Drug discovery & development possibilities for industry at SciLifeLab NMR unit and MAX IV BioMAX beamline

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SNC – main areas of activity

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Metabolomics – large sample series and multivariat analysis

Structural Biology – structure, dynamics and interactions

Solid state pharmaceutical applications with superior sensitivity (DNP-NMR)

Small molecules and standard NMR measurements – mostly local activities

Drug discovery – the topic for today

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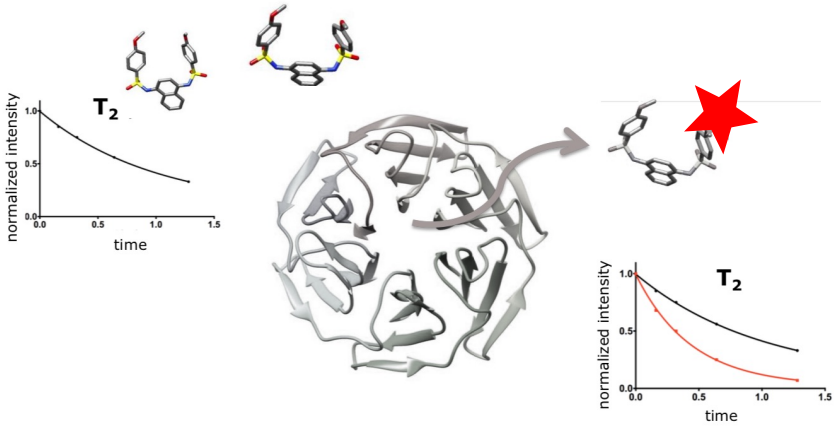
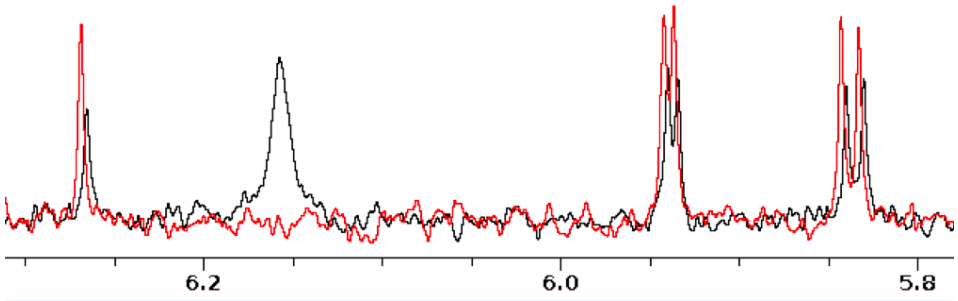
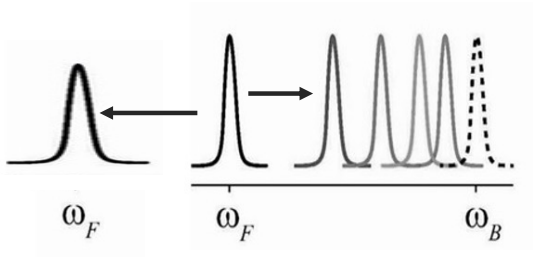
Fragment based screen by NMR



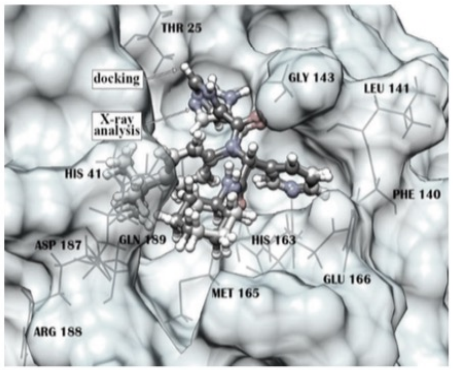
Difference in NMR relaxation properties for free and protein bound fragment.



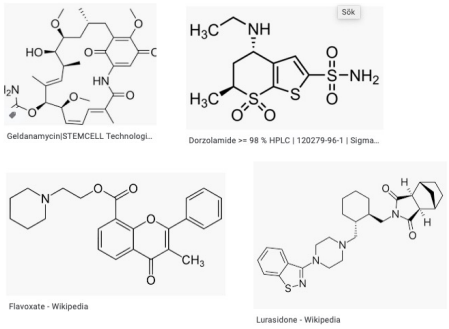
^{1}H NMR spectra fragment with and without protein.



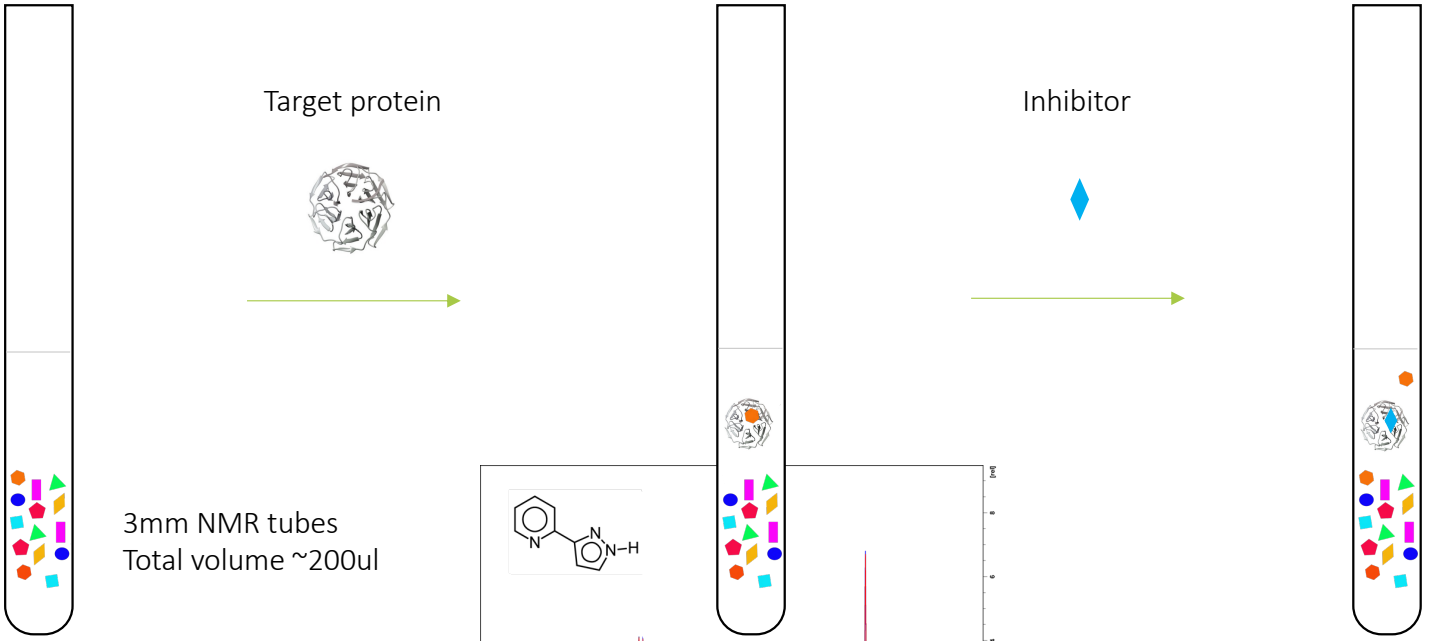
Change in chemical shift or in peak intensity.



Protein target + control



Principle for ligand detected FBS



- Fragments are pooled 10 and 10, spectra recorded
- Target protein added and spectra repeated

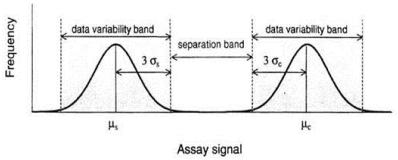
- Spectra are repeated after addition of inhibitor, should be identical to first spectrum

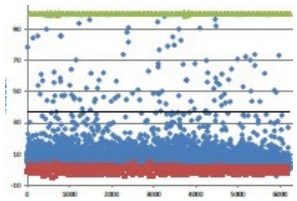
2 or 3 (if ^{19}F in fragment) different spectra needed

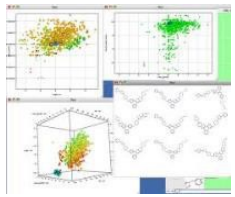
Follow up

Rerun of found hits to confirm
Redesign of fragments – CBCS

Chemical Biology Consortium Sweden
CBCS collaborative project model







- Assay design and development

PI & CBCS

Pre-project / Assay development project

- Screen

CBCS (PI)

Screening project

- Data analysis
- Hit confirmation
- In silico profiling
- etc

CBCS (PI)

- Medicinal chemistry
- Compound profiling
- Biological profiling
- Target ID /MoA

CBCS & PI

Chemistry based project

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Ligand detected – practical considerations

- 2 mg (unlabeled) protein @ 30 kDa > 0.1mM
- 4 ul of fragment @ 10 mM (In DMSO-d_6)

- 5- 10 uM protein : 50- 100 uM fragment
- 8 – 12 fragments per mix

- Spectrometer time
- NMR tubes
- Fragment library
- Control experiments
- Personell time for sample preparation and data recording
- Analysis of data upon request

Cost examples for industrial users

Bionet ^{19}F library – Screening 420 fragments excluding analysis of results

3 NMR-experiments (STD, T1rho, ^{19}F), 50 minutes run per sample => 70 h spectrometer time á 540 SEK (38000 SEK)
NMR tube cost: ~ 3000 SEK
Library cost: 5-10000 SEK
Control runs: ~5000 SEK
~130 SEK per fragment, running mixes of 10 per tube
Inhibitor experiments adds ~35 h in spectrometer = 19000
Including inhibitor experiment ~170 sek per sample
Additional 20% cost for 2 % hit rates, individual runs
Project management and laboratory work, ~20h of personnel time

150-210 SEK/fragment

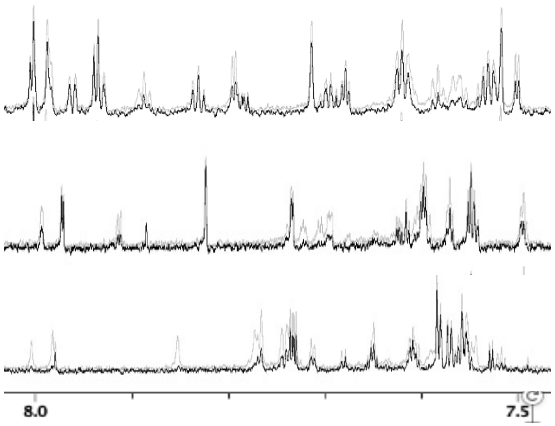
Maybridge library, 800 fragments 2 NMR experiments (STD, T1rho)

Price reduces to ~130SEK per fragment (running mixes of 10) and including inhibitor experiments

Time frame and time line

Spectrometer waiting time - ~4 weeks
Sample preparation - protein production

Planning of project – ~10 hours
Library preparation – 10-15 hours
Data recording – 3 days (scales with library size)
Data analysis – 15-20 hours



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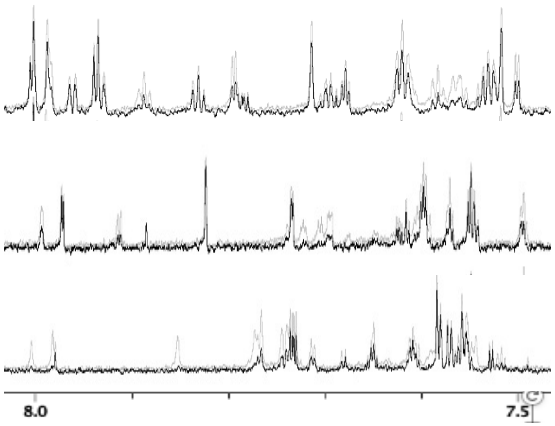

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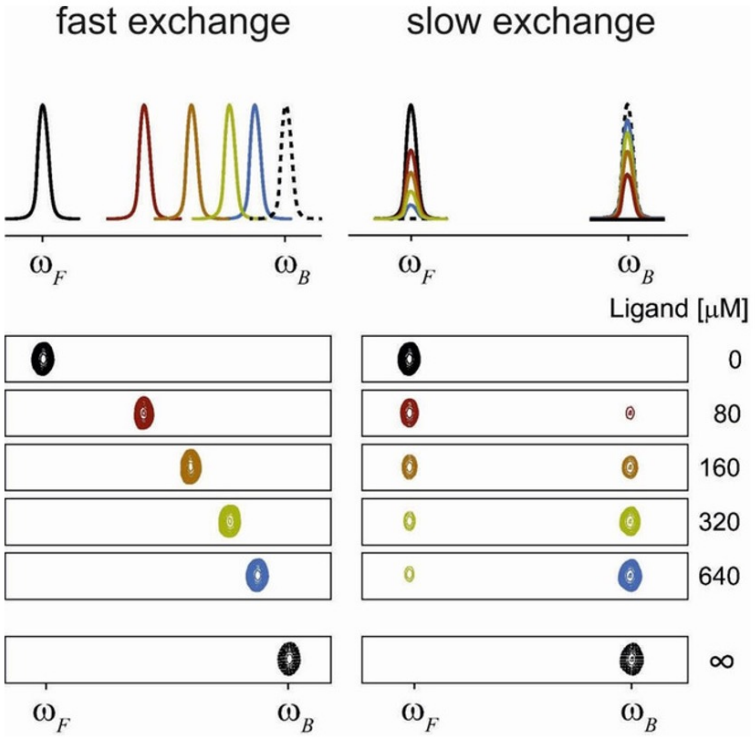
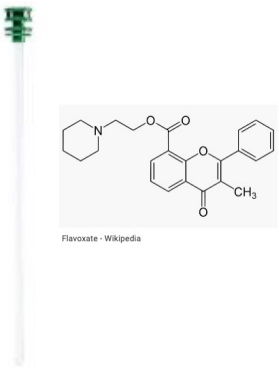
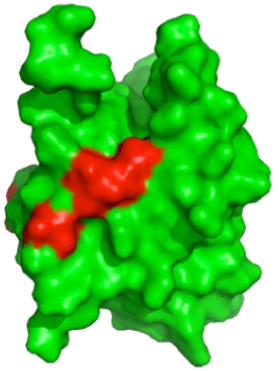
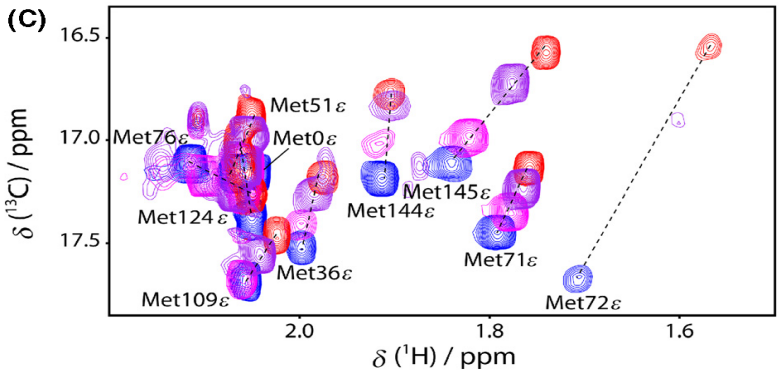
Protein detected fragment interaction



ChemPhysChem 2018, 19, 895 – 906

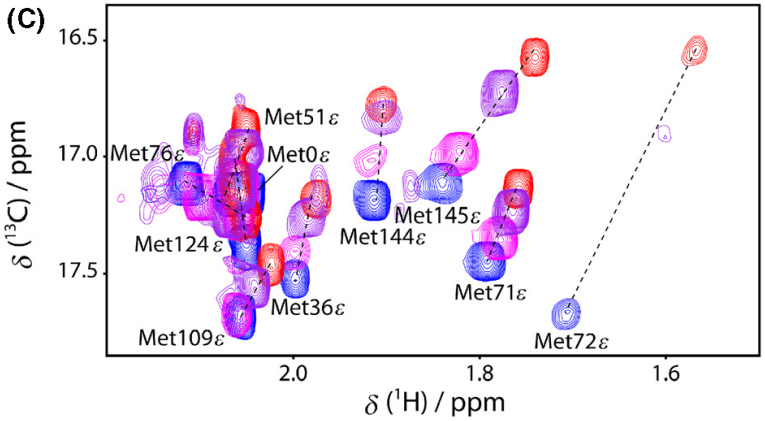






Calmodulin protein ^{1}H - ^{13}C HSQC with increasing sevoflurane

Protein detected fragment interaction - practical considerations



Single fragment : protein-detected

- >30 uM $^{15}N(^{13}C)$ protein
- 1 fragment per mix (1mM concentration, total volume 200 ul)
- >0.2 mg $^{15}N(^{13}C)$-labeled protein @ 30 kDa
- spectrometer time: 2- 8 hours
- spectrometer cost: 1- 4000 per sample

- Requires known backbone assignment to interpret data

Time frame and time line

Spectrometer waiting time - ~1-2 weeks
Sample preparation - protein production is usually time limiting

Planning of project – 2-4 hours
Sample preparation and data recording – 1-4 hours
Data analysis – 4-8 hours

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NMR FBS at SNC

NMR Fragment based screens

- Spectrometers 400 – 900 MHz, *automation and cryo probes*
- ^{19}F and ^{1}H detection optimized
- Bruker SamplePro Tube L.
- Agilent Bravo, *96 channels*
- Opentrons OT-2, *multiple dispense*

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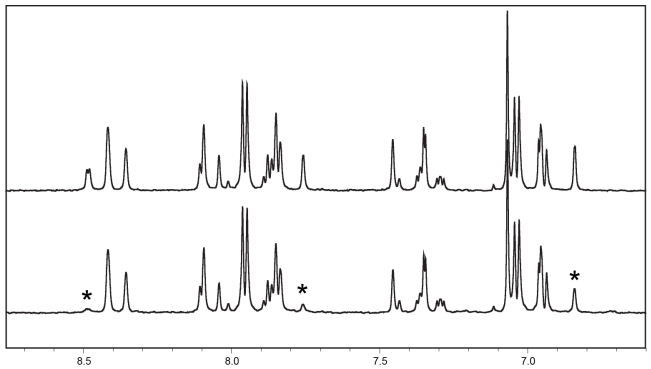
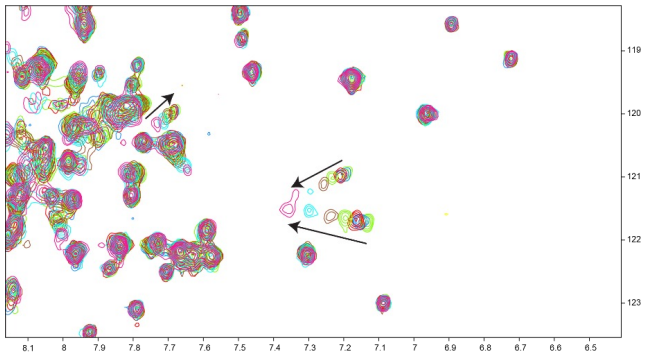
Ligand-observed experiments



Protein-observed experiments

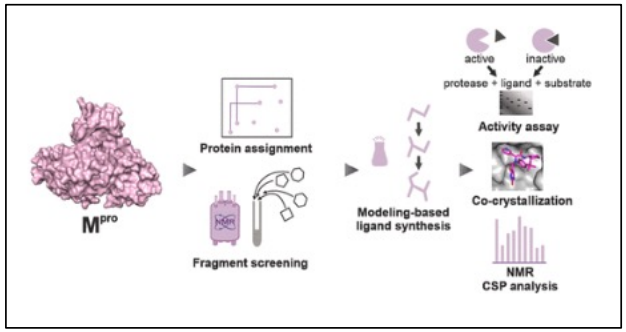


Scientific example

Targeting the Main Protease (Mpro, nsp5) by Growth of Fragment Scaffolds Exploiting Structure-Based Methodologies

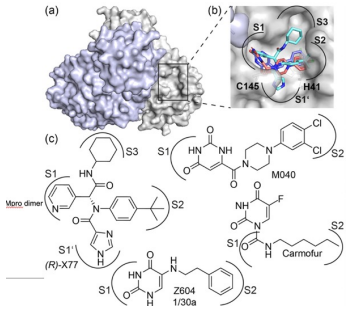
ASC Chemical Biology 2024

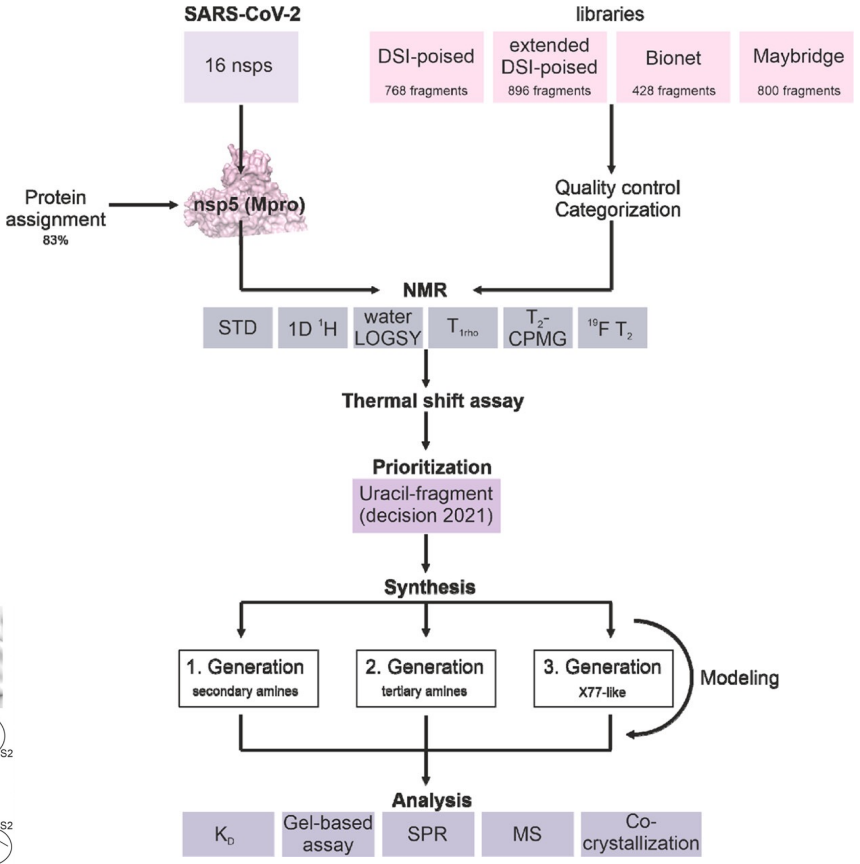
https://doi.org/10.1021/acschembio.3c00720



- Swedish NMR Centre, NMR, FBS
- CBCS fragments
- PPS, protein

- Frankfurt, protein, NMR, FBS, modelling, chemistry
- Zurich, - modelling
- Vienna, NMR, FBS
- Florence, assay
- NKI Amsterdam, SPR
- NIH, NMR, FBS
- Saverna Therapuetics – modelling





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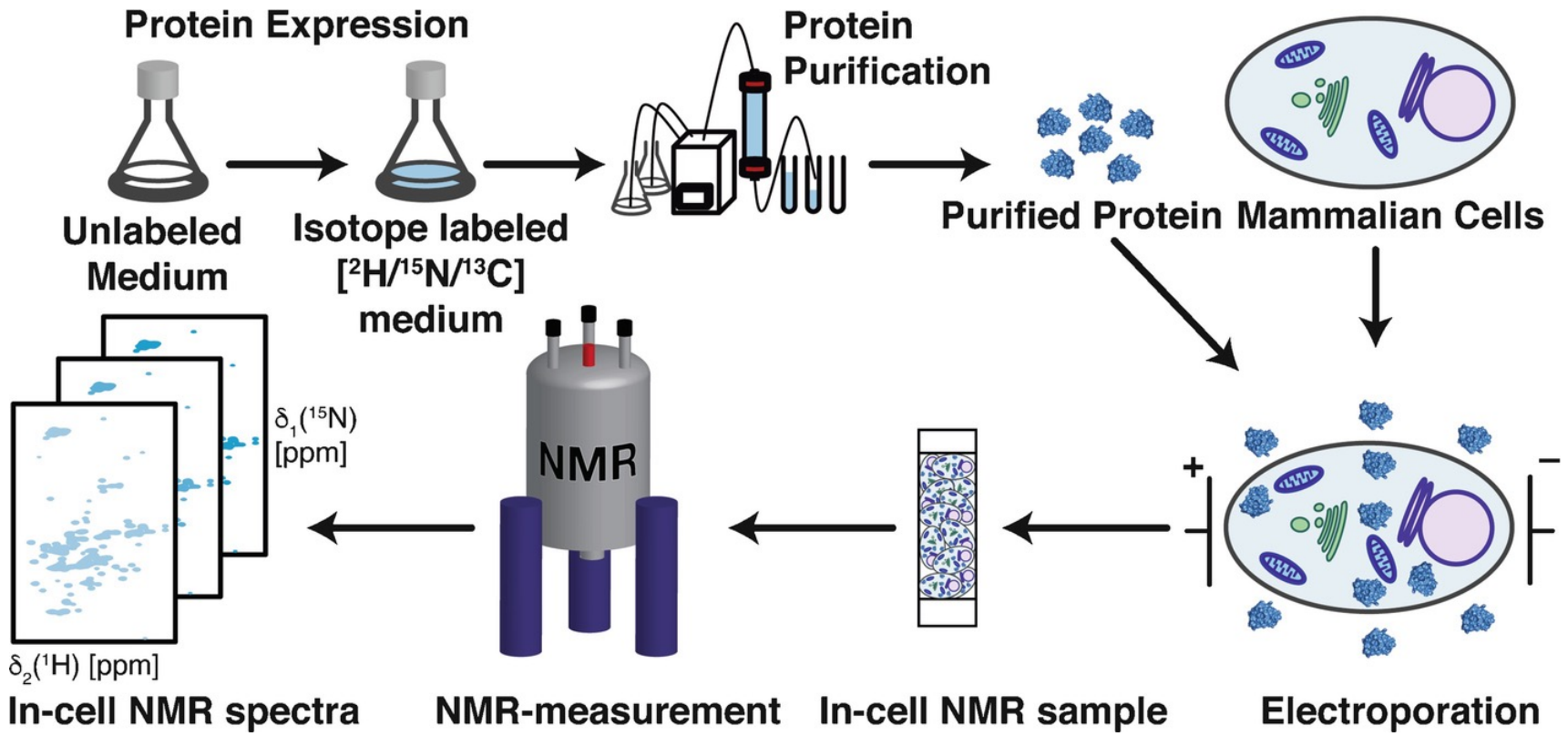
In-cell NMR



I. Matecko-Burman et. al. Recording In-Cell NMR-Spectra in Living Mammalian Cells (2020)

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Questions?

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