Molecular Dynamics Simulations of the tRNA with Modified Bases

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Basic Explanation of Nucleic Acids

1 Nitrogenous Base + 1 Sugar (ribose/deoxyribose) + 1 Phosphate = 1 Nucleotide

Nucleoside (monomer)

4 * Nucleoside = RNA or DNA strand (polymer)
RNA Structure
Modified Bases

If it’s not

adenine

uracil

guanine

cytosine

then it’s modified.

2-methylthioadenine

2-thiouracil

5-methylguanine

3-methylcytosine
Central Dogma of Molecular Biology
tRNA Secondary and Tertiary Structure

Human tRNA$_{\text{Lys},3}$
Reading the Blueprint
Modified Bases in tRNA

- Adenosine
  - 2-methylthio-N6-threonylcarbamoyl adenosine ($ms^{2}t^{6}A$)
  - 2-thiomethyl-N6-dimethylallyl adenosine ($ms^{2}l^{6}A$)
- Pseudouridine ($\psi$)
- 5-methoxycarbonylmethyl-2-thiouridine ($mcm^{5}s^{2}U$)
- Dihydouridine (D)
Human ASL$^{\text{Lys,3}}$ Requires Modified Bases for Binding

- Three modified bases are present in the anticodon-stem loop of human tRNA at positions 34, 37, and 39:
  - $\text{m}^{2}\text{t}^{6}\text{A}$ and $\text{mcm}^{5}\text{s}^{2}\text{U}$ are required to achieve wild-type binding.
Sequences of Constructs and Relative Binding Affinities

Molecular Dynamics: Overview

- Simulation of molecular motion over time
- Time dependent structural dynamics at atomic resolution
  - hydrogen bonding patterns
  - stacking patterns
  - ion binding
  - helical parameters
Molecular Dynamics: Mathematics

Newton’s Equation:

\[ F_i(t) = -\frac{\partial U(r^N_{i})}{\partial r_i} = m_i a(t) \]

Potential Function:

\[ U(r) = \sum_{\text{bonds}} K_r (r - r_{eq})^2 + \sum_{\text{angles}} K_\theta (\theta - \theta_{eq})^2 + \]

\[ + \sum_{\text{dihedrals}} \frac{V_n}{2} (1 + \cos[n\phi - \gamma]) + \]

\[ \sum_{\text{atoms}} \left[ \frac{a_{ij}}{r_{ij}^{12}} - \frac{b_{ij}}{r_{ij}^6} \right] + \sum_{\text{atoms}} \frac{q_i q_j}{\varepsilon r_{ij}} \]

Cornell et al., J. Am. Chem. Soc. 1995

Cheatham et al., Biomol. Struct. Dyn. 1999
Structures of the $\text{ASL}^{\text{Lys},3}$

**X-ray Structures**

Bénas et al., RNA, 2000

Murphy et al., Nat. Struct. Biol., 2004

**NMR Structure**

Stuart et al., Biochemistry, 2000
ms²t⁶A Prevents Displacement of U36

- without the ms²t⁶ modification, A37 displaces U36 and stacks in its place
Quantifying Displacement of U36

- Solvent accessible surface area (SASA) of U36 (only the 6-membered ring) was measured.
- When U36 was pushed out into solution, SASA rose significantly.

<table>
<thead>
<tr>
<th>ASL&lt;sub&gt;Lys,3&lt;/sub&gt;</th>
<th>SASA of U36 (Å²)</th>
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<tr>
<td>tRNA&lt;sup&gt;Lys,3&lt;/sup&gt; mcm&lt;sup&gt;6&lt;/sup&gt;s&lt;sup&gt;2&lt;/sup&gt;U34, ms&lt;sup&gt;2&lt;/sup&gt;t&lt;sup&gt;6&lt;/sup&gt;A37, ψ39 (wt)</td>
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<tr>
<td>tRNA&lt;sup&gt;Lys,3&lt;/sup&gt; (unmodified)</td>
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The Anticodon Exhibits a Stair Stepped Conformation
Quantifying Stair Stepped Configuration of Anticodon

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<th>ASL&lt;sup&gt;Lys,3&lt;/sup&gt;</th>
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Classification:

- Distance vector was decomposed into Cartesian coordinates and compared against wild type configuration.
- Vectors normal to the uridine ring planes in the anticodon were used to determine if bases were parallel.
Pseudouridine Facilitates the Formation of a Water Bridge

- In the presence of pseudouridin a water bridge forms between $\psi_{39}$ NH1 and A38 O2P through hydrogen bonding interactions
Quantification of Water Bridge

- A water bridge was considered present when:
  - hydrogen bonds existed simultaneously between a water, A38 O2P, and any hydrogen bonding donor or acceptor on U39 or NH1 if \( \psi_{39} \) was present
  - there were \( \leq 3 \) ps between consecutive interactions

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Summary

- tRNA is the bridge between nucleic acids and proteins
- Modified bases are required for human tRNA\(^{\text{Lys,3}}\) to bind to its cognate codon.
- In the absence of the ms\(^2t^6\) modification at the 37th position, adenine displaces U36 in the anticodon face.
- Pseudouridine at the 39th position facilitates the formation of a water bridge between itself and the phosphate backbone at A38.
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