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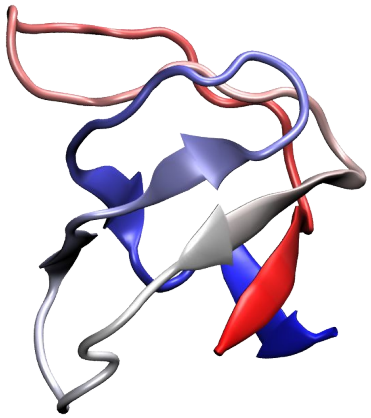
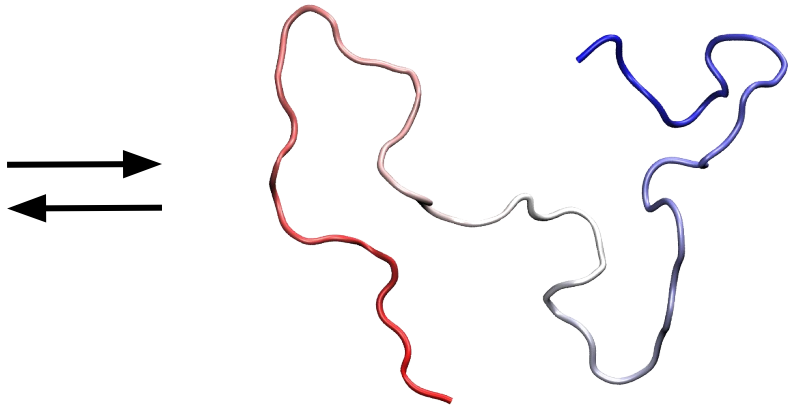
Effects of the cosolvent on the protein folding landscape

FAPESP 2020/04549-0

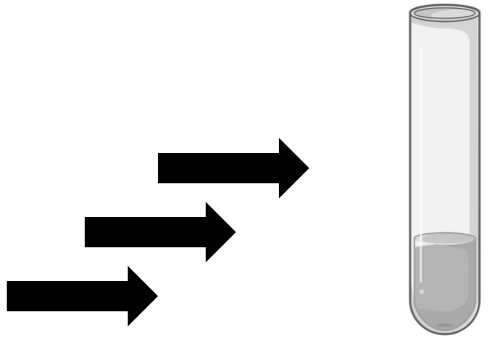
Ander Francisco Pereira
Advisor: Prof. Dr. Leandro Martínez

Overview of research

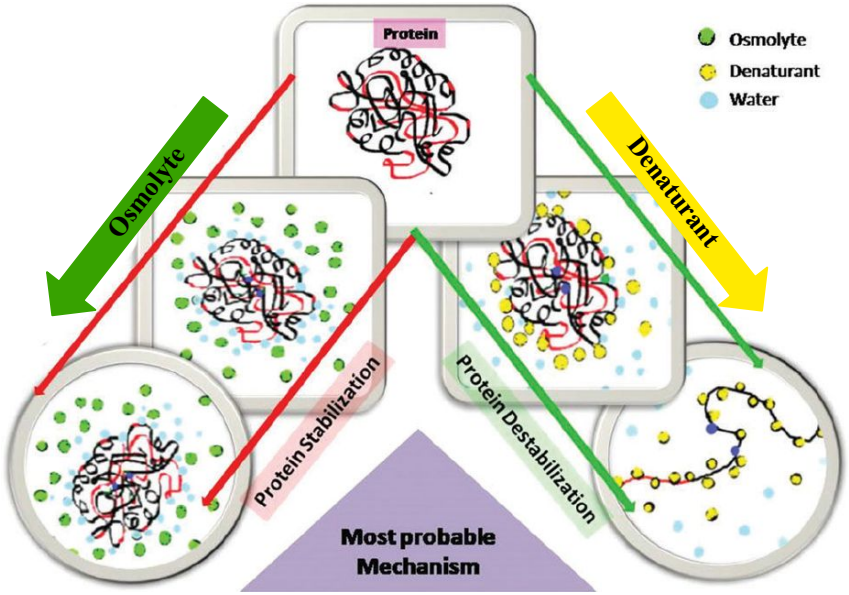




Folded state **Unfolded state**



Cosolvent

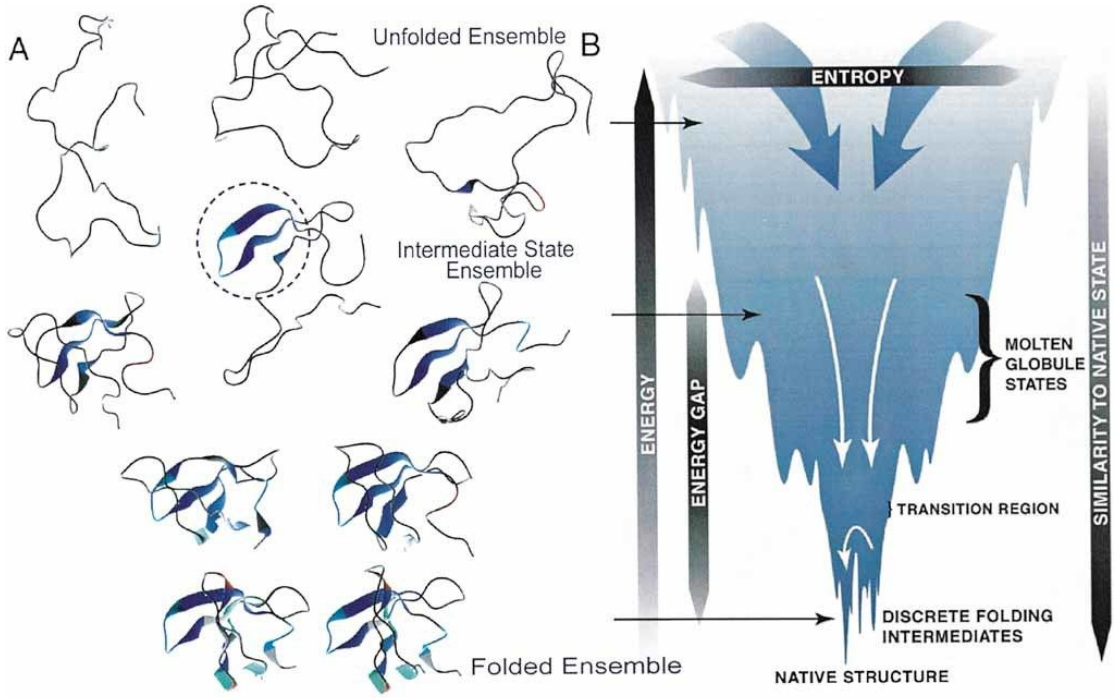


How do different cosolvents change the mechanisms of protein folding?

Rani, Anjeeta; Venkatesu, Pannuru. **Physical Chemistry Chemical Physics**, v. 20, n. 31, p. 20315-20333, 2018.

Theory of protein folding

The energy landscape theory



Structure-Based Models (SBMs)

SMOG
A WEBTOOL FOR BIOMOLECULAR SIMULATION

https://smog.rice.edu/

$$V^{SB}(\Gamma,\Gamma_0) = \sum_{bonds} \epsilon_r (r - r_0)^2 + \sum_{angles} \epsilon_\theta (\theta - \theta_0)^2$$

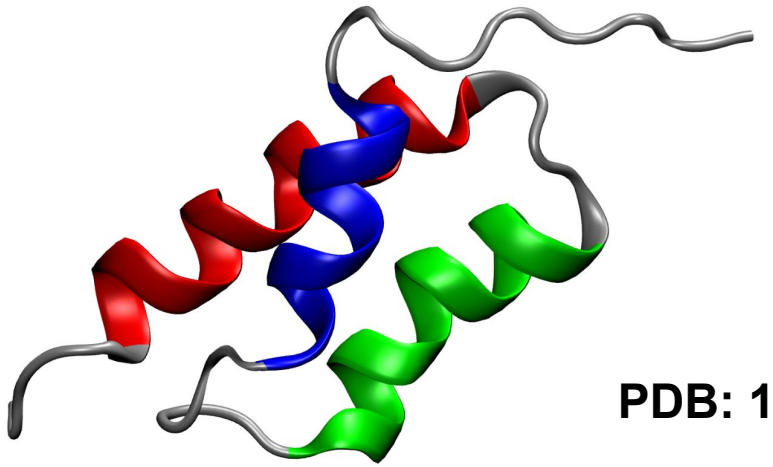
$$+ \sum_{dihedrals} \epsilon_\phi \left\{ \left[1 - cos(\phi - \phi_0)\right] + \frac{1}{2}\left[1 - cos(3(\phi - \phi_0))\right] \right\}$$

$$+ \sum_{contacts} \epsilon_C \left[5\left(\frac{d_{ij}}{r_{ij}}\right)^{12} - 6\left(\frac{d_{ij}}{r_{ij}}\right)^{10} \right] + \sum_{non-contacts} \epsilon_{NC} \left(\frac{\sigma_{NC}}{r_{ij}}\right)^{12}$$

Onuchic, José Nelson, Zaida Luthey-Schulten, and Peter G. Wolynes. **Annual review of physical chemistry**. 48.1 (1997): 545-600.
Clementi, Cecilia; Nymeyer, Hugh; Onuchic, José Nelson. **Journal of molecular biology**, v. 298, n. 5, p. 937-953, 2000.
Onuchic, José Nelson; Wolynes, Peter G. Theory of protein folding. **Current opinion in structural biology**, v. 14, n. 1, p. 70-75, 2004.

Overall picture of the BdpA folding

B domain of staphylococcal protein A:



PDB: 1BDD

(1) a a small three-helix bundle;

(2) a two-state folding mechanism;

(3) Folding occurs within ~10 μs;

(4) Helix-I is the most unstable;

(5) Helix-II is formed in the transition state;

(6) conformational transition (F-U ensemble) can involve the partial helix formation/deformation of the three helices.

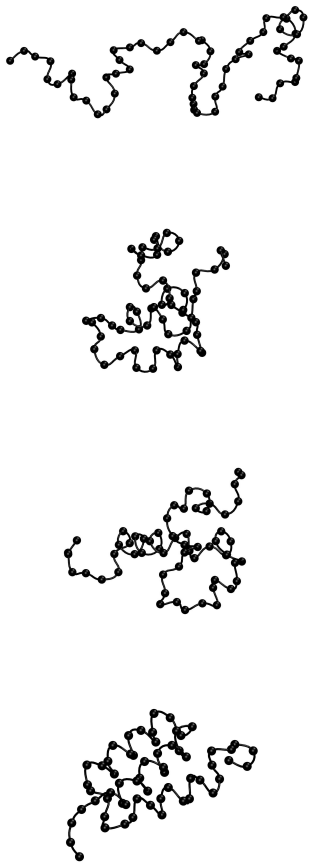
Garcia, Angel E.; Onuchic, José N. **Proceedings of the National Academy of Sciences**, v. 100, n. 24, p. 13898-13903, 2003.
Otosu, Takuhiro et al. **The Journal of Physical Chemistry B**, v. 121, n. 22, p. 5463-5473, 2017.
Santhouse, Jacqueline R. et al. **Chemical Science**, v. 13, n. 40, p. 11798-11806, 2022.

Effects of the cosolvents on the protein stability

Part1

Simulations with SBMs (C_α)

Folding ensemble

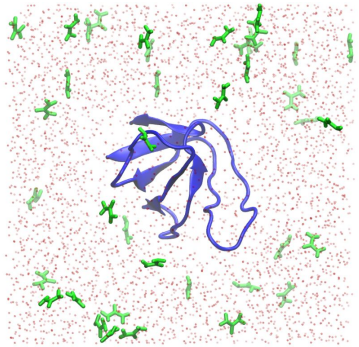


Transition structures for each protein was reconstructed as all-atom models!

A harmonic potential was applied in the C_α atoms to constrain the structures.

Part2

Atomistic simulations



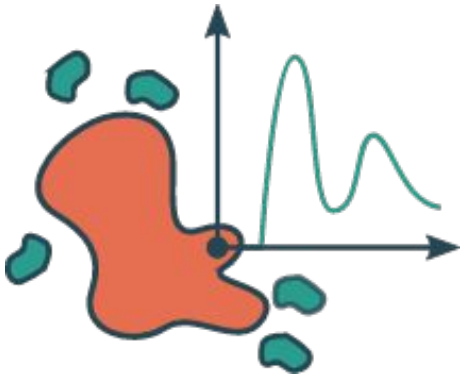
- **Protein**
- **Water**
- **Urea (or TMAO)**

...

All atom simulations in explicit solvent:

Systems	Concentration (mol L^{-1})
BdpA + TMAO	0.1, 0.2, 0.3, 0.4, and 0.5
BdpA + ureia	0.1, 0.2, 0.3, 0.4, and 0.5

Methods for describing the solvation structures

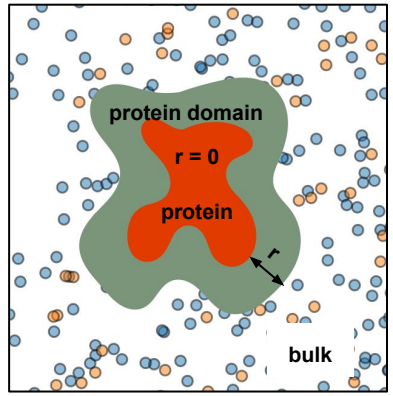


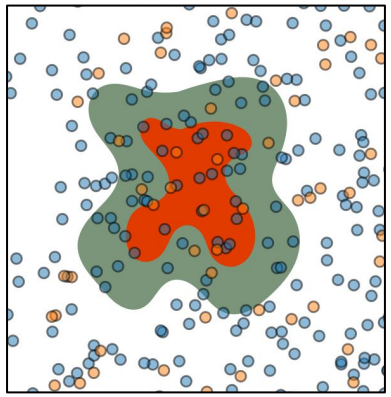
ComplexMixtures.jl
https://github.com/m3g/ComplexMixtures.jl

- **Minimum-Distance Distribution Functions (MDDF)**
  - Provide clear pictures of solvation structures of complex solutions;

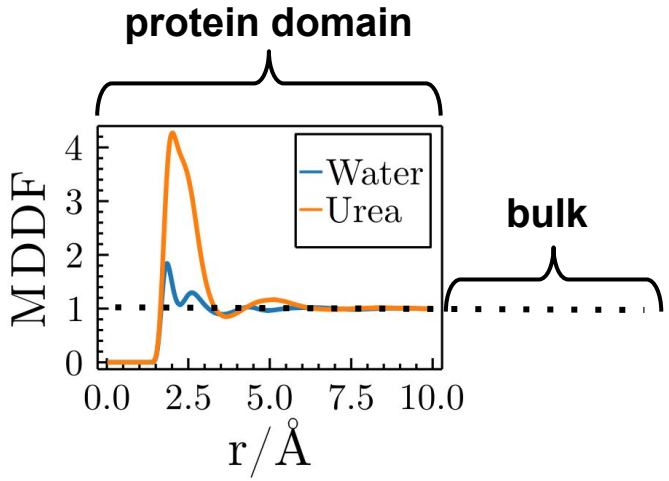
Average number density of solvent atoms

$$g_{us}^{md}(r) \equiv \frac{n_{us}(r)}{n_{us}^{*}(r)}$$



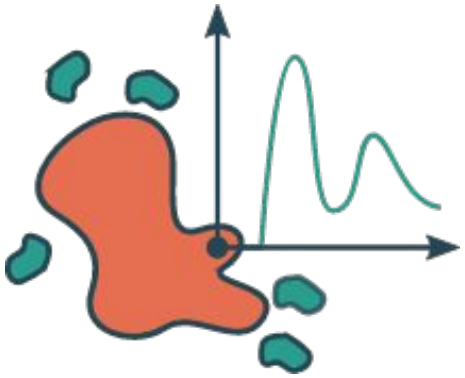


Example:



Martínez, Leandro; Shimizu, Seishi. **Journal of Chemical theory and computation**, v. 13, n. 12, p. 6358-6372, 2017.
Martínez, Leandro. **Journal of Molecular Liquids**, v. 347, p. 117945, 2022.

Methods for describing the solvation structures



ComplexMixtures.jl
https://github.com/m3g/ComplexMixtures.jl

- **Kirkwood-Buff theory**
  - Kirkwood-Buff integrals:

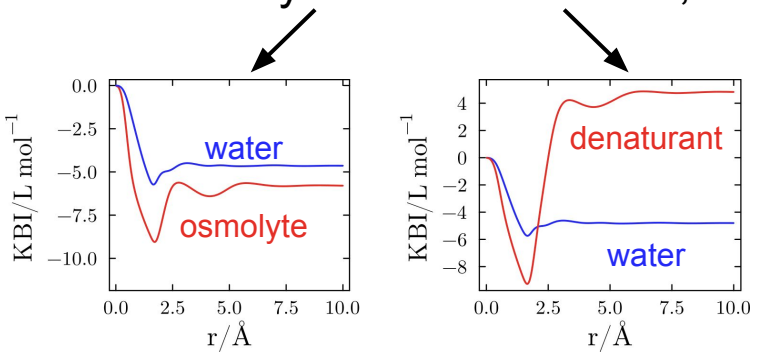
$$G_{us}(R) = \frac{1}{\rho_s}\int_0^R [n_{us}(r) - n^*_{us}(r)]S(r)\,dr$$

=

$$G_{us}(R) = \frac{1}{\rho_s}[N_{us}(R) - N^*_{us}(R)]$$

S(r) is dependent on the shape of the solute

  - General results for osmolyte and denaturant, respectively.



Preferential interaction parameters (Γ):

$$\Gamma_{uc} \approx \rho_c(G_{uc} - G_{uw})$$

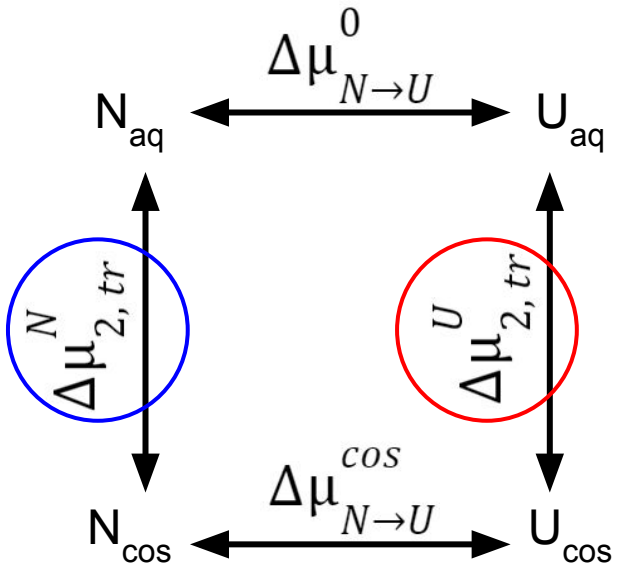
Γ > 0 → Protein is preferentially solvated by cosolvent.
Γ < 0 → Protein is preferentially hydrated.

Martínez, Leandro; Shimizu, Seishi. **Journal of Chemical theory and computation**, v. 13, n. 12, p. 6358-6372, 2017.
Martínez, Leandro. **Journal of Molecular Liquids**, v. 347, p. 117945, 2022.

Transfer Free Energy (TFE)

TFE is the total free energy of interaction of the protein with the cosolvent.

Thermodynamic cycle:



TFE can be obtained from Γ

$$(\partial\mu_2 / \partial m_3)_{m2} = -\Gamma * [\,(\partial\mu_3 / \partial m_3)_{m2}\,]$$

2 = Protein
3 = Cosolvent

Experimental parameter

$$\Gamma = \partial g_2 / \partial g_3$$

Parameter from simulation of the **native (N)** and **unfolded (U)** states at **different concentrations**.

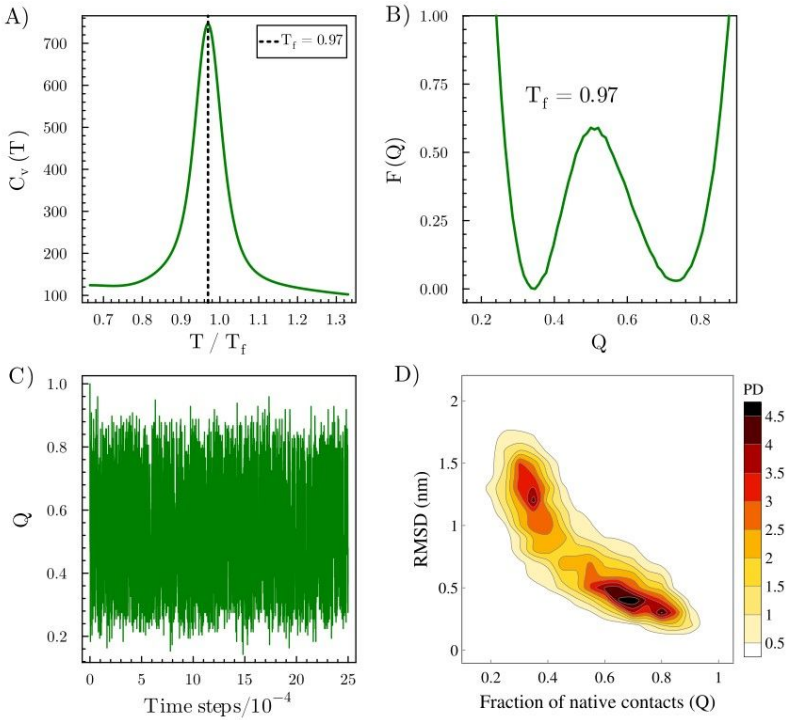
TFE is experimentally determined by:

$$\partial\Delta\mu_{2,tr} = {}^{U}\Delta\mu_{2,tr} - {}^{N}\Delta\mu_{2,tr} = \int_0^{m3} \partial\mu_2/\partial m_3\, dm_3$$

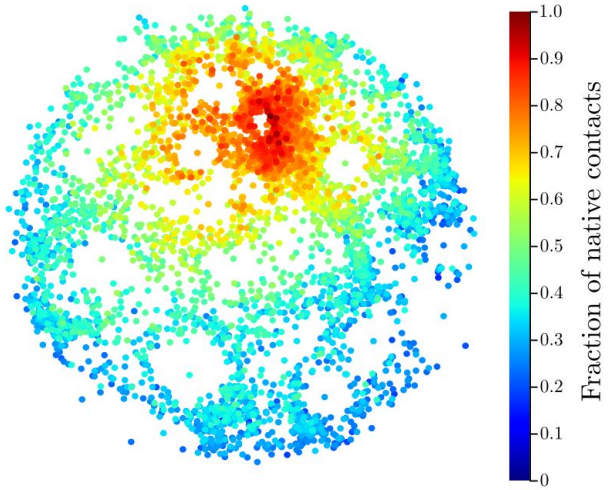
Timasheff, Serge N. **Protein stability and folding: theory and practice**. 253-269, 1995.

Protein folding visualization

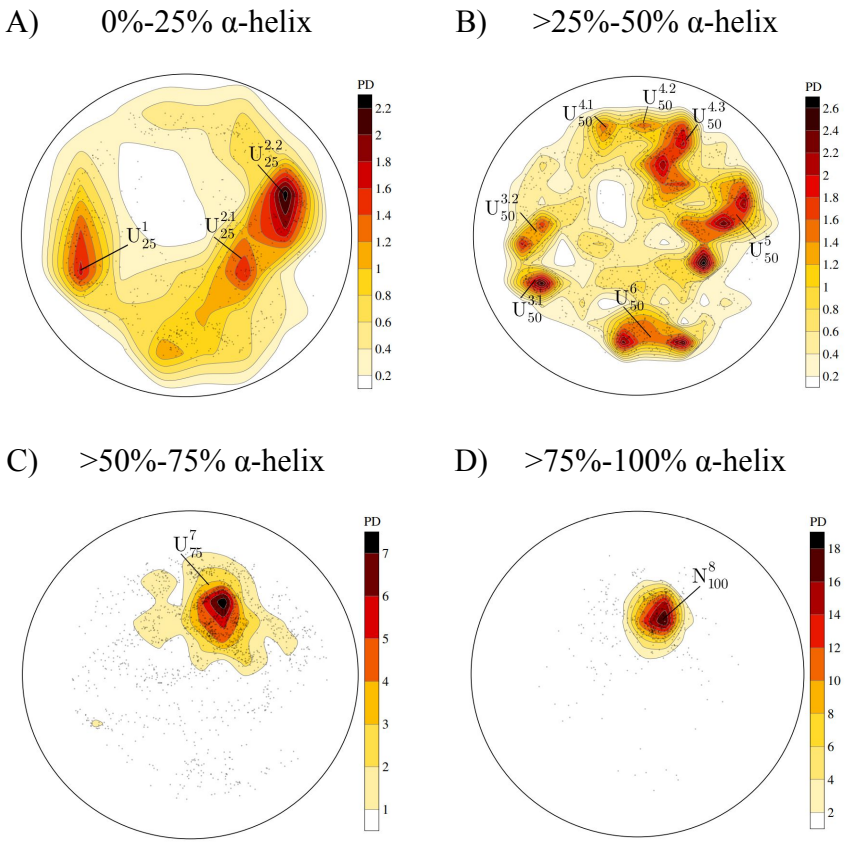
- Characterization of BdpA folding



- Visualization of the BdpA folding using ELViM



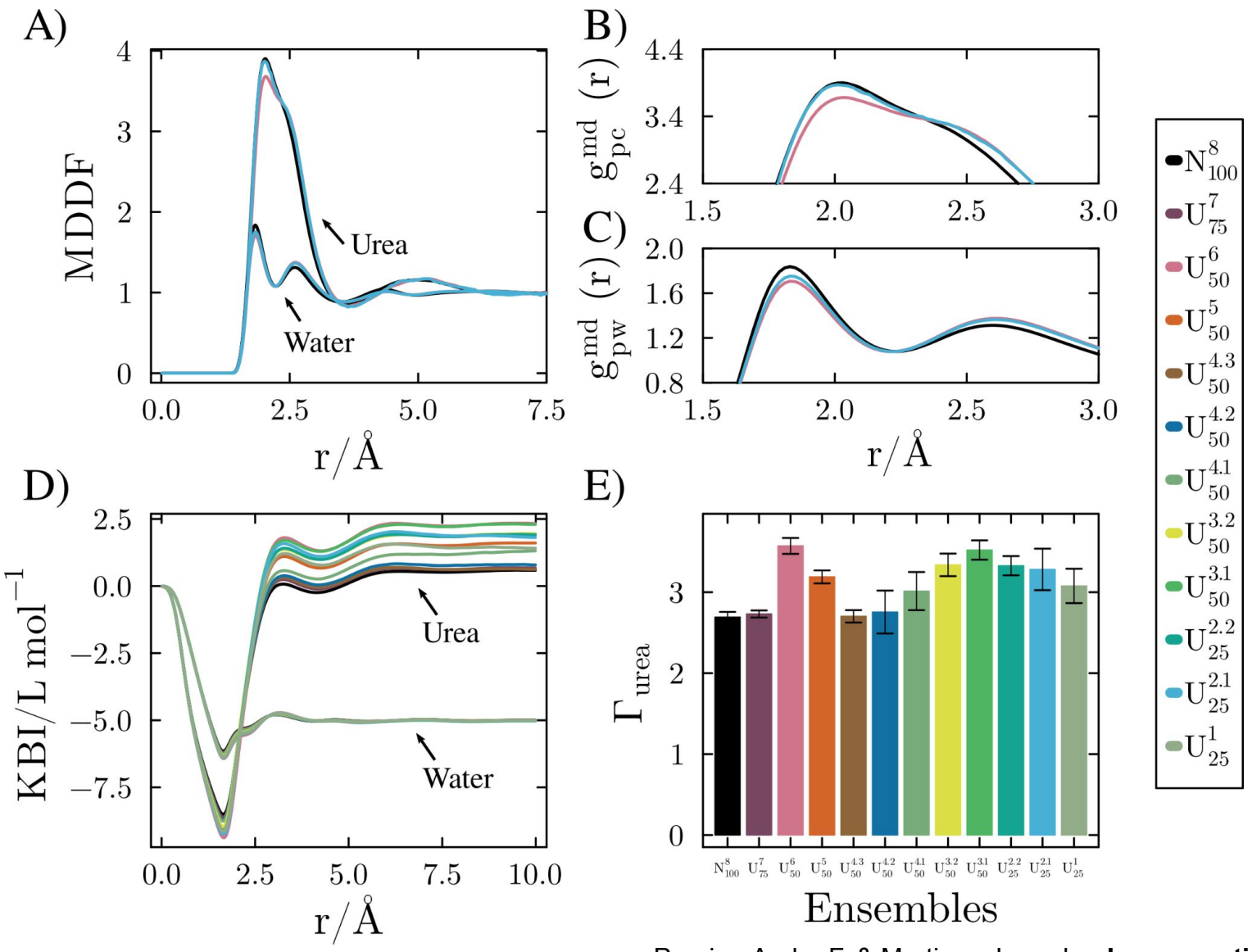
Alpha-helix content



A) The single sharp peak indicates that the two-state model is a good representation of the BdpA folding from SBMs;
B) A well-defined energy barrier separates the folded (N) and unfolded (U) states;
C) Multiple transitions between N and U are observed;
D) The contour maps of PD also capture the distinction between N and U states.

Oliveira Jr, Antonio B., Huan Yang, Paul C. Whitford, and Vitor BP Leite. **JCTC**. 15, no. 11, 6482-6490, 2019.
Pereira, Ander F., & Martínez, L. **JCIM**, 64(8), 3350-3359, 2024.

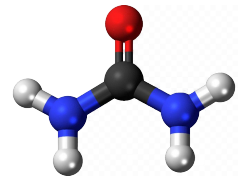
BdpA in urea ~0.5 mol L^{-1}



The density of **urea** and **water** molecules decreases in the first solvation shell of the unfolded states, but the opposite is observed in the second shell.

The Kirkwood-Buff integral (KBI) of water remains constant across all folding states.
The affinity for urea is higher in any unfolded state.

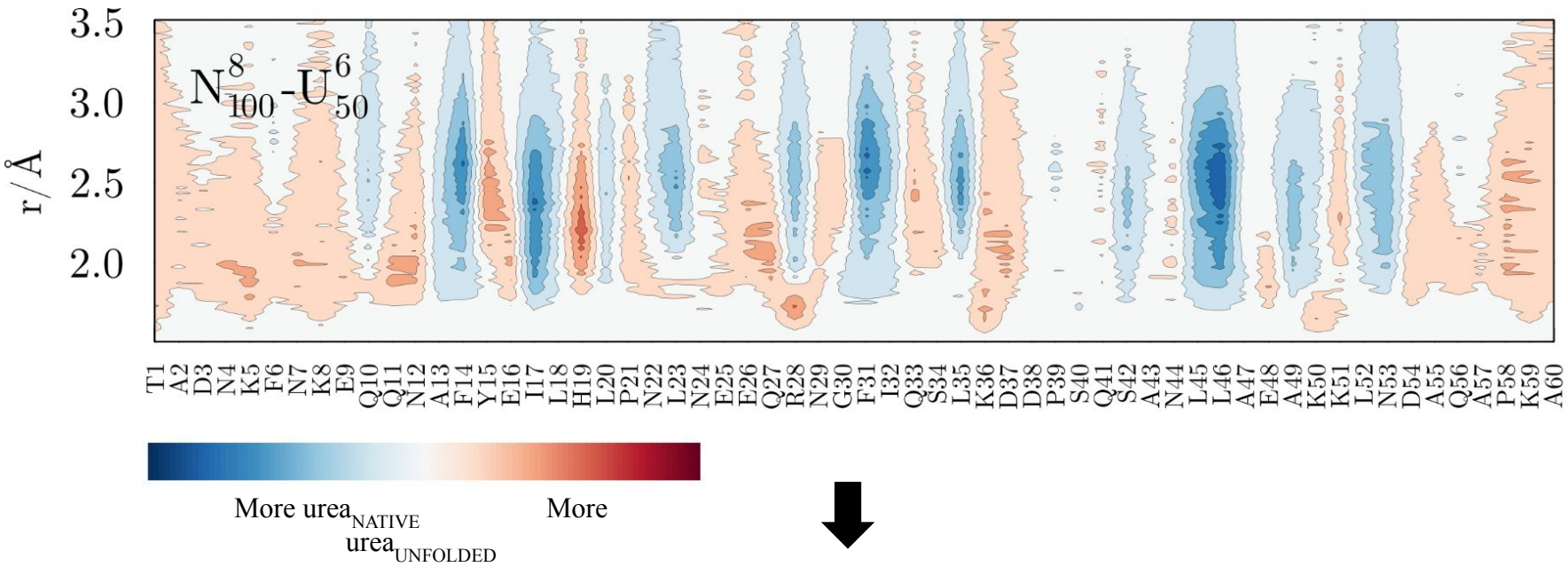
The protein is preferentially solvated by urea.



Pereira, Ander F, & Martinez, Leandro, **In preparation**, 2024.

BdpA in urea ~0.5 mol L^{-1}

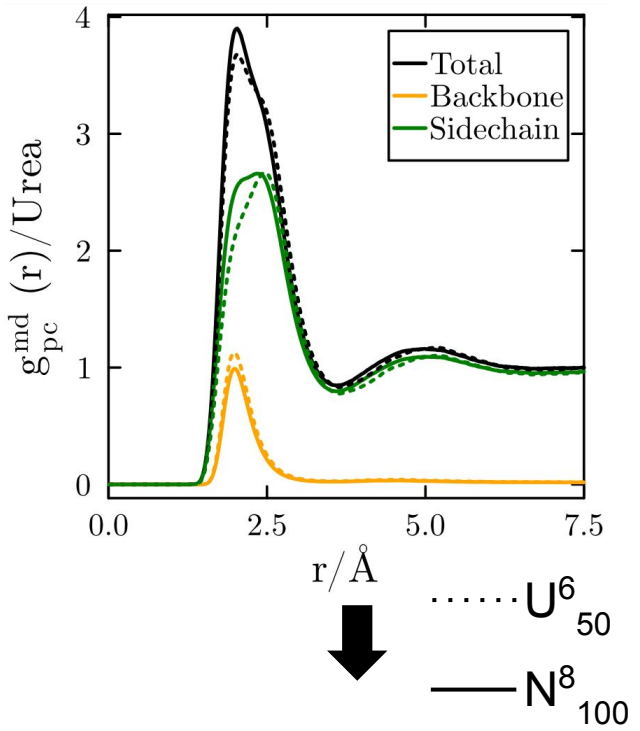
- Difference in the MDDF density of the urea in the vicinity of native (N^{8}_{100}) and unfolded (U^{6}_{50}) states.



- Urea accumulated more in the first shell (~2.0 Å) of the N^{8}_{100} state, especially around the polar, charged.

- On the other hand, for the U^{6}_{50} unfolded state, urea accumulates more around the hydrophobic residues on the second shell (~2.5 Å).

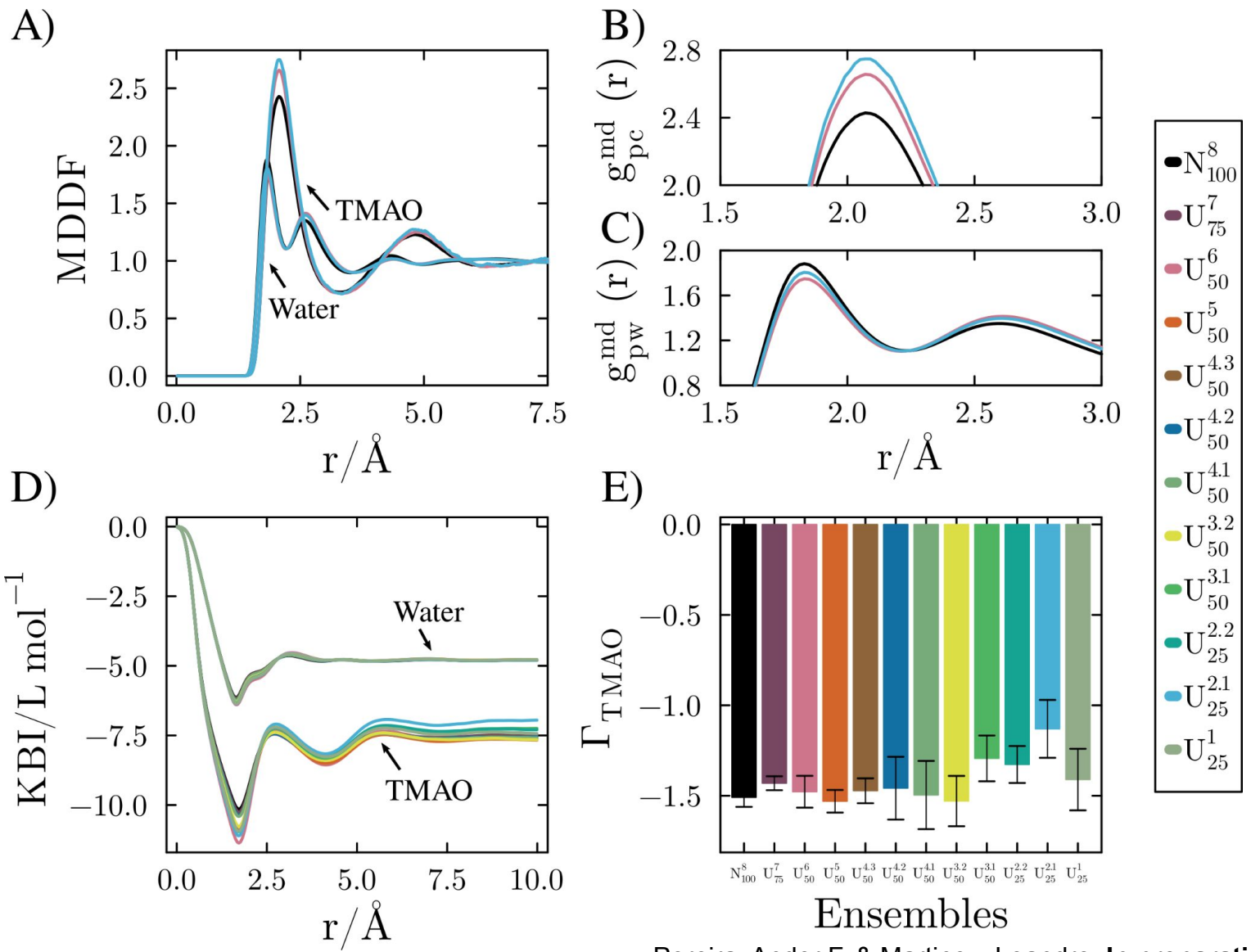
- Contributions to the total MDDF



Urea accumulates more around the backbone, and lower around the side chain of the U^{6}_{50}, compared with the N^{8}_{100}.

Pereira, Ander F, & Martinez, Leandro, **In preparation**, 2024.

BdpA in TMAO ~0.5 mol L^{-1}

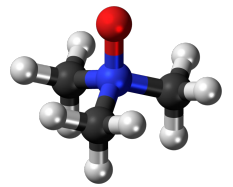


The density of **TMAO** increases in the first solvation shell of the unfolded states.

The density of **water** decreases in the first solvation shell of the unfolded states, but the opposite is observed in the second shell.

The affinity of TMAO for the unfolded states tends to be higher than for the folded state.

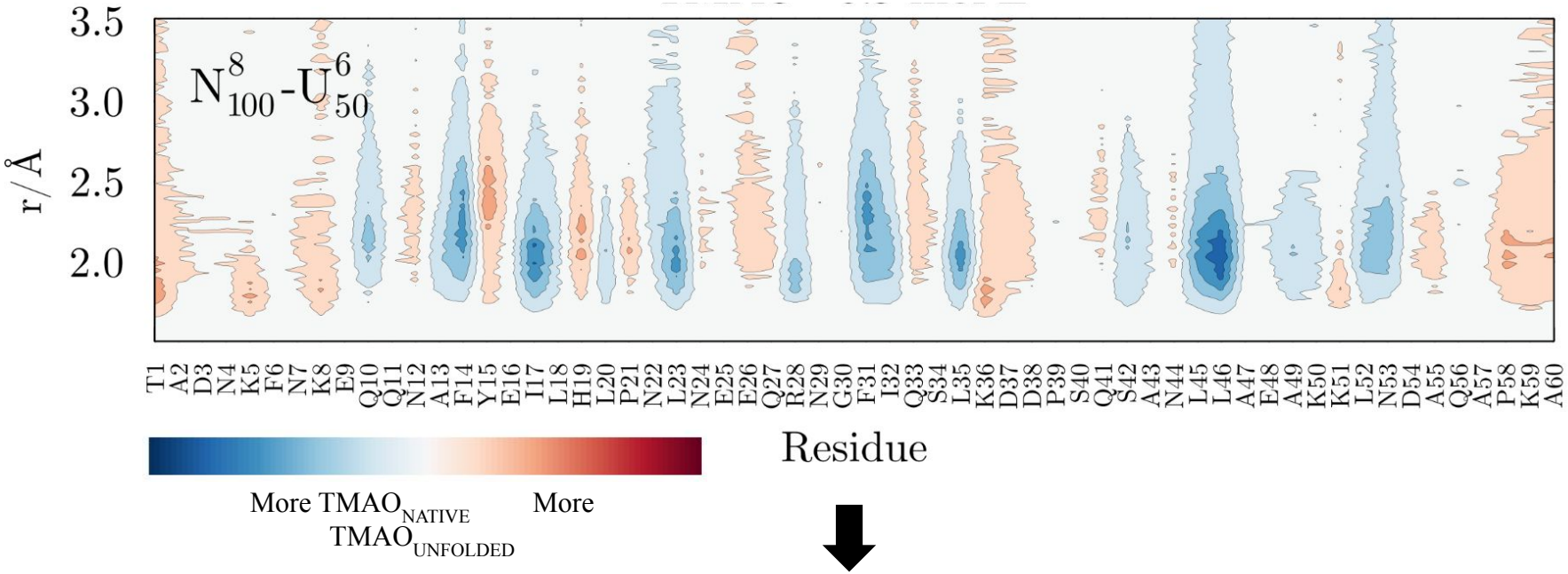
The protein is preferentially hydrated.



Pereira, Ander F, & Martinez, Leandro, **In preparation**, 2024.

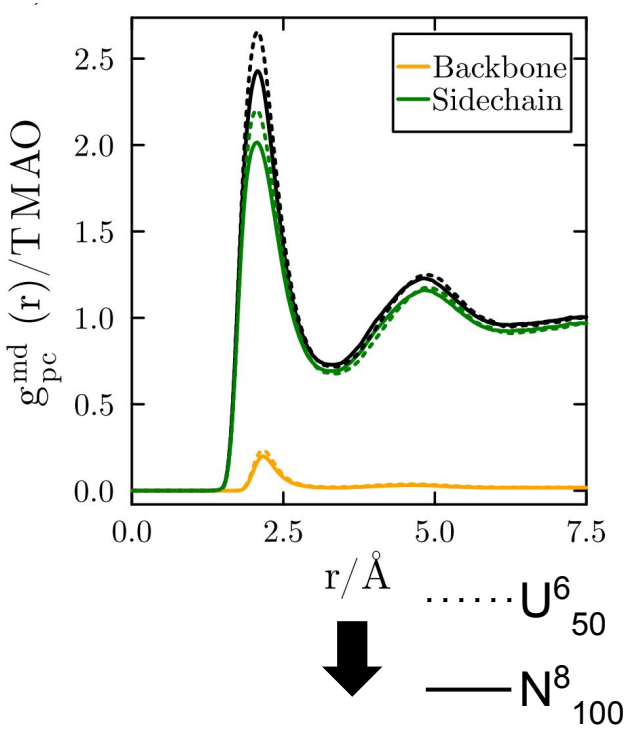
BdpA in TMAO ~0.5 mol L^{-1}

- Difference in the MDDF density of the TMAO in the vicinity of native (N^8_{100}) and unfolded (U^6_{50}) states.



- TMAO accumulated more in the first shell (~2.0 Å) of the U^6_{50} state, especially around hydrophobic residues.

- Contributions to the total MDDF

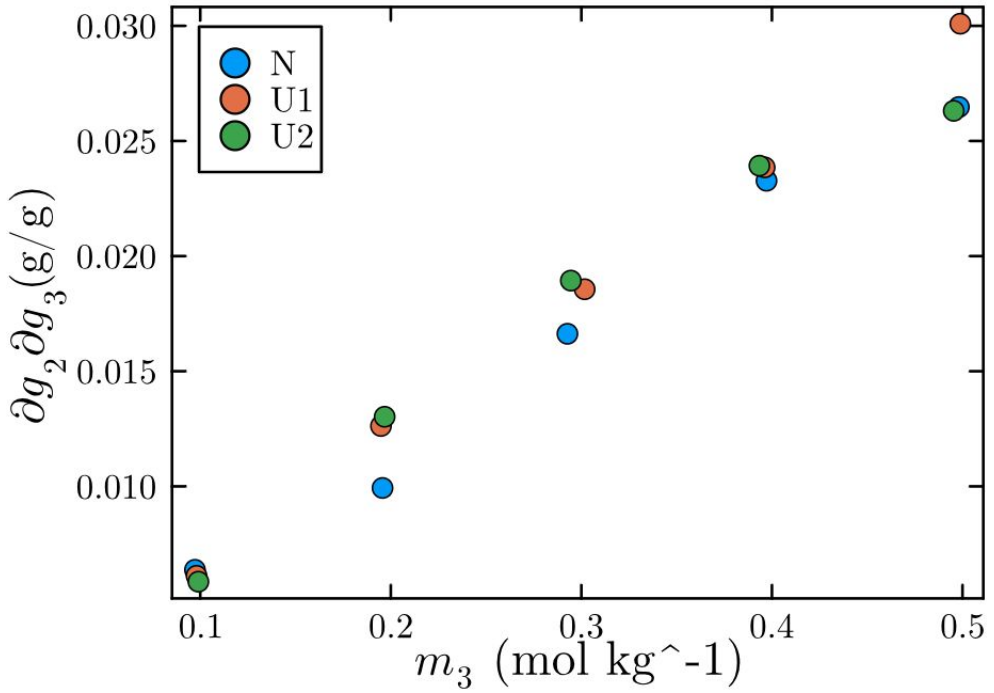


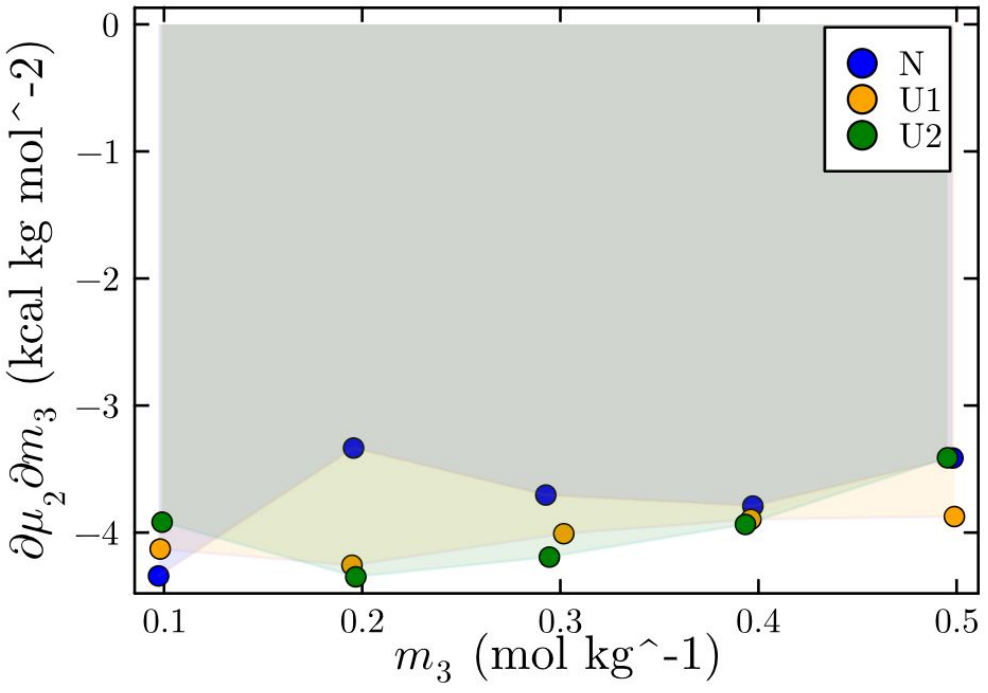
TMAO accumulate more around the side chains of the U^6_{50}, comparing with the N^8_{100}.

Pereira, Ander F, & Martinez, Leandro, **In preparation**, 2024.

Free energy of BdpA ensembles in the urea

Preferential interaction parameters: → Total free energy (Δμ):



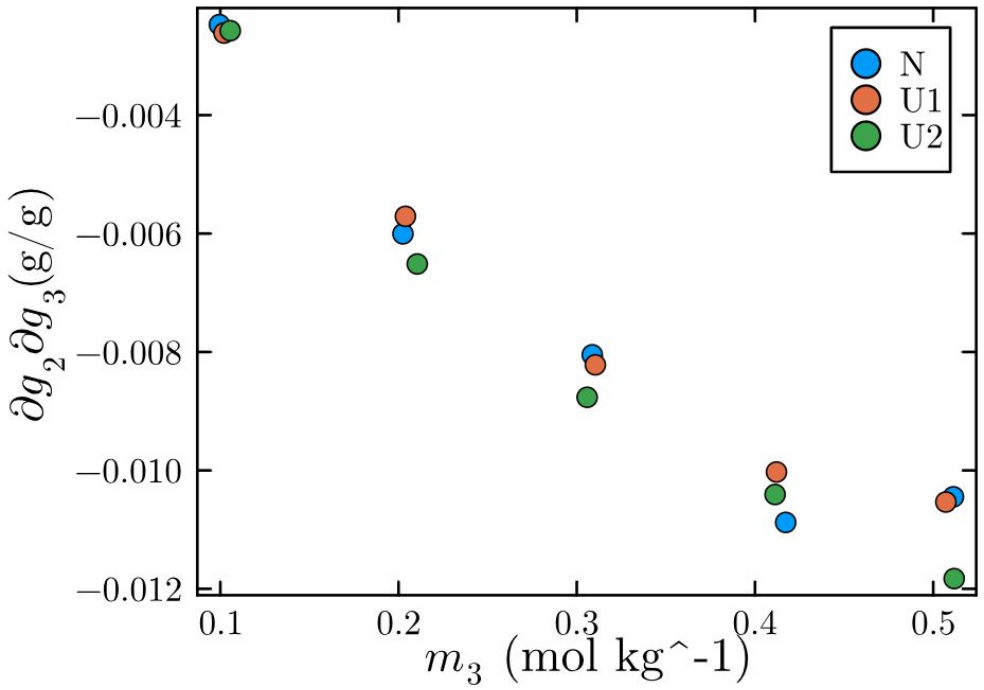


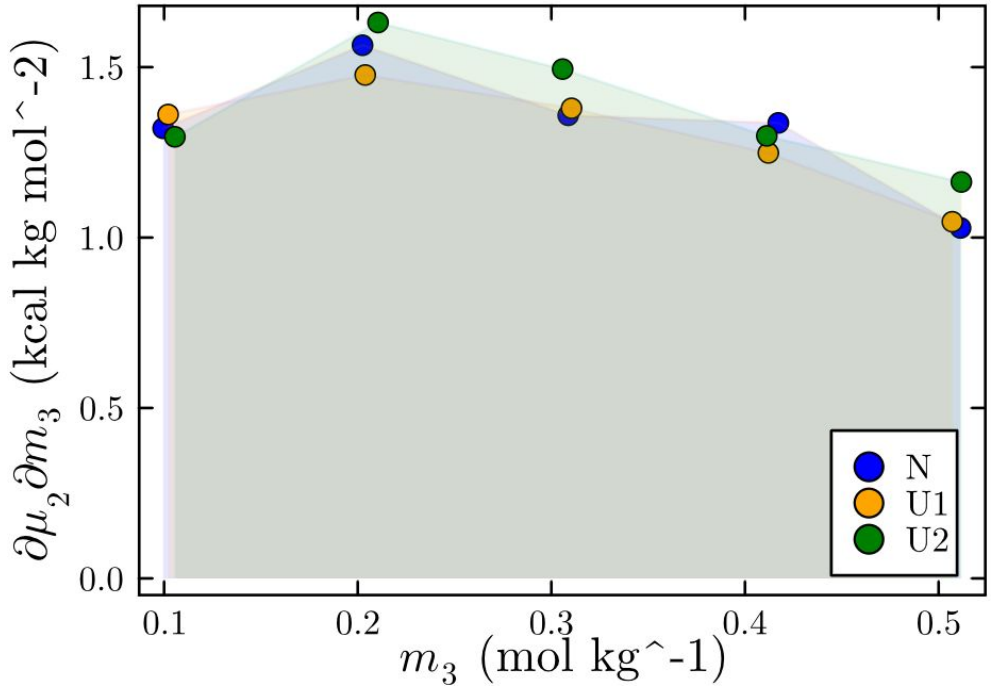
Ensemble	Δμ (kcal mol^{-1})	∂Δμ (kcal mol^{-1})	α-helix content	SASA (nm^2)
N	-1.4744	-	>75%-100%	51.26
U1	-1.6198	-0.1454	>25%-50%	64.46
U2	-1.5980	-0.1236	0%-25%	61.11

Pereira, Ander F, & Martinez, Leandro, **In preparation**, 2024. https://m3g.github.io/PereiraMartinez2024.jl

Free energy of BdpA ensembles in the TMAO

Preferential interaction parameters: → Transfer free energy (Δμ):





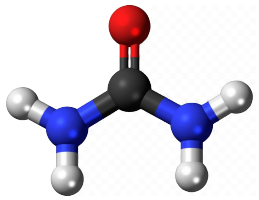
Ensemble	Δμ (kcal mol^{-1})	∂Δμ (kcal mol^{-1})	α-helix content	SASA (nm^2)
N	0.5613	-	>75%-100%	51.26
U1	0.5392	-0.02210	>25%-50%	64.46
U2	0.5734	0.01210	0%-25%	61.11

Pereira, Ander F, & Martinez, Leandro, **In preparation**, 2024. https://m3g.github.io/PereiraMartinez2024.jl

Conclusions and perspectives

- **Part 1: simulations with SBMs.**
  - show an exhaustive sampling of the folded and unfolded states of the protein, as well as the transition states.

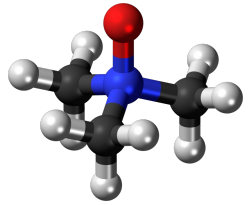
- **Part 2: atomistic simulations in explicit solvent.**
  - The MDDF and the Kirkwood-Buff theory allow us to obtain clear pictures of solvation structures.



Urea

1) accumulates on the protein surface;

2) stabilize the extended structures, with relative free energy of -**0.1454 kcal mol^{-1}**, comparing with the native state at 0.5 mol L^{-1}



TMAO

1) is preferentially excluded from the protein surface.

2) The effect is similar for both folded and unfolded states, with a slight preference for the U1 ensemble at 0.5 mol L^{-1}.

Acknowledgements

Prof. Dr Leandro Martinez
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Vinicius Piccoli (PhD)
Pamella Cristiny Carneiro da Silva (PhD)
Ana Beatriz Lima (Msc)
Brenda Kathleen de Almeida (Msc)
Lucas Verona de Araújo (IC)

M3G UNICAMP

http://m3g.iqm.unicamp.br/

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FAPESP: 2020/04549-0

CNPq
Conselho Nacional de Desenvolvimento Científico e Tecnológico

CNPq: 140853/2020-0

LinkedIn

Transfer Free Energy (TFE)

$$\Delta\mu_{2,\,tr}^{U} - \Delta\mu_{2,\,tr}^{N} = \Delta\mu_{N\rightarrow U}^{cos} - \Delta\mu_{N\rightarrow U}^{0} = m_{value}$$

Experimental parameter in the TFE equation

$$\left(\frac{\partial \mu_2}{\partial m_3}\right)_{T,P,m_2} = -\left(\frac{\partial g_3}{\partial g_2}\right)_{T,P,\mu_3} \left(\frac{RTM_2}{M_3}\right) \left(\frac{1}{m_3} + \frac{\partial \ln \gamma_3}{\partial m_3}\right)$$

How to convert g/g to mol/mol

To convert the preferential interaction parameter from units of (g/g) to units of (mol/mol), follow these steps:

Given:

- Preferential interaction parameter in units of g solute/g solvent
- You want to convert this to units of mol solute/mol solvent.

Steps:

1. **Find the molar masses:**
   - Ms: Molar mass of the solute (g/mol).
   - Mv: Molar mass of the solvent (g/mol).
2. **Use the conversion relationships:**
   - 1 g of solute = Ms1 mol of solute.
   - 1 g of solvent = Mv1 mol of solvent.
3. **Convert the preferential interaction parameter:**
   - Let Γ be the preferential interaction parameter in g solute/g solvent.
   - To convert to mol solute/mol solvent, multiply by the ratio of the molar masses:

   $$\Gamma' = \Gamma \times \frac{Ms}{Mv}$$

   where:

   - Γ′ is the preferential interaction parameter in mol solute/mol solvent.
   - Γ is the preferential interaction parameter in g solute/g solvent.

How can we determine TFE experimentally?

TFE can be obtained from preferential interaction parameter → Preferentially interaction parameters can be experimentally measured by densitometry by dialysis equilibrium or vapor pressure osmometry.

Densitometry by dialysis equilibrium is a method used to study the binding interactions between molecules. It combines dialysis, where two solutions are separated by a semipermeable membrane, with densitometry, a technique that measures the concentration of substances in solutions by analyzing their absorbance or optical density.

We can use DSC to measure the stability of protein at different cosolvent concentration

RDFs podem se experimentalmente obtidas por meio de dados provenientes de técnicas como difração de raios X (DRX) e espalhamento de nêutrons

Activity coefficients can be measured easily by vapor pressure osmometry.
The experimental parameter that I've measure before depends on the activity coefficient.