A designer FG-Nup that reconstitutes the selective transport barrier of the nuclear pore complex

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Nuclear Pore Complexes (NPCs) regulate bidirectional transport between the nucleus and the cytoplasm. Intrinsically disordered FG-Nups line the NPC lumen and form a selective barrier, where transport of most proteins is inhibited whereas specific transporter proteins freely pass. The mechanism underlying selective transport through the NPC is still debated. Here, we reconstitute the selective behaviour of the NPC bottom-up by introducing a rationally designed artificial FG-Nup that mimics natural Nups. Using QCM-D, we measure selective binding of the artificial FG-Nup brushes to the transport receptor Kap95 over cytosolic proteins such as BSA. Solid-state nanopores with the artificial FG-Nups lining their inner walls support fast translocation of Kap95 while blocking BSA, thus demonstrating selectivity. Coarse-grained molecular dynamics simulations highlight the formation of a selective meshwork with densities comparable to native NPCs. Our findings show that simple design rules can recapitulate the selective behaviour of native FG-Nups and demonstrate that no specific spacer sequence nor a spatial segregation of different FG-motif types are needed to create selective NPCs.
Nucleocytoplasmic transport is orchestrated by the nuclear pore complex (NPC), which imparts a selective barrier to biomolecules\textsuperscript{1,2}. The NPC is a large eightfold symmetric protein complex (with a size of \textasciitilde52 MDa in yeast and \textasciitilde112 MDa in vertebrates) that is embedded within the nuclear envelope and comprises \textasciitilde30 different types of Nucleoporins (Nups)\textsuperscript{3,4}. Intrinsically disordered proteins, termed phenylalanine-glycine (FG)-Nups, line the central channel of the NPC. FG-Nups are characterized by the presence of FG repeats separated by spacer sequences\textsuperscript{5} and they are highly conserved throughout species\textsuperscript{6}. FG-Nups carry out a dual function: by forming a dense barrier (100–200 mg/mL) within the NPC lumen, they allow passage of molecules in a selective manner\textsuperscript{7–10}. Small molecules can freely diffuse through, whereas larger particles are generally excluded\textsuperscript{11}.

The NPC is highly complex in its architecture and dynamics, being constituted by many different Nups that simultaneously interact with multiple transiting cargoes and NTRs. In fact, the NTRs with their cargoes may amount to almost half of the mass of the central channel, so they may be considered an intrinsic part of the NPC\textsuperscript{3}. These NPC properties complicate in vivo studies\textsuperscript{3,21–23} for which it is very challenging to identify contributions coming from individual FG-Nups\textsuperscript{24,25}. On the other hand, in vitro approaches to study nucleocytoplasmic transport using biomimetic NPC systems\textsuperscript{26–33} have thus far been limited to single native FG-Nups and mutations thereof, attempting to understand the physical behaviour of FG-Nups and their interactions with NTRs. The reliance on a few selected Nups from yeast or humans in these studies with sequences that evolved over time in different ways for each of these specific organisms makes it difficult to pinpoint the essential and minimal properties that provide FG-Nups with their specific selective functionality.

Here, we describe a bottom-up approach to studying nuclear transport selectivity, where we rationally design, synthesize, and assess artificial FG-Nups with user-defined properties that are set by an amino acid (AA) sequence that is chosen by the user. With this approach we address the question: can we build a synthetic protein that mimics the selective behaviour of native FG-Nups? By combining experiments and coarse-grained molecular dynamics (MD) simulations, we illustrate the design and synthesis of an artificial 311-residue long FG-Nup, which we coin NupX, and characterize its selective behaviour with respect to Kap95 (a well-characterized NTR from yeast, 95 kDa) versus bovine serum albumin (BSA, 66 kDa). First, we explore the interactions between Kap95 and NupX brushes with varying grafting densities using quartz crystal microbalance with dissipation monitoring (QCM-D), finding that NupX brushes bind Kap95 while showing no binding to BSA. We confirm this finding by calculating the potential of mean force (PMF) associated with the entry of Kap95 or an inert cargo into NupX brushes. Second, we explore the transport properties of NupX-functionalized solid-state nanopores and show that NupX-lined pores constitute a selective transport barrier. Similar to FG-Nups previously studied with the same technique\textsuperscript{28,31}, the NPC-mimicking nanopores allow fast and efficient passage of Kap95 molecules, while blocking transport of BSA. Coarse-grained MD simulations of NupX-functionalized nanopores highlight the formation of a dense FG-rich meshwork with similar protein densities as in native NPCs, which excludes inert molecules but allows entry and passage of Kap95.

The current work provides the proof of concept that a designer FG-Nup can reconstitute NPC-like selectivity, and the results show that no specific spacer sequence nor a spatial segregation of different FG-motifs (as observed in recent work\textsuperscript{3,34}) is required for achieving selectivity. This work lays the foundation for multiple future directions in follow-up work as the approach opens the route to systematically study the essential microscopic motifs that underlie the unique selectivity of NPCs.

**Results**

**Design of the synthetic NupX.** In the design of our synthetic NupX protein, we aim to reconstitute nuclear transport selectivity while operating under a minimal set of simple design rules. The design procedure that we outline below uses the following four rules: (i) we design a protein that incorporates the physical properties of GLFG-Nups (a specific class of essential FG-Nups that are particularly cohesive and contain many glycine-leucine-phenylalanine-glycine (GLFG)-motifs), (ii) it comprises two parts, with a cohesive domain at one end and a repulsive domain at the other end, where each domain is characterized by the ratio C/H of the number of charged and the number of hydrophobic residues, (iii) FG- and GLFG-motifs are present in an alternating and uniformly spaced fashion within the protein’s cohesive domain and (iv) the protein is intrinsically disordered throughout its full length, similar to native FG-Nups.

We implemented our design rules in a stepwise design process as follows. First, we selected and analyzed an appropriate set of native FG-Nups (design rule i), namely GLFG-Nups, which differ from other FG-Nups in terms of the type of FG repeats and the properties of the spacer regions\textsuperscript{11}. The emphasis on GLFG-Nups follows from their localization in the central channel\textsuperscript{3} of the yeast NPC (Fig. 1a), where they strongly contribute to the nuclear transport selectivity. Indeed, a small subset of GLFG-Nups (e.g., either Nup100 or Nup116 in combination with Nup145N) was shown to be essential and sufficient for cell viability\textsuperscript{21,35}. To derive the AA content of NupX, we therefore characterized the archetypical GLFG-Nup sequence by determining the AA content of the disordered regions of Nup49, Nup57, Nup145N, Nup116 and Nup100 from yeast. Of these, the most essential GLFG-Nups (i.e., Nup100, Nup116 and Nup145N) comprise a collapsed domain with a low C/H-ratio and abundance of FG/GLFG repeats, and an extended domain with a high C/H-ratio and absence of FG repeats\textsuperscript{5}. This distinction is highlighted in Fig. 1b, c, where non-FG/GLFG/charged residues are highlighted in light green and pink for the collapsed and extended domains, respectively—a colouring scheme used throughout this work. The division into two domains of these essential GLFG-Nups led us to phrase design rule ii in our design process of NupX, with each domain comprising \textasciitilde150 AA residues (see Fig. 1b, c), whereas the extended domain of NupX is of quite similar length to the corresponding extended domains of Nup100, Nup116 and Nup145N (190 residues on average), the cohesive domain is notably shorter than the collapsed domains of native GLFG-Nups (390 residues on average).

Assigning the AA content to NupX, as derived from the sequence information of the GLFG-Nups, was performed separately for the two domains: we computed the cumulative AA contents (excluding FG- and GLFG-motifs) for both the collapsed domains of all five GLFG-Nups, and for the extended domains of Nup100, Nup116 and Nup145N (design rule ii). Upon normalizing for the total length of the collapsed or extended domains of all native GLFG-Nups, this analysis resulted...
in the distributions presented in Fig. 1d, plotted separately for the collapsed (light green, top) and the extended (light red, bottom) domains. Based on these histograms, we assigned AAs to the collapsed and extended domains of NupX separately. Following design rule iii, we then placed FG- and GLFG repeats in the collapsed domain with a fixed spacer length of ten AAs. This value was chosen based on the spacer length of ~5–15 AAs in native GLFG-Nups. An analysis of the charged and hydrophobic AA content of the domains of NupX and native GLFG-Nups shows that the assigned sequence properties are indeed reproduced by our design method (Fig. 1f). Finally, the sequences of the collapsed and extended domains of NupX were repetitively...
shuffled (except for the FG- and GLFG-motifs that we kept fixed) until a desirable level of disorder was achieved (design rule iv), as predicted by PONDR36 and DISOPRED37,38 (Fig. 1g). This resulted in the NupX sequence shown in Fig. 1e. Whereas PONDR predicts one short folded segment between residues 189 and 209 (normalized position of 0.65 in Fig. 1g), additional structure prediction39 (Methods) did not yield any high-confidence folded structures for this segment.

To assess the robustness of our design procedure, we tested how permutations of the NupX sequence (which shuffle AA while retaining the FG/GLFG sequences and the definition of both domains) affect the Stokes radius Rs, as computed from one-bead-per-amino-acid (1BPA) MD simulations developed for intrinsically disordered proteins (Fig. 1h, see Methods). We found that 25 different designs for NupX (Supplementary Table 2) yielded an average Rs of 4.2 ± 0.2 nm (errors are SD). This is close to the simulated (3.9 ± 0.4 nm) and measured (3.7 ± 1.1 nm by dynamic light scattering (DLS), Supplementary Table 1) Rs value of the NupX protein design (Fig. 1e).

Summing up, using a minimal set of rules, we designed a NupX protein that incorporates the average properties that characterize GLFG-Nups.5,11 Moreover, by creating 25 different designs that all showed similar behaviour in our simulations, we showed that the physical properties such as the Stokes radius and the division of NupX into a cohesive and repulsive domain are recovered in a reliable way.

QCM-D experiments and MD simulations show selective binding of Kap95 to NupX brushes. To assess the interaction between NupX and Kap95, we employed a QCM-D, with gold-coated quartz chips and phosphate-buffered saline (PBS, pH 7.4) as running buffer, unless stated otherwise. First, C-terminus-thiolated NupX molecules were injected into the chamber at a constant flow-rate (20 μL/min) where they chemically reacted with the gold surface. Binding of NupX to the gold surface could be monitored in real time by measuring the shift in resonance frequency δf of the quartz chip (Fig. 2a). We applied the NupX coating by administering a protein concentration ranging from 100 nM to 2 μM (Supplementary Fig. 2) until a plateau in the frequency shift was reached, which typically occurred after ~1 h of incubation. To gain insight into the areal mass density of the deposited layers, we employed surface plasmon resonance (SPR) measurements (Supplementary Fig. 3), where we used the same coating protocol for consistency. From these measurements of the areal mass density, we found grafting distances of 7.7 ± 0.5 nm (mean ± SD) for chips incubated with a 60-nM NupX solution, and 2.91 ± 0.02 nm (mean ± SD) for 2 μM. In determining the grafting density from the areal mass density, we assumed a triangular lattice (since an equilateral triangulated (sometimes also denoted as hexagonal) lattice is the densest type of packing that can be described by a unique length scale that sets the grafting density). Figure 2a shows a typical frequency shift over time for the binding of 1 μM NupX to a gold surface. After the Nup-layer was formed, a 1-mercapto-11-undecylte-tri(ethyleneglycol) molecule (MUTEG), which is expected to form a ~2-nm thin passivating film17, was added to passivate any remaining bare gold that was exposed in between NupX molecules (Supplementary Fig. 4). This minimizes unintentional interactions between Kap95 and gold for subsequent binding experiments (Supplementary Fig. 5).17,18,40

Thus, after setting up a NupX-coated layer, we flushed in Kap95 at stepwise increasing concentrations (~10–3000 nM, Fig. 2d) and monitored binding to the NupX-coated surface. We observed a clear concentration-dependent amount of Kap95 molecules bound to the NupX brush. For reference, we repeated the experiment on brushes of Nsp1 (a native FG-Nup from yeast), as well as Nsp1-S, a Nsp1-mutant where the hydrophobic AA AsF, I, L, V are replaced by the hydrophilic AA Serine (S) (Fig. 2b, c). The latter was employed as a negative control since it is expected to not bind Kap95 due to the lack of FG repeats14,15. Gold surfaces coated with Nsp1 or Nsp1-S were characterized with SPR under similar coating conditions as for QCM-D, yielding grafting distances of 4.9 ± 0.1 nm for Nsp1 and 5.8 ± 0.4 nm for Nsp1-S. Upon flushing Kap95, we found, consistent with previous studies27,40, a concentration-dependent adsorption to Nsp1 brushes (Fig. 2e), whereas we did not observe any detectable interaction between Kap95 and Nsp1-S (Fig. 2f). The latter is consistent with the lack of FG repeats in the Nsp1-S sequence that makes the Nsp1-S film devoid of binding sites for Kap95. We note that non-linear effects, e.g., coverage-dependent changes in water entrapment within the layer11, are likely to affect the observed equilibrium signals, which, together with a relatively slow dissociation of Kap95 from both the NupX and Nsp1 brushes, led us to refrain from extracting a dissociation constant. Adsorbed molecules could be completely removed upon flushing 0.2 M NaOH however (Supplementary Fig. 6). Finally, we investigated whether the inert molecule BSA could bind to the NupX brush. Upon flushing 2.5 μM of BSA (Fig. 2g) we did not observe any appreciable change in the resonance frequency,
indicating that the NupX brush efficiently excludes these inert molecules. This measurement was repeated for all the grafting conditions used in this study (Supplementary Fig. 7) showing that BSA did not produce any detectable shift in frequency, while Kap95 showed clear binding to the NupX films. Importantly, the data show that the NupX brush selectively interacts with Kap95 over a range of grafting densities.

In order to study the morphology and physical properties of NupX brushes at the microscopic level, we employed coarse-grained MD simulations (see Methods), which resolved the density distribution within the NupX brush layer and the preferential adsorption of Kap95 over inert molecules of similar size such as BSA. Thirty-six NupX proteins were tethered onto a triangular lattice with a fixed spacing of 4.0 nm (Fig. 2h) or 5.7 nm (Supplementary Fig. 11), well in the range of grafting distances from 2.9 to 7.7 nm as measured by SPR. Averaged over a simulation time of 3 μs, we found that the NupX brushes with a 4.0 nm grafting distance form a laterally homogeneous meshwork with densities ranging from ~400 mg/mL near the substrate to around ~200 mg/mL in the central region and to ~300 mg/mL in the central region and to ~300 mg/mL.
near the free surface of the brush (Fig. 2i, top panel). The interface near the free surface of the brush contains the highest relative concentration of FG- and GLFG-motifs (see Fig. 2i, top panel). Notably, the protein density throughout the brush is of the same order of magnitude as the density obtained in simulations of the yeast NPC42. Upon increase of the grafting distance to 5.7 nm, we find that the NupX brush attains different and less dense conformations: the density profile plateaus at a value of 170 mg/mL and slowly decays without showing a peak density near the free surface of the brush (Supplementary Fig. 11). We translated our density profiles into height estimates in a similar fashion as other computational studies on FG-Nup brushes33,44. We consider the z-coordinates at which 90% of the protein mass is incorporated as the effective brush heights. This approach yields brush heights of 12 and 18 nm for the NupX brushes with 5.7 and 4.0 nm grafting distances, respectively. These values coincide quite well with the inflection point of the decaying tail of the density profiles in Fig. 2i and Supplementary Fig. 11.

The simulated density profiles yield notably higher brushes than expected from the Sauerbrey equation; e.g., assuming a density of the hydrated brush of ~1.05 g/mL45, one can estimate a brush height of 6.4 nm for the NupX brush in Fig. 2a (that was incubated at 1 μM, which we expect to have a grafting distance at the higher end of the values used in our simulations). Importantly, however, the Sauerbrey equation does not account for viscoelastic effects and only provides a lower limit to the brush height41. Indeed, given the dissipation-to-frequency ratio of $-0.045 \times 10^{-6}$ Hz$^{-1}$ (Supplementary Fig. 9), one expects that the actual experimental brush height will be larger than 6.4 nm, an effect also seen in other QCM-D studies of FG-Nups46,47. Notably, a quantitative difference between the NupX brush height estimations of the computational and experimental results does not affect the major conclusions of the study, namely the selective transport across biomimetic nanopores with a rationally designed artificial FG-Nup and the selective binding of Kap95 over BSA to NupX.

To assess the selective properties of the NupX brushes, we performed umbrella sampling simulations of the adsorption of Kap95 and an inert molecule to NupX brushes (Methods), again for two grafting densities of 4 and 5.7 nm. We modelled Kap95 (Supplementary Fig. 12) as an 8.5 nm sized sterically repulsive (i.e., modelling only repulsive, excluded volume interactions) particle with ten hydrophobic binding sites8,31,46,47 and a total net charge of $-170$ mg/mL and slowly decays without showing a peak density near the free surface of the brush (Supplementary Fig. 11). We translated our density profiles into height estimates in a similar fashion as other computational studies on FG-Nup brushes33,44. We consider the z-coordinates at which 90% of the protein mass is incorporated as the effective brush heights. This approach yields brush heights of 12 and 18 nm for the NupX brushes with 5.7 and 4.0 nm grafting distances, respectively. These values coincide quite well with the inflection point of the decaying tail of the density profiles in Fig. 2i and Supplementary Fig. 11.

Figure 3e, f show scatter plots of the event distributions, where the conductance blockade is plotted against dwell time for all translocation events. For the bare pore, we observe similar average amplitudes of $0.24 \pm 0.09$ and $0.20 \pm 0.05$ nS (errors are SD) for BSA and Kap95, respectively. For the NupX-coated pore, we found slightly larger but again mutually similar event amplitudes of $0.31 \pm 0.03$ and $0.27 \pm 0.03$ nS for BSA and Kap95, respectively. We found comparable translocation times through the bare pore of $0.66 \pm 0.03$ and $0.81 \pm 0.02$ ms (errors are SEM) for BSA and Kap95, respectively. For the coated pore, however, we measured longer dwell times of $5.0 \pm 0.5$ and $1.9 \pm 0.1$ ms for BSA and Kap95, respectively, which indicates that the presence of the NupX

Single-molecule translocation experiments with NupX-coated nanopores demonstrate selectivity. In order to test whether our synthetic FG-Nup does indeed form a transport barrier that mimics the selective properties of the NPC, we performed electrophysiological experiments on biomimetic nanopores28,31. These NPC mimics were built by tethering NupX proteins to the inner walls of a solid-state SiN$_x$ nanopore43,44 using self-assembled monolayer chemistry (details in Methods). Solid-state nanopores of 10–60 nm in diameter were fabricated onto a glass-supported SiN$_x$ free-standing membrane by means of TEM drilling. A buffer with 150 mM KCl, 10 mM Tris, 1 mM EDTA, at pH 7.5 was used to measure the ionic conductance through the pores, while retaining near-physiological conditions. Coating bare SiN$_x$ pores with NupX yielded a significant decrease in conductance (e.g., ~50% for ~30 nm diameter SiN$_x$ pores) of the bare-pore values, as estimated by measuring the through-pore ionic current before and after the functionalization (Supplementary Fig. 13). In addition, the current–voltage characteristic in the ±200 mV range (Supplementary Fig. 13) is linear both for the bare and NupX-coated pores, indicating that the NupX meshwork is not affected by the applied electric field at the 100 mV operating bias. To obtain more information on the NupX-coating process of our SiN$_x$ pores, we repeated the same functionalization procedure on silica-coated SPR chips (Supplementary Fig. 3), where APTES, Sulfo-SMCC and NupX coatings were independently characterized using the same coating protocol as for the SiN$_x$ nanopores, for consistency. From these experiments, we estimate an average grafting distance of $5.4 \pm 1.1$ nm between adjacent NupX molecules. Measurements of the ionic current through NupX-coated pores revealed a higher 1/f noise in the current (Supplementary Fig. 14) compared to bare pores, which we attribute to random conformational fluctuations of the Nups within the pore volume and access region53,54, similar to findings from previous studies on biomimetic nanopores28,31. To test the selective behaviour of the biomimetic nanopore, we measured translocation rates of Kap95 and BSA through bare pores of ~30–35 nm in diameter (Fig. 3a). Figure 3c shows examples of raw traces recorded for a 30 nm pore under 100 mV applied bias, when either only buffer (top), 450 nM Kap95 (middle), or 2.8 μM BSA (bottom) was added to the cis-chamber. As expected, we observed transient dips in the current through the bare pore upon injection of the proteins, which we attribute to single-molecule translocations of the analyte molecules. As is typical in nanopore experiments, translocation events yield current blockades with a characteristic amplitude and dwell time, where the former relates to the size of the molecule occupying the pore and the latter generally depends on the specific interaction between the translocating molecule and the pore wall55. Next, we repeated the experiment under identical conditions on the same pore after coating with NupX took place (Fig. 3b). Examples of typical raw traces are shown in Fig. 3d. Strikingly, Kap95 molecules could still translocate efficiently through the NupX-coated pore, whereas BSA molecules were practically blocked from transport.
molecules in the pore significantly slows down the translocation process of the passing molecules. Notably, BSA molecules were slower in translocating through the coated pore as compared to Kap95, which we attribute to the lower affinity between BSA and the NupX mesh as compared to Kap95. The transient and multivalent interactions between Kap95 and the FG repeats in the NupX meshwork lead to a reduced energy barrier as compared to BSA permeation, which may explain the observed differences in dwelling times. Repeating the same experiment on a larger 60 nm NupX-coated pore (Supplementary Fig. 15) yielded selective pores with faster translocations for both Kap95 (0.65 ± 0.05 ms) and BSA (1.6 ± 1.3 ms), consistent with the presence of an open central channel. Smaller pores (<25 nm) did not result in any detectable signal for either Kap95 or BSA, due to the poor signal-to-noise ratio attainable at such low conductances.

Most importantly, these data clearly show selectivity of the biomimetic pores. Figure 3g compares the event rate of translocations for Kap95 and BSA through bare and NupX-

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**Fig. 3 Electrical measurements on NupX-coated solid-state nanopores.**

a, b Schematic of the nanopore system before (a) and after (b) NupX functionalization. c, d Examples of raw current traces through bare (c) and NupX-coated (d) pores, recorded under 100 mV applied bias for different analyte conditions. Current traces are recorded in the presence of buffer only (top), upon addition of 450 nM Kap95 (middle) and 2.8 μM BSA (bottom). Traces were filtered at 5 kHz. e Scatter plot showing conductance blockades and dwell time distributions of translocation events of the analytes Kap95 (black, N = 506) and BSA (red, N = 387) through a bare 30 nm pore, recorded over the same time interval. f Scatter plot showing conductance blockades and dwell time distributions of translocation events of the analytes Kap95 (yellow, N = 686) and BSA (blue, N = 28) through a NupX-coated 30 nm pore, recorded over the same time interval. Top and right panels in e and f show lognormal fits to the distribution of dwell times and conductance blockades, respectively. Dashed vertical lines in top panels indicate the mean values for the dwell time distributions. g Average event rate of translocations for Kap95 through a bare pore (black), BSA through a bare pore (red), Kap95 through a NupX-coated pore (yellow) and BSA through a NupX-coated pore (blue). Error bars indicate standard deviations from independent measurements (circles) on three different pores, N = 3.
MD simulations of NupX-lined nanopores reveal their protein distribution and selectivity. We used coarse-grained MD simulations (Methods) to understand the selective properties of NupX-lined nanopores as observed in our experiments. The 20 nm height of these nanopores is the same as the SiNx membrane thickness, while we vary the diameter from 15 to 70 nm. Multiple copies of NupX are tethered to the nanopore lumen by their C-terminal domain in an equilateral triangular lattice with a spacing of 5.5 nm, based on estimates obtained from the SPR experiments (Supplementary Fig. 3, Methods). We note that the geometrical confinement by the nanopore may affect the grafting distance on the concavely curved interior pore wall (parallel to the pore axis) as compared to the planar geometry. Based on 6 μs of coarse-grained MD simulations, we obtained the protein density distribution in the (r, z)-plane (averaged over time and angle θ) within a NupX-lined nanopore of 30 nm in diameter (Fig. 4b), similar in size as the translocation experiments. High-density regions form close to the attachment sites (i.e., the four dots at each wall in Fig. 4b) and along the central axis of the nanopore. Since the triangulated lattice (comprising four rows) does not strictly exhibit a symmetry plane along the z = 0 axis, a slight asymmetry (<10% in terms of protein density) occurs between the top and bottom of the density map. From these data, we obtained a radial protein density profile, averaged over the pore height for the pore region (|z| < 10 nm, Fig. 4c), which exhibits a maximum of 230 mg/mL at the pore centre for the 30 nm NupX nanopore system and is insensitive to the aforementioned small asymmetry. This density agrees well with values in the range of 200–300 mg/mL observed in earlier computational studies of the yeast NPC central channel. We attribute the central localization of the NupX proteins to the combination of repulsion between the high C/H ratio extended domains near the pore wall and attraction between the cohesive, low C/H ratio collapsed domains of opposing NupX proteins. Since the average density in the access region (10 nm < |z| < 40 nm, Fig. 4c) is found to be low in comparison to the average density within the pore region, we conclude that the NupX proteins predominantly localize within the nanopore when the grafting distance is perturbed by ~10% in either direction (Supplementary Fig. 17) to values of 5.0 or 6.0 nm, similar density profiles are obtained. So even though the experimental grafting distance might be somewhat larger for the nanopore compared to the planar brushes due to the different geometrical confinement, similar profiles would be expected for more sparsely coated pores.

The organization of the NupX proteins inside the nanopore geometry changes notably with pore diameter (Fig. 4d, e). For large diameter pores, the density profile of NupX proteins protruding from the pore surface quite well resembles that of a planar brush (cf. Supplementary Fig. 11), resulting in a central opening for pores that are ≥45 nm. When the pore diameter reaches values <45 nm, NupX-coated nanopores are effectively sealed. This 45 nm length scale is remarkable, given the quickly decaying density profile of a planar NupX brush with a similar grafting distance (Supplementary Fig. 11). Upon further decreasing the pore diameter to values <25 nm, we find that the NupX collapsed domains are expelled from the pore region towards the access region, resulting in decreased densities in the central pore region (Fig. 4d, e). Interestingly, we find that these changes in NupX morphology as a function of pore diameter are in good qualitative agreement with predictions from earlier works on polymer-coated nanopores, which point to a curvature-dependent modulation of the brush height. More specifically, an increase in curvature (i.e., a decrease in pore diameter) of a concave brush substrate is expected to lead to a relative extension of the brush as compared to the planar geometry. In addition, attractive interactions between the cohesive head groups of NupX anchored at opposing pore walls will also contribute to the sealing of NupX pores. Finally, we note that a central opening in the NupX nanopore meshwork, present for diameters from 45 nm upwards, is consistent with the increased event frequency and translocation speed observed in large (60 nm) NupX-coated pores (Supplementary Fig. 15).

Using a relation between the local protein density and the local conductivity separately for the pore and access regions, we calculated the conductance of the NupX nanopores for varying diameters (Fig. 4d, Supplementary Fig. 18, Methods). The calculated conductance from the simulated NupX-lined pores is shown in Fig. 4f (black squares) together with the experimental conductances for bare and NupX-coated pores (open circles). Note that we adopted a critical protein density of 85 mg/mL from the earlier work on Nsp131 in our density–conductivity relation, but assume a different dependency of the local conductivity on the local protein density (Methods). Rather than assuming an abrupt complete blockage of conductance above the critical protein density of 85 mg/mL, we now use an exponential relation that provides a more gradual reduction in conductance with density. The necessity of a different density–conductance relationship indicates that the conductivity of the NupX nanopore meshwork depends non-linearly on the average protein density. Interestingly, the slope of the conductance–diameter curve for NupX-lined pores converges to that of bare pores already at relatively small pore sizes. This is due to the formation of a hole within the NupX meshwork (Fig. 4d) already in pores with diameters over 40 nm, rendering these effectively similar to bare nanopores of smaller diameter.

A spatial segregation of different types of FG-motifs, as was observed in recent computational studies, is not studied here explicitly. However, we find both types of FG-motifs localize similarly in the high-density central region within the NupX nanopore channel (Fig. 4g, h). From these distributions and the observed selective transport of these pores (Fig. 3e–g), we can infer that a spatial segregation of different FG-motifs is not required for selective transport.

Finally, in order to assess the selective properties of NupX-lined nanopores, we simulated a 30 nm diameter NupX-lined nanopore in the presence of ten Kap95 or ten inert particles. We released Kap95/inert particles in the access region at the top and recorded their location over 5 μs of simulation time (see Methods, Fig. 5c, d). The Kap95 particles entered and left the NupX meshwork and sampled the pore lumen by traversing in the z-direction (Fig. 5c). They localized preferentially at positions radially halfway between the central pore axis and the edge of the nanopore, where their time-averaged density distribution takes the shape of a concave cylindrical region, as is shown in Fig. 5a. Kap95 was found to be capable of (re-)entering and leaving the meshwork on either side (Fig. 5c). Since no external electric field
was applied, exiting and subsequent re-absorption of Kap95 into the NupX meshwork occurred and there was no directional preference for the motion of the Kap95 molecules, in contrast to the experiments. Interestingly, the NupX meshwork adapted itself to the presence of the Kap95 particles by expanding towards the access region (compare Fig. 4b and Supplementary Fig. 19): the protein density in the pore region decreased due to the presence of the Kap95, whereas the protein density increased in the access region. In contrast to the findings for Kap95, we observed that the inert particles, simulated under the same conditions, remained in the top compartment (Fig. 5b, d) and did not permeate into the NupX meshwork over the 5 μs time span of the simulation.

To quantify the selectivity of the 30 nm NupX-lined nanopores, we calculated PMF curves along the z-axis for both cargo types (Fig. 5e, see Methods). Kap95 experienced a negative free energy difference of approximately 8 kJ/mol, which amounts to a binding energy of just over 3 k_BT per Kap95. On the other hand, inert particles experience a steep energy barrier of approximately 18 kJ/mol, which corresponds to over 7 k_BT per protein. The obtained Kap95 free energy profiles are similar to those found in other simulation studies of cargo permeation through NPCs or NPC-mimicking systems. The Kap95 binding free energy differences along the nanopore axis are considerably smaller than the computed free energy profiles for NupX brushes (Fig. 2i).
**Fig. 4** Protein distribution and conductance of NupX-coated pores. a Snapshot of a biomimetic nanopore simulation. NupX proteins (following the colouring scheme of Fig. 1) were tethered with a grafting distance of 5.5 nm (yellow, top inset) to a cylindrical occlusion made of inert beads (grey). Pore diameters ranged from 15 to 70 nm, where the pore thickness was 20 nm throughout. Bottom inset: highlight of a single NupX protein (purple) within the NupX meshwork. b Axial-radial map (averaged over time and in the azimuthal direction) of the protein density within a 30-nm NupX-lined nanopore, from 6 μs simulations. Dark colours indicate regions of low density, brighter colours indicate regions of high density. The collapsed domains of the NupX proteins form a high-density central plug. The high-density regions near the pore radius (15 nm) coincide with the anchoring sites of the NupX proteins. c Density distributions (thick lines) for the pore (blue, |z| < 10 nm) and access (red, 10 nm < |z| < 40 nm) regions. Dashed curves indicate the average density within 1-nm thick slices in the z-direction. d Radial density distributions (z-averaged) for NupX-lined nanopores with diameters ranging from 15 to 70 nm (darker and lighter colours denote smaller and larger diameters, resp.). The curve for 30 nm is emphasized. An increase in pore size beyond 30 nm leads to a decrease in the pore density along the pore’s central channel. e Side-view and top-view visualizations of 20, 30, and 45 nm diameter NupX-lined nanopores. For nanopores with diameters smaller than 25 nm, the pore region density decreases due to an expulsion of the collapsed NupX domains towards the access region. For nanopore diameters larger than 40 nm, the pore density decreases and a hole forms. For nanopore diameters of 25–30 nm, the pore region is sealed by the NupX cohesive domains. f Conductance scaling for bare and NupX-coated nanopores. Open circles indicate conductance measurements for bare (red) and NupX-coated (green) pores. Squares indicate time-averaged conductance values obtained from MD simulations via a density-conductance relation (Methods). Error bars indicate the standard deviation in the conductance and are smaller than the marker. Second-order polynomial fits to the bare pore (experimental) and the simulated conductance values are included as a guide to the eye. g, h Axial-radial density maps for FG- and GLFG-motifs, respectively. Both types of motif localize in the dense central region, indicating that there is no spatial segregation of different types of FG-motifs in NupX-coated nanopores.

**Fig. 5** Effect of transporters on NupX-lined biomimetic pores. a Contour graphs of the Kap95 number density (grey contours) superimposed on the NupX protein density distributions (in the presence of Kap95) within a 30 nm NupX-lined nanopore (NupX-density follows the same colouring scheme as in Fig. 4b and is shown separately in Supplementary Fig. 19). The protein meshwork adapts (as compared to the distribution in Fig. 4b) to accommodate the permeating Kap95 particles. b Density distribution of inert particles superimposed on the NupX protein density distribution in a 30 nm diameter NupX-lined nanopore. Inert particles remain in the top compartment and do not permeate the NupX protein meshwork. c, d Simulation snapshots of 30 nm NupX-lined nanopores in the presence of Kap95 particles (c, black spheres with orange binding spots) and inert particles (d, black spheres), which were released in the top compartment. Kap95 particles enter and exit the NupX meshworks at either side of the nanopore, whereas inert particles remain in the top compartment. e PMF curves of Kap95 (blue) and inert particles (red) along the z-coordinate, obtained via Boltzmann inversion of the normalized density profile along the z-axis. The pore region coincides with an energy well of over 3 k_BT for Kap95, whereas inert particles experience a steep energy barrier of ~7 k_BT.

Most probably relates to the fact that the two studied reaction coordinates differ notably and cannot easily be compared: the reaction coordinate in Fig. 2i describes orthogonal entry into a brush that extends infinitely in the lateral direction, whereas the coordinate in Fig. 5e describes lateral entry and exit into the NupX assembly within the pore. As a result, one would not expect the free energy differences for transport through the nanopores to be similar to those obtained for entry into a brush geometry. Note that large free energy differences in our nanopores would also yield residence times that are orders of magnitude larger than the observed ~ms dwell times in our nanopore experiments (Fig. 3f). From our combined experimental and simulation results, we conclude that NupX-lined nanopores indeed reproduce the NPC’s remarkable selectivity towards Kap95.

**Discussion**

In this work, we introduced a 311-residue long artificial FG-Nup, termed NupX, that we rationally designed de novo based on the average properties of GLFG-type Nups (Nup49, Nup57, Nup100, Nup116, Nup145N) and which faithfully mimics the selective behaviour of the NPC. We experimentally found that substrates...
coated with NupX brushes of varying drafting densities bind selectively to Kap95, while they did not interact with the control protein (BSA)—a finding confirmed through coarse-grained MD simulations of the adsorption of Kap95 and inert particles. Consistent with these results, we found that Kap95 translocates through both uncoated and NupX-lined nanopores on a physiological (~ms) timescale, whereas BSA passage through the NupX-coated pores was effectively excluded. Coarse-grained MD simulations revealed how the NupX proteins form a dense (>150 mg/mL) phase that allows passage of Kap95 particles while excluding inert particles. Interestingly, we find that the high densities of the FG-rich NupX meshworks are comparable to those obtained in earlier simulation studies of yeast NPCs. A comparison of the intrinsic protein density (i.e., the protein density of an individual molecule in solution, quantified by the mass per unit Stokes volume) of NupX (219 mg/mL) with that of Nsp1 (74 mg/mL) explains why our NupX meshworks have the tendency to localize more compactly inside nanopore channels than Nsp1 in earlier work. The increased conductance of the dense NupX-lined nanopores (as compared to Nsp1) required a non-linear relation between the average protein density and the local conductivity, and indicates that the average protein density is not the only factor that describes conductivity; the dynamics of the unfolded proteins and the local charge distribution might be important as well.

The design strategy presented in this work allows us to assess the role of the AA sequence of the spacer regions in GLFG-Nups. Spacer residues were reported to be involved in the interaction interface of Nup-NTR complexes, highlighting a possible specific role of these domains in the binding of NTRs. In the current work, we assigned the positions of spacer residues along the NupX AA sequence entirely randomly, in both the collapsed, FG-rich low C/H ratio domain, and the extended high C/H ratio domain. This indicates that specific spacer sequence motifs are required to facilitate the fast and selective transport of NTRs like the spatial segregation of FG- and GLFG-motifs that was observed in earlier simulation studies of yeast NPCs. A comparison of the intrinsic protein density required to facilitate the fast and selective transport of NTRs like the spatial segregation of FG- and GLFG-motifs that was observed in earlier simulation studies of yeast NPCs. The consistency of the Stokes radii of different NupX domains with that of those obtained in earlier simulation studies of yeast NPCs. Notably, this does not rule out a different functional role for the AA sequence of the spacer regions in GLFG-Nups.

Methods

Analysis of GLFG-Nups and design of synthetic Nups. Protein sequences of *Saccharomyces cerevisiae* GLFG-type Nups (i.e., Nup100, Nup116, Nup49, Nup57 and Nup143N) were analyzed using a script custom-written with the R programming package (version 3.3.1). Following the definitions of high C/H-ratio and low C/H-ratio unfolded FG-Nup domains as given in Ref. 3, we obtained histograms of the AA frequencies in both the collapsed (low C/H-ratio) and extended (high C/H-ratio) domains. The collapsed/extended domain sequences of NupX were then assigned in three steps. First, the collapsed and extended domains of NupX were assigned equal lengths of 150 residues each. Then, by normalizing the distributions in Fig. 1d to the number of available residues within each domain, the total pool of AAs within each domain was obtained. Lastly, these AAs were randomly assigned a sequence index within each domain, with as a boundary condition the presence of FG and GLFG repeats spaced by ten residues within the low C/H-ratio domain. This approach was repeated iteratively in combination with disorder predictions using the online PONDR disorder prediction utility until a sufficiently disordered design was obtained. The final version of NupX sequence was also analyzed for secondary structure using DISOPRED and Phyre2. A 6-histidine tag was added to the N-terminus of the NupX sequence in order to facilitate protein purification (see Protein purification section). Finally, on the N-terminus a cysteine was included to allow the covalent coupling of the NupX protein to the surface.

Expression and purification of NupX and Kap95. The synthetic NupX gene (Genscript), appended with codons for an N-terminal His6-tag and a C-terminal cysteine residue, was cloned into pET28a and expressed in *Escherichia coli* ER2566 cells (New England Biolabs, bta2 lacZ:17 gene [lon] ompT gal sulA11 R(mcrCmrr)114::IS10). To load the protein into an AKTA Purifier system, the column was washed with buffer A (50 mM Tris/HCl pH 7.5, 300 mM NaCl, 8 M urea, 5 mg/mL 6-aminohexanoic acid supplemented with one tablet per 50 mL of EDTA-free Complete ULTRA protease inhibitor cocktail) and frozen as ‘nuggets’ in liquid nitrogen. Cells were lysed with a SPEX cryogenic grinder, after thawing 1.6-hexanediol was added to a final percentage of 5%, and the lysate was centrifuged for 30 min at 12500 × g in a Ti45 rotor (Beckman Coulter). The supernatant was loaded onto a 5 mL Talon column mounted in an AKTA Purifier system, the column was washed with buffer B (50 mM Tris/HCl pH 7.5, 300 mM NaCl, 300 mM urea, 5 mg/mL 6-aminohexanoic acid, 2.5% 1,6-hexanediol) and NupX was eluted with a
linear gradient of 0–200 mM imidazole. Peak fractions were pooled, diluted tenfold with buffer A lacking sodium chloride, loaded onto a 1 mL HiTrap SP sepharose HP column and NupX was eluted with a linear gradient of 0–1 M NaCl.

Kap95 was expressed as a C-terminal GST fusion protein in Escherichia coli ER2566 cells from plasmid pED4, a GEX-derived construct (kindly provided by Jaclyn Nowott) in which the thrombin cleavage site was replaced by a 3C protease cleavage site. Cells were grown in shaker flasks at 30 °C on LB medium supplemented with 100 µg/mL ampicillin, induction was induced at OD600–0.6 with 1 mM IPTG, and growth was continued overnight. Cells were harvested by centrifugation, washed with PBS, resuspended in TBT buffer (20 mM HEPES/NaOH pH 7.5, 110 mM KCl, 2 mM MgCl2, 0.1% (w/v) Tween20, 10 µg/mL CaCl2 and 1 mM benzamidine), and lysed by a cell disruptor (Constant Systems) at 20 kps. Following centrifugation for 30 min at 125,000 × g in a Ti45 rotor (Beckman Coulter), the supernatant was loaded onto a 2 mL GSTrap 4B column mounted in an Akta Pure system. The column was washed with TBT buffer, TBT + 1 M NaCl and TBT + 0.1 M ATP, and the fusion protein was eluted with TBT + 10 mM reduced glutathione. The GST moiety was cleaved off by overnight digestion with home-made 3C protease, and Kap95 was separated from GST and the protease by size exclusion chromatography on a Superdex 200 column pre-equilibrated with TBT buffer.

**QCM-D sample preparation and data acquisition.** QSense Analyzer gold- and SiN-coated quartz QCM-D chips were purchased from Bioin Scientific, Vistra Frölduna, Sweden. Prior to the experiment, chips were immersed in RCA-1 solution, which consisted of 30% Ammonium Hydroxide, 30% Hydrogen Peroxide and deionized (DI) water in 1:1.5 ratio, for ~30 min at 75 °C. This step was used to clean the surface from carbonate species, as well as to enrich the surface with hydroxyl groups in case of the SiN-coated chips. Chips were further rinsed with DI water in 1:1:5 ratio, for ~30 min at 75 °C. This step was used to reduce the thiol groups. Chips were further washed in PBS before the electrical measurement. Raw ionic current traces were recorded at 100 kHz bandwidth with an Axopatch 200B (Molecular devices) amplifier, and digitized (Digitida 1322A DAQ) at 250 kHz. Traces were monitored in real time using Clampex software (Molecular devices). Data were digitally filtered at 5 kHz using a Gaussian low-pass filter and analyzed using a custom-written Matlab script.

**SPR measurements and analysis.** All measurements were performed using a Biosnaps MP-SPR Navil™ 220A instrument equipped with two 670 nm laser diodes focused on two different spots on the sample surface. Both gold- and silicon dioxide-coated sensor slides were used (Biosnaps). Grading of proteins or MUTEX to the gold-coated or the silicon coated sensors was performed using the same protocol for the gold-coated QCM-D chips and for the nanopore chips, respectively. After the final incubation step, the chips were rinsed with milliQ water, ethanol and gently blow-dried with pure nitrogen. Immediately before measurements of the sensors in air, the backside of each sensor was cleaned by carefully rubbing lint-free lens tissue soaked in 2-propanol (Sigma Aldrich) followed by blow-drying both sides with air, the backside of each sensor was cleaned by carefully rubbing lint-free lens tissue soaked in 2-propanol (Sigma Aldrich) followed by blow-drying both sides with air, the backside of each sensor was cleaned by carefully rubbing lint-free lens tissue soaked in 2-propanol (Sigma Aldrich) followed by blow-drying both sides with air.

**Preparation of NupX-coated nanopores and current data acquisition.** Solid-state nanopores with diameters from 10 to 60 nm were drilled using TEM in glass-supported SiN-free standing membranes. Glass chips were purchased from Goeceptar. We refer to Ref. 46 for details on the fabrication of the chip substrate and free-standing membrane. Freshly drilled solid-state nanopores were rinsed with ultrapure water, ethanol, acetone, and isopropanol, followed by 2–5 min of oxygen plasma treatment, which was performed in order to further clean and activate the nanopore surface with hydroxyl groups. Next, chips were incubated in 2% APTES (3-aminopropyl-triethoxysilane) (Sigma Aldrich) in anhydrous toluene (Alfa Aesar) for 45–60 min at room temperature, shaking at 400 rpm, followed by 15 min in anhydrous toluene for washing. These two steps were performed in a glove-box under constant nitrogen stream in order to prevent the APTES from polymerizing. Then, chips were further rinsed with ultrapure water, ethanol, and heated at 110 °C for at least 30 min. This step was used to fixate the APTES layer by favouring further binding between the unreacted ethoxy groups.

The nanopore surface was completed with primary amines, which were subsequently reacted to Silfo-SMCC (sulphosuccinimidyl-4-(N-maleimidomethyl)-cyclohexane-1-carboxylate) (2 mg no-weight capsules (Pierce)), a crosslinker that contains NHS-ester (reacts to amines) and maleimide (reacts to thiols) groups at opposite ends, for >3 h at room temperature, shaking at 400 rpm. Chips were subsequently washed in PBS for 15 min and incubated with thiolated proteins for 2–3 h, which were pretreated with 5 mM TCEP for ~30 min in order to reduce the thiol groups. Chips were further washed in PBS before the electrical measurement. Raw ionic current traces were recorded at 100 kHz bandwidth with an Axopatch 200B (Molecular devices) amplifier, and digitized (Digitida 1322A DAQ) at 250 kHz. Traces were monitored in real time using Clammpex software (Molecular devices). Data were digitally filtered at 5 kHz using a Gaussian low-pass filter and analyzed using a custom-written Matlab script.

Dynamic light scattering (DLS) measurement of the hydrodynamic diameter. DLS experiments were performed using Zetasizer Nano ZS (Malvern). Cuvettes of 100 µL (Brand GMBH) were used for the measurement. All protein hydrodynamic diameters were measured in 150 mM KCl, 10 mM Tris, 1 mM EDTA, at pH 7.5, and averaged over three experiments. Mean value and standard deviation for each of the proteins used are reported in Supplementary Table 1. Proteins that contained exposed cysteines (NupX, Nsp1 and Nsp1-S) were pretreated with TCEP (present in at least 100x excess) in order to break disulfide bonds.

**Coarse-grained model for unfolded proteins.** All coarse-grained MD simulations were performed using our earlier developed 1BPA model for unfolded proteins21,26. This model maps complete AAs to single beads with a mass of 124 amu placed on the Cz position, separated by an average bond length (modelled as a stiff harmonic potential) with an equilibrium distance of 0.38 nm. Backbone potentials were assigned via an explicit coarse-grained mapping of Ramachandran data of a library of proteins that distinguishes flexible (i.e., Glycine), stiff (i.e., Proline) and regular AAs76. Non-bonded interactions between different AA residues are based on their respective hydrophobicity (normalized between 0 and 1 and based on the free energy of transfer between polar and apolar solvents) and obey the following interaction potential:

$$\Phi_{\text{ij}} = \frac{1}{\sigma} \left( \frac{r_{ij}}{\sigma} \right)^{\frac{3}{2}} \
$$

where \( e_{\text{ij}} = 10 \text{ kJ/mol} \) and \( e_{\text{ij}} = 13 \sqrt{\left(r_{ij}^3\right) / (\epsilon_{\text{ij}}^2)} \) kJ/mol, with \( e_{\text{ij}} \) the normalized (between 0 and 1) hydrophobicity of a residue i and a 0.27 a scaling exponent. The electrostatic interactions within the 1BPA model are described by a modified Coulomb law:

$$\Phi_{\text{el}} = \frac{\text{q}_{\text{i}} \text{q}_{\text{j}}}{4 \pi \varepsilon_{0} \varepsilon \left| r_{\text{ij}} \right|},$$

where the electrostatic interactions are modulated via a Debye screening constant. This form of electrostatics takes into account the salt concentration (set at 150 mM here, via a screening length \( \kappa = 1.27 \text{ nm}^{-1} \)) together with a solvent polarity at short distances via a distance-dependent dielectric constant:

$$r_{\text{ij}} = 80 \left( 1 - \frac{r_{ij}^2}{\sigma^2} \right)^{\frac{1}{2}} \left( \epsilon - 1 \right),$$

where \( \sigma = 0.25 \). Non-bonded interactions are cut-off at 2.5 nm (hydrophobic interactions) or 5.0 nm (electrostatic interactions). Since the 1BPA model operates on the relation:

$$\Gamma = \frac{pN_{\text{p}}}{M},$$

where \( p \) is the density (\( p_{\text{water}} = 1.09 \text{ g/cm}^3 \), \( p_{\text{protein}} = 1.35 \text{ g/cm}^3 \), Ref. 79), \( N_{\text{A}} \) is Avogadro’s constant, and \( M \) is the molecular weight (\( M_{\text{NupX}} = 380 \text{ Da} \), \( M_{\text{Nsp1}} = 65.7 \text{ kDa} \), \( M_{\text{Nsp1-S}} = 62.1 \text{ kDa} \), \( M_{\text{Nsp1-S}} = 32.5 \text{ kDa} \).
Density distributions and nanopore conductance from nanopore simulations.

Axi-radial density maps were obtained from NupX nanopore simulation trajectories using g_wham48 utility of GROMACS, where a bin size of 0.5 nm was used to construct number densities within a cylinder centered on the nanopore. Average densities were extracted for the pore and access regions by averaging the axi-radial density distributions over the coordinate ranges $|z| \leq 10$ nm and $10$ nm $< |z| < 40$ nm, respectively.

The conductance of X-lined nanopores was obtained by assuming that the conductance $G(d)$ is governed by a modified Hall-formula$^{11,35}$,

$$G(d) = \left( \frac{4l}{\sigma_{\text{pore}} + \frac{1}{\sigma_{\text{access}}}} \right)^{-1},$$

where $l = 20$ nm is the height of the nanopore, $d$ denotes the diameter (15–70 nm), and $\sigma_{\text{pore}}$ and $\sigma_{\text{access}}$ denote the conductivities in the pore and access regions, respectively. The conductivities in both regions can be extracted from the axi-radial density distributions by integrating and normalizing the local conductivity over the pore diameter and corresponding height ranges.

Calculating Stokes radius of NupX and variations.

Intrinsically disordered proteins were modelled using the IBA model$^{5,76}$, starting from an extended configuration. After energy minimization (steepest descent) and a brief (5 ns) equilibration step, we simulated the individual proteins for $5 \times 10^8$ steps using a timestep of 20 fs (total simulation time: 10 μs). Conformations were extracted every 10,000 frames (i.e., every 200 ps). In order to calculate the Stokes radii $(R_s)$ from the MD trajectories, we extracted protein conformations every 2 ns and applied the HYDRO+ software$^{78}$ in order to calculate the $R_s$ values. This procedure yields a total of 5000 Stokes radii per protein.

Calculating NupX brush density profiles and PMF curves of cargo adsorption.

We modelled the brush substrate as a fully triangulated (sometimes denoted as hexagonally) closed-packed array of sterically inert beads with a diameter of 3 nm. NupX proteins were tethered on top of the scaffold by their C-terminus, following an equilateral triangular lattice with a uniform grafting distance of 4.0 or 5.7 nm. A fully triangulated lattice is close-packed in two dimensions, meaning that a unique length scale sets the grafting density. The simulation box consisted of a 24 × 24 × 81.5 nm$^3$ triclinic and fully periodic unit cell (Fig. 2h). In our simulation of a less dense brush (grafting distance of 5.7 nm), the simulation box size was accordingly scaled up to 34.2 nm in the lateral dimensions. The grafting pattern of the NupX proteins was placed such as to ensure homogeneity of the NupX brush in the lateral scaled up to 34.2 nm in the lateral dimensions. The grafting pattern of the NupX surfaces using the SPR technique and should be taken as a lower limit given that SiN nanopores as cylindrically shaped occlusions in a membrane constituted Coarse-grained MD simulations of NupX-lined nanopores.
Yamada, J. et al. A bimodal distribution of two distinct categories of FG nucleoporins is required to mediate nuclear import of RanGDP. *J. Mol. Biol.* 501, 579–593 (2020).

Ghavami, A., van der Giessen, E. & Onck, P. R. Energetics of transport and kinetic analysis of multivalent binding with FG nucleoporins. *Biophys. J.* 105, 290–300 (2013).

Timney, B. L. et al. Simple rules for passive diffusion through the nuclear pore complex. *J. Cell Biol.* 110, 883–894 (1990).

Yamada, J. et al. A bimodal distribution of two distinct categories of intrinsically disordered structures with separate functions in FG nucleoporins. *Mol. Cell. Proteom.* 9, 2205–2224 (2010).

Terry, L. J. & Wente, S. R. Flexible gates: dynamic topologies and functions for nuclear pore complexes in nucleocytoplasmic transport. *Eukaryot. Cell* 8, 1814–1827 (2009).

Bayliss, R. et al. Interaction between NTFP and xFeFG-containing nucleoporins is required to mediate nuclear import of RanGDP. *J. Mol. Biol.* 293, 579–593 (1999).

Ghavami, A., van der Giessen, E. & Onck, P. R. Energetics of transport through the nuclear pore complex. *PLoS One* 11, e0148876 (2016).

Popken, P., Ghavami, A., Onck, P. R., Poolman, B. & Veenhoff, L. M. Size-dependent leak of soluble and membrane proteins through the yeast nuclear pore complex. *Mol. Biol. Cell* 26, 1386–1394 (2015).

Timney, B. L. et al. Simple rules for passive diffusion through the nuclear pore complex. *J. Cell Biol.* 206, 1016010016 (2016) https://doi.org/10.1083/jcb.201610016.

Lim, R. Y. H. et al. Flexible phenylalanine-glycine nucleoporins as entropic barriers to nucleocytoplasmic transport. *Proc. Natl Acad. Sci. USA* 103, 9512–9517 (2006).

Bayliss, R., Littlewood, T. & Stewart, M. Structural basis for the interaction between PiFG nucleoporin repeats and importin-β in nuclear trafficking. *Cell* 102, 99–108 (2000).

Rout, M. P., Aitchison, J. D., Magnasco, M. O. & Chait, B. T. Virtual gating barriers to nucleocytoplasmic transport. *Trends Cell Biol.* 16, 622–628 (2003).

Frey, S. & Görlich, D. A saturated FG-repeat hydrogel can reproduce the permeability properties of nuclear pore complexes. *Cell* 130, 512–523 (2007).

Frey, S. FG-rich repeats of nuclear pore proteins with hydrogel-like properties. *Science* 314, 815–818 (2006).

Peters, R. Translocation through the nuclear pore complex: selectivity and speed by reduction-of-dimensionality. *Traffic* 6, 421–427 (2005).

Kapinos, L. E., Schoch, R. L., Wagner, R. S., Schleicher, K. D. & Lim, R. Y. H. Karyopherin-centric control of nuclear pores based on molecular occupancy and kinetic analysis of multivalent binding with FG nucleoporins. *Biophys. J.* 106, 1751–1762 (2014).

Kapinos, L. E., Huang, B., Rencurel, C. & Lim, R. Y. H. Karyopherins regulate nuclear pore complex barrier and transport function. *J. Cell Biol.* 216, 3609–3624 (2017).

Schleicher, K. D. & Schleicher, R. K. et al. Selective transport control on molecular velcro made from intrinsically disordered proteins. *Nat. Nanotechnol.* 9, 525–530 (2014).

Lim, R. Y. H. et al. Nanomechanical basis of selective gating by the nuclear pore complex. *Science* 318, 640–643 (2007).

Adams, R. L., Timney, B. J. & Wente, S. R. A novel Saccharomyces cerevisiae FG nucleoporin mutant collection for use in nuclear pore complex functional experiments. *G3 (Beltsville)* 6, 51–58 (2016).

Yang, W., Gelles, J. & Musser, S. Imaging of single-molecule translocation through nuclear pore complexes. *Proc. Natl Acad. Sci. USA* 101, 12887–12892 (2004).

Ma, J., Goryaynov, A. & Yang, W. Super-resolution 3D tomography of interactions and competition in the nuclear pore complex. *Nat. Struct. Mol. Biol.* 23, 239–247 (2016).

Beck, M. & Hurt, E. The nuclear pore complex: understanding its function through structural insight. *Nat. Rev. Mol. Cell Biol.* 18, 73–89 (2016).

Lin, D. H. & Hoelz, A. The structure of the nuclear pore complex (an update). *Annu. Rev. Biochem.* 88, annurev-biochem-062917-011901 (2019).

Tagliazucchi, M. & Wente, S. R. Artificial nuclear pore complexes. *Nat. Nanotechnol.* 6, 433–438 (2011).

Kapinos, L. E., Schoch, R. L., Wagner, R. S., Schleicher, K. D. & Lim, R. Y. H. Karyopherin-centric control of nuclear pores based on molecular occupancy and kinetic analysis of multivalent binding with FG nucleoporins. *Biophys. J.* 106, 1751–1762 (2014).

Schmidt, H. B. & Görlich, D. Nup98 FG domains from diverse species spontaneously phase-separate into particles with nuclear pore-like permeeselectivity. *Elife* 4, e04251 (2015).

Ananth, A. N. et al. Spatial structure of disordered proteins dictates conductance and selectivity in nucleopore complex mimics. *Elife* 7, e31510 (2018).

Fisher, P. D. E. et al. A programmable DNA origami platform for organizing intrinsically disordered nucleoporins within nanopore confinement. *ACS Nano* 12, 1508–1518 (2018).

Ketterer, P. et al. DNA origami scaffold for studying intrinsically disordered proteins of the nuclear pore complex. *Nat. Commun.* 9, 902 (2018).

Huan, K., Tagliazucchi, M., Park, S. H., Rabin, Y. & Seifter, L. Nanocompartmentalization of the nuclear pore lumen. *Biophys. J.* 118, 219–231 (2020).

Zahn, R. J., Sodhi, J. S., McGuffin, L. J., Buxton, R. B. & Jones, D. T. Prediction and functional analysis of native disorder in proteins from the three kingdoms of life. *J. Mol. Biol.* 337, 635–645 (2004).

Kelley, L. A., Mezulis, S., Yates, C. M., Wass, M. N. & Sternberg, M. J. E. The Phyre2 web portal for protein modeling, prediction and analysis. *Nat. Protoc.* 7, 845–858 (2012).

Hayama, R. et al. Interactions of nuclear transport factors and surface-conjugated FG nucleoporins: insights and limitations. *PLoS One* 14, e0217897 (2019).

Reviakine, I., Johannsmann, D. & Richter, R. P. Hearing what you cannot see and visualizing what you hear: Interpreting quartz crystal microbalance data from solvated interfaces. *Anal. Chem.* 83, 8538–8848 (2011).

Wagner, R. S., Kapinos, L. E., Marshall, N. J., Stewart, M. & Lim, R. Y. H. Promiscuous binding of Karyopherinβ1 modulates FG nucleoporin barrier function and expedites NTFP transport kinetics. *Biophys. J.* 108, 918–927 (2015).

Kapinos, L. E., Huang, B., Rencurel, C. & Lim, R. Y. H. Karyopherins regulate nuclear pore complex barrier and transport function. *J. Cell Biol.* 216, 3609–3624 (2017).

Hub, J. S., de Groot, B. L. & van der Spoel, D. g. wham—a free weighted histogram analysis implementation including robust error and autocorrelation estimates. *J. Chem. Theory Comput.* 6, 3713–3720 (2010).

Kowalczyk, S. W., Grosberg, A. Y., Rabin, Y. & Dekker, C. Modeling the conductance and DNA blockade of solid-state nanopores. *Nanotechnology* 22, 315101 (2011).

Fragasso, A., Pud, S. & Dekker, C. 1/f noise in solid-state nanopores. Nanotechnology 20, 291–205 (2007).

Balan, A., Chien, C. C., Enggelke, R. & Drudic, M. Suspended solid-state membranes on glass chips with sub 1-pF capacitance for biomolecule sensing applications. *Sci. Rep.* 5, 1–8 (2015).

Kowalczyk, S. W., Grosberg, A. Y., Rabin, Y. & Dekker, C. Modeling the conductance and DNA blockade of solid-state nanopores. *Nanotechnology* 22, 315101 (2011).

Varghesevarakul, N., Song, J., Meller, A. & Greenfield, M. W. Single-molecule protein sensing in a nanopore: a tutorial. *Chem. Soc. Rev.* 47, 8512–8524 (2018).
56. Koutsionikas, A. G., Spiliopoulos, N., Anastassopoulos, D. L., Vradis, A. A. & Topraczioglu, C. Formation of polymer brushes inside cylindrical pores: a computer simulation study. J. Chem. Phys. 131, 44901 (2009).
57. Dimitrov, D. I., Milchev, A. & Binder, K. Polymer brushes in cylindrical pores: simulation versus scaling theory. J. Chem. Phys. 125, 34095 (2006).
58. Peleg, O., Tagliazucchi, M., Kröger, M., Rabin, Y. & Slezifer, I. Morphology control of hairy nanopolymers. ACS Nano 5, 4737–4747 (2011).
59. Ro, S., Gopinath lue, A. & Kim, Y. W. Interactions between a fluctuating polymer barrier and transport factors together with enzyme action are sufficient for selective and rapid transport through the nuclear pore complex. Phys. Rev. E 98, 12403 (2018).
60. Tetenbaum-Novatt, J., Hough, L. E., Mironska, R., McKenney, A. S. & Rout, M. P. Nucleocytoplasmic transport: a role for nonspecific competition in karyopherin-nucleoporin interactions. Mol. Cell. Proteomics 11, 31–46 (2012).
61. Ribbeck, K. & Görlich, D. Kinetic analysis of translocation through nuclear pore complexes. EMBO J. 20, 1320–1330 (2001).
62. Milles, S. et al. Plasticity of an ultrafast interaction between nucleoporins and nuclear transport receptors. Cell 163, 734–745 (2015).
63. Liu, S. M. & Stewart, M. Structural basis for the high-affinity binding of Nucleoporin Nup1p to the Saccharomyces cerevisiae importin-β homologue, Kap95p. J. Mol. Biol. 349, 515–525 (2005).
64. Ravhe, B. et al. Slide-and-exchange mechanism for rapid and selective transport through the nuclear pore complex. Proc. Natl Acad. Sci. USA 113, E2489–E2497 (2016).
65. Buddingh’, B. C. & van Hest, J. C. M. Artificial cells: synthetic compartments with life-like functionality and adaptivity. Acc. Chem. Res. 50, 769–777 (2017).
66. Spoelstra, W. K., Deshpande, S. & Dekker, C. Tailoring the appearance: what will synthetic cells look like? Curr. Opin. Biotechnol. 51, 47–56 (2018).
67. Das, R. K. & Pappu, R. V. Conformations of intrinsically disordered proteins are encoded by linear sequence distributions of oppositely charged residues. Proc. Natl Acad. Sci. USA 110, 13392–13397 (2013).
68. Dignon, G. L., Zheng, W., Best, R. B., Kim, Y. C. & Mittal, J. Relation between single-molecule properties and phase behavior of intrinsically disordered proteins. Proc. Natl Acad. Sci. USA 115, 9929–9934 (2018).
69. Huang, F. S., Boyken, S. E. & Baker, D. The coming of age of de novo protein design. Nature 527, 320–327 (2016).
70. Emilsson, G. et al. Strongly stretched protein resistant poly(ethylene glycol) brushes prepared by grafting-to. ACS Appl. Mater. Interfaces 7, 7505–7515 (2015).
71. Emilsson, G. et al. Surface plasmon resonance methodology for monitoring polymerization kinetics and morphology changes of brushes—evaluated with poly(N-isopropylacrylamide). Appl. Surf. Sci. 396, 384–392 (2017).
72. Ferrand-Drake Del Castillo, G., Emilsson, G. & Dahlin, A. Quantitative analysis of thickness and pH actuation of weak polyelectrolyte brushes. J. Phys. Chem. C. 122, 27516–27527 (2018).
73. Benesch, J., Askendal, A. & Tengvall, P. The determination of thickness and surface mass density of mesothick immunoprecipitate layers by null ellipsometry and protein 125Iodine labeling. J. Colloid Interface Sci. 249, 84–90 (2002).
74. Mark, J. E. Polymer Data Handbook (Oxford University Press, New York, 1999).
75. Plesa, C. & Dekker, C. Data analysis methods for solid-state nanopolymers. Nanotechnology 26, 084003 (2015).
76. Ghavami, A., van der Giessen, E. & Onck, P. R. Coarse-grained potentials for local interactions in unfocused proteins. J. Chem. Theory Comput. 9, 432–440 (2013).
77. Van Der Spoel, D. et al. GROMACS: fast, flexible, and free. J. Comput. Chem. 26, 1701–1718 (2005).
78. Ortega, A., Amorós, D. & García de la Torre, J. Prediction of hydrodynamic and other solution properties of rigid proteins from atomic- and residue-level models. Biophys. J. 101, 892–898 (2011).
79. Mishra, A. et al. The effect of FG-Nup phosphorylation on NPC selectivity: A one-bead-per-amino-acid molecular dynamics study. Int. J. Mol. Sci. 20, 396 (2019).
80. Humphrey, W., Dalke, A. & Schulten, K. VMD: visual molecular dynamics. J. Mol. Graph. 14, 33–38 (1996).

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Author contributions
A.F. and C.D. devised the experiments. H.W.d.V., E.v.d.G. and P.R.O. devised the simulations. E.O.v.d.S. cloned and purified the proteins. A.F. carried out the QCM-D and nanopore experiments and analysis. J.A. and A.D. carried out the SPR experiments and analysis. H.W.d.V. carried out the simulations and analysis. A.F., H.W.d.V., P.R.O. and C.D. wrote the manuscript.

Competing interests
The authors declare no competing interests.

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