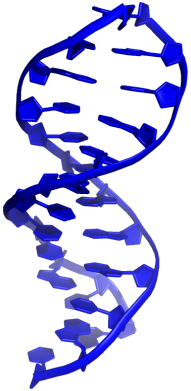
Liquid β-NMR studies of the interaction of Na and K cations with DNA G-quadruplex structures

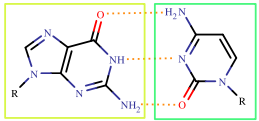
Spokesperson: Beatrice Karg, Magdalena Kowalska

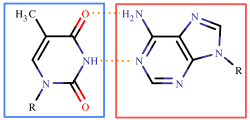
June 24, 2020

- What are DNA G-Quadruplexes and why should you care?
- What can be achieved beyond conventional NMR?

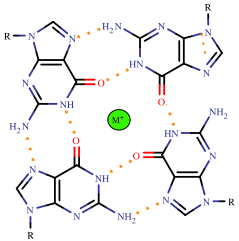
What are Quadruplexes?

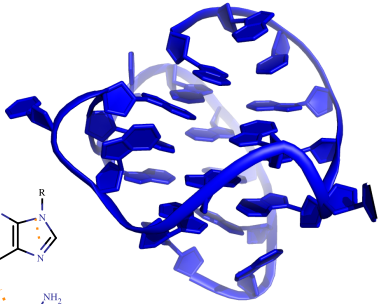






A, T, G, C





G, G, G

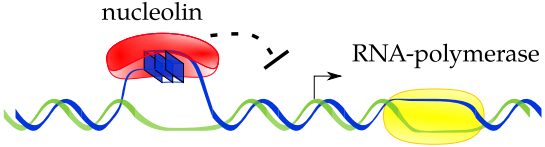
What are Quadruplexes?

PDB-Entries: 148D-Schultze 1993, 2E4I-Matsugami 2006, 2GKU-Luu 2006, 2O3M-Phan 2007, 2KZD-Lim 2010, 2LOD-Marusic 2012, 2M53-Marusic 2013, 2MBJ-Lim 2013, 2MFT-Karsisiotis 2013, 4WO3-Wei 2014, 4U5M-Schmitt 2014, 2N2D-Brcic 2015, 6ERL-Karg 2017, 5MBR-Dickerhoff 2017, 6R9K-Karg 2019

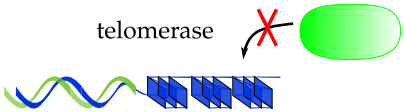
The Importance of Quadruplexes?

Biology

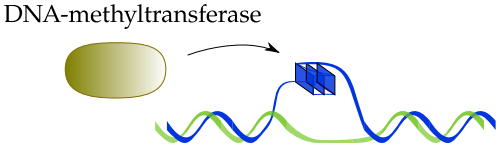
A - Transcription - Gene expression



B - Telomerelongation -> Cell death

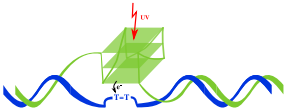


C - Epigenetics -> Gene regulation

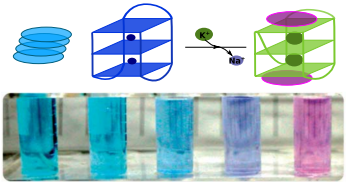


Technology

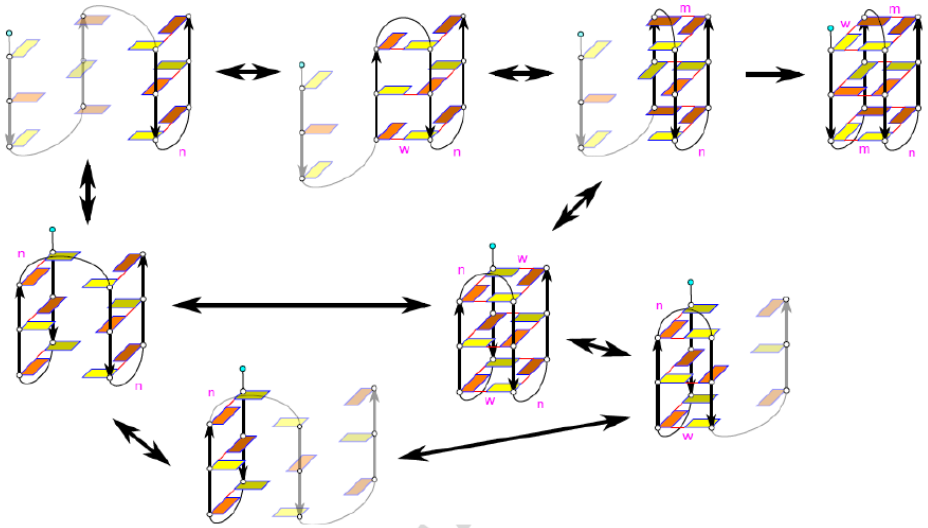
D - Damage repair -> Catalysis



E - Signal detection -> sensor design



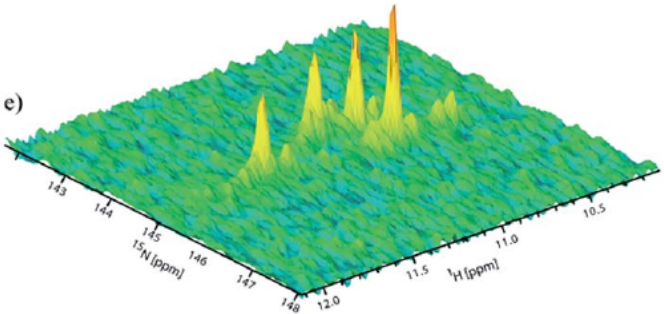
How to fold a Quadruplex



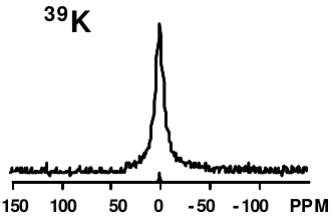
Šponer et al., Folding of guanine quadruplex molecules–funnel-like mechanism or kinetic partitioning? An overview from MD simulation studies, BBA, 2016

The Problems and the Unknowns

Signal overlap

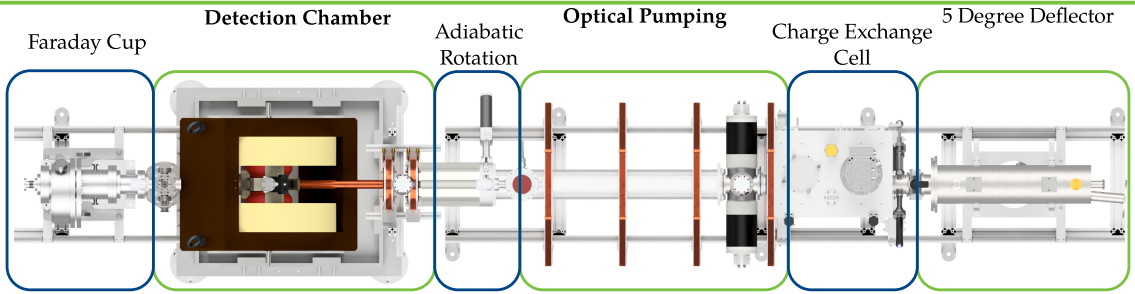


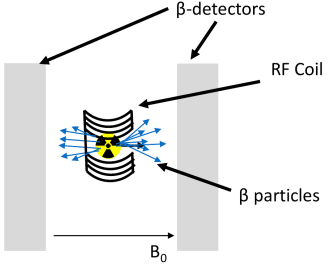
Weak & broad Resonances

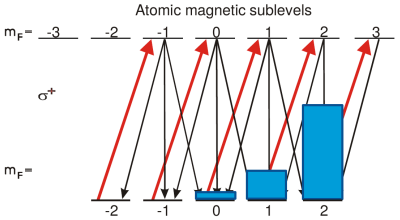


Left: Salgado et al., G-quadruplex DNA and ligand interaction in living cells using NMR spectroscopy, Chemical Science, 2015
Middle: Wong et al., Direct NMR detection of the "invisible" alkali metal cations tightly bound to G-quadruplex structures, Biochemical and Biophysical Research Communications, 2005

Sensitivity: β-NMR

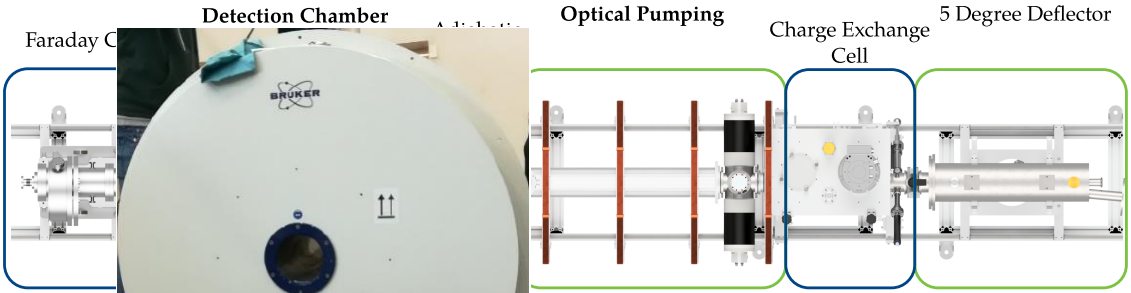






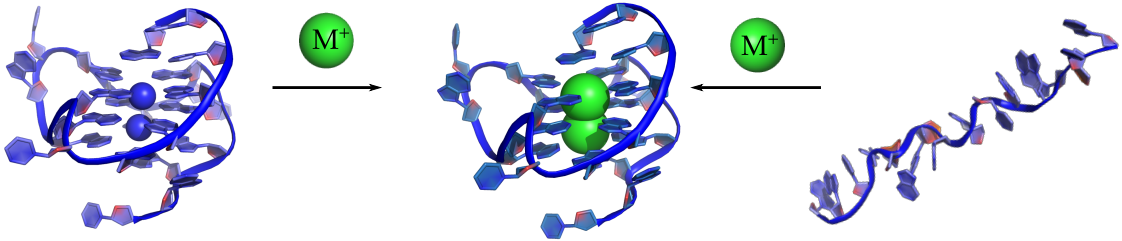
Harding et al., Magnetic moments of short-lived nuclei with part-per-million accuracy: Paving the way for applications of β-detected NMR in chemistry and biology, *submitted*

Sensitivity: β-NMR



x8 higher field

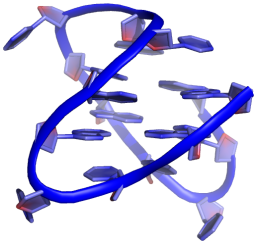
The Folding of Quadruplexes with radioactive nuclei



Nucleus	Radioactive half-life	Nuclear spin I	Observed β-asymmetry
^{26}Na	1.077 s	3	25%
^{37}K	1.237 s	3/2	8-11%
^{49}K	1.260 s	1/2	-

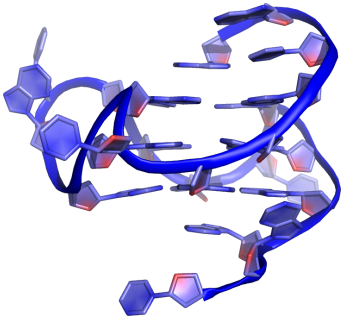
Three sequences

Thrombin Binding Aptamer (*tba*)



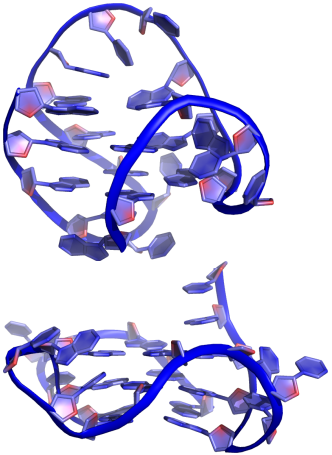
- therapeutic DNA from clinical trials
- 2 tetrads -> single ion binding site

c-myc



- oncogene promoter sequence
- most stable, fast kinetics

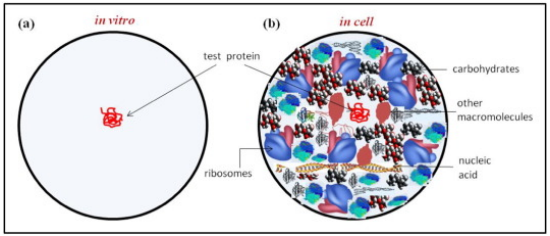
Human Telomeric (*ht*)



- native telomeric repeat
- structure cation-dependent

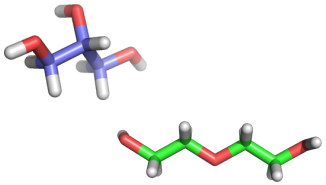
Solvents and vacuum regimes

Molecular Crowding

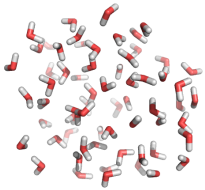


Crowding agents

Glycerol Polyethylenglycol

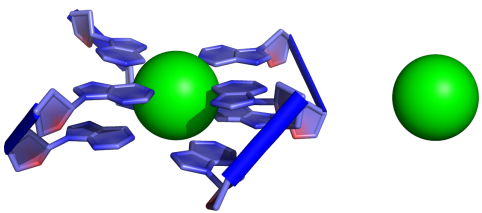


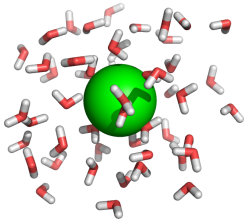
Water

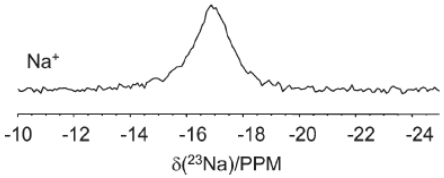


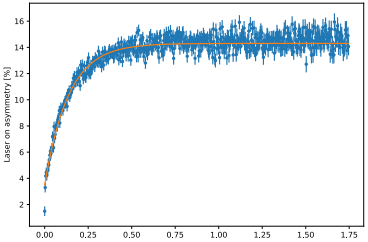
Pa ⟷ kPa

Relaxation times and chemical shift









Left: Ida et al., Direct 23Na NMR observation of mixed cations residing inside a G-quadruplex channel, Chem. Commun., 2007
Right: β-NMR measurements, T1 of 26Na in BMIM-HCOO, Oct2018

First Run: ^{26}Na with G-Quadruplexes

Samples:

- pure solvents at higher field
- Oligos: *ht*, *c-myc*
- pre-folded with ^{23}Na and unfolded
- relaxation + chemical shift

Technical:

- Ta, Ti, or UC_x targets
- yield: 10^7 ions/s
- 11 shifts
- spread over 5-6 days

Running in parallel/interchanged:

- use only every 3rd/4th proton pulse
- 4-6h to prepare and exchange biological samples

Second Run: ^{37,49}K in pure solvents

Samples:

- ^{49}K polarisation tests
- determination of magnetic moments in pure solvents

Technical:

- ^{37}K: Ti, ^{49}K: UC_x target
- yield: ^{37}K: 7×10^6 ions/s, ^{49}K: 2×10^4 ions/s
- 7 shifts
- spread over 4-5 days

Third Run: ^{37}K or ^{49}K with G-Quadruplexes

Samples:

- Oligos: *ht*, *c-myc*, *tba*
- pre-folded with ^{23}Na and unfolded
- relaxation + chemical shift

Technical:

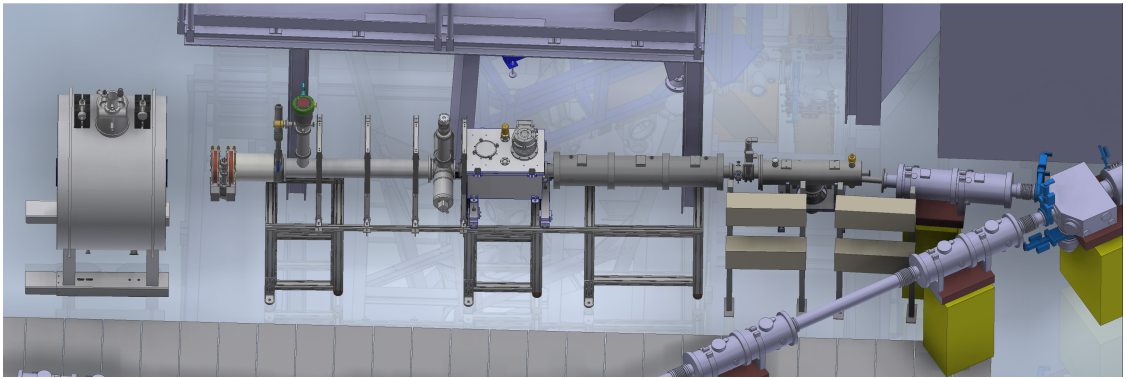
- ^{37}K: Ti, ^{49}K: UC_x target
- yield: ^{37}K: 7×10^6 ions/s, ^{49}K: 2×10^4 ions/s
- 11 shifts
- spread over 5-6 days

Summary

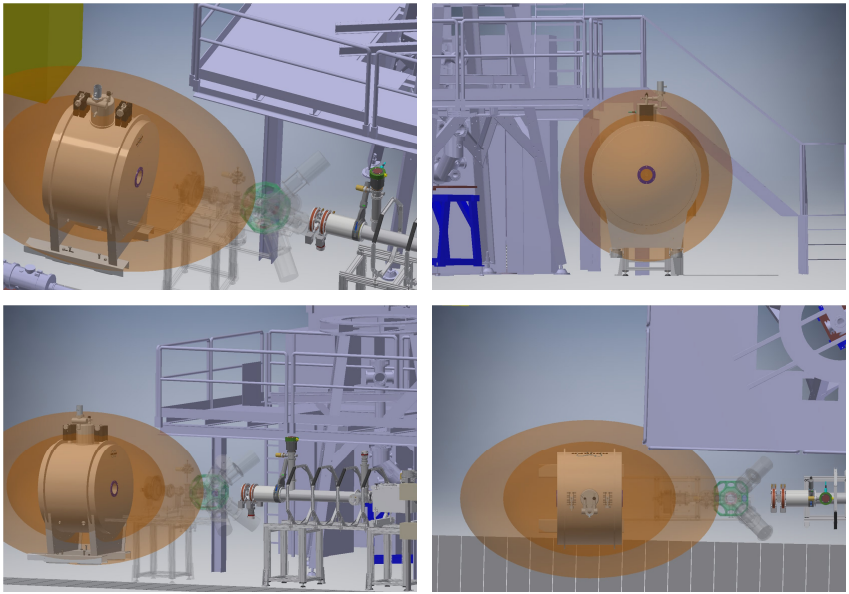
- Optical Pumping
- β-asymmetry detection
- online measurements

- high-impact biomolecules
- study dynamics and structures from a novel perspective

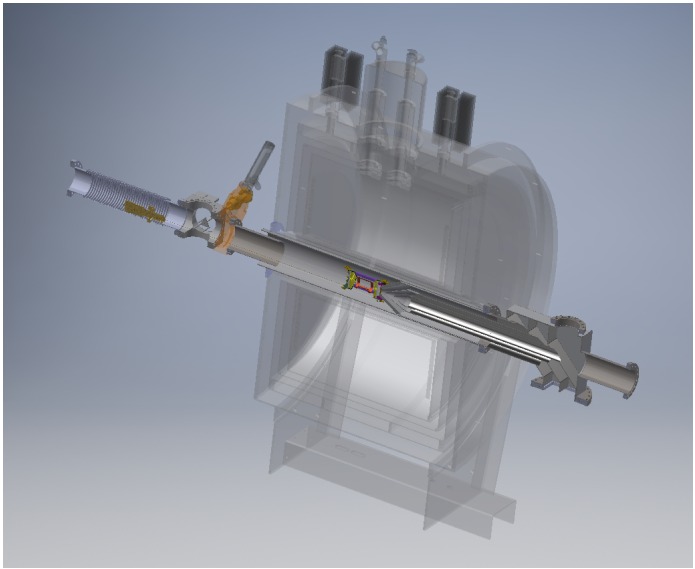
Beamline



Stray field



Inside the Magnet



Properties of stable and radioactive isotopes relevant for this proposal

Nucleus	Radioactive half-life	Nuclear spin I	Magnetic moment (μ_N)	Quadrupole moment (mb)	Observed β-asymmetry
^{23}Na	-	3/2	2.217499(7)	104	-
^{26}Na	1.077 s	3	2.849378(20)	-5	25%
^{37}K	1.237 s	3/2	0.20320(6)	100	8-11%
^{39}K	-	3/2	0.39147(3)	60	-
^{49}K	1.260 s	1/2	1.33868(8)	-	-

Harding et al., Magnetic moments of short-lived nuclei with part-per-million accuracy: Paving the way for applications of β-detected NMR in chemistry and biology, http://arxiv.org/abs/2004.02820, 2020

Shidling et al., Precision half-life measurement of the β^+ decay of ^{37}K, Phys. Rev. C, 2014

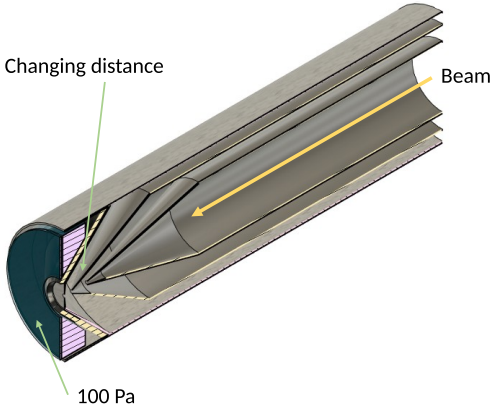
Kopf et al., Optical pumping of short lived β-radioactive isotopes and the magnetic moment of 37K, Zeitschrift für Physik, 1969

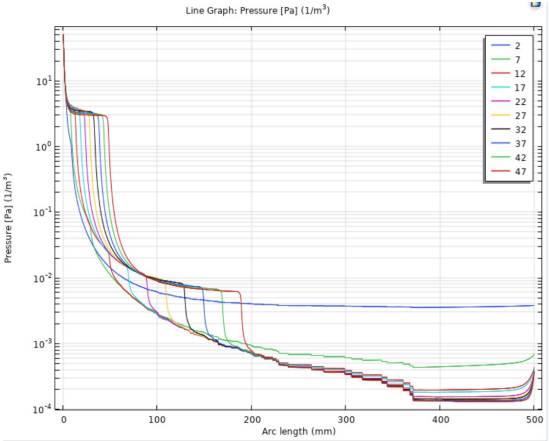
Von Platen et al., Spin exchange polarization and hfs anomaly measurement of β-active 37K, Zeitschrift für Physik, 1971

Minamisono et al., Quadrupole moment of 37K, Physics Letters B, 2008

Carraz et al., The 49K beta decay, Physics Letters B, 1982

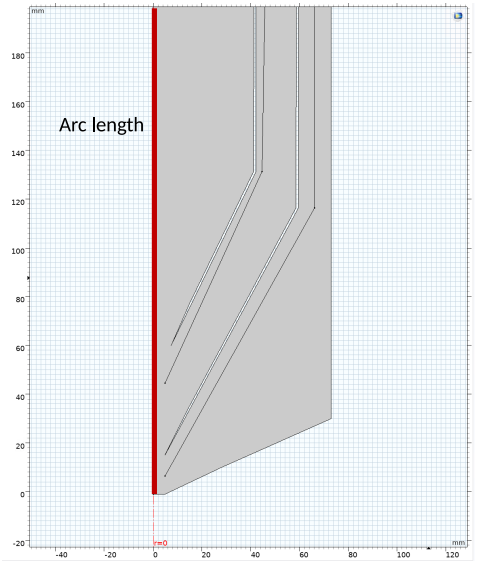
Differential Pumping (comsol)

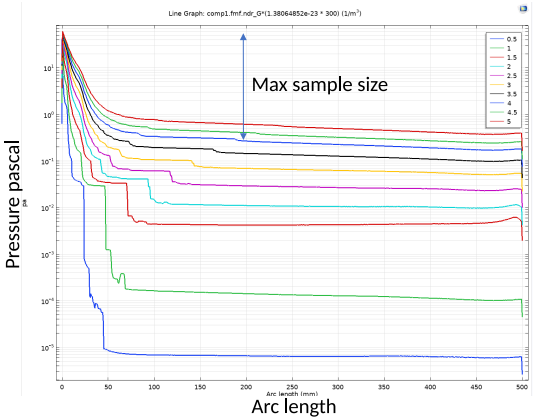




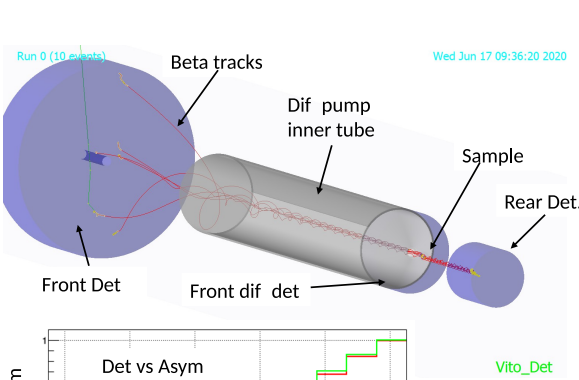
- Pressure with 2mm aperture with various distance between tubes
- 100 Pa at sample

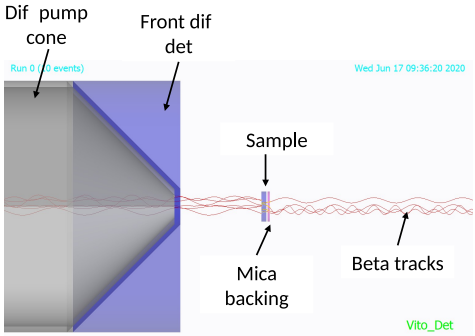
Differential Pumping - Changing Aperture size

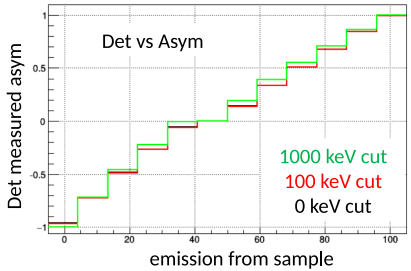




Differential Pumping - Geant4 detector simulation







- Detector array for Beta asymmetry measurements simulated in GEANT4
- 3D 4.7T Field imported from Bruker data.
- 100k events per bin
- Full beta spectra imported

Number of Shifts

- 1 shift (for each used isotope): determining the highest pressure with the differential pumping system when signals are still visible
- 1 shift at the start of every beamtime: establishing laser polarisation by HFS scans, optimising laser-atom overlap
- 0.5 shift for every change of the liquid sample): see details above
- 0.3-0.5 shift (for each solvent and G4 configuration): measuring T_1 in one liquid sample, with different parameters optimised
- 0.5-1 shift for each solvent and G4 configuration: performing several NMR scans in one liquid sample (depending on the number of peaks and observed β-asymmetry)