Protein Hydration & Protein Motion

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Nucci et al. (2011) NSMB 18, 245
Nucci et al. (2011) JACS 133, 12326

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Why study protein hydration?

- The entropy of water is the primary stabilizing force for proteins
- Protein-ligand interactions often require desolvation (“dry” interfaces)
- Protein-ligand interactions often have mediating waters (“wet” interfaces)
- The potential coupling of protein dynamics and solvent dynamics
- etc.

How can we study protein hydration?

- Computation: Dominant but somewhat suspect; few reference standards
- Crystallography: Dominant but dynamically limited & deceptive
- Dielectric relaxation
- Infrared spectroscopy
- Terahertz spectroscopy
- Neutron scattering
- Fluorescence/IR: Site-resolved probes but laborious and limited
- DNP: Site-resolved but laborious; highly complementary to NMR
- Magnetic relaxation dispersion: average property, sometimes resolved
- NMR: powerful (site resolved, comprehensive) but sample limited

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A crystallographic view: A cautionary note

Same crystal form; Same mother liquor; At RT; identical protein structure; different solvation waters (and incomplete)

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Wüthrich and coworkers (Science, 1991) proposed using NOE/ROE ratios to characterize hydration water location & dynamics

\[
\text{NOE and ROE } \propto \frac{1}{r_{HH}^6}
\]

- **NOE limited** by short residence times of hydration water
- The approach **defeated** by hydrogen exchange artifacts
- Criticized for bulk water averaging artifacts (Halle, 2003)
- Largely **abandoned** except for “structural water”

So we need a different sample with slower water, no bulk water averaging and suppressed hydrogen exchange.

*This implicitly assumes that slowing the water doesn’t qualitatively change the nature of the distribution.*
NMR spectroscopy of encapsulated protein dissolved in low viscosity fluids

- Protein pI & surfactant charge dependent
- Water loading dependent \([\text{water}] / [\text{surfactant}] \approx 10-20\)
- Salt dependent
- Equilibrium often achieved in minutes
- Preparations often stable for months/years
- Protein loading approaches 0.25 mM

Wand et al. (1998) PNAS 95, 15303

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Careful encapsulation search leads to high fidelity maintenance of structure …

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The Structure of Encapsulated Ubiquitin is Statistically Indistinguishable from the Free Solution Structure

Demonstrated:

• Native structure is maintained
• NOE- and J-based restraints used
• High resolution structure (0.25 Å rmsd backbone)
• RDCs not employed here but are available - see Valentine et al. (2006) JACS 128, 15930

Issue #1: Overcoming water dynamics

Confinement slows water dynamics ~ two orders of magnitude:
- IR
- Scattering
- Simulation
- etc.

Park et al. (2008) JPCB 112, 5279

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Issue #2: hydrogen exchange artifacts

\[ N - H + H_2O \overset{OH^-}{\rightleftharpoons} N - H + HOH \]

In bulk, hydrogen exchange corrupts NOE/ROE intensities. In the RM, HX chemistry is quenched by > 100-fold (@ same pH). Due to two factors: catalyst (OH⁻) delivered by reverse micelle exchange (µs) & slowed water dynamics.

Nucci et al. (2011) NSMB 18, 245
Issue #3: water averaging artifacts

Rendered moot since >99% of the “long distance” water is now gone!

(and no NOE interactions with the pentane/propane were found anyway)
Protein containing reverse micelles resist taking up excess water...

...suggesting that the native hydration shell is maintained i.e. thermodynamically dominant

\[ \tau_m \propto (aW_0 + b)^3 \]

\[ W_0 = [H_2O]/[\text{surfactant}] \]

Nucci et al. (2011) JBNMR 50, 421
Dozens of true protein amide hydrogen short distance interactions with water are revealed.

Ubiquitin in AOT/pentane, pH 5
Slices at the water plane (40 ms NOE/ROE mix time: linear regime)
Protein perdeuterated at carbon to eliminate $H\alpha$ under the water resonance

Nucci et al. (2011) NSMB 18, 245

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Distinguishing rigid and mobile “bound” hydration water by solution NMR

\[ \sigma_{\text{ROE}} = K \left( 2J(0) + 3J(\omega) \right) \]
\[ \sigma_{\text{NOE}} = K \left[ 6J(2\omega) - J(0) \right] \]

\[ J(\omega) = \frac{\langle O \rangle^2}{r^3} \frac{\tau_m}{1 + \omega^2 \tau_m^2} + \left( 1 - \frac{\langle O \rangle^2}{r^3} \right) \frac{\tau}{1 + \omega^2 \tau^2} \]

\[ \frac{1}{\tau} = \frac{1}{\tau_m} + \frac{1}{\tau_e} \]

ROE: Bothner-By et al. (1984) 106, 811

• Fast tumbling limit: \( \tau_m \omega \ll 1 \), \( \sigma_{\text{NOE}} / \sigma_{\text{ROE}} \rightarrow 1 \)
• Slow tumbling limit: \( \tau_m \omega \gg 1 \), \( \sigma_{\text{NOE}} / \sigma_{\text{ROE}} \rightarrow -0.5 \)
• The ROE suppresses multiple dipole-dipole transfers (spin diffusion)
• Angular disorder (O) is convolved with distance variation (r)
Hydration water dynamics correlates with the binding interface

- Hydration surface constructed using 15N- & 13C-resolved NOEs
- Rigid (blue-purple) bound water has low entropy (S)
- Dynamic (orange-red) bound water has higher entropy (S)
- High S water associated with surface not binding proteins
- Low S water associated with surface binding proteins
- Suggests the hydrophobic effect has been maximized by evolution for the protein-protein interface!

Nucci et al. (2011) JACS 133, 12326
Putting it together: Solvent slaving & what it “predicts”

- Proteins have various classes of motion
- These classes differ in the degree to which they are dependent on solvent motions

Class I: slaved to the $\alpha$ motions of solvent, which are long-range collective motions. In the absence of alpha motions in the solvent, these protein motions are impossible.

Class II: slaved to the $\beta$ motions of the hydration layer. Changes in the mobility of the hydration layer should alter the rate/magnitude of these protein motions.

Class III: non-slaved motions. These internal motions of the protein are independent of solvent. These were added relatively recently as a concession.

- There is virtually no site-resolved experimental support for this model but it is widely accepted

Dynamics as a Proxy for Entropy Parametric Relationship Between $S$ and $O^2$

Order parameter:  

- $O^2 = 1$ = fixed orientation
- $O^2 = 0$ = no preferred orientation

The simple harmonic oscillator

Li et al. (1996) Prot. Sci. 5, 2647
NMR relaxation reports on protein dynamics

\[ T_1 = \frac{\gamma_2}{2} \Delta \sigma_2 \]

\[ J(\omega) = \frac{2}{5} \left[ \frac{O^2 \tau_m}{1 + \omega^2 \tau_m^2} + \frac{(1 - O^2) \tau}{1 + \omega^2 \tau^2} \right] \]

\[ \frac{1}{T_1} = \left( \frac{\hbar^2 \gamma_H^2 \gamma_C^2}{4 r_{CH}^6} \right) (J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_H + \omega_C)) + \frac{\omega_C^2 \Delta \sigma^2}{3} J(\omega_C) \]

15N: Farrow et al. (1994) Biochem. 33, 5984
2H: Munhandiram et al. (1995) JACS. 117, 11536
13C: Ishima & Torchia (2001) JACS 123, 6164

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Side chain dynamics represented by methyl groups.

Methyl groups
– well-distributed
– numerous
– $^2$H simple relaxation

$2\text{-}3$ ps $< 30 \text{ – } 80$ ps $<< 8.5$ ns

$\tau_{\text{rot}}$, $\tau_{e}$

- $^2$H relaxation reports on C-C bond vector (sym axis)

- $O_{\text{axis}}^2 = O^2/0.111$ (geometry & time scale separation)

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Methyl groups have a wide range of $O^{2}_{axis}$ values.

Little correlation with depth of burial, amino acid type, solvent exposure, secondary structure, B-factors and other primitive correlates.


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Encapsulation generally makes the backbone more rigid.

\[ O_{NH}^2 \]

Increasing rigidity

\[ \eta_{RM}^{H_2O} \sim 50 \cdot \eta_{bulk}^{H_2O} \]

Residue #
Encapsulation generally makes the backbone motion slower.
Encapsulation causes only subtle restriction of side chain motion

\[ \eta_{RM} \sim 50 \cdot \eta_{bulk} \]

RM \( O_{axis}^2 \)
error bars from true replicates

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Are the backbone dynamics correlated with hydration water dynamics in the RM? **NO**
Are the methyl side chain dynamics correlated with hydration water dynamics in the RM? **NO**

![Graph showing the relationship between O$_2$ axis in AOT RM and NOE/ROE with Rigid Hydration and Dynamic Hydration]

*Only surface CH$_3$ shown*
Conclusions

• Encapsulation effectively eliminates the artifacts that have historically hindered detection of protein-water interactions by solution NMR methods

• Protein hydration shows heterogeneous dynamics; no obvious correlation with surface character; clustering of motional timescale apparent

• Patches of slow (long residence time) hydration water seem correlated with binding surface (one example!); suggests evolution has used the entropy of water to maximize binding free energy and to provide specificity

• Fast protein dynamics seem not slaved to solvent as predicted (one example!)
NMR Spectroscopy of Encapsulated Proteins in Low Viscosity Fluids

Recent Contributors

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Funding

Mathers, NSF & NIH

Disclosure: Ron Peterson, Brian Lefebvre & Josh Wand are Members of Daedalus Innovations, LLC

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