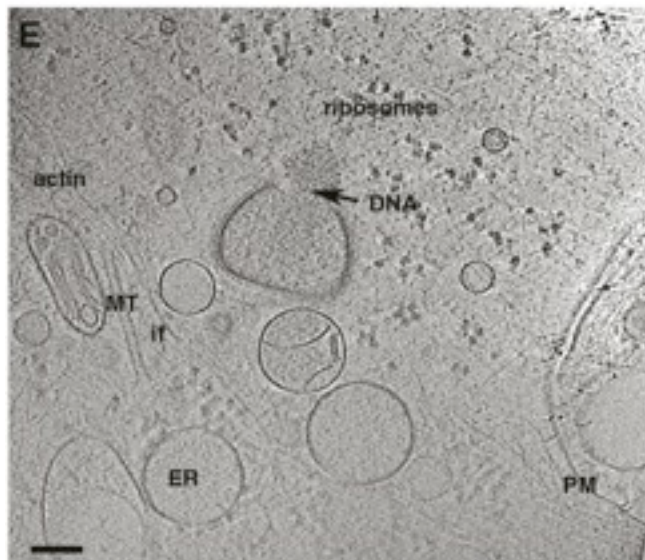
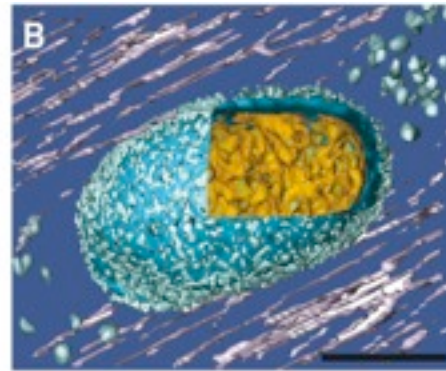
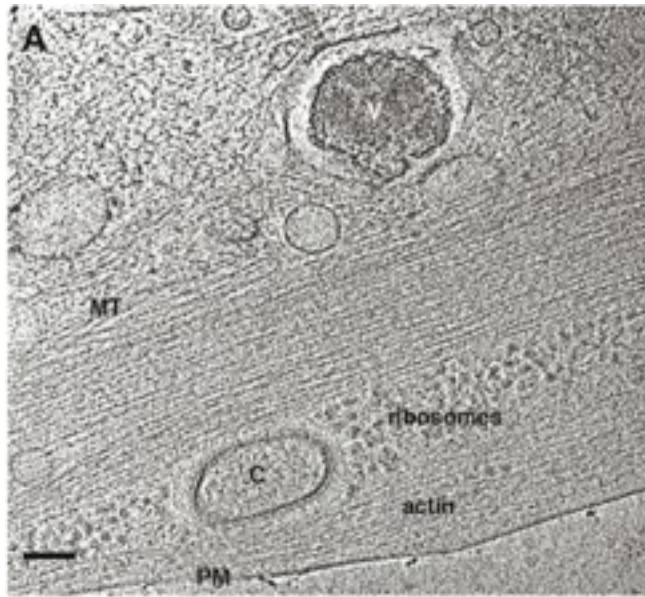


CryoET of Vaccinia Virus entry



Cyrklaff et al 2007 modified

CryoET of Vaccinia Virus entry



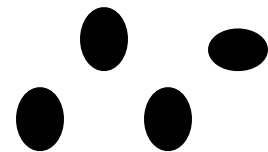
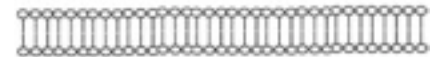
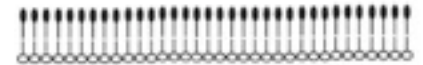
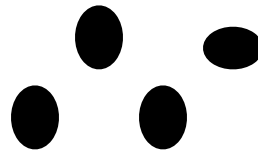
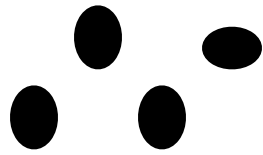
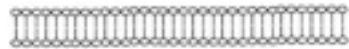
Cyrklaff et al 2007 modified

Vaccinia Virus Entry: Release of DNA

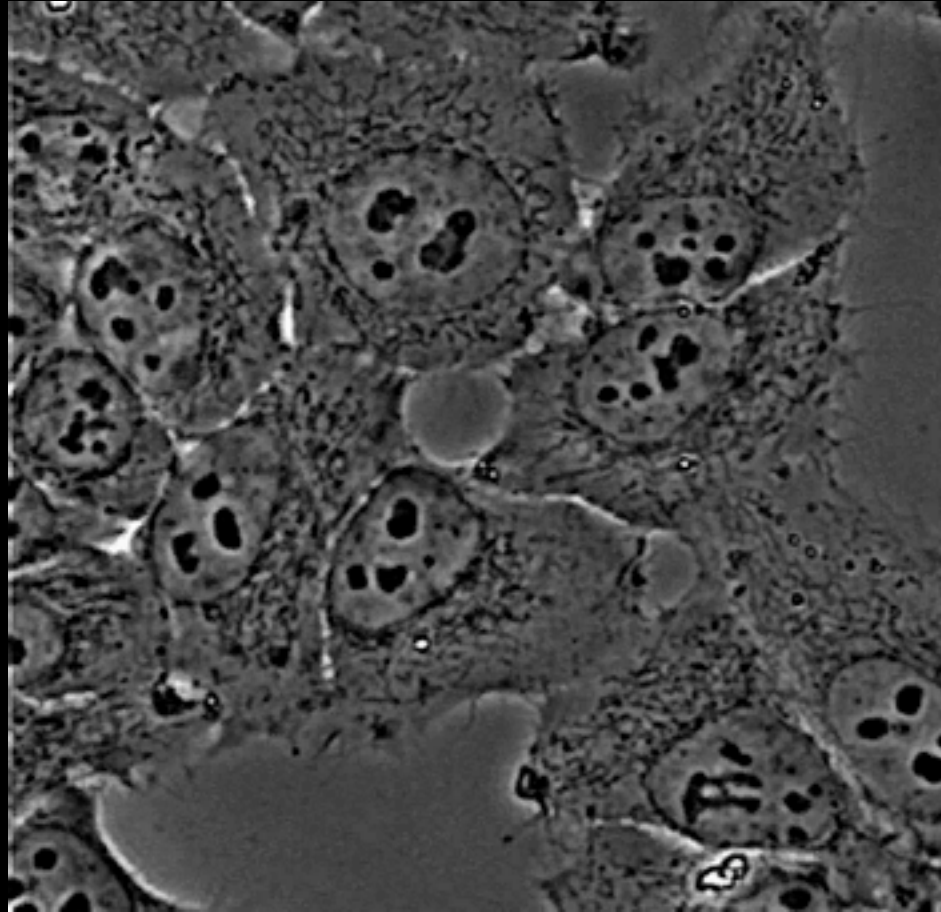


Cyrklaff et al 2007

The fundamental problem and some additional variations



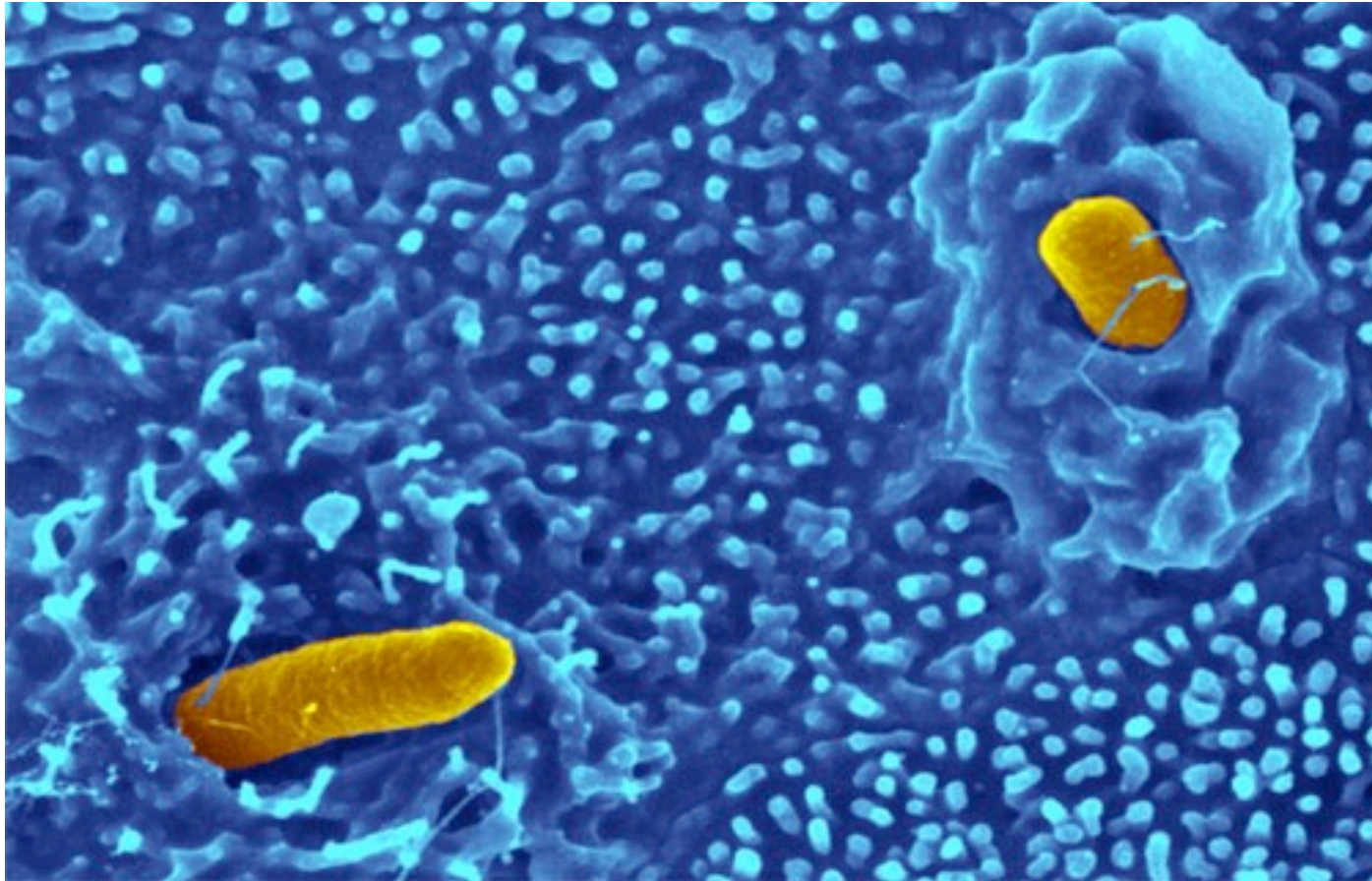
How Salmonella infects host cells



real time observation: 1.5h

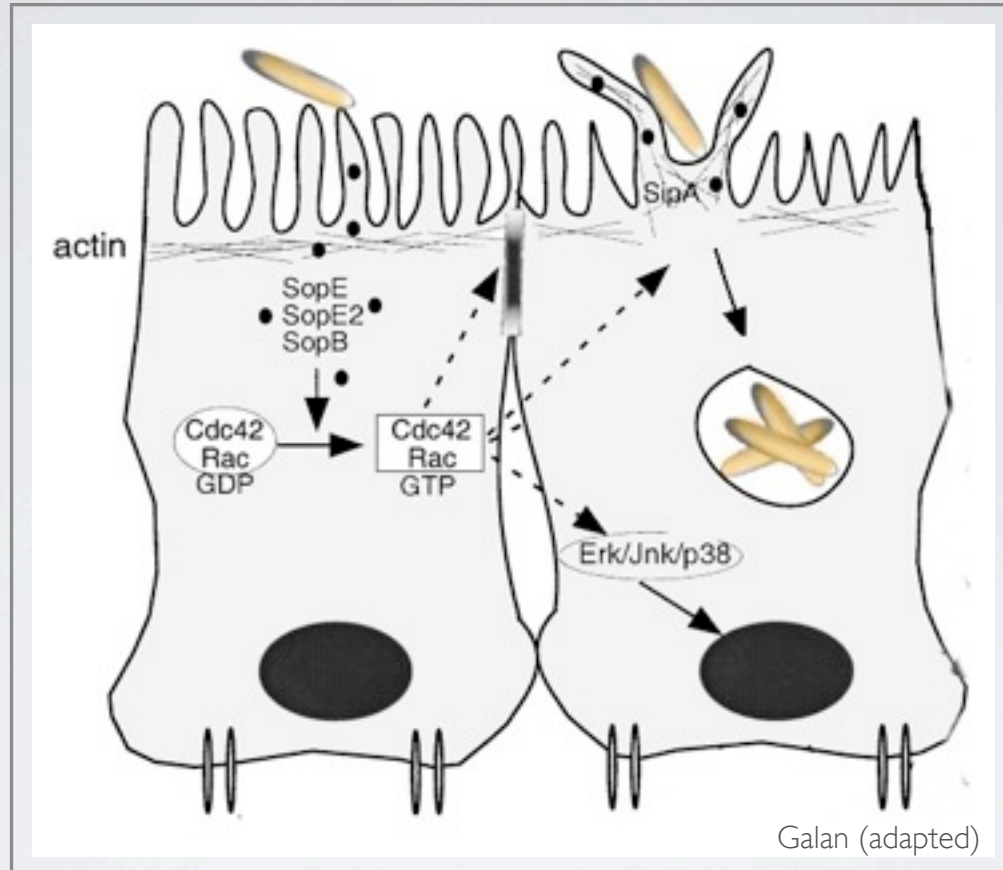
Courtesy from the GalanLab

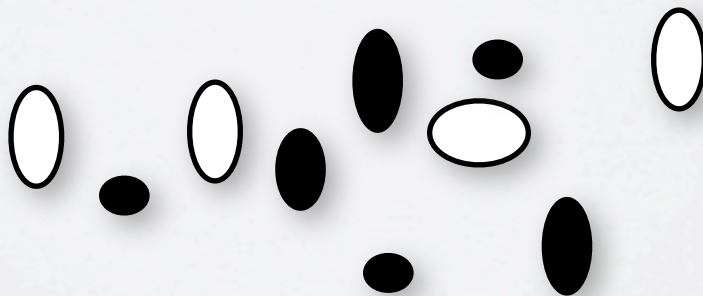
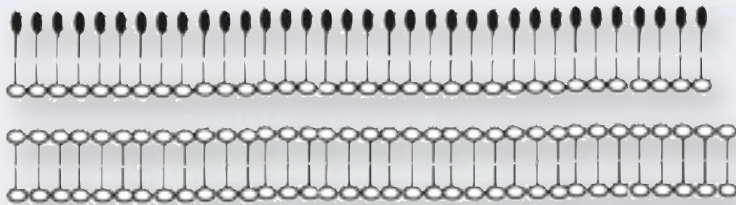
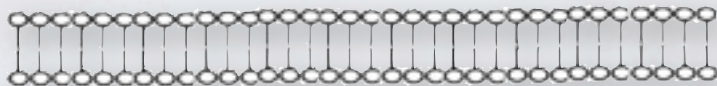
How Salmonella infects host cells

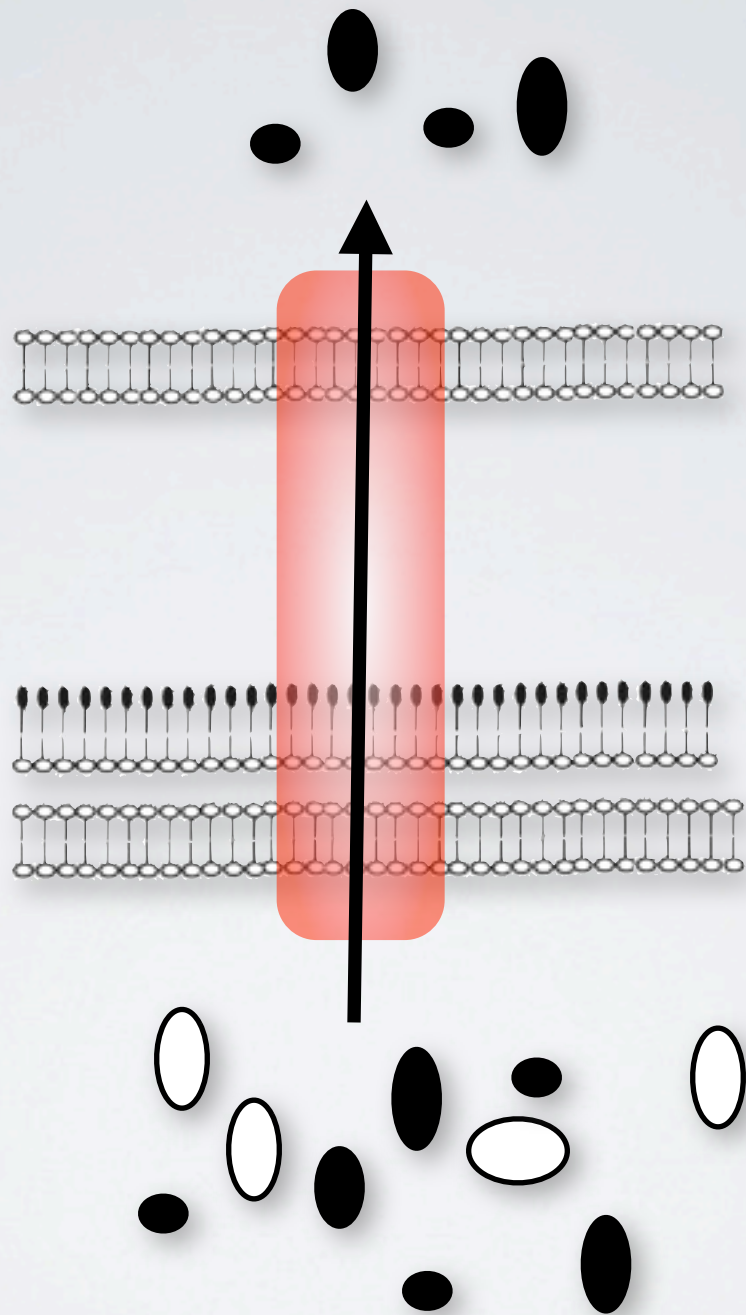


Membrane ruffling on host cell surfaces allows invasion of bacteria

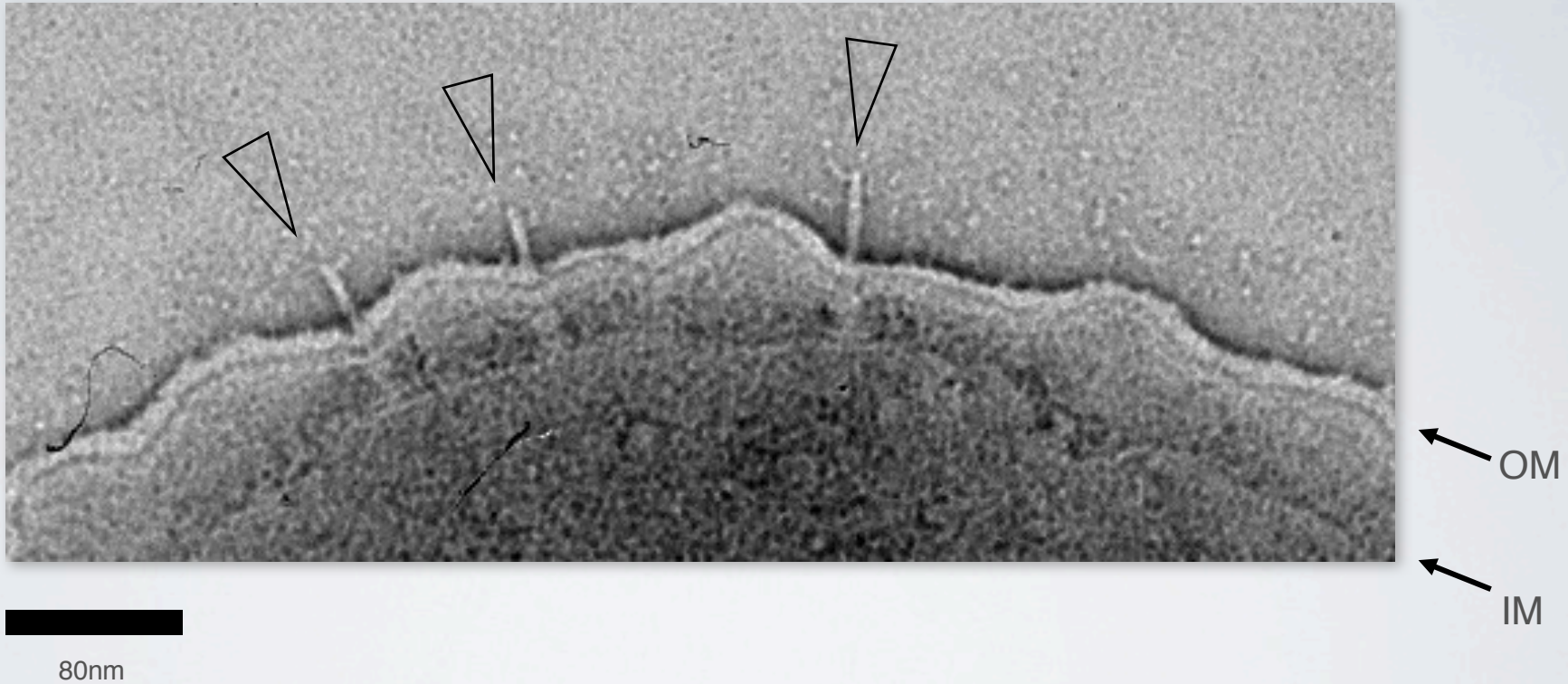
adapted from Galan 2000



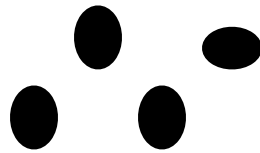
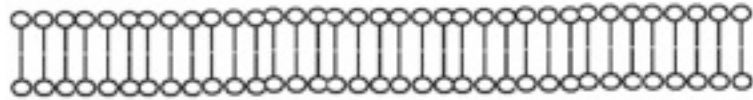
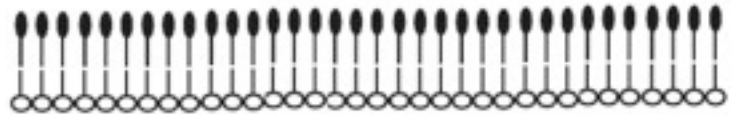
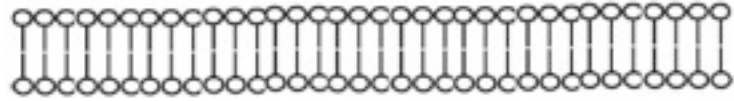




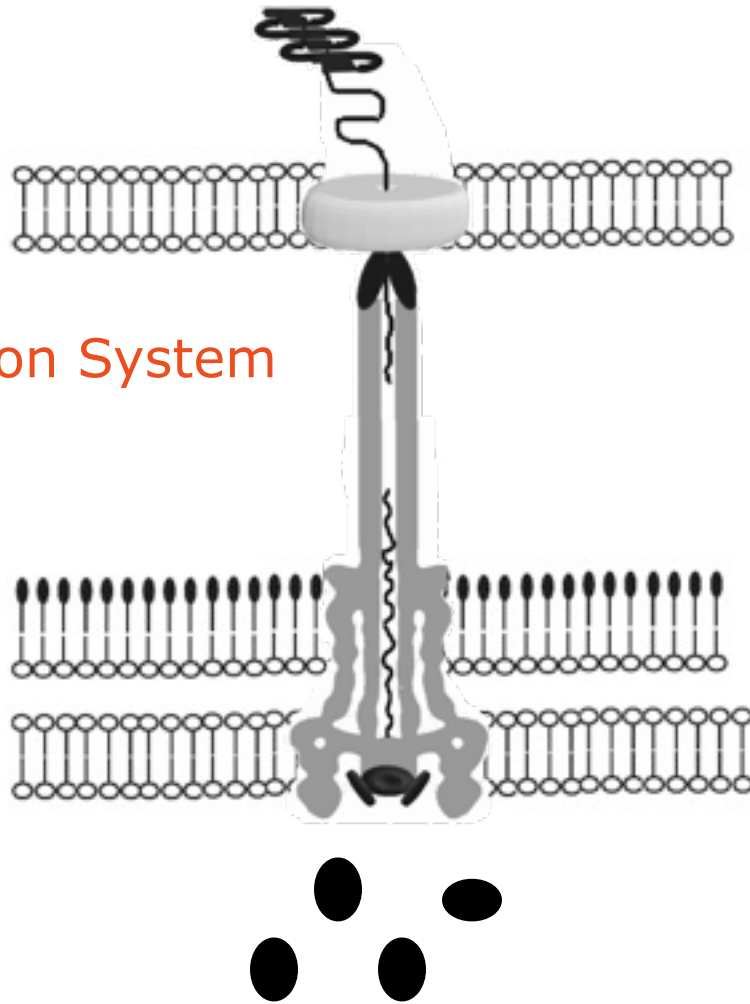
Needle-like structures are embedded in both membranes



EM analysis of osmotically shocked *Salmonella typhimurium* (PTA stained)



Type III Secretion System



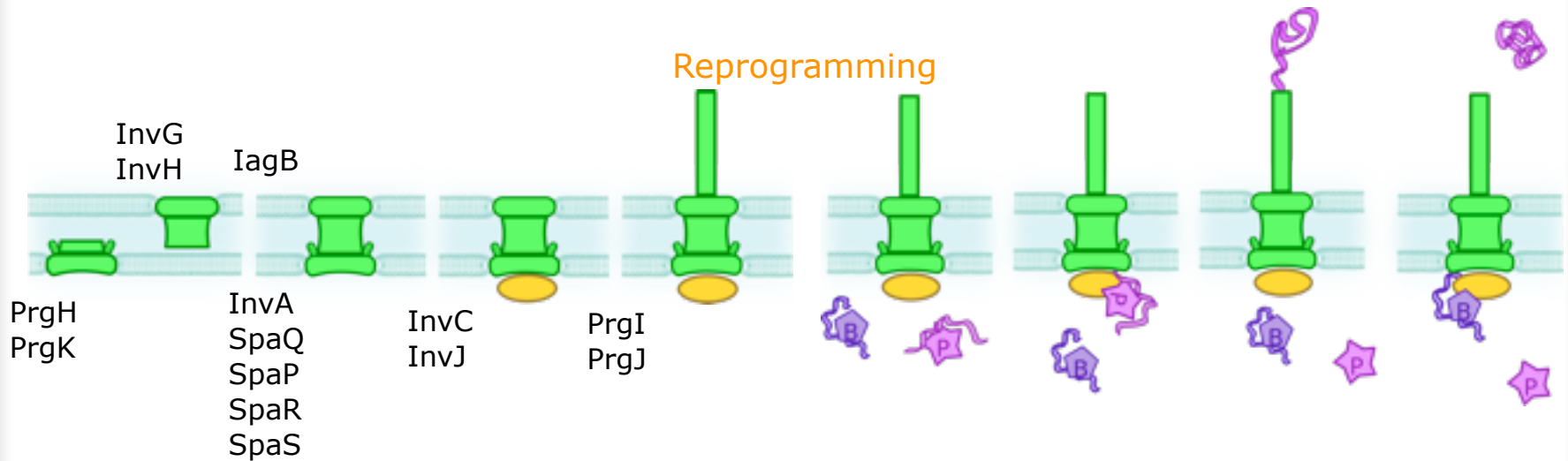
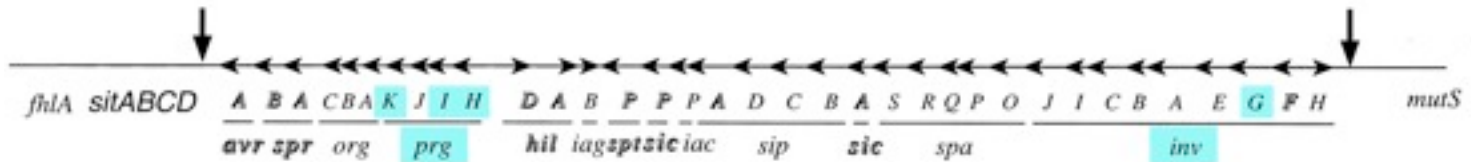
Assembly

Substrate selection

Secretion

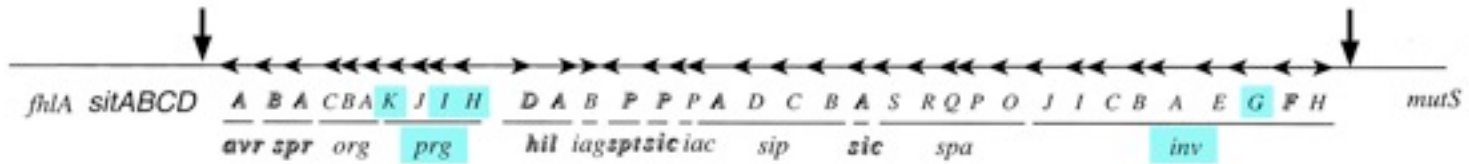
Assembly of the type III secretion system

Genes encoded on specific pathogenicity island



Assembly of the type III secretion system

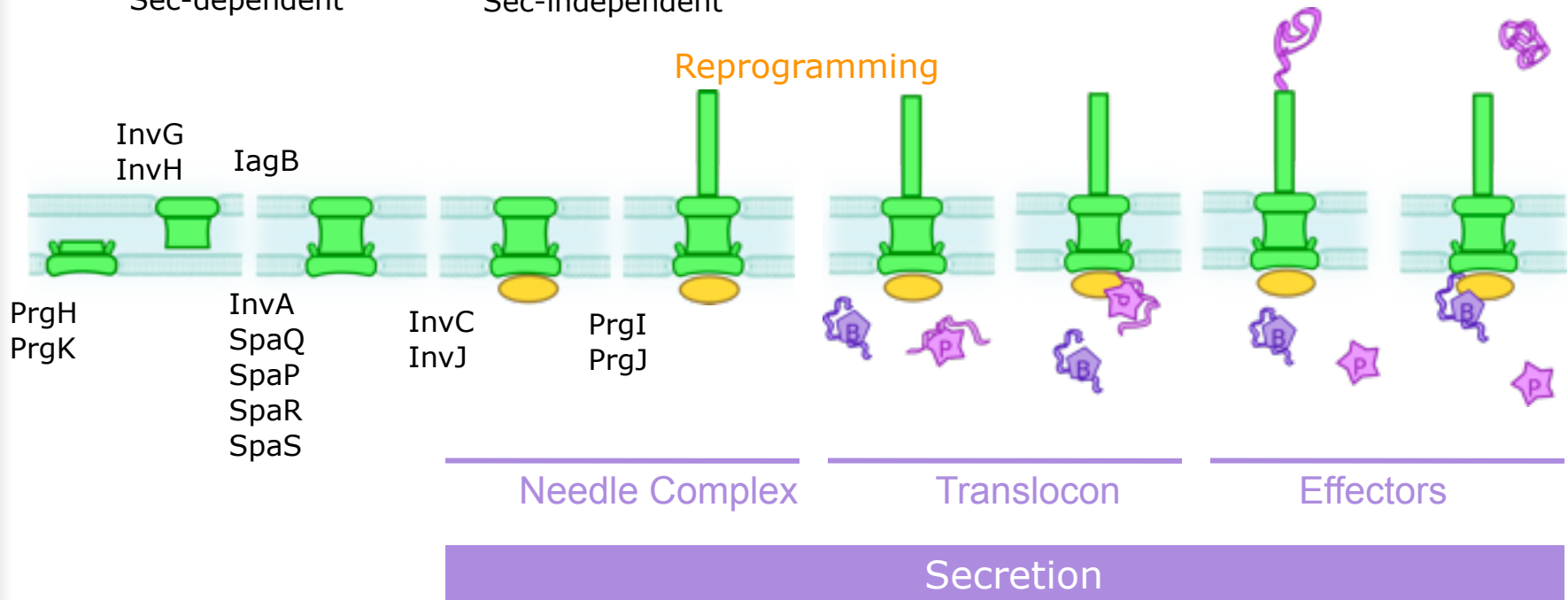
Genes encoded on specific pathogenicity island



Assembly

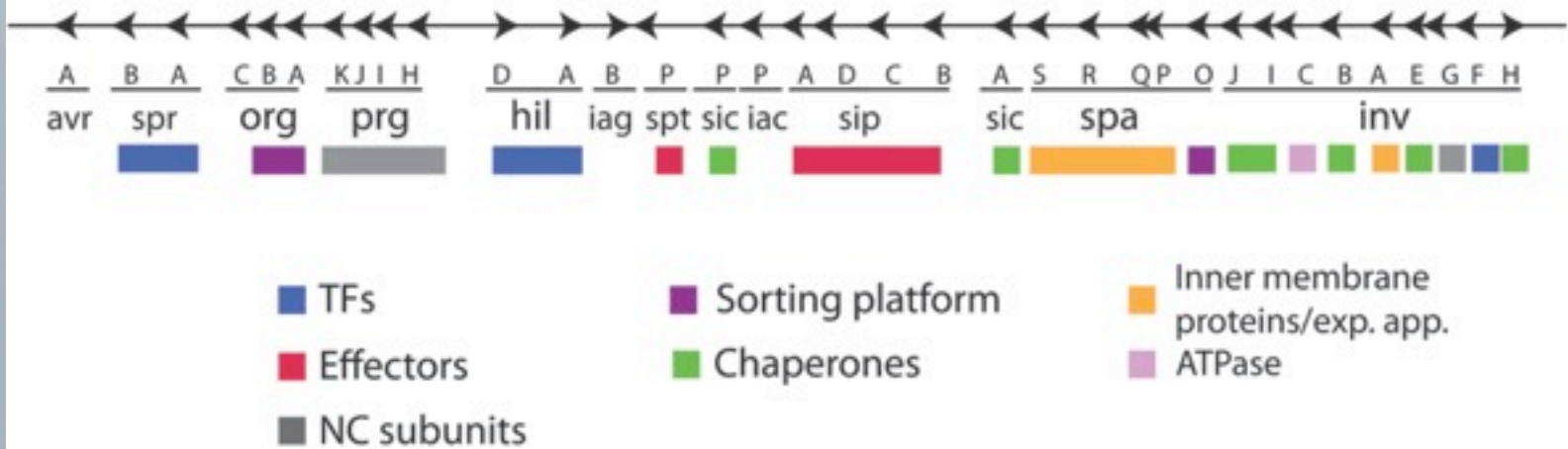
Sec-dependent

Sec-independent

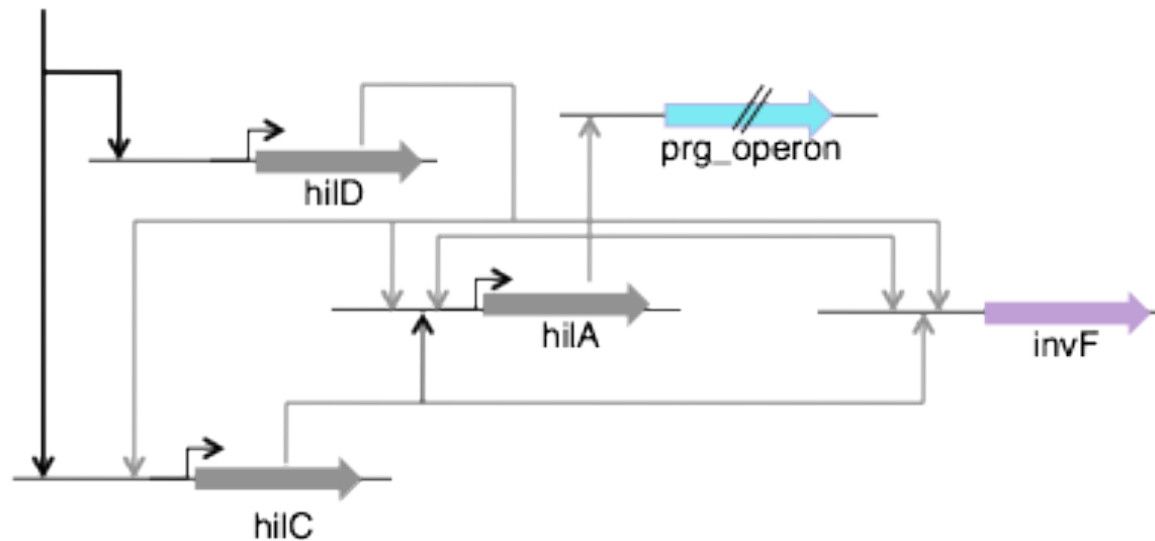


Pathogenicity Island SPI-1

SPI-1

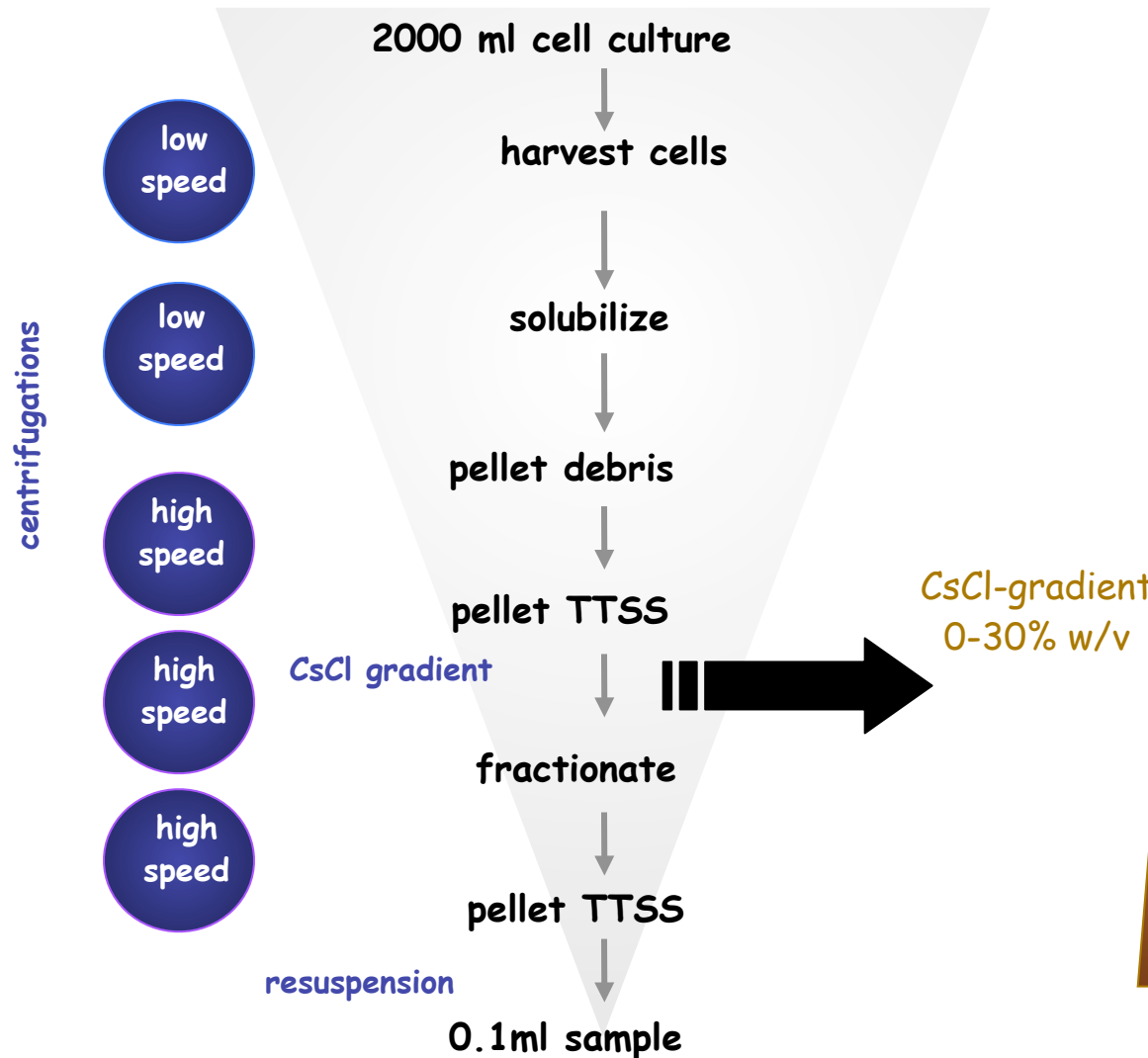


environmental cues

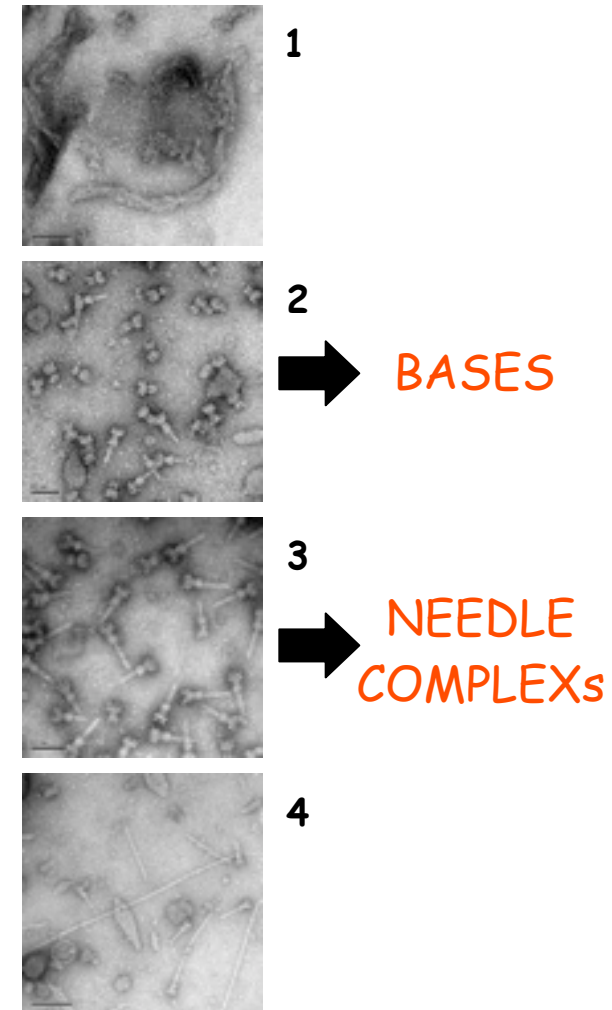


Purification of the BASE and the NEEDLE COMPLEX

Flow-chart of the purification protocol



TEM analysis of fraction:

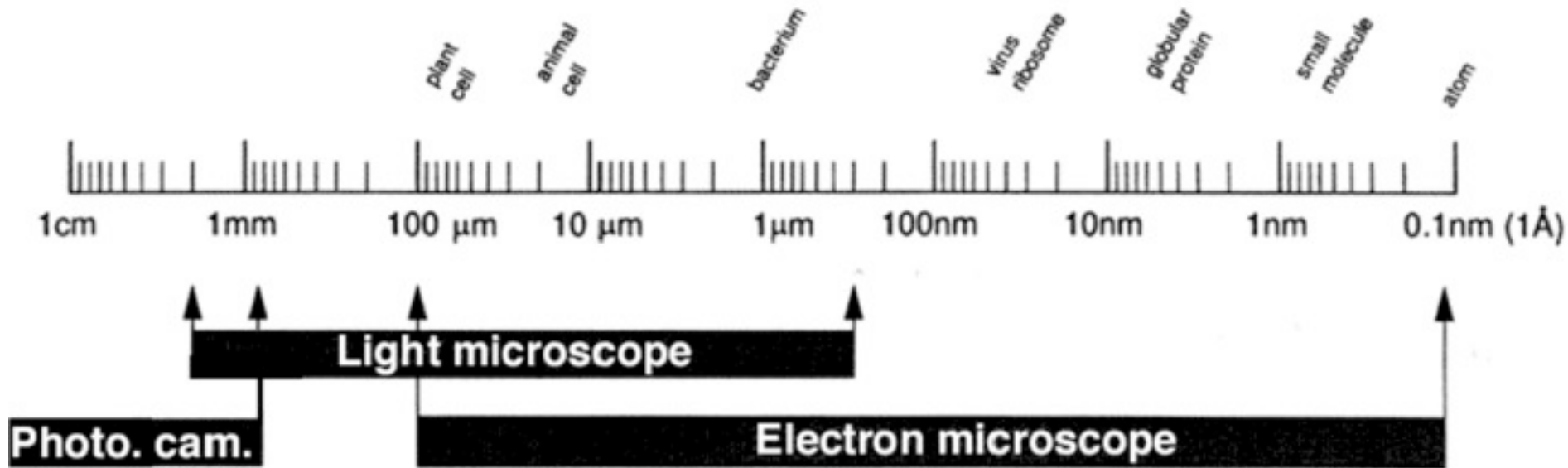
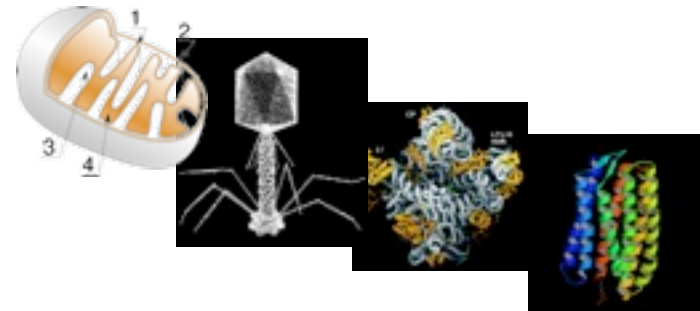


Cryo electron microscopy & Single Particle Analysis

The range of electron microscopes

CELLULAR

ATOMIC



Ernst Ruska



Prof. Ernst Ruska
1906-1988

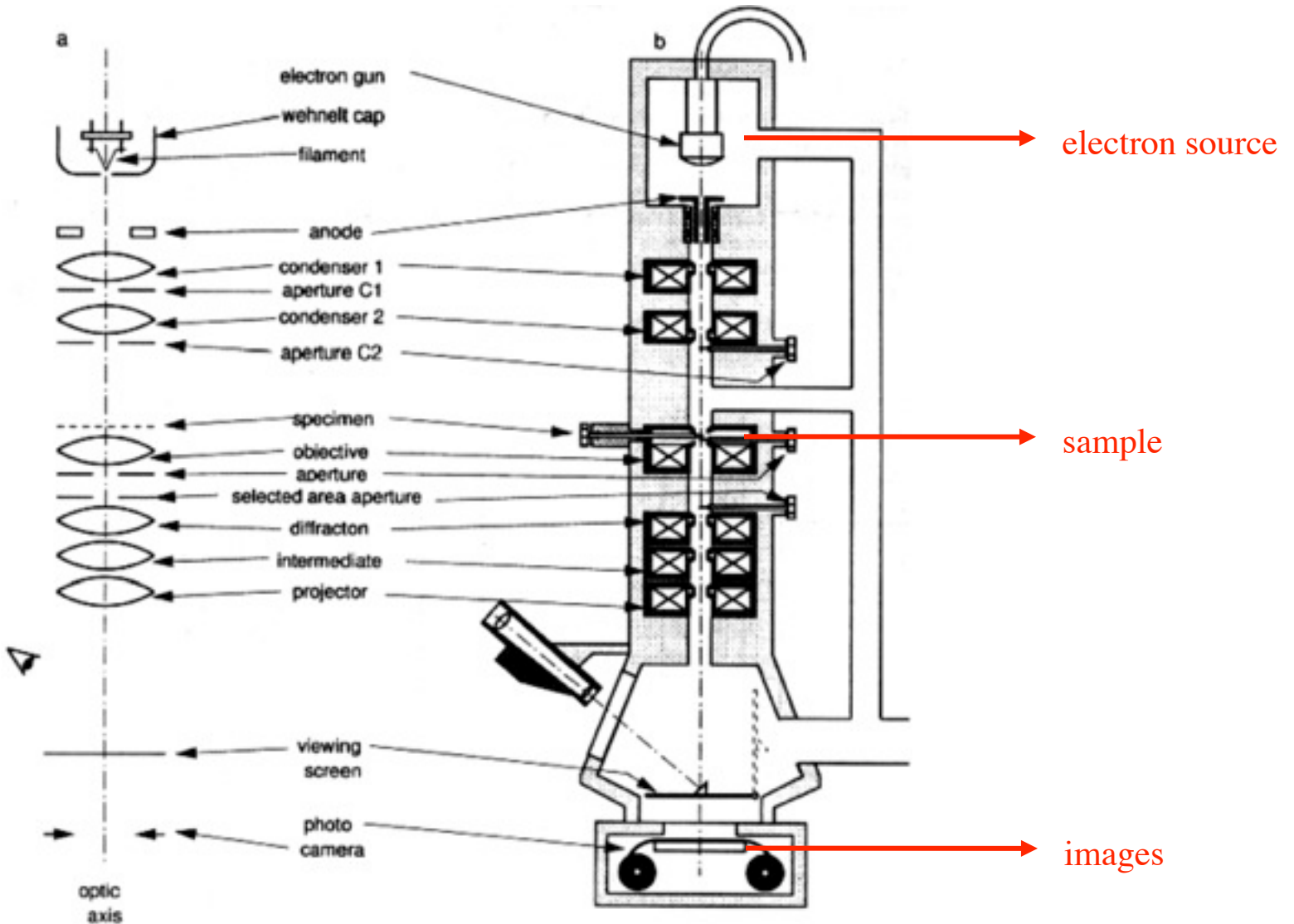
1933: 1st Electron microscope

1986: Nobel price physics
together with G. Binning and H. Rohrer

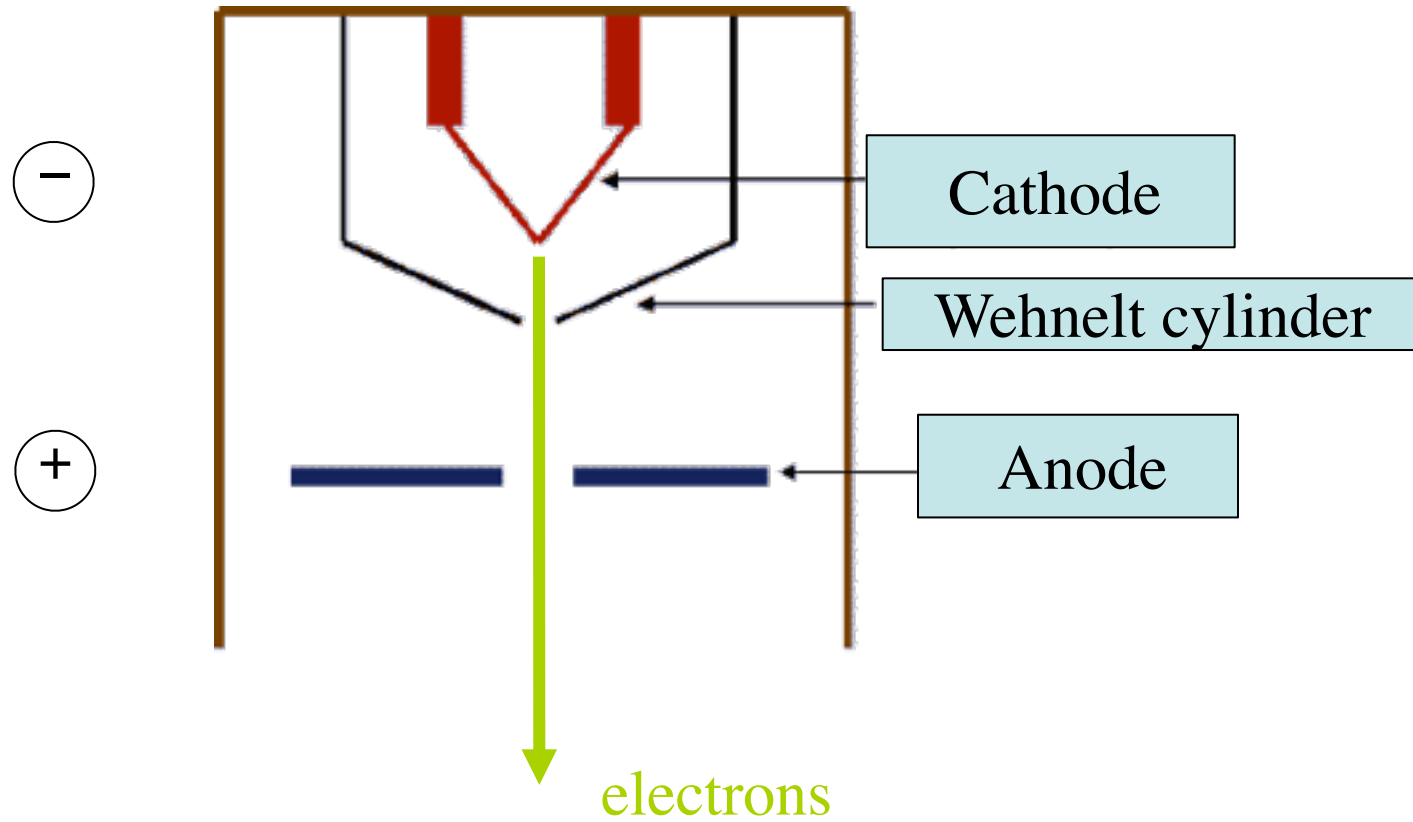
Electron Microscope



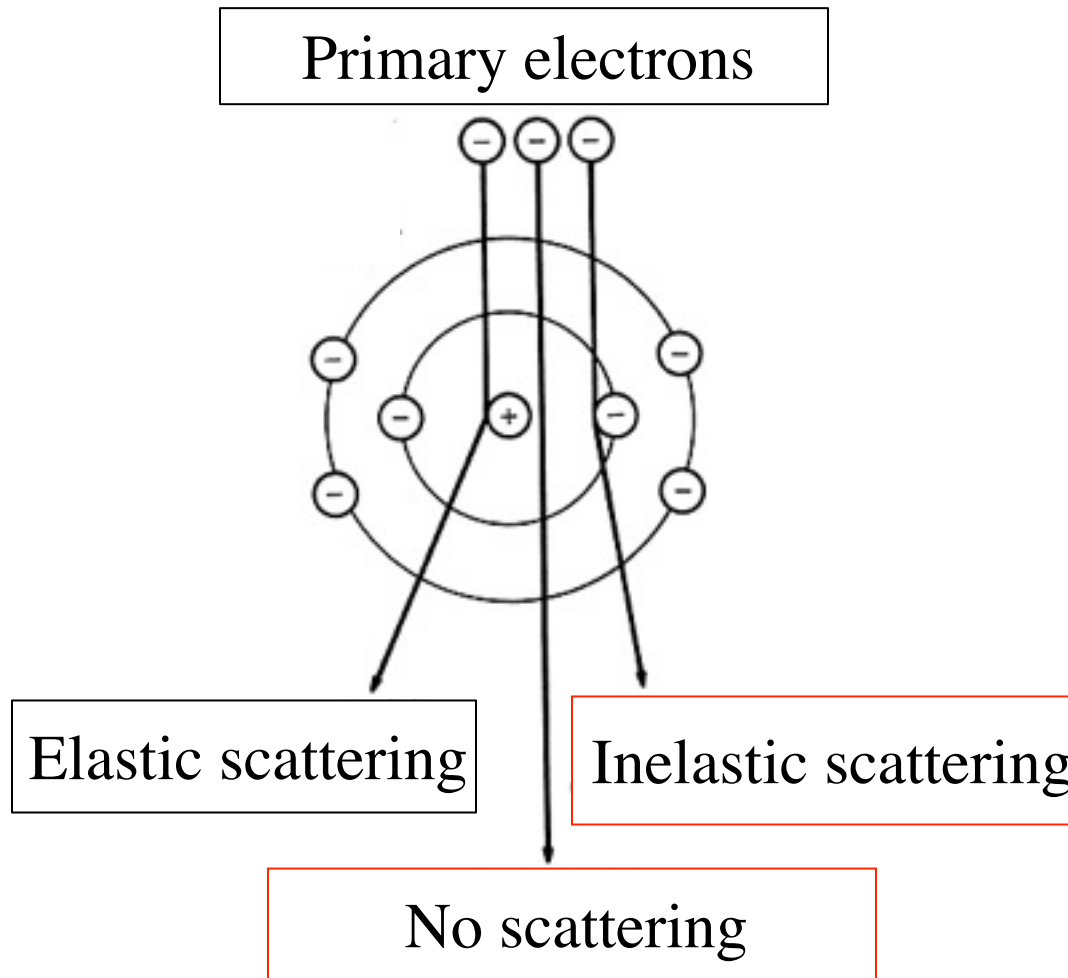
Transmission TEM



Electron Source

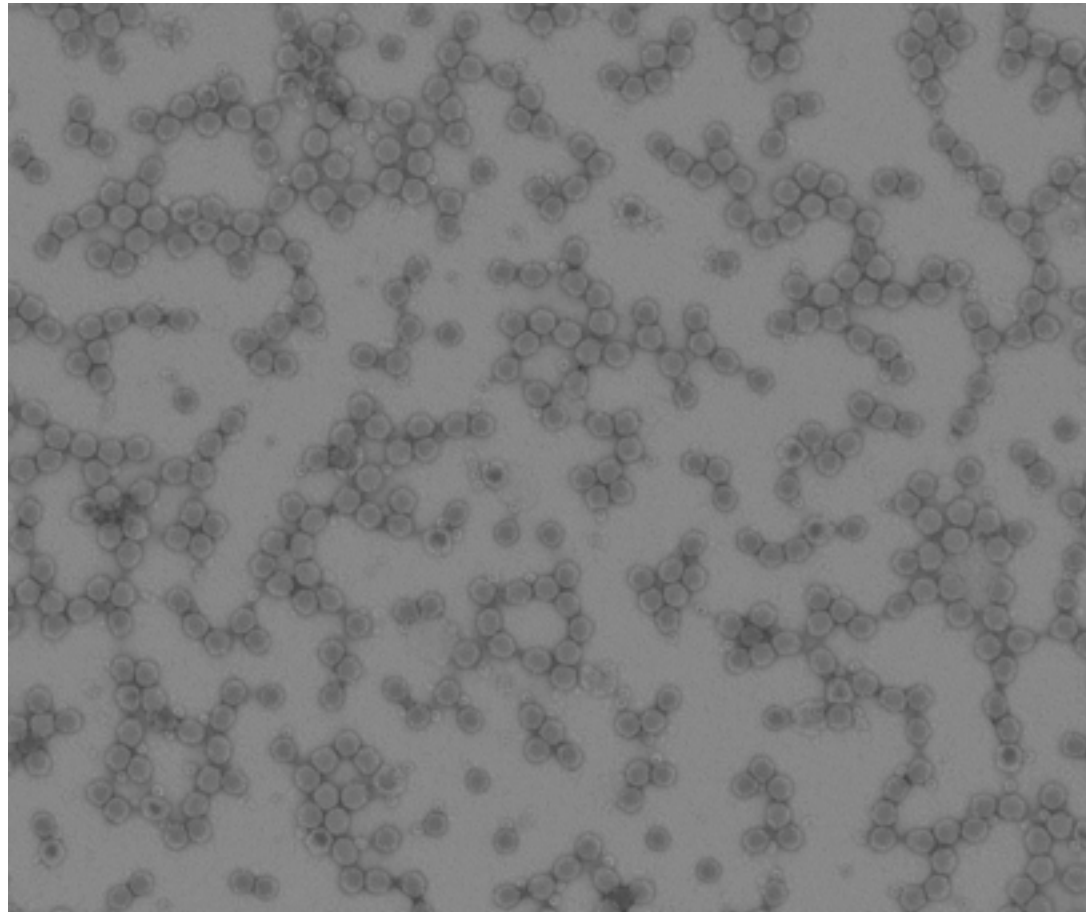


Sample: Interaction with electrons



Biological Samples

Human rhinovirus serotype 2 (d=30nm)



Periodic Table of the Elements

1 IA		New Original										18 VIIIA																																																																																																																			
1 H Hydrogen 1.00784	2 He Helium 4.002602											3 B Boron 10.811	4 C Carbon 12.011	5 N Nitrogen 14.0064	6 O Oxygen 15.999	7 F Fluorine 18.9984032	8 Ne Neon 20.1797	9 Na Sodium 22.98976928	10 Mg Magnesium 24.304	11 Al Aluminum 26.9815386	12 Si Silicon 28.0855	13 P Phosphorus 30.973761998	14 S Sulfur 32.06	15 Cl Chlorine 35.453	16 Ar Argon 39.948	17 K Potassium 39.0983	18 Ca Calcium 40.078	19 Sc Scandium 44.955912	20 Ti Titanium 47.867	21 V Vanadium 50.9415	22 Cr Chromium 51.9961	23 Mn Manganese 54.938044	24 Fe Iron 55.845	25 Co Cobalt 58.933200	26 Ni Nickel 58.6934	27 Cu Copper 63.546	28 Zn Zinc 65.408	29 Ga Gallium 69.723	30 Ge Germanium 72.64	31 As Arsenic 74.9216	32 Se Selenium 78.96	33 Br Bromine 79.904	34 Kr Krypton 83.796	35 Rb Rubidium 85.4678	36 Sr Strontium 87.62	37 Y Yttrium 88.90584	38 Zr Zirconium 91.224	39 Nb Niobium 92.90638	40 Mo Molybdenum 95.94	41 Tc Technetium 98	42 Ru Ruthenium 101.07	43 Rh Rhodium 102.90550	44 Pd Palladium 106.42	45 Ag Silver 107.8682	46 Cd Cadmium 112.411	47 In Indium 114.818	48 Sn Tin 118.710	49 Sb Antimony 121.757	50 Te Tellurium 127.6	51 I Iodine 126.90547	52 Xe Xenon 131.29	53 Cs Cesium 132.90545	54 Ba Barium 137.327	55 La Lanthanum 138.90547	56 Ce Cerium 140.12	57 Pr Praseodymium 140.90766	58 Nd Neodymium 144.242	59 Pm Promethium 145	60 Sm Samarium 150.36	61 Eu Europium 151.964	62 Gd Gadolinium 157.25	63 Tb Terbium 158.92534	64 Dy Dysprosium 162.500	65 Ho Holmium 164.93032	66 Er Erbium 167.258	67 Tm Thulium 168.93047	68 Yb Ytterbium 173.054	69 Lu Lutetium 174.967	70 Hf Hafnium 178.49	71 Ta Tantalum 180.94788	72 W Tungsten 183.84	73 Re Rhenium 186.207	74 Os Osmium 190.22	75 Ir Iridium 192.222	76 Pt Platinum 195.078	77 Au Gold 196.96657	78 Hg Mercury 200.59	79 Tl Thallium 204.3833	80 Pb Lead 207.2	81 Bi Bismuth 208.98040	82 Po Polonium 209	83 At Astatine 210	84 Rn Radon 222	85 Fr Francium 223	86 Ra Radium 226	87 Ac Actinium 227	88 Th Thorium 232.0377	89 Pa Protactinium 231.036888	90 U Uranium 238.02891	91 Np Neptunium 237	92 Pu Plutonium 244	93 Am Americium 243	94 Cm Curium 247	95 Bk Berkelium 247	96 Cf Californium 251	97 Es Einsteinium 252	98 Fm Fermium 257	99 Md Mendelevium 258	100 No Nobelium 259	101 Lr Lawrencium 260	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118

Alkali metals, Alkaline earth metals, Transition metals, Lanthanide series, Actinide series, Poor metals, Nonmetals, Noble gases, Solid, Liquid, Gas, Synthetic.

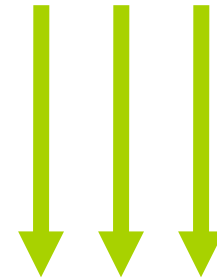
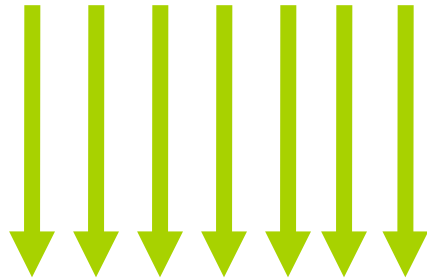
Atomic masses in parentheses are those of the most stable or common isotope.

Note: The subgroup numbers 1-10 were adopted in 1984 by the International Union of Pure and Applied Chemistry. The names of elements 112-118 are the Latin equivalents of those numbers.

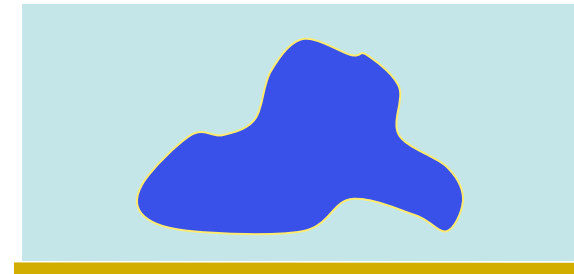
Biological Samples

Staining UAc, PTA, AM
(negative staining)

no staining



3D-volume

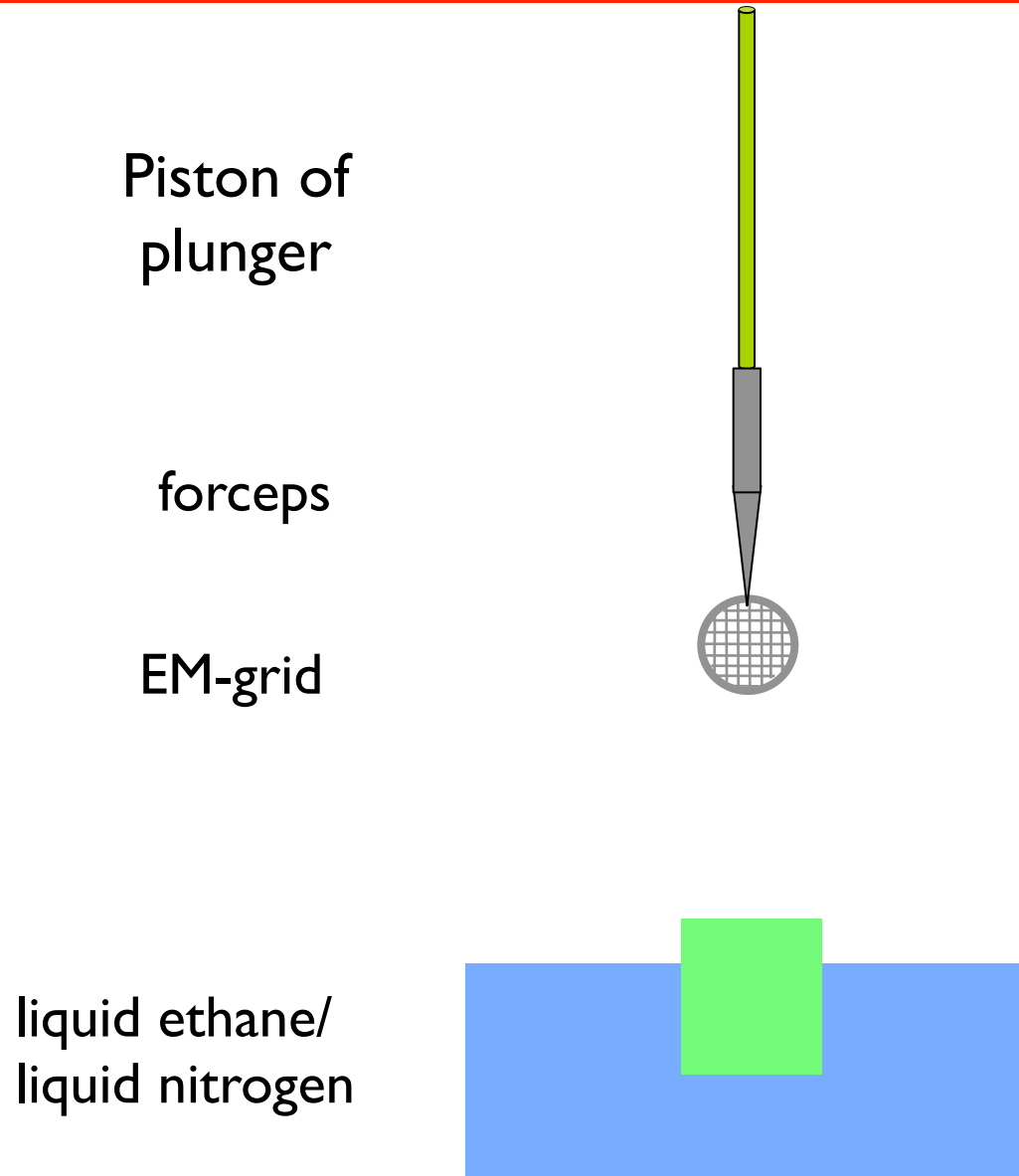


2D-Projection:

excluded volume

macromolecule

Sample vitrification



modified from V. Unger

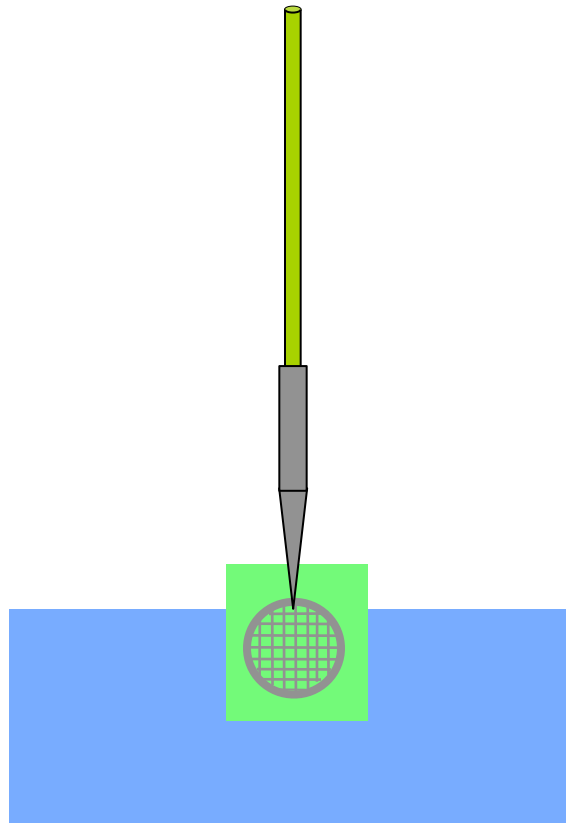
Sample vitrification

Piston of
plunger

forceps

EM-grid

liquid ethane/
liquid nitrogen



vitrification occurs
at 10^5 °C/sec

modified from V. Unger

Sample Vitrification



manual

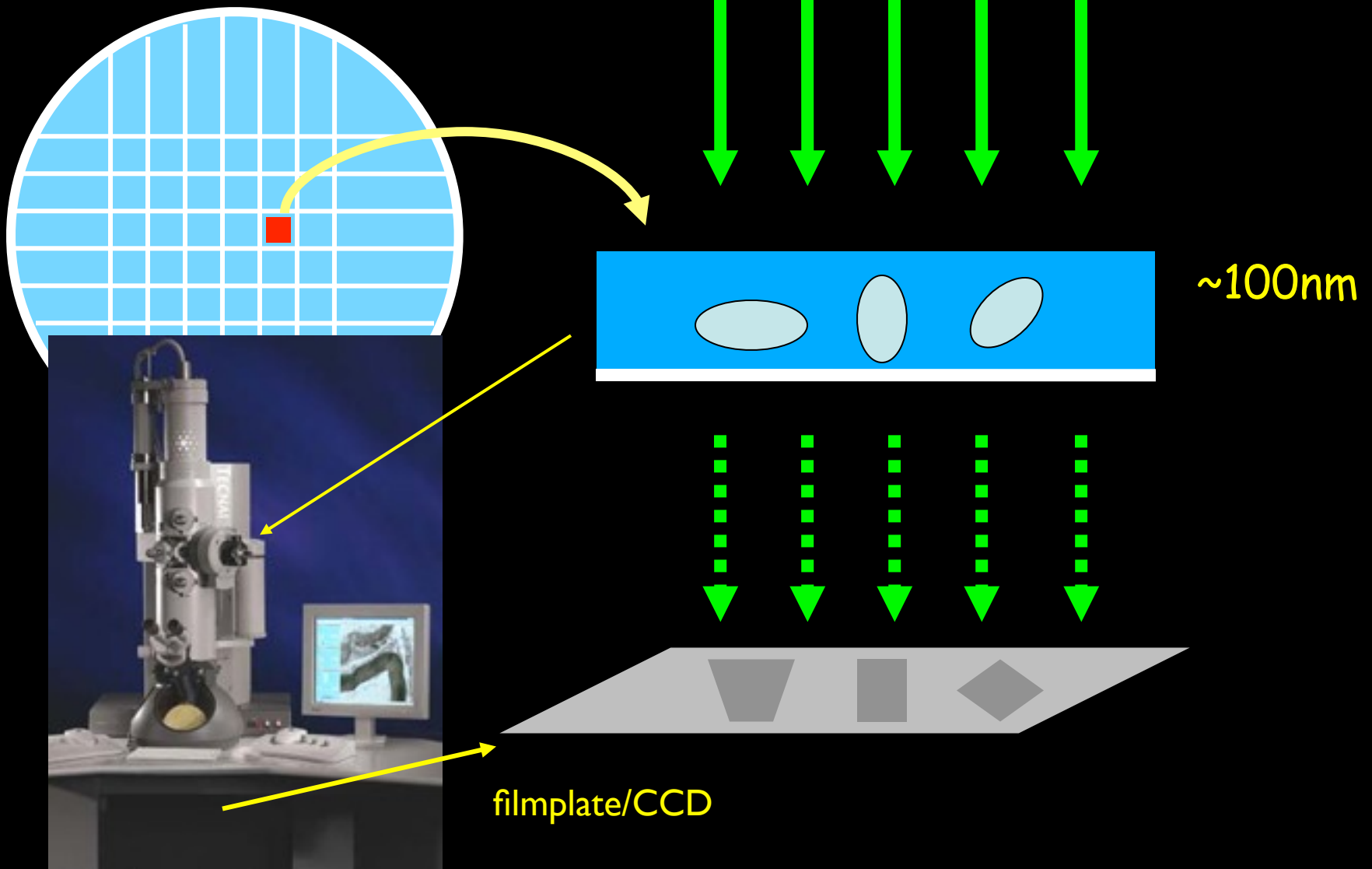


'automatic' (Vitrobot)

@ IMP/IMBA

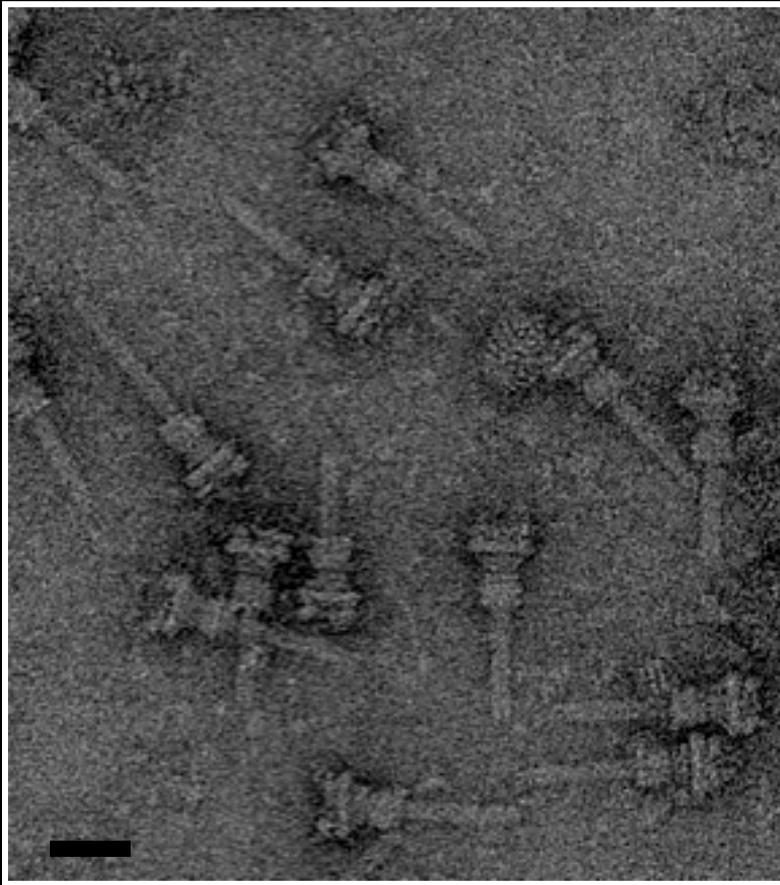


Cryo Electron Microscopy

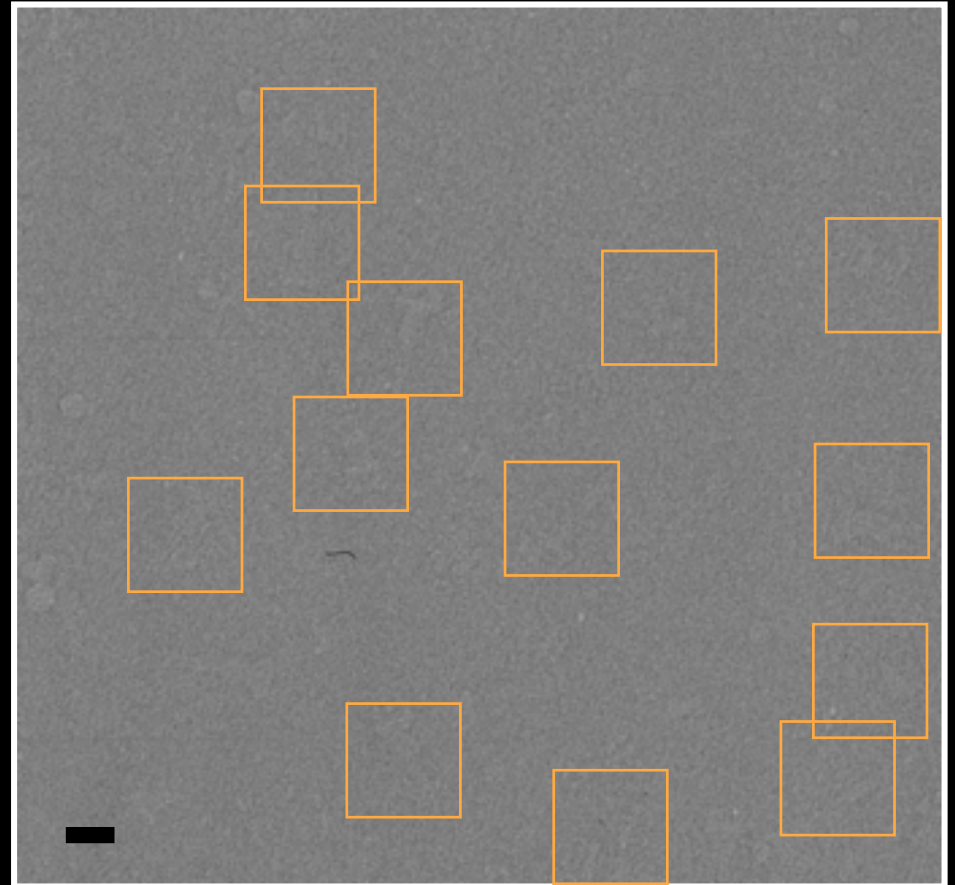


Vitrified samples show very low contrast

NEG. STAIN



CRYO

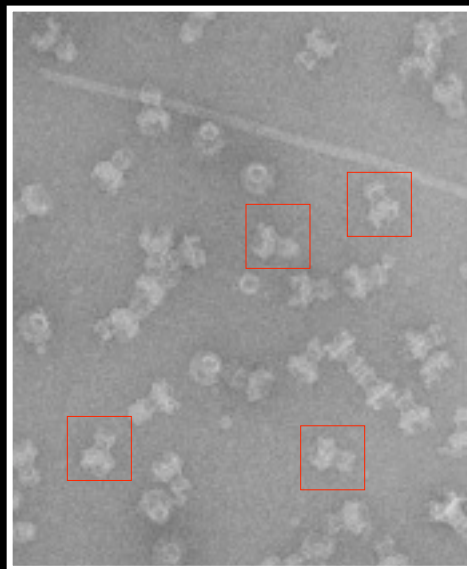


(bar 30nm)

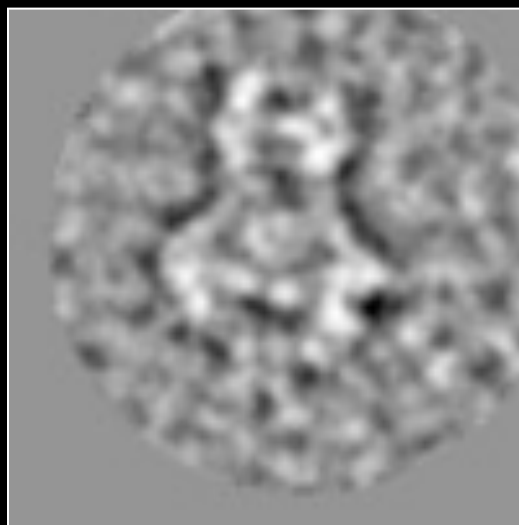
Classification: averaging improves the signal/noise ratio

Particle Selection/
Noise Reduction/
Aligning

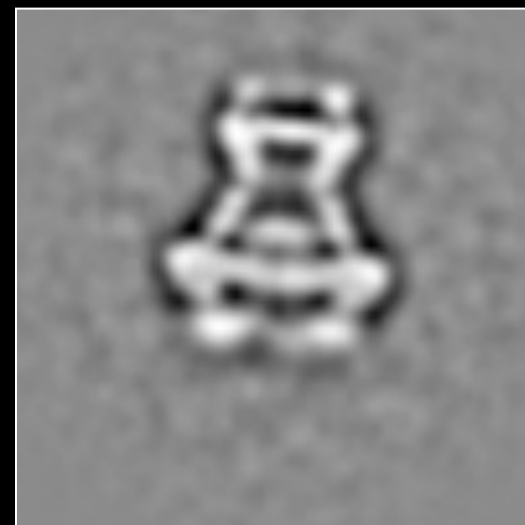
Class-Averages



original
micrograph

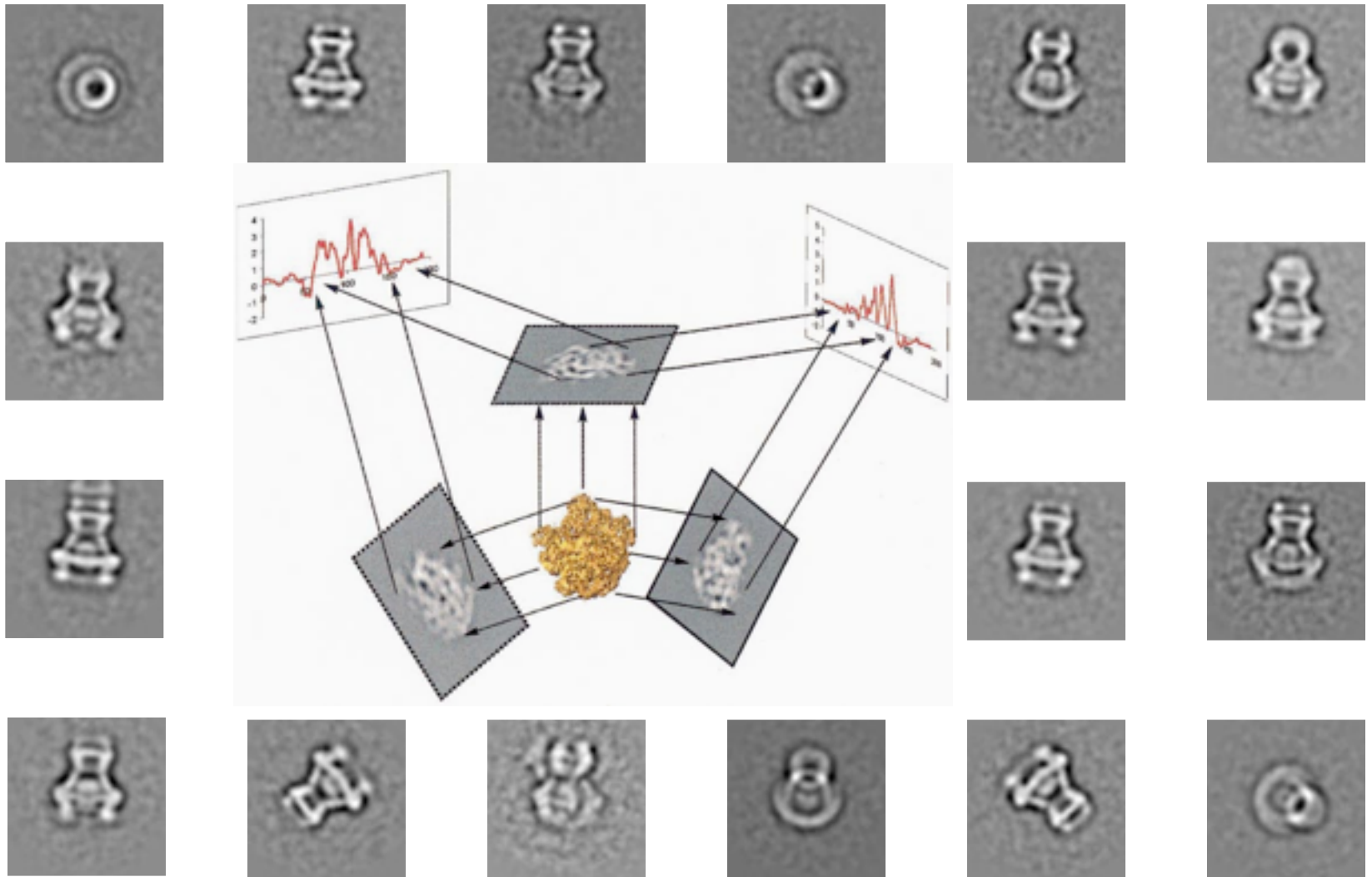


one particle

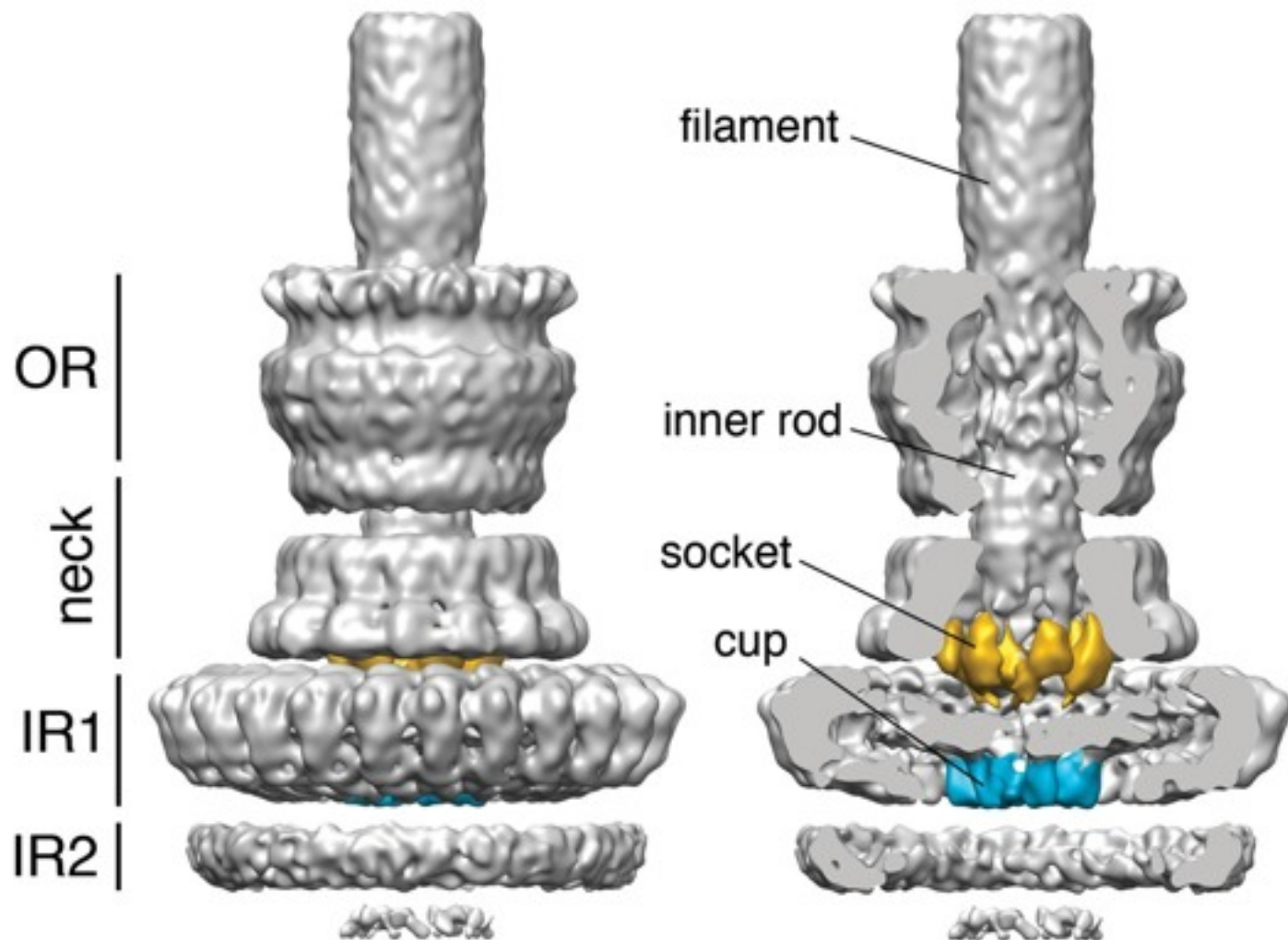


many particles
(one class)

Three-dimensional reconstitution from averaged projections



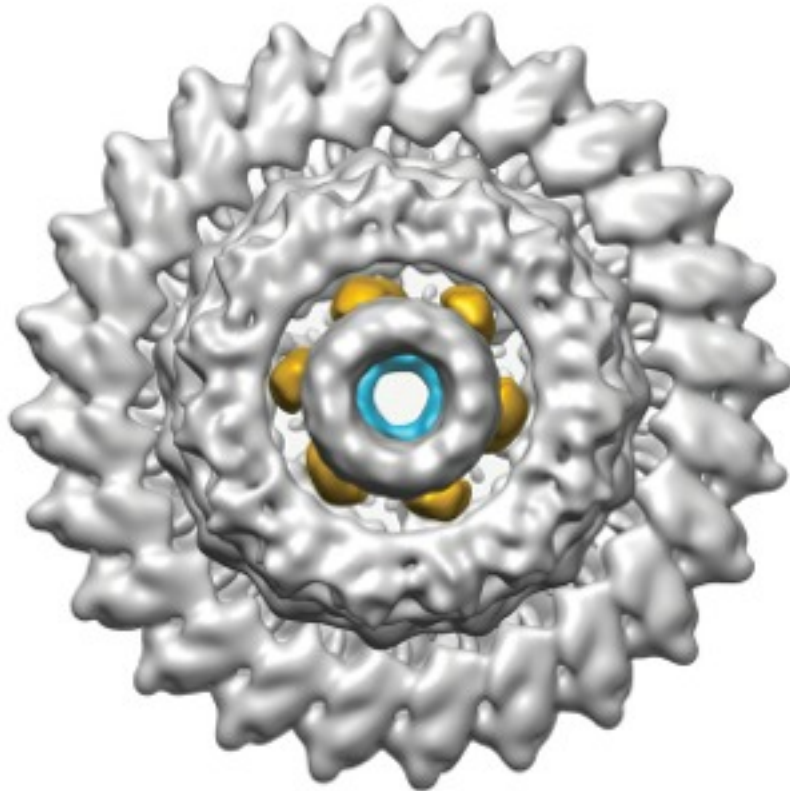
The structure of the needle complex by single particle analysis



Schraidt & Marlovits, Science 2011

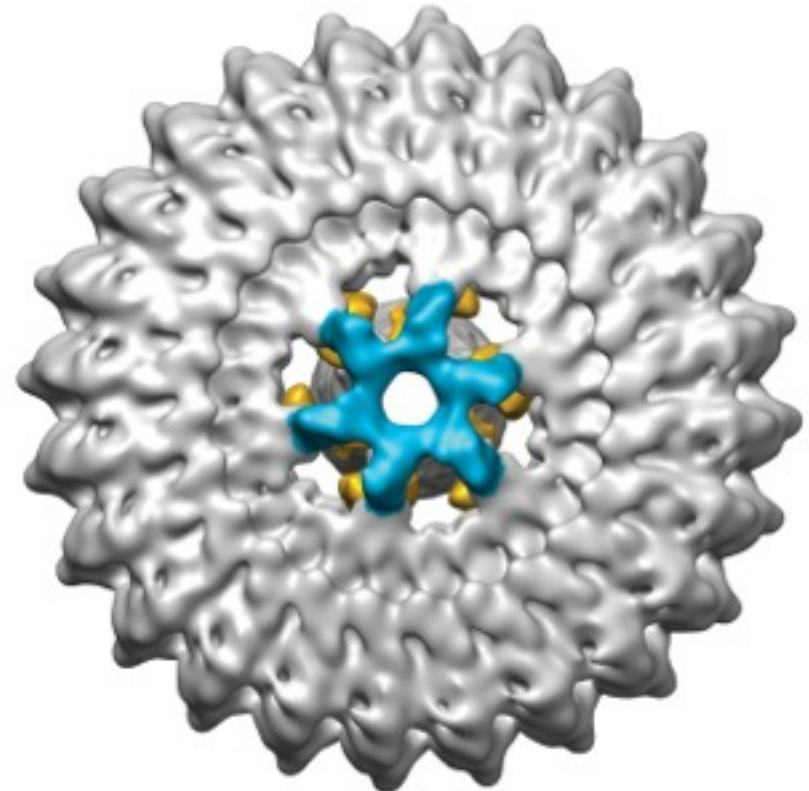
The structure of the needle complex by single particle analysis

top



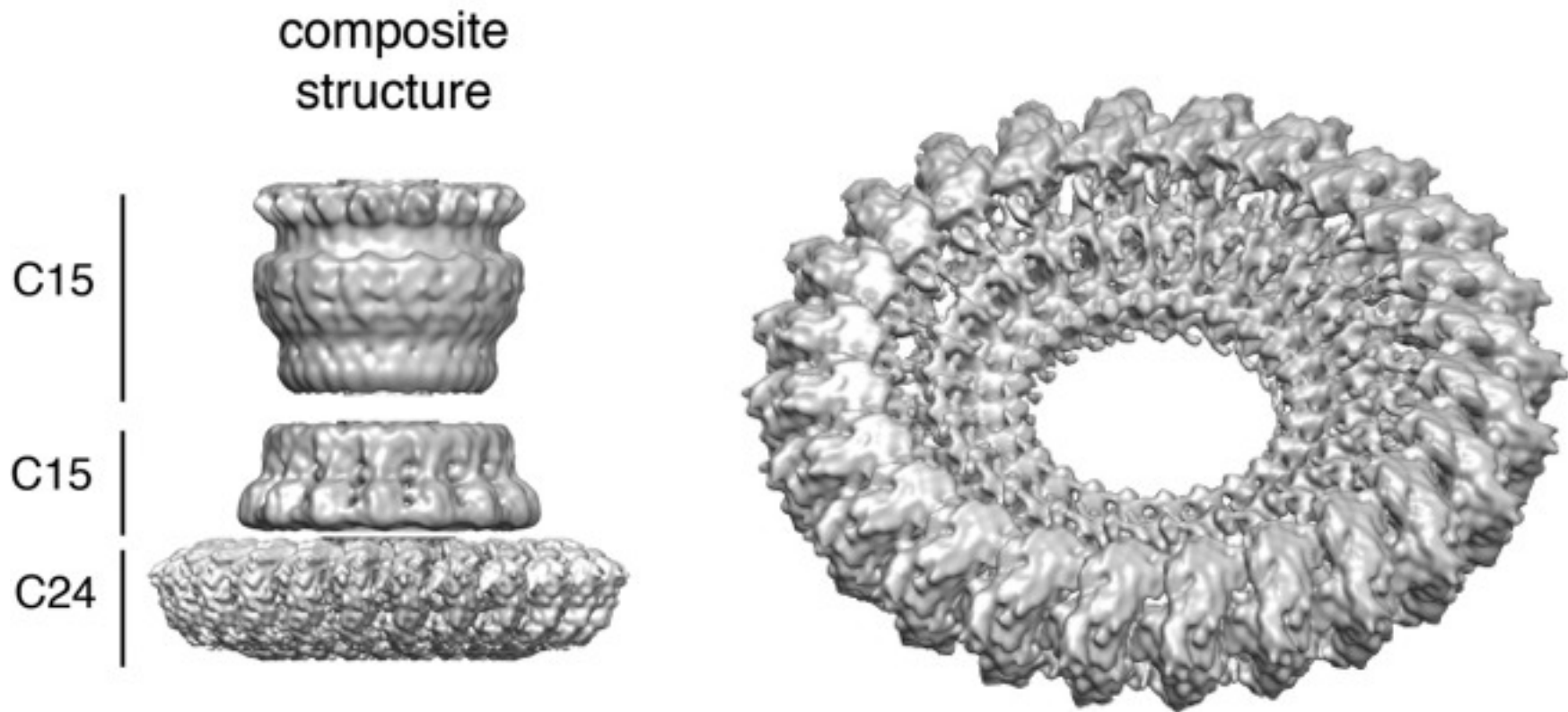
NC (C3)

bottom



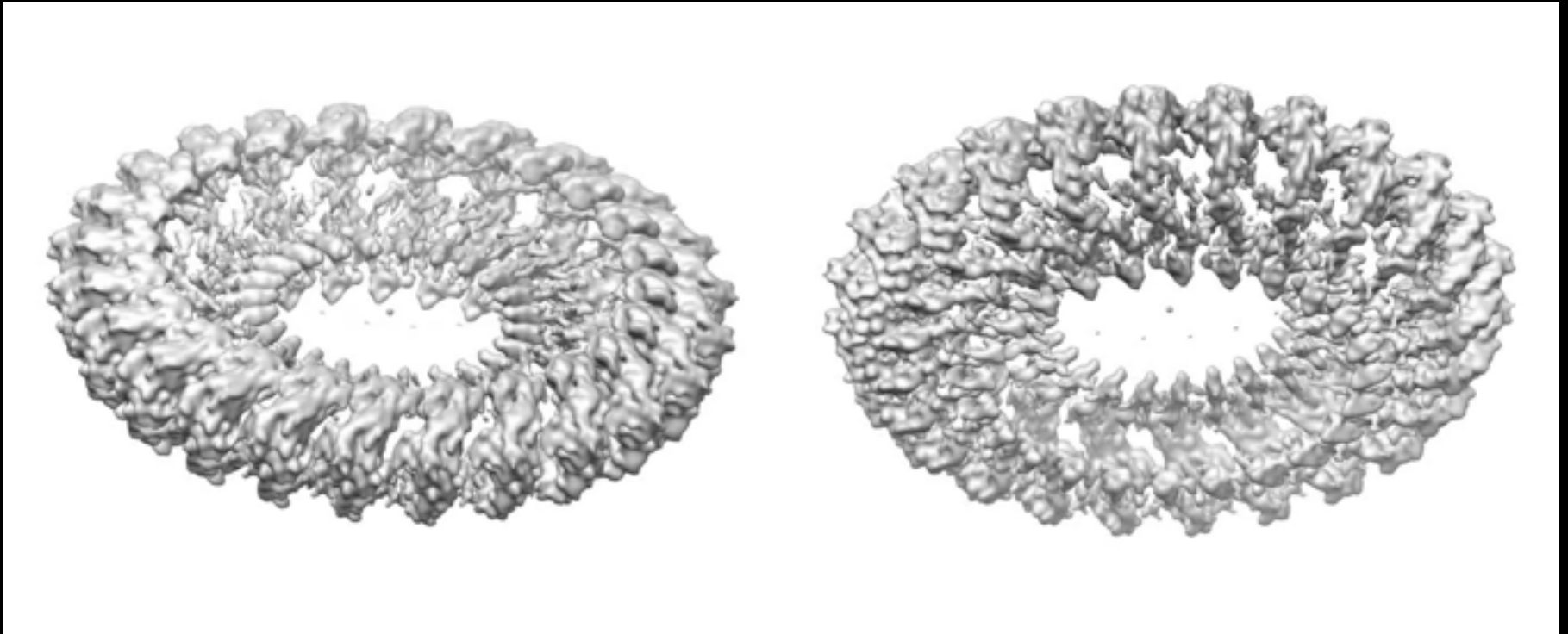
NC (C3)

The structure of the needle complex by single particle analysis



Schraidt & Marlovits, Science 2011

Concentric ring organization of the IR

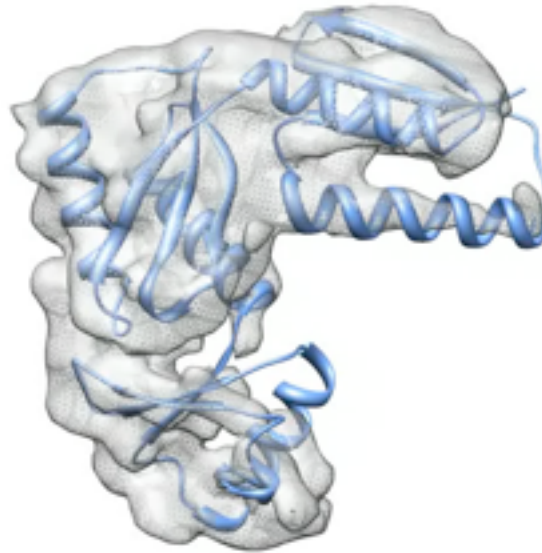


Docking of atomic structures into the EM-envelope



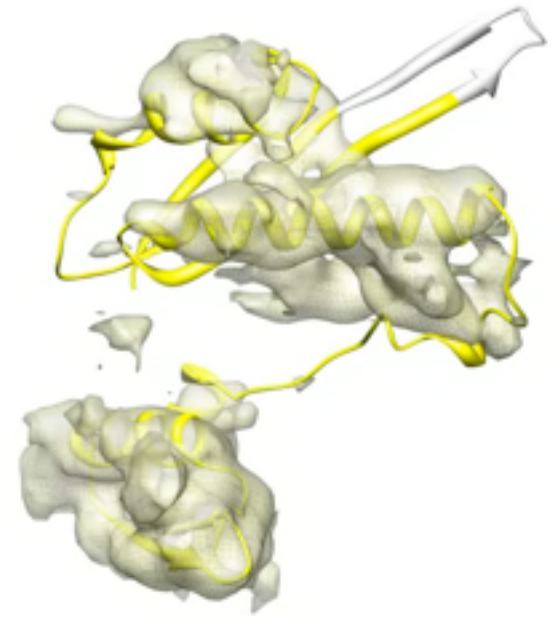
InvG

(MODEL [3GR5]:
N-terminal domain
EscC template)



PrgH

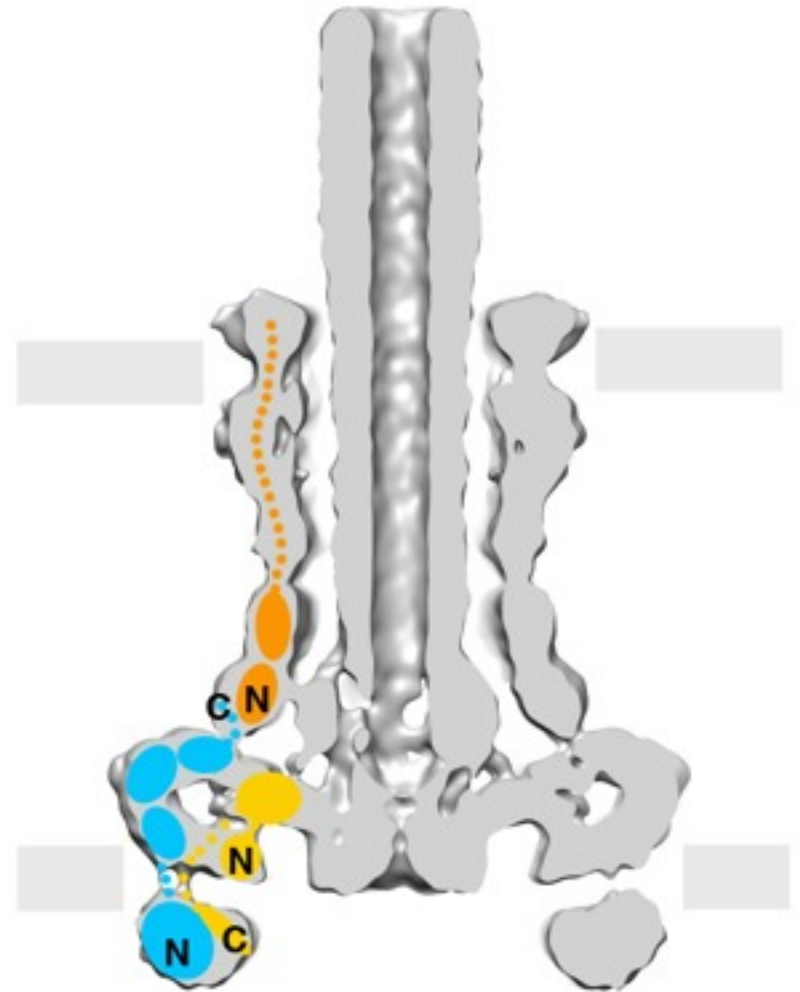
(STRUCTURE [3GR1]
C-terminal domain)



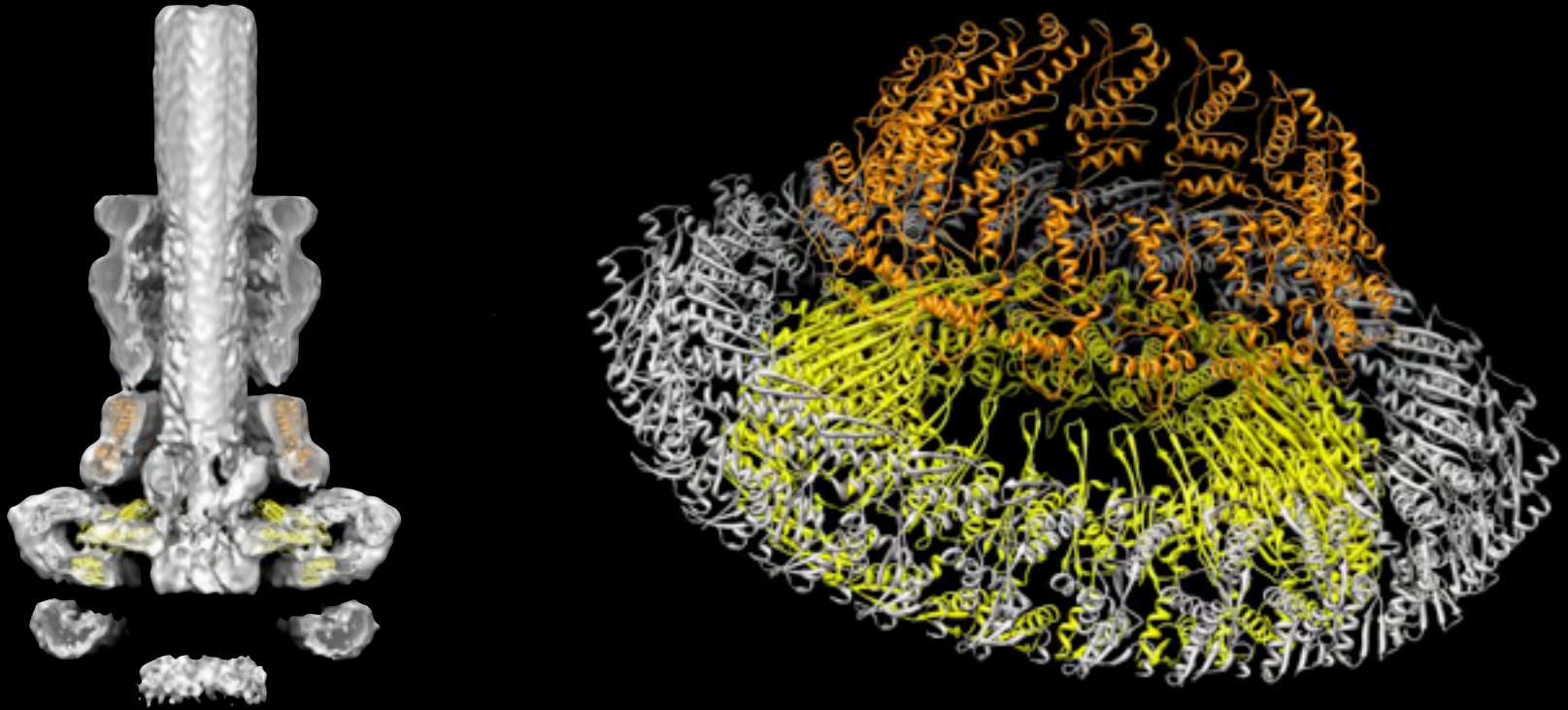
PrgK

(MODEL [1YJ7]:
N-terminal domain
EscJ template)

Topological arrangement within the periplasm:
Local Polarity: The N-termini are facing towards the inner membrane

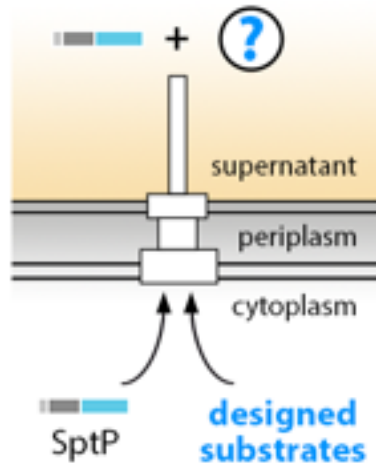


Organization of PrgH/K and InvG within the needle complex



Designed substrates are functional

Secretion test



Designed substrates



SECRETION
(supernatant)

anti SptP

anti FLAG

Set-1

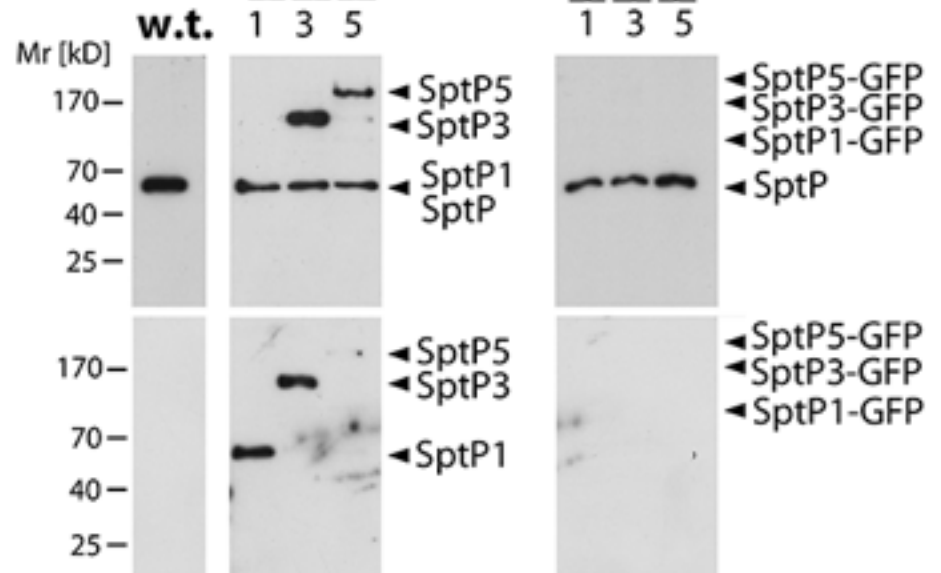
Set-2

+ pSptP

+ pSptP

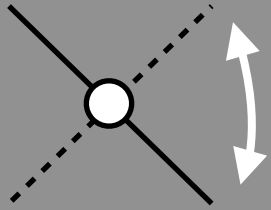
[]

[]-GFP



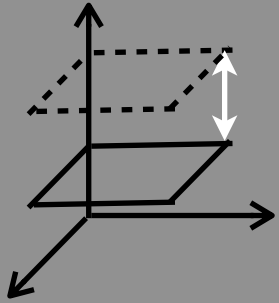
Julia Radics, Lisa Königsmaier

NCs in a cellular context: Cryo electron tomography



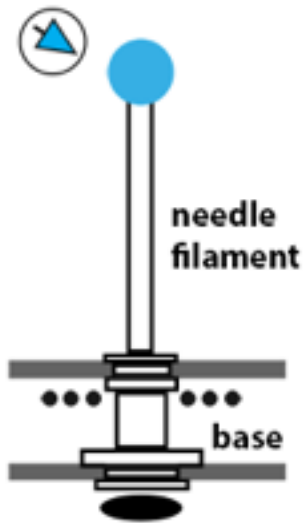
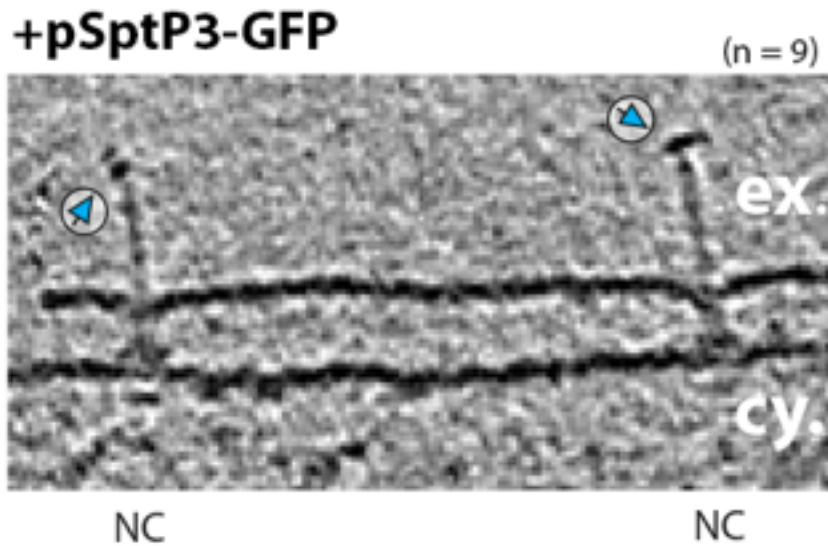
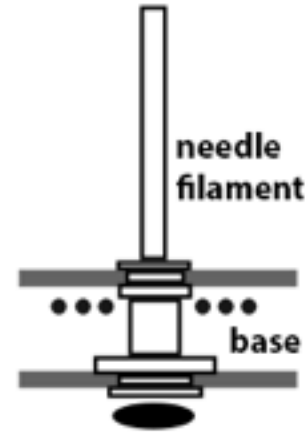
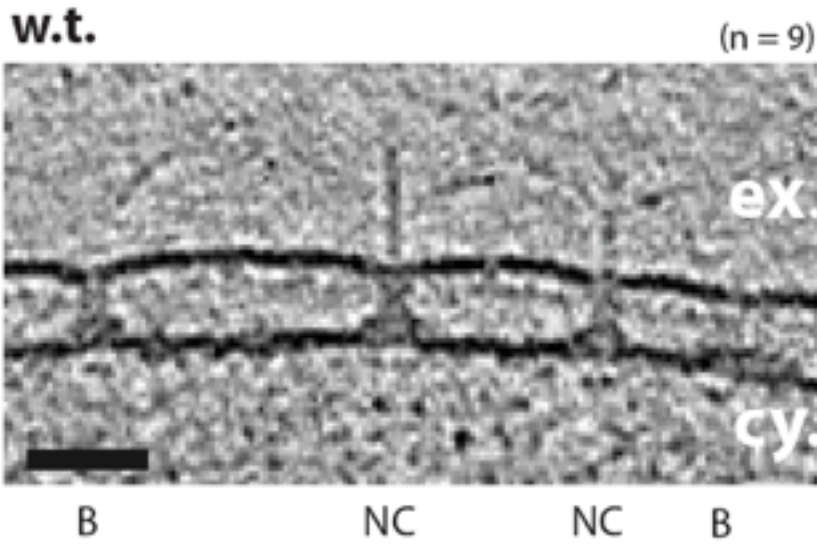
Lisa Königsmaier

NCs in a cellular context: Cryo electron tomography



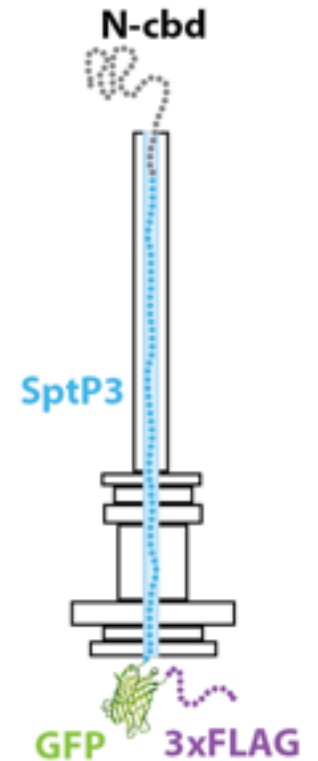
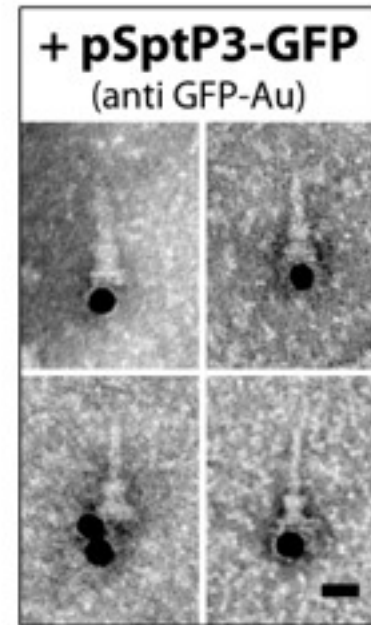
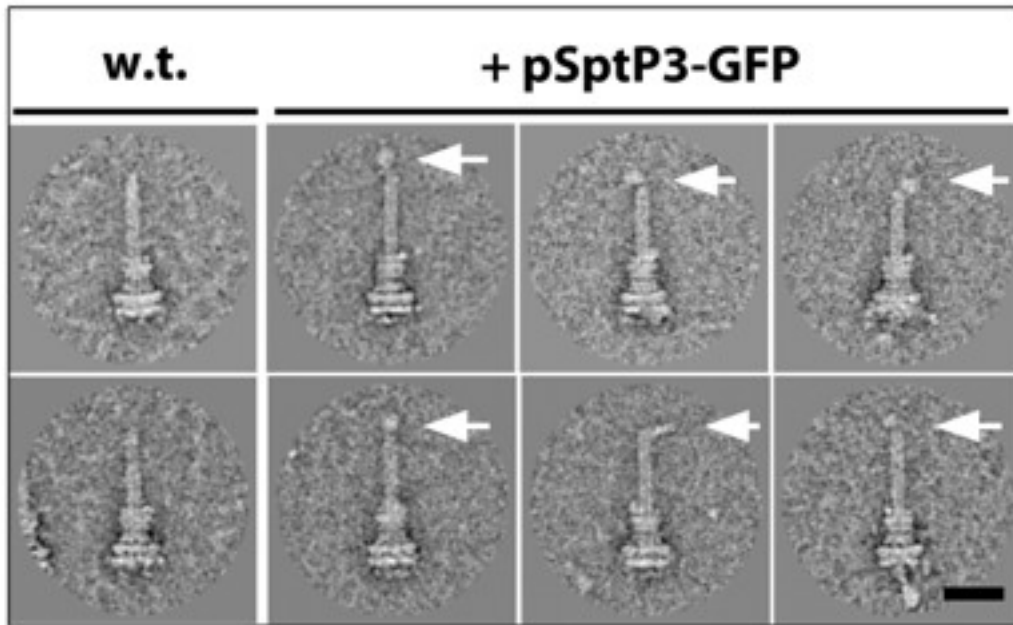
Lisa Königsmaier

Pathogenic injectisomes in action *in situ*



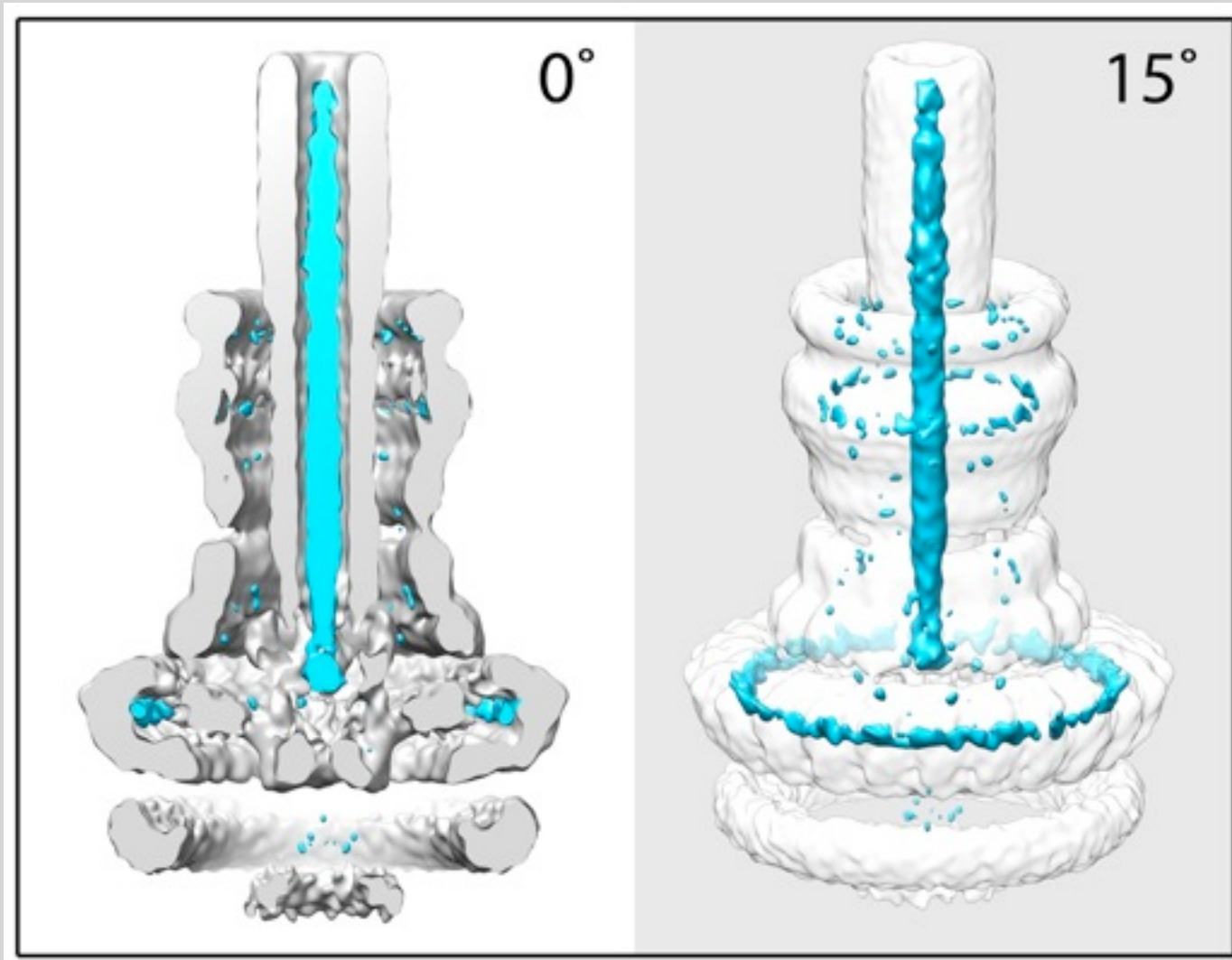
Lisa Königsmaier

Entry and exit position: Substrates are polarized within needle complexes



Julia Radics

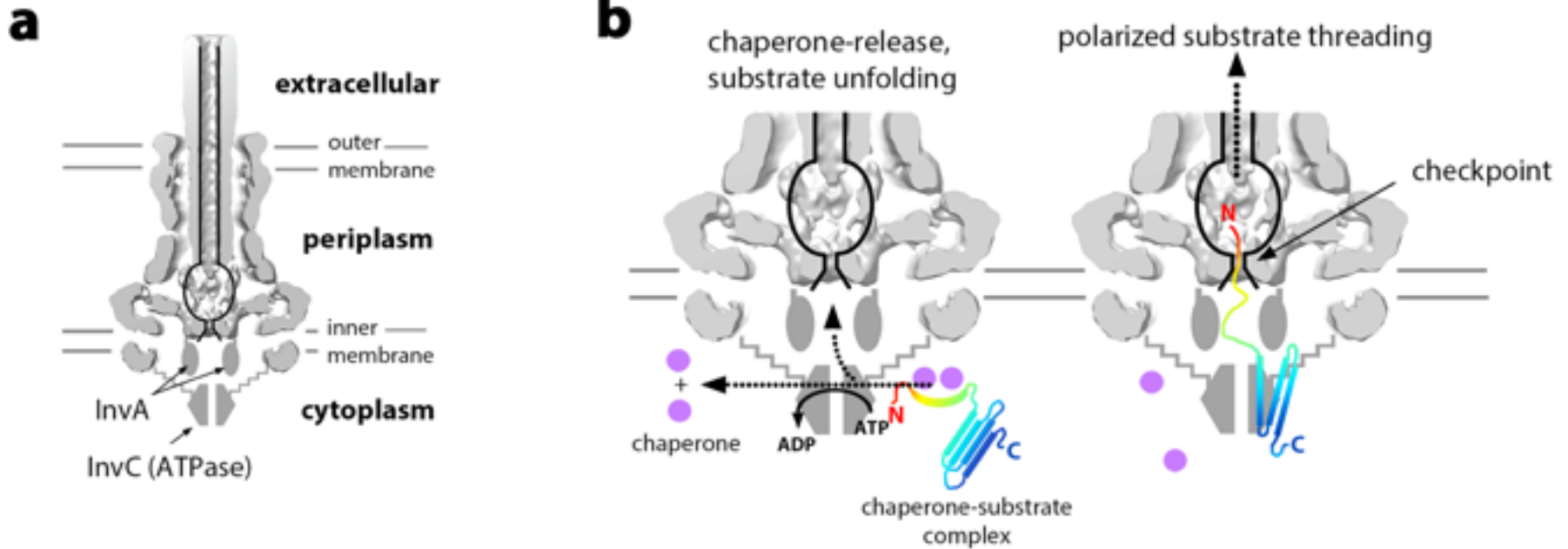
The structure of the substrate-trapped needle complex



grey: w.t. NC
blue: difference map

Julia Radics

Model of the polarized T3-secretion pathway



Julia Radics, Lisa Königsmaier

Summary and Conclusions

electron microscopy and infection biology

- single particle analysis
- electron tomography
- new software/hardware

type 3 secretion system

- is polar
- entire secretion path
- unfolding essential
- early unfolding checkpoint
- visualizing unfolded protein in situ
- high resolution