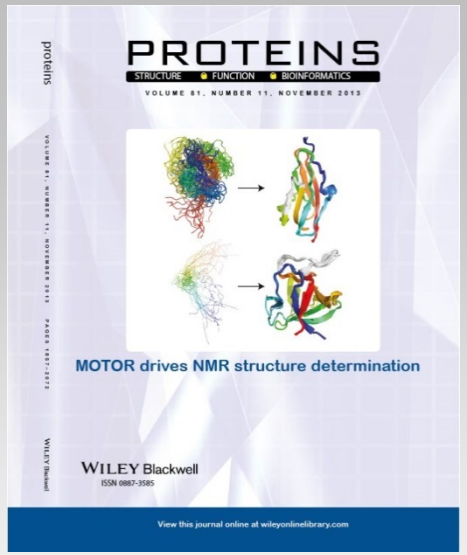
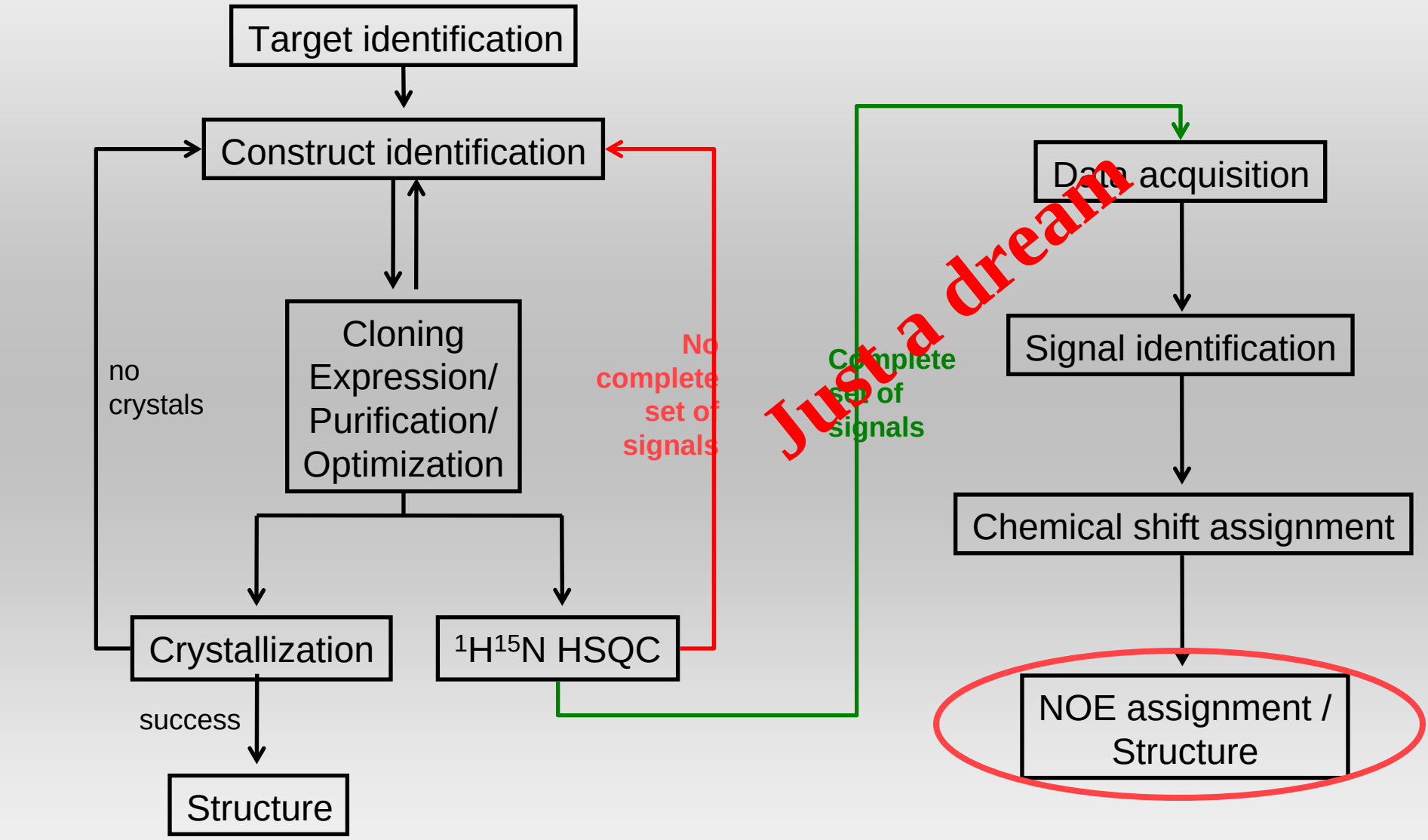
MOTOR: MOdel assisTed sOftware for NMR structure determination



Ulrich Schieborr

Protein structure determination…
(…snapshot from a dream of an NMR spectroscopist)



Practice: Structural projects reaching NMR

No crystals after:

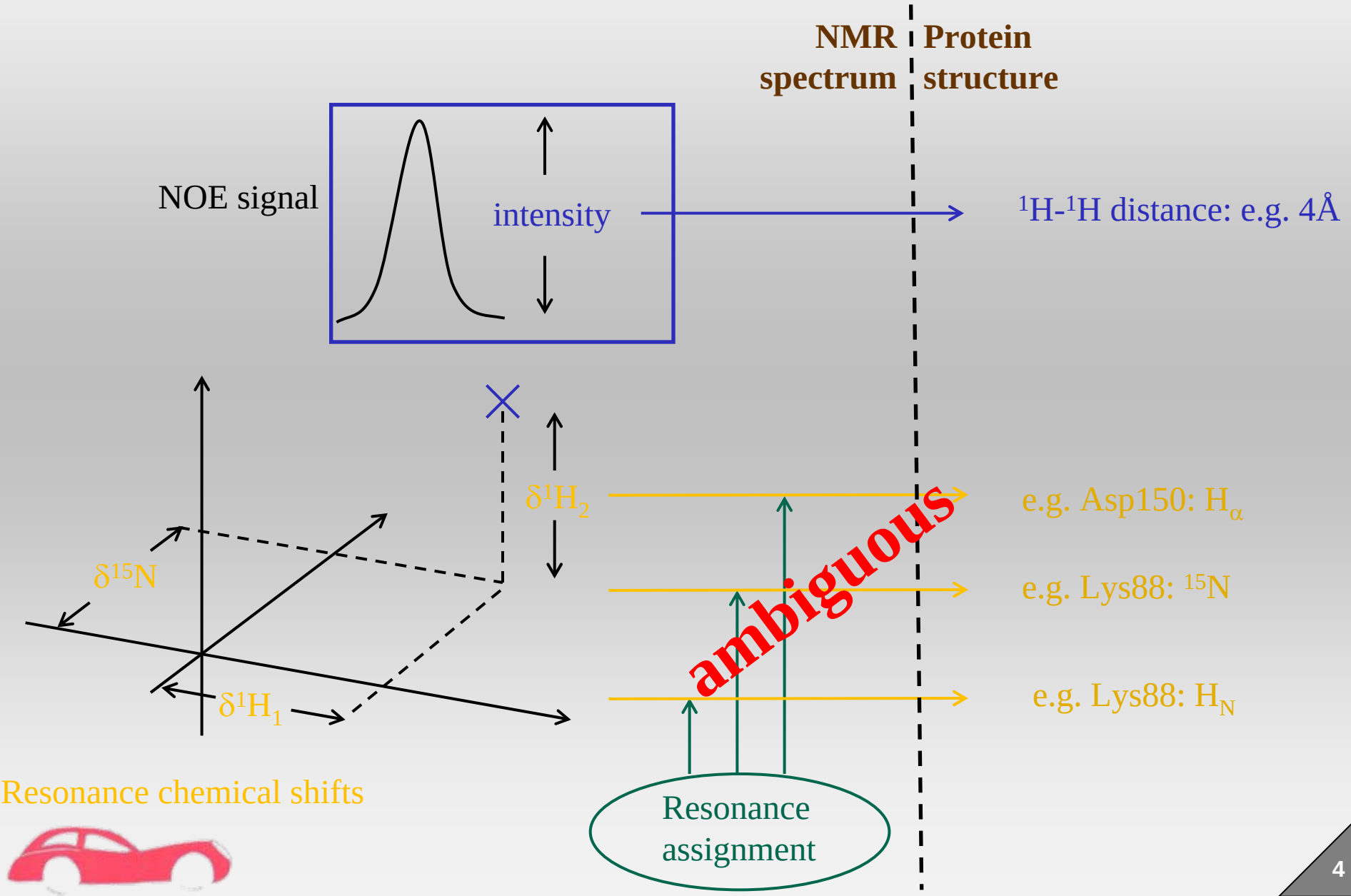
- initial trials
- construct optimization
- buffer optimization
- co-crystallization with natural ligand/co-factors
- co-crystallization with known inhibitor
- seeding
- ……..

Crystallization problems could be (amongst others) due to

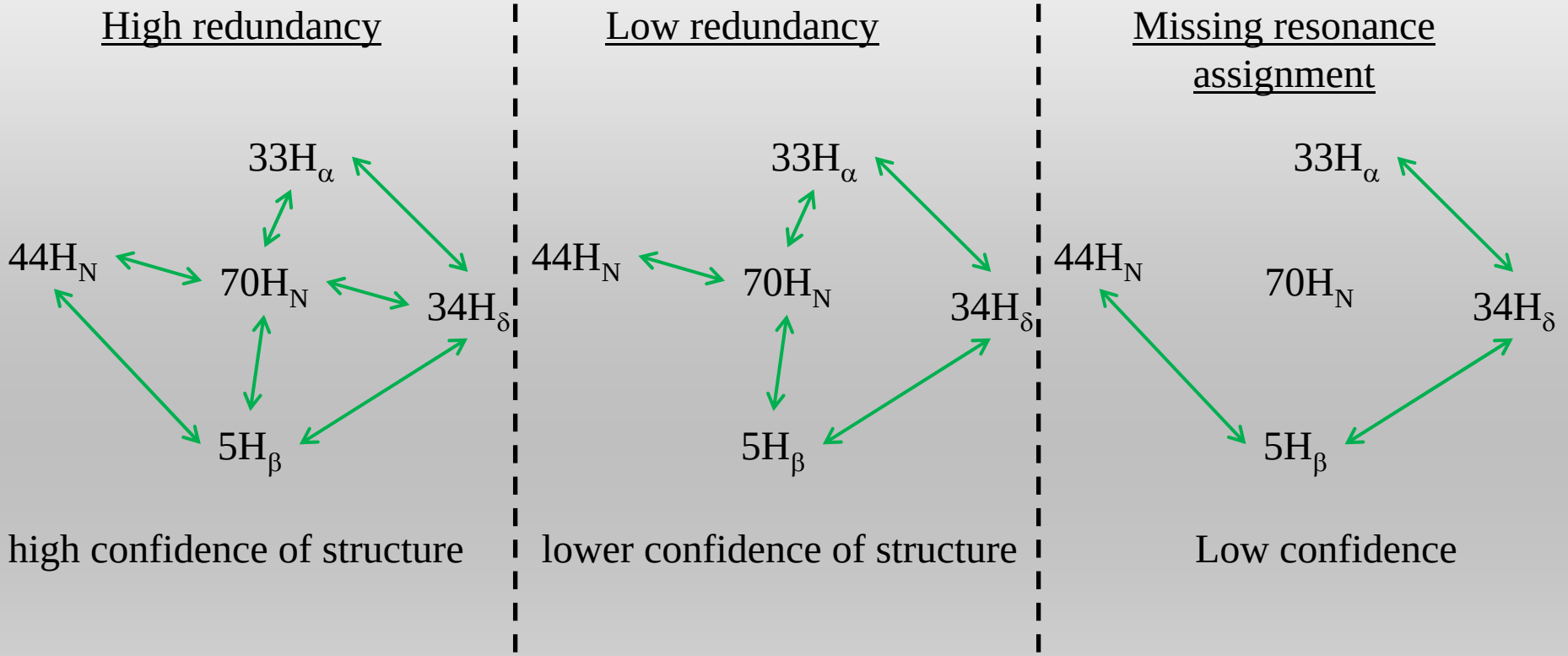
- intrinsically unstructured protein
- heterogeneous sample
- flexibility
- partially unstructured

Non-crystallizing proteins often show incomplete set of NMR signals

NOE driven structure determination



Self consistency of NOE assignment



Boot strapping NOE assignment strategies:

- Rely on self consistency in 3D space
- Need high number of NOE distances in all parts of the structure
- Fail for resonance assignment < ~90% of all nuclei

Challenges: NMR structure determination

Imperfect NOE data:
- missing peaks
- additional “noise peaks”
- imperfect peak picking
- peak overlap

Ambiguity of NOE assignment due to:
- limited precision of chemical shift assignment
- chemical shift differences in different dimensions and spectra
- (almost) same chemical shift for different nuclei

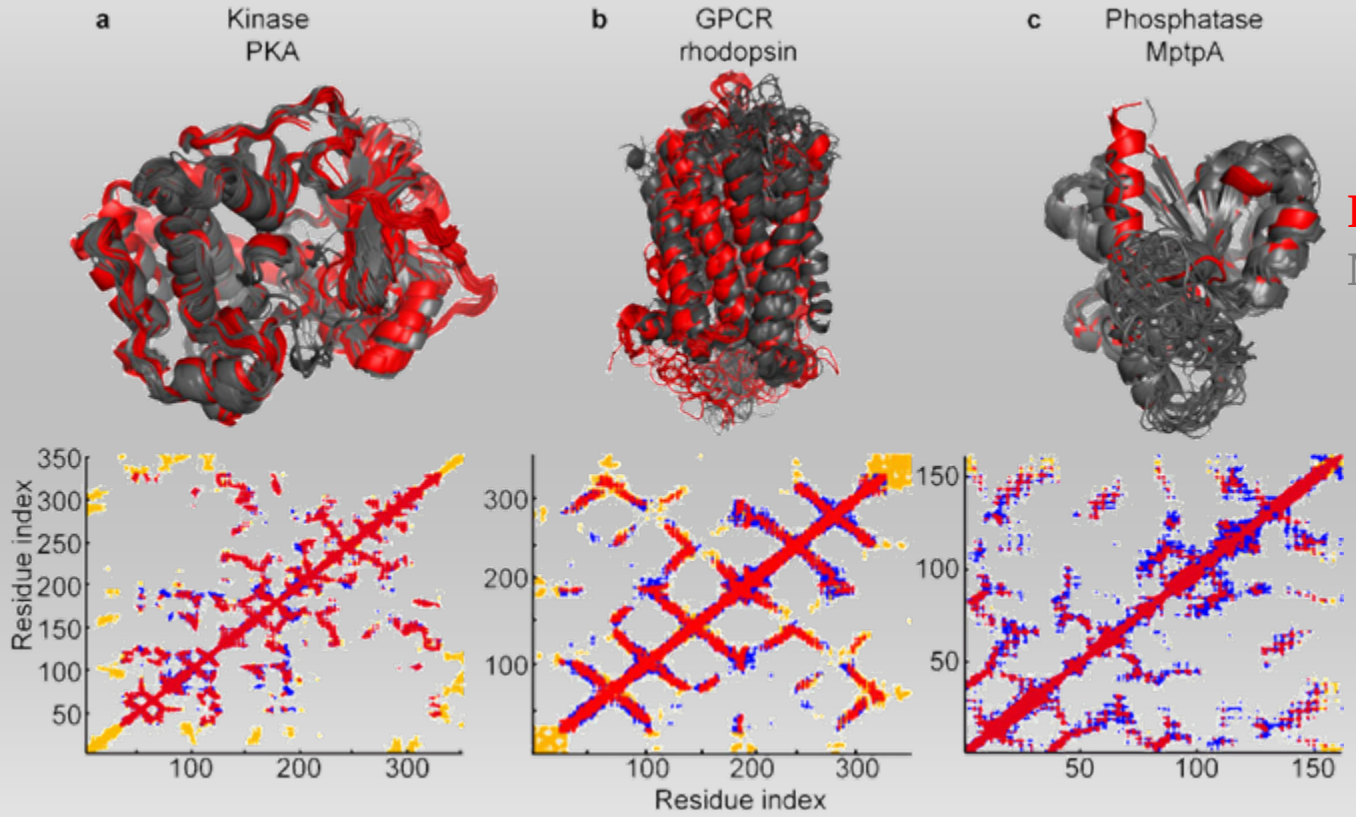
Missing chemical shift assignment due to:
- intrinsically disordered parts of the protein
- conformational heterogeneity
- conformational dynamics

MOTOR is software solving protein structures on the basis of sparse NMR data

The MOTOR approach

- Reduction of ambiguity by utilization of structural model
- No explicit reference structure used (no boot strapping)
- Instead: Global maximization of the number of self consistent NOE assignments as scoring function
- Consideration of different degrees of accuracy in measure of chemical shift values

Reduction of ambiguity: structural models



Protein structure

Model of protein structure

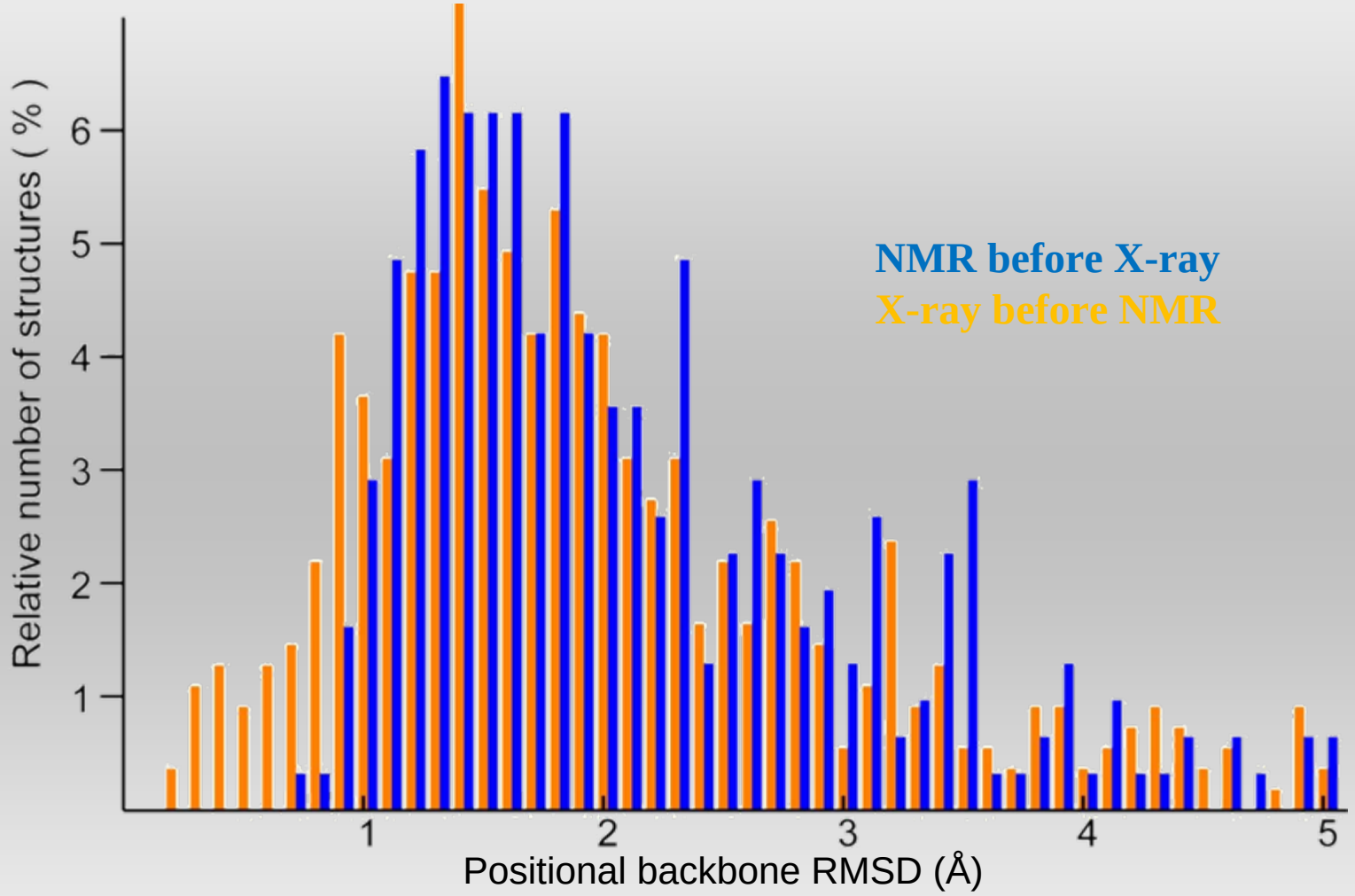
Disance <6A in both

Disance <6A only in protein

Disance <6A only in model

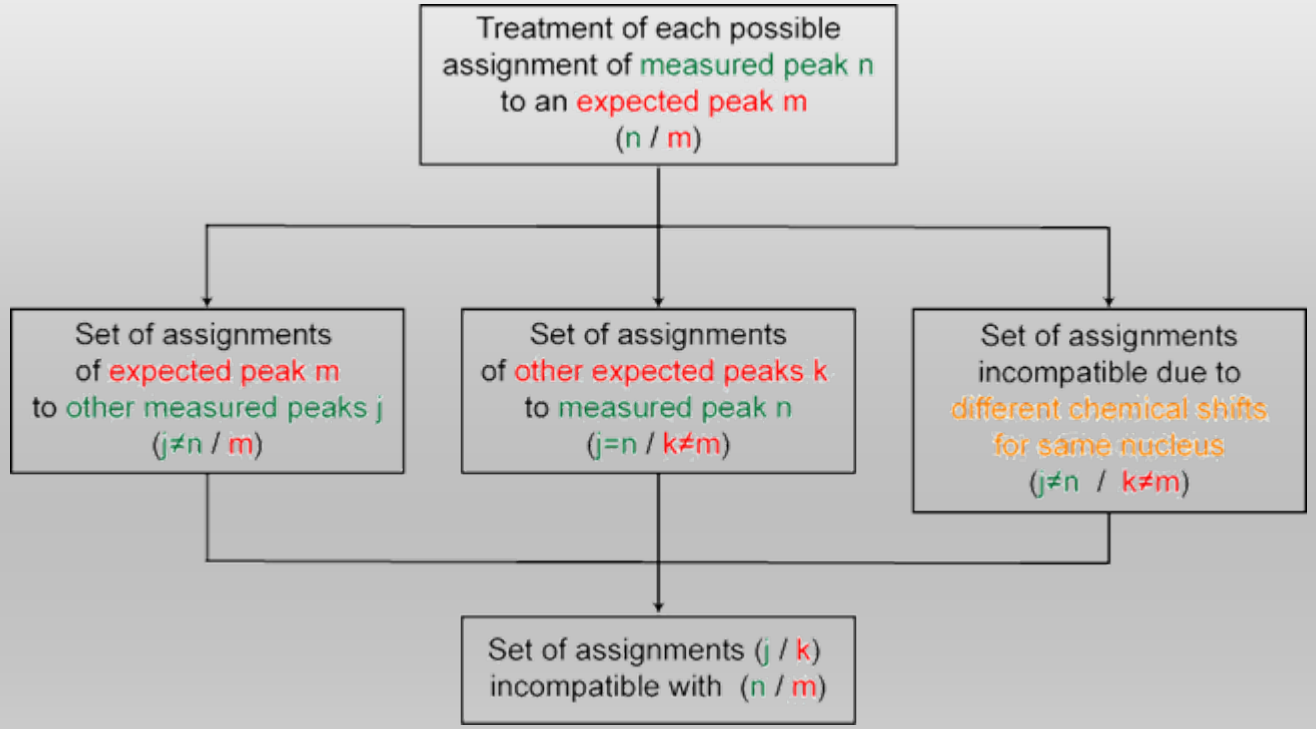
→ **Models help to predict contacts**

structural models: pdb-database analysis



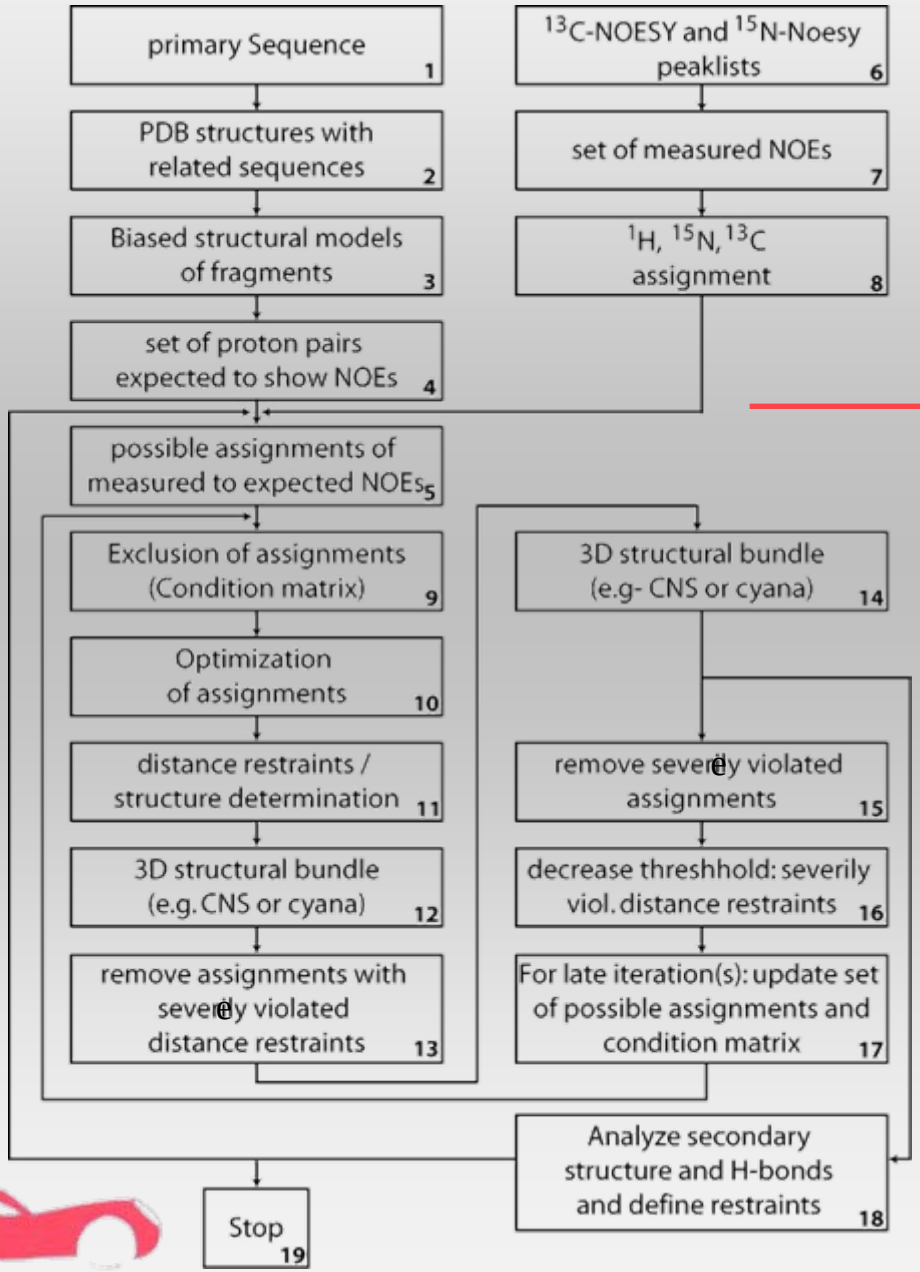
→ **Structural models have already been used and help to interpret the NMR data**

Consistency of pairs of NOE assignments



	Same spectrum		Different spectra
	Same dimension	Different dimension	
^{1}H	0.02 ppm	0.05 ppm	0.05 ppm
^{13}C	0.5 ppm	-	0.7 ppm
^{15}N	0.5 ppm	-	0.7 ppm

MOTOR: The general workflow

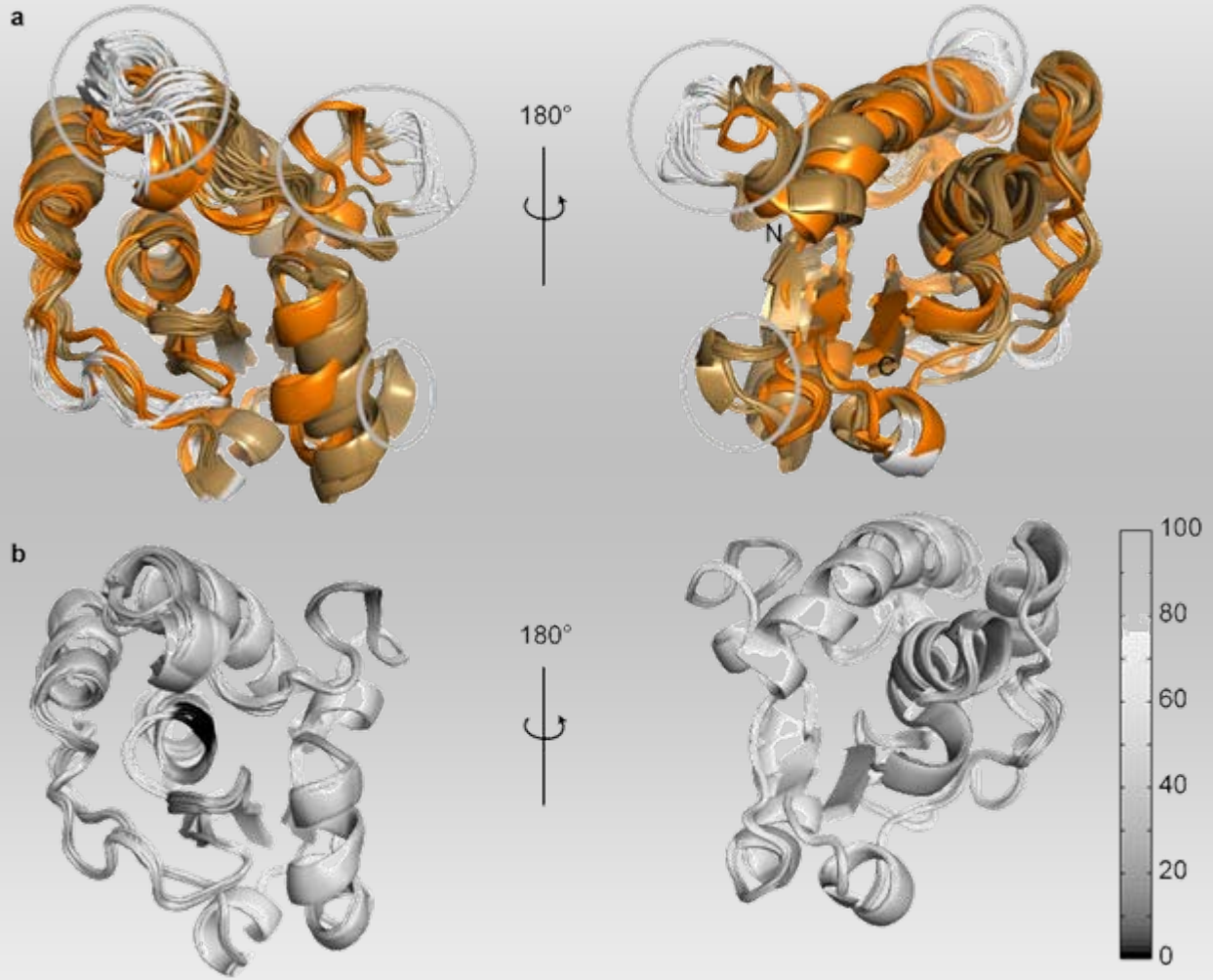


Data input and preparation

NOE-assignment and structure generation steps

Secondary structure identification

MptpA: The proof of principle



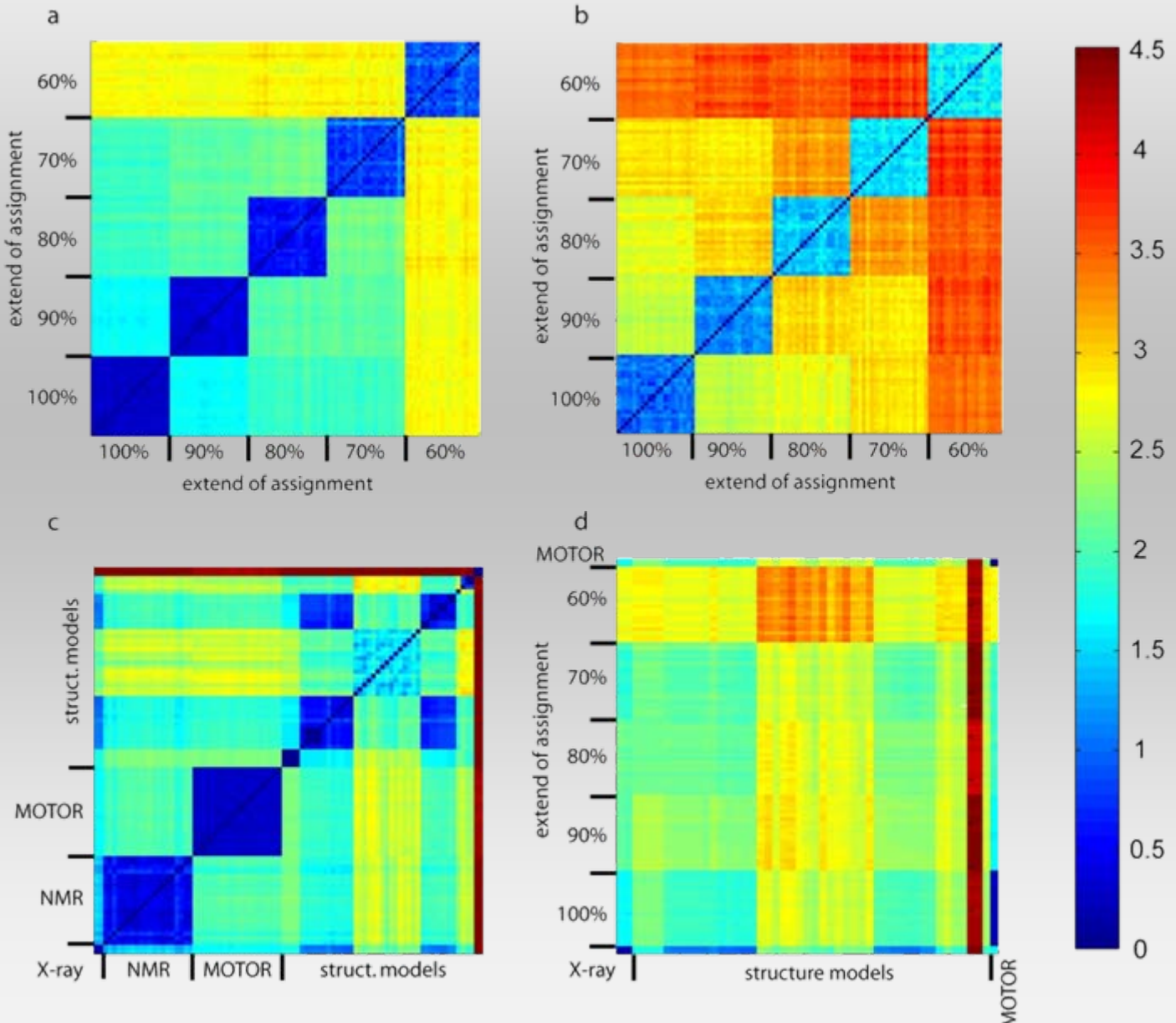
MOTOR

Reference structures

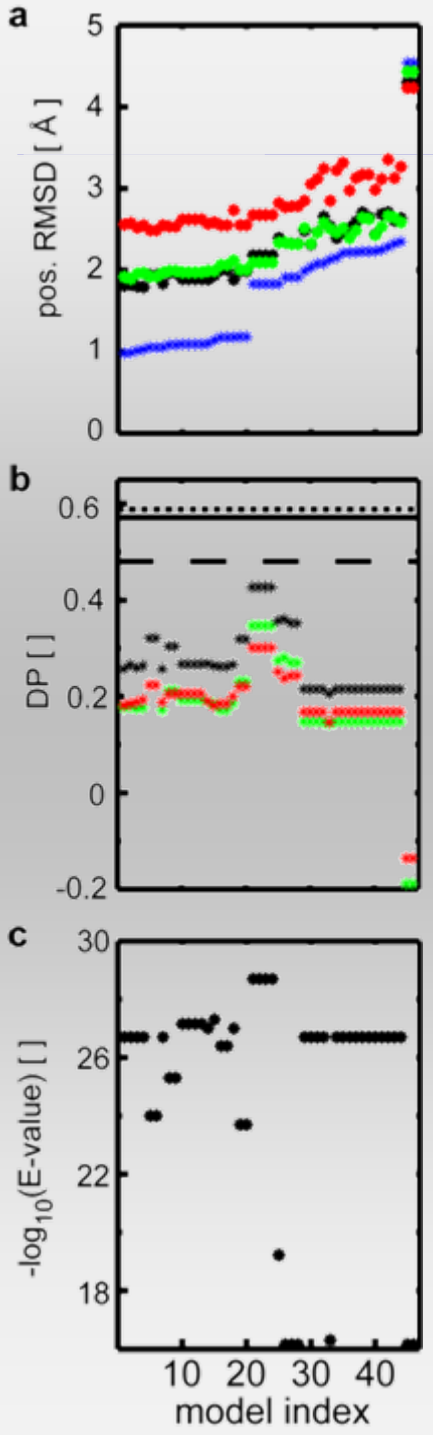
C_α B-factor >20 Å^2

MOTOR:
Assigned NOEs per residue

MptpA: Structure recognition with sparse data



MptpA: Quality control



a: pos. RMSD of models to X-ray and MOTOR (70-100%)

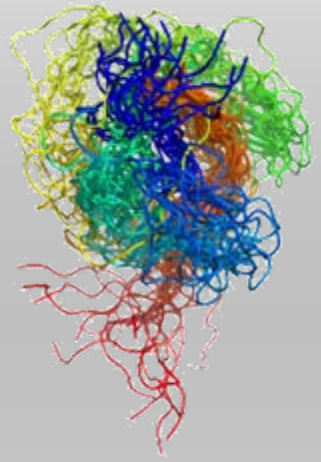
b: DP score from RPF analysis of models, MOTOR, published NMR and X-ray

c: E-values form blast analysis of model source structure prim. sequence to MptpA sequence

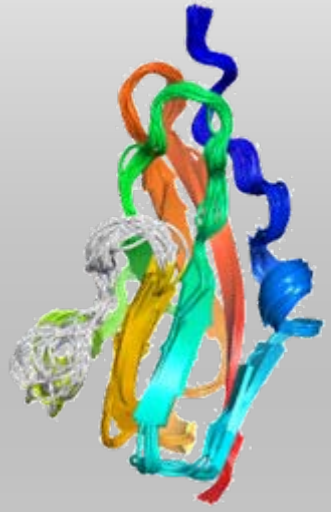
- X-ray structure
- MOTOR structures (100%)
- MOTOR structures (70%)
- MOTOR structures (60%)
- MOTOR structure (100%)
- Published NMR structure
- X-ray structure

MOTOR application: FGFR4 D2

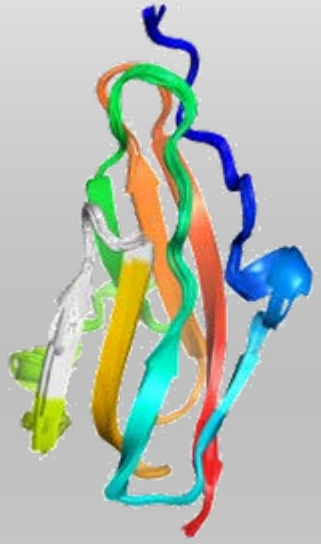
conventional structure determination



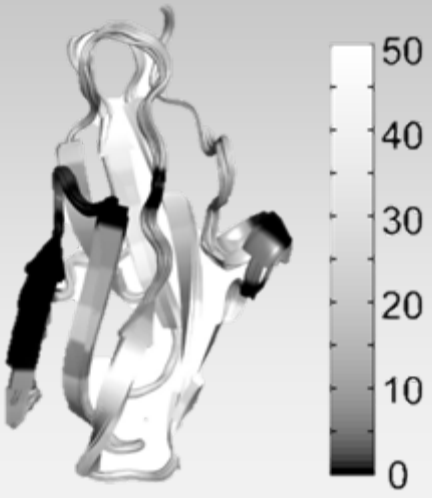
MOTOR: first calculation

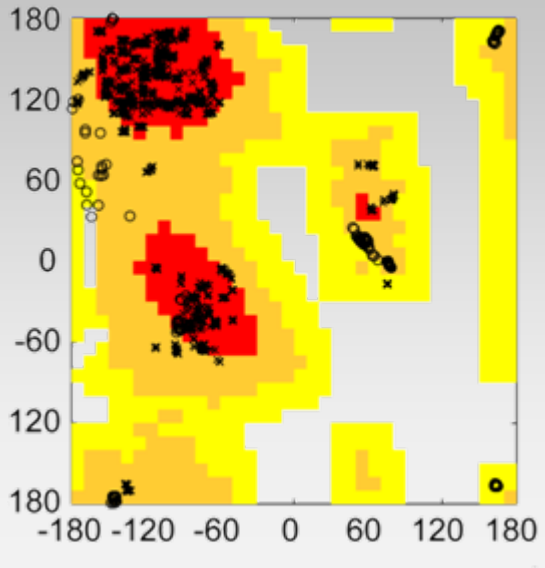


MOTOR: last calculation



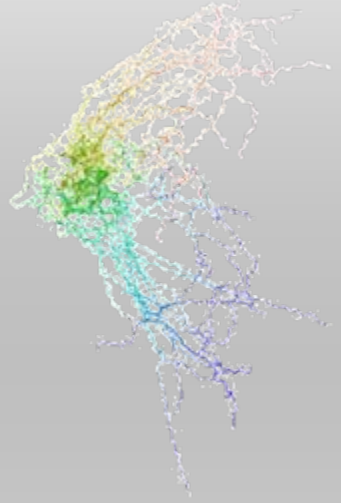
MOTOR: NOEs per residue





MOTOR application: FGF21

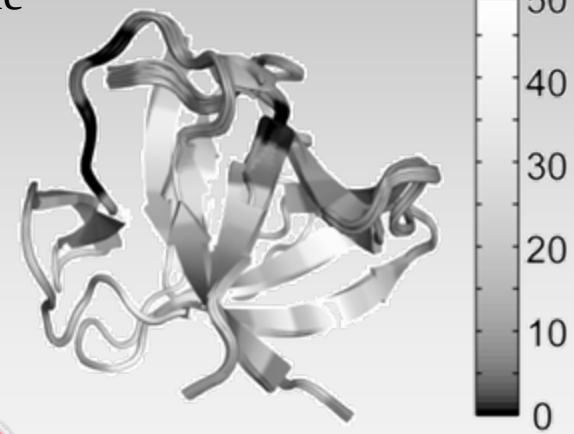
conventional structure determination

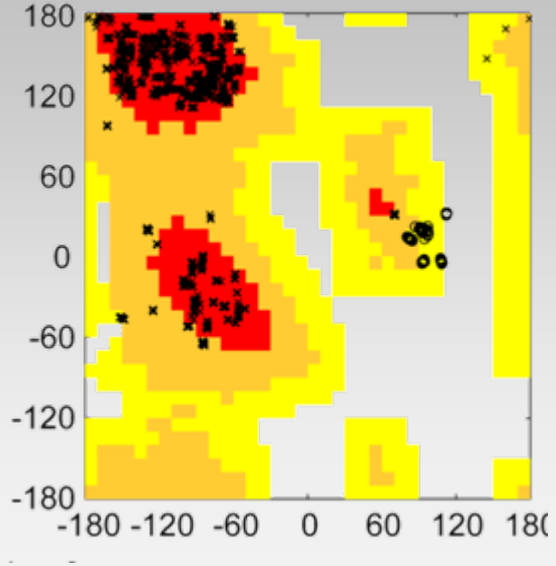


MOTOR: first calculation

MOTOR: last calculation

MOTOR: NOEs per residue





MOTOR: Minimal requirements

- 60-70% resonance assignment
- NOESY experiments (usually ^{13}C, ^{15}N and ^{13}C-aromatic)
- Protein structures with related primary sequences exist

How to start the MOTOR

Installation:

- External software
  - Structure generator (CNS or cyana)
  - Model generation: MODELER and blast
- Put the MOTOR binary to your path (e.g. /usr/local/bin/MOTOR)

Prepare calculation:

- Prepare structural models (scripts provided)
  - search for related structures (blast)
  - build structural models (MODELER)
- Optional: Run talos+ (we-nmr)
- Collect your data in one directory
  - peaks-files from the NOESYs
  - a prot-file for the sequential assignment
  - a file with the primary sequence
- edit the calculation parameter file

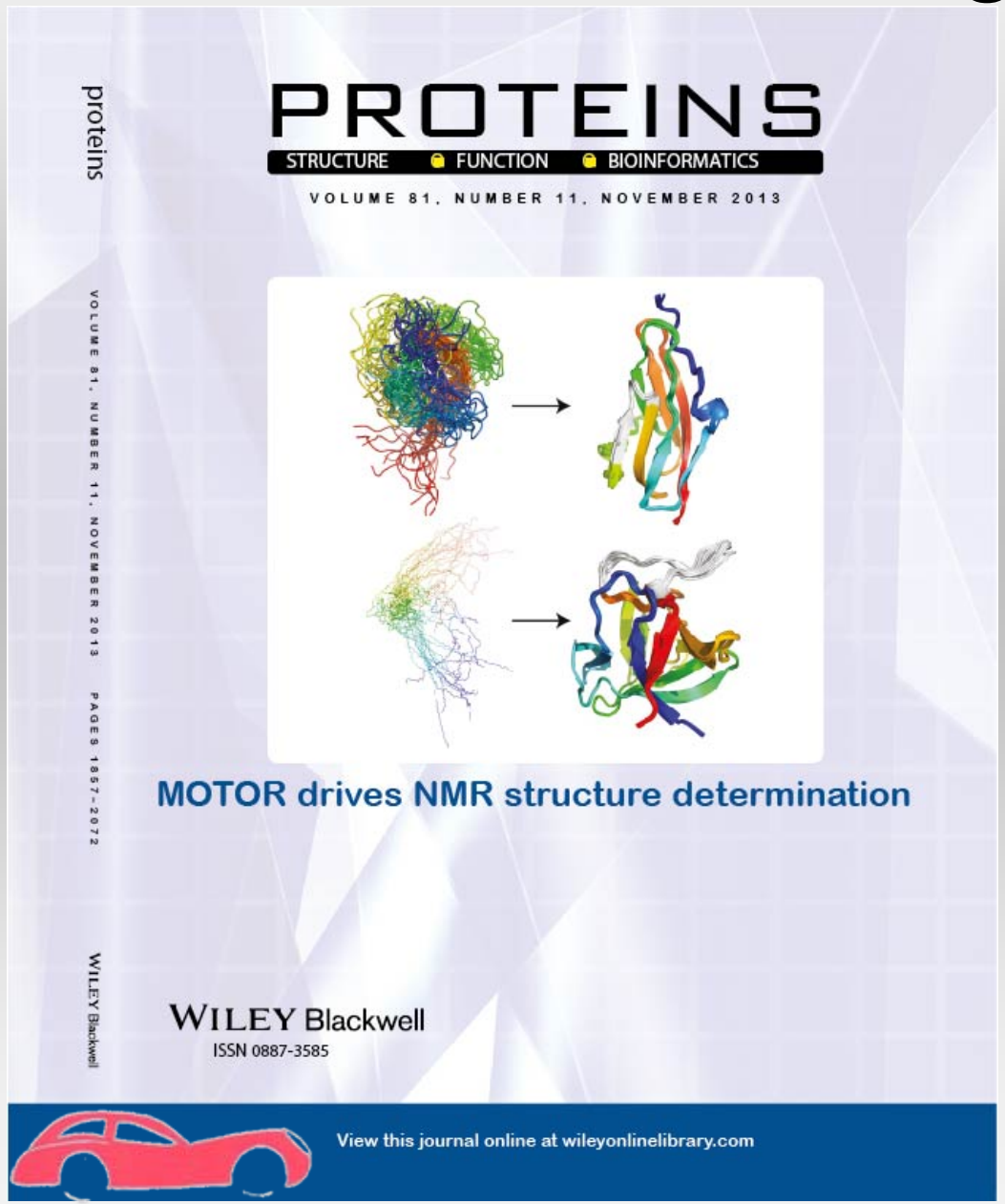
Start MOTOR:

- type “MOTOR”

Conclusions

- 70% assignment: MOTOR solves protein structures
- 60% assignment: Identification of “good models”
- Successful application of MOTOR to two *de novo* structures
- MOTOR is software free of charge for academic institutions without financial interests.
- **Collaborations very welcome**

Acknowledgements



Method discussion:
Harald Schwalbe

Molecular biology:
Krishna Saxena, Denis Kudlinzki, Santosh Gande (FGF21, FGFR4 D2), Tanja Stehle (MptpA)

NMR spectra and resonance assignment:
Sridhar Sreeramulu, Bettina Elshorst, Marcus Maurer

Backup

Observed per expected long range NOEs