WP2: NanED for life science

Jan Pieter Abrahams

Biozentrum, Basel University

Paul Scherrer Institute

We’re here!

Overview

- Goals of NanED life science work package
- Basel NanED team & infrastructure
- Introduction to protein structure
- Diffraction methods for determining protein structure
- International developments since submission of the NanED proposal
- Some adaptations to WP4

Goals of the NanED life science WP2

ESR 14:

- Experimental phasing of protein crystals by nanobeam diffraction (data collection protocols and algorithms)
- Extend the method to non-crystalline samples

ESR 15:

- Optimise data acquisition parameters for rotation & nanobeam diffraction

Swiss WP2 team

Funded by ITN:

- Senik Metinyan (ESR 14)
- Amatassalam Ben Merien (ESR 15)

Funded by SNF:

- Meng Ge (postdoc with expertise in nanocrystallography)
- Pavel Filipcik (postdoc with experience in single particle cryo-EM & nanocrystallography)
- Min Chevalier Kwon (PhD student)

Funded by PSI:

- Eric van Genderen (scientist/engineer: data collection with hybrid pixel detectors)

- Burak Demir (Master student NanoScience)

Basel WP2 infrastructure

Jeol F200

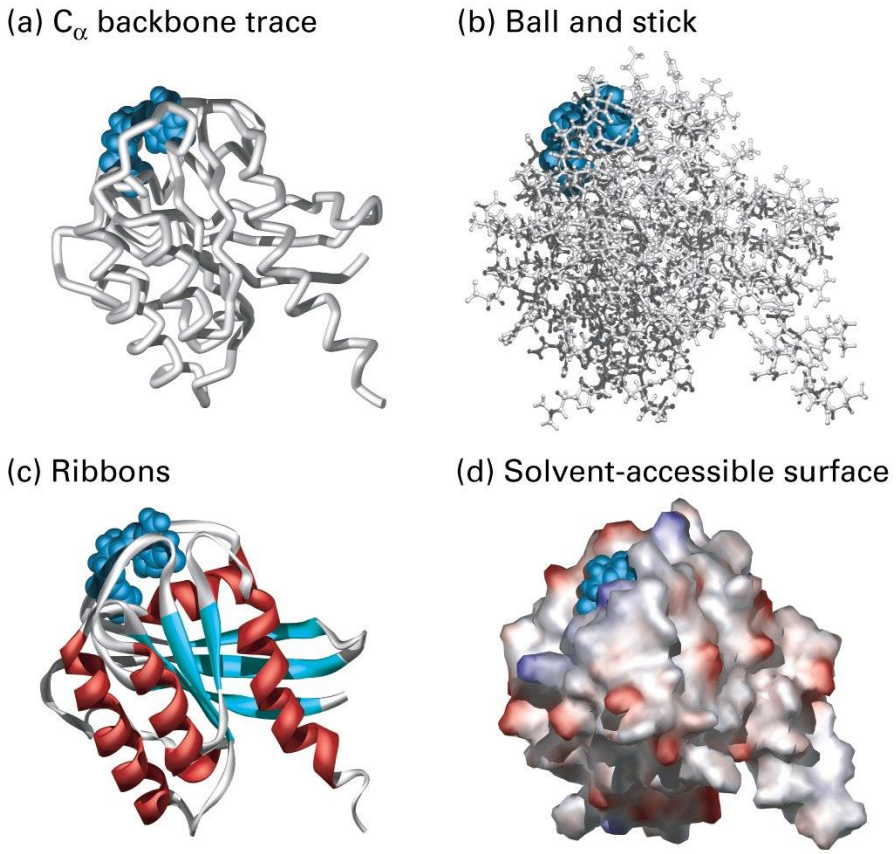
CEOS CEFID
energy filter

ASI CheeTah MR3 hybrid
pixel counting detector
(512x512; 2’000 fps)

PSI JUNGFRAU charge
integrating hybrid pixel detector
(1’024x1’536; 2’000 fps)

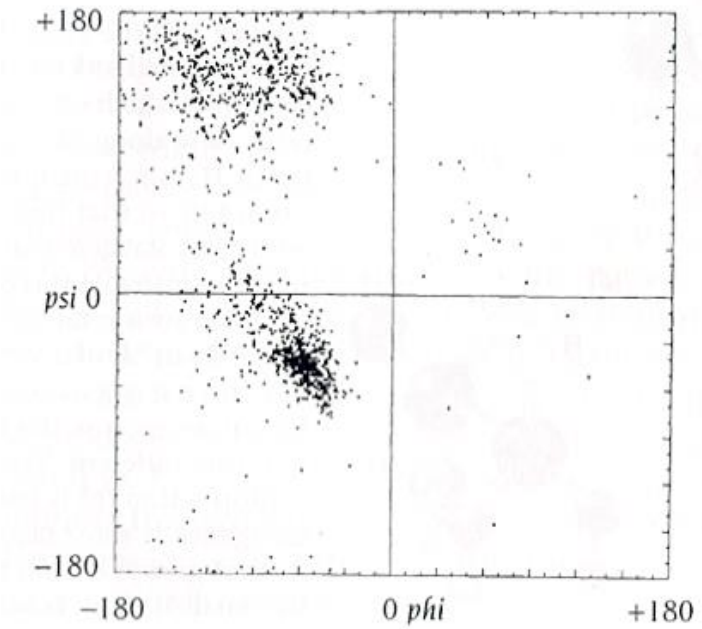
Being installed now;
ready for research:
March 2022

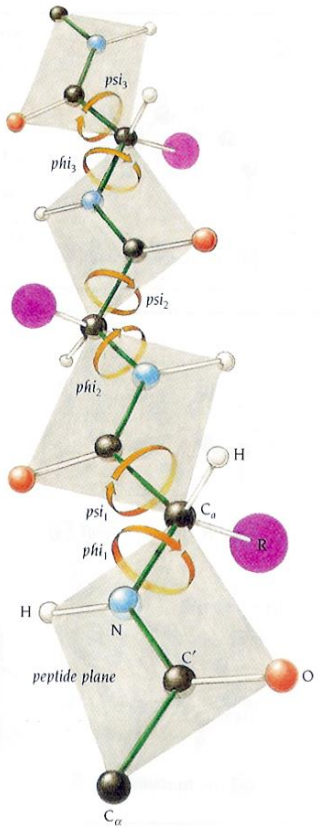
Introduction to protein structure



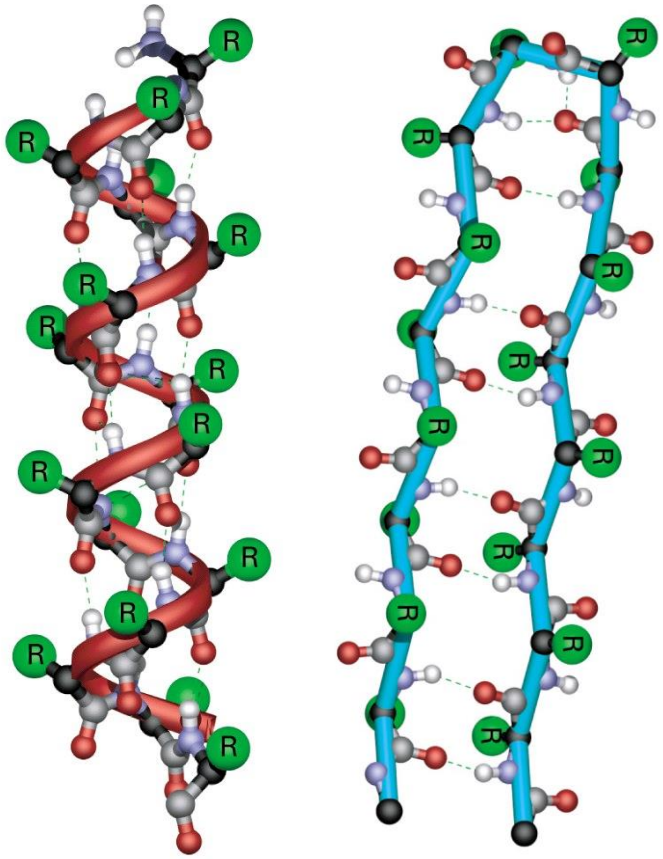
3D representations of protein structure

ϕ and ψ: torsion angles of the protein main chain bonds

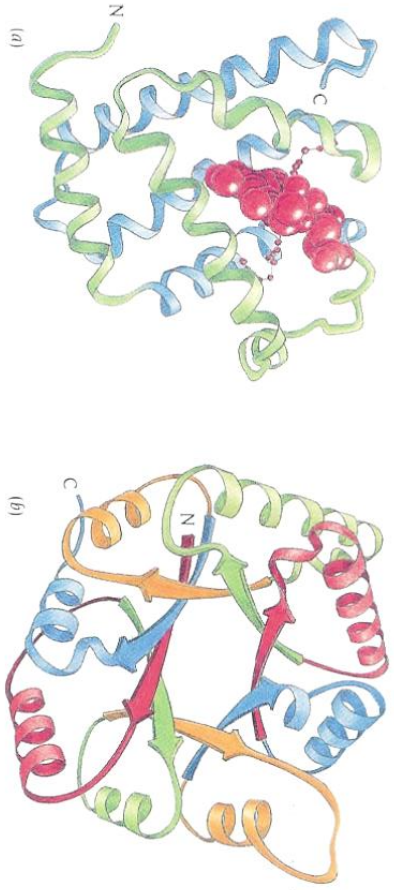




α-helix, β-sheet & loop combine into protein folding domains



α-helix, β-sheet & loop

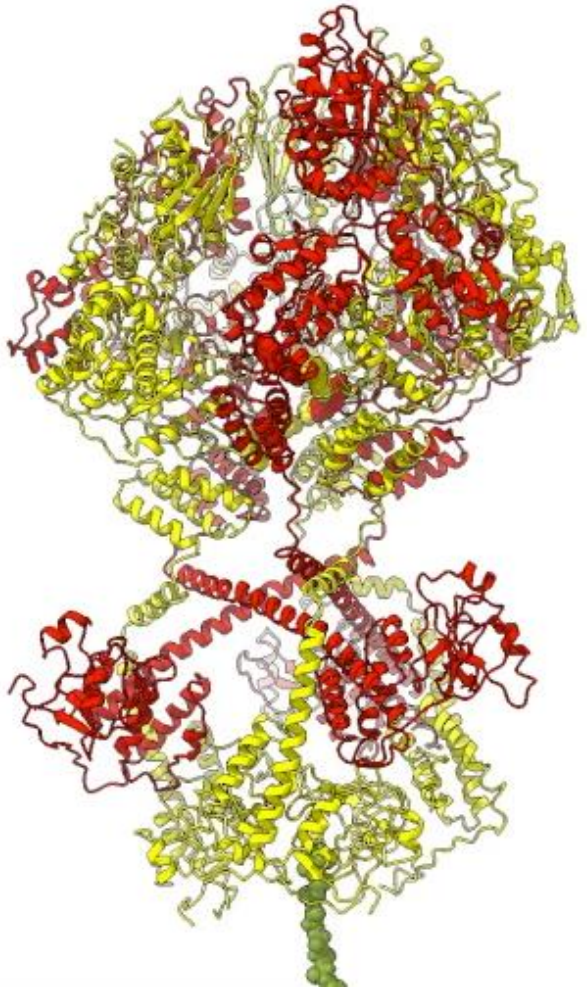


Examples of protein folds

Genome sequencing indicates there are between 2’000 and 10’000 natural protein folding patterns.

<u>Most of these folds are known</u>

Domains combine into dynamic, functional structures



Example: human mitochondrial LonP1 protease in 8 different conformations

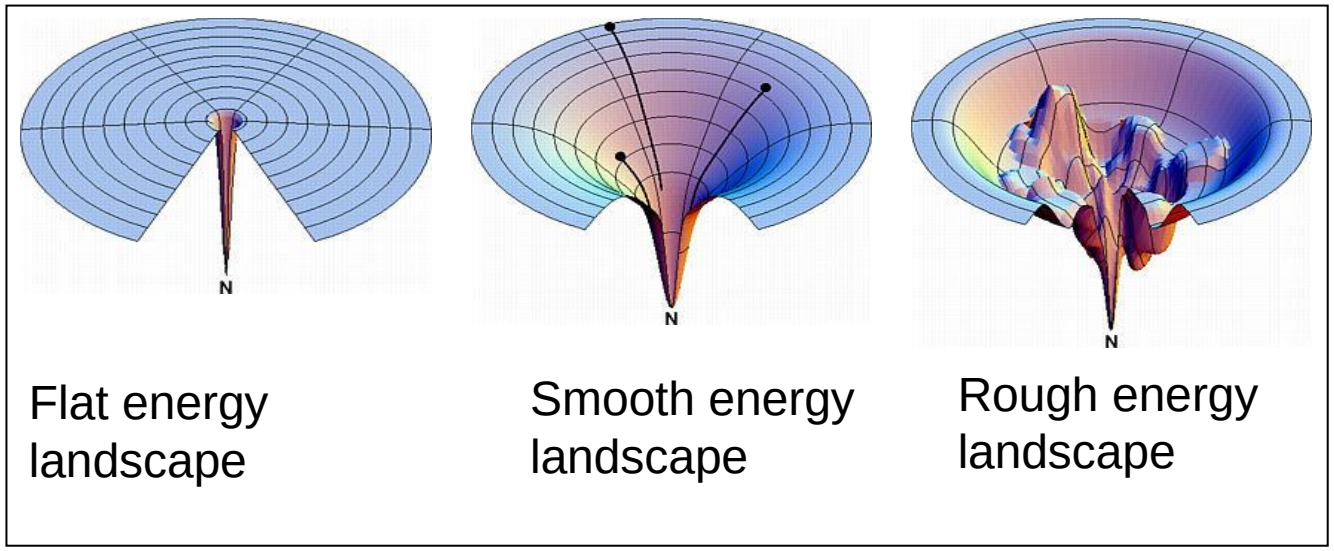
Levinthal paradox

Per amino acid there are 3 possible (ϕ, ψ) combinations: helix, sheet & loop

- A protein with N amino acids, should therefore have at least 3^{N-1} different possible conformations

- How is the proper conformation achieved???

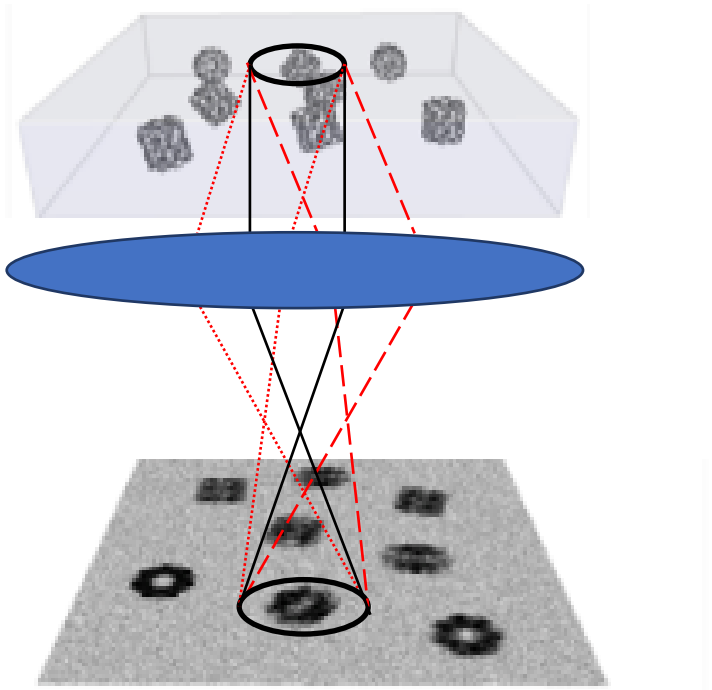
- Levinthal paradox: if all possible conformations would need to be sampled until the right one is found, the age of the universe would be too short for even a small protein to fold into its active conformation.



Solution: kinetics of folding.

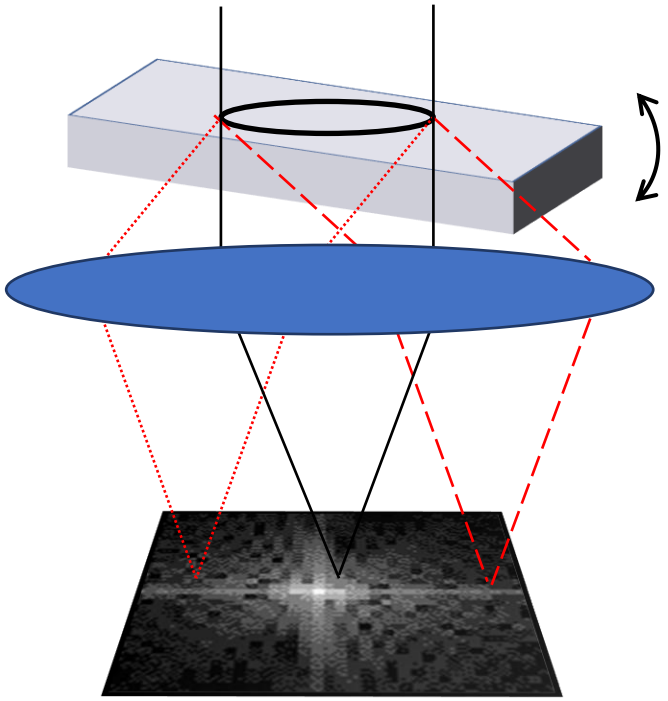
Electron diffraction methods for protein structure

Single particle cryo-EM



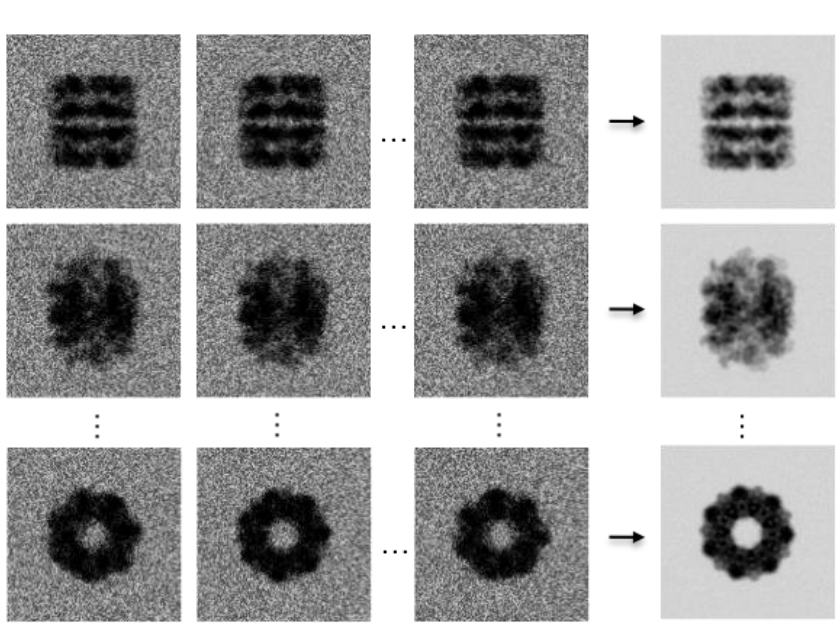
Problem: determining orientations & classes

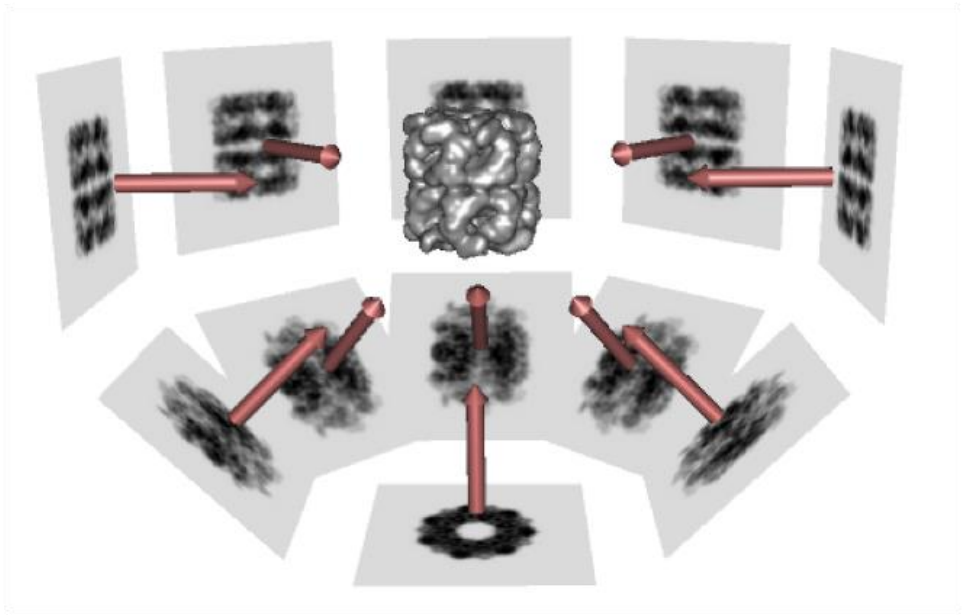
nanocrystallography



Problem: phasing diffraction pattern

Orienting & reconstructing in single particle cryo-EM

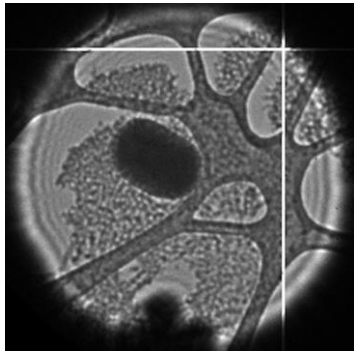




Up to a million or more individual images of the protein complexes may be required

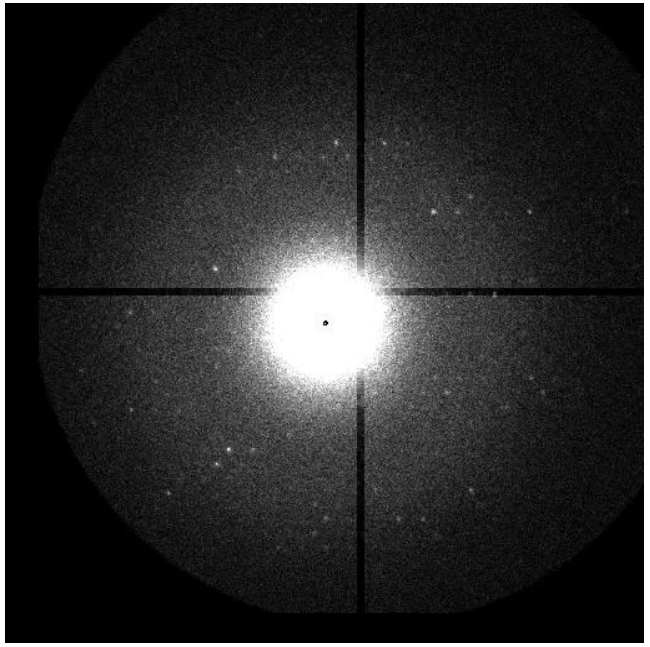
Phasing in electron nano-crystallography

Granulovirus



500 nm

Provided by
Peter Metcalf

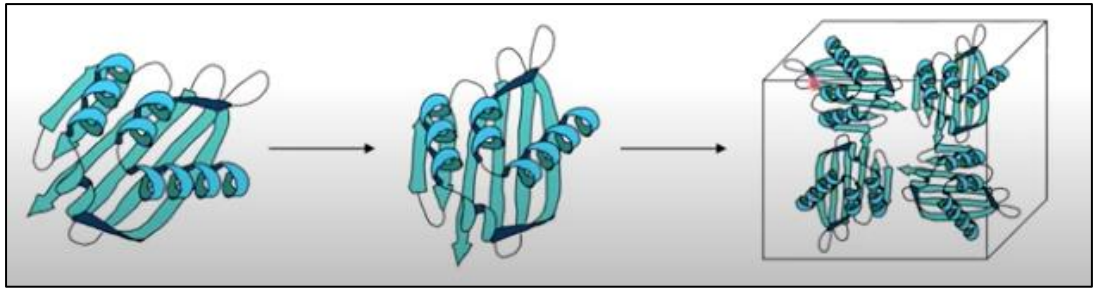


20 Deg from single crystal; ~2.4 Å

Molecular replacement for phasing diffraction data:

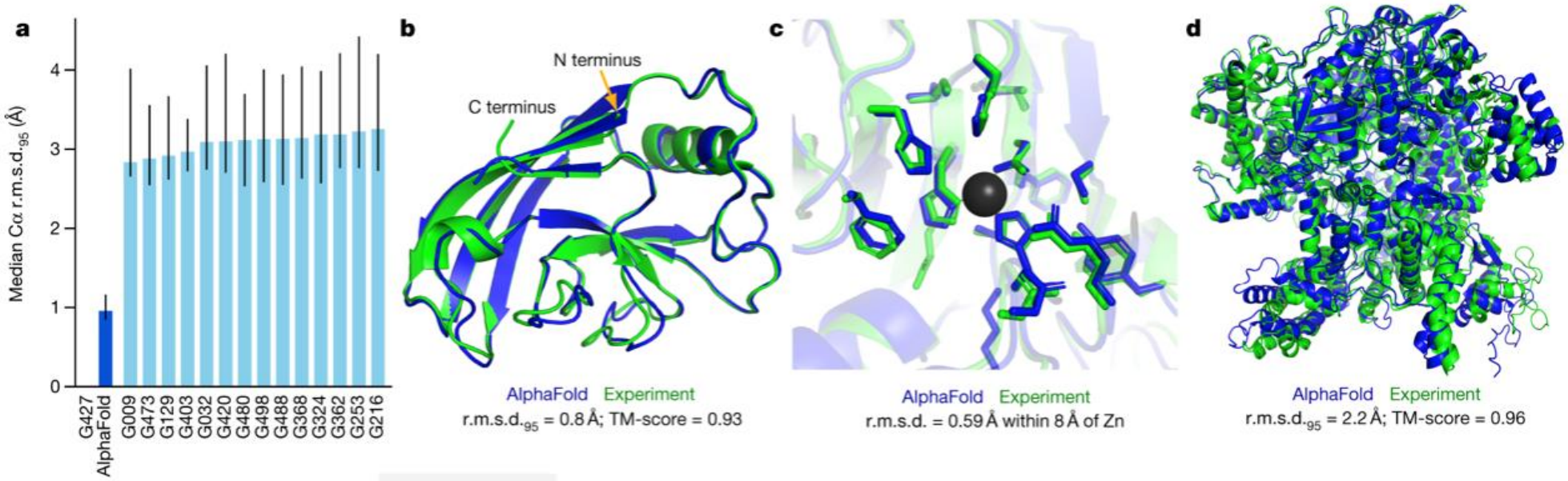
- Borrow phase information from related structure (initial model)
- Calculate difference maps (e.g. 2Fo-Fc)
- Iteratively improve model by rebuilding and refinement.

Step 1: orient the initial model in the unit cell



Knowledge of the fold solves the phase problem in protein crystallography

Recent international development (1): AlphaFold



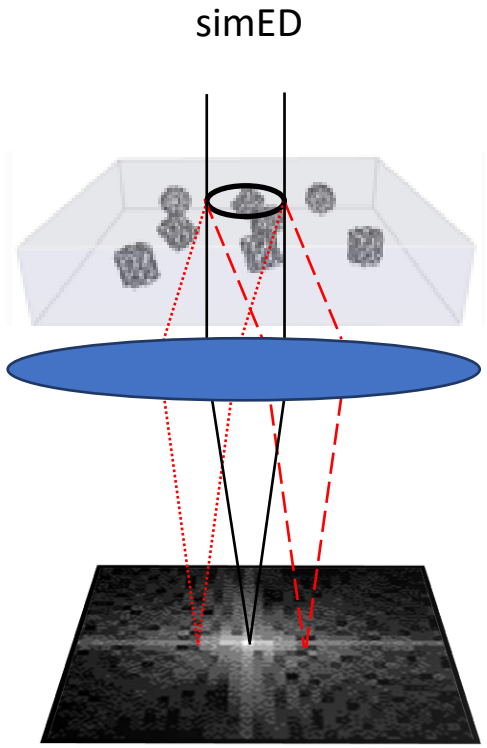
AlphaFold predicts protein structures that are as accurate as experimentally determined structures

Jumper *et al.* (2021) *Nature* **596**, 583-9

Adaptation ESR 14

Goal 1 ESR 14: Experimental phasing of protein crystals by nanobeam diffraction (data collection protocols and algorithms)

Shift focus from protein crystals to molecular complexes: single molecule nano-beam diffraction (simED)

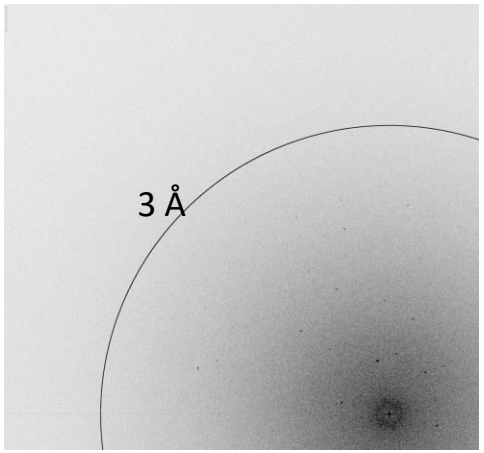


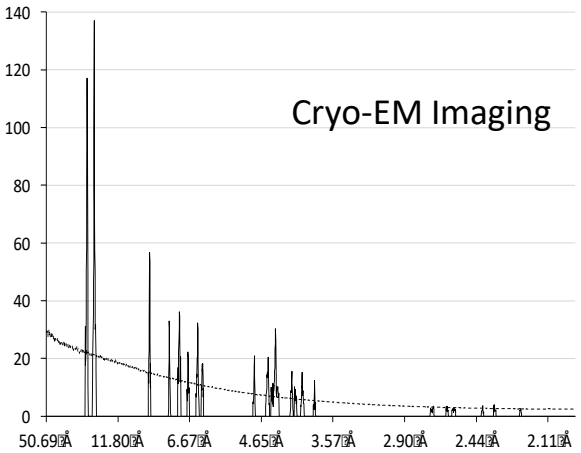
Subgoals:

- Optimal data collection (scanning & automation)
- Identifying molecule locations & orientations (deep learning / correlation)
- Phasing continuous diffraction patterns by molecular replacement
- Compare SNR to single particle cryo-EM

ED instead of EM because it improves the SNR

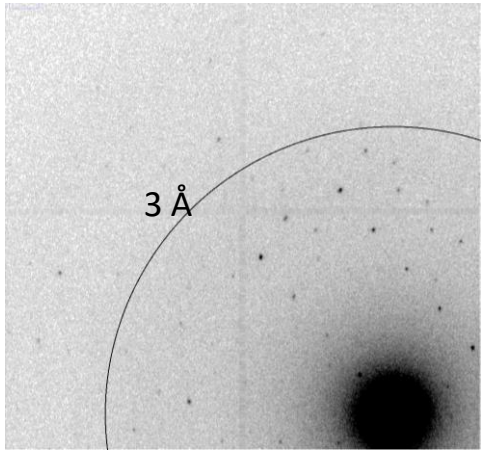
Total dose:
6 e^- / Å^2

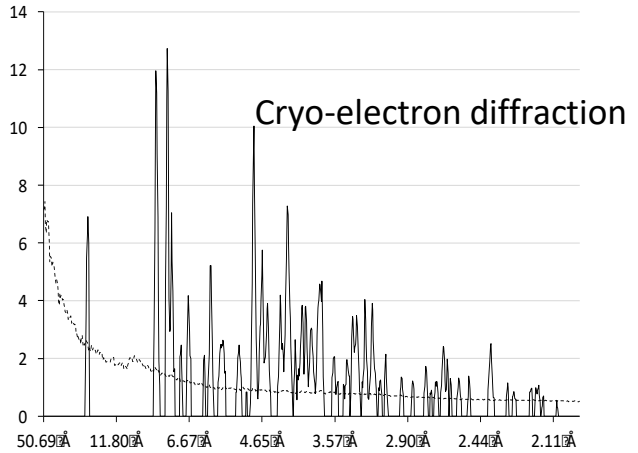




Latychevskaia &
Abrahams, 2019

Total dose:
0.06 e^- / Å^2

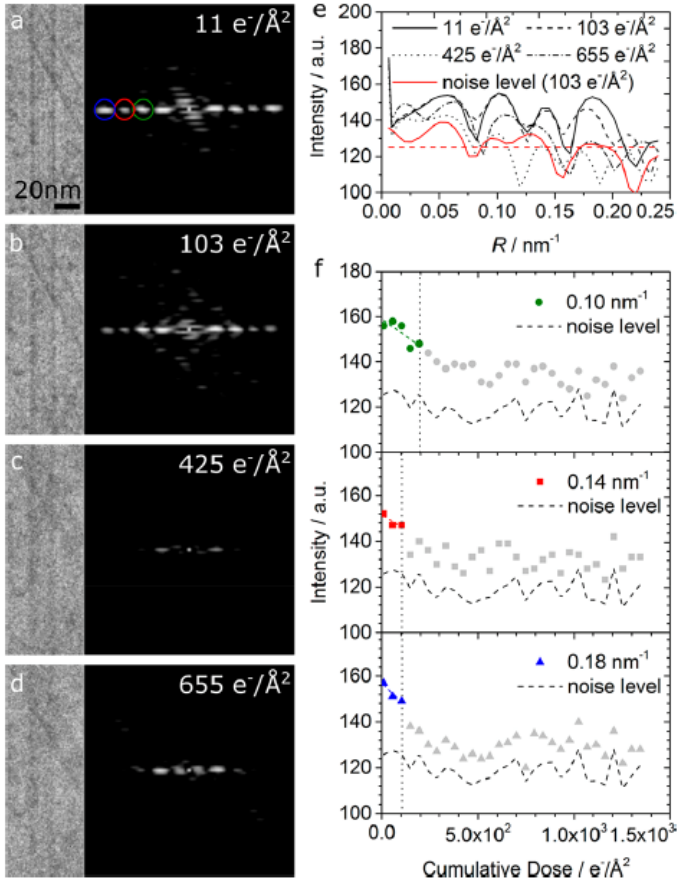




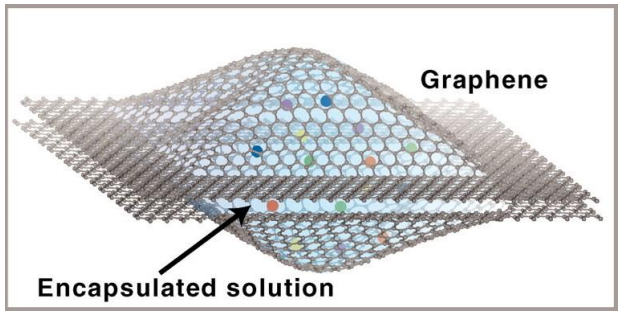
Lysozyme crystals of similar size & thickness

Nederlof et al 2013, Clabbers et al, 2017

Recent international development (2): reducing radiation damage in graphene ravioli

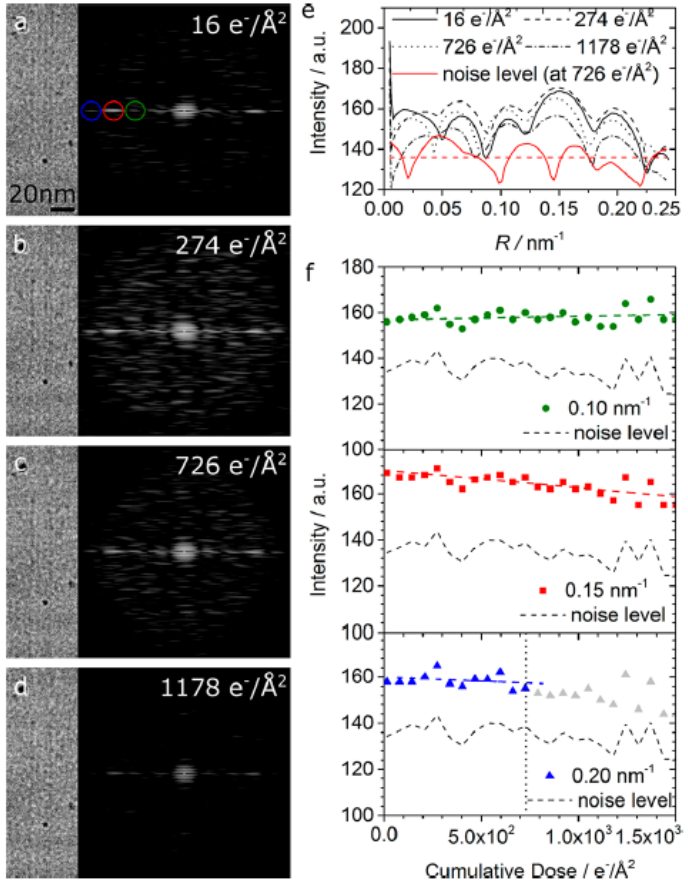


Graphene support, -270 °C, frozen hydrated sample



Niels de Jonge investigated radiation damage to microtubule protein complexes in graphene liquid cells

Keskin & de Jonge (2018) *Nano Lett.* **18,** 7435-40



Graphene liquid cell, room temperature

Adaptation ESR 15

Goal ESR 15:

- Optimise data acquisition parameters for rotation & nanobeam diffraction

Concrete step: assess ‘graphene ravioli’ for reducing radiation damage:

- Learn sample prep from the lab of Niels de Jonge
- Measure functional & structural damage using various sample preparation technologies, using Green Fluorescent Protein (GFP):
  - Functional damage: reduction of GFP fluorescence as a function of electron dose
  - Structure damage: reduction of Bragg diffraction of GFP crystals

Some conclusions

- Protein folding problem seems solved
- Nano-diffraction methods likely offer advantages in SNR over cryo-EM
- Next challenge: identify and orient proteins in complex samples (living cells?)
- Small shift in research focus required for parts of WP2
- We have a strong team and good infrastructure for the new challenges!

Thank you!