single-particle cryo-electron microscopy

technical journal club
30.05.2017

Manuela Pfammatter

outline

introduction

single-particle cryo-electron microscopy

Fernandez Leiro & Scheres, Nature, 2016

Frank, Nat Protoc, 2017

rotational states in a V-ATPase

Zhao et al., Nature, 2015

spiral architecture of Hsp104 disaggregase

Yokom et al., Nat Struct Mol Biol, 2016

conclusion & outlook

why structure determination?

protein function

▲

interactions

○

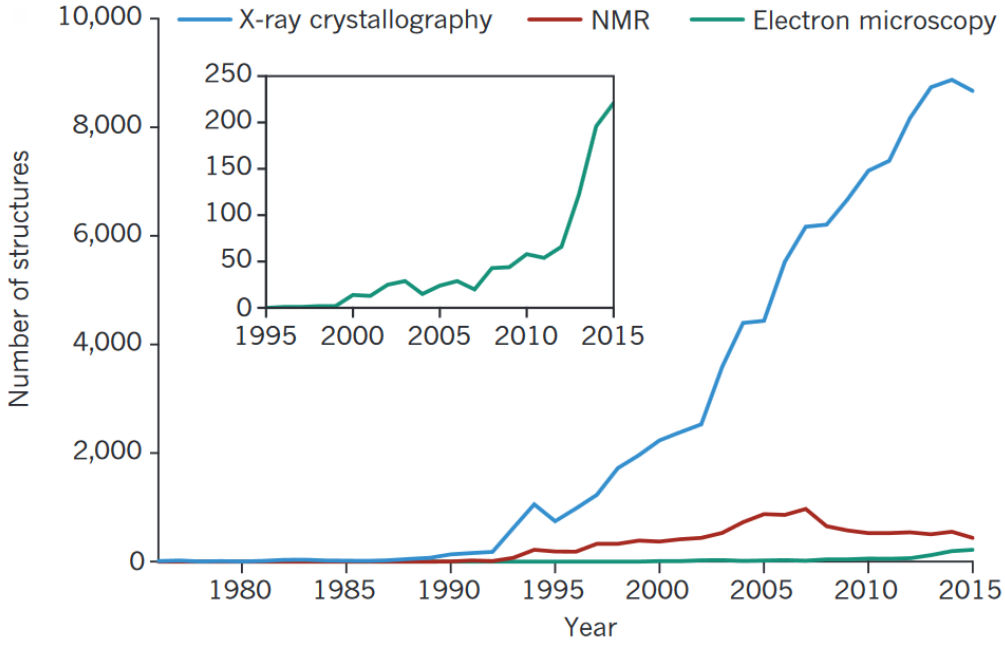
dynamics

▲

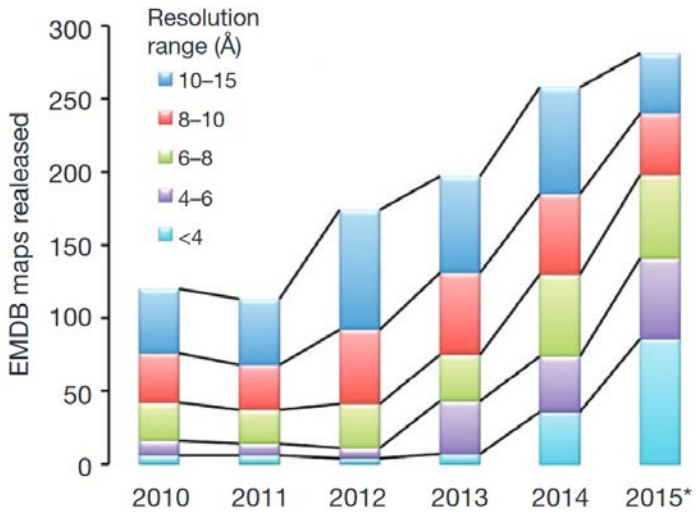
structure

elucidate **structures** to understand the **mechanisms** underlying **biological function**

methods for structure determination

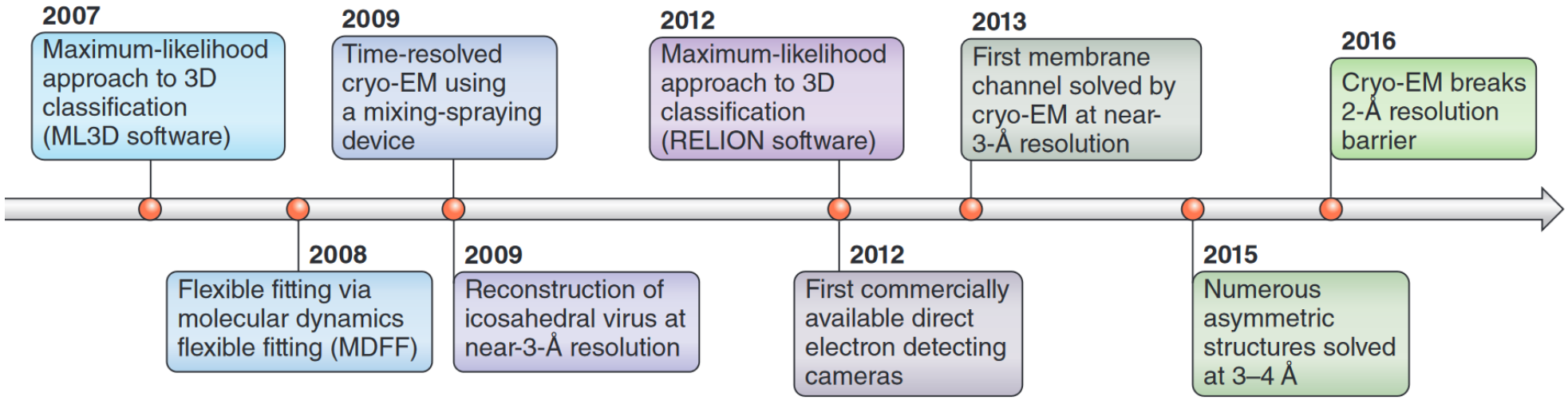


Fernandez-Leiro, 2016



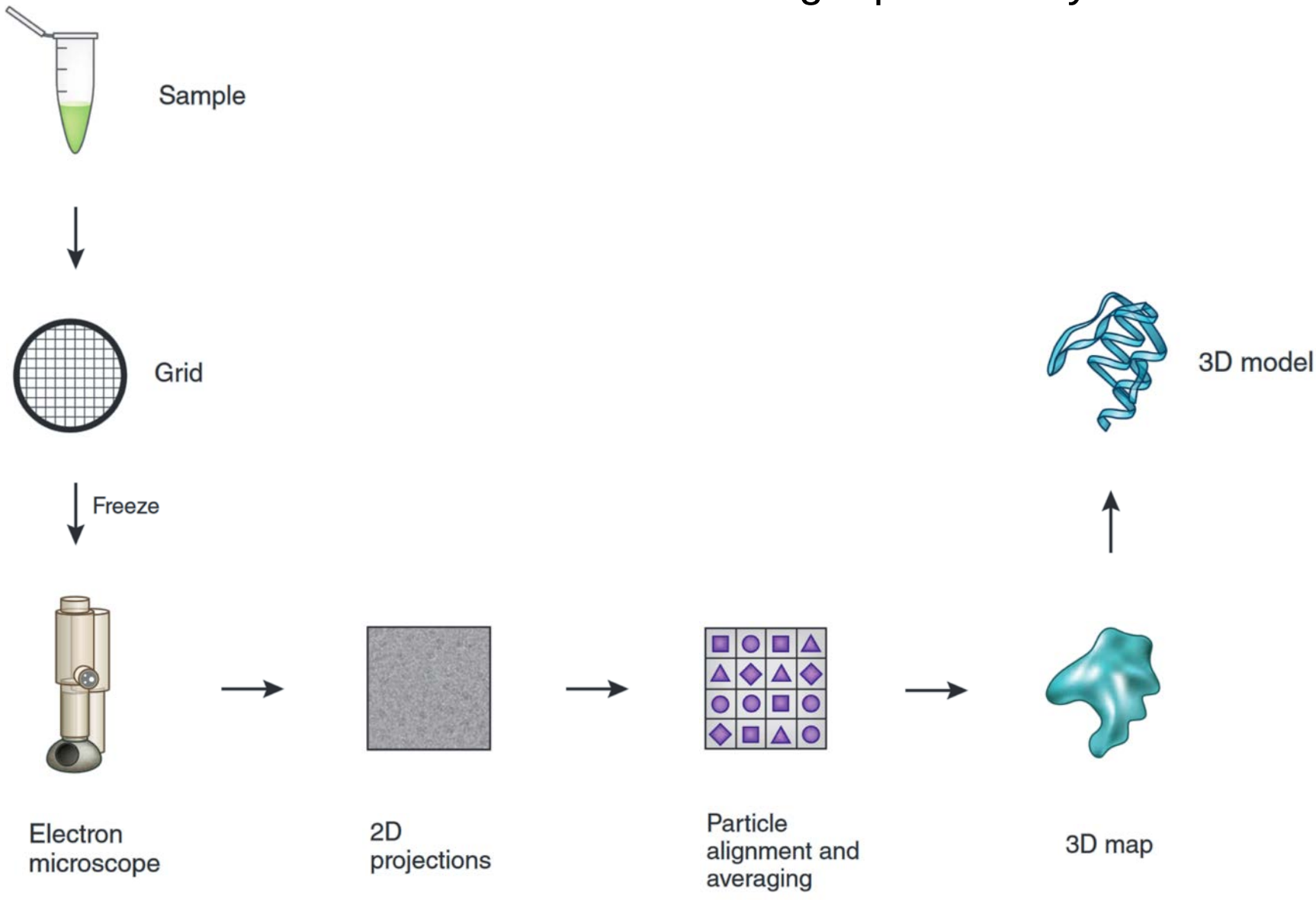
Nogales, 2016

recent advances in cryo-EM



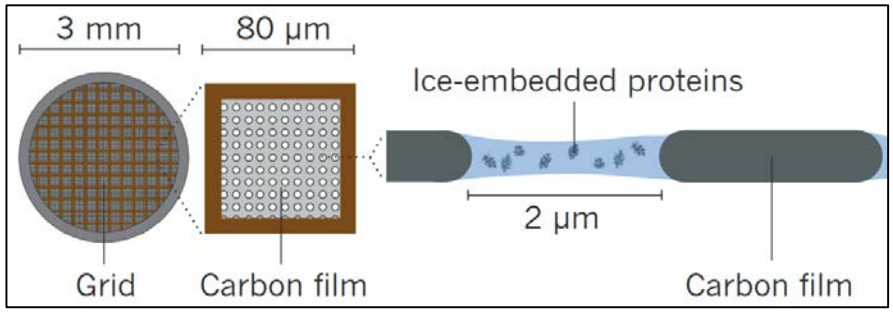
Frank, 2017

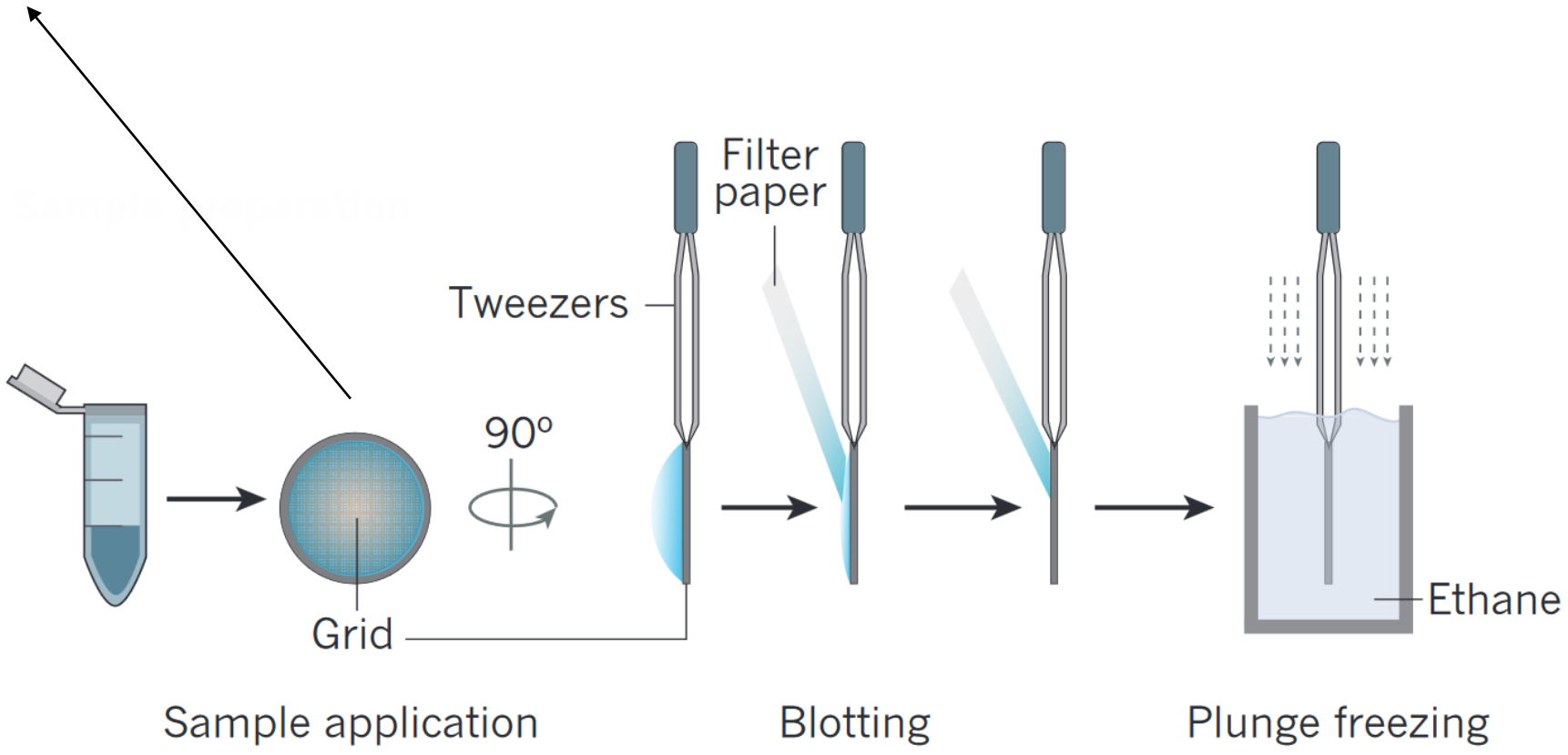
single-particle cryo-EM workflow



adapted from *Doerr, 2016*

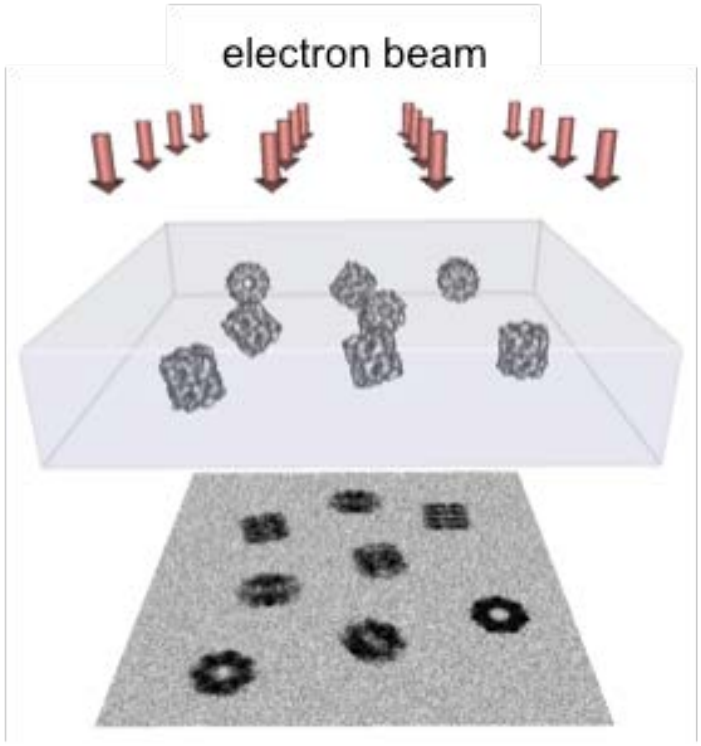
i. sample grid preparation



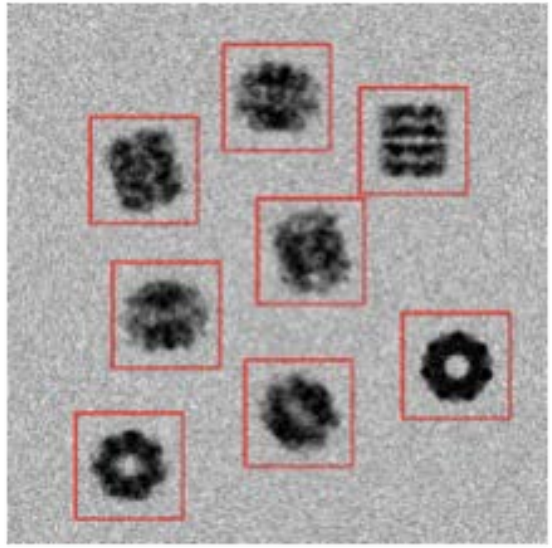


adapted from *Fernandez-Leiro, 2016*

ii. data collection: 2D projections



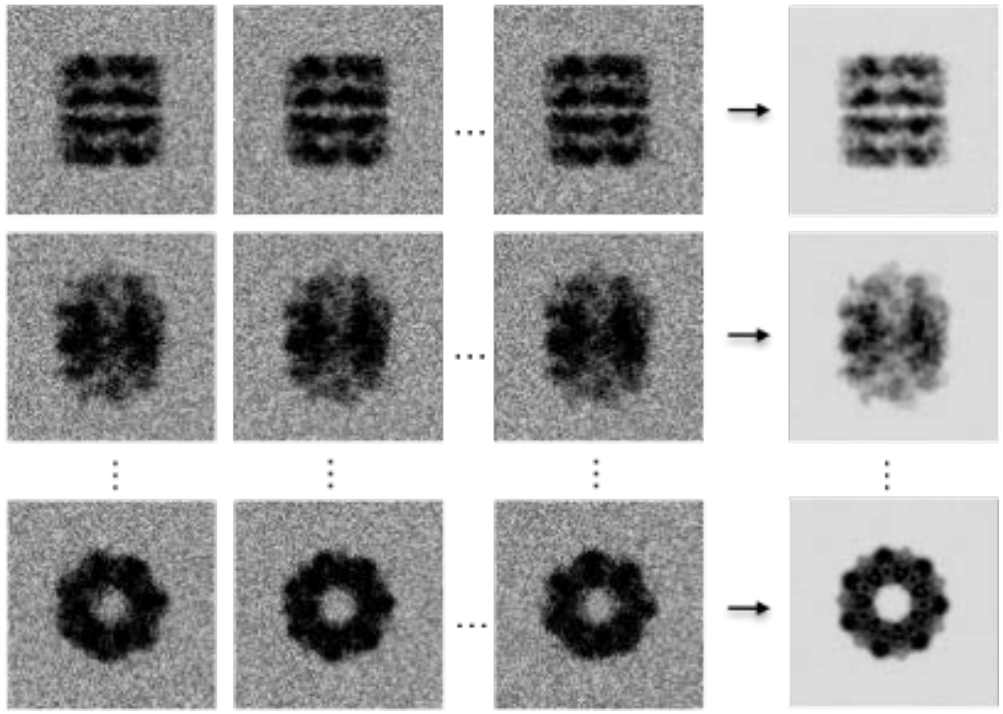
2D projections



particle boxing

adapted from *Pintilie (online)*

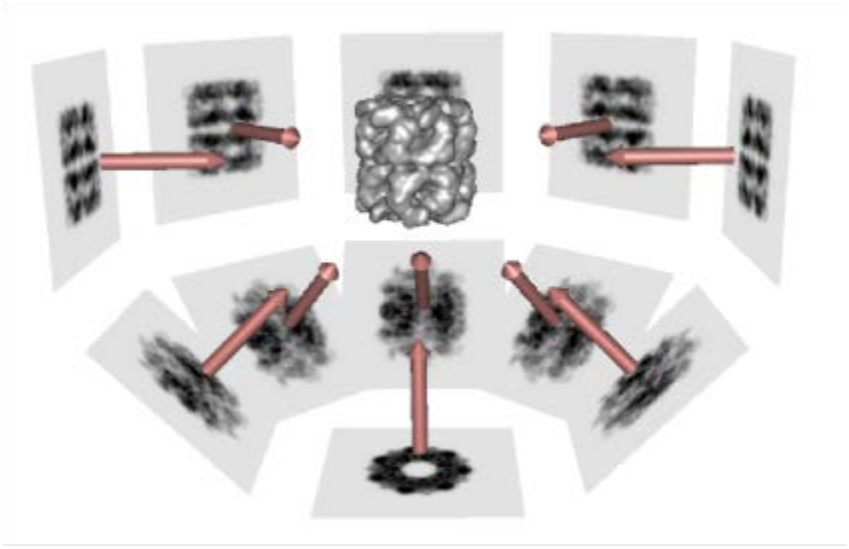
iii. data processing: particle alignment and averaging



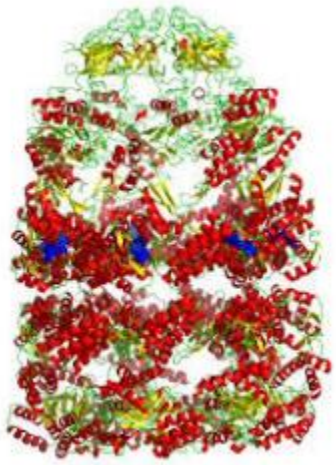
clustering averaging

adapted from *Pintilie (online)*

iv. data processing: 3D reconstruction



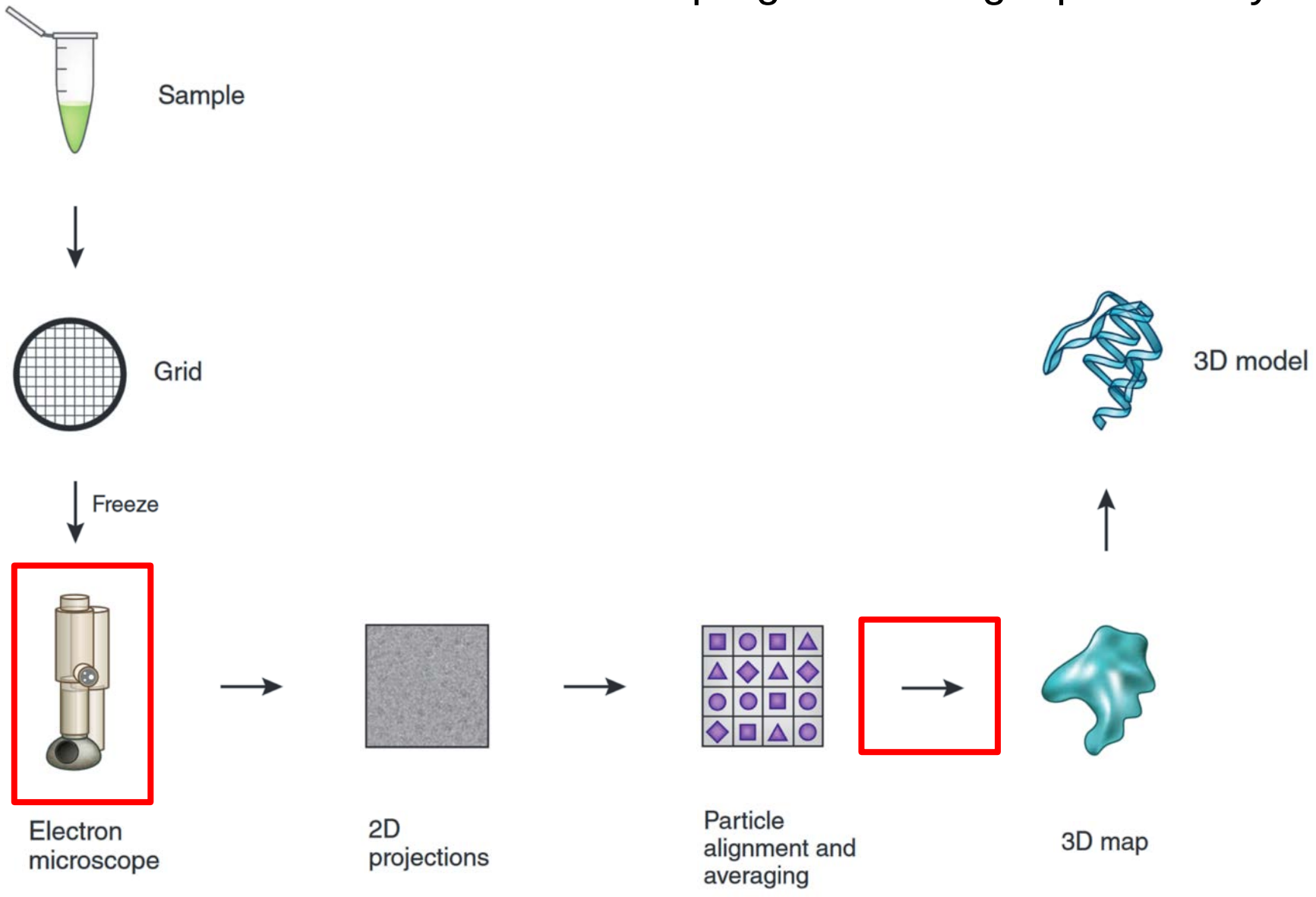
3D map



3D model

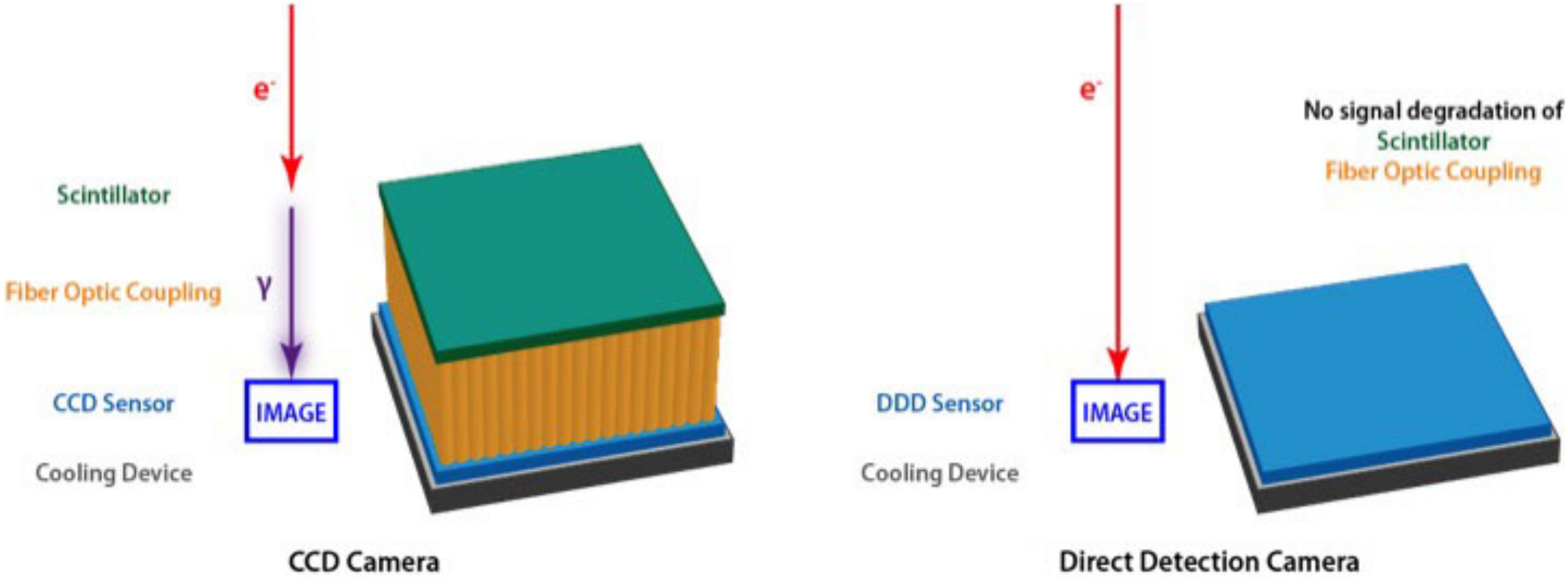
adapted from *Pintilie (online)*

recent progress in single-particle cryo-EM



adapted from *Doerr, 2016*

principle of direct detection



courtesy of Direct Electron

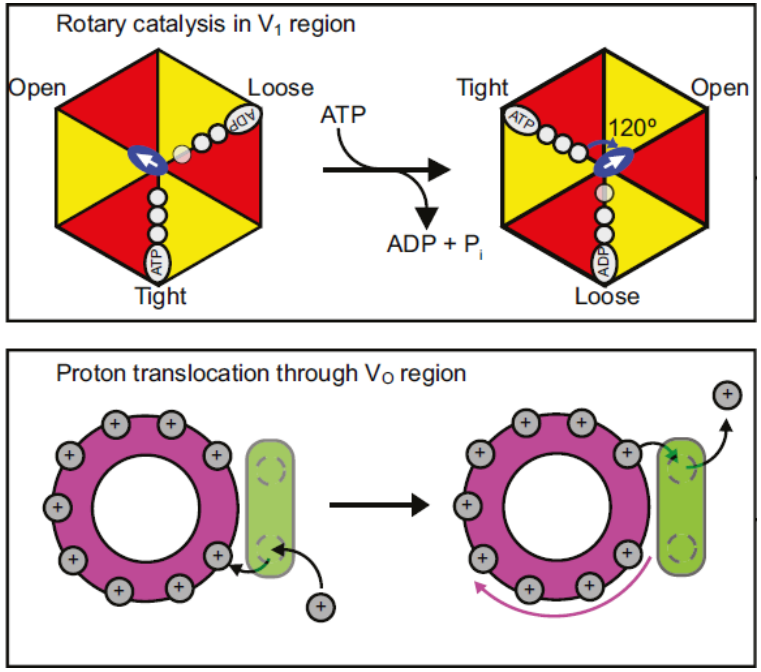
nature

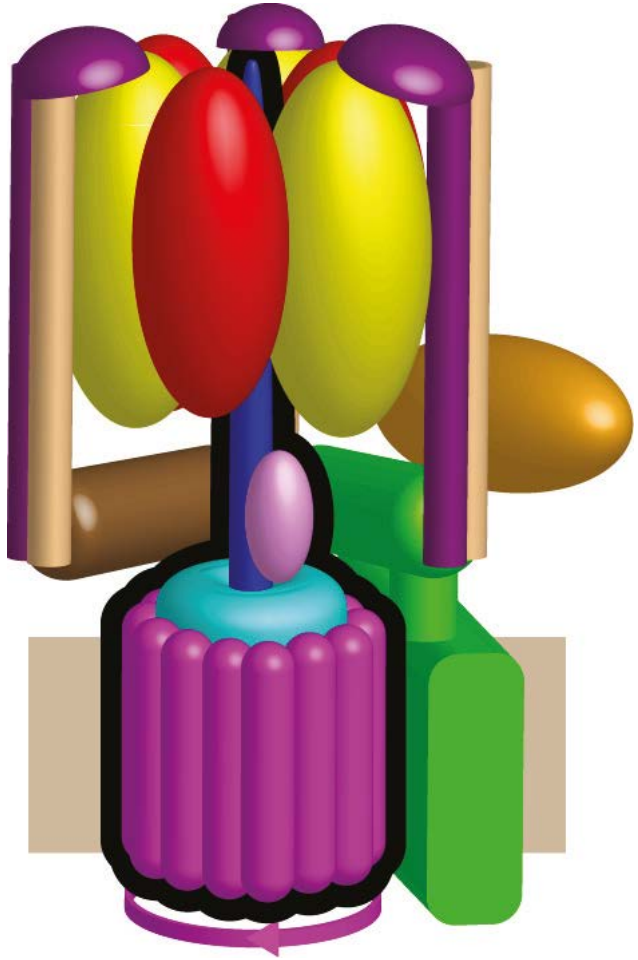
Electron cryomicroscopy observation of rotational states in a eukaryotic V–ATPase

Jianhua Zhao[1,2*], Samir Benlekbir[1*] & John L. Rubinstein[1,2,3]

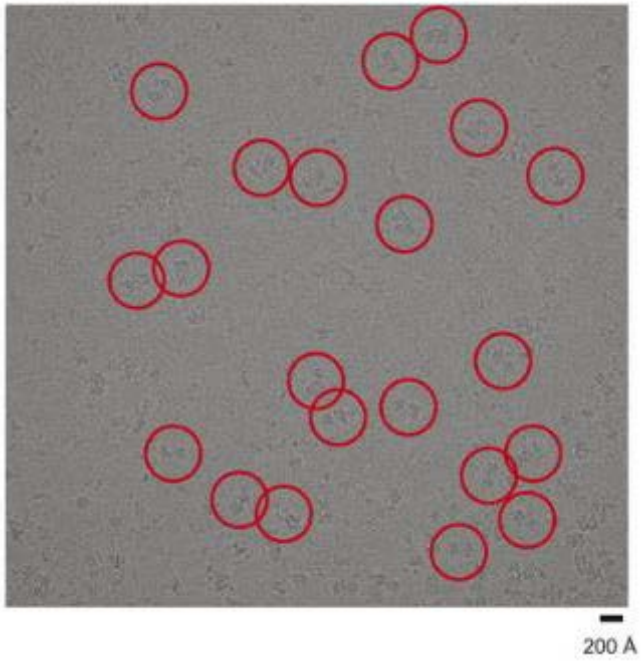
eukaryotic V-ATPase

eukaryotic vacuolar H^+-ATPase

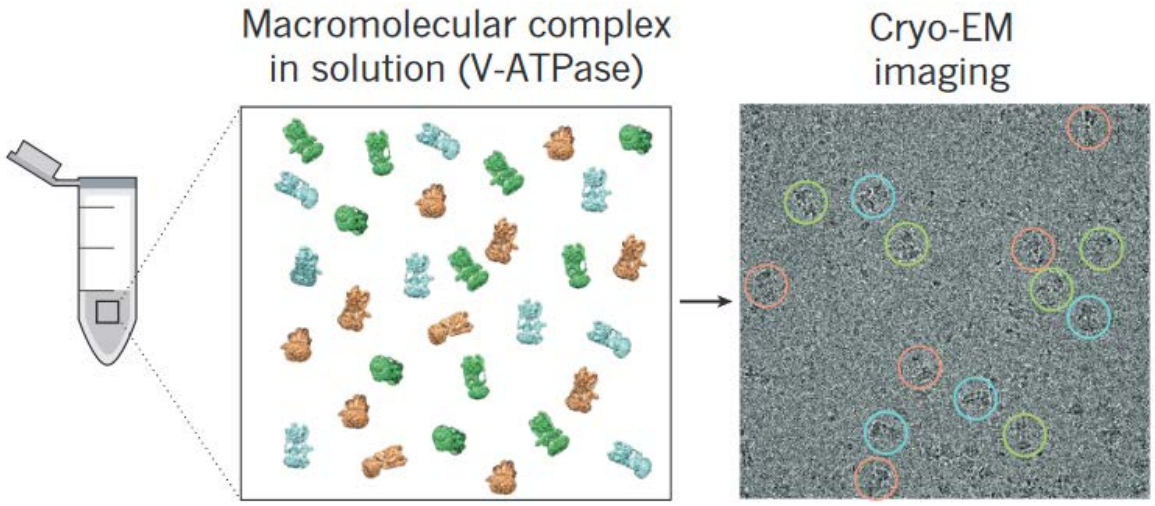




V-ATPase: data collection and processing



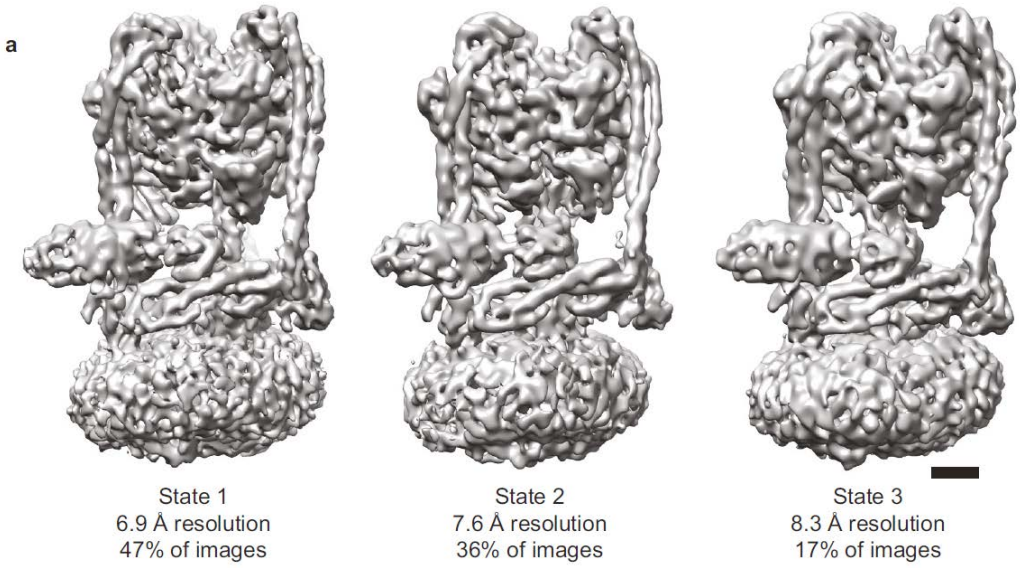
2D projection



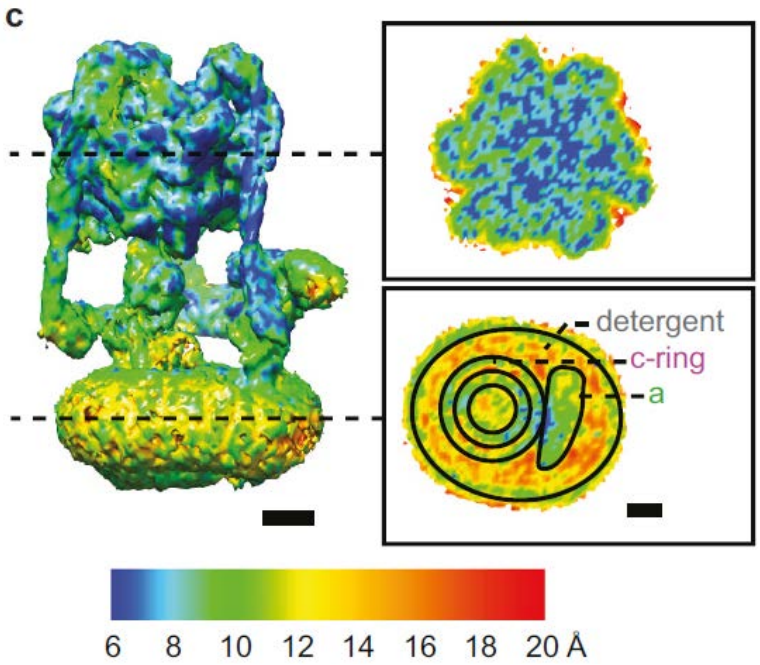
particle boxing and classification
(106 445 particles)

Zhao, 2015

V-ATPase: data processing

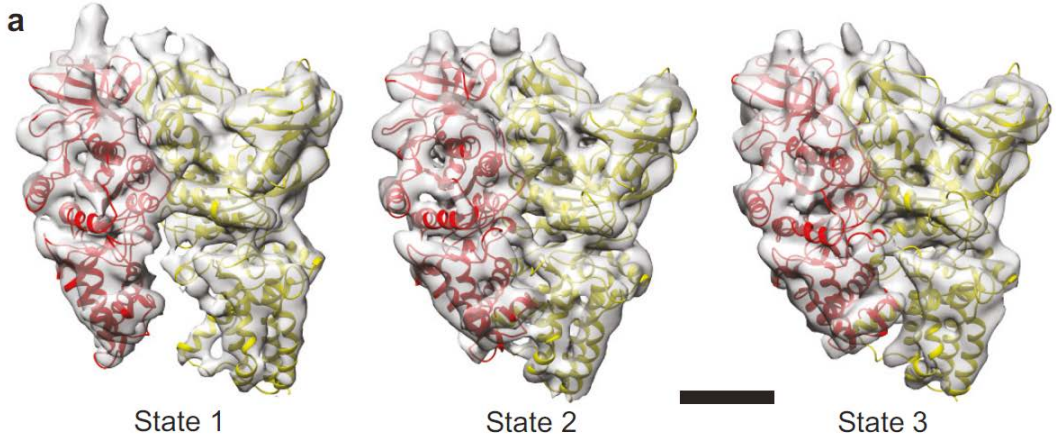


3D maps

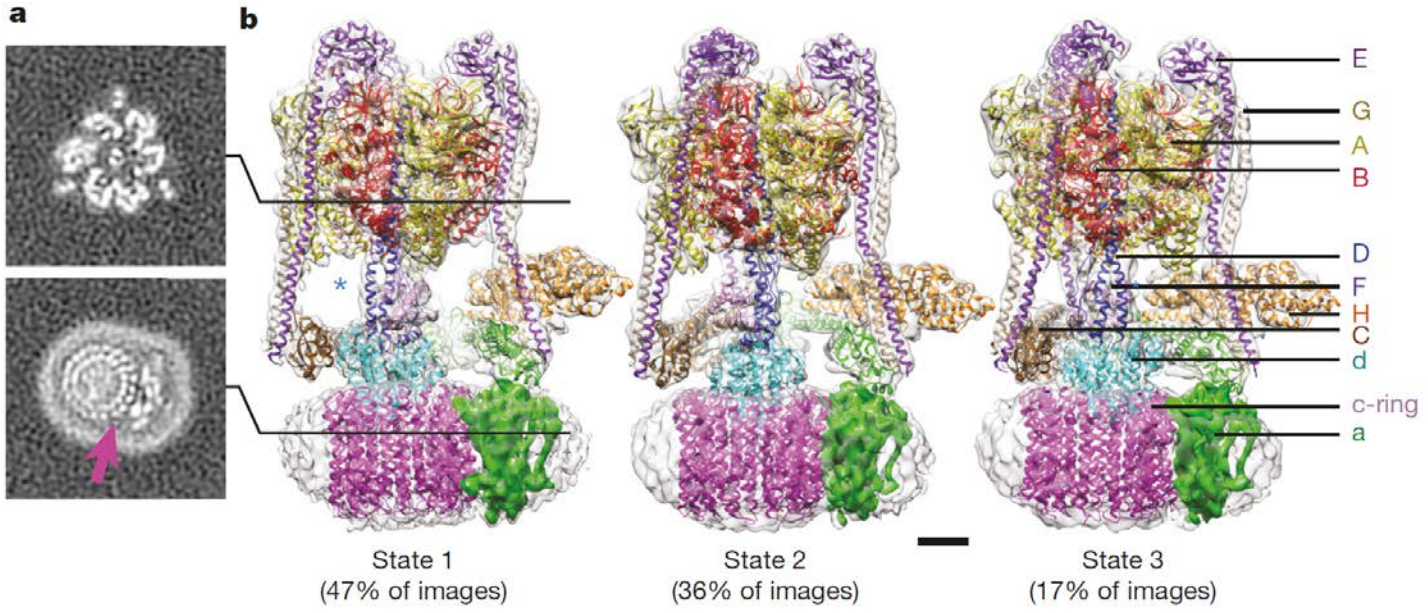


Zhao, 2015

V-ATPase: data processing

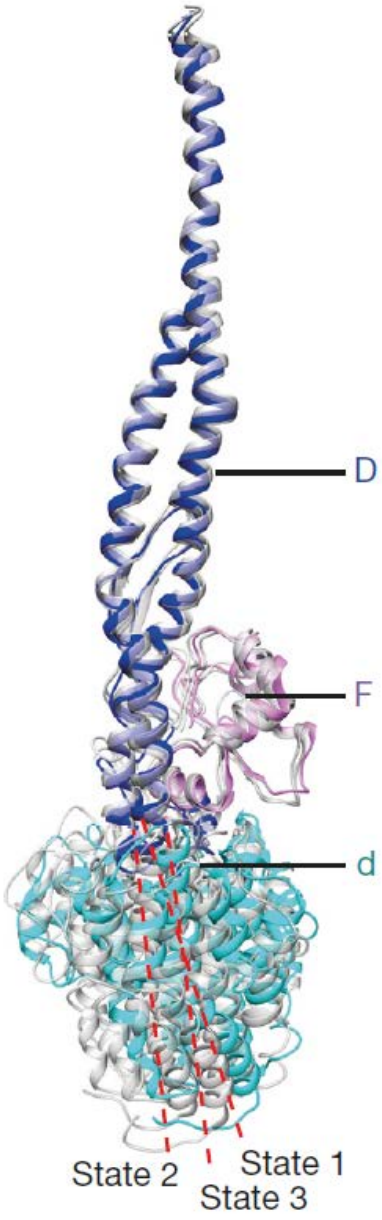


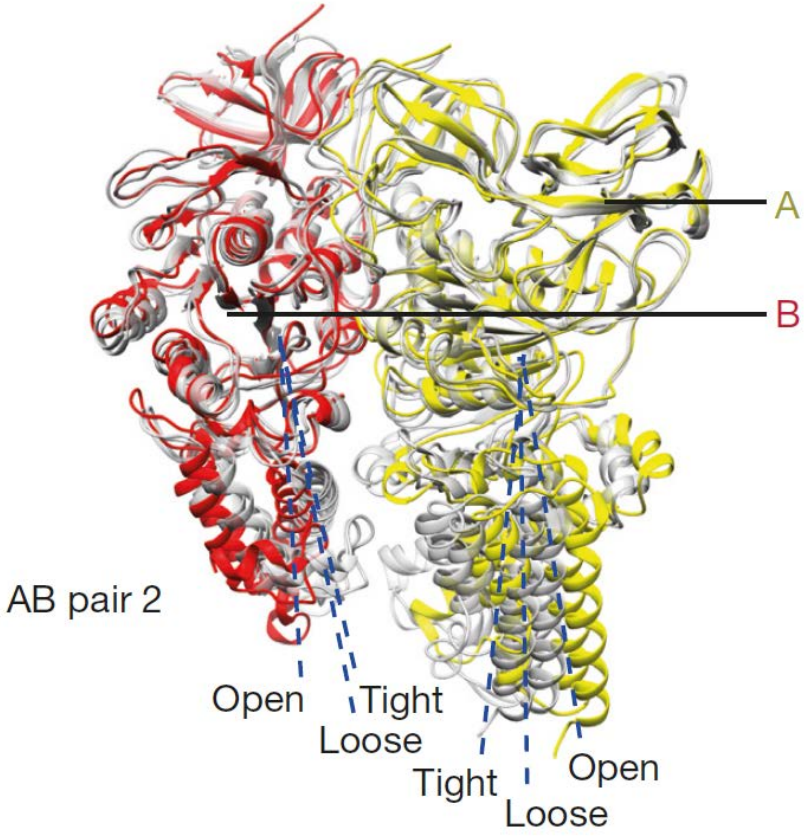
reconstruction
AB subunit



Zhao, 2015

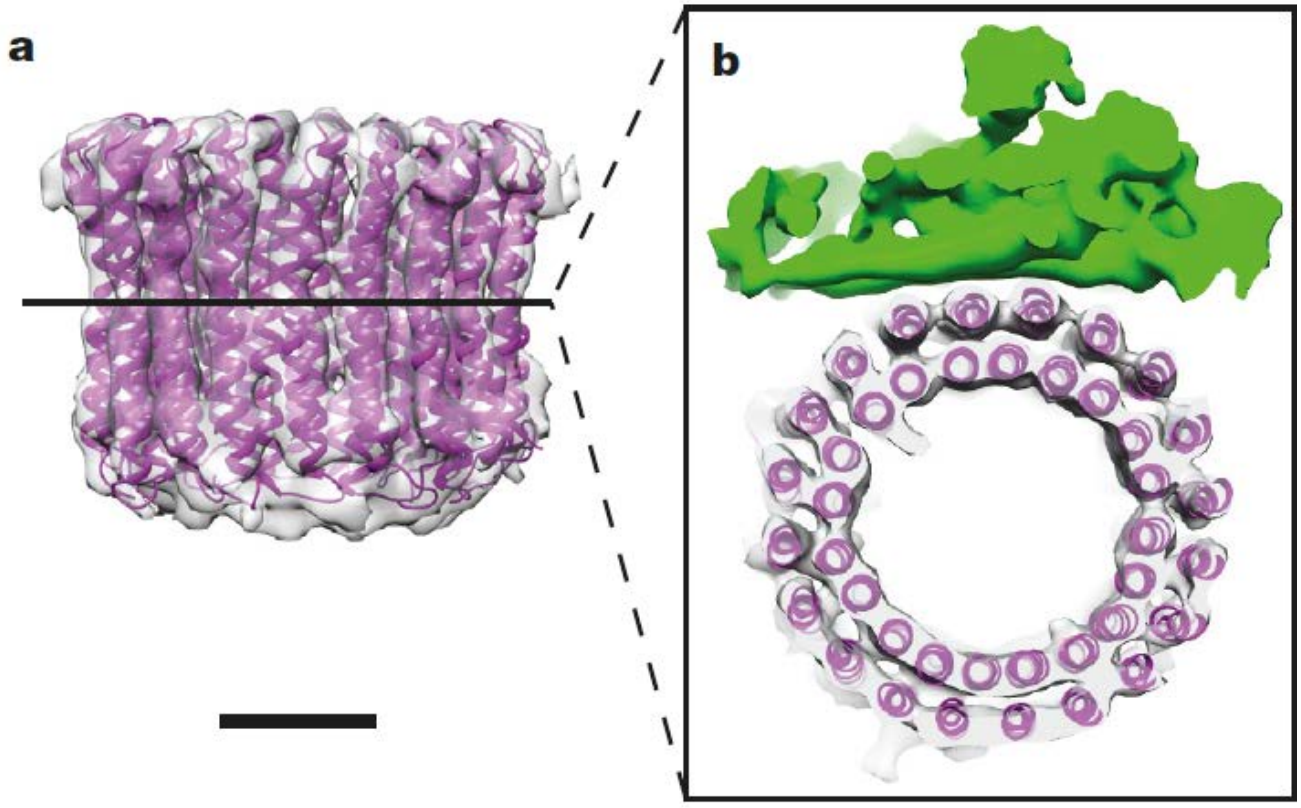
central rotor and soluble V_1 region





Zhao, 2015

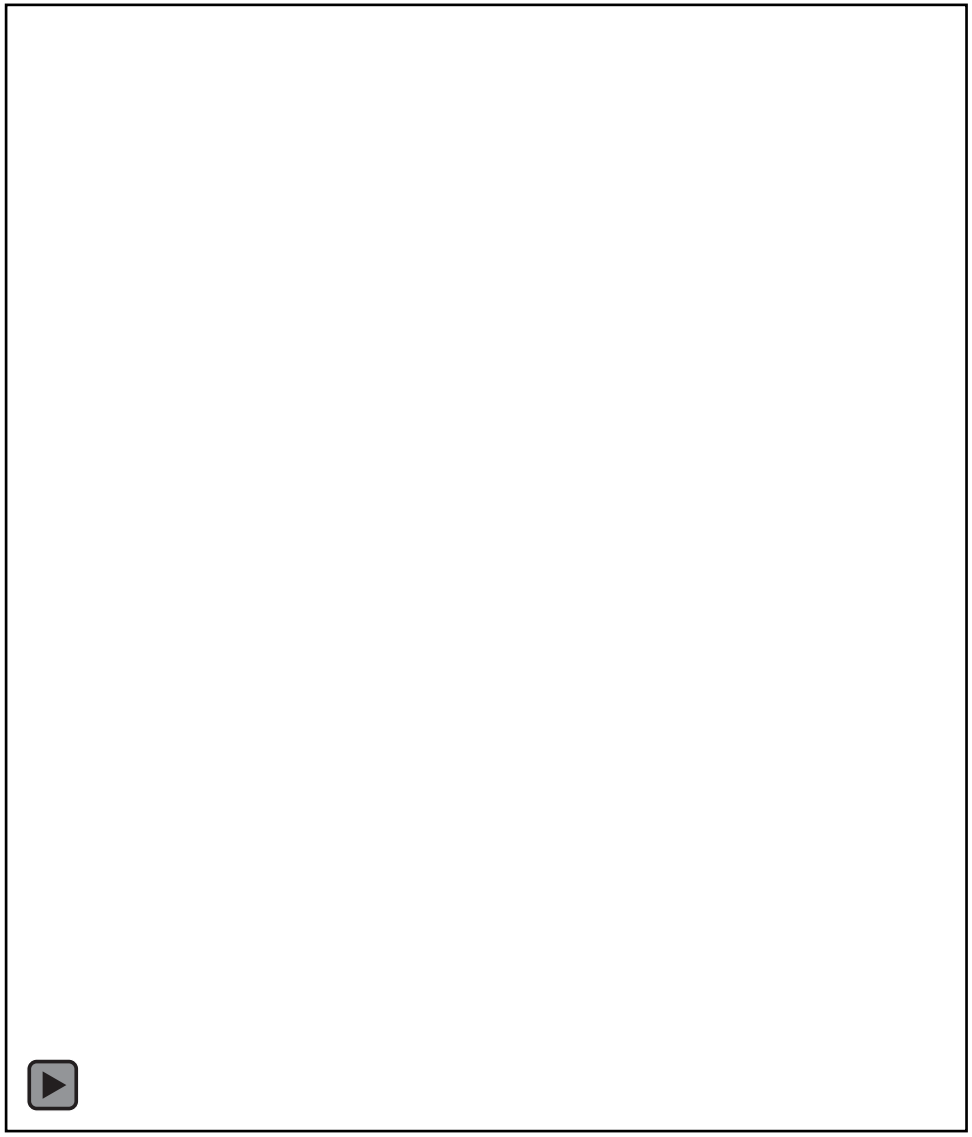
membrane-bound V_0 region



ATP:H^+ = 3:10

Zhao, 2015

structural changes occurring during rotary catalysis



Zhao, 2015

nature
structural &
molecular biology

Spiral architecture of the Hsp104 disaggregase reveals the basis for polypeptide translocation

Adam L Yokom[1,2], Stephanie N Gates[1,2], Meredith E Jackrel[3], Korrie L Mack[3,4], Min Su[1], James Shorter[3,4] & Daniel R Southworth[1]

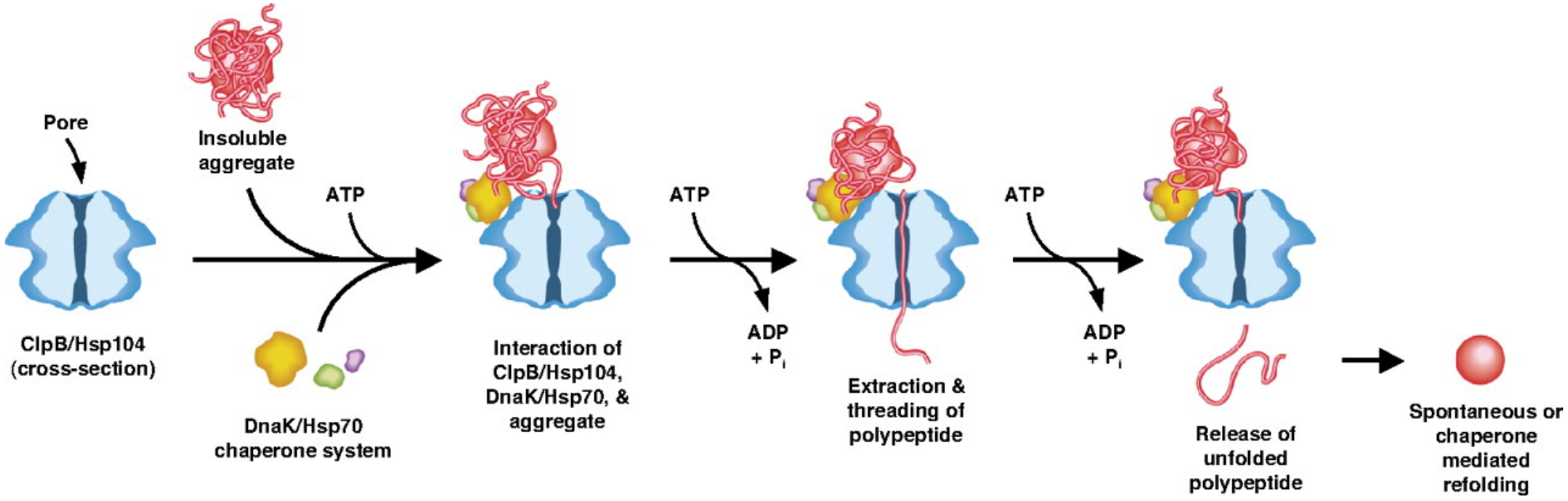
Hsp104 disaggregase

molecular chaperone, heat-shock protein

cooperation with Hsp70 in unfolding and rescuing aggregated protein

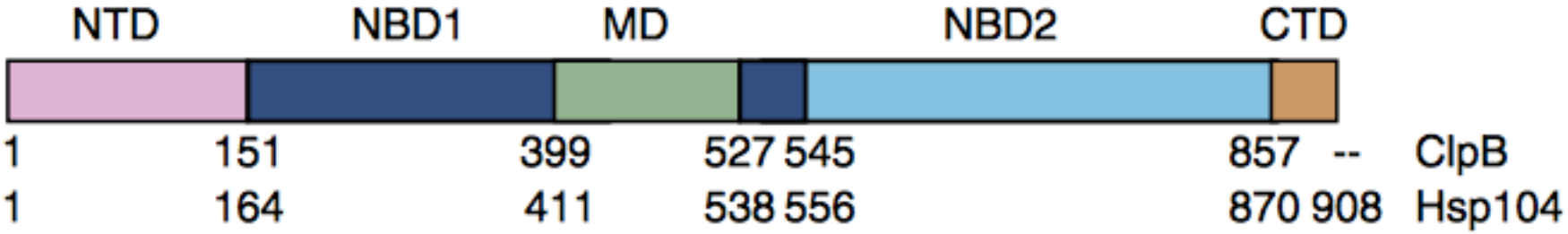
-> active translocation of polypeptide substrates through central channel

dose-dependent effects on yeast prion propagation



Doyle, 2017

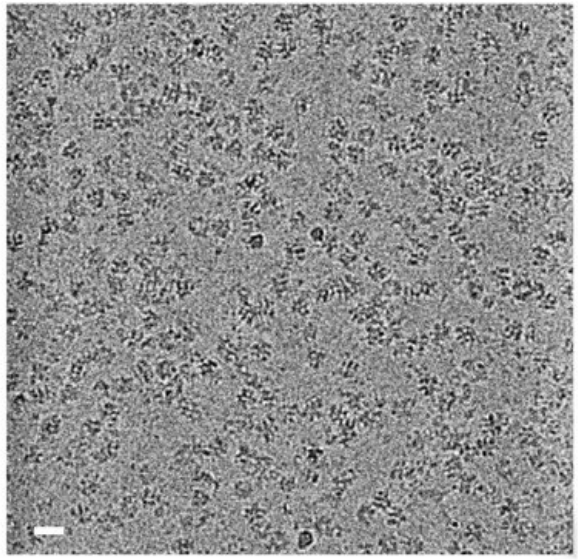
Hsp104 domain arrangement



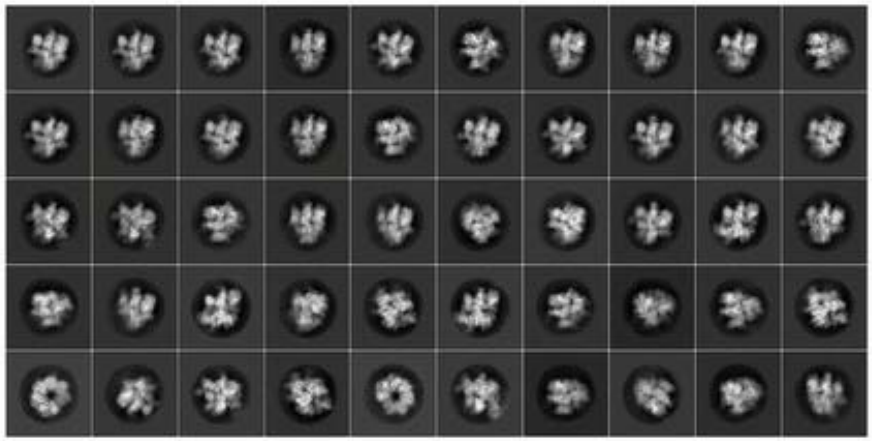
NTD	N-terminal domain	substrate engagement
NBD	nucleotide-binding domain	ATPase-binding domain, power translocation
MD	middle domain	disaggregation, interaction with Hsp70
CTD	C-terminal domain	required for hexamerisation

Yokom, 2016

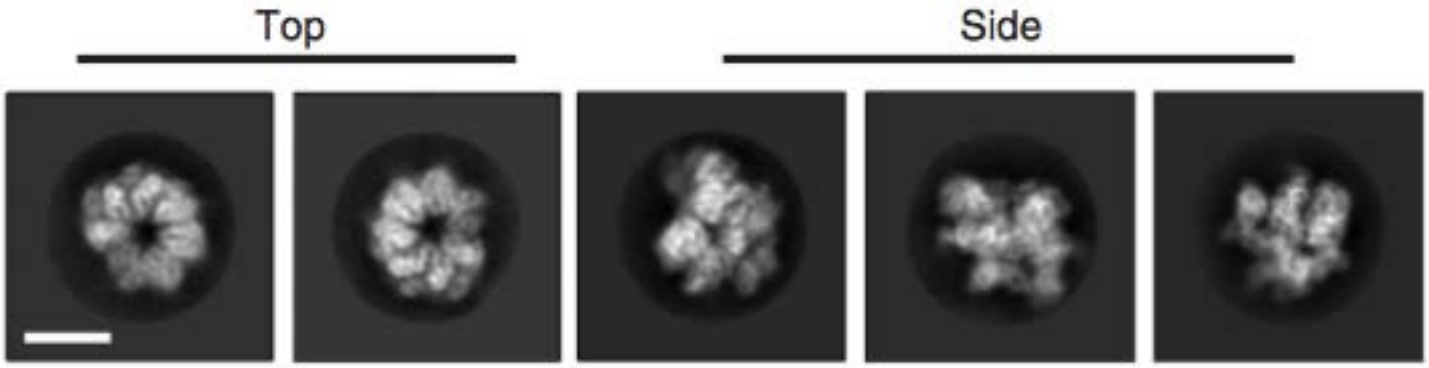
Hsp104: data collection and processing



2D projection

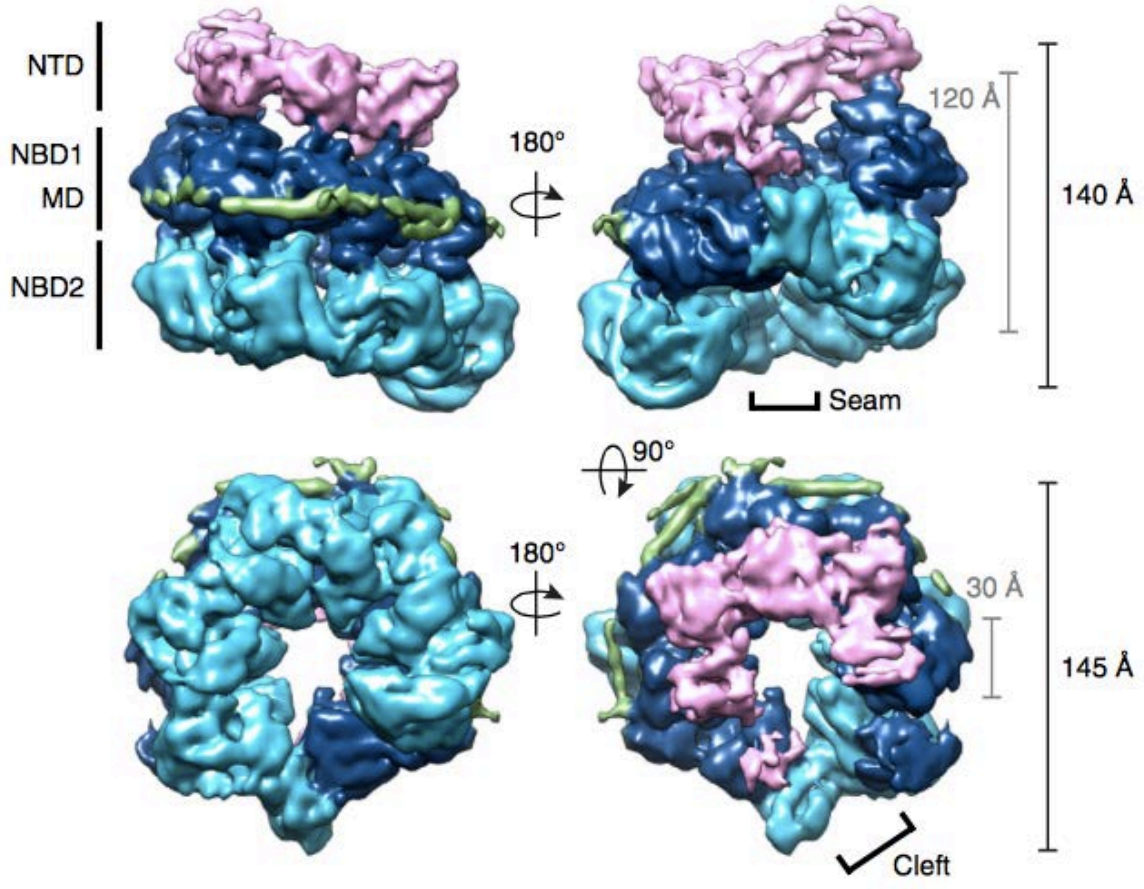


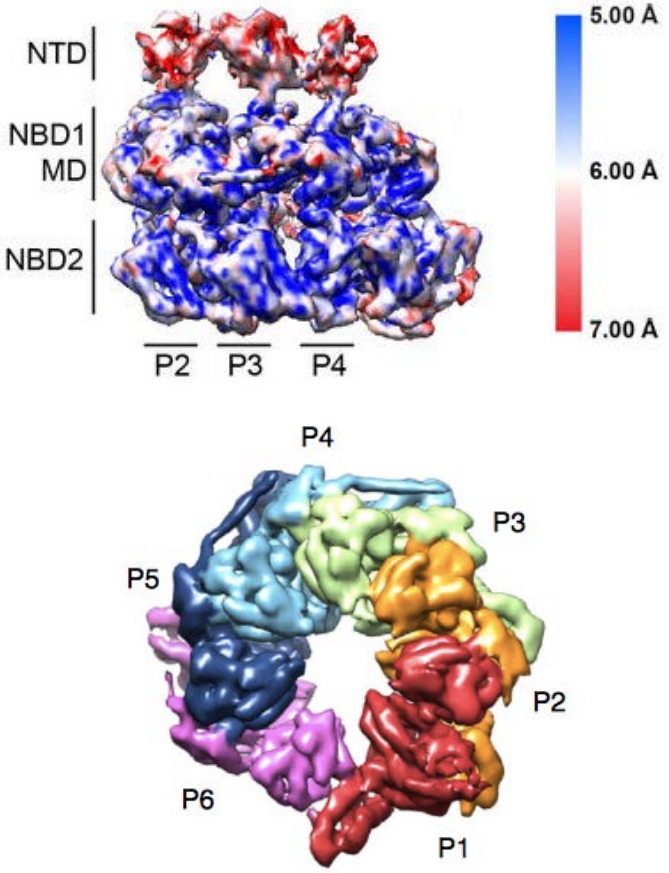
classified and averaged particles



Yokom, 2016

Hsp104: data processing

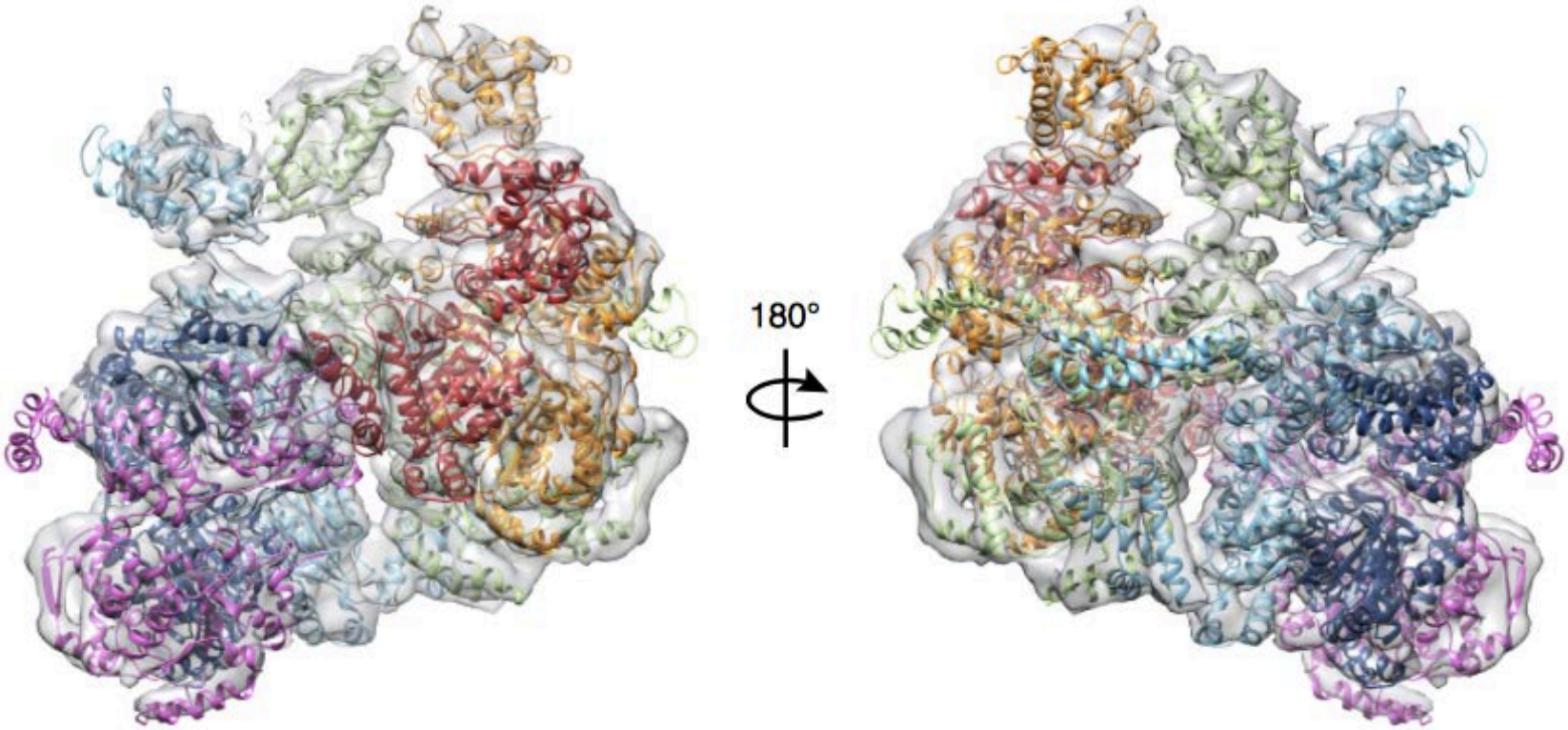




3D maps

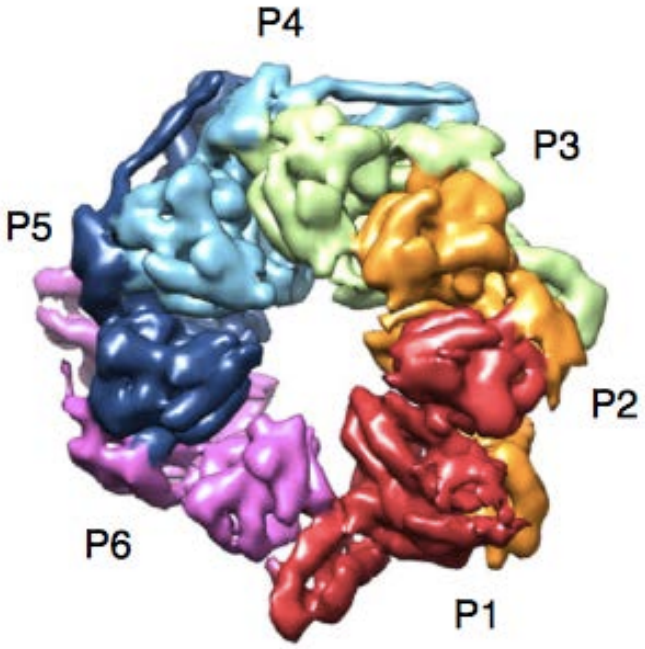
Yokom, 2016

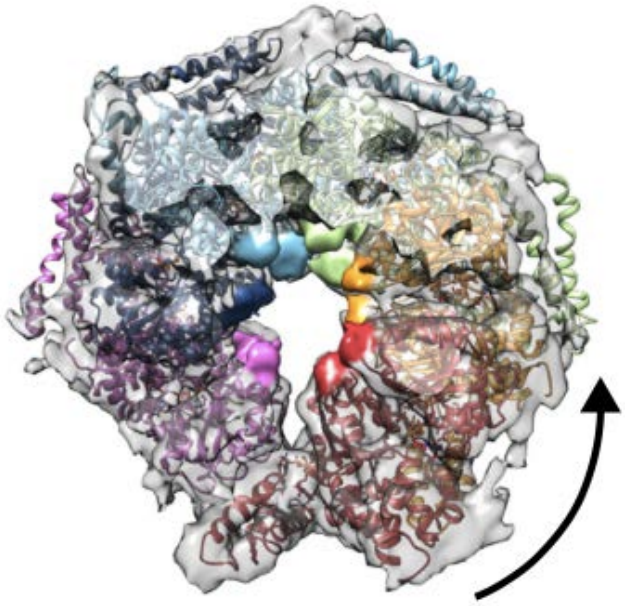
Hsp104: data processing

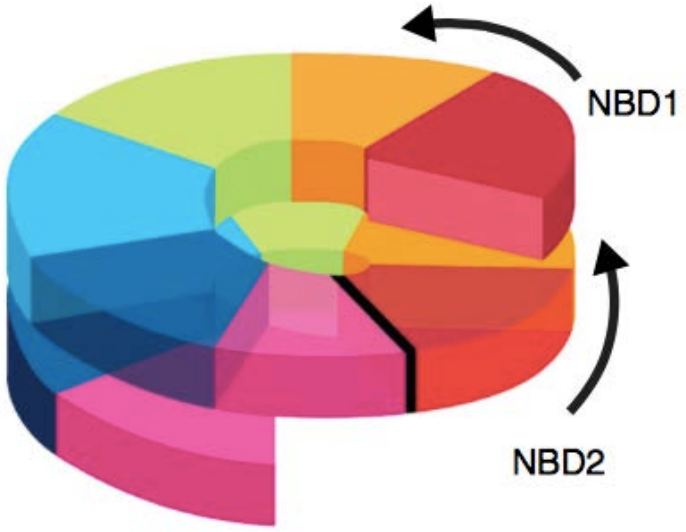


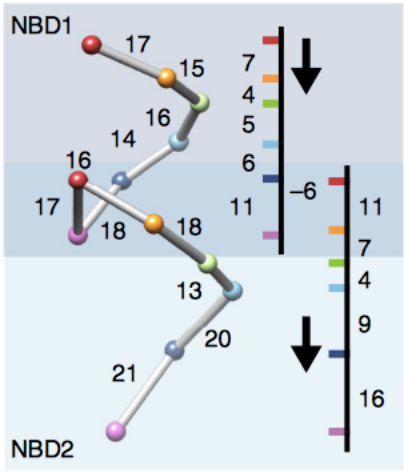
Yokom, 2016

spiral architecture

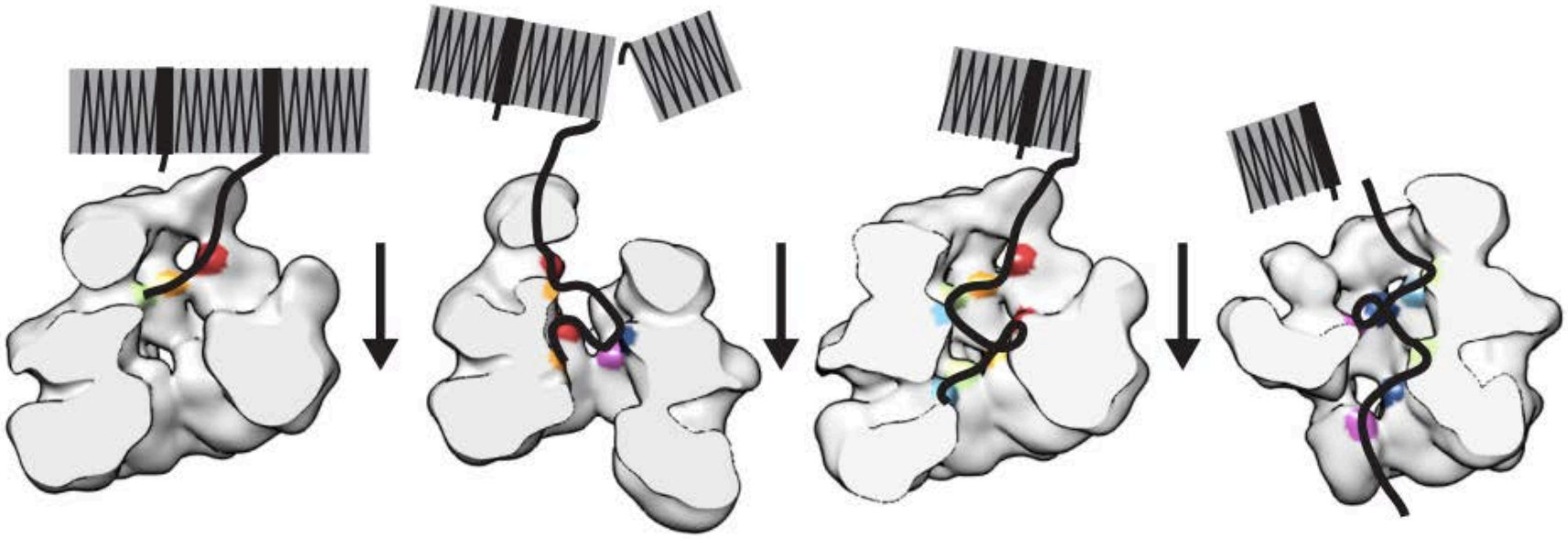








mechanism of cooperative disaggregation



cooperative disaggregation

Yokom, 2016

conclusions and outlook

+ near-atomic resolution data
+ native and hydrated structure (no contact surfaces)
+ capturing of different functional states / conformational transitions
+ flexible regions do not impede structure determination

– low signal-to-noise ration -> many images required
– time consuming and delicate sample preparation
– operation in a high vacuum
– large, expensive factilities

outlook

optimisation and automation of sample preparation

further improvements of direct electron detectors

software development

time-resolved studies