Supporting Information

Artificially Engineered Protein Hydrogels Adapted from the Nucleoporin Nsp1 for Selective Biomolecular Transport

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Materials and methods

DNA engineering: The Nsp1$^{30\text{-}591}$ gene from the Chait group was amplified by polymerase chain reaction (PCR) to prepare BamHI (*B*)-NheI (*N*)- Nsp1$^{30\text{-}591}$-SpeI (*S*)-HindIII (*H*) and *B*-*N*-cNsp1 (Nsp1$^{282\text{-}585}$)-*S*-*H*. Then, both DNA fragments were subcloned into the pET22b expression plasmid (C-terminal 6xHis tag) using BamHI and HindIII restriction sites. The pET22b vector was chosen due to the C-terminal 6xHis tag which allows isolation of full length proteins by metal affinity chromatography. *B*-*N*-1NLP-*S*-*H*, *B*-*N*-2NLP-*S*-*H*, *B*-P-EcoRI-intein[1]-*N*-cNsp1-*S*-*H*, *B*-P domain (P)-*N*-cNsp1-*S*-P-*H*, *B*-P-*N*-1NLP-*S*-P-*H* and *B*-P-*N*-2NLP-*S*-P-*H* were also prepared. Gene sequences of *B*-P domain (P)-*N*-1NLP$_{1/2}$-*S*-P-*H* and *B*-P-*N*-2NLP$_{1/2}$-*S*-P-*H* were purchased (GenScript, USA) and subcloned into the pET22b expression plasmid using BamHI and HindIII restriction sites. To prepare P-1NLP-P and P-2NLP-P, *N*-1NLP$_{1/2}$-*S* and *N*-2NLP$_{1/2}$-*S* were subcloned into pET22b-*B*-P-*N*-1NLP$_{1/2}$-*S*-P-*H* and pET22b-*B*-P-*N*-2NLP$_{1/2}$-*S*-P-*H* plasmids using the SpeI restriction enzyme site. P-cNsp1-P was prepared by subcloning *N*-cNsp1-*S* into the pET22b-*B*-P-*N*-1NLP$_{1/2}$-*S*-P-*H* plasmid using NheI and SpeI restriction sites. 1NLP and 2NLP were prepared by subcloning *N*-1NLP-*S* and *N*-2NLP-*S* from pET22b-*B*-P-*N*-1NLP-*S*-P-*H* and pET22b-*B*-P-*N*-2NLP-*S*-P-*H* plasmids into pET22b-*B*-P-*N*-1NLP$_{1/2}$-*S*-P-*H* and pET22b-*B*-P-*N*-2NLP$_{1/2}$-*S*-P-*H* plasmids using NheI and SpeI restriction enzyme sites. *B*-P-EcoRI-intein-*N* sequences were purchased

(Integrated DNA Technologies, USA) and subcloned into the pET22b-*B*-*N*-cNsp1-*S*-*H* plasmid using BamHI and NheI restriction enzyme sites. Sequences of all plasmids were confirmed by gene sequencing (Genewiz, USA). All prepared protein sequences are summarized in Figure S1.

Protein expression and purification: All prepared genes were transformed into *E.coli* OverExpress C41(DE3) cells (Lucigen, USA). For expression, a freshly-grown bacterial colony was inoculated in 10 ml LB medium with 100 μg/mL ampicillin at 37°C overnight. 10 mL of overnight culture of all samples was inoculated into 1L terrific broth (TB) media at 37°C with 100 μg/mL ampicillin until OD_{600} ~ 1. Expression was then induced overnight at room temperature with the addition of 1 mM IPTG. The cells were harvested, lysed in 50 mM Tris (pH 8), 300 mM NaCl and 8 M urea, and frozen at -80 °C. Thawed cells were sonicated, and 15 w/v% ammonium sulfate was added. Cell lysates were clarified by centrifugation (14,000 g for 30 min at 4 °C), and the proteins of interest were purified by Ni-NTA affinity chromatography under denaturing conditions with 250 mM imidazole used for protein elution. Purified samples were dialyzed against deionized (DI) water, and 20 mM Tris (pH 8) and 6 M urea were added. The proteins were further purified by ion exchange chromatography using a HiTrap Q HP column (GE Healthcare, Sweden) in an ÄKTA pure FPLC. Samples containing the desired product were dialyzed against DI water and lyophilized. P-intein-cNsp1 was prepared using the same expression condition as the other proteins, but harvested cells were resuspended in 50 mM NaH_2PO_4, 300 mM NaCl, and 10 mM imidazole and stored at -80 °C. Thawed cells were lysed by the sonication, and cell lysates clarified by centrifugation were stored overnight at 4°C at pH 7 for intein self-cleavage.[1] After adjusting the pH to 8, samples with 6xHis tags were purified by Ni-NTA under native conditions and by FPLC as described above. P-C_{30}-P protein was expressed and purified as reported previously,[2] with additional FPLC purification under denaturing

conditions. Lyophilized samples were weighed to calculate yields of samples from 1L culture (Figure 2b and Figure S3b). The purity of samples was determined to be greater than 95% by SDS-PAGE analysis (Figure 2a and Figure S3a).

For transport studies, pQE80-14xHis tag-TEV-IBB-MBP-EGFP, pQE80-14xHis tag-TEV-MBP-mCherry and pQE30-scImp β-6xHis tag from the Görlich group were transformed into SG13009 (pRep4) cells, and expression followed the protocol described elsewhere.[3] After cutting off the His-tag with TEV protease (Eton Bioscience, USA) for IBB-MBP-EGFP and MBP-mCherry, additional purification was performed for all three proteins by size exclusion chromatography in buffer containing 50 mM Tris (pH 7.5) and 200 mM NaCl. The concentration of recombinant fluorescent proteins was determined using their optical absorbance.[4]

FITC-dextran: Fluorescein isothiocyanate (FITC)−dextran with molar masses of 4, 10, 40, 70 and 150 kg/mol (catalog # 46994, FD10S, FD40, 46945 and 46946), were purchased from Sigma-Aldrich (USA). Approximate Stokes' radii of the FITC-dextran polymers were obtained from the supplier as follows: 1.4 nm (4 kg/mol), 2.3 nm (10 kg/mol), 4.5 nm (40 kg/mol), 6.0 nm (70 kg/mol) and 8.5 nm (150 kg/mol).

Hydrogel preparation: Lyophilized samples were dissolved at a concentration of 200 mg/mL in 50 mM Tris/HCl (pH7.5) and 200 mM NaCl except where otherwise noted, mixed, and allowed to gel at either room temperature or 4°C. It is known that natural nucleoporins can form homogeneous hydrogels at such high protein concentration, but at lower nucleoporin concentrations, the hydrogels lose their selective permeability function.[5, 6] For this reason, nucleoporin hydrogels, Nsp1, are typically prepared at 150-200 mg/mL concentration throughout the literature.[3, 7, 8] Since we are developing artificially engineered protein hydrogels that mimic the function of natural nucleoporin hydrogels, we have benchmarked the

nucleoporin hydrogels to these previous studies and used the concentration where it showed the selective transport property. After confirming that the designed hydrogels at 20 w/v% can show selectivity (Figure 4 and Figure S15), we checked whether the best performing gel, P-2NLP-P, can mimic this property at a lower gel concentration, 10 w/v% (Figure S15). During the hydrogel inversion test, food coloring was added in the buffer for better visualization of the test (Figure 2c).

Rheology: Oscillatory shear rheology was performed on an Anton Paar MCR-301 in Direct Strain Oscillation mode with TruGap^{TM} control. A Peltier heating system and environmental enclosure were employed to control sample temperature. Samples were loaded into a 25 mm cone-and-plate geometry with an angle of 1^{o} and sealed with a mineral oil barrier to prevent dehydration.

Raman Spectroscopy: A custom-built NIR confocal Raman inverted microscopy system[9] was used for Raman measurements. 785 nm light from a continuous-wave Ti: Sapphire laser (3900S, Spectra-Physics) pumped by a frequency-doubled Nd: YAG laser (Millennia 5sJ, Spectra-Physics) was used for the excitation. A water immersion objective lens with 1.2 NA (UPLSAPO60XWIR 60X/1.20, Olympus) was used both to focus the laser onto the sample, which is on top of quartz coverslip (043210-KJ, Alfa Aesar), and to collect the backscattered light. The Rayleigh light in the collected signal was removed by dichroic mirrors (LPD01-785RU-25×36×1.1, Semrock). Raman light was delivered to an imaging spectrograph (Holospec f/1.8i, Kaiser Optical Systems) and detected by a TE-cooled, back-illuminated, deep depleted CCD (PIXIS: 100BR_eXcelon, Princeton Instruments). The laser power at the sample plane was ca. 60 mW, and the signal was integrated for 5 seconds. 9 spectra were collected from each sample and averaged. Spectrum processing (cosmic ray removal, background subtraction and normalization) was performed by MATLAB (Mathworks) scripts.

Capillary transport measurements: 1.5 inch borosilicate capillaries with 0.9 mm inner diameters (Vitrocom 8290) were loaded by piercing pre-made hydrogels. 5 μM solutions of IBB-MBP-EGFP, MBP-mCherry, and/or importin β were injected into the capillary and sealed by a 1:1:1 mixture of vaseline, lanolin, and paraffin. Time lapses of cargo transport into the hydrogels were taken at 1 minute intervals for 1-3 hours on a Nikon Ti Eclipse inverted microscope using a Nikon CFI Plan UW 2X and Hamamatsu C11440-22CU camera. All fluorescence intensity profiles were obtained by averaging the fluorescence intensity within 100 μm slice width through the center of the gel and across the gel/buffer interface. The profiles were normalized by the bath concentration in the capillaries at the first time point. The interface between gel and buffer is assigned by a 20% change in the intensity compared to the bath fluorescence intensity as the zero point of the distance scale in each fluorescence intensity profile.

Circular dichroism spectroscopy: CD spectra were obtained on an Aviv Model 202 Circular Dichroism spectrometer. CD spectra were recorded in a quartz cell of 0.1 cm path length at 25 °C between 190 and 250 nm, using a scan rate of 12 nm/min with a wavelength step of 1 nm. cNsp1 and the NLPs were dissolved in 50 mM Tris buffer with 200 mM NaCl at pH 7.5. CD band intensities, after the buffer signal subtractions, were converted into mean residue ellipticity (MRE).

Nsp1$^{30-591}$

MDIGINSDPSTGAGAFGTGQSTFGFNNSAPNNTNNANSSITPAFGSNNTGNTAFGNSN
PTSNVFGSNNSTTNTFGSNSAGTSLFGSSSAQQTKSNGTAGGNTFGSSSLFNNSTNSNT
TKPAFGGLNFGGGNNTTPSSTGNANTSNNLFGATANANKPAFSFGATTNDDKKTEPD
KPAFSFNSSVGNKTDAQAPTTGFSFGSQLGGNKTVNEAAKPSLSFGSGSAGANPAGA
SQPEPTTNEPAKPALSFGTATSDNKTTNTTPSFSFGAKSDENKAGATSKPAFSFGAKPE
EKKDDNSSKPAFSFGAKSNEDKQDGTAKPAFSFGAKPAEKNNNETSKPAFSFGAKSD
EKKDGDASKPAFSFGAKPDENKASATSKPAFSFGAKPEEKKDDNSSKPAFSFGAKSN
EDKQDGTAKPAFSFGAKPAEKNNNETSKPAFSFGAKSDEKKDGDASKPAFSFGAKSD
EKKDSDSSKPAFSFGTKSNEKKDSGSSKPAFSFGAKPDEKKNDEVSKPAFSFGAKANE
KKESDESKSAFSFGSKPTGKEEGDGAKAAISFGAKPEEQKSSDTSKPAFTFGKLAAAL
EHHHHHH

cNsp1

MDIGINSDPGSGSGASDNKTTNTTPSFSFGAKSDENKAGATSKPAFSFGAKPEEKKDD
NSSKPAFSFGAKSNEDKQDGTAKPAFSFGAKPAEKNNNETSKPAFSFGAKSDEKKDG
DASKPAFSFGAKPDENKASATSKPAFSFGAKPEEKKDDNSSKPAFSFGAKSNEDKQD
GTAKPAFSFGAKPAEKNNNETSKPAFSFGAKSDEKKDGDASKPAFSFGAKSDEKKDS
DSSKPAFSFGTKSNEKKDSGSSKPAFSFGAKPDEKKNDEVSKPAFSFGAKANEKKESD
ESKSAFSFGSKPTGKEEGDGAKAAISFGAKPEEQKSSDTSKPAFTFGTSGSGKLAAAL
EHHHHHH

P-Intein-cNsp1

MDIGINSDPAPQMLRELQETNAALQDVRELLRQQVKEITFLKNTVMESDASGASAISG
DSLISLASTGKRVSIKDLLDEKDFEIWAINEQTMKLESAKVSRVFCTGKKLVYILKTRL
GRTIKATANHRFLTIDGWKRLDELSLKEHIALPRKLESSSLQLSPEIEKLSQSDIYWDSI
VSITETGVEEVFDLTVPGPHNFVANDIIVHNASDNKTTNTTPSFSFGAKSDENKAGAT
SKPAFSFGAKPEEKKDDNSSKPAFSFGAKSNEDKQDGTAKPAFSFGAKPAEKNNNET
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SKPAFSFGAKSNEDKQDGTAKPAFSFGAKPAEKNNNETSKPAFSFGAKSDEKKDGDA
SKPAFSFGAKSDEKKDSDSSKPAFSFGTKSNEKKDSGSSKPAFSFGAKPDEKKNDEVS
KPAFSFGAKANEKKESDESKSAFSFGSKPTGKEEGDGAKAAISFGAKPEEQKSSDTSK
PAFTFGTSGSGKLAAALEHHHHHH

1NLP

MDIGINSDPGSGASPAFSFGAKPDEKKDDDTSKPAFSFGAKPDEKKDDDTSKPAFSFG
AKPDEKKDDDTSKPAFSFGAKPDEKKDDDTSKPAFSFGAKPDEKKDDDTSKPAFSFG
AKPDEKKDDDTSKPAFSFGAKPDEKKDDDTSKPAFSFGAKPDEKKDDDTSKTSPAFS
FGAKPDEKKDDDTSKPAFSFGAKPDEKKDDDTSKPAFSFGAKPDEKKDDDTSKPAFS
FGAKPDEKKDDDTSKPAFSFGAKPDEKKDDDTSKPAFSFGAKPDEKKDDDTSKPAFS
FGAKPDEKKDDDTSKPAFSFGAKPDEKKDDDTSKTSGSGKLAAALEHHHHHH

2NLP
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AKPDEKKDSDTSKPAFSFGAKPDEKKDSDTSKPAFSFGAKPDEKKDSDTSKPAFSFGA
KPDEKKDSDTSKPAFSFGAKPDEKKDSDTSKPAFSFGAKPDEKKDSDTSKPAFSFGAK
PDEKKDSDTSKPAFSFGAKPDEKKDSDTSKTSKTSGSGKLAAALEHHHHHH

P-cNsp1-P
MDIGINSDPAPQMLRELQETNAALQDVRELLRQQVKEITFLKNTVMESDASGASDNK
TTNTTPSFSFGAKSDENKAGATSKPAFSFGAKPEEKKDDNSSKPAFSFGAKSNEDKQD
GTAKPAFSFGAKPAEKNNNETSKPAFSFGAKSDEKKDGDASKPAFSFGAKPDENKAS
ATSKPAFSFGAKPEEKKDDNSSKPAFSFGAKSNEDKQDGTAKPAFSFGAKPAEKNNN
ETSKPAFSFGAKSDEKKDGDASKPAFSFGAKSDEKKDSDSSKPAFSFGTKSNEKKDSG
SSKPAFSFGAKPDEKKNDEVSKPAFSFGAKANEKKESDESKSAFSFGSKPTGKEEGDG
AKAAISFGAKPEEQKSSDTSKPAFTFGTSAPQMLRELQETNAALQDVRELLRQQVKEI
TFLKNTVMESDASGKLAAALEHHHHHH

P-1NLP-P
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SFGAKPDEKKDDTSKPAFSFGAKPDEKKDDTSKPAFSFGAKPDEKKDDTSKPAF
SFGAKPDEKKDDTSKPAFSFGAKPDEKKDDTSKPAFSFGAKPDEKKDDTSKPAF
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AFSFGAKPDEKKDDTSKPAFSFGAKPDEKKDDTSKPAFSFGAKPDEKKDDTSKP
AFSFGAKPDEKKDDTSKTSAPQMLRELQETNAALQDVRELLRQQVKEITFLKNTV
MESDASGGKLAAALEHHHHHH

P-2NLP-P
MDIGINSDPAPQMLRELQETNAALQDVRELLRQQVKEITFLKNTVMESDASGASPAF
SFGAKPDEKKDSDTSKPAFSFGAKPDEKKDSDTSKPAFSFGAKPDEKKDSDTSKPAFS
FGAKPDEKKDSDTSKPAFSFGAKPDEKKDSDTSKPAFSFGAKPDEKKDSDTSKPAFSF
GAKPDEKKDSDTSKPAFSFGAKPDEKKDSDTSKTSPAFSFGAKPDEKKDSDTSKPAFS
FGAKPDEKKDSDTSKPAFSFGAKPDEKKDSDTSKPAFSFGAKPDEKKDSDTSKPAFSF
GAKPDEKKDSDTSKPAFSFGAKPDEKKDSDTSKPAFSFGAKPDEKKDSDTSKPAFSFG
AKPDEKKDSDTSKTSAPQMLRELQETNAALQDVRELLRQQVKEITFLKNTVMESDAS
GGKLAAALEHHHHHH

Figure S1. Protein sequences used in this study. Calculated and measured molar masses of proteins are in Table S1.

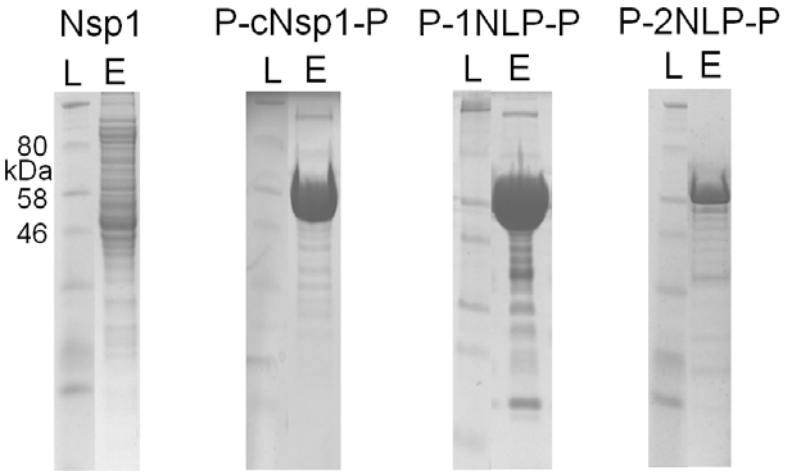


Figure S2. Protein expression levels by SDS-PAGE after the first step of protein purification. To reduce protein loss during washing step in Ni-NTA chromatography denaturing purification, the washing buffer did not contain imidazole and was prepared at pH 8, causing increased impurity during elution. After the elution, eluted proteins were run on the denaturing gels shown here (L: protein ladder; E: elution fraction). While Nsp1 (60 kg/mol), one of nuclear pore proteins, was expressed at low levels and difficult to identify on the gel, engineered proteins (P-cNsp1-P and P-NLPs-P) were highly expressed. For better purity, anion exchange chromatography was performed, with final purified products shown in Figure 2a. Note that all proteins ran slower in SDS-PAGE (Figure 2a, Figure S2-S3a) than their calculated and measured molar masses (Table S1), similar to previous reports for P-C30-P protein.[2]

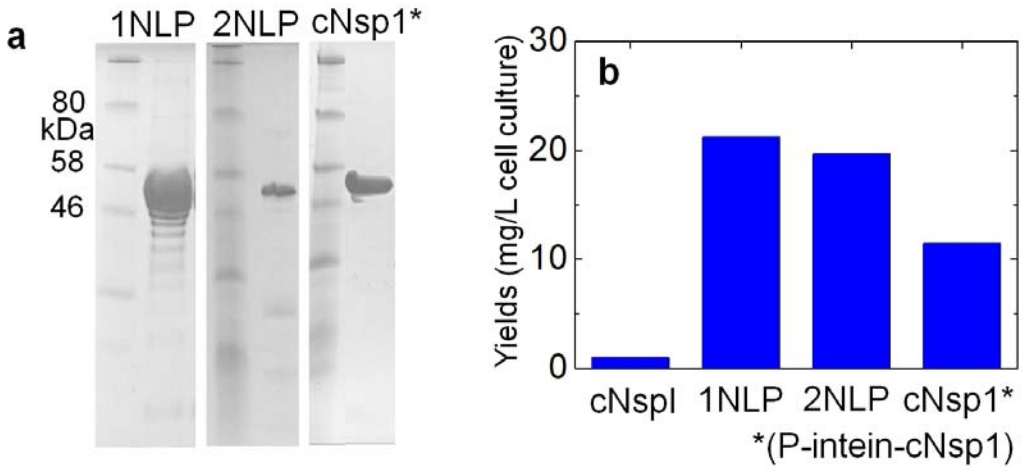


Figure S3. Biosynthesis of NLPs and cNsp1. Similar to Nsp1 expression (Figure S2), cNsp1 ($Nsp1^{282-585}$) was difficult to produce. On the other hand, both NLPs and cNsp1, as a product from P-intein-cNsp1, were synthesized in higher yields. The purity of lyophilized samples was confirmed by SDS-PAGE.

Figure S4. Hydrogel inversion tests of P-cNsp1-P in multiple buffer conditions. While cNsp1 is known not to form hydrogels,[7, 10, 11] P-cNsp1-P formed gels in all commonly used buffer conditions for Nsp1at a protein concentration of 200 mg/mL. 1: water; 2: 0.1% TFA (in water), followed by neutralization with 1/4 volume of the buffer (400 mM Tris-base, 100 mM Tris/HCl pH 7.5, 1 M NaCl)[5]; 3: 0.1% TFA (in water) and neutralized with 1/5 volume of 200 mM Tris-HCl (pH 8.5)[4, 6]; 4: 0.2% TFA (in water) and neutralized with 1/5 volume of 200 mM Tris-HCl (pH 9); 5: 100 mM phosphate buffer[7].

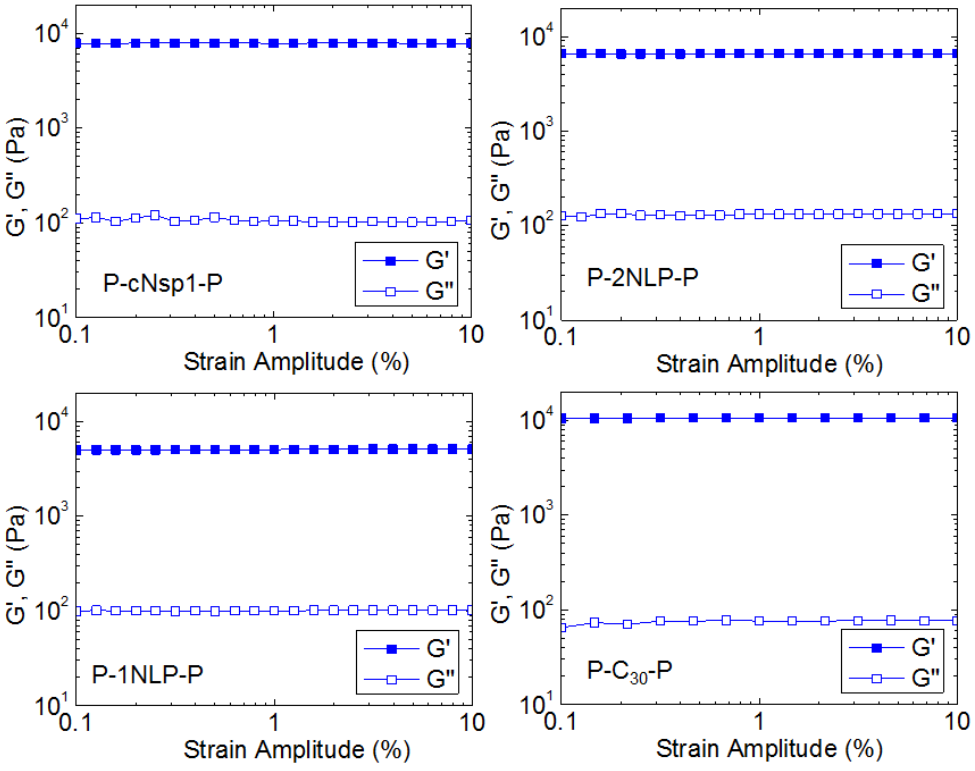


Figure S5. Strain sweep oscillatory shear rheology of 20 w/v% hydrogels at 100 rad/s and 25ºC. Frequency sweeps were performed within the linear viscoelastic range at a strain amplitude of 1%.

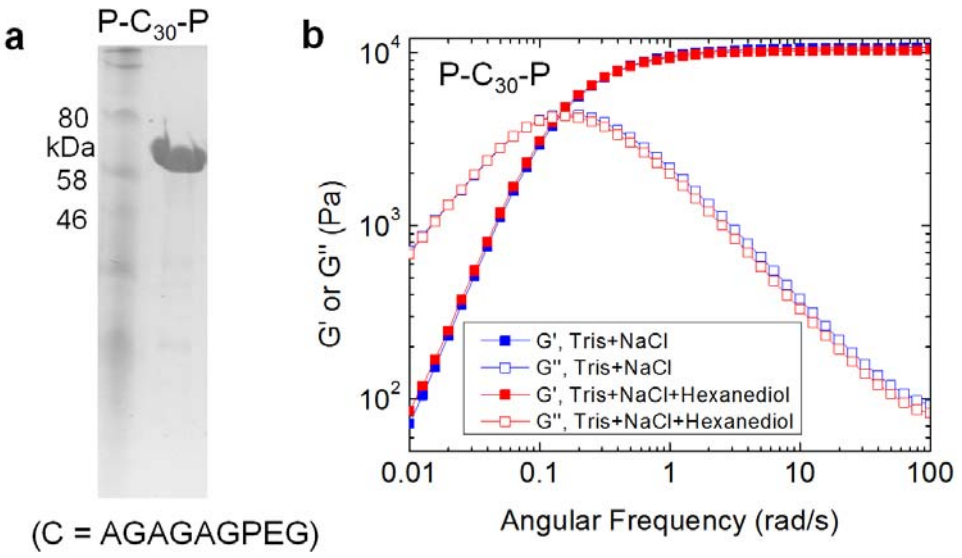


Figure S6. The 1,6-hexanediol effect on other types of physically crosslinked hydrogels with P coiled coil domains. P-C_{30}-P (37 kg/mol)[2] was synthesized and hydrogels were prepared by hydrating lyophilized samples (20 w/v%) with the buffer containing 50 mM Tris/HCl (pH 7.5) and 200 mM NaCl in the presence or absence of 10% 1,6-hexanediol. Frequency sweeps were measured at 37°C with a strain amplitude of 1%. Hexanediol did not affect the mechanical properties of P-C_{30}-P hydrogels. Note that P-C30-P protein is known to run significantly slower than its calculated and measured molar masses in SDS-PAGE.[2]

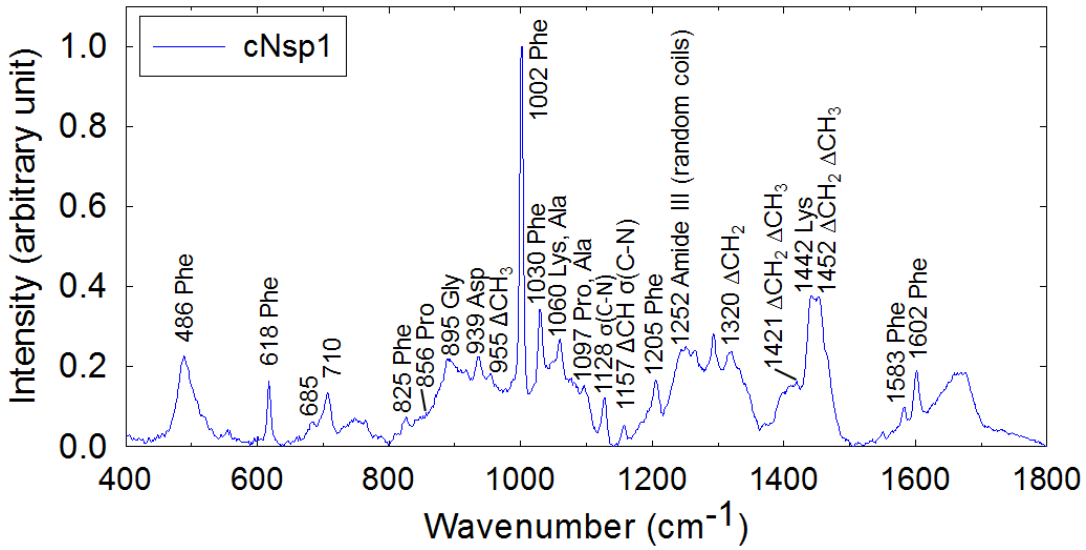


Figure S7. Raman spectra of cNsp1 (20 w/v%) with selected band assignments.[12-16] The buffer contained 50 mM Tris/HCl (pH 7.5) and 200 mM NaCl (Δ = deformation and σ = stretching).

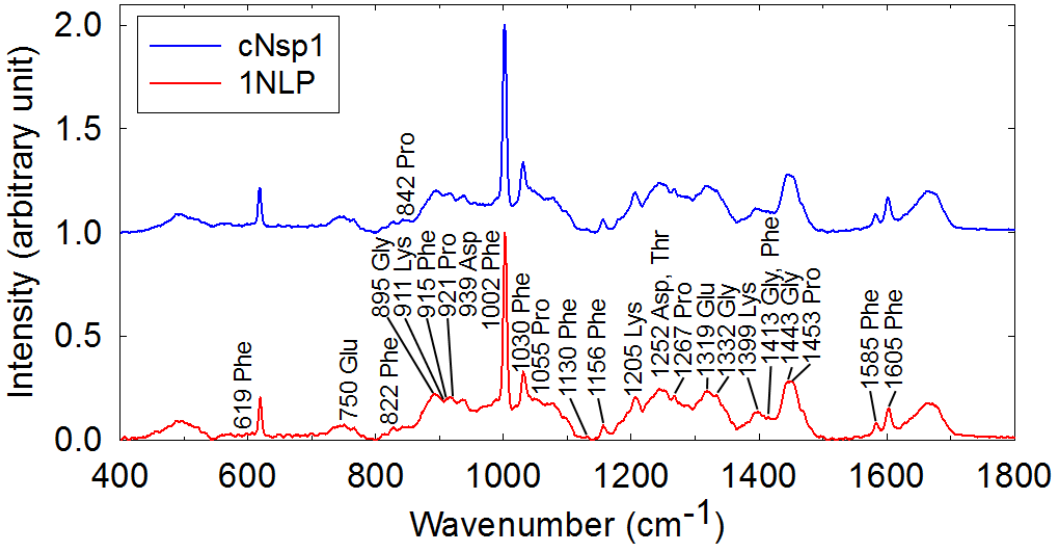


Figure S8. Raman spectra of cNsp1 and 1NLP lyophilized samples with selected band assignments.[17] The bands representing interacting moieties that were disrupted by hexanediol in solution (486 cm^{-1}, 685 cm^{-1} and 710 cm^{-1} in Figure 3e-g) were not observed in lyophilized samples.

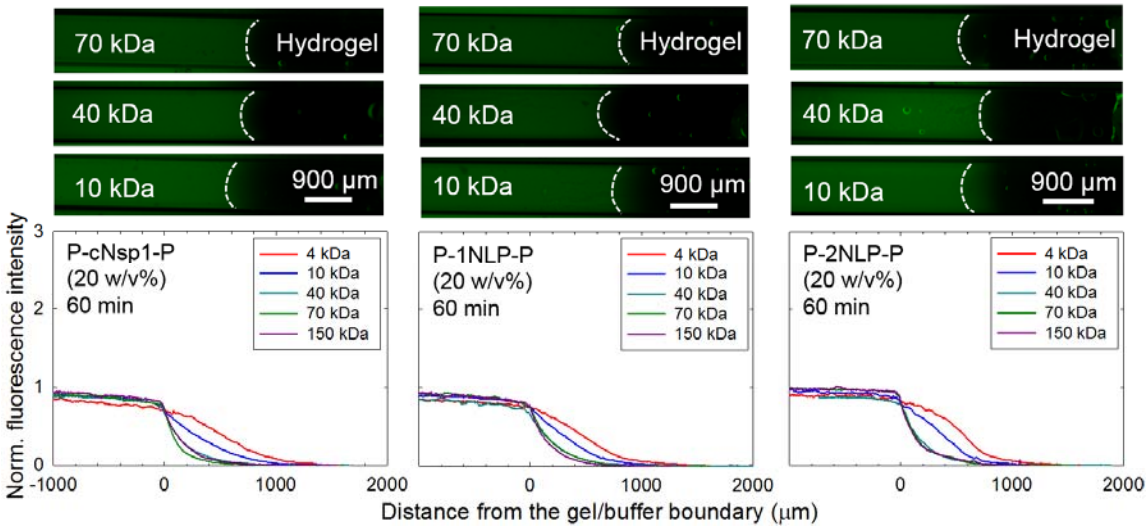


Figure S9. The pore size of designed hydrogels was measured by the dextran diffusion method. Various sizes of FITC−dextran (4, 10, 40, 70 and 150 kg/mol) were used for the fluorescence capillary assay under the same conditions as the transport measurements in Figure 4. Similar to the dextran diffusion result by Bestembayeva et al.,[18] non-interacting 40 kg/mol, 70 kg/mol and 150 kg/mol dextran probes almost remained excluded while 4 and 10 kDa pass through the hydrogels, suggesting that the pore sizes of all designed hydrogels were nearly identical. Based on Stokes' radii of the FITC-dextran polymers in the SI Methods section, the gel pore radii can be estimated between 2.3 nm and 4.5 nm. White dotted curves were drawn on the gel/buffer interface for easy visualization.

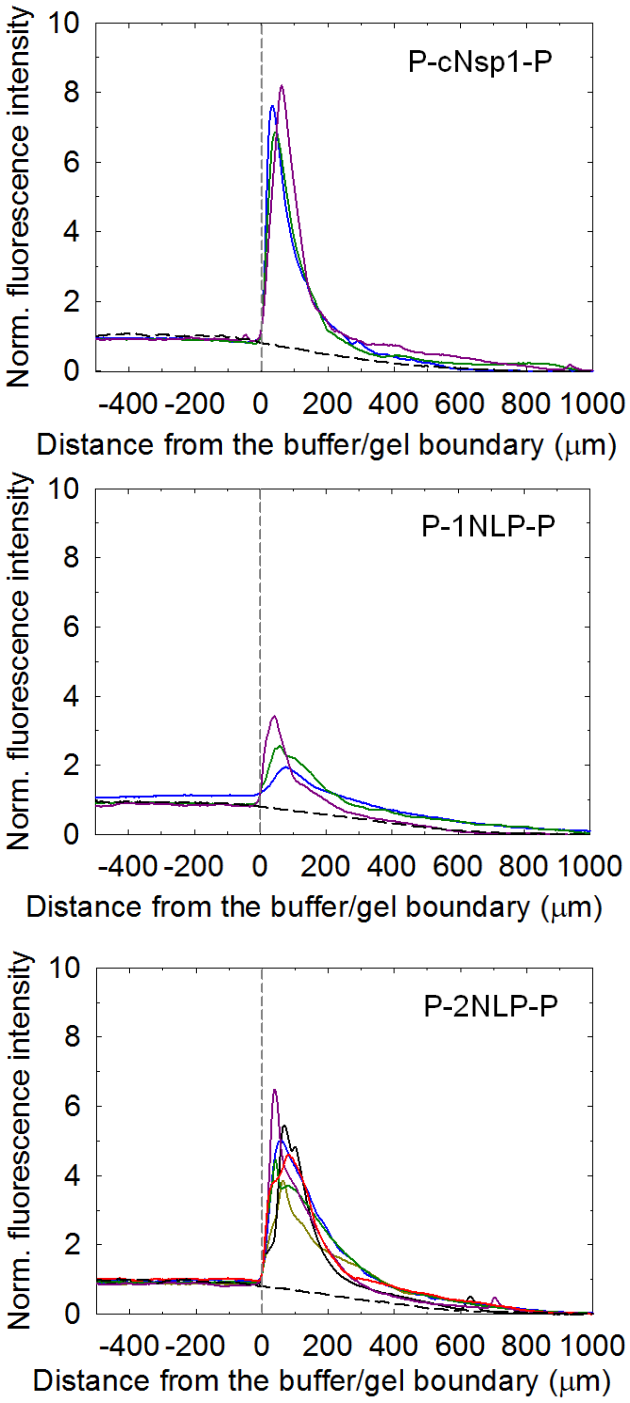


Figure S10. Examples of engineered hydrogel permeability profiles. The florescence intensity profiles on the gel at 1 hour are shown. Areas under the solid curves (> 0 μm) were calculated (Figure 4) and compared to the passive diffusion by inert molecules (black dashed curve).

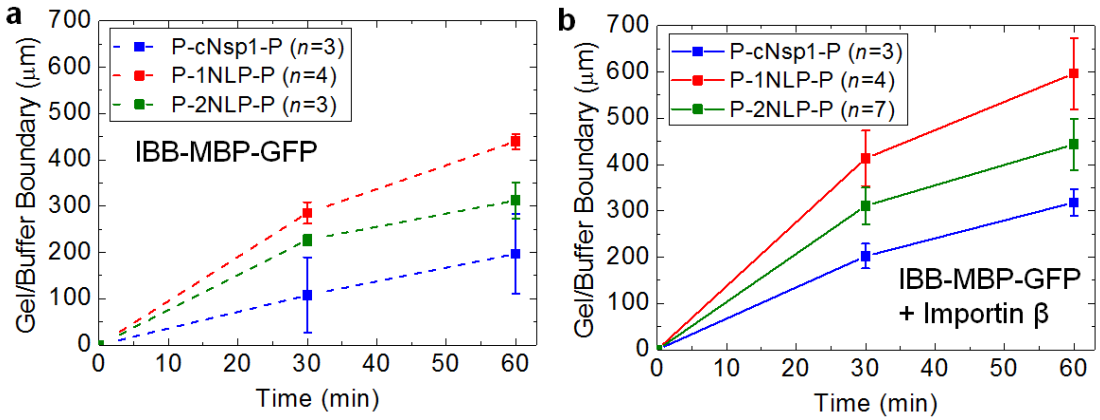
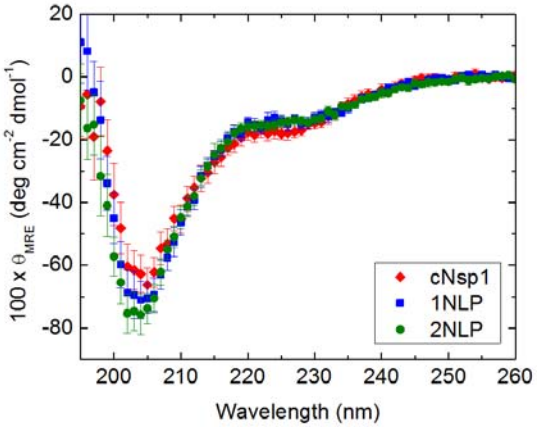


Figure S11. Gel and buffer interface changes by gel swelling during time lapse measurement of the capillary assay. The gel interface changes in Figure S10 and Figure 4c-e were measured and plotted as a function of time. Gel swelling was expected due to differences in chemical potential between the gel and the water bath, resulting in net flow into the gel (initial 20 w/v% concentration). Larger gel swelling was observed when importin β was presented in the capillary due to the additional effect of the cargo complex in increasing gel swelling. The enhanced transport rate of cargo-carrier complexes (IBB-MBP-GFP + importin β; green area in Figure 4c-e) compared to the cargo alone (gray area in Figure 4c-e) added more mass into the gel, causing larger gel swelling.



Protein	Helix 1	Helix 2	Strand 1	Strand 2	Turns	Unordered	Total	NRMSD
cNsp1	0.00	0.02	0.18	0.11	0.14	0.54	0.99	0.125
1NLP	0.00	0.02	0.19	0.12	0.18	0.47	0.98	0.141
2NLP	0.01	0.02	0.18	0.12	0.18	0.48	0.99	0.128

Figure S12. CD analysis of cNsp1 and NLPs. 5μM of the lyophilized proteins were hydrated in the buffer containing 50 mM Tris/HCl (pH 7.5) and 200 mM NaCl. By the CDSSTR method,[9] it was found that approximately 50% structures of all proteins were disordered and others were β-strand and β-turns.

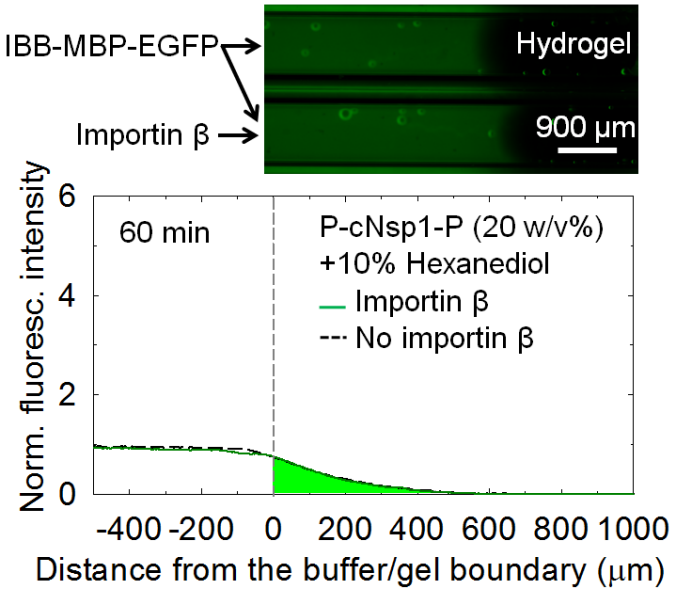


Figure S13. Fluorescence intensity measurements on P-cNsp1-P hydrogel (20 w/v%) with 10% 1,6 hexanediol in the buffer containing 50 mM Tris/HCl (pH 7.5) and 200 mM NaCl. The selective permeability function of the hydrogel was lost due to disruption of hydrophobic interactions in the gel midblock, cNsp1.

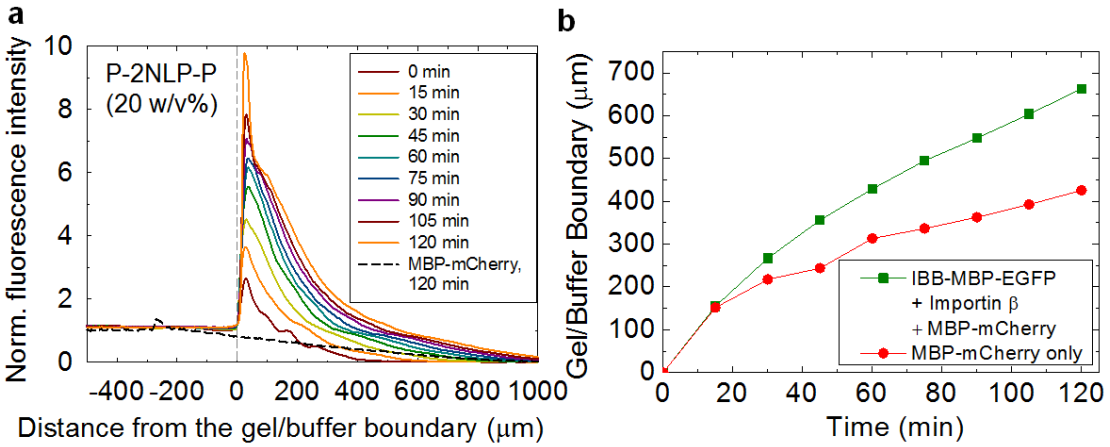


Figure S14. Time lapse measurements of P-2NLP-P gel (20 w/v%). a) Fluorescence profiles were measured to investigate the selective permeability of the gel in the mixture of IBB-MBP-EGFP, MBP-mCherry and importin β. Solid curves represent green fluorescence intensity observed in the gel. b) Gel swelling in the presence of Importin β can be inferred from the movement of the gel/buffer boundary over the course of the selective permeability tests. For the control experiment, only MBP-mCherry was also tested and the gel boundary was measured as a function of time.

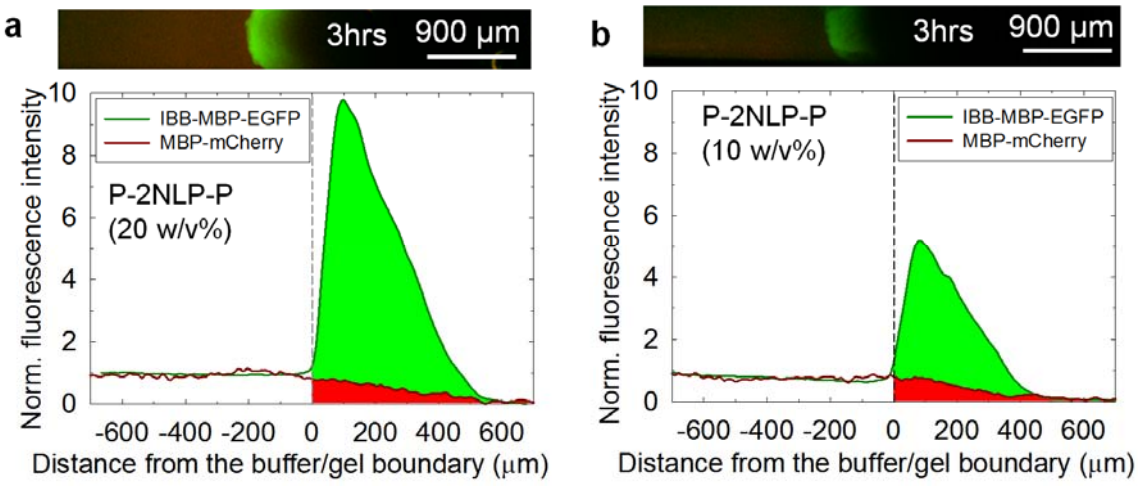


Figure S15. Selective permeability tests of P-2NLP-P gel in 20 w/v% and 10 w/v%. After finding that gels made with 2NLP have better selective accumulation than those made with1NLP and better permeability than those made with cNsp1 (Figure 4), we further investigated whether P-2NLP-P can show selective biomolecule transport at lower gel concentration, where natural FG repeat nucleoporins form inhomogeneous hydrogels with significantly reduced selective permeability.[5, 6] When hydrated in buffer at 20% and 10%, the proteins formed optically-clear gels that did not phase separate upon centrifugation, suggesting they formed macroscopically homogeneous networks under these conditions. Over three hours, the cargo-carrier complexes accumulate in the gel 10.2 and 5.2 times more than MBP-mCherry (no IBB domain), indicating that the 10 w/v% gel still shows enhanced transport of the selected biomolecules and the total accumulation depends on the number of FG sequences in the hydrogels (i.e. there are twice as many FG sequences in the 20 w/v% gel compared to the 10 w/v% gel).

Table S1. Protein molar masses measured by MALDI

	cNsp1	**1NLP**	**2NLP**	**P-cNsp1-P**	**P-1NLP-P**	**P-2NLP-P**
Measured M.M. (g/mol)	35,623	36,756	36,223	46,305	46,331	45,780
Calculated M.M. (g/mol)	35,460	36,461	36,329	45, 971	45,885	45,437

Table S2. DNA plasmids that were used in this study

Protein name	Expressed protein	Vector	Reference
$Nsp1^{30-591}$	Nsp1-His_6	pET-22b	Frey et al. (2006)[19], Jovanovic-Talisman et al. (2009)[20]
cNsp1(=$Nsp1^{274-591}$)	cNsp1-His_6	pET-22b	Ader et al. (2010)[7]
cNsp1	P-Intein-cNsp1-His_6	pET-22b	This study
1NLP	1NLP- His_6	pET-22b	This study
2NLP	2NLP- His_6	pET-22b	This study
P-cNsp1-P	P-cNsp1-P- His_6	pET-22b	This study
P-1NLP-P	P-1NLP-P- His_6	pET-22b	This study
P-2NLP-P	P-2NLP-P- His_6	pET-22b	This study
P-C_{30}-P	His_6-P-C_{30}-P	pQE-9	Olsen et al. (2010)[2]
IBB-MBP-EGFP	His_{14}-TEV-IBB-MBP-mEGFP	pQE-80	Frey and Görlich (2009)[3]
MBP-mCherry	His_{14}-TEV-MBP-mCherry	pQE-80	Frey and Görlich (2009)[3]
scImpβ	scImpβ-His_6	pQE-30	Görlich et al. (1996b)[21]

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