The Abdus Salam
International Centre for Theoretical Physics

United Nations
Educational, Scientific and
Cultural Organization

IAEA
International Atomic Energy Agency

310/1828 310/13

Workshop on Biomedical Applications of High Energy Ion Beams

Co-sponsored by: ICGEB and University of Surrey

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12-16 February 2007

Venue:
Adriatico Guest House Giambiagi Lecture Hall
ICTP, Trieste, Italy

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# Biomedical Analysis and Developing Multi-Disciplinary Collaboration

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# *Biomedical Analysis*

Karen J Kirkby

**Surrey Ion Beam Centre,**

**ATI, SEPS, University of Surrey**

# Contents

- Surrey Ion Beam Centre
- Multidisciplinary Collaboration
- Analysis of Biomedical samples
  - Protein analysis
  - Calcium phosphate analysis
  - Bio-mineralisation
  - Mass transport in cells
- Something completely different?

# Collaborative Research

# Thank You

Prof Peter Butterworth
Prof. Roger Webb
Dr Norman Kirkby
Dr Geoff Grime
Dr Russell Gwilliam
Dr Chris Jeynes

IBC Liaison RAs

Dr Inma Gomez-Morilla
Dr Melanie Webb
Dr Nianhua Peng
Mr Andy Smith

Secretarial Support

Karen Arthur

Technical Support

Adrian Cansell
Alex Royle
Mark Browton
Bevis King

PhD Students, Interns and PDRAs

Mr Jim Sharp
Mr Justin Hamilton
Mr Prashant Mistry
Mr Michael Merchant
Mr Harry Igbenehi
Ms Morgiane Richard
Ms Petra Rombouts

Dr Maxim Poporov
Dr Wojech Polak
Dr Vladimir Palatsin

Clinicians

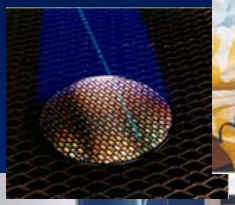
Prof Bleddyn Jones
Dr Neil Burnet
Dr Raj Jena
Prof Roger Taylor
Prof Sir Richard Thompson
Prof Pat Price

Scientists/Engineers

Prof Melvyn Folkard
Prof Boris Vojnovic
Dr Kevin Prise
Prof Jonathan Powell
Prof Roger Dale
Prof Steve Jackson
Dr Stuart Green
Dr Elspeth Garman
Dr David Vaux
Prof Alan Nehum

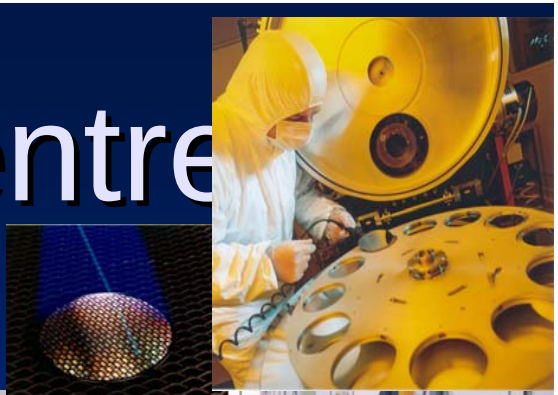
# Surrey Ion Beam Centre

- Surrey Ion Beam Centre
- EPSRC funded
- National Centre
- 3 accelerators 2keV to 10MeV
- Ion Beam modification and analysis
- 1978 UK facility for microelectronics
- Widen research base


# Surrey Ion Beam Centre

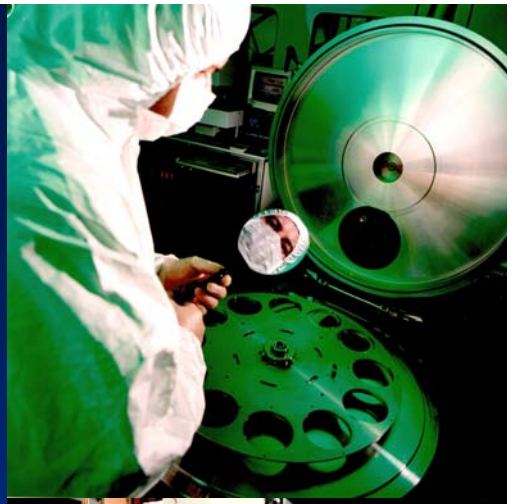
- Collaborative research UK academia & industry
- Process development
- Implantation IBMM
- Ion Beam Analysis
- New applications, MRC, BBSRC, NERC, Charities,
- Arts & Humanities

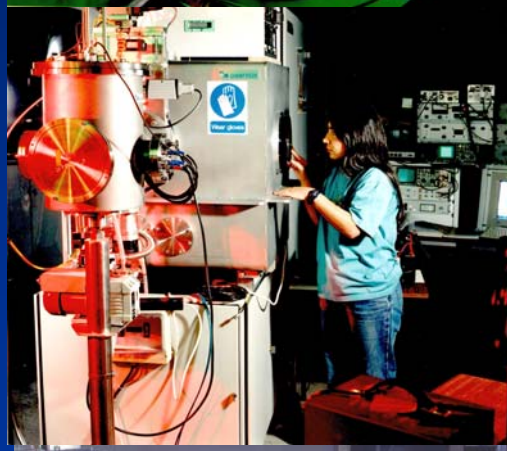




# Surrey Ion Beam Centre

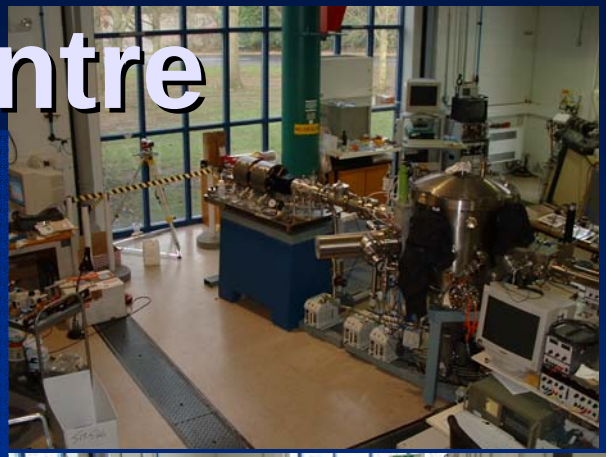
- **Controllable Materials Modification**
  - **Facilities**
    - 0.4-2MV High Energy Implanter
    - 2-200kV High Current Implanter
    - Implantation 2keV⇒4MeV (up to 10mA)
    - Sample size $mm^2$ to 40cmx40cm
    - Hot (1000$^{o}$C) or cold (~10K)
    - Sample Chambers in class 100 clean room
    - Plasma/thermal processing and metrology
  - **Applications**
    - Ion Beam Synthesis
    - Ion Implantation
    - Defect Engineering
    - Proton beam lithography



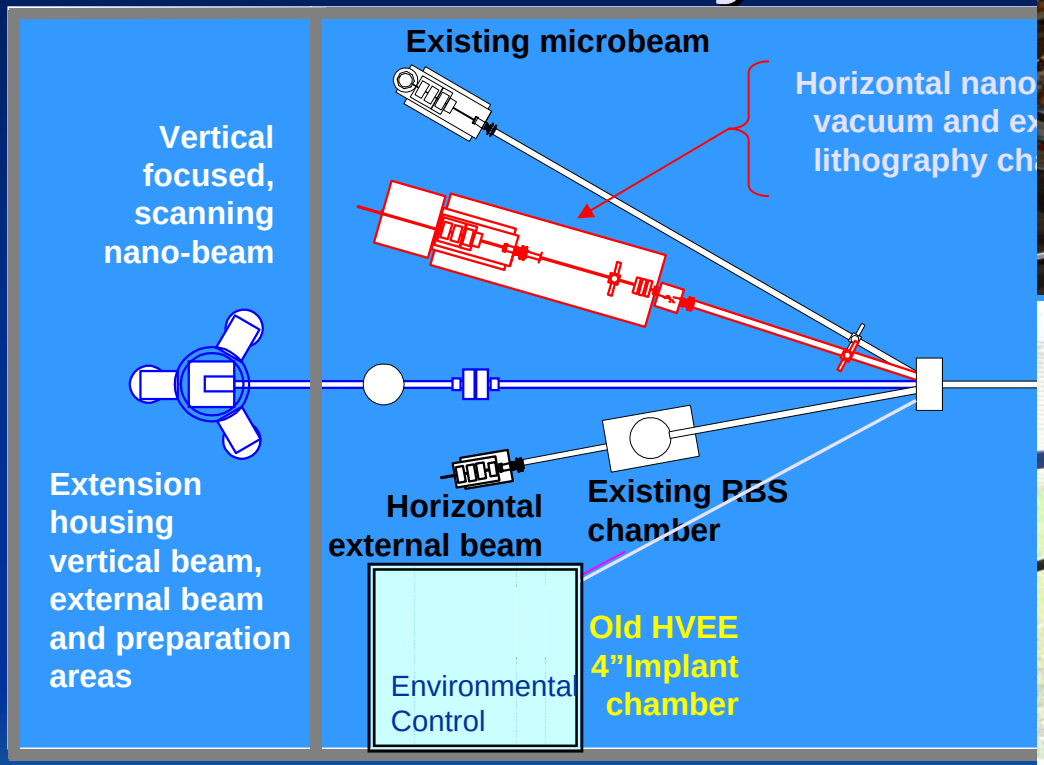


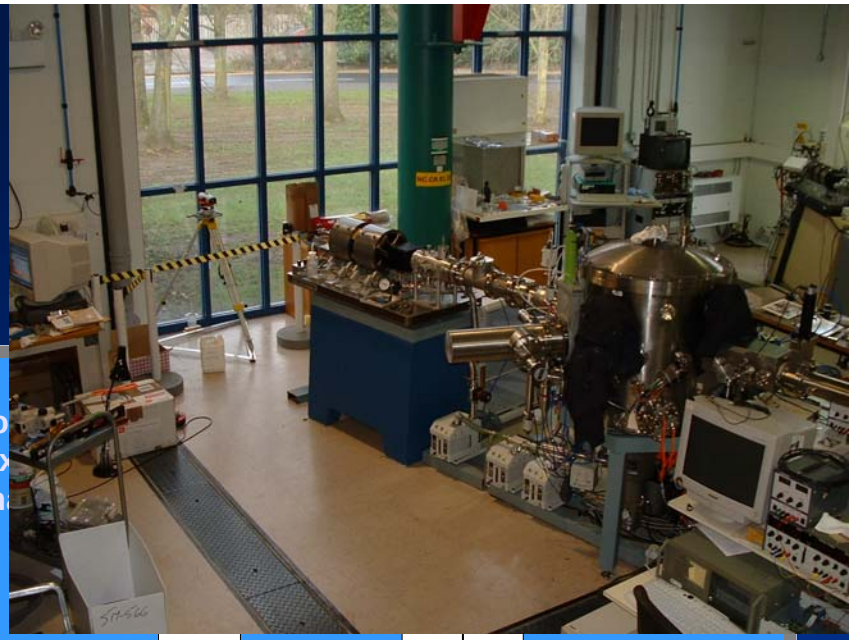
# Surrey Ion Beam Centre

- Advanced Materials Analysis
  - **Facilities**
    - 2MV Tandem
    - Techniques include RBS, EBS, ERD, PIXE, NRA
    - Sub micron size micro-beam with full scanning
    - Channelling Spectroscopy for damage analysis
    - Fully automated collection and analysis
    - External Beam for vacuum sensitive samples
  - **Applications**
    - Thin Film Depth Profiling
    - 3-D elemental composition and mapping
    - Disorder Profiling of Crystals

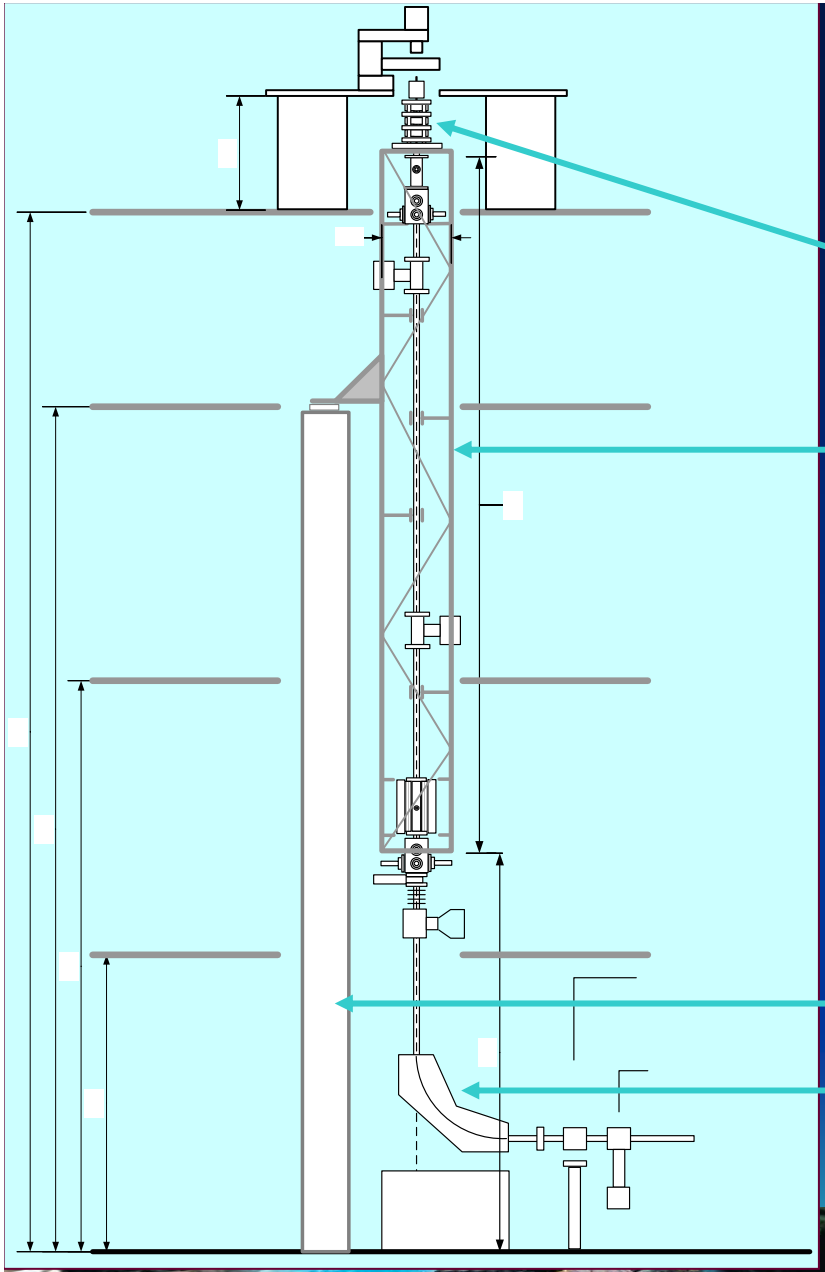


# Developments in the Stephens' Laboratory



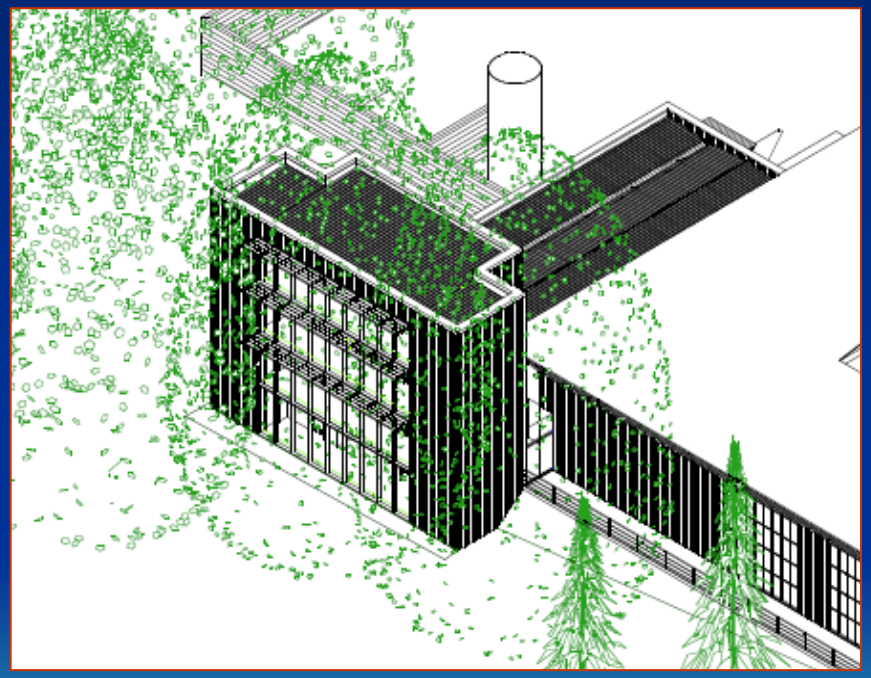



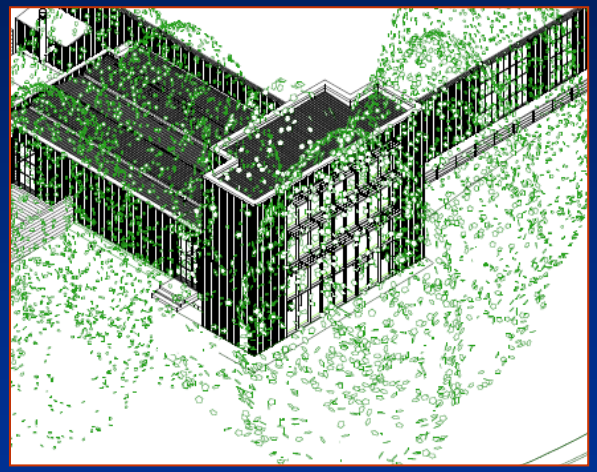
# New Developments




- H to Ar irradiation
- Living cells
- Single ion or full current
- 4 floors – 200m$^2$
- Top floor clean room and prep area

# Architect's views of the proposed new building





# Research Group

PhD Students, Interns

- Mr Jim Sharp
- Mr Justin Hamilton
- Mr Prashant Mistry
- Mr Harry Igbenehi
- Mr Max Kah
- Ms Morgiane Richard
- Ms Petra Rombouts

PDRAs

- Dr Inma Gomez-Morillla / Dr Melanie Webb
- Dr Maxim Poporov
- Dr Wojech Polak
- Dr Vladimir Palatsin
- Mr Michael Merchant
- + 2

# Applications of Ion Beams

# Applications of Ion Beams

- Ion Beam Analysis
- Proton Beam Writing
- Cell irradiation
- SIMS
- Accelerator mass spectrometry (AMS)
- Particle Therapy
- Next generation ion sources
- Device testing
- Complementary techniques

# Motivation

- Lots of applications for Ion Beams but very little interaction between communities

- UK Research network on Biomedical Applications of High Energy Ion Beams

# Aims of the Network

- Establish multidisciplinary Network and discussion forum in the UK
- Take a strategic view, look at what UK needs in terms of equipment, investment and infrastructure
- Get the funding to build the best facilities in the world
- Train the next generation of research scientists

# Achievements

- European and International
- Clinical facility for charged particle beams in UK
- Basic Technology grants (£13.5m)
- Vertical nanobeam (£1.2m Wolfson Foundation)
- Visit to European Clinical facilities
- Initiated a number of new collaborations
- This Workshop
- International Conference
- Putting people together

Modelling
NFK RPW

Particle Therapy
KJK, NFK
UK Clinicians

Cell Irradiation
KJK, GWG
GCI

Network
KJK
EPSRC,
Basic Tech

New ion beams
KJK,RPW
Basic Tech

Analysis IBA
GWG, KJK, CJ
AC,IGM
Lots

Analysis
Cluster SIMS
RPW,
Manchester

Analysis
complementary
Diamond,
Daresbury
Muons

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Manchester

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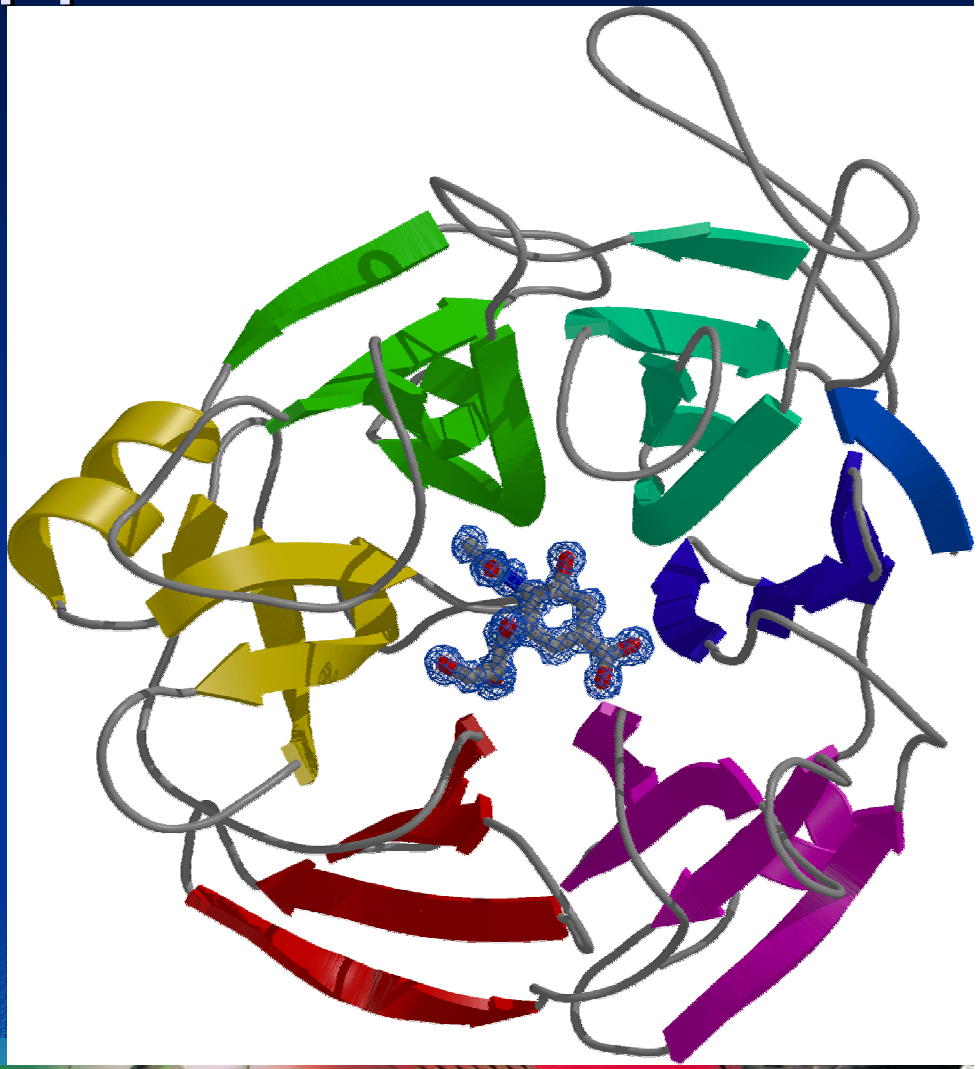
Daresbury
Muons

# Bio-medical Applications

## Analysis of Proteins

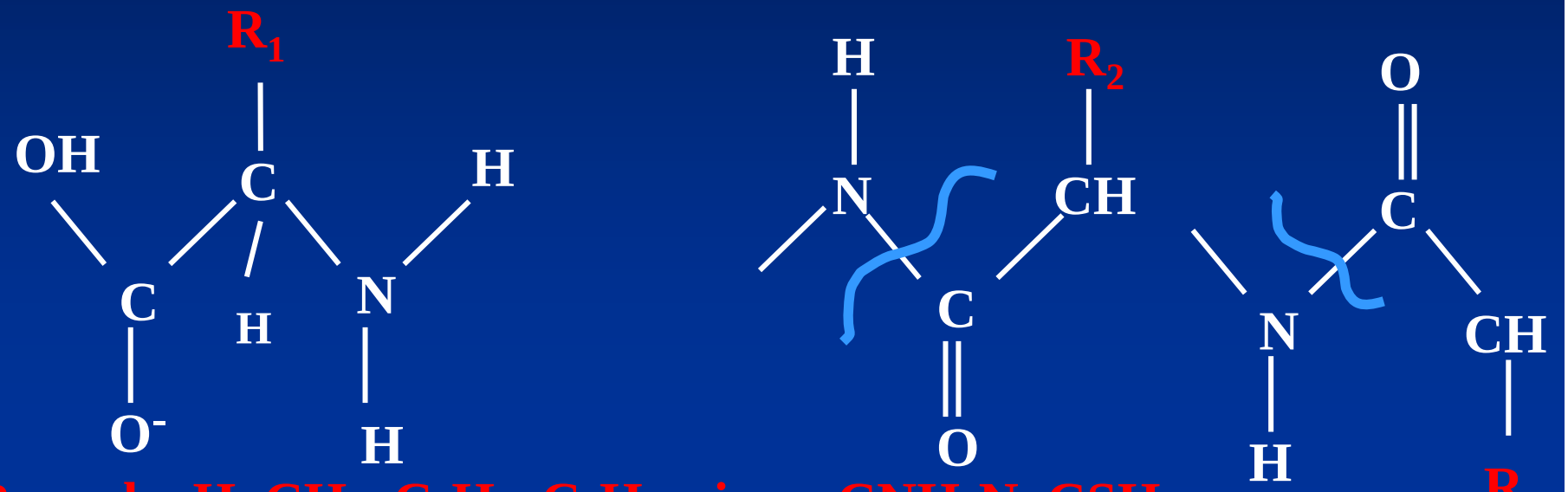


Geoff Grime (Surrey),
Elspeth Garman, University of Oxford

# WHAT IS A PROTEIN?

Made of amino acids.

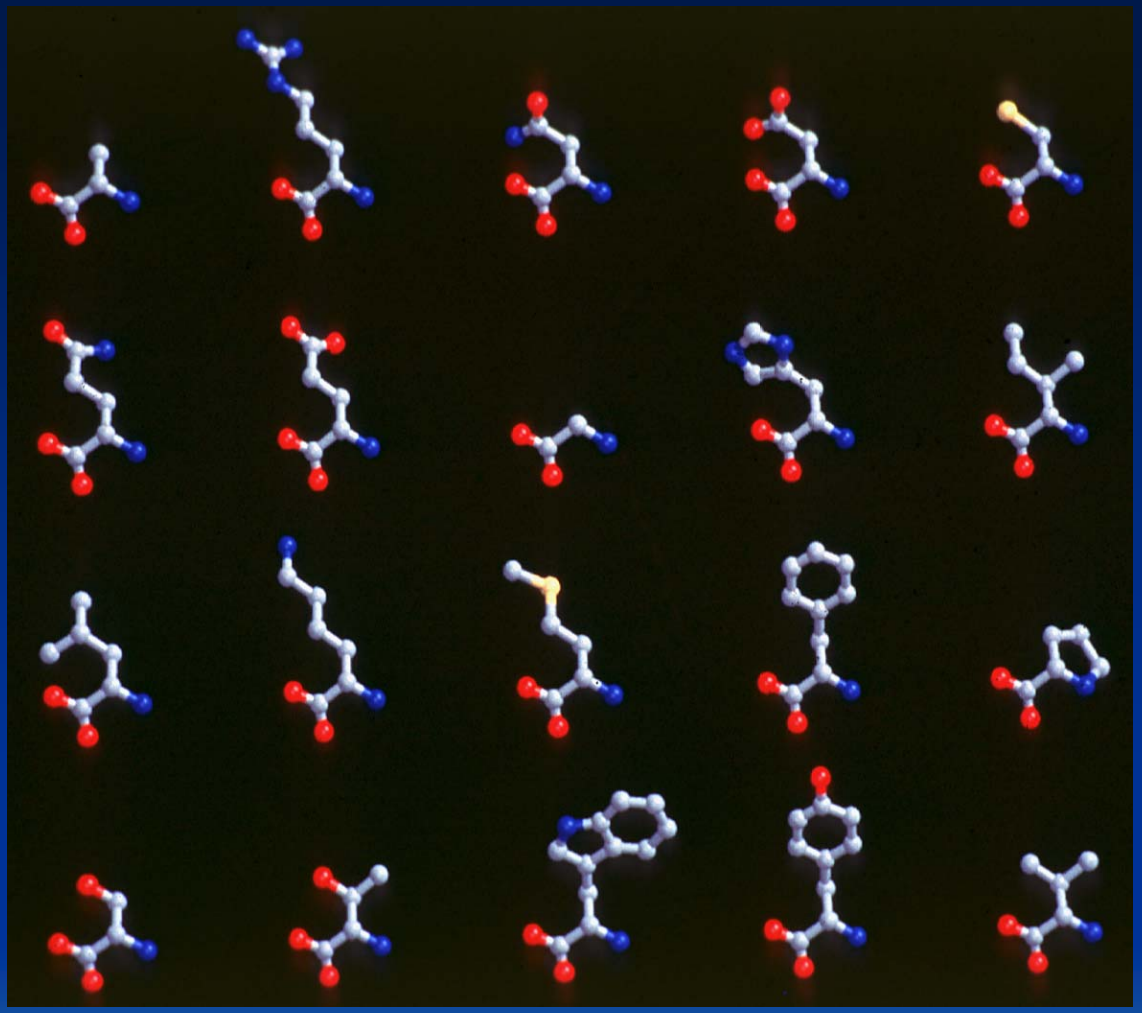
20 different `R' groups in Nature.

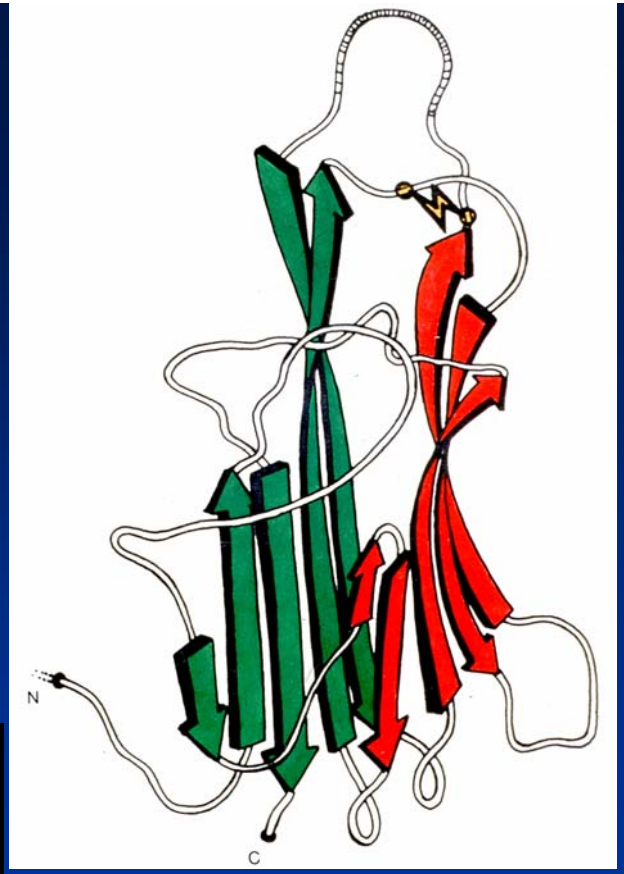


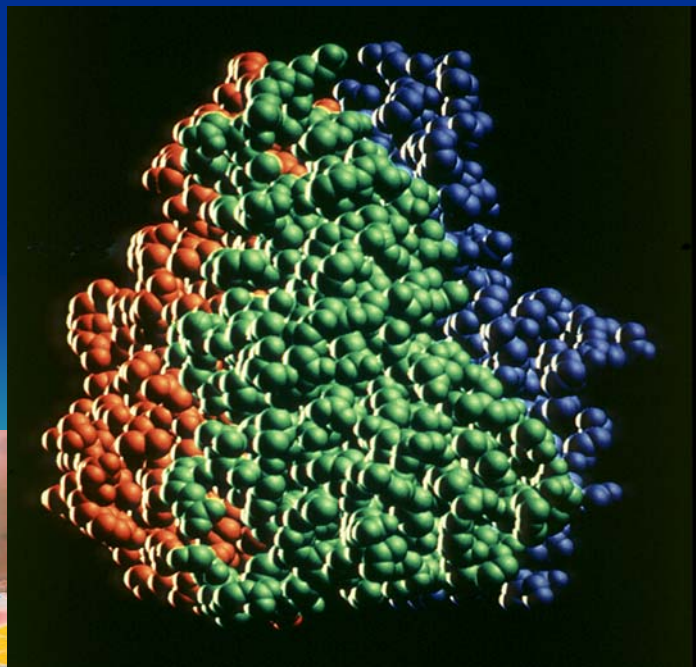

**R can be H, $CH_3$, $C_2H_5$, $C_3H_7$, rings, $CNH_4N$, $CSH_3$……**

Basic units join to form chains.

20 amino acids in Nature: methionine and cysteine contain sulphur

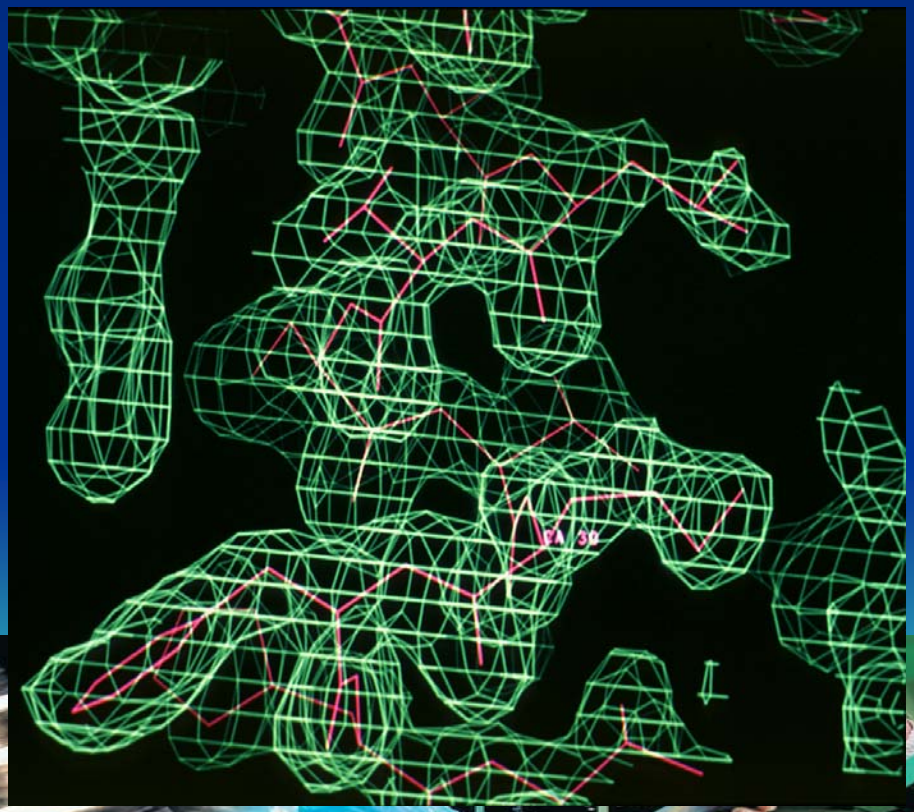



N
C

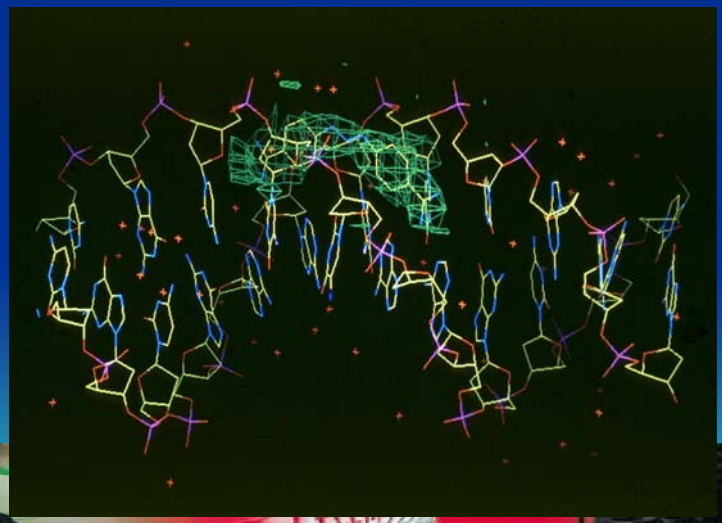


From X-ray diffraction, we get experimental electron density (green) and fit known sequence of amino acids.

Alpha helix

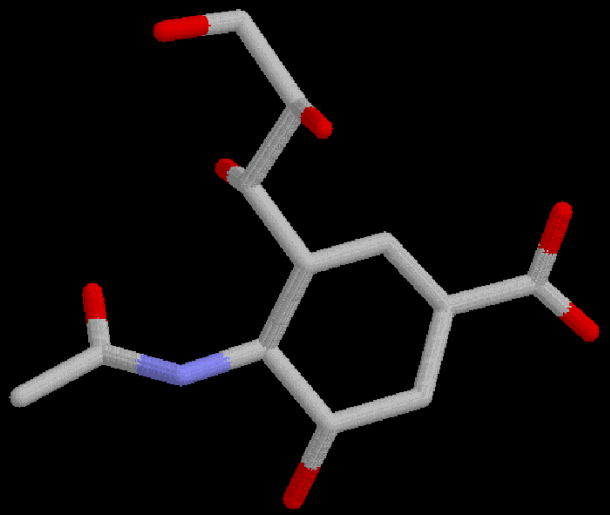


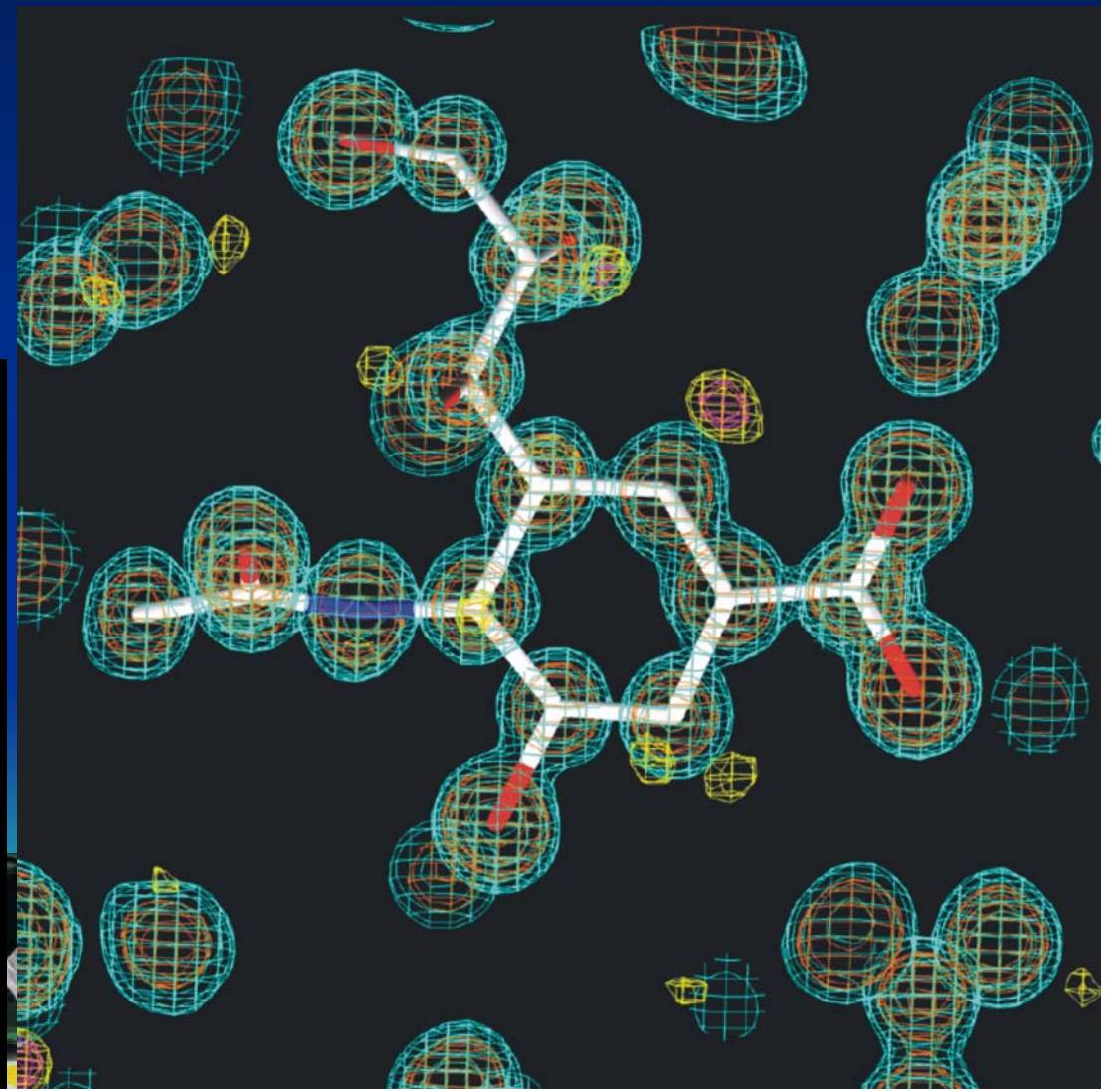
DNA + berinil

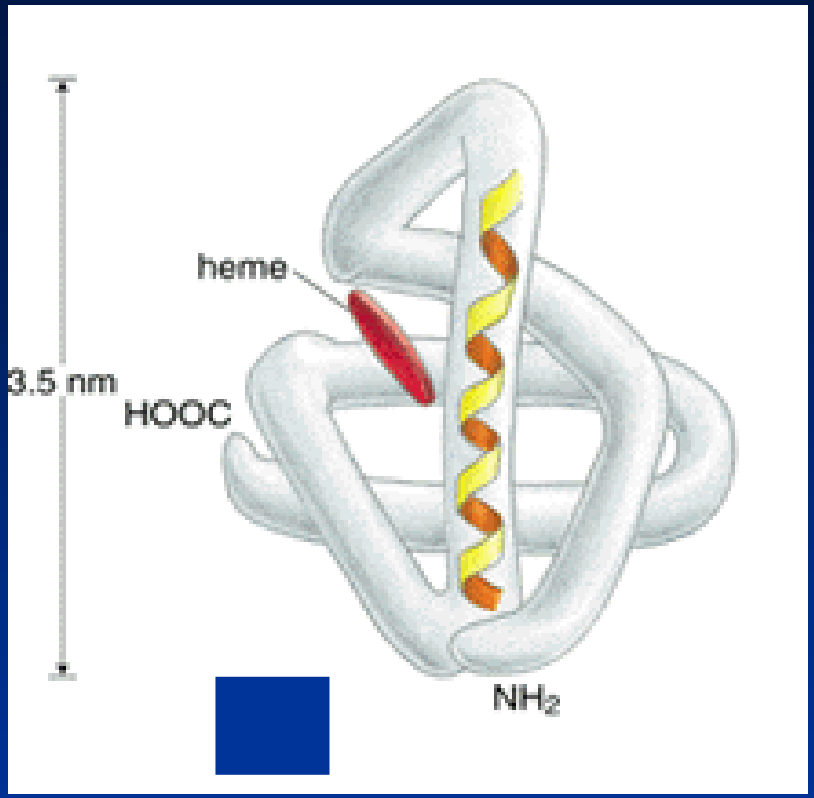


# STNA + Iso-Carba-Dana at 0.91Å

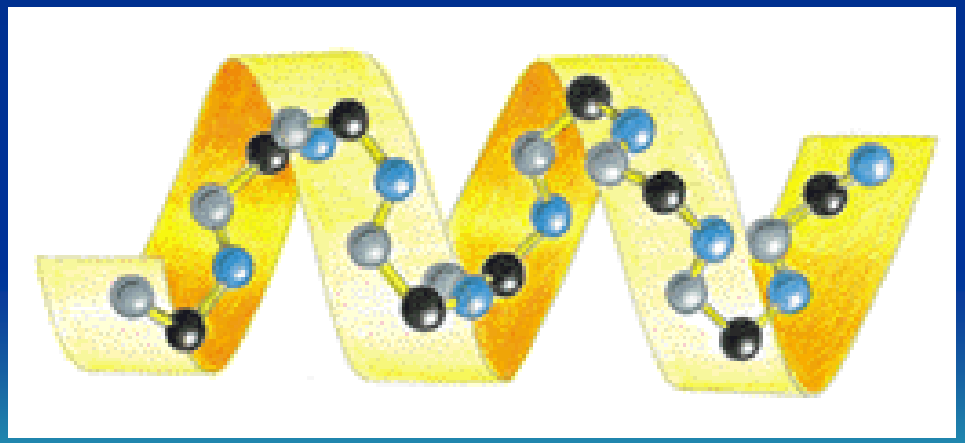
ISO-CARBA-DANA

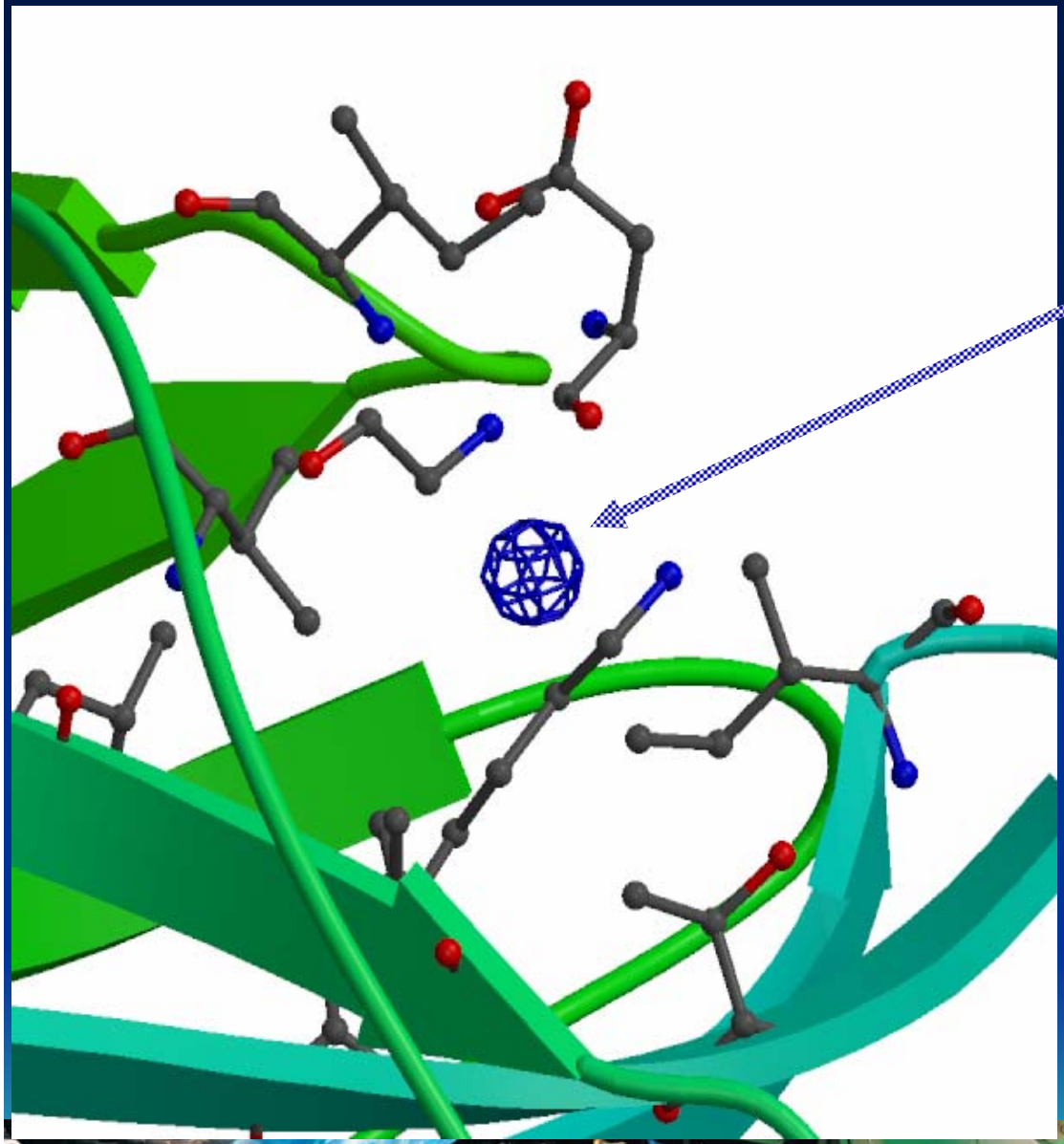







Many proteins bind additional ions for function and structure.
e.g. myoglobin has a heme which contains iron





**“Mysterious” extra electron density in difference map between model and data from x-ray crystallography**

# MicroPIXE

`Putative metal ion'

HOW CAN WE  IDENTIFY IT?

# microPIXE analysis of proteins

- Metal concentration is typically 1 atom per molecule of 10 - 100kDa (i.e. ppm)
- Quantities are usually small (microlitres of solution, 100μm crystals)
- microPIXE is ideal
- Quantitation by ratios to S (cysteine and methionine in proteins) or P (DNA/RNA) which are present in known quantities if the chemical sequence is known.

# Protein Samples

- Absolute concentrations have a large error.
- This allows us to calculate an accurate (6-10%) ratio of trace element : protein *(e.g. 1 Fe atom per protein)*

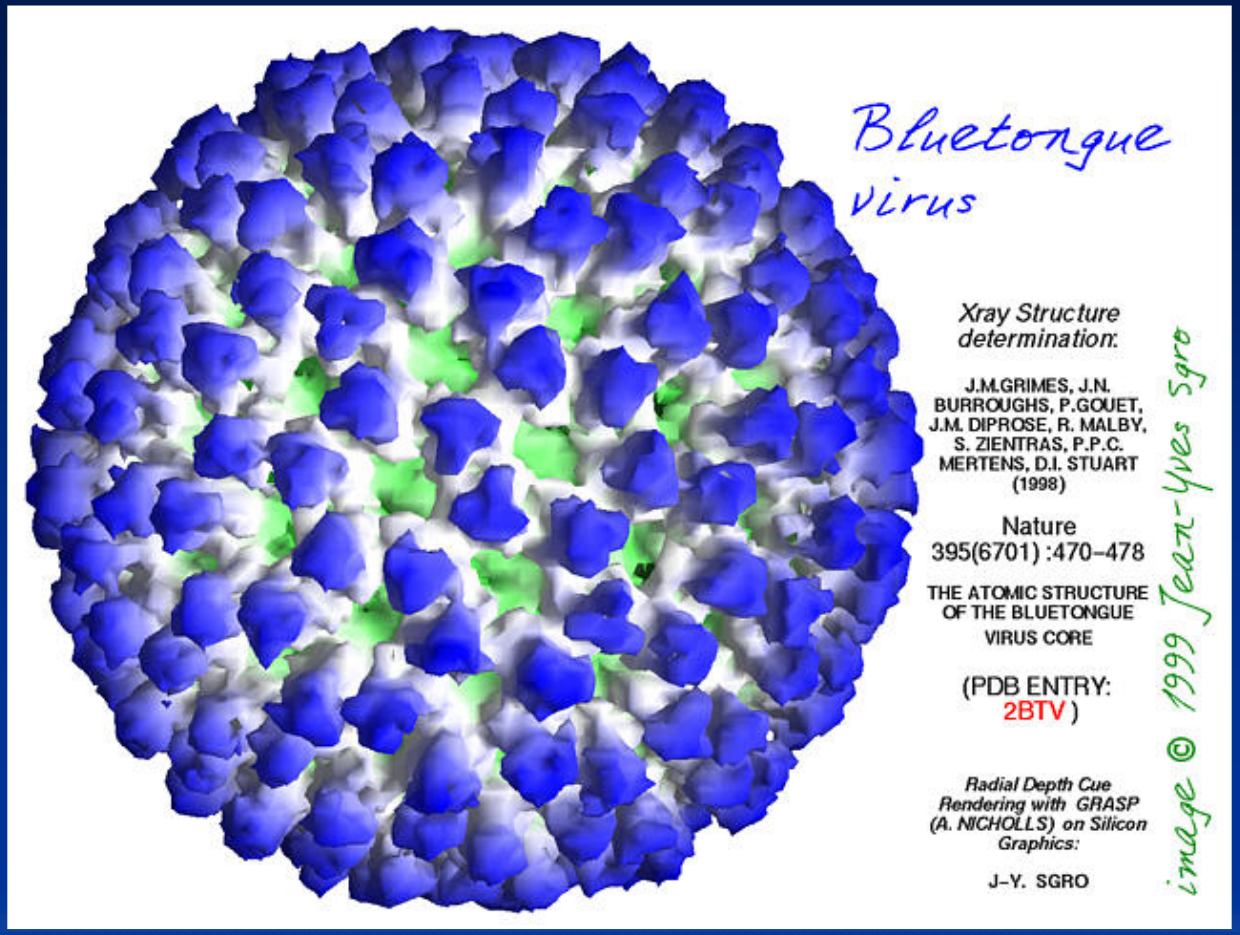
$$N_x = [C_x/C_s] \times [M_s/M_x] \times N_s$$

Number of atoms of element *x* per protein molecule = $\frac{\text{Concentration of element } x}{\text{Concentration of sulphur}} * \frac{\text{Mass of Sulphur}}{\text{Mass of element } x} *$ Number of atoms of sulphur per protein molecule

# DATA PROCESSING

- Metal concentration normalised to sulphur (or phosphorus) to give a result in atoms per protein molecule

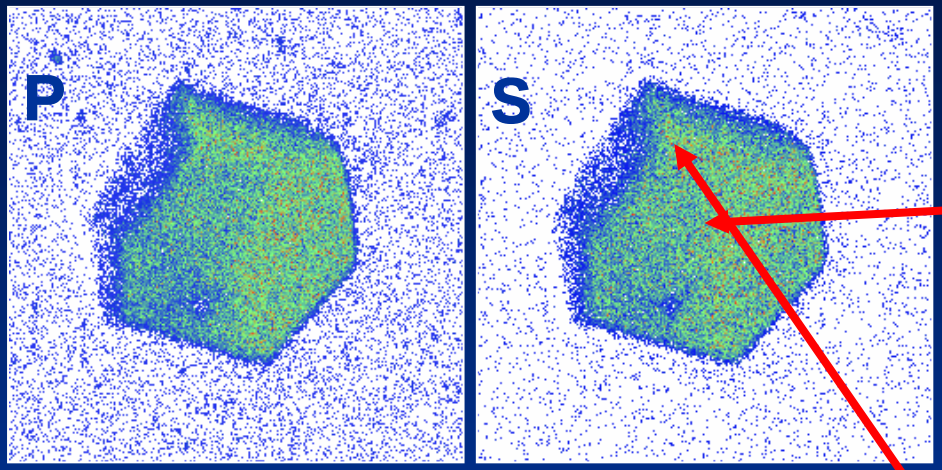
100nm

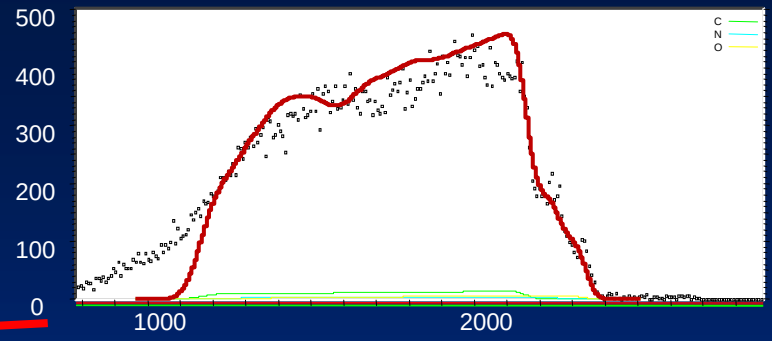



**BUT, what is the metal ion and how much of there is it??**

# Molecular structure determination by x-ray crystallography

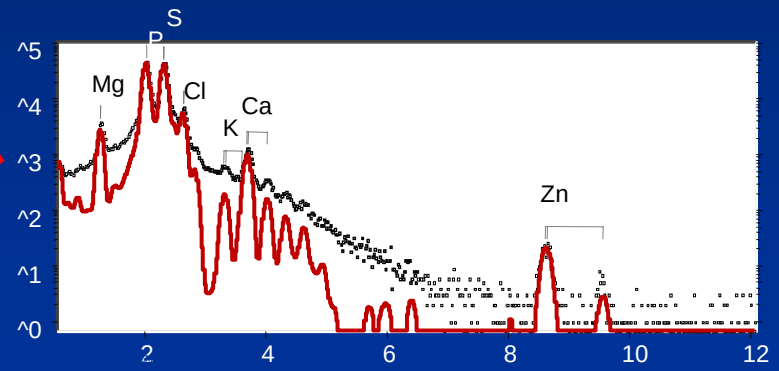
# Blue Tongue Virus crystal




RBS fit:

$C_4N_{1.25}O_{1.43}$ 20.7μm





200 μm

PIXE results:

| | |
|---|---|
| P | 1.49% |
| S | 1.17% |
| Ca | 350ppm |
| Zn | 90ppm |

Ca:S 0.02:1 atoms

Zn:S 0.004:1 atoms

P:S 1:1 atoms

EPSRC

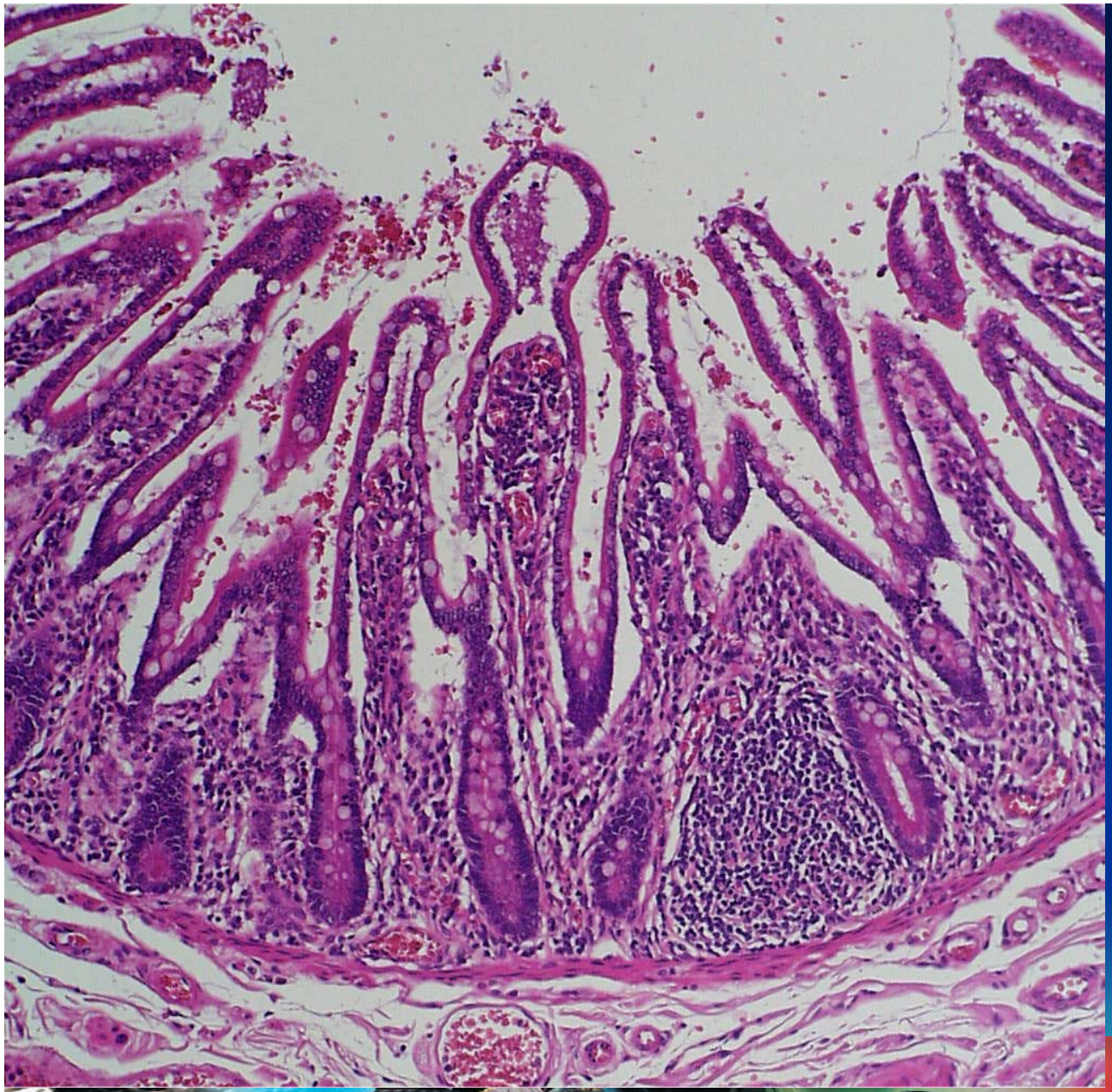
# Structural Biological Applications: Summary

- Unambiguous identification of putative ions in 3D molecular structures of a protein (e.g. distinguish Mn from Fe). This information is rarely obtainable from the diffraction experiment.
- Identification of contaminants in samples (e.g. paramagnetic ions in samples for NMR analysis).
- Determining accurate trace element stoichiometry (e.g. how many Zn atoms per protein molecule?).

# Calcium Phosphate Analysis

Jonathan Powell MRC Human Nutrition Laboratory, Cambridge, Vinay Thoree, Richard Thompson, St Thomas' Hospital

# How do our bodies cope with antigens?

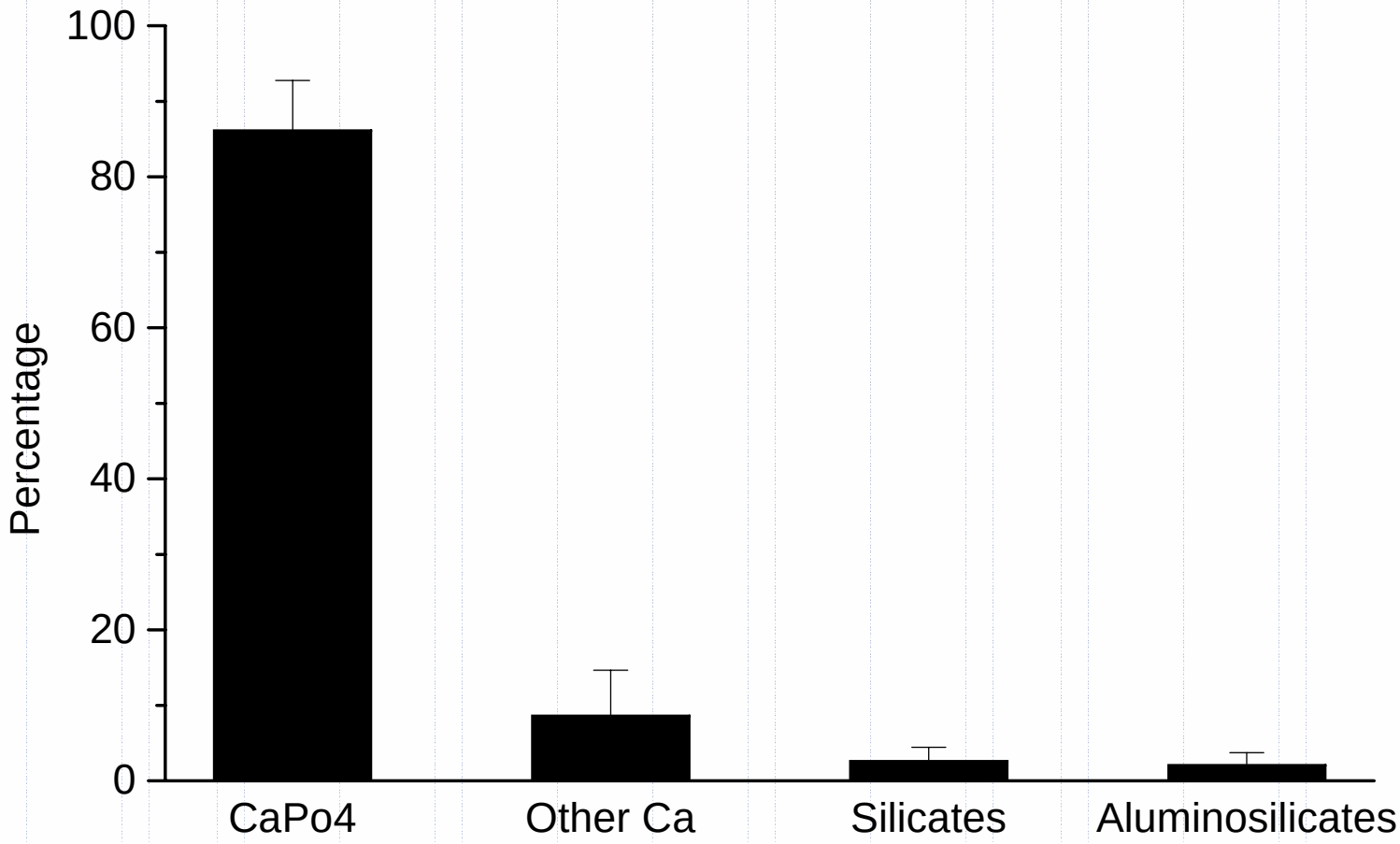


The gastrointestinal tract is an unusual organ it normally exists in harmony with a resident bacterial population. During active inflammatory bowel disease harmony is lost and active immune responses are mounted towards the resident bacteria

Effects 0.2% of population

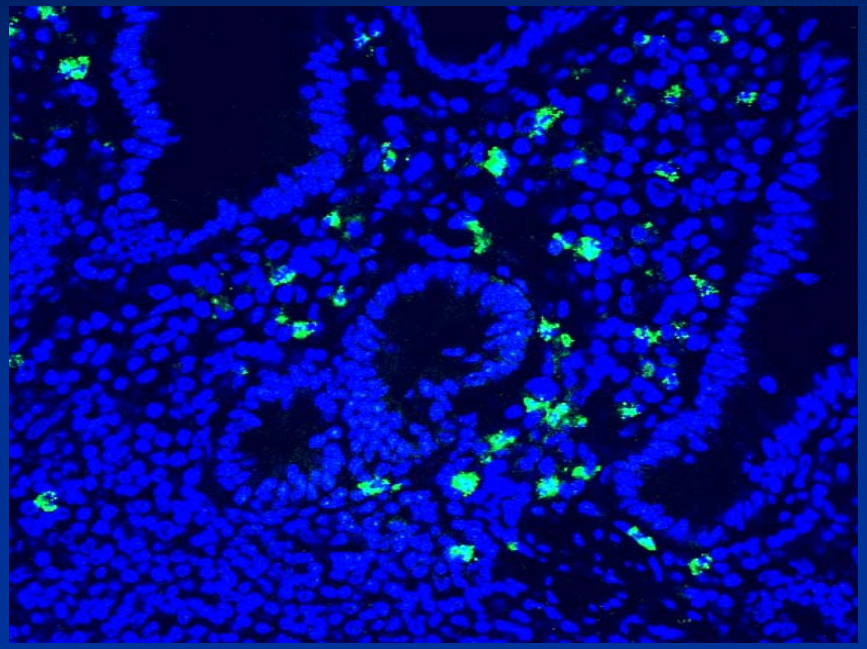
Causes and trigger unknown

# Fine particles of the gastrointestinal lumen




*Powell et al J Inorg Biochem 1999*

# Calcium Phosphate and Crohn's Disease



***Human intestinal lymphoid tissue containing cells rich in calcium phosphate microparticles as shown by calcein staining***

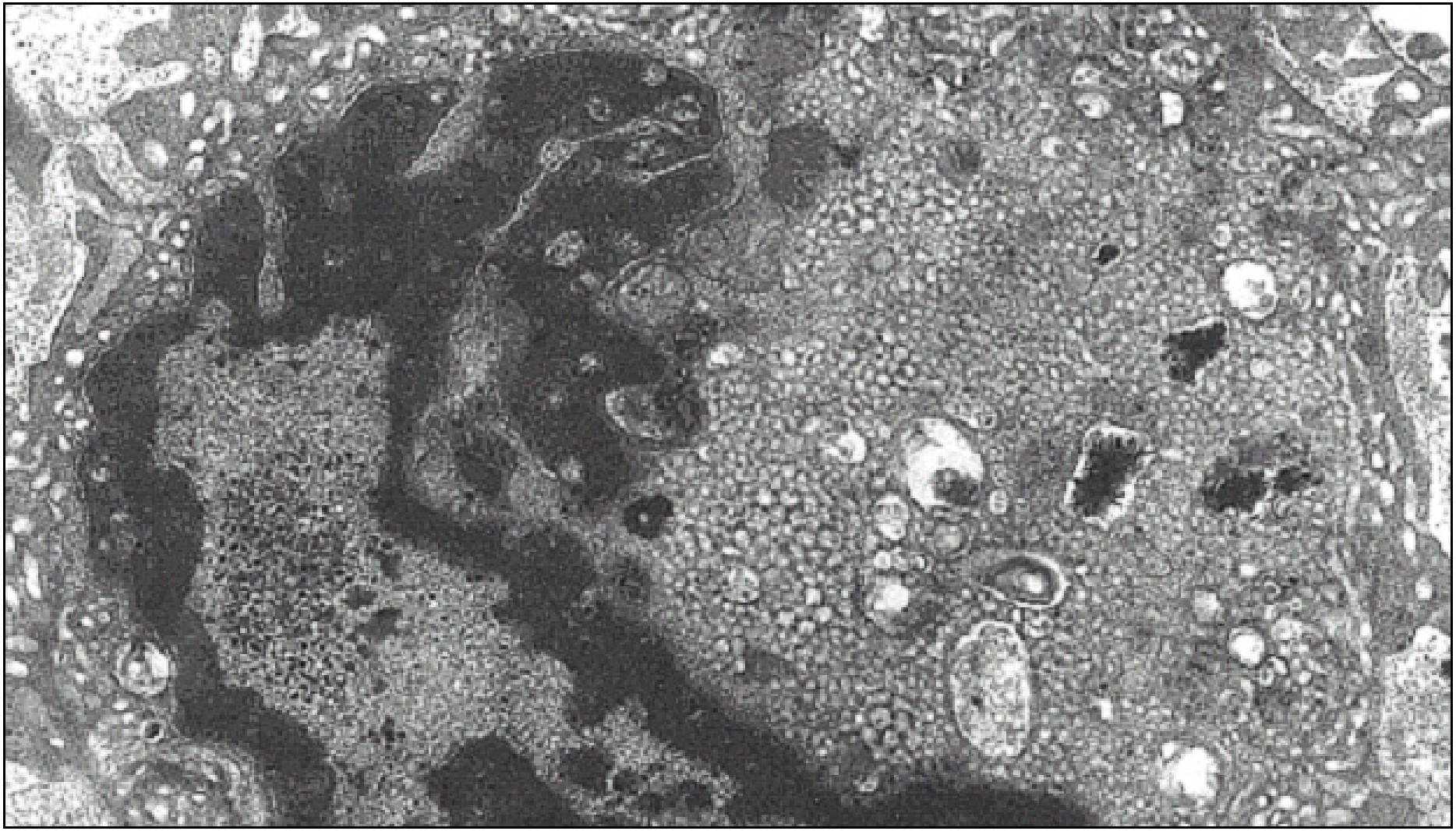
***Paticularly associated with peyers patches***

UNIVERSITY OF CAMBRIDGE

EPSRC

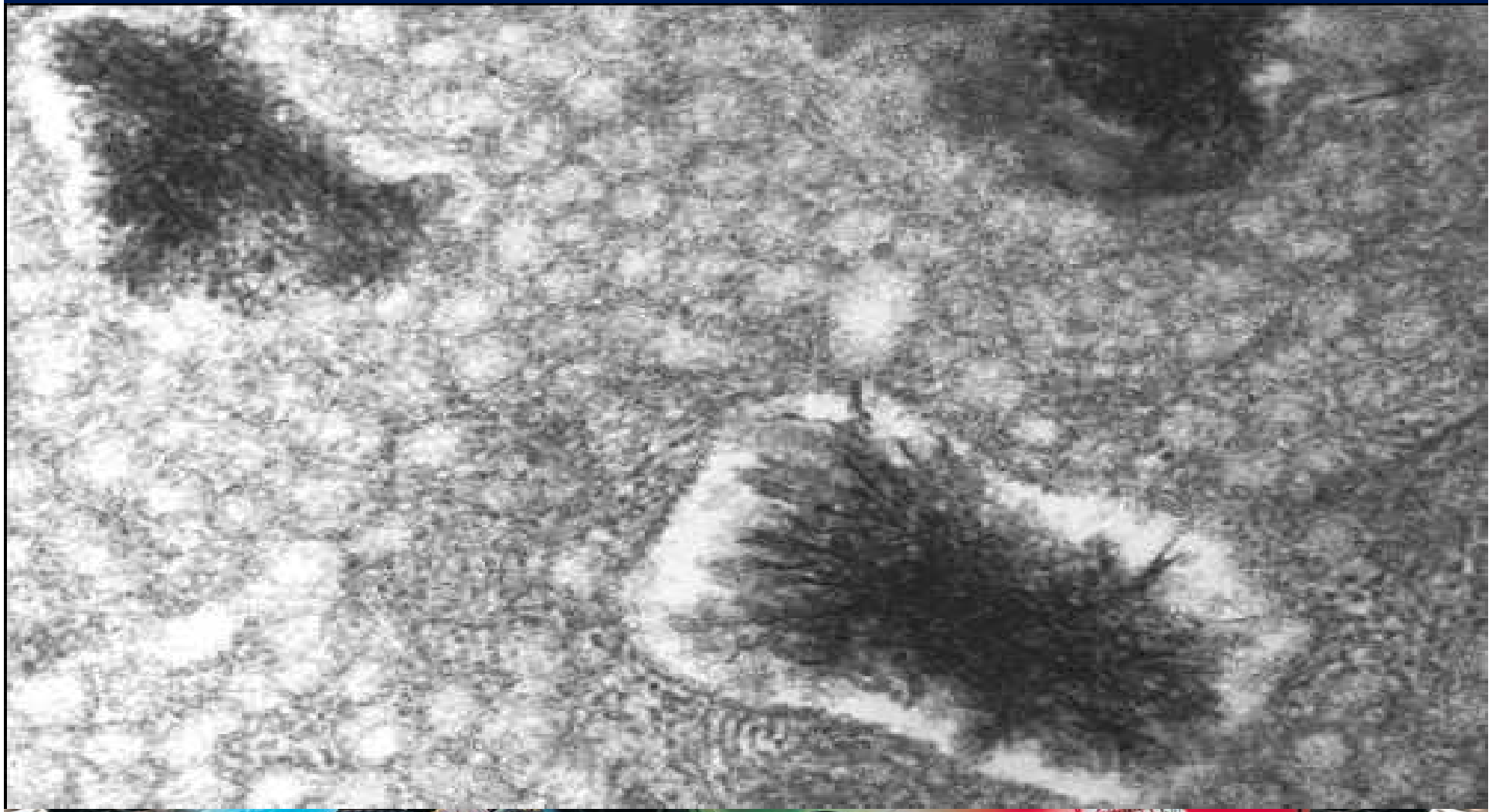
*Evans et al Gastroenterology 2002:123;1543-53*

# Calcium Phosphate in Cultured Intestinal Macrophage Phagosomes



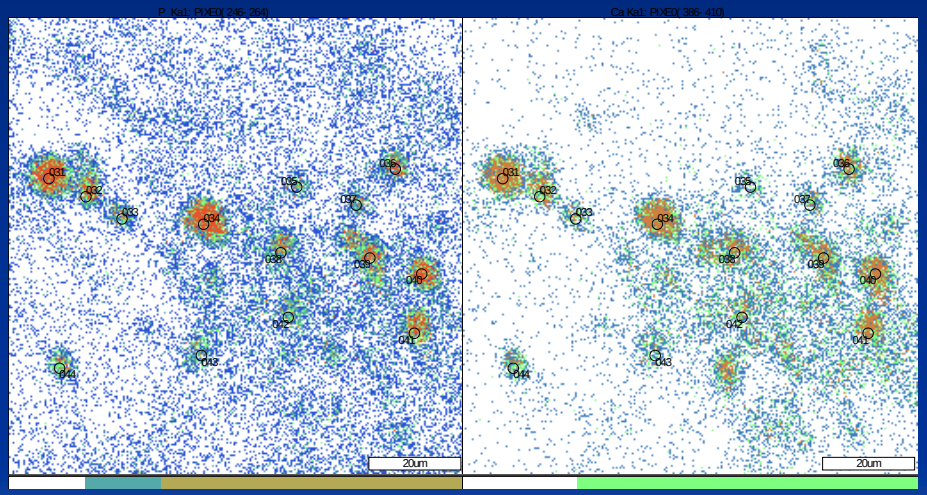
*Evans et al Gastroenterology 2002:123;1543-53*

# Calcium Phosphate in Cultured Intestinal Macrophage Phagosomes



Why is the Calcium phosphate there what does it do?

# Calcium Phosphate and Crohn's Disease




100x100μm

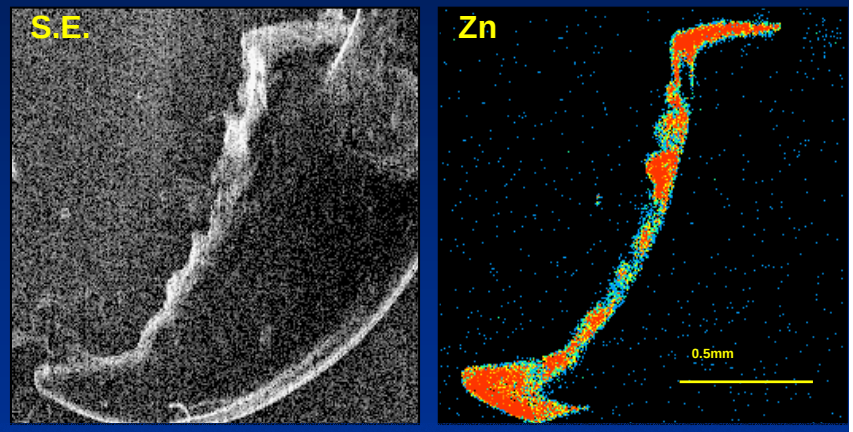
- Antigens bound in Ca phosphate particles in gut lumen
- If antigens not properly controlled diseases such as Crohn's can result
- Does diet influence this process?
- Examine Ca:P ratio
- Ca:P 0.91±0.4

# Biomineralisation

Geoff Grime, Karen Kirkby,
Inma Gomez-Morilla
Donald Quicke NHM

# Bio Hardening – Zn Mineralisation

- The mandibles of leaf cutting ants are hardened by the presence of high levels of zinc in the polymer matrix.
- In the pupa stage the mandibles have high concentrations of Mn not Zn.
- Zn is found elsewhere in the body
- Mn is known to be associated with enzymes responsible for polymerising organic materials and is likely to cause the Zn mineralisation




*Left:* Secondary electron image of mature mandible.
*Right:* PIXE image of zinc distribution

# Bio Hardening – Zn Mineralisation

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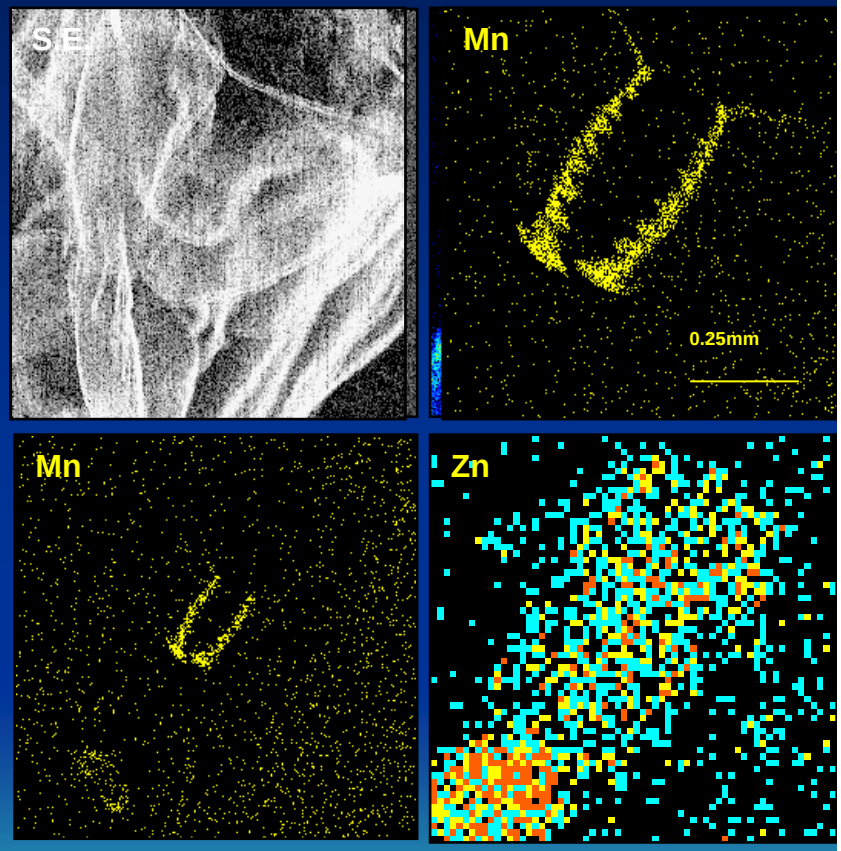
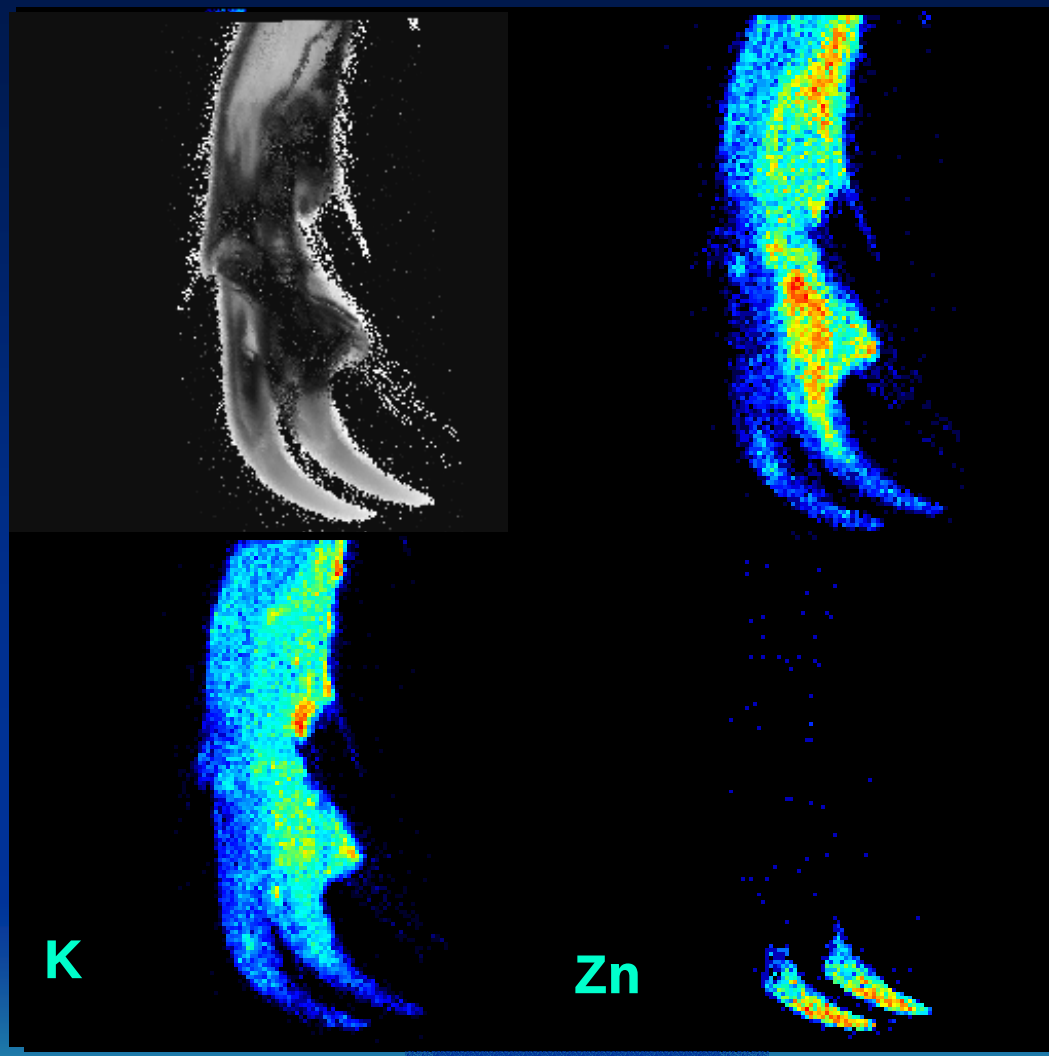



# Bio Hardening – Zn Mineralisation

- The ichneumon wasp lays its eggs within the bodies of wood boring larvae
- The ovipositor is hardened with Mn
- Its “claws” are hardened with Zn
- Traces of Mn are observed at the edges of the Zn indicating a similar process to the ant mandible




NATURAL HISTORY MUSEUM

EPSRC

# *Mass Transport in Cells*

Petra Rombouts, Inma Gomez-Morilla, Geoff W Grime, Roger P Webb, Liliana Cucenca[2], Reuben Rodriguez[2], Mark Browton, Noel Wardell[3], Brian Underwood[2], Norman F Kirkby[2] and Karen J Kirkby

**Surrey Ion Beam Centre, ATI, SEPS,**

**2 School of Engineering**

**3 School of Biomedical and Molecular Sciences**

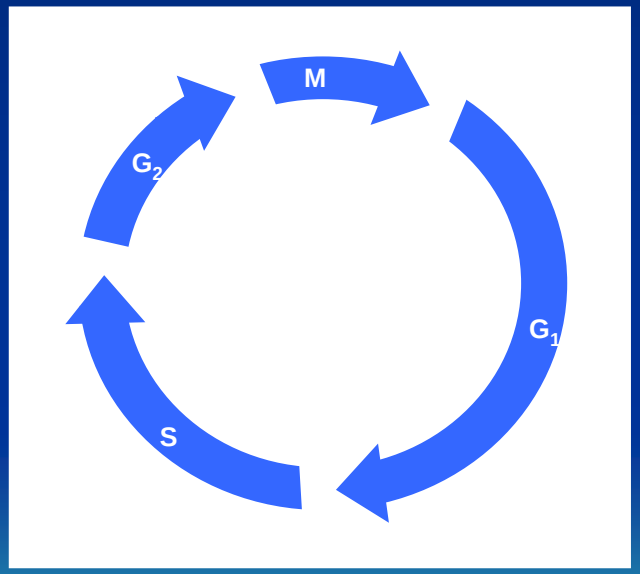
**University of Surrey**

# Motivation

- Study mass transport mechanisms of elements in cells and their surrounding media
- Input data in to models of the cell cycle e.g. CelCyMUS (**Ce**ll **cy**cle **m**odel **U**niversity of **S**urrey).
- At present CelCyMUS relies on input from experiments using populations of cells.
- But models based on the behaviour of individual cells so pressing need to validate directly individual behaviour.

# Cells and the Cell Cycle

- *Schizosaccharomyces Pombe (S pombe)*
- Edinburgh Minimal Medium 3m
- Can be used to model behaviour of mammalian cells
- Goes round cell cycle very rapidly (2 hours)



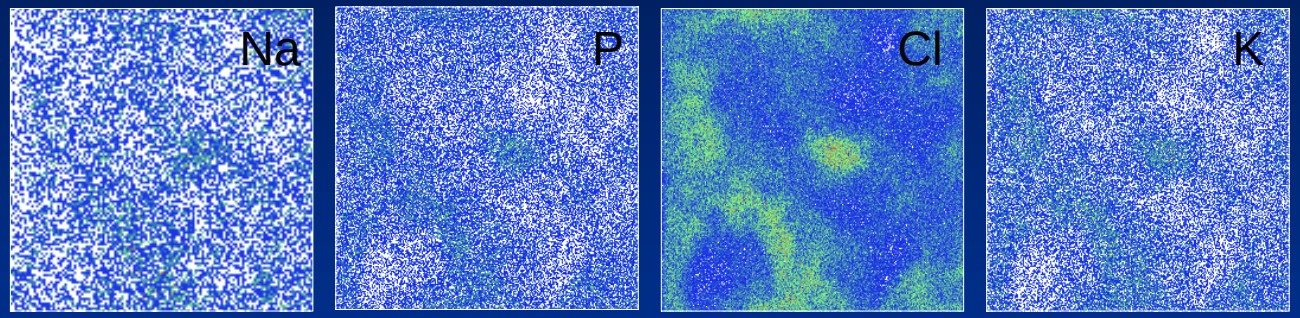

- Mitosis
- Meiosis (adverse conditions – not seen with mammalian cells)

# Sample Preparation

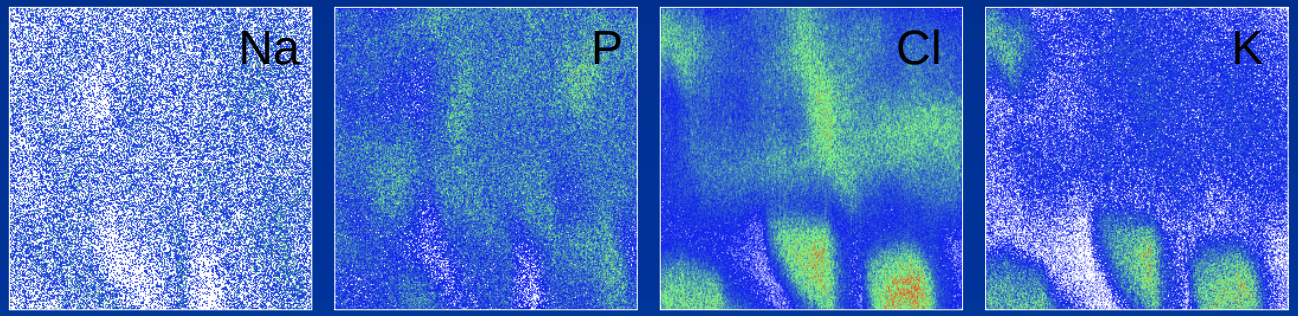
- Foils (polypropylene)
- Freezing
- Freeze drying

- Only medium
- Only cells
- Glucose solution
- Extra Mg
- Cu

# First experiments




Medium and cells
50x50 µm²

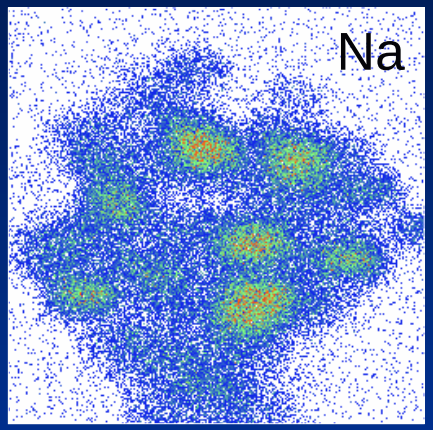


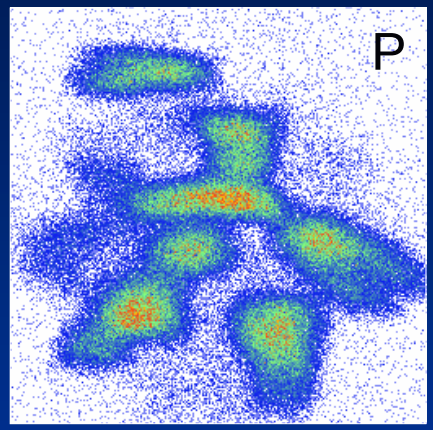

Medium without cells

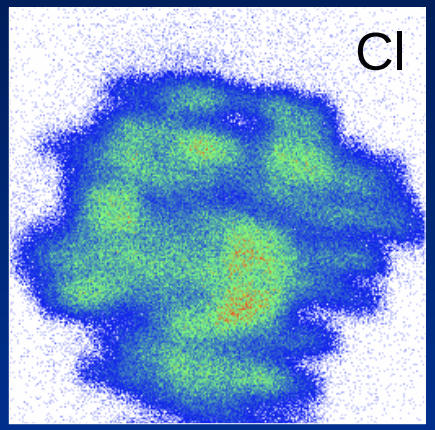
100x100 µm²

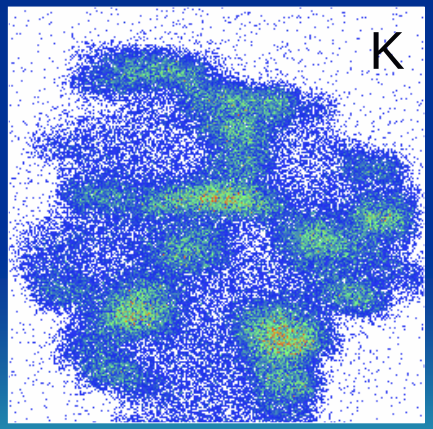
# Glucose

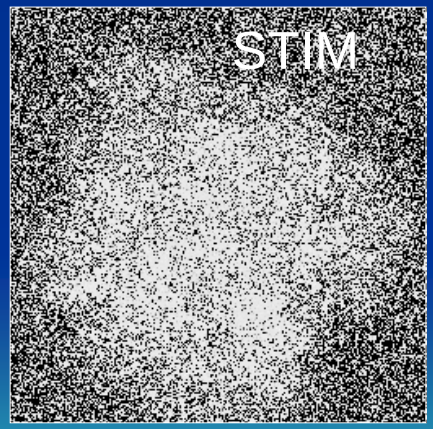
50x50 μm²

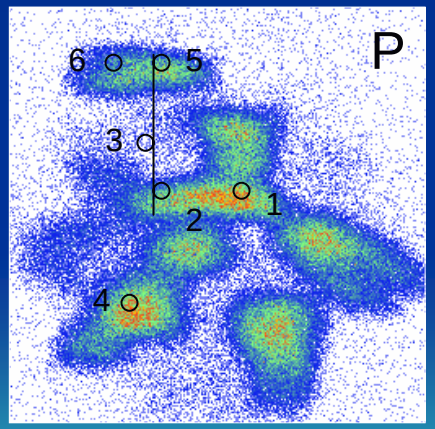


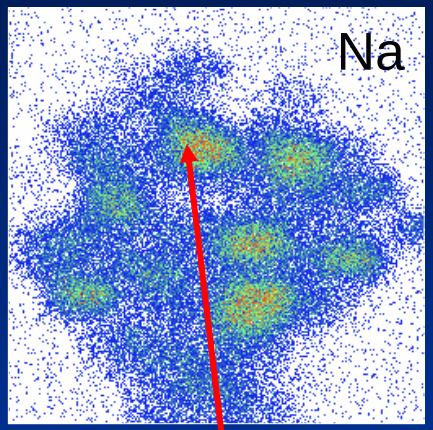



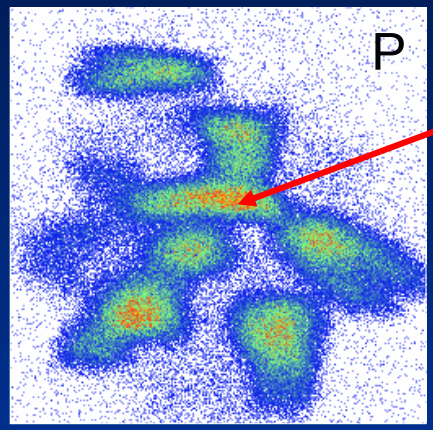




# Glucose



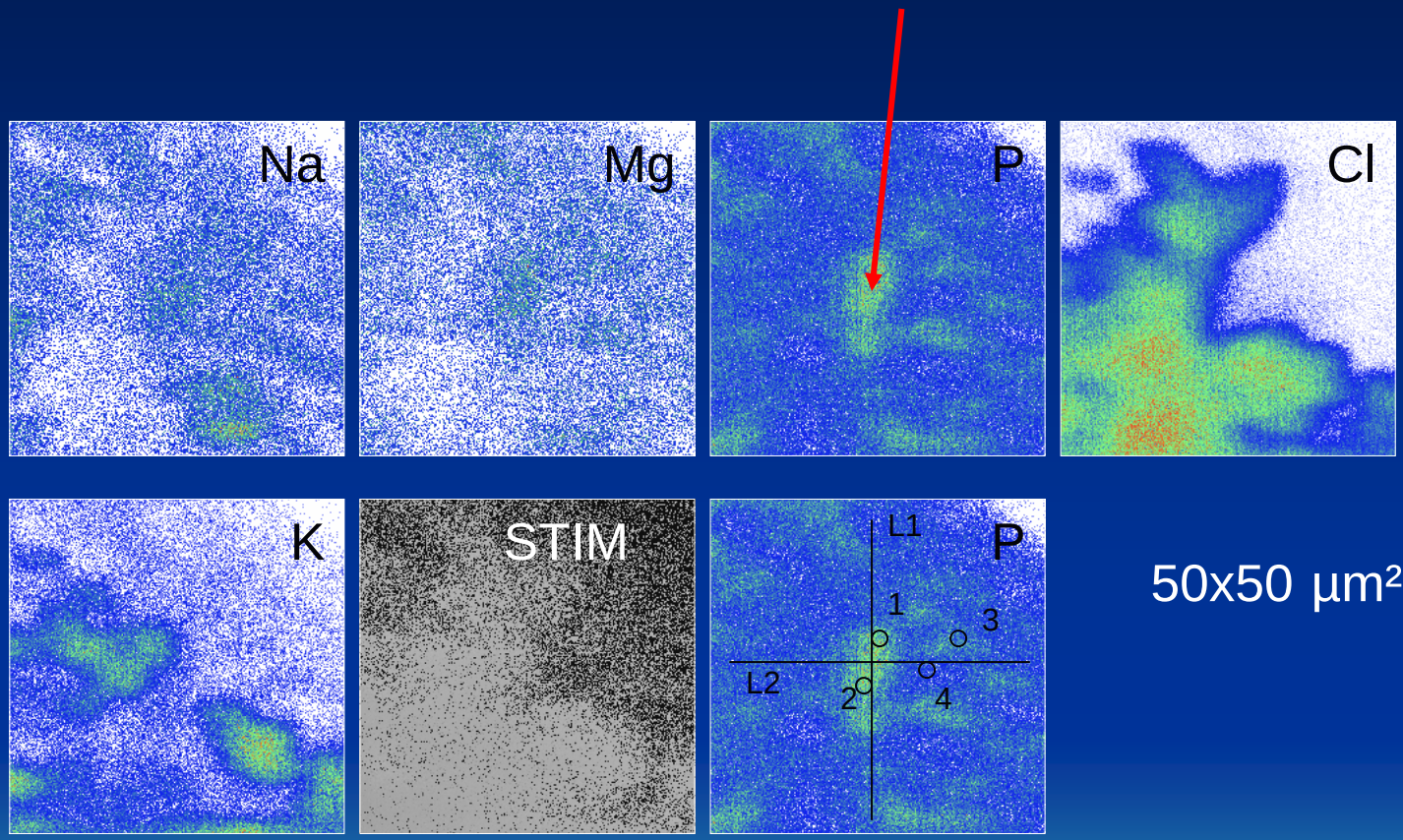




nucleus

Na and Cl adjacent to cell edge

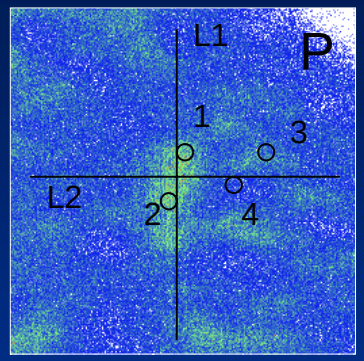
**Evidence of the cell's Na pump which pumps Na and Cl out of the cell to reduce the osmotic shock**

# Extra magnesium

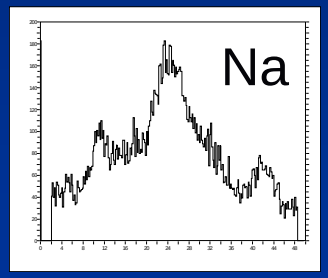


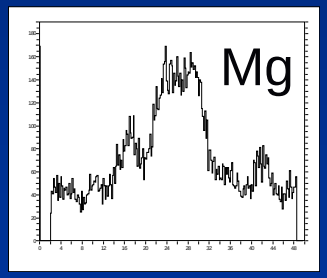

50x50 µm²

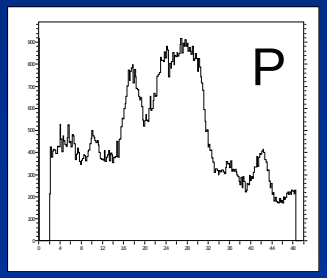
# Extra magnesium

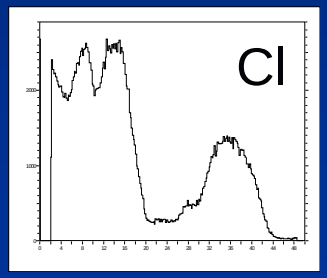


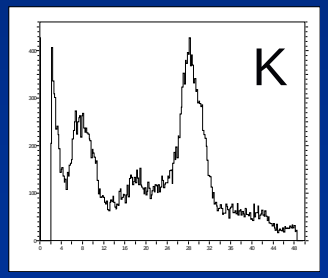

L1



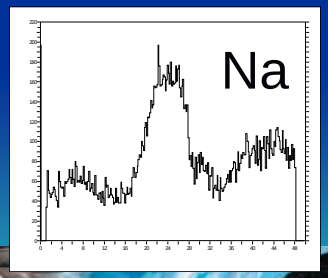


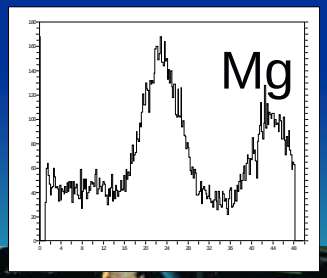


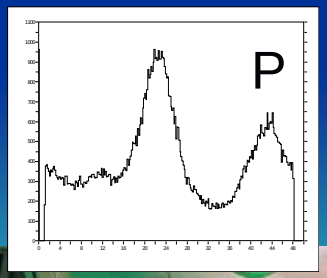


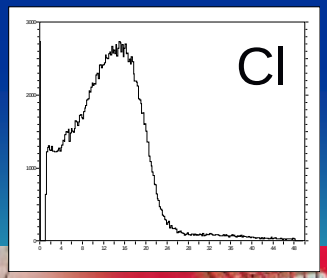


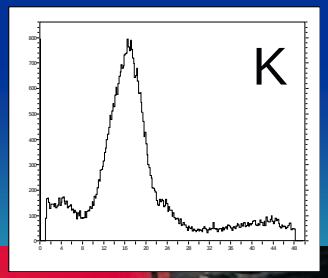

L2



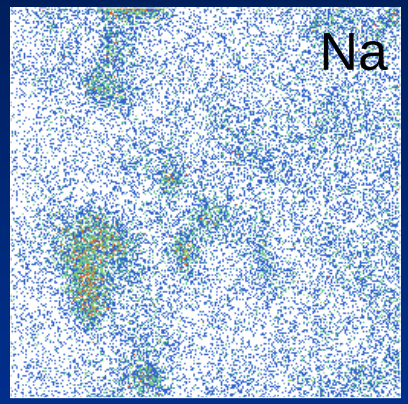


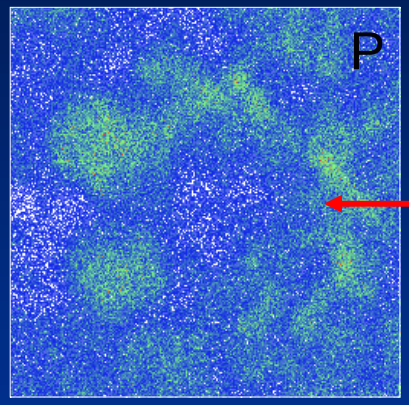


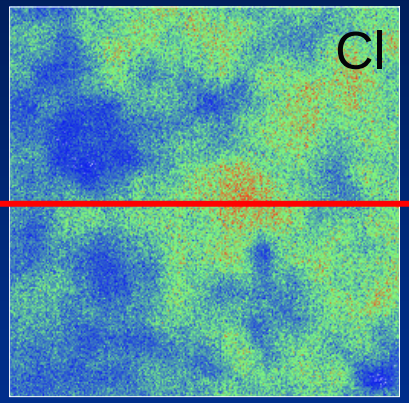




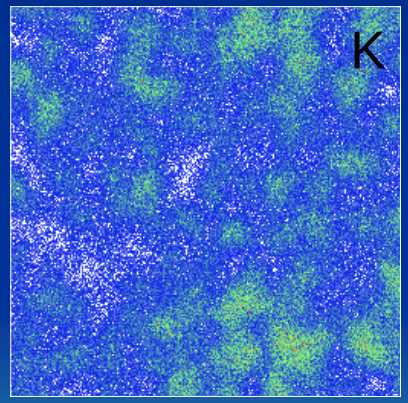

# Extra copper

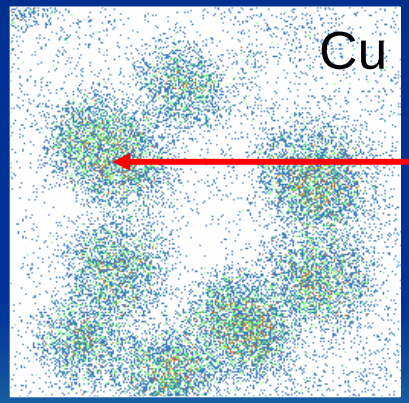


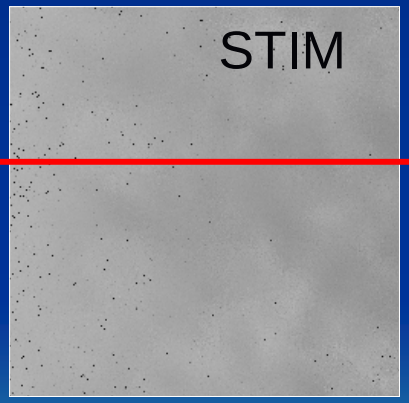




Some cells burst




Meiosis

Shrink - spores

50x50 µm²

# Conclusions

- PIXE is a suitable method for analysing cells in medium.
- Freeze drying is a appropriate preparation method for analysing cells medium.
- Cells and medium are very much alike.
- Observation of concentration gradients and mass transport
- Cells Na pump
- Onset of meiosis

# Further Research

- Requires v good co-registration of optical image and PIXE spectrum – know what you are analyzing
- Flat, thin samples.
- Improve quantitative analysis to extract information about concentration gradients.
- Measure cells in different parts of the cell cycle.

# External Beam

- External beam on samples
  - Too large
  - Too valuable
  - Too fragile
  - Too alive

# Surrey external beam facility


# External Beam analysis

Analysis of trees

# Trace element analysis

What for?

Analysis of  trace elements lead-based pigments

Difference between lead white front/back in this painting (Brindisi collection)

Analysis of ground – Sr in gypsum

# So is it a real Leonardo?

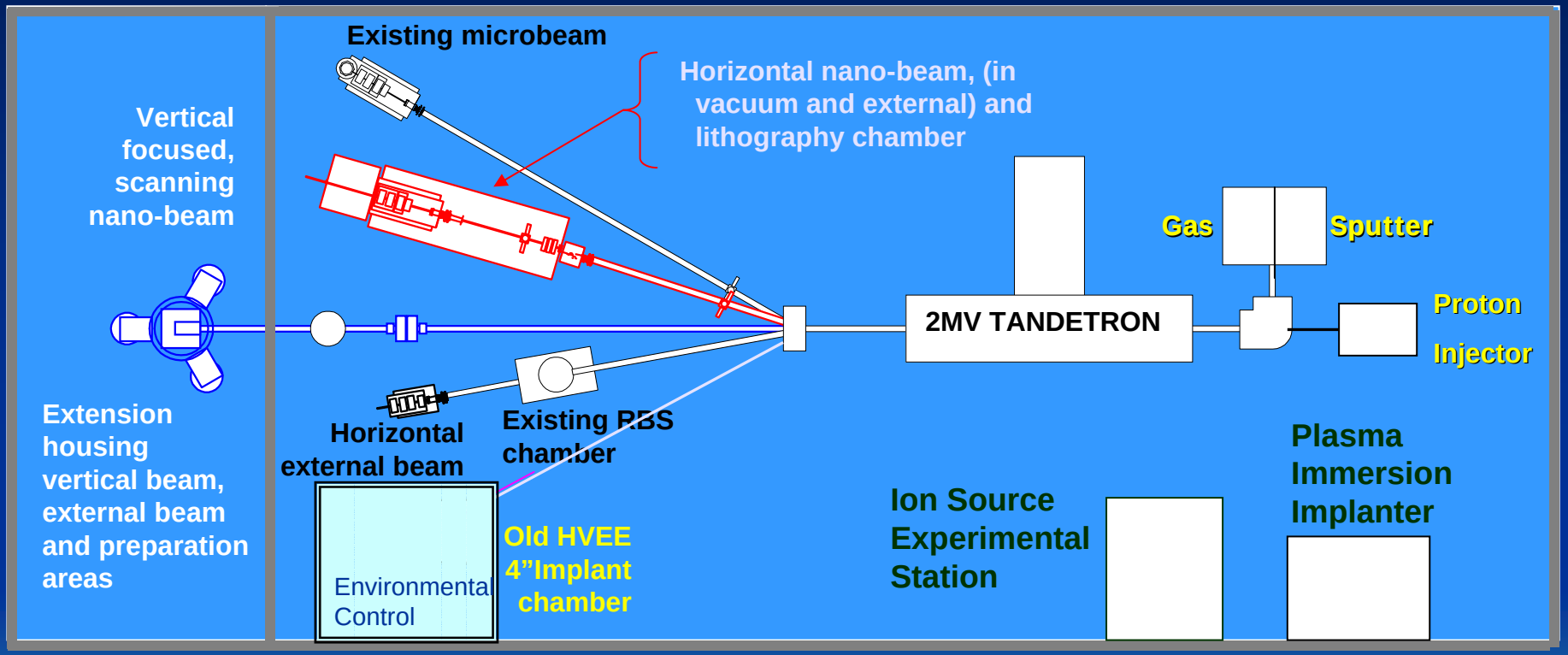

- White pigment corresponds to 16th Century Italy
- Green/brown pigment corresponds to 16th Century organo-Cu compounds
- White pigment on the back is Northern European

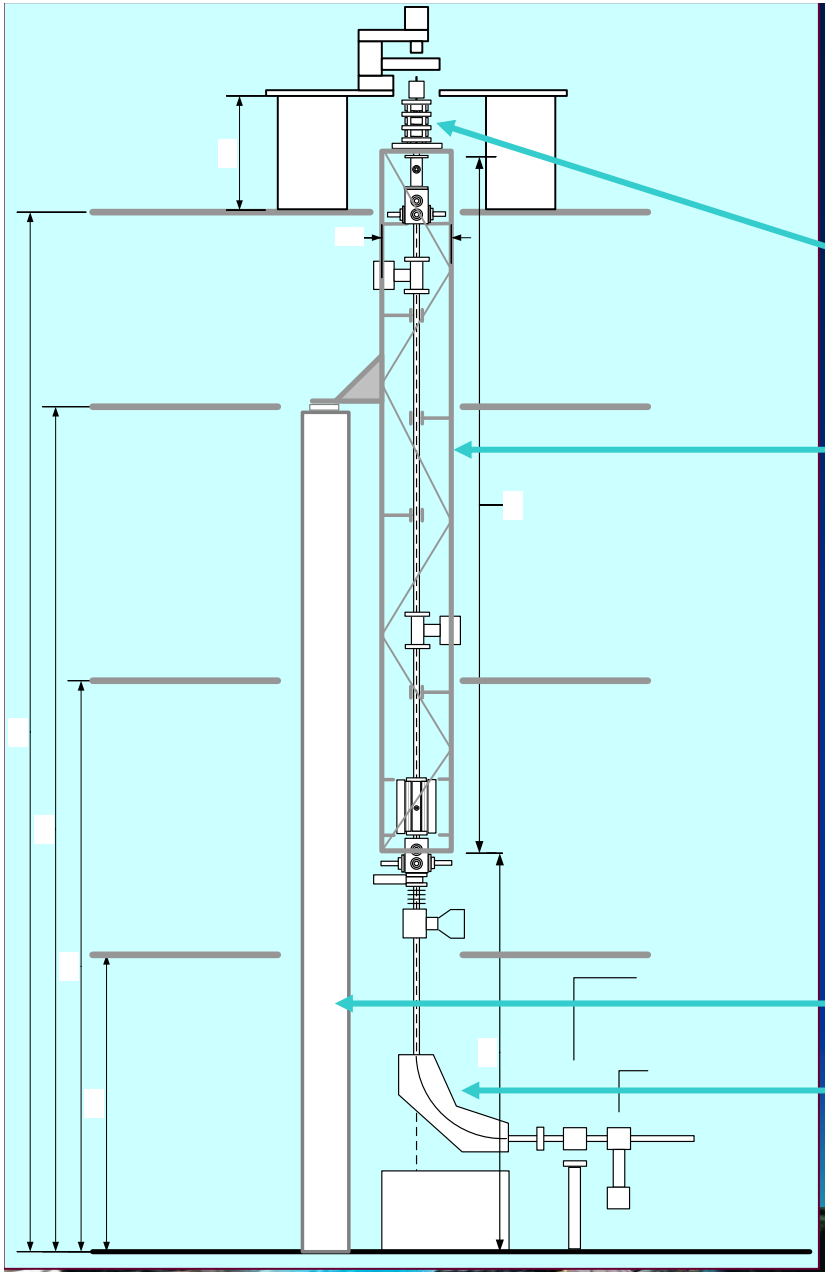
?

Thank you for listening

# Questions

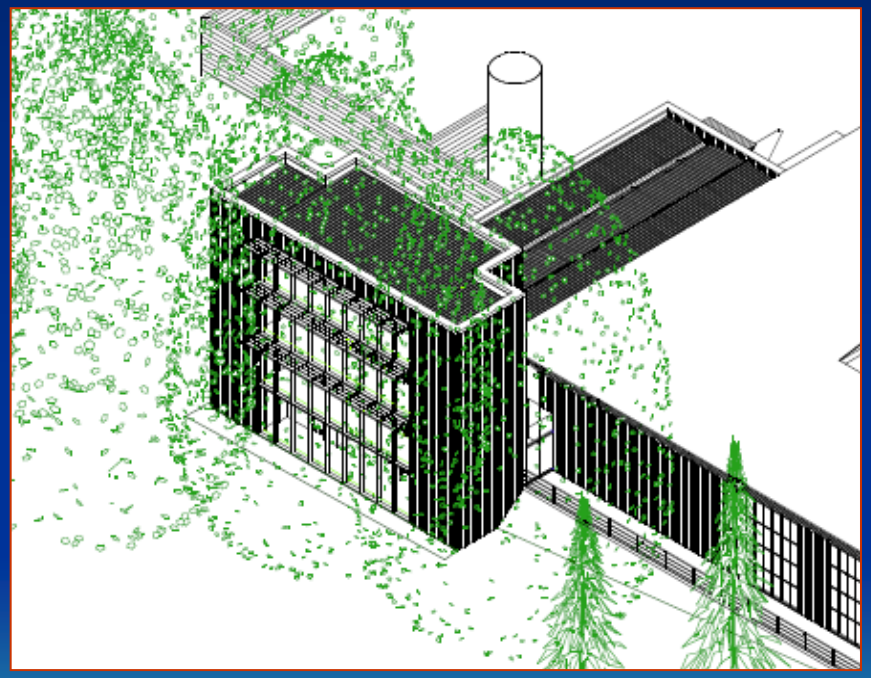
# Developments in the Stephens' Laboratory

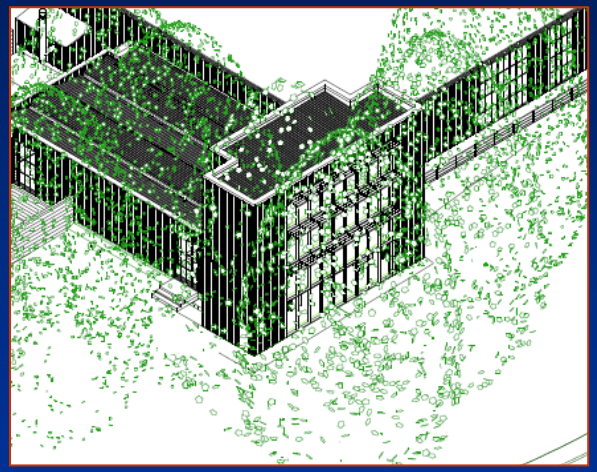



- H to Ar irradiation
- Living cells
- Single ion or full current
- 4 floors – 200m$^2$
- Top floor clean room and prep area

# Architect's views of the proposed new building





# Conclusion

- **Whole range of applications for ion beams on the micron scale**
- **Many more on the nano scale**
  - Precisely positioned single ions
  - Nano analysis
  - Nano fabrication
  - Lots of bio-medical applications for ion beam analysis on the micro and nano scale
- **Huge potential for biomedical community**