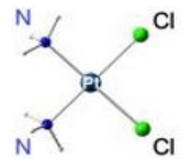
Visualizing Translation Initiation Under Stress

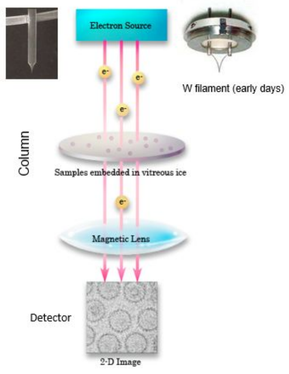
Inhibition of initiation by cisplatin

Cisplatin is a widely used anticancer drug that primarily cross-links DNA, thus suppressing cell growth. We hypothesised that cisplatin also binds ribosome RNA and inhibits translation. We studied how cisplatin affects the structure and dynamics of ribosomes. It has been shown that cisplatin is able to bind bacterial ribosomes, but this has not been studied in eukaryotes. We decided to study this issue because the therapeutic effects exerted by cisplatin on ribosomes may be the dominant of the two mechanisms.



We used **cryo-electron microscopy (cryo-EM),** a powerful method that allows to peer into the world of molecules, see their structure in a natural state, and even monitor their function in time. This method revolutionized structural biology and was awarded the Nobel Prize in Chemistry in 2017. Cisplatin is a widely used chemotherapeutic drug that binds to DNA. By assumption it also could bind to RNA, to ribosomes.

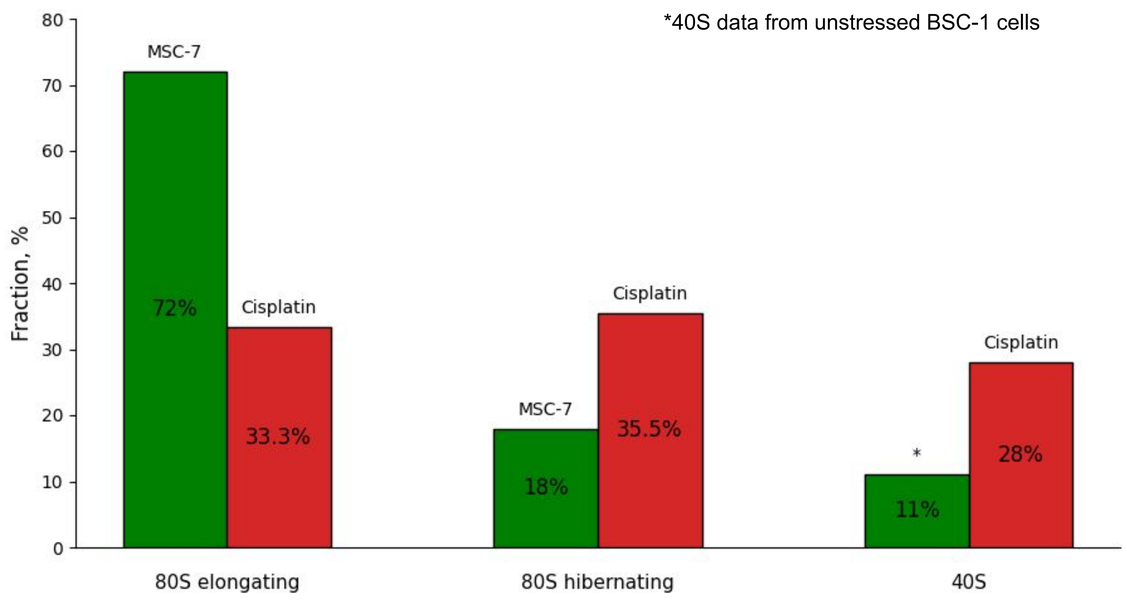




First, we localized ribosomal particles in cryo-EM micrographs from cisplatin-treated human cancer cell line MCF-7. Using two-dimensional template matching (2DTM), we identified candidate ribosomal particles by matching the experimental images to projections of a 40S ribosomal subunit structure.

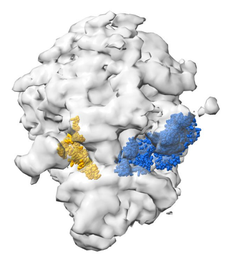


We used cisTEM to classify the particles into eight distinct 3D classes based on their structural similarity. Each class represents a structural state of the ribosome, corresponding to a distinct stage or intermediate of the translation process.

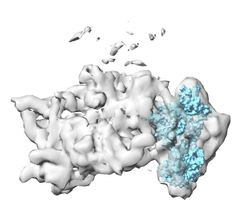




APE-site tRNA (red, purple, yellow) + eEF1α(green)



E-site tRNA (yellow) + eEF2(blue)



43S + eIF3(light blue)

We found differences between treated and normal cells in abundance of different stages of translation. Especially the amount of hibernating ribosomes is strongly increased in the cisplatin cells. Moreover, there are many more 40S subunits which are not bound to 60S subunit. This suggests that cisplatin strongly inhibits translation initiation.

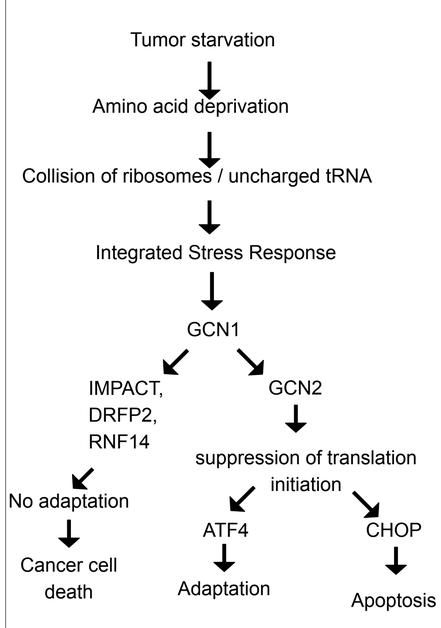
Initiation regulation by GCN2 competitors

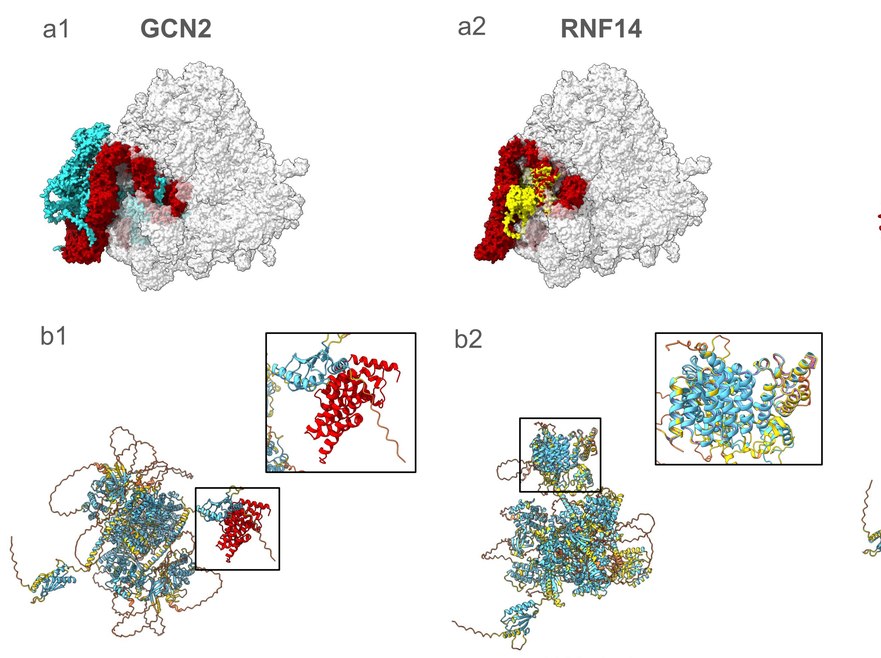
Background: Chemotherapy, immune pressure and tumor disorganization starve cancer cells of nutrients, especially amino acids. Cells adapt through the Integrated Stress Response, initiated by GCN2.

Problem: The competitors of GCN2, such as IMPACT, DRFP2 and RNF 14, lack experimental structures and it is unknown whether they bind GCN1 competitively.

Results: These proteins bind *in silico* the same position and the same amino acids of the GCN1 domain, though the predicted strength of binding differs, indicating potential competition.

Methods: AlphaFold modeled the GCN2, IMPACT, DRFP2 and RNF14 complexes with RWDBD domain of GCN1, as identified using ConSurf. We validated the models by identifying residues critical for binding and comparing clash counts between competitors.

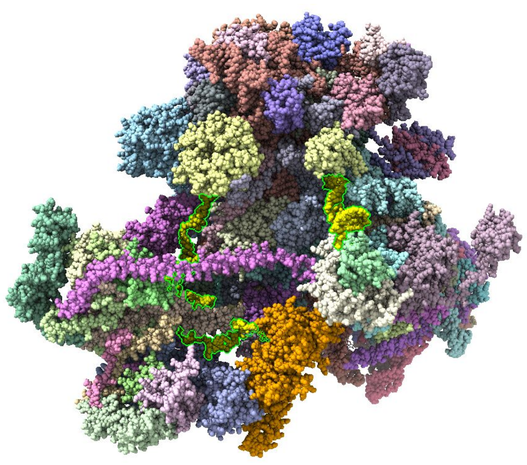




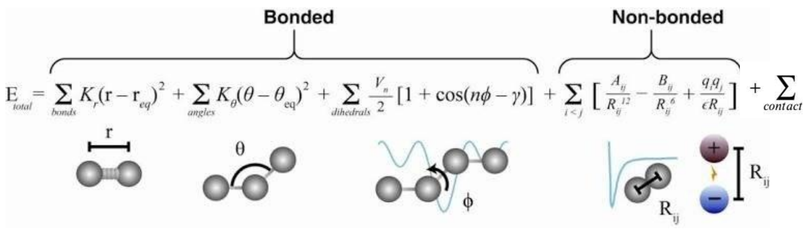
a) How the proteins can possibly look in ribosome (gray), bound to GCN1 (red); b) The clashes of competing proteins with GCN2, bound to RWDBD domain of GCN1

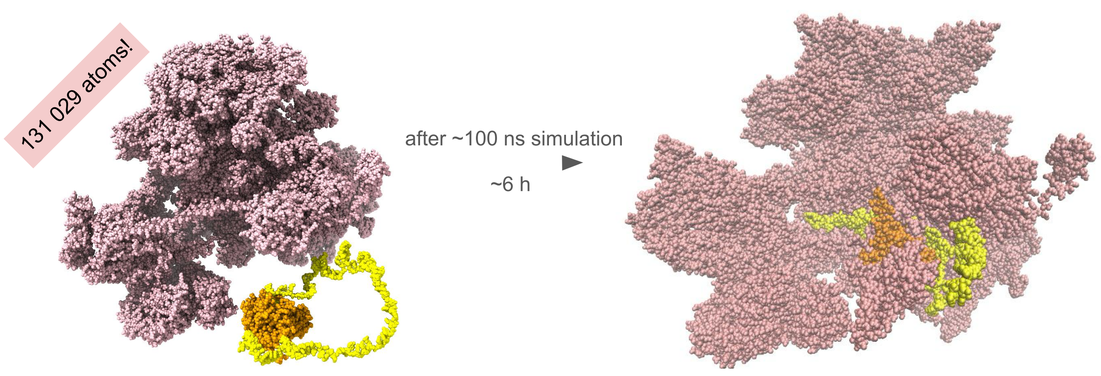
How does mRNA bind the 40S subunit to start translation?

A key question in translation initiation is how the mRNA, recruited by the cap-binding complex eIF4F, is positioned and threaded into the mRNA-binding channel of the 43S preinitiation complex (PIC) to form the 48S initiation complex.



We made a structure for the simulation using the 6ZMW (PDB) as a template. We extracted the capped mRNA together with eIF4F from the ribosome and converted the resulting structure into a SMOG-2 model, to test in an MD simulation whether and how it can find its way back into the ribosomal mRNA channel.

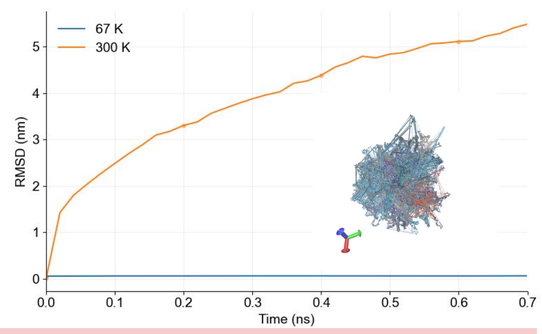




We reviewed the literature, optimized the parameters, and applied biasing forces to direct the mRNA into the channel of the small subunit. Several parameter combinations were tested in GROMACS.

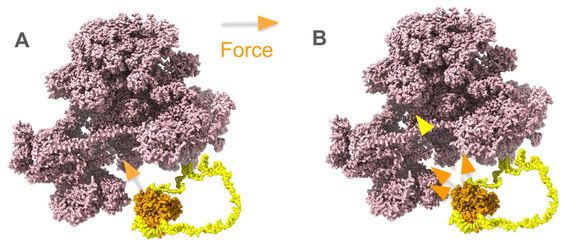
At 300 “K” (in the simulation’s reduced temperature scale), the structure became unstable and disintegrated. Lowering the temperature to 60–70 K stabilized the structure.

$$\mathrm{RMSD}(\mathbf{v},\mathbf{w}) = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\|v_i - w_i\|^2}$$



We implemented two biased MD sampling schemes: (A) steering just eIF4F toward its eIF3 contacts, and (B) adding an additional bias that guides the first mRNA nucleotides toward the entry of the mRNA channel in the preinitiation complex.

In the first nanoseconds of biased sampling, eIF4F docked into a near-native position relative to eIF3; the 5′-proximal nucleotides of the mRNA were guided toward near-native positions in the channel.



eIF3A arm movement appears unnecessary for eIF4F docking and mRNA engagement. Further simulations may provide more insight into translation-initiation dynamics.

