AIffinity
MOLECULAR DESIGN

Enhancing Drug Discovery with Nuclear Magnetic Resonance & Artificial Intelligence

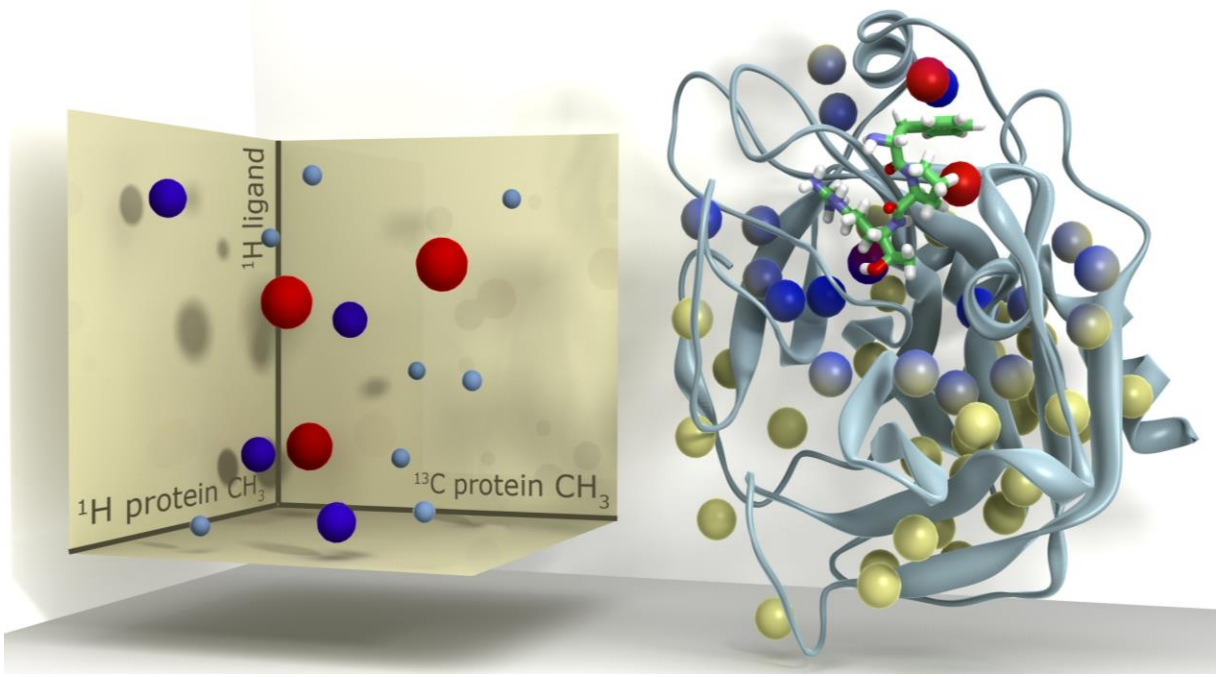
www.aiffinity.com | info@aiffinity.com

Purkyňova 127, 612 00 Brno-Medlánky, Czech Republic

Nuclear Magnetic Resonance (NMR)

Applications:

1. Protein Structure Determination
2. Conformational Dynamics Studies
3. Molecular Interaction Studies
4. Drug screening



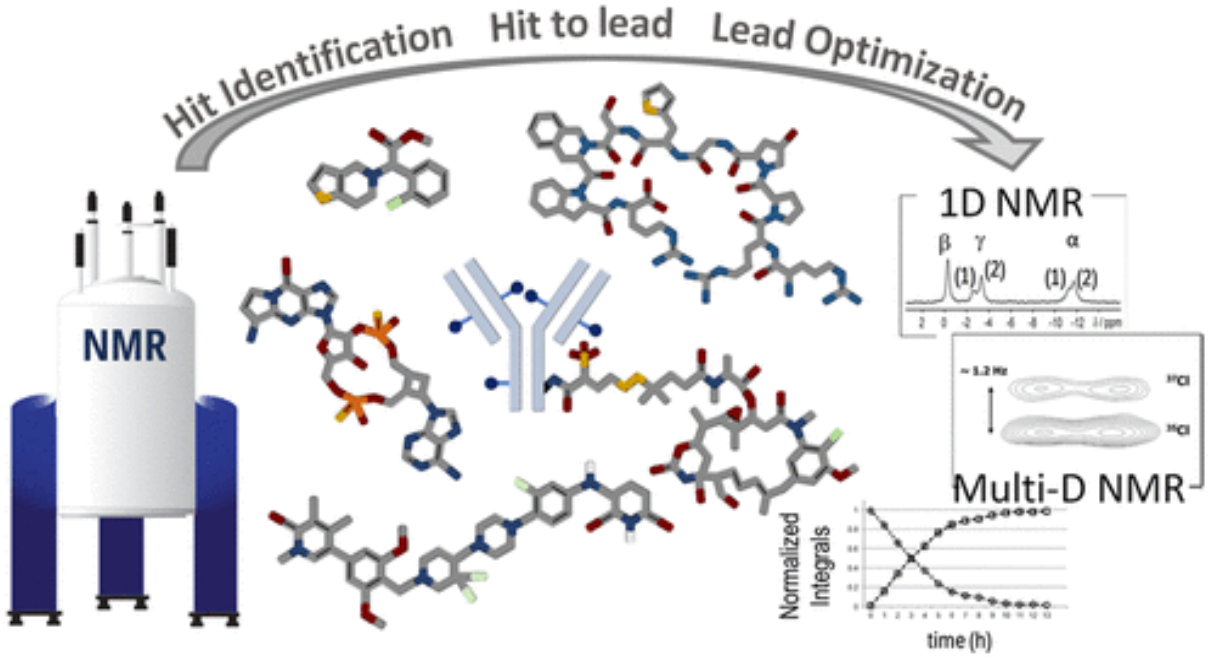
Challenges:

1. Technical Complexity
2. Instrumentation Cost
3. Resource Intensity
4. Protein Size Limitations

With **AI** and **Cheminformatics** we can navigate these challenges

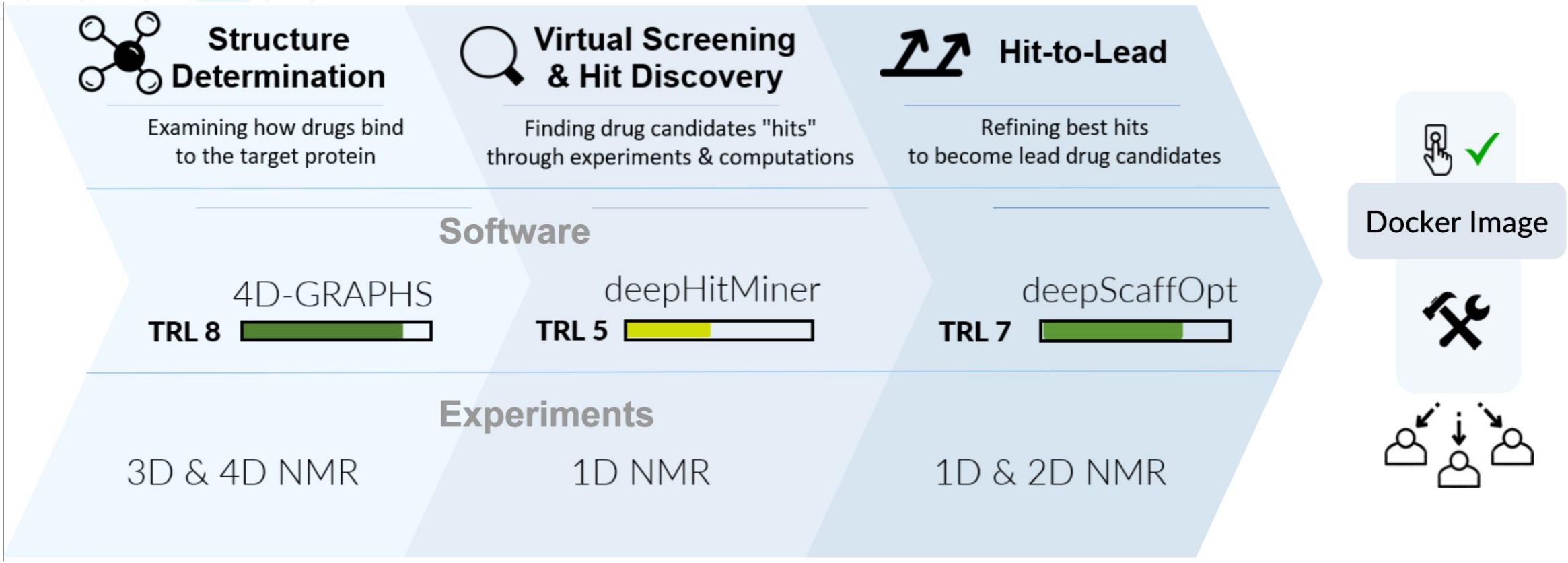
NMR in Drug Design

- Hit Identification and Hit-to-Lead.
- Interactions with ligands, peptides and other protein.
- Purity, solubility, and structural integrity of compounds.



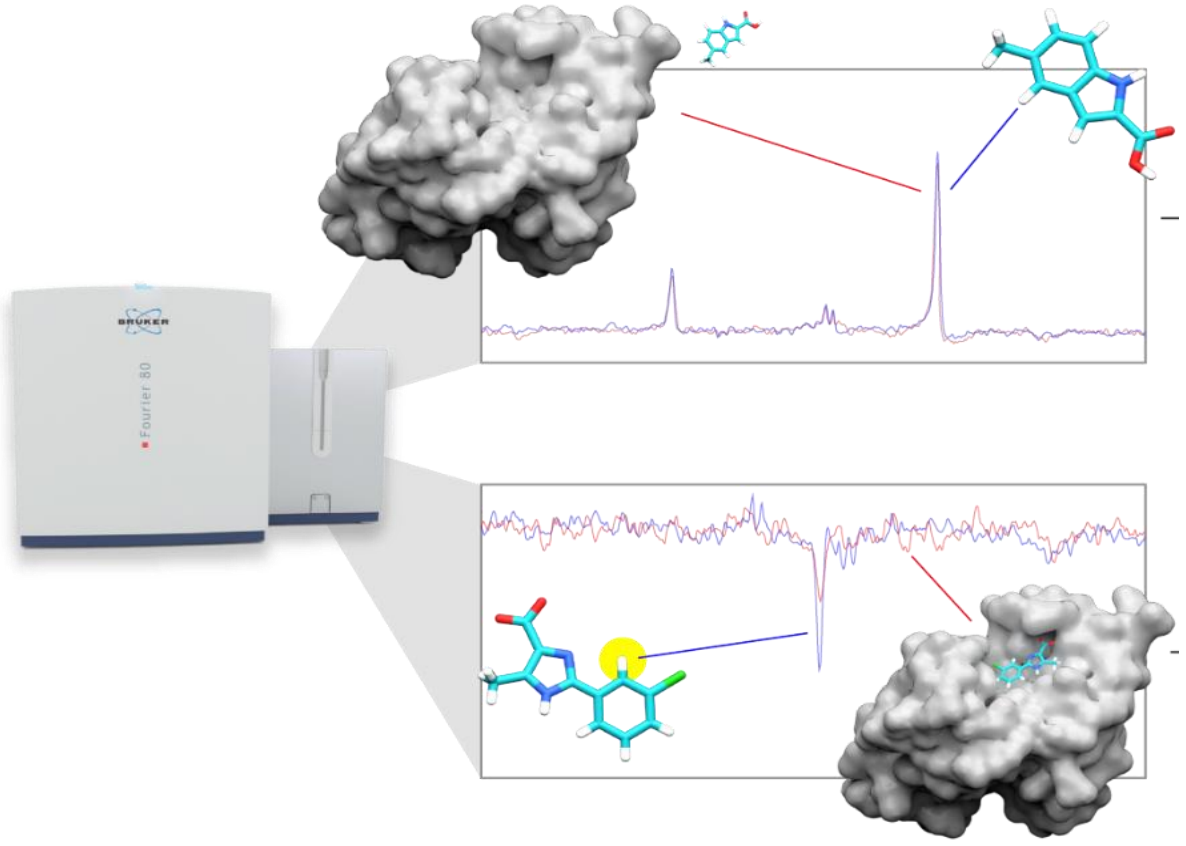
- Development of targeted protein degraders, cyclic dinucleotides, macrocyclic peptides, and antibody-drug conjugates.

Our solution: NMR-AI Integrated platform for Streamlined Target-to-Lead Process



1D NMR methods in Drug Design

- Photo-CIDNP, STD, WaterLOGSY
- Photo-CIDNP: more sensitive, faster (1,500 samples/day), benchtop NMR
- Protein 3D structure not necessary
- Give the atoms of the small molecule that interact with the protein (**ligand epitope**)
- We develop both **Generative AI** and **Virtual Screening solutions** incorporating ligand epitope infromation



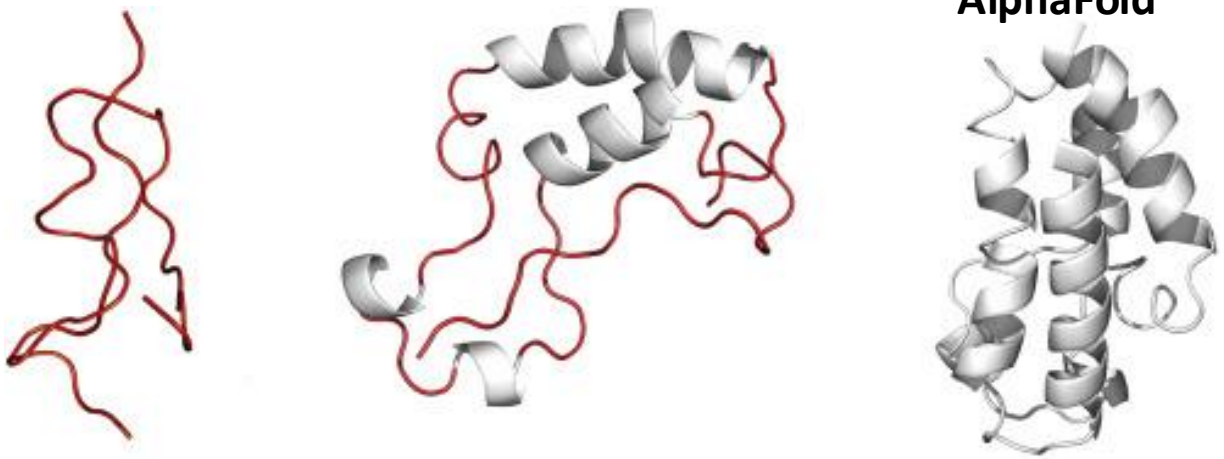
Protein NMR

Spectral
Resolution: 2D < 3D < 4D < 5D
Sensitivity: 2D > 3D > 4D > 5D
Total time: 2D < 3D < 4D < 5D
Complexity: 2D < 3D < 4D < 5D

COMPETITORS

AIffinity MOLECULAR DESIGN

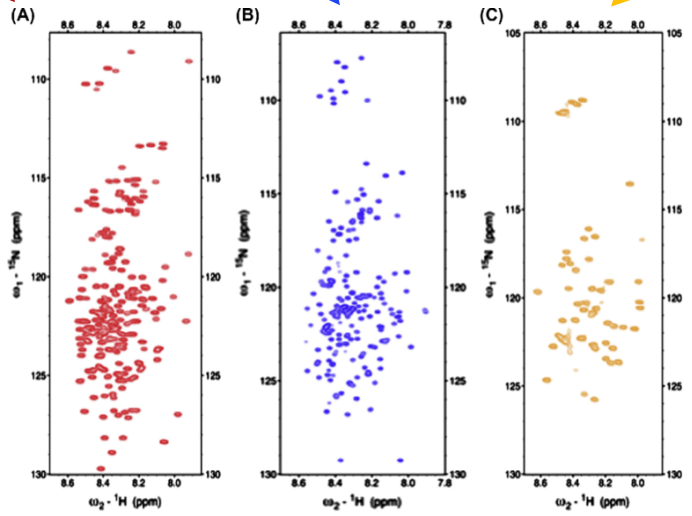
NMR **NMR** **X-ray Cryo-EM NMR AlphaFold**



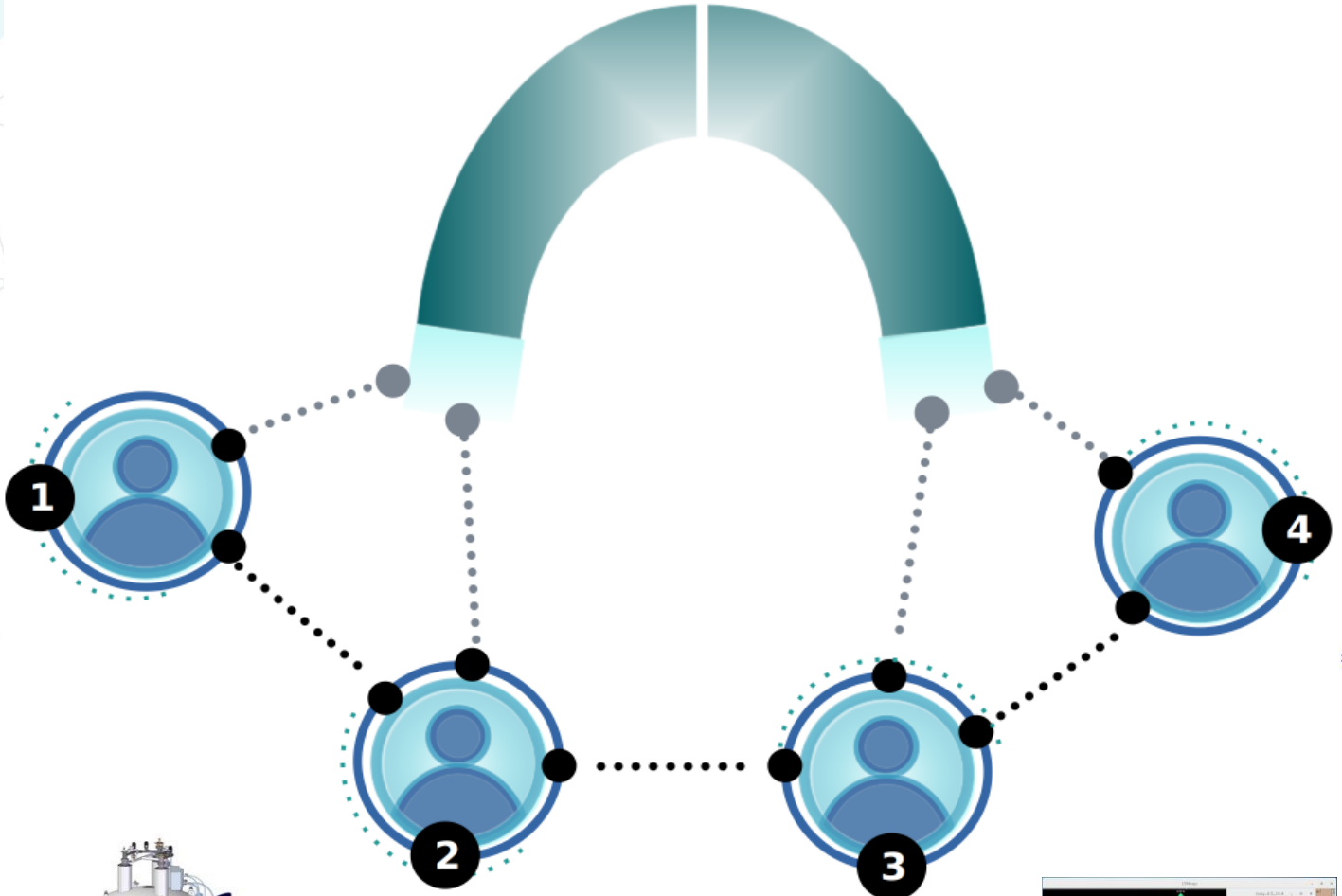
Our competitive advantages:

- Large proteins (>20 kDa)
- IDPs

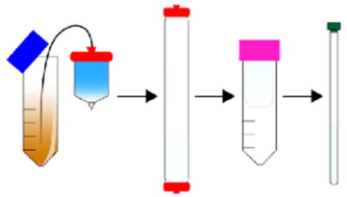
2D NMR spectrum



The Fundamental Problem of Protein NMR

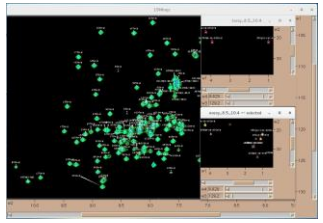


Sample preparation

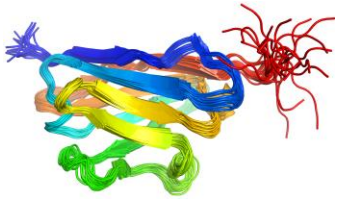


Experiment Setup & Spectra Processing

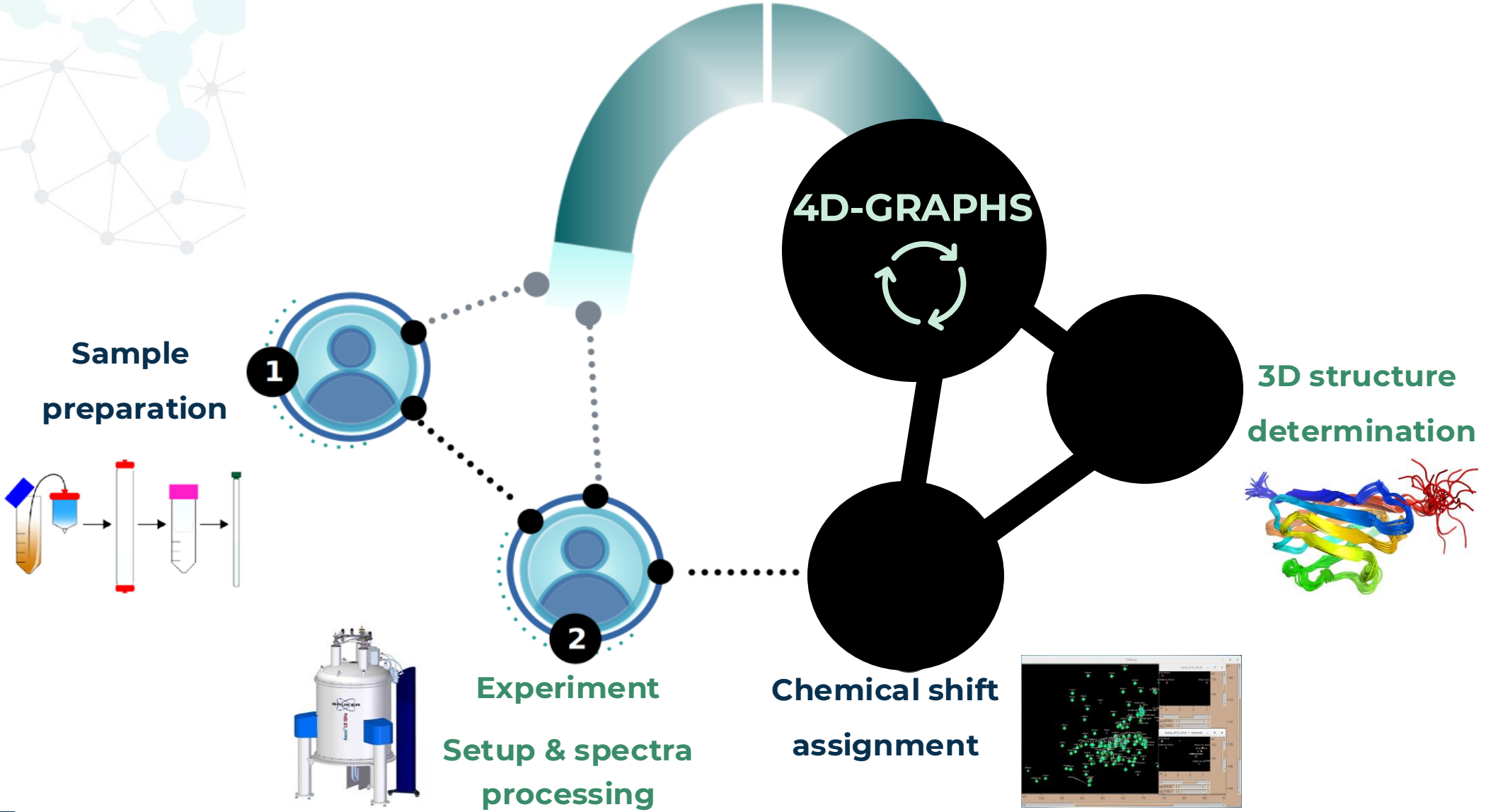
Chemical shift assignment



3D structure determination



Streamlining NMR: Reducing Human Input with 4D-GRAPHS



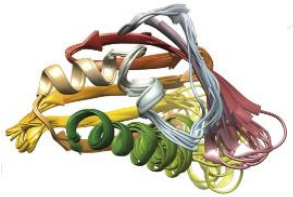
4D-CHAINS (precursor of 4D-GRAPHS) case studies

all-atom resonance assignment and structure determination from 3x 4D spectra [Evangelidis et al., 2018]

Nature Commun.

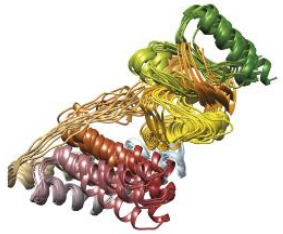
133 aa

145 aa

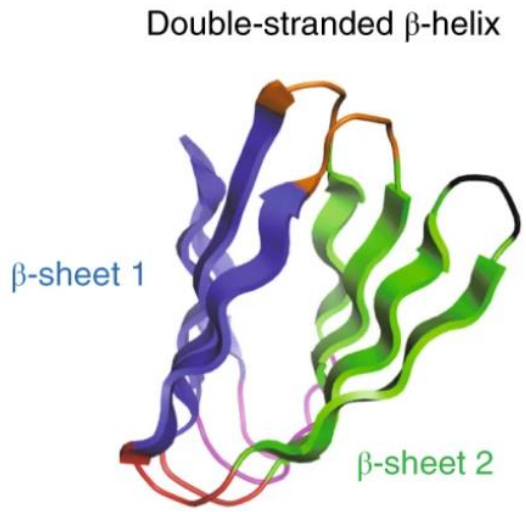


198 aa

248 aa

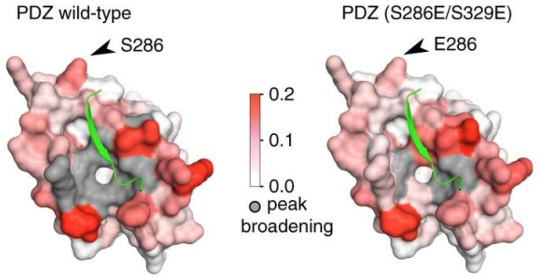


Nature Structural & Molecular Biology



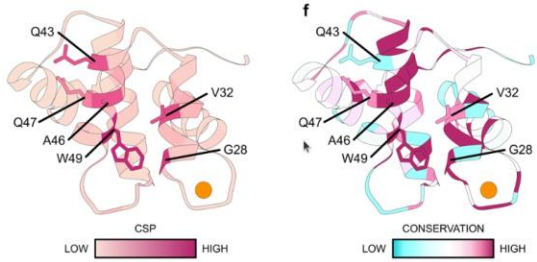
synthetic protein

Nature Commun.



protein-peptide

Nature Commun.

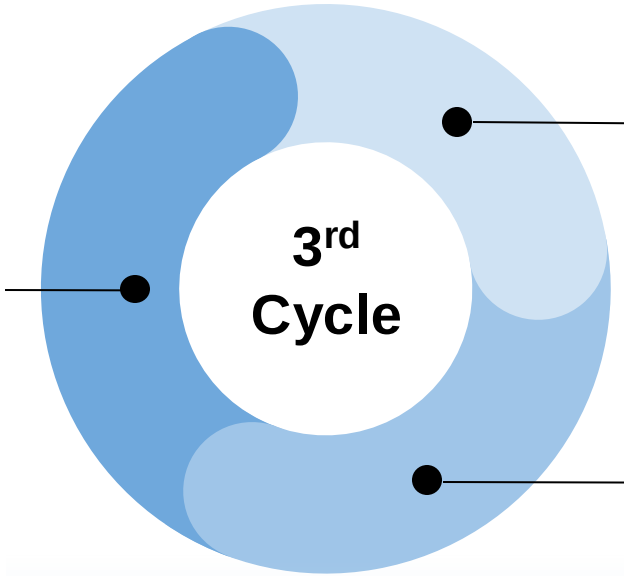


protein-protein

4D-GRAPHS: protein structure from fewer spectra

Chemical shift assignment (**4D-GRAPHS**)

4D HCNH NOESY
4D HCCH NOESY
4D HNNH NOESY
3D HNCO/ HN(CA)CO

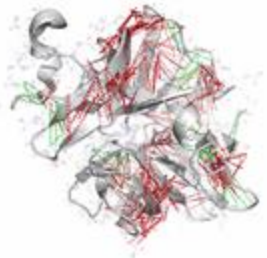


NMR restraint optimization

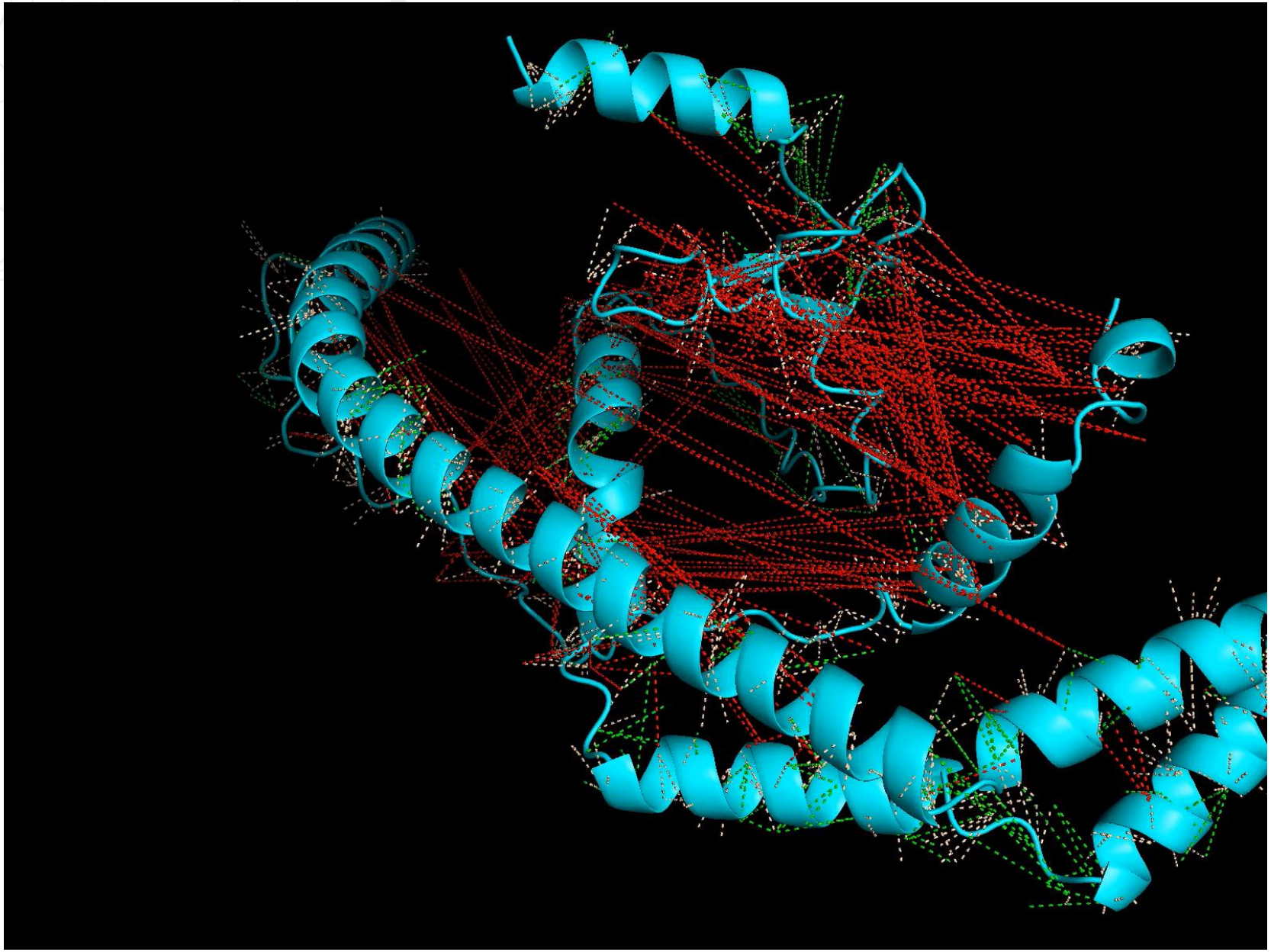
3D structure model generation

powered by OpenFold AlphaFold For

Final model



Medium-range and long-range NOE distance restraints



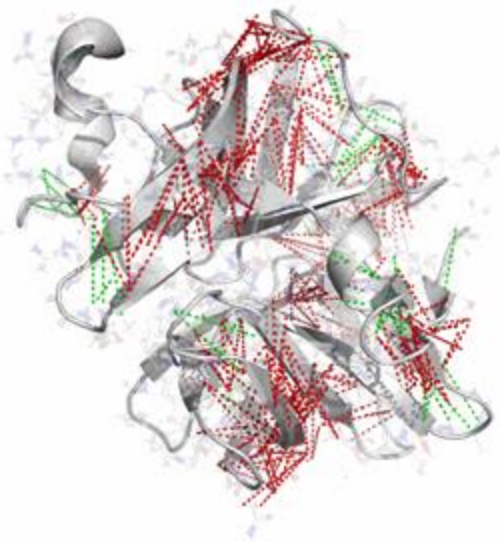
- **"Broken" OpenFold**
- **nEIt (248 amino acids)**
- **Only 4D HCNH NOESY spectrum**

1.8 mM, 20 mM sodium phosphate (pH 6.5), 100 mM NaCl, 5% D2O, 37 °C.

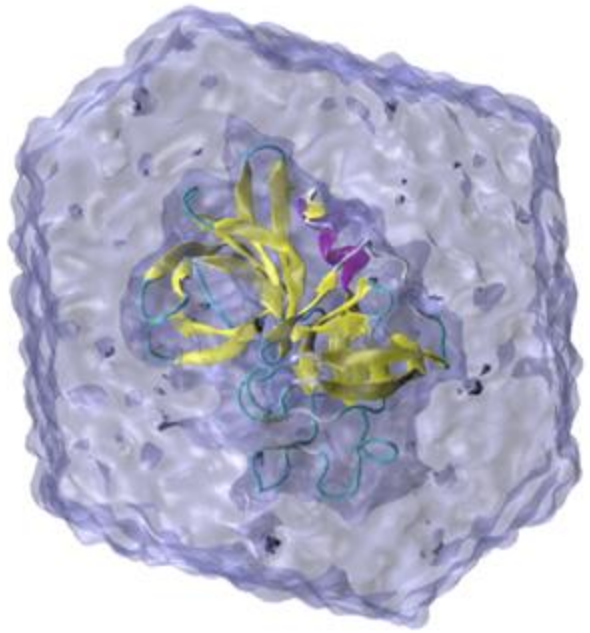
Video on YoutTube

4D-GRAPHS: protein structure from 2x 4D spectra

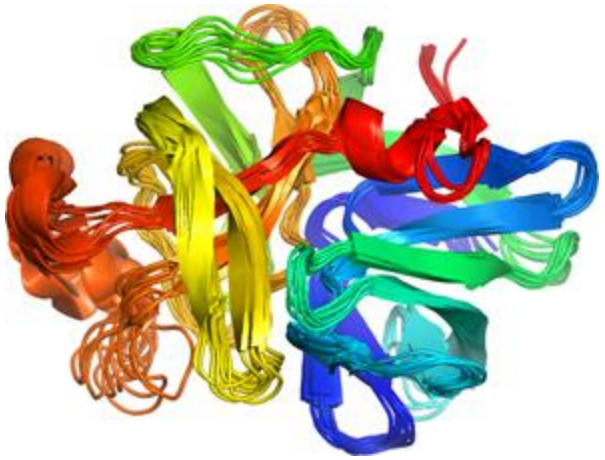
Final model



Explicit solvent equilibration

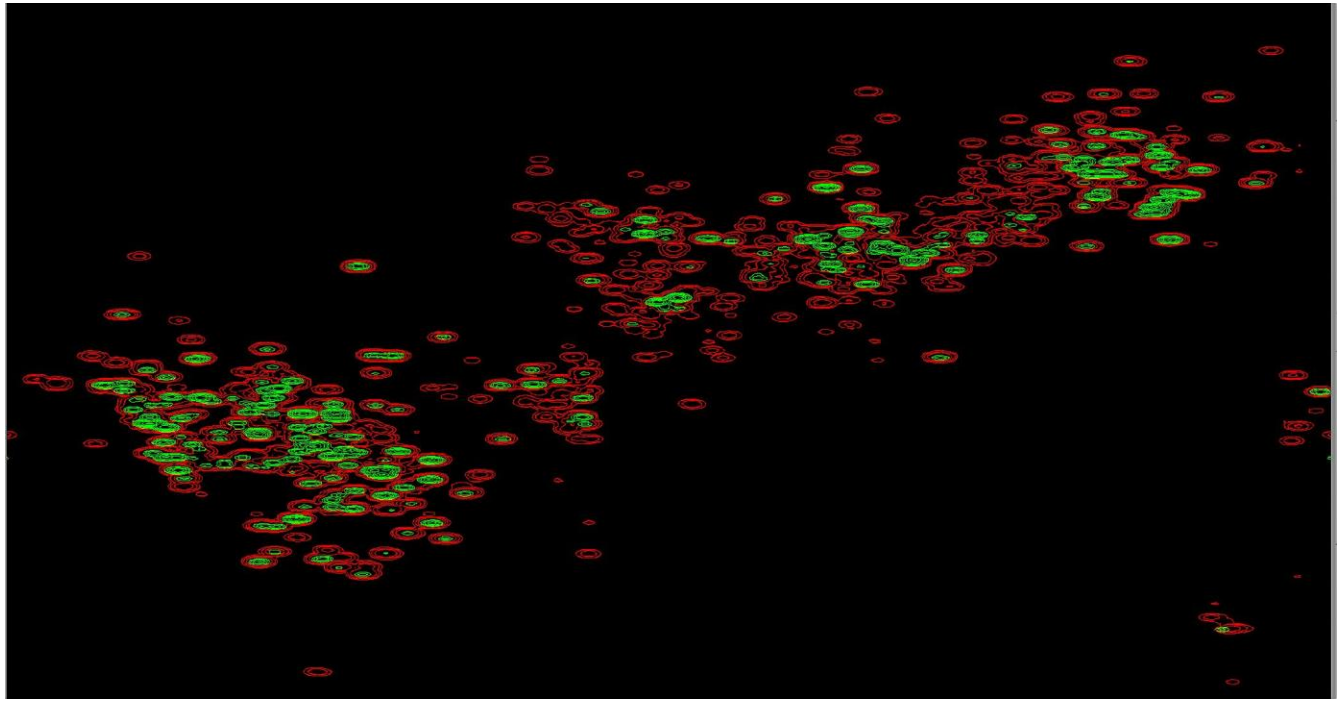


3D structure ensemble



4D Experiments have Lower Sensitivity: the case of CAII (29 kDa)

- **Overlay of HC-C projection** from 4D NOESY obtained from **273 µM** vs **600 µM** samples.
- 3D structure solved using **1x4D and 4x3D spectra on 950 MHz**: 4D HCNH NOESY, 3D HNCACB / 3D HN(CO)CACB, 3D HNCO / 3D HN(CA)CO. Total measurement time **21 days**.



Sensitive experimental protocol for backbone assignment

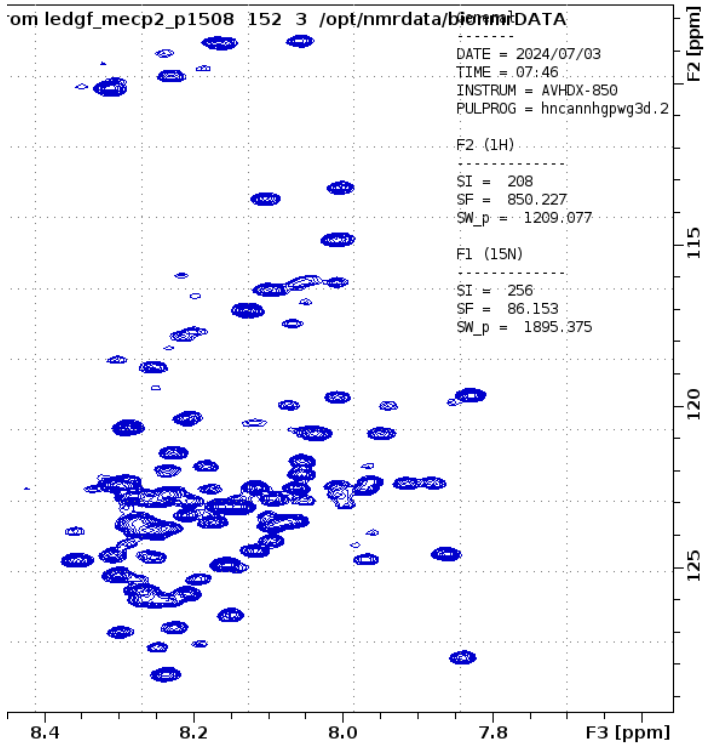
Spectrum	Visible Nuclei	Length
3D-HN(CA)NNH	N(i)-H(i)-N(i+1)	3 days
3D-H(NCA)NNH	N(i)-H(i)-H(i+1)	3 days
HNCACB	N(i-1)-H(i-1)-CA(i-1 or i)-CB(i-1 or i)	3 days
HN(CO)CACB	N(i-1)-H(i-1)-CA(i)-CB(i)	3days
HN(CA)CO	N(i-1)-H(i-1)-C'(i-1 or i)	1.5 days
HNCO	N(i-1)-H(i-1)-C'(i)	1.5 days

disordered regions; both ordered and disordered regions

- **Suitable for protein concentrations 200-400 µM**
- **Bad proton peak dispersion in IDPs leads to crowding in**

3D-H(NCA)NNH spectrum (right image).

- **If crowding is excessive, then 5D experiments must be employed.**



5D experiments for backbone assignment

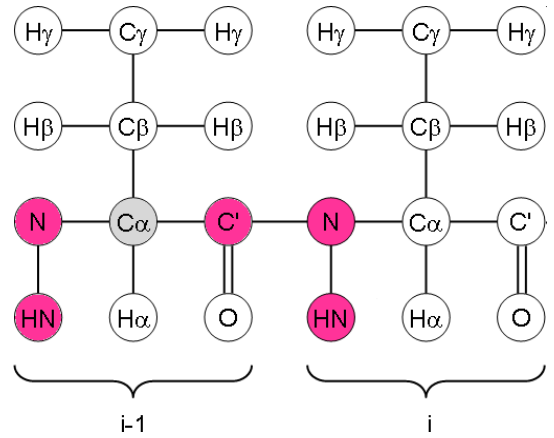
RNA polymerase δ subunit, 20.3 kDa, 172 residues, 800 µM

Disordred C-terminal region (81 residues)

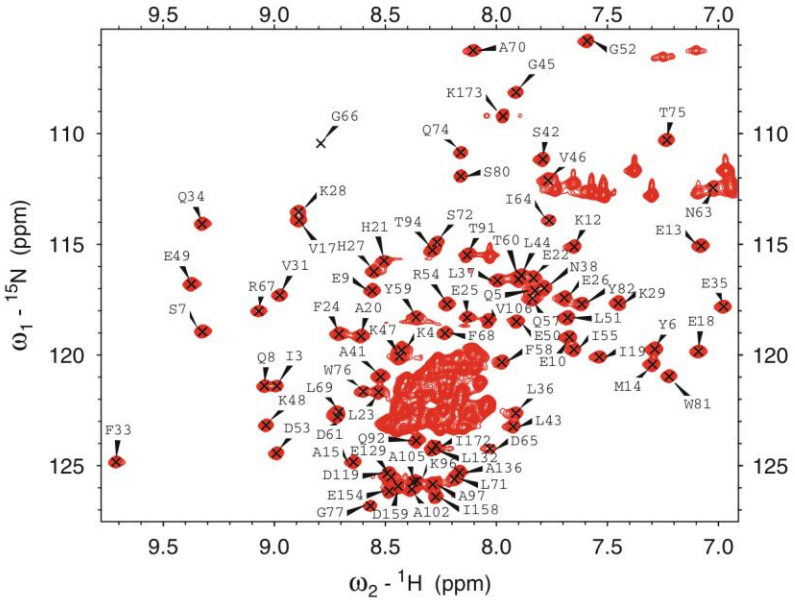
```
 GIKQYSQEE LKEMALVEIA HELFEEHKKP VPFQELLNEI
ASLLGVKKEE LGDRIAQFYT DLNIDGRFLA LSDQTWGLRS
WYPYDQLDEE TQPTVKAKKK KAKKAVEEDL DLDEFEEIDE
DDLDLDEVEE ELDLEADDFD EEDLDEDDDD LEIEEDIIDE
DDEDYDDEEE EIK
```

5D HN(CA)CONH

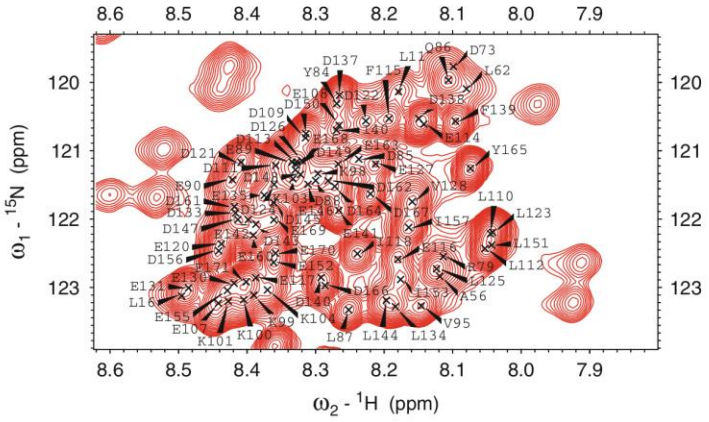
J Biomol NMR. 2010 Nov;48(3):169-77.

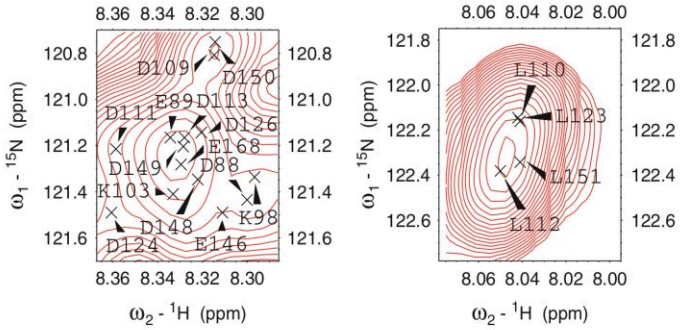


- **3D experiments for sequential assignment are inappropriate for this case.**



1H,15N-HSQC spectrum



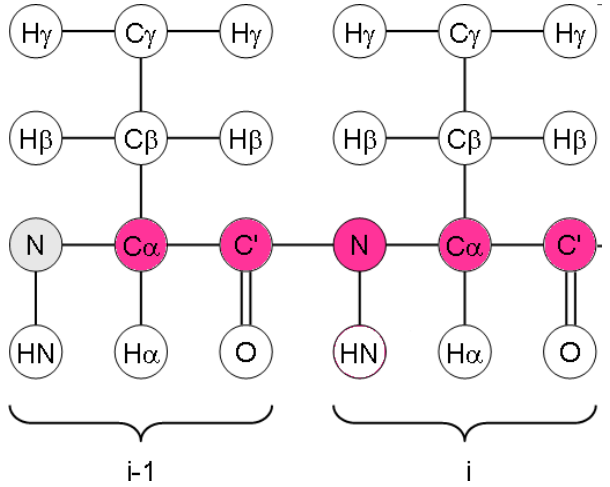


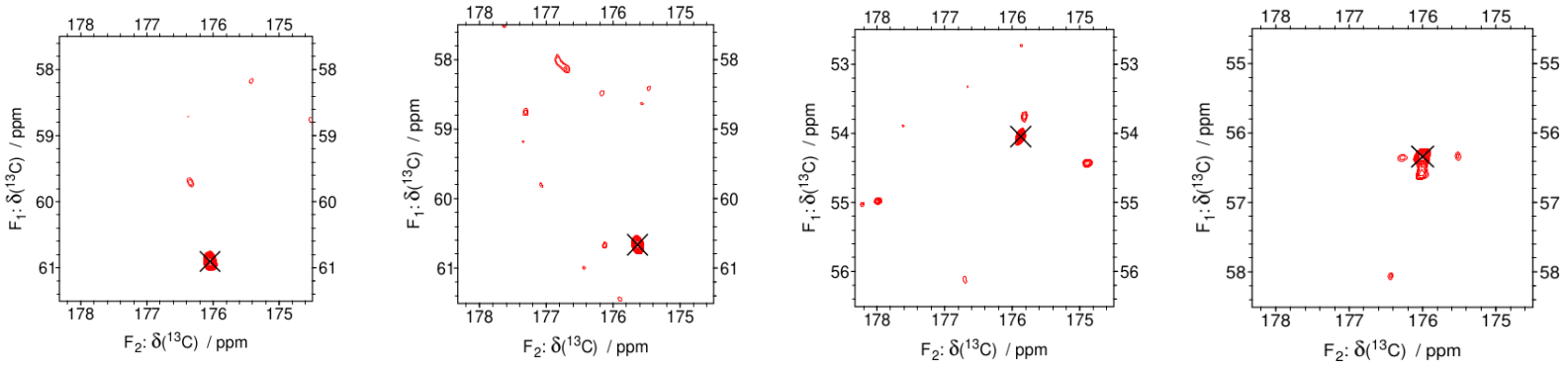
5D experiments for backbone assignment

```
 GIKQYSQEE LKEMALVEIA HELFEEHKKP VPFQELLNEI
ASLLGVKKEE LGDRIAQFYT DLNIDGRFLA LSDQTWGLRS
WYPYDQLDEE TQPTVKAKKK KAKKAVEEDL DLDEFEEIDE
DDLDLDEVEE ELDLEADDFD EEDLDEDDDD LEIEEDIIDE
DDEDYDDEEE EIK
```

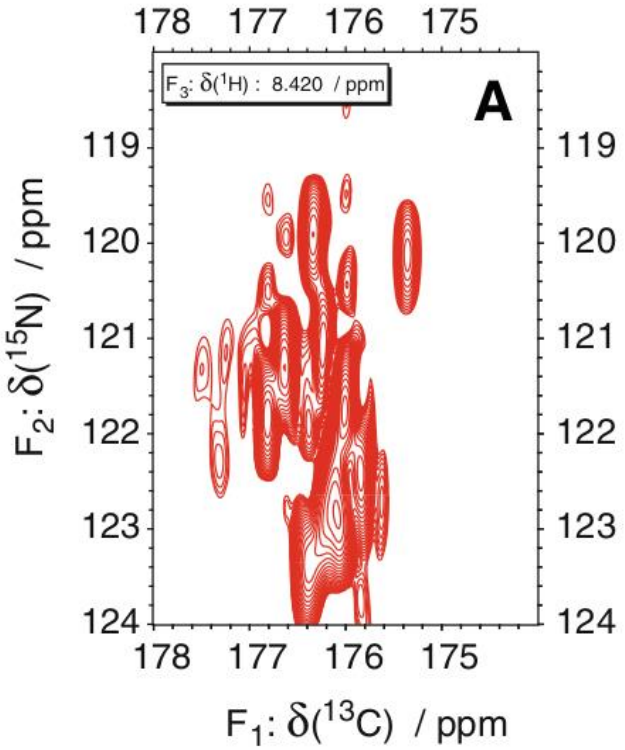
- **Sequential repeats cause severe clustering of backbone N-H peaks, even for 5D HN(CA)CONH.**

5D CACONCACO J Biomol NMR. 2011 May;50(1):1-11.





D159 E160 D161 D162



Top: Cα-C' cross-sections from 5D CACONCACO.
Right: plane from 3D HNCO showing the region around D161 and E160.

Additional 5D and 3D experiments

5D CACONCACO has **higher resolution** than **5D HN(CA)CONH** by avoiding the crowded HN dimension, but requires **higher protein concentration (>600 µM) or longer measurement**.

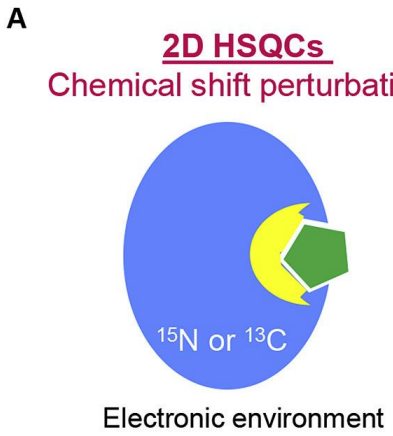
After measuring the 5D of the apo form, **3D titration experiments** can be employed to **map backbone interactions** with other molecules:

- **3D HNCO** for **5D HN(CA)CONH.**
- **3D CA(CO)NCA(CO)** for **5D CACONCACO**.

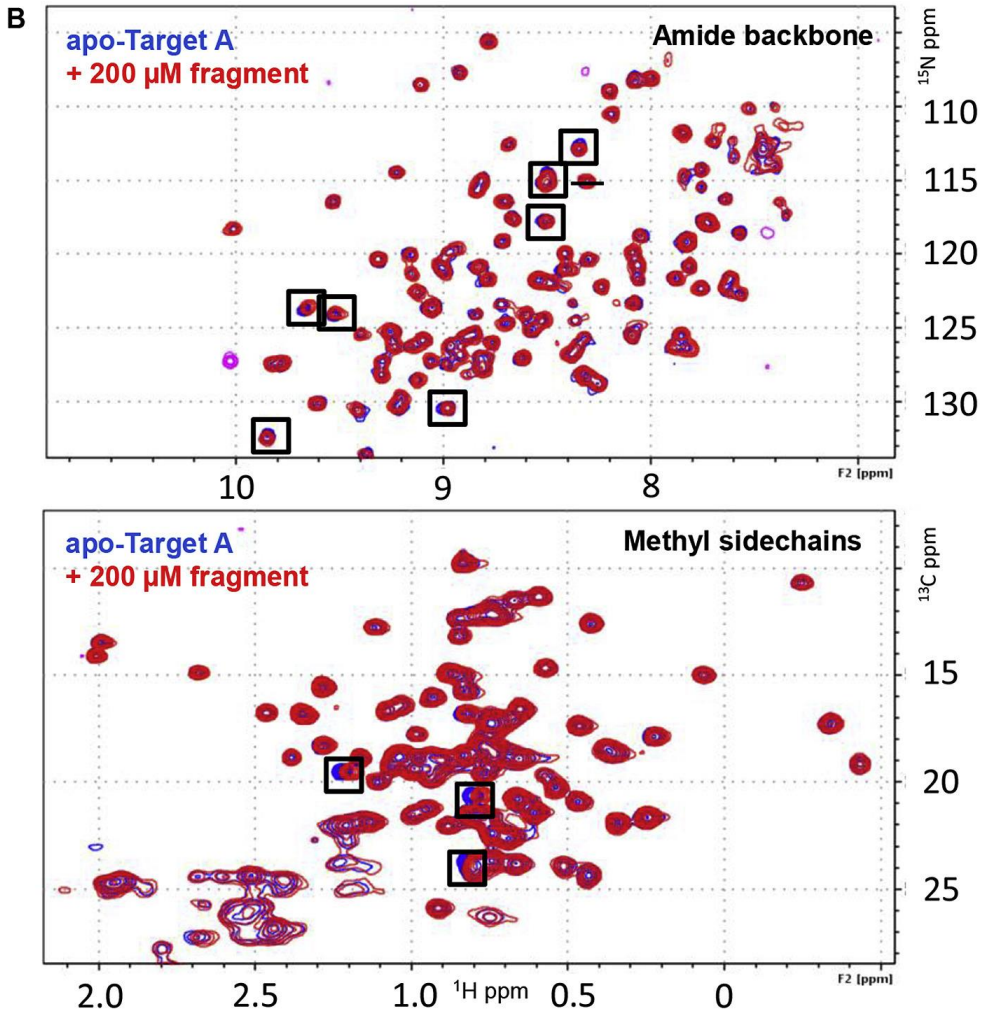
Two additional **5D experiments** provide **chemical shifts of aliphatic side chain** atoms: **HabCabCONH, HC(CC-TOCSY)CONH**

Fragment Screening by Target-Observed NMR:

(A) Cartoon depiction of target-observed NMR binding assays. Yellow regions experience chemical shift changes upon fragment binding.



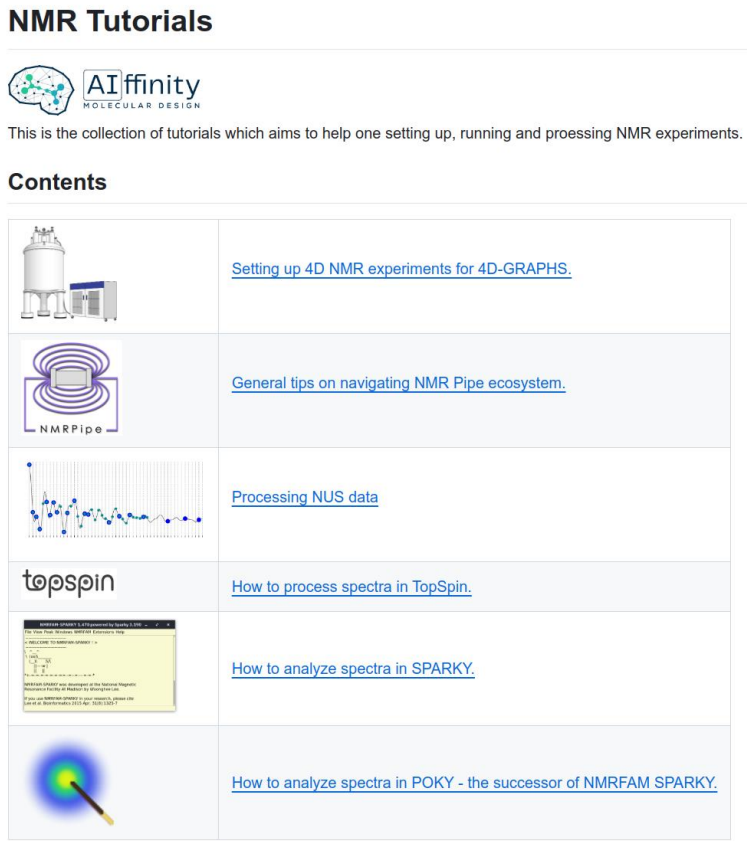
(B) 2D 1H-15N or 1H-13C HSQC experiments detect **weak** fragment binding to protein target A and map binding sites with known resonance assignments. Boxed cross-peaks indicate perturbed residues.



Arch Biochem Biophys. 2017 Aug 15:628:132-147.

AI|ffinity contributes to the NMR community

- GitHub tutorials: https://github.com/AI-ffinity/nmr_tutorials
- Topics covered:
  - Setting up 1D to 4D protein experiments in Topspin.
  - Processing non-uniformly sampled (NUS) 3D and 4D spectra.
  - Peak picking and other spectra processing with POKY.
- Future video tutorials on YouTube.



NMR Tutorials

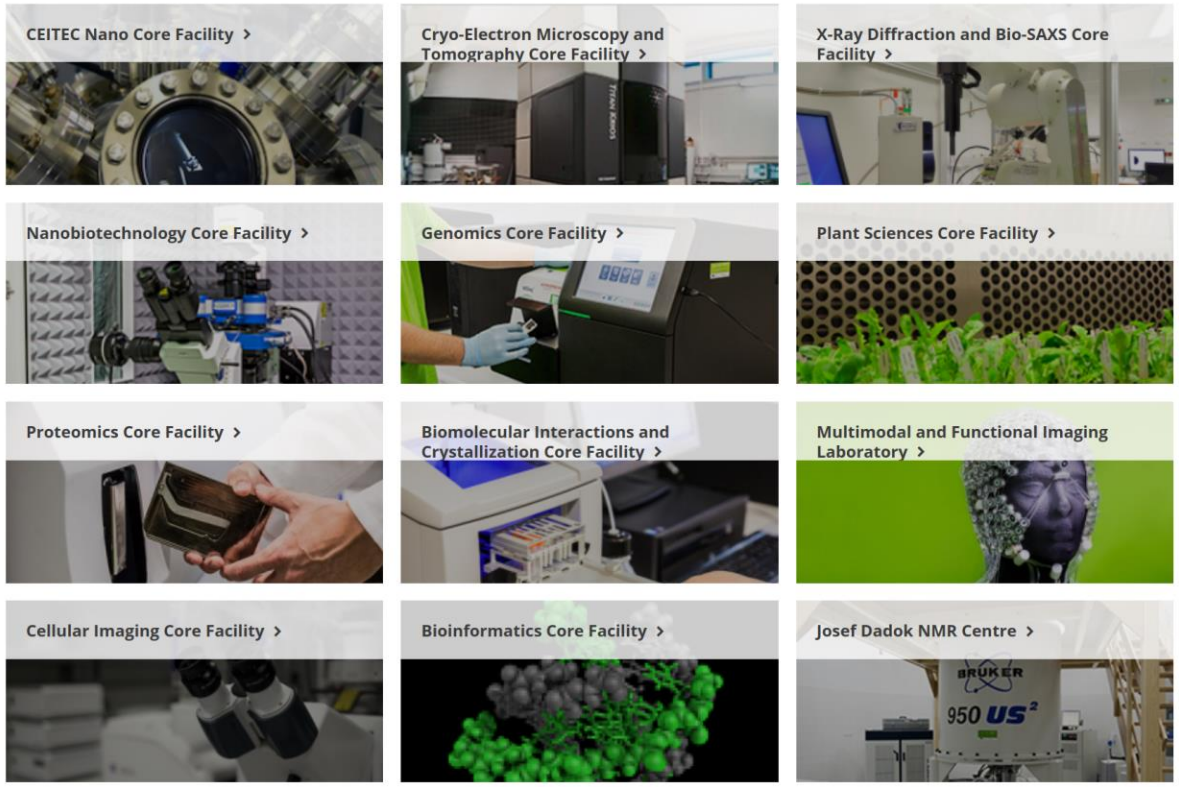
AIffinity MOLECULAR DESIGN

This is the collection of tutorials which aims to help one setting up, running and proessing NMR experiments.

Contents

	Setting up 4D NMR experiments for 4D-GRAPHS.
NMRPipe	General tips on navigating NMR Pipe ecosystem.
	Processing NUS data
topspin	How to process spectra in TopSpin.
	How to analyze spectra in SPARKY.
	How to analyze spectra in POKY - the successor of NMRFAM SPARKY.

Privileged access to full-fledged biophysical facilities



- **structure determination** (NMR, X-ray, SAXS)
- **compound screening** (MST, SPR)
- **binding validation & interactions mapping** (HDX-MS, CD, SEC, ITC)

Joint Drug Development

AIffinity MOLECULAR DESIGN

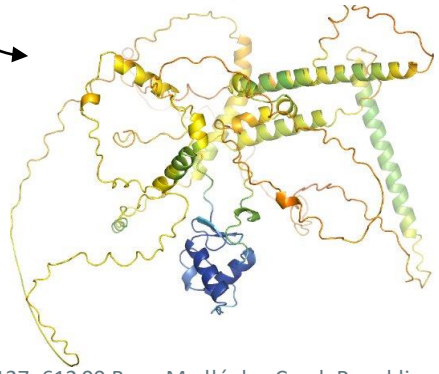
CEITEC Central European Institute of Technology BRNO | CZECH REPUBLIC

NEXMR

安宏生醫 AnHorn Medicines Innovation · New Therapeutics

MUNI SCI Department of Chemistry

Indication	Target	Drug type	Hit discovery	Hit to Lead	Lead Optimization	Pre-clinical Development
Prostate cancer	Androgen Receptor	PROTAC	→			
Colorectal cancer	Epigenetic Regulator	Small mol.	→			



AI|ffinity Team (total FTE 8.5)

Thomas Evangelidis, M.Res., M.Phil., Ph.D.
Founder, Chief Executive Officer & Chief Technology Officer

Lukasz Kozaczkiewicz, M.Sc., Ph.D., M.Eng.
Computational Biology Software Developer

Martina Poukarová, M.BA.
Back-Office Manager

Marcela Mádlíková, M.Sc.
Protein Biochemist

Vaclav Hanzl, M.Eng., Ph.D.
Chief Software Engineer

Íñigo Alcalde, M.Sc., Ph.D.
Computational Biophysics Software Developer

Jakub Jakubec, M.Sc.
PhD Student

Tereza Vučková, M.Sc.
Protein Biochemist

Alzbeta Tuerkova, M.Sc., Ph.D., M.BA.
Operations & Development Manager

Ekaterina Burakova, M.Sc., Ph.D.
NMR & Data Scientist

Khanh Nguyễn Tấn, M.Sc.
PhD Student

Scientific advisors

Pavel Polishchuk
Cheminformatics & Drug Design

Václav Veverka
NMR & Drug Design

Karel Kubíček
Protein NMR

Jiří Filipovič
High-performance computing & automatization

Daniel Svozil
Cheminformatics & Drug Design

Business advisors

Vojtěch Kadlec
UNICO

Veronika Štěpánková

AIffinity
MOLECULAR DESIGN

"Focus and excel"

Dr. Thomas Evangelidis
Founder & CEO
tevang@aiffinity.com

Supported by:

UNICO

JIC

MUNI Masaryk University

CEITEC
Central European Institute of Technology
BRNO | CZECH REPUBLIC

AI |ffinity s.r.o.
IČO 11992719
Purkyňova 127,
612 00 Brno-Medlánky,
Czech Republic

www.aiffinity.com | info@aiffinity.com