AI-Assisted

Cap Analog Discovery Platform

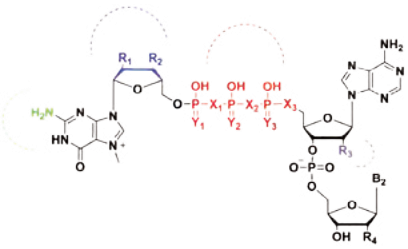
ARETERNA
A Synthgene Company

Areterna's AI-Assisted Cap Analog Discovery platform is the engine of innovation from which novel structures like CAP4 and CAP5 were constructed. Integrating a design-build-test-learn machine learning process, Areterna's Cap Discovery platform is comprised of five key steps.

1. AI-Assisted Structure Design
2. Molecular Docking Simulation
3. Result Analysis
4. Candidate Molecule Synthesis
5. Candidate Molecule Performance Testing

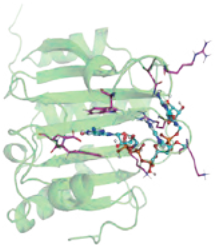
Step 1: AI-Assisted Structure Design

At the nucleus of our platform is the AI-assisted structure design, a starting point for our cap analog discovery journey. Leveraging advanced algorithms, we initiate the process with a predefined structure on the right. The AI then takes the reins, generating diverse in-silico variations. This step forms the bedrock of our approach, providing a multitude of potential candidates for further exploration.



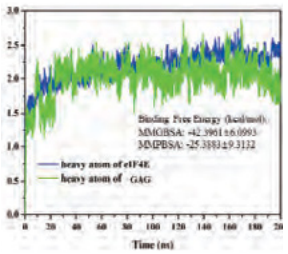
Step 2: Molecular Docking Simulation

With a plethora of in-silico designs in hand, the next step is the molecular docking simulation. Here, the small molecules designed in the previous step are subjected to rigorous scrutiny. Through intricate simulations, these molecules are fitted with the eIF4E protein, unraveling insights into their binding strength and interaction dynamics. This crucial step serves as a filter, identifying molecules with the highest potential for success.



Step 3: Result Analysis

The data generated from the molecular docking simulations undergoes a meticulous analysis. Molecules are ranked based on their binding strength to eIF4E, and this system becomes our compass guiding us toward the most promising candidates.



Step 4: Candidate Molecule Synthesis

Armed with the knowledge of the top-ranked molecules, we transition to the synthesis phase. However, practicality is a key consideration. If the molecules prove challenging to synthesize, they are deemed of lower commercial value and are consequently dropped from the pool. On the contrary, easily synthesizable candidates move forward for performance testing, setting the stage for the ultimate assessment of their efficacy.

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Step 5: Candidate Molecule Performance Testing

The culmination of our journey is the performance testing of selected molecules. Our candidates undergo a series of evaluations, beginning with mRNA synthesis. The cap analogs are used in IVT reactions and IVT parameters such as yield, purity, capping efficiency, and dsRNA content are collected. The table below shows data from 20 µl IVT reactions where CAP4 and CAP5 demonstrated comparable performance to the benchmark m7GpppAmG.

Cap Analog	mRNA Yield (µg)	mRNA Integrity	Capping Efficiency	dsRNA Ratio
m7GpppAmG	183	90.20%	98.50%	0.04%
CAP 4	187	91.30%	99.10%	0.03%
CAP 5	184	89.50%	98.90%	0.04%

Molecules demonstrating robust performance are further tested for their resistance to decapping enzymes. The table below shows the percentage of mRNA with cap on after one hour incubation with decapping enzymes. CAP4 and CAP5 clearly outperformed the benchmark m7GpppAmG.

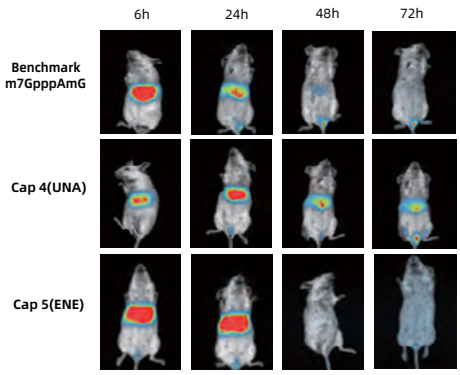
Cap Analog	Remaining Cap% -1	Remaining Cap% - 2	Remaining Cap% -3
m7GpppAmG	22.50%	19.30%	28.40%
CAP 4	77.30%	82.50%	84.00%
CAP 5	35.90%	35.20%	34.40%

The final candidates are tested for protein expression in cells using GFP construct. Finally, luciferase mRNAs are made with cap analogs and injected into mice to assess protein expression in-vivo.

The mRNA capped with m7GpppAmG showed strong luciferase signal at the 6-hour time point, but the luciferase signal was almost all gone at 48 hours. In contrast, CAP4 mRNA showed weaker expression at 6 hours, but the protein expression impressively persisted beyond 72 hours. CAP5 mRNA gave the highest protein expression initially among the 3 constructs and the strong luciferase signal carried from 6 hours to 24 hours, then died down at 48 hours.

Based on the performance data, we launched CAP4 and CAP5 as commercial products.

These and other performance data with related molecular structures will be fed into the AI system to train the model for future development. In essence, our AI-assisted cap analog discovery platform provides an engine of innovation. We will not stop at CAP4 and CAP5, more novel cap analogs will come out of the pipeline in the future.



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