Enzymatic construction of metallo-DNA

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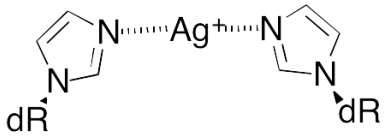
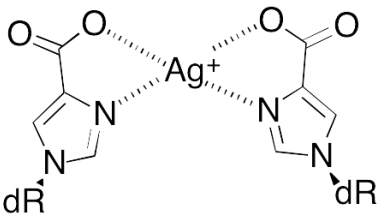
The expansion of the genetic alphabet using an artificial base pair is of high relevance in synthetic biology and could augment nucleic acid functionalities by increasing their components. Efforts in this context have produced several types of unnatural base analogs that are well tolerated by enzymes *in vivo* and *in vitro* during replication and transcription. Surprisingly, the enzymatic formation of artificial metal base pair has been vastly under-explored. These base pairs are interesting candidates for the expansion of the genetic alphabet since they are fully orthogonal to the natural Watson-Crick base pairs and marginally distort the duplex structure. Apart from the metal mediated incorporation of of **T-Hg-T** [1] or **C-Ag-T** [2] there are only a very few examples of fully orthogonal incorporations such as the **Sal-Cu-Sal** [3] or the **Pur^{DC}-Cu-3-Py** [4]. Herein, we investigated on the potential incorporation of imidazol (**dIm**) base analogs since they are known to have beneficial thermodynamic properties and only marginally distort the duplex structure.



dIm **dIm**



dClm **dClm**

A biochemical analysis allowed to show us that templated incorporation of **dImTP** occurs selectively and this analog has potential to act as an orthogonal nucleotide. However, the incorporation occurs also under metal free conditions and shows limitations regarding multiple inclusions of **dImTP** [5]. Thus, thermodynamic and minimal structural changes to the duplex are not the only factors to be considered when designing the enzymatic construction of orthogonal metal base pairs. A second generation imdiazol base analog bearing an additional carboxylic metal coordinating site (**dClm**) was synthesized to better understand the enzymatic incorporation of metal base pairs. Currently the acceptance of ligases and terminal transferases for metalllo base nucleotides is analyzed in order to study the formation of long stretches of DNA-metal chains or develop polyimidazol tags with a high affinity to metal cations [6].

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