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LA SCIENCE A PARIS

CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE

Modeling of PNA/DNA noncovalent complexes.

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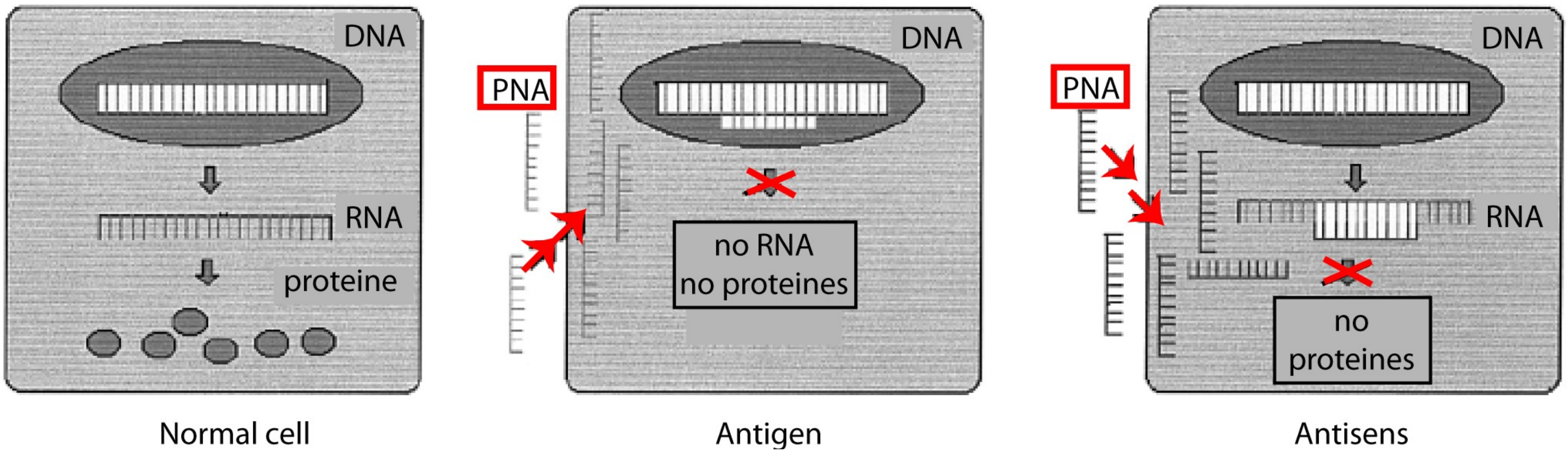
Summary

1. **Introduction**
2. **Method**
3. **Results**
    a. PNA/DNA duplexes
    b. PNA/DNA/PNA triplexes
4. **Conclusions and perspectives**

1. Introduction

PNA intermolecular interactions with DNA [2,3,4]

Potential therapeutic applications:



Other applications:

- probe for chromosomal analysis
- primers for PCR reaction

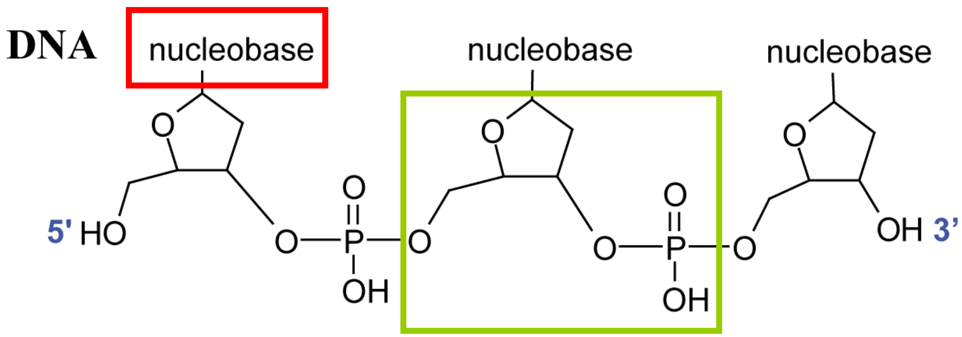
PNA = Peptide Nucleic Acid

2. Nielsen P.E. et al. *Science*. 1991, 254, 1497.
3. Ray A. et al. *The FASEB Journal*. 2000, 14, 1041. **4.** Paulasova P. et al. *Annales de Génétique, Elsevier* 2004, 47, 349.

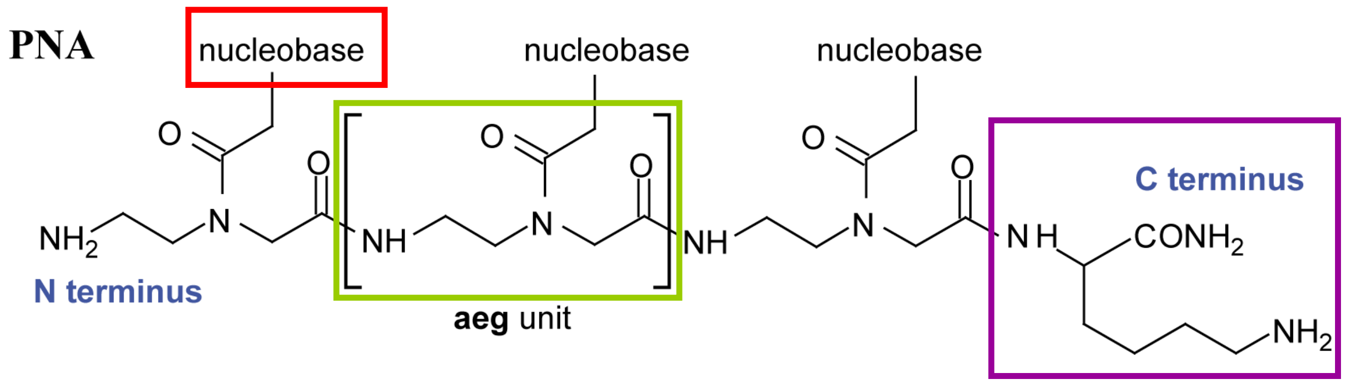
1. Introduction

What are PNA oligomers ?

« natural » oligomers



artificial oligomers
Nielsen et al.[4]

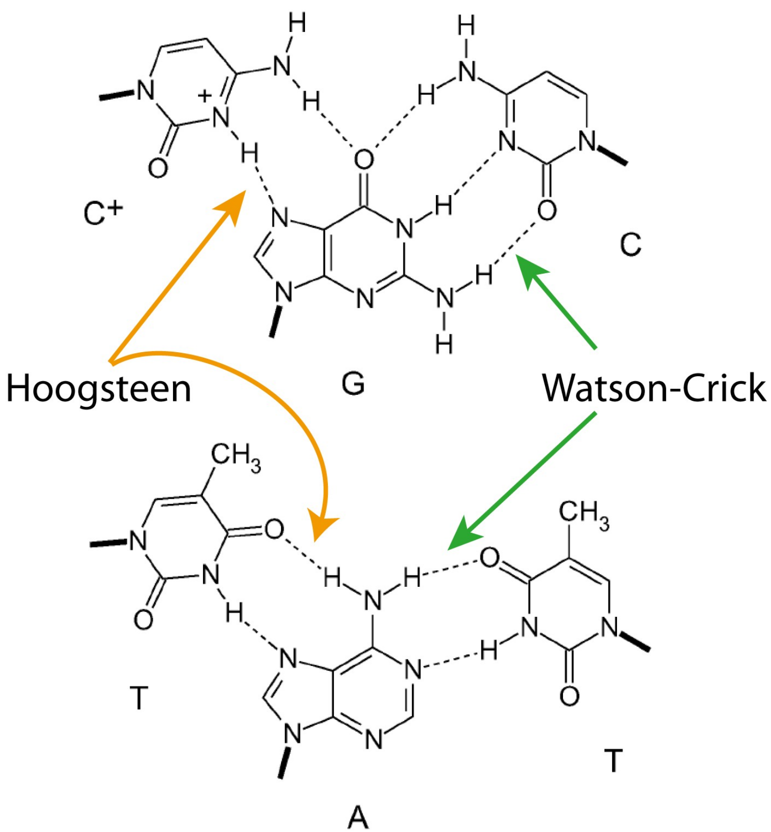


4. Nielsen P.E. et al. *Science*. 1991, 254, 1497.

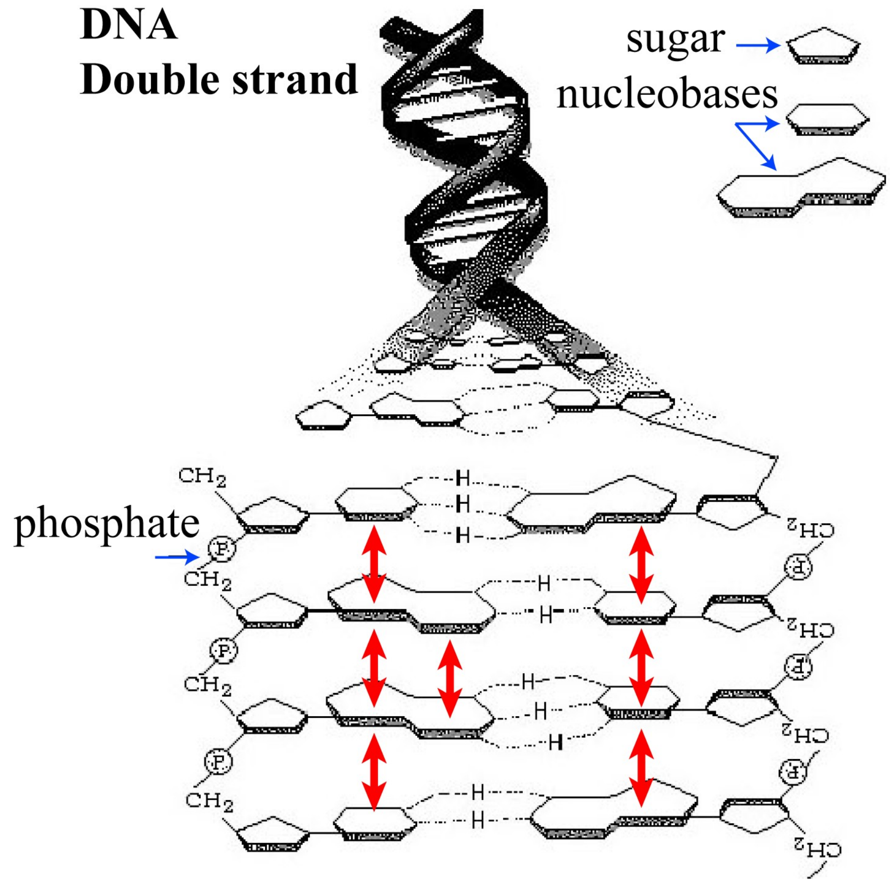
1. Introduction

How do they interact?

1. hydrogen bond (pH 6).



2. π staking : π/π noncovalent interactions due to nucleobases staking.

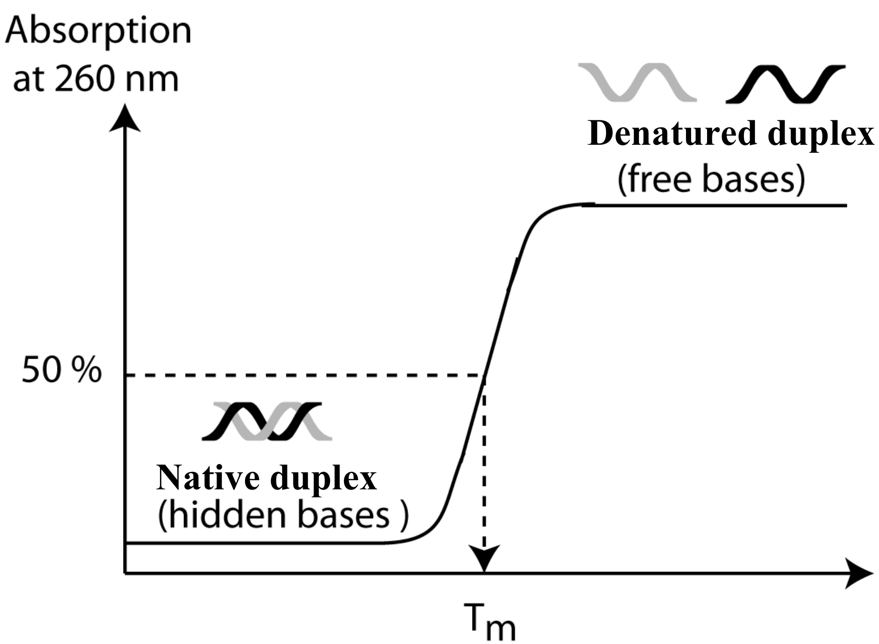


3. Hydrophobique effects (only in solution!)

1. Introduction

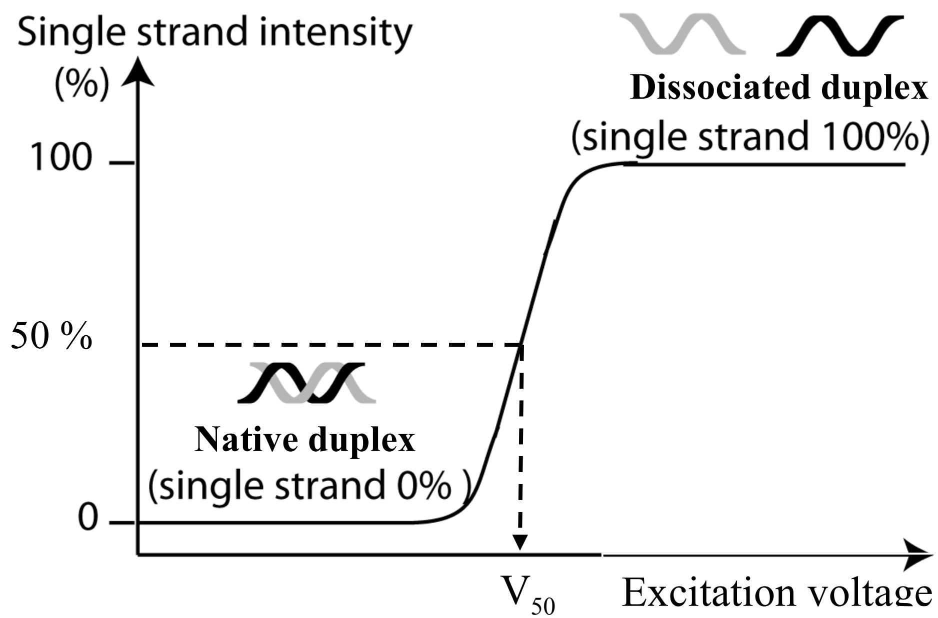
In solution

UV Spectroscopy [6]



In the gas phase

Electrospray (solvent = 100% water)
Ion trap



6. Bregant et al. Eur. J. Org. Chem. 2001, 17, 3285.
And others

7. Gabelica V. et al. J. Mass Spectrom. 2001, 36, 397.
And others …

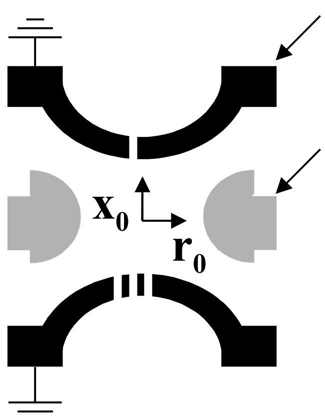
2. Method

Ion trap

Esquire 3000™ (Bruker).

Dissociation under low energy conditions

Sources: nano- and electrospray, with 100% water



Bruker™ ion trap

1. **Introduction**
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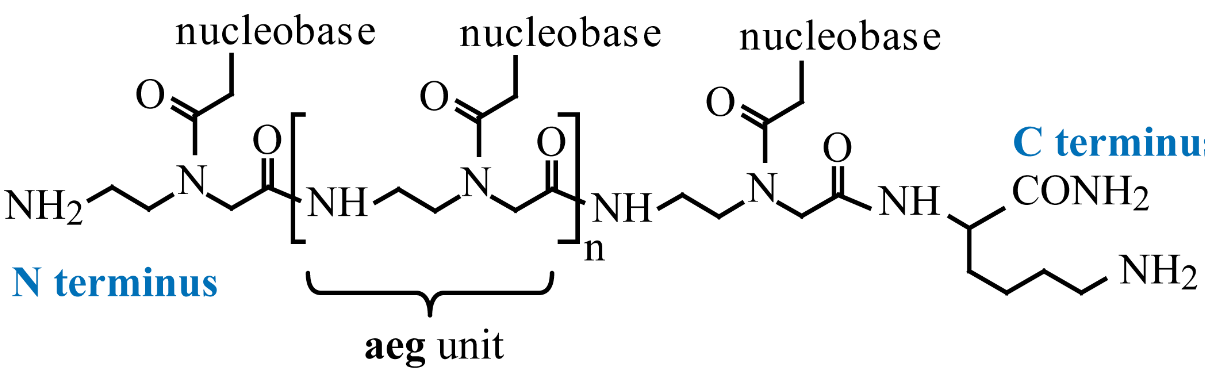
    - PNA/DNA duplexes
    - PNA/DNA/PNA triplexes
4. **Conclusions and perspectives**

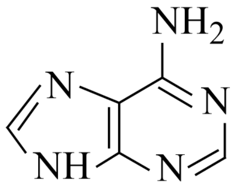
PNA/DNA duplexes

What kind of oligomers ? Various sequences !

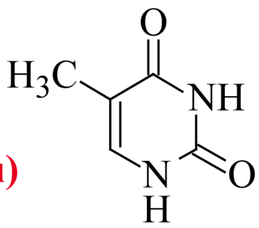


Sequence ($N_{terminal} \rightarrow C_{terminal}$)	Mw	Nomenclature
GTA GAT CAC T-Lys	2855	P_{58}
AGT GAT CTA C-Lys	2855	P_{60}
AGG TAA CGA G-Lys	2929	P_{2482}
CTC GTT ACC T-Lys	2782	P_{2484}

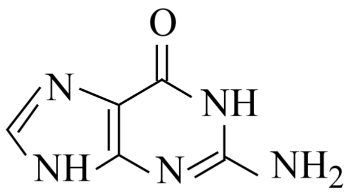
A (135 u)



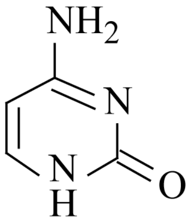
T (126 u)



G (151 u)



C (111 u)



3.a.
PNA/DNA duplexes

Orientation of their fragmentation as a function of their charge state

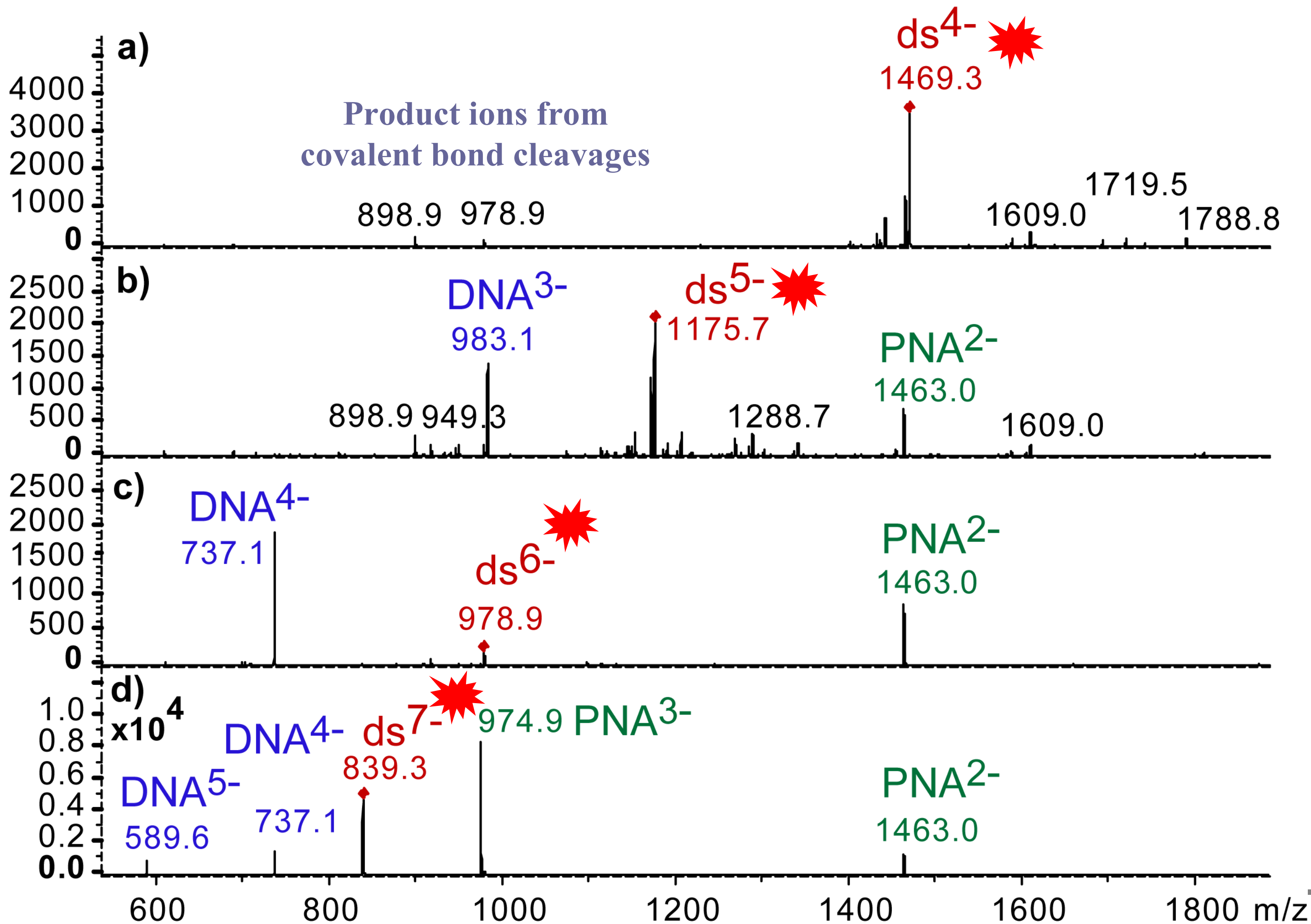
Duplex charge state

- 4 ($V_{excitation}$ = 2 V)

- 5 ($V_{excitation}$ = 1 V)

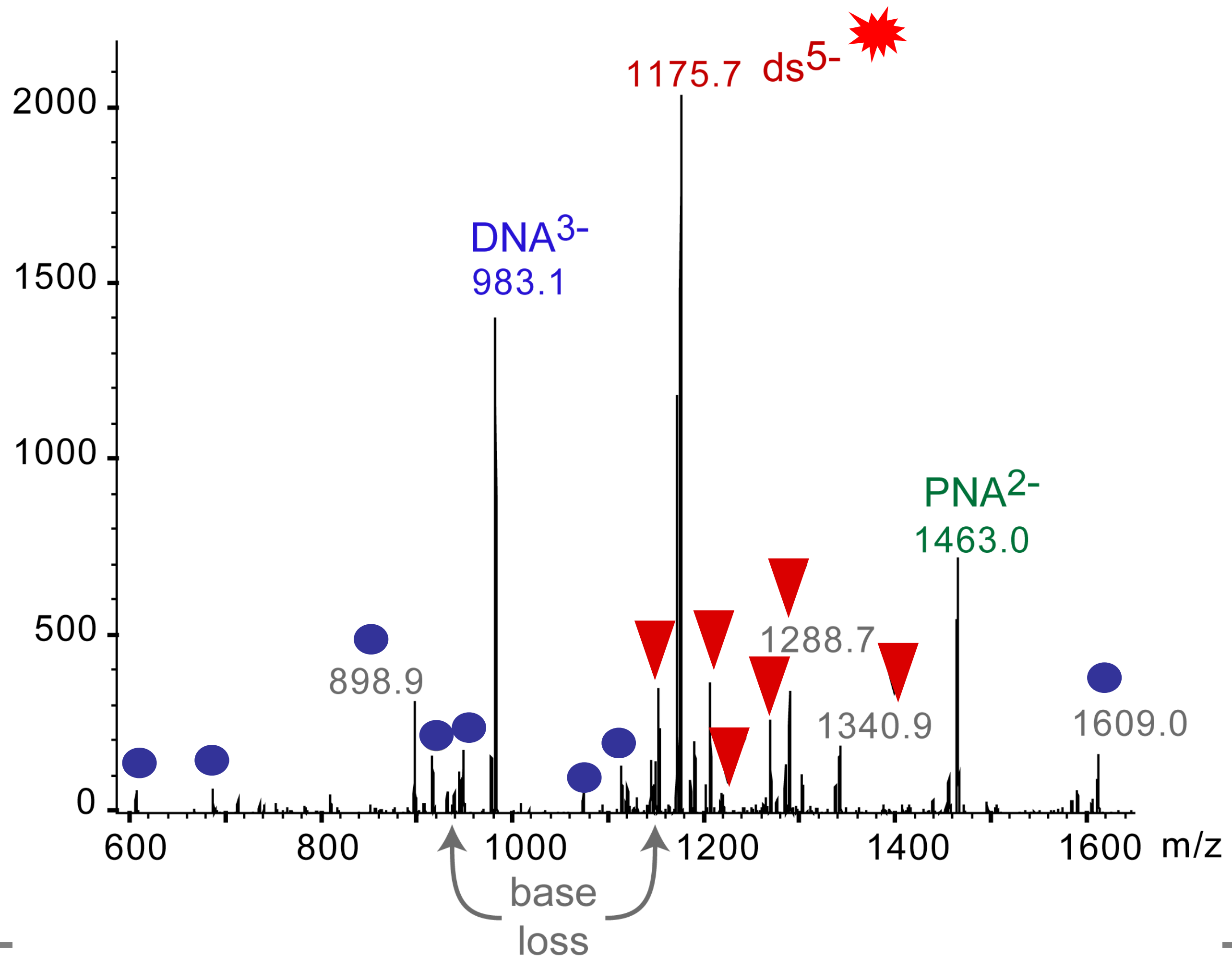
- 6 ($V_{excitation}$ = 1 V)

- 7 ($V_{excitation}$ = 0.5 V)



3.a.
PNA/DNA duplexes

Intermediate case: covalent bond cleavage and strand separation



● **[DNA sequence ions]$^{x-}$**

▼ **[ds^{5-} - DNA sequence ions^{x-}]$^{(5-x)-}$**

No PNA sequence ions!

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3.a. DNA/DNA duplexes

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DNA/DNA duplexes fragmentation orientation as a function of their charge state[13,14,15]

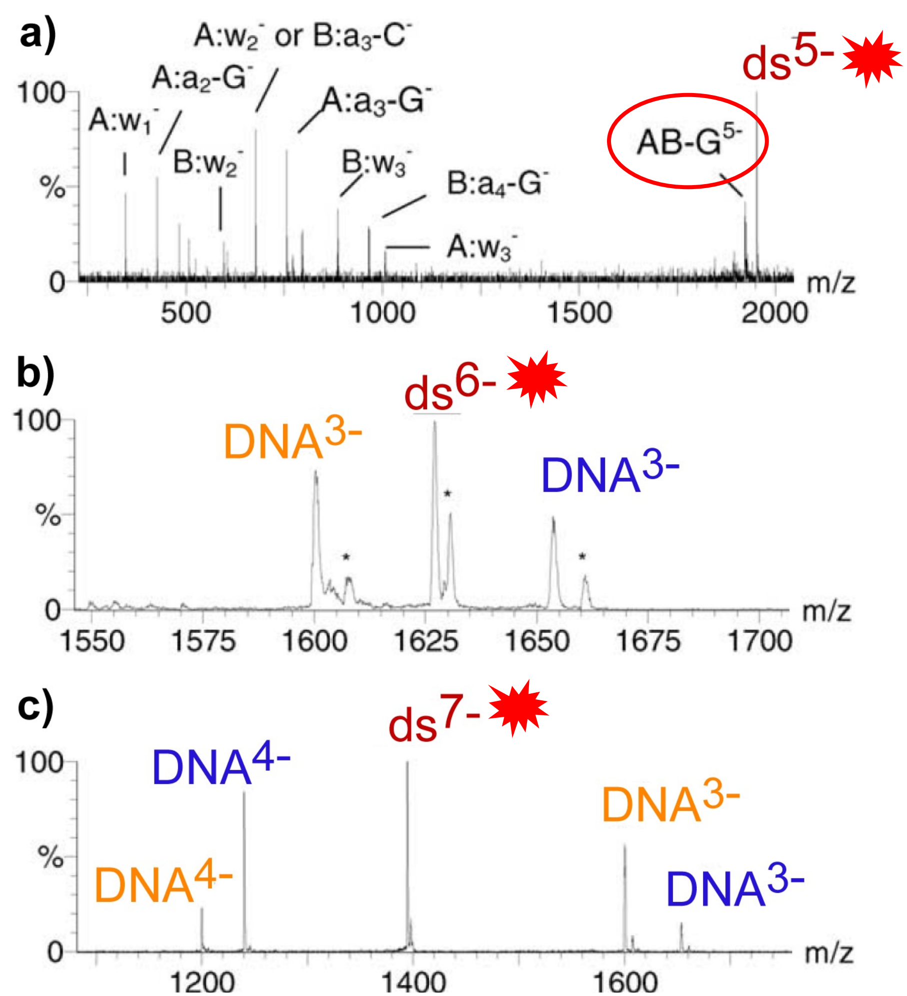
Example : **[13]** De Pauw et al. Int. J Mass Spectrom. 2002, 219, 151

Duplex charge state

- 5
(Collision energy = 30 eV)

- 6
(Collision energy = 20 eV)

- 7
(Collision energy = 14 eV)



14. Rodgers M.T. et al. *Int. J. Mass Spectrom. Ion Process.* 1994, 137, 121.
15. Wang Z. et al. *J. Am. Soc. Mass Spectrom*. 1998, 9, 693. And others ...

1. **Introduction**
2. **Method**
3. **Results**
   - PNA/DNA duplexes
   - PNA/DNA/PNA triplexes
     - Specificity
     - Fragmentation orientation
     - Gas phase and solution stability
4. **Conclusions and perspectives**

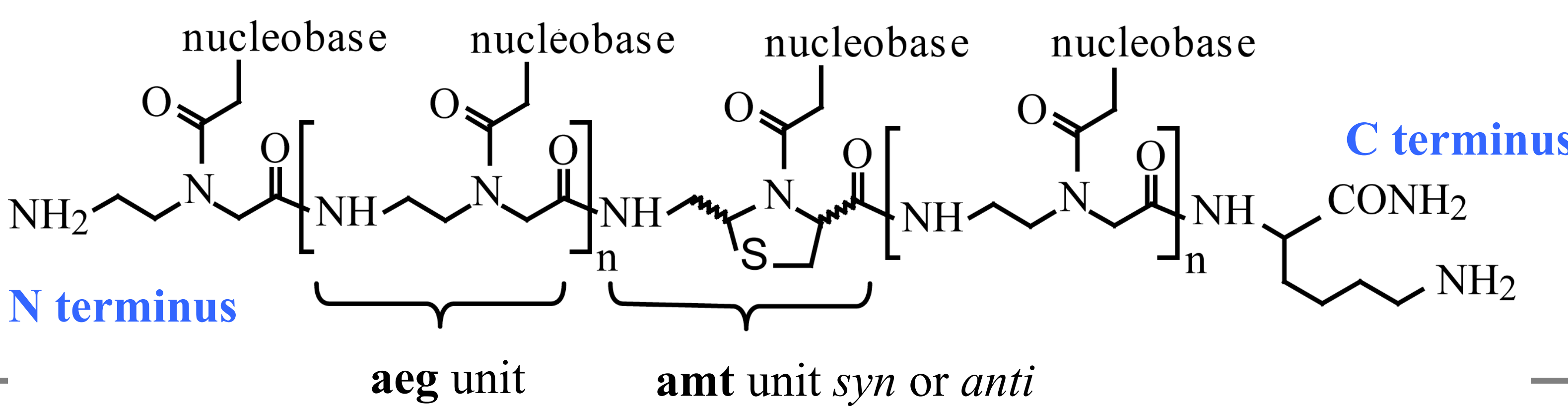
3.b.
PNA/DNA/PNA triplexes

Oligomers used for the triplexes

Modified PNA

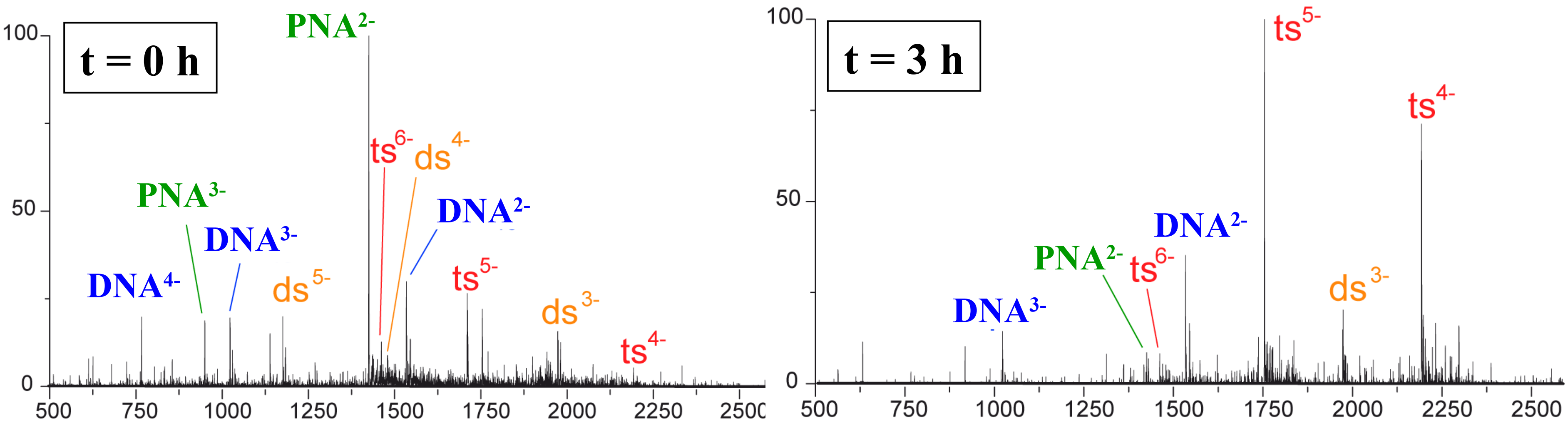
Sequence ($N_{terminus} \rightarrow C_{terminus}$)	Mw	Name
TTT TTT TTT T-Lys	2802	P_N
TTT TT$_S$T TTT T-Lys[a)]	2847	P_S
TTT TT$_A$T TTT T-Lys[b)]	2847	P_A

a) T_S : modified unit / **amt** *syn* **b)** T_A : modified unit / **amt** *anti*



3.b. PNA/DNA/PNA triplexes

Specific noncovalent triplexes

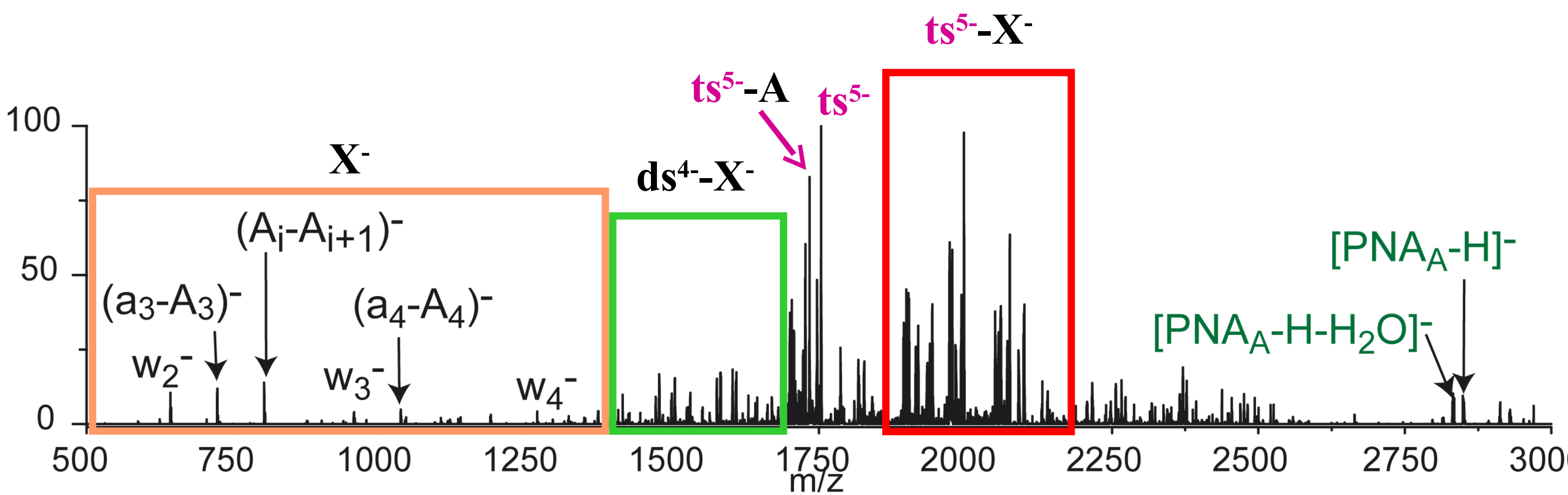


3.b.
PNA/DNA/PNA triplexes

Triplexes fragmentation orientation as a function of their charge state

Case of $[ts\text{-}nH]^{n-}$ with $n \leq 5$

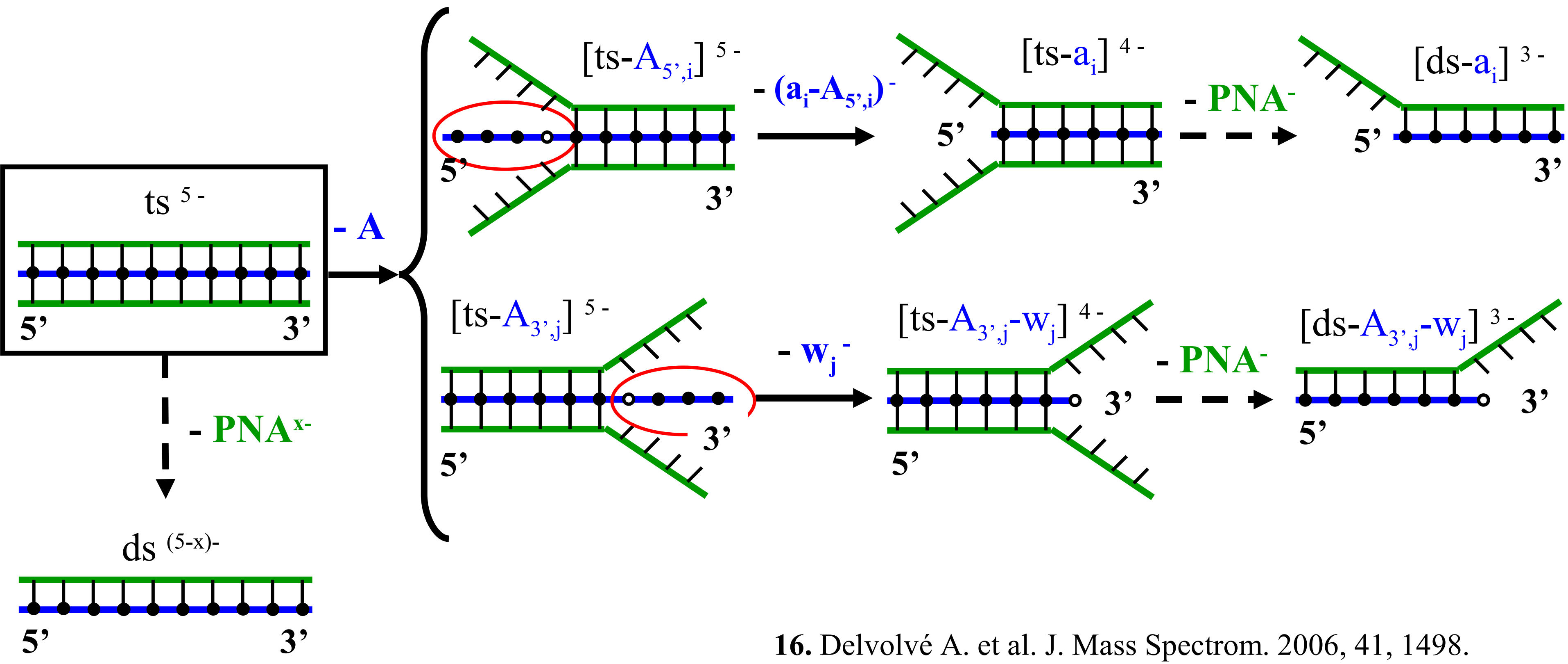
1) "no" product ions from the PNA strand; no PNA/PNA duplexes
2) PNA/DNA duplex of a minor abundance
3) $(a_i\text{-}A_i)^-$ et w_j^- product ions from the DNA strand
4) series of product ions from the loss of DNA product ions from the triplex



3.b.
PNA/DNA/PNA triplexes

A model in order to explain the ways of fragmentation[16]:

(Model based on that proposed by De Pauw et al.[17])



16. Delvolvé A. et al. J. Mass Spectrom. 2006, 41, 1498.
17. Gabelica V. et al. J. Am. Soc. Mass Spectrom. 2002, 13, 91.

3.b. PNA/DNA/PNA triplexes

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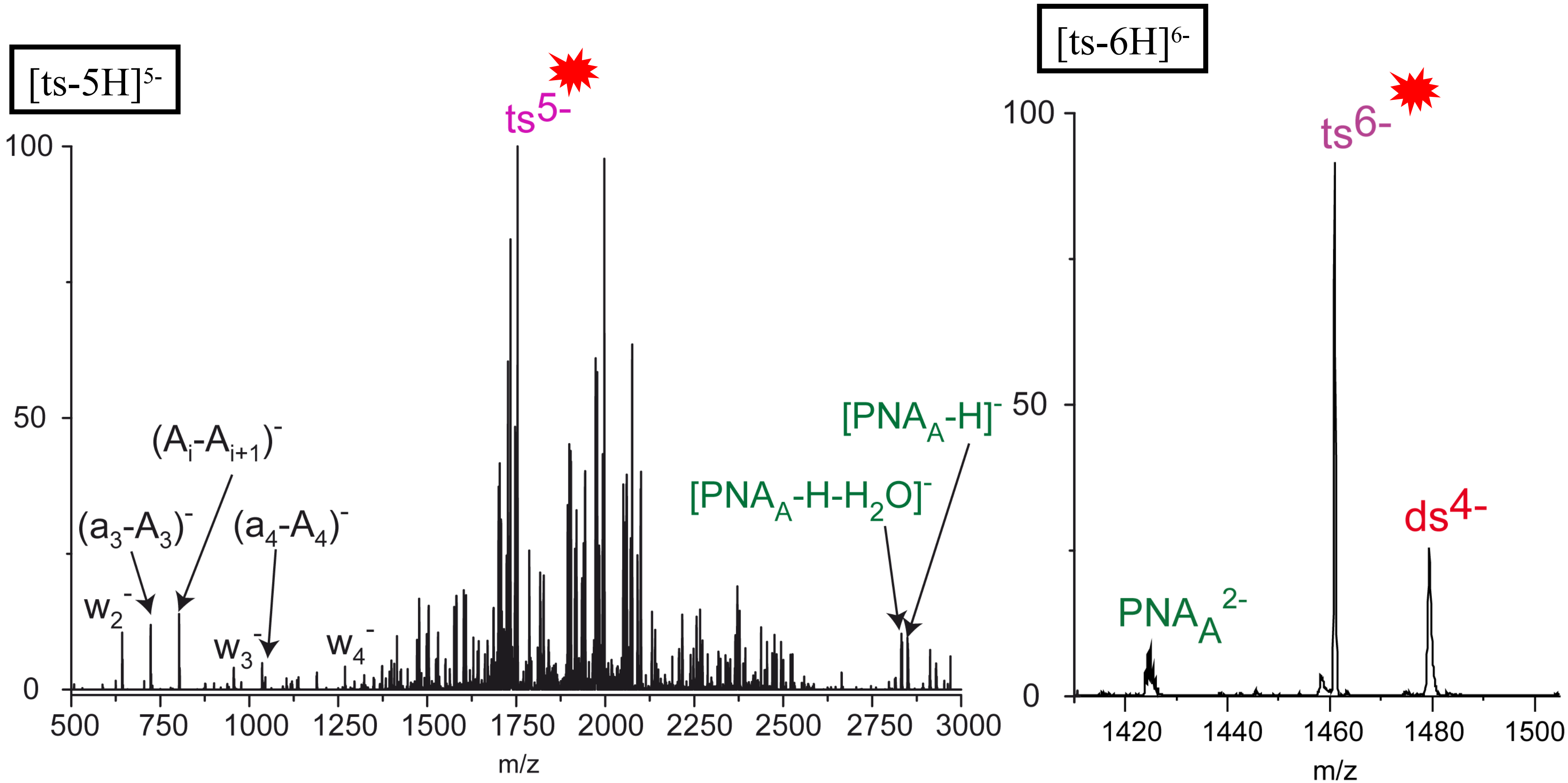
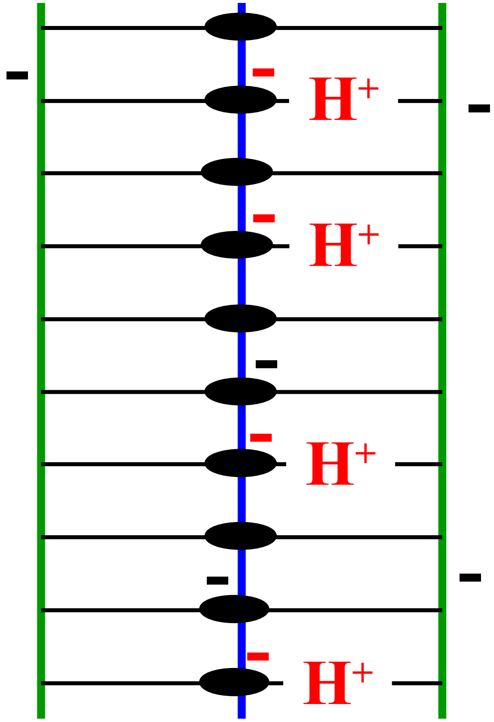
Triplexes fragmentation orientation as a function of their charge state



3.b.
PNA/DNA/PNA triplexes

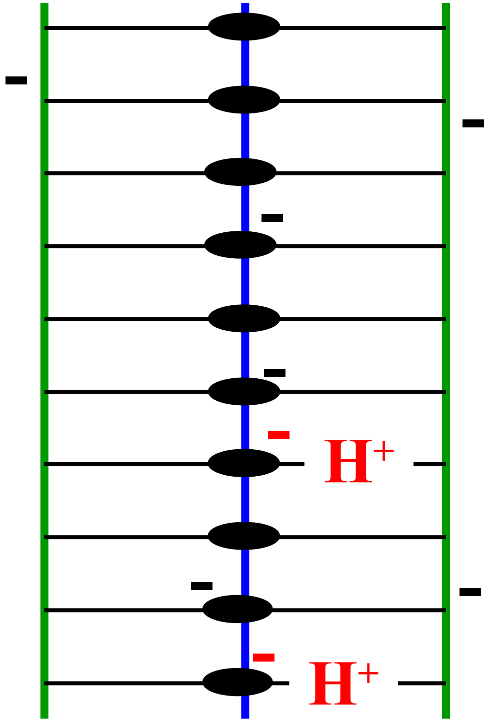
Zwitterion[16] and coulombic[16,17] repulsions model

ts 5 -



covalent bond cleavages

ts 6 -

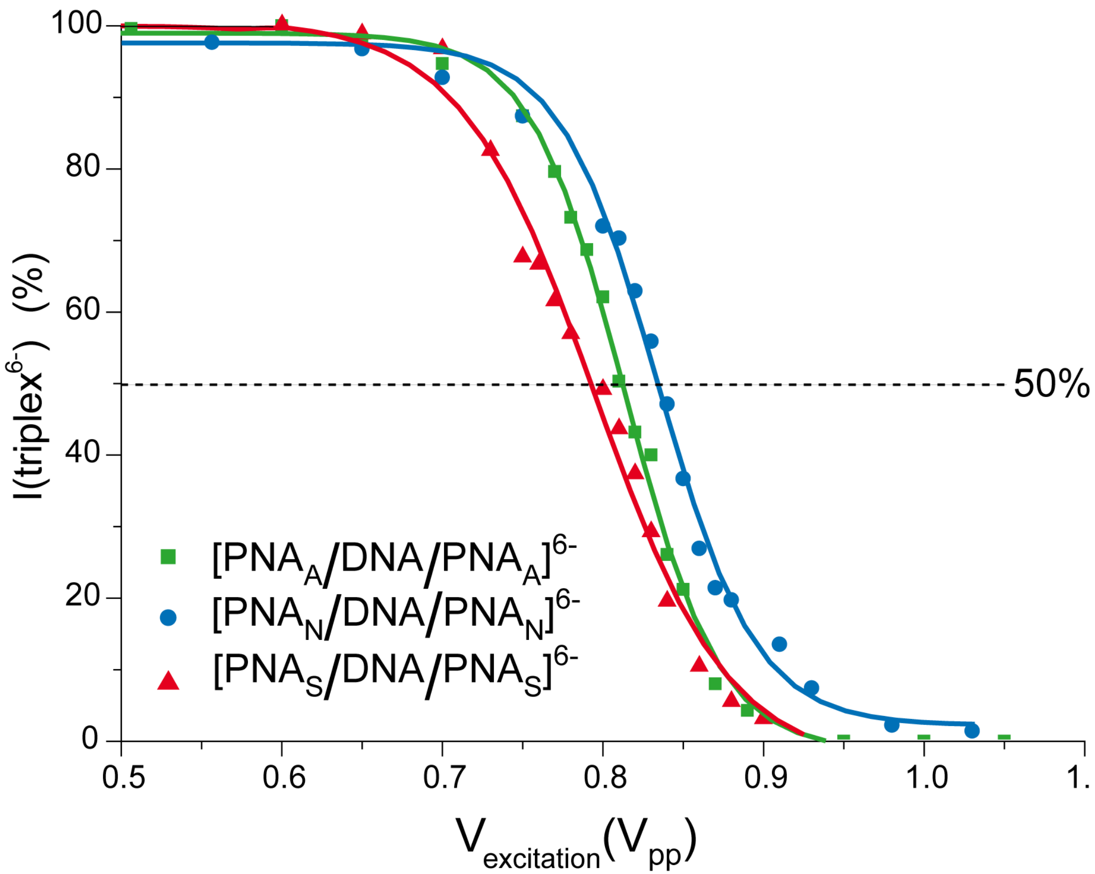


strand separation

16. Delvolvé A. et al. J. Mass Spectrom. 2006, 41, 1498.
17. Gabelica V. et al. J. Am. Soc. Mass Spectrom. 2002, 13, 91.

3.b.
Triplexes APN/ADN/APN

Gas and solution phase stability



$$ts^{6-}\,(\%) = \frac{[ts^{6-}]}{\left|[ts^{6-}] + [ds^{4-}] + [ss_{PNA}^{2-}]\right|}\, x\, 100$$

Ref. 18

triplex	Tm (°C)	V50% (V)
●	72	0,83
■	55	0,81
▲	50	0,79

18. Bregant S. et al. Eur. J. Org. Chem. 2001, 17, 3285.

4. Conclusions

<u>PNA/DNA duplexes:</u>
- **two fragmentation pathways** as a function of the **complex charge state,** as for DNA/DNA duplexes
- **no product ions** from the **PNA** strand
- **product ions** from the **DNA** strand

<u>PNA/DNA/PNA triplexes:</u>
- results **similar to PNA/DNA** duplexes
- a model : **zwitterions and coulombic repulsions**
- **similar stabilities** in the **gas phase** and in **solution** for the **studied triplexes**

4. Perspectives

Determination of PNA thermochemical parameters (acidity and proton affinity):
To better understand their reactivity.

Modeling the triplexes 3D structure:
To better understand their stability.

Exploration of other complexes by MS:
Validation of the zwitterion/coulombic repulsions model ?!

Use of other activation methods (ECD, EDD…):
Validation of the zwitterion/coulombic repulsions model ?!

Study of the relation between the solution and the gas phase:
To better understand these complexes in biological systems.

Acknowledgments

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