Possible advantages of coordinating NMR and MS-based metabolomics at Lund University

Investigation by Anders Malmendal on behalf of MoReLife

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Summary

Purpose of report

Metabolomics is the measurement of a large number of metabolite concentrations in body fluids and other biological samples, and is a strong tool in medical diagnostics and prognostics. There is no common infrastructure for metabolomics at Lund University (LU). This report aims to assess the current status for metabolomics at LU and make suggestions on how to develop an infrastructure largely based on existing resources that can accommodate the current and future needs for metabolomics analysis for researchers throughout LU.

Background

State of the art metabolomics has been performed at LU for many years. These studies are performed using mass spectrometry (MS). The complementary nuclear magnetic resonance (NMR) technique for metabolomics is not available at LU, but there is equipment available that can be used for this purpose.

Sources

Researchers at LU ranging from metabolomic experts, through users to representatives of fields where the technique can be useful were interviewed along with personnel from national facilities performing metabolomics. In order to get a feeling for the general interest in and usage of metabolomics at LU, we sent out a survey to all staff at the Faculties of Medicine and Science and at LTH.

Suggested action

Many of the interviewees expressed a strong interest in having a local expert who could advise them on how metabolomics could be useful for their project, and a local facility where the analysis can be performed and where they can get help with interpreting the results.

The best way to accommodate this and to make metabolomics available for researchers throughout LU is to form a common infrastructure (core facility) for metabolomics. This structure should contain MS- and NMR-based metabolomics and the instruments and personnel should preferably be located close to each other formally and physically. Around this structure one should have a core group where frequent users and technology experts meet regularly, a seminar series with invited and local speakers, etc.

The current and already funded equipment can form the base for a common metabolomics infrastructure. What is needed to start with, is a replacement of the old MS instrument (UHPLC-QTOF) at CRC and acquisition of the missing accessory (a cooled autosampler) on one of the NMR-spectrometers.

The extra personnel needed is one NMR metabolomic expert along with at least one technician. For flexibility, the scope of a new position in NMR could be broadened to quantitative NMR to also include closely related non-metabolite applications.

Introduction: Metabolomics by mass spectrometry and NMR

Metabolomics is a powerful tool to quantify the metabolome, i.e. all (or as many as possible) of the metabolites, found in cells, biofluids, and tissues. This gives an overview of the biochemical perturbations, and represents a shift in paradigm from hypothesis-driven studies where only one or a few compounds are measured. In contrast to the genome and proteome the metabolome is strongly affected by environmental factors such as microbiota and diet and is thus a very sensitive measure of the current state of the organism.

Based on the entire metabolite profile or a lower number of selected metabolites, disease progression or response to other types of disturbances can be monitored, and markers for early diagnosis and prognosis, and for personalized therapy selection can be identified.

The metabolite concentrations are usually measured by either mass spectrometry (MS) or nuclear magnetic resonance spectroscopy (NMR).

Mass spectrometry metabolomics. The metabolome can be looked at as an iceberg, with some metabolites at moderate to high concentrations, but the majority at extremely low levels. Thus, the detection limit of the metabolomics technique dictates how much of this iceberg that is above or below the surface. MS reaches far down (pM) into these depths of the metabolome. Furthermore, it only requires a few μL for the analysis.

Unfortunately, MS measurements have a rather low reproducibility. Hence, significant batch variations occur even when measuring on the same instrument, and direct comparison of data generated at different MS platforms generally become unfeasible. This notwithstanding, quantitative methods can be developed that cover 100 or more metabolites, but these are exceedingly expensive owing to the need for stable isotope labeled internal standards.

Instead, mass spectrometry realizes a very appropriate tool for discovery studies. Untargeted metabolomics methods may determine thousands of unique molecular features. A high-resolution mass spectrometer can determine the mass with a precision allowing for estimation of the molecular formula. In addition to this, the mass spectrometer can also fragment the analyte and measure the mass of the fragments, yielding very detailed information on unknown molecules.

NMR metabolomics is much less sensitive than MS, μM rather than pM and 100 μL or more of sample is needed. But it is cheaper, faster and much more reproducible. A standard NMR system for metabolite profiling with cooled autosampler can run 30,000 samples a year. Furthermore, as the sample is never in contact with the equipment there is no risk of contamination.

Therefore, NMR is good for screening of samples and for characterizing large cohorts. In contrast to MS, NMR can also measure lipoprotein and fatty acid classes, which may be an important feature in medical diagnostics and prognostics.

Certain standard NMR systems for metabolite profiling can be calibrated to perform identically so that spectra are independent on when and where they were acquired. A spectrum obtained in Lund will be virtually identical to spectra acquired on similar systems throughout the world. This allows the direct NMR output to be used as a measure of the metabolite profile and to be compared with cohorts analyzed at another site. Hence, results obtained from studies performed all around the world can be compared to confirm important clinical results. Furthermore, this allows for samples from longitudinal studies to be measured as they are collected and data evaluated on the fly, rather than having to wait until all samples are collected to avoid batch effects. As with MS, more advanced methods can be used to identify unknown molecular structures.

If spectra are run under these standard conditions, absolute concentrations can be measured for the most common blood and urine metabolites but also for fatty acid and lipoprotein subclasses in blood.

It is however important to note that in many cases the raw NMR spectrum (the metabolic fingerprint) rather than the derived metabolite concentrations will be the best basis for making predictions in diagnosis or prognosis, since some of the spectral information is lost when metabolite concentrations are calculated from the NMR data.

Complementarity between methods. The two methods are thus highly complementary and the choice between MS and NMR (or both) may be that between a detailed study of a biochemical mechanism and a low-cost overview of the high concentration metabolic profile with high throughput and high reproducibility.

There are several well established MS metabolomics research groups at CAS and CRC, while NMR metabolomics is unavailable at LU. However, a few groups use NMR for studying metabolism *in vitro* and *in vivo* using related methods.

Recently a number of phenome centers focused on high throughput metabolite profiling has been established internationally with more than a dozen multi-institutional hubs including the MRC-NIHR National Phenome Centre at Imperial College London and institutions in Australia, Canada, China, Japan, Singapore, Taiwan and USA. In Sweden, there are two national facilities that do metabolomics: the Swedish Metabolomics Center (SMC) in Umeå performs MS-metabolomic analyses and the Swedish NMR Center (SNC) in Göteborg performs NMR-metabolomic analyses.

Results

Is there any interest for metabolomics at LU?

Excellent metabolomics studies have been performed at LU for many years, e.g. in the groups led by Hindrik Mulder, Olle Melander, Åke Lernmark, and Peter Spégel. The samples used in these studies range from 100 β-cell samples to a large epidemiological cohort of 10000 plasma samples. The applications are mostly within diabetes and cardiovascular disease. A list of examples of projects is shown in appendix 2.

For the purpose of this investigation researchers at LU ranging from metabolomic experts, through users to representatives of fields where the technique can be useful were interviewed along with personnel from SMC and SNC (Appendix 3).

In order to get a feeling for the general interest in and usage of metabolomics at LU outside the above groups, we sent out a survey to all staff at the Faculties of Medicine and Science and LTH (appendix 4). We got 28 replies: 15 from the Faculty of Medicine, 3 from LTH, only 2 from the Faculty of Science and 8 of unknown origin. We got only one reply from Department of Biology. Despite the many applications within biological sciences there does not seem to be many researchers currently working with this at LU.

15 of the 28 have already used metabolomics: 4 each at CRC and CAS, and 3 at SMC, and the rest elsewhere. The most common topics were cancer and diabetes, but many medical fields were included and also topics like water purification. Most are interested in analyzing serum, plasma and cells, but there is also an interest in feces, urine and tissue. Likewise, most are interested in analyzing samples from human subjects, rodents and cell lines, but more exotic organisms like birds are also on the list.

There seem to be most interest for untargeted standard methods but also for special applications such as low cell numbers and metabolic flux studies.

Many expressed a strong interest in having a local expert who can advise them on what they can get out of metabolomics in their particular project, what samples to use, methods and so on. This is also a sentiment that I have met when interviewing non-experts.

Another factor that has also been mentioned is the ability to be independent of collaborators, not because of bad collaborations but in order to increase the flexibility. And that in contrast to metabolomics, there are infrastructures for genomics and proteomics at LU. Making metabolomics available would be a natural next step giving LU researchers access to all three main omics-methodologies

Current equipment at LU

Mass spectrometry. There is currently a large number of MS analyses made in the Mulder/Melander (5000 samples per year) and Spégel (~10 projects, involving >1500 samples per year) labs. It is essential for these groups to keep this operation at LU and it would be advantageous to make it available to other groups on the same condition. At the Clinical Research Center (CRC) in Malmö there is currently a QTOF. There is a service contract for this instrument that ends by the end of this year. Funding for a replacement with a newer QTOF with a 5 year service contract has been applied for within the infrastructure call at LU. At Centre for Analysis and Synthesis (CAS), Department of Chemistry there are a triple quadrupole (QQQ), an orbitrap, a QTOF (see appendix 1) that are largely available for external users. There is a service contract for the QQQ. The remaining instruments are maintained by the local technician. An application for a new Orbitrap tribrid which is a more flexible machine has also been submitted.

NMR spectroscopy. There is presently no NMR metabolomics performed at LU but there are NMR spectrometers. At the Biomedical Centre (BMC) in Lund there is a 500 MHz Varian/Agilent spectrometer. The instrument is currently being transferred to the Department of Clinical Sciences to be used for hyper polarization NMR (see below). However, up to 75% of its time may be available for metabolomic applications. Funding for the latter purpose has been applied for within the infrastructure call at the Faculty of Medical. Last year Lund University granted funds for a brand new 600 MHz Bruker spectrometer at the Department of Chemistry in Lund. A service contract has not been funded. About 50% of the time will be available for metabolomic applications on this machine. Funding for a larger 800 MHz spectrometer is applied for elsewhere.

The details are listed in appendix 5.

Future role for current infrastructures?

The current and already funded equipment can form the basis for a common metabolomics infrastructure. However, what is needed is a replacement of the old UHPLC-QTOF MS at CRC and the acquisition of a cooled autosampler for one of the NMR-spectrometers.

A cooled autosampler is a prerequisite to be able to handle the large numbers of samples used in metabolomics in a uniform and efficient way. Thus, such an autosampler needs to be bought. A SampleJet cooled autosampler from Bruker has a list price of approximately € 110 000. The Varian/Agilent brand of spectrometers is no longer available and there were never any cooled autosampler available for this brand. This means that if the 500 MHz spectrometer at BMC should be primarily used for NMR-metabolomics, it needs to be complemented with Bruker electronics in order to make it work with a Bruker autosampler. It is not obvious how much this will cost, but it may well be that it by far exceeds the cost of the autosampler. However, if this conversion can be negotiated for in the same deal as the new 600 and potential 800 MHz the price may be more limited.

There are already strong competences in MS metabolomics at CRC and CAS, and there are also strong NMR groups in the vicinity to CAS. The only competence that is needed is that in NMR metabolomics. Around these competences an organization that helps researchers at LU to get the best

suited technique or combination of techniques to solve their particular metabolomics question is needed.

How should the infrastructure be organized? What infrastructure is needed?

A common wish from many of the interviewees is a common metabolomic platform with MS and NMR, where people could come with their problem/question/ system/budget and get advice on what technique to use and on what samples and so forth. There must be efficient communication both between the people that have the knowhow of the technique and those that come with the samples and questions, and between the people that have different types of knowhow of the techniques.

The best way to organize an efficient management of the common platform is to have academic personnel that have their own research groups on the side but have allocated time at the infrastructure and technical personnel that are dedicated to the infrastructure. Preferably, they should all belong to the same department and physical unit. It would also be a good idea to train the technical personnel so that they know both how to run MS and NMR.

Should the equipment be located where the technical knowledge is or where the knowledge about the biological systems is? Keeping all equipment together is good for technical support and maintenance, but one should not move existing installations and/or break up good working environments. With technical experts the equipment is in safe hands, but they need not be "owned" by the technical experts. Keeping most of the equipment within a limited distance would be good for communication within the platform and for maintenance. This can be complemented with regular office hours close to where the users are in order to make user communication easier. Forming a core group based on frequent users and technical experts should hopefully provide a solid scaffold for regular meetings, talks etc.

Interested researchers need to be thoroughly instructed in how the samples should be collected, treated and so forth at an as early state as possible. And it should be made clear to them what type of outcome they should expect from the analysis. Fees should preferably cover running costs, wages and costs for service contracts. Without a service contract there may be a risk that the continuity in the platform suffers. There should be a green card system for people who wants to rent the equipment to run the experiments and analyze the data themselves. Service should usually be provided without coauthorship, but collaborations with coauthorship can be used when the studies are more involved, or to start up a new type of project if there is time available. There should be well-defined rules here so that that those heading the platform do not run collaborative projects just to lower the project costs

It is important that the scientists in the infrastructure have time to do their own research too, but that they then use the instruments on the same premises as the other uses. This will guarantee both that the equipment is held up-to-date and that the user fees are kept under control.

National resources and networks and other metabolomic services.

Swedish Metabolomics Center (SMC) SMC has flexible setup and provides MS-based metabolomic services at different levels. Expert users may rent instruments and run them on their own, one can get samples analyzed, and if need a new method developed. It should typically take 3 months from first communication, and costs 600 SEK/sample for 200 samples (this price is subsidized full cost is 1700-2000). Renting equipment is 2600 SEK/day.

Swedish NMR Center (SNC) The Swedish NMR Center (SNC) in Gothenburg has a standard 600 MHz NMR system for metabolite profiling with the possibility for automatic quantification of metabolites,

fatty acid and lipoprotein classes. They also have a more sensitive 800 MHz system that needs smaller sample volumes without much signal loss. There is a large capacity to run samples, but limited capacity to analyze the results. The cost at SNC is 100 SEK/sample for ordinary NMR and 150 SEK/sample with automatic quantification, for academic users (double for non-academic). 10 hours of operator/analysis time is included thereafter 700 SEK/hour.

In this context it is also relevant to mention that there are commercial services for metabolomics (Appendix 6).

What should the relation be to the national resources and networks and other similar structures

SMC in Umeå is available when extra MS capacity is needed and when specific methods available at SMC but not within the LU infrastructure are needed. SMC may also be available for larger studies.

SNC. For NMR-metabolomics there is need to have equipment to do pilot and smaller studies at LU. Larger studies can be run at SNC, but there is need for capacity to analyze the results here.

Local biobanks and Region Skåne. Metabolomics is a very adequate analysis tools for looking at metabolites in biobanked blood, urine, etc., and also as a tool for assessing the quality of the samples that are in the biobank. At SNC they have mapped the effects of sample aging and different treatments so that they to some degree can be accounted for. A "standard NMR spectrum" could also be acquired and stored with the meta data so that interested researcher can use that instead of the sample itself for further analysis. A long-term vision for NMR metabolomics is to use the "standard spectrometers" for diagnosis in the clinic.

Once an infrastructure is at the sketching table, contacts should be made to the regional biobanks and Region Skåne. Göran Karlsson from SNC is interested in making a common campaign to clarify opportunities and potential with NMR analyzes for the biobanks.

Researchers interested in metabolomics should be made aware that there may well be biobank samples that they can use, and that in some cases Region Skåne can help with providing samples for different projects.

How does the infrastructure relate to related research areas? What should be included in the infrastructure?

Supporting research areas:

Statistical analysis. Measuring many metabolites at once adds another level of intricacy to the statistical evaluation. It is thus essential that the users can be advised on how to evaluate the data statistically by univariate and multivariate methods. For some applications predictive models are also essential.

Bioinformatics. The metabolomic output is often complex and best rationalized by some kind of biochemical pathway analysis which requires expertise in the biochemistry as well.

Metabolite discovery and structure elucidation of small molecules. Whereas abundant metabolites, found on the top of the iceberg, often are of known identity, biological samples contain vast amounts of molecules at low concentrations with yet unknown identity. The same is true for some high concentration metabolites from more exotic models where the biochemistry/metabolism may be different. Therefor it is important to have MS and NMR methods to identify unknown metabolites. Collaborations with synthetic chemists may also be relevant to produce standards for unambiguous identification.

Hyperpolarization NMR (HPMR) is a novel technique to polarize molecules so that a signal can be obtained that is several orders of magnitude stronger than in conventional NMR. This gives new possibilities for obtaining metabolic fingerprints at sub-µM concentrations and opens many perspectives for studying metabolic reactions and fluxes both *in-vitro* and *in-vivo*. The HPMR technology is rapidly advancing and, as widely recognised, is able to one day become a routine tool for super-sensitive NMR metabolomics. LU has been one of the first universities, and the only in Sweden, that acquired this technology, which may provide many possibilities for synergetic projects and a unique niche for LU nationally in the field of metabolomics. Vladimir Denisov at the Department of Clinical Sciences in Lund is an expert in this field and has recently received funding in this area.

Flux analysis using isotope labelled metabolic substrates. Labelled substrates are fed into metabolism and the metabolic fate of the labels are traced by MS or NMR. HPMR can also be used to do time resolved (real-time kinetic) measurements of such processes *in vitro* or *in vivo*.

It should be stated that proteomics by MS is a totally different application and needs other equipment, software solutions, competences and personnel.

Additional research areas within quantitative NMR:

To increase the flexibility of the NMR-metabolomics platform and to accommodate more of the needs within LU it might be advisable to include additional quantitative NMR applications.

Ligand binding studies. NMR (and MS) are excellent technologies for identification and validation of interactions between small molecule ligands and various macromolecules either in competition studies or in binary mixtures. Many such applications may strongly resemble metabolomic approaches and may therefore profit from being treated within the same framework. Example: Quantitative lipid composition measurements from binding assays in lipid mixtures

Quantitative NMR measurements of other small molecules, small molecule, peptide and protein systems as a function of e.g. time. NMR is an efficient way to measure the concentration of the soluble fraction of a small molecule or smaller protein. Chemical reactions can be monitored and for larger molecules also structural rearrangements. One example is studies of protein monomers aggregating to form amyloid fibrils. The methods to analyze this data is also similar to metabolomic data.

Process control for non-biological processes. is basically the same as metabolomics as long as it is in the liquid phase and the reactions change the chemical environment of some NMR-sensitive nucleus.

Low field NMR is cheaper and smaller and may be interesting *e.g.* for online monitoring of chemical processes. These systems have lower resolution and sensitivity, but can be used to rapidly measure the concentration of different atoms, *e.g.* N, P, Na, K, Cl, H and C. Molecules can be distinguished either by size and number attached protons and in some cases from real spectra can be acquired.

How should the infrastructure be marketed and made more accessible to researchers?

The survey we made has provided quite a few interested scientists from Faculty of Medicine that were not known to the people working with metabolomics at CRC and CAS. With these there may be enough projects to start up a common metabolomic platform. A suitable next step here would be to call for a seminar for already interested researchers.

One blind spot seems to be Department of Biology, where fewer researchers show interest. To increase the interest there a good idea may be to invite metabolomic experts within biology and ecology for a

seminar so that they can see what can be done. A similar event could be held for people working with biological and chemical processes.

As mentioned above, people ask for the possibility to have someone who is interested and knowledgeable coming out and discuss the metabolomic opportunities related to their scientific projects with them. It is important to get people to understand what they will get before the analysis is started. Last but not least it is important to tell people how the samples should be collected treated and so forth at an as early stage as possible. Guidance in experimental design to avoid batch effects may also be needed.

How much staff are needed to manage the infrastructure and where should they be located?

One MS metabolomic and one NMR metabolomic expert is needed along with one or two technicians. Peter Spégel is an MS metabolomic expert and has an associate professor position at CAS. There is no NMR metabolomic expert at Lund University. The scope of a new position in NMR could be broadened to quantitative NMR in order to allow for a larger number of applications. There are currently skilled technicians working with either MS or NMR at CAS. Though they have little time available for the infra structure they could potentially train a platform technician to cover both MS and NMR. Depending on the workload and the localization of the instruments one or two technicians will be needed initially.

In what ways can LU support the infrastructure in the area?

LU can support the metabolomics infrastructure by covering costs for necessary upgrades of the existing equipment, creating positions for the new scientific and technical personnel, and potentially providing support for paying for service contracts, which will increase accessibility of the infrastructure for smaller research groups (through reducing the user fees).

In this relation it is also important to discuss how the infra structure should be maintained if a major hardware failure occurs.

Appendix 1. Uppdrag

Möjligheter för samordning inom NMR-och MS-baserad metabolomik vid LU

Utredningsuppdrag:

Möjligheter för samordning inom NMR-och MS-baserad metabolomik vid LU

Utgångspunkt:

För närvarande tillgänglig eller redan finansierad utrustning vid LU bedöms kunna med begränsade extrakostnader optimeras för att utföra high-throughput metabolomik för klinisk forskning och diagnostik (se bilaga).

Utredare

Anders Malmendal föreslås som huvudutredare. Tidigare utredning på uppdrag av Swedish NMR center (SNC) i Göteborg tas i beaktande. En referensgrupp till stöd för utredaren ska föreslås av utredaren, där sammansättningen speglar de verksamheter som berörs av utredningen.

Uppdragsbeskrivning

Det övergripande syftet är att utreda behov och förutsättningar för en samordning inom området translationell metabolomik, och att därmed ge förutsättningar för världsledande forskning vid LU.

Utredningen ska ta fram beslutsunderlag till M/LTH/N angående möjligheter till en fakultets-överskridande samordning av resurser för området metabolomik vid LU. Utredningen bör också ge konkreta förslag på vilka åtgärder som skulle behövas avseende befintlig NMR och MS infrastruktur och hur en samordning mellan eventuellt ingående parter kan göras.

Uppdraget ska redovisas senast 2019-06-07

Beaktanden:

- Utredningen bör ligga till grund för utarbetande av en strategi på LU. Ett flertal frågeställningar bör identifieras med avseende på utvärdering av nuvarande behov och tillgänglighet, samordning och framtida utvecklingsmöjligheter. information till kliniska forskarna

Frågeställningar som bör belysas:

1. Vilken utrustning finns idag inom LU, vilka är dess användningsområden och förutsättningar?
2. Vilka samordningsmöjligheter finns mellan existerande utrustningar?
3. Hur vidmakthålls existerande infrastrukturer?
4. Vilken roll kan dessa infrastrukturer spela i framtiden?
5. Hur många provanalyser kan utföras i Lund och hur mycket ska man förlita sig på SNC i Göteborg och SMC i Umeå.
6. Hur bör infrastrukturen organiseras? Vad behövs i form av infrastruktur inom området?
7. Hur förhåller sig infrastrukturen till angränsande problemställningar och var ska gränsen dras för vad som ska innefattas i infrastrukturen?
8. Hur ska infrastrukturen marknadsföras och göras mer tillgänglig för forskare?
9. Hur kan infrastrukturen kopplas till motsvarande nationella resurser och nätverk?
10. Hur mycket personal behövs för att hantera infrastrukturen och var ska de vara placerade?
11. På vilka sätt kan LU stödja infrastrukturen inom området?

Appendix 2. Examples of projects

Examples of past and current projects

Peter Spégel, Siri Malmgren, Vladimir V. Sharoyko, Isabel Goehring, Anders P. H. Danielsson, Siri Malmgren, Cecilia L. F. Nagorny, Lotta E. Anderssson, Thomas Koeck, Geoffrey W. G. Sharp, Susanne G. Straub, Claes B. Wollheim & Hindrik Mulder. *Time-resolved metabolomics analysis of β-cells implicates the pentose phosphate pathway in the control of insulin release.* Biochem. J. 450: 595–605, 2013. **Untargeted profiling of 100 β-cells samples, 50 hours of MS-time**

M. Al-Majdoub, K. Herzog, B. Daka, M. Magnusson, L. Råstam, U. Lindblad, and P. Spégel, *Population-level analysis to determine parameters that drive variation in the plasma metabolite profile*, Metabolites 8: 78, 2018. **2500 plasma samples**

L.E. Andersson, L. Scherbina, M. Al-Majdoub, N. Vishnu, C.B. Arroyo, J. Aste Carrara, C.B. Wollheim, M. Fex, H. Mulder, N. Wierup, and P. Spégel, *Glutamine-elicited secretion of glucagon-like peptide 1 is goverened by an activated glutamate dehydrogenase*, Diabetes 67: 372-384, 2018. **100 samples from beta- and L-cells**.

Filip Ottosson, Louise Brunkwall, Ulrika Ericson, Peter M. Nilsson, Peter Almgren, Céline Fernandez, Olle Melander & Marju Orho-Melander. *Connection Between BMI-Related Plasma Metabolite Profile and Gut Microbiota.* J Clin Endocrinol Metab 103: 1491–1501, 2018. **Targeted profiling of 48 metabolites in 920 plasma samples, 4 weeks of MS experiments**

Filip Ottosson, Einar Smith, Olle Melander & Céline Fernandez. *Altered Asparagine and Glutamate Homeostasis Precede Coronary Artery Disease and Type 2 Diabetes.* J Clin Endocrinol Metab 103: 3060–3069, 2018. **Targeted profiling of 35 metabolites (amino acid metabolites and acylcarnitines) in 1049 plasma samples, 6 weeks of MS experiments**

Åke Lernmark et al. *Longitudinal metabolome-wide signals in children developing islet autoantibodies in The Environmental Determinants of Diabetes in the Young (TEDDY) study.* **The metabolomes and lipidomes of TEDDY first NCC cohort subjects for IA were profiled from 10522 plasma samples**.

Examples of expected projects mentioned in the survey

Harry Björnbacka et al. *Metabolomics of plaques in cardiovascular disease.* ~70 samples 2-3 times a year.

Kazi Uddin et al. *Metabolomics in cancer metabolism*. 50-100 samples per year both targeted and untargeted metabolomics on cell lines or patient-derived xenografts and patient materials.

Caroline Isaksson et al. *Metabolic changes in birds living in an urban environment*. 200-1000 samples

Nélida Leiva Eriksson et al. *Metabolic variations in protein producing bacterial systems.* 300-400 samples per project.

Per Falås et al. *Process control for fermentation/degradation/other similar applications*, e.g. 1000 samples of ozonated waste water a year.

Potential long-term projects

Studies of large clinical cohorts and biobanks. An interesting target for high throughput metabolic profiling by NMR are the biobanks. Inspiration to the type of output that can be achievable can be obtained from Prof. Ala Korpela's work with the Finnish biobanks. One could envisage that biobank samples could be supplemented by an NMR spectrum so that the spectrum rather than a new aliquot could be analyzed when new information is needed.

Appendix 3. Interviewees

Hindrik Mulder, CRC

Olle Melander, CRC

Åke Lernmark, CRC

Vladimir Denisov, BMC

João Duarte, BMC

Roger Olsson, BMC

Andreas Edsfeldt, Department of Cardiology

Kazi Uddin, Department of Laboratory Medicine

Harry Björkbacka, Department of Clinical Sciences, Malmö

Peter Spégel, Department of Chemistry

Sara Linse, Department of Chemistry

Mikael Akke, Department of Chemistry

Nélida Leiva Eriksson, Department of Chemistry

Henrik Almqvist, Department of Chemical Engineering

Per Falås, Department of Chemical Engineering

Olena Prykhod'ko, Department of Food Technology

Caroline Isaksson, Department of Biology

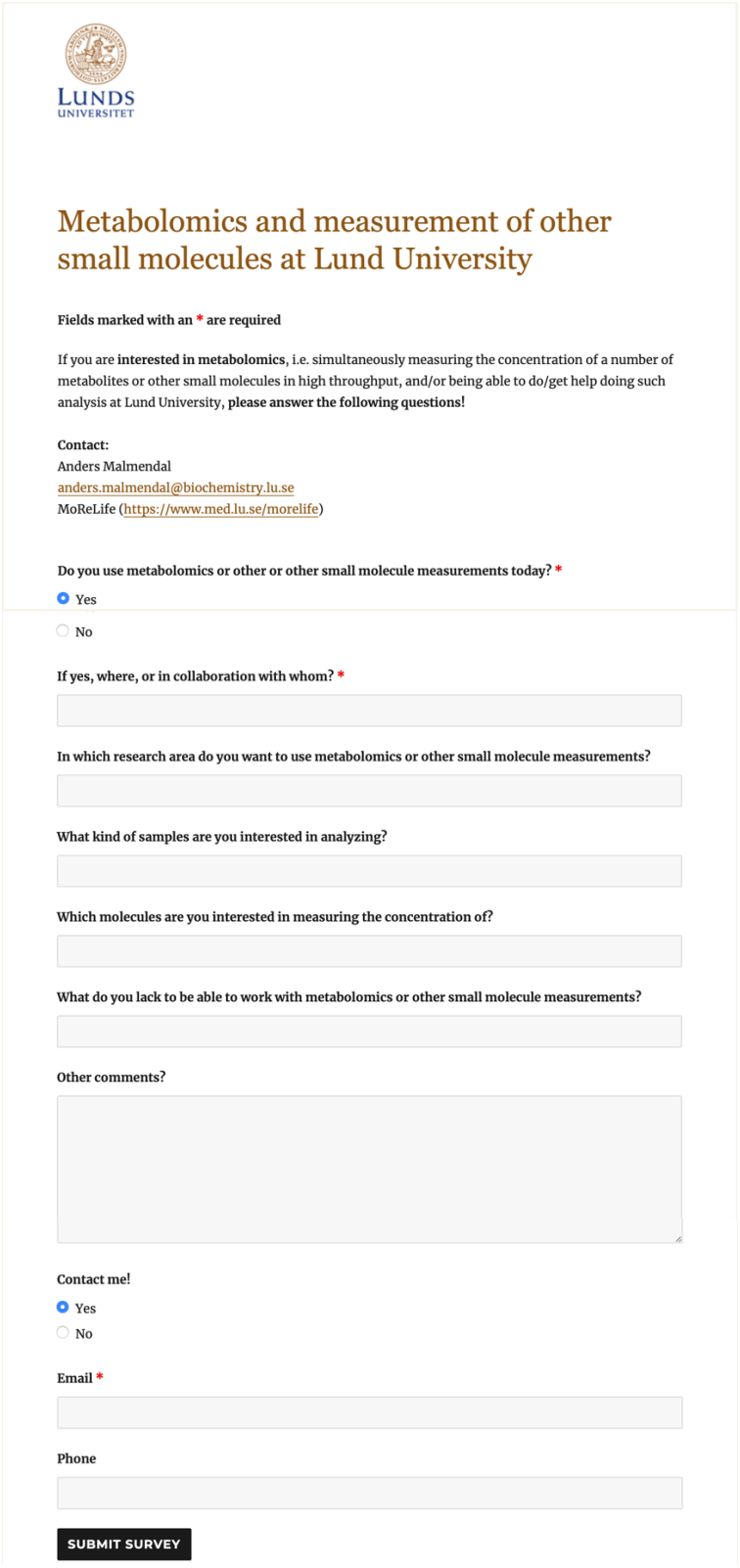
Annika Johansson & Anders Nordström, Swedish Metabolomics Center, Umeå.

Thomas Moritz, University of Copenhagen & Swedish Metabolomics Center, Umeå

Anders Pedersen, Swedish NMR Centre, Gothenburg

Morten Kjærulff Sørensen, Aarhus University

Appendix 4. The survey



LUNDS UNIVERSITET

Metabolomics and measurement of other small molecules at Lund University

Fields marked with an * are required

If you are **interested in metabolomics**, i.e. simultaneously measuring the concentration of a number of metabolites or other small molecules in high throughput, and/or being able to do/get help doing such analysis at Lund University, **please answer the following questions!**

Contact:
Anders Malmendal
anders.malmendal@biochemistry.lu.se
MoReLife (https://www.med.lu.se/morelife)

Do you use metabolomics or other or other small molecule measurements today? *

- (•) Yes
- ( ) No

If yes, where, or in collaboration with whom? *

In which research area do you want to use metabolomics or other small molecule measurements?

What kind of samples are you interested in analyzing?

Which molecules are you interested in measuring the concentration of?

What do you lack to be able to work with metabolomics or other small molecule measurements?

Other comments?

Contact me!

- (•) Yes
- ( ) No

Email *

Phone

SUBMIT SURVEY

Appendix 5. Current instrumentation

MS instrumentation at Clinical Research Center (CRC), Faculty of Medicine in Malmö

- a QTOF coupled to UHPLC
- a chip-based nanoLC.

The latter cannot be used any more since it is no longer manufactured there are no spare parts to get. There is an infrastructure application by Olle Melander for a replacement this with a newer QTOF.

MS instrumentation at Centre for Analysis and Synthesis (CAS), Department of Chemistry, Faculty of Science in Lund:

- a triple quadrupole (QQQ) mass spectrometer coupled to ultra-high-performance supercritical fluid chromatography (UHPSFC) and ultra-high performance liquid chromatography (UHPLC) (infrastructure)
- a quadrupole time-of-flight mass spectrometer (QTOF) coupled to both UHPSFC and UHPLC (available as infrastructure)
- an orbitrap Velos Pro coupled to high performance liquid chromatography (HPLC) and nanoLC (partly available as infrastructure)
- a QQQ coupled to gas chromatography (GC) and 2-3 quadrupoles (Q) coupled to GC

In addition, there are also some older mass spectrometers: QQQ, Q, QTOF, double sector. So, in total, there are about 10 mass spectrometers and about 20 LC, GC and SFC systems

There is also a sample preparation robot, vacuum centrifuge, plate sealer *etc* for semi-automated sample handling and metabolite extraction in the 96-well format, which will be available within the infrastructure.

Peter Spégel has submitted applications to replace either the orbitrap or QTOF with a new Orbitrap tribrid. It can be used for virtually everything.

NMR instrumentation at Department of Clinical Sciences, the Biomedical Center at the Faculty of Medicine in Lund

- a 500 MHz Varian/Agilent spectrometer. Up to 75% of the time is available for metabolomic applications.

NMR instrumentation at Department of Chemistry, Faculty of Science / LTH in Lund:

- Lund University has granted funds for a brand new 600 MHz Bruker spectrometer at Department of Chemistry. About 50% of the time will be available for metabolomic applications.
- Funding for a larger 800 MHz spectrometer has also been applied for.

Appendix 6. Commercial metabolomic services

Metabolon Inc is a US MS-based provider of metabolite analysis. They have a gigantic metabolite library allowing them to quantify more than 1000 metabolites. However, they can only handle a limited number of matrices and you cannot get unknown metabolite signals or raw data out. The cost per sample is on the order of € 250 for smaller series.

MS-Omics is a Danish MS-based provider of metabolomics and data analysis. They have a short lead time from receipt of samples to final report for standard analysis of blood, urine, feces, tissue, cells, bacteria, yeast, food products, fermentations and a vast range of other biological samples. Advanced multivariate data analyses available. Price examples: analysis of 50 fermentation samples for amino acids, TCA metabolites and other organic acids € 67/sample, broad coverage metabolomics of 50 fecal samples € 165/sample, quantification of short chain fatty acids in fecal water extracts: 53 euro/sample.

Nightingale Health is a Finnish NMR-based provider of metabolite analysis in blood, urine or CSF. In blood 18 small molecule metabolites, fatty acid classes, and many lipoprotein classes are quantified. In urine and CSF 61 and 28 small molecule metabolites, respectively, can be quantified. € 100/sample.

UK Biobank (a large biobank study which started 2006 in UK and is investigating contributions of genetic predisposition and environmental exposure including nutrition, lifestyle, medications etc. to the development of disease, has recently unveiled plans to analyze metabolic biomarkers in 500,000 blood samples. The project, which is expected to take 30 months, will focus on metabolic biomarkers linked to heart disease, type 2 diabetes and other common chronic diseases. It will use Nightingale's NMR-based metabolite profiling platform.