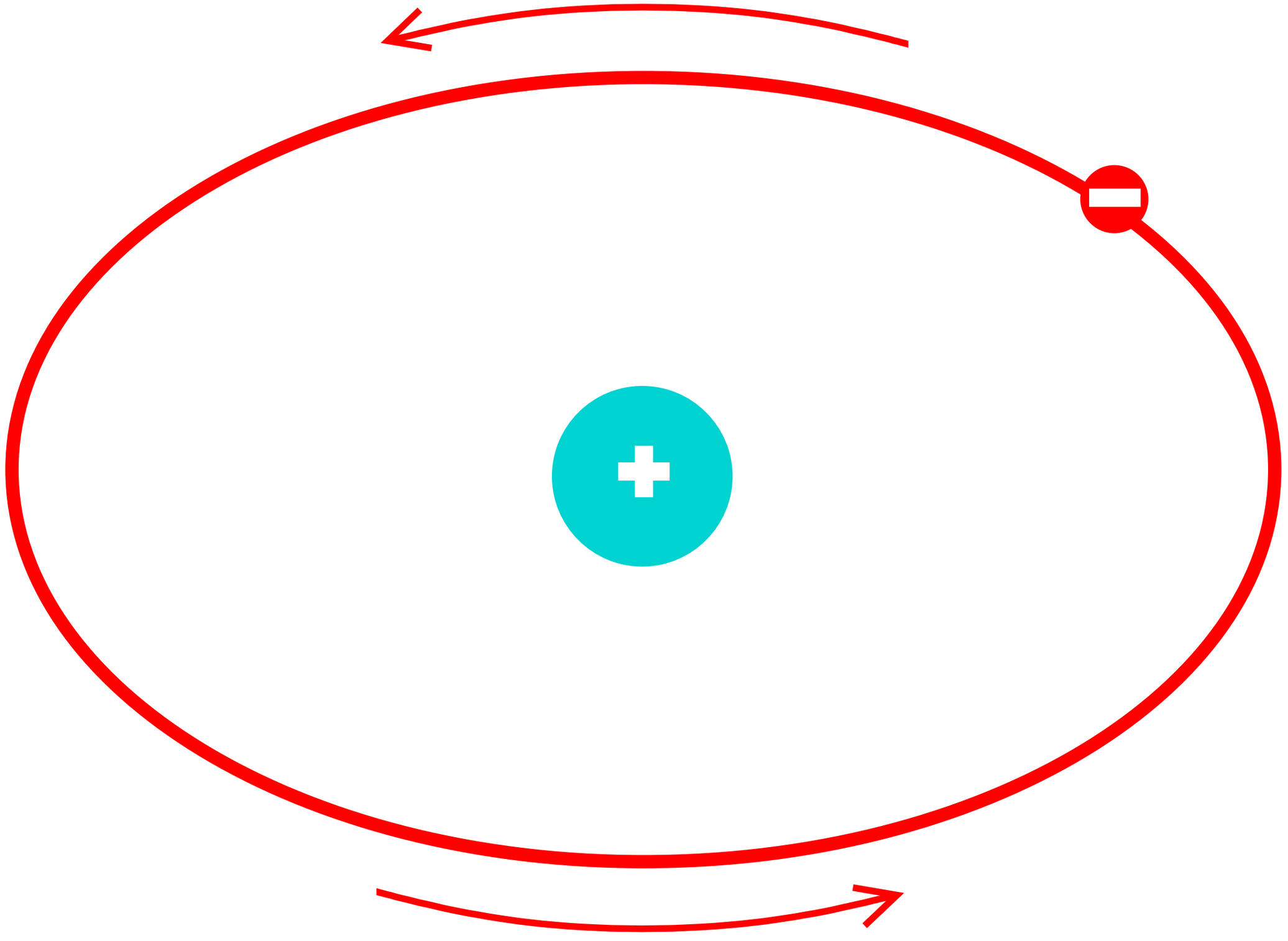
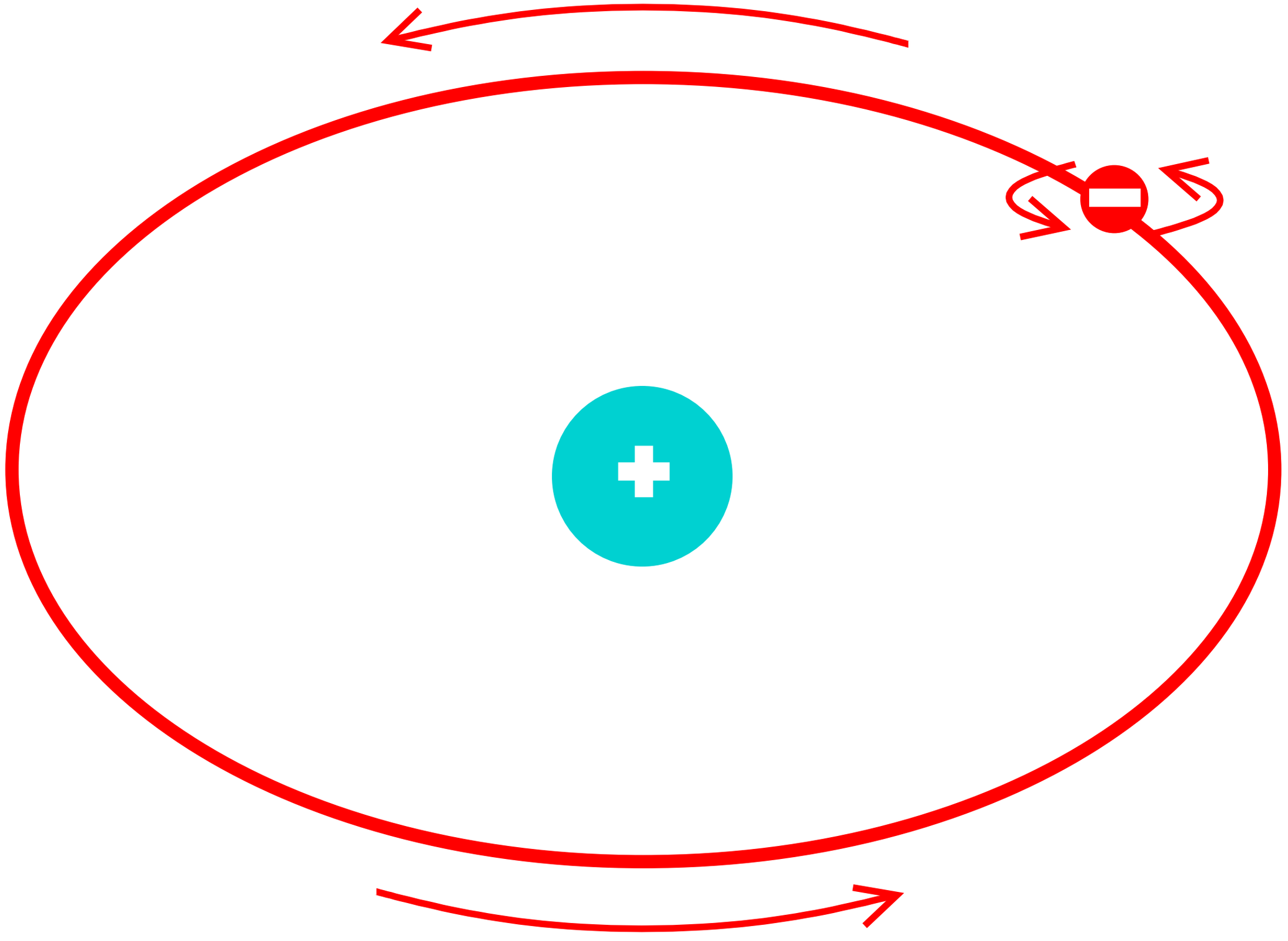
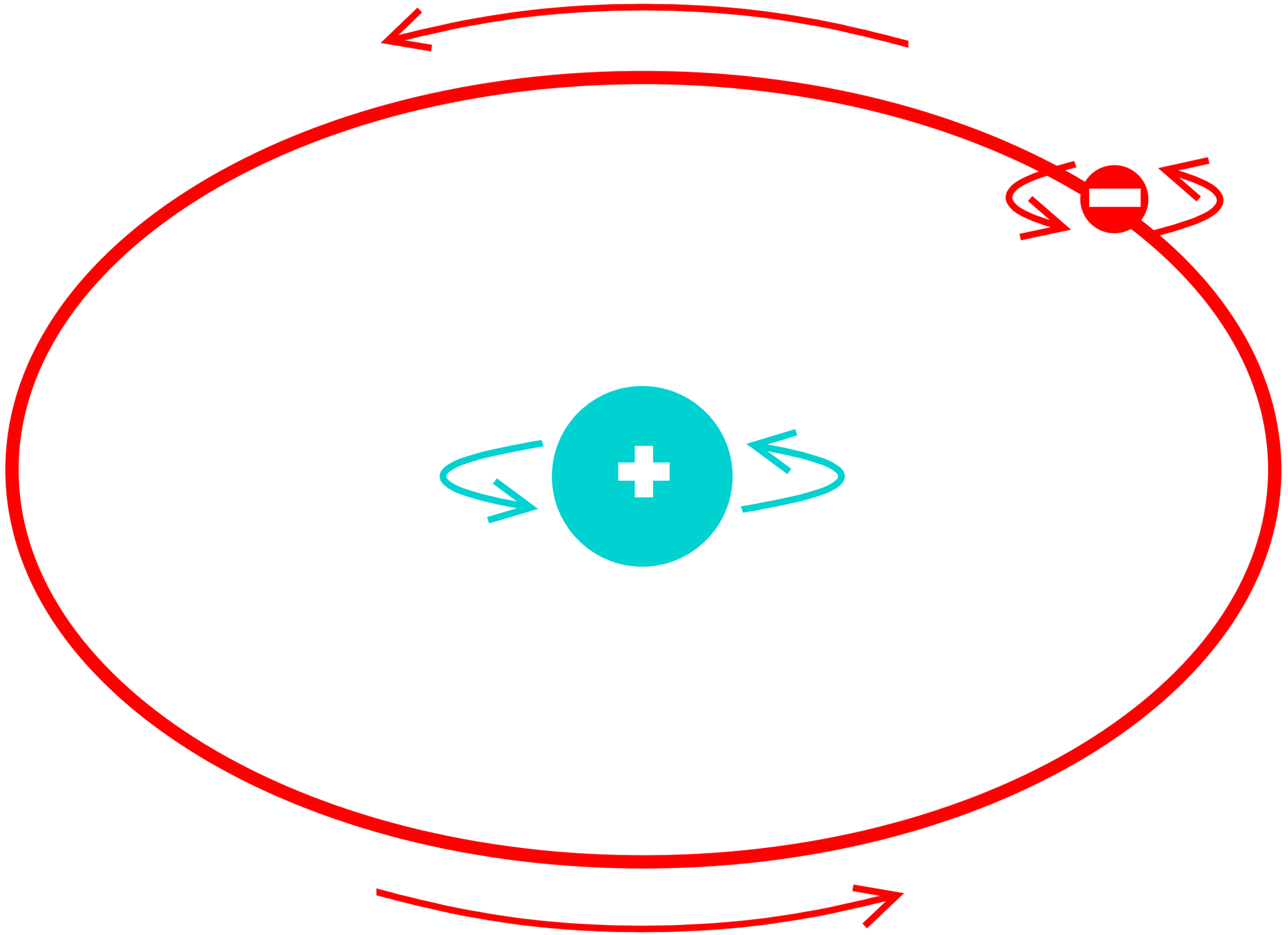
NUCLEAR MAGNETIC RESONANCE







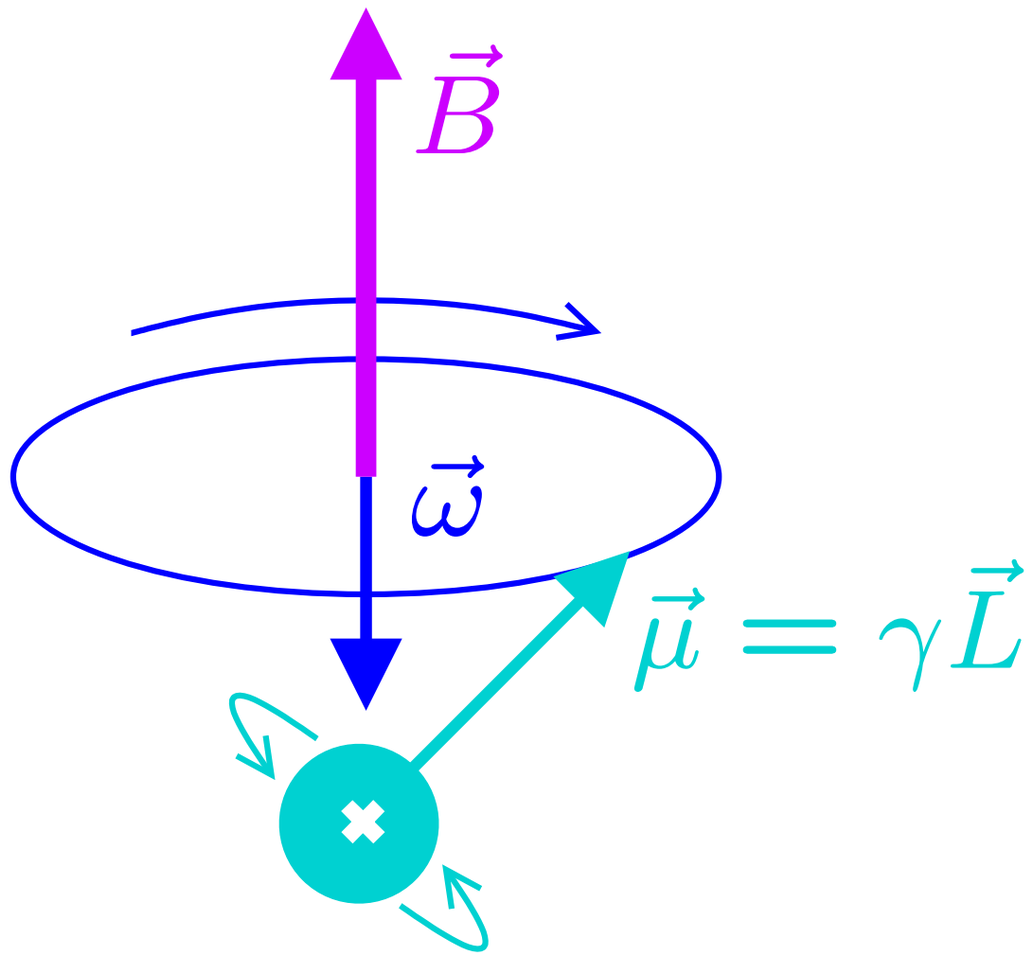
	S	$\frac{10^{-9}\gamma}{\mathrm{rad\,s^{-1}T^{-1}}}$	% v přírodě
e^-	1/2	−182,000	100
^{1}H	1/2	0,277	99,98
^{2}H	1	0,042	0,02
^{13}C	1/2	0,067	1,1
^{14}N	1	0,019	99,6
^{15}N	1/2	−0,027	0,4
^{17}O	5/2	−0,036	0,04
^{19}F	1/2	0,252	100
^{31}P	1/2	0,108	100
^{129}Xe	1/2	−0,075	24,4

Počet stacionárních stavů $= 2S + 1$

kvadrupolární jádra (příliš rychle se vrací do rovnováhy)

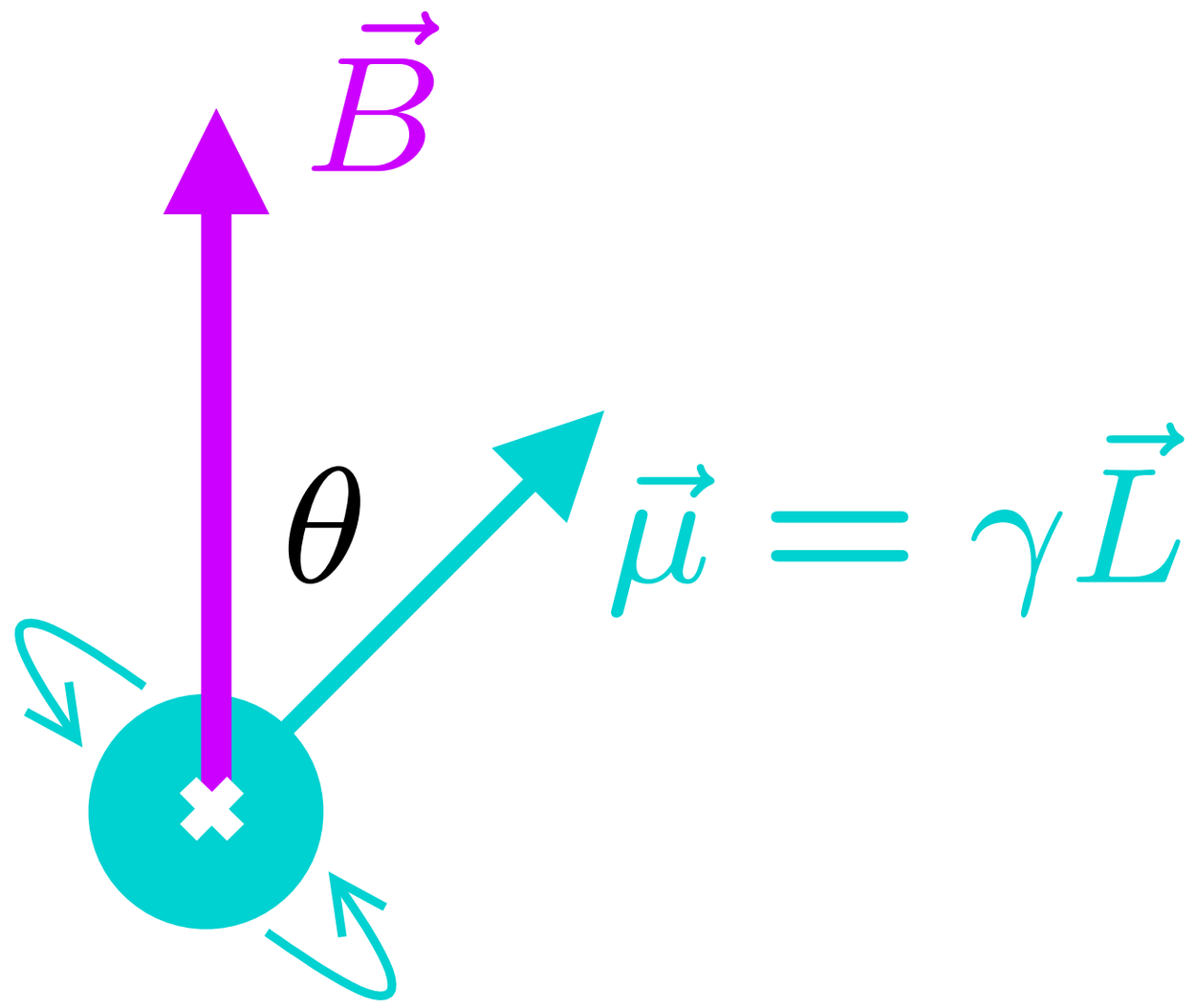
vzácné izotopy (vyžadují obohacení proteinů během exprese)

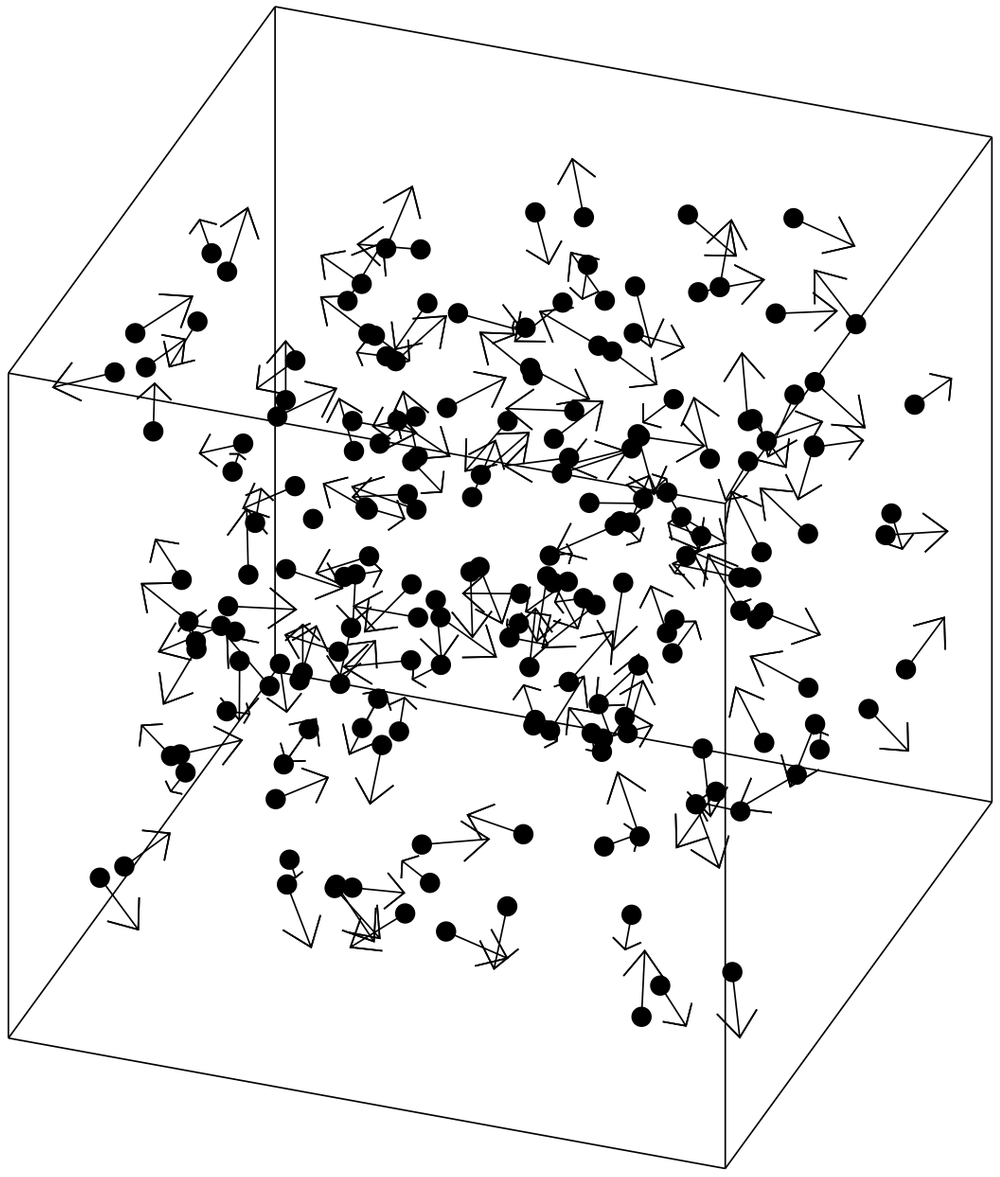
Úhlová rychlost precese $\vec{\omega} = -\gamma\vec{B}$



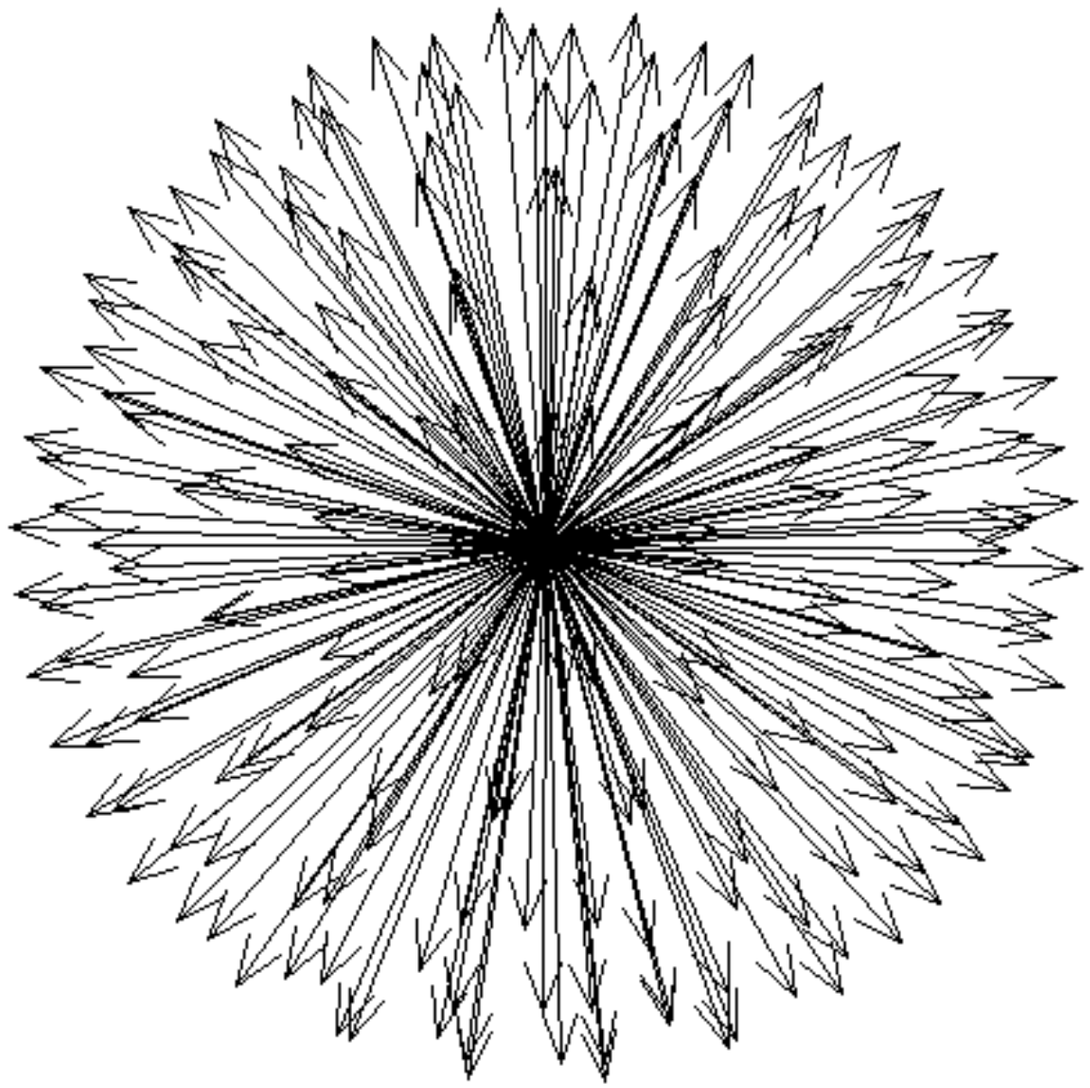
Energie magnetického momentu v poli

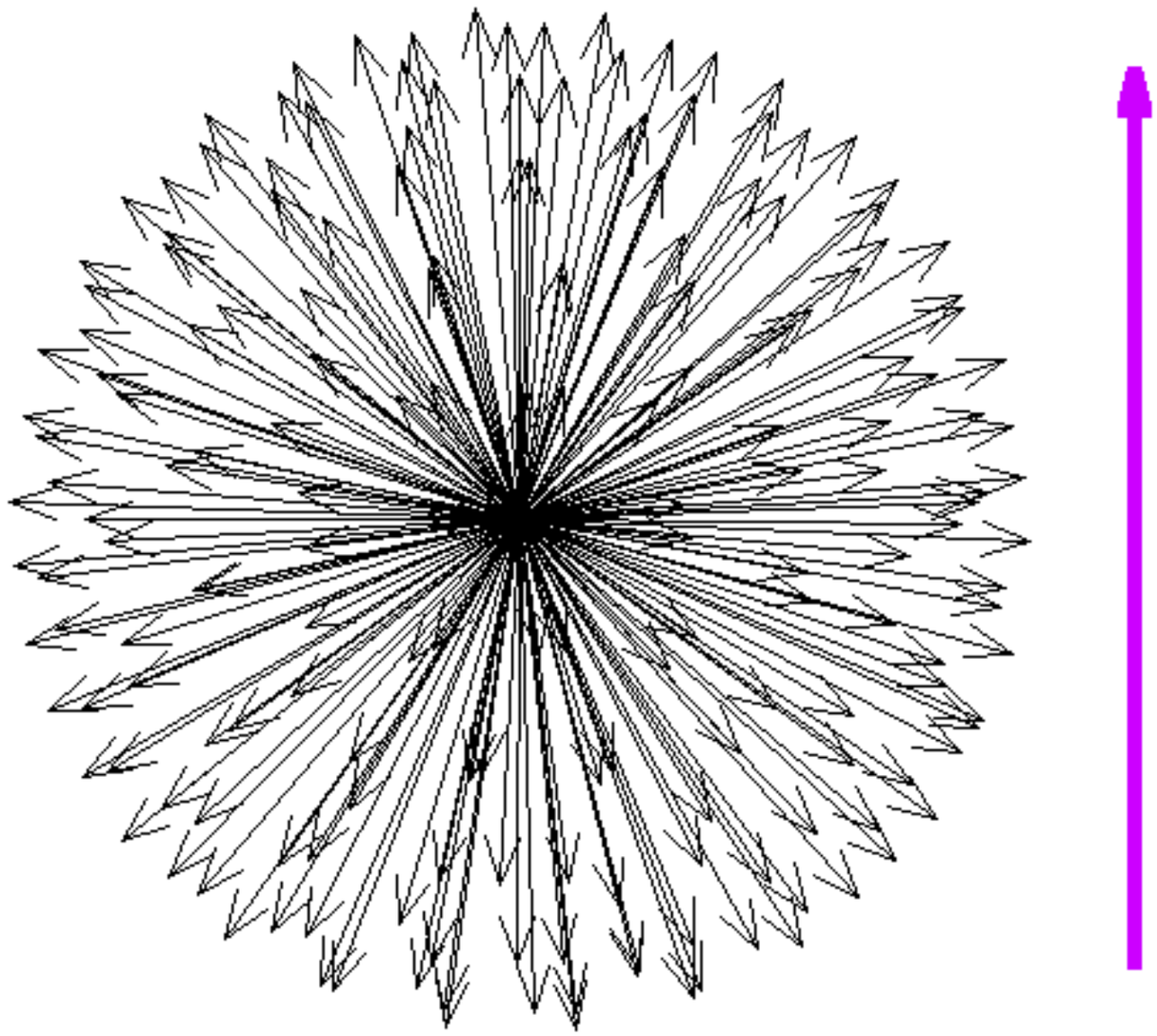
$$U = -\vec{\mu} \cdot \vec{B} = -|\mu||B| \cos\theta$$



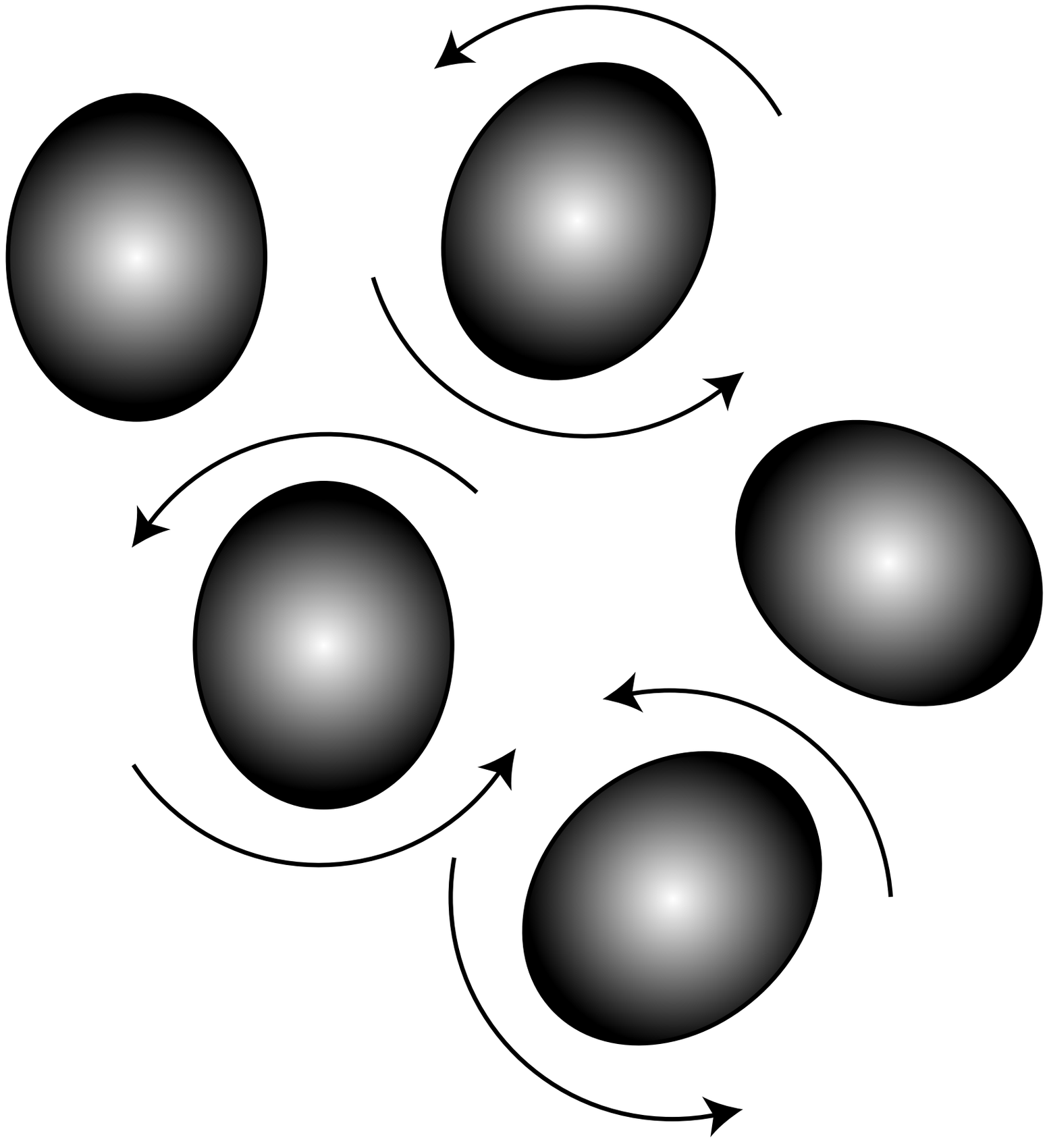


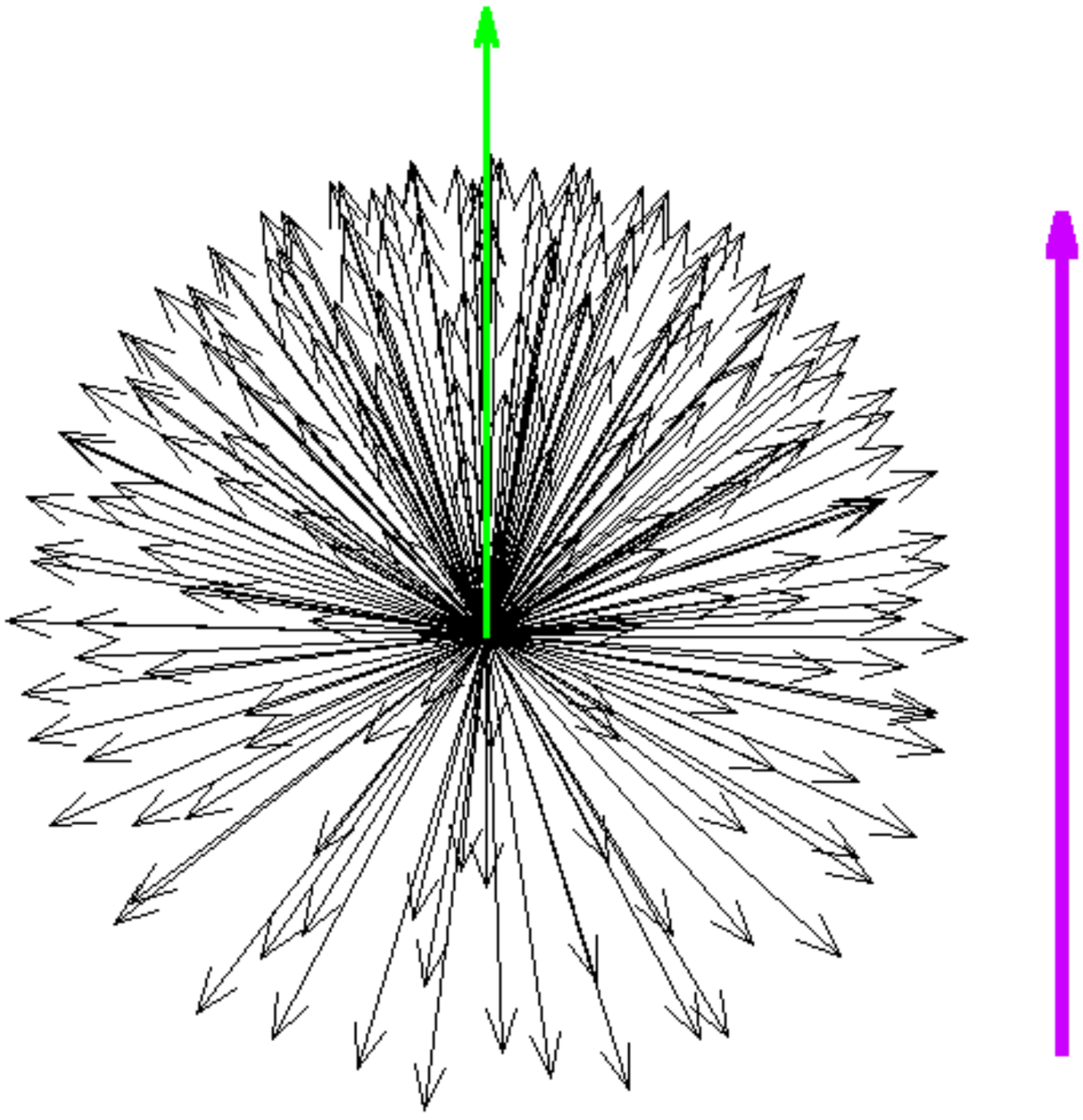
$\vec{M} = (\vec{\mu}_1 + \vec{\mu}_2 + \vec{\mu}_3 + \vec{\mu}_4 + \vec{\mu}_5 + \vec{\mu}_6 + \cdots)/V$ Magnetizace



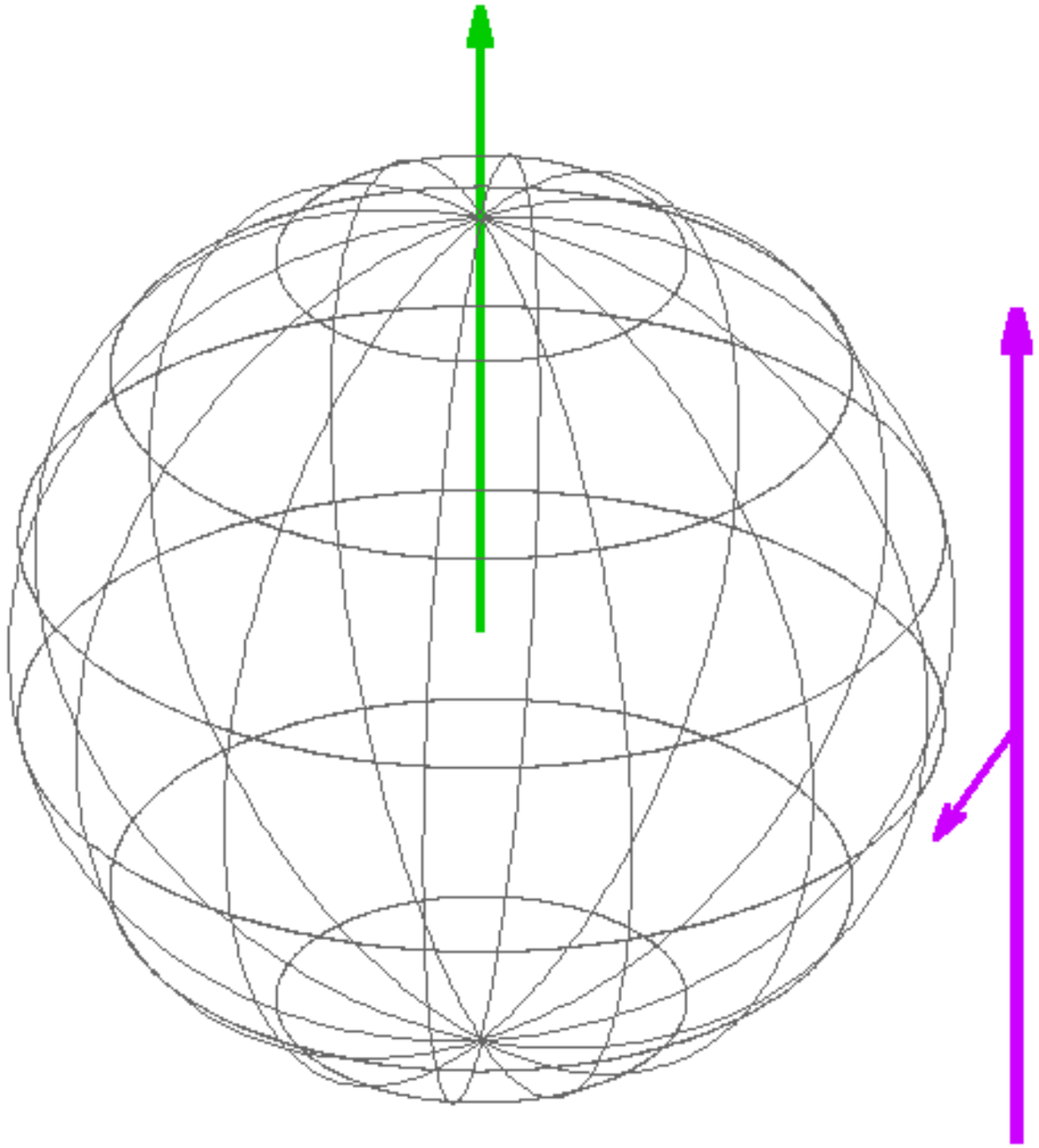


Magnetické momenty v magnetickém poli

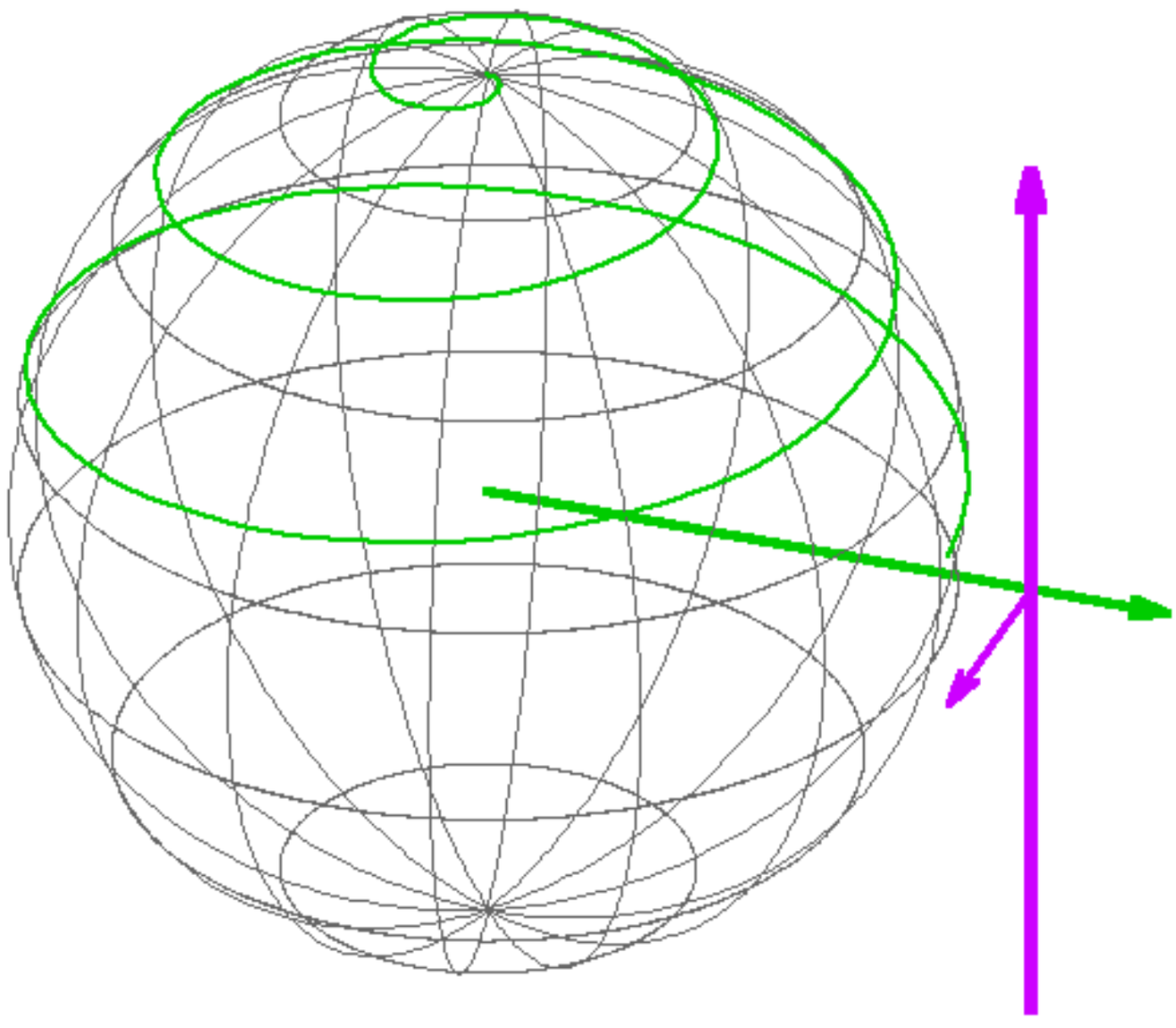


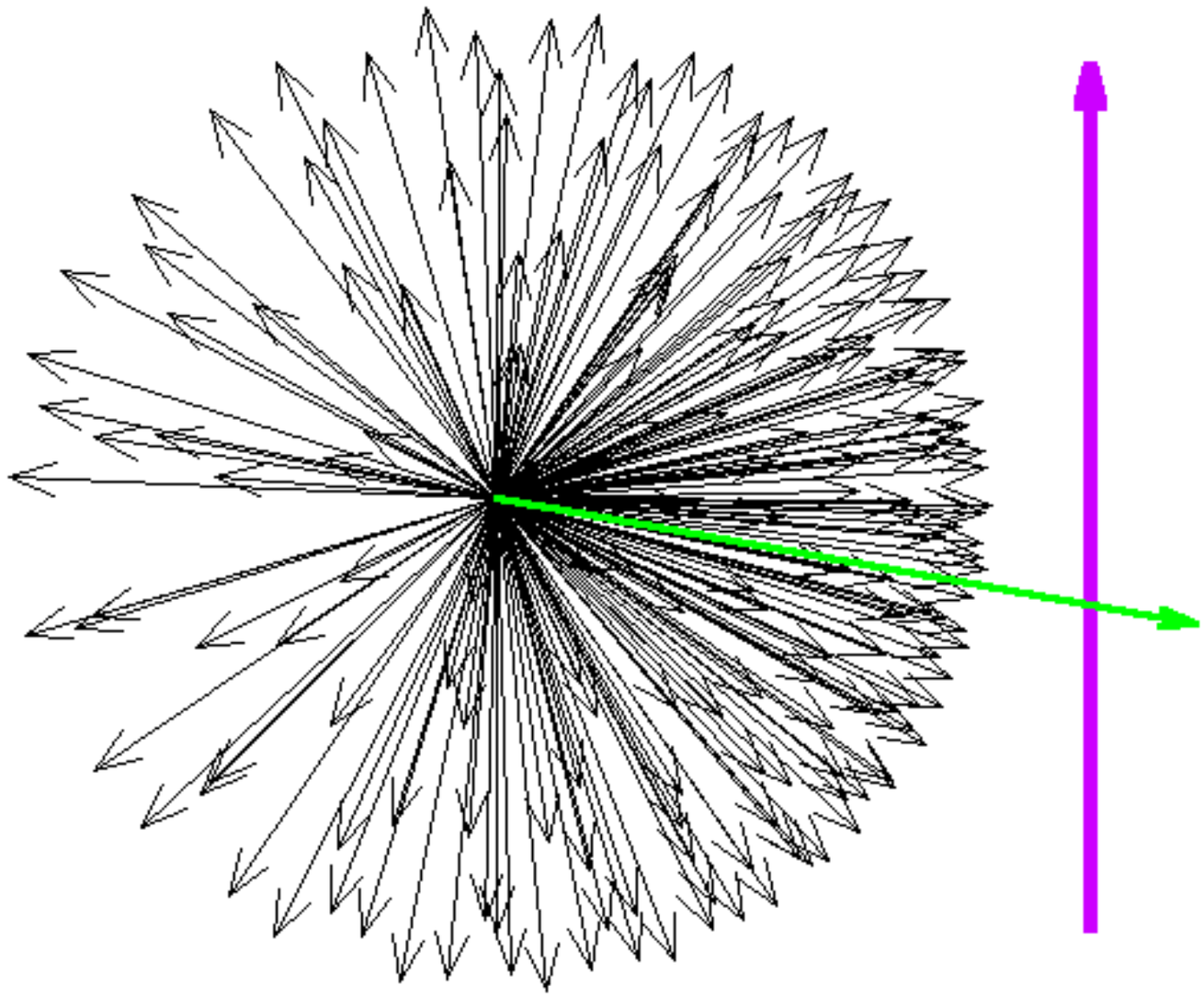


Vertikálně polarizované magnetické momenty ve vertikálním magnetickém poli

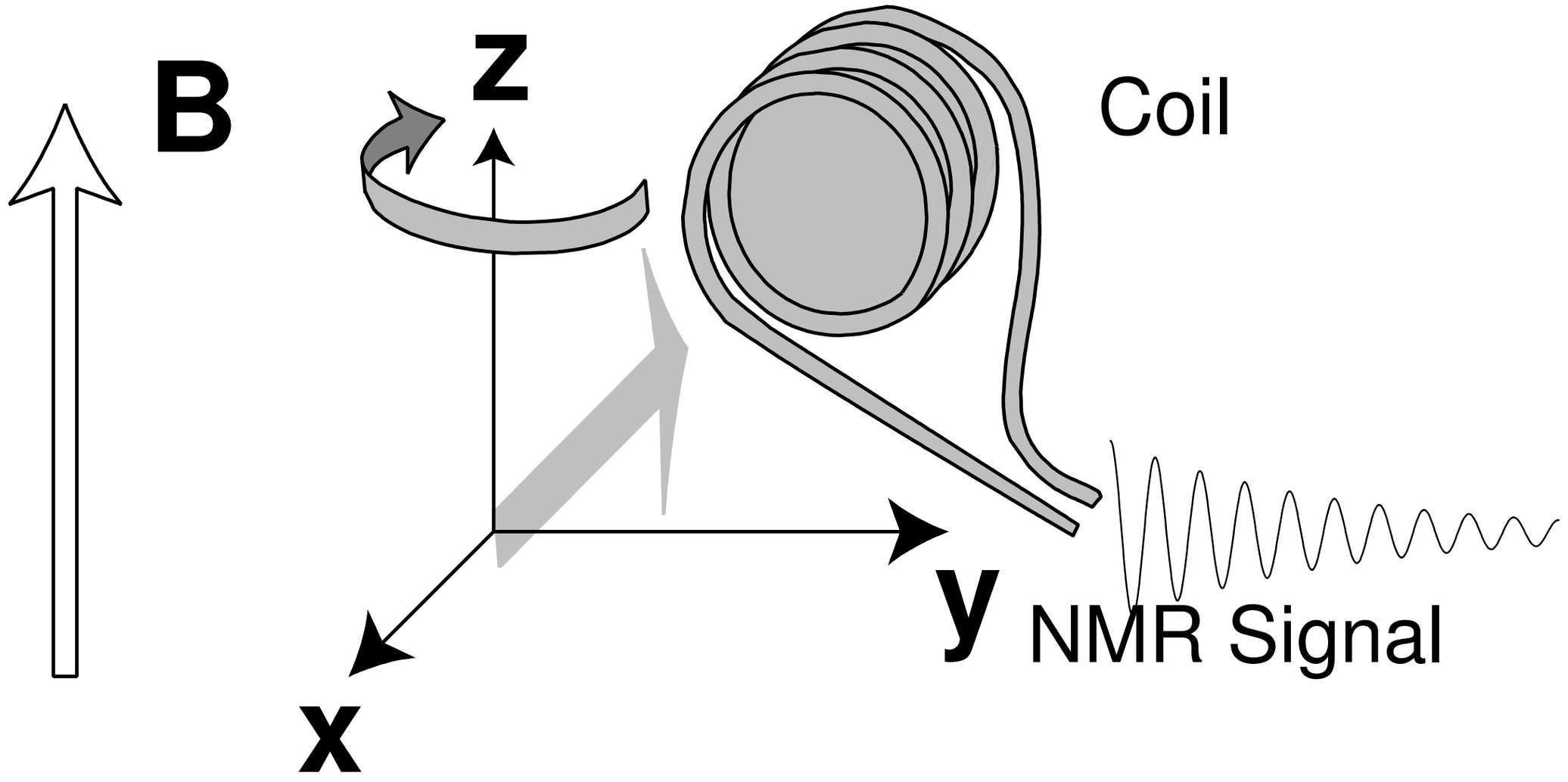


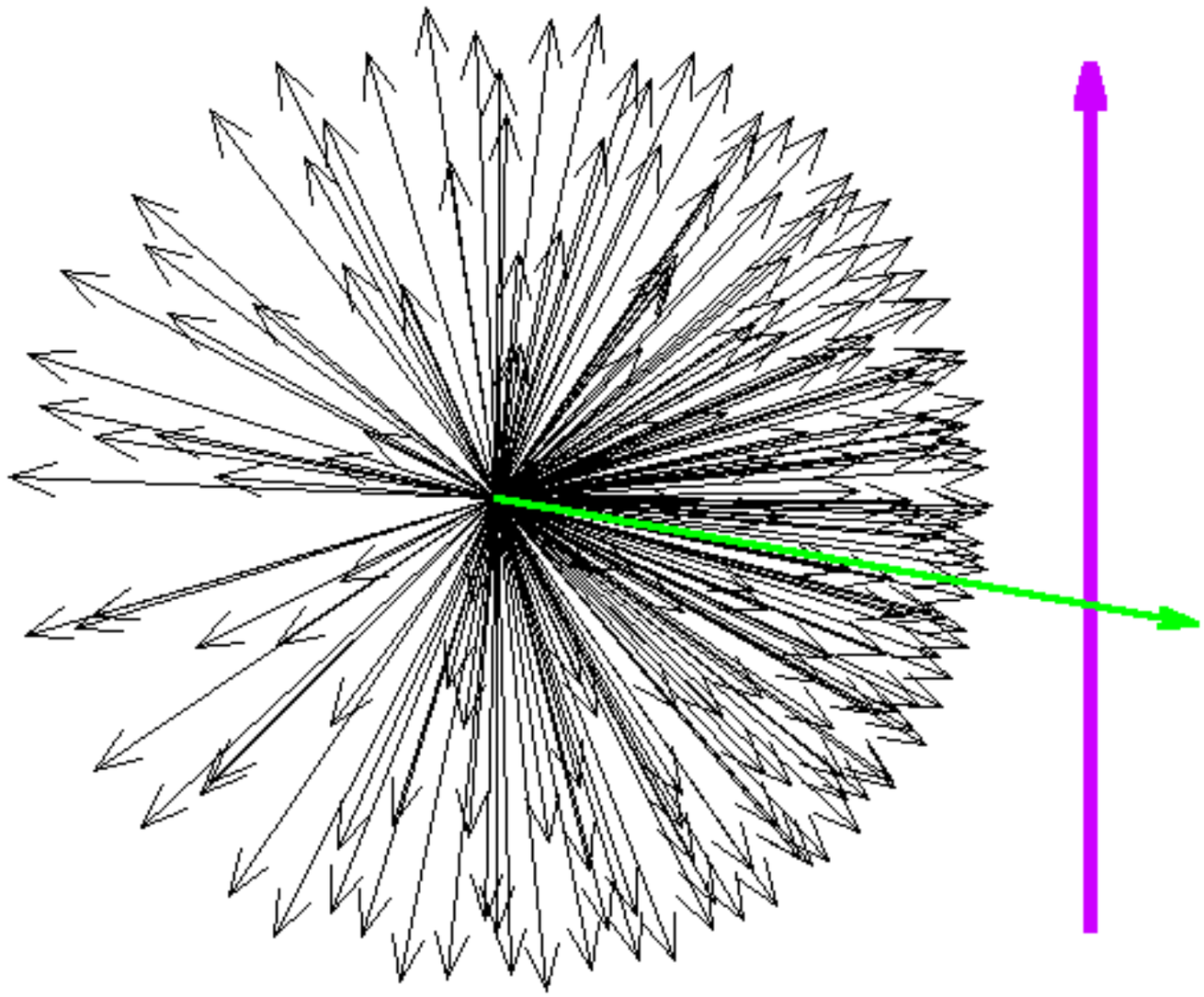
Sklopení vektoru magnetizace

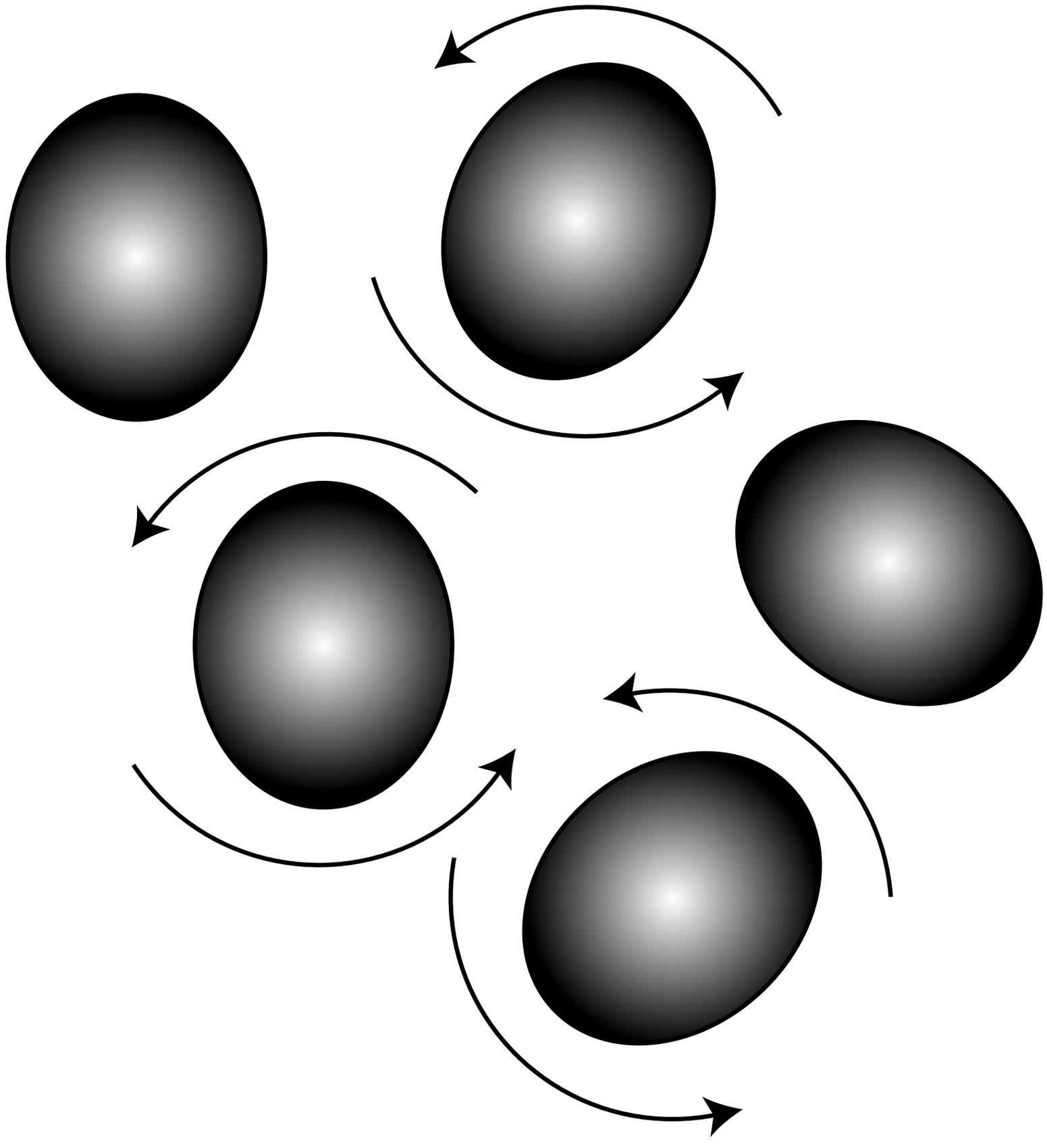


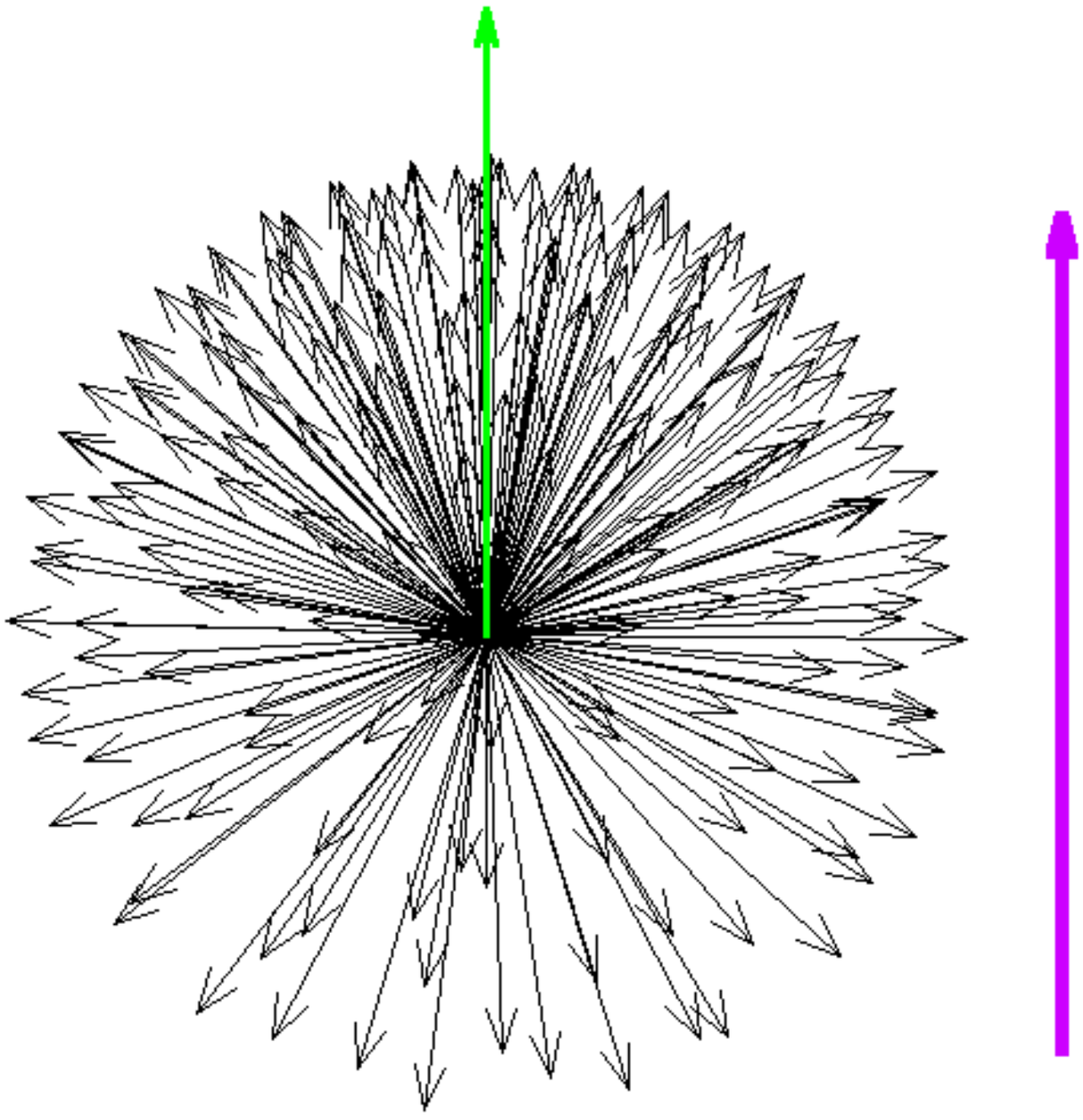


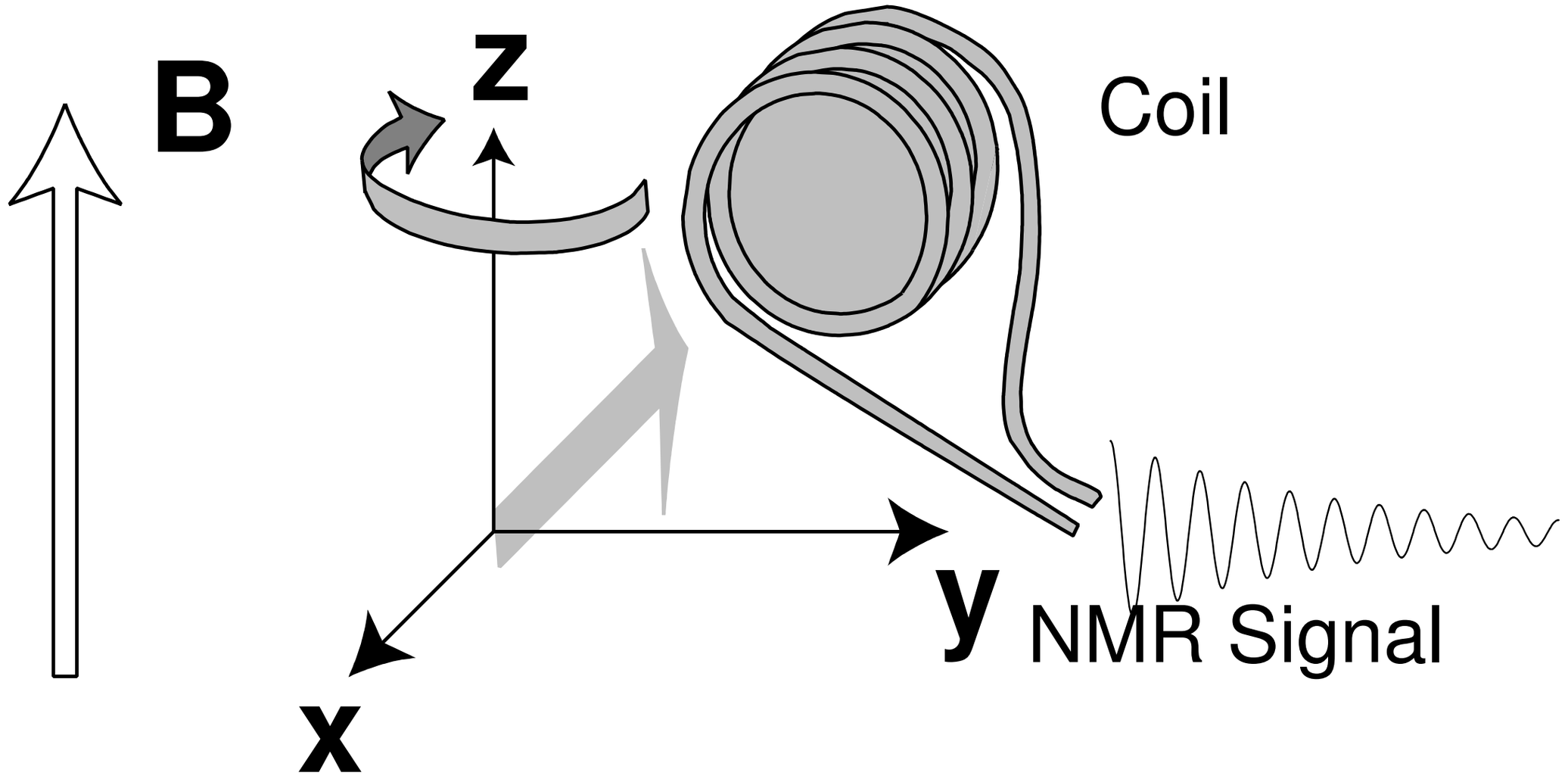
Horizontálně polarizované koherentní magnetické momenty ve vertikálním magnetickém poli

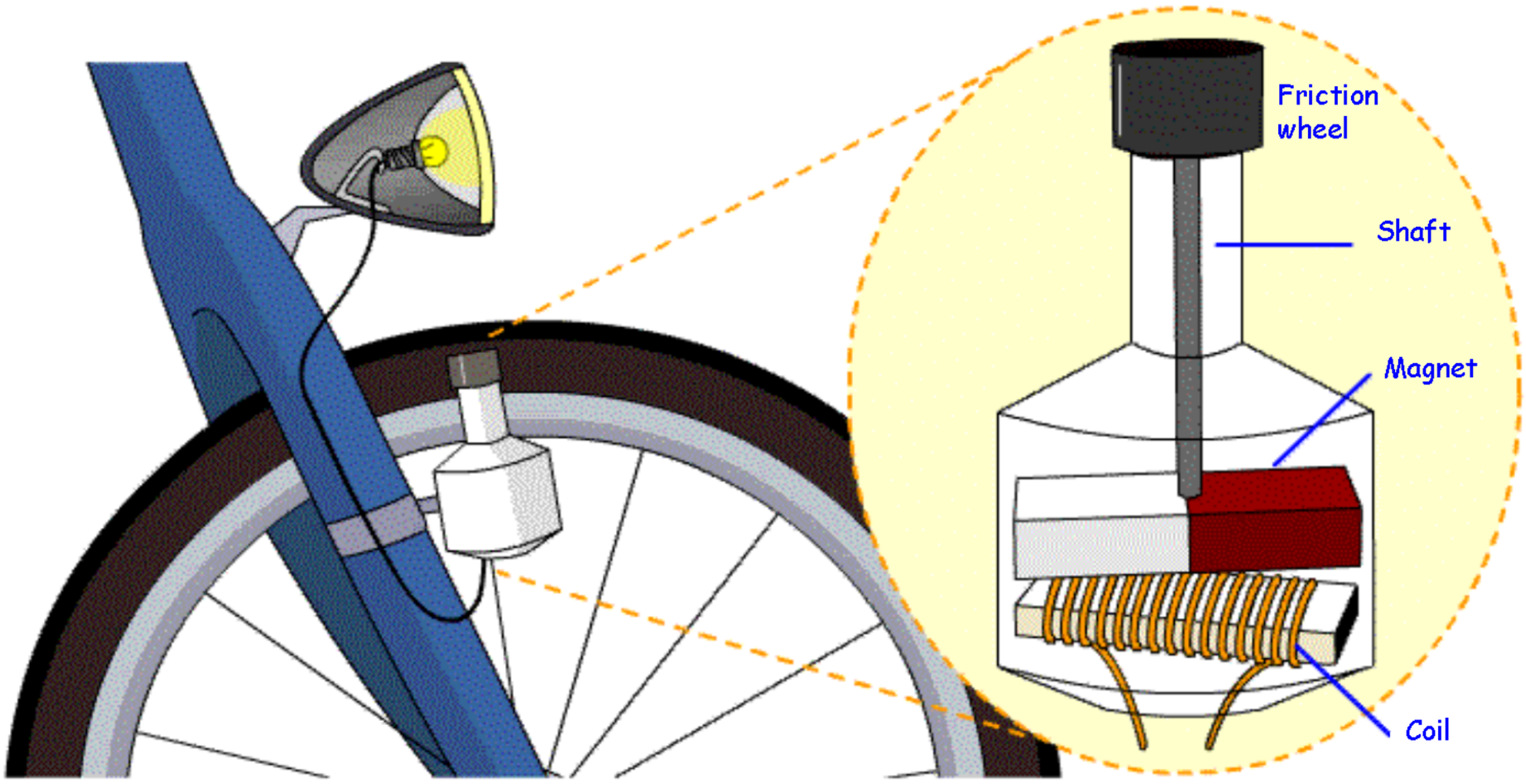

B
z
Coil
y
NMR Signal
x

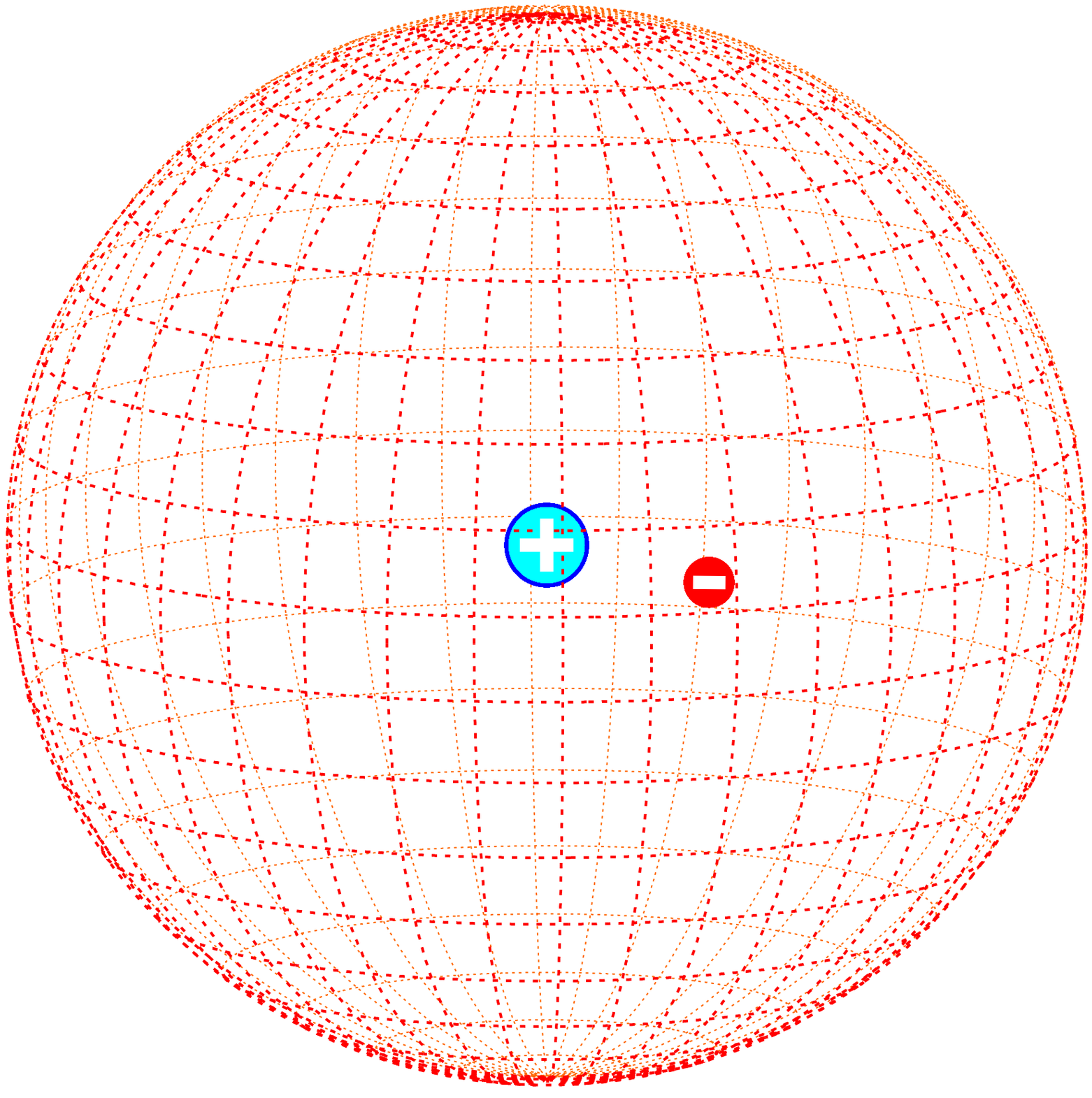


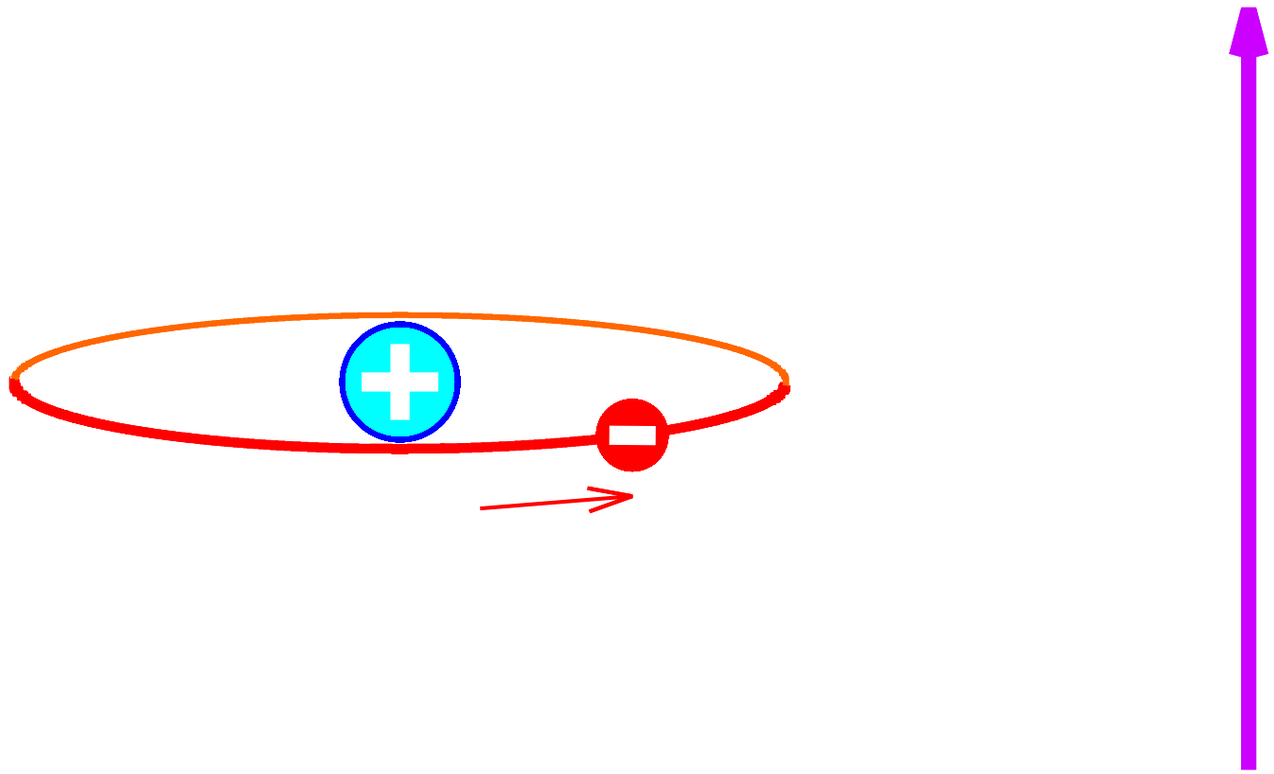


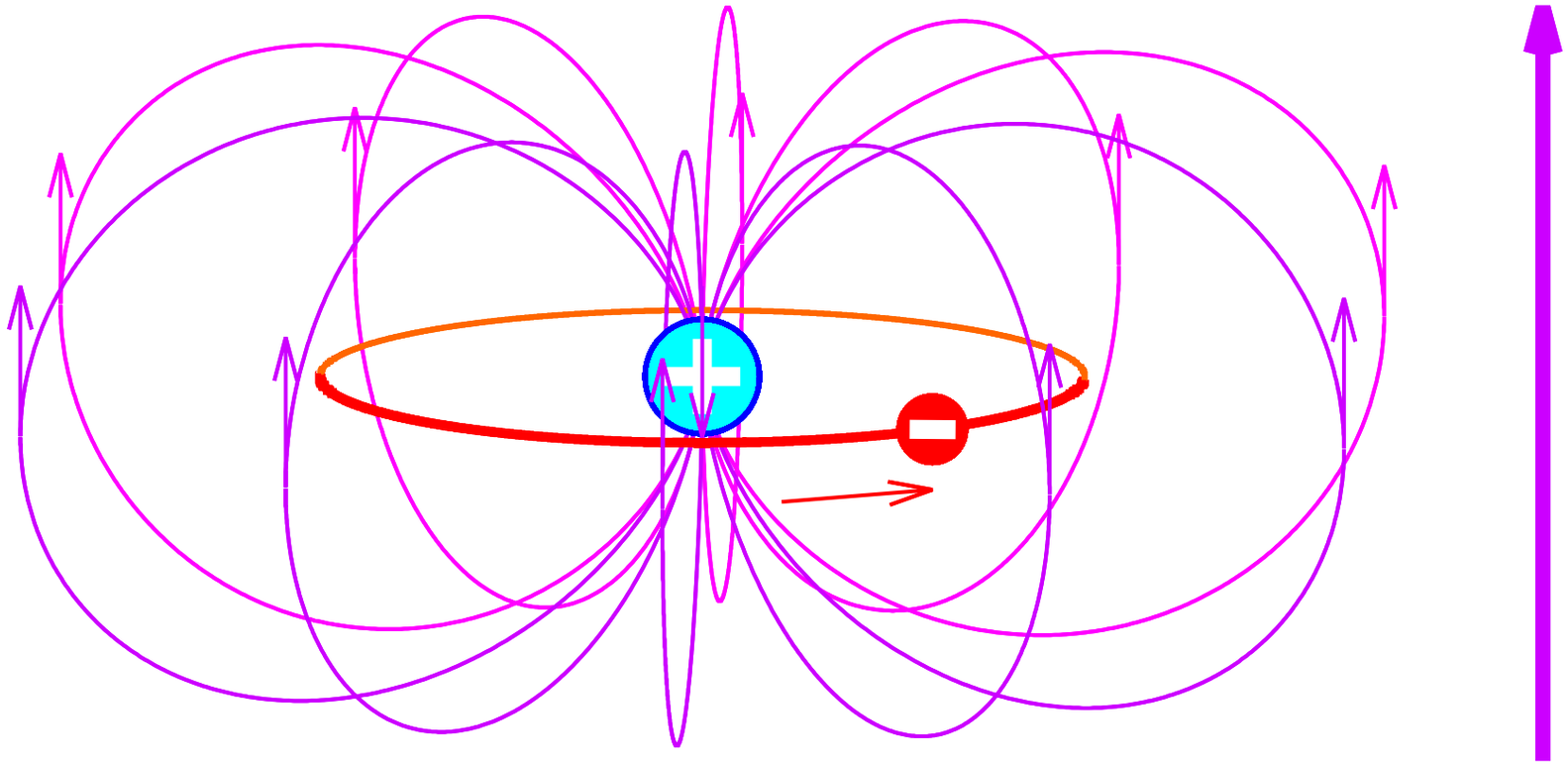


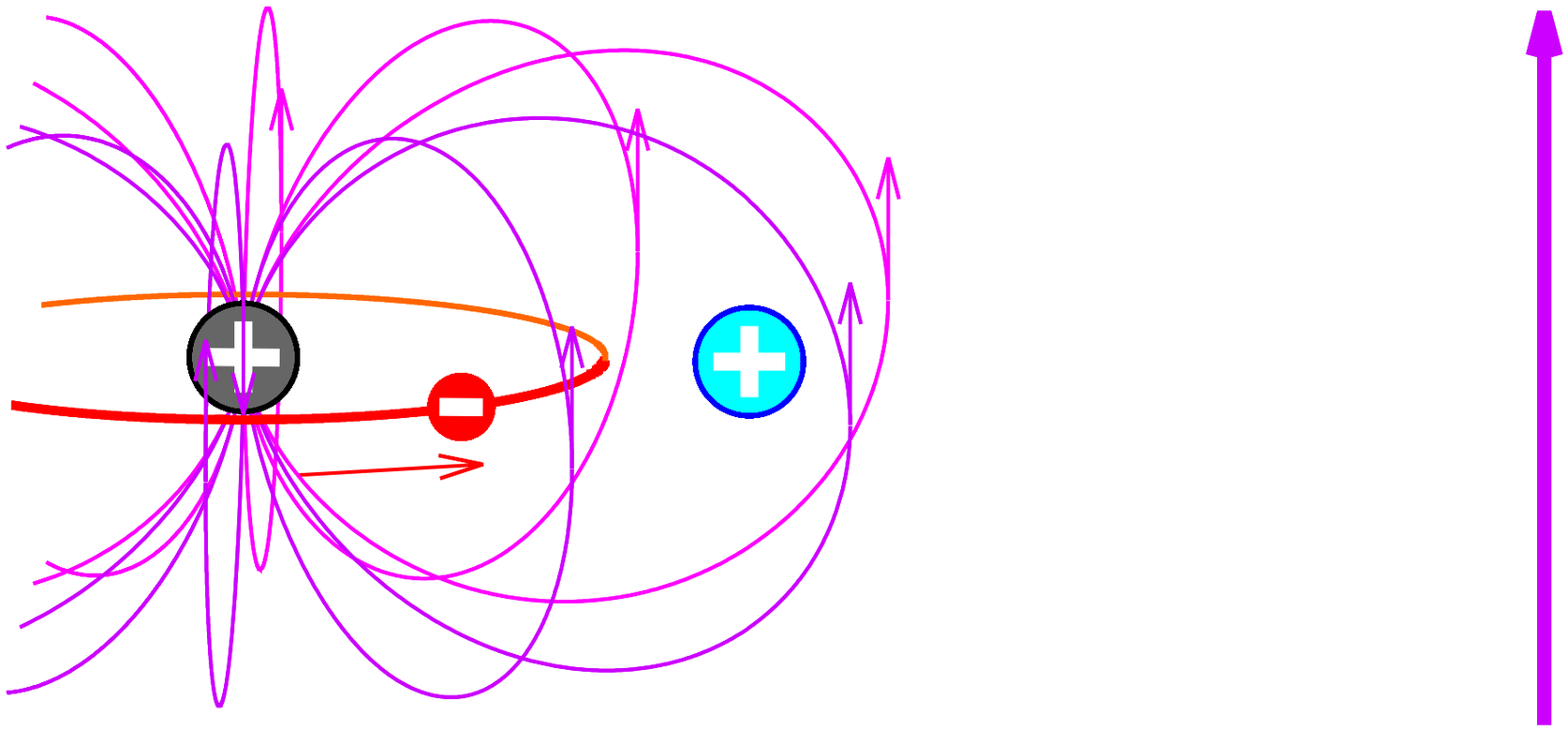

B
z
Coil
y
NMR Signal
x

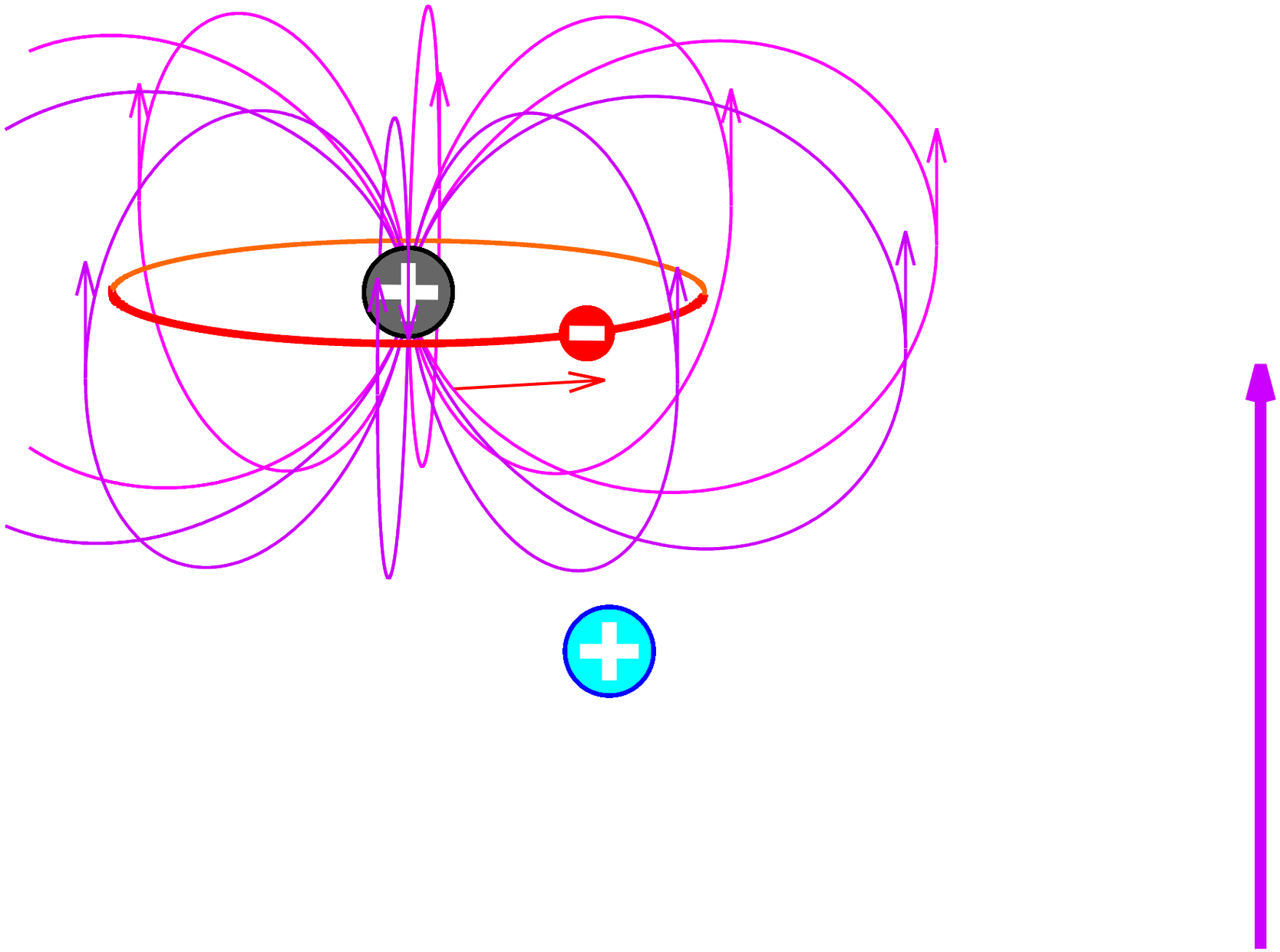

Friction wheel
Shaft
Magnet
Coil

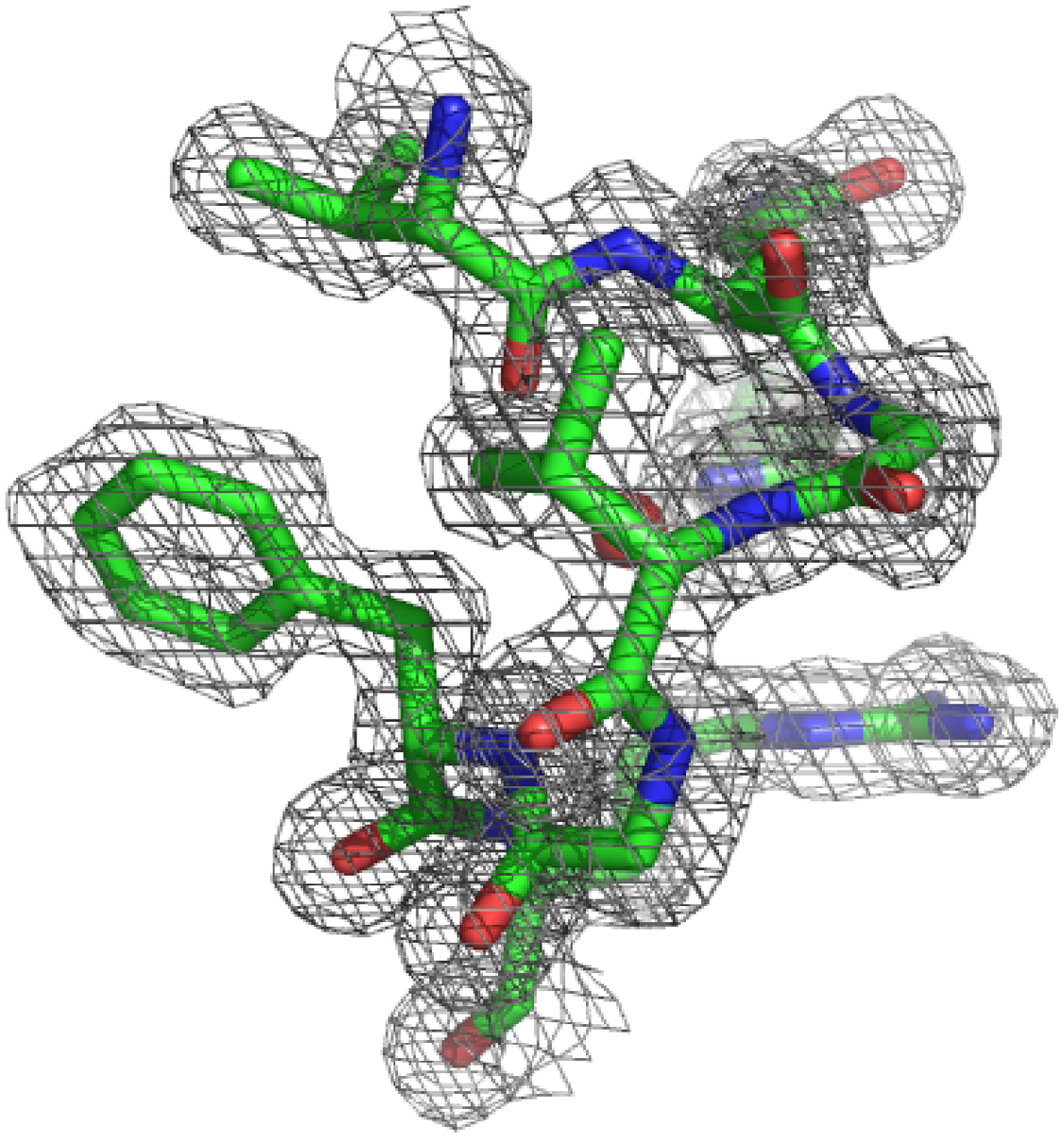


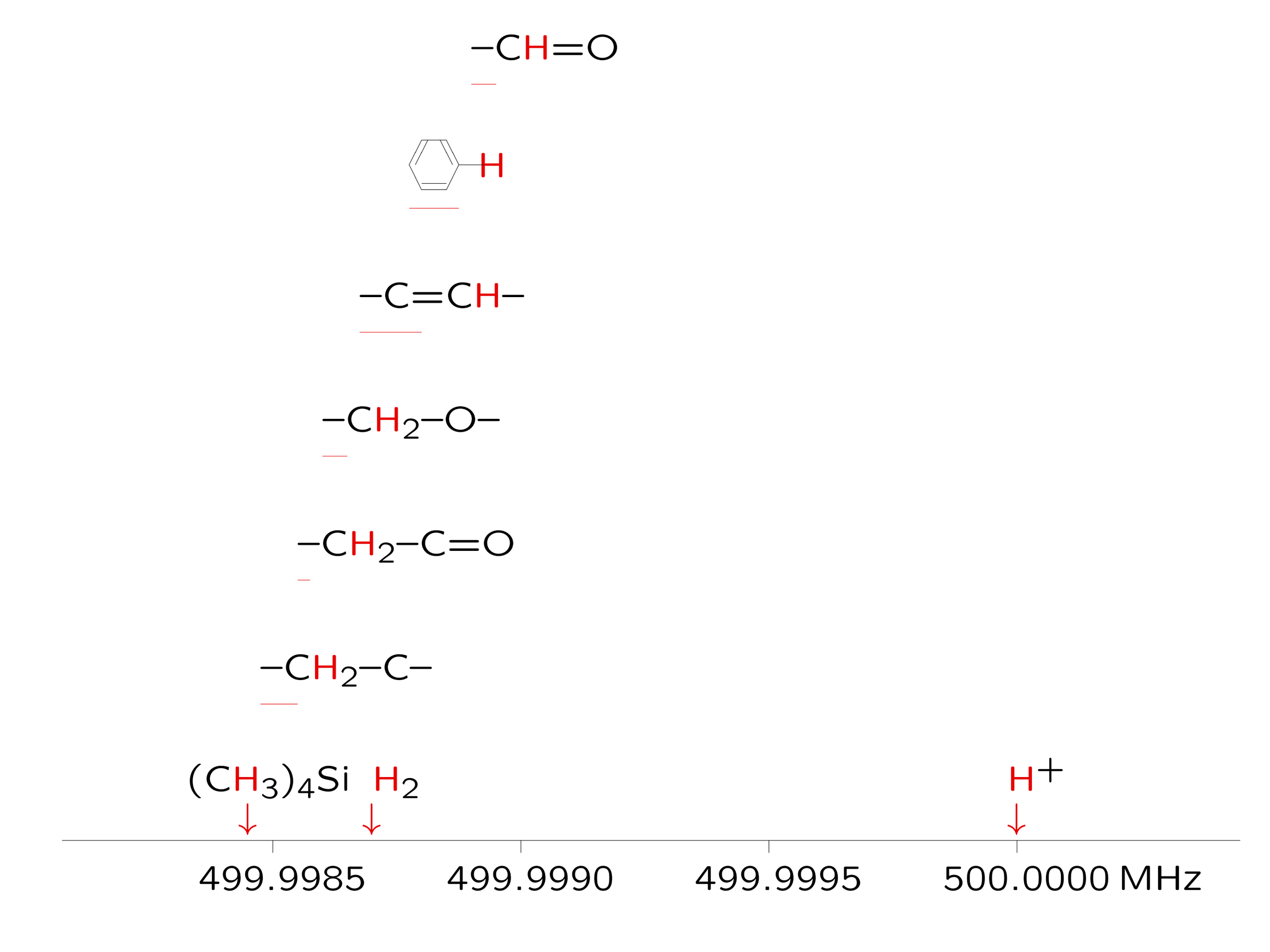


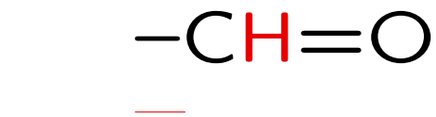


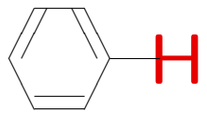


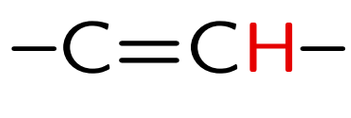


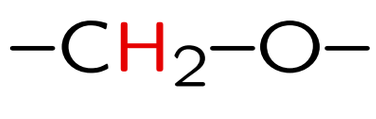


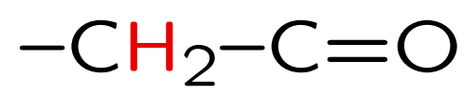

−CH=O
H
−C=CH−
−CH2−O−
−CH2−C=O
−CH2−C−
(CH3)4Si
H2
H+
499.9985
499.9990
499.9995
500.0000 MHz

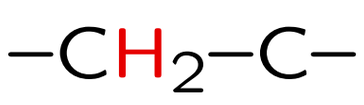

−CH=O

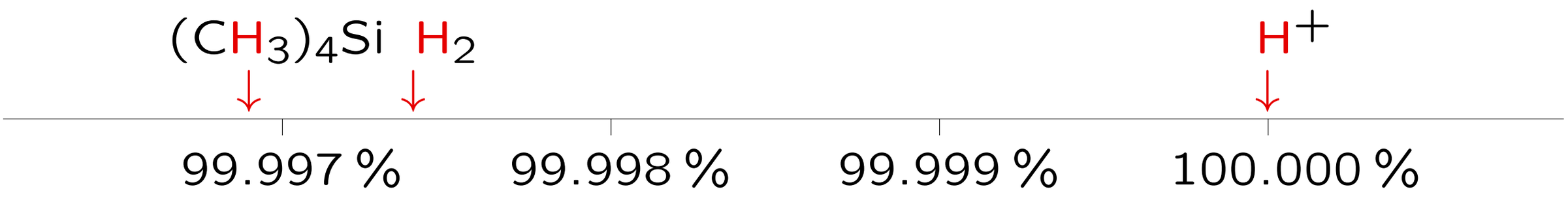

H

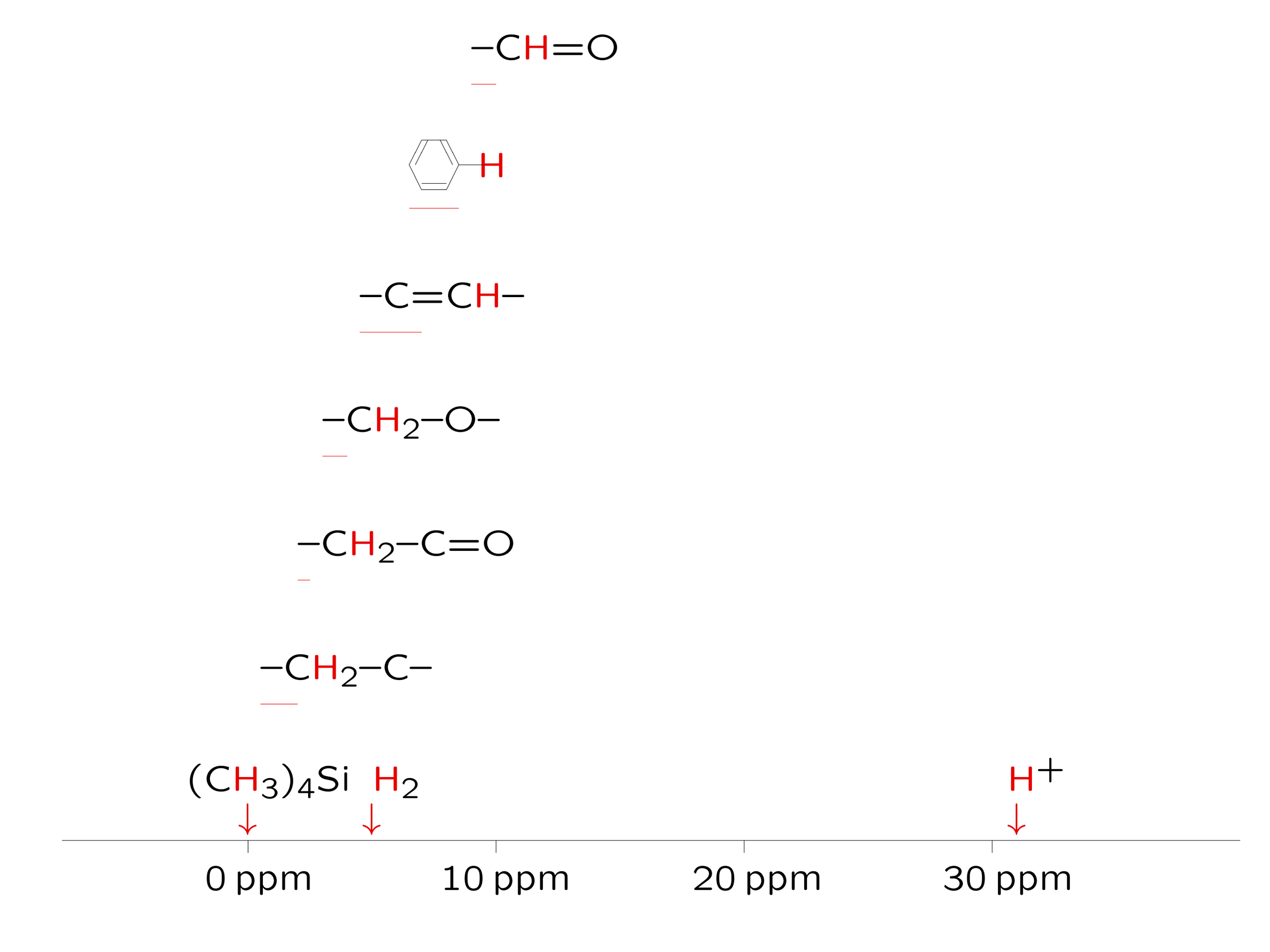

−C=CH−

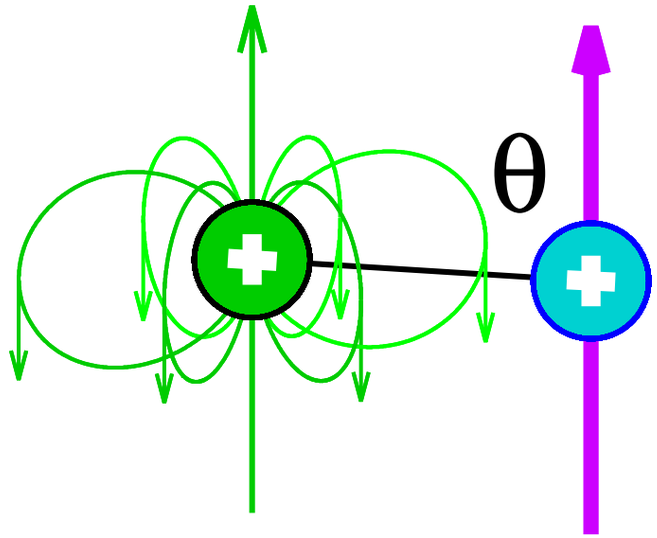

−CH2−O−

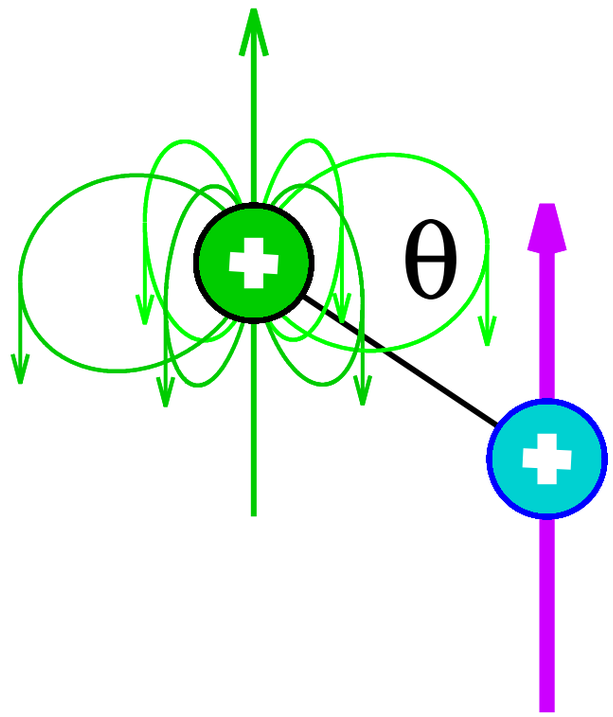

−CH2−C=O

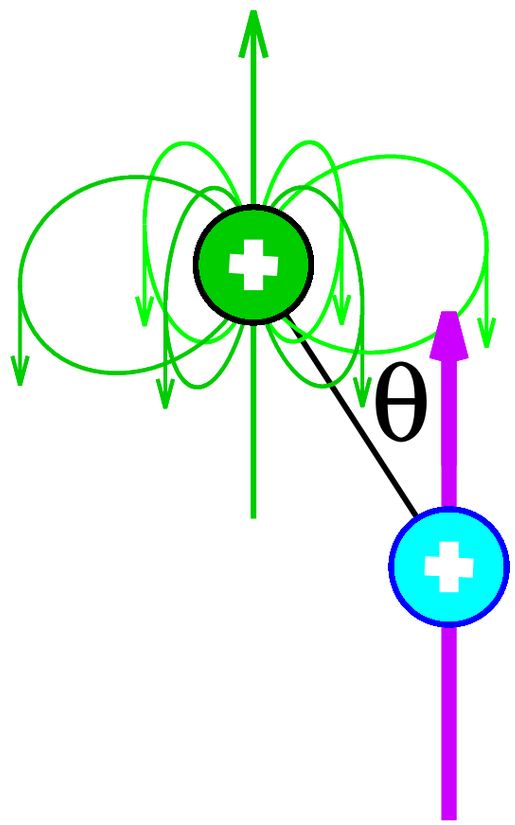

−CH2−C−

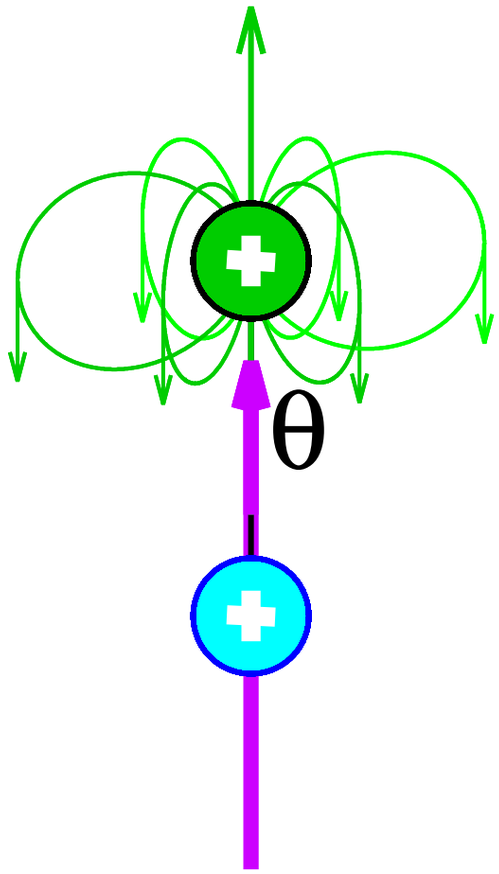

(CH3)4Si
H2
H+
99.997 %
99.998 %
99.999 %
100.000 %

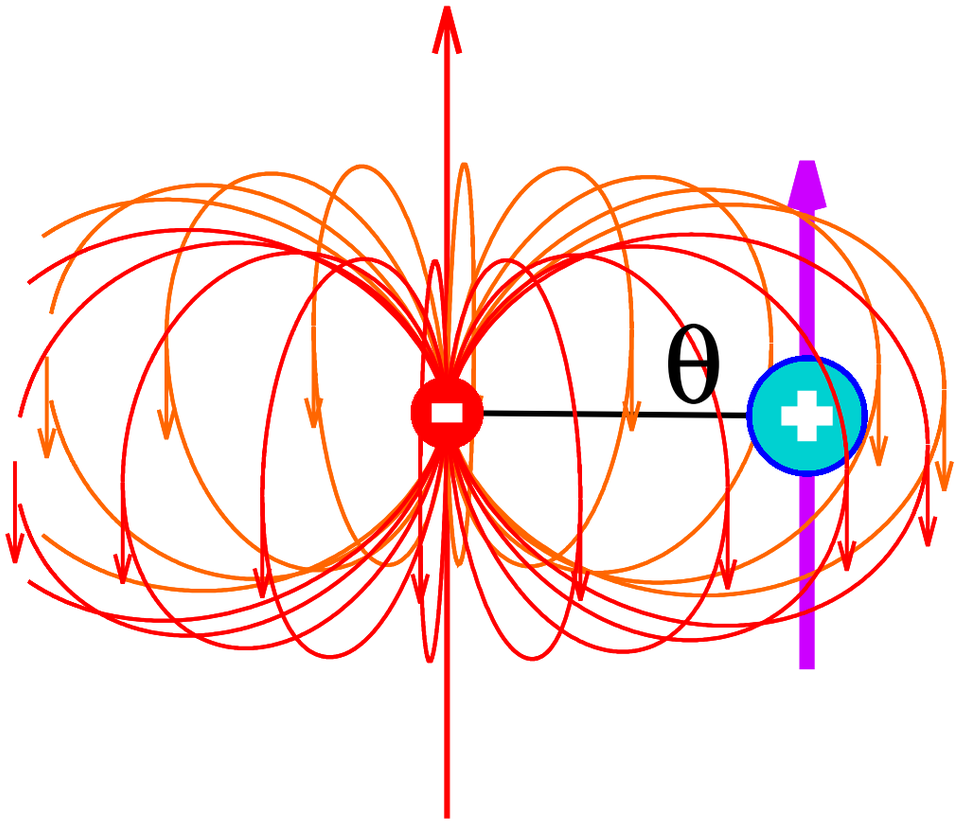

–CH=O
H
–C=CH–
–CH2–O–
–CH2–C=O
–CH2–C–
(CH3)4Si
H2
H+
0 ppm
10 ppm
20 ppm
30 ppm

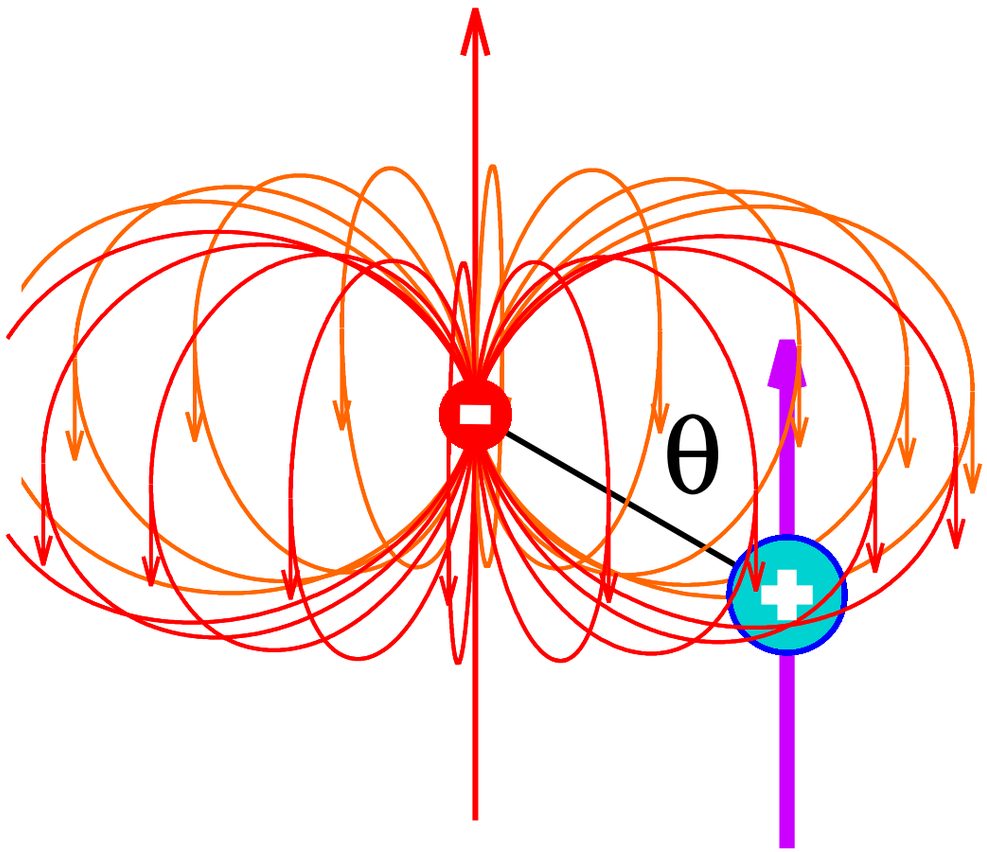

θ


θ


θ


θ


θ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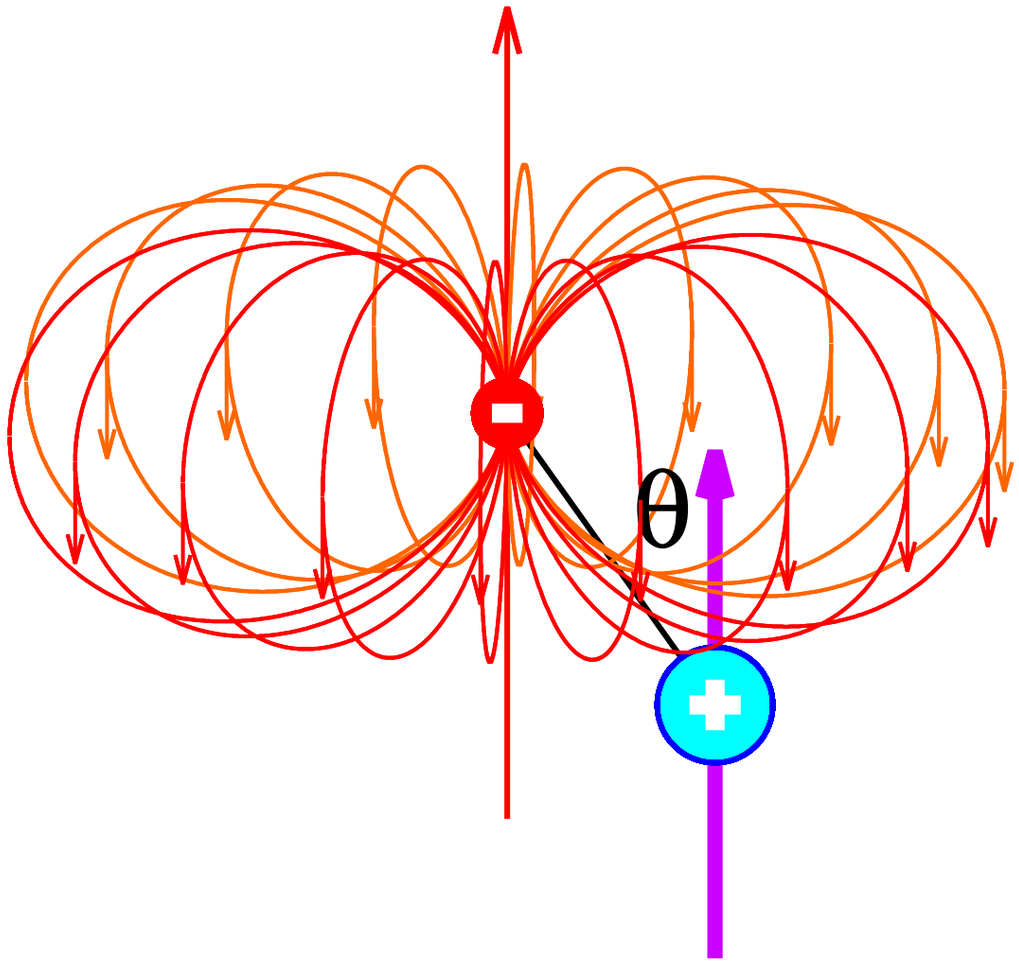
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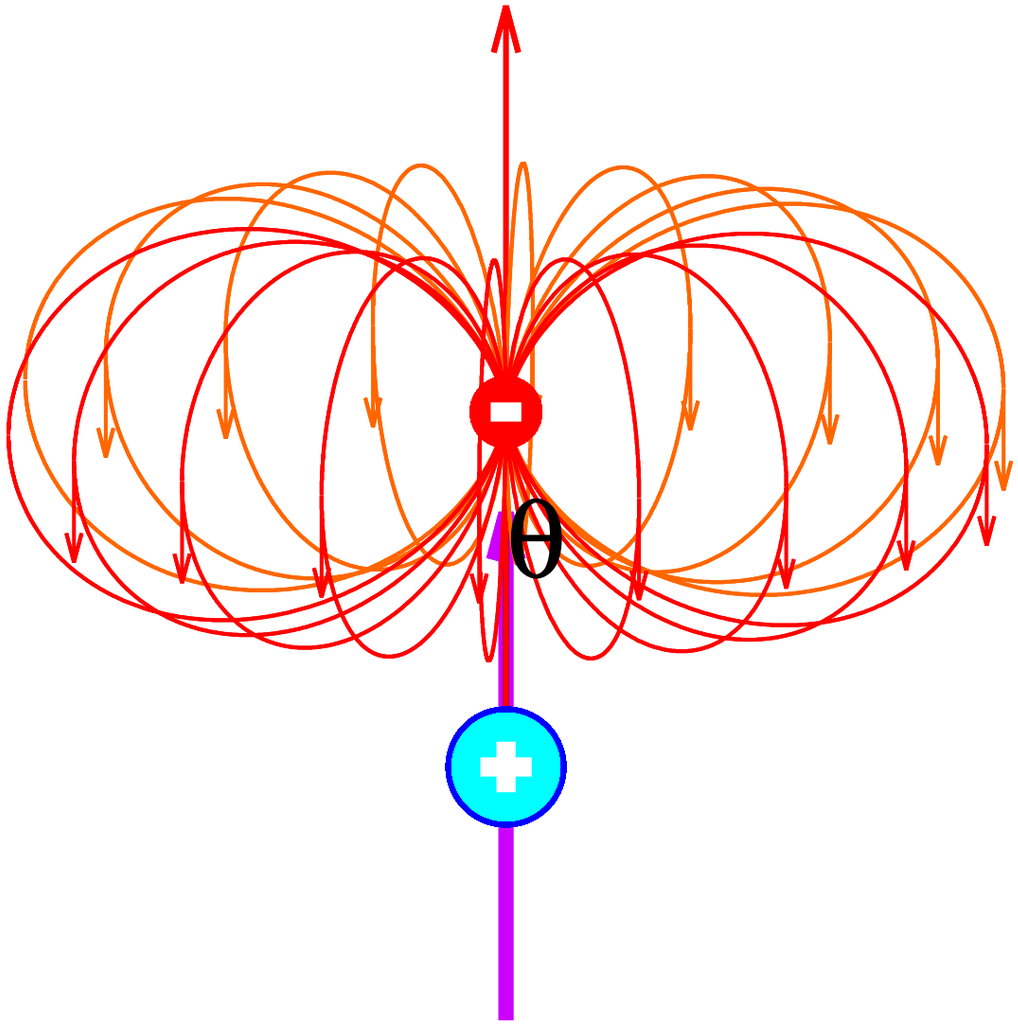
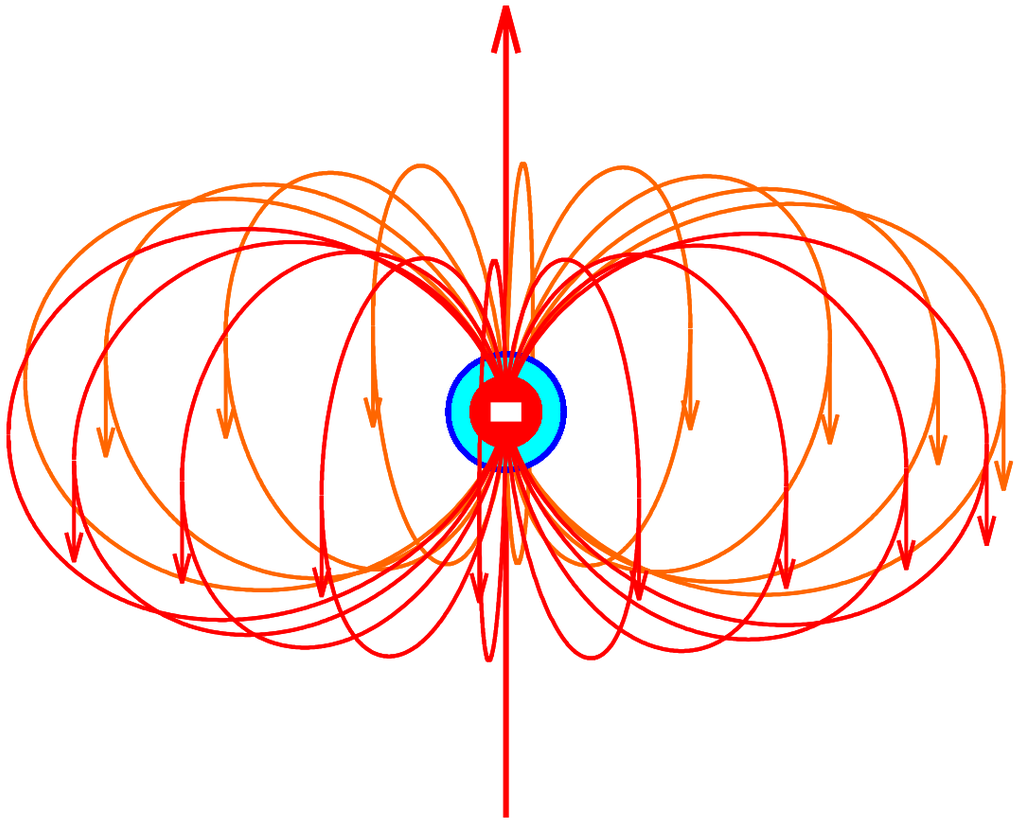
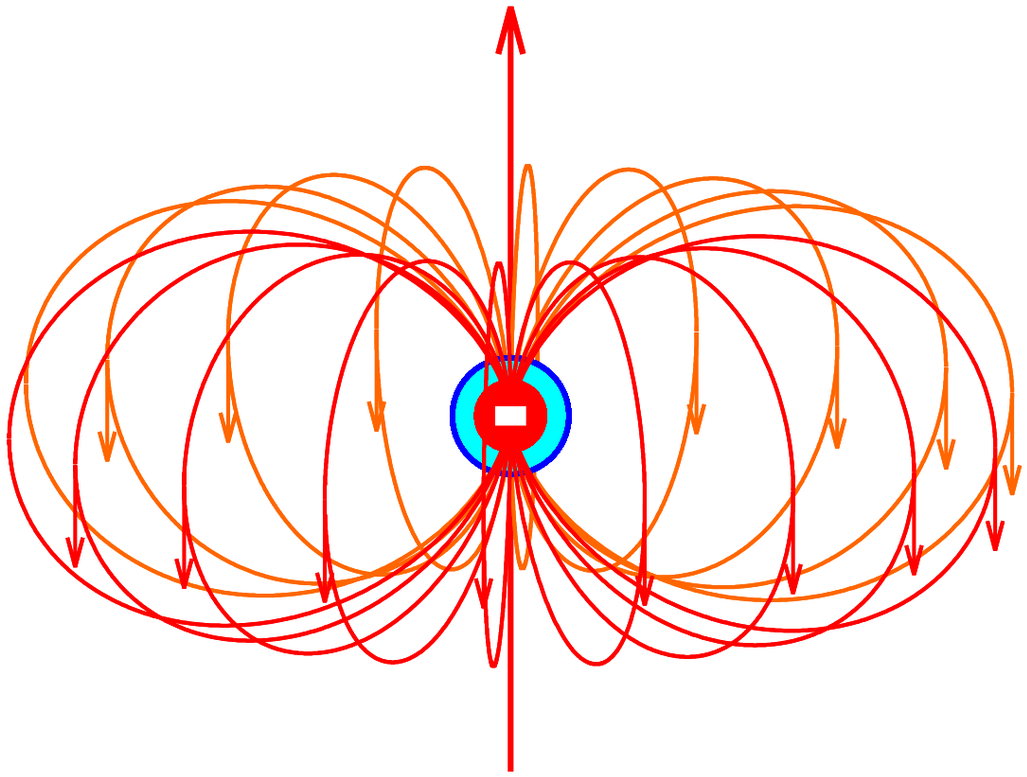
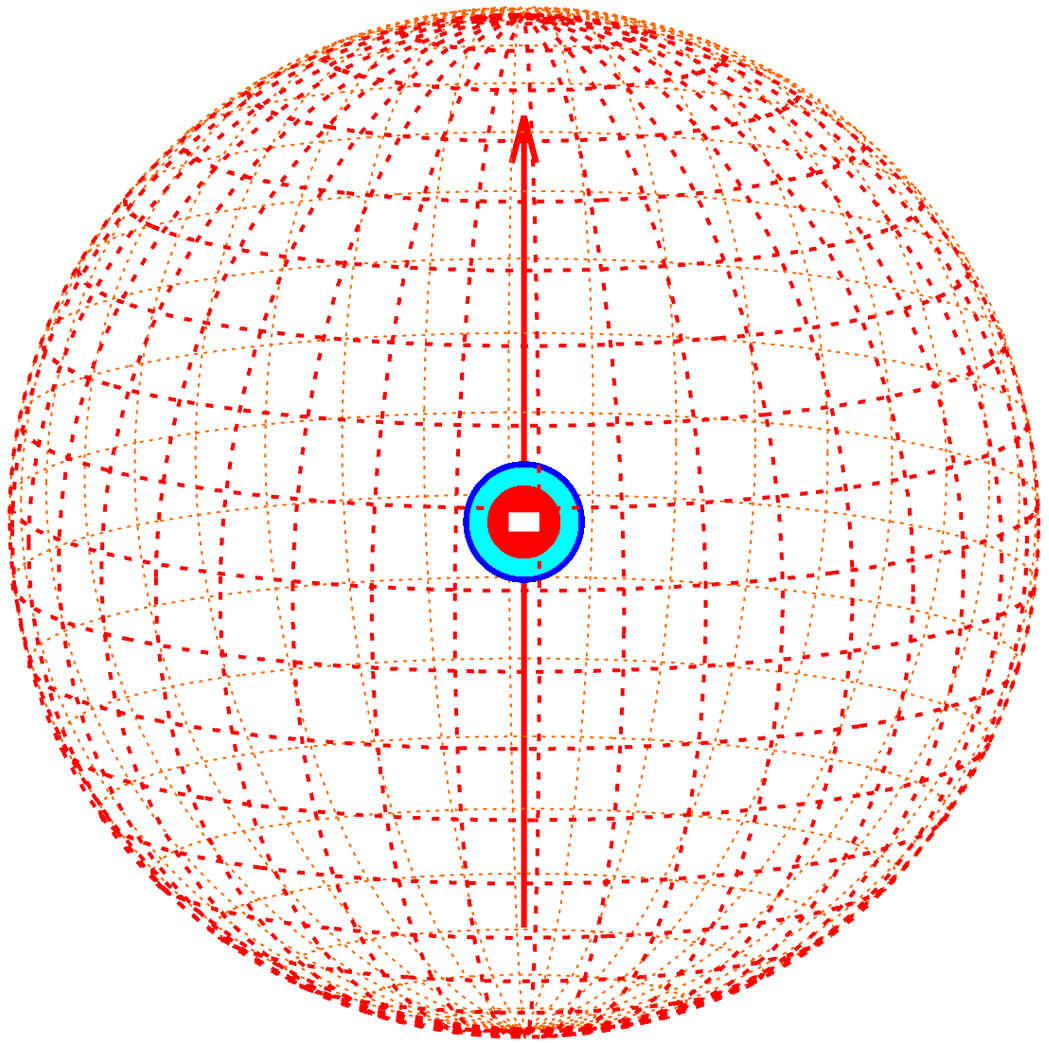
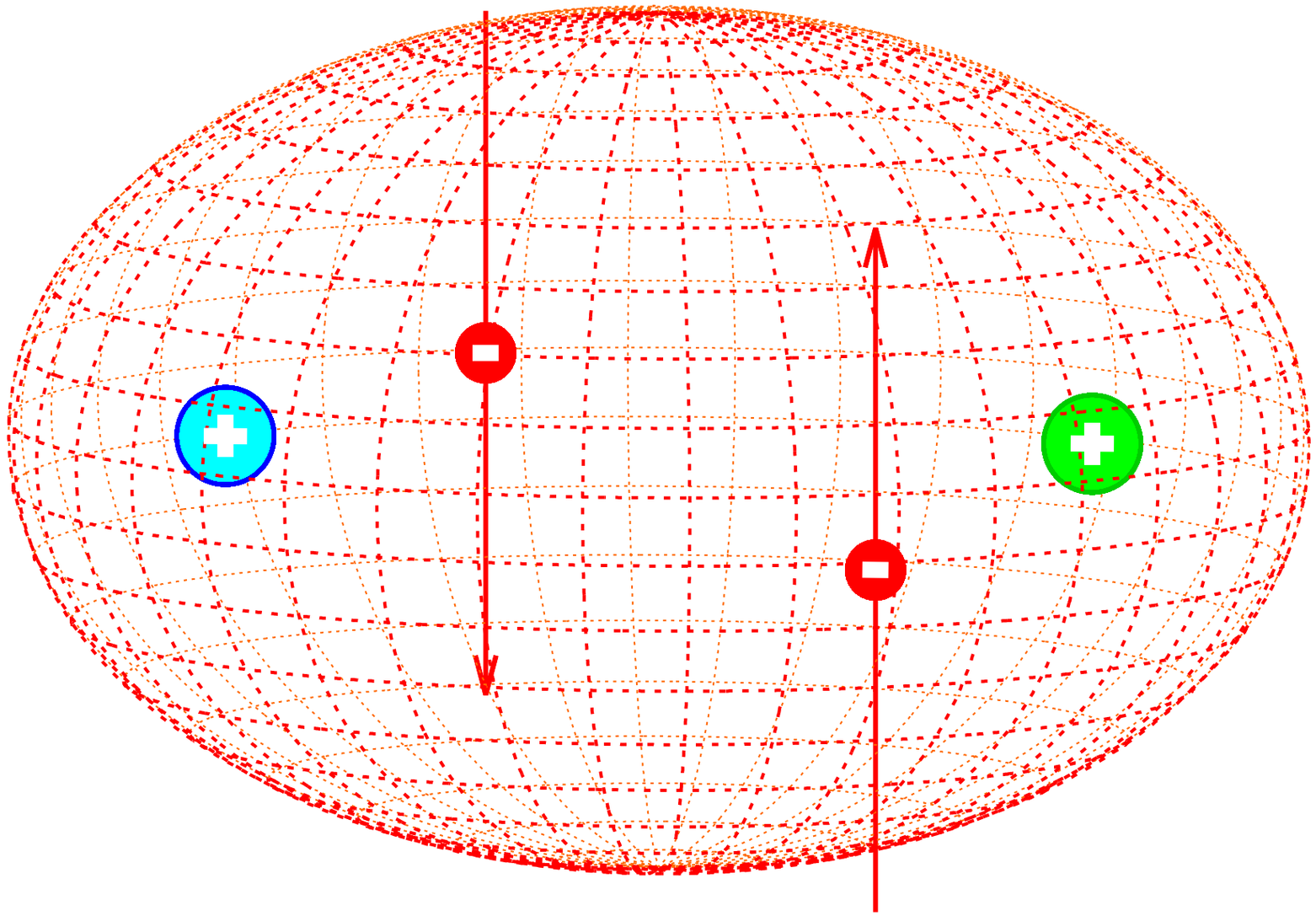

θ

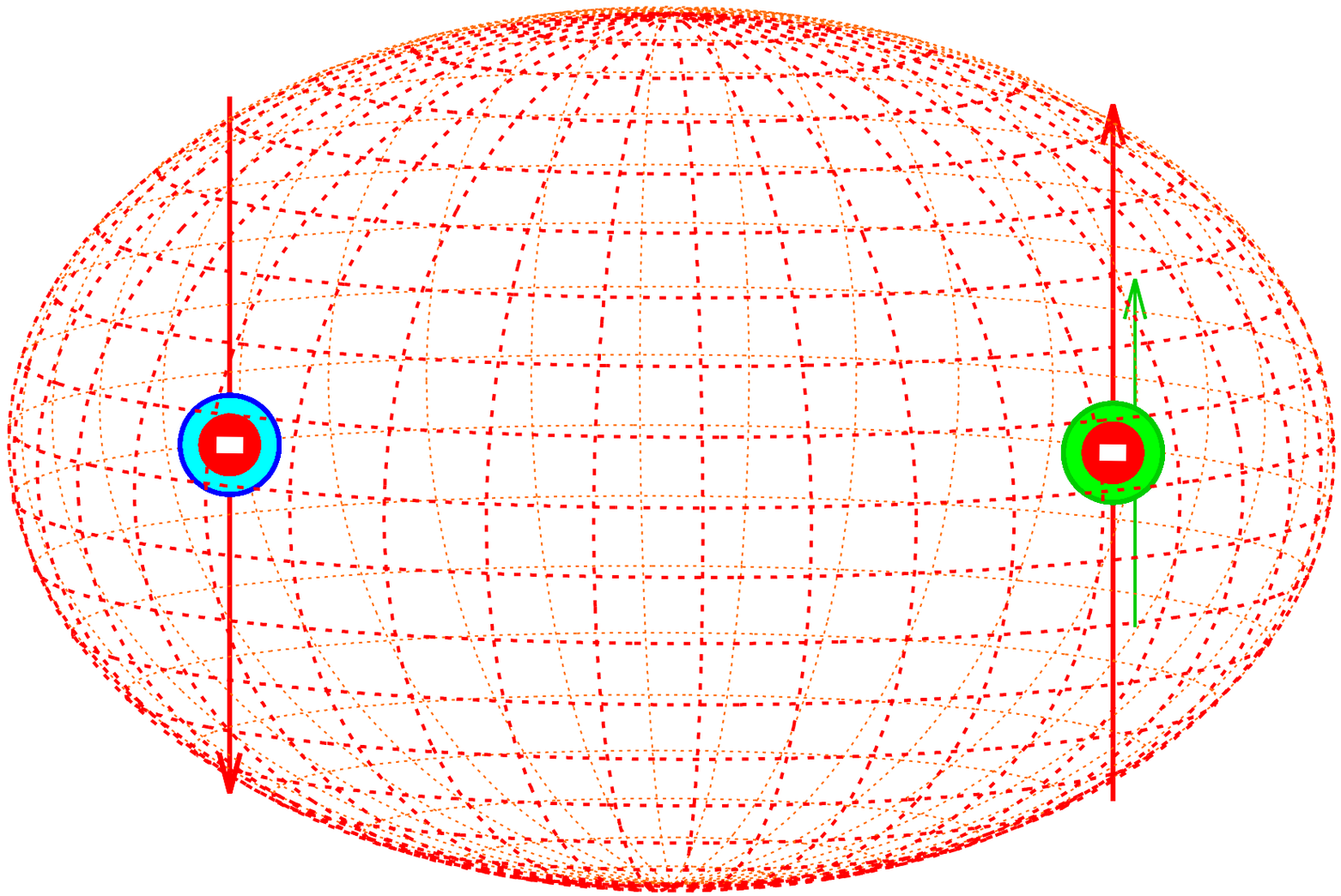


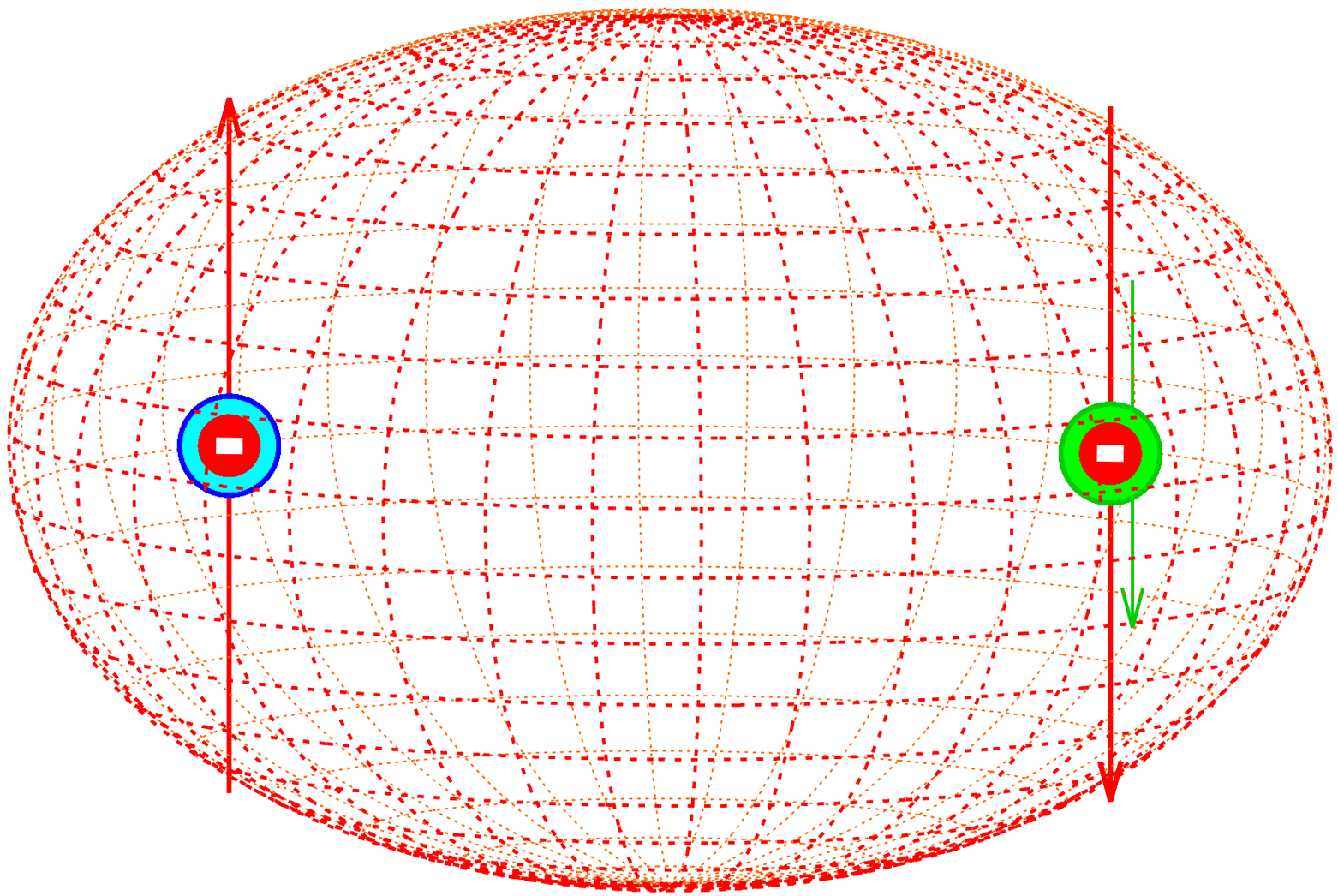
$$E = -\vec{\mu}_{\text{elektron}} \cdot \vec{\mu}_{\text{jádro}} \cdot P(\text{elektron v jádře})$$



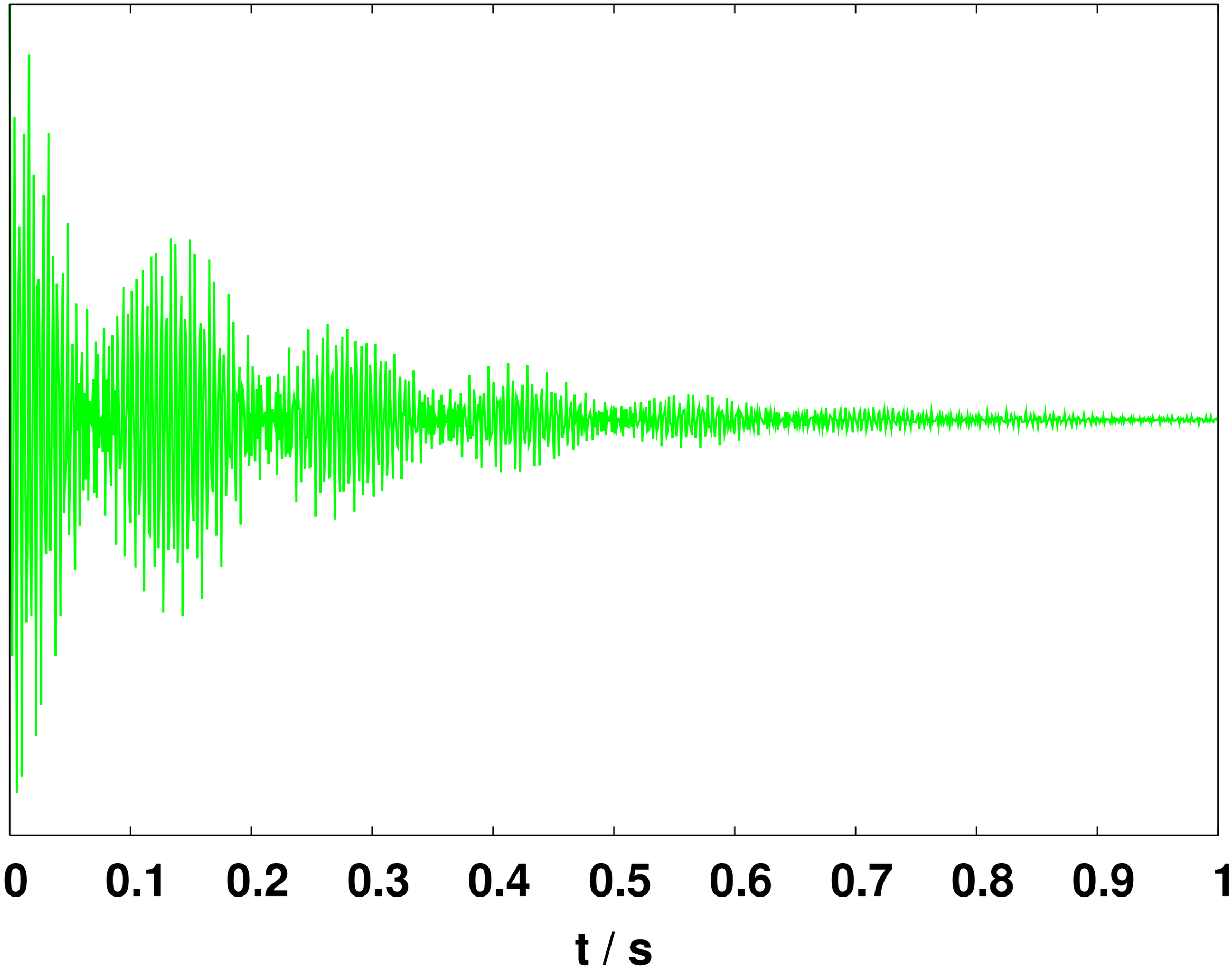








J (C–H)	130–230 Hz	
J (N–H)	90 Hz	
J (C–C)	35–55 Hz	
J (N–C)	10–15 Hz	
J (H–C–H)	14 Hz	
J (H–C–C–H)	0–14 Hz	závisí na torzním úhlu

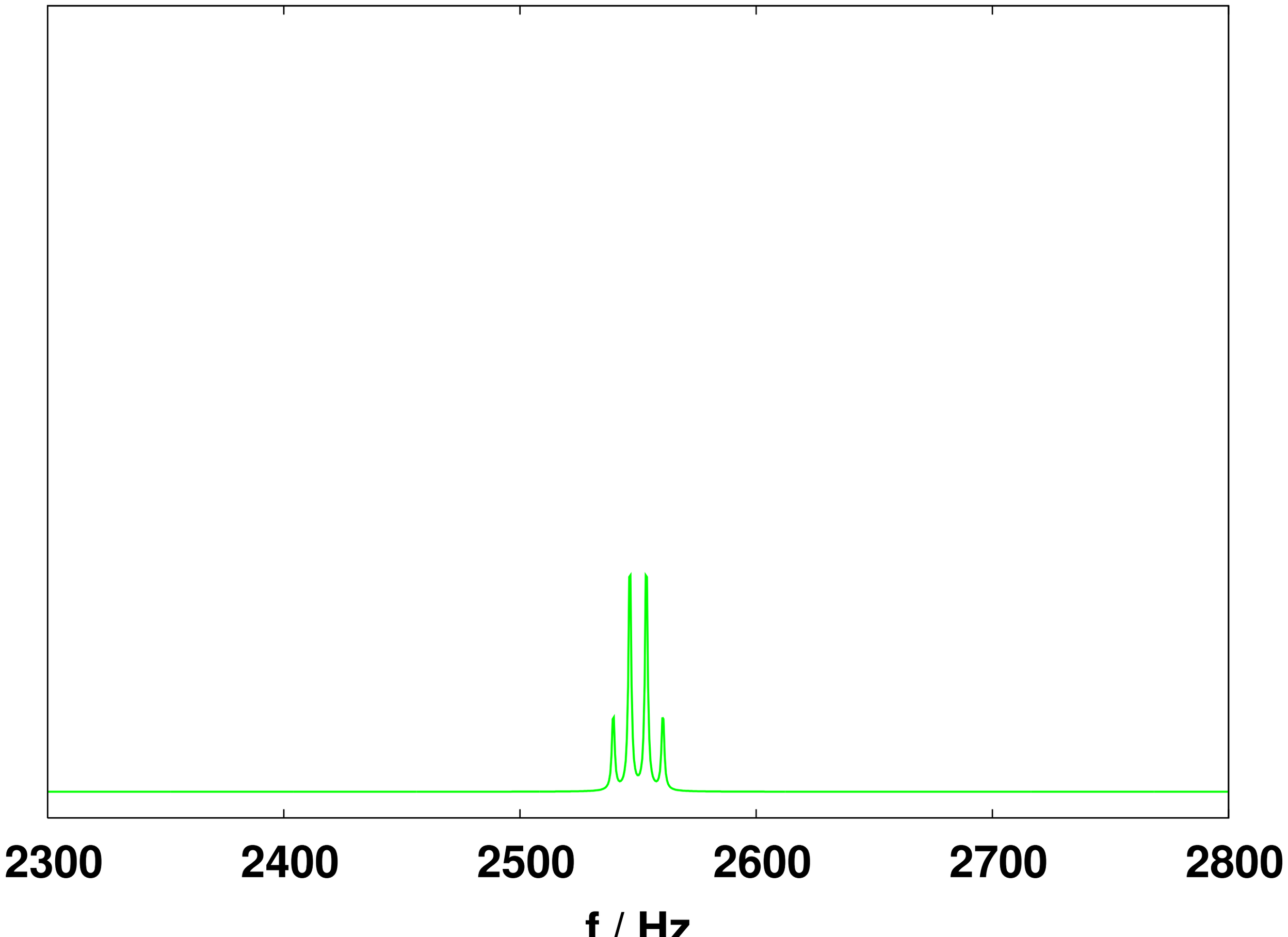

0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
t / s

-3000 -2000 -1000 0 1000 2000 3000

f / Hz

-1500 -1400 -1300 -1200 -1100 -1000

f / Hz

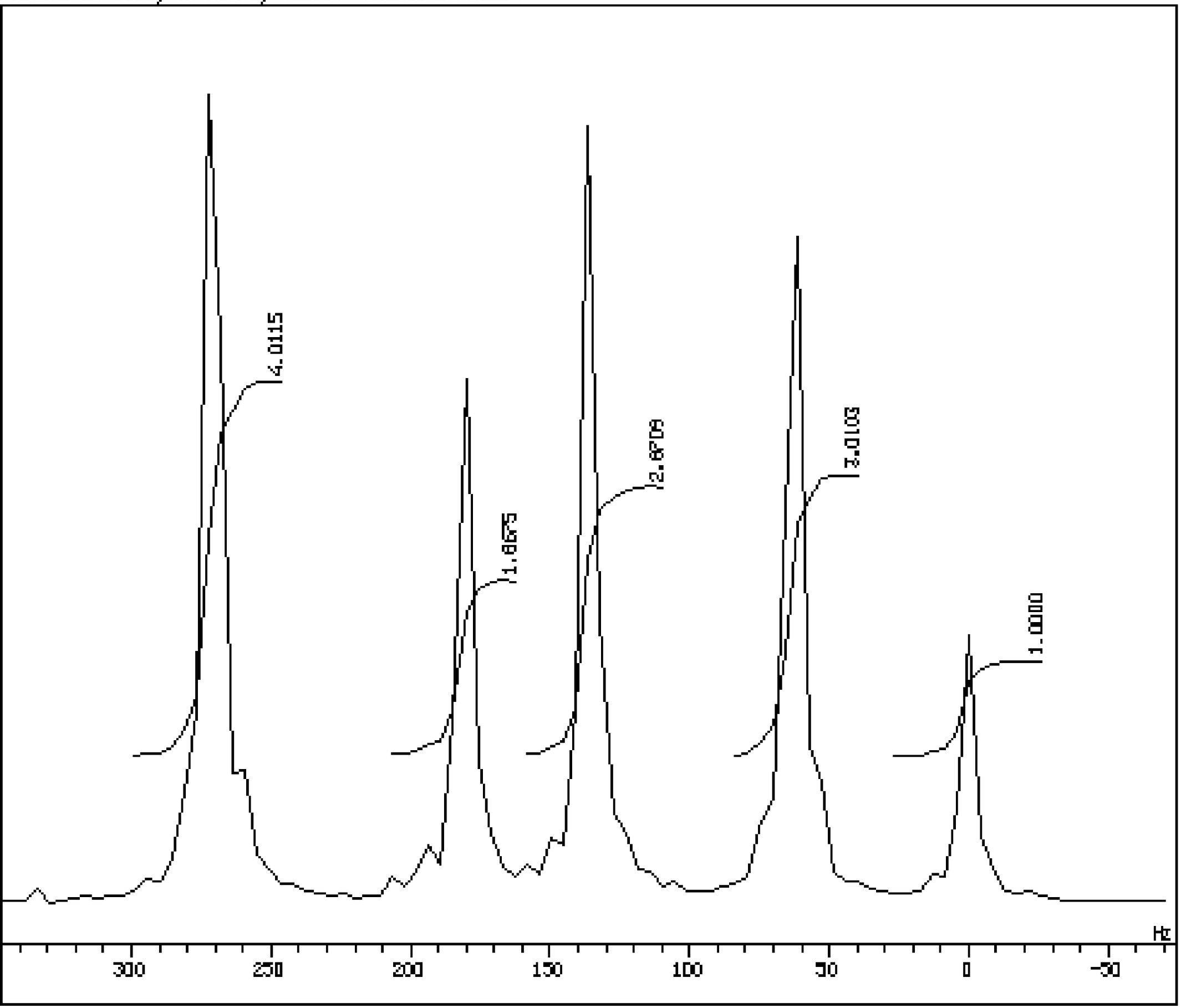

2300
2400
2500
2600
2700
2800
f / Hz

Acetaldehyd

Methyl acetaldehydu

Hänschen klein

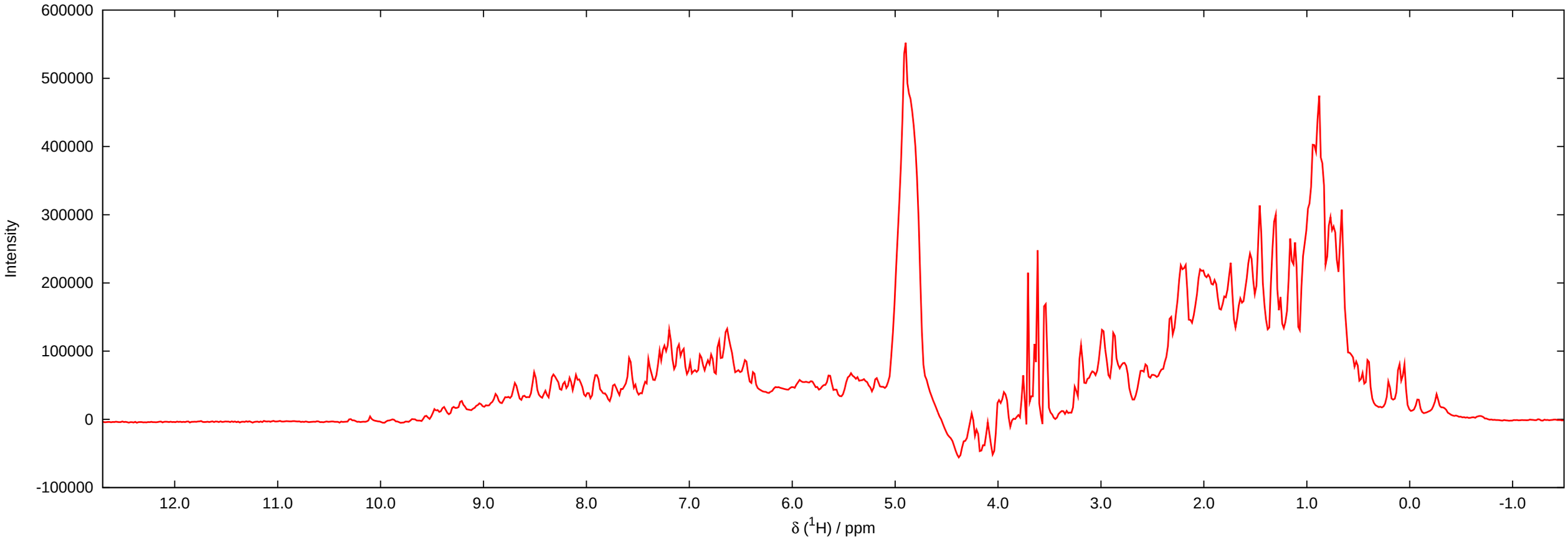
ACETONE IN C6D6, 1H H5XFG, MELODY "HAENSCHEN KLEIN"

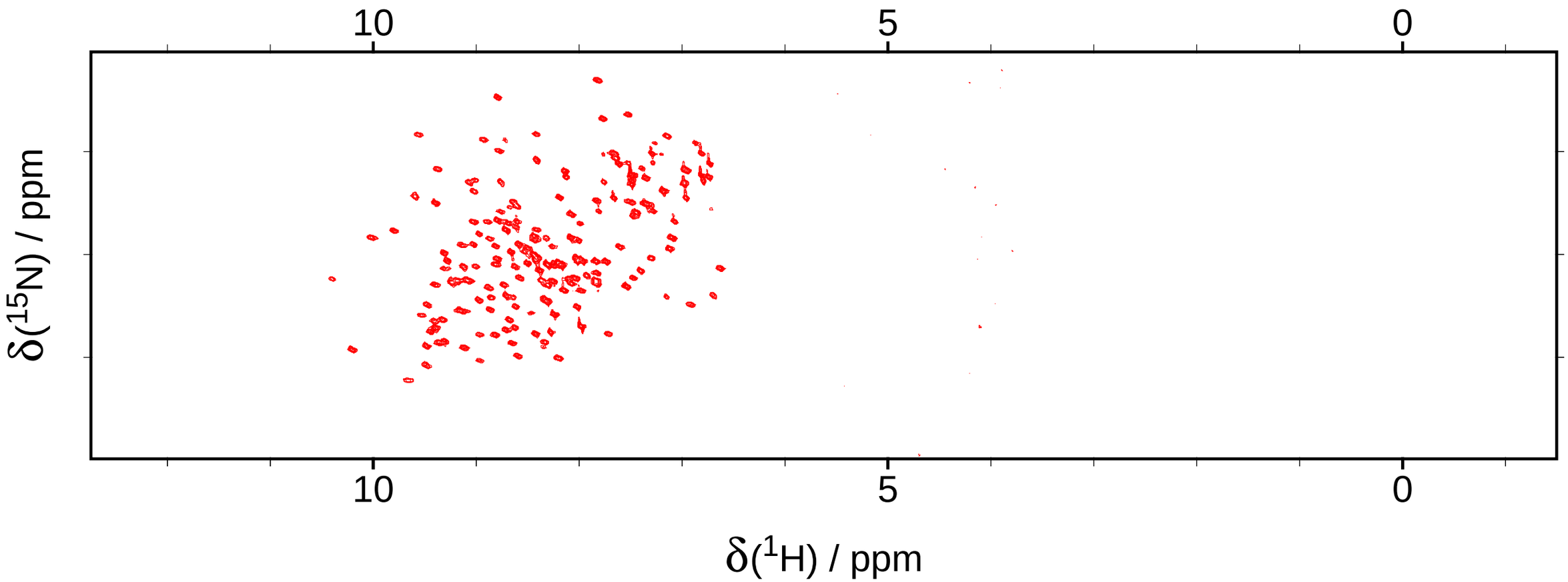


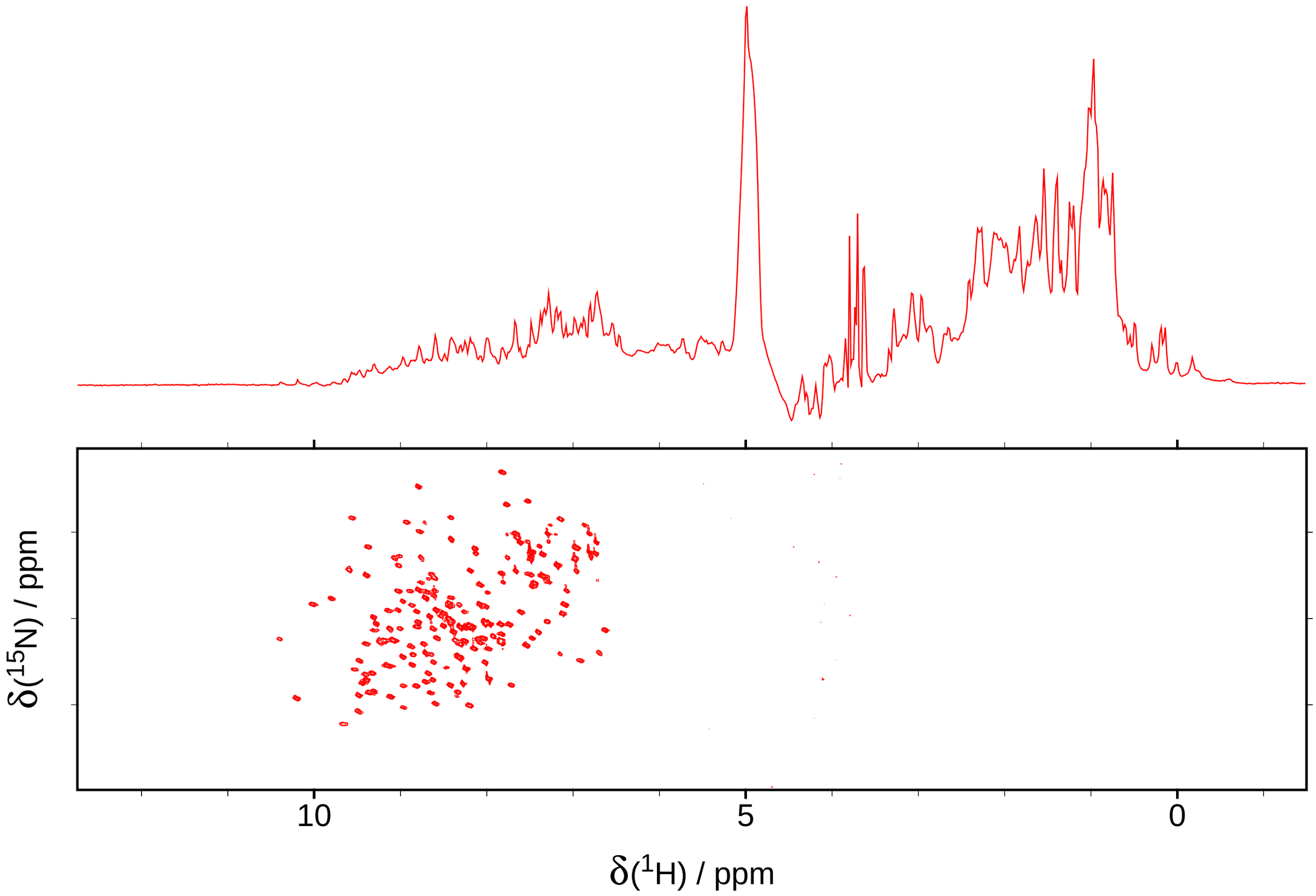
JEOL

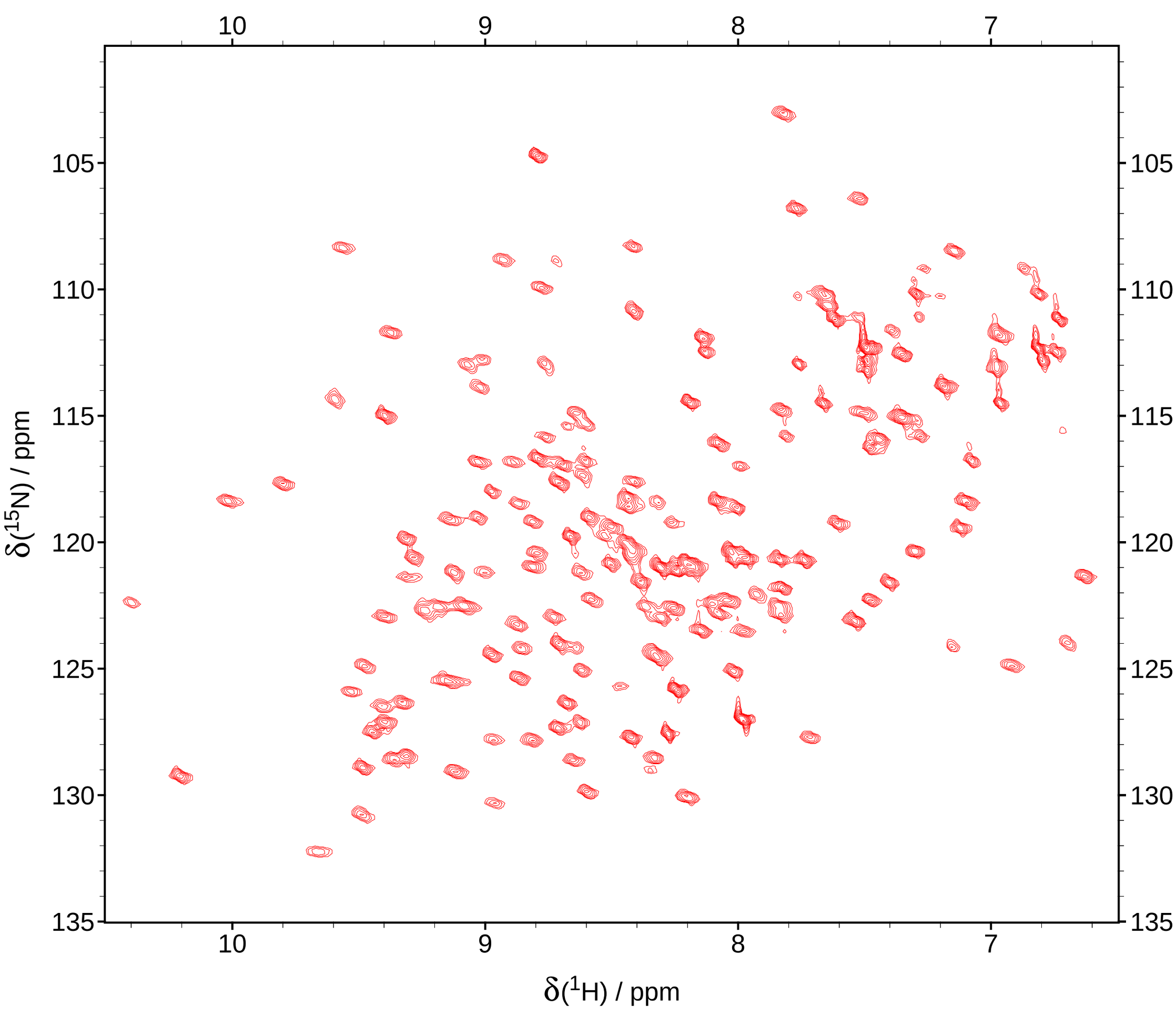
12-AUG-1996 19:16:29.54

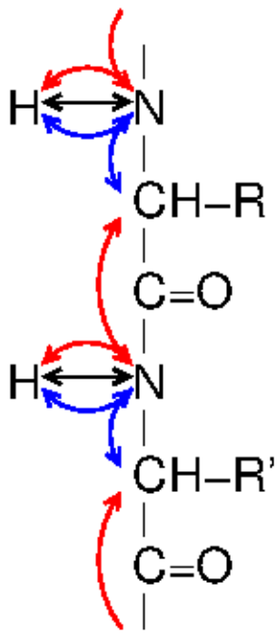
SFILE	HANS	
EXMOD	HANS	
IRMOD	NON	
POINT	2048	
SAMPO	2048	
FREQU	9000.90	Hz
FILTR	4500	Hz
SCANS	1	
DUMMY	0	
ACQTM	0.2275	sec
PD	2.7232	sec
RGAIN	10	
PW1	1.00	usec
OBNUC	1H	
OBFRQ	500.00	MHz
OBSET	160200.00	Hz
IRNUC	13C	
IRFRQ	125.65	MHz
IRSET	127958.00	Hz
IRATN	511	
IRPWH	50.0	usec
IRBP1	50	
IRBP2	6	
IRRNS	0	
TRNUC	1H	
TRFRQ	500.00	MHz
TRSET	162410.00	Hz
TRATN	511	
TRPWH	50.0	usec
TRBP1	30	
TRBP2	6	
TRRNS	0	
CTEMP	24.1	c
CSPED	11	Hz
SLVNT	C6D6	
RESOL	4.39	Hz
NMOMD	8	
BF	0.10	Hz
GF	0.00	Hz
PP	840	cp
ABSP0	-92.11	deg
ABSP1	0.00	deg
T1	0.00	x
T2	0.00	x
T3	90.00	x
T4	100.00	x
REFVL	0.00	ppm
THSP	902	
XE	421.92	Hz
XS	-676.83	Hz
YS	0.000	

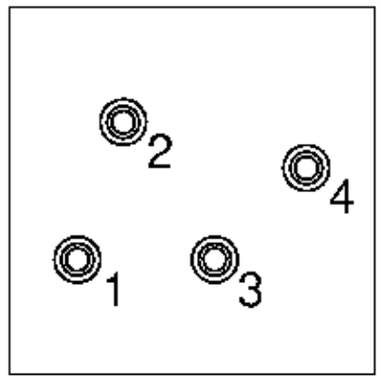

600000
500000
400000
300000
200000
100000
0
-100000
Intensity
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
δ (1H) / ppm

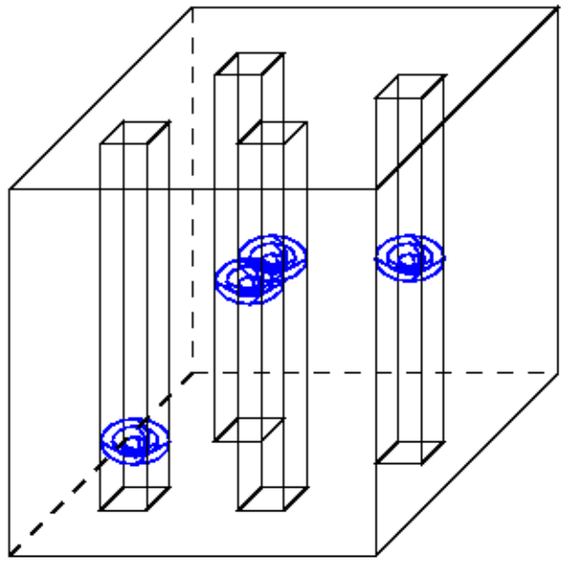

10
5
0
δ(15N) / ppm
10
5
0
δ(1H) / ppm

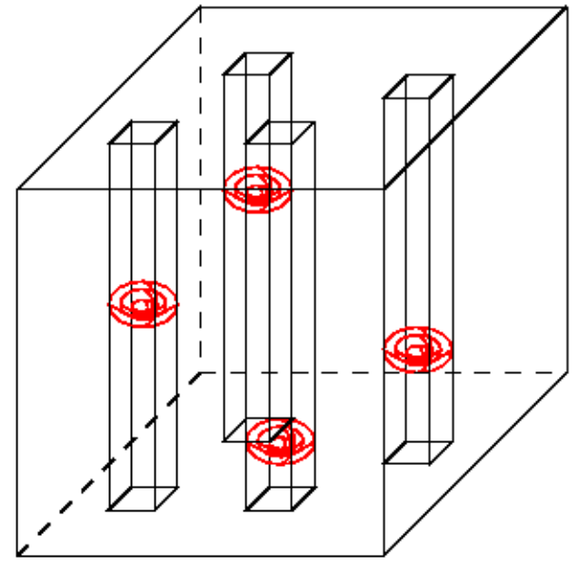

δ(15N) / ppm
10
5
0
δ(1H) / ppm

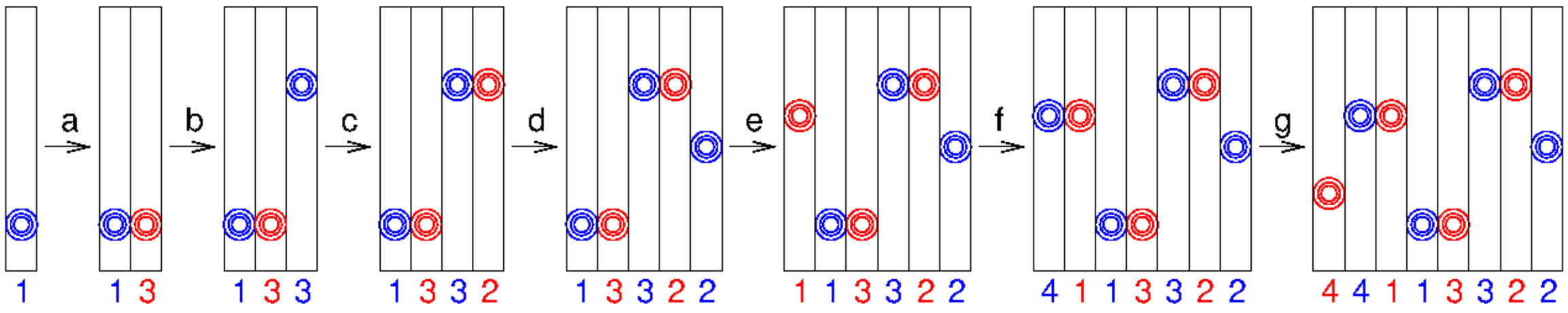

10
9
8
7
105
110
115
120
125
130
135
δ(15N) / ppm
δ(1H) / ppm

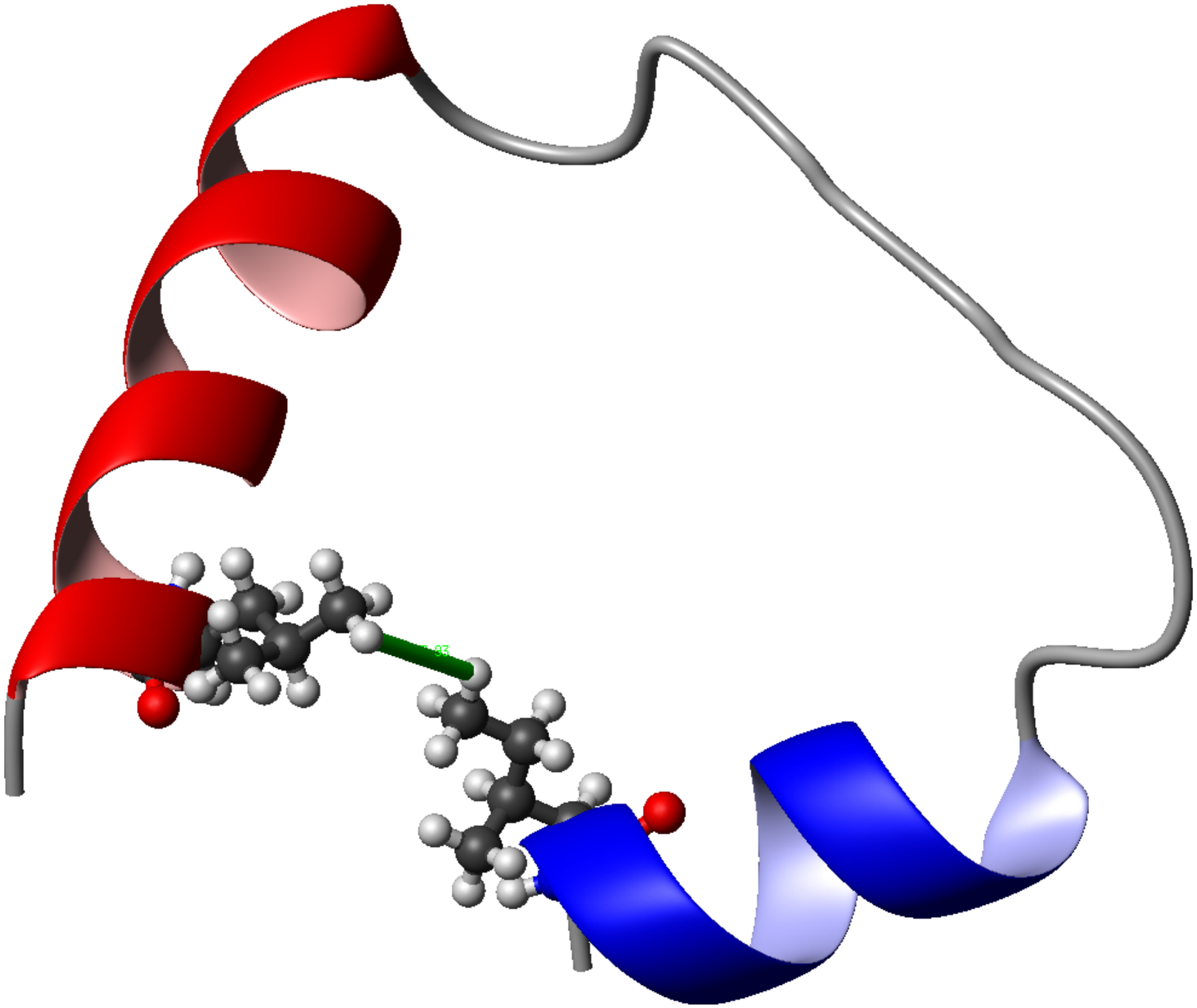

H
N
CH–R
C=O
H
N
CH–R'
C=O

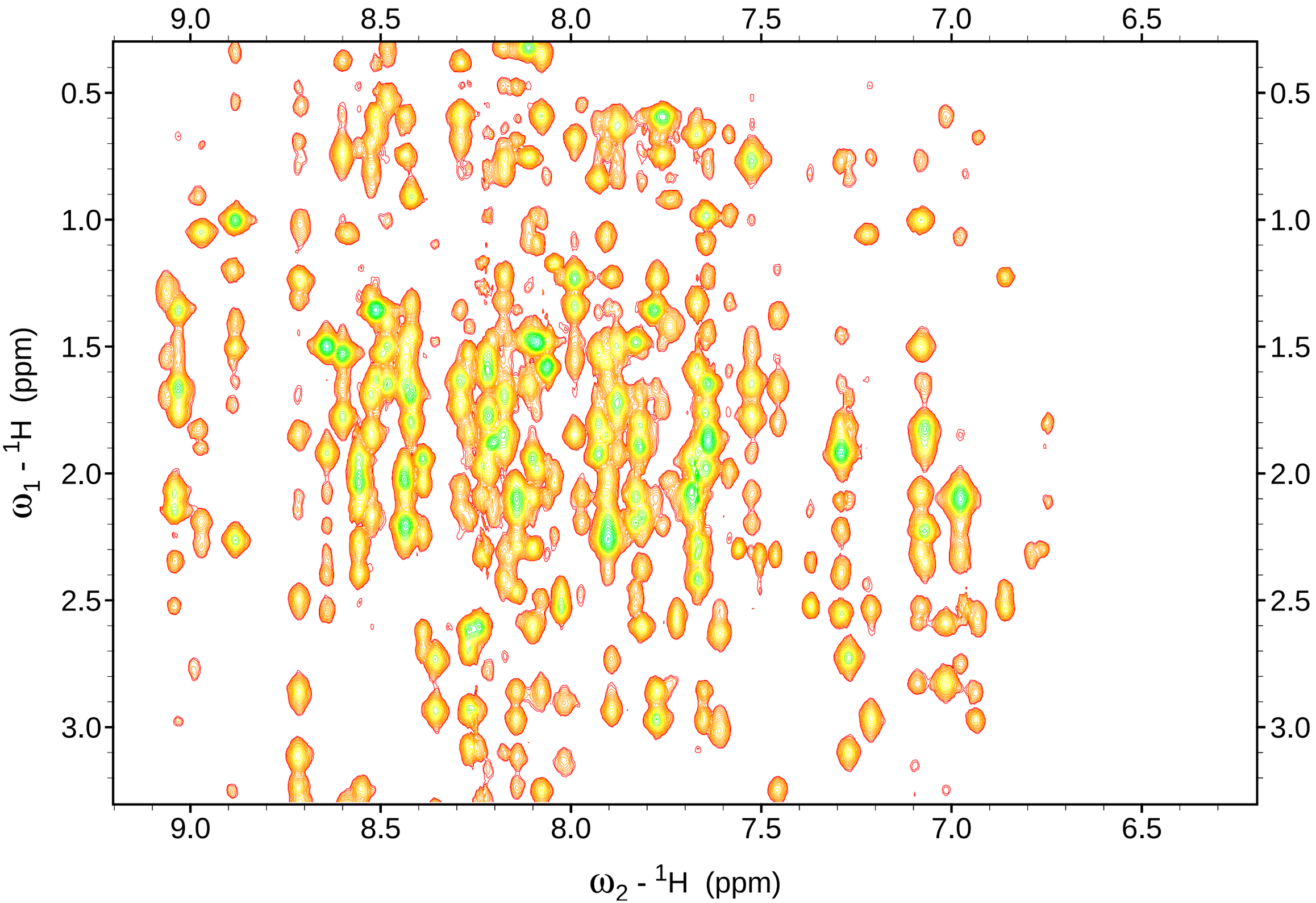

2
4
1
3

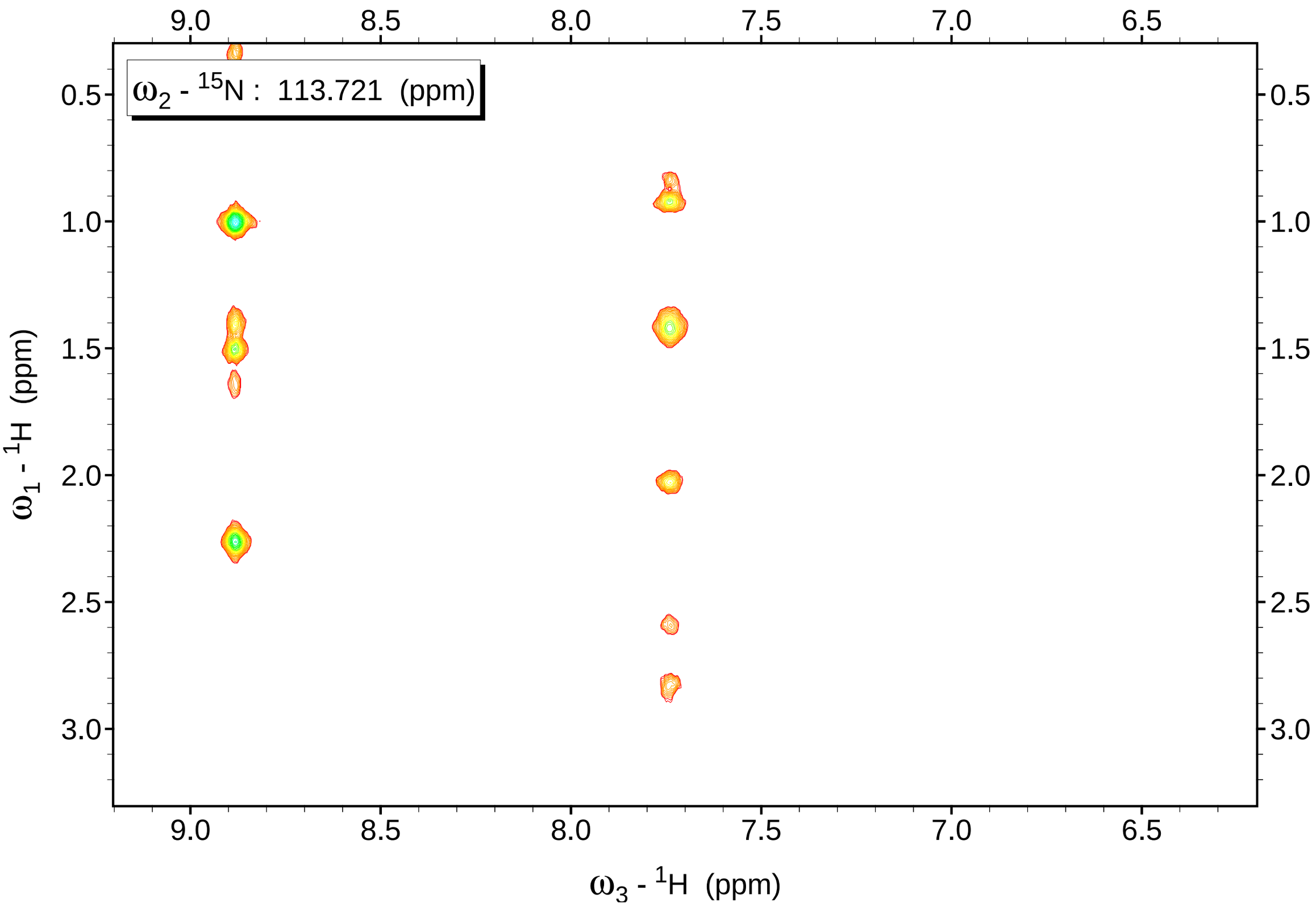


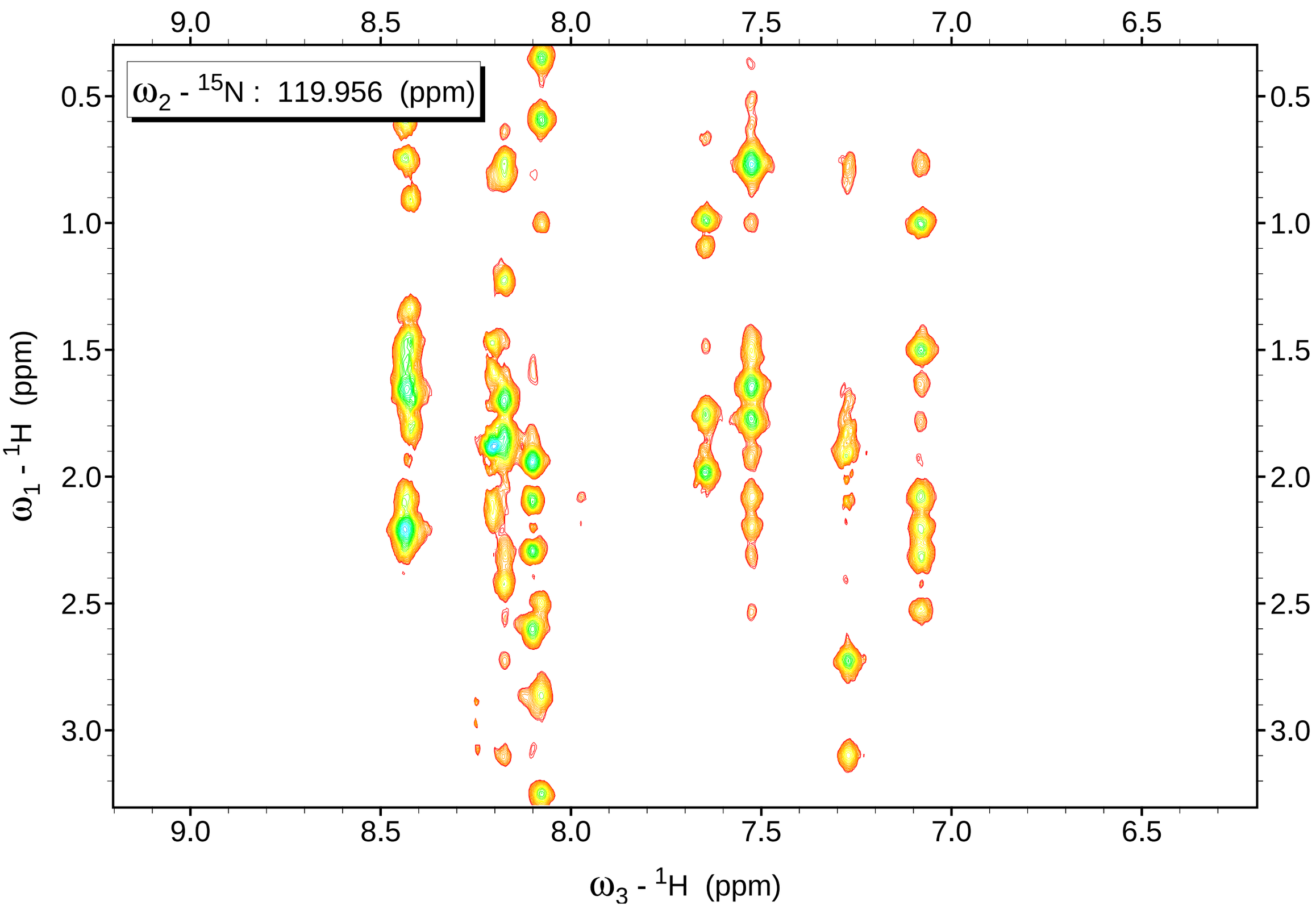


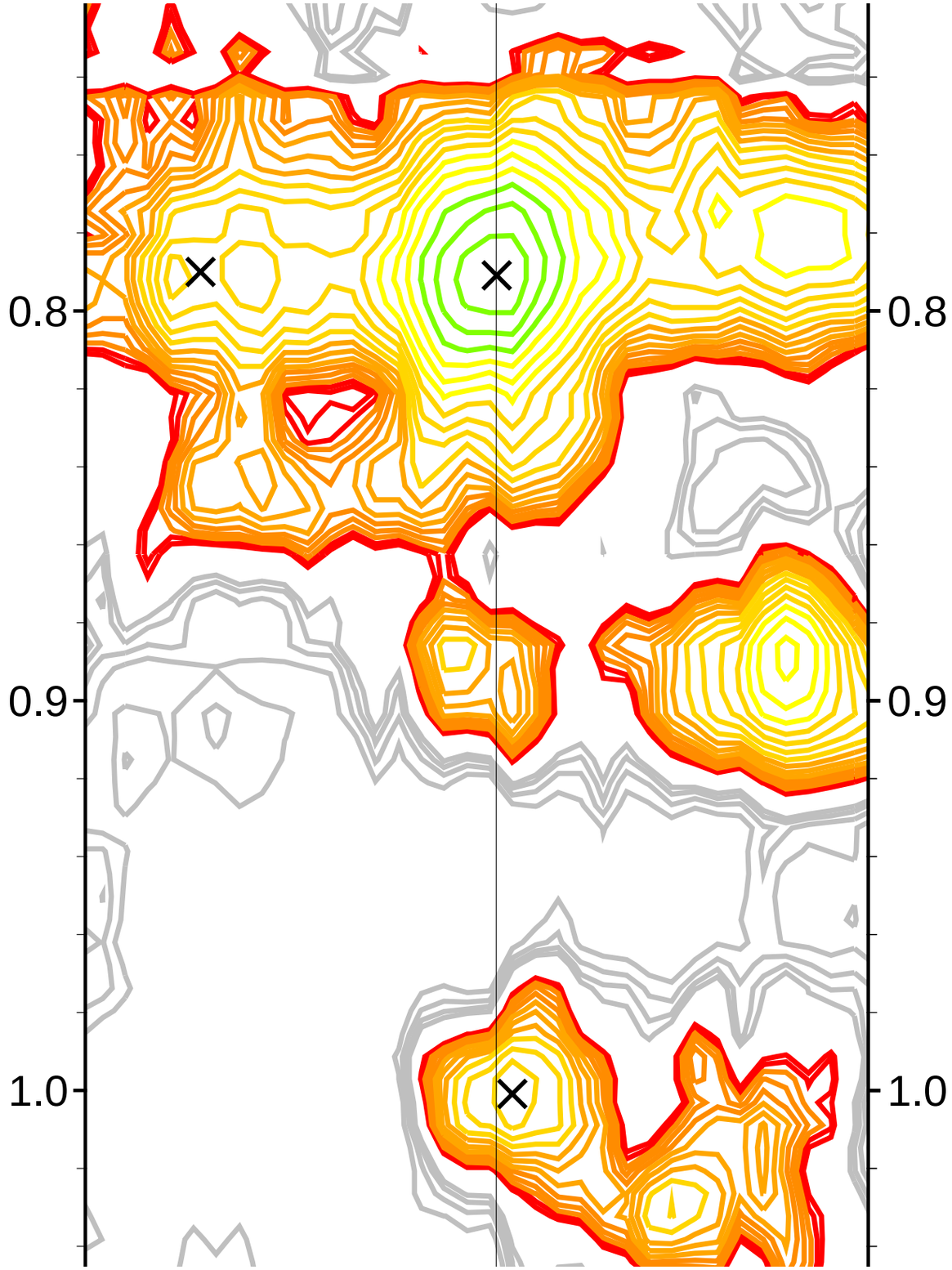

a
b
c
d
e
f
g
1
1 3
1 3 3
1 3 3 2
1 3 3 2 2
1 1 3 3 2 2
4 1 1 3 3 2 2
4 4 1 1 3 3 2 2

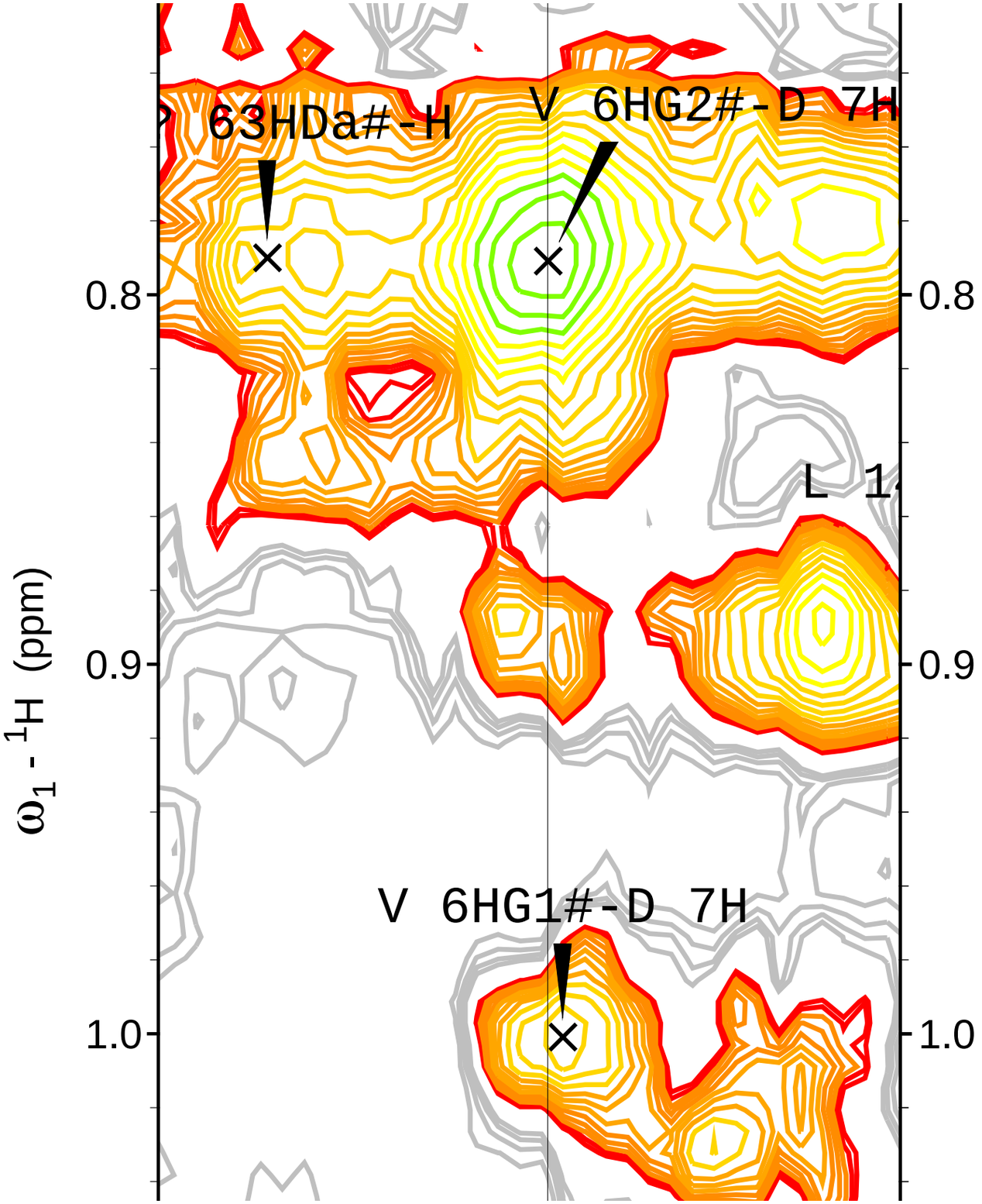



9.0
8.5
8.0
7.5
7.0
6.5
0.5
1.0
1.5
2.0
2.5
3.0
ω_1 - ^{1}H (ppm)
ω_2 - ^{1}H (ppm)


ω2 - 15N : 113.721 (ppm)
9.0
8.5
8.0
7.5
7.0
6.5
0.5
1.0
1.5
2.0
2.5
3.0
ω1 - 1H (ppm)
ω3 - 1H (ppm)


ω2 - 15N : 119.956 (ppm)
9.0
8.5
8.0
7.5
7.0
6.5
0.5
1.0
1.5
2.0
2.5
3.0
ω1 - 1H (ppm)
ω3 - 1H (ppm)

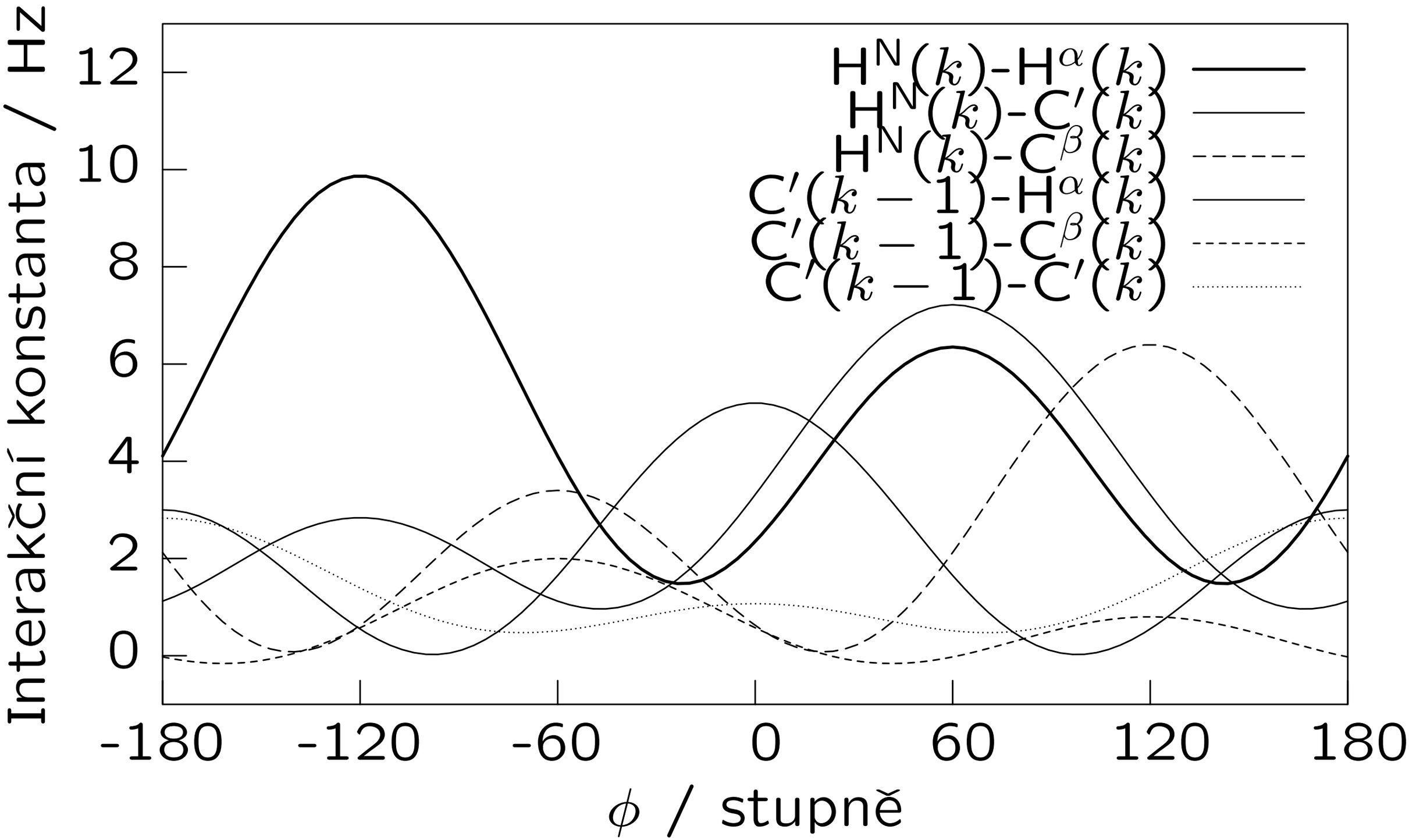

0.8
0.9
1.0
0.8
0.9
1.0
ω_1 - ^{1}H (ppm)


63HDa#-H
V 6HG2#-D 7H
V 6HG1#-D 7H
0.8
0.9
1.0
ω_1 - ^{1}H (ppm)

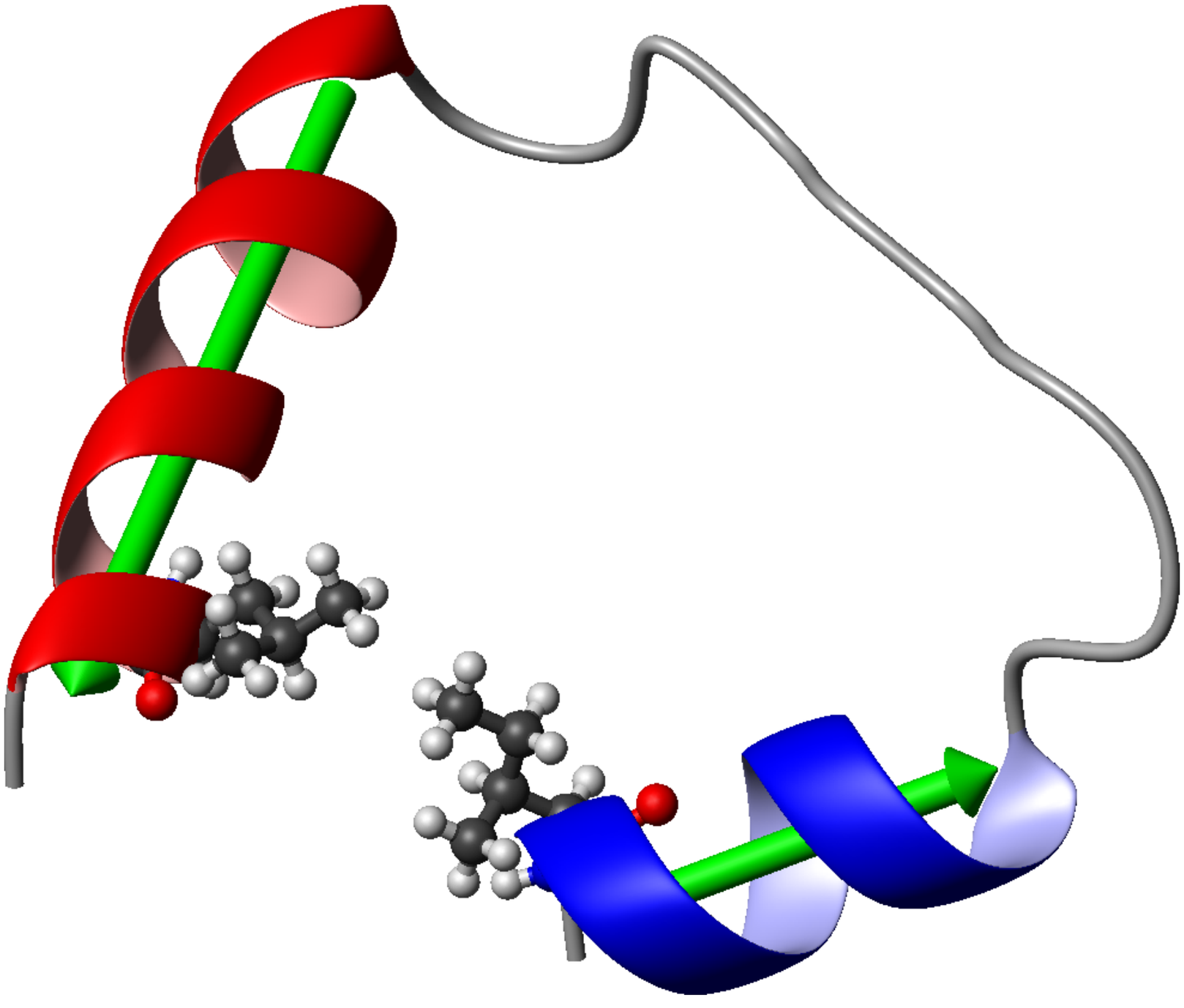
$$\frac{S}{S_{\mathrm{ref}}} = \left(\frac{r_{\mathrm{ref}}}{r}\right)^6 \tag{1}$$

$$r = r_{\mathrm{ref}} \quad \sqrt[6]{\frac{S_{\mathrm{ref}}}{S}} \tag{2}$$

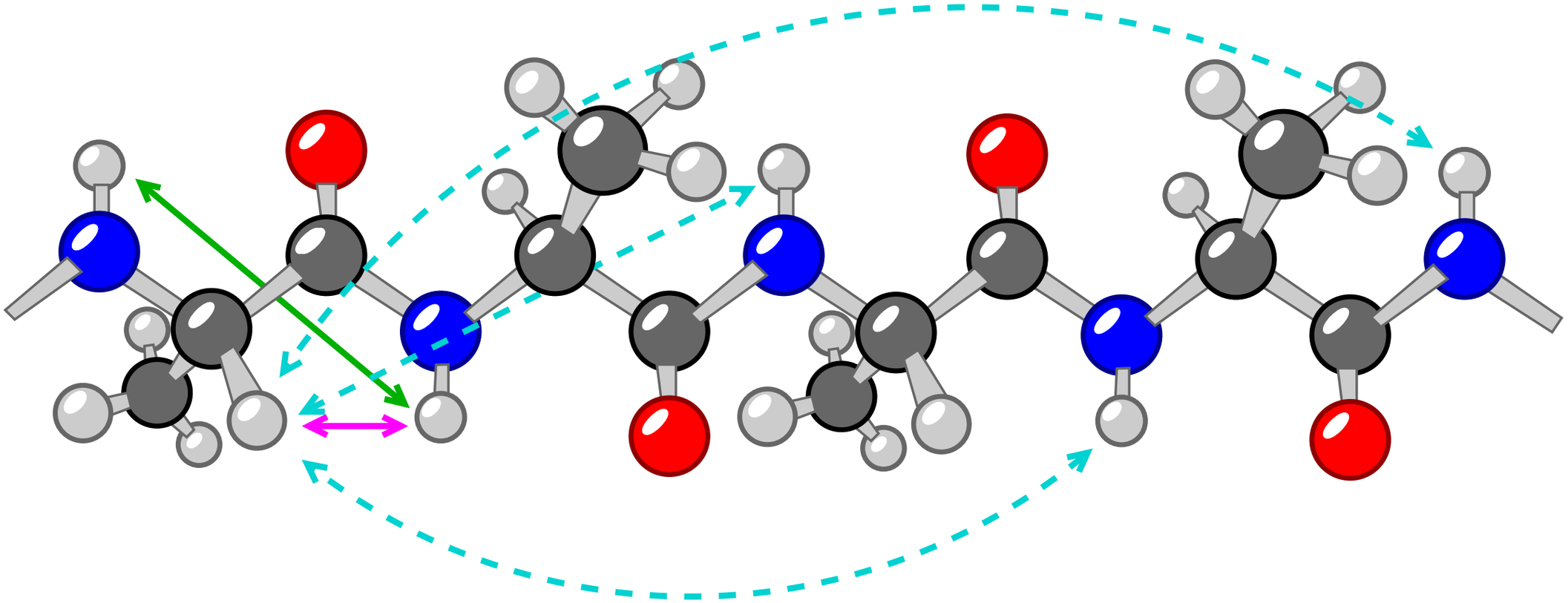
Referenční protony		vzdálenost
geminální v methylenu	H–C–H	0,17 nm
vicinální v aromatickém kruhu	H–C=C–H	0,25 nm
meta v aromatickém kruhu	H–C=CH–C–H	0,42 nm

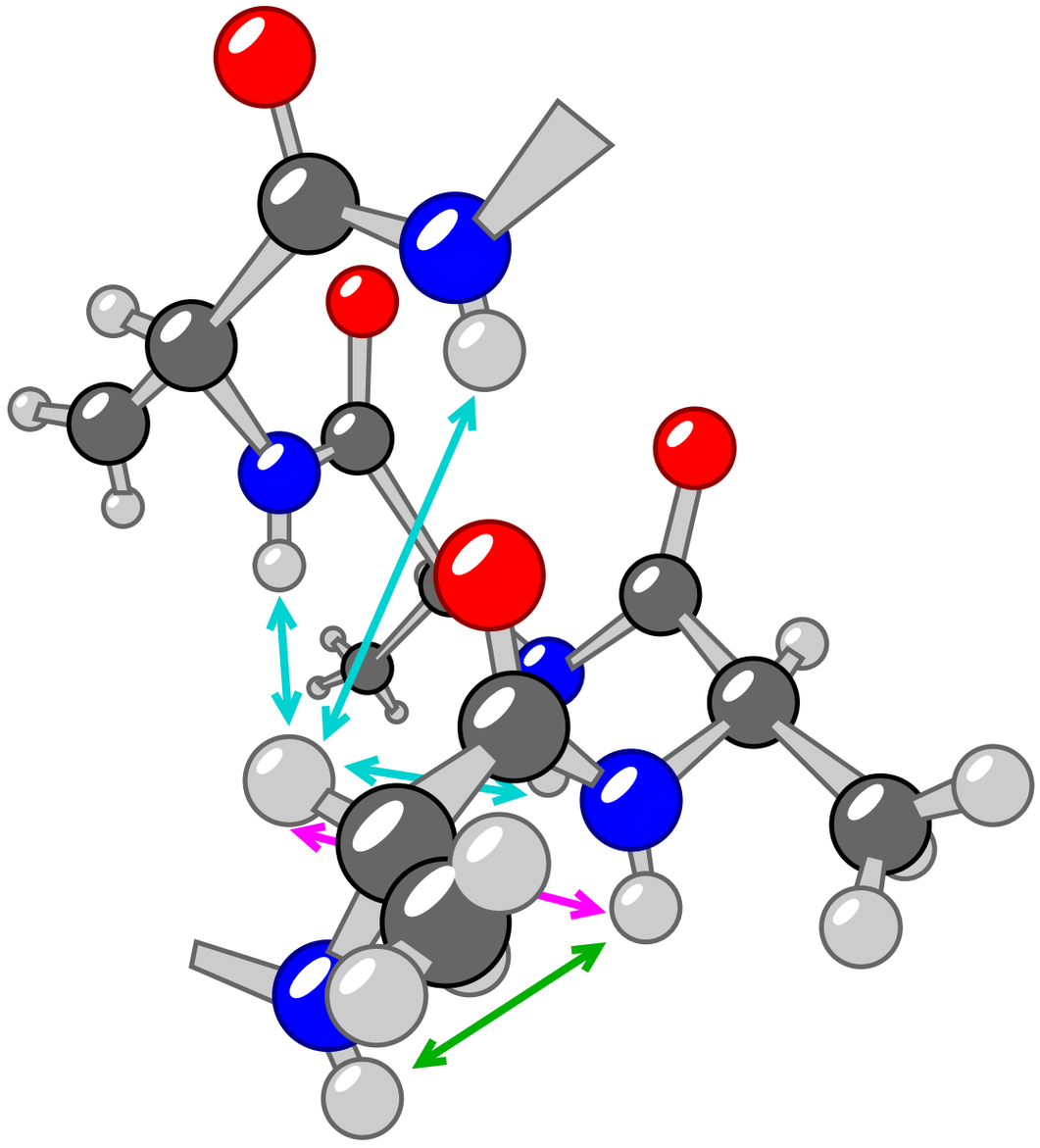

Interakční konstanta / Hz
12
10
8
6
4
2
0
-180
-120
-60
0
60
120
180
ϕ / stupně
$\mathrm{H}^{\mathrm{N}}(k)$-$\mathrm{H}^{\alpha}(k)$
$\mathrm{H}^{\mathrm{N}}(k)$-$\mathrm{C}'(k)$
$\mathrm{H}^{\mathrm{N}}(k)$-$\mathrm{C}^{\beta}(k)$
$\mathrm{C}'(k-1)$-$\mathrm{H}^{\alpha}(k)$
$\mathrm{C}'(k-1)$-$\mathrm{C}^{\beta}(k)$
$\mathrm{C}'(k-1)$-$\mathrm{C}'(k)$

Zbytkové dipólové interakce $\propto \langle 3\cos^2\theta - 1\rangle \Rightarrow$ orientace



Data	β-**list**	α-**šroubovice**
$\delta(\mathrm{C}')$, $\delta(\mathrm{C}^{\alpha})$	$\downarrow$	$\uparrow$
$\delta(\mathrm{C}^{\beta})$, $\delta(\mathrm{H}^{\alpha})$	$\uparrow$	$\downarrow$
$\lvert \mathrm{H}_i^{\alpha}\mathrm{H}_{i+1}^{\mathrm{N}} \rvert$	0,22 nm	0,35 nm
$\lvert \mathrm{H}_i^{\mathrm{N}}\mathrm{H}_{i+1}^{\mathrm{N}} \rvert$	0,40 nm	0,28 nm
$\lvert \mathrm{H}_i^{\alpha}\mathrm{H}_{i+2}^{\mathrm{N}} \rvert$	daleko	0,42 nm
$\lvert \mathrm{H}_i^{\alpha}\mathrm{H}_{i+3}^{\mathrm{N}} \rvert$	daleko	0,34 nm
$\lvert \mathrm{H}_i^{\alpha}\mathrm{H}_{i+4}^{\mathrm{N}} \rvert$	daleko	0,42 nm
${}^3J(\mathrm{H}_i^{\mathrm{N}}\mathrm{H}_i^{\alpha})$	$>$ 8 Hz	$<$ 5 Hz



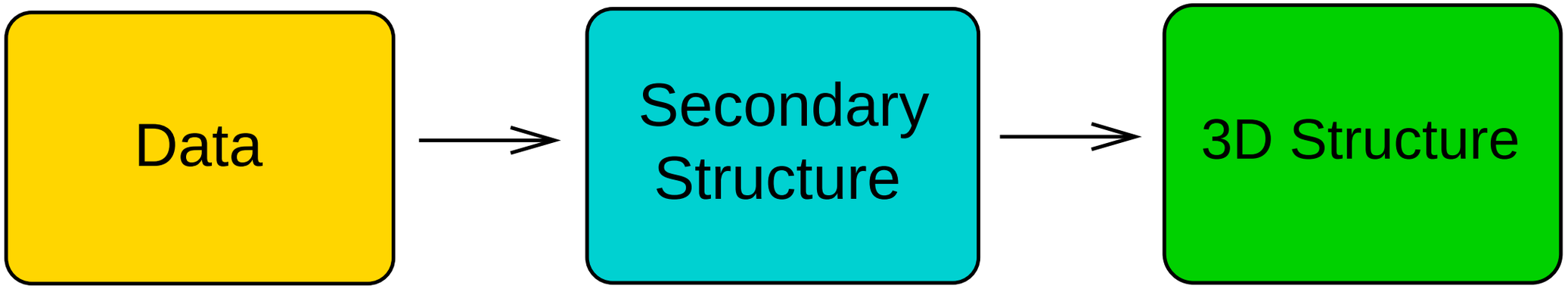


α-helix

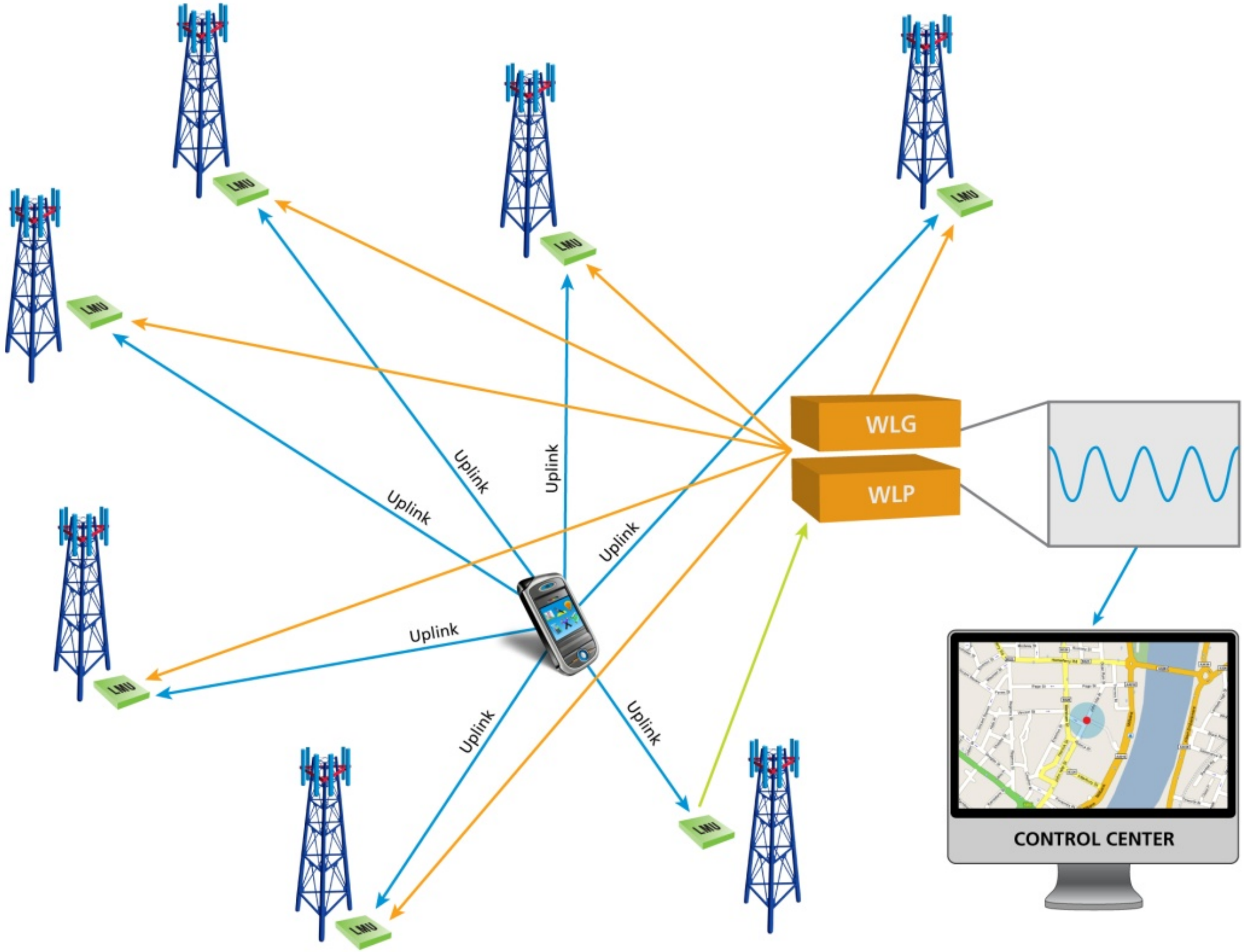
3_{10}-helix

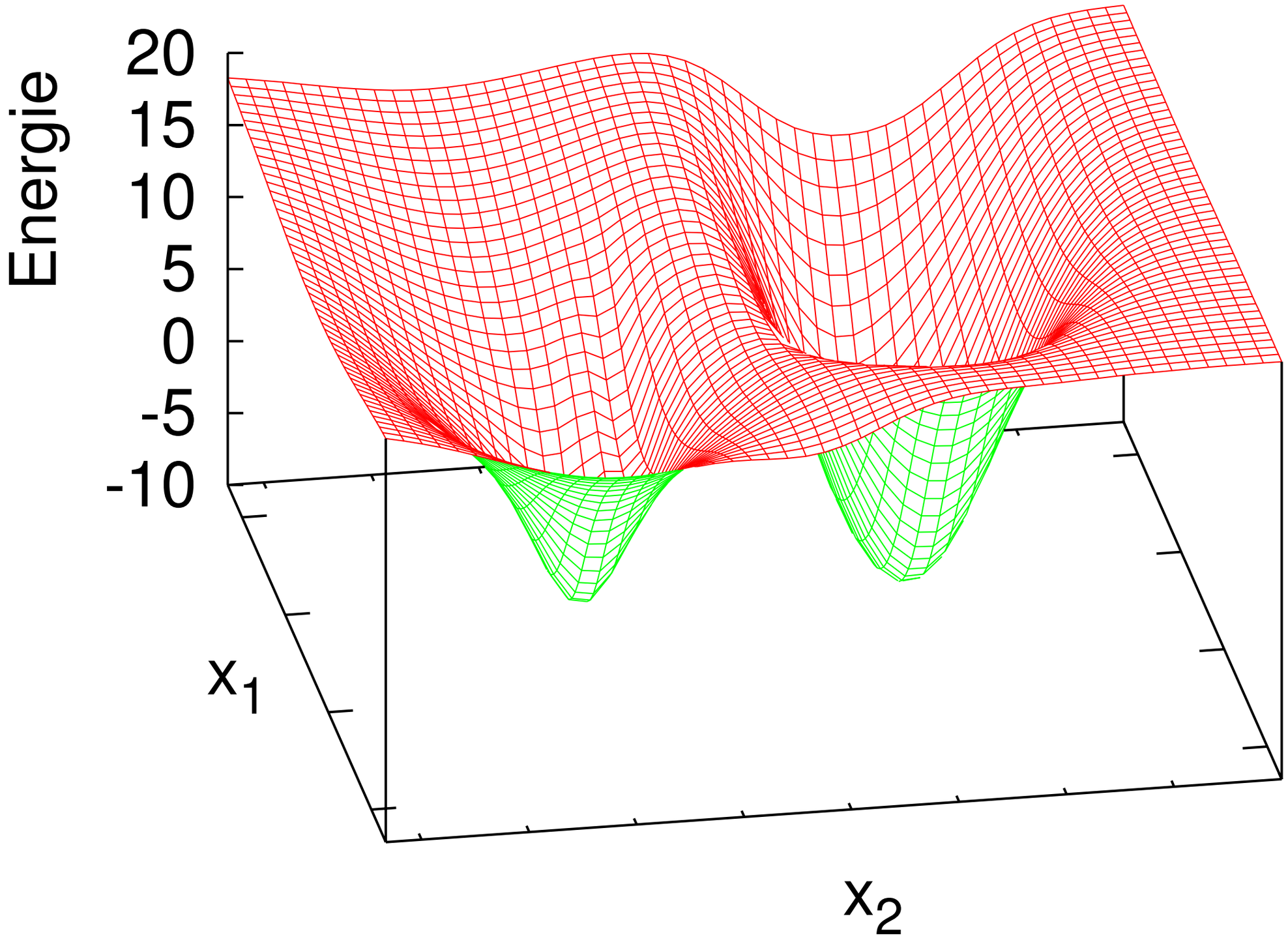
antiparallel β-sheet

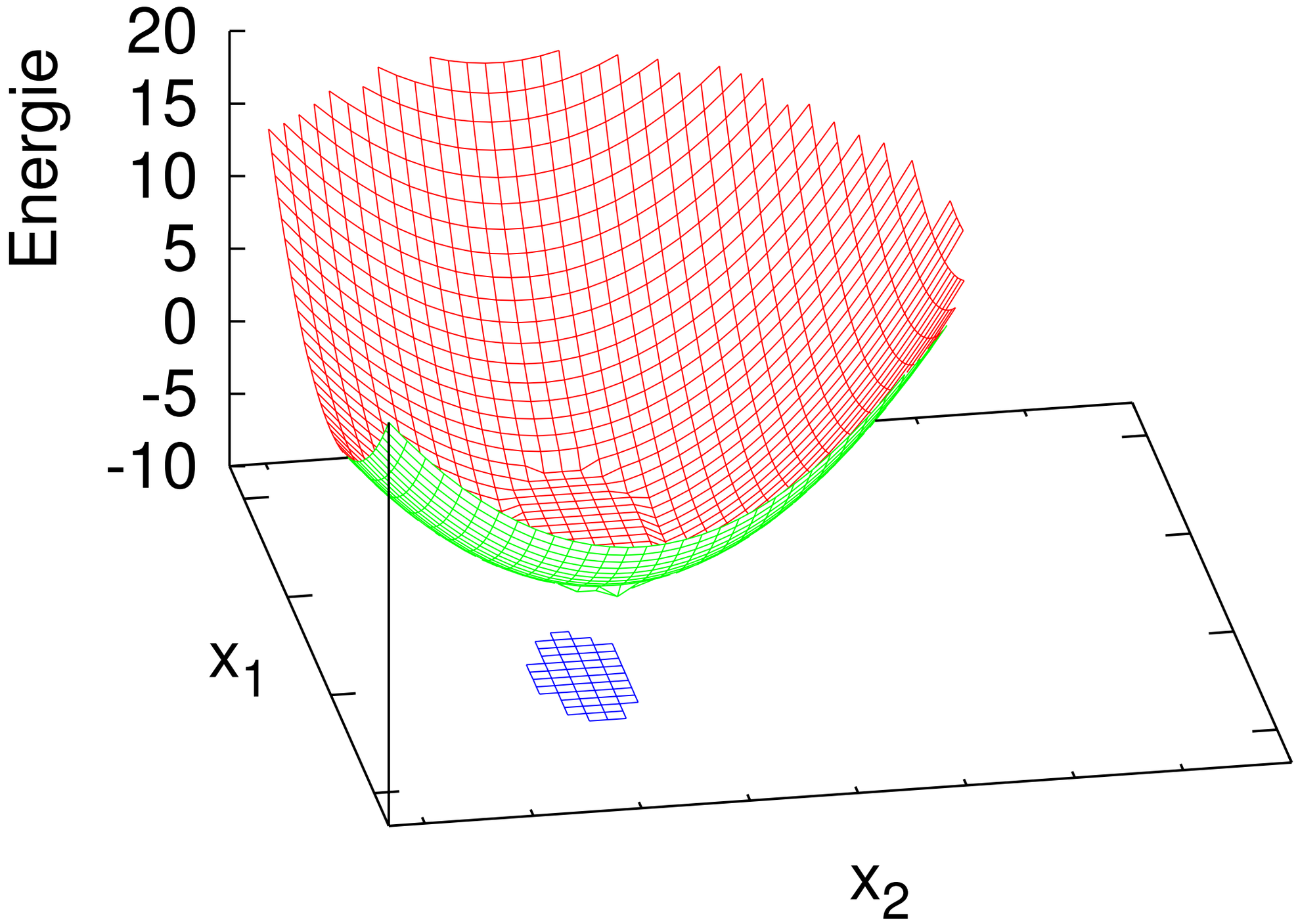
parallel β-sheet

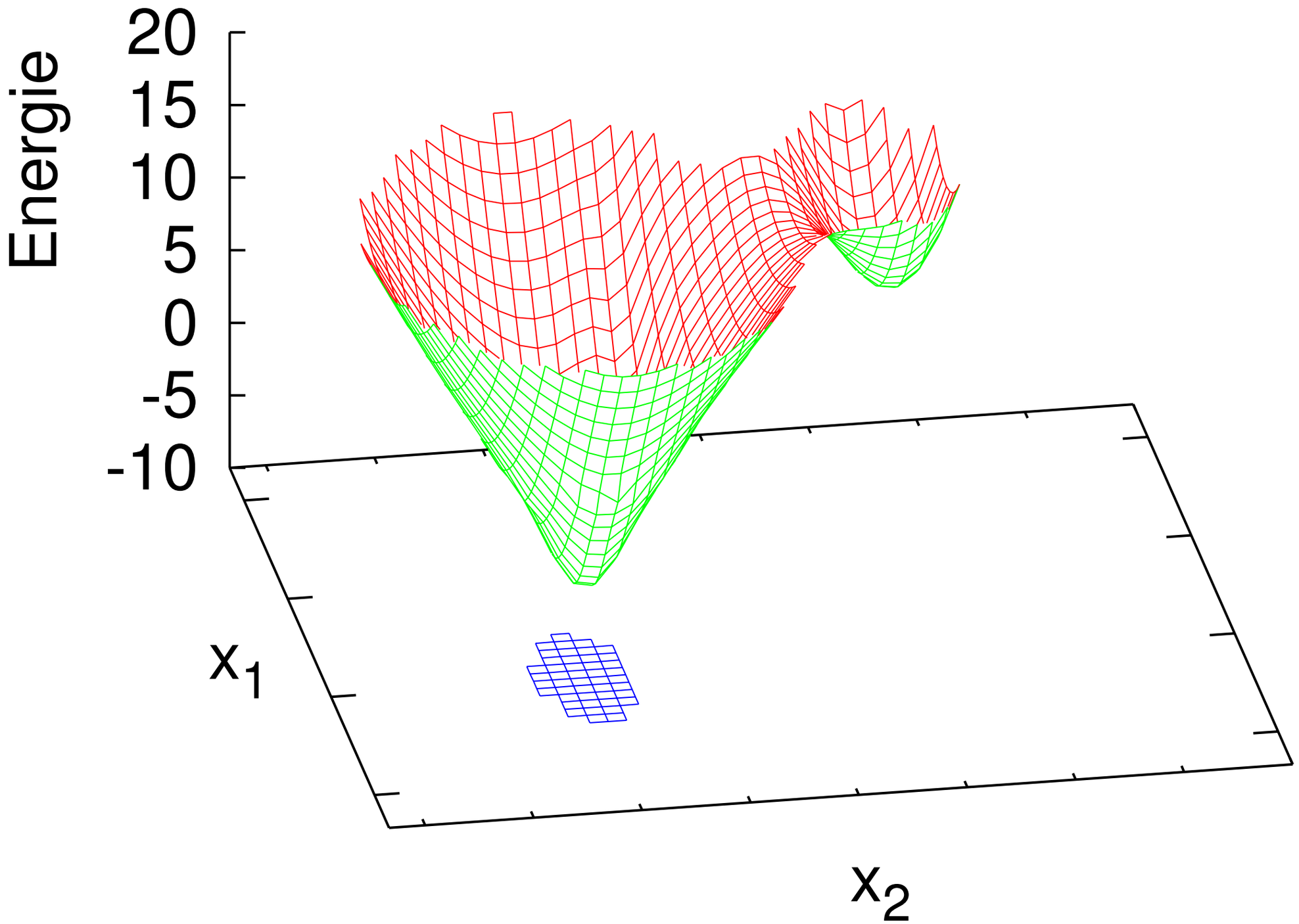

Data
Secondary Structure
3D Structure

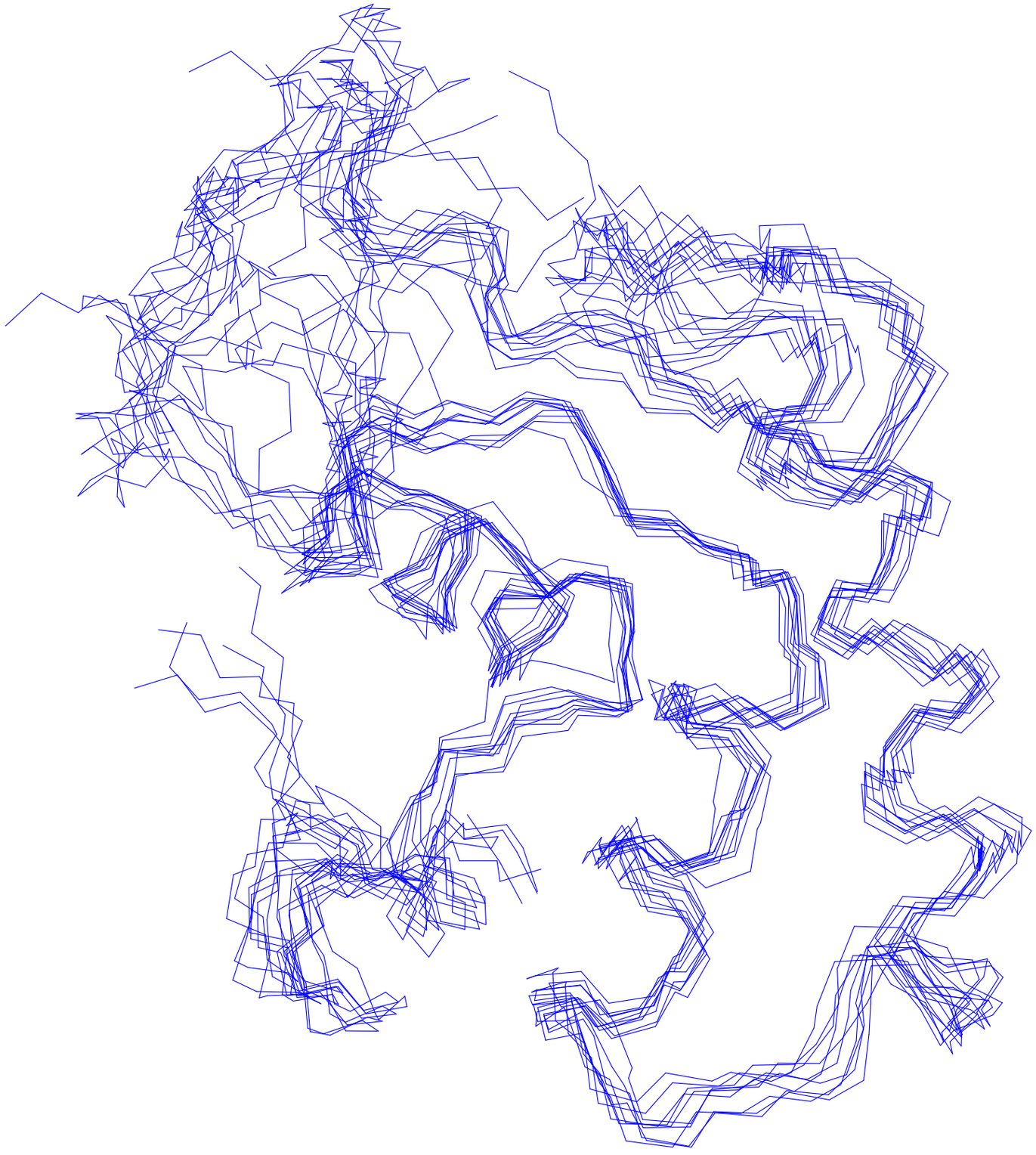

TALOS prediction
^{3}J prediction
NOE prediction
CSI prediction
10 20 30 40 50 60 70 80 90
GIKQYSQEELKEMALVEIAHELFEEHKKPVPFQELLNEIASLLGVKKEEL GDRIAQFYTDLNIDGRFLALSDQTWGLRSWYPYDQLDEETQLEHHHHHH
TALOS
^{3}J(H$^\alpha$H^N)
NN$(i, i+1)$
αN$(i, i+1)$
αN$(i, i+2)$
αN$(i, i+3)$
αN$(i, i+4)$
CO CSI
C$^\alpha$ CSI
H$^\alpha$ CSI
C$^\beta$ CSI
consensus CSI

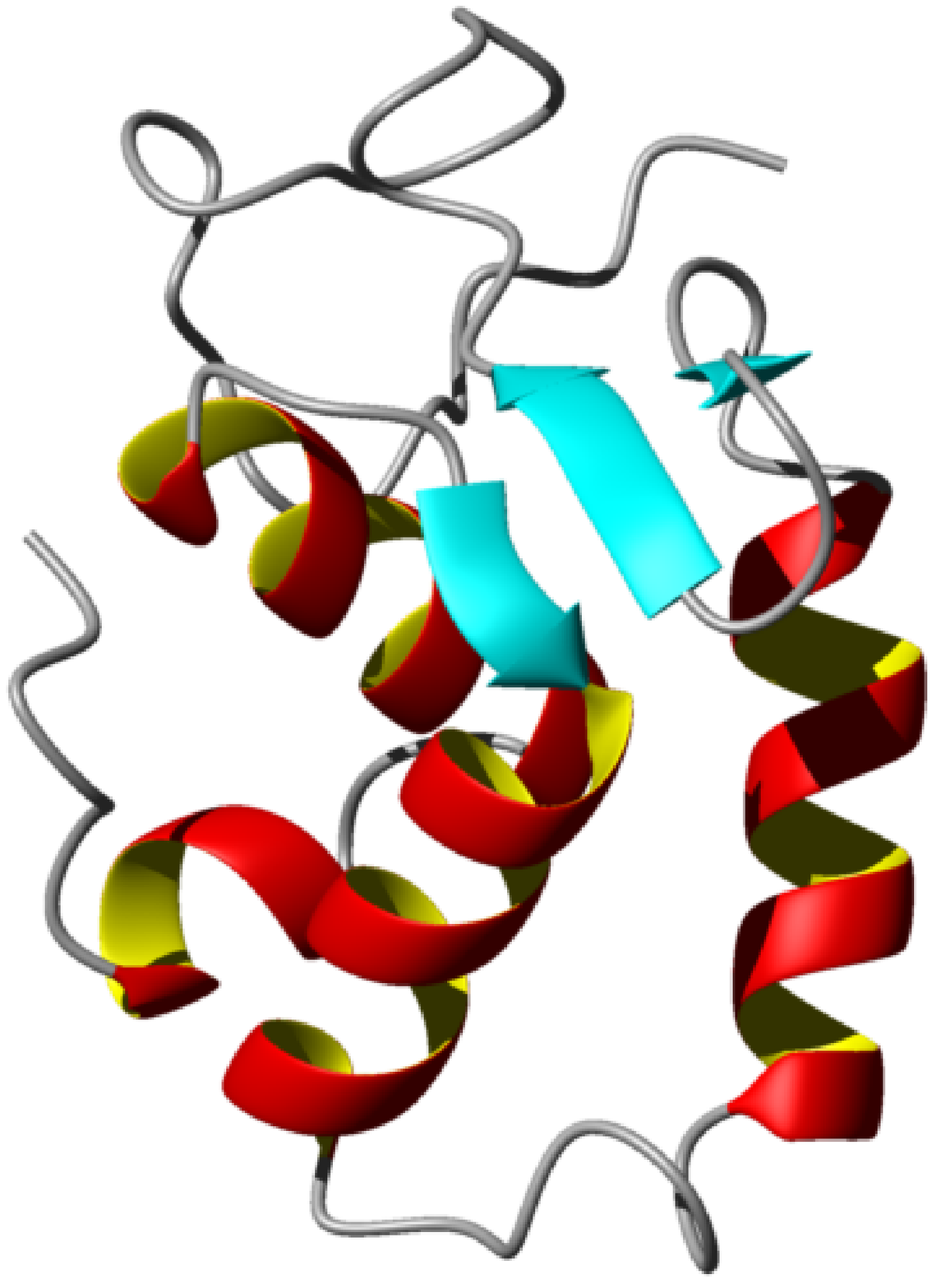

LMU
LMU
LMU
LMU
LMU
LMU
Uplink
Uplink
Uplink
Uplink
Uplink
Uplink
Uplink
WLG
WLP
CONTROL CENTER

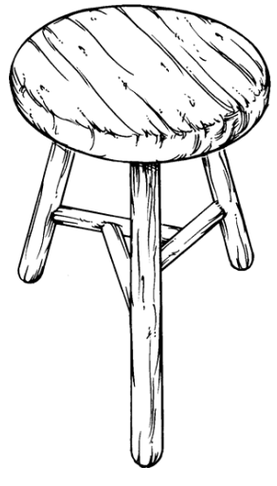

Energie
20
15
10
5
0
-5
-10
x1
x2

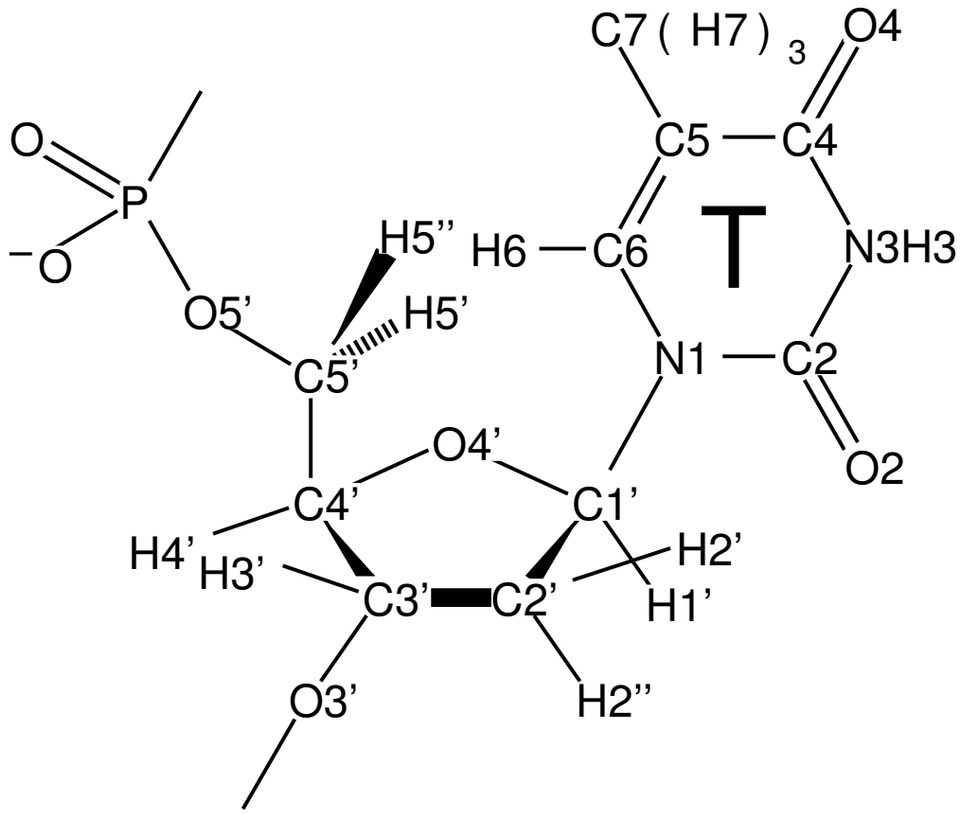

Energie
20
15
10
5
0
-5
-10
x1
x2

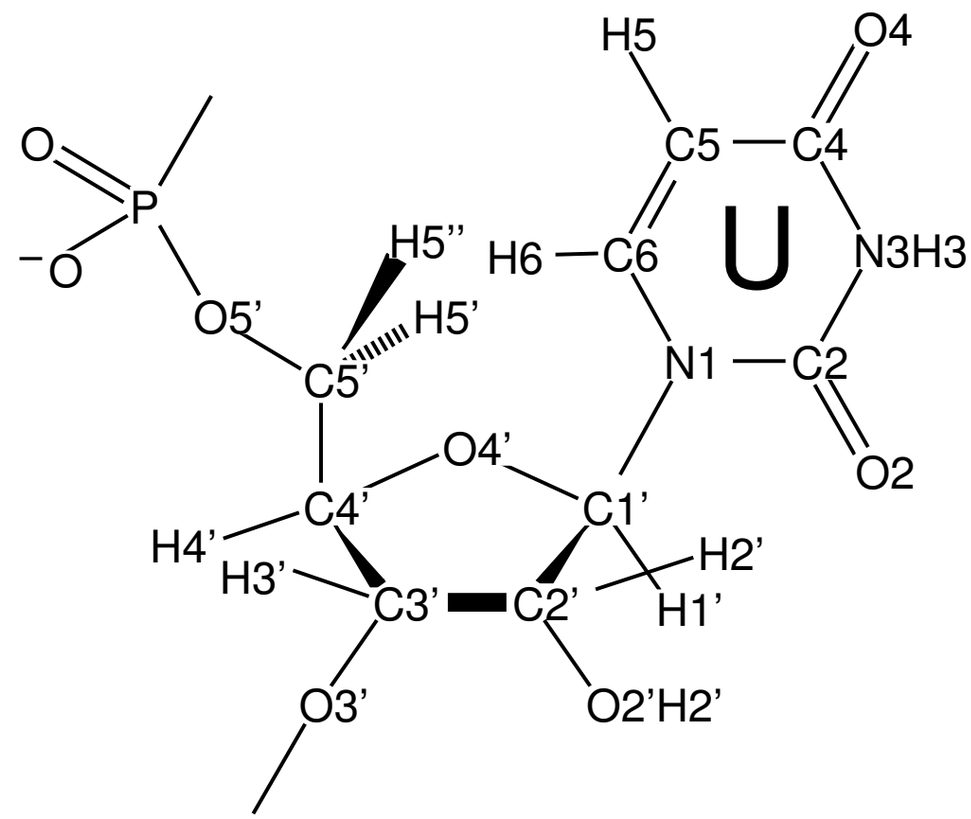

Energie
20
15
10
5
0
-5
-10
x1
x2

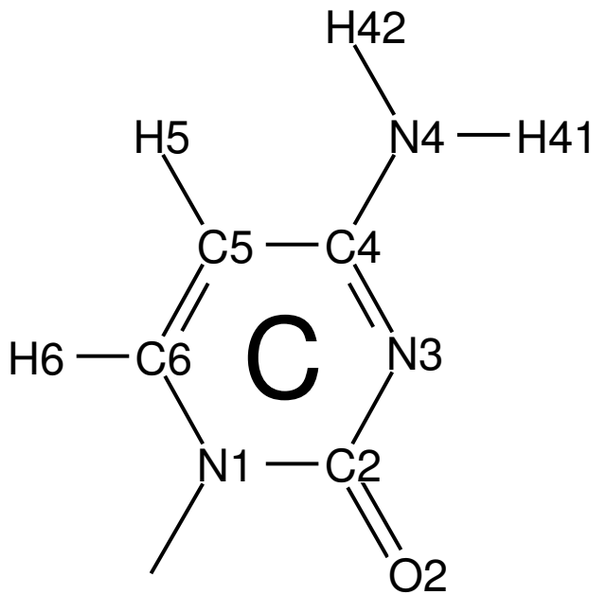


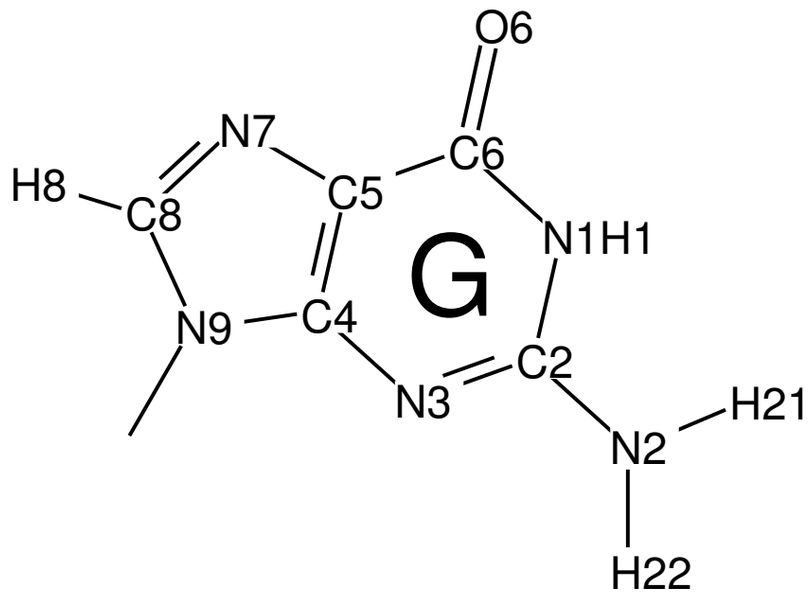


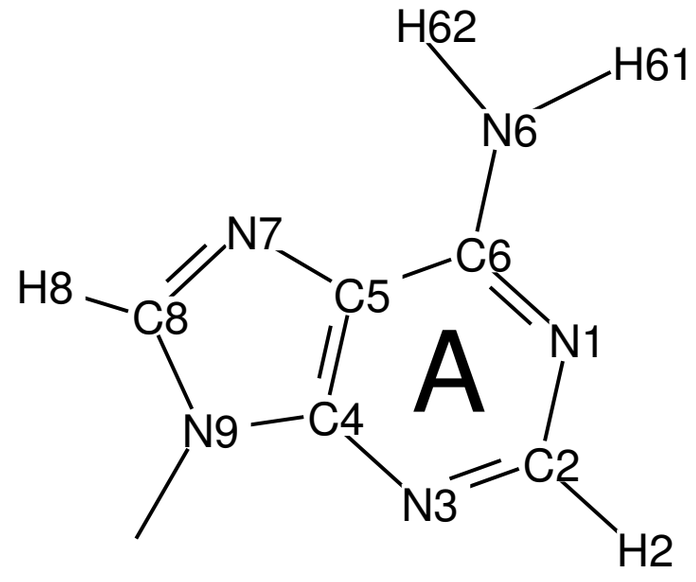



C7 (H7) 3
O4
C5
C4
O
P
O
H5''
H6
C6
T
N3H3
O5'
H5'
N1
C2
C5'
O2
O4'
C4'
C1'
H4'
H2'
H3'
C3'
C2'
H1'
O3'
H2''


H5
O4
C5
C4
O
P
O
H5''
H6
C6
U
N3H3
O5'
H5'
N1
C2
C5'
O2
O4'
C4'
C1'
H4'
H2'
H3'
C3'
C2'
H1'
O3'
O2'H2'

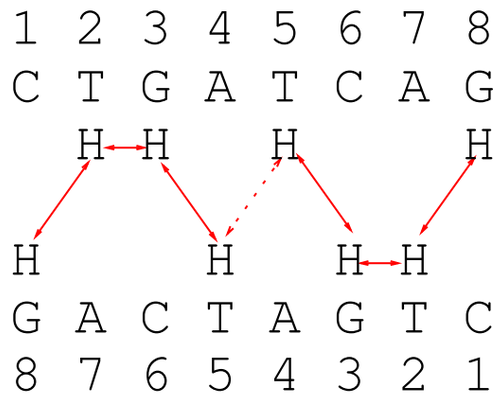

H42
H5
N4
H41
C5
C4
C
H6
C6
N3
N1
C2
O2

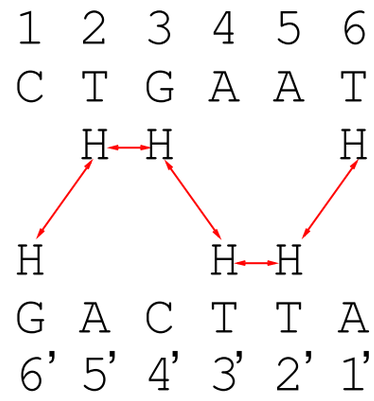

O6
N7
C6
H8
C8
C5
N1H1
G
N9
C4
N3
C2
N2
H21
H22

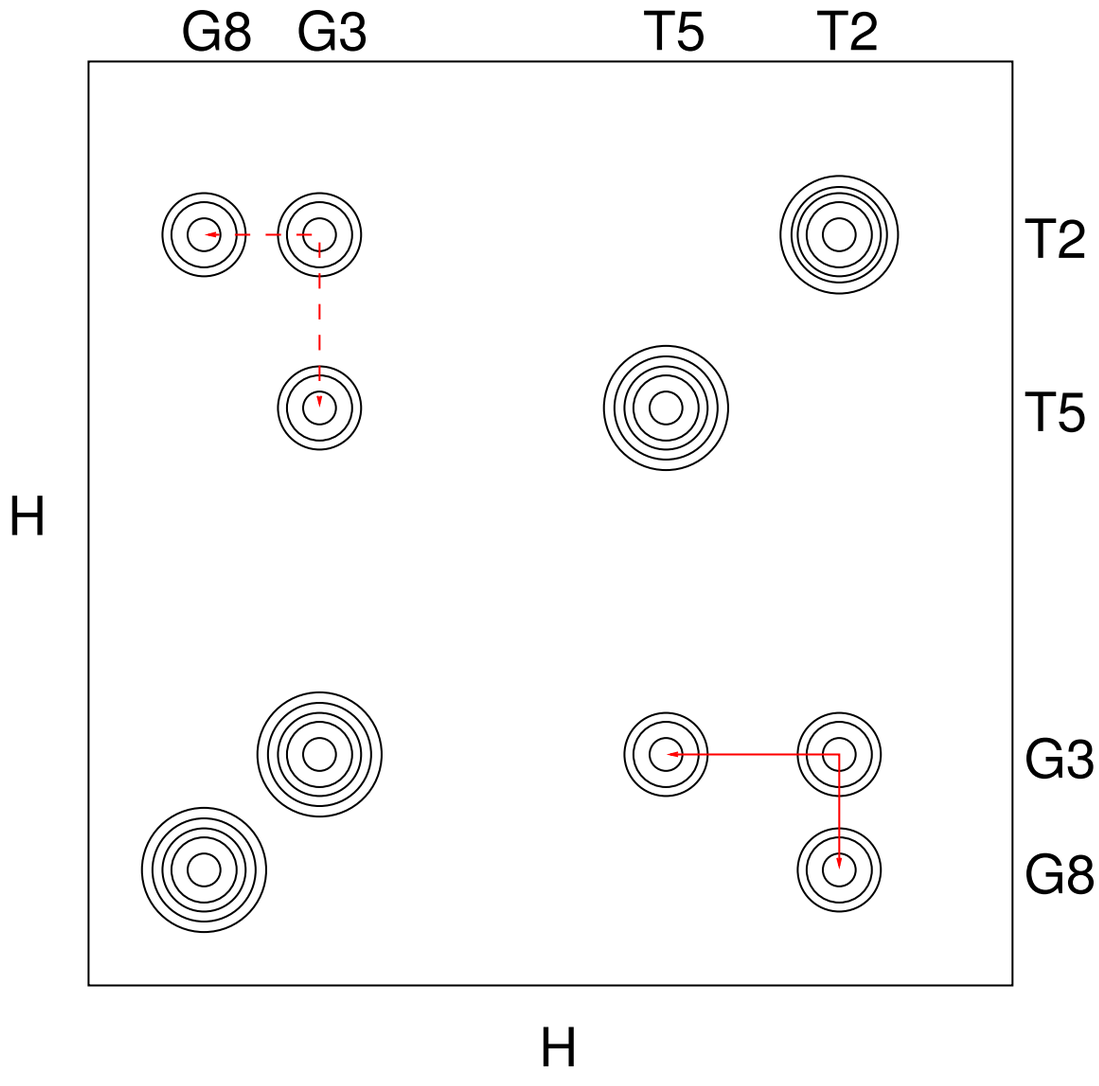

H62
H61
N6
N7
C6
H8
C8
C5
N1
A
N9
C4
N3
C2
H2

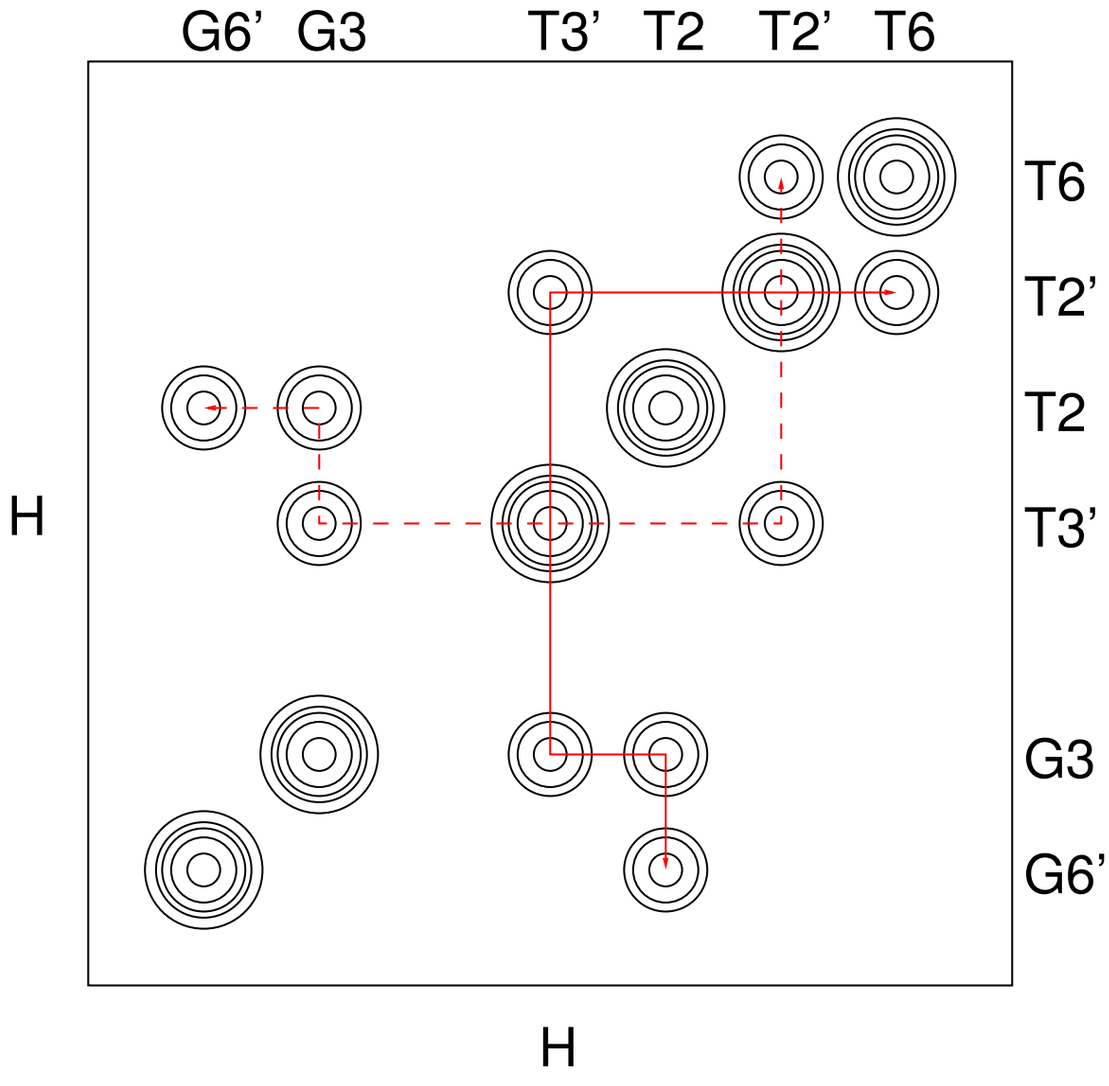
DNA double helix B

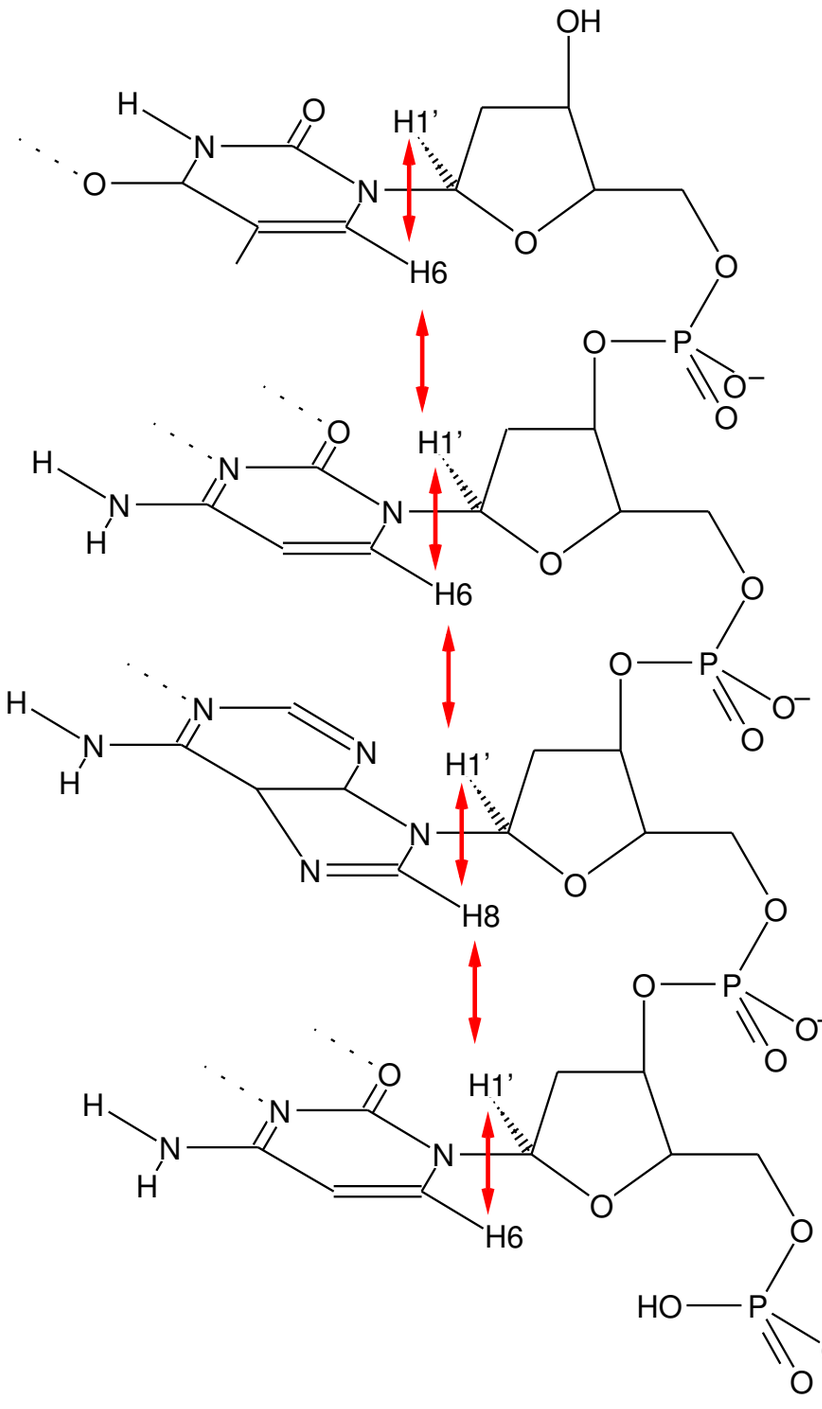
DNA double helix A

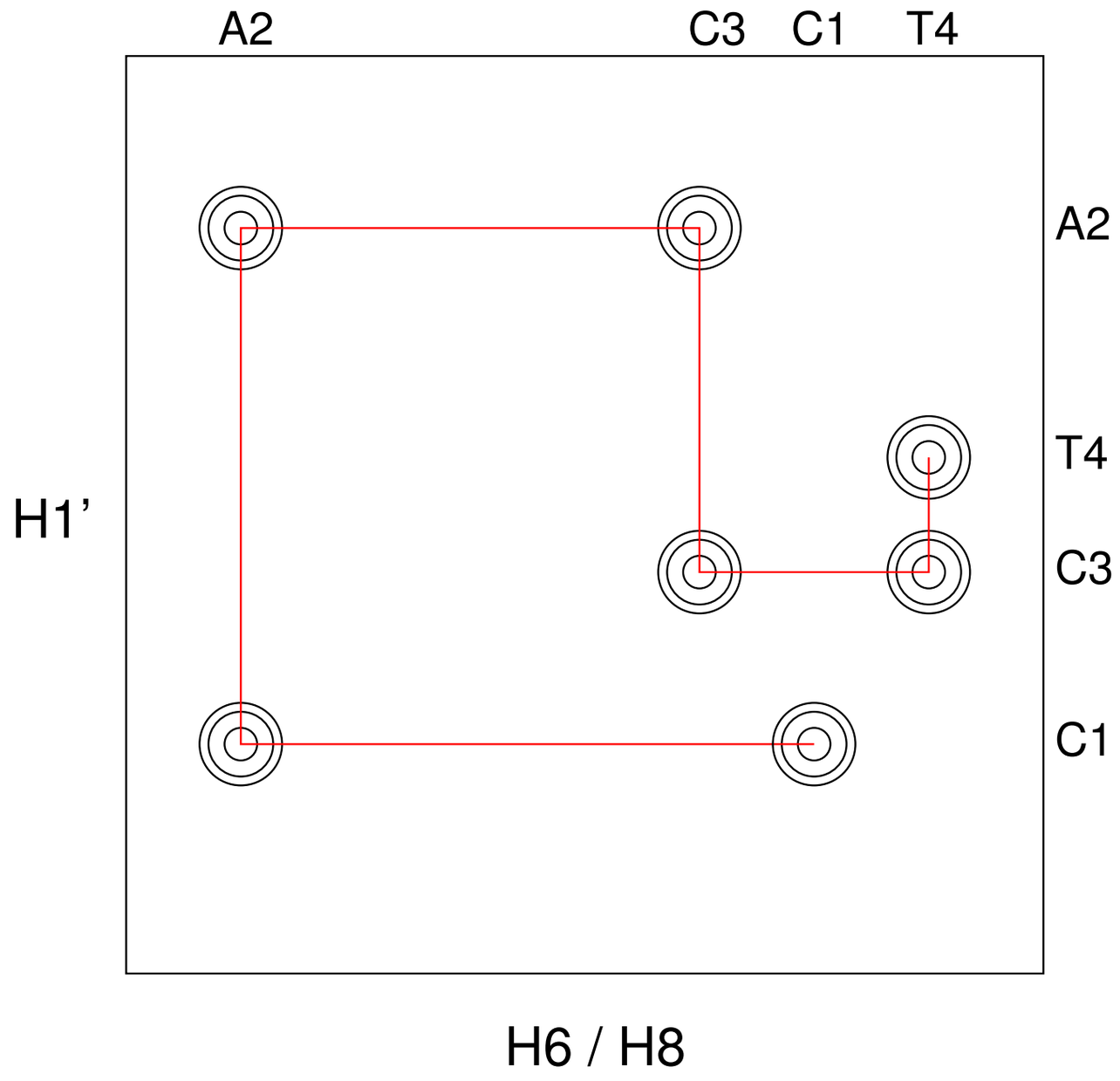

1 2 3 4 5 6 7 8
C T G A T C A G
H H H H
H H H H
G A C T A G T C
8 7 6 5 4 3 2 1


1 2 3 4 5 6
C T G A A T
H H H
H H H
G A C T T A
6' 5' 4' 3' 2' 1'


G8
G3
T5
T2
T2
T5
G3
G8
H
H

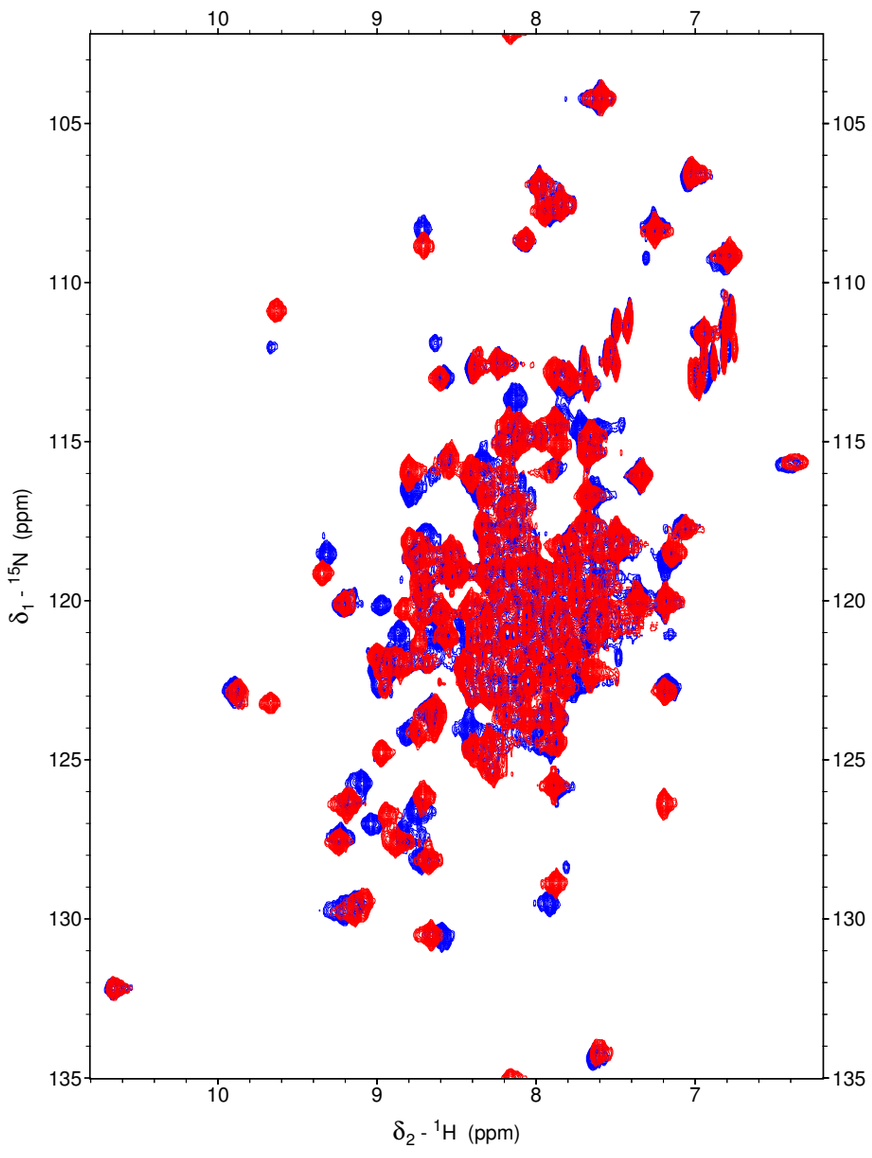

G6'
G3
T3'
T2
T2'
T6
T6
T2'
T2
T3'
G3
G6'
H
H

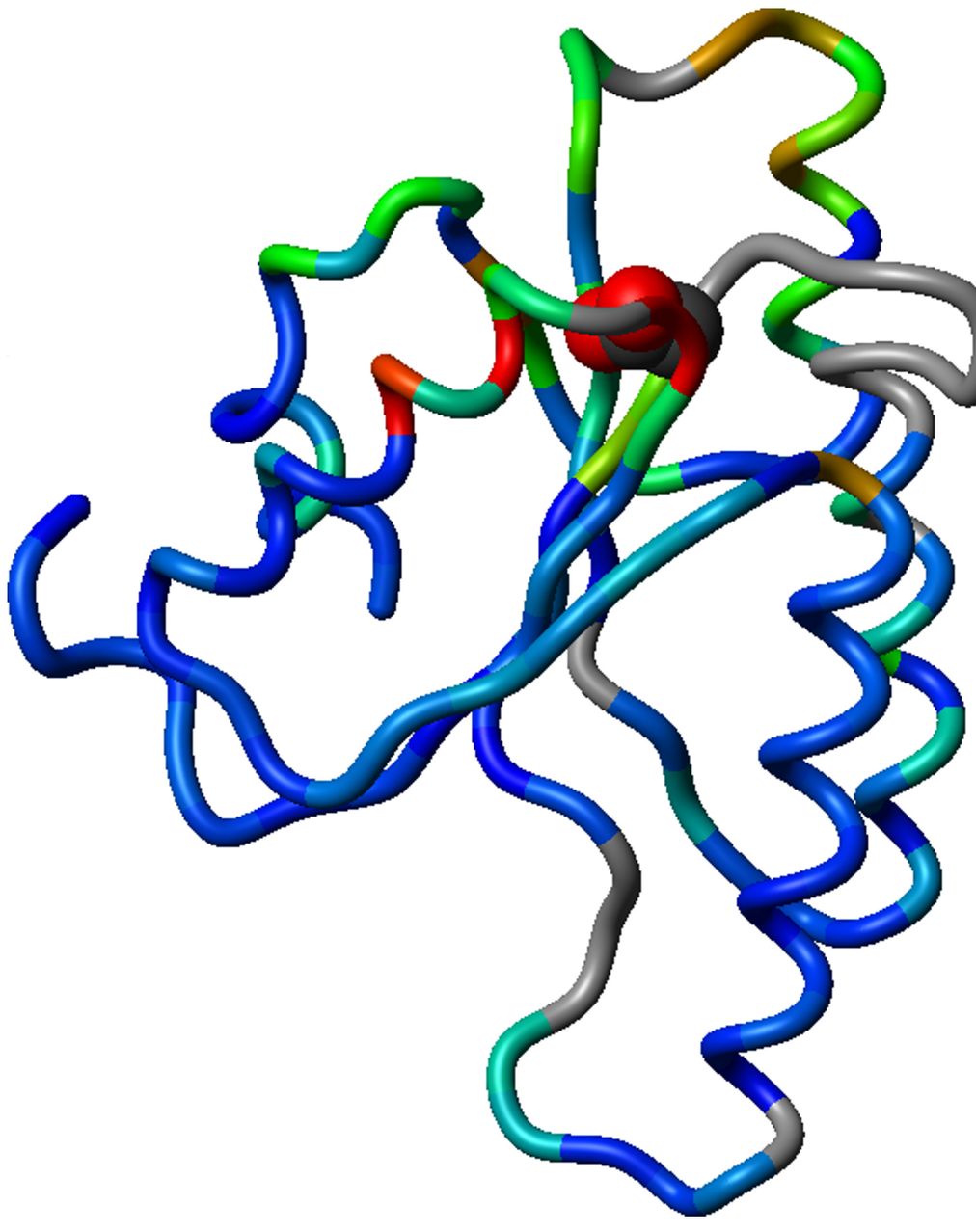

OH
H
N
O
H1’
N
O
H6
O
O
P
O
O⁻
O
H
N
H
O
N
H1’
N
O
H6
O
O
P
O
O⁻
O
H
N
H
N
N
H1’
N
N
H8
O
O
P
O
O⁻
O
H
N
H
O
N
H1’
N
O
H6
HO
P
O
O⁻
O


A2
C3
C1
T4
H1’
A2
T4
C3
C1
H6 / H8

Příklady využití NMR

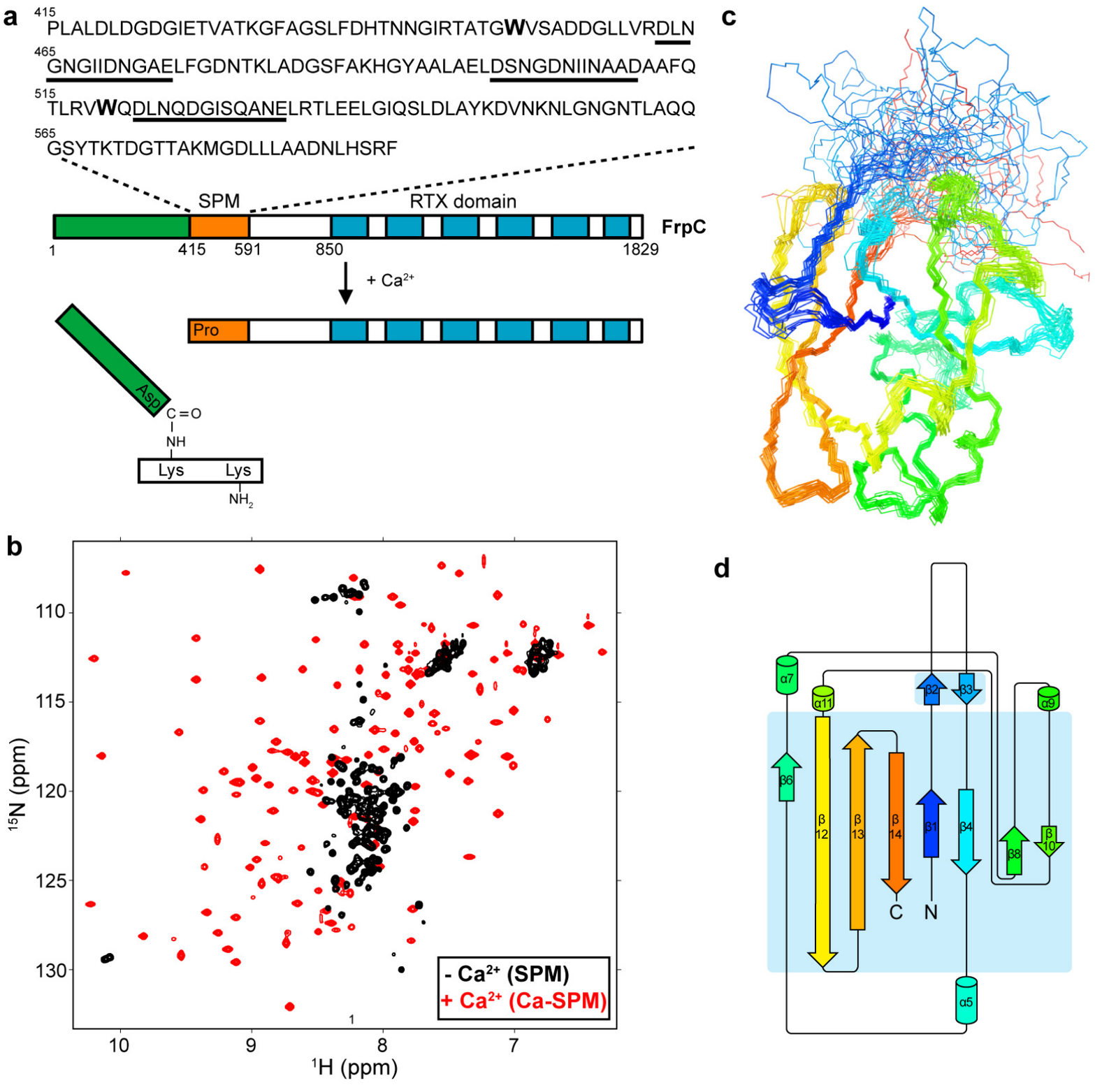
Rychlé určení vazebného místa





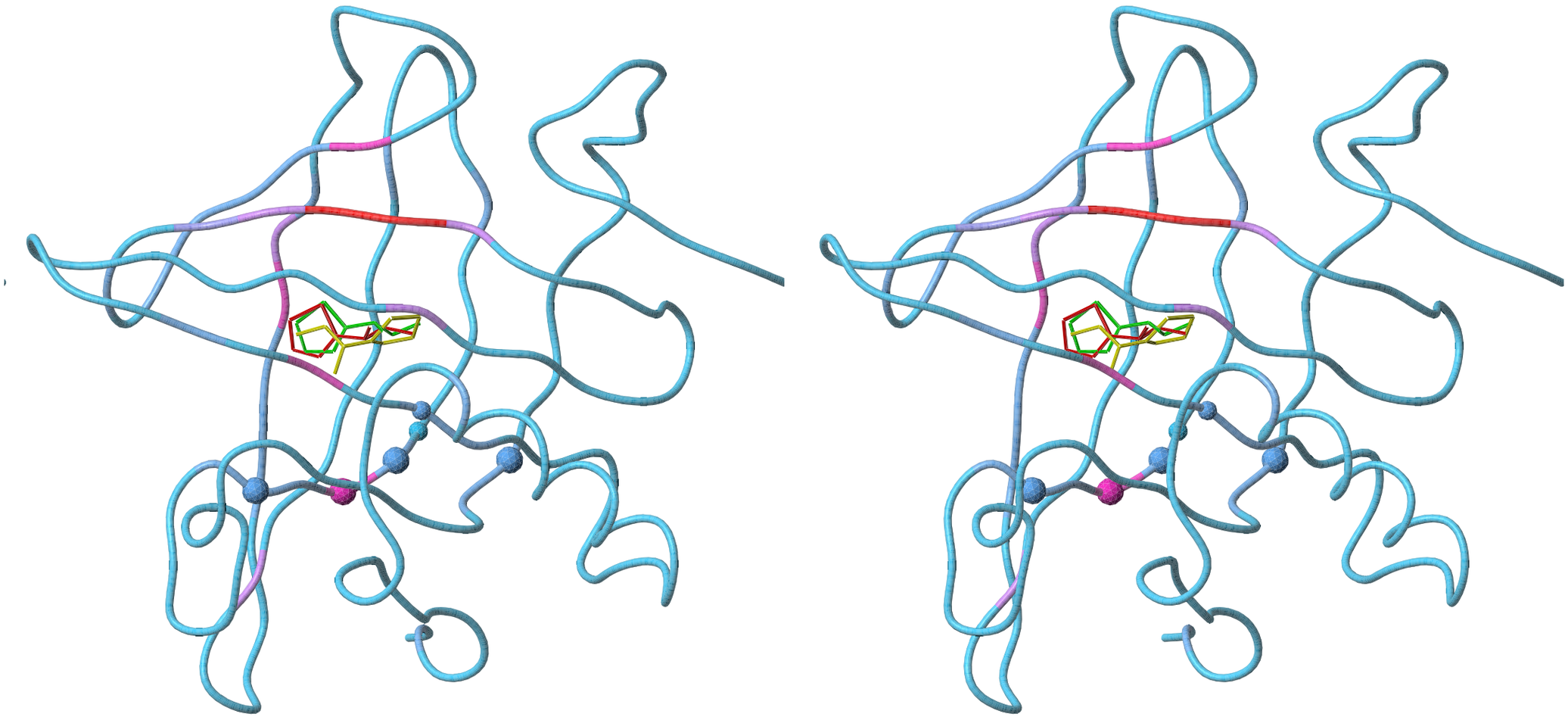
Pekárová et al., *Plant. J.* **67** (2011) 827–839.

Určení struktury proteinu, který nekrystalizuje

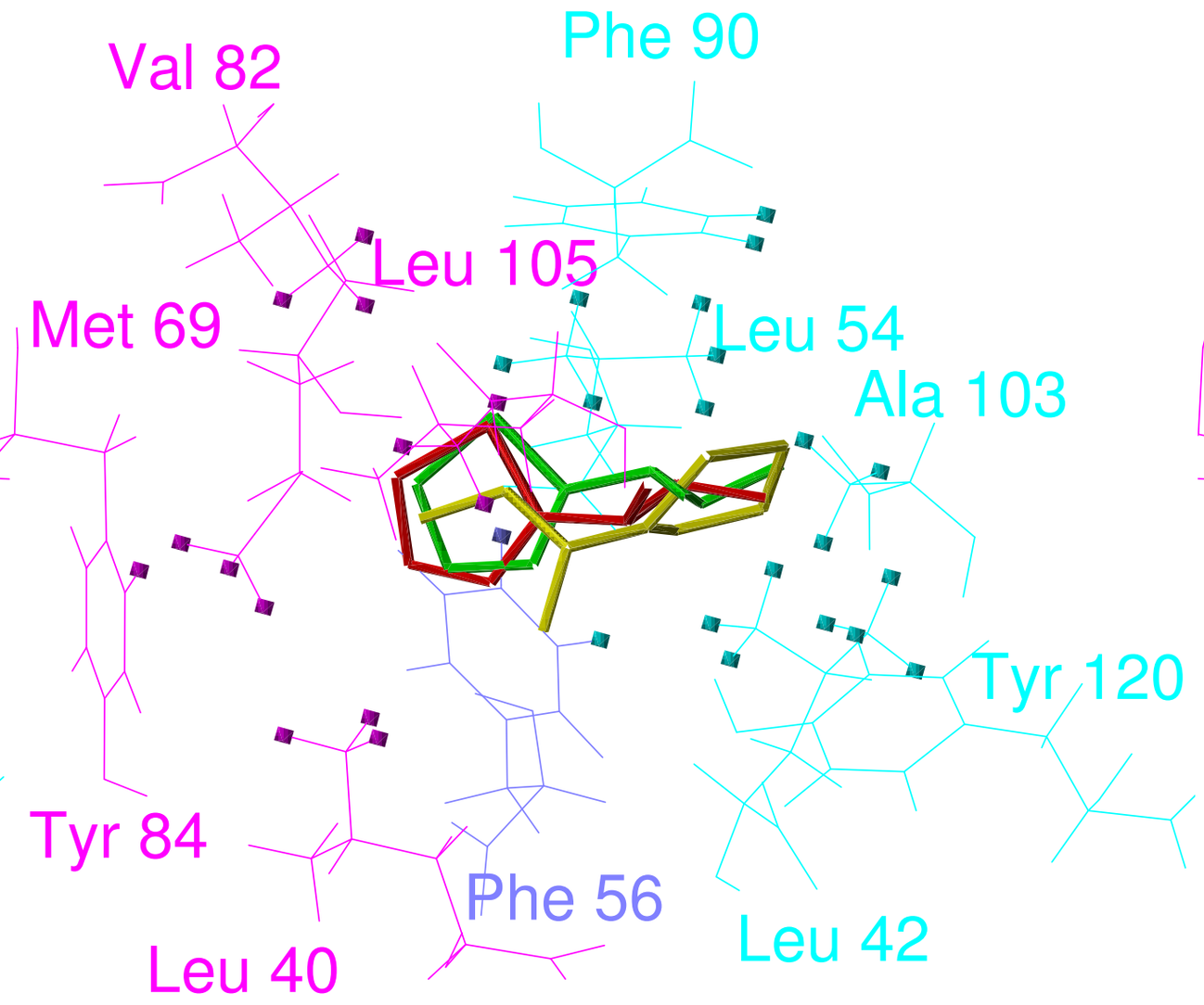


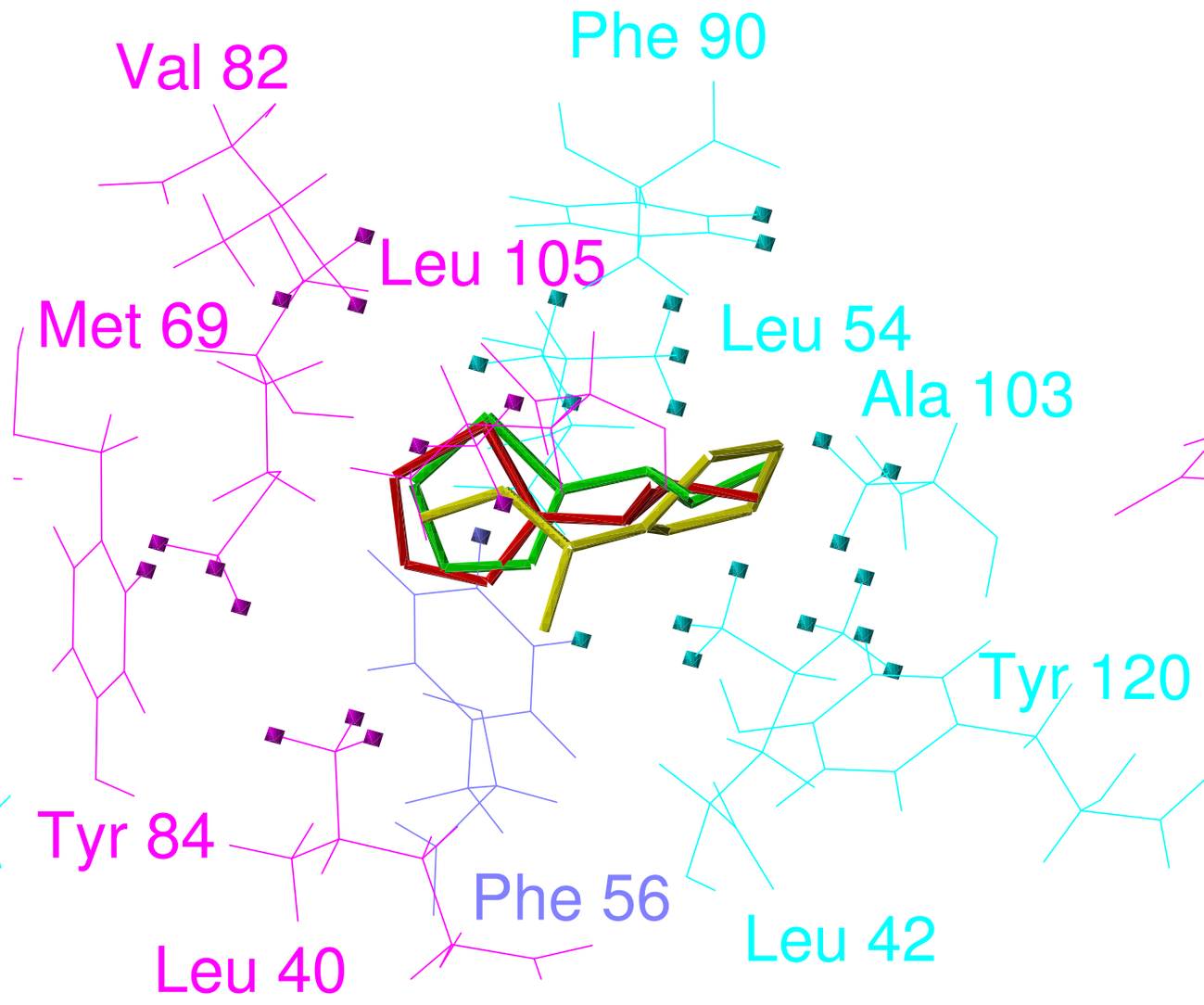
Kubáň et al., *mBio* **11** (2020) e00226-20.

Oprava chybné krystalové struktury

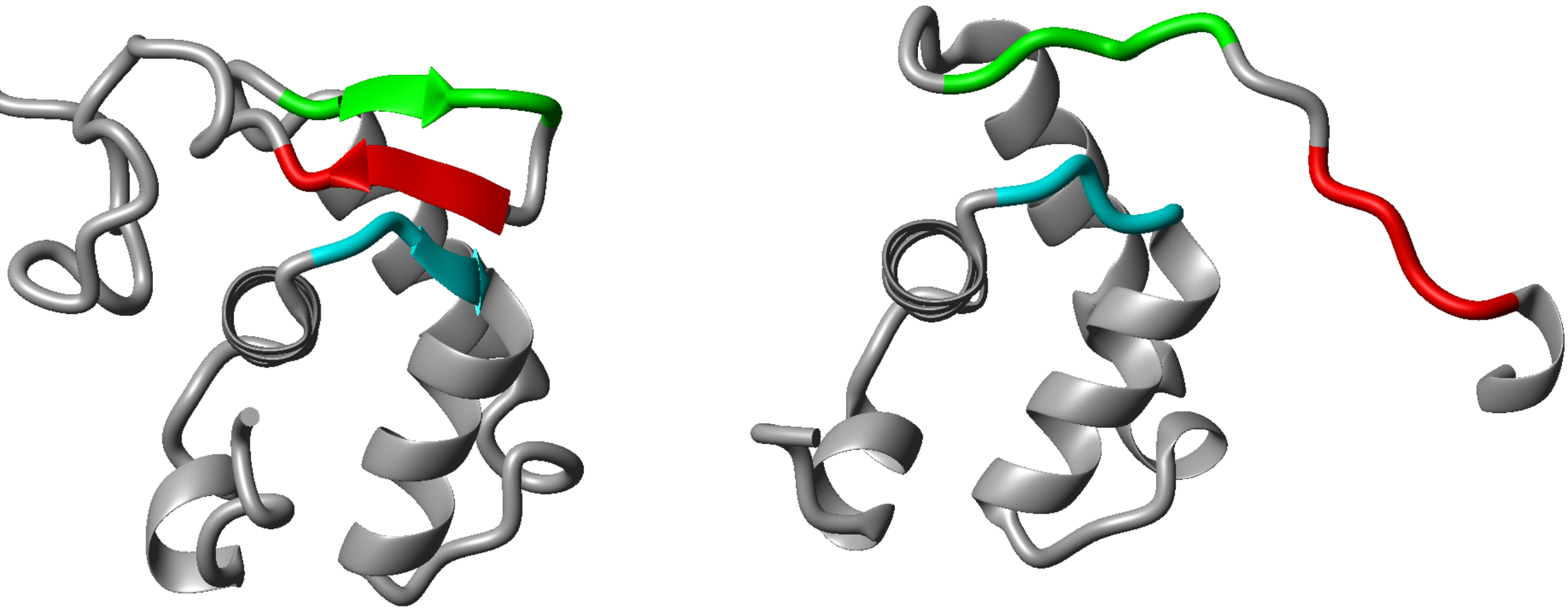


Žídek et al., *Biochemistry* **38** (1999) 9850–9861.


Val 82
Phe 90
Leu 105
Met 69
Leu 54
Ala 103
Tyr 120
Tyr 84
Phe 56
Leu 42
Leu 40

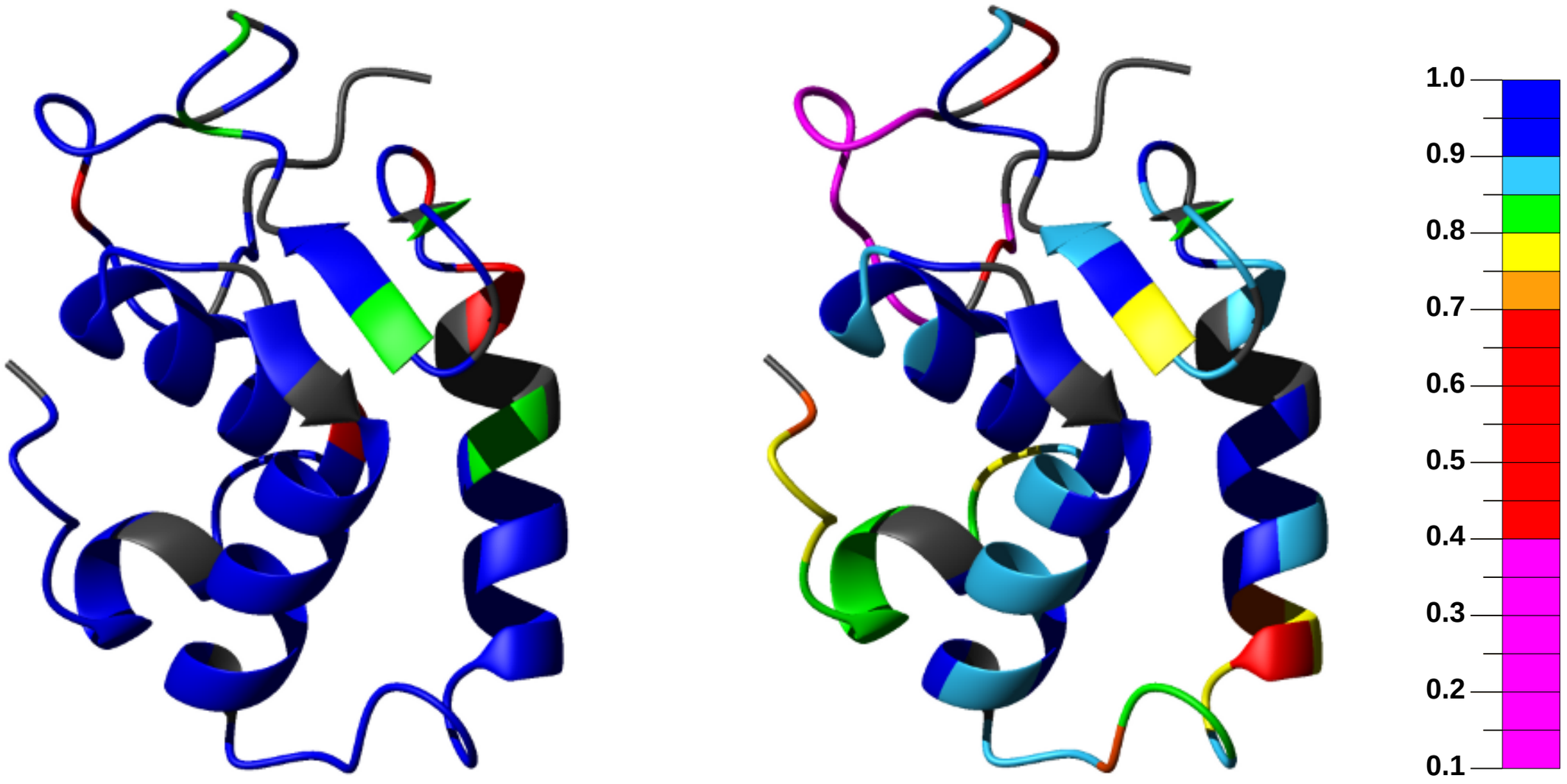

Val 82
Phe 90
Leu 105
Met 69
Leu 54
Ala 103
Tyr 120
Tyr 84
Phe 56
Leu 42
Leu 40

Určení struktury, která se liší v roztoku a krystalu



Demo et al., *J. Struct. Biol.* **187** (2014) 174–186.

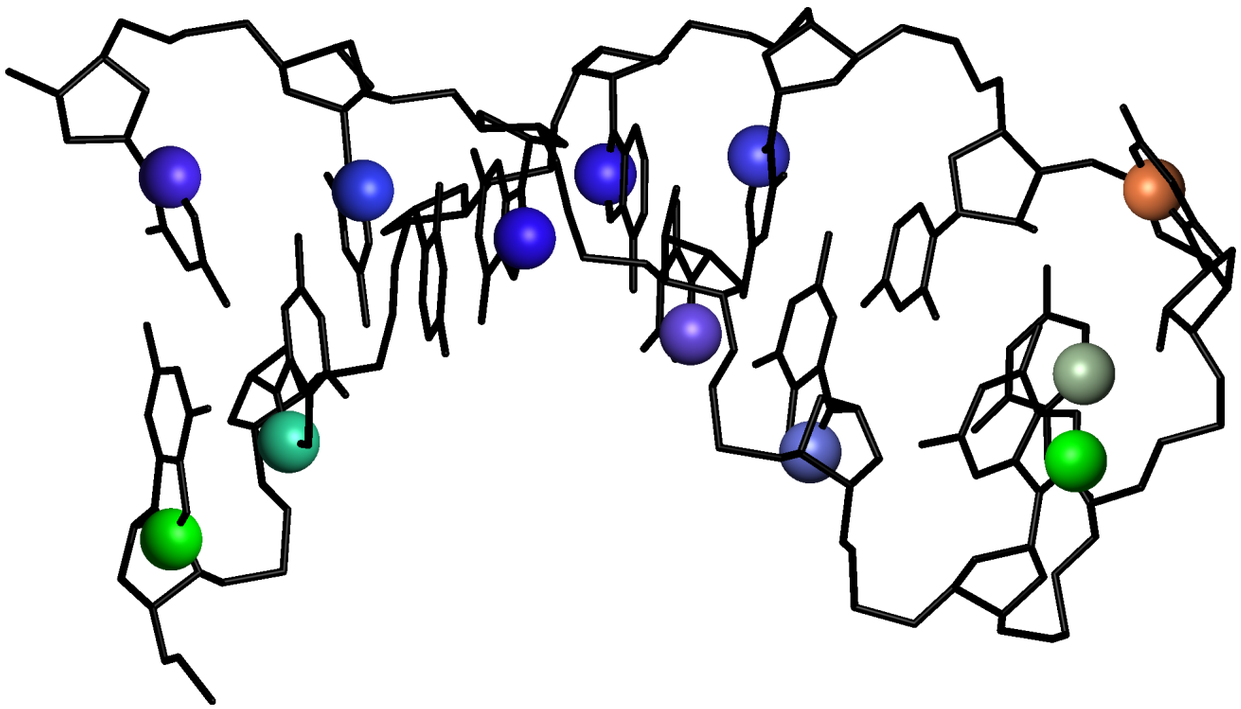
Studium pomalých (μs–ms) a rychlých (ps–ns) pohybů proteinu

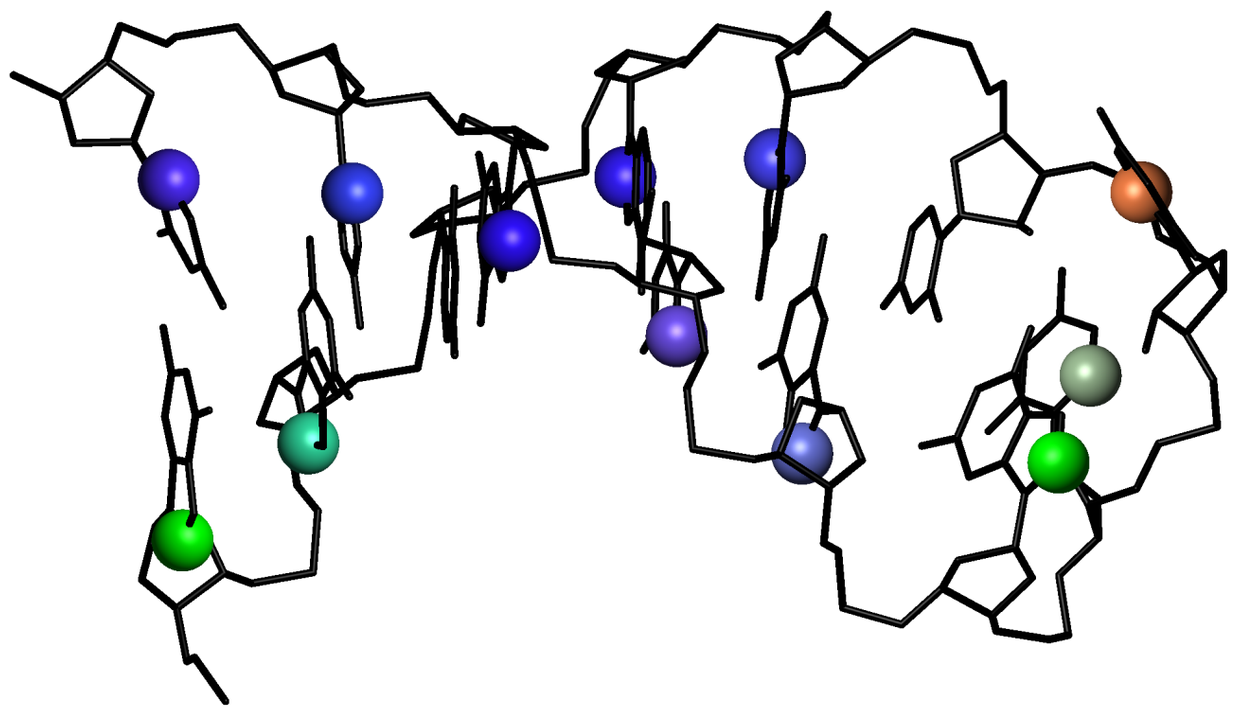


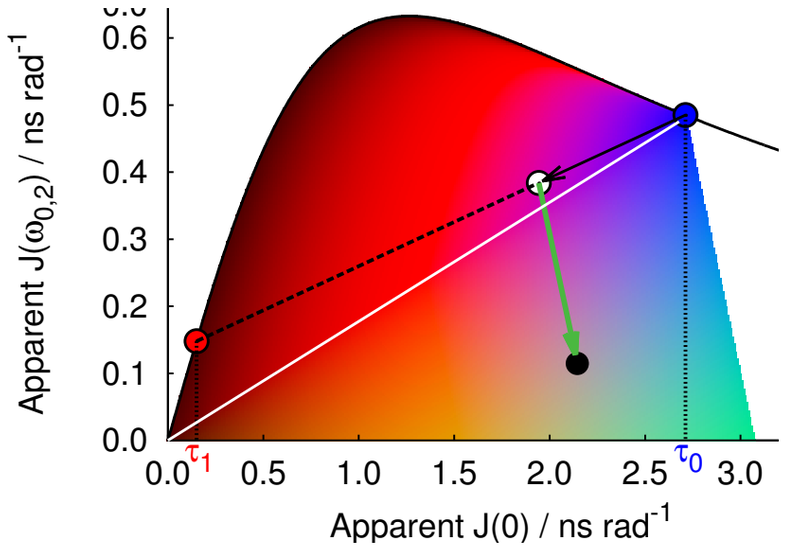
Papoušková et al., *ChemBioChem* **14** (2013) 1172–1179.

Kadeřávek et al., unpublished

Studium pomalých (μs–ms) a rychlých (ps–ns) pohybů DNA

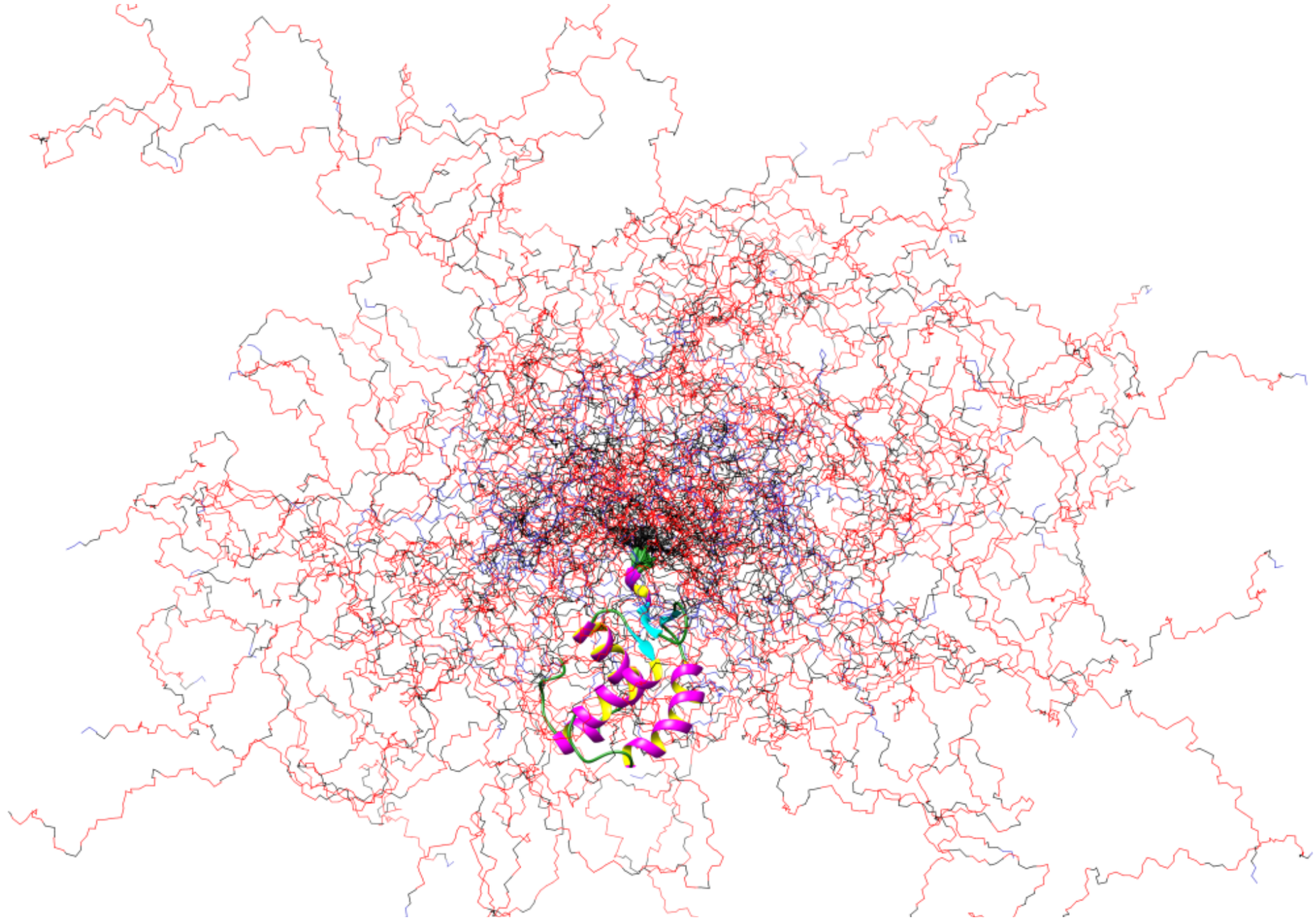




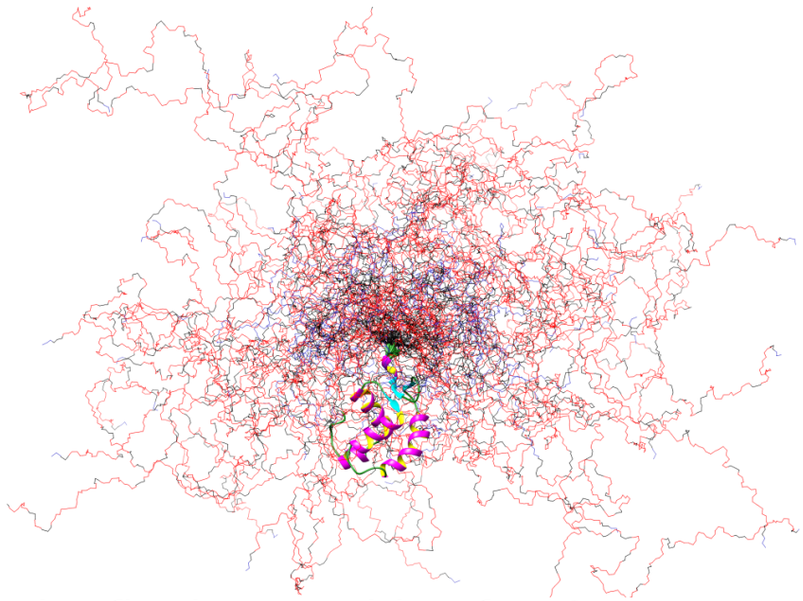


Kadeřávek et al., *J. Magn. Reson.* **266** (2016) 23–40.

Studium částečně neuspořádaného proteinu

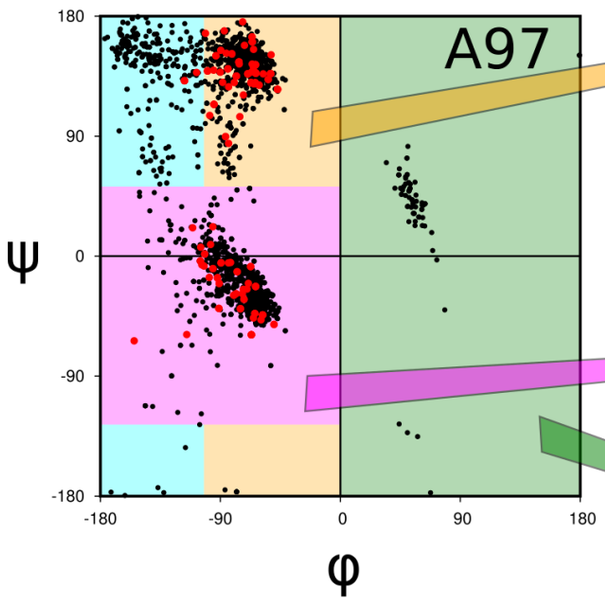


Kubáň et al., *J. Am. Chem. Soc.* **141** (2019) 16817–16828.

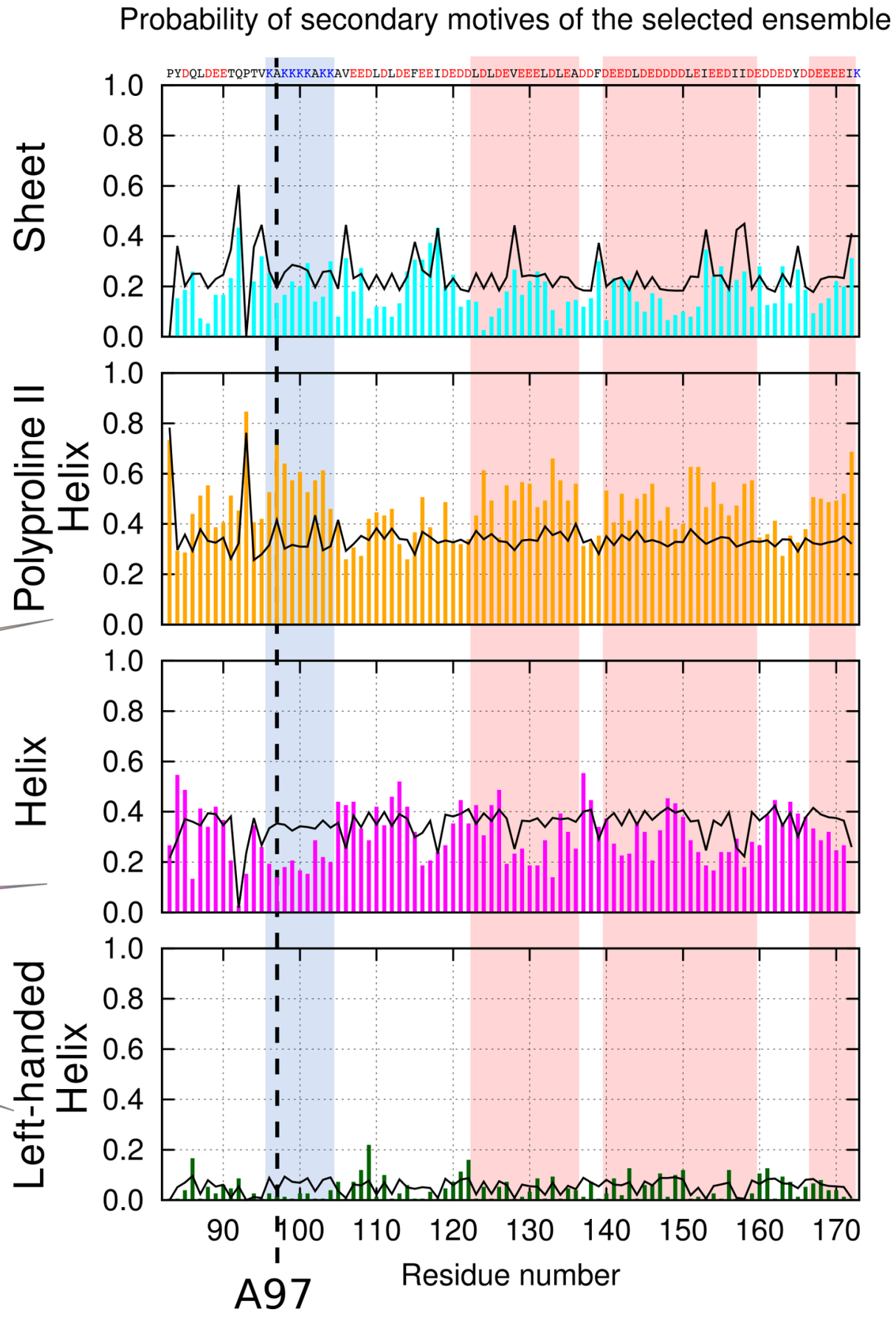


The final ensemble of 150 structures

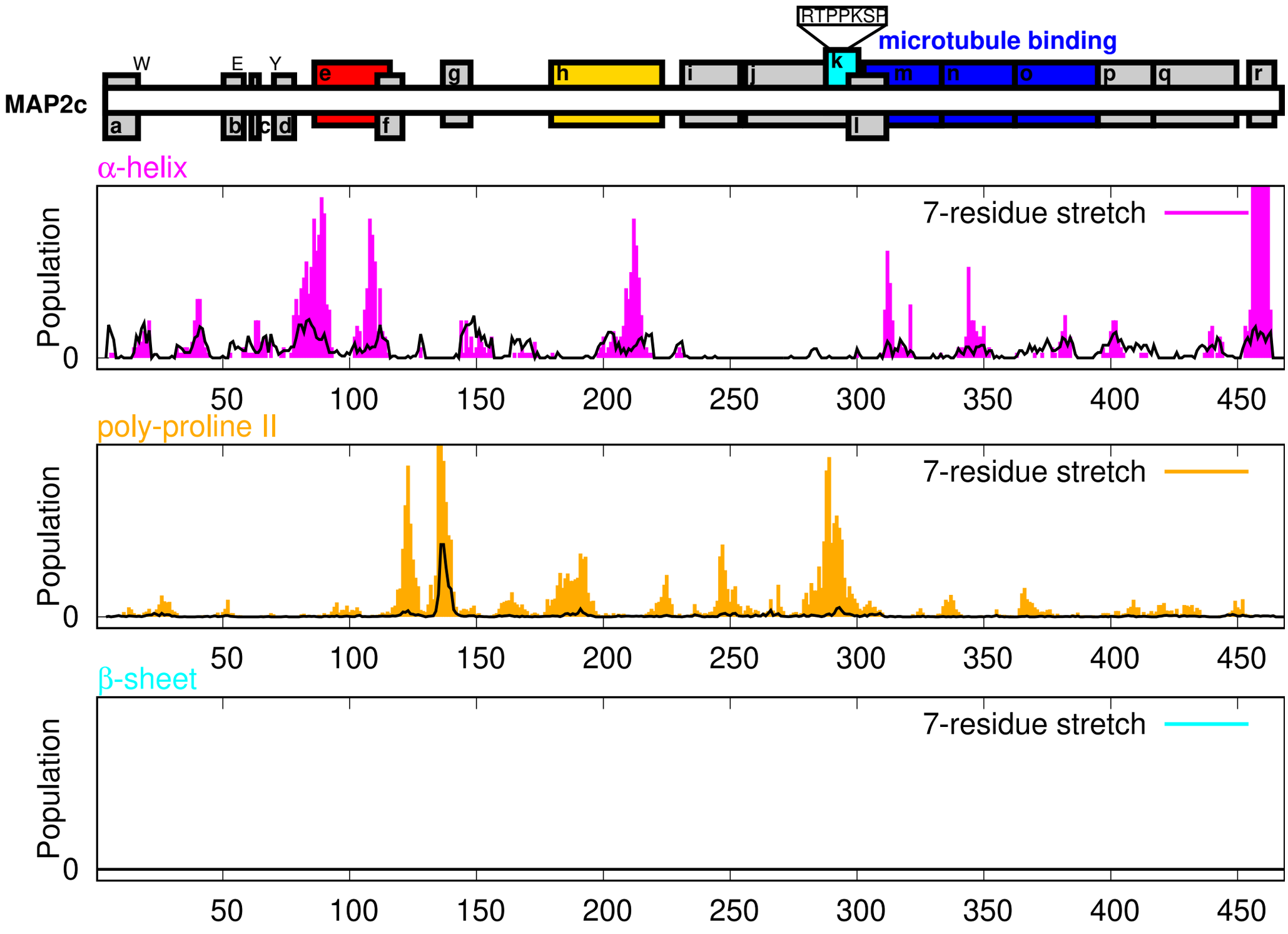
Ramachandran plot for each residue



Each individual structure is meaningless as only local conformation contains information like CS or RDC.



Studium zcela neuspořádaného proteinu



Melková et al., *J. Biol. Chem.* **293** (2018) 13297–13309.