Redox-Responsive Peptide Dimers to Stabilize and Release Therapeutic dsRNA

Jannik Keyser Paulus[a], Marvin A. Albers[b], Niall M. McLoughlin[b], Zainab M. Rashid[b], David Dulin[b] and Tom N. Grossmann[a]

a Institute of Organic and Biomolecular Chemistry, Faculty of Chemistry, Georg-August Universität Göttingen, Germany
b Department of Chemistry and Pharmaceutical Sciences and AIMMS, VU Amsterdam, The Netherlands
https://doi.org/10.17952/38EPS.2026.P095

GEORG-AUGUST-UNIVERSITÄT GÖTTINGEN

IN PUBLICA COMMODA

Motivation: Double-stranded RNA (dsRNA) is a key scaffold in antiviral defence, gene regulation, and emerging RNA therapeutics.[1] Moreover, dsRNA-based approaches can access biological targets that are otherwise difficult to modulate.

Problem: Its clinical translation is limited by rapid degradation, low uptake, and immunogenicity.[2] Current stabilization strategies offer only partial solutions and lack a broadly applicable mechanism combining protection with controlled intracellular release.

Our Solution: Natural RNA-binding proteins such as TAV2b[3] provide a structural blueprint for synthetic ligands capable of recognizing dsRNA in a sequence-independent manner. Redox-responsive peptide dimers build on this principle, offering modular RNA binders that not only engage dsRNA but can also modulate its structural and mechanical properties, including twist and flexibility, features increasingly recognized as relevant for stability and delivery. Their redox-sensitive architecture further enables environment-triggered release under intracellular reducing conditions, opening new avenues for stabilizing and deploying therapeutic dsRNA across diverse biomedical applications.

1 Design of RNA Binding Peptides

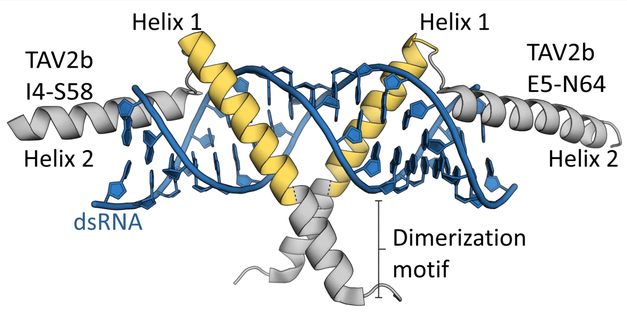


Figure 1 Crystal structure (pdb: 2zi0) of two TAV2b subunits (I4-S58 and E5-N64) bound to dsRNA (blue). Dimerization motif and helices 1 and 2 are indicated. The minimal binding motif is indicated in yellow as also shown in Figure 2A.

- TAV2b is a viral gene-silencing suppressor that binds dsRNA as a non-covalent dimer
- Starting from the native sequence, truncation and peptide stapling strategies were applied to enhance RNA binding and improve dsRNA-stabilizing properties

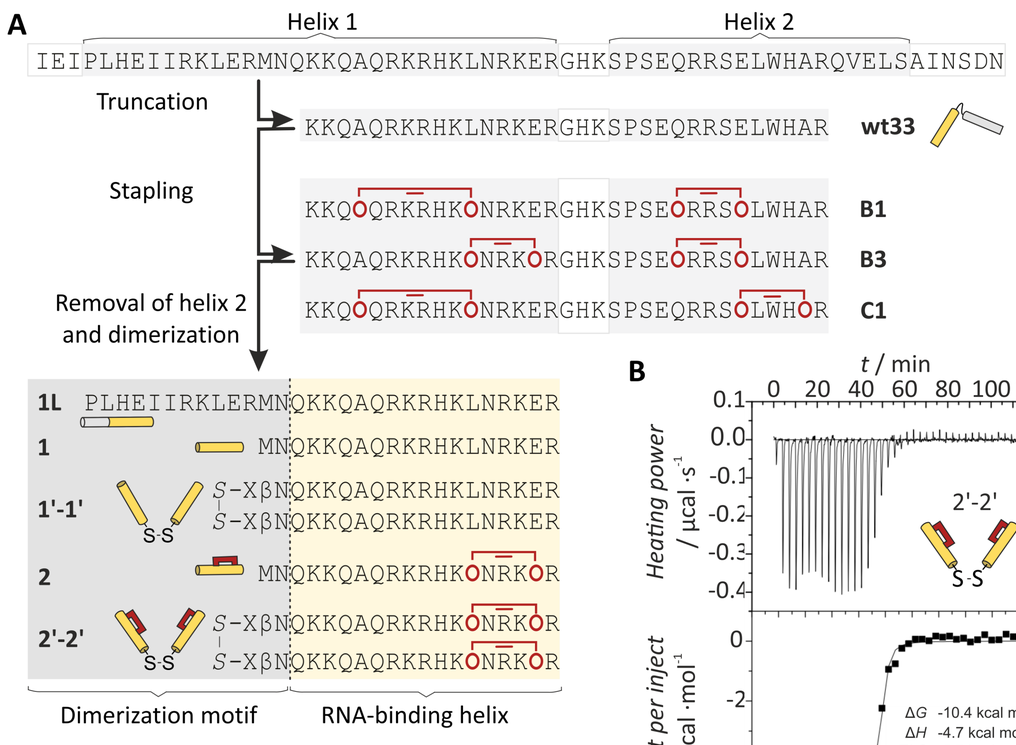


Figure 2 A) Schematic representation of the development process peptide dimer **2′-2′** starting from the native TAV2b crystal structure sequence via truncation studies and stapling of helices 1 and 2. Red O´s indicate staples. B) ITC of **2'-2'** (c= 305 µM) with miR-21 (c= 15 µM) at 30 °C in PBS. C) EMSA of miRNA-21 (c = 3 µM) incubated with **1**, **1'-1'**, **2**, **2'-2'**, **1L** and **wt33** (c= 6 µM), native PAGE, TAE-buffer, SYBR gold stain.

- **B1**, **B3**, and **C1** represent the strongest binders among a panel of 11 stapled peptides and effectively protect dsRNA from degradation[4]
- ITC measurements show that **2′-2′** binds dsRNA with high affinity (K_d = 31.7 nM), exhibiting a 10-fold stronger interaction than **wt33** (K_d = 328 nM)
- Thermal denaturation experiments reveal increased melting temperatures for RNA-peptide complexes formed by **1′-1′** and **2′-2′** compared to their monomers
- **2′-2′** binds dsRNA in a sequence-independent manner and extends the lifetime of miRNA-21 in FBS, preventing degradation for up to 6 hours in 27.5 % FBS at rt[5]

4 References

[1] K. Paunovska *et al.*, *Nat. Rev. Genet*. **2022**, 23, 265
[2] J. N. Shukla *et al.*, *RNA Biol*. **2016**, 13, 656
[3] H.-Y. Chen *et al.*, *EMBO Rep*. **2008**, 9, 754
[4] A. Kuepper *et al.*, *NAR* **2021**, 49, 22, 12622
[5] N. McLoughlin, M. A. Albers *et al.*, *Angew. Chem. Int. Ed.* **2023**, 62, e202308028
[6] Z. M. Rashid *et al.*, *JACS* **2026**, 148(8), 8298

2 RNA Uptake and Release

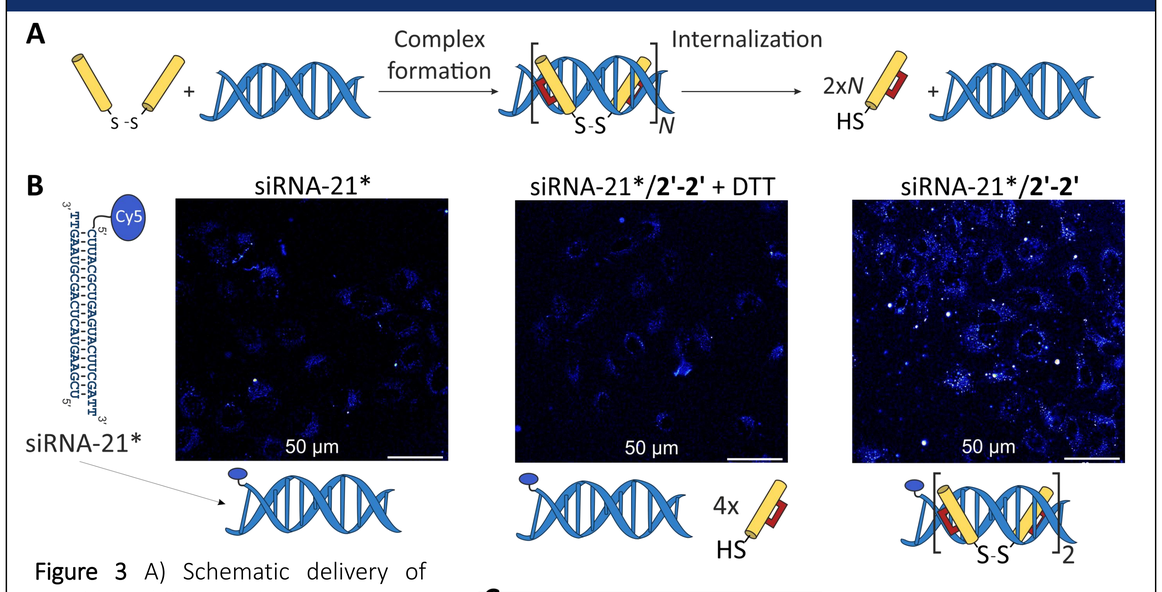
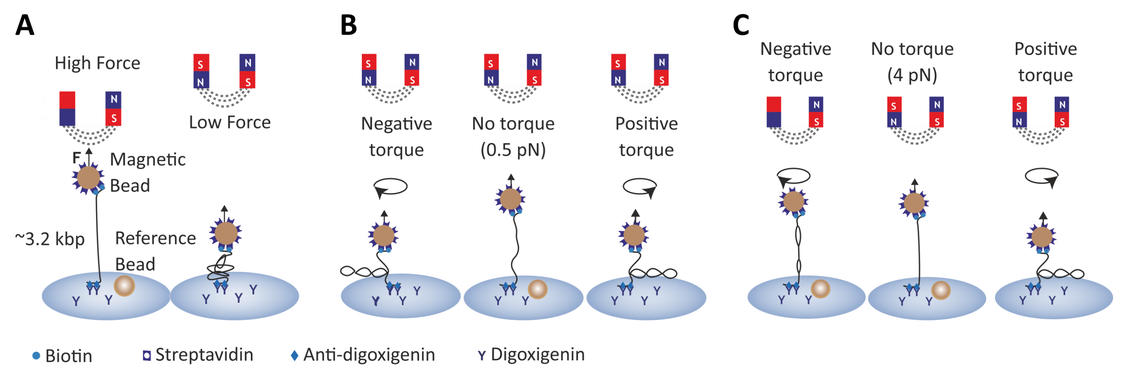


Figure 3 A) Schematic delivery of peptide stabilized RNA into cells. B) Confocal Microscopy of HEK293 cells after incubation with siRNA-21* (c=1 µM) for 1h. Only siRNA-21* (left); siRNA-21* and peptide **2′-2′**pre-treated with DTT (middle); siRNA-21* with **2′-2′** (right). C) LC-MS glutathione cleavage assay. Incubation of peptide **2′-2′** alone (left) or in complex with miRNA-21 (right) together with glutathione (5 mM).

- Cy5 labeled siRNA-21* alone showed low intracellular signal – low uptake
- Increased cellular uptake with **2'-2'**, **1′-1′** revealed similar effect on siRNA uptake
- Prior DTT treatment lowers uptake, similar behavior as shown for siRNA-21* alone
- Disulfide is cleaved under physiological glutathione concentration within 5 min
- EMSA of miRNA-21 co-incubated with peptide dimers **1′-1′** and **2′-2′**and reducing agent TCEP (c= 6, 60 and 600 µM) results in loss of binding[5]

3 Mechanical Modulation of dsRNA



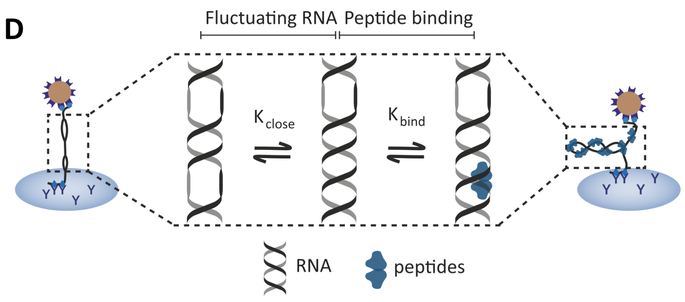


Figure 4 Schematics of magnetic tweezer assay experiments: A) force-extension; B) and C) rotation-extension experiments. D) Schematic of the two-step reaction model in which the RNA fluctuates between double-stranded and melted conformations, followed by peptide binding, which results in plectoneme formation.

- Peptide binding underwinds and elongates dsRNA, with **wt33** and **1′-1′** both increasing contour length and reducing helical twist
- **wt33** softens dsRNA, lowering persistence length and stretch modulus, and stabilizing underwound states = a flexibilizing, dynamic binder
- **1′-1′** induces dsRNA condensation at low forces, remains tightly bound, and releases only under reducing conditions = a compact, redox-controlled binder[6]

➢ Peptide dimers ideal for delivery strategies, offering high-affinity binding, compaction, and controlled cytosolic release