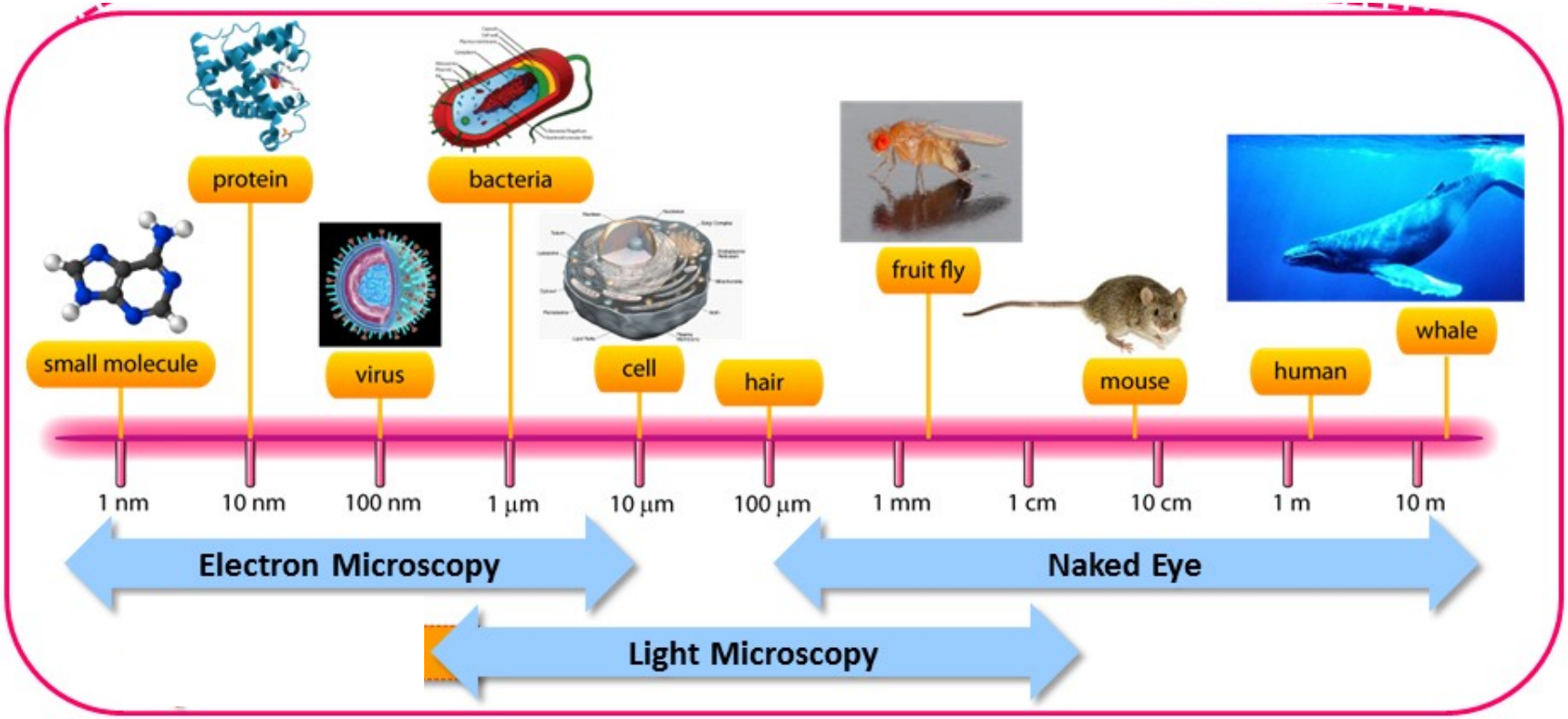
Microscopia Crioelettronica

La vita in un ghiacciolo

Indice

Le misure contano



https://www.bates.edu/gould-research-lab/research/

Limite di diffrazione di Abbe

$$d = \frac{\lambda}{2n\sin\theta} = \frac{\lambda}{2\mathrm{NA}}$$

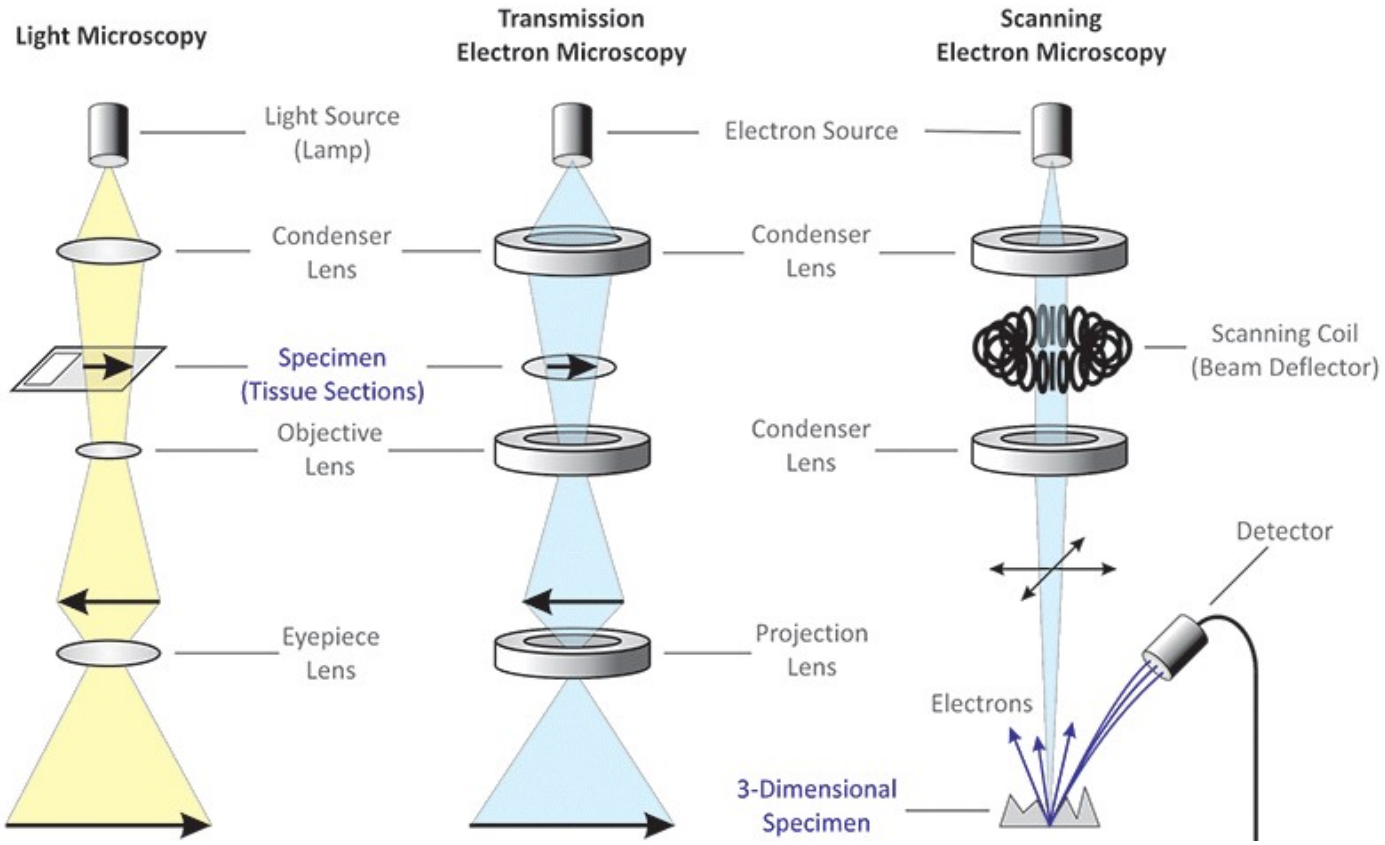
Limite di diffrazione del microscopio ottico:
~ 250 nm

Limite di diffrazione del microscopio elettronico:
~ 0.14 nm

λ: Lunghezza d'onda
n: indice di rifrazione
θ: amgolo di convergenza
NA: Apertura Numerica

https://www.ou.edu/research/electron/bmz5364/resolutn.html

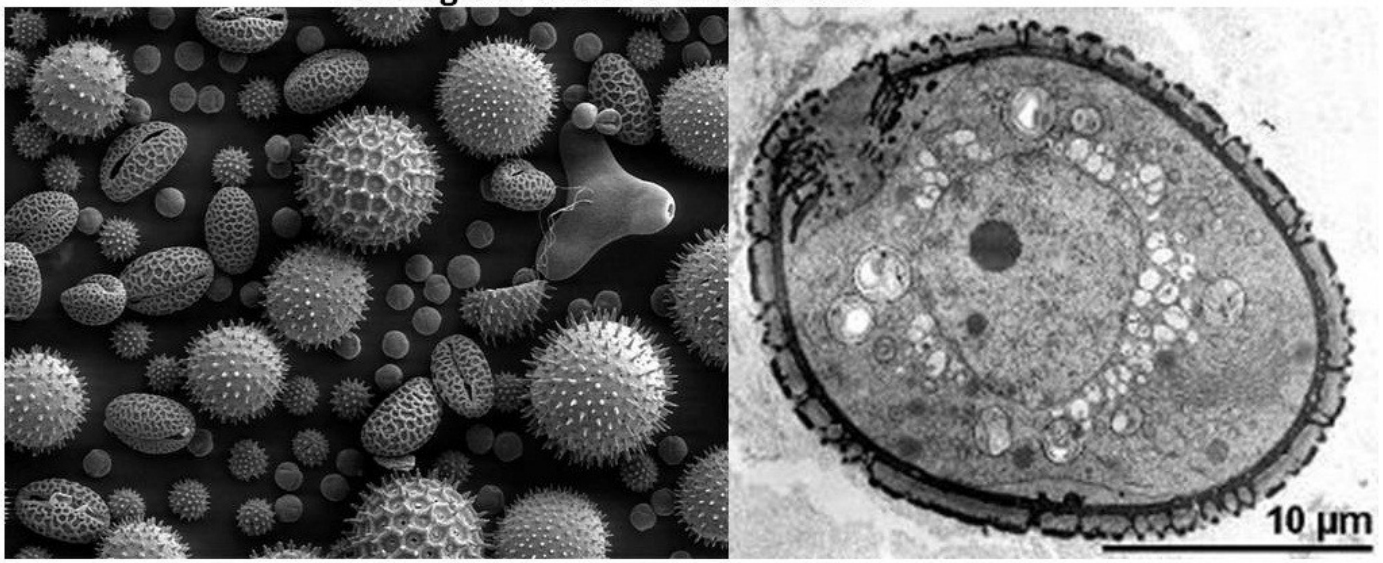
Microscopia Elettronica



https://microbiologyinfo.com/wp-content/uploads/2015/04/Light-Microscope-and-Electron-Microscope.png

Scansione vs Trasmissione

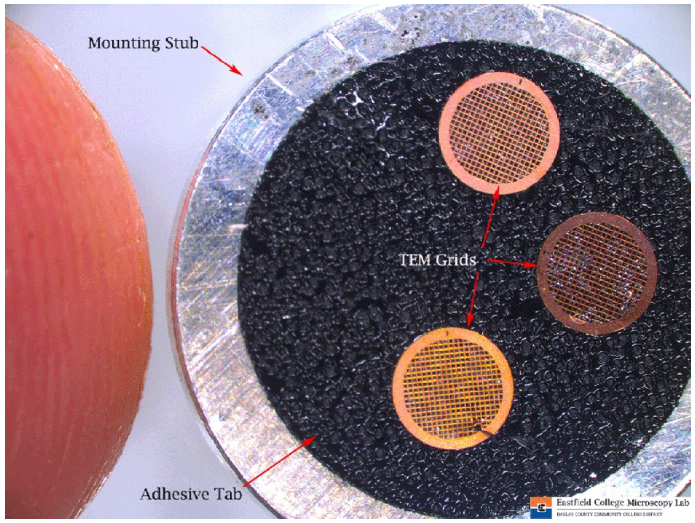
Pollen grain under SEM and TEM



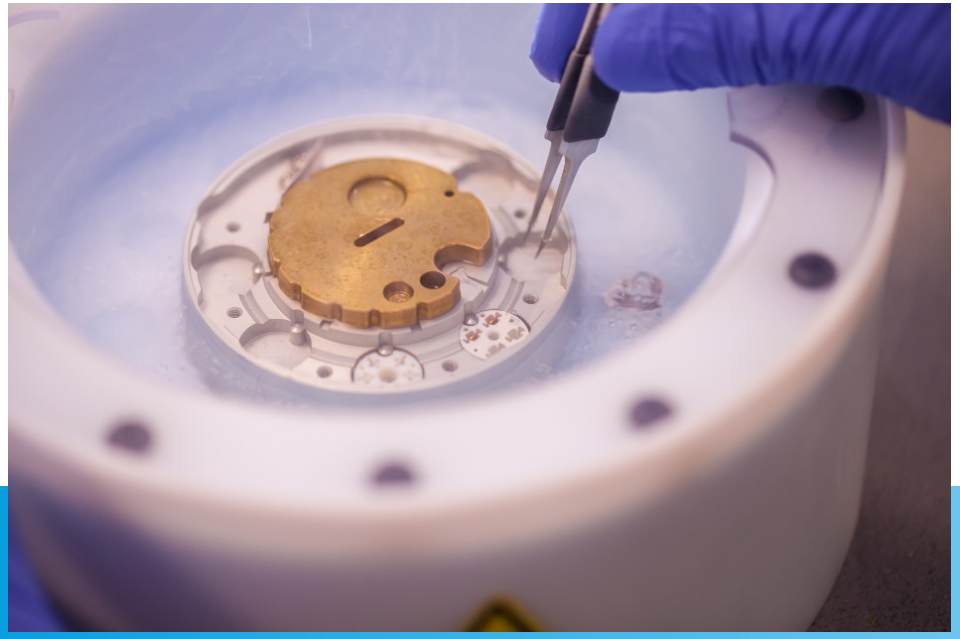
Scanning Electron Microscope (SEM) vs Transmission Electron Microscope(TEM)

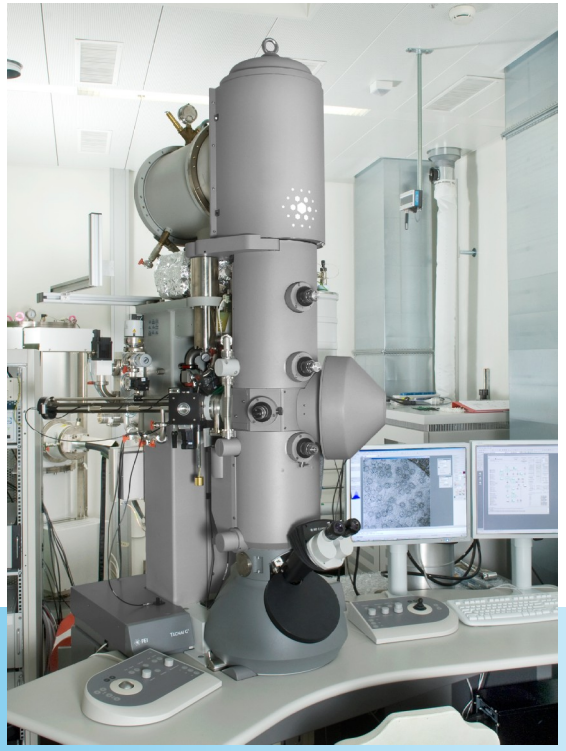
www.majordifferences.com

Microscopia criogenica elettronica



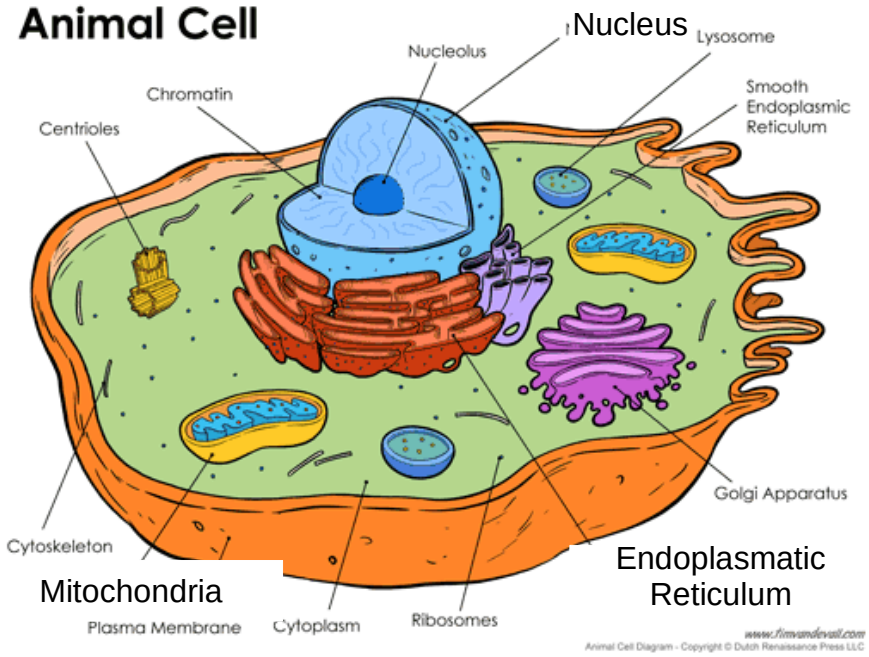
- È un TEM dotato di un serbatoio per l'azoto liquido
- L'azoto liquido raffredda il campione a temperature di da -190° fino a -140°
- Il campione viene congelato attraverso shock criotermico
- Il campione viene trasferito su una griglia porta campioni e inserito nel microscopio

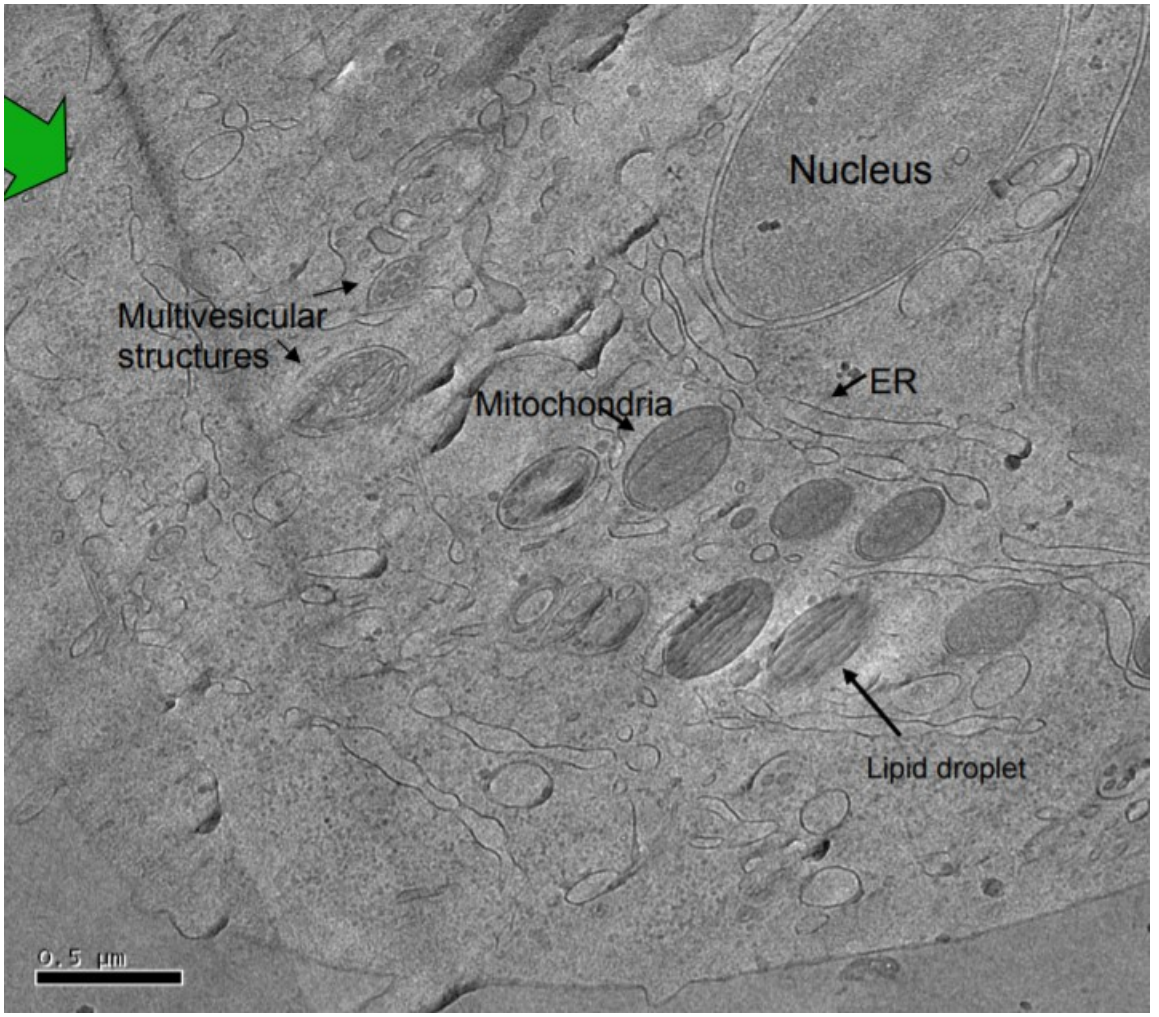




Esempio di microscopia criogenica

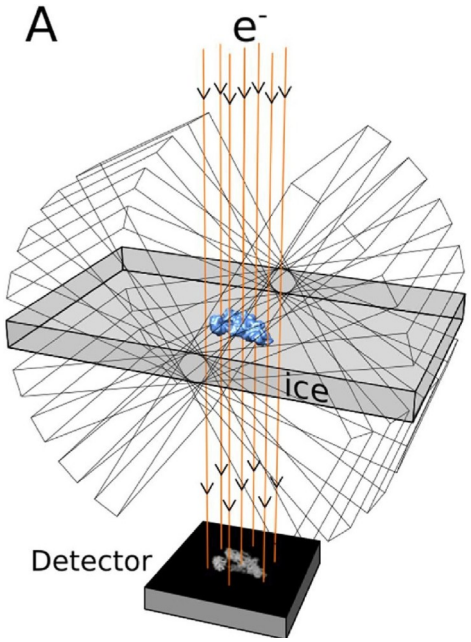
- Conserva i tessuti in uno stato idrato
- Non richiede coloranti contrastanti speciali
- Utile per analisi delle strutture molecolari

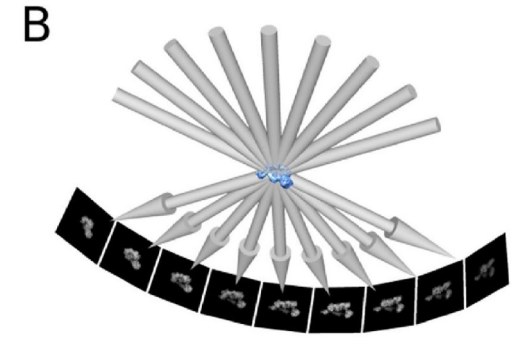


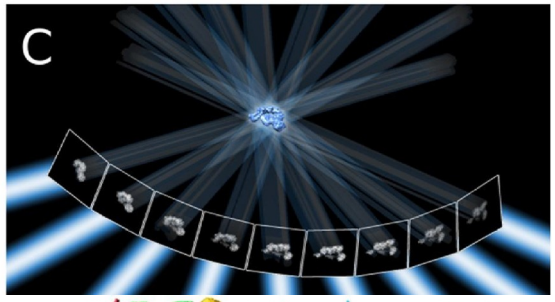


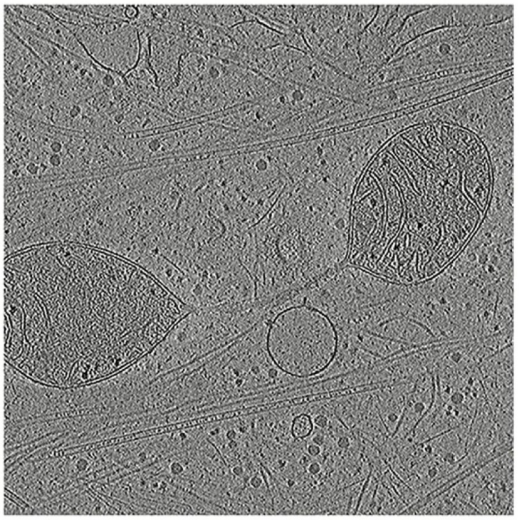
John_Briggs_El_Tomography.pdf

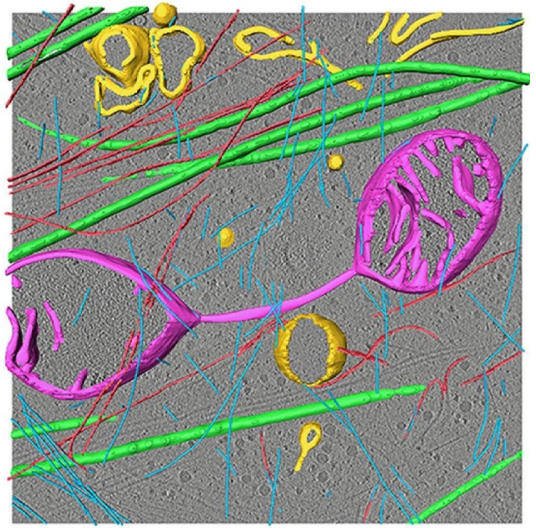
Tomografia elettronica criogenica

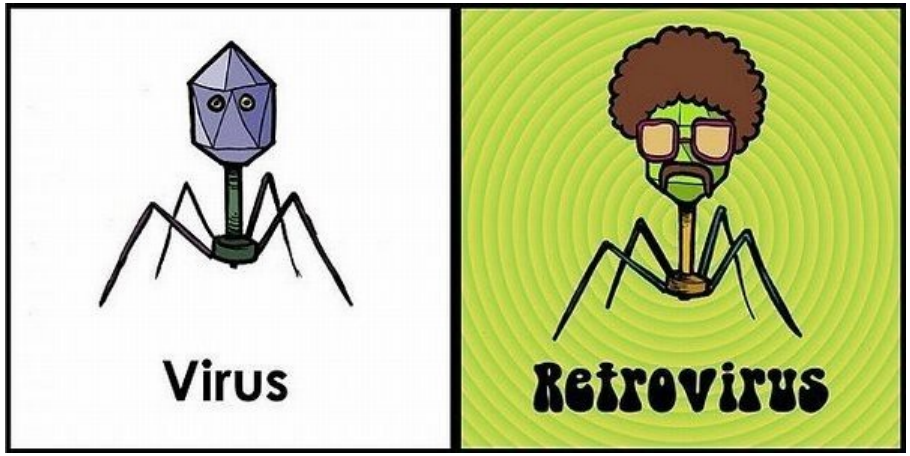


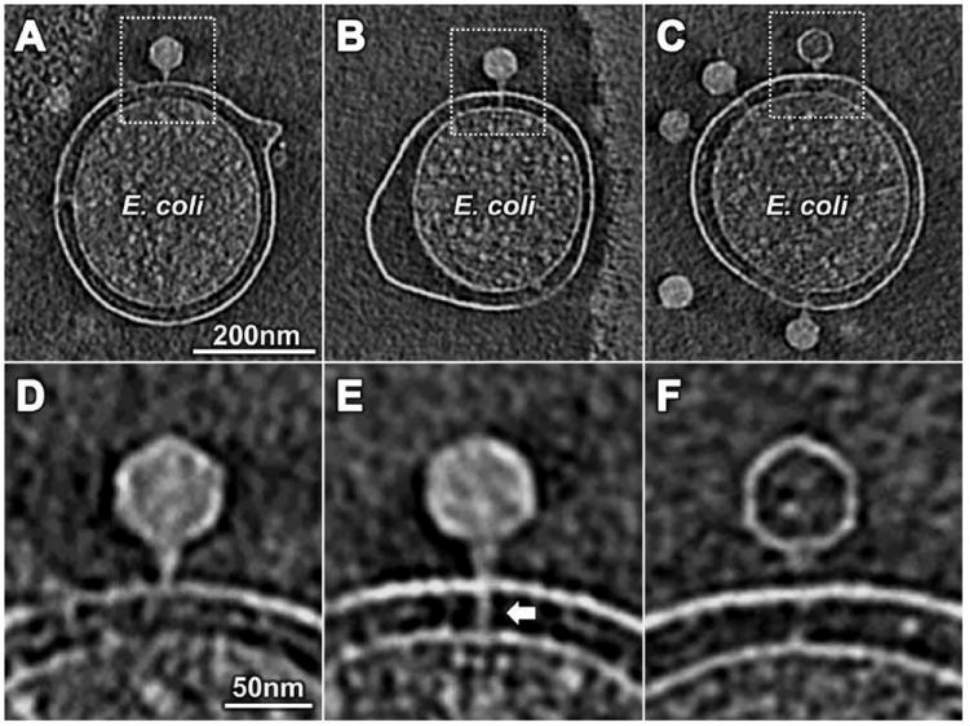








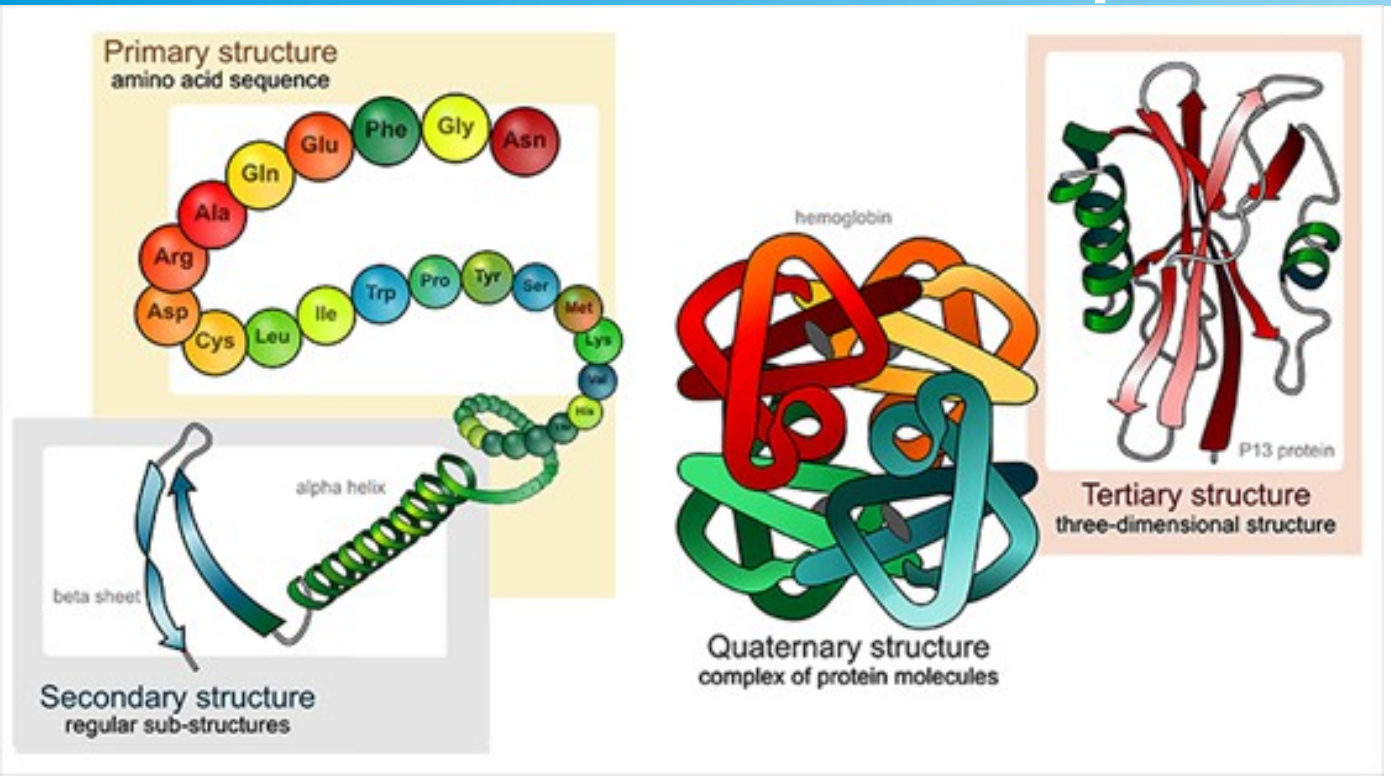


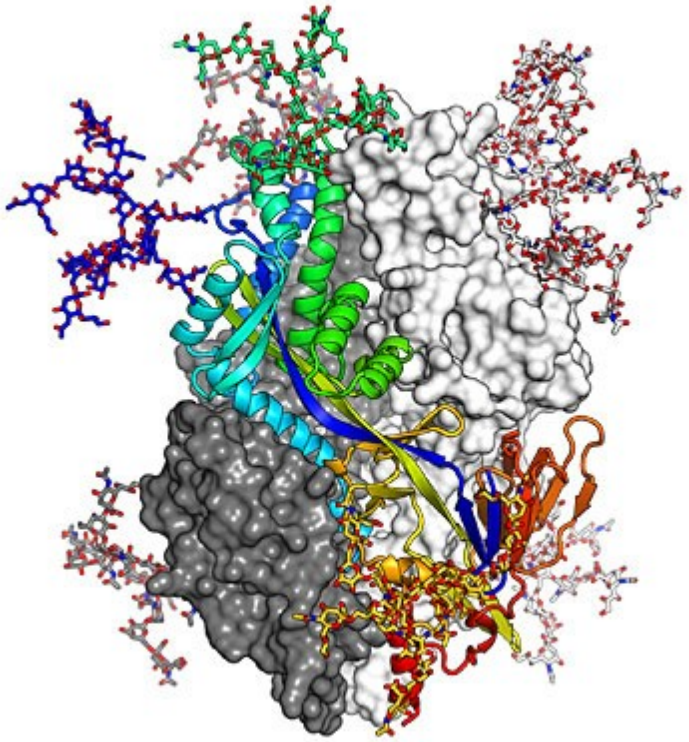


Actina (blu), membrane lipidiche (giallo), microtubuli (verde), mitocondri (viola) e filamenti intermediari (rosso).

Koning et al., Ann. Anat. (2018)
Hu et al., Science (2013)

Struttura proteine

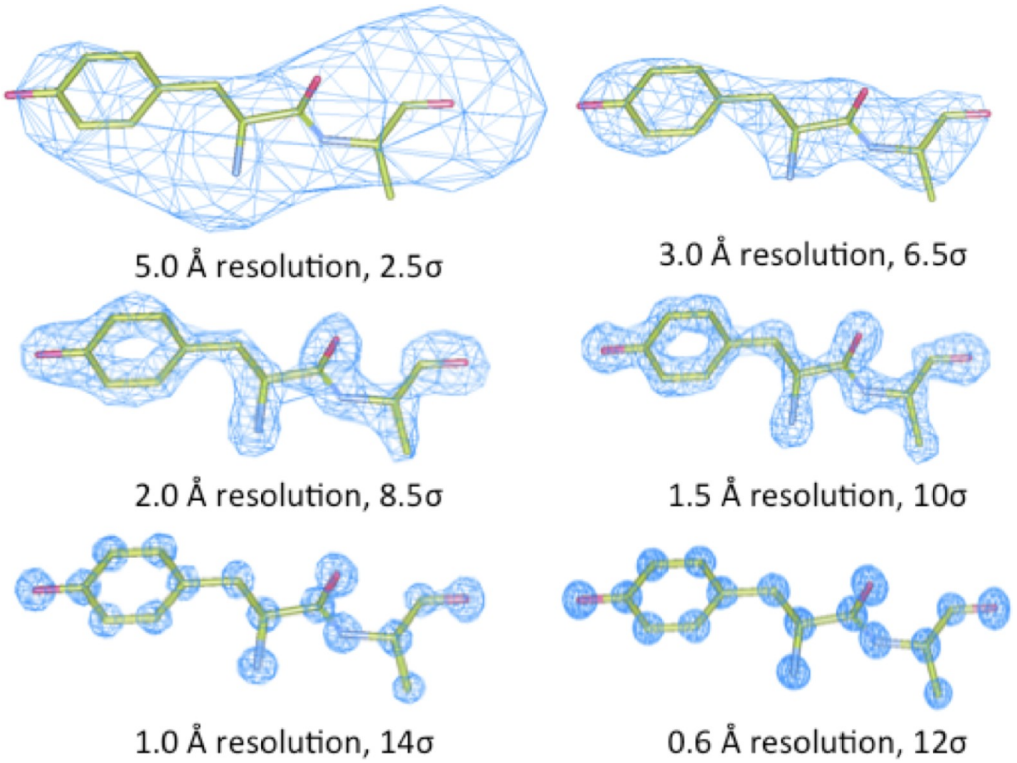


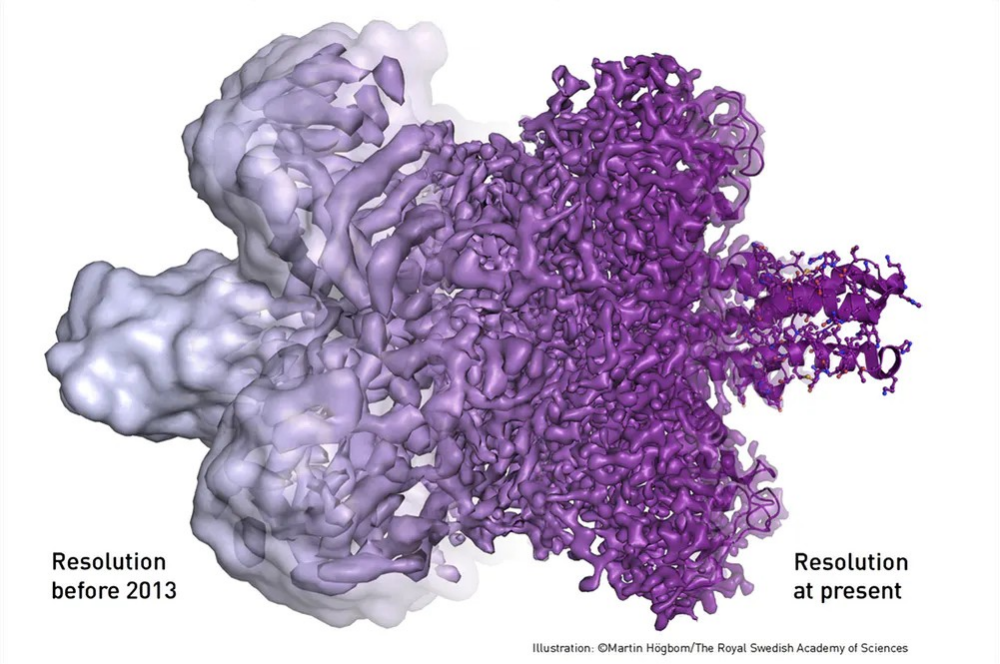


- Determinare la struttura delle proteine è una delle grandi sfide della biologia
- Le proteine sono composte da una sequenza di amminoacidi
- Gli atomi si ordinano attraverso un processo chiamato ripiegamento nello spazio tridimensionale

https://www.bnl.gov/newsroom/news.php?a=213084

Risoluzione

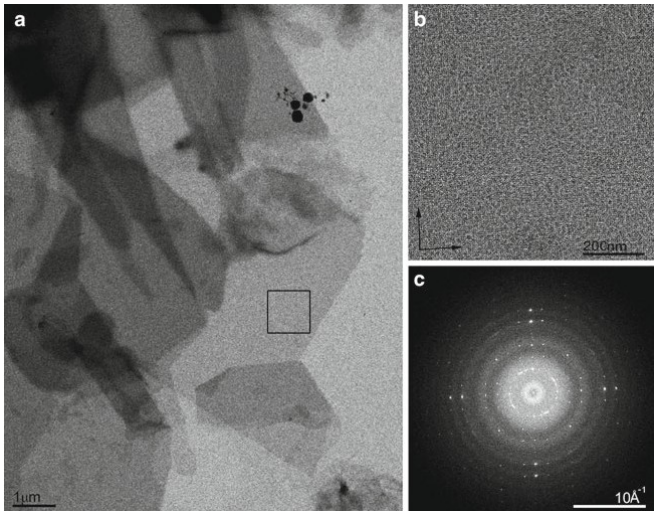


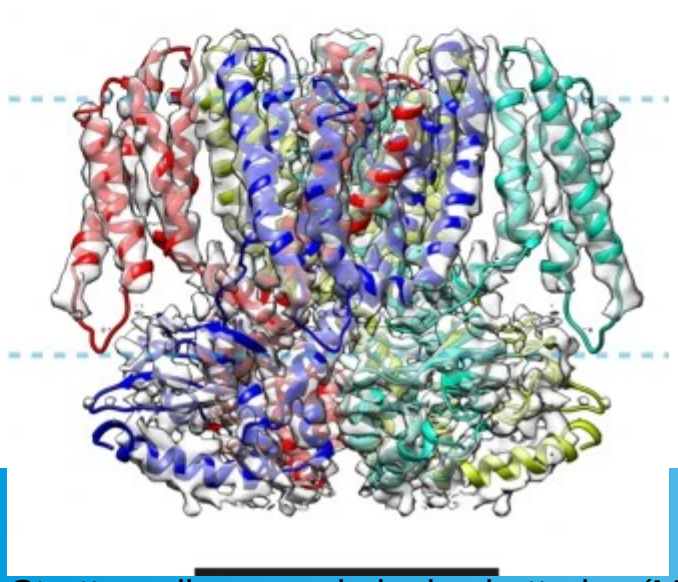


Kuster et al., Plos One (2015)
https://theconversation.com/chilled-proteins-and-3-d-images-the-cryo-electron-microscopy-technology-that-just-won-a-nobel-prize-85229

Biologia strutturale con crioEM

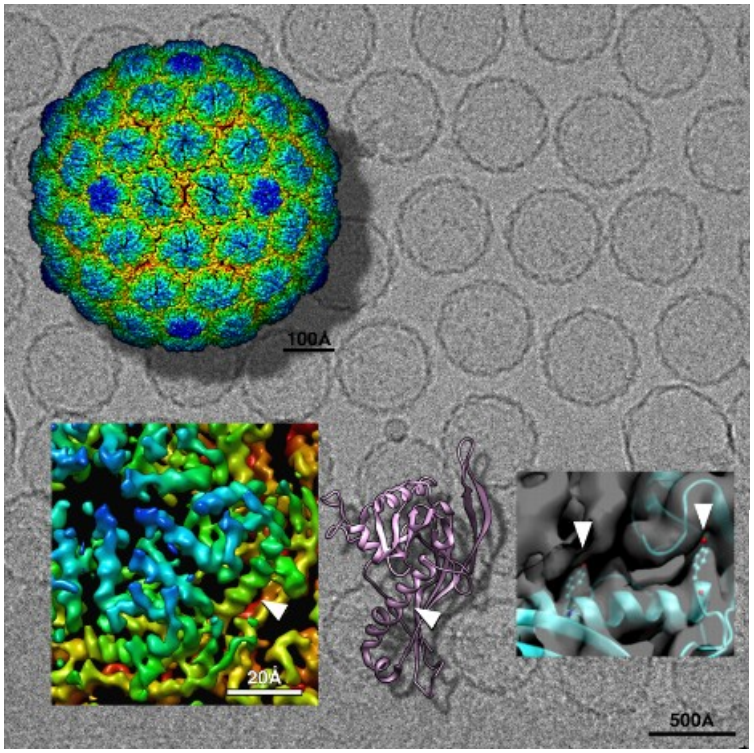
Cristallografia



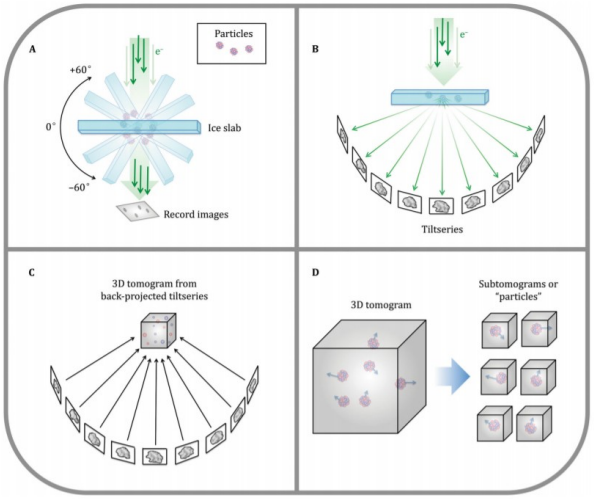


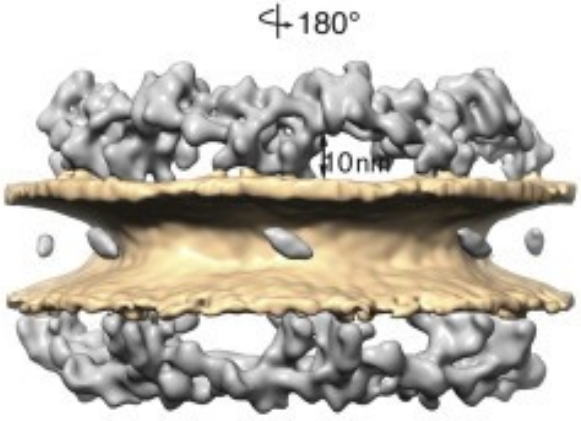
Struttura di un canale ionico batterico (MloK1)

Analisi a particelle singole



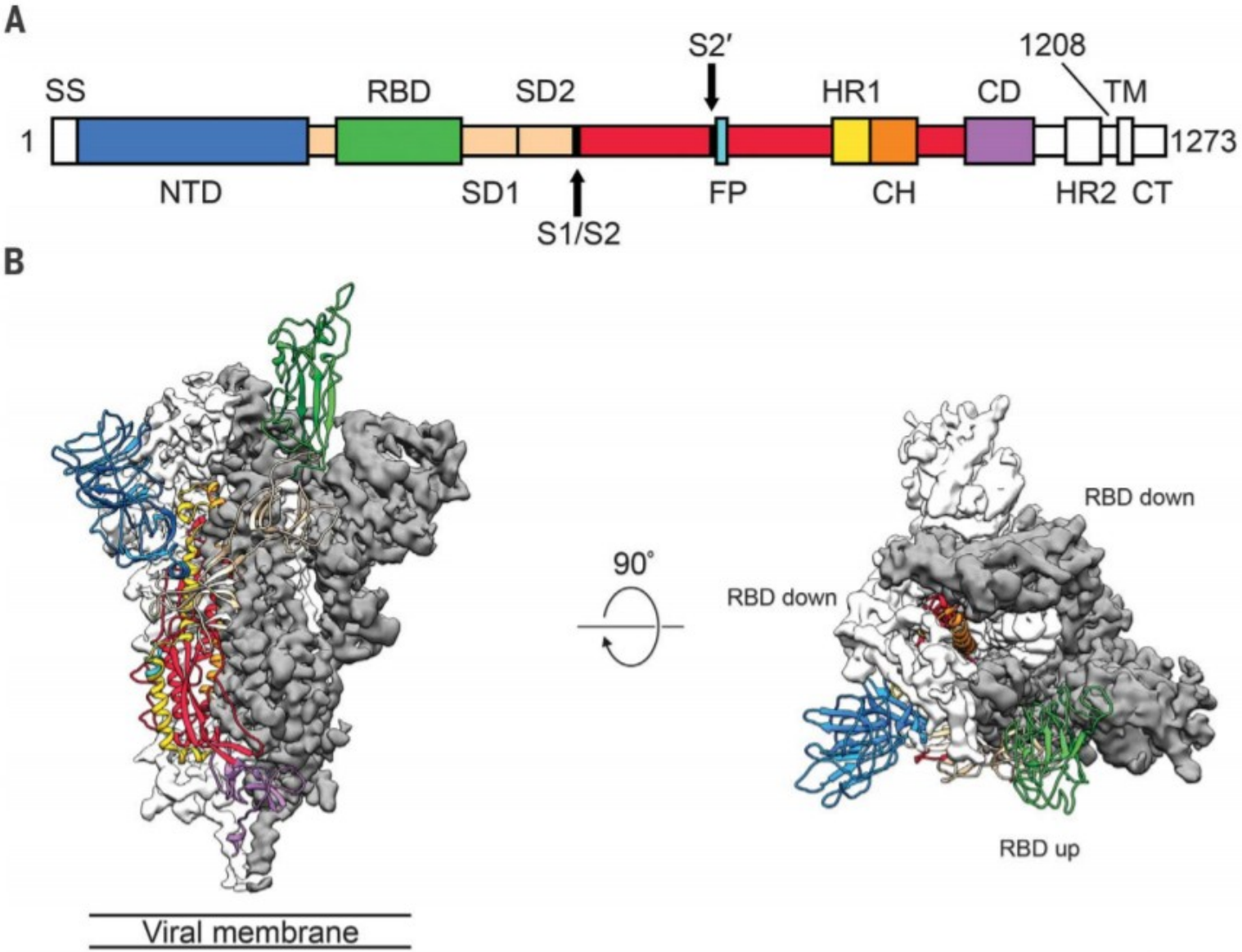
media dei sub-tomogrammi

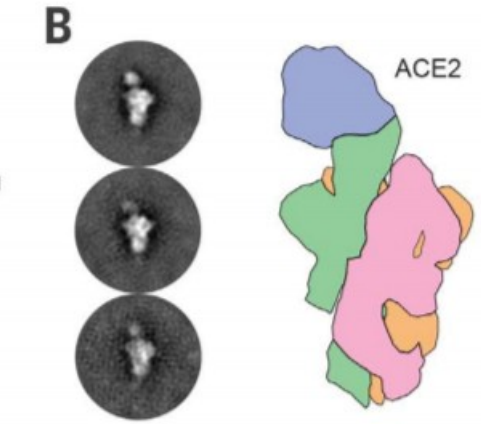


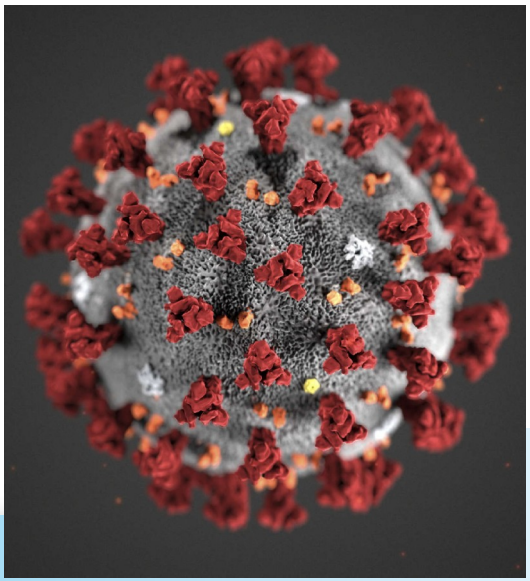


Gonen, Met. Mol. Bio. (2013)
Righetto et al., Nat Com (2019)
https://www.fei.com/life-sciences/single-particle-analysis/

Aculeo del SARS-nCoV-2







Wrapp et al., Science (2020)

Grazie per l'attenzione