RNA is special

what (else) could possibly go wrong?

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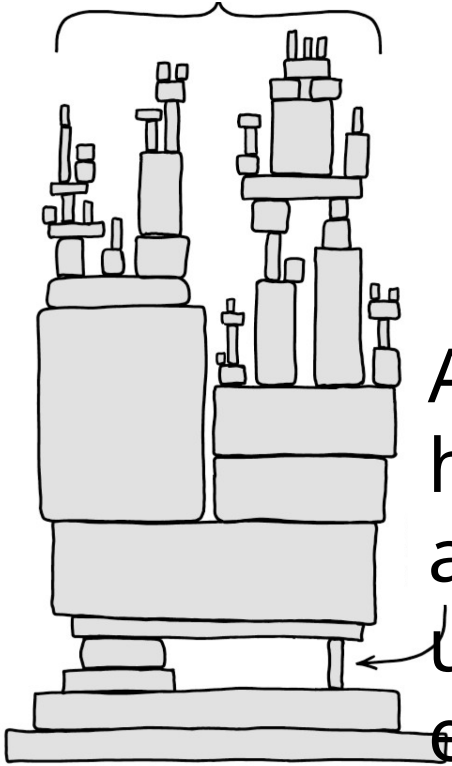
Outline

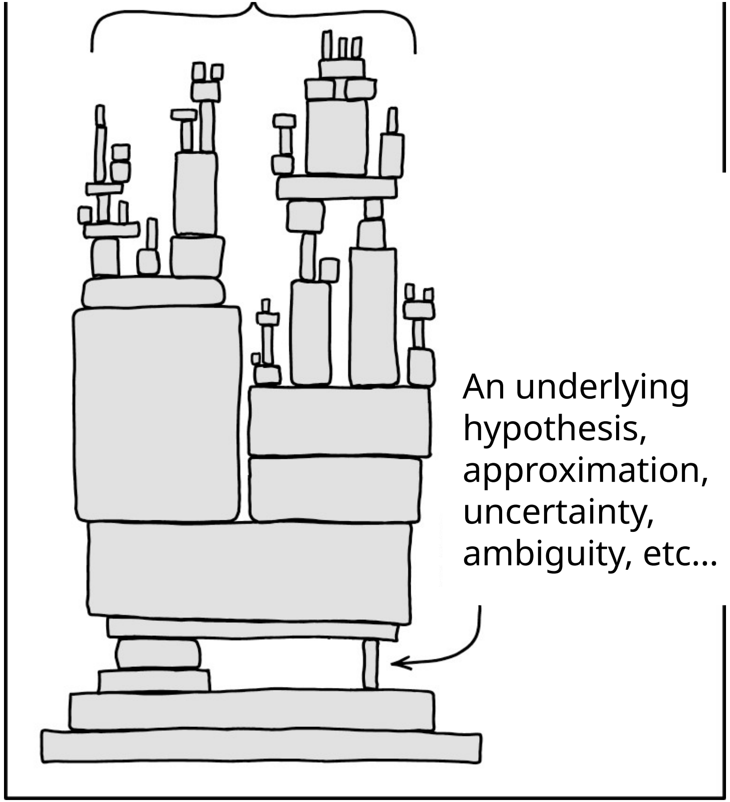
- Dependencies in research
- Importance of RNA structure/dynamics in function
- CASP16 - RNA targets
- How to help a model
- Downstream tasks

Your



An underlying hypothesis, approximation, uncertainty, ambiguity, etc…

Your

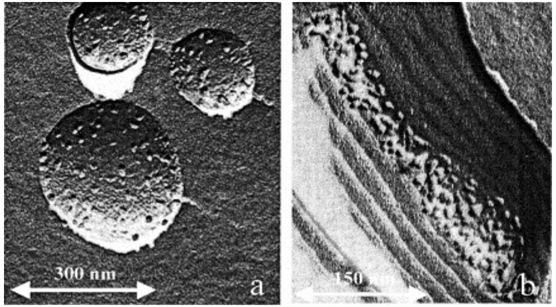


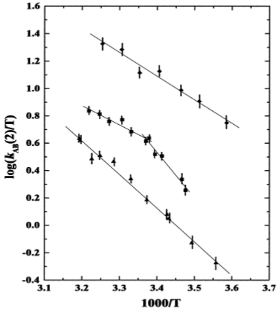
An underlying hypothesis, approximation, uncertainty, ambiguity, etc...

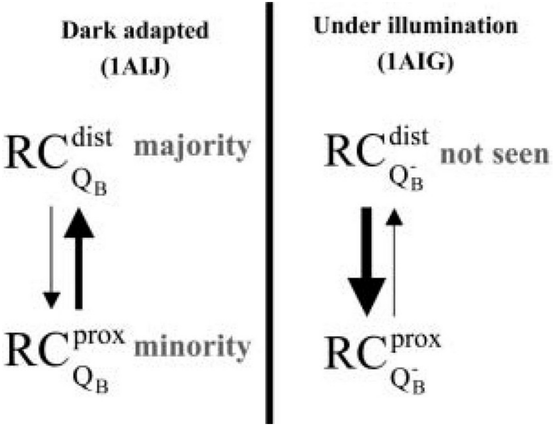
- These weaknesses are often associated with choices

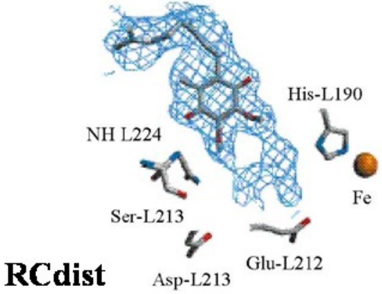
- These weaknesses need to be acknowledged
  [for the reader and for you]

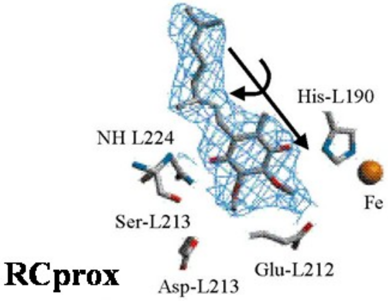
Study of the photosynthetic reaction center with a Poisson-Boltzmann approach





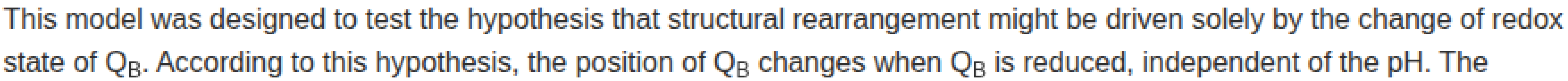




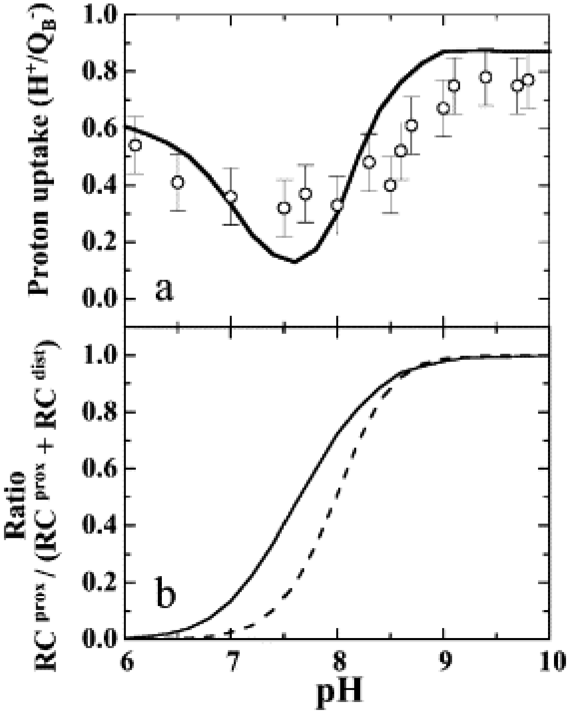


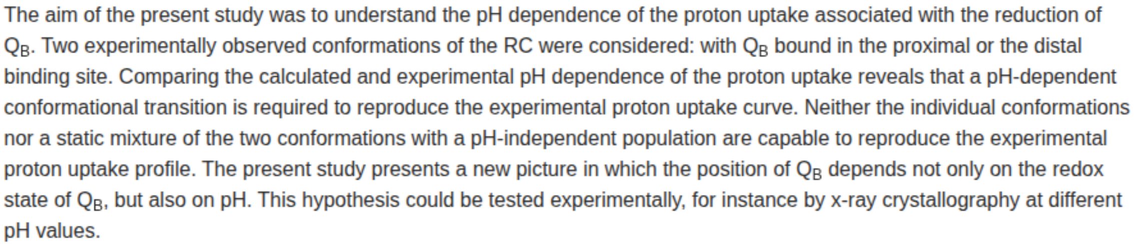
Taly, A., Sebban, P., Smith, J. C., & Ullmann, G. M. (2003). The position of QB in the photosynthetic reaction center depends on pH: a theoretical analysis of the proton uptake upon QB reduction. *Biophysical journal*, *84*(3), 2090-2098.

Conformational change as a function of pH



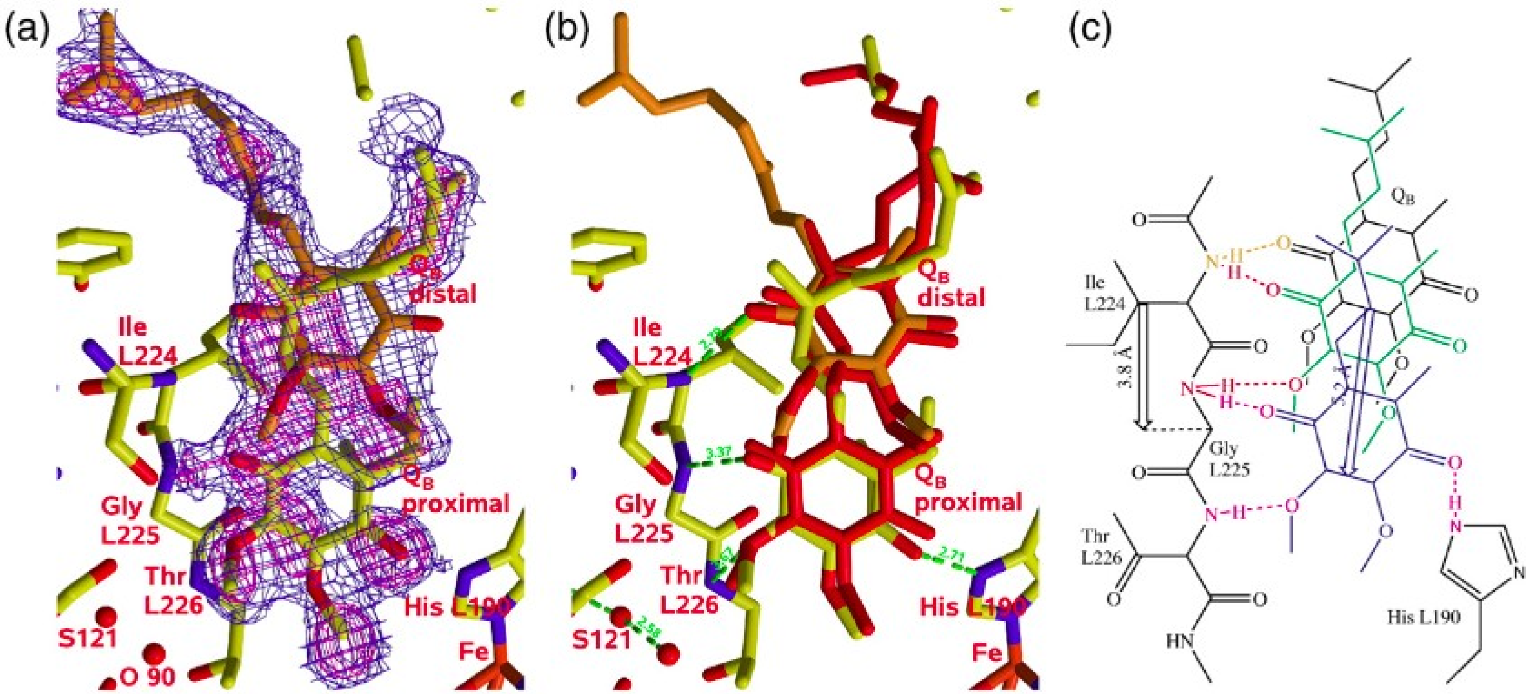
This model was designed to test the hypothesis that structural rearrangement might be driven solely by the change of redox state of Q_B. According to this hypothesis, the position of Q_B changes when Q_B is reduced, independent of the pH. The





The aim of the present study was to understand the pH dependence of the proton uptake associated with the reduction of Q_B. Two experimentally observed conformations of the RC were considered: with Q_B bound in the proximal or the distal binding site. Comparing the calculated and experimental pH dependence of the proton uptake reveals that a pH-dependent conformational transition is required to reproduce the experimental proton uptake curve. Neither the individual conformations nor a static mixture of the two conformations with a pH-independent population are capable to reproduce the experimental proton uptake profile. The present study presents a new picture in which the position of Q_B depends not only on the redox state of Q_B, but also on pH. This hypothesis could be tested experimentally, for instance by x-ray crystallography at different pH values.

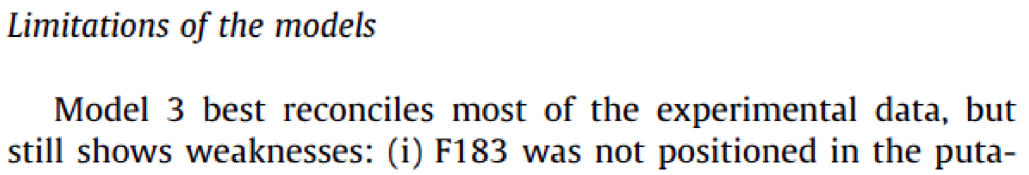
Experimental testing



pH modulates the quinone position in the photosynthetic reaction center from rhodobacter sphaeroides in the neutral and charge separated states Koepke J., Krammer E.-M., Klingen A.R., Sebban P., G.-M. Ullmann & G. Fritzsch *J. Mol. Biol.* **371**:396-409 (2007)

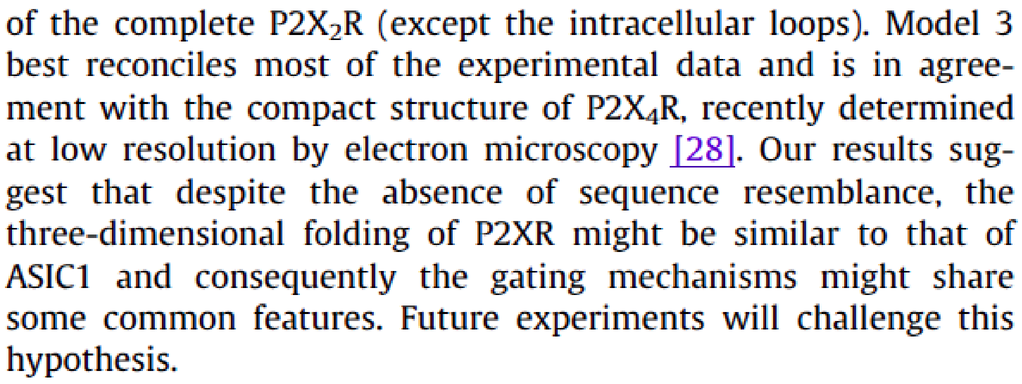
An explanation for this discrepancy is that by Taly *et al.*,[8] the semiquinone was not considered as protonatable in the illuminated state. However, as shown in Figure 5(d), the protonation state of the semiquinone Q_B influences its own binding position. Neglecting this influence will therefore modify the calculated proximal population.

A realistic model may be wrong



Limitations of the models

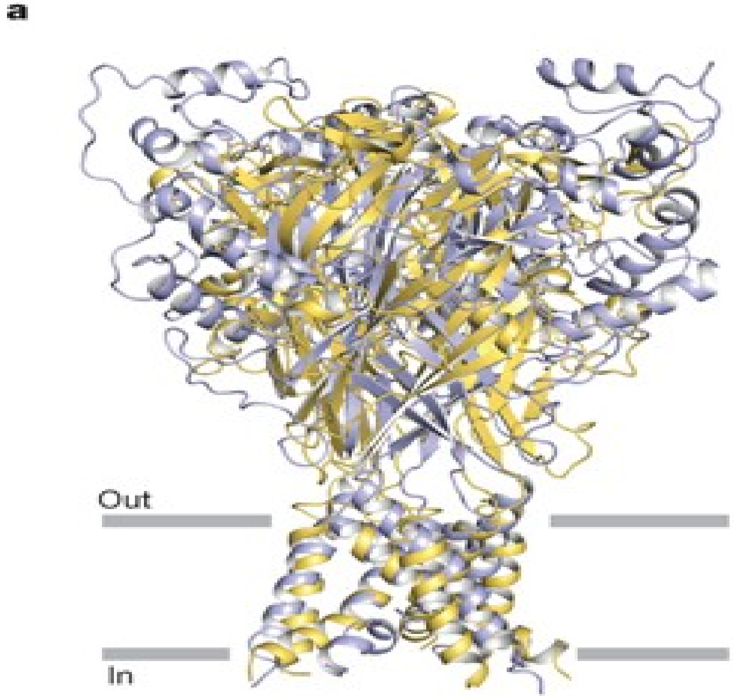
Model 3 best reconciles most of the experimental data, but still shows weaknesses: (i) F183 was not positioned in the puta-



of the complete $P2X_2R$ (except the intracellular loops). Model 3 best reconciles most of the experimental data and is in agreement with the compact structure of $P2X_4R$, recently determined at low resolution by electron microscopy [28]. Our results suggest that despite the absence of sequence resemblance, the three-dimensional folding of P2XR might be similar to that of ASIC1 and consequently the gating mechanisms might share some common features. Future experiments will challenge this hypothesis.

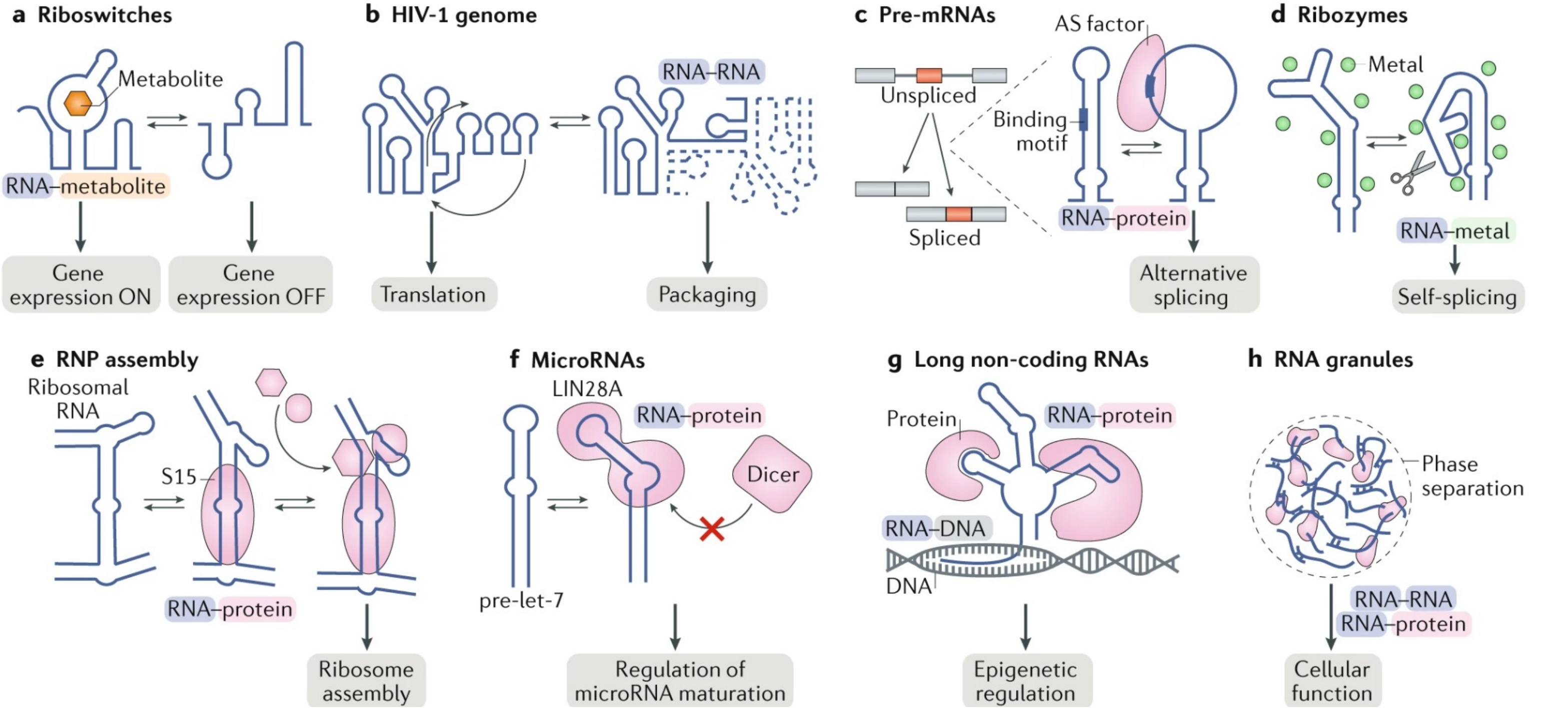
Guerlet, G., Taly, A., de Carvalho, L. P., Martz, A., Jiang, R., Specht, A., ... & Grutter, T. (2008). Comparative models of P2X2 receptor support inter-subunit ATP-binding sites. *Biochemical and biophysical research communications*, *375*(3), 405-409.

A realistic model may be wrong

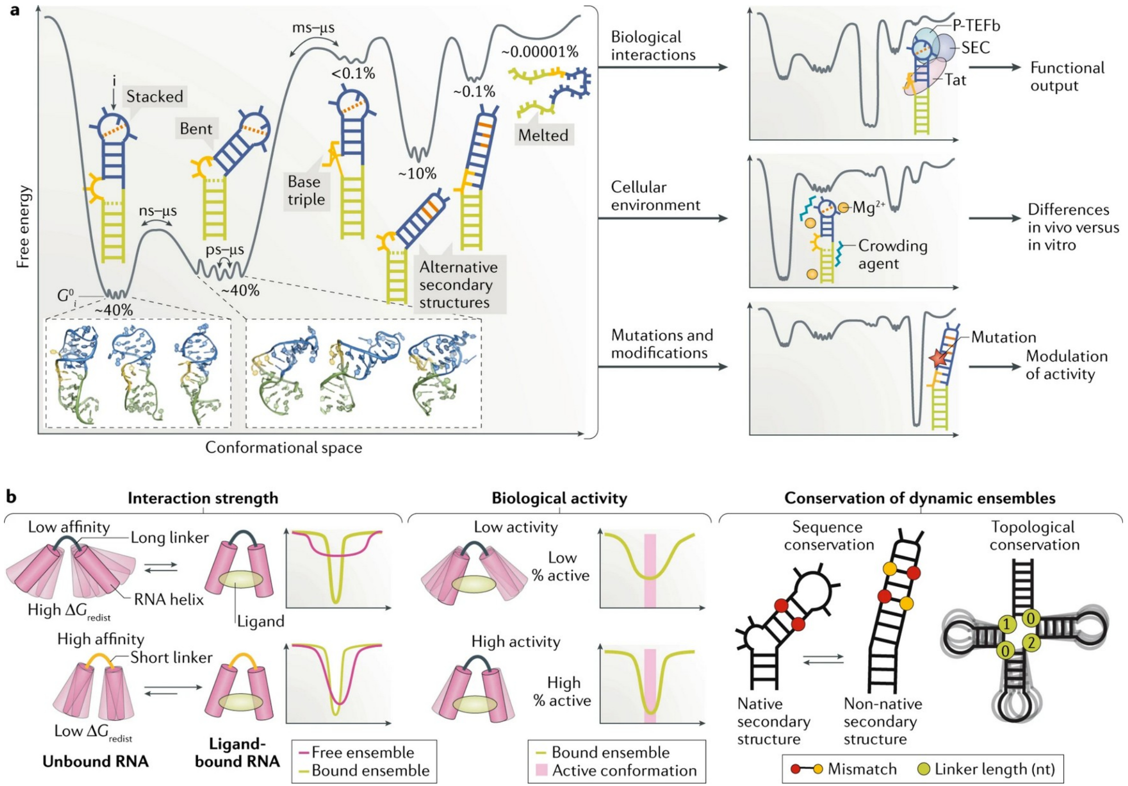


The important of RNA structure in function

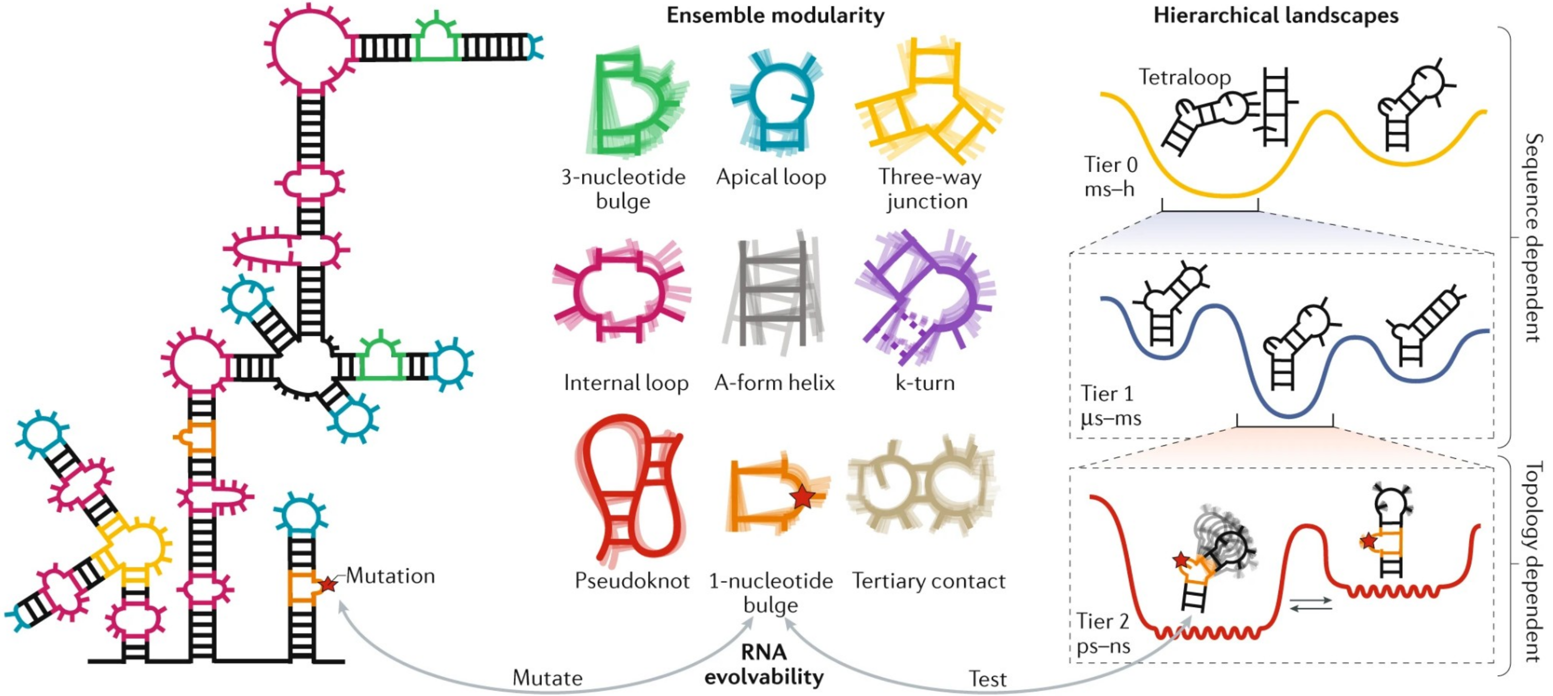
The importance of RNA structure in function



Ganser, L.R., Kelly, M.L., Herschlag, D. *et al.* The roles of structural dynamics in the cellular functions of RNAs. *Nat Rev Mol Cell Biol* 20, 474–489 (2019).

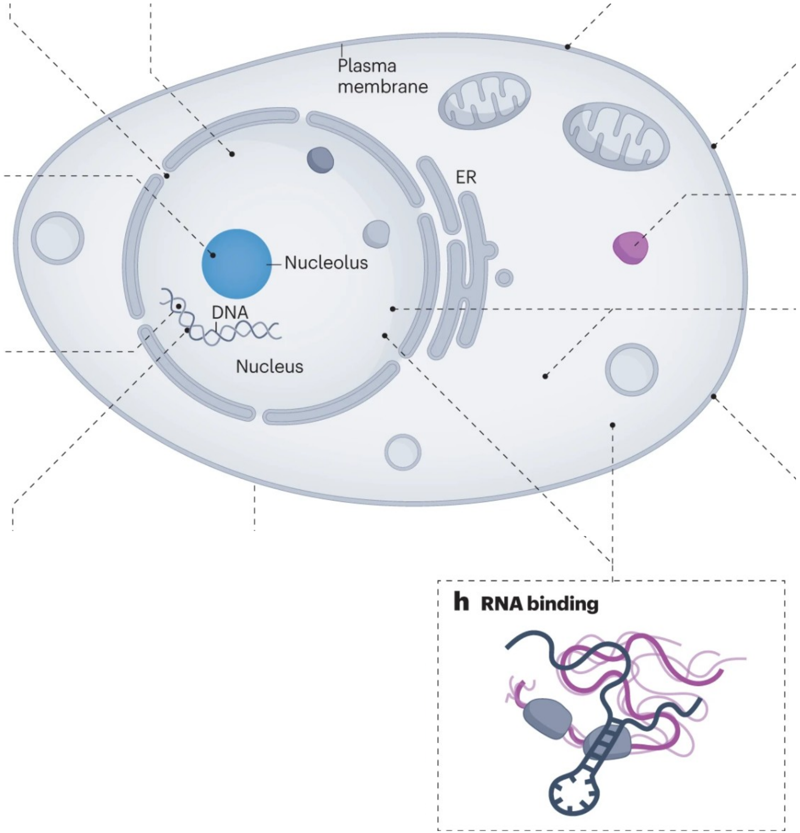


Ganser, L.R., Kelly, M.L., Herschlag, D. *et al.* The roles of structural dynamics in the cellular functions of RNAs. *Nat Rev Mol Cell Biol* 20, 474–489 (2019).



Ganser, L.R., Kelly, M.L., Herschlag, D. *et al.* The roles of structural dynamics in the cellular functions of RNAs. *Nat Rev Mol Cell Biol* 20, 474–489 (2019).

Binding to Intrinsically Disordered Proteins/Regions

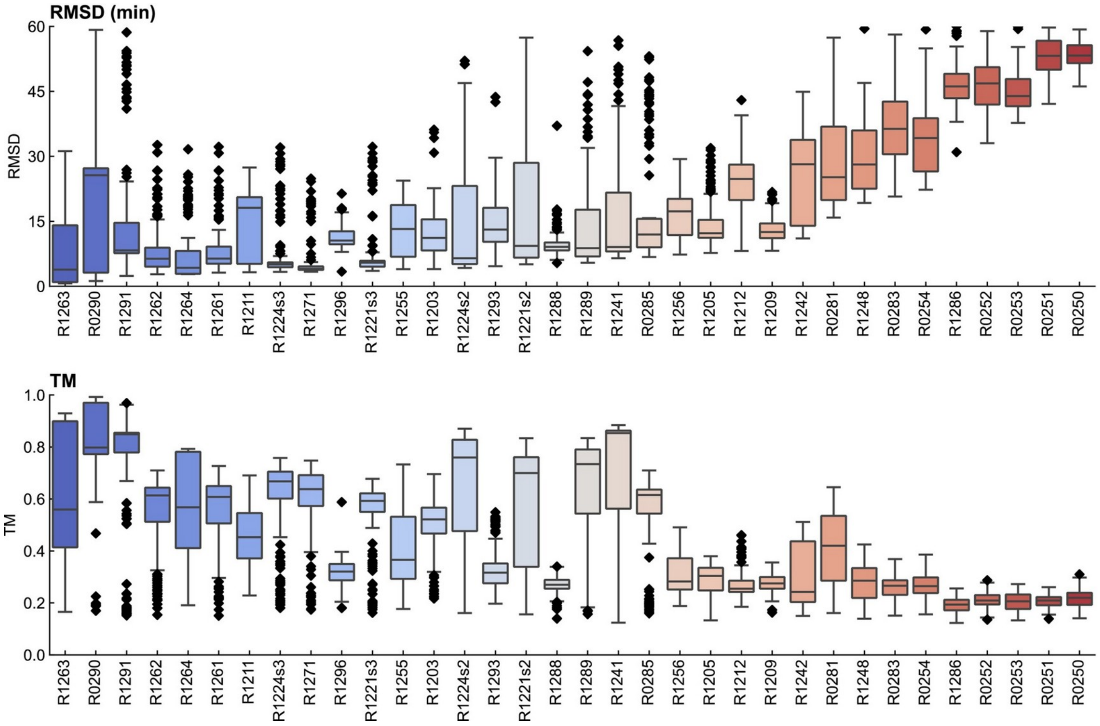


Holehouse, A.S., Kragelund, B.B. The molecular basis for cellular function of intrinsically disordered protein regions. *Nat Rev Mol Cell Biol* 25, 187–211 (2024). https://doi-org.inc.bib.cnrs.fr/10.1038/s41580-023-00673-0

RNA structure prediction

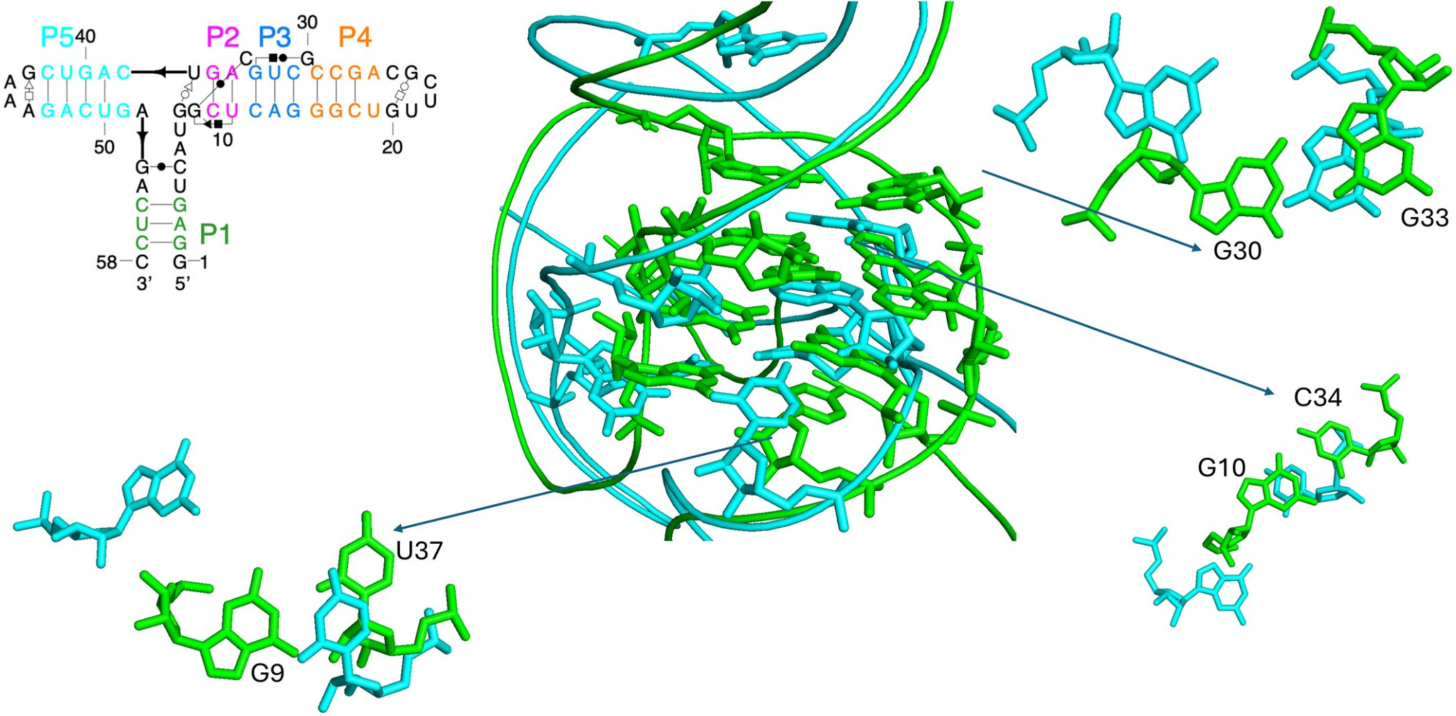
(with specific interaction: π-π vs hydrophobic interaction with ions)

CASP16



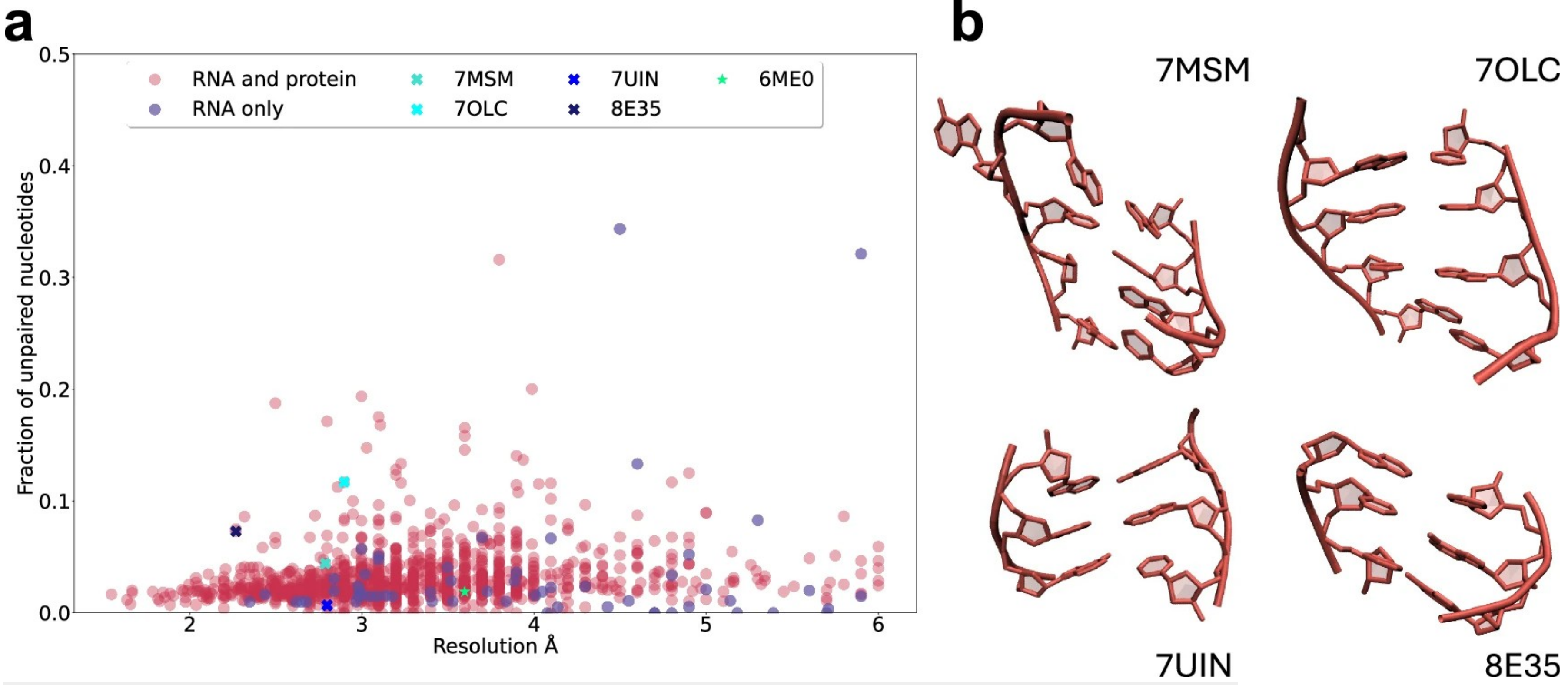
Westhof, E., Sun, H., Bu, F., & Miao, Z. (2025). The RNA-Puzzles Assessments of RNA-Only Targets in CASP16. *Proteins: Structure, Function, and Bioinformatics*.

“Although the global fold is roughly reached, the bases involved are too far apart so that none of the pairs in this network are formed in the modeled structure.”



“PDB wide analysis on mismodeled helices”

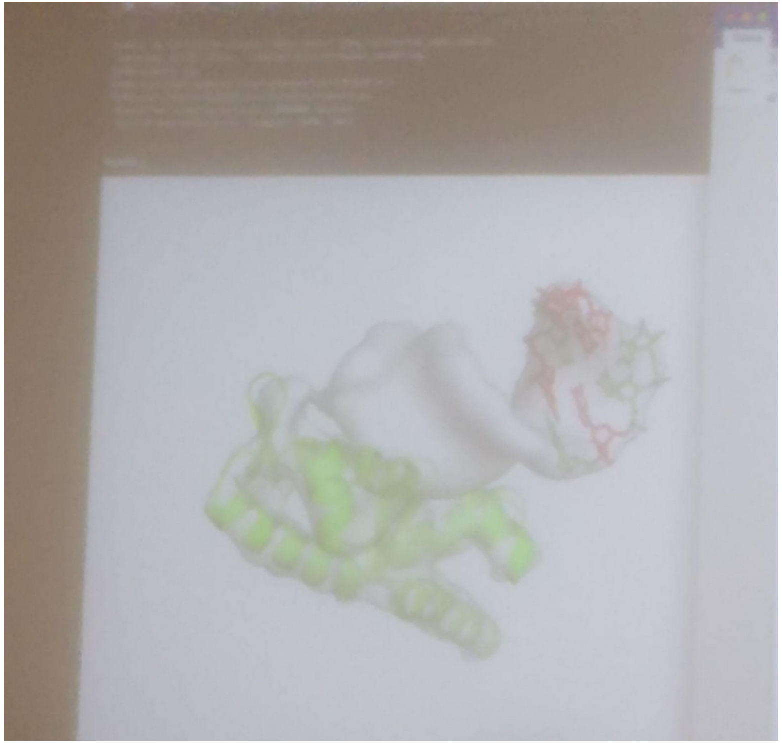
Posani, E., Janoš, P., Haack, D. *et al.* Ensemble refinement of mismodeled cryo-EM RNA structures using all-atom simulations. *Nat Commun* 16, 4549 (2025)



Examples of mis-modeled base parings, in more pathological cases (7MSM) and less serious ones (8E35).

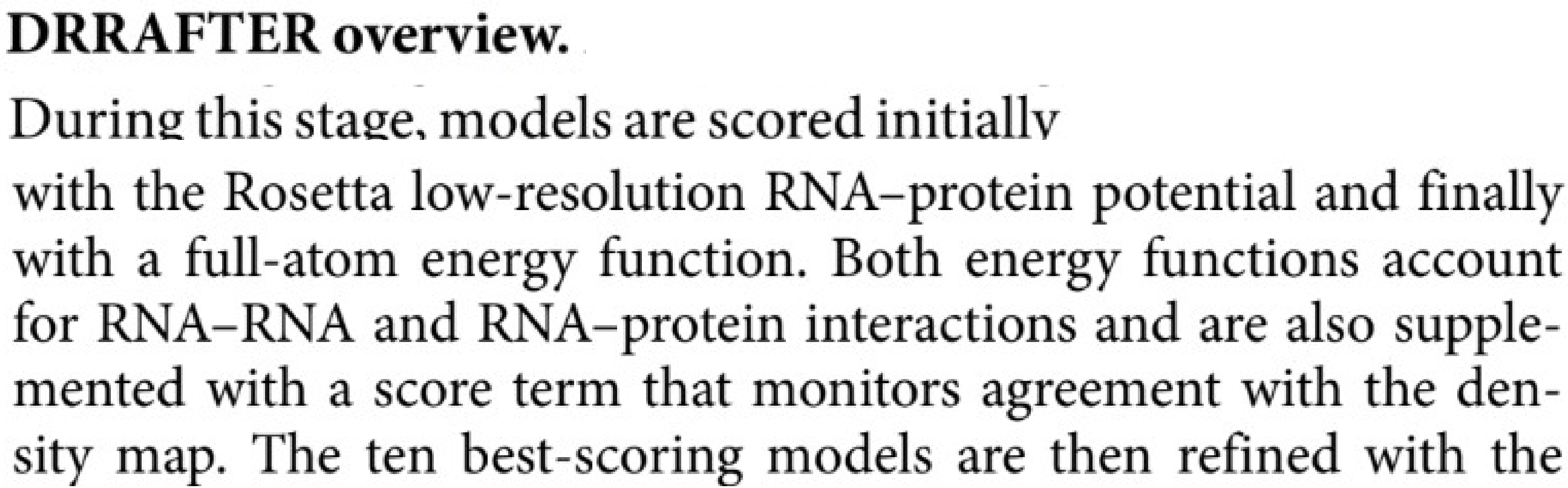
How comes the practical on Tuesday worked ?

1) Data driven!



How comes the practical worked ?

2) Force field-type scoring function



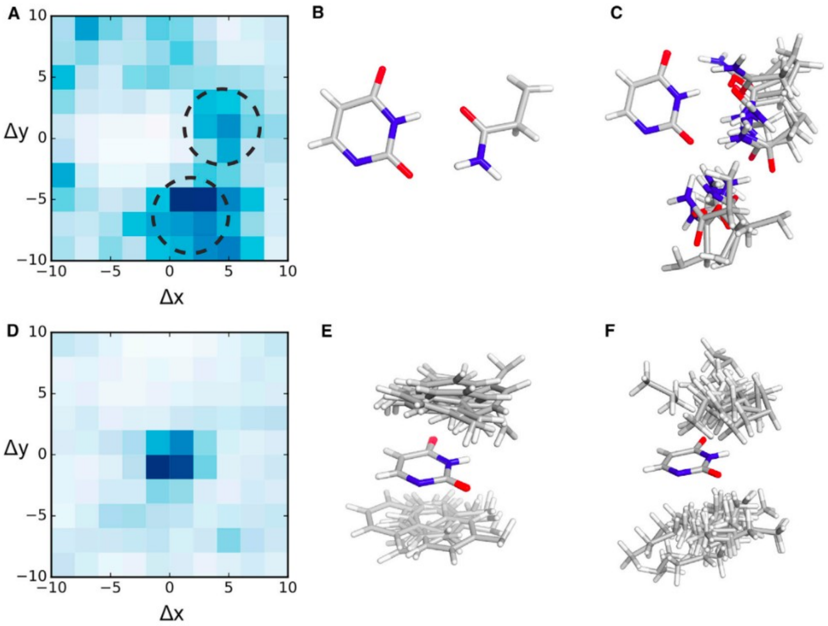
DRRAFTER overview.

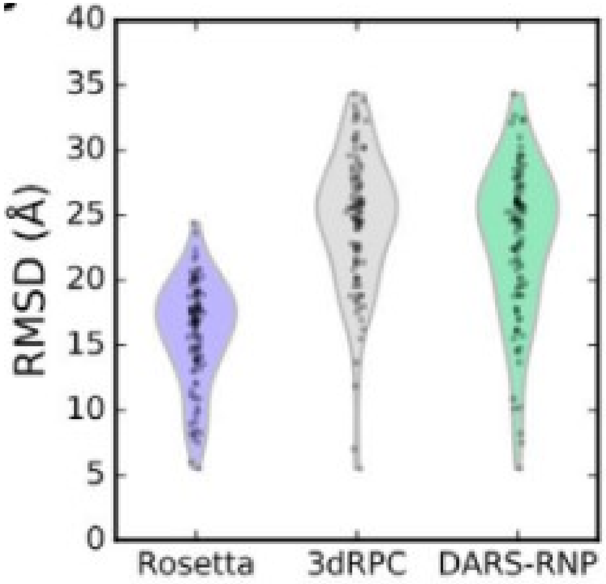
During this stage, models are scored initially with the Rosetta low-resolution RNA–protein potential and finally with a full-atom energy function. Both energy functions account for RNA–RNA and RNA–protein interactions and are also supplemented with a score term that monitors agreement with the density map. The ten best-scoring models are then refined with the

Kappel K, Liu S, Larsen KP, Skiniotis G, Puglisi EV, Puglisi JD, Zhou ZH, Zhao R, Das R. De novo computational RNA modeling into cryo-EM maps of large ribonucleoprotein complexes. *Nature Methods*. (2018). 15: 947–954.

How comes the practical worked ?

2) Force field-type scoring function

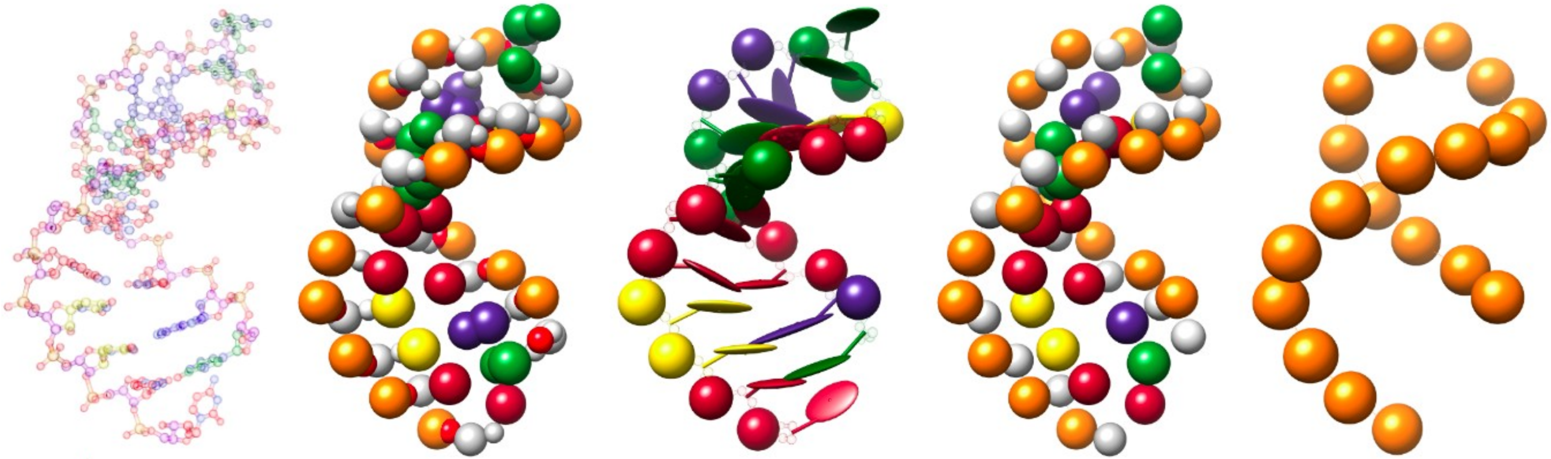




Kappel K, Das R. *Structure*. (2019). 27: 140-151

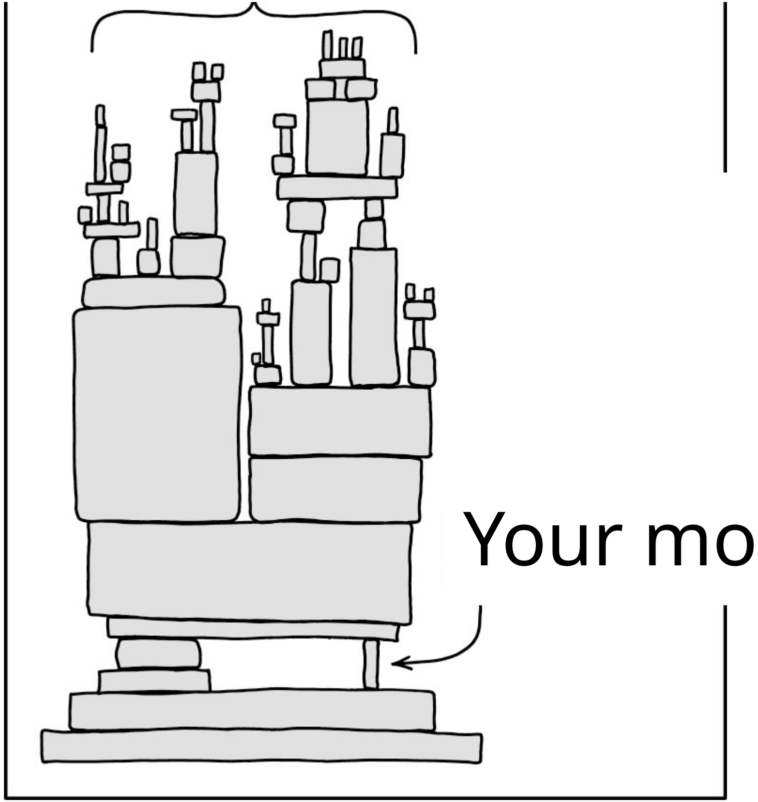
SOME COARSE GRAINED MODELS FOR RNA

ATOMISTIC	HIRE-RNA	OX-RNA	IFOLD-RNA	NAST
Full detail	6-7 beads/nt	5 interactions/nt	3 beads/nt	1 bead/nt
Force fields	Physics/Know	Thermo/Physics	Know/Thermo	Knowledge



(from Liuba slides)

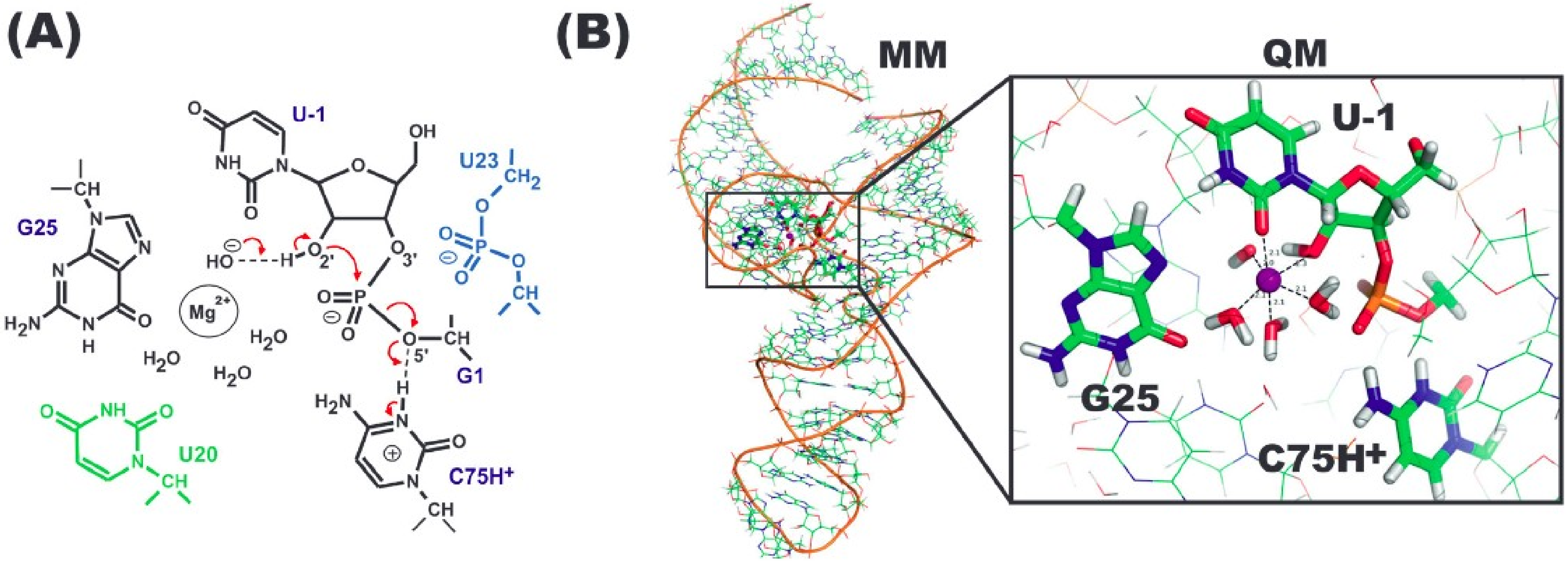
Beware of downstream calculations



Others’ work

Your model

The role of an active site Mg 2+ in HDV ribozyme self-cleavage



Mlýnský, V., Walter, N. G., Šponer, J., Otyepka, M., & Banáš, P. (2015). The role of an active site Mg 2+ in HDV ribozyme self-cleavage: insights from QM/MM calculations. *Physical Chemistry Chemical Physics*, *17*(1), 670-679.

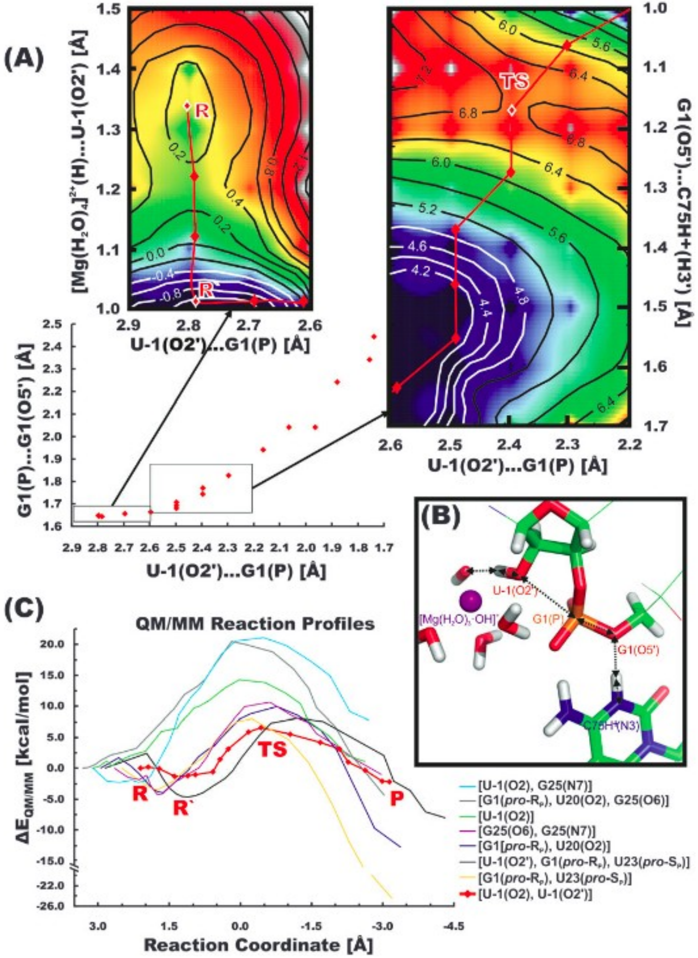
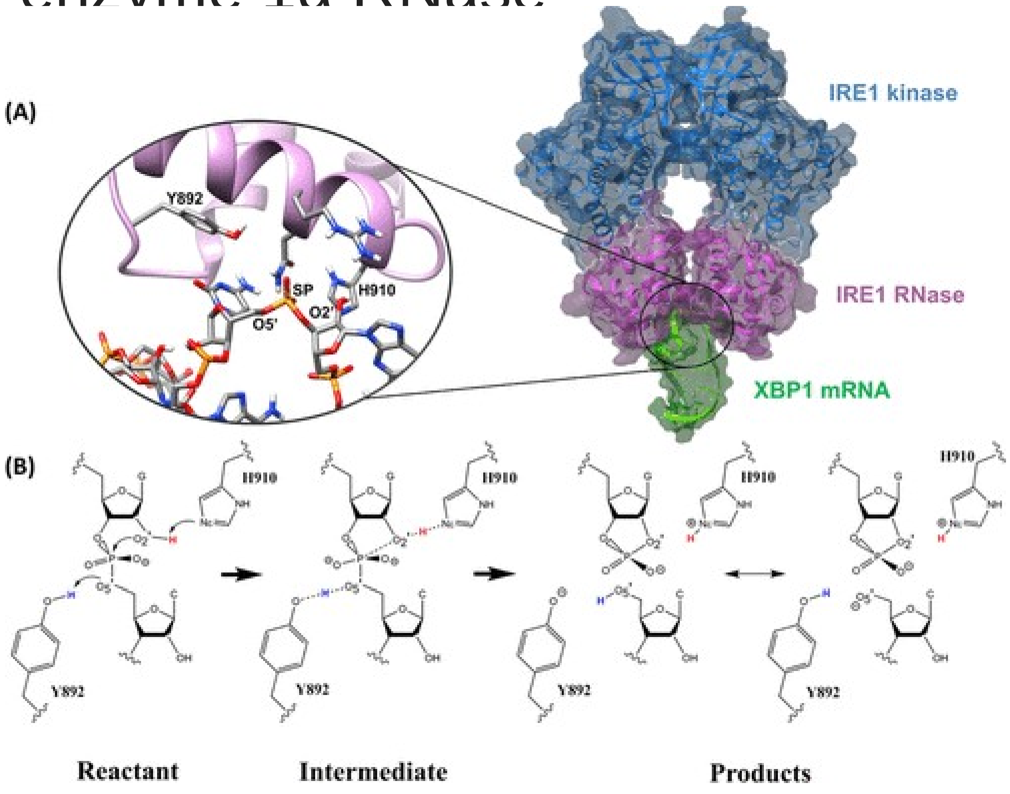


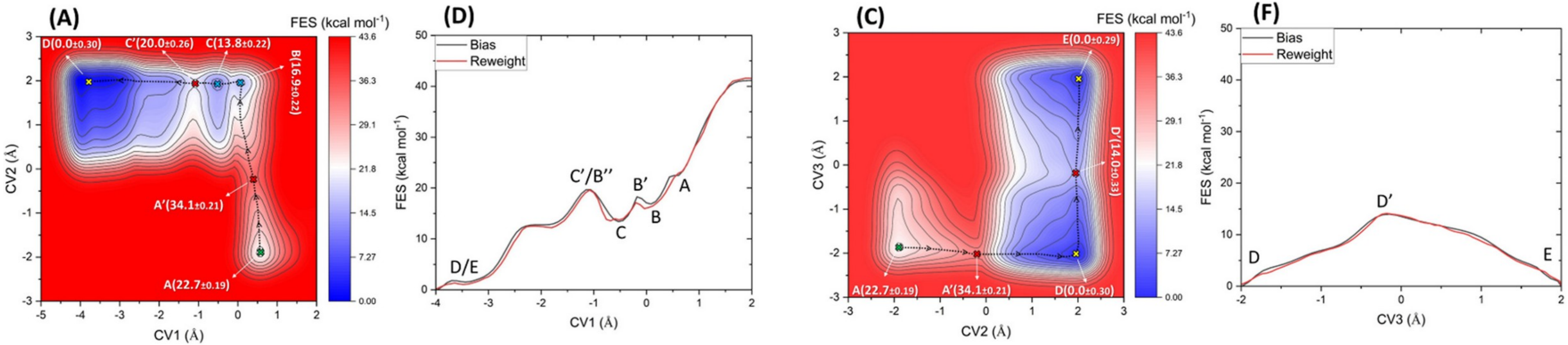
Fig. 3 The reaction pathways describing the sequential mechanism, where the first proton transfer step is separated from the subsequent nucleophilic attack. (A) Scatter chart showing optimized points along the initial reaction coordinate with double-inner-shell coordination to [U-1(O2), U-1(O2′)] as a function of the U-1(O2′)···G1(P) and G1(P)···G1(O5′) distances. Both proton transfers (black insets) were investigated further by 2D scans. The diagram on the left displays the initial proton transfer from the U-1(2′-OH) group to the deprotonated water molecule of the partially hydrated $[Mg(H_2O)_3{\cdot}OH]^+$ ion, where a pre-cleavage state R and an intermediate R′ were found. The second 2D diagram on the right represents the subsequent proton transfer from the protonated $C75H^+$ to G1(O5′), where the TS state was localized. QM/MM Gibbs energies (in kcal mol^{-1}, shown as contours and colors) were calculated at the MPW1K/6-31+G(d,p):AMBER(*ff*99bsc0χ_{OL3}) level and are depicted without any additional correction (see the Methods and Table 1). (B) The active site of the R state with double-inner-shell coordination to [U-1(O2), U-1(O2′)] highlights the key atoms and bonds involved in the cleavage reaction. (C) Calculated QM/MM (MPW1K/6-31+G(d,p):AMBER(*ff*99bsc0χ_{OL3})) energies for the eight complete reaction pathways with different coordination of the Mg^{2+} ion plotted against the reaction coordinate, *i.e.* the sum of coordinates corresponding to nucleophilic attack, first and second proton transfer defined as differences (U-1(O2′)···G1(P))−(G1(P)···G1(O5′)), $(OH^-(O)\cdots H)-(U\text{-}1(O2')\cdots H)$, and $(C75H^+(N3)\cdots H)-(G1(O5')\cdots H)$, respectively.

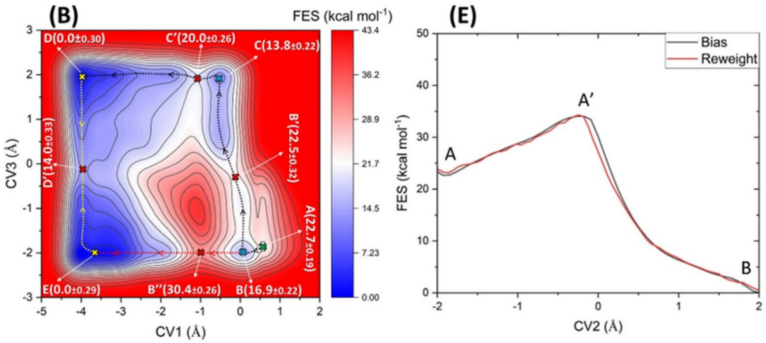
Mechanism of XBP1 mRNA cleavage by inositol requiring enzyme 1α RNase



Mahdizadeh, S. J., Palsson, E., Carlesso, A., Chevet, E., & Eriksson, L. A. (2022). QM/MM well-tempered metadynamics study of the mechanism of XBP1 mRNA cleavage by inositol requiring enzyme 1α RNase. *Journal of Chemical Information and Modeling*, *62*(17), 4247-4260.

2D projected free energy surface (FES) contour maps





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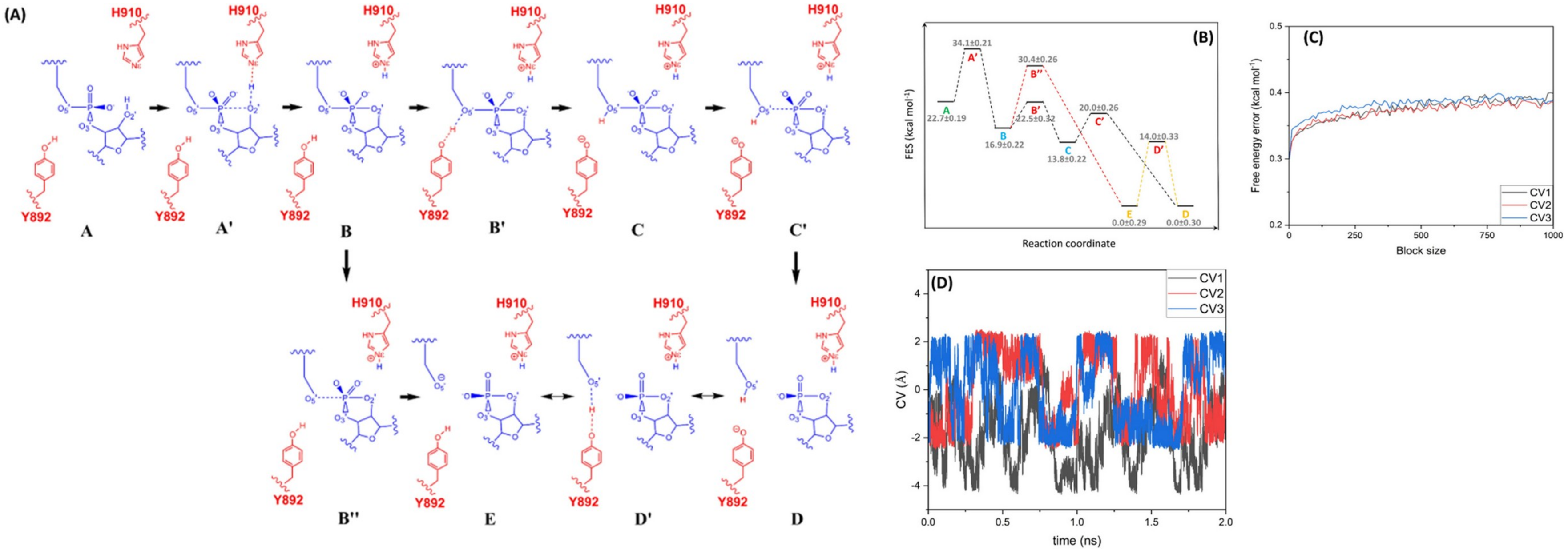
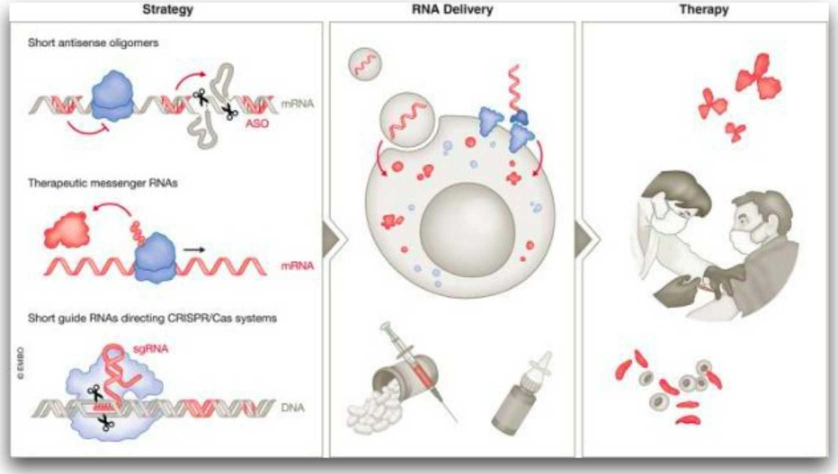
to conclude on an “Unconventional cleavage mechanism”



Mahdizadeh, S. J., Palsson, E., Carlesso, A., Chevet, E., & Eriksson, L. A. (2022). QM/MM well-tempered metadynamics study of the mechanism of XBP1 mRNA cleavage by inositol requiring enzyme 1α RNase. *Journal of Chemical Information and Modeling*, *62*(17), 4247-4260.

RNA as drug

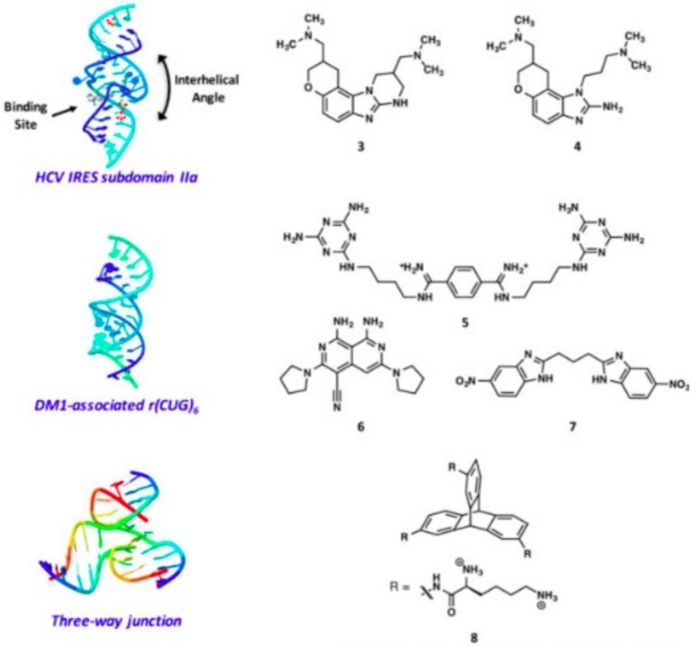
- deliver functional RNAs into cells
- produce a therapeutic effect
- mRNA vaccines, siRNA, ASO, CRISPR



EMBO J 42(21):e114760. 2023

RNA as drug target

- use drugs to bind and modify endogenous RNA



Cell Chem Biol. 23(9):1077–1090. 2016

(from Michael slides)

Take home message

- Downstream tasks often require extremely precise predictions
- Underlying methods are associated with large uncertainties/hypotheses/approximations etc…
- what could possibly go wrong?

⇒ **document what you did!!**