Molecular recognition based on nucleic acids structures: from gas phase to solution

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Context

- Development of multilevel analytical strategies
  - Screening
  - Semi-quantitation
  - Confirmation
- Requirements
  - Identification
  - Quantification
  - ISO 17025 compatible in terms of false positive, negative samples
Tools

• Sensors, bioassays, rush methods for screening
• Confirmation often requires mass spectrometry, which was problematic for non volatile, polar compounds

Electrospray ESI (nanoESI)

intact non covalent assemblies

• Concentrations: $10^{-6}$ M
  Solvent: $\text{H}_2\text{O}$
  (+ MeOH, CH$_3$CN)

• Multiply charged ions

• Quasi no fragments
Direct identification of solid phase compounds

From Leonid V. Zhigilei web site

Quadrupoles

- Filters: trajectory stability

Workshop on electronic recognition of DNA molecules
Liège, September 1-3
Quadrupolar ion traps

- Selective instability

FTMS

Workshop on electronic recognition of DNA molecules
Liège, September 1-3
**Time of flights (TOF)**

QuickTime™ et un décompresseur codec YUV420 sont requis pour visionner cette image.

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**The Mass Spectrometry Toolbox**

- M/Z determination at the nucleonic level
- Direct sampling of the solution (ESI-MS)
- Kinetics of fragmentation (breakdown curves MS-MS)
- Ion trapping
- Modes of energy transfer: CID, SID, ECD, IRMPD
- Structural information: full sequencing facility, fragmentation mechanisms, H/D exchange
- Database searching algorithms
Measuring the affinity of a chemical for the molecular target

The basic hypothesis

![Diagram showing the basic hypothesis](image)

- Full scan MS
- Electrospray

DNA as an example

1. Potentially important target for the design of new drugs, fast screening tests required
2. Oligomers are (seems to be) good models for larger structures
3. Reference methods for structures well established in solution and solid phase
4. Increasing importance of DNA in sensors as capture elements
« Historical » DNA duplexes (Watson-Crick)

Stacking

Major groove binding

Minor groove binding

Stoechiometry

1:1 or 2:1

Caution: non specific complexes

Intercalation

intercalation of planar compound

Random stacking between the base pairs preferential GC stacking
G-Quadruplexes

Q1: (TG₄T)₄
Q2: (G₄T₄G₄)₂

Sampling solution to the gas phase

Direct titration

Plots of the relative intensities of the different species (duplex, 1:1 complex, 2:1 complex) versus the drug molar fraction
Manipulating the ions in gas phase: MS/MS

Gas phase denaturation curve for the duplex GC$^5$, GC$^5$ + Hoechst, GC$^5$ + Netropsin compared to solution thermal denaturation monitored by UV

Easy differentiation of binding sites

$T_{eff}$?

V. Gabelica, E. De Pauw, Rapid Comm 14, 464, 2000

Molecular recognition by MS-MS

Intercalators: even with «random» intercalation, specificity is conserved in the gas phase

Cryptolepine

Neocryptolepine

ds+C$^5$

ds+NeoC$^5$
Structure is largely maintained

Not a proof, just a reasonable guess!

5'-AAATCGCGCGCTAAA-3'  
3'-TTTAGCGGCCGCGGT-5'  
$\Delta H_{\text{nn}} = -136.8 \text{ kcal mol}^{-1}$

5'-GGGCTATAATATCGGG-3'  
3'-CCCGATATTATAGCCC-5'  
$\Delta H_{\text{nn}} = -125.3 \text{ kcal mol}^{-1}$

5'-AGACTGTGAGTCAGTG-3'  
3'-TCTGACACTCAGTCAC-5'  
$\Delta H_{\text{nn}} = -122.6 \text{ kcal mol}^{-1}$

5'-GGGCTTTTAAAACGGG-3'  
3'-CCGGAAAATTITTGCC-5'  
$\Delta H_{\text{nn}} = -135.9 \text{ kcal mol}^{-1}$

! not expressed in the CM

Applications

Part I: Screening Drug-DNA Interactions  
Effect of the Drug Telomestatin on Telomeric Quadruplex Unfolding

Part II: H/D exchange on Quadruplexes

Part III: Modified DNA: Molecular nanoprobes

Part IV: hyphenation
Part I:
Screening Drug-DNA Interactions with sequence specificity

Human Telomeres: (TTAGGG)$_n$

Q3: GGGTTAGGGTTAGGGTTAGGG

143D: NMR
Structure (1993) 1: 263

1KF1: X-ray
Quadruplexes in Telomerase Inhibition

=> Search for drugs that stabilize the G-quadruplex conformation

Targeting G-Quadruplexes with Ligands

External Stacking

Intercalation

Groove binding

Loop Recognition
Some drug candidates

Screening Example 1

Amount bound (µM)
**Discovery of new Specific 2:1 Complex**

Manual docking and molecular dynamics

Semi-empirical model for dimer

**Screening Example 2**

11190: weaker binding, but very specific for telomeric quadruplexes

Amount bound (µM)

<table>
<thead>
<tr>
<th>0.0</th>
<th>0.2</th>
<th>0.4</th>
<th>0.6</th>
<th>0.8</th>
<th>1.0</th>
</tr>
</thead>
<tbody>
<tr>
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</table>

**Telomeric quadruplex**

1:1

**Quadruplex**

1:1

**Duplex**

1:1
Part II:

Mechanisms of action: Effect of the Drug Telomestatin on Telomeric Quadruplex Unfolding

Quenching the hybridization kinetics

Objective: To mimic drug-induced telomerase inhibition

Complementary strand $(CCCAAT)_{3}CCC$

Telomestatin

2:1

1:1

$(GGGTTA)_{3}GGG$

G-quadruplex

Duplex

Telomerase

Effect of telomestatin on the hybridization kinetics

Hybridization kinetics: without drug

Relative intensity

$\tau_{\text{hybridize}} = 532 \pm 11 \text{ s}$

Intensity of the duplex

Intensity of the quadruplex

$t = 200 \text{ s}$

$t = 2000 \text{ s}$

No drug

With telomestatin
Hybridization kinetics: with drug

Rate-limiting step = decomplexation

\[ \tau_{\text{hybridize}} = 1010 \pm 90 \, \text{s} \]

\[ \tau_{G4} = 540 \pm 30 \, \text{s} \]

1:1 cplx

2:1 cplx

Part III:

Structure (?): H/D exchange on Quadruplexes
Conformation of Quadruplexes in the Gas Phase

Preliminary experiments on H/D exchange

Previous studies on DNA non-covalent assemblies:

- General idea:
  - more compact structures have less H/D exchange sites

CD$_2$OD (deuterated methanol) in the FTICR cell ($p = 8 \times 10^{-9}$ T)

Bruker Apex-Q (Bremen, Germany)

The kinetic of deuterium incorporation was monitored (0-20 min)

- M. Vairamani and M.L. Gross, JACS (2003), 125, 42-43. (quadruplex)

**Duplex (CGCGAATTTCGCG)$_2$**

Typical: large spread
Different protons have different exchange rates

20 H/D

300 sec
50 sec
4 sec
2 sec
0 sec
**Quadruplex \([\text{TGGGGT}]_4 3\text{NH}_4^+ - 8\text{H}^+]_5^-\)**

Unexpected: Fast, Quasi no spread

**Effect of the charge and aggregation state**

<table>
<thead>
<tr>
<th>Quadruplex ([\text{TGGGGT}]_4 3\text{NH}_4^+ - 7\text{H}^+]_4^-)</th>
<th>2 components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast: 16.8 ± 0.8 H/D</td>
<td>(time constant: 2.9 ± 0.5 s)</td>
</tr>
<tr>
<td>medium: 23.2 ± 0.8 H/D</td>
<td>(time constant: 83 ± 14 s)</td>
</tr>
<tr>
<td>Total: 40 ± 1.6 H/D</td>
<td></td>
</tr>
</tbody>
</table>

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<tr>
<th>Quadruplex ([\text{TGGGGT}]_4 3\text{NH}_4^+ - 8\text{H}^+]_5^-)</th>
<th>2 components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast: 25.3 ± 0.5 H/D</td>
<td>(time constant: 3.01 ± 0.14 s)</td>
</tr>
<tr>
<td>Slow: 6.2 ± 1.2 H/D</td>
<td>(time constant: 450 ± 250 s)</td>
</tr>
<tr>
<td>Total: 31.5 ± 1.7 H/D</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Single strand ([\text{TGGGGT} - 2\text{H}^+]_2^2-)</th>
<th>1 component</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow: 7.7 ± 1.6 H/D</td>
<td>(time constant: 500 ± 250 s)</td>
</tr>
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Other duplexes and quadruplexes: typical time constant = 25-50 s
First analysis of the numbers

Per backbone (4) : max 7 H
   (5 phosphate + 2 sugar)  28
Per thymine (8) : max 1 H     8
Per guanine (4*3) : max 3H  36
Per NH$_4^+$ (3) : max 4 H  12
Per strand: max           18
Per G4:                  84

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<tr>
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<th>medium</th>
<th>Fast</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS 2-</td>
<td>7.7±1.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G4 5-</td>
<td>6.2±1.2</td>
<td>0</td>
<td>25.3±0.5</td>
</tr>
<tr>
<td>G4 4-</td>
<td></td>
<td></td>
<td>23.2±0.8</td>
</tr>
</tbody>
</table>

Charge fluctuation?

Effect of Cation replacement

[(TGGGGT)$_4$ o(3-n)NH$_4^+$ o nK$^+$-8H$^+$]$_{5^-}$

The species still exchange to the same extent => NH$_4^+$ not exchanged
Role of the cation

\[
\{(TGGGGT)_{4} + 3NH_{4}^{+} + 2H^{+}\}^{5+}: \text{fast}
\]

\[
\{(TGGGGT)_{4} + 5H^{+}\}^{5+}: \text{slow}
\]

\[\Rightarrow \text{The inner cations are essential for fast H/D exchange}\]

Inner cations are essential to provoke fast exchange (both positive and negative ion mode!). But the inner \(NH_{4}^{+}\) hydrogens are not essential for fast exchange.

**Hypothesis:** the cations included confer a rigid conformation to the quadruplex, which is highly favorable for H/D exchange.

**Next step:** influence of the drugs
Part III:

nanoprobes: Fullerene functionalized DNA

Fullerene modified DNA

Workshop on electronic recognition of DNA molecules
Liège, September 1-3
Part IV: building the sensors
The bridge between solid, liquid and gas phase

Affinity capture, SPR and MS

Surface Plasmon resonance based detection coupled with MS identification

Challenge: development of capture elements
**Biacore-MS coupling**


**New ligands: aptamers (aptare=to fit)**

Molecular « wrapping »

Molecular imprinting in solution
Aptamer capture and MALDI MS

Direct MALDI fingerprinting of bacteria
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