



Antibiotika resistent – hva nå?

Hanna-Kirsti S. Leiros

Forsker ved NorStruct

Nasjonalt kompetanseseter for struktur biologi





Oversikt

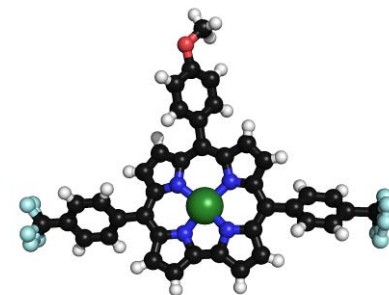
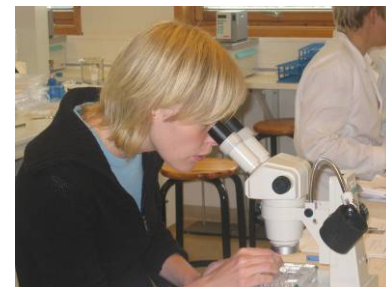
- Kjemi. NorStruct
- Vanlig fremgangs måte
 - Protein i løsning → krystall → protein struktur
- Siste forsknings resultater om antibiotika resistens





Kjemi

- Mange har et forhold til ordet, faget!
 - + eller – ladet? Kjedelig, artig
- Institutt for kjemi har ulike forsknings profiler
 - Teoretisk kjemi
 - Bioinformatikk
 - Marin bioprospektering
 - Legemiddelutvikling
 - Molekylsyntese
 - Protein strukturer

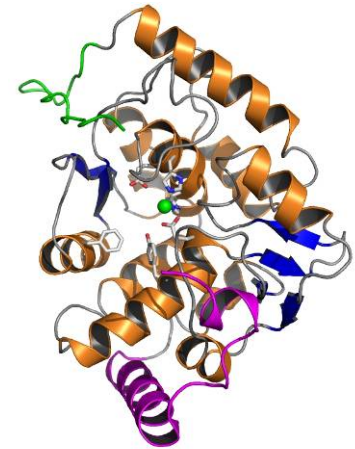




NorStruct



- Servise og kompetanse senter i protein **Struktur Biologi**
- **Hvorfor i Tromsø?**
 - Faglig dyktige i protein struktur
 - Mange års erfaring
 - Alle universiteter trenger ikke bygge opp den kompetansen vi har. Bruk oss heller!!





Service package 1: Protein production

SP1

Protein production

In SP1 NorStruct offers opportunities for subcloning, small and pilot scale expressions (up to 15 L) and purification, in addition to protein expression optimization and refolding.

The subcloning, small scale expression and purification using *E. coli* as expression host will be performed in high throughput format. Expression will also be offered using *Pichia pastoris* as expression host. The customer can select between N- and C-terminal His-tag. Expression of target proteins will be verified by SDS-PAGE and MS.



Protein expression hosts

The standard package includes:

- Cloning into expression vectors
- Small scale expression in 3 E. coli strains
- Purification using spin columns
- Medium scale (1 L) or pilot scale in fermenter followed by affinity chromatography purification

Alternatively the gene can be cloned and integrated into the genomic DNA of *P. pastoris* by homologous recombination. Five positive clones will be selected for small scale expression and purification. Pilot scale expression will be performed in a fermenter followed by affinity chromatography purification.



Summary NorStruct Services

NorStruct will continue to offer state of the art services and collaboration in structural biology techniques to the Norwegian research community. High quality expertise and services will be assured via in-house research projects and national and international partnerships.

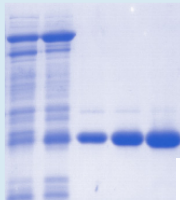
The services will be organized in three different service packages (SPs), whereby the services ordered are tailored to the customer's specific needs. The three SPs will be "Protein production" (SP1), "Structure determination" (SP2), and "Drug discovery and design" (SP3).

SP1	Subcloning, small-scale expression and purification Large scale expression and purification Protein expression optimization and refolding
SP2	Crytallization Quality control and buffer optimization Data collection and structure determination
SP3	Protein ligand complexes Molecular modelling/looking and virtual screening Protein-ligand thermodynamic studies

Protein refolding

A protein refolding package will also be available. Here commercially available kits with at least 96 different conditions will be used for refolding trials.

Customers should provide a biological activity assay which will help evaluating the refolding results.



Protein expression optimization *E. coli*

If no expression or insoluble protein is detected using vectors and strains in the standard package, customers can select the optimization package including:

- 48 different expression conditions: 8 expression vectors or 8 constructs, 3 expression strains, 2 temperatures (20°C, 37°C)
- Optimization of culture media for expression is optional.



Service package 3: Drug discovery and design

SP3

Drug discovery and design

NorStruct will through SP3 offer a new service package that covers ligand discovery and design using a combination of biophysical, computational and crystallographic techniques.

Summary NorStruct Services

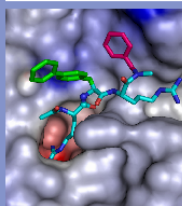
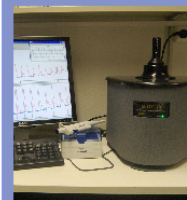
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Thermodynamics

Standard services includes characterization of ligand-receptor complexes using ultrasensitive calorimetric techniques:

- Calorimetric investigations using both Isothermal Titration (ITC) and Differential Scanning Calorimetry (DSC)
- Optimization of experimental condition suitable for calorimetry
- Complete thermodynamic characterization including determination of ΔG , ΔH , ΔS and the stoichiometry for binding reactions.



Computer modeling

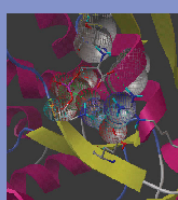
A service package for computational modeling of ligand-receptor complexes may include:

- Library construction and virtual screening
- Prediction of ADME properties
- Pharmacophore modeling
- Lead optimization using sophisticated free energy calculations.

Crystallography

NorStruct will offer a service on structural investigations in conjunction with drug design and lead optimization covering:

- High throughput structure determination of multiple ligand-receptor complexes
- Identification of weakly binding low molecular weight ligands using fragment screening for appropriate targets
- Structural studies of off-target effects, primarily serum albumin interactions
- Structure determination of putative ligands using chemical crystallography



Service package 2: Structure determination

SP2

Structure determination

Through SP2, NorStruct offers evaluation, quality control and stability/solubility optimization, crystallization experiments, data collection either using the X-ray home source or synchrotron radiation, followed by structure determination and refinement.

Quality control

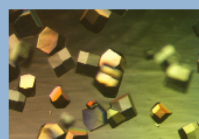
A standard service package for protein quality control and sample optimization includes:

- Sample quality control using Dynamic Light Scattering analysis (DLS) or Differential Scanning Calorimetry (DSC)
- Screening for improved solubility and stability using different buffer conditions by testing 24 different

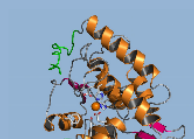
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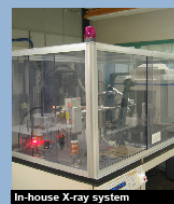


Crystallization facilities

Crystallization

NorStruct offers a unique opportunity to set up nanolitre crystallization trials with our Phoenix RE robot using very small quantities of protein. A minimum of 600 different conditions will be put up, along with subsequent optimization of crystallization conditions using the liquid handling Alchemist™ II robot.

The service is appropriate for crystallization of proteins, protein complexes and membrane proteins if soluble protein is provided.



In-house X-ray system

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Phoenix RE robot



Alchemist™ II robot

Liquid handling Alchemist™ II robot

Optimization and preparation of crystallization solutions make use of the automatic Alchemist™ II liquid handling system.

This ensures accurate pipetting volumes, less manpower and better reproducibility.

Hotel and Imager

Crystallization experiments are stored in the Gallery Plate Hotel which is coupled to our Desktop Minstrel Imager. Each crystallization drop is viewed automatically and digitally recorded, enabling rapid and detailed evaluation of the crystallization conditions.

The scheduled recording of images allows electronic documentation of the progress for each crystallization experiment.



Gallery Plate Hotel Desktop Minstrel Imager

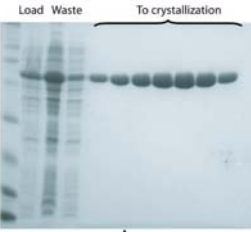
Structure determination

When protein crystals are available the service will also cover:

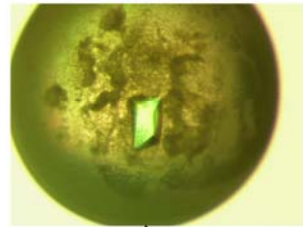
- Determination of cryogenic conditions for data collection
- Diffraction tests
- X-ray data collection at the in-house X-ray system or at a synchrotron radiation facility.

Cloning, expression and purification

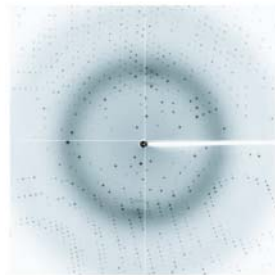
Pure protein



Crystallization

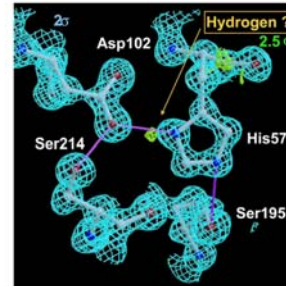


X-ray diffraction data



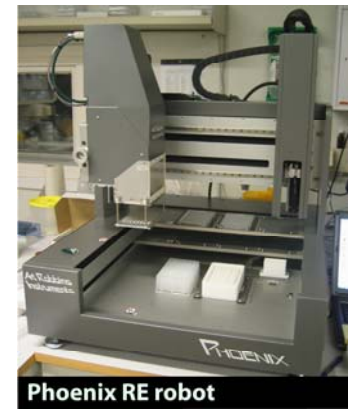
Phase problem solution

Electron density



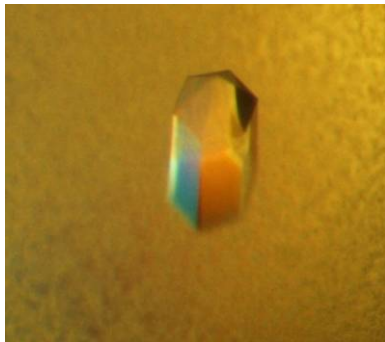
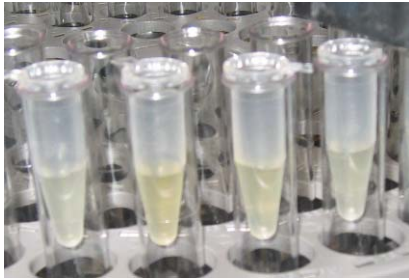
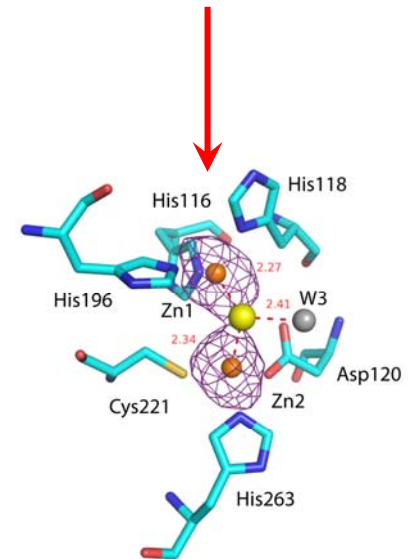
Refinement and model building

Three-dimensional structure



Her skjer **kjemien**,
Atom nivå!!

Antibiotisk resistent
proteinet





«NDM-1» kan finnes i E.coli eller andre tarmbakterier. ARKIVFOTO: INGAR HAUG STEINHOLT



Frykter spredning av ny «superbakterie»

Norske og internasjonale eksperter frykter at det antibiotika-resistente genet «NDM-1» skal spre seg. - Det er en skummel utvikling, sier lederen for Nasjonalt kompetansesenter i antibiotikaresistens.

AV EIVIND SØRLIE

Oppdatert: 12.08.10 kl. 07:10 - Opprettet: 11.08.10 kl. 20:39

- Det er en ekkel, skummel utvikling, sier Arnfinn Sundsfjord, som er leder for Nasjonalt kompetansesenter i antibiotikaresistens ved Universitetssykehuset i Nord-Norge (UNN).

I en studie publisert i det anerkjente medisinske tidsskriftet [The Lancet](#) i dag, advarer internasjonale eksperter mot en spredning av genet «NDM-1», som kan

Skriv ut Tips en venn Del

TIPS TLF: 02288 SMS/MMS: 2286 2286@aftenposten.no

AFTENPOSTEN EAVIS



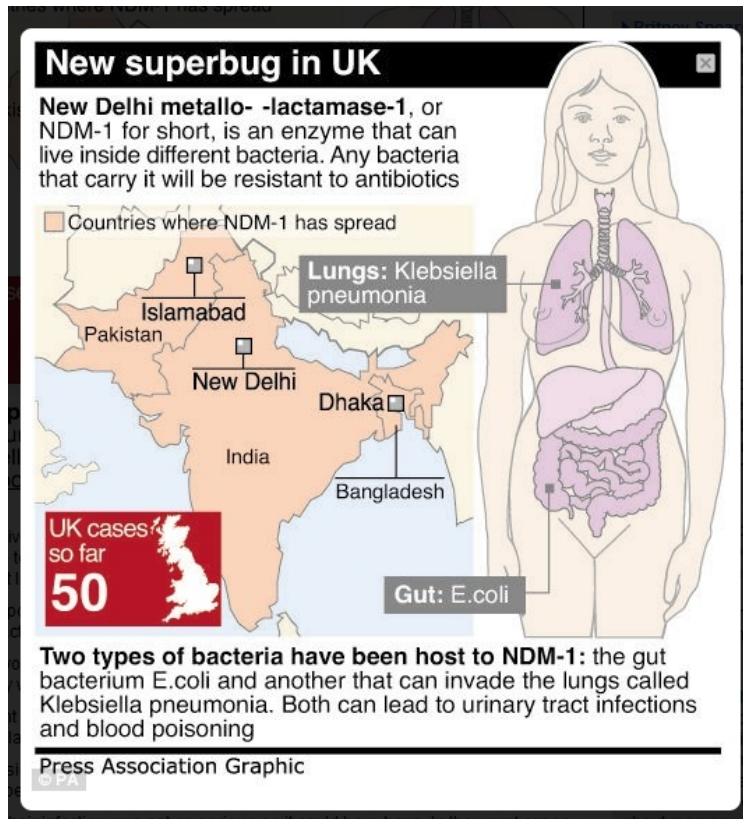
Kjøp dagens eAvis

- 12. aug 2010
- NDM-1
- Et av forskningsprosjektene

- BBC
- VG
- Aftenposten
- Daily mail
- NRK

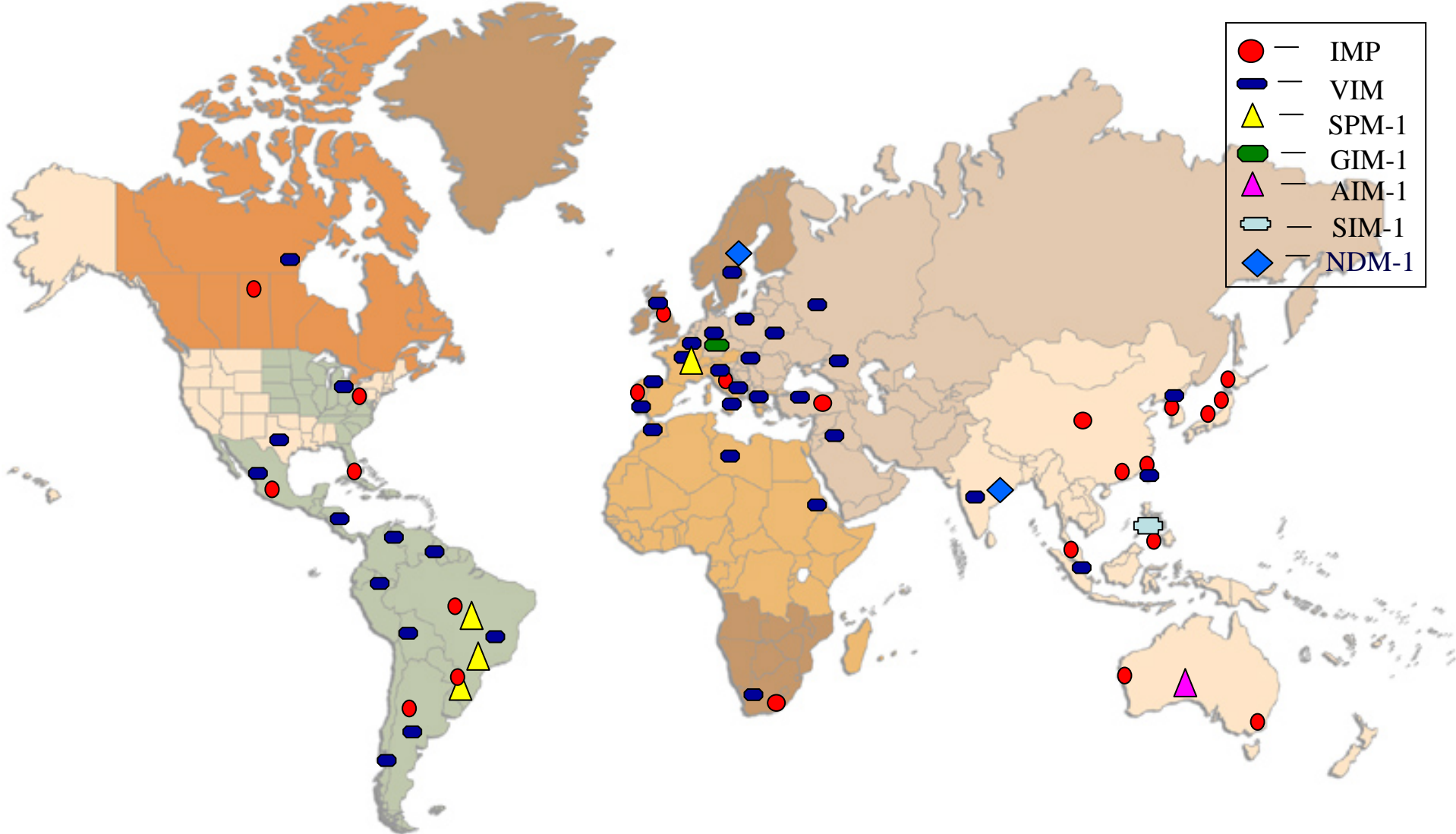


Super bakterie



- Ulike plasser i kroppen
- Spres lettere mellom ulike bakterier enn andre antibiotisk resistente proteiner

Lokalisering organismer med mobile MBL

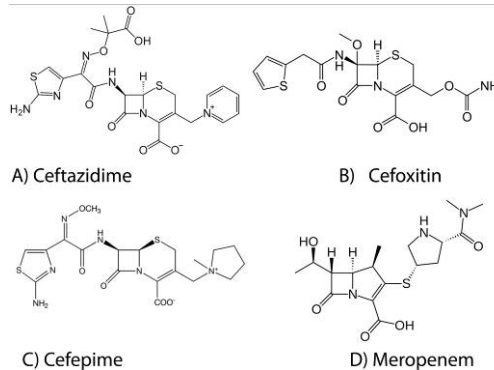
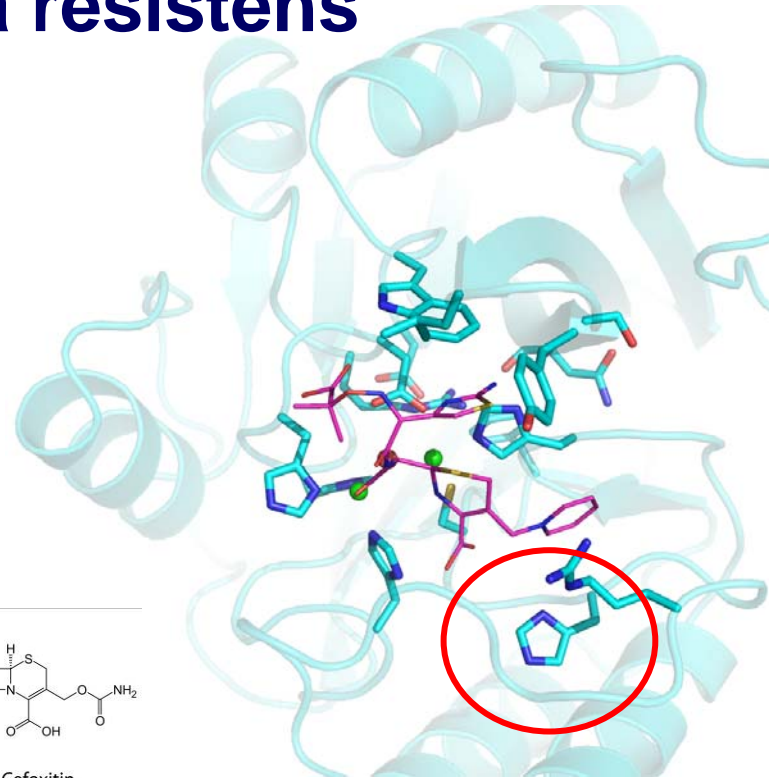
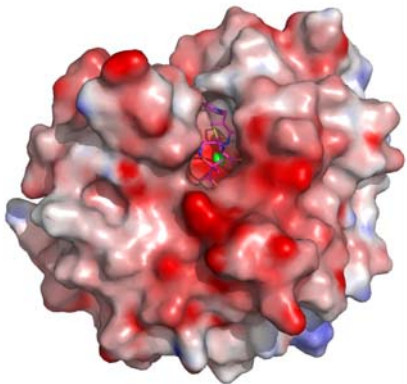
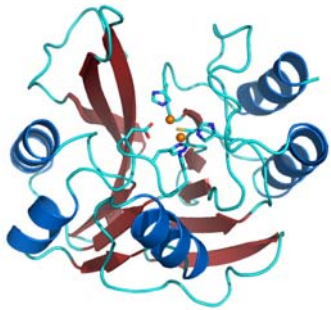
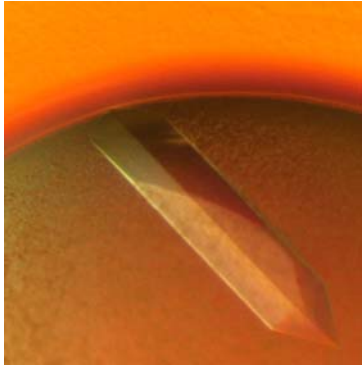




Metallo Beta laktamase (MBL) Antibiotika resistens

- Se på amino syrene
 - bygge klossene

Studere antibiotika binding

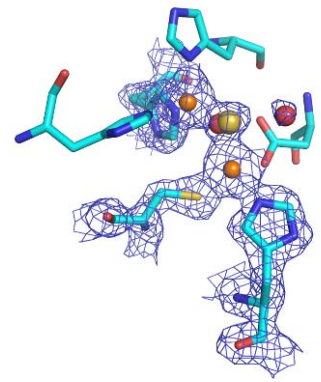




Samarbeid om Antibiotika resistens

- Kjemi, NorStruct
- Universitets sykehuset Nord-Norge (UNN),
Kompetansesentret for antibiotikaresistens (K-res)
 - Kliniske prøver, gener
- MabCent
 - Marin bioprospektering
- Lager proteinet, krystall, protein struktur.
 - Ser på **KJEMIEN** som skjer, **atom nivå**





Antibiotika resistens, Hva nå??

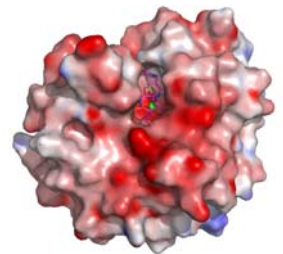
- **Fare, utfordringene**

- Folk reiser mer → Kjapper spredning av resistente bakterier
- Uvettig, unødig bruk av antibiotika

- **Hva nå??**

- Forskning er viktig.
 - Kompetansen er klar før epidemien kommer
- Pasient behandling → isolasjon, unngå spredning
- En STOR utfordring når dette rammer globalt

- Redusere medikament bruken, overvåkning i India, Hellas, m.fl.





Siste sida

- Det var forskerne sine resultater som fikk media til å omtale ”superbakterien”
 - Forskerne sa noe om økonomiske konsekvenser
 - Kanskje blir det bedre overvåking i India

