Introduction to the Scientific Community – Inorganic Chemistry

Investigation of Non-Canonical DNA with Metal Complexes by Advanced *in silico* Methods

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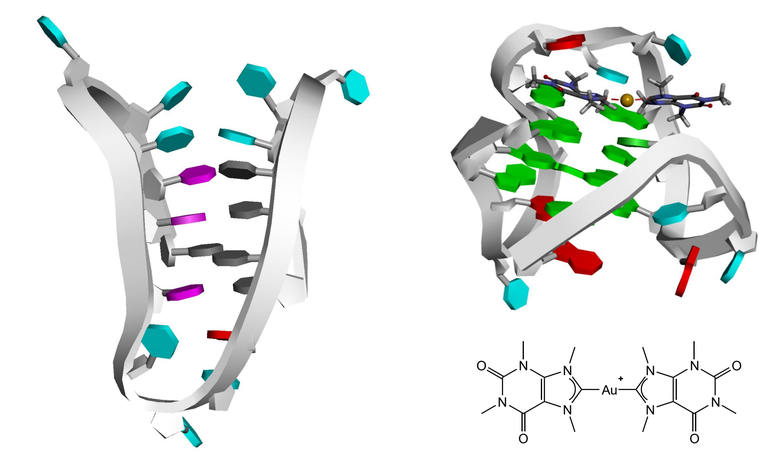
Introduction

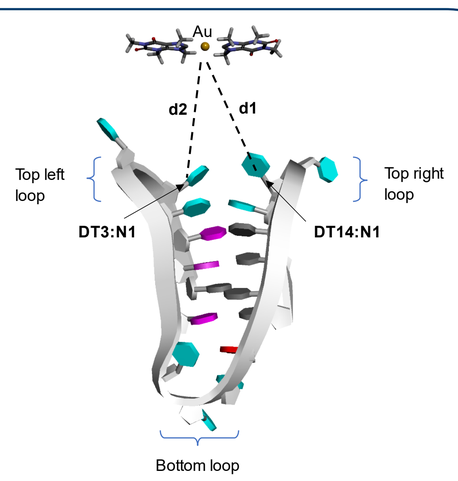
Besides the canonical DNA double helix, several non-canonical DNA secondary structures exist in the human body, two of which are the G4-quadruplex and the i-motif, both being complementary structures that can be found in certain gene promoter and telomeric DNA regions.[1] Influencing the stability of these DNA systems exerts an effect on the biological processes which they are involved in, making them an important target for small stabilizing molecules in regards to drug design.[2] So far, a stabilizing effect for the G4-quadruplex has been established computationally, as well as experimentally.[3] In this poster we present a computational study, using *in silico* Molecular Dynamics/ Metadynamics simulations, tackling the question, whether, and if so, to what extent the stabilization of a selected i-motif – a cytosine-rich non-canonical DNA structure – by the gold(I) NHC complex $AuTMX_2$ occurs.



i-motif DNA (left), G4 with $AuTMX_2$ (top right) and only the $AuTMX_2$ molecule (bottom right). DNA is represented as ribbons with polyhedra, where thymine is blue, cytosines are black and red, respectively, adenine is red, and guanine is green. $AuTMX_2$ is shown as ball and sticks (top right).

Computational Setup

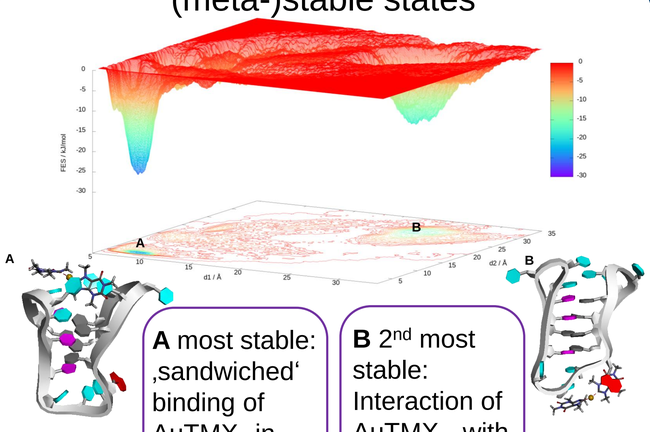
15 calculations were performed for either 100 ns or 150 ns to sample the complete trajectory and determine stable conformations through energy minima of the Free Energy Surfaces (FES) of i-motif/ $AuTMX_2$ interaction. Chosen reaction coordinates (CV) for the monitoring of the systems were the distances *d1* and *d2* between the Au(I) center and nitrogen atoms of thymine bases DT14 and DT3, respectively.



Motif setup for the MetaD calculations: i-motif with $AuTMX_2$ on top, CVs *d1* and *d2* are distances between N1 atoms of two thymine bases and the gold atom.

Metadynamics Simulations

Assignment of energy minima to (meta-)stable states



A most stable: ‚sandwiched' binding of $AuTMX_2$ in between top loops of DNA

B 2nd most stable: Interaction of $AuTMX_2$ with bottom loop of DNA

Summary of observed (meta-)stable conformations of the i-motif/ $AuTMX_2$ system

Binding mode	$\bar{x}(\Delta G) \pm \sigma$ [kJ/mol]	# of minima observed
1 'Sandwiched' stacking	-24.8 ± 7.3	8
2 Bottom loop	-15.6 ± 3.8	8
3 Top left loop	-13.3 ± 3.7	9
4 Top right loop	-14.7 ± 2.2	5
5 Parallel to long strand	-11.2 ± 1.5	3

5 (meta-)stable states identified, most stable ones are a π-stacking of $AuTMX_2$ in between the two top loops, as well as π-stacking from underneath the bottom loop of the i-motif DNA

Comparison with G4

Comparison of most stable conformations of i-motif/ $AuTMX_2$ (**S** – sandwiched, **B** – bottom loop) with most stable states of two G4/ $AuTMX_2$, one in telomeric (hTelo) and one in a gene promoter region (C-KIT1) of the DNA. [a] Binding constants calculated via $\Delta G = -RTln(K_b)$. For G4-quadruplexes, binding constants were derived from experimental ΔG_{exp}.

	i-motif	G4-quadruplex	
		hTelo[3]	C-KIT1[3]
Most stable minima	**S** -24.8 ± 7.3 kJ/mol **B** -15.6 ± 3.8 kJ/mol	**I** -37 ± 7 kJ/mol **II** -14 ± 3 kJ/mol	**I** -46 ± 3 kJ/mol **II** -12 ± 3 kJ/mol
K_b [a]	**S** $2.08 \cdot 10^4$ **B** $5.02 \cdot 10^2$	**I** $(4.36 \pm 0.49) \cdot 10^6$ **II** $(1.08 \pm 0.32) \cdot 10^2$	**I** $(2.96 \pm 0.82) \cdot 10^6$ -

Higher stabilization for interaction with G4-quadruplex: $AuTMX_2$ can planarly π-stack on top of the G4-guanine tetrad

i-motif: lower ΔG & weaker stabilization possibly by twisting of $AuTMX_2$-ligands out of planarity and the re-alignment of thymine bases of the top loops

Twisting out of planar geometry of $AuTMX_2$' caffeine ligands

Folding down of thymine bases for π-interaction with $AuTMX_2$

Conclusion

The potential of the non-canonical i-motif DNA as drug target for stabilizing molecules has been assessed with the *in silico* Molecular Dynamics/Metadynamics approach. For this, the selectively G4 stabilizing Au(I) NHC complex $AuTMX_2$ was used for the calculations of its stabilizing effect on the i-motif. The simulations rendered two distinct conformations, a stacking of $AuTMX_2$ in between the two top loops of the i-motif, as well as a stacking from underneath the bottom loop as the most stable and second most stable states, respectively. Values for the free energy ΔG, quantifying the stabilization, could not surpass the values found for the previously studied stabilization of the G4-quadruplex. Still, these findings pave the way towards further studies, possibly with other stabilizers, making the i-motif interesting for drug targeting.

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[2] Y. Chen, K. Qu, C. Zhao, L. Wu, J. Ren, J. Wang, X. Qu, *Nat. Commun*. **2012**, *3*, 1074.
[3] D. Wragg, A. de Almeida, R. Bonsignore, F. E. Kühn, S. Leoni, A. Casini, *Angew. Chem. Int. Ed.* **2018**, *57*, 14524.