Interaction of Na ions with DNA G-quadruplex structures studied directly with Na beta-NMR spectroscopy

M. Kowalska1, V. Araujo Escalona2, M. Baranowski3, J. Croese1,4, L. Cerato4, M. Bissell4, W. Gins2, F. Gustafsson2, R. Harding1,7, L. Hemmingsen8, H. Heylen1, F. Hofmann8, A. Kanellakopoulos2, V. Kocman9, M. Kozak3, M. Madurga Flores6, G. Neyens1,2, S. Pallada1, J. Plavec9, K. Szutkowski3, M. Walczak10, F. Wienholtz1, J. Wolak3, X.F. Yang11, D. Zakoucky12

1. EP-Dept, CERN, Geneva, Switzerland
2. IKS, KU Leuven, Leuven, Belgium
3. Faculty of Physics, Adam Mickiewicz University, Poznan, Poland
4. Faculty of Sciences, University of Geneva, Geneva, Switzerland
5. School of Physics and Astronomy, Manchester University, Manchester, United Kingdom
6. Dept. of Physics and Astronomy, University of Tennessee, Knoxville, Tennessee, USA
7. Department of Physics, University of York, York, United Kingdom
8. Department of Chemistry, University of Copenhagen, Copenhagen, Denmark
9. Slovenian NMR Center, National Institute of Chemistry, Ljubljana, Slovenia
10. School of Physics, Peking University, Beijing, China
11. Poznan University of Technology, Poznan, Poland
12. NPI, Czech Academy of Sciences, Rez, Czech Republic
Motivation: DNA G-quadruplexes

- DNA G-quadruplexes: Formed in guanine-rich DNA fragments
  - Present in telomeres (ends of chromosomes)
  - Present in promoter regions of many oncogenes

- Alkali metals in DNA G-quadruplexes
  - Important for their formation, stability and structural polymorphism
  - Until recently considered invisible in conventional Na+/K+ NMR

- Goal of IS601: 15 shifts to study Na-DNA interaction using ultrasensitive beta-NMR
  (5 shifts used already)

Technique: beta-NMR at VITO beamline

Up to 1e10 increased NMR sensitivity: hyperpolarization and beta-asymmetry detection

Designed, commissioned in 2017:
- Liquid Beta-NMR
- Differential pumping and transitional field
- Spin polarization
September 2017 beamtime

Online commissioning of:

- New compact beta detectors
- New NMR chamber (delayed, arrived 2 days before beamtime; vacuum leak during run)
- Liquid handling system: liquid injected and coating a substrate (30 min)
- Differential pumping system (pinholes arrived with chamber, 1-2mm off center, 1 too thick)
- New transitional magnetic field
Differential pumping tests: pinhole problems: off center and large beta background

Nevertheless, very good performance:

- 0.5 mbar: 2.5 x decrease, can go higher
- 1000 better vacuum in front of NMR chamber;
- 1e-5-1e-6 mbar in rest of beamline
December 2017 beamtime

- Improved stability and tightness of liquid-handling system
- Last pinhole out -> concentrate on resonances in good vacuum
December 2017 beamtime

B0 Field Scan along x-Axis

Improved B-field homogeneity

- Between poles: 3 ppm
- Front-back: 12 ppm
- Up-down: 3 ppm
Ionic liquid vs crystal

Same conditions
T1 in crystal >> liquid

Difference:
Narrowing in liquid due to molecule rotations
First 26Na NMR in ionic liquids

- 26Na
  - $T_{1/2} = 1.1$ s
  - $I = 3$
  - $\mu = 2.86 \mu_N$
  - $Q = -5$ mb

In comparison - 23Na:
- $I = 3/2$
- $\mu = +2.21 \mu_N$
- $Q = 100$ mb (20x larger!)

- $B_0 = 0.5$ T
- Meas. time, ca 5 min
- $T_1 = \text{ca } 150$ ms
December 2017 results

- Drift of 1 ppm/h (in 2016)
- Drift of 1.5 ppm/h (before beamtime)

Graph showing resonance position vs. time with data points for different samples.
Winter shutdown activities

- Complementary measurements on several ionic liquids with $^{23}$Na NMR
- Offline studies of short DNA sequences dissolved in ionic liquids
- Conventional NMR measurements in cell-like PEG solvent

- Modifications to the last differential pumping section to allow studies in $1 \times 10^{-3} - 1 \times 10^{-2}$ mbar: pinhole in front of chamber, not inside

- Further improvement in magnetic-field homogeneity
- Improvement of the long-term stability of the magnetic field
- Magnetic field measurement (using NMR probe) during data-taking

- Minimisation of the time required to clean the liquid handling system and inserting new samples

- Addition of temperature control and measurement of the temperature
Online plans for 2018

1st run, 9 shifts:
- Try b-NMR with ISCOOL bunched beam
- 26Na in Emim-DCA to compare to Bmim-COOH: narrower resonance? Longer relaxation time T1?
- 26Na b-NMR of DNA dissolved in Emim-DCA or Bmim-COOH: looking for change in chemical shift, T1
- Same study in another ionic liquid or other DNA

2nd run, 9 shifts:
- Systematic G-quadruplex study in best ionic liquid (identified during 1st run):
  - Change DNA concentration and or T1
- G-quadruplex study in PEG at 1e-3 (or 1e-2)mbar

Beam request:
- 26Na from Ti, Ta, or UC with surface ionization
- HRS, ISCOOL in bunch tune
- 8 additional shifts needed (10 still left from 2017)

Resolution of our system is good enough for expected chemical shifts, based on rare 23Na NMR studies:
Thank you

Funding:

ENSAR2