Structural dynamics of the GW182 silencing domain

Hydrogen-deuterium exchange fingerprint of RNA recognition motif (RRM)

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INTRODUCTION

In recent years, there have appeared a significant number of works proving that structural dynamics governs the function of biological molecules to no less extent than the protein sequence and its 3D structure. The GW182 protein is one of the crucial players in eukaryotic miRNA-mediated gene silencing, since it specifically interacts with the Argonaute (Ago) proteins and recruits the huge CCR4-NOT deadenylase complex to the targeted mRNA to trigger both translational repression at the 5' terminus and mRNA degradation from the 3' end. miRNA-dependent gene silencing by the GW182 protein is mediated by its C-terminal half called the silencing domain (SD). Structural studies of the GW182 silencing domain is challenging due to its intrinsically disordered character.

GW182s are predicted to be mostly disordered except for two globular domains: the ubiquitin associated domain and the RRM. Such protein structures are neither suitable for X-ray crystallography nor for NMR due to their dynamic character. However, quite detailed insight into the properties of proteins containing disordered regions can be provided by hydrogen-deuterium exchange mass spectrometry (HDX MS). This technique allows mapping of the stability of H-bonding networks, since the kinetics of the H/D exchange reflects the local dynamics of the protein structural elements. In this work, we reveal the structural dynamics of the human GW182 silencing domain.

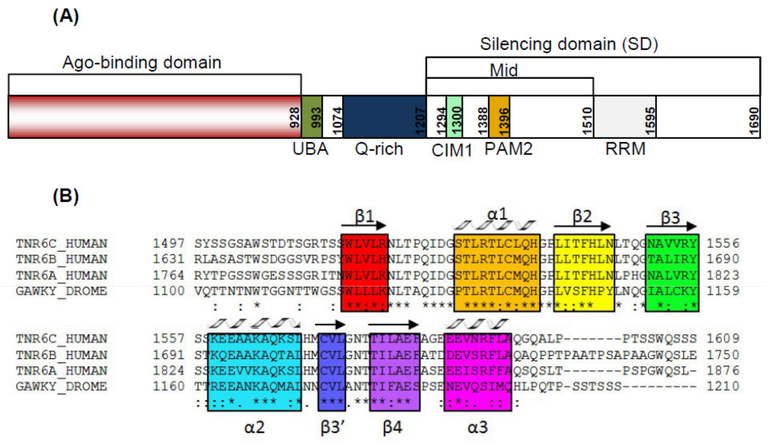


Fig. 1. (A) Schematic representation of human GW182 (TNRC6C): an Ago-binding domain, a ubiquitin associated domain (UBA), a glutamine-rich (Q-rich) region and the silencing domain (SD). SD contains a CCR4-NOT interacting motif (CIM1) [1], a PABP-interacting motif (PAM2) and RRM;
(B) Sequence alignments of the RRM domains of different GW182 isoforms from human, mouse and *Drosophila melanogaster*. Secondary structural elements are marked as color blocks corresponding to the α-helices and β-sheets shown in (C);
(C) Predicted human GW182 RRM structure shown as a ribbon colored according to the secondary structural elements in (B), aligned with the NMR *D. melanogaster* RRM structure (grey) [2].

Fig. 2. Representative mass spectra of a peptic GW182 peptide with immediately exchanged amide protons (left panels, residues 1286-1306, charge +2) encompassing the CIM1 motif and a peptide with slowly exchanging amide protons (right panels, residues1531-1543, charge +2) belonging to the most buried part of the RRM domain, at consecutive HDX time points.

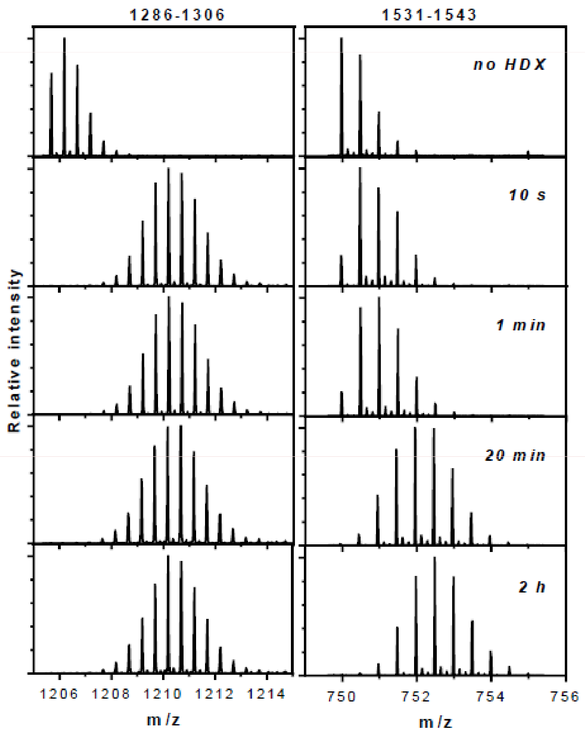


Fig. 3. Reproducible 99 % coverage of the GW182 SD sequence by redundant peptides obtained by pepsin digestion.

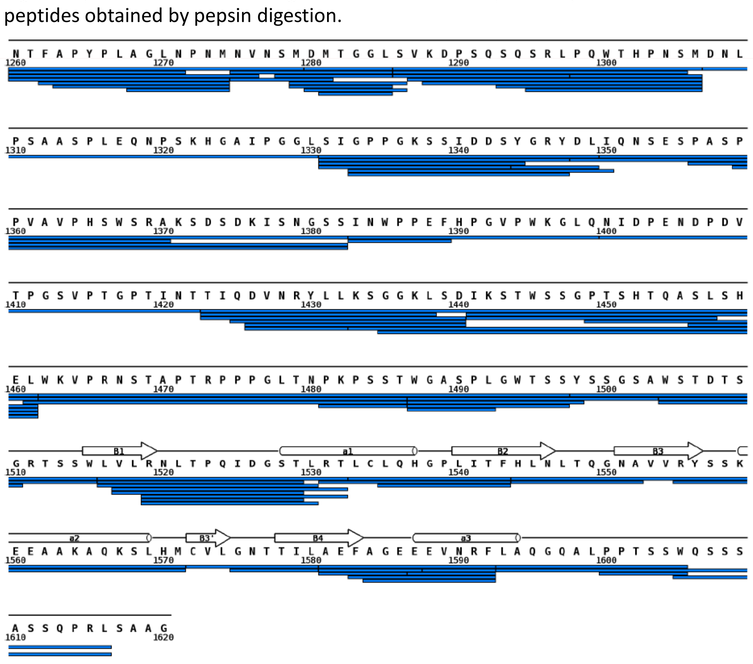


Fig. 4. (top to bottom) (A) Fraction of the exchanged population of amide hydrogens (with vertical standard errors) obtained for pepsin-generated GW182 SD10 peptides marked as horizontal black bars, after 10 seconds of deuteration, corrected for back-exchange of the individual peptides, overlayed with secondary structural elements assigned and colored as in Fig. 1A,C; (B) the same as in (A) after 1 minute, (C) after 20 minutes, and (D) after 120 minutes of deuteration.

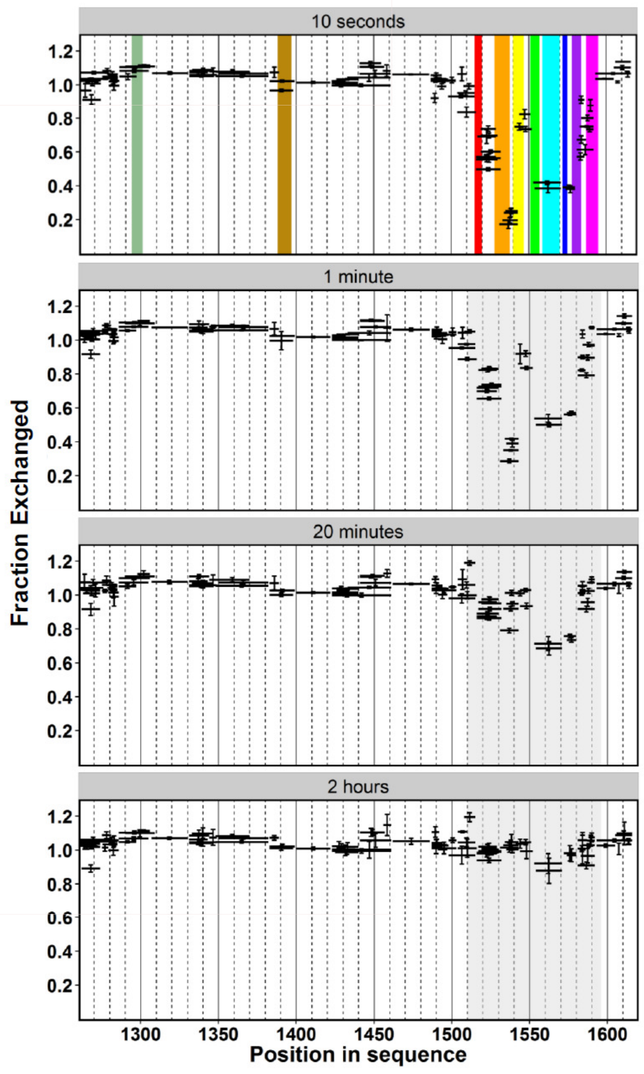


Fig. 5. Kinetic plots of hydrogen-deuterium exchange for non-redundant pepsin-generated peptides of GW182 SD10; data points represent the fraction of the amide hydrogen population exchanged to deuteria after consecutive time intevals of HDX; experimental uncertainty of the individual data points are shown as vertical bars; mono- or biexponential functions were fitted to the experimental data points. The results of the fitting are gathered in Table 1.

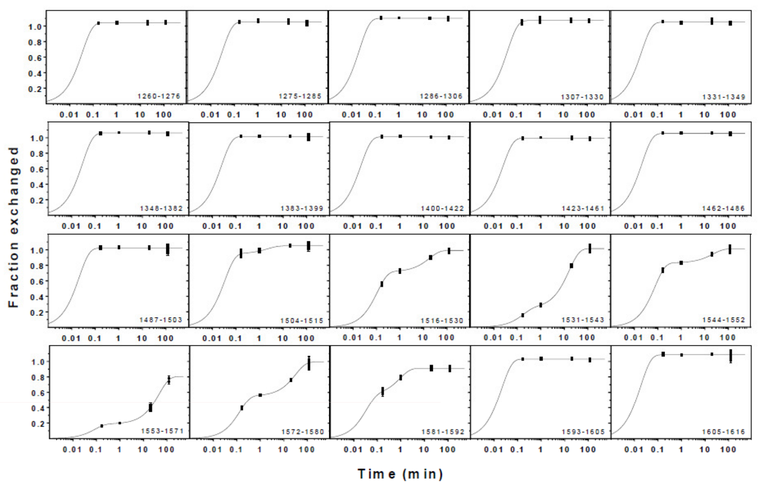


Table 1.

Kinetic parameters of hydrogen–deuterium exchange for pepsin-generated peptides of the GW182 RRM domain.

GW182	D_{inc1} (%)	k_1 (min^{-1})	D_{inc2} (%)	k_2 (min^{-1})	$\log(k_1/k_{int})$	$\log(k_2/k_{int})$
1504-1515	94.8 ± 1.6	30 [a]	10.3 ± 1.7	0.5 ± 0.3	-1.4	-3.2
1516-1530	71.5 ± 1.0	9.3 ± 0.4	27.7 ± 1.2	0.056 ± 0.006	-1.6	-3.8
1531-1543	24.1 ± 1.1	5.8 ± 0.8	77.4 ± 1.4	0.062 ± 0.002	-1.9	-3.8
1544-1552	83.0 ± 1.1	13.1 ± 0.9	18.3 ± 1.4	0.045 ± 0.009	-1.7	-4.2
1553-1571	18.7 ± 1.1	12 ± 3	62 ± 2	0.022 ± 0.002	-1.7	-4.4
1572-1580	55.2 ± 1.6	7.4 ± 0.7	44 ± 2	0.032 ± 0.005	-1.7	-4.1
1581-1592	55.9 ± 1.8	30 [a]	35.3 ± 1.9	1.08 ± 0.13	-1.0	-2.5

[a] fixed value

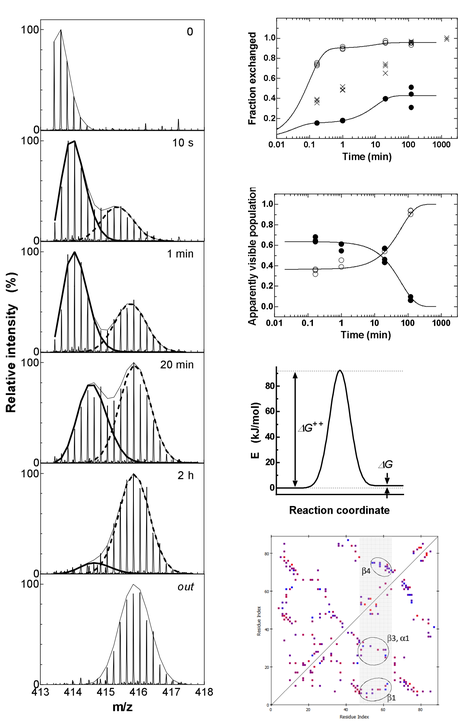
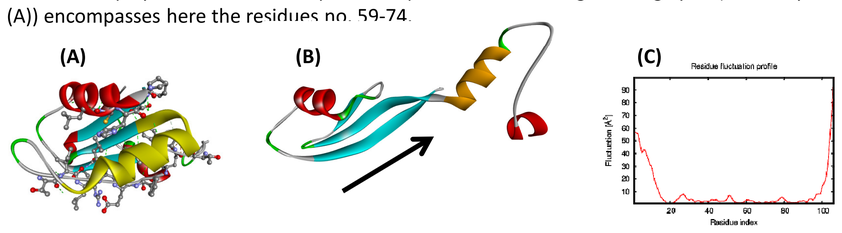


Fig. 6. (A) Bimodal isotopic envelopes for the GW182 SD RRM α2 peptide (residues 1544-1571, charge +5) after given time of H/D exchange; (—) closed conformation, (- - -) open conformation; (B) Kinetic plots of HDX for the 1544-1571 peptide; data points represent the fraction of the amide hydrogen population exchanged to deuteria after consecutive time intevals of HDX for (●) closed α2 peptide conformation, (○) open conformation, (x) average values; (C) Apparent populations in the closed and open state as seen by HDX: (●) the fraction of the population of the closed state molecules that never once underwent the change to the open conformation, (○) population of the open state together with the fraction of the population of the closed state molecules that at least once underwent the change to the open conformation; (D) Gibbs free energy barrier for the conformational equilibium of the α2 peptide; (E) Contact map (within 3.5 Å) for the side chains of the GW182 SD RRM (residues 1509-1595). The α-helix 2 region is marked as gray band. The contacts between the α2 residues with the β1, β3, β4 and α1 are encircled.

Fig. 7. (A) Human GW182 SD RRM model with the shortest α2 peptide (RYSSKEEAAKAQSLHM) that displays the bimodal isotopic envelope, marked yellow. Residues in non-covalent contacts with α2 (≤ 3.5 Å, excluding internal α2 contacts) belonging to α1, β1, β3, β4 (T15, S17, W18, L19, L21, R22, L36, C37, H40, P42, I44, T45, TILAEF(81-86), E89) shown as CPK balls-and-sticks;
(B) Crystal structure of a single RRM domain truncated from human PARN, with the α2 helix that is swapped in the RRM dimer (PDB 3CTR [3]) marked gold;
(C) The GW182 RRM flexibility profile – the domain structural fluctuations are negligible. The numbering corresponds to the RRM domain alone. The shortest peptide that contains the tight α2 helix and displays the bimodal isotopic envelope after the freezing-thawing cycle (marked yellow in (A)) encompasses here the residues no. 59-74.



CONCLUSIONS

The studies have provided the experimental proof that the silencing domain (SD) of GW182, except the RRM domain, is indeed natively unstructured. In particular, CIM1 does not display any evidence of a secondary structure.

Our work reveals the first hydrogen-deuterium exchange mass spectrometric "fingerprint" of an RNA Recognition Motif, which is one of the most frequently occurring canonical protein domains.

The GW182 RRM has a very dynamic structure, since the complete H/D exchange is achieved in 2 hours. These results indicate that water molecules can penetrate the whole domain.

We show that the RRM structure reveals the alternating HDX pattern, composed of the regions containing amide hydrogens that are more or less exchange-competent, even when the RRM domain is studied in the context of a larger fragment of the generally disordered protein, not suitable for NMR and crystallography due to its size and aggregation at higher concentrations. The experimentally determined structural dynamics of the GW182 SD RRM correlates well, in general, with the predicted α/β content.

The HDX studies suggest also that the α2 helix of the RRM can change its conformation after a freezing-thawing cycle, which might be a clue for explanation of a possible RRM aggregation pathway.

REFERENCES
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