Modelling RNA-ions interactions

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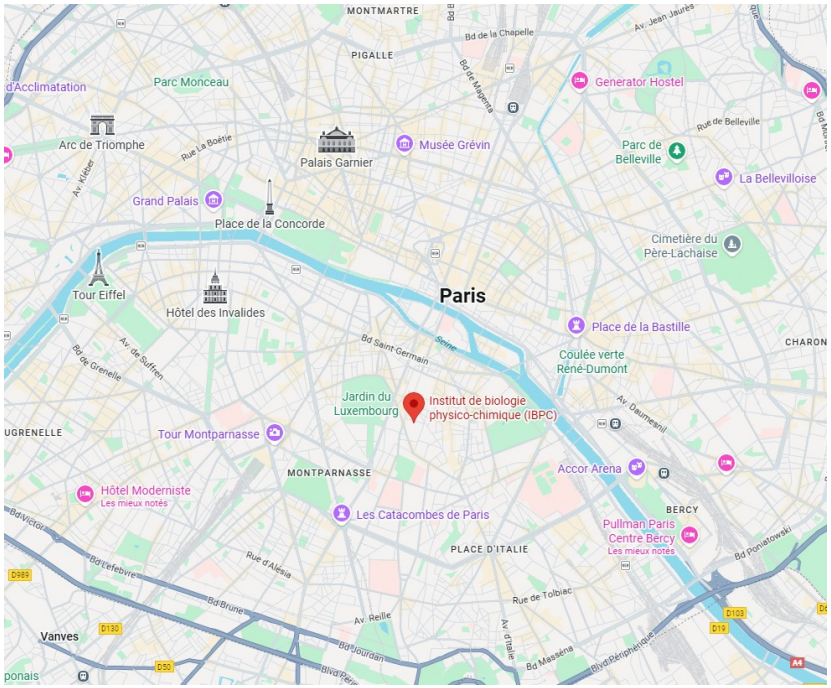
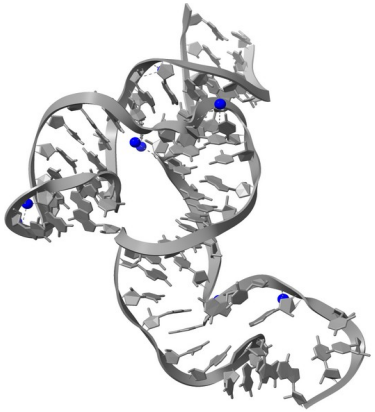


LABORATOIRE DE BIOCHIMIE THÉORIQUE (CNRS, PARIS)

Biomolecular simulations at different scales

reactivity

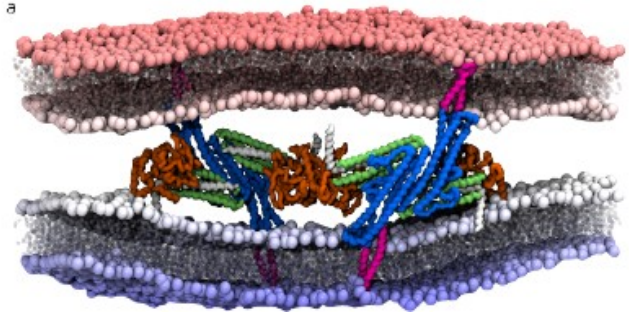


Single enzyme

QM/MM

Conformational sampling

Biomolecular assemblies



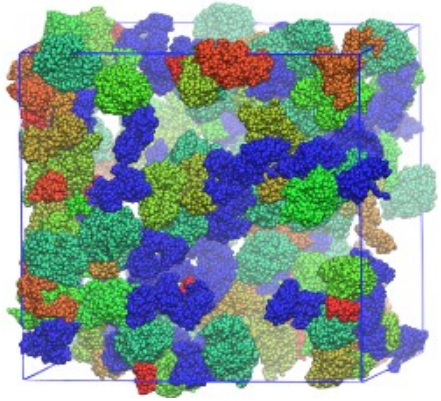
Membrane proteins, assemblies

All-atom MM

Hénin, Taly, Baaden, Sacquin-Mora,…

Crowding

Biomolecular condensates



Crowded solutions → cell?

Coarse grained

Sterpone

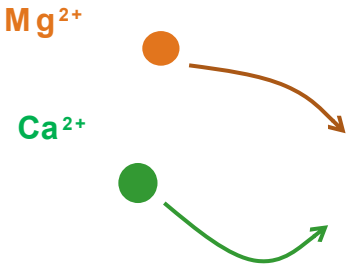
Methodological and software development

Enhanced sampling and free energy calculations (Colvars) Hénin

Visualization & virtual reality Baaden

Research Overview

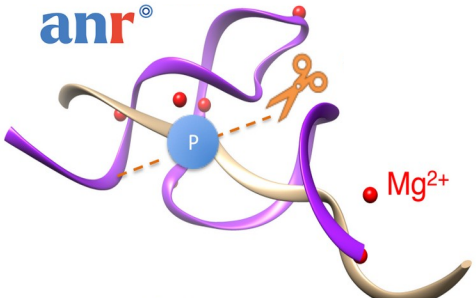
How nucleic acids interact with their complex environment (water, ions, proteins)?



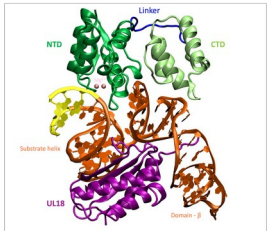
MD, Force fields, sampling, NNPs

RNA processing & reactivity

Ion effects in ribozyme catalysis



RNases

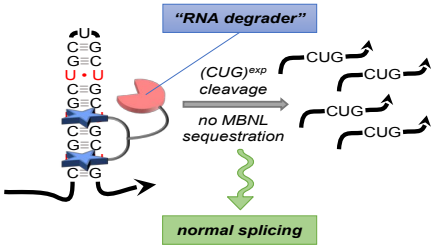


rRNA maturation

Coll. C. Tisné @ IBPC

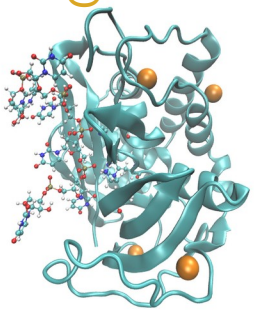
Design of artificial RNA degraders

Coll. A. Granzhan (LCBPT, UPCité)



allosteric activation of endoU

Coll. S. Campagne @ IECB



QM/MM, ML/MM, sampling

Overview « Ion-RNA interactions »

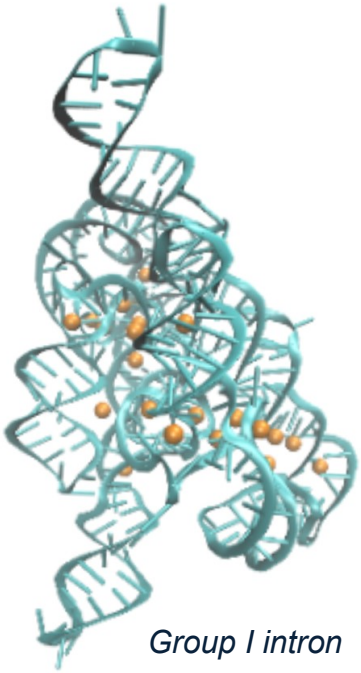
- Biological relevance of ion-RNA interactions
- Overview of experimental and theoretical
- All-atom MD simulations
  - Principles
  - Challenges
  - Force field refinement advances
  - Specific accelerated sampling strategies for ions

Biological Relevance of RNA-Ions Interactions

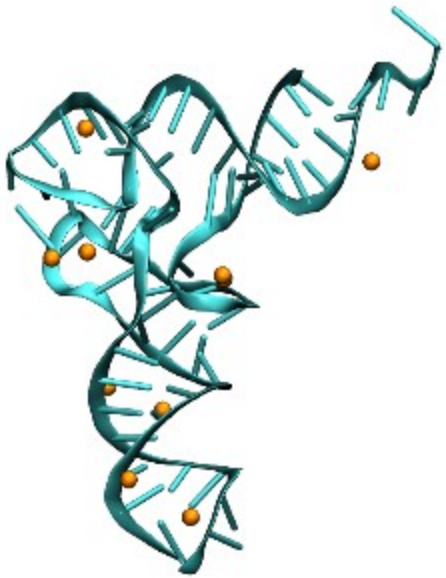
Mg^{2+} ions:

- stabilize **RNA tertiary structure**

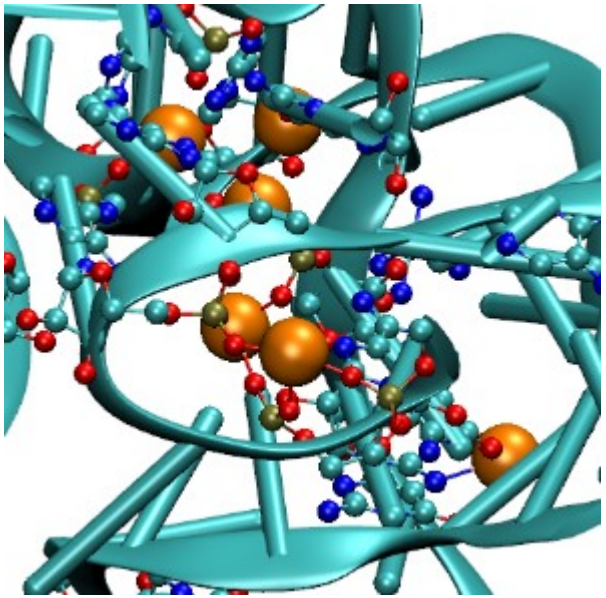
e.g. in introns, ribozymes, tRNAs



Group I intron



Phe tRNA (PDB 1EVV)



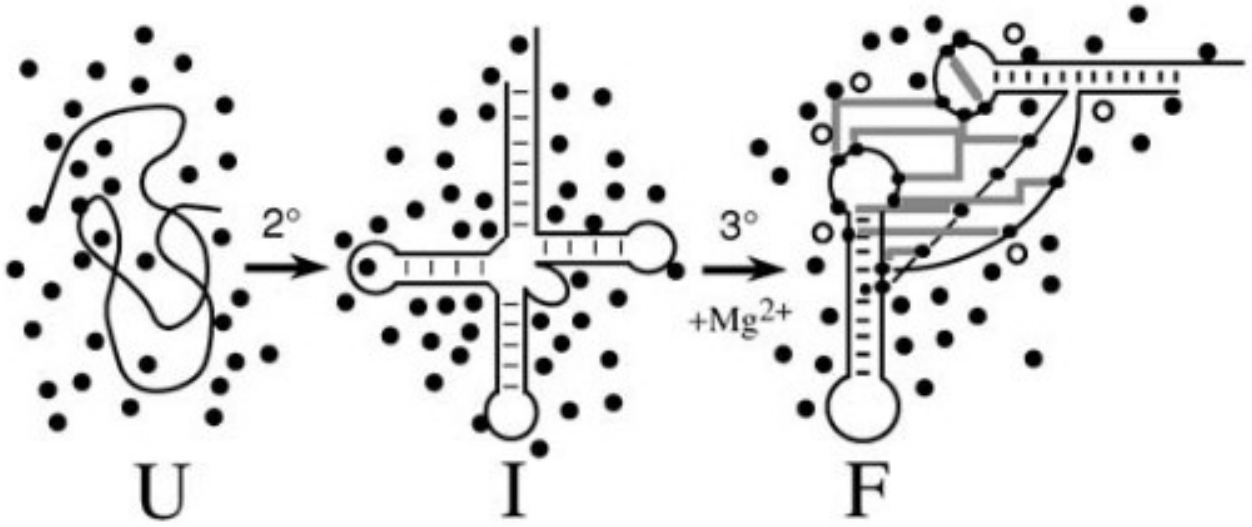
allow phosphate contacts

Draper et al., *Annu Rev Biophys Biochem Struct.*, **2005**, 34, 221
Cate JH, Gooding AR, Podell E, Zhou K, Golden BL, et al. *Science*, **1996**, 273, 1678-85
Pyle, Science, **1993**, 261, 709-714

Biological Relevance of RNA-Ions Interactions

Mg^{2+} ions:

- essential for **RNA folding**



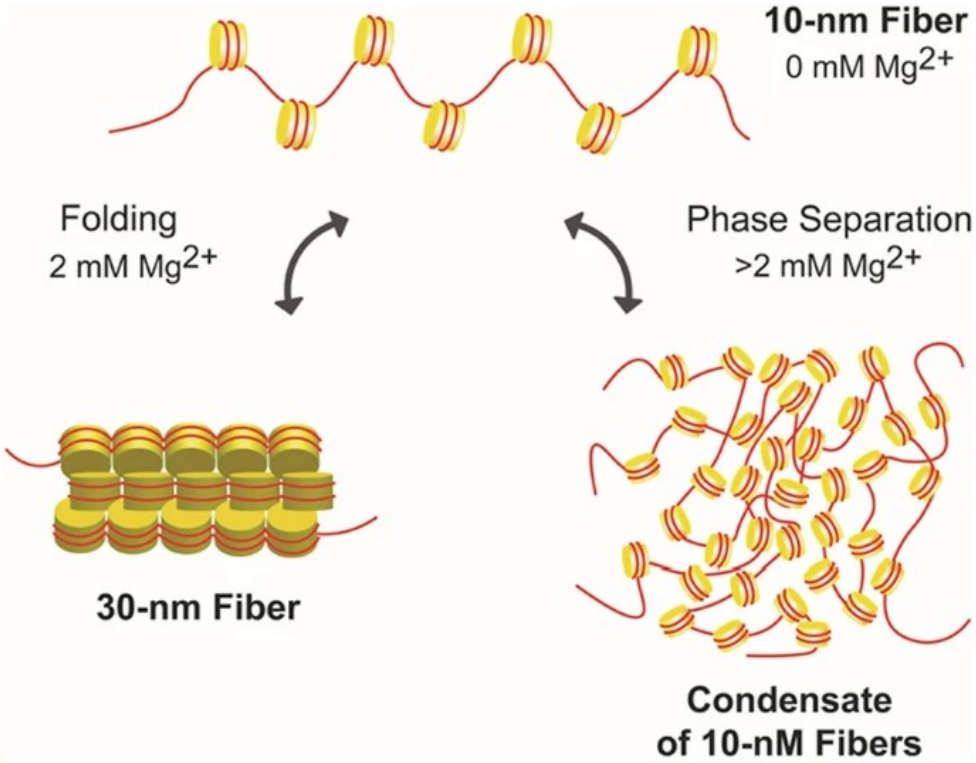
Draper et al., *Annu Rev Biophys Biochem Struct.*, **2005**, 34, 221

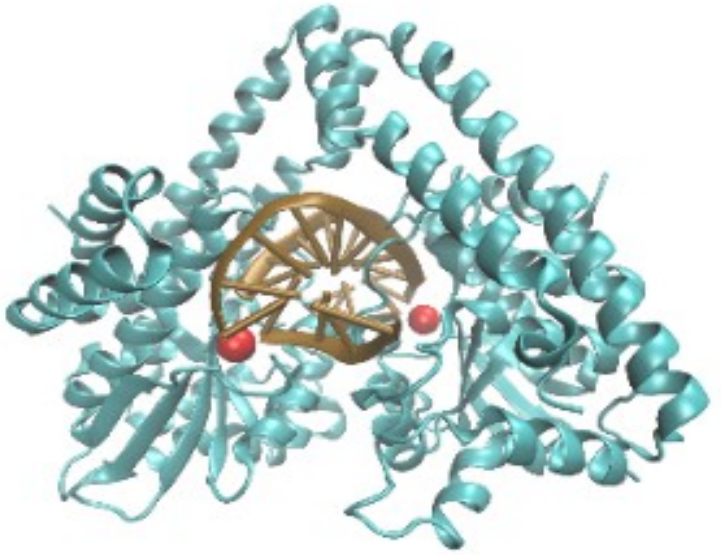
Biological Relevance of RNA-Ions Interactions

Mg^{2+} ions:

- Mediate nucleic acid – protein complex formation

- Regulate conformational state of nucleosomal arrays





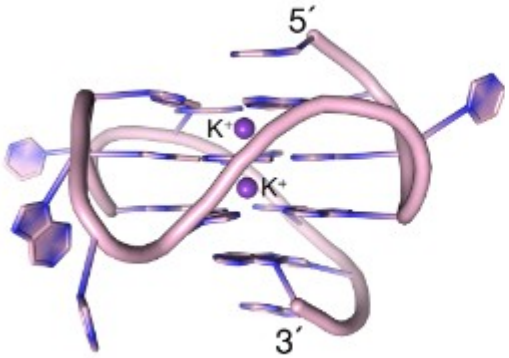
Restriction endonuclease

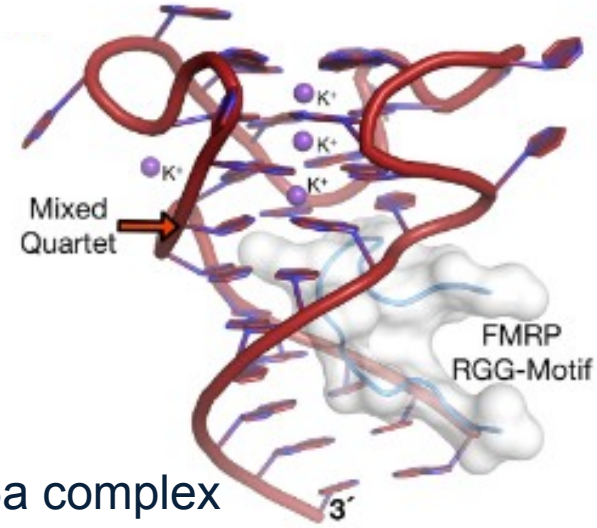
Hansen et al., *Epigenetics and Chromatin,* **2021**,

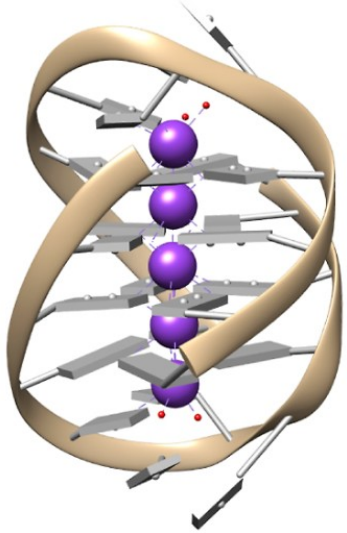
RNA –ION INTERACTIONS: WHY RELEVANT

Ions essential for stability of G-quadruplexes (G4)

K^+ ions in RNA G4s

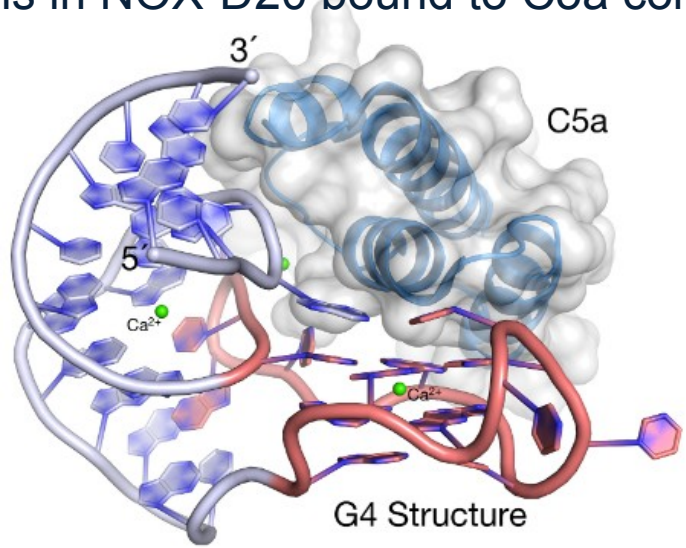






d($G_4T_4G_4$)

Ca^{2+} ions in NOX-D20 bound to C5a complex



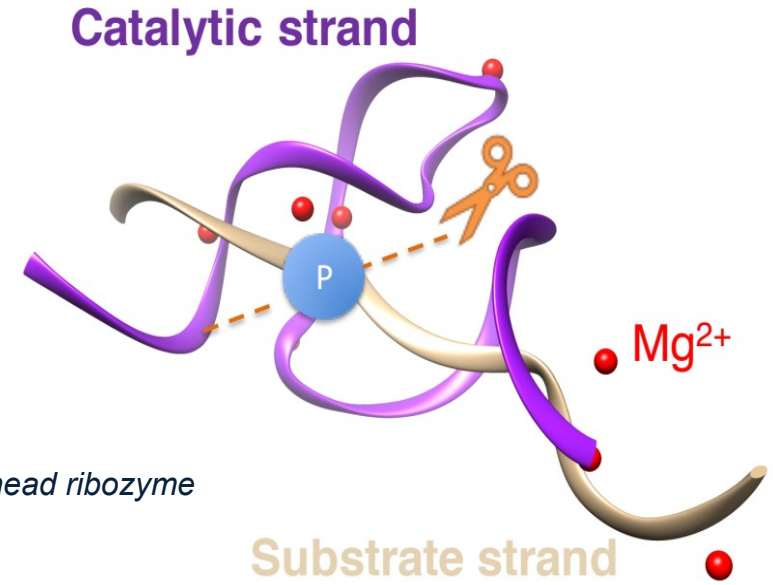
Well characterized ion effects in G4 folding, stability and polymorphism

Banco, Ferré d'Amaré, *RNA*, **2021**, 27, 390
Campbell, Neidel, **2012,** G4s and metal ions

Biological Relevance of RNA-Ions Interactions

Ion effects in ribozyme activity

- Ribozymes = RNA-based enzymes
- RNA world hypothesis
- Self-cleaving (phosphoester bond cleavage)
- Ion effects depend on ribozyme families and sequence



RzB Hammerhead ribozyme

Mg^{2+} >> Na^{+} (x10 000)
Ca^{2+} > Na^{+} (x400)

Hanna & Doudna, Curr Op Chem Biol, 2000, 4, 166
Mir…, Golden, *Biochimistry*, **2015,** 54, 5369
Roychowdhury-Saha…, Burke, *RNA*, **2006,** 12, 1846

Nucleic Acids & Ions

Ion concentrations inside a cell

~150 mM K^+

~15 mM Na^+

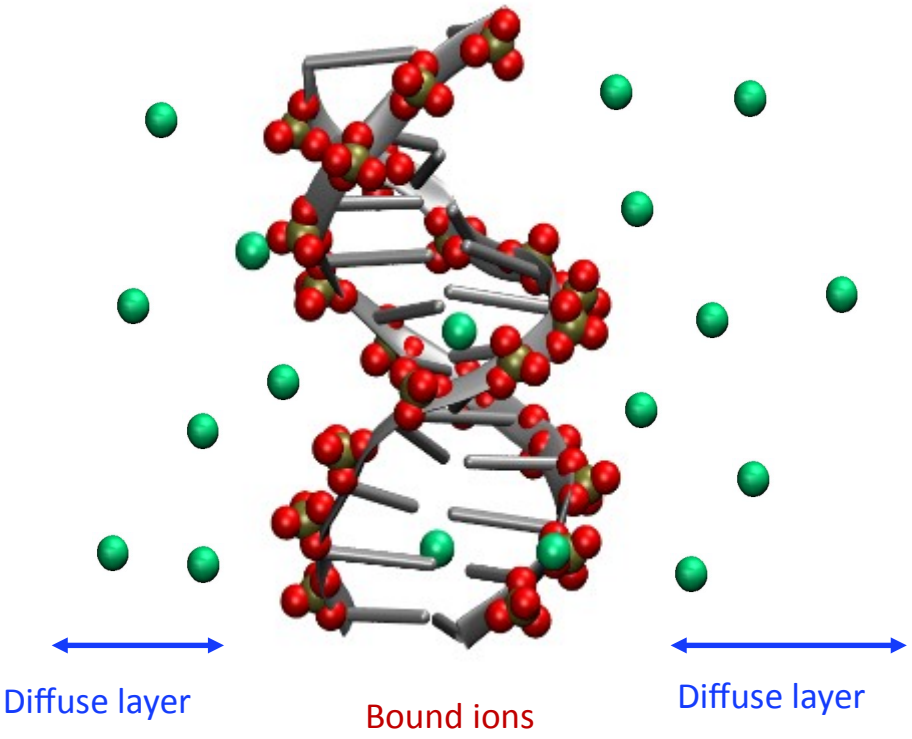
~30 mM Mg^{2+} (1 mM free)

~100 nM Ca^{2+} (signaling bursts)

Highly negatively charged biopolymers

→ « **ion atmosphere** » = **diffuse layer**

+ **Specific binding sites**, long lived interactions



Lipfert,…, Herschlag, *Annu Rev Biochem,* **2014,** 83, 813

Open questions

Structure of ion atmosphere? Competing ions K^+ vs Mg^{2+}?

Localization of binding sites ? Binding modes ? Strength?

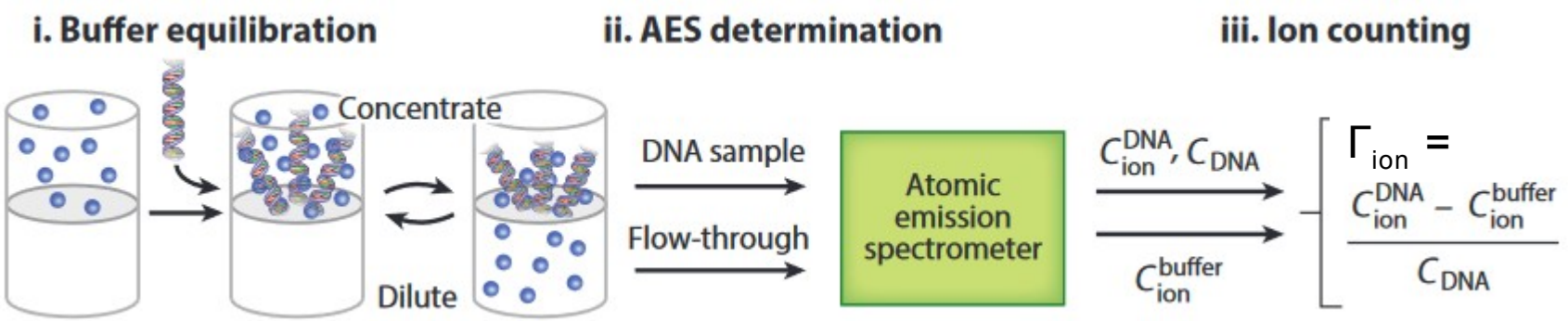
Impact on nucleic acid structure & large scale conformational changes?

PROBING THE ION ATMOSPHERE: EXPERIMENTS

Ion counting or BE-AES (Buffer equilibration – Atomic emission spectroscopy)

- equilibrium dialysis
- Comparing elemental composition of the nucleic acid and the flow-through sample

→ **Preferential interaction coefficient** Γ_{ion} = excess number of ions



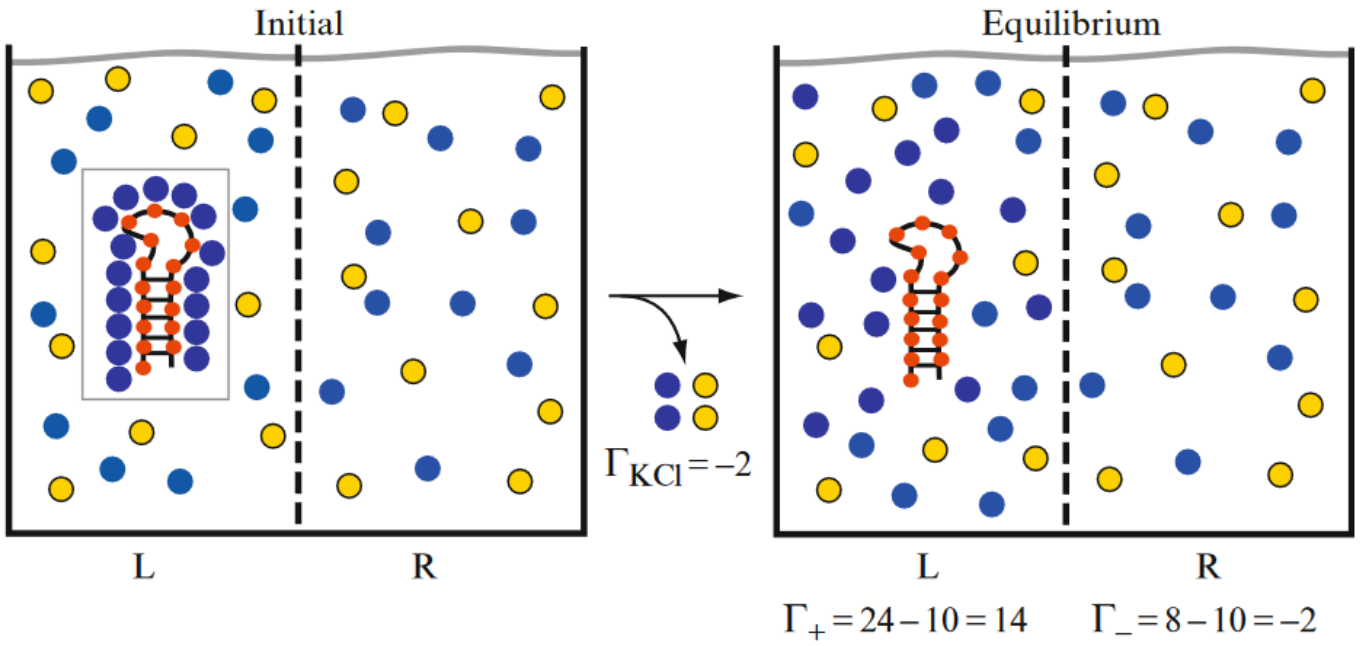
More recently: AES replaced by « inductively coupled plasma mass spectroscopy » (ICP-MS)

Leipply, Lambert, Draper, **2009**
Gebala, …, Herschlag, *JACS*, **2015**, 137, 14705

PROBING THE ION ATMOSPHERE: EXPERIMENTS

Ion counting

→ Example of determination of Γ_{ion}

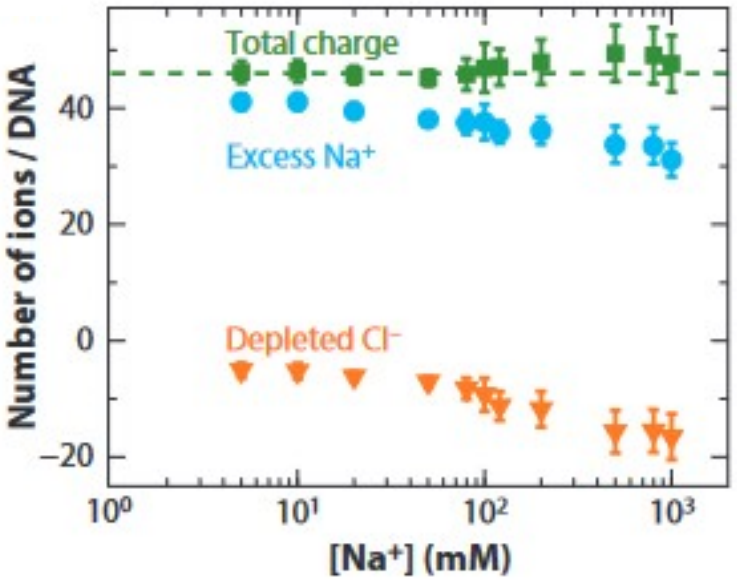


Leipply, Lambert, Draper, **2009**
Gebala, …, Herschlag, *JACS*, **2015**, 137, 14705

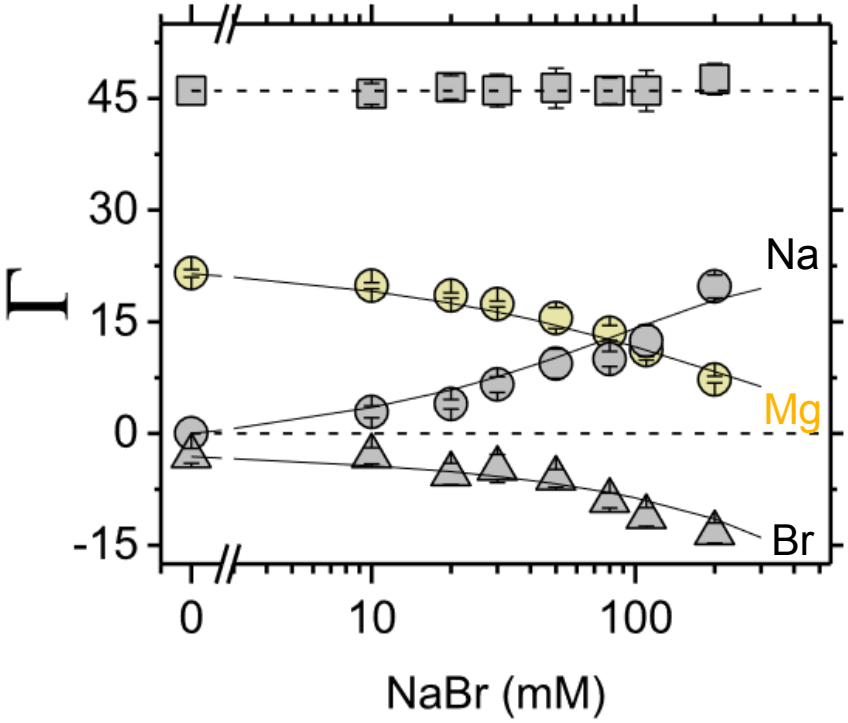
Probing the ion atmosphere: Experiments

Ion counting

24 bp DNA duplex



24 bp RNA duplex
Competition Mg^{2+} vs Na^{+}



Limitations

Lack of spatial resolution

Does not distinguish specific from diffuse binding

Leipply, Lambert, Draper, **2009**
Gebala, …, Herschlag, *JACS*, **2015**, 137, 14705
Gebala, …, Herschlag, *BiophysJ*, **2019**, 117, 1116

PROBING THE ION ATMOSPHERE: THEORY

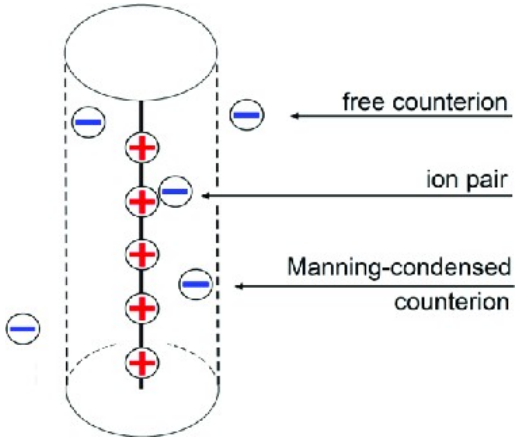
Manning theory for polyelectrolytes

Manning, 1969, Acc Chem Res

Counterion condensation onto polyions (idealized charged line)

Central idea: polyion unstable if
linear charge density > critical value

$$\Gamma = \lambda_B / l_{charge} > 1$$



l_{charge} = distance between two charges on polymer

λ_B = Bjerrum length

$$\lambda_B = \frac{e^2}{4\pi\varepsilon_0\varepsilon_r\, k_B T}$$

Typically ~7Å in water

Condensation occurs until Γ = 1

Number of **bound counterions**,
independent of ion concentration

$$f_{bound} = 1 - 1 / \Gamma$$

Manning radius? Depends on C → can be quite large

PROBING THE ION ATMOSPHERE: THEORY

Continuum electrostatics: Poisson-Boltzmann approaches

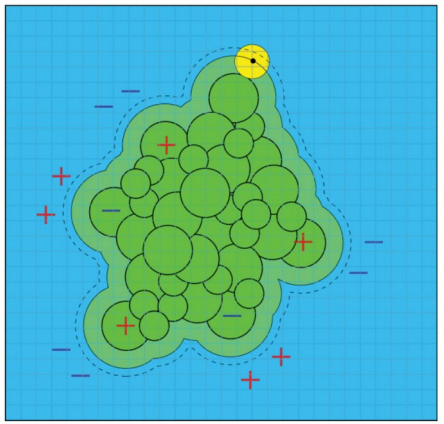
Jarayam, Beveridge, *Annu. Rev. Biophys. Biomol. Struct.*, **1996**, 25, 367

mesoscopic theory
biomolecule = low dielectric cavity, fixed charge distribution
solvent = dielectric continuum

$$\nabla \cdot [\epsilon(\mathbf{r})\nabla\psi(\mathbf{r})] + 4\pi\left[\rho^{\mathrm{f}}(\mathbf{r}) + \rho^{\mathrm{ion}}(\mathbf{r})\right] = 0$$

For weak electrostatic potential: Linearized PB equation

$$\nabla \cdot [\epsilon(\mathbf{r})\nabla\psi(\mathbf{r})] + 4\pi\rho^{\mathrm{f}}(\mathbf{r}) - \frac{8\pi e^2 I}{kT}\lambda(\mathbf{r})\psi(\mathbf{r}) = 0$$



Numerical resolution of Poisson-Boltzmann equations
→ predicts equilibrium electrostat. potential and ion distributions

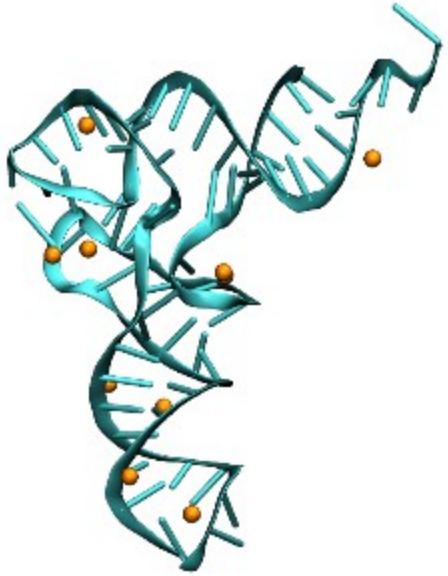
More about PB theory, hypotheses and limitations in for instance Grochowski and Trylska, Biopolymers, 2008

SPECIFIC BINDING SITES

Characterization of specific binding sites

X-ray spectroscopy

Auffinger, Westhof, *JMB*, **2000**, 300, 1113
Leonarski,…, Auffinger, *NAR*, **2025**, 53, gkae1148
Shui,…, Williams, *Structure*, **1998**, 37, 8341
Denisov, Halle, *PNAS*, **2000**, 97, 629
Hud, Polak, *Curr.Opin.Struct. Biol.* **2001**, 11, 293
Lipfert, Doniach, Das, Herschlag, **2014**
Jacobson, Saleh, *NAR*, **2017**, 45, 1596



Phe tRNA
(PDB 1EVV)

Far from solution conditions?
Crystal artefacts?
Only few ions identified? Errors in identification?

Auffinger et al, *RNA*, **2021**, 27, 243

Other approaches:
- ^{23}Na NMR

Denisov, Halle, *PNAS*, **2000**, 97, 629

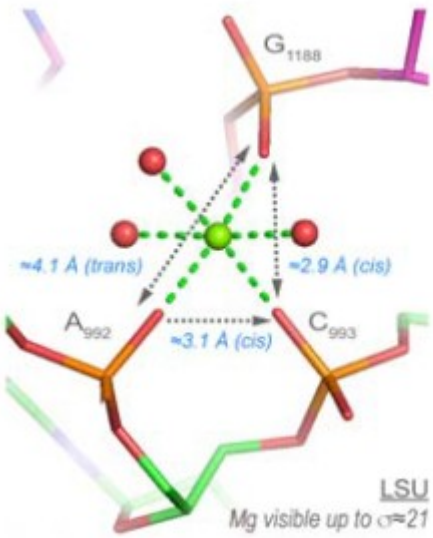
- cryoEM (few structures with high enough resolution)

SPECIFIC BINDING SITES

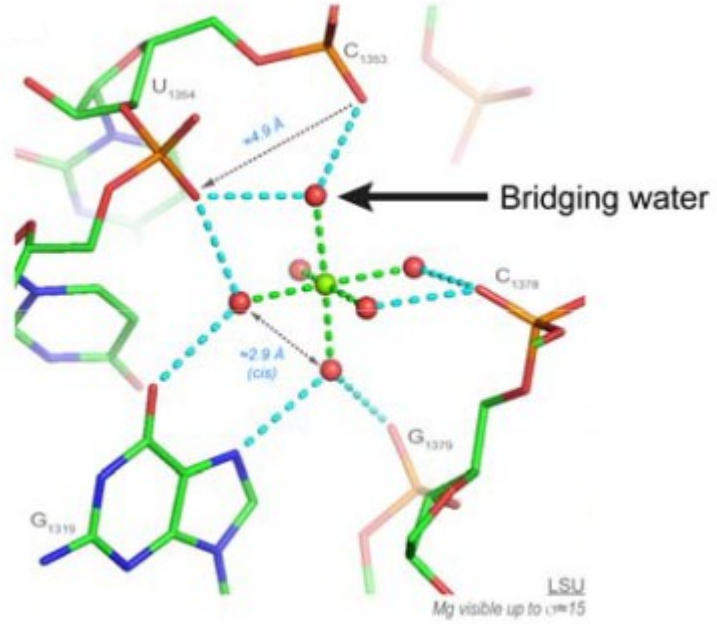
Identification of typical binding geometries

Leonarski, …, Auffinger, *NAR*, **2025,** 53,1148
Cat_wiz toolkit, Naleem… Auffinger, **2025**, *BioRXiv*

- Analysis of a 1.55A resolution ribosome cryo-EM structure
- Use geometric criteria for Mg2+ hydration and coordination geometry



Inner shell (direct contact)

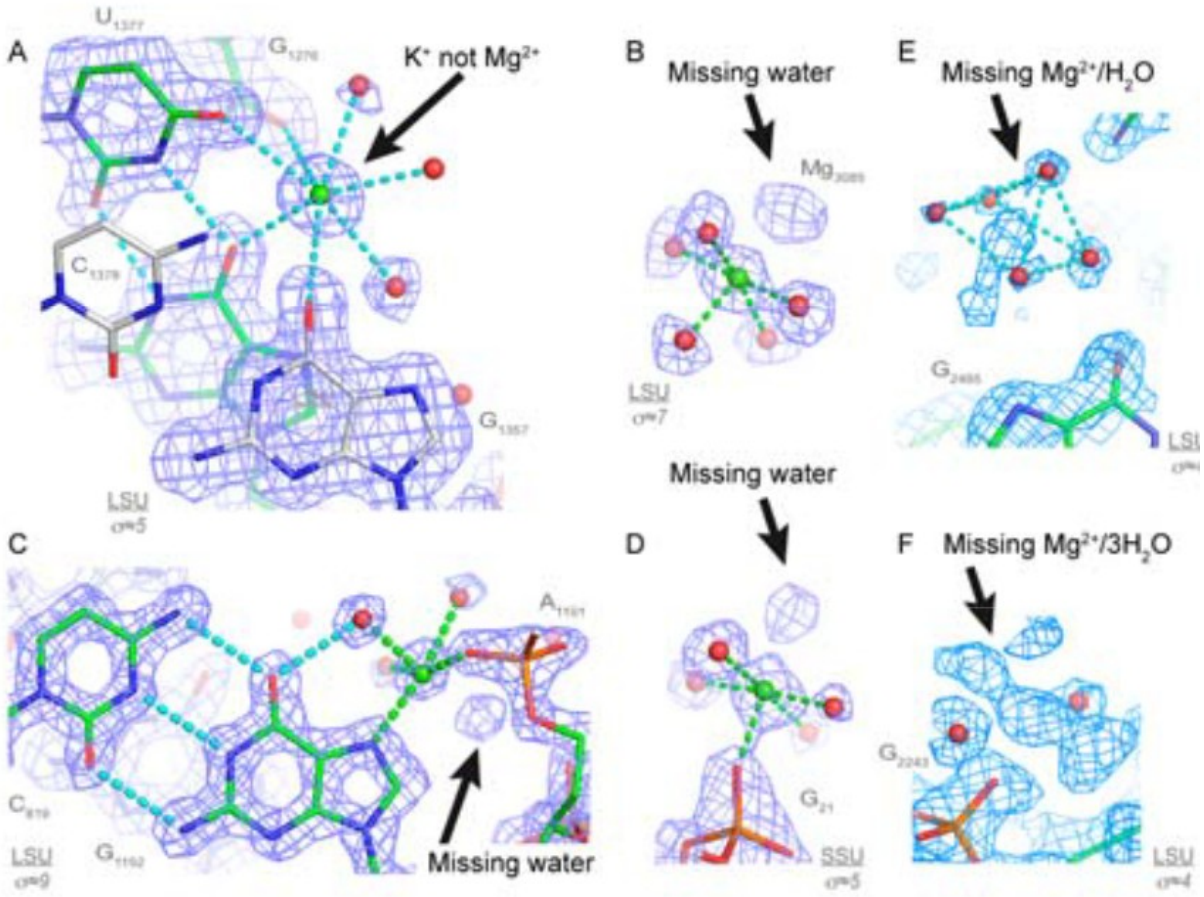


Outer shell (solvent-shared contact)

Specific Binding sites

Identification of typical binding geometries

Leonarski, …, Auffinger, *NAR*, **2025,** 53,1148
Cat_wiz toolkit, Naleem… Auffinger, **2025**, *BioRXiv*



- Correct misassignements
- Find previously unidentified ions
- Most ions still missing! 634 Mg2+/K+ ions identified
  = +1040 charges << 5000 rRNA nt

Limitations & Current Challenges

- Identification of interactions sites and occupancy / dynamics
- Molecular level description of ion binding modes & strength
- Rationalize impact on RNA properties?

Can Molecular Dynamics bring useful molecular-level insights into ion-binding?

Auffinger, Westhof, *JMB*, **2000**, 300, 1113
Rueda, …, Orozco, *Biophys J*, **2004**, 87, 800
Yoo, Aksimentiev, *JPCB*, **2012**, 116, 12946

ALL-ATOM MD SIMULATIONS: PRINCIPLE

- **System definition**

  - 1 particle = 1 atom

  - explicit solvent

- **Interaction potential between particles** $U(\mathbf{r}^N)$

$\mathbf{r}^N$: 3N coordinates { x,y,z} for N particles (atoms)

functional form + parameters = **forcefield**

- **Trajectory = numerical propagation of Newton's eq. of motion**

$$m_i \frac{\mathrm{d}^2 \boldsymbol{r}_i}{\mathrm{d}t^2} = \boldsymbol{F}_i = -\frac{\partial U(\mathbf{r}^N)}{\partial \boldsymbol{r}_i}$$

Integration step (« timestep ») Δt : typically 1 – 2 fs

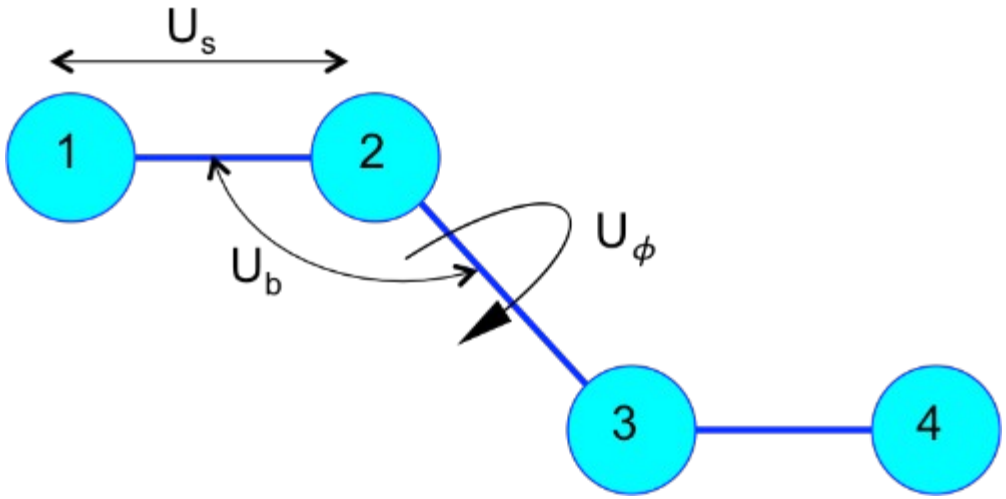
→ 10^6 iterations for 1 ns ; 10^9 iterations for 1 µs

Biomolecular (Class I) Force Fields

- Additive potentials
- Sum of bonded and non-bonded terms (no cross terms)

$$U(\mathbf{r}^N) = U_{\text{bonded}} + U_{\text{non-bonded}}$$

$$U(\mathbf{r}^N) = \underbrace{U_{\text{stretch}} + U_{\text{bend}} + U_{\text{torsion}}}_{\text{intramolecular}} + \underbrace{U_{\text{vdw}} + U_{\text{elec}}}_{\text{Intermolecular + > 3 bonds apart}}$$



Vanommeslaeghe, Guvench, MacKerell, Curr Pharm. Des., 2014, 20, 3281

BIOMOLECULAR (CLASS I) FORCE FIELDS

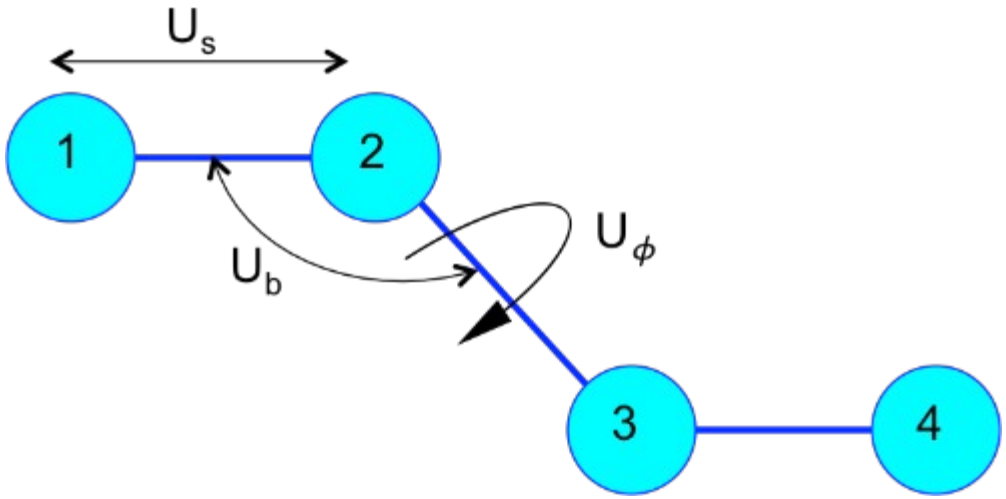
CHARMM, AMBER, OPLS, GROMOS,…

Intramolecular interactions: harmonic terms only, no anharmonicity

$$U_{\text{bonded}} = \sum_{\text{bonds}} k_s (r - r_0)^2 + \sum_{\text{angles}} k_b (\theta - \theta_0)^2 + \sum_{\text{torsions}} V_n [1 + \cos(n\phi - \delta)]$$

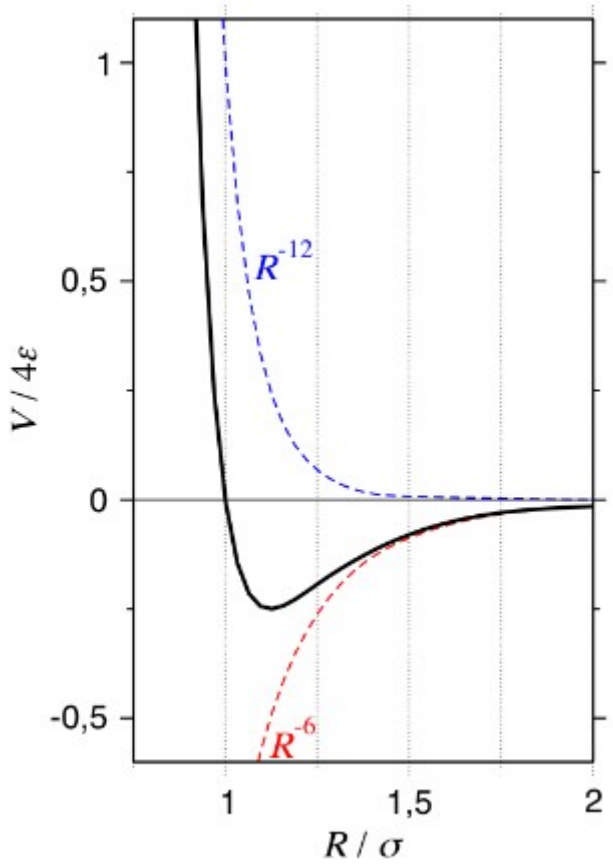
Intermolecular interactions: Coulomb + van-der-Waals

$$U_{\text{non-bonded}} = \sum_{i<j} \frac{q_i q_j}{4\pi\epsilon_0 r_{ij}} + \sum_{i<j} 4\epsilon_{ij} \left[\left(\frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right]$$



VAN DER WAALS INTERACTIONS

London dispersion forces in $1/R^6$ and repulsive wall at short distances



Lennard-Jones 6-12 potential

$$U_{\mathrm{LJ}}(r) = 4\epsilon_{ij}\left[\left(\frac{\sigma_{ij}}{r}\right)^{12} - \left(\frac{\sigma_{ij}}{r}\right)^{6}\right]$$

Combination rules (Lorentz-Berthelot)

$$\sigma_{ij} = \frac{1}{2}\left(\sigma_{ii} + \sigma_{jj}\right)$$

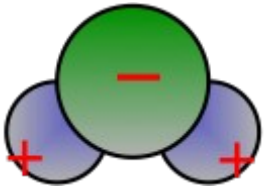
$$\varepsilon_{ij} = \sqrt{\varepsilon_{ii}\,\varepsilon_{jj}}$$

Possible to define pair-specific terms

(NBFIX approach in CHARMM)

ELECTROSTATIC INTERACTIONS

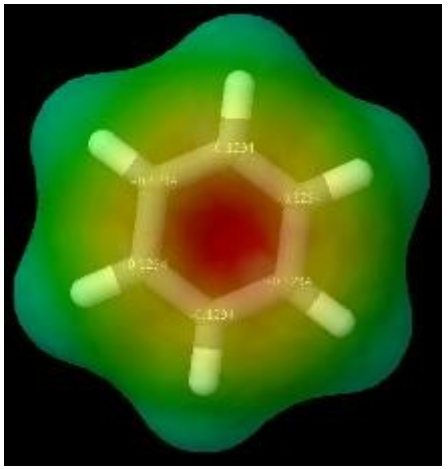
Interaction between fixed punctual atomic charges



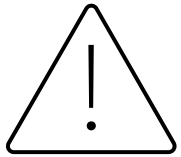
$$U_{\text{elec}} = \sum_{i<j} \frac{q_i q_j}{4\pi\epsilon_0 r_{ij}}$$

Atomic partial charge is NOT an observable (= cannot be measured)

- partitioning QM electronic density
- fit electrostatic potential
- refine vs experiments?



Different ways to derive atomic charges in most popular biomolecular forcefields (e.g. Amber vs CHARMM)



partial charges depend on polarity of the environment

→ in water, dipole moment increased by 20-50% compared to gaz phase

Biomolecular Force Fields

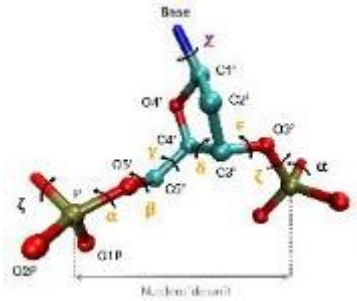
- Popular families: CHARMM, AMBER (favored for RNA)

- Different versions inside each family

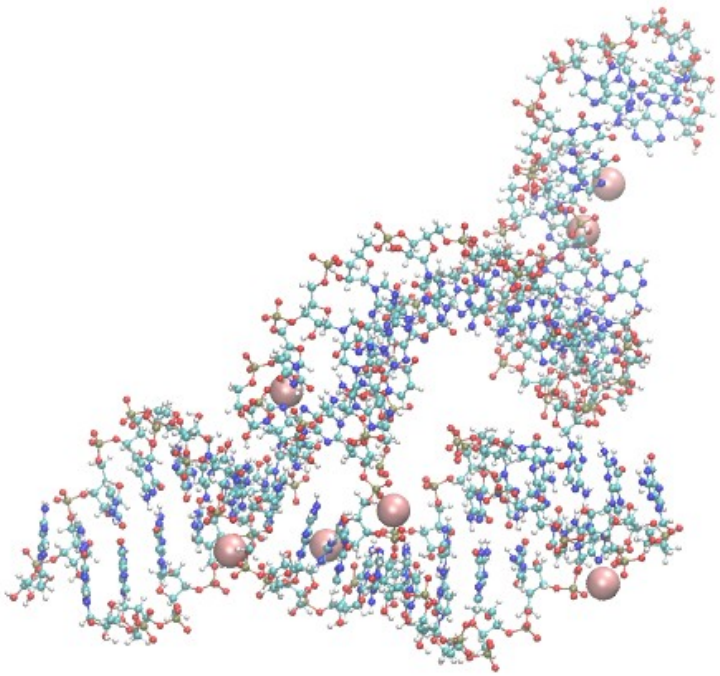
Ex: Amber force fields for DNA

Name	Modification	Notes
ff94	Original force field file	Obsolete
ff98	Modified charge set	Obsolete
ff99	Updated charge set	Foundation for all current ff's
bsc0	Barcelona α/γ backbone modification	[53]
ε/ζ OL1	ε/ζ modification for DNA	improvement for DNA, no effects for RNA [66]
χ OL4	χ modification tuned for DNA	[65]
β OL1	β dihedral modification tuned for DNA	improvement for DNA, no effects for RNA[67]
OL15	(ε/ζOL1+χOL4+βOL1)	[68]
bsc1	Update to bsc0	[69]
tumuc1	Refined electrostatic terms	[70, 71]
OL21	OL15 + α/γ modification	[72]
OL24	OL21 + δ/τ_1 modifications	[73]

Table 3.2.: *Force field name and modifications for simulating nucleic DNA. Recommended variants are listed in italics.*



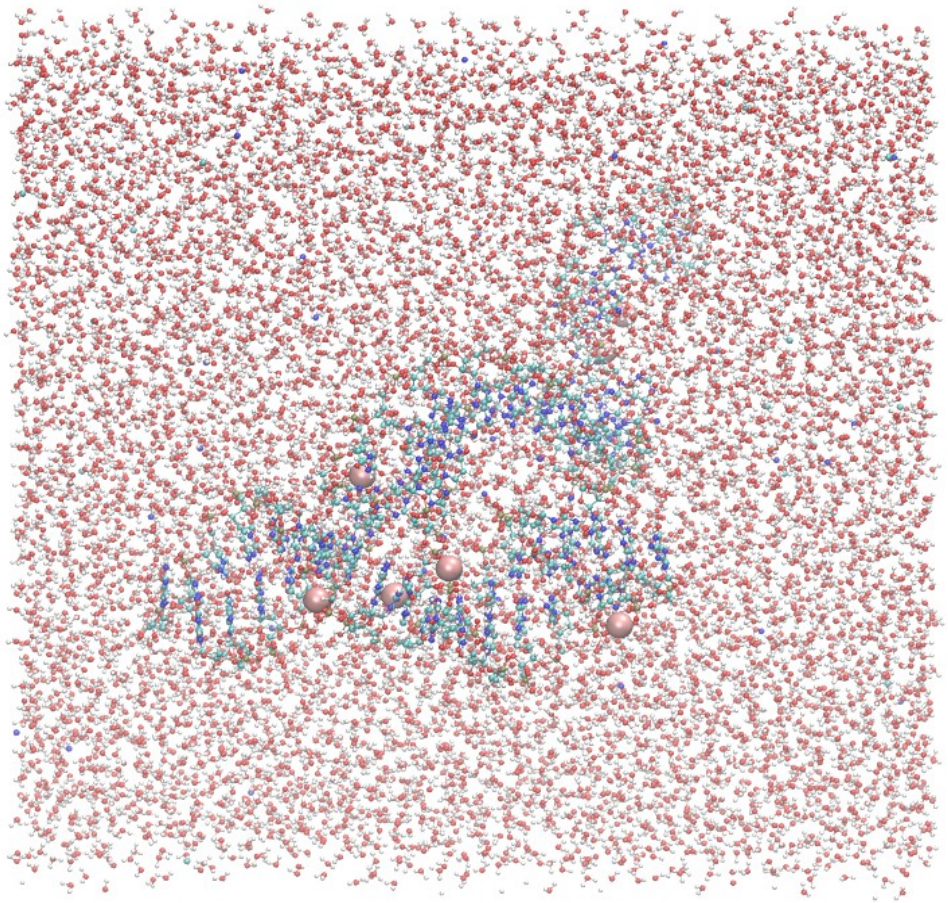
MD SIMULATIONS: ALL-ATOM DESCRIPTIONS - EXAMPLES



Hammerhead ribozyme, PDB 5EAO
RNA, 48 nucleotides, 1457 atoms
8 Mg^{2+} ions

MD SIMULATIONS: ALL-ATOM DESCRIPTIONS - EXAMPLES

Explicit solvent + ions



10 nm

~ µs simulations

97 000 atoms, 31800 water molecules , 150mM NaCl (135 Na^+, 84Cl

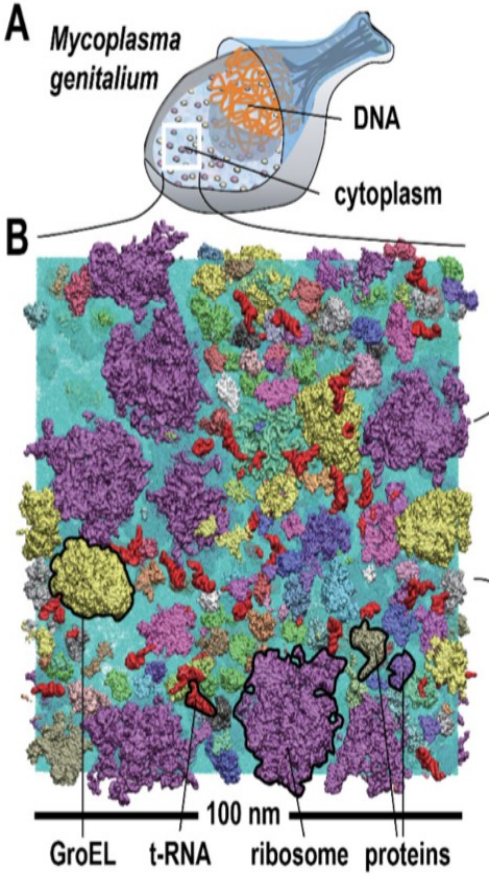
MD SIMULATIONS: ALL-ATOM DESCRIPTIONS - EXAMPLES

eLife

Biomolecular interactions modulate macromolecular structure and dynamics in atomistic model of a bacterial cytoplasm

Isseki Yu[1,2], Takaharu Mori[1,2], Tadashi Ando[3], Ryuhei Harada[4], Jaewoon Jung[4], Yuji Sugita[1,2,3,4]*, Michael Feig[3,5]*

System	MG_h	MG_{m1}
Cubic box length (nm)	99.8	48.2
Program	GENESIS	GENESIS
Simulation time	20 ns	140 ns
		number of
Ribosomes	31	3
GroELs	20	3
Proteins	1238	182
RNAs	284	28
Metabolites	41,006	5.005
Ions	214,000	23,049
Waters	26,263,505	2,944,143
Total # of atoms	103,708,785	11,737,298



https://www.youtube.com/watch?v=5JcFgj2gHx8

Yu, …, Sugita, Feig, eLife, 2016

MOLECULAR SIMULATIONS: CAN WE TRUST COMMON FORCE FIELDS?

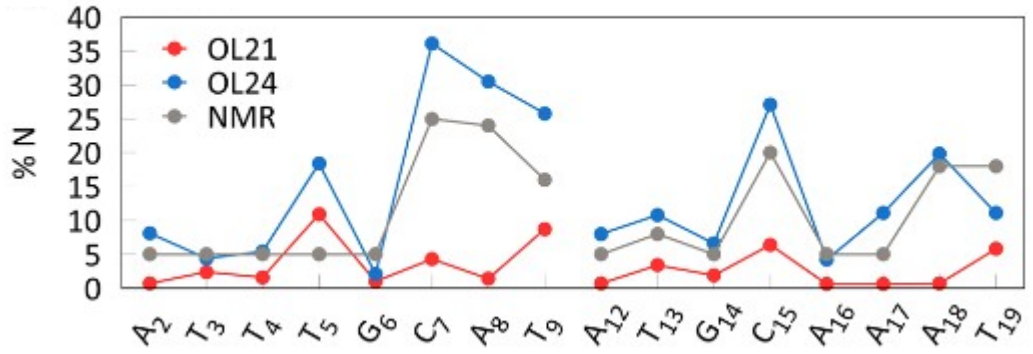
DNA & RNA force fields: mostly refined for conformational properties

Amber99ff-bsc0 / bsc1, OL corrections, CHARMM, Tumuc1…

Liebl & Zacharias, *JCTC*, **2021**, 17, 7096
Hart, …, MacKerell, *JCTC*, **2012**, 8, 348
Perez,…, Orozco, *BiophysJ,* **2007**, 3817 (bsc0); Ivani, …, Orozco, *Nat. Methods*, **2016**, 13, 55 (bsc1)
Sponer,…, Otyepka, **2018**, *ChemRev*, 118, 4177 ; Mlynsky,…, Sponer, *JCTC*, **2025**
…

Table 1. Average Structural Parameters (Last 800 ns of 1 μs Simulations) for the Dickerson–Drew Dodecamer[a]

	X-ray[b] (NMR[c])	ff99bsc0	ff99bsc0χ$_{OL4}$
minor groove width/Å	9.9 (10.5)	11.7 ± 0.8	11.1 ± 0.7
major groove width/Å	18.0 (17.6)	19.3 ± 0.7	18.6 ± 0.3
γ/deg	−113.6 (−110.8)	−119.7 ± 8.9	−115.6 ± 5.6
α/deg	−63.5 (−61.6)	−70.4 ± 4.7	−68.5 ± 3.7
β/deg	176.1 (173.5)	174.0 ± 1.5	176.1 ± 1.6
γ/deg	52.9 (50.3)	55.8 ± 2.2	55.3 ± 1.6
δ/deg	120.4 (126.7)	119.4 ± 8.4	125.4 ± 6.4
ε/deg	−181.5 (−171.6)	−173.5 ± 3.7	−176.1 ± 4.5
ζ/deg	−88.7 (−101.3)	−89.3 ± 3.5	−92.8 ± 2.1
P/deg	130.0 (136.8)	127.1 ± 14.4	138.0 ± 11.7
tm/deg	37.4 (33.4)	37.6 ± 1.4	37.4 ± 1.2
shift/Å	0.0 (0.0)	0.0 ± 0.2	0.0 ± 0.3
slide/Å	−0.08 (−0.2)	−0.5 ± 0.3	−0.4 ± 0.3
rise/Å	3.3 (3.2)	3.3 ·	
tilt/deg	−0.3 (0.0)	−0.1	
roll/deg	1.9 (3.0)	3.6 ;	
twist/deg	33.6 (35.7)	32.5	
shear/Å	−0.0 (0.0)	0.0 ;	
buckle/deg	1.2 (0.0)	−0.5	
stretch/Å	0.0 (−0.3)	−0.0	
propeller/deg	−11.4 (−17.5)	−12.	
stagger/Å	0.1 (−0.1)	0.0 ;	
opening/deg	1.8 (−1.1)	0.2 ;	
X-displacement/Å	−0.5 (−0.8)	−1.7	
Y-displacement/Å	−0.1 (0.0)	0.0 ;	
helical rise/Å	3.3 (3.2)	3.2 ;	
inclination/deg	4.1 (5.0)	7.4 ;	
tip/deg	0.6 (0.0)	0.1 ;	
helical twist/deg	34.0 (36.1)	33.7	



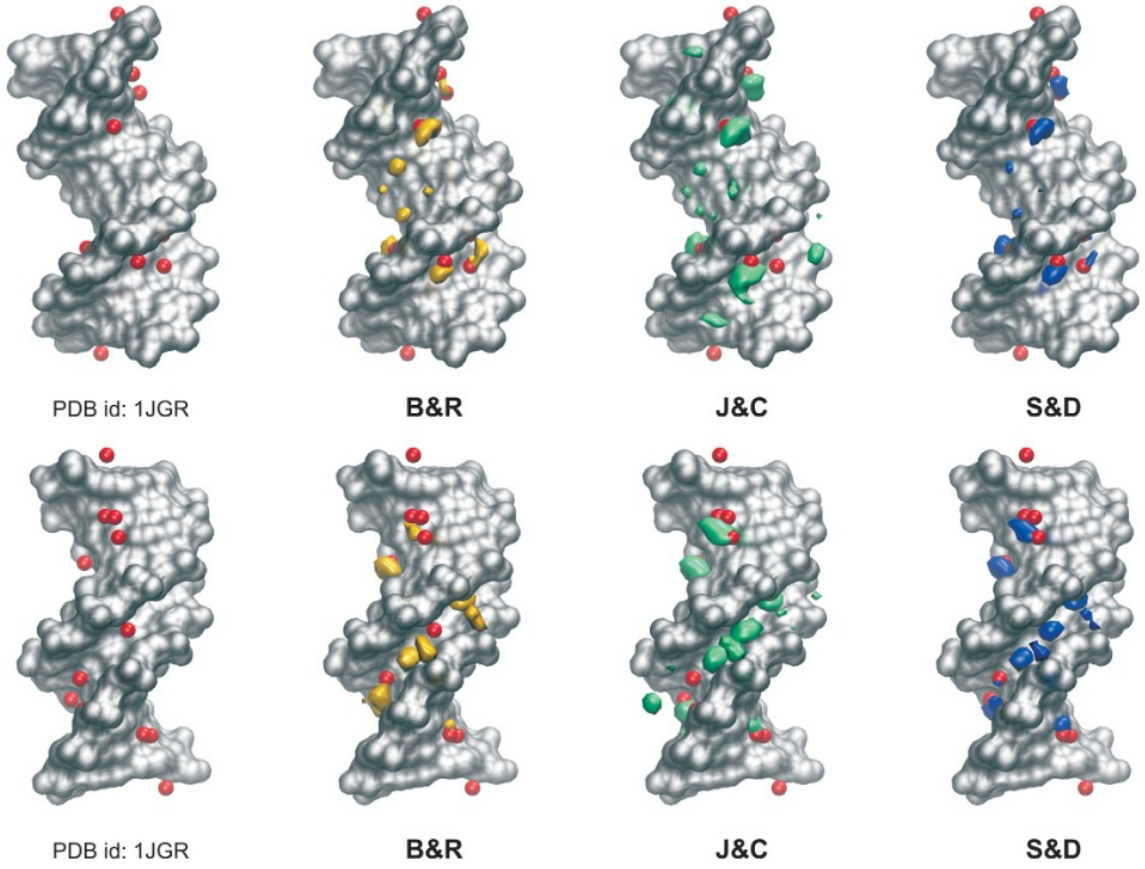
Zgarbova et al, JCTC, 2013 & 2015,…

MOLECULAR SIMULATIONS: CAN WE TRUST COMMON FORCE FIELDS?

How well is the interaction with ions captured?

- Reasonable behavior with **monovalent** ions

Pasi, …, Lavery, *NAR*, **2015**, 43, 2412
Dans,…, Orozco, *NAR*, **2016**, 44, 4052

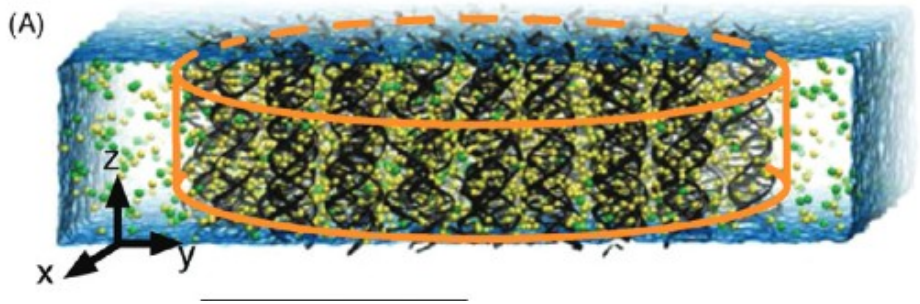


MOLECULAR SIMULATIONS: CAN WE TRUST COMMON FORCE FIELDS?

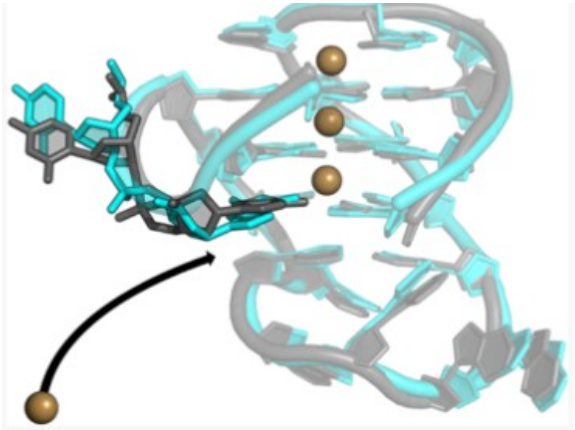
How well is the interaction with ions captured?

- **Limitations with monovalent ions**

Overbinding to phosphate groups in dense systems



Yoo, Aksimentiev, *JPCL*, **2012**, 3, 45



Recruitement of K^+ ions within G-quadruplexes
→ need polarizable ff?

Krepl, …, Sponer, *JCTC*, **2012**, 8, 2506-2520
Ratnasinghe, …, Lemkul, *JCIM*, **2020**, 60, 6476

MOLECULAR SIMULATIONS: CAN WE TRUST COMMON FORCE FIELDS?

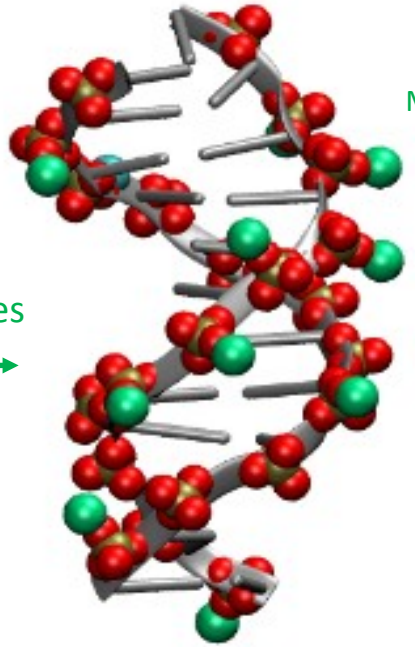
- **Divalent ions**: out-of-reach of common ff !

strong overbinding artefacts

Dans,…, Orozco, *NAR*, **2016**, 44, 4052
Sponer, …, Otyepla, *JPCL*, **2014**, 5, 1771

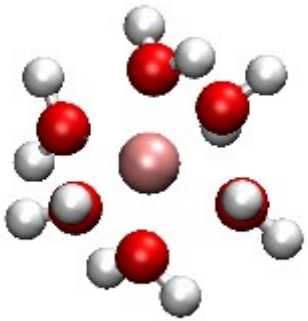
+ sampling issues

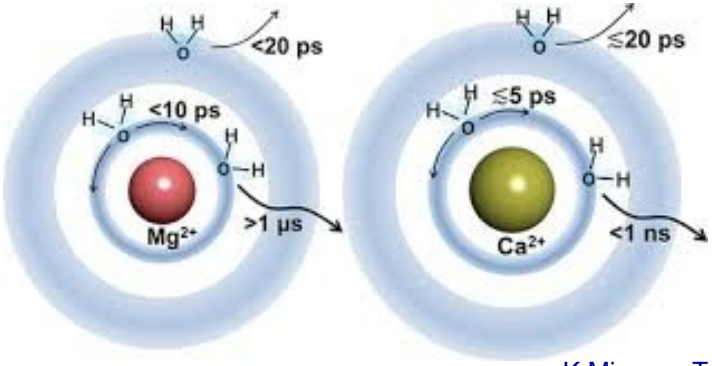
Dans,…, Orozco, *NAR*, **2016**, 44, 4052
Sponer, …, Otyepla, *JPCL*, **2014**, 5, 1771



Slow exchanges

>> µs



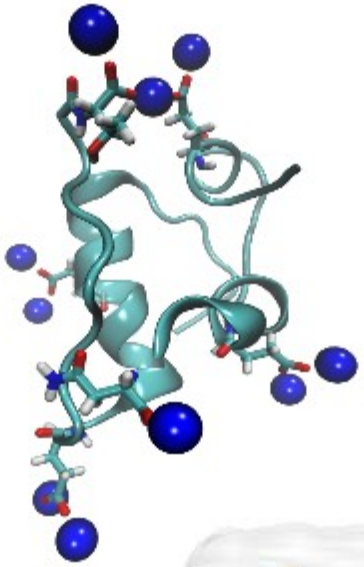


J A Cowan, *Inorg. Chem.* 1991, 30, 2740–2747
K Misawa, T Lee, S Ogawa, *BBA - Gen. Subj.* , **1982** 718, 227–229

Need for reliable force field + efficient sampling strategies!

OVERBINDING ARTEFACTS IN STANDARD FORCE FIELDS

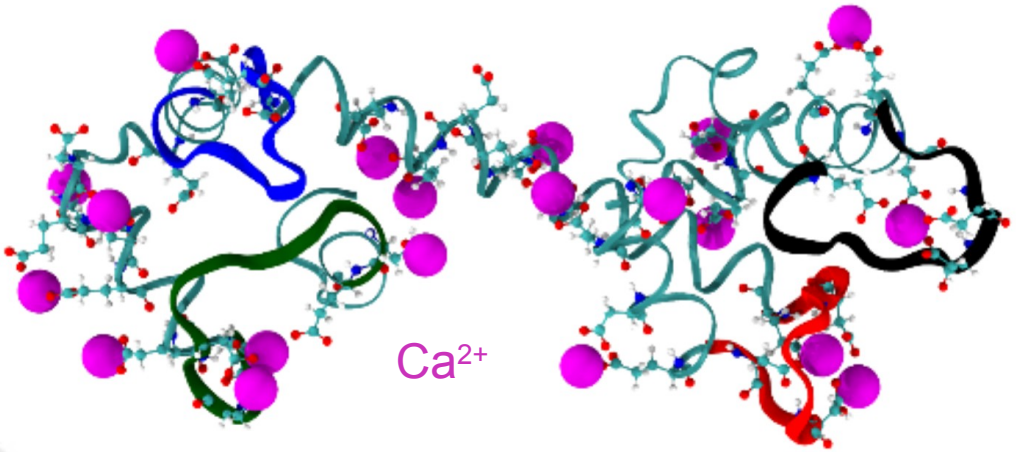
Insulin



Mg^{2+}

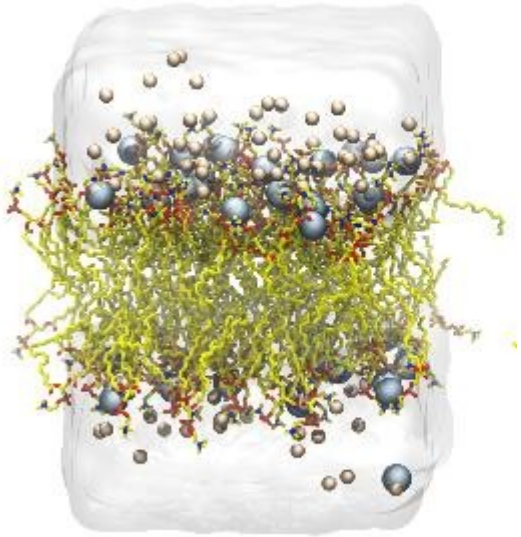
COO^-

Calmodulin

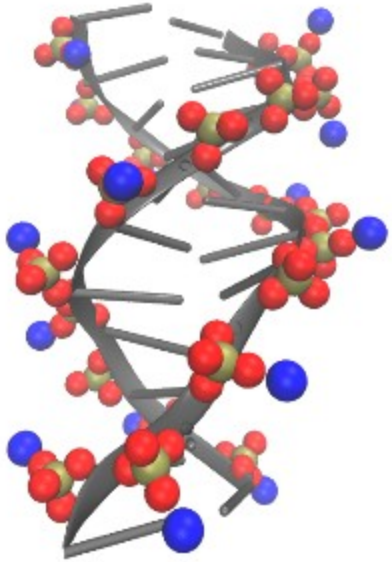


Ca^{2+}

Membranes



DNA



Accumulation of ions at biomolecular interfaces

STRATEGIES TO RECOVER MISSING ELECTRONIC POLARIZATION IN ALL ATOM SIMULATIONS

- **Standard** force fields

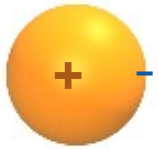
$$U_{\text{coulomb}} = \sum_{i<j} \frac{q_i q_j}{4\pi\epsilon_0 r_{ij}}$$

missing fast **electronic** polarization
= instantaneous response of electronic clouds

→ Strong **overbinding** artefacts

STRATEGIES TO RECOVER MISSING ELECTRONIC POLARIZATION IN ALL ATOM SIMULATIONS

- **Explicitly polarizable** force fields (e.g. AMOEBA, Drude)
  - **Drude** force field (Drude particles attached to heavy atoms)



    - Virtual sites carrying a partial charge q_D
    - Attached to heavy atoms via harmonic spring k_D

$$q_D = \sqrt{\alpha k_D}$$

Lemkul, Huang, Roux, MacKerell, *Chem. Rev.*, **2016**, 13, 2613-2626

  - **AMOEBA** (multipoles + induced dipoles)

Ponder, …, T. Head-Gordon, *J. Phys. Chem. B*, **2010**, 114, 2549-2564
Zhang…, Ren, *JCTC*, **2018**, 14, 2084-2108

Induced dipole on each site i

$$\mu_{i,\alpha}^{ind} = \alpha_i E_{i,\alpha} \quad (\alpha \in \{x, y, z\}) \qquad \alpha_i : \text{atomic polarizability}, \; E_{i,\alpha} : \text{electric field}$$

Mutual polarization by self-consistent iteration

$$\mu_{i,\alpha}^{ind} = \underbrace{\alpha_i \sum_{\{j\}} T_\alpha^{ij} M_j}_{\text{induced dipole on site i by the permanent multipoles of the other molecules}} + \underbrace{\alpha_i \sum_{\{j'\}} T_{\alpha,\beta}^{i,j'} \mu_{j',\beta}^{ind}}_{\text{interaction dipole on site i by the other induced dipoles}} \qquad T : \text{interaction matrix}$$

More complex parametrization

Higher computational cost

Strategies to recover missing electronic polarization in all atom simulations

- **Explicitly polarizable** force fields (e.g. AMOEBA, Drude)

STRATEGIES TO RECOVER MISSING ELECTRONIC POLARIZATION IN ALL ATOM SIMULATIONS

- **Implicit descriptions** of electronic polarization
  - **Improve ion ff with modified form of Lennard-Jones potential**

$$U_{ij}(r_{ij}) = \frac{e^2 Q_i Q_j}{r_{ij}} - \frac{C_4^{ij}}{r_{ij}^4} + \frac{C_{12}^{ij}}{r_{ij}^{12}} - \frac{C_6^{ij}}{r_{ij}^6}$$

$$= \frac{e^2 Q_i Q_j}{r_{ij}} - \frac{C_4^{ij}}{r_{ij}^4} + \varepsilon_{ij}\left[\left(\frac{R_{\min,ij}}{r_{ij}}\right)^{12} - 2\left(\frac{R_{\min,ij}}{r_{ij}}\right)^{6}\right]$$

Implicitly accounts for Ion-induced dipole interactions
Improved properties for high charge density ions

Li, Merz et al, JPCB **2015**

Not enough for ions-phosphate or ion-nucleobases interactions

Panteva, …, York, JPCB **2015**, 119, 15460

STRATEGIES TO RECOVER MISSING ELECTRONIC POLARIZATION IN ALL ATOM SIMULATIONS

- **Implicit descriptions** of electronic polarization
  - **Pair-specific Lennard-Jones** parameters (NBFIX)

Fyta, Netz, *J. Chem. Phys.*, **2011**, 136, 124103
Yoo, Aksimentiev, Phys. Chem. Chem. Phys., **2018**, 20, 8432

σ_1, ε_1 σ_2, ε_2

$$\sigma_{12} = \tfrac{1}{2}(\sigma_1+\sigma_2) \qquad \varepsilon_{12} = (\varepsilon_1\varepsilon_2)^{1/2}$$

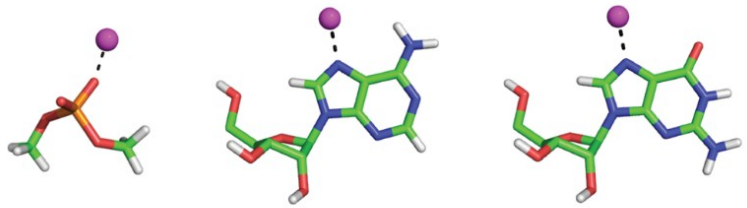
$$\sigma_{12} = \lambda_\sigma/2\,(\sigma_1+\sigma_2) \qquad \varepsilon_{12} = \lambda_\varepsilon(\varepsilon_1\varepsilon_2)^{1/2}$$

Optimized σ_{12} and ε_{12} for specific pairs
Need to be parametrized for each pair of interest

Ions – nucleic acid NBFIX terms for ion-phosphate and ion-nucleobases

- Target site-specific binding free energies derived from potentiometric pH-titration

Panteva, …, York, *JPCB* **2015**, 119, 15460
Grotz, …, Schwierz, *JCTC*, **2021**, 17, 2530



Dimethyl phosphate Adenosine Guanosine

STRATEGIES TO RECOVER MISSING ELECTRONIC POLARIZATION IN ALL ATOM SIMULATIONS

- **Implicit descriptions** of electronic polarization

« **Electronic Continuum Correction** » (ECC) = Scaled charges

$$F_{ecc} = \frac{1}{4\pi\epsilon_0} \frac{q_1' q_2'}{\epsilon_{elec} r^2}$$

q_1' q_2'

$\varepsilon_{el} \approx 1.78$ in water

→ charge scaled by $1/\sqrt{\varepsilon_{el}} \approx 0.75$

Leontyev, Stuchebrukov, *Phys. Chem. Chem. Phys.*, **2011**, 13, 2613-2626 ; Kirby, Jungwirth, *J. Phys. Chem. Lett,* **2019**, 10, 7531-7536
EDD, Javainainen, Delcroix, Jungwirth, Martinez-Seara, *J. Chem. Phys.*, **2020**, 050901

- Successfully tested on electrolytes, incl. phosphates
  scaling factor 0.75-0.85

Bruce, van der Vegt, *J. Chem. Phys.*, **2018**, 148, 222816
Puyo-Fourtine, Juillé, Hénin, Clavaguéra, EDD, *JPCB*, **2022**, 126, 4022

STRATEGIES TO RECOVER MISSING ELECTRONIC POLARIZATION IN ALL ATOM SIMULATIONS

- **Implicit descriptions** of electronic polarization

« **Electronic Continuum Correction** » (ECC) = Scaled charges

No additional cost!

Increasingly popular for proteins, phospholipids, nucleic acids (DES-Amber)

JCTC Journal of Chemical Theory and Computation

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Article

Effective Inclusion of Electronic Polarization Improves the Description of Electrostatic Interactions: The prosECCo75 Biomolecular Force Field

Ricky Nencini, Carmelo Tempra, Denys Biriukov, Miguel Riopedre-Fernandez, Victor Cruces Chamorro, Jakub Polák, Philip E. Mason, Daniel Ondo, Jan Heyda, O. H. Samuli Ollila, Pavel Jungwirth, Matti Javanainen, and Hector Martinez-Seara*

Cite This: https://doi.org/10.1021/acs.jctc.4c00743 Read Online

Development of Force Field Parameters for the Simulation of Single- and Double-Stranded DNA Molecules and DNA–Protein Complexes

Maxwell R. Tucker, Stefano Piana,* Dazhi Tan, Michael V. LeVine, and David E. Shaw*

Cite This: J. Phys. Chem. B 2022, 126, 4442–4457 Read Online

We developed two force field parameter sets: one (which we name *DES-Amber*) with charge scaling consistent with the previously reported DES-Amber protein and ion force field parameters and a second (which we name *DES-Amber SF1.0*) with no charge scaling, consistent with the previously reported DES-Amber SF1.0 protein and ion force field parameters.[8] Our

PNAS RESEARCH ARTICLE | BIOPHYSICS AND COMPUTATIONAL BIOLOGY OPEN ACCESS Check for updates

Effective polarization in potassium channel simulations: Ion conductance, occupancy, voltage response, and selectivity

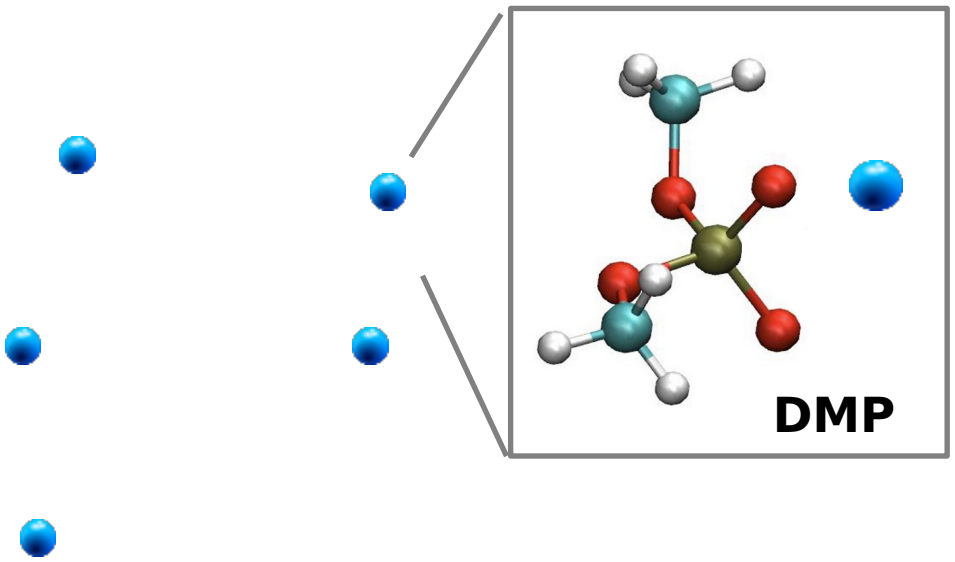
Chenggong Hui[a], Reinier de Vries[a], Wojciech Kopec[a,b,1], and Bert L. de Groot[a,1]

Can we use ECC to improve ion pairing of common nucleic acid ff?

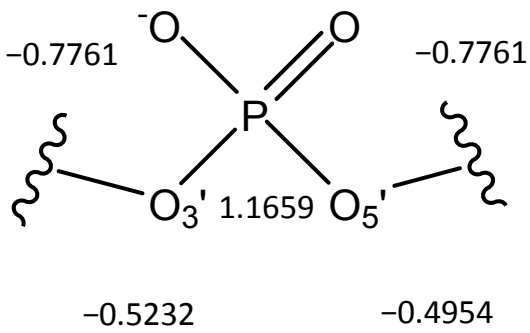
AMBER-OL15-ECC FORCE FIELD FOR DNA/RNA

Collab. P. Jurečka & M. Zgarbová, Olomouc (CZ)

Charge scaling (0.8) of the phosphate backbone



Optimized charge distribution for ion pairing with K^+ and Mg^{2+}



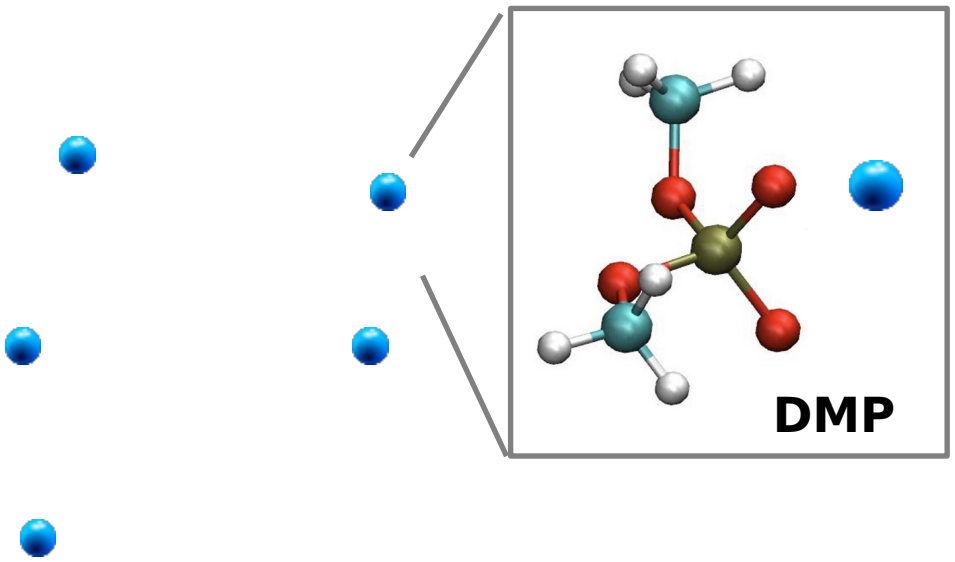
	Osmotic coeff. KDMP (0.3M)	ΔG_{bind}(DMP:Mg^{2+}) (kJ/mol)
Amber_OL15	0.88 ± 0.01	−29.8 ± 2
Expt	**0.98***	**−7.1****

*Tamaki,…, Tanihara, *BCSJ*, **1987**, 60, 1225
McDowell, …, Sutter, *Inorg Chem*, **1971, 10, 1638, for DHP:Mg^{2+}

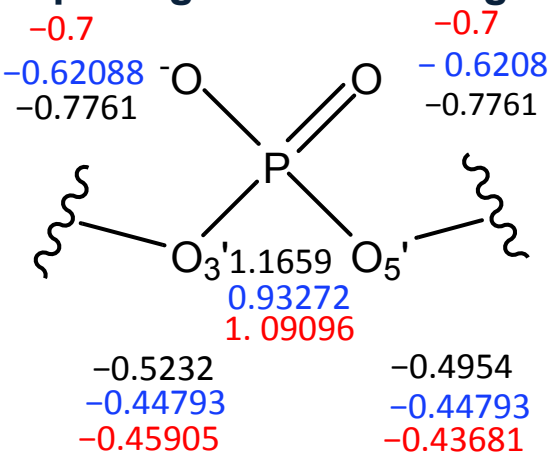
AMBER-OL15-ECC FORCE FIELD FOR DNA/RNA

Collab. P. Jurečka & M. Zgarbová, Olomouc (CZ)

Charge scaling (0.8) of the phosphate backbone



Optimized charge distribution for ion pairing with K^+ and Mg^{2+} / Ca^{2+}



	Osmotic coeff. KDMP (0.3M)	ΔG_{bind}(DMP:Mg^{2+}) (kJ/mol)
Amber_OL15	0.88 ± 0.01	−29.8 ± 2
0.8 scaling	0.93 ± 0.01	−2.8 ± 2
Amber_OL15-ECC	0.94 ± 0.01	−8.0 ± 2
Expt	**0.98***	**−7.1****

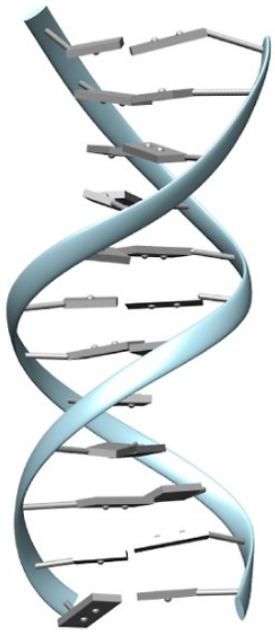
Limited experimental data
→ need more reference points

*Tamaki,…, Tanihara, *BCSJ*, **1987**, 60, 1225
McDowell, …, Sutter, *Inorg Chem*, **1971, 10, 1638, for DHP:Mg^{2+}
Sigel & Sigel, *Acc. Chem. Res.*, **2010** : ΔGbind = −3.2 kJ/mol

AMBER-OL15-ECC VALIDATION

B-DNA Dickerson-Drew dodecamer

$d(CGCGAATTCGCG)_2$



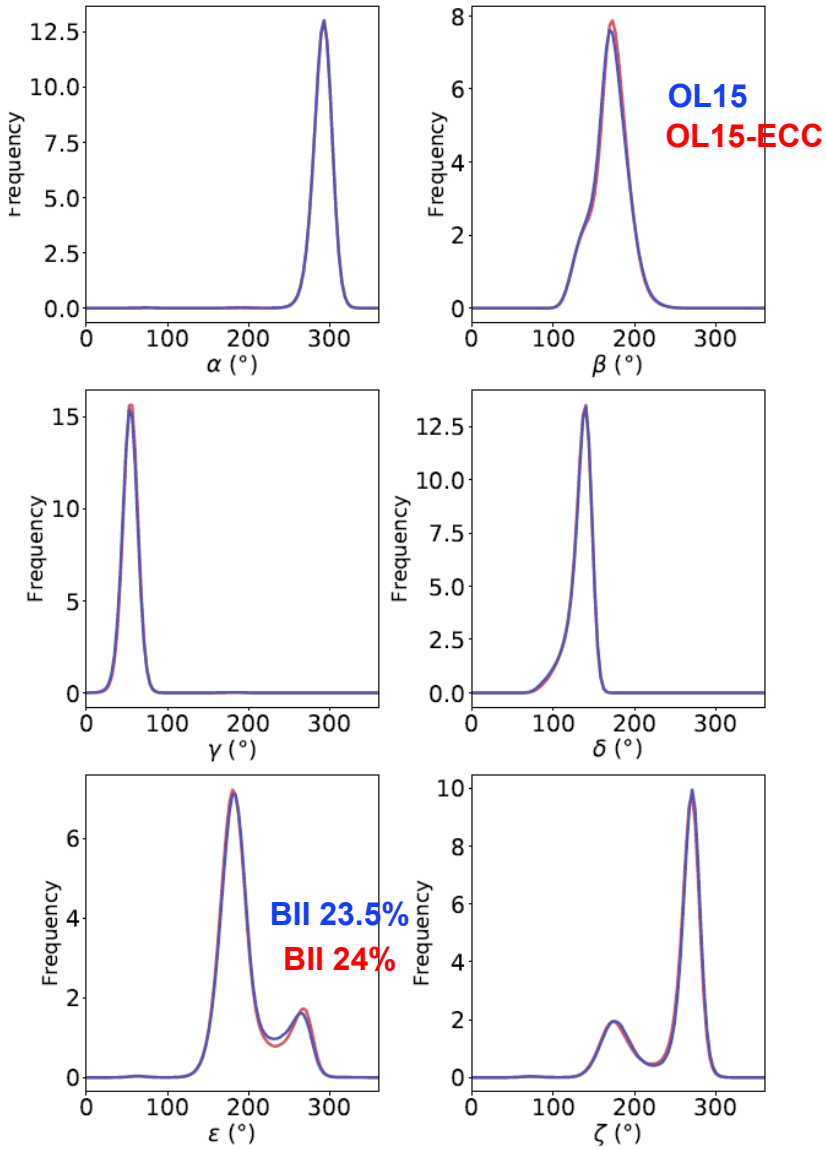
Amber-OL15 (ffbsc0-χ_{OL4}-$\varepsilon\zeta_{OL1}$-β_{OL1})

150 mM KCl, SPC/E water

3x1 μs simulations

ECC: 0.8 scaling

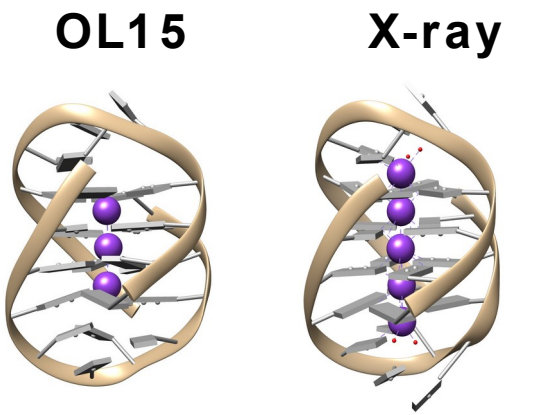
Impact of charge scaling on conformational properties?



No degradation of B-DNA structure

Puyo-Fourtine, Zgarbova, Kantin, Jurecka, EDD, **2025**, *JCTC* 21, 17, 8583–8600

DNA G-QUADRUPLEX d(G$_{4}$T$_{4}$G$_{4}$)



Improved K^+ ion retention

+ Increased loop stability

+ No degradation of backbone substates

3x1 µs simulations

OL15-ECC improves G-DNA description

→ Tests on various G4s incl. RNA-based?

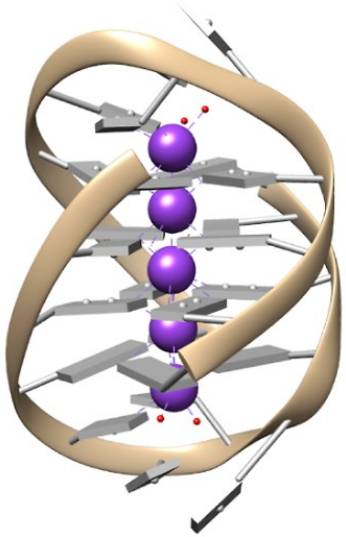
Puyo-Fourtine, Zgarbova, Kantin, Jurecka, EDD, **2025**, *JCTC* 21, 17, 8583–8600

AMBER-OL15-ECC VALIDATION

Various DNA and RNA sequences with monovalent ions

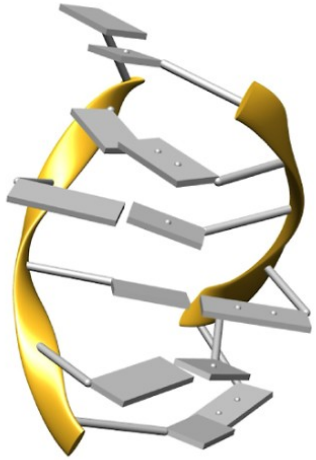
ds-DNA

Backbone substates
+ helical parms



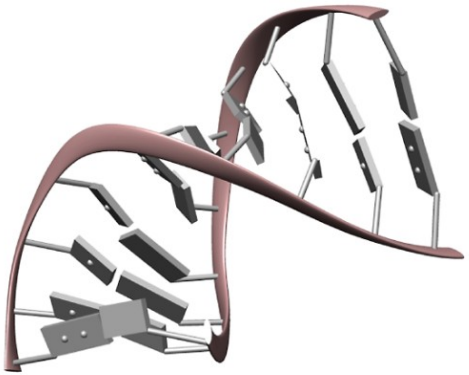
G-quadruplex

$d(G_4T_4$
all $K^+_4)$ retained



Z-DNA
(high salt)

$d(CGCGCG$
$)_2$
Backbone substates



ds-RNA
(A form)

$r(GCACCGUUG$
$G)_2$
Backbone substates
+ helical parms

ECC correction seems orthogonal to backbone dihedral refinements

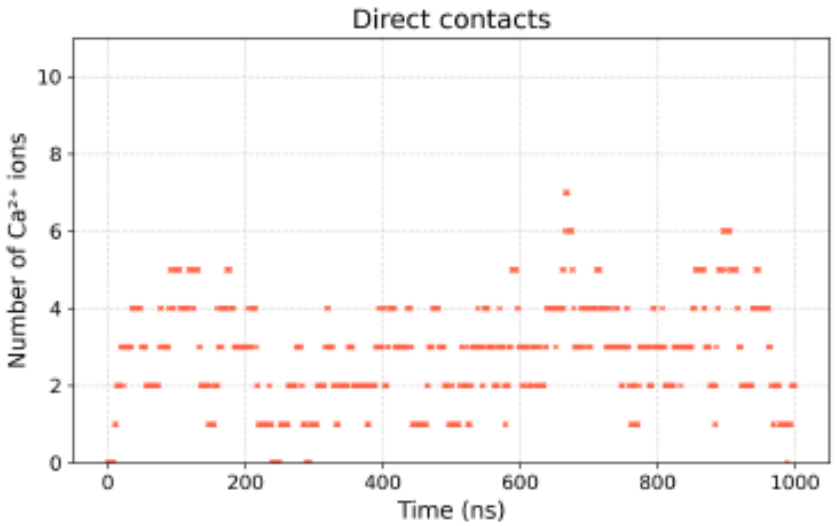
(OL21-ECC for Z-DNA)

→ Need further testing on various G4 and RNA structures

Impact on Divalent Ion Binding?

- **Ca^{2+}**

B-DNA dodecamer, 1μs, 11 Ca^{2+} ions + 150mM KCl, Amber-OL15 ff



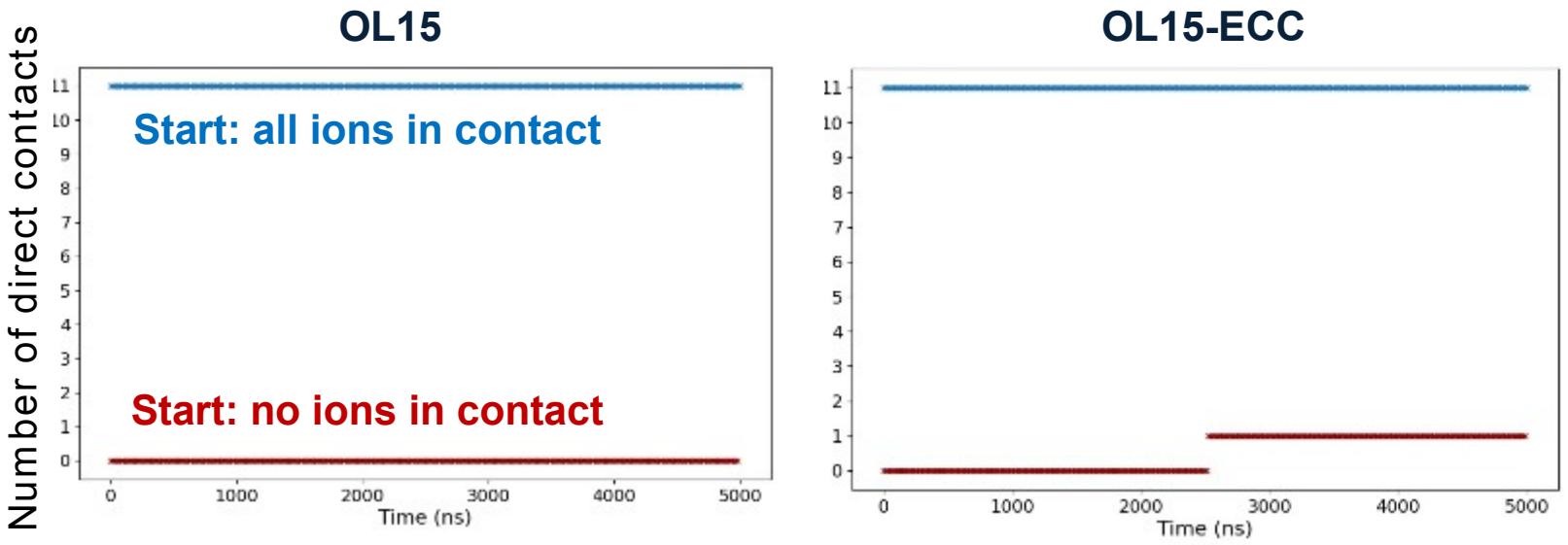
	total direct contacts	phosphate direct contacts	SShIP w. phosphates
OL15	2.8 (0.1)	2.8 (0.1)	2.8 (0.0)
OL15-ECC	1.1 (0.1)	1.1 (0.1)	1.7 (0.1)

Reduction of both direct contacts and solvent shared ion pairs

Mg^{2+} PAIRING

- **Plain MD simulations do not sample Mg^{2+} ion pairing !**

B-DNA dodecamer, Amber-OL15 force field, 5μs, 11 Mg^{2+} ions



No exchanges between contact/sship ion pairs !!

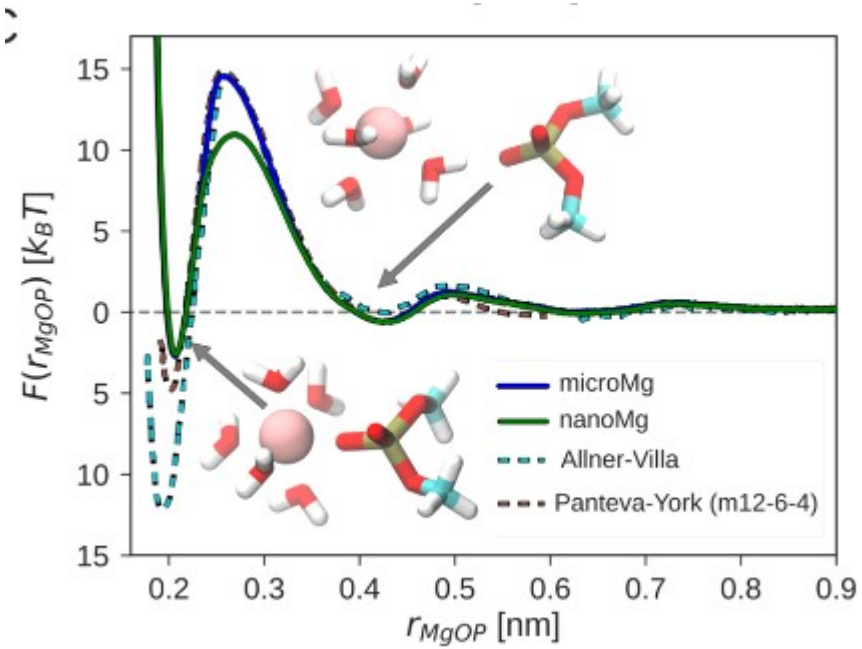
→ Need for enhanced sampling

FAST EXCHANGE ION

- **Using the pair-specific (NBFIX) approach**
- **“nanoMg” ion**

→ ion with same thermodynamic properties ΔG_{bind} but accelerated kinetics

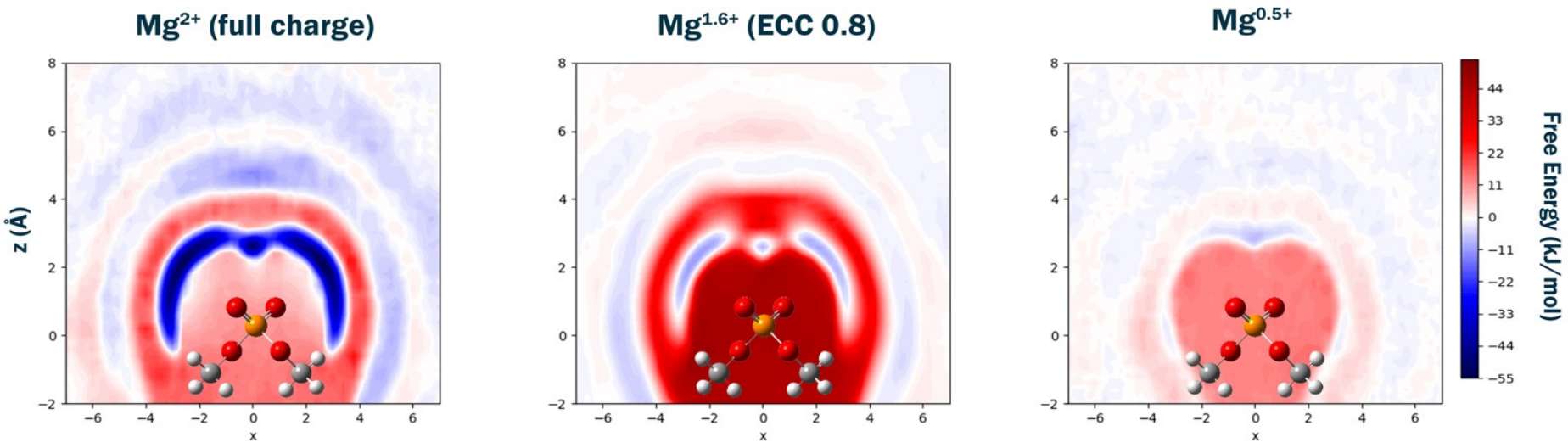
Grotz, …, Schwierz, *JCTC*, **2021**, 17, 2530



- **Limitation:** cannot reduce arbitrarily the barrier. Acceleration depends on water model.

Divalent ions binding

Slow exchange (> μs) of ligands around divalent cations (esp. Mg^{2+})
→ acute sampling issues!

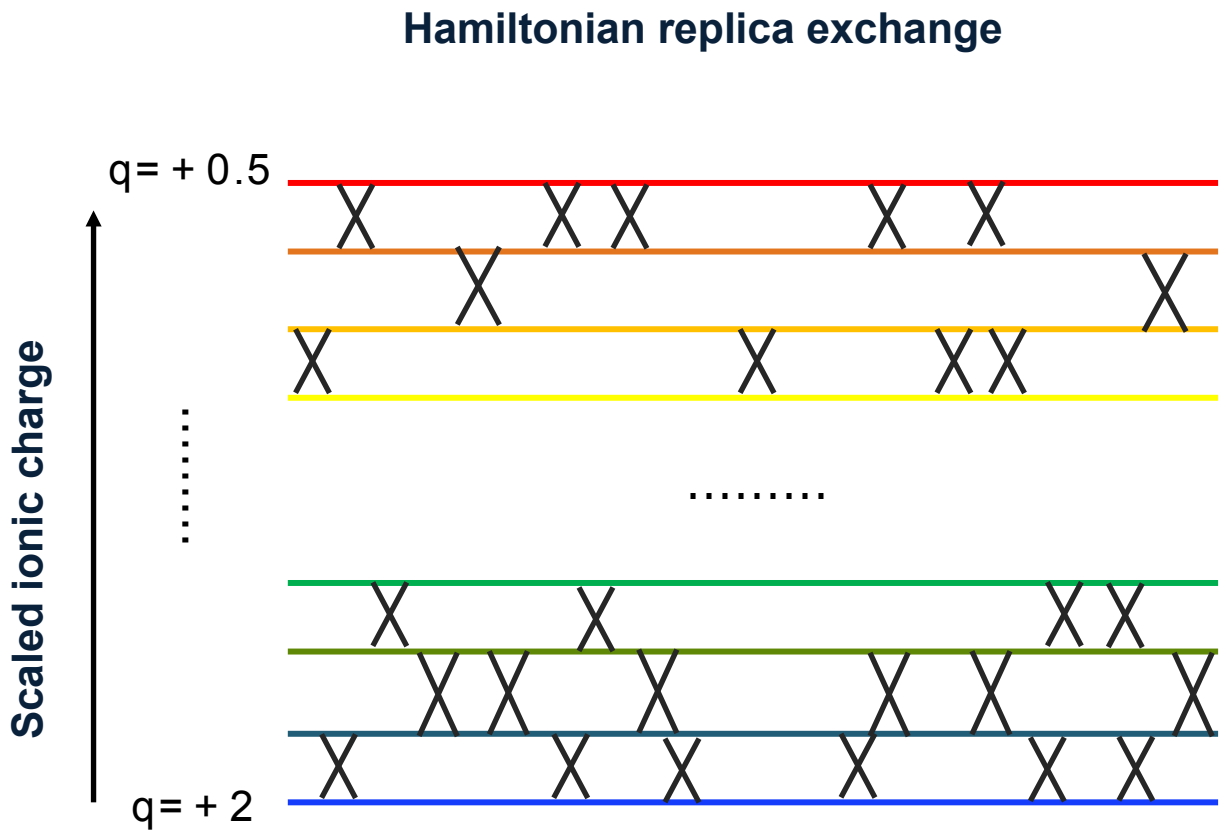


Lowering the ion charge flattens the free energy landscape

H-REX Scheme for Sampling Ion Pairing

Collab. J. Hénin @ LBT

- Charge scaling to accelerate sampling: Mg^{2+} → $Mg^{0.5+}$
- **Replica exchange** scheme
- no CV, periodic exchanges, Monte-Carlo criteria



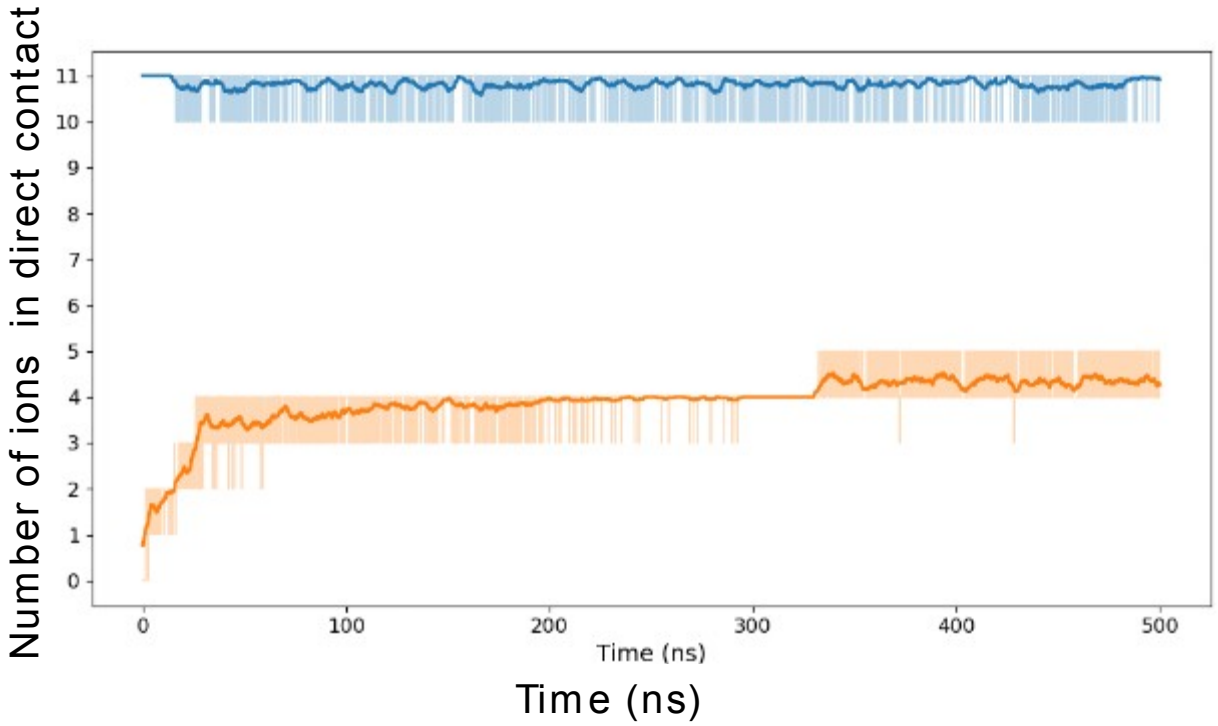
H-REX Scheme for Sampling Ion Pairing

Collab. J. Hénin @ LBT

- Charge scaling to accelerate sampling: Mg^{2+} → $Mg^{0.5+}$
- **Replica exchange** scheme

- Full charge ff → all ions should bind (ΔG_{bind} = −29.8 kJ/mol)

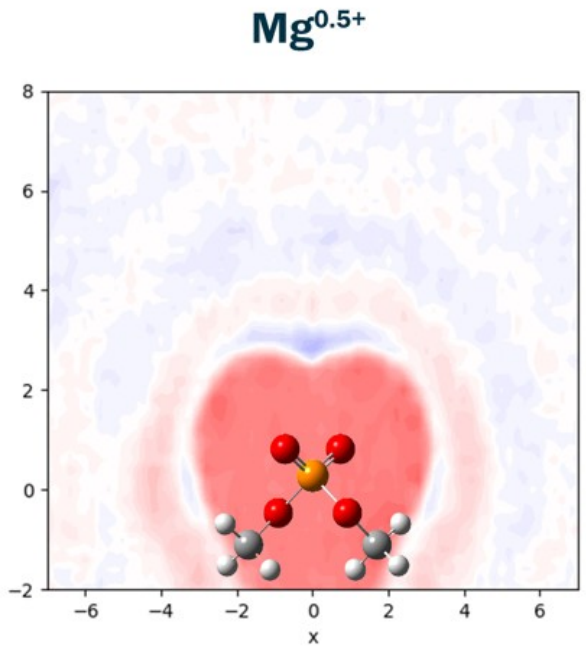
Amber-OL15,
DNA dodecamer, SPC/E water
11 Mg^{2+} ions
51 replicas, q=+2 → q=0.5
Gromacs2022.3+plumed HREX



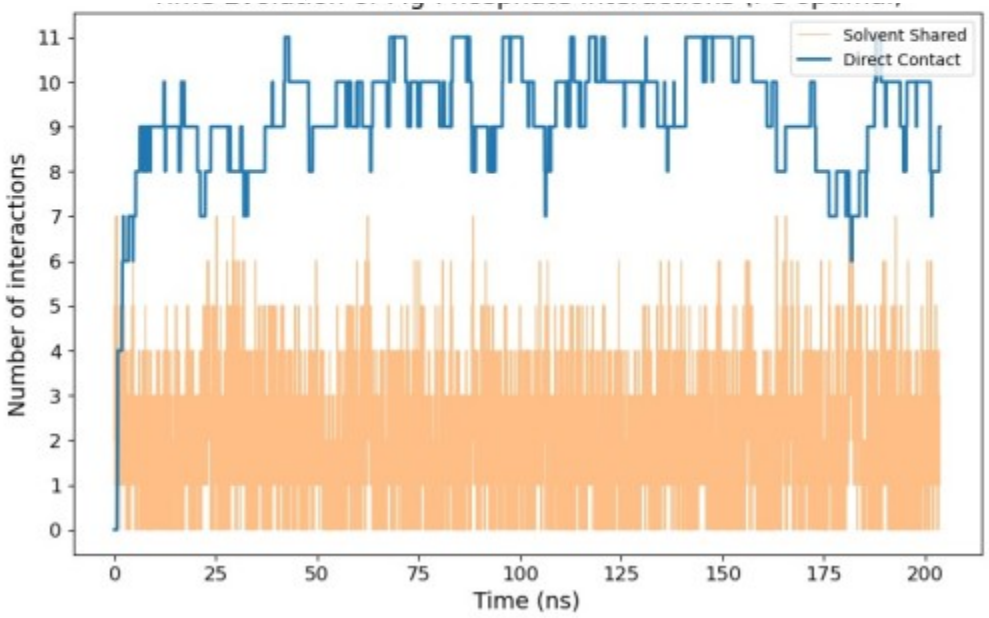
very slow convergence, not efficient

Design of Optimal ion

- **Issue**:
  → high replica: ion unbinds quickly but rarely pairs
  → design « optimal » Mg^{1+} ion with fast exchange but favorable binding



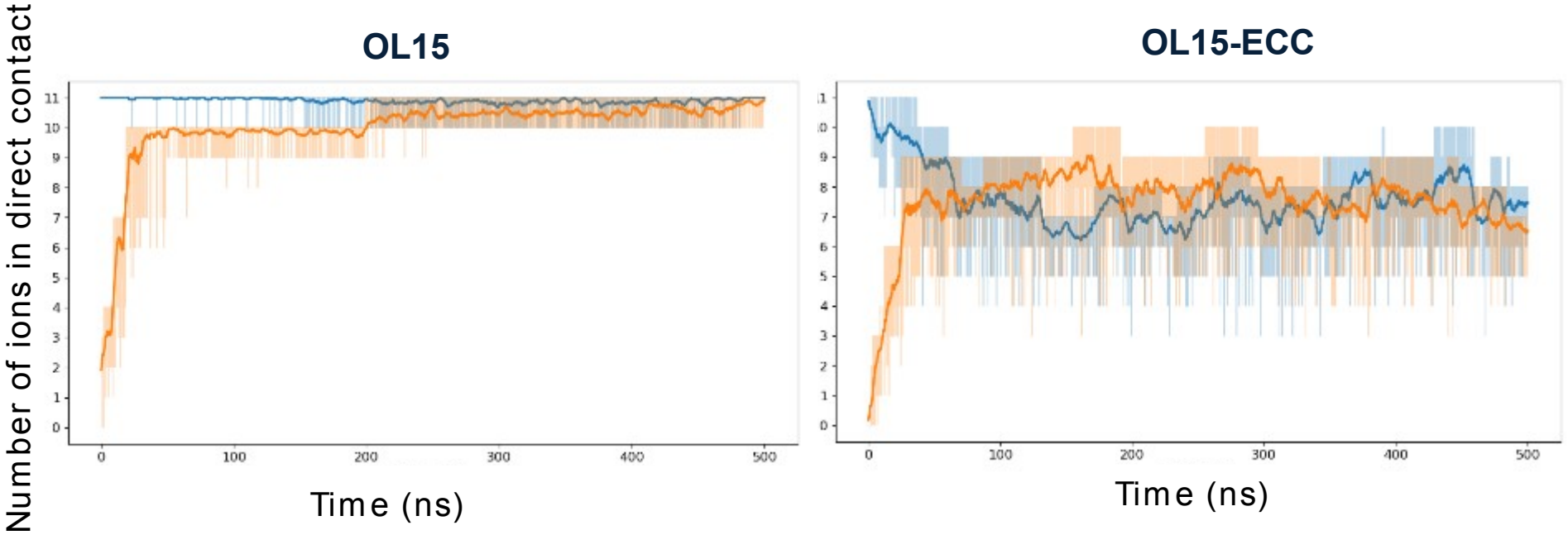
q=+1 ; ε(ion-OP) scaled 51%



H-REX Scheme for Sampling Ion Pairing

Collab. J. Hénin @ LBT

- **Solution**:
  → design « optimal » Mg^{1+} ion with fast exchange but favorable binding
  → tune q and ε(ion-OP)



Ion pairing converged in less than 200 ns!

Amber-OL15, DNA dodecamer, SPC/E water, 11 Mg^{2+} ions
OL15: 34 replicas, q=+2 → q=+1 ; ε(ion-OP) from 100 → 50% ; Gromacs2022.3+plumed HREX
OL15-ECC: 21 replicas: q=+1.6 → q=+1, ε(ion-OP) from 100 → 50%

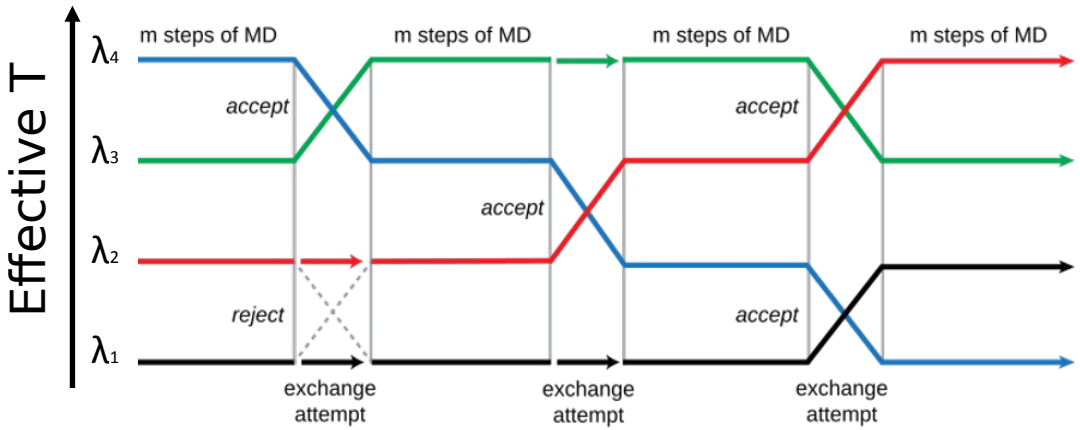
H-REX Scheme for Sampling Ion Pairing

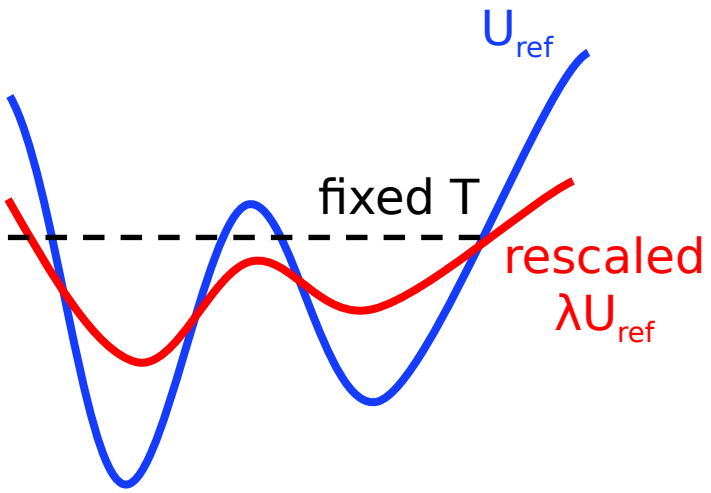
Collab. J. Hénin @ LBT

Combine ion sampling + biomolecular sampling?

→ REST2 scheme for biomolecular conformational sampling

Wang, Friesner, Berne, *J. Phys. Chem. B,* **2011**, 115, 9431-9438





$$E_m^{\mathrm{REST2}}(X) = \frac{\beta_m}{\beta_0} E_{\mathrm{pp}}(X) + \sqrt{\frac{\beta_m}{\beta_0}} E_{\mathrm{pw}}(X) + E_{\mathrm{ww}}(X)$$

Combine REST2 + ion sampling !

In progress…

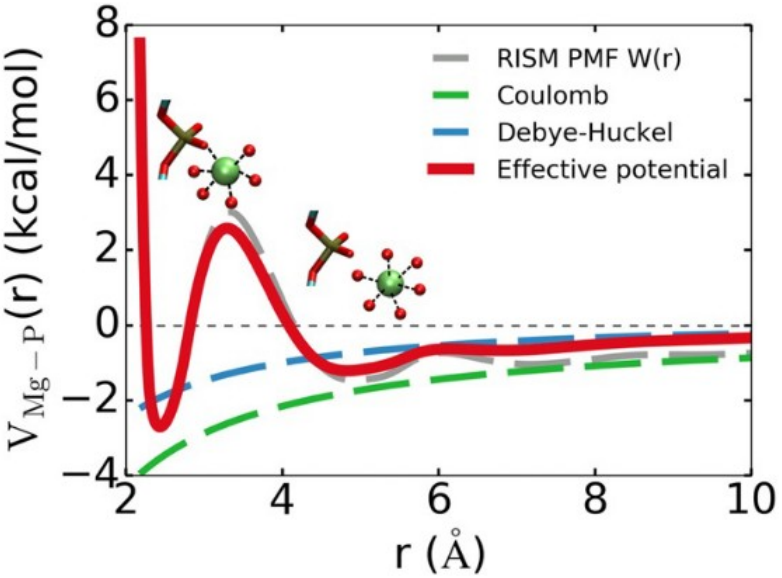
COARSE-GRAINED APPROACHES

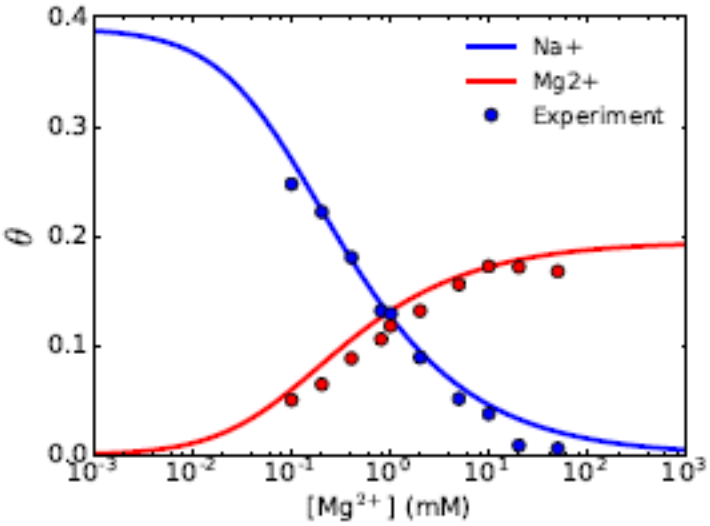
- **RNA Coarse grained description in implicit solvent**

Nguyen, Hori, Thirumalai, **2019**, *PNAS* 116, 21022

  - 3-site per nt CG description
  - Implicit monovalent ions (condensation → scaled charges on phosphate groups)
  - Explicit divalent ions

  → **Effective interaction** parametrized against statistical theory results (3D-RISM)





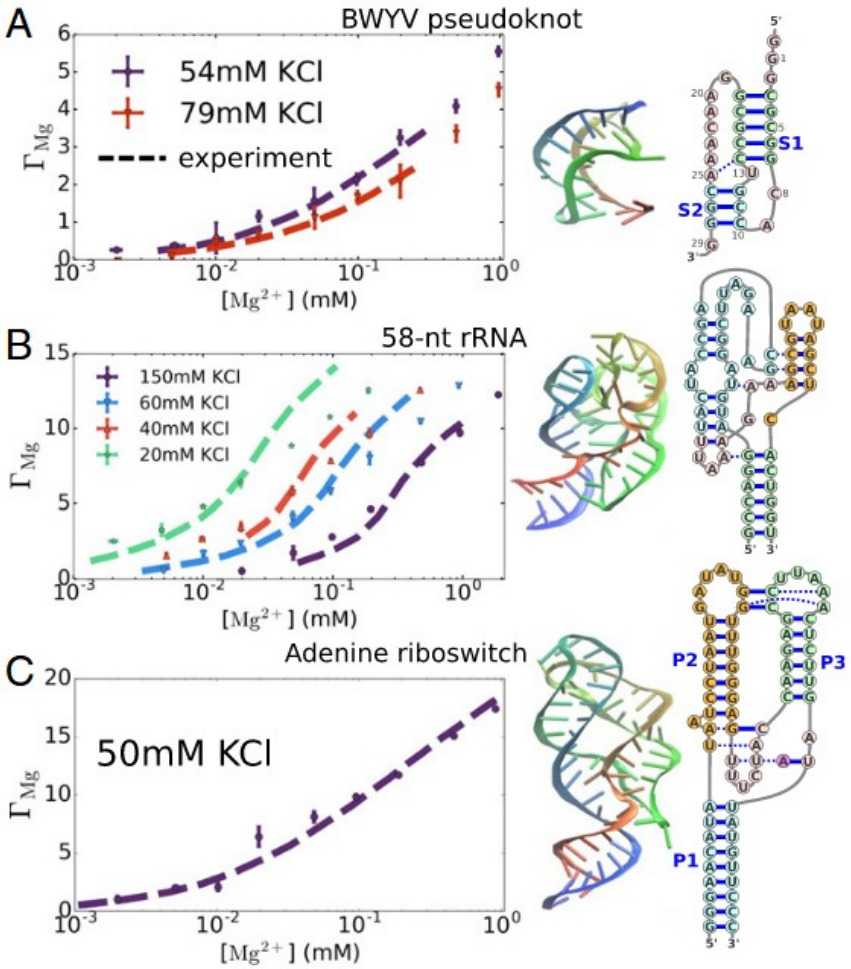
  - Validation against ion counting in DNA duplex

COARSE-GRAINED APPROACHES

- **RNA Coarse grained description in implicit solvent**

Nguyen, Hori, Thirumalai, **2019**, *PNAS* 116, 21022

  - Validation against ion counting in RNA sequences



COARSE-GRAINED APPROACHES

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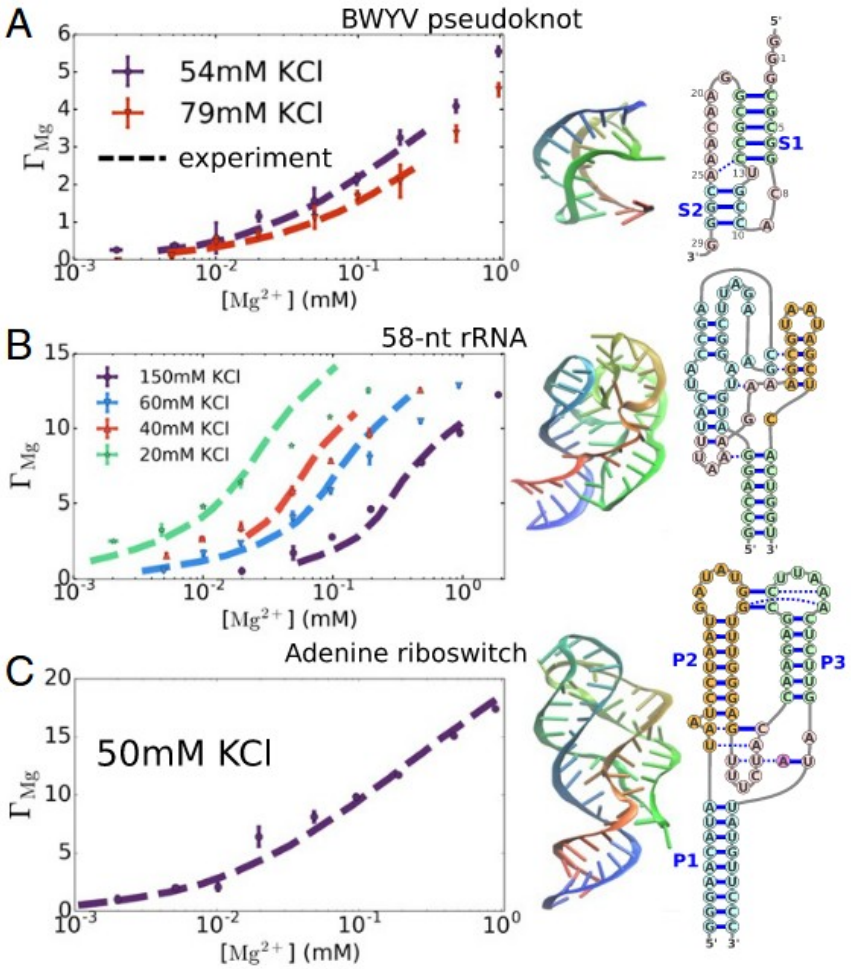


COARSE-GRAINED APPROACHES

- **RNA Coarse grained description in implicit solvent**

Nguyen, Hori, Thirumalai, **2019**, *PNAS* 116, 21022

  - Exami

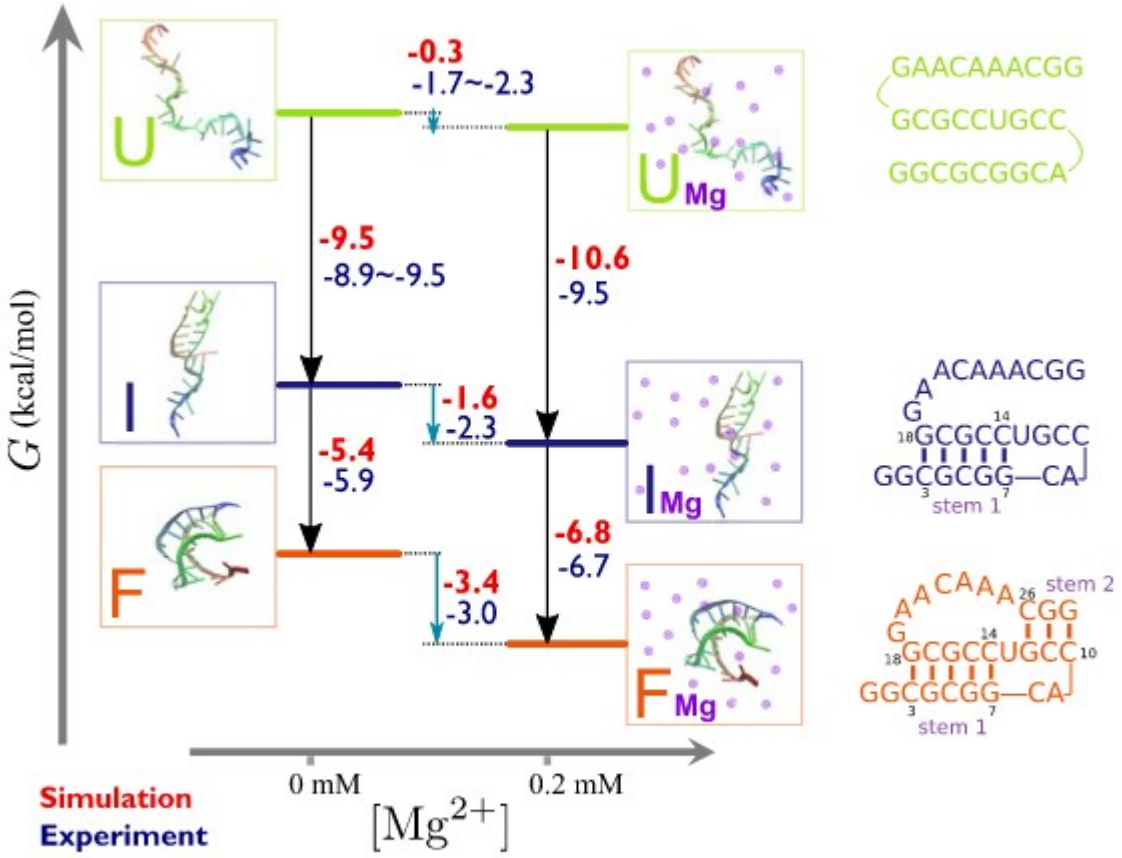
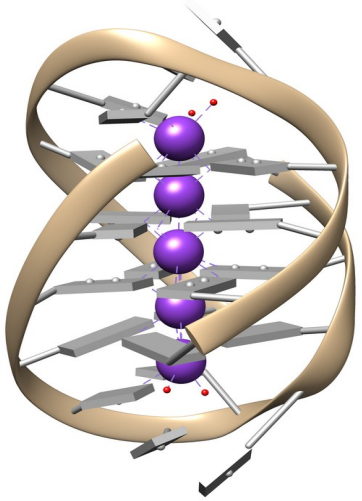


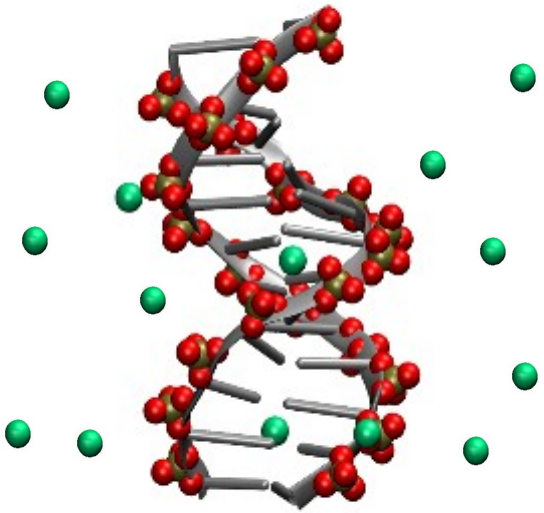
Fig. 4. Folding free energy (kcal/mol, 25 °C) diagram of BWYV pseudoknot at 54 mM KCl in the absence (*Left*) or presence (*Right*) of 0.2 mM Mg^{2+}. Theoretical values are in red and experimental values are in blue. Experimental

Conclusions & Prospects

- **Key Ion-RNA interactions**
- **Hard to probe experimentally**
- **Numerous theoretical and computational approaches**
  - Continuum theories
  - All-atom MD simulations with original force field & sampling refinements
  - Coarse-grained approaches



- **ECC force fields**
  - Very promising
  - Further testing and validation in various systems
- **Mg^{2+} sampling**
  - Emerging approaches to accelerate sampling



Acknowledgements

- **Julie Puyo-Fourtine**
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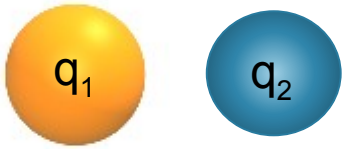
- **Laetitia Kantin**

Collaborations

- **Jérôme Hénin (LBT, IBPC)**
- **Petr Jurečka & Marie Zgarbová (Olomouc, CZ)**
- **Pavel Jungwirth (Prague, CZ) & Q-SCALING team**

HOW TO RECOVER REALISTIC ION PAIRING?

Artefacts: missing electronic polarizability



- **Explicitly Polarizable** force fields (e.g. Drude, AMOEBA)

Lemkul, MacKerell, *J.Comput.Chem.*, **2018**, 39, 2624
Zhang,…, Ponder, *JCTC*, **2018**, 14, 2084

  - Proper physics included
  - Additional parameters → harder to parametrize
  - Specific software often needed (e.g. Tinker)
  - Computationally expensive

- **Implicit approaches**

  - **Pair-specific Lennard-Jones** parameters (NBFIX)

Yoo, Aksimentiev, *JPCLett*, **2012**, 3, 45
Panteva, …, York, *JPCB* **2015**, 119, 15460

Optimized σ_{12} and ε_{12} for specific pairs
Need to be parametrized for each pair of interest

Grotz, …, Schwierz, *JCTC*, **2021**, 17, 2530

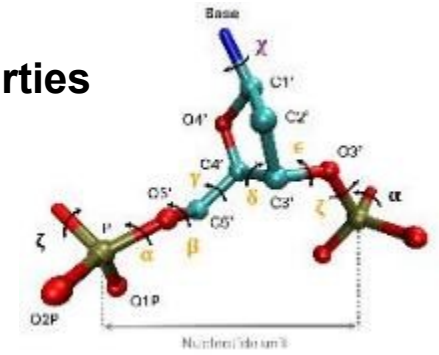
  - « **Electronic Continuum Correction** » (ECC) = scaled charges

MOLECULAR SIMULATIONS: CAN WE TRUST COMMON FORCE FIELDS?

DNA & RNA force fields: mostly refined for conformational properties

Amber99f-bsc0 / bsc1, OL corrections, CHARMM, Tumuc1…

Liebl & Zacharias, *JCTC*, **2021**, 17, 7096
Hart, …, MacKerell, *JCTC*, **2012**, 8, 348
Perez,…, Orozco, *BiophysJ*, **2007**, 3817 (bsc0); Ivani, …, Orozco, *Nat. Methods*, **2016**, 13, 55 (bsc1)
Sponer,…, Otyepka, **2018**, *ChemRev*, 118, 4177 ; Mlynsky,…, Sponer, *JCTC*, **2025**
…



Proper interaction with ions?

- Reasonable behavior of **monovalent** ions

Overbinding to phosphate groups
Unstable K^+ ions within G-quadruplexes
→ need polarizable ff?

Pasi, …, Lavery, *NAR*, **2015**, 43, 2412
Dans,…, Orozco, *NAR*, **2016**, 44, 4052

Yoo, Aksimentiev, *JPCL*, **2012**, 3, 45

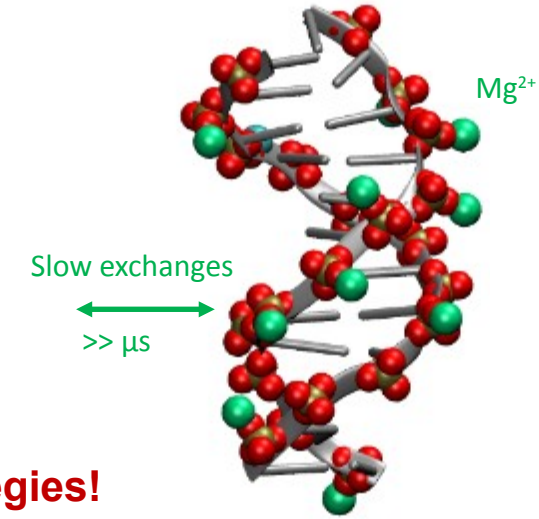
Krepl, …, Sponer, *JCTC*, **2012**, 8, 2506-2520
Ratnasinghe, …, Lemkul, *JCIM*, **2020**, 60, 6476

- **Divalent ions**: out-of-reach of common ff!

strong overbinding artefacts

Dans,…, Orozco, *NAR*, **2016**, 44, 4052
Sponer, …, Otyepka, *JPCL*, **2014**, 5, 1771

+ sampling issues



Need for reliable force field + efficient sampling strategies!

HOW TO RECOVER REALISTIC ION PAIRING?

- « **Electronic Continuum Correction** » (ECC) = scaled charges

$$|F_{ecc}| = \frac{1}{4\pi\epsilon_0} \frac{q_1 q_2}{\sqrt{\epsilon_{el}}\sqrt{\epsilon_{el}} r^2} = \frac{1}{4\pi\epsilon_0} \frac{q_1' q_2'}{r^2}$$

q_1' q_2'

→ charge scaled by $1/\sqrt{\varepsilon_{el}} \approx 0.75$

Leontyev, Stuchebrukov, *Phys. Chem. Chem. Phys.*, **2011**, 13, 2613-2626
Kirby, Jungwirth, *JPCLett*, **2019**, 10, 7531

- Successfully tested on electrolytes, incl. phosphates
  scaling factor 0.75-0.85

Bruce, van der Vegt, *J. Chem. Phys.*, **2018**, 148, 222816
MADRID2019: Zeron, …, Vega, *J.Chem.Phys.*, **2019**, 151, 134504
EDD, Javainainen, Delcroix, Jungwirth, Martinez-Seara, *J. Chem. Phys.*, **2020**, 050901
Puyo-Fourtine, Juillé, Hénin, Clavaguéra, EDD, *JPCB*, **2022**, 126, 4022

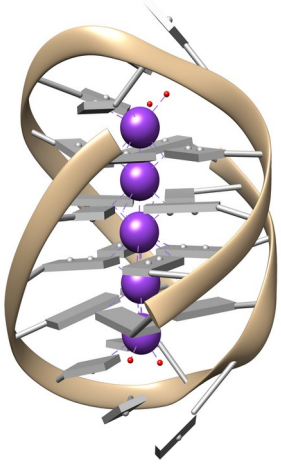
- Promising for biomolecules: proteins, phospholipids, nucleic acids (DES-Amber)
- No additional cost!
- Validation?

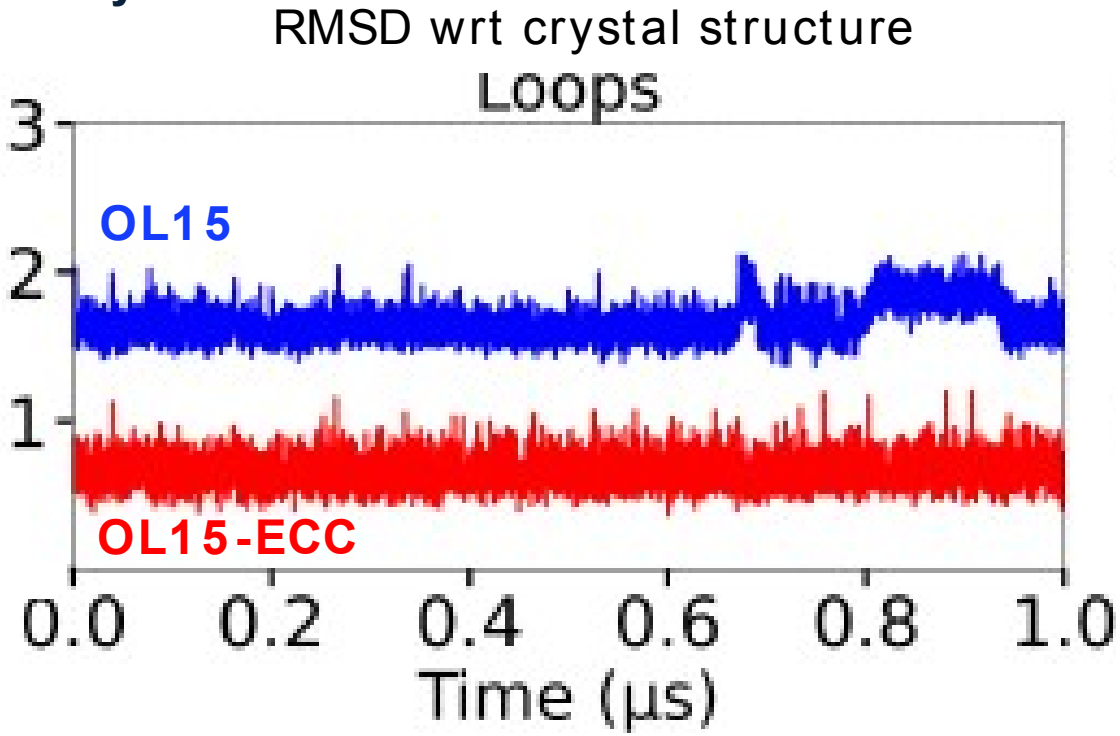
Nencini, …, Martinez-Seara, *JCTC*, **2024**, 20, 7546
Piana,…, Shaw, *JCTC*, **2020**, 16, 2494
Tucker,…, Shaw, *JPCB*, **2022**, 126, 4442

Can we use ECC to improve ion pairing of common nucleic acid ff?

DNA G-QUADRUPLEX d(G$_{4}$T$_{4}$G$_{4}$)

Increased loop stability





+ No degradation of backbone substates

OL15-ECC improves G-DNA description

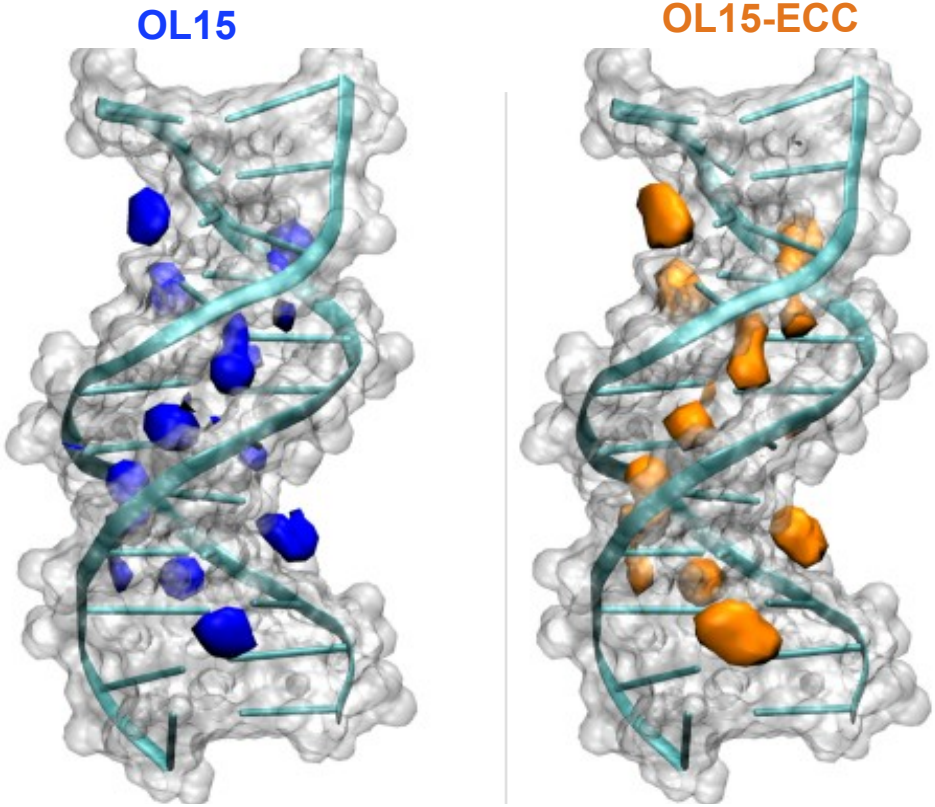
→ Tests on various G4s?

Puyo-Fourtine, Zgarbova, Kantin, Jurecka, EDD, **2025**, *JCTC* 21, 17, 8583–8600

AMBER-OL15-ECC ION BINDING

B-DNA Dickerson-Drew dodecamer

K^+ ion density maps



No change in direct contacts (inner shell)

Reduced solvent shared ion pairs (outer shell)

Puyo-Fourtine, Zgarbova, Kantin, Jurecka, EDD, **2025**, *JCTC* 21, 17, 8583–8600