NMR Core Facility –
NMR for Life national infrastructure

NMR for Life SWEDISH NMR CENTRE

SciLifeLab

KBC
Chemical Biological Centre

UMEÅ UNIVERSITY

UNIVERSITY OF GOTHENBURG

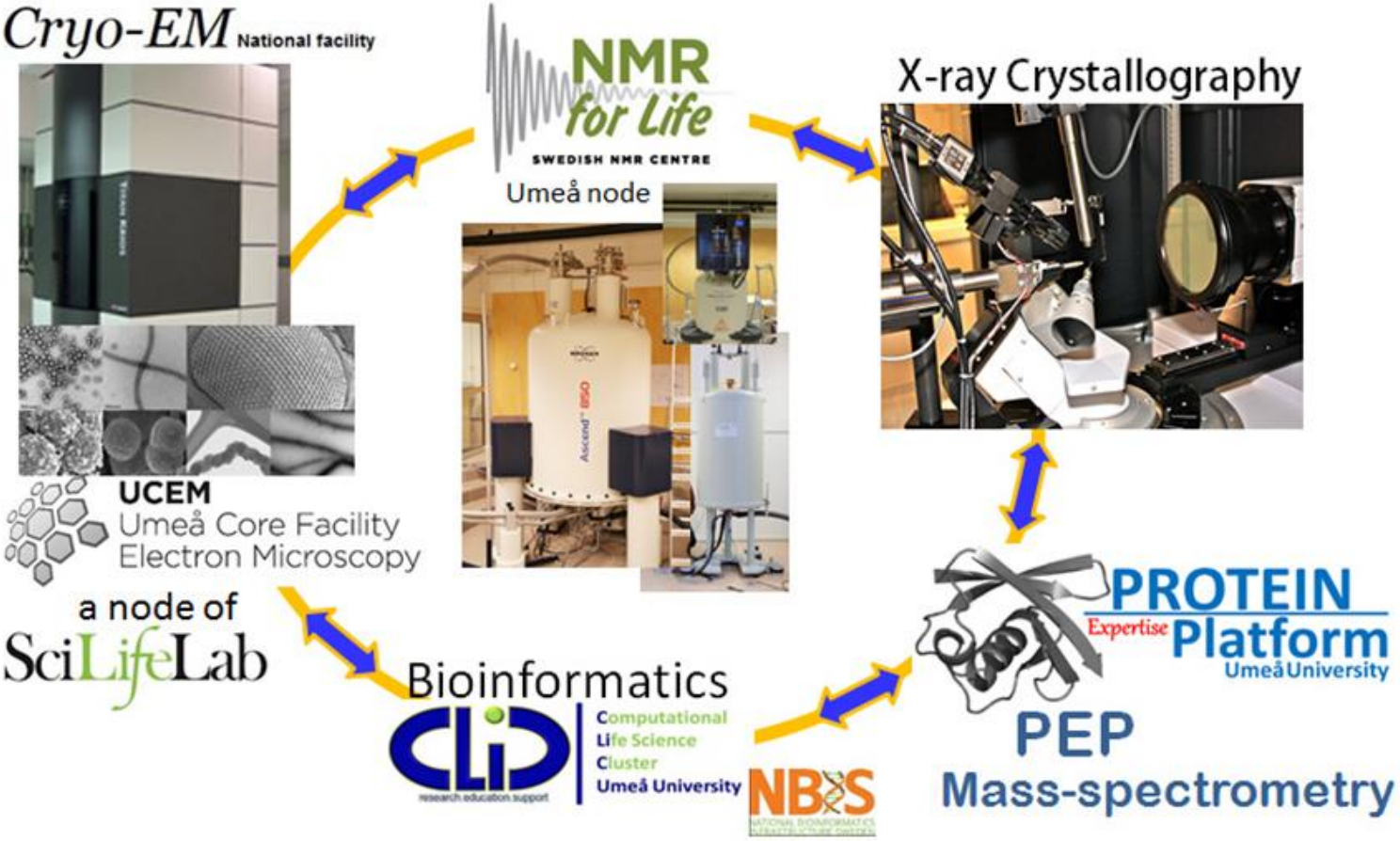
Knut och Alice Wallenbergs Stiftelse

www.umu.se/en/research/infrastructure/nmr/

https://www.scilifelab.se/facilities/swedish-nmr-centre/

Integrated Structural Biology at UMU

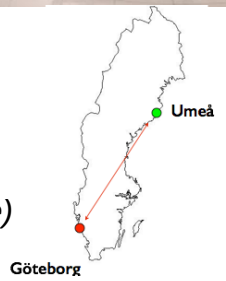
Solving challenging biological questions at atomic level



The Swedish NMR Centre

- National Research Infrastructure for NMR applications
- Structural biology, Metabolomics, Chemical biology, Enviroment, Materials
- World Class Equipment and Support

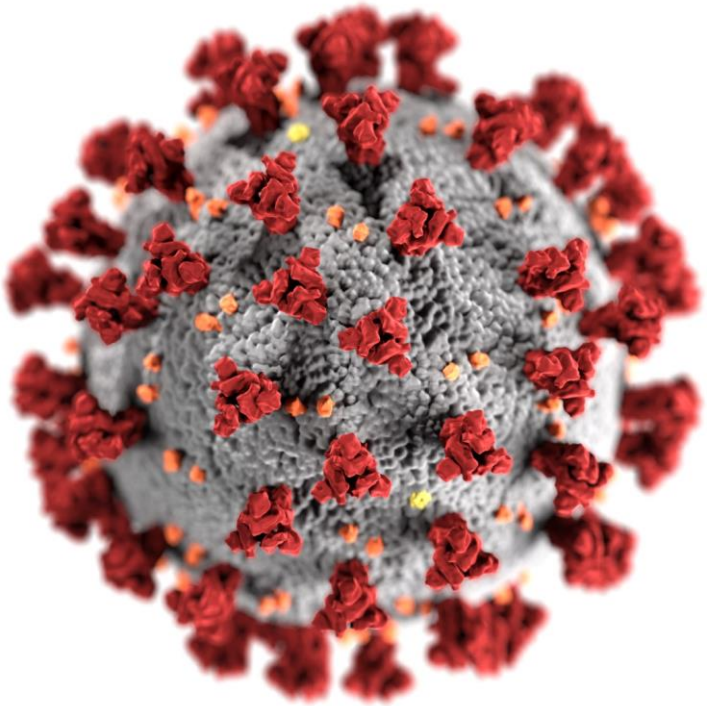
- Gothenburg node and Umeå node
- High degree of automation, automated sample changers and robots
- Continuous upgrade with state-of-the-art equipment
- National steering group *(chair M. Akke, Lund University)*
- International Evaluations *(2018, R. Bolens, Utrecht, B. Elena-Herrmann, Grenoble)*
- Recent highlight: *Burmann et al, Nature 577 (2019) 127..synuclein chaperones..*



Part of National Structural Biology Infrastructure

SciLifeLab - Cryo-EM

ESS - neutron scattering



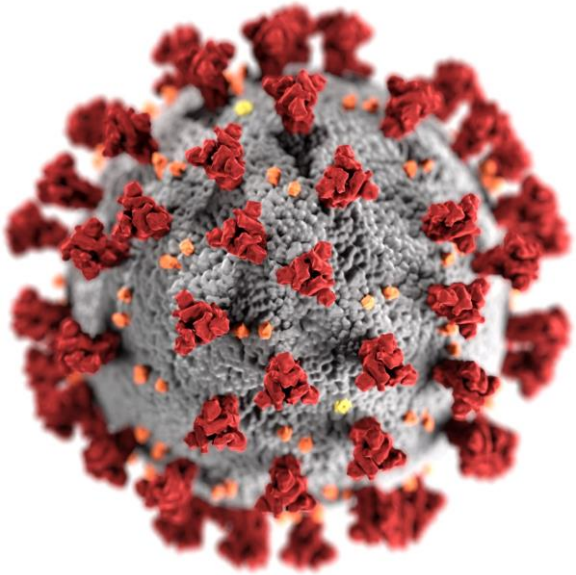
SARS CoV-2

MAX IV - X-ray

Swedish NMR Centre

Swedish NMR Centre, Göran Karlsson

ScilifeLab COVID-19 Action Plan: NMR Based Drug Discovery

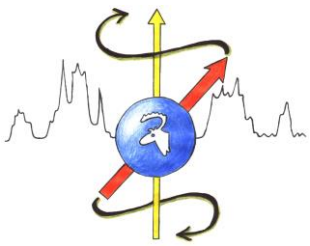


SARS CoV-2

Main aim: Identification of suitable drug candidates against Mpro (virus protease) by NMR high-throughput screening

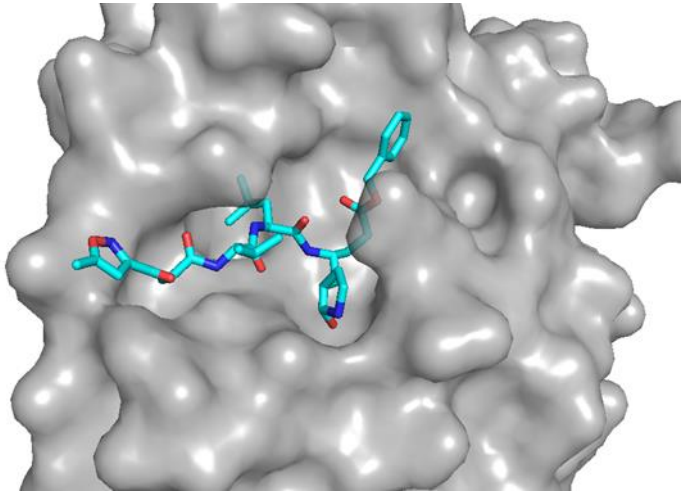
! Integration of KBC-based infrastructures !

- **PEP (Mikael L. /Uwe S.): production of proteins: main protease Mpro, Spike protein domain**
- **CBCS (Erik C.): access to large substance libraries**
- **NMR (Mattias H, team): screening of compounds against target proteins, identification of hits, validation of hits**
- **Information as input to Scilifelab (UU) coordinating drug discovery/development of COVID-19**

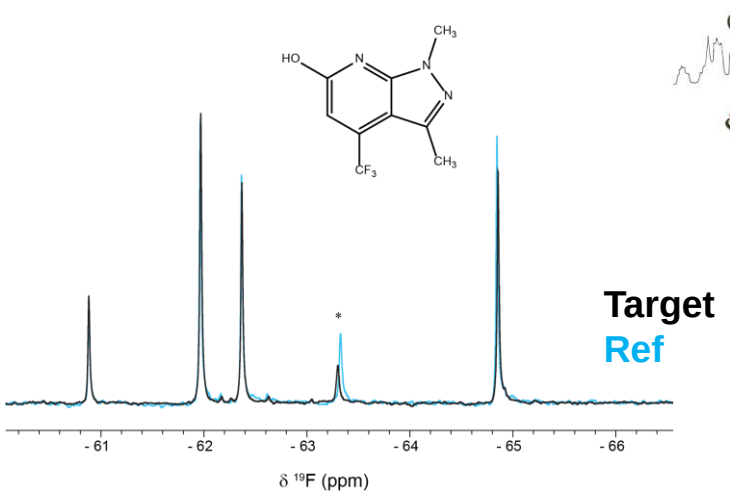


COVID-19 NMR based drug discovery: first Results

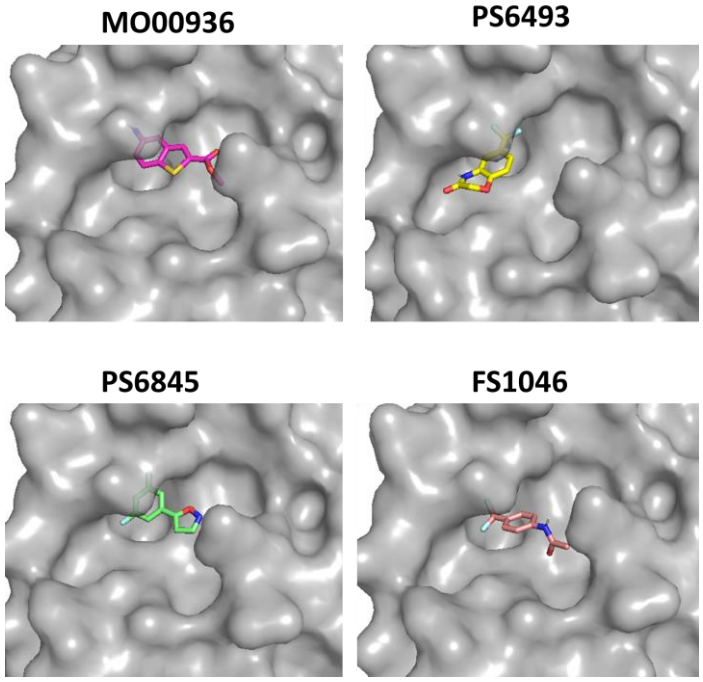
M^{pro} + α-ketoamide inhibitor



^{19}F NMR on F-containing molecules



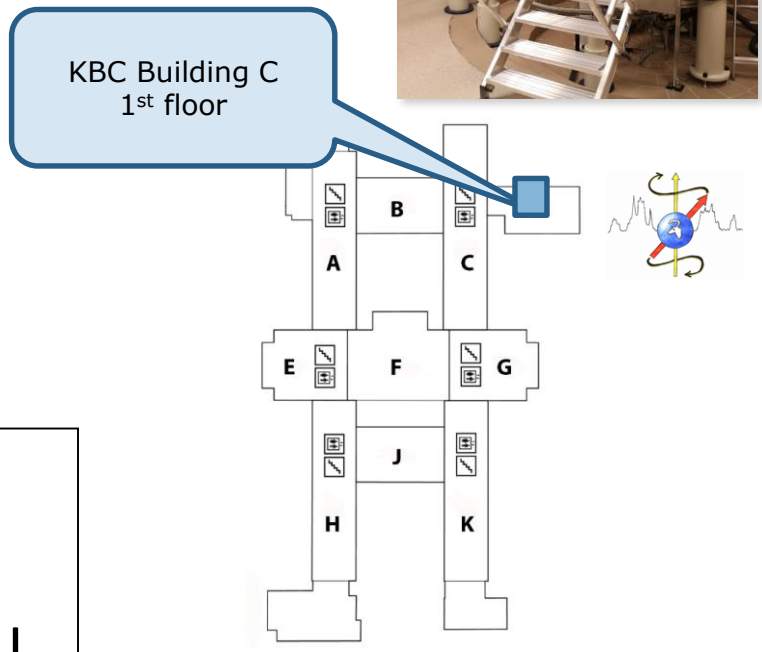
- **Successful production (with PEP) of unlabeled and labelled Mpro virus protein.**
- **Successful screening of several substance libraries.**
- **11 hit fragments have been found so far**
- **NMR characterization of Mpro protein, conformational flexibility and monomer-dimer transition (not possible by X-ray etc).**



NMR Core Facility

National “NMR for Life” Infrastructure

- **State-of-the art nordic NMR hub**
- **Solid-state and liquid NMR with 850, 600, 500, 400 MHz instruments**
- **Excellent NMR competence in many areas**



In Umeå: Several groups engage in NMR research & methodology

Nationally: Swedish NMR centre GU-UmU

International: Close contacts to EU facilities and Instruct

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Contact for service:
Tobias Sparrman, Chemistry
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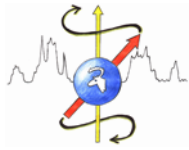
Mattias Hedenström, Chemistry
mattias.hedenstrom@umu.se

Main Research Areas/Applications

- **Structural Biology:** Proteins, RNA, membranes, enzyme dynamics
- **Plants & Environment:** Wood analysis, metabolism, soil chemistry, carbon cycling, persistent pollutants
- **Metabolomics:** Medical samples to plant cells: from liquid bodyfluids to solid tissue biopsies

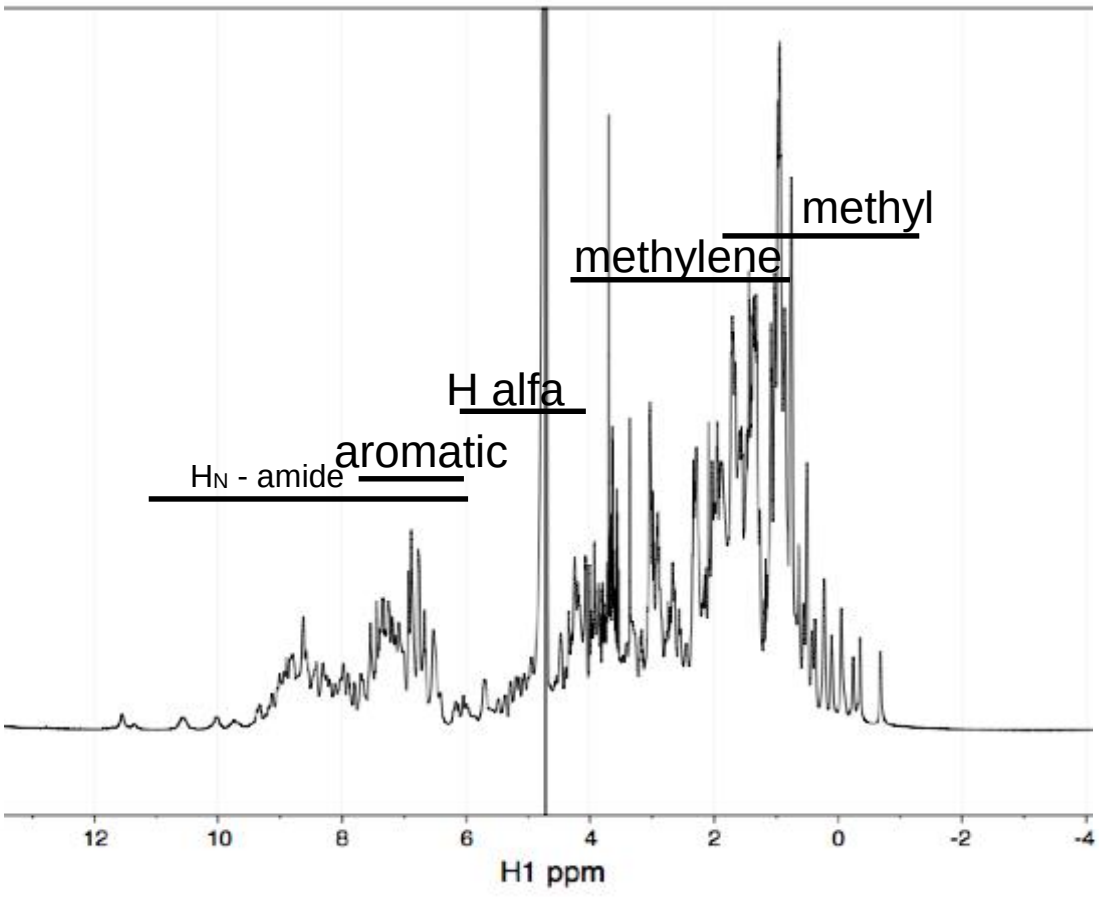
  collaboration with Swedish Metabolomics Centre (SMC)
- **Small Molecules:** Synthesis, protein-ligand interactions, fragment based screening,
- **Materials Science:** Nanoparticles, fullerenes, separation matrices

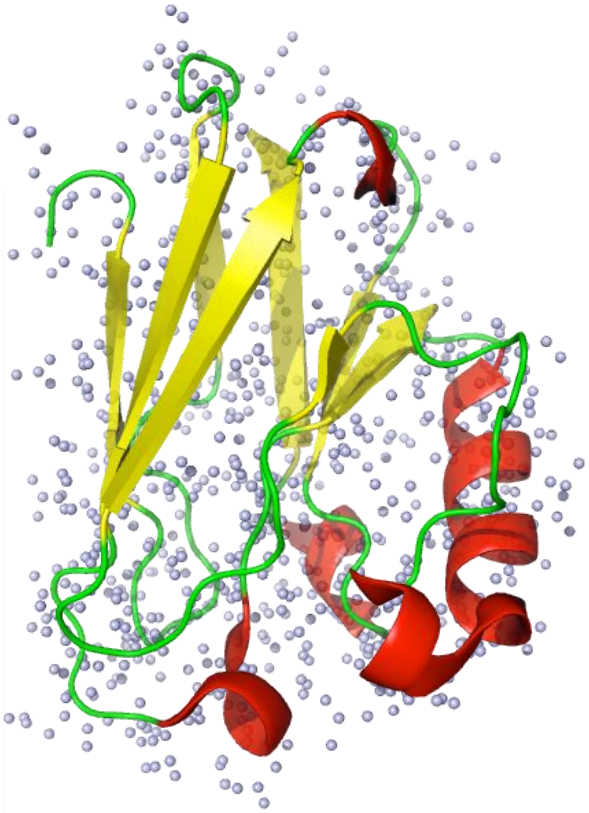
Truely interdisciplinary infrastructure



Structural Biology

labeled protein (^{1}H,^{13}C,^{15}N,^{2}H)
assign all signals
distances are measured
structure is calculated





Structural Biology

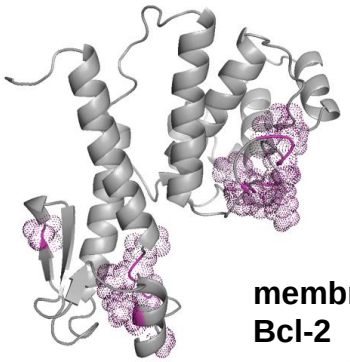
NMR does (*e.g.*)

- protein structure
- protein-protein interaction
- protein dynamics
- ligand binding
- protein folding
- reaction kinetics
- membrane proteins/lipids

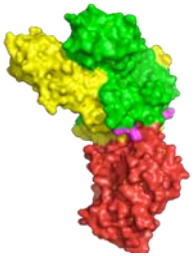
Alternative to Crystallization

- protein cannot be crystallized
- flexible tails or loops
- IDP (intrinsically disordered protein)
- amyloid fibrils and oligomers

tRNA Interaction

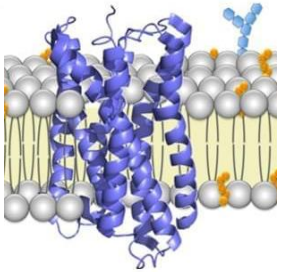


TCR+SEH

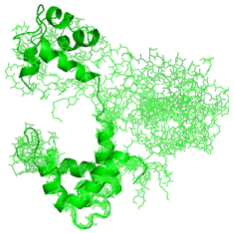


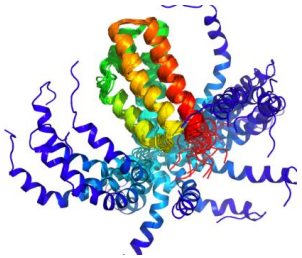
***α*-synuclein**

membrane protein Bcl-2

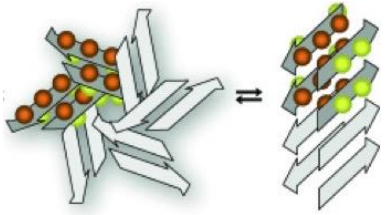


Calmodulin



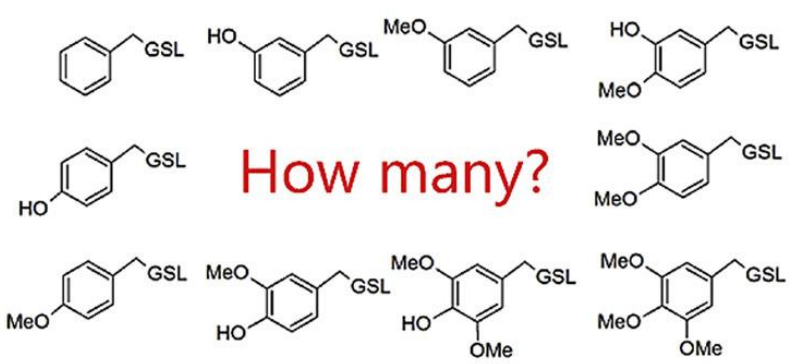


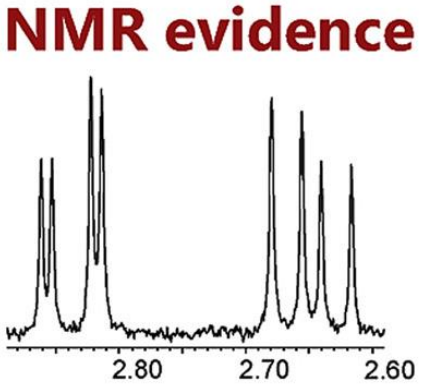
amyloids

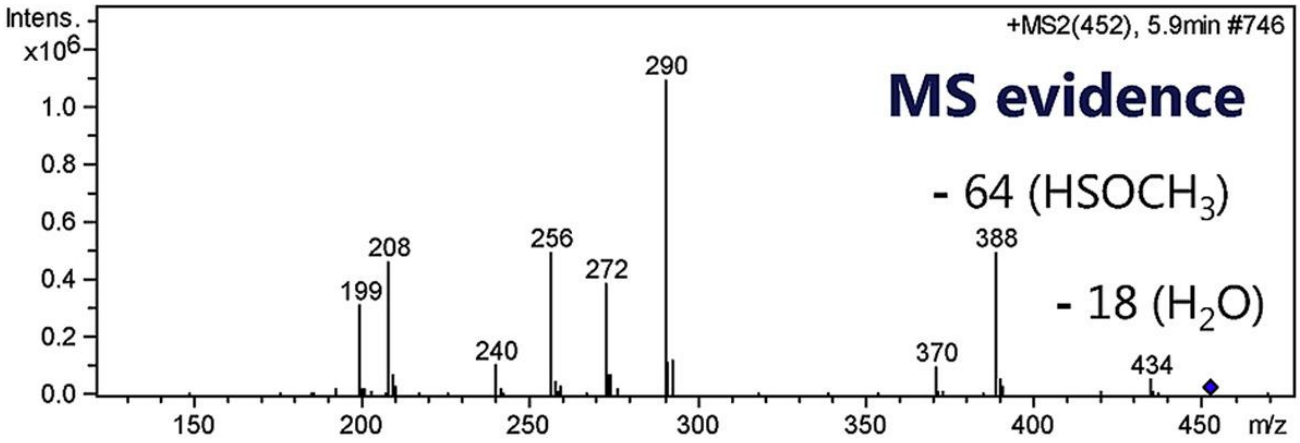


Small Molecules: Synthesis-Structure

Glucosinolates



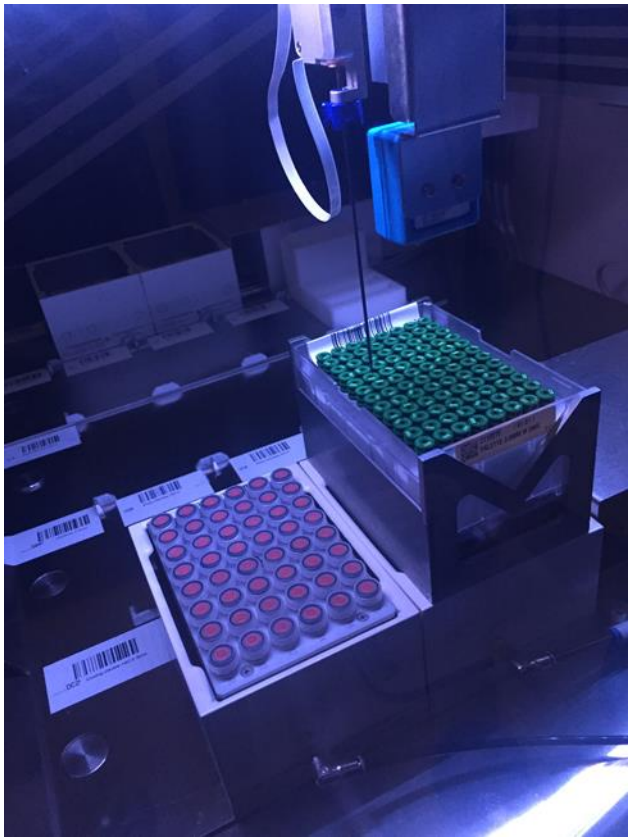




Routine NMR essential for synthesis: 400 MHz NMR dedicated for that

Chemical Biology

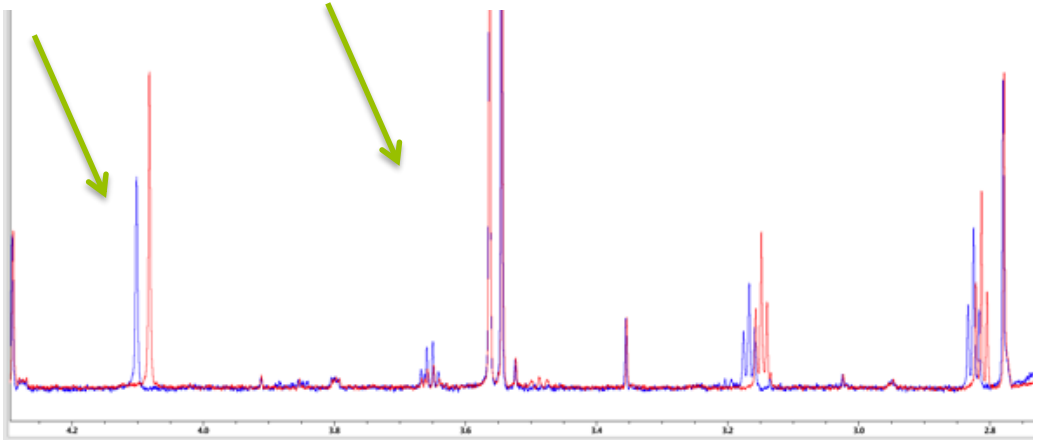
- **FBS (Fragment Based Screen)**



with LCBU/LCBKI facilities (CBCS)

Ligand detected

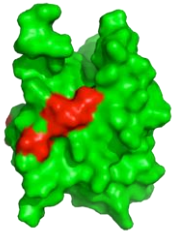
~ 2 mg unlabeled protein
- screen 1000 compounds* in 24 h



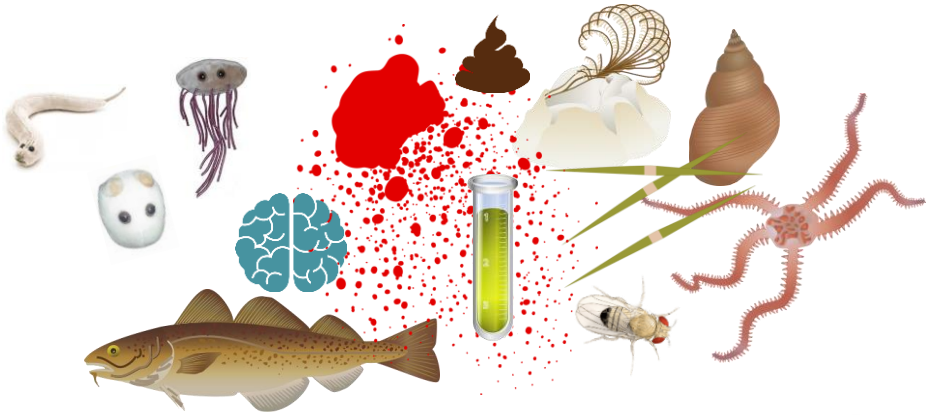
- change in intensity
- change in chemical shift

Protein detected

~ ^{15}N labelled protein ca. 1 mg
- 1 compound at the time



Metabolomics



Biofluids

– urine, serum, plasma, CSF, saliva…
– cell/tissue extracts

Biosolids

- *biopsies/tissue*
- *cell paste*
- *amyloids*
- *plant specimen*
- *environmental samples*
- *archeological samples*

NMR approach

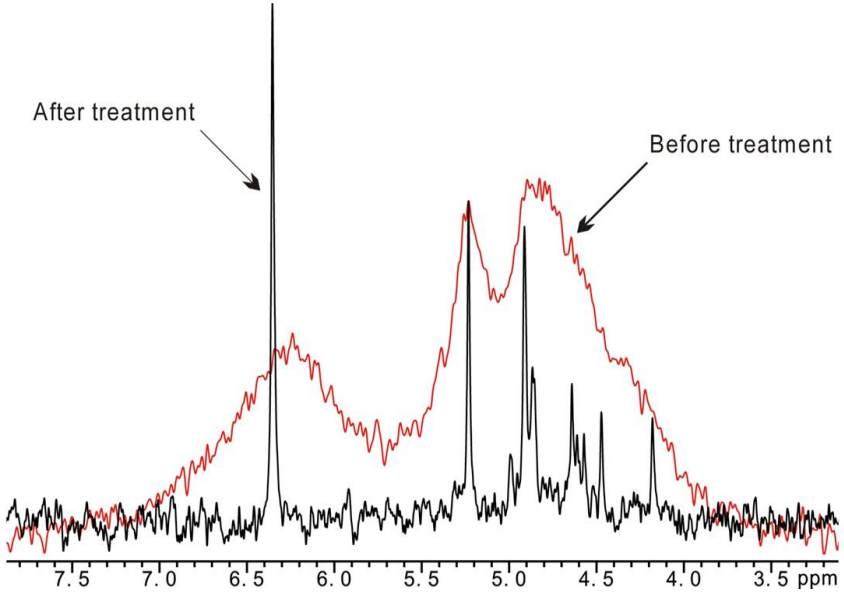
+quantification
+ identification of metabolites/biomarkers
+fast allows large sample series
+**non-destructive:** subsequent use of other methods

SciLifeLab: **Swedish Metabolomics Centre**

Environment/Ecology/Archeology

Identification of organic P-compounds in soils

Johan Vestergren EMG/SLU/Medchem

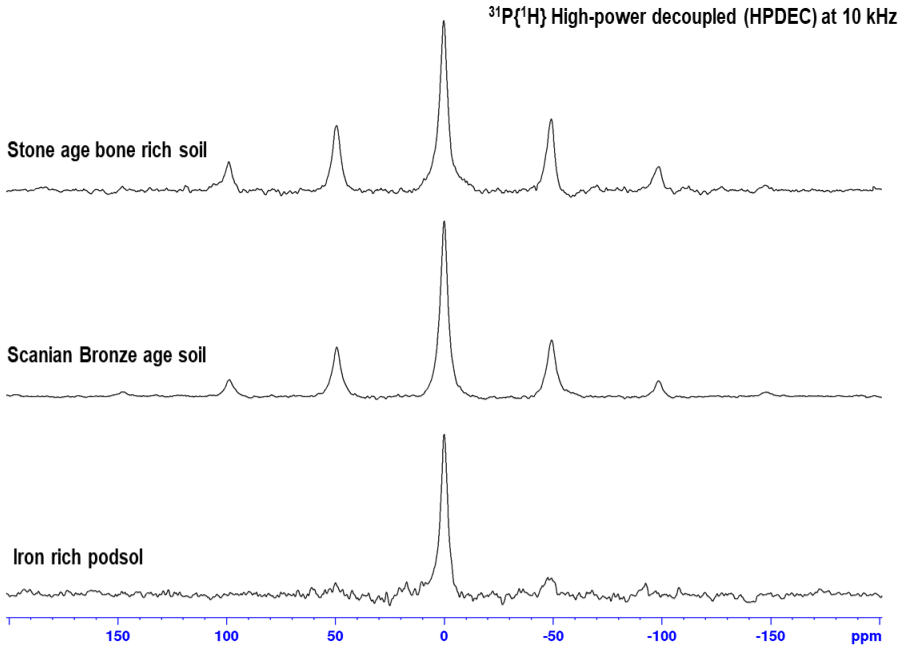


NMR: - identification of P-species
- quantification
- probing soil gradients

1D ^{31}P MAS NMR of intact Scanian Bronze age soil. Scandinavian settlements

Johan Linderholm (Miljöarkeologisks Lab. UMU)

Pool of P-species



Human Activities: - Moose for meat, leather, fur....

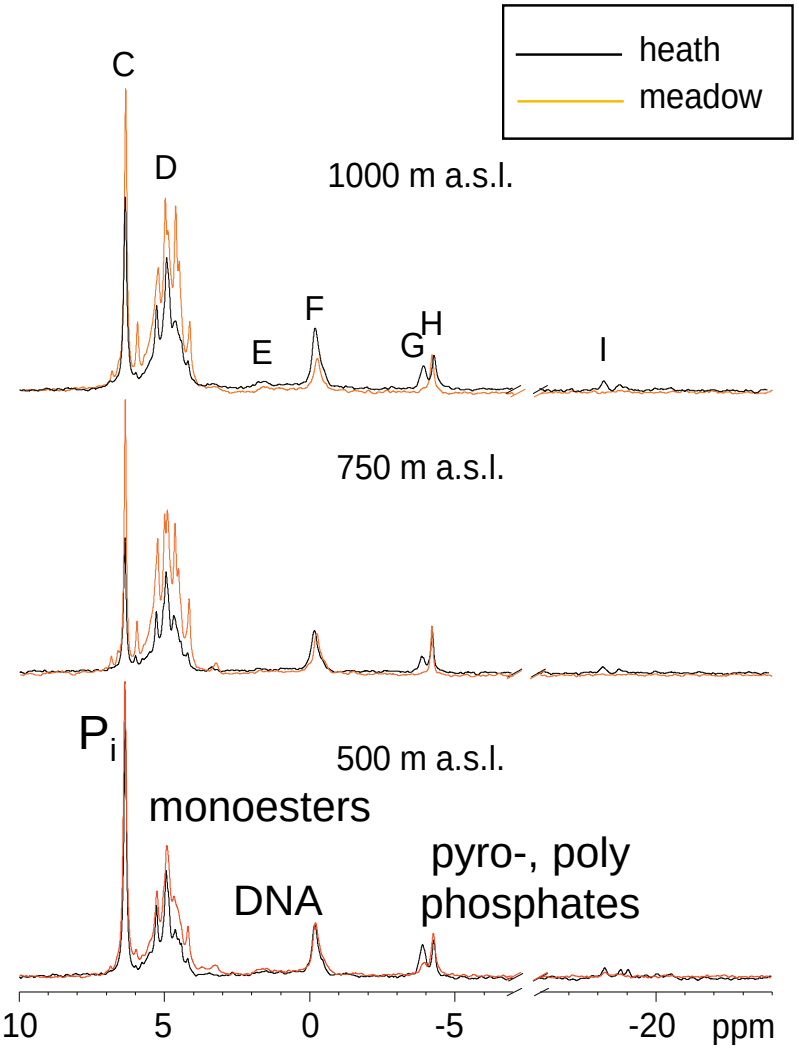
”High-Resolution Characterization of Organic Phosphorus in Soil Extracts Using 2D ^{1}H-^{31}P NMR Spectroscopy” **Vestergren J.**, Vincent AG., Jansson M., Persson P., Ilstedt U., Gröbner G., Giesler R., Schleucher J., *Environmental Science&Technology*, 2012

^{31}P NMR reveals tundra P biogeochemistry

- P composition depends on vegetation type and elevation.
- Differences in metals in soils related to differences in P.
- Climate change affects the tree line,
  this couples P speciation to C sink.

heath

meadow

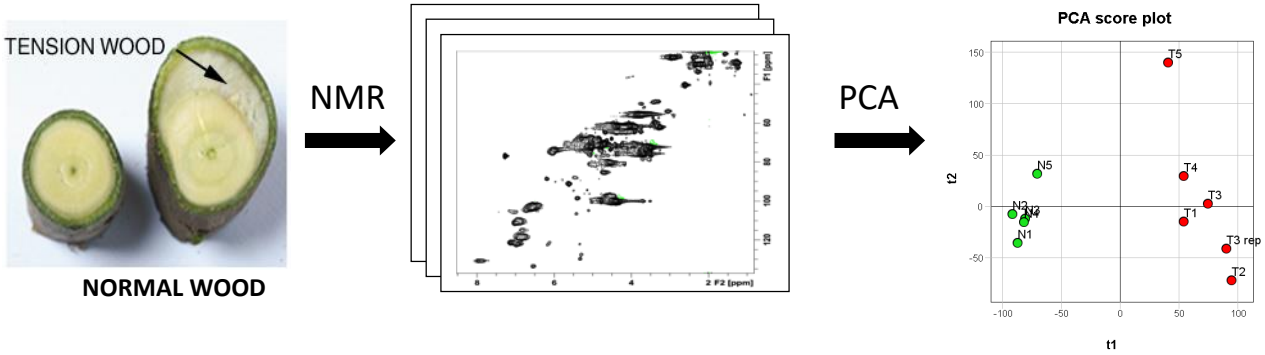


UMEÅ UNIVERSI

R. Giesler, EMG, UmU

Plant studies

1. Funcfiber – Investigation of cell wall composition in genetically modified poplar
   by 2D NMR spectroscopy and chemometrics



Hedenström, Niitylä, Mikkola

Various ongoing NMR projects with UPSC

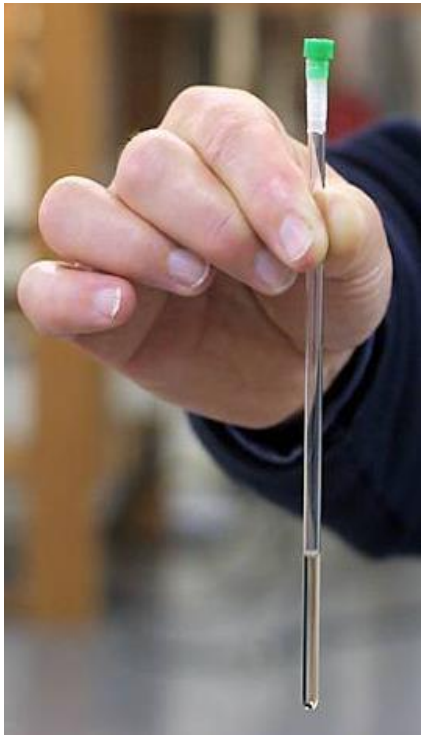
- 38 PI + group members
- 21 UMU PI + 4 SLU/Umeå
- SLU/Uppsala, Chalmers, KI, LIU, LTU, GU,UU, FOI, SU, Industry
- **Many more projects (often analysis)**

Activities of NMR infrastructure

- Local: UMU/SLU Campus
- National
- International (various projects/collaborations)
- 30+ publications 2019 from scientific contributions, plus pure instrument use
- Synergies and importance for UmU:
  Top equipment
  collaborations

Instrumentation

- 850, 600, 500, 400 MHz instruments with complementary capabilities, coordinated with GU hub.
- Sample changers on all machines for liquids and solids: standard and advanced experiments under full automation
- Robotic sample preparation,
  thousands of samples possible



850 MHz NMR Spectometer

- liquids structural biology, metabolism, protein function, biomolecular solids, tissue metabolomics
- National users distributed between GU / UmU

- Developments:
  probe for high solids resolution (ultrafast MAS), unique in Scandinavia

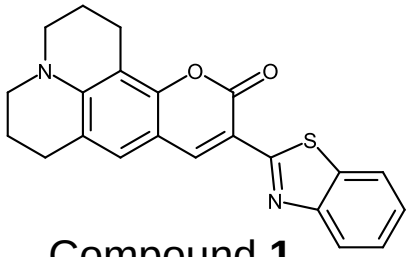
Cabinet with electronics

Helium-cooled superconducting magnet

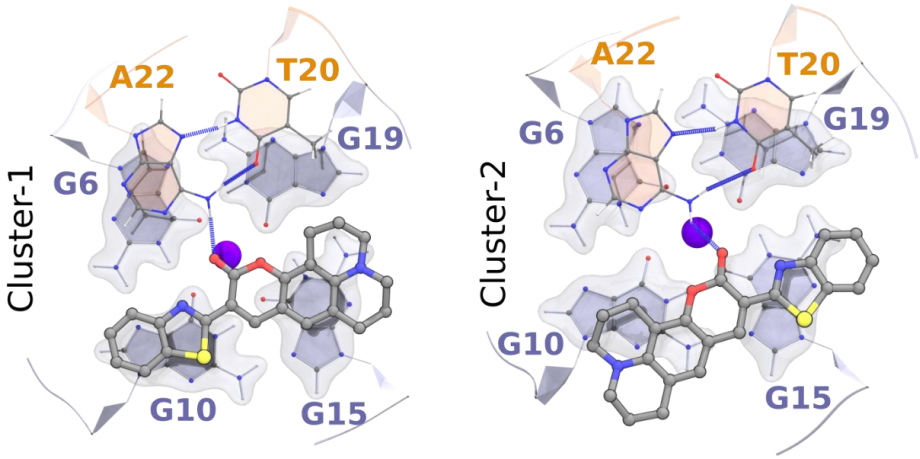
continuous maintenance and operation essential

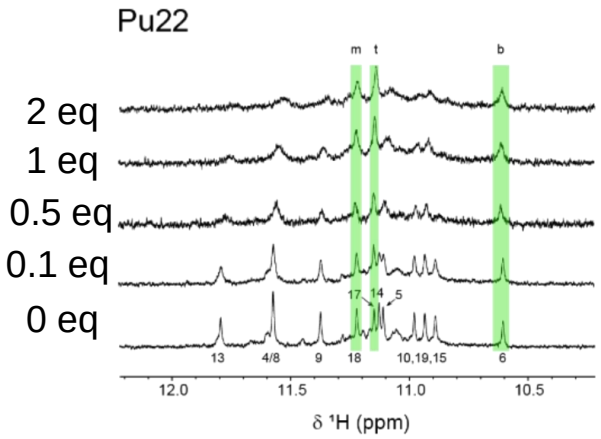
Binding Studies using NMR Titration

- G4 quadruplex DNA structures are conserved / gene regulation
- Imino protons in the tetrads serve as probes for ligand interaction
- Chemical shifts or widths of imino signals can respond to binding of coumarin-benzothiazoles ligands
- Titration of compound 1 showed that it interacts mainly with the top tetrad of the G4 structure
- The NMR results were used to calculate putative binding modes of compound **1**



Compound **1**





Sabouri / Chorell

Angewandte 2020

JACS 2020

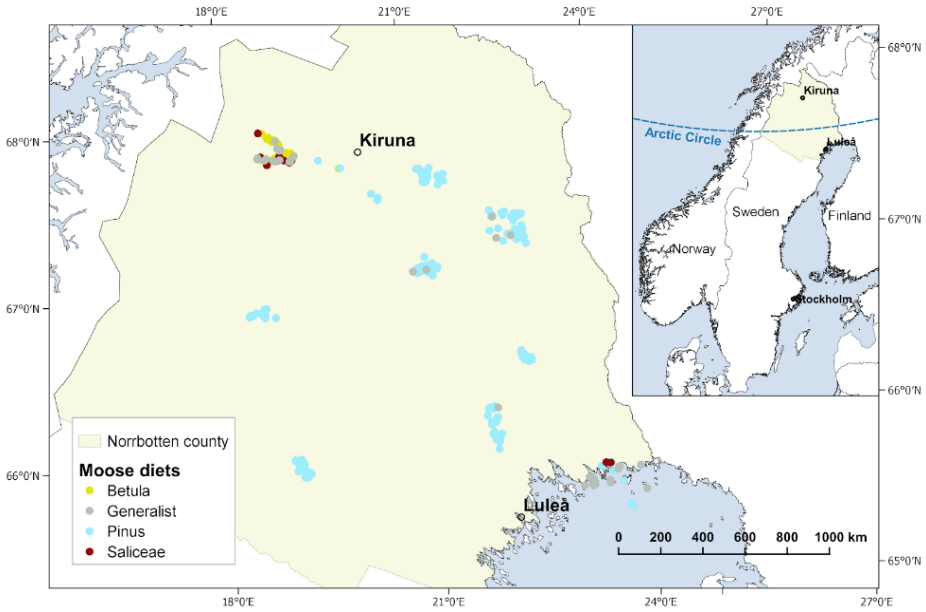
600 MHz NMR Spectometer

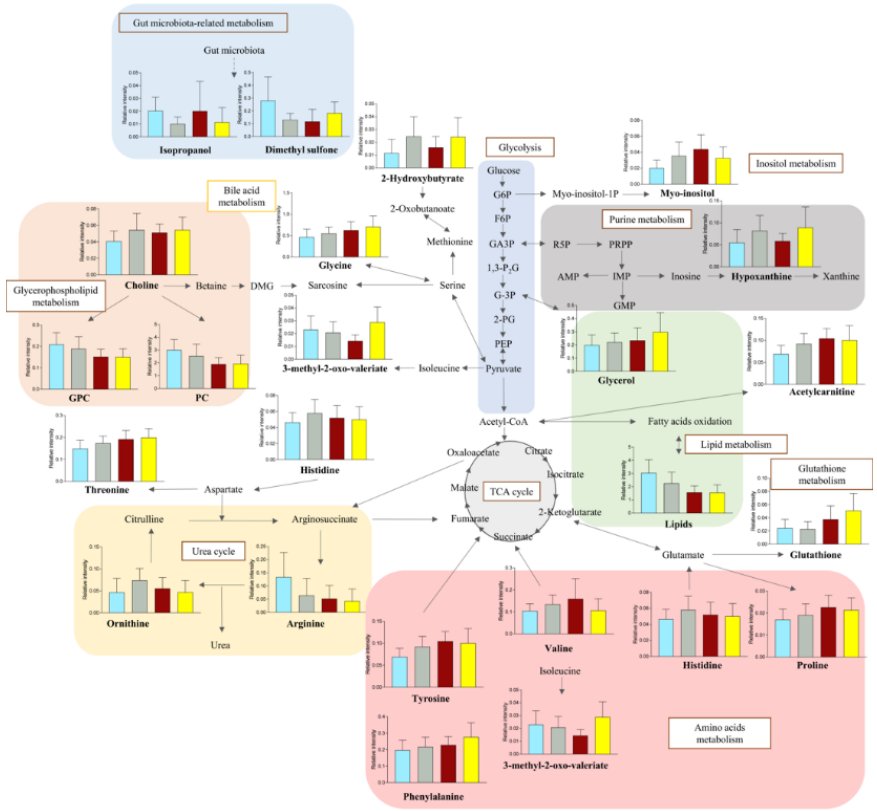
- Liquids NMR:
  metabolomics, screening, structural biology
- 500 sample capability with cooling
- 850, 600 equipped with latest probes – 5* better sensitivity but annual service needed, 300 kkr

Moose Diet, Metabolism and Movement

Christian Fohringer, Robert Spitzer, Göran Ericsson, Navinder J. Singh
Department of Wildlife, Fish, and Environmental Studies, SLU Umeå

- **Aim: Social and environmental pressures on moose populations**
- **Blood samples analysed by ^{1}H NMR metabolomics**
- **Inter-individual differences/similiarities between animals**
  - **movement patterns (resident, migratory, nomadic), diets, metabolic profiles**





Spin-off project:
Muskox comparison Zoo/Greenland

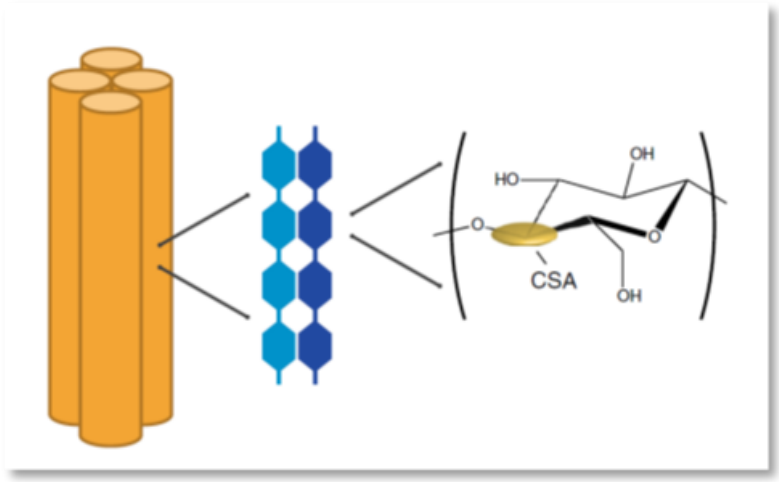
Ilona Dudka, NMRforLife

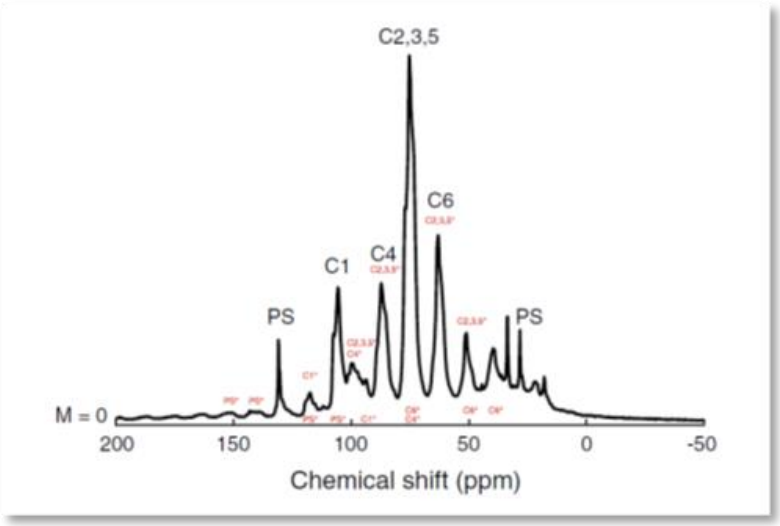
500 MHz Solid-State NMR Spectometer

- Workhorse for solids samples:
  fibres, materials science, tissue metabolomics
- Sample changer for solid samples,
  unique in Sweden
- Magnet operational since 1988,
  electronics upgraded 3*

Regenerated Cellulose: Molecular Orientation

- Molecular orientation distribution determined in a ^{13}C natural abundance Lyocell regenerated cellulose fiber bundle
- Program developed to analyze shape of NMR signals and disorder in cellulose
- Allows determination of degree of crystallinity, important parameter that differs between cotton / processed cellulose.





Svenningsson L, **Sparrman T**, Bialik E, Bernin D, Nordstierna L
Cellulose, 2019, 26, 4681

Novel Technology for Sweden

- UF-MAS NMR

  -**state-of-the-art** in structural biology, small molecules, biopsies

  -**complement** to CryoEM, X-ray & electron diffraction

  -spin at >110 kHZ, 0.7 mm rotor, sample amount ca 500 µg

  -**independent** of size, **dynamics** on molecular level

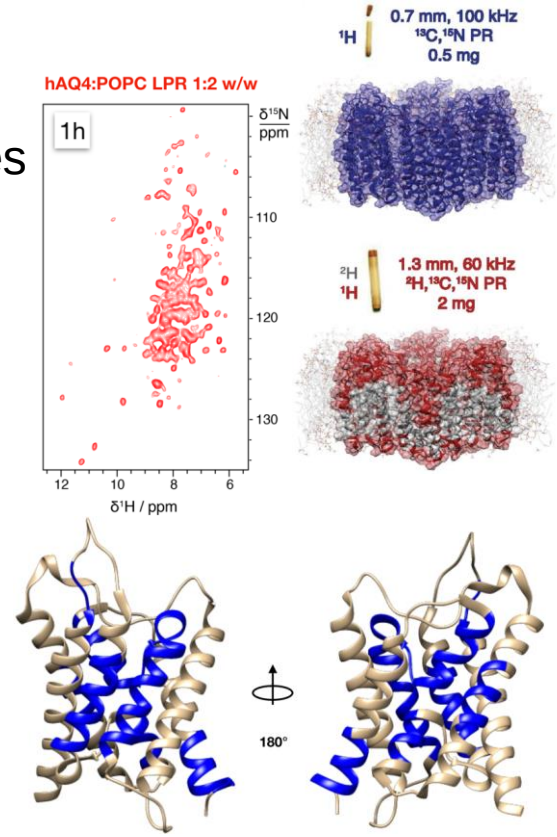
  -large proteins & proteins complexes

  -especially membrane protein in lipid bilayer

  -expertise for handling

  -installation in Q4, 2020, Swedish NMR Centre @ Umeå

  -investment, (ca 3 MSEK)

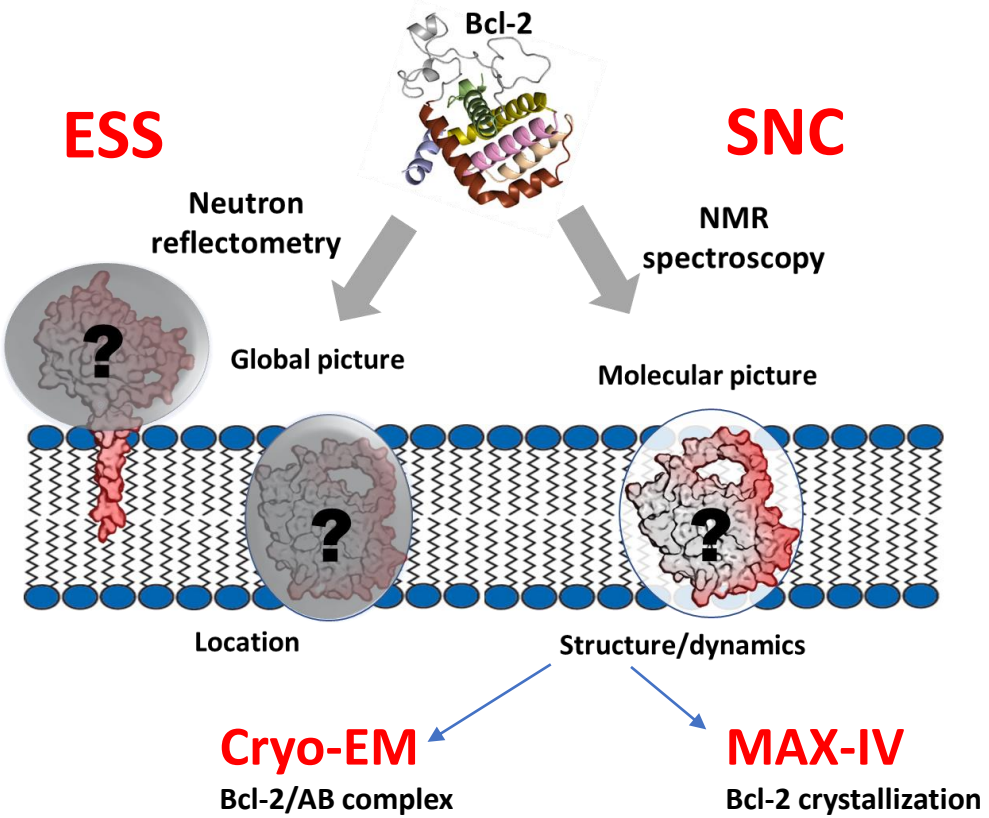


hAQP4 @ 1 GHz, collab. with G. Pintacuda, Lyon

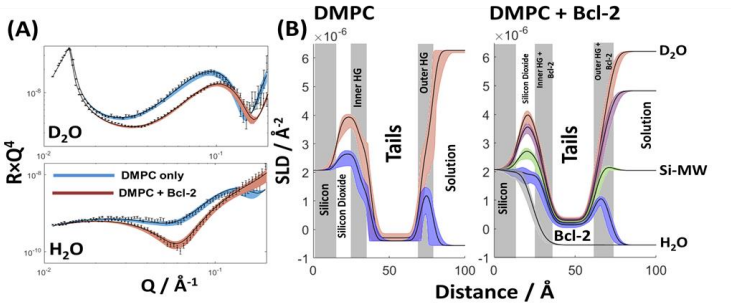
Study dynamics of water transporting pore

Combination of Methods: NMR/Neutrons/Cryo-EM/x-Ray

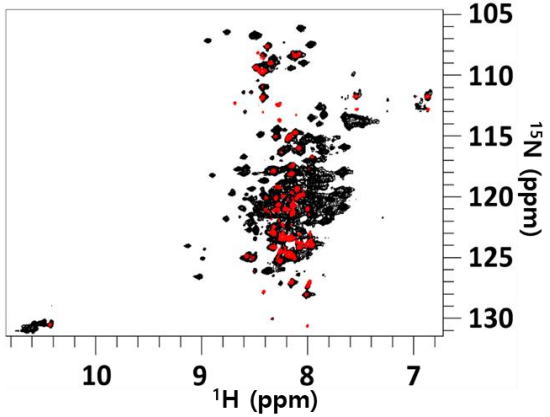
Bcl-2 membrane protein: Key player in apoptosis and cancer



ESS/ISIS: Location in the membrane

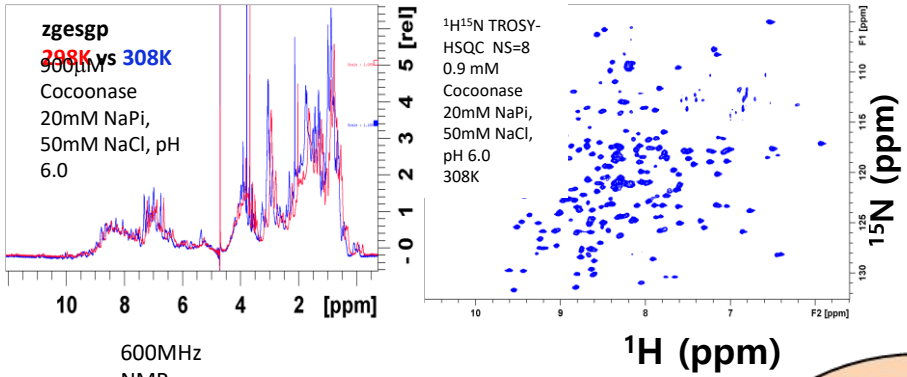


TROSY NMR: flexible IDP loop of Bcl-2



Cocoonase, a serine protease in biotechnology!

NMR data from Chanrith Phoeurk (Ph.D. Wolf-Watz)



600MHz
NMR
field

Sericulture (Silk Production)

Enzyme Technology

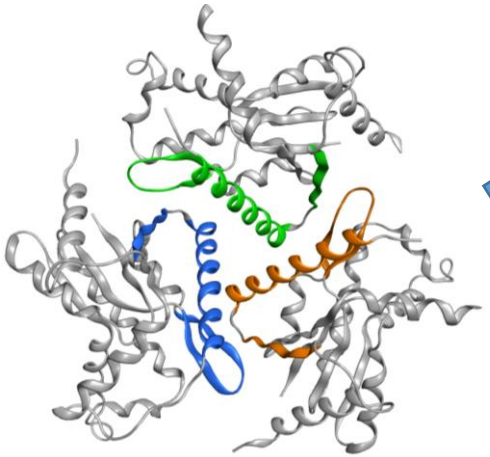
Application

- Cosmetic industry → **Silk-based cosmetic products**
- Textile industry → **Luxurious silk fibers/fabrics**
- Food industry → **Food supplements**
- Biomedical industry → **Biomaterials, pharmaceutical materials**

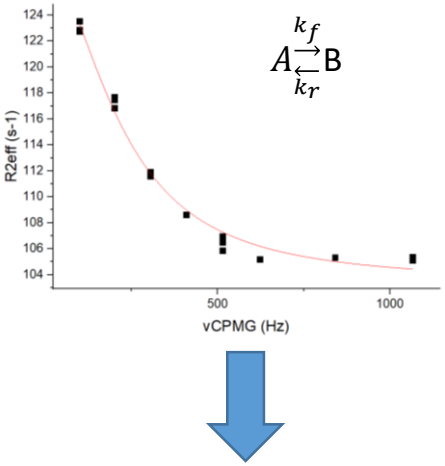
Sida
SWEDISH INTERNATIONAL
DEVELOPMENT COOPERATION AGENCY

Integration of X-ray & NMR in Enzymology

Trimeric enzyme structure solved by Elisabeth Sauer-Eriksson



Quantitative ^{19}F NMR
(good for large proteins here 70 kDa)



Shows that conformational dynamics is rate-limiting for catalysis: k_r = ***k*cat**
(data from **Apoorv Verma**)

Vetenskapsrådet

NMR main area:

Protein structures/dynamics

e.g. disordered proteins
enzyme activities
inhibitors/drugs etc.

NIH National Institutes of Health
Turning Discovery Into Health

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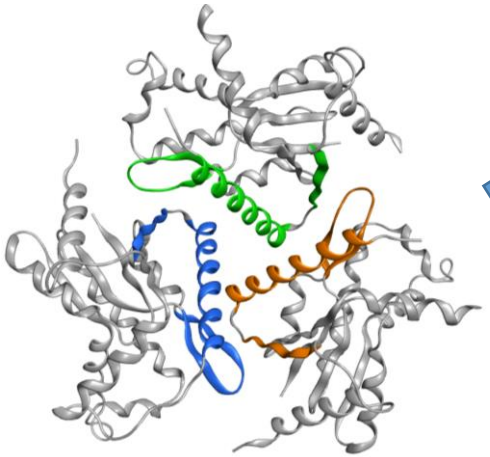

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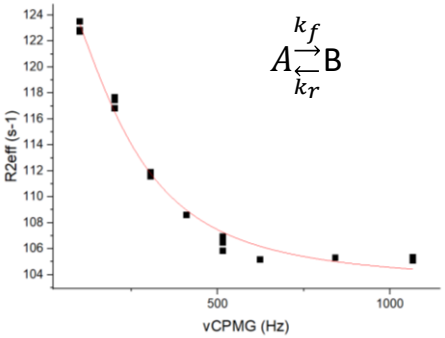

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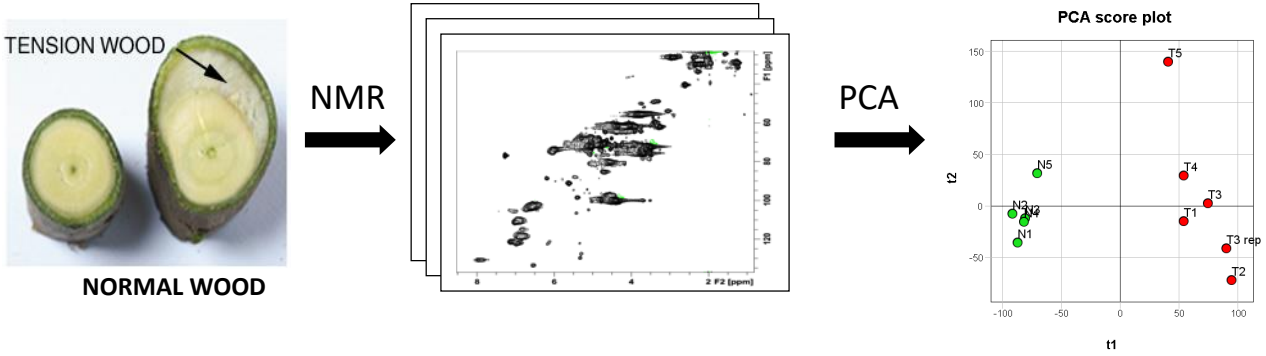
Plant studies

1. Funcfiber – Investigation of cell wall composition in genetically modified poplar
   by 2D NMR spectroscopy and chemometrics



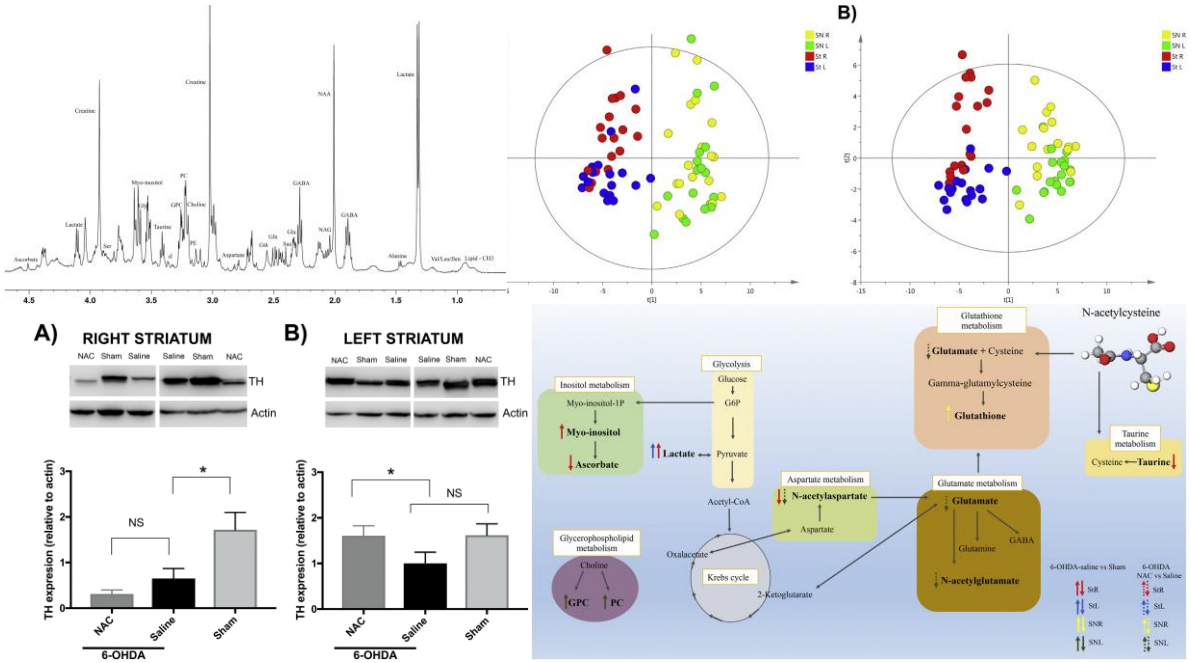
Hedenström, Niitylä, Mikkola

^{1}H NMR profiling of the 6-OHDA parkinsonian rat brain reveals metabolic alterations and signs of recovery after N-acetylcysteine treatment

Ana Virel, Ilona Dudka, Rutger Laterveer, Sara af Bjerkén

Department of Integrative Medical Biology, Umeå University, Umeå, Sweden

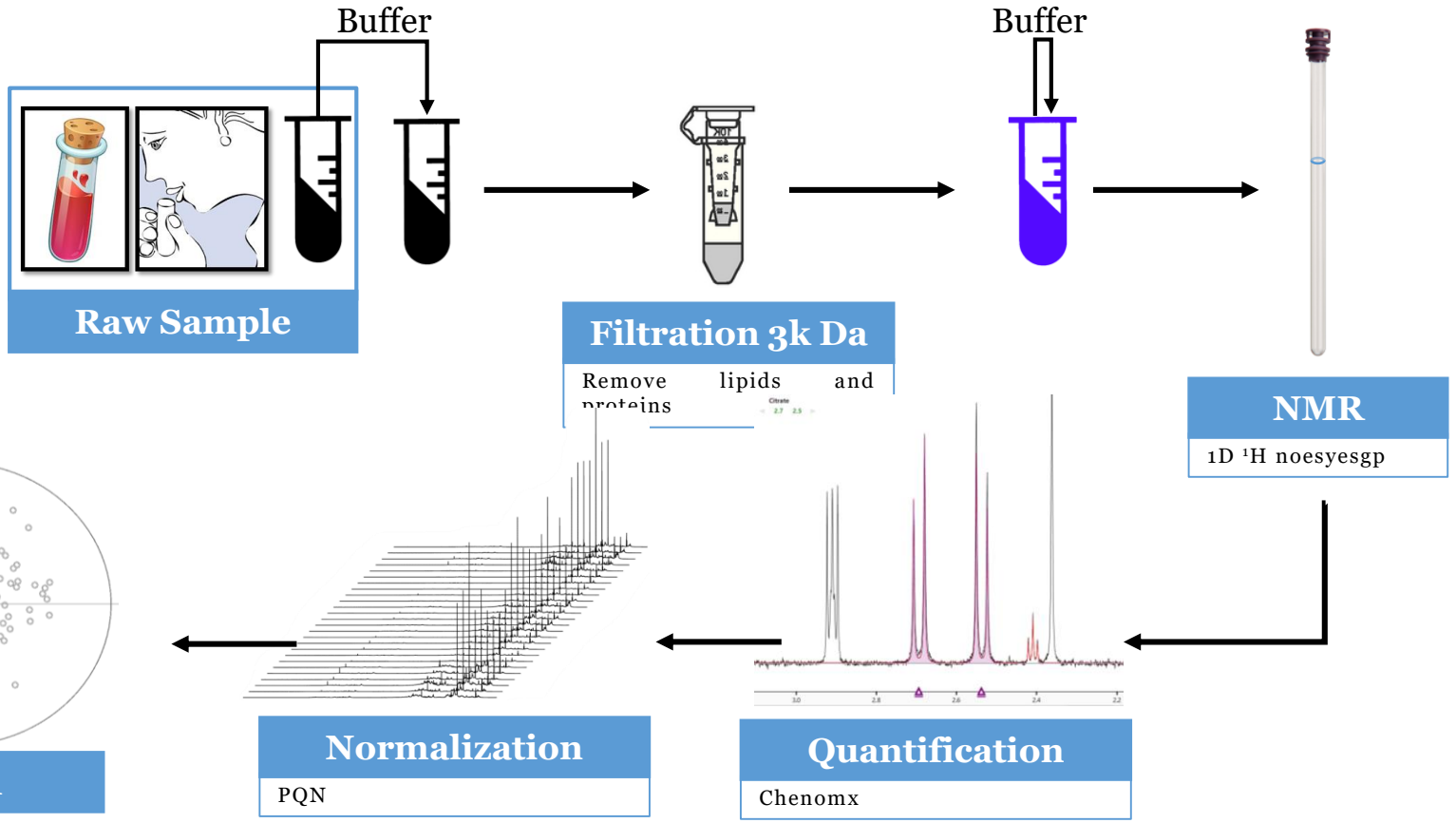
- The metabolic disturbances in the 6-hydroxydopamine (6-OHDA) hemiparkinsonian rat model were monitored and the nature and size of these metabolic alterations were analyzed.
- The results indicate that a unilateral injection of 6-OHDA into the striatum causes metabolic changes that not only affect the injected hemisphere but also the contralateral, non-lesioned side.
- We could clearly identify specific metabolic pathways that were affected, which were mostly related with oxidative stress and neurotransmission. In addition, a partial metabolic recovery by carrying out an antioxidant treatment with N-acetylcysteine (NAC) was observable.



Molecular and Cellular Neuroscience 98 (2019) 131–139

Joao Figueira/Anders Öhman:

Searching for dementia markers: Serum and Saliva NMR metabolomics



NMR - the people

- Instrument time
- Sample preparation and measurements
- Data analysis
- Scientific collaboration

Gerhard Gröbner

Jürgen Schleucher

Tobias Sparrman

Joao Figueira

Further information and applications

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Ilona Dudka

Mattias Hedenström