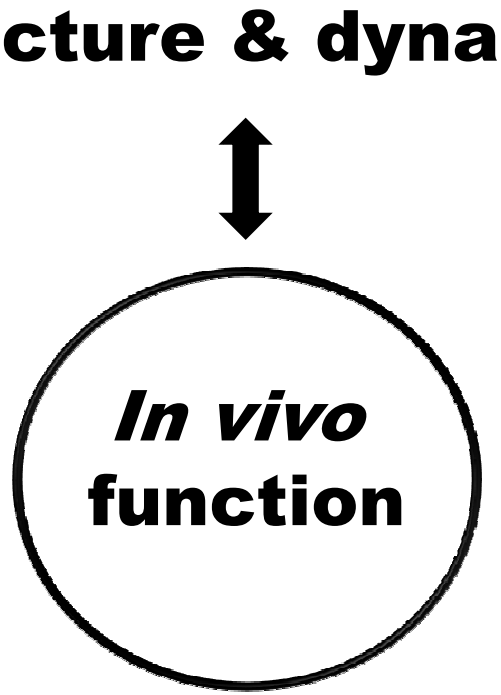
Structural Biology – a concept



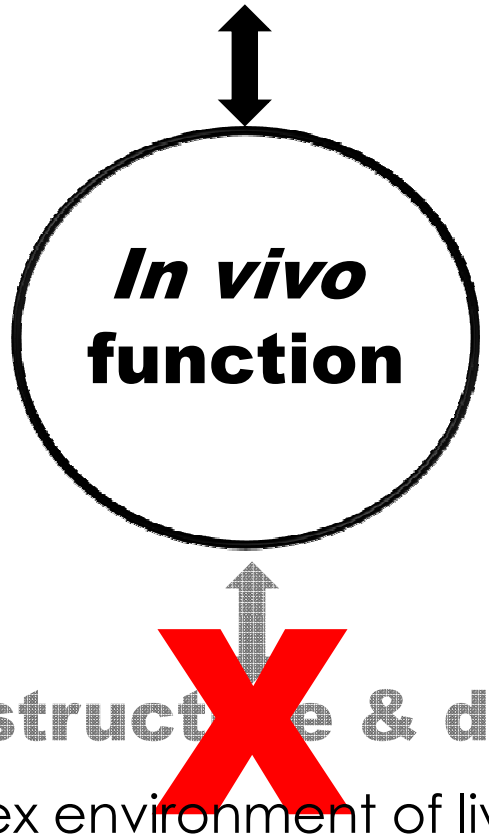
Where the structural information comes from ….

PDB Statistics – by method

Molecular Type	X-ray	NMR	EM
Nucleic acid (only)	2054	1296	35
Other	460	49	117
Protein (only)	136930	11383	3848
Protein/NA	6615	264	1069
Total	146059	12992	5069

In vitro structure & dynamics

buffered solutions, crystalline state, or deposited isolated molecules on the surface



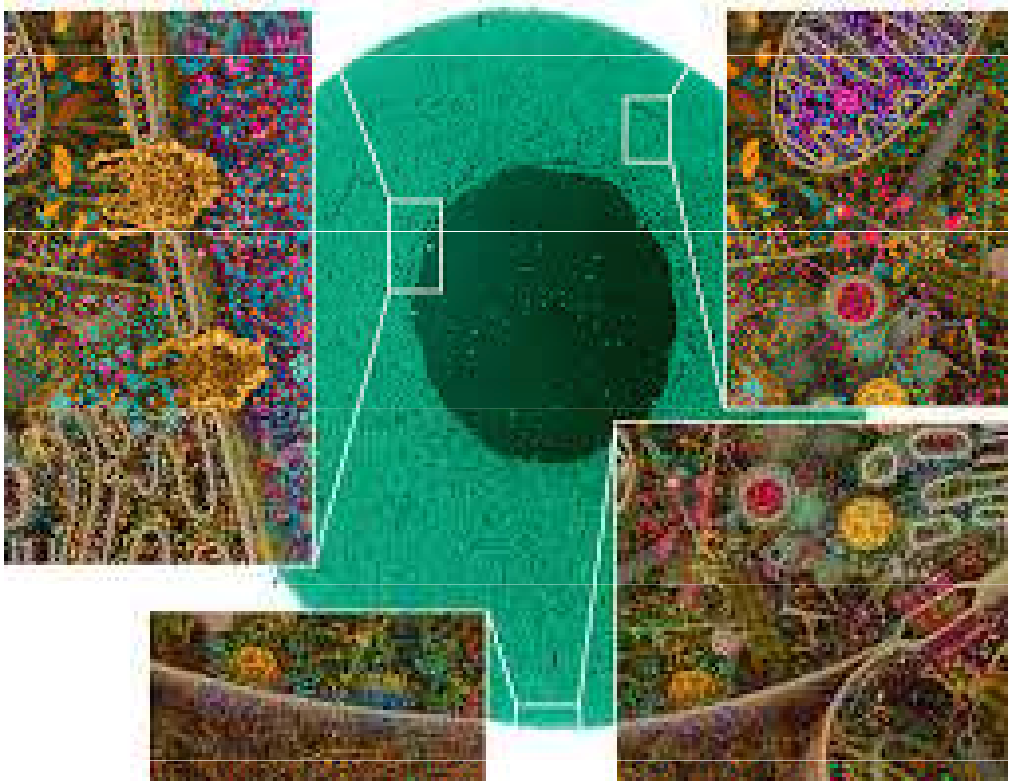
~~*In vivo* structure & dynamics~~

Complex environment of living cells

X-ray diffraction

approximation

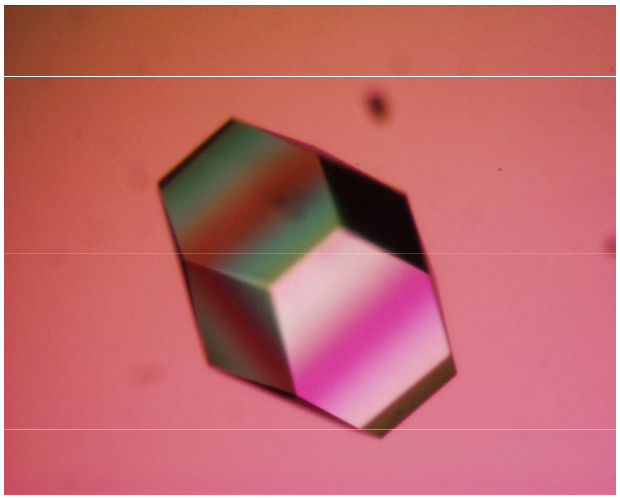
Complex heterogenous aqueous env.



37 °C

vs.

Simple homogenous anhydrous env.

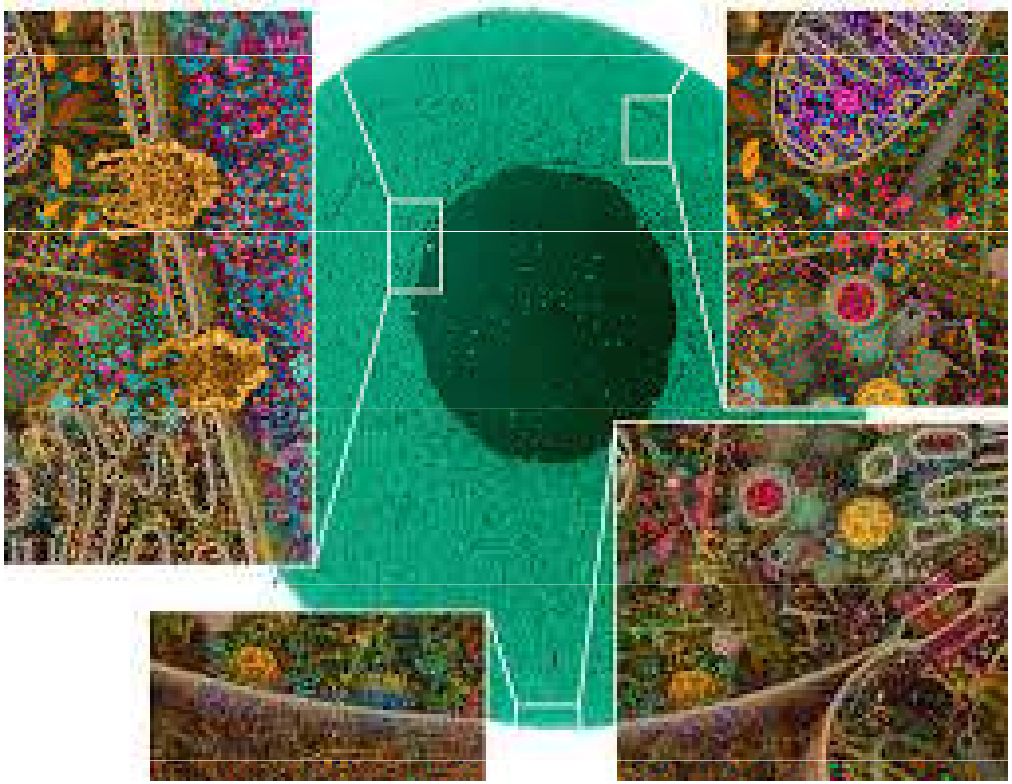


-180 °C

NMR spectroscopy

approximation

Complex heterogenous aqueous env.



37 °C

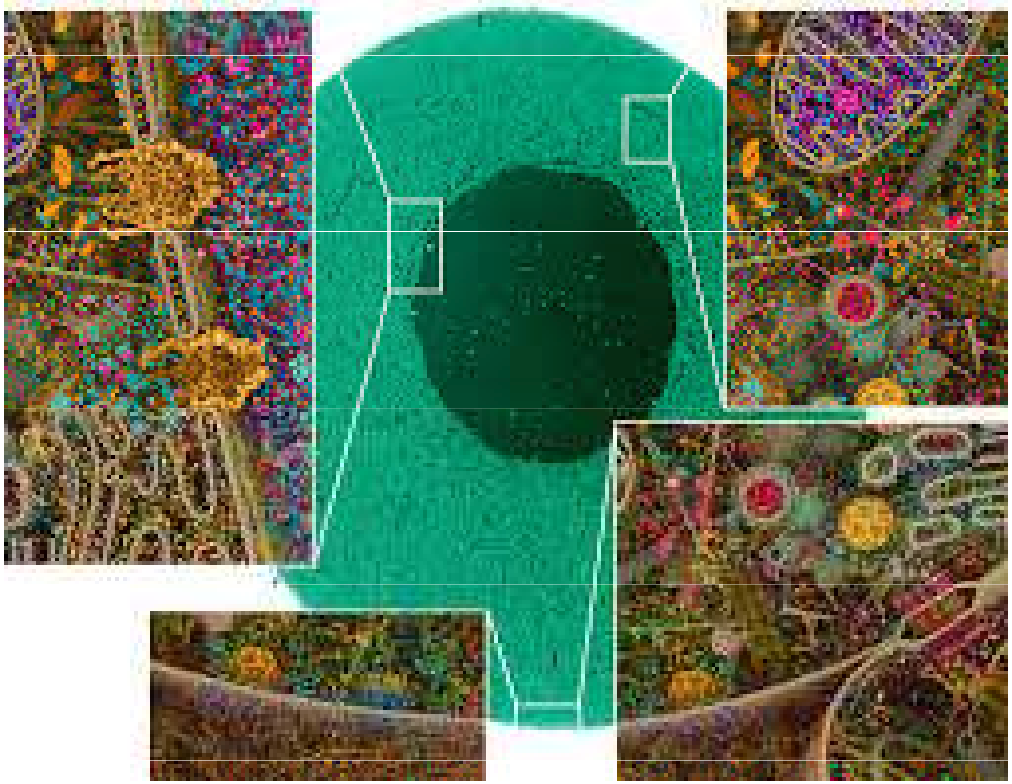
VS.

Simple homogenous aqueous env.

20 °C

Electron microscopy approximation

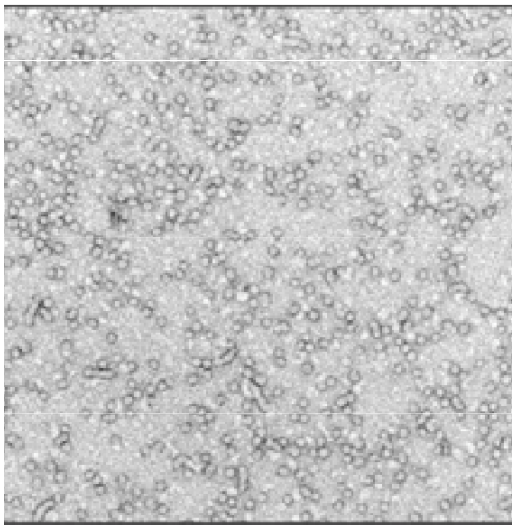
Complex heterogenous aqueous env.



37 °C

vs.

Simple homogenous aqueous env.



-180 °C

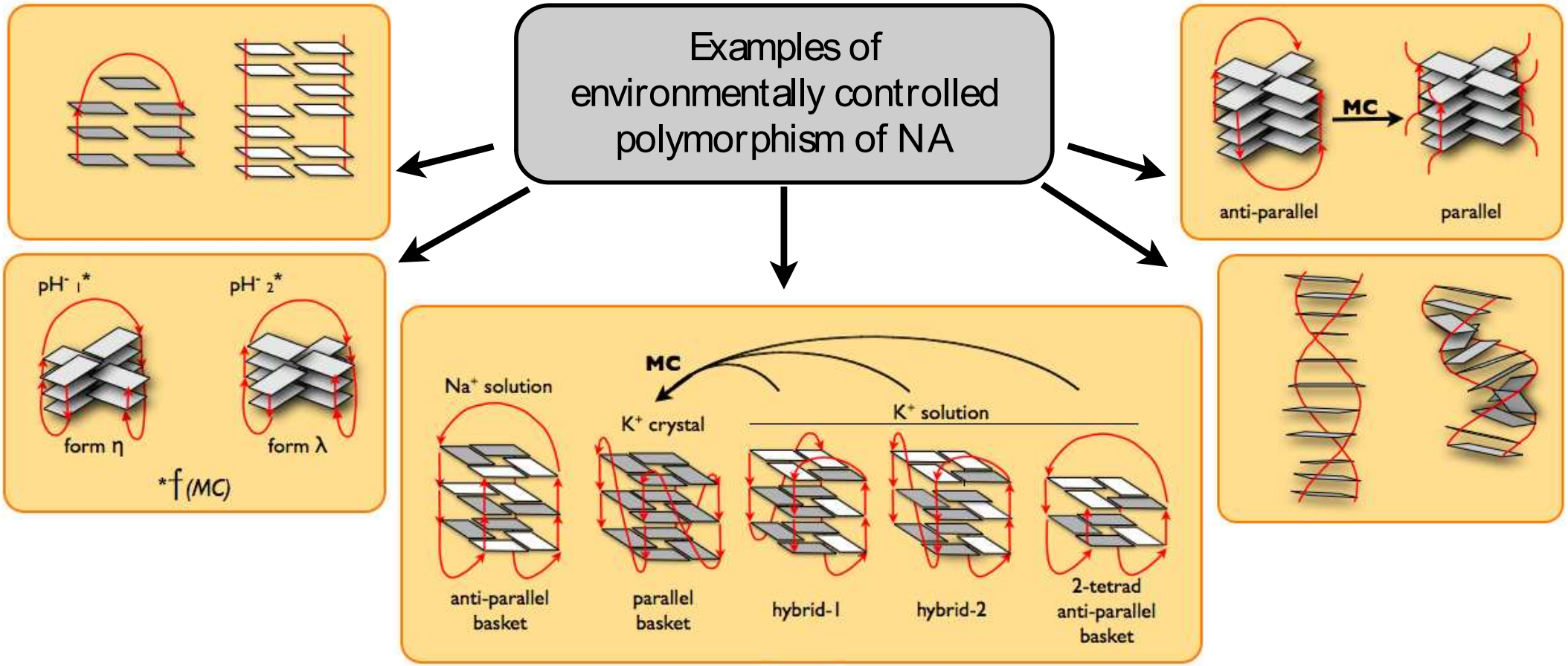
The use of these techniques imposes …

… underlying assumptions in STRUCTURAL BIOLOGY

- **structure is independent of environmental conditions**
- **thermodynamically NOT kinetically preferred conformation is responsible for biological activity.**
- **biologically active specie = lowest (free) energy structure**

- **structure is independent of environmental conditions**

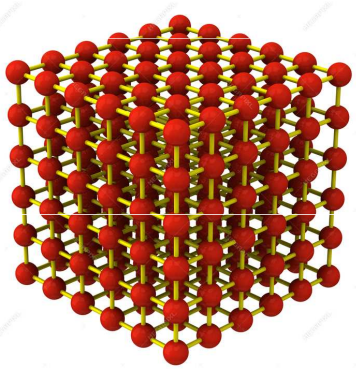
…not entirely true, particularly for DNA



(Δ pH, Δ ion strengh, ion type, MC, hydration)

X-ray – particularly prone to env. artifacts

…monocrystal production



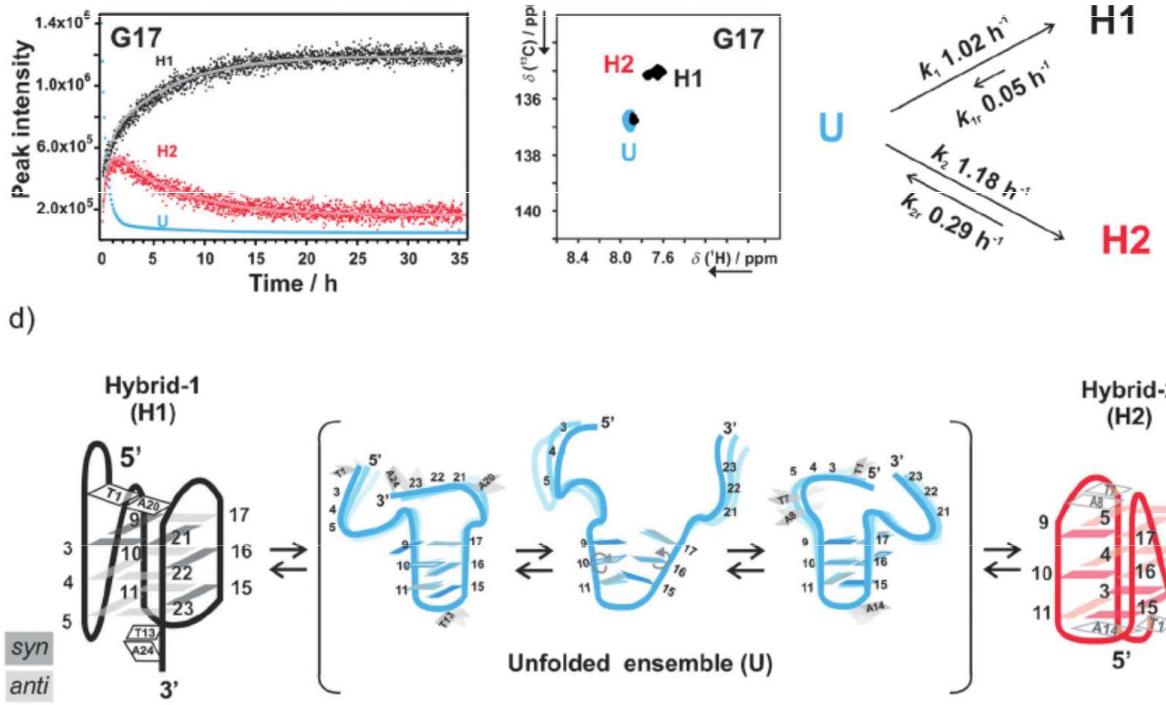
Crystal Screen™ HR2-110 Reagent Formulation

Tube #	Salt	Tube #	Buffer ◊	Tube #	Precipitant
1.	0.02 M Calcium chloride dihydrate	1.	0.1 M Sodium acetate trihydrate pH 4.6	1.	30% v/v (+/-)-2-Methyl-2,4-pentanediol
2.	None	2.	None	2.	0.4 M Potassium sodium tartrate tetrahydrate
3.	None	3.	None	3.	0.4 M Ammonium phosphate monobasic
4.	None	4.	0.1 M TRIS hydrochloride pH 8.5	4.	2.0 M Ammonium sulfate
5.	0.2 M Sodium citrate tribasic dihydrate	5.	0.1 M HEPES sodium pH 7.5	5.	30% v/v (+/-)-2-Methyl-2,4-pentanediol
6.	0.2 M Magnesium chloride hexahydrate	6.	0.1 M TRIS hydrochloride pH 8.5	6.	30% w/v Polyethylene glycol 4,000
7.	None	7.	0.1 M Sodium cacodylate trihydrate pH 6.5	7.	1.4 M Sodium acetate trihydrate
8.	0.2 M Sodium citrate tribasic dihydrate	8.	0.1 M Sodium cacodylate trihydrate pH 6.5	8.	30% v/v 2-Propanol
9.	0.2 M Ammonium acetate	9.	0.1 M Sodium citrate tribasic dihydrate pH 5.6	9.	30% w/v Polyethylene glycol 4,000
10.	0.2 M Ammonium acetate	10.	0.1 M Sodium acetate trihydrate pH 4.6	10.	30% w/v Polyethylene glycol 4,000
11.	None	11.	0.1 M Sodium citrate tribasic dihydrate pH 5.6	11.	1.0 M Ammonium phosphate monobasic
12.	0.2 M Magnesium chloride hexahydrate	12.	0.1 M HEPES sodium pH 7.5	12.	30% v/v 2-Propanol
13.	0.2 M Sodium citrate tribasic dihydrate	13.	0.1 M TRIS hydrochloride pH 8.5	13.	30% v/v Polyethylene glycol 400

- **thermodynamically NOT kinetically preferred conformation is responsible for biological activity.**

… not generally true

Biological processes (folding, interactions) t <



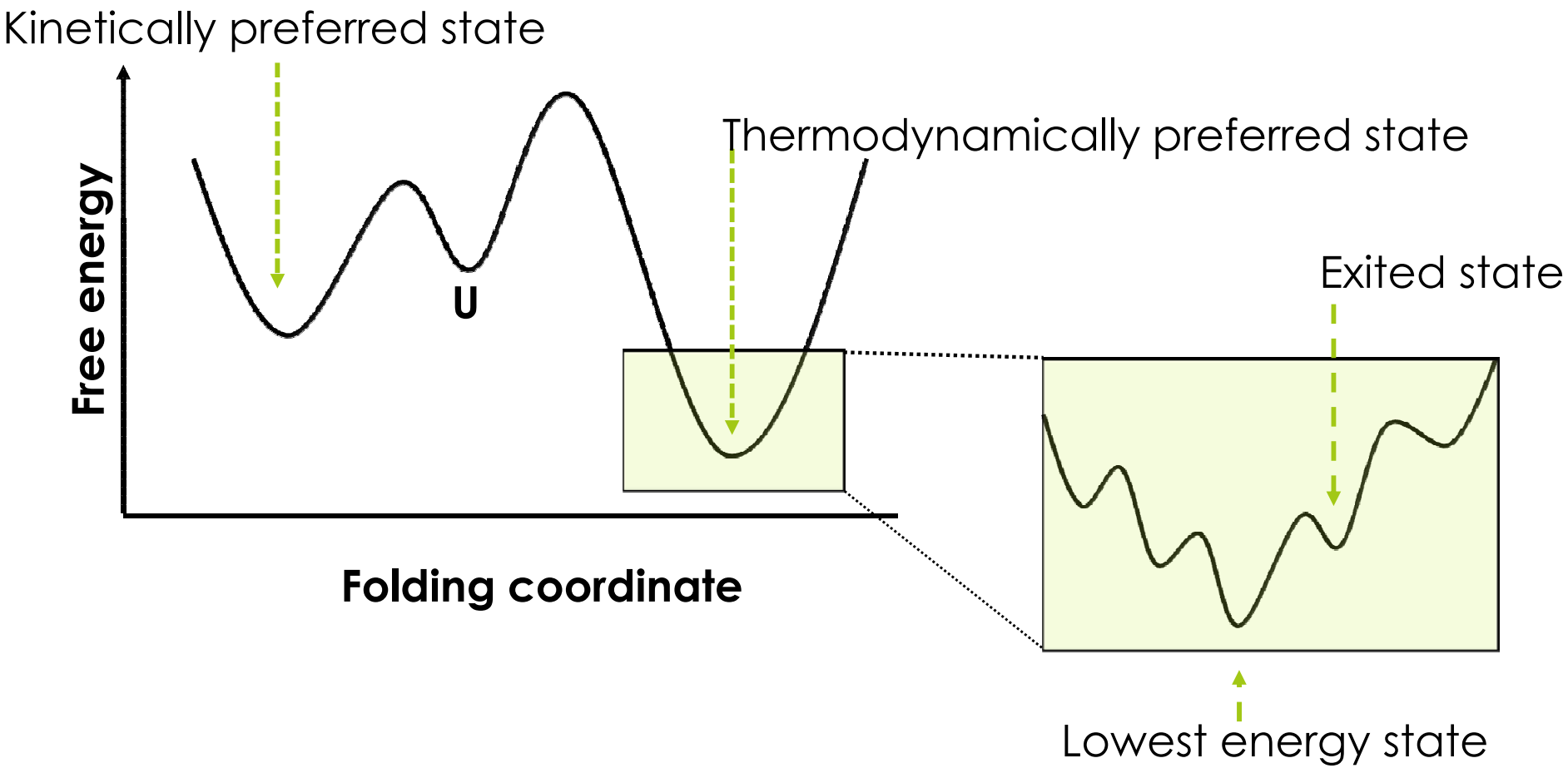
Only H2 would be observed in conventional X-ray, NMR experiment

Bessi et al. Angew Chem Int Ed Engl. 2015 Jul 13;54(29):8444-8.

- **biologically active specie = lowest (free) energy structure**

…not entirely true

…number of processes happens via conformational selection (from excited/non-visible states)

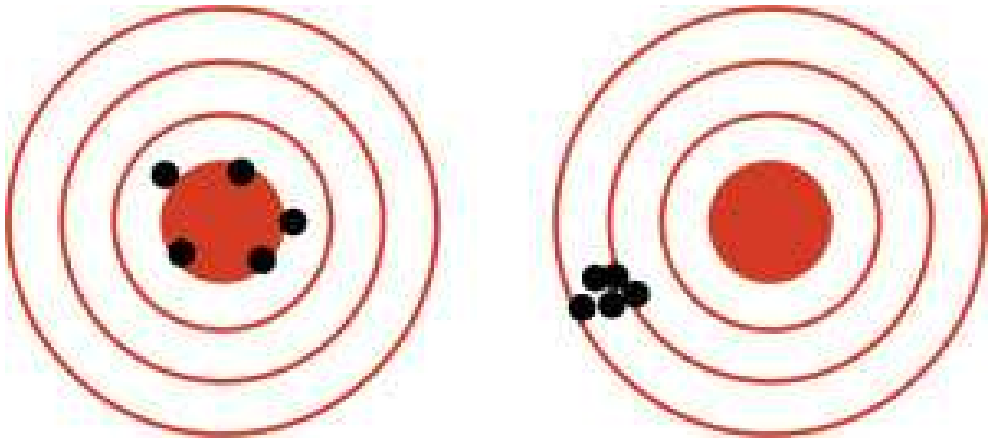


➢ Summary

…X-ray, NMR, cryoEM structures

…are biased

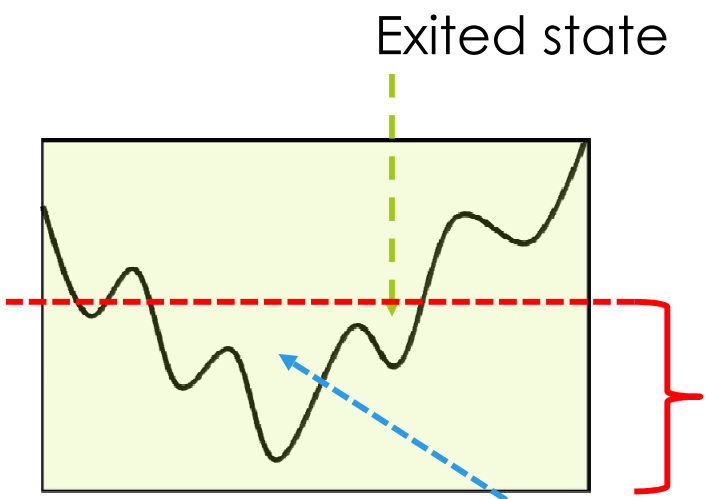
- **Towards thermodynamically preferred (lowest energy) conformation**, which is not necessarily biologically active; the bias is a function of T [°C]
- **Towards conformation specific to selected environmental conditions**
- **Parameters used to assess structure quality refers only to precision not accuracy**



➢ Summary

…in addition, NMR

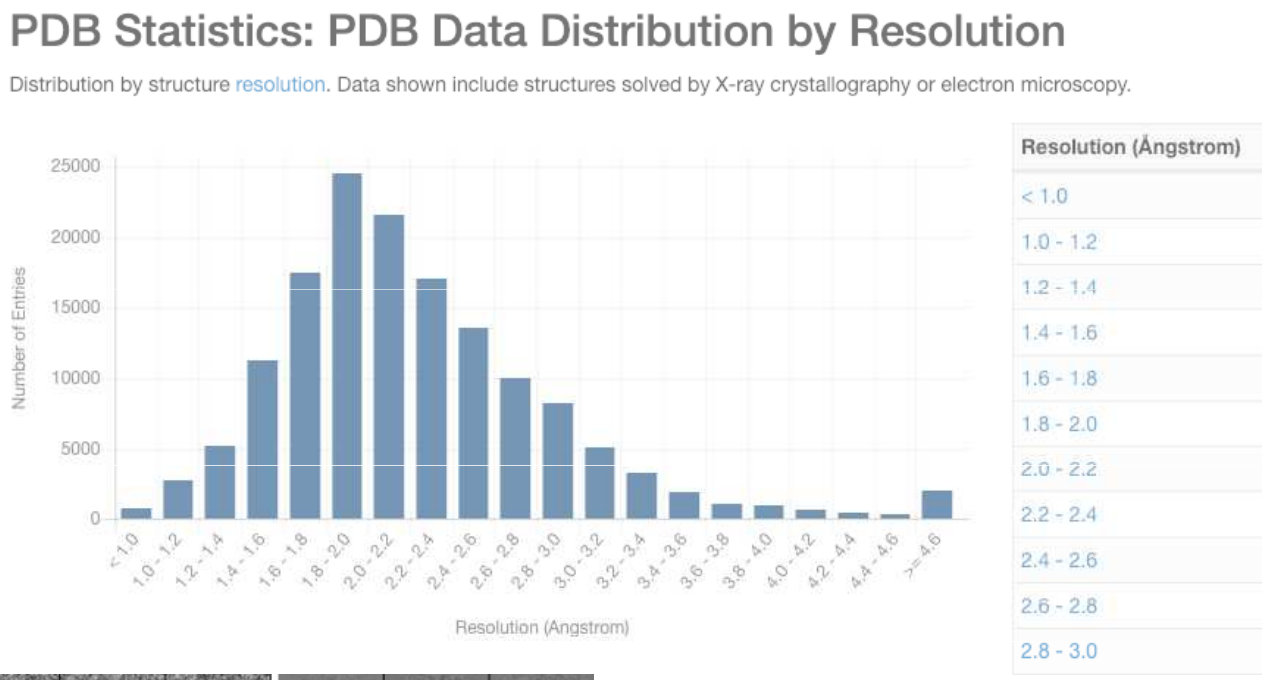
…is biased by averaging

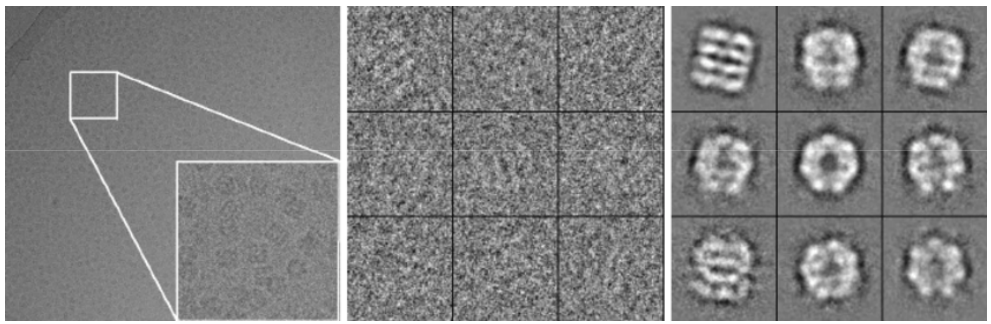


Multiple states populated
at physiological temperatures

We observe ensemble average

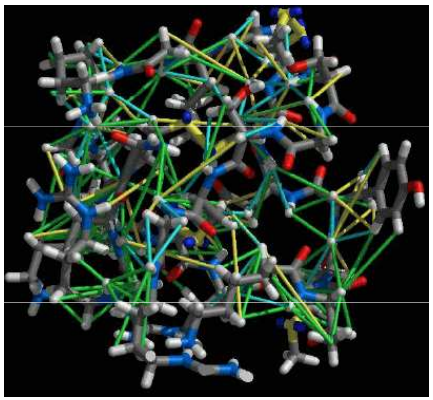
Bias from external information on local structure

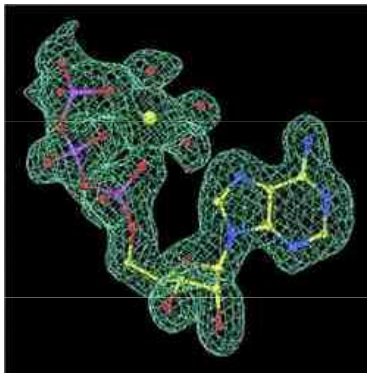




cryoEM

NMR



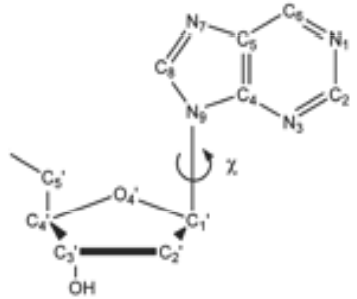


X-ray

Bias from external information on local structure

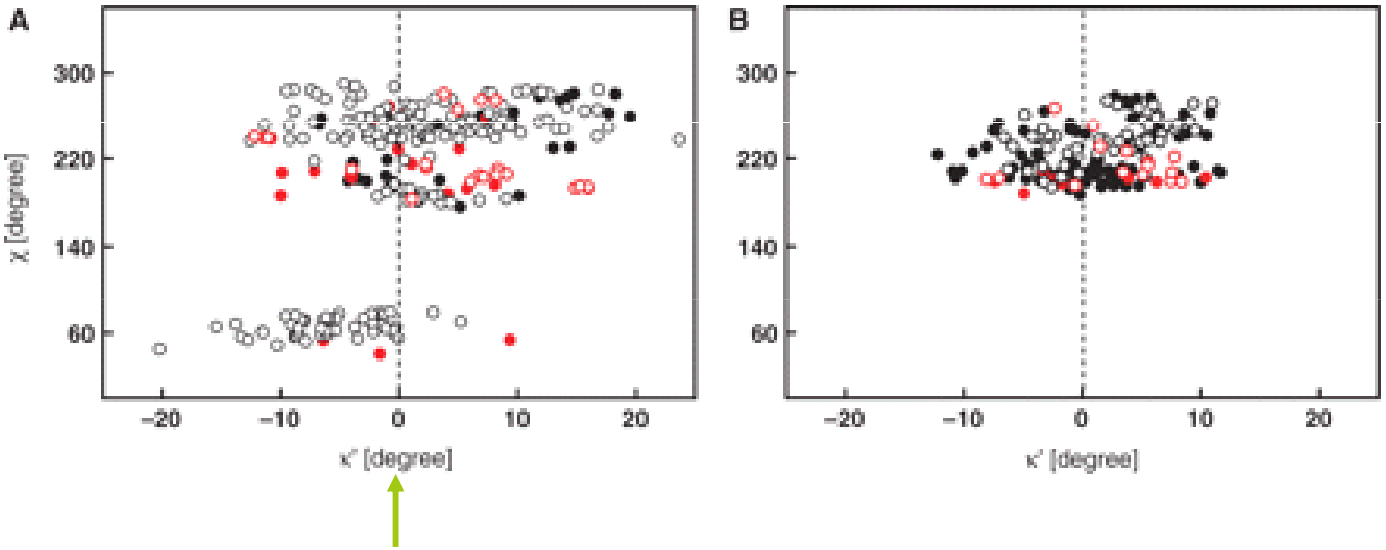
Example: Implicitly used assumption of planarity of nucleic acid bases

However, NA bases are planar only when isolated and when at 0 K.



Data from ultra-high resolution structures of nucleotides

Nucleic Acids Res, Volume 37, Issue 21, 1 November 2009, Pages 7321–7331



During structure determination we presume planarity

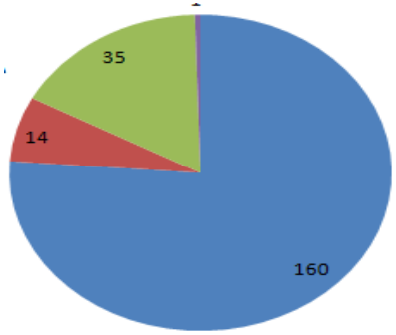
… source of the artefact in NMR – affects interpretation of NOEs, Js, RDCs

Historically based artifacts

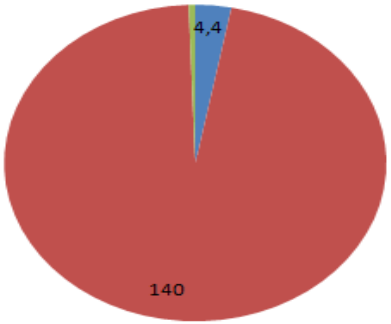
… which can be easily fixed

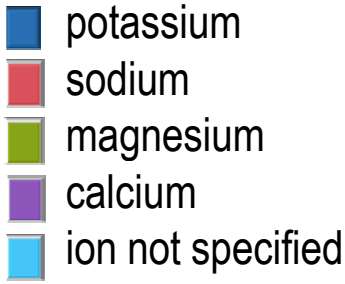
Ionic composition of:

Intracellular space

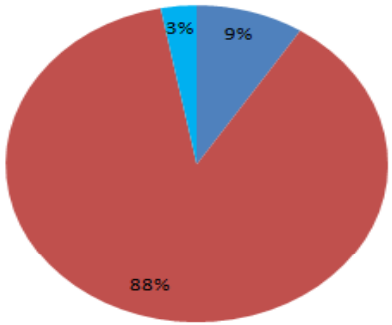


Extracellular space

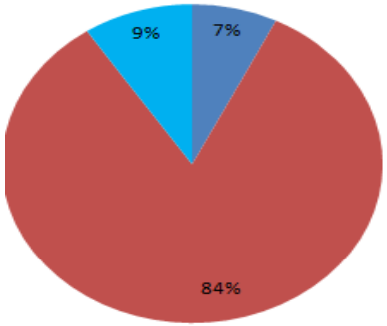




Ionic composition of buffers used for NMR studies of:



DNA

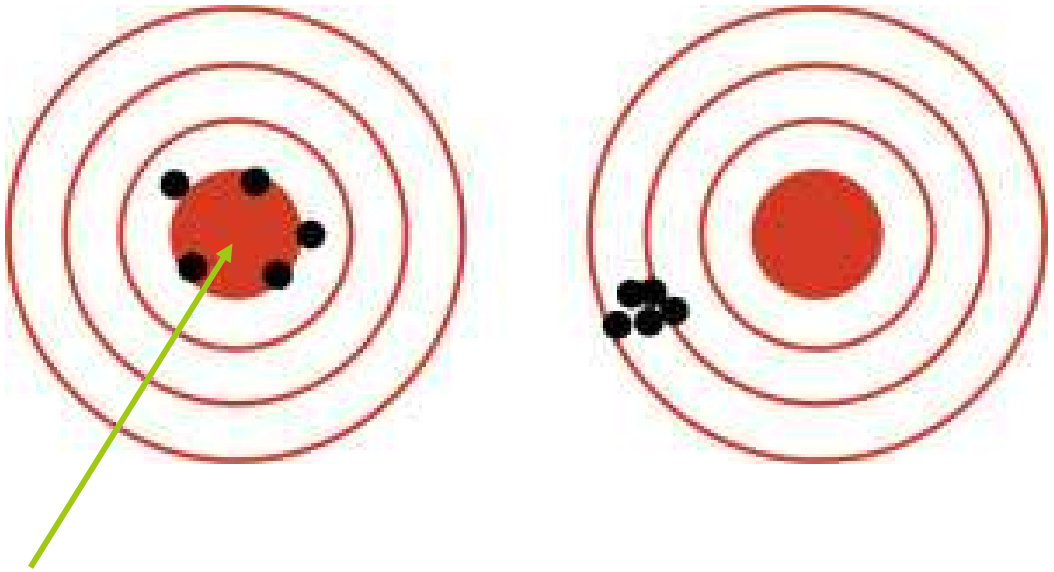


RNA

Statistics based on PDB data

Structural Biology – an issue

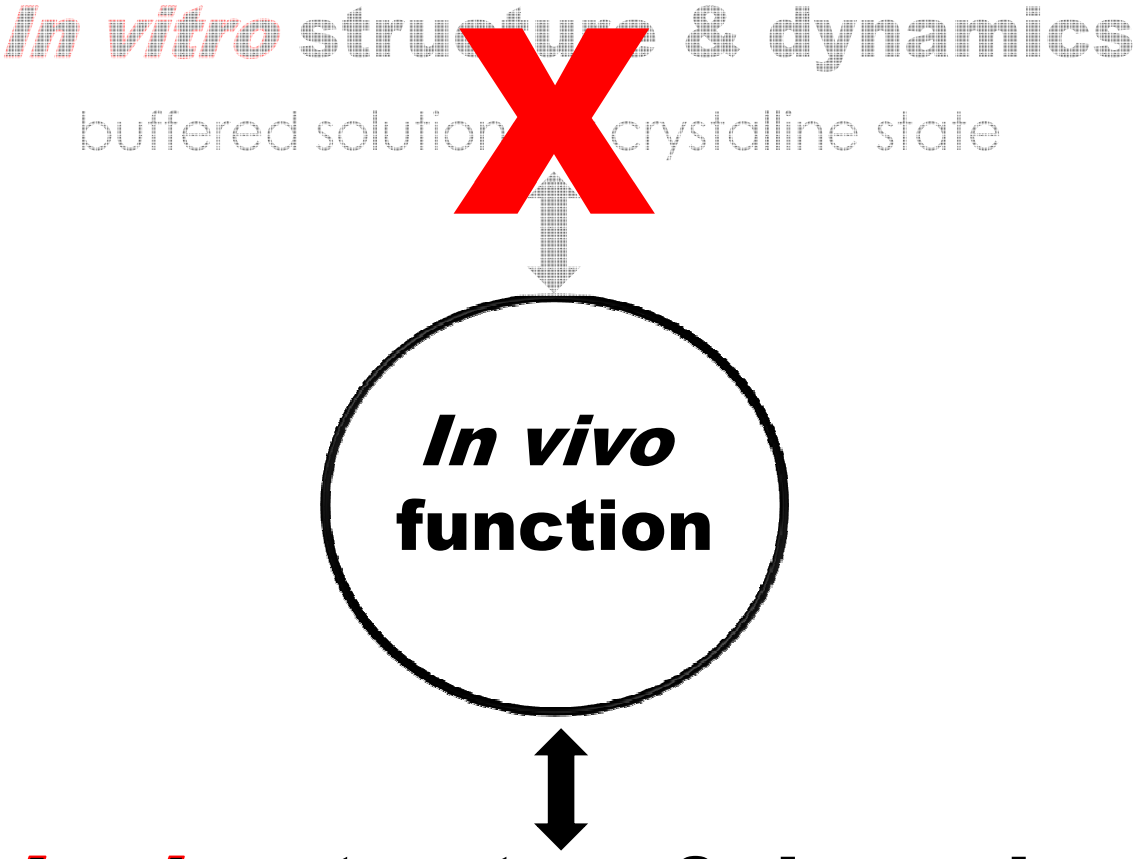
How to recognize physiologically relevant (accurate) structure?



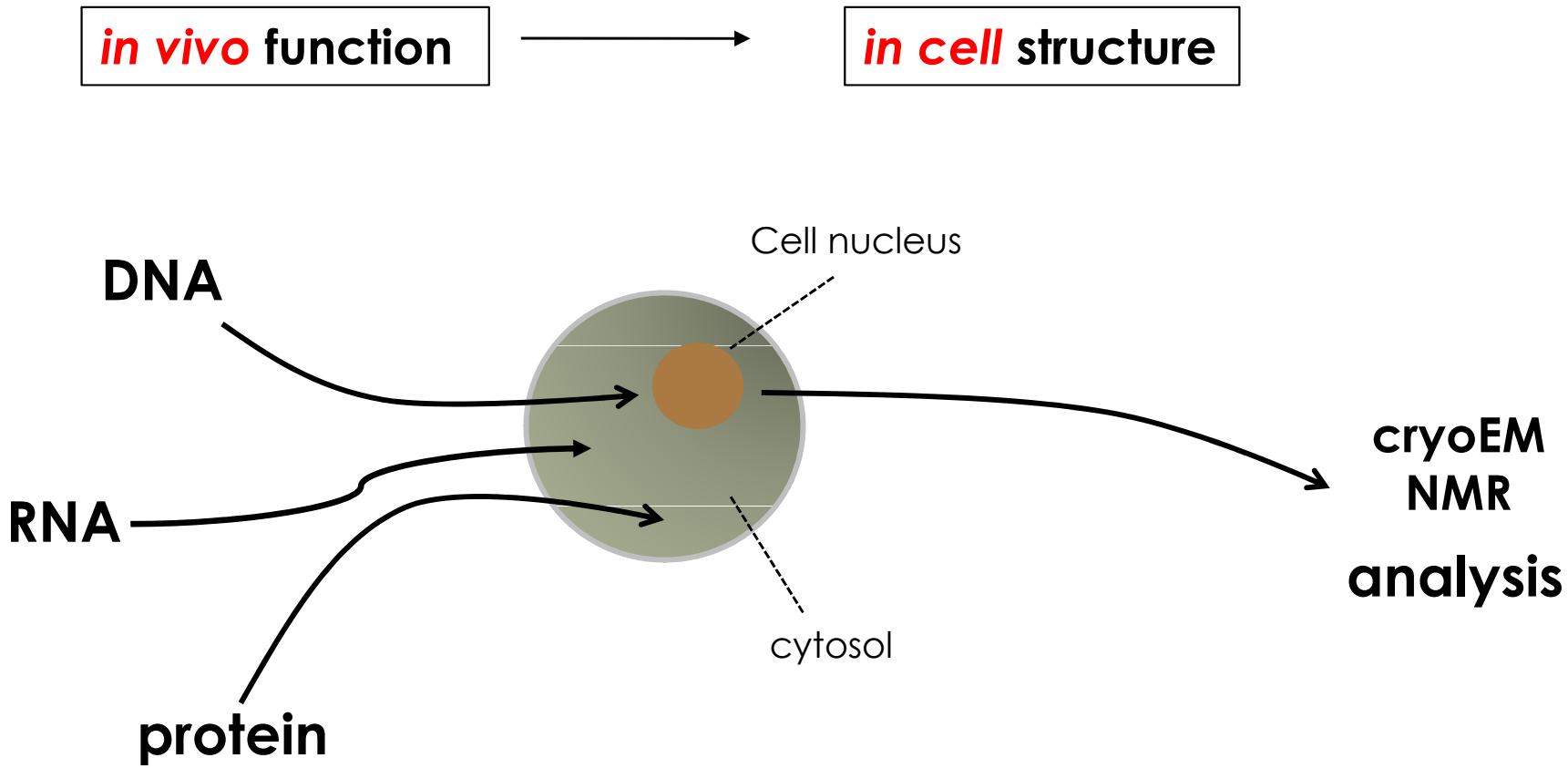
… assessment of structural accuracy presumes knowledge of reference structure

Cellular Structural Biology – a concept

… characterization of structures at physiologically relevant env. conditions



Cellular structural biology



Homework – mandatory self-reading

Plitzko et al. Structural Biology Outside the Box-Inside the Cell. *Curr Opin Struct Biol*. **2017** Oct;46:110-121

EXAM by homework

Model a 3D complex between TBA aptamer and FGF2 presuming that the TBA binds to the heparin binding site via electrostatic interactions.

Consider visualization of charge distribution on structural models of FGF2 and TBA.

e.g., Raček et al. Atomic Charge Calculator II: Web-Based Tool for the Calculation of Partial Atomic Charges. Nucleic Acids Res. 2020 May 13;gkaa367. doi: 10.1093/nar/gkaa367