Resolving the Motional Modes That Code for RNA Adaptation

*Science 2006, 311, 653-656*

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Change of conformation is requirement for biological function:

• Substrate binding
• Reaction
• Product release
Citing Articles

WEB OF KNOWLEDGE (16.10.2013)

1. Title: A hierarchy of timescales in protein dynamics is linked to enzyme catalysis
   Author(s): Henzler-Wildman, Katherine A.; Lei, Ming; Thai, Vu; et al.
   Source: NATURE Volume: 450 Issue: 7171 Pages: 913-U27 DOI: 10.1038/nature06407 Published: DEC 6 2007
   Times Cited: 333 (from All Databases)

2. Title: Visualizing spatially correlated dynamics that directs RNA conformational transitions
   Author(s): Zhang, Qi; Stelzer, Andrew C.; Fisher, Charles K.; et al.
   Source: NATURE Volume: 450 Issue: 7173 Pages: 1263-U14 DOI: 10.1038/nature06389 Published: DEC 20 2007
   Times Cited: 111 (from All Databases)

3. Title: Observing biological dynamics at atomic resolution using NMR
   Author(s): Mittermaier, Anthony K.; Kay, Lewis E.
   Source: TRENDS IN BIOCHEMICAL SCIENCES Volume: 34 Issue: 12 Pages: 601-611 DOI: 10.1016/j.tibs.2009.07.004
   Published: DEC 2009
   Times Cited: 74 (from All Databases)
Motional Modes

Complex superposition of motional modes

- Local librations (ps)
- Collective domain motions (ns to ms)
- Overall Brownian rotational diffusion (ns to ms)

Problem:
Individual contributions cannot be readily resolved when modes are physically coupled
Domain Elongation Strategy

Resolve ps local motions and ns domain motions by NMR
Domain Elongation Strategy

Similar time scales
Motional modes *coupled*
Domain Elongation Strategy

Elongation is unlabeled -> “NMR-invisible”

Hydrodynamic shape is less sensitive to domain motions

Separate time scales
Motional modes decoupled
Samples

Two regulatory RNAs from HIV-1

(E-)TAR
[(elongated) transactivation response element]

E-SL1\textsubscript{m}
[engineered form of Stem Loop 1: GAGA-tetraloop substituted for the GC-rich loop + elongated]
Elongation residues

$^{1}H$-$^{13}C$ HSQC:
Chemical shifts for E-TAR in excellent agreement with nonelongated TAR (free form/ + ARG)
[ARG: argininamide]
[Asterisks: terminal guanine (G-21, G-22) and cytosine (C+21, C+22)]
[Circles: highlighted ARG-induced chemical shift perturbation]

$^{1}H$ jump-return:
Degenerate $^{1}H$ Chemical shifts for elongation residues

Strong evidence for helical structure without effecting TAR
Results
$T_2$ vs. Resonance Intensity

**Fast motion**

**Slow motion**

*Abbildung 2.15: Zusammenhang zwischen Linienform und Relaxation*
Results: Resonance Intensities

Fig. 2. RNA dynamics by motionally decoupled NMR. (A to D) Normalized resonance intensities (peak heights) measured from nonconstant-time 2D $^1$H–$^{13}$C HSQC spectra. Shown are values for sugar C1′H1′ (diamonds) and base C2H2 (squares), C5H5 (circles), C6H6 (triangles), and C8H8 (inverted triangles) in (A) E-AU-TAR + E-GC-TAR, (B) TAR, (C) E-AU-TAR+ARG + E-GC-TAR+ARG, and (D) TAR+ARG. The intensity for each type of C-H spin is normalized to a minimum value of 0.1 independently for G-C and A-U residues. Insets show intensities for Watson-Crick residues only. The UUCG loop intensities are denoted by open symbols.
Results: Resonance Intensities

- Domain I shows only overall tumbling
- High flexible domain-domain interface
- Domain II moves collective
- UUCG loop collective and local
- Most motions not resolved in TAR but in E-TAR
Results: Resonance Intensities

(A) E-TAR

(B) TAR

(C) E-TAR + ARG

(D) TAR + ARG (argininamide)
Results: Resonance Intensities

1. Reduction in the relative intensity of most sites in domain II
2. Reduction of the mobility at the domain-domain interface (U23, A22, U40)
3. Exposes the local mobility of the UUCG loop

- Consistent with an arrests domain motions (completely undetected in TAR, detected in E-TAR)
Results: $^{15}$N Relaxation Data

Ratios ($R_2/R_1$) of imino $^{15}$N transverse ($R_2$) to longitudinal ($R_1$) relaxation rates measured for guanine (circles) and uridine (diamonds) residues in (E) E-AU-TAR + E-GC-TAR, (F) TAR, (G) E-AU-TAR+ARG + E-GC-TAR+ARG, and (H) TAR+ARG. Hydrodynamically predicted $R_2/R_1$ values are denoted by open symbols.
Results: $^{15}$N Relaxation Data

- $R_2/R_1$: domain II < domain I
  - Domain motions reorient every site in domain II relative to domain I

- $R_2/R_1$: domain II ~ domain I
  - Domain motions cannot be separated from rotational diffusion
Results: \( ^{15}\text{N} \) Relaxation Data

ARG binding increases domain II \( R_2/R_1 \)

- Arrest of domain motions

Dynamical arrest undetected!
Model-Free Analysis

Analyzing dynamics through NMR relaxation data

Rotational diffusion tensor $D_{rr}$

Rotational relaxation times $\tau_l$

PDB-file

Vectors $\vec{u}$ within the particle

Correlation function

$< P_2(t) > = < 3(\vec{u}(t) \cdot \vec{u}(0))^2 - 1 > / 2$

FT

T1, T2, NOE

Experiment

Spectral density $J_0(\omega)$

$S_f^2, S_s^2, \tau_f, \tau_s, \tau_m$
Model-Free Analysis

$S^2$: generalized order parameter (displays amplitude of internal motions)

$S^2 = 0 \rightarrow isotropic$

$S^2 = 1 \rightarrow completely\ restricted$

$S_f^2$: internal motion on the fast time scale (ps)

$S_s^2$: internal motion on the slow time scale (ns)

$\tau_f$: correlation time for internal motions on the fast time scale

$\tau_s$: correlation time for internal motions on the slow time scale

$\tau_m$: isotropic rotational correlation time of the molecule

Pertinent function in NMR for dynamics in the frequency domain
Results: Model-Free Analysis

\[ \tau_m = 18.9 \text{ ns} \quad (\tau_{\text{calc}} = 19.5 \text{ ns}) \]

excellent agreement with prediction
Results: Model-Free Analysis

\[ \tau_m = 18.9 \text{ ns} \quad (\tau_{calc} = 19.5 \text{ ns}) \]

excellent agreement with prediction

\[ \tau_f = 24 \text{ to } 33 \text{ ps} \]

\[ S_f^2 = 0.79 \text{ to } 0.89 \]

domain II displays slightly larger amplitudes
Results: Model-Free Analysis

\[ \tau_m = 18.9 \text{ ns} \ (\tau_{calc} = 19.5 \text{ ns}) \]

excellent agreement with prediction

\[ \tau_s = 1.5 \text{ to } 1.9 \text{ ns} \ (\tau_{calc} = 2.2 \text{ ns}) \]

\[ S_s^2 = 0.68 \text{ to } 0.76 \]

slower and larger amplitude domain motions for domain II

\[ \tau_f = 24 \text{ to } 33 \text{ ps} \]

\[ S_f^2 = 0.79 \text{ to } 0.89 \]

domain II displays slightly larger amplitudes
Results: Model-Free Analysis

\[ \tau_s \approx 2 \text{ ns (Domain motions)} \]
\[ \tau_{TAR} \approx 6 \text{ ns (overall rotational diffusion)} \]
\[ \tau_{m}^{E\sim TAR} \approx 20 \text{ ns (overall rotational diffusion)} \]

- Separation difficult in the absence of elongation
Results: Model-Free Analysis

- Arrests domain motions
- Uniform reduction in the librational amplitudes
Results: Model-Free Analysis

Similar local librations and collective domain motions for E-SL1_m

Fundamental motional modes for RNA structural adaptation?
RNA Adaptation

Adaptive structural changes or internal motions?

HIV-1 TAR: eight high-resolution NMR and x-ray structures
(in free form and bound to seven distinct targets)
[structures differ significantly, all-atom RMSD = 4.7 Å]
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(in free form and bound to seven distinct targets)
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Superimposed using domain I as reference

Mean angular variation $\langle \Delta \theta \rangle$
(orientation of C-H-bonds across the eight structures)

Adaptive structural changes or internal motions?
RNA Adaptation

Adaptive structural changes or internal motions?

Mean angular variation $\langle \Delta \theta \rangle$
(orientation of C-H-bonds across the eight structures)
RNA Adaptation

Adaptive structural changes or internal motions?

Mean angular variation $<\Delta \theta>$
(orientation of C-H-bonds across the eight structures)

B

$<\Delta \theta>$ (degree)

$R = 0.80$

Norm Intensity
Quellen

- H. Schwalbe, OC IV Skript, Goethe-Universität Frankfurt, **2012**
- Modelfree (version 4.0) manual
- Malcom H. Levitt, *Spin Dynamics*, second edition, John Wiley & Sons Ltd, West Sussex, **2008**
THANK YOU!