Non specificity of I-motif ligands and antibody demonstrated by Bio-Layer Interferometry (BLI)

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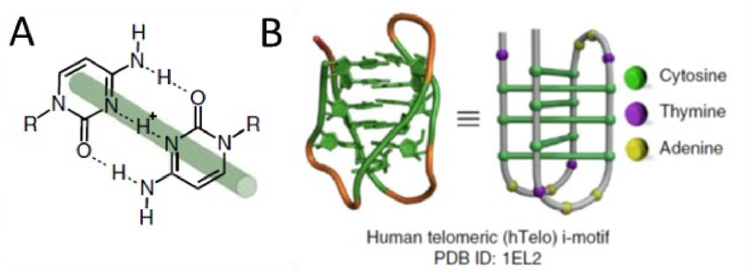
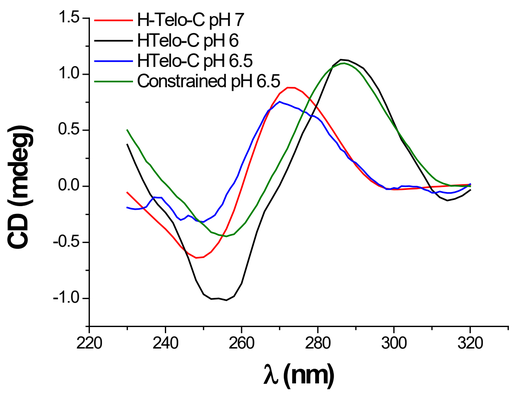
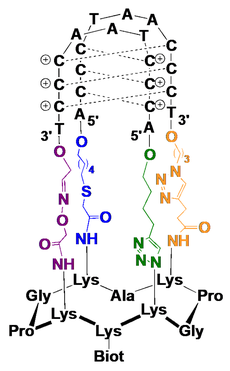


i-Motifs of DNA (hereafter, i-DNA), known *in vitro* for nearly three decades, are unusual, four-stranded structures, in which cytosines are intercalated *via* a stack of hemi-protonated C–C base pairs (CH+:C). Some of these structures have been well characterized *in vitro* and, because i-DNA may mirror other four-stranded G-rich structures (G-quadruplexes) present in gene promoters or at telomeres, their biological relevance is being investigated.

Constrained i-motif DNA

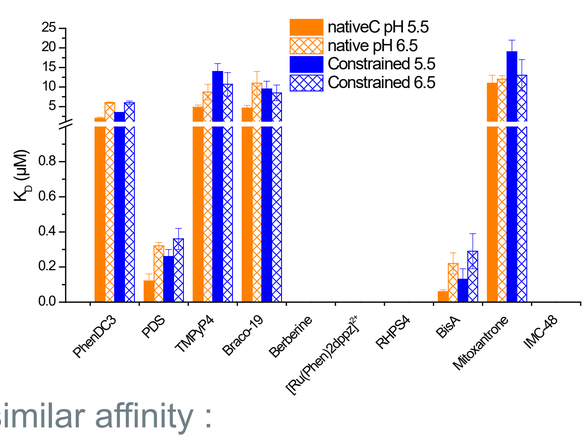


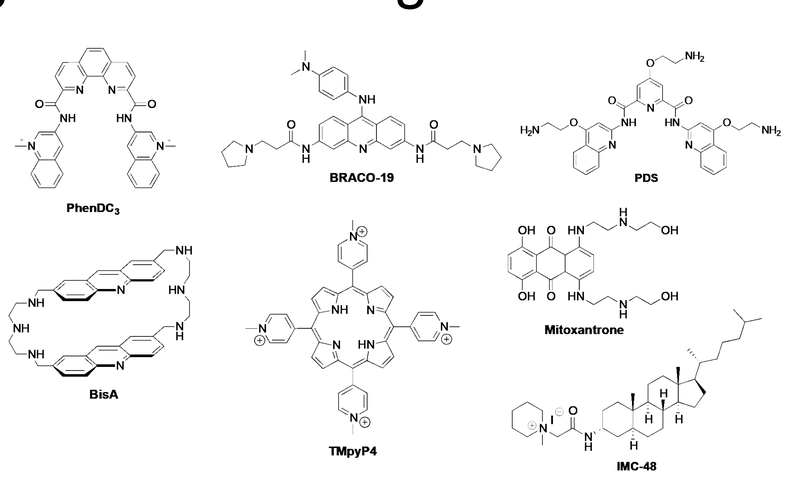
Instability of Htelo-C at neutral condition (pH ≥ 6.5).



Design of constrainsted Htelo-C base on cyclodecapeptide : **More stable**

I-motif Ligands Screening





similar affinity :

- with the reported ligands.
- with presumably folded (pH 5.5) and unfolded (pH 6.5) i-DNA sequences.
- for I-motif and duplex DNA.

A. Devaux, L. Bonnat, T. Lavergne, Eric Defrancq, Org. Biomol. Chem., ***2020****, 18, 6394-6406. https://dx.doi.org/10.1039/d0ob01311k.*

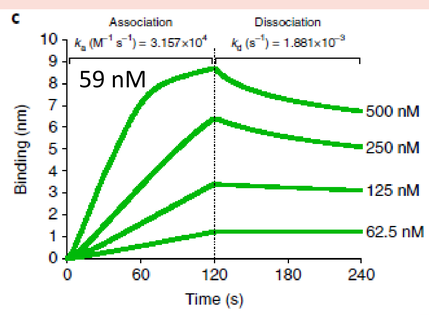
H. Bonnet, M. Morel, A. Devaux, J. Boissieras, A. Granzhan, B. Elias, T. Lavergne, J. Dejeu, E. Defrancq, Chem. Comm. ***2022****, 58, 5116. https://doi.org/10.1039/d2cc00836j.*

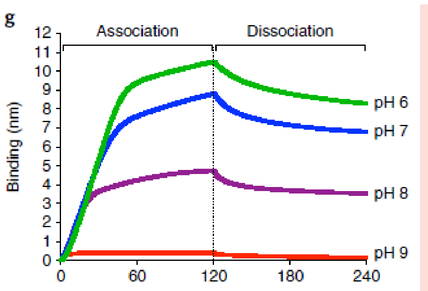
F. Berthiol, J. Boissieras, H. Bonnet, M. Pierrot, C. Philouze, J.-F. Poisson, A. Granzhan, J. Dejeu, E. Defrancq, Molecules ***2023****, 28, 682. https://doi.org/10.3390/molecules28020682*

I-mab Antibody

Previous works

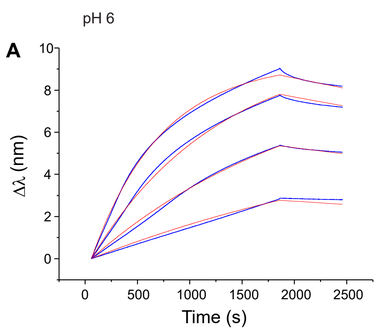
Christ et al., Nature Chemistry ***2018****, 10, 631*





Selectivity ?

Present works



Similar shape

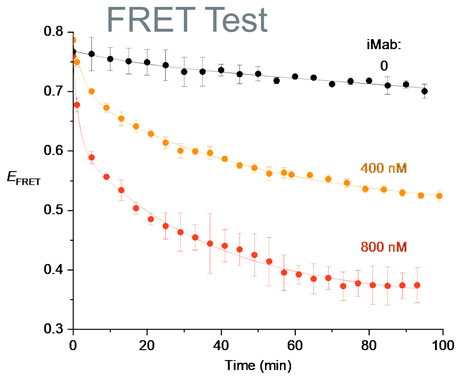
J. Boissieras, H. Bonnet, M. Fidelia Susanto, D. Gomez, A. Granzhan, E. Defrancq, J. Dejeu, Nucleic Acids Res. (2024) under review: 10.1101/2023.11.21.568054

iMab variant	pH 6.0			pH 7.5		
	k_{on} (10^3 M^{-1} s^{-1})	k_{off} (10^{-4} s^{-1})	K_D (nM)	k_{on} (10^3 M^{-1} s^{-1})	k_{off} (10^{-4} s^{-1})	K_D (nM)
scFv-His_6	3.7 ± 1.3	1.2 ± 0.0	**34 ± 10**	2.1 ± 0.5	2.4 ± 0.4	**108 ± 20**
scFv-His_6-FLAG	15 ± 6	2.7 ± 0.8	**20 ± 11**	8.1 ± 2	13 ± 3	**164 ± 40**
rabbit IgG	112 ± 27	0.1 ± 0.02	**0.1 ± 0.03**	48 ± 15	1.3 ± 0.3	**3 ± 1**

pH 6.5	k_{on} (10^3 M^{-1} s^{-1})	k_{off} (10^{-4} s^{-1})	K_D (nM)
hTeloC-27	11.0 - 11.4	5.4 - 6.7	49 - 58
Constrained	4.4 - 4.9	6.8 - 7.6	140 - 170

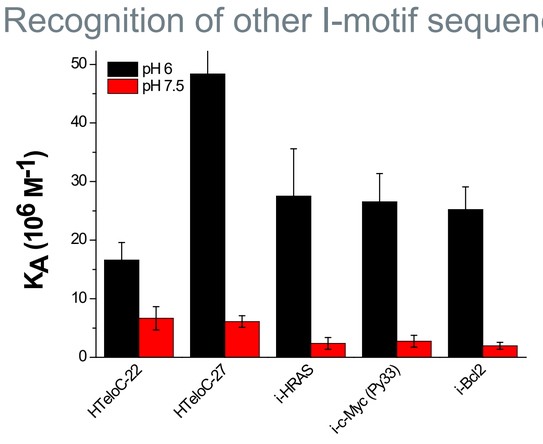
- Influence of the mAb variant
  - multivalency
- No influence of the pH
  - No specific to I-motif conformation
  - Recognition of the sequence?
  - Recognition of cytosine track?
  - Improve the folding ?

FRET Test

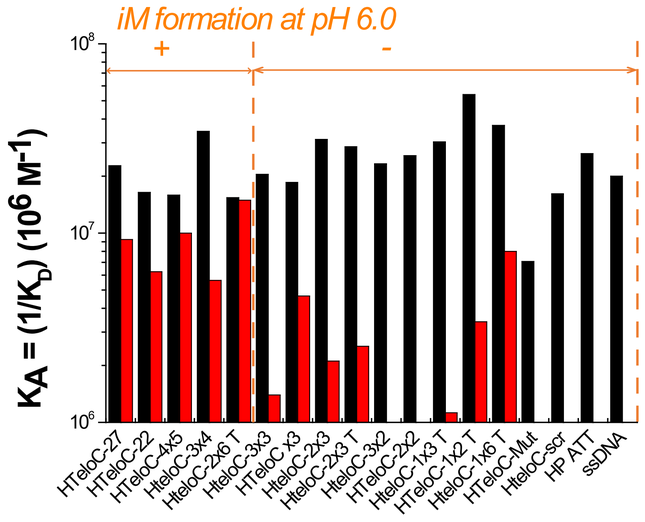


I-Mab unfolded I-motif

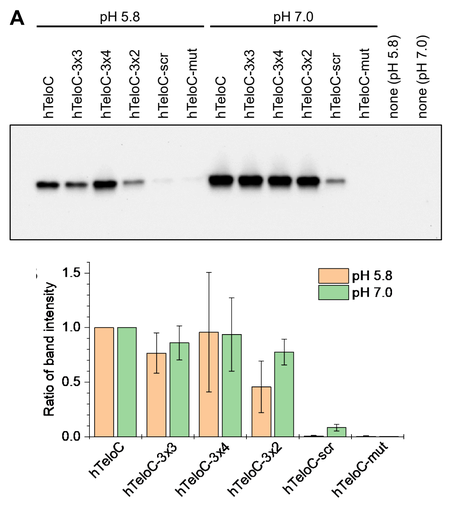
Recognition of other I-motif sequences



BLI



Pull Down Test



Recognition of no I-motif sequences

No “true” i-motif ligand and mAb !!

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