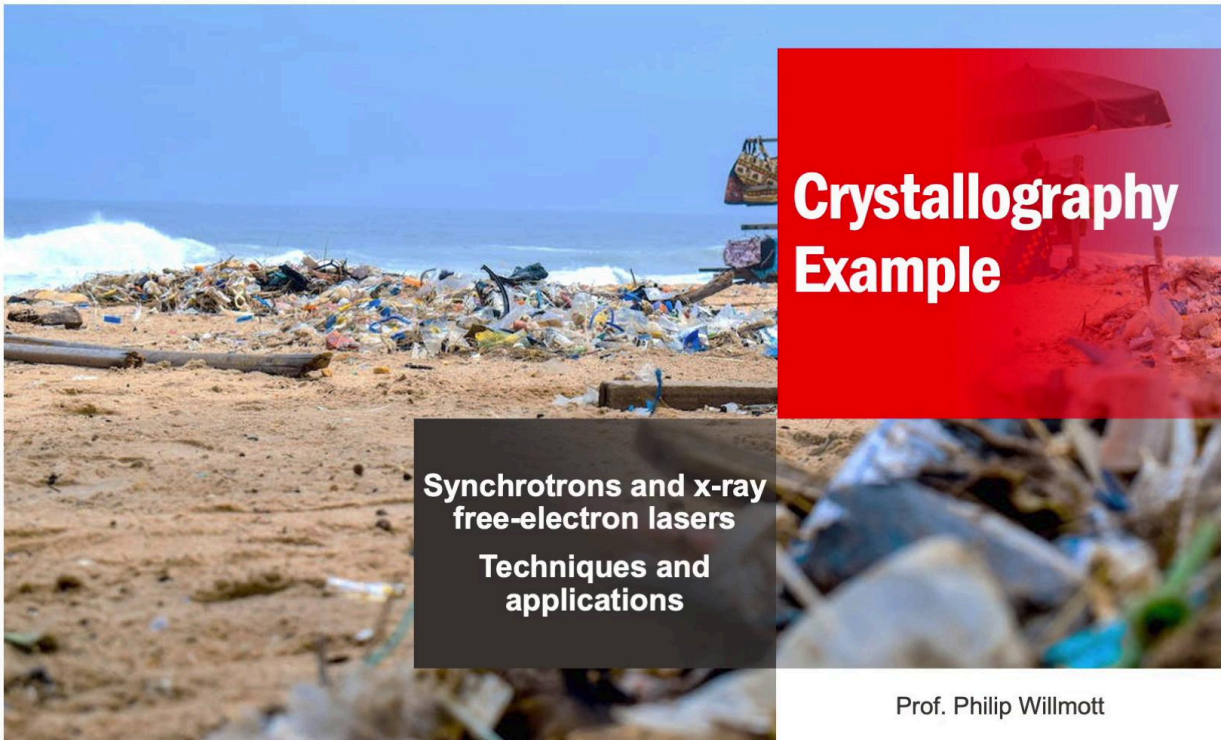
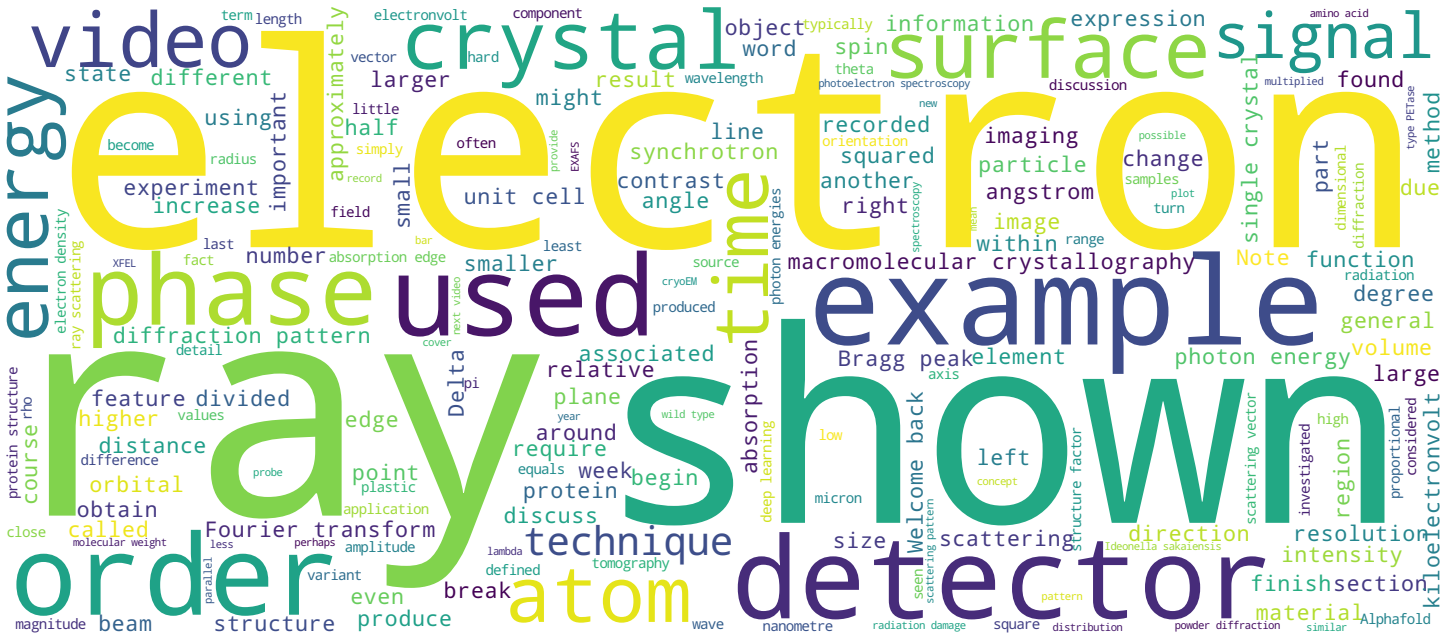




Search MOOC

Video

EPFL

Contents and objectives of this video

- Example
- The future of MX

Welcome back to the very last of this week's videos. In this video, we will finish with an example that has drawn on both conventional macromolecular crystallography and machine learning, then consider the future of macromolecular crystallography in a scientific landscape that has changed radically in the last decade.

Notes

Summary

0m 05s

Example – plastic-eating enzymes

Photo: Creative Commons

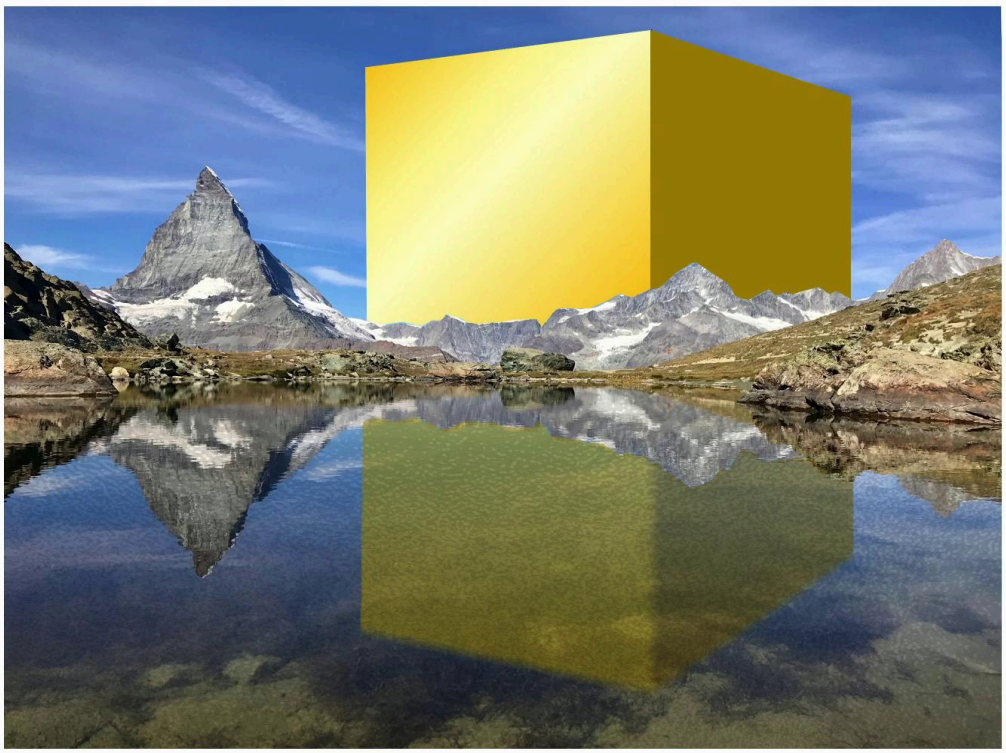
- Since 1950, 8.3 billion tons of plastic have been manufactured
  - Equivalent to a plastic cube of 2 km!!
- ¾ of all plastic ever produced is now waste
  - 9% recycled
  - 12% burnt
  - **79% waste depots**
- 8 million tons end up each year in the oceans
- e.g., Polyethylene terephthalate (PET)
  - > 2000 years to be biologically decomposed…
  - 12% of global solid waste!

The example is concerned with the production of plastic and its accumulation in the environment since the beginning of its widespread fabrication in the mid 20th century.

Notes

Summary

0m 26s



Since then, it's estimated that over 8.3 billion tonnes of plastic have been manufactured, equating to approximately 1.5 tonnes per person per lifetime. This volume of material equates to a two-kilometer-sided cube. This isn't just loose conglomerations of squished pet bottles, but a solid block with no air gaps.

Notes

Summary

0m 39s

Example – plastic-eating enzymes

Photo: Creative Commons

- Since 1950, 8.3 billion tons of plastic have been manufactured
  - Equivalent to a plastic cube of 2 km!!
- ¾ of all plastic ever produced is now waste
  - 9% recycled
  - 12% burnt
  - **79% waste depots**
- 8 million tons end up each year in the oceans
- e.g., Polyethylene terephthalate (PET)
  - > 2000 years to be biologically decomposed…
  - 12% of global solid waste!

Three quarters of all this plastic is now waste, of which almost 80 % is contained in waste depots. Notably, some 8 million tonnes end up per year in the oceans. Of all these plastics, one of the most pernicious is polyethylene terephthalate, more commonly referred to as PET. Although precise numbers are missing, it is apparent that PET requires well in excess of 2,000 years to biologically decompose. PET accounts for 12 % of all global waste.

Notes

Summary

1m 07s

Plastics, microplastics, and the environment

See e.g. https://sapea.info/topic/microplastics/

As just mentioned in the case of oceanic plastic pollution, much of plastic waste is simply abandoned, whether that be in the air, soil and sediment, fresh waters, seas, oceans, or the city streets.

Notes

Summary

1m 45s

Plastics, microplastics, and the environment

See e.g. https://sapea.info/topic/microplastics/

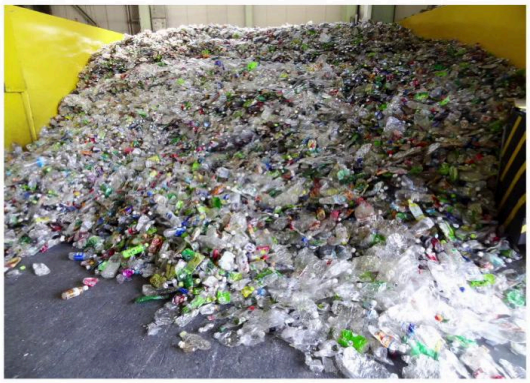
This is inevitably and inadvertently, partially taken up in the food chain, often becoming concentrated in certain organs, leading to death in many cases. We humans, at the top of the food chain as we are, are not immune to this, and the microplastics are not just found in our lungs and guts, but have also been discovered even in our blood vessels.

Notes

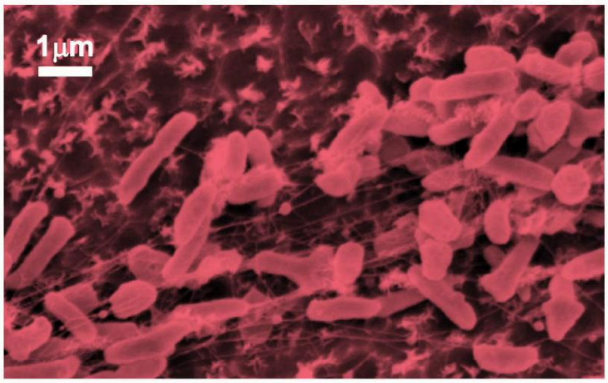
Summary

1m 59s

Curious findings in Japan



Plastic-bottle-recycling-plant, Sakai, Osaka



Ideonella sakaiensis

Austin *et al.*, PNAS **115** (2018) E4350
See also: https://www.youtube.com/watch?v=Y-GqbPmSuj4

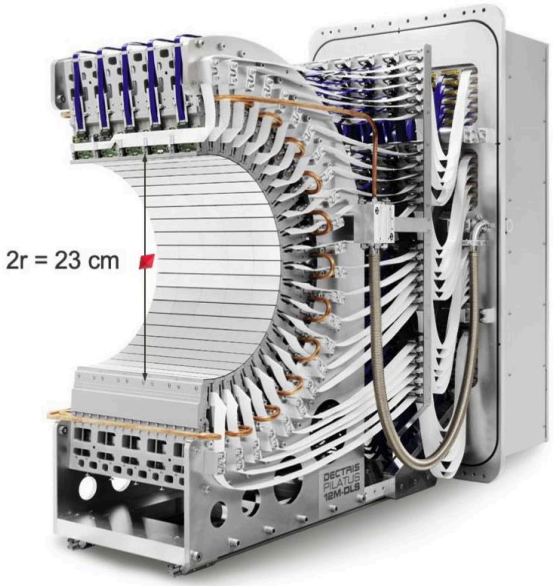
So our story begins in a plastic bottle recycling plant in Sakai, Osaka, in 2016, where a novel bacterium named Ideonella sakaiensis was discovered in a sample of PET-contaminated soil. It was shown after PET was initially degraded by Ideonella sakaiensis, the entire microbial community could set to work on it, and break 75 % of it down into carbon dioxide. So it seems it's becoming increasingly clear that nature will sooner or later exploit the carbon and energy sources that plastics offer. It was shown that the bacteria produces an enzyme, dubbed PETase, that breaks down PET into three different monomeric compounds. Furthermore, a second enzyme secreted by Ideonella sakaiensis, so-called MHETase, further breaks down one of the three direct products of PETase degradation.

Notes

Summary

2m 24s

Low-energy SAD to the rescue



Pilatus 12M detector
@ I23, Diamond Light Source

- I23 beamline, Diamond Light Source
- Low-energy photon-counting detector
  - 5 x 24 modules, 12 Mpixels
  - ± 100° curved detector surface
  - Vacuum compatible
- Diffraction sets
  - 5050 eV (2.455 Å) SAD
    - $Q_{max} = 4\pi/\lambda \sin\theta = 3.92$ Å^{-1}
    - $d_{min} = 2\pi/Q_{max} = 1.6$ Å
  - 10 keV: $Q_{max} = 7.76$ Å^{-1}, $d_{min} = 0.809$ Å
  - 12.7 keV: $Q_{max} = 9.86$ Å^{-1}, $d_{min} = 0.638$ Å

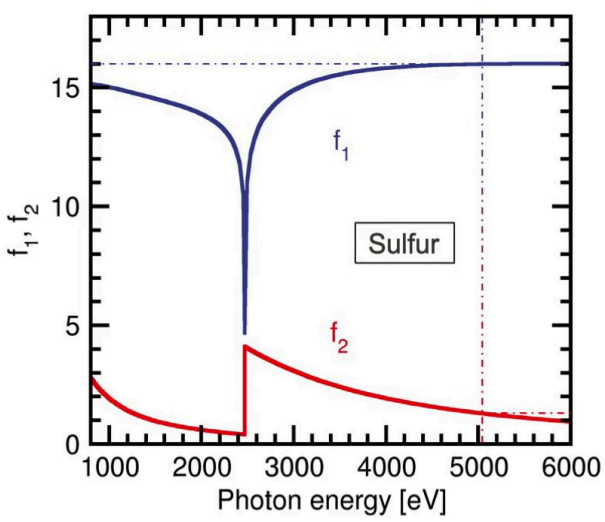
PETase crystals were grown and investigated at the Low Energy Crystallography beamline I23 at the Diamond Light Source in England. Data sets were recorded at a little over five kiloelectronvolts, and then at 10 kiloelectronvolts and then 12.7 kiloelectronvolts at the nearby I03 beamline. The detector used is quite unique as it has a cylindrical sensitive area with a radius of 11.5cm and spanning plus or minus 100 degrees. This large scattering angle allows one to access relatively large scattering vectors Q, even for low photon energies.

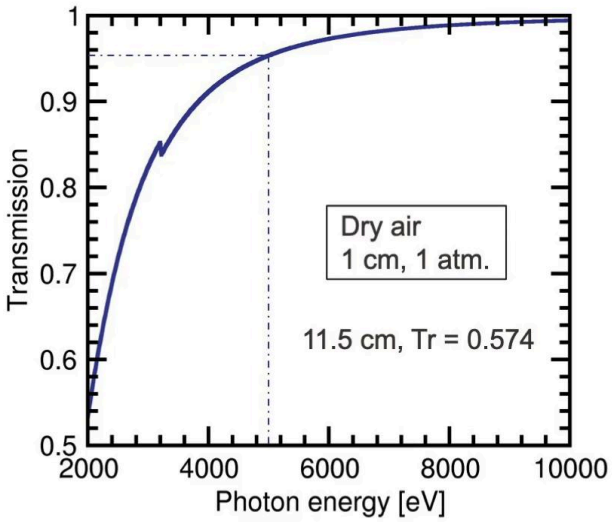
Notes

Summary

3m 29s

Low-energy SAD to the rescue



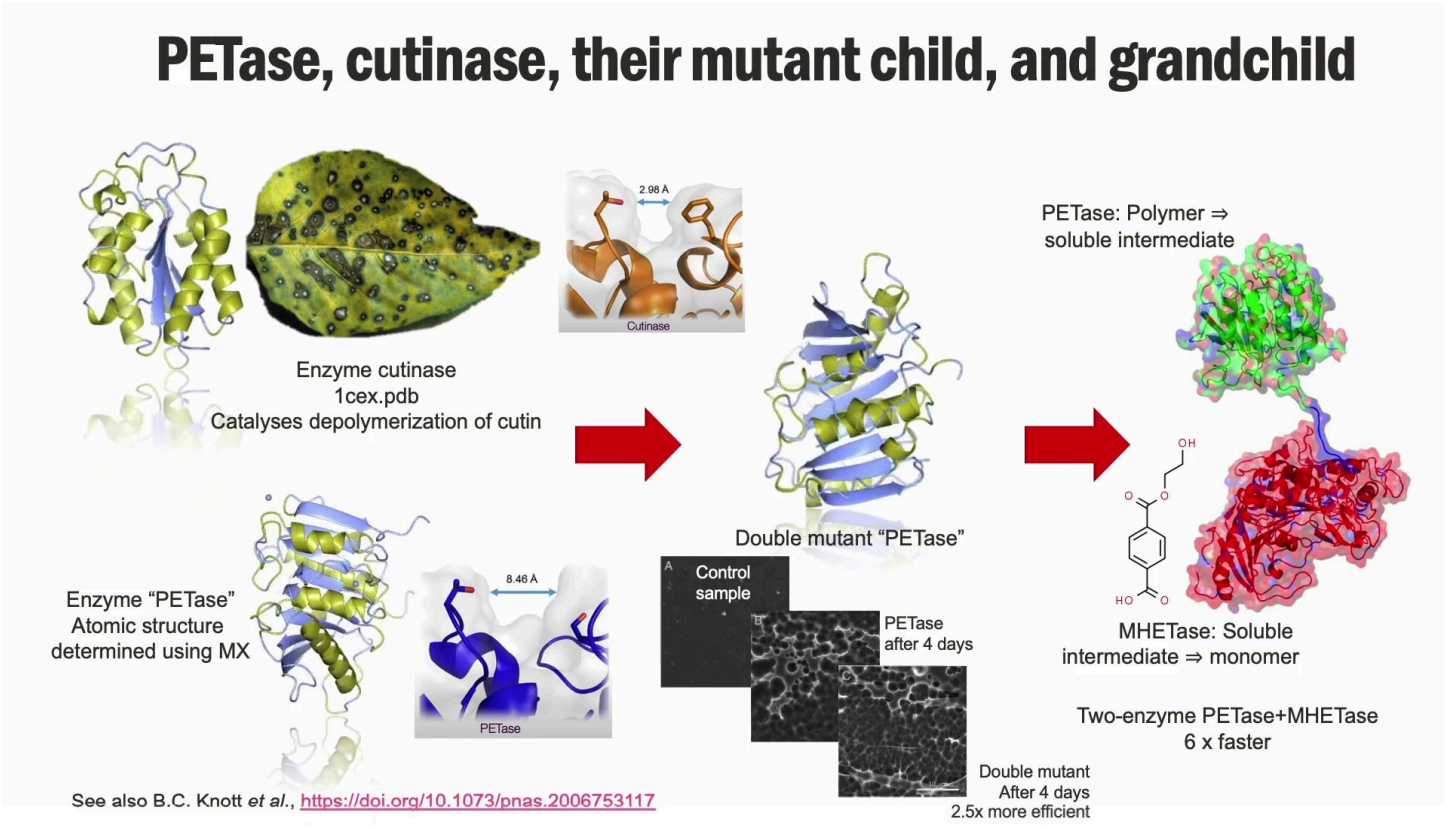


At the lower photon energy of 5.05 keV, some 2,600 electronvolts higher than the sulphur-K edge, the anomalous signal is modest. f1 in the forward direction is essentially exactly equal to the atomic number of sulphur, 16, while f2 is only about 1.3 electrons. Nonetheless, native sulphur-SAD was able to phase the data. Note that working at these low photon energies doesn't come for free. Not only is radiation damage of the crystal significantly more rapid, but the X-rays are also attenuated significantly by air. If one atmosphere dry air were used, the transmission across 11.5 cm of air would be a little under 60 %. This requires the volume between the crystal and detector to be either evacuated or filled with helium gas, which in turn can lead to denaturing of the crystal if precautions are not taken.

Notes

Summary

4m 13s



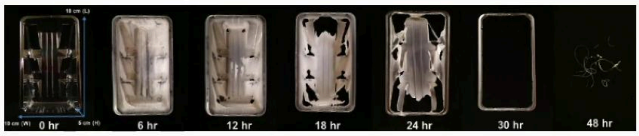
Similarities in the amino acid sequence alignments between PETase and cutinase indicated a similar structure in the active region of the enzyme responsible for the decomposition of PET. Cutinase is produced by several fungi and is used to break down the waxy protective polymer layer in leaves and plant surfaces. The active cleft site of cutinase is significantly narrower than that observed in the wild-type PETase. By mutation of two active site residues, a variant of PETase could be synthesised, with a narrower cleft width and a catalytic activity, which is shown to be two and a half times higher. Some four years later, a novel development was reported in which the synergistic actions of PETase and MHETase accelerated decomposition still further, whereby the soluble intermediates produced by PETase catalysis were further broken down into the PET monomers by MHETase mutants. This dual, enzymatic system exhibited a sixfold improvement on the wild-type PETase.

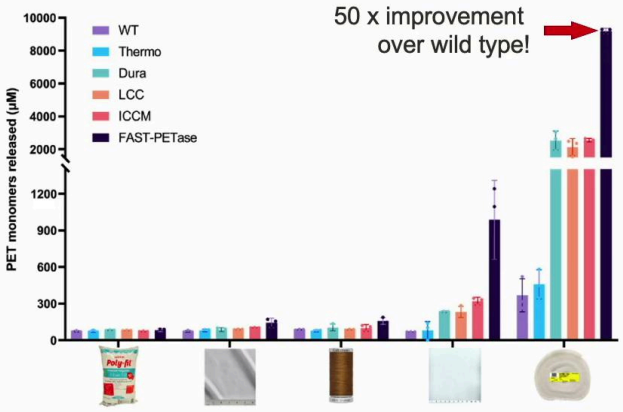
Notes

Summary

5m 18s

Deep learning further improves mutant engineering





- Previous enzymes for decomposing PET
  - Poor thermal stability and pH tolerance
  - Slow at near-RT
- MutCompute
  - Self-supervised
  - Convolutional neural network
  - Identifies non-optimal residues in wild-type structures and replaces with more stable amino acids from 19'000 sequence-diverse proteins
- 51 tested plastic products
  - RT: all completely degraded by FAST-PETase within one week
  - @ 50°C: ~ 24 hours!
- "Scanning mutagenesis"

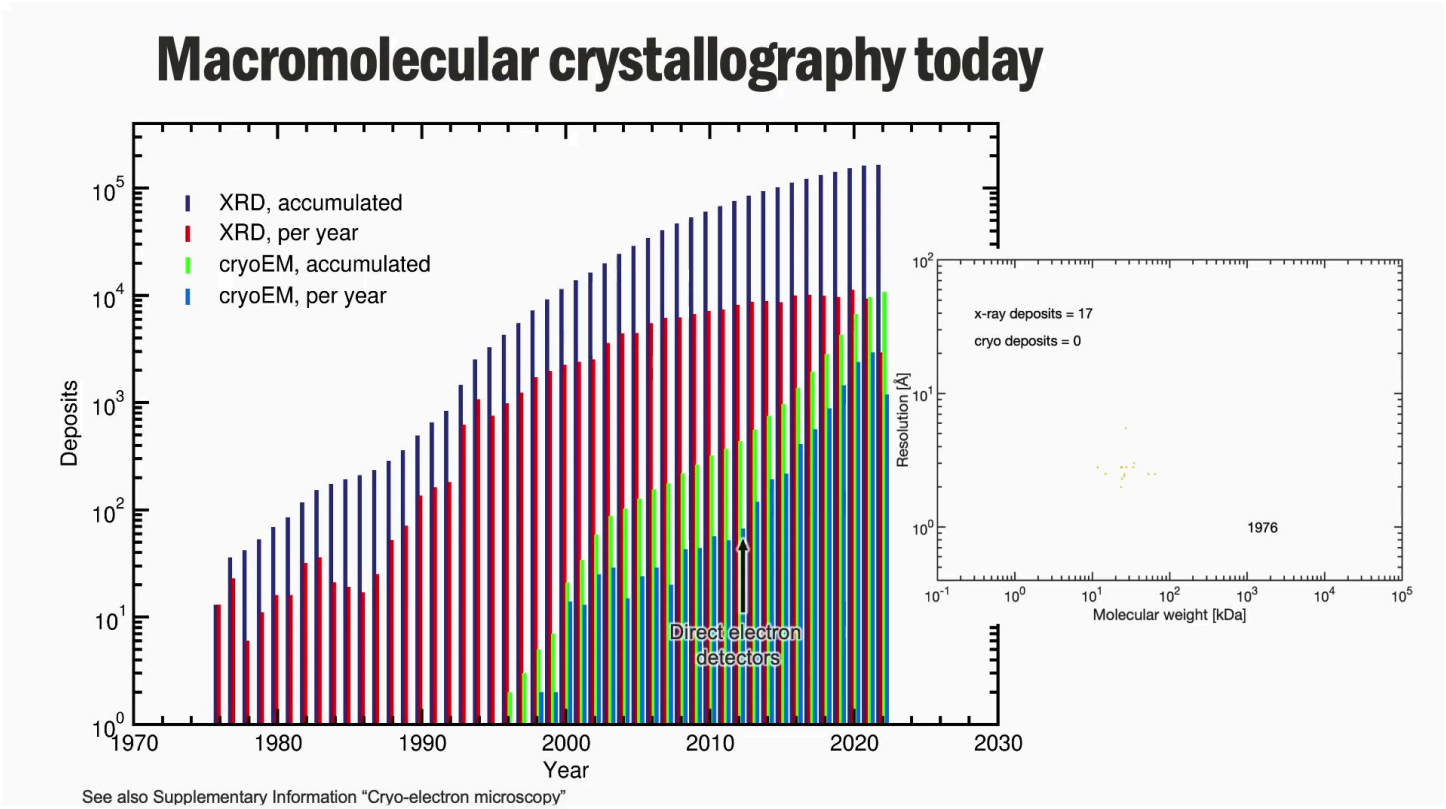
https://www.biorxiv.org/content/10.1101/2021.10.10.463845v1.full.pdf

The practical application of PET hydrolysis has been hindered by their poor thermal stability and the requirement for high processing temperatures in order to increase the reaction rate to viable values. A group from Texas reported in 2021 a variant PETase engineered by a deep learning algorithm called MutCompute. It is a self-supervised programme that learns the local chemical microenvironments of amino acids based on training of over 19,000 sequenced diverse protein structures. This enables it to predict positions within a given protein that are not optimised for their local chemical environment and replaces these with stabler alternatives. In this manner, a novel mutant protein was engineered in-silico that the authors called FAST-PETase, whereby F and A stand for functional and active respectively, were the enzyme either non-functional or inactive, it would be of no use to man nor beast. So these two go without saying. Importantly, however, it is also thermally stable and tolerant to different chemical environments, meaning that one can subject it to elevated temperatures. It outperformed wild-type PETase by well over a factor of 50 when at 50 degrees centigrade. So for example, at 50 C, FAST-PETase could completely decompose a PET bottle within 24 hours. This deep learning, scanning mutagenesis seems to have untold potential in future enzyme engineering.

Notes

Summary

6m 34s

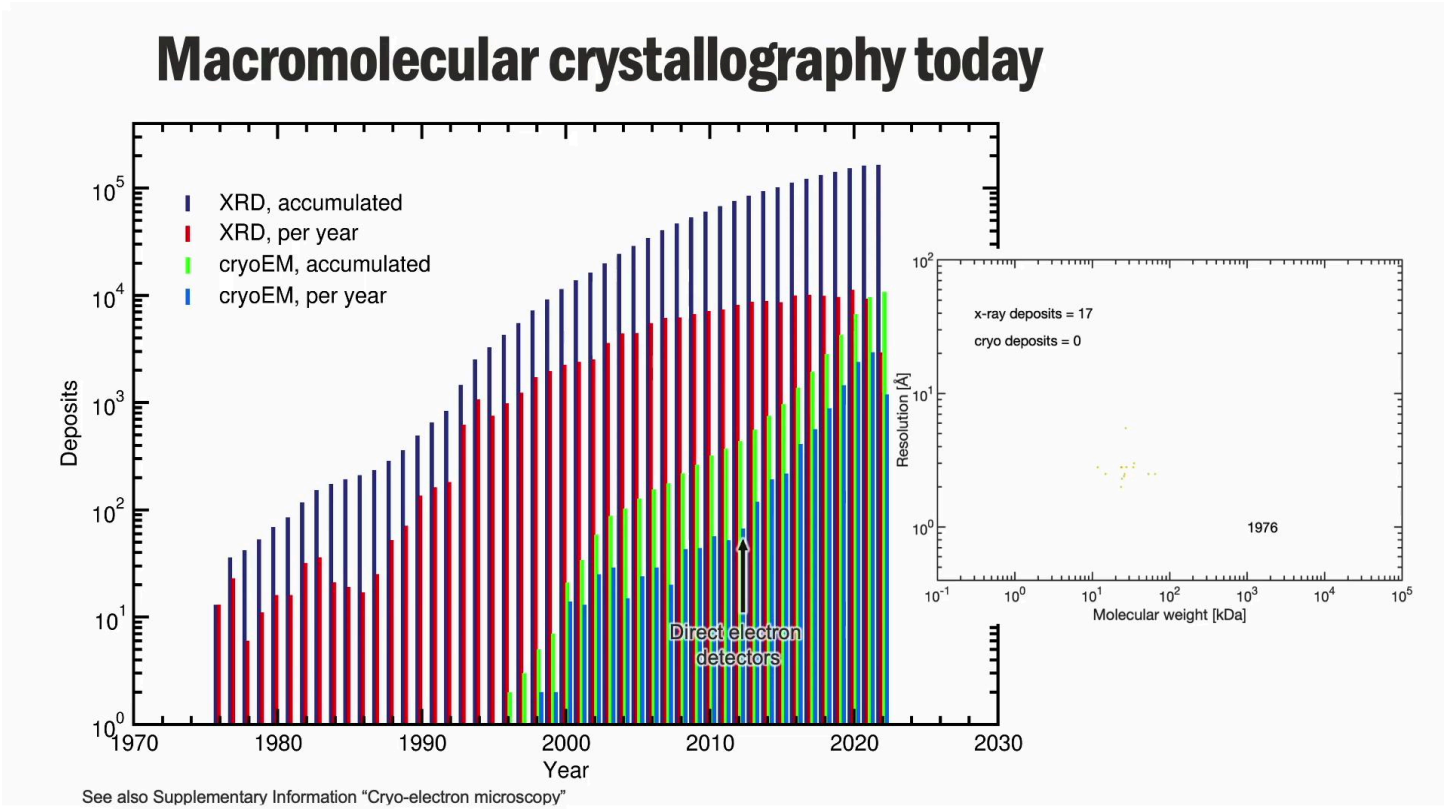


Until well into the second decade of the 21st century, macromolecular X-ray crystallography enjoyed an almost complete hegemony as a tool to determine life's molecular machinery. Because hard X-rays cannot be strongly refracted, thus precluding high magnification X-ray microscopes, the atomic scale structure of a biological molecule could only be regained by coaxing it into forming single crystals of micron size or larger, irradiating these with X-rays and then inverse Fourier transforming the resulting scattering pattern, with this latter process requiring a solution to the ubiquitous phase problem. Macromolecular crystallography has been one of the great successes in the natural sciences, garlanding several Nobel Prizes, thanks to the insights gained using this approach. The limitations of macromolecular crystallography include radiation damage, problems associated with cryo cooling, and the possibility that, in forcing a given protein to arrange itself in a regular crystalline array, its confirmation may differ from that found in nature. Indeed, many proteins that are part of a cell membrane and convey information between the confines of the cell and the external world, require their native environment to fold properly and thereby react correctly to stimuli.

Notes

Summary

8m 19s

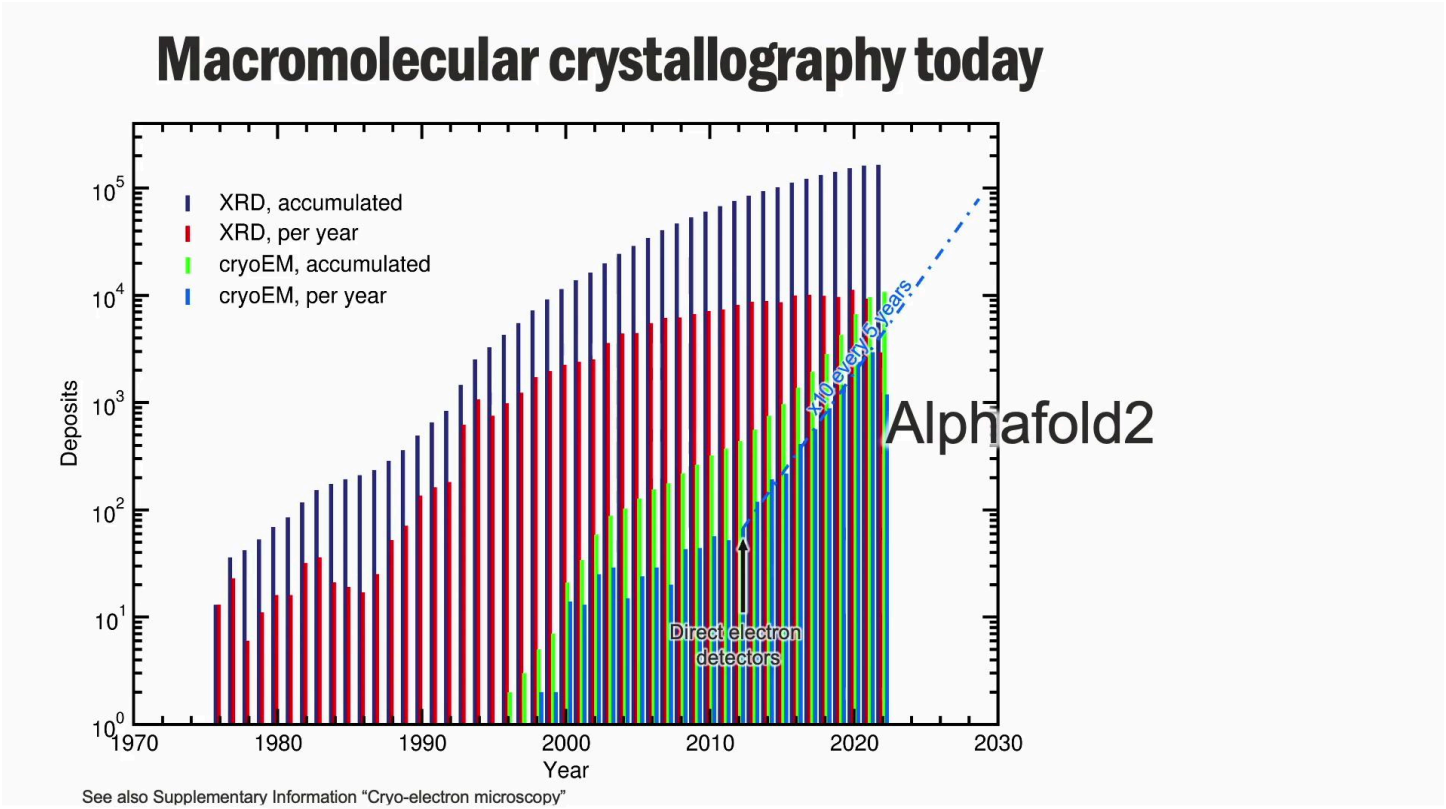


These membrane proteins are the targets for the majority of pharmaceutical drugs. Their generally hydrophobic surfaces and anisotropic orientation hinder crystallisation in an aqueous environment, and other media, such as the lipidic cubic phase or LCP, must be brought to bear on the problem. Now this brings us to perhaps the gravest bottleneck to macromolecular structure determination using diffraction, the growth of single crystals of sufficient size and quality. The newest generation of synchrotrons, DLSRs, offer a reduction in linear dimensions close to an order of magnitude, although at present, crystals much smaller than a micron remain beyond cutting edge technology. A technique which could break through this barrier would prove invaluable. In the early 2000s, a small band of determined scientists advanced major sea changes in the field of cryogenic electron microscopy, or cryoEM, in the imaging of protein structures. The improvements were largely in sample preparation, improved microscope hardware and detectors, and sophisticated algorithms for extracting the structure from the datasets.

Notes

Summary

9m 49s



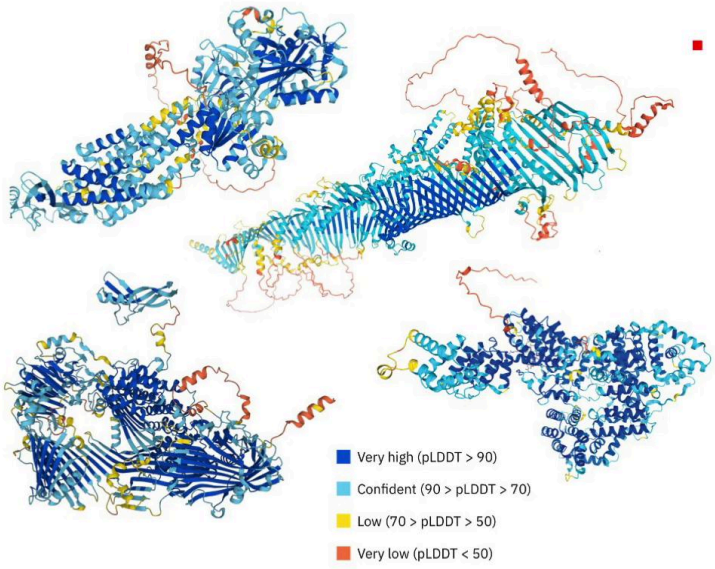
Once highly sensitive, so-called direct electron detectors were invented and implemented, the rate of discovery of protein structures at near or actual atomic resolution took off, especially for proteins with molecular weights in excess of 100,000 Daltons, as can be seen here in this plot of structure resolution versus molecular weight. Note the emergence of a cloud of cryoEM structure data points in blue, emerging after about 2015, with a resolution between three and five angstroms and molecular weights above 100,000 kilodaltons. If the present rate of cryogenic EM structures continues in the next decade as it has since 2012, cryoEM will have caught up with MX by about 2030. A more detailed overview of cryoEM is given in the supplementary materials. That all said, all bets seem to be off now Alphafold 2 has arrived, not to mention other similar deep learning algorithms which are being developed.

Notes

Summary

11m 18s

Whither macromolecular crystallography?



- Future roles of MX
  - Validation of *in-silico* predicted structures
  - Fragment screening in pharma industry
  - Time-resolved dynamics studies
    - Down to μs for SR
    - Between fs and μs for XFEL radiation
  - Kick start experimental *de novo* structure determination with Alphafold2
    - Phases retrieved through *in-silico*-derived folds and molecular replacement
    - Also for cryo-EM, especially for structures resolved to ca. 3 Å or larger

https://doi.org/10.1107/S2052252522005802
https://doi.org/10.1038/s41592-021-01380-4 and comments in this special issue of Nature Methods

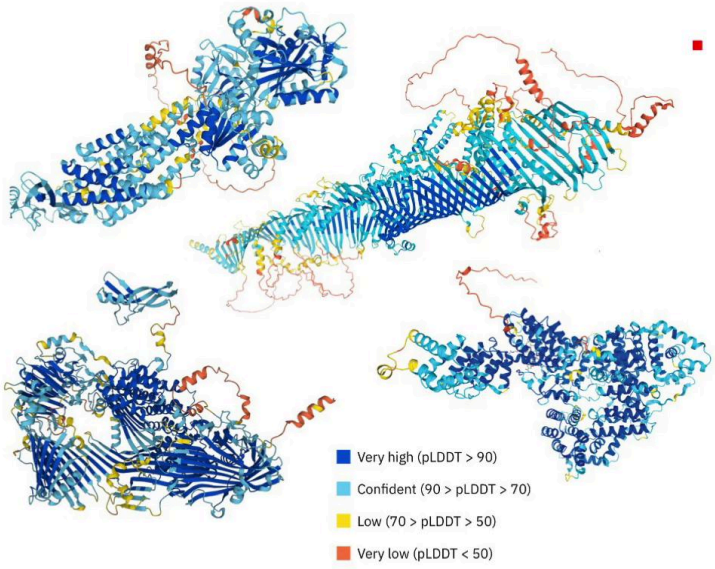
So what can we expect from macro molecular crystallography? First, it should be clearly stated that for the foreseeable future, Alphafold 2 is not a universal panacea and has some defined limitations. Presently, many proteins are to be found as complexes with other proteins or biological compounds. Although progress is being made in this direction, Alphafold 2 doesn't currently predict structures for these compound complexes. Secondly, proteins are dynamic systems and can change their confirmation depending on their local environment. Alphafold usually only predicts one of these. It also doesn't predict the effect of mutations and cannot account for proteins binding to other chemical species, such as cofactors, metals, or ligands. Protein crystallography, therefore, has a very important role still to play. Firstly, no pharmaceutical company would develop and market a drug based entirely on in-silico predictions, and experimental validation is essential in drug development programmes, not least in the task of fragment screening, in which the different impacts of small drug components must be investigated. This is a well-oiled and efficient exercise at Macromolecular Crystallography beamlines worldwide, and is an essential component of drug discovery.

Notes

Summary

12m 31s

Whither macromolecular crystallography?



- Future roles of MX
  - Validation of *in-silico* predicted structures
  - Fragment screening in pharma industry
  - Time-resolved dynamics studies
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    - Between fs and μs for XFEL radiation
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https://doi.org/10.1107/S2052252522005802
https://doi.org/10.1038/s41592-021-01380-4 and comments in this special issue of Nature Methods

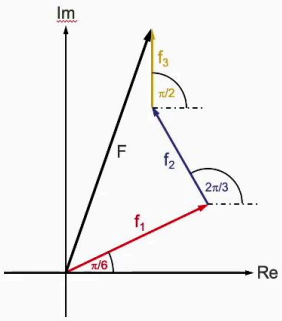
Dynamic studies of proteins down to the microsecond scale for synchrotron studies, and potentially even down to the femtosecond scale for XFELs are extremely important in understanding protein chemistry and dynamics. Finally, macromolecular crystallography in combination with in-silico derived information will be very important in de novo structure determination, not least in the field of membrane proteins. At the time this video was recorded, MX is showing no sign of ebbing, in fact, just the opposite, with demand at beamlines never being greater. The future still looks very bright for macromolecular crystallography, if perhaps the colour, if not the intensity, of the light has changed subtly.

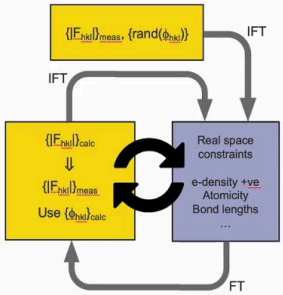
Notes

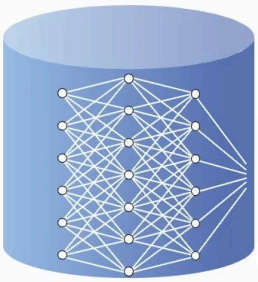
Summary

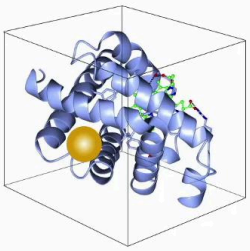
14m 07s

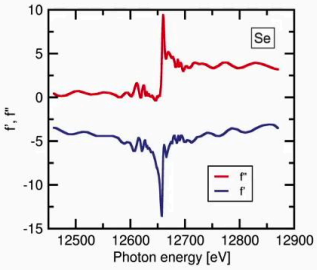
Summary of this section

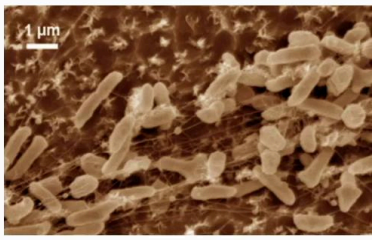












This has been quite the section. In summary, we began by looking at the phase problem in general and how we resolve this in macromolecular crystallography using traditional methods such as iterative techniques, isomorphous replacement, and anomalous diffraction. We then considered the new kid on the block, deep learning, exemplified by Alphafold 2, and finished with an example of the application of all we have covered in this section in the case of plastic degradation and decomposition.

Notes

Summary

14m 57s

Next week...

Next week, we turn to specific types of scattering and diffraction techniques, including single crystal diffraction, powder diffraction, surface diffraction, small-angle X-ray scattering, and X-ray reflectivity.

Notes

Summary

15m 30s