
Check for updates

GlmS
(Adams et al., 200
bozyme (Shechner et al., 2009
been used as a crystallization module (Ferré-D
drawback is the lack of tryptophan residues in
acid sequence. Tryptophan residues allow for
protein concentration by measuring the abso
enable the distinction between protein and salt
imaging (Meyer et al., 2015).

Thus, we designed five U1A-RBD varian
phan in their protein sequence, evaluated th
ties, solved the structures of three variants a
for soaking experiments. We also analyzed
using fluorescence imaging and monitored
after soaking the RNA into preformed
vestigated the application of an U1A va
ization of an RNA-cleaving DNAzyme in
substrate using native PAGE, solution
measurements. Further, we present an
U1A:RNA complex crystals by soakin
and cost effective alternative to gene
to co-crystallization and even more i
fragile nucleic acid molecules ren
RNA structure determination.

2. Materials and methods

2.1. Cloning and site-directed m

The *U1A* gene, encoding f
U1A-RBD (UniProtKB accessi
string by GeneArt (Thermo
additional sequence to ge
troduced by amplification
AGG TCT AGA ATG GCA
ACA AAT TTT CAT CTT
pET16b-TEV was linea
CCC CTT GGG GC-3′ a
ATG GC-3′. The plasm
Fusion HD cloning k
quencing (GATC B
mutations within t
exchange (Supple
were performed
England Biolab
proteins consi
with two ad
result of usi

2.2 Protei

Cult
were c
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Ce
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ants
A (in
ire for
(Thermo
plex were
t 180 V. For
(TBE) buffer
ucleic acid dye
ere acquired using
ornia, USA).

NA:RNA:protein complex

(GE Hea
with 50 mM HEPES
at a concentration of 50 μM was

2.6. Denaturing PAGE

To prove the presence of RNA in preformed
soaking experiments, the crystals were dissol
buffer. The samples were analyzed via denatu
the samples was carried out on 18% polyacryl
buffered with TBE for 1 h at 20 W. Imag
ChemiDoc MP System (BioRad, Hercules, C

2.7. Small angle X-ray scattering

The scattering patterns were record
SAXS system "Ganesha-Air" fro
Forschungszentrum Jülich. The X-r
(Excillum) with a liquid metal anode
with Ga-Kα radiation (wavelength λ
brilliant and a very small beam (< 20
Baden-Daettwil, Switzerland) was
patterns. All samples were sealed
diameter. Data were circular avera
and transmission corrected. The
measured at a detector distance
form factor concentrations of 5,
the corresponding buffer was s
was extrapolated to zero con
structure factor. To reconstru
was analyzed by a sequence
2017) suite. After transform
tribution, the program DA
to generate 30 low resolu
elling algorithm. A simul
compact bead model
construction did not re
and Svergun, 2003) is
culate a most probab
complex (PDB ID 6
complex were ma
illustrate that size
with the expected

2.8. NMR spect

NMR a
HD + spectr
and equipp
cryoprobe
2048x51
200 μM
in 50 m
v) D_2O
plott

2.9

0W (6SQV)
% favored and
2% favored and
9% favored and
are summarized in
ordinates for the U1A
collaboratory for Struc-
B PDB) (Berman, 2000).
for the reported crystal

cession
R70W, 6SR7 for the
obtained by soaking, and 6SQN

3. Results

3.1. Design, biosynthesis, and characterization of

To identify amino acid positions that like
and RNA-binding properties of U1A we use
1990) to search for U1A-RBD homologs th
sidues. Whereas most U1A-RBDs from *Ar*
sidues, most homologs from *Viridiplantae* c
of a phenylalanine at position 56 (Fig. S1
RNA binding as the phenylalanine stacks
complex formed by U1A and RNA (Shiel
RNA hairpin sequence is conserved in
indicates that the mutation F56W sho
U1A-RBD (Law, 2005; Shiels et al., 200
that the exchange of amino acid re
crystallization behavior and the pr
(Price et al., 1995). Since an U1A va
Y31H and Q36R has been report
properties compared to the wildty
we used this double mutant as pa
all tryptophan mutations. In ad
tions A2, H10, R70, and K98 i
genesis, since they are located
on the protein surface (Fig.
within the expression plasmi
acid sequence, we exchange
R70, and K98 by W using
then expressed in *E. coli*
yields. All proteins wer
purity using a combina
graphy steps (Fig. S2).

Next, we tested th
iants to bind RA. T
RNA hairpin sequen
analyzed the sampl
phoretic mobility
bind RNA in solu

le
tein
of the
trast in
particu-
due to the
012). In line
tography, the
ng the assembly
-resolution model
longated structural
with roughly 23 base
meter of 3 nm. The less
ith a diameter of 3 nm is
tein, therefore, we assume

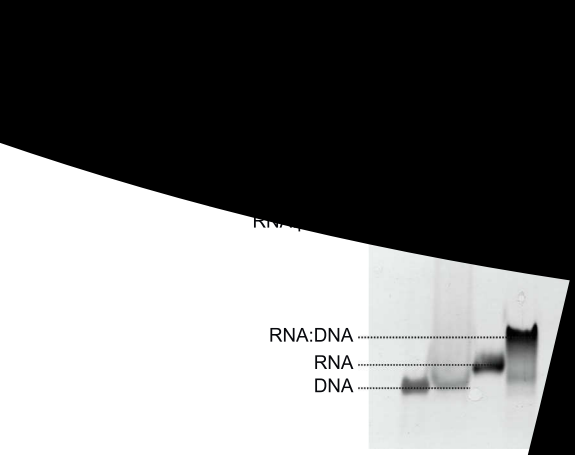

Fig. 2. Formation an
Native PAGE visuali
P: U1A protein, D:
information availa
ternary complex
black and the R
resolution shap
homonuclear
without prot
sponding t
affected b
cytosine
spectra)

that
the
To
p

NA-
es of
biolo-
ni of the
e electron
RNA-binding
some positive
der the access to
ty could not be
study of the RNA-
truct (aa 2–102) than
minus is critical for the
). Residues 93–102 form
nterface in the dimer. The

Fig. 3. Crystal structures of U1A triple mutants in
blue; R70W in cyan, and K98W in green. Biolog
K98W (D). Residues 56 and 92 are shown as s

excess electron density close to the C-te
indicate a partial formation of helix C.
rangement in U1A variant K98W may
the C-terminal residues on protein–pr

3.4. Binding of U1A variants to the U1
form

To ameliorate the process of g
we established a protocol for so
hairpin motif in combination wi
success of RNA binding exploi
tryptophan residue. We produ
R70W, and K98W, which dis
radiated with UV light (Fig.
these crystals with a solutio
motif. The RNA sequence c
a characteristic hairpin m
of the U1A protein (Tab
light shows that the cry
ment (Fig. 4, middle
crystals with the U1A
fluorescence in case
speculate that the l
cence quenching d
residue at position
consequence of a

To validate t
RNA binding to
RNA sequence
of binding to
crystals reta
RNA. In co
formed R7
tion in fl

To
bindin
specif
loadi
nuc
so
p

UV
d to
ific U1
e in case
tion of an

es of the U1A
hey are all cap-
, only the F56W
e *in crystallo*.

To investigate
protein and RNA after soaking
solved the structures of the RNA:F56W complex
by each method (subsequently referred to as
F56W:RNA$_{co\text{-}crystal}$). The best results for the soa
adding RNA to a final concentration of 1.5 mM
followed by an incubation time of 4 d at room
able to collect data and refine the structural
complex obtained by soaking to a resolution
R_{free} of 0.248/0.294. Interestingly, the space
changed from $P3_221$ for the protein in the ab
the presence of RNA. Therefore, indexing
determine whether RNA is bound to the
dition to the fluorescence quenching, a ch
is another indicator of the binding of RN
crystalline form. As a consequence, bi
quickly by indexing the crystal and dete
than collecting data, processing, phasi

The structure of F56W:RNA$_{soaked}$
viously reported double mutant U1A
via co-crystallization (Fig. 5A) (Oubr
are well-defined except the residues
the loop region of the hairpin motif
also disordered in the double m
(Oubridge et al., 1994). Interest
complex with large RNA sequen
interactions with symmetry rela
1998; Rupert, 2002). Residues
crucial for the interaction wi
resulting in a four-element-
position 56, also Y13, N16, K
binding. The electron den
position 56 is well-define
adopts the same orienta
wildtype retaining the fo

Furthermore, we so
the RNA:F56W comple
by soaking or co-crys
model to a resolution
RNA:F56W complex
with three molecul
F56W:RNA$_{soaked}$
U1A:RNA compl

Superpositio

protein in blue and RNA
as depicted in A showing
o-Fc electron density of the

acquisiti
Writing - original dra

Declaration of Competing Interest

The authors declare that they have no know
interests or personal relationships that could h
ence the work reported in this paper.

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Appendix A. Supplementary d

Supplementary data to thi
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