Nucleocapsid protein of SARS-CoV-2 phase separates into RNA-rich polymerase-containing condensates

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The etiologic agent of the Covid-19 pandemic is the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The viral membrane of SARS-CoV-2 surrounds a helical nucleocapsid in which the viral genome is encapsulated by the nucleocapsid protein. The nucleocapsid protein of SARS-CoV-2 is produced at high levels within infected cells, enhances the efficiency of viral RNA transcription, and is essential for viral replication. Here, we show that RNA induces cooperative liquid-liquid phase separation of the SARS-CoV-2 nucleocapsid protein. In agreement with its ability to phase separate in vitro, we show that the protein associates in cells with stress granules, cytoplasmic RNA/protein granules that form through liquid-liquid phase separation and are modulated by viruses to maximize replication efficiency. Liquid–liquid phase separation generates high-density protein/RNA condensates that recruit the RNA-dependent RNA polymerase complex of SARS-CoV-2 providing a mechanism for efficient transcription of viral RNA. Inhibition of RNA-induced phase separation of the nucleocapsid protein by small molecules or biologics thus can interfere with a key step in the SARS-CoV-2 replication cycle.
The etiologic agent of the Covid-19 pandemic is the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (https://www.who.int/emergencies/diseases/novel-coronavirus-2019). SARS-CoV-2 is an enveloped single-stranded, positive-sense RNA virus with a 30 kb genome, one of the largest among RNA viruses. The viral membrane of SARS-CoV-2, which contains the spike protein, a glycoprotein, and the envelope protein, surrounds a helical nucleocapsid. In the nucleocapsid, the viral genome is encapsulated by the nucleocapsid protein and thereby protected from the host cell environment. The nucleocapsid protein of human coronaviruses is produced at high levels within infected cells and is critical for virion assembly. In addition, it enhances the efficiency of sub-genomic viral RNA transcription and is essential for viral replication. Because of its importance for diagnostic and therapeutic approaches to treat Covid-19, there is an urgent need to define the molecular mechanisms that underlie the nucleocapsid protein’s fundamental viral role.

Liquid–liquid phase separation (LLPS) provides a highly cooperative mechanism to locally concentrate proteins and nucleic acids and promote cellular reactions. Recent evidence indicates that negative-sense RNA viruses, which replicate in the cytoplasm of infected cells, concentrate their replication machinery in dynamic compartments formed by LLPS of the viral structural proteins L, phosphoprotein (P), and nucleocapsid (N) protein. The genomes of positive-sense RNA viruses such as SARS-CoV-2, however, lack the genetic code for the phosphoprotein P, which is essential for LLPS in negative-sense RNA viruses.

Here we investigate liquid–liquid phase separation of the nucleocapsid protein of SARS-CoV-2 and show that nucleocapsid protein LLPS concentrates components of the SARS-CoV-2 replication machinery providing a mechanism for enhanced viral transcription and replication.

Results

LLPS of NSARS-CoV-2 and RNA into protein/RNA-dense compartments. To investigate if the N protein of SARS-CoV-2 (further termed NSARS-CoV-2; Fig. 1a) phase separates in the absence of other viral proteins, we measured the turbidity of NSARS-CoV-2 solutions at different protein concentrations. Up to 50 µM, the protein solution retained low absorbance (Fig. 1b), despite its tendency to oligomerize. Next, we tested LLPS of NSARS-CoV-2 in the presence of RNA. The 419-residue NSARS-CoV-2 contains an RNA-binding domain and a C-terminal dimerization domain embedded into long intrinsically disordered regions (Fig. 1a). The globular domains as well as the intrinsically disordered regions of coronavirus N proteins bind to RNA. At 50 µM protein concentration, the addition of 1 µM polyU (800 kDa), which we used as a substitute for viral RNA, strongly increased turbidity (Fig. 1b). Differential interference contrast (DIC) and fluorescent microscopy demonstrated the formation of spherical droplets (Fig. 1c). The droplets contained both NSARS-CoV-2 and RNA (Fig. 1c). NSARS-CoV-2/polyU droplets were robust against the presence of the aliphatic alcohol 1,6-hexanediol (Supplementary Fig. 1a). In contrast, the addition of increasing amounts of NaCl dissolved the droplets (Supplementary Fig. 1b), indicating an important role of electrostatic interactions for RNA-induced LLPS of NSARS-CoV-2. Quantification of fluorescence intensities of NSARS-CoV-2 and RNA showed that their concentration is strongly increased inside droplets (Fig. 1d), i.e., cooperative LLPS of NSARS-CoV-2 and RNA into protein/RNA-dense compartments occurs.

Next, we monitored LLPS for different NSARS-CoV-2 and polyU concentrations. According to turbidity measurements, polyU-induced LLPS started at 5–10 µM NSARS-CoV-2 (Fig. 1b and Supplementary Fig. 1c). The polyU concentration at which maximum turbidity was observed shifted to higher polyU concentrations with increasing protein concentration (Fig. 1b and Supplementary Fig. 1c). Calculation of the NSARS-CoV-2 and polyU charge showed that LLPS is strongest when charge neutralization occurs (Fig. 1b). At a given protein concentration, the turbidity increased with increasing polyU concentration, reached a maximum, and then rapidly decreased to its starting value (Fig. 1b), i.e., charge-matching RNA concentrations enable phase separation, but high RNA/protein ratios prevent LLPS of NSARS-CoV-2. The RNA-induced LLPS behavior of NSARS-CoV-2 is in agreement with the known properties of RNA-induced phase separation of prion-like RNA binding proteins.

Time-dependent transformation of NSARS-CoV-2/RNA-droplets. A characteristic property of LLPS is the liquid-like nature of phase-separated compartments. We photobleached NSARS-CoV-2 inside of NSARS-CoV-2/polyU droplets and observed rapid recovery of fluorescence (Fig. 1e). We then waited one hour and repeated fluorescence recovery after photobleaching (FRAP). At this later time point, the fluorescence recovery was best described by a bi-exponential fit consisting of two components (Fig. 1e and Supplementary Fig. 1d). In addition, the fluorescence did not fully recover (Fig. 1e), i.e., ~60% of NSARS-CoV-2 had transformed into an immobile species. The analysis shows that NSARS-CoV-2/polyU droplets change their material properties in a time-dependent manner. Because a major activity of NSARS-CoV-2 is to encapsulate RNA, the immobile fraction observed by FRAP might represent the early stages of nucleocapsid assembly. Successful nucleocapsid formation, however, also depends on the specific sequence and secondary structure of viral RNA and is therefore not expected for polyU.

SARS-CoV-2 nucleocapsid protein associates with stress granules. Stress granules (SGs) are cytoplasmic RNA/protein granules, which form through LLPS and are modulated by coronaviruses and other viruses to maximize replication efficiency. SARS-CoV-2 protein interaction mapping indicated that NSARS-CoV-2 binds the SG protein G3BP. To investigate if NSARS-CoV-2 associates with SGs, we used a previously established SG-colocalization assay. SGs were induced in HeLa cells by arsenite followed by permeabilization of the plasma membrane. Subsequently, soluble cytosolic factors were washed out and fluorescently labeled NSARS-CoV-2 was added together with Alexa Fluor 594-coupled antibody against the SG marker G3BP1. Laser scanning confocal microscopy showed arsenite-induced formation of SGs that stained for G3BP1 (Fig. 2a). FRAP of SG-associated NSARS-CoV-2 suggested the presence of three populations (Fig. 2b): a very mobile fraction with rapid fluorescence recovery, a slower diffusing component, and an immobile fraction, which does not recover its fluorescence after photobleaching (Fig. 2c). Because SGs consists of a rigid core and a dynamic shell, we attribute the different NSARS-CoV-2 diffusion properties to the localization of NSARS-CoV-2 to different sub-structures of SGs. In agreement with our findings for NSARS-CoV-2, the N protein of SARS-CoV, the causative agent of the SARS epidemic in 2002/2003, translocates to SGs in stressed SARS-CoV-infected cells.

RNA-interaction of the mutation-prone SR-region. RNA viruses have enormously high mutation rates enhancing virulence and evolvability. On June 7th 2020, already 42176 SARS-CoV-2 sequences were deposited (https://www.gisaid.org). Analysis of
the corresponding N proteins showed that the mutations are most frequent in the SR-region of NSARS-CoV-2 (Fig. 3a), which is conserved among human coronaviruses (Supplementary Fig. 2). SR-regions bind both RNA and proteins. To gain insight into the molecular properties of the SR-region of NSARS-CoV-2 and its interaction with RNA, we combined NMR spectroscopy with molecular dynamics (MD) simulations. We particularly focused on the region from A182 to S197, because it contains 9 serine and 4 arginine residues, i.e., the highest density of SR-motifs in NSARS-CoV-2 (Supplementary Fig. 2). Chemical shift analysis showed that residues A182-S197 are very dynamic with a small propensity of α-helical structure next to R189 (Fig. 3b-c).

Next, we investigated the conformational properties of A182-S197 with MD simulations. For the simulations, we used the state-of-the-art force field/water model that accurately reproduced NMR parameters of the intrinsically disordered protein α-synuclein. In agreement with the NMR analysis, residues next to R189 populate transient α-helical structure (Fig. 3c). We then performed calculations in the presence of polyU. A large number of intermolecular contacts between the arginine residues and the

Fig. 1 RNA-induced LLPS of the nucleocapsid protein of SARS-CoV-2. a Organization of NSARS-CoV-2 into two globular domains (RNA-binding domain and C-terminal dimerization domain) surrounded by long intrinsically disordered regions (IDR). The serine/arginine (SR)-rich region is conserved in coronaviruses. b Influence of RNA and protein concentration on NSARS-CoV-2/polyU-LLPS in 20 mM NaPi, pH 7.5, monitored by solution turbidity at 350 nm. Average values from three independent measurements are shown. The dashed line marks NSARS-CoV-2/polyU-concentrations at which charge neutralization occurs, assuming a charge of −1 per phosphate group. c Fluorescence and DIC microscopy of spherical droplets of 50 µM NSARS-CoV-2 and 1 µM polyU in 20 mM NaPi, pH 7.5. Fluorescently labeled RNA (green) partitioned into the droplets. Scale bar, 20 µm. Micrographs are representative of three independent biological replicates. d Increase in NSARS-CoV-2- and RNA-concentration inside of NSARS-CoV-2/polyU droplets in 20 mM NaPi, pH 7.5. Scale bars 3 µm. Micrographs are representative of three independent biological replicates. e Time-dependent change in diffusion of NSARS-CoV-2 inside polyU-induced droplets observed by FRAP. FRAP of freshly prepared droplets is shown in green, and after incubation for one hour in blue. Error bars represent the standard deviation for averaged six curves for each time point. Representative micrographs of a fresh droplet (top) and after incubation for one hour (bottom) before bleaching, after bleaching, and at the end of recovery are displayed to the right. Scale bars 10 µm.
RNA phosphate groups were observed. Most intermolecular contacts were present for R189 (Fig. 3d, red; Supplementary Fig. 3). In addition, R189 was most sensitive to the addition of polyU as observed by NMR spectroscopy (Fig. 3d, blue; Supplementary Fig. 4a). R189 is the only residue in the region from A182-S197 that is not mutated in 42176 SARS-CoV-2 sequences (Fig. 3d, gray bars), in agreement with its functional relevance.

SR-phosphorylation modulates RNA-induced phase separation. Phosphorylation of SR-domains provides functional specificity and adjustability to ribonucleoprotein formation and impairs binding of SR-domains of pre-mRNA splicing factors to protein hydrogel droplets. To gain insight into the impact of phosphorylation of the SR-region of SARS-CoV-2 on its RNA binding, we performed MD simulations of the high-density SR-stretch carrying phosphate groups at different serine residues (Supplementary Fig. 5a). Even when only a single serine, S188, was phosphorylated, the number of intra- and intermolecular peptide/peptide contacts increased (Supplementary Fig. 5b-c). Multi-site phosphorylation further raised the number of contacts (Supplementary Fig. 5b-c), and the intermolecular peptide/peptide contacts reached a maximum when three serines were phosphorylated (Supplementary Fig. 5c), i.e., when the overall charge is around zero. The phosphorylation-induced increase in intra- and intermolecular contacts is predominantly due to the formation of salt bridges between the phosphate groups and arginine side chains (Supplementary Fig. 5b-c). Because of the dense network of intra- and interpeptide salt bridges, contact formation with RNA—either polyU or a structured RNA derived from the viral genome of SARS-CoV-2—was strongly attenuated upon phosphorylation (Fig. 4a and Supplementary Fig. 6).

To validate the results from MD simulation, we phosphorylated the SR-peptide in vitro using the serine/arginine protein kinase 1 (SRPK1) and performed NMR spectroscopy. SRPK1 phosphorylates SR-motifs and is involved in a wide spectrum of cellular activities including the regulation of viral genome replication. SRPK1-phosphorylation resulted in two species, a single phosphorylation at S188 (Fig. 4b, middle) and a di-phosphorylated state, which is heterogeneously phosphorylated at four different serines (Fig. 4b, bottom; Supplementary Fig. 7). NMR titrations showed that the unmodified SR-peptide strongly interacts with polyU, but not when it is phosphorylated at S188 (Fig. 4b and Supplementary Fig. 4b). LLPS experiments further demonstrated that phosphorylation of full-length SARS-CoV-2 by SRPK1 changes its RNA-induced phase separation behavior (Fig. 4c and Supplementary Fig. 8). The maximum of RNA-induced turbidity was shifted to lower polyU-concentrations for SRPK1-phosphorylated N SARS-CoV-2 (Fig. 4c). In addition, fluorescently labeled RNA was less recruited to droplets formed by SRPK1-phosphorylated N SARS-CoV-2 (Fig. 4d). In agreement with an attenuated interaction of N SARS-CoV-2 with RNA upon SRPK1-phosphorylation, we also observed a more rapid diffusion of SRPK1-phosphorylated N SARS-CoV-2 inside of polyU-induced droplets when compared to the unmodified protein (Fig. 4e). On the other hand, SRPK1-phosphorylated N SARS-CoV-2 still colocalized with stress granules (Fig. 4d).

Nucleocapsid protein LLPS concentrates components of the SARS-CoV-2 replication machinery. LLPS provides a cooperative mechanism to locally increase protein and RNA concentrations. In addition, protein/RNA condensates can recruit additional proteins to promote reactions. To investigate if the RNA-dependent RNA polymerase (RdRp: Fig. 5a) concentrates within SARS-CoV-2 RNA droplets, we recombinantly prepared the non-structural protein (nsp) 12 of SARS-CoV-2, together with the accessory sub-units nsp7 and nsp8, which are required for transcription. First, we used nsp12, in order to investigate if the catalytic component of RdRp is recruited to N SARS-CoV-2/polyU droplets. Fluorescence microscopy revealed strong nsp12 fluorescence inside the droplets (Fig. 5b). Next, nsp12, nsp7, and nsp8 were reconstituted in a 1:1:2 stoichiometry...
together with a RNA template-product duplex, which carried fluorescein at the 5' end. The RdRp/RNA-complex was added to preformed NSARS-CoV-2/polyU droplets into which the RdRp/RNA-complex was recruited (Fig. 5c). High local concentrations of RdRp and NSARS-CoV-2 were reached (Supplementary Fig. 9).

We then investigated the influence of phosphorylation of NSARS-CoV-2 by the kinase SRPK1 on the recruitment of nsp12 and the RdRp/RNA-complex into NSARS-CoV-2/RNA droplets. The analysis showed that both nsp12 and the RdRp/RNA-complex partition less into the droplets formed by SRPK1-phosphorylated NSARS-CoV-2 (Fig. 5d). This indicates that NSARS-CoV-2 not only interacts with RNA but also directly binds to nsp12 and the nsp12/NSARS-CoV-2-interaction is attenuated by phosphorylation of the SR-region of NSARS-CoV-2. This mechanism was further supported by a more rapid recovery of fluorescence after photobleaching of nsp12 in droplets formed.
by SRPK1-phosphorylated N_{SARS-CoV-2} (Fig. 5e). The data suggest that RNA-driven condensation of N_{SARS-CoV-2} provides a mechanism for bringing together components of the viral replication machinery (Fig. 5f). In agreement with this proposed mechanism, N protein of SARS-CoV colocalizes intracellularly with replicase components30.

Discussion

Our study shows that the nucleocapsid protein of the SARS-CoV-2 virus undergoes RNA-induced liquid-liquid phase separation. Although nucleocapsid assembly can occur outside of liquid-like compartments, it was shown that the rate of assembly is increased when the nucleocapsid protein of Measles virus is concentrated through LLPS15. In addition, NSARS-CoV-2 interacts with human ribonucleoproteins21, which are found in several LLPS-driven cytosolic protein/RNA granules, suggesting that N_{SARS-CoV-2} might modulate protein/RNA granule formation in order to maximize viral replication31. In agreement with such activity, we showed that N_{SARS-CoV-2} translocates to stress granules in stressed cells.

We demonstrate that N_{SARS-CoV-2} LLPS promotes cooperative association of the RNA-dependent RNA polymerase complex with polyU RNA in vitro. This suggests that SARS-CoV-2 uses...
LLPS-based mechanisms similar to transcription hubs in cellular nuclei\cite{32,33} to enable high initiation and elongation rates during viral transcription. Because the replication machinery of coronaviruses is membrane-associated\cite{34}, it will furthermore be interesting to investigate if the SARS-CoV-2 glycoprotein M, which binds nucleocapsid protein\cite{4}, causes tethering of N\textsubscript{SARS-CoV-2}/RdRp/RNA-condensates to host cell membranes.

Taken together the data suggest that inhibition of the RNA-induced phase separation of the nucleocapsid protein of SARS-CoV-2 provides a viable and novel strategy for the design of therapeutics to treat Covid-19.

**Methods**

**Materials.** Alexa Fluor 594 conjugated anti G3BP1 antibody was purchased from Santa Cruz Biotechnology (sc-365338 AF594). Digitonin was from Merck (CAS Number 27416-86-0). Recombinant nucleocapsid protein of SARS-CoV-2 (Catalog No: Z03488-1) and SRPK1 kinase (Catalog No: PV4215) were purchased (Catalog No: Z03488-1) and SRPK1 kinase (Catalog No: PV4215) were purchased from ThermoFisher Scientific. PolyU potassium salt (800 kDa) was purchased from Sigma-Aldrich (CAS Number 27416-86-0). Recombinant nucleocapsid protein of SARS-CoV-2 (Catalog No: Z03488-1) and SRPK1 kinase (Catalog No: PV4215) were purchased from ThermoFisher Scientific. Digitonin was from Merck (CAS Number 27416-86-0). Recombinant nucleocapsid protein of SARS-CoV-2 (Catalog No: Z03488-1) and SRPK1 kinase (Catalog No: PV4215) were purchased from ThermoFisher Scientific.

**Purification of nsp12, nsp7, nsp8 and formation of RdRp complex.** Nsp12, nsp7, and nsp8 were expressed and purified as previously described\cite{35}. nsp12 was expressed in Hi5 insect cells using the pFastBac-His-MBP-SARS-CoV2-nsp12 plasmid (Addgene #15479). Cells were collected by centrifugation, resuspended in lysis buffer (300 mM NaCl, 50 mM Na-HEPES pH 7.4, 10% (v/v) glycerol, 30 mM imidazole pH 8.0, 5 mM β-mercaptoethanol, 0.284 mg ml\textsuperscript{-1} leupentin, 1.37 μg ml\textsuperscript{-1} pepstatin, 0.17 mg ml\textsuperscript{-1} PMSF and 0.33 mg ml\textsuperscript{-1} benzamidine, 3 mM MgCl\textsubscript{2}) and immediately sonicated. Lysates were cleared by centrifugation, filtered (0.8 μm), and applied to a HiTrap HP 5 ml (GE Healthcare) prepacked with amylose resin (New England Biolabs), using nickel elution buffer (300 mM NaCl, 50 mM Na-HEPES pH 7.4, 10% (v/v) glycerol, 1 mM imidazole) to elute nsp12, nsp7, and nsp8 from the amylose resin. The elution buffer was then exchanged to 20 mM NaPi, pH 8.0, 50 mM Na-HEPES pH 7.4, 10% (v/v) glycerol, 100 μM pepstatin, 0.17 mg ml\textsuperscript{-1} pepstatin, 0.17 mg ml\textsuperscript{-1} PMSF and 0.33 mg ml\textsuperscript{-1} benzamidine, 3 mM MgCl\textsubscript{2}).

The protein was eluted from the HisTrap column directly onto an XK column 16/20 (GE Healthcare), prepacked with amylose resin (New England Biolabs), using nickel elution buffer (300 mM NaCl, 50 mM Na-HEPES pH 7.4, 10% (v/v) glycerol, 1 mM imidazole) to elute nsp12, nsp7, and nsp8 from the amylose resin. The elution buffer was then exchanged to 20 mM NaPi, pH 8.0, 50 mM Na-HEPES pH 7.4, 10% (v/v) glycerol, 100 μM pepstatin, 0.17 mg ml\textsuperscript{-1} pepstatin, 0.17 mg ml\textsuperscript{-1} PMSF and 0.33 mg ml\textsuperscript{-1} benzamidine, 3 mM MgCl\textsubscript{2}).

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500 mM imidazole pH 8.0, 5 mM MgCl₂, and 5 mM β-mercaptoethanol). Protein was eluted from the amylose column using amylase elution buffer (300 mM NaCl, 50 mM Na-HEPES pH 7.4, 10% (v/v) glycerol, 1 mM TCEP). Peak fractions containing nsp12 were pooled, cleaved with His-tagged TEV (12 h), and applied to a HiTrap column to remove uncleaved nsp12, 6xHis-MBP, and TEV. The flow-through containing nsp12 was applied to a HiTrap Heparin 5 ml column (GE Healthcare). The flow-through containing nsp12 was collected, concentrated, and applied to a HiLoad 200 16/600 pg equilibrated in the size-exclusion buffer (300 mM NaCl, 20 mM Na-HEPES pH 7.4, 10% (v/v) glycerol, 1 mM MgCl₂, 1 mM TCEP). Peak fractions were pooled, concentrated until 120 µM aliquoted, flash-frozen, and stored at −80 °C until use.

Nsp7 and nsp8 were expressed in E.coli using the pET-His-ScCoV2-nsp7 plasmid (Addgene #154757) and pET-His-CoV2-nsp8 plasmid (Addgene #154758), respectively. Proteins were overexpressed in E. coli BL21 (DE3) RIL cells, grown in LB medium, collected by centrifugation, resuspended in lysis buffer (300 mM NaCl, 50 mM Na-HEPES pH 7.4, 10% (v/v) glycerol, 30 mM imidazole pH 8.0, 5 mM β-mercaptoethanol, 0.284 µg/mL – 1 leucine, 2.7µg/mL – 1 pepstatin, 0.17 mg/mL – 1 PMSF and 0.33 mg/mL – β-mercaptoethanol) and immediately sonicated. Both nsp8 and nsp7 were purified separately using the same purification procedure. Lysates were cleared by centrifugation and the supernatants applied to a HisTrap HP 5 ml column (GE Healthcare) pre-equilibrated in lysis buffer. The sample was eluted using nickel elution buffer (150 mM NaCl, 50 mM Na-HEPES pH 7.4, 10% (v/v) glycerol, 500 mM imidazole pH 8.0, and 5 mM β-mercaptoethanol) and the eluted protein dialyzed in dialysis buffer (150 mM NaCl, 50 mM Na-HEPES pH 7.4, 10% (v/v) glycerol and 5 mM β-mercaptoethanol). Protein partitioning was assayed by measuring the partition coefficient of the recruitment of 5× fluorescently labeled RNA, nsp12, or the RdRp complex recruited into NSARS-CoV-2 (used as a control group) and phosphorylated N53R-CoV2. A P value < 0.05 was set for statistical significance. The t-test was performed in Graph Prism.

Fluorescence recovery after photobleaching (FRAP). Dynamics of N53R-CoV-2 molecules in the phase-separated state were investigated by FRAP analysis. As described above, N53R-CoV-2 phase separation was induced using 50 µM N53R-CoV-2 and 1 µM polyU in 20 mM sodium phosphate buffer (NaPi), pH 7.5. For comparison of the kinetics of unmodified and SRPK1 phosphorylated N53R-CoV-2, protein, droplets of 30 µM nucleocapsid protein, 1 µM polyU and 1024 droplets were used and a total amount of 100 droplets per condition were analyzed. A two-tailed t-test was used to compare the partition coefficient values obtained for either fluorescently labeled RNA, nsp12, or the RdRp complex recruited into N53R-CoV-2 and phosphorylated N53R-CoV2. A P value < 0.05 was set for statistical significance. The t-test was performed in Graph Prism.
overnight in a buffer containing 4 mM ATP, 5 mM MgCl2, 1 mM DTT, and 5 mM EGTA. Because of the intrinsically disordered nature of the peptide, inactivation of the kinase was achieved by incubation of the sample at 65 °C for 20 min, followed by centrifugation at 15,000 × g for 30 min. Residual ATP, MgCl2, and EGTA were removed by HPLC followed by mass spectrometry. Phosphorylation of 100 µM unlabelled peptide was performed by incubation with 0.5 µM of SRPK1 kinase. The reaction mixture was incubated at 23 °C overnight in a buffer containing 8 mM ATP, 5 mM MgCl2, 1 mM DTT, and 5 mM of EGTA. Residual ATP, MgCl2, and EGTA were removed by 4 times buffer exchange using a Vivaspin 500.5 molecular weight cut-off (Sartorius, Göttingen). Samples were loaded onto a SDS-PAGE gel to confirm phosphorylation.

**In vitro phosphorylation.** Phosphorylation of a stock of 850 µM SR-peptide (1766.8 Da) was performed by incubation with 0.15 µM SRPK1 kinase at 23 °C overnight in a buffer containing 4 mM ATP, 5 mM MgCl2, 1 mM DTT, and 5 mM EGTA. Because of the intrinsically disordered nature of the peptide, inactivation of the kinase was achieved by incubation of the sample at 65 °C for 20 min, followed by centrifugation at 15,000 × g for 30 min. Residual ATP, MgCl2, and EGTA were removed by HPLC followed by mass spectrometry. Phosphorylation of 100 µM unlabelled peptide was performed by incubation with 0.5 µM of SRPK1 kinase. The reaction mixture was incubated at 23 °C overnight in a buffer containing 8 mM ATP, 5 mM MgCl2, 1 mM DTT, and 5 mM of EGTA. Residual ATP, MgCl2, and EGTA were removed by 4 times buffer exchange using a Vivaspin 500.5 molecular weight cut-off (Sartorius, Göttingen). Samples were loaded onto a SDS-PAGE gel to confirm phosphorylation.

**Molecular dynamics simulations.** Starting structures of the SR-peptides were built in the PyMOL Molecular Graphics System (Version 1.8.4.0, Schrödinger, LLC), those of the RNA molecules using the RNA modeling software SmRNASim26. Initially, the different mixtures were equilibrated with 50,000 steps of energy minimization. To further equilibrate the system, 100 ps each of volume (NVT) and pressure (NPT) equilibration were performed without position restraints in order to have different starting points in each simulation. The MD simulations were carried out in GROMACS (version 2018.3) using the AMBER99SB-ILDN force field and the TIP3P water model at a temperature of 300 K, 1 bar of pressure and with a coupling time (GT) of 0.1 ps. The mixtures were solvated in water with 150 mM NaCl ensuring overall charge neutrality. The particle mesh Ewald algorithm was used for calculation of the electrostatic term, with a radius of 16 Å for the grid-spacing and Fast Fourier Transform. The cut-off algorithm was applied for the non-coulombic potential with a radius of 10 Å. The LINCS algorithm was used to contain bonds and the non-coupled field parameters for the phosphorylated amino acids were taken from38. The number of contacts and secondary structure over the simulation trajectory were analyzed using the PyMOL Molecular Graphics System (Version 1.8.4.0, Schrödinger, LLC). To get error bars, 5 repetitions were done for each ns simulation. For 100 ns simulations the error is the standard deviation over the trajectory.

**Reporting summary.** Further information on experimental design is available in the Nature Research Reporting Summary linked to this paper.

**Data availability**

NMR assignments are available in the BMRB (code 50379). The mutation frequency from SARS-CoV-2 sequences were obtained from the database and resources of the China National Center for Bioinformation, 2019 Novel Coronavirus Resource (https://bigd.big.ac.cn/nconv?lang=en; downloaded June 7, 2020, with 42,176 genome sequences). Authors can confirm that the rest of the relevant data are included in the paper and/or its supplementary information files. Other data that support the findings of this study are available from the corresponding author upon reasonable request. Source data are provided with this paper.

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Author contributions

A.S. conducted LLPS assays; A.I.d.O. performed bioinformatic analysis, NMR spectroscopy and MD simulations; M.R. performed FRAP and stress granule experiments; A.S., A.I.d.O., M.R. and M.Z. designed the project and wrote the paper.

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