Protein-nucleotide interactions detected by solid-state NMR

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HpDnaB

ATP
or
ATP-analogues
+
ssDNA
DnaB helicases unwind double-stranded DNA

- Structural consequences of nucleotide binding (ATP & DNA)?
- Structure-function relationships in protein engines?
- How does DNA replication work on a molecular level?

Taken from: http://love-life-science.blogspot.ch/2014/09/unzipping-of-dna.html

Adapted from: http://biochem.pepperdine.edu/dokuwiki/doku.php?id=chem331:dnab_helicase
ATP-hydrolysis is coupled to molecular functioning

Walker, ..., Gay, The EMBO Journal, 1982, 1, 945-951;
The helicase from *Helicobacter pylori* forms dodecameric assemblies

**SF4 helicase**

Molecular mass of **672 kDa**, 488 aa/ monomer

Homology model for the *HpDnaB:ADP* complex (based on the *AaDnaB:ADP* crystal structure)


Questions to be addressed by solid-state NMR

Do the nucleotides bind to the protein?

Where do they bind?

What are the structural consequences of nucleotide binding?
Approaches to probe protein-nucleotide interactions

Diamagnetic NMR
(e.g. $^{31}$P MAS; $^{13}$C/$^{15}$N CSPs; $^{15}$N, $^{13}$C NCX; $^{13}$C, $^{31}$P and $^{15}$N, $^{31}$P correlations)

Dynamic nuclear polarization (DNP)

DNA binding to HpDnaB

ATP/DNA binding

Paramagnetic NMR
(e.g. substitution of the Mg$^{2+}$ cofactor by Mn$^{2+}$ or Co$^{2+}$)

EPR
(e.g. Mn$^{2+}$-Mn$^{2+}$ DEER)
Part 1

Diamagnetic solid-state NMR

*How to monitor nucleotide binding?*

*How to distinguish between bound and unbound nucleotides?*
$^{31}$P NMR to distinguish between bound and unbound nucleotides

$^{31}$P solution-state experiments allow to assign the resonances of AMP-PNP/AMP-PN

$^{31}$P direct pulsed experiments detect AMP-PNP and AMP-PN in the solution phase of the NMR rotor

$^{31}$P, $^1$H cross-polarization (CP) experiments detect bound AMP-PNP in slightly different conformations

$^{31}$P NMR: Does ssDNA bind to the helicase?

$^{31}$P **solution-state** experiments allow to detect unbound ssDNA

$^{31}$P **direct pulsed** experiments detect hydrolyzed AMP-PNP (AMP-PN) in the solution-phase of the NMR rotor

$^{31}$P, $^1$H **cross-polarization** (CP) experiments detect bound ssDNA to the helicase

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$^{31}$P chemical shifts are very sensitive to the choice of the ATP-analogue.

- $^{31}$P,$^1$H cross-polarization experiments allow to detect the bound ATP-analogues.
- Structural inhomogeneities observed upon AMP-PNP binding.
- ATP gets hydrolyzed during rotor filling.
DNA-binding monitored by $^{31}\text{P},^{1}\text{H}$ cross-polarization experiments

DnaB + ATP-analogue + ssDNA

$^{31}\text{P},^{1}\text{H}$ CP-MAS NMR @ 11.74 T
Arginine sidechains of the protein bind to ssDNA

arginine sidechain

phosphate backbone of DNA
CSPs indicate nucleotide binding & conformational switch of the CTD

HpDnaB
HpDnaB + AMP-PNP + MgCl$_2$

**Chemical-shift perturbations (CSPs)**
- Consequence of nucleotide-binding
- Related to allosteric effects

$^{13}$C-$^{13}$C 20 ms DARR @ 850 MHz

How to assign such a large system? Building block approach

Are the domains conserved? Transfer of assignments between NTD (assignment available) and FL $Hp$DnaB possible?

Wiegand, Gardiennet, ..., Terradot, Böckmann, Meier, *Biomol. NMR Assign.*, 2016, 10, 13-23;
Building block approach: Assignment of the full-length protein

Averaged absolute $^{13}$C and $^{15}$N chemical shift differences between NTD and FL HpDnaB

And the CTD? Sequential assignment of the full-length protein

Wiegand, Gardiennet, ..., Terradot, Böckmann, Meier, J. Biomol. NMR, 2016, 65, 79-86.
Part 2

Paramagnetic solid-state NMR to determine protein-ATP interactions

Where does the metal ion bind?
Paramagnetic solid-state NMR allows to identify residues in NBD

**Mn$$^{2+}$$:** Paramagnetic relaxation enhancements (PREs)

**Co$$^{2+}$$:** Pseudo-contact shifts (PCSs)

- $I_{\text{para}}/I_{\text{dia}}$ ratio for 2D $^{13}$C-$^{13}$C DARR
- $B_0 = 20.0$ T
- $T_{1e}(\text{Mn}^{2+}) = 30$ ns
- $T_{1e}(\text{Co}^{2+}) = 0.1$ ns
- $I_{\text{CP}}, I_{t1}, I_{t2}, I_{\text{DARR}}$
- experimental conditions

NMR: Diamagnetic Mg\(^{2+}\) can be substituted by paramagnetic Mn\(^{2+}\) ions

3 types of resonances:

a) Not-influenced by **AMP-PNP:Mn\(^{2+}\)** binding (e.g. 24A)

b) Attenuated upon **AMP-PNP:Mn\(^{2+}\)** binding (e.g. 228A)

c) Broadened beyond detection upon **AMP-PNP:Mn\(^{2+}\)** binding (e.g. 203A, 351A)

How to determine PREs with CCPN?

- Assign 3D spectra of diamagnetic protein sample
- Scale the spectra (use a resonance not affected by PREs, here $34V$)
- Assign 3D spectra of paramagnetic protein sample
- Extract intensities from peak assignment lists

Site-specific determination of PREs from 3D experiments

Residues with effective distances < 15 Å (distance between Cα and the two nearest metal centers)

Paramagnetic solid-state NMR allows to identify residues in NBD

Long-range distance information (> 20 Å) becomes accessible, nucleotide binding domains are identified.

Part 3

$^{31}\text{P},^{13}\text{C}$ correlation experiments to probe protein-nucleotide interactions

Where do the ATP-analogues and DNA bind?
MAS-DNP for studying protein-DNA interactions

31P-13C/15N correlations suffer from weak NMR signal

Dynamic nuclear polarization (DNP)-enhanced MAS

Collaboration with Prof. C. Copéret (ETH Zürich)

Taken from: http://www.coperetgroup.ethz.ch/research/dynamic-nuclear-polarization--dnp--.html
MAS-DNP for studying protein-DNA interactions

$HpDnaB:ADP:ssDNA$

$\rho = \left(\frac{SN_{DNP}}{SN_{NMR}}\right)$

Highest sensitivity without d8-glycerol.
2 mM AMUpol radical concentration (not optimized).

Wiegand, ..., Copéret, Böckmann, Meier, *J. Biomol. NMR*, submitted.

Collaboration with Prof. C. Copéret (ETH Zürich)
MAS-DNP for studying protein-DNA interactions

Collaboration with Prof. C. Copéret (ETH Zürich)

10.0 kHz @ 14.1 T
395 GHz gyrotron

Measurement time 21 h.

Morag, ..., Goldbourt, JACS, 2014, 136, 2292-2301;
Wiegand, ..., Copéret, Böckmann, Meier, J. Biomol. NMR, submitted.

HpDnaB:ADP:ssDNA

Lowest contour level: 2.1 times noise RMSD
Any chance for probing DNA interactions under conventional NMR conditions (in reasonable measurement time)?

CHHP 2D experiments for studying protein-DNA interactions

*HpDnaB:ADP:AIF₄⁻:ssDNA*

Measurement time 13 d.

200 μs H-H spin diffusion time
11.74 T @ 17.0 kHz
Conclusions

- $^{31}$P NMR allows to monitor nucleotide binding

- $^{13}$C/$^{15}$N CSPs highlight conformational changes

- Paramagnetic NMR allows to probe protein-ATP interactions

- DNA binding can be detected in $^{31}$P/$^{13}$C correlation experiments
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